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Factors influencing the survival of giant kelp, *Macrocystis pyrifera*, gametophytes in Stillwater Cove, Carmel Bay, California and the algal food preference of the bat star, *Asterina Miniata*, in the laboratory

Dean Francis Antonio
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Antonio, Dean Francis, M.S.

San Jose State University, 1994

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OF GIANT KELP, *MACROCYSTIS PYRIFERA*, GAMETOPHYTES IN
STILLWATER COVE, CARMEL BAY, CALIFORNIA AND THE ALGAL FOOD
PREFERENCE OF THE BAT STAR, *ASTERINA MINIATA*, IN THE LABORATORY

A Thesis

Presented to

The Faculty of Moss Landing Marine Laboratories

San Jose State University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

By

Dean Francis Antonio

May, 1994

Dean Francis Antonio

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A handwritten signature in black ink, appearing to read "Michael S. Foster", written over a horizontal line.

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ABSTRACT

FACTORS INFLUENCING THE SURVIVAL OF GIANT KELP, *MACROCYSTIS PYRIFERA*, GAMETOPHYTES IN STILLWATER COVE, CARMEL BAY, CALIFORNIA AND THE ALGAL FOOD PREFERENCE OF THE BAT STAR, *ASTERINA MINIATA*, IN THE LABORATORY

by Dean F. Antonio

Manipulative field experiments revealed that densities of newly settled zoospores and developing gametophytes of *Macrocystis pyrifera* were consistently reduced at a mean rate of 30.2% per day. Megafauna were thus implicated as the major source of gametophyte mortality because mean densities of unprotected gametophytes were significantly reduced relative to caged treatments that excluded megafauna and because no significant gametophyte mortality occurred for a treatment exposed to micro-fauna, sand scour, and sedimentation relative to controls in which these factors were excluded.

A laboratory food preference study of the abundant megafaunal sea star, *Asterina miniata*, revealed a positive association with gametophytes relative to vegetative kelp blades, benthic diatoms, and a non-food control. Gametophyte densities were significantly reduced in the experimental treatments, as in the field experiments, relative to controls where the bat star was excluded. These studies suggest that *A. miniata* may influence the abundance and distribution of giant kelp in Stillwater Cove.

DEDICATION

To my father, Dr. Adolph Antonio,
who through his brilliance, inspired me to pursue
my goals in the sciences;
and to my mother, Marie,
for her love, patience,
and support.

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TABLE OF CONTENTS

ABSTRACT	<u>page</u> iii
ACKNOWLEDGEMENTS	v
LIST OF TABLES	ix
LIST OF FIGURES	x
CHAPTER 1 FACTORS INFLUENCING THE SURVIVAL OF GIANT KELP, <i>MACROCYSTIS PYRIFERA</i> , GAMETOPHYTES IN STILLWATER COVE, CARMEL BAY, CALIFORNIA	
INTRODUCTION	1
METHODS	4
Field site description	4
Experimental approach	4
Sprophyll collection and inoculation of substrates	5
Experimental configurations of the inoculated plates	6
Site preparation	7
Physical conditions	8
Substrate choice	8
Protocol for gametophyte counts	9
Count validation	9
Rinsing of plates	10
Gametophyte development	11
Specific field studies	11
Experiment 1	11
Experiment 2	12
Transport	13

TABLE OF CONTENTS (cont.)

	<u>page</u>
Timing of seeded plate quantification	14
Statistical tests	14
RESULTS	14
DISCUSSION	16
 CHAPTER 2 THE ALGAL FOOD PREFERENCE OF THE BAT STAR, <i>ASTERINA MINIATA</i> , IN THE LABORATORY	 20
INTRODUCTION	21
METHODS	23
Experimental design	23
Physical conditions	26
Statistical analysis	26
RESULTS	27
DISCUSSION	28
Food removal	29
 CONCLUSIONS AND IMPLICATIONS OF FIELD AND LABORATORY EXPERIMENTS	 33
LITERATURE CITED	36
TABLES	41
FIGURES	50

LIST OF TABLES

<u>table</u>	<u>page</u>
1. Duration of field exposure and subsequent laboratory culture time prior to gametophyte quantification.	41
2a. Treatment exposure period vs. treatment for experiment 1.	42
2b. Means and standard errors of two-way ANOVA for experiment 1 (n = 3).	42
2c. Treatment exposure time vs. treatment (2-way ANOVA) and Ryan's Q results for experiment 1 (n/s) = not significant, (*) = means significantly differ (p<0.05).	43
3a. Exposure period and treatment analysis for experiment 2.	44
3b. Means and standard errors of two-way ANOVA for experiment 2 (n = 3).	44
3c. Treatment exposure time vs. treatment type (2-way ANOVA) and Ryan's Q results for experiment 2 (n/s) = not significant, (*) = p<0.05.	45
4. The visible invertebrates in contact with seeded acrylic plate (SAP), unseeded recruitment control (RC; for experiments 1 and 2), and cage control (CC; in experiment 2) treatments.	46
5. Association of <i>Asterina miniata</i> with three algal food types and a control (no food). Three plates of each food type were offered to two randomly selected <i>A. miniata</i> in each replicate tank (n = 6). A total of 79 observations were made for each tank over a seven day period.	47
6a. Two-level nested ANOVA's for tank effects and differences between control and experimental treatments. <i>M. pyrifera</i> gametophyte densities, diatom densities, and wet weights of <i>M. pyrifera</i> blade pieces were tested.	48
6b. Means and standard errors for the laboratory experiment.	49

LIST OF FIGURES

<u>figure</u>	<u>page</u>
1. Life history and morphology of <i>Macrocystis pyrifera</i>	50
2. Treatment configurations attached to the field site for experiments 1 and 2 (n = 3 for each treatment).	51
3. Treatments used in field experiment 2. Seeded acrylic plates (SAP's) had defined populations of giant kelp gametophytes. Other treatments included lab controls (remained in the lab) and handling controls. Exclusion cage mesh is 9.0 mm.	52
4. Gametophyte densities with increasing field exposure time for the no cage treatment of experiments 1 and 2. Error bars are standard errors of the mean.	53
5. Interaction in experiment 1. Mean gametophyte densities from two exposure periods (in hours) are plotted for three treatments. Interaction is between lab and handling control (p = 0.003).	54
6. Gametophyte density in 0.159 mm ² microscopic fields for four field treatments, three controls, and two exposure periods (n = 3). A ">" next to the treatment abbreviation indicates a significant difference (p < 0.05). Error bars are standard errors of the mean.	55
7. Design of the laboratory experiment.	56
8a. Gametophyte density for control (<i>Asterina miniata</i> excluded) and experimental (with <i>A. miniata</i> present) treatments in the lab for tanks "A" through "F". Error bars are standard errors of the mean (n = 3 for control and experimental treatments).	57

LIST OF FIGURES (cont.)

<u>figure</u>	<u>page</u>
8b. Benthic diatom density for control (<i>Asterina miniata</i> excluded) and experimental (<i>A. miniata</i> present) treatments in the lab for tanks "A" through "F". Error bars are standard errors of the mean (n = 3 for control and experimental treatments.	58
8c. Absolute weight difference of <i>Macrocystis pyrifera</i> (kelp) pieces for control (<i>Asterina miniata</i> excluded) and experimental (<i>A. miniata</i> present) treatments in the lab for tanks "A" through "F". Error bars are standard errors of the mean (n = 3 for control and experimental treatments).	59

Chapter 1

FACTORS INFLUENCING THE SURVIVAL
OF GIANT KELP, *MACROCYSTIS PYRIFERA*, GAMETOPHYTES IN
STILLWATER COVE, CARMEL BAY, CALIFORNIA

Chapter 1

FACTORS INFLUENCING THE SURVIVAL OF GIANT KELP,
MACROCYSTIS PYRIFERA, GAMETOPHYTES IN STILLWATER COVE,
CARMEL BAY, CALIFORNIA

INTRODUCTION

Many factors can affect the survival of young, germling plants. Enhancing survivorship of young terrestrial plants is an on-going concern in agriculture and forestry and has resulted in a vast number of studies. As in terrestrial plants, some macroalgae are commercially farmed and harvested. In contrast to terrestrial crops, however, relatively few studies of marine macroalgae have investigated the sources of sporling mortality (Deysher and Dean, 1986; Reed, 1989; Reed 1990b; Reed et al., 1991). These studies have focused on abiotic factors, dispersal, competition, and settlement density yet no published studies have investigated the effects of grazing or abrasion.

Giant kelp, *Macrocystis pyrifera*, is a marine macroalga whose natural populations are commercially harvested. It is a perennial brown alga that forms a surface canopy along the west coast of North and South America, southern Australia, and southern Africa (Abbot and Hollenberg, 1976). Along the west coast of North America, giant kelp has been reported from Bahia Magdalena, Baja, CA to Santa Cruz, CA (Foster and Schiel, 1985). As in other kelps (Order Laminariales), its life cycle consists of an alternation of generations that includes the growth and reproduction of microscopic gametophytes (North, 1971)(Fig. 1). Sporophylls, located just above the holdfast in adult sporophytes, release free-swimming zoospores that disperse and settle on a substrate. Upon attachment to the substrate or after three days of suspension (Reed, pers. comm.), the zoospores may develop into gametophytes.

Survival of gametophytes, their successful maturation and fertilization, and growth to the conspicuous sporophyte stage is dependent on many factors. In terrestrial, freshwater, and marine ecosystems, biological and physical factors can influence both local and large-scale distributions of organisms. Distributions of *M. pyrifera* along the central and southern California coast are also regulated by many interacting factors. Biotic and abiotic factors influencing adult sporophytic plants are well understood (North, 1971; Rosenthal et al., 1974; Foster, 1975a, 1975b; Dean and Jacobsen, 1984; Reed and Foster, 1984; Foster and Schiel, 1985; Harrold and Reed, 1985), and we know that the survival of the inconspicuous gametophyte stage is dependent on specific physical conditions of temperature, irradiance, light quality and nutrients (Luning and Neushul, 1978; Luning, 1980; Kuwbara and North, 1980; Deysher and Dean, 1986). If plants survive and grow, they may become more tolerant to both abiotic factors (light, temperature and nutrients) and biotic factors (grazing).

Although *M. pyrifera* is fertile year round and may release spores at a rate of 500,000 spores/hr (Anderson and North, 1966), successful completion of the gametophyte stage is highly variable. The highest risk to kelp plants occurs in their microscopic stages (Chapman, 1984; Reed, 1989; Reed, 1990a; Reed et al. 1991), and recruitment of giant kelp is difficult to predict due to the multitude of factors influencing their survival. Reed (1990b) suggests that much of the temporal variation in sporophyte recruitment can be caused by variation in abiotic conditions, interspecific competition, and/or the abundance of other competitors. In contrast to the long-lived terrestrial plant seeds which may germinate after the passage of much time, and a variety of conditions, *M. pyrifera* gametophytes only live a few weeks (Reed, 1990b) and require a favorable combination of biotic and abiotic conditions that must occur over a short time period.

Biological factors such as competition, grazing, digestion, and abrasion may also effect gametophyte survival. Competition among other species of kelp may influence gametophyte reproduction and recruitment (Reed et al., 1991). There are over 100 known species of gastropods that have the radula morphology capable of removing the small (~7 to ~50 μm) gametophytes from benthic substrates (Steneck and Watling, 1982). Echinoids (e.g., sea urchins), asteroids (e.g., sea stars), small crustaceans, and fish browsing may also cause mortality by digesting or abrading gametophytes (Dean et al., 1984; Harris et al., 1984). However, little is known about the rate of *in situ* gametophyte mortality and no analysis of herbivore / kelp germling associations has been investigated.

Due to the difficulty in evaluating recruitment success and assessing the survivorship of microscopic plants, other studies have largely ignored the mortality of these early stages and instead have focused on the visible appearance of small plants (Chapman, 1984; Dayton et al., 1984; Reed and Foster, 1984; Lubchenco, 1986). Other grazing studies where microscopic plants are consumed or removed may be relevant to the survivorship of kelp gametophytes. Vadas et al. (1982) noted almost 100% mortality of microscopic germlings of *Ascophyllum nodosum*, but found some plants grew to visible sizes when grazers were experimentally excluded. Size specific grazer exclusion may be an important consideration since organisms that could affect the microscopic gametophyte stages of giant kelp range from 5 μm (e.g., ciliates)(Reed, pers. comm.) to 33 cm (e.g., the gumboot chiton, *Cryptochiton stelleri*). The influence of organisms grazing and abrading benthic substrates where kelps grow and other factors that can cause *in situ* gametophyte mortality is not well understood.

The objectives of this study were to determine: (1) the rate and extent of gametophyte mortality; (2) if physical factors (ie., sedimentation, light, or water motion), biological factors (ie., grazing and/or abrading by organisms), or both are causing the

mortality during this period; and (3) the general size of the grazers causing mortality (ie., are fauna greater or smaller than 9 mm diameter).

METHODS

Field site description

Field experiments were done at a subtidal site within the kelp forest at Stillwater Cove, Carmel Bay, California (36° 34' N, 121° 56' W) and performed on May 5 - 11 (experiment 1) and November 27 - December 4, 1990 (experiment 2). Detailed descriptions of Stillwater Cove are given in Reed and Foster (1984), Foster and Schiel (1985), Schiel (1985), and Konar (1991). A single study site was chosen inside the cove approximately 100 m southwest of Pescadero rocks. It was located at a depth of 10 m on a single conglomerate rock surface (5 m wide and 2 m tall) facing east at an angle of 55°. The rock was next to a channel consisting of sand and sandstone boulders and flanked by a conglomerate pinnacle (along the northwest side) rising to within 3 m of the surface. Surface canopy in the cove is dominated by *M. pyrifera* with a sub-surface canopy consisting of *Pterygophora californica*, and an understory dominated by geniculate coralline algae (Reed and Foster, 1984). The cove is protected from both northwesterly winds (characteristic of Spring conditions) and northerly swells associated with winter storms (Reed and Foster, 1984). This site was chosen based on the presence of *M. pyrifera*, available rock surface area on which replicate experimental manipulations could be performed, a rock surface with algae and other organisms similar to other sites in the cove (Dearn, 1987), and a relatively shallow depth for sufficient light and to facilitate repetitive research diving.

Experimental approach

Artificial substrates were the primary experimental investigative tool for the study. Substrates were inoculated with *M. pyrifera* zoospores in the laboratory and "outplanted"

(ie., transplanted or attached) to the field site. Inoculated substrates provided a defined population of gametophytes upon which survival could be quantified. The initial experimental approach was to attach these defined populations of gametophytes to the field site without protection and harvest them at various times to measure the rate and duration of gametophyte mortality. A similar experiment was subsequently done at the same site (experiment 2) where plates seeded with gametophytes were shielded in several ways to differentiate the effects of various organisms and physical factors on gametophyte mortality.

The incidence of organisms directly in contact with the plates was documented to determine if particular species showed an affinity for the seeded or un-seeded acrylic plates.

Sporophyll collection and inoculation of substrates

Laboratory work was performed at Moss Landing Marine Laboratories, Hopkins Marine Station, and at the Marine Resource Laboratory at Granite Canyon. Locations varied due to the October 17, 1989 Loma Prieta earthquake and other logistical constraints. Lab studies were performed between February of 1988 and October 1991.

Giant kelp reproductive blades (sporophylls) were selected from three or more adult plants as the source of zoospores for the experiments. Sporophylls were collected, placed in a dark canvas bag, and immediately returned to the lab in a dark ice chest. Sporophylls were induced to release zoospores for inoculation or "seeding" of substrates using a combination of methods described in other studies (Neushul, 1963; North, 1974; Reed, 1989; Darrow, pers. comm.). Sporophylls were cleaned twice with a soft bristle brush in sterile, filtered seawater, and briefly rinsed in deionized water to remove epiphytes, microorganisms, and bacteria. Sporophylls were then blotted dry and placed between damp paper towels in a glass dish and chilled for one hour at 3-6°C. To induce release, sporophylls were partially submerged in sterile seawater keeping the broken ends dry to reduce mucilage fouling. With occasional swirling of the solution for 30 minutes at $15 \pm 1^\circ\text{C}$, the zoospores were gradually released

until the solution became slightly tan in color. The sporophylls were then carefully removed and density of the resulting spore solution or "inoculum" was haphazardly sampled four times with a pipette, quantified using a hemacytometer, and diluted to 1×10^4 cells ml^{-1} . This cell density was chosen because it has been shown to permit optimal sporophyte recruitment potential and allows up to 100% of female gametophytes to become mature (Reed, 1990a; Reed et al., 1991). Sterilized, sanded, acrylic plates (7.5 x 7.5 x 0.25 cm) were initially labeled and randomly distributed among two, 40 x 60 cm clean acrylic culture trays. Nine plates were uniformly spaced throughout each culture tray for subsequent verification of even settlement. Inoculum was then poured into the culture trays containing the replicate, acrylic plates until the plates were covered with 1 cm of solution. This inoculation procedure marked the beginning of the experiment. Twenty to 24 hours after inoculation, the plates were temporarily moved to a holding tray containing sterile seawater of the same temperature. The inoculum was discarded, trays and lids were washed and rinsed three times in D.I., and plates were replaced in the clean trays containing a nutrient solution.

Settlement densities of zoospores did not differ between culture trays ($p > 0.50$; $n = 9$; t-test: See "Protocol for gametophyte counts" below for the sampling method). Mean settlement densities of gametophytes (per 0.159 mm^2) were 7.4 ± 0.15 and 10.6 ± 0.09 S.E. for experiments 1 and 2, respectively.

The "recruitment control" consisted of un-seeded plates which were handled the same as the seeded plates. The recruitment control and the seeded plates (in various configurations) were outplanted to the study site in Stillwater Cove 24 - 36 hours after inoculation.

Experimental configurations of the inoculated plates

In experiment 1, only the recruitment control and the unprotected "no cage" treatment were used. In experiment 2, four configurations of seeded plates were used: (1) the "field turtle" which consisted of a seeded plate inside a covered Petri dish (this configuration was

attached to a turtle-shaped piece of acrylic which facilitated attachment to the field site); (2) the "exclusion cage" which consisted of a seeded plate inside a Vexar™ cage; (3) the "cage control" which consisted of an exclusion cage without sides housing a seeded plate; and (4) the "no cage" which was simply a seeded plate without cage protection (Fig. 2, and see experiment 2 description at the end of this methods section).

Site preparation

Thirty replicate areas (15 x 15 cm) were randomly selected and cleared one month prior to experiments 1 and 2. Sessile organisms were removed using a hammer and chisel. Cleared areas were between 1 and 3 meters from the sand and cobble bottom on the exposed rock surface. A pneumatic drill was used to make a hole in the center of each cleared area in which a plastic screw anchor was inserted for subsequent plate and cage attachment. Plates were attached to the rock (as were the cages if applicable) using stainless steel, hex head sheet metal screws and vinyl washers.

Artificial enhancement of habitat for small invertebrates was of initial concern during the field studies. However, pilot observations indicated that organisms did not appear to utilize the space between the rock and plates.

Algal abrasion and light limitation are potential sources of gametophyte mortality (Sousa et al., 1981; Reed and Foster, 1984). Therefore, algae adjacent to each cleared area were pruned back to prevent abrasion of plates during surge. Sporophylls of *Pterygophora californica* and *Macrocystis pyrifera* were removed within 10 m of the study site in order to reduce natural recruitment. Three weeks prior to experiment 1, the canopies of *M. pyrifera* and *Cystoseira osmundacea* were removed within 20 m of the study site and maintained throughout both experiments to help reduce light attenuation and to enhance gametophyte growth.

Physical conditions

Control plates kept in the lab were maintained in a still-culture nutrient solution consisting of $10 \mu\text{M PO}_4^{4-}$, $100 \mu\text{M NO}_3^-$ and $\frac{1}{2}$ trace metals in sterile seawater (Guillard and Ryther, 1962). Plates were still cultured at $13 \pm 1.5^\circ\text{C}$ with a 12/12 light / dark exposure and cool white fluorescent irradiance of $(50 \text{ to } 60 \mu\text{E m}^{-2} \text{ sec}^{-1})$ for experiment 1 and both fluorescent and incandescent irradiance $(30 \text{ to } 38 \mu\text{E m}^{-2} \text{ sec}^{-1})$ for experiment 2. Controlled incandescent irradiance only became available after experiment 1.

A surge measuring device was fabricated for the study and consisted of an epoxy reinforced plastic ball attached to a protractor by a string (See Foster et al., 1985). Surge at the field site was measured each dive day and ranged from 0 to ± 10 degrees. Field temperatures were measured with a max/min thermometer attached at the study site. This thermometer was calibrated with a VWR Scientific™ digital thermometer. Temperatures were logged and zeroed each dive day during the experimental exposure period and ranged from 11.1 to 13.3°C . Photosynthetically active radiation (PAR) was measured on May 7 and December 1, 1990 using a LI-COR™ 185-A equipped with a cosine quantum sensor (LI-190S). PAR was measured between 1130 and 1200 hours and ranged from 45.5 to $102.5 \mu\text{E m}^{-2} \text{ sec}^{-1}$ at the plate attachment site and from 37.6 to $55.4 \mu\text{E m}^{-2} \text{ sec}^{-1}$ inside a field cage.

Substrate choice

Transparent substrates were used to facilitate quantification with a compound microscope. Acrylic plates sanded with 80 grit paper were used as the preferred substrate versus frosted glass slides because the surface was relatively more heterogeneous, the acrylic plates could be drilled to facilitate attachment, and plates could be cut to any size and were relatively unbreakable. These seeded plates were used throughout the study.

Toxicity of freshly sanded acrylic plates was of initial concern. A preliminary experiment compared densities of gametophytes between freshly sanded plates and plates

sanded and soaked for one month in sterile, filtered seawater. There was no difference in gametophyte densities between these two treatments ($p > 0.50$; $n = 5$; t-test; See "Protocol for gametophyte counts" below for the sampling method).

Protocol for gametophyte counts

Gametophyte densities were quantified by scanning 10 uniformly spaced, microscopic fields of view at 400x (area of field = 0.159 mm^2). A mean gametophyte density was calculated for each plate based on this sub-sampling technique ($n = 10$ for each plate). Count consistency was maintained by counting only the pigmented, identifiable gametophytes within the microscopic field of view, counting only two gametophytes within a clump since overlapping gametophytes were difficult to differentiate, continuously adjusting the focus to cover the entire depth of field (ie., so gametophytes in micro-crevices would not be overlooked), using a specific path to consistently count the gametophytes within the field area, and logging the counts with a multi-toggle mechanical counter.

Count validation

Validation of gametophyte counts was performed throughout the study to maintain quality control. Two out of the 10 microscopic fields of view were randomly chosen and recounted after one minute. Number of gametophytes per field was logged on the mechanical counter and viewed only after the recount was complete. Several hundred newly settled zoospores and gametophytes were counted during a training period prior to the experiments. Counting accuracy for both preliminary and field experiments was 97.5% ($n = 244$ fields of view which includes counts done after field exposure). Percent accuracy of the gametophyte counts was derived by subtracting the total difference between the repeated counts from the original counts and dividing by the original count. There was no significant difference in gametophyte counts attributed to recounting inoculated plates

($p > 0.50$; $n = 5$; t-test). There was, however, a significantly higher variance associated with the recount ($p < 0.001$; F-test).

Gametophytes quantified within six days of inoculation were slightly lower (based on previous unpublished experiments and experiment 1) relative to counts made thereafter. The counting protocol did not permit counts of gametophytes that were un-pigmented and not apparently developing/growing. Therefore, gametophytes were not counted until six days after inoculation for experiment 2 in order to maintain counting consistency.

A second validation method was attempted by re-sampling seeded plates using an epifluorescent microscope at 200x. Gametophyte densities were significantly lower on the re-count apparently due to desiccation during quantification (ie., means = 6.26 gametophytes $0.159 \text{ mm}^{-2} \pm 0.28 \text{ S.E.}$ and 3.67 gametophytes $0.159 \text{ mm}^{-2} \pm 0.57 \text{ S.E.}$ for the count and recount, respectively; $p < 0.005$; t-test). Therefore, re-sampling seeded plates using the epifluorescent microscope was discontinued.

Localized desiccation of gametophytes was apparent during counts, resulting in higher variability during recounts. Therefore, all seeded plates were sacrificed after counting.

Rinsing of plates

Microorganisms (e.g., ciliates and motile algal cells) and debris collected on the field plates can contaminate the surface of seeded plates (Reed, pers. comm.). All plates were rinsed upon return to the lab to both remove debris and avoid contamination among seeded plates. Pilot studies indicated that microorganisms were effectively removed from outplanted seeded plates when rinsed with sterile, filtered seawater (ie., removed 96.9% of the organisms $\pm 3.16 \text{ S.E.}$; $p < 0.001$; $n = 10$; Mann-Whitney U Test; Zar, 1984). Effective removal was quantified by sampling ten fields of view for microorganisms and re-sampling after rinsing. In addition, numbers of gametophytes on rinsed and un-rinsed

seeded plates were counted to determine if this technique also removed gametophytes. There was no significant reduction in mean gametophyte densities due to this rinsing procedure ($p > 0.50$; $n = 6$; paired t-test). Therefore, all plates from all treatments were rinsed with sterile, filtered seawater upon return to the lab, and lab controls were also rinsed after the first field harvest. Microorganisms were not apparent on the plates or in the rinse water of seeded plates and were not quantified because the cage treatment (which allowed microorganisms to penetrate) had no significant gametophyte mortality.

Gametophyte development

Gametophyte morphology was monitored among treatments. Viable gametophytes became more pigmented and began to appear sexually dimorphic (ie., with smaller, more stringy males) after six days of field and/or lab culture. This is consistent with other researchers (Anderson, pers. comm.; Dean pers. comm.; Reed pers. comm.). The characteristic "asymmetrical dumbbell shape" of gametophytes, the relatively short field exposure, and the effective rinsing method of the seeded plates made fouling material rare and gametophytes readily distinguishable.

Specific field studies

Experiment 1

Experiment 1 was designed to measure the rate and duration of gametophyte mortality. The time of field harvesting was selected (based on field observations and quantification of gametophytes after the first harvest) so as to encompass that of highest gametophyte mortality (see below for determination of harvest time). There were three controls and one treatment: (1) lab control (seeded plates that remained in the lab); (2) handling control (seeded plates transported to the field, attached, removed, and returned to the lab during each plate attachment and harvest period - controlling for handling effects); (3) the "no cage" treatment (seeded plates which were attached to the field site); and (4)

recruitment control (un-seeded plate which controlled for natural recruitment of kelp zoospores). All controls and the treatments had equal numbers of replicates randomly attached among the 30 prepared areas. Field treatments were selected for harvest after 38 (n = 3) and 62 hours (n = 3) of field exposure. There was substantial gametophyte mortality within the first 38 hours of field exposure, consequently, the rest of the plates were field collected the following night totaling 62 hours of field exposure. The null hypotheses were: (1) there were no differences in gametophyte densities among 1, 2, and 3 above and (2) there were no differences in gametophyte survival between harvest periods.

Experiment 2

The objective of experiment 2 was to experimentally determine (using exclusion cages) which sources of mortality were reducing gametophyte densities in experiment 1 and observe associations of organisms (as in experiment 1). Four configurations of seeded plates and an unseeded recruitment control were attached to the subtidal site: (1) plates enclosed in Petri dishes "field turtles" were designed to exclude abrasion, sedimentation, and micro- and megafauna (the definitions of microfauna and megafauna for this study are mobile organisms less than 9 mm and greater than 9 mm diameter, respectively); (2) plates inside cages (exclusion cages) of 9 mm mesh designed to exclude megafauna; (3) cage controls (exclusion cages without sides) to test for artifacts associated with the cage; (4) unprotected or "no cage" plates (whose gametophytes may be subjected to all sources of mortality). Two handling controls, one for each of the two treatments ("field turtle" and "no cage"), provided a measure of gametophyte mortality due to transportation and manipulation of seeded plates in the field. An unseeded recruitment control provided a measure of natural recruitment of kelp zoospores and other potentially fouling plants (see Fig. 3 for the potential factor effects for each treatment). Field treatments were randomly attached among the 30 prepared areas. Due to the results of experiment 1 (indicating a high

rate of gametophyte mortality within 38 hours of field exposure), treatments were harvested after only 19 hours of field exposure to measure mortality during this shorter exposure period. The second harvest was done two days later totaling 69 hours of field exposure. Three replicates of each treatment were used.

The null hypotheses were: (1) there was no difference in gametophyte densities among 1 through 4 above and (2) there was no difference in gametophyte survival between harvest periods.

For both experiments 1 and 2, visible organisms associated with the no cage, cage control, and recruitment control (unseeded) treatments were noted 30 minutes after the plates were outplanted and at the onset of each dive to the site. Unknown species were collected for later identification.

Transport

All treatments other than the lab controls were transported to the field inside plastic containers (one for each treatment) in a dark ice chest filled with sterile seawater. Each plastic container contained sterile, enriched seawater in which all plates and field turtles were submerged and secured. Seeded plates were separated by vinyl washers to prevent abrasion. Water temperature was monitored every 30 minutes and slowly adjusted to ambient seawater temperature (upon outplant) or incubator temperature (upon return to the lab). Methods used to transport seeded plates were effective since no apparent gametophyte mortality could be attributed to handling effects (see results).

Treatments were harvested by placing each plate inside a Ziplock™ baggie. The baggies were carefully secured inside marked plastic containers, placed inside canvas goodie bags, and secured inside a dark ice chest upon surfacing.

Timing of seeded plate quantification

In experiment 1, all treatments were quantified three and seven days after the initiation of settlement for the 38 and 62 hour exposure periods, respectively. In experiment 2, all treatments were quantified seven days after the initiation of zoospore settlement (Table 1).

Low natural recruitment densities were measured (see results). A slight underestimate of natural recruitment may have resulted for both field experiments due to the relatively shorter culture time of the recruitment control treatment (ie., cultured for less than seven days after return from the field). Removal of kelp sori near the study site likely lowered the rate of natural recruitment.

Statistical tests

All data were tested for normality and homogeneity by plotting and using Cochran's Test for Homogeneity (Winer, 1971). Data from experiments 1 and 2 were tested using two-factor (treatment type, time), Model 1 ANOVAs and Ryan's Q for planned multiple comparisons (Day and Quinn, 1989).

RESULTS

A rapid rate of gametophyte mortality was apparent for the no cage treatment in experiments 1 and 2. Rate of gametophyte mortality was estimated at 3.71 ± 0.21 S.E. and 9.26 ± 1.33 S.E. gametophytes $0.159 \text{ mm}^{-2} \text{ day}^{-1}$ or a 50.1% and 81.7% daily reduction based on the first harvest periods of experiments 1 and 2, respectively. Overall gametophyte mortality for both experiments was 2.83 ± 0.22 S.E. gametophytes $0.159 \text{ mm}^{-2} \text{ day}^{-1}$ or a 30.2% daily reduction. This reduction in gametophyte density with increasing exposure time appeared consistent between experiments 1 and 2 (Fig. 4).

Gametophyte densities were markedly reduced in the no cage relative to controls during the 38 and 62 hour exposure periods in experiment 1. Results of the 2-way ANOVA for experiment 1 show there was a significant treatment effect but with a significant interaction (Tables 2a and 2b). A plot of these data indicated that the interaction occurred between the controls. A slight increase in gametophyte density for $t = 38$ hours relative to a decrease in gametophyte density for the $t = 62$ hours, for the lab and handling controls, suggests that the interaction was isolated between these two controls and, therefore, had minimal influence on the "no cage" / field treatment (Fig. 5; Underwood, 1981). Statistical evaluation of the no cage treatment relative to the controls was, therefore, justified and determined using pairwise multiple comparisons.

Mean gametophyte density in the "no cage" treatment was significantly less than that of the handling and lab controls in experiment 1 ($p < 0.05$) and showed significant reductions in gametophyte densities with time ($p < 0.05$; Tables 2b and 2c). The lab control for the 38 hour exposure period did not differ significantly from handling controls for both exposure periods ($p < 0.05$). The lab control had significantly higher mean gametophyte densities for the 62 hour exposure period compared to the 38 hour exposure period (ie., when quantified 6 days after inoculation, developing gametophytes became more pigmented and therefore more obvious; $p < 0.05$; Table 2c).

There was a significant time ($p = 0.0016$) and treatment ($p = 0.0004$) effect in experiment 2 with no interaction ($p = 0.9172$) (Tables 3a and 3b). Gametophyte mortality increased with decreasing cage protection and with increasing exposure duration (Fig. 6). There was a significant difference among treatments and between exposure periods (Table 3a). Multiple comparison tests indicated that there was significant gametophyte mortality for the no cage relative to the field turtle and the exclusion cage configurations for both harvest periods (Table 3c). The field turtle configuration offered the most gametophyte

protection from abrasion, grazing, sedimentation and sand scour and served as a reference treatment relative to the other cage configurations (Fig. 3). Gametophytes were significantly reduced in the no cage relative to both the exclusion cage and field turtle for both exposure periods ($p < 0.05$; Table 3c). Gametophyte densities were reduced with longer periods of exposure in both the no cage and cage control configurations (Fig. 6). Mean densities of gametophytes in the no cage and cage control treatments remained similar (Fig. 6). Mean densities for the cage control treatments were only slightly greater than the no cage treatment, therefore, a cage artifact was not detected (Table 3c).

Gametophyte densities did not significantly differ among lab, handling, and turtle controls for both exposure periods ($p < 0.05$; Fig. 6). Natural recruitment (ie., attachment of newly settled zoospores to the field site) was relatively low during the study ($\bar{x} = 0.13 \pm 0.04$ S.E. and $\bar{x} = 0.36 \pm 0.05$ S.E. per 0.159 mm^2 for experiments 1 and 2, respectively).

During the limited period of *in situ* plate observation, there was evidence that large organisms were attracted to seeded plates (Table 4). Although not statistically tested, association of organisms with unseeded recruitment controls was low relative to seeded plates for experiments 1 and 2 (Table 4). Organisms smaller than 9 mm and large enough to see without the aid of a magnifying device were not associated with any of the field treatments.

DISCUSSION

A high rate of gametophyte removal was apparent during the first 38 and 19 hours of field exposure for experiments 1 and 2, respectively (Fig. 4). A higher mortality rate during the initial 19 hours of field exposure (relative to the 38 hour exposure of experiment 1) suggests that most of the mortality occurs within the first day of outplant (Fig. 4). The

relatively higher initial density in experiment 2 may have facilitated a more efficient gametophyte removal (e.g., 81.7% daily gametophyte mortality based on the first harvest period). Gametophyte mortality continued until the remaining plates were harvested (after 62 hours of exposure for experiment 1) with only 7.6% of the original gametophyte density remaining (relative to the lab control). The similar rate of gametophyte removal between experiments 1 and 2 for the no cage treatment indicate that the factor(s) affecting the gametophytes were consistent (Fig. 4).

Significant gametophyte mortality in the no cage treatment of experiment 1 may be attributed to a multitude of biotic and abiotic factors acting over the relatively short experimental duration (Table 2c). Water motion, sedimentation, sand scour, and abrasion by drift algae, among other factors, may have contributed to the overall mortality for both time periods sampled. Although physical conditions of the study site could be considered calm and conducive for gametophyte survival and growth (i.e., low surge, low temperature and adequate irradiance), reductions in gametophyte density may be a consequence of grazing by a combination of macro- and microorganisms as well as biological and mechanical abrasion.

The results of experiment 2 suggest that large organisms (i.e., > 9.0 mm in diameter) negatively affect gametophyte survival. Enhanced gametophyte survival inside the exclusion cages indicate that certain factors possibly causing mortality were effectively reduced (Fig. 6). Slight differences in gametophyte mortality inside the exclusion cages relative to the well protected field turtles indicate that organisms, sedimentation, and debris smaller than 9 mm diameter were not significant sources of mortality during the exposure periods (Fig. 6). Microorganisms and sedimentation were, therefore, not quantified due to their lack of impact on gametophyte survival. One may speculate that non-visible organisms may have had an effect given a longer outplant duration, different habitat, and/or

temporal differences in their abundance, but these hypotheses should be experimentally investigated in future studies. Mortality in the no cage treatment relative to the exclusion cage suggests that fauna larger than 9 mm diameter were responsible for the observed mortality. Observed reductions of gametophyte densities over time in the no cage treatment also suggest that additional grazing and/or abrasion occurred between the the first and second harvests for both experiments.

Reductions in gametophyte densities can prevent sporophyte recruitment since kelps lack the ability to reproduce asexually and gametophyte proximity is necessary for fertilization (Reed, 1990; Reed et al., 1991). Although densities of gametophytes can be significantly reduced, microhabitats (e.g., crevices on rock surfaces) can serve as a gametophyte spatial refuge providing sufficient proximity for successful fertilization (ie., a minimum proximity of 1 mm; Reed, 1990).

Studies have shown that intraspecific competition can be a source of mortality in giant kelp (Reed and Foster, 1984; Dean et al., 1989). Given the high densities of gametophytes on seeded plates and the proximity required for successful fertilization, grazing and abrading megafauna may be reducing the abundance of giant kelp gametophytes and juvenile sporophytes that may otherwise be lost due to other processes including self shading (Reed and Foster, 1984).

It is unlikely that the current rate of grazing pressure can solely destroy an adult stand of giant kelp due to the plants' high rate of spore production and the spatial refuge associated with rocky bottom central California kelp forests. Grazing pressure by megafauna coupled with the lack of the required physical conditions (Deysher and Dean, 1986) may alternately result in reductions in population density or even loss of an entire stand of giant kelp. Reestablishing a kelp forest using current mariculture techniques (Barilotti, pers. comm) may be futile if grazing megafauna are sufficiently abundant.

There are many organisms greater than 9 mm diameter that may have caused reductions in gametophyte densities. Adult *Tegula* spp. are abundant on the vegetative tissue of kelps but are relatively uncommon amongst the coralline mats and subtidal benthic surfaces (Riedman et al., 1981; Dearn, 1987). Other large mollusks may be candidates for gametophyte removal but no individual species appeared to be abundant. Sea urchins, *Strongylocentrotus purpuratus* were apparent but some were trapped inside rock cavities at the study site perhaps limiting their impact on algal recruits. The bat star, *Asterina miniata*, was a conspicuous and abundant organism on the study site and in Stillwater Cove.

Chapter 2

THE ALGAL FOOD PREFERENCE OF THE BAT STAR,
ASTERINA MINIATA, IN THE LABORATORY

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INTRODUCTION

The Spinulosid sea star, *Asterina miniata* (Brandt, 1835), is a common member of the kelp forest community in central California. It is found on boulder, reef or sand substrates and is often associated with drift algae (Gerard, 1976; Schroeter, et al., 1983; Foster and Schiel, 1985; Harrold and Pearse, 1987).

Field experiments at Stillwater Cove, Pebble Beach, CA, suggest that megafauna cause significant mortality to giant kelp, *Macrocystis pyrifera*, gametophytes outplanted on acrylic plates within hours of field exposure (See chapter 1). The bat star, *Asterina miniata*, is a prominent megafaunal omnivore which uses chemical receptors for prey detection and chemically digests its food with strong acids and enzymes. It everts the cardiac stomach through its mouth, employing it as a feeding organ (Anderson, 1959; Araki, 1964). It is an abundant omnivore which has been observed with its stomach everted on substrates inoculated with algal spores and other possible algal food sources (Gerard, 1976; Dayton et al., 1984; See chapter 1) perhaps killing gametophytes.

Effects of potential grazers on the microscopic gametophyte stage of kelp are beginning to be experimentally investigated but no studies have yet been published. The high relative abundance of *A. miniata* inside Stillwater Cove ($3.7 \text{ m}^{-2} \pm 0.8 \text{ S.E.}$ for 1991 and $4.8 \text{ m}^{-2} \pm 0.7 \text{ S.E.}$ for 1992) (Foster, unpub. data), its association with algae (Gerard, 1976) and organic films on rocks (Morris et al., 1980) and its method of digestion (Anderson, 1959; Araki, 1964) suggest that it may influence the survivorship of the microscopic and juvenile stages of giant kelp (Dayton et al., 1984; Harrold and Reed, 1985).

The food preference of *A. miniata* has not been experimentally investigated with respect to microscopic algae (e.g., kelp gametophytes and benthic diatoms) and known prey with which the bat star is associated (Gerard, 1976). The food preference of *A. miniata* is of ecological interest as it may affect the kelp forest community by influencing the distribution and abundance of giant kelp (Nicotri, 1980; Dayton et al., 1984). If *A. miniata* shows a preference for gametophytes relative to other prevalent algae (e.g., benthic diatoms and drift kelp) in the artificial lab environment, then this result may suggest these animals are a source of *in situ* gametophyte mortality (See chapter 1). Even if a food preference is unsubstantiated in this study, evidence of bat star mediated mortality may still influence giant kelp. Studies have shown that marine grazers can have dramatic effects on subtidal vegetation and community structure (Paine and Vadas, 1969; Cowen et al., 1982; Fletcher, 1987; Dean et al., 1989). Hence, the consequence of bat star mediated gametophyte mortality may inhibit giant kelp recruitment and influence the dynamic kelp forest community.

Many of the common invertebrate herbivores within kelp forests are generalist feeders (Lubchenco and Gaines, 1981). Gut content analysis is a common means of determining what the particular organism has been eating and possibly its food preference. Gut content analysis of *A. miniata*, however, is impractical because only dissolved and particulate organic matter is available for analysis (Harrold, pers. comm.). Association of organisms with various flora and fauna may indicate a food preference. Although Gerard (1976) showed that *A. miniata* was associated with kelp in 60% of the observations in a central California kelp forest, this does not indicate a food preference. The preferred food may be associated with kelp in the form of an epiphyte or microscopic bacteria. Preferred food may also be unavailable (e.g., due to grazing pressure or seasonal changes in prey abundance).

Food preference of *A. miniata* in the laboratory may be determined by providing a choice among several foods (Nicotri, 1980; Watanabe, 1984; Steinberg, 1985).

Gametophytes and benthic diatoms are similar sized algal food sources that may be commonly digested by *A. miniata*. Blades of *M. pyrifera* have a completely different morphology and potentially different chemical makeup (Steinberg, pers. comm.) but are commonly associated with *A. miniata* (Gerard, 1976). A food preference for any of these algal foods may suggest that the feeding behavior is selective, not indiscriminate.

Direct observations in the field indicate the bat star leaves characteristic signs of where it everts its stomach on algae encrusted rocks. Conspicuous areas of partially digested and bleached algae are common in the central California kelp forest and may be indicative of gametophyte mortality. Alternately, this newly created space may enhance giant kelp recruitment by reducing densities of other grazers and competitors (Sousa, et al., 1981).

The purpose of this laboratory study was to determine: (1) if *Asterina miniata* shows an association with or preference for particular algae when given a choice among three algal food types relative to a no food control; and (2) if *A. miniata* cause significant mortality to or selectively remove any of the algae.

METHODS

Experimental design

This experiment was performed between March 1, 1991 and June 22, 1991 at Granite Canyon Marine Resource Laboratory. Six clean, replicate, round, fiberglass tanks were used in this experiment. Each tank had a bottom surface area of 0.9 m² and was separated into two equal sections (ie., control and experimental) by a PVC and VexarTM fence (Fig. 5). Each tank contained 21 ceramic tiles (11 x 11 cm) with sanded, acrylic

plates each measuring 7.5 x 7.5 cm and attached with new rubber bands. Three algal food types were assigned in equal numbers to 18 plates (ie., six seeded with *M. pyrifera* zoospores, six with benthic diatoms and six with known wet weights of *M. pyrifera* vegetative blades). A fourth treatment was not seeded but soaked in sterile seawater and put in the experimental side of the tanks to serve as a control for incidental association to the plate/tile. All treatments were randomly placed within each side of the tank and this configuration was duplicated for the other five tanks (Fig. 7).

Methods of sporophyll collection and seeding of acrylic plates were identical to those described in chapter 1. Benthic diatoms were derived from *Nitzschia biolata* and *Navicula zostereti* cultures used by McBride (1990) and cultured several months prior to the experiment. In order to seed the sanded acrylic plates, diatoms were scraped from 8 liter buckets, resuspended and poured through a 25 μ m filter bag to isolate the individual cells from the filaments. The single cell diatoms were counted with a hemacytometer and diluted to 1×10^4 cells ml^{-1} and plates were seeded as described for gametophytes (See chapter 1). Diatoms and gametophyte inoculation solutions were each distributed over the plates with a sterile turkey baster.

Even settlement was verified and algal cells were quantified as described in chapter 1. Each plate was sacrificed during the counting process due to variable plant mortality during counting (See chapter 1). Abundance of bacteria among treatments other than the kelp blades was qualitatively noted.

Vegetative blades of *M. pyrifera* were harvested from Stillwater Cove and remained in a holding tank for two weeks prior to the experiment. Excised pieces (2 x 1 cm) were soaked an additional 24 hours prior to the experiment. The blades were blotted dry, weighed on a digital balance, and attached to a numbered plate seasoned for two days in sterile seawater. The kelp blade, plate, and tile were also fastened together with a new

rubber band. All *M. pyrifera* blades were individually reweighed upon termination of the experiment.

Natural densities of *A. miniata* were used in the experiment (ie., two in each tank) based on density estimates inside Stillwater Cove and at Hopkins Marine Station (See Intro.). Twenty-four *A. miniata* were haphazardly collected from Stillwater Cove and maintained in a clean holding tank without visible food for one week prior to introduction into the experimental portion of each tank.

Associations of *A. miniata* with the three food types were obtained from observations made every 15 minutes for the first 2 hours of the experiment, then every hour for 24 hours mapping the location of *A. miniata* in each replicate tank. The sampling was then continued over the next six days and nights totaling 79 observation periods. An association was defined as at least one ray of *A. miniata* on the acrylic plate surface. Cardiac stomach distension was not quantitatively monitored due to the disturbance required to determine stomach condition of the asteroid.

Algal consumption / removal was assessed by removing *A. miniata* from the tanks after seven days and quantifying algal densities / weights. Each food type was compared with its respective control (where *A. miniata* was absent) by quantifying gametophyte and diatom densities and reweighing (wet weights) vegetative blades to test for differences in algal abundance.

A preliminary experiment was performed to test gametophyte / diatom survival and the optimal exposure period (ie., period necessary for *A. miniata* to explore all food types). Results of this preliminary experiment suggested that sun light was too intense for gametophyte survival in the greenhouse lab (ie., irradiance = $200 \mu\text{E m}^{-2} \text{sec}^{-1}$). Luning (1980) found similar results with other species of kelp. Therefore, all tanks were shaded which simulated subtidal light levels with a natural photoperiod. The estimated experimental

duration was seven days based on removal of gametophytes and some diatoms by *A. miniata*.

If bat stars can survive for a period of months without macroscopic food (visible food items), active foraging on any of the three algae may not be necessary to the survival of the star during the seven day food preference experiment. Therefore, if consumption is detected, then this is further evidence of an impact on developing micro-algae. It is therefore necessary to conduct a separate experiment as a test for bat star viability and persistence without food. Five individuals of *Asterina* were maintained in two tanks for 142 days. The only source of food was that which passed through a 2.0 μm filter. These tanks were cleaned with Alganox™ and fresh water bi-weekly which remove bacteria and microscopic algae.

Physical conditions

All tanks independently received filtered seawater (2.0 μm), with a turnover rate of 1.2 hours at $2 \pm 1^\circ\text{C}$ above local ambient seawater temperature. Air was bubbled into the center of each tank 10 cm below the water surface. Daytime irradiance was measured with a PAR meter (cosine collector) at the depth of 10 cm below the water surface (See chapter 1 for description of meter). Light measurements were taken among tanks on three separate dates and ranged from 9.0 to 16.0 $\mu\text{E m}^{-2} \text{sec}^{-1}$ at 1800 hours on June 13, 1991 and 15 to 38 $\mu\text{E m}^{-2} \text{sec}^{-1}$ at 1400 hours on June 15, 1991 and 15 to 25 $\mu\text{E m}^{-2} \text{sec}^{-1}$ at 1300 hours on June 18, 1991.

Statistical analysis

Chi-square was used to test for differences in the number of contacts of *A. miniata* among the three food types relative to the no-food control (See Table 5 for null hypothesis). Three separate two-level nested ANOVA's (fixed Model I) were used to test for differences among tanks and differences among treatments (ie., control vs.

experimental for gametophyte and diatom densities, and wet weights of kelp blades). Optimal experimental duration, interactive effects of herbivores, and controlling for autogenic change of the algae were investigated as described in Peterson and Renaud (1989). Cell counts were verified as described in chapter 1.

RESULTS

Analysis of the association data suggested that *A. miniata* tends to associate with *M. pyrifera* gametophytes. *A. miniata* showed a significant association with gametophytes in four of the six tanks relative to the controls (with no food) and the other algal food types (Table 5). Association with benthic diatoms and excised kelp blades was low relative to the controls (Table 5). *A. miniata* was observed dorso-ventrally flattened while on plates seeded with gametophytes. Association with the tank bottoms, walls, and wall/surface interface varied significantly among all three areas ($p < 0.05$) with an apparent preference for the wall/surface interface (e.g., based on area alone, 26.7% of *A. miniata* were expected at the wall/surface interface but 50.0% of the bat stars were found there).

Plates inoculated with benthic diatoms developed a brown film due to growth. When *A. miniata* was observed crossing over these plates, removal of the brown film by abrasion was not apparent.

A. miniata survived and were in good condition after 142 days without additions of macroscopic food.

Algal densities/weights after seven days of exposure show that gametophytes were significantly reduced when *A. miniata* was present ($p < 0.0005$) (Table 6A, 6B and Fig. 8a).

Benthic diatoms were not significantly reduced by bat stars relative to the controls (Fig. 8b), but diatom densities differed significantly among tanks ($p > 0.25$ and $0.01 < p < 0.005$, respectively).

A. miniata had no effect on the excised pieces of *M. pyrifera* blades (Tables 6a, 6b, and Fig. 8c).

DISCUSSION

Direct contact or association of *A. miniata* with gametophyte seeded plates relative to the diatoms, kelp blades, and control treatment suggest a slight food preference for gametophytes (Table 5). A physical flattening of *Asterina* to the plate surface suggested that *A. miniata* was apparently feeding. This was confirmed early in the experiment by turning over *Asterina* to see if the cardiac stomach was everted. This feeding verification procedure, however, disturbed the animal and sometimes created a response in which *A. miniata* retreated to the tank wall or wall/surface interface. Having confirmed that *A. miniata* was feeding, all subsequent observations of associations were permitted to proceed without disturbance. The number of associations with acrylic plates did not provide a measure of feeding activity because not all bat stars were directly on the plate and many were not necessarily feeding or had their cardiac stomach completely everted. These observations confirmed the need for a more thorough quantification of algal consumption.

Drift kelp is commonly seen being digested by *A. miniata* in the field (Gerard, 1976). Excised pieces of de-epiphytized kelp blades, although seasoned for 2 weeks prior to the food preference experiment, showed no evidence of bacterial decomposition. The lack of association of *A. miniata* with kelp blades may be attributed to the preference of other food types (e.g., gametophytes), unnatural excised pieces of kelp, presence of anti-grazing compounds, and/or the absence of encrusting organisms (e.g., bryozoans and epiphytes). Day and Osman (1981) suggest that bryozoans are commonly eaten by *A. miniata*. Kelp blades may have been a more attractive prey item had they included the commonly encrusting bryozoans. Drift in the field may

be relatively more desirable due to the more advanced states of decomposition and the associated epiphytes and bacteria.

Although an association of *A. miniata* with gametophyte seeded plates was apparent in four out of the six replicate tanks, it cannot be assumed that gametophytes are killed if *A. miniata* touches, passes over, or everts its stomach on a seeded plate. Therefore, it was necessary to quantify the seeded plates upon termination of the experiment in order to determine if there was *A. miniata* mediated algal removal.

Food removal

Reduced densities of gametophytes on plates exposed to *A. miniata* relative to controls where the asteroids were excluded suggests gametophytes were removed by either abrasion or chemical digestion (Tables 6a, 6b, and Fig. 8a).

As determined by the pilot study, gametophytes, benthic diatoms, and kelp blades remained on the plates upon termination of the experiment (Table 6b and Figs. 8a, 8b, and 8c). This afforded the ability to measure the rate of algal disappearance or food preference. There was a slower rate of algal removal in the lab relative to the field (See chapter 1). This can be expected due to the unnatural behavior in the artificial laboratory environment (e.g., strong association with the wall-surface interface, intra-specific interactions, less interested in feeding) or the absence of other megagrazers.

Although other studies have shown that algal spores and juveniles may survive digestion by herbivores (Santelices and Correa, 1985; Santelices et al., 1983; Lubchenco, 1986), gametophytes and diatoms affected by *A. miniata* appeared bleached and dead. Spatially distinct areas of cleared or dead gametophytes were observed while viable gametophytes remained on portions of the plates apparently not in direct contact with the cardiac stomach of *A. miniata*.

The extent of gametophyte removal by *A. miniata* may depend on various factors. The bat star's size, position on the plate, time spent on the plate, extent of stomach eversion, stomach

proximity to the gametophytes, substrate heterogeneity, and the method of gametophyte removal all may influence gametophyte survival. Localized areas of gametophyte removal were apparent upon quantification of seeded plates. There was no evidence of diatom removal when bat stars simply crossed over the brown diatom film which suggests that algal removal was by digestion rather than abrasion. Positive determination of the method used to remove the algae (ie., abrasion and digestion) could not be determined in this experiment.

Since *A. miniata* was associated with the various plates in 37% of the observations, it is interesting to note where the remaining bat stars were in the tanks (the location of individual *A. miniata* were mapped during each observation period). If one assumes *A. miniata* to be evenly distributed based on available surface area of the tank, it was surprising to find the wall/surface interface was apparently a preferred micro-habitat. This behavior is rarely observed in nature because of the high probability of becoming dislodged during changing tides and surge. Further research is clearly required to determine if this apparent wall/surface preference may be due to: (1) a possible refuge or artificial crevice, (2) an escape response from the tank, (3) a more desirable flow regime on the wall/surface interface, and/or (4) particulate food and buoyant oils/lipids on the water surface upon which it feeds (Morris et al., 1980; Ferguson, pers. comm.). Due to the strong association with the wall/surface interface and reduced densities of *A. miniata* on the tank bottom, removal of algae from the plates may be considered a conservative estimate of herbivory.

Behavior of *A. miniata* in the lab may not be representative of the natural environment. Seawater in which *M. pyrifera* was allowed to stand can be strongly stimulatory to some echinoids (Lawrence, 1975). Although *A. miniata* may use both tactile and chemical cues to locate its prey in the field (Araki, 1964), chemical detection of potential foods may be more or less difficult in the laboratory due to the artificial environment and flow regime. Some of the bat stars interacted within the tanks "fighting"

for position or apparently avoiding each other (ie., staying on opposite sides of the tank) which possibly inhibited normal foraging activity. Wobber (1975) described such agonistic interactions that results in spacial segregation of adults. Translocating bat stars to the artificial lab environment may have changed their behavior from one of normal foraging activity to a continuous escape response. *A. miniata* could apparently survive without macroscopic food based on their persistence for at least 142 days eating only the fouling microorganisms and bacteria that recruited into the tank. These artificial lab conditions, conspecific interactions and low food requirements may result in unnatural estimates of food preference and conservative estimates of food consumption.

Although *A. miniata* may cause statistically significant gametophyte mortality both in the laboratory and in nature, other factors can kill gametophytes and juvenile kelp plants. Intra-specific shading, the degree of exposure, and other physical factors can have a profound effect on giant kelp survival (Reed and Foster, 1984; Deysher and Dean, 1986; Harrold et al., 1988; Dean et al., 1989). Further reduction in gametophyte density on seeded plates is expected given longer exposure time to grazers and other environmental factors (See chapter 1). Survival of gametophytes on natural, more heterogeneous substrates may have been enhanced given a possible gametophyte refuge in micro-crevices. It is unlikely that the present abundance of *A. miniata* can solely reduce the population density of giant kelp. There are a multitude of conditions and factors that can influence the survival of both the gametophyte stage and the conspicuous adult plant. Grazing by *A. miniata*, other megainvertebrates, and some fish coupled with poor physical conditions can cause measurable reductions in population density and even prevent the reestablishment of kelp forests (Harris et al., 1984; Dean et al., 1989).

Apparent avoidance of benthic diatoms by bat stars relative to the non-food controls may suggest that there were anti-grazing compounds in these algae or it was simply not a

preferred food. Steinberg (pers. comm.) suggests that diatoms may change chemically and develop a "bad taste" during the long culture period in the lab prior to the experiment.

Further discussion of the bat star and its influence on giant kelp gametophytes as well as the concluding statements of chapter 1 are presented in the next section.

CONCLUSIONS AND IMPLICATIONS OF FIELD AND LABORATORY EXPERIMENTS

Recruitment of giant kelp to the sporophyte stage is highly variable among and within kelp forests (Deysher and Dean, 1986; Reed, 1989). The experiments performed in this study only applied to one site in Stillwater Cove during May and November of 1990. The experimental techniques used in this study, however, have wide ranging application to other kelp forests, algal communities, and ecosystems.

In this set of experiments, a consistently high rate of mortality occurred for gametophytes not shielded by various configurations of exclusion cages. Gametophyte densities in both experiments were reduced by an average of 30.2% per day. Physical factors did not limit gametophyte survival in these experiments. There was no significant gametophyte loss that may have been the result of micrograzer predation, sedimentation, or both. Megafauna larger than 9.0 mm diameter appeared to cause the greatest impact with significant reductions in gametophyte densities with as little as 19 hours of field exposure. A variety of organisms within the kelp forest may effectively reduce the densities of microscopic plants by direct or incidental grazing, digestion, and abrasion (See Pearse and Lowry, 1974 for species list; Steneck and Watling, 1982). Various organisms were observed in contact with the plates (Table 4) and their abundance may have been reduced due to the replicate clearings where plates were attached, perhaps reducing their impact on gametophytes.

Continued persistence of *M. pyrifera* in Stillwater Cove is dependent on the replacement of adult plants. This reforestation is dependent in part on a source of kelp propagules, heterogeneous rocky substrates, rapid growth of juvenile plants (to a size refuge from grazers), and "recruitment windows" of favorable physical conditions. In this study, a megafauna mediated reduction in gametophyte population density is clearly demonstrated.

The bat star, *Asterina miniata*, was an abundant, mobile organism at the study site leaving conspicuous evidence of its feeding activity. There were discrete areas at the study site and throughout the cove where understory algae had been bleached as a consequence of grazing by *A. miniata*. Although organisms may be attracted to newly cleared substrates in the field (pers. obs.), experiments suggest that *A. miniata* was apparently attracted to the kelp gametophytes as a food source. Evidence of attraction and grazing was observed when individuals migrated directly onto seeded plates and everted their cardiac stomachs. This characteristic feeding activity was apparently in response to the experimental monocultures of gametophytes or to associated bacteria. The high abundance of *A. miniata*, its conspicuous evidence of feeding activity in kelp forests, and its demonstrated preference in the laboratory for giant kelp gametophytes suggest this asteroid may reduce the recruitment density of giant kelp and influence the algal composition of kelp forests.

Removal of certain herbivores from terrestrial and marine intertidal communities may result in dramatic increases in macroflora (Nicoletti, 1977; Paine and Vadas, 1979; Jernakoff, 1985). Experimental removal or exclusion of *A. miniata* from rocky subtidal communities coupled with the physical conditions required for recruitment (Deysher and Dean, 1986) may facilitate recruitment of *M. pyrifera* through increased survival of young stages (ie., gametophytes and young sporophytes) (Nicoletti, 1977; Jernakoff, 1985).

Grazer distribution is often spatially and temporally variable, making potential effects on the algal assemblage difficult to predict (Foster and Schiel, 1985; Harrold and Reed, 1985). Even though both the lab and field studies were of short duration relative to the development time for *M. pyrifera*, the impact of *A. miniata* grazing on giant kelp gametophytes was clearly demonstrated. It may be difficult for recruiting kelp to become established where bat stars are abundant. The many physical requirements for gametophyte survival coupled with the active

foraging of *A. miniata* and other megafauna observed in nature make giant kelp recruitment to the sporophyte stage difficult to predict.

Behavioral experiments performed in the laboratory environment demonstrated that the bat stars were associated with gametophytes (in four out of six tanks) compared to benthic diatoms, kelp blades, and the non-food control. Evidence of high *in situ* gametophyte mortality seen in chapter 1 and the ability of bat stars to preferentially kill gametophytes in the lab, indicates that *A. miniata* may play an important role influencing kelp forest community structure.

The recruitment and abundance of giant kelp may be influenced in part by the foraging activity of *A. miniata*. Digestion of surfaces by bat stars may reduce the densities of recruiting kelp sporophytes while potentially creating empty patches upon which many species including zoospores can recruit. *A. miniata* has been reported to have an indirect positive effect on giant kelp recruitment by reducing the abundance of *Lytichinis anamesus*, an herbivorous urchin in southern California (Schroeter et al., 1983) known to graze on the early life stages of *Macrocystis* (Dean et al., 1988).

The dynamic web of interacting factors which influence the abundance and distribution of giant kelp can only be understood with a sound experimental approach. The laboratory and field research presented in these studies assessed only some of the factors influencing giant kelp ecology and should inspire further investigation. This field and laboratory research has clearly demonstrated the rate of *in situ* gametophyte mortality and showed that megafauna (e.g., *A. miniata*) are a potentially key source of mortality to giant kelp gametophytes. The field research presented in chapter 1, and this laboratory study will help us to understand the sources of gametophyte mortality and should provide valuable insight in determining both the factors which cause mortality to small plants and the food preferences of mobile organisms.

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Table 1. Duration of field exposure and subsequent laboratory culture time prior to gametophyte quantification.

	Hours in field	Hours cultured after field deployment	Elapsed time (days) (innoculation to final count)
Experiment 1			
Harvest 1	38	5 to 8	3
Harvest 2	62	68 to 72	7
Experiment 2			
Harvest 1	19	104 to 108	7
Harvest 2	69	66 to 70	7

Table 2a. Treatment exposure period vs. treatment for experiment 1.

ANOVA Table

Source:	df:	Sum of Squares:	Mean Square:	F-test:	P value:
EXPOSURE PERIOD (A)	1	0.009	0.009	0.044	0.8371
TREATMENT (B)	2	180.808	90.404	448.284	0.0001
AB	2	3.914	1.957	9.705	0.0031
Error	12	2.421	0.202		

Table 2b. Means and standard errors of two-way ANOVA for experiment 1
(n = 3).

Time (hours)	t = 38			t = 62	
	Treatment	mean (†)	S.E.	mean (†)	S.E.
	Lab control	7.4	0.2	8.63	0.39
	Handling control	7.7	0.17	7.3	0.15
	No cage	1.53	0.34	0.56	0.22

† Means are of gametophyte density (# / 0.159 mm²).

Table 2c. Treatment exposure time vs. treatment (2-way ANOVA) and Ryan's Q results for experiment 1 (n/s) = not significant, (*) = means significantly differ ($p < 0.05$).

Time (hours)	Treatment	t = 38			t = 62		
		Lab control	Handling control	No cage	Lab control	Handling control	No cage
t = 38	Lab control						
	Handling control	n/s					
	No cage	.	.				
t = 62	Lab control	.	.	.	.	.	.
	Handling control	n/s	n/s	.	.	.	.
	No cage	.	.	.	.	.	.

Table 3a. Exposure period and treatment analysis for experiment 2.

ANOVA Table

Source:	df:	Sum of Squares:	Mean Square:	F-test:	P value:
EXPOSURE PERIOD (A)	1	18.375	18.375	14.483	0.0016
TREATMENT (B)	3	275.908	91.969	72.488	0.0004
AB	3	0.635	0.212	0.167	0.917
Error	16	20.3	1.269		

Table 3b. Means and standard errors of two-way ANOVA for experiment 2
(n = 3).

Time (hours)	Treatment	t = 19		t = 69	
		mean	S.E.	mean	S.E.
	Lab control	11.33	0.82	12.80	0.43
	Handling control	11.00	0.37	12.60	0.78
	Turtle control	12.43	0.52	12.63	0.32
	Field turtle	11.97	0.99	10.33	0.59
	Exclusion cage	10.43	0.23	8.63	0.40
	Cage control	5.13	0.85	2.90	0.10
	No Cage	4.00	1.05	2.67	0.07

Table 3c. Treatment exposure time vs. treatment type (2-way ANOVA) and Ryan's Q results for experiment 2 (n/s) = not significant, (*) = $p < 0.05$.

Time (hours)	Treatment	t = 19				t = 69			
		Field turtle	Exclusion cage	Cage control	No cage	Field turtle	Exclusion cage	Cage control	No cage
t = 19	Field turtle								
	Exclusion cage	n/s							
	Cage control	*	*						
	No cage	*	*	n/s					
t = 69	Field turtle	n/s	n/s	*	*				
	Exclusion cage	*	n/s	*	*	n/s			
	Cage control	*	*	n/s	n/s	*	*		
	No Cage	*	*	n/s	n/s	*	*	n/s	

Table 4. The visible invertebrates in contact with seeded acrylic plate (SAP), unseeded recruitment control (RC; for experiments 1 and 2), and cage control (CC; in experiment 2) treatments.

Experiment #	Hours after outplant	Organism	Number associated with plate		
			(SAP)	(CC)	(RC)
1	1.5	<i>Cryptochiton stelleri</i>	1		0
	38	<i>Asterina miniata</i>	1		0
		<i>Acmaea mitra</i>	1		0
		<i>Tegula montereyensis</i>	1		0
		<i>Tonicella liniata</i>	1		0
	62	<i>none</i>			
2	2	<i>Asterina miniata</i> †	1	1	0
		<i>Clinocottus acuticeps</i>	0	1	0
	19	<i>Tonicella liniata</i>	1	0	0
		<i>Astraea gibberosa</i>	0	1	0
	69	<i>Asterina miniata</i>	0	0	1

† *Asterina miniata* had stomach everted while on SAP.

Table 5. Association of Asterina miniata with three algal food types and a control (no food). Three plates of each food type were offered to two randomly selected A. miniata in each replicate tank (n = 6). A total of 79 observations were made for each tank over a seven day period.

Ho = The associations of A. miniata is independent of food type.

Tank No.	No. of associations with the four treatments			No. of obs. on treatments		df	No. of obs.	chi-square calc. value	reject Ho
	control	gametophyte	diatom	kelp					
Tank A	7	16	1	1	25	3	79	24.12	yes
Tank B	4	6	0	2	12	3	79	3.67	no
Tank C	6	8	2	6	22	3	79	3.45	no
Tank D	3	11	3	4	21	3	79	8.52	yes
Tank E	1	12	2	1	16	3	79	21.51	yes
Tank F	4	13	2	4	23	3	79	12.66	yes

Table 6a. Two-level nested ANOVA's for tank effects and differences between control and experimental treatments. M. pyrifera gametophytes densities, diatoms densities, and wet weights of M. pyrifera blade pieces were tested.

	SOURCE OF VARIATION	SS	DF	MS	F	F crit	P value
Gameto- phytes	Total	68.11	35				
	Among all subgroups	43.79	11				
	Tanks	17.55	5	3.51	0.8	4.39	< 0.50
	± <u>Asterina miniata</u>	26.24	6	4.37	4.33*	2.51	0.0025<P<0.005
	Error	24.32	24	1.01			

RESULT

Ho1: There is no difference between control and experimental treatments among the six tanks (p>0.25)

Ha2: There is a difference in gametophyte densities between treatments (0.0025<p<0.005).

	SOURCE OF VARIATION	SS	DF	MS	F	F crit	P value
Benthic Diatoms	Total	6301.99	35				
	Among all subgroups	4430.09	11				
	Tanks	3983.65	5	796.74	10.71*	4.39	0.005<P<0.01
	± <u>Asterina miniata</u>	446.44	6	74.4	0.95	2.51	< 0.50
	Error	1871.85	24	77.99			

RESULT

Ho1: There is a difference between treatments among the six tanks (0.01≤p≤0.005).

Ho2: There is no difference in diatom densities between control and experimental treatments (p>0.25).

	SOURCE OF VARIATION	SS	DF	MS	F	F crit	P value
Kelp blades	Total	0.00566	35				
	Among all subgroups	0.00196	11				
	Tanks	0.0008	5	0.00016	0.842	4.39	< 0.50
	± <u>Asterina miniata</u>	0.00116	6	0.00019	1.26	2.51	< 0.50
	Error	0.0037	24	0.00015			

RESULT

Ho1: There is no difference between control and experimental treatments among the six tanks (p>0.25)

Ho2: There is no difference in kelp blade weights between control and experimental treatments (p>0.25).

Table 6b. Means and standard errors for the laboratory experiment.

	Tank A		Tank B		Tank C		Tank D		Tank E		Tank F	
	control	exp.	control	exp.	control	exp.	control	exp.	control	exp.	control	exp.
Gameto- phytes	7.07	5.43	5.87	6.03	8.01	5.90	7.10	4.70	7.80	5.87	6.27	4.60
	0.07	0.52	0.52	0.88	0.23	0.66	0.42	0.78	0.73	0.57	0.54	0.42
Benthic Diatoms †	69.07	77.37	57.00	66.67	91.45	87.70	90.40	85.83	93.45	92.07	92.90	74.30
	3.64	5.26	2.65	9.47	3.91	2.87	3.84	2.92	2.40	4.49	5.03	8.64
Kelp blades ††	0.02	0.005	0	0.02	0.01	0.01	0.015	0.025	0.01	0	0.01	0.01
	0.01	0.01	0	0	0.01	0	0.004	0.02	0.016	0	0.017	0

† Gametophytes and benthic diatom numbers are densities (number per 0.159 mm²).

†† Kelp blade data are absolute differences in weights (grams) of excised kelp pieces weighed at t = 0 and t = 7 days.

Figure 1. Life history and morphology of *Macrocystis pyrifera*.
(modified from Dawson and Foster, 1982)

Figure 2. Treatment configurations attached to the field site for experiments 1 and 2 ($n = 3$ for each treatment).

FIELD TURTLE: SAP secured inside a petri dish.
Handling controls used for this treatment.

- SEDIMENTS; - ABRASION; - MICROFAUNA;
- MEGAFUNA

EXCLUSION CAGE: SAP inside cage.

+ SEDIMENTS; + ABRASION; + MICROFAUNA;
- MEGAFUNA

CAGE CONTROL: SAP inside cage without sides.

+ SEDIMENTS; + ABRASION; + MICROFAUNA;
+ MEGAFUNA

RECRUITMENT CONTROL: Un-seeded acrylic plate.

NO CAGE: SAP exposed to all factors.

Handling control used for this treatment.

+ SEDIMENTS; + ABRASION; + MICROFAUNA;
+ MEGAFUNA

Figure 3. Treatments used in field experiment 2. Seeded acrylic plates (SAP's) had defined populations of giant kelp gametophytes. Other treatments included lab controls (remained in the lab) and handling controls. Exclusion cage mesh is 9.0 mm.

Figure 4. Gametophyte densities with increasing field exposure time for the no cage treatment of experiments 1 and 2. Error bars are standard errors of the mean.

Figure 5. Interaction in experiment 1. Mean gametophyte densities from two exposure periods (in hours) are plotted for three treatments. Interaction is between lab and handling control ($p=0.003$).

Figure 6. Gametophyte density in 0.159 mm² microscopic fields for four field treatments, three controls, and two exposure periods ($n = 3$). A ">" next to the treatment abbreviation indicates a significant difference ($p < 0.05$). Error bars are standard errors of the mean.

No. of replicate
food units in
each tank

No. of
replicate
tanks

3 of each
food type

6

Design

Figure 7. Design of the laboratory experiment.

Figure 8a. Gametophyte density for control (*Asterina miniata* excluded) and experimental (with *A. miniata* present) treatments in the lab for tanks "A" through "F". Error bars are standard errors of the mean ($n = 3$ for control and experimental treatments).

Figure 8b. Benthic diatom density for control (*Asterina miniata* excluded) and experimental (*A. miniata* present) treatments in the lab for tanks "A" through "F". Error bars are standard errors of the mean ($n = 3$ for control and experimental treatments).

Figure 8c. Absolute weight difference of *Macrocystis pyrifera* (kelp) pieces for control (*Asterina miniata* excluded) and experimental (*A. miniata* present) treatments in the lab for tanks "A" through "F". Error bars are standard errors of the mean ($n = 3$ for control and experimental treatments).