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Biology of the Marine Intertidal Mollusc Nuttallina, with Special Reference to Vertical Zonation, Taxonomy and Biogeography

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Biology of the Marine Intertidal Mollusc *Nuttallina*, with Special Reference to Vertical Zonation, Taxonomy and Biogeography

A dissertation submitted in partial satisfaction of the

requirements for the degree Doctor of Philosophy

in Marine Biology

by

Stephen Charles Piper

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  Professor Nicholas D. Holland
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1984

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1984

This dissertation is dedicated to the memory of a guy with an incredible zest for life, an insatiable curiosity about all things, a pragmatic dedication to excellence and a sense of humor, honesty, and warmth that I'll always miss, to my dad,

EDWIN MERLE PIPER, SR.

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PUBLICATIONS

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Kooyman, G. L., M. S. Castellini, D. P. Costa, J. O. Billups, and S. [C.] Piper. 1979. Diving characteristics of free-ranging Weddell seals. Antarctic Journal of the United States 14(5): 176.

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ABSTRACT OF THE DISSERTATION

Biology of the Marine Intertidal Mollusc *Nuttallina*, with Special Reference to Vertical Zonation, Taxonomy and Biogeography

by

Stephen Charles Piper
Doctor of Philosophy in Marine Biology
University of California, San Diego, 1984
Professor William A. Newman, Chairman

Variation in physical conditions that commonly occurs only over hundreds of kilometers on land or in the ocean can take place in a vertical meter or less in the intertidal zone. The abundant intertidal chiton *Nuttallina* (Mollusca: Polyplacophora) was selected for a comparative study of adaptation to the widely-disparate conditions of the high and low shore, because it occupies a broad range in the intertidal zone, from 0 to 1.8 m above MLLW.

In addition to having different population characteristics and greater body water contents, *Nuttallina* at the higher and more exposed positions on the shore tend to be much larger than low shore *Nuttallina*. A fourteen-month field study of long-term movement of tagged specimens indicated that *Nuttallina* is an excellent homer. Observed displacements are usually short and haphazardly-directed. Low shore specimens exhibit fewer and shorter displacements than do high shore ones. Thus, upshore migration does not explain the observed shore-level size gradient in *Nuttallina*. On the other hand, faster growth of tagged high shore specimens suggests that differential growth contributes to the size gradient.

Analysis of 14 presumptive loci by starch gel electrophoresis revealed that the high and low shore populations are different species. Indeed, there are three sympatric species of *Nuttallina* in California, rather than one or two as concluded by many earlier workers on the basis of external morphology alone. This work represents the first electrophoretic study on chitons.

An exhaustive search for morphological differences was facilitated by the ability to identify specimens electrophoretically. The number of gills proved to be the most useful single character for distinguishing the species. Classification functions generated by multivariate (discriminant) analysis permitted identification of the dried museum type specimens referable to *Nuttallina* and formal assignment of species names.

Nuttallina californica, a cold-water species, occurs primarily north of Point Conception, although it is found also in cold-water upwelling areas along the Pacific Coast of Baja California. *Nuttallina fluxa*, a warm-water species, occurs in high intertidal areas south of Point Conception, perhaps as far south as Bahia Magdalena. *Nuttallina kata* sp. nov. lives in the low intertidal zone primarily south of Point Conception.

CHAPTER I

GENERAL INTRODUCTION

VERTICAL INTERTIDAL ZONATION AND THE STUDY ANIMAL NUTTALLINA

The marine intertidal zone, that area of the seashore between the tides, extends worldwide and is an important marine habitat. Although it comprises only a very thin margin of the expansive ocean basins, the intertidal zone supports a tremendous biomass of animals and plants. As the boundary between the land and the sea, the intertidal zone is characterized by widely varying conditions -- nearly continuous subaerial exposure at the upper edge contrasts with ever-occurring submergence at the seaward edge while tides, waves, and topographic features contribute to an irregular gradation from terrestrial to marine conditions in between. A phenomenal diversity of microhabitat is packed into the small vertical range of the shore, and vertical zonation of intertidal animals and plants is evident everywhere in the world (e.g., Ricketts and Calvin, 1968; Stephenson and Stephenson, 1972).

The intertidal zone is an excellent arena for a variety of biological studies. Variation in physical conditions that commonly occurs only over hundreds of kilometers on land or in the ocean usually takes place over a short distance of only a meter or less in the intertidal zone. From studies on temperature acclimation of water-pumping rates in the mussel <u>Mytilus californianus</u>, Segal, Rao, and James (1953) concluded that the environmental gradient represented by a vertical meter or two in the intertidal zone is comparable to hundreds of kilometers of latitude along the California coast. Geographic separation, a factor that often complicates work in other major habitats due to problems in interpreting results as well as in carrying out the work, is avoided in intertidal studies.

Initially, the goal of the present study was to focus on environmental physiological-biochemical aspects of the biology of animals in relation to subaerial exposure. The tides effect abrupt changes from subaerial to submerged conditions, producing concomitant rapid changes in temperature, humidity/water-balance, carbon-dioxide/oxygen levels, and salinity balance, among other factors. To date, most workers have concentrated on the lethal effects of desiccation, extreme temperatures and hypo- or hyperosmotic conditions, and the physiological and/or biochemical responses of intertidal animals and plants to these phenomena. The myriad potential physiological-biochemical responses that intertidal organisms might exhibit under typical sublethal conditions during a representative subaerial exposure period have largely been neglected.

A common intertidal chiton, <u>Nuttallina</u> (Mollusca: Polyplacophora), was considered nearly ideal for the present study for a number of reasons. In Southern California, <u>Nuttallina</u> populations extend quite high in the intertidal zone, to about 1.5 to 1.8 m above mean lower low water (MLLW). Thus, at the upper end of their vertical range, <u>Nuttallina</u> specimens are subject to prolonged periods of subaerial exposure. Although several other animals, such as a barnacle, <u>Chthamalus</u>, a periwinkle, <u>Littorina</u>, and often limpets of the genus <u>Collisella</u>

(formerly Acmaea, in part) extend higher up the seashore than does Nuttallina, the latter attains much larger sizes and in this respect is much easier to work with. Further, Nuttallina occupies an extensive vertical range, from about 0 to 0.3 m above MLLW to its upper limit, so that comparisons among specimens subject to dramatically different exposure regimes at a single shore site are possible. Such comparative work can provide a useful perspective for investigating responses of high shore specimens.

Nuttallina occurs in great abundance at many locations so that the problem of obtaining sufficient numbers for study is minimized; for example, in the La Jolla, CA area, densities exceeding 2000 per m^2 have been recorded. The abundance of Nuttallina makes this animal of considerable interest as a subject for study; it is a conspicuous and important member of rocky intertidal communities, particularly on soft substrate shores in Southern California. Surprisingly, however, aside from its inclusion in several taxonomic studies and in general ecological surveys and studies, Nuttallina has been relatively neglected by researchers, even though this chiton is locally abundant in San Diego, Los Angeles, Monterey Bay and Bodega Bay, California, where institutions with prominent marine biological research programs are located.

THE FLOW OF RESEARCH: AN UNEXPECTED TURN OF EVENTS

At the outset of the present studies, Nuttallina appeared to be free of the taxonomic and/or identification problems that can complicate the study of other groups. Nuttallina was reported to be a single species north of Point Conception, namely N. californica, and a second species south of the Point, N. fluxa (Smith, 1977). Indeed, most recent workers, including taxonomists as well as ecologists, believed the two Nuttallina species to be synonymous. This belief was formalized during the course of the present studies by Ferreira (1982), who synonymized N. fluxa under N. californica in his revision of the chiton family Lepidochitonidae. Therefore, in the early phases of the present studies, it seemed reasonable to assume that all Nuttallina specimens from south of Point Conception in general, and in La Jolla, CA in particular, belonged to a single species.

The taxonomic situation of Nuttallina was in apparent contrast to that of the intertidal limpets that have been studied so extensively. Acmaeid limpets are represented by about fourteen rock-inhabiting species along the Pacific Coast of North America. There is overlap in the morphology of the different species so that individual specimens of a species are not always easily identified. For example, the mid- to high intertidal species "Collisella digitalis" is phenotypically quite plastic and exists in at least two ecotypic forms, one in beds of the stalked barnacle, Pollicipes polymerus, and the other higher on intertidal rock surfaces (Giesel, 1970). Apparently, "Collisella digitalis" exists as a sibling species pair north and south of Point Conception, CA (digitalis north, austrodigitalis south, Murphy, 1978). Furthermore, C. digitalis is often difficult to distinguish from another, sympatric, high intertidal species, C. scabra. It should be noted that as herbivorous, grazing rock dwellers, limpets bear many similarities to chitons. Thus, although limpets are in a different molluscan class from chitons, the wealth of information on limpets will be used liberally in discussing chitons throughout the present work.

My preliminary studies, on the comparative physiology of high and low shore populations of Nuttallina, produced provocative results on body water content and desiccation, but work was hampered by a persistent problem of obtaining specimens of similar size from the different shore levels. Indeed, a fairly well-developed shore-level size gradient in Nuttallina was discovered; larger specimens tended to occur at higher and more exposed positions on the shore. Further studies, on gross morphology and general population characteristics, revealed several other differences in Nuttallina with shore height. These findings prompted intensive studies on long-term movement and growth of high and low shore specimens. Results of these latter studies suggested that there is little interchange between adult members of the high and low shore populations and that high shore specimens grow faster than low shore ones. These findings prompted an electrophoretic examination of the taxonomic status of the high and low shore Nuttallina populations in La Jolla, CA.

Electrophoretic studies indicated the presence of three Nuttallina species in California, rather than just two as concluded by Smith (1977) or one as concluded by Ferreira (1982). Although morphological differences theoretically need not exist among such cryptic, or sibling, species, it was considered of paramount importance to establish some means of identifying Nuttallina specimens without the use of electrophoresis. Electrophoresis is a powerful taxonomic tool, but it is a relatively time-consuming and technical procedure so that it cannot be used routinely for identification work. Without a practicable method for identifying specimens, ecologists, as well as taxonomists, are less apt to find the species distinctions useful, rather than confusing as in the case of the limpet, Collisella austrodigitalis, another presumptive sibling species discovered by Murphy (1978) (J.H. McLean, L.A. County Museum of Natural History, in litt., March, 1981). Electrophoretic data provided an effective means of grouping specimens for morphological examination, which made it possible to establish consistent external morphological differences among the three species.

The diagnostic morphological characters for Nuttallina were used to identify dry museum type specimens and to formally assign names to the three species. The low intertidal Nuttallina population at La Jolla, CA proved to be a new, previously-undescribed species, N. kata. The high intertidal population at La Jolla, CA belonged to another species that was resurrected as the previously-described California species, N. fluxa. The third species, N. californica, occurs only rarely in La Jolla. Further study, involving both electrophoretic and external morphological identification of specimens, produced characterizations of the intertidal and biogeographic distributions of the three Nuttallina species throughout their ranges in California and Baja California.

Fortunately, sufficient morphological data had been recorded during the earlier movement and growth studies so that identifications of specimens used in those studies could be made according to the new species scheme. Data from the two studies were re-analyzed, and refined interpretations were made concerning the dynamics of high and low shore populations of Nuttallina in light of the new species scheme.

Thus, work that was aimed initially at exploring the ecological physiology of high and low shore populations of Nuttallina unexpectedly became a detailed refutation of the original well-supported assumption that high and low shore populations of Nuttallina in La Jolla and elsewhere in Southern California are conspecific. Key factors that permitted the unexpected taxonomic revision of Nuttallina were the use of vertical zonation as the framework for study and the use of electrophoresis, a powerful taxonomic tool. The taxonomic revision, in turn, allowed more refined interpretations to be made about the population dynamics of Nuttallina with respect to vertical zonation.

ORGANIZATION OF THE THESIS

Chapters III and IV, the taxonomic chapters, describe the discovery of the new species, Nuttallina kata, and the resurrection of Nuttallina fluxa. All other studies were completed before the taxonomic investigations. Those studies for which appropriate morphological data had been recorded so that identification of specimens according to the new species scheme was possible, i.e., Chapters V and VI, have been placed after the taxonomic chapters, Chapters III and IV. Studies for which appropriate diagnostic morphological data are lacking have been placed before the taxonomic chapters, in Chapter II, and in Appendices II and IV. Despite the lack of diagnostic morphological data in these latter studies, intertidal distributional information obtained in Chapter III clearly indicates that Nuttallina specimens living in the "high and dry" habitat at La Jolla belong to Nuttallina fluxa, whereas specimens in the "low" intertidal areas are primarily *Nuttallina kata* sp. nov.

Chapter II provides a general introduction to tides and marine intertidal vertical zonation. The primary study site is described and various tidal characteristics, calculated for San Diego, CA, are presented. Shore-level variation in gross morphology and population characteristics of *Nuttallina* are documented and discussed. Supplementary information is presented in Appendix I.

Chapters III and IV present a biochemical and morphological revision of *Nuttallina* as well as findings on the intertidal distribution and biogeography of the three *Nuttallina* species. Appendix III details the taxonomic status of all other species assigned to the genus *Nuttallina* in the present or the past.

Chapter V describes a long-term movement study involving two of the species, *Nuttallina fluxa* and *N. kata*. A tag was developed for the movement study and the effect of the tag on growth and mortality rates of *Nuttallina* was assessed in a laboratory study. This latter study is the subject of Appendix IV.

Chapter VI reports the results of a field study on growth in *Nuttallina fluxa* and *N. kata*. Importantly, while the results of the movement study contraindicate the possibility that an upshore migration produces the observed shore-level size gradient in *Nuttallina* at La Jolla, the growth study suggests that faster growth of the high shore species, *N. fluxa*, may be a contributing factor.

Finally, Appendix II presents findings of an investigation on the body water content of low and high shore groups of Nuttallina. The results are of interest with regard to desiccation, a factor often cited as of importance for high shore organisms.

CHAPTER II

PHYSICAL CONDITIONS AND VERTICAL ZONATION ON A SOUTHERN CALIFORNIA SHORE

INTRODUCTION

Organisms inhabiting the marine intertidal zone are subject to a unique suite of environmental conditions. Because of the action of waves and tides, intertidal organisms alternately experience marine and terrestrial conditions. The rise and fall of the tides produces a pattern such that higher intertidal organisms are exposed to air for longer periods of time than are lower ones. The abrupt gradation in environmental conditions makes the intertidal zone an excellent arena for biological studies, since a "natural experiment" is constantly in progress. Several environmental factors vary in a reasonably predictable manner up the shore over a very short horizontal distance, uncomplicated by problems of geographic separation. From studies on temperature acclimation of water-pumping rates in *Mytilus californianus*, Segal, Rao and James (1953) have concluded that the environmental gradient experienced in a vertical meter or two in the intertidal zone is comparable to that experienced in several hundred kilometers of latitude along the California coast.

The tides are highly predictable and the tidal regime of a locale can be characterized by examination of the appropriate tide tables. Tidal regimes differ considerably from locality to locality, in period type, in regularity and in range of ebb and flow. As an example of the extreme tidal pattern variability that can occur in very small geographic areas, while the tidal range of the upper Bay of Fundy, Canada is as great as 15.2 m, within a 240 km radius tidal ranges of only 15 cm occur (see Bleakney, 1972). On a relatively short stretch of the Caribbean-Gulf of Mexico coasts of Mexico and Guatemala, the four major tide types, regular and mixed diurnal and semidiurnal, can be found (Doty, 1957). Tides are not as diverse on open coastlines such as the California coast, but, too often, intertidal studies are presented without a sufficient description of local tide patterns, so that it is difficult to interpret and compare effectively the results of studies from different parts of the world.

In this chapter, the tidal regime of the southern California coast will be examined and briefly compared to that of other areas, and subaerial low tide conditions will be characterized. Particular attention will be paid to the vertical variation in predicted and actual environmental conditions. In addition, a site in La Jolla (San Diego Co.), CA, used for studies in several subsequent chapters, will be described in some detail, and the distribution and characteristics of populations of the chiton Nuttallina will be reported, with emphasis on vertical intertidal variation.

ANNUAL CYCLE OF LOW TIDES

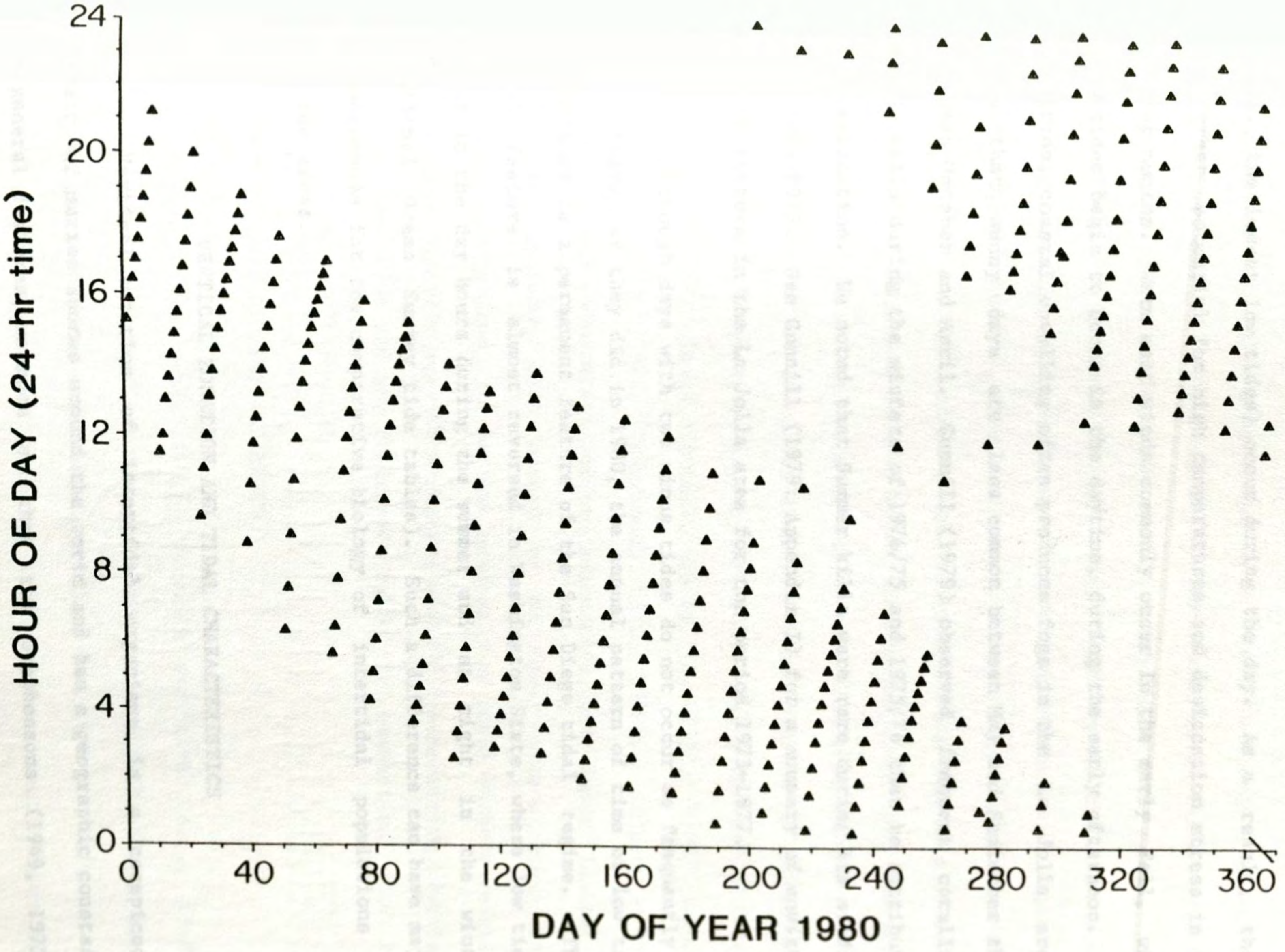
Tides along the Pacific Coast of the United States are of the mixed (or irregular) semidiurnal type. Two lows and two high tides, that is two full tidal cycles, occur each day. "Mixed" refers to the fact that the two highs as well as the lows are usually of different heights. The tides are not identical from day to day but are influenced by astronomical events with various periods (e.g., fortnight, semiannual, 8.8 years, 18.6 years; Rinehart, 1972). Since the moon is a major factor in producing the tides and it revolves around the earth on about a 28 day period (i.e., it traverses about 1/28 th of its orbit around the earth in one solar day), two full tidal cycles are completed in about 24 hours and 50 minutes, rather than in 24 hours; therefore, the tides occur approximately an hour later on successive days.

Times and heights of tides at the Broadway Pier, San Diego in 1980, compiled by the National Ocean Survey (U.S. Dept. of Commerce, formerly the U.S. Coast and Geodetic Survey), have been used to generate a plot of the time of the lowest tide for each day versus the day of the year, with the aid of a computer. The results are illustrated in Figure II-1.

The low tides not only occur later each day through a near-fortnightly period before "resetting" at a time near the one two weeks earlier, but the times of the low tides vary on an annual (solar) cycle such that, during Winter, the lowest tides occur in the afternoon-evening hours, whereas, in Summer, the lowest tides occur in the predawn-morning hours. In March-April and September-October, 1980, on several days both low tides are very low (minus) tides as a transition occurs between the different Winter and Summer times of low tide occurrence.

Variation in the times of day that low tides occur can have major consequences for the subaerial conditions that an intertidal

Figure II-1. Times of daily low tides through the year. The predicted lowest tide occurring each day in 1980 at San Diego Station (Broadway Pier) has been plotted. Predicted times and heights of tides tabulated by the National Ocean Survey (U.S. Department of Commerce), corrected to daylight savings time, have been used for calculations. Day #1 = 1 Jan 1980; day #366 = 31 Dec 1980.


HOUR OF DAY (24-hr time)
24
20
16
12
8
4
0
0
40
80
120
160
200
240
280
320
360
DAY OF YEAR 1980

organism experiences during different seasons. From September to May in San Diego, long intervals of subaerial exposure of the intertidal zone (i.e., the lowest low tides) occur during the day. As a result, there is great potential for high temperatures and desiccation stress in the Winter months. Warm east winds commonly occur in the early Fall, when low tides begin to occur in the daytime, during the early afternoon. In addition, coastal upwelling often produces fogs in the La Jolla area, such that sunny days are less common between May and September than between October and April. Gunnill (1979) observed frequent coralline algae kills during the winters of 1974/75 and 1975/76 that he attributed to desiccation. He noted that Summer kills were rare during his studies in 1973-1977. See Gunnill (1979: Appendix I) for a summary of environmental factors in the La Jolla area for the period 1973-1977.

Although days with two minus tides do not occur as frequently in most years as they did in 1980, the annual pattern of time of low tide occurrence is a permanent feature of the San Diego tidal regime. This tidal feature is almost reversed in Washington State, where low tides occur in the day hours during the summer and at night in the winter (National Ocean Survey tide tables). Such a difference can have major consequences for the comparative biology of intertidal populations in the two areas.

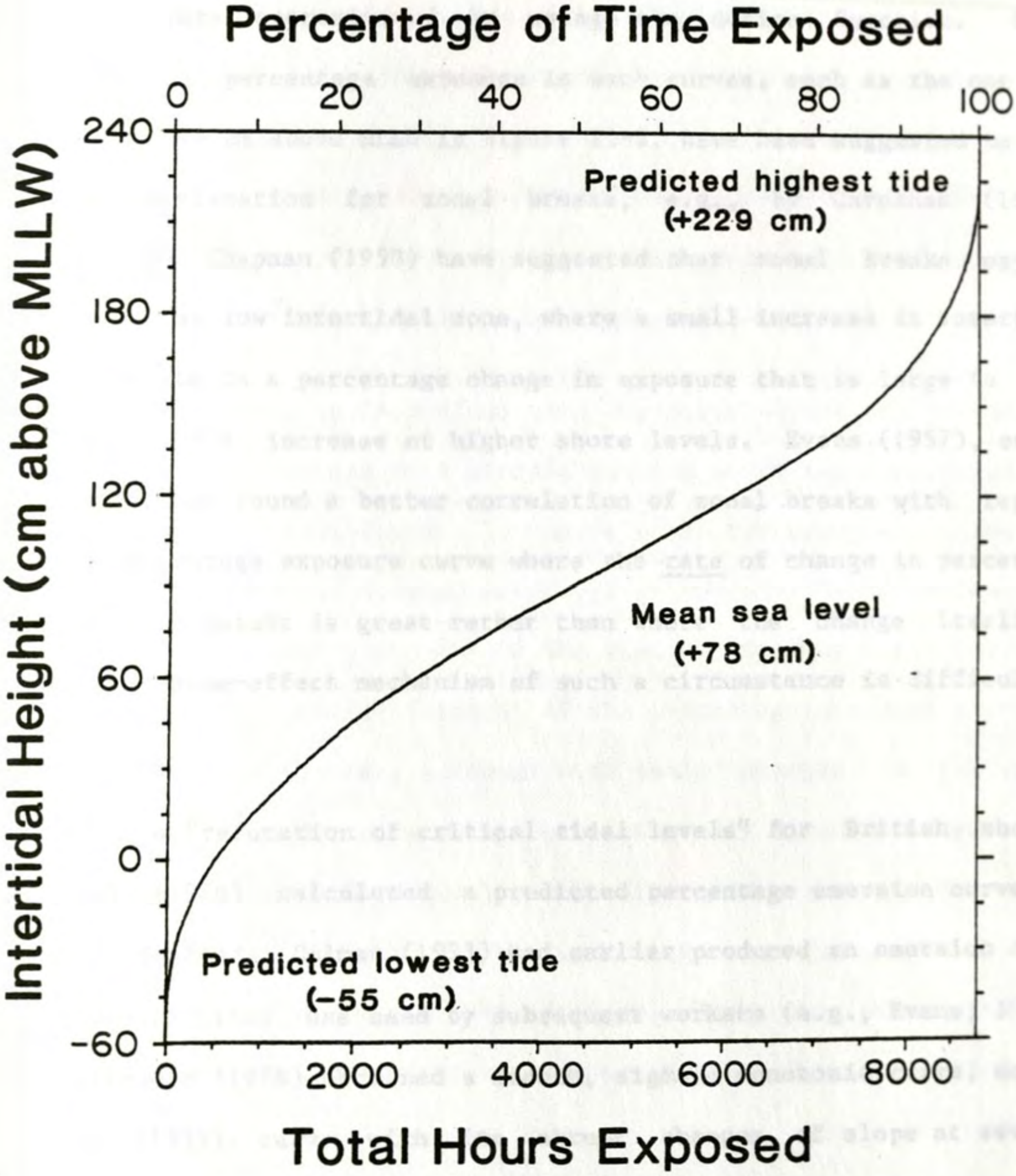
VERTICAL ZONATION AND TIDAL CHARACTERISTICS

Vertical zonation of intertidal organisms is a conspicuous aspect of marine shores around the world and has a geographic constancy in general features, so much so that the Stephensons (1949, 1972), well-traveled biologists, proposed a "universal" (later "general") scheme of intertidal zonation. Several workers have attempted to explain zonation and the conspicuous sharp breaks that occur between the zones by invoking particular aspects of the tides, often called critical tidal levels (reviews by Doty, 1957; Southward, 1958a; Lewis, 1964; Newell, 1970). It is clear that the tides themselves do not cause zonation, but that the zones are created by varying responses of organisms to environmental factors, that are either related to the tides or work in conjunction with the tides (e.g., temperature and humidity), and/or by responses of organisms to each other via competition and predation within the tidal milieu. Specifically, competition and predation are often considered to set the lower limits of distribution of intertidal organisms whereas temperature and/or desiccation are suggested to determine the upper limits (e.g., Connell, 1961a, b, 1972, 1975; Dayton, 1971; Paine, 1969, 1974; Underwood, 1973, 1979). Wolcott (1973) hypothesizes that temperature and/or desiccation set upper limits of only those organisms that border on an upper area of unexploited resources. In any case, the various proposals of early workers relating to tides and zonation provide effective means for summarizing important aspects of the tides and are discussed below in connection with the tidal regime in San Diego, CA.

PERCENTAGE OF TIME EXPOSED/SUBMERGED

Colman (1933), Hewatt (1937), Ricketts and Calvin (e.g., 1948), Dellow (1950), Beveridge and Chapman (1950), Carnahan (1952) and Evans (1957) among others have discussed intertidal zonation in relation to percentage of time exposed; see Doty (1957) for a review. Figure II-2

Figure II-2. Predicted time exposed versus intertidal height for San Diego Station (Broadway Pier) in 1980. Predicted times and heights of tides, tabulated by the National Ocean Survey (U.S. Department of Commerce) for the entire year, were used. Times and intertidal heights were interpolated when necessary using the cosine function. Calculations were performed using a Fortran 77 program on the UCSD VAX/VMS 11/780 computer system.


Percentage of Time Exposed
0
20
40
60
80
100
Intertidal Height (cm above MLLW)
240
180
120
60
0
-60
Predicted highest tide
(+229 cm)
Mean sea level
(+78 cm)
Predicted lowest tide
(−55 cm)
0
2000
4000
6000
8000
Total Hours Exposed

illustrates a percentage exposure/submergence curve calculated by computer for Broadway Pier, San Diego in 1980 from the tide tables of the National Ocean Survey (U.S. Department of Commerce) (computer program substantially modified from Lewis, 1975). Tidal height and time interpolations were approximated by using the cosine function. Rapid increases in percentage exposure in such curves, such as the one that occurs near 90 cm above MLLW in Figure II-2, have been suggested to provide an explanation for zonal breaks, e.g., by Carnahan (1952). Beveridge and Chapman (1950) have suggested that zonal breaks may be created in the low intertidal zone, where a small increase in intertidal height results in a percentage change in exposure that is large in comparison to the increase at higher shore levels. Evans (1957), on the other hand, has found a better correlation of zonal breaks with regions of the percentage exposure curve where the <u>rate</u> of change in percentage exposure with height is great rather than where the change itself is rapid; the cause-effect mechanism of such a circumstance is difficult to imagine.

In a "refutation of critical tidal levels" for British shores, Underwood (1978) calculated a predicted percentage emersion curve for Plymouth, England. Colman (1933) had earlier produced an emersion curve for Plymouth that was used by subsequent workers (e.g., Evans, 1947a, b). Underwood (1978) obtained a smooth, sigmoid monotonic curve, unlike Colman's (1933) curve with its abrupt changes of slope at several heights on the shore, and Underwood (1978) attributed the discrepancy to the difference in precision of the methods of calculation used. In addition to finding a smooth curve, Underwood (1978) presented transect data for several British shore sites indicating that the dispersion of upper and lower boundaries of species at those sites does not depart from random, i.e., no critical levels exist. However, Underwood (1978) combined data from transects separated by 5 to 10 m at each site and variation in exposure regimes among the transects quite likely contributed to the random dispersion observed. In addition, British shores are not characterized by a diurnal inequality of tides (i.e., they have equal semidiurnal tides) and Doty (1957) has emphasized that the large diurnal inequality of tides in California contribute to large breaks observed in percentage exposure curves for that coast.

In any case, it is obvious that organisms separated by only a few tens of centimeters on a steeply-sloping shore can experience very different exposure conditions. In Figure II-2, for example, we see that the 150 cm level was exposed about 86% of the time, whereas the 30 cm level was exposed only about 16% of the time at Broadway Pier, San Diego during 1980. The general features of the percentage exposure curve are constant from year to year, although with small changes in the tidal range changes in predicted exposure percentage can be expected primarily at the upper and lower boundaries of the intertidal zone. The curve presented in Figure II-2 is very similar to the curve presented in Ricketts and Calvin (e.g., in the 1948 edition), which was calculated for the six month period from January to June 1931 for "Crissy Wharf in San Francisco Bay".

Hewatt (1937) and Evans (1947a) determined the frequency of tides, either separated into low and high tides or combined, that cross given elevations of the shore and, just as for the percentage exposure curve, they found a "critical tide level" at a midshore elevation where tide frequencies peak.

MAXIMUM PERIOD OF EXPOSURE OR SUBMERGENCE

Higher shore organisms not only are exposed subaerially for a greater percentage of the time, but they are exposed for longer single periods than are lower shore inhabitants. Maximum periods of exposure at different shore heights have been discussed by several workers in relation to critical tide levels, intertidal levels where zonal breaks occur; this aspect of the tides has been elaborated on by Doty (1946). Understandably, the maximum exposure idea originated among workers along the U.S. Pacific Coast, where, unlike in many other areas, tides are characterized by a pronounced diurnal inequality of high tides and of low tides. Thus, with a small increase in elevation, an intertidal organism can experience maximal single exposure periods two to three times as long as maximal ones at lower levels, since an animal wetted by a high high tide may not be reached by the following low high tide or even by several subsequent high tides.

Figures II-3 and 4 illustrate a maximum exposure period curve determined for Broadway Pier,San Diego and the corresponding maximum submergence period curve. Although only the maximal periods have been illustrated in the figures, computer calculations reveal that similar circa-maximal periods occur quite regularly through the year. Therefore, elevational differences in maximal exposure/submergence periods are maintained throughout the year, presumably with only small changes in "break" levels with the minor changes in tidal levels that occur from

Figure II-3. Maximum predicted period of exposure versus intertidal height for San Diego Station (Broadway Pier) in 1980. Predicted times and heights of tides, tabulated by the National Ocean Survey (U.S. Department of Commerce) for the entire year, were used. Times and heights of tides were interpolated when necessary using the cosine function. Instances in which the predicted height of a high tide matched an intertidal height were regarded as submergences. Calculations were performed using a Fortran 77 program on the UCSD VAX/VMS 11/780 computer system.

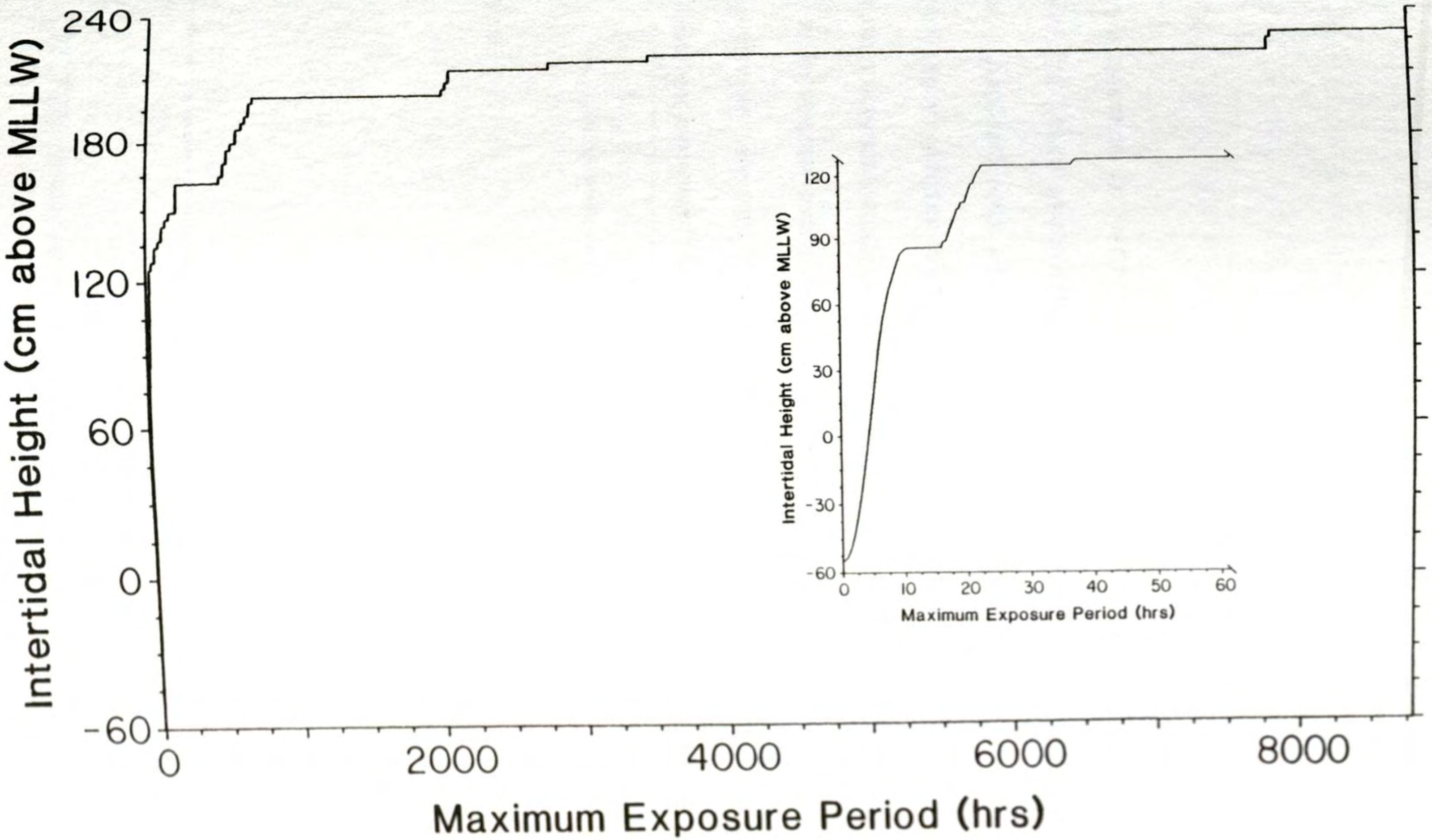
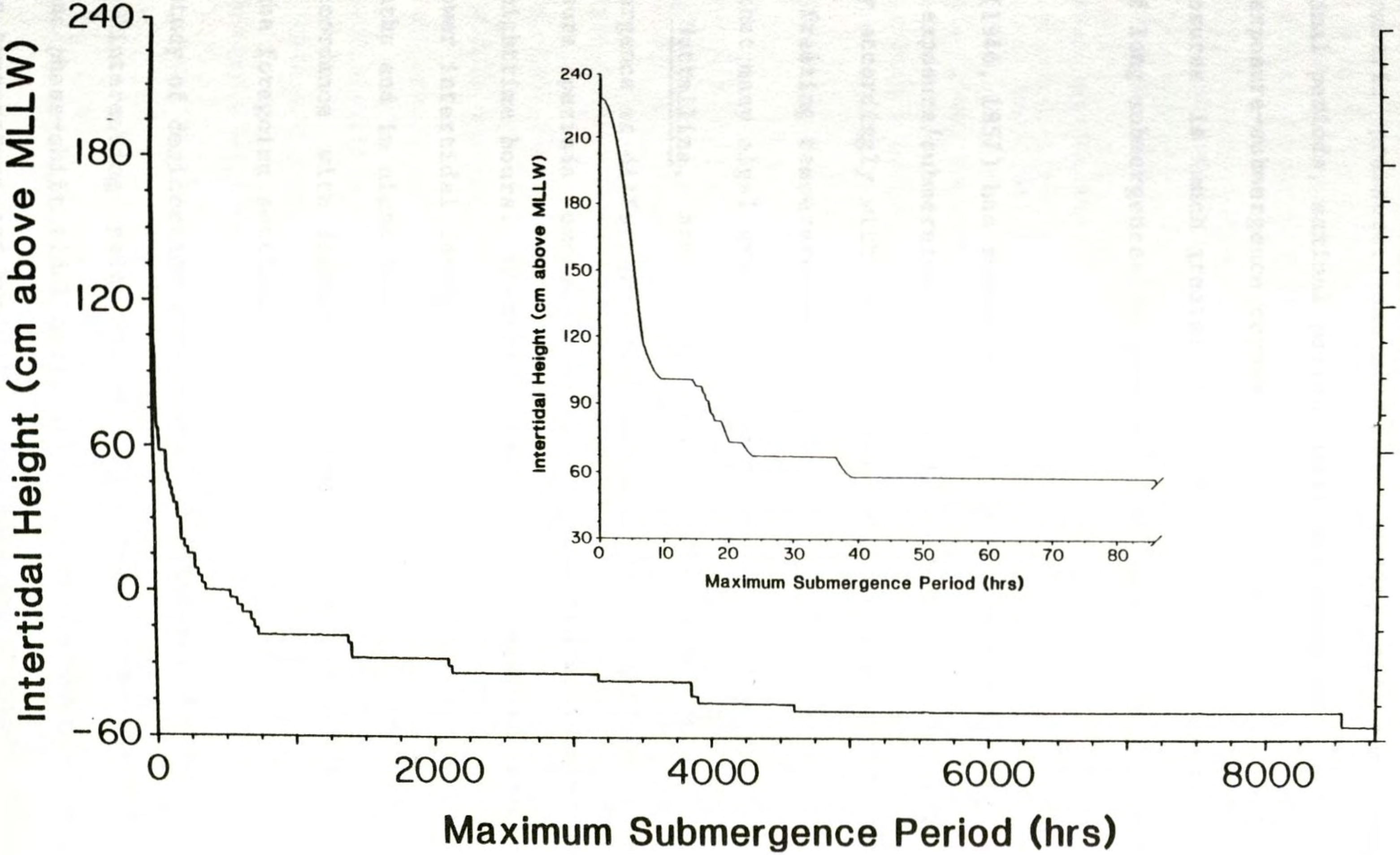

Intertidal Height (cm above MLLW)
240
180
120
60
0
-60
0
2000
4000
6000
8000
Maximum Exposure Period (hrs)
Intertidal Height (cm above MLLW)
120
90
60
30
0
-30
-60
0
10
20
30
40
50
60
Maximum Exposure Period (hrs)

Figure II-4. Maximum predicted period of submergence versus intertidal height for San Diego Station (Broadway Pier) in 1980. Predicted times and heights of tides, tabulated by the National Ocean Survey (U.S. Department of Commerce) for the entire year, were used. Times and heights of tides were interpolated when necessary using the cosine function. Instances in which the predicted height of a low tide matched an intertidal height were regarded as continued submergences. Calculations were performed using a Fortran 77 program on the UCSD VAX/VMS 11/780 computer system.


Intertidal Height (cm above MLLW)
240
180
120
60
0
-60
0
2000
4000
6000
8000
Maximum Submergence Period (hrs)
Intertidal Height (cm above MLLW)
240
210
180
150
120
90
60
30
0
10
20
30
40
50
60
70
80
Maximum Submergence Period (hrs)

year to year. Since calculations have been made from tide table predictions rather than from actual records, and because wave splash, rain and abnormal barometric pressure, among other causes, can cut short predicted maximal periods, maximal periods will not occur as calculated. However, the exposure-submergence curves indicate that the probability of long exposures is much greater at higher tide levels and that the probability of long submergences is greater at lower levels, as one would expect.

Doty (1946, 1957) has suggested that the importance of breaks in the maximal exposure/submersion curves in influencing intertidal zonation will vary accordingly with the influence of secondary factors such as high or freezing temperatures, desiccation, sunlight and rain. It can be added that many algal grazing molluscs that feed only while submerged, like Nuttallina, are likely to be affected by the number of hours of submergence at different shore levels. Above the 85 cm level, maximal exposure periods exceed 12 hours and, therefore, involve both daylight and nighttime hours. However, the shorter maximal exposure periods at lower intertidal levels tend to occur in daylight hours during winter months and in night hours during the summer months in San Diego, in accordance with findings on times of lowest tide occurrence discussed in the foregoing section.

In a study of desiccation of an intertidal limpet, Davies (1969) introduced an interesting point to be considered. Organisms may, in effect, damp and phase-shift tidal oscillations in environmental conditions, such as temperature and humidity. For example, a limpet exposed for a long period on a hot, sunny day will lose body water, but it presumably can slow the loss by clamping the protective shell closely to the substrate or by secreting a mucus sheet between the shell edge and the substrate; additionally, it may slow the water loss through tissue impermeability (see Wolcott, 1973). Thus, during an exposure period, a limpet minimizes its body water loss. However, although it is generally assumed that a limpet rapidly rehydrates during the subsequent submersion period, Davies (1969) has suggested that such re-equilibration may lag due to tissue impermeability, such that if the submersion period is fairly short, a body water deficit may be carried over into the next exposure period. Thus, the body water deficit may accumulate over successive tides. Such a notion has not been studied critically.

TIDAL CURRENT SPEED

Elmhirst (1933-1934) suggested that intertidal zonation in British seaweeds may be related to the rate of tidal currents. The sinusoidal shape of the curve of tidal height versus time for a tidal cycle reflects the fact that the greatest part of the tidal change occurs in the middle part of the tide. Thus, mid-shore organisms will experience much more rapidly-changing conditions (i.e., shock effects) than will high or low shore organisms. In support of this theory, Grubb (1936) noted bare areas and Moore and Sproston (1940) pointed out slow successional changes and minimal numbers of species in mid-tide regions subject to the rapidly changing water levels. Elmhirst's (1933-1934) shock effects probably would occur only on extremely calm days when organisms above the tide line are not wet by waves.

TEMPERATURE AND HUMIDITY DURING LOW TIDES

The Stephensons (1949, 1972) have noted that zonation occurs, both above and below the air/water interface, in ponds and lakes where there are no tides. Below the water surface there is a gradient in light penetration, temperature, and sedimentation, while, above the water surface, temperature and humidity vary. The Stephensons (1949, 1972) suggest that the tides merely serve to intensify zonation on ocean shores. We have seen that higher shore levels are subject to a greater percentage of exposure through time and that maximal exposure periods can be much longer at high than at low shore levels. The "tideless shore" concept of the Stephensons (1949) underlines the probability that the environmental conditions at different shore levels are different during any particular exposure period. Thus, the "quality" of exposure as well as the "quantity" of exposure at various shore levels is different.

Physical factors were measured at the La Jolla Seal Rock site (to be described in following sections) on 22 days during spring low low tides. Seventeen days fell in the period from 2 November 1979 to 14 April 1980 when low low tides occurred in the mid-day to afternoon, and the five remaining days (12 and 13 June 1979, 4 and 17 June 1980 and 26 September 1980) were days on which measurements were made during the early to late morning low low tide. Southern California is periodically subject to "Santa Ana winds", warm, dry winds that blow from the deserts to the east. Such winds produce relatively high air temperatures combined with low humidities. Environmental recordings made at the nearby Scripps Institution of Oceanography Pier on the days physical factors were measured at the Seal Rock study site are listed in Appendix Table 1. By chance, measurements were not made on any days with well-developed Santa Ana conditions, although a few days were characterized by marginally dry conditions. A typical day on which intertidal measurements were made was sunny and warm, but not particularly hot, with few clouds in the sky and with a breeze of up to about 5 m/sec. Only a small number of days were overcast with cool to warm winds, with sunshine occasionally breaking through the clouds.

Wind speed, temperature, and humidity [expressed as the vapor pressure deficit (see Waterhouse and Amos, 1967 for a discussion of the relative merits of using vapor pressure deficit and relative humidity as units for expressing humidity)] were measured at a site near Seal Rock, La Jolla, a site to be described in detail in following sections. Temperature was measured using a multi-channel Yellow Springs telethermometer (Model 44TC, Yellow Springs, Ohio 45387) with various probes. Needle probe (Series 400) readings required corrections of several degrees, increasing with decreasing temperature. Chiton, rock and air temperature data are presented in Table II-1. Measurements of chiton temperatures and comparison with simultaneous air and rock temperatures on a sunny, clear day indicated that chiton temperatures follow rock temperatures most closely, as has been observed in other studies (e.g., Kenny, 1958); however, the chitons may be a bit cooler than the rocks, presumably due to evaporative cooling and/or differences in solar absorption (cf., Southward, 1958b; Davies, 1970; Vermeij, 1971). Although the relationships of chiton, rock and air temperature probably varies with solar radiation, water vapor pressure deficit, wind and possibly other

TABLE II-1. Temperatures of Nuttallina specimens recorded with Yellow Springs telethermometer (Model 44TD) and needle probe (Model 513) at Seal Rock site, La Jolla, CA.[A] Chiton temperature measured by inserting probe in foot, or between valves dorsally, unless noted otherwise. Chitons in dry exposed sandstone area, unless noted.

Date	Time	Intertidal Height (cm above MLLW)	Length of Chiton (mm)	Chiton Temp (°C)	Substrate Temp (°C)	Air Temp (°C, 0.3 m above chiton)
13 JUN 1979	0900 - 1000	"HIGH"[B]	23	28.3	28.9	20.7
"	"	"	24	26.8	27.3	22.2
"	"	"	24	28.3	28.3	22.2
"	"	"	25	26.5	26.8	22.2
"	"	"	26	26.9	28.3	20.5
"	"	"	26	27.3	27.1	20.7
"	"	"	29	26.3		20.5
"	"	"	32	28.1	28.5	22.2
2 NOV 1979	1410	"LOW"[B]	25	23.4	22.7	-
"	1415	"HIGH"[B]	40	22.8	20.1	-
3 NOV 1979	1322	130	37	21.0	21.6	-
"	1325	130 (in pool)	34	22.4	26.6 (pool)[C]	-
"	1624	130		19.0 (top of girdle)		-
13 MAR 1980	1520	130	28	27.2 (in girdle)	See Note D	-
"	1524	52	19.5	26.8 (in girdle)	"	-
"	1526	52	22	24.4[E]	"	-
"	1528	52	19.5	27.4 (in girdle)	"	-
"	1536	52	25	26.0	"	-
"	1542	150	30	25.3	"	-

TABLE II-1 (cont.)

NOTES

A. Conditions and tides (times corrected to Scripps Institution of Oceanography Pier, nearby):
13 June 1979: sunny, clear sky, slight breeze. Low low tide at 609 am, -37 cm; high at 1245 pm, +131 cm.
2 November 1979: sunny, clear sky, wind to 5 knots; 707 am, +207 cm; 136 pm -18 cm; 743 pm, +171 cm.
3 November 1979: sunny,cool, slight breeze; 743 am, +216 cm; 220 pm, -30 cm; 829 pm; +165 cm.
13 March 1980: sunny, breezy; chitons from 70 cm above MLLW up are quite dry. 602 am, +183 cm; 1256 pm, -30 cm; 717 pm +143 cm.

B. "High" intertidal ∿120-265 cm above MLLW; "Low" ∿50-70 cm above MLLW. High areas were bare sandstone; low areas usually depressions surrounded with coralline algal turf.

C. Pool was ∿91 cm in diameter, 15 cm deep; temperature was of upper surface of pool, which may have been stratified.

D. Substrate temperatures nearby ranged from 22 to 25°C during these chiton temperature measurements.

E. In shaded depression.

factors, only rock temperatures were recorded routinely.

Initially, vapor pressure deficits (VPDs) were measured in the field with an electronic dewpoint hygrometer (Yellow Springs Model 91) in conjunction with a strip-chart recorder and powered by a portable Honda AC generator. The electronic hygrometer was originally selected for use because it employs small probes (~1 X 5 cm) that can be placed into microhabitats, such as chiton depressions. However, the risk of damage to the probe elements from splashed or seeping moisture was so great that the instrument could not be used routinely in this manner. Furthermore, probe response time was very slow (15-20 minutes), in breezy conditions never achieved an acceptable thermal equilibrium, and an "integrating enclosure" suggested by Yellow Springs could not be used without dramatically altering environmental conditions, so that simpler dial relative-humidity-indicating hygrometers (Airguide Model 605) were eventually employed in conjunction with the telethermometer and the VPD was calculated from relative humidity and temperature. Wind speed was measured using a hand-held Dwyer anemometer that operated on the Venturi principle. Both temperature and humidity measurements were made by applying the probe or instrument directly on the substrate near a <u>Nuttallina</u> specimen.

An attempt was made to make measurements throughout the low tide exposure period, but wave splash or surge limited the time during which measurements could be made without serious risk to the instruments. During periods of high surf, instruments were in particular danger, not so much at higher tide from the predictable beach break, but near dead low tide when a freak rush of water might reach a shore position that had been dry for hours.

Although no measurement series extended through an entire day, examination of shorter measurement series indicated that temperature and VPD traces on the shore generally followed a pattern typical of inland stations (although usually at different values). That is, during subaerial exposure, both temperature and VPD reached minima in the early morning hours, rose toward early afternoon maxima, and generally decreased toward sunset.

The highest rock temperature recorded from any shore position was 27.8°C on 2 November 1979 at 1340 hrs. The substrate at the La Jolla Seal Rock site is light in color and presumably absorbs a minimal amount of solar radiation. In addition, this substrate is coarse, porous sandstone and retains moisture for a long time. The nature of the substrate, combined with the fact that measurements were never made on extremely hot days, probably explains why a higher rock temperature was not recorded (n.b.: a dark black asphalt parking lot in La Jolla was recorded at 44.8°C in the early afternoon on 12 June 1979).

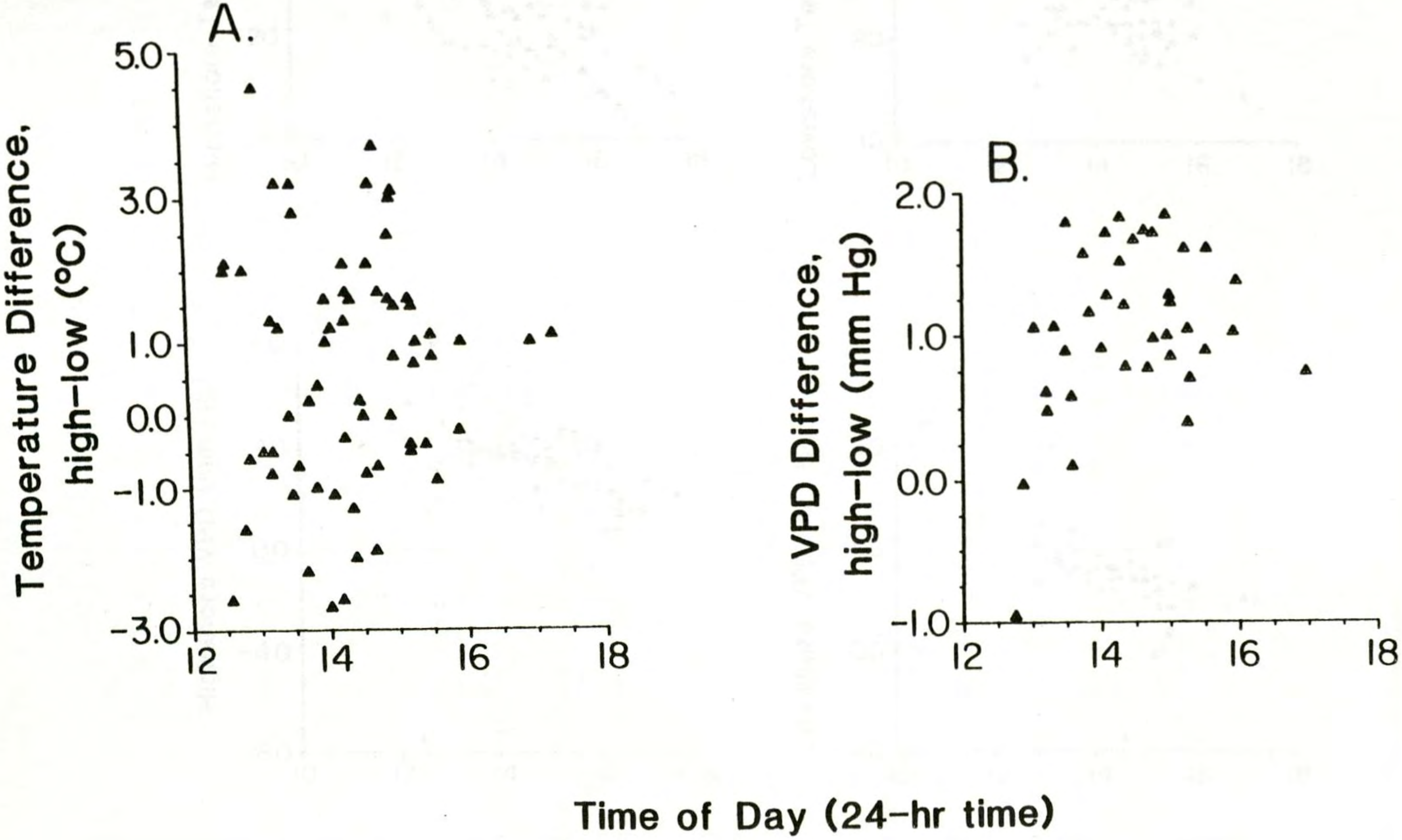
The greatest vapor pressure deficit recorded at the Seal Rock site was 8.2 mm Hg, at 1305 hrs on 3 November 1979. VPDs undoubtedly are much greater during well-developed Santa Ana conditions. Most intertidal invertebrates with tissues in contact with air theoretically will start to lose water by evaporation at about 98% relative humidity rather than at 100% RH. Such animals are usually isosmotic with seawater. If one makes calculations assuming isotonicity with seawater and using an equation from Witting (1908, p. 173), one finds that the water vapor pressure of a typical seawater solution is reduced by about 2% relative to that of pure water. This means, for example, that at 25°C an osmoconforming marine invertebrate will experience a water vapor pressure deficit at a VPD of greater than or equal to 0.5 mm Hg relative to pure water.

Virtually simultaneous measurements were made at high (~125-165 cm above MLLW) and low (~50-70 cm) shore positions on 11 of the 22 days of physical measurements. On all of the 11 days, low low tides occurred in the early to late afternoon. Figure II-5 displays the shore-level temperature and VPD differentials, and high and low shore temperatures and VPDs, for all eleven days on which comparative data are available. Mid-shore (70-125 cm above MLLW) data have not been included. Data which were not recorded simultaneously for the high and low shore are not included in Figure II-5 since events such as the sun shining through spaces between clouds and wind picking up could greatly alter temperatures and VPDs over a very short time period. Figure II-6 illustrates comparative temperature and VPD data for a day on which the shore-level VPD differential was particularly well-developed and on which the temperature differential was moderately to well-defined. Figure II-5 reveals that VPDs were quite consistently higher for the high shore, while temperatures were more variably so. On most of the days (6 of 10 for temperature; 7 of 7 for VPD), most of the substrate temperature and VPD measurements were higher (by as much as 4.5°C, 1.9 mm Hg for VPD) at the high shore than the low shore position. This situation is as might be expected, since the low shore position is the last to drain of the relatively cool seawater and is nearest the water edge at all times.

Figure II-5. Comparative substrate temperature and vapor pressure deficit data for high and low shore positions at the "Seal Rock" study site in La Jolla, CA during low tides in 1979 and 1980.

Most days were sunny and clear; see text and Appendix I. Telethermometer and electronic hygrometer probes, or dial hygrometers, were not exposed to the sun during measurements on the substrate since these apparati and the substrate absorbed solar radiation differentially. Instead, both types of apparati were shaded just long enough to allow equilibration with the environment (telethermometer: 10 seconds; hygrometers: 15-25 minutes). All hygrometers were calibrated with a sling psychrometer before and after each trip to the field.

In (A) and (B), only those measurements made virtually simultaneously at high and low shore positions (11 days during the study) are included. High positions were ~125-160 cm above MLLW and low shore positions were ~50-70 cm above MLLW, as determined using a Leitz automatic level and measuring from a nearby U.S. Coast and Geodetic Survey benchmark (see text). Measurements made at mid-intertidal positions, ~70-125 cm above MLLW, are not included in any of the plots. High-low shore vapor pressure deficit differentials are probably conservative, since low shore areas are riddled with many more deep chiton depressions than are high shore areas. As a result, low shore chitons are more often in contact with standing water at low tide, a fact not reflected in the hygrometer measurements made by placing hygrometers over, but not in, the depressions. In (C), (D), (E), and (F), all high and low shore measurements made on the days represented in (A) and (B) are plotted.


A.
Temperature Difference, high–low (°C)
5.0
3.0
1.0
0.0
−1.0
−3.0
12
14
16
18
B.
VPD Difference, high–low (mm Hg)
2.0
1.0
0.0
−1.0
12
14
16
18
Time of Day (24–hr time)

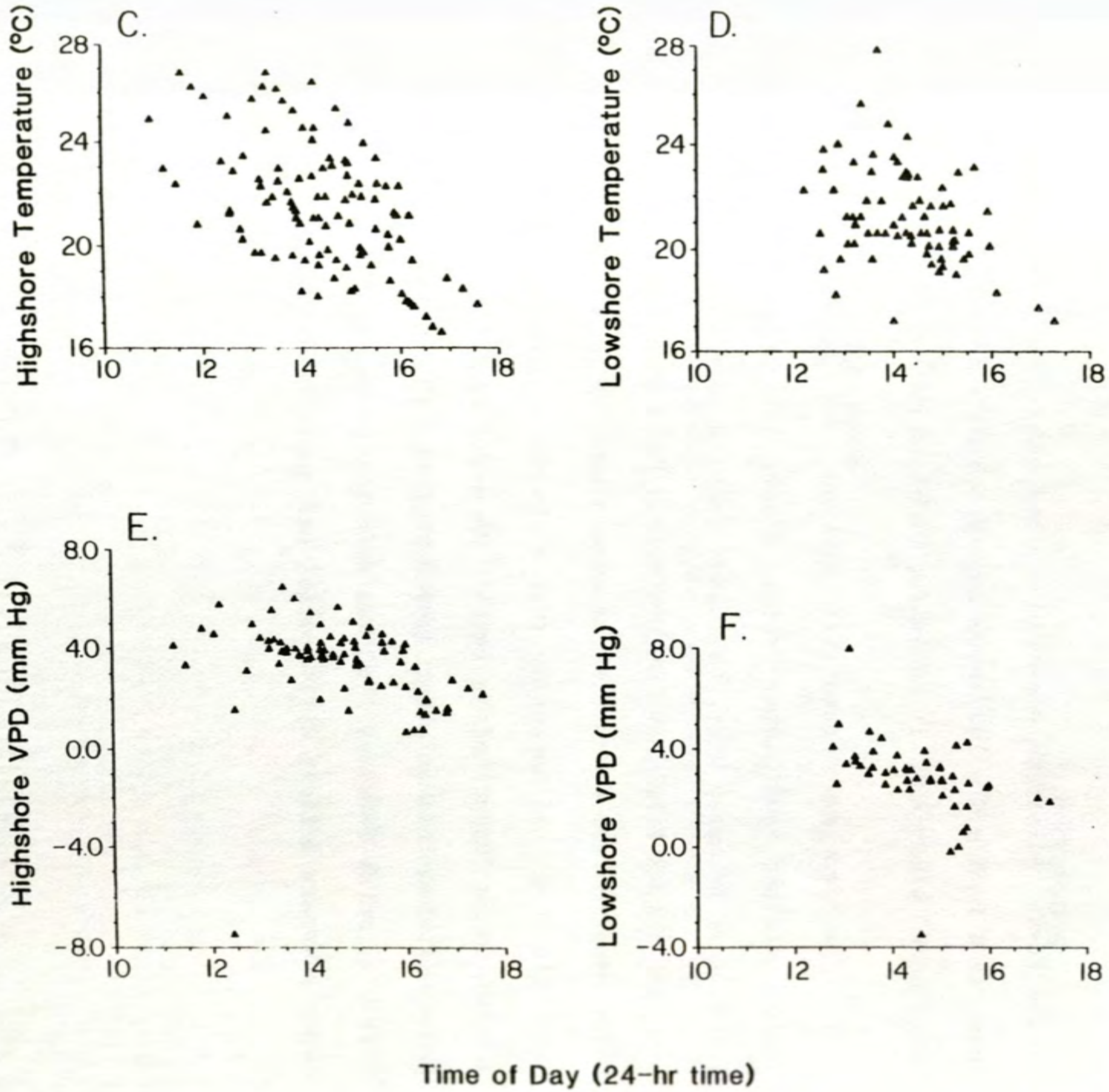
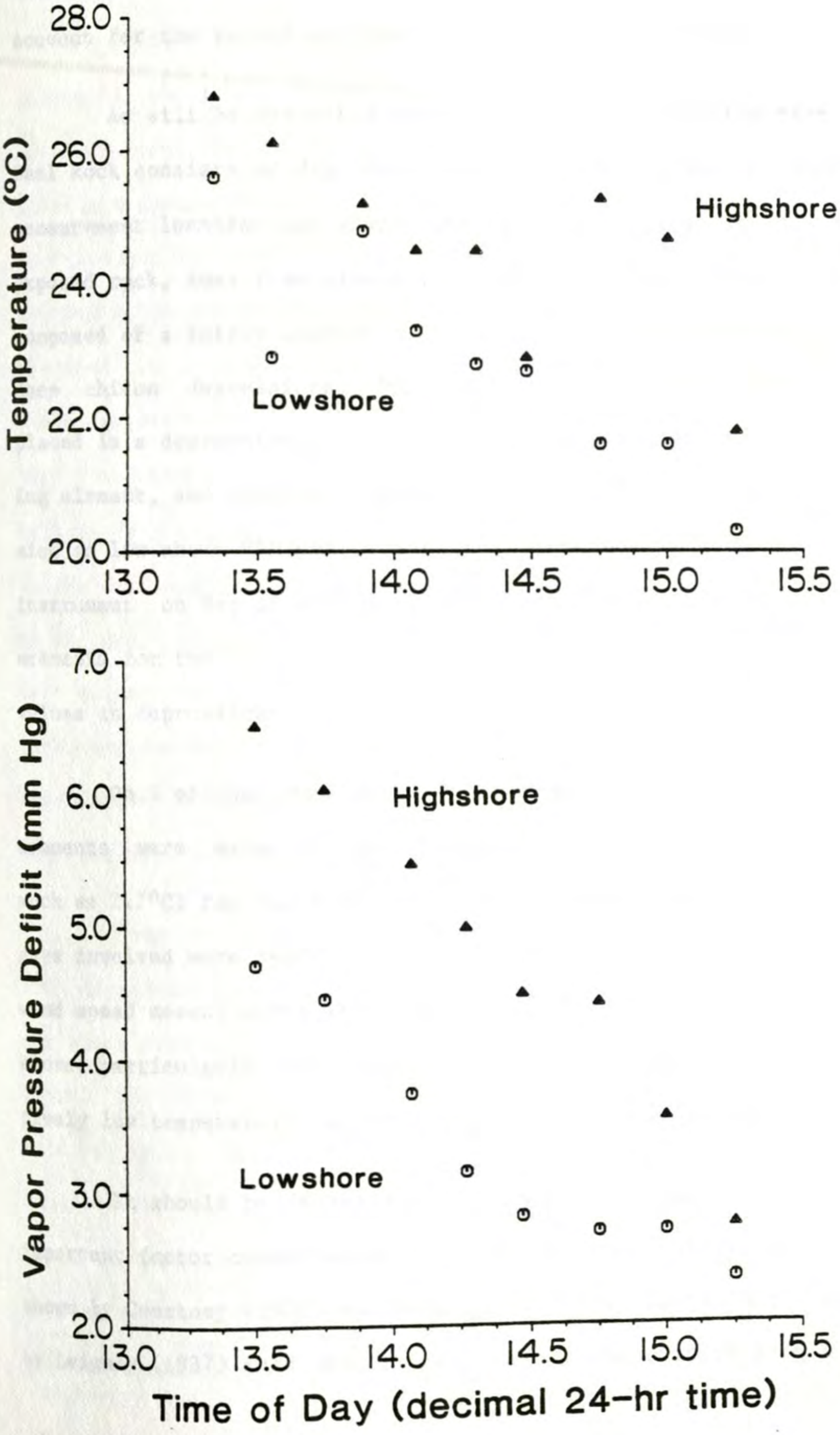

C.
Highshore Temperature (°C)
28
24
20
16
10
12
14
16
18
D.
Lowshore Temperature (°C)
28
24
20
16
10
12
14
16
18
E.
Highshore VPD (mm Hg)
8.0
4.0
0.0
-4.0
-8.0
10
12
14
16
18
F.
Lowshore VPD (mm Hg)
8.0
4.0
0.0
-4.0
10
12
14
16
18
Time of Day (24-hr time)

Figure II-6. Substrate temperatures and vapor pressure deficits measured at a high and a low shore position virtually simultaneously at the "Seal Rock" study site in La Jolla, CA on 26 February 1980.

See text and Figure II-5 caption for measurement procedures. General weather conditions: sunny, clear, slight breeze. Predicted low low tide at Broadway Pier, San Diego (-18 cm) occurred at 1329 hrs. The low shore position was approximately +63 cm above MLLW and the high shore position was about +140 cm above MLLW, as determined using a Leitz automatic level and measuring from a nearby U.S. Coast and Geodetic Survey benchmark (described in text). Compared with the measured high-low shore differentials on other days during the study, the temperature differential on 26 February 1980 was moderately- to well-defined and the vapor pressure deficit differential was quite well-developed.


Temperature (°C)
28.0
26.0
24.0
22.0
20.0
Highshore
Lowshore
13.0
13.5
14.0
14.5
15.0
15.5
Vapor Pressure Deficit (mm Hg)
7.0
6.0
5.0
4.0
3.0
2.0
Highshore
Lowshore
13.0
13.5
14.0
14.5
15.0
15.5
Time of Day (decimal 24-hr time)

The low shore coralline algal turf retains much water, which helps account for the relatively low low shore VPDs measured.

As will be described in subsequent sections, the high shore at Seal Rock consists of dry, bare sandstone and tidepools. The high shore measurement location was always chosen near a chiton on relatively bare exposed rock, away from tidepools. The low shore, on the other hand, is composed of a fairly uniform coralline algal turf and many, relatively deep chiton depressions. The electronic hygrometer probe could not be placed in a depression due to the chance of moisture ruining the detecting element, and the dial hygrometer was too large to place in a depression so low shore VPD measurements were routinely made by placing the instrument on top of the algal turf near chiton depressions. VPD measurements for the low shore area therefore are probably higher than values in depressions where Nuttallina lives.

On 3 of the remaining 4 days when comparative temperature measurements were made, a majority of the measurements were lower (by as much as 2.7°C) for the high than the low shore position. All of the days involved were typical sunny, clear days with a breeze. Comparative wind speed measurements indicated that greater wind speeds on the high shore, particularly for northerly winds, could explain some of the relatively low temperatures measured on the south-facing high shore.

It should be emphasized that wind speed has been found to be an important factor contributing to desiccation of intertidal organisms, as shown by Courtney (1972) and Coleman (1973) in particular. As suggested by Leighly (1937) (and see Wolcott, 1973), evaporation from a particular surface is proportional to both VPD and wind speed raised to a power. On four of the days on which temperature and VPD measurements were made, intertidal rocks were conspicuously dry, and although, on one of those days, winds were measured in gusts of up to only about 1.5 m/sec (~0.3 m above measurement locations), on three of the days, winds were gusting up to about 4.5 m/sec (~5.4 m/sec was the maximum windspeed measured on any day 0.3 m above measurement stations; however, wind speed was not measured on many days); wind undoubtedly enhanced evaporation. Thus, while temperatures and VPDs occasionally may be relatively lower on the high shore than the low shore, it is probable that wind often will contribute to greater evaporation from the high shore, where there generally is less protection from wind than on the low shore at Seal Rock. On one day, it seems probable that the abnormal temperature differential was caused by the fact that low shore measurements, unlike high shore ones, were made on a patch of dark brown algae that probably was heated considerably by absorbance of solar radiation. Reasons for many of the abnormal high-low shore temperature and VPD differentials are unknown.

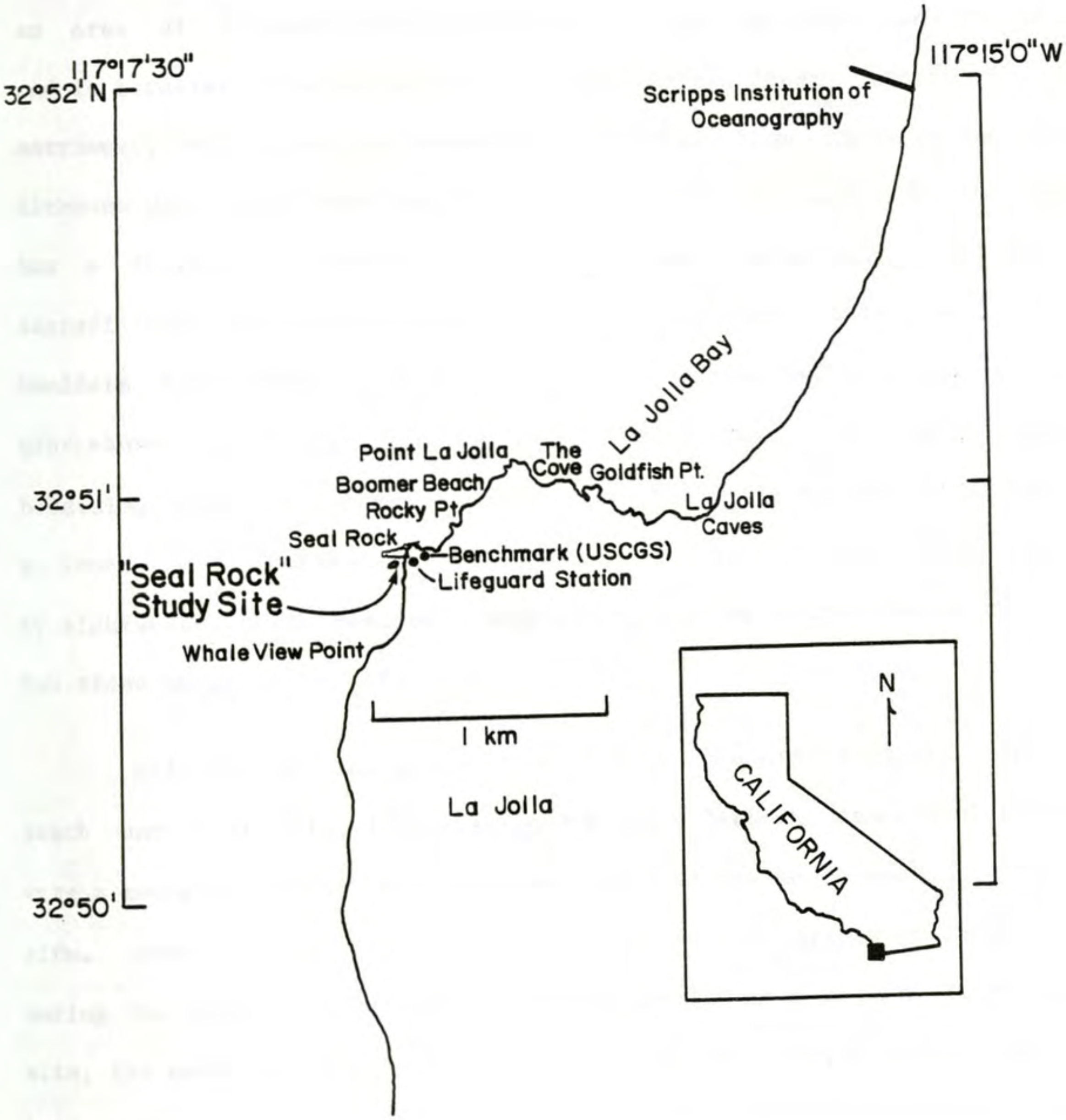
From the foregoing data, one might conclude that temperature and VPD at exposed high shore positions are most often higher than those at low shore locations on Seal Rock, La Jolla. However, for temperature at least, it is possible that during periods of warm water and relatively cold air (e.g., during the summer when low low tides occur at night), a consistently reversed gradient is produced, with higher temperatures lower on the shore. This consideration is important where acclimation studies are concerned. The complex integration of tidal exposure times, air temperatures and water temperatures at different shore heights is necessary to assess the probability of this situation. Segal (1955) has possibly provided a partial answer for the Southern California area in his study of acclimation of an intertidal limpet species at different shore heights through the year at Palos Verdes, Los Angeles, CA. Segal (1955) found that the heart rates of his limpets varied with acclimation temperature. He found that high shore specimens had lower heart rates than low shore specimens (at a particular measurement temperature) at all times of the year, and that, by reciprocally transplanting high and low shore specimens, the relationship between heart rates of high and low shore specimens could be reversed. These findings suggest that the high shore limpets experience consistently higher temperatures than the low shore ones throughout the year, including the summer months. Segal (1955) did find that the difference in heart rate between the high and low shore limpets was minimal during the summer months, though not reversed, as we might have alternatively predicted.

DESCRIPTION OF PRIMARY STUDY SITE WITH EMPHASIS ON VERTICAL ZONATION

PHYSICAL FEATURES

The south side of a large sandstone outcrop just south of Seal Rock, La Jolla, CA (32°51′N, 117°17′W) was the primary site used for studies reported on in subsequent chapters (see Fig. II-7). The outcrop consists of a soft coarse friable sandstone, which can be easily penetrated with a pocket knife, and which, due to its coarseness and porosity, retains moisture for long periods. This site was chosen for study because the intertidal area is fairly regular, without major

Figure II-7. Location of the "Seal Rock" study site on a large sandstone outcrop just south of Seal Rock, La Jolla, California. Most features are taken from the map of the La Jolla Quadrangle, mapped and published by the U.S. Geological Survey (Department of the Interior; 1967, photorevised 1975).


117°17'30"
32°52' N
32°51'
32°50'
117°15'0" W
Scripps Institution of Oceanography
La Jolla Bay
Point La Jolla
The Cove
Goldfish Pt.
Boomer Beach
Rocky Pt.
La Jolla Caves
Seal Rock
Benchmark (USCGS)
Lifeguard Station
"Seal Rock" Study Site
Whale View Point
1 km
La Jolla
N
CALIFORNIA

topographic variability, and because the study animal, Nuttallina, occurs in large numbers throughout a broad intertidal range. The site faces a smaller tall sandstone outcrop, from which it is separated by a sandy channel and beyond which is a sandy beach. The study site is in an area of exposed outer coast, but, because the site faces the beach and is bordered by a narrow surge channel and a lower outcrop on the northwest, the site is somewhat protected from waves at low tide. Although waves wash over the site from the north at high tide, the site has a fairly predictable exposure regime, relatively free of the unpredictable wave splash that occurs on coastlines littered with large boulders, for example, due to the site's protected position and its regular shore topography. Zonation is well-developed. The sandy channel bordering the south side of the study site is filled with water except at lower low tides; the channel acts as a barrier reducing disturbance by sightseers, which reaches a maximum during the winter months when low low tides occur in the afternoon.

All sides of the study site outcrop drop off abruptly, on the south and east sides into a sandy bottom. Periodic sand level changes were a conspicuous phenomenon in the channels and beach near the study site. Such changes were most dramatic during periods of large waves during the winter months; on a retaining wall to the east of the study site, the sand level changed almost 2 meters in a single 24-hour period. Sand never completely covered any parts of the study site but did occasionally cover chitons in depressions at lower shore levels for short periods. A horizontal planar ledge area bordering the study site on the south side (~0.3 m above MLLW) was most affected by sand, depressions occasionally filling with sand. Sand influx decreased rapidly with intertidal height so that upper areas were subject to very little sand influx.

GENERAL DISTRIBUTION OF BIOTA AT THE LA JOLLA SITE

Littler (1979) and Vesco (1980) have provided faunal and floral species lists for southern California rocky intertidal study sites, Ocean Beach and Whites Point, Los Angeles, CA, respectively, quite similar to the "Seal Rock" study site. Careful examination of the Seal Rock study site reveals a fairly distinct algal breakpoint at ~50 to 80 cm above MLLW [all levels determined using a Leitz automatic level Model B-4 and stadia rod marked in 50ths of ft (~0.6 cm)] which serves to define a separation of the site into upper and lower areas. The lower area is composed of a relatively uniform coralline algal turf, pitted with oblong form-fitting depressions made by Nuttallina that occurred at densities of up to about 2000 per m^2. In the study site proper, the slope of the turf area was about 15° to 40° from horizontal. The density of Nuttallina and the nature of the chiton depressions will be discussed in a subsequent section. The biotic make-up of a similar coralline algal turf community at Whites Point, Los Angeles, CA is described in detail by Vesco (1980). At Whites Point, and presumably at the Seal Rock site, foliose coralline algae, Corallina, accounts for the greatest part of algal cover of the turf, followed by an array of several red and brown algal species. The combination of a dense algal growth and chiton depressions makes the low area quite slow to drain of water at low tide; water can be observed in chiton depressions throughout many low tides. The surfaces of many of the animals in the low area, including

Nuttallina, are often overgrown with algae characteristic of the low zone.

The horizontal planar ledge area, or tidal flat, bordering the densely-populated lower area, or chiton band, is almost entirely free of chitons, although many chiton-shaped depressions occur there. Sand inundation of this area may set the lower limit of distribution of Nuttallina. The tidal flat, like the dense chiton band, has a lush stand of algae, which would suggest that food is not limiting there. Sea stars, conspicuous predators on molluscs at other sites, are absent from the lower intertidal areas of the Seal Rock study site, possibly as a result of human pressure, but more likely because the mussel beds are much better-developed at other areas around the outcrop and the usual low intertidal laminarian zone (Zone 4 of Ricketts and Calvin, 1968) is poorly developed and abbreviated by the adjoining sandy surge channel. Only one specimen of Pisaster sp. was ever observed in the study site proper over a five year period (1977-1982). Small Nuttallina specimens have been collected from at least as low as MLLW amongst boulders at Bird Rock, La Jolla, CA, indicating that Nuttallina specimens are able to exist lower in the intertidal area than they do at Seal Rock. Certainly, Nuttallina does not have an immediate physiological requirement for frequent subaerial exposure, as indicated by the fact that some fare well constantly submerged in tidepools (see Chapter V) and by the fact that others have been kept alive constantly submerged in a laboratory sea-table for several months. On the other hand, many Nuttallina specimens will crawl above a stationary water surface in the laboratory and will desiccate to death if given the opportunity, as do many limpets.

Although the breakpoint of the coralline algal turf and accompanying dense array of Nuttallina occurs primarily at about 0.5 to 0.8 m above MLLW, the turf extends farther upshore in damp areas beneath shaded ledges, in crevices, and in areas where there is water drainage from higher shore tidepools. This phenomenon suggests that the upper limit of the turf is set by water requirements. The positive correlation of great chiton densities with the presence of turf may directly result from similar requirements of the two or may reflect a cause-effect relationship between them. This correlation will be examined in Chapter III. At the lower end of the upper shore area, mussel beds are more or less well-developed in areas adjacent to the study area proper, but exist as small clumps only a few tens of centimeters in maximum diameter in the study area. The mussel beds grow slowly in size, and are sporadically decimated in periods of large waves, e.g., in the winters of 1980-81 and 1982-83. The large waves rip away big chunks of the mussel beds, and occasionally break away large segments of the underlying sandstone, leaving bare areas of rock that are rapidly colonized by opportunistic green algae.

The upper intertidal area at the Seal Rock site is composed of large areas of relatively bare sandstone presumably coated with a thin algal film. The slope of most of the high, bare area in the study site proper is from 10 to 20^{o} from horizontal. Tidepools are virtually absent, except above 150 cm above MLLW. The high tidepools are up to one or two meters in diameter and 15 to 20 cm in depth. They are relatively depauperate in fauna and flora, although encrusting coralline algae are conspicuous elements there. Although acmaeid limpets are present in good numbers, comparison with nearby, relatively unexploited localities, such as the Point Loma Reserve, San Diego Co., CA, reveals that numbers of the markedly territorial owl limpet, *Lottia gigantea*, are reduced at the Seal Rock site and that the specimens are generally smaller in size there. The diminished population of *Lottia*, likely an important species in structuring California intertidal communities (Stimson, 1970, 1973; see also Branch, 1981), is at least in part due to collection of the limpet by shore fishermen for bait (personal observations).

SHORE-LEVEL GRADIENT IN *NUTTALLINA* SIZE, DENSITY, AND BIOMASS

In collections of *Nuttallina* made at several sites in La Jolla, CA it was consistently difficult to find substantial numbers of specimens of overlapping sizes from high and low shore areas. This was a result of an obvious tendency of *Nuttallina* specimens from the higher shore, particularly in drier, exposed areas, to be of larger size. In order to document this phenomenon, a collection of *Nuttallina* along an upshore/downshore transect at the "Seal Rock" study site, was made on 7 December 1980. The transect was selected such that substantial numbers of *Nuttallina* specimens were present at all shore levels and no tidepools or large *Mytilus* beds were present. *Nuttallina* specimens were collected from four adjacent areas extending from the upper edge of the low shore, densely-populated chiton band to the upper limit of the vertical range of *Nuttallina*, i.e., from approximately 0.5 to 1.7 m above MLLW. Because of the great density of *Nuttallina* in the low shore chiton band, a smaller fifth area was randomly-selected within a 1 m^2 quadrat within the band and along the transect to represent the band population that occurs from ~0.3 to 0.6 m above MLLW. Sampling was destructive, since chitons had to be removed from the substrate for measurements, and, particularly in the lowest fifth area, where dense macroalgal turf and numerous chiton depressions occur, since all chitons in a selected area could be found only by hammering away chunks of substrate.

Chitons from the five zones were left submerged in running seawater in plastic containers for 24 hours to permit clearance of the digestive tract and full hydration. Then, all visible algae was scraped from the valves and brushed from the girdle. The intact fourth valve width (FVW) was measured to within 0.1 mm with fine-pointed dividers and a precision metric rule, as described in Chapter IV, and the blotted wet weight (BWW) was obtained by vigorously blotting the animal with tissue and weighing to the nearest 10 mg.

Results are presented in Tables II-2 and II-3 and in Figures II-8 and II-9. Tables II-2 and II-3 indicate quite clearly that Nuttallina density and total biomass decrease with increasing shore height, while FVW and BWW tend to increase with shore height. Figure II-8 illustrates the tendency toward increasing size of Nuttallina with shore height in zones 5 (lowest) through 1 (highest). Statistical comparison of FVW and BWW among the five zones bears out the zonal differences (Kruskal-Wallis, H=132, 103 for FVW, BWW, respectively, d.f.=4, $p<0.0001$ in both cases).

Smaller collections of Nuttallina, made over a time span of more than 5 years, indicate that the shore-level size gradient of Nuttallina

TABLE II-2. Density and biomass of Nuttallina at "Seal Rock" study site, La Jolla, CA on 7 December 1980.

Zone	Intertidal Height Range, cm above MLLW[A]	Habitat Type[B]	Approximate Area collected, m^2	N	Approximate density, N/m^2	Mean Blotted wet weight ± s, grams/individual	Biomass, g/m^2
1	144-168	1	4.28	58	14	1.85±1.175	26
2	109-144	1	1.01	54	53	1.48±0.969	78
3	82-109	1	0.28	53	189	0.94±0.738	178
4	52-82	1	0.18	56	311	0.68±0.543	211
5	35-52	2	0.13	137	1054	0.52±0.448	548

NOTES

A. Intertidal heights were determined ± 1 cm relative to a U.S. Geodetic Survey benchmark using a Leitz automatic level with a 22X scope. See Chapter IV for details.

B. Habitat types described in text: 1. sparsely-populated by macroinvertebrates, exposed rock, no pools, little macro-algae, not many mussels; 2. densely-populated, chitons in form-fitting depressions, damp, coralline algal turf.

TABLE II-3. Regression parameters for $\log_{10}$ transforms of blotted wet weight (BWW) versus intact fourth valve width (FVW) for Nuttallina collected in five zones along a downshore transect at the "Seal Rock" study site, La Jolla, CA on 7 December 1980, with tests for significance of deviation of regression coefficients from the value for isometry. Shore height decreases from Zone 1 to Zone 5.

Zone	N	FVW range (mm)	BWW range (grams)	Regression coefficient, b (of $\log_{10}$ FVW)	Y Intercept	Standard Error of b	Correlation	b value for isometry	Two-tailed t test	Significance
1	58	4.5-15.4	0.21-4.77	2.35	-2.09	0.085	0.96	3.00	-7.6	<0.001
2	53	3.3-14.7	0.09-3.67	2.49	-2.24	0.110	0.95	3.00	-4.6	<0.001
3	47	3.2-12.0	0.08-3.79	2.47	-2.23	0.108	0.96	3.00	-4.9	<0.001
4	54	2.0-13.2	0.02-3.44	2.94	-2.52	0.097	0.97	3.00	-0.6	>0.5
5	123	1.5-9.2	0.01-2.00	3.28	-2.76	0.063	0.98	3.00	+4.4	<0.001

Figure II-8. Fourth valve widths and blotted wet weights of *Nuttallina* specimens collected in five zones along an upshore-downshore transect at the "Seal Rock" study site, La Jolla, CA on 7 December 1980. Shore height decreases from Zone 1 to Zone 5. Intertidal height ranges of the zones are listed on the left. Zone 5 was entirely within the low-shore, densely-populated chiton band; the other four zones were contiguous and extended from the upper edge of the dense chiton band upward. Corresponding data are presented in Figure II-9 and Tables II-2 and II-3 and are explained in the text.

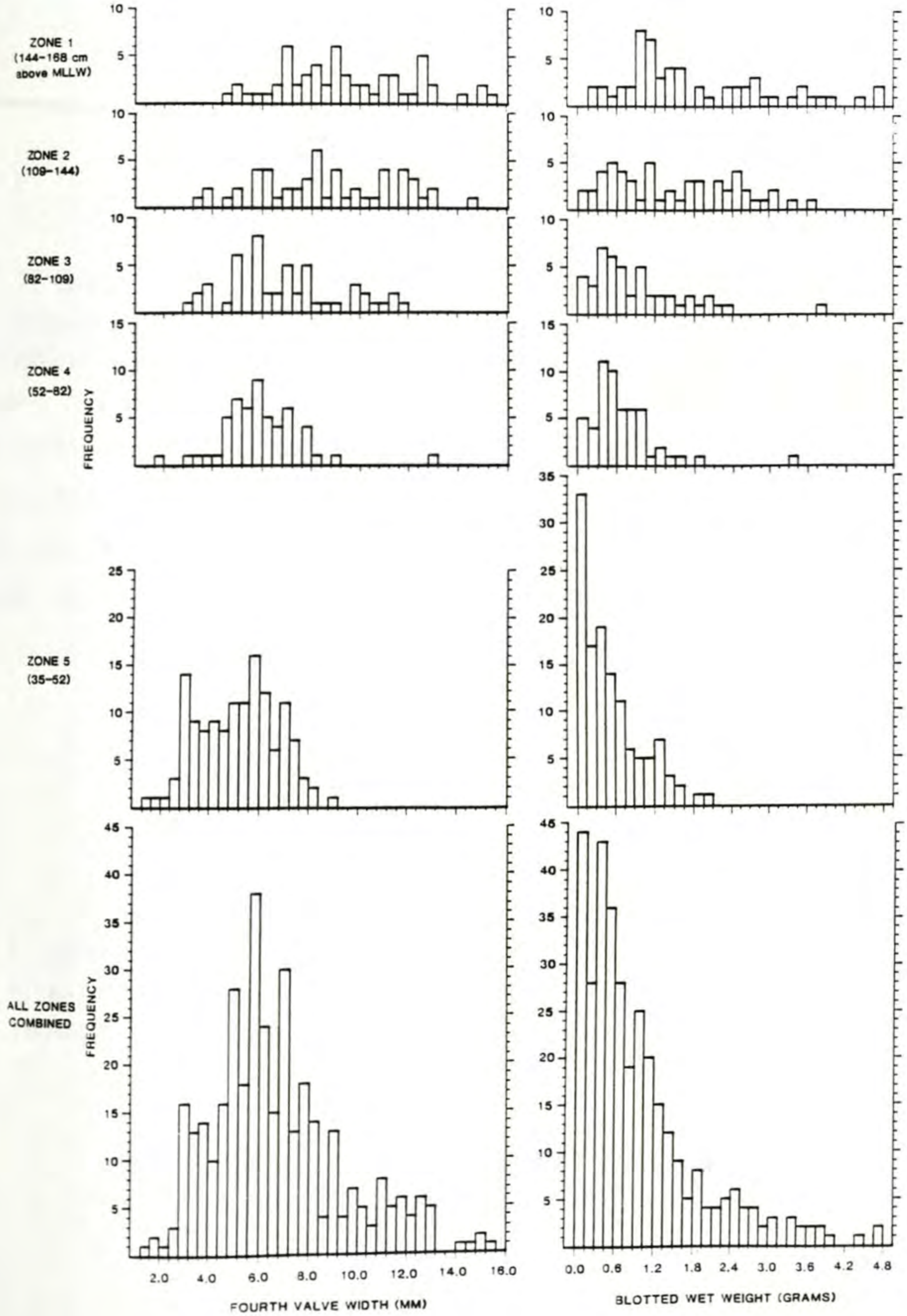
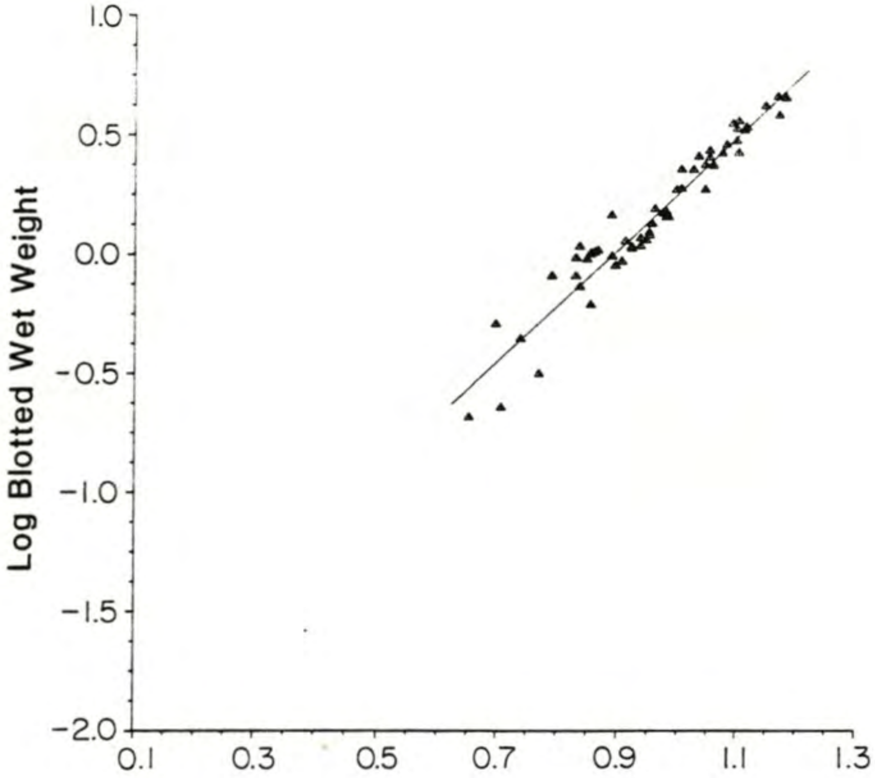
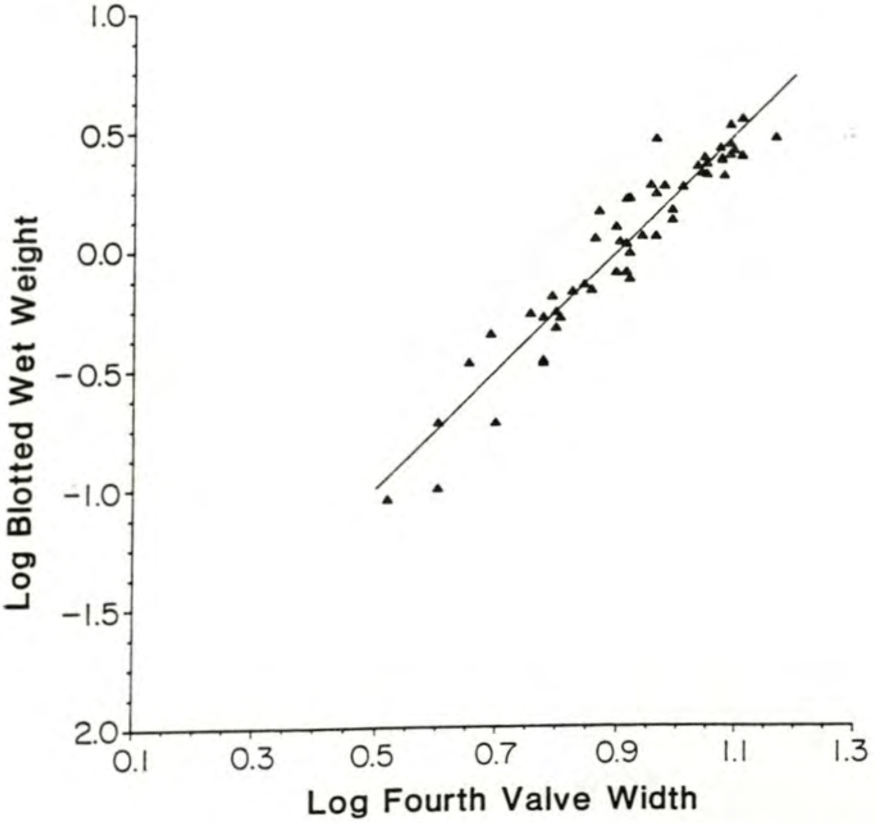

ZONE 1
(144-168 cm
above MLLW)
ZONE 2
(109-144)
ZONE 3
(82-109)
ZONE 4
(52-82)
ZONE 5
(35-52)
ALL ZONES
COMBINED
FREQUENCY
FREQUENCY
5
10
15
20
25
30
35
40
45
2.0
4.0
6.0
8.0
10.0
12.0
14.0
16.0
0.0
0.6
1.2
1.8
2.4
3.0
3.6
4.2
4.8
FOURTH VALVE WIDTH (MM)
BLOTTED WET WEIGHT (GRAMS)

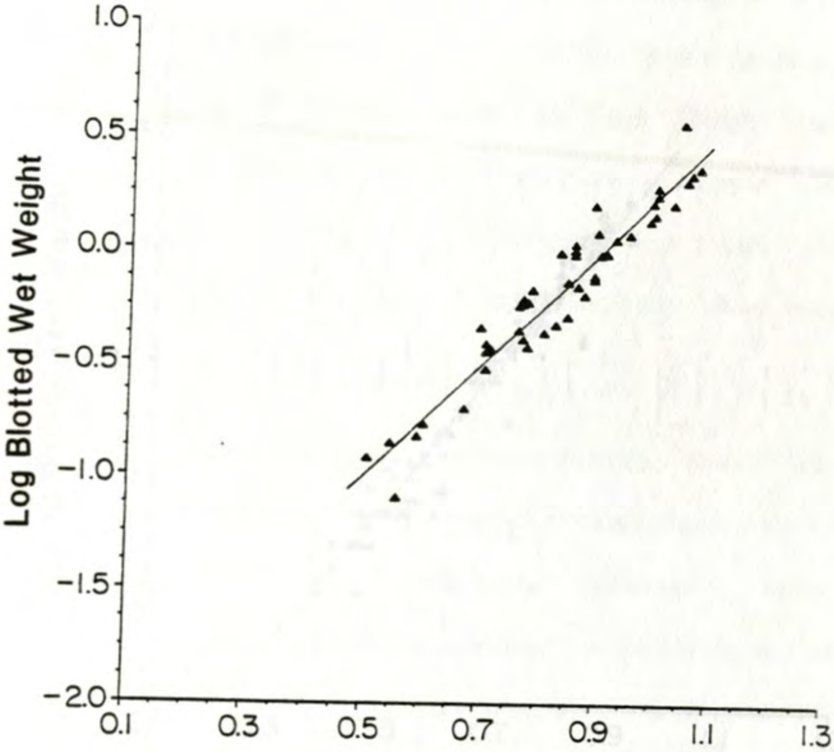
Figure II-9. Log_{10}-log_{10} plots of blotted wet weight versus fourth valve width for Nuttallina specimens collected in five zones along an upshore-downshore transect at the "Seal Rock" study site, La Jolla, CA on 7 December 1980. Specimens studied are the same as those used for Figure II-8. Corresponding data are presented in Tables II-2 and II-3 and are explained in the text and in the Figure II-8 caption.



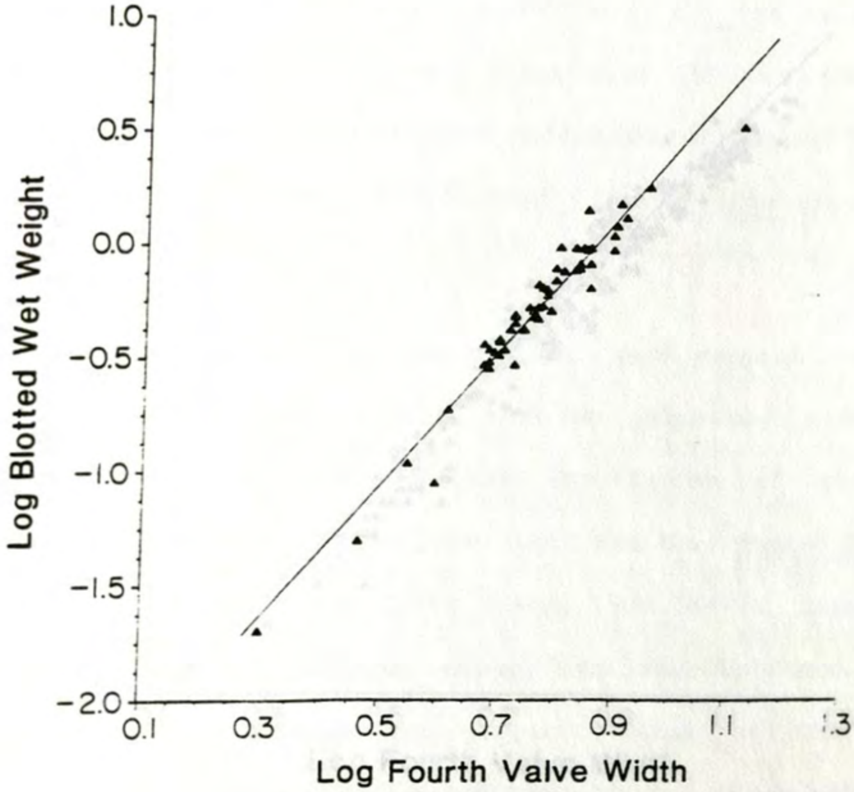
ZONE 1
(144–168 CM
ABOVE MLLW)



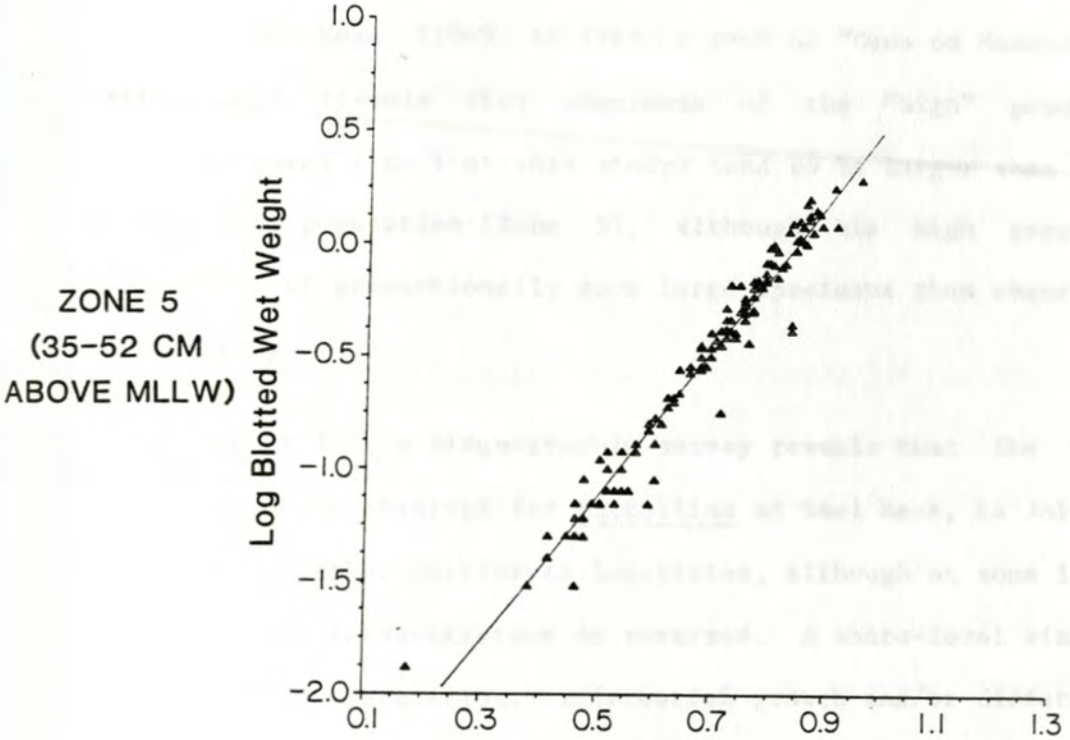
ZONE 2
(109–144 CM
ABOVE MLLW)

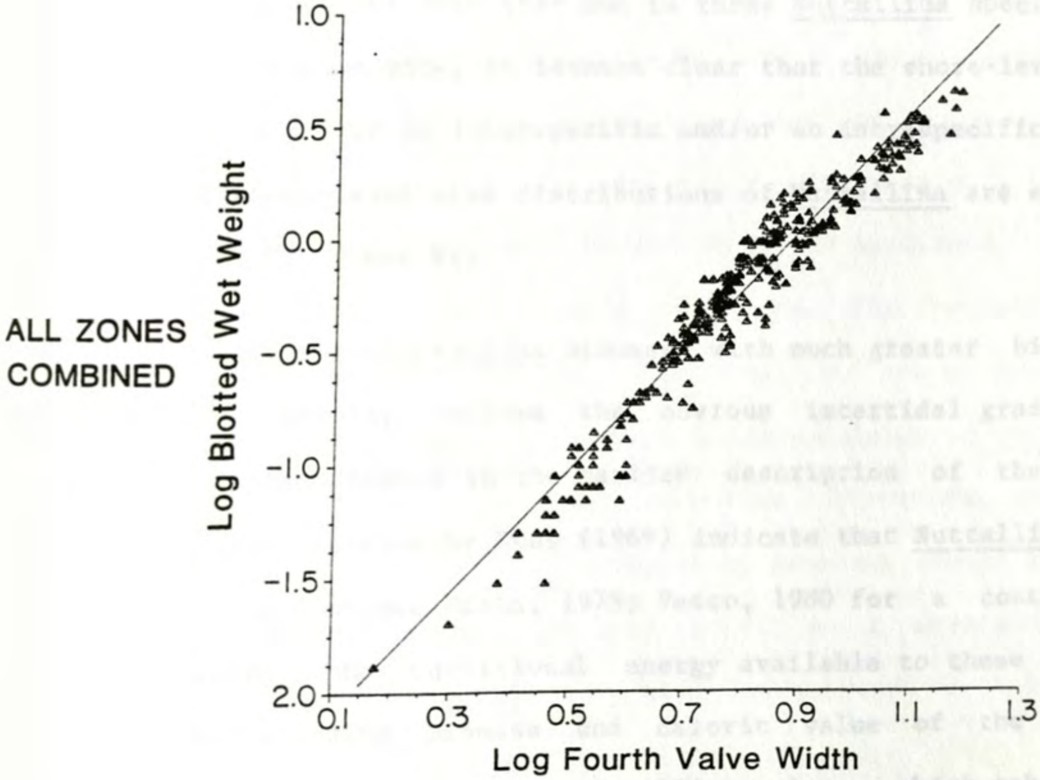


ZONE 3
(82–109 CM
ABOVE MLLW)



ZONE 4
(52–82)


ZONE 5
(35–52 CM
ABOVE MLLW)
Log Blotted Wet Weight
1.0
0.5
0.0
−0.5
−1.0
−1.5
−2.0
0.1
0.3
0.5
0.7
0.9
1.1
1.3


ALL ZONES
COMBINED
Log Blotted Wet Weight
1.0
0.5
0.0
−0.5
−1.0
−1.5
2.0
0.1
0.3
0.5
0.7
0.9
1.1
1.3
Log Fourth Valve Width

observed at Seal Rock is temporally stable. In addition, a small collection made by Kues (1969) in 1968 or 1969 at "Casa de Manana", the same site, also reveals that specimens of the "high" population (equivalent to Zones 1 to 4 of this study) tend to be larger than specimens of the "low" population (Zone 5), although his high population seems to consist of proportionally more large specimens than observed in the present study.

In Chapter III, a biogeographic survey reveals that the shore-level size gradient observed for Nuttallina at Seal Rock, La Jolla, CA commonly occurs at other California localities, although at some localities this gradient is nonexistent or reversed. A shore-level size gradient can result from migration, differential growth and/or differential mortality. In Chapter III, with the discovery of a new Nuttallina species, N. kata, and the fact that one to three Nuttallina species can occur at a particular site, it becomes clear that the shore-level size gradient can have either an interspecific and/or an intraspecific basis. The bases of shore-level size distributions of Nuttallina are examined in Chapters III, IV, V and VI.

The gradient in Nuttallina biomass, with much greater biomasses at lower shore levels, follows the obvious intertidal gradient in macroalgal biomass indicated in the earlier description of the study site. Gut content studies by Kues (1969) indicate that Nuttallina is a generalized feeder (but see Nishi, 1975; Vesco, 1980 for a contrasting view). Therefore, the nutritional energy available to these chitons depends on the standing biomass and caloric value of the algae. Although the caloric content of coralline algae, which makes up a substantial part of the low shore flora at Seal Rock, is relatively low (Paine and Vadas, 1969), it is probable that the intertidal gradient in energy available to the animals roughly parallels the gradient in standing algal biomass. Thus, the biomass of Nuttallina probably roughly parallels the intertidal gradient in available nutritional energy.

Examination of the relationship of blotted wet weight (BWW) and fourth valve width (FVW) in the chitons collected for the shore-level size distribution study reveals an interesting situation (Table II-3). BWW increases with FVW faster in lower shore Nuttallina samples; in other words, the regression coefficient of the common log transform of FVW is greater in lower shore samples (ANCOVA for equality of slopes, $F=25.0$, $p<0.0001$). Two factors might explain the changing relationship of BWW and FVW with shore height. First, a large percentage of Nuttallina specimens experience shell erosion, probably facilitated by the blue-green alga Entophysalis deusta (Nishi, 1975), and the shell makes up a large part of the BWW of a Nuttallina specimen (Appendix II). Shell erosion does not affect the FVW, since both sides of all valves are embedded in the fleshy girdle. Valves of larger specimens tend to be eroded more than those of smaller specimens. The few comparative data obtained for shell erosion of high and low shore specimens, reported in Appendix II, suggest that high shore specimens of Nuttallina experience more shell erosion than low shore ones. Therefore, the more rapid increase of BWW with FVW in the low shore area may result from the fact that the valves undergo erosion less rapidly as a specimen grows there (i.e., BWW decreases less rapidly with increasing FVW) than on the high shore.

A second factor that could explain the observed change in the rate of increase of BWW with FVW at different shore heights is the possibility that species composition changes with increasing FVW. In Chapter III, it will be shown that the low shore is occupied primarily by the newly-discovered species, *N. kata*, whereas the exposed high shore is inhabited primarily by *N. fluxa*. In Chapter IV, it will be shown that *N. fluxa* tends to have wider valves per length than does *N. kata*, and in neither species is FVW allometric with respect to length. In Chapter III, a substantial proportion of small *Nuttallina fluxa* were found among *N. kata* in small samples of young low shore chitons. Since *N. fluxa* specimens tend to possess a larger FVW per length than do *N. kata* specimens, a greater relative contribution of *N. fluxa* to samples of small chitons than to those of large chitons could explain the the greater slope of $\log_{10}$ BWW with respect to $\log_{10}$ FVW at lower shore positions. Conversely, in the higher intertidal zones (zones 1 to 3), a greater relative proportion of *N. kata* among samples of small chitons could explain the smaller slope of $\log_{10}$ BWW with respect to $\log_{10}$ FVW, but there is no evidence for such a species makeup in exposed high shore areas (Chapter III).

NUTTALLINA DEPRESSIONS IN THE SUBSTRATE

A conspicuous feature of the Seal Rock study site, and other sites in California (e.g., MacGinitie and MacGinitie, 1949, 1968a; Nishi, 1975; Vesco, 1980), is the presence of chiton-shaped depressions in which *Nuttallina* specimens often live. These depressions are often several times as deep as the chiton is high. It is quite clear that the *Nuttallina* specimens are responsible for the formation of the holes, although Anthopleura specimens are occasionally observed to inhabit similar depressions at the lower limit of the dense chiton band.

Several other chiton species, such as Acanthozostera gemmata (Thorne, 1967), Acanthochiton zelandicus and Sypharochiton pelliserpentis (Boyle, 1970), and Ceratozona angusta (Schmidt-Effing, 1980), are reported to inhabit more or less defined depressions, and several species of intertidal limpets form distinct home "scars" (see Lindberg and Dwyer, 1983; Garrity and Levings, 1983 for limpet references). The mechanism of formation of limpet home depressions has been reviewed and investigated recently by Lindberg and Dwyer (1983), who present evidence indicating that acidic mucopolysaccharides secreted from the foot and acidic mucopolysaccharides and/or carbonic anhydrase from the mantle can aid in the formation of the depression of the high intertidal homing limpet, Collisella scabra. Lindberg and Dwyer (1983) inferred the following mechanism and sequence of events in depression formation by Collisella scabra [which are similar to those of predatory gastropods (e.g., Carriker, 1969)]: 1) substratum is impregnated with acidic mucopolysaccharides secreted from the foot; 2) secretions from the mantle (acidic mucopolysaccharides and/or carbonic anhydrase) also impregnate the substratum; 3) impregnated substrate areas are recognized by the limpet and are scraped with the radula; 4) if the substratum undergoes dissolution, the depth and complexity of the depression are increased; and 5) if the substratum is harder than the radular teeth or does not undergo dissolution, the shell edge grows to conform to the substratum and no depression is formed.

Depression formation by Nuttallina apparently is accomplished very much like it is in Collisella scabra. Kues (1969) has suggested that Nuttallina uses the radula as well as "slight movements back and forth", presumably of the body, to construct its characteristic depressions. Renz (personal communication to Kues, 1969) suggested that movements of the tough girdle aid in construction of the hole. Feeding of most chitons involves rasping algae from the substrate with the radula. The radula of Nuttallina and of other chitons has teeth tipped with hard iron compounds (see Tomlinson, 1959; Lowenstam, 1962; Carefoot, 1965), and feeding usually involves incidentally removing substrate with food (Kues, 1969; Steneck and Watling, 1982; Steneck, 1983). Thus, it is not difficult to imagine the radula being used in excavating holes. Likewise, on the softer substrates such as sandstone, the girdle may help to form the depressions.

At Point Loma Ecological Reserve (Cabrillo National Monument), San Diego, Co., CA, Nuttallina specimens were found inhabiting form-fitting depressions on Mytilus at mid-intertidal levels. Examination of depressions of Nuttallina on the shells of Mytilus californianus can facilitate investigation of the mechanism of formation, as demonstrated by Lindberg and Dwyer (1983) in their study of Collisella scabra, because the structure of the depressions is well-defined and well-preserved there. Examination of depressions in the Mytilus shells only in which Nuttallina were still living and which fit the outline of the chiton closely (i.e., depressions probably formed by those chitons), provided support for both radular scraping and chemical dissolution as means of depression formation. Long radula grooves with telltale cross-hatches could be seen the length of some depressions, even along the sides of the nearly vertical walls. In other depressions, radula grooves occurred only in the immediate area of the anterior end of the chiton.

Several Nuttallina depressions had a complex structure, with different levels of different sizes, reminiscent of depressions of Collisella scabra described by Lindberg and Dwyer (1983). In several depressions, the exposed mussel shell layers were quite smooth without scratches, as if dissolved. In one case, the foot of a chiton was displaced laterally quite far from the centerline of its body; the underlying depression was particularly deep in the position underlying the displaced foot, suggesting that secretions from the foot may have been responsible. Both the foot and pallial groove of chitons are equipped with various gland cells (see review in Hyman, 1967); recently, Höglund and Rahemtulla (1977) isolated acidic mucopolysaccharides from the foot of Chiton tuberculatus. Therefore, chemical dissolution may indeed facilitate depression formation in Nuttallina and other chitons. Where chemical dissolution and radular scraping are probably not possible, such as on hard basalts, Nuttallina tends to inhabit irregularities in the rock rather than form depressions (see Chapter III) and in this is aided by the flexibility of its body.

Apparently, Nuttallina depressions can be made quite rapidly when necessary, and depressions are used by successive generations of the chitons. In laboratory observations, Renz (personal communication to Kues, 1969) noted that Nuttallina can form a hole about one-half as deep as the chiton is high in five days. At the Seal Rock site, in a flat smooth clearing I made in the low dense chiton band, I observed a colonizing chiton (~25 mm long) to make a noticeable depression in the spot it took up at the edge of the clearing in less than a month. Chiton depressions as small as 7 to 8 mm long and 1 mm deep were observed in shells of live mussels at Point Loma Reserve; if these depressions are assumed to to have been formed by a single chiton or at least to have been enlarged to the final size by the last chiton inhabiting the spot, formation of such spots can be inferred to have occurred in a few months to a year. The occurrence of depressions that are inhabited by chitons smaller than the depressions suggests that depressions are occupied by several generations of chitons. This suggestion is supported by the observed persistence and availability of empty depressions (see next section).

At the Seal Rock site, chiton depressions are well-defined and they occur at high densities within the coralline algal turf of the low chiton band; however, the depressions are often inconspicuous and occur at very low densities in the upper intertidal areas. Many chitons in the upper intertidal area occur in relatively large smooth hollows, or even on flat rock or wedged between barnacles, or near other relief not created by the chitons themselves. This differential development and occurrence of chiton depressions with shore height is likely an effect of the varying persistence of the substrate and the difference in behavior of _Nuttallina_ with shore height (see Chapter V). High shore chitons, i.e., _Nuttallina fluxa_ (see Chapter III), also are apparently quite capable of forming well-defined depressions, as evidenced by the occurrence of two small _Nuttallina fluxa_ specimens (11 and 17 mm long;

species identification confirmed by gill counts, 30 and 34 average per side, respectively; see Chapter IV) in tightly-conforming depressions on mid-high intertidal mussels at Point Loma Preserve. Depressions formed by Nuttallina in the sandstone of the low shore area at the Seal Rock site are stabilized by the encrusting and foliose algae that densely occur in the area. In addition, it appears that growth of organisms such as the sandcastle worm, Phragmatopoma, and barnacles, on the perimeter of many depressions effectively deepens them. In the barer, sparsely-populated intertidal area, on the other hand, depressions are not similarly stabilized and augmented, and, apparently, the sandstone is eroded away so that chiton sites often persist as smooth hollows much larger than the chiton. It is also probable that upper shore chiton sites are not as frequently re-occupied upon vacation as are the lower shore depressions, where chitons occur at very high densities; therefore, erosion can often easily counterbalance the activities of the chitons in high shore areas. Finally, as will be documented in Chapter V, low shore chitons are found at the same spot for much longer periods than are high shore chitons and this probably helps to explain the better definition of low shore depressions.

Nuttallina may gain several advantages by inhabiting depressions. As suggested in connection with either chitons or limpets, the depressions may act as protection against predation (e.g., Garrity and Levings, 1983), sand scouring, wave shock, osmotic stress or desiccation (see Verderber et al., 1983). Further, the depressions may facilitate territorial and/or homing behavior or may serve as a trap for drift algae (MacGinities, 1949, 1968a). The depressions potentially can facilitate functions usually served by the pallial (gill) groove in chitons and limpets, for example trapping water as an extracorporeal reserve during low tide (see Branch, 1981 review). Intertidal depressions conceivably could even serve the "brooding" function performed by the pallial grooves of a few species of intertidal molluscs (Pearse, 1979), thereby limiting dispersal and protecting the young of nominal broadcast spawners. Although some species of chitons do brood their young in the pallial grooves (Pearse, 1979), eggs or larvae have never been observed in Nuttallina depressions, and the brooding function of such depressions in other species is only speculative. The various potential functions of the depressions will be discussed and assessed further, in relation to relevant findings for Nuttallina, in the following section. Nuttallina depressions are also discussed in Chapter III in connection with biogeography, substrate type and species involved, and in Chapter V in relation to homing and territorial behavior.

DISPERSION OF NUTTALLINA AT DIFFERENT SHORE HEIGHTS

To describe the dispersion patterns of the high and low intertidal populations of Nuttallina, nearest neighbors analyses were performed using the popular formulation of this method by Clark and Evans (1954), as modified by Simberloff (1979). In this method, the observed average distance between nearest neighbors (r_{OBS}) is compared to the average expected distance (r_{EXP}) between the same number of entities distributed at random over the same area. The nearest neighbors index indicates a random dispersion if equal to 1, a uniform, or overdispersed, dispersion if greater than 1, and a clumped, or aggregated, dispersion if less than 1. Simberloff (1979) noted that, by treating biological entities as points rather than as objects of finite size, as in the classical nearest neighbors analysis such as that of Clark and Evans (1954), underestimates the expected mean nearest neighbor distance for finite entities, overestimates its error, and can lead to incorrect conclusions. Simberloff (1979) provided a formulation for finite circles and suggested that when the diameter of the objects being analyzed is larger than one-quarter to one-half the expected mean nearest neighbor distance for points, his suggested modifications can be particularly important.

Since the substrate is functionally a two-dimensional space for Nuttallina, because it must crawl over the terrain to feed or interact with other specimens, nearest neighbors analyses for Nuttallina were performed using two-dimensional formulae rather than those recently introduced for K dimensions by Clark and Evans (1979). Clark and Evans (1954) pointed out that a set of points may be random with respect to a specified area but decidedly nonrandom with respect to a large space which includes the specified area. In other words, in a heterogeneous environment, the size of the site that is selected for study is all-important. The dispersion pattern might be overdispersed within clumps contained in a larger area that exhibits aggregated dispersion overall. In the high intertidal area at the Seal Rock study site, quadrat size was chosen to be 1 m^2 to take in bare and less bare spots of substrate and small pools. The lower intertidal area at the Seal Rock site is quite uniform in appearance, and because the density of Nuttallina there is so high, three small sub-quadrats 200 cm^2 in area were randomly selected within each of three 1 m^2 quadrats for study. One small additional quadrat (20 X 20 cm) was examined in the dense chiton band area.

In the low intertidal area, where the 1 m^2 quadrats just fit within the dense chiton band vertically (~30 to 60 cm above MLLW), horizontal positions were chosen haphazardly. In the upper intertidal area, locations away from the dense chiton band at ~120 to 150 cm above MLLW were selected haphazardly in areas that were populated with reasonable numbers of <u>Nuttallina</u> specimens. All three upper intertidal quadrats contained one to three small pools approximately 30 cm in diameter, several very small (~8 cm diameter) pools and several small (~12 cm diameter) mussel clumps.

Chitons are extremely difficult to find among the depressions and dense algal growth of the low area. Thus, in the low intertidal quadrats, black and white photography with a strobe was employed to allow all of the field time to be spent selecting quadrats, carefully delineating them with string and clay, and finding and marking the individual chitons, breaking up substrate where necessary, leaving the measuring to be carefully done in the laboratory. Field and photographic measurements for the 20 X 20 cm quadrat gave similar results. Lengths of the smallest and largest chitons found were carefully measured flat, to provide a size range estimate for each quadrat. Only chitons with more than half of their total area in the quadrat were included in the quadrat density estimate and, for chitons near the quadrat boundary, nearest neighbors (N-N) distances were measured to the nearest chiton in or out of the quadrat, as suggested by Clark and Evans (1954). N-N distances were measured between centers of the fourth valves of chitons, which had been marked in the field.

The area over which a chiton can crawl is significantly greater than that calculated from the point-to-point dimensions of a quadrat. Roughness-correction factors were estimated in Chapter V by placing a string along the rock from boundary to boundary of a quadrat in several places, measuring it, dividing by the point-to-point dimension and averaging the separate estimates for each quadrat. A roughness-correction factor of 1.25 was taken as a reasonable estimate for N-N quadrats in the low dense chiton band (see Table V-1).

Quadrats in the upper intertidal area were treated differently from those on the lower shore. Due to the larger area involved and to the easier detection of chitons in the upper area, measurements were made in the field with dividers, or a string for longer distances. Roughness-correction factors were determined for each quadrat separately using the string method and averaging four measurements, two each in the x and y directions.

Results of the nearest neighbors analyses are presented in Table II-4 and Figure II-10. Although many of the quadrats are characterized by random dispersion patterns, one of the three quadrats in the high intertidal area shows a clumped dispersion, while four of the ten low quadrats exhibit an overdispersed pattern. Most quadrats with random dispersion exhibit the same tendencies (high quadrats toward clumping, low ones toward overdispersion), although the nearest neighbor index (c) was nonsignificant.

Specimens in all N-N quadrats are of a wide range of sizes and computer simulations by Simberloff (1979) suggest that allowance for

TABLE II-4. Nearest neighbor analyses of dispersion of *Nuttallina* specimens in high and low intertidal areas at Seal Rock study site, La Jolla, CA in March, April 1980 (except quadrat low 4, analyzed 15 February 1980).

Intertidal Area	Quadrat[A]	Quadrat size (pt-to-pt)	N	Density[B] (N/m^2, pt-to-pt)	Approximate Range of Chiton Lengths (mm)	$\bar{r}_{OBS}$[C] (pt-to-pt, cm)	$\bar{r}_{EXP}$ (pt-to-pt, cm)	Chiton diameter[D] (mm)	Linear Roughness Correction Factor	Roughness corrected nearest neighbor[E] index[C]	Two-Tailed Significance Level, P	Dispersion Pattern (0.05 Significance level)
HIGH	1	1 m^2	39	39	9-41	8.33	8.14	17.7	1.09	+0.29	0.77	Random
	2	1 m^2	143	143	7-40	3.53	4.48	16.6	1.10	-5.65	<<0.01	Clumped
	3	1 m^2	57	57	7-33	6.08	6.76	14.1	1.15	-1.56	0.12	Random
LOW	1-1	10 x 20 cm	35	1750	7-27	1.67	1.62	14.1	1.25	+1.35	0.18	Random
	1-2	10 x 20 cm	39	1950	6-22.5	1.54	1.42	10.6	1.25	+2.45	0.01	Over-Dispersed
	1-3	10 x 20 cm	26	1300	3.5-21	1.84	1.61	10.6	1.25	+2.37	0.02	Over-Dispersed
LOW	2-1	10 x 20 cm	42	2100	3-26.5	1.44	1.38	10.4	1.25	+1.80	0.07	Random
	2-2	10 x 20 cm	33	1650	4.5-32	1.64	1.60	12.9	1.25	+0.89	0.37	Random
	2-3	10 x 20 cm	7	350	16.5-23.5	2.81	2.94	14.1	1.25	-0.23	0.82	Random
LOW	3-1	10 x 20 cm	23	1150	7-24	1.91	1.78	12.7	1.25	+1.22	0.22	Random
	3-2	10 x 20 cm	25	1250	6-25	1.90	1.65	11.0	1.25	+2.41	0.02	Over-Dispersed
	3-3	10 x 20 cm	27	1350	7-29	1.79	1.70	12.7	1.25	+1.55	0.12	Random
LOW	4	20 x 20 cm	49	1225	6.5-29	1.87	1.73	12.6	1.25	+2.37	0.02	Over-Dispersed

NOTES

A. Three quadrats in sparsely-populated high intertidal area. Lower quadrats are in groups of three, selected randomly within a 1 m^2 area, except quadrat low 4, which was analyzed separately on 15 February 1980.

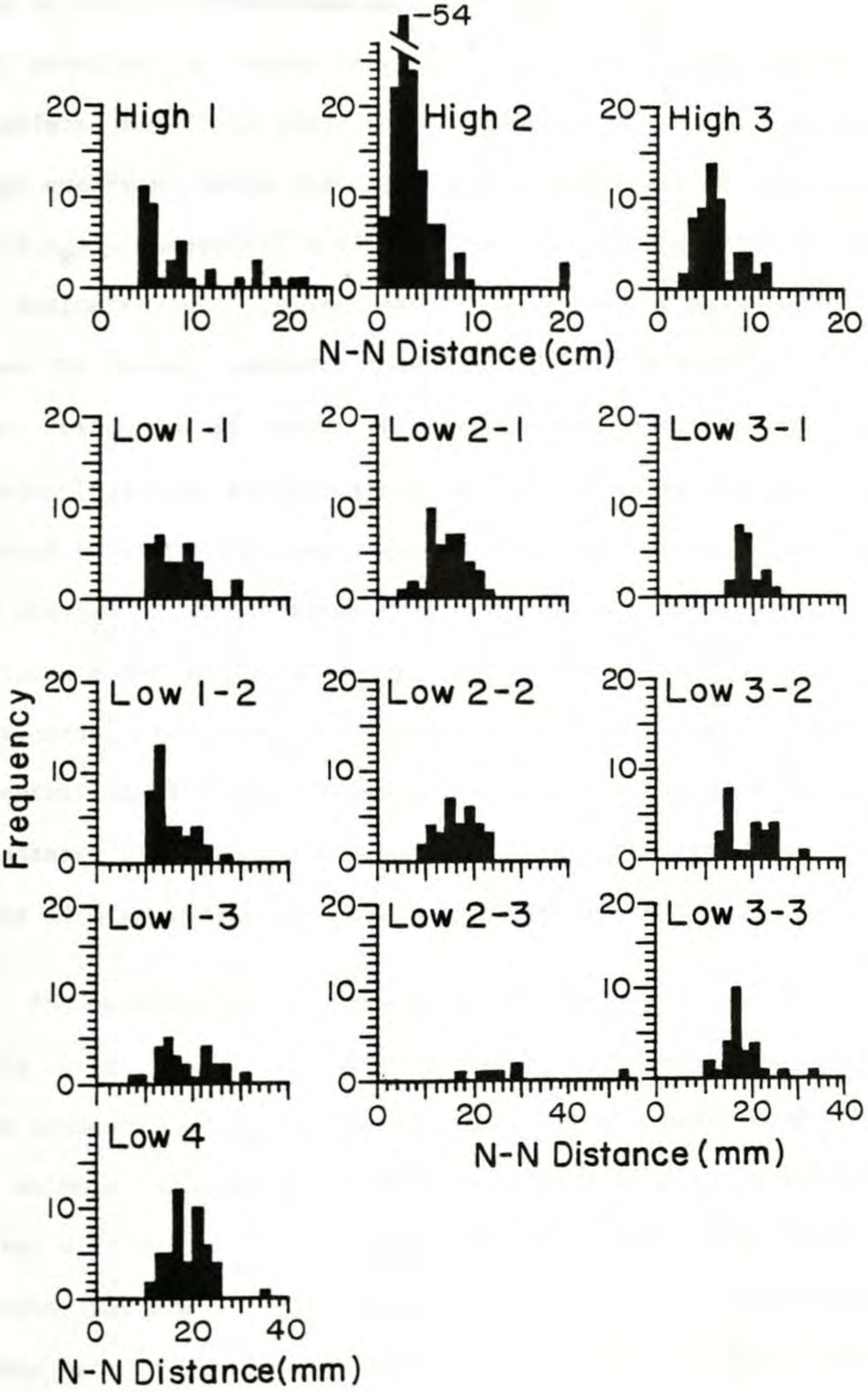
B. Density calculated using areas corresponding to point-to-point dimensions, e.g. 10 X 20 cm, not areas corrected for roughness of the substrate.

C. $\bar{r}_{OBS}$ is mean nearest neighbor point-to-point distance measured between centers of fourth valves of neighboring chitons. r's for chitons at the borders of quadrats were measured to nearest neighbor in or out of quadrat, as suggested by Clark and Evans (1954), while densities include only those *Nuttallina* more than half in quadrat boundaries. $\bar{r}_{EXP}$ (and $\sigma_{\bar{r}_{EXP}}$) determined using simulation curves in Simberloff (1979).

D. "Chiton diameter" represents the diameter of the circle with area equal to the area of a chiton of length equal to the mean of the observed length range limits, taking the area of the chiton as πab (the area of an ellipse) where a and b represent the half-length and quarter-length of the chiton.

E. "c" is the nearest neighbor statistic corrected for finite circles of equal diameter, using densities and r's corrected for roughness. The fact that animals in all quadrats were of a wide range of sizes means that the low quadrats, where the circle correction is most important ($D > 1/2\ \bar{r}_E$), are actually more overdispersed than indicated by the calculations made here assuming all animals were of the same size. A positive c represents a tendency toward overdispersion; negative c a tendency toward clumping; zero, a random dispersion.
N.B.: Subquadrat 2-3 was at the lower boundary of the low intertidal dense chiton band. Depressions in this particular subquadrat were filled with sand and many were overgrown with *Phragmatopoma*. Sand inundation therefore may explain the very low relative density in this particular quadrat.

Figure II-10. Frequency distributions of nearest neighbor (N-N) distances of Nuttallina in high and low intertidal areas at the Seal Rock study site, La Jolla, CA in March and April, 1980 (except quadrat Low 4, 15 February 1980). Low intertidal quadrats were located within the densely-populated chiton band; high quadrats were in sparsely-populated areas. Distances illustrated are point-to-point, between chiton fourth valve centers. Only in quadrat High 2 were Nuttallina specimens in contact (N=2). Corresponding data are summarized in Tables II-4 and II-5. N.B.: scale changes by a factor of 5 from high to low intertidal quadrat histograms.


-54
High 1
High 2
High 3
N-N Distance (cm)
Low 1-1
Low 2-1
Low 3-1
Low 1-2
Low 2-2
Low 3-2
Low 1-3
Low 2-3
Low 3-3
Low 4
Frequency
0
10
20
40
N-N Distance (mm)
N-N Distance(mm)

size variability tends to increase the nearest neighbor index, toward more overdispersion, i.e., toward values obtained by treating the animals as points rather than as equal-sized finite circles. Simberloff (1979) provides no means for quantitatively correcting for the size range effect, but it is clear that the effect would be negligible for the high quadrats, where the correction for circles is negligible anyway ($D < 1/2\ r_E$). Simberloff's (1979) simulations for circles of varied sizes indicate that taking into account the size range effect would increase the nearest neighbor index for the low quadrats, giving an even stronger indication of overdispersion. Additionally, it should be noted that, size-frequency distributions in the low shore dense chiton band are skewed to the right (see Figure II-8). Therefore, a size calculated as the average of the observed upper and lower size limits (the size used in this N-N analysis) overestimates the average size of chitons in a low quadrat, producing an inflated circle correction. This results in an underestimated (less overdispersed) nearest neighbor indices for the low quadrats. In summary, the tendency for overdispersion in the low quadrats is most likely underestimated considerably.

The variability in topography and habitat in the high intertial quadrats (i.e., pools, bare rock and mussel clumps) probably accounts for the tendency for <u>Nuttallina</u> to have a clumped dispersion there. These animals apparently congregate preferentially in damper habitats and areas with relief in the high intertidal zone. Despite the clumped dispersion pattern in the high intertidal area, only two <u>Nuttallina</u> specimens were in contact there. Figure II-10 displays the nearest neighbor distances observed.

Note that the size ranges of Nuttallina in the high quadrats (Table II-4) include very small chitons not observed in the high areas collected for description of the shore-level size-frequency distribution in December, 1980 (Figure II-8). This is a result of the presence of pools of substantial size in the high intertidal quadrats in the nearest neighbor analysis unlike in the size-frequency study, and is not due to the difference in time of year of the collections, as suggested by observations over several years at the site.

In the low intertidal zone, the frequency of quadrats with a random dispersion probably reflects the homogeneity of the dense chiton band in general. Instances of overdispersion in the low intertidal quadrats suggest that the chitons are spacing themselves out on the rock.

Certainly, an animal like Nuttallina that browses on algae on the substrate cannot completely cover entire intertidal areas but must leave area for algal growth. By forming depressions, Nuttallina specimens have increased the surface area of some low intertidal areas by as much as 50% (see roughness-correction factor, Table II-4), although not all of this area is available for algal growth. Table II-5 indicates that Nuttallina does not cover more than 15 to 20% of the total rough-corrected area in the low intertidal habitat, despite its high densities. In addition, not even all available depressions are constantly occupied by Nuttallina, as shown in Table II-5.

Stimson (1970, 1973), Sutherland (1970) and Branch (1975), among others (see Branch, 1981), provide evidence suggesting that various species of homing intertidal limpets compete for food or space and that

TABLE II-5. Percentage of space occupied by Nuttallina at Seal Rock site, La Jolla in February to April 1980.[A]

Intertidal Area	Quadrat	Percentage of Available Depressions Occupied by Nuttallina (Total No. of Depressions)	Roughness-corrected Area (cm^2)	Percentage of R-C Area Occupied by Nuttallina[B]
HIGH	1	-	11800	1
	2	-	12100	3
	3	-	13300	1
LOW	1-1	85(41)	312	18
	1-2	91(43)	312	11
	1-3	67(39)	312	7
LOW	2-1	89(47)	312	11
	2-2	97(34)	312	14
	2-3	58(12)	312	4
LOW	3-1	82(28)	312	9
	3-2	76(33)	312	8
	3-3	73(37)	312	11
LOW	4	-	625	10

NOTES

A. Terminology and quadrat information explained in Table II-4 and text.

B. Area occupied by chiton approximated by multiplying $\frac{\pi D^2}{4}$ by number of chitons. D (chiton diameter) and number of chitons are listed in Table II-4.

aggressive behavior, either active or ritualized (Wright, 1982), may be used to protect home areas. Similar suggestions have not been made for homing intertidal chitons, although scattered observations of aggressive encounters involving chitons have been made (see Chapter V). It is possible that the tendency for overdispersion of Nuttallina in low intertidal areas results from territoriality and/or aggressive behavior. This topic will be taken up in Chapter V.

It is important to realize that nearest neighbors analyses here have assessed positions of Nuttallina at low tide only, the period of inactivity. At high tide, Nuttallina feeds, occasionally moving relatively large distances over the substrate (Chapter V). Dispersion patterns at high tide, or feeding dispersions, may be quite unlike the low tide dispersion patterns described here, although low tide patterns are an end result of activity behavior at high tide.

The well-defined low intertidal depressions with dense algae bordering them undoubtedly help to reduce contact between individuals. No low shore individuals were observed to be in contact in nearest neighbors analyses (see Figure II-10), although at other times two individuals rarely have been observed to partially occupy the same depression for a tide or two. An individual taking up a spot on a ridge between depressions is vulnerable to a variety of biotic and abiotic factors.

Several differences in the size, density, biomass and dispersion of high and low shore populations of Nuttallina, and in the physical factors and tidal regimes that they experience, have been examined. In the next chapter, the taxonomic status of the high and low shore *Nuttallina* populations will be investigated, with unexpected results.

CHAPTER III

BIOCHEMICAL REVISION OF THE CHITON GENUS NUTTALLINA IN THE CALIFORNIAS, WITH THE DISCOVERY OF A NEW SPECIES AND RESURRECTION OF ANOTHER SPECIES, AND WITH NOTES ON INTERTIDAL DISTRIBUTION AND BIOGEOGRAPHY

INTRODUCTION

The intertidal chiton genus Nuttallina is presently considered to be represented in the northeast Pacific by a single wide-ranging species, N. californica (Nuttall, in Reeve, 1847) (Ferreira, 1982; also see Appendix III for the present disposition of other species at any time referred to Nuttallina). Nuttallina has a long taxonomic history that has been complicated by improper and/or incomprehensible citations. Apparently, early revisers, P.P. Carpenter, W.H. Dall, and H.A. Pilsbry, used information and new names contained in a massive manuscript that Carpenter produced in the 1870's but never published (cf., Bullock, 1972; Smith, 1977), and this situation has contributed to the confusion. Smith (1977) recounted much of the taxonomic history of Nuttallina in his discussion of Nuttallina (=Acanthopleura) fluxa, but he was unable to resolve satisfactorily the taxonomic bookkeeping problems for the genus.

Historically, northeast Pacific Nuttallina has intermittently been regarded as two separate species, N. californica (Nuttall, in Reeve, 1847) and N. fluxa (Carpenter, 1864). When regarded as separate species, N. californica and N. fluxa have been suggested to be distributed with a break at or near Point Conception, CA, with the former species occurring north to about the Canadian border, and the latter to the south to between Scammon's Lagoon and Punta Abreojos, Baja California (Pilsbry, 1893; Raymond, 1894; Smith, 1977). The morphological similarity of the two species has led to considerable uncertainty in their identification even by those who have accepted their separate species status (e.g., Berry, 1922); thus, it is not particularly surprising that workers have occasionally treated the two as synonyms since their descriptions (Dall, 1879; Leloup, 1940).

In recent years, most taxonomists and ecologists have had difficulty in distinguishing the two forms and/or have expressed doubt as to their status as separate species (Louda, 1972; Putnam, 1980; R. R. Seapy, Cal State Fullerton, *in litt*., 30 June 1980; Vesco, 1980) and contemporary taxonomists, with the notable exception of Smith (1977), had been content to consider *Nuttallina californica* and *Nuttallina fluxa* as conspecific (A. J. Ferreira, associate chiton specialist, California Academy of Sciences, *in litt*., 14 August 1980; J. H. McLean, curator of malacology, L.A. Co. Museum of Natural History, *in litt*., 21 April 1981). Finally, in a revision of the family Lepidochitonidae in the northeast Pacific, Ferreira (1982) recently synonymized the two *Nuttallina* species on the basis of morphological examination of many specimens from locations throughout the range of the genus.

Comparative studies of *Nuttallina* populations in La Jolla, CA revealed a number of conspicuous differences between high and low intertidal groups (described in other chapters) -- in size-frequency distribution, densities and dispersion; in homing/movement behavior and association with chiton-shaped depressions; and, preliminarily, in body water content and in growth rate. Such differences might be expected to occur between populations of a single species occurring over an extensive vertical range of the intertidal zone (i.e., over an area characterized by a steep gradient of environmental and biological factors). In fact, such differences have been observed within several intertidal species: for example, differences with intertidal height in size-frequency, density, dispersion, growth rate, and mortality in the limpet *Collisella* (= *Acmaea*) *scabra* (Sutherland, 1970), in mantle cavity water and heart rate in *Collisella* (= *Acmaea*) *limatula* (Segal, 1955), in mantle cavity and shell water in the barnacle *Tetraclita squamosa* (Suzuki and Mori, 1963), in shell morphology of the limpet *Patella vulgata* (Davies, 1969), and differences in movement on intertidal substrates with different characteristics in *Collisella digitalias* and in *Collisella scabra* (Collins, 1977). However, the finding of a substantial number of such differences in the *Nuttallina* populations of the present study raised doubt as to their taxonomic status, providing impetus for a taxonomic examination of the high and low intertidal populations.

Protein electrophoresis is one among several biochemical techniques developed within the last twenty years that have been used with success to complement traditional taxonomic methods (Avise, 1974; Shaklee et al., 1982). Whereas classical taxonomic analyses involve examination of morphological, physiological or behavioral traits for which the genetic basis is usually unclear, electrophoresis allows more direct examination of the underlying genetics by permitting the analyst to identify different proteins indicative of different alleles at gene loci. Thus, where breeding experiments are not possible or are inconclusive, protein electrophoresis can help to elucidate the reproductive relations of different groups by providing a more finely resolved picture of their genetic relationships; biological species, or evolutionary units reproductively isolated from other such units, can be detected more easily.

In most instances, biochemical analyses have confirmed the genetic isolation of groups originally recognized on the basis of breeding tests, cytological comparisons, or morphological differences. Further, there has generally been agreement between the taxonomic results generated by electrophoretic and by classical phenetic methods, such that species or populations which are most distinct on phenetic grounds are also most distinct electrophoretically (Avise, 1974). However, cases have been reported in which electrophoretic results do not match results from phenetic studies; such studies indicate a degree of independence of morphological and electrophoretic (genetic) change. Thus, in a few instances, substantial morphological variation has been accompanied by little detectable electrophoretic (genetic) divergence. For example, an electrophoretic survey of the tropical fish Hypoplectrus revealed no fixed allelic differences nor significant allele frequency differences among nine species, differentiable on the basis of distinctive color patterns, lending support for the synonymy of all nine under the name H. unicolor (Graves and Rosenblatt, 1980). Similar studies have contributed to the taxonomic simplification of the morphologically incredibly diverse pulmonate snail genus Cerion for which more than 600 species have been named (Woodruff, 1978).

In studies of this nature, conclusions as to the genetic similarity, thus reproductive integration, of different groups must be tempered with considerable caution since electrophoresis is a conservative technique. Not all nucleotide substitutions result in amino acid changes nor do all amino acid changes effect a change in net charge of proteins. Dissimilar proteins may exhibit identical electrophoretic mobilities simply because there are only a finite number of positions on an electrophoretic starch gel. Thus, there is a possibility that genetically differentiated groups will not be distinguished electrophoretically.

In the converse situation, where substantial electrophoretic (genetic) differentiation is unaccompanied by significant morphological divergence, conclusions as to the reproductive <u>isolation</u> of different groups are more likely strengthened than weakened by the conservatism of the electrophoretic technique. In several such cases, where classical taxonomic, particularly morphological, techniques have proved insufficient, electrophoresis, with its enhanced "genetic resolving" power, has been particularly effective as a taxonomic tool. Thus, in recent years, a number of sibling species, defined as "morphologically similar or identical natural populations that are reproductively isolated" (Mayr, 1963), or cryptic species, from various phyla have been detected by the application of electrophoretic techniques [e.g., in the Cnidaria (Carter and Thorpe, 1981; Bucklin and Hedgecock, 1982); Ectoprocta (Thorpe et al., 1978); Mollusca [Chambers, 1978 (Mesogastropoda); Murphy, 1978 (Archaeogastropoda)]; Annelida (Grassle and Grassle, 1976); Arthropoda (Ayala et al., 1970; Hedgecock, 1979; Dando and Southward, 1980);

Echinodermata (Manwell and Baker, 1963); and Chordata (Johnson et al., 1972)].

Electrophoretic techniques were used to examine the morphologically similar populations of Nuttallina in La Jolla. This study represents the first of its kind involving chitons. The La Jolla populations exhibited sufficient electrophoretic genetic differentiation to be regarded as three species: a high intertidal, a low intertidal, and a rarer (in La Jolla) third species. A subsequent biogeographic survey confirmed that the species normally occupy different intertidal habitats, and revealed fairly complex geographic distributions of the three Nuttallina species. These distributions are largely as would be predicted for one cold-water and two warm-water species. The complexity of the distributions has probably contributed to the uncertainty of earlier taxonomic studies.

The problem of morphological discrimination of these three abundant and conspicuous intertidal species, which are of interest to ecologists and taxonomists, is facilitated by their electrophoretic "labelling" and will be taken up in Chapter IV. Results of this study suggest that electrophoretic examination of other intertidal organisms occupying extensive vertical ranges, particularly those for which differences with intertidal height have been reported, might reveal other cryptic species.

MATERIALS AND METHODS

POPULATIONS STUDIED

Collections of Nuttallina were made between January and November, 1981 for electrophoretic, and subsequent morphological (see Chapter IV), analyses. Primary attention was given to the La Jolla, CA site that was the focus of an ongoing physiological-ecological study. Preliminary studies in La Jolla revealed consistent differences in the electrophoretic banding patterns of specimens sampled from different habitats and/or exhibiting various external morphological characters. As a result, collections at other sites were also segregated according to habitat (primarily high vs. low intertidal, but also pools where present, and other distinct habitats such as Phragmatopoma beds and coralline algal turf stands). In each habitat, a conscious effort was made to sample the full spectrum of morphological types (e.g., wide vs. narrow valves, dark vs. striped girdle), as well as the entire range of sizes present. Such a sampling scheme accents presences and absences of types in a sampling area; rarer morphs and size classes are expected to have been over-represented in the samples. Additionally, small specimens were most likely to have been missed, hidden in the foliage or substrate. Due to the constraints of time and funds, the number of individuals eventually electrophoresed was often only a subset representative of the total sample obtained from a site. Both sexes, as well as many specimens that could not be sexed, were represented in the samples, but no sex-specific electrophoretic banding patterns were detected.

Nine localities, spanning 925 air kilometers of the California and Baja California coasts, and including two of the California islands, were sampled (Figure III-1). Localities, dates of collection, and numbers of individuals electrophoresed are as follows: 1) Bodega Head, near the Bodega Marine Laboratory and near Bodega Bay, Sonoma Co., CA; 17 and 18 January 1981; N=35; 2) Gaviota, CA, isolated sandstone outcrops and boulders 4.3 km south of the Gaviota State Beach entrance; along Highway 101; 16 April 1981; N=52; 3) Whites Point, L.A., CA, study site of Vesco (1980); 3 April 1981; N=55; 4) La Jolla, CA, study site described in Chapter II; February through April, and November 1981; N=153; 5) Punta Banda, Baja CA, Mexico, SW side, 116°44.0′W, 31°44.3′N, near site designated "Papalote 16D" (Anonymous, 1960), 2 April 1981; N=41; 6) Santa Catalina Island (NW), L.A. Co., CA, eastern side of mouth of Fisherman′s Cove, University of Southern California Marine Science Center, near "West Line" of Littler (1978); 13 April 1981; N=38; 7) Santa Catalina Island (SW), at mouth on north side of Little Harbor; 14 April 1981; N=43; 8) Santa Catalina (SE), at point on southeast side of Lover′s Cove, Avalon; 15 April 1981; N=38; 9) San Nicolas Island (NW), Ventura Co., CA, north-facing shore at the west end of the island; 30 August 1981; N=12. No chitons were found at the north end of Jalama County Park, Santa Barbara Co., CA, just north of Point Conception. Live freshly-collected chitons were transported to the laboratory where they were immediately frozen and stored at -70°C, except for the specimens from Santa Catalina Island and Gaviota, which were frozen and stored at -10°C for 3 to 5 days after which they were transported packed in ice for several hours, to the laboratory, where they were immediately stored at -70°C.

Figure III-1. Geographic distribution of collection sites for the electrophoretic study and of sites of preserved collections used to supplement biogeographic information. Arrows in the inset indicate the six collection sites on the Channel Islands. Detailed information about each collection is given in Materials and Methods and in Table III-8.


125°
120°
115°
110°
40°
35°
30°
25°
20°
CAPE MENDOCINO
PT. ARENA
Bodega Bay
Bolinas
SAN FRANCISCO
Pescadero Pt
MONTEREY
Pacific Grove
POINT CONCEPTION
Shell Beach
Gaviota
SANTA BARBARA
LOS ANGELES
Whites Point
CHANNEL ISLANDS
La Jolla
SAN DIEGO
ENSENADA
Punta Banda
SAN QUINTIN
Pta. Baja
I. Guadalupe
I. San Benito
Pta. Laguna Manuela
Bahia Tortugas
PUNTA EUGENIA
Pta. Abreojos
BAHIA MAGDALENA
120°
119°
118°
34°
33°
SAN MIGUEL ISLAND
SANTA CRUZ ISLAND
SANTA ROSA I.
ANACAPA I.
USC Marine Science Center
SANTA BARBARA I.
SANTA CATALINA I.
SAN NICOLAS ISLAND
Little Harbor
Avalon
SAN CLEMENTE I.
SAN DIEGO

Notes on the major physical and ecological features of each site, particularly as compared to the La Jolla site, were recorded, and semiquantitative estimates of Nuttallina density, involving small areas (~ 0.1 m^2) within each habitat, were made. A sample of the rock substrate was collected at each site for later identification and determination of hardness on a relative scale.

Twenty-two lots of preserved specimens from 16 additional localities, from Point Arena, Mendocino Co., CA to Magdalena Bay, Baja California, were obtained from the Allan Hancock Foundation, Los Angeles, CA (AHF), the California Academy of Sciences Invertebrate Zoology Collection (CASIZ), the Los Angeles County Museum of Natural History (LACM), the San Diego Museum of Natural History (SDNH) and the Scripps Institution of Oceanography Benthic Invertebrate Collection, La Jolla, CA (SIO) to supplement the biogeographic information obtained from the electrophoretic identifications (see Figure III-1 and Table III-8 for sample information). Identifications of specimens were based on diagnostic external morphological criteria established in Chapter IV. Identifications were conservative; any specimens not clearly assignable to a particular type were categorized as questionable. Presences, but not absences or proportions of types in a sample, are significant results in the biogeographic studies because of the nature of the samples used.

ELECTROPHORESIS

Horizontal starch gel electrophoretic procedures were the same as those described by Ayala et al. (1973) with the following additions and modifications. Starch molds were 17.8x15.2x0.9 cm. To prepare for electrophoresis, 15 to 30 chitons were thawed, measured (for morphological study, Chapter IV), and dissected. The tissues were homogenized in 1 to 2 volumes of grinding buffer (0.5 M Tris-HCl, pH 7.1, 1 mM EDTA, 50 µM $NADP^+$, 1 mM β-mercaptoethanol) in either a Du-all 23 tissue homogenizer or a Diamonite mortar and pestle in an ice-water bath. Homogenates were frozen immediately in sample trays over dry ice, kept at -70°C overnight, and thawed in the morning for electrophoresis. Whatman #3 filter paper wicks, 4x9 mm, were soaked in the thawed homogenates, blotted and inserted into a slit positioned on the long side of a starch gel block for electrophoresis. The remainder of the homogenate was then stored at -70°C for any retesting that might be required. One to three previously scored Nuttallina samples were run as controls on each gel. Gels were run for 8 to 10 hours.

Preliminary testing showed that identical zymograms were obtained whether samples were homogenates of foot tissue, digestive gland, or a mixture of digestive gland and intestinal tissue; however, activity levels of some enzyms were low to negligible in one tissue or another, so all further samples included approximately equal amounts of foot tissue and digestive gland/intestine tissue, to provide reliably the complete spectrum of interpretable zymograms.

Control tests indicated that banding patterns are identical for thawed homogenates and for fresh homogenates from either frozen or live animals, although activities are slightly reduced in all enzymes except alcohol dehydrogenase in the thawed homogenates. Thus, for convenience, thawed homogenates were routinely used.

Five gel-electrode buffer systems provided the best resolution of the enzymes listed in Table III-1: systems A, B, and C of Ayala et al. (1973); system D) Gel buffer: 8 mM K_2HPO_4 and 1 mM monohydrate citric acid, pH 7.0; electrode buffer: 214 mM K_2HPO_4 and 27 mM monohydrate citric acid, pH 7.0 (Shaw and Prasad, 1970); system E) Stock solution A: 30 mM monohydrate lithium hydroxide and 190 mM boric acid, pH 8.1; stock solution B: 8 mM monohydrate citric acid and 50 mM Tris; electrode buffer: stock solution A; gel buffer: 1 to 9 mixture of stock solutions A and B (Selander and Yang, 1969). Systems A and C were regulated at about 40 mA, B at 30 mA, D at 75 mA, and E at 60 mA.

Assays for the enzymes were the same as those described by Ayala et al. (1972) and Ayala et al. (1973, 1974) with the following changes. Alkaline phosphatase: delete polyvinylpyrrolidone. Alcohol dehydrogenase: use 3 ml isopropyl alcohol and 1.5 ml amyl alcohol as substrate. Glyceraldehyde-3-phosphate dehydrogenase: use MTT rather than NBT. Hexokinase: use 900 mg glucose substrate (Tracey et al., 1975). Lactate dehydrogenase: add PMS. Peptidase: modified from Shaw and Prasad (1970); delete $MnCl_2$, substitute L-leucylglycylglycine for glycyl L-leucine, and Tris-HCl buffer, pH 8.0, for phosphate buffer, and apply as an agar overlay. 6-phosphogluconic dehydrogenase: modified from Shaw and Prasad (1970); 50 ml 0.05 M Tris-HCl, pH 8.0, 100 mg $MgCl_2$, 40 mg Na_3 6-phosphogluconate, 5 mg $NADP^+$, 3 mg PMS, 5 mg NBT. Sorbitol dehydrogenase: reagents as in Shaw and Prasad (1970), except dissolved in only 50 ml buffer. PMS was added to the appropriate stains immediately, rather than after an incubation period. After they were read, gels were photographed, and were fixed as described by Ayala et al. (1972).

TABLE III-1. Enzymes assayed with abbreviations and international code numbers, the number of presumptive loci scored, buffer systems used, and tissues showing the greatest activity in <u>Nuttallina</u>.

	Enzyme and Presumptive Loci Abbreviation	E.C. No.	Number of loci scored	Buffer System^A	Best Tissue^B
Alkaline phosphatase	Aph	3.1.3.1	1	E	F, I/DG
Alcohol dehydrogenase	Adh	1.1.1.1	1	B	I/DG
Glutamate-oxaloacetate transaminase	Got	2.6.1.1	2	B	F, I/DG
Glyceraldehyde-3-phosphate dehydrogenase	G-3-pdh	1.2.1.12	1	D	F
Hexokinase	Hk	2.7.1.1	1	B	F
Lactate dehydrogenase	Ldh	1.1.1.27	1	B	F
[L-Leucylglycylglycine] peptidase	Lggp	-	1	A	F, I/DG
Malate dehydrogenase	Mdh	1.1.1.37	2	C	F > I/DG
6-phosphogluconate dehydrogenase	6-pgdh	1.1.1.44	1	C	F, I/DG
Phosphoglucomutase	Pgm	2.7.5.1	1	C	F > I/DG
Sorbitol dehydrogenase	Sdh	1.1.1.14	2	B	I/DG

NOTES

A. Buffer systems are described under Materials and Methods.

B. Symbols for tissues are F = foot, I/DG = intestine/digestive gland; best tissues are those with greatest activity.

In the RESULTS and DISCUSSION sections, presumptive gene loci are designated by abbreviations for the enzymes following the method of Ayala et al. (1973). When there are several zones of activity for a given enzyme, a hyphenated numeral is added to designate each zone and the gene presumed to code for it. The zones are numbered in order of increasing anodal mobility. The most common allele at each locus in the Bodega population is arbitrarily designated "100". Designations for other alleles are obtained by adding (if more anodal) or subtracting (more cathodal) the number of millimeters separating the variant from the 100 allele.

RESULTS

INITIAL ELECTROPHORETIC SURVEY: GENETIC INTERPRETATION OF ZYMOGRAMS AND SEPARATION OF THREE ELECTROMORPHS

The initial survey involved Nuttallina from two different shore levels at La Jolla, "HI/DRY" from bare rock $\geq$122 cm above mean lower low water (MLLW) and "LOW" from a dense algal turf band with depressions, from about 30 to 76 cm above MLLW, and Nuttallina from the Bodega site at midtide level from about 76 to 122 cm above MLLW. Fourteen enzyme zones were resolved electrophoretically and were interpreted as genetic loci by criteria generally employed when progeny testing cannot be performed (Tracey et al., 1975). In this study, heterozygote banding patterns and Hardy-Weinberg considerations were the most important criteria used in genetic interpretations of zymograms.

Dramatic differences in the starch gel electropherograms of the three groups were obtained; five of the 14 presumptive loci surveyed indicated fixed or nonoverlapping differences among the three groups (Figure III-2 and Table III-2). Adh, represented by a single band per sample, was interpreted as a single locus, with four bands of different mobility that are nonoverlapping among the three groups. Mdh, expressing two zones of activity for each sample, was interpreted as two loci, Mdh-1 and Mdh-2. Mdh-1 was virtually monomorphic, with the exception of a single heterozygous individual that exhibited a three-banded pattern in accord with the dimeric structure of this enzyme in many other organisms (e.g., see Harris and Hopkinson, 1977; Ward, 1978). Mdh-2 expressed single bands of indentical mobility for all BODEGA and LOW specimens, while it showed single bands of faster mobility for all HI/DRY individuals. Pgm expressed one or two bands of activity per sample, with occasional quite faint subbands (satellite bands?) of slower mobility. Pgm was interpreted as a single locus with five common alleles, heterozygotes appearing as two-banded patterns in accord with the monomeric subunit structure of this enzyme in other organisms (e.g., Wilkins et al., 1978). Although this locus is characterized by a substantial degree of polymorphism and heterozygosity, bands for the BODEGA specimens never overlapped with bands of the HI/DRY and LOW groups from La Jolla. Sdh exhibited two zones of activity that were interpreted as 2 loci. Sdh-1 expressed single bands of activity that were identical in mobility for all BODEGA and LOW samples, but that were faster than the single bands of all HI/DRY chitons. Sdh-2 exhibited identical single bands for BODEGA and La Jolla LOW specimens that were slower than single bands of La Jolla HI/DRY specimens. 6-Pgdh, the only enzyme system to

Figure III-2. Electrophoretic banding patterns for the four enzyme systems (five loci) that exhibited fixed or nonoverlapping differences for the three groups, BODEGA, HI/DRY, and LOW. Each column of bands in an enzyme system represents a single specimen.

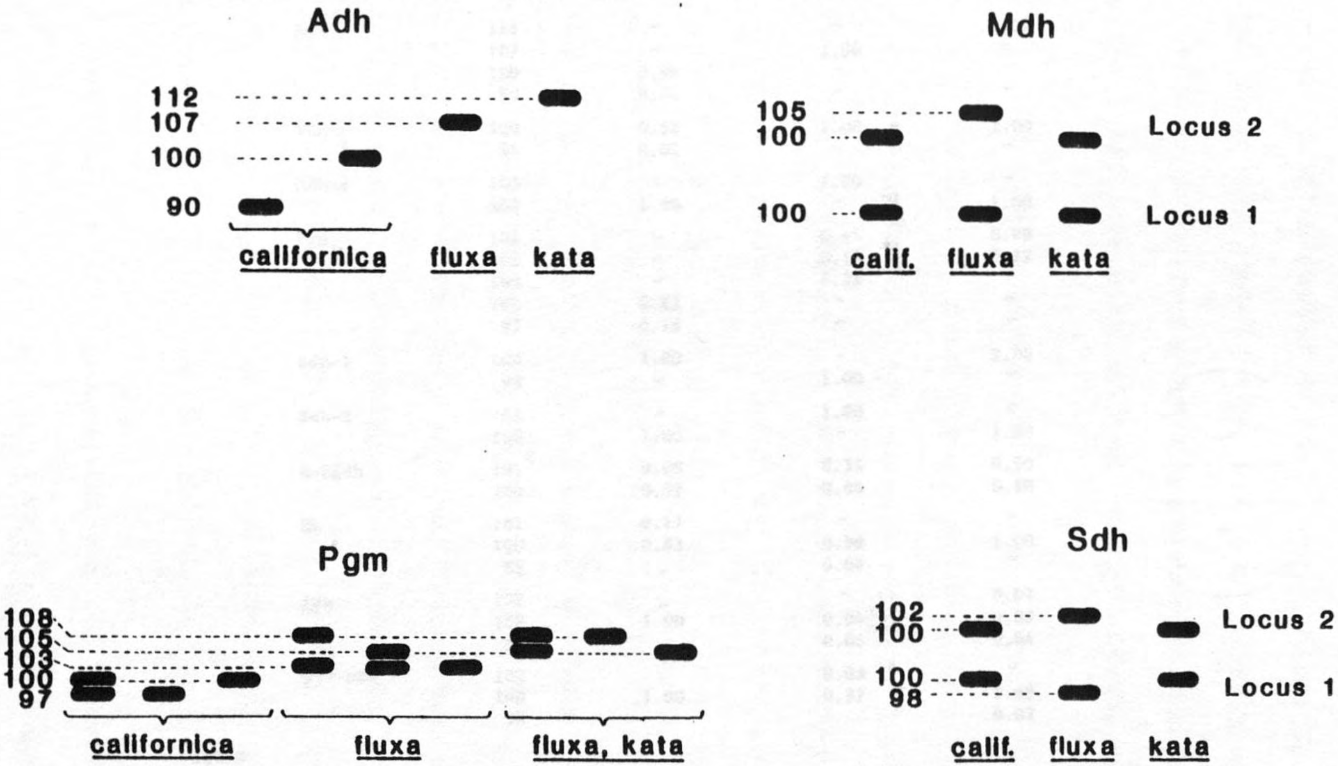

Adh
112
107
100
90
californica
fluxa
kata
Mdh
105
100
Locus 2
100
Locus 1
calif.
fluxa
kata
Pgm
108
105
103
100
97
californica
fluxa
fluxa, kata
Sdh
102
100
Locus 2
100
98
Locus 1
calif.
fluxa
kata

TABLE III-2. Allele frequencies of loci resolved in an initial survey of <u>Nuttallina</u> from the Bodega site (BODEGA) and from two shore levels at the La Jolla site (HI/DRY and LOW). Those loci not listed (Got-1, Got-2, Lggp-1, Aph) were fixed for the same allele in all 3 groups. Values next to the group description (in parentheses) indicate the average sample size per locus.[A]

Locus[B]	Allele	BODEGA(17)	HI/DRY(20)	LOW(15)
Adh	112	-	-	1.00
	107	-	1.00	-
	100	0.96	-	-
	90	0.04	-	-
Mdh-1	100	0.98	1.00	1.00
	94	0.02	-	-
Mdh-2	105	-	1.00	-
	100	1.00	-	1.00
Pgm	108	-	0.45	0.88
	105	-	0.16	0.12
	103	-	0.39	-
	100	0.82	-	-
	97	0.18	-	-
Sdh-1	100	1.00	-	1.00
	98	-	1.00	-
Sdh-2	102	-	1.00	-
	100	1.00	-	1.00
6-Pgdh	101[C]	0.08	0.35	0.90
	100	0.92	0.65	0.10
Hk	102	0.17	-	-
	100	0.83	0.98	1.00
	95	-	0.02	-
Ldh	102	-	-	0.07
	100	1.00	0.94	0.89
	98	-	0.06	0.04
G-3-pdh	102	-	0.03	-
	100	1.00	0.97	0.98
	98			0.02

NOTES

A. Loci considered only if N>6 individuals [see Gorman and Renzi (1979) concerning the relative unimportance of sample size in estimates of genetic differentiation in electrophoretic surveys].

B. Abbreviations are explained in Table III-1. -1 and -2 refer to different loci for an enzyme system. -1 represents the locus associated with the slower electrophoretic band.

C. Since 6-Pgdh runs cathodally, 101 (the most anodal allele) is closest to the origin unlike for all other enzymes here.

run cathodally, was scored as a single locus and showed a preponderance of the fast band (i.e., 100, in BODEGA and HI/DRY samples), but a greater frequency of the slow or 101 band in the LOW samples. Heterozygotes for 6-Pgdh were expressed as long barrel-shaped bands; it was not possible to accurately resolve the number of bands of the heterozygous condition, hence to infer the subunit structure of this enzyme, probably due to the small difference in mobility of the allelic variants.

The remaining seven presumptive loci, Aph, Gly-3-pdh, Got-1, Got-2, Hk, Ldh and Lggp-1, like Mdh-1, exhibited no substantial differences among the three groups. Aph showed a single band of identical mobility for all individuals. Got was represented by two zones of activity, each with identical mobility for all individuals examined, and thus was scored as two monomorphic loci, Got-1 and Got-2. Lggp expressed at least two zones of activity but only the zone of slower mobility could be resolved; Lggp-1 showed a single band of identical mobility for all individuals. Gly-3-pdh, Hk and Ldh exhibited single bands of identical mobility for almost all individuals scored, although a small amount of enzyme polymorphism was present in each. Bands of heterozygotes for Gly-3-pdh, Hk and Ldh were either insufficiently separated or too diffuse to permit reliable inferences concerning subunit structure.

The foregoing genetic interpretations of electrophoretic patterns, and the separation of the *Nuttallina* specimens examined into three different electrophoretic phenotypes (gene pools), BODEGA, HI/DRY, and LOW, are reinforced by generally close agreement between the observed phenotypic distributions and those expected assuming Hardy-

Weinberg conditions of genic equilibrium for three gene pools, computed from the data of Table III-2. The three groups clearly do not belong to only one gene pool in Hardy-Weinberg equilibrium. For example, Adh exhibited only one heterozygote (90/100) among all of the specimens examined; the absence of any 100/107 and 107/112 heterozygotes among almost all 100/100, 107/107 and 112/112 homozygotes indicates that the three groups are genetically separate. When treated as 3 separate groups, no significant departures from Hardy-Weinberg proportions are found [G goodness-of-fit test, 0.05 level, Sokal and Rohlf (1969); and see Koehn (1972) for proper degrees of freedom, i.e., 1 for 2 allele system, 3 for 3 allele system] except in Gly-3-pdh for the HI/DRY group (G statistic = 8.9, d.f. = 1) where expected frequencies for the rarer genotypic classes are prohibitively low [102/102: N_{OBS}=1, (N_{EXP}= 0.0); 100/102: N_{OBS}=0, (1.9); 100/100: N_{OBS}= 31, (30.1)]. The results of these statistical tests should be viewed with caution, since the expected frequency of the rarest genotype was never as great as 5, and thus neither X^2 nor G goodness-of-fit tests are strictly admissible [and see, for example, Fairbairn and Roff (1980) concerning the low power of the X^2 test when used in this connection for sample sizes <<200].

MORE EVIDENCE FOR THREE SEPARATE GENE POOLS AND FURTHER ELUCIDATION OF THE NATURE AND BASIS OF ELECTROPHORETIC VARIATION EXHIBITED BY THE "DIAGNOSTIC LOCI"

Further electrophoretic investigations focused on the intertidal distribution and biogeography of the three groups described above. These studies, limited to the 5 "diagnostic loci," Adh, Mdh-2, Pgm, Sdh-1 and Sdh-2, and to Mdh-1, further elucidate the nature of allelic differentiation observed among the groups. A summary of results for all individuals in the entire study (N = 467; Table III-3) shows that the three group-characteristic sets of alleles of the five diagnostic loci, found in animals at La Jolla and Bodega, maintain their integrity through all collection sites studied; that is, three and only three groups are described by the five loci.

There is a small incidence of overlap in alleles of the diagnostic loci among the three groups, but in each chiton individual involved except one, no more than one locus exhibits the overlap. The one exception shows the heterozygous condition at apparently all of the diagnostic loci, in each case for alleles characteristic of the BODEGA and HI/DRY groups. This exceptional individual from the high intertidal zone at La Jolla would then appear to be a hybrid, i.e., a backcross of individuals from the different groups, BODEGA and HI/DRY. The hybrid shows a well-resolved three-banded pattern in Mdh-2, as expected for this dimeric enzyme (100/105), barrel-shaped, "center-heavy" (presumably 3-banded as expected for a dimeric enzyme) heterozygous pattern in Sdh-1 and Sdh-2 (98/100 and 100/102 respectively), a two-banded heterozygous pattern in Pgm (100/110, and a faint band in Adh at 103 to 104, the position in which the densest middle band of a three-banded heterozygote for 100 and 107 would be found. This hybrid specimen thus appears to provide validating evidence for the genetic interpretations given for each diagnostic locus and, further, provides evidence to suggest that Adh, Mdh-2, Sdh-1 and Sdh-2 are dimeric in <u>Nuttallina</u>, as they are in other organisms. Other <u>Nuttallina</u> individuals in the study, heterozygous for one or another of the loci, confirm that Mdh-1 and Mdh-2 are

TABLE III-3. Diagnostic loci for the Nuttallina groups (N=467). Occurrence of only alleles with frequencies greater than 0.03 in at least one group are included. Dash indicates frequency of <0.005.

		BODEGA	HI/DRY	LOW
Adh	112			0.99
	107		0.99	-
	100	0.97	-	-
Mdh-2	105	0.01	0.99	-
	100	0.99	-	0.99
Pgm	≥103[A]		1.00	1.00
	100	0.87		
	97	0.13		
Sdh-1	100	0.99	0.01	0.98
	98		0.99	0.01
Sdh-2	102	0.01	0.99	0.01
	100	0.99	-	0.99

NOTES

A. Controls on Pgm gels were 100's or 97/100's. Due to the variance in run times and closeness of alleles > 100, values greater than 100 were not differentiated. It was apparent that there was one more fairly common allele at about 110 and 2 or 3 rarer alleles > 110 in addition to common alleles, 103, 105, 108 in HI/DRYs and/or LOWs.

dimeric, that Pgm is monomeric, and that Sdh-1 and Sdh-2, with barrel-shaped heterozygous banding patterns, are probably dimeric.

As in the intial survey, the genotypic frequencies of the diagnostic loci, when combined for the entire study and segregated among the three groups, meet Hardy-Weinberg expectations; although goodness-of-fit tests are not strictly admissible in all cases because of expected genotype frequencies less than 5, no significant departures from expected frequencies are found (G goodness-of-fit test; 0.05 level).

With regard to the question of an environmentally-induced versus a purely genetic basis for the electrophoretic variation observed, it is important to note that differences were observed in diagnostic loci patterns for chitons sampled at different times of the year. For example, samples taken from the La Jolla site in February through April, 1981 showed the same electrophoretic correspondence with habitat (i.e., specimens with the HI/DRY electrophoretic phenotype in the high and dry habitat; LOW types in the low intertidal habitat) as did those taken in late November, 1981. Additionally, as will be reported in following sections, Nuttallina of the three electrophoretic groups were found sympatrically in different combinations at the various collection sites and were occasionally found in the same habitat at the same shore level.

ESTIMATES OF GENETIC VARIATION AMONG THE THREE NUTTALLINA GROUPS

Traditional measures of genetic differentiation, genetic distances (D) and similarities (I; Nei, 1972), can be calculated between the three groups of Nuttallina using the data base represented by the allelic frequency data of Table III-2. Genetic distances are given in

Table III-4, and a phenogram of genetic identity of the groups is presented in Figure III-3. Ayala and Powell (1972) have pointed out that such measures, devised to quantify genetic differentiation between populations, may have the same value whether many loci possess a moderate amount of differentiation or a few loci show complete nor nearly complete differentiation. Loci at which complete differentiation exists between two populations, or "diagnostic" loci, give a clearer indication of a lack of gene flow between the two than can loci showing only partial differentiation. Therefore, the number of loci that are diagnostic among the three *Nuttallina* groups are presented in Table III-4.

INTERTIDAL DISTRIBUTION AND BIOGEOGRAPHY OF THE THREE *NUTTALLINA* GROUPS

A series of samples was collected from the La Jolla site in order to elucidate the microhabitat distribution of the three *Nuttallina* groups (see MATERIALS AND METHODS section for collection information). The distribution of smaller chitons might be expected to reflect patterns of larval settling more closely than would that of large specimens. Since only 3 small specimens were collected in the primary series of samples, an intensive search for small *Nuttallina* was undertaken later, in November, 1981. Chitons 15 mm or shorter in thawed length are referred to as "juveniles," based on personal observations of gonad maturation in La Jolla *Nuttallina* and on Ituarte's (1981) estimate for length of gonad maturation of *Nuttallina* from Santa Cruz, Co., CA (22 mm average *live* chiton total length). Samples of *Nuttallina* from various other sites in California and Baja California were taken to determine the geographic distribution of the three types of *Nuttallina* and to

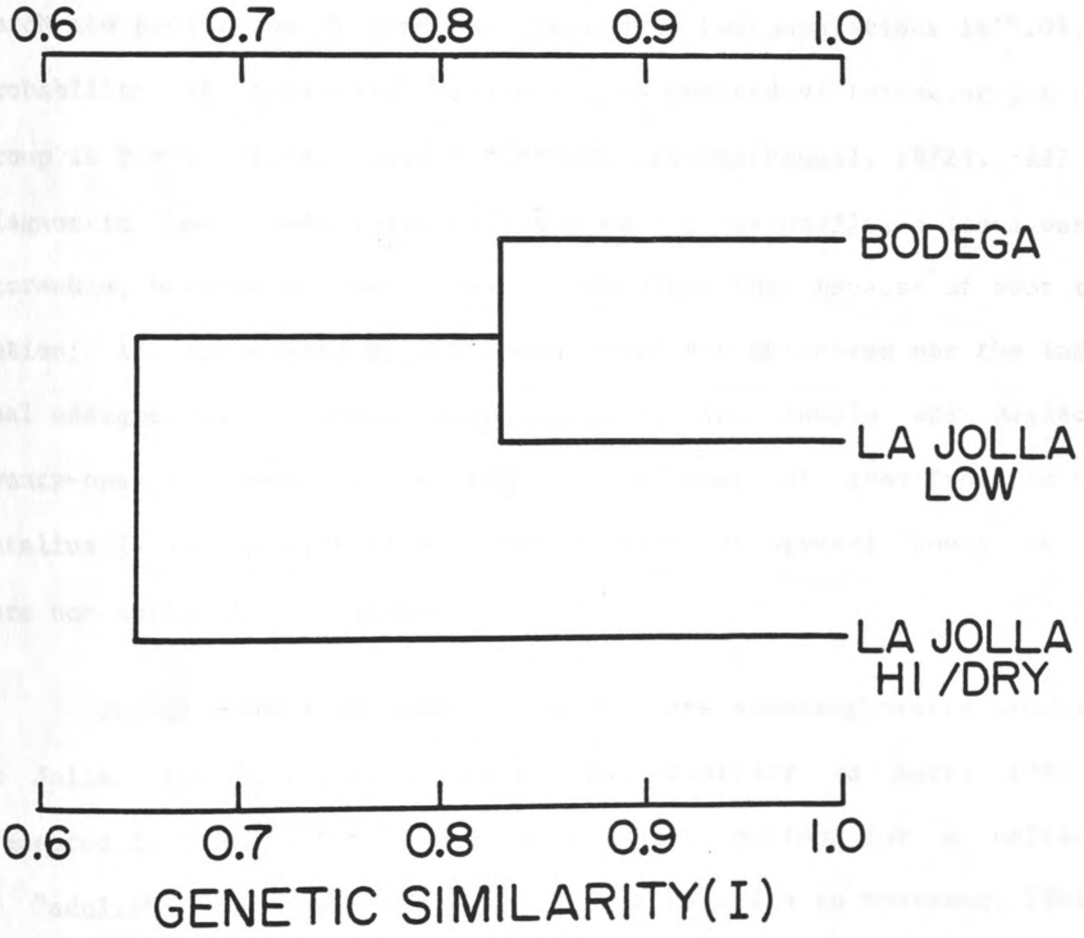
TABLE III-4. Genetic distances (Nei, 1972) ± standard error (above diagonal) and fractions (percentages in parentheses) of diagnostic loci[A] (below diagonal) between the three groups of <u>Nuttallina</u>. Both measures of genetic differentiation are based on 14 presumptive gene loci common to all 3 groups.

Populations	BODEGA	LA JOLLA HI/DRY	LA JOLLA LOW
BODEGA		0.44 ± 0.13	0.20 ± 0.08
LA JOLLA HI/DRY	5/14 (35.7%)		0.42 ± 0.13
LA JOLLA LOW	2/14 (14.3%)	4/14 (28.6%)	

NOTE

A. A locus is considered diagnostic in the present study if the proportion of shared genotypes, assuming Hardy-Weinberg equilibrium and the allelic frequencies of Table III-2 (or III-3) is 0.014 or less. That is, the probability of correctly assigning an individual to one of two groups on the basis of that locus alone is 99.3% or higher.

Figure III-3. Phenogram of genetic relationships of the three Nuttallina groups derived from calculations of Nei's (1972) coefficient of genetic similarity (I), clustered using the unweighted paired group method with arithmetic means (UPGMA). Calculations are based on the analysis of allelic products encoded by 14 presumptive loci that were expressed in all three groups of Nuttallina. Breakpoints occur at 0.65 and 0.82.


0.6
0.7
0.8
0.9
1.0
BODEGA
LA JOLLA LOW
LA JOLLA HI /DRY
0.6
0.7
0.8
0.9
1.0
GENETIC SIMILARITY(I)

further elucidate aspects of their microhabitat distribution.

Despite a small incidence of overlap in expressed alleles at individual loci, the five diagnostic loci, Adh, Mdh-2, Pgm, Sdh-1 and Sdh-2, discovered in the initial electrophoretic survey, could be used in combination to assign specimens to one of the three electromorphic groups, BODEGA type, HI/DRY type or LOW type, with virtual certainty. Virtual certainty was assured since, on the basis of only one locus for which the proportion of genotypes shared by two populations is 0.01, the probability of correctly assigning an individual to one or the other group is $P = 1 - (1/2)(0.01) = 0.995$ (Ayala and Powell, 1972). All five diagnostic loci were routinely assayed. Occasionally, a locus was not scoreable, because of low activity more often than because of poor resolution; if on retesting, the locus could not be scored nor the individual assigned to a group unambiguously, the sample was neglected. Twenty-one specimens of a total 488, most of them from the Santa Catalina Island sites that had been carried for several hours on ice, were not assignable to groups.

The microhabitat distributions of the electrophoretic groups at La Jolla, for collections made from February to April 1981, are presented in Table III-5. Microhabitat distributions for a collection of "adults" and one of "juveniles" at La Jolla in November, 1981 are presented in Table III-6. Results for the biogeographic survey, including brief physical and ecological descriptions of microhabitats at each collection site, and with Nuttallina density estimates, are presented in Table III-7. Supplementary biogeographic information, obtained through visual examination and conservative morphological diagnoses of specimens

TABLE III-5. Frequencies of electromorphic types of *Nuttallina* in intertidal microhabitats at La Jolla site in February-April 1981 collections. Numbers in parentheses are the ranges of thawed total lengths, in mm (lengths < 15 mm are listed individually).

Habitat^A	Electromorphic Type^B		
	HI/DRY	LOW	BODEGA
HIGH AND DRY	35(18.0-37.0)	3(19.0-23.0)	3(27.0-38.0)
HIGH POOLS	9(25.0-30.5)	1(24.5)	-
DENSE/SPARSE BOUNDARY AREA (MIDTIDE LEVEL)	4(24.5-35.0)	1(27.5)	-
LOW AND WET	2(11.0, 22.0)	38(12.0, 13.0, 16.0-32.0)	1(34.0)

NOTES

A. The "dense/sparse boundary" is that area that typically occurs at ~50 to 80 cm above MLLW at the La Jolla site and is described in Chapter II. "High and dry" refers to the area above the dense/sparse boundary where there are no mussels, pools, or damp patches of coralline algal turf. The substrate has few shallow indentations and is normally dry at low tide. "High pools" in the La Jolla site all occur at about 1.5 - 1.8 m above MLLW. The pools collected were all about 0.5 to 1.5 m in diameter with standing water about 3 to 15 cm deep. Patches of coralline algae occur. "Low and wet" refers to the uniform habitat below the DENSE/SPARSE boundary that is riddled with chiton-shaped depressions amongst a thick coralline-algal turf; this habitat is typically wet or damp and is described in Chapter II.

B. One hybrid specimen of HI/DRY and BODEGA (27.0 mm thawed length) occurred in high and dry habitat.

TABLE III-6. Frequencies of "Adult" (> 15 mm thawed total length) and "Juvenile" (≤ 15 mm) electromorphic types of Nuttallina at La Jolla site in November, 1981 collections. Numbers in parentheses are the ranges of thawed chiton total lengths in mm.

"ADULTS"

HABITAT[A]	ELECTROMORPHIC TYPE		
	HI/DRY	LOW	BODEGA
HIGH AND DRY	10(23.5-31.0)	-	-
HIGH AND WET	-	1(16.5)	-
HIGH POOLS	-	1(17.0)	-
LOW AND WET	-	14(15.5-30.5)	-

"JUVENILES"

HABITAT	ELECTROMORPHIC TYPE		
	HI/DRY	LOW	BODEGA
HIGH AND DRY	-	-	-
HIGH AND WET[B]	1(11.0)	10(11.0-15.0)	-
HIGH POOLS	-	6(11.0-15.0)	-
LOW AND WET	4(4.5-14.0)	7(10.0-15.0)	1(13.0)

NOTES

A. "High and Wet" refers to habitat, above the dense/sparse boundary, where there is protection of overhanging ledges, mussel clumps or stands of coralline algae. These areas are damp, often due to drainage from high pools. This habitat is occasionally riddled with chiton-shaped depressions when coralline algae is present. Other habitats are described in Table III-5.

B. "High and Wet" density of juveniles on the order of $5-10/m^2$; "Low and Wet" juvenile density possibly hundreds/m^2 -- about 1/20 m^2 was searched for juveniles in "Low and Wet" habitat.

TABLE III-7. Frequencies of electromorphic types at sites in the present study with a summary of habitat characteristics.

Site[A]	Substrate[B] (Relative Hardness)	Habitat[C]	Chiton-Shaped Depressions?	Estimated[D] Density of Nuttallina	ELECTROMORPHIC TYPE FREQUENCY (Thawed chiton total lengths in parentheses) HI/DRY	LOW	BODEGA
La Jolla	Coarse Grain, Friable Sandstone (1)	HIGH	NO	50-150/m^2	See Note E	-	-
		LOW-ALGAL TURF	YES	1000/m^2	-	-	-
Punta Banda, Baja California	Intermediate grain, intermediate composition diorite (5)	HIGH	NO	50/m^2	-	1 (22.0)	19 (21.0-35.0)
		HIGH POOL	NO	-	2 (27.0, 30.0)	1 (26.0)	1 (29.0)
		LOW AND WET	NO	50/m^2	-	-	17 (16.5-32.0)
Whites Point, Los Angeles	Diatomaceous Shale[F] (2-3)	HIGH AND DRY	NO	<25/m^2	13 (20.0-34.0)	6 (14.0-27.0)	-
		HIGH AND WET	NO	<25/m^2	3 (18.5-33.0)	3 (10.5-24.0)	-
		HIGH POOL	NO	50-75/m^2	4 (18.0-36.0)	-	-
		LOW-ALGAL TURF, *PHRAGMATOPOMA* BEDS	YES	100/m^2	1 (19.0)	10 (12.5-29.0)	-
		LOW-NO ALGAL TURF	NO	50/m^2	8 (18.0-24.0)	7 (9.5-28.0)	-
Gaviota	Friable Sandstone (2)	HIGH AND DRY	NO	10-100/m^2	26 (23.0-42.0)	-	-
		LOW AND WET	YES	200-300/m^2	26 (13.5-34.0)	-	-

TABLE III-7 (cont.)

Site^{A}	Substrate^{B} (Relative Hardness)	Habitat^{C}	Chiton-Shaped Depressions?	Estimated^{D} Density of Nuttallina	ELECTROMORPHIC TYPE FREQUENCY (Thawed lengths) HI/DRY	LOW	BODEGA
University of Southern California Marine Science Center, Santa Catalina Island	Vesicular Red Basalt (3)	HIGH	NO	<10/m^2	-	3 (17.0-26.0)	-
		MIDTIDE	YES	50-75/m^2	-	17 (15.0-29.0)	-
		LOW-ALGAL TURF	YES	<50/m^2	-	18 (12.5-26.0)	-
Little Harbor, Santa Catalina Island	Gneiss (possibly Blue Schist)	HIGH	NO	10/m^2	-	15 (13.0-28.0)	4 (28.0-32.0)
	(3; but with large quartz inclusions)	MIDTIDE	YES	50-100/m^2	-	8 (13.0-30.0)	2 (27.0, 33.0)
		LOW-ALGAL TURF	YES	50-100/m^2	-	14 (9.0-24.0)	-
Avalon, Santa Catalina Island	HIGH: Coarse-pebbled cement; LOW: fine grain, intermediate composition igneous rock (Andesite?)^{G} (4)	HIGH	NO	10/m^2		19 (17.0-32.0)	-
		LOW-MIDTIDE	YES	50/m^2	-	19 (9.0-30.0)	-
San Nicolas Island	Sandstone (?)	HIGH	NO	?	-	1 (14.5)	5 (20.0-38.0)
		LOW-ALGAL TURF	YES	?	-	3 (12.0-18.5)	3 (17.0-32.0)
Bodega Marine Laboratory, Bodega Bay, CA	?	MIDTIDE	?	?	-		35 (16.0-39.0)

TABLE III-7 (cont.)

A. The most obvious differences of each site from the La Jolla study site are as follows:

PUNTA BANDA: very diverse and plentiful assemblage of plants and animals; lower intertidal covered almost completely by *Mytilus* and *Pollicipes* with only a few clumps of foliose coralline algae. *Anthopleura elegantissima* quite dense at lower border of mussel band with *Codium*, *Pisaster*, *Anthopleura xanthogrammica*, *Strongylocentrotus*, *Egregia*, laminarian zone at successively lower levels. The outcrops drop off steeply into deep water and the intertidal here seems to sustain great wave impact.

WHITES POINT: described most recently by Vesco, 1980; large loose boulers, and underlying bedding planes with a very shallow slope (∿2 m vertical differential over a distance of 25 m); presents a broken substrate which undoubtedly produces topographic complication of zonation patterns. Algal turf zone on larger, flatter low intertidal boulders appears to be the same as in La Jolla, High pool habitat was on a tall outcrop which dropped off steeply to deep water.

GAVIOTA: large boulders and outcrops surrounded by sand; outcrops with vertical faces and flat tops where *Nuttallina* were found; foliose coralline algae were quite sparse in the lower intertidal; instead, *Gelidium*-like unidentified brown alga dominated the flora; there were some patches of encrusting coralline algae dotted by tiny holes (*Dodecaceria?*)

USC MARINE SCIENCE CENTER, CATALINA: described in detail by Littler (1978); diverse assemblage of biota; a prominent *Pelvetia* band extending to the upper end of the midtide zone; low intertidal coralline-algal-dominated turf with a tidal bench like in La Jolla, above a rapid drop-off to deep water populated by *Egregia*, *Eisenia* and various other laminarians.

LITTLE HARBOR, CATALINA: clumps of *Endocladia*, and many *Lottia* in the high area; midtide populated by a fairly dense assemblage of unidentified brown and red algae of several species; lower intertidal covered almost completely with an extremely dense coralline algal turf; rapid drop-off into laminarian zone and deep water; judging from day of collection, an area prone to high wave impact.

AVALON, CATALINA: High zone primarily coarse, weathered, somewhat bare, cement; midtide with a band of *Pelvetia* and numerous *Tetraclita*; lower zone with dense brown and red foliose algae, lots of *Tetraclita*; rapid drop-off into laminarians and deep water.

SAN NICOLAS ISLAND: many thanks to Bill Wright and Alan Shanks of Scripps Inst. of Oceanography for making this collection; limited information; apparently similar to the La Jolla site, except boulders occur in the high zone.

BODEGA: many thanks to James Trimmer of Scripps Inst. of Oceanography for making this collection; limited information: coralline algae in the collection area.

All sites other than the La Jolla site proper face the open ocean.

B. Rock samples from the sites were kindly identified by Cynthia Evans, Geological Research Division, Scripps Inst. of Oceanography. Relative hardness was determined by scratching rock samples with a knife; No. 1 represents a rock easily penetrated with a knife, 5 a rock almost unchanged by knife.

C. "High" refers to intertidal levels from about 120 cm above MLLW to about +185 cm, as judged from water level on the day of collection, or obvious algal zonation cues. "Midtide" denotes levels from about +75 to +120 cm, while "LOW" refers to levels from +75 cm down. Other notation as in Tables III-5 and III-6.

D. Densities are to be taken as rough approximations, based on counts within areas of about 0.1 m^2 considered typical of each habitat at a site, except at La Jolla where estimates are based on more rigorous counts described in Chapter II.

E. Detailed data on occurrence of electromorphic types at the La Jolla site are provided in Tables III-5 and III-6. Within any habitat at a site, the observed proportions of types favor the rarer morph because of a conscious collection bias, except at La Jolla.

F. Based on the identification provided in Vesco, 1980.

G. Possibly derived from material used to build the nearby road.

from museum preserved collections, is presented in Table III-8. A summary of both electrophoretically and morphologically-derived biogeographic findings is mapped in Figure III-4.

Numbers and thawed total length ranges of Nuttallina specimens assigned electrophoretically to each of the three groups in the entire study are as follows: BODEGA, N=91, length range = 13.0 to 39.0 mm; HI/DRY, N=148, range = 4.5 to 42.0 mm; LOW, N=227, range = 9.0 to 32.0 mm.

DISCUSSION

THREE SPECIES OF NUTTALLINA ON THE PACIFIC COAST OF NORTH AMERICA

Taxonomists and ecologists have considered Nuttallina to consist of two, and more recently of one, species on the Pacific Coast of the Americas based on external morphological examinations. However, the existence of three distinct species of Nuttallina along the west coast of California and Baja California is clearly established by the electrophoretic data obtained in this study.

The present study represents the first electrophoretic work on chitons. Therefore, no estimates of genetic distance (D) or genetic similarity (I) are available for other chiton taxa as a general guide to the genetic differentiation characteristic of different taxonomic levels within this mollusc class. However, estimates of genetic distance and similarity reported here fall in the general range of values for species, rather than for local populations or for genera, of a variety of vertebrates and Drosophila [Avise, 1976; Shaklee and Tamaru, 1981;

TABLE III-8. Supplementary biogeographic information for Nuttallina. Diagnoses conservatively made using appropriate discriminative external morphological characters discussed in Chapter IV.[A, B]

LOCATION	MORPHOLOGICAL TYPE			QUESTIONABLE IDENTIFICATIONS[C]			COMMENTS
NORTH OF POINT CONCEPTION, CA	BODEGA TYPE	HI/DRY TYPE	LOW TYPE	BODEGA → LOW	HI/DRY → LOW	LOW → HI/DRY	
Point Arena, Mendocino Co., CA pre-1914 (according to B. Roth of CAS, in litt., July 1982) CASIZ 032165[D]	4						Specimens dry, without insides
Point Arena, Mendocino Co., CA pre-1914 (according to B. Roth of CAS, in litt., July 1982) CASIZ 032166	~2						Fifteen loose valves, most look like from BODEGAs
Bodega Head, Sonoma Co., CA Exposed granite rocks 6 August 1948 AHF 1609-48	14						
Duxbury Reef, Bolinas Bay, Bolinas, CA 7 May 1966 SIO M1741	1						
Near Pescadero Point, San Mateo Co., CA Rocky shores 21 June 1968 SIO M1742	4						
Hopkins Marine Station, Pacific Grove near Monterey, CA 29 December 1977 SIO M1922, 1923, 1924	8		3			1	
Shell Beach, San Luis Obispo Co., CA. 35°09.4' N, 120° 40.6'W Intertidal, shale ledges Leg. James H. McLean, 1961-1963 LACM 61-11	7		3				
SOUTH OF POINT CONCEPTION, CA							
San Miguel Island, 0.3 miles southeast of Bay Point, Santa Barbara Co., CA. 34°02'N, 120° 18'W 21-22 August 1967 LACM 67-38	3						
San Clemente Island, Wilson Cove area, Los Angeles Co., CA. 33°N, 118°33'W. [Note in jar: "Diving off Nots Pier San Clemente Island 9/13/66"] 13 September 1966 LACM 66-51			1			1	

TABLE III-8 (cont.)

LOCATION	MORPHOLOGICAL TYPE			QUESTIONABLE IDENTIFICATIONS			COMMENTS
PACIFIC COAST OF BAJA CALIFORNIA, MEXICO	BODEGA TYPE	HI/DRY TYPE	LOW TYPE	BODEGA → LOW	HI/DRY → LOW	LOW → HI/DRY	
Punta Baja, Baja California 29°57'N, 115°48'W High intertidal 9 August 1975 SIO M1042		1					
Guadalupe Island, Baja California south end, in tide pool 7 December 1946 CASIZ 032164 (formerly CAS 32746)						4	Although Smith (1963) lists 4 adults, 3 juveniles, there are only 4 small specimens (3 intact, 1 with valves dissected) ≤10 mm in length. Small size and dry condition makes them especially difficult to identify. All four might be *N. crossota* if pale undergirdle is a reliable character for that species (see Appendix III).
Guadalupe Island, Baja California SDNH 9956						4	Specimens dry, hard to identify but not BODEGAs.
Laguna Manuela Point, Baja California 28°13.5'N, 114°11'W High rocky intertidal 4 August 1975 SIO M979		13				1	
East San Benito Island, Baja California, 28°18'38"N, 115°32'W Intertidal on rock 26 April 1950 AHF 1946-50	1	1	2		1	2	

TABLE III-8 (cont.)

LOCATION	MORPHOLOGICAL TYPE			QUESTIONABLE IDENTIFICATIONS			COMMENTS
PACIFIC COAST OF BAJA CALIFORNIA, MEXICO	BODEGA TYPE	HI/DRY TYPE	LOW TYPE	BODEGA → LOW	HI/DRY → LOW	LOW → HI/DRY	
Bahia Tortugas, Baja California 27°41'N, 114°53'W Rocky mid-intertidal 31 July 1975 SIO M952		12	1		3	3	One HI/DRY, one HI/DRY→LOW and one LOW→HI/DRY might be *N. crossota* if white/pale undergirdle is a reliable character for that species (see Appendix III). However, slightly more gills than expect for *crossota* (22-25 per side for 8-10.5 mm long animals).
Turtle Bay,[E] east of San Bartholome; Baja California 7 February 1948 SIO M570		2			1		All three might be *N. crossota* as judged by pale undergirdle although size (19-22 mm long) and many gills suggest otherwise.
Turtle Bay, Baja California 15-18 February 1969 SIO M1745		7					Five or six of these might be *N. crossota* as judged by pale undergirdle but far too many gills (26-32 per side in 13-19 mm long chitons).
Punta Abreojos, Baja California 26°44'N, 113°35'W rocky intertidal 23 July 1975 SIO M795		5					
Punta Abreojos, Baja California 26°44'N, 113°35'W Intertidal sandstone 24 July 1975 SIO M800		1					
Punta Abreojos, Baja California 26°44'N, 113°35'W "high rocks near MLLW" 25 July 1975 SIO M860			2				
Punta Abreojos, Baja California 26°43.5'N, 113°35.5'W high tide zone 27 July 1975 SIO M888		3					
Magdalena Bay, Baja California SDNH 57604					1	1	Specimens dry with no insides. Hard to identify, but based on many white girdle spines definitely not BODEGAs.

NOTES

A. Diagnoses involved visual examination of specimens. Briefly, external morphological characters corresponding to each electrophoretic phenotype are as follows (see Chapter IV):
HI/DRYs are most often distinguishable by very wide rectanguloid valves, a large proportion of white girdle spines, a relatively narrow girdle often with alternating light and dark stripes, light overall coloration and an intermediate number of gills;
LOWs by narrow valves, white and brown girdle spines, a relatively narrow girdle often with alternating dark and light stripes, light overall coloration and a small number of gills;
BODEGAs by angular valves and substantial sutural flesh encroachment, a predominance of short brown girdle spines, a relatively wide girdle with incomplete white stripes, often dark overall coloration and a large number of gills that extend up near the mouth.
Only those specimens that clearly fell in one morphological type or another were assigned to types; all others are tabulated as questionable.

B. Specimens were ethanol-preserved, unless noted otherwise.

C. Specimens tabulated as BODEGA→LOW, for example, were most likely BODEGAs but were possibly LOWs.

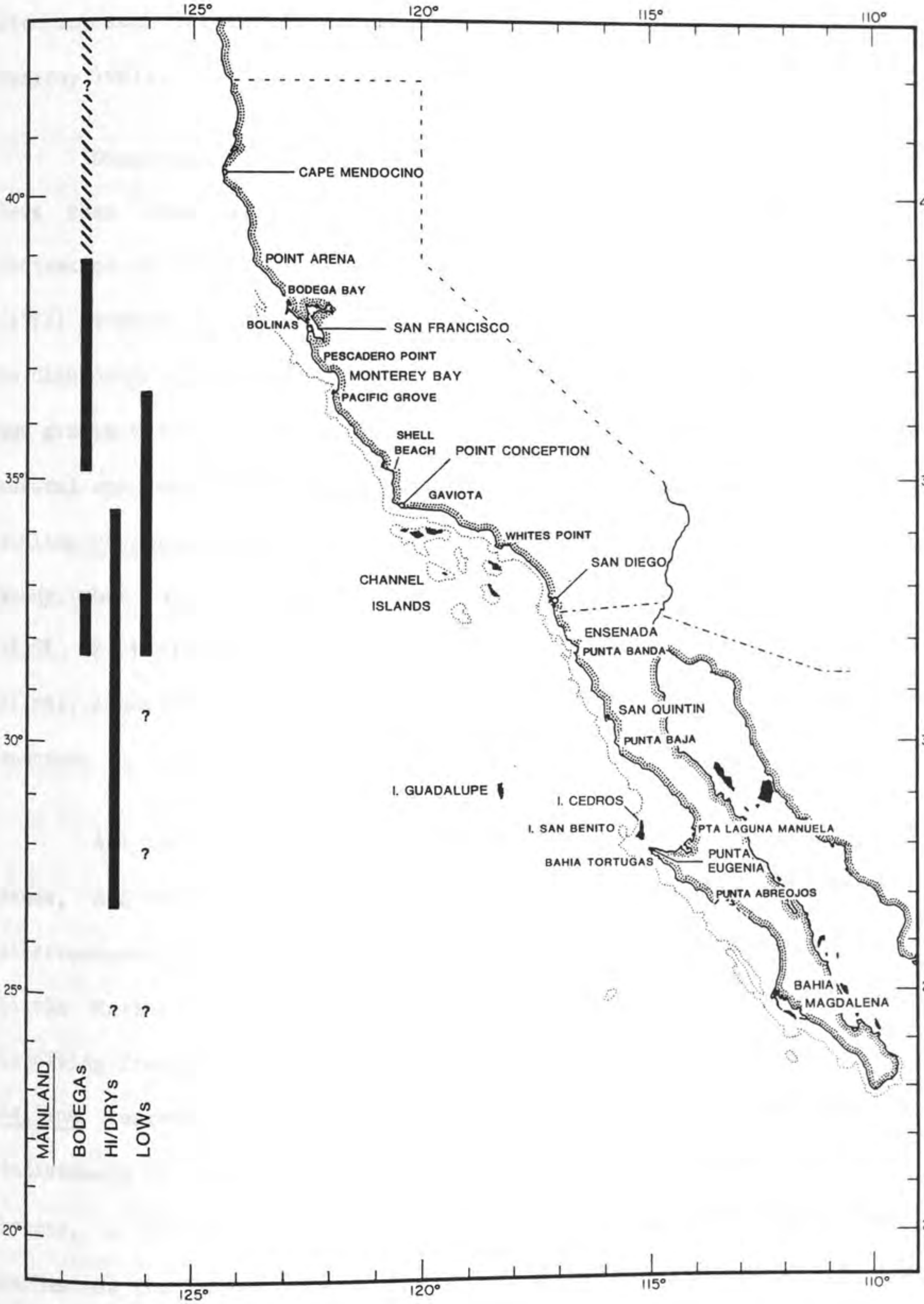
D. Abbreviations for museums as follows: Allan Hancock Foundation, Los Angeles, CA (AHF); California Academy of Sciences, Invertebrate Zoology Collection, San Francisco, CA (CASIZ); Los Angeles County Museum of Natural History, Los Angeles, CA (LACM); San Diego Museum of Natural History (SDNH); Scripps Inst. of Oceanography, Benthic Invertebrate Collection, La Jolla, San Diego Co., (SIO).

E. Apparently Bahía Tortola (27°40'N), just south of Bahía Tortugas (Brusca and Wallerstein, 1979:76), although Bahía Tortugas is Spanish for Turtle Bay.

Figure III-4. Geographic distribution of the three _Nuttallina_ groups inferred from combined results of electrophoretic diagnoses and external morphological identifications (data in Tables III-7 and III-8). Bars at the left indicate patterns of mainland occurrences of the three groups.

BODEGAs (=_N. californica_) have been reported from near the Canadian border in the past, but could be confirmed from only as far north as Point Arena, CA in the present study. Examination of specimens from Bahia Magdalena indicate the presence of HI/DRYs and/or LOWs there.

Of the six islands for which collections were obtained [San Miguel, San Nicolas, Santa Catalina and San Clemente in the Channel Islands, E. San Benito Island (near Cedros Island) and Guadalupe Island off Baja California], only Santa Catalina was extensively collected. HI/DRYs apparently are absent from Catalina Island, as well as from the other Channel Islands, whereas BODEGAs are well-represented on the western coast of Catalina Island (Little Harbor) and at San Nicolas Island, and are present at San Miguel Island. LOWs are predominant in eastern Catalina collections (USC Marine Science Center and Avalon) and are present at San Nicolas and San Clemente Islands. All three species were identified in a small collection from San Benito Island and LOWs were tentatively identified in the small Guadalupe Island collections.


125°
120°
115°
110°
40°
35°
30°
25°
20°
CAPE MENDOCINO
POINT ARENA
BODEGA BAY
BOLINAS
SAN FRANCISCO
PESCADERO POINT
MONTEREY BAY
PACIFIC GROVE
SHELL BEACH
POINT CONCEPTION
GAVIOTA
WHITES POINT
SAN DIEGO
CHANNEL ISLANDS
ENSENADA
PUNTA BANDA
SAN QUINTIN
PUNTA BAJA
I. GUADALUPE
I. CEDROS
I. SAN BENITO
PTA LAGUNA MANUELA
BAHIA TORTUGAS
PUNTA EUGENIA
PUNTA ABREOJOS
BAHIA MAGDALENA
?
MAINLAND
BODEGAs
HI/DRYs
LOWs

Shaklee et al., 1982; Thorpe, 1982 (apparently includes some data from other invertebrates and from plants); significant hazards associated with any such intergroup comparisons have been summarized by Shaklee and Tamaru, 1981].

Diagnostic loci, that is, loci for which different groups share less than some arbitrarily small percentage of genotypes, are a clear indication of a lack of gene flow between groups. Ayala and Powell (1972) report a range of 14 to 52% for the percentage of loci found to be diagnostic (allowing correct assignment of an individual to one of two groups with a 99% or greater probability) among all loci examined in several species of Drosophila; 14 to 36% of the loci were diagnostic for sibling Drosophila species alone. Results for Nuttallina in the present study, that is 14.3 to 35.7% of the loci diagnostic at a probability of 99.3% or higher for pairwise comparisons among the three groups (Table III-4), clearly fall in the range of values for Drosophila sibling species.

All three Nuttallina species co-occur at La Jolla and Punta Banda, and two of the three co-occur at three other sites collected for electrophoresis (HI/DRYs and LOWs at Whites Point; BODEGAs and LOWs at Little Harbor and San Nicolas Island; Table III-7). Such sympatry, involving free spawners (see "Biogeography" DISCUSSION section on Nuttallina reproduction), should lead to substantial numbers of hybrid individuals if there is any interbreeding. The finding of only one hybrid, a BODEGA-HI/DRY cross in La Jolla, among 467 chitons examined, emphasizes the genetic isolation among these three species. When viewed in relation to the observation that hybrid larvae can be formed from gametes of chitons of different genera, Katharina tunicata, Mopalia muscosa and Tonicella lineata [although the hybrids have not been raised to maturity (D. Bonar, personal communication to Pearse, 1979)], the finding of a single hybrid, between the BODEGA and HI/DRY Nuttallina species in this study, is even less noteworthy, especially since it is not known whether this hybrid individual was fertile.

A study of chromosomes, involving six families of chitons occurring on the west coast of North American, by Harvey (1963) provides further suggestive evidence for two of the three species. Harvey recognized two California Nuttallina species; his study included specimens that he refers to as N. californica from Monterey (N=2) and Moss Beach (N=1; presumably BODEGAs, possibly LOWs), and specimens that he refers to as N. fluxa from La Jolla (N=9) and Redondo Beach, CA (N=1; HI/DRYs and/or LOWs). Harvey (1963) reports that, although the chromosome number observed in the spermatogonial metaphase is the same in the 2 groups (haploid number = 6), the chromosomes of "fluxa" exhibit a "somewhat higher" chiasma frequency and may be slightly larger than those of "californica." White (1978) states that most species differ in their karyotypes, and has noted that such differences appear to have adaptive significance in a few cases, such as in the intertidal snail Nucella lapillus (White, 1978, p. 214). The fact that Harvey (1963) did not detect three chromosomal variants is not surprising. In his small samples, the three Nuttallina species might not have all been represented; in particular, HI/DRY or LOW may have been absent. Harvey examined only the gross features of the chromosomes, their alpha karyology, and could have missed the less conspicous differences often seen for different species. Furthermore, he only knew of two Nuttallina species and therefore was not looking for a third; it is possible he even neglected slight chromosomal differences corresponding to the third species that he might have observed, in order to preserve the simpler two-species scheme. Finally, it is possible that the chromosomes of two of the Nuttallina species are in fact similar, with no conspicuous differences, as has been found for valid species, so-called "homosequential species", in other cases (White, 1978).

Several investigators have claimed that significant changes in isozyme activities and/or electrophoretic banding patterns accompany acclimation to, or are at least triggered by, temperature (e.g., Baldwin and Hochachka, 1970; Marcus, 1977) or other environmental agents, such as food type or, possibly, day length (Oxford, 1975; Flowerdew and Crisp, 1976). Certainly, lack of corroboration for the conclusions of at least one such study (Wilson et al., 1973), and observations that many organisms show few, if any, isozyme changes with, for example, thermal acclimation (Somero, 1975; Wilson et al., 1975; Shaklee et al., 1977; Walsh, 1981), or with acclimatization to conditions at different heights on the seashore (Black and Johnson, 1981; Johnson and Black, 1982), indicate that environmentally-dependent isozyme banding pattern changes are not a general phenomenon. However, given the heterogeneity of habitat and the steep gradients of environmental factors, particularly those correlated with vertical height, in the intertidal sites of the present study, exploration of the possibility that the different electrophoretic banding patterns found for the three different "species" have been induced within a single (or two) Nuttallina species by variations in environmental conditions seems necessary.

Several lines of evidence contraindicate "environmental induction" of the electrophoretic isozyme band variations observed in the present study. If the banding patterns were "environmentally-dependent", rather than species-specific, one would expect that Nuttallina individuals occurring together on the shore when sampled, and thus presumably subject to the same environmental conditions for long periods of time, in view of the notable homing habit of Nuttallina species (see Chapter V), would have similar electromorphs. However, at the La Jolla site, where extensive microhabitat sampling was performed, chitons with the three different electrophoretic banding patterns (i.e., the three species) were occasionally found next to each other. For example, an individual with the HI/DRY banding pattern and one with the LOW pattern were collected from adjacent mid-intertidal depressions, separated by a centimeter or two. Similar cases were observed occasionally for other type pairings and for other collection sites.

Individuals exposed to "intermediate" environmental conditions, such as organisms to be found in the mid-intertidal zone, might be expected to display electrophoretic bands characteristic of both high intertidal chitons and low ones simultaneously, in parallel with the findings of Baldwin and Hochachka (1970) for trout acclimated to high, low and intermediate temperatures. No such intermediate banding pattern is associated with chitons of the mid-intertidal zone of La Jolla. In fact, as reported earlier, multiple banding patterns consisting of bands associated with more than one of the Nuttallina electromorphs are extremely rare or entirely absent at all diagnostic loci (see Table

III-3).

Nuttallina specimens collected in La Jolla at different times of the year showed similar electrophoretic patterns for the diagnostic loci (Tables III-5 and III-6). This evidence precludes the existence of "seasonal-specific" (temperature-specific?) electrophoretic band variants at these loci and eliminates the possibility that seasonal (temperature?) shifts in banding patterns, perhaps varying in timing with intertidal height, have produced the three banding patterns in a single species in this study.

Finally, as the number of diagnostic loci, encoding for enzymes with quite different functions, mounts to 5 (between the BODEGA and HI/DRY species), the probability that production of the appropriate isozymes would be turned on or off in perfect unison under various sets of environmental conditions such that three fixed sets of electrophoretic bands are always produced (i.e., the unique sets corresponding to the BODEGA, HI/DRY and LOW species shown in Figure III-2), would be expected to drop precipitously. Certainly, Shaklee et al. (1977) found that different isozymes in different tissues of the green sunfish had different responses to varying acclimation temperatures.

Thus, the three sets of electrophoretic banding patterns observed in the present study are not likely the result of "acclimatory" responses of chitons of a single Nuttallina species to environmental factors. Nor can the banding pattern differences be attributed to variations caused by sex or developmental state; both sexes and nearly the complete range of sizes of Nuttallina are represented in the present study (Tables III-5 to III-7) and are distributed among the three electromorphs. Therefore, it is concluded that the banding patterns are indicative of three Nuttallina species, which will be referred to as BODEGA, HI/DRY and LOW for the remainder of the chapter. An analysis of the external morphological features characteristic of each species, with an assignment of species names and formal descriptions, is presented in Chapter IV.

INTERPRETING DISTRIBUTIONAL DATA

A number of points should be made with regard to the interpretation of the distribution results of this study. Collections for electrophoretic diagnoses of Nuttallina groups involve samples taken during different seasons in only one year (1981) at La Jolla, and taken at only one time at all other sites. Abiotic factors and biotic factors such as larval recruitment, are likely to vary somewhat from season to season and year to year. However, since chitons sampled in this study span a wide range of sizes and, presumably, at least several year classes, conclusions made from these data are assumed to have validity of some degree of generality and permanence. Next, it should be re-emphasized that the results displayed in Tables III-5 to III-8 and in Figure III-4 most likely do not accurately reflect the relative proportions of types co-occurring in any habitat or at any collection site, particularly when they co-occur in greatly unequal proportions. At the La Jolla site, sampling was at best haphazard, but not random. At all other sampling sites in the study, collections were consciously biased in favor of the rarer morphs present. As a result, absences of types are particularly important, and findings of presences are reliable, but proportions of types are usually biased. Thus, proportions indicated by these electrophoretic data are best treated as order-of-magnitude estimates. In the museum collections used to augment biogeographic information, proportions of types likewise are probably inaccurate and, in addition, absences are not reliable, in view of the small sample sizes and necessary conservatism exercised in making identifications. However, findings of type presences in museum collections from a site are significant.

Finally, one other point must be remembered in making conclusions from the type occurrence data. Relative densities of Nuttallina must be taken into account in making any inter-habitat or inter-site comparison. For example, proportions reflected in Table III-5, taken alone, appear to indicate an abundance of HI/DRYs in the high and bare habitat at the La Jolla site and a near absence of them in the low, wet intertidal areas. However, it must be remembered that the overall density of Nuttallina in the low, wet zone is much greater than in the high intertidal zone (Table III-7, and see density studies in Chapter II, Table II-2). Using $100/m^2$ as an approximate density for Nuttallina in the high, dry area in La Jolla, and $1000/m^2$ for the low, wet area, results of Table III-5 then indicate that the density of HI/DRYs in the high and dry area is on the order of $(35/41)(100/m^2) = 85/m^2$, and in the low and wet habitat, on the order of $(2/41)(1000/m^2) = 49/m^2$. In other words, the densities of HI/DRYs in high versus low intertidal habitats are more nearly equal than proportions alone would suggest.

INTERTIDAL DISTRIBUTIONS OF THE NUTTALLINA SPECIES: LOWs

Results of intensive sampling of Nuttallina at the La Jolla site show that "adult" (>15 mm thawed total length) specimens of the LOW species represent an overwhelming majority of the adult Nuttallina in the low, wet intertidal habitat, associated with coralline algal turf and chiton-shaped depressions, and represent only a small fraction of Nuttallina in the high intertidal zone (Table III-5). Given the differences in overall Nuttallina densities at these two shore levels (Table III-7), it is clear that adult specimens of the LOW species are exceedingly abundant in the low intertidal coralline algal turf and are almost completely absent from the high shore.

Clearly, myriad factors may influence where organisms occur at any site. Factors that affect the distribution of the assemblage of species at La Jolla may be quite different from those that operate at other sites. Nevertheless, information obtained from other collection sites, where organismal patterns of distribution bear altered relations to conspicous components of the environment, can provide insight into the factors that cause and maintain the patterns seen at La Jolla. The La Jolla site is unique among the sites in this study in that it is a nearly planar, continuous outcrop with relatively few irregularities that, due to its orientation away from the open ocean, is largely protected from wave splash at low tide. This site is expected to exhibit clearer patterns of zonation than other sites, where large scale irregularities such as boulders, and direct exposure to waves can produce considerable topographic complication to vertical zonation. Despite this point and the fact that the collections at other sites were made with a conscious bias toward the rarer morphs in each habitat, the intertidal distribution of LOWs observed at La Jolla seems to hold generally at other sites in this study. The LOW species is found mainly at low intertidal levels, especially in areas with coralline algal turf and chiton-shaped depressions, and is quite sparse in high intertidal areas at all collection sites where it occurs (Table III-7).

The intertidal distribution of LOWs may merely reflect the pattern of larval settlement for the species. "Adult" Nuttallina LOWs have been found to move very little over the long term, homing to a particular spot for long periods of time (see Chapter V). If larval settling were nonselective, the number of larvae found in the high intertidal area would be expected to be much fewer than the number found low, since larval frequencies might roughly parallel the curve of submergence time versus height on the shore (see curve for San Diego in Chapter II: Figure II-2). On the other hand, it is quite possible that Nuttallina larvae settle selectively. The larvae of other chitons that occur intertidally have been shown to settle selectively. The larvae of Tonicella lineata show a specific settling response to coralline algae (Barnes and Gonor, 1973), while the larvae of Mopalia muscosa respond specifically to gamma-aminobutyric acid (GABA), that is common in many red algae, including crustose coralline algae (Morse et al., 1979). Larvae of Katharina tunicata will cease swimming and will settle rapidly in response to GABA and they will metamorphose in the presence of encrusting coralline algae (Rumrill and Cameron, 1983).

Assuming that the distribution of "juvenile" LOWs reflects larval settlement patterns more closely than does the distribution of "adults," and that recruitment patterns are more or less similar from year to year, it appears that the frequency of larval settling is greater in the low intertidal zone than in the high area at La Jolla (juveniles at hundreds/m^2 in low, 5-10/m^2 in high areas; Table III-6), as one might expect if larval settling were passive. However, during an intensive search of the high intertidal zone, juveniles were not found in the "high and dry" habitat, but only in damp areas, such as around mussels, in damp crevices and under ledges, where coralline algae is often present; if this reflects a pattern of larval settling, it is possible that a specific larval settling response, such as to coralline algae and/or GABA in damp areas or in avoidance of high, dry and bare areas, with the assemblage of sessile microflora present there providing necessary cues exist (Scheltema, 1974; Strathmann and Branscomb, 1979). Of course, larval mortality, predation and/or competition may play significant roles in effecting the patterns seen. Clearly, the larval biology of Nuttallina merits study.

In a study involving experimental manipulation of Nuttallina densities in the mid- to high intertidal zone at Santa Barbara, CA, Louda (1972) has suggested that the Nuttallina population there [probably HI/DRYs in view of the proximity to the Gaviota collection site (Table III-7) and the valve widths reported (see Chapter IV)] is self-regulating, and it is possible that intra-specific competition is involved. In support of her conclusion, Louda cites increases in survival rates of small chitons and increases in the average sizes of chitons, caused by immigration of large chitons and possibly by increases in growth rate, on rocks where Nuttallina densities were experimentally reduced. Given Louda's conclusion and the observed paucity of LOWs in the high area of La Jolla, it is tempting to suggest that LOWs are competitively excluded from high areas at La Jolla by the HI/DRYs that predominate there. This suggestion does not appear to be borne out by distributional data for other sites in the present study. At Avalon and at the USC Marine Science Center on Santa Catalina Island, LOWs occur in the absence of both HI/DRYs and BODEGAs, but LOWs only reach densities in the high intertidal areas (10-15/m^2) comparable with the densities they reach in the presence of the other species at other sites, i.e., 0-10/m^2 in the high areas at La Jolla in the presence of HI/DRYs, 5-15/m^2 at Whites Point also in the presence of HI/DRYs at low densities, and 5-10/m^2 at Little Harbor in the presence of low densities of BODEGAs (Tables III-5 to III-7). Certainly, findings of random to clumped patterns of dispersion in nearest neighbors analyses of Nuttallina in the high intertidal area at La Jolla (Chapter II: Table II-4) suggest that territorial exclusion of chitons is not occurring there.

Differential predation, particularly visual predation by birds, might be expected to account for the low densities of LOWs in higher shore areas consistently observed at all collection sites where LOWs occur. Certainly, gulls eat considerable numbers of Nuttallina in the Monterey Peninsula area, CA (Moore, 1975) and oystercatchers forage on Nuttallina at Southeast Farallon Island, CA (D. Lindberg, University of California, Santa Cruz, in litt., October, 1981) and along the Monterey Peninsula (Legg, 1954). However, although large numbers of gulls occasionally forage at the La Jolla site, particularly at low tide, no bird predation on Nuttallina is evident. No birds were ever observed eating chitons, no regurgitated "pellets" (sensu Moore, 1975) were ever found, and gulls showed no liking for medium-sized Nuttallina (20-25 mm long) thrown to them (sporadic personal observations, primarily at low tide, over four years).

The upper limit of distribution of intertidal organisms is most often suggested to be determined by physical factors, primarily by desiccation and by temperature (e.g., Connell, 1961a and b; Wolcott, 1973). The low densities of Nuttallina LOWs in the high intertidal might be related to the influence of such physical factors. LOWs are found to encroach into higher levels at La Jolla where wetter habitat, often overgrown with coralline algae, exists, but they do not reach great densities or proportions in high intertidal pools at either La Jolla or Whites Point (Tables III-5 and III-7). Thus, although desiccation may be a limiting factor, other factors are involved. High temperatures might explain the near absence of LOWs from the "high and dry" habitat, with LOW encroachment in only the cooler, wet areas of the high zone as observed (Tables III-5, III-6). However, high pools are also subject to raised temperatures and, while there is a near absence of adult LOWs there, a surprising proportion of juvenile LOWs were found in high pools in La Jolla in November (Tables III-5, III-6). Of course, the low algal biomass [food and (?)shelter for Nuttallina] in the high intertidal, possibly an effect of "harsh" physical factors, may also explain the absence of LOWs there.

LOWs are found in close association with coralline algal turf wherever it occurs. LOWs reach their highest observed densities of the study and are far and away the predominant Nuttallina species occurring in the coralline algal turf of the La Jolla site (>1000/m^2; Tables III-5 to III-7 and Chapter II: Table II-2) and of the Whites Point site (100/m^2; Table III-7). LOWs are more abundant and represent a higher proportion of the Nuttallina present in the low algal turf of Whites Point than in adjacent low intertidal areas without the algal turf (Table III-7). If this chiton-algae association is more than a coincidental correlation of the two with a third factor, it may arise through any of a number of cause-effect relationships. The algae may simply represent a food source of substantial biomass for the LOWs. In fact, analysis of gut contents of Nuttallina occurring in the low intertidal algal turf of the La Jolla site of this study (mainly LOWs) reveals that these chitons are not particularly selective feeders and that Corallina, which is a predominant algal component of the turf, is the most common alga found in LOW guts (Kues, 1969). Nishi (1975) suggests that Nuttallina prefer foliose corallines posibly because of the way in which their radulae work, in interpreting results of her analyses of gut contents of Nuttallina from the Monterey, CA area (BODEGAs and probably LOWs, see Chapter IV). However, Vesco's (1980) findings for Nuttallina occurring in the coralline algal turf at the Whites Point site (primarily LOWs, see Table III-7), indicate that detritus is the primary diet component of this chiton (74% by volume), whereas Corallina vancouveriensis, which is the most abundant alga in the lower areas of the site, comprises only 6% of the contents of guts she examined. Since coralline algae are typically low in caloric value (Paine and Vadas, 1969), the Nuttallina of her study apparently obtain little of their caloric requirements from it. At least at the Whites Point site, the coralline algal turf represents other than a primary food source for

LOWs; rather, Vesco (1980) suggests that it represents a detritus-collecting "net."

The coralline algal turf may serve to trap water and thereby reduce the risk of desiccation for LOWs. In this regard, the roles of the turf and the chiton-shaped depressions always found with it are inseparable. The holes are expected to retain more water at low tide than would a flat surface; Kues (1969) showed that body water loss from Nuttallina is much greater for chitons experimentally removed from their holes than for ones left in the chiton-shaped depressions at low tide. Interestingly, at Whites Point, the density of LOWs in low intertidal Phragmatopoma beds with the depressions but without macroscopic algae, as well as in low algal turf with chiton-shaped depressions is greater ($100/m^2$) than in low areas with neither the algae nor the depressions ($25/m^2$; Table III-7); apparently, the depressions can provide a suitable habitat for LOWs in the absence of coralline algal turf. The converse situation, that of coralline algal turf without chiton-shaped depressions, was not observed at any of the sites of the present study.

The close association of LOWs with chiton-shaped depressions suggests that the depressions are species-specific within the genus Nuttallina. However, similar chiton-shaped depressions, associated with an unidentified brown alga with Gelidium-like morphology, and with almost no coralline algae present, occur in the low intertidal sandstone at the Gaviota site, and only HI/DRYs are found in the depressions. Although LOWs might have created the depressions and subsequently been replaced by HI/DRYs, the depressions are probably better thought of as associated with low-intertidal-occurring Nuttallina in general, rather than with the LOW species itself, or even with coralline algae.

Chiton-shaped depressions occupied by Nuttallina have been found in the Monterey, CA area in Phragmatopoma and Dodecaceria beds (Nishi, 1975 and personal observations). Although samples of Nuttallina were not obtained from the Monterey area for electrophoretic identification in the present study, morphological identification of specimens in a small preserved sample collected in the locale of Nishi's Monterey Bay study site reveals the presence of both BODEGAs and LOWs (Table III-8; SIO Nos. M1922 to M1924); see Chapter IV for morphological critera used). Further evidence provided by Nishi's (1975) comments concerning identification of the Nuttallina species in her study areas indicates a good probability that LOWs are the primary species inhabiting the depressions in Monterey Bay, as is the case at most other sites in this study.

INTERTIDAL DISTRIBUTIONS OF THE NUTTALLINA SPECIES: HIDRYs

The intertidal distribution of HI/DRYs is dramatically different from that described above for LOWs. At the La Jolla study site, HI/DRYs are overwhelmingly the predominant species in the "high and dry" and the "high pool" habitats, and are nearly absent from the low intertidal areas (Tables III-5 and III-6). Even when the immense difference in overall density of Nuttallina in high versus low intertidal areas is taken into account, HI/DRYs still appear to be more dense in high areas than in low areas on the shore (Tables III-5 to III-7, and see exemplary calculations earlier in this section). At Whites Point, HI/DRYs are also the predominant species in the "high and dry" habitat, but are present in low densities (~15/m^2; Table III-7), whereas at Gaviota, the only other electrophoresis-collection site where HI/DRYs were found in substantial numbers, HI/DRYs reached densities of 10 to 100 per m^2 in the "high and dry" habitat, in the absence of LOWs and BODEGAs.

HI/DRY "juveniles", found in smaller supply than LOW "juveniles" in the November 1981 collection, apparently were more abundant in the low algal turf than in the high intertidal area, and were absent from the "high and dry" habitat at La Jolla (Table III-6). Again, as for LOWs, if one assumes that "juveniles" more closely reflect larval settlement patterns than "adults" do, these juvenile occurrences might reflect nonselective larval settlement, with many more larvae settling in low areas than high, and/or a selective system involving specific responses of larvae to damper areas with or without avoidance of "high and dry" habitats. In this connection, it is of interest to mention Louda's (1972) suggestion that Nuttallina larval settlement, probably of HI/DRYs at her site, is greatest in roughly-textured areas (like the low intertidal turf area at La Jolla) based on her findings of greater numbers of small recruits on experimentally-roughened surfaces than on smooth control rocks in Santa Barbara.

If more larvae settle low rather than high on the shore from year to year, why are the densities of HI/DRY adults in the high intertidal equal to or greater than their densities in the low area of La Jolla? An upward intertidal migration of HI/DRYs with age/size is a possibility; the tendency to find the larger HI/DRYs at higher shore levels seems to lend support to this idea (Tables III-5 and III-6). This notion is explored in Chapter V, where it is shown that at least adult Nuttallina HI/DRYs are fairly reliable homers that exhibit no consistent tendency to move in any specific direction. Differential survival with height on the shore is another possible explanation for the relatively large numbers of HI/DRYs in the high areas. In this case, it might be suggested that aspects of the coralline-algal-turf/chiton-shaped-depressions/LOW-Nuttallina species environment found in the low intertidal zone at La Jolla reduce the survivability of HI/DRYs there. In this regard, it is interesting that slightly higher densities ($25/m^2$) of HI/DRYs are found in the low zone at Whites Point where there are lower densities of LOWs ($25/m^2$), no algal turf and no chiton-shaped depressions, than are found in areas with greater LOW densities ($100/m^2$) and neither turf nor depressions (i.e., $\sim10/m^2$; Table III-7). That the presence of depressions themselves is not the primary factor in the absence of HI/DRYs is suggested by the finding of the highest densities of HI/DRYs of the present study ($200\text{-}300/m^2$) in the low intertidal area of Gaviota, where there are chiton-shaped depressions, but little coralline algae and no LOWs. Deleterious effects associated with the coralline algal turf itself such as mechanical disruption of effective movement, or predation that might be particularly intense in the coralline algal turf area may account for the lowered density of HI/DRYs in this habitat.

Competition and predation are most often suggested as the factors setting the lower limit of distribution of intertidal organisms (e.g., Connell, 1961a and b). For two species as closely-related and as apparently ecologically-similar as Nuttallina HI/DRYs and LOWs, interspecific competition may figure in their coexistence. Although there is no clear indication that the presence of HI/DRYs greatly influences the distribution of LOWs (see discussion for LOWs above), evidence exists that suggests that LOWs have an effect on the intertidal distribution of HI/DRYs. Thus, nearest neighbors analyses involving the low intertidal area in La Jolla has shown that Nuttallina, primarily LOWs, tend to be slightly "overdispersed"; this dispersion pattern is often evidence for territorial exclusion. Further, at Gaviota, where LOWs and BODEGAs are absent, the greatest densities of HI/DRYs observed in this study are found in the low intertidal zone (Table III-7). This finding suggests that although the low intertidal may be preferred by HI/DRYs, the presence of LOWs, or the operation of some factor correlated or causally-related with the presence of LOWs, at other sites often restricts their occurrence to the higher intertidal. The fact that estimated densities of Nuttallina in general tend to be greater in lower (wetter?) intertidal areas, whether numbers of HI/DRYs and LOWs occur together at a site (La Jolla and Whites Point), BODEGAs and LOWs occur together (Little Harbor), or LOWs or HI/DRYs occur alone at a site (LOWs at USC Marine Science Center and Avalon; HI/DRYs at Gaviota), suggests that the low intertidal is in some way preferable to the high zone for Nuttallina species in general.

HI/DRYs reach larger size than LOWs do (HI/DRY maximum: 42.0 mm thawed total length, N=148; probably 5 cm live length, see Chapter IV. LOW maximum: 32.0 mm thawed, N=227; probably 4 cm live, see Chapter IV). Larger size, with its concomitantly smaller surface area to volume ratio, is often noted as an advantageous character for organisms exposed to extreme temperatures and drying conditions (e.g., Boyle, 1970;

Foster, 1971; Branch, 1975); accordingly, in several intertidal mollusc populations, most notably in limpets, the larger specimens are more commonly found at higher levels in the intertidal zone (e.g., Das and Seshappa, 1948; Lewis, 1954; Frank, 1965; Blackmore, 1969; Sutherland, 1970; but see Shotwell, 1950). In addition, large size can act as a deterrent to predators such as birds, although birds have shown no obvious preference for Nuttallina of any size at the La Jolla site. HI/DRY specimens are generally larger in the high zones than in the low areas at all sites where this species occurs (Tables III-5, III-7). In contrast, LOWs in the low areas are generally equal or larger in size than LOW specimens higher on the shore.

INTERTIDAL DISTRIBUTION OF NUTTALLINA SPECIES: BODEGAs

BODEGAs were found only rarely at La Jolla (5 of 153 specimens), and in more substantial numbers at Little Harbor, San Nicolas Island, Punta Banda, and Bodega (Tables III-5, 6, 7). Infrequent occurrences and limited habitat information preclude generalizations about the characteristic habitat of BODEGAs. At Punta Banda, BODEGAs were found in about equal densities ($50/m^2$) high and low in the intertidal; no chiton-shaped depressions were present but this might reflect the fact that the substrate, i.e., diorite, was the hardest encountered in the study and to the fact that an extraordinary abundance of both plants and animals provide a multitude of depression-like "hiding places" for Nuttallina there. At La Jolla, 3 of the 5 specimens were found in the "high and dry" habitat, while at Little Harbor, BODEGAs were present in the high and midtide areas but were absent from the low algal turf. BODEGAs high on the shore tend to be equal or slightly larger in size than ones lower on the shore (Tables III-5, 7). BODEGAs found in this study were about as large as HI/DRYs (39.0 mm maximum thawed total length, N=91), but findings in Chapter IV suggest that BODEGAs attain slightly larger size than do HI/DRYs (5.5 to 7 cm live length).

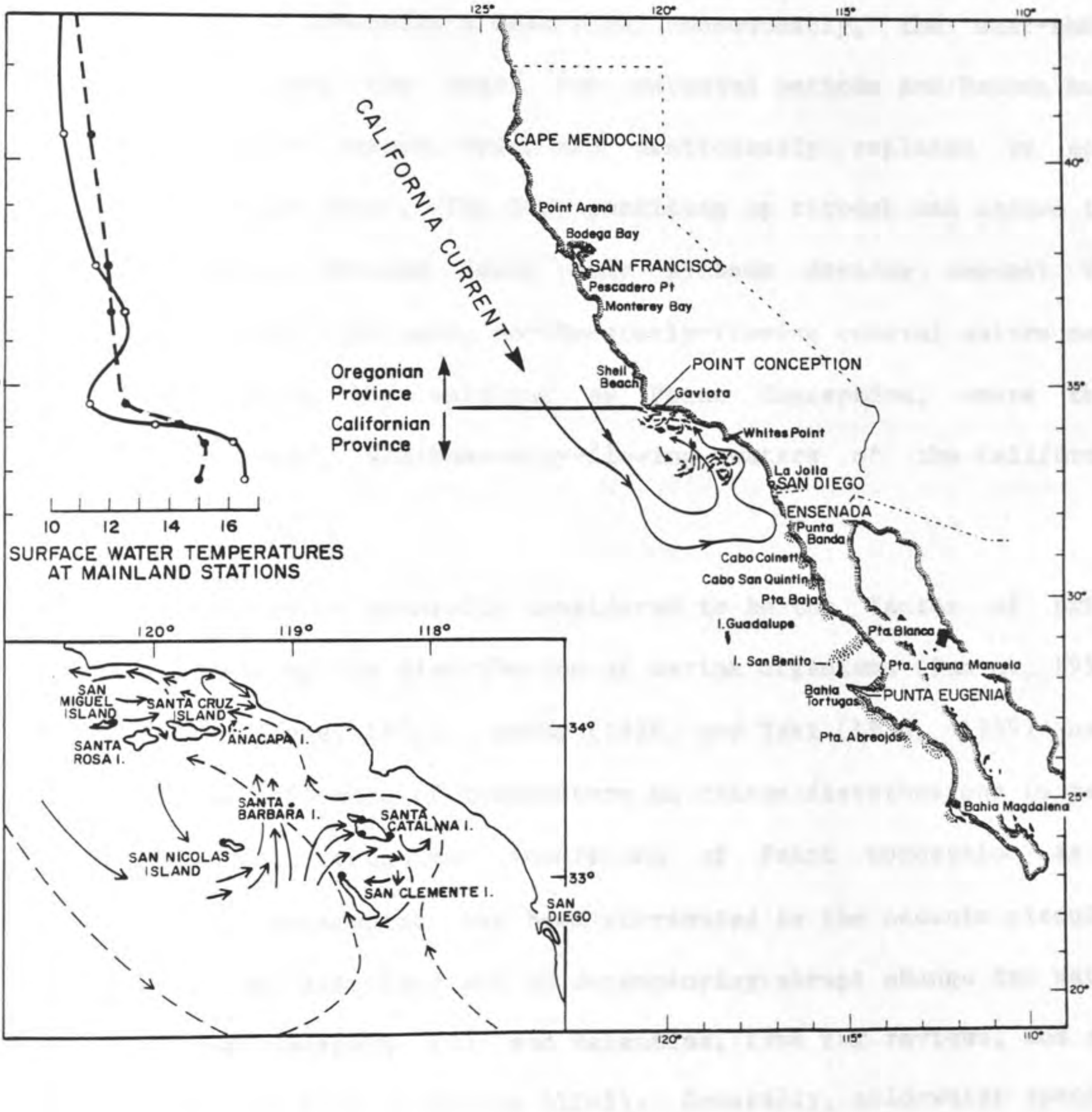
BIOGEOGRAPHY OF THE NORTHEAST PACIFIC NUTTALLINA SPECIES

The northeast Pacific coast has been divided into biogeographic zones commonly on the basis of molluscan assemblages. Each zone has characteristic faunal and floral assemblages and is generally associated with distinctive hydrographical and climatological factors (e.g., Schenk and Keen, 1936; Newell, 1948; Valentine, 1966). The collection sites of the present study fall in two adjacent provinces, the cold-temperate Oregonian Province (Point Arena, Bodega, Bolinas, Pescadero Point, Monterey, Shell Beach) and the more southern warm-temperate Californian Province (all other sites in the study), with Point Conception, CA dividing them. The span of coastline represented in this study is oceanographically and climatologically complex and this complexity is reflected in the biotic patterns of distribution (see Brusca and Wallerstein, 1979 for review).

Seapy and Littler (1980) provide a detailed description of surface current patterns off California, with emphasis on the Southern California Bight. An abbreviated account follows (see Figure III-5). Off central California, the cold California Current flows southeastward along the coast; nearshore waters are maintained at cold temperatures through spring and summer, largely by upwelling. At Point Conception, the coastline jogs abruptly to the east, but the California Current

Figure III-5. Hydrographic features of the area encompassed by collections examined in the present study.

Surface water temperatures along the California coast (graph at left) are from Sverdrup et al. (1942, p. 724); the solid line represents March to June temperatures and the broken line represents November to January temperatures. Surface current patterns of the Southern California Bight are from Seapy and Littler (1980). The inset at the bottom left shows the surface current patterns of California's Channel Islands in detail; the six collection sites on San Miguel, San Nicolas, Santa Catalina and San Clemente Islands are indicated as small solid circles. Coldwater upwelling areas in Baja California, taken from Dawson (1951), are stippled. A region of water of intermediate temperature (also stippled) extends from the Punta Blanca-Punta Maria section of the Baja central coast and impacts the San Benito Islands to the southwest.


125°
120°
115°
110°
40°
35°
30°
25°
20°
CALIFORNIA CURRENT
CAPE MENDOCINO
Point Arena
Bodega Bay
SAN FRANCISCO
Pescadero Pt
Monterey Bay
Shell Beach
POINT CONCEPTION
Gaviota
Oregonian Province
Californian Province
Whites Point
La Jolla
SAN DIEGO
ENSENADA
Punta Banda
Cabo Colnett
Cabo San Quintin
Pta. Baja
I. Guadalupe
Pta. Blanca
I. San Benito
Pta. Laguna Manuela
Bahia Tortugas
PUNTA EUGENIA
Pta. Abreojos
Bahia Magdalena
10
12
14
16
SURFACE WATER TEMPERATURES AT MAINLAND STATIONS
120°
119°
118°
34°
33°
SAN MIGUEL ISLAND
SANTA CRUZ ISLAND
SANTA ROSA I.
ANACAPA I.
SANTA BARBARA I.
SANTA CATALINA I.
SAN NICOLAS ISLAND
SAN CLEMENTE I.
SAN DIEGO

instead continues flowing southeastward to the west of the Santa Rosa-Cortez Ridge, i.e., to the west of the California islands. South of the Cortez Bank, the California Current turns eastward in a broad arc, forming the Southern California Eddy. This eddy is most strongly developed during the summer months. The northwesterly-flowing arm of the Southern California Eddy is generally a weak flow; consequently, the near-shore waters remain along the coast for extended periods and become much warmer than offshore waters, which are continuously replaced by cold California Current water. The Eddy continues up through and around the California islands; various small eddy systems develop amongst the islands. Finally, the warm, northwesterly-flowing coastal waters move as far north along the mainland as Point Conception, where they encounter the cold, southeasterly-flowing waters of the California Current.

Temperature is generally considered to be the factor of prime importance affecting the distribution of marine organisms (Gunter, 1957; Hedgpeth, 1957; Kinne, 1970). Ashby (1926) and Taki (1938; 1939) have recognized the influence of temperature on chiton distributions in particular. The well-recognized importance of Point Conception as a biogeographical breakpoint has been attributed to the oceanic circulation patterns just described and an accompanying abrupt change in water temperature (see Hedgpeth, 1957 and Valentine, 1966 for reviews, and see the temperature profile in Figure III-5). Generally, cold-water species occur north and warm-water species south of Point Conception; collections from the mainland in this study might be expected to show this general pattern. The mixed character of currents, and presumably of temperatures, in the California islands suggest that their fauna and flora might exhibit various affinities to northern cold-water and southern warm-water mainland biotic assemblages. In fact, in their study of rocky intertidal macroinvertebrates of the California islands, Seapy and Littler (1980) predicted from hydrographic patterns, and found, that San Miguel Island, bathed directly by the cold California Current, has quite strong northern affinities, and that San Nicolas Island, farther to the south and east, has northern affinities that are not quite as pronounced. Santa Catalina and San Clemente Islands, located in the path of the warmed, northerly-flowing Southern California Eddy, have strong southern warm-water affinities.

The Pacific coast of Baja California lies in warmer, more southern latitudes, and thus might be expected to support only warm-water species; however, the situation is more complex. The west coast of Baja is characterized by alternating areas of protected warm waters and of more exposed regions subject to cold water upwelling. The upwelling areas are commonly found south of headlands and support disjunct populations of cold-water plants and animals, many of which are found otherwise only north of Point Conception, CA (e.g., marine plants, Dawson, 1951; invertebrates, Emerson, 1956; fish, Hubbs, 1948; and see Figure III-5). All collections from Baja California examined in this study were from sites that are subject to upwelling (i.e., Punta Banda, Punta Baja, Bahia Tortugas, Turtle Bay, and Punta Abreojos; see Dawson, 1951 especially), except the Laguna Manuela Point site, found in the warmer waters of Bahia de Sebastian Vizcaino, Magdalena Bay, Guadalupe Island, an oceanic island found 282 kilometers off the central Baja coast, and

East San Benito Island. The San Benito Islands are bathed by a cool "tongue" of water that originates in the Punta Blanca-Punta Maria upwelling area of the mainland, and have been found to support an assemblage of marine plants and animals very similar to that found in La Jolla (Dawson, 1951).

Upwelling in Baja occurs primarily in the spring and summer; available evidence suggests that Nuttallina spawn in the spring and summer. Thus, in Nuttallina in Santa Cruz Co., CA (probably BODEGAs, possibly with LOWs), Ituarte (1981) found indications of a strong spring spawning period and another, suggested to be unusual, weak fall spawning episode. At Santa Barbara, CA, Louda (1972) noted the appearance of small chitons (probably HI/DRYs) year-round, though the greatest increase in numbers of small chitons in quadrats occurred in early to mid summer. Similarly, at La Jolla, I observed the presence of small chitons (~5-8 mm long) at several times of the year; however, Nuttallina collected from the high and low intertidal at La Jolla (HI/DRYs and LOWs) in August 1977 had much larger gonads than ones collected in September 1977, suggesting that a spawning bout occurred in August. If one assumes that Nuttallina spawn similarly at the lower latitudes of Baja California, Nuttallina larvae, presumably representing the most vulnerable life stage, would be expected to experience the spring-summer cold waters at the Baja upwelling sites of this study. Therefore, the Baja samples, except for those from Laguna Manuela Point, Magdalena Bay, and possibly Guadalupe Island, might be expected to exhibit a cold-water component, although the likelihood of this presumably would decrease with decreasing latitude.

Geographical distributions of Nuttallina species established in this study generally match predictions for one cold-water species and two warm-water species derived from a consideration of the hydrographic conditions discussed above (Figure III-4 and Tables III-5 to III-8). The complexity of distribution of the three species probably explains in part the difficulty that has been encountered in past taxonomic studies of this genus. The cold-water species, BODEGA, occurs in the apparent absence of the other two species at the cold water northern site, Bodega Head (Sonoma Co.). It is also found at Point Arena (Mendocino Co.), Bolinas (Marin Co.), Pescadero Point (San Mateo Co.), Pacific Grove (Monterey Co.), and at Shell Beach (San Luis Obispo Co.) on the mainland north of Point Conception. The distribution of BODEGA may extend north to the vicinity of the Canadian border, the reported northern limit of the range of the described cold-water Oregonian Nuttallina species, N. californica (see Chapter IV).

On the mainland south of Point Conception, BODEGAs occur in substantial proportions only at Punta Banda, the northernmost Baja California site subject to cold water upwelling. BODEGAs are poorly represented at La Jolla (5 of 153) and at the cool water site far down the Baja coast, E. San Benito Island (1 of 4). Southern submergence of BODEGAs to subtidal depths south of Point Conception, with disjunct emergences particularly in upwelling areas, might account for the mainland distribution of BODEGAs.

Although such a submergence-emergence phenomenon cannot be entirely discounted, particularly for the Baja coast, where the subtidal areas have not been extensively collected, the lack of reports of subtidal occurrences of Nuttallina in Southern California minimizes the probability of submergence-emergence. The populations of BODEGAs such as at Punta Banda and E. San Benito Island probably represent isolated outlying pockets of the species. Interestingly, Cryptochiton stelleri, a cold-water species that occurs only as far south as the northeastern Channel Islands (San Miguel and San Nicolas) today, persisted until quite recently at two isolated areas along the Baja coast, Punta Baja and Punta Camalu (Moriarty, 1980). Radiocarbon dating of valves of C.stelleri from middens at the two sites revealed ages of 160±150 and 110±150 years, respectively, whereas valves of this species from middens elsewhere on the Baja coast were dated at least several centuries earlier. Moriarty (1980) suggests that the exceptional local persistence of this cold-water species at Punta Baja and Punta Camalu can be explained by the fact that the two areas are subject to cold-water upwelling.

On the California islands, BODEGAs occur as predicted on the islands with northern affinities, San Miguel and San Nicolas Islands. BODEGAs occur unexpectedly on Santa Catalina Island, but only at the Little Harbor site, which, with its western-facing orientation, is likely to be subject to small cold water flows from the California Current (see Figure III-5).

The HI/DRY species is found only south of Point Conception, apparently in the absence of BODEGAs and LOWs at Gaviota and in substantial numbers at Whites Point and La Jolla. Although HI/DRYs appear to be scarce at the most northern Baja upwelling site, Punta Banda, they are found at all other collection sites in Baja California where positive identifications could be made -- at the warm-water Laguna Manuela Point site as well as at E. San Benito Island and all of the upwelling sites, down through Punta Abreojos. This last site represents a small southern limit extension for *Nuttallina fluxa* (= HI/DRY, Chapter IV), from Asuncion Island, central Baja California (McLean, 1978). Punta Abreojos might represent the true southern limit for HI/DRYs, since the ranges of many temperate species are known to terminate in this area. However, given the occurrence of Californian *Nuttallina* at Magdalena Bay, reported for the first time in the present study (Table III-8), and the probability that at least one of those specimens is a HI/DRY, it is probable that HI/DRYs occur as far south as Magdalena Bay, the southern limit for nearly all temperate shallow water invertebrates (Brusca and Wallerstein, 1979). Interestingly, HI/DRYs were not positively identified in any of the collections obtained from the California islands. Further sampling, especially at San Clemente, Santa Barbara, and Anacapa Islands, will reveal whether HI/DRYs are generally absent from the islands; if so, interesting questions, relating to habitat preference, competition and/or even speciation and larval dispersal, arise.

LOWs, like HI/DRYs, occur abundantly in samples south of Point Conception. However, morphological identification of *Nuttallina* in the Shell Beach and Pacific Grove (near Monterey) collections reveal the presence of LOWs at these sites, just north of Point Conception (Table III-8). Further, Ituarte's (1981) findings for a high intertidal population of large-sized *Nuttallina* and for a lower population of smaller *Nuttallina* that occur in depressions in *Phragmatopoma* beds suggest the possibility that LOWs occur in the low zone there, just north of Monterey Bay (see his figure 5 for size vs. substrate-intertidal height). Finding a warm-water species north of Point Conception, such as this, is not extraordinary. The break at Point Conception is not sharp, but rather is manifested as an overlap area, the Californian Transition Zone, with some northern species extending two to three or even more degrees in latitude south of Point Conception and some southern species penetrating two to three degrees, or more, north (Newell, 1948; Valentine, 1966; Newman, 1979). The occurrence of a warm water anomaly in the Monterey Bay area in the spring probably contributes to the extension of the ranges of some warm-water species north to this area (Sverdrup et al., 1942; see Figure III-5).

On the mainland south of Point Conception, LOWs occur in substantial numbers at Whites Point and at La Jolla. Surprisingly, LOWs are absent from (i.e., their distribution skips over) the Gaviota site. This absence might stem from the paucity of coralline algae, with which LOWs are usually associated, at this site. In Baja California, LOWs are generally found in low proportion and only at Punta Banda (2/41), and much farther south in the Bahia Tortugas area (1/22), at Punta Abreojos (2/11), and at East San Benito Island (2/4). LOWs might occur as far south as Magdalena Bay (Table III-8). The paucity or complete lack of positively-identified (i.e., not in the "questionable identification" category, Table III-8) LOWs at all Baja collection sites may reflect any of several things: 1) where indicated, samples were often from the high intertidal zone and, in any case, the high shore is usually easiest to sample, thus producing a bias against finding LOWs which usually occur low on the shore; 2) LOWs may be present in the samples, but, because of the conservatism of morphological identifications in this study, could not be positively identified; 3) LOWs might undergo southern submergence to uncollected subtidal levels, re-emerging in the cool waters of East San Benito Island and rarely in the upwelling area around Bahia Tortugas and Punta Abreojos; and 4) LOWs might not normally occur in any numbers south of the La Jolla-Punta Banda area. Further sampling is needed to judge the validity of possibilities 1 and 3, although 3, submergence to subtidal depths, is not considered likely. The fact that several preserved specimens were placed in the "LOW→HI/DRY" and "HI/DRY→LOW" questionable categories (Table III-8) suggests that possibility 2, the problem of conservatism of identifications, might have validity; electrophretic analysis of more samples would overcome this uncertainty. If the fourth possibility is true, the LOW species might qualify as a Californian Transition Zone endemic that has exploited resources not utilized due to "disharmony between overlapping [Oregonian and Californian] provincial communities" (Newman, 1979). Also, if the LOW species is truly largely absent from Baja, its latitudinal range is much shorter than that of HI/DRY; this situation would be in accord with Pielou′s (1978) prediction, which strictly applies to congeneric species with "nested" ranges, that the high intertidal species, exposed to quite variant environmental conditions, will occupy a longer latitudinal range than will the low intertidal species, which endures a smaller spectrum of environmental conditions.

On the California islands, LOWs are found at San Clemente Island and in large proportions in samples from Santa Catalina Island (100% of samples from Avalon and the USC Marine Science Center, and 86% of the sample from Little Harbor), but are absent from the small San Miguel Island sample, as expected for a warm-water species (Tables III-7 and III-8, Figure III-4). The good representation of LOWs at the San Nicolas Island site is in accord with Seapy and Littler's (1980) implication that this island should support a biotic assemblage of a slightly more warm-water character than should colder, more northern San Miguel Island.

Of course, many factors other than temperature and ocean currents can influence marine organismal biogeographic distributions. In particular, substrate is often an important factor determining where organisms are or are not found. It is interesting to note that estimated densities of <u>Nuttallina</u> in this study are generally greatest at sites with softer substrates; in fact, LOWs and HI/DRYs both reach their highest observed densities on soft sandstone, at La Jolla and Gaviota respectively (Table III-7).

In all likelihood, there is considerable genetic exchange among <u>Nuttallina</u> at sites separated by fair distances; apparent "pockets" of a single species, such as the BODEGA population at Punta Banda, are probably not isolated gene pools. Geographic distributions of the three <u>Nuttallina</u> species are probably reversibly changeable over the short term in response to warming and cooling events and trends, shifting, extending and contracting from year to year, much in the way that <u>Mytilus edulis</u> has been found to occur seasonally below Cape Hatteras on the east coast of the United States, presumably due to larval transport and recruitment in favorable seasons (Wells and Gray, 1960).

Studies of adult Nuttallina indicate a well-developed homing behavior (Chapter V), hence a small potential for dispersal of the mature chitons. However, available evidence suggests that Nuttallina are free spawners, probably with larval stages capable of considerable transport, as opposed to brooders that retain eggs or young in the pallial groove for a period of time. Personal observation of one episode of spawning in the laboratory confirms that at least one of the La Jolla Nuttallina species, probably LOW, is capable of free spawning. At 2000 hrs, 28 July 1977, three Nuttallina from the low intertidal zone at La Jolla were observed to have deposited multitudes of green eggs in seemingly sticky masses on the bottom of a slow-circulating seawater table at Scripps Institution of Oceanography. The posterior end of each chiton was near or touching an egg mass. Two steady, cloudy, opaque-white streams, presumably of sperm, were observed to issue from the posterior ends of two other Nuttallina specimens, also from the low intertidal of La Jolla. The sperm streams continued throughout the observations that lasted several minutes; the streams probably continued for a much longer period. In the field, the egg masses are probably dispersed by waves, as Okuda (1947) observed for egg strings of the chiton Cryptochiton stelleri.

Additional evidence supports the suggestion that Nuttallina species in the present study do not brood their young. In a fifteen-month-long study of the sex ratio and reproductive activity of Nuttallina in Santa Cruz Co., CA (probably BODEGAs, possibly with LOWs, see above), Ituarte (1981) apparently found no evidence for brooding nor for hermaphroditism. Similarly, personal observations of Nuttallina in La

Jolla (primarily LOWs and HI/DRYs) at various times of the year over five years indicate that the sexes are separate, and have revealed no evidence for brooding. The occurrence of at least one *Nuttallina* species on Guadalupe Island, 282 kilometers off the coast of central Baja California (Table III-8), suggests that at least one of the species is probably capable of considerable larval dispersal. Guadalupe Island is a volcanic oceanic island that has never been attached to the mainland. Finally, none of the *Nuttallina* species would be *predicted* to brood their young; most chitons known to brood are found in cold temperate or subpolar seas *and* are much smaller, usually less than 20 mm in maximum length, than *Nuttallina* species of California (Pearse, 1979). Following fertilization then, *Nuttallina* is probably like most other large temperate chitons in having a free-swimming larval stage that serves for at least short range dispersal involving several days in the plankton (Pearse, 1979).

Nuttallina larvae might delay settling for long periods in the absence of suitable substrate, as seen for the chiton *Tonicella lineata* (D. Bonar, personal communication to Pearse, 1979), and thus might possess potential for dispersal over long distances in the currents off of California. Settling *Nuttallina* might also disperse considerable distances on algal "rafts" drifting in the ocean currents. Spring speeds of the California Current off Central California are on the order of 4 to 10 km per day (Griggs, 1974). Thus, it is quite possible that the small numbers of a single *Nuttallina* species co-occurring with large numbers of one or both of the other *Nuttallina* species at sites in the present study, such as the 5 BODEGAs among 153 *Nuttallina* specimens at

La Jolla, represent specimens dispersed from other sites, rather than members of an established breeding population at the site where they are found. Certainly, if specimens occurred allopatrically, the present sympatry of the closely-related _Nuttallina_ species is clear evidence for dispersal, at least over a large time scale (Croizat et al., 1974).

SPECIATION IN THE GENUS _NUTTALLINA_

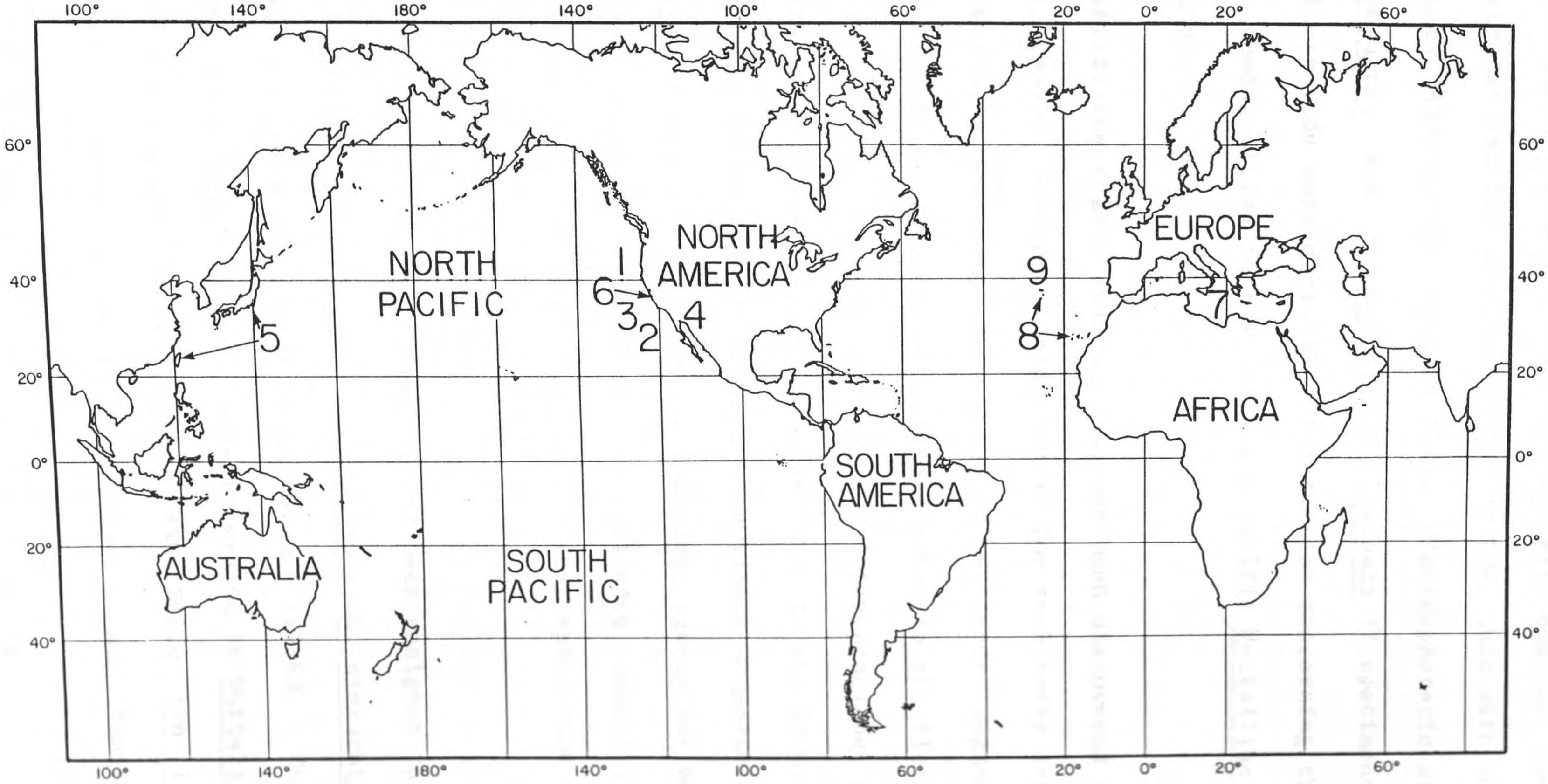
The worldwide distribution of species of the genus _Nuttallina_, as well as of species closely-related to _Nuttallina_, is presented in Figure III-6 (and see Appendix III for additional information). All known species presently assigned to the genus are living and, with the exception of _Nuttallina crossota_ Berry at the northern end of the Gulf of California, occur in temperate waters along the shores of the northern Pacific Ocean. The non-congeneric species that are apparently most closely-related to _Nuttallina_ occur in the Mediterranean Sea and the eastern Atlantic Ocean, as well as in California.

Morphological examination of specimens of _Nuttallina crossota_ from the Gulf of California suggests that this species is most closely-related to HI/DRYs, as evidenced by a predominance of white girdle spines, white and brown striping of the girdle, and quasi-rectangularity of its often short, wide valves (Appendix III). Pilsbry (1893) was unable to examine the interior of any specimens of _Chiton alternatus_ Sowerby so he could only _provisionally_ assign this species to _Nuttallina_. I have not been able to locate either type or non-type specimens of _Nuttallina alternata_ for examination (Appendix III), but the reported warm-temperate distribution of this species, in southern Japan and

Figure III-6. World distribution of living species of the genus Nuttallina and of living species in the genus Lepidochitona that are closely related to the Nuttallina species.

Fossil evidence for Nuttallina consists only of Pleistocene material from Southern California. All species occur in temperate areas of the world: (1) BODEGA (=N. californica); (2) HI/DRY (=N. fluxa); (3) LOW (=N. kata sp. nov.); (4) N. crossota Berry, 1956, Gulf of California; (5) N. alternata Sowerby, 1840, Japan and Taiwan(?); (6) Lepidochitona thomasi (Pilsbry, 1898), Monterey Bay area, CA; (7) L. (Middendorffia) corrugata (Reeve, 1848), Mediterranean Sea, south of Portugal, Spain; (8) L. (Middendorffia) piceola (Shuttleworth, 1853), Canary Islands and Azores; (9) L. simrothi (Thiele, 1902), Azores.

No living or preserved specimens of N. alternata have been located in a recent, intensive search and this species is regarded as a nomen inquirendum (see Appendix III). Nierstrasz (1905a, b) reported a single specimen of "Nuttallina scabra" from Chile, but this specimen apparently was involved in a labelling mix-up and is probably a Californian specimen. No Nuttallina specimens from Chile have been located subsequently (see Appendix III). See text for discussion.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possibly Taiwan, might indicate closer affinity with one of the warm-temperate northeast Pacific species, HI/DRY or LOW, than with the cold-temperate species, BODEGA (see Appendix III). Electrophoretic study of *Nuttallina crossota*, and of *Nuttallina alternata* if specimens can be obtained, would provide more definitive information concerning the relationships of these species to the northeast Pacific *Nuttallina* species and to each other.

Pre-Pleistocene fossil evidence has not been discovered for *Nuttallina* (Smith, 1960). However, fossil record for such rocky intertidal organisms is generally poor, and three lines of evidence suggest that speciation in *Nuttallina* might have commenced prior to the Pleistocene. First, fossil evidence for the chiton family Callistoplacidae Pilsbry, 1893, to which *Nuttallina* belongs, dates back to the Lower Miocene (Smith, 1960), about 20 to 25 m.y. ago. Such evidence suggests that the genus *Nuttallina* could have existed and commenced speciation much earlier than the Pleistocene. In addition, this evidence possibly establishes the Miocene as the limit for the earliest speciation event in *Nuttallina*.

Second, species that until recently had been assigned to *Nuttallina*, namely *Lepidochitona corrugata*, *L. piceola*, *L. simrothi*, and *L. thomasi*, exhibit a Tethyan Sea distribution (Figure III-6). The California species, *L. thomasi*, is very closely-related to *Nuttallina* (see Appendix III for a discussion of *L. thomasi*); Van Belle (*in litt.*, 6 February 1983) has concluded that the Atlantic and Mediterranean species, *L. corrugata*, *L. piceola*, and *L. simrothi*, are even more closely-related to the *Nuttallina* species than is *L. thomasi*. The extremely close relationship of these species to Nuttallina is also indicated of course by the former assignment of these species to Nuttallina.

The Tethyan distribution of the species suggests that Nuttallina might have commenced speciation well before the Pleistocene. A remarkably similar geographic distribution is exhibited by living and fossil species of the "concavus" group of Balanus barnacles (see Figure 8 of Newman, 1979; also see 1982). The fossil record for this group includes occurrences in both the Eastern Pacific and Europe as early as the Oligocene, and in northern Japan in the Mio-Pliocene.

The third line of evidence for pre-Pleistocene speciation of Nuttallina involves calculations based on the controversial "molecular clock" model of evolution. The molecular clock hypothesis has been reviewed by Thorpe (1982). The clock model predicts that individual proteins evolve at relatively constant rates, when averaged over long time periods, so that protein differences reflect divergence times between taxa. The accuracy of calculations based on the clock depends on both the degree of constancy and the calibration of the rate of protein evolution in the taxa under study. Calibrations of the clock, involving various taxa of vertebrates to date, have been performed by relating albumin immunological distances to fossil evidence (Carlson et al., 1978; and see Wilson et al., 1977). Electrophoretic genetic distances, such as D of Nei (1972), are then calibrated by virtue of their strong correlations with albumin immunological distances (see Wyles and Gorman, 1980 for a compilation). Estimates of divergence times generated in this way from the clock model can only be rough approximations. Paleontological evidence, the ultimate basis for the divergence time scale, is subject to considerable uncertainty. In addition, care must be exercised because gene loci seem to evolve at varying rates, and the calibration of, for example, Nei electrophoretic distances with time is, therefore, dependent on the mix of "fast" and "slow" evolving loci used in the study (Sarich, 1977; see Skibinski and Ward, 1982 for a slightly modified view). Also, the correlation of albumin immunological distances with electrophoretic genetic distances seems to differ among taxonomic groups (Wyles and Gorman, 1980). Finally, no invertebrates have been used in any calibration of the clock. However, Nei (1971) provided a theoretical "calibration" for the fruit fly Drosophila, reasoning that the clock runs at the rate of nucleotide substitutions, which is reasonably similar for organisms as different as procaryotes and eucaryotes. Therefore, a clock calibrated by using data from vertebrates should serve crudely for invertebrates such as chitons.

Despite the uncertainty in estimating divergence times from electrophoretic genetic distances, such estimates seem to have some validity and usefulness (Wilson et al., 1977; Vawter et al., 1980; but see Lessios, 1979, 1981). If one uses the estimates of electrophoretic distances obtained in the present study (Table III-4) in conjunction with the calibration of Sarich (1977), as modified by Carlson et al. (1978), one obtains estimates of the divergence times for the three species of this study as follows: BODEGA-HI/DRY (D=0.4), <6 million years; HI/DRY-LOW (D=0.4), <6 million years; and BODEGA-LOW (D=0.2), 2 million years. In other words, the BODEGA-LOW and HI/DRY lines split about 6 m.y. ago and the BODEGA and LOW lines separated about 2 m.y. ago (see Figure III-3). Sarich's calibration is used because his slope for the relation between estimates of albumin immunological distances (A.I.D.) and Nei distances (D), that is a D of 1.0 = 35 A.I.D. units, falls in about the middle of the range of values reported (D of 1.0 = 22 to 55 A.I.D., Wyles and Gorman, 1980). Carlson et al. (1978) modified Sarich's calibration of A.I.D. units from 1 A.I.D. unit = 0.60 m.y. to 1 unit = 0.54 m.y. Since six of the 14 loci surveyed in this study are expected to belong to the "fast evolving" class (i.e., Aph, Adh, G-3-pdh, Hk, peptidase, Pgm; see Kojima et al., 1970 and especially Johnson, 1974), divergence times are expected to be slightly lower than those for the 25/75 mix of fast/slow evolving proteins plotted by Sarich (1977), particularly when it is remembered that the time scale has been reduced by Carlson et al. (1978; so that 6 m.y. = 5.4 m.y.).

Thus, while fossil evidence suggests that speciations in Nuttallina could not have commenced earlier than the Lower Miocene, about 20 to 25 m.y. ago (i.e., the age of the earliest fossils of the Callistoplacidae), calculations based on the clock model suggest that separation of the HI/DRY line from the BODEGA-LOW line occurred about 5 to 6 m.y. ago, in the Mio-Pliocene, and the divergence of the BODEGA-LOW line, into BODEGA and LOW lines, occurred 2 m.y. ago in the early Pleistocene. During the last 25 m.y., the configuration of the circum-Pacific land masses and the general ocean surface circulation has not changed much, although the Pacific Ocean basin has narrowed somewhat in the east-west direction (van Andel, 1979). During the last 25 m.y., the climate has progressively cooled, with the notable exception of a sharp warming reversal in the middle Miocene revealed in an analysis of distributional patterns of shallow-water molluscan faunas of the middle latitudes of the marginal northeastern Pacific Ocean (Addicott, 1969). Most recently, in the last 2 or 3 m.y., the climate has been characterized by alternate cooling and warming periods accompanying glaciation episodes of the late Pliocene and Pleistocene.

Thus, if temperature is the primary factor determining the limits of the present and past distributions of Nuttallina, as has generally been assumed for most other organisms, it would seem that any pre-Pleistocene speciation events must have occurred north of, or at least at the same latitude as, the present boundaries between the species. For example, the present boundary between HI/DRYs and BODEGAs falls at about Point Conception, CA. The isotherms characteristic of waters off Point Conception today probably occurred north of Point Conception in the warmer pre-Pleistocene (i.e., pre-glaciation) past. Thus, if speciation of HI/DRY and BODEGA occurred before the Pleistocene, it probably occurred north of Point Conception, if temperature is the primary factor setting distributional limits.

The speciation represented by the north Pacific disjunction, with Nuttallina alternata on the western side and all other species on the eastern side, probably occurred when isotherms were far north of their present positions, such as during the middle Miocene, or possibly during an interglacial episode of the Pleistocene. Subsequent cooling, with a southward shift of isotherms, would then have divided the western from the eastern species.

The fact that the faunas, particularly warm-temperate ones, occurring on either side of the north Pacific are quite different at the species level argues for a pre-Pleistocene separation of _N. alternata_ from the other species of _Nuttallina_. Thus, Briggs (1974, pp. 282, 283) notes that although the Oregon and Oriental faunas are closely related at the generic level, the faunas are very different at the species level. Aside from wide-ranging arctic-boreal species, probably less than 1% of the species are shared. Keen (1941) does not provide an estimate of analogous, or closely-related, mollusc species, but _does_ estimate that more than half, and possibly as many as two-thirds, of the generic units recognized on either coast are present on the other; although nearly 2000 species occur on either side of the north Pacific, she reports that only 16 species of gastropods and pelecypods, of which she could confirm only two to four, had disjunct amphi-Pacific distributions _and_ were identical on the two sides. Bertsch and Johnson (1982) report that only 14 of 165 species of nudibranch molluscs that occur along the temperate Pacific Coast of North America also occur in Japan. Some of the 14 have continuous ranges around the perimeter of the north Pacific basin whereas the balance occur in bays and were introduced by human intervention, such as on ship bottoms or with oysters.

Taki (1938) lists seven species (_Tonicella marmorea_, _T. ruber_, _T. submarmorea_, _T. lineata_, _Mopalia ciliata_, _Cryptochiton stelleri_, and _Lepidozona mertensii_) that occur in northern Japan as well as on the North American Pacific Coast. All of these species have continuous north Pacific distributions and/or occur at subtidal depths. In discussing the molluscan fauna of the North Pacific, Dall (1921, p. 5) states that the separation between the American and Kamchatka/Okhotsk faunas has existed long enough for the development of specific differences in many originally identical forms. Of the 26 species of chitons occurring in the Sea of Japan, 22 have been assigned to genera that occur along the Pacific Coast of North America. Although eleven of the species also occur on the eastern side of the Pacific, most of these have continuous distributions through the northern Pacific and only occur in the far north near Alaska on the eastern side. Apparently, the only chiton species with a disjunct North Pacific distribution that occurs in the Sea of Japan and as far south as Oregon and California is the subtidal species, *Oldroydia percrassa* (Yakovleva, 1965, pp. 32, 33).

The present warm-temperate distribution of *Nuttallina alternata* might indicate a closer relationship to the warm temperate species, HI/DRY or LOW, than to cold temperate BODEGA. If this is the case, the time of the separation of *N. alternata* from the eastern Pacific stock probably either pre-dated the separation of BODEGAs from the BODEGA-LOW line (if *N. alternata* is most closely-related to LOWS) or pre-dated the earlier separation of the BODEGA-LOW line from the HI/DRY line (if *N. alternata* is most closely-related to HI/DRYs), since *N. alternata* most likely would have derived from the most northerly-occurring eastern Pacific *Nuttallina* population at the time (BODEGAs at present).

Speciation of *Nuttallina crossota*, which is for the most part restricted to the northern end of the Gulf of California, probably occurred following a pronounced cooling episode such as occurred during the glaciation episodes of the Pleistocene. The range(s) of the Oregonian-Californian *Nuttallina* species would have been displaced southward during a cooling episode, as the warm tropical belt underwent compression. Closure of the Panama seaway 2 to 5 m.y. ago (Woodring, 1966; Saito, 1976) precludes the possibility that Nuttallina ever entered the Caribbean during the Pleistocene. Subsequent warming, during an interglacial episode, would have resulted in a re-widening of the tropical belt and might have left a disjunct population of Nuttallina at the head of the Gulf of California as the southern limit of the range of temperate Nuttallina species retreated northward of the mouth of the Gulf of California. Disjunct populations of species at the head of the Gulf of California closely-related or supposedly identical to temperate northeast Pacific species have been observed for several other plant and animal genera (Brusca and Wallerstein, 1979). The close relationship of N. crossota to HI/DRYs suggests that speciation of the former species might have occurred after the separation of HI/DRYs from the BODEGA line.

Likewise, repeated Pleistocene glaciation is a mechanism that might have provided opportunities for similar invasion of the Oregonian/Californian Nuttallina stock from the outer coast of Baja California and California into the Gulf of California and the reciprocal events, leading to the separation of HI/DRYs from BODEGA-LOWs, and of LOWs from BODEGAs. Certainly, available fossil evidence would appear to support the idea that at least the northern cold-water species of Nuttallina, N. californica (= BODEGA, see Chapter IV), ranged at least slightly farther south during the Pleistocene than at present. Berry (1922) reports fossilized valves of N. californica, none of warm-water N. fluxa (= HI/DRY, Chapter IV), from San Pedro, CA, and in abundance from near Point Fermin, CA (just south of my Whites Point collection site). Berry recognized two living Nuttallina species from California and as a result was cautious in his fossil identifications. It is possible that, unaware of the third living species (LOW), Berry misidentified LOWs as N. californica (BODEGAs). However, the large size of two valves he mentions and the relative proportions of a "perfect specimen" intermediate valve indicate that N. californica was indeed represented in the finds. Given a sixth valve length of 8.7 mm, an average BODEGA would have a sixth valve tegmentum width of 8.8, a HI/DRY 11.2, and a LOW 8.4 mm; since the "perfect specimen" measured 9.0 mm, it was probably from N. californica.

Invasion of the Gulf probably occurred by immigration around the tip of Baja California during a cooling episode, although Garth (1960) has pointed out that marine animals might have alternatively entered the Gulf via one of three ancient water connections across the Baja California peninsula. Three areas where such a water bridge once might have existed, well north of the tip of the peninsula, are: at the level of the Magdalena Plain, at the midpeninsular level below the Sierra Vizcaino, and about the Sierra Vizcaino at the level of Scammons Lagoon.

Geographic barriers that might have brought about speciation of Nuttallina are not obvious if that speciation commenced prior to the glaciation episodes of the Pleistocene, in particular, if HI/DRYs separated from the BODEGA-LOW line on the order of 6 m.y. ago as indicated by application of the "clock model". Prior to the Pleistocene, the climate was generally warmer, so that if anything, the ranges and limits of distribution of Nuttallina are expected to have occurred at least as far north, if not farther north, than they do today. In any case, the bulk of the Gulf of California, an obvious southerly-occurring geographic barrier, did not exist prior to about 4 to 6 m.y. ago; seismic reflection and magnetic profiles indicate that most of the Gulf of California began to form as a result of ocean-floor spreading and transform faulting about 4 to 6 m.y. ago, as the Baja California peninsula was torn away from mainland Mexico (Larson et al., 1968; Moore and Buffington, 1968; Anderson, 1972; Larson, 1972). What geographic barrier could have contributed to the speciation of HI/DRY from the BODEGA-LOW line if it occurred 6 m.y. ago? The boundary between BODEGAs and HI/DRYs falls at about Point Conception today. As judged from the character of fossil evidence, February isotherms characteristic of Point Conception today (~12.5° C) occurred north, probably closer to Point Arena or Cape Mendocino, CA, 6 m.y. ago. (Durham, 1950; see Figure III-5 for the location of these geographic features today). What is the likelihood that a geographic feature such as Cape Mendocino represented an obstacle to gene flow, via associated current systems and ocean temperature differentials, sufficient to effect the separation of HI/DRYs from the BODEGA-LOW line? Certainly, Cape Mendocino might act as an obstacle to gene flow today, given its designation as a marine biotic boundary in the biogeographic analyses of Dall (1899) and Smith (1919) (and Fisher, 1887 and Schenk and Keen, 1936 place a boundary nearby, about 2 degrees latitude north of the Cape; but see Hartman and Zahary, 1983).

The north Pacific Ocean probably had a less pronounced temperature differential north to south 6 m.y. ago than at present (see van

Andel, 1979); thus, a prominence such as the Cape is not expected to have generated as great a temperature differential between the cold waters flowing from the north and any warmed counterclockwise eddy waters flowing up from the south in the past as at present. However, if Cape Mendocino area was much more prominent than it is today, a more substantial eddy system likely existed just south of this area in the past. Coastal areas west of the San Andreas and associated faults (i.e., the Pacific Plate) have been moving northwestward relative to the more eastern North American plate at a rate of about 6 cm per year for the past 11 m.y. (Atwater, 1970). Since the fault intersects the shoreline near Cape Mendocino, this section of the coastline might have jogged more abruptly east than at present, much as does the coastline south of Point Conception today. Interestingly, Schopf (1979) has suggested that current systems alone, largely independent of a strong latitudinal temperature gradient, and even in the absence of coastal geographic barriers, are entirely sufficient to produce faunal provinces.

Consideration of a role of Cape Mendocino in speciation 6 m.y. ago is roughly analogous to a consideration of the same role for Point Conception today. Today, Point Conception, designated as the boundary between the major Oregonian and Californian faunal provinces, sets the distributional limits for several species and undoubtedly affects the dispersal of at least some species that span Point Conception. Evidence suggests that the Point acts as a partial barrier to gene flow in some species. From electrophoretic evidence, Davis et al. (1981) suggest that Point Conception acts to reduce gene flow between populations of the painted greenling _Oxylebius_ occuring to the north and to the south;

this fish is known to possess a long-lived planktonic larval stage. Abrupt changes or "breaks" at Point Conception of electromorphs in populations of the limpet Collisella (Murphy, 1978; sibling species) and the cottid fish Clinocottus (Swank, 1979; cited in Davis et al., 1981) and of an internal morphological characteristic in populations of the scorpaenid reef fish Sebastes atrovirens (Love and Larson, 1978) that span the Point provide further support for the idea that the Point acts as a gene flow barrier. Similarly, Cape Mendocino might have contributed to a reduction of gene flow and thus to the speciation of marine animals such as HI/DRY-BODEGA/LOW Nuttallina spanning that prominence 6 m.y. ago. The later split of the BODEGA and LOW lines, 2 m.y. ago, might have involved a similar coastal geographic prominence, or might have involved isolations prompted by the Pleistocene cooling-warming episodes discussed earlier.

It is possible that Point Conception played a role in speciations occurring during the Pleistocene, or even prior to the Pleistocene, if present distributional limits of the Nuttallina species do not correspond to a more northerly pre-Pleistocene speciation site as expected from temperature considerations alone. Certainly, the borderland south of Point Conception has undergone substantial changes through the Cenozoic era. In general, an episode of marine transgression and basin subsidence dominated in coastal southern California during the late Miocene (Vedder and Howell, 1979). Large parts of the Ventura and Los Angeles regions were deep marine basins, and a few islands of unknown dimensions survived from a subsiding middle Miocene volcanic archipelago. Thus, the waters south of Point Conception may have extended much farther to the east and may have been more isolated than they are today. The Pliocene was a time of rapid sediment accumulation in the near shore basins. Finally, in the Pleistocene, a land bridge may or may not have connected the mainland Santa Monica Mountains with the northern Channel Islands (i.e., Anacapa, Santa Cruz, Santa Rosa and San Miguel Islands), especially during times of seawater lowering, i.e., during glaciation episodes, presenting a significant geographic barrier for marine animal populations to the north and south (Yedder and Howell, 1979; Junger and Johnson, 1979). Thus, it is possible that, in the past, Point Conception and areas to its immediate south played an even greater role in impeding gene flow between animal populations to the north and south than they do today.

If speciation has occurred allopatrically, the present distributions of Nuttallina species, with LOWs occurring in the low intertidal and HI/DRYs in the high interdidal zone, suggests that after separation of the HI/DRY and BODEGA-LOW lines, and either before or after the split of the BODEGA and LOW species, the LOWs underwent a submergence, or lowering in the intertidal position they occupied, and/or the HI/DRYs underwent an emergence, as the ranges of these two species became overlapping. Since northern species typically undergo "southern submergences" in warmer, more southern latitudes, presumably in response to temperature, it is probable that LOWs represent the more northern segment of the Nuttallina stock from which HI/DRYs and LOWs arose. In fact, LOWs may have split from BODEGAs and become a "transition zone endemic" (if it is confirmed that LOWs do not occur in Baja in any great numbers) in response to resources made available through the "disharmony" of the adjacent provincial communities of which HI/DRYs and BODEGAs were members (Newman, 1979).

As an alternative to the allopatric model, the possibility exists that HI/DRYs and LOW-BODEGAs or BODEGAs and LOWs speciated sympatrically. Thus, for example, if the original Nuttallina stock inhabited the higher shore position occupied by HI/DRYs and possibly BODEGAs today, it is possible that a mutation causing a shift in larval settling preference to a feature common to the low shore, such as to the coralline algae that LOWs are closely associated with today, established itself in the population. As mentioned earlier, the larvae of several molluscan species have been found to settle specifically in response to the gamma-aminobutyric acid (GABA) found in coralline algae. A mutation accounting for such a response to GABA, involving a shift in response from a similar "high shore" molecule such as might be associated with the characteristic high-shore microflora (see Strathmann and Branscomb, 1979), could be a simple point mutation. Since the intertidal zone is characterized by a steep vertical cline of environmental and biotic factors, a small "founder" group in the low intertidal, possessing the GABA mutation, might experience environmental cues for breeding sufficiently different from high shore ones to shift its seasonal time of breeding and thus to drastically reduce its gene flow with the parent population. Meanwhile, selective forces operating on the two groups would be different enough to cause substantial genetic differentiation and eventual reproductive isolation and speciation of the two groups. Of course, an alternative scheme might involve a parent stock in the low intertidal and a splinter group in the high intertidal.

Apparently, differential natural selection can cause genetic divergence among contiguous populations despite substantial amounts of gene flow (Ehrlich and Raven, 1969; Endler, 1973) and, in recent years, a number of models and supposed examples of sympatric and parapatric speciation have been proposed and explored (see Bush, 1975; Endler, 1977; White, 1978). In a biogeographical analysis involving algal species on both coasts of the Americas, Pielou (1978) found that congeneric species' spans overlap each other to a significantly greater degree than do those of unrelated species and that, among congeneric pairs of species, the proportion whose spans are "nested" (with the intersection of the spans being identical with the smaller span) significantly exceeds the proportion whose spans are "lapped" (with intersections of the spans forming a proper subset of each span). From these results, Pielou argues that speciation in these algae is likely "quasi-sympatric" -- that is, sister species diverge and become reproductively isolated within a single geographic region. Interestingly, White (1978) suggests that stasipatric speciation has occurred in the marine intertidal isopod species complex _Jaera_ _albifrons_, in which several species are presently sympatric, _occupying_ _different_ _zones_ _on_ _the_ _shore_; presumably, _Jaera_ broad their young, and other examples of stasipatric speciation exhibit low or moderate vagility in accord with the model of White et al. (1967). Given the likelihood that _Nuttallina_ exhibit moderate to high vagility, i.e., have larval dispersal, the stasipatric model of White et al. (1967) and sympatric models are regarded as only possible, though not probable, modes of speciation of _Nuttallina_ LOWs(-BODEGAs).

More information on Nuttallina, particularly concerning the geographic distribution of LOWs, the genetic relationships of Nuttallina from Japan, Chile and the Gulf of California to those of the present study, and the larval development and dispersal of the California Nuttallina, will undoubtedly narrow the spectrum of possible speciation schemes.

REPRODUCTIVE ISOLATING MECHANISMS

Speculation as to the isolating mechanisms that prevent introgression of the three northeast Pacific species of Nuttallina is difficult without more complete information on reproduction in this genus. However, assuming that Nuttallina specimens are generally free spawners as suggested by observations presented earlier in this paper, and in view of the observed sympatry of the species, habitat, ethological and mechanical (i.e., copulatory) isolation of reproductive adults may be eliminated as such isolating mechanisms. The lack of introgression might well result from dissynchronization of spawning, especially as might be brought about by the difference in environmental cues prevailing at the different shore levels occupied by LOWs and HI/DRYs. However, failure of gamete interactions, gametic and zygotic mortality, and young hybrid inviability cannot be ruled out as isolating mechanisms. Although the find of a hybrid between the BODEGA and HI/DRY species indicates that none of the preceding mechanisms is entirely effective for these 2 species, the fact that this hybrid was only one among 467 chitons indicates that one to all of those mechanisms, and not hybrid sterility, are primarily responsible for the lack of introgression among BODGEAs, HI/DRYs and LOWs.

THE SIGNIFICANCE OF ELECTROPHORETICALLY-DETECTED PROTEIN VARIATION

The discovery, in the present study, that closely-related *Nuttallina* species occurring at different levels of the shore (i.e., LOWs low and HI/DRYs high) possess electrophoretically-different forms of Adh, Mdh and Sdh, leads one to wonder whether these different allozymes are also functionally different in such a way that they are better suited to operate under the different conditions prevailing at the different shore positions. In other words, do the electrophoretically-detected differences in Adh, Mdh and Sdh of HI/DRYs and LOWs represent selected or neutral protein variations? In an electrophoretic and kinetic study of Ldh in congeneric species pairs of fish occurring on either side of Panama, Graves (1981) found that electrophoretic variants may or may not exhibit differences in kinetic properties that are supposedly adaptive to the different thermal regimes that the fish experience. Also, kinetic differences may or may not be associated with electrophoretically-detected variation. Graves points out that an amino acid change detected in an enzyme by electrophoresis might occur either in a part of the enzyme that affects its active site, and thus its kinetic properties, or in a part of the enzyme that does not modify its active sites. Therefore, it is not surprising that electrophoretically-detected amino acid substitutions are not necessarily indicative of functional differences in enzymes. Kinetic study of the allozymes expressed by alleles at diagnostic presumptive loci in the closely-related *Nuttallina* species of this study, and of variant allozymes in other studies where important environmental factors (that directly impinge on enzymes as do temperature and pressure) can be identified, will provide more data bearing on the question of how much of the variation found in proteins is "adaptive" and how much is "neutral." The significance of the variant allozymes to the speciation process itself in Nuttallina is impossible to determine. In general, the significance to speciation of allozymes that are found to be adaptively variant in closely-related species would likely vary in different cases. This is a topic that is difficult or, in most cases, impossible to address.

CHAPTER IV

MORPHOLOGICAL DISCRIMINATION OF THREE ELECTROPHORETICALLY-DETECTED NUTTALLINA SPECIES FROM THE PACIFIC COAST OF NORTH AMERICA, WITH THE DESCRIPTION OF A NEW SPECIES

INTRODUCTION

Electrophoresis has proven a powerful taxonomic tool with regard to the chiton genus Nuttallina of the Californias. Whereas previous workers, using traditional morphological criteria, considered the genus to include either one or two species along the northeast Pacific coast, application of electrophoretic methods not only confirmed the presence of two, but revealed the existence of a third species (Chapter III). Past attempts to define species within Nuttallina on the basis of morphological characters alone met with only limited success because of the morphological similarity among the species and the morphological variability within each species. Electrophoresis, by revealing fixed or nonoverlapping differences in the allozymes encoded by several presumptive gene loci within sympatric Nuttallina populations, clearly indicated the presence of three reproductively-isolated evolutionary units or "biological species".

For several practical reasons, it is desirable to be able to distinguish the Nuttallina species morphologically as well as electrophoretically. Nuttallina is a conspicuous and abundant component of the rocky intertidal zone and it is commonly involved in field studies. Electrophoresis is too time-consuming, expensive, and laborious a task to be used routinely in identifying specimens, and it entails the additional drawback of sacrificing, or at least partially mutilating, the specimens. Further, type specimens and other preserved museum specimens cannot be phenotyped electrophoretically. Since traditional taxonomic procedures, such as assignments of available names, are facilitated by an ability to identify type specimens, it is desirable to be able to discriminate new species on the basis of morphological characters. Certainly, theoretically, it is possible for species exhibiting electrophoretic differences to lack external morphological differences. However, discoveries of morphologically similar species (i.e., sibling or cryptic species) by biochemical methods are usually followed by the discovery of differences in morphology that are frequently subtle and/or statistical upon subsequent detailed study (e.g., Shaklee and Tamaru, 1981; Waples, 1981). Species relationships determined from electrophoretic data generally correspond well to relationships determined by morphological and other traditional types of data (Avise, 1976).

In a morphologically similar species complex such as Nuttallina, the chances for discovering morphological differences among the species are greatly enhanced when it is possible to pre-sort the specimens according to a definitive independent criterion such as electrophoretic phenotype. Use of the electrophoretic phenotype to define groups eliminates the circularity that often hampers morphological studies, that is, sorting of specimens to be examined for morphological differences by morphological means. Additionally, confounding, complex geographic and intertidal distributions, which probably hampered earlier systematic studies of Nuttallina, are eliminated from consideration. Further, in species that morphologically-overlap, there can be a tendency to admit less intraspecific variation in particular characters in an attempt to produce a clearcut division between 2 or more species. Electrophoretic "labelling" of specimens more accurately reveals the extent of morphological variation within each species and the actual success rate at which the species can be discriminated by morphological characters.

In the present study, electrophoretically-labelled specimens were examined in detail to reveal the full extent of morphological variation within each species. No single morphological character was perfectly diagnostic, although use of a combination of key morphological characters allows successful identification of virtually any Nuttallina specimen. Classification functions, generated in multivariate analyses involving gross linear measurements, provide a complementary means for discriminating the three Nuttallina species. Results of this intensive morphological examination were used to generate formal descriptions for each species reflecting the morphotype considered to be characteristic or typical of each. Armed with this morphological information, the relevant type specimens, for Nuttallina californica (Nuttall, in Reeve, 1847) and N. fluxa (Carpenter, 1864), were identified and formal names were assigned. The formal assignment of names is presented later in the present chapter. Names used to refer to the Nuttallina species in Chapter III are equivalent to formal species names as follows: BODEGA = Nuttallina californica (Nuttall, in Reeve, 1847); HI/DRY = Nuttallina fluxa (Carpenter, 1864); and LOW = Nuttallina kata sp. nov. To avoid confusion, formal species names are used throughout this chapter.

MATERIALS AND METHODS

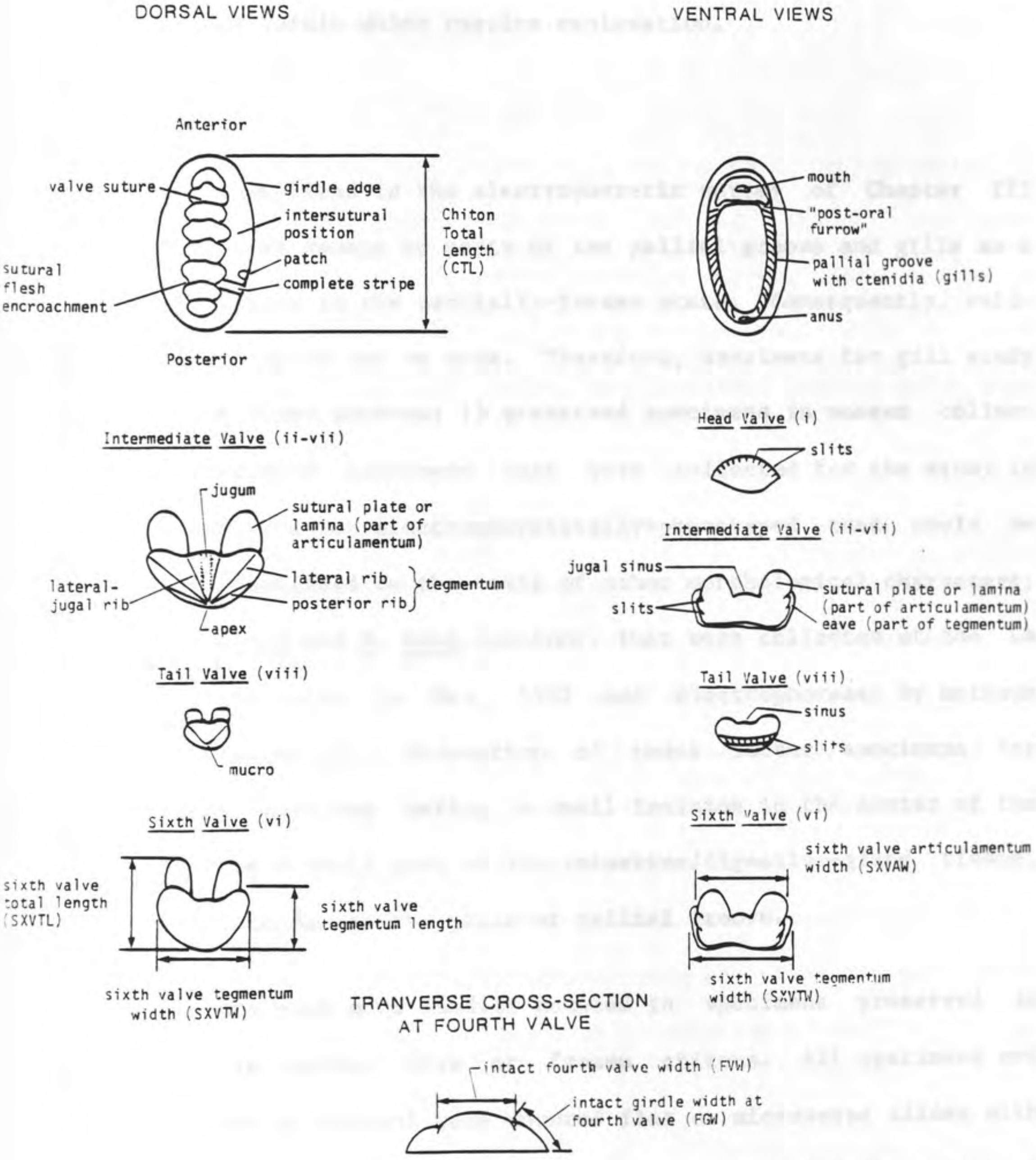
SPECIMENS STUDIED

Specimens examined in this study included chitons from La Jolla, CA, collected and electrophoretically-phenotyped in May, 1982, and many preserved museum specimens, in addition to most of the specimens collected for the electrophoretic study of Chapter III. All relevant type specimens, i.e., the holotype of *Nuttallina fluxa* (Carpenter, 1864), USNM No. 15690b, two "probable syntypes" of *Nuttallina californica* (Nuttall, *in* Reeve, 1847), ANSP No. 118697, seven "possible syntypes" of *Nuttallina californica* (Nuttall, *in* Reeve, 1847), BMNH Reg. No. 19798, and two "possible syntypes" of *Chiton scaber*, Reeve, 1847, BMNH Reg. No. 19799, were also examined. Collection dates and locations of all 633 specimens examined are listed in species descriptions below. Museum abbreviations are as follows: Academy of Natural Sciences, Philadelphia (ANSP); Allan Hancock Foundation, Los Angeles (AHF); British Museum (Natural History), London (BMNH) California Academy of Sciences, Invertebrate Zoology Collection, San Francisco (CASIZ); Los Angeles County Museum of Natural History (LACM); Scripps Institution of Oceanography Benthic Invertebrate Collection (SIO); United States National Museum (USNM).

QUALITATIVE AND MERISTIC CHARACTERS

Traditional morphological terminology for chitons, as illustrated in Smith and Carlton (1975, p. 459) and defined in Arnold (1965), has been used (see Figure IV-1). Examinations of girdle scales and spines, various valve characters including color, sculpture, shape

Figure IV-1. Chiton morphological terminology and measurements.


DORSAL VIEWS
VENTRAL VIEWS
Anterior
valve suture
girdle edge
intersutural position
patch
complete stripe
Sutural flesh encroachment
Chiton Total Length (CTL)
Posterior
mouth
"post-oral furrow"
pallial groove with ctenidia (gills)
anus
Intermediate Valve (ii-vii)
jugum
sutural plate or lamina (part of articulamentum)
lateral-jugal rib
lateral rib
posterior rib
tegmentum
apex
Head Valve (i)
slits
Intermediate Valve (ii-vii)
jugal sinus
slits
sutural plate or lamina (part of articulamentum)
eave (part of tegmentum)
Tail Valve (viii)
mucro
Tail Valve (viii)
sinus
slits
Sixth Valve (vi)
sixth valve total length (SXVTL)
sixth valve tegmentum length
sixth valve tegmentum width (SXVTW)
Sixth Valve (vi)
sixth valve articulamentum width (SXVAW)
sixth valve tegmentum width (SXVTW)
TRANVERSE CROSS-SECTION AT FOURTH VALVE
intact fourth valve width (FVW)
intact girdle width at fourth valve (FGW)

relative size and slits, gills and the radula are included in this section. Methods for the examinations are self-evident except in the case of the gills and radula which require explanation.

Gills

Chitons utilized in the electrophoretic survey of Chapter III generally sustained damage to parts of the pallial groove and gills as a result of dissection in the partially-frozen state. Consequently, reliable gill counts could not be made. Therefore, specimens for gill study were taken from other sources: 1) preserved specimens in museum collections and preserved specimens that were collected for the study in Chapter III but were not electrophoretically-phenotyped that could be unequivocally identified on the basis of other morphological characters; 2) _Nuttallina fluxa_ and _N. kata_ specimens that were collected at the La Jolla, CA study site in May, 1982 and electrophoresed by methods described in Chapter III. Dissection of these latter specimens for electrophoresis involved making a small incision in the center of the foot and removing a small part of the intestine/digestive-gland tissue, taking care not to damage the gills or pallial groove.

Gills are much more readily counted in specimens preserved in ethanol than in either live or frozen chitons. All specimens not already preserved in ethanol were mounted flat on microscope slides with rubber bands and placed in 70% ethanol for at least two days prior to study. Total ethanol-preserved chiton length including mantle was chosen as the standard measure of chiton overall size, although it is clearly subject to error involving variations in flattening and contraction and in the degree of expansion of the mantle edge. Certainly, preservation in ethanol is assumed to have similar effects on total length in all three Nuttallina species.

Alternative overall size measures, such as total volume or weight, are much less easily measured, and in fact are impossible to estimate for partially-dissected specimens, and are subject to error stemming from species-variable erosion of the valves. Measurements of individual valves bear different relations to overall size in each species (RESULTS AND DISCUSSION, Univariate Analyses) and are not a good standard of measure.

The total length of each chiton was measured to the nearest millimeter by pressing the animal firmly against a rigid precision metric rule. A few specimens from museum collections were curled and were too rigid to be flattened completely for measurement; in these cases, best "flat" estimates were made. Checks on these estimates performed by measuring the flat (ventral) surface of completely flat chitons and relating this to the measurement of total curved dorsal surface of the same chitons forced into the curled state and to the measurement of the dorsal surface of the rigid curled chitons suggests that length estimates of curled chitons were not in error by more than 2 mm (~3%) in the largest specimens.

Gill counts, for each specimen on both the left and the right sides, were performed under a dissecting microscope at about 40X magnification by leafing with a dissecting needle or pin. The "newest", most anterior gills, often appearing as small dome-shaped buds, were counted whether or not ctenidial leaflets or axes were differentiated.

Radula

Radulae from two electrophoretically-phenotyped specimens each of Nuttallina californica (from La Jolla and Bodega Head, CA), N. fluxa (La Jolla), and N. kata (La Jolla) were dissected out and placed in 10% KOH for twenty-four hours to clear away any adhering tissue. Radulae were then sputter-coated with gold and examined at low magnification (50 to 500X) on the Cambridge S-4 Scanning Electron Microscope at the Scripps Institution of Oceanography Analytical Facility. A permanent record for detailed study was obtained on black and white polaroid film.

To supplement electron microscope findings, additional radulae obtained from electrophoretically-phenotyped Nuttallina californica (from Bodega Head, 1; Little Harbor, Santa Catalina Island, 1; Punta Banda, Baja CA, Mexico, 2), N. fluxa (Gaviota, CA, 2; Whites Point, Los Angeles, CA, 2; La Jolla, 1) and N. kata (La Jolla, 3; Whites Point, 2; USC Marine Science Center, Santa Catalina Island, 2) were placed in 10% KOH for a day, stained briefly in Rose Bengal to reveal details of the transparent teeth of the radulae, and mounted on microscope slides in either Canada balsam or piccolyte. Radulae were examined under a compound microscope.

QUANTITATIVE CHARACTERS

Only data obtained from specimens electrophoretically-phenotyped in the study of Chapter III (minus San Nicolas Island specimens) were used in univariate and multivariate analyses involving various linear morphological measurements (see Figure IV-1 for terminology).

Univariate Analyses

Measurements of a wide variety of features of the valves, girdle and gross morphology of the thawed specimens were made. Intensive initial examination focused on specimens well within the geographic limits of, and thus presumably morphologically "typical" of, each species, i.e., specimens of Nuttallina fluxa and N. kata from La Jolla, CA and of N. californica from Bodega Head, CA. Characters that seemed particularly promising for differentiating among the three species in initial study were then measured in all of the remaining specimens. Measurements of chiton total length, intact fourth valve width and intact girdle width at the fourth valve were made on thawed specimens prior to electrophoresis. All other measurements were made after electrophoresis. All specimens were assigned numbers which were used throughout the study to avoid biasing those measurements made after electrophoretic species identifications.

Chitons were not bound to flat surfaces and thus curled during freezing. Thawed chiton total length (CTL) was measured to the nearest 0.5 mm by pressing the chiton firmly against a rigid precision metric rule. Intact fourth valve width (FVW) was measured to the nearest 0.1 mm by inserting fine point dividers on both sides of the intact valve at the point of maximum width and reading the divider span on a precision metric rule. Width of the girdle on one side of the fourth valve (fourth girdle width, FGW) was measured to the nearest 0.5 mm by rolling a flexible rule in contact with the girdle from the point of maximum width of the fourth valve directly to the girdle edge on the right side, or on the left if the right side was damaged. All dissected valve measurements were measured directly with vernier calipers to the nearest 0.1 mm. Valve articulamentum width refers to the maximum width of the articulamentum wherever it might occur, on the sutural plates proper or under the overhanging eaves of the tegmentum in the main body of the valve (see Figure IV-1 for terminology).

Ranges, means, standard deviations, statistical comparisons of group means, frequency histograms, and scatter plots of raw characters and characters normalized to chiton total length versus chiton total length labelled according to the geographic location of each specimen were generated for each of the characters for each species by using the Biomedical Computer Programs P-series (BMDP, developed at the Health Sciences Computing Facility, UCLA; revision date: February,1982).

Multivariate Analyses

Stepwise discriminant function analyses, utilizing only data from specimens for which complete character sets were obtained in the univariate study above, were performed using the BMDP7M program (revision date: June, 1981).

Character Interconversions

Although measurements made on dissected valves (e.g., SXVTL, SXVTW, SXVAW; see Figure IV-1) are expected to be the same whether the valves are from fresh, frozen or dried specimens, chiton total length, fourth valve width and fourth girdle width are not, since these measurements are affected by the state of contraction and rigidity of girdle tissue. To provide factors for approximate conversions of characters among the three states, fresh, frozen, and dry, 29 to 38 specimens representing a combination of all three Nuttallina species from Bodega Head, CA and La Jolla, CA were treated as follows. Live chitons were allowed to straighten undisturbed and submerged in fresh seawater and chiton total length (three times), girdle width at the fourth valve, and intact fourth valve width were measured. Chitons were then frozen at -70°C. These specimens were not bound flat to any surface but rather were allowed to curl, just as had chitons collected for electrophoresis (Chapter III). Each chiton was allowed to thaw and again chiton total length, intact fourth valve width and fourth girdle width were measured. Chitons were then bound flat to microscope slides with rubber bands and were oven-dried at 60°C for five days. Chitons treated in this way remained flat, and appeared much like dried Nuttallina type museum specimens. Chiton total length, fourth valve width and fourth girdle width were again measured to give dry values.

All measurements referred to in the RESULTS AND DISCUSSION section are of thawed specimens unless otherwise noted.

RESULTS AND DISCUSSION

QUALITATIVE AND MERISTIC CHARACTERS

Girdle Characters

Scales.

Close-up photographs of the girdles of the three species are presented in Figure IV-2. The girdle epidermis of all three species, *Nuttallina californica*, *N. fluxa*, and *N. kata*, is almost always covered with closely-set short rod-like scales; these scales are approximately 50 to 70 µm long in 25 mm long chitons. Scale color ranges from opaque white to dark brown in all three species, to nearly black in some *N. californica*. Non-white scales are typically light brown in *N. fluxa* and *N. kata* and dark brown in *N. californica*. The lighter shade of brown scales in *N. fluxa* and *N. kata* contributes to a typically overall lighter brown appearance of the girdle in these species than in most *N. californica*. However, in a rare *N. californica* specimen, the dark brown girdle scales are sparsely set in the epidermis, giving a very light brown appearance to the girdle.

Typically, both white and brown scales are present in an individual of any of the species, arranged so as to produce white stripes and patches. However, in *N. californica*, scales are occasionally all light to dark brown or black and, in *N. fluxa* and Channel Island *N. kata*, scales are rarely all rusty to dark brown. In *N. californica*, the area represented by brown and/or black scales is always much greater than that of white scales, whereas in *N. fluxa* and *N. kata*, the area

Figure IV-2 (cont.)

D. Radula, representative portion (cont.)

The radula consists of 17 teeth per transverse row in chitons. Twelve marginal teeth, including the two long spoon-shaped marginals in *Nuttallina*, occur laterally to the two tricuspid (in *Nuttallina*), large, major lateral teeth. Two winged (in *Nuttallina*) primary lateral teeth occur medially to the major laterals and flank the central tooth.

Figure IV-2 (cont.)

C. Position of the most anterior gill.

(Left) N. kata sp. nov. SIO No. M1924. Intertidal rocks at Cabrillo Point, Hopkins Marine Station, Pacific Grove, CA; 29 December 1977. Length 35 mm (in ethanol).

(Center) N. fluxa (Carpenter, 1864). Author's collection. Sandstone outcrop near Seal Rock, La Jolla, CA; 23 November 1983. Length 36 mm (in ethanol).

(Right) N. californica (Nuttall, in Reeve, 1847). SIO No. M1922. Intertidal rocks at Cabrillo Point, Hopkins Marine Station, Pacific Grove, CA; 29 December 1977. Length 36.7 mm (in ethanol).

D. Radula, representative portion.

N. kata sp. nov. Author's collection. Low intertidal sandstone rocks, La Jolla, CA. Length 30 mm (in ethanol).

The radulae of the other two Nuttallina species are similar, if not identical, to this N. kata radula. Radula at 46X magnification on a Cambridge S-4 scanning electron microscope. Radula has been sputter-coated with gold. Untreated tricuspid major lateral teeth are heavily mineralized (black), all other teeth are transparent under light microscope.

Figure IV-2 (cont.)

B. Valves, dorsal view

(Left) N. kata sp. nov. Author's collection. Sandstone outcrop near Seal Rock, La Jolla, CA; 11 July 1982. Length approximately 25 mm (in ethanol). Maximum width of fourth valve 7.5 mm. Rounded to angular valve morph as judged in intact specimen. Sutural encroachment of specimen moderately to well-developed for N. kata (min./max. ratio as low as 0.79). Sutural plate brown spots are present on most of the valves.

(Center) N. fluxa (Carpenter, 1864). Author's collection. Sandstone outcrop near Seal Rock, La Jolla, CA; 11 July 1982. Length approximately 23 mm (specimen contracted, in ethanol). Maximum width of fourth valve 8.7 mm. Valves moderately "rectangular," with fairly long tegmenta for N. fluxa. Sutural encroachment of specimen moderately to well-developed for N. fluxa (min./max. ratio as low as 0.76). Sutural plate brown spots are present on most of the valves.

(Right) N. californica (Nuttall, in Reeve, 1847). AHF 1609-48. Bodega Head, Sonoma County, CA; 6 August 1948. Length approximately 35 mm (rigidly curled, in ethanol). Maximum width of fourth valve 10.4 mm. Angular valve morph with acute divergence angle. Sutural encroachment of specimen moderately to well-developed for N. californica. Sutural plate brown spots are absent.

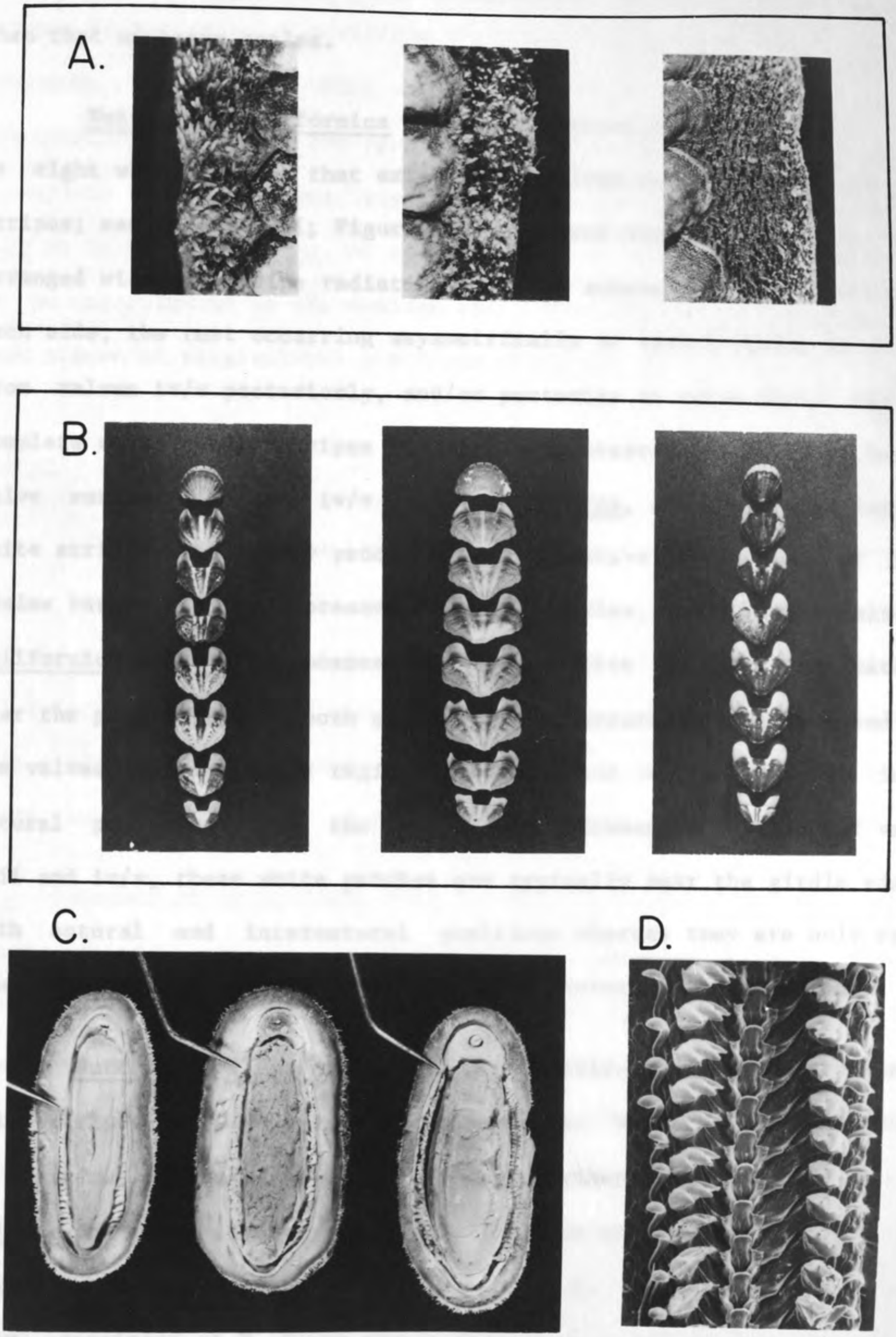
Figure IV-2. Comparative morphology of the Nuttallina species. Anterior is toward the top of the page in all figures.

A. Girdle

(Left) N. kata sp. nov. Author's collection. In low intertidal, densely-populated area with depressions and coralline algae, near Seal Rock, La Jolla, CA; 28 November 1982. Length 25 mm (in ethanol). Left girdle at valves ii-v. Note presence of both white and brown girdle spines; sutural flesh encroachment is well-developed for N. kata.

(Center) N. fluxa (Carpenter, 1864). Author's collection. High intertidal pool at Whites Point, CA; 3 April 1981. Length 32 mm (in ethanol). Right girdle at valves iii-v. Note white girdle spines and minimal sutural flesh encroachment.

(Right) N. californica (Nuttall, in Reeve, 1847). Author's collection. Sandstone outcrop near Seal Rock, La Jolla, CA; 31 March 1981. Length 47.9 mm (live). Right girdle at valves ii-v. Note predominance of brown girdle spines and extensive sutural flesh encroachment.


A.
B.
C.
D.

represented by white scales is occasionally nearly as large or greater than that of brown scales.

Nuttallina californica specimens typically possess zero to seven or eight white stripes that extend from valves to girdle edge (complete stripes; see Figure IV-1; Figure IV-7). These stripes are almost always arranged with one stripe radiating from the suture of valves i and ii at each side, the rest occurring asymmetrically or symmetrically at sutures from valves iv/v posteriorly, and/or posterior to valve viii. Distinct complete white girdle stripes have not been observed in the area between valve sutures i/ii and iv/v in N. californica, although faint complete white stripes, apparently produced by a relative sparseness of brown scales rather than by a presence of white scales, occur here rarely. N. californica quite often possess incomplete white stripes, or patches, near the girdle edge at both sutural and intersutural positions and near the valves in this girdle region at sutural and less commonly at intersutural positions. In the girdle area between the sutures of valves i/ii and iv/v, these white patches are typically near the girdle edge at both sutural and intersutural positions whereas they are only rarely found near the valves at either sutural or intersutural positions.

Nuttallina fluxa and N. kata typically possess many complete white stripes up to a maximum of over twenty. White stripes and patches in these two species are typically less distinct or contrasty than in N. californica due in part to the fact that adjacent areas typically have lighter brown scales than are found in many N. californica. Occasionally, specimens of N. fluxa and N. kata have a complete white stripe at each valve suture, three to five spaced more or less evenly anterior to valve i, and three or four posterior to valve viii, with several white patches at intersutural positions along the girdle edge. However, most specimens have fewer white stripes than this. Complete white stripes not uncommonly occur in the area between valve sutures i/ii and iv/v. Incomplete white stripes are not as commonly seen in N. fluxa and N. kata as in N. californica, or at least are too diffuse and noncontrasty to be conspicuous to the unaided eye. When present, white patches are most common at intersutural positions along the girdle edge.

Spines.

Thick, long girdle spines are interspersed seemingly haphazardly among the smaller girdle scales of specimens of each species. To the unaided eye, the girdle of N. californica typically appears smoother and "fleshier" than that of the other two species (Figures IV-2 and IV-7). This difference in appearance can be attributed to differences in the color, length and density of girdle spines among the species.

Counts of spines were made in small areas about 3.5 mm long and about 60% of the girdle width, approximately centered between valves iv and v and the girdle edge, using a dissecting microscope. Girdle areas were determined using an optical micrometer and varied from about 6 to 10 mm^2 for ten specimens of each species, ranging from about 20 to 38 mm in total length. An attempt was made to represent both extremes of girdle morphotype, i.e., "fuzzy" versus "smooth, fleshy," of each species. Specimens were from several locations. Overall spine densities were found to be quite variable and overlapping in the three species. Spine densities ranged from about 300 to 950 per cm^2. There was no obvious correlation between spine density and total length in the size range of chitons examined.

Spines, like scales, can be white or light to dark brown in all three species, to almost black in some specimens of N. californica. Rarely in N. kata, white spines with light brown annular rings were found. When results of the spine density study described above are divided according to color, differences in spine density among the species are revealed. Whereas N. fluxa and N. kata have white spine densities in the range of 200 to 700 per cm^2, N. californica have densities in the range of zero to 100 per cm^2, with an exceptional N. californica specimen from Punta Banda, Baja CA possessing about 150 per cm^2. Exceptional N. kata, from the Channel Islands, had as few as 50 white spines per cm^2. Nuttallina fluxa generally has lower brown spine densities than N. californica. N. fluxa usually have densities of brown spines in the range of zero to 100 per cm^2, although an exceptional individual from Punta Bunda had about 750 per cm^2! N. kata possessed from 200 to 650 brown spines per cm^2, with an exceptional individual from Avalon, Santa Catalina Island having less than 50 per cm^2. N. californica exhibited brown spine densities from about 400 to 850 per cm^2.

Ratios of numbers of white and brown spines are such that, in N. californica, spines are typically almost all dark brown or black, and white spines are never as numerous as brown ones. In N. fluxa, white spines typically greatly outnumber brown ones, and only very rarely do brown spines outnumber white ones. In N. kata, white spines usually are in excess over brown ones, but brown spines not uncommonly outnumber white spines, especially on the Channel Islands. Interestingly, the girdle of the N. californica/N. fluxa hybrid specimen from La Jolla (Chapter III) has a nearly equal number of brown and white spines, falling between the typical N. californica condition (very few white, many brown ones) and the typical N. fluxa condition (very few brown, many white ones).

Counts of spines involving about a third of the width of the girdle at the dorsal girdle edge generally show an increase in total spine density over densities reported above in all three species. Brown spines remain at about the same densities but white spines typically exhibit a dramatic increase, often more than doubling in N. fluxa and N. kata, and increasing from as few as zero to more than 300 per cm^2 in N. californica. Much of this white spine increase is attributable to a fringe of spines just at the girdle edge. In N. californica, when white spines are present, they are often found in tufts of several at or near the girdle edge.

Spine lengths show variation among the species. Five chiton specimens with the most "fuzzy" girdles were selected for each species. Although N. fluxa and N. kata are fairly homogeneous geographically with respect to fuzziness of the girdle, N. californica is not. N. californica from Punta Banda and the Channel Islands tend to appear more fuzzy than N. californica from Bodega Head, CA or La Jolla, CA; accordingly, N. californica specimens from the two former locations were used in this study. Specimens ranged from 25 to 38 mm in total length. For each specimen, white and brown spines of maximum length were dissected, mounted on microscope slides in piccolyte, and measured using a dissecting microscope equipped with an optical micrometer. White spines from

N. fluxa and *N. kata* fell in the same general size range, from 800 to 1050 µm in maximum length. In *N. californica*, white spines ranged slightly smaller, from 600 to 800 µm long. No obvious correlation between spine maximum length and chiton total length was observed over the chiton length range examined. Brown spines for all three species fell in about the same size range, from 400 to 850 µm in maximum length.

Thus, a combination of features contribute to the "smoother, fleshier" appearance of the typical *N. californica* girdle relative to that of the other two species. White girdle spines of *N. californica* tend to be shorter and thus less conspicuous than in the other two species. *N. californica* typically have many brown and very few white spines, and the shorter length of brown spicules in general contributes to an overall lower profile of girdle spines. Finally, the predominance of brown spines over a predominantly brown scale background tends to camouflage the spines of *N. californica*.

Girdle spine placement seems to further differentiate *N. californica* from the other two species, particularly from those *N. kata* specimens that have almost no white spines. In *N. californica*, white spines are usually found in areas with white scales throughout the girdle width. When present, white spines are also commonly found in tufts of two to five in areas with brown scales within one quarter of the distance from the girdle edge to the valves, but are only occasionally found, and only very sparsely, in areas with non-white scales more medial on the girdle than this, particularly near valves iii to vi. In *N. fluxa* and *N. kata*, white girdle spines often occur both in areas of brown and of white scales throughout the girdle width. In all three species, brown spines occur both in areas of brown and of white scales.

Valve Characters

Color.

Dissected valves from specimens of each of the three species are presented in Figure IV-2. The exposed and uneroded portion of the intact valves of *Nuttallina californica*, the tegmentum, is typically dark brown, occasionally almost black, except where jugal stripes are present (Figures IV-2 and IV-8). The uneroded tegmentum of *N. fluxa* and *N. kata* is typically light brown, occasionally dark brown (Figure IV-7). Occasionally *N. kata* and *N. fluxa* specimens possess valves, usually number v and vi, rarely vi and vii, and occasionally ii and/or iii, that are much lighter in color than the rest. These valves are either solid white or show a mosaic of black on a white background.

Quite often in intact *N. fluxa* and *N. kata*, and less often in *N. californica*, the major part of the area of the exposed tegmentum is eroded. Erosion is most severe at the central posterior edge of each valve, the oldest part of the valve, and not uncommonly involves almost the entire valve surface. Thin cross-sections of eroded valves show them to be riddled with greenish filaments [? the blue green alga *Entophysalis deusta* (cf., Nishi, 1975)], which probably contribute to the valve erosion. Due to the great degree of erosion in *N. fluxa* and *N. kata*, valves of the typical specimen are quite light brown, green and/or white overall. In *N. californica*, erosion is more often restricted to the posterior portion of the valve but occasional specimens exhibit much eroded, gray/white valves.

All three species exhibit from zero to three white stripes in the jugal area of each intermediate valve that radiate from the valve apex (see Figure IV-1). In N. fluxa, there are often three stripes or one central stripe, whereas, in N. californica, there are most often zero or two widely-spaced stripes. Specimens of N. kata show all stripe patterns frequently, with a possible predominance of individuals possessing two closely-spaced stripes. These stripes fall on or very near the three most central ribs on the valves. Judging from the smoothness of these white stripes in some cases, it appears that they are produced in at least some instances by the scraping away of the granular brown tegmentum by the posterior edge of the preceding valve. If the stripes are produced by scraping, the fact that two widely-spaced stripes are often seen in N. californica, and not in the other two species, may be an indication of a different valve geometry or valve fit. All three species show a range of intermediate valve form from low and smoothly rounded to fairly high arched, although all three most often have intermediate valves that are rounded.

The ventral surface of the valves, the articulamentum, varies from almost white through light brown, dark brown, black and/or blue in all three species. Commonly, the central portion of the undersurface is pigmentum brown or nearly black, while peripheral areas including the ventral surface of the sutural plates are white to light blue.

Raymond (1894) reported that specimens of "Nuttallina scabra" (probably both N. fluxa and N. kata of the present study) found just south of Point Conception, CA invariably possessed "a small spot of brown, placed centrally on the [dorsal] light blue surface of the sutural plates" bordering the curving tegmentum on each side, whereas specimens of "Nuttallina californica" (N. calfornica of the present study ?) north of Point Conception did not exhibit the spots, or in some cases had "indistinct clouds of a color darker than the surface of the sutural plates." In the present study, the sutural plates of dissected valves vi and vii from electrophorectically-phenotyped specimens of each species from a variety of locations were examined for the presence of "brown spots" with the following results (see Figure IV-2 for brown spot examples). The fraction of specimens of each species that possessed at least one distinct brown spot on either valve vi or viii was: N. californica, 13/76 (17%); N. fluxa, 60/130 (46%); and N. kata, 44/178 (25%). N. kata on Santa Catalina Island, CA had a conspicuously higher incidence of brown spots than did mainland N. kata: Santa Catalina Island, 36/110 (33%); mainland, 8/68 (12%). Thus, "brown spots" are not a perfect species discriminator as Raymond (1894) had hoped, but in partial accordance with his findings, results here show that the two southern species, particularly N. fluxa, more often have distinct brown spots than the northern species, N. californica.

In all three species, there is a gradation from specimens with no sutural plate brown spots or pigmentation, to those with "indistinct clouds", to those with distinct spots. Brown spots were observed to occur on one valve and not the other in single specimens, and even on one sutural plate and not the other in single valves. In a few valves, two separate brown spots were found on a single sutural plate at different distances from the tegmentum toward the anterior tip of the plate. In addition, thin cross-sections of valves occasionally showed brown layers beneath the tegmentum with the same planar orientation as the brown spots on the sutural plates. Such findings suggest that the sutural plate brown spots may be a periodic (perhaps seasonal?) phenomenon. In addition, the fact that N. kata from Santa Catalina Island exhibited a higher incidence of brown spots than did mainland N. kata specimens is interesting, particularly if such spots are composed of metal porphyrins, since such compounds in molluscan shells could conceivably have a dietary basis (cf., Comfort, 1951). Channel Island basalts are high in iron and manganese compared to mainland sandstone and shale.

Sculpture.

When not eroded, exposed valve surfaces are coarsely granulated in all three species although the tegmentum in the vicinity of the jugum may be fairly smooth and shiny. Valve i, when not eroded, generally exhibits a series of low rounded ribs radiating from the posterior central part of the valve. Intermediate valve sculpture is illustrated in Figures IV-1, IV-2, IV-7 and IV-8. The jugum and lateral ribs are often conspicuous in uneroded valves. The lateral-jugal ribs are usually broad raised areas, only rarely forming distinct ribs, usually when there are pronounced grooves lateral to the jugum and just medial to the lateral ribs. The posterior ribs often are indistinguishable from the posterior edge of the valve and do not form obvious ridges. The mucro of valve viii is usually eroded, and in all three species it is typically far posterior, subterminal to occasionally terminal.

Shape and Relative Size.

In intact specimens, intermediate valves are such that specimens of *Nuttallina californica* typically exhibit much more extensive encroachment of girdle flesh between the valves at sutures (sutural flesh encroachment), than *N. kata*, which typically show a bit more sutural flesh encroachment than *N. fluxa* (see Figures IV-2, IV-7). However, all three species exhibit a range of sutural flesh encroachment from almost none to a considerable amount. A quantitative index of sutural flesh encroachment, the ratio of the minimum straight-line distance from side to side of the intersection of two intermediate valves at a suture and the maximum width of either of the two intact intermediate valves, is as low as 0.5 for *N. californica*, and 0.6 for *N. kata*, and 0.7 for *N. fluxa*. Often *N. fluxa* and *N. kata* have ratios greater than 0.8 (i.e., have almost no sutural flesh encroachment).

Variation in sutural flesh encroachment among the three species reflects differences in the shapes of their intermediate valves (Figure IV-2). In *N. californica*, the posterior rib on either side of the valve apex generally forms a smaller angle with the jugum than does the posterior rib in *N. fluxa*, while specimens of *N. kata* exhibit intermediate angles. The angle formed with the jugum as one side and the straight line from the valve apex to the intersection of the posterior rib with the lateral valve margin as the other side represents approximately one-half of the traditional divergence angle. In *N. californica*, angles of 40 to 50° (80 to 100° divergence) are not uncommon, while in *N. fluxa*

70 to 80° angles (140 to 160° divergence) are not uncommon. Although the posterior rib, or posterior edge of the valve, is usually convex in all three species, cases of extreme sutural flesh encroachment in N. californica occasionally result from a concave posterior rib and/or a fairly abrupt turn of the tegmentum edge toward the midline anterior to the lateral rib. Most specimens of all three species exhibit a broadly-curving convex tegmentum in this area.

Although the intermediate valves of all three species exhibit a wide range of shapes, the typical overall shape of the tegmentum of uneroded intermediate valves tends to be rounded triangular in N. californica and N. kata, and in N. fluxa. from Gaviota. In N. kata, where erosion at the posterior margin of the valves is common, the overall shape of the tegmentum often appears ovoid. In N. fluxa, where the angle formed by the posterior and lateral ribs is typically greater than in the other two species, there is a tendency for the lateral edges of the valves to form straight edges nearly parallel to the jugum, so that the overall shape of the tegmentum appears more rectangular to ovoid than in the other two species. In all three species, the tegmentum of intermediate valves is wider than it is long but it tends to be longer per width in specimens of N. californica and N. kata than in N. fluxa.

Although Pilsbry (1893) indicates that the anterior edge of the tegmentum of intermediate valves of Nuttallina californica is tri-lobed, it is evident that this portion of the valves of all three species is quite variable in shape. Specimens with tri-lobed or single-lobed contours are often observed, and specimens with bi-lobed or straight outlines are not uncommonly seen.

The sutural plates of intermediate valves of all three species are not substantially different in length or degree of lateral extension. Measurements of valves vi and vii of five specimens each of *N. fluxa* and *N. kata* from La Jolla, and five specimens of *N. californica* from Bodega Head, give quite similar ratios of tegmentum length to total valve length (where total valve length = tegmentum length + sutural plate length; Table IV-1). Similarly, the extension of the sutural plates laterally, as reflected in the ratio of maximum articulamentum width to maximum tegmentum width of valves vi and vii, is comparable in all three species. Thus, as seen in Table IV-3, the ranges of this ratio for valves vi and vii, i.e., SXVAW/SXVTW and SVVAW/SVVTW respectively, are substantially the same for all three species, although the sixth valve means are statistically different.

The order of widths of the eight valves within single specimens is not different among the three species. In a survey of dissected valve tegmentum widths involving ten specimens each of *N. fluxa* and *N. kata* from La Jolla and ten specimens of *N. californica* from Bodega Head, the order of valves in decreasing tegmentum width was most often v, vi, iv, (vii, iii), ii, i, viii, although particular valves, especially v and vi, occasionally fell in different orders.

TABLE IV-1. Uneroded intermediate valve tegmentum length vs. total valve length for *Nuttallina californica* from Bodega Bay, CA and *N. fluxa* and *N. kata* from La Jolla, CA. Total valve length = tegmentum length + sutural plate length.

Species	N	Valve No.	Tegmentum Length/Total Valve Length	
			Mean ± standard deviation	Range
N. californica	5	6	0.75 ± 0.06	0.68 - 0.84
	5	7	0.75 ± 0.04	0.69 - 0.85
N. fluxa	5	6	0.77 ± 0.05	0.70 - 0.82
	5	7	0.70 ± 0.03	0.65 - 0.72
N. kata	5	6	0.71 ± 0.04	0.67 - 0.78
	5	7	0.72 ± 0.08	0.60 - 0.80

Slits.

Results of a survey of numbers of valve slits in electrophoretically-phenotyped specimens of each species taken haphazardly from several different geographic locations are presented in Figure IV-3. Intermediate valve slits, not presented in Figure IV-3, number one or two in all three species; the posterior of the two slits is occasionally present only as a punctulate slit-ray and tends to obsolescence. The number of valve i slits varies in all three species and the mode is either 10 or 11 (Figure IV-3). The number of valve viii slits also varies and tends to higher numbers in N. fluxa (mode=10) than in N. californica (mode=8) or N. kata (mode=7). No conspicuous trends in number of either valve i or valve viii slits with geographic location were evident.

The mean total lengths of specimens used in this slit survey are slightly different among species, although the ranges of the lengths are largely overlapping (Figure IV-3). This size difference tends to mask rather than accentuate differences in valve slit number trends among the species. Product-moment correlations for number of valve slits in either valve i or valve viii versus thawed total chiton length were non-significant for N. fluxa and N. kata [at $p=0.05$, two-tailed]. Product-moment and Spearman's rank correlations were both significant for N. californica, with a positive correlation of number of slits with chiton length for valve viii and a negative correlation for valve i [valve viii: $r=0.49$, $r_s=0.45$; valve i: $r=-0.54$, $r_s=-0.58$; all significant at $p=0.05$, two-tailed]. Since the average length of N. californica in this slit survey was slightly greater than for the other two species, these

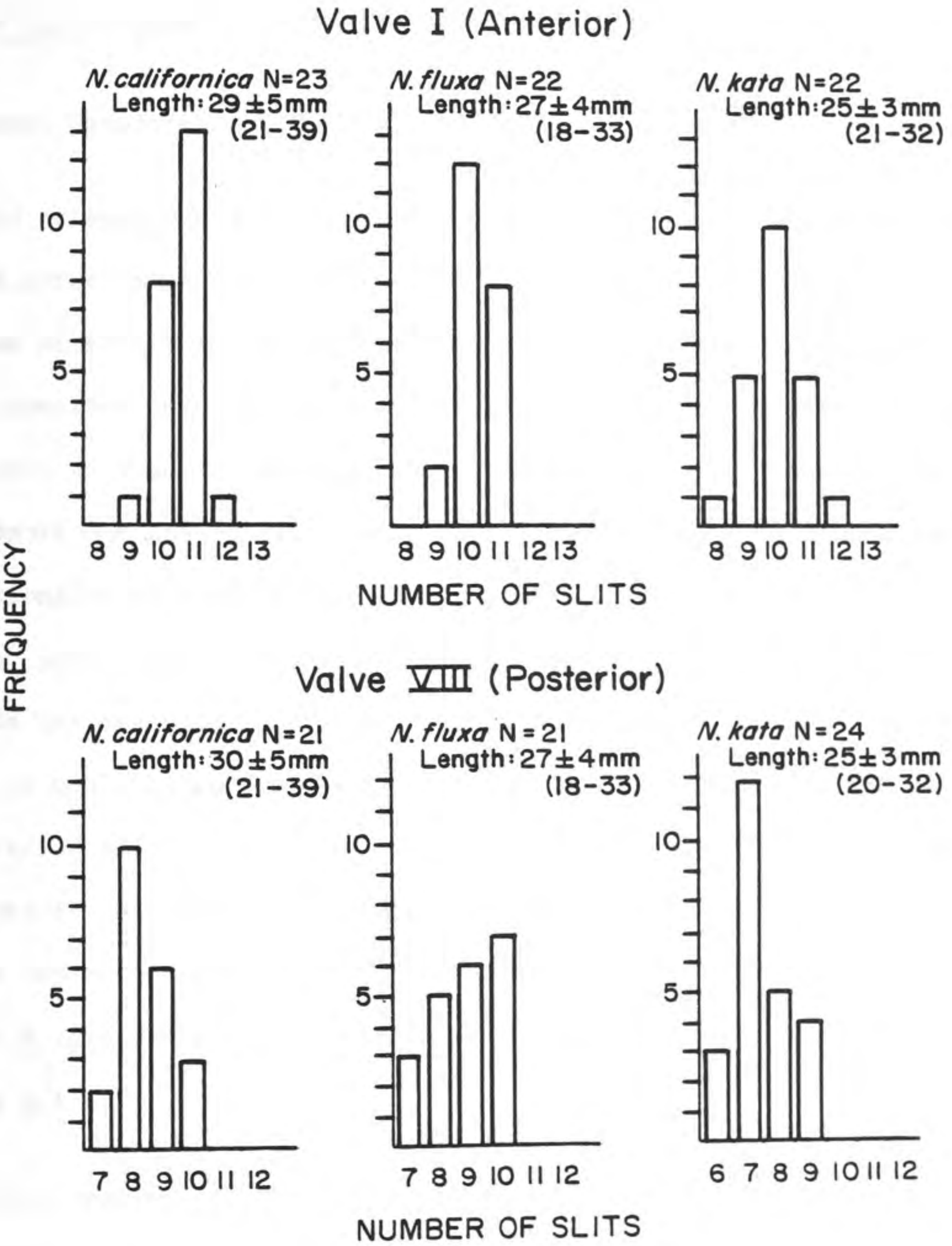
Figure IV-3. Valve slits in Nuttallina species from various geographic locations.

Numbers of each Nuttallina sp. from each geographic location were as follows:

N. californica: Bodega Head, CA, N=17; Punta Banda, Baja California, N=5; Little Harbor, Catalina Island, N=1; La Jolla, CA, N=1.

N. fluxa: La Jolla, CA, N=13; Whites Point, L.A., CA, N=3; Gaviota, CA, N=7.

N. kata: La Jolla, CA, N=12; Whites Point, CA, N=5; USC Marine Science Center, Catalina Island, N=4; Little Harbor, Catalina Island, N=2; Punta Banda, Baja California, N=1.


Valve I (Anterior)
N. californica N=23
Length: 29 ± 5mm
(21-39)
N. fluxa N=22
Length: 27 ± 4mm
(18-33)
N. kata N=22
Length: 25 ± 3mm
(21-32)
10
5
8 9 10 11 12 13
NUMBER OF SLITS
FREQUENCY
Valve VIII (Posterior)
N. californica N=21
Length: 30 ± 5mm
(21-39)
N. fluxa N=21
Length: 27 ± 4mm
(18-33)
N. kata N=24
Length: 25 ± 3mm
(20-32)
7 8 9 10 11 12
6 7 8 9 10 11 12
NUMBER OF SLITS

significant correlations are expected to have a slight effect toward masking any tendency of specimens of N. californica to have more valve i slits than in the other two species, and to have fewer valve viii slits than N. fluxa (Figure IV-3).

Renal Pores, Gonopores and Gills (Ctenidia)

An attempt was made to note the position of the renal pore and the more anterior gonopore relative to the gills of each species. Both pores were difficult to locate, particularly in specimens preserved in ethanol possibly as a result of constriction; the pores were not quite as difficult to find in live specimens anesthetized in isotonic $MgCl_2$. The gonopore was the easier pore to find because it was slightly larger and occasionally pigmented light brown rather than translucent white, but both pores were very small bumps just medial to the line of gill bases. On the basis of findings in only a few specimens for each species, it would appear that the renal pore occurs just anterior to the most posterior gill, in all three species. In N. californica, the gonopore appears to occur just anterior to the fifth most posterior gill (i.e., is separated by four gills from the renal pore), while, in N. fluxa and N. kata, the gonopore occurs between the fourth and fifth most posterior gills.

In a specimen of Nuttallina fluxa collected from the high intertidal zone at La Jolla, CA on 28 November 1982 and anesthetized in $MgCl_2$, a small green packet of eggs was observed to pass through the presumptive gonopore during manipulation, thereby lending support for the identity of the pore. The function of the more posterior "renal pore" has not been similarly confirmed, but it follows from anatomical work on other chitons (e.g., see Hyman, 1967).

Gill counts in the left versus the right sides of specimens reveal a high incidence of asymmetry in all three species (approximately 80% of specimens; Table IV-2). Most often the asymmetry involves a difference of only one or two gills between sides.

Gill counts, averaged for the left and right sides and plotted versus chiton total length, are presented in Figure IV-4. At any specified preserved length, N. californica has more gills than N. fluxa, which in turn possesses more gills than N. kata. The difference in gill numbers among the species is substantial. For example, in 25 mm long specimens, gill numbers average approximately 45 per side in N. californica, 34 in N. fluxa and 27 in N. kata.

This difference in number of gills is reflected in the positions of the most anterior and most posterior gills in each species (see Figure IV-2 for an interspecific comparison of anterior gill positions). The most anterior gill of N. californica, for all sizes examined, occurs just posterior to the intersection of the postoral furrow and the pallial groove, beneath the ii/iii valve suture or beneath valve ii. In N. kata, the most anterior gill is found much more posteriorly, typically about two-thirds to three-quarters of the distance from the anus to the postoral-furrow/pallial-groove intersection and beneath the iii/iv valve suture. The anterior gill of N. fluxa tends to occur at a position nearer that in N. californica than in N. kata, beneath valves ii or iii. In all three species, the gills are abanal; that is, there is a space

TABLE IV-2. *Nuttallina* gill count assymetry, left vs. right side

Species	N	Percent Asymmetric	Assymetry: Difference in gill number between left and right sides			
			Mean	Median	Mode	Maximum
N. californica	25	80	1.9	1.6	1,2	7
N. fluxa	30	80	1.5	1.3	1	6
N. kata	24	78	1.0	1.0	1	3

NOTE

Product-moment correlations of gill number difference (left - right side) vs. preserved total chiton length were nonsignificant for all three species (at $p = 0.05$).

Figure IV-4 (cont.)

N. fluxa: Gaviota, CA, hi/dry area, N=2; Gaviota, CA, low intertidal, N=4; Whites Point, L.A., CA, high intertidal pool, N=4; Whites Point, hi/dry area, N=2; La Jolla, CA, hi/dry area and high intertidal pools, N=9*; La Jolla, CA, low/mid and low intertidal, N=6*; Laguna Manuela Point, Baja CA (SIO M979), ?, N=3.

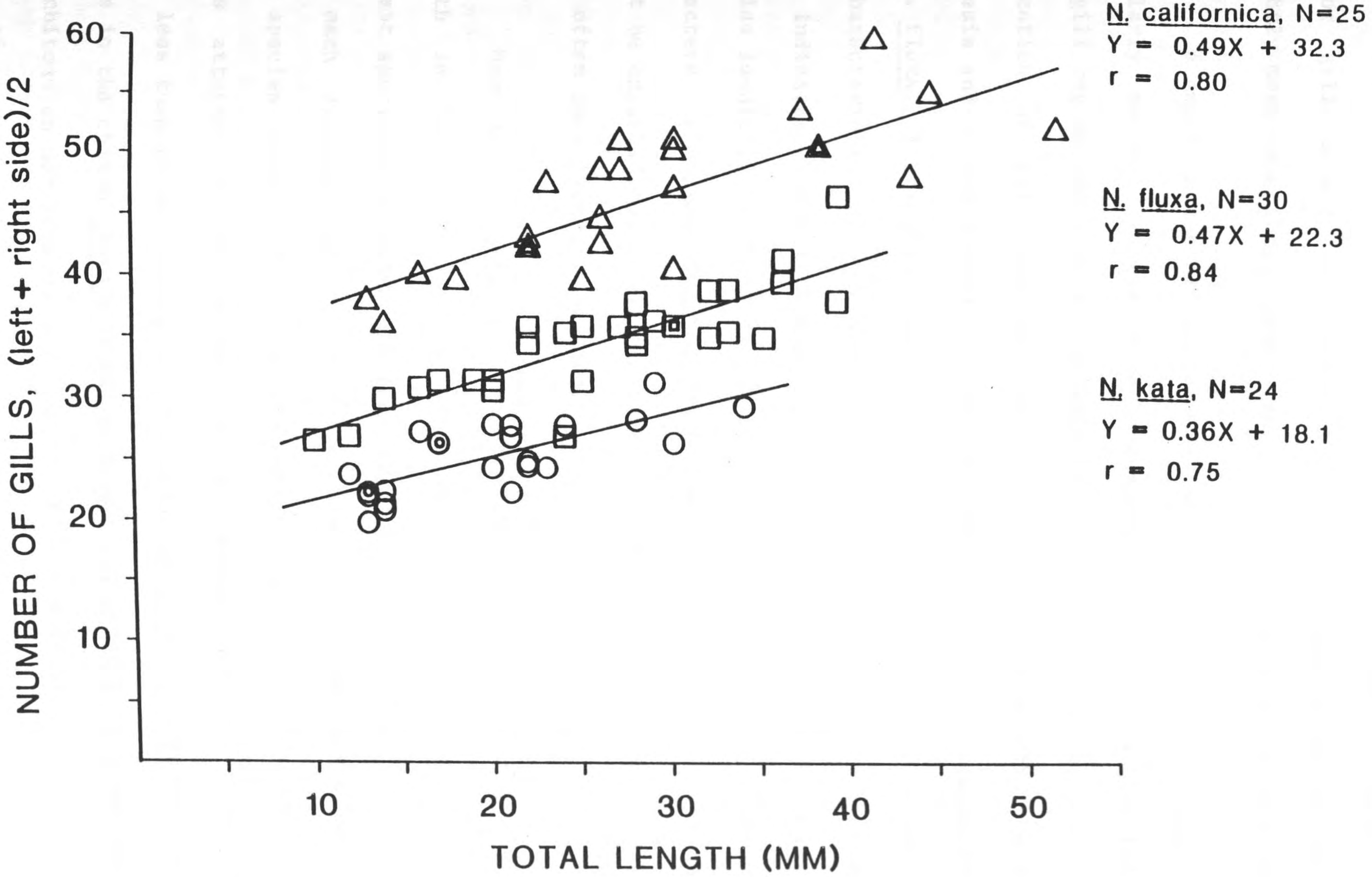
N. kata: USC Marine Science Center, Catalina Island, mid-intertidal, N=4; USC Marine Science Center, low intertidal, N=3; Avalon, Catalina Island, hi/dry area, N=1; Avalon, low/mid intertidal, N=1; Whites Point, CA, low intertidal, N=1; La Jolla, CA, high intertidal pools, N=6*; La Jolla, CA, low intertidal, N=8*.

Figure IV-4. Number of gills as a function of ethanol-preserved chiton total length in Nuttallina.

The newest, most anterior gills, often expressed as small undifferentiated dome-shaped buds, are included. Lengths refer to flat chitons; if a chiton was rigidly curled, its length was estimated. The number of gills as a function of length shows no conspicuous trend with height on shore, geographic location, or type of treatment (i.e., museum collection, non-electrophoresed-preserved, electrophoresed-preserved) in any species, as indicated by scatter plots for each factor.

Specimens by geographic location and habitat are as follows. Habitats are defined in Chapter III. Specimens for which no museum number is given are in the author's collection. Asterisks denote electrophoresed specimens.

N. californica: Bodega Head, CA, mid-intertidal, N=3; Bodega Head, CA (AHF 1609-48), ?, N=5; Bolinas, CA (SIO M1741), ?, N=1; Pescadero Pt., San Mateo Co., CA (SIO M1742), ?, N=1; Hopkins Marine Station, Pacific Grove, CA (SIO M1922), ?, N=2; Shell Beach, San Luis Obispo Co., CA (LACM 61-11), ?, N=2; San Miguel Island, CA (LACM 67-38), ?, N=1; La Jolla, CA, low-mid intertidal, N=1*; Punta Banda (so. side), Baja CA, hi/dry area, N=2; Punta Banda (so. side), Baja CA, low intertidal, N=6; E. San Benito Island, Baja CA (AHF 1946-50), ?, N=1.


N. californica, N=25
Y = 0.49X + 32.3
r = 0.80
N. fluxa, N=30
Y = 0.47X + 22.3
r = 0.84
N. kata, N=24
Y = 0.36X + 18.1
r = 0.75
NUMBER OF GILLS, (left + right side)/2
60
50
40
30
20
10
10
20
30
40
50
TOTAL LENGTH (MM)

between the anus and the most posterior gill. The base of the most posterior gill occurs under valve vii in N. californica and N. kata, and slightly more anteriorly, under the vi/vii valve suture, in N. fluxa.

A small amount of species overlap in gill numbers is seen. Particularly notable is the 24 mm long specimen of N. fluxa that falls on the gill regression line for N. kata (Figure IV-4). The species identification of this specimen was firmly established both by electrophoresis and by its extremely wide, rectangular valves, characteristic of N. fluxa. Although its most anterior gill falls beneath valve iii as is characteristic for specimens of N. fluxa, finding of such an individual indicates that gill number is not an infallible guide to Nuttallina species identification and that corroboration from other morphological characters is necessary for unequivocal identifications. Gill number might be expected to be uncharacteristically small, as in this instance, more often than large, as a result of defective development.

Mean gill numbers are significantly correlated (at p=0.05) with length in all three species, increasing with increasing length. The longest specimens examined are near the maximum preserved sizes known for each species and the difference in the maximum size examined for each species (Figure IV-4) probably reflects a real difference in the sizes attained by the species. No gill counts were made in any specimens less than 10 mm in length. The study of Hunter and Brown (1965) on gills in the chiton Chaetopleura apiculata indicates that young postlarval chitons do not possess any gills, but that within about the first year of growth (of an estimated three to six year life span), the chitons develop 17 to 20 of their maximum 25 gills per side. In contrast, Snyder and Crozier (1922) suggest that larval (?; ~0 mm fourth valve width) specimens of *Chiton tuberculatus* already possess 64 to 75 of the maximum observed 108 total gills. Clearly, if the pattern of initial rapid increase in gill number seen in *Chaetopleura apiculata* holds for the three *Nuttallina* species, a considerable amount of species overlap in gill numbers might be expected among very young chitons and gill counts would be much less useful for species identifications in specimens less than 10 mm long than for the longer ones examined in this study. On the other hand, if the pattern of gill increase is as suggested in *Chiton tuberculatus*, that is with a relatively steady increase in number through the life span, gill counts might be as useful in identifying small *Nuttallina* as big ones.

Gill number appears to be the best single morphological character for discriminating between the three *Nuttallina* species of this study. A review of available information concerning the nature of gill number variation and its significance for taxonomy of chitons seems appropriate, particularly in view of the fact that gill numbers have not been counted routinely or used in previous descriptions of chiton species. Certainly, the paucity of reports of chiton gill numbers in the literature may relate to the tedium involved in the counting process. Live specimens tend to protect their gills, making them difficult to count without sacrificing the animals in preservatives. In addition, workers occasionally work on dry museum material in which gill counts are nearly impossible. Available information concerning gill numbers in chitons comes primarily from three detailed examinations of a variety of species from several families (Snyder and Crozier, 1922; Hunter and

Brown, 1965; Johnson, 1969). In general, there is considerable variation in the number of gills in any one chiton species, much of which can be explained by the effect of size. The number of gills usually increases with length or weight in all species examined in detail, reaching a maximal number before the maximum size is attained in some species and not doing so in other species. Incidences of asymmetry in the number of gills on the left versus the right side have been found in populations of all but one (*Lepidopleurus asellus*, sample size = 6) of the more than 15 species examined, involving from 19.5 to 69% of the specimens of the species examined in detail, *Chaetopleura apiculata*, *Katharina tunicata*, *Lepidochitona cinereus*, and *Chiton tuberculatus*. Since the gill number asymmetries do not favor either the left or the right side of any species but appear to occur randomly, it appears that there is some independence of gill development on the opposite sides of a chiton.

The increase in gill number with size and growth, and the independence of gill additions on the two sides of a single growing chiton suggest possible plasticity in gill numbers and it is thus of interest to explore whether any of the environmental factors that affect the growth process might also substantially affect gill development. No evidence exists to suggest that environmental factors substantially affect gill development. Snyder and Crozier (1922) found no obvious difference between the mean gill frequencies of several size classes of populations of *Chiton tuberculatus* taken from an exposed south shore Bermudan beach and from an enclosed sound. More to the point, in the present study, specimens of each species were taken from high and low on the shore, high intertidal pools, different geographic locations and widely-different latitudes. Presumably such sampling would reveal a substantial part of the variability in gill number that could be expressed under varying environmental conditions, given the genetic constraints of the particular species involved. Environmental effects accounted for by such sampling might include different air and water temperatures, substrates and possibly food sources, emergence/submergence and oxygen regimes, solar radiation and possibly different associated microflora and parasites. It might be argued that the occurrence of *N. californica* in coldwater or higher latitudes may be responsible for their higher gill numbers compared with the two more southern, warmwater species and that if specimens of *N. californica* were transplated to the warmer, more southern latitudes they might exhibit the lower gill counts characteristic of the southern species. In fact, the "transplant experiment" has been done; for example, specimens of *N. californica* from Punta Banda, Baja CA (coldwater, lower latitude) and a specimen from La Jolla (warmwater, intermediate latitude) exhibit the same high gill counts as do specimens of *N. californica* from Bodega Head, CA (coldwater, high latitude). In fact, examination of scatter plots with specimens labelled as to position on the shore, habitat and geographic location revealed that none of the variables had an obvious, consistent effect with regard to gill number, points for each occurring haphazardly through the plots, above, at and below the regression line of gill number on preserved chiton total length for each species. Thus, although environmental factors may be responsible for some of observed variability in gill numbers via effects on gill development within each of the *Nuttallina* species, development of gills in each of the species is apparently sufficiently restrained genetically that gill numbers serve as a useful character in separating the three species.

Why have the different Nuttallina species evolved to have different gill numbers? With the discovery of the living primitive monoplacophoran, Neopilina, in 1952, there was a revival of the old theory of the origin of the molluscs, in which the multiplicity of organ systems, including the gills, in Neopilina and the chitons were considered to be primitive metameric characters indicating a segmental origin for the phylum (see Pelseneer, 1899, 1906; Naef, 1926; Lemche and Wingstrand, 1959; Fretter and Graham, 1962). However, in the chitons, the multiplied organ systems, the gills, the valves, the pedal musculature, auriculoventricular openings and a ladder-like nervous system of irregular transverse connectives, are all of different numbers and are apparently arranged independently of each other, and in particular the gills are added irregularly and independently on the two sides with growth. In view of this arrangement, the alternative hypothesis for the origin of multiple gills in chitons proposed by Yonge (1939, 1947) seems more reasonable. Yonge proposed that the multiplication of gills in the chitons, over the single pair of gills found in the "primitive mollusc," was a functional result of the elongation of the chiton body and the anterior extension of the primitively posterior mantle cavity into a pair of narrow elongated pallial grooves. If we continue along this line of reasoning, chitons with similar respiratory needs with narrower pallial grooves would be expected to have more and smaller gills than ones with more capacious pallial grooves. Pallial groove shapes and gill size have not been studied in the three Nuttallina species but it is interesting that gill numbers show a positive correlation with maximal adult length in this series of species; thus, the species with presumably the longest pallial groove, N. californica, has the most gills. Strict compliance with Yonge's hypothesis would require that N. kata, with the fewest gills and the shortest maximal length (thus the shortest pallial groove), possesses the widest pallial groove and also the largest gills.

In a study of six species of chitons, Johnson (1969) found a similar relationship between gill number and maximum adult length as was found in Nuttallina. Chiton species that reached the greatest adult size possessed more gills and relatively longer gill series at any body length than did smaller species. Crude estimates by Johnson (1969) suggested that the increased gill number of larger species was accompanied by no relative change in respiratory surface area since smaller species tended to have relatively longer gills than did larger species. If these findings also stem from a narrower pallial groove in the larger species, they would appear to support Yonge's hypothesis on chiton gill multiplication.

Clearly, the observed differences in number of gills of the three Nuttallina species might alternatively be expected to arise due to different respiratory requirements of the animals. Specimens of N. californica, with their notably robust girdles, appear to have a higher tissue(i.e., respiratory demand)/total length ratio than the two southern species, likewise have more tissue per length than N. kata, which could explain the differences in gill numbers of the three species, if surface area per gill remains unchanged.

During periods of submergence, intertidal waters are probably saturated with oxygen, and the oxygen supply is probably not limiting whether the animal occurs in warm waters (N. fluxa and N. kata) or cold waters (N. californica), barring events such as red tides. Water does not stagnate to allow local depletions in oxygen. However, respiratory requirements associated with periods of tidal emergence may be the primary consideration. Robbins (1975) has shown that Nuttallina continues to respire while exposed. Its aerial respiratory rate was found to be about three-quarters of its submerged rate at 13.5°C and apparently no oxygen debt was incurred during exposure. It is possible that the increased number of gills of the higher intertidal species, N. californica and N. fluxa, over that of the low intertidal N. kata represents an "attempt" in the two former species to achieve reasonable rates of metabolically more efficient aerobic respiration during prolonged exposure periods with gills that are primarily adapted for aquatic respiration, rather than to drastically reduce their metabolic rate or to switch to less efficient anaerobic metabolism.

Certainly, if desiccation rather than respiratory requirements is the important driving factor, an increased number of gills would not be expected in the higher intertidal species, if such an increase also represented an increase in the evaporative surface area relative to the available water volume of an animal. However, it is possible that increased numbers of gills, distributed along the entire length of the pallial groove of the higher intertidal Nuttallina fluxa and N. californica, serve to trap more water there during periods of aerial exposure (according to a volume or cube function), while only increasing the evaporative surface area slightly (according to a surface area or square function, or even a linear function if evaporation is limited by the length of the clamped girdle edge); in this way, the increased gill number might represent an adaptation to desiccation. In fact, water content studies of N. fluxa and N. kata in La Jolla, involving uniform blotting of the dorsal and ventral surfaces of the chitons, have shown that the higher shore species N. fluxa does have the higher water content (Appendix II). In any case, specific conclusions concerning gill numbers in relation to respiratory requirements or desiccation are unwarranted without information about the actual respiratory surface area represented by the gills of each species, about the relationship of total body length to live biomass, and about the pallial groove dimensions and evaporative surface areas of each Nuttallina species.

Radula

In all three Nuttallina species, the radula is approximately 35 to 40% as long as the total length of the preserved chiton. Transverse rows of dentition with well-mineralized (i.e., black, rather than red, yellow or transparent) tricuspid major lateral teeth number about 35 to 42.

Some variation was observed in the shape of the first lateral teeth and of the last marginal teeth, but the variation was found even within species and did not segregate according to species. The first lateral teeth varied from quite pointed to fairly blunt and rounded distally. This variation apparently was not due to variable tooth wear since the rounded condition was found in the newer parts of the radula as well as in the older parts. The outermost marginal teeth varied from quite narrow and separate at the edge to quite broad and fused at the edge. In view of the similarity of the radulae of the different species of Nuttallina, only one radula, from a specimen of N. kata, is presented in Figure IV-2.

Apparent differences in the food source, the algal composition, in the different habitats occupied by the Nuttallina species might have been expected to have resulted in differences in the structure of the feeding apparatus, the radula, of these chitons. In particular, the higher intertidal zone commonly inhabited by N. fluxa is covered primarily by thin films of green and blue-green algae, diatoms and patches of encrusting coralline algae. Chiton feeding in such an area necessarily involves vigorous rasping on the hard substrate and one might expect to find a radula with robust hardened teeth for scraping and teeth efficient in ingesting small particles in the chitons here. On the other hand, the low intertidal zone, commonly inhabited by N. kata, has not only algal films and crusts, but a predominance of various bushy, foliose algal forms. Chiton feeding here might involve not only scraping, but also tearing large pieces of algae. Thus, the radula of N. kata might be expected to have teeth efficient in cutting large algal pieces and long teeth for collecting and sweeping in large pieces of algae.

Studies of diet involving all three Nuttallina species have been performed. The study of Kues (1969) on "Nuttallina fluxa" in La Jolla, CA involved primarily N. fluxa ("high intertidal zone") and N. kata ("low intertidal") of the present study, and that of Vesco (1980), also on "Nuttallina fluxa", at Whites Point, CA also involved N. fluxa ("barnacle community") and N. kata ("coralline algal community"). The study of Nishi (1975) on "Nuttallina californica" in the Monterey, CA area probably involved primarily N. californica ("rocky crevices") and N. kata ("Dodecaceria beds"), as judged from her "natural history notes" and from the species composition of a Nuttallina collection from the Monterey area examined and recorded in Table III-8. The wide variety of items, plant, animal and inorganic, found in the guts of the Nuttallina species in rough proportion to their occurrence in the habitat in these studies suggest that Nuttallina acts as a fairly generalized substrate-clearer as it feeds. However, Nishi (1975) suggests that Nuttallina in her study shows a preference for bushy over encrusting coralline algae, and Kues (1969) indicates that N. fluxa prefers algal matter over barnacles in his study. In addition, these studies clearly indicate that all three species can and do ingest encrusting or film-like algae as well as bushier, large foliose algae. That all three Nuttallina species, like other chiton species (Steneck and Watling, 1982), vigorously scrape the substrate while feeding is indicated by the high gut content of sand grains from the sandstone substrate (up to 90%) in N. fluxa and N. kata (Kues, 1969; but only 1 to 2% rock in Vesco's study where substrate was harder shale). Vigorous scraping is also indicated by the presence of crustose corallines in the guts of all three species (Kues, 1969; Nishi, 1975; Vesco, 1980). All three species are capable of harvesting and ingesting large pieces of foliose algae, up to 20% of chiton length, as indicated particularly by the studies of Nishi (1975) and Vesco (1980). Thus, results of diet studies indicate that all three Nuttallina species are capable of feeding on both encrusting algae and films and on bushy, foliose forms, but that they harvest these items in approximate proportion to their frequency of occurrence in the habitat.

In view of the apparent differences in frequency of occurrence of different algal functional types in the different habitats of the Nuttallina species and the implied differences in their feeding habits, why aren't differences in the structure of their radulae found? Indeed, the radula was used by Thiele (1929) as the basis of his system of chiton classification and Bullock (1972) noted the usefulness of the radula for distinguishing species groups and convergent species in the Chitoninae. However, the structure of the chiton radula is generally fairly conservative, particularly within genera (e.g., see Yakovleva, 1965, teeth of Mopalia and Tonicella chiton species). The conservative nature of the radular structure may be a consequence of the fact that the radula serves multiple functions as a "multi-purposed tool" (Steneck and Watling, 1982), useful in feeding by scraping, sweeping and probably tearing or cutting at food sources. The chiton radula incorporates features to be found in the diverse gastropod mollusc groups that feed on various functional algal groups in a variety of ways. Thus, the two major lateral teeth, commonly with three cusps, are hardened and heavily mineralized like the teeth of docoglossan gastropod grazers (limpets), and probably function to excavate the substrate as is done by the docoglossans (see Figure IV-2). The robust buccal muscles of chitons aid in excavation (Graham, 1973). Graham (1973) indicates that chiton radulae operate like those of taenioglossan gastropods, the teeth converging toward the center during grazing. This convergence, involving the large lateral teeth, may give chitons the ability to cut and ingest larger pieces of algae. Finally, the long spoon-shaped marginal teeth, like the long and/or brushy marginal teeth of rhipidoglossan and taenioglossan gastropods, can serve to sweep in loose particles.

Apparently, the versatility of the radula of Nuttallina has provided for sufficient efficiency in dealing with the different food sources encountered by each species that substantial modification of the radular structure has not occurred. The variety of items encountered by each species may be sufficient "pressure" for maintaining a "generalized" radula that can handle many different food items.

QUANTITATIVE CHARACTERS

Univariate Analyses

Using the reasoning that animals from well within each species range would be most likely to exhibit morphology "typical" of that species, initial study was focused on specimens of Nuttallina californica from Bodega Head, CA and specimens of N. fluxa and N. kata from La Jolla, CA. Sample sizes varied from five to fifteen specimens per species for different characters. The intent of this preliminary study was to screen a considerable number of characters for their usefulness in separating the species. Then, a limited set of characters that were found to be most useful in separating the species in this study were measured in all of the specimens electrophoretically phenotyped in Chapter III except those from San Nicolas Island.

Valve erosion, which is variable among the three species, presented a problem in the study of valve measurements. Since valve erosion involves an obvious environmental component, it was decided to use data only for those dimensions virtually unaffected by erosion in a valve. Although a majority of Nuttallina valves are eroded in nature, it was hoped that by focussing on data for uneroded valve characters that the inherent (? genetic) morphology, unmodified by environmentally-caused erosion, would be revealed. Valve erosion never affects the widths of valves, just the lengths by its action on the posterior margin and apex of valves. The criteria used to judge a valve as uneroded were that the valve apex was covered with shiny tegmentum and that rib sculpture, mainly laterals, came to completion at the valve apex.

In the limited preliminary study, the elevation, tegmentum width, articulamentum width, tegmentum length and total valve length were measured for each of the eight valves of each chiton (see Figure IV-1 for terminology). Valve elevation/valve width was quite variable and overlapping among the species. However, tegmentum width or articulamentum width versus total valve length provided a fair degree of separation of species for most of the valves. Since valves vii and vi seemed to separate the species best for these ratios, these two valves were chosen for measure in further specimens. For valves vi and vii, tegmentum or articulamentum width versus tegmentum length overlapped more among the species than did tegmentum or articulamentum width versus total valve length, despite the fact that the tegmentum length/total valve length ratio was largely overlapping among the three species. Thus, total valve length rather than tegmentum length was used in the main study.

A search was made for characters in intact chitons that would serve to separate the species. The fourth valve width, as measured with fine point dividers at the maximum width of the tegmentum of the intact valve, normalized to thawed chiton total length, was found to separate the species, particularly N. fluxa and N. kata, better than did widths of other valves. In addition, the width of the girdle on one side, from the measurement location for the intact fourth valve width to the girdle edge, was found to separate N. californica from the other two species fairly well, and better than did the girdle width at the sixth valve.

Thus, as a result of preliminary study, nine characters were chosen for measurement in the full set of electrophoretically-phenotyped Nuttallina specimens. The undissected or "intact" characters, measured as described in MATERIALS AND METHODS, are: 1) thawed chiton total length including mantle (CTL); 2) intact fourth valve width (FVW); 3) girdle width on one side of intact fourth valve (FGW). The dissected uneroded valve characters measured are: 4) sixth valve total length (SXVTL); 5) sixth valve maximum tegmentum width (SXVTW); 6) sixth valve maximum articulamentum width (SXVAW); 7) seventh valve total length (SVVTL); 8) seventh valve maximum tegmentum width (SVVTW); and 9) seventh valve maximum articulamentum width (SVVAW). These characters, normalized to thawed chiton total length (CTL), and appropriate ratios thereof, are presented in Table IV-3. It should be noted that, although the maximum number of specimens for which any one character was measurable was 330, most characters were measureable for fewer cases than this, most often because of valve posterior margin erosion, sutural plate breakage, tegmentum breakage and girdle damage.

TABLE IV-3. Morphometric Data, Expressed as Percentage of Chiton Total Length, and Meaningful Ratios Thereof, for Nuttallina[A,B]

						Are Group Means Different?[C]		
Character	N. californica (=BODEGA)	N. fluxa (=HI/DRY)		N. kata (=LOW)	Are within-group variances equal? (Levene's test, p=0.05)	ANOVA, p=0.01 (within-group variances assumed equal)	Welch & Brown-Forsythe tests, p=0.01 (within-group variances not assumed equal)	Kruskal-Wallis, p=0.01 (distributions not assumed normal)
Chiton total length, CTL (mm)	16.0 - 35.0 (26.3±4.6) N=63	13.5 - 42.0 (27.1±5.0) N=104		9.0 - 32.0 (21.6±5.0) N=163	Yes	Yes	Yes	Yes
Fourth valve width, FVW	25.9 - 47.6 (32.7±4.3) N=62	22.8 - 46.8 (34.6±4.8) N=103	GAVIOTA[D] 22.8 - 40.0 (31.3±3.2) N=52 NONGAVIOTA 31.4 - 46.8 (37.9±3.6) N=51)	22.7 - 39.1 (28.7±3.0) N=154	No	Yes	Yes	Yes
Fourth girdle width, FGW	13.9 - 28.6 (20.9±3.2) N=62	9.5 - 22.2 (15.5±2.6) N=104		7.9 - 21.4 (14.3±2.9) N=163	Yes	Yes	Yes	Yes
Sixth valve total length, SXVTL	23.7 - 35.2 (29.4±2.8) N=18	21.5 - 28.1 (25.2±1.4) N=37		21.0 - 33.2 (26.7±2.3) N=60	No	Yes	Yes	Yes
Sixth valve tegmentum width, SXVTW	26.9 - 47.9 (33.0±4.3) N=50	28.9 - 45.0 (34.4±3.6) N=76	GAVIOTA 28.9 - 40.4 (32.4±2.6) N=42 NONGAVIOTA 31.8 - 45.0 (36.9±3.0) N=34	23.5 - 36.9 (29.0±2.7) N=128	No	Yes	Yes	Yes
Sixth valve articulamentum width, SXVAW	27.3 - 46.3 (33.3±3.8) N=44	27.9 - 43.6 (33.9±3.2) N=72	GAVIOTA 27.9 - 39.1 (32.2±2.4) N=40 NONGAVIOTA 33.0 - 43.6 (36.1±2.6) N=32	23.8 - 36.4 (28.8±2.5) N=125	No	Yes	Yes	Yes
Seventh valve total length, SVVTL	22.5 - 29.0 (25.8±2.0) N=9	19.3 - 22.6 * N=17		21.3 - 28.6 (24.2±1.8) N=32	Yes	Yes, p=0.026 t(pooled)	No, p=0.054 t(separate)	Yes, p=0.022 Mann-Whitney, two tailed
Seventh valve tegmentum width, SVVTW	24.4 - 45.8 (30.9±4.1) N=53	24.8 - 41.1 (30.4±3.3) N=66	GAVIOTA 24.8 - 37.4 (28.9±2.5) N=41 NONGAVIOTA 28.2 - 41.1 (32.8±3.0) N=25	21.9 - 34.8 (26.8±2.5) N=121	No	Yes	Yes	Yes

TABLE IV-3 (cont.)

Character	N. californica (=BODEGA)	N. fluxa (=HI/DRY)		N. kata (=LOW)	Are within-group variances equal? (Levene's test, p=0.05)	Are Group Means Different?^{C}		
						ANOVA, p=0.01 (within-group variances assumed equal)	Welch & Brown-Forsythe tests, p=0.01 (within-group variances not assumed equal)	Kruskal-Wallis, p=0.01 (distributions not assumed normal)
Seventh valve articulamentum width, SVVAW	26.0 - 45.3 (31.5±3.6) N=49	26.2 - 39.4 (30.6±2.8) N=66	GAVIOTA 26.2 - 36.1 (29.4±2.2) N=41 NONGAVIOTA 30.0 - 39.4 (32.7±2.3) N=25	21.9 - 34.8 (27.1±2.3) N=117	No	Yes	Yes	Yes
FCW/FVW	0.42 - 0.86 (0.64±0.10) N=62	0.29 -0.68 (0.45±0.08) N=103		0.30 - 0.86 (0.50±0.10) N=154	No	Yes	Yes	Yes
SVVTW/SVVTL	1.00 - 1.21 (1.09±0.06) N=9	1.18 - 1.51 * N=17		0.91 - 1.18 (1.07±0.07) N=31	Yes	No, p=0.57 t(pooled)	No, p=0.58 t(separate)	No, p=0.69 Mann-Whitney two tailed
SVVTW/FVW	0.83 - 1.06 (0.95±0.05) N=52	0.85 - 1.16 (0.92±0.05) N=66		0.82 - 1.05 (0.93±0.05) N=119	Yes	Yes	Yes	Yes
SVVAW/SVVTW	0.91 - 1.12 (1.02±0.05) N=49	0.92 - 1.12 (1.01±0.04) N=63		0.96 - 1.09 (1.02±0.03) N=116	No	No, p=0.43	No, p=0.48(Welch) p=0.48(B.-F.)	No
SVVAW/SVVTL	1.00 - 1.21 (1.12±0.06) N=9	1.23 - 1.54 * N=16		0.97 - 1.23 (1.11±0.07) N=31	Yes	No, p=0.52 t(pooled)	No, p=0.51 t(separate)	No, p=0.43 Mann-Whitney two tailed
SVVAW/FVW	0.82 - 1.08 (0.96±0.06) N=48	0.83 - 1.23 (0.93±0.06) N=66		0.82 - 1.08 (0.95±0.05) N=115	Yes	Yes	Yes	Yes
SXVTL/SVVTL	1.03 - 1.14 (1.08±0.04) N=6	1.12 - 1.27 (1.18±0.04) N=11		1.00 - 1.23 (1.13±0.05) N=18	Yes	Yes	Yes	Yes
SXVTW/SXVTL	1.00 - 1.24 (1.09±0.07) N=18	1.11 - 1.64 (1.34±0.13) N=37	GAVIOTA 1.11 - 1.50 (1.28±0.13) N=22 NONGAVIOTA 1.32 - 1.64 (1.41±0.08) N=15	0.93 - 1.29 (1.06±0.08) N=60	No	Yes	Yes	Yes

TABLE IV-3 (cont.)

Character	N. californica (=BODEGA)	N. fluxa (=HI/DRY)	N. kata (=LOW)	Are within-group variances equal? (Levene's test, p=0.05)	Are Groups Means Different?^C ANOVA, p=0.01 (within-group variances assumed equal)	Welch & Brown-Forsythe tests, p=0.01 (within-group variances not assumed equal)	Kruskal-Wallis, p=0.01 (distributions not assumed normal)
SXVTW/SVVTW	1.03 - 1.14 (1.08±0.02) N=44	1.07 - 1.16 (1.12±0.02) N=58	0.96 - 1.15 (1.08±0.03) N=110	No	Yes	Yes	Yes
SXVTW/FVW	0.91 - 1.15 (1.02±0.05) N=49	0.95 - 1.30 (1.02±0.06) N=76	0.90 - 1.14 (1.01±0.04) N=126	Yes	No, p=0.22	No, p=0.23(Welch) p=0.24 (B.-F.)	No
SXVAW/SXVTW	0.93 - 1.10 (1.00±0.03) N=44	0.92 -1.05 (0.98±0.03) N=72	0.92 - 1.06 (1.00±0.03) N=124	Yes	Yes	Yes	Yes
SXVAW/SXVTL	1.02 - 1.19 (1.10±0.06) N=15	1.06 - 1.59 (1.32±0.13) N=37	0.87 - 1.30 (1.06±0.08) N=59	No	Yes	Yes	Yes
SXVAW/SVVAW	1.00 - 1.10 (1.06±0.03) N=36	1.04 - 1.19 (1.09±0.02) N=56	1.00 - 1.14 (1.06±0.03) N=106	Yes	Yes	Yes	Yes
SXVAW/FVW	0.86 - 1.15 (1.01±0.06) N=43	0.90 - 1.29 (1.00±0.06) N=72	0.87 - 1.14 (1.00±0.05) N=123	Yes	No, p=0.43	No, p=0.46(Welch) p=0.45(B.-F.)	No

NOTES.

A. All measurements involved thawed specimens. Ranges are presented first, followed by means ± standard deviations in parentheses, followed by number of specimens. Valve measurements are only for virtually-uneroded valves. See text for criteria used to establish that a valve is uneroded.

B. Characters marked with an asterisk showed substantial allometric growth for these characters, tests of group means and variances were between the remaining two species N. californica and N. kata only. See Table IV-4.

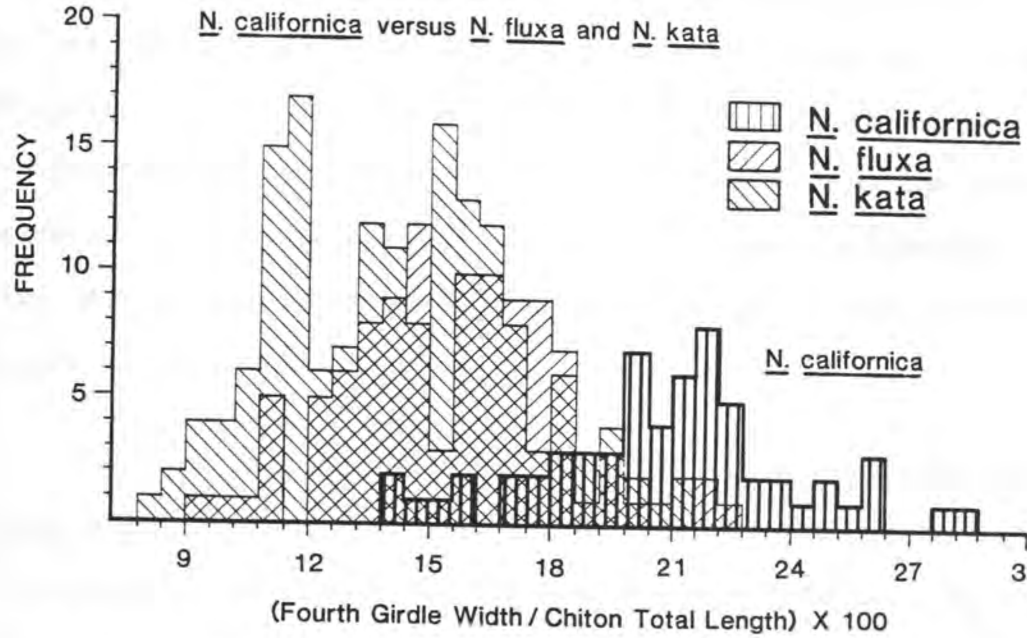
C. Statistical comparisons of group means were performed using the Biomedical Computer Programs P-series (BMDP) developed at the Health Sciences Computing Facility, UCLA. Levene's test for equal within-group variances, the Analysis of Variance (ANOVA), and Welch and Brown-Forsythe statistical tests were performed using BMDP7D (revision date: February, 1982). The Welch statistic and the Brown-Forsythe Statistic are one-way analysis of variance statistics that do not require within-group variances to be equal and are the tests of relevance when Levene's test shows within-group variances to be unequal. The probability of the Welch statistic is listed first when p>0.01. Kruskal-Wallis, the non-parametric analysis of variance, was performed using BMDP3S (revision date: February, 1982). For characters for which one species shows allometric growth, the other two species means were compared using BMDP3D (revision date: February, 1982), using a t test with variances in the two groups pooled (t pooled), and a t test in which the variance of each group is estimated separately (t separate). Where Levene's test for equal variances (BMDP3D) shows variances to be unequal, t(separate) is the relevant statistic. In addition, means for the two nonallometric species were compared using the nonparametric Mann-Whitney rank-sum test (BMDP3S).

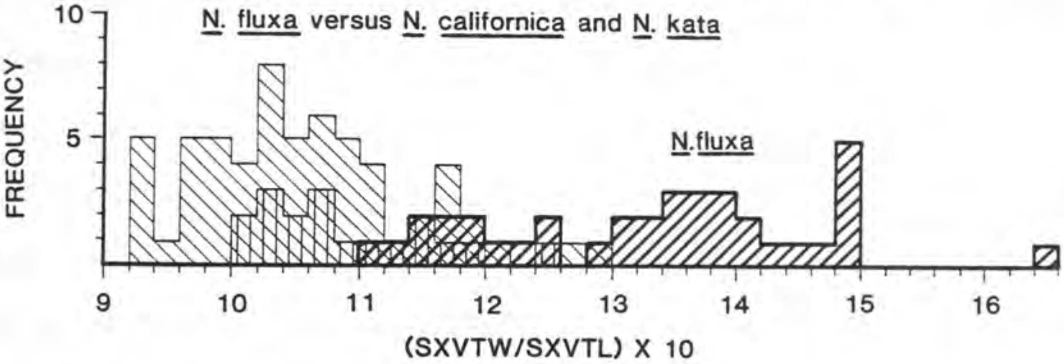
D. Nuttallina fluxa was the only species in which substantial geographic heterogeneity was detected in scatter plots of the characters. For those characters showing heterogeneity, ranges, means ± standard deviations and sample sizes are presented for specimens occurring at Gaviota, CA (GAVIOTA) and for specimens occurring at all other sites (NONCAVIOTA) separately, as well as for all N. fluxa specimens combined. T (separate) tests for comparisons of GAVIOTA vs. NONCAVIOTA N. fluxa character means (BMDP3D) were significantly different in all cases ($p<<0.001$).

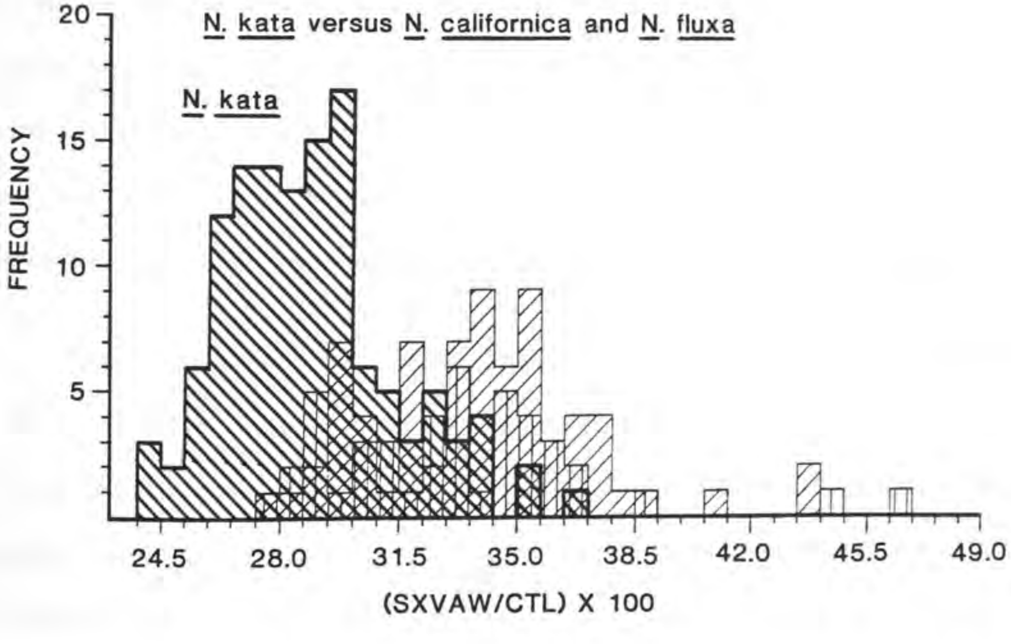
Although means for most characters are statistically different among the species, an examination of the ranges for each of the characters reveals that all characters exhibit some overlap among the three species (Table IV-3). No single character separates all three species with certainty, nor does any single character perfectly separate any one of the species from any other species. However, examination of frequency histograms for each character and ratio reveals that each species may be discriminated from the other two to a fair degree on the basis of certain characters. Thus, Nuttallina californica separates fairly well from N. fluxa and N. kata on the basis of the normalized fourth girdle width (FGW), and N. fluxa separates from N. californica and N. kata on the basis of ratios of the valve widths to valve lengths, SXVTW/SXVTL, SXVAW/SXVTL, SVVTW/SVVTL, SVVAW/SVVTL, and on the normalized seventh valve length (SVVTL). N. kata separates from N. californica and N. fluxa on the basis of normalized valve widths, SXVAW, SXVTW, SVVTW, SVVAW and FVW. Characters or ratios exemplifying the best separation of each species from the other two are presented in the histograms of Figure IV-5.

The relative narrowness of the valves of N. kata may relate to the common occurrence of this species in close-fitting depressions. Often, these depressions are deeper than the chiton and the opening of the depression is partially blocked by growths of encrusting coralline algae, Phragmatopoma tubes and/or barnacles. The openings of some holes are occluded to such an extent that it would appear that the chitons could not leave; such an observation probably led MacGinitie and MacGinitie (1968) to the conclusion that Nuttallina never leaves its

Figure IV-5. Characters exhibiting the least overlap between one *Nuttallina* species and the other two. None of these characters shows substantial allometry with chiton total length in any of the species. See text for discussion.


N. californica versus N. fluxa and N. kata
N. californica
N. fluxa
N. kata
N. californica
FREQUENCY
20
15
10
5
9
12
15
18
21
24
27
30
(Fourth Girdle Width / Chiton Total Length) X 100


N. fluxa versus N. californica and N. kata
N.fluxa
FREQUENCY
10
5
9
10
11
12
13
14
15
16
(SXVTW/SXVTL) X 10


N. kata versus N. californica and N. fluxa
N. kata
FREQUENCY
20
15
10
5
24.5
28.0
31.5
35.0
38.5
42.0
45.5
49.0
(SXVAW/CTL) X 100

depression. In fact, N. kata does leave its depression to feed at high tide (see Chapter V), and the narrowness of its valves is probably advantageous to the chiton as it goes through its contortions to exit the partially-occluded holes. It is interesting that the typically wider-valved species, N. fluxa, which does not commonly inhabit form-fitting depressions, tends to have narrower valves at Gaviota, CA where it occurs in depressions.

The relative wideness of the valves of Nuttallina fluxa and of N. californica, on the other hand, might represent an advantage in relation to predation by birds. Predation on Nuttallina by sea gulls and oystercatchers may be significant, at least in central California (Legg, 1954; Moore, 1975). Predation by birds might be particularly intense in higher intertidal areas, exposed for longer periods and lacking protective chiton depressions. The increased width of the valves of N. californica and, particularly, of N. fluxa might act as a deterrent to bird predation by lowering the upper limit to the overall size of chitons that can be swallowed. The greater maximal length of high intertidal N. californica and N. fluxa might also relate to bird predation pressure, although greater size, with its accompanying small surface area to volume ratio, is also advantageous with respect to the effects of desiccation that are more pronounced in the higher intertidal areas.

The dark-colored robust girdle of N. californica is reminiscent of that of two other conspicuous chiton species occurring in similar northern latitudes along the Pacific Coast of North America, Katharina tunicata and Cryptochiton stelleri. It is tempting to suggest that the wide robust girdle is an adaptation reflecting a greater resistance to removal from the substrate by the strong surf characteristic of the northern Pacific Coast especially during the winter. In fact, Linsenmeyer (1975) found that Katharina and Nuttallina (probably N. californica), both characteristically found in surf-exposed habitats, were more resistant to removal by lateral forces in the lab than were three chiton species normally found in lower wave impact areas. However, he indicates that the chiton foot rather than the girdle probably plays the major role in attachment to the substrate.

Geographic Variation.

Visual inspection of scatter plots for each character and ratio for each species revealed substantial geographic heterogeneity only within Nuttallina fluxa. Specimens of N. fluxa from the Gaviota, CA collecting site (see Chapter III) tended to have lower values than N. fluxa from all other sites for all valve width measurements, FVW, SXVTW, SXVAW, SVVTW, and SVVAW (Table IV-3). Group means for Gaviota versus non-Gaviota N. fluxa for each of these characters were significantly different (p=0.001, t test using separately-estimated within-group variances, BMDP3D program, revised February, 1982). This finding, of a relative valve narrowness of Gaviota N. fluxa, is interesting in view of the fact that Gaviota specimens were the only N. fluxa found closely-associated with substrate depressions (see Chapter III). The species characteristically found in form-fitting depressions, N. kata, possesses the narrowest valves of the Nuttallina. Such observations suggest the possibility that a limited cause-and-effect relationship exists in Nuttallina between narrowness of valves and factors associated with the habit of occupying intertidal depressions.

Allometry.

In systematics work, effects of allometric growth must be considered to ensure that observed group differences in characters do not stem solely from differences in overall size of the specimens examined. Here allometry is considered to be nonproportionate changes in characters with respect to another character, chiton total length (CTL). Although the ranges of total lengths (CTL) of the specimens examined are largely overlapping for the three species in this study, their means are significantly different (Table IV-3). In particular, specimens of N. kata are considerably shorter on the average than specimens of the other two species. This length difference is probably real, reflective of the fact that N. kata in the field tend to be smaller than specimens of the other two species. Each of the characters of Table IV-3 was examined for allometry to determine whether observed species differences were merely related to the difference in lengths of the species examined.

Scatter plots for each character versus chiton total length exhibited good linear fits for each species (in every case, r significant, $p<0.01$). Simple power functions provided poorer fits to the data. A character was judged to be allometric with respect to chiton total length using the following reasoning. Gould (1966) has noted that any line describing the regression of one body part on another with a significantly non-zero intercept defines a pattern of allometric growth. However, Waples (1981) has pointed out that equations with small non-zero intercepts may be of little use in systematics; allometric effects are important taxonomically only for those characters for which the regression equation explains a substantial portion of the observed variation. Thus, in this study, characters were screened for allometry by generating scatter plots of each normalized to chiton total length versus chiton total length. In a plot, a non-zero slope trend in data was taken as an indication of possible substantial allometry. A regression equation for the character versus CTL was then generated using the functional (GM) method of Ricker (1973). [The functional regression is appropriate when neither variable can be regarded as independent. The slope is the geometric mean of the slope of Y on X and the reciprocal of the slope of X on Y (conveniently calculated as the standard deviation of Y divided by the standard deviation of X)]. If the regression equation explained a substantial part of the total variation observed in that character, the character was judged to be substantially allometric. For example, the functional regression of seventh valve total length (SVVTL) on chiton total length (CTL) in *N. fluxa* predicts an increase in SVVTL of from 20.0 to 22.7% of CTL over the observed CTL range of 13.5 to 42.0 mm. Thus, allometric growth accounts for much of the observed variation in SVVTL of *N. fluxa* (19.3 to 22.6%, Table IV-3). Most characters normalized to chiton total length showed no evidence of a non-zero slope trend with chiton total length and on this basis were not further considered for substantial allometry with respect to chiton total length.

Ratios were examined for allometry in a similar manner. Scatter plots of ratios versus chiton total length generally gave very poor linear fits; no trends were evident in the plots to suggest that simple power functions would provide improved fits. One ratio in Nuttallina fluxa, SXVTL/SVVTL, and two ratios in N. kata, SVVTW/SVVTL and SXVTW/SXVTL, gave marginally significant negative product-moment correlations, but gave nonsignificant Spearman's rank correlations with chiton total length (at $p=0.05$) and were rejected from consideration for substantial allometry. FGW/FVW in N. kata exhibited a significant positive correlation with CTL (at $p=0.05$), but the functional regression equation explained only 10.5% of the observed variation in FGW/FVW; thus, FGW/FVW was judged not to be substantially allometric in N. kata.

In conclusion, if one uses somewhat subjective criteria, one finds substantial allometry in only one character and two ratios, in only one species (N. fluxa). Functional regression parameters for that character and those ratios are presented in Table IV-4. Examination of the allometric character and ratios in N. fluxa in relation to the species size differences of the specimens examined reveals that the effect of the allometry is to slightly diminish rather than enhance species differences in every case. For instance, the positive allometry observed in SVVTL for N. fluxa combined with the larger size of N. fluxa specimens examined tends to increase the mean value of SVVTL relative to those of the other two species. Thus, since the mean SVVTL in N. fluxa is lower than that of the other two species, the difference in mean values for SVVTL between N. fluxa and the other two species is diminished by allometric effects (see Table IV-3). Similarly, negative allometry in the ratios SVVTW/SVVTL and SVVAW/SVVTL of N. fluxa tends to reduce the difference in the means of these ratios between N. fluxa and the other two species since the allometry here has the effect of

TABLE IV-4. Parameters describing the functional regression on chiton total length (CTL) for the characters and ratios showing substantial allometric growth in Nuttallina [A]

Character	Species	Sample Size	Correlation Coefficient	Slope	Y-axis Intercept
SVVTL	Nuttallina fluxa	17	+0.99	+0.240	-0.5
SVVTW/SVVTL	Nuttallina fluxa	17	-0.81	-0.030	+2.0
SVVAW/SVVTL	Nuttallina fluxa	16	-0.62	-0.026	+1.9

NOTE

A. Criteria used to establish allometry were somewhat subjective and are discussed in the text. Character abbreviations are indicated in Table IV-3. All measurements are in millimeters. Regression parameters were generated using the functional (GM) method of Ricker (1973).

decreasing the higher values of N. fluxa (Table IV-3). Thus, allometry has had no substantial role in producing the observed species differences in characters and ratios presented in Table IV-3. In other words, the observed species differences in character means are not merely a reflection of group differences in absolute length of the specimens examined.

Multivariate Analyses

Although univariate analyses reveal statistically significant differences in the means for most characters among the three Nuttallina species, considerable overlap in the ranges of values for the single characters is observed (Table IV-3). For ecological as well as taxonomic work, it is desirable to be able to assign individual specimens to the different species with certainty. As indicated in Figure IV-5, such an exercise is only moderately successful in Nuttallina using characters singly, even when the task is simplified to the discrimination of specimens of one species from those of the other two. By considering the variation in several nonredundant characters simultaneously (that is, using a multivariate approach), better "resolution" of differences among specified groups can often be obtained, with the result that a higher percentage of individual specimens can be correctly assigned to the groups. Discriminant function analysis is a multivariate method by which the linear combination of several characters is generated that best discriminates among specified groups. Groups treated by this method must be specified by some a priori criterion. Nuttallina morphometric data of this study are suitable for discriminant analysis since the species groupings have been defined independently on the basis of electrophoretic phenotype (Chapter III).

The morphometric data base available for discriminant analysis is only a subset of the data presented in Table IV-3 for electrophoretically-phenotyped Nuttallina species, since only specimens for which complete morphometric data are available can be utilized. Morphometric characters used in the discriminant analysis are: 1) chiton total length (CTL); 2) intact fourth valve width (FVW); 3) intact fourth girdle width (FGW); 4) dissected sixth valve total length (SXVTL); and 6) sixth valve tegmentum width (SXVTW). Seventh valve characters were not used since their inclusion reduced the number of specimens of N. californica for which complete data were available to a sample size of only six. Specimens with data available for the sixth valve articulamentum width (SXVAW) were outnumbered by specimens with data available for SXVTW. Since these two characters were almost perfectly correlated ($r > 0.98$ for N. californica, N=44; N. fluxa, N=72 and N. kata, N=124) and inclusion of both in the analysis therefore would involve both redundancy of information and unnecessary reduction in the number of specimens with complete data, SXVTW, and not SXVAW, was used in the analysis.

Raw, nonnormalized data for each character were entered in stepwise fashion in the BMDP7M Stepwise Discriminant Analysis Program. Results of the analysis are presented in Figure IV-6 and Table IV-5. It is seen that specimens belonging to each species, or electrophoretic phenotype, form a fairly close association with other specimens of the same species, and segregate fairly well from specimens belonging to the other two species on the basis of the five morphological characters (Figure IV-6). As in the univariate analysis, no consistent effect of

Figure IV-6. Scatterplot of discriminant scores for Nuttallina specimens. Discriminant function units appear on the axes. Asterisks represent group centroids. All individuals have been phenotyped electrophoretically. Geographic locations of specimens are as follows: N. californica: Bodega Head, CA, N=8; Punta Banda, Baja CA, N=6; Little Harbor, Catalina Island, N=3; N. fluxa: La Jolla, CA, N=2; Gaviota, CA, N=22; Whites Point, Los Angeles, CA, N=13; N. kata: La Jolla, CA, N=11; Whites Point, CA, N=12; USC Marine Science Center, Catalina Island, N=13; Little Harbor, Catalina Island, N=15; Avalon, Catalina Island, N=9. Total N=114.

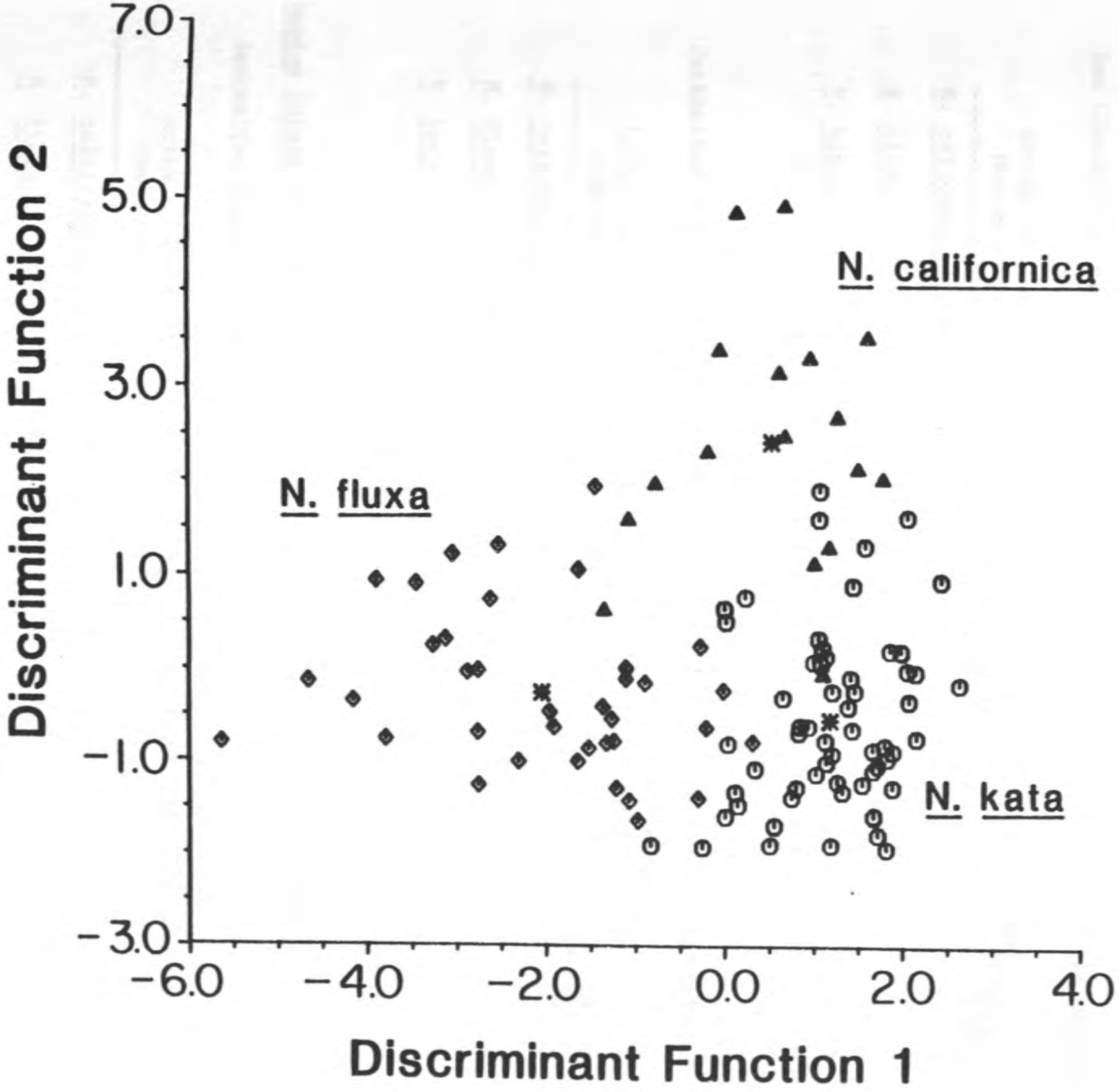

7.0
5.0
3.0
1.0
−1.0
−3.0
−6.0
−4.0
−2.0
0.0
2.0
4.0
Discriminant Function 2
Discriminant Function 1
N. californica
N. fluxa
N. kata

TABLE IV-5. Classification matrices obtained in discriminant function analyses of Nuttallina using computer program BMDP7M. Specimens are assigned to the species for which the highest classification function score is obtained.

Complete Data Set, N=114

Raw Classification Matrix (biased classification; see textfor explanation)

Actual Species Membership		Percent Correct	Number of Specimens Classified into Species		
			N. californica	N. fluxa	N. kata
N. californica,	N=17	88	15	1	1
N. fluxa,	N=37	84	1	31	5
N. kata,	N=60	92	4	1	55
		89	TOTAL (101 of 114)		

Jackknifed Classification Matrix (reduced bias; see text for explanation)

Actual Species Membership		Percent Correct	Number of Specimens Classified into Species		
			N. californica	N. fluxa	N. kata
N. californica,	N=17	82	14	2	1
N. fluxa,	N=37	84	1	31	5
N. kata,	N=60	90	5	1	54
		87	TOTAL (99 of 114)		

Random Subset of Complete Data Set, N=51 (see text for explanation)

Jackknifed Classification Matrix (reduced bias)

Actual Species Membership		Percent Correct	Number of Specimens Classified into Species		
			N. californica	N. fluxa	N. kata
N. californica,	N=17	82	14	1	2
N. fluxa,	N=19	95	0	18	1
N. kata,	N=15	87	2	0	13
		88	TOTAL (45 of 51)		

geographic location was observed in a discriminant score plot labelled according to collection site. Discriminant scores for specimens from different locations fell haphazardly around the centroid for the species.

The group means for the characters in the discriminant functions are clearly different among the three Nuttallina species ($p<0.001$, approximate "multivariate" F statistic = 33.9 for 10, 214 degrees of freedom; this statistic is a transformation of the multivariate Wilks' lambda or U statistic). More importantly, about 85 to 90% of the specimens are classified correctly according to the classification functions generated in the analysis (Table IV-5).

The raw classification matrix presented in Table IV-5 is subject to an inherent overestimate of success, produced by classifying the specimens used to generate the classification functions by those same functions. Two methods were used to produce estimates of classification success with reduced bias. One, the "jackknife-validation" procedure, provided as an option in the BMDP7M program, eliminates each specimen in turn from computations and computes the "probability of membership" of that specimen in each of the groups formed by the remaining specimens. The jackknifed classification matrix is presented in Table IV-5 and is seen to give only a slightly smaller estimate of classification success of the Nuttallina specimens (87% vs. 89%, total).

The second classification validation procedure involved dividing each species into two groups using a random-number-generating subprogram such that 20% of each species was classified according to the classification functions generated using the other 80% of the specimens. It gave overall classification success rates of 88.9, 90.5 and 89.5% in three runs. Three runs of classification of 50% of the cases using data from the other 50%, in the same manner as above, gave success rates of 83.0, 84.0 and 83.0%. The modest drop in success rate of the 50% runs is probably attributable to the substantial decrease in the amount of data used to generate the classification functions in these runs.

Thus, reduced bias estimates of classification success would appear to suggest that the classification functions generated for Nuttallina in this study using five morphological characters can be expected to correctly assign nearly 90% of specimens to one of the three Nuttallina species.

Sample sizes for the three species were considerably different in this analysis (N. californica, N=17; N. fluxa, N=37; N. kata, N=60). To investigate the effect of the different sample sizes on the classification process, a random-number generating subprogram was again used to select about 17 specimens from each species for inclusion in a discriminant analysis by the BMDP7M program. Overall classification success for this random subset of specimens was similar to that obtained for the complete data set, 88% versus 87% classified correctly, jackknifed, respectively (Table IV-5). No dramatic shift in proportions of specimens incorrectly assigned to species was noted. Whereas six of the 15 total incorrectly assigned specimens were assigned to N. kata in the full data run, three of a total six incorrectly assigned specimens were so assigned in the randomly reduced data subset run. Thus, use of different sample sizes for the three species in this analysis has had no obvious untoward effects.

Klecka (1975, p. 446) states:

> Although the theory of discriminant analysis assumes that the separate group covariance matrices are equal, this is usually not the case in practice... The chief consequence of differing covariance matrices is that cases are more likely to be classified into the group with the greatest overall dispersion, i.e., the greatest 'generalized variance' as measured by the determinant of the group covariance matrix.

Therefore, the full data set (114 specimens, 5 characters) was entered in the SPSS discriminant analysis program (Nie, 1975) to test for the equality of the three separate species covariance matrices based on both the discriminating characters and on the discriminant functions. SPSS program options were chosen so that the discriminant functions generated would be identical to those generated by the BMDP7M program (i.e., no rotation of the discriminant function axes; stepwise selection of characters using Wilks lambda as the entry level criterion). The species covariance matrices are significantly different for both characters and functions ($p<0.001$, Box's $M=99.40$, approximate $F=3.01$ for 30, 8480 d.f., and $p<0.002$, Box's $M=30.03$, approximate $F=4.83$ for 6, 21619 d.f., respectively). However, when these differences in separate species covariance matrices are taken into account, the overall classification success rate does not change. Thus, when individual group covariance matrices rather than a pooled group matrix are employed in computing probabilities of group membership (SPSS discriminant analysis program, option 14), the percentage of correctly classified specimens remains 89 (Table IV-6).

TABLE IV-6. Comparison of classifications obtained using a pooled within-group covariance matrix versus using separate group covariance matrices in the SPSS discriminant analysis computer program (Nie, 1975). Separate group covariance matrices were found to be significantly different among the three species ($p<0.001$, Box's M). No change in classification success is obtained by employing separate group covariance matrix in computing probabilities of group membership, although use of the pooled matrix gives more favorable results for *Nuttallina californica* (see text for more detail).

CLASSIFICATION MATRIX BASED ON POOLED WITHIN-GROUP COVARIANCE MATRIX

Actual Species Membership	Classified into Species		
	N. californica	*N. fluxa*	*N. kata*
N. californica	15	1	1
N. fluxa	1	31	5
N. kata	4	1	55
Classification Success	75	94	90
Overall Success	89(101 of 114)		

CLASSIFICATION MATRIX BASED ON SEPARATE GROUP COVARIANCE MATRICES

Actual Species Membership	Classified into Species		
	N. californica	*N. fluxa*	*N. kata*
N. californica	15	1	1
N. fluxa	1	34	2
N. kata	7	1	52
Classification Success	65	94	94
Overall Success	89(101 of 114)		

Classification based on separate group matrices rather than on the pooled matrix does have the effect of assigning more specimens to the species with smaller sample sizes, N. fluxa and N. californica, but only slightly increases the proportion of correct assignments to N. fluxa and N. kata, while substantially lowering the proportion to N. californica. Use of the pooled covariance matrix, as has been done above, appears to produce the more desirable classification results.

The relative contribution of each of the morphological characters to the discrimination of the three Nuttallina species can be deduced from an examination of the discriminant (not classification) functions generated in the discriminant analysis. Discriminant functions are arranged in order of decreasing importance. Function 1 explains most of the variation in the data and a difference among groups along this function or axis is more meaningful than the same absolute difference along the function 2 axis. BMDP7M and the SPSS discriminant analysis program compute eigenvalues as an indication of the relative importance of the two discriminant functions. In the present analysis of Nuttallina, discriminant function 1 has an eigenvalue of 2.19, and function 2, a value of 1.09 (Table IV-7). The cumulative proportion of total dispersion explained by function 1 is thus 2.19/(2.19 + 1.09) = 67%, and that explained by function 2 is 33%.

The absolute values of the coefficients of each standardized discriminant function indicate the relative contribution of the associated characters to that function. Coefficients for standardized discriminant functions, generated by the SPSS program, are presented in Table IV-7. In order of decreasing importance, SXVTW, SXVTL, and FVW

TABLE IV-7. Discriminant function parameters generated by the SPSS discriminant analysis computer program for the complete morphometric data set for Nuttallina. Note that the discriminant functions are standardized and are not the same as classification functions.

Variable	Coefficient	
	FUNCTION 1	FUNCTION 2
CTL	+0.13	-1.74
FVW	+1.32	-0.13
FGW	+0.22	+1.28
SXVTL	+1.88	+0.84
SXVTW	-3.80	+0.44

Eigenvalue, Function 1 = 2.19
Cumulative proportion of total dispersion = 67%

Eigenvalue, Function 2 = 1.09
Cumulative proportion of total dispersion = 33%

are seen to have the greatest contribution to the major function 1, while CTL, FGW and SXVTL have the greatest contribution to the lesser function 2. Examination of Figure IV-6, where the axes are functions 1 and 2, reveals that *N. californica* and *N. kata* occupy fairly similar positions, but are segregated from *N. fluxa* along the discriminant function 1 axis. Along the discriminant function 2 axis, *N. fluxa* and *N. kata* hold similar positions but are segregated from *N. californica*. Thus, given the relative contributions of morphological characters to each of the functions, it can be concluded that the sixth valve tegmentum width (SXVTW), and to a lesser extent the sixth valve total length (SXVTL) and fourth valve width (FVW), provide a major amount of the discrimination between *N. fluxa* and *N. californica*, but not much to the discrimination between *N. californica* and *N. kata*. On the other hand, chiton total length (CTL), fourth girdle width (FGW), and sixth valve total length (SXVTL) contribute the major amount to the discrimination between *N. californica* and *N. kata*, and substantially to the separation of *N. californica* and *N. fluxa*. In other words, *N. fluxa* and *N. kata* segregate well according to the valve characters, particularly valve widths, and *N. californica* and *N. kata* separate best on the basis of girdle width, whereas *N. californica* segregate from *N. fluxa* both on valve characters and girdle width. It should be noted that inclusion of different characters in the discriminant analysis can change the relative importance of each character for discriminating among the specified groups.

Although use of the five morphological characters, CTL, FVW, FGW, SXVTL and SXVTW, allows better separation of the three *Nuttallina* species than do fewer characters, classification functions using reduced sets of characters can be of considerable utility where data for some characters are not available. In addition, in some cases, consideration of morphological characters not used in the discriminant analysis such as girdle spine color, or consideration of habitat or geographic location, will allow elimination of one or another of the three *Nuttallina* species as a possible group for assignment of a specimen. For these situations, a series of discriminant analyses were run to generate classification functions that include fewer characters and/or that are to be used only in discrimination between two of the species. Classification functions and their estimated (jackknifed) rates of success are presented in Table IV-8.

Not surprisingly, classification success rates for discriminations involving only two species are generally better than for discrimination among all three species. Thus, when data for appropriate characters are available, *N. californica* can be separated from *N. fluxa*, or *N. fluxa* from *N. kata* with about 94% to 95% success, and *N. californica* can be discriminated from *N. kata* with about 91% success (Table IV-8).

Character Interconversions

Scatter plots involving characters of chitons different states, alive, frozen and dry, were examined for nonproportional changes with size. In all cases, the correlation of a character in different states, e.g., frozen chiton total length (CTL) versus live CTL, was highly significant whereas the correlation of the character normalized to the second state, e.g., frozen CTL/live CTL versus live CTL, was never

TABLE IV-8. Classification functions for aid in identification of *Nuttallina* specimens^. Functions were generated using the variables and species noted in the BMDP7M stepwise discriminant analysis computer program. Sample sizes were as follows: *N. californica* (=BODEGA), N=17; *N. fluxa* (=HI/DRY), N=37, and *N. kata* (=LOW), N=60.

Classification Routine	Variables Used	*Nuttallina* Species Compared	Linear Classification Function Coefficients (character measurements on thawed animals, in mm) CTL	FVW	FGW	SXVTL	SXVTW	CONSTANT	Classification Success Percentage (Jackknifed, reduced bias; see text) Individual Species	Overall
1.	CTL, FVW, FGW, SXVTL, SXVTW	*californica*	-0.06	1.60	1.88	2.14	-0.09	-19.53	82.4	86.8
		fluxa	0.88	-0.50	-2.22	-3.26	5.85	-20.19	83.8	
		kata	1.09	2.51	-1.89	1.21	-2.86	-11.77	90.0	
2.	CTL, FGW, SXVTL, SXVTW	*californica*	-0.08		2.05	1.91	1.63	-19.41	88.2	86.8
		fluxa	0.89		-2.27	-3.19	5.32	-20.18	83.8	
		kata	1.06		-1.62	0.86	-0.17	-11.47	88.3	
3.	CTL, FVW, FGW	*californica*	0.35	1.94	2.07			-19.03	88.2	78.9
		fluxa	0.72	3.30	-2.67			-18.65	73.0	
		kata	1.10	0.58	-1.70			-11.47	80.0	
4.	FVW, SXVTL, SXVTW	*californica*		2.29		2.67	-0.41	-18.94	70.6	79.8
		fluxa		-1.33		-2.35	7.35	-18.82	81.1	
		kata		1.79		2.62	-1.11	-10.16	81.7	
5.	FGW, SXVTL SXVTW	*californica*			2.01	1.78	1.53	-19.40	82.4	85.1
		fluxa			-1.74	-1.66	6.38	-19.28	83.8	
		kata			-0.99	2.69	1.11	-10.18	86.7	
6.	SXVTL, SXVTW	*californica*				2.37	2.09	-18.68	76.5	78.9
		fluxa				-2.18	5.90	-18.74	73.0	
		kata				2.39	0.83	-10.01	83.3	
7.	CTL, FVW, FGW, SXVTL, SXVTW	*californica*	-0.25	4.34	0.74	7.32	-5.47	-22.33	88.2	94.4
		fluxa	0.84	2.64	-2.68	1.24	-0.82	-17.50	97.3	
8.	CTL, FGW, SXVTL, SXVTW	*californica*	-0.38		1.54	6.25	-0.54	-21.10	88.2	94.4
		fluxa	0.76		-2.19	0.60	2.17	-17.05	97.3	
9.	CTL, FVW, FGW	*californica*	0.66	1.16	1.27			-17.29	88.2	90.7
		fluxa	1.00	2.18	-2.60			-17.36	91.9	
10.	FGW, SXVTL, SXVTW	*californica*			1.24	5.48	-0.90	-20.91	94.1	90.7
		fluxa			-1.59	2.11	2.86	-16.32	89.2	
11.	FGW, SXVTW	*californica*			2.23		2.38	-16.37	76.5	88.9
		fluxa			-1.21		4.12	-15.65	94.6	

TABLE IV-8 (cont.)

Classification Routine	Variables Used	Nuttallina Species Compared	Linear Classification Function Coefficients (character measurements on thawed animals, in mm)						Classification Success Percentage (Jackknifed, reduced bias; see text)	
			CTL	FVW	FGW	SXVTL	SXVTW	CONSTANT	Individual Species	Overall
12.	FGW	californica			4.80			-13.30	82.4	74.1
		fluxa			3.24			- 6.43	70.3	
13.	CTL, FVW, FGW, SXVTL, SXVTW	californica	0.14	0.43	2.11	-3.21	6.30	-23.76	82.4	87.0
		kata	1.02	1.33	-1.52	-1.54	1.48	-12.78	88.3	
14.	CTL, FGW, SXVTL, SXVTW	californica	0.15		2.15	-3.25	6.74	-23.75	88.2	89.6
		kata	1.04		-1.40	-1.68	2.82	-12.73	90.0	
15.	CTL, FVW, FGW	californica	0.02	4.27	1.59			-22.65	88.2	88.3
		kata	0.89	1.84	-1.73			-12.59	88.3	
16.	CTL, FGW, SXVTW	californica	-0.13		1.79		4.66	-22.91	94.1	90.9
		kata	0.89		-1.59		1.75	-12.50	90.0	
17.	CTL, FGW	californica	0.94		2.68			-19.86	82.4	88.3
		kata	1.29		-1.26			-12.07	90.0	
18.	FGW	californica			5.95			-16.30	82.4	90.9
		kata			3.24			- 5.32	93.3	
19.	CTL, FVW, FGW SXVTL	fluxa	0.91	-1.18	-2.44	-3.36	6.28	-18.42	86.5	93.8
		kata	1.31	1.96	-1.80	0.50	-2.75	-10.58	98.3	
20.	CTL, FVW, SXVTL, SXVTW	fluxa	0.73	-1.61		-3.77	6.29	-17.62	86.5	93.8
		kata	1.18	1.65		0.19	-2.74	-10.15	98.3	
21.	CTL, FVW, FGW	fluxa	0.72	2.94	-2.76			-16.62	73.0	81.4
		kata	1.18	0.07	-1.78			-10.35	86.7	
22.	FVW, SXVTL, SXVTW	fluxa		-1.76		-2.43	7.51	-17.15	86.5	93.8
		kata		1.41		2.36	-0.77	- 8.93	98.3	
23.	SXVTL, SXVTW	fluxa				-2.22	5.61	-17.00	86.5	94.8
		kata				2.19	0.76	- 8.83	100.0	
24.	SXVTW	fluxa					3.75	-16.24	70.3	78.4
		kata					2.59	- 8.10	83.3	

TABLE IV-8 (cont.)

NOTE

A. Example of use of the classification functions:
GIVEN: Specimen is one of any three of the *Nuttallina* species; measurements available are CTL, FVW, FGW (abbreviations for the characters as given in Table IV-3). This situation is covered by Classification Routine No. 3. The scores for the specimen are calculated using the three sets of linear classification function coefficients and constants listed. The specimen is most likely to belong to the species for which it gives the highest classification function score. Classification Routine No. 3 gave an overall classification success rate of about 79% and was most successful in classifying *N. californica* specimens (88%) for the original data set. It should be noted that addition of another variable does not necessarily increase the power of discrimination.

significant (at p=0.05). Thus, since little of the variability in any of the normalized characters e.g., frozen CTL/live CTL, is explained by nonproportionate changes of the characters in one state with the same characters in another state, e.g., frozen CTL with live CTL, interconversions of the characters among the different states are expressed as simple ratios rather than as regression functions. Since no obvious species differences were observed in scatter plots for any character, data for all three Nuttallina species have been pooled. Approximate conversion factors are presented in Table IV-9.

EXAMINATION OF TYPE MATERIAL

The three Nuttallina species designated as BODEGA, HI/DRY and LOW in Chapter III are here formally assigned the names that have been used throughout this chapter, Nuttallina californica (Nuttall, in Reeve, 1847), N. fluxa (Carpenter, 1864) and N. kata sp. nov., respectively, on the basis of an examination of relevant type material as follows. In addition, single hypotype specimens for N. californica (LACM No. 104357) and N. fluxa (LACM No. 104358), preserved whole in ethanol, have been deposited at LACM; hypotypes of these species also will be deposited at ANSP, BMNH, and USNM. The holotype specimen for N. kata sp. nov. (LACM No. 1734), preserved in ethanol, has been deposited at LACM and paratypes will be sent to ANSP, BMNH and USNM. Photographs of these specimens can be found in Figure IV-7.

Four separate sets of type specimens are of relevance for the formal assignment of names to the three northeast Pacific Nuttallina species of this study (see Figures IV-7 and IV-8; also see Appendix III

TABLE IV-9. Approximate factors for the interconversion of characters of *Nuttallina* among different states. Data for all three species are pooled (see text for details).

1. FROZEN/DRY [A]

Character	N	$\bar{x} \pm s$	Range	Frozen vs. Dry Values Correlation	Frozen vs. Dry Values Wilcoxon's Paired Sample Test	Dry Size Range of Character (mm)
CTL	30	1.08±0.03	1.04-1.13	0.990	p<0.001	12.0-31.0
FVW	36	1.05±0.03	0.99-1.14	0.995	p<0.001	4.3-12.8
FGW	36	1.89±0.26	1.43-2.50	0.870	p<0.001	1.0-3.5

2. FROZEN/ALIVE

Character	N	$\bar{x} \pm s$	Range	Frozen vs. Alive Values Correlation	Frozen vs. Alive Values Wilcoxon's Paired Sample Test	Alive Size Range of Character (mm)
CTL	38	0.83±0.05	0.73-0.93	0.960	p<0.001	17.0-44.0
FVW	36	1.00±0.02	0.94-1.04	0.996	N.S., p<0.20	4.4-13.1
FGW	29	0.82±0.10	0.62-1.00	0.884	p<0.001	2.5-7.5

NOTE

A. For example, the CTL for a frozen-thawed *Nuttallina* specimen is expected to be about 1.08 times that of the same specimen dry.

for the current dispositions of other Nuttallina species and names). Type material referable to Nuttallina fluxa consists of a single dry holotype specimen stored in the U.S. National Museum of Natural History (= Acanthopleura fluxa Carpenter, 1864, USNM No. 15690b). Type material attributed to Nuttallina californica consists of two lots of specimens. One lot consists of two dry "probable syntypes" stored in the collection of the Academy of Natural Sciences at Philadelphia (= Chiton californicus Nuttall, in Reeve, 1847, ANSP No. 118697). The other lot consists of seven dry "possible syntypes" and is stored at the British Museum of Natural History (= Chiton californicus Nuttall, in Reeve, 1847, BMNH Reg. No. 19798). A fourth lot of type specimens is attributed to Chiton scaber Reeve, 1847 (not Blainville, 1825). The Chiton scaber lot consists of two dry "possible syntypes" also stored at the British Museum of Natural History (BMNH Reg. No. 19799).

All four lots of presumptive type specimens have been examined carefully and observations are presented below. Results are summarized in Tables IV-10 to IV-13 and IV-15, and specimens are figured in Figures IV-7 and IV-8.

Nuttallina fluxa Holotype (USNM)

Uncertainty exists as to the exact locality of the Nuttallina fluxa holotype (USNM No. 15690b). The first mention of a locality is "Cat. Is." (i.e., Santa Catalina Island, California) (Carpenter, 1864, p. 612). The second mention (Carpenter, 1864, p. 649) is a tabular reference to the islands in the Santa Barbara group. The third (Carpenter, 1866, p. 211) is "Santa Barbara Island, Cooper." Two original

TABLE IV-10. Morphometric and qualitative characters of the holotype specimen of Nuttallina fluxa (Carpenter, 1864), USNM No. 15690b. Specimen is clearly identifiable as representative of the "HI/DRY" species.

METRIC/MERISTIC CHARACTERS

Character	Measurement or Count	Approximate frozen character equivalent using frozen/dry conversion factor from Table IV-9	Comments
Total length	14.8 cm	16.0 mm	Probably a low estimate since valves i, vii, viii are separate and anterior, posterior ends are curled
Fourth valve width	7.6 mm	8.0 mm	
Girdle width at fourth valve	1.6 mm	3.0 mm	Average for the two sides
Total width at fourth valve	9.7 mm		
Maximum width of chiton	9.7 mm		
Elevation	5.3 mm		Measured down the midline of the chiton with calipers
Disarticulated Valves			
i.			
slits	11		Square teeth
ribs	11		In line with slits; broad, low, rounded
width	6.0 mm		Measurement anterior to posterior; teeth and valve posterior partially eroded or broken
elevation	3.2		
vi.			
slits	1,1 (2,2 with slit rays)		
width(SVVTW)	7.0 mm		Low estimate since plates chipped
width(SVVAW)	7.0 mm		Low estimate since plates chipped
length(SVVTL)	4.3 mm		
elevation	2.4 mm		
viii.			
slits	9		
ribs	none obvious, eroded		
width(EVTW)	4.9 mm		
width(EVAW)	5.0 mm		Low estimate since plates broken
length(EVTL)	3.2 mm		
elevation	2.0 mm		
mucro	subterminal to terminal		

QUALITATIVE CHARACTERS

Character	Description
Valves.	
Color	Light brown; one to three white stripes on intermediate and posterior valves.
Ornamentation	Round to oblong granulation, except where eroded.
Shape	Tends to rectangular; posterior margins quite nearly perpendicular to centerline of chiton.
Girdle.	
General color	Brown to light brown.
Stripes	Full complement: 3 anterior to valve i; one at each suture; two posterior to valve vii/viii suture.
Sutural Encroachment	Minimal.

TABLE IV-11. Morphometric and qualitative characters of the "probable syntype" specimens of *Nuttallina californica* (Nuttall, in Reeve, 1847), ANSP No. 118697. The longer specimen classifies morphologically with the "BODEGA" species and has been designated herein as a paralectotype for *Nuttallina californica* (Nuttall, in Reeve, 1847). The shorter specimen cannot be identified unequivocally to species, but may be a "HI/DRY" specimen [*Nuttallina fluxa* (Carpenter, 1864)]. See text for details.

A. LARGE SPECIMEN

METRIC/MERISTIC CHARACTERS

Character	Measurement or Count	Approximate frozen character equivalent, using frozen/dry conversion factor from Table IV-9	Comments
Total length	27.2 mm	29.4 mm	Fairly flat although valve ii seems unnaturally raised
Fourth valve width	8.7 mm	9.1 mm	
Girdle width at fourth valve	3.3 mm	6.6 mm	Probably a low estimate since left girdle seems slightly contracted and is averaged in with right side
Total width at fourth valve	13.5 mm		
Maximum width of chiton	13.9 mm		
Elevation	8.0 mm		Down chiton midline, includes cardboard (<0.5 mm)
Valve i ribs	11		

QUALITATIVE CHARACTERS

Character	Description
Valves.	
Color	Dark brown, relatively uneroded.
Ornamentation	Granular throughout.
Shape	Tending to angularity; posterior margins not near to perpendicular to centerline of chiton.
Mucro (valve viii)	Terminal to subterminal.
Girdle.	Girdle in fair shape, but many spines missing due to wear.
General color	Medium brown.
Stripes	No white stripes obvious.
Spines	Most short to medium length, brown; scattered white spines at girdle margins.
Sutural Encroachment	Moderate; ratio of minimum to maximum valve width at girdle as low as 7.4/8.8=0.84 (min./max. estimate of a dry, contracted specimen expected to underestimate live or frozen min./max. value).

CHARACTERS DETERMINED BY X-RAY ANALYSIS

Character	Measurement or Count	
Slits.		
Valve i	11, possibly 10	
Valves ii-vii	2,2	
Valve viii	Cannot be determined	
Seventh valve tegmentum width (SVVTW)	ca. 8.1 mm	Erosion at posterior margin of valve; not as eroded as valve vi
Seventh valve total length (SVVTL)	ca. 6.5 mm	

TABLE IV-11 (cont.)

B. SMALL SPECIMEN

METRIC/MERISTIC CHARACTERS

Character	Measurement or Count	Approximate frozen character equivalent, using frozen/dry conversion factor from Table IV-9	
Total length	19.5 mm	21.1 mm	
Fourth valve width	7.4 mm	7.8 mm	
Girdle width at fourth valve	2.0 mm		Average of the two sides
Total width at fourth valve	10.0 mm		
Maximum width of chiton	10.0 mm		
Elevation	5.2 mm		Down centerline of chiton; a small amount of glue included
Valve i ribs	Indeterminate		Badly eroded tegmentum

QUALITATIVE CHARACTERS

Character	Description
Valves.	
Color	Light brown; valve surfaces all badly eroded; actual color indeterminate.
Ornamentation	Indeterminate.
Shape	Intermediate between angular and quadrangular; posterior of all valves badly eroded, making shape indeterminate.
Mucro (valve viii)	Eroded.
Girdle.	
General color	Medium to light brown.
Stripes	None obvious although indications of lighter areas; girdle covered with glue.
Spines	Many white spines throughout girdle; brown spines present.
Sutural encroachment	Minimal.

CHARACTERS DETERMINED BY X-RAY ANALYSIS

Character	Measurement or Count
Slits.	
Valve i	10, possibly 11
Valve ii-vii	2,2 (?)
Valve viii	9 (?)

TABLE IV-12. Morphometric and qualitative characters of the "possible syntypes" of *Chiton californicus* Nuttall in Reeve, 1847, BMNH Reg. No. 19798. The specimens have been assigned identification numbers 1 through 7; specimen length decreases with increasing identification number. Specimen No. 1 has been designated herein as the lectotype, and specimen nos. 2 through 5 have been designated as paralectotypes, of *Nuttallina californica* (Nuttall, in Reeve, 1847) (=BODEGA). Specimen No. 6 is probably a *Nuttallina fluxa* (=HI/DRY) specimen. Specimen No. 7 belongs to a different genus, possibly *Chaetopleura*.

SPECIMEN NO. 1

General Condition: dry; good; only intact valves and girdle present

METRIC/MERISTIC CHARACTERS

Character	Measurement or Count	Approximate frozen character equivalent, using frozen/dry conversion factor from Table IV-9	Comments
Total length	39.2 mm	42.3 mm	Specimen somewhat arched
Fourth valve width	10.3 mm	10.8 mm	
Girdle width at fourth valve	3.7 mm	7.0 mm	Average of two sides; curled somewhat
Total width at fourth valve	16.1 mm		
Maximum width of chiton	17.3 mm		
Elevation	9.3 mm		Specimen somewhat arched
Valve i ribs	10		

QUALITATIVE CHARACTERS

Character	Description
Valves.	
Color	Dark brown with 1 or 2 white stripes on jugal ridges of valves.
Ornamentation	Granular; posterior and lateral ribs distinct; central posterior edge of valves eroded.
Shape	Intermediate between angular and quadrangular, tending to angular.
Mucro (valve viii)	Eroded slightly but apparently terminal.
Girdle.	
General color	Medium brown.
Stripes	No white stripes.
Spines	Predominantly brown; small clumps of white spines near girdle margin.
Sutural Encroachment	Moderate; ratio of minimum to maximum valve width at girdle as low as 0.75.

TABLE IV-12 (cont.)

SPECIMEN NO. 2

General Condition: dry; good; only intact valves, girdle present

METRIC/MERISTIC CHARACTERS

Character	Measurement or Count	Approximate frozen character equivalent, using frozen/dry conversion factor from Table IV-9	Comments
Total length	38.1 mm	41.1 mm	Somewhat arched
Fourth valve width	8.1 mm	8.5 mm	
Girdle width at fourth valve	4.0 mm	7.6 mm	Average of two sides; curled somewhat
Total width at fourth valve	14.0 mm		
Maximum width of chiton	15.0 mm		
Elevation	7.9 mm		Specimen somewhat arched
Valve i ribs	∿12		

QUALITATIVE CHARACTERS

Character	Description
Valves.	
Color	Dark brown with 1 to 3 white stripes on valve jugal ridges.
Ornamentation	Finely granular; posterior and particularly lateral ribs distinct; valves virtually uneroded.
Shape	Angular.
Mucro (valve viii)	Eroded slightly but apparently terminal.
Girdle.	
General color	Light-medium brown.
Stripes	Distinct white stripes at valve i/ii and vii/viii sutures: white patches at vi/vii suture, and anterior to valve i.
Spines	Predominantly brown: many white spines near girdle margin, and a few nearer valves, usually in small clumps, and in areas of white scales.
Sutural Encroachment	Substantial: ratio of minimum to maximum valve width at girdle as low as 0.57.

TABLE IV-12 (cont.)

SPECIMEN NO. 3

General Condition: dry; good; only intact valves and girdle present

METRIC/MERISTIC CHARACTERS

Character	Measurement or Count	Approximate frozen character equivalent, using frozen/dry conversion factor from Table IV-9	Comments
Total length	35.7 mm	38.6 mm	Specimen somewhat arched
Fourth valve width	8.2 mm	8.6 mm	
Girdle width at fourth valve	4.2 mm	7.9 mm	Average of both sides; curved somewhat
Total width at fourth valve	14.4 mm		
Maximum width of chiton	14.6 mm		
Elevation	8.1 mm		Specimen arched somewhat
Valve i ribs	11		

QUALITATIVE CHARACTERS

Character	Description
Valves.	
Color	Dark brown with 2 or 3 white stripes on jugal ridges of valves.
Ornamentation	Granular; lateral ribs fairly distinct; jugal sulci fairly developed; some erosion at valve apices.
Shape	Fairly angular.
Mucro (valve viii)	Very eroded.
Girdle.	
General color	Medium brown.
Stripes	Possibly at valve i/ii suture only.
Spines	Predominantly brown; a few scattered white spines.
Sutural Encroachment	Moderate; ratio of minimum to maximum valve width at girdle as low as 0.64.

TABLE IV-12 (cont.)

SPECIMEN NO. 4

General Condition: dry; good; only intact valves, girdle present

METRIC/MERISTIC CHARACTERS

Character	Measurement or Count	Approximate frozen character equivalent, using frozen/dry conversion factor from Table IV-9	Comments
Total length	35.1 mm	37.9	Specimen somewhat arched
Fourth valve width	8.3 mm	8.7 mm	
Girdle width at fourth valve	4.8 mm	9.1 mm	Average of two sides; curled somewhat
Total width at fourth valve	14.7 mm		
Maximum width of chiton	14.9 mm		
Elevation	8.4 mm		Specimen arched considerably
Valve i ribs	∿10		

QUALITATIVE CHARACTERS

Character	Description
Valves.	
Color	Dark brown with 1 to 3 white stripes on valve jugal ridges.
Ornamentation	Granular; jugal sulci fairly well developed; some erosion at valve apices.
Shape	Fairly angular.
Mucro (valve viii)	Eroded, probably terminal.
Girdle.	
General color	Medium brown.
Stripes	White stripes at valve i/ii suture and at v/vi, vi/vii, vii/viii sutures, posterior to valve viii, and possibly anterior to valve i; many white patches near girdle margin at both sutural and intersutural positions.
Spines	Predominantly brown; a few scattered white spines.
Sutural Encroachment	Moderate; ratio of minimum to maximum valve width at girdle as low as 0.60.

TABLE IV-12 (cont.)

SPECIMEN NO. 5

General Condition: dry; good; only intact valves, girdle present

METRIC/MERISTIC CHARACTERS

Character	Measurement or Count	Approximate frozen character equivalent, using frozen/dry conversion factor from Table IV-9	Comments
Total length	27.1 mm	29.3 mm	Specimen arched somewhat
Fourth valve width	6.2 mm	6.5 mm	
Girdle width at fourth valve	3.2 mm	6.0 mm	Curled somewhat
Total width at fourth valve	10.9 mm		
Maximum width of chiton	11.5 mm		
Elevation	5.2 mm		Specimen arched somewhat
Valve i ribs	∿10		

QUALITATIVE CHARACTERS

Character	Description
Valves.	
Color	Dark brown with 1 or 2 white stripes at valve jugal ridges.
Ornamentation	Granular; lateral and posterior ribs fairly developed; small degree of erosion at valve apices.
Shape	Intermediate between angular and quadrangular, tending to angular.
Mucro (valve viii)	Uneroded; terminal.
Girdle.	
General color	Medium to light brown
Stripes	No obvious white stripes or patches.
Spines	Predominantly brown; a few scattered white spines.
Sutural Encroachment	Moderate; ratio of minimum to maximum valve width at girdle as low as 0.60.

TABLE IV-12 (cont.)

SPECIMEN NO. 6 ["M" (?) marked in black on underside of valves]

General Condition: dry; fair; only intact valves and girdle present

METRIC/MERISTIC CHARACTERS

Character	Measurement or Count	Approximate frozen character equivalent, using frozen/dry conversion factor from Table IV-9	Comments
Total length	14.6 mm	15.8 mm	Specimen arched somewhat
Fourth valve width	5.6 mm		
Girdle width at fourth valve	∿1 mm	1.9 mm	Average of two sides; curled somewhat
Total width at fourth valve	7.1 mm		
Maximum width of chiton	7.3 mm		
Elevation	4.9 mm		Specimen arched somewhat
Valve i ribs	∿10		

QUALITATIVE CHARACTERS

Character	Description
Valves.	
Color	Dark brown; no white jugal stripes.
Ornamentation	Granular; valve ribs not distinct; valve apices slightly eroded.
Shape	Quadrangular.
Mucro (valve viii)	Eroded slightly; subterminal.
Girdle.	
General color	Dark brown.
Stripes	Distinct white stripes at suture of valves i/ii, at sutures of valves v to viii, and posterior to valve viii.
Spines	White spines predominant, throughout girdle in areas of both brown and white scales; a few brown spines, mainly in areas of brown scales.
Sutural Encroachment	Minimal to nonexistent.

TABLE IV-12 (cont.)

SPECIMEN NO. 7

General Condition: dry; fair; only radula, intact valves, girdle present

METRIC/MERISTIC CHARACTERS

Character	Measurement or Count	Approximate frozen character equivalent, using frozen/dry conversion factor from Table IV-9	Comments
Total length	13.0 mm	14.0 mm	
Fourth valve width	5.4 mm	5.7 mm	
Girdle width at fourth valve	1.0 mm	1.9 mm	
Total width at fourth valve	6.6 mm		
Maximum width of chiton	7.0 mm		
Elevation	3.3 mm		Specimen arched somewhat
Valve i ribs	∿10		

QUALITATIVE CHARACTERS

Character	Description
Valves.	
Color	Mottled white to light brown; dark brown apices; medium brown valve i; no white jugal stripes.
Ornamentation	Central areas with longitudinal ribs, more or less; lateral ribs fairly distinct and nodulose; valves relatively uneroded.
Shape	Angular, tending to quadrangular.
Mucro (valve viii)	Uneroded; terminal.
Girdle.	
General color	Medium brown.
Stripes	No white stripes or patches.
Spines	Extremely sparse; predominantly light brown; most often in tufts.
Sutural Encroachment	Minimal.

TABLE IV-13. Morphometric and qualitative characters of the "possible syntypes" of *Chiton scaber* Reeve, 1847, BMNH Reg. No. 19799. The longer specimen has been designated herein as the lectotype, and the smaller one as the paralectotype, of *Chiton scaber* Reeve, 1847. Both specimens are "HI/DRY" specimens, establishing the synonymy *Chiton scaber* Reeve, 1847 = *Nuttallina fluxa* (Carpenter, 1864).

A. LARGE *C. scaber*

General Condition: dry; good; only intact valves, girdle present

METRIC/MERISTIC CHARACTERS

Character	Measurement or Count	Approximate frozen character equivalent, using frozen/dry conversion factor from Table IV-9	Comments
Total length	27.9 mm	30.1 mm	Specimen slightly arched
Fourth valve width	10.5 mm	11.0 mm	
Girdle width of fourth valve	1.8 mm	3.4 mm	
Total width at fourth valve	12.6 mm		
Maximum width of chiton	12.6 mm		Specimen fairly flat side to side
Elevation	6.5 mm		Specimen slightly arched longitudinally
Valve i ribs	11		

QUALITATIVE CHARACTERS

Character	Description
Valves.	
Color	Light to medium brown; alternating brown, white stripes on central anterior region of valves.
Ornamentation	Granular; small amount of erosion on posterior margins of valves.
Shape	Intermediate between angular, quadrangular.
Mucro (valve viii)	Eroded.
Girdle.	
General color	Medium to light brown.
Stripes	Distinct white stripes at almost all valve sutures; several complete white stripes even at valve ii to iv positions.
Spines	Many white spines throughout girdle in both white and brown scale areas very few brown spines present; many spines apparently have been rubbed off.
Sutural Encroachment	Minimal to moderate.

TABLE IV-13 (cont.)

B. SMALL *C. scaber* [small piece of paper with "217" glued to ventral part of valves]

General Condition: dry; good; only intact valves, girdle present

METRIC/MERISTIC CHARACTERS

Character	Measurement or Count	Approximate frozen character equivalent, using frozen/dry conversion factor from Table IV-9	Comments
Total length	26.5 mm	28.6 mm	Specimen flat
Fourth valve width	8.6 mm	9.0 mm	
Girdle width at fourth valve	1.5 mm	2.8 mm	
Total width at fourth valve	10.8 mm		
Maximum width of chiton	11.1 mm		Specimen slightly curved side to side
Elevation	5.3 mm		Specimen straight longitudinall
Valve i ribs	11		

QUALITATIVE CHARACTERS

Character	Description
Valves.	
Color	Light to medium brown; central areas very light brown, tending to striping.
Ornamentation	Granular; posterior and particularly lateral ribs raised and distinct; erosion minimal except on valves v, vi, vii.
Shape	Tending to angular (allowing for erosion).
Mucro (valve viii)	Slightly eroded.
Girdle.	
General color	Light brown.
Stripes	None distinct, but light areas near 3 valve sutures.
Spines	White spines throughout girdle; very few brown spines; many spines have apparently been lost, leaving holes in girdle.
Sutural Encroachment	Minimal.

TABLE IV-14. Morphometric and qualitative characters of the holotype specimen of *Nuttallina kata*, sp. nov., LACM No. 1734. Specimen was electrophoretically identified; morphological characters support the identification. Specimen is intact and preserved in ethanol. Measurements are for the specimen in ethanol.

General Condition: ethanol-preserved; good; chiton intact, except small piece of foot removed for electrophoresis; valves slightly to moderately eroded

METRIC/MERISTIC CHARACTERS

Character	Measurement or Count	Comments
Total length	34 mm	
Fourth valve width (intact)	8.6 mm	
Girdle width at fourth valve	5.5 mm	Difficult to measure; inflated tissues
Total width at fourth valve	15 mm	
Maximum width of chiton	15 mm	
Elevation	6.6 mm	
Valve i ribs	11	
Gills	28 (left), 28 (right)	Anterior gill buds underlying posterior of valve iii to iii/iv suture

QUALITATIVE CHARACTERS

Character	Description
Valves.	
Color	Medium to dark brown; one, two(?), three white stripes on intermediate and posterior valves.
Ornamentation	Granulated in most areas, where not eroded.
Shape	Angular to rounded exposed surfaces.
Mucro (valve viii)	Slightly eroded; subterminal?
Girdle.	
General color	Medium brown, except in stripe areas.
Stripes	Nearly full complement of white stripes on left side; complete stripes at i/ii, ii/iii, iii/iv, v/vi, vi/vii, vii/viii valve sutures on left side, i/ii, ii/iii, iii/iv, vii/viii on right side; no complete white stripes posterior to valve viii; one stripe in front of valve i.
Spines	Many white spines throughout girdle in both white and brown scale areas, increasing in number at girdle margin; many brown spines (fewer than white ones?) in both brown and white scale areas.
Sutural Encroachment	Moderate; ratio of minimum to maximum valve width at girdle as low as 0.75.

TABLE IV-15. Identifications of all specimens in the four relevant *Nuttallina* type lots, using multivariate classification functions from Table IV-8. Both two- and three-species classification routines have been used. Equivalent frozen values for chiton characters have been used; frozen values were estimated from dry character values using conversion factors in Table IV-9.

Type Lot Specimen	Classification Routine from Table IV-8	Classification Score			Species Identification Indicated	Confirmed or Designated Status in this work
		californica	*fluxa*	*kata*		
N. fluxa	3	8.3	11.3	5.7	*N. fluxa*	*N. fluxa*
USNM No. 15690B	9	6.4	8.3	-	*N. fluxa*	Holotype
N. californica	3	22.6	14.9	14.9	*N. californica*	*N. californica*
large specimen	15	27.3	-	18.9	*N. californica*	Paralectotype
ANSP No. 118697						
N. californica	3	11.4	12.1	9.8	*N. fluxa*	Possibly *N. fluxa*
small specimen	9	10.5	10.9	-	*N. fluxa*	
ANSP No. 118697	21	-	11.0	8.3	*N. fluxa*	
N. californica	3	31.2	28.8	29.4	*N. californica*	*N. californica*
specimen No. 1	15	35.4	-	32.8	*N. californica*	Lectotype
BMNH Reg. No. 19798						
N. californica	3	27.0	18.9	26.0	*N. californica*	*N. californica*
specimen No. 2,	15	26.6	-	26.5	*N. californica*	Paralectotype
BMNH Reg. No. 19798						
N. californica	3	27.5	16.4	22.5	*N. californica*	*N. californica*
specimen No. 3	15	27.4	-	23.9	*N. californica*	Paralectotype
BMNH Reg. No. 19798						
N. californica	3	30.0	13.0	19.8	*N. californica*	*N. californica*
specimen No. 4,	15	29.7	-	21.4	*N. californica*	Paralectotype
BMNH Reg. No. 19798						
N. californica	3	16.1	7.5	14.3	*N. californica*	*N. californica*
specimen No. 5	15	15.2	-	15.1	*N. californica*	Paralectotype
BMNH Reg. No. 19798						

TABLE IV-15 (cont.)

Type Lot Specimen	Classification Routine from Table IV-8	Classification Score *californica*	Classification Score *fluxa*	Classification Score *kata*	Species Identification Indicated	Confirmed or Designated Status in this work
N. californica	3	1.9	7.1	6.1	*N. fluxa*	Probably *N. fluxa*
specimen No. 6	21	See Note A	6.9	5.3	*N. fluxa*	
BMNH Reg. No. 19798						
N. californica	3	0.9	5.2	4.0	*N. fluxa*	Valve sculpture
specimen No. 7	9	1.0	4.1	-	*N. fluxa*	suggestive of
BMNH Reg. No. 19798	21	-	5.0	3.2	*N. fluxa*	another genus
C. scaber	3	19.9	30.2	22.2	*N. fluxa*	*C. scaber*
large specimen	21	-	28.0	19.9	*N. fluxa*	Lectotype
BMNH Reg. No. 19799						(= *N. fluxa*)
C. scaber	3	14.2	24.2	20.4	*N. fluxa*	*C. scaber*
small specimen	21	-	22.7	19.0	*N. fluxa*	Paralectotype
BMNH Reg. No. 19799						(= *N. fluxa*)

NOTE

A. White spines predominate in girdle so *fluxa* vs. *kata* classification routine is especially appropriate.

Figure IV-7 (cont.)

G. Nuttallina kata sp. nov. HOLOTYPE. LACM No. 1734. Intertidal; sandstone outcrop near Seal Rock, La Jolla, CA; 17 September 1982. Length 34 mm (in ethanol). See Table IV-14 for more information. Note complete white girdle stripes.

H. Nuttallina kata sp. nov. SIO No. M2845. Shale outcrop at foot of Camino de la Costa, La Jolla, CA; 18 January 1977. Length 35 mm (in ethanol). Note substantial erosion of several valves.

I. Nuttallina fluxa (Carpenter, 1864). SIO No. M2846. High intertidal pool at Whites Point, Los Angeles, CA; 3 April 1981. Length 32 mm (in ethanol). Note extensive erosion of valves.

Figure IV-7. Selected type and non-type material referable to *Nuttallina*. See tables and text for more information.

A. *Nuttallina californica* (Nuttall, in Reeve, 1847). HYPOTYPE. LACM No. 104357. Intertidal; Bodega Head near Bodega Marine Laboratory, Sonoma County, CA; January 1981. Length 25 mm (in ethanol). Apices of valves are eroded. Posterior girdle is damaged.

B. *Nuttallina californica* (Nuttall, in Reeve, 1847). LACM No. 61-11. Intertidal shale ledges at Shell Beach, San Luis Obispo County, CA. Length 30.5 mm (in ethanol).

C. *Nuttallina fluxa* (Carpenter, 1864). HOLOTYPE. USNM No. 15690b. Length 14.8 mm (dry). Also shown are the three disarticulated valves, i, vii, and viii. See Table IV-10 for more information.

D. *Chiton scaber* Reeve 1847 (= *Nuttallina fluxa*). LECTOTYPE (of *C. scaber*). BMNH No. 19799. "California." Length 27.9 mm (dry). See Table IV-13 for more information.

E. *Chiton scaber* Reeve, 1847 (= *Nuttallina fluxa*). PARALECTOTYPE (of *C. scaber*). BMNH No. 19799. "California." Length 26.5 mm (dry). See Table IV-13 for more information.

F. *Nuttallina fluxa* (Carpenter, 1864). HYPOTYPE. LACM No. 104358. Intertidal; shale outcrop at foot of Camino de la Costa, La Jolla, CA; 18 January 1977. Length 27 mm (in ethanol). Note well-developed valve ribs.

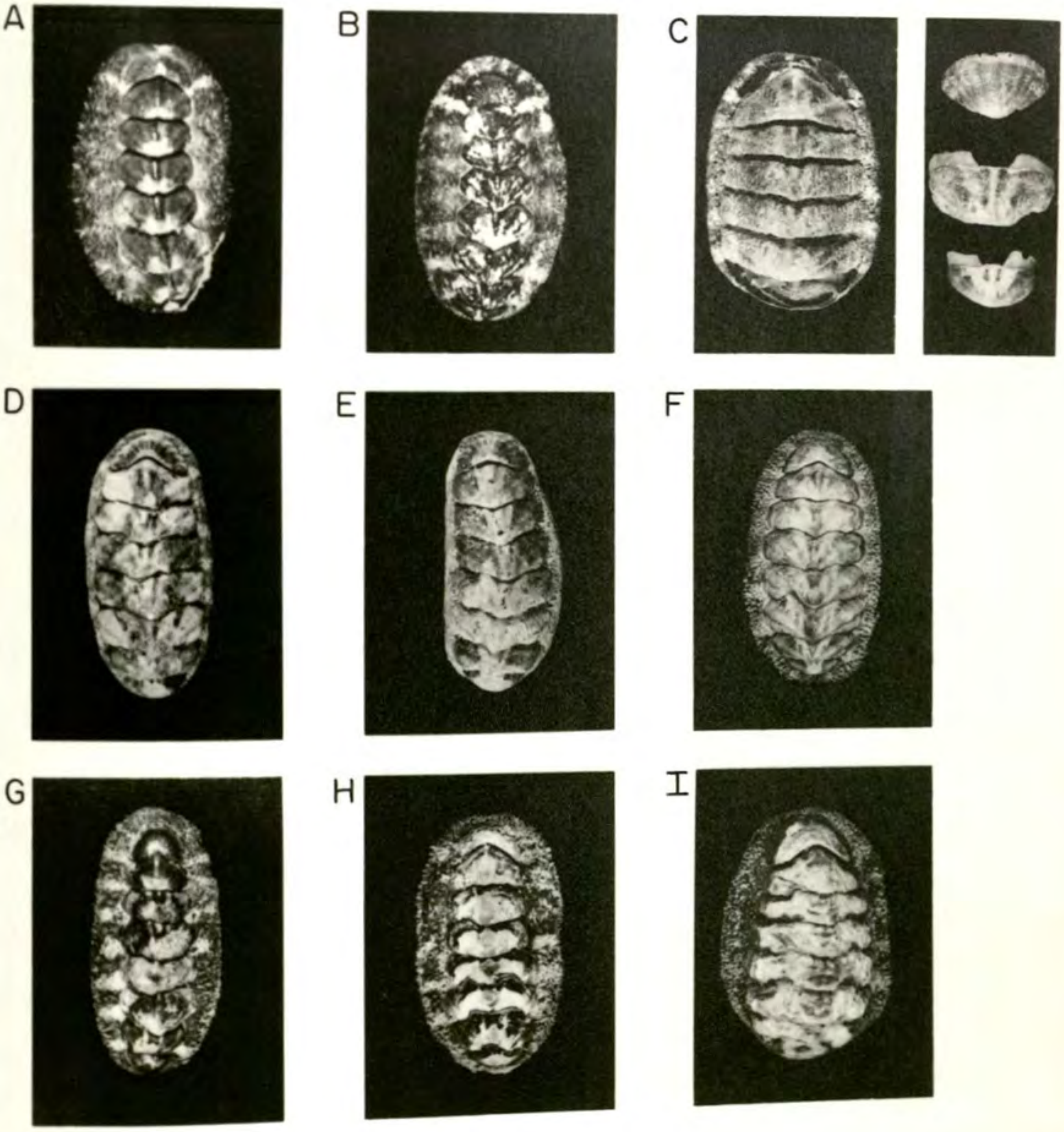

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Figure IV-8. Specimens in type lots at BMNH and ANSP referable to Nuttallina californica (Nuttall, in Reeve, 1847). The BMNH type lot consists of 7 specimens, the ANSP type lot of 2. Additional information for these lots can be found in Tables IV-11 and IV-12 and in the text.

A. Nuttallina californica. LECTOTYPE. BMNH No. 19798.
Referred to as Specimen No. 1 in text and tables. Length 39.2 mm (dry).

B. N. californica. PARALECTOTYPE. BMNH No. 19798.
Specimen No. 2. Length 38.1 mm (dry).

C. N. californica. PARALECTOTYPE. BMNH No. 19798.
Specimen No. 3. Length 35.7 mm (dry).

D. N. californica. PARALECTOTYPE. BMNH No. 19798.
Specimen No. 4. Length 35.1 mm (dry).

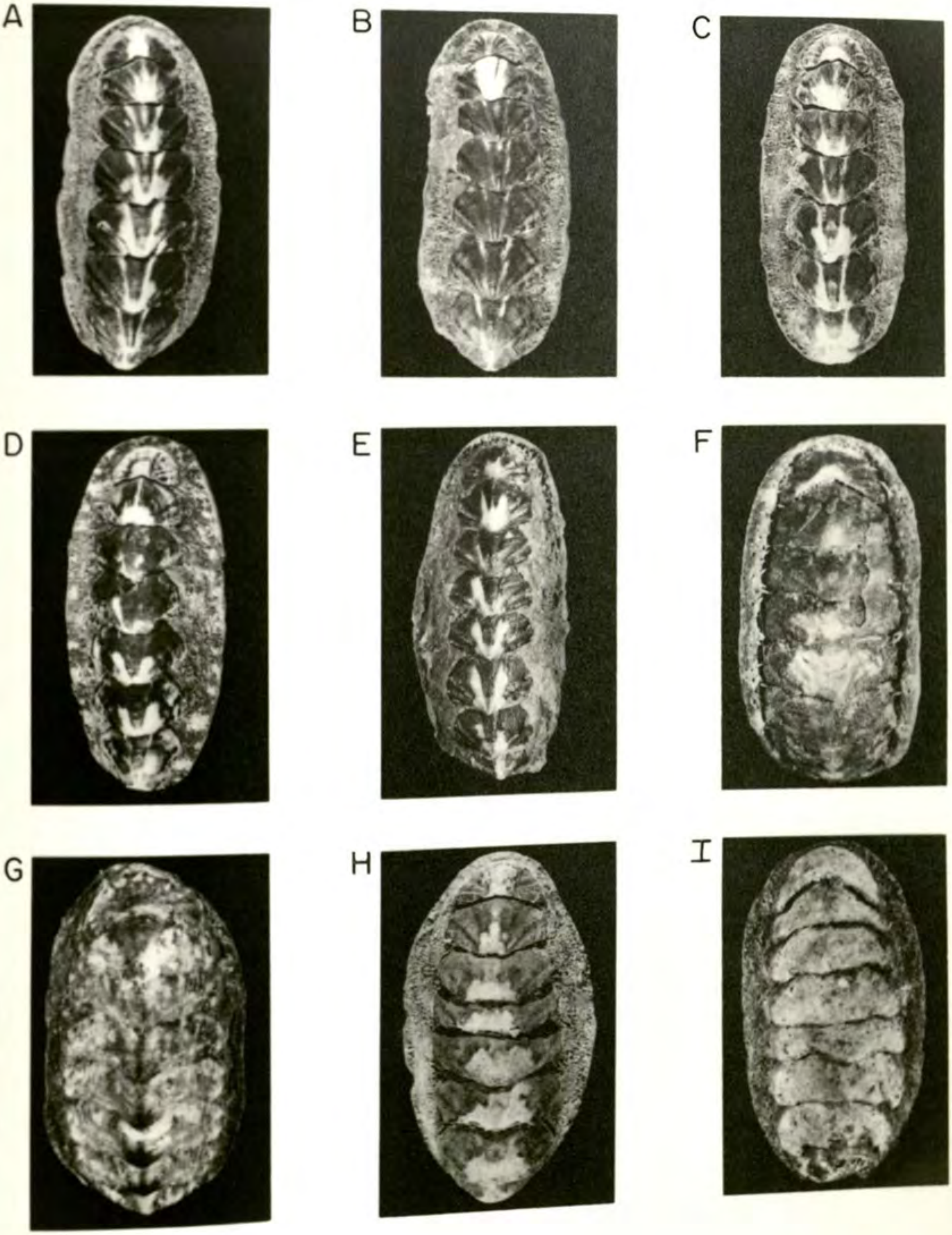
E. N. californica. PARALECTOTYPE. BMNH No. 19798.
Specimen No. 5. Length 27.1 mm (dry).

F. Nuttallina (?)fluxa (Carpenter, 1865). Probable species designation. BMNH No. 19798. Specimen No. 6. Length 14.6 mm (dry). There is a considerable amount of glue on valve surfaces.

G. (?)Chaetopleura sp. BMNH No. 19798.
Specimen No. 7. Length 13.0 mm (dry).

H. Nuttallina californica. PARALECTOTYPE. ANSP No. 118697.
Length 27.2 mm (dry).

I. Nuttallina (?)fluxa (Carpenter, 1864). Probable species designation. ANSP No. 118697. Length 19.5 mm (dry). Valves are extensively eroded.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labels with the holotype specimen state the type locality as "Sta. Barbara Cooper." However, Cooper (1867, p. 22), who probably collected the specimen and sent it to Carpenter, gives the locality as "Catalina I., or Santa Barbara I., Cal.". As the foregoing offers a choice as to type locality, Smith (1977) selected the first-cited one, Santa Catalina Island in the southern Santa Barbara Channel off Los Angeles County, California. Ferreira (1982) disregarded Smith's (1977) selection and selected Santa Barbara Island as the type locality.

In the present studies, no specimens of HI/DRY (= _Nuttallina fluxa_) were found at any of the three sites sampled around Santa Catalina Island, whereas many such specimens were identified from near Santa Barbara, CA (at Gaviota, Chapter III). The International Code of Zoological Nomenclature (ICZN, 1964) recommends using, among other choices for the information that may be used in selecting the type-locality, "the original description of the taxon" or "data accompanying the original material" (ICZN, Recommendation 72E). Therefore, I hereby select Santa Barbara (mainland), CA as the type-locality based on information on two original labels accompanying the holotype specimen.

Qualitative and quantitative observations of the _Nuttallina fluxa_ holotype specimen are presented in Table IV-10; the specimen is figured in Figure IV-7. The holotype is preserved dry, is complete and is in fairly good condition. Valves i, vii, and viii had been dissected out but are present. The foot is present, but drying has caused it to become semi-transparent, with the sutural plates of the valves visibly projecting against it. The pallial groove is indiscernible and the gill number and position of the most anterior gill cannot be determined. X-ray examination reveals that the radula is present, apparently in its entirety.

The Nuttallina fluxa holotype specimen is obviously a representative of the species referred to as HI/DRY in Chapter III. The extreme width and rectanguloid shape of the valves is never observed in BODEGAs (= N. californica) nor LOWs (= N. kata). The full complement of white stripes on the girdle is never seen in BODEGAs and the light brown color of the largely uneroded valves is almost never observed in BODEGAs. White spines are dispersed throughout the girdle unlike in BODEGAs. All three Nuttallina species not uncommonly possess 11 slits in valve i and 9 slits in valve viii as observed in the holotype.

Further support for the identification of the Nuttallina fluxa holotype as a HI/DRY comes from linear measurements. Valve vii was accessible for measurement with calipers since it was separate from the animal. Although this valve shows evidence of erosion at the posterior edge and some evidence of chipping of the sutural plates, the resultant total decrease in seventh valve total length (SVVTL) is certainly much less than 1 mm. Thus, using 5.3 mm as a conservative estimate for SVVTL (= 4.3 mm + 1 mm, see Table IV-10) and 7.0 mm for the seventh valve tegmentum width (SVVTW), the ratio SVVTW/SVVTL equals 1.3. A SVVTW/SVVTL ratio of 1.3 is well outside the range of values observed for either BODEGAs or LOWs (i.e., N. californica or N. kata, highest value = 1.21; Table IV-3), but well within the range observed for HI/DRYs (1.18-1.51).

Use of the multivariate classification functions of Table IV-8 for the available set of characters also supports the identification of the Nuttallina fluxa holotype as a HI/DRY (Table IV-15). Dried holotype total length, intact fourth valve width, and girdle width on one side of the fourth valve, converted to the equivalent frozen characters, CTL, FVW, FGW, by the conversion factors presented in Table IV-9, give 16.0, 8.0 and 3.0 mm respectively (see Table IV-10). Using classification routine 3 (Table IV-8) for CTL, FVW, and FGW, the classification function giving the highest score is that for HI/DRYs; that is, the holotype is classified as a HI/DRY. But caution must be exercised due to the variation in the frozen/dry conversion factors and the likelihood that the holotype total length is an underestimate because of the lack of support that results from the absence of three valves. 18.0 mm is probably a reasonable estimate for CTL. Using any combination of values in a range from 16 to 20 mm for CTL, 7.8 to 8.2 mm for FVW and 2.6 to 3.4 mm for FGW [the ranges used for FVW and FGW represent the (dry character)X(frozen/dry conversion factor, x ± s)], classification routine 3 still classifies the Nuttallina fluxa holotype as a HI/DRY. Thus, HI/DRYs of Chapter III can be confidently assigned the name Nuttallina fluxa (Carpenter, 1864).

Nuttallina californica (ANSP)

The oldest of the three labels accompanying the type specimens of Nuttallina californica (ANSP No. 118697) bears the note, in lettering that seems to match the lettering on the two newer labels, "EDP6120 ? From Type Lot", indicating that it is questionable whether the two specimens are actually from the type lot. A newer label with the specimens reads "probable syntypes." In reply to an inquiry about the labels, Dr. Robert Robertson, curator of molluscs at ANSP, stated that, in his opinion, Reeve, a British worker, likely never saw these specimens. The appearance of the specimens conforms to the original brief, undetailed description (Reeve, 1847), although neither specimen appears to be the N. californica (= Chiton californicus) specimen illustrated in Reeve (1847, figure 89 whole specimen and figure 89 valves vii and viii). Lacking any convincing evidence to the contrary, the two specimens are here regarded as type material attributable to Nuttallina californica (Nuttall, in Reeve, 1847). The type locality is "California," with no specific locale noted.

The specimens were examined and qualitative and quantitative observations are presented in Table IV-11; the specimens are figured in Figure IV-8. Both specimens have been preserved dry. The larger one is complete with all valves intact, with a piece of cardboard glued to the ventral surface. The valves show a small amount of erosion at the posterior margins. By detaching the cardboard at the anterior end of the specimen, it was found that at least part of the radula was present but detached, and that the foot and gills were absent, although what appeared to be a part of the intestine was present. The smaller specimen has all valves intact; most of the valve surfaces show signs of erosion, and most of the valve sculpture is gone. The foot, gills and insides are absent from the small specimen, with the posterior surfaces of the valves completely exposed.

An examination of qualitative characters of the large Nuttallina californica syntype clearly places it with the BODEGA species. The distinctive dark brown and largely uneroded tegmentum of the valves, the angularity of the valves with evidence of substantial sutural flesh encroachment, the lack of white stripes and the predominance of brown spines on a darkish-brown, extremely robust, wide girdle clearly establish this specimen as a BODEGA. The ratio of seventh valve width to length (SVVTW/SVVTL = 8.1/6.5 = 1.24) corrected for erosion in the length would fall in the range observed for BODEGAs (1.00-1.21, Table IV-3). Use of the classification functions for discriminating among the three species using CTL, FVW and FGW (classification routine 3, Table IV-8) places the large N. californica syntype specimen with BODEGAs when any combination of values within one standard deviation of the mean converted frozen CTL, FVW and FGW is used (Table IV-11; Table IV-15).

Examination of the qualitative characters of the small Nuttallina californica syntype suggests that it is not a BODEGA (Table IV-11). The presence of many white spines throughout the girdle clearly differentiates this syntype from BODEGAs. The extensive erosion of the valves, only occasionally seen in BODEGAs, and minimal sutural flesh encroachment provide further support for this conclusion. The intermediate shape of the valves and the medium brown color of the girdle without obvious stripes have been observed in specimens of all three Nuttallina species and thus offer no help in identifying the syntype. Use of the classification functions for discriminating between HI/DRYs and LOWs (Table IV-8, routine 21), or among all three species (routine 3), on the basis of converted frozen CTL, FVW, and FGW (from Table IV-11), places the small N. californica syntype with HI/DRYs, although use of high values (mean + standard deviation) of FGW places the specimen with BODEGAs (Table IV-15). In view of the numerous white spines on the girdle, the small N. californica syntype is probably a HI/DRY. In summary, the ANSP syntypes for Nuttallina californica (Nuttall, *in* Reeve, 1847) include a BODEGA (larger specimen) and probably a HI/DRY specimen.

Nuttallina californica (BMNH)

These seven specimens have been regarded as only "possible syntypes" by A. Blake (see Ferreira, 1982) and by S. Morris (*in litt*., 3 February 1983) of BMNH, because none of the seven appear to match the specimen figured with the original description (Reeve, 1847, pl. XVI, fig. 89). However, many of Reeve's (1847) drawings are from specimens in the H. Cuming collection stored in the British Museum (Dance, 1966) and these specimens are more likely to have been the ones examined by Reeve (1847) than are the ANSP specimens. The published locality is "California", which is here restricted to the shore near Bodega Marine Laboratory, Bodega Head, CA (38.3°N, 123.1°W), where the same species was positively identified in the present studies (see later).

Qualitative and quantitative observations on the specimens are presented in Table IV-12; the specimens are figured in Figure IV-8. All seven specimens have been preserved dry. Only the valves and girdle are present in all of the specimens, except in the smallest specimen in which the radula is also present. Generally, the valves are in quite good condition, not badly eroded. No foot, gills or viscera are present in any of the specimens. The specimens are referred to in order of size, the longest specimen as no. 1 and the shortest as 7, to facilitate discussion. Qualitative observations suggest that specimens no. 1 through 5 are BODEGAs, whereas 6 is possibly a HI/DRY and 7 is a representative of another chiton genus, probably Chaetopleura. General dark brown coloration of valves and girdle, predominantly brown girdle spines, moderate to extensive sutural flesh encroachment and fairly angular valves distinguish specimens no. 1 through 5 as BODEGAs. Predominant white girdle spines, numerous white girdle stripes, minimal to nonexistent sutural flesh encroachment and quadrangular valves indicate that 6 is probably a HI/DRY.

Identification based on qualitative observations are supported by use of the classification functions derived by discriminant analysis (Table IV-15). Use of classification functions for discriminating among all three Nuttallina species (routine 3; Table IV-8) or between BODEGAs and LOWs only (routine 15) on the basis of converted frozen CTL, FVW and FGW (Table IV-12), places specimens no. 1 through 4 consistently as BODEGAs, and classify specimen 5 as a BODEGA by the three species routine and a LOW by the two species routine. Use of either the three species classification functions (routine 3) or the two species functions for HI/DRYs and LOWs (routine 21) classifies specimen 6 as a HI/DRY.

Chiton scaber (BMNH)

These two specimens are labelled as only "possible syntypes" since neither matches the specimen figured with the original description (Reeve, 1847, fig. 106), although A. Blake (in litt. to Ferreira, 1982) and S. Morris (in litt., 3 February 1983) of BMNH (Mollusc Section) suggest that outlines on the original board on which the specimens had been attached indicate that a third, larger specimen is missing and it might have been the figured specimen. In addition, S. Morris (in litt., 3 February 1983) notes that her "experience with both the Cuming collection and Conch. Icon. illustrations permits [her] to justify the probability of type status [of these specimens] as established by Ms. Blake in 1979" (i.e., A. Blake in litt. to Ferreira, 1982). This lot is the only one extant that is referable to Chiton scaber Reeve, 1847; therefore, lacking evidence to the contrary, this lot is herein regarded as type material.

The originally published locality of Chiton scaber Reeve, 1847 is apparently erroneously listed as "Central America", and subsequent workers list "California" and the original specimen labels are marked "California" and "Monterey" (CA). Since Nuttallina fluxa was not found as far north as Monterey, CA in the present studies (see below and Chapter III), the type locality is here regarded simply as California.

Qualitative and quantitative observations on these two specimens are presented in Table IV-13; specimens are figured in Figure IV-7. Both specimens are preserved dry and only valves (intact) and girdle are present. Radulae, gills, foot and viscera are absent. Light to medium brown coloration of valves and girdle, predominating white spines throughout the girdle with only a few brown spines, and minimal sutural encroachment suggest that both specimens are HI/DRYs. Use of classification functions for frozen converted CTL, FVW and FGW for discriminating among all three species (Table IV-8, routine 3) and between HI/DRYs and LOWs (routine 21) indicate that both specimens, particularly the larger one, are HI/DRYs.

ASSIGNMENT OF NAMES

In the past, Chiton scaber Reeve, 1847 seems to have been treated variously as a synonym of the northern species Chiton californicus (= N. californica) as "scabra" (e.g., Carpenter, 1864; Dall, 1871), and as a synonym of the southern species Acanthopleura (= Nuttallina) fluxa Carpenter, 1864 (e.g., Pilsbry, 1893). However, as later discovered and noted by Pilsbry (1898), Chiton scaber Reeve, 1847 was preoccupied by Chiton scaber, Blainville, 1825. Chiton scaber Blainville, 1825 was a different species from the "Seas of New Holland" (Australia); it is presently assigned to a different genus, Acanthopleura (Kaas and Van Belle, 1980), and is currently a nomen inquirendum! (P. Kaas, in litt., 15 November 1982). Chiton scaber (or its derivative Nuttallina scabra) was and is not available for use in connection with the northeast Pacific Nuttallina species (Law of Homonymy, International Code of Zoological Nomenclature, 1964, Article 57, cf., Article 53, 59a).

The preoccupation of Chiton scaber and its unavailability for use with northeast Pacific Coast Nuttallina creates an interesting situation. Chiton scaber Reeve, 1847 is the type species of the genus Nuttallina by original designation (Dall, 1879). According to the International Code of Zoological Nomenclature (1964), the type species of a genus is unalterable. Thus, Chiton scaber Reeve, 1847 remains the type species of Nuttallina even though the name was preoccupied. Similar situations currently exist for other chiton genera, for example Callochiton Gray, 1847, Lepidochitona Gray, 1821, and Leptochiton Gray, 1847 (P. Kaas, in litt., 15 November 1982). Examination of the type material clearly establishes the synonymy of the type species of Nuttallina, Chiton scaber Reeve, 1847 (non Blainville, 1825), and Nuttallina fluxa (Carpenter, 1864). Article 60 (ICZN, 1964) specifies that a preoccupied name (Chiton scaber Reeve) is to be replaced by the next available name, which in this case is established by synonymy to be Nuttallina fluxa (Carpenter, 1864). This synonymy conflicts with those presented by Carpenter (1864), Smith (1977) and Ferreira (1982), all of whom designate Chiton scaber Reeve, 1847 = Chiton californicus Nuttall, in Reeve, 1847 [Smith (1977) may not have examined the type lots; Ferreira (1982) states that he did not examine the relevant type lots]. The larger Chiton scaber specimen is here selected as the lectotype of the type species Chiton scaber Reeve, 1847, the smaller one as the paralectotype.

Only two valid names remain for the West Coast Nuttallina, Nuttallina fluxa (= Acanthopleura fluxa Carpenter, 1864) and Nuttallina californica (= Chiton californicus Nuttall, in Reeve, 1847). The holotype for Nuttallina fluxa Carpenter (USNM No. 15690b) clearly matches the HI/DRY morph. Thus, Nuttallina fluxa can be applied to the southern species of Nuttallina that tends to occur higher on the shore. This designation agrees with the historical conception of N. fluxa as a southern form (see Smith, 1977).

For Nuttallina californica (Nuttall, in Reeve, 1847), the largest specimen in the BMNH lot is here designated as the lectotype and the remaining 5 specimens in both the BMNH (4) and ANSP (large one) lots that are of the same (BODEGA) morph as the lectotype are designated as paralectotypes. It should be mentioned that Ferreira (1982) selected a specimen of the *Chiton scaber* type lot at BMNH (the missing, supposed figured one) as the lectotype for *Nuttallina californica*. However, he synonymized the *Nuttallina* species that are considered in the present study and he did not examine the relevant type lots. Thus, Ferreira's (1982) lectotype designation for *Nuttallina californica* has been disregarded. The rationale for my choice of lectotype is as follows: 1) the BMNH lot is in better condition than the ANSP lot, includes more specimens that can be unambiguously identified as a particular morph, and is the lot most likely to have been examined by Reeve since it is in Britain; 2) the lectotype specimen is in generally good condition, at least as good condition as any of the other *N. californica* type material, and is unequivocally assignable to a species, the species to which the majority of the type material belongs; 3) the lectotype matches the brief description for *Chiton* (= *Nuttallina*) *californicus* in Reeve, 1847 and also in the work of the first major reviser Pilsbry (1893, p. 279), who noted in particular the presence of "... spinelets, mostly of a scorched brown color with a few white spines intermingled." Establishment of an equivalence between BODEGAs and *Nuttallina californica* type material serves to maintain the stability of the nomenclature. Pilsbry (1893), Raymond (1894) and Smith (1977) established that *Nuttallina californica* occurs north of Point Conception, CA (like BODEGAs) to Vancouver Island, whereas *Nuttallina fluxa* (HI/DRY) occurs south of Point Conception into Baja California.

No available name remains for the LOW species of *Nuttallina*, newly-discovered in Chapter III and distinguished from BODEGAs [= *Nuttallina californica* (Nuttall, *in* Reeve, 1847)] and HI/DRYs [= *Nuttallina* fluxa (Carpenter, 1864)] on the basis of electrophoretic phenotype, external morphology and natural history. LOWs are thus assigned a new name, Nuttallina kata. Type locality for this new species is the Seal Rock area, La Jolla, San Diego Co., CA.

SYSTEMATIC ACCOUNT

Phylum: Mollusca Jonston, 1650

Class: Polyplacophora Gray, 1821

Order: Neoloricata Bergenhayn, 1955

Suborder: Ischnochitonina Bergenhayn, 1930

Family: Lepidochitonidae Iredale, 1914 or Callistoplacidae Pilsbry, 1893

Genus: Nuttallina Carpenter, in Dall, 1879, p. 333

Diagnosis: "Elongate, medium sized chitons with granulose tegmentum. Head valve with low radiating ribs; intermediate valves with 2 low ribs on lateral areas; mucro definitely posterior. Sutural laminae well developed, elongate; sinus deep. Insertion teeth of posterior valve directed forward. Intermediate valves with 1 or 2 slits, the posterior one often obsolete. Eaves spongy. Girdle covered with small spiculoid scales and pointed spines." (Ferreira, 1982).

Type Species: Chiton scaber Reeve, 1847 (not Blainville, 1825) [= Nuttallina fluxa Carpenter, 1864], by original designation.

Remarks: Several major classification schemes have been proposed for chitons, e.g., Pilsbry (1892-94), Thiele (1929), Bergenhayn (1955), Smith (1960, following Berhenhayn primarily), but no scheme has gained general acceptance and a new one is currently being proposed (Van Belle, 1975-1978; Kaas and Van Belle, 1980). Taxa at the generic to ordinal level are quite variable from scheme to scheme. The genus *Nuttallina* has often been placed in Lepidochitonidae, and occasionally in the Callochitonidae or Ischnochitonidae, by workers who do not recognize Callistoplacidae, e.g., Iredale (1914), Dall (1921), Berry (1922), Thiele (1929), Leloup (1956), Thorpe (in Keen, 1971), McLean (1978), Ferreira (1982), and in the Callistoplacidae by workers who do not recognize Lepidochitonidae, e.g., Bergenhayn (1955) and Smith (1960, 1977).

The genus name *Nuttallina* had been introduced earlier by Dall, 1871 and Carpenter, 1873, based on Carpenter's unpublished manuscript, without description; thus, earlier publications are not valid as a source of the genus name (and see Smith, 1977). The type species was listed as *Chiton scaber* Reeve, 1847 in Dall (1879), which he regarded as separate from *Chiton californicus* Nuttall, *in* Reeve, 1847. However, it appears that Dall (1879) regarded *Chiton scaber* Reeve as a northern species. Pilsbry (1893) likewise regarded *Chiton californicus* Nuttall, *in* Reeve, 1847 and *Chiton scaber* Reeve, 1847 as separate species, but he designated *C. californicus* as the type species of *Nuttallina* and regarded *Chiton scaber* Reeve as a southern species. At least some of the confusion can be traced to a large unpublished manuscript produced by Carpenter in the early 1870's (cf., Bullock, 1972; Smith, 1977); several workers, including Dall, Haddon and Pilsbry, had access to the manuscript [much of it in shorthand and now stored in the U.S. National Museum of Natural History (Bullock, 1972)] and introduced several manuscript names that were later synonymized or validated.

KEY TO THE NORTHEAST PACIFIC COAST SPECIES OF NUTTALLINA

1a. Girdle spines predominantly brown . . . 2

1b. Girdle spines predominantly white . . . 3

2a. Most anterior gill or gill bud to beneath valve ii and average number of gills (left, right sides) usually greater than 35 (gill number varies with chiton length; see Figure IV-4); girdle usually dark brown to black, not fuzzy in appearance, and fairly wide (one side at valve iv ~20% of chiton total length); flesh encroachment at valve sutures moderate to quite extensive; white spines, if present, often in clumps of 2 to 5 and more numerous along girdle edge; almost never complete white girdle stripes near valves ii to iv; up to about 65 mm in live length; under defined conditions, in relation to the other two Nuttallina species, the isozymes Adh, Mdh-2, Pgm, and Sdh-2 exhibit slow mobility on electrophoretic starch gels, while Sdh-1 exhibits fast relative mobility . . . Nuttallina californica

2b. Most anterior gill or gill bud to beneath valve iii or iv and average number of gills (left, right sides) fewer than 32 (see Figure IV-4); girdle light to dark brown, fuzzy in appearance, and not wide (one side at valve iv ~15% of chiton total length); flesh encroachment at valve sutures slight to moderate; often with complete white girdle stripes near valves ii to iv; up to a maximum live length of about 42 mm; under defined conditions, in relation to the other two Nuttallina species, the isozymes Adh, Pgm, and Sdh-1 exhibit fast mobility on electrophoresis starch gels, while Mdh-2 and Sdh-2 exhibit slow relative mobility . . . Nuttallina kata.

3a. Valves often quite wide per valve length; most anterior gill or gill bud beneath valve ii or iii and average number of gills (left, right sides) usually between 26 and 45 (gill number varies with chiton length; see Figure IV-4); valves fairly quadrangular with minimal flesh encroachment at the valve sutures; up to a maximum live length of about 52 mm; under defined conditions, in relation to the other two Nuttallina species, the isozymes Pgm, Mdh-2, and Sdh-2 exhibit fast mobility on electrophoretic starch gels, while Sdh-1 exhibits slow and Adh intermediate relative mobility . . . Nuttallina fluxa.

3b. Valves narrow to medium in width per valve length; most anterior gill or gill bud beneath valve iii or iv and average number of gills (left, right sides) fewer than 32 (Figure IV-4); valves triangular to ovoid with slight to moderate flesh encroachment at the valve sutures; up to a maximum live length of about 42 mm; under defined conditions, in relation to the other two Nuttallina species, the isozymes Adh, Pgm, and Sdh-1 exhibit fast mobility on electrophoresis starch gels, while Mdh-2 and Sdh-2 exhibit slow relative mobility . . . Nuttallina kata.

SPECIES DESCRIPTIONS

Formal descriptions for the three Nuttallina species follow. Detailed information and explanation has been presented in the foregoing figures, tables and text. Descriptions of N. fluxa and N. californica omit details that do not differ substantially from the description of N. kata.

An attempt has been made to provide complete synonymies for each species. Most literature references do not provide sufficient information to allow accurate morphological identification of the specimens involved by the criteria established earlier in the present chapter; therefore, I have relied heavily on the geographic location or range cited in the reference, and on whether the worker recognized one or two Nuttallina species, in deciding which species are referred to. In general, in the absence of additional information about the chitons, references citing locations south of Point Conception, CA are considered to involve both N. fluxa and N. kata, while references to locations north of Monterey Bay, CA probably deal with N. californica. Reference to the area from Point Conception, CA to Monterey Bay, CA are considered likely to deal with N. californica, and possibly with N. kata. Examination of Nuttallina specimens collected from Guadalupe Island, Baja CA, Mexico (Chace, 1958; Smith, 1963) reveals that they might belong to N. kata, N. crossota or N. fluxa, but probably not to N. californica (see Table III-8, Chapter III). A single Nuttallina specimen reported from Chile by Nierstrasz, 1905 (also Leloup, 1956; Stuardo B., 1959) has been determined to most likely be a Nuttallina fluxa specimen from the Northern Hemisphere involved in a labelling mix-up (see Appendix III), and these references therefore are not included in synonymies.

In synonymies, a question mark preceding an author's name or a figure number denotes *possible* reference to that species. A question mark preceding "in part" denotes *certain* reference to this species and *possible* additional reference to one or both of the other *Nuttallina* species.

Nuttallina kata sp. nov. (Holotype, Figure IV-7 and summary of characters in Table IV-14; specimen with eroded valves, Figure IV-7).

Synonymy.

Acanthopleura scabra (Reeve, 1847). (?) Cooper, 1870:59, in part (Monterey Bay, CA).

Nuttallina scabra (Reeve, 1847). (?) Dall, 1871:134, in part (Monterey, CA area), Pilsbry, 1893:280-281, plt. 54, figs. 21, 22; plt. 56, figs. 19, 20 (with syns. *Chiton scaber* Reeve and *Acanthopleura fluxa* Carpenter), in part; (?) Thiele in Troschel, 1893:394, plt. 32, fig. 16 (radular dentition); (?) Wood, 1893:72, in part (Monterey Bay, CA); Raymond, 1894:133-134, in part; Keep, 1904:267, 349, in part; 1911:258, in part; 1935:29, in part; (?) Nierstrasz, 1905a:149 (Monterey, CA and "California"); Nierstrasz, 1905b:43, in part (Santa Barbara and San Diego, CA); M. Smith 1907:67, in part; (?) Lowe, 1913:28 (South Bay at Cedros Island, Baja CA, Mexico), Iredale, 1914:130 (*C. scaber* preoccupied, *A. fluxa* might be used), in part; (?).

Chiton (*Nuttallina*) *scabra* Reeve, 1847. Orcutt, 1885:544, in part (San Diego, CA).

Nuttallina californica (Nuttall, *in* Reeve, 1847). (?) Raymond, 1894:133-134, in part; (?) Berry, 1907:52, in part (listed only, Monterey, CA area); (?) Berry, 1922:409-410, 415, 418, 420, 439-441, Table 1, plt. 3, figs. 1-12, in part (Pleistocene); Johnson and Snook, 1927, 1955, 1967:562, in part, probably not fig. 662; (?) Smith and Gordon, 1948:206, in part (Monterey Bay, CA); MacGinitie and MacGinitie, 1949, 1968:386, in part, fig. 241, particularly 241B; (?) Chace, 1958:330 (Guadalupe Island, Baja CA, Mexico); Ricketts and Calvin, 1968:198-199, in part, not fig. 154; (?) Burghardt and Burghardt, 1969a:228 (less than 8 valves; Carmel, Monterey Co., CA); (?) Andrus and Legard, 1975:7, Table 1, in part (Monterey Peninsula, CA); (?) Gomez, 1975, in part (Monterey Co., CA; in association with *Cyanoplax dentiens*); (?) Linsenmeyer, 1975, in part (Monterey Peninsula, CA; resistance to removal from substrate); (?) Moore, 1975, in part (Monterey Co., CA; gull predation); (?) Nishi, 1975, in part (study on diet in Monterey Co., CA; apparently includes *N. kata* as well as *N. californica* as judged by notes on girdle spine color, and by location); (?) Piper, 1975, in part (Monterey Co., CA; blood ions); (?) Robbins, 1975, in part (Monterey Co., CA; respiration); (?) Simonsen, 1975, in part (Monterey Co., CA; osmoregulation); (?) Tenold, 1975, in part (Monterey Co., CA; tissue lipid, glycogen); Morris et al., 1980:419-420, in part, *not* fig. 16.17; (?) Ituarte, 1981, in part, (Santa Cruz Co., CA); (?) Putman, 1981:349, in part (Shell Beach, San Luis Obispo Co., CA); (?) Putman, 1982:365, in part (Diablo Cove vicinity, San Luis Obispo Co., CA).

Nuttallina fluxa (Carpenter, 1864). Pilsbry, 1898:290, in part (with syn. *Chiton scaber* Rve., 1847, not of Blainville, 1825); Dall, 1921:190, in part; (?) Berry, 1922:441, in part (Pleistocene); Oldroyd, 1927:866-867 (new name for *N. scabra* Reeve), in part; (?) Berry, 1935:89-90, 2 figs. (9 valves); A. Smith, 1947a:16, in part; 1947b:18, in part (listed only); (?) A. Smith, 1963:148 (south Guadalupe Island, Baja CA, Mexico); Kues, 1969, in part (primarily in "low intertidal"; ecology, physiology); (?) Burghardt and Burghardt, 1969a:228 (less than 8 valves; Refugio Beach, San Luis Obispo Co., CA); 1969b:32, in part, probably plt. 4, fig. 66, in color; Abbott, 1974:400, in part, (?) fig. 4694a (with syn. *Chiton scaber* Reeve, 1847, not Blainville, 1825); Allen, 1976:123, in part, (?) fig. 123; A. Smith, 1977:241-242 in part (= *Acanthopleura fluxa* Carpenter, 1864, with explanation of taxonomic history); McLean, 1978:61-62, in part, (?) fig. 34-4; Piper, 1980, in part (in low intertidal area); Putnam, 1980:129-130, in part (*N. fluxa* suggested to be junior synonym of *N. californica*); Vesco, 1980, in part (primarily in low "coralline algal community"; ecology, behavior).

Nuttallina cf. *N. fluxa* (Carpenter, 1864). (?) Berry, 1918:63, in part; (?) Chace and Chace, 1919:2, in part (Pleistocene); (?) Berry, 1922:403, 439, in part (Pleistocene).

Nuttallina californica (Reeve, 1847) var. *scabra* (Reeve, 1847). Leloup, 1940:3, 25-27, in part.

Acanthopleura fluxa Carpenter, 1864. A. Smith, 1977:241-242, in part (= *Nuttallina fluxa* Carpenter).

Description.

Chiton of moderate size, to about 4 cm maximum live length. Elongate oval in outline, typically more than twice as long as wide. Moderately elevated.

Valve surface typically mottled light brown, occasionally darker brown, one or two of the intermediate valves occasionally white or a mosaic of black on white. Erosion, particularly of posterior margins of valves, quite common, often giving the valves a white or gray to green tint. Intermediate valves with from zero to 3 white stripes on or near the jugal and jugal/lateral ribs.

Interior of valves varies from almost white through light brown, dark brown, black and/or light blue. Central portion often dark or nearly black and peripheral areas lighter, white or light blue.

All exposed dorsal valve surfaces typically granulose in uneroded specimens although occasionally jugal area may be more or less smooth. Anterior valve with low rounded radiating ribs when uneroded. Lateral areas of intermediate valves not raised but set off by low lateral ribs. Valve viii usually eroded, mucro posterior to terminal. Anterior valve possessing 8 to 12 slits (mode=10). Intermediate valves with one or two slits, the posterior of the two often only a punctulate slit-ray tending to obsolescence. Valve viii with 6 to 9 slits (mode=7).

Intermediate valves from moderately elevated to rounded or flat. Intermediate valves tend to be narrow per chiton length. The tegmentum in uneroded specimens tends to be rounded triangular in outline, erosion tending to straighten the posterior margin. Posterior margin of uneroded intermediate valves typically angular (60 to 70° angle with the jugal ridge; a 120 to 140° divergence). Anterior margin of the tegmentum often nearly straight, occasionally moderately single, double or triple lobed. Sutural flesh encroachment typically small to moderate, on either side typically 10% to 15% of the full width of an adjacent valve.

Girdle relatively narrow (a single side at the fourth valve about 15% of the chiton thawed total length). Girdle epidermis densely-set with short rod-like scales (50 to 70 µm long in 25 mm long specimens), typically forming alternating girdle stripes of white and light brown extending from the valves to the girdle edge, although the girdle is occasionally all rusty brown, especially in specimens from the Channel Islands, CA. Numerous white and light brown spines are set throughout the girdle width both in areas of white and of brown scales, giving the girdle a "fuzzy" appearance. Both white and brown spines occur in densities of 200 to 700 per cm^2; white spines typically outnumber browns but the reverse is not uncommon. Overall girdle spine densities vary from 300 to 1000 per cm^2. Spines typically increase in density near the girdle edge; white but not brown spines typically account for the increase. White spines are 800 to 1050 µm in maximum length in 30 mm long specimens, while brown girdle spines are shorter, about 400 to 850 µm in maximum length. The underside of the girdle is densely set with small scales that are a conspicuous orange-brown even in preserved specimens.

Ctenidia abanal, merobranchial. Ctenidia separated by a space from the anus and ctenidial bases extending from beneath the seventh to beneath the suture of the third and fourth valves. Gills increase in number with chiton length from about 20 in 10 mm long preserved specimens to a maximum of about 32 per side in the longest specimens. Gill numbers commonly unequal on the two sides. Inner curtain-like mantle fold lateral to the gills.

Under defined conditions, in relation to the other two _Nuttallina_ species, the isozymes Adh, Pgm and Sdh-1 exhibit a "fast mobility" phenotype on electrophoretic starch gels, while Mdh-2 and Sdh-2 exhibit "slow" relative mobility.

Distribution.

Prevalent in the Southern California Bight area, including the Channel Islands. Confirmed from as far north as Monterey Bay, CA (SIO M1924) and Shell Beach, San Luis Obispo, Co., CA (LACM 61-11). Confirmed from as far south as Punta Abreojos (SIO M860), Bahia Tortugas (SIO M952) and East San Benito Island (AHF 1946-50), Baja California, Mexico, although the next most southerly confirmed occurrence is far north of this, at Punta Banda, Baja CA, Mexico (collection for electrophoresis, Chapter III). Its frequency of occurrence from the Mexican border south remains to be elucidated.

Habitat.

Tends to occur low in the rocky intertidal zone from about 0-30 to 90 cm above mean lower low water. Occasionally found in tidepools and wetter areas higher up. Typically found in close-fitting depressions in the substrate in association with coralline algae, occasionally at very high densities (to about 1500-2000 per m^2).

Material Examined.

Type Material: Holotype (Figure IV-7). Type Locality: Seal Rock area, La Jolla, San Diego Co., CA, designated herein. 263 additional specimens. 241 specimens were electrophoretically-phenotyped: Whites Point, L.A., CA, 3 April 1981, N=26; La Jolla, CA, February through April, November 1981 and May 1982, N=96; Punta Banda, south side, Baja CA, 2 April 1981, N=2; USC Marine Science Center, Santa Catalina Island, L.A. Co., CA, 13 April 1981, N=38; Little Harbor, S. Catalina Isl., 14 April 1982, N=37; Avalon, S. Catalina Isl., 15 April 1981, N=38; San Nicolas Island (NW), Ventura Co., CA, 30 August 1981, N=4. Ten specimens were not electrophoretically-phenotyped, from collections above for electrophoretic study: USC Marine Science Center, S. Catalina Island, N=7; Avalon, S. Catalina Isl., N=2; Whites Point, CA, N=1. Twelve specimens were in preserved museum collections: SIO M1924, Hopkins Marine Station, Pacific Grove, Monterey Co., CA, 29 December 1977, N=3; LACM 61-11, Shell Beach, San Luis Obispo Co., CA, Leg J. H. McLean 1961-1963, N=3; LACM 66-51, San Clemente Island, Wilson Cove area, L.A. Co., CA, 13 September 1966, N=1; AHF 1946-50, East San Benito Island, Baja CA, 26 April 1950, N=2; SIO M952, Bahia Tortugas, 1975, N=2.

Etymology.

Named kata, the Greek for down or below, in reference to the tendency for this species to occur lower on the shore than the other northeast Pacific Nuttallina species, particularly where it co-occurs with those species. Additionally, kata is quite similar to the author's wife's name (Katy) and represents recognition and gratitude for her help in these studies.

Nuttallina fluxa (Carpenter, 1864) (Holotype, Fig. IV-7; hypotype, Fig. IV-7; specimen with badly-eroded valves, Figure IV-7).

Synonymy.

Chiton scaber Reeve, 1847, species no. 106, plt. 17, fig. 106, (not Chiton scaber Blainville, 1825, which preoccupied name, rendering it unavailable); Carpenter, 1857:290, 317, (?) 229 (compilation of reports of different authors).

Acanthopleura fluxa Carpenter, 1864:612, 649, nomen nudum; and reprint 1872:98, 135; 1866:211. Cooper, 1867:22; A. Smith, 1977:241-242, in part (= Nuttallina fluxa).

Chiton (*Nuttallina*) *scabra* Reeve, 1847. Tryon, 1883:344, plt. 86, fig. 85.

Chiton (*Nuttallina*) *scabra* Reeve, 1847. Orcutt, 1885:544, in part (San Diego, CA).

Nuttallina scabra (Reeve, 1847). Pilsbry, 1893:280-281, plt. 54, figs. 21, 22; plt. 56, figs. 19, 20 (with syns. *Chiton scaber* Reeve and *Acanthopleura fluxa* Carpenter), in part; (?) Thiele in Troschel, 1893:394, plt. 32, fig. 16 (radular dentition); Raymond, 1894:133-134, in part; Keep, 1904:267, 349, in part; 1911:258, in part; 1935:29, in part; (?) Nierstrasz, 1905a:149 ("California"); Nierstrasz, 1905b:43, in part; M. Smith 1907:67, in part; (?) Lowe, 1913:28 (South Bay, Cedros Island, Baja CA, Mexico), Iredale, 1914:130 (*C*. *scaber* preoccupied, *A*. *fluxa* might be used), in part.

Nuttallina californica (Nuttall, *in* Reeve, 1847). (?) Raymond, 1894:133-134, in part; (?) Berry, 1922:409-410, 415, 418, 420, 439-441, Table 1, plt. 3, figs. 1-12, in part (Pleistocene); Johnson and Snook, 1927, 1955, 1967:562, in part, probably not fig. 662; MacGinitie and MacGinitie, 1949, 1968:386, in part, fig. 241, primarily 241A; (?) Chace, 1958:330 (Guadalupe Island, Baja CA, Mexico); Ricketts and Calvin, 1968:198-199, in part, not fig. 154; Morris et al., 1980:419-420, in part, *not* fig. 16.17.

Nuttallina fluxa (Carpenter, 1864). Pilsbry, 1898:290, in part (with syn. *Chiton scaber* Rve., 1847, not of Blainville, 1825); Dall, 1921:190, in part; (?) Berry, 1922:441, in part (Pleistocene); Oldroyd, 1927:688- 867 (new name for *N*. *scabra* Reeve), in part; (?) Berry, 1935:89-90, 2 figs. (9 valves); A. Smith, 1947a:16, in part; 1947b:18, in part (listed only); Palmer, 1958:265-266, plt. 28, figs. 16-19 (holotype); (?) A. Smith, 1963:148 (south Guadalupe Island, Baja CA, Mexico); Kues, 1969, in part (primarily in "high intertidal"; ecology, physiology); (?) Burghardt and Burghardt, 1969a:228 (less than 8 valves; Refugio Beach, San Luis Obispo Co., CA); 1969b:32, in part, probably not plt. 4, fig. 66, in color; Louda, 1972 (ecology; probably primarily *N. fluxa* as judged by location of study and reported valve widths; Abbott, 1974:400, in part, (?) fig. 4694a (with syn. *Chiton scaber* Reeve, 1847, not Blainville, 1825); Allen, 1976:123, in part, (?) fig. 123; A. Smith, 1977:241-242, in part (= *Acanthopleura fluxa* Carpenter, 1864, with explanation of taxonomic history); McLean, 1978:61-62, in part, (?) fig. 34-4; Piper, 1980, in part (in high intertidal area primarily); Putnam, 1980:129-230, in part (*N. fluxa* suggested to be junior synonym of *N. californica*); Vesco, 1980, in part (primarily in "barnacle community"; ecology, behavior).

Nuttallina cf. *N. fluxa* (Carpenter, 1864). (?) Berry, 1918:63, in part; (?) Chace and Chace, 1919:2, in part (Pleistocene); (?) Berry, 1922:403, 439, in part (Pleistocene).

Nuttallina californica (Reeve, 1847) var. *scabra* (Reeve, 1847). Leloup, 1940:3, 25-27, in part.

Description.

Chiton of moderate size, to about 5 cm maximum live length (42 mm thawed, 39 mm preserved). Elongate oval in general outline, typically about twice as long as wide. Moderately elevated.

Intermediate valves with from zero to three white stripes, typically three or one, on or near the jugal and jugal/lateral ribs. Lateral areas of intermediate valves not raised but set off by more or less distinct broad lateral ribs. Anterior valve possessing 9 to 11 slits (mode=10). Intermediate valves with one or two slits, the posterior of the two often only a punctulate slit-ray tending to obsolescence. Valve viii with 7 to 10 slits (mode=10).

Intermediate valves most often rounded to almost flat, occasionally moderately elevated. Intermediate valves tend to be quite wide and short per chiton length, although slightly less wide in specimens from Gaviota, CA. The tegmentum in uneroded specimens tends toward a quadrate outline, erosion of the posterior margin advancing the tendency or even making the posterior concavely arcuate. Posterior margins of uneroded valves tending toward co-linearity (70 to 80° angle with the jugal ridge; a 140 to 160° divergence), although more angular in specimens from Gaviota, CA. Lateral edges of lateral areas tend toward parallel with the jugal ridge. Sutural flesh encroachment typically small to almost nonexistent, on either side typically less than 10% of the full width of an adjacent valve.

Girdle relatively narrow (a single side at the fourth valve about 15% of the thawed chiton total length). Girdle epidermis densely-set with short rod-like scales (50 to 70 µm long in 25 mm long specimens), typically forming alternating girdle stripes of white and light brown extending from valves to girdle edge, although the girdle is rarely all rusty brown. Numerous white and light brown spines are set throughout the girdle width both in areas of white and of brown scales, giving the girdle a "fuzzy" appearance. Overall spine densities are variable from 300 to 1000 per cm^2. White girdle spines typically far outnumber brown spines, although brown spines do rarely outnumber white ones. White spines typically occur in densities of 200 to 700 per cm^2 whereas brown spines usually number less than 100 per cm^2.

Ctenidia abanal, nearly holobranchial. Ctenidia separated by a space from the anus, and ctenidial bases extending from beneath the suture of valves vii and vi to beneath valve ii or iii. Gills increase in number with chiton length from about 26 gills per side in 10 mm long preserved specimens to a maximum of about 47 gills per side in the longest specimens. Gill numbers commonly unequal on the two sides. Inner curtain-like mantle fold lateral to the gills.

Under defined conditions, in relation to the other two <u>Nuttallina</u> species, the isozymes Pgm, Mdh-2 and Sdh-2 exhibit a "fast mobility" phenotype on electrophoretic starch gels, while Sdh-1 exhibits a "slow" and Adh an "intermediate" relative mobility.

Distribution.

Confirmed in this study from as far north as Gaviota, just north of Santa Barbara, CA, in abundance (in collection for electrophoresis, Chapter III), and from as far south as Punta Abreojos, Baja CA, Mexico (SIO M795; SIO M800; SIO M888). Probably occurring as far south as Magdalena Bay, Baja CA. Not found in collections examined from San Miguel, Santa Catalina, San Clemente or San Nicolas Islands of the Channel Islands off California. As a warmwater species, found in abundance on mainland southern California south of Point Conception and expected to occur particularly in warmwater embayments along the coast of Baja California. Not found in any abundance at southern side of Punta Banda, Baja CA, a coldwater upwelling area.

Habitat.

Tends to occur high on the shore, particularly when larger/older and when *N. kata* present, from about 90 to 180 cm above mean lower low water. Adults typically not in close-fitting depressions but more often near bases of mussel beds, near barnacles, in shallow hollows, in tidepools or on dry bare rock. Densities commonly less than 200 per m^2.

Material Examined.

Type Material: Holotype, *Nuttallina fluxa*, USNM 15690b (Fig. IV-7); type locality (designated herein): mainland Santa Barbara, CA. Lectotype and paralectotype of *Chiton scaber* Reeve, BMNH Reg. No. 19799 (Fig. IV-7), designated herein; type locality: "California" (label accompanying material).

221 additional specimens. 163 specimens were electrophoretically phenotyped: Gaviota, CA, 16 April 1981, N=52; Whites Point, L.A., CA, 3 April 1981, N=29; La Jolla, CA, Feb-April, Nov 1981, May 1982, N=80; Punta Banda, Baja CA, 2 April 1981, N=2. Twelve specimens were not electrophoretically phenotyped, from collections above for electrophoretic study: Gaviota, CA, N=6; Whites Point, CA, N=6. Forty-five specimens were in preserved museum collections from Baja CA, Mexico: SIO No. M1042, Punta Baja, 29°57′N, high intertidal, 9 August 1975, N=1; SIO M979, Laguna Manuela Point, high intertidal, 4 August 1975, N=13; AHF 1946-50, East San Benito Island, 26 April 1950, N=1; SIO M952, Bahia Tortugas, 27°41′N, mid-intertidal, 31 July 1975, N=12; SIO M570, Turtle Bay, East of San Bartolome, 7 February 1948, N=2; SIO M1745, Turtle Bay, 15-18 February 1969, N=7; SIO M795, SIO M800, SIO M888, Punta Abreojos, 26°44′N, 23-27 July 1975, N=9.

Nuttallina californica (Nuttall, *in* Reeve, 1847) (lectotype and four paralectotypes, BMNH Reg. No. 19798, and one additional paralectotype, ANSP No. 118697, all designated herein, Figure IV-8; hypotype, LACM No. 104357, Figure IV-7; specimen from Shell Beach, CA, LACM No. 61-11, Figure IV-7).

Synonymy.

Chiton californicus Nuttall, in Reeve, 1847, plt. 16, species no. and fig. 89, and detail of sculpture of valves vii and viii of species no. 89; Carpenter, 1857:229 (attributes species to Gould; as synonym of *C. scaber* Reeve).

Acanthopleura scabra (Reeve, 1847). Carpenter, 1864:649 and reprint 1872:135 (as syn. of *californicus*, Nutt.); Cooper, 1867:22; Cooper, 1870:59, (?) in part (Monterey Bay, CA).

Nuttallina scabra (Reeve, 1847). Dall, 1871:134, (?) in part (Monterey Bay, CA); Dall, 1879:298, 333, pl. IV, fig. 31 (radula) (with syns. *Chiton scaber* Reeve; *Chiton californicus* Nutt. in Carpenter; *Acanthopleura scabra* Carpenter; *not Chiton californicus* Nutt., *in* Reeve); Wood and Raymond, 1891:58 (San Francisco Co., CA); (?) Thiele in Troschel, 1893:394, plt. 32, fig. 16 (radular dentition); Wood, 1893:72, (?) in part (Monterey Bay, CA); (?) Nierstrasz, 1905a:149 (Monterey, CA and "California"); Packard, 1918:294-295 (San Francisco Bay, CA; confused with southern species?).

Nuttallina californica (Nuttall, *in* Reeve, 1847). Pilsbry, 1893:279-280, plt. 54, figs. 23, 24; plt. 56, figs. 12-18 (with syns. *Chiton californicus* Nuttall, *in* Reeve, 1847; *Nuttallina scabra* Carpenter, MS; Dall, 1871:134; Dall, 1879:333; *Acanthopleura scabra* Carpenter, 1864:649; Stearns [1865?], Proc. Calif. Acad. iii:346; *Nuttallina scabra* Keep, 1887:108, fig. 96; *not Chiton scaber* Reeve); Raymond, 1894:133-134, (?) in part; Pilsbry, 1898:288, 290; Keep, 1904:267, 349, fig. 298 (mistakenly called *N. scabra* which are a separate southern species);

1911:258, fig. 255; Nierstrasz, 1905b:42; Berry, 1907:52, (?) in part (listed only Monterey, CA area); Dall, 1921:189; Berry, 1922:403, 409-410, 415, 418, 420, 439-441, Table 1, plt. 3, figs. 1-12, (?) in part (Pleistocene); Oldroyd, 1924:189; 1927:866; Johnson and Snook, 1927, 1955, 1967:562, in part, (?) fig. 662; Thiele, 1929:9; Leloup, 1940:3, 23-24, 26-27, figs. 50-53, 60-61 (girdle spines, aesthetes); A. Smith, 1947a:16; 1947b:18 (listed only); Smith and Gordon, 1948:206, (?) in part (Monterey Bay, CA); MacGinitie and MacGinitie, 1949, 1968:386, in part, (?) fig. 241; Ricketts and Calvin, 1968:198-199, in part, fig. 154; (?) Burghardt and Burghardt, 1969a:228 (less than 8 valves; Carmel, Monterey Co., CA); Burghardt and Burghardt, 1969b:32, probably plt. 4, fig. 65, in color; Abbott, 1974:400, (?) fig. 4694; Andrus and Legard, 1975:7, Table 1, (?) in part (Monterey Peninsula, CA); Gomez, 1975, (?) in part (Monterey Co., CA; in association with *Cyanoplax dentiens*); Linsenmeyer, 1975, (?) in part (Monterey Peninsula, CA; resistance to removal from substrate); Moore, 1975, (?) in part (Monterey Co., CA; gull predation); Nishi, 1975, in part (study on diet, in Monterey Co., CA; apparently includes *N. kata* as well as *N. californica* as judged by notes on girdle spine color, and location); Piper, 1975, (?) in part (Monterey Co., CA; blood ions); Robbins, 1975, (?) in part (Monterey Co., CA; respiration); Simonsen, 1975, (?) in part (Monterey Co., CA; osmoregulation); A. Smith, in Smith and Carlton, 1975:462, 464, plt. 109, fig. 8; Tenold, 1975, (?) in part (Monterey Co., CA; tissue lipid, glycogen); A. Smith, 1977:241-242; Morris et al., 1980:419-420, in part, fig. 16.17; Putnam, 1980:127-129; Ituarte, 1981, (?) in part (Santa Cruz Co., CA); Putnam, 1981:349, (?) in part (Shell Beach, San Luis Obispo Co., CA); Putnam, 1982:365, (?) in part (vicinity of Diablo Cove, San Luis Obispo

Co., CA).

Nuttallina cf. N. fluxa (Carpenter, 1864). Chace and Chace, 1919:2, (?) in part (Pleistocene).

Description.

Chiton of moderate size, confirmed in the present study to greater than 5.5 cm maximum live length (51 mm preserved), possibly to 7 cm (Putnam, 1980). Elongate oval in general outline, typically more than twice as long as wide. Moderately elevated.

Valve surface typically dark brown in uneroded specimens. Erosion of posterior parts of valves not uncommon, giving the valves a gray or white coloration. Intermediate valves with from zero to three white stripes, often zero or two, on or near the jugal and jugal/lateral ribs.

Interior of valves varies from almost white through light brown, dark brown, black and/or blue. Central portion often dark brown or nearly black and peripheral areas lighter, white to light or medium blue.

All exposed dorsal valve surfaces granulose in uneroded specimens. Anterior valve with low rounded radiating ribs when uneroded. Lateral areas of intermediate valves not raised but set off by low curved lateral ribs. Ribbing occasionally quite distinct, with a jugal ridge set off from two jugal/lateral ridges by shallow sulci, and with two distinct lateral ribs, and subobsolete posterior ribs at the valve margin. Valve viii often eroded, mucro posterior to terminal. Anterior valve with 9 to 12 slits (mode=11). Intermediate valves with 1 or 2 slits, the posterior of the two sometimes only a punctulate slit-ray tending to obsolescence. Valve viii with 7 to 10 slits (mode=8).

Intermediate valves rounded to almost flat, occasionally moderately elevated. Intermediate valves tend to be long per chiton length, and occasionally moderately wide, although the wide, robust girdle usually tends to make them appear narrow to Katharina-like. The tegmentum of intermediate valves tends to be rounded triangular to very triangular, erosion tending to straighten the posterior margin, making the valves ovoid. Posterior margin of uneroded intermediate valves typically quite angular (40 to 50° angle with jugal ridge; an 80 to 100° divergence). Anterior margin of tegmentum often nearly straight, but occasionally moderately arcuate, with three or two lobes. Sutural flesh encroachment moderate to extensive, on either side 10 to as much as 25% of the full width of an adjacent valve.

Girdle moderately to quite wide at sides (a single side at the fourth valve about 20% of chiton thawed total length). Girdle epidermis usually densely set with short rod-like scales (50 to 70μm long in 25 mm long specimens), generating an overall girdle color of uniform dark brown, or forming alternating girdle stripes of white and dark brown. When present, white girdle stripes are often incomplete (i.e., do not extend from valves to girdle edge), particularly between valve sutures i/ii and iv/v, or are produced instead as patches, especially near the girdle margin at both sutural and intersutural positions. Occasionally, dark brown scales are quite sparse and produce a light brown girdle coloration. Numerous light to dark brown spines occur throughout the girdle width both in areas of brown and of white scales, whereas white spines, when present, are usually restricted to areas with white scales, except near the girdle edge. Overall girdle spine densities are about 300 to 1000 per cm^2. Brown spines are always more numerous than white ones, typically far-outnumbering them. Brown spines occur in densities of 400 to 850 per cm^2, while white spines, when present, are often in tufts of two to five, and rarely reach densities over 100 per cm^2. White spines, but not brown ones, increase in density toward the girdle edge, often occurring as a fringe at the girdle edge or as tufts of several spines. Brown spines are about 400 to 850 µm and white ones about 600 to 800 µm in maximum length in 30 mm long specimens. The predominance of shorter brown girdle spines typically gives the girdle a relatively smooth or pebbly appearance, although specimens from the Channel Islands, CA and Punta Banda, Baja CA, Mexico tend to have "fuzzier" girdles.

Ctenidia abanal, holobranchial. Ctenidia separated by a space from the anus, and the ctenidial bases extending from beneath valve vii to beneath valve ii or the valve ii/iii suture. Gills increase in number with chiton length from about 36 per side in 10 mm long preserved specimens to a maximum of about 63 per side in the longest specimens. Gill numbers are commonly unequal on the two sides. Inner curtain-like mantle fold lateral to the gills.

Under defined conditions, in relation to the other two _Nuttallina_ species, the isozymes Adh, Mdh-2, Pgm and Sdh-2 exhibit a "slow mobility" phenotype on electrophoretic starch gels, while Sdh-1 exhibits "fast" relative mobility.

Distribution.

On the mainland, primarily occurring north of Point Conception, CA, reportedly to about the Canadian border; confirmed from as far north as Point Arena, Mendocino Co., CA (CASIZ 032165, 032166) and Bodega Head, CA (collection for electrophoresis, Chapter III) in the present study. For the most part, absent from mainland southern California, and expected to be absent from warmer water areas in Baja California. Well-represented south of Point Conception on the Channel Islands, particularly the northern and western ones bathed by the California Current, and on the south side of Punta Banda, Baja CA, Mexico, and presumably other coldwater upwelling areas in northern Baja California. A single specimen confirmed from as far south as East San Benito Island, Baja CA (AHF 1946-50), although Punta Banda is the next most southerly confirmed report.

Habitat.

High to mid, possibly low, rocky intertidal areas, in areas of barnacles and mussels and/or coralline algae, in cracks, crevices or grooves, occasionally on bare rock or crustose coralline algae. Specimens high on the shore tend to be equal to or slightly larger in size than specimens from the low shore.

Material Examined.

Type Material: Lectotype and four paralectotypes, designated herein, and two other specimens, BMNH Reg. No. 19798 ; one additional paralectotype, designated herein, and one other specimen, ANSP No. 118697 (Figure IV-8); type locality: "California" (Reeve, 1847), herein restricted to Bodega Head, Sonoma Co., CA.

149 additional specimens. 91 specimens electrophoretically phenotyped: Bodega Head, CA, 17 and 18 January 1981, N=35; Little Harbor, Santa Catalina Island, L.A. Co., CA, 14 April 1981, N=6; San Nicolas Island, Ventura Co., CA, 30 August, 1981, N=8; La Jolla, CA, February-March and November 1981, N=5; Punta Banda, Baja CA, 2 April 1981, N=37. Twelve specimens were not electrophoretically phenotyped, from collections above for electrophoretic study: Bodega Head, CA, N=3; La Jolla, CA, May 1982, N=1; Punta Banda, Baja CA, N=8. Forty-four specimens were in preserved museum collections: CASIZ 032165, 032166, Point Arena, Mendocino Co., CA, pre-1914 (dry), N=6 (4 intact + valves of 1 or 2 others); AHF1609-48, Bodega Head, Sonoma Co., CA, exposed granite rocks, 6 August 1948, N=14; SIO M1741, Duxbury Reef, Bolinas, CA, 7 May 1966, N=1; SIO M1742, near Pescadero Point, San Mateo Co., CA, 21 June 1968, N=4; SIO M1922, Hopkins Marine Station Pacific Grove, Monterey Co., CA, 29 December 1977, N=8; LACM 61-11, Shell Beach, San Luis Obispo Co., CA, shale ledges, leg. J. H. McLean 1961-1963, N=7; LACM 67-38, San Miguel Island, Santa Barbara Co., CA, 21-22 August 1967, N=3; AHF1946-50, E. San Benito Island, Baja CA, 26 April 1950, N=1.

POSSIBLE EVOLUTIONARY RELATIONSHIPS OF THE THREE NUTTALLINA SPECIES: AN OVERVIEW OF ELECTROPHORETIC AND EXTERNAL MORPHOLOGICAL CHARACTERS

In Chapter III, electrophoretic analysis of fresh or frozen specimens proved to be a definitive means for classifying Nuttallina individuals to three species. Now, using external morphological characters revealed in the present study of both electrophoretically-phenotyped and nonelectrophoretically-phenotyped specimens, it is also possible to identify virtually 100% of Nuttallina individuals to species without resorting to the time-consuming and tedious task of electrophoresis. Key characters allowing discrimination among the three species include gill number and position of the most anterior gill, color and arrangement of girdle spines, color of girdle and valves, width of the girdle and extent of the sutural flesh encroachment, relative proportion and shape of the intermediate valves, and maximal chiton length. Classification functions generated in multivariate analyses of gross morphological linear measurements allow a high degree of discrimination of the Nuttallina species and can be particularly useful where other characters, such as numbers of gills, are indeterminable. In addition, habitat and geographic location considerations provide valuable clues in identifying Nuttallina specimens.

Different subsets of the key morphological characters suggest different possible affinities among the species. Thus, the single character allowing nearly perfect discrimination among all three species, gill number, suggests that Nuttallina fluxa is intermediate between N. californica and N. kata since gill number increases in the order N. kata, N. fluxa, N. californica (assuming that a similar number of gills indicates a close relationship). Consideration of the color of girdle spines suggests that *N. kata* is intermediate between *N. fluxa* and *N. californica*. *N. kata* typically has more white girdle spines than brown spines as does *N. fluxa*. However, *N. kata* specimens with a predominance of brown spines are not uncommon, especially on the Channel Islands; using girdle spine color alone, these specimens are difficult to distinguish from *N. californica*. Specimens of *N. fluxa* and *N. kata* usually have girdles with a fuzzy appearance; *N. californica* specimens typically have girdles with a smoother-appearing texture. General coloration of valves and girdle, and girdle striping, are quite similar in *N. fluxa* and *N. kata*; in this respect, both species are usually distinguishable from the darker *N. californica*. There is probably more overlap between *N. kata* and *N. californica* in coloration than between *N. fluxa* and *N. californica*.. In girdle width, *N. fluxa* and *N. kata* are again quite similar, and distinguishable from *N. californica*, and, in sutural flesh encroachment, *N. kata* seems to occupy a position intermediate between *N. fluxa* and *N. californica*. In relative proportion and shape of intermediate valves, narrow, long, occasionally angular valves would seem to place *N. kata* somewhere between *N. fluxa*, with wide, short, quadrangular valves, and *N. californica* with narrow to wide, long, angular valves. Finally, in maximum adult chiton length, *N. fluxa* occupies a position intermediate between smaller *N. kata* and larger *N. californica*.

Considered together, the key morphological characters tend to suggest that *N. kata* is intermediate between *N. fluxa* and *N. californica*. The majority of characters, all valve and girdle characters, indicate such a relationship, whereas only gill number and maximal chiton length suggest an alternate arrangement, that of *N. fluxa* intermediate between *N. kata* and *N. californica*. In one specimen in the gill study, the gill number gave an "erroneous" identification, that is gill number indicated that the chiton belonged to *N. kata* whereas its extremely wide valves and its electrophoretic phenotype placed it with *N. fluxa*; thus, valve and girdle characters might appear to be the more reliable indices of taxonomic relationship in *Nuttallina*. In fact, P. P. Carpenter and H. A. Pilsbry relied heavily on valve and girdle characters in determining chiton taxonomic relationships. In *Nuttallina*, use of valve and girdle characters rather than gill number and overall size as the basis for determining species relationships is also supported in part by evidence from the electrophoretic study.

The implications of several electrophoretic scores, or "characters", for the affinities of different species are easily combined and summarized in the single genetic distance measure, D, since each "character" represents the product of a single presumptive gene locus, whereas the taxonomic implications of several external morphological characters, each possibly involving interactions and input of several gene loci, are not as easily discerned. In Chapter III, traditional genetic measures, i.e., D or genetic distance, calculated from electrophoretic data for fourteen presumptive gene loci indicated that *N. kata* (= LOW) was intermediate between *N. californica* (= BODEGA) and *N. fluxa* (= HI/DRY).

Although both electrophoretic and external morphological considerations then appear to agree in indicating that *N. kata* is intermediate between *N. fluxa* and *N. californica*, electrophoretic data suggest that N. kata is much more closely related to N. californica than to N. fluxa, whereas, if anything, external morphological considerations indicate that N. kata is slightly closer to N. fluxa than to N. californica. That is, using all of the key external morphological characters together, it is difficult more often to tell N. kata and N. fluxa specimens apart than it is to tell either apart from N. californica specimens. Such a discrepancy in findings of electrophoresis and morphological examination is not surprising; each involves a different character or set of characters. A mosaic of selective pressures, and possibly of random, "neutral" effects, probably act in different "magnitudes" and "directions" on the different characters, producing a variety of results. Certainly, each character, morphological or electrophoretic, represents only a small part of the genome of the organism. Electrophoretic studies, such as that of Chapter III, deal only with the soluble products of structural genes, those genes which effect the synthesis of proteins that function in metabolism and biosynthesis. On the other hand, the evolutionary fate of external morphological characters may be more closely linked to the evolution of the regulatory genes, those genes responsible for patterns of structural gene activation and expression. The relative roles of structural and regulatory genes and their interaction in the evolutionary process is unclear. Interestingly, Wilson et al. (1974a, b) have suggested that there may be two types of molecular evolution — one involving structural genes, which goes on at a more or less constant rate, and a second for regulatory genes, which are primarily responsible for reproductive incompatibilities and morphological evolution. Further, some workers have related the fact that certain differences between species in composition of structural genes may be useful biochemical clocks for dating geologic times of phylogenetic branching to the possibility that most point mutations of structural genes, if not deleterious, are adaptively neutral and become fixed randomly (see Wilson et al., 1977 for review). In any case, mosaic evolution of different electrophoretic and morphological characters, neutral or selected, might be expected as long as the organisms retain sufficient coadaptation of the features to function successfully (see Stanley, 1979 for discussion of multiple gene evolution).

The conspicuous lack of concordance between evolutionary relationships of the species suggested by gill number and chiton size and those suggested by the balance of morphological characters and electrophoretic results may indicate that the two former features are particularly plastic characters and/or that they are subject to particularly intense selective pressure. The possible functional significance of gill numbers was discussed earlier. The fact that gill number and overall size show the same relationship among the Nuttallina species may indicate that the two are functionally related, as is also suggested by Johnson's (1969) finding of a correlation between gill number and maximum adult size of different chiton species. Alternatively, the covariation of gill number and overall size might reflect the fact that the two characters are simply subject to independent selective forces, or random effects, acting in the same "direction", or to related or identical selective forces.

CHAPTER V

LONG-TERM MOVEMENT STUDY ON NUTTALLINA

INTRODUCTION

In an early description of the natural history of Nuttallina, MacGinitie and MacGinitie (1949, p. 386) stated that specimens of "Nuttallina californica" (these authors apparently recognized only one Nuttallina species) possess "homing spots" that they never leave. The MacGinities noted that Nuttallina lives in deep form-fitting depressions that serve to trap seaweed debris, and they suggested that this settling debris is important in the diet of Nuttallina as "evidenced by the fact that no depressions in which Nuttallinas are living are found where the face of the rock is at an angle of 90° or more with the horizontal."

During a night watch on Nuttallina in the high intertidal area at Hopkins Marine Station near Monterey, CA, and later in the course of preliminary investigations on high and low intertidal Nuttallina populations in La Jolla, CA, it became obvious that Nuttallina is not as sedentary as MacGinitie and MacGinitie (1949) had proposed. The night watch, in April 1974, revealed that some, though not all, specimens of high intertidal Nuttallina (presumably N. californica of Chapters III, IV) leave their low-tide resting spots to graze at high tide. Weeks later, in the same locality, Nishi (1975) similarly observed specimens of "Nuttallina californica" (N. kata?, Chapter IV) to emerge from depressions in a low intertidal Dodecaceria bed when the area was awash on an incoming tide. Later, at La Jolla, it was observed that spots cleared of Nuttallina were soon reoccupied by specimens large enough to rule out the possibility that they had been newly-recruited from the plankton; that is, these chitons had moved there from elsewhere on the substrate.

As it turns out, during the last fifteen years, several short-term studies on Nuttallina, reported in unpublished student papers and as sections in master's theses, have provided a fair amount of evidence, primarily indirect, for movement in several Nuttallina populations (Table V-4). Most data have been obtained for Southern California high intertidal populations of Nuttallina fluxa, less data for N. kata (both as "Nuttallina fluxa"), and the least data has been obtained for northern California populations of N. californica (as "Nuttallina californica"; see Chapters III, IV for species designations). General conclusions that can be made from these studies are: 1) movement in Nuttallina occurs when the chitons are submerged or at least awash (i.e., not at low tide during exposure), as indicated by direct observations on N. fluxa and by comparison of chiton orientations and locations at successive low tides; 2) movement occurs during the day as well as at night, at least in Nuttallina fluxa (Kues, 1969); 3) most Nuttallina specimens are found in the same low-tide resting spot at successive low tides, over a period of several days at least, indicating that most specimens "home" or do not leave the resting spot on intervening high tides; and 4) displacements of 97 cm (or more?) are possible over a single day between successive low tides, although most observed displacements are much shorter (Table V-4).

A survey of the literature reveals that chiton species like *Nuttallina*, that move primarily while submerged or awash, are about as numerous as those that are active mainly while exposed at low tide (Table V-25). Most movements of chitons appear to be related to feeding activity. Glynn (1970) observed that the radula of *Chiton tuberculatus* was almost always extruded and in motion in moving specimens removed suddenly from the substratum, that the rasping of the radula, as observed through the glass walls of an aquarium, was closely correlated with chiton movements, and that the foregut was empty in chitons at rest, but filled rapidly after they began moving. Thorne (1967) reported that *Acanthozostera gemmata* rasps a clearly visible, uninterrupted path through encrusting algae during its nocturnal low-tide locomotory excursions. In addition, he noted that a clearly audible rasping sound was made during feeding and that shorter browsing trails were undertaken on rocks covered with thicker algal mats. Demopulos (1975) found that subtidal specimens of *Tonicella lineata* sampled at night, the time of greatest observed movement, tended to have full guts, whereas those sampled during the day did not, for very small sample sizes. *Nuttallina*, at least *N. fluxa* (Kues, 1969), is quite unlike the majority of other chitons that have been studied in that it exhibits no clear preference for movement at night. Various chiton species exhibit tendencies to move either at low tide or at high tide during night hours, but no chiton studied has shown a preference to move while exposed during daytime low tides, presumably as a result of increased dangers of desiccation or predation by visual predators such as birds during these periods.

Homing (i.e., returning to a particular resting site at the end of a feeding excursion) is quite common in the intertidal chiton species that have been studied (Table V-5). This behavior seems to have developed to varying degrees of fidelity and precision in different species, although the disparate methods employed to study and report this phenomenon make interspecific, as well as intraspecific between-site, comparisons difficult. In the only study on the mechanism of homing in chitons, Thorne (1968) concluded that Acantozostera gemmata normally returns to its homesite during feeding excursions by retracing its outgoing trail, possibly by using chemical cues from mucus it lays down. However, this chiton possesses a latent ability to home when experimentally displaced that is difficult to explain, but that might involve topographic memory or the detection of old trails around the homesite, rather than visual detection of the homesite, airborne odors from the homesite, celestial navigation or random movement. Thus, chitons are apparently like the ecologically-similar limpets that have been studied much more extensively, and for which the homing mechanism likewise is not always clear; the many limpet homing studies most often indicate that homing is effected by using chemical trails or topographic or kinaesthetic memory (e.g., Funke, 1968; Cook et al.,1969; Cook, 1969; Thomas, 1973).

Chitons are unlike limpets in that the radula has magnetic properties (S. Thorpe reported in Tomlinson, 1959) as a result of a magnetite denticle capping (Lowenstam, 1962; Carefoot, 1965; and see review of Kirschvink and Lowenstam, 1979). Lowenstam (1962) suggested that the chiton radula might possess a magnetic moment in the earth's magnetic field sufficient to "serve as a guidance system for their so-called homing instinct." This suggestion has elicited considerable interest among biologists. In laboratory experiments, various workers have been able to demonstrate that chiton behavior can be modified by strong magnetic fields (Montgomery and Smith, 1963; Ratner and Jennings, 1968; Tomlinson et al., 1980). Unfortunately, these experiments have involved magnetic fields far stronger than that of the earth (~0.5 gauss); workers have not ensured that the radula is not magnetized by such fields or demonstrated that the radula has enough virgin remanence to interact with the earth's weaker field. Indeed, Eibschutz et al. (1967) found only a very low virgin remanence in the radula of the Red Sea chiton Acanthopleura haddoni (<0.1% of the maximum possible remanence of the material, 30 emu/g) and they expressed doubt about the behavioral significance of chiton magnetite. Although Tomlinson et al. (1980) apparently have been able to show that chitons align with the magnetic field on central California shores, more study is required to establish the orientation function of the chiton radula. Certainly, the position of the magnetite on the tips of radular teeth, where it is subject to wear and "noise" as a result of feeding, would seem to be an unlikely place for an orientation mechanism. A well-innervated location would seem to offer better sensitivity to the earth's weak magnetic field. Magnetite is a very hard mineral and Carefoot's (1965) suggestion that the magnetite serves to harden the radula, and thereby slows radular wear, seems to be a better explanation for its significance.

Reports that Nuttallina specimens occasionally exhibit substantial displacements (30 to 100 cm) over a single twenty-four hour period

(Table V-4) were of concern since, for physiological studies on Nuttallina that were to be undertaken on a microhabitat scale, a key consideration for making realistic conclusions was the acclimation history of each animal. Microhabitats separated by a few centimeters to a meter or two in the intertidal zone are subject to quite different physical regimes (Chapters I, II). Since complete physiological acclimatization of an organism to conditions in its particular microhabitat might require weeks or months (e.g., Segal, 1955; and see Hochachka and Somero, 1973), for Nuttallina, it was important to establish that the chitons had inhabited the particular area in which they were collected for a reasonably long time, long enough to have acclimatized to the conditions there.

Although a Nuttallina specimen might move a substantial distance away from its low-tide resting spot while submerged at high tide, it is assumed that it is subject to virtually the same physical conditions during a high tide excursion as it would be at its resting spot, since the entire immediate area is bathed in a relatively homogeneous body of water at high tide. Only the exposed low-tide location of a chiton should be important for intertidal microhabitat acclimatization. Therefore, in the present study, the low tide resting positions of many tagged Nuttallina specimens, of a representative range of sizes and in a variety of habitats in both the high and low intertidal areas in the La Jolla study site (Chapter II), were recorded at regular intervals in order to characterize the long-term movement behavior of Nuttallina. Although long-term movement behavior has not been reported in any detail for any chitons, studies of some intertidal limpets, molluscs ecologically quite similar to chitons, have revealed a seasonal pattern of movement (see Branch, 1981 for examples). For this reason, the present study was extended through all seasons of a year.

An interesting aspect of the biology of Nuttallina at La Jolla and at some other sites is the occurrence of a shore-level size gradient such that larger specimens tend to be found higher on the shore (Chapters II, III; Kues, 1969; Ituarte, 1981). Similar intertidal size distributions have been observed for other chitons (Arey and Crozier, 1919; Boyle, 1970; Fitzgerald, 1975). Both Arey and Crozier (1919) and Boyle (1970), as well as Ituarte (1981), suggested that the size gradients might be a consequence of chitons moving to more exposed and/or higher positions on rocks as they grow. Studies on two limpet populations that are also characterized by increasing specimen size with intertidal height have provided some evidence for alternating seasonal upward and downward movements that result in net upshore displacements of the animals (Frank, 1965; Branch, 1975). Consequently, data from the present long-term study on Nuttallina were examined for indications of seasonal migrations in particular directions as well as for general tendencies toward upshore movements.

Chitons have been tagged for study for periods longer than a couple of days to several weeks in only a few previous studies (Barnawell, 1954; Boolootian, 1964; Palmer and Frank, 1974). All studies, undertaken primarily to generate growth estimates, involved relatively large chiton species (Mopalia spp. and Cryptochiton stelleri). Tagging of smaller chitons such as Nuttallina is not an easy task. Whereas none of the smaller intertidal chiton species have been tagged for study, at least two populations of fairly small intertidal limpets have been tagged with some success for long-term studies (Frank, 1965; Sutherland, 1970, but Sutherland mentioned some tag loss problems). This is undoubtedly a consequence, in part, of the relatively complex external morphology of chitons, in which the exposed shell area is divided into eight parts instead of one continuous piece as it is in the limpets; each small part (valve) of a chiton presents relatively little surface area on which to affix a tag that is free from scraping action of the next most anterior valve that occurs as the chiton moves over irregular substrate.

Workers in at least one chiton study considered tagging to be impractical (Baxter and Jones, 1978), but in that case an alternate method was available for accomplishing the goal of the study, growth rate determinations. In the present study, tagging was the only means available for characterizing movements of individual chitons in detail. Due primarily to the relatively small size of <u>Nuttallina</u>, conventional tagging methods failed and a more novel one with an acceptable loss rate was developed. The new tag had other advantages in addition to a relatively low loss rate. Details are described in the Material and Methods section as well as in Appendix IV.

When the present study was initiated, only one <u>Nuttallina</u> species was recognized from Southern California. Preliminary analysis of the results of this study suggested an absence of interchange of <u>Nuttallina</u> specimens between the densely-populated, low intertidal area and the sparsely-populated, high shore area in the La Jolla study site. This finding provided additional impetus for the subsequent investigation of the taxonomic status of the high and low Nuttallina populations. The taxonomic study resulted in the resurrection of one Nuttallina species, Nuttallina fluxa, and the discovery of a new species, Nuttallina kata sp. nov. (see Chapters III, IV). N. fluxa is found primarily in sparsely-populated, high intertidal areas and N. kata occurs most commonly in densely-populated, lower intertidal areas. Fortunately, sufficient information appropriate for distinguishing the new suite of species, particularly valve widths and chiton lengths, had been recorded during the present study so that a majority of the tagged specimens could be assigned subsequently either to the new species, N. kata., or to N. fluxa. Therefore, it has been possible to reanalyze the data obtained in the present study using the new species scheme and results are presented segregated according to species.

MATERIALS AND METHODS

THE STUDY AREA

The site used for this study is located near Seal Rock in La Jolla, CA (referred to as the Seal Rock site for convenience) and has been described in Chapter II. As described in Chapter II, the Seal Rock site has a good representation of Nuttallina in both the high and low intertidal areas. The site is afforded some protection from the human disturbance inevitable in a populated area by its separation from the shore proper, even during many low tides, by a sand-bottomed surge channel. In addition, the south-facing orientation of the site toward shore usually protects it from the full force of waves at lower tides so that work can be carried out more often than on more directly exposed shore areas nearby.

Nine squares, approximately 1 meter on a side, were laid out in two arrays separated horizontally by about four meters. Each array was laid out with two of the squares at the highest level and the remaining squares (two in one group, three in the other) extending down to the lowest intertidal levels. Each square was contiguous with at least one other square. The lowest square and part of the next lowest square in each array fell within the "densely-populated chiton band" described in Chapter II. All other squares fell primarily in the more sparsely-populated upper intertidal area.

Two criteria were used to position the two arrays of squares. First, large numbers of Nuttallina were present both in the low and in the higher intertidal levels. Second, no obvious topographic or biological barriers existed between any lower and any higher squares within the array that could hamper migration of Nuttallina between the different shore levels. A relatively regular, continuous substrate surface with only small clumps, but not continuous beds, of mussels extended from the highest to the lowest levels of both study arrays.

The squares were permanently delineated by placing 1/4" by 1" (~0.6 by 2.5 cm) vinyl anchors with stainless steel screws (Sears Roebuck Co., Chicago, Ill.) at their corners. Holes were drilled in the sandstone substrate using a masonry bit in a Skil Cordless 2002 drill (Skil Corp, Chicago, Ill.). Sea Goin' Poxy Putty (Permalite Plastics Corp., Newport Beach, CA) was used both to hold the anchor screws in place and to improve the stability of the surrounding substrate. The vinyl anchors stayed in place throughout several years of studies at this site. About once a year, new epoxy glue had to be applied to some of the screws to hold them in place since erosion of substrate around them, particularly in high areas with bare substrate, exposed as much as 1 cm of a screw in a year. Algae, barnacles and limpets had to be scraped away from the screws about twice a year to prevent the screws from being hidden, especially in the lower intertidal areas.

Several characteristics of each one meter square quadrat were measured and are reported in Table V-1. Along-shore (X) and upshore-downshore (Y) roughness indices were determined as follows. The rigid wooden quadrat frame used to locate chitons (see section on location methods below) was put into place and its four sides were projected, perpendicularly to the frame, onto the underlying rock. For the X roughness index, a string was used to follow the contour of the substrate from the rock underlying the left side of the quadrat to the position under the right side. Measurement of the string then gave the actual distance along the contour of the rock that corresponded to the 1 meter point-to-point, or straight-line, distance obtained by projecting the quadrat frame onto the substrate. For example, if the string indicated that the contour-following, or "roughness-corrected", distance was 1.20 meter, corresponding to a straight-line distance of 1.00 meters, the roughness index was 1.20/1.00=1.20. An average of three determinations at three different levels, corresponding to the top, middle and bottom of the quadrat, gave the X roughness index recorded in Table V-I. Three similar determinations in the upshore-downshore (Y) direction gave the average Y roughness index. Average tilts of each quadrat were

TABLE V-1. Characteristics of study quadrats of "Seal Rock" study site, La Jolla. Characteristics and their mode of measurement described in the text.

Quadrat Number	Intertidal Height (of rock underlying upper left corner of wood quadrat frame in place), in cm above MLLW	"X" Tilt, in degrees[A]	"Y" Tilt in degrees	"X" Roughness Index, ± SD	"Y" Roughness Index, ± SD	Compass heading of top of quadrat, in degrees (360° = magnetic north)	Chiton Habitat, Sparse or Dense[B]
1	+150	-1	-5	1.12 ± 0.03	1.22 ± 0.10	325	Sparse
2	+150	+6	+2	1.11 ± 0.03	1.15 ± 0.07	334	Sparse
3	+92	-2	-14	1.21 ± 0.04	1.22 ± 0.06	328	Sparse/Dense
4	+52	0	-14	1.18 ± 0.10	1.19 ± 0.05	324	Dense
5	+155	-2	-19	1.07 ± 0.00	1.15 ± 0.04	332	Sparse
6	+150	+2	-13	1.13 ± 0.06	1.17 ± 0.07	317	Sparse
7	+110	+3	-12	1.15 ± 0.06	1.12 ± 0.02	327	Sparse
8	+109	-2	-21	1.25 ± 0.12	1.20 ± 0.05	334	Sparse/Dense
9	+70	-3	-38	1.25 ± 0.09	1.40 ± 0.05	330	Dense

NOTES

A. A negative tilt in the "X" axis indicates that the left (west) side of the quadrat is higher than the right side and a positive one indicates that the right side is higher. A negative tilt in the "Y" axis indicates that the "bottom" edge of the quadrat is lower than the "upper" (north) edge and a positive one, that the "bottom" edge is higher

B. Habitats at the Seal Rock study site were discussed in Chapter II.

measured by placing an inclinometer (Sears) on a board placed on the wooden quadrat frame, in its position at each study quadrat. X tilt gives an indication of the average along-shore inclination of the study quadrat, while Y tilt indicates the average upshore-downshore inclination. The compass direction indicates the orientation of a line passing from the downshore to upshore sides through the middle of the quadrat frame, parallel to the Y axis. Thus, a 315° heading indicates that the upshore side of the quadrat faces northwest (with respect to magnetic north).

Vertical intertidal height above mean lower low water (MLLW) of the top left corner of the quadrat frame, the "origin", projected on the underlying rock for each study quadrat, was determined using a Leitz Automatic Level Model B-4 with a 22X magnification sighting scope and a stadia rod with 1/50 ft (~0.6 cm) divisions. A U.S. Coast and Geodetic Survey benchmark, about 100 m east of the study area, was used to determine intertidal heights within the study area. The benchmark, labelled "Roof No. 1 Seal Rock 1933", is located on top of a concrete wall east of the lifeguard station that overlooks the Children's Pool. Records at USGS, Menlo Park, CA indicate that this benchmark ("RM1") is 33.392 ft (~10.2 m) above mean sea level (National Geodetic Vertical Datum), which in turn is 2.55 ft (~0.8 m) above MLLW at nearby Scripps Institution of Oceanography pier. Thus, RM1, last checked in May, 1958, is 35.94 ft (~11.0 m) above MLLW. Surveys from the benchmark to the study area rocks and back, with four legs each, were within 0.08 ft (~2.4 cm) of each other. Determinations of relative heights within the study site, involving only 1 sighting from a reference point over a short distance, are assumed to include negligible error.

The study quadrats extended from +3 cm to +164 cm above MLLW (see Table V-1).

TAGGING

Nuttallina specimens were tagged so that individuals could be followed throughout the study. To minimize the disturbance to each chiton and to ensure that each chiton was replaced in exactly the same location and orientation in the study site, chitons were tagged in the field. Each chiton was removed carefully from the sandstone substrate, allowed to attach undisturbed on a flat submerged rock, and then its total length and FVW were measured with fine-point dividers and a precision metric rule. The chiton was then tagged with a monofilament-titanium tag (see Appendix IV) and the identifying characters stamped on the tag were recorded. The tag was always attached to the left girdle area about even with the fourth valve; constant placement of the tag in this localized area of the girdle facilitated relocating the animal if the tag was obscured by algae or substrate and facilitated determinations of whether the tag had been lost from a particular chiton or not. The precise coordinates and quadrat number were recorded for each chiton. The entire procedure, from removal to replacement and including data recording, averaged about ten to fifteen minutes per chiton.

In preliminary tests in the field, the monofilament-titanium tag proved to be preferable to standard limpet tagging techniques (whereby identification numbers are affixed to the outer surface of the shell with various glues e.g., Sutherland, 1970) for several reasons: 1) monofilament-titanium tags are not lost as rapidly as are glue tags; 2) tags were held away from the surface of the chiton and were generally easier to find in the field and were not so easily obscured by algal growths, as were tags applied to chiton valve surfaces; 3) tags were more easily read without disturbing the chiton than are glue-type tags, which often must be scraped clean of algae to be read; and 4) tags did not inhibit normal backward flexure of chitons with concomitant partial slipping of valves over each other, as do lumps of glue applied to external valve surfaces.

The effect of the monofilament-titanium tag on activity, growth and mortality of Nuttallina was assessed in extended laboratory investigations to ensure that the tag does not dramatically alter the behavior and well-being of study chitons. No obvious effect on short-term activity and growth was evident, although a slight decrease in survival time was observed in tagged versus control chitons. Details of the relatively intensive laboratory and field investigations on the tagging method and the effect of tags on Nuttallina are explained and discussed in detail in Appendix IV.

Chitons were removed from the rocks in the field for tagging and for subsequent growth measurements at about 6 month intervals. Since such manipulation could effect abnormal chiton behavior during immediately ensuing tidal cycle(s), particularly for chitons injured in the tagging process, the time interval from tagging and/or measuring to the next observation was not included in analyses of movement.

METHOD FOR DETERMINING CHITON POSITIONS

Chiton positions were generally determined during spring low low tides at two week intervals. Chitons were not touched or manipulated in any way. The study area and vicinity was carefully searched to ensure that any chitons that could not be found had died or lost their tags rather than moved a considerable distance to a new area. Positions were recorded by using a Cartesian coordinate system as follows. The rigid 1 meter square wood quadrat frame was strung with monofilament line such that it was divided into 100 small squares 10 cm on a side. The quadrat frame was aligned at the site each time using the permanent anchor screws as reference points. Alignment was accomplished by sighting down to the rock perpendicularly to the plane of the quadrat frame. The position of any chiton (actually a point at the center of the exposed surface of the fourth valve, the approximate center of the chitons) in the quadrat could be determined by sighting down through the quadrat frame and noting the distance to the right of the upper left corner of the quadrat, X cm, and the distance down from the upper left corner of the quadrat, Y cm. Distances calculated directly from these coordinates then represent straight-line, or point-to-point, distances.

Distances approximately corrected for the contour of the substrate, actual along-the-rock or "roughness-corrected" distances, was determined by applying the X roughness index of the quadrat (Table V-1) to the straight-line X distance and the Y roughness index to the Y distance. Approximate intertidal height or vertical intertidal displacement of any chiton was estimated by use of the tabulated intertidal height of the upper left corner, the "origin", of the appropriate quadrat, the X tilt angle and the X coordinate or distance, and the Y tilt angle and the Y coordinate or distance of the chiton.

The precision of this quadrat method of relocation was assessed by recording the positions of 51 small metal squares in a high (quadrat #1) and a low shore quadrat (#9), removing and re-aligning the quadrat frame, and again determining the positions of the metal pieces, without reference to the coordinates recorded initially. As expected, the distribution of straight-line distances from the first to second sets of coordinates was not significantly different from a normal distribution centered on zero (G goodness-of-fit test, 1 d.f., $p>0.25$). Straight-line distances from first to second readings averaged 0.49 cm and ranged from 0 to 1.4 cm. The upper 95% confidence limit for relocations fell at 1.26 cm, indicating that chitons in this study were relocated to within 1.26 cm with 95% confidence.

SPECIMENS STUDIED

Two hundred and five _Nuttallina_ were tagged during the study. Of the two hundred and five, forty-five specimens were observed too few times to be used in analyses; only chitons observed at least twice after tagging were included since data for the interval from tagging to the first following observation was disregarded, in order to avoid inclusion of abnormal chiton activity related to the tagging disturbance. Tagging of individuals was performed during spring low tides on 6,8,9 and 21 September, 25 and 30 October, and 3 December 1979, and on 16-18, 29,30 April, and 1 May, 1980. Chitons were selected haphazardly with respect to size and position within each quadrat, except that chitons shorter than about 16 or 17 mm in live total length were not used. Very small specimens could not be effectively tagged due to the narrow girdle area available for tagging. Size-frequency data for tagged chitons are summarized in Figure V-1.

Subsequent to and partially as a result of this study, Nuttallina was studied electrophoretically. Electrophoresis revealed the existence of three Nuttallina species in the La Jolla site used for this study, N. fluxa primarily in the high intertidal zone, a new species N. kata primarily in the low intertidal areas, and a rare (5 of 153; Chapter III) third species, N. californica that is found in greatest abundance in cold water areas primarily north of Pt. Conception, CA. In view of the low frequency of occurrence of Nuttallina californica at the La Jolla site, this species was neglected altogether in re-analyses of results of the present study.

Morphometric analysis of thawed Nuttallina specimens (Chapter IV) showed that Nuttallina fluxa tend to have wider valves per length than N. kata. Fortunately, both fourth valve width and total length had been recorded at least once for most of the tagged chitons during the present study, as part of a growth study (Chapter VI). Live total length and live fourth valve width had been recorded for 24 specimens of Nuttallina fluxa and 26 specimens of N. kata from La Jolla that were electrophoretically-phenotyped for the studies of Chapters III and IV and these data were used to generate classification functions for the discrimination of tagged live N. fluxa and N. kata (Table V-2). By using only specimens from La Jolla to generate classification functions, the potential problem of geographic heterogeneity seen in Chapter IV

Figure V-1. Initial size-frequency distributions of tagged *Nuttallina* according to quadrat. Quadrats are arranged approximately from highest (top of page) to lowest position (bottom) in the intertidal zone. Quadrats are labelled according to characteristic *Nuttallina* density, sparse or dense, as described in Chapter II.

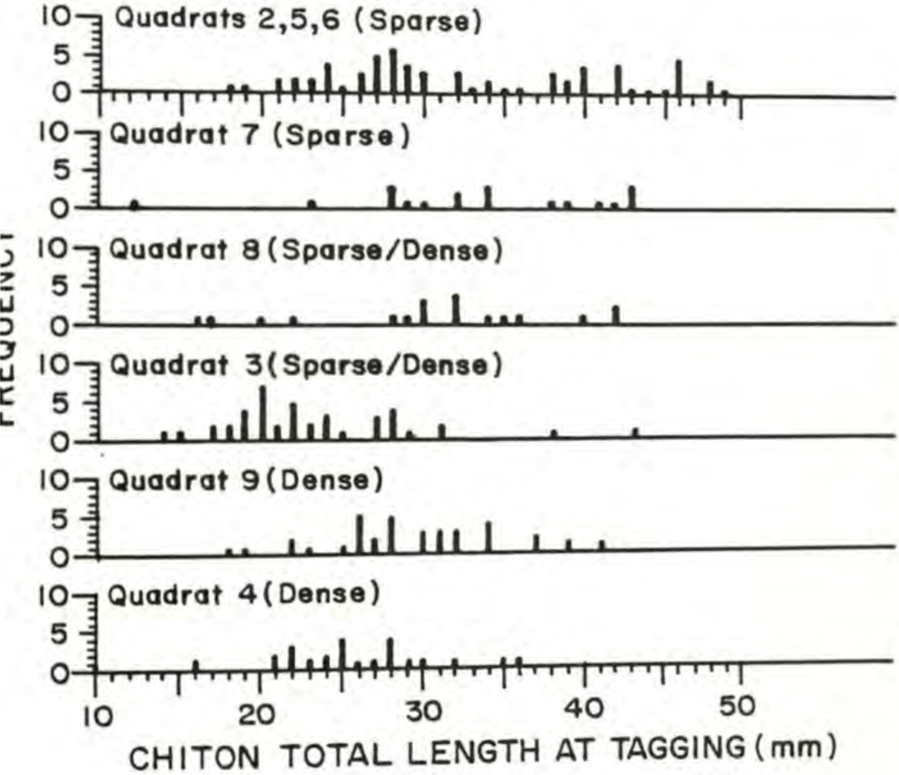

FREQUENCY
Quadrats 2,5,6 (Sparse)
Quadrat 7 (Sparse)
Quadrat 8 (Sparse/Dense)
Quadrat 3 (Sparse/Dense)
Quadrat 9 (Dense)
Quadrat 4 (Dense)
10
5
0
10
20
30
40
50
CHITON TOTAL LENGTH AT TAGGING (mm)

TABLE V-2. Classification and morphometric information for electrophoretically-phenotyped specimens of *Nuttallina fluxa* and *N. kata* from the La Jolla study site.

Species	Live Fourth Valve Width	Live Chiton Total Length	Constant	Biased Classification Success
N. fluxa	1.03	0.42	-12.87	88%
N. kata	-0.67	0.81	-9.82	

Regressions of fourth valve width on chiton length for live specimens.

N. fluxa, N = 24, r = 0.82

FVW = 0.325 CTL + 0.217 Model I regression

FVW = 0.396 CTL - 2.065 Functional regression (GM regression of Ricker, 1973)

N. kata, N = 26, r = 0.91

FVW = 0.243 CTL + 0.116 Model I regression

FVW = 0.268 CTL - 0.599 Functional regression

(Table IV-3), primarily in fourth valve widths of Gaviota and non-Gaviota specimens of N. fluxa, was eliminated, increasing the discriminating power of the classification functions for specimens of the present study.

Total length and fourth valve width data was available for 126 tagged chitons. Since multiple, successive measurements were available for some chitons, 167 cases for the 126 chitons were available for classification. Data for the 50 electrophoretically-phenotyped La Jolla chitons were entered into the SPSS Discriminant Analysis Program (Nie, 1975; version date: 15 August, 1980) using stepwise variable selection by Wilk's lambda. Classification functions generated in the analysis of these 50 grouped cases then served to classify the 167 ungrouped cases, providing probabilities of membership in N. fluxa and N. kata and probabilities that a case actually belonging to a particular species would fall that far from the group centroid, assuming a multivariate normal distribution. Classification success for the 50 grouped cases was 88% (Table V-2).

Additional information was available for identifying the tagged chitons. Close-up color slides were available for twenty-four of the chitons. In addition, three chitons had been preserved in ethanol at the end of the experiment and were available for a detailed morphological examination. Further, morphometric studies (Chapter IV) indicated that any chitons greater than 40 mm in live total length have a very high probability of belonging to Nuttallina fluxa rather than to N. kata. The longest preserved specimen of N. kata encountered in the study of Chapter IV (in the ctenidia study) was 34 mm and the longest thawed specimen of N. kata electrophoresed (Chapter III) was 32 mm (equivalent to a 39 mm live total length, see Table IV-9). Finally, the habitat of occurrence provides a valuable clue to the identity of a particular specimen. In Chapter III, it was shown that Nuttallina fluxa ("HI/DRYs") are primarily restricted to the bare, sparsely-populated high intertidal zone, whereas Nuttallina kata ("LOWs") occur mainly in the damp algal-covered densely-populated low intertidal area.

The discriminant analysis (classification) results were used as the baseline identification for the tagged specimens. The additional sources of information listed above were used to confirm and occasionally to modify discriminant analysis assignments to improve the reliability of final classifications. Technically, a chiton with a probability greater than 50% of being in one of the two species is classified into that species by the SPSS program. However, an 80% probability of membership was used as the arbitrary cut-off for assignment to a species. If the probability of membership of a tagged chiton in either of the two species was 80% or greater, only strong evidence to the contrary from other sources could cause it is to be classified as "unidentifiable". Specimens with probabilities of membership of 51% to 75% were classified to a species if evidence from other sources supported the identification and only strong indications from the other sources of evidence could lead to classification of such chitons in the species not indicated by the discriminant analysis.

With cautious use of all available evidence for each tagged chiton, 133 of the 160 tagged chitons could be assigned confidently to one of the two species (N. fluxa, N=56; N. kata, N=77; unidentifiable, N=27). Any error in assignment of the specimens would tend to obscure differences observed in the movement behavior between the two Nuttallina species.

RESULTS

TAGGED CHITON "SURVIVAL" AND DATA USED IN ANALYSES

"Survival" data (including chiton deaths and tag losses) for the chitons tagged during the study are presented in Table V-3. Tagged Nuttallina were present for 24,193 of a total possible 35,572 chiton-days, and almost one-half of the chitons present at the beginning of six month measurement intervals were also present at the ends of these intervals. Disappearance of tagged chitons between growth measurements were a consequence of both chiton deaths and of tag losses. In many cases, when a chiton could not be relocated, its last-occupied spot was vacant. Since the study site was intensively searched each time, movement to a new home site was unlikely in such cases (unless the chiton had lost its tag and was undetectable), and a vacant spot probably often signaled a chiton death. However, in two instances during the study, a rip wound was observed in the girdle of the chiton found in the spot last occupied by a tagged chiton that could not be found. In these cases, tag loss and not chiton death presumably accounted for the "disappearance" of the chiton. Chiton death rather than tag loss is thought to have accounted for most of the disappearances of tagged chitons.

Data from 160 tagged Nuttallina specimens involving 1,809 observations, separated by about 15 days on the average, for a total of

TABLE V-3. Data used in analyses of *Nuttallina* movement and "survival" of tagged *Nuttallina* specimens.[A]

Species	Habitat[B]	Number of Tagged Chitons with Usable Movement Data[C]	Total Number of Usable Observations	Total Number of Usable Observation Intervals	Days Between Successive Observations Mean (Median)[D]	Total Chiton-Days Specimens Present	Total Possible Chiton-Days[E]
Nuttallina fluxa		56	711	636	14.8(14.6)	9425	13064
	High dry sparse	41				5622	8426
	High pools	14				3241	3694
	Sparse/dense boundary	3				310	544
	Low dense	1				252	400
Nuttallina kata		77	848	753	15.8(14.8)	11860	17346
	High damp sparse	13				1374	2553
	High pools	3				894	894
	Sparse/dense boundary	4				629	966
	Low intertidal sparse, sparse/dense	3				673	793
	Low dense	55				8290	12140
Unidentifiable *Nuttallina* Specimens		27	250	222	13.1(13.5)	2908	5162
	High sparse	7				1086	1519
	High pools	11				1150	1886
	Sparse/dense boundary	4				260	781
	Low intertidal sparse, sparse/dense	3				353	549
	Low dense	2				59	427
Totals - All Species		160	1809	1611	15.1(15.0)	24193	35572

TABLE V-3 (cont.)

Species	Habitat[B]	Chiton-Days Present Vs. Total Chiton-Days Possible, Percent	Chiton-Days Present Vs. Total Chiton-Days Possible For Individuals, Mean Percent (Median, 25/75 Percentiles)	Total Possible Number of Growth Measurement Intervals[F]	Number (Percent of Total Possible) of Tagged Chitons Present at Ends of Growth Measurement Intervals	Number (Percent of Total Tagged) of Tagged Chtions Present at End of Study (Through Measurements, Other Manipulations)
Nuttallina fluxa		72.1	69.1 (72.4, 45.4/100.0)	68	31(46)	15(27)
	High dry sparse	66.7	65.8 (71.8, 21.1/100.0)	44	14(32)	10(24)
	High pools	87.7	85.5 (100.0, 71.4/100.0)	19	15(79)	5(36)
	Sparse/dense boundary	57.0	55.7 (34.7, 32.4/100.0)	3	1(33)	0(0)
	Low dense	63.0	63.0 (63.0, 63.0/63.0)	2	1(50)	0(0)
Nuttallina kata		68.4	64.9 (68.6, 32.4/100.0)	95	46(48)	19(25)
	High damp sparse	53.8	53.8 (58.1, 18.4/79.3)	13	2(15)	2(15)
	High pools	100.0	100.0 (100.0, 100.0/100.0)	5	5(100)	3(100)
	Sparse/dense boundary	65.1	56.3 (53.4, 18.4/94.2)	5	2(40)	0(0)
	Low intertidal sparse, sparse/dense	84.9	81.7 (100.0, 45.0/100.0)	4	3(75)	1(33)
	Low dense	68.3	66.0 (68.6, 34.1/100.0)	68	34(50)	13(24)
Unidentifiable *Nuttallina* Specimens		56.3	56.8 (59.2, 30.6/84.4)	28	6(21)	2(7)
	High sparse	71.5	70.0 (67.0, 43.1/100.0)	8	3(38)	2(29)
	High pools	61.0	61.0 (60.0, 31.6/78.9)	11	2(18)	0(0)
	Sparse/dense boundary	33.3	34.1 (37.8, 21.7/46.6)	4	0(0)	0(0)
	Low intertidal sparse, sparse/dense	64.3	69.2 (84.4, 23.2/100.0)	3	1(33)	0(0)
	Low dense	13.8	13.8 (13.8, 11.0/16.7)	2	0(0)	0(0)
Totals - All Species		68.0	65.0 (68.3, 35.8/100.0)	191	83(43)	36(22)

TABLE V-3 (cont.)

NOTES

A. Tagged chitons might have disappeared during this study either due to death or to loss of tag. In many cases, when a chiton could not be relocated, the spot it had last occupied was vacant, suggesting that the chiton had died. Disappearance due to loss of tag was confirmed only twice in this study, in cases where a chiton with a flesh wound in the appropriate area of the girdle still occupied the spot where the tagged chiton had been observed last.

B. Habitats described in Chapters II, III. Distinctions between high sparse damp and dry habitats were noted during the electrophoretic study of Chapter III and are reflected here for _N_. _fluxa_ and _N_. _kata_.

C. Data for the interval from tagging to the first relocation and for the interval from a growth measurement or other manipulation of chitons to the first subsequent relocation were not used, to avoid the possibility of including abnormal behavior in analyses. Forty-five unidentified _Nuttallina_ tagged in this study were observed only once, or not at all, following tagging and were not included in any of the analyses. For a chiton moving from one habitat to another during the study, days were assigned according to habitat and chiton frequency of 1 was assigned to each habitat.

D. Observation interval lengths are plotted in Figure V-2.

E. Sum of all "usable" days (see note C); likewise, all chiton-days present includes only usable days. Manipulations that required removing chitons from rocks often injured them; thus "possible days" included only intervals between nonmanipulative observation dates.

F. To April-May, 1980, then November, 1980; interval lengths varied from about 4-8 months but were usually near 6 months. Intervals for chitons that disappeared due to manipulations between measurement episodes were not included in "total possible". Specimens that were not present at the ends of such intervals were not necessarily in the study less than 4 months. Chitons present at the end of the study consisted of some tagged in Fall, 1979, some in Spring, 1980.

24,193 chiton-days, were used in analyses of movement of Nuttallina.

A summary of characteristics of data used in analyses of movement of Nuttallina is presented in Table V-3 and Figure V-2.

HOMING OR NO MOVEMENT IN NUTTALLINA?

Homing in Nuttallina would entail a return before low tide to exactly the same resting spot on a rock vacated earlier in the high tide. Since the primary purpose of this study was to characterize long-term displacements, chiton locations were routinely recorded only at low tide, during the inactivity period of Nuttallina. Lacking routine observations at high tide, the period of activity, a major assumption involved in interpreting no displacement between successive low tide observations as homing is that the chitons leave their home, or resting position, between the observations at all. Results of short-term studies on Nuttallina by myself and others clearly indicate that some, but not all, specimens of Nuttallina leave their resting sites at each high tide (Tables V-4, V-5). Kues (1969) directly observed 29 to 57% of "Nuttallina fluxa" in a high intertidal area in La Jolla, CA (presumably most were Nuttallina fluxa) to move away from their low-tide resting spots during awash periods at day or night and he suggested that many more specimens probably moved about during high tide periods during which he was unable to continue observations. Films taken by Huffman (1976) under specialized circumstances in a high intertidal area only tens of meters from my La Jolla study site clearly show that Nuttallina move away from their resting spots even during the middle of the day at high tides that barely cover them. The specific status of specimens in

Figure V-2. Observation interval lengths for Nuttallina. The length of each interval between two successive observations is plotted.

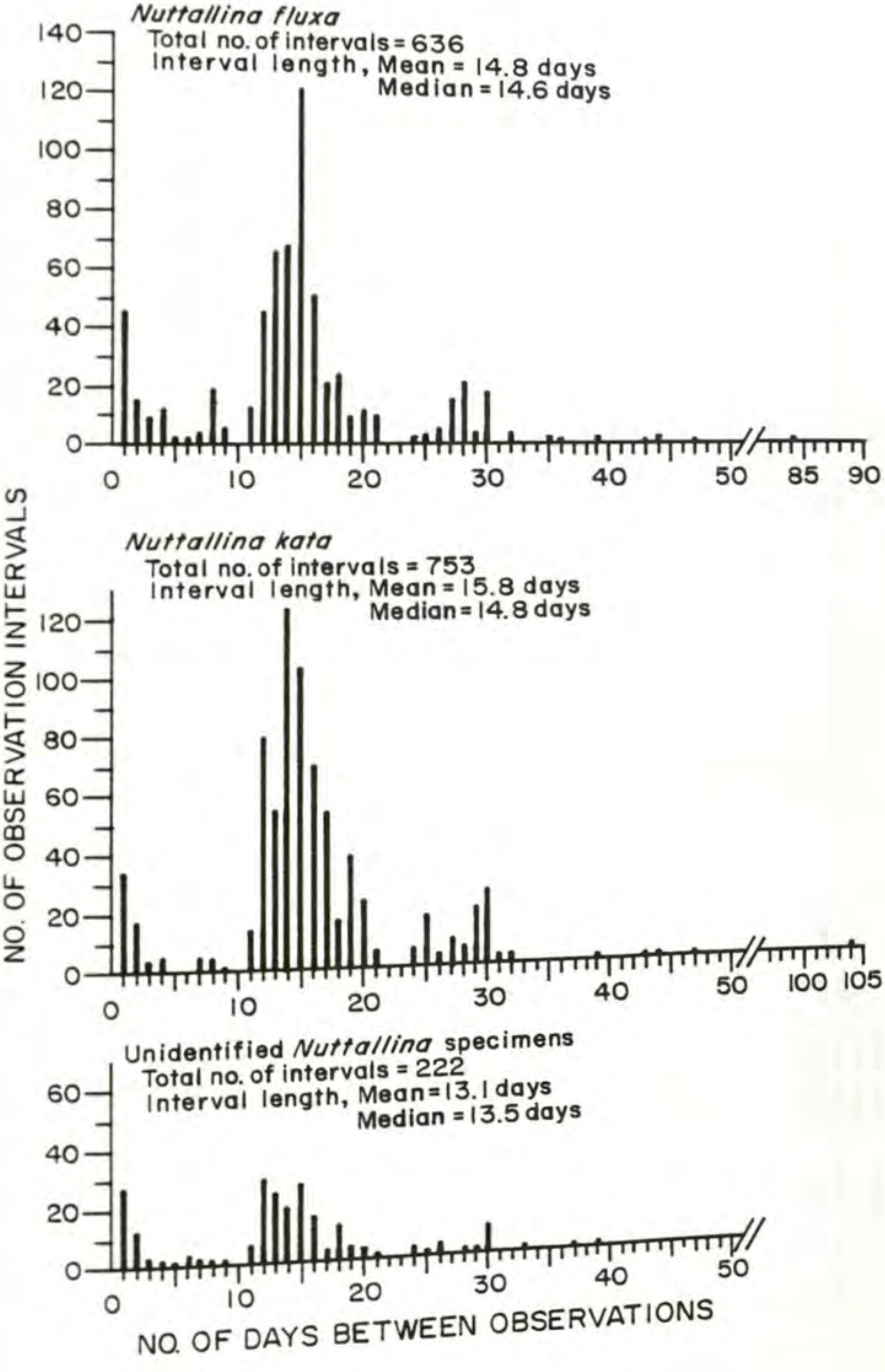

Nuttallina fluxa
Total no. of intervals = 636
Interval length, Mean = 14.8 days
Median = 14.6 days
0
20
40
60
80
100
120
140
0
10
20
30
40
50
85
90
Nuttallina kata
Total no. of intervals = 753
Interval length, Mean = 15.8 days
Median = 14.8 days
0
20
40
60
80
100
120
0
10
20
30
40
50
100
105
Unidentified *Nuttallina* specimens
Total no. of intervals = 222
Interval length, Mean = 13.1 days
Median = 13.5 days
0
20
40
60
0
10
20
30
40
50
NO. OF OBSERVATION INTERVALS
NO. OF DAYS BETWEEN OBSERVATIONS

TABLE V-4. Short-term movement studies on Nuttallina by other workers

AUTHOR	LOCATION; HABITAT	NUTTALLINA SPECIES	DATE; DURATION	PROCEDURE	RESULTS	CONCLUSIONS; COMMENTS
Kues, 1969	La Jolla, CA (Dike Rock); depressions in high intertidal area on hard sandstone boulders	"Nuttallina fluxa"; probably Nuttallina fluxa (see Chapter III)	November, 1968; 46 hours	Marked 24 chitons with paint; intermittent observations discontinued at high tide	29 to 57% of chitons out of depressions at periods awash, during day as well as at night; some chitons move 10 cm or more from homesites while submerged	Substantial percentage, if not all, of Nuttallina specimens are active when submerged, inactive in resting spots when exposed at low tide; movement apparently independent of day/night cycle
	La Jolla; same general habitat	Probably N. fluxa	10 days	Locations of 70 marked chitons recorded at low tides on 3 successive days (of 24 chitons after 10 days also)	Proportion found in same depression after one, two, three or ten days was over 80% (50 to 100% in different runs?); for 84 observations on those chitons that were in the same depression at low tides on successive days, 21 percent were at a 180% orientation , 79 percent at same orientation as originally, suggesting that at least some of the "homing" chitons moved away from homesite at high tide; nonhoming individuals up to 10 cm or more from homesite	Most Nuttallina home over short periods, while some nonhomers exhibit displacements of 10 cm or more
	Same?	N. fluxa?	1 day	Marked chitons that had homed to sites for a week, and moved various distances from homesite at low tide to unoccupied depressions nearby; location at next low tide recorded	One of 6 that moved 10 cm returned home; four of 8 that moved 5 cm returned home (others 6-13 cm from starting point)	Nuttallina apparently does not normally venture far from its homesite; if it goes too far, it takes up a new home
	La Jolla Seal Rock site; densely-populated area (~1000/m^2); sparsely-populated area (40/m^2)	"Nuttallina fluxa"; probably primarily N. kata (dense area); N. fluxa (sparse)	13 March-15 April, 1969	Cleared chitons from 400 cm^2 square in each habitat and monitored repopulation by intermittent censuses	Dense area: 41 chitons originally, 29 after one month. Sparse area: about 12 chitons originally (40/m^2), 3 after one month	Nuttallina will abandon homesites to colonize new areas

TABLE V-4 (cont.)

AUTHOR	LOCATION; HABITAT	NUTTALLINA SPECIES	DATE; DURATION	PROCEDURE	RESULTS	CONCLUSIONS; COMMENTS
Louda, 1972	Santa Barbara; pier pilings and reef rocks	"Nuttallina fluxa"; probably primarily N. fluxa (see Chapter III)	Series of 24 hr. studies: with intervening night high tides--27 March 1971, 17 March 1972; with intervening day high tides--22 Feb 1971, 25 Feb 1972, other observations	Marked chitons (Total N=116, March studies; N=100, Feb. studies) without moving them and recorded positions at successive minus low tides 24 hours apart; transplanted 20 chitons to new spots and noted positions after 24 hours	Movement apparently confined to high tide. Average displacement over 24 hrs. of undisturbed chitons in 5.0-9.9 cm interval (median in 0-4.9 cm interval); greatest displacement was 97 cm (intervening night high tide). Average displacement of transplanted chitons over 24 hrs. was 21.6 cm. Marked chitons exhibited slight tendency (not significant) for more displacement with intervening night high tides than with day ones.	Results self-explanatory
	Santa Barbara; rough rock area	N. fluxa?	16 January 1972 to 28 April 1972	Two 20x20 cm areas fenced and limpet densities in one area doubled by species; areas censused intermittently	No. of chitons in both experimental and control areas decreased from 6 to 5 during study	Increased limpet density does not cause a net movement of chitons out of fenced area although chitons can cross fences
	Santa Barbara; reef rock ~1 m. high	N. fluxa?	8 October to 2 November 1971	Censuses of chitons at different heights above sand, before and after sand level dropped	8 October: 257 chitons above 90 cm height (above MLLW) on reef (sand level); 2 November: 215 above, 45 below 90 cm height, sand level at 78 cm height on rock.	Nuttallina moves down on rock as sand retreats
Huffman, 1976 (personal communication) (E.W. Huffman, Prof. of Zoology, San Diego State University)	La Jolla, CA; near my Seal Rock study site; primarily high/dry sparsely-populated soft sandstone but some damper habitat and pools with coralline algae, all at +150 cm above MLLW	"Nuttallina fluxa"; probably primarily N. fluxa, possibly some N. kata in pools, in depressions	August, November 1976; a few hours on several different sunny days	16 mm movie film frames taken of fixed, selected areas at high tide at ~2 minute intervals; high tides were selected such that study areas were submerged by at most a few cm of water at highest tide (usually 165 cm above MLLW) and highest water occurred from 11 a.m. to 1 p.m. on sunny days, so that specimens were visible throughout the tide	Some (~50%) of specimens move away from homesites [short distances up to 35 cm(?)] during these daytime low tides; specimens generally follow the outgoing trail to return home; surprisingly, many specimens (Huffman suggests about half of the moving chitons) do not turn around as they return home [even considerable, 10 cm (?) distances] while the balance turn around to return (one specimen returned on the outgoing trail by "spiraling" back, touching the trail alternately with anterior, posterior. Thus, many specimens that are found in the same orientation before and after a high tide might have moved out and returned by backing up.	Results self-explanatory [results are from Huffman's unpublished analyses of the films as well as from my examination of several selected films]

TABLE V-4 (cont.)

AUTHOR	LOCATION; HABITAT	*NUTTALLINA* SPECIES	DATE; DURATION	PROCEDURE	RESULTS	CONCLUSIONS; COMMENTS
Bruno, 1978[A]	Bodega Marine Laboratory, Bodega Head, CA; 145 cm above MLLW	"*Nuttallina californica*"; *N. californica* (see Chapter III)	July, 1978; 8 days	Marked eight chitons in study site and marked another 8 chitons transplanted from other areas to random spots within the study area; locations noted on successive days at low tide; displaced some chitons various distances from homesites.	Chitons apparently moved only at high tide. Only 6 of 94 observations (24-hr intervals for which chitons not manipulated at the start) on 16 chitons showed displacements of 2-15 cm. Twenty-one of the 88 observations showing no displacement involved 180° orientation changes. Chitons experimentally displaced 1-7 cm from homesites returned home in 24 hrs in 4 of 6 cases.	Results self-explanatory; suggests without evidence that the chitons only move at night
King, 1980[A]	Bodega Marine Laboratory, Bodega Head, CA; high intertidal areas never completely submerged during the study	"*Nuttallina californica*"; *N. californica* (see Chapter III)	7 May to 30 May, 1980	Marked and sporadically observed chitons in two different areas; 10 or 11 specimens in each area at all times	Specimens apparently were found out of homesites only at night; it is unclear when tides occurred or when wave splash was greatest (animals apparently never completely submerged during the study). Specimens apparently moved farther from home at high tide (King's Table 4). Greatest distance any specimen was found from home was 30 cm. Animals home but some occasionally change homesites.	Results self-explanatory
Vesco, 1980	Whites Point, L.A. Co., CA; "coralline algal community" (0.4 to 0.5 m above MLLW) and "barnacle community" (0.9 to 1.2 m above MLLW)	"*Nuttallina fluxa*"; primarily *N. kata* in low area; *N. fluxa* in high area (see Chapter III)	24 to 25 May 1978; 21,22,24 July 1978	Marked 28 chitons in low area and 44 chitons in high area, and recorded orientations on successive days at low tide; other observations	Substantial tag losses (63% loss in 24 hrs) Low area: 27 of 28 observations on 16 chitons (at 24 hr intervals) show chiton in same spot; only 1 moved (2 cm). Six of 27 in same spot changed orientation 180°. High area: 11 of 17 observations on 11 chitons (at 24 hr intervals) show chiton in same spot; observed displacements were 2-6 cm, and ,in one case, 30 cm in 24 hrs. Four of the 11 in same spot changed orientation 180°. Stationary at low tide and awash.	Chitons in high area (*N. fluxa*) tend to exhibit greater displacements and "home" less frequently than ones in low area (*N. kata*). *Nuttallina* apparently move while submerged since occasional displacements are observed and chitons are stationary at low tide and while awash

NOTE.

A. Bruno (1978) and King (1980) are undergraduate student reports that contain ambiguities and lack detail; findings are presented with a note of caution.

TABLE V-5. Evidence for Short-term movement in Nuttallina in Southern California by the author

TYPE OF STUDY	LOCATION; HABITAT	NUTTALLINA SPECIES	DATE; DURATION	PROCEDURE	RESULTS
Position changes	La Jolla, CA; the Seal Rock study site; various habitats	N. fluxa and N. kata	10 September 1979 to 11 November 1980; 24 hour intervals from low tide to low tide on haphazard occasions	Positions of specimens tagged for the long-term study were recorded; probably biased toward those that moved.	Subgroup: N. kata in low intertidal densely-populated area(23 observation intervals, 12 chitons): 20 of 23: no displacement; 1 of 23: 2 cm; 1 of 23: 2.3 cm; 1 of 23: 5.0 cm.
Orientation changes	La Jolla, CA; the Seal Rock study site; high area, including dry, damp and small pool habitats, and low densely-populated area with chiton depressions and coralline algal turf	Primarily N. fluxa in high area; primarily N. kata in low area	15 to 16 December 1981 at the end of a spring low tide series(18 cm, 0 cm above MLLW, respectively); 24 hours, from 7 pm to 7 pm	Noted orientations of 21 chitons in high area, 30 chitons in low area along short transect lines at successive low tides, 24 hours apart; chitons not touched or manipulated in any way	Low area(total N=30): All in same spots- 14 with same orientation, 16 reversed orientation 180°. High area(N=21): 11 same orientation, same site; 7 reversed orientation 180°, same site; 1 changed orientation 45°, same site; 2 chitons vacated their spots
Clearing	La Jolla, CA; the Seal Rock study site; high sparsely-populated and low, densely-populated intertidal(with deep depressions) areas	Primarily N. fluxa in high area; primarily N. kata in low area	April and May, 1980; series of 24 hour studies	Removed Nuttallina specimens from resting sites at spring low tide and noted presence/absence of new animals in these sites 24 hours later at low tide	High area: Four of 58 spots occupied by new Nuttallina; one of 58 spots occupied by an Acmaeid limpet. Low area: Eight of 39 depressions occupied by new Nuttallina. Dense/sparse boundary area: 5 of 19 spots occupied by new Nuttallina; 3 of 19 spots occupied by Acmaeid limpets. Over 31 days in November, December 1980, 11 of 20 experimentally vacated spots reoccupied by Nuttallina at different shore levels; 16 Nuttallina removed from 1 m^2 area in high/dry bare habitat on 12 Sept 1977 and eighteen (ranging from 22 to 37 mm live total length) occupied this area on 18 August 1978.

TABLE V-5 (cont.)

TYPE OF STUDY	LOCATION; HABITAT	*NUTTALLINA* SPECIES	DATE; DURATION	PROCEDURE	RESULTS
Direct observations	La Jolla Seal Rock site; Gaviota site(see Chapter III)	*N. fluxa* at Gaviota; *N. fluxa* and/or *N. kata* at La Jolla	Sporadic observations over several years, particularly 1979-1981	Visual observations; chitons out of depressions usually not followed for long and movement not confirmed except where noted in "Results" column	1. 16 May 1980. La Jolla site. A few *Nuttallina* out of depressions while exposed at low tide; conditions: overcast, fairly warm, not too damp, no breeze, still dark. 2. 2 June 1980. La Jolla site. Several *Nuttallina* observed out of depressions while exposed at low tide; conditions: overcast, dewy, cool, early morning low tide, already light. 3. 11 September 1980. La Jolla site. One *Nuttallina kata* specimen (33 mm long) half in, half out of depression in upper intertidal, damp, sparse area; not entirely stationary but moving mm's; conditions:cool, no breeze, overcast dark sky, still dark. 4. 16 April 1981. Gaviota site. Many *N. fluxa* (see Chapter III) in lower intertidal out of depressions and moving while exposed; conditions: sunny early afternoon, area wet by occasional waves even at lowest tide(neap low tide, +30 cm above MLLW)

Huffman's films is not possible to determine although the bare, sparse habitat suggests that most are likely N. fluxa. In at least one case, a moving chiton emerged from a relatively deep depression surrounded by foliose coralline algae in a pool area, a microhabitat characteristic of N. kata, and this specimen might have been a specimen of N. kata. Other than the rare occasions on which I observed Nuttallina (most likely N. fluxa) moving at low tide in damp conditions (Table V-5), these are the only instances where Nuttallina have been directly observed to leave their low-tide resting spots.

A much larger body of indirect evidence also indicates that Nuttallina moves about during high tides (Tables V-4, V-5). Kues (1969), Louda (1972), Vesco (1980) and I recorded positions of marked chitons on successive low tides twenty-four hours apart; small percentages of Nuttallina fluxa (in all four studies) and smaller percentages of N. kata (Vesco, 1980 and the present study only) exhibited displacements over these periods. Since Nuttallina is immobile at low tide, it is clear that these displacements resulted from nonhoming excursions undertaken at high tide. Presumably, since many Nuttallina specimens move away from homesites and subsequently home, and therefore do not exhibit displacements on successive low tides despite having moved about, the number of instances of displaced chitons noted over 24 hour periods from low tide to low tide is a gross underestimate of the number of Nuttallina that have actually moved during the intervening high tide(s).

Additionally, Kues (1969), Vesco (1980) and I noted the orientations of chitons at 24 hour intervals, from low tide to low tide (Tables V-4, V-5). The animals often inhabit sites in which the orientation can only be one way or at 180° to that. Kues (1969) found that 21% of "Nuttallina fluxa" found in the same site on successive days had changed orientation 180°, thus had moved enough to turn around in the site at high tide. Vesco (1980) noted that 36% of N. fluxa in the same high intertidal spots on successive days had switched 180°, whereas 22% of N. kata found in the same low intertidal spots had made the change (all specimens referred to by her as "N. fluxa"). I found that 37% of N. fluxa in the same sites in high areas of the La Jolla site switched orientation 180° (and 5% switched 45°), whereas 53% of N. kata in the same spots in low areas switched 180° over a 24 hour period (Table V-5).

Huffman's (1976) films on high intertidal N. (?)fluxa provide direct evidence that the number of instances of 180° orientation changes observed in all of these studies, at least in high intertidal N. fluxa, vastly underestimate the number of chitons that actually move during intervening high tides. Huffman's (1976) films document instances of Nuttallina that move away from homesites at high tide and then return to the homesite by backing up the entire length of the outgoing trail, thus maintaining the same orientation in the homesite. Huffman (personal communication, May 1983) estimates that the frequency of occurrence of this behavior is about as great as that of turning around to return along the outgoing path (producing a 180° orientation change in the homesite). Thus it is clear that substantial percentages of Nuttallina specimens leave their homesites at high tide.

Finally, I removed Nuttallina specimens from high and low shore areas in the La Jolla site and noted the presence of new chitons in the vacated sites during the following low low tide (Table V-5). In the sparsely-populated high area, 4 of 58 experimentally-vacated sites were re-occupied by new Nuttallina, while 8 of 39 depressions in the low area were re-occupied. These data show clearly that Nuttallina move at least short distances at high tide, and more importantly, represent the most convincing data available showing that substantial percentages of low shore Nuttallina kata move completely out of their depressions at high tide. However, the fact that most low intertidal Nuttallina, in particular, occur in depressions surrounded by lush algal growth makes it difficult to discount the possibility that many of these specimens simply graze on fringing algal growth and occasionally come to rest in another orientation.

Intervals between most observations in this study averaged about 15 days in length. In view of the short-term data just presented, it would seem likely that most, if not all, Nuttallina specimens move away from their homesites at least once during a time interval of this length. Thus, animals found at the same location after an observation interval in this study will be referred to as homers, with full appreciation of the possibility that occupation of the same location at successive observations might result from zero to many true homing episodes over the many tides between observations.

RESULTS OF ANALYSES ON RAW DATA (UNWEIGHTED OBSERVATION INTERVALS OF UNEQUAL LENGTH)

Chiton positions could not be noted at perfectly regular intervals throughout this study for several reasons. Weather and wave action occasionally interfered with the observation schedule. In addition, tags were occasionally inaccessible for reading without disturbing chitons and were not always found, presumably because they were trapped out of sight between the substrate and the body of the chiton. Finally, the process of relocation of all of the study chitons generally required three consecutive low tide episodes to complete, and to avoid bias in the phase of the tide series that particular chitons were relocated, searches were started at different points in the study site area each spring tide series. Observation intervals were consequently of different lengths. Thus, analysis of chiton displacements in this study presents a problem in that observation intervals are not all of equal lengths and observations do not perfectly coincide for different chitons. In this section, unmanipulated raw data are summarized in several ways. In a subsequent section, observation intervals and real-time periods are handled such that more detailed analyses of Nuttallina displacements can be performed.

Homing (Including Instances of No Movement)

Nuttallina can be regarded as an excellent "homer". Data summarized in terms of homing are presented in Table V-6. This data summary has been generated by taking a shift in position of less than or equal to 1.26 cm between successive observations as an incidence of no displacement, i.e., homing (remember that 1.26 has been determined to be the 95% confidence limit for relocating objects using the quadrat system described in Materials and Methods). Thirty-four of the 160 tagged chitons were "perfect" homers; that is, they were found at the same spot every time they were observed during the study. If one classifies observation intervals as "homing" if the bracketing observed chiton

TABLE V- 6. Homing in Nuttallina.[A] Chitons homing for all observations are referred to as "perfect" homers; all others are referred to as "movers".

	Nuttallina fluxa	Nuttallina kata	Unidentifiable Specimens	Totals
All Chitons ("Perfect" homers and "movers")				
Total number of chitons with usable data[B]	56	77	27	160
Total number of observation intervals	636	753	222	1611
Percent of observation intervals homing	44.8%	72.2%	53.2%	58.8%
Total chiton-days observed	9425	11860	2908	24193
Percent of chiton-days homing	42.9%	71.7%	52.0%	58.1%
Number of "perfect" homing chitons (mean±s.d., range of days observed)	3 (24.7±1.15, 24-26 days)	28 (101.1±69.58, 14-213 days)	3 (62.3±50.84, 24-120 days)	34

TABLE V-6 (cont.)

	Nuttallina fluxa	Nuttallina kata	Unidentifiable Specimens	Totals
"Moving" Chitons (Total minus "perfect" homers)				
Number of "moving" chitons	53	49	24	126
Total number of observation intervals	630	590	210	1430
Percent of observation intervals homing	44.3%	64.6%	50.5%	53.6%
Total chiton-days observed	9351	9028	2721	21100
Percent of chiton-days homing	42.4%	62.9%	48.7%	52.0%
Percent of days homed by individual "moving" chitons mean±s.d. (range)	42.0±20.67% (0-88.6%)	55.4±29.12% (0-96.6%)	48.1±21.38% (0-94.3%)	48.4±25.0% (0-96.6%)
Days per homing observation interval, mean±s.d.	14.2±8.08	15.6±7.43	12.8±6.82	
Days per nonhoming observation interval, mean±s.d.	15.3±8.00	16.1±7.36	13.4±8.81	
Days per observation interval, homing and nonhoming, mean±s.d. (median, interquartile range)	14.8±8.05 (14.6, 12.3-16.5)	15.8±7.42 (14.8, 13.0-17.4)	13.1±7.80 (13.5, 10.9-16.0)	

TABLE V-6 (cont.)

	Nuttallina fluxa	Nuttallina kata	Unidentifiable Specimens
Chitons Never "Homing"[C]			
Number of specimens (ranges of number of observations and days per chiton)	4 (2-4 observations, 15-61 days)	5 (2-4 observations 7-58 days)	1 (4 observations, 57 days)

NOTES

A. Homing is simply defined as a displacement of $\leq$1.26 cm from one observation to the next. 1.26 cm represents the upper limit of the 95% confidence interval of precision for relocation of chitons by the quadrat Cartesian coordinate system. In practice, this places the break between homing and moving at $\sqrt{2}$ (=1.4) cm in this table.

B. The time interval between tagging and the first relocation, and between a measuring event (or other manipulation requiring removal of chiton from the rock) and the subsequent relocation of a chiton, was not used in any analyses since chiton behavior during these intervals may have been abnormal. Thus, 45 chitons with only 1 relocation after tagging were excluded from analyses.

C. A chiton for which successive observed positions are the same, might have occupied a different spot during that observation interval, and conversely, a chiton for which successive observations show displacement, might have homed for many tidal cycles during that interval. The assumption that a chiton has either homed or shifted positions, as indicated by successive observations, 100 percent of that time interval has been used to simplify analysis for this table.

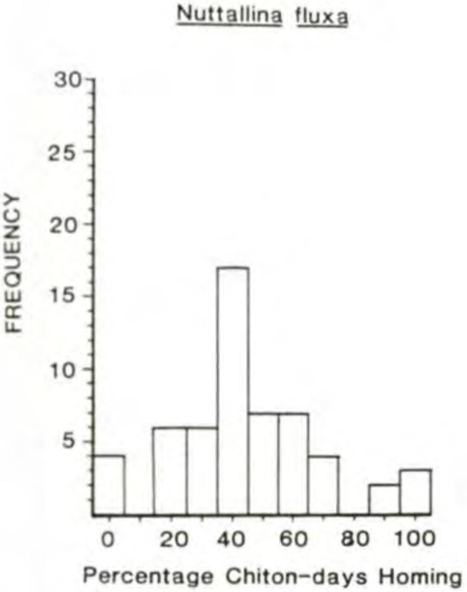
positions were within 1.26 cm of each other, homing intervals represent about 59% of the total 1,611 observation intervals (Table V-6). Recalculation of the data in terms of chiton-days involved, that is, regarding a homing interval of 15 days in length as 15 homing chiton-days, reveals that about 58% of all 24,193 chiton-days in the study were spent "homing". Such an approximation of chiton-day homing frequency is most likely an underestimate of "homing" since the probability that a chiton homed over all days of a "homing" interval is expected to be higher than that of a chiton becoming displaced over each successive day of a "moving" interval, particularly over longer intervals. While chitons might not home over all successive days of a homing interval, it is quite likely that they do. Assuming that a displacement occurs on each and every day of a "moving" interval probably considerably overestimates the number of days involving displacements, and underestimates homing days, since a shift in location accounting for all of the displacement in any observation interval could easily take place over a single tidal cycle. Both homing and moving intervals probably include days on which chitons never left their home sites while submerged at high tide.

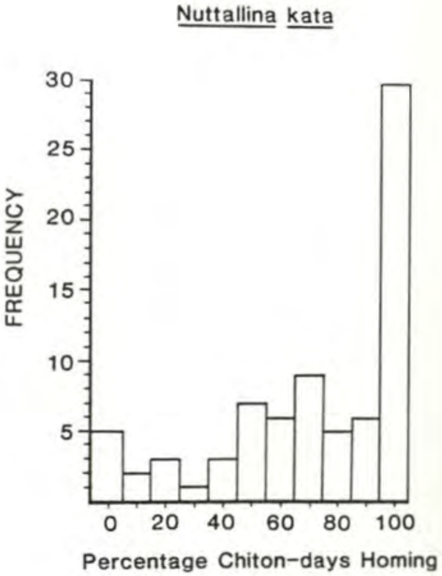
Even chitons that were not "perfect" homers, i.e., "movers", homed a considerable percentage of the time. For "movers", homing intervals represent over half (53.6%) of the total 1,430 observation intervals, and homing chiton-days comprise 52.0% of the total 21,100 chiton-days (Table V-6). Calculated on an individual basis, "movers" homed an average of 48.4% of the days they were observed.

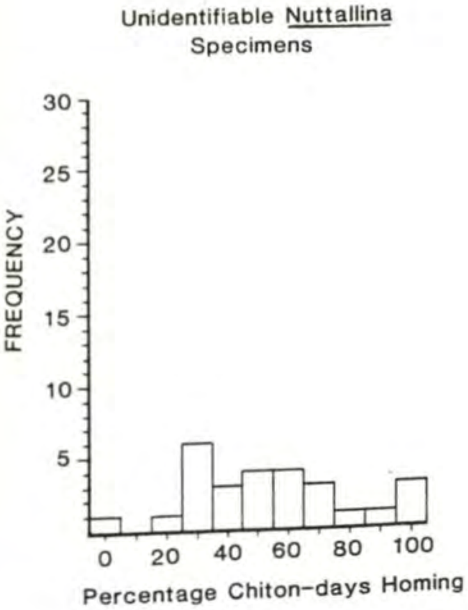
Thus, the majority of chitons in the study homed for a substantial percentage of the days they were observed. Although ten specimens never homed, i.e., were never found in the same location for two successive observations, none of these chitons was in the study for a very long period of time. The longest period such a chiton was observed was 61 days (Table V-6). There is no obvious division of the study animals into distinct "homing" and "moving" types since most animals exhibited both homing and moving intervals during the study (Figure V-3).

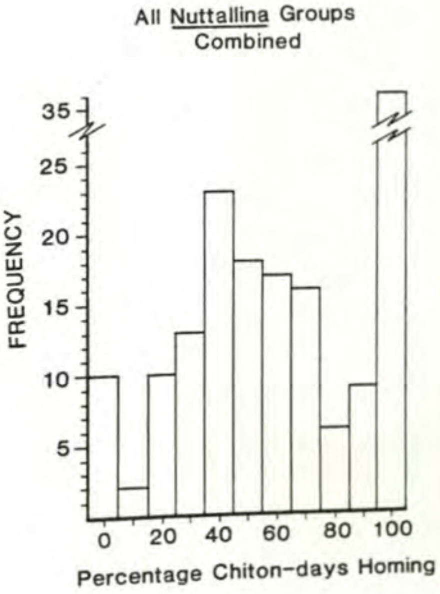
Results presented in Table V-6 clearly indicate that Nuttallina fluxa and N. kata are quite different in terms of long-term homing. In N. fluxa, only 3 of 56, or 5%, of specimens were always found at the same low tide location during the study, whereas 28 of 77 specimens of N. kata, or 36%, were never found displaced (Table V-6). These perfect homing individuals apparently are not restricted to any particular size group or habitat. Two of the three perfect homers in N. fluxa were small (20.2 and 22.2 mm length) and one was large (43.2 mm in live total length). All three specimens were found in the high dry sparse habitat (3 of 41), and no perfect homing specimens of N. fluxa were found in either high tidepools (0 of 14) or in the low dense habitat (0 of 1). However, in no habitat is the incidence of perfect homing significantly different from that expected (3/56) if all chitons are treated as equals, that is, if each is given a weight of 1 regardless of the length of time it was observed in the study (binomial test, $p>0.20$ in all cases). The 28 perfect homers in Nuttallina kata were of all sizes, from 18.7 to 39.0 mm total length, and were found in both the low dense (22 of 55) and the high damp sparse (6 of 13) intertidal areas. None of the three specimens of N. kata occurring in high intertidal pools was a perfect homer. Again, treating each chiton simply as an equal,

Figure V-3. Approximate percentage of homing chiton-days in Nuttallina individuals. Homing is defined as displacement of ≤ 1.26 cm between successive observations. All days between consecutive observations at the same location are regarded as homing days; all days between observations at different locations are regarded as moving days. As indicated in Table V-4, the number of "perfect" homing chitons, i.e., with 100% of days homing, is as follows: N. fluxa, N=3; N. kata, N=28; unidentifiable specimens, N=3. All individuals in the 0% category in this figure are true 0% homers, i.e., they exhibited displacements between all consecutive observations.


Nuttallina fluxa
FREQUENCY
30
25
20
15
10
5
0
20
40
60
80
100
Percentage Chiton-days Homing


Nuttallina kata
FREQUENCY
30
25
20
15
10
5
0
20
40
60
80
100
Percentage Chiton-days Homing


Unidentifiable Nuttallina Specimens
FREQUENCY
30
25
20
15
10
5
0
20
40
60
80
100
Percentage Chiton-days Homing


All Nuttallina Groups Combined
FREQUENCY
35
25
20
15
10
5
0
20
40
60
80
100
Percentage Chiton-days Homing

regardless of the length of time it was observed in the study, the incidence of perfect homing of N. kata is not significantly different from that expected in any habitat (expected = 28/77, binomial test, p>0.09 in all cases).

In Nuttallina fluxa, about 43% of the total chiton-days and approximately 45% of the 636 observation intervals involved no displacement, whereas, in N. kata, corresponding percentages were both about 72 (Table V-6). Similar but less pronounced species differences are found when only "movers" are included in the analysis (N. fluxa: 42 and 44; and N. kata: 63% and 65%). It can be seen that the homing percentages for the group containing unidentifiable specimens fall between those for N. fluxa and N. kata in analyses of homers plus movers, or of movers alone, as would be expected for a group comprising members of both species (Table V-6).

Distributions of percentages of chiton-days homing calculated for individual chitons also reveal species differences (Figure V-3). Whereas the distribution for Nuttallina fluxa tends to show greater frequencies around 40%, the distribution for N. kata has its greatest frequencies at 100% homing. The frequency distributions are significantly different (Kolmogorov-Smirnov two-sample test, two-tailed, p<0.001).

Displacements of Nuttallina fluxa and N. kata

Displacements of Nuttallina fluxa and of N. kata, like the homing proportions just presented, cannot be examined or compared in any detail using the raw data records, primarily because the observation intervals are of different length for different chitons (see following sections for detailed analyses), but examination of the displacement tracks of the five most mobile specimens of each species, displayed in Figures V-4 and V-5, shows a clear tendency for Nuttallina fluxa to undergo greater long-term point-to-point displacements than Nuttallina kata. The specimen tagged "1" exhibited the greatest observed displacement for Nuttallina fluxa, more than 117 cm in 143 days (Figure V-4). The chiton tagged "BH" showed the most displacement for Nuttallina kata, even though it was at the same site for observations comprising 132 of the 161 days that it was in the study. This chiton achieved most of its observed 49 cm point-to-point dispacement in a single interval 15 days long.

Upward Migration of Nuttallina?

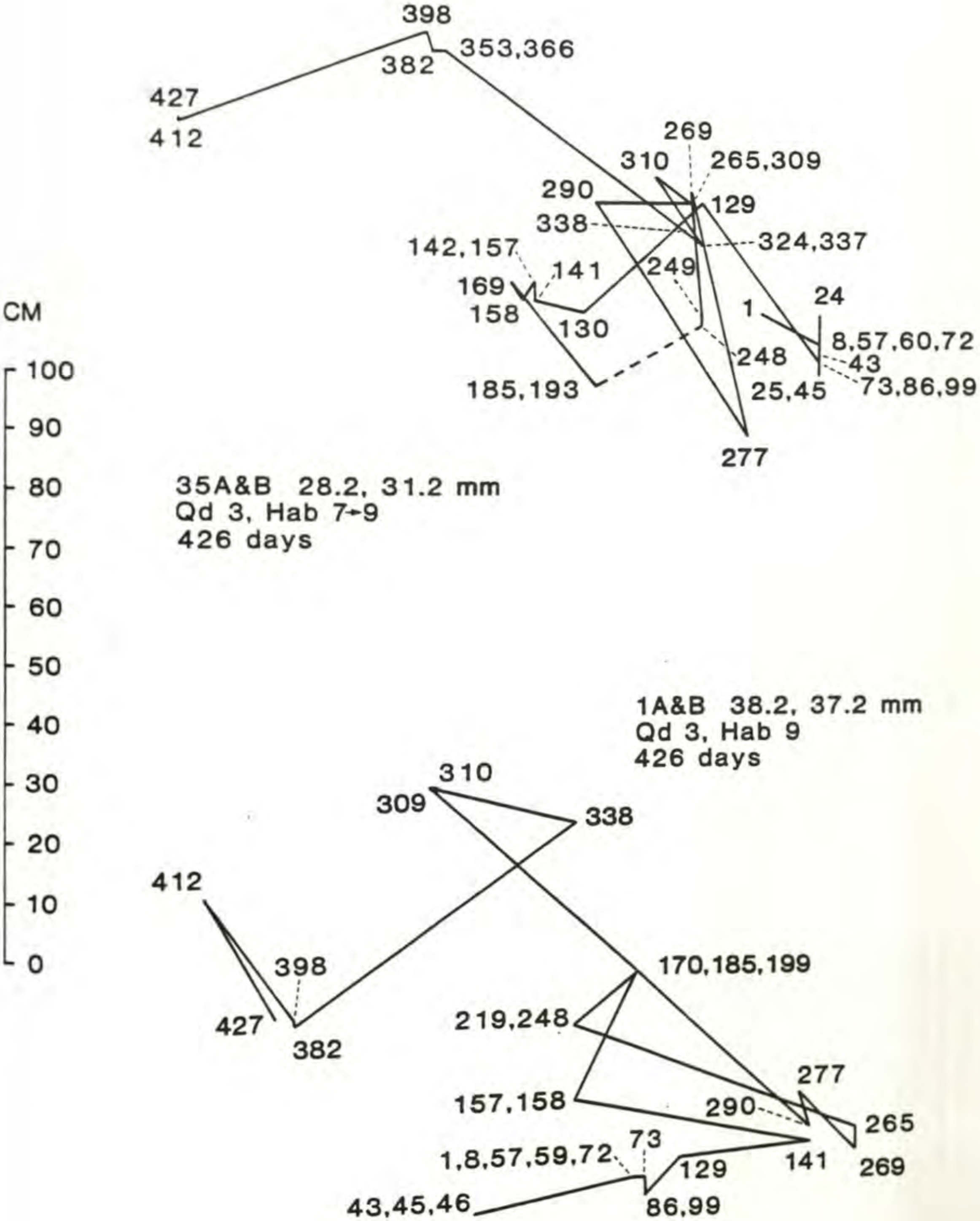
To test the possibility that the shore-level size gradient of Nuttallina, with larger specimens higher, might result from an upshore migration with age/size, I compared the positions of low shore specimens of Nuttallina fluxa and of N. kata and of unidentifiable Nuttallina specimens at the first and last usable observations of each in the study. Vertical intertidal displacements between the initial and final observed positions were estimated using the X and Y tilt angles of appropriate quadrats, listed in Table V-1.

Sixteen of the twenty-three low shore specimens of Nuttallina fluxa exhibited small net vertical displacements (Table V-7). Frequencies of specimens moving up were not significantly greater than those of specimens moving down (up=8, down=8, test for expected proportion of 0.5, one-tailed, p=0.5). Likewise, upward vertical displacements were

Figure V-4. Displacement patterns of the five tagged specimens of Nuttallina fluxa that exhibited the most displacement during the study.

Point-to-point displacements are plotted; a centimeter scale is on the left. All tracks are oriented such that the compass heading of the quadrat in which a specimen occurs is represented by the top of the page; therefore, the top of the page approximates the upshore direction. Numbers at different locations represent dates of observations. Day #1 = 10 September 1979; #200 = 27 March 1980; #429 = last day of the study, 11 November 1980.

For each specimen the following data are presented: chiton tag number; live chiton total length(s); quadrat (Qd) and habitat (Hab) in which the specimen occurred; and the number of days the specimen was in the study. Lengths are those measured at the beginning of intervals approximately 6 months long; if a chiton was in the study long enough for two measurements, the measurements are listed in order: chiton #1A&B was re-measured on day #219, chiton #3A&B on day #193, and chiton #35A&B on day #193 of the study. The bold dashed line for chiton #35A&B represents a change in location immediately following the manipulation of this animal for re-measurement. Chiton #3A&B also moved immediately after re-measurement but returned later. Chiton #1A&B did not move immediately after re-measurement. Quadrat characteristics are presented in Table V-1. Habitat #7 = mid-intertidal dense/sparse boundary; #9 = high, dry, sparsely-populated area. A change in general habitat is indicated by an arrow, e.g., Hab 7→9 indicates that the chiton moved from habitat 7 to habitat 9 during the study.


398
382
353,366
427
412
269
310
265,309
290
129
338
324,337
142,157
141
249
169
158
130
1
24
8,57,60,72
248
43
185,193
25,45
73,86,99
277
CM
100
90
80
70
60
50
40
30
20
10
0
35A&B 28.2, 31.2 mm
Qd 3, Hab 7→9
426 days
1A&B 38.2, 37.2 mm
Qd 3, Hab 9
426 days
310
309
338
412
398
427
382
170,185,199
219,248
277
157,158
290
265
73
1,8,57,59,72
129
141
269
43,45,46
86,99

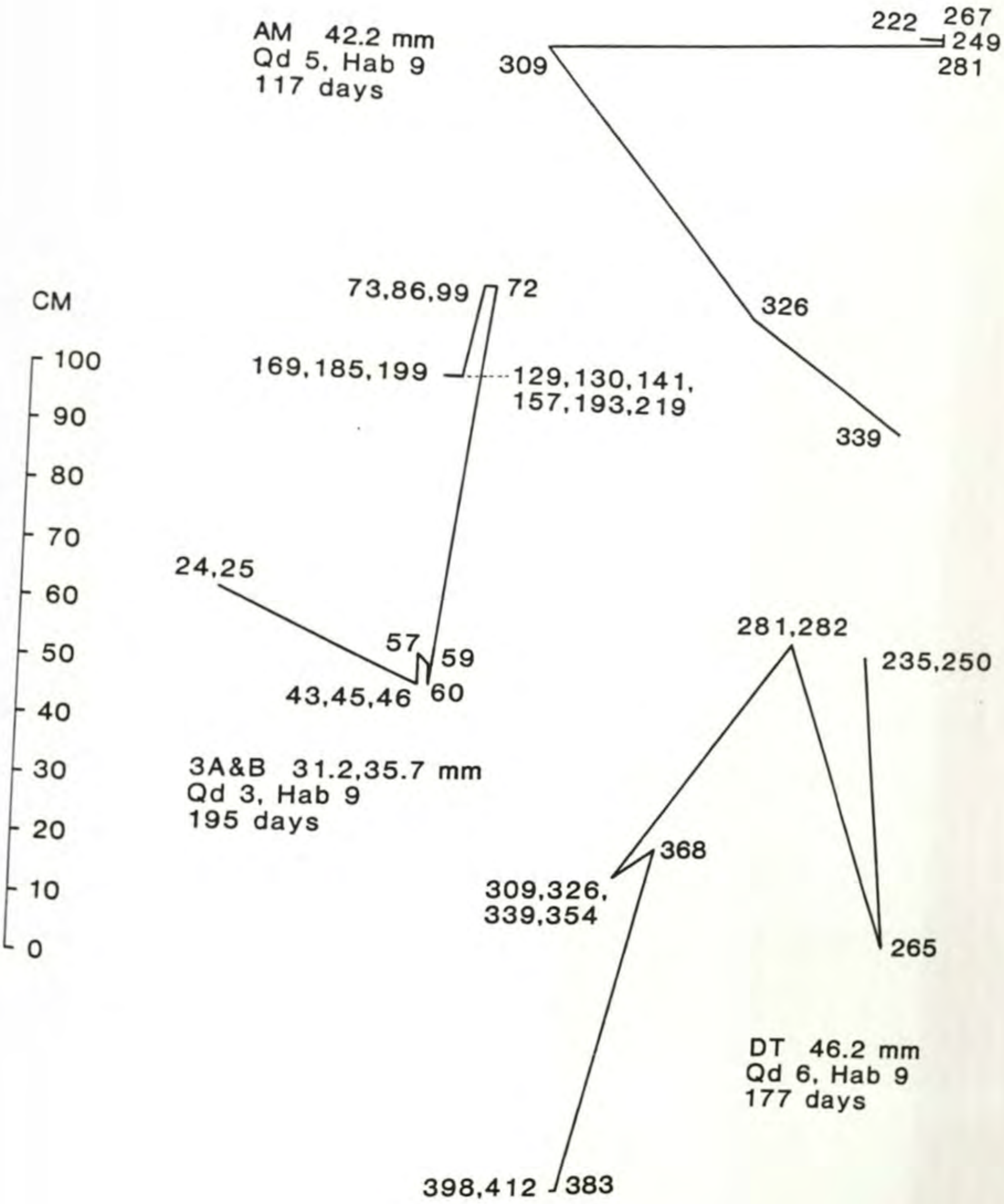

AM 42.2 mm
Qd 5, Hab 9
117 days
309
222
267
249
281
326
339
CM
100
90
80
70
60
50
40
30
20
10
0
73,86,99
72
169,185,199
129,130,141,
157,193,219
24,25
57
59
43,45,46
60
3A&B 31.2,35.7 mm
Qd 3, Hab 9
195 days
281,282
235,250
368
309,326,
339,354
265
DT 46.2 mm
Qd 6, Hab 9
177 days
398,412
383

Figure V-5. Displacement patterns of the five tagged specimens of Nuttallina kata that exhibited the most displacement during the study. See Figure V-4 caption for explanation.

Chiton #31A&B was re-measured on day #219 and chiton #73A&B on day #277 of the study. The bold dashed line for specimen #73A&B represents a change in location immediately following re-measurement. Specimen #31A&B did not move immediately after re-measurement. Habitat #4 = low intertidal, densely-populated area; #7 = mid-intertidal dense/sparse boundary; #8 = high intertidal pool; #10 = high, damp, sparsely-populated area.

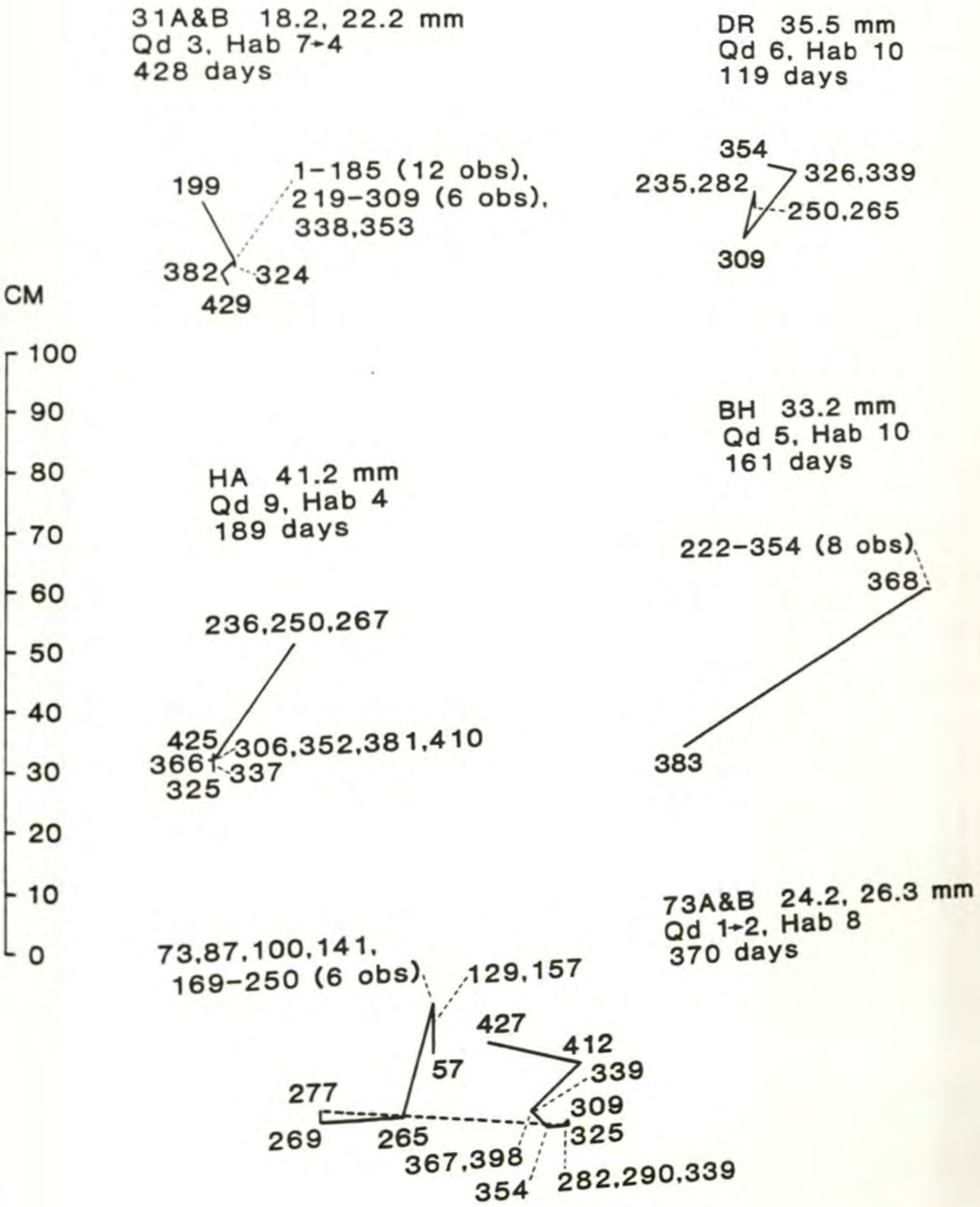
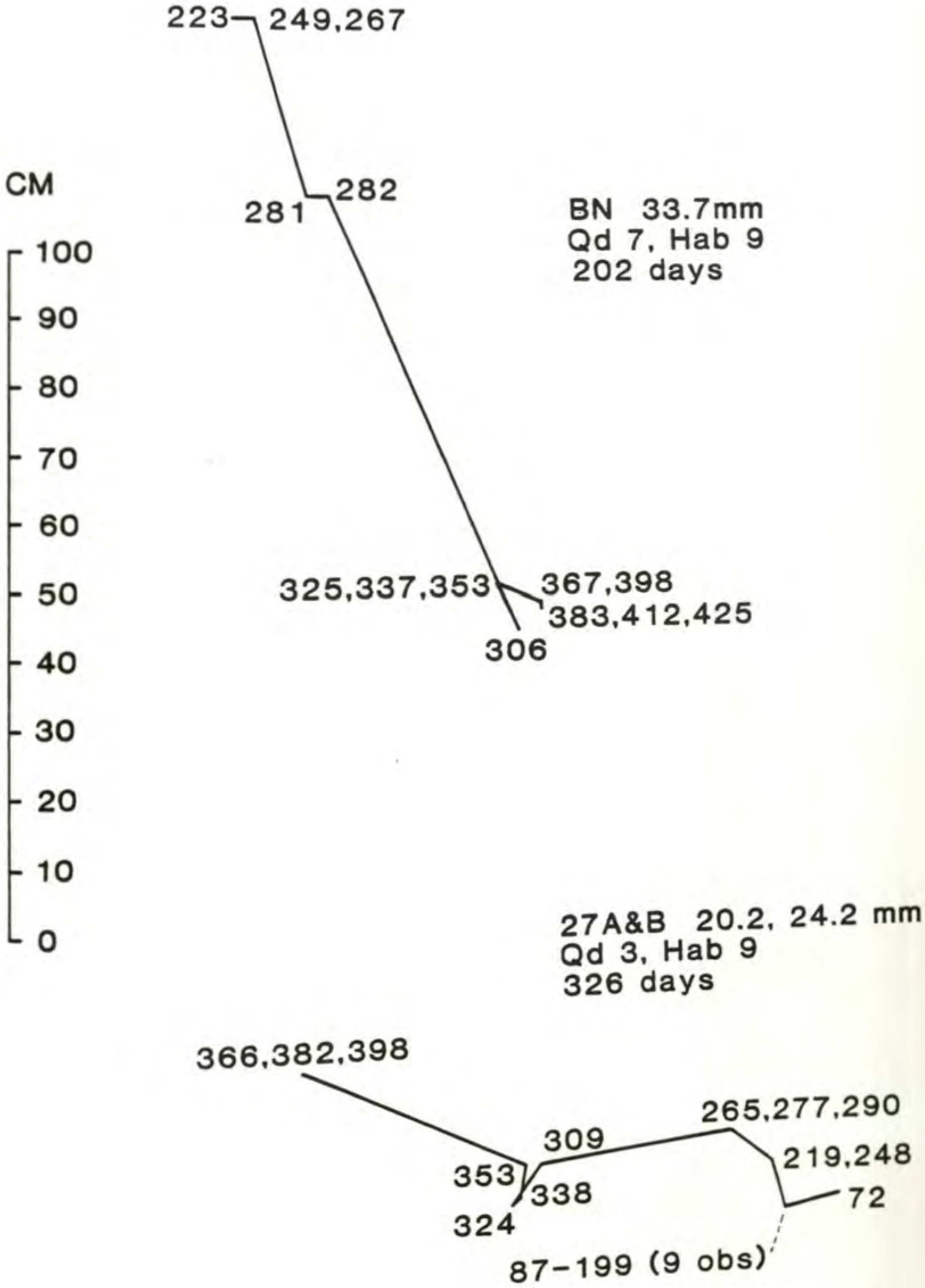

31A&B 18.2, 22.2 mm
Qd 3, Hab 7→4
428 days
199
1-185 (12 obs),
219-309 (6 obs),
338,353
382
324
429
DR 35.5 mm
Qd 6, Hab 10
119 days
354
235,282
326,339
250,265
309
CM
100
90
80
70
60
50
40
30
20
10
0
HA 41.2 mm
Qd 9, Hab 4
189 days
236,250,267
425
306,352,381,410
366
337
325
BH 33.2 mm
Qd 5, Hab 10
161 days
222-354 (8 obs)
368
383
73A&B 24.2, 26.3 mm
Qd 1→2, Hab 8
370 days
73,87,100,141,
169-250 (6 obs)
129,157
57
427
412
339
277
309
269
265
325
367,398
354
282,290,339

Figure V-6. Displacement patterns of the five tagged unidentifiable Nuttallina specimens that exhibited the most displacement during the study. See Figure V-4 caption for explanation. Specimen #27A&B did not change locations immediately after re-measurement on day #219 of the study.


223
249,267
CM
281
282
BN 33.7mm
Qd 7, Hab 9
202 days
100
90
80
70
60
50
40
30
20
10
0
325,337,353
367,398
383,412,425
306
27A&B 20.2, 24.2 mm
Qd 3, Hab 9
326 days
366,382,398
265,277,290
309
219,248
353
338
72
324
87-199 (9 obs)

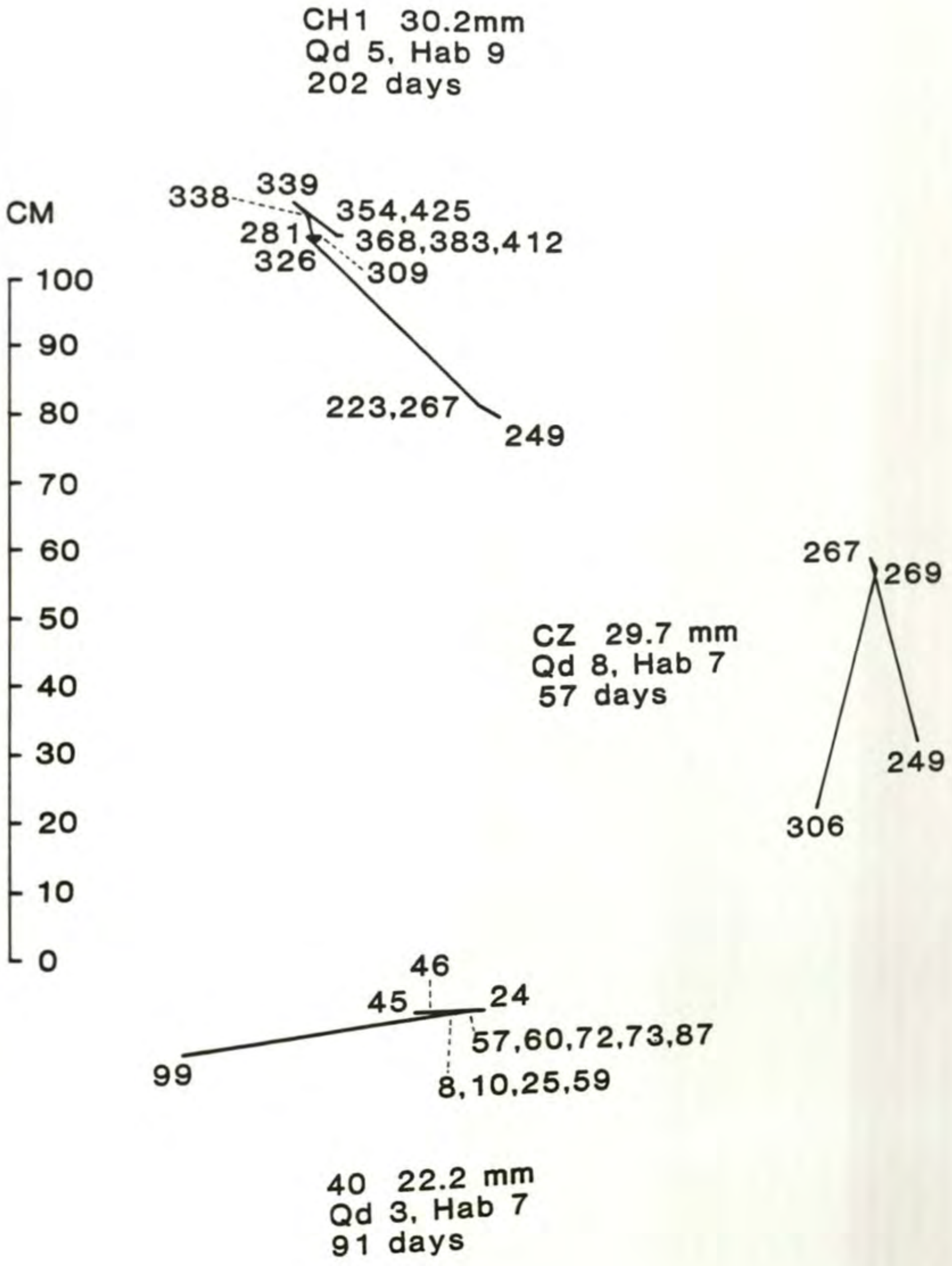

CH1 30.2mm
Qd 5, Hab 9
202 days
338
339
354,425
281
368,383,412
326
309
223,267
249
CM
100
90
80
70
60
50
40
30
20
10
0
267
269
CZ 29.7 mm
Qd 8, Hab 7
57 days
249
306
46
45
24
57,60,72,73,87
99
8,10,25,59
40 22.2 mm
Qd 3, Hab 7
91 days

TABLE V-7. Characterization of estimated[A] vertical intertidal displacements of lowshore[B] _Nuttallina_ specimens from their initial to final observed positions in the study.[C]

SPECIES	_Nuttallina fluxa_	_Nuttallina kata_	Unidentifiable _Nuttallina_ Specimens
UPPER LIMIT OF SPECIMENS CHARACTERIZED (INTERTIDAL HEIGHT ABOVE MLLW)[D]	+110 cm	+80 cm	+110 cm
TOTAL NUMBER OF CHITONS	23	55	15
I. HOMING CHITONS (≤1.26 cm displacement)			
Number (Percent of Total)	6(26)	29(53)	4(27)
Days in Study s.d.	56±46.7	122±76.6	81±52.6
II. CHITONS EXHIBITING POINT-TO-POINT DISPLACEMENT[E]			
ALL DIRECTIONS COMBINED			
Number of Chitons	17	26	11
Mean Vertical Displacement ± s.d., cm	+1.1±5.12	-0.8±2.17	-1.7±5.80
A. UPWARD DISPLACEMENTS			
Number of Chitons	8	6	2
Days in Study ± s.d.	185±124.1	204±131.0	222±147.1
Vertical Displacement			
Mean ± s.d., cm	+5.1±3.18	+0.8±0.37	+2.9±3.08
Maximum, cm	+8.5	+1.2	+5.1
Associated Point-to-point Displacement			
Mean ± s.d., cm	43.7±32.84	4.4±3.55	40.4±54.3
Maximum, cm	100.9	10.2	78.8
B. NO VERTICAL DISPLACEMENT			
Number of Chitons	1	6	3
Days in Study ± s.d.	140	188±135.4	102±75.5
Associated Point-to-point Displacement			
Mean ± s.d., cm	12.0	4.3±3.39	3.0±1.73
Maximum, cm	12.0	10.0	5.0
C. DOWNWARD DISPLACEMENT			
Number of Chitons	8	14	6
Days in Study s.d.	123±99.1	187±117.9	85±62.5
Vertical Displacement			
Mean ± s.d., cm	-2.8±3.70	-1.8±2.56	-4.1±6.94
Maximum, cm	-11.2	-10.5	-18.1
Associated Point-to-point Displacement			
Mean ± s.d., cm	22.3±22.7	5.6±5.56	44.3±50.91
Maximum, cm	66.6	21.4	96.6

TABLE V-7 (cont.)

NOTES

A. Vertical intertidal displacement of a specimen was estimated using intial and final quadrat Cartesian coordinates X and Y tilts for the appropriate quadrat listed in Table V-1.

B. The shore level used as the upper limit for specimens to be examined was set arbitrarily to include a sufficient number of specimens for statistical analysis. Lowshore specimens were expected to exhibit the greatest tendency, if any, to move upshore.

C. Specimens were in the study for variable lengths of time, but time in the study was not significantly correlated with either absolute or signed magnitudes of vertical displacement. Correlation coefficient for signed magnitudes of vertical displacement of moving chitons versus time of specimens in study: *N. fluxa*, $r = 0.186$; *N. kata*, $r = -0.003$; unidentifiable *Nuttallina*, $r = 0.453$. None significant, $p>>0.05$.

D. *N. fluxa* in quadrats 3, 4, 7-9 (see Table V-1).
N. kata in low, densely-populated area.
Unidentifiable *Nuttallina* specimens in quadrats 3, 4, 7-9.

E. For *N. fluxa*, one-tailed tests for greater upward frequencies or distances than downward ones:
Frequencies: test for expected proportion of 0.5, $p=0.5$
Vertical displacements: Kolmogorov-Smirnov two-sample test, $0.10<p<0.20$
Point-to-point displacements: Kolmogorov-Smirnov two-sample test, $0.10<p<0.20$
For *N. kata*,
Frequencies: test for expected proportion of 0.5, $p>0.9$
Vertical displacements: Kolmogorov-Smirnov two-sample test, $p>0.9$
Point-to-point displacements: Kolmogorov-Smirnov two-sample test, $0.8<p<0.9$
For unidentifiable *Nuttallina* specimens:
Frequencies: test for expected proportion of 0.5, $p>0.9$
Vertical displacements: Kolmogorov-Smirnov two-sample test, $0.7<p<0.8$
Point-to-point displacements: Kolmogorov-Smirnov two-sample test, $0.7<p<0.8$
Test for expected proportion in Dixon and Massey, 1969:100. Kolmogorov-Smirnov two-sample test, one-tailed, Siegel, 1956:135.

not significantly greater than downward ones (Kolmogorov-Smirnov two-sample test, one-tailed, $0.10<p<0.20$) nor were upward point-to-point displacements greater than downward ones (Kolmogorov-Smirnov two-sample test, one-tailed, $0.10<p<0.20$). There does appear to be a slight tendency toward longer upward-directed displacements, but the maximum upward vertical displacement is only 8.5 cm, so an upward migration of *N. fluxa* seems unlikely.

Twenty of the 55 low shore specimens of *Nuttallina kata* exhibited very small net vertical displacements (Table V-7). Similarly, for this species, the frequency of specimens exhibiting upward net displacements were not significantly greater than those showing downward ones (up=6, down=14, test for expected proportion of 0.5, one-tailed, $p>0.9$). Upward vertical displacements were not significantly greater than downward ones (Kolmogorov-Smirnov two-sample test, one-tailed, $p>0.9$) nor were upward point-to-point displacements greater than downward ones (Kolmogorov-Smirnov two-sample test, one-tailed, $0.8<p<0.9$). There does appear to be a tendency for displacements to occur more frequently and slightly greater distance downward, but the maximum downward vertical displacement observed was only 10.5 cm, and a migration thus seems not to occur.

Finally, eight of the 15 unidentifiable *Nuttallina* specimens exhibited net vertical displacements during the study (Table V-7). Again, frequencies of ascending chitons were not greater than those of descending ones (up=2, down=6, test for expected proportion of 0.5, one-tailed, $p>0.9$). Upward vertical displacements were not greater than downward ones (Kolmogorov-Smirnov two-sample test, one-tailed,

$0.7<p<0.8$) nor were upward point-to-point displacements greater than downward ones (Kolmogorov-Smirnov two-sample test, one-tailed, $0.7<p<0.8$). Again, as for N. kata, there appears to be a greater tendency for movements of slightly greater distances downward, but the maximum net downward vertical displacement was only 18.1 cm and a substantial downward migration apparently did not occur.

Analyses of raw data for all of these groups are complicated by the variability in the periods that chitons were present in the study; nevertheless, neither signed nor absolute magnitudes of net vertical displacements were significantly correlated with the number of days a specimen was in the study for any of the three groups (see Table V-7 note). In the following sections, interval lengths are standardized so that displacements of Nuttallina can be examined and compared in more detail.

RESULTS OF DETAILED ANALYSES OF DISPLACEMENTS (WEIGHTED OBSERVATION INTERVALS OF EQUAL LENGTH)

For more detailed analyses of Nuttallina displacements, unequal observation interval lengths and the lack of perfect coincidence of observations for different chitons must be dealt with or allowed for in some way since: 1) displacements are more likely to occur over longer time intervals; 2) observations at intervals of different lengths indicate different amounts of displacement for the same displacement track, unless the chitons always move in one direction, in a straight line (if not, as in Nuttallina, observations at very long intervals reveal much less displacement and detail than do extremely short intervals; and 3) displacements during particular real-time periods cannot be compared unless observations can be tailored to those real-time periods. Analyses of observed long-term displacements could be carried out using only those observation intervals of a particular length, but this method has the drawback of excluding much of the available data. For example, if analysis is restricted to intervals of 15 days, the most common interval length, the data used will represent only slightly more than 16% of all the intervals or 15% of the total chiton-days that Nuttallina was observed during the study. Clearly, if one desires to analyze displacements in particular real-time periods, rejecting observation intervals that overlap the endpoints of those periods, or if interval lengths other than 15 days are of interest, the data used will represent an even smaller fraction of the available data.

An alternative method of analysis that was used instead was preferable because it used more of the available data and allowed more flexibility in the length of intervals that could be analyzed. This method utilized "composite" observation intervals of the desired length, made up of actual observation intervals and parts of contiguous intervals using the assumption that any displacement between two successive observations has occurred linearly over the intervening time. Thus, to analyze displacements over 20 day intervals, successive, shorter observation intervals can be added together until the desired length is reached. If a single observation interval or a group of consecutive intervals add up to exactly 20 days, then the position coordinates noted at the beginning and end of this composite interval can be used to describe the nature of this 20 day displacement, just as would be done in the first analytic method, described above. However, if the consecutive intervals do not add exactly to 20 days, the observations immediately bracketing the 20 day endpoint are used to estimate unbiased coordinates for that endpoint. For example, if 20 day intervals are being analyzed and a chiton has been observed on 3 September, 20 September and 24 September, but not on 23 September, the beginning location of the chiton is taken to be the coordinates observed on 3 September, while the chiton's location at the end of the 20 day interval, on 23 September, would be estimated, by linear interpolation, as the position three-quarters of the way, in a straight line, from the coordinates observed on 20 September to those observed on 24 September.

In order to maintain independence of observation intervals in analyses by the second method just described, each observation interval or any part of it is used only once. Thus, in the foregoing example, the 20-24 September observation interval is used to estimate the coordinates on 23 September to complete the 20 day composite interval, and the remaining segment, from 23 to 24 September is discarded, the next composite observation interval starting at the 24 September observation.

In addition, to retain an idea of the total variability of displacements exhibited by the chitons, rather than taking a mean of the characteristics, e.g., distance or direction, exhibited in all intervals observed for any chiton, equal probabilities were assigned to each interval. For example, if a chiton moved 0, 2, 2 and 40 cm in four different intervals, rather than expressing this as a mean displacement of 11 cm (with a probability of 1.000), a probability distribution is set up for this chiton such that it had equal 0.250 probabilities of moving

0, 2, 2 and 40 cm. Probabilities were calculated so that the total probability for any chiton in any one of several categories to be compared, such as species, habitat or season, was exactly 1.000.

Displacements of Nuttallina Specimens

The long-term displacement records of the five specimens each of Nuttallina fluxa, of N. kata and of unidentifiable Nuttallina specimens exhibiting the most displacement (i.e., greatest magnitudes and most displacements) are presented in Figures V-4, V-5 and V-6 to give the reader an idea of the actual displacement patterns observed.

Results of analyses of displacement, using the interpolation method described above, are summarized in Tables V-8 through V-17 and in Figures V-7 through V-14. As was found in the initial analyses based simply on unweighted intervals of unequal length (see Table V-6), analyses using independent weighted observation intervals of equal length, with ending locations linearly interpolated when necessary, reveal that Nuttallina homes a considerable proportion of the time (Figure V-7 and Table V-8). It can be seen that homing proportions calculated on the basis of unweighted observation intervals of unequal length in the initial analyses, 45, 72 and 53% for N. fluxa, N. kata and unidentifiable Nuttallina specimens respectively (Table V-6), are quite similar to those calculated using weighted composite intervals of 15 days in length with interpolated endpoint locations (48%, 75% and 56% respectively, Table V-8). It is probably not coincidental that the average length of observation intervals in the study and used in the first analysis is near 15 days (Table V-6).

Table V-8. Homing proportions of *Nuttallina*, as calculated from point-to-point displacements of chitons from beginning to end of composite observation intervals. The location at the end of the composite interval was calculated by interpolation if necessary. Homing and nonhoming intervals for each chiton were weighted equally and totalled to frequency of 1.0. Expressed as chiton homing proportion ± s.d. (Number of chitons, number of intervals). Selected data are plotted in Figure V-7.

	Length of Composite Intervals, In Days				
	7	15	30	60	120
Species					
Nuttallina fluxa	0.65±0.064(56,553)	0.48±0.067(56,378)	0.36±0.067(51,216)	0.26±0.064(47,101)	0.07±0.045(34,42)
Nuttallina kata	0.86±0.039(77,694)	0.75±0.050(75,450)	0.67±0.056(71,287)	0.62±0.064(57,130)	0.57±0.074(45,55)
Unidentifiable *Nuttallina* Specimens	0.69±0.089(27,175)	0.56±0.095(27,111)	0.43±0.099(25,69)	0.26±0.104(18,28)	0.30±0.145(10,11)

Table V-9. Point-to-point, or straight-line, displacements of *Nuttallina* from beginning to end of composite observation intervals of different lengths. The location at the end of each composite interval was calculated by interpolation if necessary. Corresponding data are plotted in Figures V-8 through V-10.

Composite Observation Interval Length (days)	Number of Chitons	Number of Observation Intervals	Point-to-point Displacement (cm)					
			Mean	Median	25 to 75 Percentile Range	10 to 90 Percentile Range	95 Percentile	Maximum Displacement
Nuttallina fluxa								
7	56	553	2.5	0.7	0.0- 2.1	0.0- 6.7	13.9	31.6
15	56	378	5.4	1.4	0.0- 4.2	0.0-14.9	29.3	67.7
30	51	216	7.8	2.1	0.8- 8.5	0.0-22.2	38.2	87.7
60	47	101	11.2	3.3	1.1-15.3	0.1-31.6	44.4	64.7
120	34	42	16.4	11.5	3.0-24.4	1.9-46.6	51.7	61.7
Nuttallina kata								
7	77	694	0.6	0.0	0.0- 0.7	0.0- 1.9	3.5	22.4
15	75	450	1.2	0.0	0.0- 1.2	0.0- 3.0	5.0	48.0
30	71	287	1.5	0.3	0.0- 1.6	0.0- 4.6	7.3	17.1
60	57	130	1.9	1.0	0.0- 2.0	0.0- 7.2	10.0	16.5
120	45	55	2.6	1.0	0.2- 2.6	0.0- 7.3	11.7	22.4
Unidentifiable *Nuttallina* specimens								
7	27	175	1.8	0.0	0.0- 1.9	0.0- 5.3	11.2	23.5
15	27	111	3.1	1.0	0.0- 2.4	0.0- 8.5	14.8	43.2
30	25	69	5.1	1.5	0.0- 6.7	0.0-18.1	18.3	71.0
60	18	28	6.5	3.0	0.6- 8.0	0.0-22.4	31.9	36.2
120	10	11	21.7	5.1	1.0-33.2	0.0-79.2	90.5	90.5

Table V-10. Point-to-point displacements of Nuttallina, expressed in terms of body lengths, from beginning to end of composite observation intervals of different lengths. The point-to-point displacement of a chiton was divided by its body length to give the data presented. The location at the end of a composite interval was calculated by interpolation if necessary.

			Point-to-point Displacement, expressed in chiton body lengths					
Observation Interval Length (days)	Number of Chitons	Number of Observation Intervals	Mean	Median	25 to 75 Percentile Range	10 to 90 Percentile Range	95 Percentile	Maximum Displacement
Nuttallina fluxa								
7	56	553	0.7	0.2	0.0- 0.6	0.0- 2.1	3.6	10.5
15	56	378	1.5	0.4	0.0- 1.3	0.0- 4.5	7.8	20.3
30	51	216	2.1	0.6	0.2- 2.3	0.0- 6.3	10.4	20.8
60	47	101	3.4	0.9	0.3- 5.1	0.0- 9.1	14.2	20.7
120	34	42	4.9	2.9	0.8- 6.5	0.5-12.5	16.6	19.8
Nuttallina kata								
7	77	694	0.2	0.0	0.0- 0.2	0.0- 0.7	1.1	6.8
15	75	450	0.4	0.0	0.0- 0.5	0.0- 1.3	2.1	14.5
30	71	287	0.5	0.1	0.0- 0.6	0.0- 2.0	2.7	5.8
60	57	130	0.7	0.3	0.0- 0.8	0.0- 2.5	3.8	5.5
120	45	55	0.9	0.3	0.0- 1.0	0.0- 2.8	3.8	5.4
Unidentifiable Nuttallina specimens								
7	27	175	0.7	0.0	0.0- 0.8	0.0- 2.3	3.8	10.6
15	27	111	1.2	0.4	0.0- 1.2	0.0- 3.8	5.2	14.2
30	25	69	1.8	0.6	0.0- 2.1	0.0- 5.3	6.2	21.1
60	18	28	2.4	1.0	0.3- 2.9	0.0- 9.5	11.4	15.0
120	10	11	7.5	2.4	0.3-11.2	0.0-26.8	27.4	28.1

Table V-11. Estimated displacements of Nuttallina, corrected for roughness of the substrate, from beginning to end of composite observation intervals of different lengths. The location at the end of a composite interval was calculated by interpolation if necessary. The roughness correction was applied to the point-to-point displacement over each composite interval, using the substrate roughness indices given in Table V-1.

Observation Interval Length (days)	Number of Chitons	Number of Observation Intervals	Roughness-corrected Displacement (cm)					
			Mean	Median	25 to 75 Percentile Range	10 to 90 Percentile Range	95 Percentile	Maximum Displacement
Nuttallina fluxa								
7	56	553	2.9	0.8	0.0- 2.4	0.0- 8.2	15.7	38.4
15	56	378	6.2	1.6	0.0- 5.0	0.0-18.1	33.7	82.2
30	51	216	9.1	2.4	0.9- 9.8	0.0-25.1	42.3	97.3
60	47	101	13.2	3.6	1.3-18.7	0.1-36.2	54.0	78.6
120	34	42	19.4	13.5	3.4-27.8	2.2-54.4	62.8	75.0
Nuttallina kata								
7	77	694	0.8	0.0	0.0- 0.8	0.0- 2.3	3.9	24.5
15	75	450	1.4	0.0	0.0- 1.5	0.0- 3.5	6.0	52.4
30	71	287	1.8	0.4	0.0- 1.9	0.0- 5.8	9.5	23.1
60	57	130	2.4	1.2	0.0- 2.4	0.0- 8.5	12.5	22.3
120	45	55	3.2	1.2	0.2- 3.4	0.0- 9.2	13.8	30.2
Unidentifiable Nuttallina specimens								
7	27	175	2.1	0.0	0.0- 2.3	0.0- 6.3	13.5	28.5
15	27	111	3.6	1.1	0.0- 2.9	0.0-10.3	17.8	48.5
30	25	69	5.9	1.8	0.0- 7.7	0.0-20.1	22.2	79.8
60	18	28	7.4	3.2	0.8- 9.1	0.0-26.1	38.2	43.9
120	10	11	24.8	6.2	1.1-37.1	0.0-92.0	101.8	101.8

Table V-12. Estimated vertical intertidal displacements of *Nuttallina* over composite observation intervals of different lengths. The location at the end of a composite interval was calculated by interpolation if necessary. Vertical displacements were calculated from point-to-point displacements using "X" and "Y" tilts of quadrat areas presented in Table V-1.

				Estimated Vertical Intertidal Displacement (cm)								
	Observation Intervals Length (in days)	Number of Chitons	Number of Observation Intervals	Mean	Median	25 to 75 Percentile Range	10 to 90 Percentile Range	5 to 95 Percentile Range	Maximum Downward Displacement	Maximum Upward Displacement	Frequency Moving Downward	Frequency Moving Upward
Nuttallina fluxa	7	56	553	-0.1	0.0	-0.1 to 0.0	-0.6 to 0.3	-1.3 to 0.7	-5.9	+7.3	10.068	6.750
	15	56	378	-0.1	0.0	-0.1 to 0.0	-1.0 to 0.6	-2.9 to 1.6	-12.6	+15.5	12.664	9.422
	30	51	216	-0.2	0.0	-0.2 to 0.1	-1.4 to 0.9	-3.8 to 2.7	-19.9	+15.0	15.710	11.552
	60	47	101	+0.2	0.0	-0.4 to 0.3	-2.5 to 3.3	-3.8 to 6.0	-7.2	+12.5	15.748	14.148
	120	34	42	+0.6	0.1	-0.4 to 1.1	-2.7 to 3.6	-4.9 to 8.8	-8.2	+11.0	12.500	16.000
Nuttallina kata	7	77	694	0.0	0.0	0.0 to 0.0	-0.2 to 0.2	-0.3 to 0.3	-3.8	+1.2	3.843	4.163
	15	75	450	0.0	0.0	0.0 to 0.0	-0.3 to 0.4	-0.5 to 0.6	-8.1	+2.4	5.036	7.999
	30	71	287	0.0	0.0	0.0 to 0.0	-0.6 to 0.5	-0.8 to 0.7	-8.5	+2.5	9.107	9.382
	60	57	130	-0.2	0.0	-0.2 to 0.0	-0.7 to 0.4	-1.8 to 0.5	-8.2	+2.2	12.016	6.616
	120	45	55	-0.5	0.0	-0.5 to 0.0	-1.5 to 0.5	-2.7 to 0.5	-11.1	+0.7	12.000	6.000
Unidentifiable *Nuttallina* Specimens	7	27	175	0.0	0.0	0.0 to 0.0	-0.2 to 0.2	-0.5 to 0.4	-3.2	+4.1	3.847	3.570
	15	27	111	0.0	0.0	0.0 to 0.0	-0.6 to 0.2	-1.0 to 0.6	-8.0	+8.4	4.890	4.696
	30	25	69	+0.2	0.0	0.0 to 0.1	-0.2 to 0.5	-0.9 to 1.8	-13.3	+8.1	4.976	6.917
	60	18	28	+0.2	0.0	0.0 to 0.2	-0.2 to 0.8	-1.3 to 0.8	-5.8	+8.1	3.250	6.000
	120	10	11	-0.6	0.0	-0.3 to 0.2	-9.0 to 6.7	-16.7 to 9.3	-16.7	+9.3	3.500	2.500

Table V-13. Homing proportions of _Nuttallina_, as calculated from the greatest point-to-point displacement of chitons from the beginning location to the location at any subsequent observation within a composite observation interval. The location at the end of a composite interval was calculated by interpolation if necessary. Expressed as chiton homing proportion ± s.d. (Number of chitons, number of intervals). Selected data are plotted in Figure V-14.

	Length of Composite Observation Intervals, In Days				
Species	7	15	30	60	120
Nuttallina fluxa	0.65±0.064(56,553)	0.43±0.066(56,378)	0.27±0.062(51,216)	0.08±0.040(47,101)	0.00±0.000(34,42)
Nuttallina kata	0.86±0.039(77,694)	0.74±0.051(75,450)	0.60±0.058(71,287)	0.45±0.066(57,130)	0.33±0.070(45,55)
Unidentifiable _Nuttallina_ Specimens	0.68±0.090(27,175)	0.52±0.096(27,111)	0.34±0.095(25,69)	0.15±0.085(18,28)	0.10±0.095(10,11)

Table V-14. Greatest observed point-to-point displacements of Nuttallina from the beginning location during composite observation intervals of different lengths. The location at the end of a composite interval was calculated by interporation if necessary.

	Observation Interval Length (days)	Number of Chitons	Number of Observation Intervals	Greatest Point-to-point Displacement During Interval (cm)					
				Mean	Median	25 to 75 Percentile Range	10 to 90 Percentile Range	95 Percentile	Maximum Displacement
Nuttallina fluxa	7	56	553	2.6	0.7	0.0-2.2	0.0-6.8	13.9	31.6
	15	56	378	5.8	1.8	0.0-5.0	0.0-16.8	29.7	67.7
	30	51	216	8.5	2.2	1.0-10.0	0.0-22.2	38.2	87.7
	60	47	101	13.1	5.0	2.2-17.2	2.0-33.2	47.8	66.0
	120	34	42	19.3	13.0	5.0-28.7	3.0-50.1	56.0	66.0
Nuttallina kata	7	77	694	0.6	0.0	0.0-0.7	0.0-1.9	3.5	22.4
	15	75	450	1.3	0.0	0.0-1.4	0.0-4.0	5.3	48.0
	30	71	287	1.8	1.0	0.0-2.0	0.0-5.4	7.8	17.1
	60	57	130	2.7	1.4	0.0-3.0	0.0-8.3	10.2	16.5
	120	45	55	3.7	2.0	1.0-5.1	0.0-8.3	11.7	23.8
Unidentifiable Nuttallina Specimens	7	27	175	2.0	0.0	0.0-2.0	0.0-5.8	11.2	23.5
	15	27	111	3.7	1.2	0.0-4.0	0.0-12.0	18.4	43.2
	30	25	69	6.1	3.0	0.0-6.7	0.0-18.4	28.9	71.0
	60	18	28	8.0	4.0	2.0-9.3	1.0-22.4	31.9	36.2
	120	10	11	23.7	7.1	3.6-34.3	0.7-83.0	98.1	98.1

Table V-15. Greatest observed point-to-point displacements of Nuttallina, expressed in body lengths, from the beginning location during composite observation intervals of different lengths. The location at the end of a composite interval was calculated by interporation if necessary.

	Observation Intervals Length (days)	Number of Chitons	Number of Observation Intervals	Greatest Point-to-point Displacement During Interval, Expressed in Body Lengths					
				Mean	Median	25 to 75 Percentile Range	10 to 90 Percentile Range	95 Percentile	Maximum Displacement
Nuttallina fluxa	7	56	553	0.7	0.2	0.0-0.7	0.0-2.1	3.6	10.5
	15	56	378	1.6	0.5	0.0-1.5	0.0-4.8	8.2	20.3
	30	51	216	2.3	0.7	0.3-3.2	0.0-6.3	11.3	23.0
	60	47	101	3.9	1.5	0.7-5.2	0.4-10.4	14.2	21.1
	120	34	42	5.7	3.3	1.5-7.8	0.7-13.0	16.9	21.1
Nuttallina kata	7	77	694	0.2	0.0	0.0-0.2	0.0-0.8	1.1	6.8
	15	75	450	0.5	0.0	0.0-0.6	0.0-1.4	2.2	14.5
	30	71	287	0.6	0.3	0.0-0.7	0.0-2.5	3.1	6.1
	60	57	130	1.0	0.5	0.0-1.2	0.0-2.6	3.8	6.1
	120	45	55	1.3	0.7	0.3-2.0	0.0-3.6	4.3	5.8
Unidentifiable Nuttallina Specimens	7	27	175	0.7	0.0	0.0-0.8	0.0-2.4	3.8	10.6
	15	27	111	1.4	0.4	0.0-1.7	0.0-4.6	5.4	14.2
	30	25	69	2.2	0.9	0.0-2.2	0.0-5.4	9.7	21.1
	60	18	28	3.0	1.6	0.7-3.4	0.3-9.5	11.5	15.0
	120	10	11	8.2	3.1	1.4-11.5	0.3-28.6	29.1	29.1

Table V-16. Greatest observed point-to-point displacements of <u>Nuttallina</u>, corrected for roughness of the substrate, from the beginning location during composite observation intervals of different lengths. The location at the end of a composite interval was calculated by interpolation if necessary. The roughness correction was applied to the greatest point-to-point displacement within each composite interval, using the substrate roughness indices presented in Table V-1.

	Observation Intervals Length (days)	Number of Chitons	Number of Observation Intervals	Greatest Roughness-corrected Displacement During Interval (cm)					
				Mean	Median	25 to 75 Percentile Range	10 to 90 Percentile Range	95 Percentile	Maximum Displacement
<u>Nuttallina fluxa</u>	7	56	553	3.0	0.8	0.0-2.5	0.0-8.2	15.7	38.4
	15	56	378	6.8	2.1	0.0-6.1	0.0-19.0	34.2	82.2
	30	51	216	9.9	2.6	1.2-11.7	0.0-27.0	43.9	104.1
	60	47	101	15.4	5.6	2.5-20.1	2.1-38.8	56.4	80.2
	120	34	42	22.8	14.8	5.6-34.0	3.2-58.6	64.2	80.2
<u>Nuttallina kata</u>	7	77	694	0.8	0.0	0.0-0.9	0.0-2.4	3.9	24.5
	15	75	450	1.6	0.1	0.0-1.9	0.0-4.6	6.4	52.4
	30	71	287	2.2	1.2	0.0-2.5	0.0-6.7	9.7	23.1
	60	57	130	3.3	1.9	0.0-4.2	0.0-10.4	12.8	22.3
	120	45	55	4.5	2.4	1.2-6.2	0.0-10.5	13.8	32.4
Unidentifiable <u>Nuttallina</u> Specimens	7	27	175	2.3	0.0	0.0-2.4	0.0-6.7	13.5	28.5
	15	27	111	4.3	1.4	0.0-4.8	0.0-13.4	20.5	48.5
	30	25	69	7.0	3.4	0.0-7.7	0.0-20.5	34.7	79.8
	60	18	28	9.2	4.5	2.3-10.7	1.2-26.1	38.2	43.9
	120	10	11	27.1	8.6	4.3-38.2	0.8-96.3	110.3	110.3

TABLE V-17. Estimated vertical intertidal displacements of Nuttallina corresponding to the greatest point-to-point displacements from the beginning location during composite observation intervals of different lengths. The location at the end of a composite interval was calculated by interpolation if necessary. Vertical displacements were calculated from point-to-point displacements using "X" and "Y" tilts of quadrat areas presented in Table V-1.

	Observation Intervals Length (days)	Number of Chitons	Number of Observation Intervals	Estimated Vertical Displacement Corresponding to Greatest Point-to-Point Displacement (cm)							Frequency Moving Downward	Frequency Moving Upward
				Mean	Median	25 to 75 Percentile Range	10 to 90 Percentile Range	5 to 95 Percentile Range	Maximum Downward Displacement	Maximum Upward Displacement		
Nuttallina fluxa	7	56	553	-0.1	0.0	-0.1 to 0.0	-0.6 to 0.3	-1.3 to 0.8	-5.9	7.3	9.735	6.873
	15	56	378	-0.1	0.0	-0.2 to 0.0	-1.2 to 0.6	-2.9 to 1.6	-12.6	15.5	13.885	10.260
	30	51	216	-0.1	0.0	-0.2 to 0.2	-1.4 to 1.0	-3.8 to 2.9	-19.9	15.0	16.021	13.738
	60	47	101	+0.1	0.0	-0.7 to 0.6	-3.0 to 3.9	-4.7 to 6.4	-10.8	12.5	18.898	16.448
	120	34	42	+0.8	0.3	-0.5 to 2.0	-3.8 to 5.0	-4.9 to 11.2	-8.3	12.5	11.500	19.000
Nuttallina kata	7	77	694	0.0	0.0	0.0 to 0.0	-0.2 to 0.2	-0.3 to 0.3	-3.8	1.2	3.700	4.163
	15	75	450	0.0	0.0	0.0 to 0.0	-0.4 to 0.5	-0.5 to 0.6	-8.1	2.5	5.477	7.561
	30	71	287	-0.1	0.0	0.0 to 0.0	-0.6 to 0.6	-0.8 to 0.7	-8.5	2.5	10.163	7.876
	60	57	130	-0.3	0.0	-0.6 to 0.0	-1.2 to 0.5	-1.9 to 1.2	-8.2	2.5	17.499	8.199
	120	45	55	-0.7	0.0	-0.6 to 0.0	-2.2 to 0.6	-2.9 to 0.8	-12.3	1.2	17.500	8.000
Unidentifiable Nuttallina Specimens	7	27	175	0.0	0.0	0.0 to 0.0	-0.3 to 0.2	-0.3 to 0.5	-3.2	4.1	3.847	3.661
	15	27	111	0.0	0.0	0.0 to 0.0	-0.6 to 0.5	-1.0 to 0.7	-8.0	8.4	5.414	5.182
	30	25	69	0.4	0.0	0.0 to 0.3	-0.6 to 0.7	-0.9 to 1.8	-13.3	10.0	5.952	8.083
	60	18	28	0.2	0.0	0.0 to 0.3	-0.7 to 0.8	-1.3 to 1.6	-5.8	8.1	4.750	6.500
	120	10	11	-0.7	0.0	-0.3 to 0.5	-9.7 to 6.9	-18.1 to 9.8	-18.1	9.8	3.500	4.500

Figure V-7. Homing proportions of Nuttallina, as calculated using point-to-point displacements of chitons from beginning to end of composite observation intervals. Homing is defined as point-to-point displacement of $\leq$1.26 cm. The location at the end of the composite interval was estimated by linear interpolation if necessary. Bars represent standard errors. Corresponding data are presented in Table V-8. Values for unidentifiable Nuttallina specimens (not plotted) fall between values for N. fluxa and N. kata, as expected.

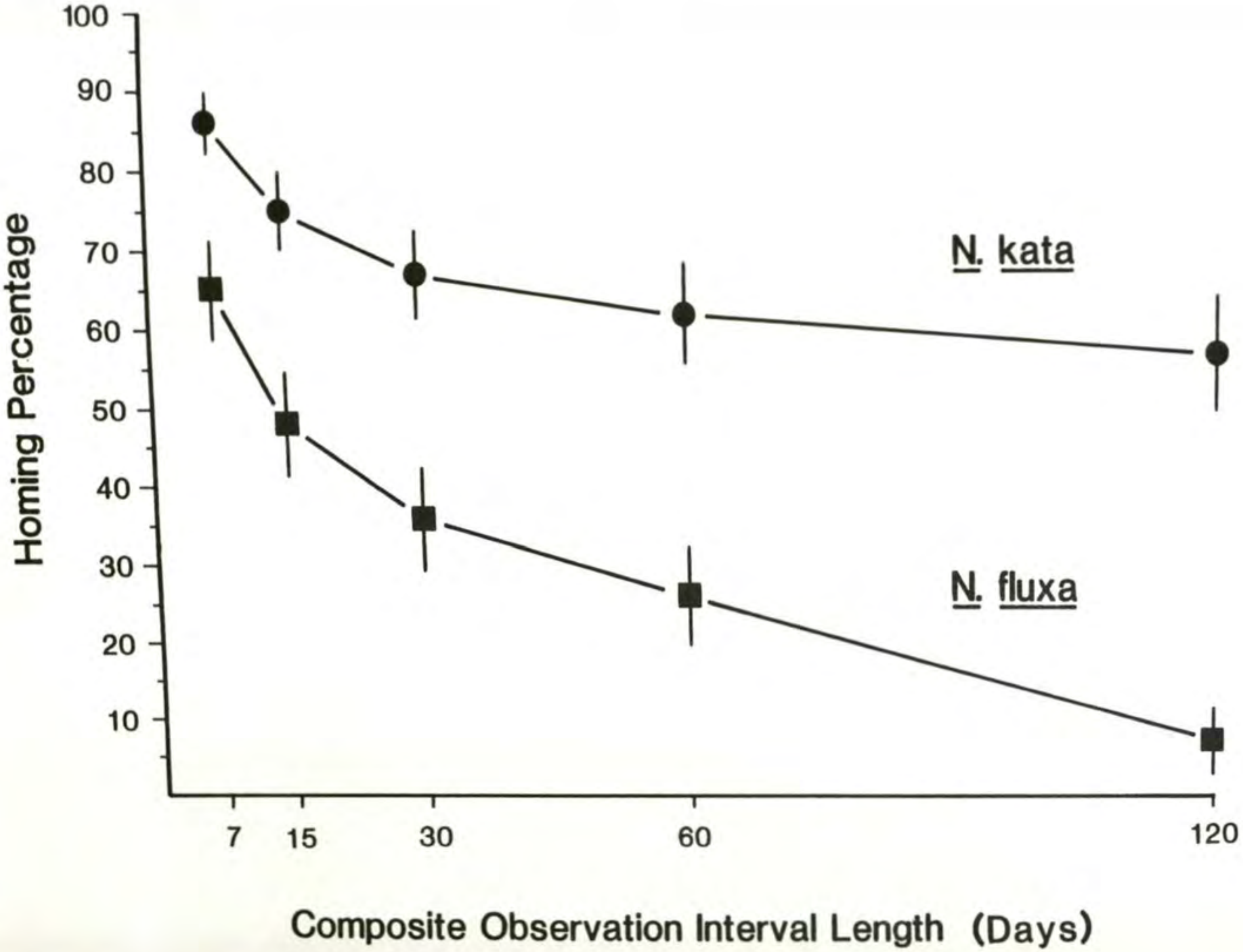

100
90
80
70
60
50
40
30
20
10
Homing Percentage
7
15
30
60
120
Composite Observation Interval Length (Days)
N. kata
N. fluxa

Figure V-8. Point-to-point displacements of <u>Nuttallina</u> <u>fluxa</u> from beginning to end of composite observation intervals of the specified lengths. Locations at the ends of composite intervals were calculated by interpolation if necessary. Homing is defined as point-to-point displacement of ≤ 1.26 cm. Homing chitons are represented by the unshaded column.

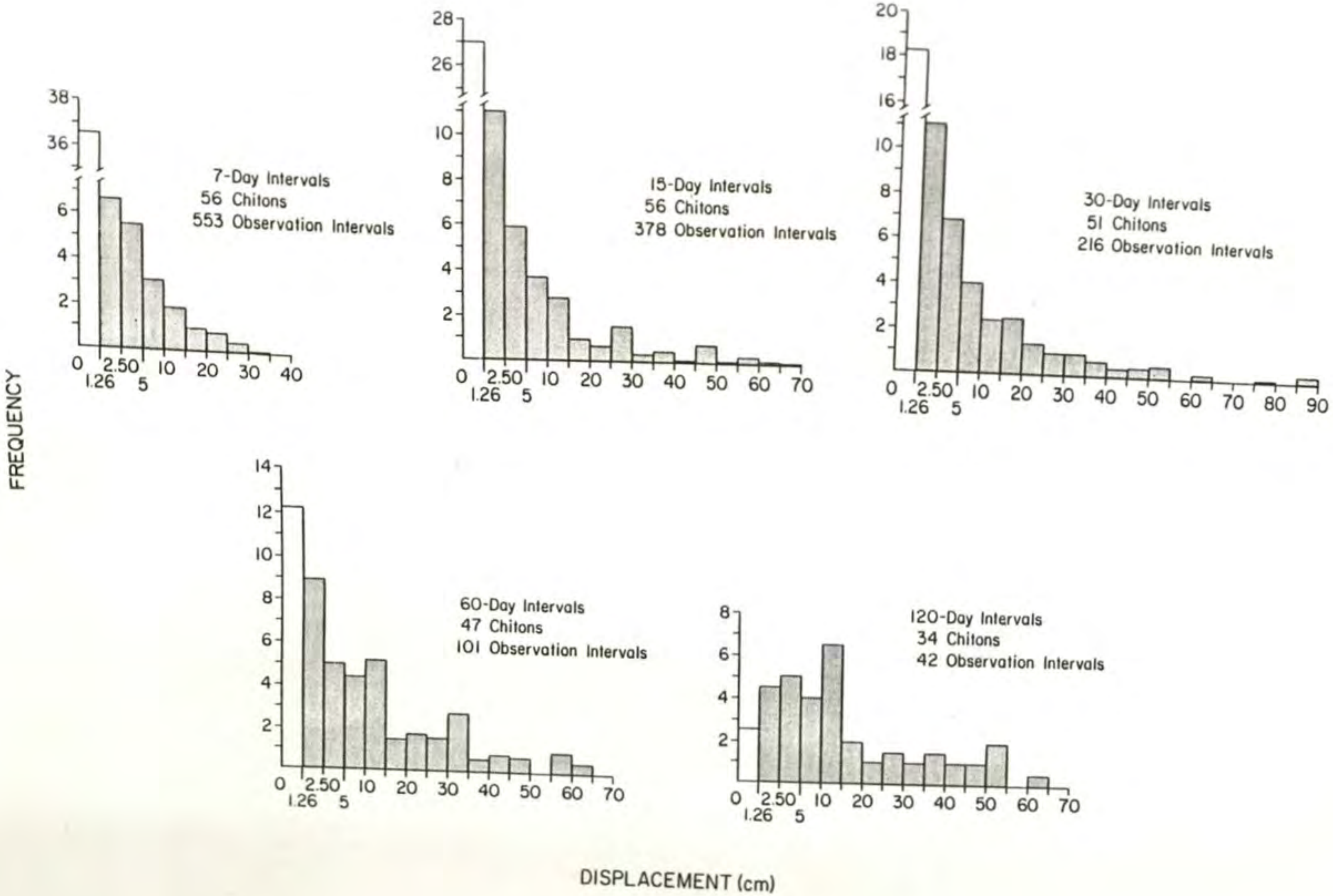

FREQUENCY
DISPLACEMENT (cm)
7-Day Intervals
56 Chitons
553 Observation Intervals
15-Day Intervals
56 Chitons
378 Observation Intervals
30-Day Intervals
51 Chitons
216 Observation Intervals
60-Day Intervals
47 Chitons
101 Observation Intervals
120-Day Intervals
34 Chitons
42 Observation Intervals
0
1.26
2.50
5
10
20
30
40
50
60
70
80
90
2
4
6
8
10
12
14
16
18
20
26
28
36
38

Figure V-9. Point-to-point displacements of Nuttallina kata from beginning to end of composite observation intervals of the specified lengths. See Figure V-8 caption. Homing chitons are represented by the unshaded column.

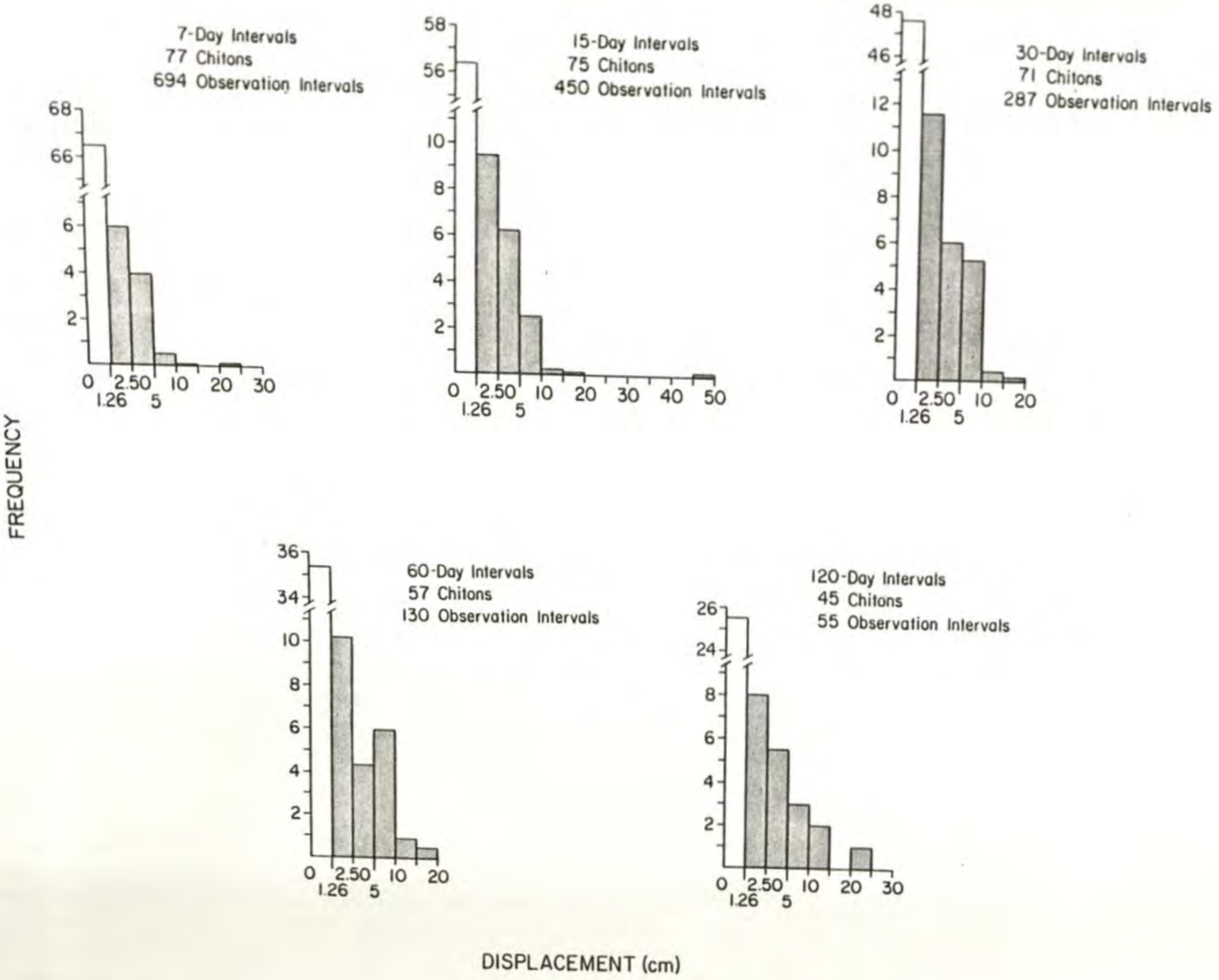

7-Day Intervals
77 Chitons
694 Observation Intervals
68
66
6
4
2
0
1.26
2.50
5
10
20
30
15-Day Intervals
75 Chitons
450 Observation Intervals
58
56
10
8
6
4
2
0
1.26
2.50
5
10
20
30
40
50
30-Day Intervals
71 Chitons
287 Observation Intervals
48
46
12
10
8
6
4
2
0
1.26
2.50
5
10
20
FREQUENCY
60-Day Intervals
57 Chitons
130 Observation Intervals
36
34
10
8
6
4
2
0
1.26
2.50
5
10
20
120-Day Intervals
45 Chitons
55 Observation Intervals
26
24
8
6
4
2
0
1.26
2.50
5
10
20
30
DISPLACEMENT (cm)

Figure V-10. Point-to-point displacements of unidentifiable Nuttallina specimens from beginning to end of composite observation intervals of the specified lengths. See Figure V-8 caption. Homing chitons are represented by the unshaded column.

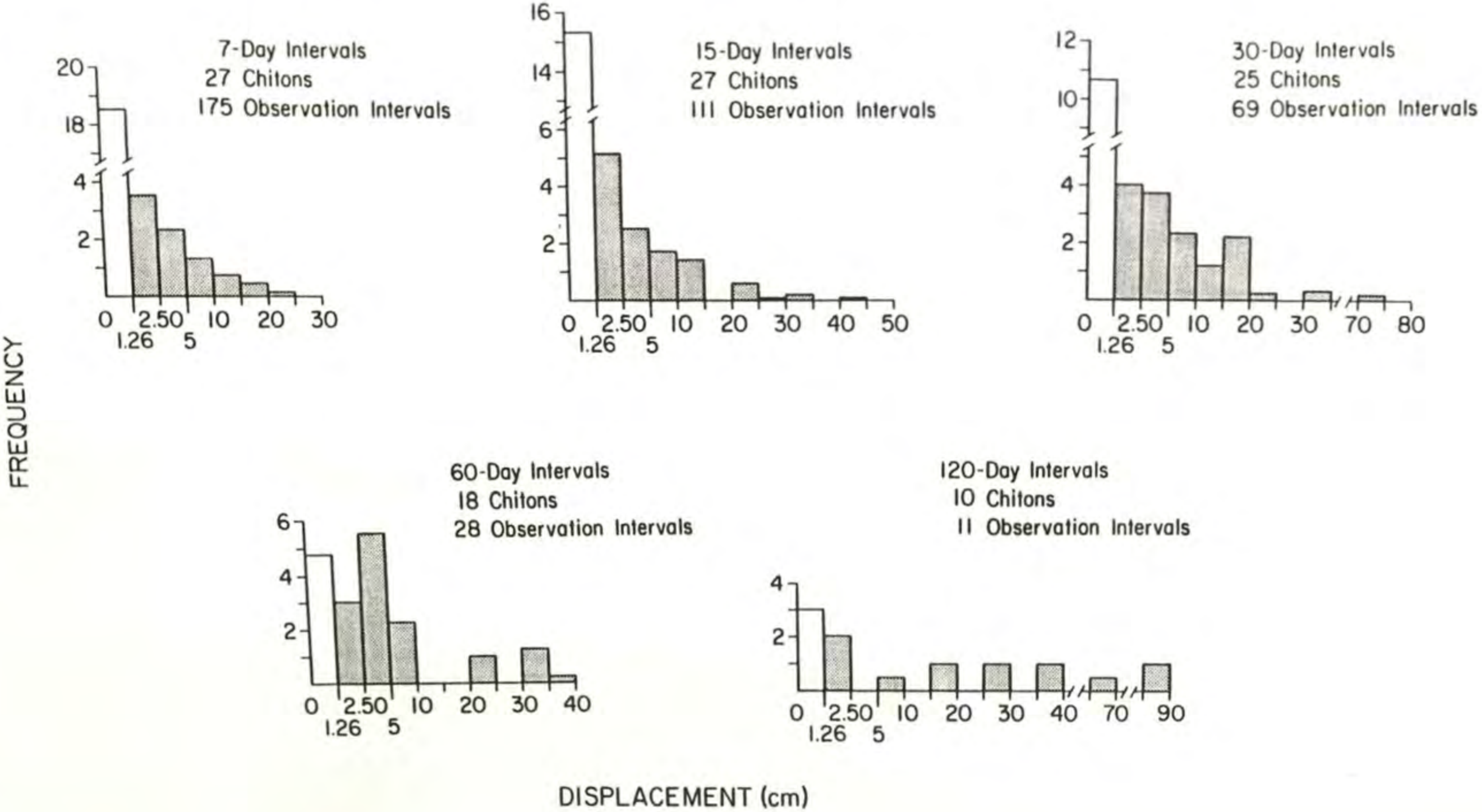

7-Day Intervals
27 Chitons
175 Observation Intervals
15-Day Intervals
27 Chitons
111 Observation Intervals
30-Day Intervals
25 Chitons
69 Observation Intervals
60-Day Intervals
18 Chitons
28 Observation Intervals
120-Day Intervals
10 Chitons
11 Observation Intervals
FREQUENCY
DISPLACEMENT (cm)
0
1.26
2.50
5
10
20
30
40
50
70
80
90
2
4
6
10
12
14
16
18
20

Figure V-11. Vertical intertidal height displacements of *Nuttallina fluxa* over composite observation intervals of the specified lengths. Vertical displacements were calculated from point-to-point displacements from the beginning to end of observation intervals, using X and Y tilts of appropriate quadrats listed in Table V-1. Homing individuals (point-to-point displacement ≤ 1.26 cm) are not included in the histograms.

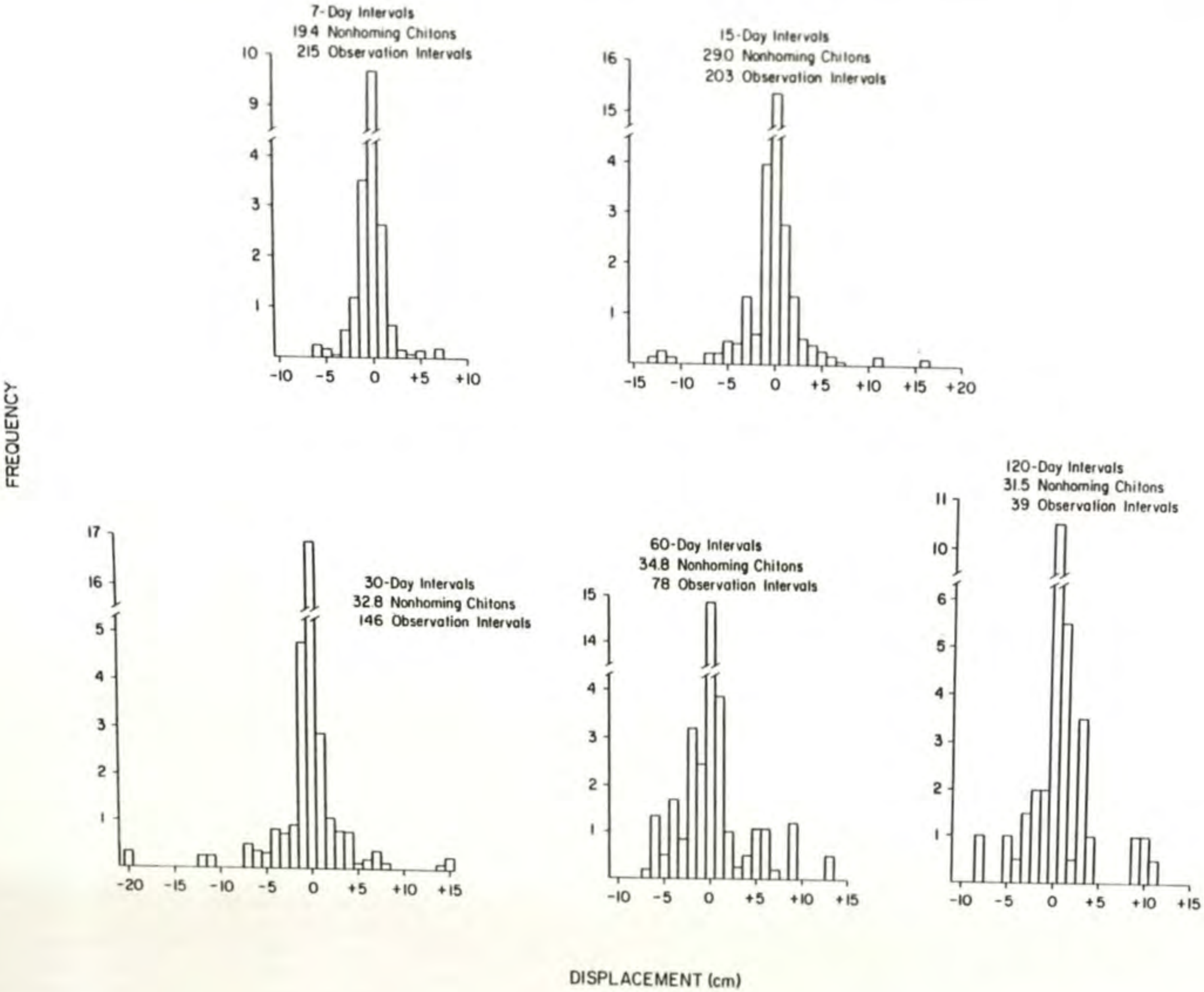

7-Day Intervals
19.4 Nonhoming Chitons
215 Observation Intervals
15-Day Intervals
29.0 Nonhoming Chitons
203 Observation Intervals
30-Day Intervals
32.8 Nonhoming Chitons
146 Observation Intervals
60-Day Intervals
34.8 Nonhoming Chitons
78 Observation Intervals
120-Day Intervals
31.5 Nonhoming Chitons
39 Observation Intervals
FREQUENCY
DISPLACEMENT (cm)
10
9
4
3
2
1
-10
-5
0
+5
+10
16
15
-15
+15
+20
17
5
-20
14
11
6

Figure V-12. Vertical intertidal height displacements of *Nuttallina kata* over composite observation intervals of the specified lengths. See Figure V-11 caption for the method of calculation. Homing individuals are not included in the histograms.

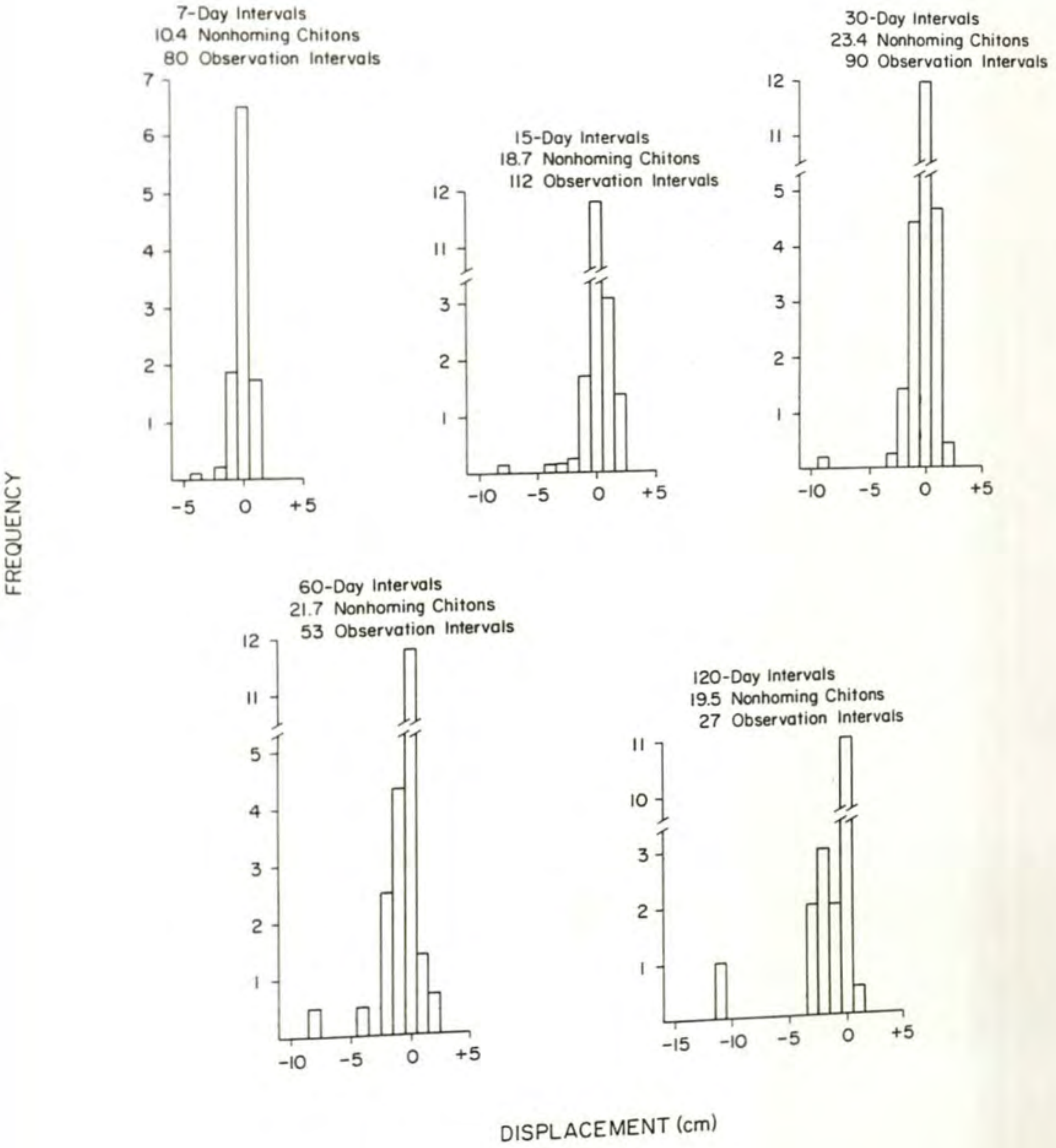

7-Day Intervals
10.4 Nonhoming Chitons
80 Observation Intervals
15-Day Intervals
18.7 Nonhoming Chitons
112 Observation Intervals
30-Day Intervals
23.4 Nonhoming Chitons
90 Observation Intervals
60-Day Intervals
21.7 Nonhoming Chitons
53 Observation Intervals
120-Day Intervals
19.5 Nonhoming Chitons
27 Observation Intervals
FREQUENCY
DISPLACEMENT (cm)
-15
-10
-5
0
+5

Figure V-13. Vertical intertidal height displacements of unidentifiable *Nuttallina* specimens over composite observation intervals of the specified lengths. See Figure V-11 caption for the method of calculation. Homing individuals are not included in the histograms.

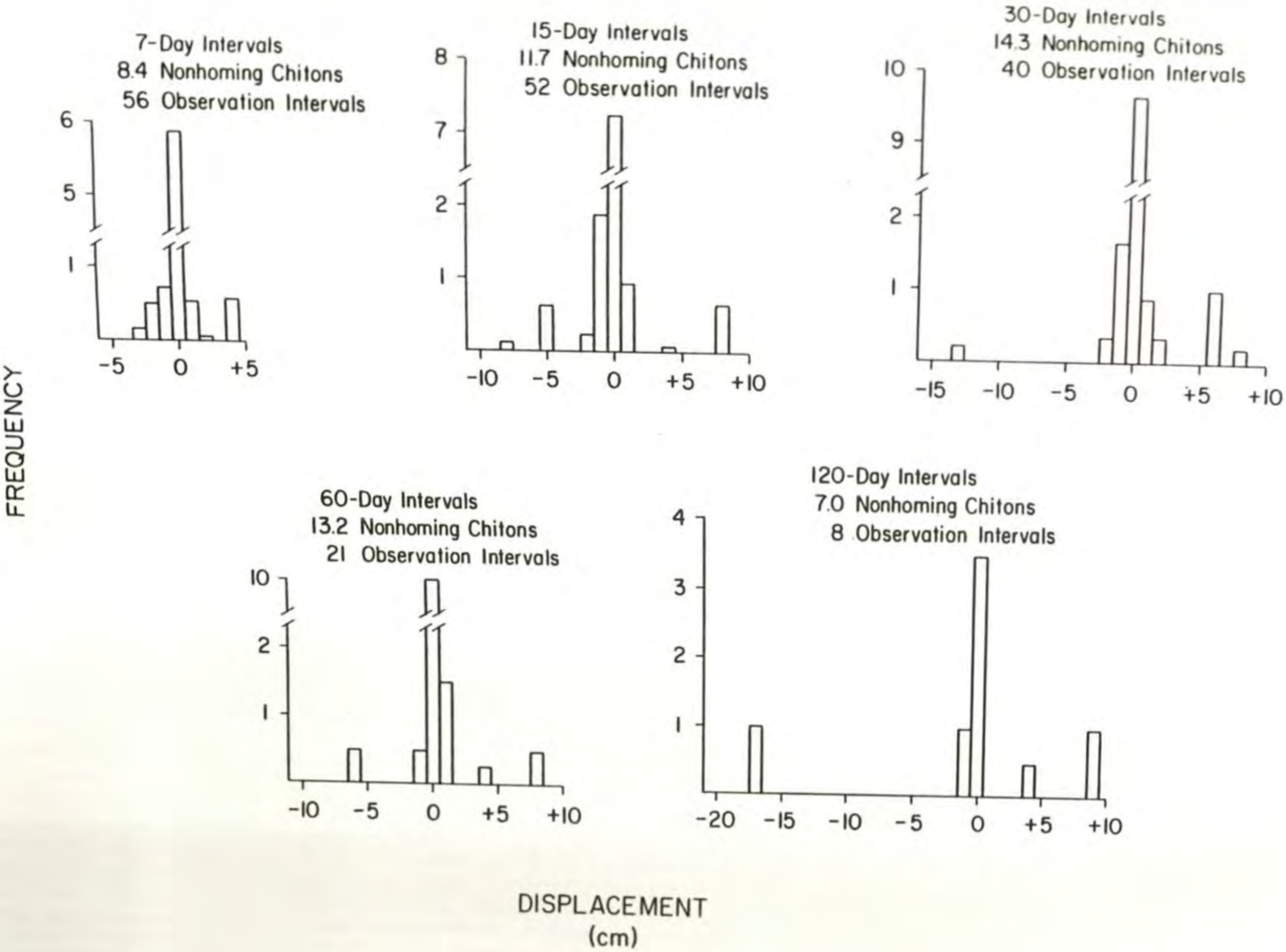
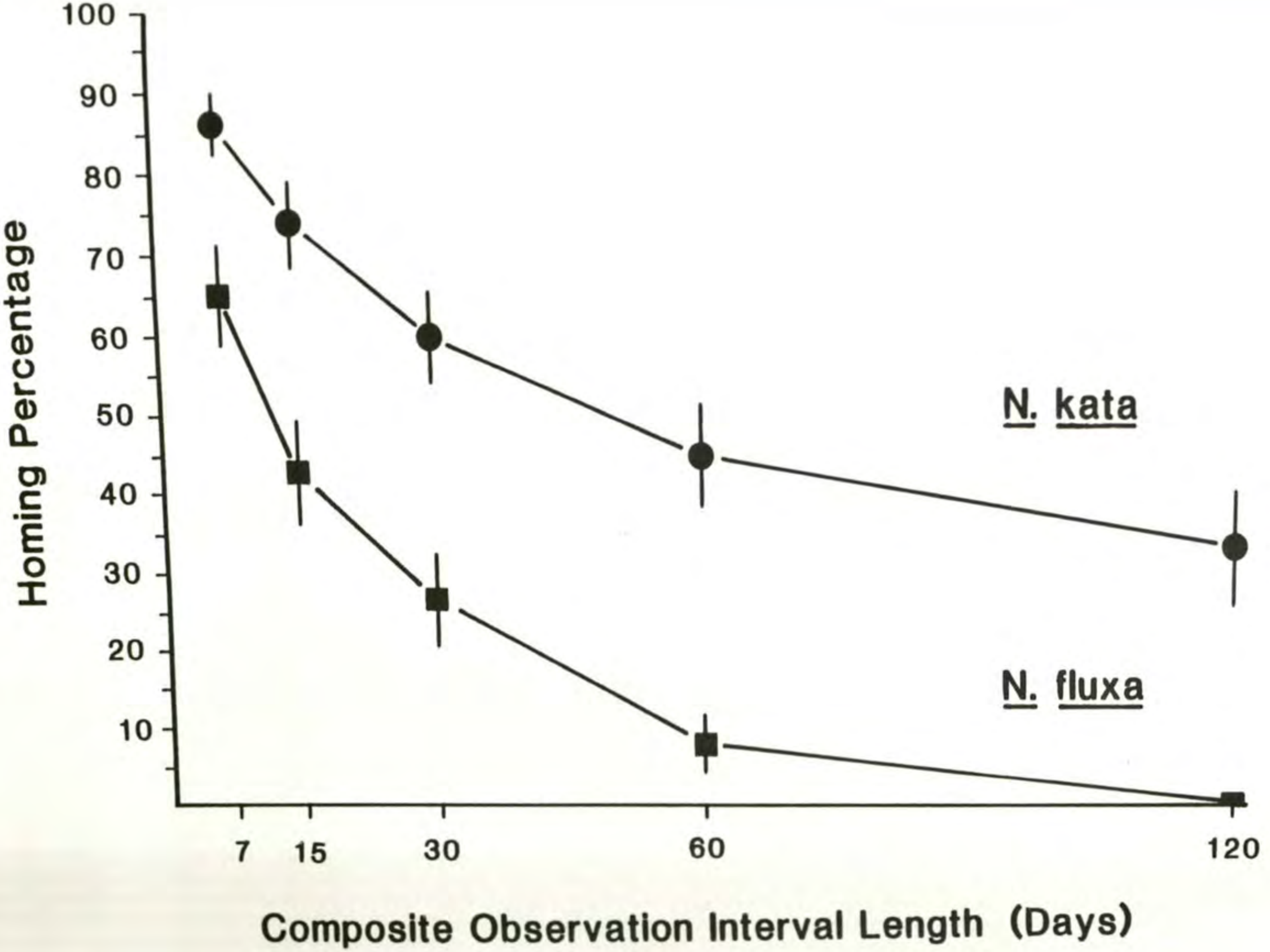

7-Day Intervals
8.4 Nonhoming Chitons
56 Observation Intervals
15-Day Intervals
11.7 Nonhoming Chitons
52 Observation Intervals
30-Day Intervals
14.3 Nonhoming Chitons
40 Observation Intervals
60-Day Intervals
13.2 Nonhoming Chitons
21 Observation Intervals
120-Day Intervals
7.0 Nonhoming Chitons
8 Observation Intervals
FREQUENCY
DISPLACEMENT
(cm)
1
2
3
4
5
6
7
8
9
10
-20
-15
-10
-5
0
+5
+10

Figure V-14. Homing proportions of Nuttallina, as calculated using the greatest point-to-point displacements of chitons from the beginning location observed during a composite observation interval of the specified length. Homing is defined as point-to-point displacement of ≤ 1.26 cm. The location at the end of the composite interval was estimated by linear interpolation if necessary. Bars represent standard errors. Corresponding data are presented in Table V-13. Values for unidentifiable Nuttallina specimens (not plotted) fall between values for N. fluxa and N. kata, as expected.


Homing Percentage
100
90
80
70
60
50
40
30
20
10
N. kata
N. fluxa
7
15
30
60
120
Composite Observation Interval Length (Days)

Homing proportions for Nuttallina, not surprisingly, decrease with increasing length of observation intervals used in analyses, and values for the unidentifiable Nuttallina group of specimens, likely composed of at least a few specimens of both N. fluxa and N. kata, are intermediate between those exhibited by Nuttallina fluxa and N. kata (Table V-8).

Point-to-point, or straight-line, displacements of Nuttallina are, not surprisingly, greater over composite observation intervals of greater length (Table V-9 and Figures V-8 through V-10). This trend is clearly shown by means, medians and other percentiles listed; however, the trend is not so evident in the maximum observed displacements, probably because these last are particularly sensitive to random events, especially for observation intervals of greater length for which smaller sample sizes are available. Point-to-point displacements were divided by the live total lengths (body lengths) of the chitons exhibiting those displacements; displacements expressed in terms of body lengths are presented in Table V-10.

Chitons occurring on substrate with great topographic relief must travel greater along-the-rock actual distances to attain the same point-to-point, or straight-line, displacements attained by chitons occurring on more planar surface. Since the irregularity of the substrate surface varied considerably over the study area, particularly between the low intertidal quadrats, where a predominance of Nuttallina kata occurred, and the high intertidal quadrats, where primarily N. fluxa occurs, "roughness-corrected" displacements were calculated and are listed in Table V-11. Displacements were "roughness-corrected" by applying X and Y roughness indices to the X and Y components of the displacement of a chiton. Roughness indices, discussed in the Materials and Methods section, are average estimated values for each quadrat and are listed in Table V-1.

In theory, in this study, roughness-corrections could increase point-to-point displacements by 7% to 40%, depending on the direction of a displacement and the quadrat in which it occurred (see Table V-1). In practice, mean displacements were increased by 14% to 33%, as revealed by a comparison of the results tabulated in Tables V-9 and V-11. Figures of frequency distributions of roughness-corrected displacements are not presented since they are quite similar in general form to those already presented for point-to-point displacements in Figures V-8 to V-10.

Vertical intertidal height displacements were estimated from the coordinates used to calculate point-to-point displacements by using the X and Y tilts of the appropriate quadrats (listed in Table V-1). Results calculated for composite observation intervals of different lengths are presented in Table V-12, with corresponding figures of frequency distributions in Figures V-11 through V-13. The vertical displacements clearly are not extensive when compared to the 550 cm plus _Nuttallina_-inhabited vertical range of the study site. Not surprisingly, the percentile ranges, such as 10 to 90, generally increase with an increase in the length of the composite observation intervals used.

The foregoing displacements were calculated using the position coordinates of a chiton at the beginning and end of a composite observation interval of a particular length (beginning-to-end method). Nuttallina does not always progress in the same general direction in going from one low tide resting position to the next, as illustrated by the records of long-term displacement displayed in Figures V-4 through 6. Therefore, displacements calculated only from positions at the beginning and end of time intervals occasionally will underestimate the maximum distance (to any new low tide resting site) a chiton ventures from its initial low tide resting spot during those time intervals. A better approximation for the greatest displacement of a chiton during a composite observation interval may be obtained by comparing the displacements from the location at the beginning of the interval to the location at each successive observation, to the end of the composite observation interval (greatest displacement method). Obviously, an estimate of this kind is dependent on the number of observations made within the composite observation interval. The more observations that are made, the better the estimate is, with the best result being obtained by observing the chiton at all low tide resting periods during a time interval.

Greatest displacements were estimated for the same composite observation intervals for which beginning-to-end displacements were calculated, and the results are presented in Tables V-13 and V-14 and Figure V-14. Point-to-point displacements of Table V-14, expressed in terms of bodylengths, with roughness-corrections applied, and in terms of vertical intertidal displacement, are presented in Tables V-15 through V-17 respectively. Comparison of Tables V-8 through V-12 with Tables V-13 through V-17 reveals that the greatest changes in results occur for the longest composite observation intervals, 120 days long, and that very little difference between results generated by the beginning-to-end and the greatest displacement methods is seen for the shorter intervals of 7 and 15 days. This is a result of the fact that most observations in this study were about 15 days apart, so that 15 day composite observation intervals included only 2 observations, whereas 120 day composite intervals included 9 different observations on the average. Even where displacements estimated from the beginning-to-end and greatest observed displacement methods differ the most, i.e., for 120 day intervals, the difference is fairly small. The mean point-to-point displacements for Nuttallina fluxa and N. kata change from 16.4 and 2.6 cm to 19.3 and 3.7 cm respectively (beginning-to-end method, Table V-9 vs. greatest displacement method, Table V-14).

Comparisons of Homing Proportions and Displacement Magnitudes of Nuttallina fluxa and N. kata

Homing proportions calculated by either the beginning-to-end or the greatest displacement method, listed in Tables V-8 and V-13 and displayed in Figures V-7 and V-14, are clearly quite different for Nuttallina fluxa and N. kata. Nuttallina kata homes a significantly greater part of the time for composite observation intervals of any length (test for difference in proportions, two-tailed, $p<0.005$ for all 10 comparisons).

Similarly, comparisons of point-to-point displacement frequency distributions, derived using both the beginning-to-end and the greatest displacement method, summarized in Tables V-9 and V-14 and displayed in

Figures V-8 and V-9, reveal significant differences between those for Nuttallina fluxa and those for N. kata, for composite observation intervals of all lengths (Kolmogorov-Smirnov two-sample test, two-sided, $p<0.001$ for observation intervals of 30, 60 and 120 days, $p<0.01$ for 15 days and $p<0.025$ for 7 day intervals). Similarly, differences in displacement frequency distributions between Nuttallina fluxa and N. kata are significant when displacements determined by either the beginning-to-end or the greatest displacement method are roughness-corrected, and when they are expressed in terms of body lengths except at the shorter observation interval lengths (Kolmogorow-Smirnov two-sample test, two-sided: roughness-corrected, generally $p<0.005$, except for 7 day intervals where $p<0.025$; in terms of body-lengths, generally $p<0.01$, and decreasing at longer observation interval lengths, and $0.05<p<0.10$ for 7 day intervals and for 15 day intervals by beginning-to-end method; but $p<0.05$ for 15 day intervals by greatest displacement method). In all cases, Nuttallina fluxa tends to exhibit longer displacements than does N. kata for composite intervals of the same length.

Characterization of Displacements of Nuttallina fluxa

Vertical Intertidal Direction of Displacements.

Homing proportions and displacement magnitudes of Nuttallina fluxa have been presented and have been compared to those of Nuttallina kata. Nuttallina fluxa does tend to show a substantial amount of long-term displacement and therefore it is of interest to determine whether these displacements show a particular orientation or whether they are more or less random.

As explained earlier, point-to-point displacements recorded for any chiton within the study area can be converted to approximate corresponding vertical intertidal displacements by using X and Y tilts determined for each 1 meter square quadrat and listed in Table V-1. Thus, each observed displacement, calculated by either the beginning-to-end or the greatest displacement method for a weighted 7, 15, 30, 60 or 120 day observation interval, can be categorized as an upward, downward or horizontal intertidal displacement. Chitons exhibiting point-to-point displacements of less than or equal to 1.26 cm were again considered homers and were not included in analyses. Frequencies of upward versus downward displacements (disregarding horizontal displacements) were compared statistically by testing the null hypothesis that the expected frequency of weighted displacements in one direction was 0.500, that is, upward displacements were as frequent as downward displacements. In no case was the observed frequency significantly different from the expected frequency of 0.5 (Table V-22; test for expected proportion, two-tailed, $p>0.42$ in all cases, except for 120 day intervals calculated by the greatest displacement method where $p=0.17$). Though not significant, the frequency of displacements in the downward direction tended to be greater for all observation interval lengths other than 120 days.

In Chapter II, it was shown that larger specimens of *Nuttallina* tend to occur higher on the shore than smaller ones. Since an upward migration of *Nuttallina* with age/size might explain the observed shore-level size gradient, upward versus downward displacement frequencies were additionally tested one-tailed for the specific hypothesis that upward frequencies exceed downward ones. Only for 120 day intervals is the frequency of upward displacements greater than that of downward displacements, and one-tailed tests of the upshore migration hypothesis are all nonsignificant (p>>0.5, except for 120 day intervals where p=0.25 for beginning-to-end method, and p=0.09 for greatest displacement method).

To test the possibility that Nuttallina fluxa exhibits greater displacements (magnitudes) either up or down the shore, the upward displacement distribution was compared to the downward displacement distribution for point-to-point displacements as well as for corresponding calculated vertical height displacements. Again, as for frequencies, no differences are found for upward versus downward displacements, either when tested in a two-tailed manner or when tested one-tailed against the specific hypothesis that upward displacements are greater than downward displacements [Kolmogorov-Smirnov two-sample tests, using the extended statistical tables in Kim and Jennrich (1970): p>>0.10, two-tailed, and p>>0.30, one-tailed, for both beginning-to-end and greatest displacement methods for intervals of 7, 15, 30, 60, 120 days].

Compass Direction of Displacements.

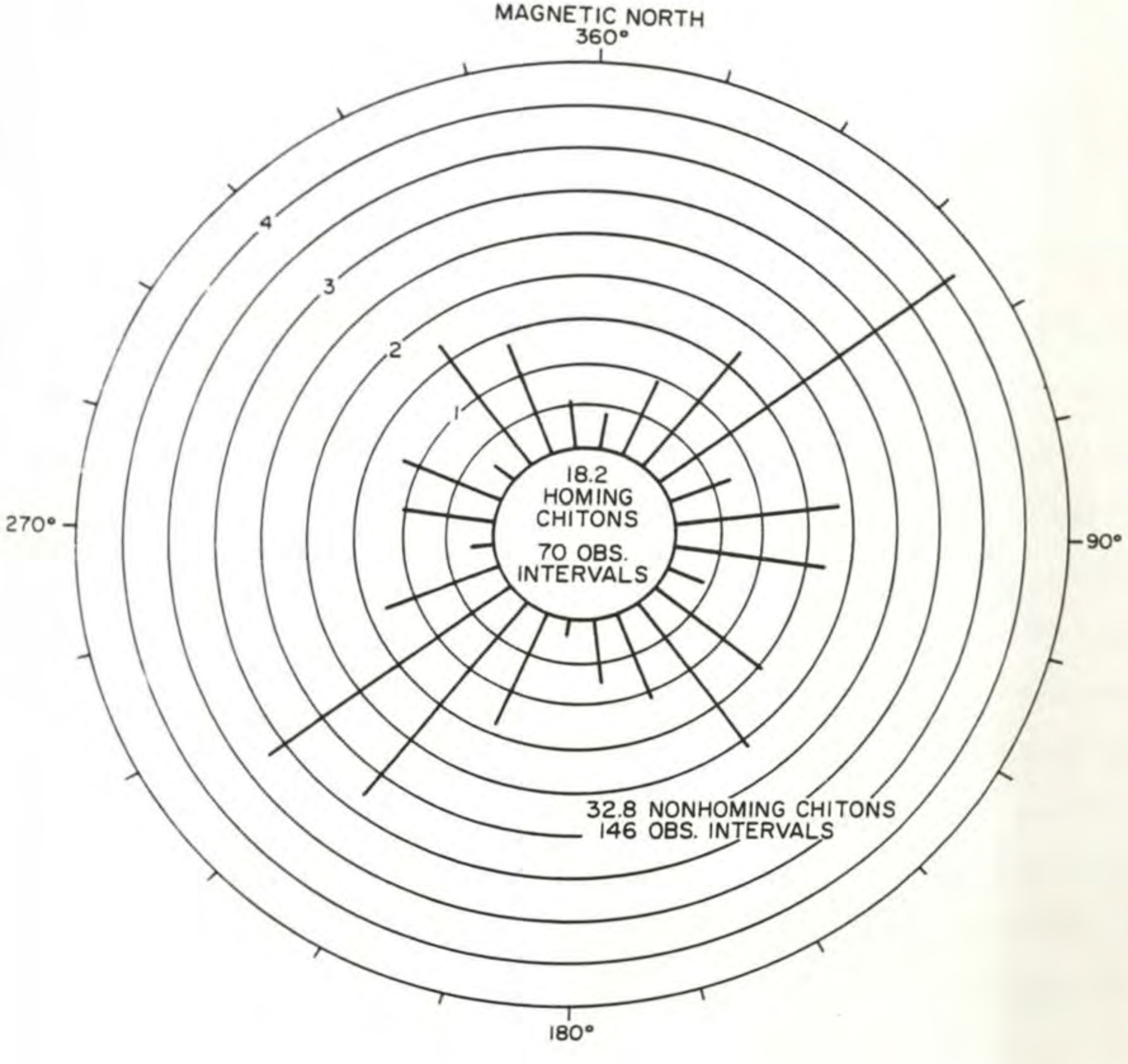
Using compass headings and X and Y tilts of quadrats (Table V-1), I calculated the compass direction, in a horizontal plane, for each displacement generated by either the beginning-to-end or greatest displacement method. At the study site, a displacement toward magnetic north is very nearly equivalent to a displacement in the upshore direction; thus, analyses of displacements with respect to general north/south directions should roughly match analyses in terms of up/down intertidal directions. However, use of compass directions allows for greater resolution of displacement directionality.

Frequencies of displacements in different compass directions are illustrated in Figure V-15 for 30 day intervals only. Two statistics for circular distributions were used to test for tendencies for displacement in particular directions. Rayleigh's test was used to test for a significant mean compass direction in displacements of <u>Nuttallina fluxa</u> (Zar, 1974, p. 317). Mean direction varied from 121 to 166°, except for the 120 day observation interval results which had mean angles of 321 and 338° (for beginning-to-end and greatest displacement methods, respectively) and for 30 and 60 day results for the greatest displacement method, where the mean angles were 28 and 278° respectively (Table V-22). In no case was the mean angle significant ($p>0.50$ for all beginning-to-end results, and for all greatest displacement results except for 120 day intervals, where $p>0.20$).

Rayleigh's test is not useful for testing particular circular distributions, such as ones with modes at 180° where it is not likely to reject the hypothesis of a uniform circular distribution. Thus, a goodness of fit test, Kuiper's modification for circular distributions of Kolmogorov's goodness-of-fit test (in Batschelet, 1965, pp. 26-27; 1970, p. 74), was also used to test the null hypothesis of a uniform circular distribution. Again, in no case could the null hypothesis be rejected [Table V-22; Kuiper circular goodness-of-fit test, $p>0.10$ (highest probability tabulated) in all cases].

Figure V-15. Circular frequency distribution of observed displacements of Nuttallina fluxa at the Seal Rock study site, La Jolla, CA for the period, 9 Sept 1979 to 11 Nov 1980.

Displacements were estimated by the beginning-to-end method for 30-day long composite observation intervals. Observations for each specimen were weighted such that the total weighting of the specimen was 1.0. Frequencies are grouped into 15° intervals, although circular statistics were calculated using 1° intervals. The scale of frequencies for nonhomers is marked on the 315° radius. The number of homing chitons and homing observation intervals is entered in the central circle of the figure. Magnetic north was taken as 360°; the declination of magnetic north at La Jolla, CA in 1980 was 13°48′ east of true north. See text for more details.


MAGNETIC NORTH
360°
270°
90°
180°
1
2
3
4
18.2
HOMING
CHITONS
70 OBS.
INTERVALS
32.8 NONHOMING CHITONS
146 OBS. INTERVALS

Finally, the magnitudes of displacements in different compass directions were compared to determine whether Nuttallina fluxa exhibits greater displacements in any specific direction. Numbers of nonhoming Nuttallina fluxa were not great enough to perform rigorous statistical tests. A grand median for all nonhoming displacements was calculated and displacements were segregated into four equal compass quadrants, with the first quadrant centered on magnetic north. The frequencies of weighted displacements falling above and below the grand median displacement were determined for each quadrant and were entered in a 2 by 4 cell contingency table. Only the analysis of 60 day interval results generated by the greatest displacement method provided sufficient numbers of nonhoming chitons to fill the eight cells (expected frequency less than 5 in two of the eight, or 25% of the cells). Point-to-point displacements were not significantly different in magnitude for the four directions (Table V-22; G test, $p>0.50$). Results for 60 day interval generated by the beginning-to-end method and results for 30 day intervals generated by both beginning-to-end and greatest displacement methods provide nearly sufficient numbers for an eight-cell G test, but reveal no difference in point-to-point displacement magnitude among the four compass quadrants (G test, $p>0.30$). Since the effect of lower-than-recommended expected cell frequencies is generally to inflate G, these findings of nonsignificant G's can be regarded as true indications of trends in the data.

Comparison of Displacements of Nuttallina fluxa in Different Habitats.

In this study, forty-one specimens of *Nuttallina fluxa* were located in the high/dry, sparsely-populated habitat, while 14 specimens were submerged in high intertidal tidepools and a single specimen occurred in the low intertidal densely-populated habitat. Point-to-point displacements of these specimens were compared using results based on 30 day observation intervals only, since intervals of this length maximized the number of nonhoming chitons, shorter interval lengths being characterized by smaller proportions of nonhoming individuals and longer interval lengths including fewer total chitons.

Results are summarized in Table V-18. The single specimen of *Nuttallina fluxa* in the low intertidal habitat homed during one of the six 30 day intervals it was observed and exhibited displacements, each of about 2 cm, in four different directions during the other 5 intervals. Homing proportions for *Nuttallina fluxa* in the other two areas, high/dry intertidal and high intertidal tidepool are quite similar, 38% and 34% of the time, respectively (test for difference in proportions, two-tailed, $p>0.75$). Similarly, there is no difference between the point-to-point displacement distributions for the two habitats (Kolmogorov-Smirnov two-sample test, two-tailed, $p>>0.10$, Kim and Jennrich, 1970).

Upward versus downward displacement frequencies are not different for specimens in the high/dry habitat (test for expected proportion of 0.5, two-tailed, $p=0.6$) nor for those in the high tidepools ($p=0.7$), although specimens in both groups tended to move downward most

TABLE V-18. Homing proportions, point-to-point displacements and vertical displacements for Nuttallina in various habitats based on 30 day composite observation intervals. Homing defined as displacement ≤ 1.26 cm. Point-to-point displacements were calculated from observed locations of chitons at the beginning and end of composite intervals. The location at the end of a composite interval was calculated by interpolation if necessary. Vertical displacements were calculated from point-to-point displacements using "X" and "Y" tilts of appropriate quadrats presented in Table V-1.

					Point-to-point Displacement (cm)					
Species	Habitat[A]	Number of Chitons	Number of 30 day Observation Intervals	Homing Proportion ± SE	Mean	Median	25 to 75 Percentile Range	10 to 90 Percentile Range	95 Percentile	Maximum Displacement
Nuttallina fluxa	Low dense	1	6	0.17±0.372	1.6	1.8	1.6-2.0	0.0-2.1	2.1	2.1
	High pools	14	77	0.34±0.126	4.5	2.2	0.2-3.6	0.0-8.0	17.3	50.6
	High dry sparse	36	127	0.38±0.081	9.6	2.0	0.9-13.2	0.0-28.0	44.7	87.7
Nuttallina kata	Low dense	52	205	0.72±0.062	1.3	0.1	0.0-1.4	0.0-3.2	7.3	17.1
	High pools	3	21	0.55±0.287	1.6	0.5	0.0-3.2	0.0-5.0	5.0	8.0
	High damp sparse	10	31	0.60±0.155	1.5	0.8	0.0-1.6	0.0-3.0	6.1	14.2
Unidentifiable Nuttallina Specimens	Low dense and sparse	4	9	0.62±0.242	1.0	0.0	0.0-1.5	0.0-1.7	8.0	8.0
	High sparse, dense and pool	21	60	0.39±0.107	5.9	2.0	0.0-7.8	0.0-18.1	18.3	71.0

TABLE V-18 (cont.)

				Estimated Vertical Intertidal Displacement (cm)				
Species	Habitat[A]	Frequency Moving Downward	Frequency Moving Upward	Mean	Median	5 to 95 Percentile Range	Maximum Downward Displacement	Maximum Upward Displacement
Nuttallina fluxa	Low dense	0.167	0.167	+0.1	0.0	-0.1 to +0.5	-0.1	+0.5
	High pools	3.754	2.760	0.0	0.0	-0.9 to +0.7	-3.5	+3.0
	High dry sparse	11.199	8.839	-0.3	0.0	-5.5 to +3.6	-19.9	+15.0
Nuttallina kata	Low dense	6.164	5.252	-0.1	0.0	-0.7 to +0.6	-8.5	+1.2
	High pools	0.875	0.375	-0.1	0.0	-1.1 to +0.4	-1.1	+0.7
	High damp sparse	1.749	1.533	0.0	0.0	-0.7 to +0.3	-1.6	+2.5
Unidentifiable *Nuttallina* Specimens	Low dense and sparse	0.000	1.250	+0.1	0.0	0.0 to +0.4	0.0	+0.4
	High sparse, dense and pool	4.976	5.667	+0.2	0.0	-0.9 to +5.8	-13.3	+8.1

NOTES

A. Habitats are as follows, and were described in Chapter II:

Low dense: low intertidal algal turf belt, densely-populated with chitons and riddled with form-fitting depressions.

High pools: high intertidal pools with standing water at all low tides; chitons always submerged.

High dry sparse: high intertidal areas, sparsely-populated with chitons, quite exposed and usually quite dry at low tide.

High damp sparse: high intertidal areas, sparsely-populated with chitons, often damp, near relief such as ledges, run-off areas, patches of algae.

[The distinction between damp and dry high sparse areas was made in the electrophoretic study Chapter III) subsequent to the present study, so that the assignment of *N. kata* specimens to damp and *N. fluxa* specimens to dry high sparse categories follows primarily from results of the electrophoretic study, with partial confirmation from subsequent examination of study site photographs with quadrat frame in place.]

Low dense and sparse: combined low dense and small sparsely-populated areas of bare rock intermingled within the low algal turf belt.

High sparse, dense and pool: combined high sparse areas, high pools and areas marginal between high sparse and dense areas. *Nuttallina fluxa* and *N. kata* specimens in areas of marginal habitats were not included in habitat analyses.

often (Table V-18). Likewise, no differences were found between upward and downward point-to-point displacement or vertical displacement magnitude distributions in either habitat (Kolmogorov-Smirnov two-sample test, two-tailed, $p >> 0.10$).

The directional pattern of point-to-point displacements does not significantly differ from a uniform circular distribution for Nuttallina fluxa in either habitat, high/dry or high tidepool (Rayleigh test, $p > 0.50$; Kuiper circular goodness-of-fit, $p >> 0.10$).

Finally, with regard to the question of an upshore migration, displacements of specimens of Nuttallina fluxa from the low shore were examined for directionality. Only one specimen of N. fluxa occurred in the lowest area on the shore, i.e., in the low dense habitat, so chitons from slightly higher ($\leq$110 cm above MLLW) adjacent sparsely-populated areas were included to bring the number of chitons included in analyses to 19. Results are summarized in Table V-19 and tests for directionality are summarized in Table V-22. Downward displacement frequencies were greater than upward displacement frequencies, but not significantly (test for expected proportion of 0.5, one-tailed for greater upward frequency, $p = 0.8$). No differences were found between upward and downward point-to-point displacement or vertical displacement distributions (Kolmogorov-Smirnov two-sample test, one-tailed for greater upward displacement magnitudes, $p > 0.7$ in both cases). Similarly, the directional pattern of point-to-point displacements does not significantly differ from a uniform circular distribution (Rayleigh test, $p > 0.50$; Kuiper, $p >> 0.10$).

TABLE V-19. Homing proportions, point-to-point displacements and vertical displacements of low-shore Nuttallina based on 30 day composite observation intervals. Homing defined as displacement ≤ 1.26 cm. Point-to-point displacements were calculated from observed locations of chitons at the beginning and end of composite intervals. The location at the end of a composite interval was calculated by interpolation if necessary. Vertical displacements were calculated from point-to-point displacements using "X" and "Y" tilts of appropriate quadrats presented in Table V-1.

					Point-to-point Displacement (cm)					
Species	Upper limit of lowshore specimens examined (intertidal height above MLLW)^{A}	Number of Chitons	Number of 30 day Observation Intervals	Homing Proportion ± SE	Mean	Median	25 to 75 Percentile Range	10 to 90 Percentile Range	95 Percentile	Maximum Displacement
<u>Nuttallina</u> <u>fluxa</u>	+110 cm	19	66	0.33±0.108	8.5	2.1	1.0-12.6	0.0-25.8	34.9	77.5
<u>Nuttallina</u> <u>kata</u>	+80 cm	52	205	0.72±0.062	1.3	0.1	0.0-1.4	0.0-3.2	7.3	17.1
Unidentifiable <u>Nuttallina</u> Specimens	+110 cm	13	35	0.44±0.138	4.7	1.5	0.0-3.7	0.0-18.3	18.3	71.0

TABLE V-19 (cont.)

Species	Frequency Moving Downward	Frequency Moving Upward	Estimated Vertical Intertidal Displacement (cm)				
			Mean	Median	5 to 95 Percentile Range	Maximum Downward Displacement	Maximum Upward Displacement
<u>Nuttallina</u> <u>fluxa</u>	7.134	3.848	-0.1	0.0	-5.5 to +3.6	-6.5	+15.0
<u>Nuttallina</u> <u>kata</u>	6.164	5.252	-0.1	0.0	-0.7 to +0.6	-8.5	+1.2
Unidentifiable <u>Nuttallina</u> Specimens	2.043	3.735	+0.2	0.0	-0.9 to +5.8	-13.3	+5.8

NOTE

A. These low-shore <u>Nuttallina</u> groups are those treated in Table V-7. See Note B for that Table. Note that the same group of <u>N</u>. <u>kata</u> is presented in Table V-18 under <u>N</u>. <u>kata</u> "Low dense".

Comparison of Displacements of Small Versus Large Specimens of Nuttallina fluxa.

In a homing species like Nuttallina fluxa, it is probable that early life stages, either larvae or young juveniles, play a large role in determining observed spatial distributions of populations. The planktonic larvae might selectively settle in suitable areas for habitation whereas the juvenile chitons might not move much at all, avoiding dangers that they are quite susceptible to, such as predation and desiccation. On the other hand, larvae might settle haphazardly and young N. fluxa might exhibit substantial long-term displacements to reach general areas to be occupied through adult life. Thus, although no information is available on larval Nuttallina settling behavior, it is of interest to compare displacements of small and large specimens of N. fluxa.

Tagging placed a lower limit on the size of Nuttallina that could be used in the study and the division of small and large chitons for analyses was set up with the goal of using the smallest chitons possible in one group and still maintaining a reasonable number for statistical purposes. Again, displacements were analyzed for 30 day intervals, for which numbers of nonhoming chitons are maximized, as explained in the previous section. Using 30 mm live total length as the line of division, analyses based on 30 day intervals included 12 small specimens and 42 large specimens.

Small ($\leq$30 mm) specimens of Nuttallina fluxa tended to home a greater proportion of the time, 40%, than did large specimens, 33%, although there was no significant difference in the two proportions (test for difference of proportions, two-tailed, p=0.6; see Table V-20). Comparison of the point-to-point displacement distributions of the two groups also revealed no differences (Kolmogorov-Smirnov two-sample test, two-tailed, p>>0.10), although the means, medians and cumulative displacement frequency distributions indicated that large specimens tend to exhibit slightly greater displacements than do small N. fluxa.

Upward versus downward displacement frequencies are not different for either large or small specimens (test for expected proportion of 0.5, two-tailed, p = 0.4, 0.8 respectively), although both groups tended to move downward most often (see Table V-20). Similarly, no differences were found between the upward and downward point-to-point displacement or vertical displacement distributions within either size group (Kolmogorov-Smirnov two-sample test, two-tailed, p>>0.10 in all cases).

The directional pattern of point-to-point displacements does not significantly differ from a uniform circular distribution for either size group of Nuttallina fluxa, small or large (Rayleigh test, p>0.50; Kuiper circular goodness-of-fit, p>>0.10; see Table V-22).

Similar analyses using 25 mm as the line of division between small and large Nuttallina fluxa groups (N = 6 and 47 respectively) gave similar results, although frequencies in the small size group were not always sufficient for statistical testing.

TABLE V-20. Homing proportions, point-to-point displacements and vertical displacements of small versus large specimens of Nuttallina, based on 30 day composite observation intervals. Where values for 25 and 30 mm lengths are listed for a species, the same data set was divided at these 2 different lengths to generate the two sets of values. Homing defined as displacement of ≤ 1.26 cm. Point-to-point displacements were calculated from observed locations of chitons at the beginning and end of composite intervals. The location at the end of a composite interval was calculated by interpolation if necessary. Vertical displacements were calculated from point-to-point displacement using "X" and "Y" tilts of appropriate quadrats, presented in Table V-1.

					Point-to-point Displacement (cm)					
Species	Live Total Length(mm)	Number of Chitons	Number of 30 day Observation Intervals	Homing Proportion ± SE	Mean	Median	25 to 75 Percentile Range	10 to 90 Percentile Range	95 Percentile	Maximum Displacement
<u>Nuttallina</u> <u>fluxa</u>	≤25.0	6	23	0.41±0.201	4.1	2.0	0.0-8.0	0.0-12.8	12.8	15.6
	≤30.0	12	51	0.40±0.142	5.0	2.0	0.0-8.7	0.0-12.8	20.7	34.0
	>25.0	47	193	0.34±0.069	8.2	2.1	0.9-8.5	0.0-23.6	38.5	87.7
	>30.0	42	165	0.33±0.072	8.9	2.2	1.0-8.8	0.0-28.0	44.2	87.7
<u>Nuttallina</u> <u>kata</u>	≤25.0	23	93	0.68±0.097	1.5	0.0	0.0-3.0	0.0-5.0	7.1	10.6
	>25.0	55	194	0.67±0.064	1.4	0.7	0.0-1.5	0.0-3.2	7.3	17.1
Unidentifiable <u>Nuttallina</u> Specimens	≤25.0	8	26	0.51±0.177	3.6	0.2	0.0-3.7	0.0-8.0	20.3	31.1
	≤30.0	19	52	0.50±0.115	4.0	1.2	0.0-3.7	0.0-15.0	18.3	31.1
	>25.0	17	43	0.39±0.118	5.8	1.7	0.0-8.0	0.0-18.1	18.3	71.0
	>30.0	6	17	0.21±0.165	8.6	3.5	1.4-8.6	0.0-18.1	33.9	71.0

TABLE V-20 (cont.)

Species	Live Total Length(mm)	Frequency Moving Downward	Frequency Moving Upward	Estimated Vertical Intertidal Displacement (cm)				
				Mean	Median	5 to 95 Percentile Range	Maximum Downward Displacement	Maximum Upward Displacement
Nuttallina fluxa	≤25.0	1.584	0.817	0.0	0.0	-1.0 to +1.0	-2.5	+2.5
	≤30.0	3.576	2.285	0.0	0.0	-2.2 to +1.0	-3.9	+6.5
	>25.0	15.947	10.909	-0.2	0.0	-3.8 to +2.7	-19.9	+15.0
	>30.0	14.489	10.532	-0.2	0.0	-4.0 to +2.9	-19.9	+15.0
Nuttallina kata	≤25.0	3.214	4.039	0.0	0.0	-0.6 to +0.8	-2.5	+2.4
	>25.0	13.702	12.687	-0.1	0.0	-0.7 to +0.6	-8.5	+2.5
Unidentifiable Nuttallina Specimens	≤25.0	2.176	0.819	-0.1	0.0	-0.9 to +0.6	-2.1	+1.8
	≤30.0	3.946	4.380	+0.3	0.0	-0.9 to +5.8	-2.1	+5.8
	>25.0	3.133	6.098	+0.3	0.0	-0.2 to +5.8	-13.3	+8.1
	>30.0	2.500	2.266	-0.1	0.0	-1.6 to +1.6	-13.3	+8.1

Comparison of Displacements of Nuttallina fluxa Among Seasons.

Analyses of the displacements of *Nuttallina fluxa* combined for the entire study, from 10 September 1979 to 11 November 1980, indicate that this species shows no directional preference in its displacements. Analyses of displacements by seasons were undertaken to determine whether *N. fluxa* might show, for example, directed movement in opposite directions during different seasons, as might be expected if, for example, this chiton migrated up and down the shore in response to seasonal variations in wave shock, temperature or desiccation.

Displacements were analyzed using 30 day composite observation intervals since this interval length provides a sufficient amount of time for substantial displacements but is short enough to include almost all possible tagged specimens. Lacking any compelling reason to do otherwise, I arbitrarily divided seasonal periods near solstices and equinoxes, splitting the year into four nearly equal time periods from 23 September 1979 to 23 September 1980.

Results are summarized in Tables V-21 and V-22. Homing versus nonhoming proportions were very similar throughout the four seasons, from about 23% to 33% (G test for independence, 3 df, $p>0.8$). Similarly, comparison of point-to-point displacement distributions among the seasons, accomplished by comparing the seasonal frequencies of chitons above and below a grand median displacement calculated for all seasons combined, revealed no significant differences (G test, $p>0.30$), although median displacements tended to be greater for Summer and Fall than for Winter and Spring.

TABLE V-21. Homing proportions, point-to-point displacements and vertical displacements of Nuttallina by season, based on 30 day composite observation intervals. Homing defined as displacement ≤ 1.26 cm. Point-to-point displacements were calculated from observed locations of chitons at the beginning and end of composite intervals. The location at the end of a composite interval was calculated by interpolation if necessary. Vertical displacements were calculated from point-to-point displacements using "X" and "Y" tilts of appropriate quadrats, presented in Table V-1.

					Point-to-point Displacement (cm)					
Species	Season^A	Number of Chitons	Number of 30 day Observation Intervals	Homing Proportion ± SE	Mean	Median	25 to 75 Percentile Range	10 to 90 Percentile Range	95 Percentile	Maximum Displacement
Nuttallina fluxa	Fall, 1979	22	51	0.23±0.089	8.6	3.0	1.9-8.4	0.0-28.4	35.3	60.5
	Winter, 1980	17	22	0.32±0.113	6.2	2.3	0.5-8.6	0.0-20.8	34.0	34.0
	Spring, 1980	42	70	0.33±0.073	7.0	2.0	0.8-8.7	0.0-22.2	40.1	50.6
	Summer, 1980	29	48	0.31±0.086	15.4	5.5	1.0-20.2	0.0-47.9	77.5	87.7
Nuttallina kata	Fall, 1979	30	53	0.53±0.091	2.0	0.0	0.0-3.0	0.0-7.1	8.0	10.0
	Winter, 1980	28	53	0.80±0.075	0.8	0.0	0.0-0.3	0.0-2.0	5.0	9.4
	Spring, 1980	64	92	0.73±0.056	1.2	0.0	0.0-1.3	0.0-3.5	7.1	17.1
	Summer, 1980	39	65	0.67±0.075	1.4	1.0	0.3-1.4	0.0-3.2	4.1	14.2
Unidentifiable Nuttallina Specimens	Fall, 1979	17	31	0.43±0.120	3.9	1.5	0.0-3.5	0.0-15.1	18.1	21.9
	Winter, 1980	7	11	0.50±0.189	1.9	0.8	0.0-1.7	0.0-5.0	10.0	10.0
	Spring, 1980	9	13	0.22±0.139	11.9	3.3	1.4-18.3	0.0-33.9	71.0	71.0
	Summer, 1980	6	10	0.25±0.177	6.5	5.3	1.5-7.4	0 5-8.6	31.1	31.1

TABLE V-21 (cont.)

Species	Season[A]	Frequency Moving Downward	Frequency Moving Upward	Estimated Vertical Intertidal Displacement (cm)				
				Mean	Median	5 to 95 Percentile Range	Maximum Downward Displacement	Maximum Upward Displacement
Nuttallina fluxa	Fall, 1979	6.830	5.831	+0.4	0.0	-2.5 to +6.5	-6.3	+15.0
	Winter, 1980	5.000	4.000	0.0	0.0	-3.9 to +2.9	-3.9	+2.9
	Spring, 1980	13.000	10.000	-0.3	0.0	-3.1 to +0.7	-10.8	+7.0
	Summer, 1980	9.000	11.000	+0.1	0.0	-5.5 to +7.9	-19.9	+13.7
Nuttallina kata	Fall, 1979	6.165	6.498	0.0	0.0	-0.8 to +0.7	-2.5	+1.2
	Winter, 1980	3.500	1.000	-0.1	0.0	-0.8 to 0.0	-2.0	+0.8
	Spring, 1980	7.000	6.500	-0.1	0.0	-1.1 to +0.7	-8.5	+1.2
	Summer, 1980	7.000	4.500	0.0	0.0	-0.7 to +0.7	-1.8	+2.5
Unidentifiable Nuttallina Specimens	Fall, 1979	4.999	3.499	-0.1	0.0	-0.8 to +0.2	-1.3	+0.4
	Winter, 1980	0.000	2.500	+0.2	0.0	0.0 to +0.4	0.0	+0.4
	Spring, 1980	1.500	5.000	+0.6	+0.4	-13.3 to +8.1	-13.3	+8.1
	Summer, 1980	1.500	3.000	+0.2	+0.3	-2.1 to +1.8	-2.1	+1.8

[A] Seasons chosen arbitrarily to approximately coincide with solstices and equinoxes, and to approximately divide the year into four equal parts.
Fall, 1979 = 9/23 - 12/23/79
Winter, 1980 = 12/23/79 - 3/24/80
Spring, 1980 = 3/24 - 6/23/80
Summer, 1980 = 6/23 - 9/23/80 .

TABLE V-22. Summary of statistical tests for directionality[A] of observed displacements of *Nuttallina fluxa*

		Test For Expected 0.5 Proportion (Up vs. Down)				Kolmogorov-Smirnov Two-Sample Test (Up vs. Down)							
	Composite Observation Interval Length (Days)	Chiton Frequency (No. of Obs. Intervals)		Z	Two-Tailed Significance	Point-to-point Displacement				Vertical Intertidal Displacement			
		Upward	Downward			Two-Sided		One-Sided (Upward Movement?)		Two-Sided		One-Sided (Upward Movement?)	
						D	P	D	P	D	P	D	P
I. All Specimens (10 Sept 1979-11 Nov 1980)													
A. Beginning-To-End Method of Analysis[B,C]	7	7(82)	10(100)	0.81	N.S.	0.13	N.S.	0.13	N.S.	0.13	N.S.	0.09	N.S.
	15	9(74)	13(88)	0.69	N.S.	0.10	N.S.	0.10	N.S.	0.14	N.S.	0.14	N.S.
	30	12(58)	16(63)	0.80	N.S.	0.12	N.S.	0.07	N.S.	0.19	N.S.	0.06	N.S.
	60	14(37)	16(32)	0.29	N.S.	0.21	N.S.	0.21	N.S.	0.19	N.S.	0.19	N.S.
	120	16(22)	13(14)	0.66	N.S.	0.28	N.S.	0.22	N.S.	0.25	N.S.	0.25	N.S.
B. Greatest Displacement Method of Analysis	7	7(84)	10(98)	0.70	N.S.	0.12	N.S.	0.12	N.S.	0.12	N.S.	0.07	N.S.
	15	10(82)	14(94)	0.40	N.S.	0.09	N.S.	0.09	N.S.	0.12	N.S.	0.05	N.S.
	30	14(64)	16(65)	0.46	N.S.	0.14	N.S.	0.03	N.S.	0.12	N.S.	0.06	N.S.
	60	16(40)	19(38)	0.41	N.S.	0.15	N.S.	0.15	N.S.	0.15	N.S.	0.15	N.S.
	120	19(23)	12(15)	1.36	0.17, N.S.	0.33	N.S.	0.08	N.S.	0.16	N.S.	0.16	N.S.
II. Within Subgroups (Beginning-To-End Method of Analysis)													
A. Habitats[D]	30												
Low Dense		0(1)	0(1)	--	--	--	--	--	--	--	--	--	--
High Dry Sparse		9(37)	11(40)	0.53	N.S.	0.23	N.S.	0.06	N.S.	0.24	N.S.	0.02	N.S.
High Tidepools		3(19)	4(19)	0.39	N.S.	0.38	N.S.	0.14	N.S.	0.24	N.S.	0.14	N.S.
Below 110 cm Above MLLW		4(17)	7(26)	0.99	N.S.	0.23	N.S.	0.23	N.S.	0.26	N.S.	0.13	N.S.
B. Sizes (Live Total Length)[E]	30												
≤25.0 mm		1(4)	1(5)	0.19	N.S.	--	--	--	--	--	--	--	--
>25.0 mm		11(54)	16(58)	0.97	0.3, N.S.	0.13	N.S.	0.06	N.S.	0.20	N.S.	0.06	N.S.
≤30.0 mm		2(10)	3(12)	0.27	N.S.	0.28	N.S.	0.28	N.S.	0.21	N.S.	0.16	N.S.
>30.0 mm		11(48)	14(51)	0.79	N.S.	0.16	N.S.	0.04	N.S.	0.20	N.S.	0.10	N.S.
C. Seasons[F]	30												
Fall, 1979		6(14)	7(16)	0.28	N.S.	0.34	N.S.	0.34	N.S.	0.25	N.S.	0.25	N.S.
Winter, 1980		4(4)	5(6)	0.33	N.S.	0.45	N.S.	0.45	N.S.	0.55	N.S.	0.55	N.S.
Spring, 1980		10(17)	13(21)	0.62	N.S.	0.32	N.S.	0.04	N.S.	0.39	N.S.	0.06	N.S.
Summer, 1980		11(18)	9(16)	0.45	N.S.	0.19	N.S.	0.19	N.S.	0.21	N.S.	0.21	N.S.

TABLE V-22 (cont.)

		Rayleigh Test				Kuiper's Goodness-Of-Fit Test (Modified for Circular Distribution From Kolmogorov-Smirnov Test)		
	Composite Observation Interval Length (Days)	Frequency of Nonhoming Chitons (No. of Obs. Intervals)	Mean Angle (Compass, Degrees)	Rayleigh's R	Significance	D^+/D^-	Kuiper's K	Significance
I. All Specimens (10 Sept 1979-11 Nov 1980)								
A. Beginning-To-End Method of Analysis [B,C]	7	19(215)	160	2.98	N.S.	0.12/-0.05	0.74	N.S.
	15	29(203)	145	2.60	N.S.	0.12/-0.03	0.82	N.S.
	30	33(146)	121	2.28	N.S.	0.11/-0.04	0.90	N.S.
	60	35(78)	161	2.16	N.S.	0.10/-0.04	0.86	N.S.
	120	32(39)	321	3.39	N.S.	0.11/-0.07	1.04	N.S.
B. Greatest Displacement Method of Analysis	7	19(217)	166	2.71	N.S.	0.11/-0.05	0.73	N.S.
	15	32(225)	141	2.97	N.S.	0.12/-0.03	0.86	N.S.
	30	37(162)	28	0.88	N.S.	0.08/-0.05	0.84	N.S.
	60	43(93)	278	1.76	N.S.	0.03/-0.08	0.76	N.S.
	120	34(42)	338	7.39	N.S.	0.08/-0.12	1.15	N.S.
II. Within Subgroups (Beginning-To-End Method of Analysis)								
A. Habitats [D]	30							
Low Dense		1(5)	--	--	--	--	--	--
High Dry Sparse		22(83)	166	2.80	N.S.	0.12/-0.04	0.77	N.S.
High Tidepools		9(54)	33	1.65	N.S.	0.17/-0.07	0.75	N.S.
Below 110 cm Above MLLW		13(49)	155	2.23	N.S.	0.20/-0.06	0.92	N.S.
B. Sizes (Live Total Length) [E]	30							
≤25.0 mm		4(14)	239	1.30	N.S.	0.16/-0.28	0.84	N.S.
>25.0 mm		31(132)	113	3.46	N.S.	0.12/-0.05	0.91	N.S.
≤30.0 mm		7(32)	218	1.82	N.S.	0.15/-0.17	0.87	N.S.
>30.0 mm		28(114)	93	3.21	N.S.	0.10/-0.04	0.79	N.S.
C. Seasons [F]	30							
Fall, 1979		17(41)	52	2.38	N.S.	0.16/-0.05	0.86	N.S.
Winter, 1980		12(14)	233	4.35	N.S.	0.16/-0.26	1.43	N.S.
Spring, 1980		28(45)	85	6.31	N.S.	0.18/-0.07	1.37	N.S.
Summer, 1980		20(34)	49	3.74	N.S.	0.18/-0.05	1.06	N.S.

TABLE V-22 (cont).

NOTES

A. Test for expected proportion from Dixon and Massey, 1969:100. Kolmogorov-Smirnov two-sample test from Siegel, 1956:127, and significance levels determined from extended tables in Kim and Jennrich (1970). Rayleigh test from Zar, 1974, using tables in Zar (and in Batschelet, 1970:64 for $N=4$ or 5). Kuiper's goodness-of-fit test for circular distributions modified from the Kolmogorov-Smirnov goodness-of-fit test, from Batschelet, 1965, 1970 using tables in Batschelet, 1974:74.

Chiton frequencies are rounded to the nearest integer but test calculations were performed using unrounded values. Corresponding numbers of observation intervals are presented to show that they exhibit trends similar to those of chiton frequencies. Partitioning chiton frequencies is expected to make tests more conservative than if frequencies were unpartitioned. Continuity corrections were not applied in tests, as these tend to make results even less significant.

Significance levels are listed if $p \leq 0.30$, except for Rayleigh's test ($p \leq 0.20$; ≤ 0.10 if $N=4$ or 5) and two-sided K-S and Kuiper's tests where p exceeded the greatest tabulated (normally near 0.10) in all cases.

B. The beginning-to-end method of analysis uses coordinates at the beginning and end of the composite observation interval of appropriate lengths for determinations of distances and directions, whereas the greatest displacement method uses the beginning coordinates and the set of observation coordinates corresponding to the greatest point-to-point displacement from the beginning coordinates observed during the composite observation interval.

C. The G test for independence (Sokal and Rohlf, 1981) was used to test the point-to-point displacements derived by beginning-to-end analyses, and based on 30 and 60 day intervals, for a tendency to be greater in particular directions. (30 and 60 day interval lengths tend to provide maximum numbers of nonhomers -- not too short for movement to occur, not long enough for great numbers of tagged chitons to disappear). Frequencies in each of four compass quadrants (I=315-45, II=45-135, III=135-225, IV=225-315° with respect to 360° magnetic north) greater than and less than the grand median displacement for all quadrants combined were entered in a 4 x 2 contingency table. Frequencies were not significantly different. Cell frequencies were not great enough for valid testing but since the effect of low cell frequencies is to inflate G, the nonsignificant result is likely an indication of an actual trend in the data.

Composite Observation Interval Length (days)	Grand Median Displacement (cm)	Frequencies (> Grand Median/ < Grand Median) in Compass Quadrants				G(3 d.f.)	Significance
		I	II	III	IV		
30	4.1	4/3	4/6	5/3	3/4	1.42	$p>0.50$
60	9.9	6/4	2/5	6/3	3/5	2.66	$p>0.30$

D. Habitats have been described in Chapter II. "Low dense" is a subset of "below 110 cm above MLLW", the latter including "high dry" areas also.

E. The same set of specimens has been divided into different size groups at 25 and 30 mm live total lengths. Thus, ≤25 mm specimens are a subset of ≤30 mm specimens and >30 mm specimens are a subset of >25 mm specimens.

F. Seasons were arbitrarily divided near solstices and equinoxes to give four approximately equal periods in the year from 23 Sept 1979 to 23 Sept 1980. Fall, 1979 = 23 Sept to 23 Dec 1979; Winter, 1980 = 23 Dec 1979 to 24 March 1980; Spring, 1980 = 24 March 1980 to 23 June 1980; Summer, 1980 = 23 June 1980 to 23 Sept 1980.

Upward and downward displacement frequencies are not different within any of the four seasons (test for expected proportion of 0.5, two-tailed, p>0.50 in all cases), although frequencies of downward displacement were slightly greater in all seasons except Summer, 1980. Similarly, no differences were found between upward and downward point-to-point displacement magnitude or vertical displacement distributions within any one of the four seasons (Kolmogorov-Smirnov two-sample test, two-tailed, p>>0.11 in all cases).

No significant directional tendency is indicated for point-to-point displacements of Nuttallina fluxa within any of the four seasons when tested against the hypothesis of a uniform circular distribution (Rayleigh test, p>0.50, except in Spring where p>0.20 and mean angle = 85°, and in Winter where p>0.20, mean angle = 233°; Kuiper circular goodness-of-fit, p>>0.10 in all cases).

Characterization of Displacements of Nuttallina kata

Analyses of displacements of Nuttallina kata were carried out in the same way as has been described for Nuttallina fluxa. Again, displacements generated by both the beginning-to-end (i.e., using coordinates at the beginning and end of each composite observation interval) and the greatest displacement methods (i.e., using the greatest point-to-point displacement from the beginning coordinates to any set of observed coordinates in a composite observation interval) for composite observation interval lengths of 7, 15, 30, 60 and 120 days were used in analyses.

Vertical Intertidal Direction of Displacements.

Frequencies of downward displacements of Nuttallina kata are not significantly different from those of upward displacements, generated by either the beginning-to-end or the greatest displacement method for 7, 15, 30, 60 or 120 day observation intervals (test for expected proportion of 0.5, two-tailed, $p>0.50$ in all cases, except for 60 day intervals where $p = 0.21$ and 0.07 for beginning-to-end and greatest displacement methods, and for 120 day intervals, where $p = 0.15$ and 0.06 for the beginning-to-end and greatest displacement methods, respectively; see Table V-23). Thus, since frequencies of upward displacements tend to be greater than those of downward ones for intervals less than 30 days long, and those of downward displacements tend to be greater for intervals greater than 30 days long, one-tailed tests for the specific hypothesis that chitons move upshore with age/size (see observations in Chapter II), are not significant ($p>0.25$ in all cases).

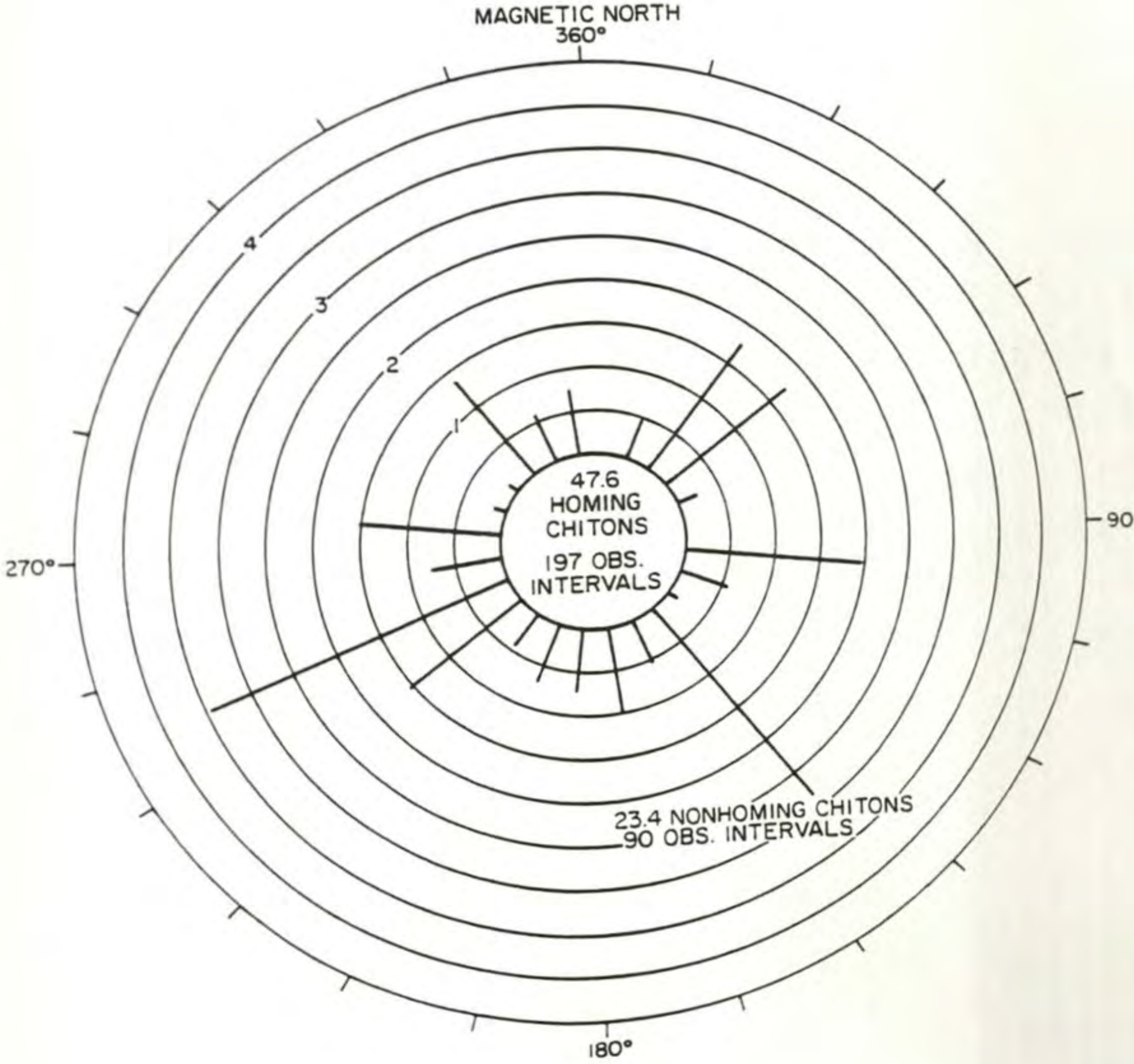
Comparisons of distributions of point-to-point displacements, and of corresponding vertical displacements, in upward versus downward directions revealed no significant differences, either when tested in a two-tailed manner or when tested one-tailed for the specific hypothesis that upward displacements are greater than downward displacements [Kolmogorov-Smirnov two-sample tests, using the extended tables of Kim and Jennrich (1970): two-tailed, $p>>0.10$, except for vertical displacements over 120 day intervals using the beginning-to-end analysis method, where $p=0.08$; and one-tailed, $p>>0.30$ for all cases using both beginning-to-end and greatest displacement methods for 7, 15, 30, 60 and 120 day intervals].

Compass Direction of Displacements.

Frequencies of displacements in different compass directions are illustrated in Figure V-16, for 30 day intervals only. As was done for *Nuttallina fluxa*, point-to-point displacements of *Nuttallina kata* were tested for a significant mean compass direction using the circular distribution statistic of Rayleigh. Mean angles for beginning-to-end and greatest displacement methods based on intervals of 7, 15, 30, 60 and 120 days varied from 106° to 267°, and in no case was the mean angle significant (Rayleigh test, $p>0.50$ for all except 60 and 120 day analyses; $p>0.20$ for beginning-to-end analysis for 60 days and for both analysis methods for 120 days, and $p>0.10$ for 60 day greatest displacement analysis; see Table V-23).

Further testing of displacement directions by Kuiper's circular goodness-of-fit statistic generally supported findings of the Rayleigh test. Directional distributions of point-to-point displacements were not significantly different from uniform circular distributions [Kuiper circular goodness-of-fit test, $p>0.10$ (highest probability tabulated in Batschelet, 1970, p. 74)], except in the case of results for 120 day observation intervals generated by the beginning-to-end method of analysis ($0.01<p<0.05$). Graphical analysis of these last results reveal that the significant test resulted from a high incidence of *Nuttallina kata*, about 56%, moving southward at headings of from 144 to 204°. This significant result should be treated with caution since it was obtained in a multiple testing situation, in which the likelihood of deriving a significant result is increased.

Figure V-16. Circular frequency distribution of observed displacements of Nuttallina kata at the Seal Rock study site, La Jolla, CA for the period, 9 Sept 1979 to 11 Nov 1980. The distribution is based on 30-day long composite observation intervals. See Figure V-15 caption for further explanation.


MAGNETIC NORTH
360°
90°
180°
270°
1
2
3
4
47.6
HOMING
CHITONS
197 OBS.
INTERVALS
23.4 NONHOMING CHITONS
90 OBS. INTERVALS

Finally, the magnitudes of point-to-point displacements of *Nuttallina kata* in different directions were compared as for *N. fluxa*, by dividing displacements in each of four compass quadrants at the grand median displacement (determined for all quadrants combined) and entering the frequencies in a 4X2 contingency table (4 direction quadrants, with the first centered on magnetic north, by 2 cells each, one above, one below the grand median). Due to the high incidence of homing in *N. kata*, numbers of nonhomers were too few for performing valid G tests, i.e., with expected frequencies of 5 in each of at least 80% of the 8 cells. However, G tests performed on results for 30 and 60 day observation intervals (for which numbers of nonhomers are greatest), generated by both the beginning-to-end and greatest displacement analytic methods, showed no significant differences in the magnitudes of displacements among the four directions (G test for independence, $p>0.80$ in all cases; see note, Table V-23). Since the effect of smaller-than-recommended expected cell frequencies in the G test is normally to inflate G, these latter test results are probably a realistic indication of the true nature of the data.

Comparison of Displacements of Specimens of Nuttallina kata *in Different Habitats*.

In this study, 55 specimens of *Nuttallina kata* were located in the damp densely-populated lower intertidal habitat, while thirteen specimens were in upper intertidal damp sparsely-populated areas, three were submerged in upper intertidal pools and 6 specimens were in border areas between the densely-populated low areas and the sparsely-populated high intertidal areas. Displacements of Nuttallina kata occurring in upper intertidal sparsely-populated areas, including tidepools, were compared to those of specimens inhabiting the low intertidal densely-populated areas using analyses based on 30 day observation intervals only, since intervals of this length maximized the available number of nonhoming chitons. Shorter interval lengths are characterized by fewer nonhoming individuals and more homers, whereas longer interval lengths allow inclusion of fewer total chitons and, as a consequence, fewer nonhoming chitons in analyses.

Results are summarized in Tables V-18, V-19 and V-23. Nuttallina kata exhibits a tendency to home less in the sparsely-populated upper intertidal areas including tidepools (59%, N=13) than in the densely-populated lower intertidal area (72%, N=52), although no difference in the homing proportions of the two groups is borne out by statistical analysis (test for difference in proportions, two-tailed, p=0.37). Similarly, there is no difference between the point-to-point displacement distributions of Nuttallina kata in the two areas (Kolmogorov-Smirnov two-sample test, two-tailed, p>>0.10, tables of Kim and Jennrich, 1970).

Upward versus downward displacement frequencies are not different for specimens of Nuttallina kata either in high intertidal areas (test for expected proportion of 0.5, two-tailed, p=0.7) or for those in low intertidal areas (p=0.8, two-tailed), although both groups tend to move downward more often. Further, no differences were found between upward and downward point-to-point displacement or vertical displacement magnitude distributions in either area (Kolmogorov-Smirnov two sample test, two-tailed, p>>0.10).

The directional pattern of point-to-point displacements does not differ significantly from a uniform circular distribution for Nuttallina kata in either high or low intertidal areas (Rayleigh test, p>0.50; Kuiper circular goodness-of-fit, p>>0.10).

Comparison of Displacements of Small Versus Large Specimens of Nuttallina kata.

Again, displacements were analyzed for 30 day intervals for which numbers of nonhoming chitons are maximized, as explained in previous sections. Division of chitons into two groups using 25 mm live total length as the dividing line, met the two goals of using the smallest chitons possible and providing a sufficient number of specimens in each group for statistical analysis.

Results are summarized in Tables V-20 and V-23. Homing proportions of small ($\leq$25 mm) and of large specimens of Nuttallina kata were virtually identical (68% and 67% respectively, test for difference of proportions, two-tailed, p>0.9). Comparison of the point-to-point displacement distributions of the two size groups revealed no differences (Kolmogorov-Smirnov two-sample test, two-tailed, p>0.10), the mean displacement being very slightly greater in the small specimens and the median displacement being slightly greater in the large specimens.

Upward versus downward displacement frequencies are not different for either small or large specimens (test for expected proportion of 0.5, two-tailed, p = 0.4, 0.6 respectively), the large specimens exhibiting a slight tendency to move downshore, and the small specimens showing a slight tendency to move upshore (Table V-20). Further, no differences were found between upward and downward point-to-point displacement or vertical displacement magnitude distributions within either size group (Kolmogorov-Smirnov two-sample test, two-tailed, $p>>0.10$ in all cases).

The directional pattern of point-to-point displacements does not significantly differ from a uniform circular distribution for either size group of *Nuttallina kata*, small or large (Rayleigh test, $p>0.50$; Kuiper circular goodness-of-fit, $p>>0.10$; Table V-23).

Comparison of Displacements of Specimens of *Nuttallina kata* Among Seasons.

As for *Nuttallina fluxa*, displacements of *Nuttallina kata* were analyzed by season to reveal any obvious directed displacements episodes, such as in opposite directions, that might not show up in an analysis for all seasons of the year combined. Again, displacements were analyzed using 30 day composite observation intervals, since this interval length provides a sufficient amount of time for substantial displacements but is short enough to include in the analysis maximal numbers of tagged specimens. As for *N. fluxa*, seasons were arbitrarily started and ended near solstices and equinoxes, dividing the year into four nearly equal time periods from 23 September 1979 to 23 September 1980.

Results are summarized in Tables V-21 and V-23. Homing proportions tended to be lower in Fall, 1979 (53%) than in the other three seasons, Winter, Spring and Summer, 1980 (80%, 73%, and 67% respectively), although differences in homing versus nonhoming frequencies among seasons were not supported statistically (G test for independence, 3 df, $p>0.10$).

Comparisons of point-to-point displacement distributions among the seasons, accomplished by comparing the seasonal frequencies of chitons above and below a grand median displacement calculated for all seasons combined, revealed significant differences (G test, 3 df, $p<0.001$). The significant result is apparently a consequence of relatively greater displacements of Nuttallina kata in Summer, 1980 and to a less marked extent in Fall, 1979, than in the other seasons, as indicated by seasonal means, medians and 75 percentiles of point-to-point displacements (Table V-21).

Upward versus downward displacement frequencies are not statistically different within any of the four seasons (test for expected proportion of 0.5, two-tailed, $p>0.24$ in all cases); frequencies of downward displacement tended to be slightly greater in all seasons except Fall, 1979. Further, no differences were found between the magnitudes of upward and downward point-to-point displacement or vertical displacement distributions within any one of the four seasons (Kolmogorov-Smirnov two-sample test, two-tailed, $p\gg0.12$ in all cases).

No significant directional tendency is indicated for point-to-point displacements of Nuttallina kata within any one of the four

TABLE V-23. Summary of statistical tests for directionality[A] of observed displacements of <u>Nuttallina</u> <u>kata</u>.

		Test For Expected 0.5 Proportion (Up vs. Down)				Kolmogorov-Smirnov Two-Sample Test (Up vs. Down)							
		Chiton Frequency (No. of Obs. Intervals)				Point-to-point Displacement				Vertical Intertidal Displacement			
	Composite Observation Interval Length (Days)	Upward	Downward	Z	Two-tailed Significance	Two-Sided		One-Sided (Upward Movement?)		Two-Sided		One-Sided (Upward Movement?)	
						D	P	D	P	D	P	D	P
I. All Specimens (10 Sept 1979-11 Nov 1980)													
A. Beginning-To-End Method of Analysis	7	4(29)	4(35)	0.11	N.S.	0.34	N.S.	0.14	N.S.	0.35	N.S.	0.02	N.S.
	15	8(45)	5(40)	0.64	N.S.	0.16	N.S.	0.16	N.S.	0.19	N.S.	0.09	N.S.
	30	9(32)	9(40)	0.06	N.S.	0.32	N.S.	0.32	N.S.	0.18	N.S.	0.07	N.S.
	60	7(15)	12(30)	1.25	0.21, N.S.	0.18	N.S.	0.07	N.S.	0.36	N.S.	0.10	N.S.
	120	6(8)	12(17)	1.42	0.15, N.S.	0.54	N.S.	0.00	N.S.	0.62	0.08, N.S.	0.00	N.S.
B. Greatest Displacement Method of Analysis	7	4(29)	4(34)	0.16	N.S.	0.39	N.S.	0.14	N.S.	0.42	N.S.	0.02	N.S.
	15	8(44)	5(47)	0.32	N.S.	0.18	N.S.	0.18	N.S.	0.16	N.S.	0.16	N.S.
	30	8(36)	10(46)	0.58	N.S.	0.35	N.S.	0.35	N.S.	0.13	N.S.	0.11	N.S.
	60	8(19)	17(41)	1.83	0.07, N.S.	0.21	N.S.	0.21	N.S.	0.28	N.S.	0.05	N.S.
	120	8(10)	18(22)	1.88	0.06, N.S.	0.26	N.S.	0.14	N.S.	0.40	N.S.	0.05	N.S.
II. Within Subgroups (Beginning-To-End Method of Analysis)													
A. Habitats[B]	30												
Low Dense (≤80 cm above MLLW)		5(23)	6(26)	0.27	N.S.	0.32	N.S.	0.32	N.S.	0.26	N.S.	0.06	N.S.
High Pools		0(2)	1(5)	0.45	N.S.	--	--	--	--	--	--	--	--
High Damp Sparse		2(3)	2(5)	0.12	N.S.	0.48	N.S.	0.48	N.S.	0.65	N.S.	0.22	N.S.
High Habitats Combined		2(5)	3(10)	0.34	N.S.	0.36	N.S.	0.36	N.S.	0.49	N.S.	0.17	N.S.
B. Sizes (live total lengths)	30												
≤25.0 mm		4(12)	2(10)	0.78	N.S.	0.42	N.S.	0.42	N.S.	0.24	N.S.	0.24	N.S.
>25.0 mm		6(21)	8(31)	0.55	N.S.	0.34	N.S.	0.34	N.S.	0.29	N.S.	0.08	N.S.
C. Seasons[C]	30												
Fall, 1979		6(11)	6(11)	0.09	N.S.	0.38	N.S.	0.38	N.S.	0.38	N.S.	0.38	N.S.
Winter, 1980		1(2)	4(7)	1.18	0.23, N.S.	0.57	N.S.	0.57	N.S.	0.50	N.S.	0.07	N.S.
Spring, 1980		6(8)	7(10)	0.14	N.S.	0.20	N.S.	0.18	N.S.	0.29	N.S.	0.00	N.S.
Summer, 1980		4(7)	7(11)	0.74	N.S.	0.35	N.S.	0.35	N.S.	0.41	N.S.	0.41	N.S.

TABLE V-23 (cont.)

	Composite Observation Interval Length (Days)	Rayleigh Test				Kuiper's Goodness-Of-Fit Test (Modified for Circular Distribution From Komogorov Smirnov Test)		
		Frequency of Nonhoming Chitons (No. of Obs. Intervals)	Mean Angle (Compass, Degrees)	Rayleigh's R	Significance	D^+/D^-	Kuiper's K	Significance
I. All Specimens (10 Sept 1979-11 Nov 1980)								
A. Beginning-To-End Method of Analysis	7	10(80)	106	0.85	N.S.	0.21/-0.03	0.77	N.S.
	15	19(112)	256	2.50	N.S.	0.16/-0.12	1.19	N.S.
	30	23(90)	204	2.77	N.S.	0.08/-0.09	0.85	N.S.
	60	22(53)	149	4.77	N.S.	0.12/-0.10	1.03	N.S.
	120	20(27)	267	4.81	N.S.	0.15/-0.25	1.76	$0.01<p<0.05$[D]
B. Greatest Displacement Method of Analysis	7	11(82)	120	0.67	N.S.	0.21/-0.03	0.79	N.S.
	15	20(124)	255	2.48	N.S.	0.16/-0.10	1.18	N.S.
	30	28(112)	199	4.42	N.S.	0.16/-0.10	1.38	N.S.
	60	32(74)	172	7.33	$0.10<p<0.20$ N.S.	0.15/-0.13	1.56	N.S.
	120	30(38)	175	6.69	N.S.	0.10/-0.15	1.37	N.S.
II. Within Subgroups (Beginning-To-End Method of Analysis)								
A. Habitats[B]	30							
Low Dense ($\le$80 cm above MLLW)		15(62)	166	1.84	N.S.	0.11/-0.10	0.79	N.S.
High Pools		1(8)	--	--	--	--	--	--
High Damp Sparse		4(10	220	1.90	N.S.	0.16/-0.30	0.92	N.S.
High Habitats Combined		5(18)	215	1.95	N.S.	0.13/-0.29	0.97	N.S.
B. Sizes (live total lengths)	30							
$\le$25.0 mm		7(28)	290	0.70	N.S.	0.14/-0.10	0.66	N.S.
>25.0 mm		18(65)	197	3.34	N.S.	0.11/-0.15	1.10	N.S.
C. Seasons[C]	30							
Fall, 1979		14(26)	229	2.66	N.S.	0.09/-0.28	1.40	N.S.
Winter, 1980		6(11)	163	2.53	N.S.	0.25/-0.22	1.09	N.S.
Spring, 1980		18(24)	206	4.12	N.S.	0.11/-0.15	1.10	N.S.
Summer, 1980		13(21)	181	1.16	N.S.	0.01/-0.16	0.61	N.S.

NOTES

A. See Notes A, B, for Table V-22. Significance levels are listed if $p \le 0.30$, except for Rayleigh's test ($p \le 0.20$; ≤ 0.10 if N=4 or 5) and two-sided K-S and Kuiper's test, where p is listed only for cases where p did not exceed the greatest probability in significance tables (normally near 0.10). G test for independence (on point-to-point displacements in different directions) performed as described in Note C, Table V-22. Cell frequencies again too low, having the effect of inflating G.

Composite Cbservation Interval Length (days)	Grand Median Displacement (cm)	Frequencies (>Grand Median/ < Grand Median) in Compass Quadrants				G(3 d.f.)	Significance
		I	II	III	IV		
30	2.6	3/2	2/3	3/3	4/4	0.15	N.S.
60	2.8	2/2	2/4	4/3	2/2	0.70	N.S.

B. "High habitats" combines all specimens in separate "high pools" and "high damp sparse" habitats.

C. See Note F in Table V-22.

D. Graphical analysis indicated that this significant result was a consequence of the fact that 56 percent of all displacements were toward the arc of the compass from 144 to 204°.

seasons when tested against a uniform circular distribution (Rayleigh test, $p>0.20$ in all cases; Kuiper circular goodness-of-fit, $p>>0.10$ in all cases; Table V-23).

Characterization of Displacements of the Unidentifiable *Nuttallina* Specimens

Analyses of displacements of the tagged specimens in this study that could not be identified to species were carried out in the same way as was done for specimens of *N. fluxa* and of *Nuttallina kata*. These analyses were undertaken to ensure that the displacement characteristics of these specimens were similar to and intermediate between those of *N. fluxa* and of *N. kata*, the species of which the unidentifiable group is composed. Analyses of the displacements of these specimens is particularly important since a few specimens in this group were unidentifiable because they occurred in the "wrong" habitat. For example, some specimens having external characters intermediate between those of the two *Nuttallina* species but slightly closer to those of *N. fluxa*, occurred in the low densely-populated area, not characteristic of *N. fluxa*, and therefore could not be identified (see Materials and Methods section). Specimens occurring in the "wrong" habitat might be those most likely to exhibit strongly directional displacements, moving to or from the "wrong" habitat in which they are found.

Again, displacements generated by both the beginning-to-end (i.e., using coordinates at the beginning and end of each composite observation interval) and the greatest displacement methods (i.e., using the greatest point-to-point displacement from the beginning coordinates to any set of coordinates observed during a composite observation interval) for composite observation interval lengths of 7, 15, 30, 60 and 120 days were used in analyses.

Vertical Intertidal Direction of Displacements.

Frequencies of downward displacements of tagged unidentifiable Nuttallina specimens combined as a group are not significantly different from those of upward displacements, generated by either the beginning-to-end or greatest displacement method using 7, 15, 30, 60 or 120 day observation intervals (test for expected proportion of 0.5, two-tailed, $p \geq 0.27$ in all cases; Table V-24). Upward frequencies are generally nearly equal to or greater than downward frequencies. One-tailed tests using the alternative that chitons move upshore with age/size (see size frequency distributions Chapter II) reveal no significant tendency for the unidentifiable Nuttallina specimens to move upshore ($p \geq 0.24$ in all cases, except for 60 day intervals analyzed by the beginning-to-end method where $p=0.14$).

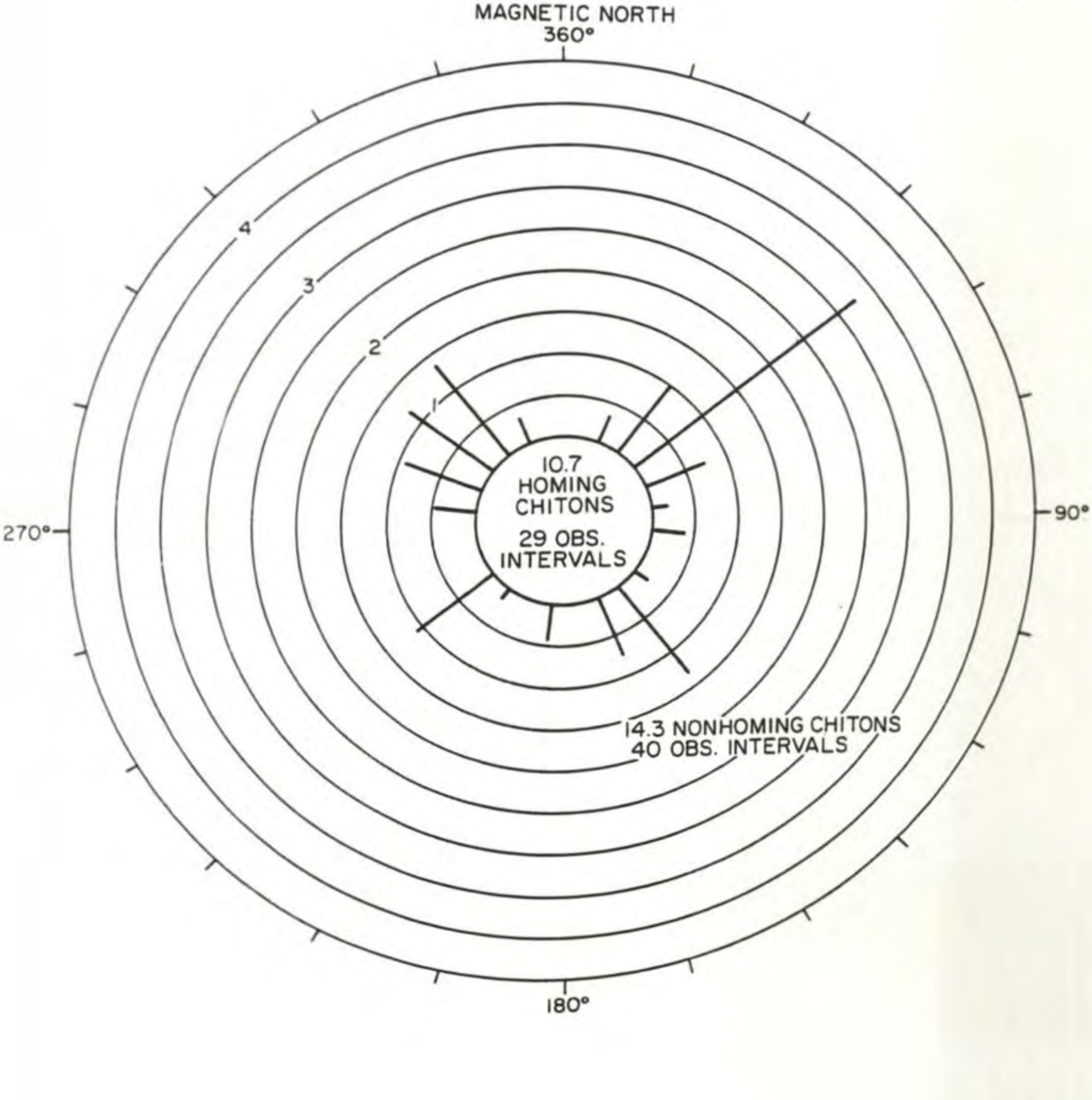
Comparisons of distributions of point-to-point displacements and of corresponding vertical displacements in upward versus downward directions revealed no significant differences, either when tested in a two-tailed manner or when tested one-tailed for the specific alternative that upward displacements are greater than downward displacements magnitudes [Kolmogorov-Smirnov two-sample tests, using extended tables of Kim and Jennrich (1970): two-tailed, $p \gg 0.10$; one-tailed, $p > 0.20$ for all cases, for both beginning-to-end and greatest displacement methods for 7, 15, 30, 60 and 120 day intervals].

Compass Direction of Displacements.

Frequencies of displacements in different compass directions are illustrated in Figure V-17, for 30 day intervals only. As was done for *Nuttallina fluxa* and *N. kata*, point-to-point displacements of unidentifiable *Nuttallina* specimens as a group were tested for a significant mean compass direction using the Rayleigh circular distribution statistic. Mean angles for beginning-to-end and greatest displacement analyses based on intervals of 7, 15, 30, 60 and 120 days were quite variable from 30 to 273°, and in no case was the mean angle significant (Rayleigh test, $p>0.20$; see Table V-24). Further testing of displacement directions by Kuiper's circular goodness-of-fit statistic supports findings of the Rayleigh test. Directional distributions of point-to-point displacements were not signifcantly different from uniform circular distributions (Kuiper circular goodness-of-fit test, $p>0.10$ in all cases; see Table V-24).

Statistical comparisons of magnitudes of point-to-point displacements of unidentifiable *Nuttallina* specimens in different directions could not be performed because of insufficient numbers of nonhoming chitons to adequately fill a 4X2 contingency table (total $N<17$ for 30 or 60 day interval results analyzed by either the beginning-to-end or the greatest displacement methods). Visual inspection of the data reveals no obvious tendency for a greater frequency of chitons to move further than the grand median displacement in any particular compass quadrant.

Figure V-17. Circular frequency distribution of observed displacements of unidentifiable Nuttallina specimens at the Seal Rock study site, La Jolla, CA for the period, 9 Sept 1979 to 11 Nov 1980. The distribution is based on 30-day long composite observation intervals. See Figure V-15 caption for further explanation.


MAGNETIC NORTH
360°
90°
180°
270°
1
2
3
4
10.7
HOMING
CHITONS
29 OBS.
INTERVALS
14.3 NONHOMING CHITONS
40 OBS. INTERVALS

Comparison of Displacements of Unidentifiable Nuttallina Specimens in Different Habitats.

In this study, five unidentifiable *Nuttallina* specimens occurred in low intertidal habitats, both densely and sparsely-populated, whereas 22 such specimens occurred in high intertidal areas, in pools or out. Displacements of specimens in different habitats were compared on the basis of 30 day observation intervals, as was previously done for specimens of *N. fluxa* and *N. kata*.

Results are summarized in Tables V-18, V-19 and V-24. Unidentifiable *Nuttallina* specimens occurring in the high intertidal areas tend to home less often than such specimens occurring in the lower intertidal areas (39% versus 62%), although the difference in homing proportions of the two groups is not borne out by statistical analysis (test for difference in proportions, two-tailed, p=0.39). Similarly, no difference between the point-to-point displacement distributions for unidentifiable *Nuttallina* specimens in the two areas is supported by statistical analysis, although specimens in the lower intertidal areas appear to exhibit shorter displacements than do specimens in the high intertidal as indicated by means, medians and cumulative frequency displacement distributions (Kolmogorov-Smirnov two-sample test, two-tailed, p>>0.10, according to tables of Kim and Jennrich, 1970).

Upward versus downward displacement frequencies are not different for unidentifiable *Nuttallina* specimens in high intertidal areas (test for expected proportion of 0.5, two-tailed, p=0.8), nor would they appear to be different for such specimens in low intertidal areas where only 3 nonhoming intervals were exhibited by 2 different chitons, resulting in 2 short upward-directed displacements (see Table V-24). Comparison of upward versus downward point-to-point displacement or vertical displacement distributions of unidentifiable Nuttallina specimens in the high intertidal area reveals no statistical difference (Kolmogorov-Smirnov two-sample test, two-tailed, $p \gg 0.100$).

Since only two of the unidentifiable Nuttallina specimens in low intertidal areas were involved in nonhoming intervals, giving a mean angle of displacement of 28°, Rayleigh and Kuiper tests of the circular distribution of displacements are not possible. The directional pattern of point-to-point displacements does not significantly differ from a uniform circular distribution for unidentifiable specimens in the high intertidal areas (Rayleigh test, $p > 0.50$; Kuiper circular goodness-of-fit, $p \gg 0.10$; Table V-24).

Finally, with regard to the question of an upshore migration, displacements of unidentifiable specimens from the low shore were examined for directionality. All specimens below 110 cm above MLLW, thirteen chitons, were included in analyses. Results are summarized in Table V-19 and tests for directionality are summarized in Table V-24. Upward displacements outnumbered downward ones, but not significantly (test for expected proportion of 0.5, one-tailed for greater upward frequency, $p=0.24$). No differences were found between upward and downward point-to-point displacement or vertical displacement distributions (Kolmogorov-Smirnov two-sample test, one-tailed for greater upward displacement magnitudes, $p > 0.5$, 0.7 respectively). Similarly, the directional pattern of point-to-point displacements does not significantly differ from a uniform circular distribution (Rayleigh test, p>0.50; Kuiper, p>>0.10).

Comparison of Displacements of Small Versus Large Unidentifiable Nuttallina Specimens.

Displacements were analyzed for 30 day intervals, for which numbers of nonhoming chitons are maximized, as explained in previous sections. Division of chitons into two groups using 25 mm live total length as the dividing line best satisfied the goal of grouping the smallest chitons possible while still providing a sufficient number of specimens in each group for statistical analyses.

Results are summarized in Table V-20. The homing proportion tends to be greater in the smaller unidentifiable Nuttallina specimens ($\leq$25 mm total length) than in the larger ones, but statistical analysis does not bear out a difference (51% versus 39% for N=8,17 respectively; test for difference of proportions, two-tailed, p=0.57). Likewise, comparison of the point-to-point displacement distributions of the two size groups revealed no differences (Kolmogorov-Smirnov two-sample test, two-tailed, p>>0.10), although the large specimens tended to move farther as indicated by means, medians and cumulative frequency displacement distributions.

Upward versus downward displacement frequencies are not different for either small or large unidentifiable Nuttallina specimens (test for expected proportion of 0.5, two-tailed, p=0.5, 0.3 respectively), the large specimens exhibiting a tendency to move upshore, the small specimens showing a slight tendency to move downshore (see Tables V-20 and V-24). Further, no differences were found between upward and downward point-to-point displacement or vertical displacement distributions within either size group (Kolmogorov-Smirnov two-sample test, two-tailed, p>0.3 in all cases).

The directional pattern of point-to-point displacements does not significantly differ from a uniform circular distribution for either size group of unidentifiable *Nuttallina* specimens, small or large (Rayleigh test, p>0.10; Kuiper circular goodness-of-fit, p>0.10).

Results obtained by dividing the unidentifiable *Nuttallina* specimens into two size groups at 30 mm total length are the same as for a division at 25 mm, except that large specimens show a slight (nonsignificant) tendency to move downshore, whereas small specimens show a nonsignificant tendency to move upshore (Table V-24).

Comparison of Displacements of Unidentifiable *Nuttallina* Specimens Among Seasons.

As was done for *Nuttallina fluxa* and *N. kata*, displacements of the unidentifiable *Nuttallina* specimens were analyzed by season to reveal any obvious differences in displacements that might be obscured by treating data for all seasons together. Again, displacements were analyzed using 30 day composite observation intervals, since this interval length provides sufficient time for substantial displacements to occur, but is short enough to include many tagged specimens in the analysis. As for the analyses involving *N. fluxa* and *N. kata*, seasons were arbitrarily started and ended near solstices and equinoxes, dividing the year into four nearly equal time periods from 23 September 1979 to 23 September 1980.

Results are summarized in Tables V-21 and V-24. Numbers of unidentifiable Nuttallina specimens were not sufficient to allow testing of the variation in homing versus nonhoming frequencies through the four seasons by a G test on a 2X4 contingency table (and in any case, G testing gave p>0.50), but homing tended to be greater in Fall, 1979 and Winter, 1980 (43% and 50% for N=17, 7 respectively) than in Spring and Summer, 1980 (22% and 25% for N=9,6 respectively).

Also, insufficient numbers of unidentifiable Nuttallina specimens were available for comparison of point-to-point displacement distributions among the seasons by comparing seasonal frequencies above and below a grand median displacement calculated for all seasons combined, and in any case, the G statistic obtained was not significant (G test for independence, 3 df, p>0.10), although displacements tend to be shorter in Fall, 1979 and Winter, 1980 than in Spring and Summer, 1980, as indicated by means, medians and cumulative frequency displacement distributions.

Upward versus downward displacement frequencies are not significantly different within any of the four seasons (test for expected proportion of 0.5, two-tailed, p>0.10 in all cases), although frequencies of upward displacement tended to be greater in all seasons except Fall, 1979, (Table V-24). Further, no differences were found between upward and downward point-to-point displacement or vertical displacement

TABLE V-24. Summary of statistical tests for directionality[A] of observed displacements in unidentifiable *Nuttallina* specimens.

	Composite Observation Interval Length (Days)	Test For Expected 0.5 Proportion (Up vs. Down)				Kolmogorov-Smirnov Two-Sample Test (Up vs. Down)							
						Point-to-point Displacement				Vertical Intertidal Displacement			
		Chiton Frequency (No. of Obs. Intervals)				Two-Sided		One-Sided (Upward Movement?)		Two-Sided		One-Sided (Upward Movement?)	
		Upward	Downward	Z	Two-Tailed Significance	D	P	D	P	D	P	D	P
I. All Specimens (10 Sept 1979- 11 Nov 1980)													
A. Beginning-To-End Method of Analysis	7	4(25)	4(23)	0.10	N.S.	0.45	N.S.	0.45	N.S.	0.17	N.S.	0.17	N.S.
	15	5(21)	5(22)	0.06	N.S.	0.46	N.S.	0.11	N.S.	0.43	N.S.	0.14	N.S.
	30	7(19)	5(14)	0.56	N.S.	0.42	N.S.	0.08	N.S.	0.30	N.S.	0.30	N.S.
	60	6(10)	3(6)	1.10	0.27, N.S.	0.29	N.S.	0.21	N.S.	0.36	N.S.	0.36	N.S.
	120	2(3)	4(4)	0.41	0.29, N.S.	0.71	N.S.	0.71	0.20<p<0.30,N.S.	0.31	N.S.	0.31	N.S.
B. Greatest Displacement Method of Analysis	7	4(26)	4(23)	0.07	N.S.	0.34	N.S.	0.34	N.S.	0.16	N.S.	0.16	N.S.
	15	5(24)	5(23)	0.08	N.S.	0.30	N.S.	0.10	N.S.	0.22	N.S.	0.12	N.S.
	30	8(23)	6(17)	0.57	N.S.	0.19	N.S.	0.10	N.S.	0.26	N.S.	0.26	N.S.
	60	6(11)	5(8)	0.72	N.S.	0.48	N.S.	0.48	0.20<p<0.30,N.S.	0.21	N.S.	0.21	N.S.
	120	4(5)	4(4)	0.35	N.S.	0.29	N.S.	0.29	N.S.	0.29	N.S.	0.29	N.S.
II. Within Subgroups (Beginning-To-End Method of Analysis)													
A. Habitats[B]	30												
Low Dense and Sparse		1(2)	0(0)	1.12	0.26, N.S.	--	--	--	--	--	--	--	--
High Sparse, Dense and Pool		6(17)	5(14)	0.21	N.S.	0.32	N.S.	0.13	N.S.	0.26	N.S.	0.26	N.S.
Below 110 cm Above MLLW		4(9)	2(5)	0.70	N.S.	0.51	N.S.	0.23	N.S.	0.32	N.S.	0.32	N.S.
B. Sizes (Live Total Length)[C]	30												
≤25.0 mm		1(4)	2(5)	0.63	N.S.	0.30	N.S.	0.24	N.S.	0.41	N.S.	0.24	N.S.
>25.0 mm		6(15)	3(9)	0.98	N.S.	0.43	N.S.	0.13	N.S.	0.42	N.S.	0.42	N.S.
≤30.0 mm		5(13)	3(8)	0.73	N.S.	0.36	N.S.	0.11	N.S.	0.24	N.S.	0.22	N.S.
>30.0 mm		2(6)	2(6)	0.02	N.S.	0.59	N.S.	0.00	N.S.	0.65	N.S.	0.65	N.S.
C. Seasons[D]	30												
Fall, 1979		3(7)	5(9)	0.51	N.S.	0.64	N.S.	0.00	N.S.	0.33	N.S.	0.21	N.S.
Winter, 1980		4(4)	0(0)	1.58	0.11, N.S.	-	-	-	-	-	-	-	-
Spring, 1980		5(6)	2(3)	1.37	0.17, N.S.	0.37	N.S.	0.37	N.S.	0.37	N.S.	0.37	N.S.
Summer, 1980		3(5)	2(3)	0.71	N.S.	0.67	N.S.	0.00	N.S.	0.33	N.S.	0.33	N.S.

TABLE V-24 (cont.)

		Rayleigh Test				Kuiper's Goodness-Of-Fit Test (Modified for Circular Distribution From Kolmogorov-Smirnov Test)		
	Composite Observation Interval Length (Days)	Frequency of Nonhoming Chitons (No. of Obs. Intervals)	Mean Angle (Compass, Degress)	Rayleigh's R	Significance	D^+/D^-	Kuiper's K	Significance
I. All Specimens (10 Sept 1979- 11 Nov 1980)								
A. Beginning-To-End Method of Analysis	7	8(56)	103	1.09	N.S.	0.16/-0.09	0.72	N.S.
	15	12(52)	127	1.71	N.S.	0.17/-0.11	0.94	N.S.
	30	14(40)	30	3.14	N.S.	0.19/-0.07	0.99	N.S.
	60	13(21)	44	2.03	N.S.	0.21/-0.11	1.14	N.S.
	120	7(8)	175	0.66	N.S.	0.19/-0.16	0.94	N.S.
B. Greatest Displacement Method of Analysis	7	9(58)	114	1.32	N.S.	0.18/-0.10	0.83	N.S.
	15	13(57)	74	2.30	N.S.	0.18/-0.06	0.90	N.S.
	30	16(47)	38	4.66	N.S.	0.19/-0.11	1.19	N.S.
	60	15(24)	227	1.98	N.S.	0.06/-0.14	0.79	N.S.
	120	9(10)	273	2.21	N.S.	0.00/-0.29	0.88	N.S.
II. Within Subgroups (Beginning-To-End Method of Analysis)								
A. Habitats^{B}	30							
Low Dense and Sparse		2(3)	--	--	--	--	--	--
High Sparse, Dense and Pool		13(37)	31	1.81	N.S.	0.14/-0.11	0.89	N.S.
Below 110 cm Above MLLW		7(18)	12	2.00	N.S.	0.20/-0.11	0.82	N.S.
B. Sizes (Live Total Length)^{C}	30							
≤25.0 mm		4(12)	193	0.99	N.S.	0.13/-0.22	0.69	N.S.
>25.0 mm		10(28)	26	4.10	0.10<p<0.20,N.S.	0.26/-0.12	1.21	N.S.
≤30.0 mm		9(27)	319	0.68	N.S.	0.06/-0.16	0.68	N.S.
>30.0 mm		5(13)	42	2.99	N.S.	0.50/-0.05	1.18	N.S.
C. Seasons^{D}	30							
Fall, 1979		10(19)	74	2.03	N.S.	0.25/-0.09	1.06	N.S.
Winter, 1980		4(4)	291	1.82	N.S.	0.09/-0.51	1.12	N.S.
Spring, 1980		7(10)	336	1.52	N.S.	0.10/-0.20	0.78	N.S.
Summer, 1980		4(8)	338	1.71	N.S.	0.18/-0.24	0.89	N.S.

NOTES

A. See Notes A, B for Table V-22. Significance levels listed if p≤0.30, except for Rayleigh's test (p≤0.20; ≤0.10 if N=4 or 5), and two-sided K-S and Kuiper's tests, where p exceeded the greatest probability tabulated (usually near 0.10) in all cases. G test for independence (point-to-point displacements in different directions) (see Note C Table V-22) not performed since frequencies far too low. E.g., for 30 day intervals, >Grand Median (=4.3 cm)/<Grand Median frequencies are: Quadrant I: 0/3; II: 4/1; III: 1/2; IV: 2/2 unpaired).

B. The "below 110 cm above MLLW" habitat contains all of the "low dense and sparse" and part of the "high sparse, dense and pool" habitats.

C. The same set of specimens was divided in two ways, at 25 and at 30 mm live total lengths; therefore ≤25 mm specimens are a subset of ≤30 mm specimens and >30 mm specimens are a subset of >25 mm chitons.

D. See note F, Table V-22.

distributions within Fall, 1979, Spring, 1980 or Summer, 1980 (Kolmogorov-Smirnov two-sample test, two-tailed, $p >> 0.14$ in all cases); in Winter, 1980, no unidentifiable Nuttallina specimens exhibited downward displacements whereas three different chitons moved short distances upshore.

No significant directional tendency is revealed for point-to-point displacements of unidentifiable Nuttallina specimens within any one of the four seasons when tested against the hypothesis of a uniform circular distribution (Rayleigh test, $p >> 0.10$ in all cases; Kuiper circular goodness-of-fit, $p >> 0.10$ in all cases).

DISCUSSION

HOMING

The outstanding feature of results of this study on the long-term movement of Nuttallina is that substantial percentages of specimens of both Nuttallina fluxa and N. kata "home" to the same low tide resting spots for considerable periods of time. Such results clearly indicate why the MacGinities (1949) were led to conclude that Nuttallina never leaves its home spot. Individuals of Nuttallina kata home an average of about 86% of 7 day intervals, decreasing to about 33% to 57% of 120 day intervals (depending on the method of analysis), while Nuttallina fluxa individuals home an average of 65% of 7 day intervals, decreasing to about 0% to 7% of 120 day intervals (Figures V-7, V-14; Tables V-8, V-13; also see Table V-6). No distinction has been, nor can be, made in this study between chitons that return to their low tide resting spots at the end of high tide feeding excursions and those that never leave the resting spot at all, since observations were made routinely only at low tide. However, evidence from several short-term studies including my own has been presented that suggests that a substantial percentage of Nuttallina specimens vacate their resting sites during any particular high tide (Tables V-4, V-5). Thus, it is probable that most of the chitons left their resting sites to feed at high tide at least once during a typical observation interval in this study (15 days long), and that most of the Nuttallina found in the same low tide resting sites after a number of days have made at least one feeding excursion away from the site and returned to it (but see later discussion concerning Nuttallina in low zone depressions surrounded by dense algal growths). For the balance of the discussion, chitons found at the same site on successive observations (most often 15 days apart) are referred to as "homers" with appreciation of the fact that some of these might not have left the homesite at all. In any case, comments referring to true homing are often equally applicable to instances where chitons might never have left the homesite.

Allowing for variations in the methods and duration of the various chiton movement studies, it is clear that Nuttallina, especially Nuttallina kata, is one of the best "homing" chitons, with regard to both fidelity and precision (Table V-25). Studies by other workers have shown that most chiton species exhibit at least a small tendency to home to particular resting spots. Accounts based on either casual observations or on more or less rigorous studies, generally a few days to a few weeks in duration, indicate that different chiton species exhibit a wide

TABLE V-25. Movement and activity patterns of chitons, segregated by geographic area.

	Location; Habitat	Author	Study Date Duration	Procedure	Homing?	Activity Pattern	Comments
1. TEMPERATE PACIFIC COAST OF NORTH AMERICA							
Cryptochiton stelleri	Oregon; intertidal and subtidal (to 10 m)	Palmer and Frank, 1974	1968-1971:3 years	Mark and recapture; locations not noted precisely	Where noted, most chitons remain within 20 m for at least 2 years	--	MacGinitie and MacGinitie (1968b) indicate that aquarium animals were strictly nocturnal; animals at 3-6°C ceased all activity. Okuda (1947) suggests that individuals in Japan might migrate to intertidal rocks from subtidal areas to breed in spring
Cyanoplax hartwegii	Pacific Grove, CA: at +0.75 to +1.2 m above MLLW; commonly under *Pelvetia*	Lyman, 1975	Spring, 1974; 50 hr and 60 hr periods (out of tidal phase by 6 or 7 hrs) for activity pattern; 14⁺ days for homing study	Positions of 13 tagged chitons recorded at hourly intervals for activity; positions of 84 tagged chitons recorded at low tides on consecutive days for homing study	Poorly-developed; e.g. only 10 of 84 in precise location for 14 days	Greatest at night; more active when dry or awash than when submerged	Homing results complicated by the fact that positions recorded in period of activity. After 35 days, several animals found 30 to 186 cm from starting position. Dark-adapted specimens found to be negatively photoactic
Cyanoplax hartwegii	Pacific Grove, CA; presumably *Pelvetia* zone, high intertidal granite	Connor, 1975	Spring, 1974; 24 hrs	Recorded position at beginning, end of 24 hour period; phase of tide, day/night not mentioned	Poorly-developed; only 2 of 22 chitons within 1 cm of start after 24 hours (average 13.4 cm: max. 35 cm)	--	--
Mopalia ciliata	Marin Co. (BCA Beach), CA; damp shale reef with gradual slope	Fitzgerald, 1975	Feb-May, 1972: 3 months	Occasional observations of location of <50 marked marked chitons; day and night observations usually at weekly intervals	No obvious homing to specific site; but 81 percent of chitons remained within 1 m of sites during study	"Moistened substrate allows the chitons to move about even during periods of aerial exposure"	No data presented for movements
Mopalia muscosa	Same as for *M. ciliata* above	Fitzgerald, 1975	Same as above	Same as above; balance of a total 50 chitons	Same as above	Same as above; small specimens in tidepools apparently more active at night than during day	1-4 cm and 4-6 cm long chitons are negatively phototactic, whereas 6-9 cm specimens apparently not. Largest (highest on shore) move least.
Mopalia muscosa	Pacific Grove, CA; 0.5 to 1.3 m above MLLW on exposed granite and in tidepool; and on cement	Smith, 1975	Spring, 1974	Positions of 8 unmarked chitons on granite recorded at $\leq$ 1 hr intervals during 10 fourteen hour periods of activity; other isolated observations; also in other habitats on 8 other chitons.	Well-developed homing in chitons on granite (69 of 71 feeding excursions) and on cement; no homing in individuals in tidepool.	Activity only when submerged or awash; only at night. Tidepool chitons active to a lesser extent at low tide at night; not active during day.	Chitons on granite left homes 4-14 times in 14 nights; most ventured 25-40 cm from home; occasionally suspend movement during night lowtide returning home before light, on incoming tide; foraging paths of different individuals generally did not overlap,
Stenoplax heathiana [="*Ischnochiton magdalensis*"]	Pacific Grove, CA; under intertidal rocks that are half-buried in sand.	Heath, 1899	--	General observations; extent of observations not clear	--	Active at night; emerge from under rocks to feed on algae	Extremely sensitive to light

TABLE V-25 (cont.)

	Location; Habitat	Author	Study Date Duration	Procedure	Homing?	Activity Pattern	Comments
Tonicella lineata	Pacific Grove, CA; subtidal, 6-10 m, and intertidal pool, ∿1.2 m above MLLW	Demopulos, 1975	Spring, 1974; 24 hour study	Subtidal: positions of 11 chitons noted at 4 hour intervals. Tidepool: positions of 15 marked chitons noted at 1 hour intervals.	Poorly-developed: 3 of 11 subtidal specimens; 3 of 15 tidepool specimens homed.	Subtidal: activity greatest at night. Tidepool: activity greatest at high tides; day/night cycle apparently not important.	Frequencies of chitons moving not reported; activity reported as average displacement/time.
II. OTHER TEMPERATE AREAS							
Chiton tuberculatus	Bermuda; Low to high intertidal rocks	Arey and Crozier, 1919; Crozier, 1921	One 24 hour period and several other periods; 1 chiton observed over 9 months	Locations of marked and unmarked chitons noted at low tide and sporadially; many isolated observations.	Homing poorly developed; small specimens wander alot and move into new areas rapidly, but intermediate and large chitons return to a "restricted area".	Move primarily when covered by tide, usually at night; but may move in damp areas. May stop if exposed at sunrise and continue in day when resubmerged.	Small specimens tend to be negatively phototactic, large specimens are photopositive and intermediate animals show light response that varies with light intensity. No detailed data presented.
"*Lepidopleurus*"(?=*Lepidochitona*) *cinereus*	Northern France; rocky shore in sandy area	Boulangé, 1927	September, 1926: April, 1927: several days and isolated observations	Positions noted when tide permitted; apparently direct observations of some excursions	Homing well-developed	--	Chitons undertood feeding
Sypharochiton pelliserpentis	New Zealand (North Island); rocky shores	Boyle, 1970	--	General observations.	Most adults home to elongatė depressions.	--	Generally, larger chitons are found higher on the shore
Sypharochiton pelliserpentis	New Zealand (North Island); occurs from high pools and high open rock (3 m above low water springs) down to top of dense brown algae (~+ 0.6 m above LWS)	Luckens, 1974	Several years in the 1960's	Incidental observations during experiments on algae-herbivore interactions.	Tend to home on upper shore but to be more free-ranging on the lower shore.	Tend to remain in homesites near edges of clear feeding areas at low tide, but apparently move at high tide,as evidenced by areas kept clear by their feeding activities.	Apparently no marked seasonal migration up or down shore
III. TROPICAL AREAS							
Acanthopleura granulata	Puerto Rico; emergent coral reef rubble,presumably 10-60 cm above mean sea level	Glynn, 1970	November, December 1968; 22 hr period and 18-24 hr period and other isolated observations	Continuously monitored 28 chitons (Nov), 27 chitons (Dec).	Home "frequently"; not always precise, chitons coming to rest few centimeters from homesite and in slightly different orientation.	Active just before twilight to midnight, decreasing to dawn; some individuals active at all hours of daylight regardless of tide.	Most feed continuously during active period but some suspend feeding for 2-8 hrs., 1 or 2 times. Some never move during 24 hours. Mean radius of feeding forays was 30 cm. Night roughly coincided with lowtide exposure in both studies but Glynn tentatively concludes day/night rather than tidal cycle is important.

TABLE V-25 (cont.)

	Location; Habitat	Author	Study Date Duration	Procedure	Homing?	Activity Pattern	Comments
Acanthopleura spinigera	Tanzania; intertidal rocks	Greenfield, 1972	--	General observations made in unpublished study by Griffiths, Greenfield	Apparently homes, to unspecified degree.	Active during high tide.	Migrates to an algal belt from rock crevices to feed.
Acanthozostera gemmata	Great Barrier Reef; intertidal	Thorne, 1967, 1968	Several days	Direct observations of marked and unmarked chitons	Homing well-developed: e.g., 28 of 31 chitons homed during one night. Orientation at home not precise, often at 180° to original orientation.	Note active during daylight hours; only active at night when exposed at low tide	Always uses outgoing path to return home but can home if displaced from trail; tend to traverse return route faster than outgoing route; most chitons in mid-intertidal ventured 20-60 cm from home (max. 200 cm), whereas those in low area, where algal growth is heavy, traveled 20 cm or less. Small specimens beneath boulders, ledges; large ones occasionally in exposed positions.
Chiton stokesii	Costa Rica Pacific Coast	Schmidt-Effing, 1980	January and March, 1976; 11 days, and other observations	--	Well-developed; e.g. 13 of 14 homed to same site for 11 days (but occasionally to mm to cm from site); orientation often changed 180° in homesite	Greatest activity at night (just after sunset, just before sunrise), usually at low tide. Longer activity period on full or new moon.	All but largest chiton left home each night; usually not more than 51 cm from homesite
Chiton tuberculatus	Puerto Rico; emergent coral reef rubble, just below mean sea level	Glynn, 1970	September and December, 1968; 18-24 hour period and 22.5 hour period, and other observations	Continuously monitored 30 chitons (Sept), 62 chitons (Dec)	Homing occurs, but not well-developed	Active at night, just after sunset and maximum shortly thereafter, decreasing till next morning	Virtually all individuals observed feeding; feed continuously with no rest periods for varying durations; mean radius of feeding forays was 45 cm; chitons of all sizes photonegative activity may be restricted in moonlight but no data for this; low tide coincided with night in at least one of the study periods so day/night vs. tidal influences not clearly separable.
Ischnochiton dispar	Costa Rica Pacific Coast	Schmidt-Effing, 1980	January and March, 1976; 11 days, and other observations	--	Apparently not precise homing, but usually returns under same rock in tidepool.	Greatest activity at night, but also active during day; primarily at low tide, very little at high tide.	Observations incidental to study of C. stokesii

range of homing fidelity and precision, from those species that rarely or never home and/or only return to general areas, such as Chiton tuberculatus (Arey and Crozier, 1919; Crozier, 1921; Glynn, 1970), Cryptochiton stelleri (Palmer and Frank, 1974), Cyanoplax hartwegii (Connor, 1975; Lyman, 1975), Tonicella lineata (Demopulos, 1975), Mopalia ciliata (Fitzgerald, 1975), and possibly Katharina tunicata (Bruno, 1978) and Ischnochiton dispar (Schmidt-Effing, 1980), to those that home frequently and/or to quite specific resting spots, such as Lepidochitona (= "Lepidopleurus") cinereus (Boulangé, 1927), Acanthozostera gemmata (Thorne, 1967, 1968), large specimens of Sypharochiton pelliserpentis (Boyle, 1970), some populations of Mopalia muscosa (Smith, 1975), Chiton stokesii (Schmidt-Effing, 1980), and possibly Acanthopleura granulata (Glynn, 1970) and Acanthopleura spinigera (Greenfield, 1972).

Generalizations about the homing fidelity of a species are not always easy to make due to the fact that considerable variation in homing fidelity apparently occurs within some chiton species, among or even within populations, at different intertidal levels, in different habitats, or among specimens of different sizes. Thus, the New Zealand chiton Sypharochiton pelliserpentis apparently homes in upper intertidal areas, but does not in low shore ones (Luckens, 1974). Mopalia muscosa was observed to home quite frequently in non-tidepool granite habitat at Monterey, CA (Smith, 1975), but not in Monterey tidepools or on a gradual-sloping damp shale reef in Marin County, CA (Smith, 1975; Fitzgerald, 1975). In addition, within a Bermudan population of Chiton tuberculatus, Arey and Crozier (1919) found that larger specimens tend to stay in restricted areas whereas small specimens wander about more freely. Among these chiton species, it is clear that *Acanthozostera gemmata* and apparently populations of *Mopalia muscosa* found in relatively dry exposed areas are excellent homers. Despite a more indirect method of study of homing in *Nuttallina*, it would appear that *Nuttallina* in La Jolla, CA compares quite favorably with these species in terms of homing. Further, the homing fidelity of *Nuttallina* equals or exceeds that of *Collisella scabra* and of *Patella*, two members of the oft-studied intertidal limpet taxon often reported to exhibit excellent homing capabilities (for *C. scabra*: e.g., Hewatt, 1940; Brant, 1950; Jessee, 1968; Haven, 1971; but see Wells, 1917 and Villee and Groody, 1940; and for *Patella*: e.g., Russell, 1907; Dearnaley et al., 1969; Cook et al., 1969 but see Orton, 1929 and Lewis, 1954).

The homing precision of *Nuttallina* is also remarkable, homing individuals coming to rest in exactly the same spot after each feeding excursion. However, unlike some populations of the well-studied homing limpets *Collisella scabra* and *Patella*, which consistently position themselves in the home depression in a unique orientation such that the rough-edged shell often fits the substrate in a "lock-and-key" arrangement (e.g., Funke, 1968; Cook et al., 1969; Lindberg and Dwyer, 1983), specimens of both *Nuttallina* species of this study occupied the resting spot in either of two orientations at an 180° angle to each other during different low tide periods. The orientation of the resting spots of *N. fluxa*, often shallow hollows in the substrate or locations near mussels, barnacles, and other relief, and particularly the orientation of the relatively deep, form-fitting depressions of *N. kata*, largely dictated the observed bipolar home positioning of individuals of these *Nuttallina* species. Such bipolar home positioning has also been described in the chitons Acanthozostera gemmata (Thorne, 1967, 1968) and Chiton stokesii (Schmidt-Effing, 1980).

THE ECOLOGICAL ADVANTAGE OF HOMING

Workers on various species of intertidal chitons and other molluscs have offered considerable speculation, and occasionally some evidence, bearing on the ecological function of the homing behavior. The suggested advantages of intertidal homing include: 1) avoidance of desiccation (e.g., Orton, 1929; Davies, 1969; Glynn, 1970; Haven, 1971; Branch, 1975; Verderber et al., 1983); 2) protection against wave-borne sand (Wolcott, 1973); 3) protection against dislodgement by waves, predators or large agonistic species (e.g., Branch, 1971, 1975, and 1981, p. 348; Wolcott, 1973; Garrity and Levings, 1983; Lindberg and Dwyer, 1983); and 4) regulation of population density and dispersion to reduce intra- and/or interspecific competition for food or space (e.g., Aitken, 1962; Stimson, 1970, 1973; Branch, 1975; Mackay and Underwood, 1977). Different intertidal mollusc species and populations have been observed to home in diverse habitats, subject to a wide variety of environmental and ecological conditions, and, Branch (1975, p. 596) has concluded, in his notable study on several Patella limpet species in South Africa, that homing and homing scars serve different primary functions in different species.

Avoidance of Desiccation?

An animal such as a limpet is expected to lose less water when exposed to air while resting on its home scar, by virtue of the close fit of its shell to its homesite, especially in the case of such limpets as _Collisella scabra_, in which the shell edge grows to conform to the unique contour of the home spot on hard insoluble substrates (cf. Lindberg and Dwyer, 1983). Of course, chitons are quite different from limpets in that they can conform to quite irregular substrates, almost anywhere on a rock, via an effective clamp-down response of the flexible girdle (Boyle, 1969). Thus, chitons are not restricted to particular spots on rocks due to the requirement of conformation of a hard shell to rock contours, as are some limpets. However, both _Nuttallina_ species of this study usually establish homesites in form-fitting depressions, in shallow hollows, at the bases of mussel beds or near barnacles, areas that usually retain moisture better than nearby areas. Kues (1969) found that 47 specimens of _Nuttallina_ situated in their "holes" in the high intertidal (probably almost all _N. fluxa_) lost an average of 10% of their body water after 6 hours of exposure at low tide, on sunny days with slight wind, whereas 16 specimens experimentally displaced from their holes to nearby rocks without depressions, but "which were in other ways identical to the rocks [the chitons] were removed from", lost an average of 54% of their body water after 6 hours of exposure under similar weather conditions. It is unclear whether Kues (1969) accounted for the water contained in the pallial grooves of the experimental chitons and for the moisture on the rock beneath the chitons in making transfers to the new rock sites, but his results suggest that homing to a suitable resting site can help to reduce desiccation in *Nuttallina*.

Avoidance of desiccation is often suggested to be the primary function of homing when intertidal animals are observed to move away from their homesites only when submerged at high tide and in cases where molluscs in drier habitats are observed to home more reliably than those in wetter habitats. For example, in studies of both chitons and limpets, high shore individuals and species often home more rigidly than low shore conspecific individuals and congeneric species [e.g., within *Patella granularis* (Branch, 1971) and within *Sypharochiton pelliserpentis* (Luckens, 1974); and between acmaeid limpet species: high *Collisella scabra* (Brant, 1950) versus low *Collisella pelta* (Villee and Groody, 1940); and see Branch, 1981, pp. 246-247] and the more rigid homing in the high shore molluscs is generally regarded as an adaptation to the greater desiccating conditions in the high intertidal zone.

In this study, findings of earlier reports (Table V-4) that *Nuttallina* moves away from its resting spot only when it is submerged at high tide were confirmed. With only four exceptions, neither *Nuttallina fluxa* nor *N. kata* was ever observed to move when exposed to air. In all four exceptional cases in which *Nuttallina* specimens were observed out of their homesites and moving, or presumably moving, at low tide, conditions were damp or wet. Two of the instances involved observations on overcast, spring and summer nights, one on an overcast, dewy early morning low tide and one observation during a neap low tide on a sunny afternoon during which occasional waves wet the area (Table V-5). Thus, the activity pattern of *Nuttallina* is as would be expected if desiccation were an important factor determining the behavior of this chiton.

If the primary function of homing were to avoid desiccation, small *Nuttallina*, with a relatively large evaporative surface area to volume ratio and presumably a high susceptibility to desiccation (see e.g., Boyle, 1970), and *Nuttallina* in dry habitats might be expected to home more reliably than large specimens and chitons occurring in damp or permanently submerged intertidal areas. As predicted by desiccation considerations, small specimens of *Nuttallina fluxa* and of the unidentifiable *Nuttallina* group tend to home a greater proportion of the time than do large chitons in these groups (Table V-20; 40% homing, for N=12, ≤30 mm in length versus 33%, N=42 large specimens, and 51%, N=8, ≤25 mm in length versus 39%, N=17 large specimens in the two groups, respectively). However, these differences were not statistically significant nor was there any difference in homing proportion between small and large specimens of *Nuttallina kata* (Table V-20; 68%, N=23 specimens, ≤25 mm in length and 67%, N=55 large specimens).

Comparisons of homing proportions observed for *Nuttallina fluxa* and *N. kata* in different habitats do not conclusively bear out predictions based on considerations of desiccation, although small sample sizes probably affect the power of the comparisons. Thus, specimens of *N. fluxa* submerged in large high intertidal pools do not home much less than *N. fluxa* in exposed high intertidal areas (Table V-18; 34%, N=14 versus 38%, N=36, 30 day intervals) and the single tagged specimen inhabiting the damp low intertidal algal turf homed for only one of the six 30 day intervals for which it was observed, less than high pool specimens of *N. fluxa*. None of these differences was statistically significant. Specimens of *Nuttallina kata* occurring in tidepools tended to home less frequently (55%, N=3) than did specimens in exposed upper intertidal areas (60%, N=10) as predicted, but N. kata in the damp low intertidal algal turf tended to home more frequently than did specimens in more exposed upper intertidal areas, contrary to expectation (72%, N=52, versus 60%, N=10, respectively, for 30 day intervals). Again, none of these differences was statistically significant.

The fact that Nuttallina fluxa does not home as frequently as Nuttallina kata (Tables V-6, V-8, V-13; Figures V-7, V-14) is a finding clearly contrary to that expected if desiccation avoidance were a primary function of the homing behavior for both of these species. A majority of tagged specimens of N. fluxa occurred in quite exposed high intertidal areas, whereas most specimens of N. kata were found in depressions in the low intertidal area in this study. Thus, Nuttallina fluxa, occurring generally in drier areas, would be expected to home much more frequently than N. kata.

A factor probably confounding any tendency for Nuttallina to home in drier habitats, as expected from desiccation considerations, is the tendency for the drier habitats to have a lower standing biomass of algal food available to browsing chitons than wetter areas. Single feeding excursions of specimens of either Nuttallina species might involve greater distances in algal-poor habitats than in algal-rich areas, as has been observed for the intertidal chiton Acanthozostera gemmata (Thorne, 1967; in low algal-rich area, most excursions less than 20 cm whereas in high algal-poor area, excursions usually between 20 and 60 cm in length) and for at least one intertidal limpet species (Lottia gigantea; Stimson, 1973). Thus, the probability of homing in response to a drive to avoid desiccation might be reduced in drier habitats as a result of the greater difficulty of homing from the greater distances covered during feeding excursions there.

In summary, data for Nuttallina, examined in relation to predictions made from desiccation considerations, does not give firm support for the idea that Nuttallina homes primarily to avoid desiccation.

Protection Against Wave-borne Sand?

In his study of several acmaeid limpets, Wolcott (1973) suggested that homing in these animals might protect them against the scouring action of wave-borne sand. Similarly, for Nuttallina, sand is probably less likely to be swept under the tough girdle (where it can injure the delicate gills) of a chiton protected in its homesite, particularly if that homesite is a depression, than of a chiton more exposed.

The site of the present study is situated on a rock outcrop that is bordered on its lower south side by a channel characterized by dramatically fluctuating levels of sand. Tens of meters to the southeast, there is a sandy beach. During six years before, during and after this study, the level of sand on the wall to the east of the study site has been observed to change substantially, up to as much as 2 meters in a single day. In addition, sand is periodically deposited in small amounts in the study site itself, notably in October, 1979 and January, February and March, 1980, during the present study, such that some chitons in depressions, particularly in lower intertidal areas, were completely covered with sand. Such sand deposition, resulting from the action of high surf, was short-lived, disappearing in a single day in March, 1980 for instance, but is a clear indication of transport of large amounts of sand across the study site. Another indication of sand impingement is the presence, during these high surf episodes, of chitons missing characteristic algal growths on their valves, probably as a result of sand blasting. Clearly, Nuttallina can avoid damage from sand scouring, by remaining in protected positions near relief, such as in depressions in the low intertidal area or near mussels, barnacles or other topographic irregularities in the upper intertidal area. Since sand is expected to have its most significant scouring action along the shore when transported by water, primarily at high tide, occupation of a suitable homesite is expected to be advantageous only to the extent that the chitons do not move around to feed at high tide during periods of great sand transport.

Protection Against Wave Shock, Predators, Agonistic Species?

Homing to appropriate spots might also provide protection against dislodgement by waves, predators and/or agonistic species. Linsenmeyer (1975) found that "Nuttallina californica" had a resistance to shear removal from a smooth Plexiglas surface greater that that of Katharina tunicata and that of Mopalia muscosa, chitons also found in surf-exposed habitats, and a resistance much greater than that of Stenoplax heathiana and that of Mopalia lignosa, two chiton species normally occurring under rocks. This resistance of Nuttallina to dislodgement is undoubtedly enhanced when it is in a high-relief homesite. However, Nuttallina moves away from its homesite only at high tide, the period of wave impact, so homing to a suitable spot can only confer an advantage to Nuttallina with respect to wave shock to the extent that it does not venture out to feed at different high tides.

Homing to appropriate spots probably provides some protection from aquatic and terrestrial predators. It is immediately obvious to one who tries to collect Nuttallina that its habit of occupying high relief spots, particularly the habit of N. kata of inhabiting deep form-fitting depressions makes it quite difficult to detach these animals from the substrate.

What are potential predators of Nuttallina? Chitons occurring on the Pacific Coast of North America are preyed upon by various predators, including: 1) birds (e.g., Legg, 1954; Glynn, 1965; Moore, 1975; D. R. Lindberg, 1981, Museum of Paleontology, UC Berkeley, in litt.); 2) seastars (e.g., Feder, 1959; Mauzey et al, 1968; Menge, 1972); 3) carnivorous gastropods (Louda, 1979); 4) fish (L. Moulton, personal communication to Palmer, 1977); and 5) possibly crabs (DeBevoise, 1975).

Of the potential predators of Nuttallina, gulls were subjectively judged to be the most abundant at the La Jolla site, but these birds showed no liking for medium-sized Nuttallina thrown to them, nor were there ever seen any of the telltale regurgitated pellets that Moore (1975) used to document substantial predation of the western gull, Larus occidentalis, on a "Nuttallina californica" population in central California. Several large shore crabs, Pachygrapsus crassipes, were resident in the study site and were observed eating from Nuttallina carcasses, but these chitons are thought to have been detached and discarded during collections. In central California, living littoral animals have been found to be the least important food source of Pachygrapsus crassipes in the series: live algae>detritus (natural and human litter)>littoral animals (Hiatt, 1948). Although Pachygrapsus is quite capable of breaking mollusc shells with its chelipeds (Chapin, 1968), this crab generally consumes intertidal animals by scavenging rather than by predation (Hiatt, 1948). Pisaster ochraceus may be a significant predator on Nuttallina populations at Santa Cruz Island in the Channel Islands of California (B. A. Menge, personal communication to Louda, 1972). However, only a single seastar, Pisaster sp., was ever observed in or near the study site over a period of four or five years. This predator probably had little impact on chitons in the study area since mussel beds were always present and Pisaster has been shown to prefer mussels over "Nuttallina californica" (probably N. fluxa of this study; Landenberger, 1968).

Since Nuttallina leaves its homesite to feed at high tide, the homesite might be expected to provide protection primarily against predators active in the area at low tide, such as seagulls, oystercatchers, turnstones and possibly crabs, rather than against aquatic predators such as fish like the sheephead (Pimelometopon pulchrum). Protection against aquatic predators would depend on the frequency and duration of feeding excursions of Nuttallina away from the homesite at high tide.

Lindberg and Dwyer (1983) suggested that homing to suitable spots might allow small limpet species to occur on the territories of larger agonistic species by preventing the small species from being shoved off the substratum. Likewise, the large territorial intertidal limpet, Lottia gigantea, might be prevented from performing its characteristic territorial bulldozing behavior (Stimson, 1970, 1973) on Nuttallina as a result of homing of the latter to high relief areas. Lottia might even be excluded completely from large areas due to a lack of suitable attachment sites for this large limpet among the closely-spaced deep oblong depressions formed particularly by homing Nuttallina kata, where these depressions extend up into the mid-intertidal range of Lottia. Lottia was observed in head-to-head contact (confrontation?) with Nuttallina in mid-intertidal areas (above the low intertidal, densely-populated chiton zone) on two low tide occasions during this study (see Table V-26), which might reflect significant interaction between the two species at high tide when both are active. However, Lottia probably has limited impact on the behavior of Nuttallina at the La Jolla study site because it occurs only in relatively small numbers there, probably as a result of sporadic collections by fishermen for bait (personal observations and confrontations). Thus, if the homing behavior of Nuttallina is a response primarily to the agonistic behavior of Lottia, it is likely a holdover of a behavior evolved in another area or another time in which Lottia was abundant.

Efficient Allocation of Resources?

Although coming to rest in appropriate sites probably does confer on Nuttallina a combination of advantages in relation to desiccation, the scouring action of wind and wave-borne sand and dislodgement by waves, predators or large agonistic species, it would seem that Nuttallina does not have to home to a unique spot to gain these advantages but rather could move from site to site. Both the low and the high intertidal areas appear to have an excess of suitable positions for the chitons. For example, in the nearest neighbors analyses performed in Spring, 1980 at the La Jolla study site (Chapter II), from 3 to 42%, ($\overline{x}$=20%) of suitable chiton depressions in nine small low intertidal quadrats were unoccupied. Higher intertidal areas also possessed many seemingly-suitable unoccupied spots near mussel beds, in pools and near barnacles. Even in the absence of extra depressions, the chitons might be expected to play "musical chairs" at the end of high tide feeding episodes, safely occupying any of several appropriate depressions, particularly in the low intertidal area where there is a fairly uniform distribution of closely-packed chiton-sized depressions.

The disadvantage of such a "musical chair" scheme, even with extra depressions available, was observed occasionally during this study. Nineteen times, pairs of chitons were observed in contact (see Table V-26); often contact occurred at a defined low tide resting spot, and since the spot was only large enough for one specimen, at least one of the chitons was in a vulnerable position, half in and half out of the depression. Such instances clearly resulted when one or both of the chitons involved had not homed to its usual spot on the rock.

It can be argued, then, that the ultimate function of the homing behavior is to ensure that <u>Nuttallina</u> specimens do not overload particular resting sites. Certainly, nearest neighbors analysis (Chapter II) has shown that <u>Nuttallina</u>, primarily <u>N</u>. <u>kata</u>, tends to be overdispersed in densely-populated low intertidal areas, as might be expected if the animals were regulating their spacing on the rock. However, similar analyses in high intertidal areas, inhabited primarily by <u>N</u>. <u>fluxa</u>, indicate a tendency toward clumped dispersion patterns, as might be

TABLE V-26. Conspicuous Nuttallina-Nuttallina and Nuttallina-Lottia contacts observed at low tide in the La Jolla, CA site near Seal Rock, during the study from 10 Sept 1979 to 11 Nov 1980

Date Contact Observed at Low Tide	Quadrat[A]	Habitat	Tagged Nuttallina (Total live length)	Mollusk Contacted[B]	Body Parts in Contact	Locations of Tagged Nuttallina Just Previous to Contact Observation Date	Locations of Tagged Nuttallina Subsequent to Contact
1. TAGGED NUTTALLINA SPECIMENS AS PARTICIPANTS							
13 Feb 1980	1	High Pool	Nuttallina fluxa (28.2 mm)	Nuttallina sp. (similar size)	--	Occupied homesite 5 cm from contact site for 3 obs, 17 Dec 1979 to 28 Jan 1980	Observed 3 cm back toward homesite on 25 Feb, 13 March; back to homesite on 26 March 1980
12 March 1980	1	High Pool	Nuttallina kata (26.2 mm)	Nuttallina sp. (slightly larger)	Head-to-head	Occupied homesite, same as contact site, for 8 obs, 20 Nov 1979 to 25 Feb 1980	Still at homesite for all obs from 26 March to 9 Nov 1980
15 May 1980	2	High Pool	Unidentifiable, probably N. fluxa (23.2 mm)	Nuttallina sp. (larger one)	--	Had homed to a site 20 cm from contact site from 20 Nov 1979 to 26 March 1980; moved to an area about 10 cm from contact site and wandered in a 4 cm diameter area for 3 obs, 16 April to 14 May 1980; moved 10 cm to point of contact	Disappeared
12 June 1980	3	High Sparse	Nuttallina fluxa (31.2 mm)	Nuttallina sp. (~20-25 mm)	--	Had homed to an area 41 cm from point of contact for 2 obs, 31 May and 4 June 1980; moved 41 cm to point of contact	Moved 46 cm to point 16 cm from 4 June site as of 25 June; moved 16 cm back to 4 June site by 14 July 1980 obs; moved 7 cm farther from contact site by 15 July 1980; only found in same site twice again, from 27 Aug to 9 Sept 1980, until end of study 9 Nov 1980
16 June 1980 17 June 1980	5	High Sparse	Nuttallina fluxa (45.7 mm)	Nuttallina sp. (similar size)	Head-to-tail Head-to-head	Occupied homesite 3 cm from contact site for 3 obs, 18 April to 4 June 1980	17 June, in head-to-head contact with large Nuttallina (same one as the previous day?) at original homesite; 14 July to 13 Aug 1980, 3 obs at 3 different sites about 2 to 3 cm from original homesite
4 June 1980	8	High sparse/dense	Nuttallina kata (29.7 mm)	Lottia (50 mm)	Head-to-head	Only in study from May to July, 1980; 15 May, at point 27 cm from contact site; 2 June, at point 2 cm from contact site	11 July 1980, 37 cm from contact site and 18 cm from 15 May 1980 site; Nuttallina disappeared after 11 July 1980

Table V-26 (cont.)

Date Contact Observed at Low Tide	Quadrat[A]	Habitat	Participants	Body Parts in Contact
II. NON-TAGGED *NUTTALLINA* SPECIMENS AS PARTICIPANTS				
13 Feb 1980	1	High pool	2 *Nuttallina* (medium-sized; 30 mm long?)	--
25 Feb 1980	3	High sparse	2 *Nuttallina* (large and medium; 35-40 and 30 mm long?)	Tail-to-tail
25 Feb 1980	3	High sparse	2 *Nuttallina* (28 mm long)	Head-to-side
13 March 1980	3	High sparse	2 *Nuttallina* (25 mm long)	Head-to-tail
16 April 1980	4	High sparse near sparse/dense boundary	2 *Nuttallina*	--
16 April 1980	2	High sparse	2 *Nuttallina* (both large; 35-40 mm?)	--
15 May 1980	3	High sparse	2 *Nuttallina*	--
15 May 1980	3	High sparse	2 *Nuttallina*	--
15 May 1980	3	High sparse	3 *Nuttallina*	--
15 May 1980	8	High sparse near sparse/dense boundary	2 *Nuttallina*	--
31 May 1980	7	High sparse	2 *Nuttallina* (both large; 35-40 mm?)	Side-to-tail
2 June 1980	8	High sparse	*Lottia* and *Nuttallina* (*Nuttallina* large; 35-40 mm long?)	Head-to-head
12 June 1980	3	High sparse	2 *Nuttallina* (~15 mm, 25 mm long)	Head-to-tail
17 June 1980	7	High sparse	2 *Nuttallina* (both large; 35-40 mm long?)	Head-to-undergirdle
11 July 1980	8	High sparse near sparse/dense boundary	2 *Nuttallina* (medium-sized; 30 mm long?)	--

NOTES.

A. See Table V-1 for quadrat characteristics

B. *Nuttallina* or *Lottia*; contacts with other limpet species were not specifically noted.

expected if animals were aggregating in areas more suitable than others. Thus, for the high intertidal chitons, homing probably functions to keep specimens in general suitable areas, such as on the damp edges of mussel beds, as well as preventing them from overloading any of the several sites within these suitable areas.

Homing, coupled with territorial behavior, has been shown to serve in the efficient allocation of resources, primarily food rather than space, in the intertidal limpet *Lottia gigantea* (Stimson, 1970, 1973) and in several South African limpets (Branch, 1975). Thus, for example, *Lottia* bulldozes intruders off of its intertidal grazing territory, maintaining an algal film "garden" on the order of 1000 cm^2 in surface area. Such behavior has not been documented in detail for any chitons, although scattered observations for the homing chiton *Mopalia muscosa* are suggestive. Smith (1975) found that in only two of fifty-three *M. muscosa* feeding forays, generally to distances of 25 to 40 centimeters from home, did a chiton enter into another's home range (determined from previous feeding paths in the study). Most interestingly, on one of these two intrusive forays, the resident *M. muscosa* left its home, approached the intruder's girdle and remained there for a half an hour, before returning home in a wide semicircular path through its home range. The intruder moved off to a new site after about an hour.

In another study, Connor (1975) observed *Mopalia muscosa* to push away actively a potential food competitor, the limpet *Collisella pelta*, in 5 of 10 trials in which the limpet was placed 1 cm from the girdle of the chiton. This behavior was observed twice in unstaged encounters in the field. In addition, *Collisella pelta* placed 1 cm from *Mopalia* muscosa tended to move further during the ensuing hour than did specimens placed 20 (or 30?) cm away. Thus, M. muscosa, a homing chiton that occurs in habitats similar to those of Nuttallina, is quite likely territorial. The intertidal chiton Katharina tunicata, also found along the Pacific Coast of North America, might also be territorial. In work on interactions between K. tunicata and limpets, R. G. Creese (personal communication to Branch, 1981) observed the chiton to push away Collisella pelta in encounters, and noted that the limpet Notoacmea scutum moves away from Katharina.

Of particular interest in regard to my field observations on Nuttallina, Branch (1975, p. 584) provides evidence that aggressive territorial encounters of the limpet Patella longicosta are postponed if the animals are left exposed by the receding tide, and are resumed with the incoming tide. In three cases, pairs of P. longicosta were found touching one another while exposed at low tide, apparently contesting algae gardens, and, in one case, after the pair had been observed motionless and touching for 3.5 hours at low tide, one limpet started pushing the other away and forced it off the intertidal garden when the incoming tide covered them again. Further study is required to determine whether the nineteen cases I noted of exposed pairs of Nuttallina touching at low tide (Table V-26), all in areas above the low densely-populated zone and a majority of which involved the head of at least one specimen touching the other chiton, are instances of suspended aggressive territorial behavior.

Another of Branch's (1975) observations, on Patella cochlear, is of interest in relation to my observations on Nuttallina kata and might help explain why pairs of *Nuttallina* were never noted to be touching in the low intertidal densely-populated zone during the present study, as might be expected (Table V-26). (It is important to point out though that chitons occurring here are quite closely spaced, and instances of touching would not be as notable as they are in the more sparsely-populated upper intertidal area). Populations of the territorial limpet, *P. cochlear*, are quite dense, home scars are clearly defined, and homing behavior is very rigid, just as for *Nuttallina kata* in the low area of the La Jolla study site. As a result of high densities, encounters in *P. cochlear* are expected quite frequently. Branch (1975, p. 585) noted that intraspecific encounters are quite mild and, despite this mildness, intruders respond quite readily and move away, leaving "one with the impression that the process [of territorial defense] has been ritualized." Further, older specimens of *P. cochlear* do not leave their scars at all, but rotate around on the scar to feed or algae in the immediate vicinity, thereby reducing the number of intraspecific encounters. Wright (1982) has described similar ritualization of behavior in the territorial intertidal limpet *Lottia*. Similarly, "mild" territorial behavior might occur in *Nuttallina kata*. In addition, although some *N. kata* clearly move away from the homesite at high tide as indicated by observed displacements and the immediate occupation of many experimentally-vacated depressions (Tables V-4, V-5), it is possible that the chitons often just rotate in their depressions while feeding on bordering algae. Such a behavior would contribute to the great frequency of "homing" success of *N. kata* observed in this study, and would suggest that observed 180° orientation reversals of low shore specimens do not always equate with excursions away from the homesite.

If the primary function of the homing behavior were the efficient partitioning of space among the chitons, this behavior would be expected to be most highly developed in areas where chitons occur most densely, such as the low intertidal zone at the study site, and less well-developed where the animals occur most sparsely, such as in high intertidal areas either submerged in tidepools or in more exposed areas. On the other hand, if food allocation were the primary function of the homing behavior, homing might be expected to be most pronounced in the high intertidal where standing algal biomass is clearly quite low, and less obvious in the low intertidal zone, where algal biomass, though in large part consisting of coralline algae which is probably of low caloric content (Paine and Vadas, 1969), is likely to be always substantial enough to meet the generalist feeding requirements (Kues, 1969) of, or at least fill the guts of Nuttallina there. A general trend in homing frequencies lending support for a space-partitioning function is seen for Nuttallina kata in low versus high damp and high pool areas (but not statistically different, Table V-20) and is seen in a comparison of Nuttallina kata, which occurs primarily in the low densely-populated zone, and N. fluxa, primarily in high sparse areas (statistically significant, Figures V-7, V-14, Tables V-6, V-8, V-13). Nuttallina fluxa had only 1 representative in the low zone and valid comparisons for this species between high and low areas cannot be made.

Of course, there are a number of differences between high and low intertidal areas that probably contribute to greater frequencies of, and longer distances covered in, feeding excursions and that might effect more frequent relocations of high intertidal Nuttallina. Such proposed differences in movement characteristics between high and low chitons could also explain the observed trends in homing frequencies between chitons in these areas. This possibility will be explored in detail in the next section.

BASIS OF HOMING BEHAVIOR IN NUTTALLINA FLUXA AND N. KATA

The frequency of "homing" in Nuttallina fluxa and N. kata is significantly different (Figures V-7, V-14; Tables V-6, V-8, V-13). Is there an intrinsic behavioral difference between Nuttallina fluxa and N. kata with regard to the tendency to home? An examination of the homing proportions of Nuttallina fluxa and N. kata co-occurring in any habitat should help to resolve what part of the differences in homing tendencies of these two species is merely dictated by differences in the habitats in which they occur. However, the power of any such comparisons is minimal because so few specimens of the two species co-occur in any habitat (Table V-18 and Chapter III). Of course, the fact that the two species occur primarily in different habitats might itself be a consequence of a behavioral difference at some life stage of these species, although it also could result from other factors, such as differential recruitment or mortality; this will be discussed later. Nevertheless, comparison of the "homing" proportion of Nuttallina fluxa with that of N. kata using specimens unequivocally-assignable to any particular habitat gives the same result for all habitats. Nuttallina kata tends to "home" more than N. fluxa in all habitats, although the difference in homing proportions is never statistically significant. Thus, in the low intertidal densely-populated habitat, data for only one specimen of N. fluxa (homing for one of six 30 day intervals, of 17%) is available for comparison with that for fifty-two specimens of *N. kata* (homing an average of 72% of intervals). In high intertidal pools, only 3 specimens of *N. kata* (homing 35%) are available for comparison with fourteen specimens of *N. fluxa* (homing 34%). Finally, if *N. fluxa* and *N. kata* occurring in high intertidal sparsely-populated non-tidepool areas are compared, even though *N. fluxa* generally occupies more exposed areas there than does *N. kata* (as indicated by results in Chapter III), *N. kata* is again seen to show a greater tendency to "home" (60%, N=10,) than *N. fluxa* (38%, N=36), although the difference again is not significant (Table V-18). It would appear that there is a small intrinsic tendency for *N. kata* to "home" more than *N. fluxa* in any given habitat.

How might differences in the habitats magnify differences in intrinsic homing tendencies of the two *Nuttallina* species? There are several obvious differences between the high intertidal area, where *Nuttallina fluxa* primarily occurs, and the low intertidal zone, where *N. kata* is primarily found. The upper intertidal area consists of large areas of almost bare rock, that support thin algal films and encrusting algae. Form-fitting chiton depressions are occasionally found in the high zone, but high intertidal chiton homesites at the La Jolla study site more often are less well-defined chiton hollows or spaces near mussels, barnacles and other areas of geologic relief. In contrast, the low intertidal zone always supported a large biomass of both encrusting and foliose algal species and almost all chitons were found in clearly-delimited, relatively deep, oblong depressions.

These habitat differences could contribute to the observed differences in "homing" of *N. fluxa* and *N. kata* in several ways. It has been observed in studies of intertidal chitons and limpets that the animals tend to traverse greater distances during feeding excursions where the standing algal biomass is lower (e.g., Thorne, 1967; Stimson, 1973), presumably to ingest quantities of food equal to those ingested in areas of greater algal biomass. In addition, the lush algal growth in the low intertidal area may have a purely mechanical effect, hampering movements of chitons. The foliose coralline algae that make up a large part of the low intertidal algal turf surround most of the chiton depressions in the low intertidal area. Chitons inhabiting the area must usually move on top of this algal assemblage in order to travel anywhere. It is quite probable that chitons are not able to attach as firmly on such an algal mat as they can on solid substrates such as the barer high intertidal rocks. Since the animals move only at high tide, when the rocks are subject to wave shock or surge, it is also probable that chitons move less effectively over the low intertidal algal turf. Thus, they might not move as far over low intertidal turf as they might over high intertidal substrates. If feeding excursion distances are shorter in areas of great algal biomass for the foregoing reasons, chitons in the low intertidal area at the La Jolla study site might be expected to home more frequently than chitons in the higher intertidal since shorter feeding excursions are expected to be associated with higher probabilities of homing success.

Only a few high tide feeding excursions of <u>Nuttallina fluxa</u> have been directly observed in their entirety, and no such excursions have been observed for <u>N. kata</u> (Table V-4); thus, direct comparisons of the lengths of feeding excursions of the two species cannot be made.

However, observations of locations of specimens of both N. fluxa and N. kata made at 24 hour intervals can be used as indirect indications of the distances normally involved in feeding excursions (Tables V-4 and V-5). The maximum displacement observed for a specimen of Nuttallina fluxa in the high intertidal zone at La Jolla was 27 cm, from one low tide position to the next, 24 hours later (for N=20 N. fluxa, 39 24-hour intervals), with another 5 N. fluxa (7 24-hr. intervals) exhibiting displacements of 5 cm or more. The maximum displacement observed for N. kata in the low intertidal area was 5 cm (for N=12 N. kata, 23 24-hr. intervals), with only two other N. kata showing any displacement at all (2 and 3 cm, for 2 intervals only). If maximum twenty-four hour displacements reflect the relative magnitudes of intervening high tide feeding excursions, it would appear that N. fluxa in high intertidal areas travel further during high tide feeding excursions than do N. kata in low intertidal areas. A difference in distances covered in feeding excursions resulting from habitat differences might then contribute to the difference in "homing" fidelity observed for N. fluxa and N. kata in this study.

Differences in frequencies of leaving the homesite could help account for differences in homing fidelity observed for Nuttallina fluxa and N. kata. During this study, chitons were not routinely observed at high tide, the period when feeding excursions take place, but direct and indirect evidence presented earlier (Tables V-4, V-5) suggested that substantial numbers of Nuttallina specimens do leave their homesites on each high tide. Chitons found at the same location for observations separated by several days have been classified as "homers" since it was considered likely that they left and returned to their homesites on at least one intervening high tide. For example, it is probable that different chitons, that have been found to home over a fifteen day observation interval, have left and returned to their homesites different numbers of times. Thus, in lieu of or in addition to making shorter feeding excursions, low intertidal chitons, in the presence of an abundant algal food supply, might simply venture away from the homesite to feed less often than high intertidal chitons.

Variations in the number of feeding excursions undertaken per time period by different individuals have been noted for at least one intertidal homing chiton species (*Mopalia muscosa*, 4 to 14 times in 14 nights; Smith, 1975) and in one homing limpet genus (e.g., *Patella*, 0 to 8 times in 14 days; Cook et al., 1969). Certainly, Kues (1969) has found that the guts of high intertidal *Nuttallina* at the La Jolla study site contain about 90% sand grains, by volume, presumably ingested in the process of rasping thin algal films, crusts and diatoms off of the soft sandstone substrate, whereas gut contents of the lower intertidal *Nuttallina* consist of more organic material, up to 50% or more by volume in some cases (but see Vesco, 1980 for Whites Point *Nuttallina*). Although the caloric value of *Corallina*, the plant Kues (1969) found most abundantly in the guts of low intertidal *Nuttallina*, is relatively low (Paine and Vadas, 1969), the dietary intake of high intertidal *Nuttallina* is probably of even lower caloric value since it includes such large percentages of inorganic material. Thus, if *N. fluxa* ingests the same, or a lesser, amount of material as *N. kata* on each feeding excursion, and if the caloric requirements of the two are similar, *Nuttallina* in the lower intertidal zone, primarily N. kata, might be expected to undertake feeding excursions less frequently than Nuttallina in the high intertidal area, primarily N. fluxa.

Another factor that may contribute significantly to the greater observed homing fidelity of the low intertidal Nuttallina kata is the fact that the homesites in the low intertidal are generally much more well-defined than those in the upper intertidal areas. Homesites in the low intertidal zone at the La Jolla site are almost all deep depressions and a chiton coming to rest a centimeter or two away from the depression at the end of a feeding excursion would usually be resting in a vulnerable position, on top of the algal turf. In the high intertidal zone, on the other hand, homesites often are not delimited precisely; a chiton coming to rest a few centimeters from its homesite, e.g., along the base of a mussel bed, often would be in a comparably suitable spot, e.g., still at the base of the mussel bed. Interestingly, there may be a feedback mechanism working between homing reliability and the delimitation of homesites; it is possible that the depressions inhabited by low intertidal N. kata are generally much more well-defined and deeper than most of those of high intertidal N. fluxa as a result of more time spent on homesites in the lower intertidal (the chitons themselves contribute to the production of the depressions, see Chapter II), because of fewer and/or shorter feeding excursions and high homing fidelity. However, many other factors such as differential erosion or long-term (historical) occupancy might be responsible for this phenomenon (see Chapter II).

OBSERVED DISPLACEMENTS OF NUTTALLINA

Although homing (or staying in place) is a conspicuous element in the behavior of Nuttallina, this chiton is not locked into an unvarying homing pattern as evidenced by the substantial number of displacements observed during this study. The majority of specimens in both Nuttallina species exhibited one or more displacements during the study, homing for varying periods of time (Table V-6; Figure V-3). Although some individuals of both species never showed any displacement and a few others were never found in the same location for any two successive observations during the study (Table V-6; Figure V-3), discrete categories of homers and nonhomers, or movers, were not evident for either N. fluxa or N. kata. Individuals of both species exhibited the full range of homing percentages, from no homing to 100% homing, with no obvious indication of a bimodality in behavior (Figure V-3).

The primary goal of this study was to provide an idea of the magnitude of displacement from low tide resting location to location that could be expected for Nuttallina. In particular, the displacement to be expected over a period of two weeks to about one month, a reasonable estimate of the amount of time required for acclimatization of animals (e.g., Hochachka and Somero, 1973), was of interest in relation to an ongoing study of the physiology of Nuttallina in different intertidal microhabitats. It was reasoned that, although Nuttallina might traverse relatively great distances over the intertidal zone to feed during high tide, the fact that all areas are bathed in seawater at this time means that the chitons will experience the same conditions, especially of temperature and "humidity", regardless of microhabitat.

However, different intertidal microhabitats are subject to different conditions during exposure at low tide (see Chapters I and II), and it is of interest whether Nuttallina can be expected to remain in the same microhabitat on successive low tides long enough to fully acclimatize to conditions at the spots in which they are finally collected.

Results from this study indicate quite clearly that a specimen of Nuttallina can generally be expected to be acclimatized to the microhabitat in which it is collected, although this microhabitat can be much more closely-defined for Nuttallina kata than for Nuttallina fluxa. Over intervals of fifteen days, 95% of the Nuttallina fluxa in this study exhibited greatest point-to-point displacements of 30 cm or less, while 95% of Nuttallina kata stayed within a 5 cm radius (Table V-14). During intervals of thirty days, 95% of specimens of Nuttallina fluxa stayed within a 38 cm radius of the starting location, while 95% of N. kata took up low tide positions no farther than 7.8 cm from starting positions. Unidentifiable Nuttallina specimens showed displacements intermediate between those of N. fluxa and N. kata, as expected for a group that likely consists of members of both of these species (see Table V-14, but also see Tables V-13 to V-17, Figure V-14, Tables V-8 to V-12, and Figures V-7 to V-13). Since displacement magnitude frequencies took the general form of negative exponential distributions (see Figures V-8 to V-10), median displacements were considerably smaller than 95 percentile displacements. For example, the median displacement for Nuttallina fluxa for both 15 and 30 day intervals was only about 2 cm as compared to 95 percentile displacements for these interval lengths of 30 and 38 cm (Table V-14, and see Figure V-8). Where sample sizes permitted, separate statistical tests revealed no significant differences in point-to-point displacement distributions compared by habitat, size or season in either species (Tables V-18 to V-21).

During this study, only two chitons, both specimens of *Nuttallina fluxa*, exhibited displacements to new habitats (broadly defined, e.g., tidepools, high intertidal dry sparsely-populated areas, low intertidal densely-populated algal turf area). Interestingly, both specimens moved from submerged positions in the same large high intertidal pool to exposed areas at its edge. One of these chitons had been found consistently in a single tidepool homesite for about 6 months (from November, 1979 to 16 May, 1980); it then made a series of moves over the next two months, until it came to rest at the new exposed spot, on 14 July, 1980, 45 cm from the original site, where it remained in an area about 5 cm in diameter for the final four months of the study (14 July to 9 November, 1980). Similarly, the other specimen remained in a tidepool area about 4 cm in diameter for four and a half months (5 November, 1979 to 26 March, 1980), and then moved to a new exposed spot 59 cm away by 31 May, 1980 to which it homed until it disappeared finally on 14 July, 1980.

One other specimen of *Nuttallina fluxa* did not switch general habitats, but did move from a location near the mid-intertidal boundary between the low densely-populated chiton zone and the upper intertidal sparsely-populated habitat to a point well up into the sparsely-populated high shore area. During the 429 day period, this chiton was observed, it moved toward and away from the boundary several times before it finally headed in a general upward-longshore direction farther into the upper intertidal sparse area (see Figure V-4 for track of this specimen, tagged #35). Finally, one small specimen of Nuttallina kata (~20 mm long, tagged #31, see Figure V-5) moved about 11 cm (point-to-point) up to the mid-intertidal dense/sparse boundary around 26 March 1980, after having homed to a lower densely-populated intertidal area for more than 6 months; it returned to lower areas by 15 April and remained in the low zone for the remainder of the study. Judging from the small incidence of Nuttallina (2 of 160) observed to change general habitat types during this fourteen month study, it is clear that one can be quite confident that particular chitons (at least those ≥15-20 mm in length) have acclimatized fully (over 2 to 4 weeks at least) to the particular habitat in which they are found.

Specimens of both Nuttallina species occasionally moved to new homesites after having homed to a site often for a considerable period of time. The changes in homesite were seemingly unpredictable over the short term, much like the process of radioactive decay. However, there might be a number of reasons for the changes in homesites. These reasons fall in three major categories: 1) the old spot might become unsuitable or unavailable, 2) another site might become more attractive than the old site, or 3) something might occur during a feeding excursion to interrupt normal return to the homesite. Evidence recorded in field notes during this study and observations of other intertidal workers can be used to explore the likelihood of the various reasons for homesite changes.

Changes in topography may result in chiton displacements, either away from sites made unsuitable or to sites that offer more protection.

For example, in February 1980 during this study, the topography of parts of the upper and middle regions of the coarse sandstone study site changed quite noticeably over a 10 day period between searches for tagged chitons. Several existing depressions were widened considerably, some high relief areas were broken up, a couple of the high intertidal pools were not as deep as before, and the roof of a small 3 inch diameter "cave" in a high pool was broken, leaving the cave and the chiton inhabiting it half-exposed. These changes probably occurred primarily as a result of the impact of large rocks thrown up by heavy surf; numbers of Stenoplax conspicua, a chiton species that occurs almost exclusively on the bottoms of rocks in sandy areas, were found exposed and injured in nearby beach pools at this time. Later, in March 1980, several small mussel patches disappeared from a high intertidal pool and from a mid-intertidal area. Also in March, many young Mytilus established themselves in the low-mid-intertidal area and, in August, much new growth of Phragmatopoma was evident in the low shore areas, likely making new protected spots available to chitons. Direct effects of most of these topography changes usually could not be documented, since tagged chitons were not often in the affected areas. However, of two tagged chitons in such areas, one Nuttallina kata in the damaged high tidepool cave, and one N. fluxa in a high tidepool at the base of a patch of mussels that was torn away, neither moved away immediately. The impact of such topographic changes is probably not equally critical at all times (both tagged chitons subsequently moved away from the impacted sites). Topographic changes undoubtedly play a role in effecting chiton displacements.

Shifting sand levels were quite obvious in areas surrounding the study site, as mentioned earlier, and small influxes of sand into the study site were sporadically noted at times of high surf in all seasons. Sand inundation was most significant in quadrats of the lowest shore areas, where the deep depressions held quantities of sand that occasionally entirely buried chitons there. Such influxes might be expected to induce the affected chitons to seek more suitable resting spots as has been noted for Chiton stokesii on rocky shores of Costa Rica (Schmidt-Effing, 1980). In fact, Louda's (1972) censuses of Nuttallina fluxa on a rocky Santa Barbara, CA reef suggest that some movements of this chiton were tied to long-term changing levels of sand. As indicated by decreasing numbers of chitons above a certain shore level in a stable population, chitons in her study apparently moved down rock surfaces as the sand level dropped over a 1 month period.

During the present study, four tagged specimens of Nuttallina kata were of special interest. The four were found completely buried by sand in low zone depressions. One of the specimens was found consistently in the same depression for the remainder of the study, even though there was still a considerable amount of sand there for two of the 4 weeks. The other three specimens were not found again after they had been buried. For one of these three specimens, the depression was empty on the next search and the chiton was not found anywhere, suggesting that the chiton had died. For the other two cases, it is unclear whether the chiton had died or the tag had been lost due to the abrasive action of the sand. It is possible that Nuttallina kata suspends high tide feeding excursions during periods of sand inundation to avoid the increased scouring action of the sand. In any case, in the low area, none of the depressions within several centimeters of a chiton are likely to be more suitable than its own, due to the presence of sand in most depressions. Sand inundation was never permanent in the study site, but rather affected low areas for only a few days to a couple of weeks, and a strategy of remaining in place and avoiding sand scouring might normally be appropriate.

Temporary sand inundation probably did little to cause immediate chiton displacements in this study and, in fact, as a result of accompanying scouring action, probably had the opposite effect on low intertidal *Nuttallina*. Louda's (1972) findings, which suggest that chiton movements accompany shifting levels of sand, are different due possibly to two factors. First, Louda's (1972) study involved not *Nuttallina kata*, but *N. fluxa*, which in this study has been found to be more likely to move than *N. kata*. Second, sand levels were dropping rather than rising in her study, so that sand scouring was probably decreasing and chitons might have been more likely to move.

It is quite possible that sand inundation sets the lower limit of distribution at the La Jolla study site. The adjoining area, below the lowest study quadrats, supports a fairly luxuriant algal turf and is pockmarked with many empty chiton depressions but, being a horizontal shelf, is persistently affected by influxes of sand.

A specimen of *Pisaster* sp. appeared in the middle area of the study site during this study. *Nuttallina*, particularly high intertidal *N. fluxa*, might move away from such a slow-moving predator as do many other molluscs (see Branch, 1981, pp. 323-325, for several intertidal limpet examples), although no such escape response has been reported in any of the studies of seastar predation on chitons (selected references cited earlier). It is not clear whether the *Pisaster* specimen directly caused displacements of *Nuttallina* in this study; one tagged chiton with a homesite at an observed position of the seastar disappeared (was eaten?) during the short 2 or 3 week stay of the *Pisaster* in the study site.

Nuttallina specimens inhabiting form-fitting depressions, primarily in the low intertidal area, might be expected to move from small home depressions to larger ones as they grow, in the way that hermit crabs switch shells. However, this would require that the chitons grow faster than the depressions can be enlarged, and the animals are capable of considerable body contraction as well as elongation, so that any displacements induced directly by growth are probably rare.

At several different times during the study, new patches of algae, e.g., *Ulva* in September and December, 1979 and May, 1980 and various unidentified brown algae at other times, became established, substantially increasing the standing algal biomass particularly in localized mid and upper intertidal areas. Such relatively dense stands of algae might cause *Nuttallina* to establish new homesites nearby. Such induced displacements were not specifically noted during the study, but observations made in early 1983 indicate that such events occur. As a result of extremely high surf during early 1983, several large sections of mussel beds and substantial chunks of substrate were torn away from mid-intertidal areas in and near the study site. By March, 1983, very conspicuous patches of rich green Ulva, typically an opportunistic colonizer (Littler, 1980), had developed in the newly-exposed sites. Close examination of these areas revealed that several Nuttallina had moved well into these areas, presumably drawn by the luxuriant growth of algal food and/or the new availability of unexploited space. Depletion of food in an area, such as might be produced by abnormally harsh desiccating conditions during periods of prolonged exposure (e.g., Seapy and Littler, 1982) or possibly by periodic sand scouring in the barer high intertidal areas, might have the opposite effect, inducing Nuttallina to move away, as was apparently observed for example by MacKay and Underwood (1977) for the intertidal limpet Cellana tramoserica.

The finding that experimentally-vacated depressions are taken up by new chitons quite frequently, more often in the densely-populated low intertidal zone than in the high shore area (Table V-5), suggests that displacements of Nuttallina might occur in natural situations when homesites are left empty as a result of the death of the occupants. A chiton might move into a vacated spot because the new site is more suitable than its old homesite and/or because it is easier to take up the new site than it is to make the return journey to its old home. If natural mortality rates are anywhere near the rate of tag losses (which include losses of tags from healthy chitons and deaths of tagged chitons) observed in this study (over 50% in 6 months; see Table V-3), then the number of Nuttallina displacements directly related to spontaneous chiton deaths could be quite substantial.

Disturbances or other eventualities during feeding excursions of Nuttallina might cause the chitons to come to rest occasionally in spots other than their own homesites. Chitons probably normally use new or old chemical trails that they lay down in leaving the homesite to return during feeding excursions (Thorne, 1968). In the relatively dense *Nuttallina* populations, feeding trails of different individuals might be expected to cross quite often, unless territorial behavior (speculated about earlier) prevents such an occurrence. On encountering other trails, feeding *Nuttallina* individuals might be expected to follow trails of other individuals to the wrong homesite on occasion, unless the trails are individualized in some way. However, in the only field investigation into the mechanisms of homing in chitons, Thorne (1968) found that when individuals of the intertidal chiton *Acanthozostera gemmata* were placed on the trails of other individuals, in four trials they never followed the "foreign" trail, even though individuals of this species were observed to exchange homesites spontaneously on other occasions in his study.

Physical disturbances might occasionally force *Nuttallina* to come to rest short of their homesites, at new sites to which they start homing. No direct evidence for such an event was obtained in this study since *Nuttallina* could not be observed routinely during their high tide feeding excursions. In her remarkable study, Smith (1975) observed an instance where a homing individual of the chiton species *Mopalia muscosa* was prevented from returning home by a rock washed onto the site. The chiton settled a short distance away, and homed to the new site for the next 15 days that it was observed. Algal debris that is often washed into the La Jolla study site might have a similar effect.

Interestingly, Westersund (1975) obtained some evidence, though not conclusive, for endogenous tidal rhythms of movement in specimens of *Mopalia muscosa* held in constant darkness in laboratory aquaria. Such rhythms might enable intertidal molluscs to "anticipate" the time of low tide so that they can return home before being exposed as has been suggested for example for the pulmonate limpet *Siphonaria normalis* which moves only when awash, and moves further and for longer periods during neap tides (long period awash) than during spring tides (shorter period awash) (Cook and Cook, 1978). Thorne (1967) suggested that the chiton *Acanthozostera gemmata* might have a "biological clock" with both a circadian and tidal periodicity that enables it to return to its homesite before dawn or an incoming tide. Dewar (1963) concluded that *Ischnochiton maorianus* exhibits an endogenous rhythm of activity (more active at night and at high tide). If shifting winds and/or a rapidly increasing barometric pressure were to cut short the "anticipated" period of submersion, *Nuttallina*, particularly in rapidly draining areas, might be required to terminate feeding excursions prematurely, coming to rest at sites short of their own homesites.

Additionally, intra- and interspecific encounters might disrupt feeding excursions of *Nuttallina*. Nineteen cases in which pairs of *Nuttallina* were observed in contact (often head-to-?) and two instances in which *Nuttallina* and the large territorial limpet *Lottia* were observed in head-to-head contact while exposed at low tide during this study have been mentioned (see Table V-26). The possibility that these occurrences represent suspended aggressive encounters, initiated while the animals were submerged, was mentioned earlier. Six of these *Nuttallina*-

Nuttallina and *Nuttallina*-*Lottia* encounters involved tagged chitons so that the locations of one of the encounter participants before and after the observed contacts are known. In five of the cases, the tagged *Nuttallina* was in contact with another mollusc at a site 3 to 41 cm away from its usual low tide resting site (see Table V-26). On observation dates immediately after the date of contact, in two of the five cases, the chiton worked its way back to the original homesite within four to six weeks. In another one of the five cases, the *Nuttallina* disappeared. In the fourth of the five cases, after contact with *Lottia* 27 cm from an initial site, one tagged *Nuttallina kata* moved 37 cm back to a point 18 cm from the intial site before it disappeared. Finally, one large *N. fluxa* was observed in contact with a *Nuttallina* 3 cm from its homesite one day, then at its homesite in head-to-head contact with a large *Nuttallina* (the same?) the following day, after which it was observed 2 to 3 cm from, but not at, the homesite.

In only one case of the six observed encounters of tagged *Nuttallina*, a specimen of *N. kata* was on its homesite, in head-to-head contact with a slightly larger *Nuttallina* specimen. The specimen of *N. kata* was found at its homesite, a well-defined depression in a high tidepool, for all observations subsequent to the observed contact.

The fact that 5 of the 6 observed encounters of tagged *Nuttallina* occurred away from the homesite, and that the tagged chiton moved back to or towards the homesite in all four cases where the chiton did not disappear is consistent with the idea that such encounters, initiated during high tide feeding excursions, occasionally interfere with returns of chitons to their homesites.

All of these encounters were observed in mid- and high intertidal sparsely-populated areas and might help account for the greater frequency of displacements observed in these areas compared with those observed in the densely-populated low tide area. Although more encounters might be expected in the densely-populated low intertidal area, the disruptive effect of such contacts might be ameliorated by "ritualization" of aggressive behavior and/or the frequency of such encounters of low shore individuals might be reduced if individuals "rotate" in place, feeding on algae fringing the home depression, as suggested earlier. It is also possible that chiton contacts are less conspicuous in the densely-populated low intertidal area than in the sparser higher areas, because the chitons are more closely-spaced in the former areas, and contacts were simply taken note of.

Two other cases were noted in which the homesite of a tagged *Nuttallina* was occupied by a new chiton and the tagged chiton was found elsewhere. In one of the cases, in quadrat 3 (described in Table V-1), a 24 mm long *N. fluxa* had homed to a single site for 5 weeks, but was found 11 cm away from this site on a subsequent observation and a new chiton had taken up its homesite. In the other case, a small "cave" in a high tidepool was occupied by a 24 mm long *Nuttallina kata*, which was replaced by a larger *Nuttallina* specimens on 31 May 1980, then by a tagged *N. fluxa* on 17 June, and finally by a 30 mm long *Nuttallina* on 29 July 1980. Such occurrences might represent cases where an intruding chiton had actively usurped another's homesite while the latter was out on a feeding foray, although the "intruder" might have simply wandered into the site when the homesite occupant did not return as usual for some reason. If the first alternative is the explanation for the change in homesite occupants, such events could account for some of the displacements observed during this study.

In the next section, the possibility that displacements in this study occur predominantly in a particular direction, i.e., that a migration occurs, is explored.

BASIS OF THE SHORE-LEVEL SIZE GRADIENT IN *NUTTALLINA*

Nuttallina exhibits a general shore-level size distribution such that specimens higher on the shore, particularly in exposed areas, tend to be larger than those lower in the intertidal zone, at La Jolla and at other sites (see Chapters II and III; Kues, 1969; Ituarte, 1981). This is not always the case, for example, where the shore topography is quite irregular and zonation is not well-developed (e.g., Dike Rock, La Jolla: Kues, 1969) and where *N. fluxa* is absent (e.g., Catalina Island: see Chapter III). Similar shore-level size gradients have been observed for other species of intertidal chitons, *Chiton tuberculatus* at Bermuda (Arey and Crozier, 1919, but see Glynn, 1970 for *C. tuberculatus* in Puerto Rico), *Sypharochiton pelliserpentis* (Boyle, 1970, but microhabitat differences often masked shore level differences), and *Mopalia muscosa* (Fitzgerald, 1975). Interestingly, Taki (1938) reported finding small specimens of several Japanese intertidal/subtidal chiton species by dredging at depths and only rarely in the intertidal zone, whereas larger specimens were commonly found in the intertidal area. Similarly, Berry (1917) noted finding small specimens of *Tonicella lineata* in dredges, but not on shore where the larger specimens were found (also see Demopulos, 1975).

The basis for observed shore-level size gradients has not been studied in chitons in any detail. Boyle (1970) found a negative correlation between rate of water loss and size in Sypharochiton pelliserpentis and suggested that the observed difference in chiton size distributions between wet and exposed surfaces was a consequence of the physical differences between the micro-habitats, such as in degrees of exposure to desiccation. He suggested that as specimens of Sypharochiton pelliserpentis grow, they move from water-filled crevice habitats to more exposed drained surfaces, where, as adults, most of the chitons take up elongate depressions to which they home. However, Boyle (1970) possessed no direct evidence to support this contention. Crozier and Arey (1918) noted a general increase in size of Chiton tuberculatus primarily from dark under-rock positions to positions fully-exposed to sunlight, with a general trend toward larger chitons up the shore. As a result of findings that small chitons, with uneroded, intact shell surfaces and light-sensing aesthetes, were negatively phototactic, while larger, much-eroded chitons were positively phototactic, these workers proposed that the observed size distribution resulted from movement of individuals to more exposed areas as their valves and aesthetes were progressively eroded away (and the chitons grew larger). Again, no long-term movement data were obtained to directly substantiate this suggestion, although Crozier and Arey (1918) did find that small specimens exhibited considerable movement, whereas larger specimens tended to remain in more restricted areas. Taki (1938) proposed that chitons gradually migrated up into the intertidal zone with increasing age to explain the large size of specimens on the shore relative to the size of those at depths, for several Japanese intertidal subtidal species; he obtained no direct evidence to support this hypothesis. Finally, Ituarte (1981) claimed without direct evidence, that small "Nuttallina californica" (N. californica or N. kata of this study) migrated to high shore areas (probably Nuttallina californica), to account for the shore-level size differences he found at a site in Santa Cruz Co., CA.

A general trend of increasing specimen size with shore height has been reported for many intertidal species of molluscs other than chitons, particularly for those species occurring in the high intertidal zone, although other mollusc species exhibit no shore-level size gradient, while still others, particularly those with low intertidal ranges, show a general decrease in specimen size with increasing intertidal height (e.g., Vermeij, 1972; Branch, 1981, p. 240). Theoretically, shore-level size gradients can result from differential growth at different intertidal heights, differential mortality and/or, in mobile species, migration. Population dynamics studies, particularly on the well-studied limpets, suggest that in different species the observed shore-level size gradients have different primary bases (see Vermeij, 1972; Underwood, 1979; Branch, 1981 for examples).

Of particular interest with respect to the present study, increasing size with intertidal height in several limpet species has been shown, with varying degrees of rigor, to result from upshore migrations with increasing age and size. In Oregon, Frank (1965) observed a net upward movement of Collisella (= Acmaea) digitalis in Fall and Winter as storm surf wet higher rock levels, and a smaller net descent in Spring as upper shore areas dried, such that the oldest and largest animals were found highest in the intertidal zone. Branch (1975) obtained weaker evidence for similar progressive upshore migrations of several South African Patella species and suggested that these limpets are limited in their upward excursions only by their tolerances to physical stresses such as desiccation. Since larger specimens generally tolerate desiccation and high temperatures better than small ones (e.g., Davies, 1969; Boyle, 1970; Wolcott, 1973), physical restraints on upward movements might lead to the observed differential zonation with respect to size.

In his study, Branch (1975) proposed that the principal advantage of upward migration is that a species can occupy a wider range over which the biomass can be spread, and hence it has more availble resources. In other words, by migrating upshore, animals in his study avoid intense competition for food and/or space in the densely-populated lower intertidal zone. Certainly, various workers have shown that patterns of movement can be modified by increased densities of intertidal limpets and by reduced stands of algal food (e.g., Frank, 1965; Mackay and Underwood, 1977).

Another obvious potential advantage of upshore migrations that might pertain in selected instances is the avoidance of aquatic predators that can forage longer or more effectively in lower shore areas than in high ones as a result of the tidal regime. It is quite possible that those factors that drive general upshore migrations are the same as those that tend to set the lower limits of the intertidal populations, most often considered to be competition and predation (e.g., Connell, 1972, 1975), whereas the factors that control and retard the upward migrations are those that also set the upper limits of the distributions of the populations, often physical factors (e.g., Wolcott, 1973).

Examination of Movement Data for Evidence of Migration Between Shore Levels: Prior to Electrophoretic Study of *Nuttallina*

At the La Jolla study site, *Nuttallina* exhibits a shore-level size gradient (with larger specimens upshore), high densities in the low intertidal areas, and low densities on the high shore. The apparent similarity of this population structure to that of the well-studied intertidal limpets that undertake migrations prompted a preliminary examination of the *Nuttallina* movement data for indications of either seasonal or constant migration upshore.

Subsequently, electrophoretic study revealed that the *Nuttallina* populations at the La Jolla site primarily consist of two separate species, *N. fluxa* (Carpenter) and *N. kata* sp. nov. In the present section, results of the preliminary examination of movement data are discussed as if the *Nuttallina* populations consist of only one species, as was originally thought. However, the new species designations are used for clarity. In the following section, the new two species scheme is incorporated in the examination to take a more refined look at the population dynamics of *Nuttallina*. This method of presentation serves to show how the movement study prompted the taxonomic study of *Nuttallina* and how the results of the taxonomic study in turn allowed a more refined interpretation of the results of the movement study.

First, as discussed in earlier sections, Nuttallina exhibits a relatively rigid homing behavior regardless of species or habitat (Tables V-6, V-8, V-13, V-18; Figures V-7, V-14). This rigid homing behavior is best-developed in chitons of the low shore, animals in which any tendency to migrate upshore should be most evident (Tables V-18, V-19). Over 30 day intervals at all times of the year, 72% of the N. kata on the low shore exhibited no displacement, and 95% moved less than 7.3 cm (Table V-18, V-19). Even over 4 month periods, nearly 60% of N. kata from all shore positions, including the more mobile high shore specimens, had not shown any displacement whatever (Table V-8). These homing data did not exhibit any significant seasonal or size-related trends (see Tables V-20, V-21).

Second, analysis of displacements generally revealed no significant tendency for Nuttallina of either species, of any size or in any habitat or season, to exhibit displacements in any particular direction (see Figures V-11, V-12, V-13, V-15, V-16, V-17, and Tables V-12, V-17, V-18, and V-20 through V-24 for data and tests). Analyses for directionality included comparisons of upward versus downward displacement frequencies, estimated vertical displacement distributions and actual point-to-point displacement distributions, and statistical tests of circular (magnetic direction) frequencies (by Rayleigh and Kuiper goodness-of-fit circular statistics), and when feasible, point-to-point displacements in different compass sectors.

Nuttallina specimens occurring low on the shore were of particular interest since they were the most likely to exhibit upshore movements (if there were an upshore migration). Statistical tests, including those comparing positions of these chitons at the beginning and end of the study, revealed no tendency for low-shore Nuttallina to move upshore (Tables V-7, V-19). Circular frequency distributions serve to illustrate the random pattern of displacements of low-shore Nuttallina specimens with respect to compass direction (Figures V-18, V-19, V-20).

Rather than exhibiting any tendency to move upshore, Nuttallina most often showed slight, though nonsignificant, tendencies toward downshore (generally southward) movement, as indicated by upward/downward frequencies and calculated mean compass headings (e.g., Tables V-12, V-17, V-22, V-23, V-24). In only one case was a significant result obtained; for all specimens of Nuttallina kata combined (120 day intervals), the tendency to move south (generally downshore) was significant by Kuiper's circular goodness-of-fit test ($p<0.05$, Table V-23). However, the significance of this result was not corroborated by the Rayleigh statistic nor by statistical comparison of upward and downward displacement frequencies (Tables V-23).

Although it is quite clear statistically that there is not a massive movement of the low shore population to the high shore, it is still quite possible that some individuals from the low shore contribute to the high shore population. At the La Jolla site, the density of chitons in the low intertidal area is quite high, up to 1400 to 2000 Nuttallina per square meter, while that in the high intertidal zone is less than 200 per square meter (Chapter II). Thus, upshore movement of only a small proportion of the low intertidal Nuttallina population could sustain high shore numbers of Nuttallina, provided that mortality rates

Figure V-18. Circular frequency distribution of observed displacements of low shore specimens of <u>Nuttallina fluxa</u> at the Seal Rock study site, La Jolla, CA for the period 9 Sept 1979 to 11 Nov 1980. Only specimens occurring below +110 cm above MLLW (in Quadrats 3, 4, 7, 8, 9 of Table V-1) are included. Displacements were estimated by the beginning-to-end method for 30-day long composite observation intervals. See Figure V-15 caption for further explanation.

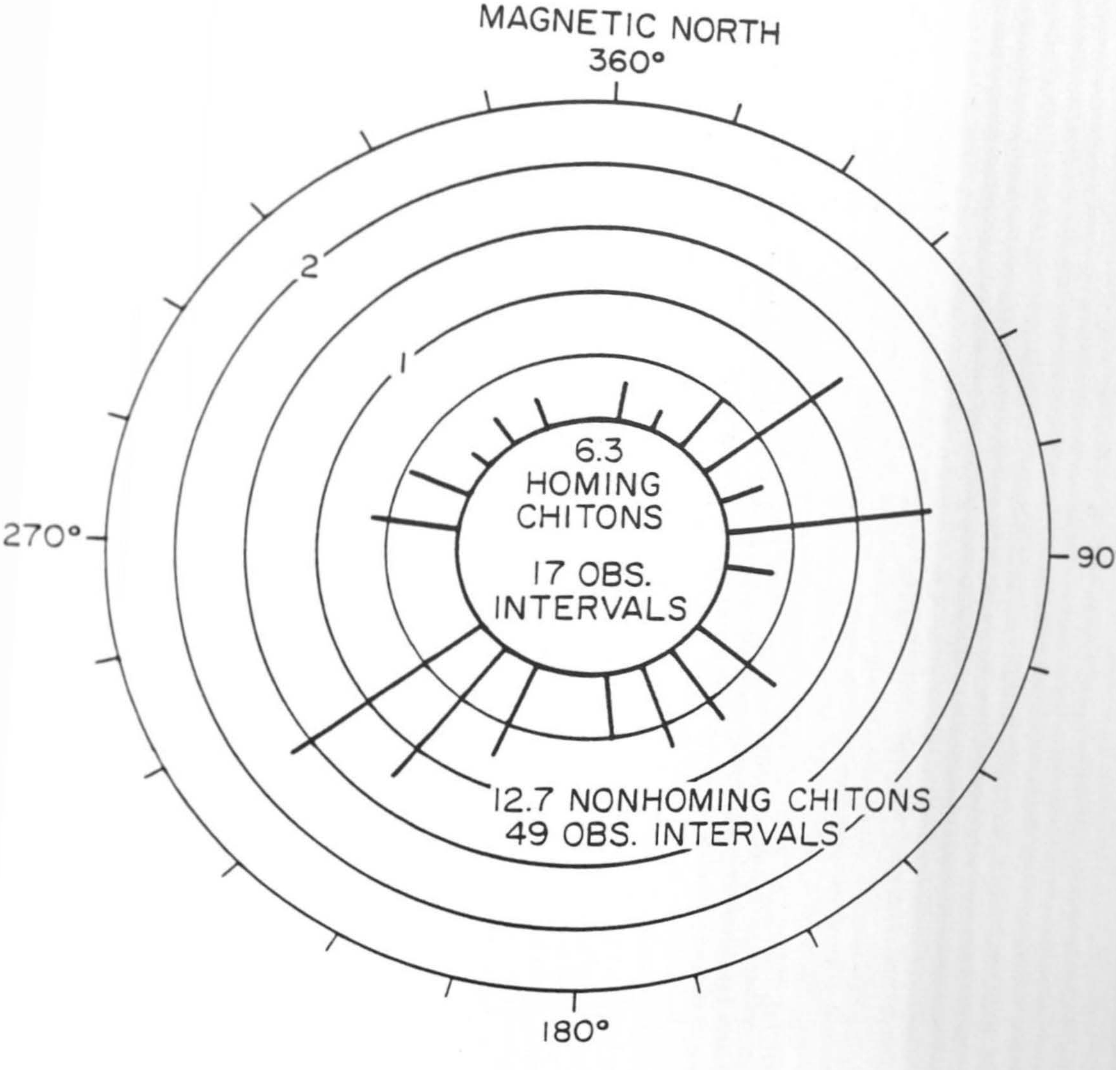

MAGNETIC NORTH
360°
270°
90°
180°
2
1
6.3
HOMING
CHITONS
17 OBS.
INTERVALS
12.7 NONHOMING CHITONS
49 OBS. INTERVALS

Figure V-19. Circular frequency distribution of observed displacements of low shore specimens of <u>Nuttallina kata</u> at the Seal Rock study site, La Jolla, CA for the period 9 Sept 1979 to 11 Nov 1980. Only specimens occurring below +80 cm above MLLW are included. See Figure V-15 caption for further explanation.

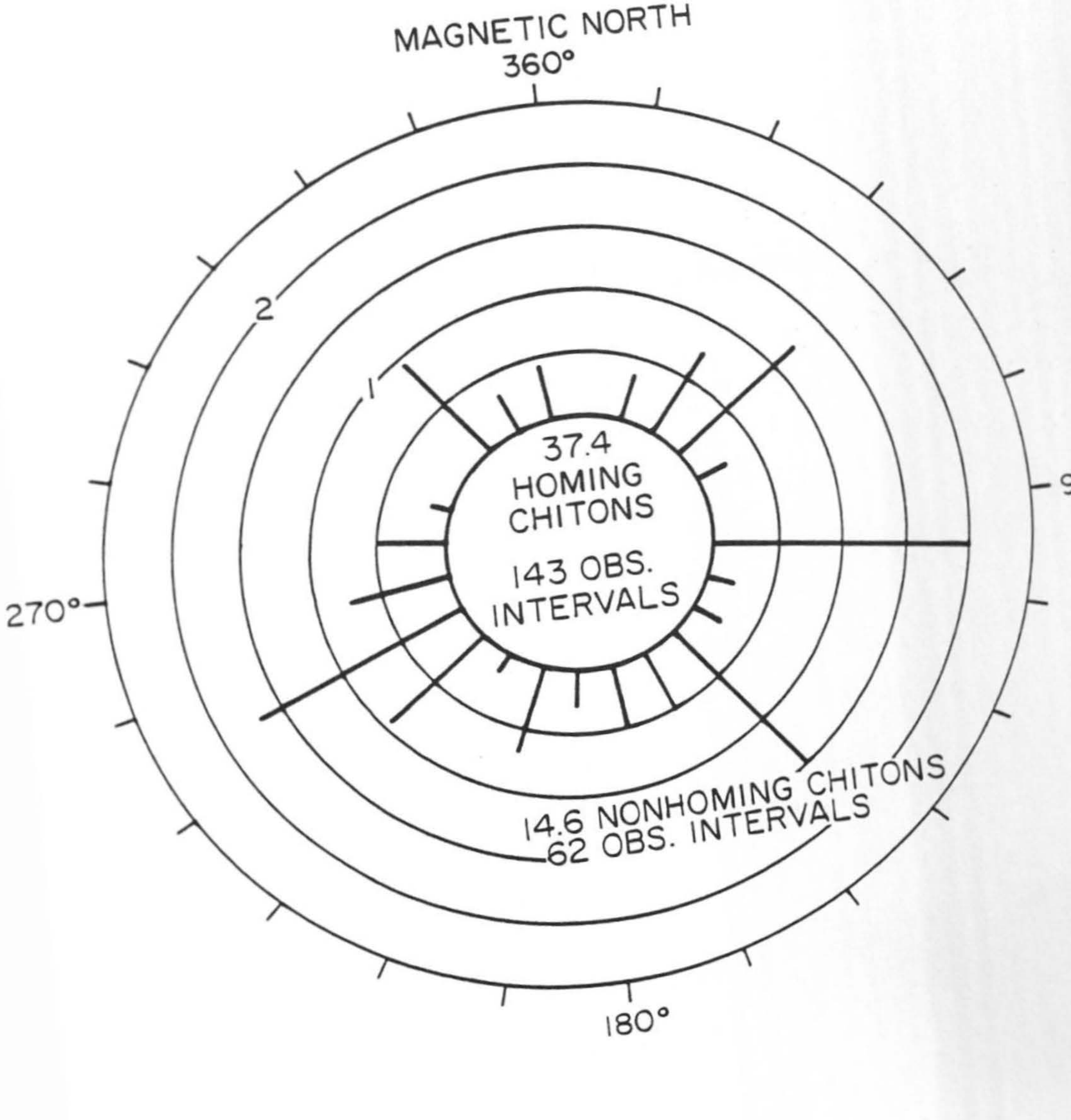
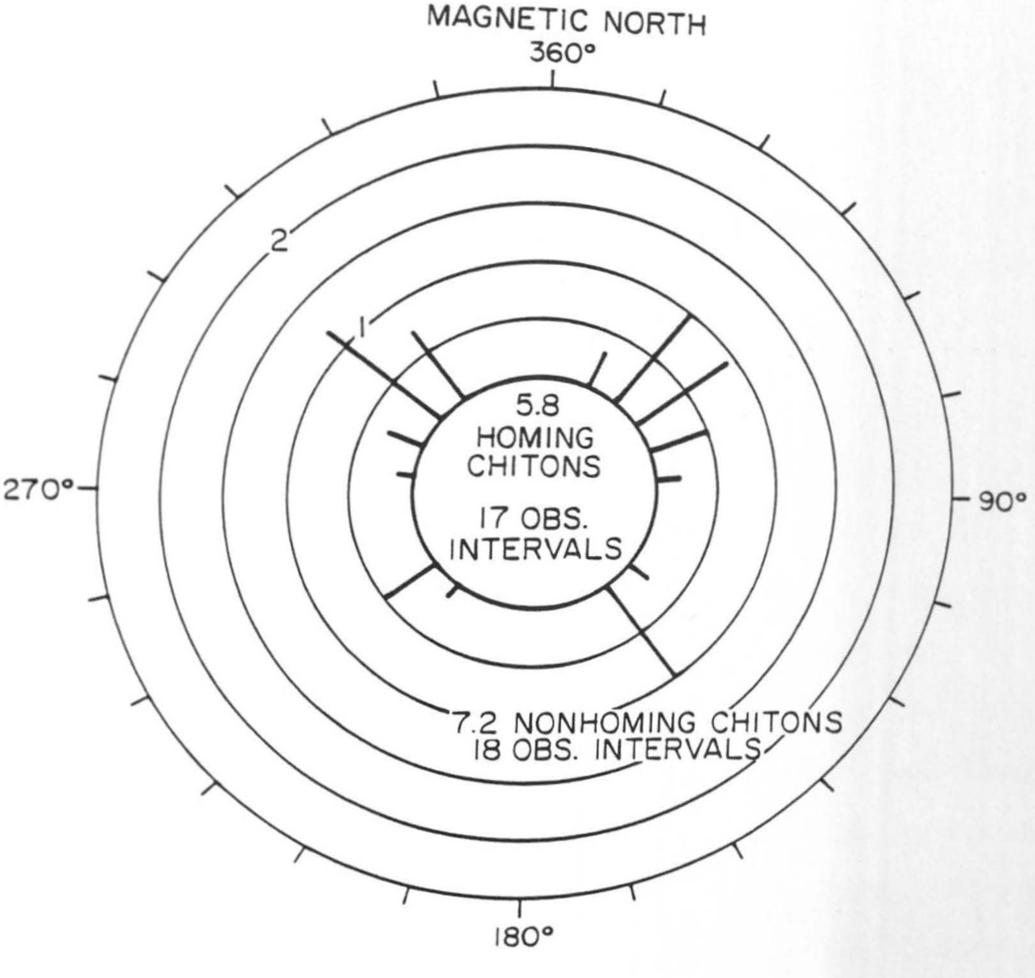

MAGNETIC NORTH
360°
90°
180°
270°
2
1
37.4
HOMING
CHITONS
143 OBS.
INTERVALS
14.6 NONHOMING CHITONS
62 OBS. INTERVALS

Figure V-20. Circular frequency distribution of observed displacements of low shore unidentifiable _Nuttallina_ specimens at the Seal Rock study site, La Jolla, CA for the period 9 Sept 1979 to 11 Nov 1980. Only specimens occurring below +110 cm above MLLW are included. See Figure V-15 caption for further explanation.


MAGNETIC NORTH
360°
90°
180°
270°
2
1
5.8
HOMING
CHITONS
17 OBS.
INTERVALS
7.2 NONHOMING CHITONS
18 OBS. INTERVALS

in the high population are, at most, not much higher than those in the low one. However, examination of movement records of individual chitons suggests that such upshore movement is not occurring. No tagged Nuttallina specimens of either species, or in the unidentifiable group, moved from the well-defined densely-populated low shore area up into the more sparsely-populated mid-shore area during the 14 months of this study. In fact, only two specimens of Nuttallina out of 160 individuals moved out of the general habitat in which they were tagged during this study. Both specimens belonged to Nuttallina fluxa, and both moved from continually-submerged positions in a large high intertidal pool to nearby exposed areas in late-spring/early-summer, 1980.

Displacement tracks of the most mobile specimens of N. fluxa, N. kata and unidentifiable Nuttallina, illustrated in Figures V-4, V-5, and V-6, generally support the contention that these chitons do not move consistently upshore. Most of the mobile specimens move about in various, and seemingly quite haphazard, directions. Specimens such as #27 in Figure V-6 move relatively unidirectionally but not far upshore. After tagging, specimen #35 (Figure V-4) moved down to the boundary between the dense and sparsely-populated areas, then moved upshore for a net vertical displacement of only about 7 cm. Specimens #1, #3, #CH1 moved considerable distances, but with only small net displacements upshore; all three started at positions high in the intertidal zone. Specimen #BN (Figure V-6) moved a considerable distance almost unidirectionally downshore from April to July, 1980, for a vertical displacement of about 19 cm; this specimen started from a position well up in the high sparsely-populated habitat. Specimens #AM and #DT (Figure V-4) also moved substantial distances downshore from positions in the high sparse habitat.

Evidence similar to mine, but always obtained over much shorter time periods and usually much less convincing, has led previous students of intertidal molluscs to conclude that migration is not an important element in the population dynamics and that differential growth or mortality must account for most of the observed shore-level size gradient (e.g., see Branch, 1975; Creese, 1980).

Taken together, the data from the present study certainly give a strong indication that Nuttallina populations from the low densely-populated band and the high sparsely-populated shore areas are relatively isolated from each other. Tagged Nuttallina did not tend to migrate upshore, and migration could not explain the shore-level size gradient, with large chitons occurring higher in the intertidal zone. The chitons show strong tendencies to home to particular sites for long periods and when they do move, movements are not in any particular direction. On the other hand, Nuttallina probably lives at least five years (see Chapter VI) and, since this study extended over a period of only 14 months, it is possible that conspicuous movements of Nuttallina to the high shore take place only every few years, rather than in every year. For example, low shore individuals might move into high areas in a year of relatively mild climatic conditions following a harsh year in which the high population suffered unusually high mortality.

Examination of Movement Data for Evidence of Migration Between Shore Levels: Subsequent to Electrophoretic Study of *Nuttallina*

Strong indications of isolation of high and low intertidal populations of adult *Nuttallina* from the present study provided additional impetus for an electrophoretic investigation into the taxonomic status of these chiton populations (see Chapter III introduction). These two *Nuttallina* populations proved to be separate species (Chapters III, IV).

The electrophoretic, or "genetic tag", results can be used now to take a more refined look at the population dynamics of *Nuttallina*. Electrophoretic findings clearly indicated that the population of *Nuttallina* specimens in the high intertidal zone, particularly in drier, more exposed areas, is composed almost entirely of *Nuttallina fluxa*, whereas the low shore population is almost all *Nuttallina kata*, a new previously undescribed species (Chapter III, particularly Tables III-5, III-6, and Chapter IV). This clearly supports the conclusion of the present study that no general upshore migration occurs in *Nuttallina* at La Jolla and, especially given the similarity of intertidal distributions of *Nuttallina fluxa* and *N. kata* at all other sites where they have been studied (Chapter III), suggests that this lack of mass migration of specimens from the low to the high shore is persistent over the years.

In view of the low mobility of adult *Nuttallina* documented in this study, it would appear that the observed intertidal distribution pattern of the two *Nuttallina* species is determined primarily by patterns of larval settling and/or by movement or mortality immediately thereafter, before the chitons reach 15 to 20 mm live total length, the minimum size that could be tagged in this study. Unfortunately, larval settling patterns are almost impossible to determine in the field since the chitons are extremely hard to locate before they reach a few mm in length and, even then, cannot be easily identified without electrophoresis (see Chapter III for a discussion of larval settling). Sporadic, intensive searches for small Nuttallina during this study did not yield many individuals, certainly not close to the magnitude of sets of small acmaeid limpets that were quite conspicuous on mid-intertidal bare rock surfaces in Summer, 1980. Louda (1972) similarly could find only very few small recruits in her ecological study of "Nuttallina fluxa" in Santa Barbara, CA (probably all N. fluxa of this study; see Chapter III remarks).

Small (~3 to 6 mm) Nuttallina found in all seasons during this study numbered only 20 to 30. These chitons were never found on mid- and high intertidal bare exposed rock surfaces, but rather in damper, more protected areas, primarily in the low intertidal algal turf and in damp low mid-intertidal areas, but also in damp high-mid areas and in a high shore tidepool. Unfortunately, these juveniles were not collected and can not be assigned to the two Nuttallina species discovered subsequently. After the present study, during the electrophoretic study, an intensive search for juveniles in November, 1981 again yielded very few small chitons, most greater than 6 mm in length (Chapter III: Table III-6). Like the small Nuttallina found in 1979 and 1980, these juveniles were all living in wet areas at the La Jolla site. It might not be surprising that four of the five juveniles determined electrophoretically to be Nuttallina fluxa ("HI/DRY" morph) occurred in the low, wet densely-populated algal turf area, but it certainly is surprising that the twenty-three electrophoretically-identified N. kata juveniles occurred nearly equally often in "high, wet", "high pool" and "low, wet" habitats (Chapter III: Table III-6). These juvenile distributions are nearly the reverse of the adult intertidal distributions!

Dynamics of the Intertidal Distribution of Nuttallina fluxa

It is difficult to make firm conclusions without knowledge of relative mortality rates in high shore areas, but if these distributional findings reliably represent persistent patterns of recruitment (i.e., 1 of 5 Nuttallina fluxa juveniles in high area), it would appear that individuals of Nuttallina fluxa recruited in the low intertidal areas must move upshore, presumably before they attain 15 to 20 mm in length. However, small Nuttallina would be expected to home even more reliably than adult Nuttallina, if the tendency to home holds a constant relationship with size (this is certainly not always true in other species); in this study, N. fluxa less than 25 mm in length, like small specimens of N. kata and small unidentifiable specimens, tended to home more frequently than did larger specimens (Table V-20). Even so, it is possible that those juveniles that do move, move in a more directed manner upshore than do the adults examined in this study.

An alternative to juvenile upshore movement that could explain the disparity in observed intertidal distributions of juvenile and adult specimens of N. fluxa is that recruitment of N. fluxa to high damp areas is greater at other times than in these studies. Certainly, settlement seems to be variable at the Seal Rock site; I noted a particularly heavy set of small Nuttallina specimens (in the lower intertidal area, not high) on 12 September 1977, but I had trouble finding any such specimens the year of the present study. Certainly, recruitment of intertidal gastropods varies considerably from year to year and place to place (Underwood, 1979). Another possibility is that mortality rates of N. fluxa in high areas are much lower than in low areas, and a relatively small proportion of recruits to high areas maintains a substantial population of adult *N. fluxa* there.

What can we say about the presence of adult *Nuttallina fluxa* in high, dry areas, where juvenile *N. fluxa* are never found? Mortality rates may be low enough in the high, dry exposed areas so that very few specimens need to move there from high damp areas and pools to maintain the low density of relatively large *N. fluxa* found there. Some evidence for these proposed movements between high intertidal habitats was obtained in the present study; the only two *Nuttallina* specimens observed to change general habitats during this study were both *N. fluxa* of about 35 mm in length that moved out of a high tidepool at a time when conditions were fairly mild, i.e., in May and July when exposure at low low tides occurs at night, and homed to nearby exposed areas on bare rock for the remainder of the study. Generally, large specimens of intertidal molluscs can withstand desiccation and high temperatures better than smaller conspecifics (e.g., Davies, 1969; Boyle, 1970; Wolcott, 1973; Branch, 1981); this probably explains why larger specimens of *N. fluxa* are more apt to be found in drier, more exposed areas of the high shore.

Dynamics of the Intertidal Distribution of Nuttallina kata

In *Nuttallina kata*, for which electrophoretically-identified juveniles ($\leq$15 mm) were found fairly equally at all levels of the shore in damp habitats (Tables III-5 and III-6), juveniles might move downshore to produce the adult distribution pattern (with *N. kata* primarily in low areas). A tendency for downshore (southward) movement, though usually nonsignificant, was observed in many subgroups of tagged adult *N. kata* in this study (e.g., Tables V-12, V-18, V-19, V-20, V-21, V-23), although not for small specimens ($\leq$25 mm, Table V-20). In fact, tagged *N. kata* of all sizes homed extremely reliably in this study, and a more appealing explanation for the adult/juvenile distribution disparity would seem to be that mortality rates of *N. kata* are greater in upper intertidal areas than in the low area, the eventual habitat of most adult *N. kata*. The maintenance of adult *Nuttallina* distribution patterns is discussed in Chapter III.

Differential Growth and/or Differential Mortality

The electrophoretic study (Chapter III) has clearly revealed that the shore-level size gradient *Nuttallina* results from species differences as well as from differences within *N. fluxa* itself. In *Nuttallina* sampled from many sites as well as from La Jolla, *N. fluxa* was found to attain larger size, 50 to 55 mm in length, than *N. kata*, 40 to 45 mm length. In addition, at La Jolla as well as at several other sites, *N. fluxa* tends to be larger in size in the high intertidal than in the low. In contrast, at La Jolla and elsewhere, *N. kata* tends to attain larger size in low areas than in high ones (Chapter III: Tables

III-5 to III-7). Although small recruits of Nuttallina fluxa might move into higher damp areas from low area settling sites as suggested earlier, it seems that directed movements do not account for much of the general shore-level size gradient in Nuttallina, with the largest specimens occurring in the highest shore areas.

Thus, interspecific differences in growth rates and/or mortality, possibly superimposed on shore-level differences within species in growth and/or mortality, must account for most of the general shore-level size gradient in Nuttallina observed at La Jolla. Results from a field growth study indicate that the high shore species, N. fluxa, probably does grow faster than the low shore species, N. kata, thereby contributing to the observed shore-level size gradient (see Chapter VI).

Mortality rates for high and low intertidal Nuttallina are not known reliably. "Survival" data, presented in Table V-3, represent maximum estimates for mortality rates, since tag losses as well as chiton deaths are included in the estimates. Sample sizes were limited due to the considerable time required to relocate specimens, while ensuring that animals that could not be found were not anywhere on the shore, with tag still attached. Size differences between the largest subgroups, high intertidal N. fluxa (N=41) and low intertidal N. kata (N=55), contribute most to the observed shore-level size gradient. "Survival" times for these two subgroups were quite similar (about 66% of total possible chiton-days for individuals of both; Table V-3), but mortality rates for the two can not be concluded to be similar without knowledge of comparative tag loss rates in the two areas and the size-specific structure of mortalities.

To obtain reliable mortality estimates, many hundreds of tagged chitons of all sizes and in all representative habitats would have to be monitored; a reliable estimate of tag losses would have to be obtained simultaneously. This would be valuable information, particularly if it could be obtained for several years, under a variety of climatic conditions. Unfortunately, this process would be extremely time-consuming in Nuttallina; it is especially difficult to relocate chitons in the low algal turf area and re-finding all tagged chitons in this study generally took three low-tide periods every two weeks.

No data are available for the comparative dynamics of high and low shore populations of other chiton species; however, for the well-studied intertidal limpets, evidence suggests that both differential growth and differential mortality can contribute to shore-level size gradients of nonmigrating species (Underwood, 1979; Branch, 1981). In particular, high and low intertidal populations of Collisella scabra in Sutherland's (1970) study have many of the characteristics of Nuttallina populations in La Jolla, except that the Nuttallina high and low populations have been found to be separate species. Sutherland (1970) found that C. scabra increases in size with vertical intertidal height. Like Nuttallina populations, those of C. scabra are fairly dense and exhibit random to uniform dispersion in low intertidal areas, and are more sparse with clumped dispersion in high shore areas. Sutherland (1970) found that growth in high intertidal C. scabra was greater than in the lower intertidal limpets because of low limpet densities, and growth was seasonal, presumably increasing and decreasing with food supply. Mortality rates on the high shore were relatively low as were recruitment rates, and catastrophic mortality occasionally occurred. In low intertidal C. scabra, on the other hand, growth was slower presumably as a result of higher densities of limpets, and growth was more constant, presumably because food was more constantly available. Mortality rates in the low population were relatively high and the probability of catastrophic mortality was lower.

Sutherland (1970) did not study the movement behavior of Collisella scabra, but rather assumed that "there was no mixing between populations from different intertidal heights", primarily on evidence contained in an unpublished student report (Brant, 1950). Brant's (1950) study indicated that greater than 95% of the excursions of Collisella scabra away from homesites concluded in homing. Sutherland (1970) used this evidence to account for limpets that had lost their tags (more were lost in the low zone than the high in his study) in determining mortality rates. Limpets that could not be found after having homed to a site for about 4 months were recorded as documented deaths. However, it is important to realize that Brant's (1950) study: 1) extended only over a 23 day period in July, 1950, and no individual was followed longer than 5 days, 2) revealed spatial heterogeneity in movement, with the percentage of animals homing over a 3 to 5 day period varying from 82 to 100%, 3) included only high intertidal areas, above 3.9 ft (119 cm) above MLLW, at a different site (Monterey, CA) and with apparently much lower limpet densities (400 vs. 1400 in Sutherland's lower zone) than Sutherland's (Bodega Bay, CA), and 4) probably included primarily large limpets since, where noted, no specimen was smaller than 11 mm in length [vs. a minimum of 4 mm in Sutherland's (1970) study].

Brant (1950) recorded a much larger frequency of nonhoming events in an area where limpet densities were experimentally increased and noted an increasing tendency for longer foraging movements under natural conditions as his study progressed. Wells (1917; Acmaea spectrum=Collisella scabra) and Villee and Groody (1940) observed some specimens of Collisella scabra to exhibit fairly substantial displacements to new homesites, and Choat (1977) recorded a small upward migration of Collisella scabra on vertical pilings in February in his study at Santa Barbara, CA.

Given the foregoing facts, it is probable that there was more movement of specimens than Sutherland (1970) appreciated, and that there was considerable mixing of individuals from different heights, particularly as a result of movement from lower levels that were very densely-populated with small limpets, to higher ones. Since tag loss was a greater problem in the lower zone in Sutherland's (1970) study, it is possible that deaths "documented" by assuming homing in this area were inflated relative to those in the high area as a result of unappreciated movement frequencies (thus partially accounting for the higher observed mortality rate in the lower zone). Lewis and Bowman (1975) raised an additional question as to whether the lower growth rates Sutherland (1970) recorded in his lower zone 2 were due to between-habitat differences (barnacles vs. no barnacles) similar to the ones they noted in the limpet Patella vulgata even at similar tidal levels, rather than primarily to greater limpet densities and lower food per limpet as Sutherland's (1970) limpet density manipulation experiment suggested. In addition, Sutherland's (1970) conclusion that the largest high zone specimens of Collisella scabra live longer (11 versus 7 years) and grow faster than the largest lower zone limpets is interesting in that it is contrary to findings of the majority of other workers, who find longevity to be inversely related to growth rate (e.g., Fischer-Piette, 1948; Comfort, 1957; Frank, 1965; Lewis and Bowman, 1975).

An increase in intertidal height usually corresponds with a decrease in the growth rate of a mollusc, other factors being equal (presumably as a result of decreasing time available for feeding; see Branch, 1981, p. 299 for limpet examples). However, where the density of the lower intertidal population of a species is much greater than that of the higher intertidal population, lower growth rates and higher mortality rates of the lower intertidal population, as documented by Sutherland (1970), might be common. Other possible examples of this latter situation could be the Patella study by Lewis and Bowman (1975) and the study of Creese (1980). Creese (1980) found growth rates to be greater and mortality rates to be lower in a sparsely-occurring, high intertidal population of the limpet, Notoacmea petterdi, than in a more densely-occurring, low intertidal one. In Nuttallina in the present study, where the low intertidal chitons similarly occur much more densely than do the high ones, similar differences in growth and mortality rates with intertidal height might account for the observed shore-level size gradient. Studies on growth of Nuttallina in the field will be described in the next chapter.

CHAPTER VI

FIELD GROWTH STUDIES ON NUTTALLINA

INTRODUCTION

Although Nuttallina is abundant in many rocky shore areas, very little information is available on growth in Southern California specimens of this chiton. Harvey (1963) presented data on growth of twelve specimens of "Nuttallina fluxa" (N. fluxa and/or N. kata) held in an aquarium at Scripps Institution of Oceanography for 9 months and Louda (1972) obtained growth data for twenty specimens of "Nuttallina fluxa" (probably N. fluxa) observed over 4 1/2 months on a rocky shore near Santa Barbara, CA. No general conclusions about growth rates or age/size relationships in Nuttallina can be made from these studies. The MacGinities (1949, 1968, p. 386) have guessed that Nuttallina lives at least twenty or twenty-five years with no supporting evidence.

The present study was initiated to obtain a clearer idea of the growth rate and age of a representative specimen of Nuttallina found on the shore. A reliable estimate of age of typical specimens can be of much utility in understanding the population dynamics of Nuttallina populations. For example, if a typical Nuttallina specimen lives for 25 years rather than 5 years, turnover rates in a particular shore area can be concluded to be much slower, with possibly profound consequences for predicted migration and recruitment rates.

Growth rates and ages of a variety of molluscan species have been estimated by several different techniques, including analyses of temporally-sequential size-frequency distributions (e.g., Glynn, 1970; Baxter and Jones, 1978), internal or external shell growth line counts in unmanipulated specimens or in specimens injected with tetracycline HCl [e.g., Crozier, 1918a,b; Nakahara, 1961; Baxter and Jones, 1978; Kahn and Pompea, 1978 but see Nature 279(5712)], shell natural radionuclide ratios (Turekian et al., 1975, 1979), $^{18}O/^{16}O$ shell profiles (e.g., Wefer and Killingley, 1980), and mark/recapture studies (e.g., Boolootian, 1964; Palmer and Frank, 1974; Brousseau, 1979). Each method has its own advantages and disadvantages, some of which have been discussed by Haskin (1954), Wilbur and Owen (1964) and Rhoads and Lutz (1980).

Relatively few extensive growth studies have been undertaken on chitons and growth estimates for most species comprise guesses, educated or otherwise. A summary of sources of growth data for chitons is presented in Table VI-5. Chiton growth studies have employed several techniques but conclusions made in these studies have often been questionable, or even contraindicated in other studies. It is clear that, due to variation in biology from species to species, particular methods of growth estimation that are practicable for one species are not useful for other species. Accordingly, in the present study, the feasibility of several techniques was examined before it was determined that the mark/recapture method, though labor-intensive, was the most practical for growth estimation in Nuttallina.

At the time the present study was carried out, the *Nuttallina* populations were thought to consist of one species. Specimens were subsequently assigned to the two species, *Nuttallina fluxa* and *N. kata*, using morphological criteria as in Chapter V, on discovery that the study area contained primarily two *Nuttallina* species.

MATERIALS AND METHODS

Analyses of size-frequency distributions sampled sequentially in time seem to be unreliable for obtaining growth rate estimates for *Nuttallina* populations in La Jolla, CA. This method is not reliable unless growth is fairly rapid and uniform throughout the population and recruitment is confined to a relatively short period of the year; year class modes must be clearly indicated in a size-frequency distribution, or the number of year classes present must be known from other evidence, in order to obtain a reliable growth estimate by this method (e.g., see MacDonald and Pitcher, 1979). In addition, for fairly immobile intertidal species, for which interhabitat growth differences can be significant (e.g., Lewis and Bowman, 1975), size-frequency distributions ideally should be determined nondestructively (i.e., leaving specimens alive in situ and the habitat unaltered) in the same site over time.

It is impossible to obtain size-frequency data for *Nuttallina*, particularly for low-shore *N. kata*, in a nondestructive manner as required for effective application of the size-frequency technique. Chitons cannot be reliably measured without moving them from the substrate, and some specimens cannot be detected without partially destroying the habitat; removal of chitons from the substrate, particularly those chitons that inhabit form-fitting depressions, for measurement is necessarily associated with increased mortality and destruction of the habitat.

The size-frequency distribution for *Nuttallina* determined in Fall, 1980 (see Chapter II) does not reveal the clearly polymodal population structure needed to determine growth rates, without supplementary data, in a species such as *Nuttallina* which undoubtedly lives for more than two or three years. Preliminary results and problems with applying the size-frequency analytic technique to *Nuttallina* populations indicated that the great amount of labor required for this method was not justifiable.

Shell growth lines were not useful for growth rate estimation in *Nuttallina*. Both external and internal shell growth lines can indicate growth rates in molluscs (e.g., Hughes, 1970; Seed, 1973; Kenny, 1977; Jones et al., 1978). Since different time periods have been ascribed to growth lines in various molluscs and "check" lines can be caused by disturbances such as handling and wave shock (see Wilbur and Owen, 1964; Kennish, 1980), a common problem in growth studies utilizing shell growth lines is to determine the amount of time corresponding to a single line and to ensure that the lines are produced regularly with time.

Only external shell lines have been used to estimate growth rates in chitons, with varying degrees of success (Crozier, 1918a,b; Arey and Crozier, 1919; MacGinitie and MacGinitie, 1968b; Baxter and Jones, 1978, but see Glynn, 1970; Palmer and Frank, 1974). In *Nuttallina*, on the external shell surface, grooves occasionally occur in the tegmentum near, and paralleling, the anterior growing edge, and, occasionally, there are striations in the sutural plates, but such features do not occur frequently enough to permit study of their temporal significance or their regularity with time. In addition, erosion of the external valve surface, probably facilitated by the blue-green alga *Entophysalis deusta* (Nishi, 1975), limits the number of *Nuttallina* specimens for which a complete growth record is present in the external shell layer.

Microscopic examination of thin cross-sections of *Nuttallina* valves, cut in various planes, sagittally, transversely, and radially from the apex, with a slow speed diamond saw (Isomet 11-1180, Buehler Ltd, Evanston, Ill.), very rarely revealed internal structures that could be construed as periodic. For example, an 8 mm long radial section of an intermediate valve possessed three long, dark brown wedges, alternating with two white stripes, that passed from a ventral white band up toward a more dorsal white band, all contained within the articulamentum layer of the valve. No such alternating structure was observed more than rarely in the articulamentum. Neither could any internal periodic structure be discerned in the outer tegmentum shell layer, as would be expected by analogy (homology?: Beedham and Trueman, 1967) with the growth lines observed in the outer layer of clam shells, for example. Certainly, the complexity of polyplacophoran shell growth, with layers being formed at several surfaces simultaneously and with sensory-related aesthete channels penetrating the tegmentum layer vertically (see Baxter and Jones, 1981), increases the difficulty of discerning internal periodic structures. Even if periodic shell structures could be found, a supplemental technique would have to be used to calibrate them with time.

The isotopic ratio of oxygen ($^{18}O/^{16}O$) incorporated biologically into calcite or aragonite varies predictably with temperature and salinity. This ratio has been used, among other things, to estimate growth rates and age in individual animals that occur in areas subject to fluctuations in ocean temperature (e.g., Wefer and Killingley, 1980). Ocean waters at La Jolla, CA show a large and regular annual fluctuation in temperature, without substantial complication from salinity fluctuations; good records of salinity make it possible to estimate water oxygen isotope composition quite accurately. Shell oxygen isotope profiles of an intertidal mollusc, *Mytilus californianus*, at La Jolla reflect seasonal ocean temperature changes with high fidelity (Killingley and Berger, 1979) and indicate that this technique might work well for other molluscs in the area.

The oxygen isotope technique seems ideal for investigation of growth in individual *Nuttallina* specimens from La Jolla, CA, although the method is technologically-demanding and quite time consuming, i.e., 20-30 minutes per sample. were on a mass spectrometer by Using a mass spectrometer and methods described in Killingley and Berger (1979), Killingley (Geological Research Division at Scripps Institution of Oceanography) analyzed shell samples taken from four *Nuttallina* specimens, for which collection dates are known. Calcium carbonate in *Nuttallina* was assumed to be in the form of aragonite as it is in another chiton (Boggild, 1930). Analyses can be performed on shell samples as small as 2 mg with a precision of about 0.1 per mil, although most samples were nearer 5 mg in size.

The mark/recapture technique has been used previously with the most success on relatively large species of chiton (Mopalia muscosa, Boolootian, 1964; Cryptochiton stelleri, Palmer and Frank, 1974). Workers on small chiton species have noted the unfeasibility of the technique (e.g., Baxter and Jones, 1978). However, with the development of an effective tag (see Appendix IV), this method proved practical, though labor-intensive, for application to the relatively small Nuttallina species. Specimens tagged for study of long-term displacements (Chapter V) were measured carefully for growth rate determinations.

Growth measurements of the tagged chitons included those of live chiton total length (CTL) and intact fourth valve width (FVW). Although weight measurements have been used effectively to monitor growth in Cryptochiton stelleri, in which the valves are covered by epidermis (Palmer and Frank, 1974), this type of measurement was not practical for Nuttallina because: 1) it would entail removing an occasionally substantial valve algal overgrowth by vigorous scraping, with consequent disturbance to the specimen and potential alteration of its long-term behavior including feeding (sensory aesthetes are present in the valves); 2) it would involve bringing animals into the laboratory for a period of time to allow sufficient time for emptying of the digestive tract; and 3) it would entail vigorous blotting of the chitons with accompanying increases in mortality rates, in order to obtain reproducible measurements, unless provisions for weighing animals in water were arranged.

CTL measurements were made in the field as follows. The specimen was carefully removed from its site, was placed on a flat rock in a shallow tidepool so that it was half-submerged (specimens relax more rapidly when placed in water), and was left undisturbed for several minutes. Its CTL was then measured to 0.1 mm using a pair of fine-pointed dividers and transferring to a precision metric rule. CTL was taken as the average of at least three such measurements. In some instances, if a chiton were disturbed by a measurement, it would not fully relax again. In such cases, only relaxed measurements were used.

Preliminary measurements revealed relatively slow growth rates, so sampling intervals were increased to approximately six months in length during the study. This measurement regime helped to minimize disturbance to the chitons, but had the disadvantage that a substantial proportion of study animals were lost between samples, thereby reducing the amount of growth data obtained.

Part way through the study, it was discovered that the FVW could be measured in a live chiton with precision and relative ease. Fine points of dividers could be inserted medially to the fleshy girdle and, with care, could be made to straddle the maximum width of the fourth valve at its lateral edges. The FVW was then determined to 0.1 mm by transferring to a precision metric rule. The FVW was the average of at least two such measurements for a chiton. FVW growth estimates were obtained only for the last half of the fourteen month study, from about May to November 1980.

RESULTS

$^{18}O/^{16}O$ DETERMINATIONS

No firm conclusions concerning growth rates and longevity of Nuttallina specimens can be made with the shell $^{18}O/^{16}O$ data obtained to date. However, this technique has been used on relatively few species of animals and is still largely at an exploratory stage of development. Since data obtained by this method are relatively scarce, a presentation of findings here seems worthwhile.

$^{18}O/^{16}O$ measurements on twenty-three shell samples from four Nuttallina specimens have been made to date, and data are presented in Table VI-1 and Figure VI-1. As expected, measurements for the Bodega Bay specimen reflect growth of the shell in much colder water than is found in La Jolla, CA. The Nuttallina specimen collected from the south side of Punta Banda, Baja CA, Mexico, an area of seasonal cold-water upwelling, also seems to have grown in relatively cool water. These data lend support for the conclusion (in Chapter III) that Nuttallina californica is a cold-water species, relative to N. fluxa and N. kata.

The series of twelve samples obtained along a line from the anterior edge of the sutural plate toward the shell apex in the specimen of Nuttallina kata do not reveal substantial variation in temperature. Ideally, a series of ^{18}O samples taken from a shell such as this should reflect fluctuations of temperature spanning a large proportion of the observed annual variation in surface water at the collection location, such that a complete cycle of variation would unequivocally represent a year of growth.

TABLE VI-1. Oxygen isotope data for samples from fourth valves of Nuttallina. Since tidepool temperatures are variable, and often much different from ocean temperatures, none of the specimens was from a tidepool. Analytic procedures are described in Killingley and Berger, 1979. Standard carbonate was PDB (Pee Dee Belemnite). The chiton valves are assumed to consist of aragonite (Boggild, 1930).

Species (determined by electrophoresis)	Collection Locality	Collection Date	Habitat[A]	Chiton Measurements, mm (CTL=chiton total length; FVW=fourth valve width)	Sample location in "sutural plate" series, in mm from anterior edge[B] (overall distance from anterior edge of sutural plate to valve apex in parentheses)	Sutural plate (articulamentum) isotope data $d^{18}O$	Sutural plate (articulamentum) isotope data $d^{13}C$	Sample location in valve main body series, in mm from jugal sinus edge[C] (overall distance from jugal sinus to valve apex in parentheses)	Valve main body (articulamentum layer) isotope data $\delta^{18}O$	Valve main body (articulamentum layer) isotope data $\delta^{13}C$
Nuttallina californica	Punta Banda, Baja CA (Chapter III collection site)	2 April 1981	High intertidal	CTL: 33.0 (frozen) FVW: 12.0	0.5	0.43	0.38			
					1.5	0.62	0.40			
					(8 mm)					
Nuttallina californica	Bodega Bay, CA (Chapter III collection site)	January 1981	High-mid area	CTL: 39 (frozen) FVW: 12.6	0.5	1.73	-0.50			
					1.5	1.51	-0.72			
					(10 mm)					
Nuttallina fluxa	La Jolla, CA (Seal Rock)	4 February 1981	High, exposed rocks	CTL: 31 (frozen) FVW: 15.8				0.3	-0.35	0.78
								0.8	0.06	0.17
								1.5	-0.05	0.39
								(8 mm)		
Nuttallina kata	La Jolla, CA (Seal Rock)	15 February 1981	Low-mid, densely-populated area, shallow depression	CTL: 35 (live) FVW: 9.2	1.0	-0.16	-0.99	0.3	0.09	-0.61
					1.8	-0.16	-1.30	1.0	0.72	0.05
					2.2	-0.25	-1.46	1.9	-0.19	-0.89
					2.6	-0.10	-0.83	2.8	0.09	-1.00
					3.0	-0.15	-1.49	(6.4)		
					3.4	-0.10	-1.65			
					3.8	-0.31	-1.85			
					4.2	-0.24	-1.67			
					4.5	-0.41	-2.24			
					5.0	-0.12	-1.81			
					5.3	-0.05	-2.51			
					5.6	0.00	-2.74			
					(10 mm)					

NOTES

A. Habitats described in Chapters II and III.

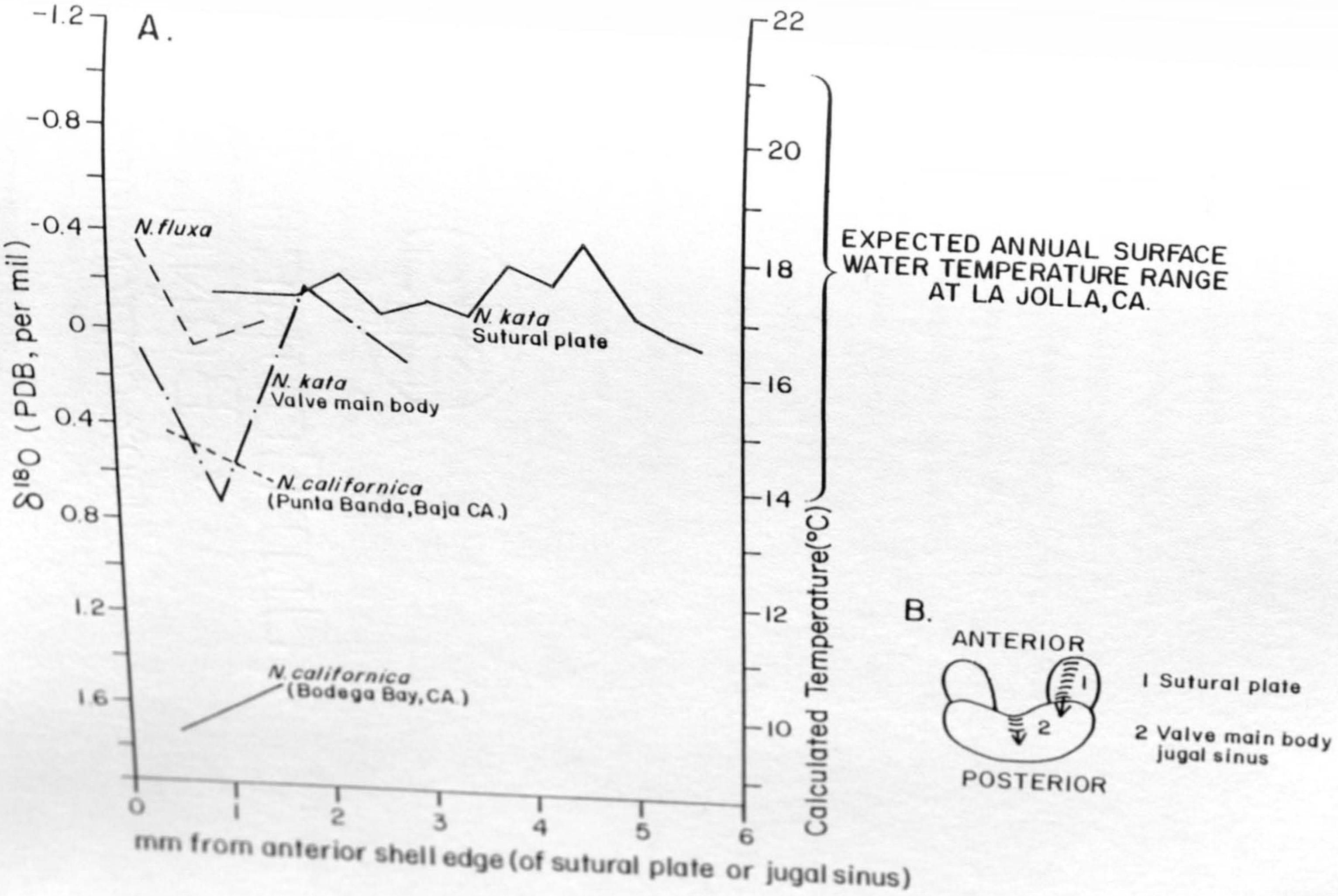
B. The anterior edge of the sutural plate is the growing edge (the newest material). Sutural plate locations were measured from the anterior edge toward the valve apex. For N. kata, the "sutural plate" series of samples extended into the valve main body, and the tegmentum layer had to be removed to sample the articulamentum layer there.

C. Locations on the valve main body were measured from the jugal sinus toward the valve apex.

Figure VI-1 $^{18}O/^{16}O$ analyses of fourth valve samples from *Nuttallina* specimens.

Corresponding data, including appropriate overall dimensions, are presented in Table VI-1. All samples were taken from the shell articulamentum layer; overlying tegmentum was filed away if necessary. Samples for *N. fluxa* were from the valve main body; samples for both specimens of *N. californica* were from the sutural plates. *N. kata* samples were from both the sutural plate and the valve main body, as indicated.

Temperature scale in (A) assumes that the *Nuttallina* valves are aragonite and that $\delta^{18}O$ of La Jolla seawater is -0.6 ‰. Aragonite is 0.6 ‰ heavier than calcite. The mollusc paleotemperature equation of Epstein et al. (1953) was used for conversions. Shell sample locations are illustrated in (B). Similar sample locations do not necessarily coincide in time for different specimens or for different layers of the fourth valve of a single chiton.


A.
-1.2
-0.8
-0.4
0
0.4
0.8
1.2
1.6
δ¹⁸O (PDB, per mil)
22
20
18
16
14
12
10
Calculated Temperature(°C)
0
1
2
3
4
5
6
mm from anterior shell edge (of sutural plate or jugal sinus)
N. fluxa
N. kata
Sutural plate
N. kata
Valve main body
N. californica
(Punta Banda, Baja CA.)
N. californica
(Bodega Bay, CA.)
EXPECTED ANNUAL SURFACE
WATER TEMPERATURE RANGE
AT LA JOLLA, CA.
B.
ANTERIOR
POSTERIOR
1
2
1 Sutural plate
2 Valve main body
jugal sinus

The most promising shell samples came from the Nuttallina kata valve main body (Table VI-1; Figure VI-1). In this series, considerable $^{18}O/^{16}O$ fluctuation is revealed, despite the fact that the valve main body is considerably shorter (i.e., the time axis is more compressed) than the sutural plate-to-apex dimension and despite the fact that the samples were more widely-spaced than were those in the sutural plate sample series (in which little $^{18}O/^{16}O$ variation was revealed). Clearly, because the upswing in the $^{18}O/^{16}O$ "cycle" (signifying a downswing in temperature) is demarcated by only a single sample, the single apparent cycle (Figure VI-1) could represent a conglomeration of several cycles.

GROWTH OF TAGGED SPECIMENS

Results of laboratory studies presented in Appendix IV indicate that the growth of tagged and untagged Nuttallina specimens are not different; thus, the tag has no obvious effect on growth of this chiton.

Growth measurements of live chiton total length (CTL) and/or intact fourth valve width (FVW) were obtained in the field for 58 Nuttallina specimens, assigned by morphological criteria (Chapter IV) as follows: 24 Nuttallina fluxa, 31 N. kata and three unidentifiable specimens. Measurement intervals varied from 164 to 230 days in length (mean=203), starting in September to December 1979 and terminating in March to June 1980 ("Winter") or starting in March to June 1980 and terminating in November 1980 ("Summer"). Change in CTL was measured for 39 specimens for one interval, Winter or Summer, and for 15 specimens for two intervals, while changes in FVW were determined for 32 specimens, Summer only. CTLs of specimens of Nuttallina fluxa, for which CTL growth measurements were obtained, ranged from 22.2 to 50.0 mm, while specimens of N. kata ranged from 18.2 to 34.7 mm, and unidentifiable specimens ranged from 20.2 to 36.0 mm. FVWs ranged from 8.1 to 15.7 mm for N. fluxa, 5.2 to 9.2 mm for N. kata and 9.2 to 9.9 for unidentifiable Nuttallina specimens. Examination of the growth data reveals considerable variability; in addition, there are a few instances of negative growth.

In previous studies, six growth curves have been used to describe the growth of animals and plants: the exponential, power, Gompertz, logistic, Bertalanffy, and Krüger curves (see Kaufmann, 1981; Krüger, 1962). Various formulations of these curves were examined in an attempt to describe Nuttallina growth data. The Krüger growth function (Krüger, 1962), which describes an asymptotic growth curve, has not been widely-used and was not used in the present study. Although high-order polynomials might provide better fits to the growth data, they, unlike the growth curve formulations, are unsuitable for extrapolations to unstudied sizes of animals and for comparisons between populations (Haukioja and Hakala, 1980). Therefore, such polynomials were not explored for use in the present study.

Variations of Ford-Walford plots, which allow plotting of growth data in straight lines, have previously been used to determine parameters of the Gompertz, logistic and Bertalanffy curves from cross-sectional data (e.g., Walford, 1946; Gulland, 1965; Theisen, 1973; Yamaguchi, 1975), but a disadvantage of these plots is that the time interval for all of the data points must be the same, i.e., data taken with different elapsed time interval lengths cannot be pooled. Van Devender (1978) and Kaufmann (1981) present methods that permit use of measurement intervals of variable lengths in analyses employing various growth curves formulated as straight lines. Both workers employ differential equations of the growth curve formulations, Van Devender (1978) expressing growth rate in terms of size for the Bertalanffy formulation, and Kaufmann (1981) expressing size-specific growth rate estimates in terms of size to investigate fits to the Gompertz, logistic, Bertalanffy, power and exponential growth models.

The techniques of Van Devender (1978) and Kaufmann (1981) were used in the present study to determine which growth model best described growth data obtained for tagged <u>Nuttallina</u> specimens. Specifically, data were plotted using the following axes: 1) $(S_2 - S_1)/\Delta t$ versus size ["size" used was the initial size, S_1, or the arithmetic mean of the initial and final sizes, $(S_1+S_2)/2$, or the geometric mean size, $\sqrt{(S_1 X S_2)}$, in different plots]; 2) G versus $\ln \sqrt{(S_1 X S_2)}$; 3) G versus $\sqrt{(S_1 X S_2)}$, where S_1 and S_2 are the initial and final CTLs (or FVWs) in mm, t is the time in days, and G is the size-specific growth rate, dS/Sdt, estimated as $(\ln S_2 - \ln S_1)/\Delta t$. The axes in 1) are those associated with the differential equation of the Bertalanffy formulation (Van Devender, 1978), those in 2) are those for the differential form of the Gompertz and exponential curves (exponential indicated by a line with a zero slope) suggested by Kaufmann (1981), and those in 3) are associated with the differential form of the logistic growth formula, also suggested by Kaufmann (1981). Axes associated with the differential form of the power growth curve [i.e., ln G versus $\ln \sqrt{(S_1 X S_2)}$] were not useful, since, for cases of negative growth as obtained in the present study, the ln G transformation entails taking the logarithm of a negative number.

Variables on neither axis, growth rate nor size, have been measured without error. Regressions in such cases are of Model II type. The appropriate treatment of Model II regressions is controversial; where the regressions are to be used for predictive relationships, as in this study, Sokal and Rohlf (1981, p. 549) suggest using simple linear regression techniques (Model I) for which statistical methods are well-studied. Such usage is fairly common in growth studies and other morphological work (see Ricker, 1973; Van Devender, 1978; Kaufmann, 1981), and will be followed in the present study.

All significant Model I regressions obtained in bivariate plots of data for CTL and FVW on axes listed above, along with corresponding geometric mean (or reduced major axis; Ricker, 1973) regression parameters, are listed in Table VI-2. Multiple, i.e., two, growth estimates for a single chiton were averaged on the appropriate axes to provide one estimate per chiton. It is not surprising that several growth models fit particular <u>Nuttallina</u> data subsets well since relationships between growth rate and size are not strong in the present study (see Kaufmann, 1981). The significant regressions listed in Table VI-2 reflect the relationship observed most often in growth studies involving adult specimens of a wide range of sizes -- smaller specimens exhibit greater growth rates.

TABLE VI-2. Regression statistics for <u>Nuttallina</u> growth using various models.

Growth data were plotted on axes appropriate to differential equations for the Bertalanffy, Gompertz (and exponential), power, and logistic growth models using BMDP6D (Dixon and Brown, 1981). Only significant regressions are listed ($p<0.05$, two-tailed); although all are also significant at $p=0.05$, one-tailed). Beneath parameters for each Model I regression are listed the corresponding functional regression parameters (e.g., regression No. 1 = Model I regression; No. 1A = corresponding functional or geometric mean regression parameters). $G = (\ln S_2 - \ln S_1)/\Delta t$ (i.e., estimate for the size-specific growth rate); $E\text{-}2 = \times 10^{-2}$ (etc.); S = live total length or fourth valve width, in mm (appropriate section of table); t = time, in days.

Regression Number	*Nuttallina* Species	Growth Model	Dependent Variable	Independent Variable	N	r	Slope	Y intercept	Range, independent variable	Mean, dependent variable	Mean, independent variable
TOTAL LENGTHS -- FOR CHITONS PRESENT THROUGH ONE GROWTH INTERVAL (150-235 DAYS)											
1	*fluxa*	Bertalanffy	$(S_2-S_1)/\Delta t$	S_1	23	-0.43	-6.65E-4	3.42E-2	23.6-48.2	1.13E-2	34.5
1A							-1.56E-3	6.51E-2			
2	*fluxa*	Gompertz	G	$\ln\sqrt{S_2S_1}$	23	-0.42	-7.72E-4	3.09E-3	3.22-3.90	3.42E-4	3.56
2A							-1.85E-3	6.93E-3			
3	*fluxa*	Logistic	G	$\sqrt{S_2S_1}$	23	-0.42	-2.16E-5	1.11E-3	25.2-49.7	3.42E-4	35.6
3A							-5.18E-5	2.19E-3			
4	*fluxa*	Bertalanffy	G	$1/\sqrt{S_2S_1}$	23	+0.41	+2.66E-2	-4.26E-4	2.01E-2 to 3.99E-2	3.42E-4	2.89E-2
4A							+6.41E-2	-1.51E-3			
5	*kata*	Bertalanffy	$(S_2-S_1)/\Delta t$	S_1	28	-0.47	-1.50E-3	4.88E-2	20.3-34.6	8.88E-3	26.7
5A							-3.18E-3	9.36E-2			

Table VI-2 (cont.)

Regression Number	*Nuttallina* Species	Growth Model	Dependent Variable	Independent Variable	N	r	Slope	Y intercept	Range, independent variable	Mean, dependent variable	Mean, independent variable
FOURTH VALVE WIDTHS -- "SUMMER" INTERVAL ONLY (150-222 DAYS)											
6	*fluxa*	Bertalanffy	$(S_2-S_1)/\Delta t$	S_1	14	-0.67	-3.90E-4	5.99E-3	8.1-15.8	1.12E-3	12.5
6A							-5.83E-4	8.39E-3			
7	*fluxa*	Bertalanffy	$(S_2-S_1)/\Delta t$	$(S_1+S_2)/2$	14	-0.64	-3.84E-4	5.95E-3	8.3-15.6	1.12E-3	12.6
7A							-6.02E-4	8.70E-3			
8	*fluxa*	Bertalanffy	$(S_2-S_1)/\Delta t$	$\sqrt{S_2S_1}$	14	-0.64	-3.84E-4	5.95E-3	8.3-15.6	1.12E-3	12.6
8A							-6.02E-4	8.70E-3			
9	*fluxa*	Gompertz	G	$\ln\sqrt{S_2S_1}$	14	-0.74	-4.48E-4	1.23E-3	2.11-2.74	1.01E-4	2.52
9A							-6.04E-4	1.62E-3			
10	*fluxa*	Logistic	G	$\sqrt{S_2S_1}$	14	-0.77	-4.02E-5	6.07E-4	8.3-15.6	1.01E-4	12.6
10A							-5.20E-5	7.56E-4			
11	*fluxa*	Bertalanffy	G	$1/\sqrt{S_2S_1}$	14	+0.70	+4.77E-3	-2.88E-4	0.064-0.122	1.01E-4	8.16E-2
11A							+6.76E-3	-4.51E-4			
12	*kata*	Bertalanffy	$(S_2-S_1)/\Delta t$	S_1	14	-0.69	-6.98E-4	6.43E-3	5.2-9.2	1.49E-3	7.1
12A							-1.01E-3	8.61E-3			
13	*kata*	Bertalanffy	$(S_2-S_1)/\Delta t$	$(S_1+S_2)/2$	14	-0.64	-6.92E-4	6.48E-3	5.3-9.1	1.49E-3	7.2
13A							-1.08E-3	9.30E-3			

Table VI-2 (cont.)

Regression Number	*Nuttallina* Species	Growth Model	Dependent Variable	Independent Variable	N	r	Slope	Y intercept	Range, independent variable	Mean, dependent variable	Mean, independent variable
FOURTH VALVE WIDTHS (cont.)											
14	*kata*	Bertalanffy	$(S_2-S_1)/\Delta t$	$\sqrt{S_2S_1}$	14	-0.64	-6.92E-4	6.48E-3	5.3-9.1	1.49E-3	7.2
14A							-1.08E-3	9.29E-3			
15	*kata*	Gompertz	G	$\ln\sqrt{S_1S_2}$	14	-0.72	-8.31E-4	1.86E-3	1.67-2.21	2.29E-4	1.96
15A							-1.16E-3	2.50E-3			
16	*kata*	Logistic	G	$\sqrt{S_1S_2}$	14	-0.74	-1.21E-4	1.10E-3	5.3-9.1	2.29E-4	7.2
16A							-1.63E-4	1.40E-3			
17	*kata*	Bertalanffy	G	$1/\sqrt{S_1S_2}$	14	+0.69	+5.54E-3	-5.59E-4	0.110-0.188	2.29E-4	1.42E-1
17A							+8.02E-3	-9.13E-4			
TOTAL LENGTHS -- FOR CHITONS PRESENT THROUGH BOTH GROWTH INTERVALS (342-434 DAYS TOTAL)											
18	*fluxa*	Bertalanffy	$(S_2-S_1)/\Delta t$	S_1	7	-0.70	-6.92E-4	3.38E-2	22.2-40.2	1.33E-2	29.5
19	*fluxa*	Gompertz	G	$\ln\sqrt{S_2S_1}$	7	-0.75	-8.92E-4	3.52E-3	3.22-3.70	4.35E-4	3.46
20	*fluxa*	Logistic	G	$\sqrt{S_1S_2}$	7	-0.78	-2.91E-5	1.37E-3	24.8-40.5	4.35E-4	32.0
21	*fluxa*	Bertalanffy	G	$1/\sqrt{S_1S_2}$	7	+0.71	+2.65E-2	-4.12E-4	2.48E-2 to 4.02E-2	4.35E-4	3.19E-2
22	*fluxa*	Power	ln G	$\ln\sqrt{S_1S_2}$	7	-0.73	-4.20E0	6.58E0	3.22-3.70	-7.95E0	3.46
23	*kata*	No significant fit	$(S_2-S_1)/\Delta t$	S_1	8	-0.19	--	--	18.2-28.2	1.32E-2	23.0

NOTE.

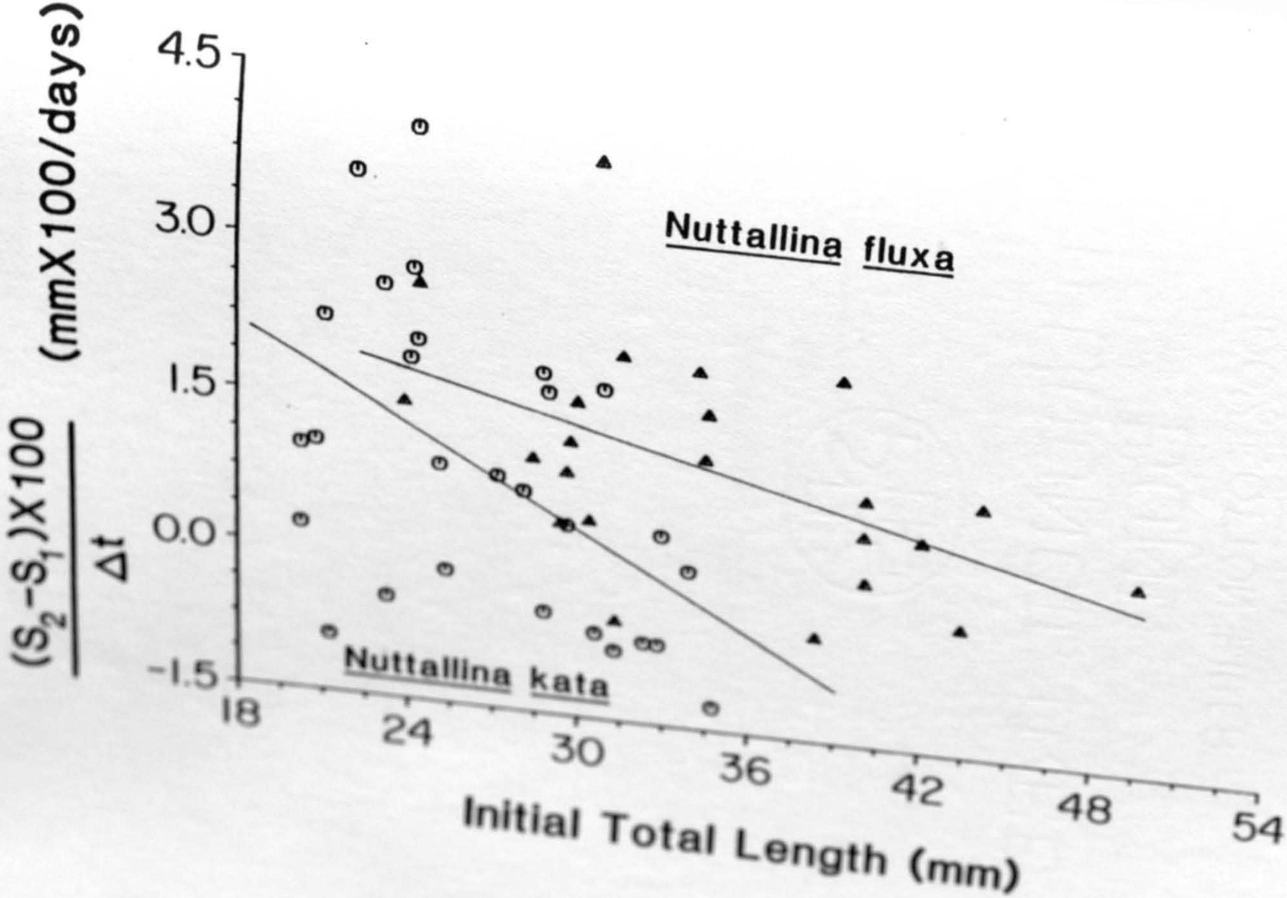
A. For chitons present through both growth intervals, the observed growth increments during the two intervals have been averaged (unweighted) on the appropriate axes to give a single growth interval. Residuals were examined using BMDP1R (Dixon and Brown, 1981) (see text). Use of the initial size, as the independent variable, in regression nos. 1, 5, 6, 12, and 18, systematically underestimates actual instantaneous growth rates. Underestimates vary with growth rate and the length of the time interval between growth estimates (Yamaguchi, 1975). Underestimate factors are ~0.92 for *N. fluxa* CTLs, 0.85 for *N. kata* CTLs, 0.95 for *N. fluxa* FVWs, 0.92 for *N. kata* FVWs, and 0.85 for *N. fluxa* CTL (year-long intervals).

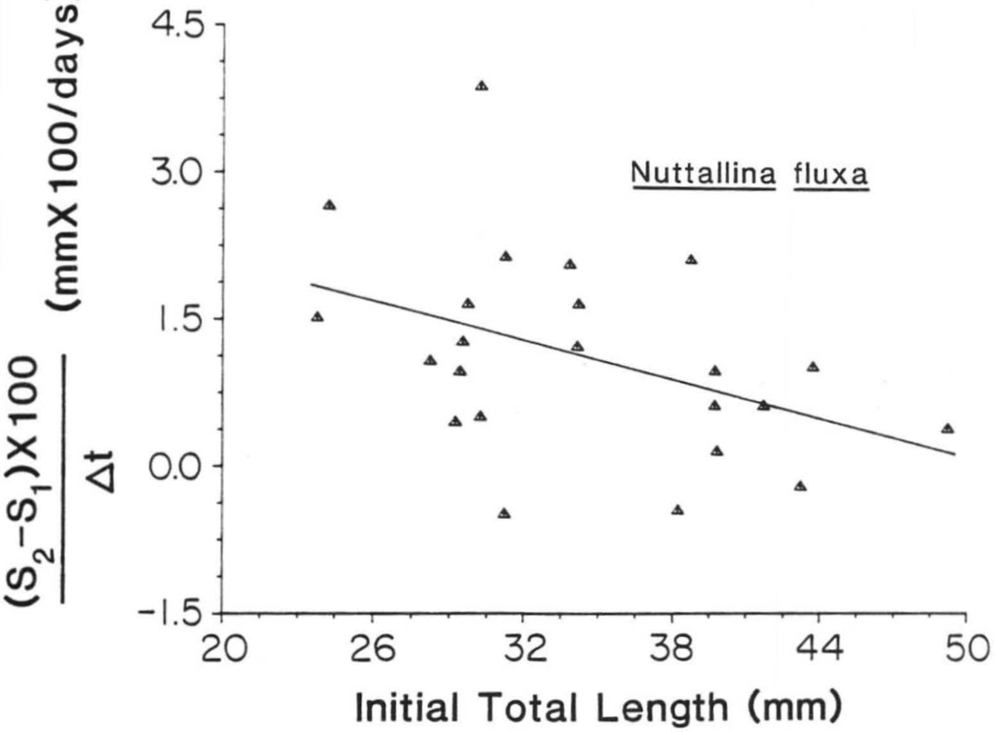
LIVE CHITON TOTAL LENGTH (CTL) GROWTH

The best linear fit for CTL growth data, for both Nuttallina fluxa and N. kata, was obtained on the Bertalanffy axes, $(S_2-S_1)/\Delta t$ versus S_1. For N. kata, these axes gave the only significant regression, whereas, for N. fluxa, four pairs of axes gave similar fits (Table VI-2). Examination of residuals (using BMDP1R computer program, Dixon and Brown, 1981, revision date: April, 1982) revealed that residuals were normally distributed in all cases. Tests for heteroscedasticity (Zar, 1974, p. 223) were not significant, although variances appear to be greater for small sizes (no transformation is available to remedy this situation easily).

Bivariate plots for the best regressions (Figure VI-2) reveal the great variability in growth observed among specimens of each species. Instances of negative growth were disconcerting at first, but a review of the literature suggested that such cases are real (see Discussion section). The great variability within each species indicates that the regressions are of limited use in describing growth of individual specimens, but such regressions can be used for describing growth of the studied populations and for comparing the populations. Using the best CTL regressions for Nuttallina fluxa and N. kata, i.e., relationships of $(S_2-S_1)/\Delta t$ and S_1, the growth of these two species were compared using an analysis of covariance (ANCOVA; BMDP1V computer program, revision date: April, 1982) and results are presented in Table VI-3 (Analysis No. 1). Analysis No. 1, in which the requirement for homogeneous slopes is satisfied ($p=0.18$, N.S.), clearly shows that the initial size-adjusted mean growth rate for Nuttallina fluxa is much greater

Figure VI-2. Field total length (CTL) growth in tagged specimens of *Nuttallina fluxa* and *N. kata* at the Seal Rock study site, La Jolla, CA. Axes correspond to a variation of the Bertalanffy growth model. See Table VI-2 for regression parameters. Considerable individual variability in growth was observed. Size and species distinctions help to explain some of the observed variability in growth. S_1 and S_2 are initial and final live chiton total lengths respectively; Δt is elapsed time in days. See text for details.


(mmX100/days)
$\frac{(S_2-S_1)X100}{\Delta t}$
4.5
3.0
1.5
0.0
-1.5
18
24
30
36
42
48
54
Initial Total Length (mm)
Nuttallina fluxa
Nuttallina kata


4.5
3.0
1.5
0.0
-1.5
20
26
32
38
44
50
$\frac{(S_2-S_1)X100}{\Delta t}$ (mmX100/days)
Nuttallina fluxa
Initial Total Length (mm)

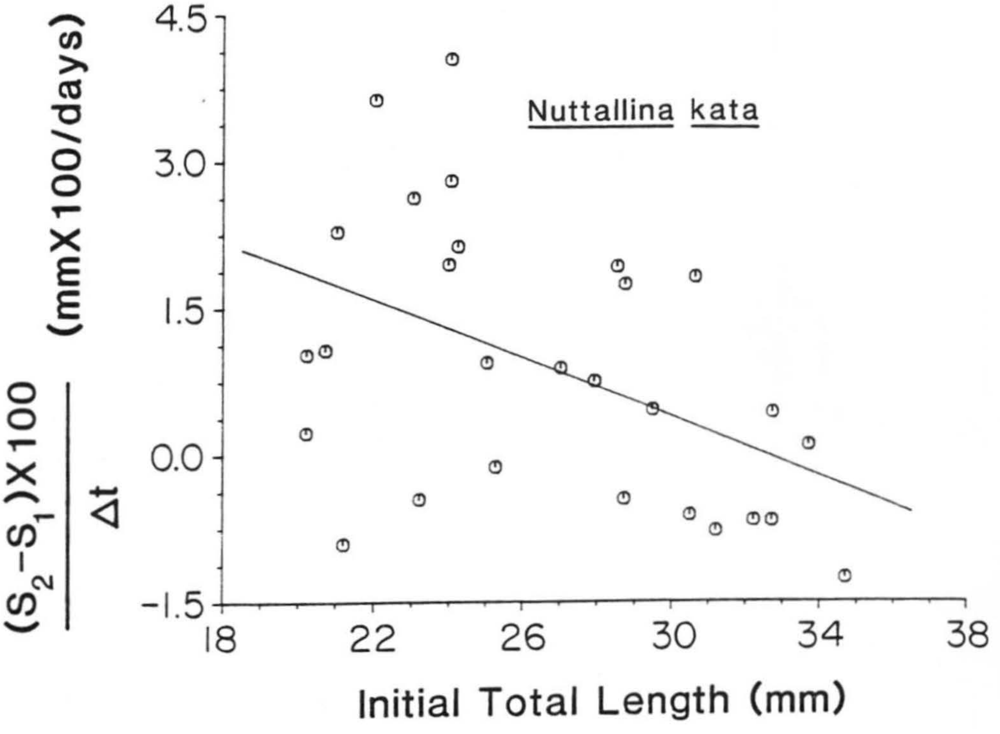

4.5
3.0
1.5
0.0
-1.5
18
22
26
30
34
38
$\frac{(S_2-S_1)X100}{\Delta t}$ (mmX100/days)
Nuttallina kata
Initial Total Length (mm)

TABLE VI-3. Results of comparisons of growth rates of *Nuttallina fluxa* and *Nuttallina kata* by ANCOVAs. ANCOVAs were performed using the BMDP1V computer program (Dixon and Brown, 1981; revision date: April, 1982). The dependent variable and covariate corresponding to the Bertalanffy growth model for length and the logistic model for valve width data were used in these analyses, since these models were found to adequately describe growth in the *Nuttallina* species (see Table VI-2). S_1 = initial size (CTL or FVW); S_2 = final size; G = size-specific growth rate estimated as $(\ln S_2 - \ln S_1)/\Delta t$. S in mm, t in days.

			Dependent Variable			Covariate			Significance Levels, P[A]		
Analysis No.	Species	N	Form	Unadjusted Mean	Adjusted Mean	Form	Unadjusted Mean	Adjusted Mean	Equality of Adjusted Means, of Dependent Variable (i.e., growth)	Zero Slope	Equality of Slopes
Total Length Growth Data											
1.	*Nuttallina fluxa*	23	$\frac{S_2-S_1}{\Delta t}$	0.0113	0.0154	S_1	34.5	30.2	0.02	0.002	0.18
	Nuttallina kata	28		0.0089	0.0055		26.7	30.2			
2.[B]	*Nuttallina fluxa*	30	$\frac{S_2-S_1}{\Delta t}$	0.0116	0.0163	S_1	33.8	29.7	0.006	0.0001	0.14
	Nuttallina kata	36		0.0097	0.0058		26.4	29.7			
3.	*Nuttallina fluxa*										
	"WINTER"	16	$\frac{S_2-S_1}{\Delta t}$	0.0153	0.0127	S_1	30.2	33.8	0.66	0.07	0.14
	"SUMMER"	14		0.0074	0.0104		37.8	33.8			
4.	*Nuttallina kata*										
	"WINTER"	21	$\frac{S_2-S_1}{\Delta t}$	0.0163	0.0137	S_1	23.9	26.4	0.10	0.08	0.70
	"SUMMER"	15		0.0005	0.0041		29.8	26.4			
Fourth Valve Width Growth Data ("SUMMER" only)											
5.	*Nuttallina fluxa*	14	G	1.0×10^{-4}	2.7×10^{-4}	$\sqrt{S_1S_2}$	12.6	9.9	0.03[D]	0.0003	0.01
	Nuttallina kata	14		2.3×10^{-4}	0.6×10^{-4}		7.2	9.9			

TABLE VI-3 (cont.)

NOTES

A. A $p \leq 0.05$ indicates that means are different, slopes are not zero, or slopes are not equal.

B. Analyses nos. 1 and 2 include the same *N. fluxa* and *N. kata* specimens, but the data have been analyzed differently. In no. 1, when two measurements were made for a chiton, i.e. both a "WINTER" and a "SUMMER" growth measurement, the measurements were averaged on the appropriate axes to yield one value for the chiton. In analaysis no. 2, when two measurements had been obtained for a chiton, the measurements were treated as independent data. The two methods yield similar results.

C. "WINTER" denotes the period starting in September to December 1979 and terminating in March to June 1980. "SUMMER" represented the period starting in March to June 1980 and terminating in November 1980.

D. In analysis no. 5, the test for a difference in the adjusted means of the dependent variable is invalidated by the inequality of slopes of data for the two species.

than that for N. kata (15.4 vs. 5.5 µm/day, $p<0.02$).

However, results of Analysis No. 1 (Table VI-3) are not entirely convincing since the ranges of the independent variable, initial size, for the two species are considerably different (Figure VI-2). Regressions describing the growth rate-size relationship in a certain size range do not necessarily describe this relationship in other size ranges of a population (see Kaufmann, 1981). An alternative approach for the species growth rate comparison is to use a simpler statistical test on data in a size range for which there is substantial overlap between the two species. Inspection of Figure VI-2 indicates that growth rate data for the range of initial lengths from 26.5 to 34.5 mm, noninclusive, is suitable for a conservative species comparison. Again, the CTL growth rate of Nuttallina fluxa is concluded to be greater than that of N. kata for specimens in the same initial CTL range [Mann-Whitney U test, Siegel, 1956, p. 124, $N_{\underline{kata}}= 13$, $N_{\underline{fluxa}}= 12$, $p=0.03$, two-tailed, corrected for ties].

CTL growth rates of Nuttallina fluxa and N. kata during Winter and Summer were not statistically different. However, Summer growth rates tend to be lower, particularly in Nuttallina kata (ANCOVA Analyses No. 3 and 4, Table VI-3).

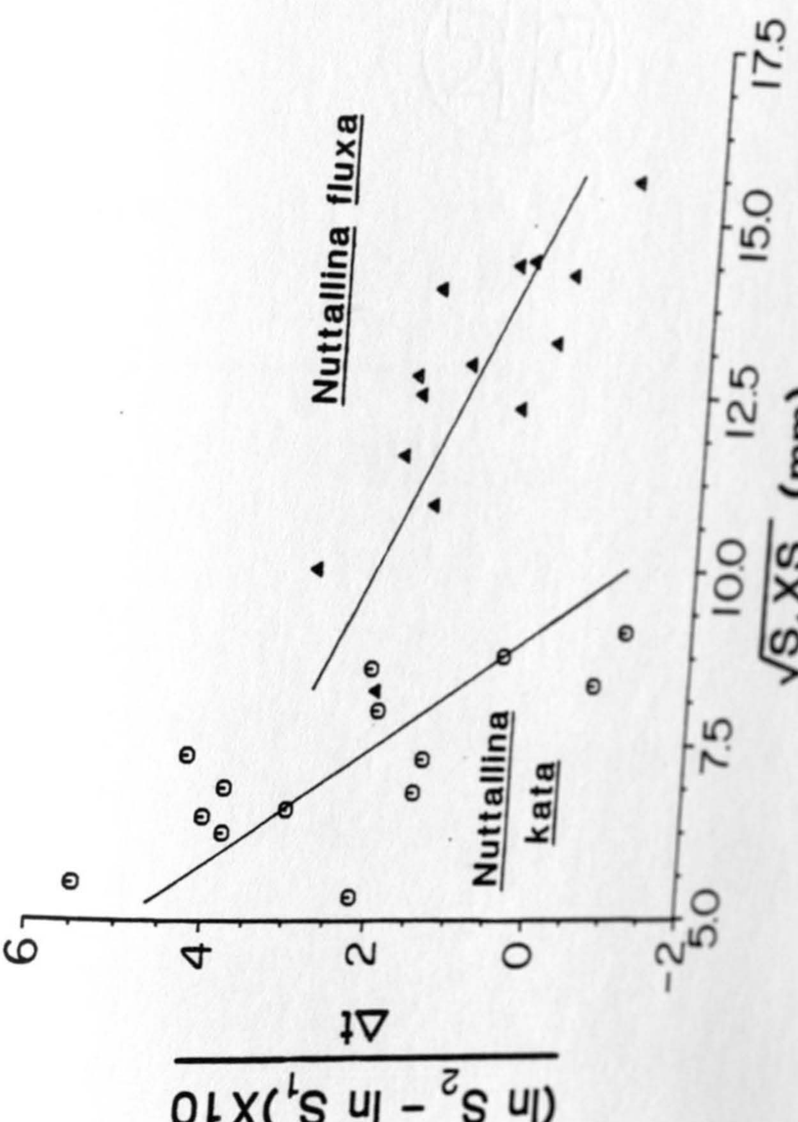
Examination of residuals of growth rate estimates on the regressions for the separate Nuttallina species in Figure VI-2 does not reveal a conspicuous tendency for positive or negative residual valves to be associated with particular habitats for either species. Growth estimates for Nuttallina kata were primarily for specimens in the low intertidal densely-populated habitat (23 of 28), whereas growth rate estimates for N. fluxa were mainly for specimens in the high shore area (21 of 23, with 8 of the 21 from high shore tidepools).

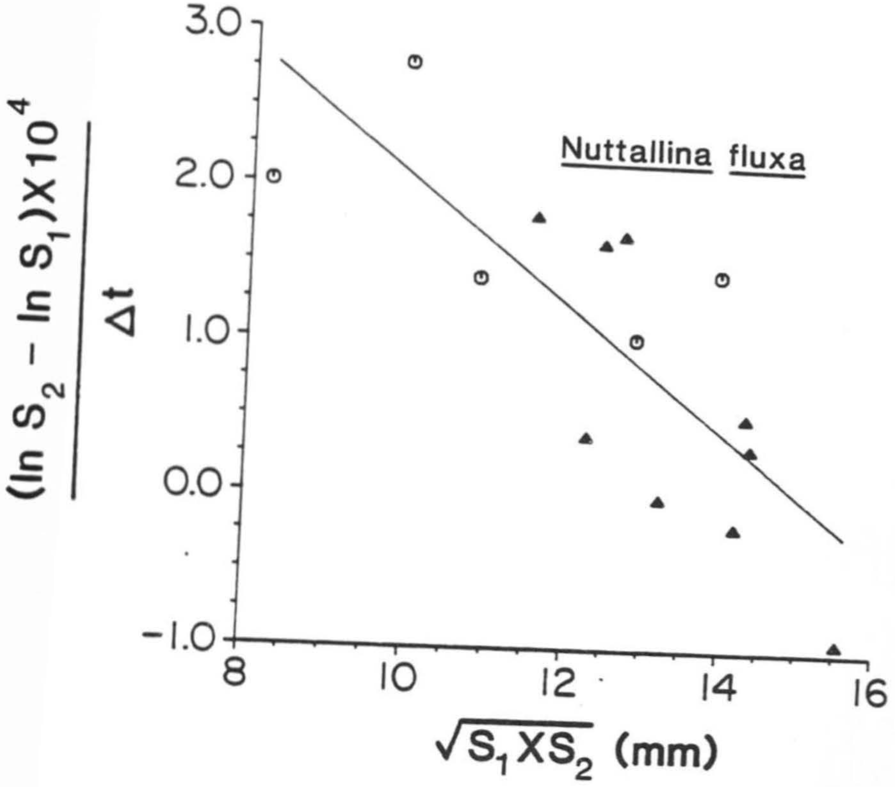
INTACT FOURTH VALVE WIDTH (FVW) GROWTH

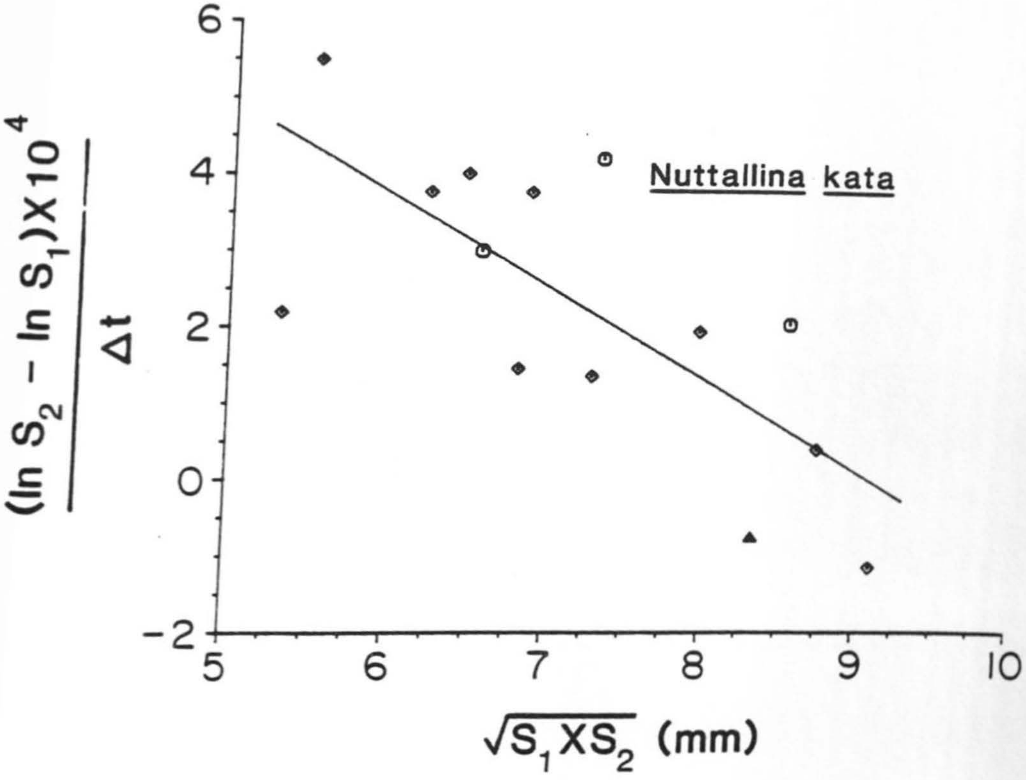
All growth models except the exponential one (power curve again not used because of cases of negative growth) provided good fits to the FVW growth data obtained for both Nuttallina species (Table VI-2). Data plotted on axes associated with the logistic growth model (suggested by Kaufmann, 1981) are presented in Figure VI-3. It is obvious that there is even less overlap for FVW ranges of study specimens of the two species than for CTLs, as expected from the fact that the tendency for Nuttallina fluxa to possess wider valves per length than N. kata (see Chapter IV) was used as the morphological criterion for assigning the study specimens to species.

Comparison of the FVW growth rates of Nuttallina fluxa and N. kata by ANCOVA, using dependent and covariate variables appropriate for the differential form of the logistic growth function, indicates that the slopes for the two species are significantly different; thus, comparisons of adjusted growth rates for the species by ANCOVA are invalid (Table VI-3: Analysis No. 5). ANCOVAs, with dependent and covariate variables appropriate for the other growth models, yield results similar to those of Analysis No. 5 (i.e., adjusted mean growth rates indicated to be different), with the difference that the slopes for the two species are not significantly different. The significant dependence of growth rate on FVW and the lack of substantial overlap in FVWs for

Figure VI-3. Field fourth valve width growth in tagged specimens of *Nuttallina fluxa* and *N. kata* at the Seal Rock study site, La Jolla, CA, for the "Summer" season only (see text). Axes are those suggested by Kaufmann (1981) for the logistic growth model. See Table VI-2 for regression parameters. Size and species distinctions help to explain some of the observed variability in growth. S_1 and S_2 are initial and final intact fourth valve widths respectively; Δt is elapsed time in days. Triangles = sparsely-populated, non-pool, high intertidal area; circles = high intertidal pool habitat; diamonds = densely-populated, low intertidal area.


(ln S_2 – ln S_1)X10^4 / Δt
6
4
2
0
-2
5.0
7.5
10.0
12.5
15.0
17.5
$\sqrt{S_1 X S_2}$ (mm)
Nuttallina kata
Nuttallina fluxa


3.0
2.0
1.0
0.0
−1.0
8
10
12
14
16
Nuttallina fluxa
$\frac{(\ln S_2 - \ln S_1) \times 10^4}{\Delta t}$
$\sqrt{S_1 X S_2}$ (mm)


6
4
2
0
−2
5
6
7
8
9
10
Nuttallina kata
$\frac{(\ln S_2 - \ln S_1) \times 10^4}{\Delta t}$
$\sqrt{S_1 X S_2}$ (mm)

specimens from the two species precludes effective comparison of the FVW growth data by statistical techniques other than ANCOVA.

FVW growth data plotted in Figure VI-3 reveal the habitats corresponding with specific data points. Within each species, it can be seen that growth rate estimates in different habitats generally conform to the regression line for all habitats combined. The two diverging prongs in the figure (toward lower growth rates, greater widths) for the two species are composed of values primarily from different habitats (low intertidal densely-populated habitat for the N. kata prong, high intertidal areas for the N. fluxa prong). Therefore, the apparent difference in growth patterns of the two species may have primarily an environmental basis rather than a genetic one. However, the single FVW growth rate datum for a specimen of Nuttallina kata occurring in the high intertidal non-pool habitat (Figure VI-3) contraindicates this suggestion.

ASYMPTOTIC SIZE AND TIME/AGE ESTIMATES

The largest specimens of Nuttallina fluxa and N. kata found in the field have been about 52 and 42 mm long, respectively (see Chapter IV). Asymptotic length estimates obtained from the Table VI-2 regressions (Table VI-4) agree, within the associated standard error intervals calculated by inverse prediction, with maximal sizes observed in the field. The fact that the asymptotic length estimate for Nuttallina kata is fairly low may reflect abnormally low growth rates, at least for large specimens during the study.

TABLE VI-4. Elapsed times associated with selected length increments, and estimated asymptotic lengths, of Nuttallina species. Regressions from Table VI-2 were used. Elapsed time estimates are most reliable for sizes near the observed mean of the independent variable. Use of initial size as the independent variable introduces a systematic bias tending to produce overestimates of elapsed times, since growth rates were determined over finite time intervals. S_1 = initial length, mm; S_2 = final length, mm; Δt = elapsed days.

Species	N	Observed Range of Initial Lengths, mm	Predicted Dependent Variable $((S_2-S_1)/\Delta t)$ at independent variable mean	Independent Variable (S_1), mean	Estimated Asymptotic Length ± S.E., mm[A]	Maximum S.E. of dependent variable within observed range of independent variable	Predicted time (yrs) elapsed over observed range of initial lengths (Range associated with maximum S.E. of dependent variable)
DATA FOR APPROXIMATE 6 MONTH INTERVALS							
N. fluxa	23	23.8 - 49.2	$9.9E^{-}3$	34.5	51 ± 15.0	$2.16E^{-}3$ @ X=49.2 mm	7.0 (5.8-9.0)
		25 - 30[B]	$1.6E^{-}2$	34.5		$2.07E^{-}3$ @ X=25.0 mm	0.9 (0.8-1.0)
N. kata	28	20.2 - 34.7	$7.6E^{-}3$	26.7	33 ± 8.8	$2.54E^{-}3$ @ X=34.7 mm	5.2 (3.9-7.8)
		25 - 30	$7.5E^{-}3$	26.7		$2.45E^{-}3$ @ X=30.0 mm	1.8 (1.4-2.7)
DATA FOR APPROXIMATE YEAR INTERVALS							
N. fluxa	7	22.2 - 40.2	$1.2E^{-}2$	29.5	49 ± 7.1	$1.66E^{-}3$ @ X=40.2 mm	4.0 (3.6-4.7)
		25 - 30	$1.5E^{-}2$	29.5		$1.67E^{-}3$ @ X=25.0 mm	0.9 (0.8-1.0)
N. kata	8	18.2 - 28.2	$1.3E^{-}2$	Regression Not significant, $p > 0.5$		$7.76E^{-}3$	2.1 (1.3-5.0)

NOTE

A. Obtained by inverse prediction, Zar, 1974:213-214.

B. Values estimated for 25-30 mm since growth for specimens in this size range was observed for both species and the values obtained allow for more direct comparisons of elapsed times between the species.

The asymptotic length estimate for Nuttallina fluxa, obtained using only CTL growth data for specimens present for two growth intervals (343 to 434 days total), agrees well with the estimate derived from single growth interval data (Table VI-4; 49 mm and 51 mm, respectively). Similar circa-year-interval growth data for Nuttallina kata did not show a significant relationship with size, i.e., the slope was not significantly different from zero, so that an estimate for the asymptotic length can not be made.

Elapsed time and age estimates can be associated with Nuttallina sizes using the regressions in Table VI-2 and Figures VI-2 and VI-3. Such estimates are most reliable within the range of observed sizes, for estimating time elapsed during growth from one size to another. One has to be cautious in extrapolating to sizes out of the observed size range, since the relationship of growth rate to size is not necessarily constant for Nuttallina of all sizes.

Elapsed time estimates for the entire observed initial size range for each species and for a size range of overlap (25 to 30 mm CTL) between the two Nuttallina species are presented in Table VI-4. These estimates were made using regressions listed in Table VI-2. Since growth rates decrease with increasing size, elapsed time estimates made for a size range near the asymptotic size will be greater than for a range far below the asymptotic size. For example, in N. kata, the estimated elapsed time for CTL growth from 20.2 to 34.7 mm (5.2 years) is expected to be much greater than that for the same size increment at smaller sizes (e.g., 5.7 to 20.2 mm). This effect undoubtedly contributes to the fact that the estimated elapsed time for growth of

Nuttallina kata from 25 to 30 mm in length is greater than it is for N. fluxa (1.8 versus 0.9 years).

Absolute ages of Nuttallina specimens are of more interest than are elapsed times for growth between different adult sizes. Fabens (1965) has developed a computer program for deriving an age-size Bertalanffy growth curve from data sets like those of the present study, that consist of pairs of recorded sizes and the time intervals between them. The Fabens (1965) program can be used to describe the CTL growth data for Nuttallina fluxa and N. kata since growth data for both species give significant fits to the Bertalanffy growth model (Table VI-2).

In order to describe the Nuttallina CTL data by Fabens' (1965) program, it must be assumed that the growth of chitons less than 20 mm in length is also adequately described by the Bertalanffy growth model, since data were not obtained for such specimens in the present study. Such as assumption is partially justified in view of the applicability of the Bertalanffy model for other molluscs that have been studied (e.g., see Brousseau, 1979, but see Kaufmann, 1981, p. 299). Fabens' (1965) program requires that the age be supplied for at least one specimen of known size, to permit calculation of the constant of integration that is needed to generate the age/size Bertalanffy growth curve. Preferably, an age and its corresponding size within the range of observed sizes should be supplied (see Kaufmann, 1981), rather than a size at birth as is suggested by Fabens (1965). Such information is not available for either of the Nuttallina species. Therefore, a range of sizes that reasonably might be expected to describe a one-year-old specimen of Nuttallina were supplied to the Fabens (1965) program to set constants of integration for different curves. By this procedure, a family of curves was generated for each species. Use of different constants of integration in this way has no effect on either the shape of the growth curve or the estimated asymptotic size (Kaufmann, 1981); the sole effect of varying the constant of integration is to translate the growth curve along the abscissa, i.e., the age axis.

Families of Bertalanffy age-length curves for Nuttallina fluxa and N. kata, generated with Fabens' (1965) program, are presented in Figure VI-4. As can be seen, use of different constants of integration produces its largest effect on the variation in age-length relationships at short lengths, and produces progressively smaller variations at longer lengths, e.g., in the range of lengths observed in the present study. The Bertalanffy curves indicate that most Nuttallina specimens in the present study are less than 10 years old, if indeed growth of specimens shorter than 20 mm in length can be described by the Bertalanffy model, as can the growth of the larger specimens.

Linear plots on axes suggested by Kaufmann (1981) and residual analyses indicated that the logistic growth model best described the FVW growth data of both Nuttallina species (see Table VI-2). Age-FVW growth curves, estimated using the logistic growth model parameters of Table VI-2, for Nuttallina fluxa and N. kata are presented in Figure VI-5. Use of the logistic model to describe the growth of Nuttallina entails the assumption that the growth of chitons smaller than those observed in the present study, i.e., less than 8.1 mm FVW for N. fluxa and 5.2 mm FVW for N. kata, like that of the observed specimens, can be described adequately by the logistic growth model. Kaufmann (1981) has noted that

Figure VI-4. Bertalanffy age/total-length curves for *Nuttallina fluxa* and *N. kata* at La Jolla, CA.

Curves were generated from the total length data of Figure VI-2 using the computer program developed by Fabens (1965). Since an age cannot be supplied for *Nuttallina* at a particular size as required to estimate the constant of integration, 5, 10 and 15 mm have been taken as reasonable estimates of chiton total length at one year to estimate the integration constant and to generate a family of three curves for each species. The curve for which a 10 mm long specimen is taken to be 1 year old falls in the middle of each family of curves. See text for details.

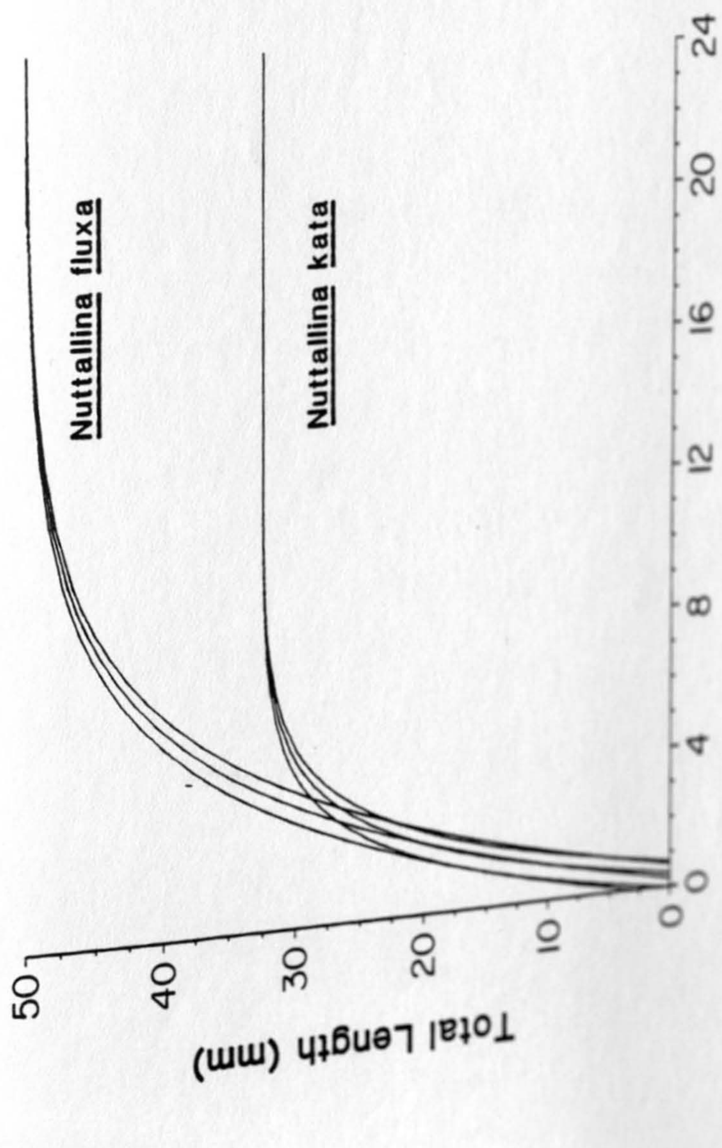
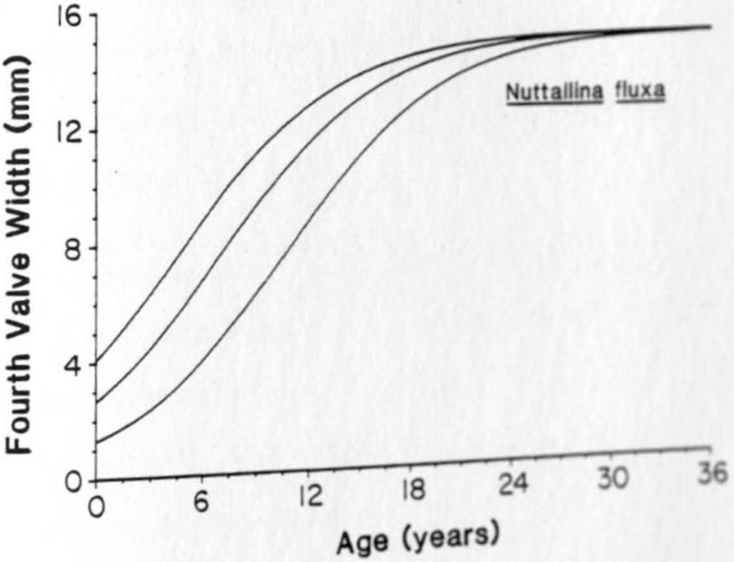
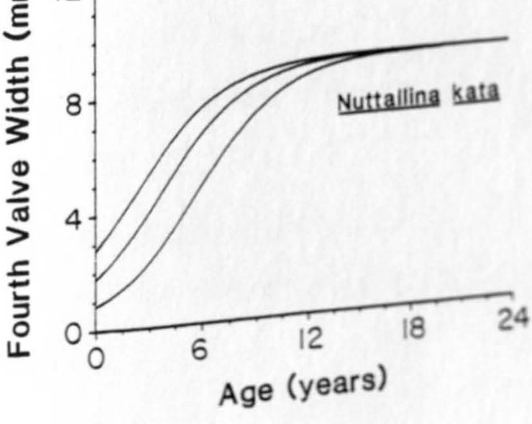

Total Length (mm)
50
40
30
20
10
0
0
4
8
12
16
20
24
Age (years)
Nuttallina fluxa
Nuttallina kata

Figure VI-5. Logistic age/fourth-valve-width curves for *Nuttallina fluxa* and *N. kata* at La Jolla, CA.

Curves were generated from the fourth-valve-width (FVW) growth data of Figure VI-3, using suggestions of Kaufmann (1981). The correction factor explored by Kaufmann (1981) is negligible for both species. Since an age cannot be supplied for *Nuttallina* at a particular size, as required to estimate the constant of integration, a family of three reasonable curves has been generated for each species. For *N. fluxa*, FVWs of 1.6, 3.2 and 4.8 mm at one year were used to estimate the integration constants; for *N. kata*, FVWs of 1.2, 2.4 and 3.6 mm at one year were used. These values approximately correspond to live total lengths (CTLs) of 5, 10 and 15 mm (used in Figure VI-4), as indicated by calculations using data from Table IV-3 (with Table IV-9 conversions) or from the Model I regressions in Table V-2. FVW data, unlike CTL data, were obtained only for the "Summer" season, during which growth rates are lowest. This seasonal bias helps to explain why age estimates obtained from FVW data are higher than those from CTL data. See text for details.


Fourth Valve Width (mm)
16
12
8
4
0
0
6
12
18
24
30
36
Age (years)
Nuttallina fluxa


Fourth Valve Width (mm)
12
8
4
0
0
6
12
18
24
Age (years)
Nuttallina kata

growth rate analyses utilizing axes suggested in his paper entail application of a factor to correct for a systematic underestimate of the size-specific growth rate. Correction factors for the logistic growth model vary with size and are difficult to estimate. Kaufmann (1981) has supplied correction factors for the Gompertz growth model, and calculation of these for Nuttallina fluxa and N. kata from parameters listed in Table VI-2 reveals that the corrections involved are much less than 0.5% in the worst case, i.e., for the longest time interval between FVW measurements, 222 days.

Again, since an age is not known for a specimen of any size, the constant of integration cannot be estimated. Thus, a family of age/FVW logistic curves corresponding to a range of integration constants are presented for each species. For the logistic curve, as for the Bertalanffy curve, the effect of changing the constant of integration is to translate the curve along the abscissa, i.e., the age axis; the shape of the curve and its asymptote remain unchanged. As can be seen by comparing the general shapes of the logistic and the Bertalanffy families of curves (Figure VI-4; VI-5), age/size relationships at intermediate sizes are much more dramatically influenced by the choice of the constant of integration for the logistic curve than they are for the Bertalanffy curve. This is a consequence of the occurrence of an inflection in the logistic curve (at half asymptotic size), and the lack of one in the Bertalanffy curve. Translation of an inflected curve along the abscissa substantially alters x-y relationships (translation of the Gompertz curve has an intermediate effect, since this curve has an inflection at a relatively small size, $0.37\ S_{\infty}$).

Therefore, it is desirable to supply a reasonable guess for a post-settlement FVW and a corresponding age to set the constant of integration, and thereby to obtain a particular logistic age-FVW curve from among the family of curves (Figure VI-5). Unfortunately, _Nuttallina_ species do not appear to have a restricted spawning period so that year classes are not obvious. The smallest _Nuttallina_ specimens ever observed in the field were 3 to 6 mm long, and a 10 mm length is probably a reasonable estimate for a one-year-old specimen. If one uses morphometric data and appropriate conversion factors listed in Chapter IV (Tables IV-3 and IV-9; non-Gaviota data for _N. fluxa_) or the predictive regression presented in Chapter V (Table V-2), FVWs for one-year-old chitons are then estimated to be 3.2 mm for _N. fluxa_ and 2.4 mm for _N. kata_. FVW growth curves for the two species, generated with integration constants determined from these FVW-age estimates, are illustrated in Figure VI-5. These curves indicate that a specimen of _Nuttallina fluxa_ with a FVW of 11 mm (3/4 asymptotic FVW) is about 11 years old, and a specimen of _N. kata_ with a 7 mm FVW (about 3/4 asymptotic FVW) is about 7 years old.

Since FVW is not allometric with respect to CTL in either _Nuttallina fluxa_ or _N. kata_ (see Chapter IV, Table IV-3), age estimates derived from FVW growth data should be similar to those obtained from CTL growth data. However, FVW growth analyses give rise to older age estimates than do CTL analyses (compare Figures VI-4 and VI-5). In addition to contributions from sampling and measurement error, this discrepancy is probably a consequence of the fact that FVW measurements, unlike CTL ones, were obtained only for the "Summer" period, during which the Nuttallina species tend to exhibit slower growth rates (see Winter-Summer growth rate comparisons, Table VI-3).

DISCUSSION

Several techniques have been used to determine growth rates of molluscs, including internal and external shell growth line counts, shell natural radionuclide ratios, size-frequency analyses, mark and recapture, and more recently $^{18}O/^{16}O$ shell profiles. The feasibility of the different techniques varies with the biology of the species studied. For Nuttallina, the mark/recapture procedure and possibly $^{18}O/^{16}O$ shell profiles appear to be the only practicable techniques available for growth rate estimation.

Background information on $^{18}O/^{16}O$ ratios has been presented by Rye and Sommer (1980). Although $^{18}O/^{16}O$ shell profiles have been used with success for determining Mytilus californianus growth patterns in the general vicinity of the present study (Killingley and Berger, 1979), preliminary $^{18}O/^{16}O$ determinations on serial samples from the valves of La Jolla Nuttallina have not revealed the expected conspicuous annual temperature cycles requisite for ageing specimens, nor have the temperatures estimated from the $^{18}O/^{16}O$ measurements spanned a major portion of the expected annual surface ocean temperature range of the La Jolla area, except in samples from the main valve area of the specimen of Nuttallina kata (Figure VI-1).

Several factors might explain the minimal temperature variation reflected in the shell samples: 1) adequate sampling resolution has not been achieved and each sample consists of shell deposited over a large part of a year, i.e., annual temperature cycle, such that the sampling has produced temperature averaging; 2) shell material deposited serially with time has not been sampled; 3) shell is deposited only while a specimen is subject to temperatures over a narrow range, e.g., 16.5 to 18.5°C; or 4) shell is not only deposited while the animal is immersed in seawater, and therefore aerial temperatures confound the shell temperature record. The most likely explanations are that sampling resolution has not been adequate and/or that shell growth occurs during tidal emersion.

Certainly, Nuttallina shells and their annual growth increments are considerably smaller than those of molluscs and barnacles previously aged by the $^{18}O/^{16}O$ technique (see Wefer and Killingley, 1980; Killingley and Newman, 1982). The use of a 0.5 mm diameter dental drill coupled with the capability for precise analyses of small samples (<0.5 mg) permitted sampling at very close intervals, but the complexity of shell growth apparently limits the amount of temporally-homogeneous shell material available. In the sutural plate area, shell is deposited not just at the anterior edge but presumably also ventrally and dorsally, so that successive shell layers are juxtaposed in a dorso-ventral as well as an anterio-posterior axis (see Baxter and Jones, 1981). It is particularly likely that the sample series from the sutural plate of Nuttallina kata is temporally-sequential; this is indicated by the ^{13}C trend, with higher values (i.e., values nearer equilibrium) in newer shell, as has been found in other organisms (e.g., Mulcahy et al., 1979).

There are many examples of molluscs that stop or slow growth in colder water during the winter months (e.g., Palmer and Frank, 1974; Wefer and Killingley, 1980; and see Allen, 1963), but it is highly improbable that the small temperature variation reflected in $^{18}O/^{16}O$ determinations results from such a phenomenon in *Nuttallina*. Both *Nuttallina* species of the present study have extensive latitudinal ranges, from Monterey Bay and Point Conception to southern Baja California, which indicates a capacity to thrive in a broad range of temperatures. In addition, mark/recapture results suggests that, if anything, growth may slow not in the colder months, i.e., the Winter interval, but in the warmer Summer interval (Table VI-3).

The possibility that intertidal molluscs grow when exposed to air is untested. However, the intertidal barnacle *Semibalanus* (= *Balanus*) *balanoides* apparently grows only when immersed (Bourget and Crisp, 1975). Further, tidal growth rings in intertidal mussels and other bivalves may result from shell dissolution caused by the build-up of acidic anaerobic metabolites when these animals are exposed to air (Lutz and Rhoads, 1977, 1980). If anaerobiosis is an important factor determining whether or not shell is deposited, it might be that higher shore species, that respire in air better than in water in some cases, unlike lower shore species (see, e.g., Murdoch and Shumway, 1980), and thus might avoid the necessity of accumulating acidic anaerobic end-products, deposit rather than dissolve shell when exposed to air. Certainly, land snails deposit shell while exposed to air. If *Nuttallina* does deposit shell while exposed to air, an uninterpretable temperature record, reflected in the $^{18}O/^{16}O$ profile, would result in the La Jolla area. The ocean surface temperatures in La Jolla follow a relatively simple annual pattern with the lowest values in January-March and the highest values in August-September (Anonymous, 1980, 1981). However, the lowest low tides, i.e., when intertidal animals are exposed to air for long periods, occur in the afternoon in Winter (warm air, cool ocean temperatures) and at night in Summer (cool air, warm ocean temperatures). Thus, the inclusion of tides into the environmental scheme, reflected in shell deposition during aerial exposure, would produce an intertidal temperature record much more complex than that produced by the surface ocean water itself.

The fact that the average temperature reflected in the sutural plate series from N. kata falls at about the annual average surface water temperature value for La Jolla, about 17°C (Figure VI-1; Anonymous, 1980, 1981), could reflect either poor sampling resolution, i.e., temperature averaging, or a contribution of shell deposited during aerial exposure. However, the series of four samples from the valve main body of the same chiton exhibit a different pattern, with substantial variation in ^{18}O values (Figure VI-1; Table VI-1); this discrepancy indicates that the relative lack of useful variation in $^{18}O/^{16}O$ values for the sutural plate is a sampling problem.

The most promising $^{18}O/^{16}O$ results were obtained by sampling the valve main body of Nuttallina kata; in samples from the valve main body, there is a suggestion of a cycle in the plotted points (Figure VI-1). Clearly, since the downphase of the temperature cycle is demarcated by only a single sample, it is not safe to assume that the apparent single cycle is not a serendipitous conglomeration of points from several temperature cycles. If the apparent cycle is interpreted as a single annual temperature cycle, these results would indicate that this 35 mm long specimen of N. kata was 2 to 3 years old, with one annual cycle per 2 mm of a shell with an overall dimension of 6.4 mm. However, it is odd that the single apparent cycle involves only the lower part of the observed La Jolla surface temperature range, and age estimates obtained using the mark/recapture technique (discussed later) indicate that a 35 mm long specimen of N. kata is probably much older than 2 or 3 years. While the low age estimate suggested by $^{18}O/^{16}O$ measurements is within the potential range of values indicated by the mark/recapture method, it is probable that the single apparent $^{18}O/^{16}O$ cycle in Figure VI-1 is made up of more cycles, i.e., that even for the most promising $^{18}O/^{16}O$ results obtained to date, sampling resolution may be inadequate.

$^{18}O/^{16}O$ studies on Nuttallina have not been exhaustive. In future work, samples can be obtained at closer intervals in the articulamentum layer of the valve main body. The outer shell tegmental layer has been avoided in initial studies because it consists of an organic matrix and contains much of the shell nerve tissue, i.e., aesthetes, in addition to aragonite. However, the fact that apparent growth lines occasionally occur in the external sculpturing of Nuttallina valves indicates that the outer tegmental layer may contain an easily interpretable $^{18}O/^{16}O$ record and that it should be the object of future sampling. Certainly, successful $^{18}O/^{16}O$ analyses would provide valuable information for Nuttallina that cannot be obtained easily by any other method. Such analyses, which are in progress, should indicate whether poor sampling resolution or some other factor, such as subaerial shell growth, is an insurmountable obstacle for the effective use of $^{18}O/^{16}O$ determinations for the estimation of growth rates of Nuttallina individuals.

In the present study, mark/recapture growth measurements were obtained for more chiton specimens (N=61) than in any previous study, except in that of Palmer and Frank (1974) for the giant gumboot chiton, Cryptochiton stelleri. Considerable individual variability in growth was observed in the present study (Figures VI-2, VI-3). Such a phenomenon is commonly indicated in growth studies on intertidal molluscs where raw data are presented (e.g., Boolootian, 1964; Daly, 1975; Bretos, 1978). Interestingly, different specimens of the intertidal snail Thais lamellosa from a single population exhibited growth rates varying by a factor of more than 2, even when food, population density, and temperature were kept constant (A. R. Palmer, personal communication to Vermeij, 1980). In addition, in the present study, instances of negative growth were observed, as has been observed for other chitons and limpets in the field (e.g., Boolootian, 1964; Palmer and Frank, 1974; Daly, 1975), as well as for Nuttallina specimens maintained in the laboratory (Appendix IV).

Regression analysis indicates that some of the observed variability in growth of Nuttallina can be explained by the effect of size. Larger specimens exhibit slower growth rates in both N. fluxa and N. kata. This size effect is quite common in animals and various mathematical formulae have been used to describe several such growth-size patterns in individual organisms. Among these formulae are the Bertalanffy

(Bertalanffy, 1938, 1960), Gompertz (Winsor, 1932), and logistic (Verhulst, 1838; Robertson, 1923) asymptotic growth models. Fits of these growth models, and of the exponential and power growth models (Kaufmann, 1981), to the *Nuttallina* growth data were examined by regression analysis, using dependent and independent variables from the differential equations of the models, as suggested by Van Devender (1978) and Kaufmann (1981). Due to the individual variability in the growth data, none of the models described growth rates of total length (CTL) with respect to CTL well. Several of the models described the CTL growth data of *Nuttallina fluxa* about equally, probably as a result of the variability of the data and of the fact that less than the entire range of sizes of *Nuttallina* were studied (Table VI-2; see Kaufmann, 1981). Examination of residuals indicated that a variant of the Bertalanffy growth model provided the best fit for CTL growth of both *N. fluxa* and *N. kata*. Fourth valve width (FVW) growth data exhibited much less unexplained variability than did the CTL data, and though several models provided similar fits to the data, the logistic growth model provided the best description for data for both *N. fluxa* and *N. kata* (Table VI-2).

By describing the *Nuttallina* growth rate data in terms of the linear formulations of the growth models above, one can compare the growth rates of *Nuttallina fluxa* and *N. kata* effectively, as well as demonstrate that the growth rates are size-dependent. ANCOVAs, with size (CTL or FVW) as the covariate (Table VI-3: Analyses No. 1 and 5), suggest that *N. fluxa* and *N. kata* in this study were growing quite differently. In particular, Analysis No. 1 (Table VI-3) suggests that specimens of N. fluxa grow faster than those of N. kata, when growth rates are adjusted to a CTL of 30 mm. This result indicates that the intertidal size gradient observed for Nuttallina, with larger specimens (primarily N. fluxa) in the high intertidal and smaller specimens (primarily N. kata) on the lower shore (Chapters II,III,V), is a consequence of differential growth rates, at least in part. Of the other potential factors contributing to the shore-level size gradient, migration upshore has been discounted (Chapter V) whereas differential mortality has not been studied.

ANCOVA No. 5 (Table VI-3) for FVW growth data, with FVW as the covariate, reveals that the slope for the growth rate versus FVW relationship is quite different for Nuttallina fluxa and N. kata. Although CTL and particularly FVW growth rate comparisons between the two species are compromised somewhat by limited ranges of overlap in size between the species, a substantial portion of the variation in growth rate data is apparently explained by species differences (Figures VI-2 and VI-3). Because of the strong species-habitat associations, however, it is difficult to separate habitat effects on growth rates from purely genetic effects. Discussion of Nuttallina ages, later, will reveal that detecting growth rate differences between N. fluxa and N. kata was probably partially a fortuitous result of using the larger specimens of both species. Certainly, species growth rate comparisons of specimens of all sizes combined would have revealed differences, but comparisons for small specimens of the species only might have revealed similar growth rates as is suggested by extrapolations to small sizes, e.g., see Figures VI-4 and VI-5.

What ages are indicated for *Nuttallina* specimens by the mark/recapture data? $^{18}O/^{16}O$ measurements reported in the present study indicated that a 35 mm long specimen of *N. kata* was at least 3 years old (Figure VI-1) and MacGinitie and MacGinitie (1949, 1968, p. 386) have proffered a guess of "at least twenty or twenty-five years" for the age of the oldest *Nuttallina* specimens. However, since both *Nuttallina fluxa* and *N. kata* appear to approach an asymptotic size, or at least grow so slowly as large specimens that they reach a functional size asymptote, as do most other animals that have been studied, an age estimate such as that of the MacGinities' (1968) is of limited utility and testability. Examination of Figure VI-4 reveals that an "ideal" *Nuttallina* specimen of near-asymptotic size can vary greatly in age, on the order of tens of years.

More useful and testable age estimates can be made for sizes substantially smaller than the asymptotic value. The most reliable of such estimates that can be obtained from the present study are those of the average time required to grow from one size to another within the size range actually observed, as has been done in Table VI-4. Further, using the assumption that the growth of small individuals, that could not be monitored during this study due to the restraints of the marking technique, can be described adequately by the growth model that fits the observed growth of the larger individuals, absolute ages can be assigned to specimens of particular sizes. Age estimates derived from CTL and FVW data in this manner indicate that most specimens of both *Nuttallina* species are less than 10 years old (Figures VI-4, VI-5). This estimate is testable and can be useful because it allows one to make fairly refined conclusions about the biology of the animal. In particular, population dynamics studies can benefit by this age estimate since it permits population turnover rates to be estimated.

The great variability in individual growth rates observed in this study indicates that specimens of a particular size may be of considerably different ages. Thus, it is clear that the $^{18}O/^{16}O$ technique, or a similar technique that can allow one to age a particular individual, can be indispensable where detailed knowledge of the age structure of a group of specimens are required.

Limited data for growth of Nuttallina in Southern California have been obtained in only two other studies. Louda (1972) obtained sixth valve width growth measurements for twelve specimens of "Nuttallina fluxa" (probably N. fluxa of the present study) subjected to experimental field conditions of reduced Nuttallina density and complete limpet removal, i.e., conditions potentially conducive to abnormally rapid growth. Louda (1972) recorded two instances of negative growth, a phenomenon observed also in the present study. Regression analysis of Louda's (1972) data revealed a poor relationship between sixth valve width and growth rate ($r<0.2$ for all axes used in the present study). The specimens, ranging in initial sixth valve width from 7.9 to 11.9 mm, grew from an average width of 9.6 to 10.4 mm in 139 days, from 30 November 1971 to 17 April 1972. This rate of growth is much greater than that observed for N. fluxa FVW in the present study (Table VI-2, Regression No. 10). Assuming that the sixth valve width of N. fluxa is not allometric with respect to the fourth valve width, the higher growth rate observed in Louda's (1972) study can be explained by her experimental conditions, expected to be favorable to fast growth, and possibly by the fact that the study was undertaken in Winter, which findings in my study suggested may be more favorable to Nuttallina growth than Summer, when my FVW measurements were obtained.

Harvey (1963) obtained growth data for specimens of "Nuttallina fluxa" (N. fluxa and/or N. kata of this study?) held in an aquarium from 3 September 1961 to 1 June 1962. Regression analyses of his data revealed positive relationships between CTL growth rate and CTL, but since initial CTLs only varied from 8.0 to 11.3 mm, the growth data can be pooled. During the 271 days between measurements, the twenty chitons in his study grew from 9.5 (S.D.=1.12) to 15.8 (S.D.=1.81) mm in average length. Clearly, these chitons, held in an experimental aquarium, could exhibit growth rates much different from rates they might attain in the field. The chiton specimens observed by Harvey (1963) were much smaller than those of the present study and comparison of their growth rates with those of specimens of the present study requires extensive extrapolation. As might be expected for chitons held in the laboratory, small Nuttallina in Harvey's (1963) study tended to exhibit lower growth rates (0.023 mm/day) than predicted from the regressions for large field specimens of the present study (0.028 and 0.034 mm/day for N. fluxa and N. kata respectively, using CTL regressions in Table VI-2). Age estimates obtained from a combination of Harvey's (1963) data and that of the present study are similar to those made from growth data of the present study alone. For CTL growth data of either N. fluxa or N. kata combined with the Harvey (1963) data, the Gompertz growth model provides a better fit than does the Bertalanffy model and the Gompertz curve indicates that most specimens of either species are less than 10 years old.

How do growth rate estimates for Nuttallina compare with those for other chitons? Examination of the literature on chiton growth reveals that the growth estimates available for most chiton species, and presented in popular works such as MacGinitie and MacGinitie (1968) and Morris et al. (1980), are based on little or no direct evidence. The growth data available for chitons, with corresponding growth rate estimates, are summarized in Table VI-5.

It appears that strict growth rate comparisons between chiton species in different studies are not presently feasible. The growth data available for any particular chiton species are generally scanty, making it difficult to generalize and, particularly, to predict the effects that season and locality have on growth rates. Unfortunately, in cases where different workers have studied a species, growth estimates have been quite different, apparently as a result of the difference in techniques used. For example, in a study of Chiton tuberculatus in Puerto Rico, Panama, and Bermuda, Glynn (1970), using size-frequency analyses primarily, concluded that the mean life span of the chiton is probably near 2 years, rather than 8 years as concluded by Crozier (1918a,b) and Arey and Crozier (1919) who used external shell groove counts primarily. Glynn (1970) found that specimens from one of his study locations had a groove number to length relationship similar to that of specimens from Crozier's location, which suggests that chitons from both areas had similar growth rates. However, Glynn (1970) was unable to determine Crozier's method of calibration, which he presumed

TABLE VI-5. Growth Information Available for Chitons. Studies are arranged according to the primary means utilized to obtain growth estimates

Species	Source	Location; Habitat	Sample Size	Initial Size Range of study specimens	Growth Rate	Date; duration	Maximum size attained (source if another study)^A	Comments
I. GROWTH DATA OBTAINED BY MARK/RECAPTURE OR ISOLATION								
Cryptochiton stelleri	Palmer and Frank, 1974	Oregon; intertidal and subtidal to 10 m	228	~50-1300 g (most 500-800g)	Mean growth=90-155g/yr for specimens 300-800g	Variable duration; 3 month to 3 yr intervals in 1960's, early 1970's	1300 g ?	Estimates: 400 g chiton=5 years old; 800 g=8 years; 1200 g=16 years. Growth quite variable; many instances of negative growth in winter. Unable to use external shell lines to age chitons as had the MacGinities (1968b). Tag loss rate high and variable.
Mopalia ciliata, *M. hindsii*, *M. muscosa*	Barnawell, 1954	San Francisco Bay, CA; intertidal	8 (17 recaptures)	--	*M. ciliata*: 11-40 mm in length per year; *M. hindsii*: 20-50 mm/yr; *M. muscosa*: 15-34 mm/yr	Seven month period	*M. ciliata*: 7.5 cm in maximum length; *M. hindsii*: 10 cm; *M. muscosa*: 9 cm (Morris et al, 1980)	Sample size extremely small
Mopalia muscosa	Boolootian, 1964	Santa Monica Bay, CA; intertidal	31	Not reported	Most specimens lost weight; a few specimens exhibited shell growth	One to thirteen months	9 cm long (Morris et al, 1980)	31 chitons recovered out of 176 marked and released; no growth exhibited by other specimens held in lab.

TABLE VI-5 (cont.)

Species	Source	Location; Habitat	Sample Size	Initial Size Range of study specimens	Growth Rate	Date; duration	Maximum size attained (source, if another study)	Comments
"Nuttallina fluxa"	Harvey, 1963	La Jolla, CA; experimental aquarium at Scripps Inst. of Oceanography; maintained under fluorescent light, no simulated tides but a wave generator used	20	8.0-11.3 mm total length	Very poor (nonsignificant) negative correlation of length and growth rate. Mean growth from 9.5 to 15.8 mm in length in 271 days	3 September 1961 to 1 June 1962	42 mm long (N. kata); 52 mm long (N. fluxa) (present study; see comments)	Specimens apparently from southern California, and probably N. kata and/or N. fluxa. Perfect correlation of initial and final length suggests that specimens were identified only by size rank
"Nuttallina fluxa"	Louda, 1972	Santa Barbara, CA; mid-upper rocky intertidal; all limpets removed	12	7.9-11.9 mm sixth valve width	Very poor (nonsignificant) positive correlation between size and growth rate; mean growth from 9.6 to 10.4 mm sixth valve width in 139 days	30 November 1971 to 17 April 1972	16.0 mm fourth valve width,~52 mm length for N. fluxa (present study)	All limpets removed so growth rate probably greater than normal (size-frequency studies on small selected areas, with N<<25, under various conditions over 4 to 6 months gave no clear evidence for growth; may be confounded by differential immigration, emigration; specimens probably N. fluxa of the present study (see Chapter III)
Tonicella lineata	Barnes, 1972	Oregon (Yaquina Head); high intertidal pool	12	1.4-3.2 cm length	Mean growth=0.4 cm/11 months (0.1-0.9 cm)	30 April 1968 to 13 March 1969 (11 months)	50 mm length	Suggests that 5-6 mm long specimens about 1 year old from these data and from the fact that spawning occurs in April and 2 specimens,5 and 5.7 mm long, observed in May, Recapture rate 25%
		Laboratory	10	Size at settlement	To 0.75 mm (0.65-0.83)	45 days	--	--

TABLE VI-5 (cont.)

Species	Source	Location; Habitat	Sample Size	Initial Size Range of study specimens	Growth Rate	Date; duration	Maximum size attained (source if another study)^	Comments
II. GROWTH DATA OBTAINED BY "DIRECT OBSERVATIONS" IN THE LAB OR FIELD								
Chaetopleura apiculata	Grave, 1932	Massachusetts, USA; subtidal, 7.6-9.1 m	--	--	0.5 mm length 10 days after metamorphosis; 4-5 mm length: 50 days; 10-12 mm: 1 year; 25-29 mm: 3 years	--	30 mm length ?	Unclear in report as to how long actually observed in laboratory; after 50 days may be guesses. Suggests lives to about 4 years
Cryptochiton stelleri	Heath, 1905	Monterey Bay,CA	--	--	12-25 mm length in 3-4 months; up to 75-95 mm in 1 year; 120-175 mm in 2 years	--	33 cm length (Morris et al,1980)	Heath's (1905) account is not detailed but he apparently observed specimens in isolated situations in the Monterey Bay area
Katharina tunicata	Heath, 1905	Monterey Bay, CA; tidepools near mean tide level	--	"young"	25 mm length, 1 yr; 33-36 mm, 2 yr; 55 mm, 3 yr	--	12 cm length (Morris et al,1980)	Heath (1905) notes that the growth rate of *K. tunicata* also appears to be characteristic of *Mopalia muscosa*, *Mopalia lignosa*, *Ischnochiton regularis*, *Stenoplax heathiana* (=*Ischnochiton magdalensis*), and *Lepidozona* (=*Ischnochiton*) *cooperi*.
Lepidochitona dentiens (=*Trachydermon raymondi*)	Heath, 1905	Monterey Bay, CA	--	--	To 8-10 mm length in 1 year	--	20 mm length (Morris et al,1980)	See comments above
Lepidochitona dentiens (=*Nuttallina thomasi*)	Heath, 1905	Monterey Bay, CA	--	--	To 12 mm in about 1.5 yrs	--	12 mm length	Maximum length reported is for "*Nuttallina thomasi*", not *Lepidochitona dentiens*; *thomasi* is undoubtedly a *Lepidochitona* but probably not *dentiens* (see Appendix III)

TABLE VI-5 (cont.)

Species	Source	Location; Habitat	Sample Size	Initial Size Range of study specimens	Growth Rate	Date; duration	Maximum size attained (source if another study)^A	Comments
III. GROWTH DATA OBTAINED PRIMARILY FROM SIZE-FREQUENCY ANALYSES								
Chiton tuberculatus and *Acanthopleura granulata*	Glynn, 1970	Puerto Rico and Panama; coral reef intertidal	Generally 100-150 per collection at two month intervals	Most size classes up to 80 mm length	30-40 mm length/yr for specimens in 0-30 mm range	Main study 14 months in 1967-1968	~90 mm long, both species	Some direct observations supplemented size-frequency analysis. Size frequency analysis unconvincing; specimens collected from different areas in successive collections. Faster growth in smaller individuals! Suggests that most of the specimens in the 40-60 mm length range are about 2 years old (cf., Crozier, 1918a,b study). Was unable to interpret external growth lines as had Crozier.
Lepidochitona cinereus	Baxter and Jones, 1978	Eastern Scotland; intertidal mean tide level±0.5 m	--	3-22 mm length	Uninfected specimens to maximum length (~20 mm) in 5-6 years	Main study October,1975 to October,1976	19.6 mm length for uninfected specimens; 28.1 mm for infected specimens	Counts of external shell winter growth check lines aided interpretation of length-frequency data. Specimens infected with protozoan *Minchinia chitonis* grew faster than uninfected specimens in larger size classes
Lepidopleurus asellus	Christiansen, 1954	Norway; 12-16 m subtidal	--	1-16 mm length	Lives more than 1 year	October 1949 to September 1950	16 mm length ?	Data were from dredged samples
"*Nuttallina californica*"	Ituarte, 1981	Santa Cruz Co.,CA; mid-intertidal reef	Average of 141 per monthly (nondestructive) sample	Primarily ~10-35 mm length	Mean growth of about 10-15 mm length/yr for different size classes	June 1978-June 1979	60 mm length (70 mm?, Putnam, 1980)	Size-frequency histograms on which analyses are based generally do not show clear modes; suggests maximum length of 60 mm attained in about 4 years; specimens probably *N. californica* of the present study (see Chapter III)

TABLE VI-5 (cont.)

Species	Source	Location; Habitat	Sample Size	Initial Size Range of study specimens	Growth Rate	Date; duration	Maximum size attained (source if another study)[A]	Comments
IV. GROWTH DATA OBTAINED USING PRIMARILY COUNTS OF EXTERNAL SHELL GROWTH LINES								
Chiton tuberculatus	Crozier, 1918a,b; Arey and Crozier, 1919	Bermuda; intertidal	881+ specimens	--	To ~10 cm length in 12 years; mean longevity of about 8 years	--	~10 cm length	Length-frequency inspection aided interpretation of external shell growth lines. Modal age in population studies of about 4-6 years. Glynn (1970) suggests this chiton grows much faster.
Cryptochiton stelleri	MacGinitie and MacGinitie, 1968b	Washington State, USA; low intertidal to subtidal	--	--	7-8 inch (~18-20 cm) length: 20 years old; maximum age >25 years	--	33 cm length (Morris et al, 1980)	Relationship of growth lines and age not confirmed; Palmer and Frank (1974) suggest *C. stelleri* from Oregon generally much younger

NOTE: A. Maximum size attained by the population or species (parentheses: source if from another study).

would entail some adjustment for the fact that specimens of 5 to 6 years possess more than 20 grooves. It is clear that different techniques account for the different age estimates obtained in these two studies.

The growth rate estimate that Heath (1905) proffered for *Cryptochiton stelleri*, apparently on the basis of direct observations in Monterey Bay, CA, is much greater than that obtained by the MacGinities (1968b) for this species in Washington State, U.S.A. using external shell line counts [175 mm length attained in 2 years, and 7 to 8 inches (~18-20 cm) attained in 20 years, respectively). From their mark/recapture data, Palmer and Frank (1974) concluded that a 400 g (15-20 cm long?) *Cryptochiton* specimen from Oregon was about 5 years old, which agrees better with Heath's (1905) estimate than with that of the MacGinities (1968b). Although these estimates may reflect real latitudinal growth differences, with slow-growing, long-living populations occurring in colder northern waters, it is curious that Palmer and Frank (1974) were not able to detect unambiguous growth lines in *Cryptochiton*; this problem attaches some doubt to the MacGinities' (1968b) indirect ageing method and the particularly high age estimate that they obtained using it.

Despite the problems preventing strict interspecific growth comparisons, the results of the chiton growth studies, summarized in Table VI-5, generally suggest that specimens of small chiton species, less than 30 mm in maximum length, e.g., *Chaetopleura apiculata*, *Lepidochitona cinereus*, *L. dentiens*, *L. thomasi*, and *Lepidopleurus asellus*, attain their maximum sizes in five years or fewer, whereas specimens of larger species, e.g., *Chiton tuberculatus*, *Cryptochiton stelleri*, *Ischnochiton* spp., *Katharina tunicata*, *Lepidozona cooperi*, *Mopalia* spp., and *Stenoplax heathiana*, are more likely to attain maximum sizes in 5 to 10 or more years. The conclusion of the present study, that most specimens of the medium-sized *Nuttallina* species, *N. fluxa* and *N. kata*, are in the 5 to 10 year old range, appears to agree with the tentative age estimates that are available for other chiton species.

APPENDIX I

SELECTED MARINE ENVIRONMENTAL RECORDS AT LA JOLLA, CALIFORNIA

The "Seal Rock" study site, described in Chapter II, is 3.1 km from the end of the Scripps Institution of Oceanography Pier, La Jolla, CA (Lat. 32°52′N., Long. 117°15′W.) across La Jolla Bay. Daily readings of environmental conditions are made at the end of the Scripps Pier by Scripps Aquarium personnel. These data are compiled and analyzed as part of the shore stations temperature program of the Marine Life Research Group (MLRG) at Scripps Institution of Oceanography. Data from the original MLRG data sheets for each of the 22 days in 1979 and 1980 on which environmental recordings were made at the "Seal Rock" study site (Chapter II) are presented in Appendix Table 1. Codes used in Appendix Table 1 are explained in Appendix Table 2.

APPENDIX TABLE 1. Selected Environmental Records for Scripps Institution of Oceanography Pier Collected for the Marine Life Research Group Shore Temperature Program in 1979 and 1980.[A]

		Air Temp °F			Water Temp °C		Wind		Sling Hygrometer				Clouds				Swell			
Date	Time	Max	Min	Pres.	Surf.	Bottom	Dir	Spd (kts)	Dry Bulb °F	Wet Bulb	Rel Hum	Weather	Type	Amt	VIS	SEA	Dir	Per. (sec)	Ht. (ft.)	Rainfall (in.)
12 June 1979	0830	81.0	67.0	71.0	18.8	18.8	360	2	71.0	66.0	77.0	02	-	0	6	1	270	6	1	0.00
13 June 1979	1000	77.0	66.0	71.0	20.5	18.7	320	3	71.0	68.0	86.0	02	-	0	6	2	280	6	2-3	0.00
2 Nov 1979	0956	64.0	54.0	60.0	16.3	15.8	-	0	60.0	56.0	78.0	02	0	1	7	2	260	10	2	0.00
3 Nov 1979	1010	66.0	54.0	63.0	16.5	16.5	243	2	63.0	60.0	84.0	03	4	4	7	1	250	12	1	0.00
19 Nov 1979	1030	65.0	53.0	58.0	15.3	15.3	310	3	58.0	51.0	61.0	02	-	0	7	3	290	10	3	0.00
12 Feb 1980	1130	66.0	48.0	60.0	14.9	14.9	310	5	60.0	52.0	58.0	02	1, 2	1	7	1	290	12	1-2	0.00
13 Feb 1980	1000	64.0	56.0	60.0	14.7	14.7	90	4	60.0	55.0	73.0	59	6	8	6	1	220	14	1	0.05
25 Feb 1980	0955	64.0	52.0	60.0	16.1	16.0	-	0	60.0	56.0	78.0	02	1	3	7	1	270	13	1	0.00
26 Feb 1980	1030	67.0	57.0	66.0	16.3	16.2	-	0	66.0	57.0	57.0	02	2	1	9	2	270	8	1	0.00
27 Feb 1980	1030	70.0	57.0	65.0	16.2	16.2	-	0	65.0	57.0	61.0	02	5	1	8	2	270	11	2	0.00
12 Mar 1980	0900	63.0	56.0	60.0	16.0	16.0	130	4	60.0	52.0	65.0	02	-	0	8	2	260	7	3	0.00
13 Mar 1980	-- No Scripps Record --																			
14 Mar 1980	1045	64.0	55.0	61.0	16.2	15.9	270	4	61.0	57.0	78.0	02	-	0	7	2	260	7	2	0.00
17 Mar 1980	1100	67.0	54.0	64.0	16.0	15.8	290	2	64.0	55.0	56.0	02	-	0	9	1	270	10	1	0.00
26 Mar 1980	1030	61.0	43.0	53.0	15.7	15.3	280	2	53.0	48.0	69.0	02	8	1	9	3	270	7	3	0.22
27 Mar 1980	1100	61.0	52.0	49.0	15.0	15.0	220	15	59.0	53.0	67.0	02	8	5	7	2	270	7	2	0.00
28 Mar 1980	-- No Scripps Record --																			
11 Apr 1980	1000	65.0	58.0	60.0	15.5	15.5	220	3	60.0	58.0	89.0	10	-	0	2	2	270	10	5	0.00
14 Apr 1980	0930	72.0	55.0	65.0	16.0	14.0	-	0	65.0	58.0	66.0	02	1	1	7	1	290	8	1	0.00
4 June 1980	1048	61.5	58.0	59.5	17.5	16.5	270	5-6	59.5	57.5	89.0	02	0	2	6	2	270	8	3	0.00
17 June 1980	1145	65.0	59.0	-	16.5	15.0	220	0.5	63.0	58.0	74.0	03	6	8	4	2	280	8	2-3	0.00
26 Sep 1980	0730	60.0	61.0	64.0	19.0	18.8	270	1	64.0	62.0	90.0	45	0	9	4	2	240	7	2	0.00

NOTE

A. Codes used are explained in Appendix Table 2.

APPENDIX TABLE 2. Weather, cloud, visibility and sea codes used in Appendix Table 1.

WEATHER CODE

01	Clouds generally dissolving or becoming less developed
02	State of sky on the whole unchanged
03	Clouds generally forming or developing
10	Light fog (visibility 1100 yds or more)
45	Fog, sky not discernible, no appreciable change during preceding hour
59	Drizzle and rain, moderate or heavy at time of observation

CLOUD TYPE CODE

0	Stratus or fractostratus
1	Cirrus
2	Cirrostratus
3	Cirrocumulus
4	Altocumulus
5	Altostratus
6	Stratocumulus
7	Nimbostratus
8	Cumulus or fractocumulus
9	Cumulonimbus

VISIBILITY CODE

0	Dense fog, 50 yds
1	Thick fog, 200 yds
2	Fog, 400 yds
3	Moderate fog, 1000 yds
4	Thin fog or mist, 1 mile
5	Visibility poor, 2 miles
6	Visibility moderate, 5 miles
7	Visibility good, 10 miles
8	Visibility very good, 30 miles
9	Visibility excellent, over 30 miles

CLOUD COVER (AMOUNT) CODE

0	No clouds
1	Less than or equal to 1/10 sky cover
2	2/10 and 3/10
3	4/10
4	5/10
5	6/10
6	7/10 and 8/10
7	9/10 and 9/10 plus
8	10/10
9	Sky obscured

SEA CODE

0	Flat calm
1	Less than 1 ft
2	1 to 3 ft
3	3 to 5 ft
4	5 to 8 ft
5	8 to 12 ft
6	12 to 20 ft
7	20 to 40 ft
8	40 ft and over
9	Very rough, confused sea

APPENDIX II

BODY WATER CONTENT OF HIGH AND LOW SHORE SPECIMENS OF NUTTALLINA

INTRODUCTION

Morphological analyses of high and low shore Nuttallina were undertaken to provide background data for intended studies on the physiology of these groups. Water content data for Nuttallina have been reported previously by Kues (1969) in a general physiological-ecological study on La Jolla, CA populations and by Simonsen (1975) in a study on osmoregulation in high and low groups of "Nuttallina californica" at Monterey, CA. Water content data were obtained in the present study by procedures varying from those of the earlier studies and are presented in a different manner. The results are of interest with respect to desiccation.

MATERIALS AND METHODS

Nuttallina specimens were collected from high and low shore levels for body water analysis on several different occasions. Collection information is presented in Appendix Table 3. In the laboratory, algae and attached animals were removed from the chitons; encrusting algae was scraped from the valve surfaces. The chitons were assigned accession numbers, so that there was no possibility of biasing measurements, and were kept submerged in running sea water in clean glass containers for 24 to 48 hours, to allow full hydration and to allow gut clearance. The

APPENDIX TABLE 3. Nuttallina collected for water content analyses[A]

Collection Date	Location[B]	Approximate Intertidal Level (Above MLLW)	Group Reference Name	Habitat Description
27 JULY 1977	Bird Rock, La Jolla	0.7 m	JULY HIGH	Horizontal planar area at base of large outcrop; chitons in depressions; algae sparse; some brown algae.
27 JULY 1977	Bird Rock	0.4 m	JULY LOW	Horizontal area; chitons in depressions surrounded by thick foliose coralline algal mat.
5 AUG 1977	Bird Rock	0.7 m	AUG HIGH	Same site as JULY HIGH.
5 AUG 1977	Bird Rock	0.4 m	AUG LOW	Same site as JULY LOW.
12 SEPT 1977	Seal Rock, La Jolla	1.6 m	SEPT HIGH	Bare sparsely-populated sandstone; northwest-facing.
12 SEPT 1977	Seal Rock	0.3 m	SEPT LOW	Chitons in form-fitting depressions; dense coralline algal turf.
17 OCT 1977	Seal Rock → Lab[C]	1.6 m	OCT HIGH	Same collection area as SEPT HIGH.
17 OCT 1977	Seal Rock → Lab[C]	0.3 m	OCT LOW	Same collection area as SEPT LOW.
27 JAN 1978	Near Seal Rock	1.2 m–1.7 m	JAN HIGH	North-northwest facing vertical face of large solitary outcrop; relatively bare sandstone.

APPENDIX TABLE 3 (cont.)

NOTES

A. JULY LOW, AUG LOW, SEPT LOW and OCT LOW are probably primarily *Nuttallina kata*; SEPT HIGH, OCT HIGH and JAN HIGH are primarily *N. fluxa*, and JULY HIGH and AUG HIGH may have been primarily *N. fluxa* but with some *N. kata* (see Chapters II and III for habitats of *Nuttallina* spp.)

B. Seal Rock refers to the primary La Jolla site used throughout the present studies on *Nuttallina* (see Chapter II description). The Bird Rock area has been described by Gunnill (1979).

C. "17 OCT 1977" chitons were collected with those of "12 SEPT 1977" at Seal Rock on 12 SEPT 1977; the October specimens were a subsample of the Sept collection that was kept submerged in running sea water in the lab until 17 OCT 1977, for an acclimation study.

OCTOBER group of chitons was collected with the SEPT 1977 group, but, unlike the SEPTEMBER group, was acclimated in the laboratory for over a month to investigate the nature of body water contents. Chitons were kept submerged in running sea water at 18 to 21°C and laboratory fluorescent lights usually were kept on from 800 to 1800 hrs.

For all except the JULY groups of chitons which were not dissected, individuals were removed from the sea water one at a time for measurement and dissection. On removal from the seawater, each chiton was immediately blotted with paper tissue. The foot was pressed firmly with the usual result that urine(?) was released (as evidenced by the release of a substantial amount of water after the chiton had been blotted well). Blotting was continued until the outline of the pallial groove was distinctly outlined on the paper tissue. The chiton was then weighed to the nearest 10 mg to give total blotted weight. Valves were then dissected away from the tissue (except for JULY groups), blotted, and weighed to give the wet valve weight. Valves and tissues were dried separately in an oven to constant weight (usually about 30 hours). Various drying temperatures were used to determine whether results were temperature-dependent. Dried valves and dried tissues were weighed, except for the AUGUST groups, for which the valves were not dried. For the JULY groups, chitons were dried to constant weight at 60°C, weighed, dried at 120°C, and reweighed. Wet tissue weights were determined as the total blotted weight minus wet valve weight. Water weights were determined by subtraction of appropriate components. For example, valve water = wet valves - dry valves, and tissue water = wet tissue - dry tissue.

An attempt to sex chitons by gonad contents was made for all but the JULY and JANUARY groups. Valve erosion was quantified by determining the number of valves affected and the approximate percentage of total surface area involved.

Data were analyzed using various computer programs in the Biomedical Computer Program (BMDP) statistical package (Dixon and Brown, 1981) on the University of California, San Diego VAX/VMS-11/780 computer system.

RESULTS

Water content results are summarized in Appendix Table 4. Bivariate scatter plots generated by BMDP6D revealed generally strong positive linear correlations between chiton body component weights and total chiton blotted weight ($r>0.97$ for almost all cases, see table notes). Statistical tests revealed only one case of allometry (i.e., Y intercept not equal to zero for a linear regression, see Gould, 1966), involving the diminutive valve water component (<1% of total blotted weight). Thus, the results were conveniently, and reliably, expressed as percentages of total blotted weight, since variation with total weight explained most of the variation in the different components.

Analyses of covariance (ANCOVAs) were performed to compare water contents of the different groups (see Appendix Table 5). In some cases, slopes for different groups initially were significantly different; in all such cases, examination of residuals revealed an extreme outlier, usually one and no more than two (see Appendix Table 5), that when

APPENDIX TABLE 4. Summary of data for body water content analyses, expressed as percentages of total blotted weight except where indicated otherwise[A]

Group	Sample Size	Total Blotted Weight ±S.D. (Range, g.)	WHOLE CHITON				VALVES (WITHOUT TISSUE)			
			Dry Chiton (60°C)	Water (60°C)	Dry Chiton (120°C)	Water (120°C)	Wet Valves	Dry Valves (60°C)	Valve Water (60°C)	Valve Water (60°C) As Percent Of Wet Valves
JULY HIGH	28	1.66±0.772 (0.28-3.27)	51.8±3.1 (44.1-57.2)	48.2±3.1 (42.8-55.9)	50.9±3.2 (42.9-56.3)	49.1±3.2 (43.7-57.1)				
JULY LOW	26	1.45±0.615 (0.24-2.91)	54.4±3.0 (48.1-59.5)	45.6±3.0 (40.5-51.9)	53.2±3.0 (46.4-58.5)	46.8±3.0 (41.5-53.6)				
AUG HIGH	10	1.62±0.732 (0.42-2.72)					40.8±2.8 (37.0-44.2)			
AUG LOW	10	1.30±0.602 (0.62-2.46)					45.4±3.6 (38.7-50.2)			
SEPT HIGH	10	3.06±0.948 (1.80-5.20)	53.8±2.1 (50.1-56.3)	46.2±2.1 (43.7-49.9)			41.5±2.3 (36.6-44.7)	40.7±2.3 (35.9-43.8)	0.9±0.2 (0.6-1.2)	2.1±0.5 (1.4-2.8)
SEPT LOW	10	1.22±0.625 (0.07-2.08)	52.2±2.3 (49.2-54.9)	47.8±2.3 (45.1-50.8)			36.0±4.2 (29.0-41.9)	35.2±4.0 (29.0-40.7)	0.9±0.4 (0.0-1.3)	2.4±1.0 (0.0-3.5)
OCT HIGH	10	1.87±0.876 (0.98-3.46)	56.3±2.4 (53.3-60.2)	43.7±2.4 (39.8-46.7)			43.1±2.1 (40.6-47.3)	42.7±2.0 (40.1-46.6)	0.5±0.2 (0.2-0.7)	1.1±0.4 (0.5-1.7)
OCT LOW	10	0.52±0.384 (0.12-1.22)	55.3±3.2 (50.7-59.6)	44.7±3.2 (40.4-49.3)			37.6±5.7 (28.2-48.0)	37.7±5.2 (30.5-47.2)	0.0±1.3 (-2.3-1.3)	-0.3±3.8 (-8.1-3.5)
JAN HIGH	8	1.05±0.350 (0.58-1.69)	53.0±2.2 (50.3-56.9)	47.0±2.2 (43.1-49.7)			40.1±2.4 (35.3-42.9)	39.5±2.3 (34.9-41.8)	0.6±0.3[B] (0.3-1.0)	1.4±0.6 (0.8-2.4)

APPENDIX TABLE 4 (cont.)

		TISSUE (WITHOUT VALVES)						
Group	Sample Size	Wet Tissue	Dry Tissue (60°C)	Tissue Water (60°C)	Tissue Water (60°C) As Percent Of Wet Tissue	Dry Tissue (105°)	Tissue Water (105°C)	Tissue Water (105°C) As Percent Of Wet Tissue
JULY HIGH	28							
JULY LOW	26							
AUG HIGH	10	59.2±2.8 (55.8-63.0)				14.5±1.5 (12.7-16.8)	44.7±2.0 (42.3-49.1)	75.6±1.9 (72.4-77.9)
AUG LOW	10	54.6±3.6 (49.8-61.3)				14.8±1.6 (13.0-18.4)	39.8±2.4 (36.8-43.8)	73.0±1.6 (69.9-75.0)
SEPT HIGH	10	58.5±2.3 (55.3-63.4)	13.1±1.1 (11.2-14.6)	45.3±2.2 (42.8-49.3)	77.5±1.7 (75.2-81.5)			
SEPT LOW	10	64.0±4.2 (58.1-71.0)	17.1±2.9 (14.2-24.6)	46.9±2.3 (43.9-50.0)	73.4±2.9 (65.3-75.5)			
OCT HIGH	10	56.9±2.1 (52.7-59.4)	13.7±0.9 (12.0-14.9)	43.2±2.5 (39.2-46.3)	75.9±2.0 (73.2-79.4)			
OCT LOW	10	62.4±5.7 (52.0-71.8)	17.6±3.1 (12.3-22.9)	44.7±3.4 (39.7-48.9)	71.9±2.9 (67.1-76.3)			
JAN HIGH	8	59.9±2.4 (57.1-64.7)	13.5±1.7 (11.1-16.3)	46.5±2.2 (42.8-49.3)	77.6±2.6 (72.4-80.9)			

NOTES

A. All percentages summarize relationships with $r \geq 0.97$ and with Y-intercept = 0, with the following exception. Valve water wt. correlations with total blotted chiton weight and with wet valve wt. were 0.62 (statistically nonsignificant) for SEPT HIGH, and 0.83-0.94 (significant, $p<0.05$) for all other groups.

B. Y intercept statistically different from 0; thus allometric even though best described by a straight line (see Gould, 1966). The functional regression (Ricker, 1973) is: [Valve water wt. in g (60°C)] = 0.013 [total blotted wt.] - 0.007.

APPENDIX TABLE 5. Analyses of Covariance on water and valve weight data for Nuttallina. ANCOVAs were performed by the MBDPIV computer program (revision date: April, 1982). In a few cases, analysis of residuals revealed that unequal slopes between groups were due to a single outlier, which was removed to correct the situation (in only one case, for SEPT HIGH value weight analyses, two outliers were removed).

Groups Compared	Dependent variable adjusted mean, g. (unadjusted mean)	Covariate adjusted mean, g. (unadjusted mean)	N	Significance of test for equality of adjusted means of dependent variable
	Absolute water wt. (60°C)	Total dry wt. (60°C)	54	0.01
JULY HIGH	0.77(0.80)	0.83(0.86)		
JULY LOW	0.69(0.66)	(0.79)		
	Absolute water wt. (120°C)	Total dry wt. (120°C)	54	0.01
JULY HIGH	0.78(0.81)	0.81(0.84)		
JULY LOW	0.71(0.68)	(0.78)		
	Tissue water wt. (105°C)	Dry tissue wt. (105°C)	20	0.01
AUG HIGH	0.66(0.73)	0.21(0.23)		
AUG LOW	0.57(0.51)	(0.19)		
	Wet valve wt.	Dry tissue wt. (105°C)	20	0.26
AUG HIGH	0.60(0.66)	0.21(0.23)		
AUG LOW	0.66(0.60)	(0.19)		
	Tissue water wt. (60°C)	Dry tissue wt. (60°C)	19	0.001
SEPT HIGH	0.98(1.26)	0.28(0.37)		
SEPT LOW	0.83(0.57)	(0.20)		
	Wet valve wt.	Dry tissue wt. (60°C)	17	0.002
SEPT HIGH	0.97(1.25)	0.28(0.39)		
SEPT LOW	0.67(0.42)	(0.18)		
	Tissue water wt. (60°C)	Dry tissue wt (60°C)	19	0.01
OCT HIGH	0.50(0.74)	0.16(0.24)		
OCT LOW	0.44(0.23)	(0.08)		

APPENDIX TABLE 5 (cont.)

Groups Compared	Dependent variable adjusted mean, g. (unadjusted mean)	Covariate adjusted mean, g. (unadjusted mean)	N	Significance of test for equality of adjusted means of dependent variable
	Wet valve wt.	Dry tissue wt. (60°C)	19	0.02
OCT HIGH	0.56(0.81)	0.17(0.26)		
OCT LOW	0.46(0.18)	(0.08)		
	Tissue water wt. (60°C)	Dry tissue wt. (60°C)	39	0.005
SEPT HIGH	0.81(1.26)	0.22(0.37)		
SEPT LOW	0.65(0.57)	(0.20)		
OCT HIGH	0.71(0.81)	(0.26)		
OCT LOW	0.65(0.23)	(0.08)		
	Wet valve wt.	Dry tissue wt. (60°C)	37	0.0005
SEPT HIGH	0.77(1.25)	0.22(0.39)		
SEPT LOW	0.53(0.42)	(0.18)		
OCT HIGH	0.71(0.81)	(0.26)		
OCT LOW	0.60(0.21)	(0.08)		
	Tissue water wt. (60°C)	Dry tissue wt. (60°C)	27	0.14
SEPT HIGH	0.90(1.26)	0.26(0.37)		
OCT HIGH	0.82(0.81)	(0.26)		
JAN HIGH	0.88(0.49)	(0.14)		
	Wet valve wt.	Dry tissue wt. (60°C)	26	0.16
SEPT HIGH	0.73(1.19)	0.25(0.37)		
OCT HIGH	0.82(0.74)	(0.23)		
JAN HIGH	0.85(0.43)	(0.14)		

removed rendered slopes nonsignificantly different, as required for a comparison of adjusted group means by ANCOVA.

In every collection, high shore specimens had greater water contents than low specimens. The same result, high specimens with more water, is obtained, whether animals are dried at 60, 105, or 120°C, although water fractions are higher for higher temperatures as expected (see Appendix Table 4).

In the first collection (JULY groups), chitons were not dissected. However, the valve contribution to the total weight is substantial and variable valve erosion in different groups might substantially affect results. Thus, all later groups were dissected in order to determine the relative contributions of valve and tissue weights. When water is expressed as a percentage of total blotted chiton weight for the SEPT and OCT groups, water is higher in the low shore groups than in the high ones (valves were not dried for AUGUST groups so comparable values are not available). However, in both SEPT and OCT groups, the valves of the HIGH groups comprise a significantly greater proportion of the total weight (see ANCOVAs, Appendix Table 5, and see Appendix Table 4). Thus, when water is expressed in terms of only tissue weight, a more functional relationship, again it is found that water content of the HIGH group is greater in both SEPT and OCT as well as in the AUGUST collection (see Appendix Tables 4, 5).

It is difficult to obtain reliable valve thicknesses, but it is clear that higher shore <u>Nuttallina</u> (<u>N</u>. <u>fluxa</u>) tend to have wider valves (see Chapter IV), which helps to explain the difference in valve contributions between SEPT and OCT HIGH and LOW groups. However, valves contribute about equally to AUG HIGH and LOW chiton weights (see Appendix Table 5) and this may be explained in part by differential erosion. Valve erosion is difficult to quantify, but for the AUGUST collection, the valves of AUG HIGH chitons apparently were eroded more than were those of AUG LOW specimens. AUG HIGH chitons were eroded on from 4 to 8 valves ($\bar{x}$=6.5), over nearly 50% of the valve surface ($\bar{x}$=47%), whereas the AUG LOW chitons were eroded on from 0 to 8 valves ($\bar{x}$=4.0) over a quarter of the total valve surface area ($\bar{x}$=23%) (HIGH vs. LOW erosion, $p<0.025$ for both measures, two-tailed t test). Subjectively, valve erosion of low intertidal chitons at Seal Rock appears to be more extensive than that recorded in the AUGUST Bird Rock collection.

Observed differences in body water content do not appear to stem from differences in sex or reproductive state of the chitons (Appendix Table 6). Not only are the male, female and unsexable categories fairly equally represented between HIGH and LOW groups for any collection date, but examination of scatter plots reveal no tendency for any category to occur consistently above or below the regression line for tissue water weight versus dry tissue weight for a group as a whole (but sample sizes not large enough for a G test among the categories by position relative to regression line).

The OCT groups, which were kept submerged in a laboratory water table for over a month, represent an experiment in acclimation, intended to investigate the basis for water differences in high and low intertidal chitons. If the body water differences were not found between OCTOBER high and low shore groups acclimated to identical conditions,

APPENDIX TABLE 6. Summary of sex and reproductive state of *Nuttallina* collected for water content study. No attempt was made to sexually-identify JULY or JAN groups. "Unsexable" generally includes chitons with no substantial amount of gonad products.

Collection Group	Male	Female	Unsexable
AUG HIGH	4	4	2
AUG LOW	3	5	2
SEPT HIGH	2	1	7
SEPT LOW	0	1	9
OCT HIGH	2	1	7
OCT LOW	0	0	10

this might reflect a physiological rather than a genetic basis for the differences. An ANCOVA of tissue water weight (on covariate dry tissue weight, Appendix Table 5) reveals that the two groups have significantly different mean adjusted tissue water weights, thus suggesting that the water differences might be genetically-fixed. An ANCOVA including all SEPT and OCT HIGH and LOW groups also reveals a significant water difference among the groups, but pairwise t tests, provided by the BMDP1V program for exploratory purposes, suggest that the difference is primarily attributable to the SEPT HIGH group. The SEPT HIGH group is different from the other three groups which are fairly similar. The conclusiveness of this experiment is diminished by the fact that specimens in the HIGH and LOW groups for SEPT and for OCT were of considerably different sizes; size differences among individuals at different shore levels at Seal Rock have been documented (Chapter II), and thus are not merely an artifact of collection. An additional problem with the experiment was that the specimens in the OCT groups were smaller than their SEPT counterparts, possibly as a result of weight loss due to starvation in the laboratory.

Finally, no body water content differences were found among the three comparable groups analyzed in different months, SEPT HIGH, OCT HIGH, and JAN HIGH, although again size differences among specimens in the different groups were a problem.

DISCUSSION

High shore Nuttallina tend to have higher tissue water contents than do low shore specimens. Simonsen (1975) reports the opposite relationship in her study (61% low versus 56% high) but she reported her values in terms of whole chiton weights and, as shown in the previous section, inclusion of the substantial and variable shell fraction to the weight can yield misleading results. Water percentages reported by Simonsen (1975) are higher than those presented in this study (43.7 to 48.2% when calculated in the same manner), presumably as a result of less vigorous blotting prior to initial weighing, and possibly as a result of the fact that her study involved a different Nuttallina species, Nuttallina californica. Kues (1969) does not provide data for comparison of high and low populations, but calculations from his data for "extreme high intertidal" populations of Nuttallina occurring on hard sandstone (which dries rapidly and may simulate "high") versus those on coarse sandstone (which dries slowly and may simulate "low") reveal a slighter higher percentage of water in the former than the latter group (43.9 and 42.7% for N = 84, 93 respectively, significantly different, t test, two-tailed, p=0.025). Thus, his results are similar to those of the present study, although valves are included in his reported weights.

Differences in body water content of fully-hydrated Nuttallina from different shore levels observed in this study were somewhat surprising when initially discovered. Subsequent to and as a partial result of this discovery, I have been able to demonstrate that high and low shore Nuttallina are different species (Chapters III,IV).

Therefore, the body water differences may be explained on a genetic basis. It appears that, although interpretation of the September-October "acclimation" experiment results was not straightforward as a result of different specimen sizes in the various groups, the correct conclusion may have been obtained; that is, water content differences are probably not due to acclimation phenomena related to differences in shore height or substrate. The HIGH and LOW groups, in September and October, probably represent highly segregated collections of the two different species, Nuttallina fluxa (high) and N. kata (low), since the animals were collected in the characteristic habitats of these species, high/dry and low dense, respectively (see Chapter III).

For the JULY and AUGUST groups, it is less clear whether two species are involved. The HIGH groups were collected at 70 cm above MLLW, within the normal range of N. kata and the HIGH and LOW groups were of similar sizes, a situation unlikely to occur where N. kata and N. fluxa are collected at any site (Chapter III). However, the Bird Rock HIGH collection site looks like the high, sparsely-populated habitat where N. fluxa characteristically occurs at the Seal Rock site (see Chapter II), but at a low intertidal level; therefore, the Bird Rock high intertidal groups collected in the present study might comprise substantial proportions of N. fluxa.

Might differences in the high and low shore specimens of Nuttallina unrelated to the species differences still help to explain the observed disparity in water contents? Could the water content differences result from differences in reproductive state? A breakdown of water content results by species and reproductive state (gravid, nongravid) indicates that the differences do not arise from differences in reproductive state. Although Giese and Hart (1967) have reported higher water percentages in testes than in ovaries of specimens of the chiton Katharina tunicata in all seasons (average difference = 5.3% of gonad wet weight), the gonad represented a maximum of only 15%, and a minimum of 1%, of the total body weight, so that this sex difference amounted to a maximum of only about a 1 to 1.5% difference in water content when expressed in terms of shell-free chiton wet weight. Giese and Hart (1967) indicate that the water contents of all other body components of Katharina are similar between the sexes.

The observed water difference in Nuttallina may result from a difference in nutritional state of high shore and low shore chitons. The low total wet weights of the OCTOBER groups relative to the SEPTEMBER groups collected from the field at the same time may have resulted in part from starvation of the former while they were held in the laboratory. Both of the OCTOBER groups have lower water contents (as tissue water relative to wet tissue) than their SEPTEMBER counterparts (Appendix Table 4). In early stages of starvation glycogen stores are often reduced; glycogen is associated with relatively great amounts of water (Prosser, 1973). Low shore Nuttallina specimens that occur in very high densities at the Seal Rock site occasionally might experience prolonged periods of relative starvation that could help explain their low body water content relative to high shore specimens (n.b.: Sutherland, 1970 explained faster growth in higher shore limpets by this same line of reasoning; however, algal standing biomass is quite high in the present collection sites, weighing against the suggestion of starvation in the low zone). However, Giese and Araki (1962) report that two species of chitons, *Katharina tunicata* and *Mopalia hindsii*, have high amounts of lipids, and relatively low amounts of glycogen, and they suggest that water contents of at least the foot, gut, radular muscle and gonads of twelve-week starved *Katharina* are essentially the same (or even higher in the mantle) as in unstarved chitons. Their method of water determination consisted of draining rather than blotting the organs, and these workers note that their water determinations are subject to error.

Another explanation for the observed water content differences in high and low intertidal *Nuttallina* presented itself during detailed morphological studies of the two species involved, *Nuttallina fluxa* and *N. kata* (Chapter IV). *Nuttallina fluxa* tends to have more gills per body length than does *N. kata* (see Chapter IV). Since wet weights were determined by blotting the entire chiton including the gill area, more gills in *Nuttallina fluxa* (the high shore species) may have trapped more water, contributing to the higher observed water content of specimens of this species. If so, this phenomenon might also be of functional significance in the field, allowing high shore specimens to retain needed water for longer periods by trapping greater amounts of water in the roof of the gill chamber as desiccation progresses.

Several workers have suggested that the ability of various intertidal animals to withstand desiccation for extended periods is enhanced by stores of "extra" water, variously called extravisceral, extracorporeal or mantle cavity water, held within the shell outside the body tissues (gastropods: Abe, 1931; Shotwell, 1950; Segal, 1956; Segal and Dehnel, 1962; Klekowski, 1963; Seapy and Hoppe, 1973; Dehnel, 1978; but see Wolcott, 1973, and see Von Brand et al., 1957 for relative losses of mantle cavity and tissue water; bivalves: e.g., Coleman, 1973; barnacles: e.g., Suzuki and Mori, 1963; Newman, 1967). *Nuttallina*, particularly low shore specimens of *Nuttallina kata*, often occurs in deep, form-fitting depressions that can trap a large "extravisceral" store of water (Chapter II). However, those specimens expected to experience the most desiccation are those in high shore positions on less contoured rock that does not hold much moisture, and it is for these high shore specimens, presumably *Nuttallina fluxa*, for which the greatest internal (tissue) "stores" of water were found in this study, as expected if a high body water content has functional significance for desiccation.

Horn (1982) recently reported results for high and low shore groups of the chiton *Sypharochiton pelliserpentis* similar to those of the present study. Although his statistical methods were not optimal, Horn (1982) found that high shore groups of the chiton had higher body water contents (expressed as percentages of shell-free wet weights) than did low ones. In addition, although he found no significant relationship between gill number and dry body weight [my studies (Chapter IV) suggest that gill number might give a significant relationship with the cube root of dry body weight], his results indicate slightly greater (but nonsignificant) dry gill weights in high shore than in low shore chitons, a situation roughly similar to that observed in *Nuttallina*.

In addition, Horn (1982) found high shore chitons to be able to withstand desiccation better than low shore specimens; high shore chitons lost water less rapidly and survived a greater water loss (as a percentage of total body water) than did low shore chitons. Horn (1982) found lethal water losses (as a percentage of body water) for Sypharochiton pelliserpentis on the order of 50 to 65% (for 1 g chitons) and Boyle (1970) found levels of 75% for the same chiton species using different methods. Similarly, Kues (1969) concluded that lethal water loss levels for high shore Nuttallina fluxa were about 50 to 55% of total body water [for ~2 g animals, roughly equivalent to Horn's (1982) shell-free 1 g chitons].

Horn (1982) found that Sypharochiton pelliserpentis is tolerant of a level of desiccation stress generally greater than the stress experienced in the field, a phenomenon reported in previous studies of several intertidal molluscs (see Underwood, 1979; Branch, 1981 for examples). However, it is likely that there is often an interplay of physical factors in the field, and that particular factors, such as desiccation, are lethal at lower levels in combination with other factors than when tested individually, as emphasized by Alderdice (1972), Vernberg (1975) and Kennedy (1976), for example. In addition, the rate at which desiccation occurs may be critical. In any case, higher water content, greater desiccation tolerance and greater desiccation resistance probably function as a hedge against periods of abnormally low humidity and/or of high winds, such as occasionally occur in Southern California when winter afternoon low low tides coincide with warm, dry east winds.

APPENDIX III

DISPOSITION OF SPECIES ASSIGNED TO THE CHITON GENUS *NUTTALLINA*

SPECIES CURRENTLY ASSIGNED TO *NUTTALLINA*

Nuttallina crossota Berry, 1956

Type locality: Puerto Penasco, Sonora, Mexico (31°20′ N., 113°33′ W.). Largely restricted to the northern end of the Gulf of California, from San Felipe to Bahia de Los Angeles, and Puerto Penasco to Guaymas, Mexico (Keen, 1971). Specimens in this geographic range that are attributed to *N. crossota* (= *N. mexicana*) in the SDNH collection are all preserved dry: SDNH No. 63076; No. 64026; No. 23618a (N=10); No. 45770 (N=2); No. 63071; No. 23673 ("*Nuttallina mexicana*"); and No. 68720 (N=2). Three specimens at SDNH were collected outside of the above-mentioned range and are all preserved dry: SDNH No. 23576, La Paz, Baja CA, February 1939, N=1 ("*Nuttallina* cf. *crossota*"); SDNH No. 23677, Magdalena Bay, Baja CA, specimen missing ("*Nuttallina* cf. *crossota*"); SDNH No. 68702, Acapulco, Mexico, N=1 ("*Nuttallina crossota*"). In addition, Smith (1961) reports taking a single specimen at "Puerto Ballandra Bay, north of La Paz" and notes that Lowe collected a single specimen on West San Benito Island in 1932 (SDNH No. 23685; not found at SDNH, July 1982).

Berry (1956) notes that this species is distinguishable by its small size (maximum length about 18 mm, Keen, 1971; Ferreira, 1982 mentions a specimen 23 mm in length), short sutural laminae, generally narrow eaves, stubby teeth of valve viii, minuteness of the prevailing girdle spines, and the presence of sutural spines in tufts. Pilsbry (unpublished notes presented in Smith, 1961), commenting on the species relationships of Nuttallina crossota (= N. mexicana), states that it is:

> A much smaller species than N. fluxa Cpr., more elevated, with smaller girdle spines than in young fluxa of the same size. The mucro of valve viii is more posterior; in young fluxa equal in length to N. mexicana the mucro is before the posterior margin of the valve. In N. thomasi Pils. the posterior valve is wider with the mucro situated much less posteriorly and the girdle spines are far smaller.

Ferreira (1982) recently concluded that Nuttallina crossota was indeed distinct from the northeast Pacific coast Nuttallina species. For the former species, he noted the following characteristics: 1) smaller size; 2) lighter coloration; 3) girdle spines white to tan; 4) bolder radial ribs in the anterior valve and lateral areas of intermediate valves, although ribs subdued in some specimens; 5) tegmentum more coarsely granose; 6) white articulamentum; and 7) habitat, favoring the undersides of rocks in quiet water.

Personal examination of type material stored at SDNH, i.e., the paratype of N. crossota Berry, 1956 at SDNH (SDNH No. 11398; labelled "1251" on foot; locality: reef at west end of bight, west of Puerto Penasco, Sonora, Mexico; leg S. S. Berry, 9 March 1949), proved of limited usefulness due to the dried condition and small size of the specimen (9 mm length; slightly curled). Likewise, a dried specimen of "Nuttallina mexicana Pils." (SDNH No. 23673; locality: Guaymas, Mexico; collector H.N. Lowe, January, 1930; ~9 mm length curled; =N. crossota, see next section) was of little utility. However, examination of an ethanol-preserved lot of *Nuttallina crossota* from 2 miles (~3.2 km) north of San Felipe (SIO No. M1744) leads me to the conclusion that *N. crossota* is more closely related to *N. fluxa* and *N. kata* than to *N. californica*. The specimens, unlike *N. californica*, all have valves of light pink, white or brown, and girdles with alternating white and light-brown stripes. Girdle spines are long and most often white or light brown, unlike the stubbier, dark-brown spines seen in typical *N. californica*. Unlike in *N. fluxa* or *N. kata*, however, the spines are fairly sparse and are often produced in tufts of 2 to 5. Scales on the underside of the girdle are pure white, unlike in West Coast *Nuttallina* in which these scales are distinctly orange/brown, even in ethanol-preserved specimens. The constancy of this feature in *N. crossota* was largely confirmed by an examination of specimens at SDNH, where all the specimens are dry, although in some cases drying seemed to have caused some darkening of the undergirdle scales. Ferreira (1982) described these scales as "transparent" in the specimens he examined.

Valve shape of the specimens (SIO No. M1744) tends toward the rectangular, short, wide condition commonly observed in *N. fluxa*; likewise, the hypotype from Bahia de los Angeles, figured in Smith (1961), shows the rectanguloid condition, but with prominent jugal ridges that are not seen in any *Nuttallina* from the Pacific Coast. On the other hand, the specimen figured in Keen (1971), and Berry's (1956) note that intermediate valves are V-shaped, suggest a valve morphology closer to that of *N. kata*. Most dry specimens at SDNH, listed in the opening paragraph, had squarish valves although a few had more angular valves;

specimens exhibited a range of valve widths from narrow to fairly wide.

Gills were counted in the two largest specimens of the San Felipe collection (SIO No. M1744; 12 and 14 mm preserved total length) and the averages for the left and right sides were 15.5 and 18.5, respectively (both gave asymmetrical gill counts). Reference to on Figure IV-5 reveals that these specimens have fewer gills than the three northeast Pacific coast species but are closest in number to *N. kata*. It is difficult to evaluate the genetic implications of this finding in view of the allopatry of the compared species and the possible effect of environmental factors, such as temperature, on the process of addition of new gills during growth. It is interesting that Johnson's (1969) finding of a positive correlation between number of gills and maximum adult length is followed in the four *Nuttallina* species.

The radular teeth of *Nuttallina crossota* are indistinguishable from those examined from specimens of northeast Pacific *Nuttallina* (Chapter IV), as judged from Figure 90 in Ferreira (1982) for a specimen of *N. crossota* from Puerto Penasco (LACM No. 63-56). Radular characteristics are generally conservative within chiton genera (see Chapter IV).

Nuttallina crossota seems to warrant species status separate from the three northeast Pacific *Nuttallina* species, particularly in view of the tufty arrangement of its girdle spines, its small size, and, provisionally, the white color of the scales of its undergirdle. The predominance of white girdle spines, and rectangularity and large relative width of valves in specimens examined suggests a fairly close relationship with *N. fluxa*, while the angularity of valves in some specimens and the low gill counts suggest an affinity to *N. kata*. If the white color of undergirdle scales is confirmed as a distinctive specific character for *N. crossota*, four specimens from Guadalupe Island, Baja CA (CASIZ No. 032164), three from Bahia Tortugas, Baja CA (SIO No. M952), from Turtle Bay (SIO No. M570 and SIO No. M1745) might be *N. crossota*, although at present they have been tentatively identified as *N. fluxa* or *N. kata* primarily on the basis of gill numbers (Chapter III: Table III-8). An electrophoretic study of *Nuttallina crossota* would help resolve the nature of the interrelationship of this species with those studied in Chapters III and IV.

Nuttallina mexicana Pilsbry, Pilsbry and Lowe, 1932 (*nomen nudum*; synonymized with *N. crossota*)

From Guaymas, Mexico. The manuscript describing this species was lost and never published. Later, *N. mexicana* was listed in Pilsbry and Lowe (1932) without description, and thus is to be considered a *nomen nudum*. Smith (1961) determined that *N. mexicana* was later named and described by S. S. Berry as *Nuttallina crossota* Berry, 1956. Thus, he synonymized *N. mexicana* under *N. crossota*. Ferreira (1982) includes *N. mexicana* in his synonymy for *N. crossota*. See earlier discussion of *N. crossota* for more information.

Nuttallina alternata (Sowerby, 1840)

From Japan. This species was described as *Chiton alternatus* by Sowerby, and was provisionally assigned to *Nuttallina* by Pilsbry (1893).

The type specimen(s) were listed as stored in the H. Cuming collection by Sowerby (1840). Much of the Cuming collection is now stored in the British Museum of Natural History (BMNH), but no type, possible type or even nontype material referable to *Nuttallina*, or *Chiton*, *alternatus* can be located at BMNH (Ms S. Morris, BMNH, *in litt.*, 28 September 1982). R. C. Bullock (University of Rhode Island, *in litt.*, 5 November 1982) examined the entire chiton collection at BMNH in recent years, making extensive photographic records, and found no record of any of Sowerby's species; he remarked that some Cuming material, including type material, has shown up in Australia, and some in Denmark. An inquiry sent to W. Ponder (Australian Museum, Sydney) on 22 November 1982 has not been answered; no workers in Denmark have been contacted. W. K. Emerson (*in litt.*, 22 October 1982) remarked that the National Museum of Wales (Cathays Park, Cardiff) stores the Melville-Tomlin collection, which contains some of the Cuming material, although he notes that many of the specimens described in the Conchological Illustrations by Sowerby (1847) went to private collections and have been lost to science. An inquiry sent to P. G. Oliver at the National Museum of Wales on 28 October 1982 has not been answered.

Pilsbry (1893) apparently examined a dried specimen of *Chiton alternatus* in making the provisional assignment to *Nuttallina*, but no specimen referable to *Nuttallina*, or *Chiton*, *alternatus* has been located at the Academy of Natural Sciences in Philadelphia (R. Robertson and M. A. Garback of ANSP, <u>in litt</u>., 9 December 1982).

<u>Nuttallina alternata</u> has been listed most recently from "Japan; Hachijo-jima(?); Kotosho (=Botel Tobago), Formosa(?)" by Is. Taki (1962), and Iw. Taki (1964), although relevant specimens were apparently all confiscated when Is. Taki was taken as a prisoner of war, were all left in Shanghai, China at the end of World War II and are lost (Is. Taki, 1962; P. Kaas, <u>in litt</u>., 15 November 1982). Is. Taki has passed away, but an inquiry to Iw. Taki (Kyoto, reply 14 August 1982) turned up no specimens nor any new information about the species. Similarly, correspondence with other workers in Japan (T. Okutani, National Science Museum, Tokyo, reply 29 July 1982; J. T. Moyer, Tatsuo Tanaka Mem. Biol. Station, Miyake-jima, 30 July 1982) and Taiwan (K. Y. Lai, Taiwan Museum, Taipei, 20 January 1983; K. Y. Soong, Inst. of Zoology, Academia Sinica, Taipei, 18 December 1982) turned up no specimens or new reports of such specimens. Cardin (1976) reported four species of chitons taken in a shelling expedition to Hachijo-jima, none of which belonged to <u>Nuttallina</u>.

Finally, correspondence with other mollusc and chiton specialists, including R. H. Jensen (Delaware Museum of Natural History, 17 November 1982), J. Rosewater (U.S. National Museum of Natural History, 22 October 1982), W. K. Emerson (American Museum of Natural History, 22 October 1982), K. J. Boss (Museum of Comparative Zoology, Cambridge, Mass, 16 November 1982), B. Roth (Calif. Acad. of Sciences, 17 November 1982) in the United States, and P. Kaas (Rijksmuseum van Natuurlijke Historie, Nederland, 15 November 1982), R. A. Van Belle (Belgium, 6

February 1983) and P. Bouchet (Mus. Nat. Hist. Naturelle, Paris, 30 November 1982) in Europe, turned up no specimens nor any information about specimens, type or nontype, referable to *Nuttallina*, or *Chiton*, *alternata*. Therefore, *Nuttallina alternata* must be regarded as a *nomen inquirendum*.

Pilsbry (1893) noted that *N. alternata* seems to agree with the other *Nuttallina* species in the dorsal and lateral ribs of the intermediate valves and the ribs of the anterior valve, but that the interior of *N. alternata* is unknown. Available figures of *Nuttallina alternata* (Pilsbry, 1893, plts. 54, figs. 25-27; Sowerby, 1840, Conch. Ill., fig. 141), showing a narrowish girdle with alternating light and dark brown stripes, and showing fairly rectangular, wide, short valves are highly reminiscent of *N. fluxa*. The description provided by Pilsbry (1893, taken from Sowerby) does little to elucidate the relationship of *N. alternata* with the northeast Pacific *Nuttallina* species, although the "minute spines" on the girdle and the general dark olive-green color are more suggestive of *N. californica* than *N. fluxa* or *N. kata*. The alternately black and white banded girdle spines mentioned in the description have only been observed in *N. kata*.

On the basis of what is known for other mollusc and fish genera on the two sides of the Pacific (Keen, 1941; Yakovleva, 1965; Briggs, 1974), the fact that *N. alternata* has a warm temperate distribution suggests that there is only a moderate probability that this chiton is synonymous with any of the North American species. Thus, Briggs (1974) has noted that the fish fauna south of the Aleutian Province of the southeastern Bering Sea in the Oregon Province and south of the Eastern

Kamchatka Province of the southwestern Bering Sea in the Orient are very different, and that aside from the wide-ranging, arctic-boreal species, probably less than 1% of the species are shared. Of course, at the generic level, the two sides of the North Pacific are very closely-related. Although Keen (1941) gives no estimate of the total number of closely-related, or analogous, species occurring in California and Japan, she estimates that one half to two-thirds of the molluscan generic units are shared by the two sides of the north Pacific; however, Keen (1941) found very few pelecypod or gastropod molluscs to be identical in California and Japan.

If specimens should become available that are referable to *Nuttallina alternata*, electrophoretic study (of fresh or frozen specimens) or morphological examination (of well-preserved specimens) would help clarify the relationship of this species to the other *Nuttallina* species.

"*Nuttallina scabra*" (Reeve, 1847) or (Carpenter, 1864)

The name *Nuttallina scabra* has been used in reference to both the southern and northern California *Nuttallina* species, as well as to a (?)Chilean specimen; workers apparently recognizing only one Californian species have also used this name. See the synonymies for *N. kata*, *N. fluxa* and *N. californica*, and the Assignment of Names section in Chapter IV.

The species name is most commonly suggested to derive from *Chiton scaber* Reeve, 1847, although there is some question as to whether it might derive instead from Acanthopleura scabra Carpenter, 1864 (see Smith, 1977). In either case, the name Nuttallina scabra is invalid. Chiton scaber Reeve, 1847 is a junior homonym of Chiton scaber Blainville, 1825, a different species from the "Seas of New Holland" that is now in the family Acanthochitonidae. In 1864, Carpenter presented Acanthopleura scabra, without adequate description, as a synonym of Chiton californicus Nuttall, in Reeve, 1847; the species name was attributed by Carpenter (1864) himself to Reeve (see Smith, 1977).

"Nuttallina scabra" from Chile (also see preceding discussion for N. scabra)

Nierstrasz (1905a) reports one specimen of "Nuttallina scabra" from Chile in the collection of the Leyden Museum of Natural History. No specific location is mentioned. Listings of Nuttallina scabra from Chile in Nierstrasz (1905b), Leloup (1940, 1956) and Stuardo B. (1959) are all based on the single specimen in the Leyden Museum reported by Nierstrasz (1905a).

A request for Nierstrasz's Chilean specimen of Nuttallina from the Leyden Museum of Natural History produced the following reply from Dr. Piet Kaas, a chiton expert associated with the museum (in litt., 12 May 1982):

> the specimen under discussion is a badly preserved, dry and partly disarticulated *N. californica* (Reeve), valves VII and VIII missing [Kaas is unaware of the distinctions among the three *Nuttallina* species, so his identification can be regarded only as *Nuttallina* sp.]. The original label reads '*Chiton barnesi* Gray. H. B. Preston, 1902. Chile.' Another (typewritten) label, probably Nierstrasz's, reads '*Nuttallina scabra* Rve. Chili. H. B. Preston, 1905.'

Kaas believes that since Preston was a recognized dealer of natural history specimens, whose identifications and localities are generally correct, there must have been a labelling mix-up before the specimen was registered in the Leyden Museum collection, i.e., either the label or the specimen was mislaid.

On reception of the specimen for examination, it was found to be accompanied by five labels (including two newer ones apparently written by Kaas). Of the three older labels, only the "*Radsia barnesii*" (=*Chiton barnesi*) label mentioned above stated "Chile" unequivocally (and "?*Nuttallina*" on the reverse), whereas two "*Nuttallina scabra*" labels noted "?Chili", indicating that the location was questionable.

Personal examination of the dry specimen reveals it to be identical or at least closely-related to *Nuttallina fluxa*. The valves are short, wide and light brown in color, and sutural flesh encroachment is negligible, as is typical in *N. fluxa*; however, the valves are more angular than in most *N. fluxa*. Valve i has 11 slits, and viii has 10 slits as is often seen in *N. fluxa*. The girdle is fairly narrow and has short white spines through out.

The critical question remains whether the specimen, a *Nuttallina*, is actually from Chile or whether the label presently with the specimen was originally with another specimen, possibly one of *Chiton* *barnesi* from Chile, while the *Nuttallina* specimen was actually from North America. Certainly, workers on the Lund University Chile Expedition (L.U.C.E.) of 1948-1949 (Leloup, 1956) did not turn up any new specimens of *Nuttallina* in Chile, and Stuardo B. (1959), who formulated a key for Chilean chitons based on his experience with the fauna around Valparaiso and based on examination of the L.U.C.E. collection at Bergen, Norway, did not report any new specimens of *Nuttallina*. *Nuttallina* is not included in the checklists of Boudet R. (1945) or of Carcelles and Williams (1951), which include Chilean chitons and other molluscs, nor did any specimens of *Nuttallina* turn up in the Royal Society Expedition to southern Chile (42 to 55°S, Dell, 1971).

Information obtained from Chilean workers supports the conclusion that the report of *Nuttallina* from Chile is erroneous. P. Sanchez R. (Pontificia Universidad Catolica de Chile, Santiago; *in litt.*, 14 December 1982) reports that members of his marine biological group have had much experience in the intertidal and shallow subtidal along most of the Chilean coast and have never encountered *Nuttallina*. He also reports that N. Bahamonde (long-time curator of marine invertebrates at the National Museum) does not know of any *Nuttallina* specimens from Chile. Similarly, J. Stuardo B. (Universidad de Concepcion, Concepcion, *in litt.*, 27 December 1982) notes that he has never seen the Nierstrasz specimen of "*N. scabra*", nor has he seen any *Nuttallina* specimens either near Valparaiso or in southern Chile. Therefore, it seems reasonable to conclude that *Nuttallina* does not occur in the Southern Hemisphere.

CHITON SPECIES PRESENTLY ASSIGNED TO GENERA OTHER THAN NUTTALLINA, BUT PREVIOUSLY ASSIGNED TO NUTTALLINA

Nuttallina thomasi Pilsbry, 1898

This species is properly assigned to the genus Lepidochitona. Distribution: from Monterey, CA in small tidepools four to eight feet (~1.2 to 2.4 m) above mean tide mark (Pilsbry, 1898). In describing this species, Pilsbry (1898) comments as follows: "This species differs markedly from N. californica ('Nutt.' Rve.) and N. fluxa (Cpr.) in the shorter valves, lack of radial ribs on the anterior and intermediate valves, and more anterior mucro of the posterior valve, and the single slitting of the valves ii to vii...". Pilsbry [Pilsbry's unpublished notes reported in Smith (1961)] noted that in comparison to Nuttallina fluxa and Nuttallina crossota (= N. mexicana), "In N. thomasi Pils. the posterior valve is wider with the mucro situated much less posteriorly and the girdle spines are far smaller."

Ferreira (1982, and in litt., 3 April 1982) has synonymized N. thomasi under Lepidochitona dentiens (Gould, 1846). D. Eernisse (University of California, Santa Cruz, personal communication, 7 January 1983) also believes N. thomasi to be a Lepidochitona. Eernisse is presently working on the problem. He bases his conclusion on: 1) the similarity of valves of N. thomasi and L. dentiens revealed in studies involving discriminant analysis; 2) the similarity of SEM-visualized egg morphology of N. thomasi and that of Lepidochitona; and 3) the dissimilarity of eggs of N. thomasi and those of "Nuttallina californica" (i.e., Nuttallina from Santa Cruz, CA; Eernisse, in litt., 27 October 1981).

However, Eernisse and Pearse (1980) and Eernisse (in litt., 27 October 1981) have found L. thomasi to be a gonochoric brooder and, at present, unlike Ferreira (1982), consider it to be a different species from the larger, gonochoric, free-spawning species, Lepidochitona dentiens. D. Eernisse (personal communication, 7 January 1983) presently bases the dentiens/thomasi distinction on: 1) egg morphology; 2) reproductive mode: dentiens a free spawner, thomasi a brooder of eggs and young; 3) valve morphology; 4) girdle morphology: thomasi with girdle spines, dentiens never with true spines; 5) electrophoresis: fixed allelic differences at a few of the 17 loci surveyed; and 6) ecology: thomasi occurs higher in the intertidal, with rare but locally-abundant populations.

Nuttallina allantophora Dall, 1919

Las Animas Bay, Baja California. This species has been placed in synonymy with the western Pacific species Liolophura japonica (Lischke, 1873) by Smith (1977). N. allantophora is based on a single specimen from Las Animas Bay, Baja and Keen (1971, p. 908) states that the type locality is probably in error. Both H. A. Pilsbry and S. S. Berry suspected that Dall had placed this specimen in the wrong genus (see Smith, 1977). Pilsbry [unpublished notes recorded in Smith (1961)] noted that "'Nuttallina' allantophora ... [is quite unlike Nuttallina crossota (= N. mexicana)] and those [Nuttallina] previously known from

California in sculpture, and indeed [its] reference to Nuttallina appears doubtful ... The presence of 'partly ocular' pustules in [Nuttallina allantophora] ... [is] certainly [a] strange feature for Nuttallina!" Smith (1977) made the reassignment to Liolophura, noting the presence of short, blunt spines and a tail valve with an unslit, shelf-like insertion plate characteristic of the genus Liolophura Pilsbry 1893. See Smith (1977) for a complete synonymy.

Nuttallina magdalena Dall, 1919

From Magdalena Bay, Baja California. H. A. Pilsbry expressed doubt as to the generic assignment of this species. Pilsbry [unpublished notes recorded in Smith (1961)] stated that "...'N.' magdalena Dall [is] quite unlike [Nuttallina crossota (= N. mexicana)] and [Nuttallina] previously known from California in sculpture, and indeed [its] reference to Nuttallina appears doubtful ... [The] beaded, Pallochiton-like sculpture of N. magdalena [is] certainly [a] strange feature for Nuttallina!"

Smith (1977) placed this species in synonymy with Chaetopleura (Pallochiton) lanuginosa (Carpenter in Dall, 1879). This action followed the transfer of N. magdalena to Chaetopleura by Keen (1958) on the advice of S. S. Berry (see Smith, 1977). See Smith (1977) for a complete synonymy.

Nuttallina piceola or *Nuttallina* (*Middendorffia*) *piceolus* (Shuttleworth, 1853)

From the Canary Islands and the Azores (Kaas and Van Belle, 1981). Reassigned to the genus *Middendorffia* by Bergenhayn (1931) and finally to *Lepidochitona* (*Lepidochitona*) *piceola* (Shuttleworth, 1853) by Kaas and Van Belle (1981). See Kaas and Van Belle (1981) for a complete synonymy.

Nuttallina (*Middendorffia*) *cinerea* (Poli, 1791)

From the Mediterranean Sea, southern coast of Portugal and Spain. Assigned to *Lepidochitona* (*Lepidochitona*) *corrugata* (Reeve, 1848) by Kaas and Van Belle (1981), although many authors prefer to use the older name *Middendorffia caprearum* (Scacchi, 1836). See Kaas and Van Belle (1981) for a complete synonymy.

Nuttallina (*Middendorffia*) *simrothi* (Thiele, 1902)

From the Azores only. Assigned to *Lepidochitona* (*Lepidochitona*) *simrothi* (Thiele, 1902) by Kaas and Van Belle (1981), which see for full synonymy.

APPENDIX IV

DEVELOPMENT OF A CHITON TAG AND ASSESSMENT OF ITS EFFECT ON THE BEHAVIOR, GROWTH, AND MORTALITY RATE OF NUTTALLINA

AN EFFECTIVE TAGGING SYSTEM

The primary requirement for a tag for Nuttallina was that it last for long periods of time, preferably more than a year. Mutilation methods commonly used to mark animals, i.e., cutting notches in the flesh in different places (e.g., in Chiton tuberculatus, Crozier and Arey, 1918), bleaching the flesh, "branding" the flesh by freezing, or tattooing the flesh by scratching and applying India ink (c.f., Southwood, 1978), were avoided because they do not allow easy individual identification of hundreds of small animals, they can change or become overgrown during a long-term study, and/or they are susceptible to confusion with natural markings, cuts, gouges, or colors often seen on Nuttallina specimens in the field.

A number of intertidal tagging studies have been carried out successfully with chitons and limpets by using numbers drawn on glue or identification disks glued to the shell (e.g., Sutherland, 1970; Lyman, 1975). Therefore, various glues were investigated for their feasibility in tagging Nuttallina. In the laboratory, specimens were blotted dry and different glues were applied to the valves. The glues were either used alone or were used to attach small plastic identification disks to the valves. Preliminary testing revealed that epoxy glues (Sea Goin'

Poxy Putty, Permalite Plastics Corp., Newport Beach, CA; Pettit Polypoxy Underwater Patching Compound) stuck to the chiton valves and plastic identification disks more reliably than did cyanoacrylate-based glues (Zip-Grip 10, Devcon Corp., Danvers, MA; Crazy Glue 3, Duro Corp.). Apparently, the cyanoacrylate glues failed because the chiton valves are slightly porous and/or rough and, even though valves were blotted dry, sufficient moisture remained to prevent the cyanoacrylate glues from working. Demopulos (1975), Fulton (1975), and Lyman (1975) have previously used Zip-Grip 10 with apparent success in tagging various species of chitons for short-term studies. However, Vesco (1980) reports losing about 63% of the tags she had placed on Nuttallina in the field with this glue within a 24 hour interval! Pettit Polypoxy attached disks to valves very well and had the advantage of being an inconspicuous gray color, but it cured only to a soft rubbery condition and therefore was easily deformed and easily removed. Sea Goin' Poxy Putty, on the other hand, cured to rock hardness and was expected to resist sand abrasion well; however, it was difficult to apply to small localized areas, such as chiton valves, because of its putty-like consistency. Sea Goin' Poxy Putty is bright white when applied, but changes in a day or two to a less conspicuous dull yellow color. It was considered a good choice for tagging.

Tests of Sea Goin' Poxy Putty in the field gave poor results. Nuttallina were tagged by blotting the valves dry and applying a small plastic disk to a valve with Sea Goin' Poxy Putty. Animals were tagged in the field rather than in the laboratory so that they could be replaced in the same location and orientation as that in which they had been found. Tagging in the field also eliminated the unnecessary disturbance to the chitons that can result from transporting them to and from the laboratory and leaving them in the laboratory for at least one tidal cycle. Of 128 Nuttallina specimens tagged in this way on 21 and 22 June 1978, only 28 were relocated less than a week later, from 25 to 27 June 1978. Less than 2 months later, on 18 August 1978, only 2 chitons could be found with plastic disks and glue still intact; another three animals had what appeared to be the remains of small amounts of glue on their valves. The fact that several of the glued tags were in various stages of falling off on 25 to 27 June 1978 suggested that most of the glue tag losses that occurred over the two month trial period were due to detachment of the tags rather than to deaths of the chitons.

In a concurrent study, eighteen chitons were tagged on 23 June 1978 with annealed Monel wire, 0.020 in. (~0.5 mm) in diameter, forming a loop, with the wire entering and exiting the flesh of the dorsal surface of the girdle, and with the ends twisted together. Only ten wire-tagged chitons could be located on 25 June 1978, and only two of these were relocated on 18 August 1978. Loss of wire-tagged Nuttallina might have resulted from death due to injury to the chitons as well as from tag pull-outs.

These preliminary field tagging trials provided some valuable information. First, tagging with epoxy glue sets a lower limit to the size of the chitons that can be tagged. Unlike in limpets, where the shell is one continuous surface, in chitons the shell is divided into 8 separate plates that can move with respect to each other. Whereas in a 10 mm long limpet, the minimum shell surface dimension might be 10 mm, in a chiton 10 mm long, the minimum dimension of exposed surface provided by even the largest valve, its length, is at most 1 mm. In addition, in chitons, the fact that valves move over each other when the chiton flexes its anterior or posterior end upward. Therefore, it is obvious that a glue tag on a valve of a chiton is at high risk of failure. Further, it is exceedingly difficult to apply Sea Goin′ Poxy Putty to the valves without having it overlap at least 2 adjacent valves. Thus, when the chiton moves, the tendency for separate valves to move relative to each other increases the probability that a glue tag will be dislodged. Finally, the fact that many *Nuttallina* specimens have valves that are eroded and crumbly to some extent, riddled with filaments of the (?)blue-green alga *Entophysalis deusta* (Nishi, 1975; and see Appendix II), means that tags attached to the valve surface will often be lost unless a large amount of the valve surface is sanded away prior to the application of the tag. When glue tags *do* stay in place, they probably hamper free movement of at least the smaller chitons to some extent via restrictions on independent movements of the valves.

Another important finding from the initial tag trial indicates that any small tag attached to a valve surface will have limited utility for use with *Nuttallina* for another reason in addition to those described above. During the two months that elapsed from tagging to the second relocation of glue-tagged chitons, a considerable amount of new algae became established on the valves of the chitons. Thus, even where tags were still attached, they were difficult, or even impossible to detect, under the new algal growth. Clearing the algae away from the valves during each relocation attempt is not an acceptable solution to the problem since such a process would entail subjecting many tagged chitons to considerable chronic disturbance, in addition to taking up considerable amounts of time unnecessarily checking untagged chitons for tags.

An additional potential problem with the epoxy glue tags on chiton valve surfaces is suggested by findings of studies on valve structure and chiton behavior. Chitons possess sensory organs in the dorsal valve surface called aesthetes that have been suggested to function in light reception (Boyle, 1977) and alterations of the valve surface might elicit abnormal chiton behavior. For example, interference with the integrity of the shell surface of _Ischnochiton maorianus_ by abrasion or masking affects the rate at which this chiton orients to light stimuli, although it does not eliminate the light response (Boyle, 1972). In addition, Arey and Crozier (1919) have suggested that _Chiton tuberculatus_ migrates to more exposed habitats on the shore as it ages and gradually becomes positively phototactic, which they suggest is a result of progressive erosion of the valve surface with a consequent loss of intact aesthetes and an alteration of the negative phototactic response exhibited by the young specimens. Thus, although the importance of the aesthetes to the behavior of _Nuttallina_ has not been assessed (and is probably minimal?), the application of any tag to the valve surface has potential for altering the normal behavior, including long-term movement patterns, of this chiton.

Although epoxy glue tag efficiency probably could have been increased by allowing the glue to cure longer in exposure to air, glue tags were not tested further because of the problems of limited valve space, crumbliness of valves, and particularly of algal overgrowth. Similarly, consideration of other tagging methods involving the valve surface, particularly that of drilling small pits in the valves in a coded arrangement and filling them with epoxy, was abandoned, primarily because of the problem of algal overgrowths.

Attaching a tag to the girdle rather than to the valve surface of a chiton eliminates all of the problems discussed above. Applying such a tag so that it extends away from the chiton surface almost entirely eliminates the problem of algal overgrowth. The annealed Monel wire tags used in the preliminary field tests met several of the requirements for an effective chiton tag. However, wire tag returns were low; only two of the eighteen Monel tags could be relocated after the two month trial in the field. As judged from wire-tagging trials in the laboratory, a substantial percentage of the losses were probably due to chiton deaths caused by injury sustained while the tags were being applied. The fact that eight of the eighteen wire tags disappeared in only two days in the field supports this conclusion. Corrosion or pull-outs of the firmly-embedded wire tags probably accounted for few of the tag losses in the field trial.

The wire tags had obvious disadvantages that could be ameliorated. First, they were rigid. Any movement of the chiton involving flexing of the girdle is resisted by the tag. The wire tag is probably a source of considerable disturbance to a moving chiton. In addition, the rigidity of the tag probably increases the probability that it will eventually tear out or cut through flesh. Second, even though the tags were of corrosion-resistant Monel and probably worked well for short periods, they were apt to corrode and fall out eventually over periods of several months. In addition, Monel wire may be toxic and irritating to chitons.

Monofilament fishing line lacks many of the drawbacks of wire for use in tagging. It is available in a wide range of flexibilities and strengths (diameters) and it is biologically-inert. In addition, monofilament is unaltered by long periods of submersion in seawater (H. Fahrner, Customer Service, Maxima Fishing Line Manufacturing Co. KG, Geretsried, West Germany, <u>in litt</u>., 1979). Threading of the monofilament through the tough girdle can be achieved by piercing the girdle with a stainless steel syringe needle of appropriate size bent into an arc and threading the monofilament through the needle, after which the needle can be removed, leaving the monofilament in place. By threading the needle in and out the dorsal surface of the girdle, damage to the gills and unnecessary damage to the girdle itself can be avoided. This method leaves unaffected the ventral surface of the girdle that is used in movement and clamping to the substrate.

The size of monofilament line used represented a compromise between maximum flexibility and minimum damage to the girdle (small diameter, low test strength) and resistance to parting by abrasion and low probability of cutting through flesh (large diameter, high strength). A survey of many available monofilament fishing lines revealed that Maxima (Maxima Manufacturing Co., Geretsried, West Germany) and Stren (E.I. DuPont de Nemours and Co.,Inc, Wilmington, Delaware) fishing lines have the minimum diameter per pound test strength. Four pound test line [0.008 in. (~0.2 mm) diameter] was found to be the best compromise for diameter, flexibility, and strength. Lines of smaller diameter were too flexible to thread easily through syringe needles; water tension was enough to prevent threading at times. 26 gauge stainless steel syringe needles were the smallest needles that would allow four pound monofilament to be easily threaded. Needles could be bent after inserting 0.010 in. (~0.25 mm) diameter music wire to prevent collapse of the needle wall.

Titanium was the material used for the identification disk to be threaded on the monofilament. Plastic was not used because it is more vulnerable to sand abrasion, and usually becomes brittle and crumbles after several months in the field. Titanium is superior to stainless steel for intertidal use because it has an outstanding resistance to corrosion by seawater and is lighter in weight. Although aluminum is even lighter than titanium, it is less resistant to both sand abrasion and corrosion. Fouling organisms can adhere to titanium but they cause no pitting or corrosion of the metal (Anonymous, undated). For chiton tags, three-sixteenths in. (~4.8 mm) diameter disks of 0.005 in. (~0.13 mm) thick titanium shim stock made with a metal hole punch) were each stamped with two one-sixteenth in. (~1.6 mm) high numbers or letters and were perforated with a high speed drill [0.016 in. (~0.4 mm) bit]. The small disks were then ground down, leaving only the stamped characters. Disks made in this manner weighed less than 5 mg. Disks were attached to chitons with monofilament using a surgeon's knot. Some slack was left in the monofilament loop to allow for growth of the chiton, and to ensure that the tag stood away from the surface of the chiton.

ASSESSMENT OF THE EFFECT OF THE TAG ON THE ACTIVITY AND WELL-BEING OF *NUTTALLINA*

Since the monofilament tagging system involves inflicting a small wound on each chiton, the effect of the tag on the activity and health of *Nuttallina* was assessed. Ten *Nuttallina* specimens were tagged and were placed near the center of a water table; ten untagged *Nuttallina* were also placed in the center of the same water table. The behavior of chitons in the two groups was similar. All specimens clamped well to the table in a short time and moved to the base of the walls of the table within three hours. Subsequently, both tagged and untagged chitons moved considerable distances around the table from time to time, coming to rest in new locations. Two tagged chitons died within a week of tagging, probably from injuries sustained during tagging, but all other specimens were alive and in good condition after one month.

A more rigorous investigation of the effect of the tag on growth and mortality of *Nuttallina*, as indicators of the general well-being of the chitons, was performed. A total of more than 200 *Nuttallina* were collected from the low and high intertidal areas of the La Jolla (Seal Rock) study site during the study. Each specimen was treated as follows immediately after collection. Algae and attached animals such as barnacles were removed. The intact fourth valve width (FVW; see description of this character in Chapter IV) was measured three times. The chiton was then blotted dry with paper tissue and was weighed to the nearest 0.01 g (as in Appendix II). Animals were then ranked according to weight, keeping low and high intertidal chitons separate, and were paired according to weight. One of each chiton pair was selected randomly for tagging, using weights as identifying indices, so that the tagged animal was randomly lighter or heavier than the untagged animal, if not the same weight. Eighty-two healthy pairs of Nuttallina, one tagged and the other untagged in each pair, were generated during the study.

Chiton cages were made by drilling many small holes in clear plastic freezer containers 0.57 liter in capacity (12x8x6.5 cm; "Superseal Clears", #6512, Eagle Affiliates, Brooklyn, NY) and, after the cages had been allowed to age in seawater and algae had established itself on the cages, each chiton pair was placed in a cage. Cages were placed in a laboratory seawater table and in an outside tank with running seawater. In the laboratory, a diurnal tidal cycle was generated by emptying water from the water table for 3 to 6 hours per day throughout most of the study. Laboratory fluorescent lights were generally on from about 800 to 1800 hrs. Cages in the outside tank were left continually submerged and were subject to the natural light cycle.

Thirty-five pairs of chitons were started on 14 to 16 July 1980. Since an intense bloom of Gymnodinium flavum fouled the seawater system at the end of that month and killed a majority of the chitons, 30 new pairs were started on 8 and 9 August 1980 and another 17 pairs were started on 4 and 5 September 1980. Thirty-two of the total 164 chitons were still alive when the experiment was terminated on 1 April 1981. The average chiton survived for about 95 of an average possible 241 days in this study, including chitons killed by the Gymnodinium bloom and taking the study termination date as the "mortality" date of chitons alive at the end of the study.

Since earlier experimentation revealed that a number of chitons die within a week after tagging, presumably due to injury sustained during tagging, no chiton pairs involving chitons that had died in fewer than 7 days were included in comparisons of survival characteristics of specimens in this study. Using the number of days survived as the relevant score for each chiton of a pair, the survival times of untagged chitons were only slightly, but significantly, longer than those of tagged chitons (Sign Test: longer, N=29; shorter, N=23; equal, N=15; p=0.24, one-tailed; Wilcoxon Matched-Pairs Signed Ranks Test, z=-1.85, p=0.03, one-tailed).

In the same chitons, successive measurements of fourth valve widths (FVW) and blotted wet weights allowed assessment of comparative growth of tagged and untagged chitons in pairs. Both types of measurements were made at intervals of 80 to 128 days during the 261 day study. Initial FVWs of chitons in the study ranged from 4.0 to 12.9 mm. Chitons in both tagged and untagged categories showed positive FVW growth during the study [Sign Test (positive vs. negative growth): tagged chitons, N=28, p<0.001, two-tailed; untagged chitons, N=25, p=0.002, two-tailed; Wilcoxon's Matched-Pair Signed Ranks Test on absolute growth in mm per day: tagged, p=0.006, two-tailed; untagged, p=0.0006, two-tailed]. For all chitons combined, the average initial FVW was 8.2 mm and the average final FVW was 8.4 mm, after an average period of 109 days. Comparison of tagged versus untagged chitons in pairs revealed no significant difference in absolute growth of FVW in mm per day (Sign Test: N=25 with 4 ties, p=0.19, one-tailed; Wilcoxon's Matched-Pair

Signed Ranks Test: p=0.24, one-tailed).

Surprisingly, whereas FVW measurements revealed positive growth during the study period, blotted wet weight measurements revealed negative growth, a significant loss in weight of the chitons. Growth of valves and tissue in these animals are apparently independent processes. Results obtained in a growth study by Baxter and Jones (1978) similarly indicated an independence in growth of these two body components in the chiton Lepidochitona cinereus. Initial blotted wet weights of the chitons in the present study ranged from 0.32 to 4.54 g. Chitons within both tagged and untagged groups lost weight during the study [Sign Test (positive vs. negative weight change): tagged, N=25, p<<0.001, two-tailed; untagged, N=25, p<<0.001, two-tailed; Wilcoxon's Matched-Pair Signed Ranks Test on absolute growth in grams per day: tagged, p<<0.001, two-tailed; untagged, p<<0.001, two-tailed]. For all chitons combined, the average initial blotted weight was 1.73 g and the final blotted weight was 1.44 g, for an average loss of about 17% of weight over an average period of 113 days.

It is possible that algal food was limiting during the study and/or that conditions in the laboratory were harsher, by virtue of being abnormal, than those normally experienced by the chitons in the field. However, in Oregon, Palmer and Frank (1974) noted that large percentages of short-term weight changes of tagged Cryptochiton stelleri in their field study were negative during winter months, and Boolootian (1964) reported that of 31 marked specimens of the chiton Mopalia muscosa recaptured after periods of from 1 to 13 months, twenty-three lost up to 36% in body weight. Of course, these weight losses may be tied to spawning. Weight losses may be common in the field.

Comparison of tagged and untagged chitons in pairs in the present study revealed no significant difference in absolute growth in grams of blotted weight per day (Sign Test: N=25 with 2 ties, p=0.50, one-tailed; Wilcoxon's Matched-Pair Signed Ranks Test: p=0.48, one-tailed).

In conclusion, tagging appears to have only a minor effect on the health of *Nuttallina* as indicated by a small increase in the mortality rate and no detectable change in the growth rate of tagged specimens in comparison with untagged specimens in the laboratory. In addition, it should be emphasized that the weight of the tag is negligible and the small drag that the tag produces during submergence undoubtedly falls well within the range normally experienced by *Nuttallina* specimens in the field, since they are subject to fouling by algae and barnacles. Injury associated with the tag also falls within the natural range of experience of *Nuttallina*. Specimens in the field have been observed with mutilated girdles, presumably resulting from grazing by other herbivores, as judged from observations in the laboratory, and possibly from wave-borne rocks and sand. One chiton observed in the field had a group of three small specimens of the stalked barnacle *Pollicipes* growing from the girdle. Since findings of the present study reveal that tagging has no obvious effect on short-term behavior or on growth in the laboratory, and has only a slight effect on the longevity of *Nuttallina*, no dramatic effect of tags on long-term movement behavior is expected in the field.

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