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Evolutionary Processes contributing to Population Structure in the Rockfishes of the Subgenus Rosicola: Implications for Fishery Management, Stock Assessment and Prioritization of Future Analyses of Structure in the Genus Sebastes.

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by

John Edward Budrick

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Committee in charge:

Professor Richard S. Dodd, Chair
Professor George Roderick
Professor Rauri C.K. Bowie

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Abstract

Evolutionary Processes contributing to Population Structure in the Rockfishes of the Subgenus *Rosicola*: Implications for Fishery Management, Stock Assessment and Prioritization of Future Analyses of Structure in the Genus *Sebastes*

by

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This study was undertaken to identify population structure in the three rockfish species of the subgenus *Rosicola* through genetic analysis of six microsatelite loci applied to individuals sampled from 13 locations across the range of each species from Vancouver, British Columbia and San Martin Island, Mexico. Sampling each species throughout their respective ranges allowed for a more comprehensive and conclusive analysis of structure to inform proper stratification of stock assessments, catch tracking and management of these stocks. In addition, modeling potential evolutionary scenarios and testing for evolutionary processes shaping the current population structure were undertaken to better understand factors contributing to population structure and speciation in the subgenus. Results of this study were compared to previous research on members of this and other subgenera within the genus *Sebastes* to identify concordance in the phylogeography of subgenera or consistent processes between species indicative of common mechanisms contributing to genetic variation. Patterns observed were used to infer prioritization of future testing for population structure in the genus *Sebastes* and research to determine whether they are predictive.

No significant population structure was identified in the canary rockfish *S. pinniger* (Gill 1864) or the sunset rockfish *S. crocotulus* (Hyde *et al.* 2008). Three genetically distinct genetic clusters indicative of separate populations were identified in the vermilion rockfish *S. miniatus* (Jordan and Gilbert 1880) and population structure correlated with latitude and depth. While populations were found to overlap in their distribution to some extent, population structure was consistent with the boundary between the San Diegan and Oregonian biogeographic provinces near Point Conception, California. Additional population structure in the Southern California Bight south of Point Conception was correlated with adult depth distribution with a break around 60 m (30 fathoms (fm)). Assignment tests indicated that the young of year of each population as well as *S. miniatus* co-occur and reside in kelp forests before making ontogenetic migration to deeper depths with age. The deepest distributed of these clusters commonly occurs in depths of up to 100 m (50 fm), beyond which *S. crocotulus* was the most common species

(Hyde *et al.* 2007). This population structure related to depth poses issues for stratification of historical data used in stock assessment and allocation of catch to each population as identifying characteristics have not been identified and management is already confounded by the presence of the recently identified cryptic species *S. crocotulus.*

Previous studies hypothesized that speciation between *S. crocotulus* and *S. miniatus* was the result of paedomorphsis in the form of concatenated migration to deeper depth isolating populations by depth arising from the greater area of nearshore habitat around the channel islands during early Pliocene glaciations when sea level falls as much as 300 ft. around 2.3 MYA (Hyde *et al.* 2008). Tests were conducted for repetition of such a pattern in forming population structure observed in *S. miniatus*. Timing of divergence in *S. miniatus* populations identified using assignment tests was estimated to be in the late Pleistocene only 102,429 (95% CI: 22,884 – 248,933) thousand years ago between clusters 1 and 2 and 255,141 (95% CI: 80,080 - 592,847) thousand years ago for their common ancestor and cluster 3. This is consistent with timing of glaciations, but results indicate northward expansion of a population, which may have occurred during an interglacial period. Our analyses indicate that mutation contributes significantly to population structure between species in the subgenus, but does not contribute to population structure in *S. miniatus* indicating that it is due to more recent drift or selection alone.

Population structure in *S. miniatus* consistent with northward migration of *S. miniatus* with lower genetic diversity indicative of a founder effect and private alleles accumulated in the time since divergence. No signal of bottleneck was present in any of the species or populations, though a model reflecting a reduction in population size in the northern population was found to have a high likelihood and posterior probability in Approximate Bayesian Computation analysis of potential evolutionary scenarios. It is possible that reductions in population size occurred too many generations ago to be detected using methods based on relationships between number of alleles and heterozygosity, given expansion of the population since its initial isolation. The results of modeling evolutionary processes indicated potential for gene flow between the northern population and deeper southern population potentially resulting in the shallow southern population. This may be the result of shared alleles from incomplete lineage sorting rather than admixture, or admixture occurred in the distant past as little evidence hybridization was observed in tests for individuals of hybrid classes. The southern shallow population had higher genetic diversity indicating that it may be an older stable population, in potential glacial refugia to the south. These results are also consistent with the possibility that population structure in *S. miniatus* as the result of northward expansion during the interglacial period.

Though the presence of a distinct deeper population in the southern California bight is consistent with paedomorphosis posed as the mechanism resulting in divergence between *S. miniatus* and *S. crocotulus* in Hyde *et al.* (2008), the processes leading to the division of adults on the basis of depth is still uncertain between the shallower and deeper southern population. We postulated that migration or shifts in abundance over time to

glacial refugia in the south during glaciations may be associated with shifts to deeper depths to align with lower temperatures experienced at higher latitudes during the interglacial. Adults of each population would be potentially isolated from the shallower distributed progenitor taking advantage of ample nearshore habitat in the Southern California Bight during glaciations (Jacobs *et al.* 2007, Hyde *et al.* 2008). Having shifted abundance to the south and taken on adaptations to deeper depths, the now more deeply distributed population may remain isolated from the southerly shallow populations.

While concordance was imperfect, analogous structure in other Subgenera within the *Sebastes* including the *Sebastosomas* (Burford and Bernardi 2008), *Pteropodus* (Li *et al.* 2006) and *Sebastomus* (Rocha-Oliveras *et al.* 1999) that reflect population structure north and south of Point Conception as well as cryptic population structure with depth in cowcod (Hess *et al.* 2014), which we observed in the *Rosicola*. In other subgenera, differentiation is commonly accompanied by color variation and many of the sister species with similar indices of differentiation to populations identified in *S. miniatus* are identified as separate species due to their disparate coloration. This may in part be the result of sexual selection facilitated by internal fertilization (Gunderson and Vetter 2006).

Commonalities provide guidance on prioritizing future research to focus on species with distributions bridging Point Conception and a broad range of depth distributions. In addition, species with variable coloration should be considered for analysis as the line between species may be blurry but somewhat visible to the eye, needing only be confirmed by genetic analysis that can detect differentiation that in this case was indicative of a relatively recently diverged sister species (Frable *et al.* 2015, Narum *et al.* 2004). Nearshore species that release their larvae in the "sticky water" closer to shore that is slower moving due to turbulence and shear forces slowing its movement in the prevailing currents (Gunderson and Vetter 2006; Berntson and Moran 2009). This may contribute to maintenance of populations structure due to relatively inhibited larval transport compared to congeners in deeper waters spawning during periods of stronger flow of currents along the coast. These results provide guidance on selection of species for further analysis within the subgenus to identify what might be population structure or cryptic species that should be accounted for in stock assessments and management where possible. Nearshore species with large ranges bridging Point Conception, California, spawning in the spring during maximal upwelling, with broad depth range and with a large degree of variability in coloration are most likely to harbor genetic variation of interest to management and their analysis may provide further understanding the speciation process in this diverse genus.

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Dedication

This work is dedicated to my colleagues, friends and family here and gone for the support and encouragement they provided during the course of completing this endeavor.

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University of California at Berkeley, Minor in Forestry, 2000.

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Environmental Scientist, California Department of Fish and Wildlife, Marine Region, Representative to the Scientific and Statistical Committee of the Pacific Fishery Management Council, 10/2015 - Present.

Associate Marine/Fisheries Biologist, California Department of Fish and Wildlife, Marine Region, Representative to the Groundfish Management Team of the Pacific Fishery Management Council, 06/2007 – 09/2010, 01/2012 – 09/2015.

Environmental Scientist, California Department of Fish and Wildlife, Marine Region, Field Supervisor and Lead for the California Recreational Fishery Survey, 09/2010 - 01/2012.

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Founding Board Member, Waterside Workshops-Street Level Cycles and Berkeley Boat House Programs, Berkeley California, Inception in 01/2007 to 10/2014.

Federal Contractor/Graduate Student Collaborator, National Marine Fisheries Service, 05/2003 - 05/2006.

Executive Director, Fishery Enhancement and Research Foundation, 10/2002 - 5/2006.

Graduate Student Instructor, UC Berkeley, Tree Taxonomy and Growth (2 Semesters), Forest Genetics (2 Semesters), American Wildlife (1 Semester) and Molecular Ecology (1 Semester).

Fishery Technician, Pacific States Marine Fisheries Commission, Marine Recreational Fishery Statistical Survey, 02/2001- 04/2005.

Scientific Aid, California Department of Fish and Wildlife, San Francisco Bay Herring Management Project, 11/2001 - 08/2002.

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Introduction

The United States west coast groundfish fishery supports a multi-million dollar fishing industry providing employment and contributing to the greater economic vitality of coastal communities (PFMC 2012). The passage of the Fishery Management and Conservation Act of 1976 provided low interest loans for the purchase of vessels by domestic fishermen and acted as a mechanism to help exclude foreign vessels, a strategy that ultimately resulted in overcapitalization (McEvoy 1986). This overcapitalization during the 1970s and 1980s combined with a lack of appropriate regulation and environmental conditions associated with a high temperature phase of the pacific decadal oscillation during the 1980s and 1990s resulted in the depletion of many bottom dwelling species referred to as groundfish stocks (PFMC 2012). In January of 2000 the West Coast Groundfish Fishery Disaster was declared by the U.S. Secretary of State. Severe restrictions on harvest were put in place to begin rebuilding overfished stocks, resulting in economic hardship and decline of some local fishing communities (PFMC 2012).

The genus *Sebastes* is the most speciose fish genus on the Pacific coast of North America with over 67 species (Love *et al.* 2002). These species comprise the majority of the species in the in the West Coast Groundfish Fishery Management Plan (WCGFMP) of the Pacific Fishery Management Council (PFMC). This genus includes species that are extremely vulnerable to overfishing due to their slow growth rates, late age of maturation and variable reproductive success between years (Waples *et al.* 2009). Several stocks have been identified as overfished including the canary rockfish *S. pinniger*, darkblotched rockfish *S. crameri*, Pacific ocean perch *S. alutus*, cowcod *S. levis*, yelloweye rockfish *S. ruberimus* and bocaccio *S. pauscispinus* (PFMC 2012).

The status of many groundfish stocks managed by the PFMC under the WCGFMP have been assessed and of those, ten species were deemed overfished including the aforementioned seven species of rockfish. Rebuilding plans for overfished species are based on stock assessments that sometimes assume populations are panmictic interbreeding populations or management regions for stocks are implemented on the basis of biological or political boundaries. Genetic analyses of populations can provide evidence of population structure that should be accounted for in stock assessments to allow accurate accounting of population dynamics for species subject to fishing mortality and to help ensure sustainable harvest levels across the species distributional range (Gundersen and Vetter 2006). Of the more than 90 species in the WCGFMP, few have been the subject of genetic analysis of population structure to delimit separate stocks for management, including most of the assessed stocks of the rockfish of the genus *Sebastes* (Waples *et al.* 2009).

Members of the *Sebastes* are characterized by internal fertilization and gestation, followed by a primitive form of live birth called lecithotrophy with some degree of direct maternal energetic contribution to development while in the ovary and hatching just prior to parturition as larvae are extruded into the environment (Love *et al.* 2002). Larvae may travel for 3-4 months and up to 200 miles in currents as members of the planktonic community before settling on benthic habitat (Love *et al.* 2002). Timing of release of

larvae may strongly influence the direction and magnitude of gene flow within the California Current, Alaskan Gyre and Southern California Eddy (Gunderson and Vetter 2006). The distance of larval dispersal is determined in part by the strength and direction of currents during the pelagic larval phase, but their distribution in the water column due to buoyancy, swimming behavior or larval survival ultimately determines the range limits of distribution (Gunderson and Vetter 2006).

Larval survival is determined in part by the temperature tolerance of the species, prey availability, predation rates and advection offshore at jets among other biological tolerances and requirements (Cowen R.K. and S. Sponaugle 2009, Gaines *et al.* 2010, Kinlan *et al.* 2005). The effective migration distance of larvae and adults and effective reproduction in the newly established habitat determine the potential for range extension or persistence with fluctuating environmental conditions at all time scales (Palumbi 2003, Sotka and Palumbi 2006). The current depth and latitudinal distribution of a given species may not reflect the historical distribution given the range of environmental conditions during glacial cycles, thus the factors contributing to speciation in allopatry due to vicariance or founder effects can be difficult to identify (Jacobs *et al.* 2004, Longhurst 2007, Bigg *et al.* 2008).

Detection of consistent genetic breaks among species in the *Sebastes* has been elusive. Although Point Conception, Cape Mendocino and Cape Blanco have been implicated as barriers to gene flow as a result of differential water temperatures, divergent currents, or jets where larvae are advected offshore (Gunderson and Vetter 2006). A combination of physical and biological factors may result in differential patterns of population structure within the range of each species. Evidence for genetic breaks at these locations varies across species and between genetic markers within a species (Cope 2004, Burford and Larson 2007, Gharrett 2005). However, there may be common mechanisms of speciation or barriers to gene flow that would result in genetic breaks between populations that are ubiquitous across species as evidenced by phylogeographic concordance.

Understanding the current distribution of genetic variation in the three species of rockfish in the subgenus *Rosicola* including the previously overfished and now recently rebuilt stock of *S. pinniger* (Gill 1864, Figure 1, c.) can provide information pertinent to proper management and a starting point in examining micro-evolutionary forces contributing to speciation in the subgenus and population structure within each species. Cryptic species continue to be identified within *Sebastes* and include *S. miniatus* the vermilion rockfish (Jordan and Gilbert 1880, Figure 1, a. and b.) and the recently identified *S. crocotulus* the sunset rockfish (Hyde *et al.* 2008, Figure 1, d.) within the subgenus *Rosicola* that may indicate the mechanism by which speciation occurs (Hyde *et al.* 2008). Cryptic species present a complication for management as stock assessment and tracking of landings cannot account for each species since they are difficult discriminate visually. Even within *S. miniatus*, there is considerable variation in coloration, making identification confusing for those trying to identify members of each species.

a.) *S. miniatus* (solid color morph)

b.) *S. miniatus* (mottled color morph)

c.) *S. pinniger*

d.) *S. crocotulus*

Figure 1. Pictures of: a.) *Sebastes miniatus* (solid color morph), b.) *S. miniatus* (mottled color morph) c.) *S. pinniger*, d.) *S. crocotulus*.

In this study, the population structure in the three *Sebastes* species within the subgenus *Rosicola* including *S. pinniger* an overfished species, *S. miniatus* and its cryptic sister species *S. crocotulus* are analyzed with the same six microsatellite loci across their known range (Figure 2). This primary purpose of this research is to delineate individual breeding populations, which is essential to the proper assessment and management of groundfish stocks to help ensure sustainable yields, prevent overfishing and rebuild overfished stocks. We also seek to elucidate the underlying evolutionary forces that have driven microevolution in the *Rosicola* as this genus serves as a good model for understanding the radiation and speciation in the *Sebastes* rockfish as a whole and test for concordance in these processes by comparing our results to previous studies in other subgenera.

The three species within the subgenus *Rosicola* have different latitudinal and depth distributions that, as a group, extend across the Aleutian, Oregonian and San Diegan biogeographic regions (Love *et al.* 2002). Our study provides an analysis of population structure across the distribution of each species over a larger geographic range and higher spatial resolution than examined in previous studies with this class of genetic markers. This provides an opportunity to evaluate the coastwide patterns of population structure for closely related species with differing life history characteristics (Love *et al.* 2002). Samples were collected from throughout the known range of each species to allow a comprehensive analysis of population structure to inform management and to provide context for further analysis of the evolutionary mechanisms underlying existing structure

or lack there of (Figure 2). Previous studies of species in the *Rosicola* have either provided conflicting results between markers as with canary rockfish (Wishard *et al.* 1980; Gomez-Uchida 2006), covered a more limited portion of the geographic range (Gomez-Uchida 2006; Sivasundar and Palumbi 2010), or used only a single mtDNA locus representing only the maternal lineage (Hyde *et al.* 2009).

This study was undertaken to improve our understanding of the geographic and bathymetric discontinuities in gene flow within species of the subgenus *Rosicola* through more thorough sampling and analysis of population structure throughout the range of each of the three species. Furthermore, we test various models representing possible evolutionary scenarios to gain further insight in to the forces shaping population structure and speciation in the subgenus *Rosicola*. Lastly, we review literature from previous studies and identify commonalities that may be indicative of shared and repeated patterns of genetic differentiation associated with geographic breaks or evolutionary forces shaping population structure in the *Sebastes* as a whole. The intended outcome increased knowledge of population structure to inform stratification of stock assessments and management of these species, an improved understanding of the evolutionary process in *Sebastes* and determination of patterns aiding in selection of high priority species for future analysis are identified.

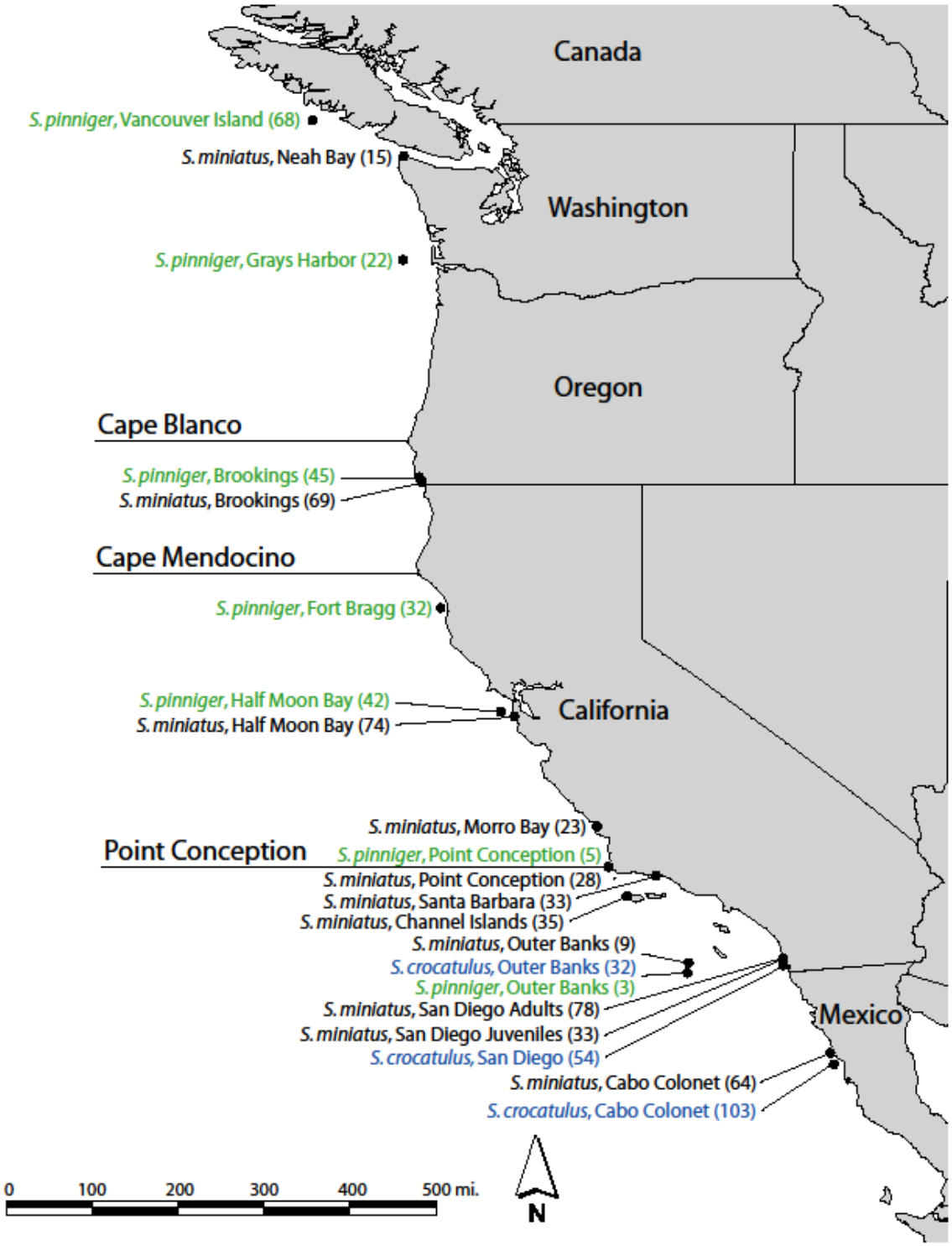

Figure 2. Chart of the sample locations and the number of individuals sampled for each species, *S. miniatus* (black), *S. crocotulus* (blue) and *S. pinniger* (green).

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Population Genetic Analysis of the Overfished Rockfish Species *Sebastes pinniger* and Implications for Management

Abstract

In this study we tested for population structure in the overfished canary rockfish, *Sebastes pinniger* (Gill 1864), as well as for the presence of genetic bottlenecks indicative of severe reductions in population size. Such reductions could result from either overfishing of this stock during from the 1960s to 1990s by foreighn and domestic fleets (Thorson and Wetzel 2015) or earlier natural declines in abundance during the Pleistocene or halocene period during glacial cycles (Jacobs et *al.* 2004). The results of our analysis using six microsatellite loci were interpreted with consideration of previous research either conducted with different genetic markers or sampled over a more limited extent of the range. Tests for heterozygote excess using the program Bottleneck (Cornuet and Luikart 1997) did not detect evidence of genetic bottlenecks resulting from overfishing of its population size to less than 10% of its historical spawning stock biomass. The duration or severity of the decline may not have been long enough for alleles to be lost in sufficient number to result in detectable genetic bottlenecks. No significant population structure was identified between sample locations in tests for significant indices of genetic differentiation (F_{ST} <0.007) or in clustering algorithms employed in the programs BAPS (Corrander *et al.* 2004) or Structure (Pritchard *et al.* 2000).

Private alleles were more prevalent at sample locations at the edges of the sampled range of the species distribution near Vancouver Island, Canada and Half Moon Bay, California, which may be correlated with traits of adaptive significance at the extremes of the species tolerance to environmental conditions. Localized depletion at the edges of the species range should be avoided to prevent potential loss of locally adapted genetic variants. If private alleles are physically linked to loci coding for adaptive traits resulting from strong environmental selection, individuals from the center of the range may be less able to effectively migrate to the edges of the species range to repopulate the margins of their distribution. The current assessment treats the species as a single population, but explicitly tracks differences in age-structure and stock status in each of three spatial strata consistent with state boundaries (PFMC 2016). This model structure is supported by our findings, though managers should consider the need to avoid localized depletion at the edges of the species core range. The distribution of this species extends over political borders shared with Canada and the lack of genetic population structure may have implications for assessment and management that may benefit from collaborative efforts to properly manage this straddling fish stock.

Introduction

The purpose of this study is to identify and delineate population structure and test for evidence of genetic bottlenecks in the canary rockfish *S. pinniger* (Gill 1864), a recently rebuilt fishstock that had been severally overfished. The stock has been assessed and managed as a single unit from the Mexican border to the Canadian border (Thorson and Wetzel 2016), though the most recent assessment tracks differences in age-structure and stock status in spatial strata consistent with state boundaries. This allows the assessment

to account for variation in exploitation rates among states due to differences in regulatory histories. Previous studies of population structure using genetic methods have not provided conclusive or consistent results, which we attempt to resolve in this study. A previous allozyme study of *S. pinniger* identified slight but statistically significant population structure off the Washington coast at one locus (Wishard *et al.* 1980). However, no significant population structure among microsatellite loci was detected over a restricted portion of the geographic range north of Fort Bragg, California (Gomez-Uchida and Banks 2006).

No studies have been conducted on the southern portion of the range south of Fort Bragg, California, where *S. pinniger* is commonly encountered to Point Conception and with lower frequency as far south as Baja California, Mexico. The canary rockfish, *S. pinniger,* is a schooling species, found in depths from 10 fm to 150 fm, predominantly distributed in the colder Aleutian and Oregonian biogeographic provinces, from the Gulf of Alaska to Point Conception, California. It is rarely encountered in the San Diegan biogeographic province to the south of Point Conception (Love *et al.* 2002). There are a number of aspects of the life history of *S. pinniger* pertinent to understanding the results of population genetic analysis.

Spawning takes place in winter and larvae are pelagic for three to four months before settling on and around shallow reefs where they remain for three years before going through an ontogenetic migration to progressively greater depths (to 300 meters) as they age (Love *et al.*2002, Wallace and Cope 2011). Unlike many other members of the genus *Sebastes* that are more solitary, *S. pinniger* exhibit schooling behavior and has been found to move large distances as adults, with up to 326 kilometers between location of tagging to recapture in tagging studies (Love *et al.* 2002). The long larval duration and timing of larval release prior to spring upwelling, in conjunction with increased southward flow of the California current is conducive to gene flow along the coast. This, as well as the large distance of adult movement, led us to hypothesize that canary rockfish would exhibit a lower degree of genetic differentiation than more sedentary, long-lived, late maturing and solitary species of the genus *Sebastes*.

In addition to analysis of population structure, we examined whether there is a detectable genetic bottleneck due to recent declines in abundance of the stock, which was estimated to have been reduced to 10.8% of historical biomass in the mid 1990s (Wallace and Cope 2011). The stock was declared overfished in 1999, when it was discovered that the spawning stock biomass fell below the threshold value of 25% of unfished levels for rockfish, forming the basis for its overfished status (Wallace and Cope 2011). To facilitate rebuilding of the stock to above 40% of unfished biomass at which the stock is considered healthy, severe reductions in harvest limits were implemented and abided by through implementation of reduced season lengths, lower trip limits, gear restrictions and area closures to minimize interactions with this and other overfished shelf rockfish species (PFMC 2012). The most recent assessment indicates that the stock has rebuilt to above this threshold level and is now above 56% of its historical spawning stock biomass (Thorson and Wetzel 2006). We test whether this magnitude of reduction in abundance

or a previous severe reduction in population size due to natural population size fluctuations has been sufficient to lead to a detectable signal of a genetic bottleneck.

Testing for population structure in *S. pinniger* is necessary to confirm that the current assessment model structure, which assumes a single stock, is appropriate; in light of the high potential for adult movement observed in tagging studies, in addition to larval dispersal potential (Love *et al. 2002*). Adult migration and larval dispersal may contribute to the ability of distant populations to replenish one another over time. Further tests for a pattern of isolation by distance between sampled locations along the coast were conducted to help inform the degree to which distant individuals may replenish heavily harvested areas. By examining the distribution of allelic variation, inbreeding and heterozygosity, we identify the genetic diversity, potential range extensions and patterns indicative of the approximate center of the species range given expectations of stable abundance and newly established populations. The intent is to use genetic data from microsatelite markers to inform aspects of the biology of the species as well as its assessment and identify management implications to the extent possible given the available data.

Methods

Sample collection

White muscle tissue or pectoral fin clips were taken from 209 individuals and preserved in 95% ethanol for later DNA extraction. Fish were collected from long-line research surveys, recreational catch, spear fishing and from the National Marine Fisheries Service Triennial Trawl Survey, primarily sampled at five locations from Vancouver Island, Canada to Half Moon Bay, California. An additional five specimens were collected in the vicinity of Point Conception, California and three specimens from the Outer Banks off San Diego, California, which were included in assignment tests to test for structure at the southern extent of the range where sample size was low due to rarity. All samples were collected after fish were deceased, having being caught by licensed anglers or permitted sampling programs occurring prior to accessessing tissue samples for our study and were thus exempt from UC Berkeley Animal Care and Use Committee Protocols per discussion with a representative. Tissue samples and extracted DNA are available for confirmation of our results or further analysis. The location name, state/country, average latitude and longitude, three digit alpha code for the population used in further analyses and number of samples are provided in Table 1.

It was assumed that specimens from the recreational fishery were caught no further than 30 miles north or south of the port at which they were sampled as most trips north of Point Conception are day trips with limited range to maximize fishing time. To standardize the geographic scope of sampling locations, groups of individuals within 60 miles of one another were selected from the National Marine Fisheries Service sample archive to form putative populations for analysis. Aggregation of these samples resulted in seven putative populations for analysis. The samples consisted of individuals representing multiple year classes, and are more representative of inter-generational stability in genetic variation (Waples 1998).

Table 1. Sampled putative populations, alpha codes for sampled locations, number of specimens of *S. pinniger* and average latitude and longitude of capture at each sample location. A chart displaying the sample locations is provided in the overview section.

Sample Location	State / Country	Alpha Code	Individuals	Latitude	Longitude
Vancouver Island	Canada	VCI	68	49.065	-126.403
Grays Harbor	Washington	GHB	22	46.353	-124.644
Brookings	Oregon	BRK	45	42.139	-124.334
Fort Bragg	California	FTB	32	39.599	-123.914
Half Moon Bay	California	HMP	42	37.583	-122.742
Point Conception	California	PTP	5	34.504	-120.612
Outer Banks	California	OBP	3	32.503	-118.703

DNA extraction

DNA extraction was performed using several protocols. For most samples, DNA was extracted using the Doyle and Doyle (1987) extraction method, as modified by Cullings (1992), with an additional proteinase K digestion step prior to chloroform DNA purification and subsequent isopropyl alcohol, sodium acetate and ethanol precipitation. Many of the samples were extracted using a standard proteinase K digestion followed by a lithium chloride:chloroform nucleic acid purification and subsequent ethanol precipitation (Gemmel and Akiyama 1996). DNA from the remaining samples was extracted using either the DNeasy kit (QIAGEN) following the manufacturer's protocol or by use of a Chelex (Bio-Rad Laboratories) boiling technique (Hyde *et al.* 2005). Extraction method did not affect results in test genotyping of extractions of the same individual.

DNA amplification

Nine microsatellite loci were chosen from the libraries developed by Gomez-Uchida *et al*. (2003) and Westerman *et al*. (2005) and tested on a group of specimens from throughout the species range. Of these loci, six were selected for easy, reliable scoring, and moderate polymorphism for all three species in the subgenus. All microsatellite loci were amplified by polymerase chain reaction (PCR) following the conditions described in Gomez-Uchida *et al*. (2003) and Westerman *et al*. (2005). Fluorescently labeled PCR products were sized using an ABI 3130XL Genetic Analyzer with the ROX 500 size standard (Applied Biosystems) and scored using Genemapper version 3.7 software (Applied Biosystems).

Data quality tests

Tests for evidence of scoring error due to stuttering, large allele dropout and null alleles were conducted using Microchecker (van Oosterhaut *et al.* 2004). The program uses a Monte Carlo simulation method to generate expected homozygote and heterozygote allele size frequency differences. Hardy-Weinberg theory of equilibrium was used to estimate expected allele frequencies and the frequency of any null alleles. Significant deviation (*P*

< 0.05) from expected values would indicate the presence in introduced error due to a missing allele (i.e. an allele smaller or larger than the size standard used).

Markov Chain Monte Carlo Simulations (Guo and Thompson 1992) with 100,000 Markov chain steps and 3,000 dememorization steps were used to test for deviation from Hardy Weinberg Equilibrium (HWE) in Arlequin (Excoffier *et al.* 2005) for each locus within each population and with all populations combined. Probabilities were corrected using the sequential Bonferroni procedure (Rice 1989). Log-likelihood ratio-tests with the empirical distribution obtained by a permutation procedure (Excoffier and Slatkin 1998) were used to test for linkage disequilibrium (LD) between loci under the null hypothesis of linkage equilibrium in Arlequin (Excoffier *et al.* 2005). Simultaneous tests were adjusted using a Bonferroni adjustment with an initial α level of 0.05 (Rice 1989).

Identification of population structure

Indices of genetic differentiation and clustering methods were employed to identify genetic population structure within each species. Pairwise F_{ST} (Weir and Cockerham 1984) was estimated between sampled populations in the program Arlequin (Excoffier *et al.* 1995). Tests for significance were also conducted with the null distribution of pairwise F_{ST} values under the hypothesis of no difference between the populations obtained by permuting alleles among populations. The *P*-value of the test is the proportion of permutations leading to an F_{ST} value larger or equal to the observed one.

The question becomes whether a given magnitude of F_{ST} constitute populations as defined by molecular criteria for deviation from panmixis. Among these criteria are thresholds for the number of clusters, migration rates and measures of genetic differentiation as measured by F_{ST} that constitute genetically differentiated populations with independent demographic processes. Relative to migration, two criteria defined by Waples and Gaggliotti (2006) regarding indices of genetic differentiation constituting biologically meaningful population structure were evaluated. The first is referred to as EV 3 in which populations exchange less than 5 migrants per generation corresponding to an approximate $F_{ST} = 0.05$ and the second less restrictive criteria EV 4 in which less than 25 migrants per generation are exchanged, corresponding to an $F_{ST} = 0.01$. Both criteria can be associated with evidence of departures from panmixia (Waples and Gaggliotti 2006). Considering the greater potential for migration of larvae in the marine environment the less stringent EV4 of $F_{ST} = 0.01$ corresponding to 25 migrants per generation may be sufficient to detect population structure. We compared the F_{ST} values for comparisons between populations to examine whether the thresholds are exceeded indicating the presence of significant population structure.

The program STRUCTURE (Pritchard *et al.* 2000) is a Bayesian algorithm used to identify the number of clusters of samples (K) or populations that minimize deviation from Hardy-Weinberg Equilibrium and Linkage Disequilibrium. The optimal number of clusters (K) of samples results in the highest negative log-likelihood value (Pritchard *et al.* 2000). The analysis was conducted with K set from 1 (a single panmictic population) to the number of sample locations analyzed as putative populations. We inferred population division (number of *K* populations) by performing 10 independent runs of

each *K* with a burn-in of 10,000 iterations, and 1,000,000 iterations of the Gibbs sampler. The admixture model with correlated frequencies was used without prior population information. An ad hoc statistic ΔK based on the rate of change in the log probability of data between successive K values from STRUCTURE was used as further evidence of the number of clusters in the sample set (Evanno *et al.* 2005).

A second clustering method, BAPS (Corrander *et al.* 2004), was used to determine which combination of predetermined samples is best supported by the data based on the criteria of maximizing heterogeneity between groups and homogeneity within groups. The program uses importance sampling to approximate posterior probabilities performing an exact Bayesian analysis by enumerative calculation to determine the expected number of clusters. The optimal number of populations is assumed to be the partition with the highest posterior probability. Burn-in was conducted with 10,000 iterations followed by 1,000,000 steps after burn-in. Values of K clusters from one to the total number of sampled locations were examined to identify the number of clusters that provided the model with the maximum Bayesian posterior probability. Analysis of clustering groups of individuals was followed by an analysis of admixture based on mixture clustering.

Analysis of genetic diversity, inbreeding and private alleles

The distribution of genetic variation and the degree of inbreeding, the average heterozygosity, number of alleles, allelic richness reflecting the number of alleles normalized by sample size, the fraction of total alleles in each group, the inbreeding coefficient F_{IS} in each population were analyzed using the program FSTAT (Goudet 1995). Allelic diversity and the number of private alleles between sample locations were compared to examine whether: 1) sample locations or clusters have higher allelic diversity indicative of centers of distribution or, 2) a greater number of private alleles indicative of isolation and divergence, or selection and adaptation at the edge of their distribution. An analysis of the inbreeding coefficient F_{IS} was conducted across sample locations to determine whether the degree of inbreeding differs among regions.

Tests for genetic bottlenecks from recent severe reductions in population size

Following a severe reduction in population size referred to as a "bottleneck", gaps in the distribution of allelic lengths can occur due to loss of alleles and heterozygote excess can occur depending on the variability of the locus and the time that has passed since the bottleneck began. We tested for heterozygote excess, indicative of recent reductions in population size in a sample location that may reflect overfishing or a recent extreme natural fluctuation in population size, using the program Bottleneck (Cornuet and Luikart 1997). The fact that heterozygosity deficiency can occur with loci evolving under the strict one step single mutation model (SMM) presents potential problems for the detection of bottlenecks when using tests for heterozygosity excess (Cornuet and Luikart 1997). The test may not be significant even in a bottlenecked population because some loci, evolving under the strict SMM and with large effective population size, exhibit heterozygosity deficiency. Consequently, a significant heterozygote excess for selectively neutral markers should be detected only in populations having experienced a severe recent prolonged population size reduction.

We applied three statistical tests for excess heterozygosity in the program Bottleneck. The first test is based on sign tests on the difference (observed - expected) in heterozygosity across all loci (L) in which there is expected to be an equal probability L/2 of deficit or excess in a population in mutation drift equilibrium with no bottleneck. For a sampled population a significant deviation from L/2 can be detected assuming a binomial distribution of outcomes. A second test takes into account the magnitude of heterozygote excess/deficiency with a null hypothesis that the difference between observed and expected heterozygosity is random and the expected value is equal to zero for all loci for a population in mutation drift equilibrium. If the null hypothesis is rejected with a one tailed test for heterozygote excess ($P<0.05$), the alternative hypothesis is that heterozygote excess exists. The overall power of this parametric test is generally higher than that of the first non-parametric test, but both tests are slightly more conservative in rejecting the null hypothesis when the single mutation model is assumed (Cornuet and Luikart 1997). Both test for significant deviations from the expected heterozygosity that can result from the more rapid loss of alleles due to reduction in population size for which a lower heterozygosity would be expected than is observed. Estimates of expected values were made using 100,000 replications in the program Bottleneck with the provided data.

Results

Data quality tests

The test for allelic dropout, stutter and null alleles using Microchecker did not identify evidence of scoring errors or allelic dropout, as estimated values of homozygosity and heterozygosity were within the range of expected values from the Monte Carlo simulations. Exact tests for deviation from HWE at the species level revealed no significant deviation ($P < 0.05$) at any loci, while at a population level the few instances of significant deviation from HWE and LD were observed at a greater number of loci further to the south towards Half Moon Bay (Table 2). Deviation from HWE and the presence of LD between sets of loci were inconsistent among populations (Table 2).

Table 2. Results for tests for Linkage Disequilibrium and deviation from Hardy-Weinberg Equilibrium in *S. pinniger*, sampled in each location.

Population	Loci Deviating from HWE	Pairs of Loci Exhibiting Significant Linkage Disequilibrium
Vancouver Island	None	3-6
Grays Harbor	None	None
Brookings	3	1-5, 3-6
Fort Bragg	None	4-5, 3-6
Half Moon Bay	2, 4	1-4, 2-4, 4-6

Identification of population structure

No significant population structure was detected when Bayesian analyses were performed in STRUCTURE or in a population level mixture analysis in BAPS. Though significant linkage disequilibrium was found between two pairs of loci, range-wide, analysis with the program STRUCTURE indicated that a single population model, with K = 1 resulted in the highest negative log-likelihood value (LnP(D) = -4711). The population level mixture analysis in BAPS supports the inference from STRUCTURE, which did not identify population structure.

A mixture analysis at the individual level in BAPS resulted in the highest log maximum likelihood for K = 3. However, admixture analysis of the distribution of individuals assigned to each cluster showed that they were interspersed between sampled locations, with no clear indication of patterns in the distribution of the clusters, as seen in Figure 1. In addition, the proportion of ancestry from STRUCTURE with K = 3 is equal in all individuals across sample locations (Figure 2), indicative of a single population, contradicting results from the mixture analysis at the individual level in BAPS. The low and non-significant pairwise F_{ST}-values between sample locations ($F_{ST} < 0.005$, Table 3) are well below the thresholds of $F_{ST} = 0.05$ or 0.01 for deviation from panmixis between sample locations according to the criteria of Waples and Gaggliotti (2006), consistent with the absence of population structure observed in STRUCTURE.

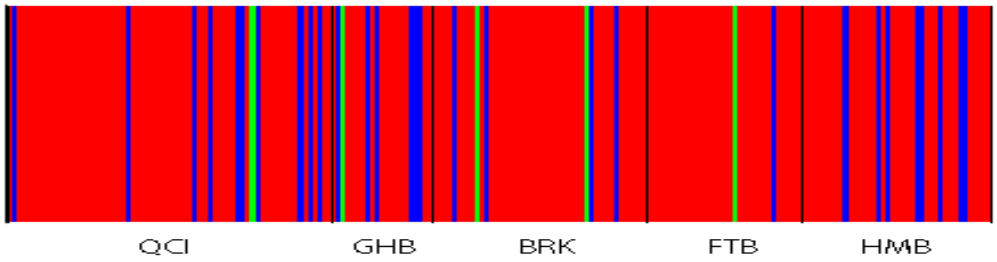

Figure 1. Posterior probability of assignment of each individual to each cluster represented by the differing colored bars from admixture analysis of BAPS for K = 3 clusters conducted at an individual level for *S. pinniger*.

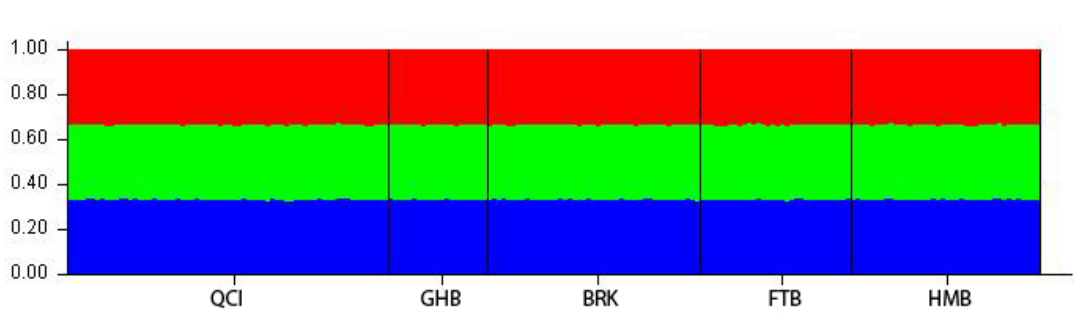

Figure 2. Proportion of ancestry from STRUCTURE for *S. pinniger* with three clusters (K = 3).

Table 3. Pairwise F_{ST}-values between sampled locations and corresponding *P*-values for *S. pinniger* ($P < 0.05$, Bonferroni Corrected: $\alpha = 0.05/6 = 0.0083$).

Pop 1	Pop 2	F_{ST}	*P* value
VCI	GHB	-0.00511	0.937
VCI	BRK	-0.00024	0.459
VCI	FTB	-0.00098	0.613
VCI	HMB	0.00146	0.279
GHB	BRK	-0.00011	0.414
GHB	FTB	-0.00587	0.847
GHB	HMB	-0.01038	0.982
BRK	FTB	-0.00348	0.739
BRK	HMB	0.00019	0.360
FTB	HMB	0.00111	0.378

Tests for Isolation-by-Distance using a mantel test (Mantel, 1967) (Table 4) did not recover a significant correlation ($P < 0.05$) of genetic differentiation with geographic distance, as measured by linearized $F_{ST,}$ with distance between sample locations in *S. pinniger*. Many of the F_{ST}-values estimated in FSTAT were negative (nearly zero) or extremely low ($F_{ST}<0.001$). The linear regression of linearized F_{ST} with distance is very close to zero with a very weak slope (Figure 3). This would suggest that there is no or weak genetic differentiation with distance between Vancouver Island, Canada and Half Moon Bay, California populations of *S. pinniger*, indicative of high gene flow among the sampled locations.

Table 4. Results of mantel tests for correlation between F_{ST} and distance in kilometers (km) between sampled locations.

Parameter	F_{ST} vs. km.	F_{ST} vs. ln (km.)	ln (F_{ST}) vs. km.	ln (F_{ST}) vs. ln (km.)
r	0.07420	-0.09500	0.13180	-0.08240
p-value	0.47400	0.63200	0.29500	0.54600
Slope	0.00001	-0.01230	0.00127	-1.49000
R^2	0.00550	0.00902	0.01740	0.00679

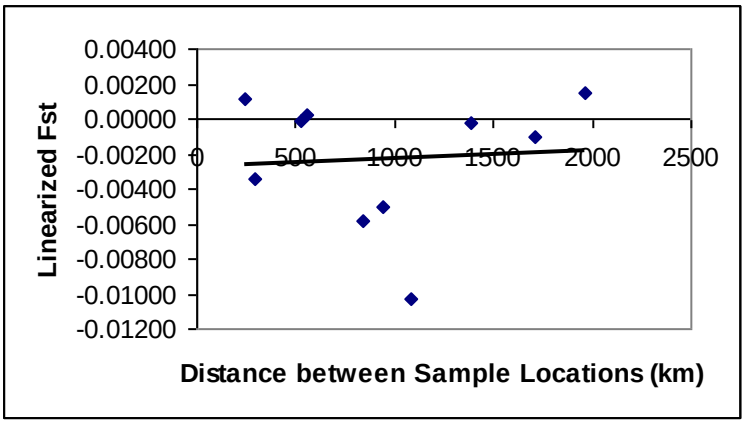

Figure 3. Genetic differentiation measured by linearized F_{ST} with the natural log of geographic distance in kilometers (km) between sampled locations of *S. pinniger*.

Evaluation of genetic diversity and inbreeding

The highest number of private alleles were found in the Half Moon Bay and Vancouver Island samples, near the edges of the sampled range. This is consistent with expectations of adaptation to more extreme conditions at the edge of the species' range, which may have occurred at loci linked to the neutral microsatellite loci used in this study (Table 5). Alternatively, the presence of private alleles may be the result of reduced geneflow at the edge of the species range resulting in genetic drift. The greatest number of alleles was found in the Vancouver Islands, though the allelic diversity was marginally higher closer to the center of the range in Fort Bragg. The lowest allelic diversity was observed in Half Moon Bay. Grays Harbor had the least sampled alleles and highest F_{IS}, but had high allelic diversity (Table 5), though there were only 22 sampled individuals in Grays Harbor and random sampling error could explain this discrepancy.

Table 5. The number of private alleles, average heterozygosity, number of alleles, allelic diversity, fraction of total alleles sampled and inbreeding coefficient F_{IS}, for each sample location.

Sample Location	Private Alleles	Average Heterozygosity	Alleles	Allelic Diversity	Fraction of Alleles	F_{IS}
Vancouver Island	6	0.74	84	56.1	0.8	0.01
Grays Harbor	1	0.76	59	56.1	0.6	0.07
Brookings	1	0.71	73	54.7	0.7	0.00
Fort Bragg	4	0.74	71	58.0	0.7	-0.03
Half Moon Bay	6	0.72	68	53.0	0.7	0.03
All	-	-	104	55.8	1.0	-

Tests for genetic bottlenecks resulting from recent severe reductions in population size
No evidence of population bottlenecks were detected at any of the sample locations (Table 6). The sign test showed heterozygote excess at three loci in the Grays Harbor sample location, though the remaining tests showed no significant deviation from expected results for Wilcoxen or standard rank tests, while mode shift was normal without a pattern indicative of a bottleneck. The inconsistent sign test results recovered for half the loci at Grays Harbor may in part be an artifact of random sampling error given the low sample size at this location.

Table 6. The observed heterozygosity, expected heterozygosity and results of sign, standard difference, signed rank tests as well as evidence of a mode shift in allele distributions from the program Bottleneck for each sample location.

Sample Location	Ho	He	Sign Test	Standard Difference Test	Wilcoxen Test	Mode Shift
Vancouver Island	0.74	0.82	0/6	Deficient	Deficient	Normal
Grays Harbor	0.75	0.77	3/6	Non-Sig.	Non-Sig.	Normal
Brookings	0.71	0.78	0/6	Deficient	Deficient	Normal
Fort Bragg	0.74	0.79	1/6	Deficient	Deficient	Normal
Half Moon Bay	0.72	0.77	1/6	Deficient	Deficient	Normal

Discussion

Tests for population structure and comparison to previous research
Tests for population structure using STRUCTURE, BAPS and genetic differentiation as measured by F_{ST} indicate a lack of population structure among sampled locations. Despite having sampled across the majority of the distribution of *S. pinniger*, no significant population structure was detected with the genetic markers employed. Here we compare the results to those found in previous population genetic research conducted over a more limited geographic range and with other genetic markers.

In an allozyme study conducted by Wishard *et al.* (1980) a variant of the PGI-2 allozyme locus was absent in samples north of Central Oregon, indicating a potential barrier to gene flow near Cape Blanco hypothesized to be due to isolation as a result of an offshore jet in the vicinity or divergence of the Alaskan and California currents. No evidence of such structure was identified in our study despite having sampled multiple locations north and south of Cape Blanco. In research conducted by Wishard *et al.* (1980) differentiation in a variant of the PGM locus was found to be correlated with depth, indicating an association of the genotype with bathymetry thought to have adaptive significance. Alternatively, the presence of this variant to the north is consistent with our findings of private alleles to the north of the range of the species, which support the supposition that private alleles are indicative of selection at the northern portion of the range despite association with bathemetery. While the program BAPS at the individual level identified three clusters within *S. pinniger*, we found no geographic pattern in the clusters, though bathymetric association was not tested due to a lack of data associated with depth of

capture and no evidence of structure with other methods. Genetic differentiation measured by pairwise F_{ST}-values was very low and population structure was absent in STRUCTURE and BAPS analyses conducted over the majority of the species range. Thus our results suggest high gene flow over large distances in *S. pinniger* and the presence of a single genetic management unit within the primary range of the species.

Though the range of *S. pinniger* extends between the Shelikof Strait in the western Gulf of Alaska and Cabo Colonet, Mexico, its abundance declines north of British Columbia, Canada, and south of Point Conception, California. Our study sampled its primary range, leaving approximately 400 miles to the north and 150 miles to the south at the edges of its distribution unrepresented in our study. While population structure may occur in un-sampled regions outside its primary range, population structure in the core of species range sampled in this study was lacking. The low F_{ST}-values corresponding to high gene flow in our study makes the presence of population structure of importance to defining population structure for management purposes beyond the sampled range unlikely. One consideration of biological interest and potential importance to conservation is the potential for selection at the edges of the species range that may result in alleles of adaptive significance or the result of genetic drift due to reduced geneflow.

Our results are consistent with those of Gomez-Uchida (2006), which found no significant genetic differentiation at 10 microsatellite loci in samples from northern Washington to Fort Bragg as evidenced by low F_{ST} (<0.001). In addition, no clustering was observed in a multi-dimensional scaling analysis, nor was an isolation-by-distance pattern observed between sample locations, also consistent with the results of our study. Our study did not identify evidence of population structure in samples collected closer to the southern extent of the range in the Half Moon Bay as measured by F_{ST} or in cluster analyses or in samples collected in the California bight in cluster analyses. The results indicate that there is no geographic population structure; and, significant structure based on depth or at the unsampled edges of the species range is unlikely given extremely low F_{ST}-values (less than 0.01) indicating migration of far more than 50 migrants per generation between sample locations used in the criteria for population structure from Waples and Gaggliotti (2006). In addition, the results from the program structure and the relatively low number of loci exhibiting deviation from HWE and LD, indicate a single panmictic population. This is not unsurprising taking into account the potential for migration in the adult and larval life history stages.

Evaluation of genetic diversity, inbreeding and distribution of private alleles

Genetic variation at the edges of the species range pertinent to conservation may exist as a result of strong selection at the extremes of the species' environmental tolerances and could result in local variation of adaptive significance. The presence of a higher number of private alleles at the edges of the species range provides evidence that this may be the case in *S. pinniger*, though this may be due to other reasons and the number of private alleles is not adjusted for sample size (Table 5). While these private alleles are present in the farthest extent of the range sampled in this study, the lack of genetic differentiation would indicate that gene flow is still occurring with the core population. Though microsatellites are neutral non-coding segments, they can be physically linked on a

chromosome with segments coding for traits under selection or a suite of associated characteristics adapted to local conditions at the extremes of a species range. The presence of variants of the PGI-2 allozyme locus off central Oregon in the study conducted by Wishard (1980) is consistent with the possibility that selection is occurring at the northern end of the range, which may be of adaptive significance.

This may provide a conservation concern and the impetus to maintain a sufficient effective population size to retain genetic diversity at the extremes of the latitudinal range of *S. pinniger*. Given that these alleles are relatively uncommon or absent in other parts of the range, if individuals bearing these locally adapted alleles are depleted, individuals from elsewhere may not effectively replenish the population at the edges of the range without the traits the alleles impart or negate the evolutionary potential for range expansion or resilience to climate change they may represent. Further study using thousands of loci such as restriction site associated DNA or exome-capture to test for the presence of selection across latitude may be of use in further substantiating the potential for localized adaptation of adaptive significance worthy of concerted efforts to conserve.

Tests for genetic bottlenecks from recent severe reductions in population size
According to the most recent stock assessment, canary rockfish were lightly exploited until the early 1940s, when catches increased with accelerated rate of decline during the late 1970s, reaching a low of 9.7 percent of unfished biomass in the mid-1990s (PFMC 2011). The stock was declared overfished in 1999, dropping below the threshold of 25% historical spawning stock. Since then the stock has been subject to a rebuilding plan requiring greatly reduced harvest and has grown to the current stock status of 56 percent of the unfished biomass in 2015 and is now considered rebuilt, having rebuilt to a stock size of 40% in 2006 (PFMC 2016). The faster than expected rebuilding in the most recent assessment is in part due to greatly reduced fishing mortality with management under the rebuilding plan, stronger than usual recent recruitment, updated model parameters including stock productivity and changes to the way natural mortality is accounted for in the assessment (PFMC 2016).

The results of our study indicate that the magnitude of declines in historical spawning stock biomass did not result in a detectable signal of population bottleneck indicated by heterozygote excess given the markers and methods employed. Marine fish populations are often made up of thousands if not millions of individuals, thus declines to 25% of the historic unfished population size considered to be overfished or even below 10% of unfished biomass, as is the case for *S. pinniger* may not represent a decline sufficient to result in a detectable genetic bottleneck. In addition, species of the genus the *Sebastes* have been found to exhibit multiple paternity despite internal fertilization, which may increase the potential of the population to maintain greater allelic variation with fewer individuals (Hyde *et al.* 2008).

The tests for heterozygosity excess can detect bottlenecks for a window of time after a bottleneck has been initiated, since mutations eventually fill the gaps between expected and observed heterozygosity. Power analyses and theoretical models suggest that a bottleneck of $Ne = 50$ is likely to be detectable for 25-250 generations (0.25 - 2.5 times

2Ne) after the initiation of a population reduction (Cornuet and Luikart 1997). Thus only recent historical population declines are detectable, but the severity and duration must be sufficient to reduce the number of alleles and heterozygote excess indicative of a bottleneck, which we did not detect. Given that these fish reach sexual maturity at 6 years old (Love *et al.* 2002), only 2 to 3 generations have passed since the deepest decline in numbers of individuals; thus detectable reductions in allelic diversity may not have accrued over such a short time span.

Tests for bottlenecks rely on the assumption that each sample is representative of a well defined population, with no immigration, no population substructure, and that loci are selectively neutral, which appear to be met given the results of our study regarding population structure and use of microsatellite loci which are neutral markers. While detection of declines due to exploitation was of particular interest, bottlenecks may not have occurred as a result of such declines due to the short duration or insufficient reduction in population size. Concomitantly, severe extended non-anthropogenic reductions in population size that cause rarer alleles to be lost may not have occurred to sufficient magnitude and duration to result in significant genetic bottlenecks detectable with the methods employed.

Considerations for stock assessment and management

For the purposes of stock assessment, the absence of significant population structure supports the current practice of assessing and managing the species as a single unit. These results are also of interest to co-management of canary rockfish between Canada and the United States as the stock straddles geographic boundaries and management may benefit from collaborative research and assessment efforts. While genetic differentiation between sampled locations is not evident with the available data, managers should be mindful of the need to prevent localized depletion at the edge of the species range. This outcome may have the potential to reduce local productivity as the presence of private alleles may reflect localized adaptation at linked loci and adaptive alleles found at the edge of the species range may not be replenished from elsewhere in the event of depletion. Thus individuals from the center of the range may not effectively repopulate the edges of the range should the private alleles identified with selectively neutral microsatelite markers be linked to those with adaptive significance subject to selection. Further research regarding the presence of private alleles by examining functional enzymes or their coding regions using genomic methods to test for adaptive genetic variants at the edges of the range, the conservation of which would be facilitated by preventing excessive harvest at the edges of the species range.

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Analysis of Population Structure in the recently recognized Cryptic Species *S. crocotulus* and Implications for Fishery Management

Abstract

In this study we test for population structure in the recently-identified sunset rockfish *S. crocotulus*, the newly discovered cryptic sister-species to the vermilion rockfish *Sebastes miniatus*Jordan & Gilbert (1880), using six microsatellite loci. In addition, we discuss the management implications of the existence of this newly-identified species. The earlier collaborative study that identified *S. crocotulus* as a separate species (Hyde *et al*. 2008) was conducted to examine differences in the distribution of the two species and the degree of genetic differentiation between species and found evidence of separation in the depth distribution of adults.

No significant population structure was identified between sample locations in *S. crocotulus* ($F_{ST} < 0.005$), which was predominantly distributed at depths greater than 50 fm (100 m) south of Point Conception, consistent with the finding of the previous study by Hyde *et al.* (2008). Extensive sampling north of Point Conception did not identify individuals of *S. crocotulus*, simplifying assessment of *S. miniatus* in the region. The differential depth distribution of this cryptic species south of Point Conception presents challenges in stratifying historical removals of each species through harvest.

Stratification of management areas, stock assessment and catch tracking may be necessary to account for the presence of the cryptic species *S. crocotulus* as well as *S. miniatus*. In addition, this presents issues for representativeness of catch per unit of effort used to reflect the demographic trajectories of each stock in assessments of population status and sustainable harvest levels. The distribution of both species across the U.S./Mexico border raises considerations regarding collaborative assessment and management as straddling fish stocks. In addition, the depth distribution of *S. miniatus* appears to be limited to shallower depths than previously understood, whereas the more deeply distributed cryptic sister species *S. crocotulus* should remain in its current designation in the shelf rockfish stock complex.

Introduction

Many of the assessed stocks of rockfish of the genus *Sebastes* in the Pacific Coast Groundfish Management Plan lack population genetic analyses to identify separate management units (Waples *et al.* 2009). Genetic analyses of populations can provide evidence of population structure that should be accounted for in stock assessments to allow accurate accounting of population dynamics of species subject to fishing mortality and to help ensure sustainable harvest levels (Gundersen and Vetter 2006). The purpose of this study is to identify and delineate population structure in *S. crocotulus* (Hyde *et al.* 2008), a previously unidentified cryptic sister-species to *S. miniatus*. The newly discovered cryptic species *S. crocotulus* is primarily distributed from south of Point Conception to Southern Baja, Mexico, in waters deeper than 50 fm (100 m) (Hyde *et al.* 2008). Presently, there are no published analyses of population structure within the described range of *S. crocotulus*.

The isolation of sister-species by adult depth distribution has been ascribed to isolation resulting from paedomorphosis in the form of the concatenation of ontogenetic migration to deeper depths (Hyde *et al.* 2008). While the current depth distribution of *S. crocotulus* may suggest such a mechanism for speciation, this pattern may have arisen under previous geographic isolation with subsequent secondary parapatry with continued isolation of adults by depth. Continued isolation may also be reinforced through sexual selection as rockfish reproduce through internal fertilization and exhibit courtship behavior (Gunderson and Vetter 2006).

While the sister-species appear to be subject to parapatric isolation of adults by depth distribution, there may be hybridization at the margin of their respective depth distributions, which we examine with the available microsatellite data. Genetic assignment tests were used to examine whether post-settlement young of year juveniles sampled in San Diego included both *S. crocotulus* and *S. miniatus* indicating use of the same kelp forest habitat as juveniles. The limited range of *S. crocotulus* in the San Diegan biogeographic province provides limited range over which selection or genetic drift can result in differential alleles over its distribution. As such, population structure was expected to be limited in *S. crocotulus* leading us to hypothesize that a single panmictic population is present.

Methods

Sample Collection, DNA Extraction, DNA Amplification and Data Quality Tests

Sample collection, DNA extraction, DNA amplification and data quality tests were undertaken following the methods described in the preceding chapter. A total of 671 fish originally identified in the field as *S. miniatus* were collected from 1994 to 2005 between Neah Bay, Washington and San Martin Island, Mexico (Figure 1, Table 1). All samples were collected after fish were deceased, having being caught by licensed anglers or permitted sampling programs occurring prior to accessessing tissue samples for our study and were thus exempt from UC Berkeley Animal Care and Use Committee Protocols per discussion with a representative. Tissue samples and extracted DNA are available for confirmation of our results or further analysis.

Because *S. crocotulus* (Hyde *et al.* 2008), is difficult to differentiate from *S. miniatus* in the field, genetic assignment methods were used to separate samples of each species. Of the fish originally identified as *S. miniatus*, 189 were assigned to *S. crocotulus.* To standardize the geographic scope of sampling locations, individuals within 60 miles of one another were selected from the National Marine Fisheries Service sample archive to form putative populations for analysis. These were found from Point Conception, California, to San Martin, Mexico, but were only sampled in sufficient sample sizes for analysis of individuals from San Diego, the Outer Banks and Cabo Colonet. An additional five adult specimens were collected further north in the vicinity of Point Conception. Young of year juveniles were also collected in San Diego in the vicinity of Scripps Institute of Oceanography within 10 fm (20 m) using hand nets while scuba diving and analyzed for comparison to adults. The remaining samples consisted of individuals representing multiple year classes, and are more representative of inter-generational stability in genetic variation (Waples 1998). The location name,

state/country, average latitude and longitude, three digit alpha code for the population used in further analyses and number of samples are provided in Table 1.

Table 1. The name of sampled putative populations of *S. crocotulus*, alpha codes for sampled locations, number of specimens and average latitude and longitude of capture for each species and sample location.

Sample Location	State / Country	Alpha Code	Individuals	Latitude	Longitude
Santa Diego	California	SDR	54	32.790	-117.259
Outer Banks	California	OBM	32	32.711	-119.090
Cabo Colonet	Mexico	CCM	103	30.745	-116.258

Analysis of population structure

A number of methods were employed to identify genetic population structure within each species. The program STRUCTURE (Pritchard *et al.* 2000) is a Bayesian algorithm used to identify the number of clusters of samples (K) or populations that minimize deviation from Hardy-Weinberg Equilibrium and Linkage Disequilibrium. The optimal number of clusters (K) of samples results is inferred from the highest negative log-likelihood value (Pritchard *et al.* 2000). Initially, an analysis with both species combined was performed by setting the number of clusters (K) equal to 2, to differentiate individuals of the cryptic species *S. crocotulus* from *S. miniatus* and confirm that sampled individuals were correctly assigned prior to further analysis.

Each species was subsequently analyzed independently with K set from 1 (a single panmictic population) to the number of putative sampled populations. Following (Pritchard *et al.* 2000), the most likely number of clusters is given by the value of K with the highest negative log-likelihood value. We inferred population division (number of *K* populations) by performing 10 independent runs of each *K* with a burn-in of 10,000 iterations, and 1,000,000 iterations of the Gibbs sampler. The admixture model with correlated frequencies was used without prior population information. An ad hoc statistic ΔK based on the rate of change in the log probability of data between successive K values from STRUCTURE was used as further evidence of the number of clusters in the sample set (Evanno *et al.* 2005).

A second clustering method BAPS (Corrander *et al.* 2004) was used to determine which combination of predetermined samples is best supported by the data based on the criteria of maximizing heterogeneity between groups and homogeneity within groups. The program uses importance sampling to approximate posterior probabilities performing an exact Bayesian analysis by enumerative calculation to determine the expected number of clusters. The optimal number of populations is assumed to be the partition with the highest posterior probability. Burn-in was conducted with 10,000 iterations followed by 1,000,000 steps after burn-in. Values of K from one to the total number of sampled locations for each species were examined to identify the number of clusters that underscore the model with the maximum Bayesian posterior probability. Analysis of

clustering groups of individuals was followed by an analysis of admixture based on mixture clustering.

Pairwise F_{ST} (Weir and Cockerham 1984) was estimated between sampled populations using the program Arlequin (Excoffier *et al.* 1995). Tests for significance were also conducted with the null distribution of pairwise F_{ST}-values under the hypothesis of no difference between the populations obtained by permuting alleles among populations. The *P*-value of the test is the proportion of permutations leading to an F_{ST}-value larger or equal to that observed.

The question becomes whether F_{ST}-values estimated for a given pair of populations indicate the presence of biologically meaningful populations as defined by molecular criteria for deviation from panmixis (Waples and Gaggliotti 2006). These include thresholds for genetic differentiation as measured by F_{ST} that constitute genetically differentiated populations with independent demographic processes. Two criteria were evaluated, EV 3 in which populations exchange less than 5 migrants per generation corresponding to an $F_{ST} = 0.05$ and EV 4 in which less than 25 migrants per generation are exchanged, corresponding to an $F_{ST} = 0.01$. These criteria can be associated with evidence of departures from panmixia (Waples and Gaggliotti 2006). The less stringent EV4 of $F_{ST} = 0.01$ may be sufficient to detect population structure, considering the greater potential for migration of larvae in the marine environment. The F_{ST}-values for comparisons between populations were compared to examine whether the thresholds are exceeded, indicating the presence of significant population structure.

Test for co-occurrence of post-settlement young of year of S. crocotulus and S. miniatus in kelp forest habitat

A total of 40 young of year individuals originally identified as individuals of *S. miniatus* were sampled in kelp forest habitat off of San Diego in depths less than 10 fm (20 m). Collectors did not know how to identify individuals of the two cryptic species among these specimens. The assignment of post-settlement young of year in STRUCTURE from the analysis with two clusters including adults and juveniles of *S. crocotulus* and *S. miniatus* were examined to determine whether the two species co-occur as juveniles in the sampled kelp habitat. If all populations or species are present within the sampled habitat, this would indicate that they all use the same habitat as young of the year.

Analysis of genetic diversity, inbreeding and private alleles

We examined the distribution of genetic variation, degree of inbreeding, the average heterozygosity, number of alleles, allelic richness reflecting the number of alleles normalized by sample size, the fraction of total alleles, the inbreeding coefficient F_{IS} in each sample location using the program FSTAT (Goudet 2002). We compared allelic diversity and the number of private alleles among sample locations to examine whether sample locations or clusters have higher allelic diversity indicative of centers of distribution or a greater number of private alleles indicative of drift due to isolation or adaptation at the edge of the distribution. Conversely, we examined whether sample locations exhibited lower genetic diversity indicative of founder events or severe bottlenecks. The analysis of the inbreeding coefficient F_{IS} was conducted across sample

locations to determine whether the degree of inbreeding or lack there of varies across regions.

Results

Data quality tests

The test for allelic dropout, stutter and null alleles using Microchecker did not identify evidence of scoring errors, as estimated values of homozygosity and heterozygosity were within the range of expected values from the Monte Carlo simulations. Exact tests for deviation from HWE at a species level revealed significant deviation ($p < 0.05$) at one locus. Significant linkage disequilibrium ($p < 0.05$) was identified between three pairs of loci (Table 2). Deviation from HWE and LD may be due to random sampling error, sampling of multiple non-interbreeding populations, or physical linkage. Deviation from HWE and the presence of LD was inconsistent across populations indicating that the deviation may be due to a Wahlund effect from sampling multiple non-interbreeding populations (Table 2). Coastal locations in San Diego and Cabo Colonet displayed a greater frequency of deviation from HWE and LD than offshore samples at the Outer Banks where none was observed.

Table 2. Results of tests for Linkage Disequilibrium and deviation from Hardy-Weinberg Equilibrium in *S. crocotulus* sampled in each location.

Population	Loci Deviating from HWE	Pairs of Loci Exhibiting Significant Linkage Disequilibrium
Outer Banks	NA	2-5
San Diego	1, 2, 5, 6	1-2, 1-4, 1-5, 2-4, 2-5, 3-5, 4-5, 4-6
Cabo Colonet	3	1-4, 1-5, 1-6, 3-4, 4-6

Analysis of population structure

Analysis with the program STRUCTURE indicated that the model reflecting a single panmictic population (K = 1) provides the highest negative log-likelihood value (Table 3) despite the presence of some linkage disequilibrium and deviation from HWE. Higher values of K result in equal proportions of ancestry in all populations indicating no detectable population structure (Figure 1). Similarly, the BAPS population level mixture analysis of sample locations, found a single panmictic population (K = 1) had the highest log maximum likelihood value indicating no detectable population structure.

Although the BAPS mixture analysis at the individual level found two populations (K = 2) having the highest log maximum likelihood, the admixture analysis of the distribution of individuals assigned to each cluster were interspersed between sampled locations with no clear geographic patterns of distribution of the clusters as seen in Figure 2. This is contrary to the results of analysis in the program STRUCTURE, population level analysis in BAPS and the very low F_{ST} values between sampled locations indicative of a single panmictic population. Low and non-significant F_{ST} values were estimated for *S. crocotulus* between San Diego and Cabo Colonet ($F_{ST} = 0.003$, $p = 0.108$), San Diego and

the Outer Banks (F_{ST} = 0.0045, p = 0.045) and low but marginally non-significant F_{ST} values between the most distant sampled populations of the Outer Banks and Cabo Colonet (F_{ST} = 0.006, p = 0.009, α = 0.05/6 = 0.0083). The F_{ST}-values were well below the thresholds of F_{ST} = 0.05 or 0.01 for deviation from panmixis between sample locations from Waples and Gaggliotti (2006). The preponderance of results indicates the presence of a single panmictic population.

Table 3. Average maximum likelihood values and variance for each number of clusters K for *S. crocotulus* from 10 runs at each value of K.

Clusters K	LnP(D)	Var[LnP(D)]
1	-4492	43
2	-4588	269
3	-4763	637

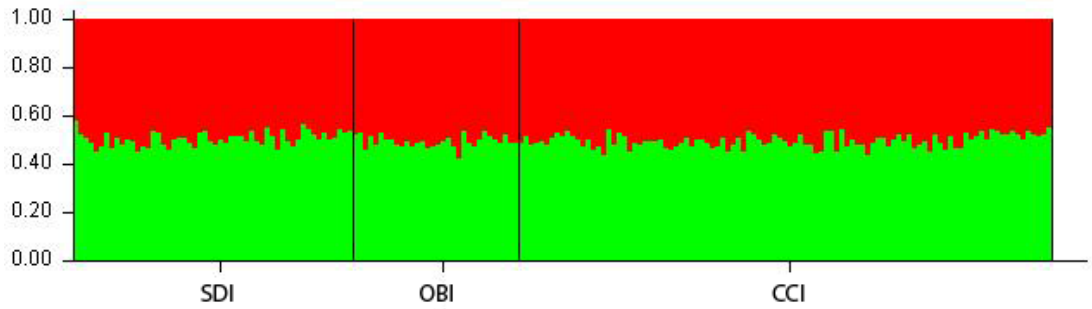

Figure 1. Proportion of ancestry from STRUCTURE for *S. crocotulus* with two clusters (K = 2).

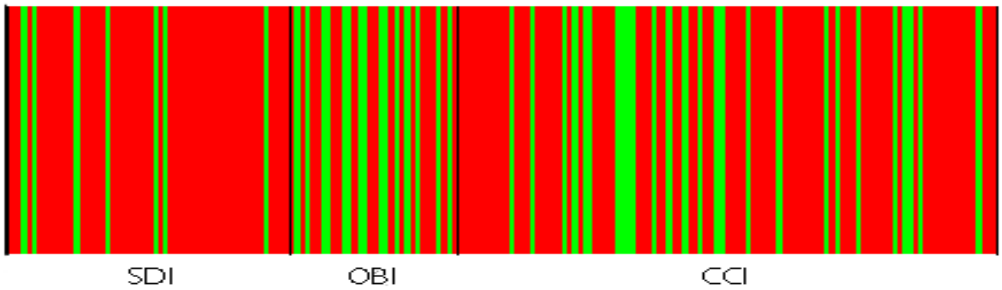

Figure 2. Posterior probability of assignment of each individual to each cluster represented by the differing colored bars from admixture analysis of BAPS for K = 2 clusters conducted at an individual level for *S. crocotulus*.

Test for co-occurrence of post-settlement young of year of S. crocotulus and S. miniatus in kelp forest habitat

Though the 40 specimens of post-settlement young of year *S. crocotulus* and *S. miniatus* were sampled in waters less than 10 fm (20 m) in depth, individuals undergo ontogenetic migration to different depths as they grow and mature (Hyde *et al.* 2008). Individuals assigned to both species were present in the samples collected in the vicinity of San Diego. Of the 40 specimens, 33 assigned to *S. miniatus* and seven to *S. crocotulus*. This indicates that post-settlement young of year of both species utilize kelp forest habitat on shallow reefs, with subsequent ontogenetic migration to deeper depths as they mature.

Analysis of genetic diversity, inbreeding and private alleles

The greatest number of private alleles and fraction of alleles were observed in Cabo Colonet, the most southerly sample location, with more than double the number of private alleles observed than elsewhere (Table 4). The highest average heterozygosity and allelic diversity were observed at the outer banks followed by Cabo Colonet (Table 4). In San Diego, the average heterozygosity, number of alleles, fraction of alleles and allelic diversity adjusted to the smallest population size were appreciably lower than elsewhere, while F_{IS}, the inbreeding coefficient was three times higher than the other sample locations, though generally low compared to the maximum value of 1 (Table 4). While the allelic richness was normalized for sample size, the sample size may have implications for the number of private alleles and fractions of alleles as the number of sampled individuals varies greatly among sample locations and may be too small for allele saturation to be reached in San Diego where the fewest individuals were sampled (Table 4).

Table 4. The number of individuals sampled, private alleles, average heterozygosity, number of alleles, allelic diversity, fraction of total alleles sampled and inbreeding coefficient F_{IS} in each sampled location.

Sample Location	Number of Samples	Private Alleles	Average He	Alleles	Fraction of Alleles	Allelic Diversity	F_{IS}
Outer Banks	54	5	0.71	84	0.84	72.2	0.02
San Diego	32	2	0.48	66	0.66	65.5	0.06
Cabo Colonet	103	14	0.61	91	0.91	69.2	0.02
All	189	-	-	100	-	70.4	-

Discussion

Analysis of population structure

Hyde *et al.* (2008) found that *S. crocotulus* is primarily distributed south of Point Conception. Analysis of the mitochondrial control region in specimens assumed by Sivasundar and Palumbi (2010) to represent *S. miniatus* also identified two divergent lineages in specimens sampled in Santa Barbara, reflecting the presence of the cryptic species *S. crocotulus*. The distribution of *S. crocotulus* observed in our study was consistent with that of Hyde *et al.* (2008) and Sivasundar and Palumbi (2010). We obtained sufficient sample sizes for analysis at the Outer Banks, San Diego and Cabo

Colonet. In assigning sampled individuals to species for further analysis, one specimen of *S. crocotulus* was sampled in Santa Barbara and another at the Channel Islands even though numerous specimens of *S. miniatus* were sampled at these locations. Our results indicate that *S. crocotulus* were rare in the northern extent of the Southern California Bight and were absent at more northerly latitudes. Examination of the depth distribution of adults provided results consistent with the reported depth distribution of greater than 50 fm (100 m) reported in Hyde *et al.* (2008), as would be expected given that the two studies use many of the same samples. Both the low genetic differentiation estimated with F_{ST} and results from analysis in the program STRUCTURE indicate a single panmictic population.

Though F_{ST}-values were very low (<0.006) and were not significant, F_{ST} was highest for comparisons of the other two sampled locations with the Outer Banks. The species has a relatively small geographic range and only three of the sampled locations had sufficient sample size for analysis of putative populations, limiting the spatial extent and resolution of the analysis. Our study included sample locations covering the majority of the range of individuals previously described as *S. miniatus*. While its range extends to Isla San Benito, Mexico, this is only 200 miles south of Cabo Colonet, the southernmost sample location in our study. To the north of Point Conception, individuals previously identified as *S. miniatus*, were common only in the shallow portion of their depth range, confirming the absence of *S. crocotulus*, which occupies the deeper portion of the presumed range of these cryptic species and is only found to the south in our study; this is consistent with the described depth and latitude description prior to identification of the cryptic sister species (Love *et al*. 2002). Given the very low F_{ST}-values among sampled locations, it is unlikely that strong population structure is present within the limited extent of its range further to the south. Sampling further to the south and at more locations may provide insight into the degree of isolation-by-distance in the species, though F_{ST}-values are so low in our study that gene flow appears to be high and it seems unlikely that population structure of concern to management will be found within the species.

Implications for stock assessment and fishery management

The absence of detectable population structure in *S. crocotulus* has a number of implications for fishery management. The prevalence of *S. miniatus* north and presence of *S. crocotulus* south of Point Conception provides a basis for independent stock assessment and catch accounting in each region. Historically, individuals encountered south of Point Conception were identified as *S. miniatus*, but since such individuals could have been either *S. miniatus* or *S. crocotulus*, accurate assessments of each stock based on past catches will be difficult to carry out. The contribution of each to past and present catch and indices of abundance using stock assessments may be confounded by the presence of two species and the proportion of each would need to be determined to facilitate assessment of individual stocks.

The differential depth distribution of *S. crocotulus* south of Point Conception may make it possible for the proportion of catch by depth to be used to apportion historical catch south of Point Conception. Data informing apportionment of catch or indices of abundance between species may not be available for sectors that historically harvested

substantial quantities of what was assumed to be *S. miniatus* or a portion of the catch time series. Fluctuations in recruitment could cause the proportion of each cryptic species taken in the fishery to vary through time. In addition, as noted in Hyde *et al.* (2008) and confirmed in our study, depth restrictions have redistributed fishing effort away from adult *S. crocotulus*, which is predominantly distributed in depths greater than the current depth restrictions and onto *S. miniatus* and juvenile *S. crocotulus* in waters less than 50 fm (100 m). This may have increased the proportion of *S. miniatus* in the recent catch since depth restrictions were implemented in 2000 and may result in differential depletion of each species. Differential exploitation of *S. miniatus* and *S. crocotulus* has implications for stock assessment and fishery management that will pose difficulties in assessing each species south of Point Conception where they co-occur. This may introduce an error if the stocks were assessed as a complex assuming similar exploitation rates.

The question still remains as to whether the two species have different growth rates, have experienced similar exploitation history and whether they have similar recruitment patterns all of which would affect the respective demographic trajectories of each stock and thus potentially the status, trend and scale resulting from stock assessments. Such issues may hamper independent assessment of each stock south of Point Conception and necessitate assessment of a complex of cryptic species limiting the ability to independently manage each stock. If trends in recruitment, growth rates or exploitation history differ significantly, this may result in error in assessment results when the species are treated as a complex that may have implications for sustainable harvest of either stock using status determination or abundance estimates from such as assessment. Testing the assumption that these parameters do not differ between species would be valuable in weighing the degree to which they may pose an issue for management in the context of assessment as a complex managed as a single unit.

Considerations for composition of stock complexes

Species within the Pacific Coast Groundfish Fishery Management Plan are divided into nearshore, shelf, and slope rockfish stock complexes or are managed outside of complexes as individual stocks if assessed as overfished or if management outside the complex reduces the chances overfishing component stocks (PFMC 2012). The complexes are further divided north and south of 40°10’ N. latitude off of Cape Mendocino, California. The vermilion rockfish *S. miniatus* was included in the shelf rockfish complex both north and south of 40°10’ N. latitude, prior to the discovery of its cryptic sister species *S. crocotulus* occupying deeper depths. The predominant depth distribution of *S. miniatus* in less than 50 fm (100 m) as revealed in Hyde *et al.* (2008) and further elucidated in this study indicates that its depth distribution is more similar to species included in the nearshore rockfish complex, to which technically, it should be reassigned. Given the predominant distribution of adult *S. crocotulus* in depths greater than 50 fm (100 m), once it is added to the management plan, it should be assigned to the shelf rockfish complex south of 40°10’ N. latitude or south of Point Conception alone if a new complex management line is implemented as recommended.

Broader management considerations

Population structure with depth and across borders of major biogeographic regions or other barriers to gene flow are evident in other species within the genus *Sebastes* (Burford and Bernardi 2008, Gunderson and Vetter 2006). The implications for assessment and proper management are important to recognize and address. Population structure may exist in numerous species that has not yet been brought to light or addressed in management, posing potential flaws that could result in mis-informed management. As such, further research to identify population structure and align management lines with biological boundaries to facilitate estimation of catch, assessment and management of individual stocks should be a priority for future research. Such research is especially important in the case of broadly distributed species whose ranges bridge the aforementioned barriers or occupy a broad range of depths as was the case in *S. miniatus* as previously recognized. These are stocks for which cryptic species or population structure are more likely to be present, but unaccounted for in management.

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Analysis of Genetic Population Structure with Latitude, Longitude and Depth in the Widely Distributed Vermilion Rockfish, *S. miniatus*, and Implications for Fishery Management

Abstract

In this study we test for population structure in the vermilion rockfish, *S. miniatus* (Jordan and Gilbert 1880), along the Pacific coast of North America from Neah Bay, Washington, to Cabo Colonet, Mexico. The results of our analysis using six microsatellite loci were interpreted in light of previous research conducted with different genetic markers, sampling over a limited extent of their range or with few sample locations limiting the spatial resolution of the analyses. Through our more extensive sampling and interpretation of all available research, we provide a more comprehensive understanding of the geographic and bathymetric distribution of population structure to inform proper assessment and management.

Significant population structure was identified among sample locations in this and previous studies. Analysis of genetic differentiation, cluster analysis, and assignment tests identified three groups within *S. miniatus.* This study confirms a strong genetic break at Point Conception and resolves apparent structure between two populations in the south with differential adult depth distributions. Northern cluster 1, identified in each of two assignment methods, was primarily distributed north of Point Conception in depths less than 30 fathoms (fm). The more southerly cluster 2 was most common south of Point Conception, in depths less than 30 fm, though this group was infrequently encountered further north. Cluster 3 was predominantly found south of Point Conception in depths between 10 and 50 fm, but rarely encountered north of Point Conception. The cryptic sister-species *S. crocotulus* was predominantly distributed in depths greater than 50 fm south of Point Conception.

Additional stratification of management areas, stock assessment, and catch tracking may be necessary to account for population structure in *S. miniatus* and the presence of the cryptic species *S. crocotulus*. Stratifying stock assessments at Point Conception will allow cluster 1 in *S. miniatus* to be assessed separately from the other two clusters identified, as well as *S. crocotulus*. The differential depth distribution of cluster 2, cluster 3 and *S. crocotulus* south of Point Conception presents challenges in stratifying historical catch and catch per unit of effort used to reflect the demographic trajectories of each stock in assessments of population status and sustainable harvest levels. The issues with stratification are further complicated by ontogenetic migration from shallow reefs to greater depths occupied by adults of each species, resulting in sympatry of populations at all but the greatest depths occupied by *S. crocotulus*. The distribution of clusters 2 and cluster 3 extend over political borders shared with Mexico and may have implications for management that should be considered for management under a straddling fish stocks agreement. In addition, the depth distribution of *S. miniatus* is more similar to that of species assigned to the nearshore stock complex used in management despite currently being assigned to the shelf rockfish complex, due to the deeper depth distribution of the

cryptic sister-species *S. crocotulus.* This has implications for structuring of stock complexes used in management and permitting commercial harvest of nearshore stocks.

Introduction

Understanding the current distribution of genetic variation provides information pertinent to proper management and a starting point in examining micro-evolutionary forces contributing to speciation and population structure. The purpose of this study is to identify and delineate population structure in *Sebastes miniatus* (Jordan and Gilbert 1880). This solitary rockfish species is predominantly distributed at depths less than 50 fm (100 m) (Hyde *et al.* 2008). Its range extends from Montague Island in Prince William Sound, Alaska, to Isla San Benito, Baja California, although it is relatively uncommon north of California (Love *et al.* 2002).

Population structure in *S. miniatus* has been identified using 15 microsatellite loci in specimens from Santa Barbara and Monterey, California, although the low spatial resolution of the study prevented delineation of populations (Sivasundar and Palumbi 2010) from those of *S. crocotulus* its cryptic sister species. A range-wide analysis of mitochondrial sequence data for cytochrome-b in *S. miniatus* identified *g*enetic breaks at Point Conception, the Washington Coast, Cape Mendocino, within the Southern California Bight at Santa Monica, and at Cabo Colonet (Hyde *et al.* 2009). Mitochondrial DNA has a lower effective population size than bi-parentally inherited diploid genomic markers leading to a rate of genetic drift and lineage sorting that is four times that of bi-parentally inherited nuclear markers. While informative of population structure, mitochondrial DNA is maternally inherited as a single non-recombining unit and reflects a single gene lineage, which may differ from somatic genes with bi-parental inheritance (Hoarau *et al.* 2004).

Detection of consistent genetic breaks in species of *Sebastes* has been elusive. Although Point Conception, Cape Mendocino and Cape Blanco have been implicated as barriers to gene flow as a result of differential water temperatures, divergent currents, or jets where larvae are advected offshore (Gunderson and Vetter 2006), evidence for genetic breaks at these locations varies across species and among genetic markers within a species (Cope 2004, Burford and Larson 2007, Gharrett 2005). However, there may be some common mechanisms of speciation or barriers to gene flow that result in genetic breaks that are ubiquitous among species with similar life history traits. A combination of physical and biological factors may result in differential patterns of population structure within the range of a species.

Members of the genus *Sebastes* are characterized by a primitive form of live birth called lecithotrophy, although there is a degree of direct maternal energetic contribution to developing embryos within the ovary. Larvae may travel for 3-4 months and up to 200 miles in currents as members of the planktonic community before settling on benthic habitat (Love *et al.* 2002). Timing of release of larvae may strongly influence the direction and magnitude of gene flow within the California Current, Alaskan Gyre and Southern California Eddy (Gunderson and Vetter 2006). The distance of larval dispersal is determined in part by the strength and direction of currents during the pelagic larval

phase, but their distribution in the water column due to buoyancy, swimming behavior or larval survival may ultimately determine their range of distribution (Gunderson and Vetter 2006).

Larval survival is determined in part by the temperature tolerance of the species, food availability, predation rates and advection offshore at jets, among other biological tolerances and requirements (Cowen R.K. and S. Sponaugle 2009, Gaines *et al.* 2010, Kinlan *et al.* 2005). The effective migration distance of larvae and adults and effective reproduction in the newly established habitat determine the potential for range extension, or persistence with fluctuating environment conditions at all time scales (Palumbi 2003, Sotka and Palumbi 2006). The current depth and latitudinal distribution of a given species may not reflect the historical distribution in which divergence began, thus the factors contributing to speciation in allopatry due to vicariance or founder effects can be difficult to identify (Jacobs *et al.* 2004, Longhurst 2007, Bigg *et al.* 2008).

The primary objective of this study is to identify and delineate population structure pertinent to management using microsatellite loci, and to compare our results with previous research. Our study provides an analysis of population structure across the distribution of *S. miniatus*, over a larger geographic range and higher spatial resolution than examined in previous studies with this class of genetic marker. Previous studies have covered a more limited portion of the geographic range (Sivasundar and Palumbi 2010), or used only a single mtDNA locus (Hyde *et al.* 2009).

The present study was undertaken to improve our understanding of the biogeographic and bathymetric discontinuities in gene flow by sampling throughout the range of *S. miniatus*. Here we test the following hypotheses with a view to understanding population structure for fishery management:

1. Divergence between cryptic species *S. miniatus* and *S. crocotulus* was hypothesized to have occurred through paedomorphosis in the form of concatenation of ontogenetic migration to deeper depths in *S. crocotulus* resulting in differential adult depth distribution shallower and deeper than 50 fm, respectively (Hyde *et al.* 2008). We tested for population structure based on depth in *S. miniatus* as evidenced from correlation of population structure with differential depth distribution. Such a pattern would be consistent with the hypothesis of repeated genetic differentiation as a result of isolation of adult spawning populations by depth.

2. Population structure in *S. miniatus* will be consistent with major biogeographic breaks along the coast at Point Conception, Cape Mendocino, Cape Blanco and within the southern California bight. These are the boundaries of biogeographic compartments along the coast identified in Longhurst (2007) found to be consistent with the results of mtDNA analyses by Hyde and Vetter (2009).

3. Genetic assignment tests will indicate that post-settlement young of year juveniles sampled in San Diego will include both *S. crocotulus* and *S. miniatus* or sub-populations indicating that they co-occur in kelp forests as juveniles.

Methods

Sample Collection, DNA Extraction, DNA Amplification and Data Quality Tests

Sample collection, DNA extraction, DNA amplification and data quality tests were undertaken following the methods described in the first chapter. A total of 671 fish identified in the field as *S. miniatus* were collected from 1994 to 2005 between Neah Bay, Washington, and San Martin Island, Mexico (Table 1). We employed genetic assignment methods to distinguish actual specimens of *S. miniatus* from the newly-recognized species *S. crocotulus*, and determined that 461 of the originally-collected specimens were indeed *S. miniatus*. Details of the collection locations and sample sizes are shown in Table 1. A greater number of locations were sampled in the vicinity of Point Conception near the major biogeographic break identified with cytochrome-b (Hyde and Vetter 2009) to evaluate population structure in the vicinity at a higher geographic resolution.

It was assumed that specimens from the recreational fishery were caught no further than 30 miles North or South of the port from which they were sampled. To standardize the geographic scope of sampling locations, individuals within 60 miles of one another were selected from the National Marine Fisheries Service sample archive to form putative populations for analysis with a similar spatial resolution. Aggregation of these samples resulted in ten putative sample populations for analysis. Of the specimens collected in San Diego, 40 were young of year fish under 100 mm in length. These specimens were included in a separate putative population for comparison to the adult population in San Diego. The remaining samples consisted of individuals representing multiple year classes, and are more representative of inter-generational stability in genetic variation (Waples 1998). All samples were collected after fish were deceased, having being caught by licensed anglers or permitted sampling programs occurring prior to accessessing tissue samples for our study and were thus exempt from UC Berkeley Animal Care and Use Committee Protocols per discussion with a representative. Tissue samples and extracted DNA are available for confirmation of our results or further analysis.

Table 1. The name of sampled putative populations, alpha codes for sampled locations, number of specimens and average latitude and longitude of capture for each species and sample location.

Sample Location	State / Country	Alpha Code	Number of Individuals	Latitude	Longitude
Neah Bay	Washington	NEA	15	48.367	-124.629
Brookings	Oregon	BRK	69	42.044	-124.273
Half Moon Bay	California	HMB	74	37.502	-122.481
Morro Bay	California	MOB	23	35.358	-120.868
Point Conception	California	PTC	28	34.577	-120.647
Channel Islands	California	CIS	35	34.010	-120.292
Santa Barbara	California	SBR	33	34.398	-119.722
Outer Banks	California	OBM	9	32.517	-119.115
San Diego Adults	California	SDA	78	32.664	-117.242
San Diego Juveniles	California	SDJ	33	32.664	-117.242
Cabo Colonet	Mexico	CCO	64	30.962	-116.330

Identification of population structure

A number of methods were employed to identify genetic population structure. The program STRUCTURE (Pritchard *et al.* 2000) is a Bayesian algorithm used to identify the number of clusters of samples (K) or populations that minimize deviation from Hardy-Weinberg Equilibrium and Linkage Disequilibrium. The optimal number of clusters (K) of samples results in highest negative log-likelihood value (Pritchard *et al.* 2000). Initially, an analysis was performed by setting the number of clusters (K) to 2, to differentiate individuals of cryptic species *S. crocotulus* from *S. miniatus* prior to further species specific analysis.

Individuals assigned to *S. miniatus* were subsequently analyzed independently with K set from 1 (a single panmictic population) to the number of putative sampled populations. Following Pritchard *et al.* (2000), the most likely number of clusters is given by the value of K with the highest negative log-likelihood value. We inferred population division (number of *K* populations) by performing 10 independent runs of each *K* with a burn-in of 10,000 iterations, and 1,000,000 iterations of the Gibbs sampler. The admixture model with correlated frequencies was used without prior population information. An ad hoc statistic ΔK based on the rate of change in the log probability of data between successive K values from STRUCTURE was used as further evidence of the number of clusters in the sample set (Evanno *et al.* 2005).

Individuals were assigned to clusters based on their proportion of ancestry estimated by STRUCTURE. Individuals were assigned to a cluster if their ancestry in that cluster exceeded 50% for K = 2 and 33% for K = 3 since these two models were found to be the most likely, as explained in the results section. This cutoff was applied since a more stringent cutoff would result in many samples being assigned as intermediate, and thus being excluded from analysis. This would potentially bias the results since groups may share alleles due to incomplete lineage sorting or hybridization. The resulting clusters

were tested for significant reductions in linkage disequilibrium and deviations from HWE and LD in Arlequin. The resulting number of significant deviations from HWE and pairs of loci in LD were compared between K = 1, K = 2 and K = 3 to evaluate the degree of reduction resulting from the increased number of assumed clusters.

A second clustering method, BAPS (Corrander *et al.* 2004), was used to determine which combination of predetermined samples is best supported by the data based on the criteria of maximizing heterogeneity between groups and homogeneity within groups. The program uses importance sampling to approximate posterior probabilities performing an exact Bayesian analysis by enumerative calculation to determine the expected number of clusters. The optimal number of populations is assumed to be the partition with the highest posterior probability. Burn in was conducted with 10,000 iterations followed by 1,000,000 steps after burn in. Values of K clusters from one to the total number of sampled locations for each species were examined to identify the number of clusters that provided the model with the maximum Bayesian posterior probability. Analysis of clustering groups of individuals was followed by an analysis of admixture based on mixture clustering. An iterative reanalysis of individual identified clusters was conducted until no additional clusters could be identified.

Pairwise F_{ST} (Weir and Cockerham 1984) was estimated between sampled populations and *S. miniatus* clusters identified by STRUCTURE with K = 2 and K = 3 in the program Arlequin (Excoffier *et al.* 1995). Tests for significance were also conducted with the null distribution of pairwise F_{ST} values under the hypothesis of no difference between the populations obtained by permuting alleles among populations. The *P*-value of the test is the proportion of permutations leading to an F_{ST} value larger or equal to the observed one. Differentiation as measured by F_{ST} values was also calculated between groups of individuals assigned to clusters identified in STRUCTURE analysis of K = 3 in each sampled population with a sample size greater than five individuals in a cluster to identify significant differentiation.

Molecular criteria for deviation from panmixis have been developed to inform whether F_{ST} estimated for a given pair of populations indicate populations as defined by (Waples and Gaggliotti 2006). Thresholds for genetic differentiation as measured by F_{ST} relative to migration rates and measures provide a threshold for comparisons that constitute genetically differentiated populations with independent demographic processes. Two criteria were evaluated relative to migration, EV 3 in which populations exchange less than 5 migrants per generation corresponding to an $F_{ST} = 0.05$ and EV 4 in which less than 25 migrants per generation are exchanged, corresponding to an $F_{ST} = 0.01$. Evidence of departures from panmixia is associated with thresholds provided under each of the criteria (Waples and Gaggliotti 2006). There is greater potential for migration of larvae in the marine environment, thus the less stringent EV4 of $F_{ST} = 0.01$ may be sufficient to detect population structure. We examine whether the thresholds are exceeded by comparing the F_{ST} values between populations for each species to determine whether significant population structure is present.

Comparison of cluster assignment proportions between adults and juveniles between populations and between cryptic species were conducted to determine if the adults and juveniles are in proportional abundance assuming random sampling. Tests for significant genetic differentiation as measured by F_{ST} values were conducted between samples of adults and juveniles to examine whether those assigned to the same cluster showed less genetic differentiation than those assigned to others as expected. The F_{ST}-values were compared to test for stability of population structure identified in adults and test for differentiation in a given cluster between generations at the same sample location.

Test for co-occurrence of post settlement young of year S. crocotulus and S. miniatus and differentiation between adults and juveniles in San Diego

A total of 40 young of year individuals were sampled in kelp forest habitat off of San Diego in depths less than 20 fm without knowledge of what species or population they belonged to. The assignment of post-settlement young of year in STRUCTURE from the analysis with four clusters including adults and juveniles of *S. crocotulus* and *S. miniatus* were examined to determine whether they co-occur in the sampled kelp habitat. If all populations or species are present within the sampled habitat, this would indicate that they all use the same habitat as young of year. We also examined the number of young of year and adults in each cluster or species in San Diego to determine whether they were proportional.

Tests for correlation of assignment of individuals to clusters in S. miniatus with depth, longitude and latitude of capture

The distribution of various rockfishes varies with latitude and depth along the coast, with some species more abundant at certain depths during their life history (Love *et al.* 2002). Previous research identified the cryptic species *S. crocotulus*, which occupies a deeper depth distribution as adults as the sister species to *S. miniatus* (Hyde *et al.* 2008). To examine the possibility of a repeated pattern of differentiation within the subgenus, we tested for similar structure within *S. miniatus*. Data for the depth, latitude and longitude of capture were used as independent variables with cluster assignment as the dependent variable.

A total of 255 *S. miniatus* specimens assigned to clusters from Structure K = 3 from Morro Bay to Cabo Colonet in depths within 80 fm (146.3 m) were used to test for patterns in the geographical distribution of clusters. Contingency tests and logistic regression were conducted to test for significant correlations of cluster assignment frequency with depth, latitude and longitude in the sub-region. Univariate logistic regression and generalized linear regression with continuous values of depth in fathoms, latitude and longitude in degrees and tenths of minutes were conducted. In addition, contingency tests were conducted using 10 fm depth bins as well as latitude and longitude binned in whole degrees equivalent to 60 nautical mile intervals.

Tests for co-occurrence of population structure with locations expected to pose barriers to gene flow

The genetic structure was investigated using an analysis of molecular variance (AMOVA) framework in Arlequin (Excoffier 1995) as initially defined by Cockerham

(1969, 1973), and extended by others (Weir and Cockerham 1984; Long 1986). To identify genetic structure we tested the null hypothesis of homogeneity between 1) all sampled locations, 2) populations sampled North and South of the location of offshore jets at Cape Blanco and Cape Mendocino, 3) the break between the San Diegan and Oregonian biogeographic regions at Point Conception, and 4) groups of samples assigned to clusters from BAPS and STRUCTURE K = 3. The resulting genetic variance at each hierarchical level as well as covariance components was used to compute fixation indices (F_{ST}) and the inbreeding coefficient (F_{IS}). The indices were then compared between groupings to examine the degree of genetic differentiation and population genetic structure. The significance of the fixation indices was tested using a non-parametric permutation approach described in Excoffier *et al.* (1992).

Sequential groupings of populations to the north and south of each pair of sampled locations were also analyzed in an AMOVA framework (sensu Buonaccorsi *et al.* 2005) to identify areas where groupings were genetically homogeneous and between which areas were heterogeneous. Adjacent sample pooling analysis was conducted with sequential grouping of populations north and south of a given sample location. The F_{ST} and F_{IS} values were calculated with each combination of sample locations. Increased F_{ST} values, lower F_{IS} values and a higher proportion of variance among sample locations in the AMOVA are expected when sample locations including homogeneous populations are combined. Once the location of maximum coast-wide homogeneity was identified, a repeated analysis was conducted with samples to the north and south in a further evaluation of population sub-structure.

Results

Data quality tests

The test for allelic dropout, stutter and null alleles using Microchecker did not identify evidence of scoring errors, as estimated values of homozygosity and heterozygosity were within the range of expected values from the Monte Carlo simulations. Exact tests for deviation from HWE at a species level revealed significant deviation ($P < 0.05$) at four loci. Significant linkage disequilibrium ($P < 0.05$) was identified between ten pairs of loci in *S. miniatus* as a whole and within many putative sample populations (Table 2). Deviation from HWE and LD may be due to random sampling error, sampling of multiple non-interbreeding populations or physical linkage. Deviation from HWE and the presence of LD was inconsistent across populations, indicating that the deviation may be due to a Wahlund effect from sampling multiple non-interbreeding populations (Table 2). The greatest degree of linkage disequilibrium was observed when individuals from all sampled populations were combined, indicating important deviation from panmixis. The number of loci exhibiting deviation from HWE and LD was higher in Half Moon Bay and San Diego, indicating that these may be locations where non-randomly interbreeding populations were sampled.

Table 2. Results of tests for Linkage Disequilibrium and deviation from Hardy-Weinberg Equilibrium in each sampled location. Note that individual numbers reflect the numerical nomenclature applied to each locus rather than the number of loci reflecting HWE and LD.

Population	Loci Deviating from HWE	Pairs of Loci Exhibiting Significant Linkage Disequilibrium
Neah Bay	NA	4-5
Brookings	1	NA
Half Moon Bay	1, 2	1-2, 1-3, 1-5, 2-3, 3-4, 4-5
Morro Bay	NA	3-5
Point Conception	1, 5	4-6
Channel Islands	5	1-5
Santa Barbara	6	NA
Outer Banks	5	NA
San Diego Adults	4	1-4, 1-6, 2-4, 2-5, 2-6
San Diego Juveniles	NA	3-6, 5-6
Cabo Colonet	1	4-5, 4-6

Tests for population structure

Clustering analyses and assignment tests

The program STRUCTURE identified three clusters (K = 3) as the most likely population structure in *S. miniatus* (Ln P(D) = -10,875). The model with two clusters (K = 2) had the second highest negative log-likelihood. The mean second order rate of change in negative log-likelihood values with delta K (Evanno *et al.* 2005) confirmed that the model with three clusters was the optimal clustering of samples. The number of individuals assigned to cluster 1 declined from Morro Bay southwards and the majority of individuals were assigned to clusters 2 and 3 south of Point Conception (Figure 1). While both models showed that most individuals had some degree of shared proportion of ancestry and individuals assigned to each of the clusters occurred throughout the range (Figures 2 and 3), this may be due to a combination of incomplete lineage sorting, interbreeding, and migration. The pattern of population structure at Point Conception was clear in both models and the presence of two clusters predominantly distributed south of Point Conception was apparent in the K = 3 model (Figure 3). There was no discernible pattern in the spatial distribution of southerly individuals from clusters 2 and 3 south of Point Conception other than a higher prevalence of cluster 3 in the Santa Barbara Channel, Offshore Banks and Cabo Colonet where some samples were collected from greater depths (Figure 1). The STRUCTURE K = 3 model resulted in the assignment of individuals to clusters that most closely met the expectations of randomly breeding populations. The clusters identified with the K = 3 model are explored in further analyses.

The assignment of individuals to clusters in BAPS and STRUCTURE were largely consistent, with assignment of only a few individuals to different clusters at each location with the exception of the Channel Islands (Table 3). In the BAPS mixture analysis at the population level, the model for two populations (K = 2) had the highest log-maximum likelihood value reflecting a geographic break in population structure between Half Moon Bay and Morro Bay. The two clusters are divided by a transition zone between Morro Bay and the Channel Islands where individuals from each cluster are found. When a fixed K of 3 was applied in BAPS in a population level analysis, the break was evident at Point Conception and only the Outer Banks samples were identified as being assigned to the third cluster, with only partial posterior probability of assignment of individuals to cluster 3 elsewhere, which differed from the individual level analysis.

The individual level analysis in BAPS identified three populations (K = 3) as having the highest log maximum likelihood value assigning individuals south of Point Conception to groups consistent with the results from STRUCTURE with three populations (K = 3) (Table 3). For the sake of comparison to the population level model and the results from STRUCTURE, the admixture analysis with the K = 2 model at the individual level was analyzed. The results in Figure 4 showed a less abrupt break in population structure at Point Conception than the population level analysis in BAPS described above and more individuals assigned to cluster 1 to the south and fewer cluster 2 individuals to the north than in the results from STRUCTURE K = 2 seen in Figure 1. As with the results of STRUCTURE K = 3, when three clusters were analyzed in BAPS, cluster three was prevalent in Santa Barbara, the Outer Banks and Cabo Colonet, while cluster 2 was more prevalent in the Channel Islands, San Diego and the transition zone with cluster 1 between Morro Bay and Point Conception (Figure 5).

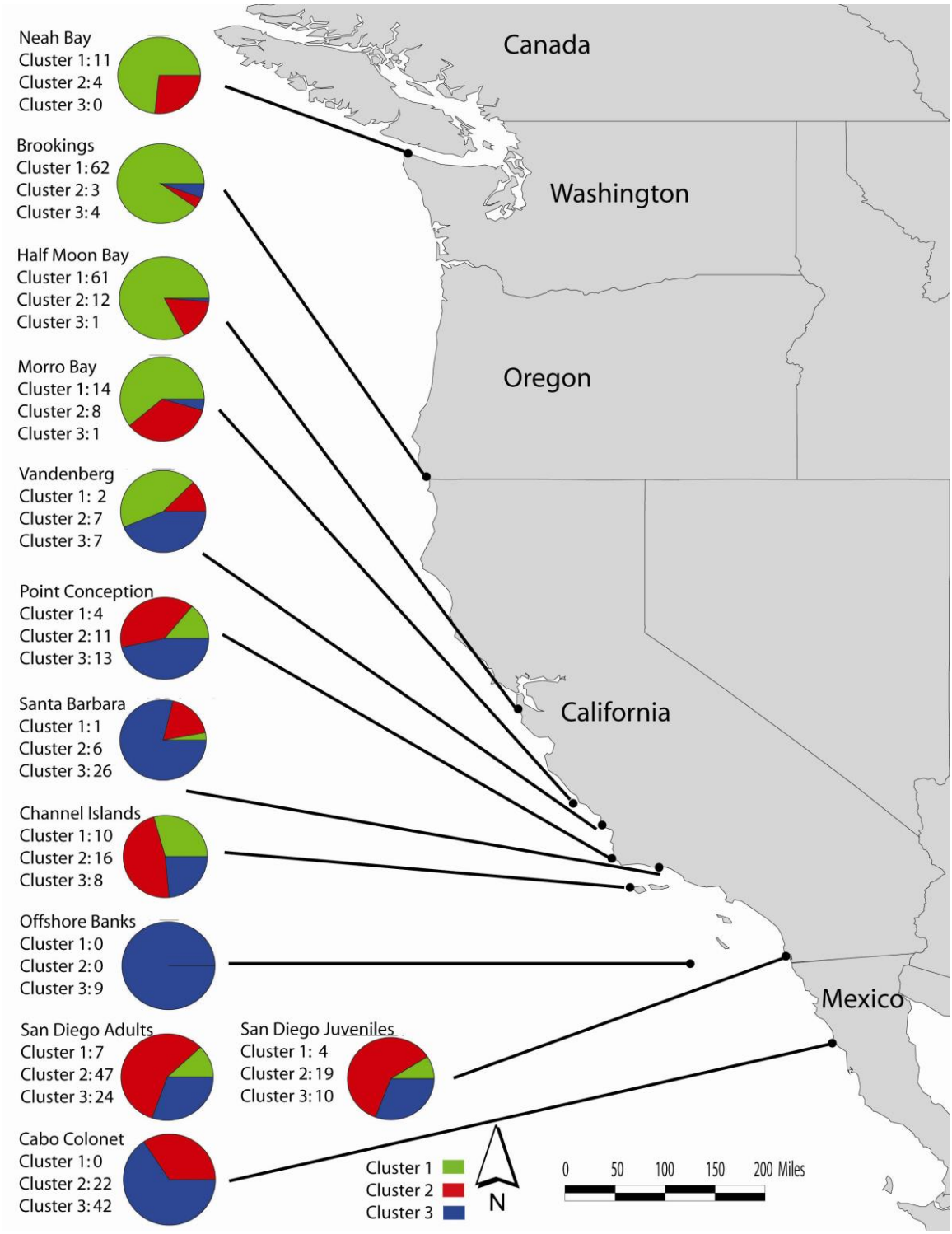

Figure 1. Pie charts displaying the proportion of *S. miniatus* that were assigned to each of the clusters from STRUCTURE assuming three clusters (K = 3) coastwide. The location indicated by the dot on the chart is placed at the average latitude and longitude of the samples in the area.

Table 3. Number of *S. miniatus* individuals for each sampled location assigned to clusters resulting from three assumed clusters (K = 3) in STRUCTURE and BAPS.

	BAPS K = 3 Cluster			STRUCTURE K = 3 Cluster		
Population	**1**	**2**	**3**	**1**	**2**	**3**
Neah Bay	11	4	0	11	4	0
Brookings	60	4	5	62	3	4
Half Moon Bay	58	15	1	61	12	1
Morro Bay	13	10	0	14	8	1
Point Conception	4	13	11	4	11	13
Channel Islands	8	19	7	16	10	8
Santa Barbara	1	7	25	1	6	26
Outer Banks	0	0	9	0	0	9
San Diego Adults	8	45	25	7	47	24
San Diego Juveniles	4	19	10	4	19	10
Cabo Colonet	1	24	39	0	22	42

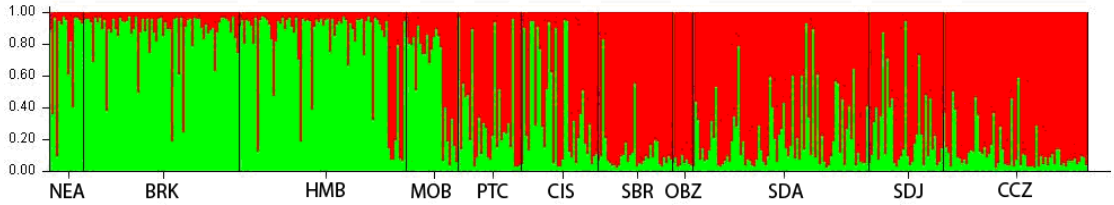

Figure 2. Proportion of ancestry from STRUCTURE for each *S. miniatus* individual with two clusters (K = 2).

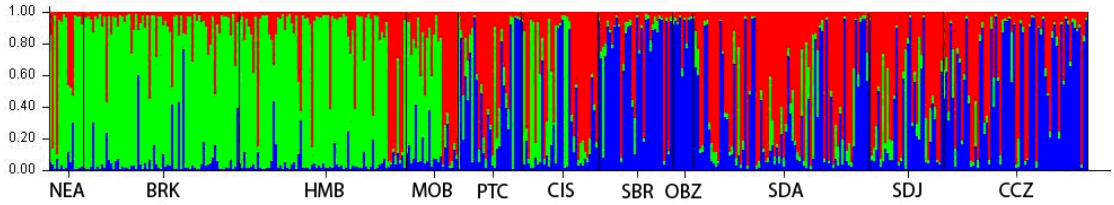

Figure 3. Proportion of ancestry from STRUCTURE for each *S. miniatus* individual with three clusters (K = 3).

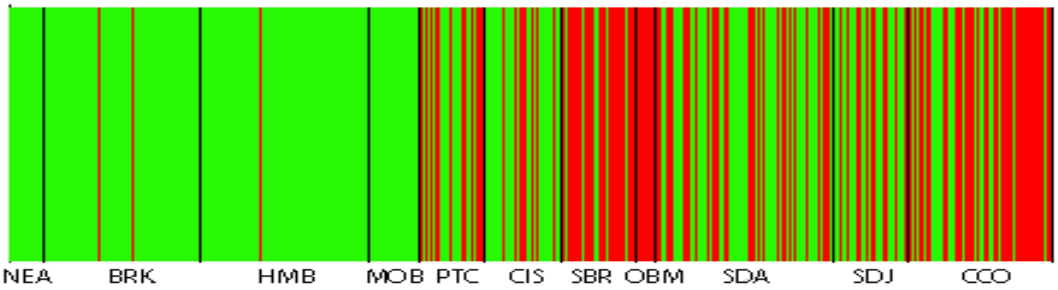

Figure 4. Results of admixture analysis from BAPS for K = 2 clusters conducted at an individual level for *S. miniatus*. Posterior probability of assignment of each individual from each sample location to each cluster is represented by the differing colored bars.

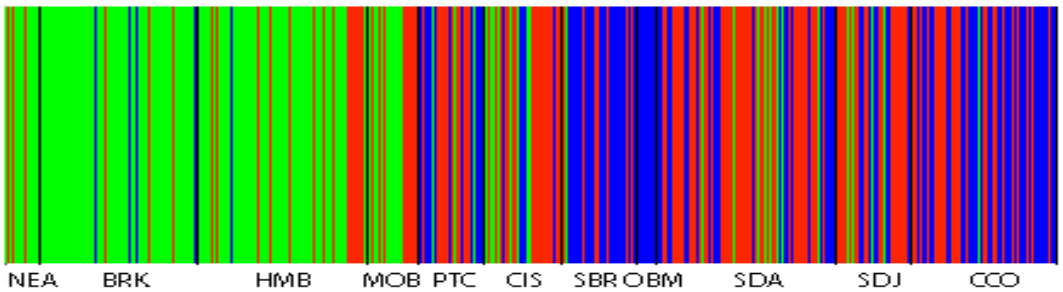

Figure 5. Results of admixture analysis from BAPS for K = 3 clusters conducted at an individual level for *S. miniatus*. Posterior probability of assignment of each individual from each sample location to each cluster is represented by the differing colored bars.

Indices of differentiation F_{ST}

Significant genetic differentiation as measured by F_{ST} was evident in *S. miniatus* with strong population structure between sample locations north and south of Point Conception (Table 4). In addition, the F_{ST}-values were elevated in comparisons of the Outer Banks and Santa Barbara to other sample locations, which may be due to the prevalence of individuals assigned to cluster 3 identified in the Outer Banks and Santa Barbara (Table 4). There are elevated levels of genetic differentiation as measured by F_{ST} between Neah Bay and other sample locations north of Point Conception, though this may be due in part to the low sample size in Neah Bay.

The F_{ST}-values resulting from pairwise comparison of all individuals assigned to clusters from analysis in STRUCTURE with K = 3 were all significant ($P < 0.05$) and indicated greater genetic differentiation between the more northerly distributed cluster 1 and cluster 3 ($F_{ST} = 0.080$) than between cluster 1 and cluster 2 ($F_{ST} = 0.055$) or cluster 2 and cluster 3 ($F_{ST} = 0.028$). Relative to migration the two criteria evaluated, EV 3 in which populations exchange less than 5 migrants per generation corresponding to an $F_{ST} = 0.05$ and EV 4 in which less than 25 migrants per generation are exchanged, corresponding to

an F_{ST} = 0.01, were exceeded as threshold values indicating significant population structure.

Within cluster 1 comparisons between sample locations resulted in only a few moderate and significant F_{ST}-values between Neah Bay and other sample locations (NEA1 vs. HMB1 (0.030), MOB1 (0.075) and CIS1 (0.052)), as was also the case for Morro Bay (MOB1 vs. BRK1 (0.027) and HMB1 (0.016)). This indicates the potential for residual population structure within cluster 1 at Neah Bay and a transition to alleles from the other two clusters in waters of Morro Bay, although the sample size of only 17 individuals in Neah Bay introduces the possibility that this structure may be due to random sampling error. The remaining comparisons in cluster 1 showed a lack of significant differentiation between sample locations. Mostly low and non-significant F_{ST}-values were observed in comparisons among sample locations for samples assigned to clusters 2 and cluster 3.

The F_{ST}-values for comparisons between cluster 1 and cluster 2 between sampled locations indicates genetic differentiation increases with distance between sampled locations except in sites with low sample sizes (Table 5). Genetic differentiation was significant between sample locations for cluster 1 and 2 north of Point Conception, but not in comparisons south of Point Conception where sample sizes for cluster 1 were low (Table 5). Higher F_{ST}-values and significant differentiation were observed between cluster 1 and 3, consistent with population level analyses prior to assignment as a result of the prevalence of each cluster between regions (Table 6). In comparisons between cluster 1 north of Point Conception to cluster 2 south of Point Conception (Table 5) and all but the most northerly samples 3 (Table 6), EV 3 (F_{ST} = 0.05) and EV 4 (F_{ST} = 0.01) were exceeded between most sample locations.

Comparisons for cluster 2 and cluster 3 showed mostly weak and non-significant genetic differentiation between sample locations north of Point Conception where sample sizes were low, but moderate and significant comparisons to the south where sample sizes were greater (Table 7). The highest F_{ST}-values were observed in comparisons with the Outer Banks where only individuals from cluster 3 were found. The high and significant F_{ST}-values for most comparisons between cluster 2 samples from the Channel Islands, San Diego and Cabo Colonet and cluster 3 sample locations, which may be due in part to the higher sample sizes for each cluster at these locations. Genetic differentiation is significant between sample locations south of Point Conception in the center of their distribution where larger sample sizes were available indicating sympatric or parapatric genetically divergent populations within *a priori* sample locations (Table 7). The threshold of EV 3 (F_{ST} = 0.05) was seldom exceeded, while EV 4 (F_{ST} = 0.01) was more frequently exceeded indicating more moderate, but potentially meaningful population structure between cluster 2 and 3 at the sample locations toward the center of their range in the Channel Islands, San Diego and Cabo Colonet with larger sample sizes south of Point Conception.

The presence of differentiation between panmictic populations suggests population structure on a finer scale than the 60 mile area (included in each sampling location) can resolve. This may be evidence of secondary contact of divergent populations with

limited subsequent gene flow. This would be consistent with isolation-by-depth as a factor in population structure between cluster 2 and 3 as with *S. miniatus* and *S. crocotulus* (Hyde *et al.* 2008). Comparison of results with migration rate thresholds reflects population structure identified in Structure K = 3 with population structure north and south of Point Conception and in the southern California Bight between cluster 2 and 3, with additional population structure between Neah Bay and other locations north of Point Conception.

Genetic differentiation measured by F_{ST} between young of year *S. miniatus* assigned to clusters indicated moderate levels of differentiation, though only comparisons between cluster 1 and cluster 3 were significant ($P < 0.05$, Bonferroni Corrected: $\alpha = 0.05/6 = 0.0083$), in part due to the low sample size with only four juveniles from cluster 1 (cluster 1 vs. cluster 2, $F_{ST} = 0.0103$, $p = 0.342$; cluster 1 vs. cluster 3, $F_{ST} = 0.059$, $p = 0.009$; cluster 2 vs. cluster 3, $F_{ST} = 0.0189$, $p = 0.045$). Prior to assignment to clusters, the F_{ST} values between juveniles and adults sampled in San Diego, California were low and non-significant ($F_{ST} = -0.0021$, $p = 0.766$). Comparisons of adults to juveniles in each cluster also indicated no significant differentiation between generations in San Diego (Adult 1 vs. Juvenile 1, $F_{ST} = -0.0007$, $p = 0.496$; Adult 2 vs. Juvenile 2, $F_{ST} = -0.0009$, $p = 0.685$, Adult 3 vs. Juvenile 3, $F_{ST} = -0.0021$, $p = 0.550$). The comparisons between cluster 1 and cluster 2 resulted in elevated but non-significant values (Adult 1 vs. Juvenile 2, $F_{ST} = 0.0151$, $p = 0.144$; Juvenile 1 vs. Adult 2, = F_{ST} 0.0264 ($p = 0.027$). Comparisons between cluster 1 and cluster 3 (Adult 1 vs. Juvenile 3, $F_{ST} = 0.0431$, 0.009; Juvenile 1 vs. Adult 3, $F_{ST} = 0.0493$, $p = 0.009$) were elevated and significant as was also the case for comparisons between cluster 2 and cluster 3 (Adult 2 vs. Juvenile 3, $F_{ST} = 0.0290$, $p = 0.000$); Juvenile 2 vs. Adult 3, $F_{ST} = 0.0206$, $p = 0.009$). The results are consistent with comparisons between adults of each cluster, indicating intergenerational stability in differentiation between species and populations.

Table 4. F_{ST}-values and *P*-values in parentheses for comparisons of each of the sampled locations of *S. miniatus* (P < 0.05, Bonferroni Corrected: $\alpha = 0.05/6 = 0.0083$). Gray highlight indicates as statistically significant result.

Pop	NEA	BRK	HMB	MOB	PTC	CIS	SBR	OBZ	SDA	SDJ
BRK	0.0108 (0.036)	0 (*)								
HMB	0.0128 (0.054)	0.0031 (0.045)	0 (*)							
MOB	0.0409 (0)	0.0235 (0)	0.0083 (0.045)	0 (*)						
PTC	0.0403 (0)	0.0362 (0)	0.0198 (0)	0.0011 (0.297)	0 (*)					
CIS	0.0545 (0)	0.0388 (0)	0.0239 (0)	0.0011 (0.432)	-0.0042 (0.882)	0 (*)				
SBR	0.1063 (0)	0.0835 (0)	0.0684 (0)	0.0261 (0)	0.0113 (0.009)	0.0126 (0.009)	0 (*)			
OBZ	0.1364 (0)	0.1156 (0)	0.0910 (0)	0.0372 (0.009)	0.0185 (0.063)	0.0285 (0)	0.0047 (0.306)	0 (*)		
SDA	0.0570 (0)	0.0485 (0)	0.0363 (0)	0.0090 (0.009)	-0.0024 (0.865)	-0.0003 (0.568)	0.0144 (0)	0.0285 (0)	0 (*)	
SDJ	0.0572 (0)	0.0550 (0)	0.0381 (0)	0.0117 (0)	-0.0045 (0.856)	-0.0012 (0.514)	0.0139 (0)	0.0222 (0)	-0.0021 (0.766)	0 (*)
CCZ	0.0738 (0)	0.0661 (0)	0.05113 (0)	0.0185 (0)	0.0021 (0.288)	0.0078 (0.009)	0.0015 (0.288)	0.016 (0.063)	0.0041 (0.063)	0.0027 (0.162)

Table 5. F_{ST}-values and *P*-values for comparisons of each of the populations of *S. miniatus* with a STRUCTURE model with three populations (K = 3) for cluster 1 vs. cluster 2 individuals sampled at each sample location (P < 0.05, Bonferroni Corrected: α = 0.05/6 = 0.0083). Gray highlight indicates as statistically significant result.

Pop/ Cluster (N)	NEA_1 (11)	BRK_1 (62)	HMB_1 (61)	MOB_1 (14)	PTC_1 (10)	CIS_1 (8)	SDA_1 (7)	SDJ_1 (4)
NEA_2 (4)	0.0823 (0)	0.0457 (0)	0.0374 (0.054)	0.0553 (0.009)	0.0551 (0.189)	0.0620 (0)	-0.0051 (0.559)	0.0256 (0.414)
BRK_2 (3)	0.1286 (0)	0.0462 (0)	0.0392 (0.153)	0.0261 (0.099)	0.0622 (0.288)	-0.0099 (0.513)	0.0354 (0.144)	0.0642 (0.189)
HMB_2 (12)	0.0883 (0)	0.0574 (0)	0.0572 (0)	0.0458 (0.009)	0.0426 (0.027)	0.0366 (0.009)	0.0199 (0.054)	0.0119 (0.261)
MOB_2 (8)	0.1026 (0)	0.0693 (0)	0.0645 (0)	0.0644 (0)	0.0316 (0.162)	0.0554 (0)	0.0273 (0.054)	-0.0031 (0.612)
PTC_2 (11)	0.0819 (0)	0.0411 (0)	0.0312 (0)	0.0323 (0.009)	0.0153 (0.234)	0.0241 (0.009)	0.0144 (0.144)	-0.0046 (0.577)
CIS_2 (16)	0.1147 (0)	0.0742 (0)	0.0648 (0)	0.0571 (0)	0.0509 (0.054)	0.0629 (0)	0.0439 (0.009)	0.0132 (0.207)
SBR_2 (6)	0.1245 (0)	0.0804 (0)	0.0764 (0)	0.0411 (0)	0.08342 (0)	0.0376 (0.009	0.0622 (0)	0.0132 (0.387)
SDA_2 (47)	0.1045 (0)	0.0636 (0)	0.0603 (0)	0.0500 (0)	0.03754 (0)	0.0516 (0)	0.0279 (0.009)	0.0151 (0.144)
SDJ_2 (19)	0.1007 (0)	0.0705 (0)	0.0646 (0)	0.0519 (0)	0.0534 (0.009)	0.0462 (0)	0.0264 (0.027)	0.0103 (0.342)
CCZ_2 (22)	0.1079 (0)	0.0666 (0)	0.0627 (0)	0.0437 (0)	0.0557 (0.009)	0.0481 (0)	0.0337 (0.009)	0.0105 (0.306)

Table 6. F_{ST}-values and *P*-values for comparisons of each of the populations of *S. miniatus* with a STRUCTURE model with three populations (K = 3) for cluster 1 vs. cluster 3 individuals sampled at each sample location (P < 0.05, Bonferroni Corrected: α = 0.05/6 = 0.0083). Gray highlight indicates as statistically significant result.

Pop/ Cluster (N)	NEA_1 (11)	BRK_1 (62)	HMB_1 (61)	MOB_1 (14)	PTC_1 (10)	CIS_1 (8)	SDA_1 (7)	SDJ_1 (4)
BRK_3 (4)	0.0701 (0.045)	0.0183 (0.117)	0.0177 (0.1982)	0.0217 (0.144)	0.0128 (0.369)	-0.0031 (0.568)	-0.0191 (0.730)	-0.0196 (0.874)
PTC_3 (13)	0.1073 (0)	0.0740 (0)	0.0669 (0)	0.0487 (0)	0.0913 (0)	0.0543 (0)	0.0374 (0)	0.0313 (0.027)
CIS_3 (8)	0.1646 (0)	0.1152 (0)	0.1048 (0)	0.0801 (0)	0.1089 0)	0.0880 (0)	0.0677 (0.009)	0.084 (0.009)
SBR_3 (26)	0.1569 (0)	0.1022 (0)	0.099 (0)	0.0612 (0)	0.1116 (0)	0.0689 (0)	0.0688 (0)	0.0808 (0)
OBZ_3 (9)	0.1829 (0)	0.1274 (0)	0.1128 (0)	0.0765 (0)	0.1164 (0)	0.0914 (0)	0.0868 (0.009)	0.0769 (0.027)
SDA_3 (24)	0.1223 (0)	0.0870 (0)	0.0840 (0)	0.0513 (0)	0.0965 (0)	0.0625 (0)	0.0407 (0.009)	0.0493 (0.009)
SDJ_3 (10)	0.1349 (0)	0.0957 (0)	0.0848 (0)	0.0667 (0)	0.0950 (0)	0.0701 (0)	0.0431 (0.009)	0.0589 (0.009)
CCZ_3 (42)	0.1219 (0)	0.0874 (0)	0.0838 (0)	0.0570 (0)	0.0926 (0)	0.0623 (0)	0.0454 (0)	0.0500 (0)

Table 7. F_{ST}-values and *P*-values for comparisons of each of the populations of *S. miniatus* with a STRUCTURE model (K = 3) for cluster 2 vs. cluster 3 individuals sampled at each sample location (P < 0.05, Bonferroni Corrected: α = 0.05/6 = 0.0083). Gray highlight indicates as statistically significant result.

Pop/ Cluster (N)	NEA2 (4)	BRK2 (3)	HMB2 (12)	MOB2 (8)	PTC2 (11)	CIS2 (16)	SBR2 (6)	SDA2 (47)	SDJ2 (19)	CCZ2 (22)
BRK3 (4)	0.0230 (0.315)	-0.0716 (0.991)	0.0152 (0.207)	-0.0089 (0.721)	-0.0273 (0.901)	0.0058 (0.378)	0.0105 (0.387)	-0.0006 (0.451)	-0.0108 (0.639)	0.0169 (0.270)
PTC3 (13)	0.0079 (0.189)	0.0335 (0.027)	0.0066 (0.198)	0.0298 (0)	0.0219 (0.009)	0.0315 (0)	-0.0078 (0.612)	0.0386 (0)	0.0243 (0.009)	0.0273 (0)
CIS3 (8)	0.0345 (0.135)	-0.0063 (0.766)	0.0131 (0.171)	0.0171 (0.216)	0.0199 (0.081)	0.0433 (0)	0.0375 (0.081)	0.0399 (0)	0.0313 (0)	0.0475 (0)
SBR3 (26)	0.0579 (0)	0.0228 (0.189)	0.0228 (0.009)	0.0343 (0)	0.028 (0.027)	0.0422 (0)	0.0140 (0.153)	0.041 (0)	0.0303 (0)	0.0387 (0)
OBZ3 (9)	0.0548 (0.045)	0.0299 (0.189)	0.0365 (0.009)	0.0219 (0.126)	0.0191 (0.198)	0.0468 (0)	0.0135 (0.333)	0.0506 (0)	0.0346 (0)	0.0488 (0)
SDA3 (24)	0.0277 (0.081)	0.0203 (0.243)	0.0116 (0.063)	0.0234 (0.018)	0.0122 (0.081)	0.0349 (0)	0.0027 (0.396)	0.0363 (0)	0.0206 (0.009)	0.0298 (0)
SDJ3 (10)	0.0039 (0.387)	0.0116 (0.180)	0.0111 (0.171)	0.0112 (0.135)	0.0042 (0.306)	0.0279 (0.009)	0.0072 (0.216)	0.0290 (0)	0.0189 (0.045)	0.0264 (0.018)
CCZ3 (42)	0.0265 (0.018)	0.0207 (0.162)	0.0145 (0.036)	0.0231 (0.036)	0.0134 (0.036)	0.0285 (0)	0.0002 (0.496)	0.0339 (0)	0.0193 (0.009)	0.0260 (0)

Test for co-occurrence of post-settlement young of year of S. crocotulus and S. miniatus assigned to clusters identified with STRUCTURE and differentiation between adults and juveniles in San Diego

Though the 40 specimens of post-settlement young of year *S. crocotulus* and *S. miniatus* were sampled in waters less than 10 fm, individuals undergo ontogenetic migration to different depths as they grow and mature (Love *et al.* 2002). While individuals assigned to both species and all three clusters in *S. miniatus* were present in the samples collected in the vicinity of San Diego, only four individuals from cluster 1 were present. The remaining post-settlement juveniles included 19 cluster 2 individuals, 10 cluster 3 individuals and seven *S. crocotulus*. The proportion of sampled juvenile *S. miniatus* resembled very closely the composition of the adults and juveniles (cluster 1:cluster 2:cluster 3, adults 10:60:30, juvenile 12:58:30). This indicates that recruitment and presence of young on the reef is proportional to the relative abundance of adults in each cluster, possibly due to recruitment in proportion to the relative abundance of adults and consistent recruitment between clusters. When *S. crocotulus* was included, the proportions of adults and juveniles assigned to populations reflected a higher proportion of *S. crocotulus* in adults (cluster 1:cluster 2:cluster 3:*S. crocotulus*, adult 5:36:18:41 and juvenile 8:37:20:35).

Tests for correlation of assignment of S. miniatus individuals to clusters from STRUCTURE with depth, longitude and latitude of capture

To the north of Point Conception, cluster 1 is prevalent with cluster 2 encountered in lower frequency, while cluster 3 is very uncommon north of the sample location near Vandenberg Air Force Base (Figure 1). There were no clear geographic patterns in the distribution of *S. miniatus* cluster 2 and cluster 3 within the Southern California Bight where clusters co-occur, other than the prevalence of cluster 2 in the Channel Islands and San Diego and cluster 3 in Santa Barbara and Cabo Colonet and the Outer Banks (Figure 6). The Morro Bay sample location that contains samples primarily encountered in the vicinity of Morro Bay were combined with samples collected in the vicinity of Vandenberg Airforce base approximately 40 nautical miles to the south for analysis with indices of genetic differentiation (F_{ST}), due to their proximity. When samples collected near Vandenberg Air Force Base were separated from the Morro Bay sample, it was apparent that cluster 1 was more prevalent in Morro Bay than near Vandenberg to the south, where cluster 3 was prevalent in Figure 6. Inclusion of the cluster 3 individuals from Vandenberg in the Morro Bay may explain the higher F_{ST}-values for comparisons of Morro Bay with other sample locations north of Point Conception prior to assignment of individuals to clusters as seen in Table 4. The depth distribution of sampling may be a confounding factor relative to the latitude of sampled locations.

Both clusters 1 and 2 individuals are more commonly encountered in depths between 10 and 30 fm (60 m) with some cluster 2 observed in up to 50 fm (100 m), while cluster 3 is predominantly distributed in 10 to 50 fm (20 m to 100 m), but were encountered in depths up to 80 fm (160 m) (Figure 7). Plots of the frequency of individuals encountered in 10 fm bins at each sample location shown in Figure 8 indicate that cluster 1 is predominantly distributed north of Point Conception in waters shallower than 30 fm (60 m), while cluster 2 is primarily distributed south of Point Conception in depths less than

30 (60 m), cluster 3 is mostly found south of Point Conception in depths between 10 fm (20 m) and 50 fm (100 m) and adult *S. crocotulus* are found predominantly in deeper waters.

The univariate logistic fit of cluster assignment with numeric continuous values of depth in fathoms as well as latitude and longitude to examine spatial distribution resulted in significant ($p < 0.0001$) difference in log-likelihood and improved fit compared to the model without the explanatory variables. The model with depth resulted in the greatest difference in log-likelihood and explained the highest proportion of variance (-log-likelihood $\Delta = 39.9$, $R^2 = 0.16$, Figure 9 D.), then latitude (-log-likelihood $\Delta = 19$, $R^2 = 0.08$, Figure 9 E.) and weaker correlation with longitude (-log-likelihood $\Delta = 0.07$, $R^2 = 0.07$, Figure 9 F.). Plots of the logistic probability of encounter of each cluster with depth, latitude and longitude are reflected by the proportion of the probability reflected above and below blue lines delineating the relative probability of clusters 1, 2 and 3 provided in Figure 9.

The significant chi-square probability ($p < 0.0001$) of encounter of members of each cluster with depth, latitude and longitude indicates a significant correlation of the proportion of each cluster relative to these variables. The parameter estimates, describing the rate of change in the probability of encounter, indicated a decreasing probability of encountering cluster 1 compared to cluster 3 with depth (parameter estimate = -0.178), an increasing probability at higher latitudes (parameter estimate = 0.868) and decreasing probability with increasing longitude (parameter estimate = -0.689). The chi-square probabilities were also significant for the declining relative probability of observing cluster 2 relative to cluster 3 with depth (parameter estimate = -0.089) and increased with longitude (parameter estimate = 0.169), though no significant correlation with latitude over the subregion was found.

The univariate contingency test for the correlation of cluster assignment with ordinal binned magnitude for depth in 10 fathoms bins, latitude by degree bin and longitude by degree bin, resulted in significant likelihood ratio tests and Pearson correlation coefficient values ($P < 0.0001$) for depth (-log-likelihood $\Delta = 44.6$, $R^2 = 0.18$), latitude (-log-likelihood $\Delta = 44.1$, $R^2 = 0.18$) and longitude (-log-likelihood $\Delta = 36.6$, $R^2 = 0.15$). Mosaic plots of the relative probability of encounter of each cluster with depth, latitude and longitude are provided in Figure 9.

The multivariate logistic fit of cluster by depth, latitude and longitude as numeric continuous values resulted in a significant -log-likelihood difference of 59.7 (Prob > Chi-square <0.0001, $R^2 = 0.24$) compared to the base model. The parameter estimates were only significant for the relative probability of observing cluster 1 compared to cluster 3 (parameter estimate = -0.15, $P < 0.001$) with depth, while latitude and longitude were not significant. The relative likelihood of encountering cluster 2 compared to cluster 3 decreased with depth (parameter estimate = -0.09, $P < 0.001$), increased with latitude (parameter estimate = 0.81, $P = 0.0016$) and increased with longitude (0.58, $P = 0.0158$). The Wald Test chi-square was significant for depth (40.099, $P < 0.001$), latitude (9.96, $P = 0.0068$) and longitude (7.57, $P = 0.0227$).

The multivariate logistic fit of cluster with depth in fathoms, latitude and longitude as numeric continuous values, resulted in a significant log-likelihood change of 100.36 compared to the base model ($P < 0.0001$, $R^2 = 0.3983$). The model treated each bin as a categorical variable and results in higher sum of squares due to the large number of variables in the model. Though many of the comparisons were not valid due to a lack of data across bins or were non-significant, those that were significant indicate points of change in the distribution of each cluster with depth, latitude and longitude. The relative probability of observing cluster 1 relative to cluster 3 decreased significantly between 20-30 fm (40-60 m) and 40-50 fm (80-100 m) (parameter = -4.7, P = 0.0003), increased between 35 and 36 degree bins (parameter = 3.6, P = 0.021) and decreased with longitude between the -120 and -119 degree bins (parameter = -13.8, P = 0.000). The relative probability of observing cluster 2 compared to cluster 3 decreased significantly with depth from 20-30 fm (40-60 m) to 40-50 fm (80-100 m) (parameter = -3.2, P = 0.003) and decreased from the -120 to -121 degree longitude bin, though no significant correlation assignment at each latitude in the Southern California Bight. The Wald test chi-square indicated that only depth was a significant factor within the sub-region examined (Wald Test Chi-Square = 21.3, P = 0.0194).

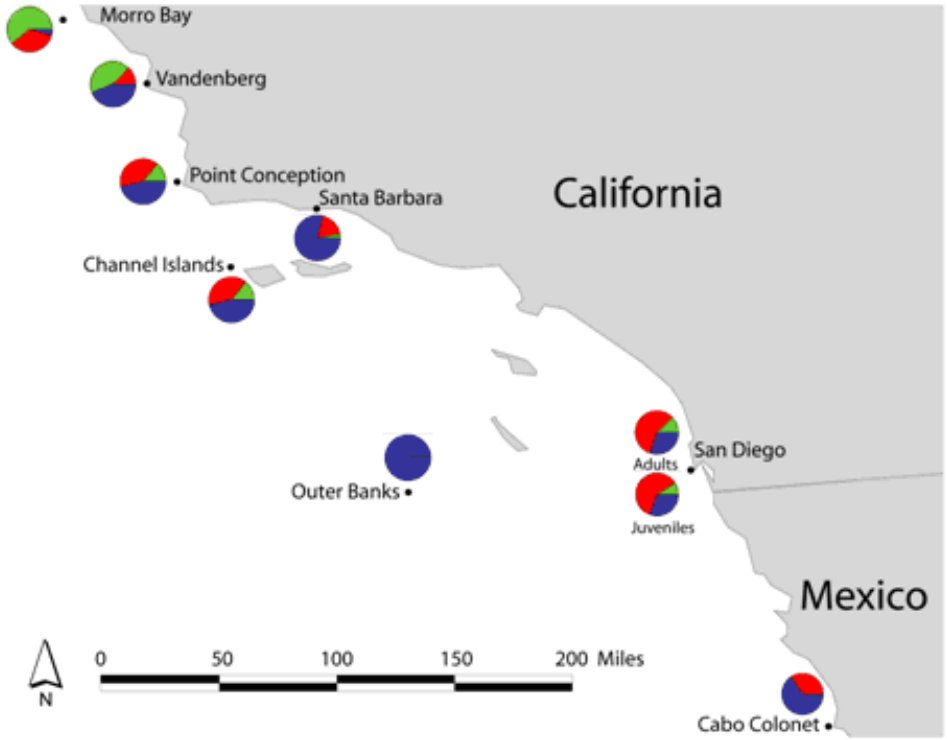

Figure 6. Pie charts displaying the proportion of *S. miniatus* that were assigned to each of the clusters from STRUCTURE assuming three clusters (K = 3) from Morro Bay to Cabo Colonet used in the tests for correlation cluster assignment with variables of interest.

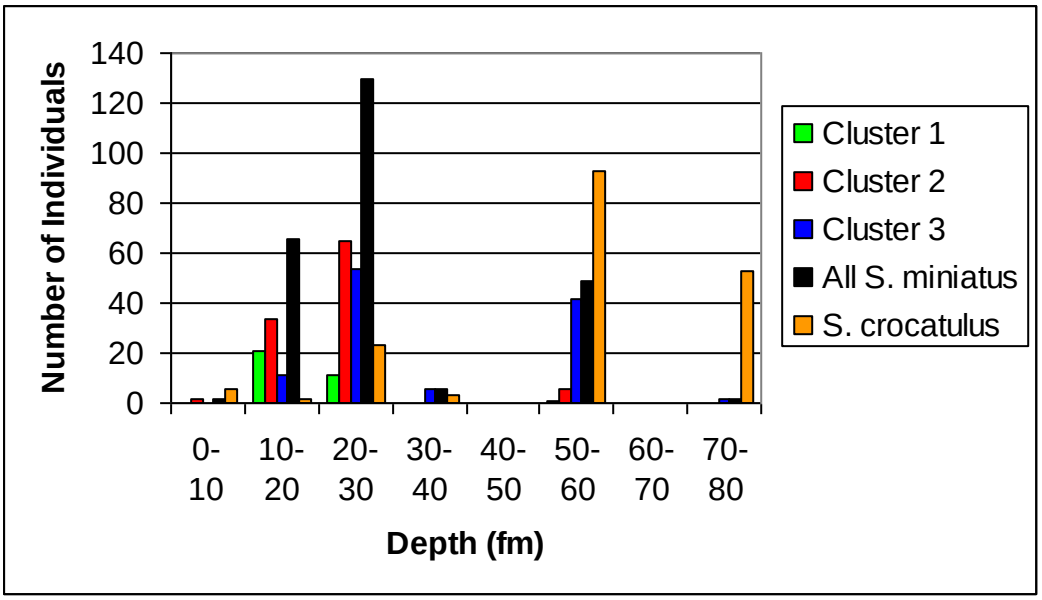

Figure 7. Frequency of sampled *S. miniatus* and individuals assigned to cluster with STRUCTURE (K = 3) with 10 fm (20 m) depth increments.

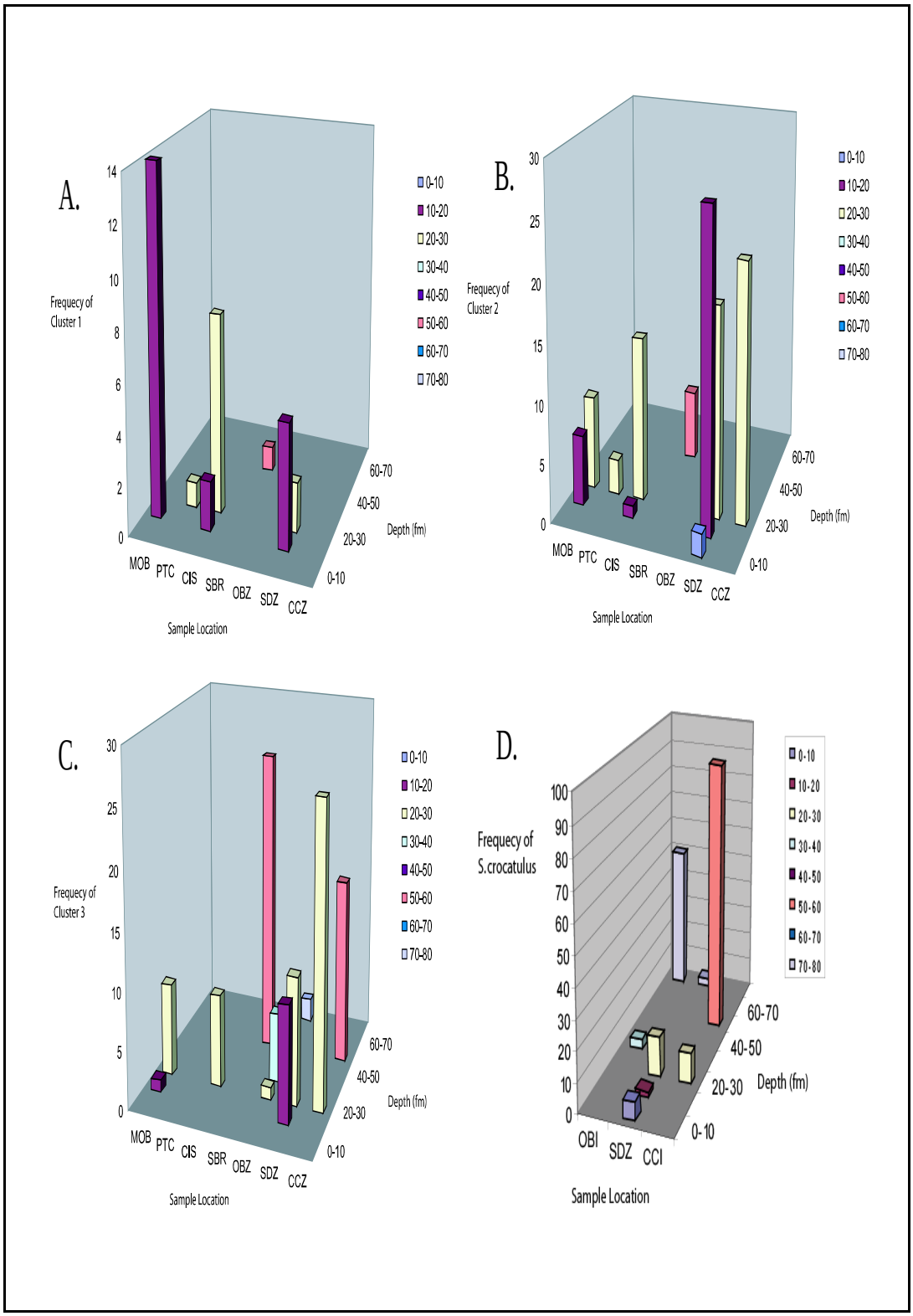

Figure 8. Frequency of *S. crocotulus* and *S. miniatus* individuals from Morro Bay, California to Cabo Colonet, Mexico assigned to cluster using STRUCTURE (K=3): A.) Cluster 1, B.) Cluster 2, C.) Cluster 3 and D.) *S. crocotulus* in each 10 fm (20 m) depth bin at each sampled location. See Table 1 for location codes.

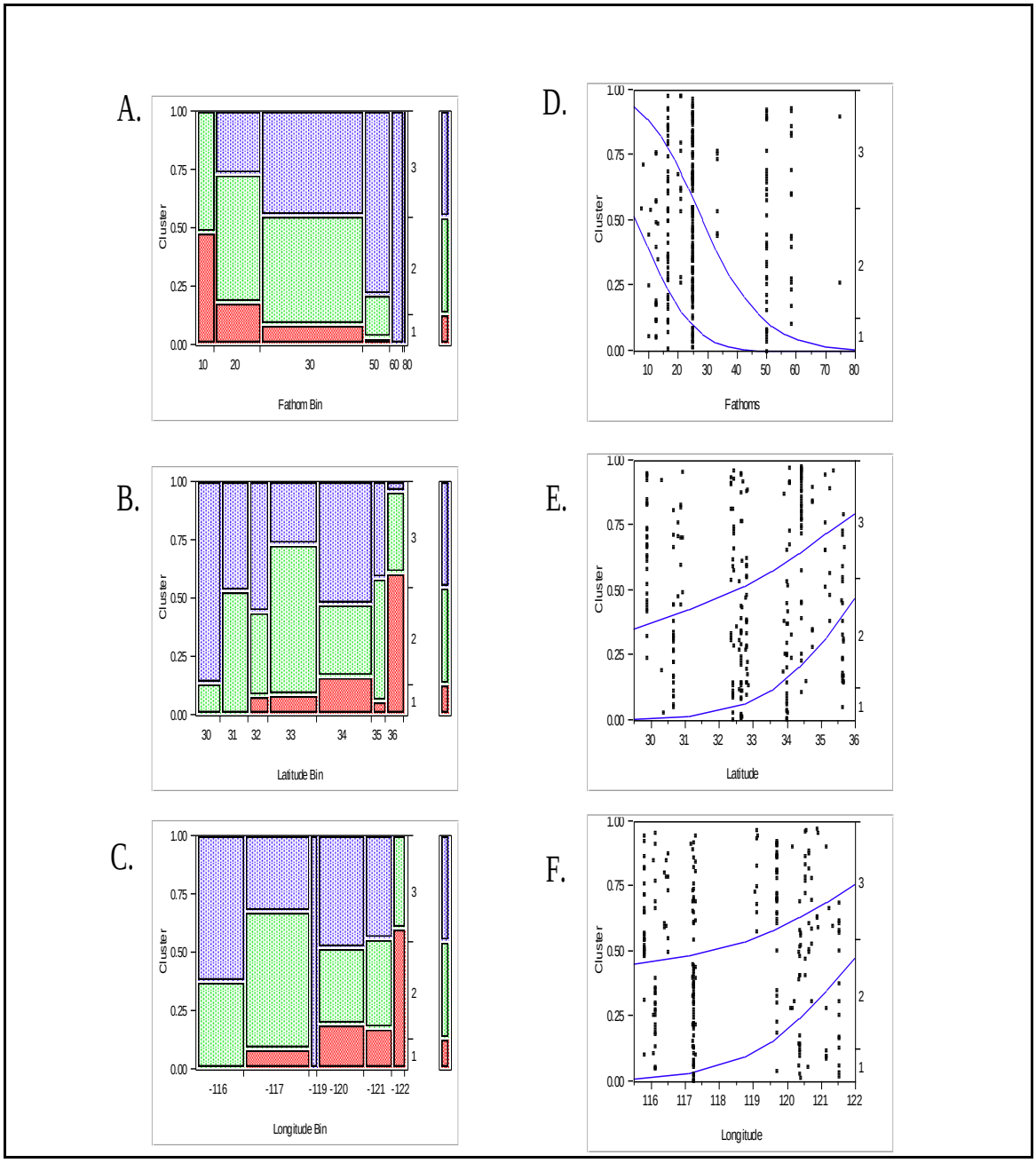

Figure 9. Mosaic plots from contingency tests indicating the probability of encountering an individual from each cluster identified in STRUCTURE (K = 3) in each Depth (A), Latitude (B) and Longitude (C) bin. Probability of encountering an individual from each cluster from univariate logistic regression analysis with continuous values of Depth (D), Latitude (E) and Longitude (D) as delineated by the blue lines between clusters. Values below the lower line correspond to the probability of encountering cluster 1, the difference between the first line and the second indicates the probability of encountering cluster 2, while values above the second line indicates the probability of encountering cluster 3. Cluster 1 is red, cluster 2 is green and cluster 3 is blue in A through C.

Tests for co-occurrence of population structure with locations expected to pose barriers to gene flow

Partitioning of variation among sampled putative populations in Table 1 explained a lower percentage of the variation between groups (2.8%, F_{ST} = 0.03) and resulted in higher percentage of variation within groups (2.4%, F_{IS} = 0.02), than partitioning by a geographic boundary at Point Conception (4.2% between, F_{ST} = 0.04; 2.8% within, F_{IS} = 0.03). The results of partitioning among regions north and south of Point Conception explained less of the variation between groups and resulted in a lesser reduction in the percent variation within groups compared to stratification by clusters identified in BAPS and STRUCTURE (Table 8). The results of the AMOVA analysis indicate that the highest percentage of variation between groups was found with STRUCTURE K = 2 (6%, F_{ST} = 0.06), followed by STRUCTURE K = 3 (5.6%, F_{ST} = 0.06) and BAPS K = 3 (5.3%, F_{ST} = 0.05). The lowest percent variation within groupings was provided by STRUCTURE K = 3 (1.1%, F_{IS} = 0.01) followed by BAPS K = 3 (1.3%, F_{IS} = 0.01) and STRUCTURE K = 2 (1.8%, F_{IS} = 0.02). Grouping of samples with BAPS K = 2 explains less of the variation between populations (4.2%, F_{ST} = 0.04) and results in a higher percentage of variation within populations (2.9%, F_{ST} = 0.03), than other clustering results. The results for BAPS K = 2 are comparable to that found with a break at Point Conception and the distribution of individuals were largely consistent with this break.

An AMOVA conducted with samples partitioned south of Brooking as well as Point Conception to test for population structure at Cape Mendocino explained 4.3% of the variance between and resulted in 3.1% variation within populations, with an F_{ST} of 0.05 and F_{IS} of 0.03. An AMOVA conducted with samples partitioned south of Neah Bay to test for population structure at Cape Blanco as well as Point Conception explained 4.3% of the variance between groups and 3.0% variation within groups, an F_{ST} of 0.04 and a F_{IS} of 0.03. While a second partition at these locations results in a slight increase in the percent variation between groups and a higher F_{ST} value, the percent variation within groups increased compared to partitioning at Point Conception alone and resulted in a higher F_{IS} value.

The results of AMOVA with either Brookings and Neah Bay or Neah Bay alone separated from the remaining clusters from STRUCTURE K = 3 accounted for a lower percentage of variation between sample locations (5.4 %, F_{ST} = 0.05) and resulted in higher percentage of variation within populations (1.5%, F_{IS} = 0.02), than assignment tests with K = 3 alone. This indicates that the groupings accounting for population structure at Cape Mendocino and Cape Blanco do not appreciably improve the explanatory ability of the model and indicate weak or no population structure. The co-occurrence of individuals assigned to each cluster in *a priori* samples across boundaries limits the ability of geographic groupings at sample location level to resolve population structure. This analysis confirms that assignment methods accounting for population structure at Point Conception and between two populations isolated by depth in the Southern California Bight explain a higher proportion of the variation between populations and results in a lower proportion of variation within populations than geographic groupings alone.

The AMOVA analysis with sequential grouping north and south of each sample location confirms that the main division of population structure is in the vicinity of Point Conception (Table 9, Figure 10). The lowest F_{IS} values and highest F_{ST} values (Figure 10) as well as the greatest percent variation between populations from division of samples south of Half Moon Bay and similar results were observed with division south of Morro Bay (Table 9). A secondary peak in F_{IS} and F_{ST} was observed when samples were divided south of Santa Barbara, where cluster 3 individuals from STRUCTURE analysis increase in prevalence (Table 9, Figure 10).

When the AMOVA analysis with sequential grouping was repeated for only samples north or south of Point Conception to test for additional structure within groupings, no substantial structure was identified south of Point Conception as indicated by very low F_{ST} values and percentages of variation between sample locations as well as a lack of variation in F_{IS} values. This may be due in part to the co-occurrence of cluster 2 or cluster 3 in each of the sampled locations. There was only moderate genetic differentiation between sample locations to the north of Point Conception apparent from slightly elevated F_{ST} values between samples divided south of Neah Bay and south of Half Moon Bay (Table 9). The presence of a higher proportion of individuals assigned to cluster 3 included in the Morro Bay sample, reflect population structure already identified in the major break at Point Conception where cluster 3 was more common.

Table 8. Results of AMOVA analysis with six combinations separate groupings of *S. miniatus* samples corresponding to sample locations, North and South of Point Conception, North and South of Point Conception with the Outer Banks separated, groups resulting from STRUCTURE and BAPS cluster analyses with K = 2, K = 3.

Source of Variation	Variable	Sample Location	North and South of Pt. Conception	North of Pt. Conception vs. Outer Banks and Santa Barbara	Structure K=2	Structure K=3	BAPS K=2	BAPS K=3
Among Groups	d.f.	10	1	2	**1**	2	1	2
	Sum of Squares	78.4	46.7	52.6	**68.7**	87.1	44.6	82.4
	Variance Components	0.07	0.10	0.09	**0.15**	0.14	0.10	0.13
	Percentage of Variation	2.8	4.2	3.8	**6.0**	5.6	4.2	5.3
	F_{ST}	0.03	0.04	0.04	**0.06**	0.06	0.04	0.05
Within Groups	d.f.	449	458	457	458	**457**	458	457
	Sum of Squares	1066.6	1098.3	1092.4	1076.3	**1057.9**	1100.4	1062.6
	Variance Components	0.06	0.07	0.06	0.04	**0.03**	0.07	0.03
	Percentage of Variation	2.4	2.8	2.6	1.8	**1.1**	2.9	1.3
	F_{IS}	0.02	0.03	0.03	0.02	**0.01**	0.03	0.01

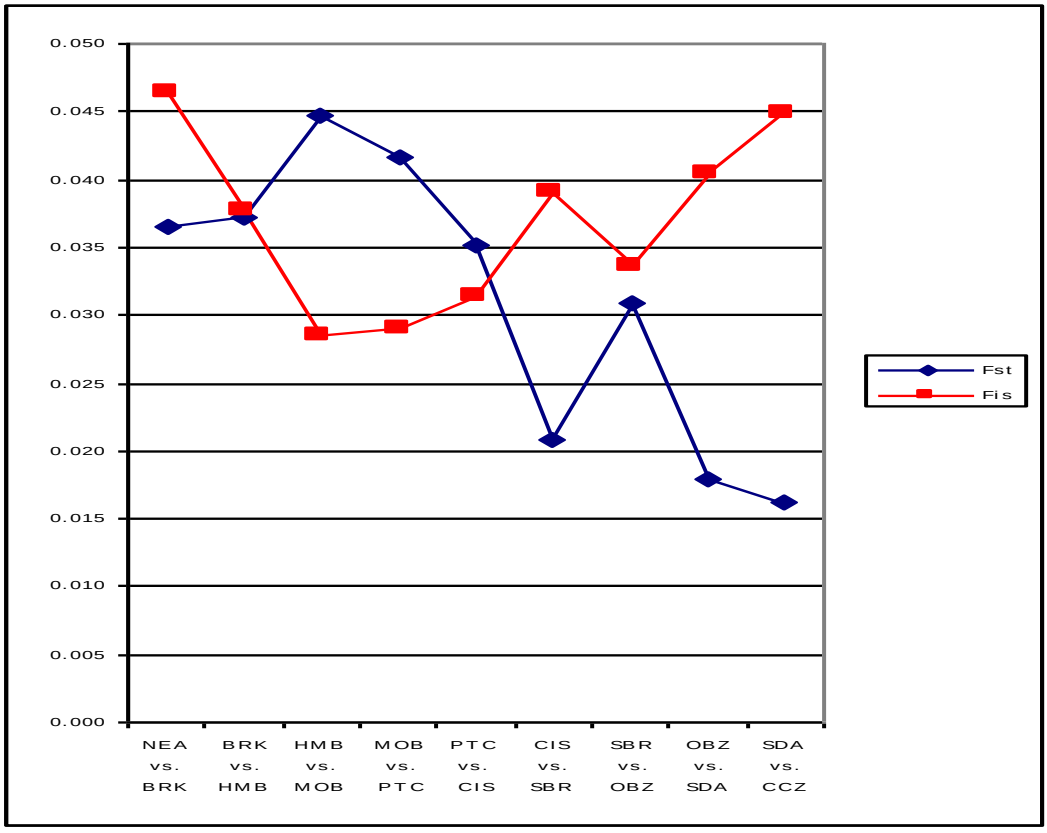

Figure 10. F_{IS} (red) and F_{ST} (blue) values from sequential groupings of populations north and south of sampled locations coastwide.

Table 9. F_{IS} values, F_{ST} values and percent variation between populations from AMOVA analysis from sequential groupings of populations north and south of sampled locations coastwide, north of Point Conception and south of Point Conception.

Region	North vs. South Populations	Percent Variation Between	F_{ST}	F_{IS}
Coastwide	NEA vs. BRK	3.58	0.0365	0.046
	BRK vs. HMB	3.71	0.0372	0.038
	HMB vs. MOB	**4.47**	**0.0448**	**0.029**
	MOB vs. PTC	**4.15**	**0.0416**	**0.029**
	PTC vs. CIS	3.51	0.0352	0.031
	CIS vs. SBR	2.07	0.0208	0.039
	SBR vs. OBZ	***3.08***	***0.0308***	***0.034***
	OBZ vs. SDA	1.78	0.0179	0.041
	SDA vs. CCZ	1.60	0.0162	0.045
North of Point Conception	NEA vs. BRK	1.27	0.0123	0.035
	BRK vs. HMB	0.68	0.0070	0.035
	HMB vs. MOB	**1.62**	**0.0166**	**0.036**
South of Point Conception	PTC vs. CIS	-0.19	-0.0017	0.023
	CIS vs. SBR	0.21	0.0021	0.022
	SBR vs. OBZ	0.06	0.0007	0.023
	OBZ vs. SDA	0.17	0.0017	0.022
	SDA vs. CCZ	**0.25**	**0.0025**	**0.022**

Discussion

Identification of population structure and resolution of conflicts with previous studies

Examination of patterns of population structure using Structure, BAPS and genetic differentiation as measured by F_{ST} provided clear indications of the degree of population structure in *S. miniatus*. Our study shows prominent population structure in *S. miniatus* in the vicinity of Point Conception. Additional population structure is present within the Southern California Bight based on depth and some structure identified in the north potentially a result of low sample size at Neah Bay. We determined that structure within the Southern California Bight is attributable to the presence of two populations with differing depth distributions. Here we review the results and compare them to those found in previous population genetic research conducted over a more limited geographic range or with other genetic markers.

Analysis of 15 microsatellites in limited sampling between Santa Barbara and Monterey, California by Sivasundar and Palumbi (2010) did not detect significant population structure within *S. miniatus*, potentially due to low sample size. Though we used fewer loci, the greater number of sample locations over a greater proportion of its range and at

higher spatial resolution allowed us to identify and delimit additional population structure. We detected strong population structure at Point Conception and within the Southern California Bight based on depth.

The strong break in population structure within *S. miniatus* in the vicinity of Point Conception observed in our study is consistent with analysis of mitochondrial cytochrome b by Hyde *et al.* (2009). Cluster analysis with BAPS and STRUCTURE identified three populations, with cluster 1 primarily distributed to the north of Point Conception and two populations south of Point Conception that appeared to be sympatric at the geographic resolution of 60 miles provided by *a priori* sampling. No significant population structure was identified between Half Moon Bay and Brookings with either microsatellites in our study or with mitochondrial DNA in Hyde *et al.* (2009), indicating that Cape Mendocino does not present a barrier to gene flow. Lesser differentiation within the northern population between Brookings and Neah Bay as indicated by elevated and significant F_{ST} values, is consistent with the results of Hyde *et al.* (2009), though sample sizes at Neah Bay were low in both studies due to infrequent encounters when sampling at this latitude.

Though *S. miniatus* is relatively uncommon north of California, its range extends more than 500 miles to the north of Neah Bay to Prince William Sound, Alaska. The additional population structure in cluster 1 between Brookings and Neah Bay may reflect adaptations to differential environmental conditions at the northern edge of its range including water temperature in the Aleutian biogeographic region (Gunderson and Vetter 2006). Analysis of samples from further north would provide conclusive evidence of the presence or absence of population structure at the northern edge of the range of *S. miniatus*. The relative absence of the species in the region in question makes further exploration of population structure of limited utility to management of the species as a whole. This may reflect that Neah Bay may be beyond the core reproductive range under the current environmental conditions.

Prior to assignment to clusters, elevated F_{ST} values were observed in comparisons to the Outer Banks, Santa Barbara and Cabo Colonet and other sample locations south of Point Conception. Similar population structure was identified south of Point Conception with cytochrome b (Hyde *et al.* 2009) and was hypothesized to be associated with biogeographic compartments identified by Longhurst (2007), though our analysis provides an alternative hypothesis. The proportion of individuals assigned to a given cluster at each location may be the cause of the population structure identified between sample locations south of Point Conception in Hyde *et al*. (2009). When individuals in each sample location were not assigned to cluster prior to analysis with microsatellites in our study there was a similar appearance of geographic population structure south of Point Conception to that observed with cytochrome b.

Comparisons of F_{ST} between sample locations for individuals assigned to differing clusters again indicated biologically meaningful population structure north and south of Point Conception. For comparisons of sample locations for cluster 1 to cluster 2 and cluster 3, strong structure exceeding both thresholds EV 3 and EV 4 appeared to center

around Point Conception. In comparisons between cluster 2 and cluster 3, significant population structure was only found at sample locations toward the center of their ranges south of Point Conception where higher sample sizes were available. In these comparisons, only the EV 4 (F_{ST} = 0.01) criterion for comparisons between sample locations is frequently exceeded indicating that there is more moderate, but biologically meaningful population structure between these locations suggesting more recent divergence, continued gene flow between clusters or misassignments between clusters carried forward in estimates of F_{ST}. The results were largely consistent with the distribution of individuals assigned to clusters using assignment methods.

After individuals were assigned to clusters, tests for genetic differentiation measured with F_{ST} between each sample location for individuals assigned to the same cluster resulted in low and non-significant estimates of F_{ST}. The lack of differentiation between sample locations south of Point Conception for individuals assigned to the same population indicates a high degree of gene flow through migration between sample locations. This indicates that population structure identified in cytochrome b may be the result of not having first assigned individuals in each population to their respective clusters. Thus unrecognized population structure at a finer spatial resolution exists in each sample location. This is reflected in the presence of individuals of each cluster identified using the program STRUCTURE in each location in our study. Differing proportions of individuals belonging to each cluster may have been sampled resulting in the observed population structure in Hyde *et al*. (2009), using cytochrome b. In actuality, population structure may be the result of differential depth distribution and structure identified may have been an artifact of the proportion of fish sampled at each depth with weak differentiation between individuals in each cluster between sample locations. Had individuals been assigned to cluster using microsatellites prior to analysis with cytochrome b, population structure may no longer be observed between individuals assigned to the same cluster in each population. Such an exercise might confirm significant and potentially biologically meaningful population structure between samples within a sample location identified by clustering of individuals using STRUCTURE and BAPS.

Correlation of population structure with depth

The clusters identified in STRUCTURE with K = 3 were significantly correlated with depth. The elevated and significant differentiation as measured by F_{ST} between individuals assigned to different clusters south of Point Conception indicates the potential for secondary contact of previously isolated non-interbreeding populations with adaptations to differential depths or associated temperatures. Adults of cluster 2 occurred primarily at depths less than 30 fm and adults of cluster 3 at depths primarily between 10-50 fm. Alternatively, differentiation may have arisen through parapatric isolation of adults by depth, as proposed by Hyde *et al.* (2008) as a mechanism for divergence between *S. miniatus* and its cryptic sister species *S. crocotulus*. The correlation of each cluster with depth results in population structure at a higher resolution than the 60 mile by 60 mile sampling locations. Apparent population structure between *a priori* sample locations may be an artifact of differential sampling of depths at each sample location. This emphasizes the need to account for depth as well as latitude and longitude in

analyses of population structure in the *Sebastes* when the subject species is distributed over a broad depth range.

As noted in Hyde *et al.* (2009), parturition in *S. miniatus* primarily occurs in fall and early winter with a peak month in November during the weakest alongshore flow and upwelling in the California Current. The weak poleward flow at the time of parturition may reduce gene flow and contribute to increased population structure in *S. miniatus* compared to its congeners that release larvae during peak upwelling in the spring promoting greater gene flow along the coast in these species. The correlation of cluster 2 and cluster 3 with different depths may reflect secondary contact of previously isolated populations previously occupying different latitudes and adapted to different depths as hypothesized by Hyde *et al.* (2008). Isolation of adults by depth, differences in mating behavior or timing of reproduction may provide complete or partial barriers to mating on secondary contact. Alternatively, population structure may be due to hybridization with *S. crocotulus* resulting in cluster 3 or infrequent hybridization, a subject for further research.

Tests for co-occurrence of population structure with locations expected to pose barriers to gene flow

The AMOVA conducted by Hyde *et al.* (2009) with individuals from Kyoquot Sound, Canada to San Quintin, Mexico reported significant partitioning of genetic variance across the biogeographic boundary at Point Conception with additional barriers at Cape Mendocino, Punta Colonet, Santa Monica Bay and along the coast of Washington. Hyde *et al.* (2009) interpreted the patterns observed in the AMOVA analysis to indicate population structure consistent with geographic compartments along the California Current Province noted in Longhurst (2007). Our results indicate that population structure observed south of Point Conception is likely due to the presence of population structure with depth between cluster 2 and cluster 3. Sampling of different proportions of individuals from each cluster at each sample location may be due to the depth of sampling efforts contributing to the appearance of population structure at Santa Monica Bay and Punta Colonet observed in mtDNA while both clusters may occur in each location in actuality. We did not identify significant population structure between Half Moon Bay and Brookings on either side of Cape Mendocino, which was also the case with cytochrome b as indicated by low Phi_{ST} values (Hyde *et al.* 2009); despite this, Cape Mendocino was still identified as a barrier in that study. Genetic differentiation measured by $\mathbf{F_{ST}}$ identified population structure between Neah Bay and Brookings as was also the case with cytochrome b (Hyde *et al.* 2009), though sample sizes at Neah Bay were low in both studies. This would suggest that population structure is not consistent with the compartments identified by Longhurst (2007).

Samples north of Point Conception are primarily assigned to cluster 1 in STRUCTURE and BAPS in both K = 2 or K = 3 models. The biogeographic break and associated differential water temperatures at Point Conception may pose a persistent barrier to effective migration or larval dispersal with selection acting to prevent successful recruitment across biogeographic and temperature regimes. The population structure reflected in cluster 1 and 2 across Point Conception may in part be a result of differential

adaptation to disparate water temperature regimes or other ecological parameters in each biogeographic province. Genetic differentiation at the northern edge of the distribution of *S. miniatus* at Neah Bay may reflect selection or isolation due to the divergence of the northward flow of the Alaskan Current off of Northern Washington and southward flow of the California Current at lower latitudes or be due to random sampling error. The timing of parturition in *S. miniatus* coincides with a lull in the upwelling during the fall and winter allowing larval dispersal (Longhurst 2007), which may prevent larval dispersal across Point Conception. Effective migration of adults unaffected by advection between regions may not be sufficient to prevent genetic drift due to isolating mechanisms such as breeding behavior.

Implications for fishery management

Considerations for stock assessment

The presence of population structure identified within *S. miniatus* has a number of implications for fishery management. The prevalence of separate populations of *S. miniatus* north and south of Point Conception justifies independent stock assessment and catch accounting in each region. The majority of the individuals sampled north of Point Conception were assigned to the northern population in cluster 1 allowing assessment and management of this population separately from the other two clusters of *S. miniatus* and *S. crocotulus,* which complicate assessment to the south. Historically, the catch south of Point Conception was identified as *S. miniatus,* but could have been members of three genetic groups. This presents difficulties in conducting accurate assessments of each. The contribution of each to removals and indices of abundance used to measure trends in population size would need to be determined to facilitate assessment of individual populations or species. If such differences cannot be resolved, assessments may have to be conducted at a complex level combining the three populations as well *S. crocotulus* south of Point Conception. Current management using depth restrictions to avoid contact with overfished species such as cowcod may place disproportionate fishing effort on *S. miniatus.* This presents a disconnect between the scale of the assessment based on a complex relative to the removals of any one species or population with implications for sustainable harvest without precautionary measures.

Given the differential depth distribution of cryptic species south of Point Conception the proportion of catch by depth could potentially be used to apportion historical catch south of Point Conception. Such data may not be available for sectors that historically harvested substantial quantities of "vermilion rockfish" or a portion of the catch time series. Fluctuations in recruitment could cause the proportion of each species or population taken in the fishery to vary through time. In addition, as noted in *Hyde et al.* (2008) and confirmed in our study, depth restrictions have redistributed fishing effort into shallower depths, away from adult *S. crocotulus,* which is predominantly distributed in depths greater than the current depth restrictions and onto *S. miniatus* and juvenile *S. crocotulus* in waters less than the 60 fm depth restriction. This may have increased the proportion of *S. miniatus* in the catch since depth restrictions were implemented in 2000 and cause differential status of each species in terms of depletion.

Inability to discriminate between species and populations in historical catch and trends in abundance would potentially prevent accurate assessment of each stock. The question still remains as to whether the cryptic species or populations of *S. miniatus* have different growth rates, age at maturation have experienced similar exploitation history and whether they have similar recruitment patterns. These life history characteristics would potentially affect the respective demographic trajectories of each stock and thus the status, trend and scale resulting from stock assessments, affecting the ability to accurately assess stocks as a complex. Should the catch histories or indices of abundance not be stratified to be representative preventing individual assessment of stocks and stocks be found to have differential life history characteristics, this would pose a potential error in assessment of a complex; this would present a potential impasse or require buffers sufficiently large to address uncertainty and prevent overharvest.

Potential changes to spatial boundaries and composition of stock complexes
Species within the Groundfish Fishery Management Plan are divided into nearshore, shelf, and slope rockfish stock complexes or are managed outside of complexes as individual stocks if assessed as overfished or management outside the complex reduces the chances overfishing a component species in the stock complex. The complexes are further divided north and south of 40°10' N. latitude off of Cape Mendocino, California. Our study did not identify population structure at Cape Mendocino and would suggest a differential scheme of management lines might better align with the geographic distribution of *S. miniatus*. The vermilion rockfish *S. miniatus* is currently included in the minor shelf rockfish complex both north and south of 40°10' N. latitude, prior to the discovery of its cryptic sister species *S. crocotulus*. The predominant depth distribution of *S. miniatus* in less than 50 fm as revealed Hyde *et al.* 2008 and further elucidated in this study indicates that its depth distribution is more similar to species included in the nearshore rockfish complex, to which it theoretically should be reassigned, while *S. crocotulus*, which occupies deeper depths would remain in the shelf rockfish complex.

Assessment could be stratified at Point Conception, 34°27' N. latitude, to manage the northern cluster 1 population apart from southerly shallow cluster 2 and deep water cluster 3 populations as well as *S. crocotulus*. Thus implementation of a second management line at 34°27' N. latitude at Point Conception would better align regulation of take through differential management measures and trip limits with the population structure identified. Removing *S. miniatus* from the minor shelf rockfish complex with *S. crocatulus* may pose some management issues as catch tracking cannot be undertaken to discriminate between populations within *S. miniatus* or *S. crocotulus* to the south of point Conception. To the north of Point Conception, *S. miniatus* could be removed from the complex and given a separate annual catch target to keep catch within sustainable levels. Given the predominant distribution of adult *S. crocotulus* in depths greater than 50 fm, hypothetically it should be assigned to the shelf rockfish complex south of 40°10' N. latitude. Given the inability to differentiate between *S. miniatus* and *S. crocotulus* south of point Conception, unless this and other issues with historical catch and indices of abundance allowing individual assessment of each stock can be resolved, they may need to remain in a the minor shelf rockfish complex. To the south they could be assessed and managed as a complex and an ACT set based on the assessment to the north to manage

cluster 1 within sustainable levels to avoid complications with the current management scheme.

Considerations for the nearshore permitting system

Identification of *S. miniatus* as a nearshore species may pose potential complications for the current management context within which the commercial fishery in California manages nearshore species. At present there are shallower and deeper nearshore permits within limited access program. Currently as a shelf rockfish, the open access fishery can retain what is referred to as vermilion rockfish presumed to be *S. miniatus* shoreward of the non-trawl rockfish conservation area line. Were vermilion rockfish to be redesignated as a nearshore rockfish, this may exclude the open fishery from fishing for retaining them, while providing exclusive access to holders of the deeper nearshore permit holders. While this may present a potential means of curtailing take of vermilion rockfish, it would pose complications to management that may take years to resolve. In addition, the presence of *S. crocotulus* as a shelf rockfish and the inability to differentiate between them in the catch poses an issue in management and enforcement unless simpler means of discriminating between the two species are identified. Although the caudal peduncle depth and eye diameter to length ratio and number of gill rakers have been identified as means to differentiate between species, these are not readily employed by fishermen in the field and more easily applied characteristics need to be identified to facilitate identification in the field in addition to potential issues discussed above regarding individual assessment and composition of stock complexes before differential management could be implemented.

Overarching considerations for management and future research

While identification of population structure is essential for proper stock assessment and fishery management, dealing with the ramifications of the findings may not be straight forward. As identified in our study, cryptic species and population structure can pose a number of complications. Our study points to the need to collect data not only on the location of sampled individuals included in analysis, but also the depth of capture as population structure can occur on a finer resolution than the areas used in groupings identified as putative populations due to the potential for bathometric isolation of adults. Use of clustering methods can be used to identify such otherwise latent population structure and depth of capture can be used to test for correlation of depth of capture with assignment to test for such structure.

Ancillary data on meristic counts or morphometic data garnered from specimens or their photographs that might assist in identification of identification characteristics that would aid in later visual discrimination of populations or cryptic species. Alternatively, directed sampling to obtain samples that can later be genotyped and analyzed for such characteristics can be undertaken to minimize effort in the field at the time of collection. In addition, collection of otoliths, lengths and ovaries from a number of individuals gentotyped to be from each respective grouping can be analyzed to determine whether significant differences in growth or age at maturation or recruitment patterns are apparent, though such an effort may require significantly higher sample sizes. Future

research to find identifying characterstics and test for differences in life history are a high prioritity for future research to inform field sampling and assessment.

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Phylogeography of the Subgenus *Rosicola* and Analysis of Processes contributing to Population Structure and Speciation

Abstract

To better understand the evolutionary processes contributing to speciation and population structure in the subgenus *Rosicola*, we carried out a number of genetic analyses using six microsatelite loci applied to samples of *S. miniatus* (Jordan and Gilbert 1880), *S. crocotulus(Hyde et al. 2008)* and *S. pinniger* (Gill 1864) collected from the Queen Charlotte Islands, Canada to San Martin Island Mexico. We found a significant effect of mutation on genetic differentiation among the three species within the *Rosicola*, but not to differentiation among three clusters within *S. miniatus* detected using the program STRUCTURE, suggesting deep divergence among species, and recent divergence among clusters within *S. miniatus*. This topology is consistent with that observed in Hyde *et al.* (2008) with deeper divergence between *S. pinniger* and *S. crocotulus* (2.7 MYA, Hyde et al. 2008), followed by divergence of *S. crocotulus* and *S. miniatus* (2.3 MYA). Phylogeographic analysis of Cavalli-Sforza chord distance indicates that individuals in each population assigned to the three genetic clusters of *S. miniatus*, form reciprocally monophyletic groups, indicating cohesion of individuals assigned to each cluster between sample locations supporting evidence of population structure.

Timing of divergence between *S. pinniger* and *S. crocotulus* was used to calibrate results of the timing of divergence from a coalescent model of an evolutionary scenario in DIY ABC for the three distinct clusters of *S. miniatus* that were identified with the program STRUCTURE. The model used in estimating divergence time supported the most recent divergence between *S. miniatus* clusters 1 and 2 from a common ancestor that diverged earlier from *S. miniatus* cluster 3, with both divergent events taking place during the late and mid Pleistocene, respectively. This is consistent with theories that populations inside and outside the Channel Islands became isolated during glacial periods and expanded northwards in the interglacial period with potential for divergence and subsequent speciation through founder events and differential selection pressures outside of glacial refugia.

The lower genetic diversity in the northern cluster 1 of *S. miniatus* and support for a model reflecting population size reduction may be the result of northward expansion, though no bottleneck was detected in any of the populations or species. The elevated diversity of the shallow southerly distributed cluster 2 indicates a more stable population that may be distributed in more southerly refugia during glaciations. No hybridization was detected, suggesting a lack of interbreeding on secondary contact. Further analyses with additional loci and approaches may bring further clarity to the speciation processes and evolutionary forces that contributed to the formation of population structure in the *Rosicola* and the higher diversity observed in the genus *Sebastes* in the North-Eastern Pacific as a whole.

Introduction

The North-Eastern Pacific is known for high biodiversity in numerous marine taxa, thought to be the result of a number factors including: 1) geomorphology interacting with sea level changes during glaciations affecting currents, 2) contemporary variation in water temperature and productivity across latitudes, 3) a period of protracted upwelling allowing differential timing of successful reproduction, 4) numerous bays and estuaries supporting natal homing and divergence of estuarine species and 5) exchange of species within the taxonomically diverse West Pacific during interglacial periods (Jacobs *et al.* 2009). Of the four representative genera of the family *Scorpaenidae* residing in the North-Eastern Pacific, the *Sebastes* are by far the most specious with at least 69 identified species from the Aleutian Islands to Mexico (Love *et al.* 2002). *Sebastes* diversity in this region is the greatest world wide, despite the genus having originated in the Western Pacific near Japan (Hyde and Vetter 2007).

Life history characteristics of the genus *Sebastes* may contribute to their high diversity including: 1) internal fertilization allowing mate selection that is exhibited in courtship behavior, 2) relatively low adult movement making larval dispersal distance the primary driver of migration and 3) the apparent associations with restricted ranges of latitude and depth (Gunderson and Vetter 2006, Hyde and Vetter 2007, Love *et al.* 2002). Though evidence of hybridization between species in the *Sebastes* has been identified between three species in Puget Sound (Seeb *et al. 1998*), many *Sebastes* have been observed to exhibit species specific courtship behavior that may pose a barrier to mating between sister species after secondary contact (Love *et al.* 2002). In addition, differential timing of breeding, depth distribution of adults or adaptation to environmental variables affecting latitudinal distribution may help maintain isolation (Gunderson and Vetter 2006). Despite sharing these characteristics, species diversity is far lower in other parts of the world (Love *et al.* 2002, Hyde and Vetter 2007), suggesting that the geomorphology or environmental conditions in the North-Eastern Pacific contribute to greater rates of speciation.

There is no evidence of secondary invasion from the Western Pacific Ocean during recent interglacial periods (Hyde and Vetter 2007, Li *et al.* 2006, Johns and Avise 1998). The most complete phylogenetic analysis of the genus *Sebastes* showed that eastern and western Pacific species are monophyletic indicating no secondary exchange of species from the North-Western Pacific Ocean since the original introduction during the middle Miocene approximately five million years ago (Hyde and Vetter 2007). Hyde and Vetter (2007) proposed that speciation occurs as species re-occupy habitat after glacial retreat, or by vicariant isolation processes on smaller geographic scales due to isolation through sea level change resulting in barriers formed by the Channel Islands and alteration of oceanic currents.

Most sister species within the *Sebastes* are distributed in close geographic proximity (Hyde and Vetter 2007) and differences in the distribution of sister species within a subgenus are associated with latitude and depth *(*Li *et al.* 2006, Narum *et al.* 2004 Cope 2004, Burford and Bernardi 2008, Hyde *et al.* 2008, Gharett *et al.* 2005). Within the subgenus *Pteropodus*, *S. carnatus* is distributed farther to the north and in deeper water

than its sister species *S. chrysomelas* (Narum *et al.* 2004). In the subgenus *Sebastosomus*, *S. entomelas* is found at deeper depths and further north than *S. mystinus,* while within *S. mystinus,* a second recently discovered cryptic species is distributed further north and in deeper depths than the other with a transition zone near Cape Mendocino (Cope 2004, Burford and Bernardi 2008, Frable *et al.* 2015). The repeated spatial pattern of differing depth and latitude distribution of sister taxa and populations may point to common recurring mechanisms of speciation across sub-genera (Burford and Bernardi 2008). Such patterns are also observed in the subgenus *Rosicola* (Hyde *et al.* 2008). The three species within the subgenus *Rosicola* have variable latitudinal ranges from Alaska to Isla San Benito, Baja California extending across Alaska, Oregonian and San Diegan biogeographic regions. Their depth distributions are associated with ontogenetic shifts from shallow reefs to both the nearshore waters within 50 fm (100 m) in the case of *S. miniatus* and across the shelf for *S. crocotulus*, to depths reaching 200 fm (400m) for *S. pinniger* as adults (Love *et al.* 2002).

Cytochrome b sequence data and coalescence modeling indicate deepest divergence in the subgenus *Rosicola* dates to the Miocene 2.7 million years ago (MYA) between *S. pinniger* and the most recent common ancestor of *S. crocotulus* (Hyde *et al*. 2008). Secondary divergence of *S. miniatus* from a recently identified cryptic species *S. crocotulus* was dated at 2.3 MYA (Hyde *et al*. 2008). Paedomorphosis, defined as speciation through the loss of a trait, was proposed by Hyde *et al*. (2008) as the mode of speciation between *S. miniatus* and *S. crocotulus* through truncation of the adult depth distribution in the most recent common ancestor of the two species. Sea level decline and barriers to gene flow during glacial maxima as well as increased upwelling during this period have been implicated in the speciation process leading to divergence (Hyde and Vetter 2007, Hyde *et al.* 2008). Though this theory is consistent with the current depth distribution of the two species in the Southern California bight, where they co-occur, there is still a question of why this difference would arise. Speciation mechanisms leading to the differential depth and latitude distribution of the sister species may be evident in the phylogeography of sister species and population structure within species.

The depth and timing of divergence between the three recognized species was examined in our collaborative research in Hyde *et al.* (2008), but the pattern of population structure that we have detected within *S. miniatus* provides additional perspectives on micro-evolutionary processes within the genus *Sebastes* as a whole. The results of the spatial analysis of population structure in the preceding chapters provide a starting point for posing and answering questions regarding the evolutionary processes driving speciation and population structure in the subgenus *Rosicola*. The pattern of population structure raises questions regarding demographic history of the populations, which we test using the same six microsatelites applied to all species.

Of the theories regarding speciation, we focus particular attention on tests for evidence of recent speciation consistent with Pleistocene glaciations. Changes in sea level during glaciations may be driving vicariance in species distributed within the Southern California Bight resulting from expansion of the Channel Islands with decline in sea level followed by northward expansion during interglacial period and subsequent speciation

within the genus *Sebastes* (Hyde and Vetter 2007, Li *et al.* 2006, Johns and Avise 1998, Gunderson and Vetter 2006, Mangel *et al.* 2007, Steffanson *et al.*2009). Thus we explore the possibility that the observed structure reflects a repeated pattern of speciation associated with sea level decline during glacial maxima and northward expansion during interglacial periods with isolation from refugia and/ or selection leading to divergence. We hypothesize that estimates of time of divergence of populations within *S. miniatus* will coincide with more recent glacial cycles during the Pleistocene. This would substantiate the hypothesis of Hyde *et al.* (2008) that isolation of populations inside and outside of the Channel Islands as a result of decreased sea level at glacial maxima resulted in multiple vicariance and northward expansion events within the *Sebastes*.

To provide context for further consideration of speciation mechanisms, population structure of other species analyzed within the *Rosicola* was evaluated to elucidate the population structure the subgenus and the magnitude of genetic differentiation between species and populations. Tests for significant contribution of mutation to genetic differentiation between species and populations were conducted to test the hypothesis that mutation would contribute significantly to differentiation among species, but not among clusters assumed to reflect more recently diverged populations within *S. miniatus*. We analyzed the phylogeography of the subgenus *Rosicola* to provide a basis for discerning phylogenetic relationships in the context of their geographic distributions to test the hypothesis that individuals of each species and genetic clusters reflecting populations within *S. miniatus* would be nearer to one another in the topology supporting reciprocal monophyly among them and that more proximate populations would be more closely associated in terms of genetic distance. Using phylogeographic relationships as a starting place we conducted further analysis of time since divergence, migration rates and historical population sizes to test the hypothesis that differentiation originated during Pleistocene glacial maxima.

In addition, the following analyses of demographic processes were undertaken to identify phenomena contributing to population structure or maintaining differentiation resulting from prior isolating mechanisms. Measures of genetic diversity were examined to identify areas with higher allelic diversity and heterozygosity, with the hypothesis that the southern portions of populations range will have higher diversity indicative of probable centers of distribution with stable population sizes. Inbreeding coefficients were estimated to test for mating between related individuals to test the hypothesis that the northern extent of each species will have higher inbreeding coefficients consistent with founder events. Tests were conducted for heterozygote excess indicative of recent severe reductions in population size or "bottlenecks" in each population or species to test the hypothesis that sample locations at the northern extent of the range will reflect a bottleneck consistent with founder effects. We hypothesize that private alleles will be more common at the edges of the species range indicative genetic drift or mutations potentially associated with adaptation to local conditions after generations of isolation. Tests were made for hybridization or introgression between species or populations in the *Rosicola* to test the hypothesis that no interbreeding is occurring on secondary contact. The overall intent of these analyses is to provide a broader understanding of the micro-

evolutionary forces shaping the rapid radiation of species in the *Sebastes* of the North-Eastern Pacific.

Our results are interpreted in light of proposed speciation mechanisms in the genus *Sebastes*, the current understanding of the phylogenetic relationships and population structure within the subgenus *Rosicola* and evidence of recent speciation through Pleistocene vicariance within the genus *Sebastes* in other studies (Hyde and Vetter 2007, Li *et al.* 2006, Johns and Avise 1998, Gunderson and Vetter 2006, Mangel *et al.* 2007, Steffanson *et al.* 2009). The timing and extent of divergence at the species and population level within the subgenus *Rosicola* was compared to that in other sub-genera of the *Sebastes* to identify concordance supporting common recent speciation mechanism (Burford and Bernardi 2008, Li *et al.* 2006, Danielsdottir 2008, Rocha-Olivares and Vetter 1999). Thus we examine whether the interaction of the geomorphology of the California coast and changes in sea level related to climate change during glacial and interglacial periods may have contributed to the diversity of the *Sebastes* observed in the vicinity of Point Conception and the higher diversity in the North-Eastern Pacific as a whole.

Methods

Sample Collection, DNA Extraction, DNA Amplification and Data Quality Tests

Sample collection, DNA extraction, DNA amplification and data quality tests were undertaken following the methods described in the first chapter. While *S. pinniger* were easily identified to species visually, cluster analysis of individuals identified as *S. miniatus* using STRUCTURE was conducted to identify individuals of its cryptic sister species *S. crocotulus* for further analysis. These efforts yielded 189 *S. crocotulus*, 461 *S. miniatus* and 209 individuals of *S. pinniger* for analysis. A chart of the sampled locations including the number of samples collected at each site is provided in Figure 1. For each of the sampled locations, the location name, state/country, three digit alpha code for the population used in further analyses, average latitude and longitude, number of samples are provided in Table 1. All samples were collected after fish were deceased, having being caught by licensed anglers or permitted sampling programs occurring prior to accessessing tissue samples for our study and were thus exempt from UC Berkeley Animal Care and Use Committee Protocols per discussion with a representative. Tissue samples and extracted DNA are available for confirmation of our results or further analysis.

Table 1. Name of sampled putative populations, alpha codes for sampled locations, number of sampled individuals and average latitude and longitude of capture for each species and sample location.

Species	Sample Location	State / Country	Alpha Code	Individuals	Latitude	Longitude
S. pinniger	Queen Charlotte Islands	Canada	QCI	68	49.065	-126.403
	Grays Harbor	Washington	GHB	22	46.353	-124.644
	Brookings	Oregon	BRK	45	42.139	-124.334
	Fort Bragg	California	FTB	32	39.599	-123.914
	Half Moon Bay	California	HMP	42	37.583	-122.742
S. miniatus	Neah Bay	Washington	NEA	15	48.367	-124.629
	Brookings	Oregon	BRK	69	42.044	-124.273
	Half Moon Bay	California	HMB	74	37.502	-122.481
	Morro Bay	California	MOB	23	35.358	-120.868
	Point Conception	California	PTC	28	34.577	-120.647
	Channel Islands	California	CIS	35	34.010	-120.292
	Santa Barbara	California	SBR	33	34.398	-119.722
	Outer Banks	California	OBM	9	32.517	-119.115
	San Diego Adults	California	SDA	78	32.664	-117.242
	San Diego Juveniles	California	SDJ	33	32.664	-117.242
	Cabo Colonet	Mexico	CCO	64	30.962	-116.330
S. crocotulus	Outer Banks	California	SDI	32	32.790	-117.259
	San Diego	California	OBI	54	32.711	-119.090
	Cabo Colonet	California	CCI	103	30.745	-116.258

Figure 1. Chart of the sample locations and the number of individuals sampled for each species, *S. miniatus* (black), *S. crocotulus* (blue) and *S. pinniger* (green).

Population structure in the subgenus Rosicola informative of the sequence and rooting structure of phylogenetic relationships

Population structure across all species within the *Rosicola* was evaluated using the program STRUCTURE (Pritchard 2000) and Bayesian Analysis of Population Structure BAPS2 (Corrander 2004). Both algorithms were analyzed from zero to five clusters (K = 5) to elucidate the population structure and degree of shared ancestry among species in the subgenus assuming three clusters within *S. miniatus* identified in analyses conducted in the preceding chapter. The proportion of ancestry of each individual assigned to each species was used to assign individuals to a cluster or population and the resulting groupings analyzed in the program STRUCTURE with K = 5 to test for evidence of shared ancestry indicative of incomplete lineage sorting, hybridization or introgression.

Genetic differentiation measured by F_{ST} between species and groups of individuals assigned to clusters in STRUCTURE assumed to reflect populations of *S. miniatus* were estimated and compared to determine the relative depth of differentiation between species and populations. Genetic differentiation estimated by F_{ST} measures differences in allelic identity, as compared to R_{ST}, which also accounts for differences in the size of alleles due to mutation. In combination with the results of phylogeographic analyses, the results of these analyses provide a foundation for structuring evolutionary scenarios in DIY ABC to make inferences about population history using approximate Bayesian computation (ABC).

Tests for significant contribution of mutation to differentiation in the subgenus Rosicola

Tests for significant contribution of mutation to genetic differentiation between *S. crocotulus*, *S. miniatus* and *S. pinniger* were carried out in Spage DI (Hardy and Vekmans 2002), by comparing R_{ST} with permuted R_{ST} (ρR_{ST}). By permuting alleles, ρR_{ST} becomes equivalent to F_{ST} estimated from unordered alleles. Spage DI calculates the significance of differences between R_{ST} and ρR_{ST} and therefore allows a test of whether differentiation due to mutation leading to the ordered sequence of alleles is significantly greater than differentiation due to drift alone. Permutation tests were also carried out within species including clusters identified in *S. miniatus*.

Phylogeography of the subgenus Rosicola

The Cavalli-Sforza chord measure of genetic distance (Cavalli-Sforza and Edwards 1967) was calculated with the microsatellite data and visualized with Unweighted Pair Group with Arithmetic Mean (UPGMA) methods in the program Phylip (Felsenstein 1973). To evaluate the phylogeography of species in the subgenus *Rosicola* and three clusters within *S. miniatus* identified in STRUCTURE, the optimal tree topology of groups of individuals assigned to species or clusters in each sample location was examined. Samples assigned to each cluster were aggregated within sampled locations to test for reciprocal monophyly among groups of individuals assigned to a cluster in each sample location. The same analysis was conducted at a species level as well as with samples assigned using the STRUCTURE results for K = 5 to include three groups within *S. miniatus* to examine whether assignment appreciably changed tree topology and apparent phylogeography of the subgenus.

Evaluating models of evolutionary scenarios for the subgenus Rosicola and tests for consistency of timing of divergence between populations of S. miniatus with Pleistocene glacial maxima.

One focus of our study is to determine the historical timing of divergence of populations of *S. miniatus* to test for consistency with the hypothesis of speciation through vicariance during glaciations (Hyde and Vetter 2007). We hypothesized that abrupt high magnitude changes in sea level that accompanied multiple glaciations in the Pleistocene and early Holocene may have contributed to recent population structure within *S. miniatus*. To test this hypothesis we used Approximate Bayesian Computation (ABC) in the program DIY ABC (Cornuet *et al.* 2014) to evaluate alternative models of population history.

In practice, the ABC approach can be summarized in three successive steps (Excoffier *et al.* 2005): i) generating simulated data sets, ii) selecting simulated data sets closest to observed data set and iii) estimating posterior distributions of parameters through a local linear regression procedure. We tested evolutionary scenarios to identify which was the most likely according to the ABC criteria by simulating one sample summary statistics including mean number of alleles, mean genetic diversity and mean size variance as well as three sample summary statistics of F_{ST}, classification index and $\delta\mu^2$.

Each scenario includes a historical model describing how the sampled populations are connected to their common ancestor and a mutational model describing how allelic states of the studied genes are changing along their genealogical trees. Two measures of posterior probabilities of scenarios were employed for comparing different models (hereafter named scenarios) that can explain observed data. The first measure is simply the relative proportion of each scenario in the simulated data sets closest to observed data sets (Miller *et al.* 2005, Pascual *et al.* 2007). The second measure is obtained by a logistic regression of each scenario probability on the deviations between simulated and observed summary statistics (Fagundes *et al.* 2007, Beaumont 2008). To assess precision of parameter estimation for time of divergence and effective populations, we computed the relative median of the absolute error (RMAE) on 500 pseudo-observed data sets simulated under the best-fit scenario (Cornuet *et al.* 2014). The Type 1 and Type 2 error rates were also measured for each Scenario were also estimated to assess the confidence in each scenario.

A number of models explored combinations the order of divergence of the three clusters of *S. miniatus* identified in structure alone and with the other two species in the subgenus *Rosicola*. This included 1.) alternation of *S. crocotulus* and *S. pinniger* as most basal species, 2.) various combinations of the order of divergence of *S. miniatus* clusters relative to one another and 3.) models exploring admixture between *S. miniatus* populations as well as 4.) fluctuations in effective population size after divergence 5.) models lacking admixture of fluctuations in effective population size after divergence. The topology and sequence of divergence identified through preliminary analyses as having the highest posterior priority and logistic regression results, was analyzed further. The first scenario evaluated the divergence of species and populations without severe reduction in population size or admixture, which would reflect differentiation of continuously distributed most recent common ancestor, consistent with divergence

through selection or isolating mechanisms driving genetic drift. The second scenario reflected severe reduction in population size after divergence indicative of potential founder events with small populations isolated outside the Channel islands during a glacial period or founder populations expanding northward in an interglacial period. The third reflects stable population size in all populations with admixture between the northern clusters 1 and southern deep cluster 3 of *S. miniatus* resulting in cluster 2. The analogous analyses were carried out for the subgenus as a whole and the three clusters of *S. miniatus* alone. Parameters used in the analysis are provided in Table 2 below.

For the three scenarios with the highest posterior probability, the time of divergence between species and structure in *S. miniatus* were estimated and compared to the timing of declines in sea level during the Pleistocene glaciation. The timing of divergence between *S. crocotulus* and *S. pinniger* 2.7 MYA from Hyde *et al.* (2007) was used to calibrate the estimate of timing with an assumption of 10 years mean generation time for *S. miniatus* (Love *et al.* 2002*).* The number of generations between species divergence times was estimated for each scenario and timing since divergence was calibrated against the ratio of the number of years estimated for *S. crocotulus* and *S. pinniger* relative to the 2.7 MYA time since divergence estimated with mtDNA from Hyde *et al.* (2007). Timing of divergence consistent with major changes in sea level associated with glacial maxima would support vicariance resulting from the barrier to gene flow posed by the Channel Islands and subsequent genetic differentiation could contribute to divergence. We hypothesize that the estimation of the timing of genetic divergence of this population will reflect a Pleistocene origin consistent with the hypothesis of isolation and subsequent divergence during glacial maxima or interglacial periods.

Table 2. Parameter/Prior/Assumptions used in modeling scenarios for analysis in ABC-DIY.

Parameter/ Prior/ Assumption	Scenario 4	Scenario 10	Scenario 8	Scenario 6	Scenario 2
Mean Mutation Rate	Uniform: 1E-004 - 1E-003	Uniform: 1E-004 - 1E-003	Uniform: 1E-004 - 1E-003	Uniform: 1E-004 - 1E-003	Uniform: 1E-004 - 1E-003
Individual locus mutation rate	Gamma: 1E-005 - 1E-002	Gamma: 1E-005 - 1E-002	Gamma: 1E-005 - 1E-002	Gamma: 1E-005 - 1E-002	Gamma: 1E-005 - 1E-002
Mean Coefficient P	Uniform: 1E-001 - 1E-001	Uniform: 1E-001 - 1E-001	Uniform: 1E-001 - 1E-001	Uniform: 1E-001 - 1E-001	Uniform: 1E-001 - 1E-001
Individual locus coefficient P	Gamma: 1E-002 - 1E-001	Gamma: 1E-002 - 1E-001	Gamma: 1E-002 - 1E-001	Gamma: 1E-002 - 1E-001	Gamma: 1E-002 - 1E-001
Mean SNI Rate	Log-u: 1E-008 - 1E-005	Log-u: 1E-008 - 1E-005	Log-u: 1E-008 - 1E-005	Log-u: 1E-008 - 1E-005	Log-u: 1E-008 - 1E-005
Individual locus SNI Rate	Gamma: 1E-009 - 1E-004	Gamma: 1E-009 - 1E-004	Gamma: 1E-009 - 1E-004	Gamma: 1E-009 - 1E-004	Gamma: 1E-009 - 1E-004
Effective Population Size	Uniform: 10 - 10000	Uniform: 10 - 10000	Uniform: 10 - 10000	Uniform: 10 - 10000	Uniform: 10 - 10000
Time in Generation	Uniform: 10 - 10000	Uniform: 10 - 10000	Uniform: 10 - 10000	Uniform: 10 - 10000	Uniform: 10 - 10000
Admixture Coefficient	Uniform: 0.001 - 0.999	Uniform: 0.001 - 0.999	Uniform: 0.001 - 0.999	Uniform: 0.001 - 0.999	Uniform: 0.001 - 0.999
N1	Uniform: 10 - 10000	Uniform: 10 - 10000	Uniform: 10 - 10000	Uniform: 10 - 10000	Uniform: 10 - 10000
N2	Uniform: 10 - 10000	Uniform: 10 - 10000	Uniform: 10 - 10000	Uniform: 10 - 10000	Uniform: 10 - 10000
N3	Uniform: 10 - 10000	Uniform: 10 - 10000	Uniform: 10 - 10000	Uniform: 10 - 10000	Uniform: 10 - 10000
N4	Uniform: 10 - 10000	Uniform: 10 - 10000	NA	NA	NA
N5	Uniform: 10 - 10000	Uniform: 10 - 10000	NA	NA	NA
t1	Uniform: 10 - 10000	Uniform: 10 - 10000	Uniform: 10 - 10000	Uniform: 10 - 10000	Uniform: 10 - 10000
t2	Uniform: 10 - 10000	Uniform: 10 - 10000	Uniform: 10 - 10000	Uniform: 10 - 10000	Uniform: 10 - 10000
t3	Uniform: 10 - 10000	Uniform: 10 - 10000	NA	NA	NA
t4	Uniform: 10 - 10000	Uniform: 10 - 10000	NA	NA	NA
t1-db	NA	NA	Uniform: 10 - 10000	NA	NA
t2-db	NA	NA	Uniform: 10 - 10000	NA	NA
ta	NA	NA	NA	Uniform: 10 - 10000	NA
td	NA	NA	NA	Uniform: 10 - 10000	NA
ra	NA	NA	NA	Uniform: 0.001 - 0.999	NA
1-ra	NA	NA	NA	Uniform: 0.001 - 0.999	NA
1-r1	NA	Uniform: 0.001 - 0.999	NA	NA	NA
r1	NA	Uniform: 0.001 - 0.999	NA	NA	NA

Analysis of Demographic Processes

Evaluation of genetic diversity and inbreeding

To examine the distribution of genetic variation and the degree of inbreeding in each species, cluster identified in *S. miniatus* and sample location, the average heterozygosity, number of alleles, allelic richness reflecting the number of alleles normalized by sample size, the fraction of total alleles in each group, the inbreeding coefficient F_{IS} were estimated using the program FSTAT (Goudet 2001). We compared allelic diversity, the number of private alleles and effective population size between sample locations within each species or cluster in *S. miniatus* identified in STRUCTURE to examine whether sample locations or cluster or species have higher allelic diversity or a greater number of private alleles indicative of large stable populations that may be older or are remnants of refugia during glaciations. Conversely, we examined whether populations had lower genetic diversity and lacked private alleles indicative of founder events or severe bottlenecks. The analysis of the inbreeding coefficient F_{IS} was conducted across sample locations to determine whether the degree of inbreeding or lack there of varies across regions.

Tests for recent severe reductions in population size

Following a severe reduction in population size referred to as a "bottleneck", gaps in the distribution of allelic lengths can occur due to loss of alleles and heterozygote excess can occur depending on the variability of the locus and the time that has passed since the bottleneck began. We test for heterozygote excess indicative of recent reductions in population size in each species and population in the *Rosicola* that may reflect overfishing or extreme natural fluctuation in population size. We tested for heterozygote excess in each species and population in the *Rosicola* using the program Bottleneck (Luikart *et al.* 1998).

We applied three statistical tests for excess heterozygosity using the program Bottleneck. The first test is based on sign tests on the difference (observed - expected) in heterozygosity across all loci (L), in which there is expected to be an equal probability L/2 of deficit or excess in a population in mutation drift equilibrium with no bottleneck. For a sampled population, a significant deviation from L/2 can be detected assuming a binomial distribution of outcomes. A second test takes into account the magnitude of heterozygosity excess/deficiency with a null hypothesis that the difference between observed and expected heterozygosity is random and the expected value is equal to zero for all loci for a population in mutation drift equilibrium. If the null hypothesis is rejected with a one-tailed test for heterozygote excess ($P < 0.05$), the alternative hypothesis is that heterozygote excess exists. The overall power of this parametric test is generally higher than that of the first non-parametric test, but both tests are slightly more conservative in rejecting the null hypothesis when the SMM is assumed (Cornuet and Luikart 1997). Both test for significant deviations from the expected heterozygosity that can result from the more rapid loss of alleles due to reduction in population size for which a lower heterozygosity would be expected than is observed. Estimates of expected values were made using 1000 replications with the provided data in the program Bottleneck.

Heterozygote deficiency can occur with loci evolving under the strict one step SMM, which presents potential problems for the detection of bottlenecks when using tests for heterozygote excess. The test may not be significant even in a bottlenecked population because some loci, evolving under the strict SMM and with large effective population size exhibit heterozygote deficiency. Consequently, a significant heterozygote excess for selectively neutral markers should be detected only in populations having experienced a recent severe reduction in population size.

Tests for hybridization between species or populations in the Rosicola

We tested for hybridization or introgression among species in the subgenus *Rosicola* and populations in the *S. miniatus* to determine whether they are interbreeding. To do so, we used the program STRUCTURE (K = 5) to identify individuals with a high estimated probability of ancestry in more than one species or cluster identified in *S. miniatus*. In addition, we use the program New Hybrid and allele frequency data to assign individuals to pure, hybrid F1 and F2 or back-cross categories using Bayesian methods. Both Jeffrey's and normal priors on frequency distributions in a given locus were applied. Burn-in was conducted with 10,000 iterations followed by 100,000 iterations thereafter.

Results

Population structure in the subgenus Rosicola informative of the sequence and rooting structure of phylogenetic relationships

The high and significant (P<0.00001) F_{ST} values among each of the species in the subgenus reflected deep divergence consistent with the findings for Hyde *et al.* (2008). Differentiation between *S. pinniger* and *S. crocotulus* (F_{ST} = 0.233) was greater than between *S. miniatus* and *S. pinniger* (F_{ST} = 0.175) or *S. crocotulus* and *S. miniatus* (F_{ST} = 0.142), indicating an older and deeper divergence. The results of comparisons between *S. miniatus* clusters from STRUCTURE K = 5 to other members of the subgenus showed decreasing F_{ST} values from cluster 1 through cluster 3 in pairwise comparison to *S. crocotulus* and *S. pinniger* (Table 2). Comparisons between clusters indicate greatest genetic differentiation between cluster 1 and cluster 3 (F_{ST} = 0.080), more moderate differentiation between cluster 1 and cluster 2 (F_{ST} = 0.055) and least differentiation between clusters 2 and cluster 3 (F_{ST} = 0.028).

The low proportion of ancestry contributed from other species in results of STRUCTURE analysis (K = 5) reflecting three clusters in *S. miniatus* and the other two species shown in Figure 2 indicates a low rate of shared alleles between species. The low posterior probability of assignment in the admixture analysis at the individual level in BAPS in Figure 2 provides similar results. A greater proportion of ancestry from cluster 2 than cluster 3 was observed in cluster 1, while a greater proportion of cluster 2 than cluster 1 was observed in cluster 3 (Figure 2). A greater proportion of *S. crocotulus* was observed in cluster 2 and cluster 3 than in cluster 1, though little was observed in general. These results are consistent with their geographic distribution, distance between core distributions and timing of divergence and magnitude of genetic differentiation as measured by F_{ST}. The sharing of alleles resulting in the contribution of a proportion of ancestry or admixture is in part due to incomplete lineage sorting or hybridization, the latter of which we examine below. Almost no posterior mean estimate of the proportion

of the genome other than primary species or population was observed in the results of BAPS other than one individual with contributions from cluster 2 and cluster 3 and one individual of *S. crocotulus* with cluster 1 (Figure 3).

Table 3. F_{ST} for pair-wise comparison between each *S. crocotulus*, *S. miniatus* and each of the clusters of *S. miniatus* identified with STRUCTURE K = 5 from all sampling locations combined. All results were significant with *P* < 0.0000 level (P < 0.05, Bonferonni Corrected: α = 0.05/6 = 0.0083).

Species / Cluster 1	Species / Cluster 2	F_{ST}
S. miniatus Cluster 1	*S. pinniger*	0.206
S. miniatus Cluster 2	*S. pinniger*	0.194
S. miniatus Cluster 3	*S. pinniger*	0.187
S. crocotulus	*S. pinniger*	0.232
S. crocotulus	*S. miniatus* Cluster 1	0.192
S. crocotulus	*S. miniatus* Cluster 2	0.147
S. crocotulus	*S. miniatus* Cluster 3	0.139
S. miniatus Cluster 2	*S. miniatus* Cluster 1	0.055
S. miniatus Cluster 3	*S. miniatus* Cluster 1	0.080
S. miniatus Cluster 3	*S. miniatus* Cluster 2	0.028

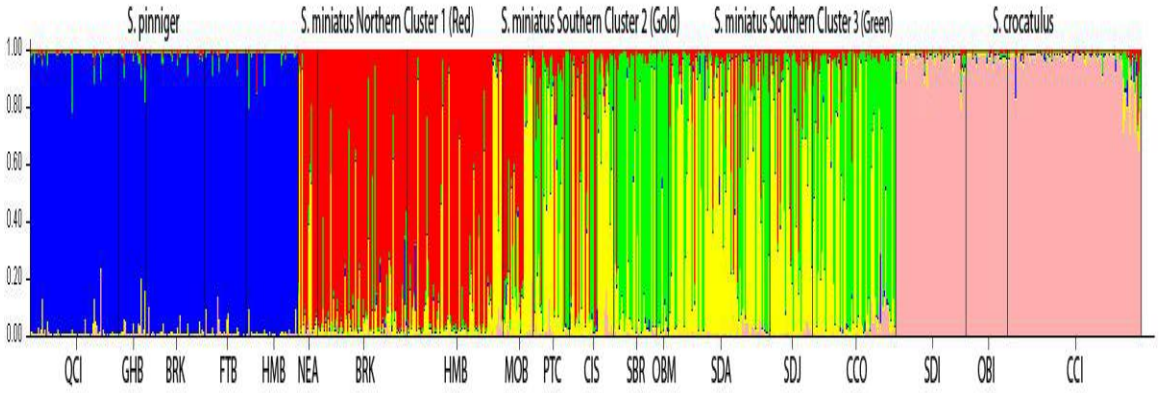

Figure 2. Proportion of ancestry from STRUCTURE for species in the subgenus *Rosicola* with five clusters (K = 5) at each sample location indicated by three digit alpha code. The assignments to *S. miniatus* Cluster 1 (red), Cluster 2 (yellow) and Cluster 3 (green) are indicated by greater than 33% proportion of ancestry to a given cluster.

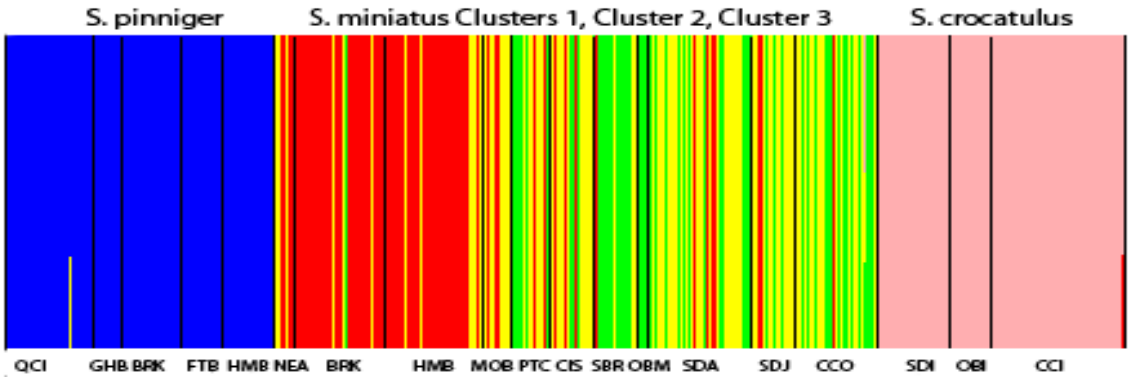

Figure 3. Results of admixture analysis from BAPS for K = 5 clusters conducted at an individual level for all three species of the subgenus *Rosicola* found to have the highest negative log maximum likelihood. Posterior probability of assignment of each individual to each cluster is represented by the differing colored bars.

Tests for significant contribution of mutation to differentiation in the subgenus Rosicola

The null hypothesis that mutation does not contribute to differentiation, but rather differentiation is due to genetic drift alone is valid when a permutation test for alleles between populations results in failure to reject the null hypothesis that the R_{ST} for permutated alleles (ρR_{ST}) is not significantly different from the observed R_{ST} using a one-tailed t-test ($P < 0.05$, Bonferroni Corrected: $\alpha = 0.05/6 = 0.0083$). Permutation tests between *S. pinniger*, *S. miniatus* and S. *crocotulus*, indicate a significant contribution of mutation to divergence between species (Table 4). The result is not significant for comparison of *S. miniatus* and *S. pinniger* when the Bonferroni correction for multiple comparisons is applied (Table 4).

When an analysis was conducted among *S. miniatus* clusters identified using the STRUCTURE model K = 5, no significant contribution of mutation to genetic structure was detected among clusters when corrections for multiple comparisons were applied (Table 5). Comparisons between *S. miniatus* clusters and *S. pinniger* were significant with the exception of cluster 2. All tests for contribution of mutation to differentiation with *S. crocotulus* were significant.

Table 4. The R_{ST} value and *P*-value for test that R_{ST} is significantly greater than ρR_{ST} resulting from permutation of alleles between species conducted in Spage DI (P < 0.05, Bonferroni Corrected: α = 0.05/6 = 0.0083).

Species 1	Species 2	R_{ST}	Mean Permuted Value	P(1-sided test, H1: obs. > exp.)
S. pinniger	*S. miniatus*	0.3872	0.1678	0.0195
S. pinniger	*S. crocotulus*	0.5079	0.1524	0.0002
S. miniatus	*S. crocotulus*	0.4645	0.1359	<0.0001

Table 5. The R_{ST} value and *P*-value for test that R_{ST} is significantly greater than ρR_{ST} resulting from permutation of alleles between species or *S. miniatus* clusters identified in STRUCTURE model K = 5 conducted in Spage DI (P < 0.05, Bonferroni Corrected: α = 0.05/6 = 0.0083).

Species / Cluster	Species / Cluster	R_{ST}	Mean Permuted Value	P(1-sided test, H1: obs>exp)
S. pinniger	*S. miniatus Cluster 1*	0.5220	0.2000	0.0018
S. pinniger	*S. miniatus Cluster 2*	0.3551	0.1738	0.048
S. pinniger	*S. miniatus Cluster 3*	0.3933	0.1574	0.0064
S. pinniger	*S. crocotulus*	0.5079	0.1543	0.0002
S. miniatus Cluster 1	*S. miniatus Cluster 2*	0.0789	0.0501	0.1704
S. miniatus Cluster 1	*S. miniatus Cluster 3*	0.1516	0.0768	0.0434
S. miniatus Cluster 1	*S. crocotulus*	0.4739	0.1865	0.0026
S. miniatus Cluster 2	*S. miniatus Cluster 3*	0.0367	0.0298	0.3196
S. miniatus Cluster 2	*S. crocotulus*	0.4020	0.1404	0.0015
S. miniatus Cluster 3	*S. crocotulus*	0.4963	0.1284	<0.0001

Phylogeography of the subgenus Rosicola

The results from all three species are useful in establishing the proper rooting, sequence of divergence and testing for reciprocal monophyly among species as well as clusters identified within *S. miniatus*. The UPGMA tree of the Cavalli-Sforza chord distance for individuals of each species in the subgenus *Rosicola* from each sample location is provided in Figure 4. In examining the topology of the UPGMA trees, no strong population structure was evident within *S. crocotulus* or *S. pinniger*. The lower Cavalli-Sforza chord measure values and resulting proximity in the tree topology between proximate geographic locations in these species suggests the presence of isolation by distance from weak genetic structure with distance between populations with the exception of *S. pinniger* in Grays Harbor (Figure 4). Grays Harbor is most proximate to Vancouver Islands in geographic distance but they are unexpectedly distant in the tree topology. This may be the result of the relatively low sample size in Grays Harbor (N = 22) rather than substantial genetic differentiation of individuals in Grays Harbor from the remainder of the coast.

Interpretation of tree topology in the UPGMA diagram of Cavalli-Sforza index of genetic differentiation provides some insight on the phylogeography of each of the species in the *Rosicola* and clusters identified in *S. miniatus*. Population structure within *S. miniatus* appears to be distributed north and south of Point Conception in the analysis of sampled locations without considering clusters observed in the program STRUCTURE (Figure 4). It appears that additional population structure may exist in offshore populations as evidenced by the distant position of the outer banks branch where cluster 3 was prevalent relative to the remainder of the sample locations south of Point Conception providing an indication of additional population structure before clustering with STRUCTURE.

When sampled *S. miniatus* were assigned to separate clusters with STRUCTURE K = 5 in each sample location and Cavalli-Sforza chord measure calculated, the pattern

observed in UPGMA clustering reflects a similar phylogeographic pattern to that identified without assignment to clusters as seen in Figure 5. The structure identified north and south of Point Conception was subsumed by clusters. A greater number of cluster 1 individuals were sampled in locations north of Point Conception, while clusters 2 and cluster 3 from STRUCTURE analysis with K = 5 analysis were found in southern sample locations. When the sample size for a given cluster within a sample location was less than four, the cluster was excluded from analysis. In many locations, south of Point Conception, there were not enough individuals assigned to cluster 1 and the same was true to the north of Point Conception for samples of cluster 2 or cluster 3. All clusters in each sample location were reciprocally monophyletic for K = 5 with the exception of cluster 2 and cluster 3 from Brookings, which grouped together and apart from the remainder (Figure 5). The sample sizes within these sites were still very low for some clusters, thus the topology of these locations should be interpreted with caution since placement in the topology may be the result of random sampling error rather than the true distribution of alleles within a given sample location.

In the UPGMA tree from structure K = 5, within cluster 1 branches, Neah Bay and samples from south of Point Conception appeared to be distant from the remaining locations from Brookings to Morro Bay where the highest number of individuals assigned to this cluster were found (Figure 5). There was pronounced distance within cluster 2 between Brookings or Neah Bay and the other samples while other samples from north of Point Conception were also somewhat distant from the remaining samples to the south where the majority of cluster 2 individuals were found. Within cluster 3, outer banks and channel island samples were somewhat distant from the mainland samples from south of Point Conception.

There is an apparent replete pattern in topology of species within the *Rosicola* and between clusters within *S. miniatus* with geographic distribution indicative of concordance reflecting a repeated mechanism of speciation. As discussed in chapter 3, cluster 3 occupies a deeper adult depth distribution than the other two clusters and is most common in latitudes analogous to the more deeply diverged *S. crocotulus*. In addition, *S. miniatus* individuals assigned to cluster1 occupy similar latitudes to *S. pinniger*, while cluster 2 holds an intermediate position both genetically and geographically between the two clusters. Thus cluster 1 may be to cluster 2 within *S. miniatus* what *S. pinniger* is to *S. crocotulus* in terms of repeated patterns of speciation. Further analysis of the depth distribution and latitudinal distribution of each cluster may indicate the underlying mechanisms for this repeated pattern.

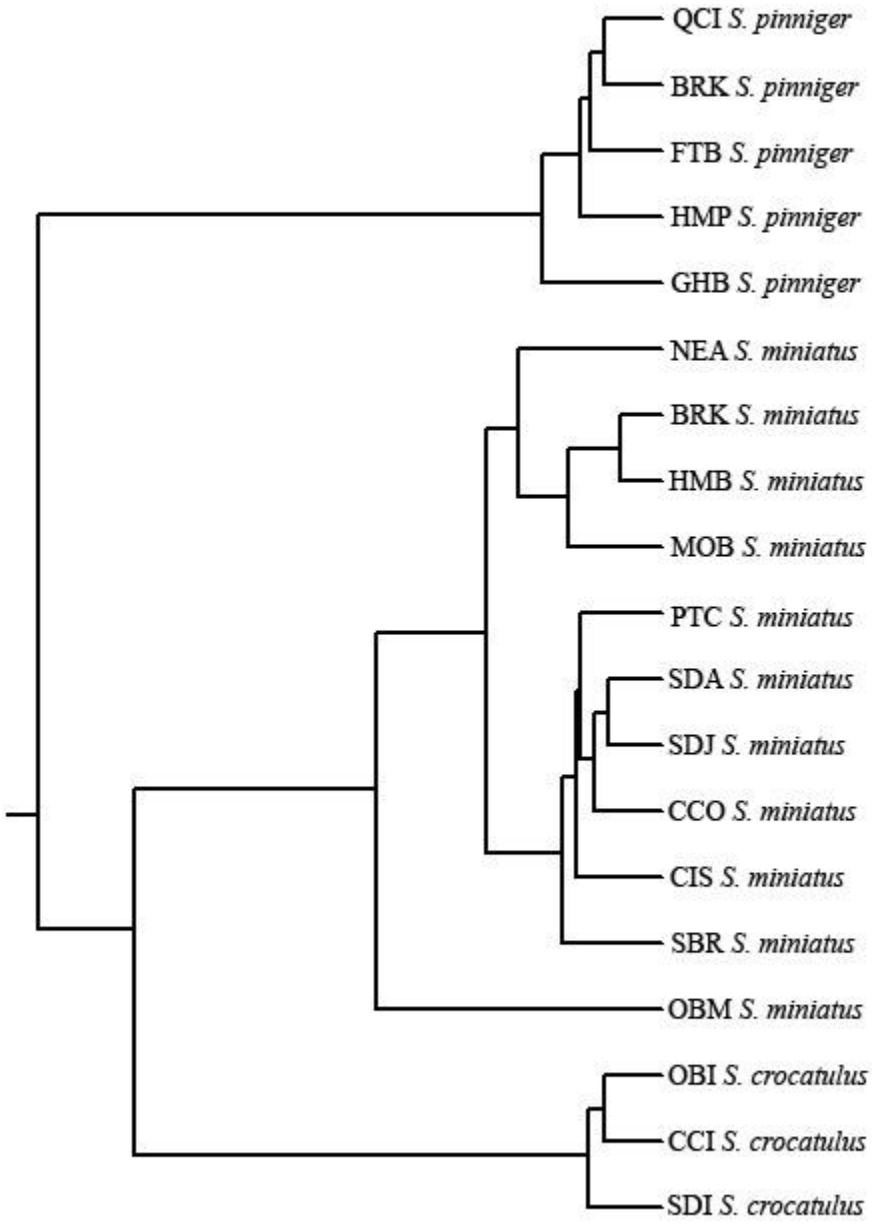

Figure 4. UPGMA diagram of Cavalli-Sforza chord measure (Cavalli-Sforza and Edwards, 1967) for all sampled locations for each species in the subgenus *Rosicola*.

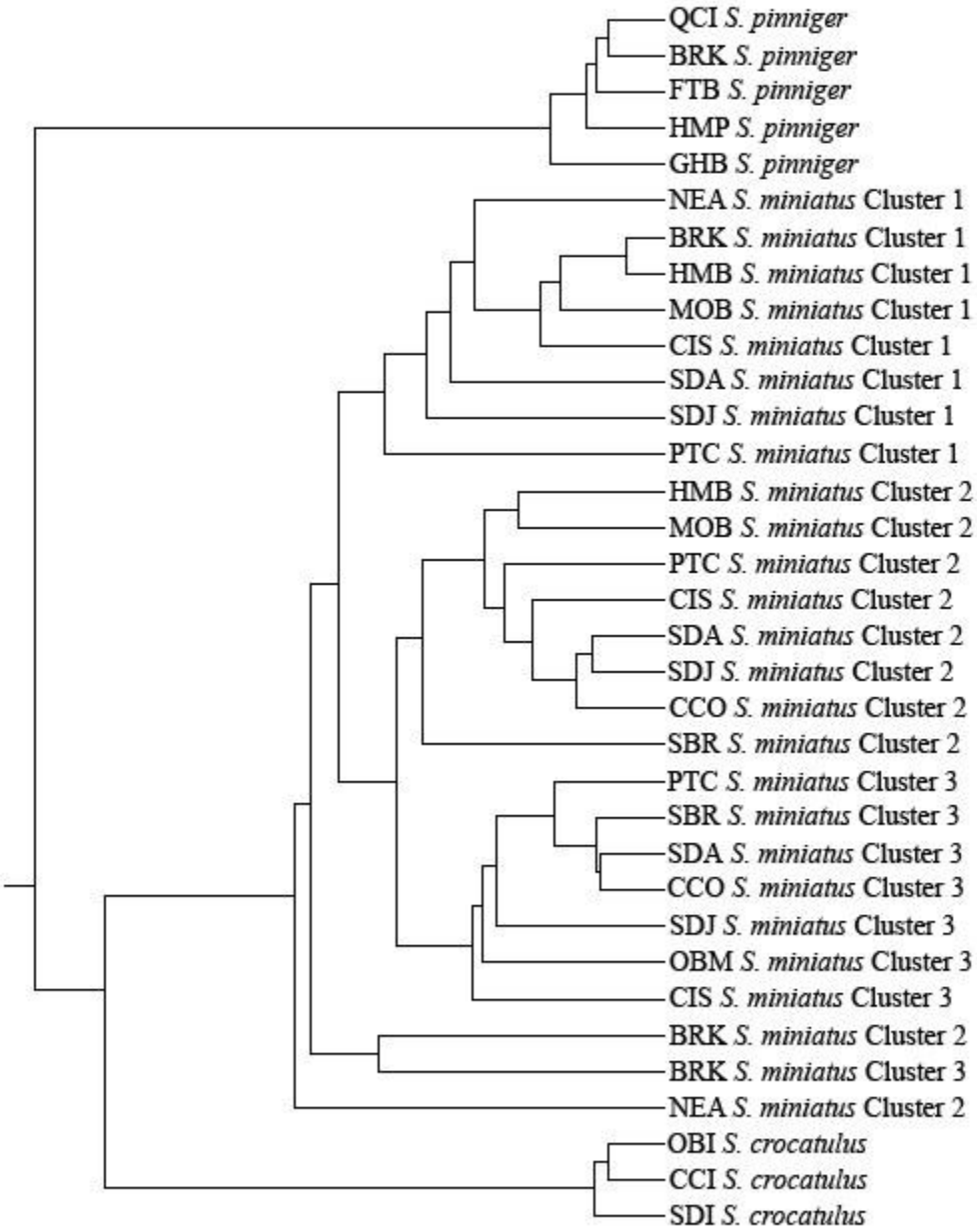

Figure 5. UPGMA diagram of Cavalli-Sforza chord measure (Cavalli-Sforza and Edwards, 1967) for all sampled locations for each species in subgenus *Rosicola* with *S. miniatus* assigned to three clusters from Structure K = 5 within each sampled location.

Evaluating models of evolutionary scenarios and tests for consistency of timing of divergence between populations of S. miniatus with Pleistocene glacial maxima.
Scenarios reflecting divergence of *S. pinniger* and *S. crocotulus* with subsequent divergence of *S. miniatus* from *S. pinniger* as opposed to *S. crocotulus* were not supported by the posterior probabilities or logistic analysis. Scenarios consistent with *S. crocotulus* sharing a most recent common ancestor with *S. miniatus* with significantly higher values were better supported by posterior probabilities and logistic regression results. The model with *S. pinniger* as the most basal species with the subsequent divergence between *S. miniatus* and *S. crocotulus* resulted in significantly higher logistic regression results and were consistent with the order of divergence in Hyde *et al.* (2008) using mitochondrial DNA sequence data, though posterior probabilities were not significantly different between scenarios. Within *S. miniatus* scenarios, logistic method results supported earliest divergence of cluster 1 and cluster 3 both in analyses at the level of the entire subgenus as well as the clusters alone, though there were not significant differences between results for the posterior probability of scenarios.

Scenarios with basal divergence between *S. crocotulus* and *S. pinniger*, subsequent divergence between *S. miniatus* cluster 3 and most recent common ancestors of cluster 1 and 2 and most recent divergence between clusters 1 and 2, were analyzed further. Though posterior probabilities and likelihood method results at the subgenus level as well as between clusters identified in *S. miniatus* consistent with this underlying topology were not significantly different among scenarios reflecting admixture, population size reduction or without either, the best supported order of divergence was the same (Figures 6 through 10). While the ordering divergence in the topology within the subgenus is well supported, the analysis was only conducted with six microsatelite loci and there was no significant differences between scenarios reflecting admixture, reduction in population size or lack there of could not be detected. The scenario without admixture or reduction in population size was the best supported by the higher values from the logistic method, while the absolute values for the posterior probability results supported the admixture scenario.

The posterior probability (Table 6) for admixture between cluster 1 and 3 resulting in cluster 2 (scenario 10, Figure 7 for the subgenus and scenario 6 for *S. miniatus* alone, Figure 8) was slightly higher than divergence without admixture or changes in population size (scenario 4, Figure 8 for the subgenus and scenario 2, Figure 7 for *S. miniatus* alone), though better supported by the logistic method. This could in deed be the result of continued hybridization or introgression between clusters 1 and 3 or gene flow between cluster 1 or cluster 3 with cluster 2 rather than gene flow between population 1 and 3 directly. Alternatively, it slightly higher posterior probability of the admixture scenario may in part be due to incomplete lineage sorting between clusters, given the greater support by the logistic regression method for the scenario without admixture or reduction in population size after divergence.

The scenario exploring reduction in population size with divergence between clusters of *S. miniatus* (scenario 8, Figure 10), was less well supported by either posterior probability or logistic methods (Table 6). The greater support for divergence without severe

reductions in population size or admixture (Scenarios 2 for *S. miniatus* clusters and 4 for the full subgenus) would indicate that divergence occurred without founder events or bottlenecks and admixture has not occurred after divergence of clusters of *S. miniatus* despite the potential for secondary contact (Table 6). The Type 1 and Type 2 error rates for each scenario are also provided in Table 6.

The F_{ST}-values for comparisons of the three clusters identified in *S. miniatus* indicate clusters 2 and 3 are the least differentiated, followed 1 and 2 and the highest differentiation is present between 1 and 3. Contrary to this pattern, the best supported scenario reflects the deepest divergence between cluster 3 and the most recent common ancestor of clusters 1 and 2, while 1 and 2 are more recently diverged. The topology observed in the best supported scenario may be in part due driven by the greater differentiation indicated by the higher F_{ST} between clusters 1 and 3, which is consistent deeper divergence between cluster 3 from the common ancestor of clusters 1 and 2. Introgression or lower greater degree of incomplete lineage sorting at the shared margins of the range of 1 and 2 forming an area of admixture between populations could explain the somewhat confounded results. Additional analyses in New Hybrid will allow further examination of contemporary gene flow between populations.

The results for generations since divergence between clusters in *S. miniatus* from scenario 4, without admixture or changes in effective population size which was best supported was further analyzed to produce estimates of timing of divergence in years for comparison to the timing of periods of glaciations. Timing of divergence appears consistent with the Pleistocene though the specific stage of the Pleistocene let alone a particular glaciation event may be difficult to determine given the uncertainty in the estimate. Timing of divergence in scenario 4 indicates the most recent divergence between the shallow distributed northerly cluster 1 and cluster 2 is estimated to be about the early Pleistocene around 102,429 years ago (95% CI: 22,884 – 248,933) years ago, consistent with the mid Tarantian or early Ionian stage. The timing of divergence between cluster 3 and the common ancestor of the other two clusters is estimated to have occurred 255,141 years ago (95% CI: 80,080 - 592,847), which places divergence in the early Tarantian or mid-Ionian stage of the Pleistocene. The timing of divergence between *S. crocotulus* and *S. miniatus* 1.26 (0.50-2.27) Million Years Ago (MYA) in scenario 4 from microsatelite data, is more recent than the estimate directly from sequence based estimates at 2.3 MYA from Hyde *et al.* (2008).

In scenario 4, the effective population size of cluster 2 is greater than the other two *S. miniatus* clusters and *S. crocotulus* (Table 7). This might indicate that cluster 2 has a larger pool of genetic diversity or individuals as a result of its prevalence to the south in what was likely warmer water refugia during glacial maxima, whereas population 1 which is most northerly has a lower effective population size. The effective population size in the thousands of individuals would indicate that a small fraction of the population is contributing to future generations given far higher population size estimates from recent stock assessments (PFMC 2014). Reduction in population size would be consistent with interglacial expansion scenario for founder effects as populations move north during an interglacial period. Results for the effective population size in generations and timing

of divergence under each of the scenarios are provided in Table 7. While the scenario reflecting a severe reduction in population size after divergence was not the best supported scenario, the best supported scenario without admixture of severe reduction show lower effective population size in the more northerly distributed cluster 1. This is consistent with lower population size that may be expected with a more recent diverged population at the northern extent of its range or a founder event. The relative median absolute error estimates (RMAE) were generally low, though they were elevated for the timing of divergence for *S. miniatus* cluster 3 from the other two and for the effective population size of cluster 1 (Table 7).

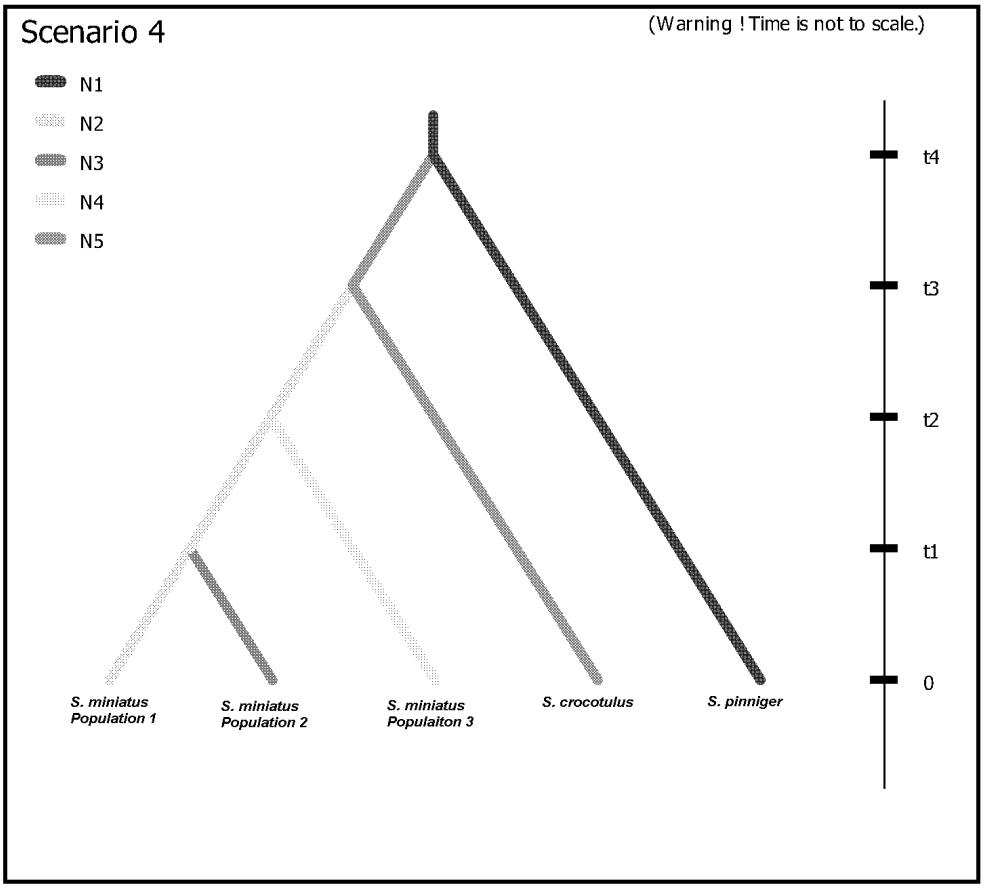

Figure 6. Scenario of evolutionary history of the *Rosicola* reflecting no admixture or reduction in population size.

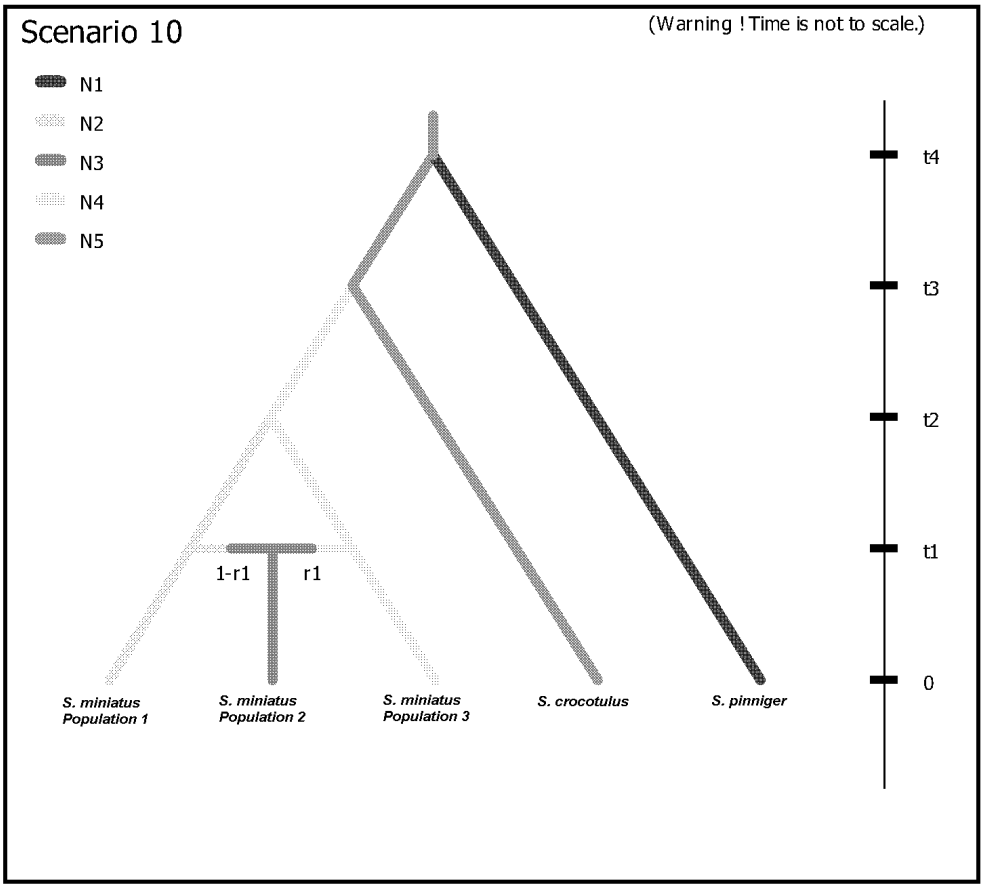

Figure 7. Scenario of evolutionary history of the *Rosicola* showing admixture between population 1 and 3 resulting in population 2.

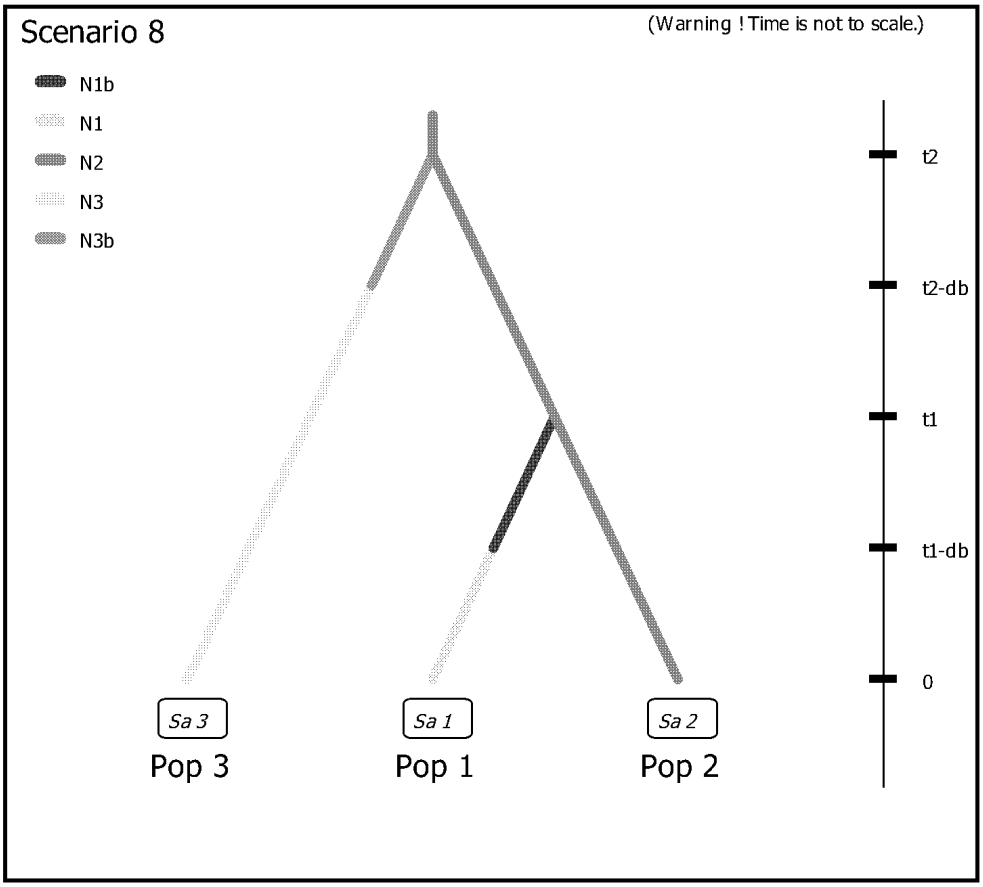

Figure 8. Scenario of evolutionary history showing a change in population size subsequent to divergence of population 3 from population 2 and following the later divergence of population 1 from population 2.

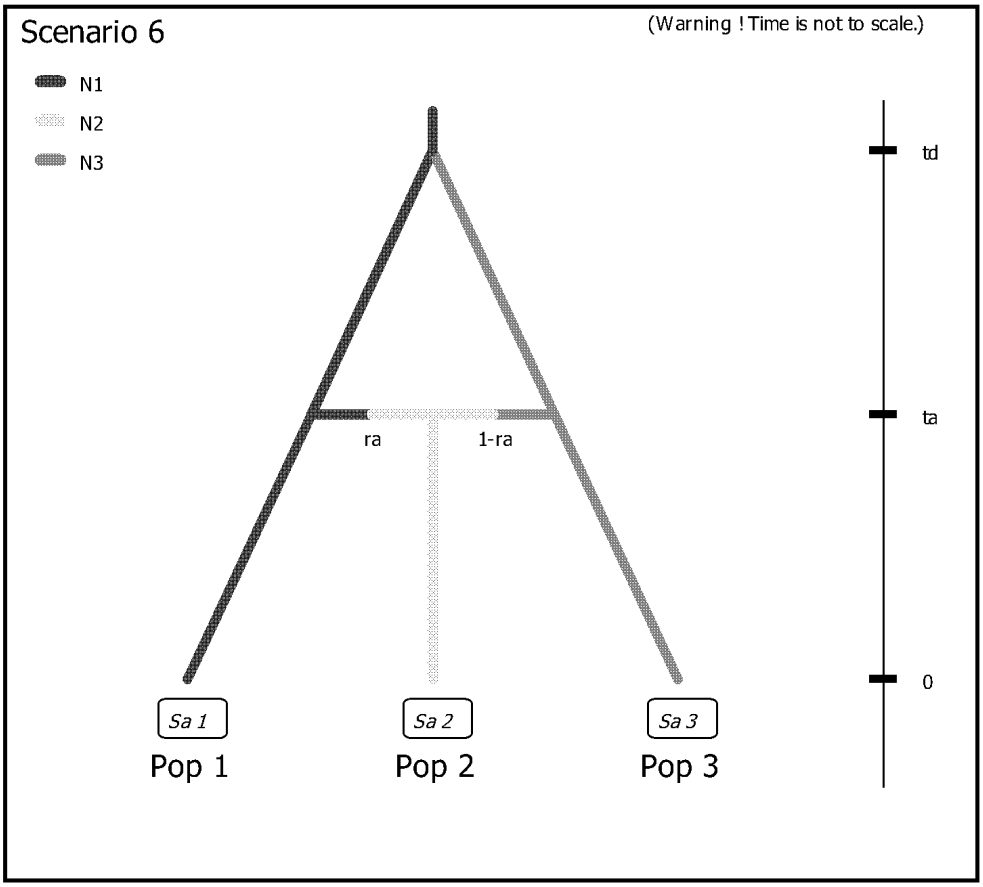

Figure 9. Scenario of evolutionary history of the populations of *S. miniatus* identified with STRUCTURE K = 3 showing admixture between population 1 and 3 resulting in population 2.

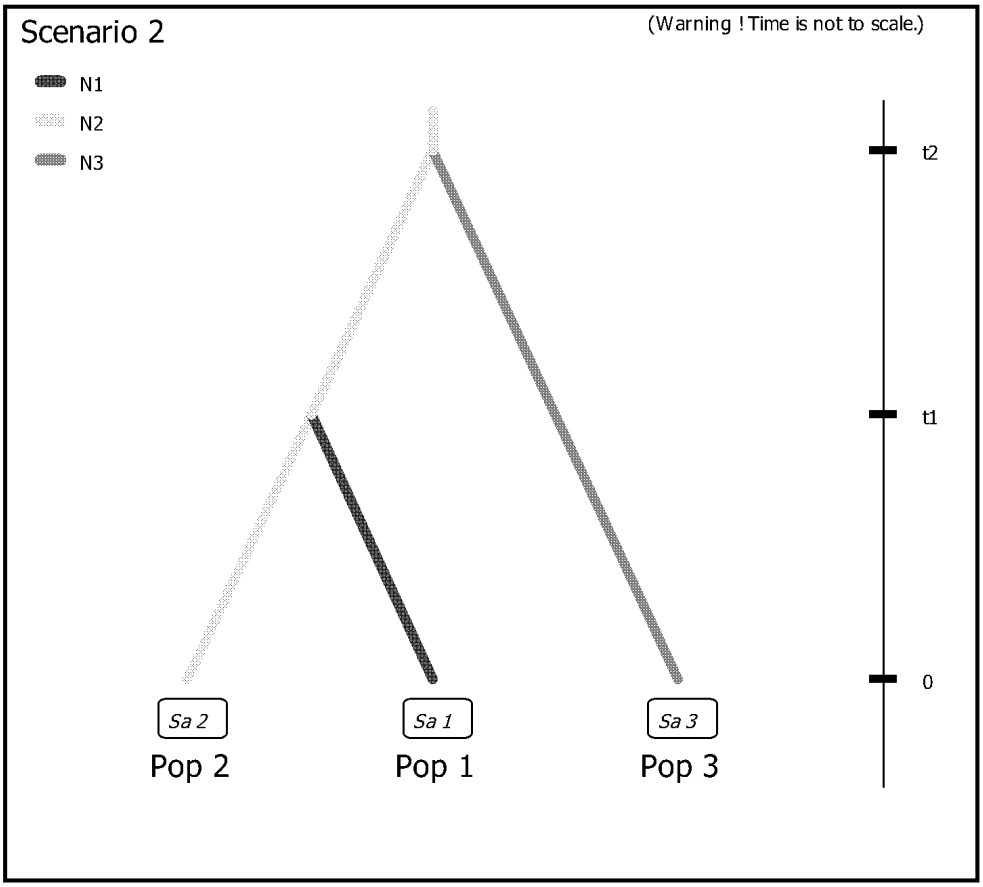

Figure 10. Scenario of evolutionary history of the populations of *S. miniatus* identified with STRUCTURE K = 3 showing divergence between population 3 and population 2 with subsequent divergence of population 1 from population 2.

Table 6. Results of posterior probability and likelihood methods of scenario selection, associated 95% credibility intervals and Type 1/Type2 error estimates for each scenario.

Group	Scenario	Posterior Probability [95% CI]	Type 1 Error Rate	Type 2 Error Rate	Logistic Approach [95% CI]	Type 1 Error Rate	Type 2 Error Rate
Subgenus Rosicola	4	0.096 [0.000-0.225]	0.542	0.169	0.318 [0.203-0.439]	0.546	0.142
	10	0.111 [0.000-0.249]	0.654	0.110	0.294 [0.188-0.399]	0.562	0.148
S. miniatus Clusters	2	0.097 [0.000-0.226]	0.646	0.126	0.255 [0.159-0.350]	0.592	0.137
	6	0.110 [0.000-0.247]	0.472	0.165	0.190 [0.122-0.258]	0.416	0.148
	8	0.095 [0.000-0.224]	0.570	0.137	0.112 [0.054-0.171]	0.530	0.143

Table7. Parameter values and relative median of the absolute error for estimates of parameter values from scenarios reflecting the evolutionary history of the *Rosicola* as a whole and between clusters identified in *S. miniatus* alone identified as having the highest posterior probability and logistic regression values.

	Rosicola				***S. miniatus* Clusters**					
	Scenario 4		**Scenario 10**		**Scenario 2**		**Scenario 6**		**Scenario 8**	
Parameter	**Value 95% CI**	**RMAE**	**Value 95% CI**	**RMAE**	**Value 95% CI**	**RMAE**	**Value 95% CI**	**RMAE**	**Value 95% CI**	**RMAE**
Ne *S. pinniger*	6520 [3630-9290]	0.160	7280 [5030-9410]	0.162	NA	NA	NA	NA	NA	NA
Ne *S. miniatus Cluster 1*	3280 [1130-7010]	0.263	5800 [2860-9060]	0.265	4520 [1530-8340]	0.209	6330 [3490-9350]	0.196	5410 [803-9660]	0.148
Ne *S. miniatus Cluster 2*	6810 [3320-9510]	0.111	7220 [4280-9590]	0.170	6740 [3460-9310]	0.197	7220 [4100-9630]	0.178	7990 [5530-9800]	0.186
Ne *S. miniatus Cluster 3*	5830 [2690-8940]	0.208	6220 [3400-9120]	0.197	7080 [4100-9530]	0.255	6490 [3400-9230]	0.249	5230 [783-9430]	0.162
Ne *S. crocotulus*	4680 [2170-7860]	0.223	5780 [3090-8930]	0.249	NA	NA	NA	NA	NA	NA
t1	330 [71.2-802]	0.260	343 [73.6-793]	0.300	239 [56.8-588]	0.199	NA	NA	213 [47.9-523]	0.205
t2	822 [258-1910]	0.302	1230 [379-2740]	0.378	636 [94.7-217]	0.414	NA	NA	918 [133-3070]	0.389
t3	4060 [1620-7340]	0.229	5410 [2700-8320]	0.271	NA	NA	NA	NA	NA	NA
t4	7410 [4060-9750]	0.109	8240 [5480-9890]	0.169	NA	NA	NA	NA	NA	NA
ta	NA	NA	NA	NA	NA	NA	247 [47.7-59.5]	0.263	NA	NA
td	NA	NA	NA	NA	NA	NA	1120 [189-3690]	0.244	NA	NA
ra	NA	NA	NA	NA	NA	NA	0.523 [0.12 - 0.90]	0.695	NA	NA

Analysis of Demographic Processes

Evaluation of genetic diversity and inbreeding

In *S. pinniger*, the greatest number of private alleles was found at the edges of the sampled range and the greatest numbers of alleles were found in the Vancouver Islands, Canada, while allelic diversity was marginally higher in Fort Bragg (Table 8). The lowest allelic diversity adjusted for sample size was observed in Half Moon Bay, while the least sampled alleles and highest F_{IS} was found in Grays Harbor where allelic diversity adjusted for sample size was the second lowest (Table 8). There were only 22 sampled individuals in Grays Harbor, which may explain this discrepancy. The lower allelic diversity at the edge of the range as well as a higher number of private alleles at the southern edge of the range in Half Moon Bay is consistent with expectations of adaptation in response to selection at the edge of the range or potentially the higher number of private alleles are the result of the remnant population in the southern refugia.

Sample locations to the south of Half Moon Bay show greater allelic diversity *in S. miniatus*, with the exception of the Outer Banks location in which only nine individuals were sampled and in which F_{IS} was high (Table 8). The higher allelic diversity further to the south may be the result of large stable populations across evolutionary time suggesting either the center of distribution or glacial refugia in each species and population. At the edge of the range in Neah Bay, the allelic diversity was lower and F_{IS} was elevated as well, again, in part this could be due to a low sample size as only 15 individuals were sampled. Brookings, Half Moon Bay, the Channel Islands, San Diego Adults and Cabo Colonet had the greatest number of private alleles. The highest number of private alleles was observed at the southern edge of the range in Cabo Colonet and high diversity elsewhere south of Conception other than the Outer Banks and Juveniles in San Diego, indicating the possible location of former refugia inside the southern California bight during past glaciation events (Table 8). The more centrally distributed cluster 2 had the highest number of private alleles and allelic diversity indicating the potential for this to be a historically stable population distributed in glacial refugia in suitable water temperatures to the south or inside the Southern California bight. Juveniles off San Diego had the lowest allelic diversity of the adult population indicating the potential for a sweepstakes effect resulting from survival of only larvae from individuals that spawned during a period when survival was favored as proposed for species with high fecundity by Hedgecock *et al.* (2011), thus reducing diversity observed in any one year class at a given location. The unique alleles in Half Moon Bay and Brookings, may have arisen since interglacial expansion north of Point Conception after the last glacial maximum.

Table 8. The number of sampled individuals, private alleles, average heterozygosity, number of alleles, allelic diversity, fraction of total alleles sampled and inbreeding coefficient F_{IS} in each species, population or sample location. Results noted in the text are highlighted.

Analysis	Subgroup	Private Alleles	Private Alleles between *S. miniatus* Clusters	Average He	Alleles	Allelic Diversity	Fraction of Alleles	F_{IS}
S. pinniger	Vancouver Island	*6*	-	0.74	84	56.1	0.8	0.01
	Grays Harbor	1	-	0.76	59	56.1	0.6	0.07
	Brookings	1	-	0.71	73	54.7	0.7	0.00
	Fort Bragg	4	-	0.74	71	58.0	0.7	-0.03
	Half Moon Bay	*6*	-	0.72	68	53.0	0.7	0.03
	All	-	-	-	104	55.8	1.0	-
S. miniatus	Neah Bay	0	-	0.76	45	38.1	0.4	*0.14*
	Brookings	3	-	0.78	66	39.6	0.6	0.01
	Half Moon Bay	2	-	0.78	70	39.0	0.6	0.05
	Morro Bay	0	-	0.79	62	42.7	0.5	0.02
	Point Conception	1	-	0.82	72	46.7	0.6	0.02
	Channel Islands	3	-	0.79	72	43.6	0.6	0.04
	Santa Barbara	1	-	0.79	71	43.4	0.6	0.03
	Outer Banks	1	-	0.78	38	38.0	0.3	*0.14*
	San Diego Adults	3	-	0.79	85	45.2	0.7	0.03
	San Diego Juveniles	1	-	0.79	66	42.3	0.6	0.01
	Cabo Colonet	4	-	0.80	80	44.2	0.7	0.03
	All	-	-	-	118	44.7	1.0	-
S. crocotulus	Outer Banks	5	-	0.71	84	72.2	-	0.02
	San Diego	2	-	*0.48*	66	65.5	-	0.06
	Cabo Colonet	14	-	0.61	91	69.2	-	0.02
	All	-	-	-	100	70.4	-	-
Across Species	*S. pinniger*	21	-	0.73	104	102.8	-	0.01
	S. miniatus	11	-	0.81	118	102.3	-	0.06
	S. crocotulus	16	-	0.73	100	99.8	-	0.03
	All	-	-	-	158	137.9	-	-
Structure K = 5	*S. pinniger*	21	-	0.73	104	96.2	-	0.01
	S. miniatus Cluster 1	1	7	0.76	*75*	*71.5*	-	0.03
	S. miniatus Cluster 2	2	*19*	0.78	93	92.3	-	0.02
	S. miniatus Cluster 3	3	15	0.80	81	80.4	-	0.03
	S. crocotulus	17	-	0.73	100	94.1	-	0.03
	All	-	-	-	158	130.6	-	-

Tests for recent severe reductions in population size

The results of standard difference and signed rank tests for bottlenecks were negative resulting either in heterozygote deficit or non-significant excess and normal mode shift patterns. Results of these analyses are provided in Table 9 below.

Table 9. The expected heterozygosity, observed heterozygosity and results of sign, standard difference and signed rank tests as well as evidence of a mode shift in allele distributions from the program Bottleneck for each species, population and sample location.

Analysis	Subgroup	Ho	He	Sign Test	Standard Difference Test	Wilcoxen Test	Mode Shift
S. pinniger	Vancouver Island	0.74	0.82	0/6	Deficient	Deficient	Normal
	Grays Harbor	0.75	0.77	3/6	Non-Sig.	Non-Sig.	Normal
	Brookings	0.71	0.78	0/6	Deficient	Deficient	Normal
	Fort Bragg	0.74	0.79	1/6	Deficient	Deficient	Normal
	Half Moon Bay	0.72	0.77	1/6	Deficient	Deficient	Normal
S. miniatus	Neah Bay	0.75	0.81	0/6	Deficient	Deficient	Normal
	Brookings	0.78	0.85	0/6	Deficient	Deficient	Normal
	Half Moon Bay	0.78	0.85	1/6	Deficient	Deficient	Normal
	Morro Bay	0.79	0.86	1/6	Deficient	Deficient	Normal
	Point Conception	0.82	0.87	1/6	Deficient	Deficient	Normal
	Channel Islands	0.79	0.86	1/6	Deficient	Deficient	Normal
	Santa Barbara	0.79	0.87	1/6	Deficient	Deficient	Normal
	Outer Banks	0.77	0.79	1/6	Deficient	Deficient	Normal
	San Diego	0.79	0.87	1/6	Deficient	Deficient	Normal
	San Diego Juv.	0.79	0.85	0/6	Deficient	Deficient	Normal
	Cabo Colonet	0.80	0.87	1/6	Deficient	Deficient	Normal
S. crocotulus	Outer Banks	0.73	0.82	0/6	Deficient	Deficient	Normal
	San Diego	0.70	0.81	0/6	Deficient	Deficient	Normal
	Cabo Colonet	0.73	0.86	1/6	Deficient	Deficient	Normal
Rosicola	*S. pinniger*	0.73	0.83	0/6	Deficient	Deficient	Normal
	S. miniatus	0.81	0.90	0/6	Deficient	Deficient	Normal
	S. crocotulus	0.73	0.87	1/6	Deficient	Deficient	Normal
S. miniatus Structure K = 3	*S. miniatus* Cluster 1	0.76	0.85	1/6	Deficient	Deficient	Normal
	S. miniatus Cluster 2	0.78	0.88	1/6	Deficient	Deficient	Normal
	S. miniatus Cluster 3	0.80	0.87	1/6	Deficient	Deficient	Normal

Hybridization in the Subgenus Rosicola

The northerly distributed *S. miniatus* cluster 1 from STRUCTURE K = 5 was relatively uncommon south of Point Conception and the most southern deepwater STRUCTURE cluster 3 was primarily distributed south of Point Conception. As a result our analysis of hybridization was conducted on the basis of clusters in *S. miniatus* as well as at a higher spatial resolution to areas of high geographic overlap between species or clusters. Individuals were only assigned to pure categories with high posterior probabilities in the New Hybrids (Anderson and Thompson 2002) analysis with a dirichlet prior without

training pure frequencies using structure assignments, indicating no hybrid individuals between clusters of *S. miniatus* or any of the congeners. The analysis with a normal prior resulted in only a few individuals being assigned to hybrid categories and even then, with relatively low posterior probabilities.

When structure results for K = 3 were used to specify the frequency distributions of pure individuals, the results were similar to those found without assigning individuals, with individuals being assigned one of the two pure strains with high posterior probabilities, most with a posterior probability of 0.98 or greater. No hybrid classes were detected using allele frequency distributions from the structure K = 3 clusters for the cluster 1 and the most cluster 3 with ancestry greater than 0.90 in STRUCTURE analysis to train frequencies of "pure" individuals. All individuals from southern cluster 2 have the highest posterior probability of being from the pure southerly population rather than any hybrid class.

Analyses between each of the species within the subgenus and between the Southern clusters of *S. miniatus* and *S. crocotulus* indicate that no detectable hybridization is currently occurring between any of the recognized members of the subgenus or clusters of *S. miniatus*. The *S. miniatus* K = 3 cluster 2 individuals do not appear to be hybrids of cluster 1 and cluster 3 as hypothesized in DIY ABC scenarios 10 for the full subgenus (Figure 7) and scenario 6 for *S. miniatus* alone (Figure 9). The cluster 2 and cluster 3 individuals appear to be clustered together. Even when individuals with high probability of ancestry in any one cluster were used as pure classes to train the algorithm on prior information regarding allele frequencies in each cluster on individuals strongly assigned to a given cluster, the results remained the same, no hybrid individuals were identified. These results may indicate that no hybridization is occurring between populations of *S. miniatus*. Alternatively, the sampling, analyzed loci or the number of loci are insufficient to identify the hybrid individuals that may actually exist.

Discussion

In this study we explored a number of factors related to the current genetic diversity, spatial distribution of genetic variation, hybridization, evolutionary models of genetic variation and timing of divergence in the subgenus *Rosicola*. The phylogeography of the species in the *Rosicola* and distribution of genetic differentiation *S. miniatus* in our study is consistent with previous findings and expands on what is known about the nature and timing of evolutionary processes contributing to its formation. Interpretation of the results provides insight to guide prioritization of other species for genetic analysis and points the direction for future research to resolve remaining questions.

Mutation is contributing significantly to genetic differentiation between the three species in the subgenus, combined with very high and significant indices of genetic differentiation, which along with the lack of hybridization and admixture in BAPS and STRUCURE analyses indicate three deeply diverged species with no clear signs of introgression on secondary contact. The three clusters identified using the program STRUCTURE identified what are assumed to be three populations of *S. miniatus* identified by adherence to Hardy Weinberg and linkage equilibrium expected in

randomly breeding populations in panmixis. Timing of divergence was consistent with the hypothesized period in the Pleistocene and early Holocene, during periods subject to glaciation affecting sea level, currents and water temperature along the Pacific coast of North America (Jacobs *et al.* 2004).

Evidence of northward expansion in cluster 1 and a potential founder effect was reflected in the lower allelic diversity, smaller effective population size and topology of scenarios supported by modeling in DIY ABC. In addition, the northerly distribution of cluster 1, the presence of private alleles and population size reduction subsequent to divergence was not excluded as a potential scenario of evolutionary history, all consistent with this population potentially being the result of a northward expansion during an interglacial period (Figure 9). There was no signal of a bottleneck in any of the populations or species, though the change in population size associated with northward expansion may have been sufficiently far in the past for tests focused on relationships between allelic diversity and heterozygosity to no longer be able to detect a bottleneck if it occurred, as equilibrium may have resumed. Alternatively, if a bottleneck did occur, it may not have been of sufficient depth or duration to significantly affect heterozygosity relative to allelic diversity.

Though F_{ST} and R_{ST} values would indicate a closer relationship between individuals assigned to cluster 2 and cluster 3, the preferred models from DIY ABC showed earlier divergence from cluster 3 consistent with indices of abundance. An alternative model reflects admixture between cluster 1 and 3 producing cluster 2, though New Hybrid produced very few instances of individuals assigned to hybrid classes; mainly F2 and backcrossed individuals mostly reflecting low posterior probability of assignment to a given class. It is possible that there were once ancient hybrid events that are now far enough in the past that hybridization is no longer detected. There is also the possibility that the six microsatelite loci employed may not have been sufficiently informative to clearly identify hybrid individuals and more loci may be necessary to identify hybrid classes. While we did not identify individuals with consistent hybrid genotypes in New Hybrid, the program STRUCTURE does show some sharing of posterior probability of ancestry among the *S. miniatus* populations, with relatively low shared probability of ancestry between species consistent with the depth of divergence.

Though we have referred to the clusters within *S. miniatus* as being populations, the line between populations and species is not definitive and some could be considered incipient species given the relative magnitude of divergence between clusters identified with the program STRUCTURE. The gopher rockfish *S. carnatus* and its sister species the black and yellow rockfish *S. chrysomelas* have similar coloration patterns and an F_{ST} of 0.046, measured using some of the same loci employed in our study. This level of lower than the magnitude of differentiation between *S. miniatus* clusters 1 and 3 with an F_{ST} of 0.080 or the F_{ST} of 0.055 between clusters 1 and 2, while only slightly higher than the F_{ST} of 0.028 between clusters 2 and 3, indicative of strong differentiation on par with levels recognized between species in the *Sebastes*. Though, we perceive their bright red coloration and gestalt appearance as indicating the same species, differences observed between solid brick red and mottled orange-red coloration have been dismissed as

variation within the species; the depth of differentiation may point to separate species or populations at the least. Unfortunately, neither photos of the original subject fish nor carcasses were taken to allow testing for associations of cluster assignments with coloration or meristics or morphometric characters. Thus *S. crocotulus* may not be the only cryptic species within the *Rosicola*, though similarity in appearance makes accounting for the presence of separate populations or species in management difficult if not impossible.

Concordance with other subgenera in the Sebastes

Most sister species within the *Sebastes* are distributed in close geographic proximity (Hyde and Vetter 2007) and differences in the distribution of sister species within a subgenus are associated with latitude and depth *(*Li *et al.* 2006, Narum *et al.* 2004, Cope 2004, Burford and Bernardi 2008, Hyde *et al.* 2008, Gharett *et al.* 2005). Within the subgenus *Pteropodus*, *S. carnatus* is distributed farther to the north and in deeper water than its sister species *S. chrysomelas* (Narum 2004). In the subgenus *Sebastosomus, S. entomelas* is found at deeper depths and further north than *S. mystinus,* while within *S. mystinus,* a second population or incipient species is distributed further north and in deeper depths than the other with a transition zone near Cape Mendocino (Cope 2004, Burford and Bernardi 2008). The repeated spatial pattern of differing depth and latitude distribution of sister taxa and populations may point to a common recurring mechanism of speciation across sub-genera (Burford and Bernardi 2008). Such patterns are also observed in the subgenus *Rosicola* (Hyde *et al.* 2008).

Within a single species, individuals are often distributed in deeper depths in the southern portion of their range than to the north where they occupy shallower depths (Love *et al.* 2002). Over time genetic differentiation as a result of isolation at the extremes of the range may result in population structure. During the subsequent glaciation, population centers of the more northerly adapted population may shift south and overlap in latitude of the southern population, but adults may align with colder water in deeper depths in southerly latitudes. The extent of differentiation through isolation by distance during the interglacial may be sufficient prevent interbreeding on secondary contact during an interglacial period (ie cluster 1). Thus the apparent pattern of paedomorphosis due to concatenation of depth distribution may be the result of northward expansion during glaciation and subsequent southerly range shifts aligning with colder water in deeper depths than the source population (cluster 2) in regufia of the California bight occupying the same latitude during glacial maxima. Alternatively, isolation in side and outside of the channel islands by separate currents inshore and offshore minimizing gene flow and differential environmental conditions may promote adaptation may lead to differentiation (ie cluster 3). These potential mechanisms are nonexclusive and could result in speciation in both glaciations through partial isolation under vicariance and adaptation to environmental conditions between water bodies posed by the Channel Islands and interglacial periods through northward expansion, isolation by distance and adaptation to cooler water temperatures.

Demographic Processes

Concerns were expressed by Hyde *et al.* (2008) regarding redistribution of fishing effort onto *S. miniatus* since the year 2000 when the Rockfish Conservation Area was implemented that has restricted fishing to less than 60 fm to minimize bycatch of overfished rockfish species that occurring in deeper depths including cowcod and bocaccio. Marine fish populations are often made up of thousands if not millions of individuals, thus declines to 25% of the population size considered to be overfished under the Pacific Fishery Management Council criteria for minimum stock size threshold may not have declined to a population size resulting in a detectable genetic bottleneck. In addition, many marine fish species including the *Sebastes*, exhibiting multiple paternities despite internal fertilization, are polygamous, thus maintaining greater allelic variation with fewer individuals. Though the effective population sizes from DIY ABC analysis indicated only thousands of individuals, given the late age of maturation of the *Rosicola*, a large proportion of the population may be composed of juvenile fish not currently contributing to the effective population size, but is part of the census population actively fished years before maturing to be part of Ne.

Detection of genetic bottlenecks due to exploitation was of particular interest, but more extreme non-anthropogenic reductions in population size can occur due to climate shifts. In either case, no evidence for bottlenecks was found at the population or species level. The tests for heterozygosity excess can detect bottlenecks for a window of time after a bottleneck has been initiated since mutations eventually fill the gaps between expected and observed heterozygosity. Power analyses and theoretical models suggest that a bottleneck of $Ne = 50$ is likely to be detectable for 25-250 generations (0.25 - 2.5 times 2Ne) after the initiation of a population reduction. Thus only recent historical population declines are detectable. Though populations may not have declined to a level resulting in detectable heterozygote excess, less severe reductions in population size can still increase the rate of genetic drift contributing to differentiation or rates of divergence under selection. These factors may contribute to population structure and later speciation during shifts in ranges of stocks or divergence at the edges of a species range during a glacial maximum as changing environmental conditions may contribute to the rate of drift, hastening divergence.

Hybridization in the subgenus

The lack of assignment of individuals to hybrid categories may reflect difficulty in identifying hybrid individuals with existing loci or number of loci if they exist. This applies to species as well as cluster level analyses. This may be indicative of potential incipient speciation in *S. miniatus* with similar spatial pattern of population structure as *S. crocotulus*, *S. pinniger* and *S. miniatus*. If populations are no longer interbreeding, this would provide additional support for independent management and implicate potential behavioral characteristics or spatial separation of adults creating barriers to mating on secondary contact. Though hybridization has been detected in other species within the *Sebastes* of the subgenus *Pteropodus* (Seeb *et al.* 1998, Buonacorsi 2005), our study did not detect hybridization or introgression among species in the subgenus *Rosicola* or clusters of *S. miniatus*.

Population structure observed in *S. miniatus* cluster 3 may have arisen from isolation outside the Channel Island chain or in separate refugia from cluster 2 within the Southern California Bight during recent glacial maxima resulting in divergence and secondary contact with or without hybridization between populations with different primary adult depth distributions. Though the overlap in depth distribution may bring gravid females of shallower dwelling clusters into contact with earlier maturing males of deeper dwelling clusters as well as *S. crocotulus* providing potential for hybridization, our analysis did not detect any. Postzygotic isolation mechanisms such as asynchrony in timing or the location of spawning, differences in courtship behavior or morphological characteristics may prevent mating between clusters of *S. miniatus* and the other two species on secondary contact.

Theoretical considerations and topics for further research

We theorize that differential depth distribution interpreted as paedomorphosis by concatenation of depth distribution in Hyde *et al.* (2008) may have arose under allopatric separation of populations subject to differential temperature regimes inside and outside the Channel Islands chain during Pleistocene glacial maxima. The current depth and latitudinal distribution of population structure may be associated with temperatures at depth consistent with refugia inside and outside the channel island chain during the last glacial maximum. Subsequently, the distribution of species can shift over time either within a generation through adult movement or over generations through larval settlement to where it is best adapted and most prolific making it difficult to determine the circumstances under which speciation took place in relatively continuous marine environment.

An alternative but non-exclusive hypothesis is that northward expansion from glacial refugia and subsequent founder effects and differential selection/adaptation to differential temperature regimes at higher latitudes resulted in population structure in the latitudinal axis. Northward expansion during interglacial periods into shallower northerly depths with warmer water temperatures more similar to those found in refugia during glacial periods may have left a pattern of gene surfing due to the increase in the frequency of alleles for new traits adaptive to conditions at the northern extreme of their distribution (Edmonds *et al.* 2004; Hallatschek and Nelson 2008). This may be an additional factor contributing to genetic differentiation and eventual speciation in the *Sebastes*.

In combination, vicariance during glacial periods followed by northward expansion of populations during interglacial periods may both contribute to the speciation process. The cyclic nature of the glaciation process during historical and recent glaciations may have resulted in repeated speciation events throughout the genus *Sebastes*. Expansion with subsequent isolation by distance or barrier may not have succeeded or there may not be sufficient isolation outside of the Channel Islands with subsequent introgression or extirpation event, any of which could result in inconsistencies across subgenera instead of concordance. Differences in life history and distribution within a subgenus may also be more or less conducive to gene flow or isolation affecting formation of population structure and subsequent speciation. As a result, though the resulting pattern in the topology of their phylogeography may vary, there is potential for similar cyclical drivers

to form repeated speciation events further diversifying the genus with successive glaciations.

Analysis of sequence data or single nucleotide polymorphisms with complementary methods may provide further insight into processes analyzed herein or a greater number of loci to provide greater power to detect processes for which conclusive results could not be derived with the few loci available in this study. Feed and temperature trials in mericultural experiments exploring the effects of water temperature on survival of larvae and adults to explore metabolic tolerances of each species and populations identified in *S. miniatus* may support hypotheses related to adaptation and alignment with specific water temperatures. Additional research into the distribution of larvae of each cluster identified in *S. miniatus* and the other congeners in the *Rosicola* would provide further support regarding temperature tolerances and the distribution of populations or species. Ecological niche modeling using this tolerance data and climatological data on water temperature could be used to explore these scenarios from a bioenergetic perspective of tolerance testing as conducted in Bigg *et al.* (2008) for Atlantic Cod to provide a means to further test proposed hypotheses related to water temperature tolerances and the distribution of the clusters identified in S. miniatus and the other congeners of the subgenus Rosicola. While our study provides additional insight into the factors contributing to population structure in the subgenus Rosicola, there are inherent difficulties of identifying evolutionary mechanisms in the continuous marine environment. This requires analysis of multiple markers and application complementary methods provide a more comprehensive understanding of factors contributing to population evolution and diversity observed in the genus *Sebastes* in the North-Eastern Pacific.

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Summary

In this study, the population structure of three species in the subgenus *Rosicola* were analyzed with the same six microsatellite loci across their known range. This research was aimed at properly delineating individual breeding populations, which is essential to the proper assessment and management groundfish stocks to ensure sustainable yields, prevent overfishing and rebuild overfished stocks. This provided an opportunity to evaluate the coastwide pattern of population structure for closely related species with differing life history characteristics such as presence or absence of schooling behavior and timing of spawning affecting gene flow. In addition, the three species within the subgenus *Rosicola* have different latitudinal and depth distributions, though as a group, they extended across the Aleutian, Oregonian and San Diegan biogeographic regions subject to differing currents and environmental conditions including water temperature. Samples of each species were collected from throughout the known range of each species to allow a comprehensive analysis of population structure to inform management and to provide context for further analysis of the evolutionary mechanisms that may contribute to population structure or lack there of.

We also sought to elucidate the underlying evolutionary forces that have driven microevolution in the *Rosicola* as a model for understanding speciation in the *Sebastes* as a whole and test for concordance in these processes by comparing our results to previous studies in other subgenera. To this end, we tested various models representing possible evolutionary scenarios to gain further insight in to the forces shaping population structure and speciation in the *Rosicola*. In the interest of providing a comprehensive analysis we reviewed literature from previous studies and identify commonalities that may be indicative of shared and repeated patterns of genetic differentiation associated with geographic breaks or evolutionary forces shaping population structure in the *Sebastes* as a whole. The intended outcome was a better understanding of population structure for use in assessment of abundance and management of these species. In addition, we sought to provide an improved understanding of the evolutionary process in the *Sebastes* and patterns aiding in selection of high priority species for future analysis.

In chapter 1, no population structure was identified in *S. pinniger* in indices of differentiation or clustering methods employed in BAPS and STRUCTURE. Though private alleles were identified in the northern extent of their range, this schooling species known to move large distances as adults as well as timing of spawning consistent with maximum upwelling and southward flow of the California current provided results indicative of high gene flow and limited population structure. Despite the population having been assessed to have recently been fished to below levels considered to provide maximum yield, there was no evidence of significant genetic bottlenecks from exploitation or non-anthropogenic causes.

Similarly the results of analysis of *S. crocotulus* the recently identified cryptic species to *S. miniatus* (Hyde *et al.* 2008) displayed negligible population structure across its limited range south of Point Conception. Its more solitary demersal life history and spawning

during late fall when upwelling is weaker would suggest the potential for more restricted gene flow. To the contrary, the gyre formed by currents within the Southern California bight may broadly distribute its larvae and its distribution in deeper waters less subject to friction from bottom substrate forming "sticky water" in shallower depths may facilitate gene flow and result in the lack of apparent population structure. The range of the stock from Point Conception to the Baja peninsula may be sufficiently limited that adult migration or larval movement has resulted in a relatively uniform distribution of genetic variation.

The more solitary but broadly distributed *S. miniatus* appears to contain residual population structure even after having accounted for the presence of the cryptic species *S. crocotulus.* Not only was latitudinal population structure identified, but genetic structure was also identified with depth. Point Conception was again identified as the location of strong population structure with cluster 1 primarily distributed to the north and cluster 3 primarily distributed to the south. Cluster 2 is distributed in an intermediate depth and latitude to cluster 1 and cluster 3. While the depth and latitudinal distribution may be intermediate, tests for hybridization in the program New Hybrids did not identify hybrid individuals among clusters identified in STRUCTURE or between species with the available loci.

Similar spatial patterns genetic variation have been identified in other subgenera as discussed in Chapter 4. The pattern of genetic differentiation in other subgenera was more easily detected as a result of more apparent differential coloration of individuals of each species, though recently diverged cryptic sister species have also been identified in blue rockfish, *S. mystinus* (Cope 2004, Burford and Bernardi 2008). The coloration of *S. miniatus* and *S. crocotulus* varies to a lesser extent from more solid to blotchy and red to orange, within a given region, but had largely been dismissed as color variation within a single species. Perhaps the degree of differences in coloration have been constrained by the benefit derived from reddish coloration since red light is the first wavelength to be filtered the visible spectrum in marine waters, leaving them camouflaged at deeper depths. It is possible that what color variation is observed may have an underlying genetic component. Future research could focus on identifying meristic, morphometric or visual differences that can be used in the field to easily discriminate between species and populations. Though there known differences in coloration, the range of gill raker counts and eye width and caudal peduncle height ratios to length identified in Hyde *et al.* (2008), they do not lend themselves to efficient identification by anglers, fishermen or samplers in the field.

The resulting difficulties in stratifying historical catch or assigning encountered individual to species or populations in developing indices of abundance hampers stock assessment efforts. Should identification methods be developed or genetic methods be used to discriminate between them, this may improve stock assessments in the future, but data from the past cannot be resolved limiting the time series available. Identification of individuals belonging to each stock or species in the future would require more extensive training of field samplers should characteristics be developed or expensive laboratory testing in a sustained effort to develop a time series of catch and indices of abundance to

inform assessments. While this would improve prospects for a sufficiently informed stock assessment in the future, historical catch data and encounter rates informing indices of abundance are difficult if not impossible to reconcile. At present the only option may be to assess the stock north and south of Point Conception and treat the stock to the south as a complex containing clusters 2, 3 and *S. miniatus,* assuming the same growth rates, recruitment patterns and age of maturation between them. Differential exploitation due to current depth restrictions may result in violation of the assumption that they have been harvested at the same rate, potentially leaving *S. miniatus* more depleted than *S. crocotulus*. Harvest rates may be set at precautionary levels to account for the uncertainty in assessment results, while efforts are made to improve knowledge catch and the stock composition to improve future assessments. Separate assessment of cluster 1 north of Point Conception may be possible and an annual catch target set in the area north of Point Conception below the aggregate catch limit for the complex to restrict catch of this stock.

Our results point out the need to test for genetic differentiation by depth as well as latitude, distance or other factors contributing to isolation along the coast such as diverging currents etc.. While the interaction of life history, geomorphology and oceanographic factors contribute to the observed population structure, some commonalities may exist. We tested several evolutionary scenarios to identify the order of divergence within the subgenus and potential scenarios leading to divergence. While the order of divergence was apparent, our study was inconclusive relative to the potential for admixture to contribute to population structure, reduction in effective population size after divergence or divergence between stable populations due to isolation during glacial and interglacial cycles, the latter was supported, having the highest logistic regression result. Thus isolation of populations inside and outside the Channel Islands during glaciations and northward expansion with isolation by distance and local adaptation during interglacial periods followed by a lack of hybridization on secondary contact are potential mechanisms contributing to the development of genetic divergence leading to speciation in this and other subgenera. Though concordance is identified in other subgenera, results are not directly comparable in part due to the possibility that divergence may not have prevented admixture on secondary contact or that genetically diverged population segments have been lost. Recent glaciations during the Pleistocene and Holocene have provided opportunities for repeated cycles of differentiation resulting in population structure, which may lead to speciation. Our estimates of the timing of divergence between clusters identified in *S. miniatus* are consistent with the Pleistocene, making it plausible that such mechanisms have contributed to population observed in this species.

Previous attempts by Berntson and Moran (2009) indicated that species found in shallower depths subject to friction from bottom structure on currents referred to as "sticky water" were more likely to have restricted geneflow and thus more likely to display population structure, thus nearshore rockfish species may be a higher priority for future analysis. Variability in coloration across a species range however subtle may be another indication of underlying population structure as may be the case for *S. miniatus* and *S. crocotulus*, thus species like the copper rockfish *S. carnatus*. The results of our

study indicate that the greatest potential for latent population structure to be identified is in species with broad depth distributions and geographic range making such species a priority. In addition, species with ranges that cross Point Conception and other landmarks consistent with changes in the direction or currents or biogeographic regions along the coast may have greater potential for population structure. Given the presence of private alleles to the north in *S. pinniger* and cluster 1 of *S. miniatus*, species distributed on either side of the divergence between the California and Alaskan current could be a priority for further analysis should the species be commonly encountered north into waters of Alaska including the China rockfish *S. nebulosus* and Quillback rockfish *S. maliger* (Love *et al.* 2002), which have not been analyzed to date.

First sampling a substantial number of individuals from a number of species at the extremes of their range to identify the presence of structure on a range wide basis and at the extremes of its depth distribution in each location may be the most efficient approach to test for the presence of population structure. This can be followed by further sampling at a higher spatial resolution across their range to better delineate population structure at a finer scale informing stratification of assessment and management. This two step process may be a more efficient means of identifying structure rather than initial higher resolution analysis of fewer species from the outset.

In addition to historical events, contemporary migration can maintain and reinforce population structure in the *Sebastes* during either larval or adult life history stages (Love *et al.* 2002). Larvae travel in the upper water column with the current for 3-4 months before settling out on reefs or other substrate where they will grow to adults (Moser and Boehlert 1991). Alaskan Current moves north from central Oregon diverging from southward flowing California Current in the Oregonian biogeographic province (Jacobs 2004). Within the California bight the California current a shears off the coast and weakens near point Conception while a gyre is formed within the bight moving northward along the coast shoreward of the Channel Islands in the San Diegan biogeographic province (Jacobs *et al.* 2004). Attention to the distribution of the species relative to these currents, the timing of spawning and other life history characteristics may also provide information pertinent to prioritization of species for future analysis.

Migration during the larval phase varies by species and depends on the seasonal timing of parturition, strength of the current and the duration of the larval stage. Environmental conditions can determine the distribution of species across the pacific coast and allow or prohibit migration between regions depending on the environmental tolerances of each species, which may change as will conditions over time. The interplay between environmental conditions, geomorphology of the coast and life history of the species and its tolerances and ability to adapt to new environments will determine the distribution of the species, fluctuations in recruitment and population structure that should be accounted for in fishery management, especially when stock complexes are used in management. Further analysis of the distribution of juvenile rockfish of *S. crocotulus* and clusters identified in *S. miniatus* clusters as well as feeding trials with water temperature as well as ecological distribution modeling (Bigg *et al.* 2008; Robinson *et al.* 2011) may be of use in identifying the environmental tolerances of each species. This research may provide further information indicative of conditions that contribute to the distribution of the species in the *Rosicola* and

similar research on other species in the *Sebastes* may help inform a broader framework to explain the potential distribution and conditions contributing to divergence during glacial cycles.

A comprehensive ecological distribution modeling analysis with a phylogeographic basis provided by the results of the phylogeny in Hyde (2007) combined with distribution information compiled in Love *et al.* (2002) could may further elucidate mechanisms involved in speciation and formation of population structure. In addition, such research would better inform prioritization of future genetic analysis of the remaining species within the more the 67 species in the Sebastes as candidates for genetic analysis. Continued research to understand the mechanisms contributing to speciation in the *Sebastes* and continued efforts to identify population structure in the *Sebastes* are essential for accurate assessment and management of species of this magnificently diverse genus.

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