THE EFFECTS OF RISING OCEAN TEMPERATURE AND PCO2 ON
THE PHYSIOLOGY AND GROWTH OF GIANT KELP, MACROCYSTIS
PYRIFERA, AND GRAZING BY PURPLE URCHINS,
STRONGYLOCENTROTUS PURPURATUS

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The Effects of Rising Ocean Temperature and pCO2 on the Physiology and
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ABSTRACT OF THE THESIS

The Effects of Rising Ocean Temperature and pCO2 on the Physiology and Growth of Giant Kelp, *Macrocystis pyrifera*, and Grazing by Purple Urchins, *Strongylocentrotus purpuratus*

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As climate change rapidly alters the world’s oceans, marine life will have to acclimate and/or adapt to warmer and more acidic conditions. While there is a growing body of literature on the individual effects of elevated temperature and CO2 on marine biota, few studies have examined the synergistic effects of these factors, especially regarding how they impact species interactions. In coastal environments of temperate latitudes, forests of kelp (large brown seaweeds in the Order Laminariales) provide habitat and food for numerous species, support enhanced biodiversity, and provide important ecosystem services. Consequently, impacts to these important ecosystem engineers can have disproportionately large effects on coastal ecosystem functioning. To determine how climate change might impact kelp forest ecosystems, I examined two of the more conspicuous and ecologically important kelp forest species, namely the giant kelp, *Macrocystis pyrifera*, and the purple sea urchin, *Strongylocentrotus purpuratus*. First, I performed three separate experiments in order to determine the effects of elevated temperature and pCO2 on *M. pyrifera* growth and photosynthetic performance. In my first experiment I cultured *M. pyrifera* meristematic tissues under three pCO2 levels (500, 1000, 1500 μatm CO2) and examined how this impacted their growth, steady-state photosynthetic oxygen evolution, and changes in their tissue carbon:nitrogen ratios. In my second experiment, I used a fully factorial design with two temperatures (12°C and 15°C) and two pCO2 levels (500 μatm and 1500 μatm CO2), and examined how these impacted kelp growth, steady-state photosynthetic carbon uptake, and tissue carbon:nitrogen ratios. In my third experiment, I used the same fully factorial design (12°C and 15°C; 500 μatm and 1500 μatm CO2), but examined changes in kelp photosynthetic pigment composition and carbonic anhydrase activity (an estimate of their ability to use HCO3- in photosynthesis). Counter to my expectations, elevating only pCO2 in the water had no effect on kelp growth rates, photosynthesis or tissue carbon:nitrogen ratios in either of the first two experiments. In contrast, in the second experiment, elevating only seawater temperature resulted in a significant reduction in both photosynthesis and growth, and an increase in tissue carbon:nitrogen ratios. However, when seawater temperature and pCO2 were increased together, the kelps exhibited significant increases in photosynthesis and growth relative to the other treatments. This suggested that rising ocean temperatures may interact with rising pCO2 to elicit responses that are different than when either of these factors is increased by itself. In my third experiment, elevating pCO2 in the water significantly reduced carbonic anhydrase activity, suggesting a reduction in HCO3- based...
photosynthesis (i.e. a down regulation of carbon concentrating mechanisms) and an increase in CO\textsubscript{2}-based photosynthesis. In contrast, elevating temperature and/or CO\textsubscript{2} alone had little-to-no impact on photosynthetic pigment concentrations. Following the experiments on \textit{M. pyrifer\textsubscript{a}}, I then examined how climate change will impact the interactions between \textit{S. purpur\textsubscript{a}tus} and \textit{M. pyrifer\textsubscript{a}}. Here, I cultured these two species separately under both “present day” conditions (i.e. 12\textdegree\text{C} and 500 µatm CO\textsubscript{2}) and “future” conditions (i.e. 15\textdegree\text{C} and 1500 µatm CO\textsubscript{2}) for three months. During this period, urchins were fed kelp from either their own water conditions or the alternate conditions, resulting in a fully factorial design with four treatment combinations (urchins held under either present day or future conditions being fed kelps grown under either present day or future conditions). My results indicate that urchins held under future conditions exhibited reduced feeding and growth rates, and smaller gonads than urchins held under present day conditions regardless of the conditions in which their food was grown. In contrast, urchins held under present day conditions and fed kelp grown under future conditions showed higher feeding and growth rates compared to similar urchins fed kelps grown under present day conditions. Together, my data suggest that \textit{M. pyrifer\textsubscript{a}} may benefit physiologically from a warmer, more acidic (i.e. higher pCO\textsubscript{2}) ocean while \textit{S. purpur\textsubscript{a}tus} will likely be impacted negatively. Given that \textit{S. purpur\textsubscript{a}tus} can exert a strong deterministic influence on \textit{M. pyrifer\textsubscript{a}} distribution and abundance, changes to either of their populations that might arise from climate change can alter how they interact and thus have serious consequences for many coastal environments.
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CHAPTER 1

INTRODUCTION

Anthropogenic climate change is rapidly impacting the world’s oceans, altering seawater chemistry by making them warmer and more acidic (Feely et al. 2004; Solomon et al. 2007). Atmospheric CO₂ concentrations have been rising since the Industrial Revolution, surpassing 400 ppm CO₂ in 2013 and resulting in a 1°C rise in average global atmospheric temperature (Solomon et al. 2007). During this period, sea surface temperatures have also increased, causing shifts in species ranges and changes in community assemblages as warmer temperatures move into higher latitudes (Beaugrand et al. 2002; Perry et al. 2005). In addition to ocean warming, climate change is also altering the chemistry of the ocean through the process of ocean acidification. Approximately 30% of all anthropogenic CO₂ emissions have been absorbed by the oceans, causing a 0.1 decrease in average ocean pH over the past century and a shift in the distribution of ocean carbon speciation, i.e. decreasing levels of carbonate (CO₃²⁻) and increasing levels of free CO₂ (Feely et al. 2004; Doney et al. 2009). By the end of this century, average sea surface temperature and ocean CO₂ partial pressure (pCO₂) are predicted to increase by an additional 3-4°C and ~500 μatm, respectively (Solomon et al. 2007). This will cause an additional decrease in ocean pH by 0.3-0.4 units (Caldeira & Wickett 2003) and undoubtedly negatively impact marine species on a global scale.

Increases in ocean temperature, pCO₂ and acidity are expected to have far reaching impacts on ocean life, threatening the stability of marine ecosystems and reducing their ecosystem services, which by some estimates constitute 60% of all global ecosystem services (Costanza et al. 1997). Consequently, over the last decade there has been a near exponential increase in the number of studies examining the impacts of ocean climate change on marine organisms across a wide range of taxonomic groups (see Wernberg et al. 2012; Kroeker et al. 2013 for reviews). For example, numerous studies have examined the effects of elevated temperature and pCO₂ on echinoderms (Miles et al. 2007; Gooding et al. 2009; Ries et al. 2009; Dupont et al. 2010), crustaceans (Landes & Zimmer 2012), fish (Munday et al. 2009;
Ferrari et al. 2011), and coralline algae (Kuffner et al. 2008; Martin and Gattuso 2009). Generally, increases in temperature below an organism’s thermal maximum can cause increases in basal metabolic rates (Gillooly et al. 2002; Brown et al. 2004), increased growth and feeding (Gooding et al. 2009; O’Connor 2009), and shorter larval development times (Brennand et al. 2009; Padilla et al. 2013). Increases in pCO$_2$ can also cause increases in metabolic rates (Wood et al. 2008; Spicer et al. 2011) as well as changes to internal acid/base balances (Miles et al. 2007; Catarino et al. 2012). As increases in both temperature and pCO$_2$ can have positive effects on metabolism and growth, it has been suggested that some organisms may benefit from climate change (Hendricks et al. 2010). However, calcifying marine organisms will likely be negatively impacted by ocean acidification, as decreases in pH and CO$_3^{2-}$ concentrations reduce calcification rates, growth and survival in a wide range of calcifying organisms (e.g. Gooding et al. 2009; Martin and Gattuso 2009; Johnson and Carpenter 2012; Courteny et al. 2013). Despite our growing understanding of the effect of ocean climate change, serious gaps in our knowledge remain for most species, especially in coastal ecosystems. For instance, the predicted increases in seawater temperature and pCO$_2$ (Solomon et al. 2007) are based on models for the open-ocean where values are expected to track atmospheric concentrations, and therefore they may not apply to coastal ecosystems where these values are much more dynamic. In coastal ecosystems, ambient pCO$_2$ is strongly impacted by local rates of photosynthesis and respiration within the benthic communities, resulting in large diurnal variation in pCO$_2$ and pH (Hofmann et al. 2011; Edwards and Kim unpublished data). Thus, many organisms may already be experiencing pCO$_2$ concentrations comparable to those predicted for the open ocean by the end of the century (Solomon et al. 2007). Furthermore, the vast majority of climate change studies have focused on the individual effects of either temperature or pCO$_2$ on organism physiology, despite the fact that most marine organisms will be exposed to concurrent increases in both variables as the climate continues to change. In fact, in a meta-analysis of 110 marine climate change studies published between 2000 and 2009, Wernberg et al. (2012) found that less than 10% of these studies examined the impacts of simultaneous increases in both temperature and CO$_2$. Such single factor studies ignore possible interactive effects between climate change stressors (Crain et al. 2008), making it difficult to predict how temperature and pCO$_2$ will ultimately impact marine organisms. Finally, the majority of
studies have focused on either physiological or demographic impacts to single species, leaving us with relatively little understanding of how climate change will impact species interactions. Changes in such interactions may reduce or ameliorate the impacts of a stressor on an organism, ultimately resulting in outcomes different from what single species studies would predict. For example, while growth in some plants may increase under higher temperature conditions, herbivore feeding rates may also increase thereby negating the positive effects on the plant (O’Connor 2009). As a result, care must be taken when extrapolating the physiological effects on single species to community level impacts on the ecosystem as a whole. This is especially important for the complex ecosystems in the coastal environment, where community structure is strongly influenced by species interactions.

Kelp forests are among the most productive and diverse ecosystems in the world. Along the southern coast of California, USA, the dominant canopy-forming kelp is *Macrocystis pyrifera*, the giant kelp. It grows at depths up to 30 meters and can reach up to 50 meters in length (Dayton 1985). The main thallus of the kelp forms a large three dimensional structure that provides food and habitat for numerous species of invertebrates, fish and marine mammals, some of which are exclusively associated with kelp forests (Dayton 1985). In addition, *M. pyrifera* forests support production in nearby environments through the export of energy in the form of drift kelp and Particulate Organic Carbon (POC), with nearly 60% of carbon in some intertidal environments originating within nearby kelp forests (Duggins *et al.* 1989). Many kelp forests are notable in that they tend to be dominated by a single type of herbivore, stronglycentroid sea urchins (Dayton 1985), and the structure of the kelp forest community is often determined by the interaction between sea urchins and kelp (Harrold & Reed 1985). When food supply is high, urchins generally are not active foragers, but rather inhabit protected microhabitats such as rock crevices where they subsist primarily by capturing drift kelp (Dean *et al.* 1984, Harrold & Reed 1985). However, when supplies of drift kelp are low or when the urchins are freed from predation pressure, they often emerge from their protected crevices and actively on feed on attached macroalgae (Estes & Palmisano 1974; Cowen 1983; Harrold & Reed 1985). During these periods, urchin populations can form large mobile aggregations called “fronts” (Dean *et al.* 1984) that denude large swaths of kelp from the benthos and create urchin barrens (Estes & Palmisano 1974; Harrold & Reed 1985). Urchins in these fronts often show signs of food
limitation such as smaller gonads (Dean et al. 1984), suggesting that changes in the quantity or quality of their food supply may be instrumental in the formation of barrens. Once established urchin barrens can be remarkably stable, capable of persisting for years or decades (Mann 1977; Keats 1991; Leinaas & Christie 1996). Along the coast of California, one the most abundant species of urchin is the purple sea urchin Strongylocentrotus purpuratus, which consumes a wide variety of algae including kelp (Kenner 1992). Given the interactions between S. purpuratus and M. pyrifera can play an important role in structuring kelp forest communities, changes in the strength of this interaction that may occur due to climate change could have serious consequences for coastal ecosystems.

Due to their ubiquity across the globe and the ease with which they can be handled, the effects of climate change have been studied most extensively on benthic invertebrates such as sea urchins (Wernberg et al. 2012). Like most organisms with calcium carbonate skeletons, sea urchins are expected to be vulnerable to ocean acidification due to decreases in CO$_3^{2-}$ concentrations and higher carbonate dissolution rates in more acidic waters (Doney et al. 2009). However, the degree to which they are vulnerable is equivocal, as studies have shown variable responses in different species (Ries et al. 2009) and different age classes within species. In particular, juvenile and larval stages are expected to be impacted most by ocean acidification (Portner 2008; Dupont et al. 2010). For example, sea urchin larvae held under elevated pCO$_2$ have shown decreases in size (Padilla et al. 2013), deformation of skeletal structures (Kurihara & Shirayama 2004; Kurihara et al. 2004; Brennand et al. 2009), and down regulation of the genes responsible for the construction of their skeletons (Todgham & Hofman 2009; Padilla et al. 2013). In contrast, fewer studies have examined how adults will respond to climate change, but those that have suggest that adult urchins are more robust to changes in seawater pCO$_2$ (Dupont et al. 2010). Even so, elevated pCO$_2$ is still expected to negatively impact urchin internal acid/base balances (Miles et al. 2007, Catarino et al. 2012) and rates of calcification (Ries et al. 2009; Courtney et al. 2013) in some species, while positively impacting certain metabolic rates (Spicer et al. 2011). In addition, interactions between temperature and pCO$_2$ may change how different life stages respond. For instance, while elevated temperature has been shown to ameliorate the negative effects of elevated pCO$_2$ in urchin larvae (Brennand et al. 2009), adult life stages (which have smaller windows of thermal tolerance and operate closer to their thermal maximums
than juvenile stages) may be pushed beyond their thermal limits making them more vulnerable to the negative effects of acidification (Portner 2008).

While studies on the impacts of climate change on invertebrates over the past decade have become increasingly common, relatively few studies have examined the effects of climate change on macroalgae (Wernberg et al. 2012), and those that have largely focused on the impacts on calcifying algae (Kroeker et al. 2013). Consequently, in spite of their importance as ecosystem engineers, the effects of climate change on non-calcifying macroalgae, such as M. pyrifer, are relatively unknown. In fact, a recent review by Harley et al. (2012) summarized the current understanding of how the various life stages of M. pyrifer are likely to respond to future increases in temperature or pCO$_2$. They concluded that, due to the lack of data, predictions for how the majority of life stages will be impacted by climate change cannot be made, while also noting that information on the interactive effects of elevated temperature and pCO$_2$ are non-existent for M. pyrifer. Elevating pCO$_2$ alone may result in positive impacts by way of carbon fertilization, as the majority of marine macroalgae appear to be carbon limited under present-day pCO$_2$ conditions, and thus they are expected to show increases in growth and photosynthesis under elevated levels of pCO$_2$ (Koch et al. 2013). However, short-term increases in photosynthesis from increased CO$_2$ concentrations do not always result in increased growth rates over longer periods (Israel & Hophy 2002). Likewise, the effects of temperature on macroalgal photosynthesis are strongly dependent on the duration of exposure. Short-term incubations under higher temperatures generally increase photosynthetic rate as the higher temperatures speed up enzymatic reactions (Davison 1987). Longer-term incubations that allow time for acclimation often show the opposite effects, with algae raised under higher temperatures showing lower rates of photosynthesis than those raised under lower temperatures (Davison et al. 1991). However, these negative effects can be ameliorated by acclimation of enzymes associated with carbon reduction in the Calvin Cycle, thereby allowing algae raised in high temperatures to exhibit rates of photosynthesis similar to those in algae raised in low temperatures (Davison et al. 1991). Acclimation to future ocean temperatures, therefore, may allow some macroalgae to maintain levels of growth and primary productivity seen under present-day conditions, though how temperature and pCO$_2$ will interact synergistically remains unknown.
The purpose of my study was two-fold. First, I determined how *M. pyrifera* will respond physiologically to increases in pCO$_2$ and temperature. Second, I determined how feeding and growth of *S. purpuratus* will be affected by increases in sea temperature and pCO$_2$. For the first part of my study, I performed three separate experiments, each with goal of answering one of three primary questions: (1) What is the effect of elevated ocean pCO$_2$ on *M. pyrifera* photosynthetic performance? (2) What are the individual and synergistic effects of elevated pCO$_2$ and temperature on *M. pyrifera* growth and photosynthetic performance (3) What are the effects of elevated pCO$_2$ and temperature on *M. pyrifera* photosynthetic pigment composition and bicarbonate usage in photosynthesis (*i.e.* carbonic anhydrase activity)?

In the first experiment, I raised *M. pyrifera* meristematic tissues under three pCO$_2$ concentrations approximating present day benthic pCO$_2$ levels in the kelp forest from where the meristems were collected (500 μatm CO$_2$), and two different pCO$_2$ levels predicted for the ocean by the end of the 21st century (1000 μatm CO$_2$ and 1500 μatm CO$_2$) (Solomon *et al.* 2007). For the second and third experiments, I raised meristems under a fully factorial design with two temperature levels (12°C and 15°C) and two CO$_2$ levels (500 μatm and 1500 μatm). For the second part of my study, I assessed how climate change will impact growth and feeding in *S. purpuratus*, with attention to decoupling the direct impacts of climate change on the urchins themselves from the indirect effects of the urchins eating kelps raised under predicted future conditions. For this experiment, I again used a fully factorial experimental design, with *M. pyrifera* and *S. purpuratus* raised independently under both “present day” conditions (i.e. 12°C, 500 μatm pCO$_2$) or “future” conditions (i.e. 15°C, 1500 μatm pCO$_2$), and the urchins then fed kelps raised under both conditions. In addition to growth and feeding, I was interested in seeing how climate change conditions would affect urchin gonad size. Sea urchin gonads are sites of energy storage and low gonad indices can be caused by starvation conditions, such as found within urchins in “barren grounds” (Harrold & Reed 1985), or by a low quality diet (Lemire & Himmelman 1996).
CHAPTER 2

MATERIALS AND METHODS

FIRST EXPERIMENT: EFFECTS OF ELEVATED pCO₂ ON M. PYRIFERA PHOTOSYNTHESIS

In my first experiment, conducted between May 2012 and July 2012, I evaluated the effects of elevated pCO₂ alone on the photosynthetic performance of *Macrocystis pyriforma* (C. Agardh). To do this, meristematic tissues were collected from 72 randomly selected *M. pyriforma* sporophytes at a depth of 12-15 m near the center of the Point Loma kelp forest, San Diego, CA using SCUBA. The meristems were brought to the surface and immediately placed in a dark cooler filled with seawater and transported to San Diego State’s Coastal Marine Institute Laboratory. Once at the laboratory, the tissues were trimmed to a weight of approximately 5.5 g and placed into nine 18.5 L acrylic mesocosms (n = 6 per mesocosm). Small lead fishing weights were attached to the base of each meristem to keep them on the mesocosm bottom. Each of the nine mesocosms was connected to one of three 100 gallon holding tanks (n = 3 mesocosms per holding tank) filled with seawater that was collected at the beginning of the experiment from a coastal site ~20 km north of our collection site. Seawater pCO₂ levels within each holding tank were adjusted to create three treatments; 500 µatm pCO₂, 1000 µatm pCO₂ and 1500 µatm pCO₂. The 500 µatm pCO₂ treatment was chosen to represent the average yearly pCO₂ values observed within the Point Loma kelp forest (Edwards & Kim unpublished data), while the 1000 µatm pCO₂ and 1500 µatm pCO₂ treatments represent different predictions for average atmospheric CO₂ levels at the end of the 21st century (Solomon *et al.* 2007). To maintain desired pCO₂ levels in the 500 µatm pCO₂ tank, an aquarium air pump was placed outside the building along the exterior wall where the mesocosms were located, and the air hose brought into building to the site of the holding tanks. This pumped ambient air from the exterior of the building into the holding tanks where it was mixed with the seawater using a Venturi injector. For the 1000 µatm pCO₂ and 1500 µatm pCO₂ treatments, two certified mixtures of CO₂ and air (1005 ppm pCO₂ and 1485 ppm pCO₂) were purchased from Praxair, San Diego, and bubbled into the
holding tanks using Venturri injectors. To ensure pCO$_2$ levels remained at the levels desired, water samples were taken from the outflow lines of the holding tanks on each day and analyzed using potentiometric titration to determine Total Alkalinity (TA) and total inorganic carbon (C$_{T}$) within the tank water. These values were then used to calculate the pCO$_2$ within the tanks using the program CO2.SYS. To limit gas exchange between the tanks and atmosphere, the mesocosms were sealed, thereby closing the circuit between them and the holding tanks. Temperature in the tanks was maintained at 12°C, the average temperature within the Point Loma kelp forest during the study, and was controlled using aquarium chillers. Each chiller was connected to a control box which constantly monitored temperature within the tanks using an attached underwater thermometer. Light above the mesocosms was provided by full spectrum fluorescent bulbs set on a 12:12 cycle, with light levels set at ~15-20 μmol photons, approximately the same levels the kelps experienced at the depths where they were collected (Edwards & Kim unpublished data). To ensure algae were not nutrient limited during the experiment, nitrate within the water was measured every 3-4 days using a Lachat QuickChem 8000 FIA and used as a proxy for the total nutrient profile within the holding tanks. If nitrate levels were found to be lower than 1-2 μM, 1-2 ml of Proline algae fertilizer (Guillards) were added to the holding tanks.

To determine if _M. pyrifera_ growth varied between the three pCO$_2$ treatments, changes in meristem weight were measured weekly over the course of the six-week experiment. The effect of elevated pCO$_2$ on _M. pyrifera_ growth was determined by comparing the average change in kelp weight among the treatments over the course of the experiment. Four weeks into the experiment, one of the pumps that circulate the water between the holding tanks and mesocosms in the 1500 μatm pCO$_2$ treatment failed, resulting in a drop in water flow to one mesocosm and the loss of all six meristems. These meristems were excluded from the final growth rate analysis.

To determine the effects of elevated pCO$_2$ on _M. pyrifera_ photosynthesis, photosynthetic oxygen evolution was measured at the end of the six-week experiment. As temperature is known to affect photosynthetic rates in algae, measurements were conducted in a temperature-controlled room set at 12°C. Measurements were made over the course of three days, with measurements for _M. pyrifera_ in the 500, 1000, and 1500 μatm pCO$_2$ treatments occurring on the first, second and third days, respectively. On the morning of
each day, three 500 ml BOD bottles were filled with seawater taken from the tank outflow line of the treatment being measured and the initial oxygen concentrations within the water in each bottle was measured using a Unisec PA2000 Picoammeter. Also on each day, a section was excised from the middle portion of the most basal blade on three separate M. pyrifera meristems. Each blade section was patted dry, weighed and placed into one of the three BOD bottles. Bottles were placed within a water bath within the temperature-controlled room and cultured for two hours under a full-spectrum irradiance of 100 μmol photons. To adjust light levels to 100 μmol photons, bottles were placed in specially designed boxes with window tinting of different transparencies. To prevent boundary layer formation, which can reduce carbon and nutrient fluxes and interfere with carbon uptake mass transport into the kelp meristems, water motion within each bottle was maintained using magnetic stirring plates. Each bottle was placed on top of a submersible magnetic stirring plate that was powered by a submersible aquarium pump. Stirring plates spun small magnetic bars (1cm x 2mm) within the bottles, agitating the water and preventing the formation of boundary layers. At the end of the two hour incubation, the M. pyrifera meristems were removed from the bottles and the oxygen concentration within the bottles was again measured. Following this, blade sections were taken from all meristems, again excised from the middle portion of the most basal blade, for determination of carbon and nitrogen content. Specifically, blade sections were placed on a sheet of non-stick aluminum foil, allowed to dry overnight at 80º, then ground to a fine powder in a Wiley Mill plant grinder. Total carbon and total nitrogen content of the tissue samples were measured using a Costech ECS 410 Elemental Analyzer and the ratio calculated as (% carbon/% nitrogen). All data met assumptions of normality and homoscedasity. Data for growth rate, oxygen evolution and carbon:nitrogen ratio were all separately analyzed using one-factor ANOVA’s, with pCO₂ concentration as a factor.

SECOND EXPERIMENT: SYNERGISTIC EFFECTS OF ELEVATED TEMPERATURE AND pCO₂ ON M. PYRIFERA GROWTH AND PHOTOSYNTHESIS

In my second experiment, conducted between September 2013 and October 2013, I examined the individual and synergistic effects of elevated temperature and pCO₂ on M. pyrifera physiology. To do this, M. pyrifera meristems were collected and brought to the laboratory as described for my first experiment above. These were trimmed to a weight of
approximately 7 grams before being placed into one of four 90 L acrylic mesocosms (n = 18 per mesocosm). The mesocosm were established as described above with the exception that a fourth holding tank and mesocosm line was added. Specifically, each mesocosm was connected to one of four 100 gallon holding tanks filled with seawater that was collected at the beginning of the experiment from a coastal site ~20 km north of our study site. Seawater temperature and pCO$_2$ levels within each holding tank were adjusted to make four orthogonal treatment combinations; a “present day” treatment at 12°C and 500 µatm pCO$_2$, an “elevated temperature” treatment at 15°C and 500µatm pCO$_2$, an “elevated CO$_2$” treatment at 12°C and 1500µatm pCO$_2$, and a “future” treatment at 15°C and 1500µatm pCO$_2$. The “present day” conditions were selected to represent the ambient temperature and CO$_2$ levels within the Point Loma kelp forest during the experiment, while the levels for the experimental treatments were selected based on predicted increases in temperature and pCO$_2$ for the end of the 21st century (Solomon et al. 2007). Temperature, CO$_2$, light and nutrient levels within tanks were measured and maintained in the same manner as the first experiment. Meristems were held within mesocosms for one month. During that time, wet weight of meristems was measured weekly and growth rates were again determined as the change in average meristem weight in each treatment over the course of the experiment. Carbon:nitrogen ratios were measured in the manner described for the first experiment, with blade sections dried overnight at 80°C, ground to powder and measured in a Costech ECS 410 Elemental Analyzer.

To determine the effects of elevated temperature and pCO$_2$ on $M$. pyrifera, photosynthesis, photosynthetic carbon uptake by the meristems was measured at the end of the one-month experiment. This was conducted over the course of four days, with meristems from different treatments measured on different days as described above. To ensure temperature remained constant, measurements were made within the temperature-controlled room, with the temperature of the room set to the temperature of the mesocosm treatment. On the morning of each day, eighteen 500ml BOD bottles were filled with seawater taken from the same treatment as the meristems being measured, sealed, and stored in darkness within the cold room before being used. To determine each treatment’s starting Total Inorganic Carbon (TIC), a separate sample was taken from the outflow line of the holding tank and measured using potentiometric titration. Also on each day, a section was excised
from the middle portion of the most basal blade on three separate *M. pyrifera* meristems in the treatment being evaluated. Each blade section was patted dry, weighed and placed into one of the three glass BOD bottles. These bottles were then placed upright in a water bath within the temperature-controlled room, and photosynthetic carbon uptake by the blades was measured under seven irradiances (0, 5, 10, 15, 30, 75, 125 µmols photons) for one hour each. Starting with the 0 µmols photons irradiance level (which was used to determine rates of dark respiration), the blades were allowed to incubate for one hour, after which the blade sections were removed and the TIC within the bottles measured using potentiometric titration as described above. Carbon uptake was calculated as the difference between TIC at the end of one hour versus the treatment’s starting TIC, standardized to the weight of the blade section. The blade sections were then placed in new bottles (that had been stored in the dark as described above) and the process repeated for the next greater irradiance. Light was provided by compact fluorescent bulbs hung directly above the bottles and light levels were adjusted in two ways. To achieve the lower light levels of 5, 10, and 15 µmols photons, discs constructed of window tinting of different shading properties were placed over the bottles, reducing the amount of light reaching the kelp blade. To achieve the higher light levels, the tinting was removed and the light bulbs moved closer to the bottles. Prior to each successive increase in irradiance, the light levels were re-measured using a Li-Cor light meter to ensure light was at the desired levels. To prevent the formation of boundary layers, magnetic stirring bars were placed into each bottle. Stirring bars were powered magnetic stirring plates placed under the bottles, each plate powered by an aquarium pump within the water bath as described above. For each treatment the carbon uptake at each light level was plotted and fit using Platt *et al.* (1975). From these best fit lines, the maximum rate of photosynthesis (P$_{\text{max}}$) and the photosynthetic efficiency (α) of each treatment were calculated. All data met assumptions of normality and homoscedasticity. Growth rate, carbon uptake and C:N ratio data were separately analyzed using two factor ANOVA’s, with temperature and pCO$_2$ concentration as the factors.
THIRD EXPERIMENT: EFFECTS OF TEMPERATURE AND pCO₂ ON PHOTOSYNTHETIC PIGMENTS AND CA ACTIVITY

A third experiment was conducted in May 2013 to determine how elevated temperature and pCO₂ impact specific physiological characters in *M. pyrifera*. The water within the mesocosm holding tanks was replaced and the experiment ran for two weeks and meristems were collected and held in the fully orthogonal treatment design as described in the second experiment. The two-week time frame was selected because the previous experiment had shown differences in meristem growth rate between treatments over this time period, and it was believed that two weeks would be sufficient time for temperature and pCO₂ induced changes in physiology to occur. Temperature, pCO₂ concentrations, and light were maintained in the same manner described above. Due to the equipment failure of the Lachat QuickChem 8000 FIA nutrient analyzer, I was unable to measure nitrate concentrations within the treatment tanks over the course of the experiment. In lieu of measurement, 1-2 mL of the Proline growth fertilizer were added to the treatment tanks ever 2-4 days, as the previous experiments had shown that this schedule was sufficient to maintain nitrate above saturating levels. Thus, while I did not measure nitrate, it is unlikely that meristems were nutrient limited over the course of the experiment.

As changes in photosynthetic rate can be caused by changes in the concentrations of photosynthetic pigments, I examined the effects of elevated temperature and pCO₂ on the concentrations of chlorophyll a, chlorophyll c and fucoxanthin within meristems. Pigments were extracted from the meristems at the end of the two-week experiment using the DMSO method (Gerard 1990). Specifically, 2.3 cm² disks of meristematic tissue were excised from the most basal blade of each meristem, approximately 3 cm above the pneumatocyst. Disks were wrapped in tin foil and frozen in a -80°C until ready for measurement. Fucoxanthin and chlorophyll c were extracted by placing disks in 4 ml of dimethyl/sulfoxide (DMSO) for 5 minutes. Disks were then removed and chlorophyll a was extracted by placing the disks in 2 ml of MeOH for 15 minutes. Distilled water was added to the DMSO extract (DMSO:water=4:1), and absorbance was measured in a Molecular Devices SpectraMax 190 at 480, 582, 631, and 665 nm. Distilled water and acetone were added to the MeOH extract (acetone:MeOH:water = 3:1:1), and absorbance was determined at 470, 581, 631, and 664 nm. Concentrations were then calculated using the equations of Seely *et al.* (1972).
The reaction rates of photosynthetic enzymes can exert a strong effect on overall rates of photosynthesis, and I evaluated the potential effect of elevated temperature and pCO$_2$ on the enzyme Carbonic Anhydrase (CA). In marine photoautotrophs, CA allows the utilization of HCO$_3^-$ as a photosynthetic carbon source by catalyzing the transformation of HCO$_3^-$ in CO$_2$ within the cell where it becomes available for binding to Rubisco during the Calvin Cycle. I examined differences in CA activity within meristems that were held under different pCO$_2$ and temperature conditions by measuring changes in photosynthetic oxygen evolution before versus after inhibition of CA. To do this, a water jacketed incubation chamber was filled with seawater from the treatment of the meristem to be measured. To maintain the temperature of the seawater at the desired level, the water jacketed chamber was connected to a temperature-controlled water circulator that was set to the treatment temperature. As I was interested in how differences in irradiance might synergistically alter how _M. pyrifera_ responds to elevated temperature and pCO$_2$, I performed two inhibition experiments, one under 20 μmol photons irradiance and the other under 125 μmol photons irradiance. In both experiments, oxygen concentration within the incubation chamber was measured using a YSI Pro BOD sensor connected to a YSI Pro ODO processor set to record oxygen within the chamber every second for the duration of the experiment. Blade sections were taken from the middle portion of the second most basal blade of meristems within each treatment. Three blade sections were used from each treatment. At the beginning of each CA inhibition experiment, the light source was turned on and changes in oxygen within the chamber due to photosynthesis were measured for 10 – 20 minutes. At this point, 163 μL of acetazolamide (AZ) were injected into the chamber, and oxygen was again measured for another 10 – 20 minutes. Acetazolamide is a known inhibitor of carbonic anhydrase, and the effects of acetazolamide on oxygen evolution have been used to infer HCO$_3^-$ usage in several species of algae (Bjork _et al._ 1993; Mercado _et al._ 1998; Zou and Gao 2009). We used separate linear regressions to determine the slope of the change in oxygen concentration during the time periods before (B$_{slope}$) and after (A$_{slope}$) addition of AZ, with slopes standardized to the weight of the blade section. Relative activity of CA, an indication of CO$_2$-based versus HCO$_3^-$-based photosynthesis was then calculated as (B$_{slope}$ – A$_{slope}$)/B$_{slope}$, which yielding a value indicating the degree to which the rate of oxygen evolution was altered by inhibiting CA. Specifically, values closer to zero indicated little or no difference after inhibition,
suggesting little CA activity and thus low HCO$_3$ usage, with progressively higher values indicating progressively higher rates of CA activity and thus high HCO$_3$’ usage. All data met assumptions of normality and homoscedasity. Concentrations and ratios of photosynthetic pigments were analyzed with two factor ANOVA’s, with temperature and pCO$_2$ as the factors. Carbonic anhydrase activity was analyzed using a three factor ANOVA, with temperature, pCO$_2$ and light level as the factors.

**Effects of Temperature and pCO$_2$ on Sea Urchin Growth, Gonad Index and Feeding Rates**

To determine how temperature and CO$_2$-induced changes in *M. pyrifera* will impact urchin growth, gonad index and feeding rates, a three-month feeding study was performed from January to March 2013. The four 100-gallon holding tanks were refilled with new seawater and assigned to one of two water treatments; two tanks were established to mimic “present day” conditions (12°C and 500 µatm pCO$_2$) and two tanks were established to mimic “future” conditions (15°C and 1500 µatm CO$_2$). Because the presence of the urchins appeared to impact long-term stability of CO$_2$ and nitrogen within the closed-circuit mesocosm system, seawater within the holding tanks was replaced one month into the experiment and every two weeks after that, with the temperature and pCO$_2$ levels within the holding tanks adjusted in the manner described above. However, in this experiment, each holding tank was connected to two mesocosms, one large (90 L) and one small (18.5 L). Kelp was held in the large mesocosms while urchins were held in the small mesocosms. This resulted in a total of eight mesocosms, with two large and two small mesocosms for each water treatment. Light was provided to the large mesocosms in the same manner and at the same levels as the previous experiments, while the small mesocosms were placed below our seawater table and out of direct light to simulate the natural conditions, in crevices and under boulders, in which urchins occur.

Thirty-six purple urchins, *Stronglyocentrotus purpuratus* (Stimpson), with test diameters between 3 and 5 cm were collected from a depth of 12 meters near the middle of the Point Loma kelp forest using SCUBA. They were immediately placed in a dark cooler filled with chilled sea water and transported to the San Diego State University Coastal and Marine Institute Laboratory where they were placed in a holding tank that was connected to a flow through seawater system, and starved for three weeks. This was done to standardize
their nutritional condition, as food can remain within the urchin digestive system for up to two weeks. After the starvation period, the urchins were divided into four groups of nine urchins each, and each group was assigned to one of the four small mesocosms. Each small mesocosm was subsequently divided into nine, equally sized compartments using Vexar mesh, with each individual compartment holding a single urchin. The initial size (test diameter) and total weights were recorded for each urchin. Test size was measured with Vernier calipers to the nearest 0.1 mm. To measure initial weights, each urchin was shaken for 10 seconds in order to remove excess water, and weighed to the nearest 0.01 g. One week before the urchins were placed into the small mesocosms, meristems of *M. pyrifera* were collected from the Point Loma Kelp forest and brought to the laboratory in the manner described in the previous experiments. Approximately 14 meristems were placed into each of the large mesocosms, creating four groups of kelp within two water treatments (present-day and future). Meristems were allowed to acclimate to their new water conditions for one week before being fed to the urchins. This time period was selected because the previous experiment had shown differences in meristem growth between these treatments during this period of time. Once feeding had begun, new scimitars were collected weekly and acclimated before being fed to the urchins. Although effort was made to allow all meristems to acclimate for one week prior to feeding, occasionally poor weather conditions prevented dive operations and collections had to be deferred. Consequently, on occasion the meristems were acclimated to their new water conditions for less than a week. To determine how changes in kelp physiology induced by increased temperature and pCO$_2$ impact urchin growth, the urchin groups were assigned one of two feeding regimens. One “present day” group and one “future” group were fed kelp meristems from their own respective water treatments (e.g. urchins held under present-day conditions were fed kelps acclimated to present-day conditions), while the second “present day” and “future” groups were fed kelp from the opposite water treatment (e.g. urchins held under present-day conditions were fed kelps acclimated to future conditions). This combination of the two water treatments and two feeding treatments resulted in four final treatments; present day urchins fed present day kelp (hereafter $P_U P_K$); present day urchins fed future kelp ($P_U F_K$); future urchins fed present day kelp ($F_U P_K$); and future urchins fed future kelp ($F_U F_K$).
Urchins in the mesocosms were fed once per day for three months. Each day, a single meristem was removed from each of the large mesocosms, its blades weighed, and cut into roughly equal sections. One blade section was then placed into each small compartment that held the individual urchins within the small mesocosms according to the treatment design describe above. After 24 hours, any remaining pieces of the blade sections were removed from each compartment and the urchins were given new blade sections from a new meristem. To avoid experimental artifacts that may arise from feeding urchins kelp from the same large mesocosm, each day selection of the meristems alternated between the large mesocosms for that water treatment. Urchin feces were siphoned out of the small mesocosms daily to prevent their accumulation and to prevent the buildup of nitrogenous waste in the water. Urchins were fed continuously throughout the experiment, with the exception of one three-week interruption approximately six weeks after the experiment began. This interruption was caused by poor weather conditions which made it impossible to collect kelp for the feedings. Feeding rates for each urchin group were measured one month after the start of the experiment, one day after the aforementioned three-week interruption, and two weeks after the interruption. Though the interruption was not planned, this arrangement allowed me to examine how each urchin’s condition (no feeding vs. every day feeding) altered the effect their assigned water and kelp treatments had on feeding rate. On the days I measured feeding rate, urchins were removed from their compartments before feeding and weighed. Urchins were then placed back in their compartments and fed meristem sections of known weight and allowed to eat for 24 hours. At the end of this time period, any remaining blade pieces were removed and reweighed. Feeding rates were calculated as the difference in blade weight before and after the feeding period per gram of urchin weight. At the end of the study, the ending test size and total weight of each urchin was measured in the manner described above. Urchins were then dissected and their gonads, somatic tissue, and tests weighed. From these measurements I calculated each urchin’s gonad index as (gonad weight/total weight) x 100. All data met assumptions of normality and homoscedasity. Growth rate, feeding rate and gonad indices data were analyzed using two-factor ANOVA’s, with urchin treatment (“present day” vs. “future”) and kelp treatment as the factors (“present day” vs. “future”).
CHAPTER 3

RESULTS

FIRST EXPERIMENT: EFFECTS OF ELEVATED pCO2 ON M. pyrifera PHOTOSYNTHESIS

I established three separate treatments to examine the effects of elevated pCO2 on M. pyrifera growth and photosynthetic performance. Seawater pCO2 conditions within the 100-gallon holding tanks remained within the desired levels during the experiment. Specifically, pCO2 levels within the tanks varied between 300 and 600 μatm pCO2 in the 500 μatm pCO2 tank, between 800 and 1000 μatm pCO2 in the 1000 pCO2 tank, and between 1200 and 1500 μatm in the 1500 μatm pCO2 tank (Figure 1). Likewise, seawater temperature did not vary from 12°C by more than 1°C on any one day during the experiment.

![CO2 Concentration](image)

Figure 1. Daily seawater pCO2 levels within the three 100-gallon holding tanks during the 6-week experiment.

Growth of the Macroystis pyrifera meristems held within the mesocosms did not vary significantly among the three seawater pCO2 treatments (ANOVA: F = 0.086, p = 0.918, df = 2, 45). Specifically, the average weight of the meristems did not vary among the three pCO2 treatments on any of the dates it was measured, nor did the average change in weight that was measured between dates over the course of the experiment (Figure 2). Likewise, carbon:nitrogen ratios within the M. pyrifera tissues also did not vary among the
Figure 2. Change in weight of *Macrocystis pyrifera* meristems (mean ± s.e.) over the course of six weeks in the first experiment. pCO$_2$ condition had no significant effect on growth of meristems. N = 18 for the 500 and 1000 µatm pCO$_2$ treatments, and n = 12 for the 1500 µatm pCO$_2$ treatment.

three seawater pCO$_2$ treatments (F = 0.410, p = 0.666, df = 2, 45) (Figure 3), nor did photosynthetic oxygen evolution by the meristems (F = 0.065, p = 0.93, df = 2, 45) (Figure 4). Together, this indicated that elevating pCO$_2$ alone did not have a significant impact on *M. pyrifera* growth or photosynthetic performance.

SECOND EXPERIMENT: SYNERGISTIC EFFECTS OF ELEVATED TEMPERATURE AND pCO$_2$ ON *M. PYRIFERA* GROWTH AND PHOTOSYNTHESIS

Seawater pCO$_2$ and temperature within the 100-gallon holding tanks approximated the desired levels during the experiment. Specifically, seawater temperature within the mesocosms did not vary from their desired levels by more than 1°C on any day during the course of the experiment. Further, pCO$_2$ levels within the two 500 µatm pCO$_2$ treatments (12°C and 15°C) ranged between 400 – 600 µatm pCO$_2$, while levels in the two 1500 µatm pCO$_2$ treatments ranged between 1200 – 1500 µatm pCO$_2$ (Figure 5).

Unlike with the effects of pCO$_2$ alone, growth rates of the *M. pyrifera* meristems within the experimental mesocosms varied significantly between the pCO$_2$ treatments (ANOVA: F = 29.21, p < 0.001, df = 1, 58) but not between the two temperatures (F = 1.31, p = 0.25, df = 1, 58). However, these two factors interacted such that the effect of pCO$_2$
Figure 3. Carbon to nitrogen ratios (mean + s.e.) of *Macrocystis pyrifera* meristems in the first experiment. No significant differences were observed in meristem C:N ratio due to pCO$_2$ treatment. N = 18 for the 500 and 1000 µatm pCO$_2$ treatments, and n = 12 for the 1500 µatm pCO$_2$ treatment.

Figure 4. Photosynthesis measured as rates of O$_2$ evolution (mean + s.e.) of *Macrocystis pyrifera* meristems under three pCO$_2$ levels. Blade sections were taken from the middle portion of the most basal blade of the meristem (n = 3 per treatment) and O$_2$ evolution was measured for two hours under 100 µmol photons at 12°C.
Figure 5. Daily seawater pCO$_2$ measured using potentiometric titration in the four mesocosms during the one-month experiment. Temperature and pCO$_2$ levels for treatments were: present day (12°C and 500 µatm pCO$_2$), elevated temperature (15°C and 500 µatm pCO$_2$), elevated pCO$_2$ (12°C and 1500 µatm pCO$_2$) and future (15°C and 1500 µatm pCO$_2$).

depended on temperature (pCO$_2$ x temperature interaction: F = 27.09, p < 0.001, df = 1, 58) suggesting a synergistic relationship (Table 1). Specifically, meristems within the elevated temperature alone treatment (i.e. 15°C and 500 µatm pCO$_2$) exhibited the lowest growth rates of all the treatment combinations, and by the end of the one-month experiment, showed biomass loss due to tissue deterioration when all other treatments continued to show biomass gain (Figure 6). In contrast, meristems in the “future” treatment where both temperature and pCO$_2$ were elevated (i.e. 15°C and 1500 µatm pCO$_2$) exhibited the highest growth rates compared to the other three kelp treatments (Figure 6). Growth rates of meristems in both the “present day” control treatment (i.e. 12°C and 500 µatm pCO$_2$) and in the elevated pCO$_2$ alone treatment (i.e. 12°C and 1500 µatm pCO$_2$) did not vary significantly from each other (Figure 6).

Table 1. Results of Two Factor Model I ANOVA Testing the Effects of Temperature and pCO$_2$ on M. pyrifera Growth Rates

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Figure 6. Growth rate (mean ± se) of *Macrocystis pyrifera* meristems during one month in the experimental mesocosms. The mesocosms followed a fully orthogonal design with two temperature levels (12°C and 15°C) and two pCO$_2$ levels (500 μatm and 1500 μatm) (n = 18).

Photosynthetic performance of *Macrocystis pyrifera* meristems, measured by carbon uptake, varied among the different seawater treatments (Figure 7). Specifically, meristems grown under future conditions (i.e. 15°C and 1500 μatm pCO$_2$) showed the highest maximum rates of photosynthesis (P$_{max}$) of any treatment and were more than twice those observed under the “present day” conditions. In fact, P$_{max}$ was significantly greater under future conditions than either present-day conditions (Tukey’s: p = 0.016, df = 3, 9), elevated pCO$_2$ conditions (Tukey’s: p = 0.045, df = 3, 9), or elevated temperature conditions (Tukey’s: p = 0.002, df = 3, 9). These results were similar to the observed differences in growth rates and indicates that *M. pyrifera* may perform better physiologically under future climate change conditions than they do today. In contrast, P$_{max}$ did not vary significantly among *M. pyrifera* meristems grown in mesocosms under present day conditions versus conditions where only pCO$_2$ was elevated (Tukey’s: p = 0.56, df = 3, 9), though there was a non-
significant increase in $P_{\text{max}}$ under elevated pCO$_2$. Likewise, $P_{\text{max}}$ also did not vary among meristems grown under present day and elevated temperature conditions (Tukey’s: $p = 0.11$, $df = 3$, 9), though there was a non-significant decrease under elevated temperature. In contrast, $P_{\text{max}}$ did vary significantly among meristems grown in the mesocosms where only pCO$_2$ was elevated compared to those grown in mesocosms where only temperature was elevated (Tukey’s: $p = 0.04$, $df = 3$, 9), likely due to the observed non-significant differences between meristems of these two treatments and the control present day treatment. Photosynthetic efficiency under non-saturating irradiances ($\alpha$) did not differ between $M. pyrifera$ meristems grown under present day and elevated CO$_2$ conditions (Tukey’s: $p = 0.21$, $df = 3$, 9). However, $\alpha$ in meristems grown under future conditions were significantly lower ($p = 0.05$, $df = 3$, 9) than in meristems grown under present day conditions, while $\alpha$ in
meristems grown under elevated temperature conditions alone was significantly lower than in meristems grown under either present day conditions (p = 0.002, df = 3, 9), elevated pCO$_2$ (p = 0.011, df = 3, 9) or future (p = 0.039, df = 3, 9) conditions. Together, these findings supported my earlier results that changes in temperature and pCO$_2$ interact synergistically to influence photosynthesis, with the negative effects of elevated temperature being ameliorated by higher pCO$_2$.

*Macrocystis pyrifera* tissue carbon:nitrogen ratios varied significantly between present day and future pCO$_2$ conditions (ANOVA: F = 10.164, p = 0.003, df = 1, 45) but not between the two temperature treatments (F = 2.67, p = 0.109, df = 1, 45). However, pCO$_2$ and temperature interacted such that the overall effect of elevated pCO$_2$ depended on the temperature (Temp x pCO$_2$ interaction: F = 7.996, p = 0.007, df = 1, 45). Specifically, C:N ratios in the meristem tissues did not differ significantly between *M. pyrifera* grown under present day, elevated pCO$_2$ conditions, or future conditions, but did exhibit significantly higher C:N ratios under elevated temperature compared to present day conditions (Tukey’s: p = 0.05, df = 3, 45), elevated pCO$_2$ conditions (p = 0.04, df = 3, 45), or future conditions (p = 0.005, df = 3, 45) (Figure 8). This difference was primarily due to elevated carbon content, as little difference in nitrogen content was observed (data not shown).

**Third Experiment: Effect of Temperature and pCO$_2$ on Photosynthetic Pigments and CA Activity**

Over the course of the third experiment, growth rates of meristems differed significantly between the two temperature treatments (ANOVA: F = 8.117, p = 0.007, df = 1, 36), but not the two pCO$_2$ treatments (F = 2.528, p = 0.121, df = 1, 36), and these two factors did not interact (F = 2.319, p = 0.137, df = 1, 36) (Figure 9). Specifically, growth rates in meristems grown under elevated temperature conditions were significantly lower than those in meristems grown under either present day (Tukey’s: p = 0.019) or elevated pCO$_2$ (p = 0.017) conditions, but were not significantly differ from those in meristems grown under future conditions (p = 0.142). Further, growth rates were not significantly different between the present day, elevated CO$_2$, or future treatments (p > 0.05). Together, this suggests that elevating temperature by itself results in reduced growth in *M. pyrifera*, but increasing temperature and pCO$_2$ together may ameliorate these effects.
Figure 8. Carbon:Nitrogen ratios (mean ± se) of *Macrocystis pyrifera* meristems grown under different temperatures and pCO$_2$ conditions in the second experiment. C:N ratio was significantly higher (p < 0.05) meristems under elevated temperature compared to meristems in other treatments.

The concentration of chlorophyll a did not differ between *M. pyrifera* meristems grown under the two temperature (ANOVA: F = 0.750, p = 0.394, df = 1, 27) or pCO$_2$ conditions (F = 1.161, p = 0.291, df = 1, 27), nor was there any interaction between the two factors (F = 0.013, p = 0.91, df = 1, 27). Similarly, neither temperature (ANOVA: F = 0.493, p = 0.489, df = 1, 27) nor pCO$_2$ (F = 0.001, p = 0.98, df = 1, 27) had an effect on either chlorophyll c or fucoxanthin (ANOVA: Temp, F = 1.835, p = 0.187, df = 1, 27; pCO$_2$, F = 0.385, p = 0.540, df = 1, 27) concentrations (Figure 10). Although total concentrations of pigment did not differ between meristems of different treatments, it was possible that either temperature or pCO$_2$ could alter the ratios of these pigments within meristems. Ratios of Chl a/Chl c, Chl c/fucoxanthin and Chl a/fucoxanthin were calculated using the concentration data above. Temperature and pCO$_2$ had no effect on the ratio of Chla/Chlc (ANOVA: Temp, F = 0.770, p = 0.380, df = 1, 27; pCO$_2$, F = 1.454, p = 0.239, df = 1, 27; Temp x pCO$_2$, F = 1.545, p = 0.225, df = 1,27) (Figure 11A), or on either Chla/fucoxanthin (ANOVA: Temp, F = 1.058, p = 0.313, df = 1, 27; pCO$_2$, F = 0.072, p = 0.790, df = 1, 27; Temp x pCO$_2$, F = 0.290, p = 0.594, df = 1, 27), or Chlc/fucoxanthin (ANOVA: Temp, F = 0.796, p = 0.380, df
Figure 9. Growth rates (mean ± se) of *Macrocystis pyrifera* meristems grown under two temperature and two pCO$_2$ conditions in the third experiment. Experiment used a fully orthogonal design of two temperature levels (12°C and 15°C) and two CO$_2$ levels (500 µatm and 1500 µatm pCO$_2$). Values are based on the difference in meristem weights between the first and last day of the experiment (n = 10).

= 1, 27; pCO$_2$, F = 0.047, p = 0.830, df = 1, 27; Temp x pCO$_2$, F = 1.609, p = 0.216, df = 1, 27) (Figure 11B).

Carbonic Anhydrase (CA) activity, which describes the relative reliance on HCO$_3^-$ use in photosynthesis, was analyzed using a three factor ANOVA, with temperature, pCO$_2$ and light level as factors (Table 2). CA activity was significantly affected by pCO$_2$ for *M. pyrifera* meristems measured under low light conditions (20 µmol photons) (ANOVA: F = 13.66, p = 0.008, df = 1, 15). However, it did not differ between temperatures (F = 0.36, p = 0.56, df = 1, 15) (Figure 12A). Further, these two factors did not interact with each other (temperature x pCO$_2$ interaction: F = 0.39, p = 0.54, df = 1, 15), suggesting the effect of pCO$_2$ on CA activity was not temperature-dependent. In contrast, when measured under high light conditions (125 µmol photons), neither pCO$_2$ (ANOVA: F = 2.79, p = 0.13, df = 1, 15) nor temperature (F = 0.44, p = 0.52) had a significant effect on CA activity within the *M. pyrifera* meristems (Figure 12B). However, irradiance itself exerted a significant effect on
Figure 10. Concentrations of photosynthetic pigments within *Macrocystis pyrifera* tissue (mean + se) (N = 8). Pigments were extracted using DMSO and measured spectrophotometrically. There was no significant effect of temperature conditions, pCO$_2$ conditions or temperature-pCO$_2$ interaction on pigment concentrations. Chl a = chlorophyll a, chl c = chlorophyll c, fuco = fucoxanthin.

CA activity (ANOVA: F = 14.420, p = 0.002, df = 1, 15), with HCO$_3^-$ utilization being greater under 120 umols than under 20 µmols. This effect of light on CA activity was not influenced by temperature (ANOVA: F = 0.746, p = 0.401, df = 1, 15) or pCO$_2$ (ANOVA: F = 0.536, p = 0.475, df = 1, 15) nor was there any interaction between the three factors of light, temperature and pCO$_2$ (ANOVA: F= 1.538, p = 0.233, df =1, 15).

**Effects of Temperature and pCO$_2$ on Sea Urchin Growth, Gonad Index and Feeding Rates**

Growth rates of *Strongylocentrotus purpuratus* differed significantly between individuals held under present-day and future conditions (ANOVA: F ratio = 14.79, p = 0.001, df = 1, 24). Specifically, *S. purpuratus* raised under present day conditions (i.e. 12°C and 500 µatm pCO$_2$) grew significantly faster than urchins raised under future conditions (15°C and 1500 µatm pCO$_2$) regardless of the conditions under which their food was grown (Figure 13). Specifically, while urchins held under present day conditions showed higher total weight at the end of the experiment, urchins under future conditions showed either no
Figure 11. Ratios of photosynthetic pigments within *Macrocystis pyrifera* tissues (mean + se) (N = 8). No significant effects of temperature, pCO$_2$ or temperature-pCO$_2$ interactions were seen on the ratio of Chl a/Chl c (A), Chl a/Fuco, or Chl c/Fuco (B). Chl a = chlorophyll a, chl c = chlorophyll c, fuco = fucoxanthin.
Table 2. Results of the Three Factor Model I ANOVA Testing the Effects of Temperature, pCO₂ and Light Level on Carbonic Anhydrase Activity in M. pyrifera Meristems

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III SS</th>
<th>Df</th>
<th>Mean Squares</th>
<th>F-ratio</th>
<th>p-value</th>
</tr>
</thead>
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<tr>
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<td>0.888</td>
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<tr>
<td>Temperature</td>
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<td>1</td>
<td>0.003</td>
<td>0.039</td>
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<tr>
<td>pCO₂</td>
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<td>1</td>
<td>0.755</td>
<td>11.452</td>
<td>0.004</td>
</tr>
<tr>
<td>Light x Temperature</td>
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<td>1</td>
<td>0.050</td>
<td>0.755</td>
<td>0.399</td>
</tr>
<tr>
<td>Light x pCO₂</td>
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<td>1</td>
<td>0.036</td>
<td>0.549</td>
<td>0.470</td>
</tr>
<tr>
<td>Temperature x pCO₂</td>
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<td>1</td>
<td>0.015</td>
<td>0.225</td>
<td>0.642</td>
</tr>
<tr>
<td>Light x Temperature x pCO₂</td>
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<td>1</td>
<td>0.092</td>
<td>1.391</td>
<td>0.257</td>
</tr>
<tr>
<td>Error</td>
<td>0.989</td>
<td>15</td>
<td>0.066</td>
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<td></td>
</tr>
</tbody>
</table>

change in total weight or decreases in weight by the end of three months. Further, the conditions under which their food (M. pyrifera) was grown also had a significant effect on urchin growth, with the urchins feeding on M. pyrifera raised under future conditions growing significantly more rapidly than urchins feeding on M. pyrifera raised under present day conditions (ANOVA: F = 4.39, p = 0.047, df = 1, 24) regardless of the conditions under which the urchin were held (ANOVA: urchin x kelp: F ratio = 0.001, p = 0.98, df = 1, 24). In addition, urchin gonad indices were significantly impacted by the conditions under which the urchins were held, with higher indices observed in urchins held under present day conditions than urchins held under future conditions (ANOVA: F = 3.243, p = 0.09, df = 1, 24) (Figure 14). In contrast to the total growth weight of the urchins, no effect of the conditions under which food was grown was observed on urchin gonad size (ANOVA: F = 0.057, p = 0.831, df = 1). In addition, there was no significant interaction between urchin treatment and kelp treatment on gonad size (ANOVA: F = .085, p = 0.76, df = 1, 24).

In the first feeding trial, the conditions urchins were held under had a significant effect on urchin feeding rate (ANOVA: F = 10.29, p = 0.003, df = 1, 32), with urchins held under present day conditions exhibiting significantly greater feeding rates than urchins held under future conditions (Figure 15A). In contrast, and similar to patterns observed in urchin gonads, the conditions under which their food, M. pyrifera, was held did not impact urchin feeding rates (F = 2.41, p = 0.13, df = 1, 32), although there was a non-significant increase in feeding rates among urchins fed kelp from future conditions. The effect of urchin conditions on feeding rate did not vary depending on the conditions kelp were raised under (Urchin x
Figure 12. Differences in carbonic anhydrase activity in *Macrocystis pyrifera* meristems when grown between treatments (mean ± se, N= 3), under (A) 20 μmol photons and (B) 125 μmol photons. CA activity was measured as the difference between the rate of oxygen evolution before (B_slope) and after (A_slope) application of CA inhibitor, calculated as ((B_slope – A_slope)/B_slope) per gram of algae.
Figure 13. Mean growth (changes in total weight, ± se) of *Strongylocentrotus purpuratus* held under present-day and future temperature and pCO₂ conditions. Left-hand bars represent *S. purpuratus* held under present day conditions (i.e. 12°C and 500 µatm pCO₂) and fed kelp grown under both present day (P₀P₀K) and future (P₀F₀K) (i.e. 15°C and 1500 µatm pCO₂) conditions. Right-hand bars represent *S. purpuratus* held under future conditions and fed kelp grown under both present-day (F₀P₀K) and future (F₀F₀K) conditions. Urchins were weighed at the beginning and end of the three-month study. Sample size for P₀P₀K, P₀F₀K, F₀P₀K and F₀F₀K is N = 9, N = 8, N = 7 and N = 5, respectively.

Figure 14. Gonad indices (mean ± s.e.) of *S. purpuratus* from different sea urchin and kelp treatments. Gonads were separated from other urchin tissue, dried of excess water and weighed. Gonad index was significantly different between urchin treatments (p = 0.09). Data was calculated (gonad size/test size) x 100.
Figure 15. Feeding rates, measured as the amount of kelp consumed per g urchin per 24 hours, of sea urchins at (A) one month and (B) two months after the start of the three month urchin incubation. Kelp pieces were weighed prior to feeding and placed into individual urchin compartments. Urchins were allowed to feed for 24 hours, after which any remaining kelp was removed and reweighed. Data are means ± se. N = 9 for all treatments in A. In B, N for P_U_P_K, P_U_F_K, F_U_P_K, and F_U_F_K was N = 9, N = 8, N = 8 and N = 5, respectively.
Kelp interaction: F ratio = 0.35, p = 0.55, df = 1, 32). In the second feeding trial, none of the factors had a significant impact on feeding rate (ANOVA: Urchin, F = 0.19, p = 0.66; Kelp, F = 0.29, p = 0.59; Urchin x Kelp, F = 0.49, p = 0.48). This feeding trial took place immediately following a three week starvation, and all urchin groups consumed amounts of kelp (roughly 0.6 g on average) comparable to the highest feeding rates seen in the first feeding trial (data not shown). Similarly, the third feeding experiment revealed no effects of either the conditions under which the urchin were held (ANOVA: F = 1.66, p = 0.20, df = 1, 26), or the conditions under which their food was grown (F = 2.90, p = 0.10, df = 1, 26) on S. purpuratus feeding rate, nor did these factors interact (urchin x kelp: F = 0.40, p = 0.53 df = 1, 26), (Figure 15B). Interestingly, feeding rates of urchin groups in the third trial were generally lower compared to the first two feeding trials. Consequently, while the conditions under which M. pyrifera is grown may impact urchin feeding rates, these patterns can be weak and often not significant. However, one trend that was observed is that urchins held under future conditions may exhibit reduced feeding, especially when feeding on kelps grown under future conditions.
CHAPTER 4

DISCUSSION

As anthropogenic emissions of greenhouse gases continue to increase, the world’s oceans will progressively become warmer and more acidic (Calderia & Wickett 2003; Sabine et al. 2004; Solomon et al. 2007). Marine organisms will not only have to adapt to these concomitant increases in temperature, acidity and pCO$_2$, but also to changes in biotic interactions as climate change alters community structure and organism physiology/behavior. To date the majority of climate change studies have focused on the individual effects of temperature and/or pCO$_2$ on single species (reviewed in Wernberg et al. 2012), but using these studies to make predictions of how ecosystems will respond to future climate change is problematic. One reason for this is that environmental stressors, such as increases in temperature and/or pCO$_2$, may not support the same relationships in all circumstances, but instead may interact in complex ways depending on their relative strength and on the organisms in which they are examined (Crain et al. 2008). In addition, the direct effects of these stressors on organism physiology may be altered by changes in the strength of species interactions (O’Connor 2009), resulting in outcomes different than those observed when species are studied in isolation. In this study, I demonstrated that the physiological responses of the giant kelp, *Macrocystis pyrifera*, to increases in temperature and pCO$_2$, such as those predicted by climate change models (Solomon et al. 2007), vary greatly between situations where these factors are examined individually versus together. In addition, while increases in temperature and pCO$_2$ negatively affected growth and feeding rates in the purple sea urchin, *Stronglycentrotus purpuratus*, these effects were at least partially ameliorated by the effects of increased temperature and pCO$_2$ on the *M. pyrifera* on which the urchins feed. Whether kelp or sea urchins will respond similarly under natural settings is unclear, but these data do highlight the importance of conducting ecologically realistic studies of climate change that consider the interactive effects of multiple stressors and species interactions, and therefore have important implications for the future of coastal kelp forest ecosystems.
Despite its worldwide distribution and its status as an important habitat ecosystem engineer (Dayton 1985), relatively little is known about how *M. pyrifera* will respond to a warmer, more acidic ocean (reviewed in Harley et al. 2012). Given this lack of information, my first goal in this thesis was to determine how *M. pyrifera* will respond physiologically to increases in ocean pCO$_2$. In my first experiment, elevating seawater CO$_2$ to either 1000 or 1500 μatm pCO$_2$ had no discernible effects on the growth rate or photosynthetic performance of *M. pyrifera* meristems compared to present-day conditions (500 μatm pCO$_2$). In fact, growth, photosynthetic oxygen evolution, and tissue carbon:nitrogen ratios of the *M. pyrifera* meristems grown in the experimental mesocosms did not differ between any of the pCO$_2$ treatments over the course of the experiment. This suggests that *M. pyrifera* may be carbon-saturated under current pCO$_2$ levels and is unlikely to respond significantly to changes in ocean pCO$_2$ alone. This is likely due to the ability of *M. pyrifera* to convert bicarbonate (HCO$_3^-$), which compromises ~90% of all carbon in the ocean and is not limiting, into CO$_2$ for use as a carbon source in photosynthesis (Parker 1965). In my second experiment, I examined how simultaneous changes in seawater temperature and pCO$_2$ will impact *M. pyrifera* growth and photosynthetic performance. To examine the interactive effects of increased temperature and pCO$_2$, I compared *M. pyrifera* growth and photosynthetic performance between present day CO$_2$ values (500 μatm pCO$_2$) and the highest values used in my first experiment (1500 μatm pCO$_2$), and between a low temperatures (12°C) such as those that approximate current temperatures in the kelp forest and high temperatures (15°C) which represent possible future ocean temperature in the kelp forest. As in my first experiment, *M. pyrifera* meristems held under elevated pCO$_2$ alone showed little-to-no changes in growth rate, photosynthetic performance, or tissue C:N ratio relative to meristems held under present-day conditions. In contrast, *M. pyrifera* growth, photosynthetic performance, and tissue C:N ratios were strongly impacted by elevated temperature. Specifically, *M. pyrifera* raised under elevated temperature alone exhibited reduced growth, lower maximum rates of photosynthesis under saturating irradiances ($P_{max}$), and reduced photosynthetic efficiency under non-saturating irradiances ($\alpha$) relative to meristems held under either present-day, elevated pCO$_2$, or future conditions. In addition, by the end of the experiment, significant tissue deterioration was observed in the *M. pyrifera* held under elevated temperature, which resulted in a decrease in thallus mass compared to *M. pyrifera* in
all other treatment combinations where the *M. pyrifer*a continued to grow throughout the experiment. Perhaps most interesting is that *M. pyrifer*a raised under conditions where both temperature and pCO$_2$ were increased together (i.e. future conditions) exhibited the fastest growth and greatest photosynthetic performance. In fact, $P_{\text{max}}$ in *M. pyrifer*a raised under future conditions was nearly double that of *M. pyrifer*a raised under present day conditions. This may have been due to the effect of temperature on RuBisCO activity during photosynthetic carbon reduction (i.e. the Calvin Cycle). Higher temperatures are known to increase the reaction rate of RuBisCO and, at least under present-day pCO$_2$ conditions, to increase oxygenase activity which reduces CO$_2$ binding. Thus, it is possible that *M. pyrifer*a raised under elevated temperatures suffered losses of photosynthetic carbon reduction due to higher rates of photorespiration. In contrast, *M. pyrifer*a raised under higher pCO$_2$ concentrations presumably exhibited diminished oxygenase activity by saturating the binding sites on RuBisCO with CO$_2$, while the increased reaction rates induced by higher temperatures allowed *M. pyrifer*a to take advantage of the increased CO$_2$ concentrations by increasing the rate of CO$_2$ binding. Thus, together this suggests that *M. pyrifer*a may benefit physiologically from simultaneous increases in both temperature and pCO$_2$ relative to present-day conditions or conditions where only temperature or pCO$_2$ are increased alone. These data suggest that the interactive effects of pCO$_2$ and temperature may be quite different from their individual effects and thus simultaneous increases in both factors may have significant consequences for ecosystem health that cannot be predicted from studies where only one is considered.

To evaluate specific physiological changes within *M. pyrifer*a that may have been responsible for the significant effects of increased temperature and pCO$_2$ on *M. pyrifer*a growth and photosynthesis, I examined possible changes in photosynthetic *M. pyrifer*a pigment concentration and carbonic anhydrase activity. In doing so, I found no significant differences in the concentrations of chlorophyll $a$, chlorophyll $c$ or fucoxanthin, nor any changes in the ratios among these pigments within *M. pyrifer*a raised under different temperature and pCO$_2$ combinations. This suggests that the observed differences in growth rates and photosynthetic performance between temperature and pCO$_2$ treatments were not due to differences in light absorption by the photosystems. However, significant differences were observed in the activity of carbonic anhydrase between *M. pyrifer*a raised under
different temperature and pCO$_2$ treatments, at least when examined under low irradiance (20 µmol photons). Specifically, under low light $M.~pyrifer a$ raised under high pCO$_2$ (1500 µatm pCO$_2$) exhibited little–to-no change in photosynthetic oxygen evolution following the inhibition of carbonic anhydrase activity by exposure to acetazolamide, regardless of the temperature in which it was examined. This suggests that $M.~pyrifer a$, under elevated pCO$_2$, was not utilizing HCO$_3^-$ as a carbon source in photosynthesis but was instead relying on diffuse uptake of CO$_2$. In contrast, $M.~pyrifer a$ raised under low pCO$_2$ (500 µatm pCO$_2$) exhibited significant decreases in photosynthetic oxygen evolution following exposure to acetazolamide, a pattern that was not impacted by differences in temperature. Interestingly, this pattern was not observed when CA activity of $M.~pyrifer a$ was examined under higher light levels. Specifically, exposure to acetazolamide under saturating irradiances (125 mmol photons) resulted in decreased photosynthetic oxygen evolution across all temperature and pCO$_2$ concentrations examined. This suggests that $M.~pyrifer a$ from all these treatments were utilizing HCO$_3^-$ as a carbon source in photosynthesis. In addition, the values for the degree of inhibition were much higher under high light compared to low, with the differences between the two light levels likely due to the higher overall rate of photosynthesis and demand for CO$_2$ at the site of ribulose 1,5-bisphosphate carboxylase (RuBisCo) in the saturating light experiment. Since light levels above my mesocosms were held at 15 – 20 µmol photons, these results suggest that the $M.~pyrifer a$ raised under high pCO$_2$ may have been down-regulating production of carbonic anhydrase during the duration of the experiment. If true, this would allow the meristems to utilize the energy that would have otherwise gone towards carbonic anhydrase production for growth which, when coupled to likely increased carboxylase activity of RuBisCO under elevated temperature and pCO$_2$, may partially explain why $M.~pyrifer a$ grew fastest under the future conditions when held under low light. When exposed to high light, photosynthesis increased and the need for additional CO$_2$ at the site of RuBisCO exceed what could be provided by diffuse CO$_2$, resulting in increased carbonic anhydrase activity. Though not significant, these results seemed to be more pronounced at higher temperatures, when enzymatic activity of Calvin cycle enzymes, including RuBisCO, was likely increased. Thus, the reliance of $M.~pyrifer a$ on HCO$_3^-$ for photosynthesis appears to be dependent on multiple factors, being greatest when irradiance and temperature are elevated and pCO$_2$ is low (i.e. when photosynthesis can become carbon
limited) and least when irradiance and temperature are low and pCO$_2$ is increased (i.e. when photosynthesis is not likely to be carbon limited). Together, this suggests that the impacts of climate change on *M. pyrifera* growth and photosynthetic performance may be strongly dependent on multiple characteristics of the habitat in which it occurs.

Curiously, although the impacts of temperature and pCO$_2$ on *M. pyrifera* growth and photosynthesis were significant and clear in my second experiment, differences in growth rates between future conditions and present-day conditions were not observed the third experiment. The reason for this is not clear, as the two experiments had the same experimental design in terms of irradiance, temperature and pCO$_2$ concentrations. However, it is possible that given the different seasons in which the *M. pyrifera* meristems were collected, this difference may be the result of intrinsic seasonal variation within the *M. pyrifera* meristems themselves (Luning 1993). In fact, at the time of collection for the third experiment, the *M. pyrifera* in the field were senescent and thus appeared in poor physiological condition (personal observation) which may account for their different response to elevated temperature and pCO$_2$ compared to *M. pyrifera* in the second experiment. This, however, remains to be tested.

In addition to altering organism physiology, environmental stressors such as increased temperature and/or pCO$_2$ can alter the manner in which species interact. In my experiment examining *Strongylocentrotus purpuratus*, I wanted to determine the effects of elevated temperature and pCO$_2$ on sea urchin growth and feeding, and then decouple this from the effects of the urchins eating food (*M. pyrifera*) also raised under these conditions. Given that climate change can alter *M. pyrifera* growth and physiology, this essentially compares the direct effects of climate change on the urchins versus the indirect effects of eating food that was impacted by climate change. I found that urchins held under higher temperatures and pCO$_2$ (i.e. future conditions) exhibited significantly lower growth rates, reduced feeding rates, and smaller gonad indices than urchins held under present day conditions. It is unclear which factor, temperature or pCO$_2$, was responsible for these differences and given logistic constraints of working in a small mesocosm system, my experimental design was not able to separate them for this experiment. However, metabolic rates are known to increase with temperature across a wide range of organisms (Gillooly *et al.* 2002), and increased metabolic costs associated with elevated pCO$_2$ have been observed
in sea urchins (Spicer et al. 2011; Catarino et al. 2012) and other echinoderms (Wood et al. 2008). Thus, the decrease in growth rate may be due to changes in physiology brought on by increases in either temperature or pCO$_2$. Physiological changes, such as increased metabolism, might also explain the lower gonad indices in the urchins held under future conditions, as higher metabolic costs would leave less energy available for gonad production and energy storage. In fact, other studies have observed decreases in gonad size under elevated pCO$_2$ (Siikavoupio et al. 2007; Kurihara 2008), supporting this idea. An alternative explanation, though obviously not mutually exclusive, is that the lower growth rates of urchins held under future conditions are due to lower feeding rates in those urchins. This is somewhat surprising given that feeding rates, like other processes tied to metabolism, are generally thought to increase with increasing temperature (Brown et al. 2004), though elevated pCO$_2$ has been shown to decrease feeding rates in sea urchins (Siikavoupio et al. 2007). Perhaps the most interesting result of my study was that the direct effects of elevated temperature and pCO$_2$ on the urchins were, at least partially, ameliorated by differences in their diets, with _M. pyrifera_ raised in future conditions allowing significantly higher growth and feeding rates in the urchins that fed on them. One possible explanation for this is that urchins were engaging in compensatory feeding, wherein animals subsisting on lower quality food consume greater quantities in order to ameliorate the negative effects of a diet low in nutrients or calories (Cruz Rivera & Hay 2000). However, analysis of _M. pyrifera_ tissue C:N ratios, a generally good metric of food quality in seaweeds, showed no significant difference between individuals raised under present day and future conditions.

The potential for elevated temperature and pCO$_2$ to cause phase shifts in kelp forest ecosystems by benefiting some species more than other has been noted in other studies (Connell & Russel 2010), and the results of my study have important implications for the future of kelp forest ecosystems. The increased rates of _M. pyrifera_ photosynthetic carbon uptake and growth suggest that, at least physiologically, _M. pyrifera_ sporophytes may benefit from a changing climate, while _S. purpuratus_ may show negative responses. These negative responses, however, may be partially ameliorated if the urchins benefit by feeding on the _M. pyrifera_ under these conditions. Therefore, since kelp-urchin interactions play a dominant role in structuring kelp forest communities (Mann 1977; Dayton 1985; Harold & Reed 1985; Leinaas & Christie 1996), changes in the strength of these interactions in the future could
alter the composition of the kelp forest. For example, phase shifts from an urchin dominated barren grounds to kelp dominated algal forests have been observed to result from a decrease in herbivory pressure, such as from the removal of herbivores by predation (Estes & Palmisano 1974) or from mass mortality (Leinaas & Christie 1996). The observed lowered urchin feeding rates under future conditions suggest a possible decrease in overall herbivory pressure under predicted future conditions. These data, combined with higher growth rates of *M. pyrifera*, suggests a potential for greater resiliency in kelp forests following disturbances or in previously urchin-dominated barren grounds as the climate warms.

However, an alternative possibility is suggested by the smaller gonad size in urchins raised under future conditions and eating kelp raised under future conditions. Here, small gonad sizes can be indicative of a low quality diet (Lemire & Himmelman 1996) or starvation conditions, and are often found in individual urchins inhabiting barren grounds (Dean *et al.* 1984; Harrold & Reed 1985). These barren grounds are generally believed to form when the demand of drift kelp exceeds supply, causing urchins to emerge from their cryptic habitats and actively forage. At these times urchins can form aggregations that consume and thereby exclude all macroalgae in large areas of the benthos. If the smaller gonad indices seen in our future conditions indicate that urchins were in fact starving, climate change may lead to an increase in active foraging among urchins, exacerbating the detrimental effect they have on macroalgal growth and increasing the occurrence of barren grounds.

In order to predict the future effects of climate change on marine communities, studies must begin to take into account the synergistic effects of multiple stressors and species interactions. In this study, I demonstrated the importance of this by showing how the response of individual organisms to climate change conditions varies when each climate factor is examined alone or when they are examined together. One of the more important findings was that while increases in temperature and/or pCO$_2$ alone may lead to negative or no impacts to *M. pyrifera*, respectively, increasing these two variables together results in positive impacts to *M. pyrifera*. Likewise, the effects on one species (e.g. *S. purpuratus*) may be negative while the impacts to another species (e.g. *M. pyrifera*) may be positive, thereby altering species interactions. If these interactions are important to either species (e.g. by grazing or predation), the ultimate impacts of climate change may be more complicated than they are when each species is considered alone. Thus, it is recommended to expand
climate change studies to include a range of environmental variables on a suite of interacting species.
REFERENCES


