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# Trophic interactions among *Chlorostoma brunnea*, *Macrocystis pyrifera*, and fungi

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TROPHIC INTERACTIONS AMONG *CHLOROSTOMA BRUNNEA*, *MACROCYSTIS*  
*PYRIFERA*, AND FUNGI

A Thesis

Presented to

The Faculty of the Moss Landing Marine Laboratory

San José State University

In Partial Fulfillment

Of the Requirements for the Degree

Master of Science

by

Selena M. McMillan

May 2010

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The Designated Thesis Committee Approves the Thesis Titled

TROPHIC INTERACTIONS AMONG *CHLOROSTOMA BRUNNEA*, *MACROCYSTIS*  
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## ABSTRACT

### TROPHIC INTERACTIONS AMONG *CHLOROSTOMA BRUNNEA*, *MACROCYSTIS PYRIFERA*, AND FUNGI

by Selena M. McMillan

The purpose of this study was to evaluate how one of the most abundant kelp forest herbivores in central California, the trochid snail *Chlorostoma brunnea*, affects the productivity and survivorship of the giant kelp *Macrocystis pyrifera* within central California. The effects of this turban snail species were investigated using experimental field manipulations of snail abundance on *Macrocystis* sporophytes and supplementary laboratory experiments. Experimental field manipulation of *C. brunnea* densities (0-450 snails per sporophyte) revealed an overcompensation of growth by *Macrocystis* in response to moderate snail densities. This finding is consistent with a terrestrial growth premise, the Grazing Optimization Hypothesis. Laboratory feeding experiments also demonstrated an overcompensatory response of *Macrocystis* to *C. brunnea* grazing. These experiments identified marine fungi growing on *Macrocystis* as a potential primary food source for *C. brunnea*. The effects of *C. brunnea* grazing on fungal biomass produced an inverse relationship; fungal biomass was significantly less when *C. brunnea* grazed at moderate densities. These results indicate that the interaction between marine fungi and *C. brunnea* may serve as a potential mechanism for compensatory growth in *Macrocystis*. As moderately abundant snails remove fungi, *Macrocystis* may attain a greater growth rate.

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## INTRODUCTION TO THESIS

The giant kelp, *Macrocystis pyrifera*, is a large subtidal alga that forms extensive beds along the coastlines of New Zealand, southern Australia, North and South America, and South Africa (Graham et al. 2007). Giant kelp forests form complex structures that host numerous associated species such as fish, arthropods, echinoderms, molluscs, mammals, and other algae (Rosenthal et al. 1974). Intraspecific and interspecific interactions have been well studied in these giant kelp systems (North 1971, Dayton 1985a, b, Foster and Schiel 1985, North 1994, Steneck et al. 2002). A more thorough understanding of the strength of trophic interactions, however, is essential to determine the overall dynamics of the kelp forest community (North 1971, Dayton 1985a, Foster and Schiel 1985, Estes and Duggins 1995).

Interactions between *Macrocystis pyrifera* and its grazers is a subject well studied in southern California (e.g., Dean et al. 1984, Dayton 1985a, Ebeling et al. 1985, Harrold and Reed 1985, Davenport and Anderson 2007), but less focus on these relationships has been applied to central California (Pearse and Hines 1979, Cowen et al. 1982). The dominant grazers of giant kelp in southern California include the sea urchins *Strongylocentrotus purpuratus*, *S. franciscanus*, *Lytechinus anamesus* and *Centrostephanus coronatus* (the latter only occurs south of Point Conception). These urchins can completely remove kelp forests in southern California causing urchin barrens (Ebeling et al. 1985, Harrold and Reed 1985). In situations where the urchins do not cause barrens, a greater abundance of urchins may cause a less diverse system through the removal of some algal species (Graham 2004). In central California, however, sea

urchins are preyed upon by the sunflower star *Pycnopodia helianthoides*, the wolf eel *Anarrichtheys ocellatus*, and sea otters *Enhydra lutris*, (which are non-existent south of Pt. Conception with the exception of a translocated population at San Nicolas Island) (Graham et al. 2006). With the presence of these predators, urchins in central California never reach the densities necessary to overgraze *Macrocystis* (Watanabe and Harrold 1991). In fact, these grazers tend to consume mostly drift algae and do not graze directly on attached *Macrocystis* (Lowry and Pearse 1973, Reed and Foster 1984, Foster and Schiel 1985, Harrold and Reed 1985, Harrold and Pearse 1987).

In the central Californian kelp forests, many intermediate herbivorous species prey on adult *Macrocystis* sporophytes such as snails, limpets, isopods, and amphipods (Foster and Schiel 1985). These mesograzers live and feed directly on the *Macrocystis* tissues and may indirectly affect the alga by causing the removal of all or parts of the sporophyte (Foster and Schiel 1985). These indirect effects may be generated through the weakening of tissues by creating wounds that attract epiphytes and fungal and bacterial infections, which could lead to loss of blades, fronds, or holdfasts (Foster and Schiel 1985).

Only a handful of researchers have examined sublethal effects of herbivores on kelps (Kain 1963, Black 1976, Graham 2002, Davenport and Anderson 2007). Although intermediate grazers may not have a detrimental impact on kelp like grazers such as urchins (Dayton 1985a), the effects on growth and reproduction may affect the overall health of the kelp and the population dynamics of the kelp forest (Foster and Schiel 1985, Davenport and Anderson 2007). Therefore, the first goal of this project was to determine

the effects of intermediate grazers on the productivity and reproductive potential of *Macrocystis pyrifera*. Formally of the genus *Tegula*, the most conspicuous of these grazers is the assemblage of trochid snails, *Chlorostoma brunnea*, *C. montereyi*, and *Promartynia pulligo* (Watanabe 1984).

It has been suggested that wounding by grazers can reduce biomass and may reduce the fitness of some algal species especially when the wounding occurs before disturbance (Dayton 1985a, Foster and Schiel 1985, Toth and Pavia 2005). By removing biomass through grazing, especially at times of lesser production or disturbance of *Macrocystis pyrifera*, turban snails could reduce growth rates and increase sporophyte mortality (Foster and Schiel 1985). Additionally, terrestrial studies that have simulated or used actual grazing by insects on single leaves have shown reduction in photosynthetic rates in the remaining tissue of the grazed leaf if tissue damage exceeded a threshold level (Hall and Ferree 1975, 1976, Poston et al. 1976). Grazed *Macrocystis* blades, therefore, may have lesser photosynthetic rate than non-grazed blades causing reduced production.

Alternatively, several researchers have shown an increase in plant photosynthesis and growth after grazing, which ultimately led to the development of the grazing optimization hypothesis (GOH) (Eaton 1931, Pearson 1965, Kumar and Joshi 1972, Hodgkinson 1974, Detling et al. 1979, McNaughton 1979). Researchers investigating interactions between herbivores and their algal prey have traditionally focused on negative linear relationships (i.e., all grazing was detrimental to the algae grazed). However, recent research was designed to investigate alternate interactions. One study

on autotrophic microcosms did demonstrate effects (due to grazing intensity) similar to the GOH predictions. In that study, the introduction of the herbivorous fish (*Notropis spilopterus*) increased net primary productivity of phytoplankton (predominantly *Spirogyra*) (Cooper 1973). Furthermore, the enhancement of net primary productivity was positively correlated with herbivore biomass up to a certain threshold and then inversely correlated with increasing herbivores. This relationship approximated the first derivative of a sigmoid population growth model and the GOH curve (Figure 1).

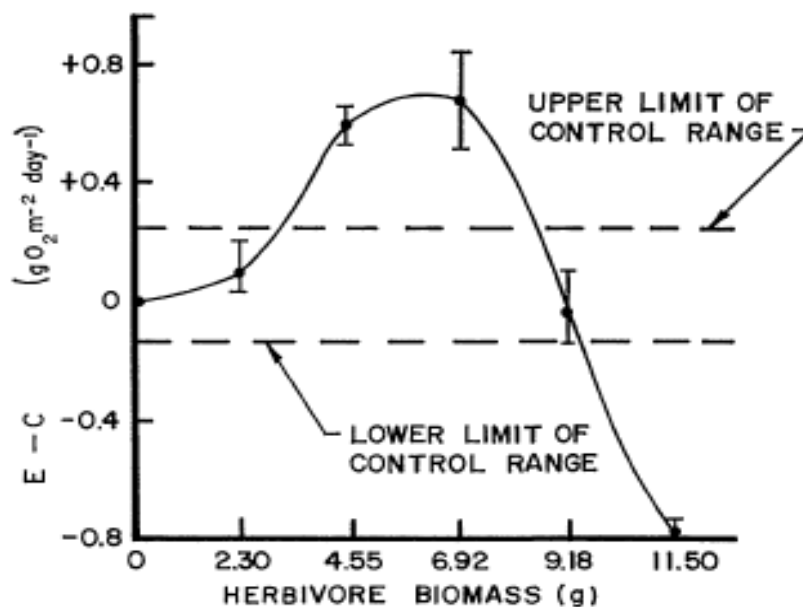


Figure 1: Difference in net primary productivity ( $\text{g O}_2/\text{m}^2/\text{day}$ ) in experimental (E) microcosms and control (C) microcosms vs. grazing biomass of *Notropis spilopterus* (Cooper 1973).

One of the main controversies surrounding compensatory growth, or positive response of plant growth due to herbivory, is the lack of mechanisms found that would induce and sustain the compensation (Belsky 1986, Belsky et al. 1993). Some of the mechanisms discussed were an increase of photosynthetic rate of residual tissue, an



allocation of current photosynthate to new leaf blades, removal of older or senescent tissue, increased light availability to sub-canopy tissue, and addition of available nutrients to the plant by grazing herbivores (Belsky et al. 1993, de Mazancourt et al. 1998). Other possible mechanisms may include epiphyte removal and reduction in viral, bacterial, or fungal pathogens in the plant/algal tissues. Considerable debate continues regarding whether such mechanisms have been clearly demonstrated (Belsky et. al 1993, de Mazancourt 1998, Agrawal 2000, Hawkes and Sullivan 2001). The goals of this study were to test for the existence of compensatory growth in *Macrocystis* (Chapter I) and if it existed, to investigate possible mechanisms behind such compensation (Chapter II).

Recent ecological investigations have revealed novel relationships between plants and snail grazers (Silliman and Newell 2003). Some snails formally believed to be grazing primarily on plant material were actually grazing on fungal pathogens. Therefore, as a possible compensatory mechanism, I explored whether fungi were present in the living blade tissues of *Macrocystis* and whether fungal biomass was affected by *C. brunnea* grazing. If evidence of a trophic interaction between the turban snail and fungal biomass was found, conclusions may be made about the role of fungi as a potential food source for *C. brunnea* and the interaction as a possible mechanism for compensatory growth of *Macrocystis*.

Application of the grazing optimization hypothesis (GOH) to the turban snail-*Macrocystis* system may provide new insights into the dynamics of algae-grazer interactions. More specifically, the GOH would predict that moderate grazing by *Chlorostoma brunnea* on *Macrocystis pyrifera* has a positive effect on growth and

reproductive potential of the alga. If the GOH explains the dynamics of this interaction better than traditional negative linear responses, *Macrocystis* productivity will increase with increasing densities of *C. brunnea* grazing, then after a certain grazer density is reached, decrease with increased densities of *C. brunnea*. This would help explain the paucity of observations regarding negative effects of these abundant grazers on *Macrocystis* populations, and introduce a new approach for examining effects of grazers on algae in marine systems.

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## CHAPTER I

### *CHLOROSTOMA BRUNNEA* GRAZING EFFECTS ON THE PRODUCTIVITY OF THE GIANT KELP, *MACROCYSTIS PYRIFERA*, IN CENTRAL CALIFORNIA

#### ABSTRACT

The Growth Optimization Hypothesis (GOH) explains overcompensatory growth in terrestrial plants in the presence of grazing. In this study, this hypothesis was tested for the first time in the nearshore marine environment using the giant kelp *Macrocystis pyrifera* and the trochid snail *Chlorostoma brunnea* as a model. A range of densities of *C. brunnea* (0-450 snails/sporophyte) was applied in field manipulations of 10 *Macrocystis* sporophytes within Stillwater Cove, Carmel, California, and again, in supplementary laboratory experiments. The second order polynomial relationship revealed field and laboratory studies support the GOH of terrestrial biology and counter the traditional negative linear response expected in macroalgal-grazer interactions. This indicates a mutualistic relationship between *Macrocystis* and moderate turban snail densities within the central California giant kelp forest system.



## INTRODUCTION

The most accepted view concerning the effects of grazers on plant and algal production is that of a deleterious impact. This negative linear relationship between herbivores and their prey has been demonstrated numerous times in terrestrial (Belsky 1986, Crawley 1997, Bigger and Marvier 1998) and algal biology (Lubchenco and Gaines 1981). Recently, scientists have found that some plants and algae can resist or tolerate the effects of herbivory (Lubchenco and Gaines 1981, Bryant et al. 1983, Belsky 1986). Positive response of plant growth to herbivory has been defined as compensation (Belsky 1986). Plants and algae compensate for grazing and that compensation can even alleviate the potential harmful effects of herbivory (Kumar and Joshi 1972, Vikery 1972, Chew 1974, Dyer 1975, McNaughton 1976, 1979a, Owen and Weigert 1976, Dyer et al. 1982, McNaughton 1983, Maschinski and Whitham 1989, Vail 1992).

Recently, new hypotheses have been created to explain compensatory growth. One such hypothesis states that plants can compensate for lesser levels of grazing intensity until a certain level of herbivory is reached, leading to a threshold of herbivory effects (McNaughton 1979a). Additionally, a second hypothesis has emerged stating that moderate grazing intensity leads to overcompensation by plants, whereas less levels and greater levels of herbivory cause decreased production (Dyer 1975). This hypothesis has been implied or expressed in several terrestrial studies (Eaton 1931, Taylor and Bardner 1968, Kumar and Joshi 1972, Vickery 1972, Chew 1974, Harris 1974, Dyer and Bokhari 1976, McNaughton 1976, 1979a, b). This has led to the creation of the grazing optimization hypothesis (GOH), which states that several possibilities can occur due to

herbivory under different grazing intensities (Hilbert et al. 1981). With minor amounts of grazing, an enhancement in relative growth rate can lead to increased net primary production or overcompensation (Figure 2A). At moderate levels of grazing intensity, major increases in relative growth rate can occur without a significant increase in production (Figure 2, level of optimal grazing). Plants growing at their maximum relative growth rate may not respond positively and may be able to sustain less grazing than plants with less than maximum growth rates (Figure 2B). The greater the grazing intensity, the less likely an increase of production will occur, and the greater the response that is required for a positive effect to be evident (Figure 2, undercompensation). The GOH may also be useful for explaining responses of autotrophs to mesograzers in the marine environment.

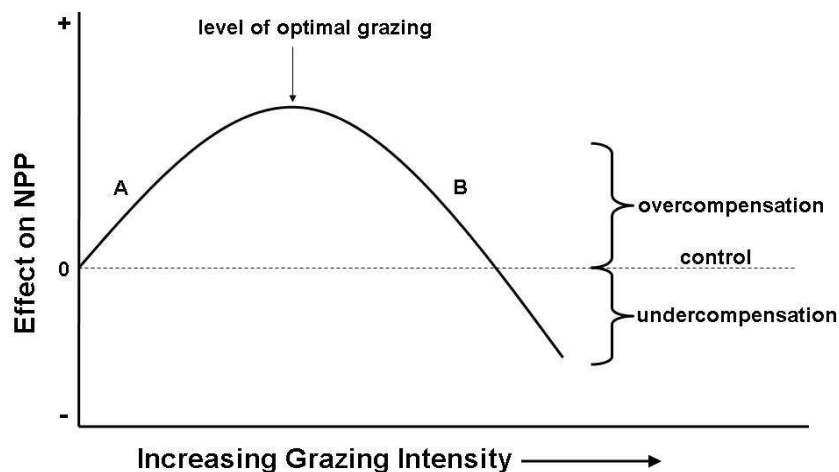


Figure 2: The grazing optimization hypothesis curve shows the change in production [effect on net primary production (NPP)] due to grazing. Control represents level of production in the absence of grazers. Overcompensation represents production higher than that in the absence of grazers, and undercompensation is lowered production compared to the control. Increasing production is represented by the curve at point A, and decreasing production by point B. Adapted from Belski 1986.

Still contentious in the plant biology field, compensatory views on production due to herbivory have rarely been applied to the marine system (Vermeij 1983, Littler et al. 1995). A handful of recent studies have examined the existence of compensatory growth in marine plants and macroalgae. The seagrass, *Posidonia oceanica* indicated compensation of growth after simulated grazing (Vergés et al. 2008), and it has been suggested that seagrass beds were compensated for green turtle grazing through removal of detrital material away from the beds, reducing anoxia of the sediments (Jackson 2001). Research conducted on coralline algae revealed compensatory growth by the algae when exposed to moderate grazing intensity. Compensation in growth by the algae was due to possible epiphyte removal by the grazers (Littler et al. 1995). More recently, a study conducted in Chile indicated that the brown alga, *Macrocystis integrifolia* compensated for grazing by the amphipod, *Peramphithoe femorata*, through a reallocation of resources (translocation) from grazed to ungrazed portions of the alga (Cerda et al. 2009).

A model system for studying possible compensatory growth strategies in the marine environment is the relationship between the giant kelp, *Macrocystis pyrifera*, and its grazer *Chlorostoma brunnea*, one of three species of turban snails that prey on *Macrocystis* in central California. Three species of turban snails (*Chlorostoma brunnea*, *C. montereyi*, and *Promartynia pulligo*) graze directly on attached *Macrocystis pyrifera* and are highly abundant in central California (Watanabe 1984a), with densities of 150 to 350 turban snails per *Macrocystis* sporophyte (Watanabe 1984a, Table 1). These herbivores use *Macrocystis* as their preferred food source and shelter from benthic

predators such as *Pisaster giganteus* and *Pycnopodia helianthoides* (Watanabe 1984b). Although mortality of an adult *Macrocystis* sporophyte by these mesograzers has not been described, indirect effects may alter the structure of the kelp forest. Species that graze on *Macrocystis* adults that do not directly remove individuals may, through the weakening of tissues, leave the sporophytes vulnerable to surge, epiphytes, and bacterial infections thereby, indirectly removing all or parts of the sporophyte (Foster and Schiel 1985). Grazing also may lead to a reduction in reproductive potential by removing reproductive blades or causing stress to the sporophyte, initiating reduction in production of sori in favor of allocation of materials for new growth (Graham 2002). Reduction in sporophylls and reduction in sorus area on existing sporophylls would lead to loss of zoospore production, therefore, a decrease in reproductive potential (Graham 2002).

Giant kelp forest communities are considered one of the most productive communities within the marine environment (McFarland and Prescott 1959, McLean 1962), and *Macrocystis* yields between 350g-1500g C m<sup>-2</sup>year<sup>-1</sup> within the shallow California temperate seas (Mann 1982). Gross anatomy of the *Macrocystis* sporophyte, or individual, includes the holdfast, stipes, blades, and pneumatocysts. Holdfasts are made of finger-like projections called haptera that attach the sporophyte to the substrata. Stipes crop up from the holdfast and are dichotomously branched giving rise to the apical meristem from which blades grow. Pneumatocysts are gas-filled sections that connect the blades to the stipes and allow the stipes and blades to extend vertically in the water column. Photosynthesis occurs in all areas of the sporophyte with the majority of production occurring within the biomass of the seasonally extensive canopy that is

created at the water's surface (North 1994). Growth rates (elongation rates) of *Macrocystis* fronds (stipes + blades + pneumatocysts) are as great as 5.6 - 8.0 percent per day in southern California (North 1971b) and 2.7 - 6.8 percent per day in central California (Phillips et al. 1988), with the highest growth rates occurring during periods of greatest nutrient concentrations (usually during winter-spring, or upwelling periods) (Zimmerman and Kremer 1986).

Fronds grow continuously until the end of their life span (about 6-9 months), at which point the apical scimitar is no longer evident, but is replaced by a terminal blade (North 1971a, Gerard 1976, Lobban 1978). Once the frond stops growing, it begins to senesce and is replaced by juvenile fronds. Translocation of growth materials generally occurs from the older dying frond to the new frond initials growing up from the base of the parent frond (Lobban and Harrison 1994). Senescence of blade material can also occur through grazing damage and through the invasion of microbial pathogens within the laminae (North 1979b, Lobban and Harrison 1994).

The reproductive parts of the *Macrocystis pyrifera* sporophyte include the sporophylls (blades bearing sporangia found at the base of the sporophyte) and the sorus (distinct area on the sporophyll which bears sporangia) (North 1994). Sporophyll production (density, size, and fertility) is linked directly to zoospore production; therefore, sporophyll condition can be a proxy for reproductive potential in a *Macrocystis* sporophyte (Graham 2002).

In southern California, the effects of grazing by the amphipod, *Amphithoe humeralis*, on the blades of *Macrocystis* caused a prolonged reduction of reproductive

potential through sterility of sporophylls (Graham 2002). Therefore, turban snails, like *A. humeralis*, may cause diminution of sporophylls or reduce sorus area causing a decrease in reproductive potential. Such grazing effects by turban snails have been observed in Carmel Bay, central California, on the kelp *Pterygophora californica* (Foster and Schiel 1985). During times of greater turban snail densities, sporophyll growth was prevented or impeded by turban snail grazing. Therefore, grazing by turban snails may lead to a similar loss in reproductive potential of *Macrocystis*.

The effects of mesograzers, such as *Chlorostoma brunnea*, on *Macrocystis* and other kelps have not been investigated thoroughly because mesograzers' size and activity make it difficult for density manipulation in the field (Lobban and Harrison 1994, Davenport and Anderson 2007). Also, the lack of experimental studies in the kelp forest system is likely due to the difficulty in designing a way to test the effects of grazing on the production and fitness of *Macrocystis* in situ (Duffy and Hay 2000, Graham 2002). Although extremely abundant, *C. brunnea*, *C. montereyi* and *Promartynia pulligo* have been considered to have negligible effects on *Macrocystis* production (Foster and Schiel 1985). No researchers, however, have examined the effects of these turban snails on *Macrocystis* growth or reproduction and the paucity of effects observed in the field could be due to a possible compensatory growth by the alga in response to herbivory. Therefore, the primary objective of this study was to examine the effects of *C. brunnea* grazing on *Macrocystis* and test whether they were negative, or positive. If effects were found to be positive, the second objective was to test whether they indicated either compensatory growth (or threshold model), or overcompensation of growth (the grazing

optimization curve). In the presence of this abundant herbivore, it would seem that evidence of a grazing effect would be evident on the growth rate or reproductive potential of the *Macrocystis* sporophyte. However, if an effect is not evident, this would indicate that *Macrocystis* can sustain this abundant gastropod without any negative or positive impacts.

## METHODS

### *Study site*

Field experiments and collections occurred at Stillwater Cove, in Carmel Bay, California (36°34'N, 121°56'W), which is located on the southern coast of the Monterey Peninsula and contained a kelp bed well-protected from storm swell and a substrate of moderate-relief sandstone, conglomerate, and lava (Reed and Foster, 1984; Figure 3). *Macrocystis pyrifera* (giant kelp) was the dominant surface canopy and grew at depths of up to 30 meters. This location contained a high abundance of all study species (Hunt 1977; McMillan unpublished data). This particular site has been the subject of many scientific studies, and was in close proximity to a previous study on turban snails and *Macrocystis* (Hunt 1977).



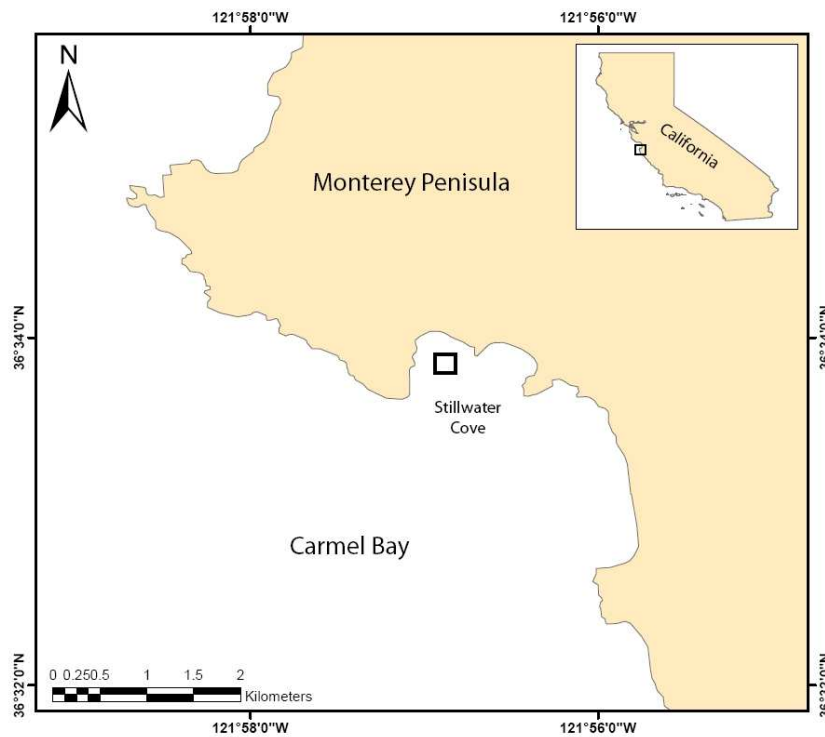


Figure 3: Map of Carmel Bay, Carmel, California. Study site is indicated by the black square within Stillwater Cove. Map courtesy of Kristen Hunter-Thomas.

### *Distribution of turban snail species within Stillwater Cove*

In order to evaluate the abundance, density, and size distribution of turban snails on *Macrocystis pyrifera* individuals within the study site, SCUBA surveys were conducted in November 2007, on randomly selected *Macrocystis* sporophytes (n=6) between depths of 7-12m within Stillwater Cove. Depth of each *Macrocystis* individual surveyed was determined, and the number of stipes longer than one meter were counted and recorded. All turban snails were collected by hand, measured, and identified to species. Snails were separated by placing them into a series of four 19-liter buckets with 2.5, 2.0, 1.5cm diameter holes drilled into the bottom; the bottom bucket had no holes.

The bucket with no drilled holes was used on the bottom tier to collect all snails less than 1.5cm in diameter. These four sizes were chosen to distinguish between juveniles (<1.5cm) and sexually mature adults (>1.5) (adapted from Watanabe 1984a); sexually mature snails were then separated into three size bins to estimate average size for each species (>2.5cm, 2.0-2.5cm, 1.5-2.0cm). Once snails were identified and measured, they were returned to the water. Turban snail assemblages on *Macrocystis* were assessed, as total number of each species per stipe; there was no significant difference in mean densities among the three snail species (ANOVA:  $F_{2,15} = 0.033$ ,  $p = 0.978$ ). The average density of snails per sporophyte was 255.5 ( $\pm 30.1$ SE) and the dominant size was 2.0-2.5cm (Table 1, Appendix A). Conversely, Watanabe (1984a) found that population densities of *Chlorostoma brunnea* and *Promartynia pulligo* within the nearby kelp bed of Hopkins Marine Reserve (HMR) were similar overall, but *P. pulligo* was observed at a higher rate on sporophytes at the same depths that I surveyed at Stillwater Cove (7-12m) (1984a). *Chlorostoma montereyi* were not found with high frequency at HMR and were considered rare overall.

Table 1: Mean ( $\pm$ SE) number of turban snails per *Macrocystis pyrifera* sporophyte (n= 6) by species and size (cm) found in Stillwater Cove, Carmel, California.

Snail species	Shell diameter (cm)	Mean No./sporophyte
<i>Promartynia pulligo</i> (n=511)	>2.5	0.17 (0.17)
	2-2.5	41.50 (12.51)
	1.5-2	31.17 (7.57)
	<1.5	12.33 (3.11)
	Total	85.17 (19.24)
<i>Chlorostoma montereyi</i> (n=494)	>2.5	2.17 (0.70)
	2-2.5	44.33 (10.75)
	1.5-2	21.83 (3.36)
	<1.5	14.0 (3.10)
	Total	82.33 (12.71)
<i>Chlorostoma brunnea</i> (n=528)	>2.5	0.17 (0.17)
	2-2.5	34.17 (8.15)
	1.5-2	33.00 (6.15)
	<1.5	20.67 (8.58)
	Total	88.00 (14.15)
Mean No. of Total Snails/ Sporophyte		255.50 (30.10)

To reduce confounding factors related to using multiple species of snails in my study, I chose to use only one of the three subtidal turban snail species present. *Chlorostoma brunnea* had significantly greater per capita consumption rates (75.12 mg/snail/day) when compared with *C. montereyi* and *Promartynia pulligo* (45.25 mg/snail/day and 48.44 mg/snail/day, respectively; Watanabe 1984b); therefore, if a grazing effect was present, it would likely be observed with *C. brunnea*. Therefore, *C. brunnea* at 2.0-2.5cm in diameter (the mean snail diameter found in preliminary surveys) was selected for all experimental manipulations.

*Field experiments of Chlorostoma brunnea grazing on Macrocystis pyrifera*

The effects of turban snail herbivory on the growth rate, reproduction, and survival of *Macrocystis* were quantified using a field experiment. Twenty *Macrocystis* individuals were selected and tagged using bicycle tape marked with numbers 1-10 and attached to the holdfast via a zip tie. All sporophytes were located in Stillwater Cove and were used to create 10 treatment levels, 5 artifact controls, and 5 controls (Figure 4). Sporophytes occurred at similar holdfast depths (~8 meters) and were in close proximity to each other, yet far enough apart to reduce mixing of fronds at the surface canopy (~10 meters). All peripheral *Macrocystis* sporophytes were removed within 10 meters of each individual used in the study. This limited the amount of emigration and immigration of the snails through the canopy (Watanabe 1984a). Extraneous sporophytes were bundled, tagged with a buoy, and then stipes were severed at the holdfast sending the individuals to the surface intact. All detached sporophytes were exported from the site to reduce the amount of drift material and potential tangling with experimental sporophytes.

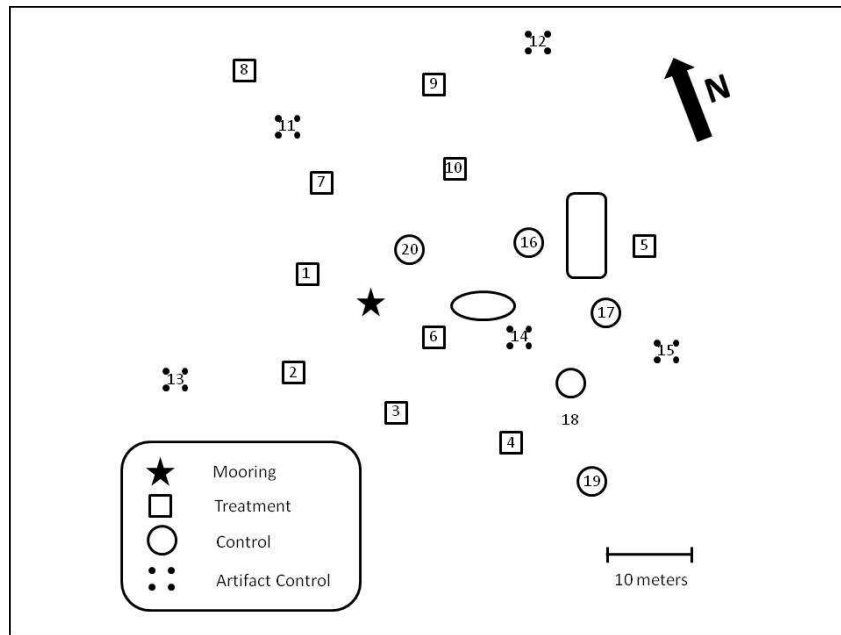


Figure 4: Study site within Stillwater Cove, Carmel Bay, Carmel, California, indicating controls, artifact controls, and treatment.

Cages ( $1\text{m}^2$ ) were placed around the base of each experimental kelp plant to reduce immigration of snails and emigration of *C. brunnea*. Each cage was constructed of a  $\frac{1}{2}$ " copper frame impregnated with rebar for increased durability and weight. Mollusks have an aversion to copper and will not crawl across it (Johnson 1992, McMillan 2009). The frame was elevated on four legs (20cm in height) that were used to secure the cage to the surrounding substrate via stainless steel eyebolts drilled into the substrate and secured with marine epoxy (Figure 5a). On each frame, 6.5cm mesh nylon netting was attached and formed a "skirt" around each holdfast. The skirt was cinched midway around the holdfast preventing snails from climbing on or off the sporophyte via the holdfast, creating a moat around the base (holdfast) of the sporophyte (McMillan 2009; Figure 5b). Each of the five artifact controls also were treated with copper cages

but with no netting attached to the holdfast to allow free movement of snails on and off the sporophyte. This treatment allowed for detection of any effects of the copper cage on *Macrocystis* physiology (Figure 5c). The control sporophytes were not manipulated in any way except for the removal of periphery sporophytes within 10 meters of the individuals.

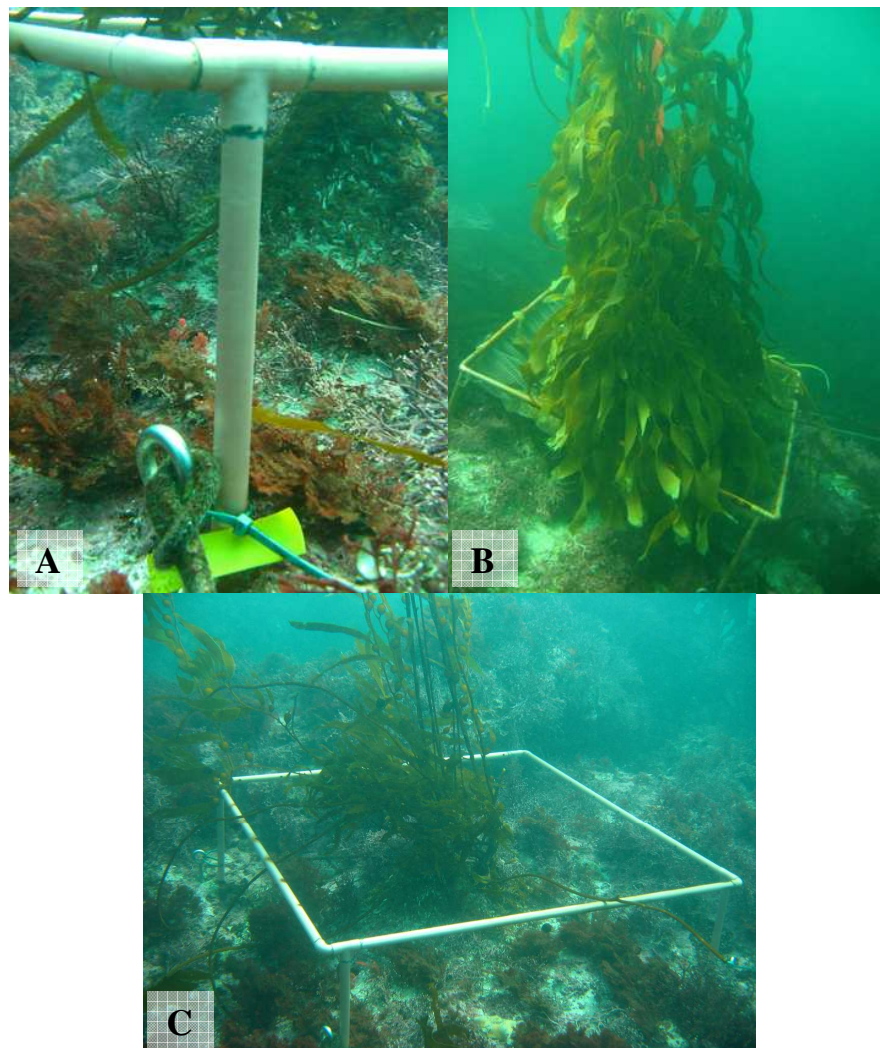


Figure 5: Images of copper inclusion/exclusion cages installed in Stillwater Cove, Carmel, California in the fall of 2007. Images include: A) picture of cage leg attached to eyebolt and secured to substrate, B) treatment cage with mesh, and C) artifact control cage with no mesh. (Images A and B from McMillan 2009).

The ten treatment sporophytes were randomly stocked with *C. brunnea* as follows: 0, 50, 100, 150, 200, 250, 300, 350, 400 and 450 snails per sporophyte with the median density of 250 snails, reflecting the average density of snails found in previous surveys (see above). The snails used to stock the kelp sporophytes were collected from the kelp forest within Stillwater Cove near the experimental site. As with surveys of turban snails, *Macrocystis* sporophytes were randomly chosen and all snails collected, sorted by size and species, and all *C. brunnea* between 2.0cm and 2.5cm (mean size of *C. brunnea* individuals collected within Stillwater Cove) were used to stock the treatments. The number of snails was monitored monthly to insure it remained constant for each treatment throughout the experiment (McMillan 2009).

#### *Macrocystis pyrifera growth and reproductive potential*

Five fronds were tagged on each sporophyte with numbered spiral poultry bands to identify and track growth rates of individual fronds. Throughout the experiment, frond loss was recorded and new fronds were tagged to maintain at least five fronds on each sporophyte. Growth was determined by measuring the length (to nearest 5cm) of each tagged frond from the base of the frond to the beginning of the apical meristem for each sporophyte.

To determine changes in growth, existence of reproductive sporophylls, and reproductive potential, surveys of all *Macrocystis* individuals were conducted bi-weekly between September 12<sup>th</sup> and January 11<sup>th</sup> of 2007. All treatment plants were relieved of all conspicuous gastropods to ready the sporophytes for stocking. Pre-stocking surveys

were conducted between September 12<sup>th</sup> and October 31<sup>st</sup>, before snails were added and all results were interpreted as growth, and reproductive potential of plants with natural *C. brunnea* densities. The sporophytes were cleared and stocked between October 31<sup>st</sup> and November 11<sup>th</sup> and surveyed until December 4<sup>th</sup> at which time a large storm destroyed and/or removed all cages and the experiment was concluded. Post-disturbance surveys were conducted January 2<sup>nd</sup> and January 11<sup>th</sup> of 2008.

For the field experiment, more than one value was recorded for each sporophyte, (growth rate for individual fronds). Therefore, I used the mean of the multiple values for each growth variable per sporophyte for statistical analysis, and each sporophyte was considered as one replicate. Growth of *Macrocystis* individuals across all treatments (treatments, artifact controls, and controls) were examined before the manipulation of snail densities and grew as predicted by previous studies of *Macrocystis* growth (North 1971). Frond elongation rates (m/frond/day) were significantly correlated with initial frond lengths. There was a significant positive relationship between growth rates of all tagged fronds and initial frond lengths; however, it was not exponential ( $F = 99.104$ ,  $df = 120$   $R^2 = 0.452$ ,  $p < 0.001$ ; Figure 6a). Therefore, all growth rates were determined using the standardized formula:

$$\text{Standardized Growth Rate} = \frac{\text{Ending Length} - \text{Initial Length}}{\text{Initial Length} * \text{Days}}$$

where ending and initial lengths were measured in meters (to nearest 5 cm) and time was measured in days.



Once standardized, the initial growth rates of all fronds were not significantly different ( $F = 0.010$ ,  $df = 120$ ,  $R^2 < 0.001$ ,  $p = 0.92$ ; Figure 6b), therefore, could be analyzed for changes in growth rates due to treatment effects.

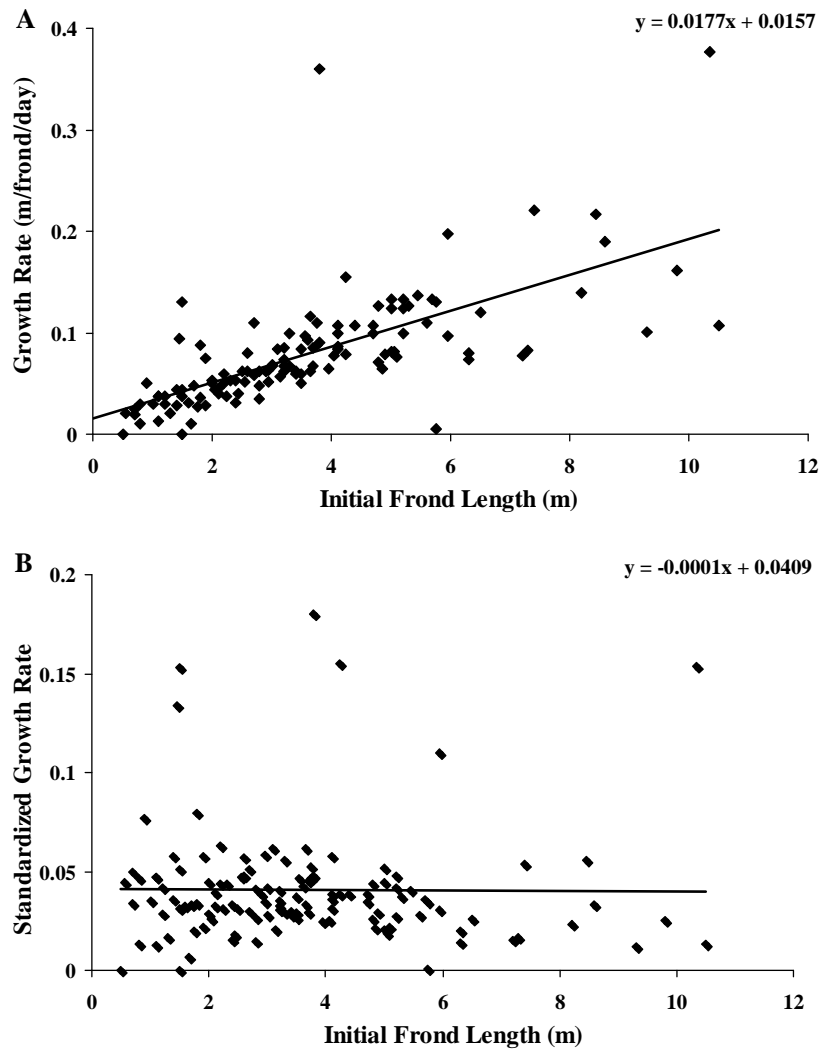


Figure 6: *Macrocyctis pyrifera* growth prior to manipulation of snail densities on all (control artifact control and treatment) sporophytes. Graphs are as follows: A) relationship of growth rates (m/frond/day) to initial frond lengths of all sporophytes and B) relationship between standardized growth rates of fronds to initial frond lengths for all sporophytes.

Artifact controls and controls were examined for differences among sporophytes for each treatment and between treatments to investigate a possible cage effect using a one-way ANOVA ( $\alpha = 0.05$ ). Homogeneity of variance was tested using Levene's test and normality with a Kolmogorov-Smirnov test.

Sporophylls were examined for sori presence, and reproductive potential was quantified by estimating the sorus area of haphazardly chosen reproductive sporophylls on each individual (Graham 2002). The overall sporophyll sorus area of each sporophyte (sporophyll condition) was quantified using the following designated values: 0 = no sori present, 1 = sori appeared at pneumatocyst end of sporophylls, 2 = sori found primarily in the middle of sporophylls, 3 = sori appeared at the end of sporophylls, 4 = sori covered the entire length of sporophylls, 5 = sori covered entire length of sporophylls and sporophylls were sloughing. These conditions (with condition 5 having the greatest reproductive potential) were used to compare reproductive potential among experimental groups (treatment, control, and artifact control) and over time.

Reproductive potential was examined by analyzing the relationship of sporophyll condition to treatment levels before and after the manipulation of snail densities. For example, if *C. brunnea* grazing negatively affected reproductive potential, a change from a greater condition to a lesser condition would have indicated a reduction in sori, therefore, a loss in reproductive potential (e.g., condition 5 to condition 2). If the relationship between reproductive potential and *C. brunnea* grazing reflects the grazing optimization hypothesis, a second-order polynomial curve would indicate that at lesser and greater densities of snails, lesser sporophyll condition occurred, whereas at moderate

densities of snails, I would expect greater sporophyll condition, meaning an increased reproductive potential.

*Laboratory experiments of Chlorostoma brunnea grazing on Macrocystis pyrifera growth*

Laboratory experiments were conducted to better assess the strength of the effect of varying densities of *Chlorostoma brunnea* on the biomass and growth rate of *Macrocystis pyrifera*. The laboratory environment minimized environmental stressors *Macrocystis* individuals may incur in the field such as incumbent weather and herbivory by other grazers. *C. brunnea* and *Macrocystis* individuals were collected from the field site, Stillwater Cove in April 2009. Snails were placed in indoor aquaria for one week to acclimate to laboratory conditions. During the holding period, additional *Macrocystis* material was made available to the snails to ensure they were well fed. *Macrocystis* sporophytes were collected, weighed, measured, photographed, and placed in outdoor mesocosms within 48 hours of collection.

Sixteen outdoor 208-liter tanks plumbed with running seawater housed the study subjects during the experiment. A sprinkler system and bubbler wands were used in each tank to reduce the desiccation of canopy fronds and increase water circulation. Three young sporophytes of *Macrocystis* (1-2 meters in height) were attached to holdfast holders on the bottom of each mesocosm.

Four densities of *C. brunnea* (0, 30, 60, and 120 individuals/tank of 2-2.5cm aperture diameter) were replicated in 4 tanks each. To determine whether the amount of snails in the experimental tanks was reflective of densities observed in the field, a post-

hoc evaluation of biomass to snail abundance ratio was conducted. On November 24, 2010, 48 fronds from four *Macrocystis* sporophytes were collected from Stillwater Cove. The fronds were brought to Moss Landing Marine Laboratories, measured (to the nearest 5cm) and weighed (to the nearest 0.5 kg). Regression analysis indicated a significant linear relationship between frond length and frond weight ( $F = 119.6$ ,  $df = 45$ ,  $R^2 = 0.727$ ,  $p < 0.001$ ; Figure 7). The slope of 101.3 g/m for the regression was less than a previously recorded value of 260 g/m for California (Nyman et al. 1993). However, the latter value was recorded for fronds during summer (June) and in southern California where production values are considerably greater (North 1994).

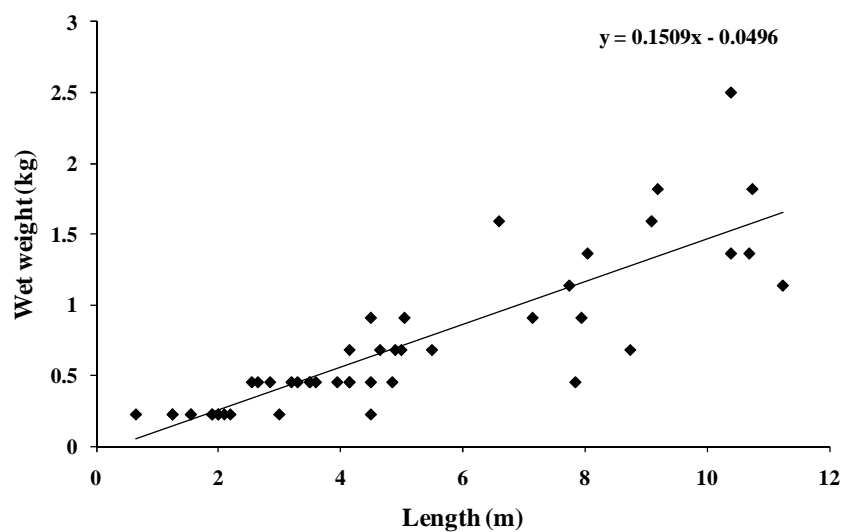


Figure 7: Relationship between *Macrocystis pyrifera* frond length (m) and wet weight (kg) of fronds on November 24, 2010. (n = 48)

The equation of the regression line ( $y = 0.1509x - 0.0496$ ) was then used to determine the average snail density per kilogram of *Macrocystis* biomass. Using the average length of *Macrocystis* fronds from my experimental sporophytes (4.4m) and the

average density of snails per sporophyte from my previous snails surveys (255.5 snails/sporophyte), I determined that the average number of *Chlorostoma brunnea* per tank should be about 11 snails. However, after a preliminary experiment, it was noted that only about 20-30% of the number of stocked snails remained on the *Macrocystis* material after several days (personal observation). This reflects the finding by Watanabe (1984b) that 70% of 1,500 turban snails tagged and released on kelp sporophytes to move off of those individuals within 15 days. Therefore, the densities of *C. brunnea* used in this experiment were not excessive.

At the initiation of the experiment (April 5, 2009), all sporophytes were weighed wet, and all fronds on each sporophyte were tagged with numbered spiral poultry bands to identify and track growth rates of individual fronds. Growth was measured using the methods described previously for the field experiment. Weight and length measurements occurred one week after the initiation of the experiment (April 12, 2009) and again at the termination of the experiment on April 15, 2009.

For the laboratory experiments, more than one value was recorded (growth rate for individual fronds) for each tank. Therefore, I used the mean of multiple values for each growth variable per tank for statistical analysis, and each tank was considered as one replicate. Growth rates (m/day) were significantly correlated with initial frond lengths; however, unlike the sporophytes used in the field experiment (0.5m – 11m), the laboratory individuals ranged from 0.14m – 2.0m in length, therefore, did not follow the same pattern as the fronds measured in the field. Therefore, all laboratory growth rates were standardized using:

$$\text{Standardized Growth Rate} = \frac{\text{Ending Length}}{\text{Initial Length} * \text{Time Elapsed}}$$

where ending and initial lengths were measured in meters and time was measured in days.

Any treatments with less than three data points at the end of the experiment were removed from the analysis. All results for growth were analyzed using a regression analysis (SPSS 16.0,  $\alpha = 0.05$ ) to test for either: 1) a linear relationship or 2) the relationship that approximated the first derivative of a sigmoid population growth model and the GOH curve.

A significant positive linear regression would indicate that *C. brunnea* grazing had a positive impact on growth and/or reproductive potential, whereas a negative linear response would indicate the traditional grazer-macroalgae relationship as found in most herbivory studies. A significant regression line that followed a positive second-order polynomial relationship would indicate that the grazing by *C. brunnea* on *Macrocystis* growth and/or reproductive potential was consistent the GOH curve.

To determine loss of *Macrocystis* tissue due to a range of densities of *C. brunnea*, biomass measurements taken in the laboratory experiment were calculated as percent biomass loss. This loss of biomass would indicate a loss in production; therefore, represent an additional measure of productivity to test the effects of snail grazing on *Macrocystis*. Data were analyzed using a one-way Analysis of Variance (ANOVA) followed by Tukey's post-hoc test to detect differences among treatments (SPSS 16.0,  $\alpha = 0.05$ ). Homogeneity of variance was tested using Levene's test and normality with a Kolmogorov-Smirnof test.

## RESULTS

### *Determining effects of Chlorostoma brunnea grazing on Macrocystis pyrifera growth and reproductive potential (field experiment)*

The average standardized growth rate (SGR) was  $(0.019 \pm 0.002 \text{ SE})$  for all treatment sporophytes before the manipulation of *Chlorostoma brunnea* densities. There was no relationship between Standardized Growth Rate and snail densities for linear ( $F = 0.601$ ,  $df = 8$ ,  $R^2 = 0.07$ ,  $p = 0.46$ ) or non-linear ( $F = 2.568$ ,  $df = 7$ ,  $R^2 = 0.423$ ,  $p = 0.146$ ) trends, indicating no pre-existing bias in the data (Figure 8).

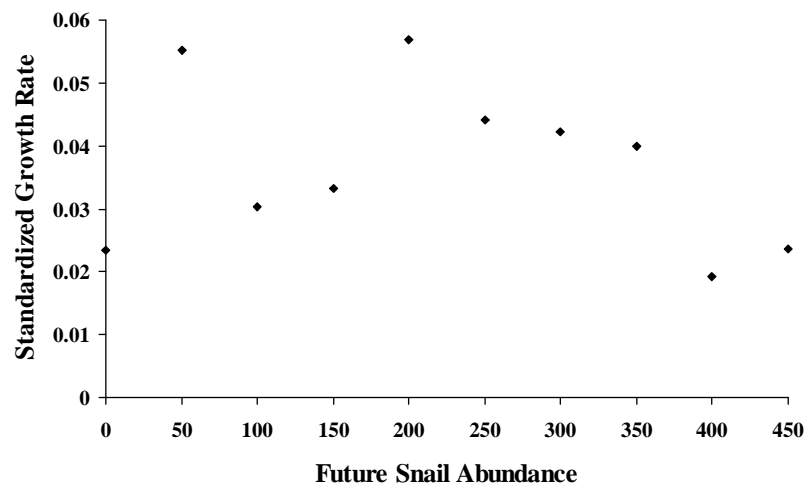


Figure 8: Standardized growth rates of each sporophyte and the snail densities with which they will be stocked. No pattern of standardized growth rate was evident among treatment sporophytes prior to stocking.

There were no significant differences in SGRs among the control or the artifact control sporophytes, so both treatments were grouped for analyses between the two controls (Appendix B). SGR was significantly less for the artifact control sporophytes

(0.022±0.003 SE) than the controls (0.097±0.011 SE) indicating a possible effect of copper on *Macrocystis* growth ( $F_{1,28} = 39.159$ ,  $p < 0.001$ ).

Due to the unexpected termination of the experiment by an extremely destructive winter storm (Lewitsky et al. 2008), the amount of time that elapsed from implementation of varying snail densities on the treatment sporophytes and the last sampling event of the experiment was less than one month. Therefore, the standardized growth rates of each sporophyte from the final sampling event (last two weeks) was subtracted from the initial sampling event (first two weeks) to determine the difference in frond elongation rates among treatment individuals. The data for the treatment sporophyte with 350 snails was removed from the analysis (< 3 data points available). There was no significant linear trend ( $F = 0.143$ ,  $df = 7$ ,  $R^2 = 0.201$ ,  $p = 0.716$ ), however, the second-order polynomial regression was significant ( $F = 9.042$ ,  $df = 6$ ,  $R^2 = 0.751$ ,  $p = 0.015$ ; Figure 9), mimicking the GOH curve. As *C. brunnea* densities increased, the frond elongation rate of *Macrocystis* increased from negative values (meaning lesser growth than the initial sampling event) until moderate densities of snails were reached (250 snails) where the greatest growth was positive relative to initial values. Standardized growth rate then decreased with increasing snail densities. The difference in growth was near zero for the moderate densities of snails, but standardized growth rate was less than zero for all other densities.



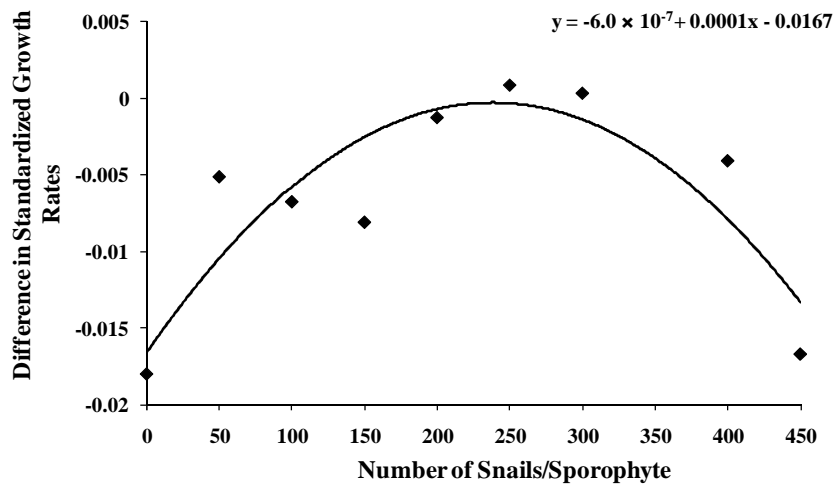


Figure 9: Difference in standardized growth rates sporophytes from initial (sampling period 1) and ending (sampling period 2) sampling dates (November 14<sup>th</sup> and 28<sup>th</sup> of 2008) after manipulation of snail densities in Stillwater Cove plotted against the number of stocked snails.

Reproductive potential of treatment sporophytes had no significant relationship with snail densities (Figure 10). All sporophylls were quantified as 4 or 5 during the initial sampling event after stocking. The majority of the sporophytes remained a 4 (sori covered the entire length of sporophylls) or a 5 (sori covered entire length of sporophylls and sporophylls were sloughing) during the second sampling event. However, I observed a loss in reproductive potential for the sporophyte stocked with 100 *Chlorostoma brunnea* (from 4 to 0, meaning the sori covered the sporophylls to no sori were present). This loss is not explained by the snail density pattern (i.e., one would expect to see sori losses due to greater grazing pressure). The largest change in reproductive potential occurred after the winter storm; however, no pattern due to prior grazer abundance was detected. In fact, the sporophyte with the greatest stocked snail abundance (450 *C. brunnea*) maintained its reproductive/sloughing state (sporophyll condition = 5).

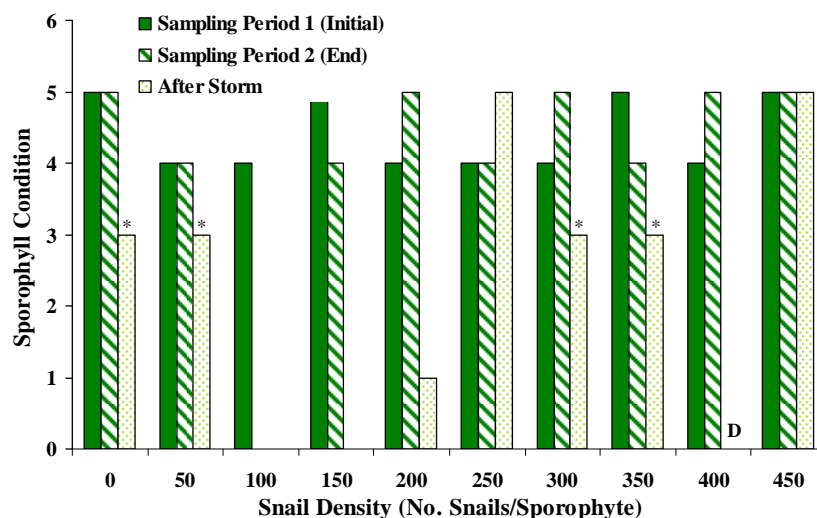


Figure 10: Quantitative condition of sporophytes from initial (sampling period 1) and ending (sampling period 2) sampling dates (November 14<sup>th</sup> and 28<sup>th</sup> of 2008) after addition of snail densities and after the decadal storm (January 2<sup>nd</sup>, 2008). All sporophytes with a sporophyll condition of 5 and all bars with an asterisk had sloughing sporophylls. The letter “D” in the graph indicates a sporophyte removal due to the storm.

*Effects of Chlorostoma brunnea densities on Macrocystis pyrifera growth*  
(Laboratory experiments)

*Macrocystis* growth rates in outdoor mesocosms were minimal (mean = 0.007 m/frond/day; SE  $\pm$  0.0006) relative to field experiments (mean = 0.072 m/frond/day; SE  $\pm$  0.005); therefore, detection of differences between treatments was less pronounced. Still, the results of the laboratory experiment mirrored the findings of the field snail manipulations. No linear relationship was found between *C. brunnea* grazing and standardized growth rate of *Macrocystis* sporophytes ( $F = 1.182$ ,  $df = 14$ ,  $R^2 = 0.078$ ,  $p = 0.295$ ). However, snail densities affected growth significantly when data were analyzed with a second-order polynomial regression ( $F = 4.362$ ,  $df = 13$ ,  $R^2 = 0.402$ ,  $p = 0.036$ ; Figure 11). As grazing intensities increased from zero (through the addition of snails),

production increased and was greatest at moderate snail densities (30-60 snails/tank).

Growth decreased as snail densities increased to 120 snails/tank, indicating a density at which *Macrocystis* cannot compensate for the grazing.

This overcompensation is evident as the curve is higher at moderate snail densities than at zero snails and high densities.

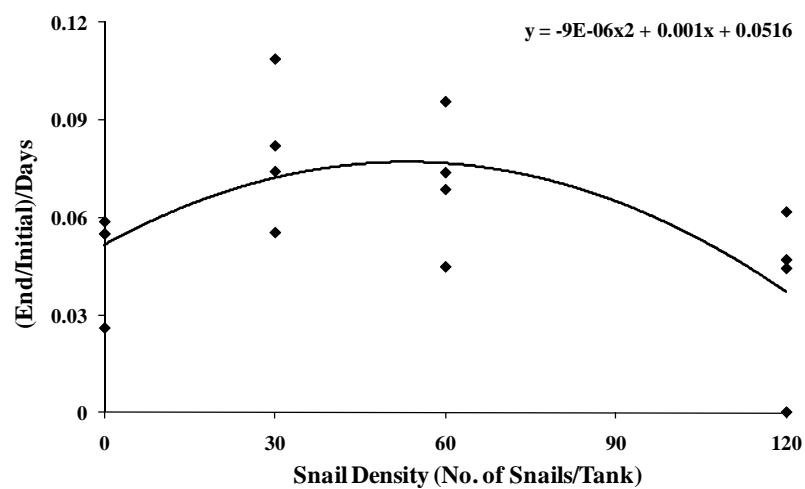


Figure 11: Standardized growth rate of *Macrocystis pyrifera* in each mesocosm plotted against number of snails in corresponding tanks.

Percentage loss for *Macrocystis pyrifera* biomass for each mesocosm treatment of varying snail densities during the entire experimental period was significantly different ( $F_{3,12} = 5.881$ ,  $p = 0.01$ ; Figure 11). Percentage biomass loss was significantly greater for the tanks with the greatest number of *C. brunnea* (120 snails) (Tukey's Honestly-Significant-Difference Test). No significance difference was found among any of the other treatments.

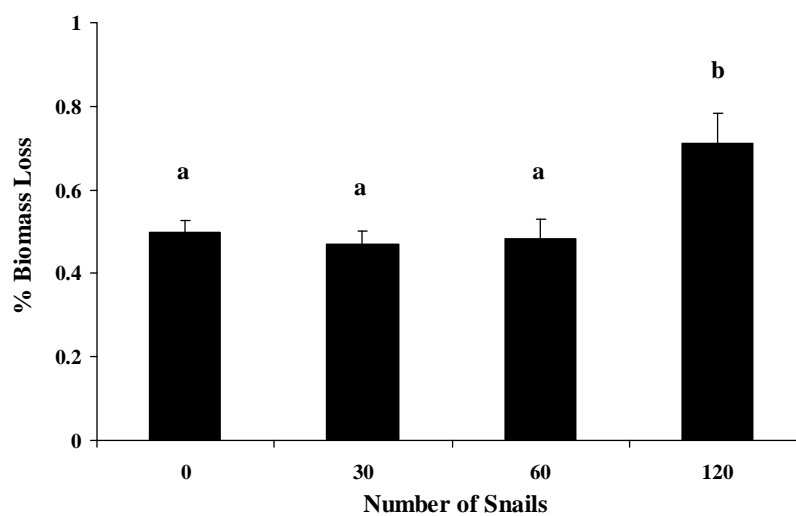


Figure 12: Percentage biomass loss in mesocosms with 0, 30, 60, and 120 snails per tank. Letters represent significant ( $\alpha = 0.05$ ) differences between treatments.

## DISCUSSION

During the fall season, within Stillwater Cove, *Macrocystis* sporophytes overcompensated for grazing by the trochid snail, *Chlorostoma brunnea* at moderate densities and had lesser productivity at low and high snail densities. After a large, winter storm occurred, evidence of hindrance by turban snail grazing on sporophyte recovery was observed on several *Macrocystis* individuals as previously described (Foster and Schiel 1985, personal observation). Laboratory experiments conducted during spring indicated a similar overcompensation of growth by *Macrocystis* in the presence of low to moderate *C. brunnea* densities. These studies indicate that the grazing optimization hypothesis may best explain the interaction between a macroalga and a mesograzer in central California giant kelp systems.

Previous researchers of primary production in *Macrocystis* have shown a positive linear relationship between growth rates and frond lengths (North 1971b). Growth rates of *Macrocystis* sporophytes, before the manipulation of *C. brunnea* densities, were consistent with those results. Once a standardization equation was applied to the growth rates, no pattern was evident and variability was nominal. This indicated that the sporophytes were growing at similar rates and experiencing similar biotic factors, therefore, would respond to effects of grazing by varying densities of *C. brunnea* independent of other variables. Any changes seen in growth rates by the treatment sporophytes would be due to the manipulation of snail densities on the individuals.

The differences between the SGR of the artifact control sporophytes and the control sporophytes indicated a possible effect of copper on *Macrocystis* growth.

Additionally, the SGR of the treatment sporophytes before the manipulation of snail densities was similar to the SGR of the artifact control sporophytes (0.019 and 0.022, respectively). However, all treatment sporophytes experienced the same copper effect; therefore, the differences in growth rate indicated by the experiment were due to the manipulation of snail densities.

After the experimental addition of snail densities to treatment sporophytes, SGR of *Macrocystis* followed the grazing optimization hypothesis curve. This finding did not follow the traditional negative linear response expected by grazers on macroalgae, but rather, demonstrated the greatest overcompensation of growth by kelp in the presence of moderate snail densities (200-300 snails/sporophyte; Figure 9) when compared with growth in the absence of snails. The results were represented as a difference between the two post-treatment sampling events to demonstrate how growth changed over time with the manipulation of snail densities. Negative numbers represented those sporophytes that had a loss of productivity between the two sampling dates, whereas the sporophytes that had a difference in standardized growth rates (SGR) approaching or around zero did not change from the initial to the ending measurements (no or little difference in rate of production). The latter results were observed in snail densities that reflected the average number of snails found per sporophyte in a previous survey within Stillwater Cove (Table 1). At densities found in nature, growth of *Macrocystis* was not compromised; however, the reduction of growth at low and high densities of snails relative to the average densities alluded to a mutualistic relationship between *Macrocystis* and these grazers. With *C. brunnea* at moderate densities, production of *Macrocystis* was optimized relative

to production at lesser and greater grazing intensities. More importantly, *Macrocystis* production at moderate densities of snails was greater than production without snails. This indicates compensation by *Macrocystis* for the natural grazer intensities found within the cove. Without these moderate densities of snails, one would expect to see productivity rates drop reflecting those found at the low and high stocked snail densities in the experiment.

To compare growth rates for the treatment, *Macrocystis* sporophytes for the months of October (prior to manipulation of *C. brunnea* densities) and November (after the manipulation) of 2007 to previously recorded growth rates for the area, I used growth rates obtained from a study conducted at Hopkins Marine Reserve (HMR), Monterey, California from 1985-1989 (Watanabe, unpublished data). These data were calculated using the instantaneous daily rate equation:

$$\text{IDR} = 100 * \ln (L1 / L0) / \text{Days}$$

where I assumed exponential growth, and L0 is beginning length and L1 is end length.

The average of the rates obtained by Watanabe for October and November were compared with an average of the two sampling dates (10/16/2007 and 10/31/2007) for October and the two sampling dates 11/14/2007 and 11/28/2007) for November.

Instantaneous daily growth rate (IDR) for treatment sporophytes before manipulation of snail densities (October) was  $2.770 \text{ SE} \pm 0.104$  and after (November) was  $1.683 \text{ SE} \pm 0.085$ . Watanabe's reported greater IDR's for HMR at  $3.73 \text{ SE} \pm 0.249$  for October and  $3.30 \text{ SE} \pm 0.158$  for November. The difference in growth rates was not surprising given that total nitrogen concentrations for Stillwater Cove usually are less than those for

Hopkins Marine Reserve and may limit growth of *Macrocystis* during fall (Jackson 2005, PISCO unpublished data). The IDR between the October and November months of the experimental sporophytes reflected loss of production due to manipulation of snail densities.

Reproductive potential indicated little change during the experiment. This lack of relationship between grazer density and sori development can be explained by the short time frame in which the experiment took place (28 days). A previous study indicated that sporophylls with greater levels of grazing by the amphipod, *Amphithoe humeralis*, did not have complete loss in reproductive potential for 3 months, at which time a sudden temperature change may have attributed more to the sterility of the sporophylls than the influx of grazing (Graham 2000). Similarly, between my two sampling periods there was no overall loss in reproductive potential. However, the appearance of a decadal storm disturbance at the end of the experimental period initiated a loss of sori area, reflecting the speed of transition as observed by Graham (2000) with an extreme temperature change.

Laboratory experiments demonstrated a similar pattern as the field manipulations. The growth rates for the laboratory sporophytes were considerably less, but this was due to the small size of the sporophytes compared with field individuals, the translocation from the field at 3m depth to a small 0.5m tall tank, and the differences in irradiance. The range of snail densities in the outdoor mesocosms did not directly reflect the snail abundances applied to the sporophytes in the field, but did reflect grazing intensity by the snails (only 20-30% of stocking densities remained on the *Macrocystis* fronds within the



mesocosms). The relationship between SGRs for *Macrocystis* and the four *C. brunnea* densities supported the grazing optimization hypothesis, and provided more evidence of the positive trophic interaction that was induced by moderate grazing intensities.

Growth rates were greatest at moderate snail densities but percentage biomass loss indicated only compensation rather than overcompensation. The percentage biomass loss indicated no difference in loss of sporophyte frond material until the greatest densities of snails. Conversely, growth was found to be at its highest in the tanks with 30 and 60 snails. Therefore, one would assume biomass loss in those tanks would be less because production was greater. However, if the loss of biomass was not different between the tanks with no snails and the tanks where snail grazing was occurring at low and moderate levels, one can interpret this pattern as follows: 1) loss of tissues in fronds without grazing may be due to removal of older, senescent material; 2) loss of biomass in tanks with snails may be due to removal of epiphytic or endophytic growth that, through removal, enhances the productivity of the sporophyte. This level of productivity versus biomass loss indicates that the *Macrocystis* was compensating for the grazing by *C. brunnea*, except at greater stocking densities (120 snails), where biomass loss was greater than the other tanks and compensation of grazing did not occur.

The overcompensatory growth response observed in *Macrocystis* due to *C. brunnea* grazing in the field and in the laboratory could be attributed to many possible mechanisms. Within the realm of terrestrial plant biology, mutualistic evolutionary partnerships between plants and herbivores may explain this type of growth (McNaughton 1983); however the subject is under much scrutiny (Bergelson and

Crawley 1992, Belsky et al. 1993, Aarssen 1995). Other possibilities include: removal of senescent material by the snail, thereby allowing the *Macrocystis* individual to reallocate materials to growing parts of the sporophyte (Sargent and Lantrip 1952, Thrower 1967, Langer 1972, Schmitz and Lobban 1976, Lobban 1978, Manley 1984); removal of terminal, canopy forming fronds that would allow for increased light availability to the sub-canopy fronds (Lobban 1978, Luning, 1981 ; Reed and Foster, 1984); removal of epiphytic or endophytic organisms from blade material (Littler et al. 1995, Aumack et al. unpublished), thus increasing photosynthetic capabilities; or an increase of nitrogen availability through the excretion of ammonium levels by *C. brunnea* in close proximity to *Macrocystis* could lead to an increase in production (Hurd et al. 1994).

Studies of terrestrial plants have demonstrated preferential removal of old leaf tissue by grazers (Langer 1972) which created greater light intensity on younger previously shaded tissues (Jameson 1963). Turban snails graze more frequently on senescent blades than non-senescent material (Hunt 1977, McMillan personal observation); additionally, *Promartynia pulligo* prefers older material of some algae to younger material (Durante and Chia 1991). Senescent kelp material may have lesser C:N ratios than non-senescent material making senescent blades more nutritionally valuable to grazers (Yee et al. unpublished data). By removal of this senescent material, plants may redirect (translocate) material needed for growth to other areas of the sporophyte (McNaughton 1979). Also, through removal of this material, the individual kelp sporophyte may be less likely removed by winter storms due to the removal of extraneous fronds that may cause drag in high wave activity (Black 1976, Graham 1997).

Epiphytic fouling can lead to reduced photosynthetic ability and gas exchange, leading to lowered productivity rates of the algal host (Dodds 1991). For example, a reduction in productivity during the months of greatest growth potential was observed in *Macrocystis integrifolia* in British Columbia due to an increase in epiphytism on the fronds of the alga (Lobban 1978). Recent studies have indicated a trophic interaction between epiphytes, algae, and grazers. Grazing by the chiton, *Choneplax lata* on the crustose coralline alga *Porolithon pachydermum* increased biomass by removing competitive filamentous algae and increasing meristematic activity through the radulations of the grazing activity (Littler et al. 1995). Another study, conducted in the Western Arctic Peninsula, indicated that some algae, in the presence of amphipod grazers, had lesser epiphytic fouling and greater photo-efficiencies than algae without grazers (Aumack 2009). *Chlorostoma brunnea* grazing could potentially remove epi- and/or endobionts from the photosynthetic tissues of *Macrocystis*, hence increasing production. This could be done preferentially (snails preferring epiphytes more than *Macrocystis* tissue) or secondarily (epiphyte removal occurring only as a bi-product of snail grazing).

An increase in growth of *Macrocystis* due to nitrogen availability through the excretion of ammonium by encrusting hydroids occurred in New Zealand, when levels of nitrogen were limiting (Hepburn and Hurd 2005). The subtidal turban snails that graze on *Macrocystis* may also contribute to the total nitrogen available to the sporophyte. However, nitrogen availability within Monterey Bay was rarely limiting for *Macrocystis*

growth (Watanabe, unpublished data); therefore, ammonium enrichment by the snails was probably not the mechanism for overcompensation in growth.

Overcompensation by marine algae has not been demonstrated before this study. This does not mean that this trophic interaction does not occur in other marine systems. Recently, compensatory growth occurred in the temperate seagrass *Posidonia oceanica* in response to simulated grazing (Vérges et al. 2008). Growth rates of the seagrass shoots at four levels of grazing (none, low, moderate, and high) reflected that of overcompensation and the grazing optimization hypothesis. These results were not interpreted by the authors as evidence of overcompensation, which was probably due to the lack of interdisciplinary information shared between terrestrial and marine biology disciplines. Application of the grazing optimization hypothesis to a marine system is a novel approach to explaining positive effects of grazers on marine plants and algae. Current research into herbivore effects is usually conducted with only two levels of grazing (grazers present and no grazers). Further studies using the GOH as a model for compensatory growth relationships should be conducted within the herbivore-marine algae systems using a range of grazer densities..

The growth rate of *Macrocystis* is dependent on light, temperature, and nutrient availability, which are dynamic abiotic factors (Clendenning 1971, Jackson 1977). During summer, the rates of photosynthesis and growth in *Macrocystis* decreases (Clendenning 1971, Jackson 1977). However, greater temperatures increase consumption rates in many grazers including several species of turban snails (Leighton 1971, Yee and Murray 2003). This would suggest that in seasons of greater temperatures and lesser

nutrient availability, kelp growth would decrease, but turban snail grazing would increase leading to a more pronounced effect. Furthermore, during winter, high wave action due to storms tends to rip out *Macrocystis* fronds, damaging the plant and decreasing biomass (Seymour et al. 1989, Graham et al. 1997, Utter and Denny 1995). During this time, recovery of *Macrocystis* individuals may be hindered by turban snail grazing. Due to this seasonality component, it is important that future studies be conducted during all seasons (for at least one year) to capture any effects of season on turban snail grazing and *Macrocystis* production, fitness, and reproduction.

Perhaps *C. brunnea* is not preferentially grazing on senescent material, but the grazed material begins to senesce once the blade is grazed. Wounding by grazers may induce production of fungal and bacterial infections causing biomass loss through breakage of material weakened by infections (Foster and Schiel 1985). A species of periwinkle snail, *Littoraria irrorata*, grazing on live salt-marsh cordgrass, *Spartina alterniflora*, caused a proliferation of fungal pathogenic material (Silliman and Zieman 2001). The snails then used the fungi and senescing tissue as a primary food source rather than the living tissues of the plant. This interaction could possibly occur in the turban snail-*Macrocystis* system. Grazing scars on otherwise healthy blades indicated senescing tissue around the area of the grazer-induced wound (personal observation). Therefore, an investigation of possible grazer-induced fungal or bacterial infections would offer evidence of possible snail-pathogen interactions on the blades of *Macrocystis*.

To determine if the trophic interaction between *C. brunnea* and *Macrocystis* is a type of mutualistic association, studies should be designed to concentrate not only on the fitness of *Macrocystis* sporophytes, but the fitness of the snails when overcompensation by *Macrocystis* occurs. Mutualism is considered an interaction in which both species benefit from the relationship as opposed to those of that species that are not a part of the association (Agrawal 2000). Also, investigation into sustainability of this mutualism would indicate whether this interaction is a true mutualism (occurring all the time), or more likely, a conditional mutualism, where the association is only mutually beneficial under certain conditions. A study conducted for several seasons could capture the effects of turban snails on *Macrocystis* under different environmental conditions and under different *Macrocystis* production rates. I suspect that in times of greater production (i.e., the spring upwelling season), the effects of turban snails are negligible. However, in late summer, when production is lesser, the effects of these herbivores may be strong and a mutualistic interaction between *Macrocystis* and the turban snail species apparent.

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## CHAPTER II

### THE ROLE OF FUNGI IN THE TURBAN SNAIL-*MACROCYSTIS* SYSTEM

#### ABSTRACT

Researchers of trophic interactions in marine systems have traditionally investigated macroscopic organisms. Recent studies, however, have indicated strong associations among snails, marine plants, and fungal pathogens. Mesocosm experiments were conducted to investigate if snail grazing affects fungal biomass on the giant kelp, *Macrocystis pyrifera*, and how fungal biomass varied with temperature and densities of *Chlorostoma brunnea* (an abundant marine snail). These variables were manipulated and differences were examined in *Macrocystis* biomass, growth rates, and fungal biomass among treatments of high/low temperatures, snail presence/absence, and varying snail densities. In the presence of moderate densities of *C. brunnea*, *Macrocystis* remained intact, whereas fungal biomass was significantly less than treatments with no snails. However, at greater densities of *C. brunnea*, snails grazed directly on *Macrocystis* causing the degradation of the alga, and increasing fungal biomass. At moderate densities, the snail is a consumer of the fungi, and the *Macrocystis* acts as fungal substrate. Field surveys indicated significant differences in fungal biomass among wave exposure, bottom and canopy blades, and grazed and ungrazed blades of *Macrocystis*. These differences indicated interactions between *Macrocystis* and fungal pathogens that may be directly affected by turban snail grazing.



## INTRODUCTION

The examination of trophic interactions is important for understanding the positive and negative biological forces that affect organisms within an ecosystem (Paine 1980, Menge 1992, Forester et al. 1999, Bascompte et al. 2005). For years, researchers have examined primarily interactions that can induce lethal effects (Mann 1982, Strong 1992), and little investigation has been applied to the secondary interactions or indirect effects that may affect ecological communities (Paine 1980, Molis et al. 2010). Recent researchers have examined these formally unexplored relationships and found interactions (formally considered weak) that play strong roles in the top-down and bottom-up forces that drive population dynamics (Power 1992, Silliman and Zieman 2001).

A rarely investigated interaction in the marine system is that between fungal pathogens and algae. Few mycologists and phycologists have examined the ecology of marine fungi or how their presence might affect infected organisms and have only merely documented their existence on algal matter (Kohlmeyer and Kohlmeyer 1979). Fungal matter was identified on subtidal algae from beach rack; therefore, collection may have occurred after the algae started to rot on shore (S. Schatz, personal communication). Marine fungal pathogens may be strictly detritus feeders or saprophages, and do not have a direct effect on living algae or animals (Schatz 1984). Investigators have recently begun studying enzyme production in certain species of fungi and whether such fungi are capable of degrading live tissue, rather than simply digesting senescent tissue or detritus

(Chesters and Bull 1963, Wainwright 1980, Wainwright and Sherbrock-Cox 1981, Schaumann and Weide 1990).

Recent studies have indicated fungi to have a strong interaction with snails and marine plants. Discovered in salt marsh systems, this snail-fungal-plant interaction was exhibited as grazer-induced wounds on the salt marsh grass, *Spartina alterniflora*, induced by the gastropod, *Littoraria irrorata* (Silliman and Newell 2003). These wounds facilitated fungal invasions, which led to drastic decreases in plant biomass, and were recognized as important controlling mechanisms to salt marsh populations where this interaction occurred (Silliman and Newell 2003). In some terrestrial systems, pathogens and mesograzers may share the same host plant, and can trophically interact affecting the primary food source for the other species (Silliman and Newell 2003, Hatcher et al. 2004, Stout et al. 2006).

Additionally, researchers have shown that certain marine gastropods graze preferentially on algae that are infected with fungal pathogens (Wilson and Knoyle 1961, Kohlmeyer and Kohlmeyer 1979). For example, *Chondrus crispus*, when infected by the fungus, *Didymospheria danica*, is attacked by marine mollusks at the site of infection (Wilson and Knoyle 1961). Higher fungi can produce metabolites and enzymes that may provide nutrients for some marine organisms (Block et al. 1973, Kirk et al. 1974, Gessner 1980, Schatz 1984). We have few data about marine fungi as a potential food source for grazers and further study is warranted (Schatz 1984).

The giant kelp, *Macrocystis pyrifera*, is one of the main organisms in kelp forests worldwide, and is considered the largest marine alga (Foster and Schiel 1985, Graham et

al. 2008). A brown alga (Phaeophyceae), the *Macrocystis* sporophyte is constructed of vegetative fronds anchored to the substrate by a holdfast and held upright in the water column through gas-filled pneumatocysts located at the base of each blade or laminae (Lobban 1978). This alga forms a complex habitat that is host to numerous species relationships between producers (e.g., red foliose algae, corallines, kelps and other brown algae) and consumers (e.g., predators, grazers, planktivores, and detritivores) (Graham et al. 2008). Studies of trophic interactions in kelp forests have traditionally involved macroscopic organisms (Pace et. al 1999, Graham 2004). Several researchers, however, have suggested a need for further scientific investigations into relationships that involve biological pathogens (North 1979, Kohlmeyer 1979, Schatz 1984, Hyde et al. 1998, Silliman and Newell 2003). Biological pathogens that affect kelp are regulated by environmental variability (North 1971), anthropogenic influences (Andrews 1976), and biotic agents such as fungi (Kohlmeyer 1969, Schatz 1984, Apt 1988), bacteria (Andrews 1976, Apt 1988) and endophytic algae (Andrews 1977, Yoshida and Akiyama 1979, Apt 1988). I investigated the existence, proliferation, and trophic relationship between marine fungi present on *Macrocystis pyrifera* and an abundant grazer, the turban snail, within central California.

Three species of turban snails, *Chlorostoma brunnea*, *C. montereyi* and *Promartynia pulligo* graze on giant kelp in central California (Watanabe 1984 alb). These snails preferentially graze on giant kelp senescent material (Hunt 1977, McMillan personal observation), which has been suggested to host degradative fungal, viral and bacterial pathogens (North 1979). Interactions between these snails and fungal pathogens

on the *Macrocystis* sporophyte have been largely overlooked, but may cause weakening and removal of *Macrocystis* when combined with environmental factors (Foster and Schiel 1985).

Temperature is likely important in increasing degradation of *Macrocystis* by fungal pathogens (North 1979). Senescence and decay increase with greater summer temperatures, and an increase in temperature can increase the rate of biogenic infections. These changes in temperature can cause large epidemics of rotting fronds within a kelp stand (McFarland and Prescott 1959, North 1971a, North and Clendenning 1971, North 1979). Loss of nutrients also may hasten senescence, and it is not always possible to determine whether unhealthy appearance results from natural senescence or because of pathogenic invasions (North 1979). The first goal of this study, therefore, was to determine if fungal pathogens exist on living tissue of *Macrocystis pyrifera* and whether turban snail grazing and/or temperature affect fungal growth and the growth and biomass of *Macrocystis*. The second objective was to determine how a range of turban snail densities affects the fungus-snail kelp interaction. The third objective was to determine the turban snails' affinity for senescent *Macrocystis* blade material more than fresh laminae. The fourth objective of this study was to determine if wounds created on blades of *Macrocystis* by turban snails in the field had evidence of greater fungal biomass than non-grazed blade material. The final objective of this study was to determine the amount of fungal biomass occurring spatially in regards to turban snail abundance (between sheltered and more exposed sites) and locality on the sporophytes. The snails more often are found grazing in the canopy of the *Macrocystis* kelp forest; therefore, one would

expect to see differences between lower blades and blade material occurring in the canopy of the *Macrocystis* sporophyte. By determining where fungal bionts exist at the highest densities spatially on the sporophyte and in relation to snail densities, more information could be garnered about the relationship of fungi to *Macrocystis* and snails at different sites and along the frond. A recent study demonstrated that *Chlorostoma brunnea* grazing at moderate densities optimized growth of *Macrocystis*. This study compliments that previous research by evaluating the interaction between fungi and *C. brunnea* as a possible mechanism behind compensatory growth in *Macrocystis* fronds.

## METHODS

### *Influence of temperature and grazers on *Macrocystis pyrifera* growth and fungal biomass*

Outdoor mesocosm experiments were conducted in 16, 210-liter tanks plumbed with flowing unfiltered seawater and supplied with bubblers for improved water circulation and small sprinklers to reduce sun scorching of *Macrocystis* canopy blades (Figure 13). All field collections occurred at Stillwater Cove, Carmel, California on SCUBA at 4 and 8 meters depth. A 2 X 2 factorial design with two levels of temperature (high and low) and two levels of grazing (snails present and snails absent) was used to determine the effects of snail grazing and temperature on *Macrocystis* growth and fungal biomass during March 2008. The four treatments were designated as follows: 1) greater temperature-with snails, 2) lesser temperature-with snails, 3) greater temperature-without snails, 4) lesser temperatures-without snails. Greater and lesser temperatures were alternated among the sixteen tanks. Eight tanks were heated by 500 watt heaters suspended from the top of the tanks keeping the water at 14.1°C ( $\pm 0.13$  SE). Lesser temperatures were regulated at 12.4°C ( $\pm 0.05$  SE) in the other 8 tanks by using a closed circuit system of chiller-cooled freshwater running through 3 meters of aluminum pipe coiled along the inside of the tanks. These temperatures reflected the mean high, 13.8°C ( $\pm 0.09$  SE), and mean low, 12.8°C ( $\pm 0.07$ ) daily temperatures within the Monterey Bay for 2007 (from the NOAA National Buoy Data Center). Four tanks of greater temperature and four tanks of lesser temperature were randomly stocked with 50 *Chlorostoma brunnea* (grazer treatment), and the other eight tanks contained no snails. The experiment was conducted for 14 days and upon termination, all *Macrocystis*

material was measured, weighed for wet weight, and plugs were taken from the blades for fungal analysis.



Figure 13: Picture of outdoor mesocosms used in laboratory experiments.

Whole *Macrocystis* sporophytes were selected with the following characteristics: 1-2 meters in height; apical meristem was intact for all fronds; and in good condition (few grazing scars and little to no deterioration of the blades). To reduce confounding factors of using all three turban snail species in the laboratory experiments, only *Chlorostoma brunnea* was used in the experiments. Snails of 2-2.5cm were collected, brought to the Moss Landing Marine Laboratories, and placed in aquaria for at least one week to acclimate; *Macrocystis* tissue was fed to snails to limit starvation. *Macrocystis* sporophytes were weighed (wet weight) and placed in a holdfast holder (2-3

sporophytes/holder) at the bottom of each mesocosm. Each tank contained between 614g and 1040g wet weight of *Macrocystis* sporophytes with a mean mass of 782.81g ( $\pm 28.64$  SE) in each tank, and biomass was not significantly different among treatments ( $F = 1.58$ ,  $df = 3$ ,  $P = 0.25$ ).

All fronds were tagged with numbered spiral poultry bands, and length of fronds was determined by measuring each tagged frond to the nearest centimeter from the top of the holdfast to the base of the apical scimitar (the terminal laminae).

*Macrocystis* growth rates (m/day) for all mesocosm experiments were significantly correlated with initial frond lengths. Therefore, all growth rates were standardized using:

$$\text{Standardized Growth Rate} = \frac{\text{Ending Length}}{\text{Initial Length} * \text{Time Elapsed}}$$

where ending and initial lengths were measured in centimeters and time was measured in days.

#### *Determination of fungal biomass*

Fungal biomass for all experiments and surveys was estimated from ergosterol content of kelp material as described in Gulis and Suberkropp (2006). Sets of 15, 10-mm plugs were extracted from *Macrocystis* blade material at the laboratory, preserved in methanol, and stored at  $-20^{\circ}\text{C}$  until extraction. Samples were extracted with alcoholic KOH; lipids were partitioned into pentane, evaporated to dryness, reconstituted in methanol, and filtered. Ergosterol was quantified with HPLC (Shimadzu, Columbia, MD) equipped with Whatman Partisphere C18 column and an ultraviolet detector set at



282 nm and compared with external ergosterol standards. Sets of 5, 10 mm plugs also were extracted for ash-free dry mass (AFDM) analysis. Samples were dried in a 50°C drying oven, weighed and then placed in a muffle furnace at 500°C where it was oxidized, or ashed for four hours. The sample was then reweighed and the difference between the dried sample and the ashed sample was the AFDM. Once determined, the amount of ergosterol detected was divided by the AFDM of the relevant sample. The final unit for fungal biomass, therefore, was milligrams of fungi per gram of AFDM.

More than one value was recorded (growth rate for individual *Macrocystis* fronds) for each tank. Therefore, I used the mean of multiple values for each tank for statistical analysis, and each tank was considered as one replicate,  $n = 4$ . Biomass measurements recorded in the laboratory experiment were calculated as percentage biomass loss. Differences in response variables were assessed using a two-way Analysis of Variance (ANOVA) followed by Tukey's post-hoc test to test for differences among treatments (SPSS 16.0,  $\alpha = 0.05$ ), except when a significant interaction between variables was found in which case a planned pairwise comparison among means was tested using Fisher's least significant difference method (Fisher's LSD, SPSS 16.0,  $\alpha = 0.05$ ). Homogeneity of variance was tested using Levene's test and normality with Kolmogorov-Smirnov test. When appropriate, an arcsine transformation was used to normalize data. With respect to the assumptions of homogeneity of variances, the ANOVA was considered robust to differences in variances when replication was equal (Zar 1999).

### *Varying snail densities effects on fungal biomass*

In an additional mesocosm experiment, varying levels of *Chlorostoma brunnea* densities were used to study gastropod grazing on fungal biomass. Snails were placed in a range of densities in 8 of the 16 tanks (10, 20, 40, 60, 80, 100, 120, 140 individuals/tank); the other eight tanks contained no snails. The tanks had flowing seawater with an average temperature of 11.6°C ( $\pm 0.1$ SE); however, surface temperatures of the tanks reached much greater temperatures (personal observation). This experiment began June 11<sup>th</sup>, 2008 and lasted for 12 days. Upon termination of the experiment, all *Macrocystis* frond material was measured, wet weight determined, and plugs were taken from the blades for fungal analysis. To test the relationship among a range of densities of turban snails (*C. brunnea*) and *Macrocystis* growth, biomass loss, and fungal biomass, data were analyzed using a linear and non-linear regression analysis to determine the best relationship (SPSS 16.0,  $\alpha = 0.05$ ).

### *Snail feeding preference experiments*

To determine whether *Chlorostoma brunnea* preferred old (senescent) versus new (non-senescent) *Macrocystis* blade material, *C. brunnea* and *Macrocystis* individuals were collected from Stillwater Cove, Carmel, CA. *C. brunnea* was acclimated for 48 hours in aquaria and supplied with food (fresh *Macrocystis* tissue) to limit starvation. One blade of each old and new material was weighed after blotting dry and placed in each aquaria. Each 38-liter aquarium was fabricated with partitions creating 5 sections per aquarium. Ten *C. brunnea* were placed in each section of aquaria and each partition

was treated as a replicate for old and new material treated with snails. The experiment was then repeated without snails to represent a control (i.e., no snails). The tanks were supplied with flowing sea water and the experiment was conducted for 48 hours for each treatment. At the termination of the experiment, all *Macrocystis* blades were weighed wet, and differences in response variables between age of frond and snail presence/absence were tested using a two-way Analysis of Variance (ANOVA; SPSS 16.0,  $\alpha = 0.05$ ). Variance components were calculated to evaluate magnitude of effects for significant factors ( $p < 0.05$ ) (Winer 1971, Graham and Edwards 2001). Homogeneity of variance was tested using Levene's test and normality using a Kolmogorov-Smirnov test. With respect to the assumptions of homogeneity of variances, the ANOVA is considered robust to differences in variances when replication is equal (Zar 1999).

#### *Field surveys*

To determine whether fungal biomass varied with different wave exposures and at different parts of the *Macrocystis* sporophyte (bottom, middle, canopy), surveys were conducted at two sites along the central California coastline. Sampling occurred at Stillwater Cove, a large, sheltered *Macrocystis* kelp forest (Reed and Foster, 1984), and Pescadero Point, an exposed kelp bed, experiencing high waves and currents, just outside and north of Stillwater Cove within Carmel Bay, Carmel, California (Andrews 1945). Pescadero Point is at the extreme northern end of Carmel Bay and has been characterized as a kelp bed that is exposed to the open ocean (Andrews 1945). Four *Macrocystis*

sporophytes from each site were selected from between 10 and 13m depth, stipe numbers were counted, and all conspicuous gastropods were collected from each sporophyte. Blades were haphazardly collected from the bottom, middle, and canopy of each surveyed *Macrocystis* individual. Snails were counted, measured (<1.5, 1.5-2.0, 2.0-2.5, and >2.0cm size bins), and identified to species before they were released back into the water. All *Macrocystis* material was brought back to the laboratory for fungal biomass analysis where plugs were removed from the blades. Differences in fungal biomass between sites (Pescadero Point and Stillwater Cove) and among positions (bottom, middle, top) were determined using two-way ANOVA followed by Tukey's post-hoc test to test for differences among treatments (SPSS 16.0,  $\alpha = 0.05$ ).

To examine differences in fungal biomass between wounds on *Macrocystis* laminae created by turban snail grazing and areas of no scarring, blades with and without turban snail grazing wounds were collected from sporophytes with holdfasts at 6m depth at Stillwater Cove. Blades with snail grazing were determined by the presence of rasping scars visible on the surface of the blade created by turban snail grazing. Blades with no grazing had no visible scarring (Figure 14). *Macrocystis* blades were transported back to the laboratory for fungal biomass analysis where plugs were randomly collected from the blades and processed for fungal biomass analysis (see above). A one-way ANOVA was used to test for differences in mean fungal biomass between grazed and ungrazed *Macrocystis* blade material. Homogeneity of variance was tested with a Levene's test and normality was tested with a Kolmogorov-Smirnov tests. When appropriate, an arcsine transformation was used to normalize the data.



Figure 14: Picture of ungrazed (top) and grazed (bottom) *Macrocyctis pyrifera* blades.

## RESULTS

### *Effects of temperature and grazers on marine fungi and *Macrocystis pyrifera* growth*

Marine fungi were detected among the living tissues of *Macrocystis*. Fungal biomass was greater in samples with no snails than those treated with snails ( $F_{1,12} = 22.665$ ,  $p < 0.001$ ; Table 2), but there was no significant difference between temperature treatments ( $F_{1,12} = 0.051$ ,  $p = 0.208$ ; Table 2; Figure 15a). There was no significant interaction for fungal biomass ( $F_{1,12} = 0.031$ ,  $P = 0.321$ ; Table 2); standardized growth rates of *Macrocystis*, however, were significant for the interaction term (snails x temperature;  $F_{1,12} = 7.113$ ,  $p = 0.021$ ; Table 2). A pairwise comparison of the interaction term indicated that the SGR of *Macrocystis* was significantly greater in the treatment with increased temperature with snails treatment than in the treatment with greater temperature without snails and cold temperatures without snails treatment ( $p = 0.029$ ; Table 3; Figure 15b). In the presence of *Chlorostoma brunnea*, *Macrocystis* percentage biomass loss was significantly higher than in the treatments without snails, although the kelp remained intact ( $F_{1,12} = 6.707$ ,  $p = 0.237$ ; Figure 15c). Biomass loss in the absence of snails was due to senescence of fronds, suggesting that removal of fungi by snail grazing reduced frond decay. Temperature did not affect biomass loss of *Macrocystis* fronds in the experiment ( $F_{1,12} = 0.584$ ,  $p = 0.46$ ; Figure 15c).

Table 2: Results of a two-way ANOVA for fungal biomass, growth, and percent biomass lost in *Macrocystis pyrifera*. Significant results are bolded ( $\alpha < 0.05$ ).

Variable	Sources	df	MS	F	P
<b>Fungal Biomass</b>					
Between Subjects	Snails	1	0.648	22.665	<b>0.0005</b>
	Temperature	1	0.051	1.771	0.2080
	Snails X Temperature	1	0.031	1.071	0.3211
	Error	12	0.029		
<b>Growth</b>					
Between Subjects	Snails	1	<0.001	0.094	0.765
	Temperature	1	<0.001	0.697	0.4201
	Snails X Temperature	1	0.004	7.113	<b>0.0205</b>
	Error	12	0.001		
<b>Percent Biomass Loss</b>					
Between Subjects	Snails	1	0.152	6.707	<b>0.0237</b>
	Temperature	1	0.013	0.584	0.4597
	Snails X Temperature	1	0.002	0.099	0.7580
	Error	12	0.023		

Table 3: Results of Fisher's Least-Significant-Difference Test for interaction term of growth of *Macrocystis pyrifera*. For temperature 1 = hot, 2 = cold. For snails 1 = no snails, 2 = snails. Significant results are bolded ( $\alpha < 0.05$ ).

Temperature(i)* Snails(i-j)	Temperature(j)* Snails(j-i)	Difference	P	95.0% Confidence Interval	
				Lower	Upper
1*1	1*2	-0.028	0.121	-0.064	0.008
1*1	2*1	-0.041	<b>0.029</b>	-0.077	-0.005
1*1	2*2	-0.006	0.715	-0.042	0.03
1*2	2*1	-0.013	0.436	-0.049	0.023
1*2	2*2	0.021	0.22	-0.015	0.057
2*1	2*2	0.035	0.057	-0.001	0.071

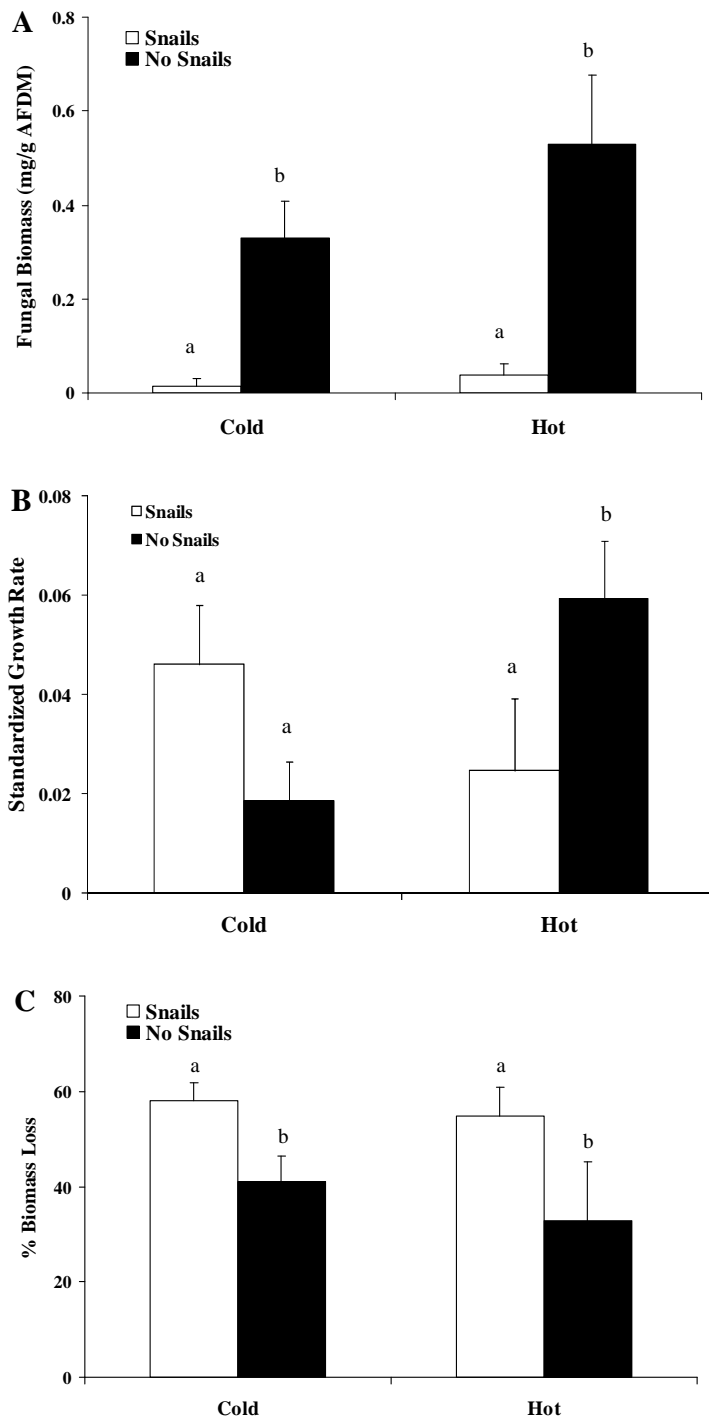


Figure 15: Results of mesocosm experiments that involved cold and high temperature tanks with or without snails with the following response variables: A) Mean fungal biomass on *Macrocystis* blades, B) Standardized growth rate of *Macrocystis* fronds, C) Percent biomass loss of *Macrocystis* material. (Error bars are +SE)



### *Effects of variable snail densities on fungal biomass*

The effects of snail densities on fungal biomass were insignificant for both a linear ( $p = 0.915$ ,  $R^2=0.116$ ) and a nonlinear regression, although, there was a weak nonlinear effect of snail density on fungal biomass ( $p = 0.077$ ,  $R^2=0.575$ ; Figure 16). Fungal biomass was generally minimal at lesser to moderate snail densities and greatest at greater snail densities reached. At greater densities of *C. brunnea*, snails grazed directly on *Macrocystis* causing the degradation of the alga, corresponding with a subsequent increase in fungal biomass.

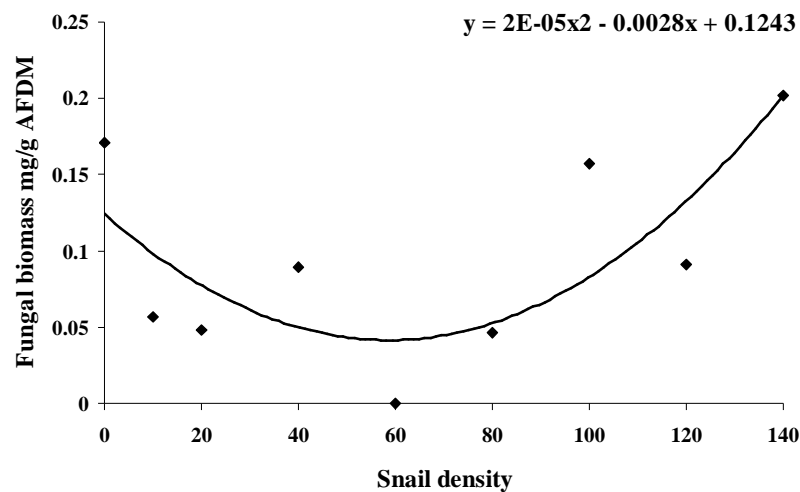


Figure 16: Nonlinear regression associated with the relationship between fungal biomass and a range of *Chlorostoma brunnea* densities on *Macrocystis*.

The effect of *C. brunnea* grazing on standardized growth rates (SGR) of *Macrocystis* in these experiments was not determined. Due to the timing of the experiment (mid-June), exposure of *Macrocystis* sporophytes to extreme sunlight at the surface of the tanks caused desiccation of the canopy blades and lead to senescence of

most of the apical meristems. There was not enough data (length measurements) available, therefore, to determine SGR for most of the fronds in the mesocosms. The relationship between loss of *Macrocystis* biomass loss and varying snail densities was not significant for linear ( $p = 0.285$ ,  $R^2 = 0.161$ ) or nonlinear regressions ( $p = 0.478$ ,  $R^2 = 0.218$ ; Figure 17).

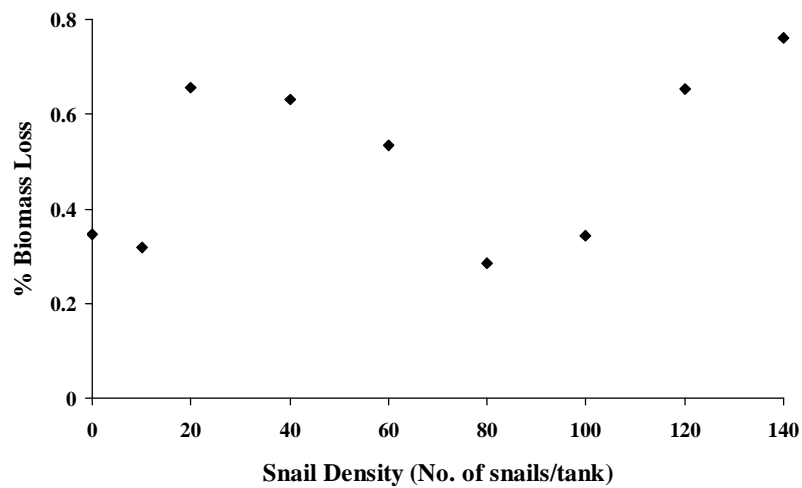


Figure 17: Scatterplot of *Chlorostoma brunnea* density versus percentage biomass loss of *Macrocystis pyrifera*.

#### *Snail feeding preference experiments*

Feeding experiments indicated differences between *Chlorostoma brunnea* grazing on senescent and non-senescent blades, snail presence (snails) and absence (control), and the interaction between the two treatments (Figure 18, Table 4). An evaluation of the magnitude of effects showed that the effect of age ( $\omega = 0.45$ ) was greater than either snail treatment ( $\omega = 0.21$ ) or the interaction of the two terms ( $\omega = 0.16$ ) (Table 5). Change in

biomass was determined mostly by the condition of the blade then by snail presence or absence. Biomass actually increased for senescent *Macrocystis* blades with no grazing by snails and senescent blades with snails had less biomass loss than non-senescent blades without snails. However, when snails were present, they always caused more loss of biomass than when snails were absent ( $1.84\text{g} \pm 0.221 \text{ SE}$ ;  $0.404\text{g} \pm 0.03 \text{ SE}$ ).

Table 4: Results of a two-way ANOVA for change in *Macrocystis pyrifera* biomass in non-senescent and senescent blades in the presence and absence of *Chlorostoma brunnea*. Significant results are bolded ( $\alpha < 0.05$ ).

Variable	Source	df	MS	F	P
Biomass					
Between Subjects	Snails	1	6.294	12.744	<b>0.003</b>
	Age	1	12.609	25.528	<b>&lt; 0.001</b>
	Snails X Age	1	2.578	5.219	<b>0.036</b>
	Error	16	0.494		

Table 5: Results of an analysis of the magnitude of effects for change in *Macrocystis pyrifera* biomass in non-senescent and senescent blades in the presence and absence of *Chlorostoma brunnea*.

Magnitude of Effects	Component	GD <sup>2</sup>
Snails	0.58	0.21
Age	1.21	0.45
Snails X Age	0.42	0.16
E	0.494	0.18
Total	2.704	1

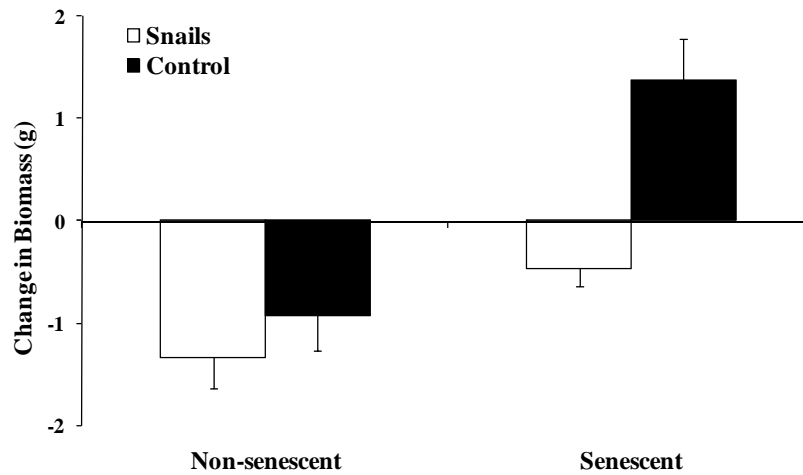


Figure 18: Change in *Macrocystis pyrifera* biomass as a function of condition (senescent and non-senescent blades) and *Chlorostoma brunnea* presence (snails) or absence (control). (Error bars represent  $\pm$ SE).

### Field surveys

The results of the field survey indicated no interaction of fungal biomass between the two sites (Pescadero Point and Stillwater Cove) and *Macrocystis* sporophyte positions (bottom, middle, and top) ( $F_{1,18} = 1.491$ ,  $P = 0.252$ ; Table 6). Fungal biomass was significantly different among locations on the sporophyte ( $F_{2,18} = 4.035$ ,  $P = 0.036$  Figure 19) and post-hoc analysis indicated a significant difference between bottom and canopy blades at Pescadero Point and Stillwater Cove ( $p = 0.03$ , Tukey test, Appendix C).

Table 6: Results of a two-way ANOVA for fungal biomass in *Macrocystis pyrifera* from bottom, middle and canopy blades (position) at Stillwater Cove and Pescadero Point (site). Significant results are bolded ( $\alpha < 0.05$ ).

Source	df	MS	F	P
Site	1	0	0.855	0.367
Position	2	0.001	4.035	<b>0.036</b>
Site X Position	2	0	1.491	0.252
Error	18	0		

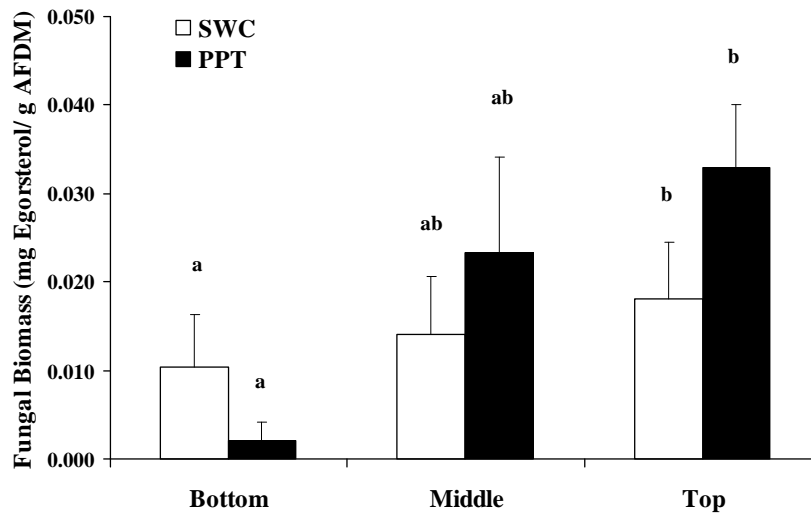


Figure 19: Fungal biomass for *Macrocyctis pyrifera* blade material found at Pescadero Point (PPT) and Stillwater Cove (SWC) at the bottom, middle, and top (canopy) of sporophytes. Letters represent significant differences.

Turban snail (*Chlorostoma brunnea*, *C. montereyi* and *Promartynia pulligo*)

densities were significantly greater on *Macrocyctis* sporophytes at Stillwater Cove than at Pescadero Point, the more exposed location ( $F_{1,6} = 113.481$ ,  $P < 0.001$ , Figure 20a; Appendix D). Mean densities of turban snail on sporophytes were 10.84 ( $\pm 0.661$  SE) snails per stipe in Stillwater Cove and 1.93 ( $\pm 0.297$  SE) snails per stipe at Pescadero Point. No significant difference among snail species at each site was found, but there was a significant difference between sites (Figure 20b; Appendix E).

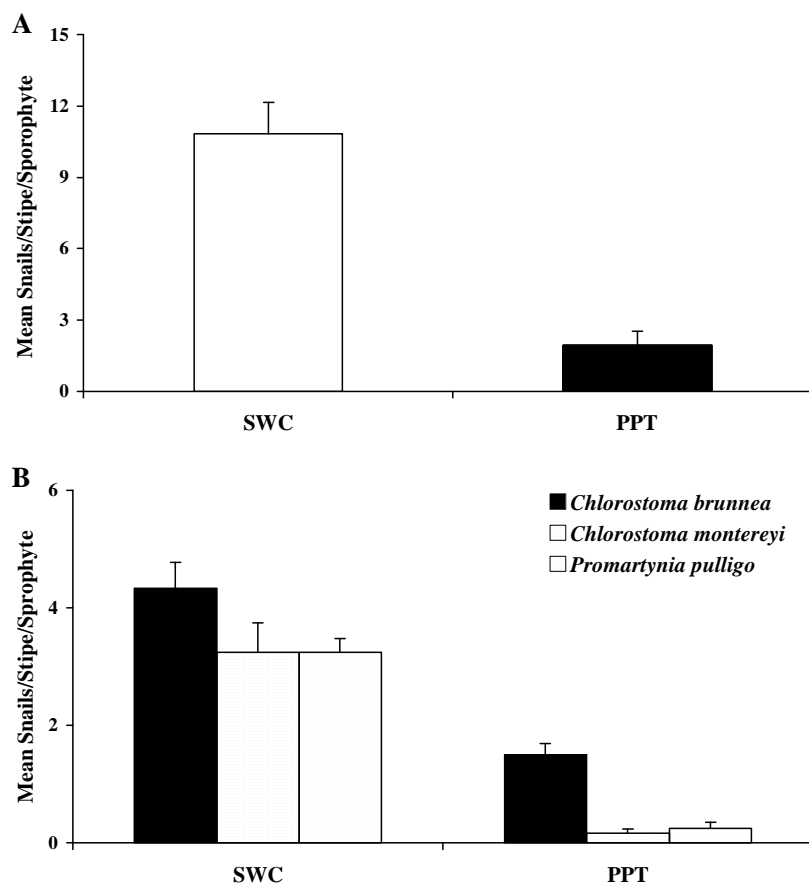


Figure 20: Results for snail abundance and distribution from field survey. A) Mean number of turban snails per stipe on *Macrocyctis* sporophytes surveyed in Stillwater Cove and Pescadero Point. Abundance of turban snails was significantly greater in Stillwater Cove ( $\pm$ SE). B) Mean number of each species of turban snail per stipe per sporophyte surveyed in Stillwater Cove and Pescadero Point. (Error bars represent  $\pm$ SE).

Surveys of *Macrocyctis* blades with and without turban snail grazing scars in Stillwater Cove indicated significantly greater amount of fungal biomass on those blades with grazing scars (0.339mg ergosterol/g AFDM  $\pm$  0.038 SE) than without (0.108 ergosterol/g AFDM  $\pm$  0.008 SE)( $F_{1,7} = 45.002$ ,  $P < 0.001$ ).

## DISCUSSION

Laboratory experiments and field surveys demonstrated that fungal bionts occur on living *Macrocystis pyrifera* frond material. I also found that turban snail grazing affects fungal biomass, and wounding by turban grazing can increase fungal pathogens within *Macrocystis* blade material. These results indicated that trophic interactions do exist among these algal, molluscan and fungal species.

Temperature did not affect fungal biomass. The difference in temperature between the treatments was not great (12°C and 14°C), and the difference in temperature treatments may have not been adequate to produce an effect on fungal biomass. Presence of snails and did decrease fungal biomass, suggesting *Chlorostoma brunnea* consumed fungi either primarily or secondarily when present on *Macrocystis*.

Standardized growth rate (SGR) was significantly greater at 14°C than 12°C when snails were present but the SGR was less at 14°C when snails were absent. *Macrocystis* growth rates are optimal at greater temperatures (Clendenning and Sargent 1971), and a previous study indicated that growth rates also were optimized at moderate densities of *Chlorostoma brunnea* (Chapter 1). Additionally, it has been suggested that warmer temperatures induce senescence and proliferate biological pathogens (North 1979); therefore, *C. brunnea* may have removed senescent material and fungal pathogens through grazing thereby relocating growth materials to the growing parts of the *Macrocystis* sporophyte (Lobban and Harrison 1994).

Percentage biomass loss of *Macrocystis* was significantly greater in the presence of snails. The average rate of consumption by *C. brunnea* on *Macrocystis* was an

estimated 0.075 grams per day (Watanabe 1984a). With 50 snails stocked in the tanks, predicted total consumption rate per tank (if all snails were all feeding on the *Macrocystis* material) was 3.750 grams of material per day. An average of about 14 grams of material was removed per tank per day (almost 4 times the amount of material, however, expected to be consumed). The material in the tanks was not replaced; therefore the sporophytes were allowed to senesce and accumulate fungal pathogens unlike previous studies. In feeding experiments, Watanabe (1984a) found the snails that fed on *Macrocystis* had lesser growth and gonadal development than those fed on a mixed algal diet. In this experiment, however, *Macrocystis* tissue was replaced frequently (once every 6-10 days) and no deterioration of algal material was observed. If the *Macrocystis* tissue became senescent, the snail growth may have been enhanced due to the ingestion of fungal pathogens. Silliman and Newell (2003) found that snail growth was enhanced through the consumption of plant material that contained a greater biomass of fungi. The palatability of senescent material also may be greater allowing snails to consume the material at a greater rate thus increasing growth and gonad indices. This would explain why snails consumed more material in my experiment than in earlier feeding experiments (Watanabe 1984a).

*Chlorostoma brunnea* reduced fungal biomass at moderate densities of snails relative to higher and lower densities, although the pattern was weak. This pattern was opposite the observed of a previous study in which *Macrocystis* growth was greater at moderate densities of *C. brunnea* than at lesser and greater densities. This could indicate a preference for fungal pathogens by the snails. At lesser snail densities, fungal biomass



was greater, but as snail densities increased to moderate densities, fungal biomass decreased, indicating that snail grazing was controlling fungal pathogens. As densities increased further, snails began to graze directly on the *Macrocystis*, increasing wounding and senescence of frond material, subsequently increasing fungal biomass. The relationship between *Chlorostoma brunnea* and the unidentified marine fungi resembles the associations previously reported for salt marsh systems at greater, yet naturally occurring, snail densities (Silliman and Newell 2003). At moderate densities, the snails consumed the fungi, and the *Macrocystis* acted as a fungal substrate.

Changes in *Macrocystis* biomass in the snail density experiment were not significant for any regression, however, at the point at which snails were controlling fungi (at 60 snails/tank), biomass loss decreased, indicating snails could have been grazing directly on fungal biomass and increasing growth of non-infected frond material. Percentage biomass loss then increased with greater densities of snails possibly indicating proliferation of fungi and loss of biomass due to grazing and senescence. The effect of fungi on the physiology of *Macrocystis pyrifera* has yet to be determined. This interaction must be investigated to determine if the effect of *C. brunnea* on fungal pathogens inhibits any potentially negative impact the fungi has on *Macrocystis* production and the overall effect of these interactions on *Macrocystis* populations through time.

Fungal biomass was greater in the canopy versus the lower blades of *Macrocystis* sporophytes at both sites. Older fronds were usually found at the canopy and degradation of older blades occurs more frequently in the canopy of *Macrocystis* (personal

observation). Therefore, it was not surprising that fungal pathogens were found at greater amounts in the canopy than in subcanopy blade material. Observations of greater epiphytic growth and senescence at the top half *Macrocystis* sporophytes indicated that grazing by turban snails may control the epiphytism on *Macrocystis* sporophytes. Marine fungi, along with other biogenic pathogens, such as bacteria and yeast, are important in the formation of the biofilm that is the foundation for other fouling organisms (epiphytes) (Holmstrom and Kjellberg 1994). With the removal of this layer, large-scale biofouling cannot occur (Hellio et al. 2000).

By removing biofilm on the surface of *Macrocystis* material through grazing, turban snails may inadvertently scar the laminae, creating a wound by which an invasion of biotic pathogens can enter the cells (Silliman and Newell 2003), consume the laminarin (Schatz 1984) and proliferate, thus causing a breakdown of cell walls. This could possibly reduce the effects of phenolics or chemical defenses of the *Macrocystis* blade material allowing for greater palatability of the blades for the snails. Through this proliferation, snails may initiate and encourage the growth of fungi in viable algal tissues (Silliman and Newell 2003).

The survey of grazer wounds induced by turban snails demonstrated a significantly greater fungal biomass surrounding the wound than in areas of no wounding on the blades of *Macrocystis*. This indicated that grazing may open up areas on the blades for fungal infections and that a mutualistic relationship between fungi and turban snails may be occurring. Through wounding, snails may proliferate fungal infections and consume senescent material caused by the degradation of *Macrocystis* cells by the algae.

Obligate fungi usually reside in the tissues of its algal host and in turn can create a successional process by which the fungi can induce microbial colonization by other fungi to produce detritus (Schatz 1984). Some higher marine fungi, such as *Dendryphiella salina*, use laminarin as a carbon source (Tubaki 1969), and can degrade alginates, which are found in *Macrocystis* (Zimmerman and Kremer 1986, Lobban and Harrison 1994, Wainwright, 1980; Wainwright and Sherbrock-Cox 1981). Not only can this fungus degrade the algae, but some higher fungi actually produce degradative enzymes and metabolites that could provide a nutrient source for grazers (Block et al. 1973, Kirk et al. 1974), making it a preferred food source (Schatz 1984, Silliman and Newell 2003). A previous study on fungal infected tissues of *Laminaria saccharina* total nitrogen was be greater in infected tissue than non infected tissue of *L. saccharina* indicating greater nutrient availability (Schatz 1984). Furthermore, the preference of *C. brunnea* for senescent over non-senescent blades in this study indicated that the above may be true.

This kelp-grazer-fungal interaction may not cause complete removal of the *Macrocystis* but may help provide the macroalgal detritus necessary for many kelp forest species to survive (Linley et al. 1981, Dunton and Schell 1987, Duggins et al. 1989). Fungi may play an integral part in ecological interactions in marine systems and therefore more studies should be developed to further investigate these roles (Golubic et al. 2005). Most fungal pathogens are specific to their hosts (Kohlmeyer 1979); therefore, it would be interesting to cultivate this fungus, or fungi, associated *Macrocystis* tissues and determine if it is a new species specific to *Macrocystis* or a suite of species available to infect the kelp's living tissues.

Taking into account the possibility that fungal pathogens create a biofilm that allows for a foundation by which algae and animals can settle, and grazers can remove that biofilm if only grazing superficially on *Macrocystis* fronds, removal of that biofilm can, therefore reduce the amount of fungal pathogens on the blade. At greater densities, grazers induce wounds on the *Macrocystis* that encourages fungal growth. It would be expected as densities of turban snails within the central Californian kelp forest increase from zero to moderate densities/grazing intensities, *Macrocystis* fronds would experience greater growth potential as fungal pathogens and epiphytes were removed from the photosynthetic blades of the sporophyte. As grazing intensities increased, however, more grazing scars would occur, proliferating fungi, and in turn tipping the balance of a seemingly mutualistic relationship between snails and *Macrocystis* to a point where the effect of grazers and fungi were detrimental to *Macrocystis* growth.

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## THESIS CONCLUSION

This novel application of a traditionally terrestrial hypothesis to a marine system provides insight into a trophic interaction that was previously designated as being non-consequential. This new discovery, that *Chlorostoma brunnea* affects *Macrocystis pyrifera* in a positive way through growth optimization of the marine alga, could lead to further research on other algal-grazer interactions. Furthermore, this study suggests a possible mechanism behind the overcompensation of *Macrocystis pyrifera* growth to grazing by *C. brunnea*. This mechanism, the likely consumption of fungi by *C. brunnea* from the blades of *Macrocystis*, introduces a new trophic player into the grazer-kelp system. This type of trophic interaction has only previously been studied in salt-marsh and seagrass systems, and never in context with compensatory growth.

## APPENDICES

Appendix A: Results of one-way ANOVA for snail abundance for three species of turban snails in Stillwater Cove.

	Sum of Squares	df	Mean Square	F	p
Between Groups	96.333	2	48.167	0.033	0.968
Within Groups	21960.17	15	1464.011		
Total	22056.5	17			

Appendix B: Results of one-way ANOVAs for standardized growth rate for the *Macrocystis pyrifera* sporophyte (A) artifact controls and (B) controls.

<b>A</b>	Sum of Squares	df	Mean Square	F	p
Between Groups	0.001	2	0.000	3.280	0.080
Within Groups	0.001	10	0.000		
Total	0.002	12			

<b>B</b>	Sum of Squares	df	Mean Square	F	p
Between Groups	0.014	3	0.005	3.249	0.057
Within Groups	0.018	13	0.001		
Total	0.032	16			

Appendix C: Results of Tukey test performed on position *Macrocystis* blade collected for fungal biomass analysis.

LOCATION(i)	LOCATION(j)	Difference	p	95.0% Confidence Interval	
				Lower	Upper
1	2	-0.012	0.199	-0.03	0.005
1	3	-0.019	0.03	-0.037	-0.002
2	3	-0.007	0.582	-0.025	0.011

Appendix D: Results of one-way ANOVA for mean turban snails per stipe per sporophyte between two sites, Stillwater Cove and Pescadero Point.

Source	Sum of Squares	df	Mean Squares	F-ratio	p
PPT_SWC	158.806	1	158.806	113.481	< 0.001
Error	8.396	6	1.399		

Appendix E: Results of two-way ANOVA for mean turban snail species per stipe per sporophyte between two sites, Stillwater Cove and Pescadero Point.

Tests of Between-Subjects Effects						
Dependent Variable: Snail Abundance						
Source	Sum of Squares	df	Mean Square	F	p	
Corrected Model	34.798	5	6.959	3.37538	0.025	
Intercept	70.38	1	70.38	34.1345	< 0.001	
Site	27.766	1	27.766	13.4666	0.002	
Species	6.825	2	3.413	1.65511	0.219	
Site * Species	0.206	2	0.103	0.05002	0.951	
Error	37.113	18	2.062			
Total	142.29	24				