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UNIVERSITY OF CALIFORNIA, SAN DIEGO

Planktonic Patterns and Processes in the Giant Kelp *Macrocystis pyrifera*

A dissertation submitted in partial satisfaction for the degree

Doctor of Philosophy in Oceanography

by

Michael Hall Graham

Committee in charge:

Paul K. Dayton, Chair

Nicholas D. Holland

Lisa A. Levin

B. Greg Mitchell

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2000

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To the historians of nature ...

who, with no intent of fame and fortune, tirelessly
seek to better understand the nature of our world.

**Its only an island if you
look at it from the water ...**

- Martin Brody (1975)

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VITA

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- Graham, M. H. 1996. Effect of high irradiance on recruitment of the giant kelp, *Macrocystis* (Phaeophyta), to shallow water. *Journal of Phycology* 32:903-906.
- Graham, M. H., C. Harrold, S. Lisin, K. Light, J. Watanabe, and M. S. Foster. 1997. Population dynamics of *Macrocystis pyrifera* along a wave exposure gradient. *Marine Ecology Progress Series* 148:269-279.
- Graham, M. H. 1997. Factors determining the upper limit of giant kelp, *Macrocystis pyrifera*, along the Monterey Peninsula, central California, USA. *Journal of Experimental Marine Biology and Ecology* 218:127-149.
- Graham, M. H. 1999. Where will all the PhDs go? *Trends in Ecology and Evolution* 5:208.
- Graham, M. H. and B. G. Mitchell. 1999. Obtaining absorption spectra from individual macroalgal spores using microphotometry. *Hydrobiologia* 398/399:231-239.
- Graham, M. H. 1999. Identification of kelp zoospores from in situ plankton samples. *Marine Biology* 135:709-720.

ABSTRACT OF THE DISSERTATION

Planktonic Patterns and Processes in the Giant Kelp *Macrocystis pyrifera*

by

Michael H. Graham

Doctor of Philosophy in Oceanography

University of California, San Diego, 2000

Professor Paul K. Dayton, Chair

Propagule supply is fundamental in regulating the strength of demographic and genetic interactions in natural populations. In marine systems, recent studies focusing on benthic fish and invertebrate species with long planktonic durations have found that propagule production and supply are de-coupled by physical transport processes. Most benthic marine populations therefore have been considered demographically open, whereby recruitment is driven by remote propagule production. Few studies have focused on species with shorter planktonic durations (e. g., seaweeds). I developed techniques for *in situ* sampling and identification of kelp zoospores and used them to study coupling between giant kelp (*Macrocystis pyrifera*) zoospore production and supply in the Point Loma kelp forest during 1999. The techniques were based on instrumentation for (1) concentrating and isolating planktonic particles from sea water and (2) obtaining absorption spectra from individual kelp zoospores. Absorption spectra were found to be species-specific and useful for classifying individual zoospores among southern California kelp taxa. As such, giant

kelp zoospores were quantified from sea water collected in the forest interior at intervals spanning minutes to months. Temporal variability in zoospore concentration was random and relatively constant at intervals < 24 hr, but highly structured at longer time scales with large fluctuations reflecting changes in adult reproductive condition. Reproductive biomass, fertility, and size-structure of adults found within 100 m² of the zoospore sampling location explained greater than 75% of temporal variability in zoospore supply. The tight coupling between zoospore production and supply was due to a lack of strong currents in the forest interior; drag of adult plants dampened flow keeping zoospores close to their release sites. Coupling was validated at two additional interior sites. Lower plant densities along the forest edges, however, resulted in rapid uni-directional flows likely transporting zoospores far from adults. Here, sampling indicated that zoospore supply was decoupled from local zoospore production. These results therefore suggest that kelp populations are not simply open or closed, but that the extent of reproductive coupling likely exists along a continuum, due to the scale-dependent contribution of local versus remote propagule supply.

Chapter I

Introduction to the dissertation

“I know few things more surprising than to see this plant [giant kelp, *Macrocystis pyrifera*] growing and flourishing from amidst those great breakers of the western ocean, which no masses of rock, let it be ever so hard, can long resist”

- Charles Darwin, Voyage of the Beagle (1839)

The cohesion of biological species is maintained by demographic and genetic interactions that act across broad temporal and spatial scales (Grosberg and Cunningham 2000). Such interactions occur among life-history stages, among individuals within populations, and even among the distinct populations that comprise a species distribution. My primary research interests are in the processes that regulate the strength of these interactions. It is the breakdown of demographic and genetic exchange that ultimately results in population subdivision, divergence, and eventually speciation or extinction. I study kelps because they provide a system amenable to empirical investigation of these interactions across relevant ecological scales.

At the population scale, researchers have often asked whether species are demographically open or closed (reviewed in Caley et al. 1996). In open populations, recruitment comes from remotely produced propagules, and recruitment and local reproduction are generally independent of each other. In closed populations, however, recruitment comes from locally produced propagules, and as such recruitment is coupled to local reproduction. The dichotomous distinction between open versus closed population dynamics has been considered important, as it helps to describe the extent to which different

populations within a species are demographically and genetically connected (Caley et al. 1996, Cowen et al. 2000).

These population-level interactions ultimately depend on both (1) recruitment rates and (2) the origin of recruits. In marine systems, recent studies focusing on benthic fish and invertebrates with long planktonic durations have found that propagule production and supply are de-coupled by physical transport processes (e. g., Roughgarden et al. 1988, Wing et al. 1998, Shanks et al. 2000), although some species have evolved mechanisms by which larvae are retained near adults (e. g., Jones et al. 1999, Swearer et al. 1999, Cowen et al. 2000). Most benthic marine populations therefore have been considered demographically open, whereby recruitment is driven by remote propagule production (Caley et al. 1996).

Kelps and other seaweeds have life histories to similar those of most benthic marine animals, as they alternate between distinct benthic and planktonic stages (Figure 1). Adult benthic sporophytes release microscopic zoospores into the plankton, which settle onto hard substrate where they germinate into either male or female gametophytes. After a week or so, gametes are released, fertilization occurs, and a new benthic sporophyte generation grows to adult size. The difference between kelps and most fish or invertebrates, however, is that the kelp planktonic dispersal stage lasts only as long as it takes zoospores to reach suitable settlement substrate, which can be anywhere from minutes to a couple of days (B. Gaylord et al. manuscript in review). Given this shorter planktonic duration, the demographic interactions within and among kelp populations may be inherently different than those popularized by contemporary studies.

The planktonic nature of many marine propagules results in a strong link between dispersal and oceanographic variability. The diverse morphological design of marine propagules and the great variety of marine life histories further suggest broad temporal and spatial variability in the strength of this linkage. Physical transport due to turbulence,

internal waves, and tides, as well as propagule swimming and diel cues to reproductive output, can result in significant variability in dispersal at scales of minutes to days; the occurrence of storms, current variability, and lunar cycles act on dispersal at the scale of days to months; seasonal shifts in oceanographic climate and adult reproduction, grazing outbreaks, and dormancy are relevant at scales of months to seasons; ENSO, annual fluctuations in adult density, climate change, and habitat loss work from years to decades; and changes in eustatic sea level, plate tectonics, glaciation, and population extinctions and migrations act at centuries and beyond. That dispersal variability can exist across such broad temporal and spatial scales, even within individual species, argues against a dichotomous “open versus closed” approach to population dynamics. In fact, the scale at which some species or populations are open may simply mark the scale at which others are closed. A fundamental goal of my research was to visualize the continuum across which the strength of interactions among individuals vary within a species.

The giant kelp (*Macrocystis pyrifera*) provides a model system for investigating the continuity of demographic interactions within marine species. Giant kelp has a truly global distribution, ranging from central to Baja California in the Northern Hemisphere, and from Chile and Argentina to South Africa, Australia, New Zealand, and many sub-Antarctic islands in the Southern Hemisphere. This distribution is not continuous, however, with discrete populations of various sizes isolated by as little as 50 m to over 1000's kms. Furthermore, the ecological and economic importance of giant kelp has fueled extensive laboratory research on giant kelp life histories, gametogenesis, fertilization, and propagule characteristics (reviewed in North 1994), and field research on giant kelp distribution and abundance (reviewed in Foster and Schiel 1985). Yet, our knowledge of giant kelp propagule transport, propagule origin, and the link between propagule production, supply, and recruitment is poor.

The primary limitation to studies of kelp planktonic processes has been the lack of techniques for studying plankton abundance *in situ*. Planktonic propagule abundance has never been determined directly for any seaweed species. This is because seaweed propagules are microscopic and generally featureless, and are thus hard to isolate from other particles in plankton samples. The only previous studies of seaweed dispersal have been limited to either laboratory studies of propagule characteristics (e. g., swimming, sinking, photosynthesis; Hoffman and Camus 1989, Reed et al. 1992, Beach et al. 1995, Reed et al. 1999) or field studies where propagules were allowed to settle and grow to a macroscopic and identifiable size (Hruby and Norton 1979, Amsler and Searles 1980, Hoffman and Ugarte 1985, Zechman and Mathieson 1985, Reed et al. 1988, Fredriksen et al. 1995, Santelices et al. 1995). The signal of dispersal and propagule transport, however, is thought to be blurred in these indirect studies due to post-settlement processes (Norton 1992). My research goals, therefore, required the development of new techniques for sampling, isolating, and identifying kelp zoospores from *in situ* plankton samples. Description and validation of these techniques comprise **Chapters II & III** of the dissertation.

The logic of my dissertation research was to build a small-scale mechanistic model of the extent of coupling between giant kelp zoospore production and supply, and then study the generality of this model in space and time. The lack of previous field studies on planktonic propagules of kelps necessitated the identification of spatial and temporal patterns in zoospore supply as a first step. By studying temporal variability in zoospore supply at a fixed site over a broad range in time I was able to untangle spatial effects from the concomitant effects of time. These studies are presented in **Chapter IV**. My goal was not simply to describe variability in zoospore supply, but to begin to understand the underlying processes that regulate zoospore production. Small-scale processes that regulate the reproductive output of giant kelp populations were studied in **Chapter V**. Finally, the studies were designed to specifically address the continuity of giant kelp demographic

interactions in space and time, and as such, the research may serve as a base to build future models of the dynamics of marine populations.

This study was designed to be a constructive and multi-disciplinary foray into previously untapped research areas in seaweed population dynamics. The research broke technological bounds to address questions that have long been of interests to phycologists, and ecologists in general. Yet, in the end, I posed more questions than I answered.

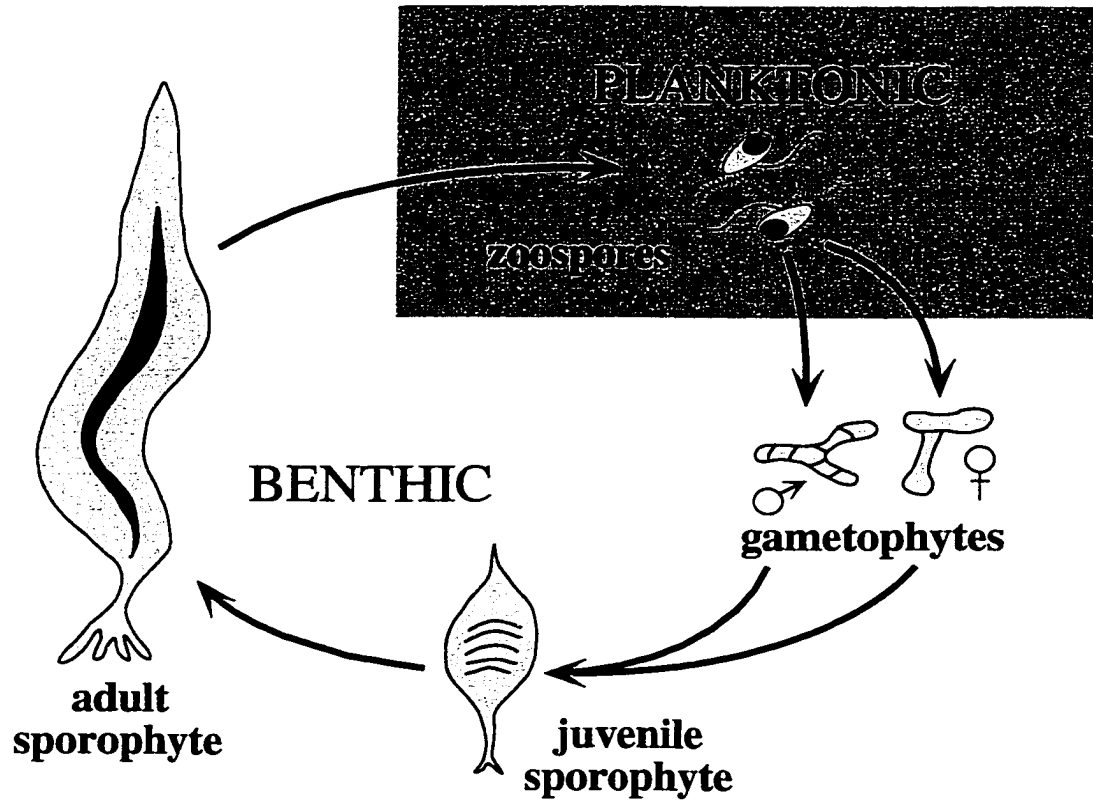


Figure 1-1. Typical kelp life-history.

Literature Cited

- Amsler, C. D., and R. B. Searles. 1980. Vertical distribution of seaweed spores in a water column offshore of North Carolina. *Journal of Phycology* 16:617-619.
- Beach, K. S., C. M. Smith, T. Michael, and H. Shin. 1995. Photosynthesis in reproductive unicells of *Ulva fasciata* and *Enteromorpha flexuosa*: implications for ecological success. *Marine Ecology Progress Series* 125:229-237.
- Caley, M. J., M. H. Carr, M. A. Hixon, T. P. Hughes, G. P. Jones, and B. A. Menge. 1996. Recruitment and the local dynamics of open marine populations. *Annual Review of Ecology and Systematics* 27:477-500.
- Cowen, R. K., K. M. M. Lwiza, S. Sponaugle, C. B. Paris, and D. B. Olson. 2000. Connectivity of marine populations: Open or closed? *Science* 287:857-859.
- Foster, M. S., and D. R. Schiel. 1985. The ecology of giant kelp forests in California: a community profile. United States Fish and Wildlife Service Biological Report 85:1-152.
- Fredriksen, S., K. Sjutun, T. E. Lein, and J. Rueness. 1995. Spore dispersal in *Laminaria hyperborea* (Laminariales, Phaeophyceae). *Sarsia* 80:47-54.
- Grosberg, R. K. and C. W. Cunningham. In press. Genetic structure in the sea: from populations to communities. Pages xxx-xxx in M. D. Bertness, S. Gaines and M. E. Hay, editors. *Marine Community Ecology*. Sinauer Associates, Sunderland.
- Hoffman, A. J., and P. Camus. 1989. Sinking rates and viability of spores from benthic algae in central Chile. *Journal of Experimental Marine Biology and Ecology* 126:281-191.
- Hoffman, A. J., and R. Ugarte. 1985. The arrival of propagules of marine macroalgae in the intertidal zone. *Journal of Experimental Marine Biology and Ecology* 92:83-95.
- Hruby, T., and T. A. Norton. 1979. Algal colonization on rocky shores in the Firth of Clyde. *Journal of Ecology* 67:65-77.
- Jones, G. P., M. J. Milicich, M. J. Emslie, and C. Lunow. 1999. Self-recruitment in a coral reef fish population. *Nature* 402:802-804.

- North, W. J. 1994. Review of *Macrocystis* biology. Pages 447-527 in I. Akatsuka, editor. *Biology of Economic Algae*. Academic Publishing, The Hague.
- Norton, T. A. 1992. Dispersal by macroalgae. *British Phycological Journal* 27:293-301.
- Reed, D. C., C. D. Amsler, and A. W. Ebeling. 1992. Dispersal in kelps: factors affecting spore swimming and competency. *Ecology* 73:1577-1585.
- Reed, D. C., M. A. Brzezinski, D. A. Coury, W. M. Graham, and R. L. Petty. 1999. Neutral lipids in macroalgal spores and their role in swimming. *Marine Biology* 133:737-744.
- Reed, D. C., D. R. Laur, and A. W. Ebeling. 1988. Variation in algal dispersal and recruitment: the importance of episodic events. *Ecological Monographs* 58:321-335.
- Roughgarden, J., S. Gaines, and H. Possingham. 1988. Recruitment dynamics in complex life cycles. *Science* 241:1460-1466.
- Santelices, B., A. J. Hoffman, D. Aedo, M. Bobadilla, and R. Otaiza. 1995. A bank of microscopic forms on disturbed boulders and stones in tide pools. *Marine Ecology Progress Series* 129:215-228.
- Shanks, A. L., J. Largier, L. Brink, J. Brubaker, and R. Hooff. 2000. Demonstration of the onshore transport of larval invertebrates by the shoreward movement of an upwelling front. *Limnology and Oceanography*. 45:230-236.
- Swearer, S. E., J. E. Caselle, D. W. Lea, and R. R. Warner. 1999. Larval retention and recruitment in an island population of a coral-reef fish. *Nature* 402:799-802.
- Wing, S. R., L. W. Botsford, S. V. Ralston, and J. L. Largier. 1998. Meroplanktonic distribution and circulation in a coastal retention zone of the northern California upwelling system. *Limnology and Oceanography* 43:1710-1721.
- Zechman, F. W., and A. C. Mathieson. 1985. The distribution of seaweed propagules in estuarine, coastal and offshore waters of New Hampshire, U S A. *Botanica Marina* 28:283-294.

Chapter II

Obtaining absorption spectra from individual macroalgal spores using microphotometry

Abstract

Information on the ecophysiology of macroalgal planktonic propagules (e. g., spores) has been hard to obtain, given their small size and low concentration in the water column. Studies of the photo-physiology of macroalgal spores, for example, have been limited by the need to aggregate many spores into bulk samples for analysis. Subsequently, physiological variability among spores (e. g., pigment concentration, absorption characteristics) is lost, and taxonomic comparisons from multi-taxa samples are impossible. Here I present a technique that utilizes a spectral microphotometer to produce visible (400-800 nm) absorption spectra from individual particles; the particles in this case are macroalgal spores. The microphotometer consists of a microscope fitted with a monochromator and spectrophotometer. After mounting spores from laboratory or field suspensions onto transparent membrane filters, absorption characteristics of individual spores, or even individual plastids, can be evaluated independently from the remaining particles in the sample. Use of transparent rather than opaque membrane filters allows for determination of absorption spectra, as well as more traditional microscopic analyses (e. g., bright field, dark field, epi-fluorescence). Glutaraldehyde fixation and cold storage (-10°C) were found to be appropriate for maintaining the integrity of absorption spectra for at least 3 days. To demonstrate the utility of microphotometry for macroalgal studies, absorption spectra were obtained and analyzed from spores of various kelps and filamentous red algae.

Introduction

As typical of most benthic marine organisms, marine macroalgal life histories alternate between separate benthic and planktonic stages. Although considerable information is available concerning the ecophysiology of macroalgal benthic stages (e. g., Lobban & Harrison 1994), laboratory studies have provided only minimal information on the ecophysiology of macroalgal planktonic propagules (Amsler & Neushul 1991, Brzezinski et al. 1993, Beach et al. 1995), while the ecophysiology of the propagules *in situ* remains completely unknown. This dearth of information concerning macroalgal propagules is primarily due to (1) difficulties in sampling and identifying the small and often rare propagules, and (2) technological limitations that prohibit the analysis of propagules on an individual basis. As an example of the latter, recent laboratory studies of the photo-physiology of brown (Amsler & Neushul 1991) and green macroalgal zoospores (Beach et al. 1995) required the analysis of bulk zoospore samples in order to utilize traditional phycological methods (e. g., spectrophotometry, fluorometry, HPLC, light/dark incubations). As such, variability in physiological variables (e. g., pigment concentrations, absorption spectra) among individual zoospores, of the same taxa or between taxa, is averaged away. Flow cytometry has been used to study some aspects of the physiology of individual kelp zoospores (Brzezinski et al. 1993), however, the large sample sizes required for analysis and the inability to observe directly the particles being analyzed makes this method impractical for most applications, especially those utilizing multi-taxa field samples.

Microphotometric methods have been developed for determining *in vivo* absorption spectra for individual microalgal cells (Iturriaga et al. 1988). These microphotometric systems typically consist of a microscope fitted with a spectrophotometer and scanning monochromator, and work by aligning a transparent aperture over individual particles through which spectral light transmittance (or absorbance) is measured. The result is continuous transmission (or absorption) spectra over a range of wavelengths determined by

the optical characteristics of the system. Although application of such methods by phytoplankton ecologists has been described (Iturriaga et al. 1988, Iturriaga & Siegel 1989, Carpenter et al. 1990, 1991, Bidigare et al. 1993, Robinson et al. 1995, Stephens 1995), microphotometry has yet to be used in macroalgal studies.

Here I present a refined method for obtaining absorption spectra from macroalgal spores using microphotometry. The method was developed to provide absorption spectra for species-specific identification of individual kelp zoospores from *in situ* water samples, and is part of a larger investigation into the role of pre-settlement processes on the dynamics of subtidal kelp populations. Microphotometry, however, should prove useful to those interested in general photo-physiology of macroalgal spores and thalli. The method includes description of: (1) preparation (fixation and mounting) of macroalgal spore samples for use with the microphotometry system; (2) procedures for obtaining absorption spectra from mounted spore samples; (3) techniques for storing mounted spore samples that preserve the integrity of spore absorption characteristics. In addition, I reveal the advantage of microphotometry over traditional spectrophotometric methods by examining absorption spectra from: (1) individual zoospores of two co-occurring species of subtidal kelp (*Macrocystis pyrifera* (L.) C. Agardh and *Pterygophora californica* Ruprecht); and (2) individual tetraspores within attached tetrasporangia of two species of filamentous red algae (*Callithamnion biserialatum* Kylin and *Scagelia pylaisaei* [Montagne] Wynne).

Methods

Sample preparation

Kelp zoospores - *Macrocystis* and *Pterygophora* sporophylls were collected from 15 m depth within the Pt. Loma kelp forest, southern California, USA in April 1997.

Sporophylls with sori were trimmed, wrapped in moist paper towels, sealed in dry plastic bags, and transported to the laboratory in coolers at ambient temperature. Zoospore

suspensions were created 4 to 6 hours later by re-immersing sporophylls in 500 ml of 15°C 0.2 μm filtered seawater (FSW) under cool white fluorescent lighting. Zoospores were fixed by mixing 1 ml aliquots of zoospore suspensions with 4 ml of 2.5% glutaraldehyde in FSW (2% final concentration); aliquots were taken from the upper 1 cm of zoospore suspensions in order to sample motile zoospores only. Fixed zoospore samples were vacuum filtered onto Whatman Cyclopore transparent polycarbonate membrane filters (25 mm filter diameter; 1.0 μm pore diameter) under low pressure (< 1 cm of mercury). The last 1 mm of suspension was released from vacuum pressure and gravity filtered in order to minimize zoospore damage (Iturriaga & Siegel 1989). Filters were air-dried until all visible moisture had evaporated, and mounted (zoospores up) between glass microscope slides and cover slips using Cargille fluorescence microscopy immersion oil (Type FF). Zoospore movement on the filters during mounting was minimized by coating slides and coverslips with immersion oil before placement of the filters. Coverslips were sealed to the slides using clear nail polish.

Red algal tetraspores - Tetrasporangial thalli of *Callithamnion* and *Scagelia* were supplied from San Juan Islands, Washington, USA by Dr. David Garbary (Garbary et al. 1999). The plants were collected subtidally from 5 - 10 m depth. All thalli were fixed in 2% glutaraldehyde and gravity filtered onto Whatman Cyclopore transparent polycarbonate membrane filters (25 mm filter diameter; 3.0 μm pore diameter). Filters were mounted to microscope slides as described above for kelp zoospores.

Microphotometry

The microphotometric system consisted of a universal microscope (Olympus Provis AX70, Lake Success, New York) equipped with a Nano500 diode-array spectrophotometer and a halogen light source (Nanometrics, Sunnyvale, California). A circular aperture,

through which irradiance was sampled, was placed between the objective and monochromator. Light passing through the sample and aperture was projected onto the monochromator (at the focal plane) which dispersed the spectrum onto a linear diode-array detector. This system recorded relative spectral irradiance over a range of 400 - 800 nm (1.2 nm intervals).

Irradiance transmitted through each spore, $I_{s(\lambda)}$ was determined by placing the aperture either directly over individual kelp zoospore plastids (kelp zoospores have single discoid plastids, Figure 2-1; Henry & Cole 1982) or within individual red algal tetraspores (Figure 2-2). To facilitate alignment, the sample image was superimposed with an image of the aperture onto a video system; a monitor allowed the aperture to be maneuvered precisely along the sample without using the oculars. Irradiance incident on the spore (the blank), $I_{b(\lambda)}$ was determined by placing the aperture over an adjacent particle-free area without changing the focus. Background current noise was measured prior to sampling each mounted filter and was subtracted from each spectral scan of that filter (both $I_{s(\lambda)}$ and $I_{b(\lambda)}$). Absorbance (optical density) for each scan was determined using:

$$A_{\lambda} = -\log(I_{s(\lambda)}/I_{b(\lambda)}),$$

where A_{λ} is the absorbance at a specified wavelength, λ . The system required < 5 s to obtain a final absorption spectra. All measurements were made in a dark room. Measurements for kelp zoospores were made with a 100x oil-immersion objective, while measurements for red algal tetraspores were made with a 40x objective. The aperture had a 1.83 μm diameter and 2.63 μm^2 cross-sectional area when using the 100x oil-immersion objective, and a 4.53 μm diameter and 16.18 μm^2 cross-sectional area when using the 40x objective. For kelp zoospores, plastid diameter varied greatly between species (Figure 2-1, see Results) and A_{λ} was affected by the cross-sectional area of the aperture occupied by the plastids; only *Pterygophora* plastids were larger than the aperture. All red algal tetraspores were larger than the aperture. Thus, absorption spectra obtained by the above method

represent true *in vivo* individual-particle (plastid) absorption spectra (*sensu* Iturriaga et al. 1988) only for *Pterygophora* and the red algae. However, since the aperture diameter was fixed and dependent only on the level of magnification, resulting absorption spectra at the same magnification for different cells can be compared within and among species.

Smoothing of each final absorption spectrum was done using the Savitsky-Golay algorithm which performs a least-squares polynomial fit to a specified number of convolution points (Savitsky & Golay 1964, Steinier et al. 1972, Madden 1978). I empirically determined that second-degree polynomials and 15 convolution points were best for ensuring that absorption peaks were not “over-smoothed” (Figure 2-3) (see Wilson & Polo 1981 for method). Use of 15 convolution points in the smoothing process subsequently truncated the absorption spectra from 400 - 800 nm to 408 - 792 nm. Although photon scattering was shown to be low for similar microphotometric systems (Iturriaga et al. 1988, Iturriaga & Siegel 1989, Stephens 1995), A_{750} was subtracted from all A_{λ} values to compensate for any photon loss due to scattering (Iturriaga et al. 1988). Thus, A_{λ} are reported from 408 - 750 nm only, above which absorbance was assumed to be zero (Figure 2-3).

Storage effects

Experiments were done to test the effect of storage duration on absorption spectra of *Macrocystis* and *Pterygophora* zoospores. These taxa were chosen for the storage duration experiments because fertile plants were readily available and preliminary observations suggested that, among brown and red macroalgae, absorption spectra of kelp zoospores were the most vulnerable to storage degradation. Zoospore suspensions from sporophylls of 1 *Macrocystis* sporophyte and 1 *Pterygophora* sporophyte were used to create 14 mounted zoospore samples for each species, as described above. Sampling from only 1 plant per species was necessary to control for potential variability among plants. Storage

duration treatments consisted of mounted zoospore samples analyzed 0, 1, 2, 3, 4, 7, and 10 days after mounting. Absorption spectra for the 0 day treatments were obtained within 1 h of mounting, whereas all other treatments were stored for the allotted time duration in the dark at -10°C . For each species, absorption spectra were determined for 20 randomly chosen (undamaged) zoospores in each of 2 replicate samples per treatment.

The effect of storage duration on zoospore absorption spectra was analyzed graphically for each species by plotting average A_{λ} (averaged over all 40 zoospores for each of the 7 storage duration treatments) as a function of wavelength. Significant differences among treatments and controls (0 day treatments) were determined by comparing average A_{λ} for each treatment with 99% confidence intervals of the average A_{λ} for the controls. Treatments falling outside these 99% confidence regions at any wavelength were considered significantly different from the controls and therefore unsuitable durations for sample storage. In addition, nested analysis of variance (ANOVA) was used to test the effect of storage duration (fixed factor; 7 levels) and sample replication (random factor nested within storage duration; 2 levels) on A_{438} (dependent variable). The primary use of these analyses was to determine whether A_{438} differed among replicate samples; non-significant results of the nested factor for both *Macrocystis* and *Pterygophora* (i. e. no significant difference among samples per treatment; see **Results**) support the use of the 40 individual zoospores per treatment as independent replicates for the initial analysis of storage duration on A_{λ} (Underwood 1997). Cochran's test and analysis of residuals indicated that the assumptions of homogeneity of variances, independence, and normality of error terms were met.

Results

Mounting quality

The mounting procedure was highly effective in producing undamaged spore samples

for use with microphotometry. Very few kelp zoospores (< 1%) showed signs of damage from the filtration process. The method used to apply filters and coverslips to the slides appeared to minimize movement of the kelp zoospores on the filters; clumping was not evident and the distribution of zoospore densities did not significantly differ from a normal distribution (mean \pm SD: 55.63 ± 8.81 ; Kolmogorov-Smirnov chi-square: $N = 40$, maximum difference = 0.0752, Lilliefors probability = 0.86). Further, resulting sample mounts were very stable (zoospores did not move during microphotometric analyses). Mounted samples of red algal tetrasporangial thalli were also very stable, with branches and tetrasporangia lying flat and rarely overlapping. The transparent membrane filters were truly transparent (absorption between 408 and 750 nm was insignificant, Figure 2-3) and the addition of immersion oil between the filter and coverslip enhanced the detail of the samples. Consequently, it was very easy to visualize plastids in each zoospore for both kelp species (Figure 2-1) or to distinguish individual tetraspores within tetrasporangia (Figure 2-2), facilitating the positioning of the aperture directly over samples when obtaining absorption spectra. Occasionally, samples were located over filter pores resulting in enhanced apparent absorbance at wavelengths > 700 nm, presumably due to increased scattered light errors; such absorption spectra were discarded.

Absorption spectra

Kelp zoospores - *Macrocystis* and *Pterygophora* zoospore plastids sampled immediately (i. e. 0 day control treatments) exhibited absorption spectra of similar shape to those previously described for adult kelps (Smith & Alberte 1994, Grzymski et al. 1997) (Figure 2-4). Presence of chlorophyll *a* was indicated by absorption peaks around 438 and 673 nm and a shoulder between 618 and 620 nm, whereas presence of chlorophyll *c* was indicated by high absorption between 460 and 470 nm and a distinct peak at 634 nm (Rowan 1989). Fucoxanthin also could be identified by multiple shoulders between 485 and 560 nm and a

small peak at 584 nm (Smith & Alberte 1994). Although absorption spectra shape was similar between *Macrocystis* and *Pterygophora* zoospore plastids, magnitude of absorption spectra differed greatly between the two taxa (Figure 2-4). For example, absorbance at 438 nm was greater for *Pterygophora* (mean \pm SD: 0.433 ± 0.031 , $n = 20$) than for *Macrocystis* (mean \pm SD: 0.191 ± 0.020 , $n = 20$), and A_λ averaged between 408 and 750 nm was ≈ 2.3 times higher for *Pterygophora* (0.144) than *Macrocystis* (0.062). Range in absorbance at 438 nm (*Macrocystis*: 0.144 - 0.232; *Pterygophora*: 0.377 - 0.506) and 673 nm (*Macrocystis*: 0.101 - 0.182; *Pterygophora*: 0.257 - 0.382), and 99% confidence regions of control treatments at wavelengths < 700 nm (Figure 2-4), did not overlap. In addition, plastid size varied significantly between the two species with plastid diameter (μm) being greater for *Pterygophora* (mean \pm SD: 2.10 ± 0.15 ; $n = 20$) than for *Macrocystis* (mean \pm SD: 1.30 ± 0.14 ; $n = 20$) (t-test: $t = 17.22$, $df = 38$, $p < 0.0001$).

For both *Macrocystis* and *Pterygophora*, magnitude of absorption spectra varied significantly among zoospores sampled immediately and those stored in the dark at -10°C for various durations (Figure 2-4). For *Macrocystis*, average absorbance of zoospores stored for 1, 2 and 3 days were well within 99% confidence intervals of those sampled immediately (0 day control treatment) at all wavelengths, whereas average absorbance of zoospores sampled immediately were significantly higher than those sampled after 4, 7 and 10 days at wavelengths > 430 nm. For *Pterygophora*, only average absorbance from zoospores sampled after 10 days differed significantly from that of the control, although average absorbance from zoospores sampled after 7 days were at the edge of 99% confidence intervals of the control at all wavelengths (Figure 2-4). These results were supported by the ANOVAs used to test the effect of storage duration and sample replication on A_{438} . The effect of storage duration on A_{438} was significant for both species (*Macrocystis*: $F = 39.22$; $df = 6, 7$; $p < 0.001$, *Pterygophora*: $F = 10.14$; $df = 6, 7$; $p < 0.001$). The effect of the nested factor (sample replication) on A_{438} , however, was non-

significant for both species (*Macrocystis* sample replication effect: $F = 0.49$; $df = 7, 266$; $p = 0.841$, *Pterygophora* sample replication effect: $F = 1.12$; $df = 7, 266$; $p = 0.348$).

Red algal tetraspores - The tetraspores analyzed from *Callithamnion* and *Scagelia* were within cruciate tetrasporangia still attached to the adult thalli (Figure 2-2); three absorption spectra were obtained within each of three tetraspores for a single tetrasporangium per species. The shape of these absorption spectra indicated absorption characteristics similar to those previously published for red algae (Smith & Alberte 1994) (Figure 2-5). Presence of chlorophyll *a* was again identified by absorption peaks near 435 and 673 nm and between 612 and 620 nm. Multiple peaks were observed between 450 and 600 nm indicating the presence of rhodophycean phycoerythrin (Smith & Alberte 1994). As found by Smith & Alberte (1994) for other rhodophyte taxa, absorbance between 450 and 600 nm was similar to absorbance at 438 nm for *Scagelia* (Figure 2-5), although absorbance between 450 and 600 nm for *Callithamnion* often exceeded absorbance at 438 nm. Both shape and magnitude of absorption spectra varied greatly between *Callithamnion* and *Scagelia* tetraspores (Figure 2-5). Average absorbance (\pm SD; $n = 3$) at the blue (435 nm; *Callithamnion*: 0.404 ± 0.023 ; *Scagelia*: 0.329 ± 0.115) and red peaks (673 nm; *Callithamnion*: 0.189 ± 0.014 ; *Scagelia*: 0.168 ± 0.072) was similar between the two species. Differences between *Callithamnion* and *Scagelia* in absorbance due to rhodophycean phycoerythrin (i. e. between 450 and 600 nm), however, were dramatic (Figure 2-5); average absorbance was higher for *Callithamnion* at all wavelengths between 450 and 550 nm. Some absorbance peaks were observed only for *Callithamnion* (e. g., 448 and 462 nm), whereas others were observed only for *Scagelia* (e. g., 552 nm). In addition, among-tetraspore variability in absorbance was greater at all wavelengths for *Scagelia* than for *Callithamnion* (Figure 2-5).

Discussion

The results of this study indicate that microphotometry can be used reliably to obtain absorption spectra from macroalgal spores, whether the spores have been released or are still within attached sporangia. The microphotometric system produced accurate and precise absorption spectra in a short period of time (< 5 s per spore), and the absorption spectra suggested spore pigment compositions similar to those described previously for adult macroalgae (Smith & Alberte 1994, Grzymalski et al. 1997). The transparent membrane filters were found to be extremely stable for mounting samples, such that locating and sampling individual spores was very easy. Due to the high stability of the mount, spore samples remained directly beneath the aperture for the entire duration of spectral scans. Unlike other “transparent” membrane filters, the Whatman filters used in this study were truly transparent resulting in very high optical quality and detail, and should find use in studies other than microphotometry where visualization of filtered samples is necessary (e. g., as an alternative to the traditional method of estimating spore concentrations using hemacytometers). Finally, variability in the quality of sample mounts was shown to have little effect on resulting absorption spectra (i. e. sample replication factor in ANOVAs was non-significant), suggesting that results for any given spore suspension are reproducible among different samples.

Storage of mounted glutaraldehyde-fixed kelp zoospore samples in the dark at -10°C was found to preserve the integrity of absorption spectra for at least 3 days for both *Macrocystis* and *Pterygophora*, similar to results previously described for storage of phytoplankton (Jeffrey et al. 1997). This is ample time to allow for timely and efficient analysis of multiple spore samples using microphotometry (i. e. samples do not have to be analyzed immediately). Although glutaraldehyde fixation was satisfactory for my purposes, recent evidence suggests that fixation with paraformaldehyde may allow for greater storage durations (Troussellier et al. 1995). Since my primary use of microphotometry will be for

analysis of field samples, it was decided that storage of multiple water samples using liquid nitrogen would not be feasible given the limited space available on small boats to accommodate the relatively large storage containers. However, future efforts to utilize on-board liquid nitrogen storage may also extend the viability of kelp zoospore samples beyond that described herein (Vaulot et al. 1989, Jeffrey et al. 1997).

Among-taxa comparisons of absorption spectra indicated that microphotometry may be useful in addressing a variety of questions regarding macroalgal spore photo-physiology. Kelp zoospore absorption spectra were quantitatively different between *Macrocystis* and *Pterygophora*, two species often co-existing in subtidal habitats of the northeast Pacific. My results are consistent with previous work showing differences in pigment concentration (chlorophyll *a*) per zoospore for different kelp species (Amsler & Neushul 1991). Exactly why pigment concentrations and subsequent absorption spectra of kelp zoospores differ between species is not clear. Differences in plastid diameter between species probably may explain much of the variation in absorption spectra since *Macrocystis* plastids ($1.34 \mu\text{m}^2$ average area) occupied an average of only 50.9% of the total area of that sampled by the microphotometer ($2.63 \mu\text{m}^2$ area), whereas all *Pterygophora* plastids ($2.72 \mu\text{m}^2$ minimum area) occupied 100% of the aperture when perfectly centered. Still, functional significance of larger zoospore plastids in *Pterygophora* relative to *Macrocystis* is unknown. Amsler & Neushul (1991) hypothesized that among-species differences in pigment concentrations and photosynthetic capabilities of kelp zoospores may be adaptations favouring increased growth and survival of the microscopic benthic stages. Given application of my microphotometric method to kelp zoospores from field samples, additional research into the ecophysiology of kelp zoospores, and early microscopic stages, will be possible.

Among-taxa comparisons of red algal tetraspores were also interesting. Absorption spectra shape and magnitude differed greatly between *Callithamnion* and *Scagelia* tetraspores. Given that my data represent true *in vivo* individual-particle absorption spectra

for these taxa, I suggest that differences observed between *Callithamnion* and *Scagelia* were due to variable pigment compositions, as well as variable pigment concentrations. It was surprising to observe such high variability in putative pigment composition among closely related taxa (both species within the family Ceramiaceae). Further, the high variability in magnitude of absorption spectra observed among *Scagelia* tetraspores suggests that pigment concentration may differ even among tetraspores within the same tetrasporangium. Additional work is necessary to critically test this hypothesis. Regardless of the conclusions that can be drawn from the data presented here, my study demonstrates the utility of microphotometry as a tool for isolating absorption characteristics of individual macroalgal cells; whether they be free-living propagules such as spores, from macroalgal thalli such as spores within attached sporangia, or even macroalgal vegetative cells.

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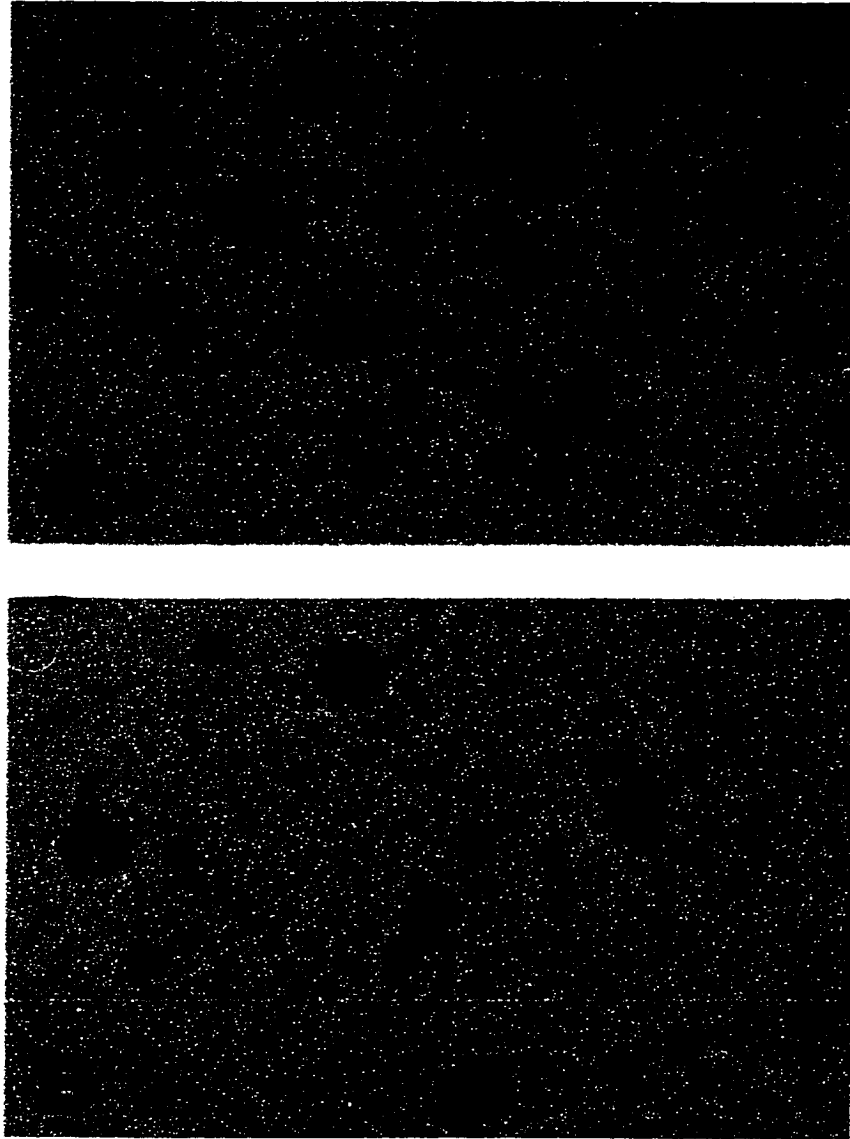


Figure 2-1. Light microscopy photographs of *Macrocyctis pyrifera* and *Pterygophora californica* zoospores mounted onto transparent membrane filters. A. *Macrocyctis* zoospores, and B. *Pterygophora* zoospores, each showing the location of the single discoid plastids within each zoospore. Flagella are visible for some zoospores of each species; retention of these presumably fragile structures suggests that zoospores suffered little damage during mounting. *Pterygophora* zoospore plastids are larger than those of *Macrocyctis*. For scale, black filled circles are the pores of the membrane filters and have diameters of 1 μm . Magnification was 1000x for both photographs.

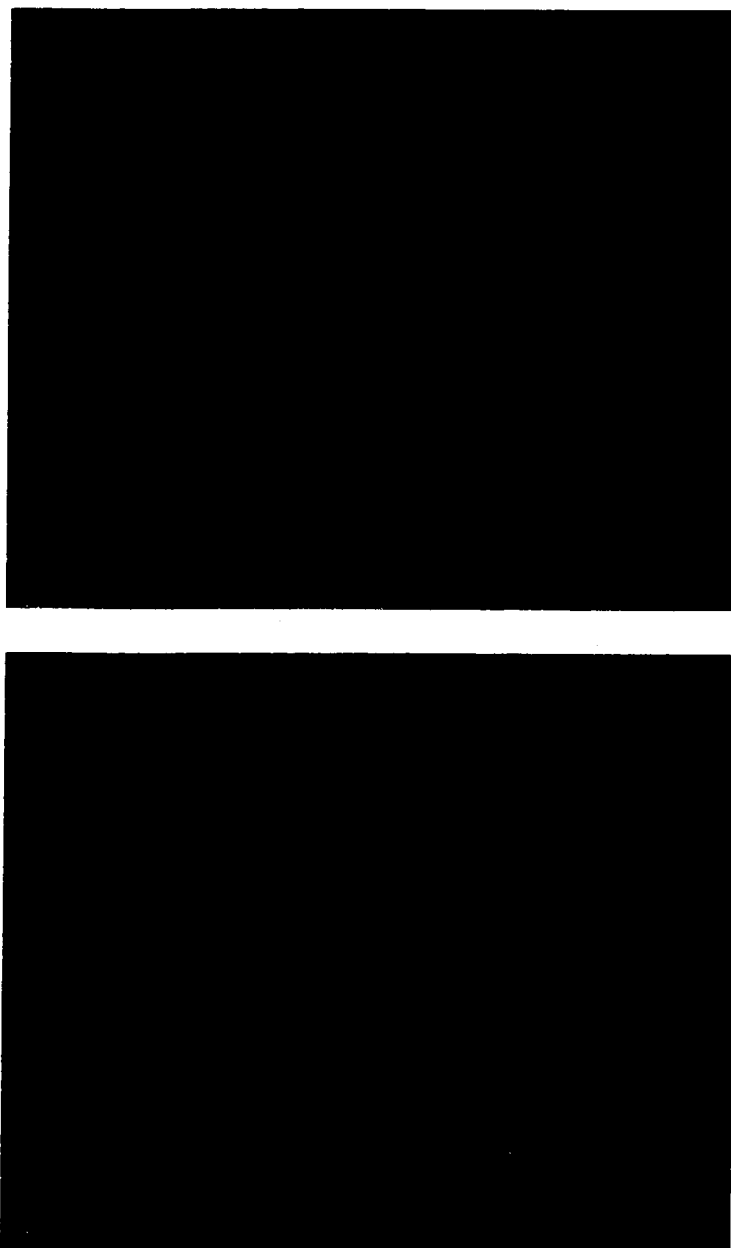


Figure 2-2. Light microscopy photographs of *Callithamnion biseriatum* and *Scagelia pylaisaei* tetrasporangial thalli mounted onto transparent membrane filters. A. *Callithamnion* tetrasporangia, and B. *Scagelia* tetrasporangia, each showing the distinction between individual tetraspores. Magnification was 400x for both photographs.

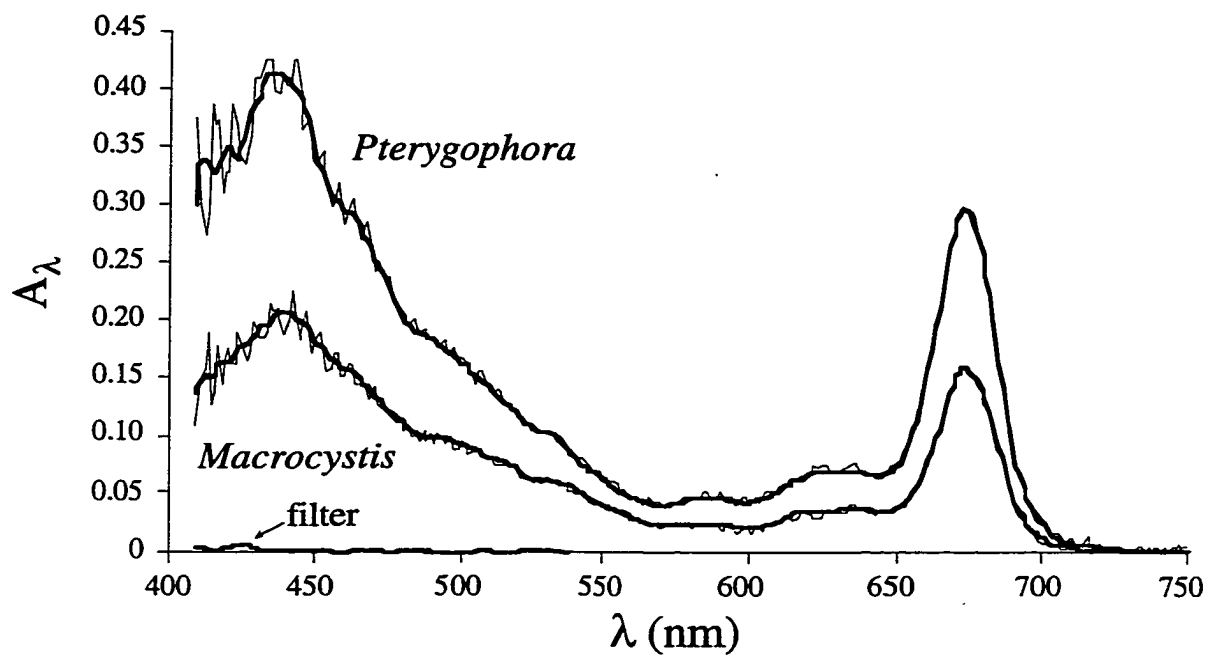


Figure 2-3. Typical absorption spectra (A_λ as a function of wavelength, λ) for single zoospore plastids of *Macrocyctis pyrifera* and *Pterygophora californica*. Thin lines are unsmoothed spectra and thick lines are Savitsky-Golay smoothed spectra. The thin line barely visible along the abscissa is average A_λ of an unused filter relative to air ($n = 20$). A_λ for the filter shows a small peak at 422 nm, but was negligible at all other wavelengths.

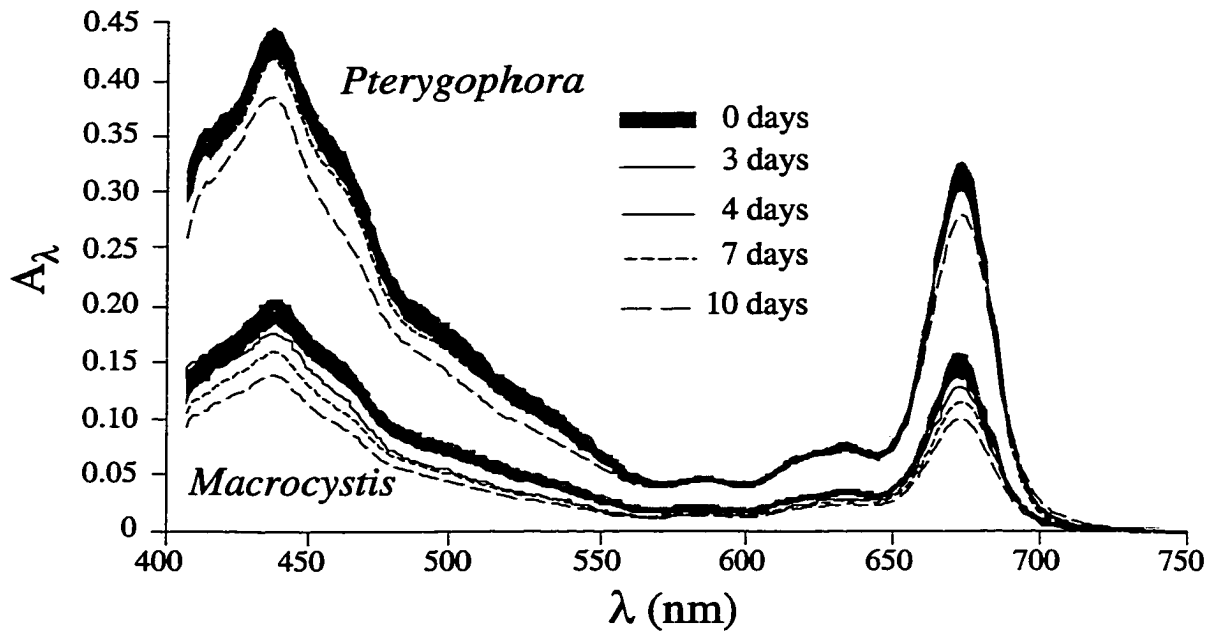


Figure 2-4. Effect of storage duration on absorption spectra for *Macrocystis pyrifera* and *Pterygophora californica* zoospores. Gray shading indicates 99% confidence regions around average A_λ for those zoospores sampled immediately after fixation (controls). Lines indicate average A_λ for those zoospores sampled after various durations of storage at -10°C ; average A_λ for the 0, 1, and 2 day treatments could not be distinguished visually and for clarity were not included in the figure. Note: average A_λ for the 3 & 4 day *Pterygophora* treatments perfectly overlap. Since average A_λ per treatment did not significantly differ among replicate samples ($n = 20$) for either species (see text), data represent average A_λ pooled for all zoospores per treatment ($n = 40$).

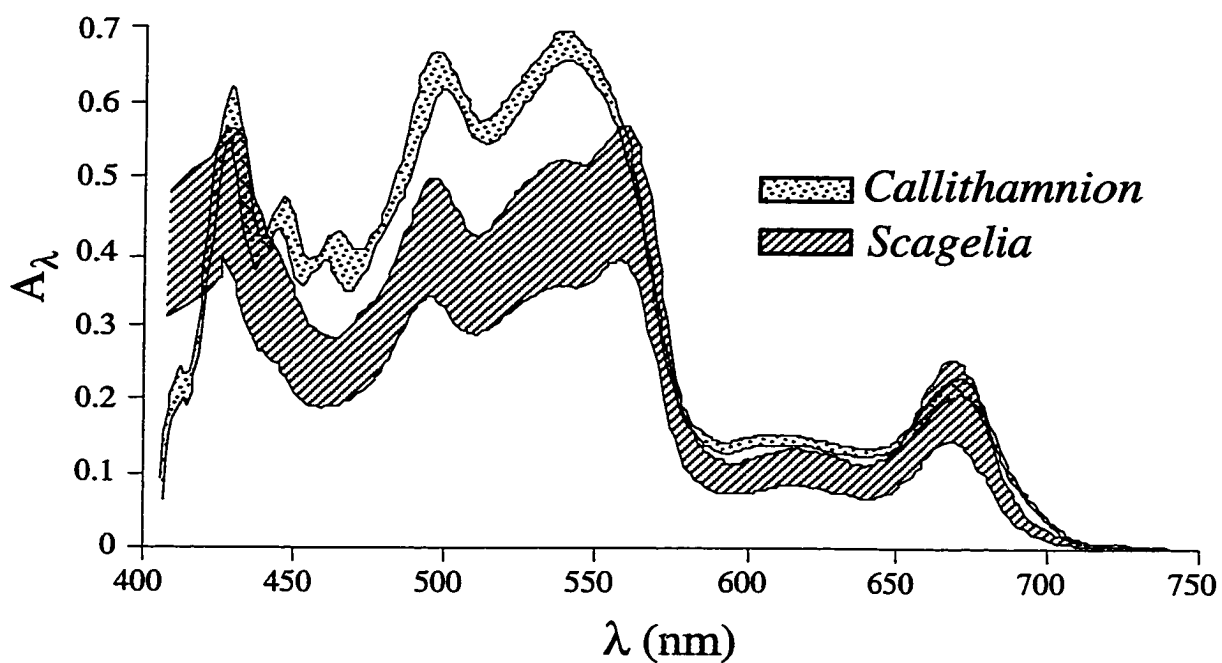


Figure 2-5. Absorption spectra for *Callithamnion biseriatum* and *Scagelia pylaisae* tetraspores. Data are 95% confidence regions around average A_λ for 3 tetraspores within a single cruciate tetrasporangium per species.

Literature Cited

- Amsler, C. D., and M. Neushul. 1991. Photosynthetic physiology and chemical composition of spores of the kelps *Macrocystis pyrifera*, *Nereocystis luetkeana*, *Laminaria farlowii*, and *Pterygophora californica* (Phaeophyceae). *Journal of Phycology* 27:26-34.
- Beach, K. S., C. M. Smith, T. Michael, and H. Shin. 1995. Photosynthesis in reproductive unicells of *Ulva fasciata* and *Enteromorpha flexuosa*: implications for ecological success. *Marine Ecology Progress Series* 125:229-237.
- Bidigare, R. R., M. E. Ondrusek, M. C. Kennicutt II, R. Iturriaga, H. R. Harvey, R. R. Hoham, and S. A. Macko. 1993. Evidence for a photoprotective function for secondary carotenoids of snow algae. *Journal of Phycology* 29:427-434.
- Brzezinski, M. A., D. C. Reed, and C. D. Amsler. 1993. Neutral lipids as major storage products in zoospores of the giant kelp *Macrocystis pyrifera* (Phaeophyceae). *Journal of Phycology* 29:16-23.
- Carpenter, E. J., J. Chang, M. Cotrell, J. Schubauer, H. W. Paerl, B. M. Bebout, and D. G. Capone. 1990. Re-evaluation of nitrogenase oxygen-protective mechanism in the planktonic marine cyanobacterium *Trichodesmium*. *Marine Ecology Progress Series* 65:151-158.
- Carpenter, E. J., J. Chang, and L. P. Shapiro. 1991. Green and blue fluorescing dinoflagellates in Bahamian waters. *Marine Biology* 108:145-149.
- Garbary, D. J., K. Y. Kim, T. Klinger, and D. Duggins. 1999. Preliminary observations on the development of kelp gametophytes endophytic in red algae. *Hydrobiologia* 398/99:247-252.
- Grzymiski, J., G. Johnsen, and E. Sakshaug. 1997. The significance of intracellular self-shading on the biooptical properties of brown, red, and green macroalgae. *Journal of Phycology* 33:408-414.
- Henry, E. C., and K. M. Cole. 1982. Ultrastructure of swimmers in the Laminariales (Phaeophyceae). I. Zoospores. *Journal of Phycology* 18:550-569.
- Iturriaga, R., B. G. Mitchell, and D. A. Kiefer. 1988. Microphotometric analysis of

- individual particle absorption spectra. *Limnology and Oceanography* 33:128-135.
- Iturriaga, R., and D. A. Siegel. 1989. Microphotometric characterization of phytoplankton and detrital absorption properties of the Sargasso Sea. *Limnology and Oceanography* 34:1706-1726.
- Jeffrey, S. W., R. F. C. Mantoura, and S. W. Wright. 1997. *Phytoplankton pigments in oceanography: guidelines to modern methods*. UNESCO Publishing, Paris.
- Lobban, C. S., and P. J. Harrison. 1994. *Seaweed ecology and physiology*. Cambridge University Press, Cambridge.
- Madden, H. H. 1978. Comments on the Savitsky-Golay convolution method for least-squares fit smoothing and differentiation of digital data. *Analytical Chemistry* 50:1383-1386.
- Robinson, D. H., K. R. Arrigo, R. Iturriaga, and C. W. Sullivan. 1995. Microalgal light-harvesting in extreme low-light environments in McMurdo Sound, Antarctica. *Journal of Phycology* 31:508-520.
- Rowan, K. S. 1989. *Photosynthetic Pigments of Algae*. Cambridge University Press, Cambridge.
- Savitsky, A., and M. J. E. Golay. 1964. Smoothing and differentiation of data by simplified least squares procedures. *Analytical Chemistry* 36:1627-1639.
- Smith, C. M., and R. S. Alberte. 1994. Characterization of *in vivo* absorption features of chlorophyte, phaeophyte and rhodophyte algal species. *Marine Biology* 118:511-521.
- Steinier, J., Y. Termonia, and J. Deltour. 1972. Comments on smoothing and differentiation of data by simplified least square procedures. *Analytical Chemistry* 44:1906-1909.
- Stephens, F. C. 1995. Variability of spectral absorption efficiency within living cells of *Pyrocystis lunula* (Dinophyta). *Marine Biology* 122:325-331.
- Troussellier, M., C. Courties, and S. Zettelmaier. 1995. Flow cytometric analysis of coastal lagoon bacterioplankton and picophytoplankton: fixation and storage effects. *Estuarine and Coastal Shelf Science* 40:621-633.

- Underwood, A. J. 1997. *Experiments in ecology: their logical design and interpretation using analysis of variance*. Cambridge University Press, Cambridge
- Vaulot, D., C. Courties, and F. Partensky. 1989. A simple method to preserve oceanic phytoplankton for flow cytometric analysis. *Cytometry* 10:629-635.
- Wilson, P. D., and S. R. Polo. 1981. Polynomial filters of any degree. *Journal of the Optical Society of America* 71:599-603.

Chapter III

Identification of kelp zoospores from *in situ* plankton samples

Abstract

Studies of the link between planktonic and benthic stages of marine macroalgae have been hampered by the inability to identify planktonic stages from field samples. Here I describe a microphotometric method for identifying kelp zoospores from *in situ* plankton samples based on light absorbance characteristics of zoospore plastids. Absorption spectra were obtained from plastids within *Macrocystis pyrifera*, *Pterygophora californica*, *Eisenia arborea* and *Laminaria farlowii* zoospores released in the laboratory from adult specimens collected in the field; these are the dominant subtidal kelps in most southern California kelp forests. Absorption spectra were highly specific to each kelp species. Average absorbance decreased from *P. californica* to *E. arborea* to *L. farlowii* to *M. pyrifera* at most wavelengths between 400 and 750 nm. Discriminant function analyses provided a discriminant rule, based on zoospore plastid absorbance values at 25 different wavelengths, to be used for classifying unknown zoospores among the four kelp species. Cross-validation techniques indicated that the discriminant rule could successfully identify 97.8% *M. pyrifera*, 96.4% *P. californica*, 86.2% *E. arborea* and 69.7% *L. farlowii* zoospores. Most misidentified *E. arborea* zoospores were identified as *L. farlowii*, and vice-versa; when the relatively rare *E. arborea* was pooled with *L. farlowii* and treated as a single complex, the discriminant rule could identify 98.2% *M. pyrifera*, 97.4% *P. californica* and 89.5% *E. arborea/L. farlowii* zoospores. Coupled with a protocol for isolating kelp zoospores from non-kelp cells, this discriminant rule was used to determine kelp zoospore abundances and species-compositions of *in situ* plankton samples. This is the first description of a method for identifying macroalgal zoospores or spores from field samples.

Introduction

Recruitment and dynamics of macroalgal benthic (post-settlement) stages are reasonably well studied, yet very little is known about processes affecting macroalgal planktonic (pre-settlement) stages in the water column. Most macroalgal recruitment is probably regulated, at least in part, by the supply and arrival of viable propagules (e. g., spores, zoospores, and zygotes) to suitable substrates (Hoffman 1987, Santelices 1990, Amsler et al. 1992, Norton 1992). Therefore, variability in spatial and temporal distributions of planktonic stages in the water column may have pronounced effects on macroalgal population dynamics, as has been observed for other marine organisms (e. g., Gaines et al. 1985, Roughgarden et al. 1988). However, few *in situ* patterns of macroalgal propagule abundance have been described (Hruby and Norton 1979, Amsler and Searles 1980, Hoffman and Ugarte 1985, Zechman and Mathieson 1985, Fredriksen et al. 1995, Santelices et al. 1995).

This lack of information concerning the distribution of macroalgal planktonic stages results from difficulties in sampling propagule abundance in the field (Norton 1992). For example, sampling and identification of the small and relatively featureless zoospores of kelps (Laminariales: Phaeophyceae) have been especially problematic (Anderson and North 1966, Reed 1990); species-specific identification of microscopic kelp pre- and post-settlement stages is not currently possible. Of the few pioneering studies that attempted to evaluate kelp zoospore abundance in the water column (Hoffman and Ugarte 1985, Zechman and Mathieson 1985, Fredriksen et al. 1995), all measured laboratory zoospore settlement rates and used them as a proxies for *in situ* zoospore abundance. These researchers collected water samples from nearshore habitats and allowed zoospores to settle and grow on artificial substrata in the laboratory. Kelp post-settlement stages (e. g., germlings, gametophytes and sporophytes) were distinguished from those of other brown algae by eye or by using conventional light microscopy; identifications were made only to the ordinal level. Due to variability in zoospore settlement success and post-settlement

growth and mortality, such studies provide only indirect estimates of the distribution of kelp zoospores in the water column (Norton 1992). Three additional studies used similar techniques to investigate *in situ* patterns of macroalgal propagule abundance although kelp zoospore abundance was not specifically targeted (Hruby and Norton 1979, Amsler and Searles 1980, Santelices et al. 1995). Consequently, despite these heroic efforts, the direct link between dynamics of planktonic and benthic stages has not been investigated for kelps or most other macroalgae (but see Deysher and Norton 1982 and Kendrick and Walker 1991 for exceptions using the large and distinct propagules of *Sargassum* sp.).

Although species-specific identification of kelp zoospores has yet to be realized, among-species variability in zoospore photosynthetic physiology may provide a basis for identifications. Amsler and Neushul (1991) found that zoospore pigment concentrations (chlorophyll *a* concentration per zoospore) differed significantly among four species of subtidal kelp (species in order of increasing chlorophyll *a* per zoospore: *Macrocystis pyrifera*, *Nereocystis luetkeana*, *Laminaria farlowii*, *Pterygophora californica*). Given differences in cellular pigment concentration, light absorbance characteristics of zoospores may also differ from one species to the next (Rowan 1989), providing a potential method for identifying kelp zoospores.

If zoospores collected in the field are to be identified on the basis of light absorbance characteristics, absorption spectra must be obtained from individual zoospores. Graham and Mitchell (1999) recently described a microphotometric method for determining absorption spectra for individual macroalgal cells. The microphotometer consisted of a conventional light microscope fitted with a spectrophotometer and measured spectral light transmittance (and thus absorbance) through individual particles. By focusing the microphotometer over plastids within kelp zoospores, we obtained precise absorption spectra for individual zoospores (kelps have only a single discoid plastid per zoospore) and verified that absorption spectra from individual *Macrocystis pyrifera* and *Pterygophora*

californica zoospores did, in fact, reflect the pattern of cellular pigment concentrations observed by Amsler and Neushul (1991).

The purpose of the present study was to determine if plastid absorption spectra could be used to identify kelp zoospores from *in situ* plankton samples. Here I present: (1) absorption spectra for laboratory suspensions of zoospores from four dominant species of southern California subtidal kelps; (2) an identification algorithm used to discriminate zoospores among species; (3) various cross-validations of the identification algorithm; and (4) a protocol for distinguishing kelp zoospores from non-kelp cells obtained from *in situ* plankton samples. Kelp zoospore abundances and species-compositions from field samples are also presented here, although specific hypotheses concerning the distribution of kelp zoospores in the water column will be described elsewhere. This is the first description of a method for identifying macroalgal zoospores or spores from field samples, and the method is currently being used in an investigation of the role of pre-settlement processes on the dynamics of subtidal kelp populations.

Methods

Sample collection and preparation

The four kelp species of interest were *Macrocystis pyrifera* (L.) C. Agardh, *Pterygophora californica* Ruprecht, *Eisenia arborea* Areschoug and *Laminaria farlowii* Setchell, as they are the dominant subtidal kelps in southern California (Dayton et al. 1992). Since each genus is represented in southern California by a single species, these taxa will be referred to as: *Macrocystis*, *Pterygophora*, *Eisenia* and *Laminaria*. *Egrecia menziesii* Areschoug, *Agarum fimbriatum* Harvey and *Pelagophycus porra* Setchell are also distributed in southern California but are only observed in very shallow or deep habitats. Zoospore production (sporogenesis) and release occur in areas of sporangial aggregation (sori) that are borne either directly on undifferentiated adult blades (*Laminaria* and *Eisenia*)

or on specialized blades (sporophylls; *Macrocystis* and *Pterygophora*). Sporogenous tissue samples were collected for each species on many dates between April 1997 and June 1998 within the Point Loma kelp forest in San Diego County, California, USA (32°42'N; 117°16'W). The goal was to account for as much spatio-temporal variability in zoospore plastid absorption spectra as possible. Samples were usually collected every 2 weeks by SCUBA, except during winter when storms prohibited sampling (see Figure 3-1 for sampling frequency). When possible, samples were collected from three adult plants per species per sampling date. *Macrocystis* samples were collected on each sampling date, whereas the availability of *Pterygophora*, *Eisenia* and *Laminaria* samples was determined by the local abundance of these species and their reproductive seasonality. Most samples were collected along a depth gradient from 8 to 21 m within the central portion of this large (~ 1 x 7 km) kelp forest although samples were also collected in the northern and southern sections of the forest (2 - 3 km from the center).

Sporogenous tissue samples were wrapped in moist paper towels, sealed in dry plastic bags, and transported to the laboratory in coolers. Zoospore suspensions were created 4 to 6 h later by re-immersing sori for less than 1 h in 500 ml of 15°C 0.2 µm filtered seawater (FSW) under cool white fluorescent lighting. Released zoospores were fixed by mixing 1-ml aliquots of zoospore suspensions with 4-ml of 2.5% buffered glutaraldehyde in FSW (2% final concentration) (Henry and Cole 1982a). Aliquots were taken from the upper 1 cm of zoospore suspensions in order to sample motile zoospores only. Fixed zoospore samples were vacuum filtered onto Cyclopore™ transparent polycarbonate membrane filters (CTPM filters; 25 mm filter diameter; 1.0 µm pore diameter; Whatman Int. Ltd., Maidstone, England) under low vacuum (< 1 cm of mercury). The last 1 mm of suspension was released from vacuum and gravity filtered to minimize zoospore damage. Filters were air-dried until all visible moisture had evaporated and mounted (zoospores up) between glass microscope slides and cover slips using low-fluorescence microscopy immersion oil.

Mounted zoospore samples were stored at -10°C until absorption spectra were obtained from the zoospores. Sample storage was never longer than 4 hours; I found that kelp zoospores stored under these conditions maintained the integrity of absorption spectra for at least 4 days (Graham and Mitchell 1999, **Chapter II**).

Obtaining absorption spectra

Absorption spectra were determined for the single plastids within individual zoospores using microphotometry according to the methods described in **Chapter II** (Graham and Mitchell 1999). Only pyriform-shaped zoospores were sampled, presumably limiting analyses to zoospores that were swimming prior to fixation (Reed et al. 1992). The microphotometer consisted of a conventional light microscope (Olympus[®] Provis[™] AX70, Lake Success, New York) equipped with a Nano500[™] diode-array spectrophotometer (Nanometrics[®], Sunnyvale, California) and a halogen light source. A circular aperture was placed inline between the objective and spectrophotometer, and light passing through the sample and aperture was detected by the spectrophotometer. Irradiance transmitted through each plastid, $I_{s(\lambda)}$ was determined by placing the aperture directly over the plastid. Irradiance incident on the plastid (the blank), $I_{b(\lambda)}$ was determined by placing the aperture over an adjacent particle-free area without changing the focus. Background current noise was measured prior to sampling each mounted filter and was subtracted from each spectral scan of that filter (both $I_{s(\lambda)}$ and $I_{b(\lambda)}$). Absorbance (optical density) for each scan was determined using:

$$A_{\lambda} = -\log(I_{s(\lambda)}/I_{b(\lambda)}),$$

where A_{λ} is the absorbance at a specified wavelength, λ . This system required 3 s to obtain an absorption spectrum over a range of 400 - 800 nm (1.2 nm intervals) from an individual kelp zoospore plastid. All measurements were made in a dark room using a Plan Fluorite[™] 100x oil-immersion objective. Spectral smoothing and adjustments for light

scattering were done according to **Chapter II** (Graham and Mitchell 1999); absorption spectra were scaled to zero at 750 nm and are reported from 400 - 750 nm only, above which absorbance was assumed to be zero.

Identification scheme

Plastid absorption spectra were obtained from 15 zoospores for each sample (adult plant) on the first 2 sampling dates, and from 20 zoospores for each sample on the remaining sampling dates. Absorption spectra for each sample were randomly split into 2 data sets: a Model data set (total for all samples: 450 *Macrocystis*, 200 *Pterygophora*, 80 *Eisenia* and 76 *Laminaria* spectra) and a Test data set (total for all samples: 445 *Macrocystis*, 195 *Pterygophora*, 80 *Eisenia* and 73 *Laminaria* spectra). The Model data set was analysed using linear discriminant function analysis (DFA) of absorbance at 25 different wavelengths (every 12.1 nm between 408.1 and 698.7 nm) to classify individual zoospores among the 4 kelp species. Prior probabilities were not included in the analysis. The DFA resulted in a significant discriminant rule which consisted of one algorithm for each of the 4 species; each algorithm included a coefficient for each absorbance wavelength and a constant. When the constants and coefficients were applied to an absorption spectrum, the algorithm with the largest numerical value indicated the species-identity of the spectrum and thus the identity of the respective zoospore.

Cross-validation

The identification algorithm was cross-validated against two different data sets that contained absorption spectra for which zoospore species identities were known. First, the identification algorithm was applied to the Test data set which was collected simultaneously with the Model data set. This validation provided a general estimate as to how well the algorithm could predict zoospore identities between April 1997 and June 1998, the timespan

over which the Model and Test data sets were collected. The estimate was used to assess the overall rigor of the identification algorithm for predicting zoospore identities. Second, the identification algorithm was applied to a data set that consisted of zoospore absorption spectra collected after June 1998 (the Future data set). Due to the reproductive seasonality of *Pterygophora*, *Eisenia* and *Laminaria*, this data set included several *Macrocystis* samples, a few *Pterygophora* and *Laminaria* samples, but no *Eisenia* samples. Although limited in scope, the results of the Future data set validation were used to estimate the ability of the identification algorithm to predict zoospore identities from future samples.

Distinguishing kelp zoospores from non-kelp cells

Kelp zoospores were distinguished from non-kelp cells by criteria based on published descriptions of particle size, morphology, and ultrastructure for the various planktonic cells likely to be encountered in field samples (Table 3-1). Since *Macrocystis*, *Pterygophora*, *Eisenia* and *Laminaria* zoospores are very small with a typical width of 3 μm and lengths up to 7 μm (Henry and Cole 1982a, Graham and Mitchell 1999, **Chapter II**), sieving plankton samples through a 10 μm mesh eliminates the large non-kelp cells from samples, and small non-kelp cells less than 1 μm length are not retained on the CTPM filters. After removing non-kelp cells on the basis of size, I could distinguish kelp zoospores from most other cells in field samples by their pyriform shape, single discoid plastids, and lack of eyespots (Table 3-1). These features are easily observed using transmitted-light and blue-excitation epi-fluorescence microscopy. Any Chlorophyta zoids that have not been removed from samples based on size, morphology or ultrastructure can be distinguished from kelp zoospores by the lack of chlorophyll *c* and fucoxanthin peaks in the plastid absorption spectra (Table 3-1). Most phaeophyceae taxa (brown algae) have sperm characterized by > 1 plastid and an eyespot (Fritsch 1945, Dodge 1973, Henry and Cole 1982b, Kawai 1992) although a few oogamous phaeophyceae taxa have pyriform sperm with single plastids and

no eyespots (examples described in Fritsch 1945, Dodge 1973, Henry and Cole 1982b, Kawai 1992, van den Hoek et al. 1995). Such sperm are thus similar in size, morphology and ultrastructure to kelp zoospores. However, since the male gametophytes of these taxa are benthic and microscopic (Fritsch 1945, van den Hoek et al. 1995), significant numbers of such sperm would probably never exit the subtidal benthic boundary layer to confound plankton samples.

The precision with which criteria in Table 3-1 could be used to distinguish kelp zoospores from non-kelp cells in field samples was also quantified. Twenty liters of raw seawater were collected at the Scripps Institution of Oceanography (SIO) pier from 5 m depth during October 1998 and January 1999; SIO pier-water has a high diversity of non-kelp cells (M. H. Graham personal observation) and is > 0.5 km from the nearest source of kelp zoospores (a very low density population of intertidal *Eisenia*). *Macrocystis* zoospore suspensions (of varying zoospore density) were fluorescently labeled with Calcofluor™ White M2R (a UV-excitation cellulose stain; Sigma Chemical Company, St. Louis, MO), passed through a sample concentrating apparatus (described below), and vacuum filtered onto CTPM filters (47 mm filter diameter; 1 μ m pore diameter). Fifty ml of FSW were passed through the filter to remove residual stain. SIO pier-water was then passed through the sample concentrating apparatus and vacuum filtered onto the same filters as the zoospores. The filters were mounted for microphotometry as described above, with ten mounted filters created in both October 1998 and January 1999. Mounted filters contained fluorescently-labeled *Macrocystis* zoospores overlain with unlabeled SIO pier-water; controls indicated that this procedure did not label cells from the SIO pier-water samples. The kelp zoospores were distinguished from non-kelp cells using transmitted-light and blue-excitation epi-fluorescence microscopy according to criteria in Table 3-1. Zoospores were counted in a single horizontal 9.36 mm² swath for each filter to give an “estimated” zoospore density (# zoospores/swath). UV-excitation epi-fluorescence microscopy was

then used to illuminate stained zoospores to give the density of “labeled” zoospores in the exact same swath. The relationship between “estimated” and “labeled” zoospore density evaluated the efficacy of the discrimination criteria in Table 3-1.

Analysis of field samples

To prove the utility of the identification technique, I determined kelp zoospore abundances and species-compositions for *in situ* plankton samples. Four replicate 2-L plankton samples were collected on each of two dates in October 1998 and one date in February 1999 at 15 m depth using a pump from a boat in the central portion of the Point Loma kelp forest. Plankton samples were stored in 99.9% opaque HDPE containers and fixed in 0.5% buffered glutaraldehyde to kill motile zoospores. Approximately 50 seconds were required to collect a 2-L sample, and the 4 replicate samples per date were separated by at least 15 minutes. Plankton samples were stored on ice, transported to the laboratory, and passed through nested sieves of 333, 90, and 10 μm mesh to eliminate large non-kelp cells. Sample filtration and mounting were facilitated by concentrating the plankton samples using a Centramate™ PE tangential-flow filtration system with a 0.8 μm Omega™ cassette (Pall Filtron®, Northborough, MA). This sample-concentrating apparatus was able to condense plankton samples of up to 100-L down to 60-ml (it took less than 4 minutes to concentrate a 2-L sample); I have never observed kelp zoospores in the waste water or physically damaged kelp zoospores in concentrated samples. The 60-ml concentrated samples were then vacuum-filtered onto CTPM filters (47 mm filter diameter; 1 μm pore diameter). Thus each filter contained the cells from a single 2-L sample. One ml of 2% buffered glutaraldehyde was added at the end of filtration to preserve the samples. Filters were mounted as described above and stored at -10°C for up to 3 days (Graham and Mitchell 1999, **Chapter II**). Among 1-L, 2-L, 4-L and 6-L plankton samples, 2-L plankton samples were found to be the best compromise between analytical precision and efficiency.

The procedure for estimating kelp zoospore abundance and species-composition was to: (1) determine total kelp zoospore density per sample (# zoospores/L) by counting all kelp zoospores (using criteria in Table 3-1) in five horizontal 9.36 mm² swaths for each filter, standardizing the counts to total filter area (1017.88 mm²; # zoospores/2-L), and dividing by 2; and (2) identify 20 haphazardly located kelp zoospores per filter using criteria in Table 3-1 followed by microphotometry and the identification algorithm. Resulting species-compositions were multiplied by the estimated zoospore densities to give densities of zoospores per kelp species. I determined that the minimum detectable total kelp zoospore abundance using this technique (i. e. only 1 zoospore counted in the 5 swaths per filter) was 11 zoospores/L.

Statistical analyses

In addition to the DFA, various statistical analyses were conducted on the absorption spectra. First, Lilliefors tests and analyses of histograms were done to determine the distribution of plastid absorbance at the peak wavelength (438 nm) for each of the *Pterygophora*, *Eisenia* and *Laminaria* zoospore samples, and 23 of the 46 *Macrocystis* zoospore samples. All samples analyzed had approximately normal distributions for absorbance at 438 nm (all Lilliefors probabilities > 0.1). Second, nested analysis of variance (ANOVA) was used to test the effect of Date (random factor) and Plant (random factor nested within Date) on plastid absorbance at 438 nm for each of the four kelp species. *Macrocystis* and *Pterygophora* analyses were limited to dates when 3 plants were collected (*Macrocystis*: 10 dates; *Pterygophora*: 5 dates), whereas *Eisenia* and *Laminaria* analyses were limited to dates when 2 plants were collected (*Eisenia*: 4 dates; *Laminaria*: 3 dates). Analysis of model residuals indicated that the assumptions of normality, homoscedasticity, and independence of error terms were met for each of the 4 nested ANOVAs. Percent of total variance in plastid absorbance (estimated at 438 nm) explained

by Date and Plant effects was determined according to Thompson and Moore (1963) and Winer et al. (1991). Given the random nature of the Date factor, multiple comparisons were not conducted. Finally, because both “estimated” and “labeled” zoospore densities were potentially measured with observer-error, the relationship between “estimated” and “labeled” zoospore density was determined using major-axis regression (via principal components) with individual filters serving as replicates (Sokal and Rohlf 1981). All analyses were done using SYSTAT (Macintosh v. 5.2.1).

Results

Reproductive timing

Temporal patterns in the onset of reproduction for adult *Macrocystis*, *Pterygophora*, *Eisenia* and *Laminaria* at the Point Loma kelp forest were similar to those previously published (Neushul 1963, McPeak 1981, Dayton et al. 1999). Reproductive *Macrocystis* adults were found at the sample collection sites throughout the study. Further, zoospores were obtained from *Macrocystis* sori collected on each sampling date (Figure 3-1), indicating that the Point Loma *Macrocystis* population was “continuously” reproductive. Conversely, Point Loma *Pterygophora*, *Eisenia* and *Laminaria* populations were distinctly “seasonal” in reproduction (Figure 3-1). These three taxa exhibited 4 - 5 month reproductive seasons, with the onset of sorus development generally occurring around June for *Eisenia*, September for *Laminaria*, and November for *Pterygophora* (Dayton et al. 1999, M. H. Graham personal observation). Zoospores, however, could not be obtained from *Pterygophora*, *Eisenia* or *Laminaria* sori that were collected during the first 2 months or so of their reproductive season, suggesting that, although sori may be present on adult plants, variable sporangial development in these seasonally-reproducing taxa limits the time period for potential release of zoospores to later in the reproductive season.

Within- and among-taxa variability in plastid absorbance

Temporal variability in zoospore plastid absorbance estimates (measured at the 438 nm peak) was significant for *Macrocystis* and *Pterygophora* but not significant for *Eisenia* and *Laminaria* (Figure 3-1; Date effect in Table 3-2). *Macrocystis* absorbences fluctuated randomly among dates between April 1997 and June 1998. Although highest *Macrocystis* plastid absorbences were observed during April and May 1997, just prior to the onset of warm-water El Nino conditions in August 1997, absorbences (averaged per date) were not correlated with average bottom temperature measured at 15 m depth (averaged between dates) (Figure 3-2). However, a pattern of greater variability in absorbance and higher maximum absorbences, with decreasing average bottom temperature, was observed for *Macrocystis* zoospores (Figure 3-2). Thus, although low absorbences were observed at all temperatures, high absorbences became more likely as temperature decreased. Among-date fluctuations in *Pterygophora* plastid absorbences between April 1997 and June 1998 showed a significant pattern of higher absorbences during cold periods (April-May 1997 and May 1998) and lower absorbences during warm periods (January-April 1998) (Figure 3-2). *Eisenia* and *Laminaria* plastid absorbences fluctuated little among dates between April 1997 and June 1998 (Figure 3-1).

Plastid absorbences (estimated at 438 nm) varied significantly among plants sampled on a given sampling date for *Macrocystis*, *Pterygophora* and *Eisenia*, but were not significant for *Laminaria* (Figure 3-1; Plant effect in Table 3-2). Plant effects for *Macrocystis* and *Pterygophora*, however, accounted for less total variability in plastid absorbences (estimated at 438 nm) than Date effects for these species (Table 3-2). Although Date effects were also stronger than Plant effects for *Eisenia*, the greatest amount of total variability in *Eisenia* plastid absorbance was explained by within-plant effects (i. e. Error) (Table 3-2). The greatest variability in *Laminaria* plastid absorbance was also explained by within-plant variability (i. e. Error), with little variability explained by either Date or Plant effects

(Table 3-2). Consequently, magnitude of plastid absorbences appeared to be driven primarily by among-date variability for *Macrocystis* and *Pterygophora*, among-date and within-plant variability for *Eisenia*, and within-plant variability for *Laminaria*. Although these patterns are well supported by the data presented here, rigorous comparisons between the seasonally-reproducing *Pterygophora*, *Eisenia* and *Laminaria*, and continuously-reproducing *Macrocystis* will require a longer time series.

Despite the described patterns of variability in absorbance among dates and plants, plastid absorption spectra were highly specific to each taxa (Figure 3-3). Absorbences between 400 - 500 nm and 575 - 700 nm decreased from *Pterygophora* to *Eisenia* to *Laminaria* to *Macrocystis*, whereas absorbences between 500 - 575 nm decreased from *Eisenia* to *Pterygophora* to *Laminaria* to *Macrocystis*. The switch in order for *Pterygophora* and *Eisenia* between 500 - 575 nm was observed for nearly all of the *Pterygophora* and *Eisenia* spectra. Except for *Pterygophora* and *Eisenia* in the region of 475 - 575 nm, 99.9% confidence intervals for each species rarely overlapped (Figure 3-3). All absorption spectra exhibited the various peaks and shoulders attributed to typical kelp pigment composition (i. e. chlorophylls *a* & *c*, fucoxanthin) as described in **Chapter II** (Graham and Mitchell 1999).

Zoospore identification and cross-validation

Zoospores were successfully classified among the 4 kelp species using DFA. Cross-validation of the identification algorithm against the Test data set indicated that the algorithm was a robust predictor of the identity of zoospores beyond those used to create the algorithm (Table 3-3); 97.8% of *Macrocystis*, 96.4% of *Pterygophora*, 86.3% of *Eisenia* and 69.9% of *Laminaria* zoospores in the Test data set were correctly identified. The results, however, indicated that most misidentified *Eisenia* zoospores were identified as *Laminaria*, and vice-versa (Table 3-3). *Eisenia* is currently absent at the study sites where

in situ plankton samples are being taken (Chapter IV), and relatively rare in the Point Loma kelp forest in general (Dayton et al. 1992, 1999). When *Eisenia* and *Laminaria* were treated as a single complex, the ability of the identification algorithm to predict zoospore identities was greatly improved (Table 3-3); 98.2% of *Macrocystis*, 97.4% of *Pterygophora* and 89.5% of *Eisenia/Laminaria* zoospores were correctly identified. Composition of the final identification algorithm is given in Table 3-4. The identification algorithm was also successful in identifying most of the kelp zoospores in the Future data set (i. e. after June 1998) although the sample size for this validation was limited (Table 3-5); 95.6% of *Macrocystis*, 100.0% of *Pterygophora* and 90.0% of *Eisenia/Laminaria* zoospores were correctly identified. Although adequate zoospore samples could not be collected from the deep-water kelps *Agarum fimbriatum* and *Pelagophycus porra* to allow their inclusion in this identification algorithm, a limited number of zoospores have been analyzed (~10 per species) and suggest that plastid absorbences for both species are lower at all wavelengths than presented here for *Macrocystis* (Figure 3-3). If this pattern is real then any *A. fimbriatum* or *P. porra* zoospores present in *in situ* plankton samples would likely be identified as *Macrocystis*. Zoospore plastid absorbences remain unknown for *Egregia menziesii*.

The protocol developed for discriminating between kelp zoospores and non-kelp cells was very precise. The relationship between the density of zoospores estimated using the criteria in Table 3-1 (“estimated” zoospore density) and the true density of zoospores (“labeled” zoospore density) was highly significant (Figure 3-4). The slope was estimated to be 0.99 and the confidence intervals (CI) around the slope were relatively tight and contained 1 (upper CI: 1.17; lower CI: 0.83); the intercept was estimated to be -1.02 (CI for the intercept were not determined). A slope of unity would be expected for a perfect relationship between “estimated” and “labeled” zoospore density. Thus, the density of zoospores estimated using the criteria in Table 1 appeared to predict accurately the true

density of kelp zoospores on the filters. In a few cases, “estimated” and “labeled” zoospore densities differed by 1 - 2 zoospores, possibly due to difficulty in tracking *exactly* the same swath for the two density estimates. In one case, however, the “estimated” zoospore density was much greater than the “labeled” zoospore density. This outlying datum could indicate: (1) the presence of non-kelp cells that could not be distinguished from kelp zoospores and were thus counted as “estimated” zoospores but not “labeled” zoospores; or (2) incomplete fluorescent-labeling of kelp zoospores in the sample. That only 1 of 20 data points showed this pattern suggests that the presence of confounding non-kelp cells was unlikely, since an aggregation of non-kelp cells in a single sample would not be expected. Incomplete labeling of a single sample, however, was possible since each of the 20 samples were labeled with individual aliquots of fluorescent stain. Therefore, the observed errors in the relationship between “estimated” and “labeled” zoospore density probably resulted from errors in the validation technique rather than errors in the discrimination criteria.

Zoospore abundance in field samples

Kelp zoospore densities were highly variable both within and among field sampling dates. Total kelp zoospore densities were an order of magnitude higher in February 1999 than on either date in October 1998, and variability in zoospore density (SD) increased greatly with increasing zoospore density (Table 3-6). Although within-date variability in kelp zoospore density was high, standard deviations in total kelp zoospore density did not exceed the average density on any given date.

Zoospore species-compositions were also highly variable within and among sampling dates. *Macrocystis* zoospores dominated plankton samples on all sampling dates, whereas *Pterygophora* and *Eisenia/Laminaria* zoospores were only observed in the February 1999 samples (Table 3-6). Again, given the current low abundance of *Eisenia* in the Point Loma

kelp forest, *Eisenia/Laminaria* zoospores were assumed to be primarily *Laminaria*. Occurrence of *Pterygophora* and *Eisenia/Laminaria* zoospores in only the February 1999 samples corresponds well with the reproductive seasonality previously described for these taxa (Figure 3-1). As with total kelp zoospore density, the density of *Macrocystis* zoospores increased by one order of magnitude from October 1998 to February 1999, and the standard deviation in *Macrocystis* zoospore density was greater on dates with higher average densities (Table 3-6). *Eisenia/Laminaria* zoospore densities estimated during February 1999 were lower than for *Macrocystis* on that date but greater than the density of *Macrocystis* zoospores estimated in early October 1998. Besides the dates when no *Pterygophora* and *Eisenia/Laminaria* zoospores were observed, the density of *Pterygophora* zoospores estimated in February 1999 was the lowest found for any of the three taxa during the study. Further, standard deviations in *Pterygophora* and *Eisenia/Laminaria* zoospore density were greater than average densities for the February 1999 sample (Table 3-6).

Discussion

The specific factors responsible for the observed among-species differences in absorption spectra, and thus the success of the identification algorithm, were unclear. In **Chapter II** (Graham and Mitchell 1999), I reported that *Pterygophora* plastids were significantly larger than *Macrocystis* plastids and suggested that differences in the area of plastids occupied by the microphotometer aperture accounted for absorption spectra specific to these species. We found that *Pterygophora* plastids always occupied greater than 100% of the aperture, whereas *Macrocystis* plastids occupied an average of only 50.9%. Thus, even if pigment concentrations per unit of plastid volume were the same, *Pterygophora* plastids would have greater apparent absorbance than *Macrocystis*. Additional observations, however, indicate that 99% of all *Eisenia* plastids also occupy greater than 100% of the

aperture (M. H. Graham unpublished data). Still, the identification algorithm presented here was successful in discriminating between most *Pterygophora* and *Eisenia* zoospores based on plastid absorption spectra. Therefore, observed differences in absorption spectra between *Pterygophora* and *Eisenia* cannot be due to differences in the area of plastids occupied by the microphotometer aperture, as was previously suggested for *Macrocystis* and *Pterygophora* (Graham and Mitchell 1999, Chapter II). Further, *Laminaria* and *Eisenia* also differ markedly in plastid size, with *Laminaria* having smaller plastids (M. H. Graham personal observation). Yet, *Laminaria* and *Eisenia* have very similar plastid absorption spectra as evidenced by the poor ability of the identification algorithm to discriminate among zoospores of these two species. This spectral pattern suggests that the smaller size of *Laminaria* plastids must be compensated by greater pigment concentration per unit of plastid volume than for *Eisenia*. Additional studies that directly compare zoospore plastid pigment concentration to plastid absorbance are needed to better understand the observed among-species variability in zoospore absorption spectra.

Variability in zoospore plastid absorbance is likely to have ecological ramifications. Differences observed among the 4 kelp species studied (i. e. order of plastid absorbances: *Macrocystis* > *Laminaria* > *Eisenia* > *Pterygophora*) corresponded well with the results of Amsler and Neushul (1991), who found that zoospore-specific chlorophyll *a* concentrations and photosynthesis increased significantly from *Macrocystis* to *Laminaria* to *Pterygophora* (*Eisenia* zoospores were not studied). Thus, the among-species differences in zoospore plastid absorbance that I observed likely represent differences in photosynthetic physiology, and might ultimately be expressed as differences in zoospore swimming durations, zoospore settlement and germination success, or photosynthetic potential of early kelp germlings. Data presented by Reed et al. (1992, Figure 3-1) suggested that the maximal time that *Pterygophora* zoospores can swim is longer than for *Macrocystis*. Reed et al. (1992) also found that zoospore settlement success for *Pterygophora* was statistically

greater than for *Macrocystis*. Finally, the onset of gametogenesis and subsequent fertilization was demonstrated to be more rapid for *Pterygophora* than *Macrocystis*, thus affording *Pterygophora* a competitive edge in situations when zoospores of both species settled simultaneously (Reed 1990). Despite these preliminary results, it remains to be determined whether variability in plastid absorbance can be used to estimate variability in zoospore photosynthetic physiology.

The negative relationships between plastid absorbance and bottom temperature that were observed for *Macrocystis* and *Pterygophora* are interesting because, at least in the case of *Pterygophora*, effects of bottom temperature on zoospore physiology were not expected. In coastal regions, seawater nitrogen concentrations increase sharply as water temperature decreases, providing increased nutrients for uptake by kelps (Zimmerman and Robertson 1985). Kelp pigments can be significant nitrogen sinks (Rowan 1989, Lobban and Harrison 1994), and pigment concentrations have been shown to be related to seawater nitrate concentration (Smith et al. 1983). Reed et al. (1996), however, had previously shown that, although adult C/N ratios were highly variable over time for both *Macrocystis* and *Pterygophora*, correlations between bottom temperature and zoospore C/N ratios were weak for *Macrocystis* and non-significant for *Pterygophora*. Their hypothesis was that *Pterygophora*, and to a lesser extent *Macrocystis*, was maintaining zoospore quality (i. e. stable chemical composition) at the expense of zoospore quantity or the quality of the adult thalli. My results, however, show that at least one indicator of zoospore quality (plastid pigment concentration or absorbance) is strongly affected by temperature for these species. During periods of high temperature, and thus low nutrient concentrations, kelps may shift the allocation of nitrogen within zoospores (e. g., from plastid pigments to amino acids or other protein reserves). If such within-zoospore variation in C/N ratios does occur, it would be detected by the spatially discrete aperture of the microphotometer but not by bulk analyses of zoospore chemical composition.

Despite the apparent robustness of the zoospore identification algorithm to temporal fluctuations in plastid absorbance, the magnitude of large-scale variability in plastid absorbance remains unknown. *Macrocystis*, *Pterygophora*, *Eisenia* and *Laminaria* sporophyll samples were collected throughout the Point Loma kelp forest but were not collected from other regions. Therefore, this identification algorithm is specific only to these species at Point Loma, and it is not known whether the algorithm can be used for identifying kelp zoospores from field samples taken in other kelp forests. In fact, the patterns described for *Macrocystis* and *Pterygophora* zoospore plastid absorbances versus bottom temperatures suggest that different absorption spectra signatures would be observed for these species in regions that are warmer or colder, or more nutrient poor or rich, than Point Loma (e. g., outside the Southern California Bight), although the general among-species pattern in plastid absorbance will likely be observed (*Pterygophora* > *Eisenia* > *Laminaria* > *Macrocystis*). Consequently, the identification algorithm presented here must be re-validated or modified before being applied to kelps in other geographic regions.

The methodology for discriminating kelp zoospores from non-kelp cells in field samples, however, should be immediately applicable wherever kelps are present. Most of the morphological and ultrastructural features of kelp zoospores that distinguish them from the other cells are phylogenetically conserved and have been known for over 50 years (Fritsch 1945) with few taxonomic changes during that time. One exception is the recent conclusion that the families of “primitive” Laminariales (Chordaceae, Phyllariaceae, and Pseudochordaceae) possess eyespots in conjunction with their single zoospore plastids (Henry and South 1987, Flores-Moya and Henry 1998). As such, these taxa may also be easily distinguished from “advanced” kelp zoospores in regions where the two groups co-occur. The methodology for distinguishing between kelp and non-kelp cells is also often highly redundant. For example, many different criteria exist for discriminating kelp zoospores from most other Heterokontophytes, such as multiple plastids and the presence

of eyespots (Table 3-1). Consequently, researchers may be able to fine-tune the identification methodology for use with equipment specific to their analytical facility (e. g., limited access to fluorescence microscopes).

In some cases, the methodology for species-specific kelp zoospore identifications may be simplified beyond that presented here. For example, had only *Macrocystis* and *Pterygophora* been present at Point Loma, kelp zoospore identifications could possibly have been made on the basis of plastid size alone, without the need to use microphotometry. New techniques for estimating plastid size, however, would need to be developed to overcome the imprecision of plastid sizes estimated using light microscopy (Graham and Mitchell 1999, **Chapter II**). Testing and modification of the method described here for kelp zoospore identification is also a continuing process. The use of *relative* zoospore species-compositions biases species-specific zoospore abundances due to the “unit-sum” constraint (i. e. the summed composition of all components equals 1) (Aitchison 1986). By determining the identity of exactly 20 zoospores per sample, I found the lowest detectable abundance of zoospores per kelp species to be 54/L in October 1998, but increasing to 943/L in February 1999. In order to achieve a constant lowest detectable zoospore abundance, the sample size for making future species-composition determinations should be a function of the observed total kelp zoospore abundance. Further, studies are currently underway to determine over what spatial (meters to kilometers) and temporal (minutes to months) scales kelp zoospore abundance is most variable, for the purpose of estimating an optimal sample size, interval and duration for monitoring kelp zoospore abundance.

Despite these limitations, however, the basic methodology described here for *in situ* sampling and identification of kelp zoospores is robust and may find broad application in the study of macroalgal planktonic stages. Much of the protocol for isolating and identifying kelp zoospores from plankton samples consists of simple modifications of standard procedures utilized by ecologists for the study of nearshore phytoplankton

assemblages and, as such, the necessary analytical materials should be readily available. Furthermore, the features that distinguish zoospores of different kelp species from each other, as well as from non-kelp cells, are linked to physiological traits that are likely related to the fitness of kelp microscopic stages (e. g., photosynthetic physiology or potential for phototaxis; Fritsch 1945, Henry and Cole 1982a, Kawai 1992) and thus may be stable under strong selective pressure. If intense competition among macroalgal microscopic stages is common, photo-physiological features unique to planktonic propagules of other macroalgal taxa may also await discovery. The ecology of macroalgal planktonic stages is a wide-open field of study with both basic and applied scientific implications (e. g., dispersal, recruitment, gene flow, population maintenance and population restoration). By combining basic principles of macroalgal propagule physiology with modern technological advances (e. g., microphotometry), ecologists may finally have a rigorous tool for investigating the role of planktonic processes in the life history and ecology of marine macroalgae.

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Table 3-1. Criteria for distinguishing kelp zoospores (Laminariales) from non-kelp cells in field samples. Primary characters for discriminating each taxa from kelp zoospores are in bold. Morphology and presence of eyespot (transmitted-light and blue-excitation epi-fluorescence microscopy) were easiest to determine, followed by number of plastids (blue-excitation epi-fluorescence microscopy) and pigment composition (microphotometry). "Laminariales" include all kelp genera in families Alariaceae, Laminariaceae and Lessoniaceae. "All other orders" of phaeophyceae taxa include genera in Ectocarpales, Chordariales, Scytosiphonales, Dictyosiphonales, Sphacelariales, Desmarestiales and Sporochneales (systematics according to Van den Hoek et al. 1995) (*GA* gametes; *SP* spores; *ZD* zoids; *ZO* zoospores; *ZY* zygotes; *P* present; *U* uncommon; *a* absent).

Taxa	Stage	Morphology	Eyespot	Plastids	Dominant pigments	Source
Chlorophyta						
Bryopsidophyce	GA	asymmetrical	P	1	chl <i>a + b</i>	2, 5
Bryopsidophyce	ZY	asymmetrical	?	?	chl <i>a + b</i>	"
Chlorophyceae	ZD	asymmetrical	P	1	chl <i>a + b</i>	"
Cladophorophyce	GA	asymmetrical	P	1	chl <i>a + b</i>	"
Cladophorophyce	ZY	asymmetrical	?	?	chl <i>a + b</i>	"
Prasinophyceae	ZD	scaled	P	1	chl <i>a + b</i>	"
Ulvophyceae	GA	asymmetrical	P	1	chl <i>a + b</i>	"
Ulvophyceae	ZY	asymmetrical	?	2	chl <i>a + b</i>	"
Cryptophyta	ZD	furrowed	U	1 - 2	chl <i>a + c</i> , phycobilins	2, 5, 6
Dinophyta	ZD	thecate	U	varies	chl <i>a + c</i> , xanthophylls	"
Euglenophyta	ZD	elongate	P	varies	chl <i>a + b</i>	"
Haptophyta						
Haptophyceae						
Coccosphaerales	ZD	coccoliths	a	1 - 2	chl <i>a + c</i> , xanthophylls	"
Isochrysidales	ZD	spherical	a	2	chl <i>a + c</i> , xanthophylls	"
Isochrysidales	ZD	spherical	a	2	chl <i>a + c</i> , xanthophylls	"
Pavlovales	ZD	elongate	P	2	chl <i>a + c</i> , xanthophylls	"
Prymnesiales	ZD	scaled	a	2	chl <i>a + c</i> , xanthophylls	"
Heterokontophyta						
Bacillariophyceae	GA	pyriform	P	varies	chl <i>a + c</i> , xanthophylls	2, 5, 6
Chrysophyceae						
Chrysosphaerales	ZD	spherical	P	2	chl <i>a + c</i> , xanthophylls	"
Mallomonadales	ZD	scaled	a	2	chl <i>a + c</i> , xanthophylls	"

Table 3-1. (continued).

Heterokontophyta						
Ochromonadales						
Chromulinace	ZD	spherical	P	2	chl <i>a + c</i> , xanthophylls	“
Chrysococcace	ZD	loricate	P	2	chl <i>a + c</i> , xanthophylls	“
Dynobryace	ZD	loricate	P	2	chl <i>a + c</i> , xanthophylls	“
Ochromonadace						
<u>Boekelovia</u>	ZD	triangular	P	?	chl <i>a + c</i> , xanthophylls	“
<u>Ochromonas</u>	ZD	asymmetrical	P	1 - 2	chl <i>a + c</i> , xanthophylls	“
<u>Sphaleromantis</u>	ZD	scaled	P	1	chl <i>a + c</i> , xanthophylls	“
Dictyochophyce	ZD	siliceous	P	0 or > 2	chl <i>a + c</i> , xanthophylls	“
Phaeophyceae						
Laminariales	ZO	pyriform	a	1	chl <i>a + c</i> , xanthophylls	3, 4
Fucales	ZY	spherical	a	many	chl <i>a + c</i> , xanthophylls	5
Dictyotales	SP	spherical	a	many	chl <i>a + c</i> , xanthophylls	1, 4, 5
Dictyotales	GA	pyriform	a	many ^a	chl <i>a + c</i> , xanthophylls	“
all other orders	ZO	pyriform	P	varies	chl <i>a + c</i> , xanthophylls	“
all other orders	GA ^b	pyriform	P	varies	chl <i>a + c</i> , xanthophylls	“
Raphidophyceae	ZD	pyriform	P	many	chl <i>a + c</i> , xanthophylls	2, 5, 6
Sarcinochrysid	ZD	pyriform	?	2	chl <i>a + c</i> , xanthophylls	5, 6
Rhodophyta	SP	spherical	a	many	chl <i>a</i> , phycobilins	2, 5

^a Dictyotalean spermatozoid plastids are small and extremely pale compared to larger, more pigmented kelp plastids (Fritsch 1945, Phillips et al. 1990)

^b Non-oogamous orders only

¹ Fritsch (1945), ² Dodge (1973), ³ Henry and Cole (1982a), ⁴ Kawai (1992), ⁵ Van den Hoek et al. (1995), ⁶ Tomas (1997)

Table 3-2. *Macrocystis pyrifera*, *Pterygophora californica*, *Eisenia arborea* and *Laminaria farlowii*. Results of nested ANOVAs testing for effects of Date and Plant (within Date) variability on zoospore plastid-absorbance at 438 nm (SS sums of squares; MS mean square; % percent of total variability in zoospore plastid-absorbance explained by specific source).

Source	SS	df	MS	F	P	%
<i>Macrocystis</i>						
Date	0.334	9	0.037	21.96	< 0.0001	53.3
Plant	0.034	20	0.002	3.72	< 0.0001	5.6
Error	0.246	540	< 0.001			41.1
<i>Pterygophora</i>						
Date	0.959	4	0.240	54.98	< 0.0001	71.6
Plant	0.044	10	0.004	3.10	0.001	2.7
Error	0.380	270	0.001			25.7
<i>Eisenia</i>						
Date	0.140	3	0.047	4.96	0.078	33.6
Plant	0.038	4	0.009	6.53	< 0.0001	14.4
Error	0.219	152	0.001			52.0
<i>Laminaria</i>						
Date	0.002	2	0.001	0.13	0.882	0
Plant	0.019	3	0.006	2.54	0.060	3.1
Error	0.278	114	0.002			96.9

Table 3-3. *Macrocystis pyrifera*, *Pterygophora californica*, *Eisenia arborea* and *Laminaria farlowii*. Validation of discriminant function analysis (DFA) against data (Test) collected simultaneously with those used to create discriminant rule (Model). DFA for identification of all four kelp species. [Along rows true identity of zoospores; along columns identity predicted by DFA; values are percentages (frequencies); frequencies in vertical "total" column sample sizes included in DFA for each species] Validations were created by applying significant discriminant rule (arising from DFA) to individual absorption spectra.

True identity	Predicted identity				Total
	<i>Macrocystis</i>	<i>Pterygophora</i>	<i>Eisenia</i>	<i>Laminaria</i>	
<i>Macrocystis</i>	97.75(435)	0(0)	0.67(3)	1.57(7)	100.00(445)
<i>Pterygophora</i>	0(0)	96.41(188)	1.54(3)	2.05(4)	100.00(195)
<i>Eisenia</i>	2.50(2)	0(0)	86.25(69)	11.25(9)	100.00(80)
<i>Laminaria</i>	10.96(8)	2.74(2)	16.44(12)	69.86(51)	100.00(73)
Total	100.00(445)	97.44(190)	108.75(87)	97.26(71)	100.00(793)

Table 3-4. *Macrocystis pyrifera*, *Pterygophora californica*, *Eisenia arborea* and *Laminaria farlowii*. Validation of discriminant function analysis (DFA) against data (Test) collected simultaneously with those used to create discriminant rule (Model). *Eisenia* and *Laminaria* were not distinguished from each other in DFA. [Along rows true identity of zoospores; along columns identity predicted by DFA; values are percentages (frequencies); frequencies in vertical "total" column sample sizes included in DFA for each species] Validations were created by applying significant discriminant rule (arising from DFA) to individual absorption spectra.

True identity	Predicted identity			Total
	<i>Macrocystis</i>	<i>Pterygophora</i>	<i>Eisenia/Laminaria</i>	
<i>Macrocystis</i>	98.20(437)	0(0)	1.78(8)	100.00(445)
<i>Pterygophora</i>	0(0)	97.44(190)	2.56(5)	100.00(195)
<i>Eisenia/Laminaria</i>	8.49(13)	1.96(3)	89.54(137)	100.00(153)
Total	98.88(450)	98.97(193)	98.04(150)	100.00(793)

Table 3-5. *Macrocystis pyrifera*, *Pterygophora californica*, *Eisenia arborea* and *Laminaria farlowii*. Identification algorithms created by DFA. Columns contain algorithm coefficients and constants; *Eisenia* and *Laminaria* were not distinguished from each other in DFA. To identify kelp zoospores, each algorithm is independently applied to a single zoospore absorption spectrum by multiplying coefficients by associated absorbances, summing coefficient/absorbance products for all wavelengths, and adding constant. Algorithm with largest value for given zoospore absorption spectrum indicates identity of zoospore.

wavelength	Species		
coefficients(nm)	<i>Macrocystis</i>	<i>Pterygophora</i>	<i>Eisenia/Laminaria</i>
408.1	-0.003	68.243	10.202
420.2	-71.272	-94.100	-71.381
432.3	69.308	113.504	69.972
444.4	128.036	6.545	170.310
456.5	-5.628	235.629	138.214
468.6	-53.525	127.893	-237.358
480.7	-52.889	-120.108	-13.419
492.9	-27.484	-214.286	-92.198
504.9	164.066	245.604	203.443
517.1	46.931	223.885	359.492
529.2	-56.682	57.674	-164.031
541.3	21.243	-130.150	-34.215
553.4	-355.694	-916.055	-228.628
565.5	-110.670	-656.635	-646.440
577.6	372.495	378.183	758.584
589.7	638.661	998.982	501.938
601.8	-555.929	-324.163	-809.231
613.9	-321.883	604.410	-132.949
626.0	-49.263	-263.434	149.993
638.2	-113.718	-680.119	-91.389
650.3	284.092	409.687	96.778
662.4	41.674	62.055	137.829
674.5	20.632	-50.758	-64.719
686.6	-97.668	-55.471	-167.180
698.7	534.351	1116.598	864.935
constant	-11.653	-71.629	-29.244

Table 3-6. *Macrocystis pyrifera*, *Pterygophora californica*, *Eisenia arborea* and *Laminaria farlowii*. Validation of DFA against data collected after June 1998 (Future). *Eisenia* and *Laminaria* were not distinguished from each other in DFA. *E. arborea* data were not available; therefore *Eisenia/Laminaria* data represent *Laminaria* spectra only. Further details as in legend to Table 3-3.

True identity	Predicted identity			Total
	<i>Macrocystis</i>	<i>Pterygophora</i>	<i>Eisenia/Laminaria</i>	
<i>Macrocystis</i>	95.60(239)	0(0)	4.40(11)	100.00(250)
<i>Pterygophora</i>	0(0)	100.00(80)	0(0)	100.00(80)
<i>Eisenia/Laminaria</i>	10.00(3)	0(0)	90.00(27)	100.00(30)
Total	96.80(242)	100.00(80)	126.67(38)	100.00(360)

Table 3-7. *Macrocystis pyrifera*, *Pterygophora californica*, *Eisenia arborea* and *Laminaria farlowii*. Total kelp zoospore-density (no·l⁻¹) and species composition from 2-liter *in situ* plankton samples. Density estimates are means (\pm SD), n = 4; densities have been rounded to the nearest whole zoospore. *Eisenia* and *Laminaria* zoospores were not discriminated from each other.

Date	Total kelp	<i>Macrocystis</i>	<i>Pterygophora</i>	<i>Eisenia/Laminaria</i>
13 October 1998	1360 (58)	1360 (58)	0	0
24 October 1998	5274 (2253)	5274 (2253)	0	0
2 February 1999	18868 (10357)	16423 (8834)	288 (576)	2157 (2931)

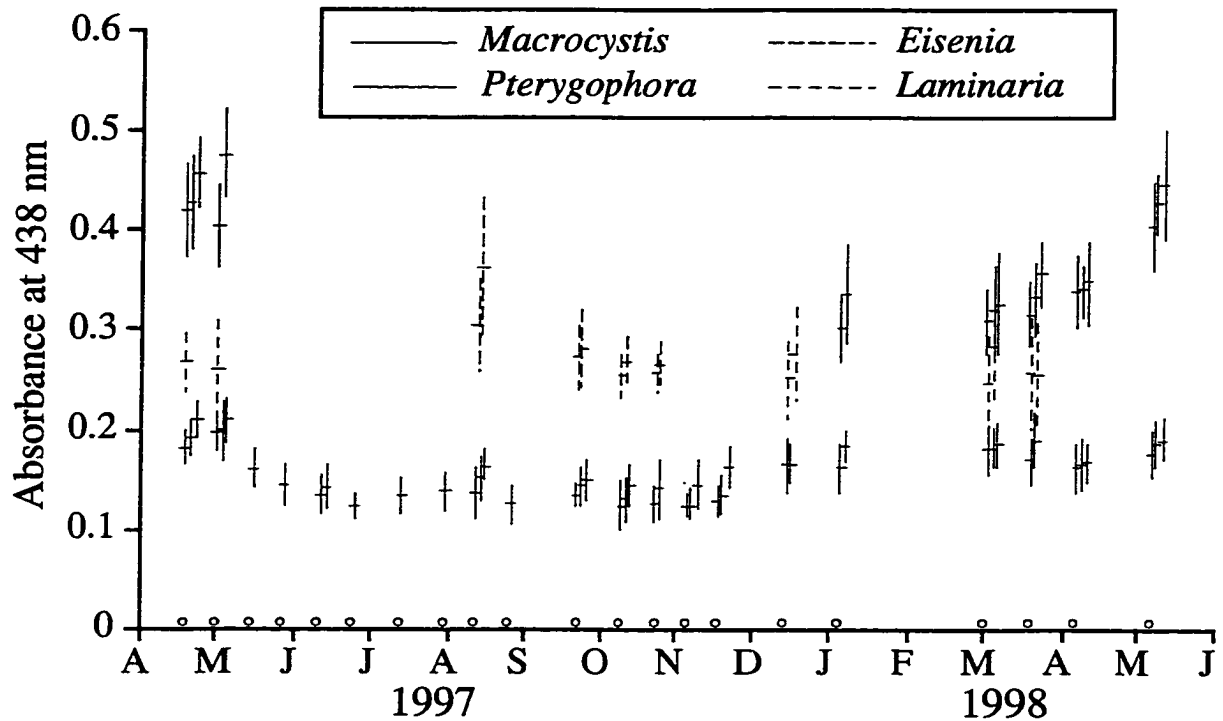


Figure 3-1. *Macrocystis pyrifera*, *Pterygophora californica*, *Eisenia arborea* and *Laminaria farlowii*. Time-series of zoospore plastid-absorbance measured at 438 nm between April 1997 and June 1998 (circles along x-axis sampling dates). Data are means \pm standard deviations. Number of zoospores analyzed per species per date was 15 for the first two sampling dates and 20 for subsequent dates. Up to three samples were collected for each species on each sampling date; when multiple samples were collected on a given date, the data were staggered in order to visualize overlapping error bars.

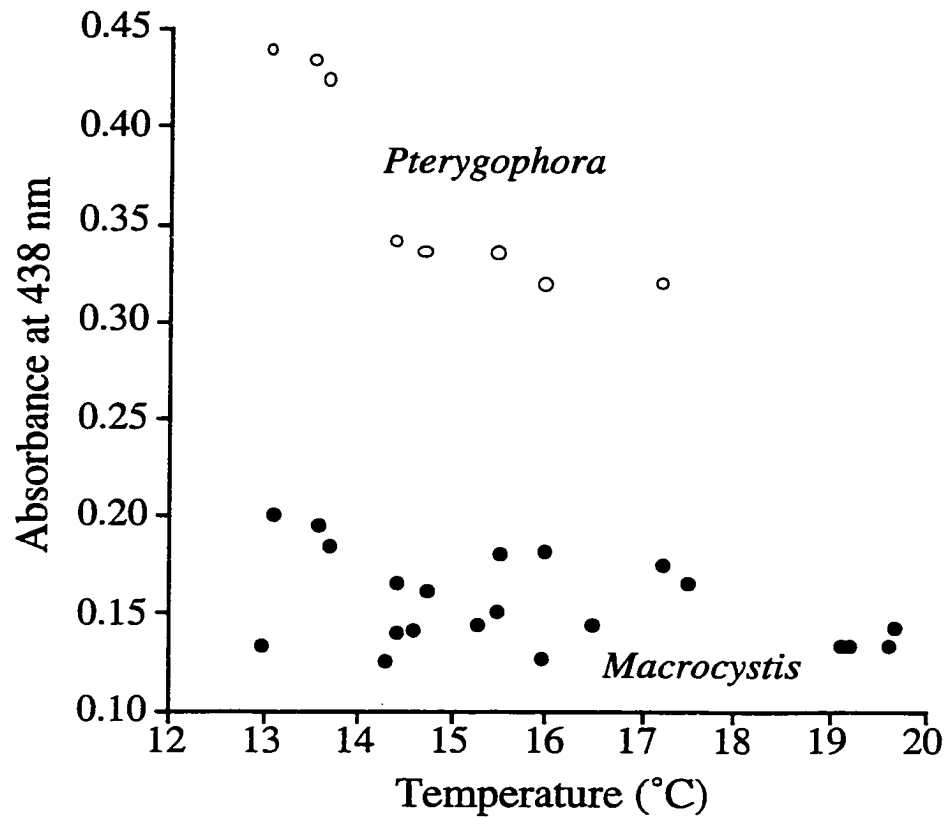


Figure 3-2. *Macrocystis pyrifera* and *Pterygophora californica*. Relationship between average zoospore plastid-absorbance measured at 438 nm and average bottom temperature. Absorbances were averaged across all samples for a given date per species; bottom temperatures represent daily temperature measurements averaged across all days between sampling periods. Pearson product-moment correlations: $r = -0.37$, $n = 21$, $p = 0.083$ for *Macrocystis*; $r = -0.86$, $n = 8$, $p = 0.006$ for *Pterygophora*.

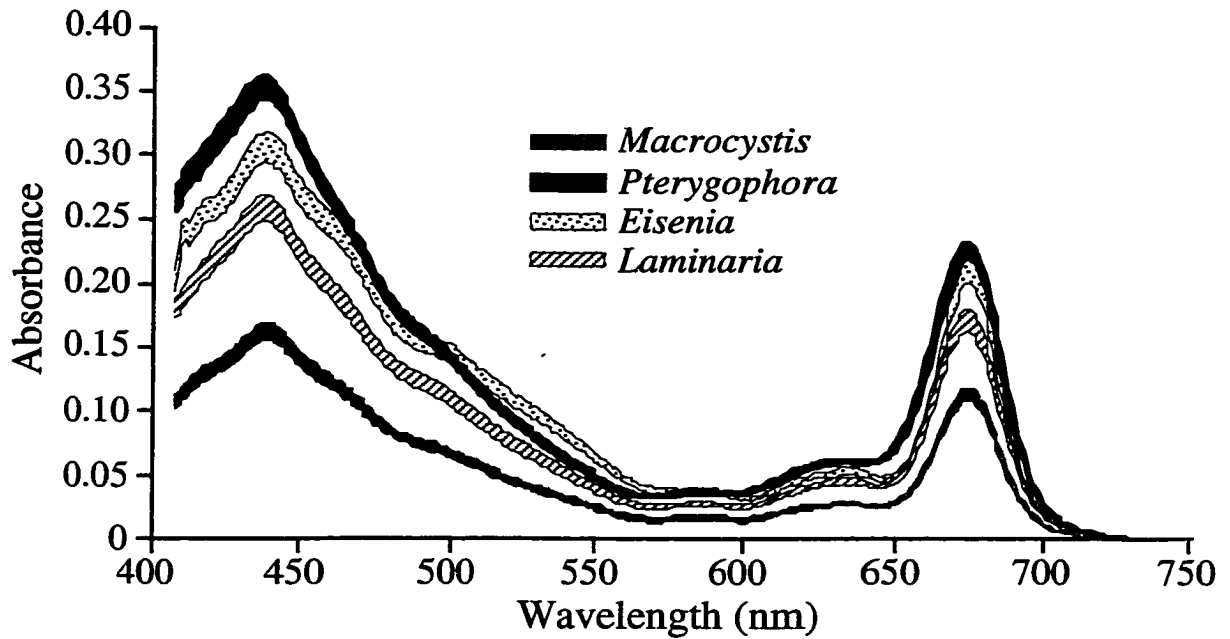


Figure 3-3. *Macrocystis pyrifera* ($n = 450$), *Pterygophora californica* ($n = 200$), *Eisenia arborea* ($n = 80$) and *Laminaria farlowii* ($n = 76$). Zoospore plastid-absorbance as a function of wavelength. Data represent 99.9% confidence intervals around average absorption spectrum for each species. Data are from Model data set used to create identification algorithm.

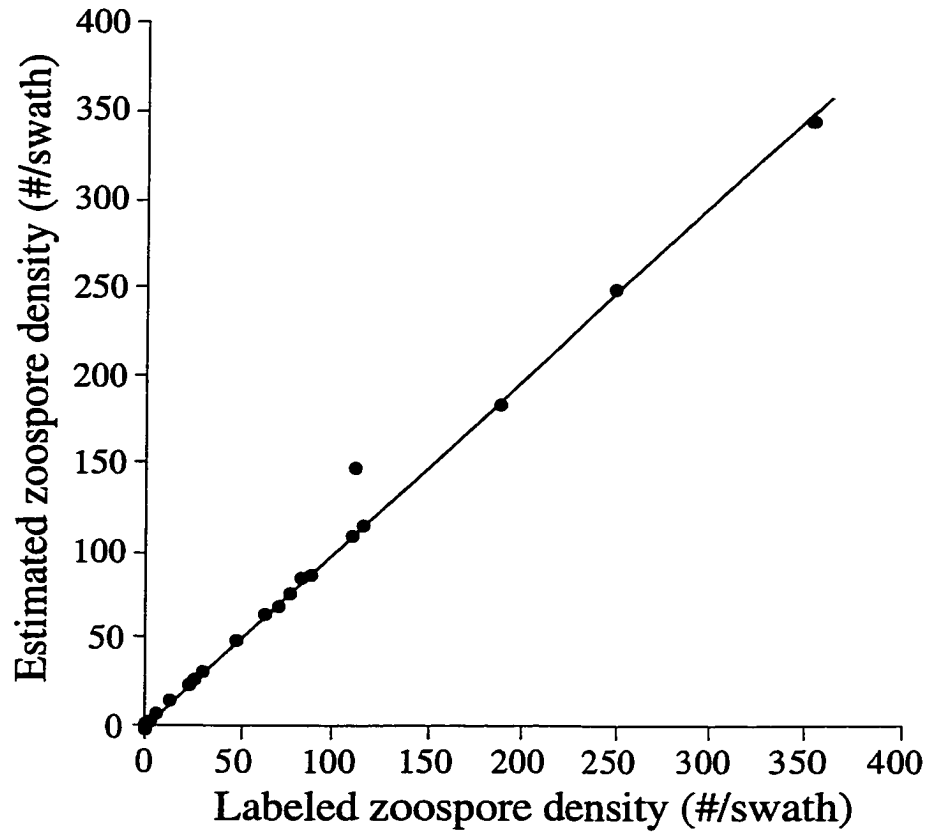


Figure 3-4. *Macrocystis pyrifera*. Relationship between true density of zoospores in simulated field sample (“labeled” zoospore density) and density of zoospores estimated using discrimination criteria in Table 1 (“estimated” zoospore density). Fitted line for major-axis regression equation is shown: $[(\text{labeled}) = -1.02 + 0.99 \cdot (\text{estimated})]; n = 20$.

Literature Cited

- Aitchison, J. 1986. *The statistical analysis of compositional data*. Chapman & Hall, New York.
- Amsler, C. D., and M. Neushul. 1991. Photosynthetic physiology and chemical composition of spores of the kelps *Macrocystis pyrifera*, *Nereocystis luetkeana*, *Laminaria farlowii*, and *Pterygophora californica* (Phaeophyceae). *Journal of Phycology* 27:26-34.
- Amsler, C. D., D. C. Reed, and M. Neushul. 1992. The microclimate inhabited by macroalgal propagules. *British Phycological Journal* 27:253-270.
- Amsler, C. D., and R. B. Searles. 1980. Vertical distribution of seaweed spores in a water column offshore of North Carolina. *Journal of Phycology* 16:617-619.
- Anderson, E. K., and W. J. North. 1966. *In situ* studies of spore production and dispersal in the giant kelp, *Macrocystis*. *Proceedings of the International Seaweed Symposium* 5:73-86.
- Dayton, P. K., M. J. Tegner, P. E. Parnell, and P. B. Edwards. 1992. Temporal and spatial patterns of disturbance and recovery in a kelp forest community. *Ecological Monographs* 62:421-445.
- Dayton, P. K., M. J. Tegner, P. B. Edwards, and K. L. Riser. 1999. Temporal and spatial scales of kelp demography: the role of oceanographic climate. *Ecological Monographs* 69:219-250.
- Deysher, L., and T. A. Norton. 1982. Dispersal and colonization in *Sargassum muticum* (Yendo) Fensholt. *Journal of Experimental Marine Biology and Ecology* 56:179-195.
- Dodge, J. D. 1973. *The fine structure of algal cells*. Academic Press, London.
- Flores-Moya, A., and E. C. Henry. 1998. Gametophyte and first stages of the sporophyte of *Phyllariopsis purpurascens* (Laminariales, Phaeophyta). *Phycologia* 37:398-401.
- Fredriksen, S., K. Sjotun, T. E. Lein, and J. Rueness. 1995. Spore dispersal in *Laminaria hyperborea* (Laminariales, Phaeophyceae). *Sarsia* 80:47-54.

- Fritsch, F. E. 1945. The structure and reproduction of algae. Cambridge University Press, London.
- Gaines, S., S. Brown, and J. Roughgarden. 1985. Spatial variations in larval concentrations as a cause of spatial variation in settlement for the barnacle, *Balanus glandula*. *Oecologia* 67:267-272.
- Graham, M. H., and B. G. Mitchell. 1999. Obtaining absorption spectra from individual macroalgal spores using microphotometry. *Hydrobiologia* 398/399:231-239.
- Henry, E. C., and K. M. Cole. 1982a. Ultrastructure of swarmers in the Laminariales (Phaeophyceae). I. Zoospores. *Journal of Phycology* 18:550-569.
- Henry, E. C., and K. M. Cole. 1982b. Ultrastructure of swarmers in the Laminariales (Phaeophyceae). II. Sperm. *Journal of Phycology* 18:570-579.
- Henry, E. C., and G. R. South. 1987. *Phyllariopsis* gen. nov. and a reappraisal of the Phyllariaceae Tilden 1935 (Laminariales, Phaeophyceae). *Phycologia* 26:9-16.
- Hoffman, A. J. 1987. The arrival of seaweed propagules at the shore: a review. *Botanica Marina* 30:151-165.
- Hoffman, A. J., and R. Ugarte. 1985. The arrival of propagules of marine macroalgae in the intertidal zone. *Journal of Experimental Marine Biology and Ecology* 92:83-95.
- Hruby, T., and T. A. Norton. 1979. Algal colonization on rocky shores in the Firth of Clyde. *Journal of Ecology* 67:65-77.
- Kawai, H. 1992. A summary of the morphology of chloroplasts and flagellated cells in the Phaeophyceae. *Korean Journal of Phycology* 7:33-43.
- Kendrick, G. A., and D. I. Walker. 1991. Dispersal distances for propagules of *Sargassum spinuligerum* (Sargassaceae, Phaeophyta) measured directly by vital staining and venturi suction sampling. *Marine Ecology Progress Series* 79:133-138.
- Lobban, C. S., and P. J. Harrison. 1994. Seaweed ecology and physiology. Cambridge University Press, Cambridge.
- McPeak, R. H. 1981. Fruiting in several species of Laminariales from southern California.

- Proceedings of the International Seaweed Symposium 8:404-409.
- Neushul, M. 1963. Studies on the giant kelp, *Macrocystis*. II. Reproduction. American Journal of Botany 50:354-359.
- Norton, T. A. 1992. Dispersal by macroalgae. British Phycological Journal 27:293-301.
- Phillips, J. A., M. N. Clayton, I Maier, W. Boland, D. G. Müller. 1990. Sexual reproduction in *Dictyota diemensis* (Dictyotales, Phaeophyta). Phycologia 29:367-379.
- Reed, D. C. 1990. The effects of variable settlement and early recruitment on patterns of kelp recruitment. Ecology 71:776-787.
- Reed, D. C., C. D. Amsler, and A. W. Ebeling. 1992. Dispersal in kelps: factors affecting spore swimming and competency. Ecology 73:1577-1585.
- Reed, D. C., A. W. Ebeling, T. W. Anderson, and M. Anghera. 1996. Differential reproductive responses to fluctuating resources in two seaweeds with different reproductive strategies. Ecology 77:300-316.
- Roughgarden, J., S. Gaines, and H. Possingham. 1988. Recruitment dynamics in complex life cycles. Science 241:1460-1466.
- Rowan, K. S. 1989. Photosynthetic pigments of algae. Cambridge University Press, Cambridge.
- Santelices, B. 1990. Patterns of reproduction, dispersal and recruitment in seaweeds. Oceanography Marine Biology Annual Review 28:177-276.
- Santelices, B., A. J. Hoffman, D. Aedo, M. Bobadilla, and R. Otaiza. 1995. A bank of microscopic forms on disturbed boulders and stones in tide pools. Marine Ecology Progress Series 129:215-228.
- Smith, R. G., W. N. Wheeler, and L. M. Srivastava. 1983. Seasonal photosynthetic performance of *Macrocystis integrifolia* (Phaeophyceae). Journal of Phycology 19:352-359.
- Sokal, R. R., and F. J. Rohlf. 1981. Biometry. W. H. Freeman & Co., New York.

Thompson, W. A., and J. R. Moore. 1963. Non-negative estimates of variance components. *Technometrics* 5:441-449.

Tomas, C. R. (editor). 1997. *Identifying marine phytoplankton*. Academic Press, San Diego.

van den Hoek, C., D. G. Mann, and H. M. Jahns. 1995. *Algae: an introduction to phycology*. Cambridge University Press, Cambridge.

Winer, B. J., D. R. Brown, K. M. Michels. 1991. *Statistical principles in experimental design*. McGraw-Hill, Inc., New York.

Zechman, F. W., and A. C. Mathieson. 1985. The distribution of seaweed propagules in estuarine, coastal and offshore waters of New Hampshire, U. S. A. *Botanica Marina* 28:283-294.

Zimmerman, R. C., and D. L. Robertson. 1985. Effects of El Nino on local hydrography and growth of the giant kelp, *Macrocystis pyrifera*, at Santa Catalina Island, California. *Limnology and Oceanography* 30:1298-1302.

Chapter IV

Scale-dependent effects of physical transport and local reproduction on giant kelp zoospore supply

Abstract

Propagule dispersal is fundamental in regulating the strength of demographic and genetic interactions between individuals both within and among populations. For benthic marine species with planktonic propagules, variability in dispersal is dependent primarily on the duration propagules remain in the plankton and the flow characteristics of the environment. Most studies have centered on invertebrate and fish species with relatively long planktonic durations (weeks to months), subsequently focusing attention on the role of broad-scale physical transport processes in regulating propagule supply and dispersal. I studied spatiotemporal variability in zoospore supply of a seaweed with a short planktonic duration (few days), giant kelp *Macrocystis pyrifera*. Planktonic propagules were filtered from sea water pumped from a few centimeters above the substratum in a Southern California kelp forest in 1999. Kelp zoospores unequivocally identified spectrophotometrically as those of *Macrocystis pyrifera* were quantified. Collections were made in the center of the kelp forest at twenty 1-min intervals on two dates and at twenty 10-min and at sixteen 100-min intervals on one date each. At the same location, approximately three samples per month were collected from late February through mid November. In addition, zoospore concentrations at the center of the forest were compared (1) with those from the seaward and landward edge of the forest on eight dates and (2) with those from the north-central and south-central part of the forest on seven dates. Time series analyses over intervals < 24 hr showed a relatively constant zoospore concentration randomly dispersed about the average. In contrast, temporal variability in zoospore concentration over longer

time scales was highly structured, with large fluctuations evidently reflecting relative changes in the reproductive condition of the adult kelp. The tight coupling between zoospore supply and local reproduction in the center of the forest appeared to be driven by low net current displacement due to the drag of adult plants, keeping zoospores close to their release site; coupling between zoospore supply and local reproduction was validated at the two additional central sites separated by 1 km. Lower plant densities along the seaward and landward edge of the forest, however, had more limited effects on flow. Here, flows were rapid and uni-directional, transporting zoospores far from the adults that released them, and de-coupling zoospore supply from local reproduction. The results suggest that the size of and location within kelp populations is an important determinant of the importance of local reproduction to zoospore supply. Variability in strength of interactions between individuals within and among kelp populations is therefore scale-dependent, blurring the distinction between open and closed population dynamics.

Introduction

It has recently been shown that marine species exist along a continuum of population subdivision, with most exhibiting demographic and genetic structure on regional, and sometimes even local, spatial scales (Palumbi 1995, Burton 1998, Bohonak 1999, Jones et al. 1999, Swearer et al. 1999, Cowen et al. 2000, Grosberg and Cunningham 2000). The extent to which marine species display such structure depends upon the strength of demographic and genetic interactions among constituent populations, individuals, and even life stages. For organisms that alternate between distinct benthic and planktonic life stages, much attention has been focused on physical/biological interactions that link the dynamics of the two stages. For example, “supply-side” studies have emphasized the importance of propagule supply in regulating recruitment, colonization, and connectivity among marine invertebrate and fish populations, and have often been successful in explaining significant

variability in population dynamics and structure (Lewin 1986, Roughgarden et al. 1988, Underwood and Fairweather 1989, Gaines and Lafferty 1995). These studies have also led to the realization that the interactions that regulate demographic and genetic structure in marine species can be extremely variable in time and space. Exploration of the underlying dynamics of these interactions and the processes that drive them is crucial to understanding physical and biological control of the organization of marine communities.

The functional role of propagule supply in the population dynamics of marine organisms is generally considered to be constrained by biological characteristics of propagules and physical characteristics of the environment. Most larvae of non-brooding marine invertebrates and fish are not competent for settlement until some time after release and many can maintain position in the water column by swimming or buoyancy regulation (e. g., Victor 1991, Young 1995). Consequently, larvae may remain in the plankton for weeks to months prior to settlement, facilitating their transport by hydrodynamic forces that vary over broad spatial and temporal scales (Roughgarden et al. 1988, Victor 1991, Shanks 1995, Downes and Keough 1998). In some taxa, the supply of larvae competent for settlement is de-coupled from local demographic and reproductive processes because the larvae are advected far from the adults that released them (Roughgarden et al. 1988, Wing et al. 1998, Shanks et al. 2000). However, in other taxa, physical transport processes (e. g., currents, eddies, or fronts) retain larvae near the site of release, coupling larval supply to local reproduction (Jones et al. 1999, Swearer et al. 1999). As such, marine invertebrate and fish populations are generally considered to be either “open”, with recruitment determined primarily by the supply of larvae from remote locations, or “closed”, with a stronger link to local larval sources (Sale 1991, Gaines and Lafferty 1995, Caley et al. 1996, Cowen et al. 2000).

The dynamics of kelp populations offer an interesting contrast to supply-side models developed for invertebrates and fish. Unlike most larvae, kelp propagules (zoospores) are

able to settle immediately upon release from adults, with most settlement likely occurring within days of release (Reed et al. 1992). Zoospore planktonic duration therefore depends primarily on the time it takes to reach suitable settlement substrate. Kelp zoospores are small ($\sim 3 \times 7 \mu\text{m}$) with very slow sinking rates (Hoffman and Camus 1989, Amsler et al. 1992, B. Gaylord et al., manuscript in review) and extremely low Reynolds numbers ($\sim 2.5 \times 10^{-4}$); consequently they have been modeled as passive planktonic particles (Gaylord et al., manuscript in review). As such, settlement is thought to be dependent upon the advective and diffusive transport of zoospores to the sea floor which is regulated primarily by hydrodynamic processes in the water column and the height at which zoospores are released (Hoffman 1987, Santelices 1990, B. Gaylord et al., manuscript in review). Both hydrodynamics and zoospore release height can vary greatly within and among kelp assemblages and their resident kelp taxa. Despite their relatively short plankton duration, competent kelp propagules therefore may settle close to the adults that released them when currents are weak, dispersing farther when currents are strong. As such, kelps provide a unique opportunity to test prevalent themes in supply-side ecology, in particular the coupling/de-coupling of propagule supply to local reproduction and the distinction between “open” and “closed” population dynamics.

The general focus of previous supply-side studies on organisms with long planktonic durations has effectively directed attention to large-scale physical transport processes that vary over broad scales (days to months, and 100's of meters to 1000's of kilometers), such as upwelling relaxation events, eddies, and variations in current velocity and direction (Gaines et al. 1985, Roughgarden et al. 1988, Wing et al. 1998, Morgan et al. 2000, Shanks et al. 2000). Field studies conducted at smaller-scales have been less common (Grosberg 1982, Booth 1991, Minchinton and Scheibling 1991, Pineda 1991, Miron et al. 1995, Pineda 1999). The immediate settlement competency of kelp zoospores and minimum settlement density required for recruitment (Reed et al. 1991), however, suggest that small-

scale temporal and spatial patchiness in zoospore supply may contribute to variability in kelp recruitment. Characterization of the combined role of small- and large-scale processes in regulating kelp zoospore supply may also help to resolve contemporary debates concerning kelp dispersal. Field studies that have shown kelp zoospores can disperse over long distances > 4 km (Reed et al. 1988) seemingly contradict other studies that have promoted kelps as predominantly short-distance dispersers $< 10^3$ m (Anderson and North 1966, Dayton et al. 1984, Reed et al. 1988, Fredriksen et al. 1995). Given the potentially broad range of time kelp zoospores spend in the plankton and the high temporal and spatial variability observed in nearshore hydrodynamic processes (Jackson and Winant 1983, Denny 1988, Jackson 1997, Washburn et al. 1999) a discrete distinction between short- and long-distance kelp dispersal seems unrealistic. Rather, kelp dispersal most likely varies continuously as a function of physical and biological processes. Yet the debate between short- versus long-distance dispersal is not trivial; it has great implications for kelp meta-population dynamics, gene flow, and evolution.

Understanding the relevant temporal and spatial scales over which kelp zoospore supply varies, and the combination of physical and biological processes that regulate that variability, is fundamental to studying the role of remote versus local reproduction to kelp population dynamics. Kelp recruitment following massive disturbances that remove all local adults must come from remote zoospore production, drifting sporogenous tissue, or dormant recruits. This may not be the case, however, in established kelp forests where a relatively continuous distribution of healthy kelp plants provides abundant local zoospore sources. In such kelp forests, which are common along the southern California coast and offshore islands (North and Hubbs 1968, North et al. 1993), variability in zoospore supply will likely be due to a combination of physical transport and reproductive processes acting over a variety of temporal and spatial scales.

This study considers some fundamental questions in supply-side ecology relevant to the

population dynamics of giant kelp (*Macrocystis pyrifera*) within the largest continuous kelp forest in southern California. The primary goal was to give a detailed and accurate description of the natural variability in giant kelp zoospore supply and the physical/biological processes important in regulating this variability: an adequate availability of propagules for settlement is considered vital to seaweed recruitment (Hruby and Norton 1979, Hoffman and Ugarte 1985, Hoffman 1987, Santelices 1990, Santelices et al. 1995). I specifically asked: Over what temporal and spatial scales is giant kelp zoospore supply most variable? What is the extent of coupling between zoospore supply and local reproductive processes? What are the relative contributions of physical transport and local reproduction to zoospore supply? Do these contributions vary over time and space? Are kelp populations open or closed? The study provides a view of planktonic processes in benthic organisms that differs strikingly from current supply-side models because it emphasizes variable coupling between propagule supply and local reproduction, rapid responses to environmental stochasticity, and novel interactions between giant kelp and its environment.

Study System

The research was done within the central portion of the Point Loma kelp forest located offshore of San Diego, California, USA (Figure 4-1). This large kelp forest (~1 x 8 km) has been extensively studied since the early 1950's (reviewed in North 1971) and is the site of continuous ecological study since 1971 (Dayton et al. 1984, Dayton and Tegner 1984, Dayton et al. 1992, Tegner et al. 1996, 1997, Dayton et al. 1999). The kelp forest grows on a submerged rocky terrace that is flat and gradually sloping with isolated regions of high vertical relief (rocks, pinnacles, and ledges), and is bound by sand in deep water (~30 m) and to the north and south by the mouths of Mission Bay and San Diego Bay. Previous studies have provided extensive information on hydrographic conditions at Point Loma, as well as the abundance, distribution, size, and overall health of the resident kelp assemblage,

facilitating this study of physical and biological regulation of giant kelp zoospore supply.

The Point Loma kelp forest lies at the southern end of the Southern California Bight where the oceanographic climate is strongly influenced by variability in the flow of the California Countercurrent (Hickey 1993). The California Countercurrent bathes the Point Loma kelp forest in high-salinity, high-temperature, and low-nutrient water during summer and fall, interrupted by periods of wind-driven upwelling during winter and spring (Tegner et al. 1997). Average sea-surface and bottom temperatures peak during late summer at $\sim 21^{\circ}\text{C}$ and 16°C , respectively, and nitrate concentrations generally fall to less than $1\ \mu\text{M}$. During spring upwelling events average sea-surface and bottom temperatures drop to less than 14°C and 12°C , with nitrate concentrations often exceeding $10\ \mu\text{M}$. These seasonal differences in temperature and nutrients are of fundamental importance in regulating giant kelp survival, growth, reproduction, and recruitment in southern California (Dean and Jacobsen 1984, Zimmerman and Robertson 1985, Deysher and Dean 1986a, Deysher and Dean 1986b, Zimmerman and Kremer 1986, Dayton et al. 1999) and can be exacerbated/ameliorated during El Niño/La Niña events (Dean and Jacobsen 1986, Tegner et al. 1997, Dayton et al. 1999).

Currents within the Point Loma kelp forest are greatly modified by the kelp assemblage itself. Attached adult giant kelp plants exert drag on the water decreasing the velocity of both across- and along-shore currents within the forest relative to outside (Jackson and Winant 1983, Jackson 1997). Jackson (1997) found that along-shore currents on the outer edge of the kelp forest are an order of magnitude greater ($2.1 \pm 9.9\ \text{cm/s}$) than in the center of the forest ($0.2 \pm 2.0\ \text{cm/s}$). Across-shore currents are also dampened within the forest with average velocities in the center ranging from 0.2 ± 0.7 to $0.8 \pm 1.4\ \text{cm/s}$. Both across- and along-shore currents at Point Loma are oscillatory, and although instantaneous velocities commonly exceed $20\ \text{cm/s}$, *net* horizontal water displacement is low (Jackson 1997, M. H. Graham unpublished data). Peak current velocities generally occur at high-

frequencies and are likely due to surface gravity waves (swell and surfbeat), but lower-frequency oscillatory flows (internal waves and tides) can be important. Jackson (1997) estimated that internal waves could generate oscillating excursions of water up to 600 m into the Point Loma kelp forest over a 24 hr period, and variable-frequency internal waves propagate across-shore during much of the year. The hydrodynamic regime within the Point Loma kelp forest is therefore dominated by across- and along-shore flows of extremely low net displacement, but with oscillating horizontal excursions ranging from 1 to 100's of meters.

Experimental studies have clearly demonstrated the competitive dominance of giant kelp and its role in regulating kelp assemblage structure (Dayton et al. 1999). Giant kelp population dynamics at Point Loma is driven by pulses of strong recruitment following severe physical disturbance (due to storms or ENSO; Dayton et al. 1992). The generally low frequency of such events at Point Loma (3-7 years) allows for long periods of self-thinning that ultimately determine plant size and density (Dayton et al. 1992, Graham et al. 1997, Tegner et al. 1997). Multiple overlapping cohorts at different stages of self-thinning can result in high variability in giant kelp size and density at small spatial scales (**Chapter V**). In giant kelp, zoospores are released from sporogenous tissue (sori) located on specialized blades (sporophylls) at the base of the plants (~0.5 m off the substrate). Reed (1987) demonstrated that plant biomass was related to the biomass of sporophylls, effectively linking reproductive output to plant size. Reproductive giant kelp plants can be found throughout the year at Point Loma (Graham 1999, **Chapter III**), except during the height of severe ENSO events, and giant kelp populations in this region can therefore be considered as *continuously* reproductive (Neushul 1963, McPeak 1981, Graham 1999, **Chapter III**). Individual plants, however, cycle between fertility (sori present) and sterility (sori absent) due to fluctuations in plant biomass (**Chapter V**). The potentially continuous reproduction of giant kelp at Point Loma contrasts with the distinctly seasonal reproduction

of *Eisenia arborea*, *Laminaria farlowii*, and *Pterygophora californica* (McPeak 1981, Graham 1999, **Chapter III**), as well as that of most other kelp taxa in the world (Hoffman 1987, Santelices 1990).

These physical and biological characteristics of the Point Loma kelp forest dictated many aspects of the study design. The broad temporal range of hydrodynamic forcing at Point Loma and the likely sharp seasonal transition in the abundance and reproductive condition of giant kelp plants suggested variability in zoospore supply would be best characterized by sampling over scales from minutes to months; more broad temporal variability in demography and reproduction are addressed in **Chapter V**. The well-documented across- and along-shore flows further warranted a spatial sampling design that could partition and compare across- and along-shore variability in zoospore supply. The resulting data on spatiotemporal variability in zoospore supply were used, in conjunction with hydrographic, demographic, and reproductive surveys, to determine the extent to which the supply of giant kelp zoospores was regulated by physical transport and local reproduction.

Methods

Study sites

An across-/along-shore array of five study sites was created in the middle of the Point Loma kelp forest (Figure 4-1b). The three sites of the along-shore leg ran along the 15 m isobath (North, Central, and South) with the across-shore leg adding additional sites at 12 m (East) and 18 m (West). Most studies were done at Central which was the junction of the two legs. Each site was separated from its neighbor by 300-500 m and marked with a permanent 100 m² circular leadline grid (11.3 m diameter); North and South were the most distant from each other, separated by 900 m. Buoy lines were mounted to stainless steel eyebolts on steel plates at the center of each site. All sites were on flat rocky substrate with

low vertical relief. Giant kelp, *Laminaria farlowii*, and *Pterygophora californica* were abundant at each site, whereas only 1 adult *Eisenia arborea* was found and only at Central. No other kelp species were observed within a ~100 m radius of each study site. Turf algal cover varied according to depth with > 50% cover at the shallow site (East), 15-30% cover at intermediate depths (North, Central, and South), and < 5% cover at the deep site (West).

Sampling and identification of kelp zoospores

In situ plankton samples were collected using a subtidal pumping system described in **Chapter III** (Graham 1999). Briefly, the system consisted of a 25 m long hose (1.5 cm diameter) connected to a diaphragm pump that was operated from a small boat using a marine battery. The submerged end of the hose was fitted with a right-angle nozzle to direct horizontal intake, and rigged with a detachable clip that could be secured to permanent hardware at the base of the buoy lines and released remotely by a cable leading to the boat. The nozzle opening was located ~3 cm above the substrate. Pumped water was passed through 1 mm mesh, fixed immediately using 0.5% buffered formaldehyde, and stored in 2-L 99.9%-opaque high-density polyethylene containers. It took approximately 40 s to collect a 2-L sample at depths less than 20 m. Sample containers were transported to the laboratory on ice in coolers.

Plankton samples were processed within 6 hrs of returning to the laboratory. Samples were pre-filtered through 333 μm , 90 μm , and 10 μm nested sieves and concentrated using a tangential-flow filtration unit fitted with a 1 μm cassette (Graham 1999, **Chapter III**). Filtration retained greater than 99.99% of particles larger than 1 μm diameter and resulted in 40-60 ml concentrated samples. Concentrated samples were vacuum-filtered onto 47 mm diameter transparent membrane filters (1 μm pore diameter), preserved with ~5 ml of 2.0% buffered glutaraldehyde, and mounted onto glass microscope slides using immersion oil (Graham and Mitchell 1999, **Chapter II**). Each mounted filter contained all particles

between 1-10 μm diameter from a single 2-L sample. Mounted filters were stored in the dark at $< -10^{\circ}\text{C}$ for up to 2 days before analysis (Graham and Mitchell 1999, **Chapter II**). Density of giant kelp zoospores ($\#/L$) was estimated microscopically for each sample (see Graham 1999, **Chapter III**). Giant kelp zoospores were distinguished from those of *Pterygophora californica*, *Laminaria farlowii*, and *Eisenia arborea* based on species-specific absorption spectra of plastids within the zoospores, obtained by microphotometry (Graham 1999, Graham and Mitchell 1999, Chapters 2 and 3). This method has a minimum detectable zoospore density of 11 zoospores/L and a validated accuracy for giant kelp zoospores of greater than 98% (Graham 1999, **Chapter III**).

Hydrographic conditions

In the absence of direct current measurements, certain aspects of nearshore hydrodynamic processes can be studied by spatially and temporally explicit hydrographic surveys. Submersible sensors (Stowaway[®] Tidbit; Onset Computer Corp., Bourne, MA) continuously measured sea-surface and bottom temperatures at each site and mid-depth temperatures at East, Central and West. Temperature was measured every 15 minutes and sampling was synchronized among the 5 sites for all sensors. Synchronization allowed for the quantification of internal wave propagation based on horizontal and vertical variability in temperature (Pineda 1999). Tidal heights for Point Loma were determined from tide tables. Significant wave height was measured every 5 minutes from a deepwater buoy (183 m depth) located directly offshore of the study sites (Buoy #09101, Coastal Data Information Program, Scripps Institution of Oceanography).

Demography and reproduction

All identifiable giant kelp plants were mapped within the circular grids at each site. Plants greater than 10 cm length were marked with plastic tags attached using small cable

ties. Graham et al. (1997) found that this tagging method had a low tag loss rate and did not affect or cause mortality. Tags were replaced as they were over-grown.

For a given site, all plants were censused on each sampling date to determine giant kelp density, size-structure, and reproductive condition. Plant size was quantified as the number of fronds greater than 2 m length (Dayton et al. 1992). Plant fertility was based on soral presence and quality: sori were absent, present (non-sloughing), or present (sloughing). Sloughing is a condition when sori are vigorously releasing zoospores and can easily be distinguished from non-sloughing sori based on the presence of white tattered sporophylls (Neushul 1963). It was not possible to count all sporophylls on all plants. Since sporophylls are bundled in a single location on each plant, bundle size was quantified for each plant as simply small or large, with small sporophyll bundles having < 20 individual sporophyll blades.

Zoospore supply

Due to the passive transport of kelp zoospores with moving water, variability in zoospore density will result from changes in the concentration of zoospores as well as advection of the water. My approach to studying temporal variability in zoospore supply was to fix the sampling location (at Central) and collect various time series of zoospore density over a broad temporal range. Logistical constraints of studying nearshore plankton necessitated the use of different sampling schemes for two discrete temporal scales: within-day (≤ 24 hrs) and among-day (> 24 hrs). Within-day time series were collected during four independent sampling bouts in 1999: two bouts sampled every minute for 20 minutes (June 16 & September 1); one bout sampled every 10 minutes for 200 minutes (July 15); and one bout sampled every 100 minutes for 1600 minutes (November 16). These different bouts were designed to detect variability in zoospore density due to surface waves, internal waves, and tides, respectively. An among-day time series was collected by taking individual

samples on 26 dates spanning a period of 262 days in 1999 (February 28 to November 16); the single samples taken on each date allowed for direct comparison of among- and within-day variability. Two additional plankton samples were collected on each of the 26 among-day sampling dates providing an estimate of *average* daily zoospore density. This was used in conjunction with the demographic surveys to test the relationship between giant kelp zoospore supply and local reproduction.

Spatial variability in zoospore supply was studied by comparing zoospore density among the various sites of the across-/along-shore array. The hypothesis that zoospore density varied significantly across-shore was tested by collecting replicate plankton samples at East, Central, and West on 8 dates during 1999, whereas significant across-shore differences were tested by sampling at North, Central, and South on 7 different dates during 1999 ($n = 3$ per site per date).

Statistical analyses

Repeated sampling at a fixed site (Central) was used to identify temporal patterns in zoospore supply. Simple linear and non-linear (≤ 3 rd-order polynomials) regressions tested for temporal trends in within-day time series at Central. Kolmogorov-Smirnoff goodness-of-fit tests compared the distribution of both within- and among-day sample estimates to that predicted by Poisson (random) distributions. The goal was to detect non-random structure in the data that would suggest patchiness in zoospore supply at specific temporal scales. Equal spacing of within-day samples allowed for traditional time series analyses by autocorrelation. Unequal spacing of among-day samples precluded autocorrelation analyses as well as the incorporation of both within- and among-day time series into a single spectral analysis (e. g., Fourier transform). Instead, coefficients of variation (CV) were estimated for all pairs of samples *within* each of the 5 sampling bouts (4 within-day, 1 among-day), resulting in estimates of standardized variance for temporal

intervals ranging from 1 minute to 262 days. CV values for pairs of data points are essentially estimates of percent change. Replicate CV estimates for a given time interval \leq 24 hours were averaged; CV estimates for intervals $>$ 24 hours were pooled every 7 days and averaged. The relationship between standardized variance of zoospore supply and time interval was analyzed graphically.

The relationship between zoospore supply and local reproduction at Central was tested using sequential regression (Tabachnick and Fidell 1996). Demographic and reproductive explanatory variables were generally correlated among each other, violating the assumption of orthogonality. In sequential regression explanatory variables were given priorities which dictated the order they entered a forward-stepping multiple regression model. Priorities were based on partial regression coefficients that took into account effects of all potential explanatory variables. Significance of the explanatory variable with the highest priority was based on variability it explained in the response. Significance of lower priority variables was sequentially determined based on variability they explained in the response that was not explained by variables already in the model. Only significant explanatory variables ($P \leq 0.15$) were retained in the final model. Since the sequential model was fit directly to the Central data, the form of the resulting regression model may not be general. The final *parameterized* regression model therefore was cross-validated against independent data collected both along- (North and South; $n = 7$) and across-shore (East and West; $n = 8$), by applying the intercept and regression coefficients estimated by the sequential regression (fit of cross-validation was estimated by r^2).

Across- and along-shore patterns in zoospore supply were tested separately by comparing all sites using Type II analysis of variance (ANOVA). The response variable was daily zoospore density ($n = 3$ per site per date). Both across- and along-shore ANOVA models tested for main effects and interactions of Date and Site. Since all sites were randomly chosen in the across- and along-shore directions, and sampling dates were

dictated by weather, both Date and Site were treated as random factors. Variance components and magnitude of effects (% variance explained) were estimated for all main effects and interactions (Graham and Edwards in press). Significant Date effects indicated synchrony in zoospore supply among the sampling sites, whereas significant Site effects indicated site-specificity in zoospore supply and significant Date * Site interactions indicated that site-specificity in zoospore supply was time-dependent. All interactions proved to be significant and planned-comparisons tested for significant *uni-directional* across- (East > Central > West or East < Central < West) and along-shore (North > Central > South or North < Central < South) trends in zoospore density on each sampling date using sequential Bonferroni contrasts (Rice 1989). These uni-directional trends represented 2 of 9 possible combinations of sites either across- or along-shore (22%).

Zoospore density estimates were square-root transformed prior to sequential regression and ANOVA analyses. Linearity, independence, normality, and equality of error terms were validated by analysis of residuals. All statistics were done using Systat 9.0.

Results

Temporal variability in zoospore supply

Within-day samples of zoospore density were randomly distributed and did not exhibit general increasing or decreasing trends, providing little evidence of underlying physical/biological structure in zoospore supply at time scales ≤ 24 hrs (Figure 4-2). For each of the 4 within-day sampling bouts, zoospore density did not vary significantly as a function of time (linear/non-linear regression: all $P > 0.25$) nor did sample estimates differ significantly from those predicted by Poisson distributions (all $P > 0.4$). None of the within-day time series were auto-correlated ($P > 0.1$ at all time lags). High-frequency sampling of zoospore density therefore suggested a relatively fixed and constant zoospore supply at temporal scales ≤ 24 hours with sample estimates *randomly* dispersed about the

average density.

Variability of among-day samples in zoospore density differed strongly from within-day time series. Zoospore density was not constant with time but fluctuated episodically over the 262 days of the study (Figure 4-3). Days with high zoospore density occurred only during the spring, and sample estimates differed significantly from those predicted by a Poisson distribution ($P < 0.0001$). The variance/mean ratio was $\gg 1000$ indicating that the among-day distribution of zoospore density was extremely contagious. Contrary to temporal scales ≤ 24 hours, these patterns suggested that among-day variability in zoospore density was driven primarily by changes in the magnitude of zoospore supply with *non-random* zoospore dispersion.

These functional differences between within- and among-day variability were supported by combined analyses that indicated variability in zoospore density increased with increasing temporal scale. Coefficients of variation for the within-day time series did not differ significantly among each other (1 minute data: $CV = 0.18$ & 0.35 ; 10 minute data: $CV = 0.26$; 100 minute data: $CV = 0.48$; Cochran's $C = 0.37$, $P > 0.05$), but were significantly less than observed among days ($CV = 1.48$; Cochran's $C = 0.54$, $P < 0.01$). Further, the combined CV spectral analysis indicated that variability (percent change) in zoospore density increased exponentially as time intervals between sample collection exceeded 24 hours (Figure 4-4). This "reddened" spectrum of temporal variance in zoospore density suggested that the *primary* structure in zoospore supply was regulated by processes acting over temporal scales > 24 hours.

Zoospore supply versus local reproduction

Analysis of replicate samples taken on each date at Central allowed for a more detailed investigation of among-day variability in zoospore supply. Average daily zoospore density varied more than 2 orders-of-magnitude from as little as ~ 250 zoospores/L to over 54000

zoospores/L (Figure 4-5). Zoospore density was initially high during March and early April 1999 before falling to less than 1500 zoospores/L in mid-late April. Zoospore density rebounded during spring/summer and then remained relatively low from July to the end of the study in November. Within-day variability in among-day time series (variance among the 3 replicate daily samples) was generally low, with high variability observed only in early spring.

Peak estimates in average daily zoospore density during spring suggested a likely relationship between zoospore supply and local reproduction, as reproductive condition of adult giant kelp is generally high during periods of spring upwelling (Reed et al. 1996). Although density of all fertile plants (both sloughing and non-sloughing) was positively correlated with average daily zoospore density ($r = 0.52$), the density of sloughing plants alone exhibited the strongest relationship with zoospore density ($r = 0.59$). Further, the correlation between average daily zoospore density and the density of sloughing plants with large sporophyll bundles was stronger ($r = 0.69$) than for plants with small bundles ($r = 0.26$). As such, only the density of sloughing plants with large sporophyll bundles was considered in subsequent analyses (referred to simply as sloughing plants). Preliminary graphical analyses suggested that size-structure of sloughing plants explained additional among-day variability in zoospore density and thus, density of sloughing plants was partitioned into 5 size classes: ≤ 8 stipes, 9-15 stipes, 16-20 stipes, 21-25 stipes, and ≥ 26 stipes. Detailed analyses of the transition of plants between (1) sterility, fertility, and sloughing, (2) large and small sporophyll bundles, and (3) different size-classes are given in **Chapter V**.

Density of sloughing plants within these 5 size classes were collinear to various extent (Table 4-1), necessitating the use of sequential regression (principal components regression gave similar results as described here, although less intuitive). Sequential regression identified significant positive relationships between average daily zoospore density and the

density of sloughing plants in the ≤ 8 , 9-15, and 21-25 stipe size classes (Figure 4-5; Table 4-2). Variability in zoospore density was best predicted by changes in the number of small sloughing plants (≤ 8 stipes). Increasingly larger size classes explained decreasing amounts of variability in zoospore density not already explained by the smallest size class. A functional relationship between size class structure and zoospore density, however, was not inferred since the density and size of local reproductive plants was not manipulated; the observed relationship simply represents the best available predictive model. Local reproduction ultimately explained 77% of the total among-day variability in zoospore density (Figure 4-6a; Table 4-2). Cross-validation with data from the other 4 sites supported the generality of this strong relationship between zoospore supply and local reproduction (Figure 4-6b; $r^2 = 0.61$). This was despite the fact that the data for cross-validation came from sites spanning a relatively broad depth gradient and geographical area ($\sim 1 \times 1$ km) with a correspondingly broad range of among-site variability in plant density and reproductive condition. Data from the along-shore sites (North and South), however, fit the parameterized model ($r^2 = 0.78$) better than the across-shore sites (East and West; $r^2 = 0.34$) (Figure 6b).

Significant relationships were not detected between average daily zoospore density and any of the hydrographic factors measured during the study. Daily estimates of average significant wave height, sea-surface and bottom water temperature, within-day variability in mid-depth water temperature (used as an index of internal wave activity), and tidal highs, lows, and range were insignificant predictors of the residual variability in zoospore density that was not explained by local reproduction (all $P > 0.25$). Within-day variability in zoospore density (standard deviation of 3 replicate daily samples) was also not related to any hydrographic factors (all $P > 0.25$).

Spatial variability in zoospore supply

Across- and along-shore patterns in zoospore density were complex (Figures 4-7 and 4-8). The main effects of Date and Site were significant and strong both across- and along-shore, with Date effects explaining 52-77% of the total variability in zoospore density (Table 4-3). Date * Site interactions were also significant and strong both across- and along-shore, although interactions generally explained less variability in zoospore density than the strongest main effects (Table 4-3). Main effects and interactions together explained 89% of across-shore variability in zoospore density and 94% of along-shore variability. Total variability in zoospore density (σ^2) was greater across-shore (3114.5) than along-shore (2513.7).

The strong and validated relationship between zoospore density and the density of sloughing plants suggested that much of the variability in zoospore density may simply be due to among-date and among-site variability in local reproduction. As such, the linear model resulting from the sequential regression analysis was used to predict average daily zoospore density at each of the 5 sampling sites on each of the sampling dates. These predicted values were then subtracted from each daily replicate sample to give estimates of zoospore density not explained by local reproduction (“adjusted” zoospore density). This technique essentially “partialled-out” the effects of local reproduction on zoospore density, allowing the effects of across- and along-shore physical transport to be tested directly.

As predicted, partialling-out the effect of local reproduction on zoospore density greatly reduced the total variability in zoospore density observed across- and along-shore (Figures 4-7 and 4-8; Table 4-4). Across-shore variance decreased 54% from 3114.5 to 1417.0 and along-shore variance decreased 82% from 2513.7 to 448.7, reflecting the cross-validation results (Figure 4-6b). Error variance was unchanged since the same predicted average daily zoospore density was subtracted from each of the within-day samples at a given site. The reduction in total variability in zoospore density was due primarily to large

decreases in the main effects of Date and Site. For the across-shore model, among-date variance decreased 62% from 1632.2 to 618.3 but remained significant, whereas among-site variance decreased 100% from 361.9 to 0. For the along-shore model, among-date variance decreased 98% from 1934 to 30 and among-site variance decreased 74% from 207.3 to 54.1, both going from highly significant to highly non-significant.

In contrast, interaction terms remained significant even after removing the effects of local reproduction on zoospore density (Table 4-4). Across-shore variance due to Date * Site interactions decreased 41% from 788.1 to 466.4 whereas along-shore variance due to Date * Site interactions decreased only 3% from 233.6 to 225.8. Although these *adjusted* interaction terms explained only 15% and 9% of the *unadjusted* total variability in zoospore density across- and along-shore, respectively, they suggested that physical transport is likely a component of the observed variability in zoospore supply. Significant uni-directional, among-site gradients in zoospore density consistent with physical transport were observed during 2 of 8 across-shore sampling dates and 0 of 7 along-shore sampling dates (Figures 4-7 and 4-8). Hydrographic data were used to estimate the direction of across-shore water motion due to tides and internal waves, and it was found that the instance of significant increases in adjusted zoospore density from East to West corresponded to a falling tide whereas the instance of significant decreases from East to West corresponded to a rising tide (Figure 4-7). The probability that uni-directional trends would be observed on 2 of 8 dates by random chance alone, however, was high ($P = 0.30$; based on binomial distribution with $p = 0.22$, $n = 2$, $N = 8$).

Discussion

Giant kelp's standing as a foundation species in nearshore habitats of California is due primarily to its superior competitive strength and its ability to recruit rapidly to open substrate following physical and biological disturbance (North 1971, Foster and Schiel

1985, North 1994, Dayton et al. 1999). This study has demonstrated that even during periods of low adult density and reproductive condition, giant kelp zoospores are continuously present in the water column at Point Loma, giving this population the potential for year-round recruitment. More interesting, however, was the indication that variability in zoospore supply was complex, due to both physical and biological processes acting on different temporal and spatial scales. The data suggested variable coupling between reproduction and zoospore supply, with direct implications to the concentration and genetic makeup of the zoospores. Here I discuss (1) how the dynamic linkage between giant kelp zoospore supply, demography, and reproduction is regulated by hydrodynamics at small spatial scales, and (2) that these processes can be scaled up to explain the ability of the Point Loma kelp forest to modify directly the relative contribution of local versus remote zoospore production on zoospore supply.

Most variability in zoospore supply at Central occurred over temporal scales greater than 24 hours and was explained by local reproduction. Temporal and spatial variability in demography and reproduction will therefore directly translate into variability in zoospore supply, leading to the “reddening” of the zoospore density spectrum with increasing temporal scale. Reproductive condition of adult giant kelp plants changes rapidly (days to weeks) due to variability in plant biomass or environmental conditions (**Chapter V**), contributing to the increased variability in zoospore supply observed at these temporal scales. At scales of weeks to months, changes in the abundance and reproduction of plants due to grazing (**Chapter V**), storm mortality, recruitment, and seasonal changes in oceanographic conditions, further drove variability in zoospore supply. Additional increases in variability of zoospore supply would be expected with increasing temporal scale, as ENSO events and decadal shifts in oceanographic climate are experienced, although these phenomena lie beyond the scope of this study. The well-established sensitivity of giant kelp to disturbance and changes in environmental conditions (Dayton et al. 1984, Zimmerman

and Robertson 1985, Zimmerman and Kremer 1986, Reed 1987, Dayton et al. 1992, Reed et al. 1996, Graham et al. 1997, Dayton et al. 1999), in conjunction with the tight reproductive coupling, ultimately resulted in a rapid response of giant kelp zoospore supply to environmental stochasticity.

Variability in zoospore supply over temporal scales less than 24 hours was much less structured. Although currents in the center of the Point Loma kelp forest have very low net across- and along-shore displacement, they oscillate at relatively high instantaneous horizontal and vertical velocities (Jackson 1997, M. H. Graham unpublished data) enhancing the advection of zoospores from sporophylls to the substrate (~0.5 m). Random-walk models of zoospore dispersal under such hydrodynamic forcing predict that zoospores simply slosh back and forth with little net displacement from the adult plants that released them (M. H. Graham unpublished data). Specifically, zoospores are predicted to be found (in all directions) closest to the plants that release them, with the shape of the resulting 2-dimensional dispersal curve constrained by the extent of turbulence. This pattern of restricted zoospore dispersal would result in the tight coupling observed between among-day temporal variability in zoospore supply and local reproduction. At Point Loma, the spatial distribution of adult giant kelp plants and variability in their reproductive condition are non-uniform (**Chapter V**), suggesting that in a localized region of the kelp forest there will be a mosaic of multiple overlapping zoospore dispersal curves of different shapes. Further deformation of the dispersal curves by oscillating flow (and therefore turbulence) likely produced the random dispersion of high-frequency zoospore density estimates around average values.

There was evidence that long-distance physical transport processes contributed at least partly to variability in zoospore abundance at Central. Twenty-three percent of among-day variability in zoospore abundance remained unexplained by local reproduction. Clearly, the turbulent nature of the water column will likely keep some giant kelp zoospores extended

long enough to be transported away from the adults that produced them (B. Gaylord et al., manuscript in review). Drifting reproductive plants or sporogenous tissue may also provide a remotely-produced zoospore source (Dayton et al. 1984, Dayton 1985). Over time, zoospores will accumulate to provide a background abundance onto which newly released zoospores are continuously added. The intercept of the sequential regression analysis estimated this background zoospore abundance to be 717 zoospores/L (Table 4-2). Although weak relative to the tight local reproductive coupling previously described, this link between long-distance dispersal and zoospore supply would effectively smooth the impacts of small-scale variability in adult density and reproductive condition on zoospore abundance.

A primary goal of the study was to determine if variability in zoospore abundance that was not due to local reproduction could be explained by the transport of large concentrations of zoospores by surface waves, internal waves, and tides. The effect of local reproduction on zoospore supply explained most of the significant main effects of Site and some of the significant Date * Site interactions. Although significant Date * Site interactions were still present after partialling-out the effects of local reproduction, uni-directional across-shore patterns were consistent with the predicted direction of physical transport on only 2 of 8 sampling dates. The high probability of these patterns occurring simply by random chance alone, however, suggests that across-shore processes (e. g., surface and internal waves and tides) were not an important component of zoospore transport.

The highly significant and strong main effects of Date detected during across- and along-shore sampling of zoospore density suggested some level of synchrony in zoospore supply among sites which were separated by up to 1 km. Reproductive synchrony is an important process as it can increase the concentration of zoospores in the water column, lessen the effects of dilution during zoospore dispersal (Reed et al. 1997), and contribute to

background zoospore abundances. Synchrony can result from rapid synchronous release of zoospores from adult plants (Reed et al. 1997), long-distance zoospore dispersal (Reed et al. 1988), or long-term synchronous changes in adult reproductive condition. Synchronous release of zoospores and long-distance zoospore dispersal, however, were not supported by the data. Partialling-out the effects of local reproduction on zoospore supply decreased the among-date variance by 62 to 98%, indicating that the main effects of Date were due primarily to temporal variability in the local density of reproductive giant kelp plants. The most probable explanation for the observed synchrony is therefore an external constraint on local reproduction, homogeneous at least across the spatial scales addressed during this study. That is, a general pattern of increased zoospore supply as oceanographic conditions conducive to good kelp growth and reproduction become established at Point Loma, and decreased zoospore supply as conditions deteriorate or broad-scale grazing occurs (Chapter V). Still, Reed et al. (1997) proposed that synchronous release of zoospores was triggered by the occurrence of storms. During this study it was impossible to sample zoospore abundance until 2-3 days after storms had subsided, and thus storm signals may have been missed. Storm-induced release of zoospores did not appear to not be important in explaining the observed reproductive synchrony.

Cross-validation indicated that the coupling observed at Central between temporal variability in local reproduction and zoospore supply was strong at other sites *within* the kelp forest, but weak *outside* the kelp forest. Within the forest, observed patterns of temporal and spatial variability in zoospore supply corresponded well with the low-displacement oscillating current regime previously described (Jackson 1997). The North, South, and Central study sites were all greater than 0.5 km from both the offshore and inshore edges of the forest, exceeding the minimum size of kelp forests that Jackson and Winant (1983) estimated was needed to significantly dampen both across- and along-shore flows (~100 m). Net displacement of across- and along-shore currents increases offshore

and onshore from the center of the kelp forest (Jackson 1997), and as such, the relative contribution of physical transport versus local reproduction to variability in zoospore supply should be greatest along the outer edges of the kelp forest. This hypothesis was strongly supported by the 2-fold decrease in the amount of variability in zoospore density explained by local reproduction at East and West relative to North and South, as estimated during cross-validation of the sequential regression model.

This pattern of increased reproductive coupling with decreased net current displacement helps to explain the apparent controversy of whether kelp dispersal occurs over primarily short or long distances. Dayton et al. (1984) described a rapid decrease in the density of giant kelp recruits with increasing distance from adult plants in a clearing in the Point Loma kelp forest, suggesting that most zoospore dispersal (or at least survival following dispersal, settlement, and fertilization) was limited to within 10 m of the nearest adult giant kelp plants. Again, data from this study strongly support predominantly short distance kelp dispersal within the Point Loma kelp forest. In contrast, Reed et al. (1988) observed settlement of *Pterygophora californica* zoospores over 4 km from the nearest known zoospore source. Their study was conducted in Santa Barbara, California, in a region of sustained unidirectional along-shore currents (> 5 cm/s) and high net displacement (Washburn et al. 1999), where the probability of long-distance physical transport is likely much greater than within the Point Loma kelp forest. Net current displacement of this magnitude is similar to that estimated by Jackson (1997) outside of the Point Loma kelp forest. As such, although reproductive coupling is tight in the center of the Point Loma kelp forest, zoospores produced by plants along the perimeter of the forest may be physically transported long distances.

The interaction between kelp assemblage size, net current displacement, and reproductive coupling may also have significant consequences for kelp population dynamics. Following initial colonization, a kelp assemblage will be too small to significantly dampen currents and

modify net current displacement (Jackson and Winant 1983, Jackson 1997). Subsequently, the contribution of local reproduction to zoospore supply will be small due to the advection of zoospores away from the assemblage, limiting the potential of the kelp population to seed itself yet increasing the percentage of zoospores capable of colonizing new habitat. As the density of kelp plants increases, so does the effect of the kelp assemblage on net current displacement, gradually increasing the retention of zoospores and the contribution of local reproduction. As such, there likely exists a threshold in kelp assemblage size above which more zoospores are retained locally than transported away. Subsequent increases in self-seeding may therefore result in increased recruitment success and recovery of populations following disturbance. If true, large kelp assemblages may be more stable than small assemblages, an hypothesis that awaits rigorous testing. It is important that species-specific techniques are developed for estimating giant kelp settlement in mixed-species assemblages to test the relationship between giant kelp recruitment and zoospore supply. The pattern of increased zoospore retention relative to zoospore leakage with increasing population size would also hold as one moves from outside to within a given population.

Although the goals of this study were primarily ecological, the results have broad evolutionary implications. Tight reproductive coupling observed in the center of the Point Loma kelp forest suggests that subsequent recruits will not be displaced far from their parents. Consequently, these juveniles will have a high probability of experiencing the same selective pressures as the adults, suggesting a potential for adaptation of kelp populations to local environmental conditions. Tight reproductive coupling and short-distance dispersal will also likely increase rates of self-fertilization and may lead to small-scale genetic structure within kelp forests. At the same time, however, some zoospores will leak out of the local population, even under the weakest current conditions. The question as to whether resulting gene flow is enough to homogenize such small-scale genetic structure can be tested genetically by sampling giant kelp juveniles and adults within and along the outer

edges of populations.

As for whether kelp populations are “open” or “closed”, for giant kelp, the answer is not as simple as the question. Although adult plants of similar reproductive condition likely produce similar amounts of zoospores, zoospore dispersal is strongly dependent on temporal and spatial variability in hydrodynamics. Furthermore, variability in adult size, distribution, and abundance significantly modifies the flow characteristics of the physical environment. As such, this taxon has a unique and important role in determining the fate of its propagules and in regulating demographic and genetic exchanges within and among populations. Given both stochastic (environmental) and deterministic (biological) constraints on giant kelp populations, a more continuous view of the strength and extent to which populations interact is warranted.

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Table 4-1. VIF-Correlation matrix of density of sloughing giant kelp plants in 5 size-classes. Data along diagonal are variance-inflation-factors ($VIF = 1/(1-R_i^{2*})$, where R_i^{2*} is the coefficient of determination when variable i is regressed against all other variables) (Tabachnick and Fidell 1996). Off-diagonal data are Pearson product-moment correlations (r) between pairs of variables. VIF values ≥ 2 and r values ≥ 0.3 represented high collinearity among the size classes which served as explanatory variables in subsequent sequential regression analyses (Table 4-2).

	≤ 8 stipes	9-15 stipes	16-20 stipes	21-25 stipes	≥ 26 stipes
≤ 8 stipes	1.49	-	-	-	-
9-15 stipes	0.41	2.74	-	-	-
16-20 stipes	0.05	0.66	2.03	-	-
21-25 stipes	0.44	0.49	0.26	1.50	-
≥ 26 stipes	-0.05	0.32	0.35	0.05	1.24

Table 4-2. Sequential regression analysis of the effects of local reproduction on average daily zoospore density. Explanatory variables were the density of sloughing giant kelp plants in 5 size-classes (same as in Table 4-1). Assigned priority in which explanatory variables entered analysis: ≤ 8 stipes, 9-15 stipes, 21-25 stipes, 16-20 stipes, ≥ 26 stipes. Density of sloughing plants in the 16-20 and ≥ 26 stipe size classes were insignificant predictors of average daily zoospore density ($P \geq 0.15$) and are not included here.

Variable	β	SE	t	P	r^2
Constant	26.78	10.27	2.61	0.0173	-
≤ 8 stipes	58.02	8.37	6.93	<0.0001	0.585
9-15 stipes	4.40	1.34	3.27	0.0040	0.130
21-25 stipes	14.32	6.82	2.10	0.0495	0.054

Analysis of Variance						
Source	SS	df	MS	F	P	R^2
Regression	40798.9	3	13599.6	21.04	<0.0001	0.769
Error	12282.8	19	646.5			

Table 4-3. Model II ANOVAs testing the effects of Date, Site, and Date * Site on average daily zoospore density sampled a) across-shore and b) along-shore. F-ratios for the main effects of Date and Site utilized the interaction MS in the denominator, whereas the F-ratio for the interaction utilized the Error MS in the denominator. σ^2 equals the variance contribution (variance component) of individual main effects, interactions, or error to the response. % equals percent variance contribution relative to total σ^2 . N = 72 for across-shore analysis and N = 63 for along-shore analysis.

Source	SS	df	MS	F	P	σ^2	%
a) Date	121708.0	7	17386.9	6.45	0.0016	1632.2	52.4
Site	22760.2	2	11380.1	4.22	0.0368	361.9	11.6
Date * Site	37754.2	14	2696.7	8.12	<0.0001	788.1	25.3
Error	15948.6	48	332.3	-	-	332.3	10.7
Total						3114.5	100.0
b) Date	109742.0	6	18290.4	21.79	<0.0001	1934.0	76.9
Site	10384.1	2	5192.0	6.18	0.0143	207.3	8.3
Date * Site	10073.6	12	839.5	6.05	<0.0001	233.6	9.3
Error	5831.3	42	138.8	-	-	138.8	5.5
Total						2513.7	100.0

Table 4-4. Model II ANOVAs testing the effects of Date, Site, and Date * Site on average daily zoospore density sampled a) across-shore and b) along-shore, after the effects of local reproduction on zoospore density were removed. Zoospore density data were adjusted according to the final parameterized sequential regression model (Table 4-2). F-ratios for the main effects of Date and Site utilized the interaction MS in the denominator, whereas the F-ratio for the interaction utilized the Error MS in the denominator. σ^2 equals the variance contribution (variance component) of individual main effects, interactions, or error to the response. % equals percent variance contribution relative to *unadjusted* total σ^2 (Table 4-3). The negative variance component for across-shore Site effects was remedied using the “pool-the-minimum-violator” technique (Graham and Edwards in press). N = 72 for across-shore analysis and N = 63 for along-shore analysis.

Source	SS	df	MS	F	P	σ^2	%
a) Date	51074.0	7	7296.3	4.02	0.0129	618.3	19.9
Site	2299.9	2	1149.9	0.63	0.5451	0	0
Date * Site	25402.1	14	1814.4	5.46	<0.0001	466.4	15.0
Error	15949.4	48	332.3	-	-	332.3	10.7
Total						1417.0	45.6
b) Date	6516.1	6	1086.1	1.33	0.3167	30.0	1.2
Site	3906.2	2	1953.1	2.39	0.1338	54.1	2.2
Date * Site	9793.0	12	816.1	5.88	<0.0001	225.8	9.0
Error	5831.6	42	138.8	-	-	138.8	5.5
Total						448.7	17.9

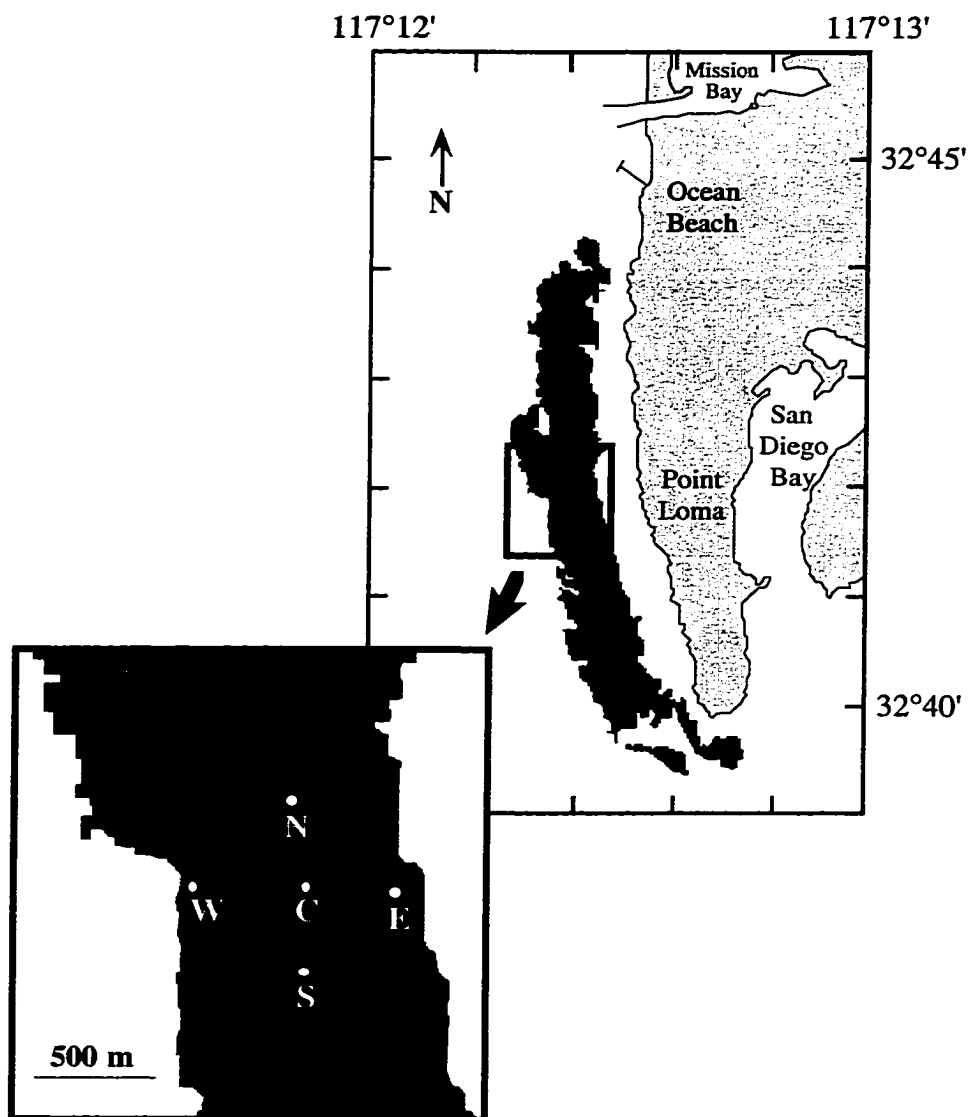


Figure 4-1. Map of study sites at Point Loma. Shaded area represents extent of giant kelp canopy on August 12, 1996.

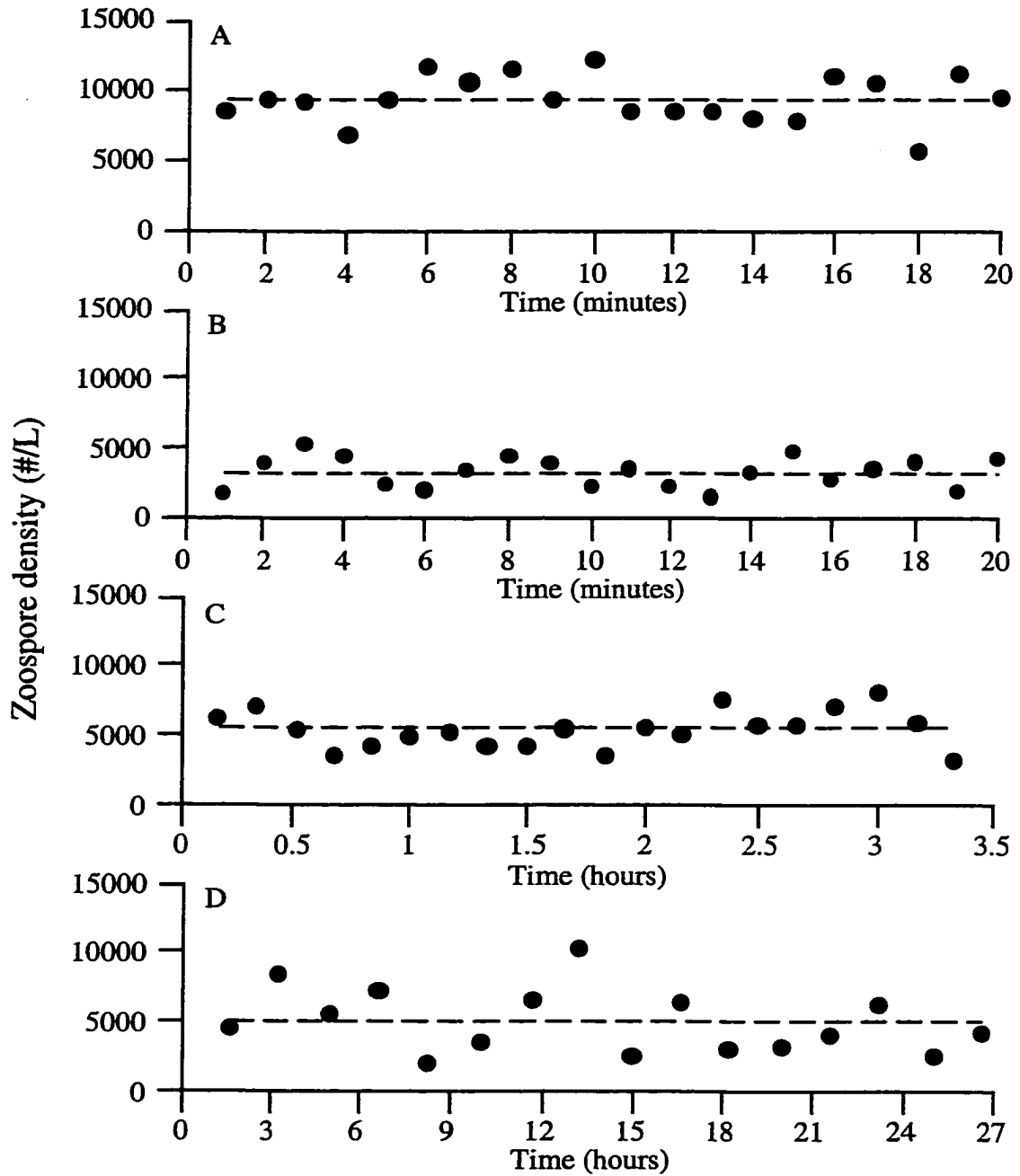


Figure 4-2. Various within-day time series of zoospore density taken at Central in 1999. Sampling intervals were: 1 minute (A & B), 10 minutes (C), and 100 minutes (D). The dashed line represents the average zoospore density for each time series.

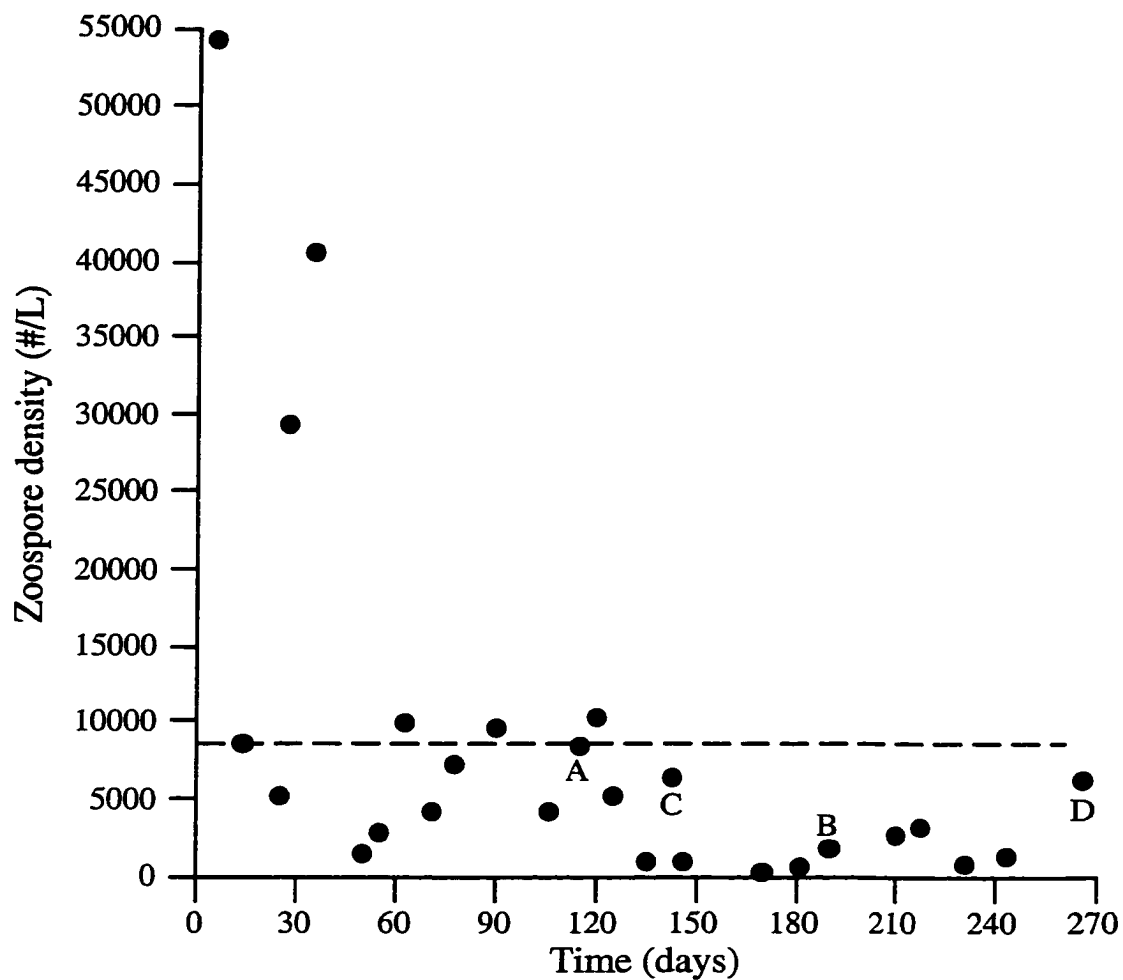


Figure 4-3. Among-day time series of zoospore density taken at Central in 1999. The dashed line represents the average zoospore density. Letters represent within-day sampling dates (Figure 4-2).

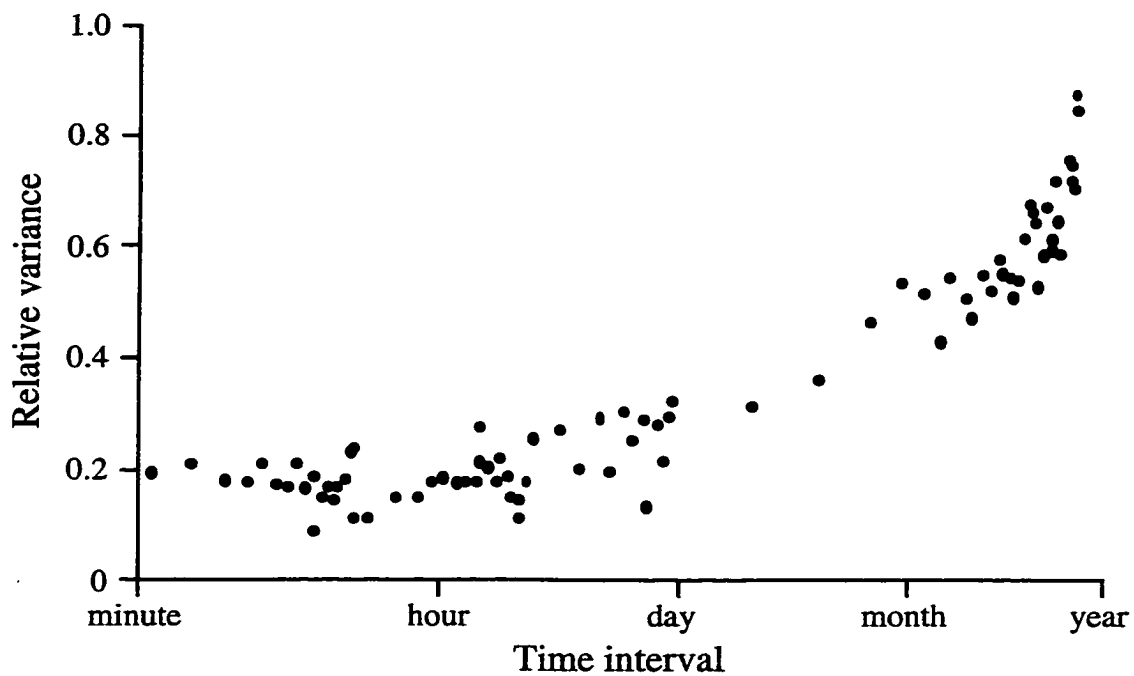


Figure 4-4. Combined within- and among-day spectral analysis of zoospore density. Standardized variances (% change) represent average coefficients of variation (CV) of zoospore density from pairs of samples separated by various time intervals.

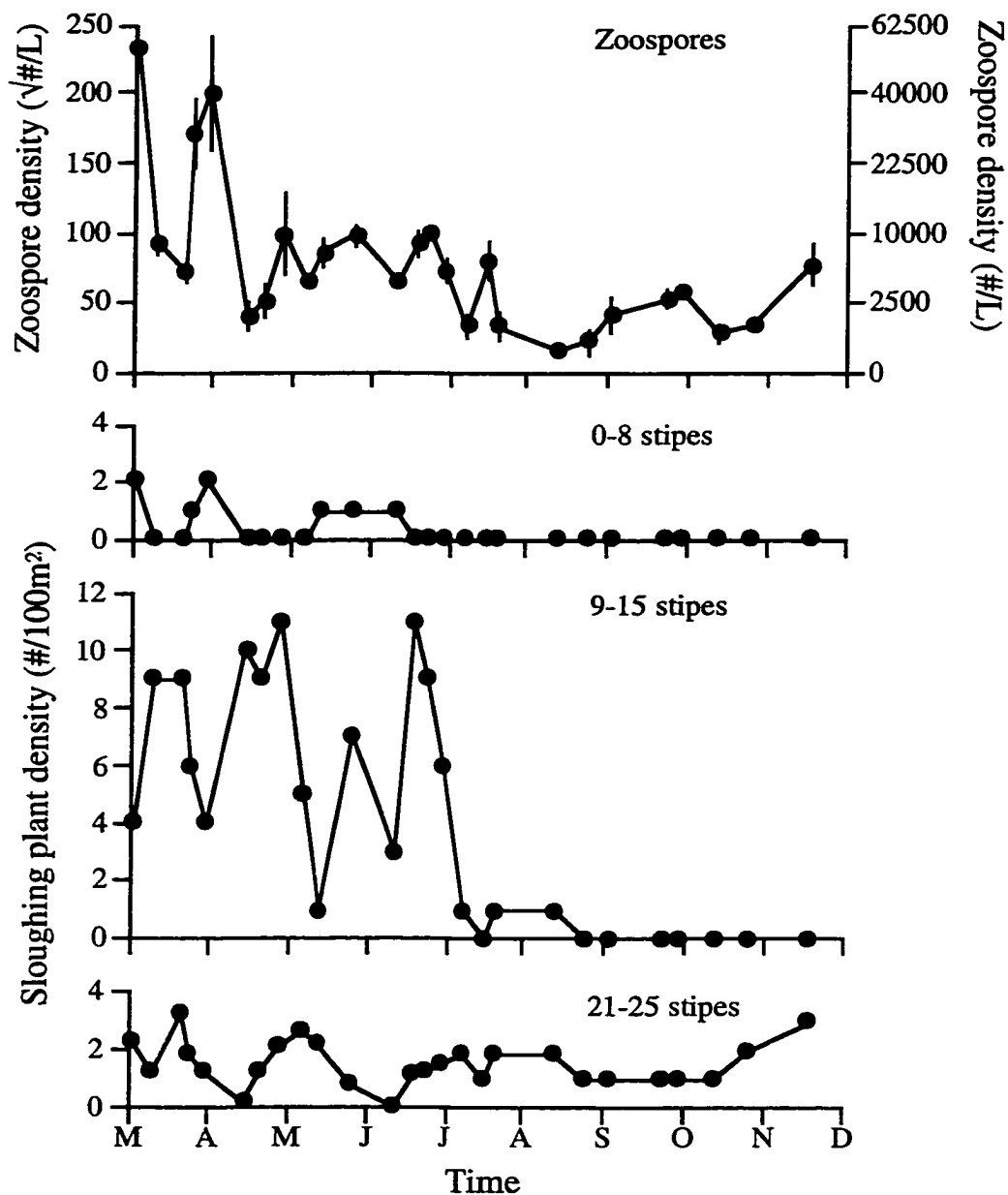


Figure 4-5. Among-day patterns of temporal variability in average daily zoospore density, and the density of sloughing giant kelp plants in ≤ 8 stipe, 9-15 stipe, and 21-25 stipe size classes. Collinearity of sloughing plant density among the 3 size classes has been removed according to priorities used in the sequential regression analyses (Table 4-2). Note different y-axes in A represent square-root-transformed and untransformed zoospore densities. Error bars are standard deviations.

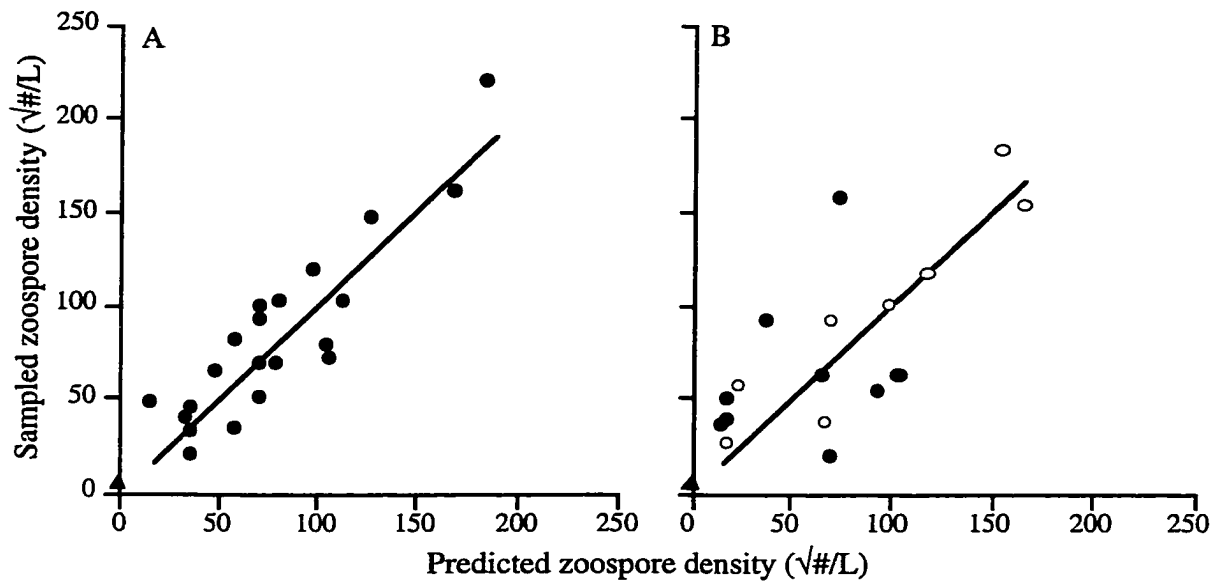


Figure 4-6. Relationship between sample average daily zoospore density (square-root-transformed) and average daily zoospore density predicted by sequential regression model (square-root-transformed) at Central (A) and North, South, East, and West (B). B represents cross-validation of the sequential regression model (Table 4-2). Lines represent 1:1 fit of sampled versus predicted zoospore density. Triangles are averages of all zoospore density samples taken on dates when sloughing plant density was zero in all size classes, as these are essentially replicates of the y-intercept. Hollow circles in B represent samples from North and South; solid circles are samples from East and West.

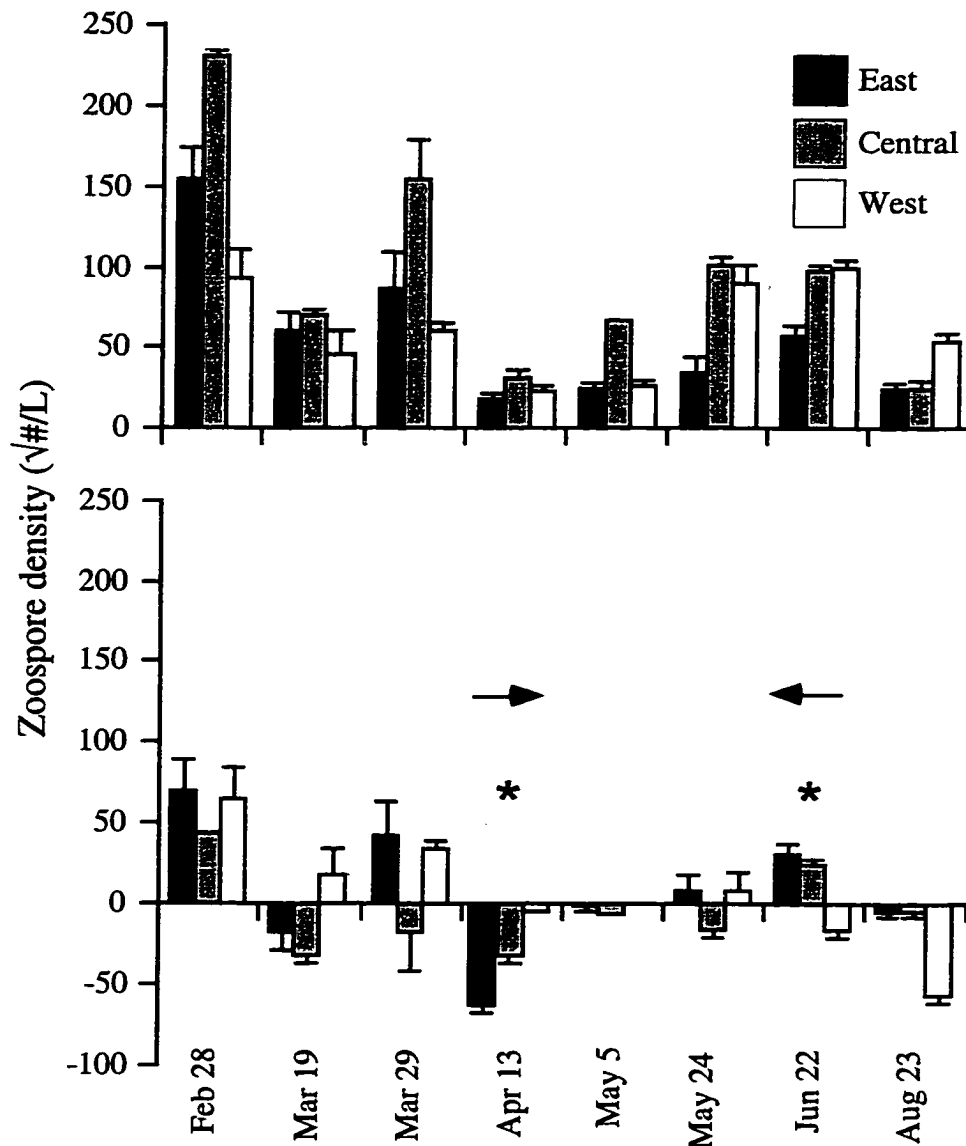


Figure 4-7. Across-shore patterns of among-date and -site variability in average daily zoospore density (top) (square-root-transformed) and adjusted average daily zoospore density (bottom) (i. e. after effects of local reproduction were partialled-out) (square-root-transformed). Asterisks indicate dates when uni-directional gradients in adjusted zoospore density were significant, and arrows indicate tidal directions on those dates. Error bars are standard errors.

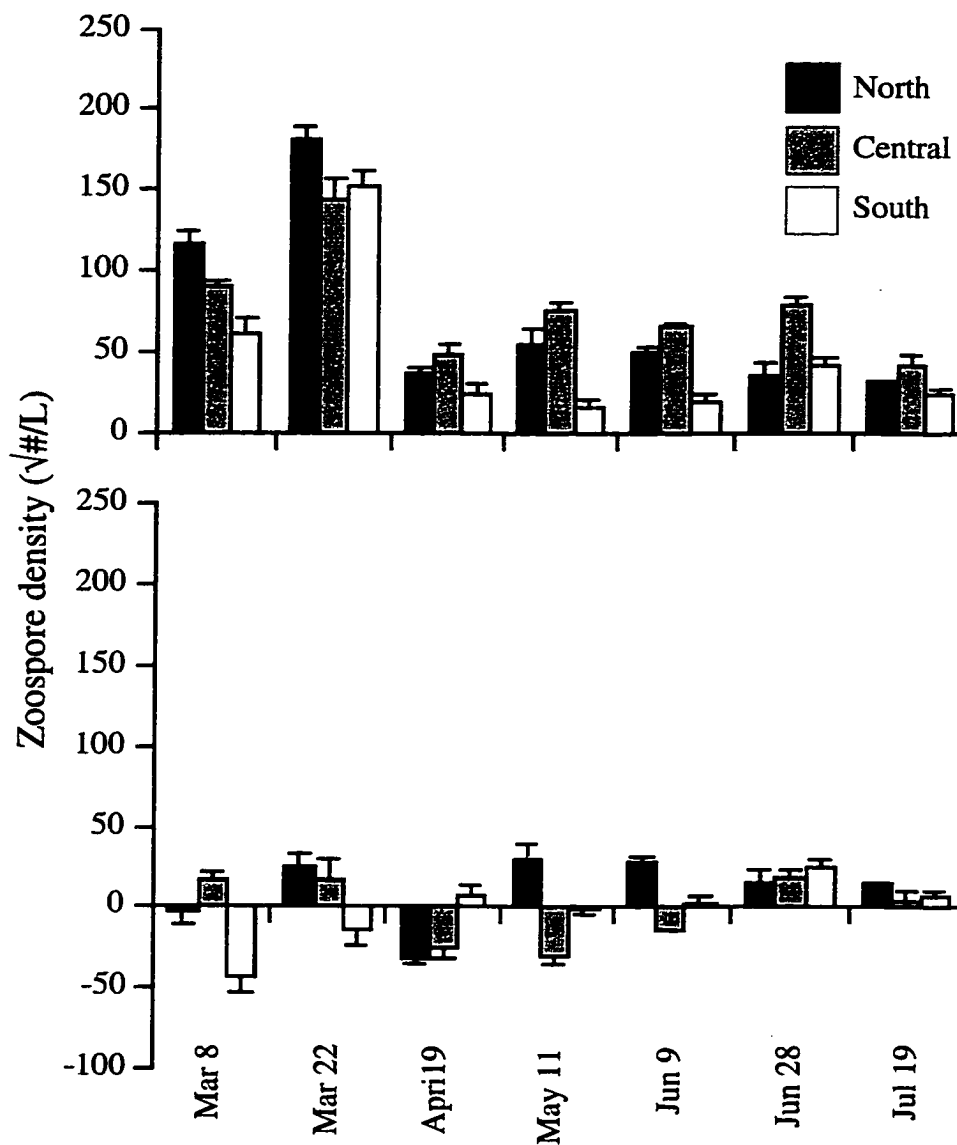


Figure 4-8. Along-shore patterns of among-date and -site variability in average daily zoospore density (top) (square-root-transformed) and adjusted average daily zoospore density (bottom) (i. e. after effects of local reproduction were partialled-out) (square-root-transformed). Error bars are standard errors.

Literature Cited

- Amsler, C. D., D. C. Reed, and M. Neushul. 1992. The microclimate inhabited by macroalgal propagules. *British Phycological Journal* 27:253-270.
- Anderson, E. K., and W. J. North. 1966. *In situ* studies of spore production and dispersal in the giant kelp, *Macrocystis*. *Proceedings of the International Seaweed Symposium* 5:73-86
- Bohonak, A. J. 1999. Dispersal, gene flow, and population structure. *Quarterly Review of Biology* 74:21-45.
- Booth, D. J. 1991. The effects of sampling frequency on estimates of recruitment of the domino damselfish *Dascyllus albisella* Gill. *Journal of Experimental Marine Biology and Ecology* 145:149-160.
- Burton, R. S. 1998. Intraspecific phylogeography across the Point Conception biogeographic boundary. *Evolution* 52:734-745.
- Caley, M. J., M. H. Carr, M. A. Hixon, T. P. Hughes, G. P. Jones, and B. A. Menge. 1996. Recruitment and the local dynamics of open marine populations. *Annual Review of Ecology and Systematics* 27:477-500.
- Cowen, R. K., K. M. M. Lwiza, S. Sponaugle, C. B. Paris, and D. B. Olson. 2000. Connectivity of marine populations: Open or closed? *Science* 287:857-859.
- Dayton, P. K., V. Currie, T. Gerrodette, B. D. Keller, R. Rosenthal, and D. Ven Tresca. 1984. Patch dynamics and stability of some California kelp communities. *Ecological Monographs* 54:253-289.
- Dayton, P. K., and M. J. Tegner. 1984. Catastrophic storms, El Nino, and patch stability in a southern California kelp community. *Science* 224:283-285.
- Dayton, P. K., M. J. Tegner, P. B. Edwards, and K. L. Riser. 1999. Temporal and spatial scales of kelp demography: the role of oceanographic climate. *Ecological Monographs* 69:219-250.
- Dayton, P. K., M. J. Tegner, P. E. Parnell, and P. B. Edwards. 1992. Temporal and spatial patterns of disturbance and recovery in a kelp forest community. *Ecological Monographs*

- 62:421-445.
- Dean, T. A., and F. R. Jacobsen. 1984. Growth of juvenile *Macrocystis pyrifera* (Laminariales) in relation to environmental factors. *Marine Biology* 83:301-311.
- Dean, T. A., and F. R. Jacobsen. 1986. Nutrient-limited growth of juvenile kelp, *Macrocystis pyrifera*, during the 1982-1984 "El Nino" in southern California. *Marine Biology* 90:597-601.
- Denny, M. W. 1988. *Biology and the Mechanics of the Wave-Swept Environment*. Princeton University Press, Princeton, New Jersey.
- Deysher, L. E., and T. A. Dean. 1986a. In situ recruitment of sporophytes of the giant kelp, *Macrocystis pyrifera* (L) C Agardh: effects of physical factors. *Journal of Experimental Marine Biology and Ecology* 103:41-63.
- Deysher, L. E., and T. A. Dean. 1986b. Interactive effects of light and temperature on sporophyte production in the giant kelp *Macrocystis pyrifera*. *Marine Biology* 93:17-20.
- Downes, B. J., and M. J. Keough. 1998. Scaling of colonization processes in streams: Parallels and lessons from marine hard substrata. *Australian Journal of Ecology* 23:8-26.
- Foster, M. S., and D. R. Schiel. 1985. The ecology of giant kelp forests in California: a community profile. United States Fish and Wildlife Service Biological Report 85:1-152.
- Fredriksen, S., K. Sjøtun, T. E. Lein, and J. Rueness. 1995. Spore dispersal in *Laminaria hyperborea* (Laminariales, Phaeophyceae). *Sarsia* 80:47-54.
- Gaines, S., S. Brown, and J. Roughgarden. 1985. Spatial variations in larval concentrations as a cause of spatial variation in settlement for the barnacle, *Balanus glandula*. *Oecologia* 67:267-272.
- Gaines, S. D., and K. D. Lafferty. 1995. Modeling the dynamics of marine species: the importance of incorporating larval dispersal. Pages 389-412 in L. R. McEdwards, editor. *Ecology of Marine Invertebrate Larvae*. CRC Press, Boca Rotan.
- Graham, M. H. 1999. Identification of kelp zoospores from in situ plankton samples. *Marine Biology* 135:709-720.

- Graham, M. H., C. Harrold, S. Lisin, K. Light, J. M. Watanabe, and M. S. Foster. 1997. Population dynamics of giant kelp *Macrocystis pyrifera* along a wave exposure gradient. *Marine Ecology Progress Series* 148:269-279.
- Graham, M. H., and M. S. Edwards. In press. Statistical significance vs. fit: estimating the importance of individual factors in ecological analysis of variance. *Oikos*.
- Graham, M. H., and B. G. Mitchell. 1999. Obtaining absorption spectra from individual macroalgal spores using microphotometry. *Hydrobiologia* 398-399:231-239.
- Grosberg, R. K. 1982. Intertidal zonation of barnacles: the influence of planktonic zonation of larvae on vertical distribution of adults. *Ecology* 63:894-899.
- Grosberg, R. K. and C. W. Cunningham. In press. Genetic structure in the sea: from populations to communities. Pages xxx-xxx in M. D. Bertness, S. Gaines and M. E. Hay, editors. *Marine Community Ecology*. Sinauer Associates, Sunderland.
- Hickey, B. M. 1993. Physical Oceanography. Pages 19-70 in M. D. Dailey, D. J. Reish, and J. W. Anderson, editors. *Ecology of the Southern California Bight: a synthesis and interpretation*. University of California, Berkeley.
- Hoffman, A. J. 1987. The arrival of seaweed propagules at the shore: a review. *Botanica Marina* 30:151-165.
- Hoffman, A. J., and P. Camus. 1989. Sinking rates and viability of spores from benthic algae in central Chile. *Journal of Experimental Marine Biology and Ecology* 126:281-191.
- Hoffman, A. J., and R. Ugarte. 1985. The arrival of propagules of marine macroalgae in the intertidal zone. *Journal of Experimental Marine Biology and Ecology* 92:83-95.
- Hruby, T., and T. A. Norton. 1979. Algal colonization on rocky shores in the Firth of Clyde. *Journal of Ecology* 67:65-77.
- Jackson, G. A. 1997. Currents in the high drag environment of a coastal kelp stand off California. *Continental Shelf Research* 17:1913-1928.
- Jackson, G. A., and C. D. Winant. 1983. Effect of a kelp forest on coastal currents. *Continental Shelf Research* 2:75-80.

- Jones, G. P., M. J. Milicich, M. J. Emslie, and C. Lunow. 1999. Self-recruitment in a coral reef fish population. *Nature* 402:802-804.
- Lewin, R. 1986. Supply-side ecology. *Science* 234:25-27.
- McPeak, R. H. 1981. Fruiting in several species of Laminariales from southern California. *Proceedings of the International Seaweed Symposium* 8:404-409
- Minchinton, T. E., and R. E. Scheibling. 1991. The influence of larval supply and settlement on the population structure of barnacles. *Ecology* 72:1867-1879.
- Miron, G., B. Boudreau, and E. Bourget. 1995. Use of larval supply in benthic ecology: testing correlations between larval supply and larval settlement. *Marine Ecology Progress Series* 124:301-305.
- Morgan, L. E., S. R. Wing, L. W. Botsford, C. J. Lundquist, and J. M. Diehl. 2000. Spatial variability in red sea urchin (*Strongylocentrotus franciscanus*) recruitment in northern California. *Fisheries Oceanography* 9:83-98.
- Neushul, M. 1963. Studies on the giant kelp, *Macrocystis* II Reproduction. *Journal of Botany* 50:354-359.
- North, W. J. 1971. The biology of giant kelp beds (*Macrocystis*) in California: Introduction and background. *Nova Hedwigia* 32:1-68.
- North, W. J. 1994. Review of *Macrocystis* biology. Pages 447-527 in I. Akatsuka, editor. *Biology of Economic Algae*. Academic Publishing, The Hague.
- North, W. J., and C. L. Hubbs, editors. 1968. Utilization of kelp-bed resources in southern California. *State of California Resources Agency and Fish and Game*, Sacramento.
- North, W. J., D. E. James, and L. G. Jones. 1993. History of kelp beds (*Macrocystis*) in Orange and San Diego Counties, California. *Hydrobiologia* 260/261:277-283.
- Palumbi, S. R. 1995. Using genetics as an indirect estimator of larval dispersal. Pages 369-387 in L. McEdward, editor. *Ecology of Marine Invertebrate Larvae*. CRC Press, New York.

- Pineda, J. 1991. Predictable upwelling and the shoreward transport of planktonic larvae by internal tidal bores. *Science* 253:548-551.
- Pineda, J. 1999. Circulation and larval distribution in internal tidal bore warm fronts. *Limnology and Oceanography* 44:1400-1414.
- Reed, D. C. 1987. Factors affecting the production of sporophylls in the giant kelp *Macrocystis pyrifera* (L) C Ag. *Journal of Experimental Marine Biology and Ecology* 113:61-69.
- Reed, D. C., C. D. Amsler, and A. W. Ebeling. 1992. Dispersal in kelps: factors affecting spore swimming and competency. *Ecology* 73:1577-1585.
- Reed, D. C., T. W. Anderson, A. W. Ebeling, and M. Anghera. 1997. The role of reproductive synchrony in the colonization potential of kelp. *Ecology* 78:2443-2457.
- Reed, D. C., M. A. Brzezinski, D. A. Coury, W. M. Graham, and R. L. Petty. 1999. Neutral lipids in macroalgal spores and their role in swimming. *Marine Biology* 133:737-744.
- Reed, D. C., A. W. Ebeling, T. W. Anderson, and M. Anghera. 1996. Differential reproductive responses to fluctuating resources in two seaweeds with different reproductive strategies. *Ecology* 77:300-316.
- Reed, D. C., D. R. Laur, and A. W. Ebeling. 1988. Variation in algal dispersal and recruitment: the importance of episodic events. *Ecological Monographs* 58:321-335.
- Reed, D. C., M. Neushul, and A. W. Ebeling. 1991. Role of settlement density on gametophyte growth and reproduction in the kelps *Pterygophora californica* and *Macrocystis pyrifera* (Phaeophyceae). *Journal of Phycology* 27:361-366.
- Rice, W. R. 1989. Analyzing tables of statistical tests. *Evolution* 43:223-225.
- Roughgarden, J., S. Gaines, and H. Possingham. 1988. Recruitment dynamics in complex life cycles. *Science* 241:1460-1466.
- Sale, P. F. 1991. Reef fish communities: open nonequilibrium systems. Pages 564-598 in P. F. Sale, editor. *The Ecology of Fishes on Coral Reefs*. Academic Press, Inc., San Diego.
- Santelices, B. 1990. Patterns of reproduction, dispersal and recruitment in seaweeds.

Oceanography and Marine Biology Annual Review 28:177-276.

Santelices, B., A. J. Hoffman, D. Aedo, M. Bobadilla, and R. Otaiza. 1995. A bank of microscopic forms on disturbed boulders and stones in tide pools. *Marine Ecology Progress Series* 129:215-228.

Shanks, A. L. 1995. Mechanisms of cross-shelf dispersal of larval invertebrates and fish. Pages 323-367 *in* L. R. McEdwards, editor. *Ecology of Marine Invertebrate Larvae*. CRC Press, Boca Rotan, Florida, USA.

Shanks, A. L., J. Largier, L. Brink, J. Brubaker, and R. Hooff. 2000. Demonstration of the onshore transport of larval invertebrates by the shoreward movement of an upwelling front. *Limnology and Oceanography*. 45:230-236.

Swearer, S. E., J. E. Caselle, D. W. Lea, and R. R. Warner. 1999. Larval retention and recruitment in an island population of a coral-reef fish. *Nature* 402:799-802.

Tabachnick, B. G., and L. S. Fidell. 1996. *Using multivariate statistics*, 3rd ed edition. HarperCollins College Publishers, New York.

Tegner, M. J., P. K. Dayton, P. B. Edwards, and K. L. Riser. 1996. Is there evidence for long-term climatic change in southern California kelp forests? *CalCOFI Reports* 37:111-126.

Tegner, M. J., P. K. Dayton, P. B. Edwards, and K. L. Riser. 1997. Large-scale, low-frequency oceanographic effects on kelp forest succession: a tale of two cohorts. *Marine Ecology Progress Series* 146:117-134.

Underwood, A. J., and P. G. Fairweather. 1989. Supply-side ecology and benthic marine assemblages. *Trends in Ecology and Evolution* 4:16-20.

Victor, B. C. 1991. Settlement strategies and biogeography of reef fishes. Pages 231-260 *in* P. F. Sale, editor. *The Ecology of Fishes on Coral Reefs*. Academic Press, Inc., San Diego.

Wing, S. R., L. W. Botsford, S. V. Ralston, and J. L. Largier. 1998. Meroplanktonic distribution and circulation in a coastal retention zone of the northern California upwelling system. *Limnology and Oceanography* 43:1710-1721.

Young, C. M. 1995. Behavior and locomotion during the dispersal phase of larval life.

Pages 249-277 in L. R. McEdwards, editor. *Ecology of Marine Invertebrate Larvae*. CRC Press, Boca Rotan.

Zimmerman, R. C., and J. N. Kremer. 1986. In situ growth and chemical composition of the giant kelp, *Macrocystis pyrifera*: response to temporal changes in ambient nutrient availability. *Marine Ecology Progress Series* 27:277-285.

Zimmerman, R. C., and D. L. Robertson. 1985. Effects of El Nino on local hydrography and growth of the giant kelp, *Macrocystis pyrifera*, at Santa Catalina Island, California. *Limnology and Oceanography* 30:1298-1302.

Chapter V

Importance of small-scale reproductive processes to propagule output of giant kelp

Abstract

Most marine species for which population dynamics have been studied possess propagules with relatively long planktonic durations, essentially de-coupling propagule supply from local reproduction. In such species, small-scale demographic and reproductive processes are likely of little importance to propagule supply in the overall population. It has recently been found, however, that propagule production and supply of the giant kelp *Macrocystis pyrifera* are tightly coupled in the center of the Point Loma kelp forest (in southern California), suggesting that small-scale processes may be important to variability in propagule output. This study details patterns of small-scale temporal and spatial variability in giant kelp demography and reproduction. Specific goals were to: (1) quantify the transition of adult plants among different size-classes and reproductive conditions; (2) study physical and biological factors that regulate such transitions; and (3) analyze spatial patterns in adult size-distribution and reproductive condition. Zoospore supply varied over 2 orders-of-magnitude from March 1999 to November 1999 despite an essentially constant density of adult plants, suggesting a limited importance of plant recruitment and mortality to reproductive output of the population. Instead, changes in zoospore supply were due to the rapid transition of giant kelp plants into and out of the highest reproductive condition (sloughing), when zoospores are vigorously being released from sori. Sloughing plants, however, proved to be physiologically vulnerable to disturbances, with all plants at the study site completely losing sloughing sori during summer 1999. The result was decreased reproductive potential for the population, and decreased zoospore supply, despite the

continuous presence of adult plants with sporophylls and non-sloughing sori. This vulnerability was highest for smaller relative to larger size-classes. The decreased reproductive condition was caused by an infestation of grazing amphipods that effectively removed most plant biomass without increasing plant mortality. The loss of sloughing sori therefore represented either an overall decrease in plant production or a trade-off between plant growth and reproduction as photosynthetic resource were diverted to increase biomass. The movement of grazers from plant to plant resulted in a patchy distribution of sloughing plants, and subsequently, patchy recruitment. These results indicate that giant kelp reproduction is highly variable in space and time and rapidly responds to biomass loss, with even small-scale disturbances impacting giant kelp recruitment.

Introduction

Recruitment variability is an important component of the dynamics of natural populations and can be regulated by various physical and biological factors that constrain reproductive output, dispersal, fertilization success, and juvenile growth. In marine systems, recent research on recruitment processes has focused on understanding variability in propagule supply, as the arrival of propagules to suitable substrate is an important bottleneck in the recruitment success of many taxa (Underwood and Denley 1984, Hoffman 1987, Roughgarden et al. 1988, Underwood and Fairweather 1989, Santelices 1990, Vadas et al. 1992, Gaines and Lafferty 1995, Caley et al. 1996). Moreover, the planktonic nature of many marine propagules has prompted some to suggest that marine populations are generally “open”, with remote propagule sources largely fueling recruitment (Caley et al. 1996). Recruitment variability in such open marine systems is essentially de-coupled from local propagule production. Given the diffusive nature of planktonic dispersal, it is likely that in open populations, spatial and temporal variability in the abundance and reproductive condition of adults will be of little consequence to local recruitment (Caley et al. 1996). It is

therefore not surprising that detailed data on local demography and reproduction have not been the primary focus of recruitment studies of marine organisms.

It has recently been shown that the supply of giant kelp (*Macrocystis pyrifera*) propagules (zoospores) can be tightly coupled to local zoospore production in the Point Kelp forest in southern California (**Chapter IV**). In the absence of uni-directional currents, most giant kelp zoospores are retained near the adult plants that released them. Such conditions occur in the center of the Point Kelp forest where the high drag of giant kelp plants significantly modifies flow (Jackson and Winant 1983, Jackson 1997). In **Chapter IV**, I found that in the Point Loma kelp forest (the largest in southern California), abundance, size-class structure, and reproductive condition of local adults (i. e. within 100 m² area) explained greater than 75% of within-year variability in giant kelp zoospore supply. This tight coupling between zoospore production and supply suggests that local, high-frequency changes in adult abundance, size, and fertility may be important in regulating giant kelp zoospore supply, and thus, recruitment.

Most research to date on seaweed reproduction has centered on functional comparisons between annual and perennial taxa (reviewed in Santelices 1990). This literature is rich, with significant contributions on adaptive differences between seasonal versus continuous reproduction (Reed et al. 1996), internal versus external cues for gamete/spore production and release (Reed 1987, Amsler and Neushul 1989, Lüning and Dieck 1989, Reed et al. 1996, Brawley et al. 1999), trade-offs between reproduction and growth (Pfister 1992, Van Patten and Yarish 1993), pre- versus post-settlement processes (Lotze et al. 1999, Worm et al. 1999, Lotze and Schramm 2000, Lotze et al. 2000), and opportunistic versus late-successional life-histories (Cubit 1984, Sousa 1984). Except for a few studies in intertidal systems (e. g., Johnson and Brawley 1998), however, most research has been conducted at temporal scales of seasons to years. As such, the importance of high-frequency fluctuations in seaweed reproduction to population dynamics may be unrealized, particularly with

continuously reproductive taxa like giant kelp that show tight coupling between zoospore production and supply.

This study describes broad-scale spatial (m's to km's) and temporal (days to years) variability in giant kelp demography and reproduction, with particular focus on the smallest scales. The research stems from the previous identification of adult size-class structure and reproductive condition as key factors in predicting variability in zoospore supply. Specific goals of the current study were to: (1) quantify the transition of adult plants among different size-classes and reproductive conditions; (2) study physical and biological factors that regulate such transitions; and (3) analyze spatial patterns in adult size-distribution and reproductive condition. In addition, the detailed demographic mapping of this study allowed for comparisons of local reproduction and subsequent recruitment. The results demonstrate that quantifying small-scale demographic and reproductive patterns can be important for understanding the dynamics of marine populations.

Methods

Study design

The research was done at 5 sites within the central portion of the Point Loma kelp forest located offshore of San Diego, California, USA (32°42'N, 117°16'W). One site (Central) was used for weekly studies of density, size-class structure, and reproduction at a spatial scale of m's, whereas the other 4 sites (8 m, 12 m, 15 m, and 18 m) were sampled monthly for studies of reproduction across a broad depth gradient at a scale of 100's of m's. Central was located along the 15 m isobath and was marked with a permanent 100 m² circular leadline grid (11.3 m diameter); physical and biological characteristics of this site were detailed in **Chapter IV**. The other 4 sites were arranged perpendicular to shore, marked with 4 parallel 25 x 4 m leadline swaths, and were named after the isobaths on which they were located; these sites have been maintained for much of the 70's, 80's, and

90's, and are part of a long-term study of kelp ecology at Point Loma (described in Dayton et al. 1984, Dayton et al. 1992, Dayton et al. 1999). All 5 sites were on flat rocky substrate with low vertical relief and generally contained giant kelp, sub-canopy kelps (*Pterygophora californica* and *Eisenia arborea*) and prostrate kelps (*Laminaria farlowii*).

Demography and reproduction

Spatial and temporal studies at the smallest scales investigated were limited to Central. All identifiable giant kelp greater than 10 cm length were tagged and mapped within the circular grid. All plants were censused on each of 26 sampling dates between February 28, 1999 and November 16, 1999 to determine giant kelp density, size-class structure, and reproductive condition. Plant size was quantified as the number of fronds greater than 2 m length (Dayton et al. 1992, Dayton et al. 1999, **Chapter IV**). Plant fertility was based on the presence and quality of sori (aggregations of sporangia borne on specialized sporophylls): sori were absent (sterile), present (fertile), or present (sloughing). Sloughing is a condition in which sori are present and vigorously releasing zoospores; sloughing sori can easily be distinguished from fertile sori based on the presence of white tattered sporophylls (Neushul 1963, **Chapter IV**). Size of the single sporophyll bundle per plant was quantified as small or large, with small sporophyll bundles having < 20 individual sporophyll blades (**Chapter IV**). Plants were considered to be recruits on the sampling date that they were first tagged and to be mortalities on the date they were observed missing (Graham et al. 1997). Upon recruitment, plants were designated as juveniles until sori were first observed, after which they were adults regardless of the presence or absence of sori. This avoids the "sliding" distinction of juveniles versus adults that occurs when adulthood is based on plant size, as plant size is highly variable (Dayton et al. 1992, Graham 1997, Dayton et al. 1999). Juvenile data were not analyzed here.

In **Chapter IV**, I found that the abundance of sloughing plants with large sporophyll

bundles was the best predictor of temporal variability in giant kelp zoospore supply. Specifically, the abundance of either non-sloughing plants (i. e. fertile + sterile) or plants with small sporophyll bundles (regardless of sori presence or quality) did not explain a significant amount of variability in zoospore supply that was not already explained by the abundance of sloughing plants with large sporophyll bundles. Explanatory power of the zoospore supply model was further enhanced by partitioning sloughing plants with large sporophyll bundles into five size-classes: ≤ 8 , 9-15, 16-20, 21-25, and ≥ 26 stipes per plant. Of these, the ≤ 8 , 9-15, and 21-25 stipe size-classes were found to be most important. Analyses of the Central data in this study were designed therefore to quantify the transition of giant kelp plants into sloughing condition, large sporophyll bundles, and between the five size-classes.

Demography and reproduction at 8 m, 12 m, 15 m, and 18 m were studied differently than at Central. Sampling at these sites was not designed to provide complete demographic maps or to assess variability in giant kelp abundance, but rather to track general long-term changes in reproductive condition at each site. The data were used here to provide a time-series of the percentage of giant kelp plants that were sloughing. Adult giant kelp were haphazardly tagged and mapped along the permanent swaths on various dates between July 1997 and March 2000, with the goal of maintaining at least 10 tagged plants per site. The number of tagged plants therefore varied with time, and briefly fell to zero during the 1997-98 ENSO due to high plant mortality. Sori presence and quality were quantified as above; although changes in sporophyll bundle size were not analyzed. Only plants with large sporophyll bundles at the time of initial tagging were included here.

Zoospore supply

Zoospore supply at Central was estimated from 2-L *in situ* plankton samples collected using a subtidal pump mounted at a fixed location less than 3 cm from the substrate

(see Chapters 2, 3, and 4 for detailed sampling protocol). Three samples were taken on each of the 26 dates that plant demography and reproduction were censused. Plankton samples were processed according to **Chapter IV**, with the density of giant kelp zoospores (#/L) estimated microscopically for each sample (Graham 1999, Graham and Mitchell 1999, Chapters 2 and 3). This method has a minimum detectable zoospore density of 11 zoospores/L and a validated accuracy for giant kelp zoospores of greater than 98% (Graham 1999, **Chapter III**).

Hydrographic conditions

Surface and bottom temperatures were measured at Central every 15 min between February 28, 1999 and November 16, 1999 using Stowaway[®] Tidbit submersible sensors (Onset Computer Corp., Bourne, MA). *In situ* nitrate concentrations were estimated from 250 ml bottom-water samples collected at the 15 m site (within 75 m of Central) on 6 dates between April 14, 1999 and November 24, 1999. Water samples were transported to the laboratory in a cooler, passed through glass fiber filters, frozen, and later analyzed for nitrate concentrations using a Skalar SanPlus[®] continuous-flow AutoAnalyzer[®] and a modified procedure of Armstrong et al. (1967). Significant wave height was measured every 5 minutes from a deepwater buoy (183 m depth) located directly offshore of the study sites (Buoy #09101, Coastal Data Information Program, Scripps Institution of Oceanography).

Results

The 1997-98 ENSO resulted in 100% mortality of giant kelp at Central by Spring 1998, as well as throughout much of the Point Loma kelp forest (Dayton et al. 1999). The onset of strong La Niña conditions in Summer/Fall 1998, and the return of low temperatures and high nutrient concentrations, fueled extensive giant kelp recruitment. Density of adult giant kelp subsequently reached 21 plants/100 m² by March 1999 and remained between 21 and

23 plants/100 m² until the end of the study (Figure 5-1). Giant kelp biomass, however, appeared to be much lower than carrying-capacity as the total number of stipes/100 m² increased from ~ 255 in March to over 420 by December (Figure 5-1). Adult self-thinning, characteristic of mature giant kelp assemblages at Point Loma (Dayton et al. 1984, Dayton et al. 1992, Graham et al. 1997), was not evident. Thus, the giant kelp assemblage at Central was recovering from the 1997-98 ENSO during the entire study, with stable adult densities and steady increases in plant size. This pattern was observed at many sites throughout the Point Loma kelp forest (**Chapter IV**). Despite the relatively constant total density of local adult giant kelp, zoospore density at Central varied more than 2 orders-of-magnitude during the study, from over 54000 zoospores/L in March to as little as ~250 zoospores/L in August (Figure 5-1). The link between local zoospore production and supply, as described in **Chapter IV**, was therefore due to changes in reproductive condition of adult plants, rather than recruitment and mortalities.

Most adult giant kelp at Central possessed sporophylls throughout the study. The only two adults observed without sporophylls died during Fall 1999, with sporophyll loss occurring during the few weeks prior to mortality. Sporophyll bundle size varied randomly with time (Figure 5-2), as there was no increase or decrease (linear or \leq 3rd order polynomial) in the number of plants with large bundles with time (all P values \geq 0.25), and no relationship between sporophyll bundle size and zoospore density (**Chapter IV**). The random variability in bundle size was due to fluctuations of individual adult plants. Most plants with small bundles remained so throughout the study, whereas plants with large bundles often lost and then regained sporophyll biomass. An episode of rapid loss and recovery of sporophyll bundle size was apparent during July/August 1999 (Figure 5-2).

The general condition of sori on plants with large sporophyll bundles shifted from predominantly sloughing at the beginning of the study to predominantly sterile by the end, subsequently decreasing zoospore density (Figures 5-1 & 5-3). Zoospore density was

highest in Spring 1999 during the peak abundance of plants with sloughing sori; 100% of adult plants with large sporophyll bundles were sloughing during March and April 1999. May, June, and early-July 1999 saw a moderate transition of plants from sloughing to fertile condition, with sterile plants remaining rare during this time. The abundance of sterile plants increased rapidly after mid-July, corresponding to a decrease in sloughing plants (Figure 5-3). Sterile plants dominated the local population until the end of the study, with a complete absence of sloughing plants during September 1999 when sloughing plants increased slightly. Zoospore density was low following the sharp decrease in sloughing plants during late-July 1999 (Figures 5-1 & 5-3).

This shift in abundance of sloughing to non-sloughing plants (i. e. fertile + sterile) was size-dependent. The 9-15 stipe size-class accounted for the highest number of sloughing plants during the beginning of the study, yet was the first size-class to transition into non-sloughing condition (Figure 5-4). Few plants in the 16-20, 21-25, and ≥ 26 stipe size-classes had transitioned into non-sloughing condition by July, after which a large increase in non-sloughing plants occurred for each of these size-classes. The decreased abundance of the ≤ 8 stipe size-class over the same time period represented growth to the 9-15 stipe size-class, not a change in reproductive condition. During July and August the 16-20 and 21-25 stipe size-classes represented most of the sloughing plants. Decreased abundance of non-sloughing plants in the 9-15 stipe size-class from September to November, however, was not due to transitions back to sloughing condition, but rather simply shifts to either smaller (≤ 8 stipes) or larger (16-20 stipes) non-sloughing size-classes. Transitions back to sloughing condition during October and November were limited to 1 plant in each of the 16-20 and 21-25 stipe size-classes.

General patterns emerged when the transition of adult plants among size-classes and reproductive conditions was determined for different seasons during 1999 (Figure 5-5). Spring 1999 saw seemingly random shifts in plant size and reproductive condition, with

many plants remaining unchanged in size and reproduction. In Summer 1999, however, all sloughing plants shifted to non-sloughing condition, and both increases and decreases in plant size were observed; again, many plants remained unchanged in size and reproduction. During Fall 1999, most transitions were due to increases in size, with many plants remaining unchanged in size and reproduction. A few plants in Fall 1999 increased in both size and reproduction, and a few also died due to apparent senescence; senescing plants were sterile and of the smallest size-class.

Changes in plant reproductive condition followed the pattern of sori development described by Neushul (1963). Sporophylls grew continuously from a meristematic region at their attachment to supporting fronds. As sloughing ceased, the white tattered sori were lost off the ends of the sporophylls. Sori production often persisted in the absence of sloughing, in which case continued sporophyll growth resulted in extensive cover of fertile, but not sloughing, sori (e. g., mid-July; Figure 5-3). If sori production ceased entirely, fertile sori were lost off the ends of the sporophylls as well, resulting in complete sterility despite the presence of often large sporophyll bundles (e. g., late-August to October; Figure 5-3). The actual transition of sori from fertility to sterility was easily observed, as the edge of fertile sori was clearly distinct from sterile tissue (see Neushul 1963 for greater detail of this transition). The shift from sterile to sloughing sporophylls was simply the reverse. Fertile sori began to be produced on sporophylls, increasing in size as sporophylls continued to grow, subsequently leading to the production of sloughing sori (e. g., November; Figure 5-3). The transition from sterility to fertility to sloughing, and back again, did not always occur simultaneously for all sporophylls on a given plant, although that was generally the case.

The speed with which plants transitioned between different reproductive conditions was therefore strongly dependent on sporophyll growth rates. Although I did not estimate sporophyll growth during this study, the demographic data indicated that the transition

between reproductive conditions was rapid. For example, 7 of 17 plants in early-May and 8 of 12 plants in early-July were lost from sloughing condition over a period of less than 10 days (Figures 5-3 and 5-6). Shifts from non-sloughing to sloughing condition during late-May (9 to 14 plants in < 14 days) and late-June and (9 of 14 plants in < 10 days) were just as dramatic. The complete transition of sloughing plants to sterility, however, was necessarily slower than the simple loss of sloughing sori, as plants first needed to cycle through fertile sori conditions.

The general shift from high to low reproductive condition was due primarily to decreased production of sloughing sori following early-July (Figures 5-4 & 5-5). The drop in July of sloughing plant density corresponded to a brief 1-week peak in sea-surface temperature above 20°C (Figure 5-7). Bottom temperatures, however, were below 15.5°C throughout the study, keeping bottom nitrate concentrations greater than 2 μM during the summer and fall (Figure 5-7). Hydrographic conditions in the Point Loma kelp forest during 1999 were therefore not as severe as in typical summers (Tegner et al. 1996, Tegner et al. 1997, Dayton et al. 1999), suggesting that hydrography was not the primary factor driving the large decrease in reproductive condition. Furthermore, if a seasonal shift in oceanographic climate was responsible for the poor production of sloughing sori, then the patterns seen at Central should have been observed throughout the kelp forest. Although sloughing plants were rare at the 12 m site during late-Summer/Fall 1999, they were common at the 15 m site (< 75 m from Central) and dominated the 8 m and 18 m sites during this period (Figure 5-8). The high percentage of sloughing plants at 8 m, 12 m, 15 m, and 18 m sites during late-Summer/Fall 1997 further suggests that decreased production of sloughing sori is not common during these seasons (Figure 5-8). Decreased reproductive condition due to biomass loss from wave disturbance was also unlikely given the low significant wave heights measured off Point Loma during summer and fall (Figure 5-8).

The rapid shift in plant reproductive condition that started in July, appeared to be driven in part by loss of frond biomass due to an infestation of grazing amphipods. In late-April, large numbers of amphipods were observed curling blades on adult giant kelp fronds. By May, signs of direct amphipod grazing were apparent, although most damage was localized onto a few plants. By mid-June, most plants had amphipod damage with some showing complete loss of blade biomass; the retention of intact pneumatocysts kept damaged fronds afloat despite the lack of blade biomass, negating a sharp decrease in stipe density during this period (Figure 5-1). The 2-3 plants that continued sloughing until late-August were the least damaged. In early-July, amphipods were observed feeding on sporophyll blades, directly resulting in the rapid (albeit brief) decrease in sporophyll bundle size during late-July (Figure 5-2); amphipod damage to other kelp taxa (e. g., *Laminaria farlowii* and *Pterygophora californica*) and was observed during July. By the time sporophyll bundle sizes had recovered, sloughing plants were completely absent (Figure 5-3). A giant kelp surface canopy was absent at Central from mid-June to mid-August 1999, a seasonal time period when canopies are usually extensive in southern California giant kelp forests (North et al. 1993, Tegner et al. 1996). Although direct biomass estimates were not made, enhanced production of healthy blades was observed on each sampling date from August through November. Again, Fall 1999 was a period of general increases in plant size (Figure 5-5). By October, there were no signs of amphipod damage. Similar patterns of localized, yet severe amphipod grazing were observed throughout the Point Loma kelp forest.

The distribution of giant kelp plants within Central was contagious, as most plants were aggregated in the northwest and southeast regions of the site (Figure 5-9). The spread of the amphipod infestation throughout the site resulted in a distinct spatial pattern for the general transition of plants from high to low reproductive condition. Amphipod densities were first noticed to be extremely high on two individuals in May, one each in the northwest and southeast regions (Figure 5-9). The decrease in reproductive condition spread to

neighboring plants until by September, no plants with large sporophyll bundles were sloughing. The last plant to lose sloughing sori in August was also the first plant to begin sloughing again in October. This plant was the farthest from the two plants that were initially infested. Although none of the adult plants in the northwest region returned to sloughing condition following the decrease in amphipod grazing, most of these plants had grown in size and transitioned into fertile condition by November.

The distribution of giant kelp recruits at Central mimicked the distribution of adults (Figure 5-10). Again, most recruits were aggregated in the northwest and southeast regions of the site. The lack of recruitment during May, June, and July was due to amphipods directly grazing blade stage giant kelp prior to the time the plants were large enough to receive a tag (i. e. < 10 cm). Blade stage giant kelp were observed at Central on all 26 sampling dates from March through November. None of the recruits observed during this study had grown to adult size by November; the two additions to the adult population during spring were from juveniles already present at the site during March. Furthermore, the density of giant kelp recruits within the 12 regions of the leadline grid at Central was not correlated with the density the other kelp taxa, *Laminaria farlowii* and *Pterygophora californica*, in the same regions (both P's ≥ 0.4).

Discussion

Year-round reproduction in the perennial giant kelp *Macrocystis pyrifera* is considered a potential life-history advantage over other perennial, yet seasonally reproducing, subtidal kelp taxa in southern California (McPeak 1981, North 1994, Reed et al. 1996, Dayton et al. 1999). Seasonal sori production in *Pterygophora californica*, *Laminaria farlowii*, and *Eisenia arborea*, all common in the Point Loma kelp forest, is likely cued by endogenous rhythms entrained to steadfast environmental clocks (e. g., daylength; Amsler and Neushul 1989, Lüning and Dieck 1989, tom Dieck (Bartsch) 1991). Such "rigid" seasonal

reproduction may allow these kelps to anticipate environmental conditions (e. g., spring upwelling) that enhance propagule settlement, fertilization, and recruitment success. In contrast, the ecological success of giant kelp may be attributed to its potential for year-round reproduction, and thus recruitment, whereby giant kelp can take advantage of newly created open space when others can not. This study has shown that the reproductive output of giant kelp (1) responds rapidly to environmental variability, (2) is highly vulnerable to physical and biological disturbance, and (3) is structured at small temporal and spatial scales.

Given that zoospore supply is tightly linked to the abundance of plants with high reproductive condition, variability in plant density will obviously be important in regulating zoospore supply. Giant kelp density in California is variable over a wide range of temporal and spatial scales (Cowen et al. 1982, Foster 1982, Dayton et al. 1984, 1992, 1999, Graham 1997, Graham et al. 1997) and can change rapidly, and predictably, especially during the recruitment and self-thinning stage of post-disturbance recovery (Dayton et al. 1984, Dayton et al. 1992, Graham et al. 1997). The demographic studies conducted at Central during 1999, however, clearly demonstrated that variability in reproduction alone can drive the observed 2 orders-of-magnitude variability in giant kelp zoospore density.

Much of the variability in giant kelp reproductive condition appears to be linked to sporophyll growth rates. If a giant kelp plant is producing sloughing sori, then sorus surface area per sporophyll, and thus the standing stock of sporangia, will be determined by the length of the sporophylls. Sporangia standing stock will therefore vary as sori are lost to erosion off the tips of sporophylls and new sporophyll tissue is created. The other kelp taxa in southern California create discrete sori on individual sporophyll blades that are not growing. Such species therefore have a less variable standing stock of sporangia per plant and a more constant overall rate of zoospore production. One exception is *Laminaria farlowii* in which sori are produced on the single blade that makes up the plant. Long-term reproductive data however indicate that periods of *L. farlowii* blade growth and sori

production do not overlap in time (Dayton et al 1999, P. K. Dayton, M. J. Tegner, K. L. Riser unpublished data). As such, maximum zoospore production per plant will be fixed before reproduction begins. The ecological and evolutionary consequences of these two distinct forms of kelp sporophyll growth have yet to be considered. Furthermore, it is still unclear exactly how fast giant kelp sporophylls grow, and whether sporophyll growth is linked to plant biomass, and thus vulnerable to external cues, or rather cued by internal endogenous rhythms. These are important areas for future research, especially given the newfound link between small-scale patterns in zoospore production and supply.

Giant kelp plants in the highest reproductive condition (i. e. with sloughing sori) appear to have the greatest contribution to zoospore supply. This is interesting, as most previous studies of giant kelp reproduction have focused on either the abundance of sporophylls or the simple presence of sori (Reed 1987, Reed et al. 1996, Reed et al. 1997, Dayton et al. 1999). Although zoospores were present in the water column during periods when sloughing plants were absent, changes in the abundance of plants in lower reproductive conditions did not reflect variability in zoospore production. That sloughing plants were present at nearby sites when they were absent at Central, suggests such distant sources of sloughing plants likely provided a background supply of zoospores.

The link between the abundance of sloughing plants and zoospore supply is important because the production of sloughing sori appears to be more sensitive to environmental stress than simply the production of any sori. Thus, although giant kelp has the potential for year-round zoospore production, it also has the potential for periods of low reproductive condition and decreased zoospore output, triggered by environmental variability. This vulnerability, however, was not constant for all plants; smaller size-classes seemed most susceptible to decreases in reproductive condition and less likely to recover. Furthermore, the decreased reproductive condition that occurred as plants increased biomass in response to grazing suggests that a trade-off exists between frond growth and reproduction.

Although the plants consistently had large sporophyll bundles, frond and blade growth may have diverted resources away from the production of sloughing sori, and therefore accounted for decreased reproductive output.

The link between plant size and reproductive condition likely resulted in the patchy distribution of sloughing plants in space and time, and suggests that individual reproductive histories are important to the reproductive output of the overall population. Grazer damage, entanglement with drifting plants, and other stochastic localized disturbances will promote small-scale variability in plant reproductive condition. The resulting patterns are on top of those produced by large-scale processes like general shifts in oceanographic climate, broad-scale wave-disturbance, or large grazing events. Given the tight link between zoospore production and supply, at least within the weak current environment of the kelp forest interior, variability in zoospore supply will represent the combined contribution of reproductive processes across a continuum of scales.

The amphipod grazing event described during this study deserves further discussion. Similar infestations have been described following previous ENSO events (Dayton et al. 1984, Tegner and Dayton 1987). One possible explanation is that the loss of kelp biomass during ENSO results in the displacement to other habitats of fishes that generally occupy kelp canopies. Many of these fishes (e. g., señorita, kelp perch, and juvenile kelp bass) feed on grazing invertebrates that inhabit the kelp canopy, as well as other benthic vegetation (Bernstein and Jung 1979, Dayton 1985). In the absence of these “picker” fish, grazing invertebrate populations can grow unchecked (Bernstein and Jung 1979). Presumably such infestations are halted by the return of picker fishes or some aspect of the life-histories of the invertebrates that causes a depression in their population numbers. Thus, given that grazer infestations have followed two of the most recent ENSO events (Dayton et al. 1984, Tegner and Dayton 1987, this study), the resulting small-scale spatial variability in giant kelp reproduction may be a common characteristic of the post-ENSO recovery of giant kelp

populations. The impact of grazing infestations on giant kelp population dynamics, however, can not be assessed until the relationship between variable zoospore supply and subsequent recruitment has been studied.

The similarity observed during this study between small-scale spatial distribution of reproductive giant kelp plants and that of giant kelp recruits supports previous hypotheses that realized giant kelp zoospore dispersal is limited (Anderson and North 1966, Dayton et al. 1984). Although zoospores were continuously in the water column at Central, recruitment success was highest near established plants. It is unknown whether this pattern is due to pre-settlement processes that affect the arrival of zoospores to the substrate, or settlement and post-settlement processes that regulate germling survival, fertilization success, and the growth of recruits to macroscopic size. The data presented here suggest that the potential for post-settlement mortality should have been the same for all species of kelp, yet giant kelp recruits were rarely observed distant from established adults. Studies of the relative role of pre- versus post-settlement processes in regulating giant kelp recruitment are needed.

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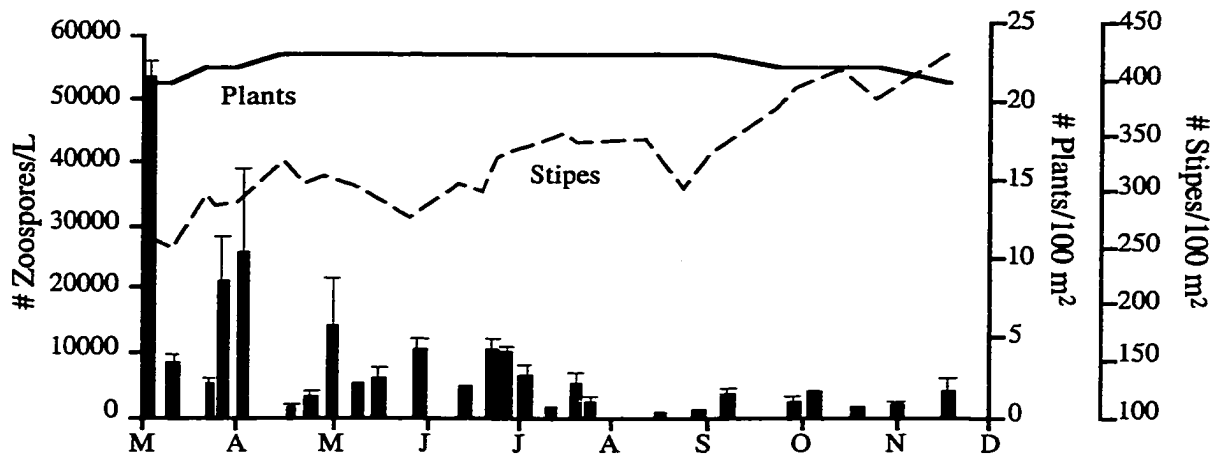


Figure 5-1. Temporal variability in giant kelp zoospore density, adult plant density, and adult stipe density at Central during 1999. Zoospore data are means \pm 1 SD ($n = 3$). Plant and stipe density data are totals observed within the 100 m² sampling site.

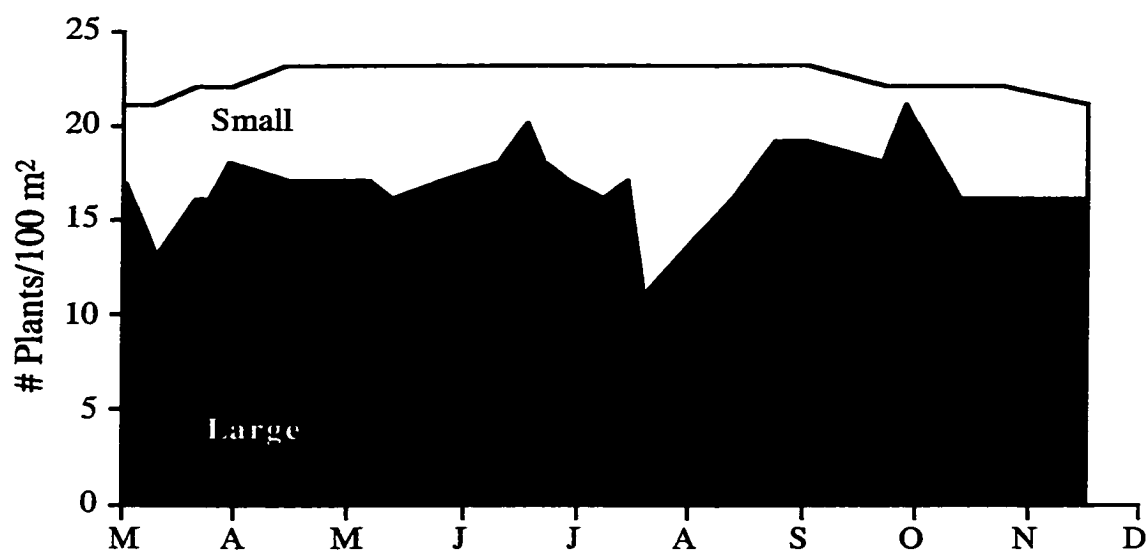


Figure 5-2. Temporal variability in the density of adult giant kelp plants with small and large sporophyll bundles at Central during 1999. Plant density data are totals observed within the 100 m² sampling site.

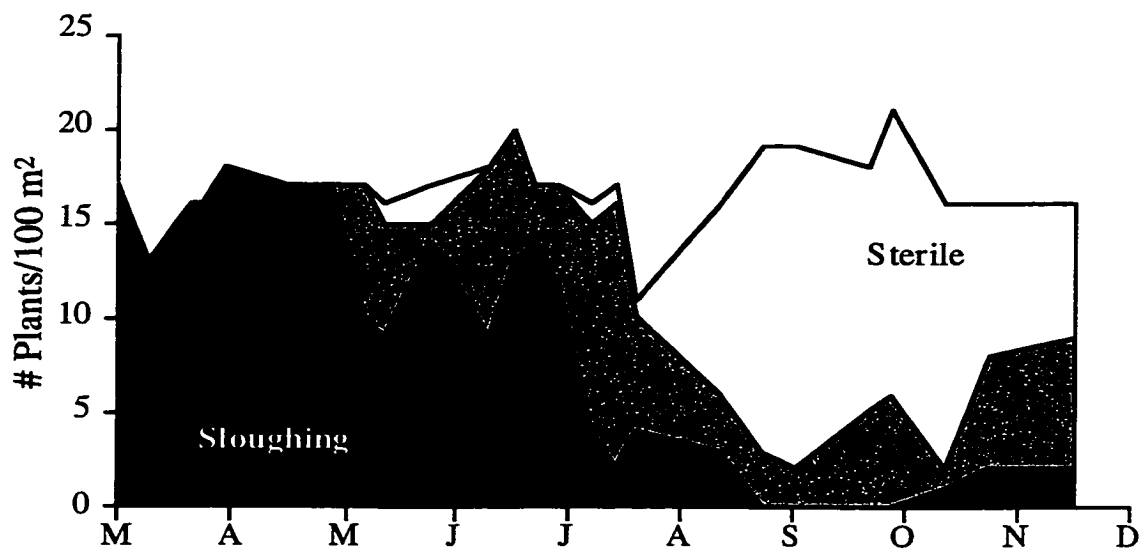


Figure 5-3. Temporal variability in the density of adult giant kelp plants with large sporophyll bundles and either sterile, fertile, or sloughing sori at Central during 1999. Plant density data are totals observed within the 100 m² sampling site.

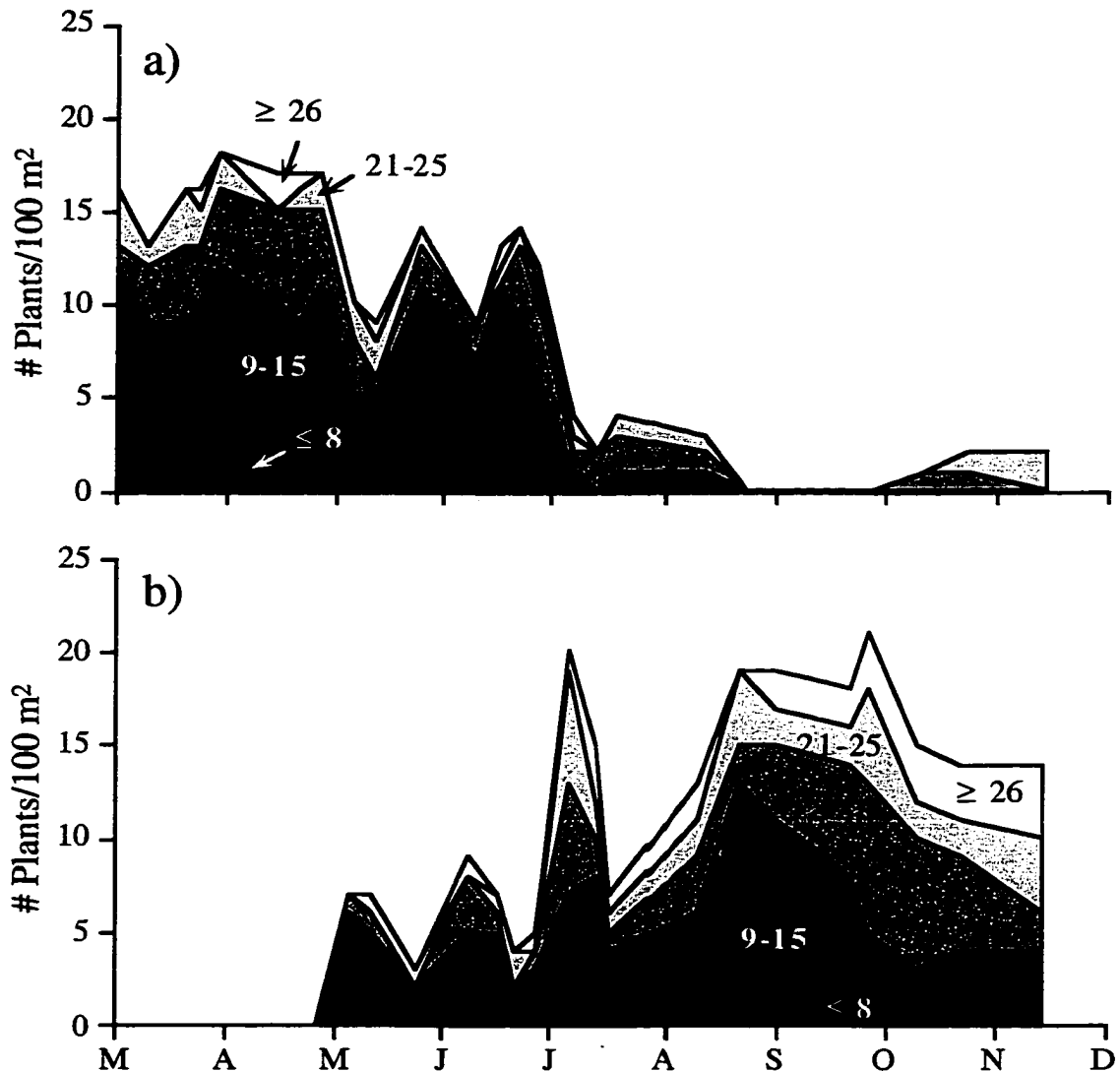


Figure 5-4. Temporal variability in the density of adult giant kelp plants with large sporophyll bundles in each of five size-classes at Central during 1999. a) all plants with sloughing sori; b) all plants with non-sloughing (i. e. sterile + fertile) sori. Size-classes represent the total number of stipes per plant. Plant density data are totals observed within the 100 m² sampling site.

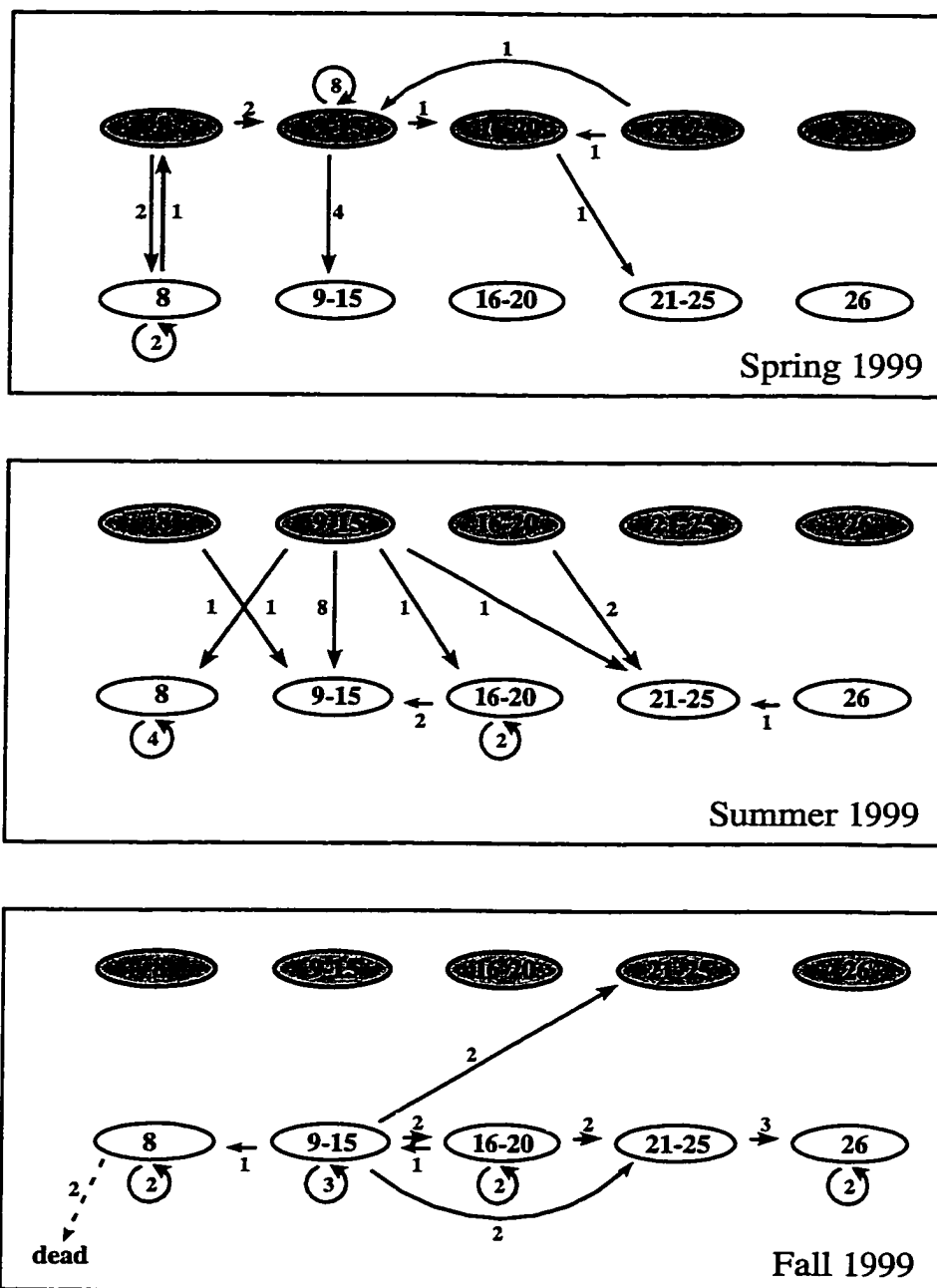


Figure 5-5. Transition of adult giant kelp plants among size-classes and reproductive conditions at Central during 1999. Ovals represent the five different size-classes based on the total number of stipes per plant; shaded ovals are plants with sloughing sori, open ovals are plants with non-sloughing (i. e. sterile + fertile) sori. Arrows represent transitions. Numbers next to arrows represent the number of plants making each form of transition. Spring transitions occurred from February 28 to May 24; Summer transitions occurred from June 9 to August 23; Fall transitions occurred from September 1 to November 16.

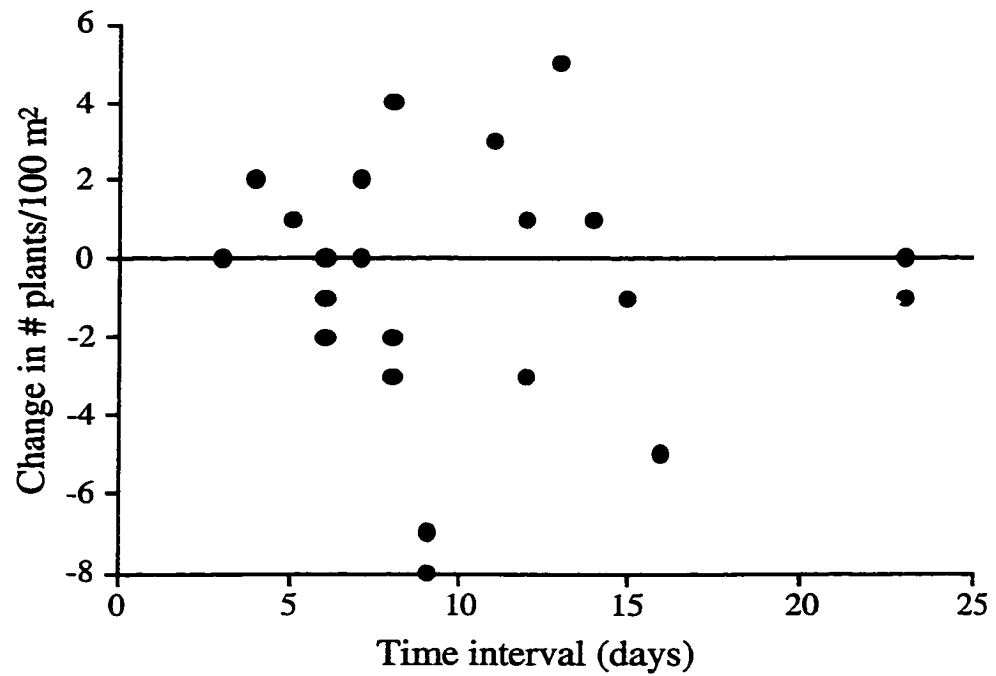


Figure 5-6. Absolute change in the density of adult giant kelp plants with large sporophyll bundles and sloughing sori as a function of the time interval between density estimates.

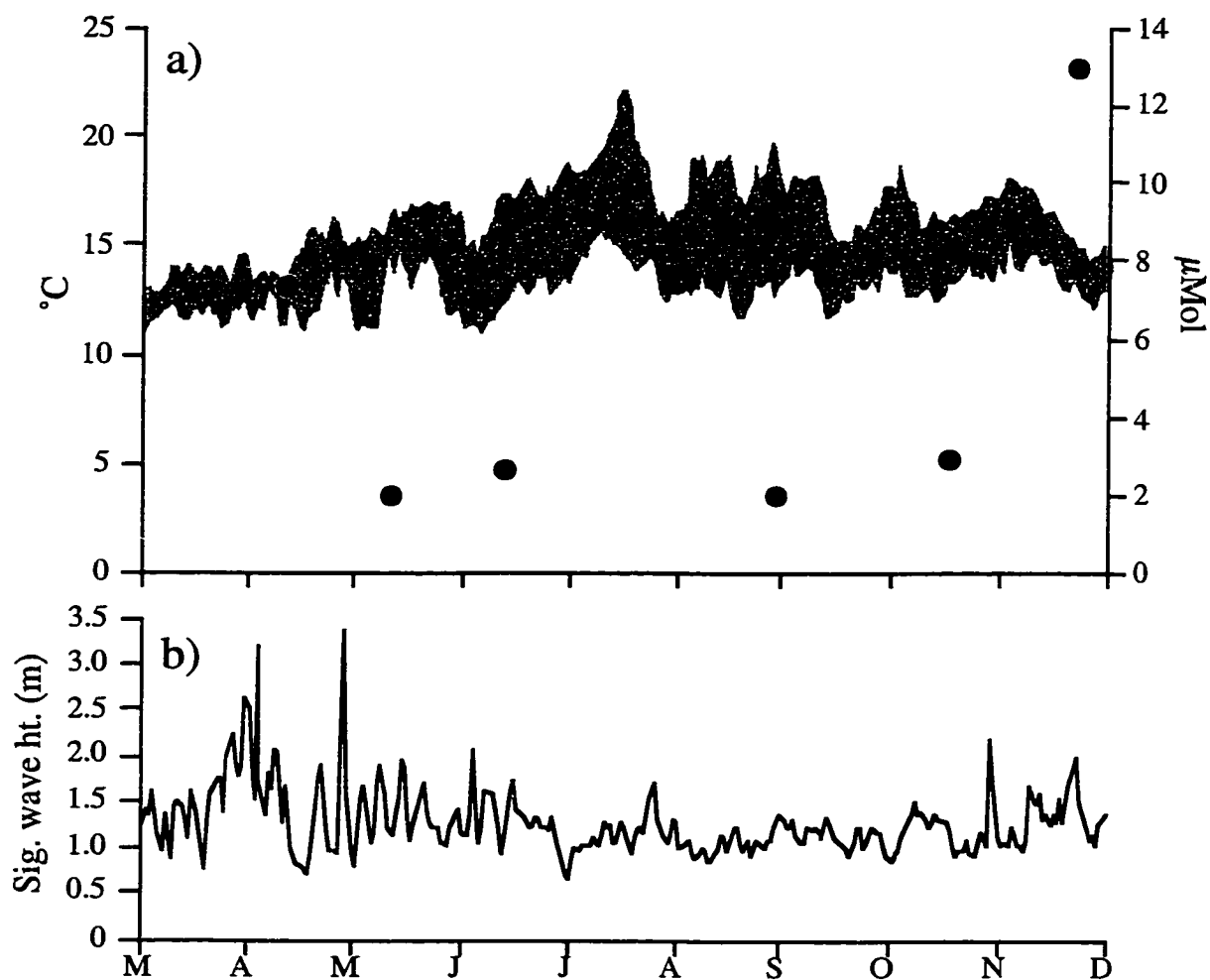


Figure 5-7. Temporal variability in a) the range of daily sea-surface to -bottom temperature (shaded region) and bi-monthly nitrate concentration and b) daily significant wave height during 1999. Temperature data are from Central; nutrient data are from the 15 m site ~ 75 m from Central; wave data are from offshore of the Point Loma kelp forest.

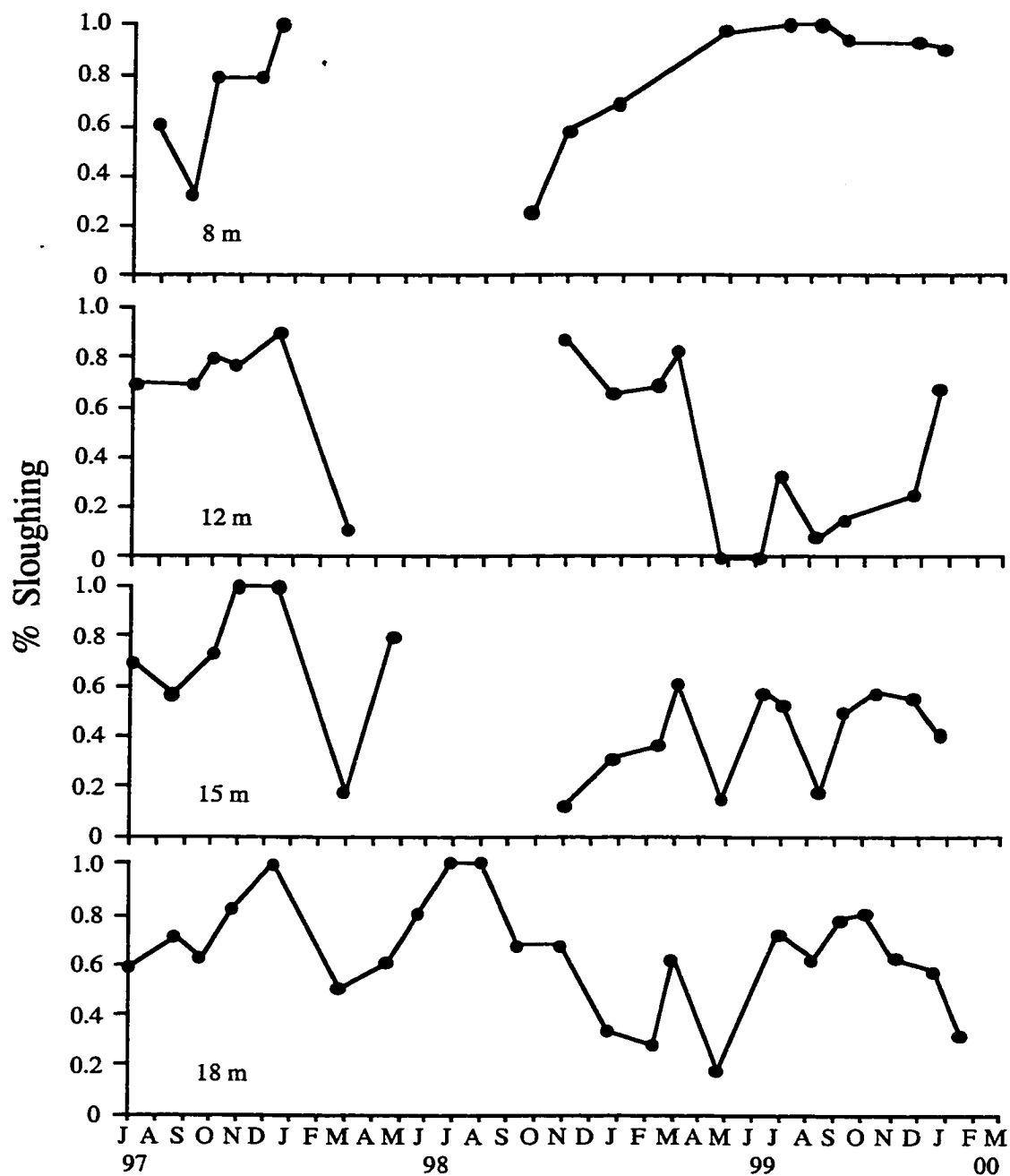


Figure 5-8. Temporal variability in the percent of tagged adult giant kelp plants that had sloughing sori at the 8 m, 12 m, 15 m, and 18 m sites from 1997 to 2000. Gaps in time series represent dates during ENSO 1997/98 went no adult giant kelp were present at the study sites.

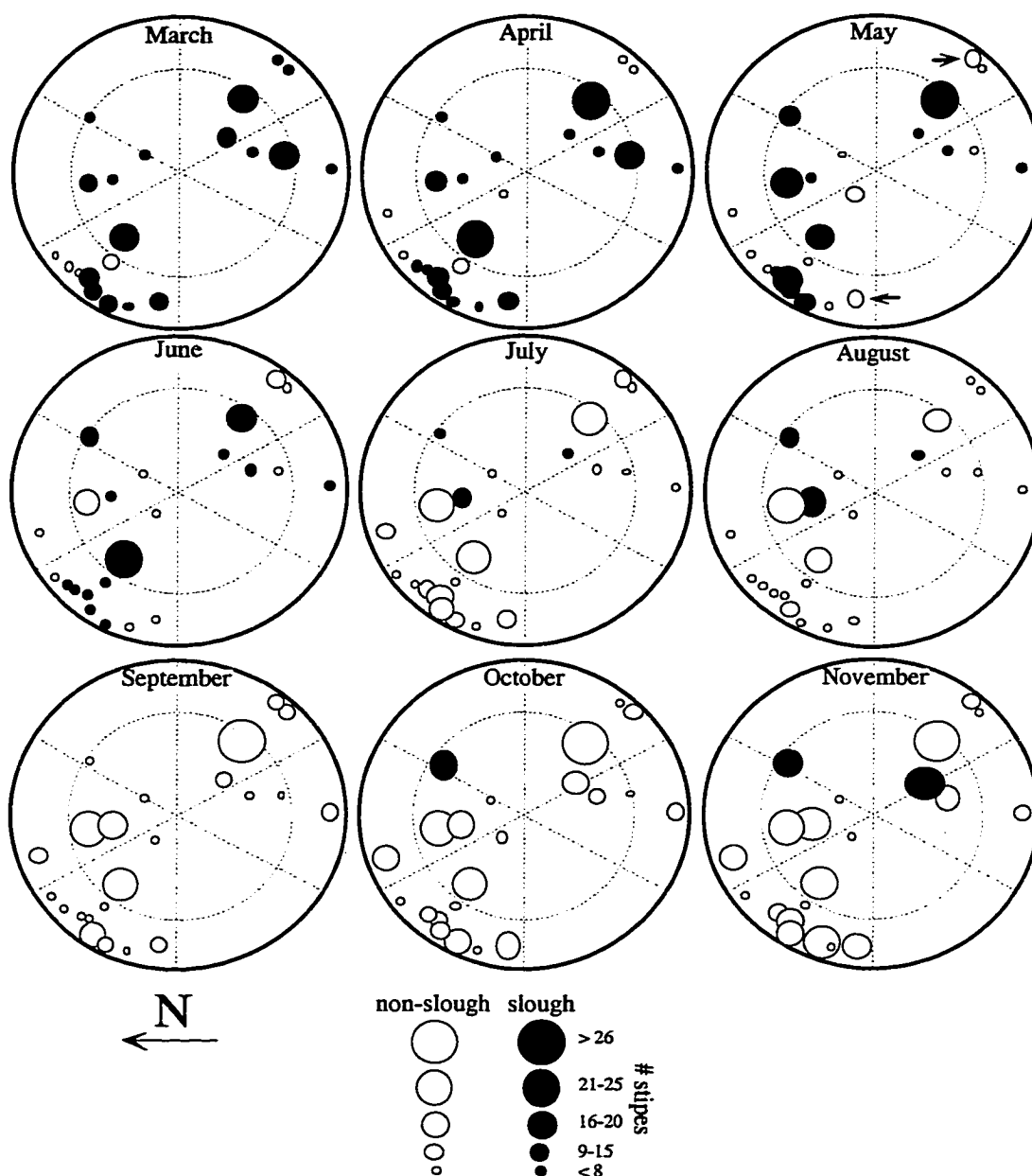


Figure 5-9. Monthly spatial maps of adult giant kelp plants at Central during 1999. The maps represent median size and reproductive condition of the multiple censuses during each month. Grey lines represent the position of the leadline sampling grid; study site diameter equals 11.3 m. Arrows during May point to the two plants on which amphipod grazing was first observed.

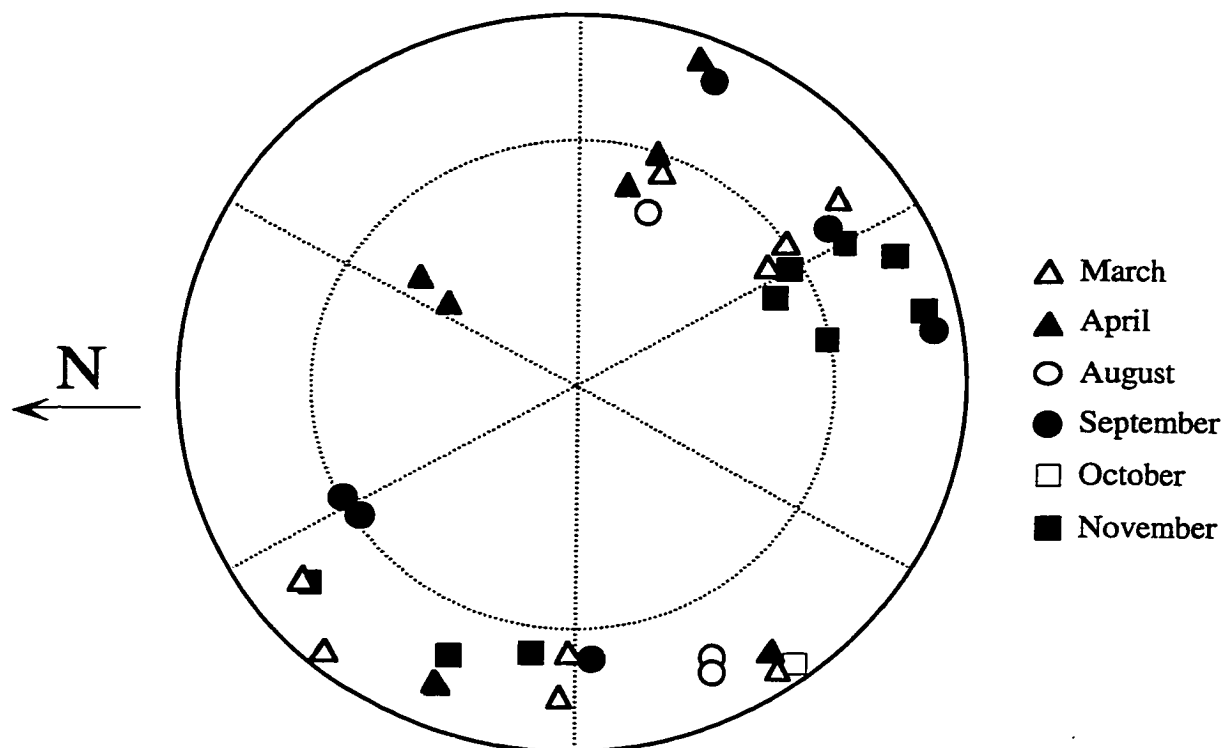


Figure 5-10. Spatial map of giant kelp recruitment at Central during 1999. Recruits are only those plants large enough to receive a tag (> 10 cm), and are only plotted for the month during which they recruited. Recruits were not observed during May, June, or July. Position and orientation of the sampling grid is the same as in Figure 5-8.

Literature Cited

- Amsler, C. D., and M. Neushul. 1989. Diel periodicity of spore release from the kelp *Nereocystis luetkeana* (Mertens) Postels et Ruprecht. *Journal of Experimental Marine Biology and Ecology* 134:117-127.
- Anderson, E. K., W. J. North. 1966. *In situ* studies of spore production and dispersal in the giant kelp, *Macrocystis*. *Proceedings of the International Seaweed Symposium* 5:73-86.
- Armstrong, F. A. J., C. A. Stearns, and J. D. H. Strickland. 1967. The measurement of upwelling and subsequent biological processes by means of the Technicon Autoanalyzer and associated equipment. *Deep-Sea Research* 14:381-389.
- Barrales, H. L., and C. S. Lobban. 1975. The comparative ecology of *Macrocystis pyrifera*, with emphasis on the forests of Chubut, Argentina. *Journal of Ecology* 63:657-677.
- Bernstein, B. L., and N. Jung. 1979. Selective pressures and coevolution in a kelp canopy community in southern California. *Ecological Monographs* 49:335-355.
- Brawley, S. H., L. E. Johnson, G. A. Pearson, V. Speransky, R. Li, and S. Serrao. 1999. Gamete release at low tide in furoid algae: maladaptive or advantageous? *American Zoologist* 39:218-229.
- Caley, M. J., M. H. Carr, M. A. Hixon, T. P. Hughes, G. P. Jones, and B. A. Menge. 1996. Recruitment and the local dynamics of open marine populations. *Annual Review of Ecology and Systematics* 27:477-500.
- Cowen, R. F., C. R. Agegian, and M. S. Foster. 1982. The maintenance of community structure in a central California giant kelp forest. *Journal of Experimental Marine Biology and Ecology* 64:189-201.
- Cubit, J. D. 1984. Herbivory and the seasonal abundance of algae on a high intertidal rocky shore. *Ecology* 65:1904-1917.
- Dayton, P. K. 1985. Ecology of kelp communities. *Annual Review of Ecology and Systematics* 16:215-245.
- Dayton, P. K., V. Currie, T. Gerrodette, B. D. Keller, R. Rosenthal, D. Ven Tresca. 1984. Patch dynamics and stability of some California kelp communities. *Ecological*

Monographs 54:253-289.

Dayton, P. K., and M. J. Tegner. 1984. Catastrophic storms, El Nino, and patch stability in a southern California kelp community. *Science* 224:283-285.

Dayton, P. K., M. J. Tegner, P. B. Edwards, and K. L. Riser. 1999. Temporal and spatial scales of kelp demography: the role of oceanographic climate. *Ecological Monographs* 69:219-250.

Dayton, P. K., M. J. Tegner, P. E. Parnell, P. B. Edwards. 1992. Temporal and spatial patterns of disturbance and recovery in a kelp forest community. *Ecological Monographs* 62:421-445.

Ebeling, A. W., D. R. Laur, R. J. Rowley. 1985. Severe storm disturbances and reversal of community structure in a southern California kelp forest. *Marine Biology* 84:287-294.

Foster, M. S. 1982. The regulation of macroalgal associations in kelp forests. Pages 185-205 in L. Srivastava, editor. *Synthetic and Degradative Processes in Marine Macrophytes*. Walter de Gruyter & Co., Berlin.

Gaines, S. D., and K. D. Lafferty. 1995. Modeling the dynamics of marine species: the importance of incorporating larval dispersal. Pages 389-412 in L. R. McEdwards, editor. *Ecology of Marine Invertebrate Larvae*. CRC Press, Boca Rotan.

Graham, M. H. 1997. Factors determining the upper limit of giant kelp, *Macrocystis pyrifera* Agardh, along the Monterey Peninsula, central California, USA. *Journal of Experimental Marine Biology and Ecology* 218:127-149.

Graham, M. H. 1999. Identification of kelp zoospores from in situ plankton samples. *Marine Biology* 135:709-720.

Graham, M. H., C. Harrold, S. Lisin, K. Light, J. M. Watanabe, and M. S. Foster. 1997. Population dynamics of giant kelp *Macrocystis pyrifera* along a wave exposure gradient. *Marine Ecology Progress Series* 148:269-279.

Graham, M. H., and B. G. Mitchell. 1999. Obtaining absorption spectra from individual macroalgal spores using microphotometry. *Hydrobiologia* 398-399:231-239.

Harrold, C., J. Watanabe, S. Lisin. 1988. Spatial variation in the structure of kelp forest

communities along a wave exposure gradient. *Marine Ecology* 9:131-156.

Hoffman, A. J. 1987. The arrival of seaweed propagules at the shore: a review. *Botanica Marina* 30:151-165.

Jackson, G. A. 1997. Currents in the high drag environment of a coastal kelp stand off California. *Continental Shelf Research* 17:1913-1928.

Jackson, G. A., and C. D. Winant. 1983. Effect of a kelp forest on coastal currents. *Continental Shelf Research* 2:75-80.

Johnson, L. E., and S. H. Brawley. 1998. Dispersal and recruitment of a canopy-forming intertidal alga: The relative roles of propagule availability and post-settlement processes. *Oecologia* 117:517-526.

Ladah, L. B., J. A. Zertuche-Gonzalez, and G. Hernandez-Carmona. 1999. Giant kelp (*Macrocystis pyrifera*, Phaeophyceae) recruitment near its southern limit in Baja California after mass disappearance during ENSO 1997-1998. *Journal of Phycology* 35:1106-1112.

Lotze, H. K., and W. Schramm. 2000. Ecophysiological traits explain species dominance patterns in macroalgal blooms. *Journal of Phycology* V36:287-295.

Lotze, H. K., W. Schramm, D. Schories, B. Worm. 1999. Control of macroalgal blooms at early developmental stages: *Pilayella littoralis* versus *Enteromorpha* spp. *Oecologia* 119:46-54.

Lotze, H. K., B. Worm, U. Sommer. 2000. Propagule banks, herbivory and nutrient supply control population development and dominance patterns in macroalgal blooms. *Oikos* 89:46-58.

Lüning, K., I. tom Dieck. 1989. Environmental triggers in algal seasonality. *Botanica Marina* 32:389-397.

McPeak, R. H. 1981. Fruiting in several species of Laminariales from southern California. *Proceedings of the International Seaweed Symposium* 8:404-409.

Neushul, M. 1963. Studies on the giant kelp, *Macrocystis* II Reproduction. *Journal of Botany* 50:354-359.

- North, W. J. 1994. Review of *Macrocystis* biology. Pages 447-527 in I. Akatsuka, editor. *Biology of Economic Algae*. Academic Publishing, The Hague.
- North, W. J., D. E. James, and L. G. Jones. 1993. History of kelp beds (*Macrocystis*) in Orange and San Diego Counties, California. *Hydrobiologia* 260/261:277-283.
- Pearson, G. A., E. A. Serrao, and S. H. Brawley. 1998. Control of gamete release in fucoid algae: sensing hydrodynamic conditions via carbon acquisition. *Ecology* 79:1725-1739.
- Pfister, C. A. 1992. Costs of reproduction in an intertidal kelp: patterns of allocation and life history consequences. *Ecology* 73:1586-1596.
- Reed, D. C. 1987. Factors affecting the production of sporophylls in the giant kelp *Macrocystis pyrifera* (L) C Ag. *Journal of Experimental Marine Biology and Ecology* 113:61-69.
- Reed, D. C., T. W. Anderson, A. W. Ebeling, and M. Anghera. 1997. The role of reproductive synchrony in the colonization potential of kelp. *Ecology* 78:2443-2457.
- Reed, D. C., A. W. Ebeling, T. W. Anderson, and M. Anghera. 1996. Differential reproductive responses to fluctuating resources in two seaweeds with different reproductive strategies. *Ecology* 77:300-316.
- Roughgarden, J., S. Gaines, and H. Possingham. 1988. Recruitment dynamics in complex life cycles. *Science* 241:1460-1466.
- Santelices, B. 1990. Patterns of reproduction, dispersal and recruitment in seaweeds. *Oceanography and Marine Biology Annual Review* 28:177-276.
- Sousa, W. P. 1984. Intertidal mosaics: patch size, propagule availability, and spatially variable patterns of succession. *Ecology* 65:1918-1935.
- Tegner, M. J., and P. K. Dayton. 1987. El Niño effects on southern California kelp forest communities. *Advances in Ecological Research* 17:243-279.
- Tegner, M. J., P. K. Dayton, P. B. Edwards, K. L. Riser. 1996. Is there evidence for long-term climatic change in southern California kelp forests? *CalCOFI Reports* 37:111-126.

- Tegner, M. J., P. K. Dayton, P. B. Edwards, K. L. Riser. 1997. Large-scale, low-frequency oceanographic effects on kelp forest succession: a tale of two cohorts. *Marine Ecology Progress Series* 146:117-134.
- tom Dieck (Bartsch), I. 1991. Circannual growth rhythm and photoperiodic sorus induction in the kelp *Laminaria setchellii* (Phaeophyta). *Journal of Phycology* 27:341-350.
- Underwood, A. J., and E. J. Denley. 1984. Paradigms, explanations, and generalizations in models for the structure of intertidal communities on rocky shores. Pages 151-180 in D. R. Strong, D. S. Simberloff, L. G. Abele, and A. B. Thistle, editors. *Ecological Communities: Conceptual Issues and the Evidence*. Princeton University Press, Princeton.
- Underwood, A. J., and P. G. Fairweather. 1989. Supply-side ecology and benthic marine assemblages. *Trends in Ecology and Evolution* 4:16-20.
- Vadas, R. L., Sr., S. Johnson, and T. A. Norton. 1992. Recruitment and mortality of early post-settlement stages of benthic algae. *British Phycological Journal* 27:331-351.
- Van Patten, M. S., and C. Yarish. 1993. Allocation of blade surface to reproduction in *Laminaria longicuris* of Long Island Sound, USA. *Hydrobiologia* 260-261:173-181.
- Worm, B., H. K. Lotze, C. Bostrom, R. Engkvist, V. Labanauskas, U. Sommer. 1999. Marine diversity shift linked to interactions among grazers, nutrients and propagule banks. *Marine Ecology Progress Series*:309-314.

Chapter VI

Conclusion to the dissertation

The realization that coupling between giant kelp zoospore production and supply is dependent on physical properties of the populations themselves, broadens our view of giant kelp as a foundation species in nearshore marine communities. This species not only provides the structure and energy for one of the most productive marine ecosystems in the world (Barnes and Hughes 1988), but as it now appears may also regulate its own distribution and abundance through feedback mechanisms involved in dispersal. The flow-mediated coupling that was identified in this study will also likely affect the dispersal capabilities of other kelp forest seaweeds and animals. Giant kelp essentially creates areas of low net current displacement in coastal regions that are generally characterized by extensive along-shore flows, and therefore may significantly alter the dynamics of entire assemblages of nearshore marine organisms.

The modification of physical and biological environmental characteristics by benthic marine organisms is well known (Denny 1988, Koehl 1996). Kelps in particular have been shown to be significant habitat modifiers, with the drag of plants deforming flow (Jackson and Winant 1983, Eckman et al. 1989, Jackson 1997), plant senescence fueling the production of particulate and dissolved organic matter (Duggins et al. 1989, Duggins and Eckman 1997), and canopies shading light (Dayton 1975, Reed and Foster 1984, Kennelly 1989, Schroeter et al. 1995, Graham et al. 1997, Edwards 1998, Dayton et al. 1999). For giant kelp, research has focused primarily on the effect of canopy shading on algal recruitment and population structure (Reed and Foster 1984, Graham et al. 1997, Edwards 1998, Dayton et al. 1999). Ecologists, however, have long been aware, at least qualitatively, that giant kelp populations can also affect ocean currents (Jackson and Winant 1983,

Tegner and Dayton 1987, Jackson 1997). The large stationary plants of a kelp forest impose drag on the water and result in the dampening of both along- and across-shore flows within, and diversion of currents around, giant kelp populations (Jackson and Winant 1983). To date, application of this knowledge has been limited to studies of sediment transport and beach nourishment (Elwany and Flick 1996), larval transport of kelp forest inhabitants (Schroeter et al. 1996), and flow-mediated nutrient transport (Jackson 1997). The suggestion that flow modification regulates kelp propagule supply is unique to this study.

The high temporal and spatial variability in coastal flows within and between giant kelp forests also supports a continuous rather than dichotomous view of population connectivity. In highly connected open systems, propagule retention will be low with most propagules transported away from the site of production, whereas in poorly connected closed systems, propagule retention will be high. But these two levels of population connectivity are simply upper and lower bounds of a continuum (Figure 6-1). In fact, the two different strengths of reproductive coupling observed during this study (77% within the Point Loma kelp forest and 38% along the edges) highlight the fact that natural populations may lie *within* the continuum rather than at one extreme of connectivity or the other. What is more interesting, however, is where populations, species, or functional taxonomic groupings are positioned along this continuum, and what processes regulate whether these positions are constant or variable in space and time.

The continuum of population connectivity can be conceptualized for individual giant kelp populations based on the spatially variable reproductive coupling observed during this study, and the flow modification studies of Jackson and Winant (1983) and Jackson (1997). As stated in **Chapter IV**, sites along the outer edge of kelp forests experience greater uni-directional flows than in the center of the forests, resulting in a gradient of decreasing net current displacement towards the forest interior. This decrease in net current

displacement appears to result in increased coupling between zoospore production and supply, and thus increased zoospore retention (Figure 6-2). An important additional component of this conceptual model is the observation of Jackson and Winant (1983) and Jackson (1997) that the size of kelp forests is vital to determining the extent of flow modification. Small kelp forests will not have the capacity to dampen flows to the same extent that larger forests can. As such, a decreasing trend in net current displacement, and increasing trend in zoospore retention, should also exist as populations grow in size (Figure 6-2). That is, zoospore production and supply should not be as tightly coupled in the center of small populations as in the center of large populations.

This conceptual model immediately spawns two new questions for investigation: (1) what is the spatial scale or threshold for flow modification by giant kelp plants?; and (2) how can patterns of spatial variability in reproductive coupling be used to model demographic exchanges within and among giant kelp populations? The first question needs to be addressed by extensive empirical studies of along- and across-shore flows throughout kelp forests of different sizes. It is important to understand quantitatively the extent to which individual plants interact to modify flows over broad spatial scales. Furthermore, we need to understand how this modification varies temporally as currents fluctuate, stratification of the water column changes, and plants gain and lose biomass. The second question can not be addressed without a better understanding of these aspects of flow modification. Even then, however, the modeling of demographic exchanges will be challenging. Studies will need to step away from simply estimating the extent to which zoospore production and supply are coupled, and begin to predict the direct effects of flow modification on zoospore dispersal distances. For example, what is the probability that zoospores will be exchanged between the edge and center of giant kelp populations? If zoospores are able to escape forest interiors, where do they go? Can they penetrate the interiors of other populations? Are demographic interactions *between* populations limited

only to exchanges among individuals along the edges? Does variability in net current displacement also regulate settlement success? The answers to these questions are important as they will allow for the modeling of giant kelp in California as a meta-population. That is, they will provide the necessary information for predicting the demographic exchanges within and among discrete populations.

The conceptual model of zoospore retention versus flow modification also leads directly to certain ecological and evolutionary hypotheses. First, increased zoospore retention in forest interiors should lead to an increased ratio of local versus remote contributions to propagule supply. This hypothesis has been indirectly tested during this study, but warrants further investigation either through experimental removal of local adults or the development of techniques for unequivocally distinguishing locally from remotely produced zoospores. Second, the retention of zoospores within kelp forests should enhance zoospore settlement rates (Anderson and North 1966). This hypothesis, however, can not be tested until techniques are developed to identify settled giant kelp zoospores from those of other kelp taxa. Such development is currently under way. Third, if zoospore settlement is actually enhanced due to decreased net current displacement in forest interiors, then fertilization and recruitment success should also be enhanced; Reed et al. (1991) clearly demonstrated that a threshold in zoospore settlement existed below which kelp recruitment is not possible. Thus, recruitment should be less stable along the edges of kelp forests relative to the centers, as well as within small forests relative to larger ones. Fortunately, resources are currently available to test this hypothesis. North et al. (1993) published a time series of population sizes (based on the area of remotely sensed surface canopies) for numerous giant kelp populations in southern California. A plot of standardized temporal variability in population size versus the maximum size of each population revealed that larger giant kelp forests are in fact more temporally stable than smaller forests (Figure 6-3). Whether this observation reflects the dynamics of my conceptual model requires further

investigation. These data do, however, suggest that such questions can be feasibly tested. Finally, that zoospores should be retained near the adults that released them, suggests that self-fertilization and inbreeding will be enhanced, and gene flow dampened, in the interior of kelp populations relative to the exterior. Molecular markers necessary to test such small-scale differences in genetic similarity are currently under development (e. g., microsatellites), and may be the ultimate tool for studying the continuum of population connectivity.

The demographic analyses conducted in **Chapters IV & V** will be an important component of future meta-population models, as they will be necessary for determining the amount of zoospores produced in any given region, as well as help to predict temporal variability in population structure. Further work, however, is necessary before either of these goals can be realized. First, the demographic and reproductive model developed for predicting zoospore supply from local populations was based on observational studies. As such, it was not possible to tease apart the collinearities observed in apparent zoospore production among the different plant size classes (**Chapter IV**). That is, although only 3 size classes were found to be most important in predicting local zoospore supply, the other size classes were also clearly producing zoospores; this zoospore production was simply correlated with that of the 3 most-important size classes and considered statistically redundant. The model will therefore incorrectly predict a complete loss of zoospores from the system when these 3 most-important size classes are absent. The goal of this study, however, was simply to identify a linkage between local zoospore production and supply. Future studies that manipulate plant size and density will be vital to refining this model. Second, the transitions of plants between different size classes and reproductive conditions was found to be highly stochastic, and determined primarily by the disturbance history of individual plants (**Chapter V**). Consequently, these data do not fit well into traditional deterministic population modeling techniques (e. g., Caswell 1989). Such efforts will require longer time series of the size and reproduction of individual plants.

The results of this study do, however, in one way support the utility of deterministic modeling analyses of giant kelp population dynamics. The fact that giant kelp populations appear to be reproductively coupled at some non-trivial spatial scale again suggests that adult population structure can be used to predict much of the supply of propagules to the local population. This linkage is vital to deterministic matrix models as it is the only direct connection between adults and recruitment (Figure 6-4). Furthermore, by validating the strength of the benthic-planktonic transition in the giant kelp life history, the results of this study should facilitate subsequent investigation into planktonic-benthic and benthic-benthic transitions, and thus promote the collection of data that will ultimately close the giant kelp life history (Figure 6-4). The role of zoospore supply in regulating zoospore settlement is completely unknown, and the various post-settlement processes thought to be important to giant kelp recruitment (e. g., dormancy; inter-specific competition; grazing mortality) are only beginning to be studied (Deysher and Dean 1986, Reed 1990, Leonard 1994, Reed et al. 1997). Still, with the advances made herein, the life history of giant kelp represents the most well understood of all seaweed taxa, and I anticipate that this species will be our best chance to form a complete view of how seaweeds interact with their environment to regulate their ecology and evolution.

The results of this study will also have ramifications beyond the population-level scales to which I limited my research. Many environmental processes, spanning broad temporal and spatial scales, act to modify available giant kelp habitat, and thus giant kelp distribution and abundance. ENSO, decadal shifts in oceanographic climate, substrate availability, eustatic sea level changes, and vicariance due to plate tectonics may all constrain the size and isolation of giant kelp forests, and thus control the upper limit to population connectivity. As we better understand how reproductive coupling, and thus demographic exchanges, vary in time and space, we can begin to scale up our investigations from individual populations to meta-populations and ultimately global species distributions. We will then be able to study

the temporal and spatial scales over which populations diverge and converge, and in the end, better understand the biological and physical processes that regulate speciation and extinction. The success of my studies with giant kelp suggest that such a macroscopic view of organism ecology and evolution is obtainable. We simply need to develop and ask appropriate and rigorous questions, continue to utilize the latest technological advances to address these questions, and stop worrying about our failure to meet preconceived notions as to how nature works. All answers to the right questions lead to enlightenment!

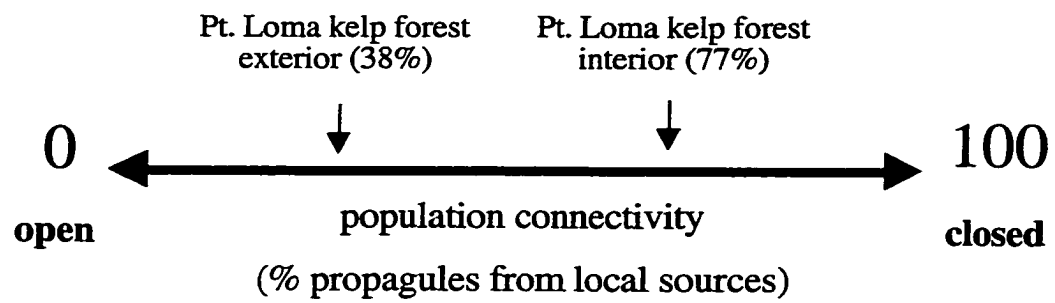


Figure 6-1. Conceptual diagram of the continuum of population connectivity.

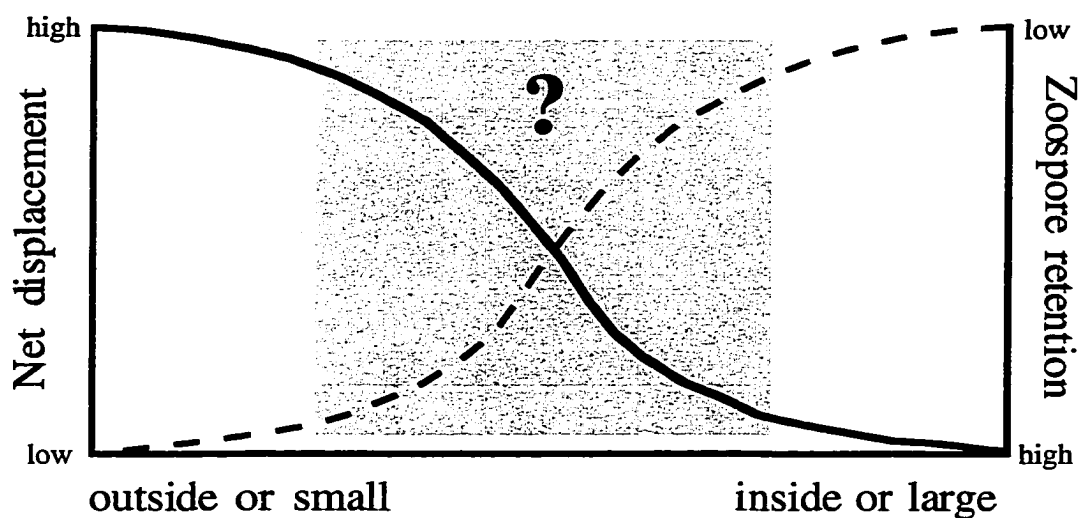


Figure 6-2. Conceptual diagram of the relationship between net current displacement, giant kelp zoospore retention, and the size of or location within giant kelp populations. Although data presented herein (**Chapter IV**) suggest that the relationship between net current displacement and zoospore retention is linear, the form of the relationship between net current displacement and population size or location is unknown (as indicated by the shading).

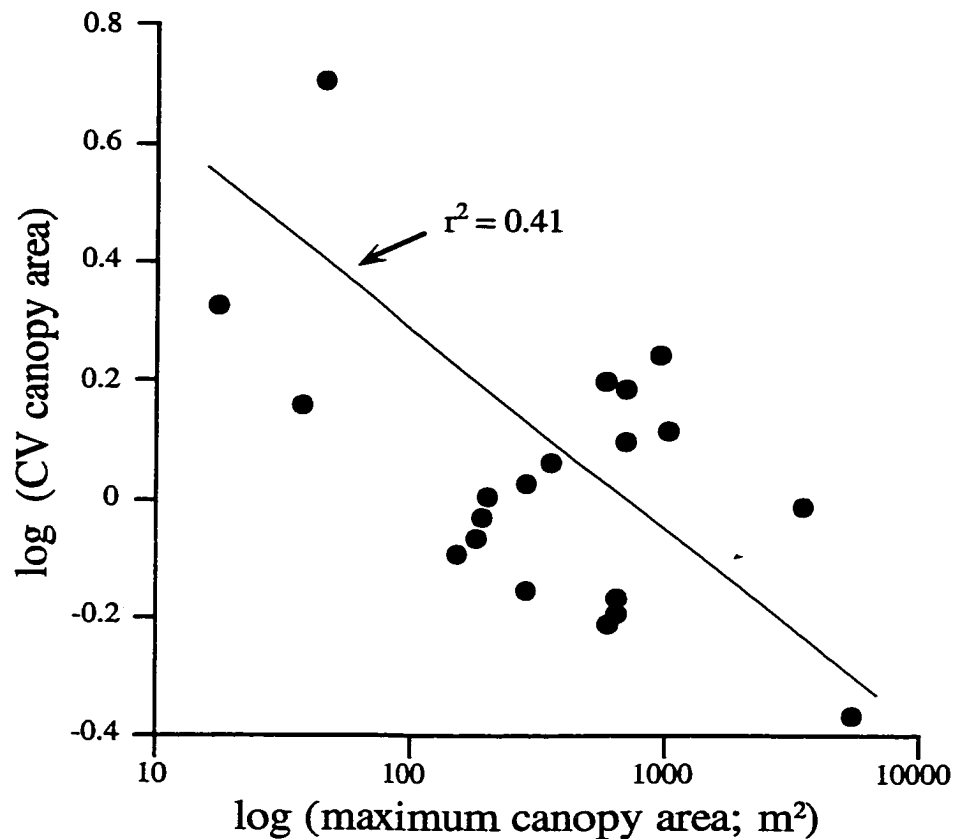


Figure 6-3. Relationship between temporal variability in population size and maximum population size for 20 southern California giant kelp forests from 1967 to 1991. Population size was estimated each year from infra-red aerial photographs of giant kelp canopies. Variability in canopy area was standardized to average canopy area for each population using coefficients of variation (CV) to allow comparisons among populations. r^2 represents the amount of temporal variability in canopy area that can be explained by maximum canopy area using linear regression ($P = 0.013$). The Point Loma kelp forest was the largest kelp forest used in this study (datum in lower right corner). Raw data were from North et al. (1993).

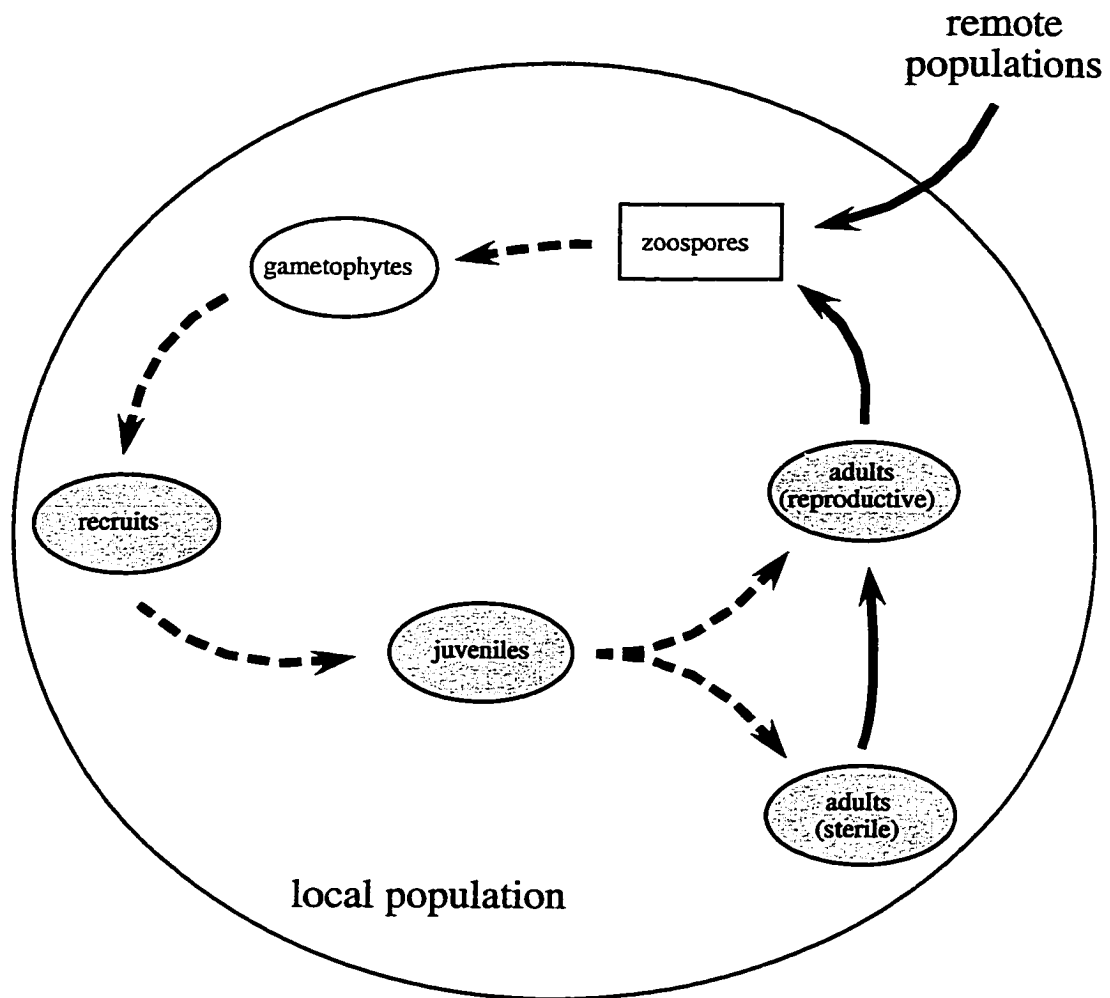


Figure 6-4. Linkages among important stages in the kelp life-history as it relates to adult population dynamics. Ovals represent benthic stages; the square represents planktonic stages; filled ovals represent sporophytes. Solid arrows represent links investigated in this study.

Literature Cited

- Anderson, E. K., W. J. North. 1966. *In situ* studies of spore production and dispersal in the giant kelp, *Macrocystis*. Proceedings of the International Seaweed Symposium 5:73-86.
- Barnes, R. S. K. and R. N. Hughes. 1988. An Introduction to Marine Ecology. Blackwell Scientific Publications, Oxford.
- Caswell, H. 1989. Matrix Population Models : Construction, Analysis, and Interpretation. Sinauer Associates, Sunderland.
- Dayton, P. K. 1975. Experimental studies of algal canopy interactions in a sea otter-dominated kelp community at Amchitka Island, Alaska. Fishery Bulletin 73:230-237.
- Dayton, P. K., M. J. Tegner, P. B. Edwards, and K. L. Riser. 1999. Temporal and spatial scales of kelp demography: the role of oceanographic climate. Ecological Monographs 69:219-250.
- Denny, M. W. 1988. Biology and the Mechanics of the Wave-Swept Environment. Princeton University Press, Princeton.
- Deysher, L. E., and T. A. Dean. 1986. In situ recruitment of sporophytes of the giant kelp, *Macrocystis pyrifera* (L) C Agardh: effects of physical factors. Journal of Experimental Marine Biology and Ecology 103:41-63.
- Duggins, D. O., and J. E. Eckman. 1997. Is kelp detritus a good food for suspension feeders? Effects of kelp species, age and secondary metabolites. Marine Biology 128:489-495.
- Duggins, D. O., C. A. Simenstad, and J. A. Estes. 1989. Magnification of secondary production by kelp detritus in coastal marine ecosystems. Science 245:170-173.
- Eckman, J. E., D. O. Duggins, and A. T. Sewell. 1989. Ecology of understory kelp environments. I. Effects of kelps on flow and particle transport near the bottom. Journal of Experimental Marine Biology and Ecology 129:173-187.
- Edwards, M. S. 1998. Effects of long-term kelp canopy exclusion on the abundance of the annual alga *Desmarestia ligulata* (light F). Journal of Experimental Marine Biology and Ecology 228:309-326.

- Elwany, M. H. S., and R. E. Flick. 1996. Relationship between kelp beds and beach width in southern California. *Journal of Waterway, Port, Coastal, and Ocean Engineering* 122:34-37.
- Graham, M. H., C. Harrold, S. Lisin, K. Light, J. M. Watanabe, and M. S. Foster. 1997. Population dynamics of giant kelp *Macrocystis pyrifera* along a wave exposure gradient. *Marine Ecology Progress Series* 148:269-279.
- Jackson, G. A. 1997. Currents in the high drag environment of a coastal kelp stand off California. *Continental Shelf Research* 17:1913-1928.
- Jackson, G. A., and C. D. Winant. 1983. Effect of a kelp forest on coastal currents. *Continental Shelf Research* 2:75-80.
- Kennelly, S. J. 1989. Effects of kelp canopies on understory species due to shade and scour. *Marine Ecology Progress Series* 50:215-224.
- Koehl, M. A. R. 1996. When does morphology matter? *Annual Review of Ecology and Systematics* 27:501-542.
- Leonard, G. H. 1994. Effect of the bat star *Asterina miniata* (Brandt) on recruitment of the giant kelp *Macrocystis pyrifera* C Agardh. *Journal of Experimental Marine Biology and Ecology* 179:81-98.
- North, W. J., D. E. James, and L. G. Jones. 1993. History of kelp beds (*Macrocystis*) in Orange and San Diego Counties, California. *Hydrobiologia* 260/261:277-283.
- Reed, D. C. 1990. The effects of variable settlement and early recruitment on patterns of kelp recruitment. *Ecology* 71:776-787.
- Reed, D. C., T. W. Anderson, A. W. Ebeling, and M. Anghera. 1997. The role of reproductive synchrony in the colonization potential of kelp. *Ecology* 78:2443-2457.
- Reed, D. C., M. Neushul, and A. W. Ebeling. 1991. Role of settlement density on gametophyte growth and reproduction in the kelps *Pterygophora californica* and *Macrocystis pyrifera* (Phaeophyceae). *Journal of Phycology* 27:361-366.

Reed, D. C., and M. S. Foster. 1984. The effects of canopy shading on algal recruitment and growth in a giant kelp forest. *Ecology* 65:937-948.

Schroeter, S. C., T. A. Dean, K. Thies, and J. D. Dixon. 1995. Effects of shading by adults on the growth of blade-stage *Macrocystis pyrifera* (Phaeophyta) during and after the 1982-1984 El Niño. *Journal of Phycology* 31:697-702.

Schroeter, S. C., J. D. Dixon, T. A. Ebert, and J. V. Rankin. 1996. Effects of kelp forests *Macrocystis pyrifera* on the larval distribution and settlement of red and purple sea urchins *Strongylocentrotus franciscanus* and *S. purpuratus*. *Marine Ecology Progress Series* 133:125-134.

Tegner, M. J., and P. K. Dayton. 1987. El Niño effects on southern California kelp forest communities. *Advances in Ecological Research* 17:243-279.