AN ABSTRACT OF THE DISSERTATION OF

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Title: Effects of Environmental Stress on Intertidal Mussel Reproduction

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Environmental stress can negatively affect the ability of organisms to reproduce. Energetic trade-offs exist in all organisms, and under stress, energy may be allocated away from reproduction and towards physiological defense and repair mechanisms. The rocky intertidal environment is ideal for investigating the influence of environmental stress, as organisms are exposed to both terrestrial and marine conditions due to tidal fluctuation. Aerial exposure at low tide can lead to high temperature, desiccation, and oxidative stress. Stress in the intertidal zone increases along a vertical gradient, as organisms in the high intertidal are exposed to air for longer periods of time than those in the low intertidal.

Mussels are typically the dominant space-occupiers on temperate rocky shores, and they span the vertical gradient by occupying the entire mid-zone, from the low intertidal, which is a relatively low-stress environment, to the high intertidal, which is a high-stress environment. In this dissertation, we compared growth, reproduction, physiological defenses, pigmentation, and survival of mussels from the low-stress and high-stress regimes. We also compared energy allocation towards reproduction in mussels across a gradient of food availability between sites on the central Oregon coast. Findings indicate that growth and energy allocation towards reproduction are reduced in the high edge of the mussel bed, and physiological defenses are increased. A pattern was revealed where mussels in the high edge of the mussel bed are accumulating high concentrations of carotenoid pigments into their gonadal tissues, which was previously thought to be a secondary sex characteristic of females. This suggests that mussels may be incorporating carotenoids into their gonads in an effort to protect their gametes from damage by oxygen free radicals generated during aerial exposure.

Results of this research have implications for intertidal systems under climate change scenarios, as extreme aerial temperature events are predicted to increase in frequency and severity, and changes in oceanic circulation may also occur. Based on the findings presented in this dissertation, increases in aerial temperature could potentially lead to decreased energy allocation towards reproduction, changes in spawning time, and reduced survival of adult mussels.

Effects of Environmental Stress on Intertidal Mussel Reproduction

by

Laura E. Petes

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APPROVED:

Co-Major Professor, representing Zoology

Co-Major Professor, representing Zoology

Chair of the Department of Zoology

Dean of the Graduate School

I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

Laura E. Petes, Author

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TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

TABLE OF CONTENTS (Continued)

LIST OF FIGURES

LIST OF FIGURES (Continued)

LIST OF FIGURES (Continued)

LIST OF TABLES

LIST OF APPENDIX FIGURES

Effects of Environmental Stress on Intertidal Mussel Reproduction

Chapter 1: General Introduction

Energetic trade-offs can occur in all organisms upon exposure to stress, as energy is reallocated from growth and reproduction towards defense and repair mechanisms in order to increase chances of survival (e.g. Stearns 1992, Schreck et al. 2001). Reproduction is relatively costly and can limit energy available for immune function and defenses against stress (e.g. Stearns 1992). In addition, damaging oxygen free radicals are generated as byproducts during metabolic processes involved in gamete maturation (Harshman and Zera 2007). Increased reproduction can therefore lead to increased oxidative damage (Alonso-Alvarez et al. 2004a) and decreased survival (Wang et al. 2001) under conditions of oxidative stress. Many organisms compromise their chances of successful reproduction by releasing their gametes in response to stress (e.g. Schreck et al. 2001, Philippart et al. 2003) or by resorbing their gametes into their gonads rather than spawning (e.g. Webb et al. 1999). However, if present reproduction is compromised, future fitness may be enhanced, as chances of adult survival increase due to increased energy available for defense and repair. In addition, if gametes released are mature and viable but adult mortality occurs, offspring can result, and genetic material will be passed on to future generations.

The rocky intertidal zone is an inherently stressful but readily accessible environment, and it is therefore a useful system for studying the effects of stress. Intertidal organisms are subject to marine conditions during immersion at high tide and terrestrial conditions during emersion at low tide (e.g. Denny and Paine 1998). Environmental stress in the intertidal zone increases along a vertical gradient, as organisms in the high zone of the intertidal are exposed to aerial conditions for a longer period of time at low tide than organisms in the low zone (Davenport and Davenport 2005). Aerial exposure can lead to potentially high thermal and desiccation stress (e.g. Helmuth et al. 2002), as well as the production of oxygen radicals through metabolic processes (e.g. Abele et al. 1998).

Mussels are ideal organisms to study for investigating environmental stress in the rocky intertidal zone. They are the dominant competitors for space on temperate rocky shores around the world (Seed and Suchanek 1992). Mussels span the vertical gradient of stress in the intertidal, as they inhabit the entire mid-zone. Mussels at the tidal height of the lower edge of the mussel bed are exposed to aerial conditions ~15% of the time during a typical spring-neap tidal cycle, whereas mussels at the height of the upper edge are exposed ~50% of the time (Davenport and Davenport 2005).

While there have been several studies examining the effects of environmental stress on physiology of mussels (e.g. Helmuth and Hofmann 2001, Halpin et al. 2004), there have been few attempts to determine how stress influences reproduction in this system.

In this dissertation, I pose the following overarching questions for investigating the effects of stress on intertidal mussels:

1. How does environmental stress affect growth, defense, and survival?
2. What are the effects of environmental stress on timing of spawning and energy allocation towards reproduction?
3. How does reproduction vary along a gradient of food availability?
4. What are the effects of environmental stress on pigmentation of gonadal tissue?

To address these questions, I conducted field experiments and observational studies in the rocky intertidal zones of Oregon and New Zealand. I combined field measurements of growth and reproduction with laboratory analyses of reproduction and physiological indicators of stress.

In Chapter 2, *Intertidal stress decreases survival, growth, reproduction, and parasite load in New Zealand mussels*, my co-authors and I test the effects of environmental stress on two intertidal mussel species on the south island of New Zealand. Based on their natural distributions in the intertidal zone, one mussel species appeared to be more thermotolerant than the other. I conducted a field experiment in which both species were reciprocally transplanted between the low (relatively low-stress) and high (relatively high-stress) edges of the mussel bed. The purpose of this experiment was to determine how stress affects growth, spawning time, energy allocation towards reproduction, and parasite load in mussels. A natural heat stress event during the experiment created an opportunity to quantify both sublethal stress effects and species-specific mortality.

In Chapter 3, *Effects of environmental stress on intertidal mussels and their sea star predators*, my colleagues and I conducted a field transplant experiment on the central Oregon coast. The experiment was set in the context of environmental stress models of community ecology theory (e.g. Menge and Sutherland 1976, 1987), which propose that mobile predators will be more affected by stress than their sessile prey. The theories suggest that mobile consumers typically utilize behavioral escape mechanisms, whereas sessile prey have adopted physiological mechanisms for coping with stress. Growth, heat shock protein (Hsp) production, and stage of maturity were examined in mussels over time, and weight, Hsp production, and mortality were quantified in sea stars. Potential relationships between Hsp and reproduction were investigated.

In Chapter 4, *Environmental stress influences patterns of reproduction and tissue color in rocky intertidal mussels*, we conducted observational field surveys of intertidal mussel reproduction at 4 sites on the Oregon coast across two environmental gradients: 1) a within-site vertical gradient of stress and 2) a between-site gradient of food availability. The purpose of this study was to determine how stress and food availability affect energy allocation towards reproduction and timing of spawning in mussels. This study also examined a pattern where both female and male mussels in the high edge of the mussel bed had orange gonadal tissue, which had never before been documented for males.

In Chapter 5, *Physiological ecology of reproduction in the intertidal mussel* Mytilus californianus: *mechanistic responses to environmental stress*, my co-authors and I investigated the patterns found in Chapter 4 in more detail, using field surveys, laboratory pigment measurements, and a field transplant experiment. Observational studies of timing of spawning, energy allocation towards reproduction, and gonadal tissue pigmentation were conducted along both the vertical stress gradient and the food-availability gradient at two sites on the Oregon coast. We hypothesized that the orange coloration found in gonadal tissue in Chapter 4 was due to the presence of carotenoid pigments, and we performed laboratory investigations in Chapter 5 to quantify these pigments. A field transplant experiment was performed to determine how environmental stress affects mussel growth, reproduction, and carotenoid content. We propose that high concentrations of carotenoids may be accumulated in high-edge mussel gonads to protect their gametes from damaging oxygen free radicals.

The rocky intertidal system is an ideal environment for documenting potential effects of climate change, as organisms are exposed to both marine and terrestrial conditions daily. All four years during which I collected data for this dissertation, 2003-2006, fell in the top six highest global average annual temperatures ever recorded since the 1890's, and 2005 was the hottest global year on record (NASA 2006). Organisms in the upper edge of the intertidal zone are already living close to or at their physiological tolerance limits (e.g. Tsuchiya 1983, Helmuth et al. 2002). These organisms are adapted to coping with environmental stress but are likely to suffer mortality from extreme aerial temperature events that are predicted to increase in frequency and severity (e.g. Houghton et al. 2001).

Predicted changes in oceanic circulation under climate change (e.g. Lubchenco et al. 1993, Harley et al. 2006) could alter food availability for adult and larval intertidal organisms and could potentially affect chances that larvae will be successfully transported back to shore. In addition to 2005 being the hottest global year on record, the summer of 2005 was characterized by oceanic circulation anomalies off the Oregon coast, as the spring transition to upwelling occurred in July (almost three months late), resulting in decreased food supply and low recruitment of juveniles onshore (Barth et al. 2007). This delay was subsequently followed by intense upwelling and a hypoxic event (Chan et al. in prep.). Seasonal hypoxia began off the Oregon coast in 2002 and has since occurred every summer, with 2006 having the largest hypoxic event with the longest duration (Chan et al. in prep.). These anomalies in oceanic circulation are consistent with predictions of climate change. It is critical to study vulnerable habitats, such as the intertidal system, to gain insight into potential current and future effects of climate change.

Chapter 2:

Intertidal stress decreases survival, growth, reproduction, and parasite load in New Zealand mussels

Laura E. Petes, Bruce A. Menge, and Gayle D. Murphy

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Abstract:

To test the effects of environmental stress on mussel growth, reproduction, and parasite load, reciprocal transplants of two New Zealand mussel species, *Mytilus galloprovincialis* and *Perna canaliculus*, were performed between the high (high-stress) and low (low-stress) elevation edges of an intertidal mussel bed in New Zealand. Mussels transplanted to the high edge of the mussel bed exhibited slower growth and lower mass of reproductive tissue, indicating that stress impairs the ability of these organisms to grow and reproduce. Parasitic pea crabs were more prevalent in *M. galloprovincialis* than in *P. canaliculus*, showing that infestation rate is species-specific. Overall, *M. galloprovincialis* grew more slowly than *P. canaliculus* but invested more relative energy towards reproduction, which may help explain how these two species coexist in this habitat. An anomalous high aerial temperature event led to differential mortality of the two mussel species in the field, revealing that *P. canaliculus* is less thermotolerant than *M. galloprovincialis*. These results suggest that the abundance of *P. canaliculus*, the competitive dominant on New Zealand rocky shores, could decrease in the face of global climate change, dramatically altering intertidal community structure.

Introduction:

Global temperatures have risen 0.6˚C during the past century and are predicted to continue rising by 1.4-5.8˚C over the next century (Houghton et al. 2001). Expected alterations to the physical environment under climate change scenarios include potential sea level rise, warming of both air and water temperatures, alterations to oceanic circulation, and increasing frequency and severity of storms (e.g. Lubchenco et al. 1993, Houghton et al. 2001). Climate change may also lead to numerous biological consequences for organisms, including phenological shifts and alterations to species ranges (Parmesan and Yohe 2003). Marine ecosystems such as coral reefs (Hoegh-Guldberg 1999), kelp forests (Schiel et al. 2004), and the rocky intertidal zone (Sagarin et al. 1999) have already exhibited shifts in community structure in the face of warming temperatures. It is likely that environmental stress will increase under scenarios of climate change, because increasing frequency and severity of aerial temperature events are predicted to occur (Houghton et al. 2001).

The rocky intertidal zone is an ideal model system for examining the effects of environmental stress, as the physical environment is inherently stressful to the biota that live in this habitat. Intertidal organisms are exposed to both terrestrial and marine conditions on a daily basis, and timing of aerial exposure varies with the tidal cycle (Denny and Paine 1998, Helmuth 1999, Helmuth et al. 2002). Upper distributional limits in the intertidal zone are thought to be determined by desiccation and thermal stress, whereas lower limits are typically established by species interactions (e.g. Connell 1961, Paine 1966, Paine 1974, Lubchenco 1980). While environmental stress in the upper limit of the rocky intertidal zone is driven by a synergistic combination of temperature, desiccation, and decreased food supply that potentially result from exposure at low tide, high aerial temperatures appear to cause the most dramatic sublethal (e.g. Helmuth and Hofmann 2001) and lethal (Tsuchiya 1983) physiological consequences for intertidal organisms.

Mytilid mussels are dominant space-occupiers on temperate rocky shores throughout the world (e.g. Seed and Suchanek 1992) and span the entire vertical area between the low zone, an area of relatively low thermal and desiccation stress, and the high zone, a potentially high-stress environment. High aerial temperatures during low tide can lead to protein damage (e.g. Helmuth and Hofmann 2001, Halpin et al. 2004), reduced growth (e.g. Menge et al. 2002a), and mass mortality (Tsuchiya 1983) in mussels at the upper edge of the mussel bed. However, very little is known about the effects of environmental stress on intertidal mussel reproduction.

Reproduction can be inhibited by sublethal environmental stress, because animals reallocate energy away from gamete production and towards defense and repair mechanisms (e.g. Michalek-Wagner and Willis 2001). Timing of reproduction can be modulated by thermal stress, potentially having consequences of asynchrony and overall decreased fertilization and recruitment success (Walther et al. 2002, Philippart et al. 2003). In addition, pathogens can reduce fecundity of invertebrates (e.g. Petes et al. 2003). All of these factors can lead to a decreased number of propagules that supply adult populations and communities.

As the climate warms and environmental stress increases, the number and severity of parasite and pathogen infections will likely increase (Harvell et al. 2002). However, predicting which player in a host-parasite interaction will be affected more negatively by environmental stress is difficult, as parasites could be more susceptible to stress than their hosts (Lafferty and Kuris 1999, Lafferty and Holt 2003). Pea crab infestation can be a common occurrence in bivalves worldwide (Haines et al. 1994, Tablado and Gappa 1995, O'Beirn and Walker 1999). Pea crabs are kleptoparasites that reside within mussel shells and feed off host mucous food strings, potentially decreasing host growth and reproduction (Anderson 1975, Tablado and Gappa 1995, O'Beirn and Walker 1999).

Pea crabs exhibit patterns of intertidal zonation along with their mussel hosts. Houghton (1963) found that pea crab infection in *Mytilus edulis* decreases from the low-shore to the mid-shore. It is possible that lower tidal height allows for longer invasion time for crabs (Kruczynski 1974) or higher food availability (Haines et al. 1994), because immersion time is longer at low tide. Alternatively, pea crabs may be less tolerant of stress than their bivalve hosts and therefore unable to survive aerial exposure higher on the shore.

On the east coast of the South Island of New Zealand, rocky shores are dominated by mussels in the mid-zone of the rocky intertidal (Knox 1953, Menge et al. 1999). The two dominant species are *Perna canaliculus* G. and *Mytilus galloprovincialis* L. (Knox 1953, Menge et al. 1999). Both species undergo gametogenesis throughout the year and exhibit both late winter and late summer spawning events (Kennedy 1977, Buchanan 2001). These mussel species can host the pea crab *Pinnotheres novaezelandiae* F. (Hickman 1978, Stevens 1990). Both mussel species span the vertical gradient between the low zone and the high zone, and *P. canaliculus* appears to be the competitive dominant, growing large (~15 cm) at the lower edge of its distribution. However, this species is found only in crevices in the very extreme upper edge of the mussel bed, suggesting that it is less thermotolerant than *M. galloprovincialis* (Kennedy 1976).

The purpose of this study was to determine the effects of environmental stress on two intertidal mussel species. We predicted that under conditions of high stress, mussels would exhibit decreased growth, reproduction, and parasite load. We also predicted that *P. canaliculus* would show the greatest response to sublethal stress. Species-specific responses to stress could provide insight into future alterations to community structure under scenarios of global climate change.

Materials and Methods:

This study was conducted on the east coast of the South Island of New Zealand, specifically at “Box Thumb” (43º34’ S, 172º48’ E) on Godley Head of the Banks Peninsula near Christchurch. Box Thumb is a small peninsula with a basaltic substrate. Macroalgae and *P. canaliculus* dominate the low zone, *M. galloprovincialis* and *P. canaliculus* dominate the mid zone, and barnacles dominate the high zone (Menge et al. 1999, Menge et al. in press). Tides are mixed semidiurnal, and low tides often occur during the daytime throughout the summer season, providing the potential for high thermal and desiccation stress.

Temperature recording

Two TidbiT temperature loggers (Onset Computer Corp., Pocahassett, MA, USA) were deployed in the intertidal zone: one logger was placed above the high edge of the mussel bed ("high-zone logger") and the other was placed in the middle of the mussel bed ("mid-zone logger") on Oct. 26, 2001; these loggers recorded hourly temperatures (air or water depending on tidal cycle) each Oct.-Feb. through Feb. 25, 2005.

Surveys of mussel reproduction and parasite load

Surveys of reproduction in natural mussel populations were conducted monthly during the experiment (Dec. 2004-Feb. 2005). Mussels of two species (*M. galloprovincialis* and *P. canaliculus*) were haphazardly collected from the lower edge (~+1.0 m MLLW; "low edge") and upper edge (~+2.0 m MLLW; "high edge") of the mussel bed (n=50 of both species, both edges; total mussels per month=200). All mussels collected were in the same size class (approximately 5-7 cm in length) to minimize the influence of size variation. Mussels were opened and assessed for the presence of pea crabs.

In a subsample of mussels (n=10 of both species, both zones; total mussels each month=40), gonadal tissue and somatic tissue (including gills, hepatopancreas, and adductor muscles) were separated and weighed. From these tissues, a gonadosomatic index (GSI; e.g. Roff 1992) was calculated:

$$GSI=(\text{gonadal tissue weight})/(\text{gonadal}+\text{somatic tissue weight})$$

Gonadal tissue from each mussel was collected and placed into 10% formalin in seawater for fixation prior to histological processing to assess spawning activity.

Field transplantation and sampling

Mussels of both species were collected haphazardly from the low and high edges of the mussel bed on Oct. 23, 2004. All mussels collected were between 5-7 cm in length. Prior to transplantation, the posterior margin of the shell of each individual was notched with a file; a scar forms at the site of notching, and new shell growth can subsequently be measured from this point (Menge et al. 2004). Growth rate (mm new growth per day) was calculated as:

Growth rate=(New shell growth/Initial length)/(Days since experiment initiation)

Mussels were transplanted into mixed-species plots of 40 individuals (20 *M. galloprovincialis* and 20 *P. canaliculus* per plot). The mussels were reciprocally transplanted between edges and within edges of the mussel bed. Between-edge transplants were conducted from low-to-high (“LH”; n=4 plots) and high-to-low (“HL”; n=4) edges of the mussel bed. Within-edge transplants were conducted from low-to-low (“LL”; n=4 plots) and high-to-high (“HH”; n=4) edges to control for the effect of transplantation stress. Mussels were placed ventral-side down against the rocks to allow for byssal thread attachment using plastic-mesh cages ~20 x 20cm with 10-cm-wide borders (Menge et al. 1994). Mussels were held under mesh for six weeks to allow for firm attachment prior to mesh removal, which occurred on Dec. 8, 2004.

Monthly sampling was conducted three times from Dec. 2004-Feb. 2005. Each month, a subsample of four mussels of each species was removed from each plot. For every individual, growth rate was measured, GSI was calculated, and gonadal tissue was fixed for histological processing. Each mussel was also examined for the presence of pea crabs.

Histological processing and analyses

Gonadal tissues were dehydrated, embedded in paraffin wax, sliced to 7 µm thickness, and stained with Mayer's haemalum and eosin according to Luna (1968). Each slide was examined under a compound microscope at 100-400x (Leica DMLS, Leica Microsystems, Inc., Bannockburn, IL, USA). Spawned-out mussels were identified by lack of gametes and only lipid storage tissue remaining.

Mortality event

In January 2005, high aerial temperatures occurred in Christchurch. During this event, high mussel mortality was discernible in the intertidal zone by the presence of gaping mussels with bits of tissue remaining at Box Thumb. To quantify this event, the numbers of dead and alive mussels of both species were counted in five $0.25m^2$ quadrats in the low and high edges of the mussel bed. The event also led to high mortality of experimental mussels in the high-edge plots, and as a consequence, individuals from these treatments were unavailable for collection during the February sampling date.

Statistical analyses

All analyses were performed using JMP 6.0 (SAS Institute Inc., Cary, NC, USA, 2005). For the surveys of reproduction, gonadosomatic index (GSI) data were analyzed as a 3-factor ANOVA, with time, edge, and species as explanatory variables. For experimental mussels, growth rate, GSI, percentage of mussels spawned-out, and pea crab prevalence were analyzed separately with RM-MANOVA, using species, edge, and species x edge interactions as explanatory variables for December and January. Treatment comparisons were subsequently performed using RM-MANOVA within an edge for Dec.-Feb. for low-edge treatments (LL and HL) and Dec.-Jan. for high-edge treatments (HH and LH). Data were examined for normality and the presence of outliers. To meet the assumption of normality, growth rate data were square-root transformed, and all percentage data (percentage of mussels spawned-out and pea crab prevalence) were arcsine-square root transformed.

Results:

Temperature

Temperatures were consistently higher above the high edge of the mussel bed than in the mussel bed (Fig. 2.1), showing that temperatures were higher on average with increasing tidal height in the intertidal zone. While temperatures recorded by these loggers may deviate slightly from mussel body temperatures (e.g. Helmuth and Hofmann 2001), the recorded temperatures accurately represented aerial temperatures recorded by the New Zealand government for this location and time period (NIWA 2004, 2005) and capture the magnitude of the aerial temperature event. Extreme high temperatures occurred in January 2005 both in the intertidal zone (Fig. 2.2) and in the city of Christchurch (NIWA 2005). The mid-zone temperature logger recorded a high of 36.9˚C in the mussel bed during low tide on Jan. 15, 2005 (Fig. 2.2a). For three days in a row (Jan. 13-15), this logger recorded temperatures above 31˚C, and this was the only time period that this temperature was reached in the four months of temperature recording. In addition, in the previous three years, 31˚C was never reached in the same location during these months (Fig. 2.2a). Whereas the highest weekly average for 2001-2004 was 23˚C, a weekly average high temperature of 30˚C was reached the week of Jan. 10-16, 2005, indicating the extreme and prolonged heat wave (Fig. 2.2b). This heat event followed below-average temperatures in late Dec. 2004 through early Jan. 2005 (Fig. 2.2b, NIWA 2004).

Mussel reproduction and parasite load surveys

Surveys conducted on both mussel species from Dec.-Feb. at the higher and lower edges of the mussel bed adjacent to experimental plots revealed that *M. galloprovincialis* had higher gonadosomatic indices (GSI) than *P. canaliculus* (ANOVA: $F_{1,108}=113.73$, $p<0.0001$), indicating more relative energy allocation towards reproduction. High-edge mussels had higher GSI than the low-edge mussels ($F_{1,108}=13.97$, $p=0.0003$), but a time x edge interaction ($F_{2,108}=3.71$, $p=0.03$) reflected that high-edge mussels had an overall decrease in GSI over the summer sampling period. Spawning activity was higher in the high edge of the mussel bed in *M. galloprovincialis*, and the drop in GSI over the summer likely resulted from loss of gametes through spawning (Table 2.1).

Surveys of natural populations at Box Thumb during the experiment indicated that pea crab infection rates were consistently higher in *M. galloprovincialis* (46-52%; Table 2.2) than in *P. canaliculus* (8-20%; Table 2.2) at the low edge of the mussel bed throughout the summer. At the high edge, the same pattern held, but infection rates were very low overall (0-10%; Table 2.2).

Experimental results: Mussel growth rate

Regardless of their origin, growth rates of experimental mussels were higher in low-edge (LL and HL) treatments than high-edge (LH and HH) treatments ($F_{1,28}$=71.49, $p<0.0001$; Fig. 2.3; Table 2.3). Growth was faster in *P. canaliculus* than in *M. galloprovincialis* ($F_{1,28}$=194.60, $p<0.0001$; Fig. 2.3). There was also an edge x species interaction ($F_{1,28}$=12.30, p=0.002), indicating that growth was dependent on both species and location. In addition, growth rate marginally increased over time ($F_{1,28}$=5.00, p=0.04). Within the high-edge treatments, growth rate did not vary between species ($F_{1,12}$=1.77, p=0.21), and HH and LH growth rates were the same ($F_{1,12}$=0.28, p=0.61), but growth rate increased over time ($F_{1,12}$=12.83, p=0.004). Within the low-edge treatments, growth rate was the same in LL and HL mussels ($F_{1,12}$=12.30, p=0.77), but growth was faster in *P. canaliculus* than in *M. galloprovincialis* ($F_{1,12}$=24.01, p=0.0004). Overall, growth rates did not change with time ($F_{2,11}$=0.84, p=0.46); however, a time x species interaction ($F_{2,11}$=6.02, p=0.02) indicated that growth rate decreased in *P. canaliculus* between January and February and increased in *M. galloprovincialis* during the same time period.

Gonadosomatic index (GSI)

Gonadosomatic indices (GSI) were higher in *M. galloprovincialis* than in *P. canaliculus* ($F_{1,28}$=45.39, p<0.0001; Fig. 2.4; Table 2.3) in both the high ($F_{1,12}$=88.74, p<0.0001) and low ($F_{1,12}$=33.82, p<0.0001) edges. The low-edge treatments had higher GSI than the high-edge treatments ($F_{1,28}$=22.12, p<0.0001; Fig. 2.4). GSI decreased between December and January ($F_{1,28}$=9.72, p=0.004). In the high edge, the HH treatment mussels had higher GSI than the LH treatment ($F_{1,12}$=19.77, p=0.0008). GSI decreased with time in the high edge ($F_{1,12}$=14.41, p=0.002), and a time x treatment x species interaction ($F_{1,12}$=7.37, p=0.02) showed that GSI decreased in LH *M. galloprovincialis* between December and January. In the low-edge treatments, GSI was higher in HL mussels than LL mussels in February ($t_{1,12}$=7.77, p<0.0001).

Spawning in mussels

Histological analyses indicated that a higher percentage of mussels were spawned-out in the high-edge treatments than in the low-edge treatments ($F_{1,28}$=13.91, p=0.0009; Fig. 2.5; Table 2.3). The two mussel species did not differ in spawning activity ($F_{1,28}$=1.44, p=0.24), and time did not affect spawning ($F_{1,28}$=1.75, p=0.20). Within the high edge, spawning activity was higher in the LH treatment than the HH treatment ($F_{1,12}$=5.85, p=0.03). Within the low edge, there were no species ($F_{1,12}$=1.80, p=0.20) or treatment ($F_{1,12}$=3.87, p=0.07) effects, but spawning activity increased over the course of the summer ($F_{2,11}$=6.99, p=0.01).

Prevalence of pea crab infection

Overall, pea crab infection rate was higher in *M. galloprovincialis* than in *P. canaliculus* ($F_{1,28}$=33.92, p<0.0001; Fig. 2.6; Table 2.3) in both the high ($F_{1,12}$=7.25, p=0.02) and low ($F_{1,12}$=24.20, p=0.0004) edges of the mussel bed. Infection rates in the low and high edges did not differ ($F_{1,28}$=2.47, p=0.13), although there was a trend towards higher infection in low-edge treatments. Pea crab infection rates did not change with time ($F_{1,28}$=0.10, p=0.75). In addition, pea crab prevalence was higher in the LL treatment than the HL treatment ($F_{1,28}$=33.92, p=0.03).

Temperature-related mortality event

No mortality (0%) of either mussel species resulted at the low edge of the mussel bed from the natural thermal stress event in Christchurch. However, in the high edge, 35.4% ($\underline{+}$5.0% s.e.) of *P. canaliculus* and 3.4% ($\underline{+}$1.2% s.e.) of *M. galloprovincialis* were found dead and gaping.

Discussion:

In the New Zealand rocky intertidal system, environmental stress led to decreased growth rates, reduced energy allocation towards reproduction, and increased spawning activity in two species of mussels. One species (*P. canaliculus*) was more negatively affected by stress and suffered mortality as a result. At the lower edge of the mussel bed, *P. canaliculus* dominates due to its rapid growth rate and high competitive ability (Menge et al. in press). However, under extreme high temperatures, survival of *P. canaliculus* higher on the shore was reduced, in strong contrast to the minimal effect on *M. galloprovincialis*. This result suggests that *M. galloprovincialis* is more tolerant of thermal and desiccation stress. The allocation of more energy towards reproduction, indicated by higher gonadosomatic indices (GSI), further indicates that *M. galloprovincialis* is less sensitive to environmental stress. The complementary tradeoff strategies, faster growth by *P. canaliculus* and greater stress tolerance in *M. galloprovincialis*, may underlie the coexistence of these two species in the rocky intertidal zone.

Effects of transplantation revealed a plastic, inducible response to stress. Growth rates of mussels of both species transplanted from the low to high edge of the mussel bed decreased and were identical to the within-high edge mussels, indicating that environmental stress in the upper intertidal led immediately to slower growth rates. A high percentage of mussels (44% of *M. galloprovincialis* and 31% of *P. canaliculus*) transplanted from the low to the high edge had spawned all of their gametes by January. This is in contrast to the within-low edge treatment, in which few mussels (13% of *M. galloprovincialis* and 6% of *P. canaliculus*) had spawned gametes by the same month. Stress affects many aspects of reproduction, including timing of spawning, gamete quality, and fertilization success (e.g. Schreck et al. 2001). It is possible that in this system, earlier spawning time could be a response to an increase in stress. It is possible that high-edge mussels are releasing gametes to reallocate energy away from reproduction and towards defense and repair mechanisms that increase their likelihood of survival.

The predictions of global climate change scenarios are complex and include alterations to oceanic circulation, increasing frequency and severity of storms and aerial temperature events, and sea level rise (e.g. Lubchenco et al. 1993, Houghton et al. 2001). All of these factors could potentially affect intertidal communities. This study documented strong sublethal (growth and reproduction) and lethal effects of aerial temperature on intertidal mussels. Warming temperatures likely resulting from climate change have already been shown to affect reproduction and recruitment in bivalves (Philippart et al. 2003) and may have already affected mussel bed community diversity (Smith et al. 2006). Although releasing gametes in response to sudden increases in stress, as documented here, has potential adaptive value, temperature-dependent spawning of bivalves can lead to a mismatch with phytoplankton blooms, leaving larvae food-deficient and potentially decreasing larval survivorship (Philippart et al. 2003). Hence, spawning induced by environmental stress in New Zealand mussels could possibly lead to increased larval mortality and lower recruitment rates of juveniles.

Environmental stress can increase parasite mortality and therefore decrease the impact of the parasite on the host (Lafferty and Kuris 1999, Lafferty and Holt 2003). Monthly surveys of pea crab prevalence revealed low infestation rates in the high-edge mussels. This is consistent with other studies (e.g. Houghton 1963) that show strong patterns of pea crab zonation. After the high temperature event in January, 2 of the 16 pea crabs found in low-edge experimental mussels during February sampling were dead. This phenomenon had not been seen throughout the rest of the experiment and suggests heat-related mortality. In addition, all pea crabs living in the high-edge mussels likely died along with their hosts during the mussel mortality event.

In the face of increasing environmental stress under global climate change scenarios (Houghton et al. 2001), species will be differentially affected by warming temperatures (e.g. Schiel et al. 2004). Many intertidal organisms are already living close to or at their physiological thermal tolerance limit (Stillman and Somero 2000, Somero 2002). The upper distributional limit of mussel beds fluctuates with long-term patterns of emersion time and would move down under scenarios of increasing aerial temperatures (Denny and Paine 1998, Harley et al. 2006). The anomalous temperature event in this study surpassed the tolerance limit of *P. canaliculus* living at the upper edge of their distribution, killing approximately one-third of the population. While we recognize that the sublethal and lethal responses of mussels in this study were not a direct response to climate change, the findings reveal likely consequences of climate change on a community-dominant group. As a result of future high temperature events, alterations in community structure could occur, as *P. canaliculus* distribution in the intertidal could shift lower along the vertical stress gradient, and *M. galloprovincialis* would subsequently dominate the upper edge of the mussel bed even more strongly.

Under increasing global temperatures and particularly higher variance in temperature (Houghton et al. 2001), it is likely that the number of acute aerial temperature events will increase. The high temperature on the day of the mortality event in this study was the 3rd highest January temperature for this area of New Zealand since records began in 1939 (NIWA 2005). This followed immediately after the coldest December in Christchurch since temperature measurements started in 1953 (NIWA 2004). It is likely that some species will be unable to adapt quickly enough to defend themselves from a rapidly changing and unpredictable climatic regime (Houghton et al. 2001). This study provides some insight into potential alterations in intertidal species interactions that could arise as a consequence of global climate change.

Acknowledgements:

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Table 2.1. Spawning activity (% of mussels spawned-out) for high and low edge surveys of the mussel bed (n=9-10 mussels) from Dec. 2004 to Feb. 2005.

Treatment	**Species**	**8-Dec**	**7-Jan**	**5-Feb**
High edge survey	*M. galloprovincialis*	20%	50%	60%
	P. canaliculus	10%	0%	20%
Low edge survey	*M. galloprovincialis*	0%	20%	10%
	P. canaliculus	10%	10%	10%

Table 2.2. Pea crab prevalence (% of mussels infected) for high and low edge surveys of the mussel bed (n=50 mussels) from Dec. 2004 to Feb. 2005.

Treatment	**Species**	**8-Dec**	**7-Jan**	**5-Feb**
High edge survey	*M. galloprovincialis*	6%	10%	4%
	P. canaliculus	0%	0%	2%
Low edge survey	*M. galloprovincialis*	52%	46%	48%
	P. canaliculus	8%	20%	8%

Table 2.3. Results of repeated-measures MANOVA tests for growth, GSI, percentage of mussels spawned-out, and pea crab prevalence. Comparisons were performed between edges and subsequently within edges. All results for P<0.10 are displayed. Results of interaction terms with P>0.10 are not shown. Values for P<0.05 are bolded.

Response variable	Comparison	Parameter	df	F	P
Growth rate	Between edges	Species	1,28	194.60	**<0.0001**
		Edge	1,28	71.49	**<0.0001**
		Edge x Species	1,28	12.30	**0.002**
		Time	1,28	5.00	**0.04**
	Within high edge	Species	1,12	1.77	0.21
		Treatment	1,12	0.28	0.61
		Time	1,12	12.83	**0.004**
	Within low edge	Species	1,12	24.01	**0.0004**
		Treatment	1,12	0.09	0.77
		Time	2,11	0.84	0.46
		Time x Species	2,11	6.02	**0.02**
Gonadosomatic index (GSI)	Between edges	Species	1,28	45.39	**<0.0001**
		Edge	1,28	22.12	**<0.0001**
		Time	1,28	9.72	**0.004**
		Time x Species	1,28	3.56	0.07
	Within high edge	Species	1,12	88.74	**<0.0001**
		Treatment	1,12	19.77	**0.0008**
		Time	1,12	14.41	**0.002**
		Time x Treatment x Species	1,12	7.37	**0.02**
	Within low edge	Species	1,12	33.82	**<0.0001**
		Treatment	1,12	1.67	0.22
		Time x Species	2,11	3.08	0.09
		Time x Treatment	2,11	10.21	**0.003**
Percentage of mussels spawned-out	Between edges	Species	1,28	1.44	0.24
		Edge	1,28	13.91	**0.0009**
	Within high edge	Species	1,12	0.57	0.47
		Treatment	1,12	5.85	**0.03**
	Within low edge	Species	1,12	1.80	0.20
		Treatment	1,12	3.87	0.07
		Time	2,11	6.99	**0.01**

Table 2.3 (Continued) Results of repeated-measures MANOVA tests for growth, GSI, percentage of mussels spawned-out, and pea crab prevalence. Comparisons were performed between edges and subsequently within edges. All results for P<0.10 are displayed. Results of interaction terms with P>0.10 are not shown. Values for P<0.05 are bolded.

Percentage of mussels infected by pea crabs	Between edges	Species	1,28	33.92	**<0.0001**
		Edge	1,28	2.47	0.13
	Within high edge	Species	1,12	7.25	**0.02**
		Treatment	1,12	0.67	0.43
		Time x Treatment x Species	1,12	3.16	0.10
	Within low edge	Species	1,12	24.20	**0.0004**
		Treatment	1,12	6.42	**0.03**

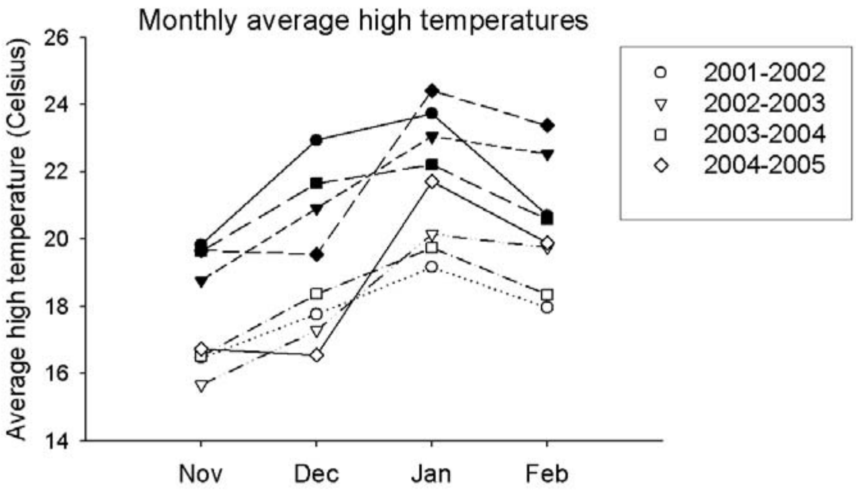
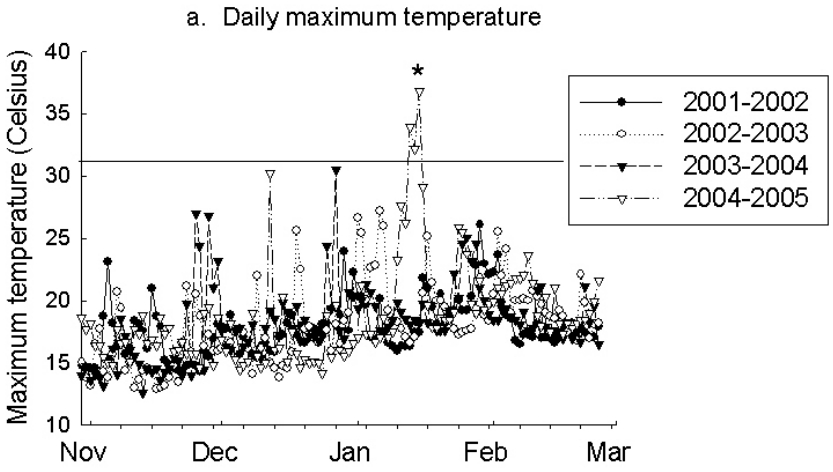


Fig. 2.1. Monthly average high temperatures from high-zone logger (dark symbols) and mid-zone logger (white symbols) for Nov.-Feb. of 2001-2005.


a. Daily maximum temperature

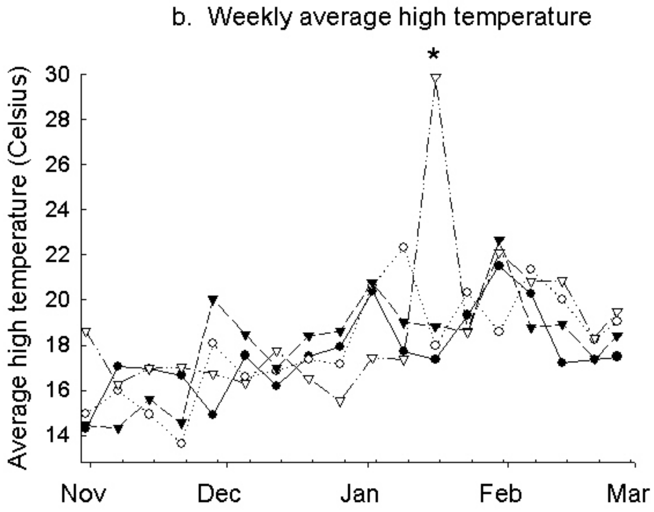

b. Weekly average high temperature

Fig. 2.2. a. Daily maximum temperatures and **b.** average maximum weekly temperatures for mid-zone temperature logger from October-February of 2001-2005. Asterisks denote heat stress event in Jan. 2005. Line in Fig. 2.2a shows 31°C, which was the record temperature hit between 2001-2005.

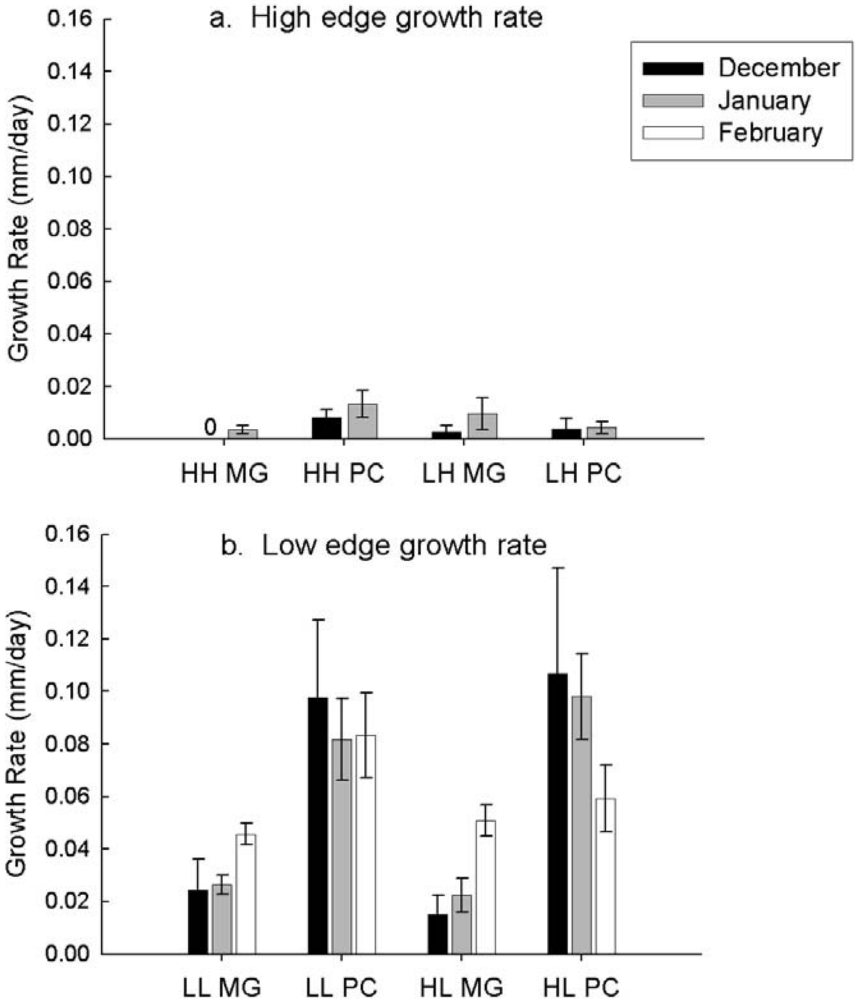


Fig. 2.3. Growth rates of two mussel species for **a.** high-edge and **b.** low-edge treatments. Mussels: MG=*Mytilus galloprovincialis*, PC=*Perna canaliculus*; transplant treatments: HH=high-to-high, LH=low-to-high, LL=low-to-low, HL=high-to-low edge. Error bars = standard error. Zero denotes no growth.

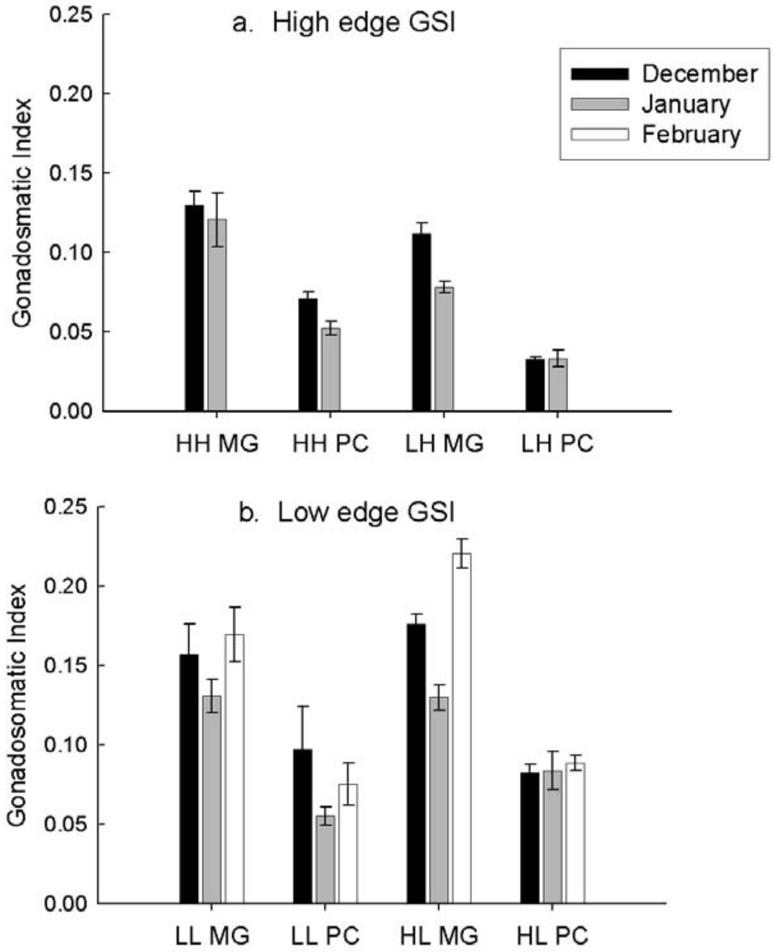


Fig. 2.4. Gonadosomatic indices (GSI) for **a.** high-edge and **b.** low-edge treatments. Mussels: MG=*Mytilus galloprovincialis*, PC=*Perna canaliculus*; transplant treatments: HH=high-to-high, LH=low-to-high, LL=low-to-low, HL=high-to-low edge. Error bars = standard error.

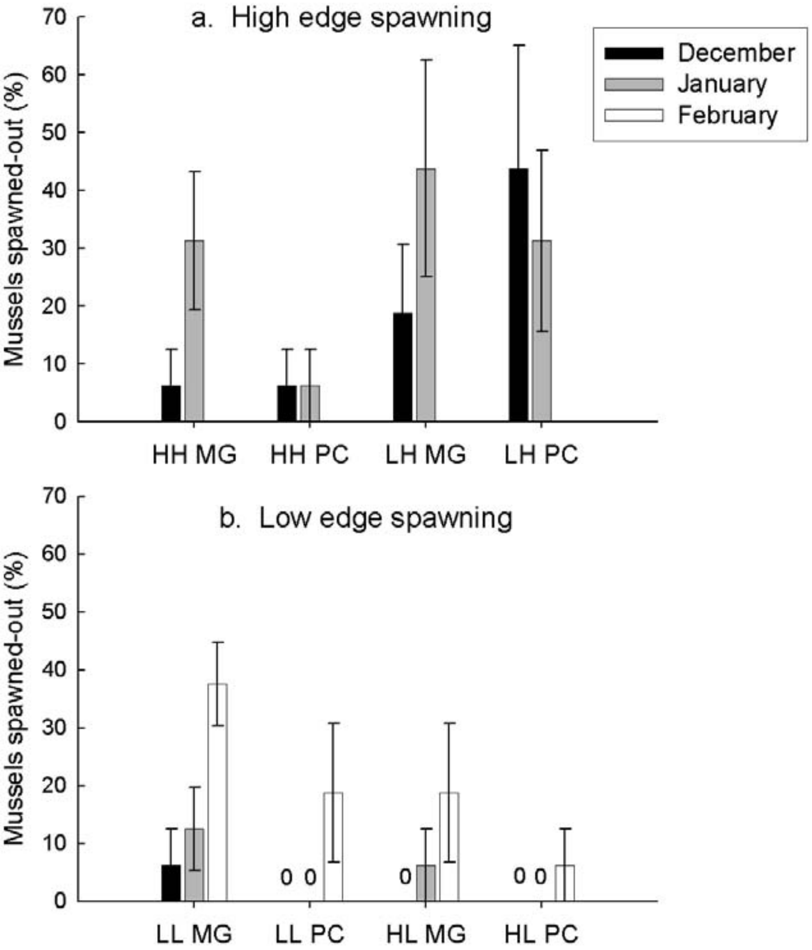


Fig. 2.5. Percentage of spawned-out mussels for **a.** high-edge and **b.** low-edge treatments. Mussels: MG=*Mytilus galloprovincialis*, PC=*Perna canaliculus*; transplant treatments: HH=high-to-high, LH=low-to-high, LL=low-to-low, HL=high-to-low edge. Error bars = standard error. Zeros indicate no spawned-out mussels.

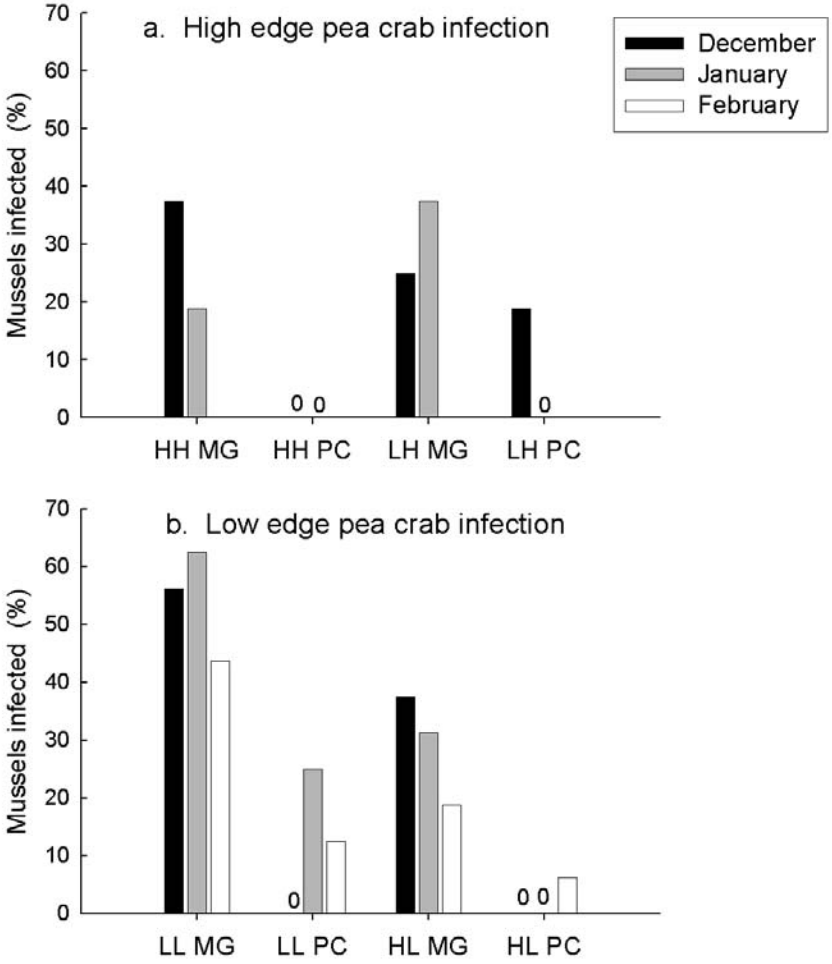


Fig. 2.6. Percentage of mussels infected with pea crabs for **a.** high-edge and **b.** low-edge treatments. Mussels: MG=*Mytilus galloprovincialis*, PC=*Perna canaliculus*; transplant treatments: HH=high-to-high, LH=low-to-high, LL=low-to-low, HL=high-to-low edge. Zeros indicate no pea crabs.

Chapter 3:

Effects of environmental stress on intertidal mussels and their sea star predators

Laura E. Petes, Morgan E. Packard, Ruth H. Milston-Clements,
Tracey S. Momoda, and Bruce A. Menge

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Abstract:

Consumer stress models of ecological theory predict that predators are more susceptible to stress than their prey. Intertidal mussels, *Mytilus californianus*, span a vertical stress gradient from the low zone (lower stress) to the high zone (higher thermal and desiccation stress) while their sea star predators, *Pisaster ochraceus*, range from the low zone only into the lower edge of the mussel zone. In summer 2003, we tested the responses to environmental stress of sea stars and mussels in an experiment conducted on the Oregon coast. Mussels were transplanted from the middle of the mussel bed to cages in the low and high edges of the mussel bed. Sea star predators were added to half of the mussel cages. Mussels and sea stars were sampled between June and August for indicators of sublethal stress. Mussel growth was measured, and tissues were collected for heat shock protein (Hsp70) analyses and histological analyses of reproduction. Sea stars were weighed, and tissues were sampled for Hsp70 analyses. Mussels in the high-edge cages had higher levels of total Hsp70 and exhibited spawning activity earlier in the summer than mussels in the low-edge cages. Mussels with higher levels of Hsp70 had lower reproductive potential, indicating a possible trade-off between Hsp production and reproduction. Sea stars suffered high mortality in the high edge, and low-edge sea stars lost weight, suggesting that they were sublethally stressed. Results of this study indicate that intertidal stress affected the mobile predator more than its sessile prey, which is consistent with predictions of consumer stress models.

Introduction:

Ecologists have proposed multiple models to predict how environmental stress affects species interactions and community structure (e.g. Connell 1975, Menge and Sutherland 1976, 1987, Menge and Olson 1990, Bruno et al. 2003). Consumer stress models predict that mobile top predators are more affected by environmental stress than their sessile prey (Connell 1975, Menge and Sutherland 1976, Menge and Farrell 1989). Sessile organisms are likely to face the highest probability of exposure to physiological stress due to their immobility (Menge and Sutherland 1976, 1987) and thus are predicted to be better adapted to stressful conditions. In contrast, due to their mobility, relatively large predators are predicted to be capable of behaviorally avoiding stressful conditions. These traits (mobility and size), through adaptive trade-offs, may make them more susceptible to environmental stress. Manifestations of exposure to stressful conditions by predators include sheltering during periods of stress, with consequent reduced feeding (Menge and Sutherland 1976, 1987, Menge et al. 2002a). In contrast, prey stress models predict that prey will be more stressed than their predators (see Menge and Olson 1990). Such models are more applicable to situations where predators are small relative to their prey (Menge and Olson 1990, Menge et al. 2002a). Few empirical tests of environmental stress models have been performed in the field, but evidence has been documented that supports both predator (e.g. Menge 1978a,b, McClanahan 1992, Menge et al. 2002a) and prey (e.g. Louda and Collinge 1992, Trowbridge 1998) stress models, suggesting that interactions may be context- or system-dependent.

The rocky intertidal zone is an ideal system for investigating environmental stress gradients and responses of predators and prey. The intertidal zone is a harsh environment for organisms, because they are exposed to both aerial and aquatic conditions on a daily and tidal basis (e.g. Denny and Paine 1998, Helmuth 1999, Helmuth et al. 2002). Weather and climatic fluctuation can generate substantial variation under both conditions, with aerial temperatures ranging between freezing and hot and aquatic conditions ranging between calm to severely wave-swept. On the west coast of North America, the sea star *Pisaster ochraceus* is a keystone predator in rocky intertidal systems. If sea stars are removed, their principal prey, the mussel *Mytilus californianus*, colonizes and dominates the low intertidal zone, thereby decreasing species diversity (Paine 1966, 1974, Menge et al. 1994). Sea stars respond to higher water temperatures by increasing their feeding rate on mussels (Sanford 1999), one example of a temperature-related effect on intertidal invertebrate function.

Mytilid mussels are the competitive dominants for space on temperate rocky shores throughout the world (Seed 1976, Seed and Suchanek 1992). Upper distributional limits in the rocky intertidal zone are thought to be determined by desiccation and thermal stress (Connell 1972), whereas lower limits are frequently set by species interactions, including predation pressure (Connell 1961, Paine 1974, Lubchenco and Menge 1978). Mussels span this entire vertical range, from the lower intertidal (low-stress) to the high intertidal (high-stress environment) zone. Recently, several intertidal studies have documented consequences of this vertical stress gradient to intertidal invertebrates using physiological tools (e.g. Roberts et al. 1997, Dahlhoff et al. 2001, 2002, Tomanek and Sanford 2003, Halpin et al. 2004). However, the sublethal effects of stress on these organisms are still relatively unstudied.

Several techniques can be utilized to quantify the physiological effects of sublethal stress on organisms. Heat shock proteins (Hsp) act as molecular chaperones in the cell (Lindquist 1986). They refold denatured stress-damaged structural proteins and can be induced as a defense against tissue-damaging heat stress (e.g. Buckley et al. 2001, Halpin et al. 2004). However, Hsp synthesis is costly (Feder and Hofmann 1999), as Hsp do not contribute to growth and reproduction, and their synthesis may interfere with production of more critical proteins under thermal stress (Helmuth and Hofmann 2001), potentially reducing scope for growth (Roberts et al. 1997). Inducible (Hsp72; 72kDa) and constitutive (Hsc75; 75kDa) isoforms of Hsp70 can be examined to determine both background levels of heat shock proteins and levels of Hsp induced upon exposure to thermal stress (Hofmann and Somero 1995, Helmuth and Hofmann 2001, Halpin et al. 2002). The level of total Hsp70 produced appears to be the best predictor for thermotolerance (Sorte and Hofmann 2005).

Reproduction can also be used as an indicator of sublethal stress (Michalek-Wagner and Willis 2001, Schreck et al. 2001). The process of reproduction shows effects of stress earlier than many other biological processes. Repeated acute stress can decrease quality of gametes (Burdon and Müller 1987, Campbell et al. 1992), and chronic stress may lead to spawning failure (Bromage 1995). If the fecundity of a population is lowered, there may be a decreased supply of new propagules that maintain adult populations and communities (Brokordt et al. 2000). Sublethal thermal stress on adult mussels leads to reduced survival of gametes through embryogenesis, which causes a decline in ecological fitness of individuals in the population (Bayne 1972). However, little is known about the effects of physiological repair mechanisms on reproductive potential.

This study tested the effects of environmental stress on two important players in the intertidal system, the predatory sea star *Pisaster ochraceus* and its principal prey, the mussel *Mytilus californianus*.

Materials and Methods:

A field experiment was initiated at Strawberry Hill (44º25'N, 124º12'W) on the central Oregon coast in April 2003.

Experimental set-up

All mussels were collected from Bob Creek, OR (44º24'N, 124º11'W), approximately 1 km south of Strawberry Hill on April 13, 2003. Each individual was haphazardly collected from the intermediate vertical height of the mussel bed (not the high or low edges) to select mussels that had been previously exposed to average levels of thermal and desiccation stress. After collection, each mussel (initial size ~4-6 cm) was notched with a file at the posterior tip of the shell. New shell growth can be measured past the point of notching due to "scar" formation (Menge et al. 2004). Mussels were held in ambient seawater tanks overnight at Oregon State University's Hatfield Marine Science Center in Newport, OR.

On April 14, 2003 at Strawberry Hill, mussels were placed into stainless steel mesh cages (38 x 38 x 15 cm) that were fastened to the rock using lag screws screwed into wall anchors placed in holes drilled in the rock. Cages were established in the low and high edges of the mussel bed with the following three treatments:

a. Sea stars and no mussel food source
b. Sea stars with mussel food source
c. Mussels and no sea star predators

With 3 treatments x 2 edges x 4 replicates, a total of 24 cages were deployed. Fifty mussels were placed into each mussel cage (treatments b. and c.) and were covered with plastic vexar mesh for four weeks to allow time for attachment and growth of new byssal threads for adhesion onto the substrate (Menge et al. 2004). On May 19, 2003, the plastic mesh was removed from the mussels, and three weighed and measured sea stars were added to each sea star cage (treatments a. and b.). Sea star average initial wet mass per cage was 319.6 g (±10.4 g s.e.), and average standard length (madreporite to the tip of the opposite arm) was 12.0 cm (±0.2 cm s.e.). All cages were covered with plastic vexar mesh lids in order to prevent sea stars from escaping and to provide similar thermal environments to all treatments. This design was likely to have moderated thermal and desiccation conditions in the cages relative to natural surfaces, but a proper test of the effects of stress required that experimental conditions be as nearly identical as possible.

To evaluate the similarity of conditions in cages and to monitor thermal environment as perceived by mussels, temperature was quantified within cages.

Temperature logger-containing mussel mimics (“Robomussels”) were created by embedding TidbiT temperature loggers (Onset Computer Corp., Pocasset, MA) in epoxy and black resin molded into the shape of a mussel according to Helmuth and Hofmann (2001). These loggers accurately simulate mussel body temperature to within 2°C (Gilman et al. 2006). Two “Robomussels” were deployed in both low-edge and high-edge cages. Temperatures (aerial or aquatic, depending on tidal cycles) were recorded every 6 minutes over the course of the entire experiment.

Field sampling protocol

Cages were sampled every four weeks from early June through late August 2003, for a total of four sampling periods: June 2, July 2, July 31, and August 28. In treatment b. (+sea stars, +mussels), mussels were replaced as necessary during the experiment, as their numbers were depleted due to sea star feeding. These supplemental mussels were attached to the inside of the cage in a mesh bag with holes large enough for sea stars to feed through (L. Petes, personal observation).

During each sampling period, three mussels were collected from the treatment c. (-sea stars, +mussels) cages and were dissected and measured. Gonadal tissue was excised and placed into 10% formalin in seawater for fixation. Gill tissue from each individual was removed and flash-frozen in liquid nitrogen for transport back to a -80°C freezer at Oregon State University (Corvallis, OR) prior to heat shock protein analyses. Each month, every sea star was removed from the sea star cages (treatments a. and b.) and was weighed in the field. A small sample of tube feet tissue was removed from each individual and flash-frozen in liquid nitrogen for transport to the –80°C freezer in Corvallis. Sea stars were placed back into their cages immediately after sampling.

Histological processing and analyses

Mussel gonadal tissues were dehydrated, embedded in paraffin wax, sectioned to a thickness of 7 µm, and stained with hematoxylin and eosin according to Luna (1968). Each slide was examined under a compound microscope at 100-400x (Leica DMLS, Leica Microsystems, Inc., Bannockburn, IL). Sex was identified for each individual, and stage of maturity was assessed using the following categories:

FEMALES:

1- resting stage (no gametes)
2- pre-vitellogenic – oogonia present
3- early vitellogenesis – few yolk platelets in primary oocytes
4- mid-vitellogenesis - more yolk platelets accumulated, germinal vesicle is central
5- ripe – full of yolk, germinal vesicle migrating towards animal pole
6- early ovulaton - some spawning activity indicated by empty follicles
7- mid-ovulation - few ripe oocytes remaining, many empty follicles
8- post-ovulation - completely spawned-out, all follicles empty

MALES:

1- resting stage (no gametes)
2- pre-meiotic – spermatogonia present in testicular cysts
3- early meiosis - spermatogonia and primary spermatocytes
4- mid-meiosis - spermatogonia, primary and secondary spermatocytes, spermatids
5- ripe - all of the above plus spermatozoa
6- early spermiation - some spawning activity, some spermatozoa missing
7- late spermiation - mostly spawned, few spermatozoa remaining
8- post-spermiation - completely spawned-out, empty testicular cysts

Reproductive potential was quantified only for females, because male tissue was composed of masses of testicular cysts that were variable in size, maturity, and cell density. For each female, the number of vitellogenic oocytes within a field of view at 400x was counted for 10 randomized areas of gonadal tissue per slide, and an average "reproductive potential" was calculated from these areas.

Heat shock protein analyses in mussels

Mussel gill tissues were homogenized in a Tris-SDS buffer (50 mM Tris-HCl pH 6.8, 4% SDS, 1 mM EDTA, 1 mM PMSF) at a weight:volume ratio of 1:4. Samples were then heated at 100°C for 5 min. and centrifuged at 14,000 g for 15 min. as in Halpin et al. (2004). The supernatant was decanted, stored at -80°C, and used for the remainder of the analyses. Protein concentrations of samples were determined using modified Bradford protein assays according to the manufacturer's instructions (Pierce: Coomassie Plus-200). Ten µg of total protein were loaded for each sample onto 7.5% SDS-polyacrylamide gels (VWR: 53225-106) adjacent to 10 µg Hsc70 protein positive control (StressGen: SPP-751) and a Kaleidoscope pre-stained molecular weight marker (BioRad: 161-0324). Bands were resolved using electrophoresis (running buffer: 25 mM Tris, 192 mM glycine, 0.1% SDS) at 125 V for 100 min. Gels were transferred electrophoretically in buffer (25 mM Tris, 192 mM glycine, 20% methanol) onto PVDF membranes (Fisher Scientific: IPVH00010) at 100 V for 60 min.

Western blotting was performed after transfer according to Tomanek and Sanford (2003). Membranes were incubated at a 1:2500 dilution with monocolonal rat antibody (IgG) against Hsp70 (Affinity Bioreagent: MA3-001). After washing, membranes were incubated with rabbit-anti-rat bridging antibody (Vector: AI-4000) at a dilution of 1:2000. After more washing, they were incubated with horseradish-peroxidase protein A solution (BioRad) at 1:5000. To visualize bands, membranes were developed using chemiluminescence detection (Pierce: SuperSignal) and exposure of blots to X-ray film. Induced (72kDa) and constitutive (75kDa) bands of Hsp70 were quantified from developed film with densitometry and ImageQuaNT software (Molecular Dynamics). Sample band intensities were divided by band intensities of Hsc70 positive controls for each gel in order to allow comparison between gels (see Fig. 3.1a for an image of a Western blot).

Heat shock protein analyses in sea stars

Sea star tube feet tissues were homogenized as described above for mussels except at a weight:volume ratio of 1:1. Gel electrophoresis was performed as described above. Due to technical difficulties that arose with the Affinity rat anti-Hsp70 primary antibody, a different primary antibody (Hsp70 rabbit polyclonal; Stressgen: SPA-757) at a 1:1,000 dilution and secondary antibody (ECL anti-rabbit IgG, HRP-conjugated; Amersham: NA934V) at 1:5,000 were used for Western blotting of sea star samples. These antibodies only allowed for detection of total

Hsp70 as one large band, which was quantified as described above (see Fig. 3.1b for an image of a Western blot).

Statistical analyses

Monthly average temperatures (aerial and aquatic) and monthly average high temperatures (aerial) were calculated from Robomussel temperature data.

Experimental data were analyzed in JMP 6.0 (SAS, Inc., Cary, NC); repeated-measures multivariate analysis of variance (RM-MANOVA) tests were performed on the four collection time points.

For mussels, edge (low vs. high) was used as the explanatory variable, and Hsp (total, induced, and constitutive), stage of maturity, and reproductive potential were separately analyzed as response variables. Mussel growth was analyzed for only initial and final sampling time points. Data were examined for normality and the presence of outliers. Mussel Hsp70 (total, inducible, and constitutive), stage of maturity, and reproductive potential data were ln-transformed prior to analyses to improve normality. Even after transformation, some time points did not have normally distributed variance for total and induced Hsp70. A regression was also performed between reproductive potential and total Hsp70 (both ln-transformed) for pooled data from mussel averages for all time points to determine if there was a trade-off between Hsp70 production and reproductive potential.

For sea stars, edge (low vs. high) and treatment (+mussels, -mussels) were used as explanatory variables in RM- MANOVA to examine the mortality response.

Due to high mortality in the high edge (see below), only low-edge sea stars were analyzed for weight and total Hsp production, with treatment as the explanatory variable. Two sets of sea stars were accidentally replaced into the wrong cages during the second sampling date, and these two cages were subsequently eliminated from analyses. Sea star mortality data (proportion dead) were arcsine-square root transformed, and Hsp70 data were ln-transformed to meet assumptions of normality. Multiple transformations were attempted with weight data, but none resulted in a normal distribution, and untransformed data were therefore analyzed.

Results:

Robomussel temperatures

Average monthly temperatures, which incorporated both aerial and aquatic temperatures, were higher at the high edge of the mussel bed (Fig. 3.2). Average monthly high temperatures (aerial) were also consistently higher at the high edge of the mussel bed (Fig. 3.2).

Mussel responses to environmental stress

There was no significant effect of edge on mussel growth (Table 3.1; $F_{1,6}$=0.61, p=0.46); however, growth did increase in both low and high edges over time ($F_{1,6}$=18.41, p=0.005). Total Hsp70 production for mussels was higher in the high edge than in the low edge (Fig. 3.3; Table 3.1; $F_{1,6}$=5.72, p=0.05) and did not change over time ($F_{3,4}$=1.98, p=0.26). The inducible isoform (Hsp72) of Hsp70 was the same in high-edge mussels as in low-edge mussels (Fig. 3.3; Table 3.1; $F_{1,6}$=3.34, p=0.11), and there was no change in this isoform across time ($F_{3,4}$=0.62, p=0.64). Constitutive Hsp isoform (Hsc75) production was the same regardless of edge in the high-edge mussels than in the low-edge mussels (Fig. 3.3; Table 3.1; $F_{1,6}$=4.09, p=0.09), but production fluctuated with time ($F_{3,4}$=15.31, p=0.01).

Stage of reproductive maturity was higher in high-edge mussels than low-edge mussels (Fig. 3.4a; Table 3.1; $F_{1,6}$=15.55, p=0.008), reflecting increased spawning activity. Stage of maturity increased marginally over the course of the summer ($F_{3,4}$=5.24, p=0.07), with spawning occurring at both edges. Reproductive potential was higher in low-edge mussels than in high-edge mussels (Fig. 3.4b; Table 3.1; $F_{1,5}$=13.78, p=0.01), indicating a higher number of ripe gametes that may be spawned. There was a significant negative relationship between total Hsp70 and reproductive potential when data were pooled across both edges and all time points, suggesting a possible trade-off between Hsp70 production and reproduction (R^2=0.16, $F_{1,29}$=5.33, p=0.03).

Sea star responses to environmental stress

Sea stars at the high edge of the mussel bed suffered high mortality (Fig. 3.5; Table 3.1; $F_{1,10}$=22.37, p=0.0008), and mortality increased with time ($F_{3,8}$=5.60, p=0.02). Food availability ($\pm$mussels) had no effect on mortality (Fig. 3.5; Table 3.1; $F_{1,10}$=0.54, p=0.48). Due to high mortality in high-edge sea stars, the high-edge treatments were omitted from subsequent analyses.

Low-edge sea stars were analyzed for weight and Hsp70 production. Due to the influence of high winds on the balance in the field on the first sampling date, sea star weights were obtained for only the last three sampling dates. Weight was higher in sea stars in the +mussel treatment (treatment b.; Fig. 3.6a; Table 3.1; $F_{1,3}$=14.74, p=0.03), showing that sea stars were feeding. However, weight decreased in all animals over time ($F_{2,2}$=24.41, p=0.04), indicating potential sublethal stress and reduced feeding activity. Total Hsp70 production was the same in low-edge sea stars regardless of food availability (Fig. 3.6b; Table 3.1; $F_{1,3}$=0.37, p=0.58) or time ($F_{3,1}$=1.91, p=0.48).

Discussion:

Consumer stress models predict that mobile consumers will be less able to defend themselves from stress and that sessile prey will be better adapted (Connell 1975, Menge and Sutherland 1976, Menge and Farrell 1989). The results of this study are consistent with the predictions of these models. As expected, predatory sea stars exhibited high mortality under stressful conditions. Further, sublethal stress effects were also evident, because all sea stars including those with a mussel food source lost weight throughout the experiment. While mussels exhibited physiological signs of sublethal stress (increased Hsp production), no heat-related mortality was documented, as no gaping shells were present in cages without predators (L. Petes, personal observation).

The high mortality of sea stars compared to the low mortality of mussels indicates that sea stars were unable to survive the same level of environmental stress as their sessile prey. However, it is important to note that caging sea stars imposed an artificial regime on their behavior, as they would typically avoid thermal stress by crawling into spatial refuges (crevices, etc.) or retreating to the lower shore. Other mobile organisms exhibit up-regulation of Hsp and no mortality when transplanted above their vertical range into cages in the rocky intertidal zone (Tomanek and Somero 2003). This suggests that some mobile animals in the intertidal zone use Hsp to defend themselves from physiological damage. High mortality of sea stars, in contrast, may indicate that sea stars do not effectively use Hsp as a defense to combat tissue-damaging heat stress. The few surviving sea stars that were available for tissue sampling from high-edge treatments showed no increase in Hsp production (data not shown). Sea stars likely invest a majority of their energy in moving to spatial refuges in order to avoid thermal and desiccation stress.

Mussels, as sessile organisms, have to rely instead on defenses against stress to prevent physiological damage or mortality from occurring. Mussels increased production of Hsp as a plastic response to stress. This is consistent with Halpin et al. (2004), who found that transplanted mussels increased Hsp production in the high intertidal zone. While there were no significant differences in this study in production of inducible and constitutive Hsp70 between edges, these isoforms were consistently higher in mussels from the high edge. This suggests that the “constitutive” isoform may actually be up-regulated in response to increased stress, and therefore its constitutive definition should be questioned. Total Hsp, the most reliable metric of thermal tolerance (Sorte and Hofmann 2005), was significantly higher in high-edge mussels.

Timing of reproduction and reproductive potential were strongly influenced by environmental stress. Mussels transplanted to the high edge showed increased spawning activity and therefore a decrease in the number of ripe gametes maintained in their gonadal tissue (lower reproductive potential). Many organisms exhibit accelerated maturity (e.g. Dethier et al. 2005) or spawn in response to stress (Schreck et al. 2001, Philippart et al. 2003, Chapter 2). When this occurs, gametes that are spawned may not be mature and viable (Schreck et al. 2001) or may be released asynchronously from the rest of the population (Philippart et al. 2003). This would represent wasted energy invested in reproduction and may be a last-resort option for an organism facing mortality.

Few, if any, field studies have been conducted to examine the effects of Hsp production on reproduction. Almost all studies of Hsp effects on reproduction have been conducted in the laboratory on fruit flies (e.g. Krebs and Loeschcke 1994), and the majority of these studies find that organisms eliciting a heat shock response exhibit reduced fecundity. In this experiment, there was a negative correlation between Hsp production and reproductive potential in mussels. Hsp production is costly, as it requires extensive energy and interferes with normal cell function (Feder and Hofmann 1999, Sørensen et al. 2003). Within organisms, there appears to be tight regulation of the balance between the cost of Hsp and the benefit of fertility (Krebs and Loeschcke 1994, Sørensen et al. 2003). Given that the relationship documented in this study was only correlative, the evidence for energetic trade-offs between Hsp production and reproduction should be investigated further.

Many intertidal organisms are already living at their physiological limit for stress (Hofmann and Somero 1995, Tomanek 2002). The rocky intertidal zone is a habitat especially vulnerable to climate change, as organisms are exposed to both terrestrial and marine conditions on a daily and tidal basis (e.g. Denny and Paine 1998, Helmuth 1999, Sagarin et al. 1999, Helmuth et al. 2002). As global temperatures continue to rise (Houghton et al. 2001), it is likely that only organisms with the ability to successfully respond to stress will survive. Results of this study indicate that sessile organisms in the intertidal zone can survive high aerial temperatures. However, sessile organisms living at their tolerance limit may exhibit mortality if defense mechanisms are insufficient for coping with unpredictable acute or chronic thermal stress events (e.g. Chapter 2) and may therefore be more vulnerable to climate change. In addition, as temperatures increase, it is possible that mussel populations will be at a disadvantage relative to their mobile predators. Rising sea levels are likely to extend the vertical range of foraging sea stars, and feeding rates of *P. ochraceus* should increase with increasing water temperature (Sanford 1999). However, according to consumer stress models and the results of this study, if sea stars are also exposed to higher aerial temperatures during low tides, feeding activity may be reduced, which could decrease the interaction strength between sea stars and mussels.

Overall, it seems likely that major shifts in intertidal community structure may result from warming, although it is difficult to predict which effects might prevail. Clearly, understanding the influence of sublethal stress in intertidal invertebrates could provide insights into future alterations in the physiology and ecology of marine organisms as a result of climate change (e.g. Helmuth and Hofmann 2001, Helmuth et al. 2002).

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Table 3.1. Results of repeated-measures multivariate analysis of variance tests for mussels (*Mytilus californianus*) and sea stars (*Pisaster ochraceus*). Comparisons were performed between edges for mussels. Sea star comparisons were made between both treatments and edges for mortality but were only made between treatments (within the low edge) for weight and total Hsp70. Parameters and values with p≤0.05 are in bold.

Organism	Response variable	Parameter	df	F	P
Mytilus californianus	**Growth**	Edge	1,6	0.61	0.46
		Time	1,6	18.41	**0.005**
		Time x Edge	1,6	0.61	0.46
	Total Hsp70 (ln-transformed)	**Edge**	1,6	5.72	**0.05**
		Time	3,4	1.98	0.26
		Time x Edge	3,4	2.91	0.16
	Induced Hsp70 (ln-transformed)	Edge	1,6	3.34	0.11
		Time	3,4	0.62	0.64
		Time x Edge	3,4	2.15	0.24
	Constitutive Hsp70 (ln-transformed)	Edge	1,6	4.09	0.09
		Time	3,4	15.31	**0.01**
		Time x Edge	3,4	0.49	0.71
	Stage of Maturity (ln-transformed)	**Edge**	1,6	15.55	**0.008**
		Time	3,4	5.24	0.07
		Time x Edge	3,4	1.58	0.33
	Reproductive Potential (ln-transformed)	**Edge**	1,5	13.78	**0.01**
		Time	3,3	1.78	0.32
		Time x Edge	3,3	2.79	0.21
Pisaster ochraceus	**Mortality** (arcsin-square root transformed)	**Edge**	1,10	22.37	**0.0008**
		Treatment	1,10	0.54	0.48
		Treatment x Edge	1,10	0.80	0.39
		Time	3,8	5.60	**0.02**
		Time x Edge	3,8	0.62	0.62
		Time x Treatment	3,8	0.70	0.58
		Time x Treatment x Edge	3,8	0.63	0.62
	Weight	**Treatment**	1,3	14.74	**0.03**
		Time	2,2	24.41	**0.04**
		Time x Treatment	2,2	5.05	0.16
	Total Hsp70 (ln-transformed)	Treatment	1,3	0.37	0.58
		Time	3,1	1.91	0.48
		Time x Treatment	3,1	5.39	0.30

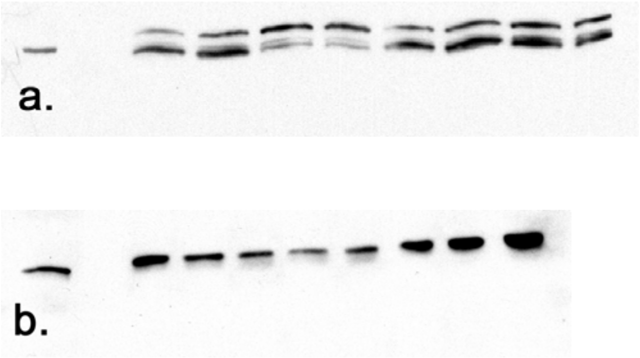

Fig. 3.1. Western blot analyses of heat shock proteins from **a.** mussels and **b.** sea stars. The developed blots were exposed to X-ray film for 30 s. Left-most lane represents Hsc70 standard. The other eight lanes represent individual samples.

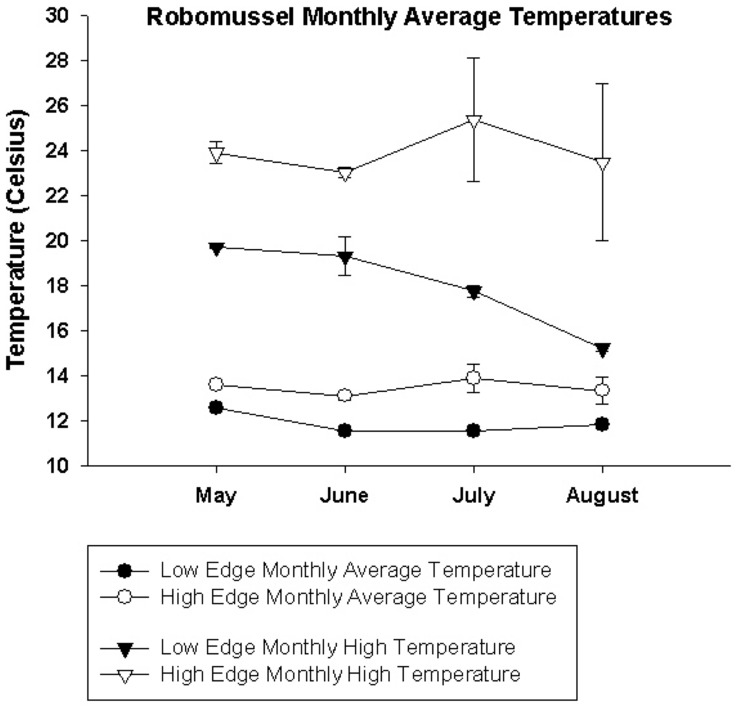

Fig. 3.2. Average monthly temperatures and average monthly high temperatures for Robomussels deployed in cages. Y-error bars denote standard error.

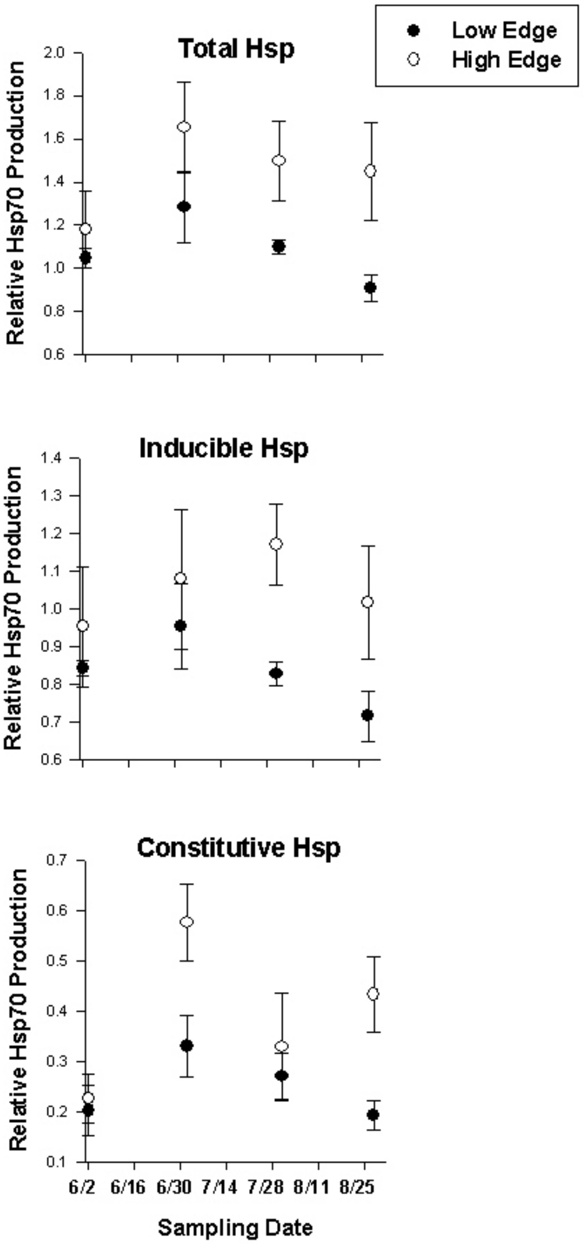

Fig. 3.3. Average heat shock protein production in mussels over the course of the summer. Total Hsp70 levels, inducible Hsp70 levels, and constitutive Hsp70 levels. Y-error bars denote standard error of n=4 cages per edge.

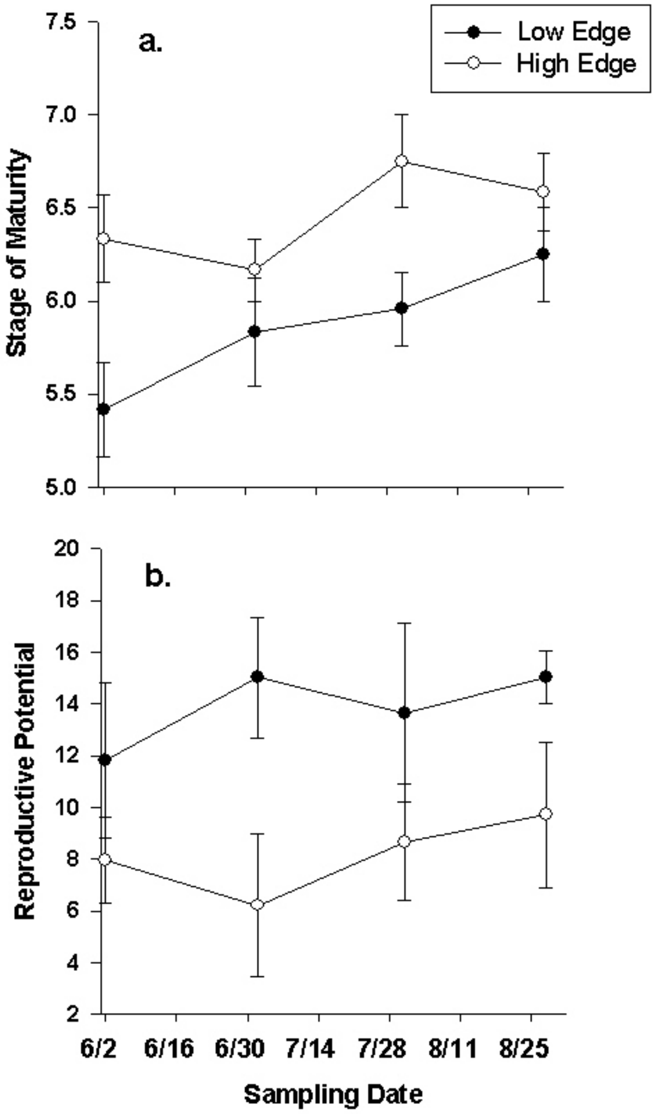

Fig. 3.4. Average **a.** stage of maturity and **b.** reproductive potential of mussels. Reproductive potential is the number of vitellogenic oocytes per ocular field-of-view at 400x. Y-error bars denote standard error of n=3-4 cages per edge.

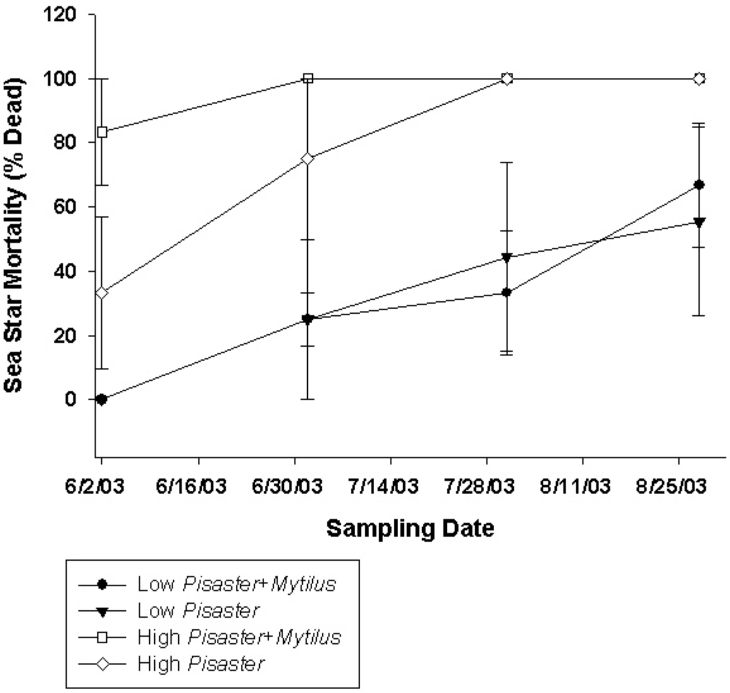

Fig. 3.5. Mortality of sea stars (average percentage dead) in each treatment. Y-error bars denote standard error of n=4 cages per treatment.

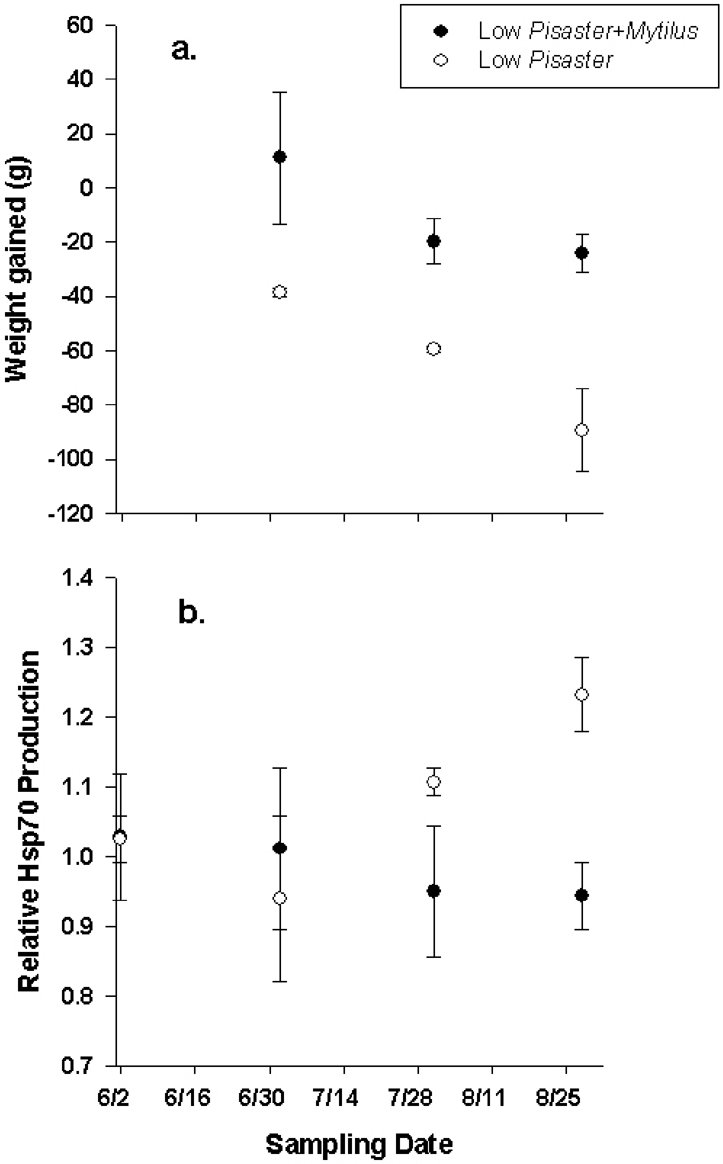

Fig. 3.6. Average **a.** weight gain and **b.** relative Hsp70 production of low-edge sea stars. Y-error bars denote standard error of n=2-4 cages per treatment per time point.

Chapter 4:

Environmental stress influences patterns of reproduction and tissue color in rocky intertidal mussels

Laura E. Petes, Bruce A. Menge, Francis Chan, and Molly A.H. Webb

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Abstract:

Stress in the rocky intertidal zone is caused by a number of factors including extreme temperatures and reduced food availability during aerial exposure at low tide. The duration and severity of each of these factors, both alone and in combination, can influence the ecology and physiology of intertidal organisms. Patterns of mussel reproduction and gonadal tissue coloration were examined along two environmental axes: (1) a vertical (tidal height) stress gradient within each of 4 sites on the central Oregon coast and (2) a food-availability gradient across the 4 sites. Food availability appeared to affect timing of reproduction, but there was no relationship between food and gonadal tissue color. Mussels at the stressful high edge of the mussel bed spawned earlier in the summer than mussels at the low edge, indicating that stress appears to influence timing of reproduction. Surprisingly, both male and female mussels at the high edge contained high concentrations of orange pigmentation in their gonadal tissue. This finding is contrary to conventional wisdom that mussel sex can be determined visually by gonad color (males = white, females = orange). Given that males had orange gonads at the high edge of the mussel bed, field classification of mussel sex based on tissue color is likely to be inaccurate. This pattern allows us to formulate a working hypothesis where pigments are accumulated in gonadal tissue to protect gametes from damaging oxygen radicals generated as a result of aerial exposure at low tide.

Introduction:

Environmental stress is defined as any characteristic of the physical environment that causes changes in biochemical reactions by approaching or exceeding physiological tolerance limits of organisms (Menge and Sutherland 1976). Stress can lead to a suite of negative consequences, including reduced growth, changes in metabolic rate, decreased fecundity, and mortality (e.g. Schreck et al. 2001). Environmental stress decreases reproductive success in many different organisms, as energy is typically allocated away from reproduction and towards defense and repair mechanisms to increase chances of survival (Dobson and Smith 2000, Schreck et al. 2001).

The rocky intertidal zone is an excellent system for investigating environmental stress gradients and their consequences (e.g. Menge et al. 2002a). The intertidal environment is inherently stressful, because organisms are exposed to both marine conditions when immersed at high tide and terrestrial conditions when emersed at low tide (e.g. Helmuth and Hofmann 2001, Davenport and Davenport 2005). Environmental stress in the intertidal zone is likely a combination of reduced time for feeding, increased exposure to aerial temperatures, and increased desiccation during emersion at low tide. Stress increases along a vertical gradient in the intertidal zone, from the relatively low-stress low zone to the higher-stress high zone, as aerial emersion time increases with increasing height on the shore (Davenport and Davenport 2005). The lower limits of species distributions in the intertidal zone are typically thought to be determined by species interactions (e.g. Connell 1961, Paine

1974), whereas the upper limits appear to be set by physiological tolerance limits (Connell 1972; see Lubchenco and Cubit 1980 for exception).

Mytilid mussels are competitive dominants for space on many temperate rocky shores (Seed and Suchanek 1992). They span the vertical stress gradient in the intertidal, inhabiting the entire mid-intertidal zone, from the upper edge of the low zone to the lower edge of the high zone. Mussels in the high edge of the mussel bed live close to their physiological tolerance limits and can exhibit sublethal (e.g. Helmuth and Hofmann 2001) or lethal (Tsuchiya 1983, Chapter 2) signs of stress at these margins. Mussels are gonochoric broadcast-spawners that are capable of reproducing throughout the year (Seed and Suchanek 1992). For decades, rocky intertidal ecologists have identified mussel sex (male vs. female) in the field based on the color of the gonadal tissue (Chipperfield 1953, Seed and Suchanek 1992). Males have been identified by the presence of creamy white gonadal tissue, whereas ripe females typically have been identified by the presence of bright orange gonads (Chipperfield 1953, Seed and Suchanek 1992). This difference in tissue coloration has been attributed to higher levels of carotenoid pigments in females than in males (Scheer 1940, Campbell 1969). Carotenoids can help to defend organisms against damaging singlet oxygen radicals generated as metabolic byproducts during exposure to stress, because these pigments can bind to singlet oxygen and convert it to less-harmful hydrogen peroxide (Miki 1991). However, the relationship between environmental stress and gonadal tissue color has never before been explored in intertidal mussels. Because mussels are filter-feeders, they obtain carotenoids from the phytoplankton they consume (Campbell 1969). Mussels have the ability to selectively uptake specific carotenoids from phytoplankton and assimilate these pigments into their gonadal tissue (Campbell 1970).

Two Capes on the central Oregon coast are known to differ in their oceanographic regimes and bottom topography, creating persistent differences in nearshore primary production and therefore availability of food for mussels and other filter-feeders (Menge et al. 2002b, 2004, Leslie et al. 2005). Cape Perpetua has relatively high productivity, indicated by high chlorophyll-*a* (chl-*a*) concentration (Menge et al. 1997, 2002b, 2004), which is a good estimate of phytoplankton abundance in nearshore marine systems (Menge et al. 1997). This high abundance of food leads to rapid growth rates of many filter-feeding invertebrates, such as mussels (Menge et al. 2002b, 2004). In contrast, Cape Foulweather (~80 km North) has relatively low phytoplankton abundance and consequently slower growth rates of sessile invertebrates (Menge et al. 1997, 2002b, 2004). These contrasting food regimes have been correlated with differential reproductive success of onshore adults, with adult barnacles from Cape Perpetua producing more larvae, presumably as a result of greater availability of food (Leslie et al. 2005).

The purpose of this study was to test the following three hypotheses in intertidal mussels:

1. Food availability affects timing of reproduction.
2. Increased food availability leads to more orange coloration in gonadal tissue.

3. Stress influences timing of reproduction and tissue coloration.

Materials and Methods:

To investigate how environmental stress and food influence patterns of reproduction and gonadal tissue color, mussels were collected monthly between May and September 2004 from four field sites on the central Oregon coast. Two of the sites, Fogarty Creek ("FC"; 44.84°N, 124.06°W) and Boiler Bay ("BB"; 44.83°N, 124.06°W), are located at Cape Foulweather. Fogarty Creek is ~0.5 km North of Boiler Bay. The other two sites, Yachats Beach ("YB"; 44.32°N, 124.12°W) and Strawberry Hill ("SH"; 44.25°N, 124.12°W), are located ~80 km South at Cape Perpetua. Yachats Beach is ~6 km North of Strawberry Hill.

*Chlorophyll-*a *and temperature measurements*

Phytoplankton concentration was estimated using chlorophyll-*a* (chl-*a*) to quantify the food source for mussels. Surf zone chl-*a* measurements were collected once per month between May and August 2004 on the same day at all four field sites as described in Menge et al. (2004). Briefly, replicate bottle samples (n=3) of water were taken from shore and filtered in the field through combusted Whatman GF/F glass-fiber filters. Chl-*a* concentration was determined using a Turner Designs TD-700 fluorometer (Turner Designs, Sunnyvale, CA, USA) according to Welshmeyer (1994). The fluorometer was calibrated with a chl-*a* standard from Sigma Chemical Company (St. Louis, MO, USA).

One TidbiT temperature logger (Onset Computer Corp., Pocahassett, MA, USA) was deployed in the middle of the mussel bed at each site (FC, BB, YB, and SH) to record temperature (aerial or aquatic depending on tidal cycle) every 10 minutes from May 1 – Sept. 30, 2004. At SH, two temperature loggers were deployed from April 1 – Sept. 30, 2004 to document differences in temperature between the middle and the lower edge of the mussel bed.

Field surveys of reproduction and tissue color

At each site, mussels (*Mytilus californianus*) between 5-7 cm in length were collected. This species reaches reproductive maturity at a size of 2.5 cm (Suchanek 1981), and therefore all of these individuals were large enough to have attained reproductive maturity. Mussels were collected from the high (~+2.0 m above MLLW) and low (~+1.0 m above MLLW) edges of the mussel bed (n=15 from each edge) and dissected in the field. Gonadal tissue color was scored on a 1-3 scale with 1=white, 2=peach, and 3=orange (see Fig. A1 in Appendix), and sex (male, female, or unknown) was assessed based on tissue color. Gonadal tissue was subsequently removed and placed into 10% formalin in seawater for histological processing to confirm sex and assess stage of maturity.

Histological analyses of sex and stage of maturity

Gonadal tissues were dehydrated, embedded in paraffin wax, sliced to 7 µm thickness, and stained with hematoxylin and eosin according to Luna (1968). Slides were examined under a compound microscope at 100-400 x (Leica DMLS, Leica Microsystems, Inc., Bannockburn, IL). Sex (male or female) was identified on each slide, and stage of maturity was assigned into the same categories as in Chapter 3.

Statistical analyses

Survey data were analyzed with a 4-factor analysis of variance (ANOVA) using JMP 6.0 (SAS Institute, Inc., Cary, NC, USA) statistical software. Month, edge, site, sex, and all interactions were examined as explanatory variables, and stage of maturity and color were analyzed separately as continuous response variables. Residual and normal probability plots were examined for the presence of outliers and for normality. Both stage of maturity and color responses were ln-transformed to meet assumptions of normality and independent error terms for all analyses. Sample sizes were unbalanced, because sex could not be accurately determined in the field prior to histological processing, and therefore between 4 and 11 individuals of each sex resulted from the 15 mussels collected in each edge during each time point. To create balanced sample sizes so that assumptions of ANOVA would not be violated, subsets of data ($n=4$) were randomly selected for ANOVA (Quinn and Keough 2002). Categories were compared with Tukey-Kramer honest significant difference (HSD) tests based on least square means estimated from main effects or interaction terms at $p<0.05$ (Quinn and Keough 2002). The relationship between color and stage of maturity was also examined to see how color changes with spawning activity. Two exploratory 4-factor ANOVAs were then performed to determine differences between

Capes by testing the effects of month, edge, Cape, sex, and all possible interactions on stage and color.

A subsequent analysis was performed to quantify what percentage of mussels would have been sexed incorrectly based on the field color system. The percentage of mussels incorrectly sexed was calculated as follows: if color score was higher than a "2" (peach) and the mussel was a male, it would have been incorrectly classified as a female. If color score was lower than a "2" and the mussel was a female, it would have been incorrectly identified as a male. If color was a "2", the sex would have been classified as "unknown" in the field. Data were arcsin-sqrt-transformed prior to analyses to improve normality. An ANOVA was performed across pooled time points testing the effects of site, edge, sex, and the edge x sex interaction on the percentage of mussels that would be inaccurately sexed based on color. Categories were compared with Tukey-Kramer HSD tests based on least square means estimated from main effects or interaction terms at $p<0.05$ (Quinn and Keough 2002).

Results:

Chlorophyll-a:

Chlorophyll-*a* was typically lower at the Cape Foulweather sites (FC and BB) than the Cape Perpetua sites (YB and SH), and there was a pulse of high phytoplankton in July at all sites (Fig. 4.1a).

Temperature across sites:

Monthly average high temperature (calculated from daily highs of temperature loggers in the middle of the mussel bed) was consistent between May and July and decreased slightly in August and September (Fig. 4.1b). There were some differences in temperature between the four sites, with BB and SH being hotter on average than FC and YB. This could either be due to true between-site differences or could instead be the result of microhabitat temperature differences due to placement of loggers (e.g. Helmuth and Hofmann 2001). Because there was only one logger at each site, it is difficult to determine whether between-site temperature differences are valid. For this study, we will focus on summer temperature trends across all sites, rather than at any individual sites.

Temperature in the vertical stress gradient:

Temperature was consistently higher on average in the middle of the mussel bed than the low edge (Fig. 4.2a). Calculations of monthly average temperatures (from daily averages) and monthly average high temperatures (from daily maxima) revealed the same pattern, with higher temperatures reached by the mid-zone logger than the low-zone logger (Fig. 4.2b).

Patterns of reproduction and tissue color

In general, stage of maturity was higher in high-edge mussels, indicating that more spawning had occurred in the high-edge mussels than the low-edge mussels ($F_{1,240}$=4.29, p=0.04; Fig. 4.3a; see Table 4.1 for complete results of ANOVA). Stage was marginally lower at FC than at SH overall, suggesting less summer spawning activity at FC ($F_{3,240}$=2.82, p=0.04). Stage was also higher overall in males than females ($F_{1,240}$=15.17, p=0.0001), although this is likely due to the fact that it is easier to see the disappearance of sperm from the center of testicular cysts than when a fraction of oocytes have been spawned. Stage of maturity was higher in YB and SH high-edge mussels than high-edge FC and low-edge YB mussels ($F_{3,240}$=7.99, p<0.0001).

Spawning activity increased throughout the summer ($F_{4,240}$=44.86, p<0.0001), with most individuals being mature in May and June, beginning to spawn in July and August, and having spawned many of their gametes by September. Spawning was initiated earlier in the summer in the high edge of the mussel bed than the low edge ($F_{4,240}$=4.28, p=0.002). BB mussels began spawning earlier than did the mussels at other sites ($F_{12,240}$=10.16, p<0.0001). High-edge males at the end of the summer had exhibited more spawning activity by the end of the summer than high-edge males and females from both edges at the beginning of the summer (Fig. 4.4a; $F_{4,240}$=3.11, p=0.02). The most mature mussels were found in the low edge of BB in July, YB in May, and FC and SH in June; the most spawned-out mussels were found in the high edges of YB and SH in July and SH in September (Fig. 4.5a; $F_{12,240}$=2.64, p=0.002). A month x sex x site interaction ($F_{12,240}$=2.23, p=0.01) showed that the most mature mussels were BB males in July, and the most spawning had occurred in BB males in September and SH males in July (Fig. 4.6a).

Color was higher in the high-edge mussels than the low-edge mussels ($F_{1,240}$=56.78, p<0.0001; Fig. 4.3b; see Table 4.1 for complete results of ANOVA). Females had higher overall levels of orange coloration than males ($F_{1,240}$=84.82, p<0.0001), but there were no differences in color between high-edge males and females, which were all more orange than low-edge males ($F_{1,240}$=52.53, p<0.0001). BB had the most orange coloration, and FC had the least ($F_{3,240}$=4.91, p=0.003).

Overall, color decreased through time, with May and June having higher levels of orange coloration than July, August, and September ($F_{4,240}$=27.71, p<0.0001). In addition, color varied with time across the sites ($F_{12,240}$=4.41, p<0.0001), with the highest level of orange coloration at YB in June and the lowest levels at FC in August and September. Color varied with sex over time ($F_{4,240}$=4.15, p=0.003), as females in May and June had the highest levels of orange coloration, and males in July through September had the lowest levels. A relationship between sex, edge, and month on color (Fig. 4.4b; $F_{4,240}$=3.19, p=0.01) revealed that females from both edges had more orange coloration earlier in the summer than low-edge males later in the summer. A relationship between site, edge, and month on color (Fig. 4.5b; $F_{12,240}$=2.07, p=0.02) showed that the highest orange coloration was found in mussels in the high edge at YB in June and SH and FC in May, and the lowest was in the low edge at FC in August and September. FC females in May and June and YB females in June had the most orange coloration, whereas FC males in August had the least (Fig. 4.6b; $F_{12,240}$=2.80, p=0.001).

Stage of maturity increased with a decrease in color ($F_{1,318}$=30.73, $p<0.0001$), although the linear fit was weak (R^2=0.09). This result suggests that there may be a slight decrease in gonadal tissue color with an increase in spawning activity.

The ANOVAs performed with Cape rather than site as an explanatory variable elucidated several of the complex differences described above (see Table 4.2 for complete ANOVA results). Mussels at Cape Perpetua began spawning earlier than mussels at Cape Foulweather ($F_{4,280}$=11.45, $p<0.0001$). Mussels in the high edge at Cape Perpetua exhibited the same amount of spawning activity overall as mussels in the low edge at Cape Foulweather but spawned more than mussels in the high edge at Cape Foulweather and in the low edge at Cape Perpetua ($F_{1,280}$=18.50, $p<0.0001$). Mussels in the high edge at Cape Perpetua in July through September and in the low edge at Foulweather in September had exhibited more spawning activity than mussels in both edges at Cape Perpetua in May and June ($F_{4,280}$=5.12, p=0.0005). There were few effects of Cape on color, although mussels at Capes Perpetua and Foulweather in May and June had the most orange coloration, and mussels at Cape Foulweather in August and September had the least ($F_{4,280}$=2.70, p=0.03). Also, females at Capes Perpetua and Foulweather in May and June (prior to spawning) were the most orange, and males at Cape Perpetua in May and at Cape Foulweather in July and August were the least orange ($F_{4,280}$=3.17, p=0.01).

Accuracy of using tissue color to identify sex of mussels

Mussels in the high edge of the mussel bed would have been inaccurately sexed based on color more frequently than mussels in the low edge ($F_{1,9}=22.82$, $p=0.001$). More males than females were incorrectly sexed based on color ($F_{1,9}=7.00$, $p=0.03$). High-edge males were sexed incorrectly 60.1% (±1.4%) of the time, which was much more often ($F_{1,9}=25.22$, $p=0.0007$) than low-edge males (6.5% ±3.1%), low-edge females (14.8% ±4.8%), and high-edge females (13.7 ±4.5%). There were no differences in the ability to correctly sex mussels based on color between sites ($F_{3,9}=1.13$, $p=0.39$).

If the percentage classified as "unknown" was added to the percentage scored incorrectly, the same differences resulted, except that the percentage misclassified increased as follows: high-edge males (86.0% ±3.8%) were misidentified more frequently than low-edge males (43.5% ±1.7%), high-edge females (38.3% ±5.6%), and low-edge females (28.1% ±6.9%).

Discussion:

The interactions between food availability, temperature, and stress play a critical role in the timing and success of reproduction in many different organisms (e.g. Schreck et al. 2001, Philippart et al. 2003, Leslie et al. 2005). Environmental stress in the rocky intertidal zone is likely due to synergistic interactions between decreased food availability, high temperatures, and desiccation during long periods of aerial exposure (e.g. Connell 1961). This study showed that both reproduction and tissue color vary along vertical stress gradients in the rocky intertidal zone. Gonadal maturation in bivalves often coincides with increasing temperatures and peak phytoplankton production in the summer, and spawning typically occurs after this gonadal growth period (Sastry 1966). The same temporal pattern in reproduction was revealed in this study, particularly in the high-edge mussels, as spawning increased over the summer.

Food availability

The food regime (chl-*a*) was related to timing of reproduction, but there was no relationship between food and gonadal tissue color. Phytoplankton (chl-*a*) concentrations were higher at the Cape Perpetua sites than the Cape Foulweather sites, which is consistent with previous studies documenting a persistent difference in food availability between these areas of the Oregon coast (Menge et al. 1997, 2002b, 2004, Leslie et al. 2005). Mussels at Cape Perpetua sites spawned earlier in the summer, suggesting that mussel populations at these sites had acquired sufficient nutrients and energy to reproduce earlier in the season. All sites showed some spawning activity during the phytoplankton bloom in July, although spawning also occurred both before and after this peak. Perhaps mussels in this environment are not food-limited, as the Oregon coast has high phytoplankton abundance due to intermittent upwelling (Menge et al. 2002b), and therefore they do not show a strong temporal response to pulses in food availability. While differences in chl-*a* were found between Capes, there were no differences in gonadal tissue color between sites, suggesting that at least at larger scales, tissue color may not change in response to food. However, tissue color did decrease over time as spawning activity increased, indicating that pigments may be lost with release of gametes.

Temperature

There was a relationship between thermal environment and timing of reproduction in the mussel bed. Spawning activity increased in all mussels throughout the summer. High-edge mussels spawned earlier than mussels in the low edge. This may be partially due to greater exposure to high summer temperatures in the high edge during aerial emersion. It is possible that mussels in high-stress environments expend so much energy towards physiological defenses against stress (e.g. Helmuth and Hofmann 2001, Halpin et al. 2004) that they do not have enough energy available for maintenance of gametes during the summer. Spawning can occur as a response to thermal stress (e.g. Schreck et al. 2001, Chapter 2) and can lead to asynchrony and decreased fertilization success in populations (Philippart et al. 2003). Further research needs to be conducted to determine whether gametes spawned from high-edge mussels are viable and whether enough gametes are released synchronously into the water column to ensure fertilization success. In addition, spawning in the early summer (June) may result in larvae that are held offshore due to seasonal upwelling that occurs off the Oregon coast in May through August (e.g. Barth et al. 2007). Mussel recruitment in Oregon peaks in the late summer through late fall months, when relaxation events and downwelling currents occur with greater frequency, and propagules are delivered back to shore as a result of these events (e.g. Barth et al. 2007). Larvae resulting from high-edge spawning events may fail to be transported back onshore due to the timing of these oceanographic processes and could therefore represent wasted reproductive effort.

Color of gonadal tissue

Environmental stress (low-edge vs. high-edge environment) strongly influenced patterns of gonadal tissue coloration. High-edge mussels had higher incidence of orange coloration than mussels from the low edge of the mussel bed. The most striking and consistent difference was found in gonadal pigmentation between low-edge and high-edge males. High-edge males had just as much orange coloration in their gonadal tissue as females. Well over half of the males from the high edge would have been scored incorrectly based on tissue color, indicating that this is an inaccurate method for assigning sex to *Mytilus californianus*, particularly in the high edge of the mussel bed where the pattern of high orange coloration is most distinct. Using visual classification based on tissue color would have resulted in the incorrect interpretation that sex ratios are skewed, with a majority of mussels in the high edge being female. Based on these results, visual identification of sex should be examined for accuracy in other mussel species. As orange coloration in mussels is due to the presence of carotenoid pigments (Campbell 1969, 1970), it appears that male mussels are accumulating high levels of carotenoids into their gonadal tissue (see Fig. A2 in Appendix for a photograph of high-edge male with orange tissue). This phenomenon has never been documented before and has interesting implications with regards to the relationship between carotenoid pigments and resistance to potential oxidative stress.

Reactive oxygen species are generated in intertidal organisms as a consequence of thermal stress experienced during aerial exposure at low tide (e.g. Abele et al. 1998), and these oxygen radicals can be extremely damaging to DNA, lipids, and proteins (Di Mascio et al. 1991). Carotenoid pigments are known for their antioxidant properties, as they can bind to singlet oxygen radicals and convert them to less-damaging hydrogen peroxide (Miki 1991). It is therefore possible that mussels in the high edge of the mussel bed are accumulating high concentrations of carotenoid pigments to protect their gametes from oxidative damage.

Conclusions

Timing of intertidal mussel reproduction was related to both food availability and environmental stress. Mussels at sites with higher food availability spawned earlier in the summer, suggesting that they may have more energy available for reproduction. However, mussels in the high edge spawned earlier in the summer than mussels in the low edge, indicating that spawning is possibly a stress response to high aerial temperatures. Gonadal tissue color in both females and males was consistently orange in mussels from the high edge, suggesting that not only is visual identification of sex in mussels inaccurate but that mussels may be accumulating carotenoid pigments to defend their gametes from harmful oxygen radicals.

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Table 4.1. Results of 4-factor ANOVA on random balanced subset of data from surveys examining effects of month, site, edge, sex, and all possible interactions on color and stage of maturity (both ln-transformed). All values with $p \leq 0.05$ are in boldface.

	Stage of Maturity				**Color**			
Parameter	**df**	**SS**	**F**	**P**	**df**	**SS**	**F**	**P**
Month	4	1.53	44.86	**<0.0001**	4	6.11	27.71	**<0.0001**
Site	3	0.07	2.82	**0.04**	3	0.81	4.91	**0.003**
Edge	1	0.04	4.29	**0.04**	1	3.13	56.78	**<0.0001**
Sex	1	0.13	15.17	**0.0001**	1	4.68	84.82	**<0.0001**
Edge x Site	3	0.20	7.99	**<0.0001**	3	0.33	2.01	0.11
Sex x Site	3	0.01	0.46	0.71	3	0.19	1.14	0.33
Sex x Edge	1	0.00	0.03	0.87	1	2.90	52.53	**<0.0001**
Edge x Site x Sex	3	0.02	0.68	0.57	3	0.42	2.55	0.06
Month x Site	12	1.04	10.16	**<0.0001**	12	2.92	4.41	**<0.0001**
Month x Edge	4	0.15	4.28	**0.002**	4	0.12	0.53	0.71
Month x Sex	4	0.08	2.43	**0.05**	4	0.92	4.15	**0.003**
Month x Sex x Edge	4	0.11	3.11	**0.02**	4	0.71	3.19	**0.01**
Month x Site x Edge	12	0.27	2.64	**0.002**	12	1.37	2.07	**0.02**
Month x Sex x Site	12	0.23	2.23	**0.01**	12	1.86	2.80	**0.001**
Month x Sex x Site x Edge	12	0.18	1.71	0.07	12	1.08	1.63	0.08
Error	240	2.05			240	13.25		
Total	319	6.12		**<0.0001**	319	40.80		**<0.0001**

Table 4.2. Results of 4-factor ANOVA from random subset of data from surveys examining effects of month, Cape, edge, sex, and all possible interactions on color and stage of maturity (both ln-transformed). All values with $p \leq 0.05$ are in boldface.

	Stage of Maturity				**Color**			
Parameter	**df**	**SS**	**F**	**P**	**df**	**SS**	**F**	**P**
Month	4	1.53	35.47	**<0.0001**	4	6.12	21.94	**<0.0001**
Cape	1	0.04	3.25	0.07	1	0.001	0.02	0.88
Edge	1	0.04	3.40	0.07	1	3.13	44.95	**<0.0001**
Sex	1	0.13	12.00	**0.0006**	1	4.68	67.15	**<0.0001**
Edge x Cape	1	0.20	18.50	**<0.0001**	1	0.07	1.01	0.32
Sex x Cape	1	0.00	0.003	0.95	1	0.06	0.87	0.35
Sex x Edge	1	0.00	0.02	0.88	1	2.90	41.59	**<0.0001**
Edge x Cape x Sex	1	0.001	0.07	0.80	1	0.12	1.66	0.20
Month x Cape	4	0.50	11.45	**<0.0001**	4	0.75	2.69	**0.03**
Month x Edge	4	0.15	3.39	**0.01**	4	0.12	0.42	0.80
Month x Sex	4	0.08	1.92	0.11	4	0.92	3.29	**0.01**
Month x Sex x Edge	4	0.11	2.46	**0.05**	4	0.71	2.53	**0.04**
Month x Cape x Edge	4	0.22	5.12	**0.0005**	4	0.40	1.42	0.23
Month x Sex x Cape	4	0.08	1.89	0.11	4	0.88	3.17	**0.01**
Month x Sex x Cape x Edge	4	0.015	0.35	0.84	4	0.43	1.54	0.19
Error	280	3.03			280	19.52		
Total	319	6.12		**<0.0001**	319	40.80		**<0.0001**

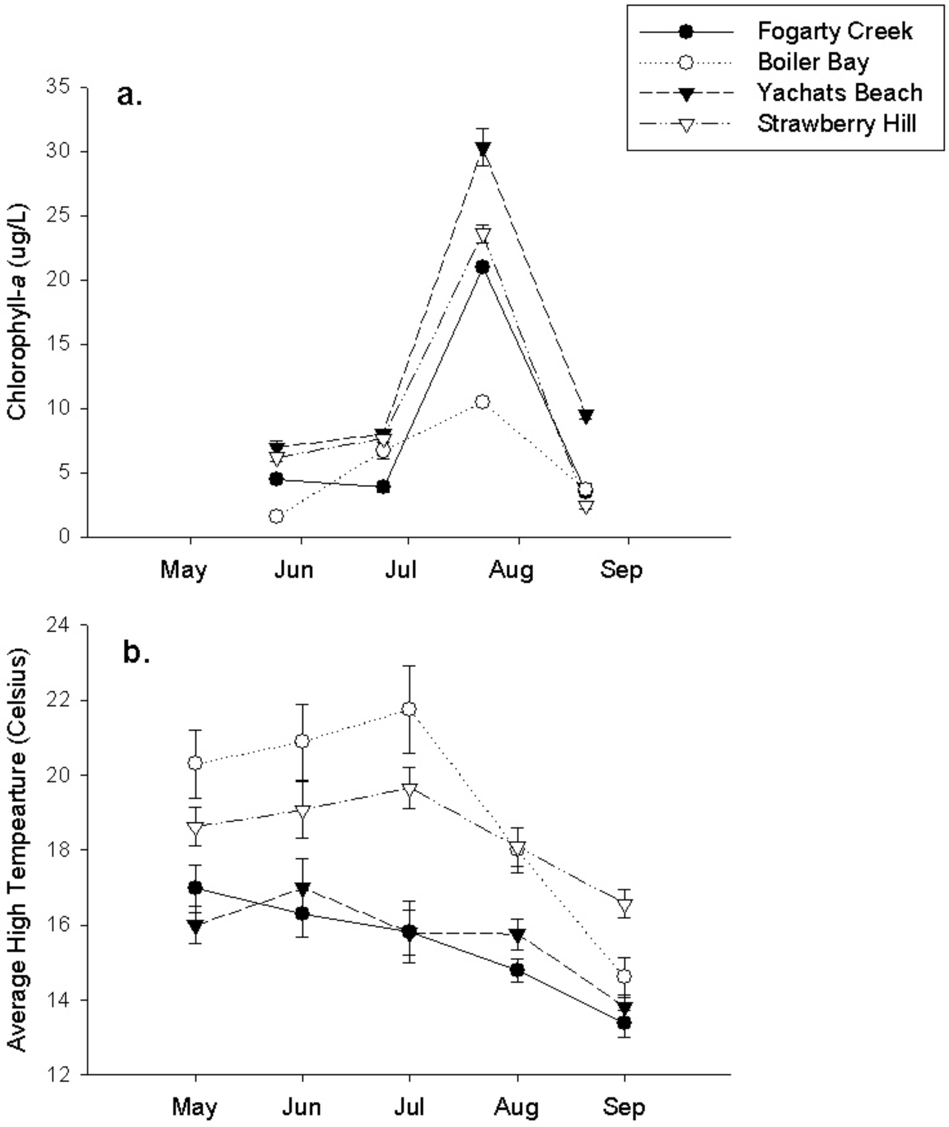

Fig. 4.1. Chlorophyll-*a* (chl-*a*) and temperature measurements at all four field sites from May-Sept. 2004. **a.** Monthly chl-*a* measurements. (Y-error bars are standard error of replicate bottle samples.) **b.** Monthly average high temperature based on daily high temperatures. (Y-error bars are standard error of daily highs.)

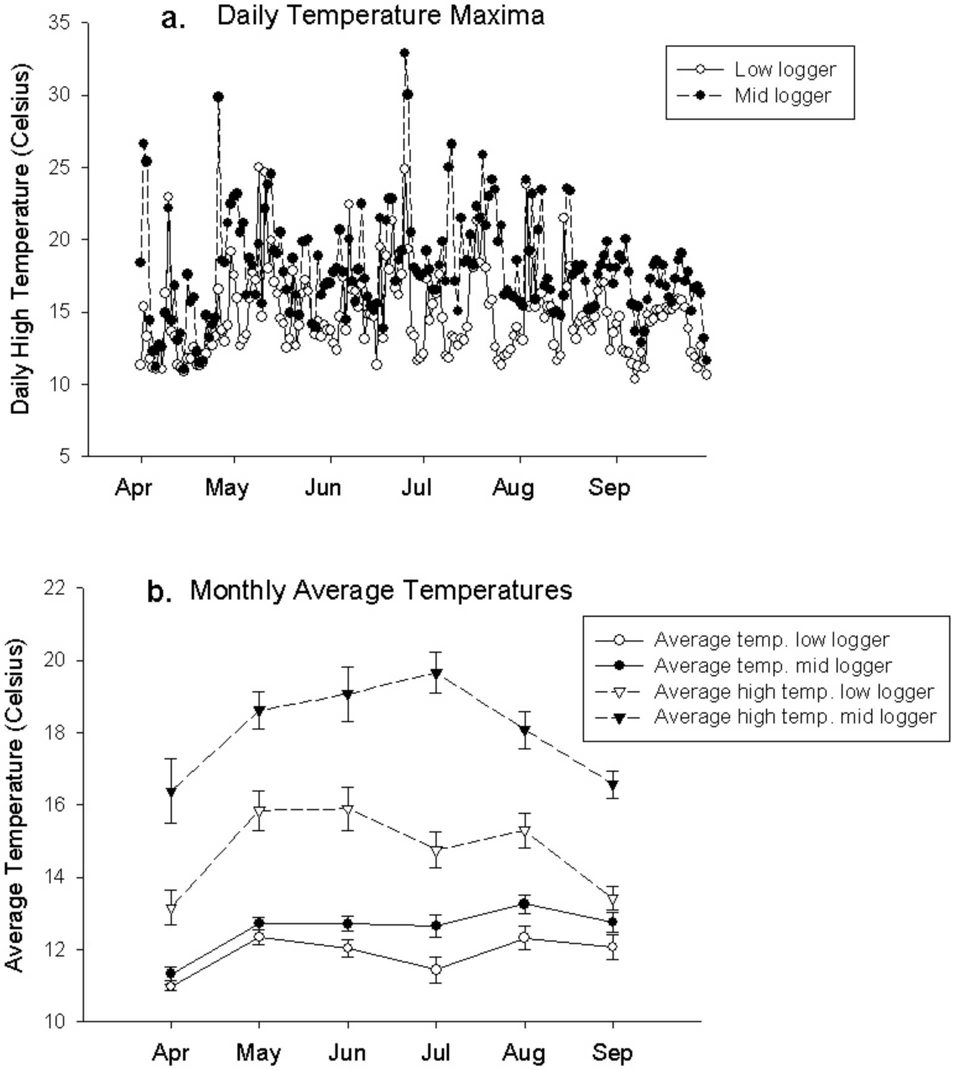

Fig. 4.2. Temperature measurements from SH temperature loggers. **a.** Daily maxima from low and mid-zone April 1 – Sept. 30, 2004. **b.** Average monthly temperatures and average monthly high temperatures calculated from daily recordings in low and mid-zone loggers (Y-error bars are standard error of daily average or daily highs.).

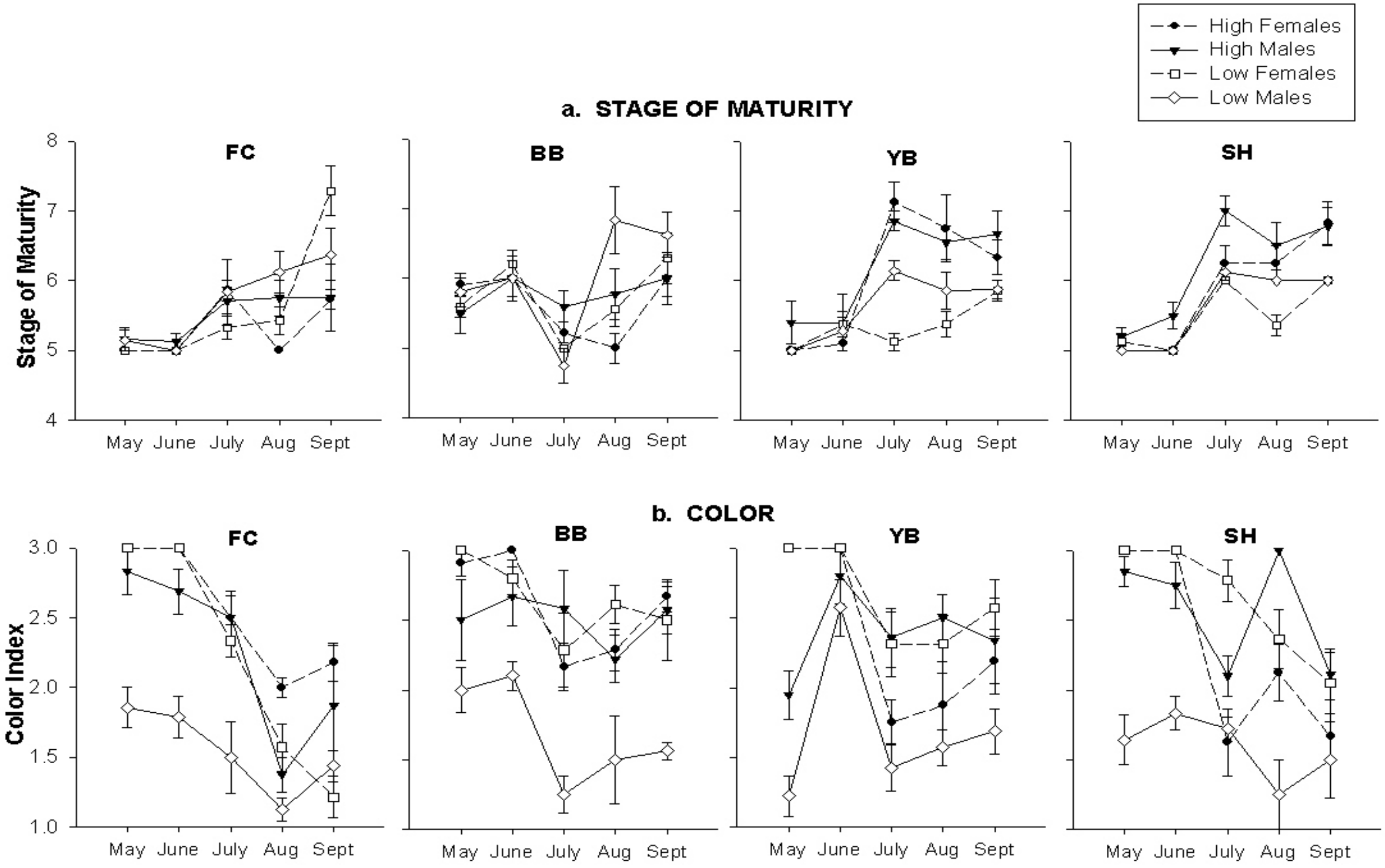

Fig. 4.3. Average **a.** stage of maturity and **b.** color index (1=white, 2=peach, 3=orange) of mussels collected from four field sites in May-Sept. 2004. Y-error bars are standard error.

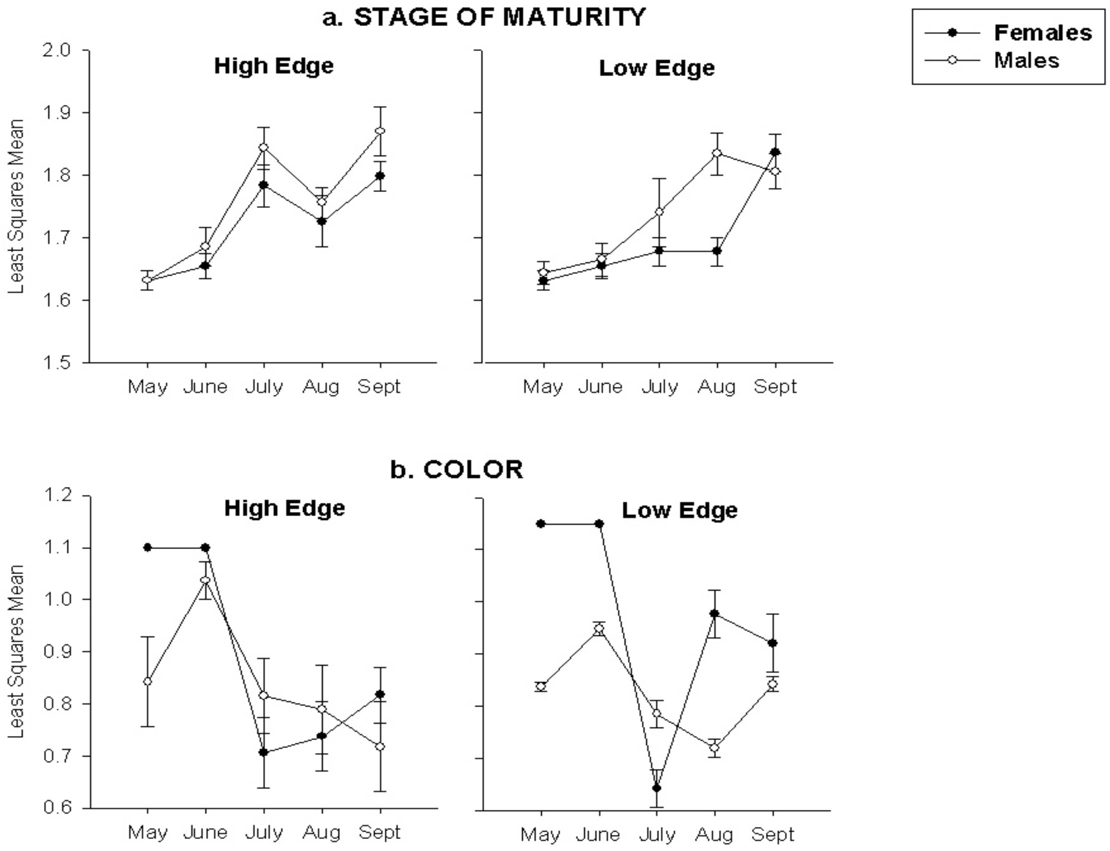

Fig. 4.4. Least squares means of ln-transformed **a.** stage of maturity and **b.** color index for 3-way month x sex x edge interaction. Y-error bars are standard error.

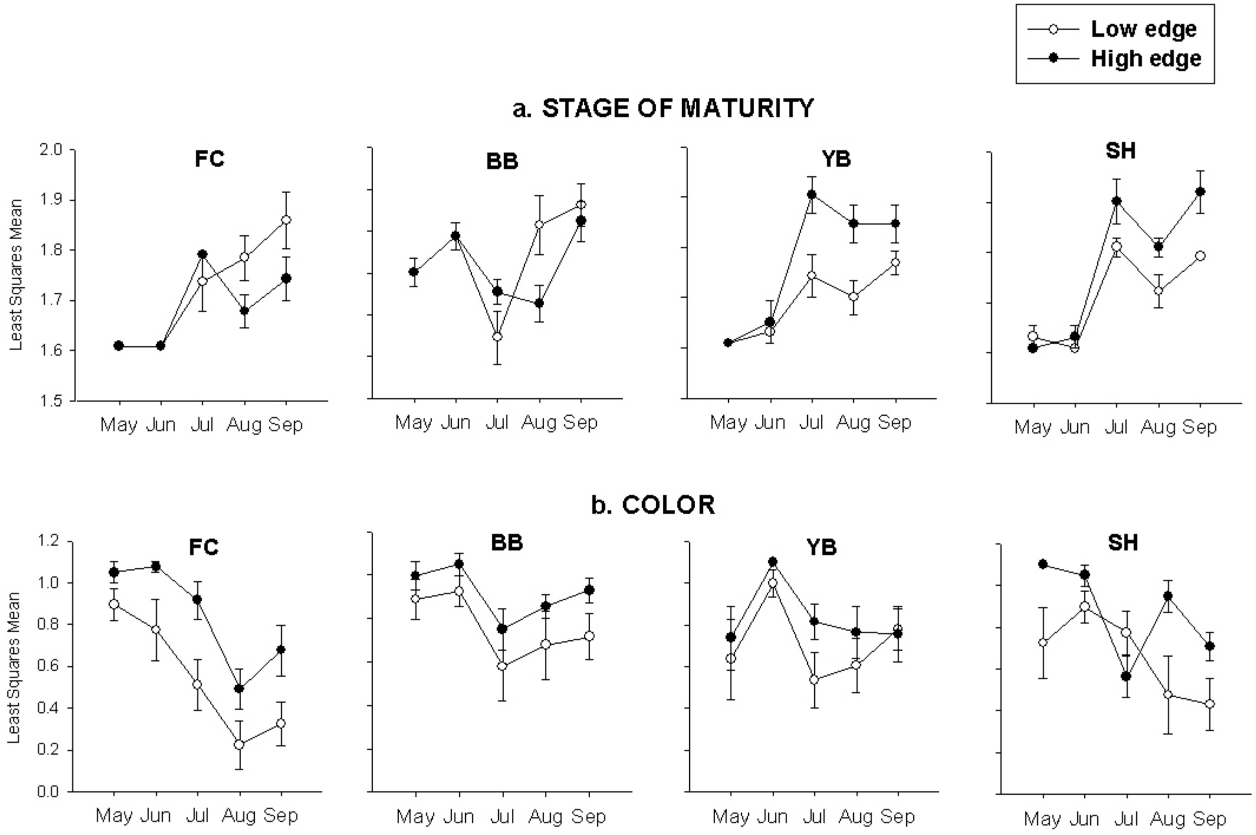

Fig. 4.5. Least squares means of ln-transformed **a.** stage of maturity and **b.** color index for 3-way month x site x edge interaction. Y-error bars are standard error.

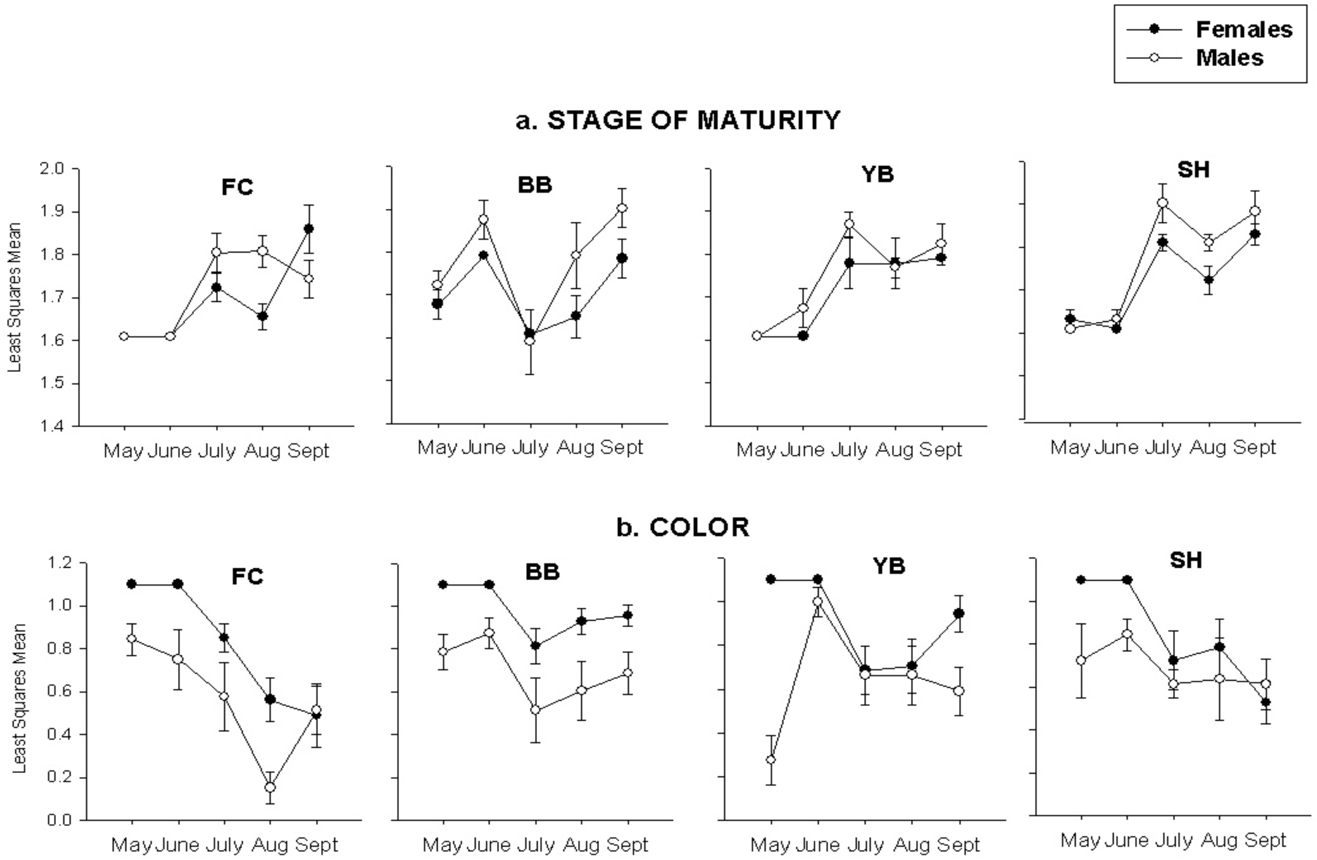

Fig. 4.6. Least squares means of ln-transformed **a.** stage of maturity and **b.** color index for 3-way month x sex x site interaction. Y-error bars are standard error.

Chapter 5:

Physiological ecology of reproduction in the intertidal mussel *Mytilus californianus*: mechanistic responses to environmental stress

Laura E. Petes, Bruce A. Menge, and Alyssa L. Harris

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Abstract:

Environmental stress can negatively affect ecological performance (growth, reproduction, and survival), and thus understanding how organisms respond to stress is critically important. Assessing the influence of stress on ecological systems was long hampered by the difficulty of quantifying sublethal stress, but the recent incorporation of physiological tools into ecological methods has begun to elucidate these effects. This study examined the influence of stress on the physiological ecology of reproduction in the dominant rocky intertidal mussel *Mytilus californianus* on the Oregon coast. Stress in the intertidal zone increases along a vertical gradient, with higher physical stress occurring in the higher intertidal zone, both due to reduced feeding time and longer exposure to aerial conditions (temperature, desiccation, etc.). Reproduction was compared in mussels from the low and high vertical edges of the mussel bed at two sites that differ in primary productivity. High-edge mussels invested less energy in reproduction and also spawned all of their gametes in the early summer, whereas low-edge mussels spawned small batches of gametes throughout the year. High-edge mussels accumulated high concentrations of carotenoid pigments into their gonads, potentially to protect gametes from damaging oxidative stress experienced during aerial exposure. The oxygen-radical quenching pigment zeaxanthin was the most concentrated carotenoid in gonads. High levels of carotenoids have never been documented in male mussels and were previously considered a characteristic of females. A reciprocal transplant experiment revealed plastic responses in growth and reproduction to increased stress. In contrast,

carotenoid concentration did not increase in response to stress, suggesting that this is a relatively conserved trait. Increased aerial temperatures and alterations in oceanographic regimes under climate change are likely to greatly modify environmental regimes for intertidal organisms. The all-or-nothing spawning strategy of high-edge mussels is risky, as it could result in a mismatch between timing of reproduction, food availability, and larval transport to shore, the latter two of which are dependent on oceanographic processes that have recently exhibited increasing unpredictability. Our results suggest that under increasing stress, energy will be allocated away from reproduction towards costly physiological defenses, such as carotenoid accumulation, potentially leading to consequences for population dynamics.

Introduction:

The negative effects of environmental stress on organisms have been documented in many systems (e.g. Moberg 1991, Schreck et al. 2001). Physiological trade-offs exist in all plants and animals, because each individual only has a certain amount of energy available to maintain normal physiological processes, such as growth, reproduction, metabolism, and immune function (Stearns 1992). When stress occurs, organisms must reallocate energy away from normal processes towards defense and repair mechanisms in order to survive. Reproduction is energetically expensive (Williams 1966), and reproductive processes may therefore be compromised under stressful conditions in an attempt to devote more energy towards survival (Wingfield and Sapolsky 2003). Impaired or suppressed reproduction has large negative consequences for population dynamics, and in the most extreme cases, for species persistence, as fitness could be compromised. The interactions between stress and reproduction have been widely documented in vertebrates, from fish (e.g. Schreck et al. 2001), to birds (e.g. Ots and Hőrak 1996), amphibians and reptiles (e.g. Moore and Jessop 2003), and mammals (e.g. Moburg 1991), but the effects of environmental stress on invertebrates are more relatively unknown. While there have been several studies investigating the effects of stress on physiology of intertidal organisms (e.g. Helmuth and Hofmann 2001, Sukhotin et al. 2002), the effects on reproduction are poorly understood.

Environmental stress in the rocky intertidal zone

The rocky intertidal zone is an ideal system for examining the effects of environmental stress, as it is a naturally harsh environment. All organisms living in this environment experience both marine conditions when immersed in water and terrestrial conditions when emersed in air on a daily tidal basis (e.g. Denny and Paine 1998, Helmuth et al. 2002). Environmental stress increases along a vertical gradient in the rocky intertidal zone, with relatively low stress in the low intertidal and relatively high stress in the high intertidal, as organisms in the high intertidal are exposed to aerial conditions for a longer period of time at low tide (Connell 1972). Stress in the high intertidal is the result of both decreased food availability and increased thermal stress at low tide. Intertidal organisms living at their upper limit exhibit physiological consequences of exposure to environmental stress, such as reduced growth (Menge et al. 2002a), decreased metabolic rate (Bayne et al. 1976), and mortality (Tsuchiya 1983, Chapter 2).

The influence of temperature and oceanographic regime on the Oregon intertidal zone

Extreme low tides ("spring" tides) occur on the Oregon coast during daylight hours in the spring and summer months and can therefore lead to high thermal and desiccation stress for intertidal organisms (Helmuth et al. 2002). In the summer, because of this timing, the Oregon intertidal system can be a more stressful environment than the intertidal system in, for example, southern California, where extreme summertime low tides occur at night (Helmuth et al. 2002). This "mosaic" pattern of thermal stress along the West Coast of North America creates interesting predictions for intertidal systems under climate change and indicates that the Oregon intertidal system may be among the most vulnerable environments to predicted increases in aerial temperature (Helmuth et al. 2002).

The oceanographic regime can underlie patterns of food abundance and juvenile recruitment success for intertidal organisms. Two Capes on the central Oregon coast have differing oceanographic conditions and coastal shelf topography that create differences in primary productivity (e.g. Menge et al. 2002b, 2004, Leslie et al. 2005). Cape Perpetua has relatively high productivity, indicated by high chlorophyll-*a* (chl-*a*) concentrations, a proxy for both phytoplankton abundance and productivity (Menge 2000). In contrast, Cape Foulweather (~80 km North) has relatively low phytoplankton abundance (e.g. Menge et al. 2002b, 2004). These contrasting food regimes lead to more rapid growth rates of filter-feeding invertebrates, such as mussels, at Cape Perpetua than at Cape Foulweather (Menge et al. 2002b, 2004). However, set in the context of the entire West Coast of North America, both central Oregon sites have relatively high primary productivity compared with sites in California (Menge et al. 2004, Barth et al. 2007). In situations of persistent upwelling, such as those in northern California, propagules are transported offshore and are not delivered back onshore. The intermittent-upwelling regime of the Oregon coast, in which upwelling events alternate with relaxation periods, allows for larvae and phytoplankton to be transported shoreward (Menge et al. 2004).

Predictions for the intertidal environment under climate change scenarios

Predictions of global climate change include warming of air and water temperatures, changes in oceanic circulation, increased intensity of upwelling events, and sea-level rise (Lubchenco et al. 1993, Houghton et al. 2001). The rocky intertidal environment is particularly vulnerable to climate change, as organisms are already living close to or at their physiological tolerance threshold (Tsuchiya 1983). The upper distributional limit for a species in the intertidal zone may shift lower due to extreme aerial temperature events, as mortality risk increases for individuals at the upper edge of their normal vertical range (Helmuth et al. 2002, Harley et al. 2006). Sublethal consequences of climate change in the intertidal zone are also likely to occur. Mismatches in temperature-induced spawning events and phytoplankton food supply for larvae have already been documented (Philippart et al. 2003). Changes in oceanic circulation, and the potential for increased intensity of upwelling events, may lead to transport of larvae and phytoplankton offshore with little return of propagules back onshore (e.g. Lubchenco et al. 1993, Harley et al. 2006). All of these effects would have potentially large negative consequences for intertidal systems. It is important to gain a better understanding of the effects of environmental stress and food supply on intertidal organisms for enhanced understanding of how climate change may affect these systems in the future.

Rocky intertidal mussels

Mussels are the dominant competitors for space in many temperate rocky intertidal systems around the world (e.g. Paine 1966, 1974, Seed 1976, Seed and Suchanek 1992). They typically span the vertical stress gradient by inhabiting the entire mid-zone of the intertidal. The lower edge of the mussel bed is a relatively low-stress environment, and the upper edge of the mussel bed is an area of relatively high stress due to potentially long periods of aerial exposure (Seed and Suchanek 1992). Organisms at the tidal height (~ +1.0 m above MLLW) of the low edge are exposed to the air on average ~15% of the time during a typical spring-neap tidal cycle, whereas organisms in the high edge (~ +2.0 m above MLLW) can be exposed up to ~50% of the time (Davenport and Davenport 2005). Because they are sessile, mussels do not have the ability to avoid stress and therefore use physiological defenses, such as increased heat shock protein production (e.g. Halpin et al. 2004, Chapter 3), to increase their chances of survival.

Mytilus californianus is a common and ubiquitous mussel species on wave-exposed shores along the West Coast of North America, spanning a latitudinal range from Baja California through the Aleutian Islands in Alaska. Across most of this range, it is competitively dominant to other space occupiers (e.g. Paine 1966, 1974) due to its relatively large size, rapid growth rate, production of large numbers of gametes, and high recruitment success (e.g. Sastry 1979). Mytilid mussels are gonochoric broadcast spawners, and temperature and food availability can drive the timing and success of reproduction (Sastry 1966, 1979). Previous research on *M. californianus* suggested that this species releases gametes throughout the year with no predictable seasonal cycle (Suchanek 1981), using a "dribble-spawning" strategy. A study conducted on the central Oregon coast discovered that a majority of both female and male *M. californianus* in the stressful high edge of the mussel bed had brightly-pigmented orange gonadal tissue (Chapter 4). Prior to that study, the color of the gonadal tissue had been assumed to be a reliable indicator of sex, with males having white tissue and females having orange tissue (Scheer 1940, Chipperfield 1953). Orange color in gonadal tissue of females is due to the presence of carotenoid pigments (Campbell 1969), but orange gonadal tissue and high concentrations of carotenoid pigments have never before been documented in male mussels.

Carotenoid pigments and their role in defense against oxidative stress

Carotenoid pigments are well known for their function as antioxidants and their critical role in defense against damaging oxygen free radicals (e.g. Miki 1991). When oxygen is metabolized during normal oxidation of food and generation of energy, harmful oxygen radicals are formed as byproducts (Livingstone 2003). These reactive oxygen species (ROS) can cause damage to DNA, proteins, carbohydrates, and lipids (e.g. Di Mascio et al. 1991). Carotenoids can bind to harmful singlet oxygen radicals ($^1O_2$) and convert them into less-damaging hydrogen peroxide (H_2O_2; Di Mascio et al. 1991, Miki 1991). While this can lead to deterioration of the pigments, other molecules are prevented from damage by ROS (Obermüller et al. 2005). Animals must obtain carotenoid pigments through their food and subsequently modify them into animal pigments, as they cannot endogenously synthesize carotenoids (Buchecker 1982). Mussels selectively uptake and assimilate specific carotenoid pigments from their phytoplankton food source into gonadal and somatic tissues (Campbell 1969, 1970). Seasonality of spawning and changes in food availability can affect the quantity of carotenoids present in mussel gonads (Scheer 1940, Campbell 1969, 1970). However, the effects of environmental stress on carotenoid content in intertidal mussels are unknown. In addition to carotenoids, other low molecular weight scavengers and specific antioxidant enzymes, including superoxide dismutase (SOD), defend organisms from free radicals (Livingstone 2003). SOD scavenges superoxide anion radicals ($O_2^{\cdot -}$) and converts them into hydrogen peroxide (H_2O_2; Halliwell and Gutteridge 1999).

Oxidative stress can occur in intertidal organisms during aerial exposure. Mussels exposed to aerial conditions at low tide experience potentially high temperature (e.g. Helmuth et al. 2002), desiccation (e.g. Bertness et al. 2006), and ultraviolet-B (UV-B) radiation (e.g. Abele-Oeschger et al. 1997) stress. Any of these physical conditions in the intertidal system could induce metabolic processes that generate oxygen radicals.

In Chapter 4, we reported that at the low edge of the mussel bed, most males have white gonadal tissue and females have orange gonadal tissue, but that both sexes at the high edge tend to have orange gonadal tissue. We hypothesized that this color pattern may be due to accumulation of high concentrations of carotenoid pigments in high-edge mussels. However, this study was limited to a single summer, based on visual assessment of tissue color alone, and no experiments were performed to test the carotenoid hypothesis. The question remains whether the orange coloration in males is due to the presence of carotenoids and whether this pattern is consistent across time and spawning seasonality. It is also unknown whether carotenoid content of mussels changes in response to stress.

The purpose of this study was to examine how environmental stress affects energy allocation towards reproduction, timing of spawning, and carotenoid content in gonadal tissues of intertidal mussels. The following hypotheses were tested in the Oregon intertidal zone:

1. Temperature influences timing of reproduction in intertidal mussels.

2. Food availability affects energy allocation towards reproduction and carotenoid content of gonadal tissue.

3. Mussels in high-stress environments have less energy available for reproduction.

4. Environmental stress leads to increased defenses against oxidative damage (increased carotenoid content and SOD activity).

5. Mussels respond plastically to environmental stress.

Materials and Methods:

To evaluate how reproduction and carotenoid content vary with sex, edge (environmental stress), and site (food availability), a variety of both environmental and biological parameters were quantified as described below.

Field sites for surveys of reproduction and carotenoid content:

Environmental stress (temperature), food (chlorophyll-*a*), and mussel reproductive processes were investigated at two sites on the central Oregon coast: Fogarty Creek ("FC"; 44.84°N, 124.06°W) on Cape Foulweather and Strawberry Hill ("SH"; 44.25°N, 124.12°W) on Cape Perpetua.

Temperature recording:

To measure seasonality of temperature throughout the year, one TidbiT temperature logger (Onset Computer Corp., Pocahassett, MA, USA) was deployed in the middle of the mussel bed (mid zone) at both FC and SH. These loggers recorded continuous temperature every two minutes from May 1, 2005-July 31, 2006. Two breaks in continuous recording occurred at SH between May 27-June 24 and Sept. 18-Oct. 17, 2005 due to equipment failure. Thermal data during these two time periods were therefore unavailable for SH.

To determine the differences in temperature between edges of the mussel bed, on Nov. 15, 2005 at FC, we deployed two "Robomussels" (mussel mimics consisting of a TidbiT logger embedded within epoxy and resin; see Helmuth and Hofmann 2001) each at the low and high edges of the mussel bed. These temperature loggers accurately simulate mussel body temperature to within 2°C (Helmuth and Hofmann 2001, Gilman et al. 2006). The loggers recorded temperature (aerial or aquatic depending on the tidal cycle) every 10 minutes for 225 days until June 27, 2006. The battery of one logger in the low edge failed on March 27, 2006, and data after this date were therefore only available from one low-edge logger.

*Chlorophyll-*a *(chl-*a*) measurements:*

To quantify the food source for mussels, phytoplankton concentration was estimated using chlorophyll-*a* (chl-*a*). Surf-zone chl-*a* measurements were collected one to three times per month between May and August in 2005 and 2006 at SH and FC as described in Menge et al. (2004). Replicate bottle samples (n=3) of water were taken from shore and filtered in the field through combusted Whatman GF/F glass-fiber filters. Chl-*a* concentration of samples was determined using a Turner Designs TD-700 fluorometer (Turner Designs, Sunnyvale, CA, USA) according to

Welshmeyer (1994). The fluorometer was calibrated with a chl-*a* standard from Sigma Chemical Company (St. Louis, MO, USA).

Field surveys:

To evaluate how reproductive condition varied between sexes, edges, and sites through time, mussels were collected every other month between May 2005 and July 2006. Mussels between 4-7 cm in length were collected from the high (~+2.0 m above MLLW) and low (~+1.0 m above MLLW) edges of the mussel bed (n=15-50 from each edge) and were dissected in the field. Tissue color was scored on a 1-3 scale with 1=white, 2=peach, and 3=orange as in Chapter 4 (see Fig. A1 in Appendix A). Gonadal tissue was removed and placed into 10% formalin in seawater for histological processing.

Histological analyses of sex and stage of maturity:

To evaluate sex and stage of maturity using microscopy, gonadal tissues were dehydrated, embedded in paraffin wax, sliced to 7 µm thickness, and stained with hematoxylin and eosin according to Luna (1968). Slides were examined under a compound microscope at 100-400x (Leica DMLS, Leica Microsystems, Inc., Bannockburn, IL). Sex (male or female) was identified on each slide, and stage of maturity was assigned into categories according to Chapter 3.

Gonadosomatic index (GSI):

To determine how stress and food influence energy allocation towards reproduction, beginning with the September 2005 collection, gonadal and somatic tissues were excised from the mussels, and tissues were weighed prior to gonadal tissue fixation to develop a gonadosomatic index (GSI; e.g. Roff 1992):

$$GSI=(\text{gonadal tissue weight})/(\text{gonadal}+\text{somatic tissue weight})$$

During every other collection (July 2005, Nov. 2005, March 2006, July 2006), gonadal tissues from each individual were divided after being weighed, such that half of the tissue was preserved for histological analyses as described above, and the other half was flash-frozen in liquid nitrogen for transport back to a –80˚C freezer in Corvallis, OR for storage prior to carotenoid pigment extraction. In July 2005, extra males were collected from both the low and high edges of the mussel bed (n=7 from each), and tissues were divided into thirds: one third was preserved for histological analyses, the second third was flash-frozen for carotenoid extraction, and the remainder was flash-frozen for superoxide dismutase (SOD) assays (described below).

Carotenoid extraction and spectrophotometry:

To determine how carotenoid content varies with sex, as well as with food and stress, gonadal tissues (0.04-0.20 g in weight) were thawed on ice and subsequently homogenized in 2 mL 90% HPLC-grade acetone. The volume of acetone was then brought up to 4 mL. Samples were extracted at –20˚C for three hours prior to reading pigment levels. Tubes were centrifuged at 5,000 rpm for 5 minutes. Supernatant was removed and transferred to a quartz cuvette (1-cm path length and 3-mL volume) for reading. Absorbance was quantified using a UV-1201 Shimadzu UV-Vis spectrophotometer (Shimadzu Scientific Instruments, Columbia, MD, USA). Spectral scans were performed from 380-580 nm (see Fig. B1 in Appendix B for representative spectra). Carotenoid levels were corrected by tissue weight prior to analyses. Because zeaxanthin was found to be the dominant carotenoid (see Results), the wavelength of 452 nm was selected for analyses of carotenoid concentration according to Britton (1995).

High-performance liquid chromatography (HPLC):

To determine which specific carotenoid pigments were present in mussel gonads, males and females in both edges (n=6) from the July 2006 sampling period at SH were analyzed using HPLC. Samples were homogenized as described above for spectrophotometry and were stored at –20˚C overnight (~17 hours) for pigment extraction prior to HPLC. Samples were analyzed with reversed-phase HPLC using a Waters Alliance 2690 separation module (Waters Corporation, Milford, MA, USA) equipped with an Allsphere C8 3 µm column with a C8 Allsphere guard column (Alltech Associates, Deerfield, IL, USA). Solvent A consisted of 75% 0.5N aqueous ammonium acetate and 25% methanol. Solvent B consisted of 100% methanol. Pigments were detected with a Waters 996 photodiode array detector (Waters Corporation) set at 436 nm and were eluted within approximately 25 min. using the following gradient from 100% A to 100% B at a flow rate of 1 mL min^{-1}: (min; %A, %B) = (0; 100,0), (20; 35,65), (30; 25,75); (35; 0,100). Peak absorbance was measured at 436 nm and calculated relative to the chl-*a* response at 436 nm. Results were compared to retention times and spectra of carotenoid standards in addition to published spectra.

Superoxide dismutase (SOD) assays:

To determine how environmental stress affects antioxidant enzyme activity, mussel gonadal tissues were thawed and homogenized in a Tris-HCl Buffer (0.25 M sucrose, 10mM Tris, 1mM EDTA, pH 7.5) at a weight:volume ratio of 1:4. Samples were cold-centrifuged at 10,000g for 15 min. at 4ºC. The supernatant was decanted, stored at -80ºC, and used for the remainder of the analyses. Protein content of samples was determined by using modified Bradford protein assays according to instructions from the manufacturer (Pierce: Coomassie-Plus-200, Rockford, IL, USA). Superoxide dismutase (SOD; EC 1.15.1.1) levels were assayed using a kit (S311-10) from Dojindo Molecular Technologies, Inc. (Gaithersburg, MD, USA) according to manufacturer's instruction. Briefly, this protocol quantifies SOD inhibition of xanthine oxidase activity using colorimetric analysis. Samples were diluted to a ratio of 1:32 and run in triplicate along with a standard curve of SOD standard (Sigma-Aldrich S2515, St. Louis, MO, USA). Inhibition was quantified with a microplate reader at 450 nm, and sample values were compared to standards and corrected by protein content.

Field transplant experiment:

To test if mussel reproduction and carotenoid content respond plastically to alterations in environmental stress, a field transplant experiment was conducted at SH in summer 2004. Mussels between 5-8 cm in length were haphazardly collected from the lower edge ("low edge") and upper edge ("high edge") of the mussel bed on April 9, 2004. Before transplantation, each mussel was notched for growth with a file according to Menge et al. (2004); subsequent shell growth can be measured from the point of scar formation. Daily growth rate was calculated as:

Growth rate=(New shell growth/Initial length)/(Days since beginning of experiment)

Mussels were transplanted into plots of 40 individuals in three transplant treatments:

a. **Within-edge transplants:** Mussels transplanted within an edge, Low-to-Low edge ("LL") and High-to-High ("HH") edge.

b. **Between-edge transplants:** Mussels transplanted between edges**,** Low-to-High ("LH") edge and High-to-Low ("HL") edge.

c. ***In situ*****:** Mussels marked and not transplanted to control for potential effects of transplantation stress.

There were four replicates of each treatment in each edge for a total of 24 experimental plots. Mussels were placed ventral-side down and were covered with plastic mesh to allow for byssal thread attachment according to Menge et al. (2004). After six weeks, the mesh was removed from all plots.

Each month between May and August 2004, three mussels were removed from each plot. These mussels were used to quantify shell growth and were subsequently dissected and scored for color on the 1-3 scale. Gonadosomatic index (GSI) was calculated, and tissues were collected for pigment and histological analyses as described above. In the laboratory, carotenoid pigments were quantified using spectrophotometry, and sex and stage of maturity were assessed with histology.

Statistical analyses for field surveys:

Field surveys were analyzed with 4-factor analysis of variance (ANOVA) using JMP 6.0 (SAS Institute, Inc., Cary, NC, USA) statistical software. Month, edge, site, sex, and all interactions were examined as explanatory variables, and color, stage of maturity, GSI, and carotenoid pigments (from spectrophotometry) were examined as separate response variables. Residual and normal probability plots were examined for the presence of outliers and for normality. All response variables were ln-transformed to improve normality. Due to unbalanced sample sizes in some analyses, subsets of data ($n=6$) were randomly selected to create balanced sample sizes for ANOVA (Quinn and Keough 2002). Categories were compared with Tukey-Kramer honest significant difference (HSD) tests based on least square means estimated from main effects or interaction terms at $p<0.05$.

The relationship between color and carotenoids was examined for data pooled across time points to determine the accuracy of the field color scoring system in categorizing carotenoid concentration. The relationship between GSI and stage of maturity was also examined to determine if mussels that are spawned-out have lower GSI than those that are ripe. A regression was performed separately for females and males to determine the relationship between stage of maturity and carotenoid content.

HPLC data were analyzed using two-factor ANOVA with sex and edge as explanatory variables, and each of the three major carotenoid pigments were investigated as separate response variables. Because these data were based on percentages, they were arcsin-square-root-transformed to improve normality.

Superoxide dismutase (SOD) data were analyzed using one-way ANOVA. Edge was used as the explanatory variable, and SOD (Units per µg protein) and carotenoid content were investigated separately as response variables. Both response variables were ln-transformed prior to analysis. The linear relationship between SOD and carotenoids was then examined.

Statistical analyses for field transplant experiment:

Data from the field transplant experiment were analyzed with repeated-measures multivariate analysis of variance (RM-MANOVA). High mussel mortality occurred in the low-edge transplants due to sea star predation events in mid-June, and these plots were therefore omitted from analyses. Data were analyzed three ways:

1. Between edges for all time points for *in situ* plots.
2. Between edges and treatments for the first two collection periods (prior to predator-induced mortality).
3. Within the high edge across all treatments and time points.

Growth rate, GSI, stage of maturity, color, and carotenoid pigments were examined as separate response variables. Data were examined for normality and the presence of outliers. GSI, stage of maturity, color, and carotenoid pigments were ln-transformed to improve normality, and growth rates were square-root-transformed. No growth rate or GSI information was collected during the first time point (May), and thus this time point was omitted for these two parameters. For these parameters in analysis #2 (described above), data were analyzed for the second time point (June) as an analysis of variance (ANOVA) without repeated-measures.

Because strong between-sex differences in carotenoid content were later discovered, females and males were also examined separately by averaging the carotenoid level of the 0-3 individuals of each sex in each experimental plot. RM-MANOVA analysis was not possible with the resulting degrees of freedom, and therefore May and June were analyzed separately for sex, treatment, and sex x treatment interactions. While we acknowledge that this analysis involves statistical pseudoreplication, it was performed simply for the purpose of exploring patterns in the data, and we draw no firm conclusions from the results.

Results:

Temperature:

Monthly average high temperatures were highest in the late spring to early summer (May-June 2005) at both FC and SH (Fig. 5.1). Temperatures decreased and reached a minimum in December 2005 before steadily increasing to reach a second maximum in May 2006. Both FC and SH followed this same pattern through time, although SH temperatures were higher on average than FC temperatures.

Temperatures differed between the low and high edges of the mussel bed (Fig. 5.2). Average monthly temperatures were lower at the high edge in the winter months and higher at the high edge in the spring and summer months (Fig. 5.2a). Such patterns are due to seasonal changes coupled with longer periods of aerial exposure in the high edge than the low edge. The low-edge Robomussels recorded temperatures more similar to water temperature than did the high-edge Robomussels (Fig. 5.2). In spring and summer months, average monthly high temperatures (based on daily high temperatures) were up to twice as high at the high edge than at the low edge of the mussel bed (e.g. 28.49±1.30°C at the high edge and 14.78°C at the low edge for the month of June; Fig. 5.2b). High temperatures at the high edge of the mussel bed reached 30°C 27 times between April 15-June 27, 2006 compared to only 1 time at the low edge. This included a period of eight consecutive days that high-edge loggers reached temperatures above 30°C during a spring tide series from June 17-24, 2006. As costly heat shock protein production is induced in mussels at temperatures above 30°C (Halpin et al. 2004), these data indicate that high-edge mussels are frequently exposed to stressful thermal conditions.

Chlorophyll-a *(chl*-a*):*

Chl-*a* concentration was consistently higher at SH than FC (Fig. 5.3), which is consistent with previous studies conducted in this area of the Oregon coast (Menge et al. 2002b, 2004, Barth et al. 2007). In addition, the peak in phytoplankton concentration was later in 2005 (August) compared to the more typical timing of the July peak in 2006. This later peak was due to delayed upwelling in summer 2005 on the Oregon coast (Barth et al. 2007).

Field surveys:

There were many significant 2-way and 3-way interactions with time (month), which are all found in Tables 5.1 and 5.2. For the purpose of the Results section, we will focus on the interactions that were the most biologically relevant.

Stage of maturity varied between both sites and edges over time. Spawning activity reached a peak at SH in September 2005 and at FC in November 2005, revealing a relationship between site and spawning time ($F_{7,320}$=6.72, p<0.0001, Fig. 5.4, see Table 5.1 for complete ANOVA results). Stage of maturity fluctuated more with time in the high-edge than the low-edge mussels ($F_{7,320}$=3.69, p=0.0008). SH high-edge mussels had two spawning pulses, one in July-Sept. 2005 and one in March 2006, whereas FC high-edge mussels only spawned during the first pulse ($F_{7,320}$=2.64, p=0.01). Stage of maturity was higher overall at FC than SH ($F_{1,320}$=9.28, p=0.003) and higher overall in the high-edge mussels than the low-edge mussels, indicating more spawning activity at the high edge ($F_{1,320}$=18.62, p<0.0001). Stage of maturity was also higher in males than females ($F_{1,320}$=26.27, p<0.0001), although this is likely due to the scoring systems, as it is easier to see the disappearance of sperm from the center of testicular cysts than when a fraction of oocytes have been spawned.

Gonadosomatic index (GSI) fluctuated with time and varied between sites and edges. Low-edge GSI decreased between Nov. 2005 and March 2006, then increased through July 2006, whereas high-edge GSI increased between Nov. 2005 and Jan. 2006, then leveled off ($F_{5,240}$=4.59, p=0.0005, Fig. 5.5, Table 5.1). GSI was higher overall at SH than at FC ($F_{1,240}$=21.13, p<0.0001), indicating more energy allocation towards reproduction at this site. GSI was higher overall in the low-edge mussels than the high-edge mussels ($F_{1,240}$=65.55, p<0.0001), suggesting that mussels in higher-food, lower-stress environments dedicate more relative energy towards reproduction. GSI was lower in females than in males ($F_{1,240}$ =20.00, p<0.0001).

Color, as assessed visually in the field, was strongly dependent on sex, edge, site, and time of year. Color was higher in the low-edge mussels at SH than the low-edge mussels at FC ($F_{1,320}$=8.36, p=0.004, Fig. 5.6, see Table 5.2 for complete ANOVA results). Females at SH were more orange than females at FC ($F_{1,320}$=12.32, p=0.0005). Color decreased in females from May through Nov. 2005, remained constant, then increased from March through July 2006; in males, color increased from May through Nov. 2005, then remained constant ($F_{7,320}$=5.64, p<0.0001). Females at SH showed a decrease in color between May 2005 and March 2006, then an increase until July 2006, whereas color in females at FC remained relatively constant until March 2006, when color began to increase ($F_{7,320}$=2.84, p=0.007). Color in low-edge males at SH increased with time, and color in low-edge males at FC remained relatively constant; color in low-edge females at SH changed more through time than at FC ($F_{7,320}$=2.63, p=0.01). Color was higher overall at SH than at FC ($F_{1,320}$=18.20, p<0.0001) and was higher overall in the high-edge mussels than the low-edge mussels ($F_{1,320}$=118.70, p<0.0001). Color was higher in females than in males ($F_{1,320}$=34.41, p<0.0001), and color was higher in high-edge males than females and low-edge males ($F_{1,320}$=69.95, p<0.0001).

Carotenoid content (based on spectrophotometry) varied between edges, sites, and sexes over time. Carotenoid content was higher in SH females than SH males and FC mussels of both sexes ($F_{1,160}$=4.54, p=0.03, Table 5.2, Fig. 5.7). SH and FC high-edge mussels had higher carotenoid levels than SH low-edge mussels, which were higher than FC low-edge mussels ($F_{1,160}$=8.46, p=0.004). Carotenoid pigments changed through time, with July of 2005 and 2006 having higher levels than Nov. 2005 or March 2006 ($F_{3,160}$=3.96, p=0.009). Pigments were highest in July 2005 and 2006 at SH and lowest in July 2006 at FC ($F_{3,160}$=3.70, p=0.01). Carotenoids varied with month and sex, as the highest levels were seen in females in July 2005 and 2006, and the lowest levels were in March 2006 females and July 2006 males ($F_{3,160}$=3.53, p=0.01). Carotenoid content was higher overall at SH than at FC ($F_{1,160}$=11.41, p=0.0009) and higher overall in the high-edge mussels than the low-edge mussels ($F_{1,160}$=96.49, p<0.0001). Overall, carotenoid pigment concentrations were higher in females than in males ($F_{1,160}$=9.42, p=0.003), and pigments were higher in high-edge males than females from both edges, which had higher levels than low-edge males ($F_{1,160}$=48.10, p<0.0001).

Carotenoid content (pooled across time), increased with field color score ($F_{1,190}$=327.00, p<0.0001, R^2=0.63), indicating that the field scoring system may be a good proxy for carotenoid content:

$$\ln \text{Carotenoid Content} = 0.05 + 1.60\,(\ln \text{Color Index Score})$$

However, with untransformed data, there was a funnel-shape to the relationship of color vs. carotenoid content, suggesting that when color was orange in the field (scored as a “3”), it was difficult to determine carotenoid pigment concentrations without performing spectrophotometric analyses.

GSI (pooled across time) decreased as stage of maturity increased ($F_{1,286}$=30.42, $p<0.0001$), suggesting that GSI may be somewhat indicative of spawning activity. However, the R^2 was low (0.10), showing that the linear relationship ($ln\ GSI = -0.11-1.11(ln\ Stage\ of\ Maturity)$) is weak.

Sex-specific differences in the relationship between stage of maturity and carotenoid content were revealed. For female mussels, carotenoid content pooled across time decreased as stage of maturity increased ($F_{1,94}$=10.93, $p=0.001$), indicating that female mussels that had spawned more had lower levels of pigment in their gonadal tissue, but the linear relationship was weak (R^2=0.11). In contrast, for males, carotenoid content pooled over time increased with increasing stage ($F_{1,94}$=7.49, $p=0.007$), showing that as spawning increased, concentration of carotenoids increased in male gonads, although this relationship was also weak (R^2=0.07). These sex-specific differences are likely due to the fact that mussel oocytes are pigmented, but sperm are relatively free of carotenoids (Scheer 1940, Campbell 1969, 1970). Thus, in females, as oocytes are shed from gonadal tissue, carotenoid concentration decreases, whereas in males, when sperm are spawned, carotenoid concentration increases. Maternal provisioning of carotenoid pigments in other organisms is thought to protect oocytes and resulting embryos from damaging UV-B radiation (e.g. Lamare and Hofmann 2004).

High-performance liquid chromatography (HPLC):

HPLC analyses revealed three major carotenoid pigments in mussel gonads. The presence of zeaxanthin was confirmed with comparison to zeaxanthin standard retention times and spectra. Alloxanthin was tentatively identified based on similar retention times and spectra as samples thought to contain this pigment, but no standard was available. The third carotenoid (Appendix B), based on evidence from other published studies of mussel carotenoids, is likely to be mytiloxanthin (Scheer 1940, Campbell 1970). These three pigments combined comprised between 79-92% of total carotenoids in all samples examined (Fig. 5.8), whereas no other pigment was responsible for more than 5% of the total carotenoids. The pigment likely to be mytiloxanthin was found in lower concentrations in the high-edge mussels than the low-edge mussels ($F_{1,20}$=6.22, p=0.02; Table 5.3), and there was less of this pigment in males than in females ($F_{1,20}$=9.13, p=0.007). In contrast, alloxanthin was more concentrated in high-edge mussels ($F_{1,20}$=6.37, p=0.02) and males ($F_{1,20}$=6.70, p=0.02). There were no significant differences in zeaxanthin concentrations between edges ($F_{1,20}$=0.00, p=0.97) or sexes, although zeaxanthin was marginally higher in males than in females ($F_{1,20}$=3.58, p=0.07).

Superoxide dismutase (SOD) activity:

There was no difference in SOD activity between males from the high (70.27$\pm$5.57 Units/mg protein) and low (62.10$\pm$4.43 Units/mg protein) edges of the mussel bed ($F_{1,12}$=1.25, p=0.28). Although carotenoid content in gonadal tissues was higher in high-edge males (relative absorbance at 452nm: 6.42±1.01/g tissue) than low-edge (2.11±0.30/g tissue) males ($F_{1,12}$=21.66, p=0.0006), carotenoid content and SOD activity were unrelated within individuals (R^2=0.05, $F_{1,12}$=0.66, p=0.43).

Field transplant experiment:

Growth rate was higher at the low edge than the high edge for *in situ* plots ($F_{1,6}$=12.72, p=0.01, Table 5.4, Fig. 5.9a), but growth rate did not vary over time ($F_{2,5}$=0.04, p=0.96). Between edges and treatments in June, growth rate was higher in the low edge than in the high edge (df=1, t=9.11, p=0.009), and there was no difference between treatments (df=2, t=1.13, p=0.35). Within the high edge, there were no differences in growth rate between treatments ($F_{2,5}$=1.57, p=0.30) or across time ($F_{2,4}$=0.93, p=0.47).

Stage of maturity was higher in the high-edge *in situ* plots than the low-edge plots ($F_{1,6}$=52.86, p=0.0003, Table 5.4, Fig. 5.9b), indicating more spawning activity in the high edge. In addition, stage of maturity increased over time ($F_{3,4}$=128.70, p=0.0002) in both edges. For May and June comparisons of both edges and all treatments, stage of maturity was higher in high-edge treatments ($F_{1,15}$=18.35, p=0.0007) than low-edge treatments, and treatments did not differ ($F_{2,15}$=0.11, p=0.90). Stage of maturity (spawning activity) also increased over time ($F_{1,15}$=80.07, p<0.0001), and stage increased more rapidly in high-edge treatments than low-edge treatments (time x edge: $F_{1,15}$=9.27, p=0.008). Within the high edge, the treatments did not differ ($F_{2,5}$=3.01, p=0.14), and stage of maturity increased over time ($F_{3,3}$=32.10, p=0.009).

Gonadosomatic index (GSI) was higher at the low edge than the high edge for *in situ* plots ($F_{1,6}$=13.66, p=0.01, Table 5.4, Fig. 5.9c), indicating higher energy allocation towards reproduction in low-edge mussels. GSI increased over time in low-edge mussels ($F_{2,5}$=15.78, p=0.007). In June, GSI was higher in the low-edge treatments (df=1, t=5.78, p=0.03) than the high-edge treatments, and treatments did not differ (df=2, t=0.18, p=0.83). Within the high edge across all time points, GSI did not differ between treatments ($F_{2,5}$=0.32, p=0.74) or across time ($F_{4,8}$=0.94, p=0.46).

Color was the same between edges for the *in situ* plots ($F_{1,6}$=0.02, p=0.90, Table 5.4, Fig. 5.10a). Color decreased over time ($F_{3,4}$=8.65, p=0.03) and decreased more in the high-edge *in situ* plots than the low-edge plots over time ($F_{3,4}$=8.70, p=0.03). For all treatments in May and June, there was less color in *in situ* low-edge mussels than other treatments (edge x treatment: $F_{2,15}$=5.64, p=0.01). Within the high edge across all time points and treatments, color did not vary with treatment ($F_{2,5}$=0.43, p=0.68) or time ($F_{3,3}$=1.71, p=0.34).

Carotenoid pigment levels were the same in low-edge and high-edge *in situ* plots ($F_{1,6}$=0.01, p=0.92, Table 5.4, Fig. 5.10b) and did not change with time ($F_{3,4}$=0.24, p=0.86). In May and June for all treatments, carotenoid concentrations were higher in *in situ* high-edge treatments than other treatments (edge x treatment: $F_{2,15}$=4.73, p=0.03) but did not vary with time ($F_{1,15}$=0.34, p=0.57). At the high edge, carotenoid levels did not vary between treatments ($F_{2,5}$=1.43, p=0.32) or over time ($F_{3,3}$=0.27, p=0.84).

When carotenoid pigments were examined separately in males and females for each time point, several differences were revealed. For all treatments in May, females overall had higher carotenoid levels than males ($F_{1,32}$=71.35, p<0.0001), and HL and *in situ* high-edge treatments had higher levels than LL ($F_{5,32}$=4.75, p=0.002), suggesting that mussels did not lose pigments when transplanted into the low edge. In addition, *in situ* females from both edges, HL and HH females had the highest levels of carotenoid pigments, and *in situ* low-edge, LL, and LH males had the lowest levels ($F_{5,32}$=2.47, p=0.05). The same pattern held true in June, with females having higher levels than males ($F_{1,20}$=6.80, p=0.02), and the *in situ* high-edge treatment having higher levels than the LH treatment ($F_{5,20}$=3.33, p=0.02), suggesting that mussels did not accumulate pigments after being transplanted into the high edge.

Discussion:

Energetic trade-offs exist in all organisms, and in the face of stress, growth and reproduction can be compromised to conserve energy for physiological defenses that increase chances of survival (Stearns 1992). The results of this study indicate that this is likely to be the case for intertidal mussels, as mussels allocated less energy towards reproduction under stress and invested energy in the inefficient (Fox and Hopkins 1966) and energetically expensive (Brush 1990, Hill 1996) process of carotenoid pigment assimilation. Our study is the most comprehensive investigation of the reproductive ecology and physiology of intertidal mussels conducted to date. This study has also generated several new insights, modifying earlier interpretations of reproduction in *Mytilus californianus*. One of these was the belief that gonadal tissue color was a reliable indicator of sex in mussels. Our analyses suggest that overall, the match between tissue color and sex is variable, and in particular, that high-edge male mussels would have been reliably identified as females if tissue color was the sole categorizing factor. Second, prior work indicated that *M. californianus* is a "dribble spawner," releasing only a portion of their total available gametes during each of multiple, small spawning events (Suchanek 1981). Our study shows that this is true only for mussels lower on the shore; mussels at the high edge of the mussel bed released all gametes at once. Third, our analyses reveal that the effects of environmental stress are complex, varying with time of year, between sites with differing food availability, and between high and low edges of the mussel bed. Below we discuss these points and place the results in the context of both environmental stress and implications for mussels under predicted climate change scenarios.

Environmental stress effects on reproduction:

This study documented complex and strong effects of environmental stress on reproduction. Mussels at the high edge of the mussel bed showed increased spawning activity and accelerated timing of spawning when compared to low-edge mussels. This pattern has been documented before, with a higher percentage of mussels at the upper edge of their distribution having spawned than mussels lower on the shore

(Campbell 1969). Thermal stress can lead to spawning (e.g. Schreck et al. 2001, Chapter 2). In addition, mussels experiencing food shortage cannot maintain ripe gametes (Bayne and Thompson 1970). It is possible that mussels at the high edge do not have enough energy to maintain ripe gametes in their tissues due to a combination of stressors, including lack of food and potentially high aerial temperatures, and therefore release their gametes prematurely.

The interaction between food availability and temperature plays a critical role in the timing and success of reproduction. Gonadal growth in bivalves often occurs with increasing temperature and peak phytoplankton production in the summer, and spawning occurs after this growth period (Sastry 1966). SH had higher chl-*a* levels than FC, and GSI was higher in mussels at SH than FC, indicating that these animals were dedicating relatively more energy towards reproduction. SH mussels spawned earlier in the summer than mussels at FC, which could either be due to more energy stores or potentially as a response to higher thermal stress. In addition, mussels at SH exhibited two pulses in spawning activity throughout one annual cycle, whereas FC only had one, suggesting that mussels at SH had more energy stored for spawning. GSI was higher in low-edge mussels than high-edge mussels, indicating that mussels at the low edge have more energy available for reproduction, as they have higher food availability and a reduced need to produce physiological defenses against stress than mussels at the high edge of the mussel bed.

The relationship between temperature-induced spawning and oceanography has important implications for the future success of mussel larvae. Aerial temperature appeared to affect timing of reproduction in mussels. The highest intertidal temperatures were recorded in May and June, and a majority of spawning activity in the high-edge mussels occurred between May and July, showing a relationship between high temperatures and spawning seasonality. Seasonal intermittent upwelling leading to offshore transport of propagules typically begins in mid-April and intensifies in July off the Oregon coast, and peak recruitment of mussel juveniles normally occurs between the end of the summer and the end of autumn during upwelling relaxation when larvae are delivered back onshore (Connolly et al. 2001). Early spawning of adults under thermal stress could lead to a mismatch of larval production with the peak in phytoplankton that serves as a food supply, causing potentially starved larvae (Philippart et al. 2003).

The summers of both 2005 and 2006 were characterized by anomalies in oceanic circulation off the Oregon coast. In 2005, the transition to upwelling occurred in July, two months later than normal, leading to warmer water and reduced food availability (Barth et al. 2007). Upwelling in the summer of 2006 was so strong and persistent that it led to a large, offshore hypoxic event (Chan et al. in prep.). High-edge and low-edge mussels exhibited contrasting reproductive strategies of either a single mass spawning (high-edge mussels) or repeated “dribble spawning” (low-edge mussels), each of which has interesting implications. Mussels at the high edge during the summertime spawned out all of their gametes, rather than only a fraction, which is an indication that spawning may be an attempt to conserve energy for physiological defenses. However, the strategy of releasing all gametes at the same time is risky in this environment, as survival of larvae and successful return of propagules back onshore rely on the timing of oceanographic processes. Given the recent anomalies in oceanic circulation off the Oregon coast, the strategy of consistent low levels of spawning (“dribble spawning”) exhibited by low-edge mussels throughout the year may therefore be more advantageous in this system.

Environmental stress affected both growth and reproduction of mussels. The transplant experiment revealed that the high-stress environment at the high edge of the mussel bed (higher temperatures, lower food availability) led to decreased growth rates, decreased energy allocation towards reproduction (lower GSI), and increased spawning activity in intertidal mussels. All of these findings indicate that mussels exhibit a plastic response to stress, as there were strong effects of edge but no differences between treatments. This finding is consistent with other studies (e.g. Halpin et al. 2004, Chapter 3), which have documented up-regulation of mussel physiological defenses when exposed to increasing stress.

Food and environmental stress effects on carotenoid pigments:

Both food supply and environmental stress affected carotenoid content in mussels. Carotenoid concentration was higher in mussels at SH than FC, which is consistent with patterns of higher chl-*a* levels at Cape Perpetua than at Cape Foulweather (Menge et al. 2002b, 2004, Leslie et al. 2005). Mussel carotenoid content has been shown to fluctuate with changes in phytoplankton density (Jensen and Sakshaug 1970). This suggests that not only are phytoplankton more abundant at

SH than FC, but that the mussels at SH are accumulating more phytoplankton pigments into their tissues. Mussels are very efficient at removing carotenoids from the water column (Rodhouse et al. 1985) and can selectively assimilate specific carotenoid pigments from their food source (Campbell 1970, Jensen and Sakshaug 1970). However, while chl-*a* levels fluctuated with time in this study, carotenoid content tended to remain relatively constant, indicating that mussels likely maintain carotenoids in their tissues. This maintenance of carotenoid pigments was also documented in an earlier laboratory study, which showed that carotenoids are not easily lost from mussel gonadal tissue except upon starvation (Scheer 1940).

Differences in food availability between the high edge and low edge of the mussel bed may have affected carotenoid concentration. Mussels (*Mytilus edulis*) feed 97-99% of the time when in water (Loosanoff 1942), and there is no difference in feeding rates of low-edge and high-edge mussels when underwater (Jørgensen 1960). However, there is a difference in immersion time during which mussels can feed, with low-edge mussels immersed ~35% more of the time than high-edge mussels during a typical spring-neap tidal cycle (Davenport and Davenport 2005). Campbell (1969) found that mussels with higher food availability have higher levels of carotenoid pigments, which implies that mussels lower on the shore should have higher concentrations than mussels higher on the shore. This is opposite of the patterns documented in this study and suggests an alternative explanation for carotenoid accumulation in high-edge mussels, possibly to protect gametes from oxidative damage.

Carotenoid pigments varied with sex, stress, and stage of maturity. Carotenoid content was always lowest in low-edge males, whereas high-edge males had either higher or the same concentration of carotenoids in their gonadal tissues as females. This reveals the striking and consistent difference in gonadal tissue pigmentation between low-edge and high-edge males. Carotenoid concentration was higher in spawned-out males than in ripe males, showing that the majority of color is found in the storage tissue of the male gonad, as sperm cells are not pigmented (Scheer 1940). The tissue of mussel gonads contains high concentrations of lipids and functions in both storage and gametogenesis (Sastry 1979). Females, in contrast, exhibited a decrease in carotenoids with spawning due to the loss of pigmented oocytes (Scheer 1940) from gonadal tissue.

There were few differences in color and carotenoid content in the transplant experiment. This is likely due in part to the strong sex-specific differences in carotenoid content, which had to be averaged for the purpose of the sampling design. In addition, in conjunction with results from the field surveys, it is possible that carotenoid content does not respond quickly to changes in stress. If the transplant experiment had been conducted for several years rather than several months, an increase in carotenoid concentration in mussels transplanted from low-stress to high-stress environments may have resulted. There could be a genetic component to differences in carotenoid content between edges of the mussel bed as a result of selective mortality, although evidence for significant genetic differentiation between mussels from low and high edges of the intertidal zone is controversial (Levinton and

Koehn 1976, Levinton and Suchanek 1978). Given the consistently high concentrations of carotenoid pigments in high-edge mussels and the lack of temporal fluctuation in carotenoids with changing phytoplankton abundance, it is possible that carotenoids are not limiting in this environment. The Oregon coast is an area of extremely high productivity due to intermittent upwelling that results in a high abundance of phytoplankton being supplied as food to onshore organisms (e.g. Menge et al. 2004). However, it is also possible that while carotenoids may not be limiting, accumulation of carotenoid pigments is costly in mussels, which could help to explain why low-edge males do not incorporate high levels of carotenoids into their tissues. It has been shown in other systems that metabolic transformations of carotenoid pigments from the diet are inefficient (Fox and Hopkins 1966) and energetically expensive (Brush 1990, Hill 1996).

The major carotenoid pigments found in gonadal tissues of *Mytilus californianus* were zeaxanthin, alloxanthin, and likely mytiloxanthin, which are consistent with findings from previous research on mussel carotenoids (Campbell 1970). Accumulation of hypophasic carotenoids (pigments with two or more hydroxyl groups), such as zeaxanthin, was previously considered to be a true secondary sex characteristic, as this phenomenon had only been documented in females (Scheer 1940). In this study, however, accumulation of zeaxanthin occurred equally in male and female mussels, indicating that this is not a secondary sex characteristic. Earlier research on *M. californianus* found that alloxanthin and mytiloxanthin were much more concentrated than zeaxanthin (Campbell 1970). In contrast, this study found highest concentrations of zeaxanthin and lower concentrations of the other two pigments. There are several explanations for why this may be the case. Campbell's study (1970) was conducted in southern California. It is possible that differences in carotenoids are attributable to differences in phytoplankton food sources between California and Oregon (e.g. Menge et al. 2004). Another explanation is that selective accumulation of zeaxanthin may be occurring in Oregon mussels to protect gametes from oxidative damage, particularly given that low tides occur during daylight hours in the Oregon summers, in contrast to nighttime timing of low tides in southern California (e.g. Helmuth et al. 2002).

Food availability can influence total carotenoid content and levels of individual pigments in mussel gonadal tissues (Scheer 1940, Campbell 1970). All mussels at the high edge of the mussel bed maintained high concentrations of carotenoid pigments in their tissues compared to the low-edge mussels regardless of season or spawning activity, indicating that carotenoids may not be limiting in this system. However, differences in individual pigments were seen that could reflect the differences in food availability between edges. Alloxanthin and zeaxanthin are still found in high concentrations when mussels are starved, whereas mytiloxanthin decreases (Scheer 1940). Mussels at the high edge of the mussel bed have shorter periods of time in which to feed (Davenport and Davenport 2005) and are therefore relatively food-limited compared to low-edge mussels. The decreased concentrations of the potential mytiloxanthin pigment found in high-edge mussels may therefore indicate that these mussels experience starvation due to longer periods of emersion at low tide.

Each of the three major carotenoids found in mussel gonadal tissue serves a diversity of functions in different organisms. Alloxanthin is an acetylenic carotenoid that originates in Cryptophyceae (cryptomonads) and is accumulated from this food source into tissues and egg cells of bivalves, gastropods, and fish (Shahidi et al. 1998). This carotenoid also functions as a light-harvesting pigment in plants (Siefermann-Harms 1987). Mytiloxanthin is typically found in Mytilid mussels (Liaanen-Jensen 1991), but its functions are relatively unknown. Zeaxanthin is a xanthophyll derived from β-carotene that is found in marine algae, crustaceans, echinoderms, fish, and mollusks and is often accumulated in ovaries (Miki et al. 1982, Shahidi et al. 1998). Zeaxanthin has high activity as an antioxidant and quenches singlet oxygen radicals twice as effectively as β-carotene (Miki 1991, Woodall et al. 1996). In plants, zeaxanthin prevents accumulation of damaging ROS that lead to lipid peroxidation and protects thylakoid membrane lipids from damage by heat stress (Havaux and Niyogi 1999). In the human retina, high concentrations of zeaxanthin lead to decreased risk of macular degeneration and cataracts (Moeller et al. 2000). It is therefore likely that zeaxanthin also plays an important antioxidant role in intertidal mussels.

Carotenoid pigments and oxidative stress:

Maintaining high concentrations of carotenoid pigments in gonadal tissue may be an adaptive response to stress in an attempt to protect gametes from oxidative damage. ROS can be produced as a result of thermal stress experienced during aerial exposure in intertidal organisms (Abele et al. 1998). Carotenoid pigments help defend organisms against oxidative damage (Miki et al. 1982, Miki 1991) and could serve an important role in protecting gametes from damage after release by the adults. Lamare and Hoffman (2004) found that embryos resulting from sea urchin gonads with high carotenoid concentrations showed lower sensitivity to UV radiation than embryos fertilized from females with low carotenoid levels in their gonadal tissues. ROS are generated during oocyte maturation, and carotenoids help prevent peroxidation of fatty acids from occurring during this process (Liñán-Cabello et al. 2004). Dietary supplementation of carotenoids can lead to increased antioxidant defenses (Alonso-Alvarez et al. 2004b) and egg yolk that has decreased susceptibility to lipid peroxidation (Blount et al. 2002). Male mussels at the high edge of the mussel bed may accumulate carotenoid pigments into their gonadal tissues to prevent lipid peroxidation from occurring in the lipid-rich storage tissue that surrounds the gametes.

Hypoxia and anoxia can occur in intertidal organisms during aerial exposure at extreme low tides (Brinkhoff et al. 1983, McMahon 1988, Altieri 2006). As a result of hypoxic conditions, anaerobic end products are accumulated into mussel tissues (Widdows et al. 1979). Mussel carotenoproteins are activated in nerve cells during periods of gradual anoxia (Paparo and Murphy 1978), and only mussels with high carotenoid content in their nervous tissue can successfully withstand hypoxia (Karnaukhov 1979). Carotenoids in high-edge mussels may therefore help these individuals survive under stressful conditions, including hypoxia.

The “free radical theory of aging” proposes that changes in aging cells are related to free radical reactions and resulting oxidative damage to membrane lipids (Harman 1981). More recent theories suggest that cellular aging is related to a decreased ability to inactivate free radicals, as activity of protective antioxidant systems decreases with age (e.g. Videla et al. 1987). Decrease antioxidant enzyme activity in older mussels makes them more susceptible to oxidative damage than younger individuals (Viarengo et al. 1991), which is consistent with the free radical theories of aging. Growth rates of mussels are slower in the high edge of the mussel bed than the low edge, as shown from the transplant experiment as well as other studies (e.g. Menge et al. 2002a, Chapter 2). Because the mussels collected in the field survey from the low and high edges were of the same size class, it is very likely that the mussels from the high edge were older than mussels from the low edge. This could indicate that the older mussels have simply had a longer period of time in which to accumulate carotenoids into their tissues. However, a previous study (Viarengo et al. 1991) showed that carotenoid content in mussels decreases with age, suggesting that this is likely not the case. Alternatively, older mussels may continuously assimilate carotenoid pigments to compensate for decreased metabolic rate and antioxidant enzyme performance.

The finding that superoxide dismutase (SOD) activity was not different between mussels from the low and high edges of the mussel bed was surprising. There are several possible explanations for why this was the case. Gonadal tissue may not be the most enzymatically-active tissue in mussels and therefore may not have exhibited increased enzyme activity. However, gonadal tissue is known to have high lipid peroxidizing enzyme activity (Musgrave et al. 1987), indicating that this possibility is unlikely. In addition, other studies have found no difference in mussel SOD levels in response to various stressors (e.g. Livingstone et al. 1990, Viarengo et al. 1991). There also appears to be no effect of age on SOD activity in mussels (Viarengo et al. 1991, Sukhotin et al. 2002). It is also possible that high levels of carotenoids in high-edge mussels are effective enough at scavenging free radicals that SOD is not used as a defense in this system.

Conclusions:

The results of these studies elucidate the complex effects of environmental stress on reproduction and pigmentation in rocky intertidal mussels. Mussels inhabiting relatively high-stress areas of the intertidal zone and those transplanted to the high-stress environment exhibited early spawning and lower energy investment in reproduction, showing that mussel reproduction responds plastically to environmental stress. In addition, mussel gonads in the high edge of the mussel bed contained higher concentrations of carotenoid pigments, a potentially costly defense, than mussel gonads in lower-stress conditions. Mussels in this study exhibited energetic trade-offs (e.g. Stearns 1992) under stress, as energy towards growth and reproduction was reduced, and energy towards physiological defenses (carotenoids) was increased. Early spawning and reduced energy allocation towards reproduction could affect future population sizes of mussels, whose beds provide critical habitat for hundreds of intertidal species (Seed and Suchanek 1992). These findings have important implications for alterations to oceanic circulation and increasing thermal stress predicted to occur under global climate change scenarios (e.g. Lubchenco et al. 1993, Houghton et al. 2001). The combination of increasing unpredictability of oceanographic processes with stress-induced spawning could lead to reduced larval survival and decreased recruitment success. This has implications for the potential effects of climate change on fitness in this system, as successful reproduction could be compromised by stress. In addition, the intertidal zone is an already stressful environment in which organisms are living close to or at their physiological tolerance limits (e.g. Tsuchiya 1983). Even slight increases in aerial temperature could have serious sublethal and lethal consequences for these organisms.

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Table 5.1. Results of 4-factor ANOVA on data subset from field surveys examining effects of month, site, edge, and sex on stage of maturity and gonadosomatic index. Both responses were ln-transformed. All values with p<0.05 are in boldface.

	Stage of Maturity				**Gonadosomatic Index**			
Parameter	**df**	**SS**	**F**	**P**	**df**	**SS**	**F**	**P**
Month	7	1.44	22.64	**<0.0001**	5	2.40	3.56	**0.004**
Site	1	0.08	9.28	**0.003**	1	2.87	21.33	**<0.0001**
Edge	1	0.17	18.62	**<0.0001**	1	8.82	65.55	**<0.0001**
Sex	1	0.24	26.27	**<0.0001**	1	2.69	20.00	**<0.0001**
Edge x Site	1	0.007	0.75	0.39	1	0.04	0.28	0.60
Sex x Site	1	0.005	0.53	0.47	1	0.07	0.50	0.48
Sex x Edge	1	0.000	0.01	0.92	1	0.01	0.10	0.75
Edge x Site x Sex	1	0.000	0.003	0.95	1	0.11	0.84	0.36
Month x Site	7	0.43	6.72	**<0.0001**	5	1.26	1.87	0.10
Month x Edge	7	0.23	3.69	**0.0008**	5	3.10	4.59	**0.0005**
Month x Sex	7	0.06	0.90	0.50	5	1.12	1.67	0.14
Month x Sex x Edge	7	0.08	1.33	0.24	5	0.90	1.33	0.25
Month x Site x Edge	7	0.17	2.64	**0.01**	5	1.44	2.14	0.06
Month x Sex x Site	7	0.22	3.50	**0.001**	5	0.54	0.81	0.55
Month x Sex x Site x Edge	7	0.05	0.81	0.58	5	0.35	0.51	0.76
Error	320	2.91			240	32.30		
Total	383	6.10		**<0.0001**	287	58.01		**<0.0001**

Table 5.2. Results of 4-factor ANOVA on data subset from field surveys examining effects of month, site, edge, and sex on color and carotenoid pigments. Both responses were ln-transformed. All values with p<0.05 are in boldface.

	Color				**Carotenoids**			
Parameter	**df**	**SS**	**F**	**P**	**df**	**SS**	**F**	**P**
Month	7	0.92	2.10	**0.05**	3	2.69	3.96	**0.009**
Site	1	1.14	18.20	**<0.0001**	1	2.58	11.41	**0.0009**
Edge	1	7.46	118.70	**<0.0001**	1	21.79	96.49	**<0.0001**
Sex	1	2.16	34.41	**<0.0001**	1	2.13	9.42	**0.003**
Edge x Site	1	0.53	8.36	**0.004**	1	1.91	8.46	**0.004**
Sex x Site	1	0.77	12.32	**0.0005**	1	1.03	4.54	**0.03**
Sex x Edge	1	4.39	69.95	**<0.0001**	1	10.86	48.10	**<0.0001**
Edge x Site x Sex	1	0.01	0.23	0.63	1	0.18	0.81	0.37
Month x Site	7	0.41	0.94	0.47	3	2.50	3.70	**0.01**
Month x Edge	7	0.27	0.61	0.74	3	1.41	2.08	0.11
Month x Sex	7	2.48	5.64	**<0.0001**	3	2.39	3.53	**0.01**
Month x Sex x Edge	7	0.65	1.47	0.18	3	0.71	1.04	0.37
Month x Site x Edge	7	0.44	1.00	0.43	3	0.69	1.02	0.38
Month x Sex x Site	7	1.25	2.84	**0.007**	3	2.36	3.48	**0.02**
Month x Sex x Site x Edge	7	1.16	2.63	**0.01**	3	1.57	2.32	0.08
Error	320	20.10			160	36.13		
Total	383	44.15		**<0.0001**	191	90.93		**<0.0001**

Table 5.3. Results of 2-factor ANOVA testing effects of sex and edge on three major carotenoid pigments. All values with p<0.05 are in boldface.

	Mytiloxanthin				**Alloxanthin**				**Zeaxanthin**			
Parameter	**df**	**SS**	**F**	**P**	**df**	**SS**	**F**	**P**	**df**	**SS**	**F**	**P**
Edge	1	0.09	6.22	**0.02**	1	0.04	6.47	**0.02**	1	0.00	0.002	0.97
Sex	1	0.13	9.13	**0.007**	1	0.04	6.70	**0.02**	1	0.01	3.58	0.07
Sex x Edge	1	0.0008	0.06	0.81	1	0.0006	0.09	0.77	1	0.00	0.07	0.79
Error	20	0.28			20	0.12			20	0.06		
Total	23	0.49		**0.009**	23	0.20		**0.02**	23	0.07		0.33

Table 5.4. Results of repeated-measures MANOVA tests for growth rate, stage of maturity, GSI, color, and carotenoids in the field transplant experiment. Comparisons were performed between edges for *in situ* mussels, between edges and treatments for the first two time points, and within the high edge for all treatments and time points. Values for p<0.05 are in boldface.

Comparison	**Parameter**	**Growth Rate**			**Stage of Maturity**			**Gonadosomatic Index**			**Color**			**Carotenoids**		
		df	**F**	**P**	**df**	**F**	**P**	**df**	**F**	**P**	**df**	**F**	**P**	**df**	**F**	**P**
Between edges (*in situ* plots)	Edge	1,6	12.72	**0.01**	1,6	52.86	**0.0003**	1,6	13.66	**0.01**	1,6	0.02	0.90	1,6	0.01	0.92
	Time	2,5	0.04	0.96	3,4	128.70	**0.0002**	2,5	15.78	**0.007**	3,4	8.65	**0.03**	3,4	0.24	0.86
	Time x Edge	2,5	0.15	0.87	3,4	3.13	0.15	2,5	1.75	0.27	3,4	8.70	**0.03**	3,4	0.81	0.55
Between edges (for first two collection periods)	Edge				1,15	18.35	**0.0007**				1,15	0.00	1.00	1,15	0.05	0.82
	Treatment				2,15	0.11	0.90				2,15	0.20	0.82	2,15	0.97	0.40
	Edge x Treatment				2,15	0.76	0.48				2,15	5.64	**0.01**	2,15	4.73	**0.03**
	Time				1,15	80.07	**<0.0001**				1,15	2.62	0.13	1,15	0.34	0.57
	Time x Edge				1,15	9.27	**0.008**				1,15	0.00	0.99	1,15	0.17	0.69
	Time x Treatment				2,15	0.89	0.43				2,15	0.93	0.42	2,15	0.13	0.88
	Time x Edge x Treatment				2,15	2.95	0.08				2,15	0.85	0.45	2,15	0.87	0.44
Within high edge	Treatment	2,5	1.57	0.30	2,5	1.43	0.32	2,5	0.32	0.74	2,5	0.43	0.68	2,5	1.40	0.33
	Time	2,4	0.93	0.47	3,3	0.27	0.84	2,4	0.94	0.46	3,3	1.71	0.34	3,3	0.27	0.84
	Time x Treatment	4,8	0.35	0.84	6,6	0.82	0.60	4,8	1.23	0.37	6,6	0.32	0.90	6,6	0.83	0.58

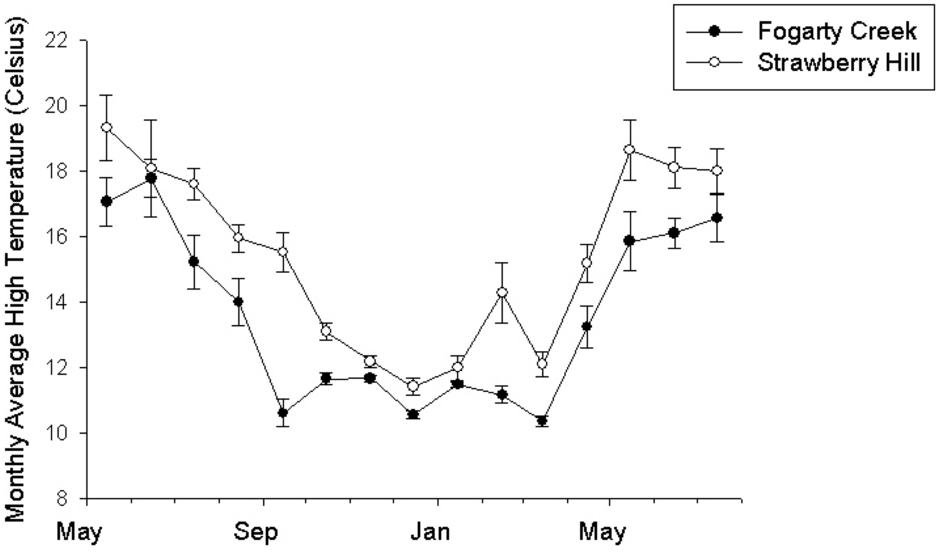

Fig. 5.1. Monthly average high temperature in the mid zone at FC and SH based on daily high temperatures. Y-error bars are standard errors of daily high temperatures.

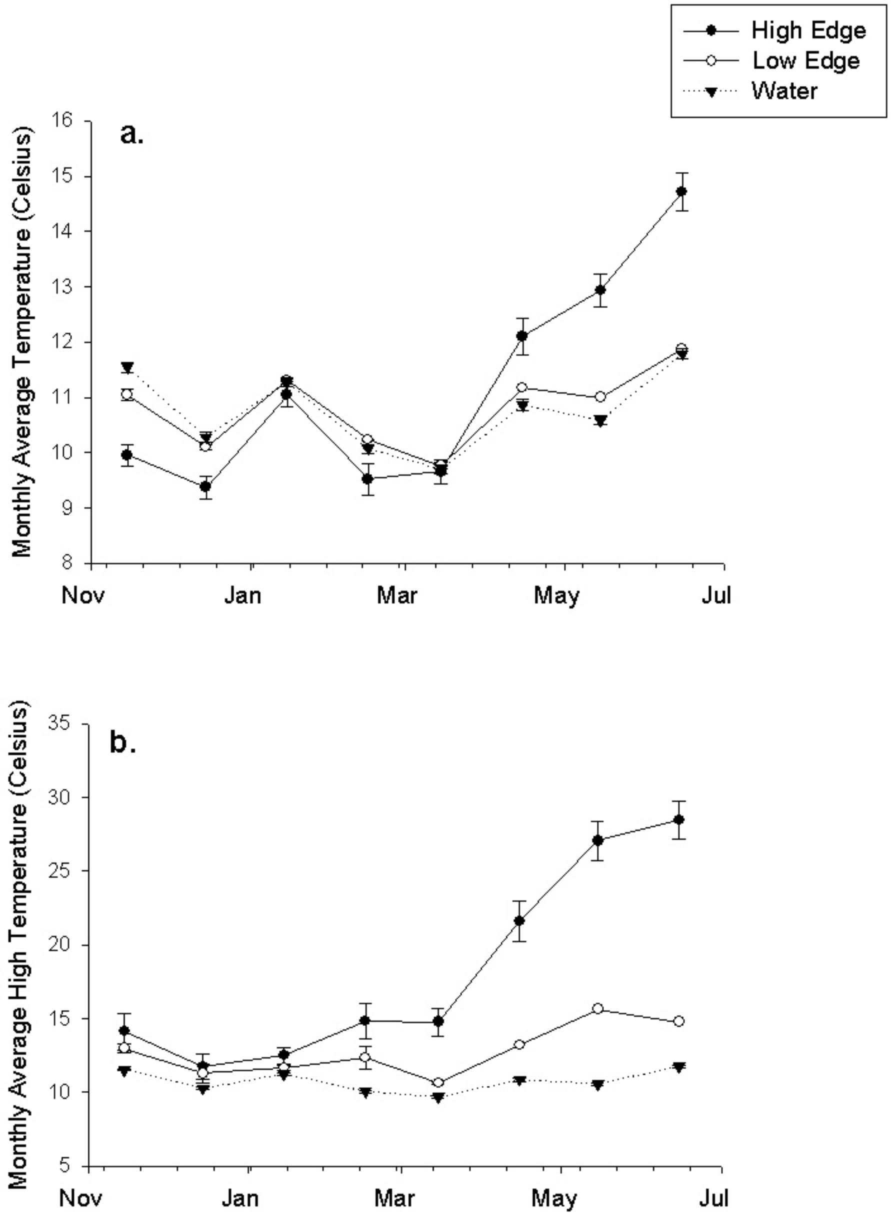

Fig. 5.2. Average temperatures in low and high edges of the mussel bed at FC from Nov. 2005-June 2006. **a**. Monthly average temperature based on averages of daily temperatures. **b**. Monthly average high temperature based on averages of daily high temperatures. Y-error bars are standard error of 2 temperature loggers.

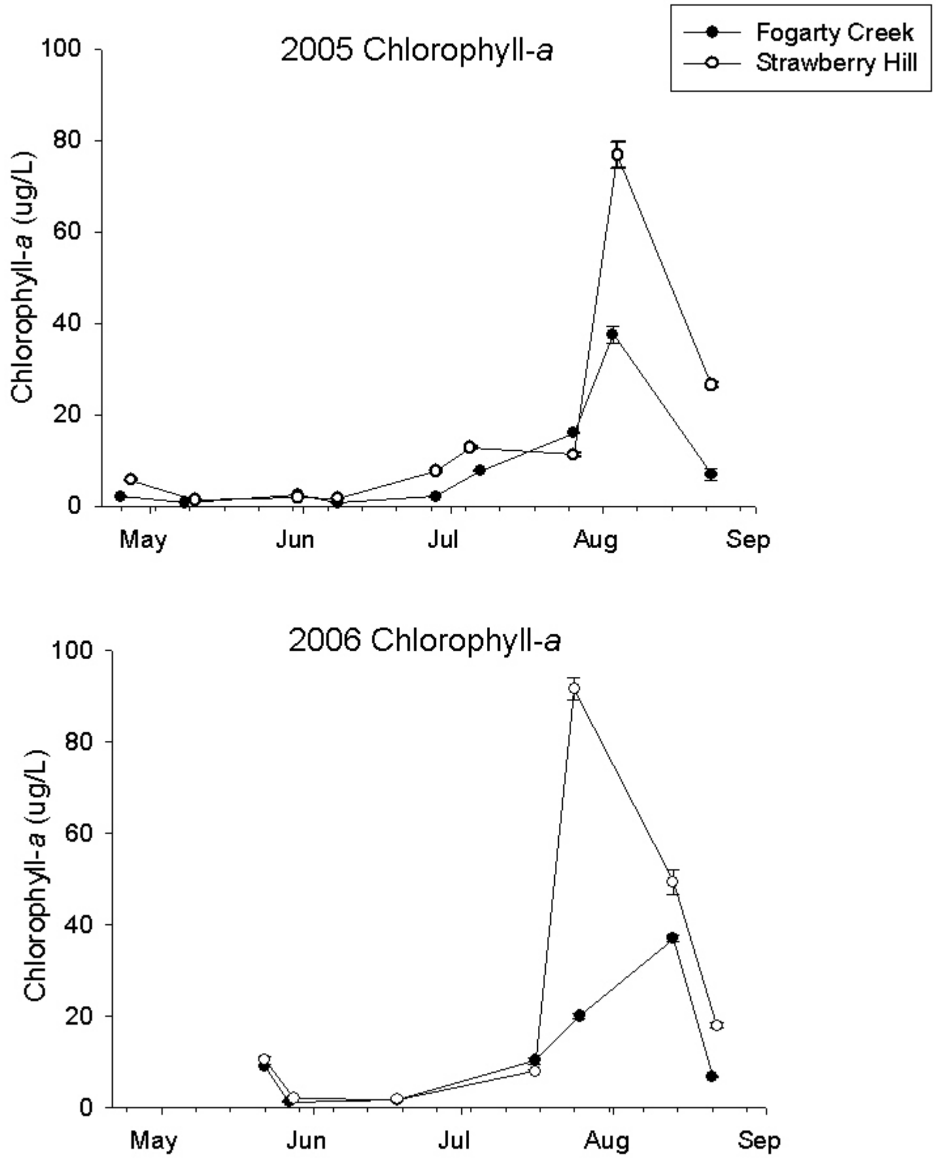

Fig. 5.3. Chlorophyll-*a* levels at FC and SH in 2005 and 2006. Y-error bars are standard error.

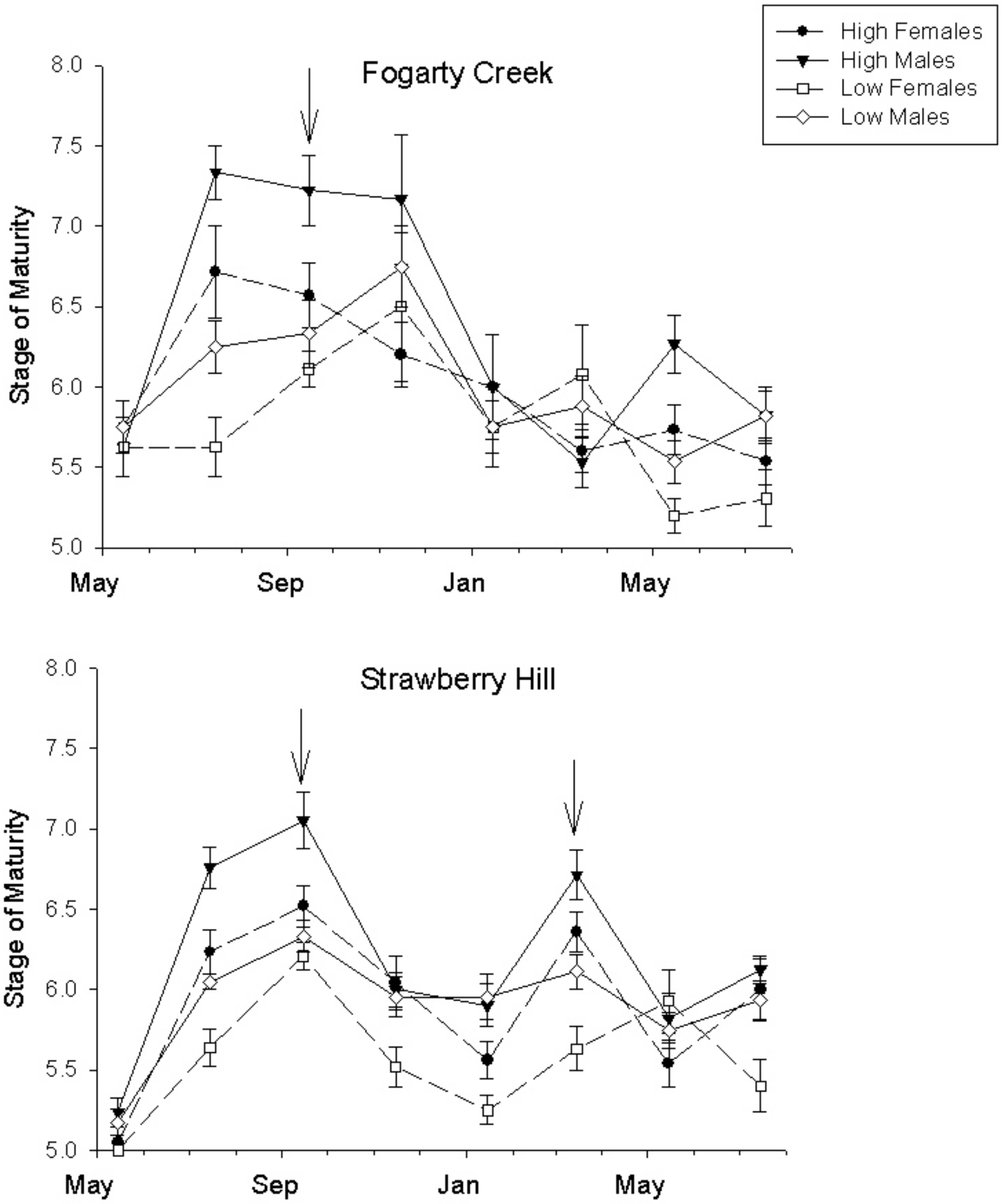

Fig. 5.4. Average stage of maturity of mussels collected from FC and SH in May 2005-July 2006. Y-error bars are standard error. Arrows indicate when spawning events had occurred.

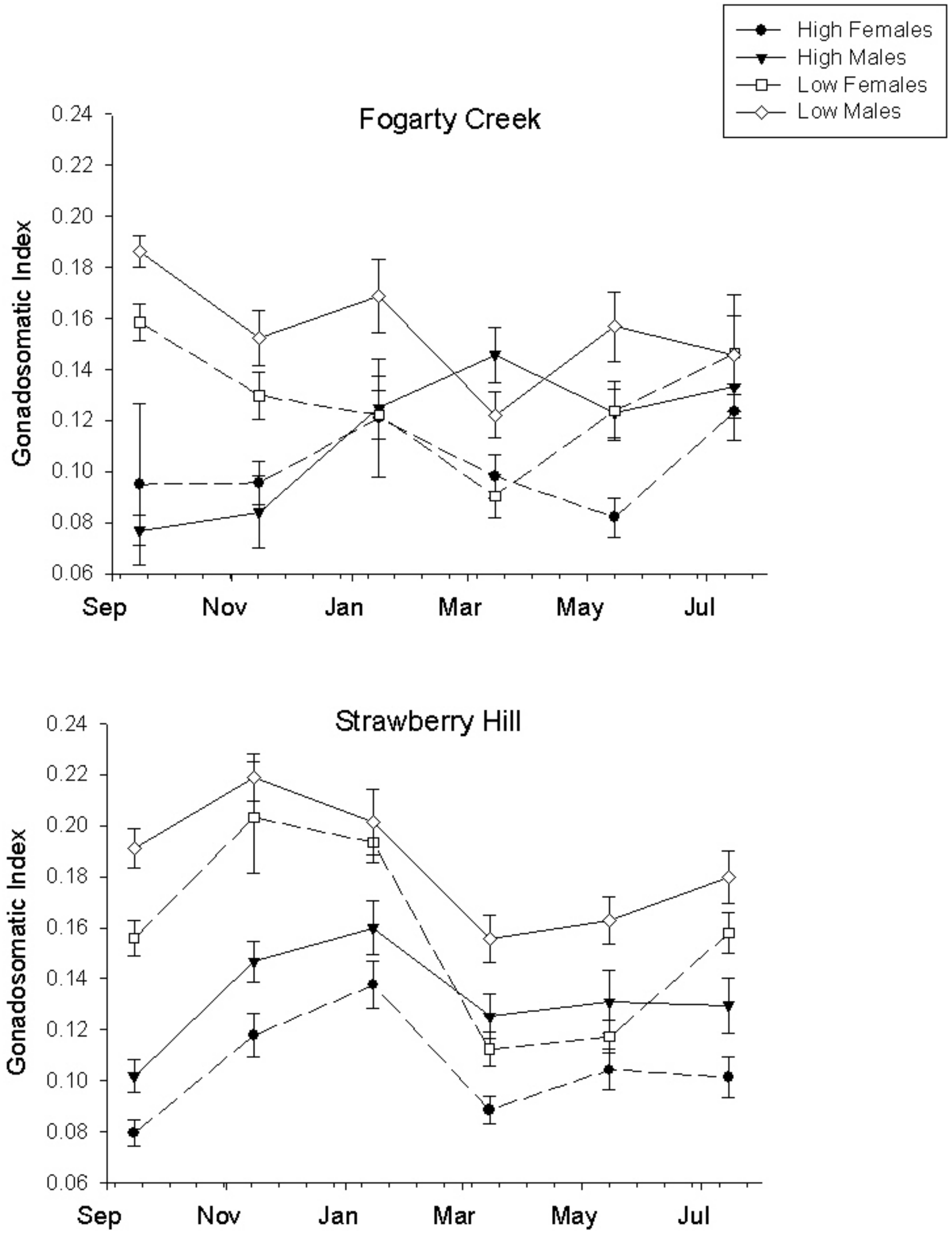

Fig. 5.5. Average gonadosomatic index (GSI) of mussels collected from FC and SH in September 2005-July 2006. Y-error bars are standard error.

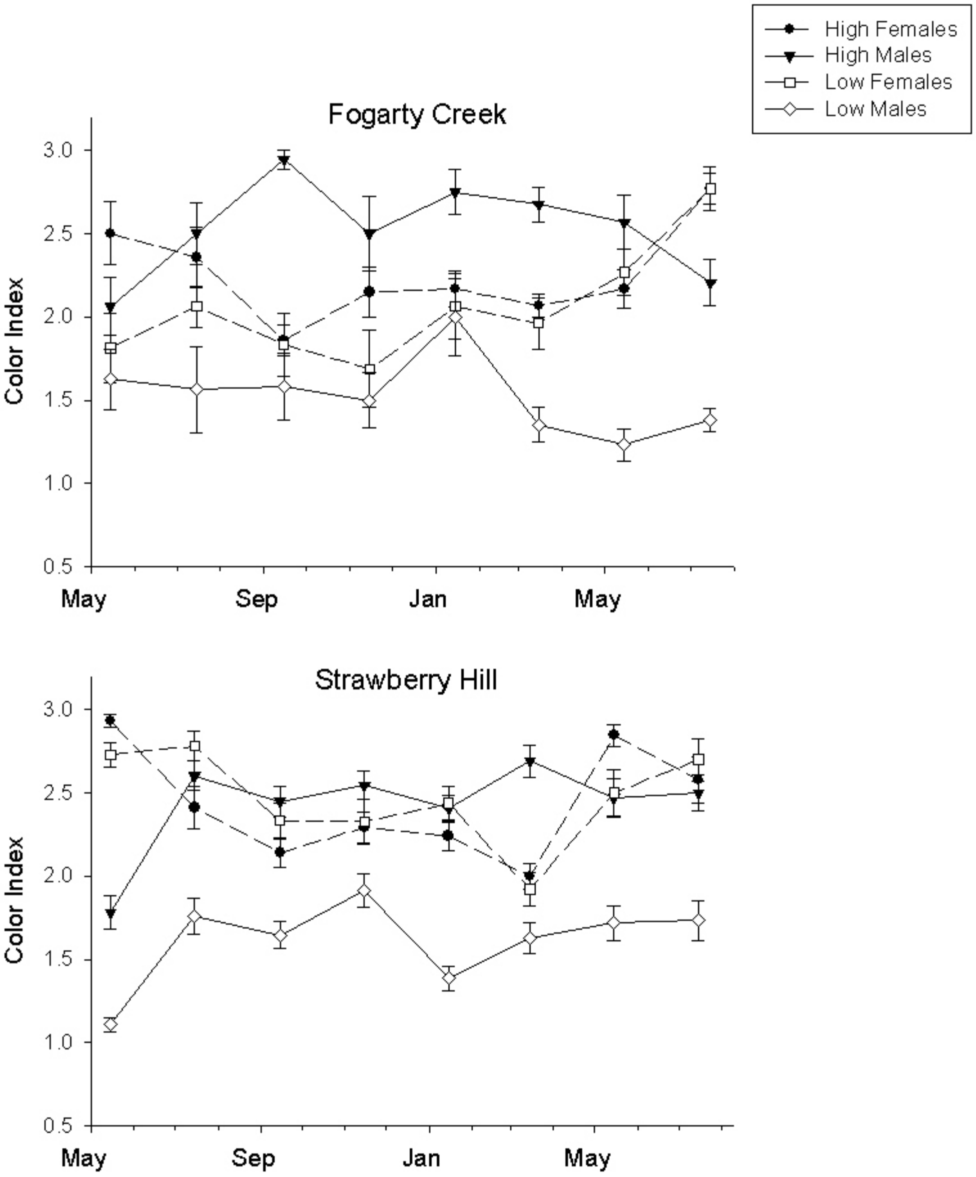

Fig. 5.6. Average color index (1=white, 2=peach, 3=orange) of mussels collected from FC and SH in May 2005-July 2006. Y-error bars are standard error.

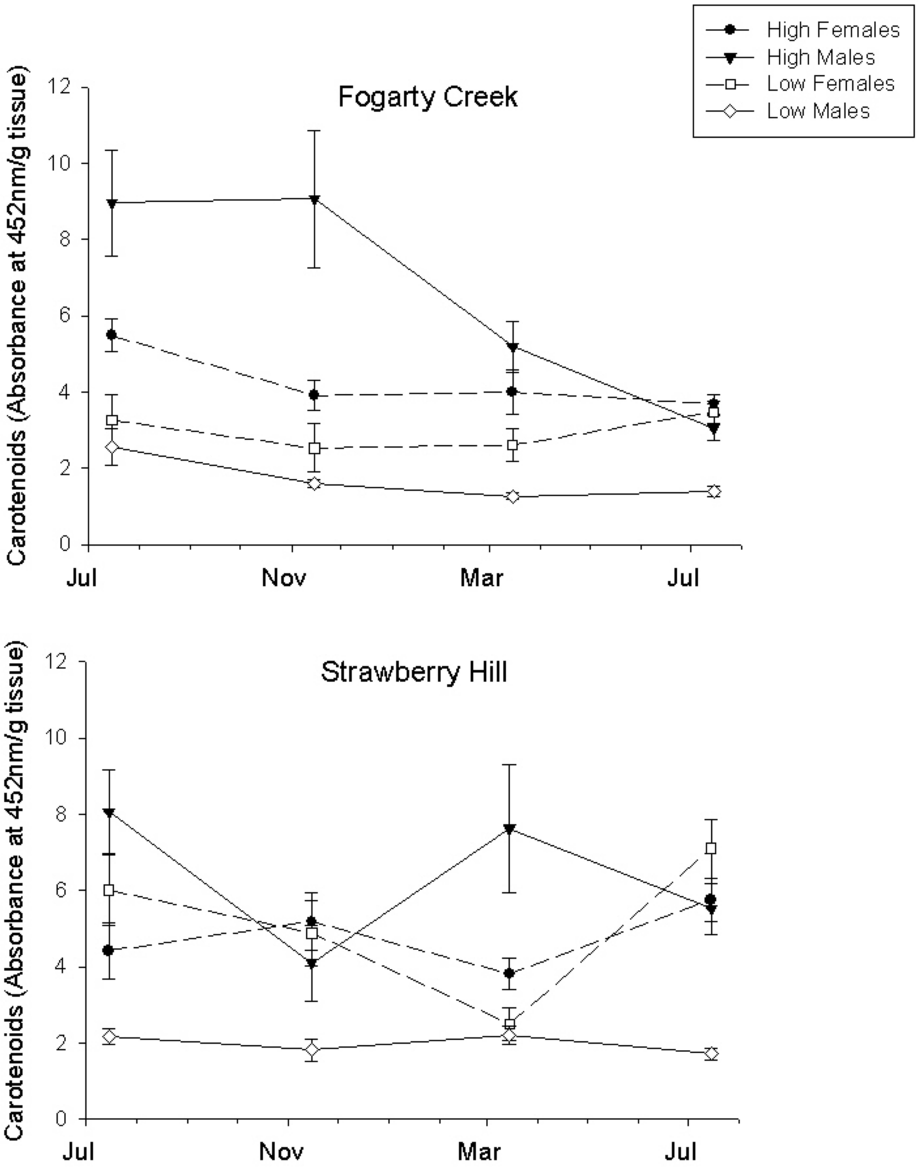

Fig. 5.7. Average carotenoid pigment content of mussels collected from FC and SH in July 2005-July 2006. Y-error bars are standard error.

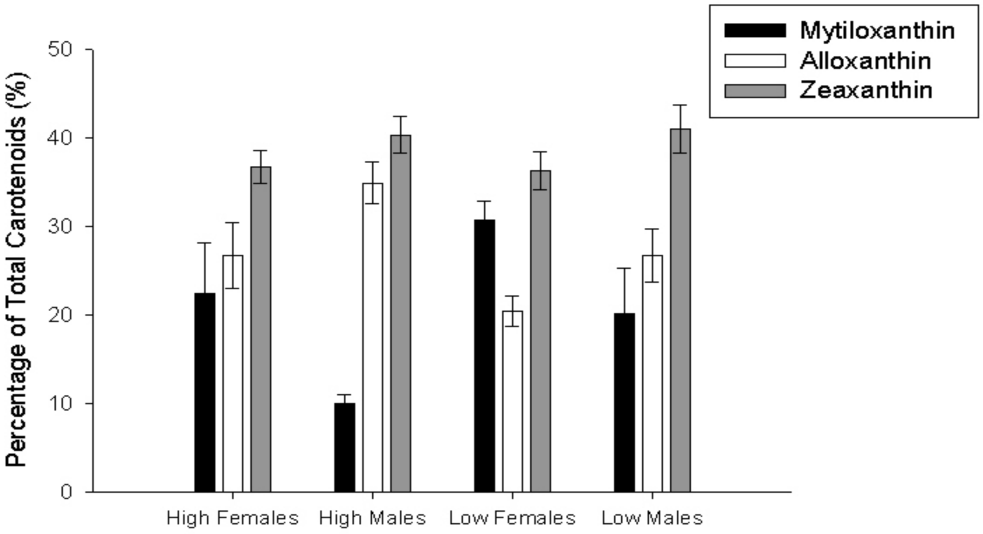

Fig. 5.8. Average percentage of each of the three major carotenoid pigments (alloxanthin, mytiloxanthin, zeaxanthin) out of total carotenoids. Y-error bars are standard error.

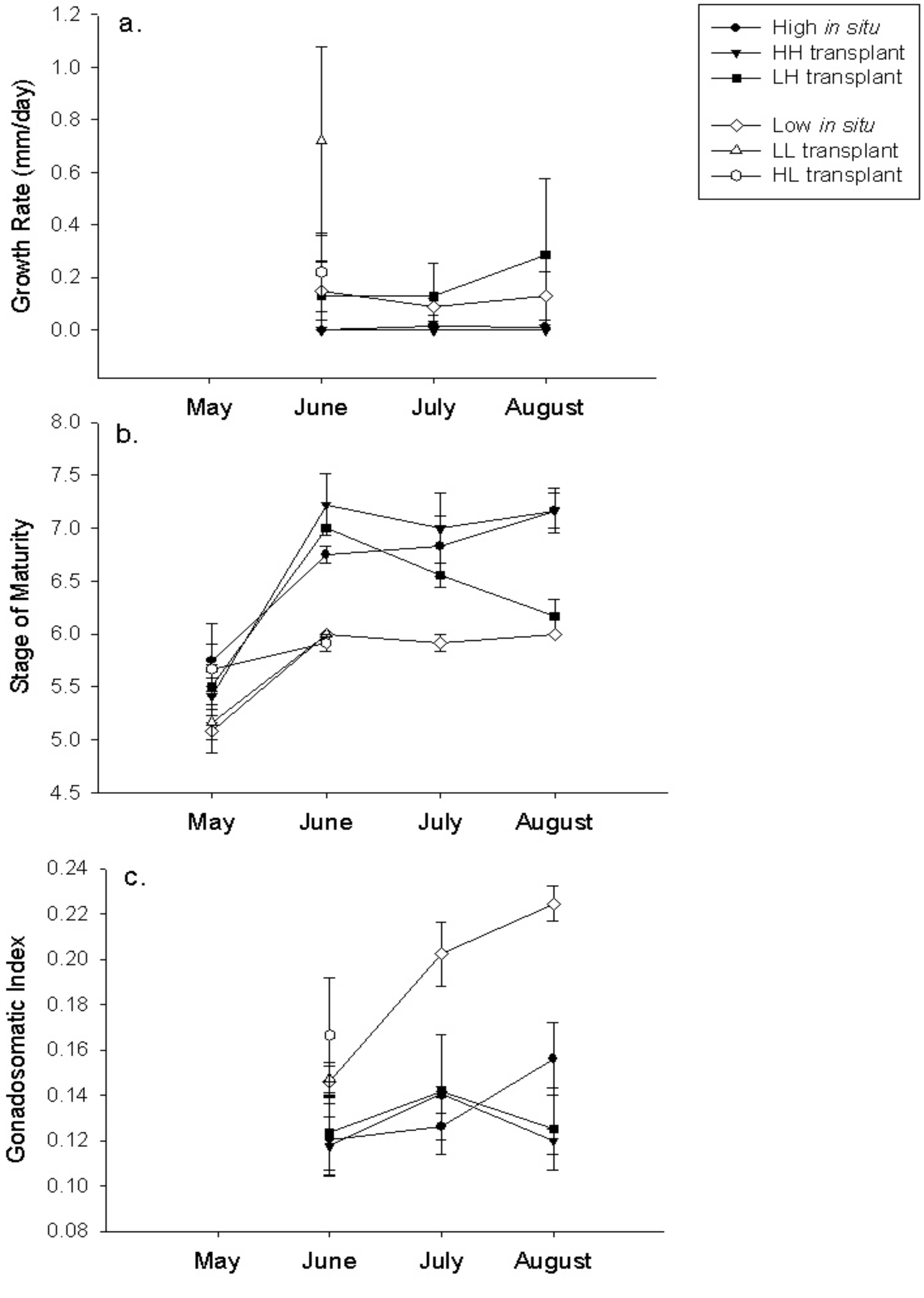

Fig. 5.9. Average **a.** growth rate, **b.** stage of maturity, and **c.** gonadosomatic index (GSI) of mussels from transplant experiment conducted between May-August 2004. Y-error bars are standard error.

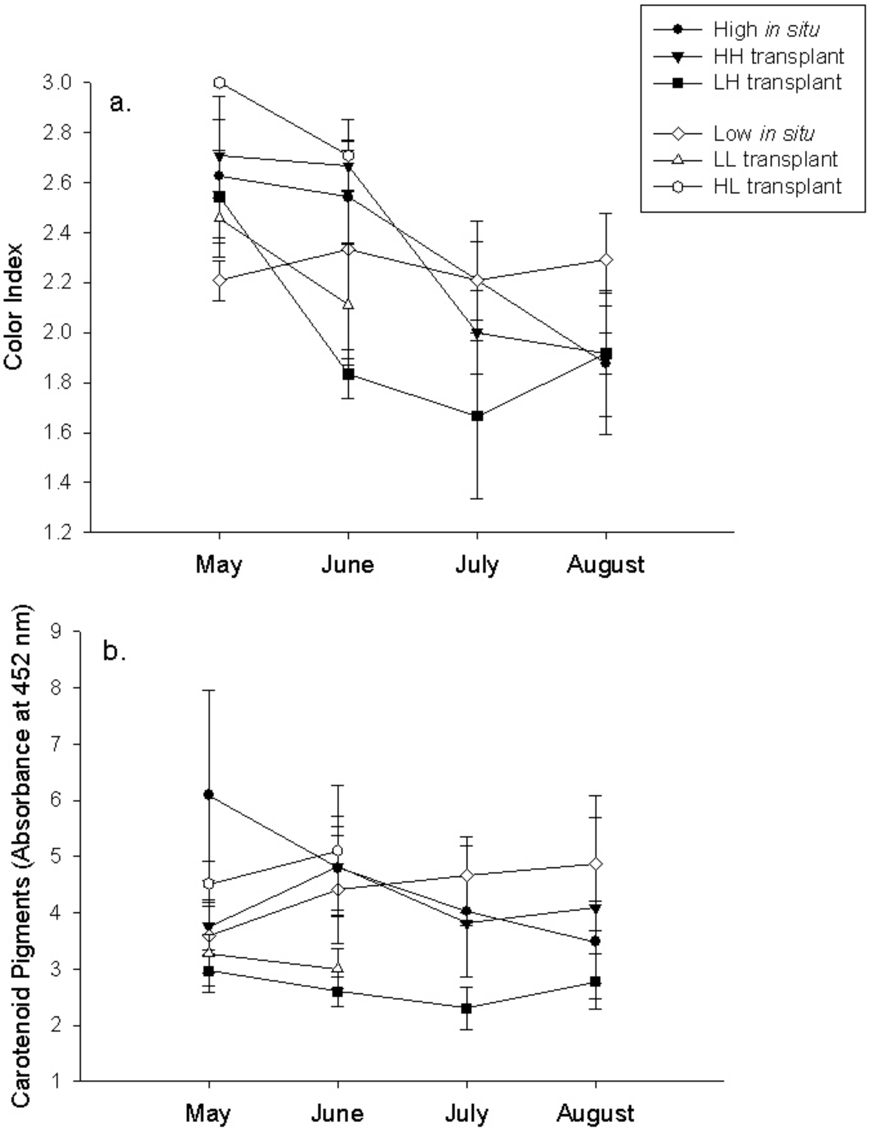

Fig. 5.10. Average **a.** color index and **b.** carotenoid pigment level of mussels from transplant experiment conducted between May-August 2004. Y-error bars are standard error.

Chapter 6: General Conclusion

The studies presented in this dissertation represent an attempt to gain enhanced understanding of how environmental stress affects the ecology and physiology of intertidal mussels. My results suggest that energetic trade-offs do occur in intertidal mussels and that reproduction may be compromised under situations of stress in an attempt to conserve energy for physiological defenses that increase the chance of survival. This concluding chapter integrates the findings to address the overarching questions that I posed in Chapter 1, then places them in the context of global climate change.

1. How does environmental stress affect growth, defense, and survival?

Overall, mussels responded plastically to stress in their growth, production of physiological defenses, and survival. Transplant experiments conducted in Chapters 2 and 5 showed that mussels in both New Zealand and Oregon grew more slowly when moved to the high edge of the intertidal zone. This is likely due to a combination of increased exposure to aerial temperatures at low tide and decreased time for feeding at high tide. In Chapter 2, we documented high mortality in the high edge of the mussel bed after an extreme aerial temperature event in New Zealand for both experimental mussels and mussels living naturally in the high edge. In addition, this mortality event was species-specific, with one species (*Perna canaliculus*) suffering much higher mortality than the other (*Mytilus galloprovincialis*). In Chapter 3, we found that Oregon mussels increased production of heat shock proteins, which are indicators of stress, upon transplantation to the high edge of the mussel bed.

2. What are the effects of environmental stress on timing of spawning and energy allocation towards reproduction?

Environmental stress led to increased spawning activity in all studies conducted for this dissertation. This occurred in both high-edge mussels in their natural environment and in mussels experimentally transplanted to the high edge, indicating that this is a plastic response. The majority of spawning activity in high-edge mussels occurred at the beginning of the summer when aerial temperatures began to increase, suggesting that mussels may spawn their gametes to conserve energy for physiological defenses against stress in summer months. Alternatively, high-edge mussels may be relying on high aerial temperature as a proximate cue for spawning. However, as discussed in Chapter 5, releasing all gametes simultaneously may not be the best strategy for successful reproduction, as it relies on predictable timing of oceanographic events to supply food for larvae and return them back to shore for settlement. The continuous "dribble-spawning" strategy exhibited by low-edge mussels may therefore be more effective, as it is more likely that some of these gametes and resulting larvae will survive and be returned to shore.

As documented in Chapters 2 and 5, environmental stress affected energy allocation towards reproduction (measured as gonadosomatic index, or GSI). Mussels either from or transplanted to the high edge of the mussel bed had lower GSI than low-edge mussels, suggesting that stress influences energy available for reproduction. This was seen in both New Zealand and Oregon for all three species of mussels investigated.

3. How does reproduction vary along a gradient of food availability?

In Chapter 5, we found that mussels at the sites with higher primary productivity (in Cape Perpetua) invested relatively more energy into reproduction than mussels at sites with lower primary productivity (in Cape Foulweather). This is consistent with the findings of Leslie et al. (2005) for barnacle reproduction. In addition, in Chapter 4, we revealed an interesting pattern where high-edge mussels at the Cape Perpetua sites spawn earlier than sites at Cape Foulweather.

4. What are the effects of environmental stress on pigmentation of gonadal tissue?

In Chapter 4, we showed that both female and male mussels in the high edge of the mussel bed had brightly-pigmented gonadal tissue, and this pattern was documented across time at all four sites on the central Oregon coast. In Chapter 5, we determined that the orange coloration was due to high concentrations of carotenoid pigments that had been incorporated into the gonads. A field transplant experiment suggested that this is not a plastic response over the period of a few months, as carotenoids did not increase upon transplantation to the high edge. However, it is possible that carotenoid accumulation is a longer-term response to stress and that it potentially takes months or years to accumulate high concentrations of pigments. Carotenoid pigments are known for their antioxidant properties, as they have the ability to quench harmful singlet oxygen radicals (Miki 1991). Intertidal organisms are exposed to potentially high temperatures (e.g. Helmuth et al. 2002), desiccation (Bertness et al. 2006), ultraviolet-B radiation (e.g. Abele-Oeschger et al. 1997), and periods of hypoxia (e.g. Altieri 2006) during aerial exposure at low tide, all of which could lead to metabolic processes that generate oxygen radicals. It is therefore possible that mussels in the stressful areas of the intertidal zone are accumulating carotenoid pigments to defend their gametes from damaging free radicals.

The overall conclusions of my research are that environmental stress affects many aspects of mussel ecology and physiology. Stress leads to trade-offs, with less energy invested into growth and reproduction, and more energy allocated towards physiological defense and repair mechanisms (e.g. heat shock protein production). In the relatively high-stress high edge of the mussel bed, mussels spawn early in the summer and release a majority of their gametes simultaneously, whereas mussels in the low edge “dribble spawn” continuously, a strategy that likely requires more energy but is less risky for future success of gametes and survival of larvae. Mussels in the high edge of the mussel bed accumulate high concentrations of carotenoids into their gonadal tissues, potentially to protect their gametes from oxidative damage. Extreme temperature stress can lead to mussel mortality events, and these events can have larger effects on species that are less thermotolerant.

There are many unknowns that still remain from this research. It would be interesting to examine whether gametes spawned in response to temperature stress are actually viable. Also, higher resolution investigations (at a timescale of days, rather than months) into the relationships between phytoplankton abundance, timing of spawning events, larval duration, larval feeding behavior, and timing of recruitment may help elucidate whether or not larvae are food-limited in this system and what factors affect recruitment success. Many unanswered questions arise from the carotenoid research. Are gametes spawned from mussels with high carotenoid content more resistant to oxidative damage, and are they more viable? Would mussels with low carotenoid content survive in the high edge of the mussel bed, and would they reproduce successfully? How long does it take for mussels in the high edge to accumulate carotenoid pigments? A combination of field and laboratory experiments may tease apart some of the mechanisms behind carotenoid accumulation, as well as the functional role of carotenoids in the intertidal zone. In addition to these questions, what factors make certain mussel species more vulnerable to high aerial temperatures than others, and how will warming temperatures affect these communities?

The results of my dissertation lend some insight into predictions for intertidal systems under global climate change scenarios. Predicted alterations under climate change include increasing frequency and severity of high aerial temperature events (IPCC 2007), rising sea levels, warming ocean temperatures, and potential changes in oceanic circulation, including increased frequency and intensity of coastal upwelling events (e.g. Bakun 1990, Lubchenco et al. 1993, Harley et al. 2006). While there have been many predictions, there are few data available on how changes in temperature and oceanic circulation will affect population dynamics of intertidal communities.

Warming temperatures and changes to oceanic circulation both occurred during the course of my dissertation research. Four of the six hottest global years ever recorded since the 1890's were the years 2003-2006 (NASA 2006). In the Pacific Northwest, temperatures during this time were warmer than average in 2004 and 2006, and while 2004 and 2005 were relatively dry, 2006 had one of the wettest winters on record (NOAA 2006). In New Zealand, the high aerial temperature recorded during the mussel mortality event in Chapter 2 was the 3rd highest January temperature for Christchurch since records began in 1939 (NIWA 2005). The overall warming trend and increasing severity of high aerial temperature events are both consistent with climate change predictions (IPCC 2007). Some species will not be able to adapt quickly enough to defend themselves from a rapidly changing and unpredictable climate (Houghton et al. 2001), and this was observed in the species-specific mortality of intertidal mussels in Chapter 2. Changes in oceanic circulation have also occurred on the central Oregon coast within the past several years. Unprecedented seasonal hypoxia in the inner shelf began in the summer of 2002 off the Oregon coast (Grantham et al. 2004) and has since occurred all of the 5 past summers, with 2006 having the most severe and longest duration of hypoxia ever recorded for this region (Chan et al. in prep.). In addition, the summer of 2005 was characterized by delayed upwelling, which altered timing of food availability and recruitment for intertidal organisms (Barth et al. 2007). These anomalies in oceanic circulation are consistent with predictions from climate change scenarios.

Based on the results of my dissertation research, I offer some potential predictions for intertidal mussels under climate change:

1. If temperature-induced spawning occurs due to increased frequency of extreme aerial temperature events, gametes that are spawned prematurely may not yet be viable or, if successfully fertilized, may have insufficient food supply as larvae.

2. Larvae resulting from summer high-edge spawning in May and June could be pushed far offshore due to seasonal upwelling events, which are predicted to increase in severity (e.g. Lubchenco et al. 1993), and may not be successfully returned to shore for settlement.

3. Mussel species may be differentially affected by warm aerial temperatures, as certain species appear to be more thermotolerant than others.

4. Mortality in the high edge of the mussel bed from thermal stress (as seen in New Zealand) could result in a downward shift in the upper limit of mussels in the intertidal zone. In addition, as sea level rises, zones are expected to move upwards on the shore (Lubchenco et al. 1993). Therefore, the combination of both upward shifts of the low intertidal and downward shifts of the high intertidal will likely result in an overall narrowing of the intertidal zone (Lubchenco et al. 1993, Harley et al. 2006).

The results of my dissertation represent some of the first findings for how key elements of mussel population dynamics may be affected under climate change. By integrating marine community ecology with physiological tools, I was able to gain a better understanding for how mussels are affected sublethally by environmental stress. Mussels are the dominant space-occupiers on many rocky shores throughout the world and build beds that provide critical habitat for hundreds of intertidal species

(Seed and Suchanek 1992). If mussel reproductive and recruitment success are reduced, and adults suffer temperature-induced mortality, decreased abundance of mussels on rocky shores could result. A decline in the spatially complex habitat of the mussel bed could lead to an overall decrease in intertidal biodiversity.

Bibliography

Abele, D., B. Burlando, A. Viarengo, and H.O. Pörtner. 1998. Exposure to elevated temperatures and hydrogen peroxide elicits oxidative stress and antioxidant response in the Antarctic intertidal limpet *Nacella concinna*. Comparative Biochemistry and Physiology **120B**: 425-435.

Abele-Oeschger, D., H. Tug, and R. Röttgers. 1997. Dynamics of UV-driven hydrogen peroxide formation on an intertidal sandflat. Limnology and Oceanography **42**(6): 1406-1415.

Alonso-Alvarez, C., S. Bertrand, G. Devevey, J. Prost, B. Faivre, and G. Sorci. 2004a. Increased susceptibility to oxidative stress as a proximate cost of reproduction. Ecology Letters **7**: 363-368.

Alonso-Alvarez, C., S. Bertrand, G. Devevey, M. Gaillard, J. Prost, B. Faivre, and G. Sorci. 2004b. An experimental test of the dose-dependent effect of carotenoids and immune activation on sexual signals and antioxidant activity. The American Naturalist **164**(5): 651-659.

Altieri, A.H. 2006. Inducible variation in hypoxia tolerance across the intertidal-subtidal distribution of the blue mussel *Mytilus edulis*. Marine Ecology Progress Series **325**: 295-300.

Anderson, G. 1975. The effects of intertidal height and the parasitic crustacean *Fabia subquadrata* Dana on the nutrition and reproductive capacity of the California sea mussel *Mytilus californianus* Conrad. The Veliger 17(3): 299-306.

Bakun, A. 1990. Global climate change and intensification of coastal ocean upwelling. Science **247**: 198-201.

Barth, J.A., B.A. Menge, J. Lubchenco, F. Chan, J.M. Bane, A.R. Kirincich, M.A. McManus, K.J. Nielsen, and L. Washburn. 2007. Delayed upwelling alters nearshore coastal ocean ecosystems in the northern California current. Proceedings of the National Academy of Sciences USA 104(10): 3719-3724.

Bayne, B.L. and R.J. Thompson. 1970. Some physiological consequences of keeping *Mytilus edulis* in the laboratory. Helgoland Marine Research **20**: 526-552.

Bayne, B.L. 1972. Some effects of stress in the adult on the larval development of *Mytilus edulis*. Nature **237**: 459.

Bayne, B.L., C.J. Bayne, T.C. Carefoot, and R.J. Thompson. 1976. Physiological ecology of *Mytilus californianus* Conrad. 1. Metabolism and energy-balance. Oecologia **22**: 211-228.

Bertness, M.D., C.M. Crain, B.R. Silliman, M.C. Bazterrica, M.V. Reyna, F. Hildago, and J.K. Farina. 2006. The community structure of Western Atlantic Patagonian rocky shores. Ecological Monographs **76**(3): 439-460.

Blount, J.D., P.F. Surai, D.C. Houston, and A.P. Møller. 2002. Patterns of yolk enrichment with dietary carotenoids in gulls: the roles of pigment acquisition and utilization. Functional Ecology **16**: 445-453.

Brinkhoff, W., K. Stockmann, and M. Grieshaber. 1983. Natural occurrence of anaerobiosis in mollusks from intertidal habitats. Oecologia **57**: 151-155.

Britton, G. 1995. UV/visible spectroscopy. Pages 13-63 *in* G. Britton, S. Liaaen-Jensen, and H. Pfander, editors. Carotenoids: spectroscopy, Vol. 1B. Birkhäuser Verlag, Basel, CH.

Brokordt, K.B., J.H. Himmelman, and H. Guderley. 2000. Reproductive investment reduces recuperation from escape responses in the tropical scallop *Euvola ziczac*. Marine Biology **137**(5-6): 857-865.

Bromage, N.R. 1995. Broodstock management and seed quality – general considerations. Pages 1-24 *in* N.R. Bromage and R.J. Roberts, editors. Broodstock management and egg and larval quality. Blackwell Science Inc., London, UK.

Bruno, J.F., J.J. Stachowicz, and M.D. Bertness. 2003. Inclusion of facilitation into ecological theory. Trends in Ecology and Evolution **18**: 119-125.

Brush, A.H. 1990. Metabolism of carotenoid pigments in birds. Federation of American Societies for Experimental Biology Journal **4**: 2969-2977.

Buchanan, S. 2001. Measuring reproductive condition in the Greenshell^{TM} mussel *Perna canaliculus*. New Zealand Journal of Marine and Freshwater Research **35**: 859-870.

Buchecker, R. 1982. A chemist's view of animal carotenoids. Pages 175-193 *in* G. Britton and T.W. Goodwin, editors. Carotenoid chemistry and biochemistry. Pergamon Press, Oxford, UK.

Buckley, B.A., M.E. Owen, and G.E. Hofmann. 2001. Adjusting the thermostat: the threshod induction temperature for the heat shock response in intertidal mussels (genus *Mytilus*) changes as a function of thermal history. Journal of Experimental Biology **204**: 3571-3579.

Burdon, J.J. and W.J. Müller. 1987. Measuring the cost of resistance to *Puccinia coronata* Cda in *Avena fatua* L. Journal of Applied Ecology **24**: 191-200.

Campbell, S.A. 1969. Seasonal cycles in the carotenoid content in *Mytilus edulis*. Marine Biology **4**: 227-232.

Campbell, S.A. 1970. The carotenoid pigments of *Mytilus edulis* and *Mytilus californianus*. Comparative Biochemistry and Physiology **32**: 97-115.

Campbell, P.M., T.G. Pottinger, and J.P. Sumpter. 1992. Stress reduces the quality of gametes produced by rainbow trout. Biology of Reproduction **47**: 1140-1150.

Chipperfield, P.N.J. 1953. Observations on the breeding and settlement of *Mytilus edulis* in British waters. Journal of the Marine Biological Association of the UK **32**: 449-476.

Connell, J.H. 1961. The influence of interspecific competition and other factors on the distribution of the barnacle *Chthamalus stellatus*. Ecology **42**(4): 710-723.

Connell, J.H. 1972. Community interactions on marine rocky intertidal shores. Annual Review of Ecology and Systematics **3**: 169-192.

Connell, J.H. 1975. Some mechanisms producing structure in natural communities: a model and evidence from field experiments. Pages 460-490 *in* M.L. Cody and J. Diamond, editors. Ecology and evolution of communities. Harvard University Press, Cambridge, USA.

Connolly, S.R., B.A. Menge, and J. Roughgarden. 2001. A latitudinal gradient in recruitment of intertidal invertebrates in the Northeast Pacific Ocean. Ecology **82**(7): 1799-1813.

Dahlhoff, E.P., B.A. Buckley, and B.A. Menge. 2001. Physiology of the rocky intertidal predator *Nucella ostrina* along an environmental stress gradient. Ecology **82**(10): 2816-2829.

Dahlhoff, E.P., J.H. Stillman, and B.A. Menge. 2002. Physiological community ecology: variation in metabolic activity of ecologically important rocky intertidal invertebrates along environmental gradients. Integrative and Comparative Biology **42**(4): 862-871.

Davenport, J. and J.L. Davenport. 2005. Effects of shore height, wave exposure and geographical distance on thermal niche width of intertidal fauna. Marine Ecology Progress Series **292**: 41-50.

Denny, M.W. and R.T. Paine. 1998. Celestial mechanics, sea-level changes, and intertidal ecology. Biological Bulletin **194**: 108-115.

Dethier, M.N., S.L. Williams, and A. Freeman. 2005. Seaweeds under stress: manipulated stress and herbivory affect critical life-history functions. Ecological Monographs **75**(3): 403-418.

DiMascio, P., M.E. Murphy, and H. Sies. 1991. Antioxidant defense systems: the role of carotenoids, tocopherol, and thiols. The American Journal of Clinical Nutrition **53**: 194S-200S.

Dobson, H. and R.F. Smith. 2000. What is stress, and how does it affect reproduction? Animal Reproduction Science **60**: 743-752.

Feder, M.E. and G.E. Hofmann. 1999. Heat-shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology. Annual Review of Physiology **61**: 243-282.

Fox, D.L. and T.S. Hopkins. 1966. Comparative metabolic fractionation of carotenoids in three flamingo species. Comparative Biochemistry and Physiology **17**: 841-856.

Gilman, S.E., D.S. Wethey, and B. Helmuth. 2006. Variation in the sensitivity of organismal body temperature to climate change over local and geographic scales. Proceedings of the National Academy of Sciences USA **103**(25): 9560-9565.

Grantham, B.A., F. Chan, K.J. Nielsen, D.S. Fox, J.A. Barth, A. Huyer, J. Lubchenco, and B.A. Menge. 2004. Upwelling-driven nearshore hypoxia signals ecosystem and oceanographic changes in the northeast Pacific. Nature **429**: 749-754.

Haines, C.M.C., M. Edmunds, and A.R. Pewsey. 1994. The pea crab, *Pinnotheres pisum* (Linnaeus, 1767), and its association with the common mussel, *Mytilus edulis* (Linnaeus, 1758), in the Solent (UK). Journal of Shellfish Research **13**(1): 5-10.

Halliwell, B. and J.M.C. Gutteridge. 1999. Free radicals in biology and medicine. Oxford University Press, Oxford, UK.

Halpin, P.M., C.J. Sorte, G.E. Hofmann, and B.A. Menge. 2002. Patterns of variation in levels of Hsp70 in natural rocky shore populations from microscales to mesoscales. Integrative and Comparative Biology **42**(4): 815-824.

Halpin, P.M., B.A. Menge, and G.E. Hofmann. 2004. Experimental demonstration of plasticity in the heat shock response of the intertidal mussel *Mytilus californianus*. Marine Ecology Progress Series **276**: 137-145.

Harley, C.D.G., A.R. Hughes, K.M. Hultgren, B.G. Miner, C.J.B. Sorte, C.S. Thornber, L.F. Rodriguez, L. Tomanek, and S.L. Williams. 2006. The impacts of climate change in coastal marine systems. Ecology Letters **9**: 228-241.

Harman, D. 1981. The aging process. Proceedings of the National Academy of Sciences USA **78**: 7124-7128.

Harshman, L.G. and A.J. Zera. 2007. The cost of reproduction: the devil in the details. Trends in Ecology and Evolution **22**(2): 80-86.

Harvell, C.D., C.E. Mitchell, J.R. Ward, S. Altizer, A.P. Dobson, R.S. Ostfeld, and M.D. Samuel. 2002. Climate warming and disease risks for terrestrial and marine biota. Science **296**: 2158-2162.

Havaux, M. and K.K. Niyogi. 1999. The violaxanthin cycle protects plants from photooxidative damage by more than one mechanism. Proceedings of the National Academy of Sciences USA **96**: 8762-8767.

Helmuth, B. 1999. Thermal biology of rocky intertidal mussels: quantifying body temperatures using climatological data. Ecology **80**: 15-34.

Helmuth, B. and G.E. Hofmann. 2001. Microhabitats, thermal heterogeneity, and patterns of physiological stress in the rocky intertidal zone. Biological Bulletin **201**: 374-384.

Helmuth, B., C.D.G. Harley, P. Halpin, M. O'Donnell, G.E. Hofmann, and C.A. Blanchette. 2002. Climate change and latitudinal patterns of intertidal thermal stress. Science **298**: 1015-1017.

Hickman, R.W. 1978. Incidence of a pea crab and a trematode in cultivated and natural green-lipped mussels. New Zealand Journal of Marine and Freshwater Research **12**(2): 211-215.

Hill, G.E. 1996. Redness as a measure of the production cost of ornamental coloration. Ethology, Ecology, and Evolution **8**: 157-175.

Hoegh-Guldberg, O. 1999. Climate change, coral bleaching, and future of the world's coral reefs. Marine and Freshwater Research **50**: 839-866.

Hofmann, G.E. and G.N. 1995. Evidence for protein damage at environmental temperatures: seasonal changes in levels of ubiquitin conjugates and Hsp70 in the intertidal mussel *Mytilus trossulus*. Journal of Experimental Biology **198**: 1509-1518.

Houghton, D. 1963. The relationship between tidal level and the occurrence of *Pinnotheres pisum* in *Mytilus edulis*. Journal of Animal Ecology **32**: 253-257.

Houghton, J.T., Y. Ding, D.J. Griggs, M. Noguer, P.J. van der Linden, and D. Xiaosu, editors. 2001. Climate Change 2001: the Scientific Basis. Cambridge University Press, Cambridge, UK.

IPCC 2007. Climate change 2007: the physical science basis. Summary for policymakers. http://www.ipcc.ch/SPM2feb07.pdf, accessed March 27, 2007.

Jensen, A. and E. Sakshaug. 1970. Producer-consumer relationships in the sea. II. Correlation between *Mytilus* pigmentation and the density and composition of phytoplanktonic populations in inshore waters. Journal of Experimental Marine Biology and Ecology **5**: 246-253.

Jørgensen, C.B. 1960. Efficiency of particle retention and rate of water transport in undisturbed lamellibranches. Journal du Conseil Permanent International pour l'Exploration de la Mer **25**: 94-116.

Karnaukhov, V.N. 1979. The role of filtrator mollusks rich in carotenoids in the self-cleaning of fresh waters. Symposia Biologica Hungaria **19**: 151-167.

Kennedy, V.S. 1976. Desiccation, higher temperatures and upper intertidal limits of three species of sea mussels (Mollusca: Bivalvia) in New Zealand. Marine Biology **35**: 127-137.

Kennedy, V.S. 1977. Reproduction in *Mytilus edulis aoteanus* and *Aulacomya maoriana* (Mollusca: Bivalvia) from Taylors Mistake, New Zealand. New Zealand Journal of Marine and Freshwater Research **11**: 255-267.

Knox, G.A. 1953. The intertidal ecology of Taylor's Mistake, Banks Peninsula. Transactions of the Royal Society of New Zealand **81**: 189-220.

Krebs, R.A. and V. Loeschcke. 1994. Costs and benefits of activation of the heat-shock response in *Drosophila melanogaster*. Functional Ecology **8**: 730-737.

Kruczynski, W.L. 1974. Relationship between depth and occurrence of pea crabs, *Pinnotheres maculatus*, in blue mussels, *Mytilus edulis* in the vicinity of Woods Hole, Massachusetts. Chesapeake Science **15**: 167-169.

Lafferty, K.D. and A.M. Kuris. 1999. How environmental stress affects the impacts of parasites. Limnology and Oceanography **44:** 925-931.

Lafferty, K.D. and R.D. Holt. 2003. How should environmental stress affect the population dynamics of disease? Ecology Letters **6**: 654-664.

Lamare, M.D. and J. Hoffman. 2004. Natural variation of carotenoids in the eggs and gonads of the echinoid genus, *Strongylocentrotus*: implications for their role in ultraviolet radiation protection. Journal of Experimental Marine Biology and Ecology **312**: 215-233.

Leslie, H.M., E.N. Breck, J. Lubchenco, and B.A. Menge. 2005. Barnacle reproductive hotspots linked to nearshore ocean conditions. Proceedings of the National Academy of Sciences USA **102**(30): 10534-10539.

Levinton, J.S. and R.K. Koehn. 1976. Population genetics of mussels. Pages 357-384 *in* B.L. Bayne, editor. Marine mussels, their ecology and physiology. Cambridge University Press, Cambridge, UK.

Levinton, J.S. and T.H. Suchanek. 1978. Geographic variation, niche breadth and genetic differentiation at different geographic scales in the mussels *Mytilus californianus* and *M. edulis*. Marine Biology **49**(4): 363-375.

Liaaen-Jensen, S. 1991. Marine carotenoids: recent progress. Pure and Applied Chemistry **63**(1): 1-12.

Liñán-Cabello, M.A., R. Medina-Zendejas, M. Sánchez-Barajas, and A.M. Herrera. 2004. Effects of carotenoids and retinol in oocytes maturation of crayfish *Cherax quadrucarinatus*. Aquaculture Research **35**: 905-911.

Lindquist, S. 1986. The heat-shock response. Annual Review of Biochemistry **55**: 1151-1191.

Livingstone, D.R., P. Garcia Martinez, X. Michel, J.F. Narbonne, S. O'Hara, D.

Ribera, and G.W. Winston. 1990. Oxyradical production as a pollution-mediated mechanism of toxicity in the common mussel, *Mytilus edulis* L., and other molluscs. Functional Ecology **4**(3): 415-424.

Livingstone, D.R. 2003. Oxidative stress in aquatic organisms in relation to pollution and aquaculture. Revue de Médecine Vétérinaire **154**(6): 427-430.

Loosanoff, V.L. 1942. Shell movements of the edible mussel, *Mytilus edulis* (L.) in relation to temperature. Ecology **23**: 231-244.

Louda, M. and S.K. Collinge. 1992. Plant resistance to insect herbivores: a field test of the environmental stress hypothesis. Ecology **73**(1): 153-169.

Lubchenco, J. and B.A. Menge. 1978. Community development and persistence in a low rocky intertidal zone. Ecological Monographs **59**: 67-94.

Lubchenco, J. 1980. Algal zonation in the New England rocky intertidal community: an experimental analysis. Ecology **61**: 333-344.

Lubchenco, J. and J. Cubit. 1980. Heteromorphic life histories of certain marine algae as adaptations to variations in herbivory. Ecology **61**(3): 676-687.

Lubchenco, J., S.A. Navarrete, B.N. Tissot, and J.C. Castilla. 1993. Possible ecological responses to global climate change: nearshore benthic biota of northeastern Pacific coastal ecosystems. Pages 147-166 *in* H.A. Mooney, E.R. Fuentes, and B.I. Kronberg, editors. Earth System Responses to Global Change. Academic Press, San Diego, USA.

Luna, L.G. 1968. Manual of Histological Staining Methods of the Armed Forces Institute of Pathology. McGraw-Hill Book Co., New York, USA.

McClanahan, T.R. 1992. Epibenthic gastropods of the middle Florida Keys: the role of habitat and environmental stress on assemblage composition. Journal of Experimental Marine Biology and Ecology **160**(2): 169-190.

McMahon, B.R. 1988. Physiological responses to oxygen depletion in intertidal animals. American Zoologist **28**: 39-53.

Menge, B.A. and J.P. Sutherland. 1976. Species diversity gradients: synthesis of the roles of predation, competition, and temporal heterogeneity. The American Naturalist **110**(973): 351-369.

Menge, B.A. 1978a. Predation intensity in a rocky intertidal community.

Relation between predator foraging activity and environmental harshness. Oecologia **34**: 1-16.

Menge, B.A. 1978b. Predation intensity in a rocky intertidal community. Effect of algal canopy, wave action and desiccation on predator feeding rates. Oecologia **34**: 17-35.

Menge, B.A. and J.P. Sutherland. 1987. Community regulation: variation in disturbance, competition, and predation in relation to environmental stress and recruitment. The American Naturalist **130**(5): 730-757.

Menge, B.A. and T.M. Farrell. 1989. Community structure and interaction webs in shallow marine hard-bottom communities: tests of an environmental stress model. Advances in Ecological Research **19**: 189-262.

Menge, B.A. and A.M. Olson. 1990. Role of scale and environmental factors in regulation of community structure. Trends in Ecology and Evolution **5**(2): 52-57.

Menge, B.A., E.L. Berlow, C.A. Blanchette, S.A. Navarrete, and S.B. Yamada. 1994. The keystone species concept: variation in interaction strength in a rocky intertidal habitat. Ecological Monographs **64**: 249-286.

Menge, B.A., B.A. Daley, P.A. Wheeler, E.P. Dahlhoff, E. Sanford, and P.T. Strub. 1997. Benthic-pelagic links and rocky intertidal communities: bottom-up effects on top-down control? Proceedings of the National Academy of Sciences USA **94**: 14530-14535.

Menge, B.A., B.A. Daley, J. Lubchenco, E. Sanford, E. Dahlhoff, P.M. Halpin, G. Hudson, and J.L. Burnaford. 1999. Top-down and bottom-up regulation of New Zealand rocky intertidal communities. Ecological Monographs **69**(3): 297-330.

Menge, B.A. 2000. Top-down and bottom-up community regulation in marine rocky intertidal habitats. Journal of Experimental Marine Biology and Ecology **250**: 257-289.

Menge, B.A., A.M. Olson, and E.P. Dahlhoff. 2002a. Environmental stress, bottom-up effects, and community dynamics: integrating molecular-physiological and ecological approaches. Integrative and Comparative Biology **42**: 892-908.

Menge, B.A., E. Sanford, B.A. Daley, T.L. Freidenburg, G. Hudson, and J.

Lubchenco. 2002b. Inter-hemispheric comparison of bottom-up effects on community structure: insights revealed using the comparative-experimental approach. Ecological Research **17**: 1-16.

Menge, B.A., C. Blanchette, P. Raimondi, T. Freidenberg, S. Gaines, J. Lubchenco, D. Lohse, G. Hudson, M. Foley, and J. Pamplin. 2004. Species interaction strength: testing model predictions along an upwelling gradient. Ecological Monographs **74**(4): 663-684.

Menge, B.A., Daley, B.A., Sanford, E., Dahlhoff, E.P., Lubchenco, J. In press. Mussel zonation in New Zealand: integrative ecophysiological approach. Marine Ecology Progress Series.

Michalek-Wagner, K. and B.L. Willis. 2001. Impacts of bleaching on the soft coral *Lobophytum compactum*. I. Fecundity, fertilization and offspring viability. Coral Reefs **19**: 231-239.

Miki, W., K. Yamaguchi, and S. Konosu. 1982. Comparison of carotenoids in the ovaries of marine fish and shellfish. Comparative Biochemistry and Physiology Part B: **71**: 7-11.

Miki, W. 1991. Biological functions and activities of animal carotenoids. Pure and Applied Chemistry **63**(1): 141-146.

Moburg, G.P. 1991. How behavioral stress disrupts the endocrine control of reproduction in domestic animals. Journal of Dairy Science **74**(1): 304-311.

Moeller, S.M., P.F. Jacques, and J.B. Blumberg. 2000. The potential role of dietary xanthophylls in cataract and age-related macular degeneration. Journal of the American College of Nutrition **19**: 522S-527S.

Moore, I.T. and T.S. Jessop. 2003. Stress, reproduction, and adrenocortical modulation in amphibians and reptiles. Hormones and Behavior **43**: 39-47.

Musgrave, M.A., S.P. Gould, and R.F. Ablett. 1987. Enzymatic lipid peroxidation in the gonadal and hepatopancreatic microsomal fraction of cultivated mussels (*Mytilus edulis* L.). Journal of Food Science **52**(3): 609-612.

NASA. 2006. 2006 was Earth's fifth warmest year. http://www.nasa.gov/vision/earth/environment/2006_warm.html, accessed on March 20, 2007.

NOAA. 2006. National Weather Service Forecast Office, Portland, OR. Unique local climate data. http://www.weather.gov/climate/local_data.php?wfo=pqr, accessed on Apr. 4, 2007.

NIWA. 2004. New Zealand national climate summary – the year 2004. http://www.niwascience.co.nz/ncc/cs/aclimsum_04.pdf/view_pdf, accessed on Dec. 2, 2006.

NIWA. 2005. New Zealand national climate summary – the year 2005. http://www.niwascience.co.nz/ncc/cs/aclimsum_05.pdf/view_pdf, accessed on Dec. 2, 2006.

O'Beirn, F.X. and R.L. Walker. 1999. Pea crab, *Pinnotheres ostreum* Say, 1817, in the eastern oyster *Crassostrea virginica* (Gmelin, 1791): prevalence and apparent adverse effects on oyster gonad development. The Veliger **42**(1): 17-20.

Obermüller, B., U. Karsten, and D. Abele. 2005. Response of oxidative stress parameters and sunscreening compounds in Arctic amphipods during experimental exposure to maximal natural UVB radiation. Journal of Experimental Marine Biology and Ecology **323**: 100-117.

Ots, I. And P. Hőrak. 1996. Great tits *Parus major* trade health for reproduction. Biological Sciences **263**(1376): 1443-1447.

Paine, R.T. 1966. Food web complexity and species diversity. The American Naturalist **100**: 65-75.

Paine, R.T. 1974. Intertidal community structure: experimental studies on the relationship between a dominant competitor and its principal predator. Oecologia **15**: 93-120.

Paparo, A.A. and J.A. Murphy. 1978. Cilioexcitatory and cilioinhibitory processes initiated by light on two identifiable neuronal pigments in *Mytilus edulis*. The Journal of Experimental Biology **76**: 47-61.

Parmesan, C. and G. Yohe. 2003. A globally coherent fingerprint of climate change impact across natural systems. Nature **421**: 37-42.

Petes, L.E., C.D. Harvell, E.C. Peters, M.A.H. Webb, and K.M. Mullen. 2003. Pathogens induce melanization and compromise reproduction in Caribbean sea fans. Marine Ecology Progress Series **264**: 167-171.

Philippart, C.J.M., H.M. van Aken, J.J. Beukema, O.G. Bos, G.C. Cadée, and R.

Dekker. 2003. Climate-related changes in recruitment of the bivalve *Macoma balthica*. Limnology and Oceanography **48**(6): 2171-2185.

Quinn, G.P. and M.J. Keough. 2002. Experimental design and data analysis for biologists. Cambridge University Press, Cambridge, UK.

Roberts, D.A., G.E. Hofmann, and G.N. Somero. 1997. Heat-Shock protein expression in *Mytilus californianus*: acclimatization (seasonal and tidal-height comparisons) and acclimation effects. Biological Bulletin **192**: 309-320.

Rodhouse, P.G., C.M. Roden, M.P. Hensey, and T.H. Ryan. 1985. Production of mussels, *Mytilus edulis*, in suspended culture and estimates of carbon and nitrogen flow: Killary Harbour, Ireland. Journal of the Marine Biological Association of the United Kingdom **65**: 55-68.

Roff, D.A. 1992. Evolution of life histories: theory and analysis. Chapman & Hall, New York, USA.

Sagarin, R.D., J.P. Barry, S.E. Gilman, and C.H. Baxter. 1999. Climate-related change in an intertidal community over short and long time scales. Ecological Monographs **69**(4): 465-490.

Sanford, E. 1999. Regulation of keystone predation by small changes in ocean temperature. Science **283**: 2095-2097.

Sastry, A.N. 1966. Temperature effects in reproduction of the Bay scallop, *Aequipecten irradians* Lamarck. Biological Bulletin **130**: 118-134.

Sastry, A.N. 1979. Pelecypoda (excluding Ostreidae). Pages 113-292 *in* A.C. Giese and J.S. Pearse, editors. Reproduction of marine invertebrates, Vol.5. Academic Press, New York, USA.

Scheer, B.T. 1940. Some features of the metabolism of the carotenoid pigments in the California sea mussel (*Mytilus californianus*). Journal of Biological Chemistry **136**: 275-299.

Schiel, D.R., J.R. Steinbeck, and M.S. Foster. 2004. Ten years of induced ocean warming causes comprehensive changes in marine benthic communities. Ecology **85**: 1833-1839.

Schreck, C.B., W. Contreras-Sanchez, and M.S. Fitzpatrick. 2001. Effects of stress on fish reproduction, gamete quality, and progeny. Aquaculture **197**: 3-24.

Seed, R. 1976. Ecology. Pages 13-65 *in* B.L. Bayne, editor. Marine mussels: their ecology and physiology. Cambridge University Press, Cambridge, UK.

Seed, R. and T.H. Suchanek. 1992. Population and community ecology of *Mytilus*. Pages 87-169 *in* E. Gosling, editor. The Mussel *Mytilus*: Ecology, Physiology, Genetics, and Culture. Elsevier Science Publ., Amsterdam, NL.

Shahidi, F., Metusalach, and J.A. Brown. 1998. Carotenoid pigments in seafoods and aquaculture. Critical Reviews in Food Science **38**(1): 1-67.

Siefermann-Harms, D. 1987. The light-harvesting and protective function of carotenoids in photosynthetic membranes. Physiologia Plantarum **69**: 561-568.

Smith, J.R., P. Fong, and R.F. Ambrose. 2006. Dramatic declines in mussel bed community diversity: response to climate change. Ecology **87**(5): 1153-1161.

Somero, G.N. 2002. Thermal physiology and vertical zonation of intertidal animals: optima, limits, and costs of living. Integrative and Comparative Biology **42**(4): 780-789.

Sørensen, J.G., T.N. Kristensen, and V. Loeschcke. 2003. The evolutionary and ecological role of heat shock proteins. Ecology Letters **6**(11): 1025-1037.

Sorte, C.J.B. and G.E. Hofmann. 2005. Thermotolerance and heat-shock protein expression in Northeastern Pacific *Nucella* species with different biogeographical ranges. Marine Biology **146**(5): 985-993.

Stearns, S.C. 1992. The evolution of life histories. Oxford University Press, Oxford, UK.

Stevens, P.M. 1990. Specificity of host recognition of individuals from different host races of symbiotic pea crabs (Decapoda: Pinnotheridae). Journal of Experimental Marine Biology and Ecology **143**(3): 193-207.

Stillman, J.H. and G.N. Somero. 2000. A comparative analysis of the upper thermal tolerance limits of eastern Pacific porcelain crabs, Genus *Petrolisthes*: influences of latitude, vertical zonation, acclimation, and phylogeny. Physiological and Biochemical Zoology **73**: 200-208.

Suchanek, R. 1981. The role of disturbance in the evolution of life history strategies in the intertidal mussels *Mytilus edulis* and *Mytilus californianus*. Oecologia **50**: 143-152.

Sukhotin, A.A., D. Abele, and H.-O. Portner. 2002. Growth, metabolism and lipid peroxidation in *Mytilus edulis*: age and size effects. Marine Ecology Progress Series **226**: 223-234.

Tablado, A. and J.L. Gappa. 1995. Host-parasite relationships between the mussel, *Mytilus edulis* L., and the pea crab, *Tumidotheres maculates* (Say), in the southwestern Atlantic. Journal of Shellfish Research **14**(2): 417-423.

Tomanek, L. 2002. The heat-shock response: its variation, regulation and ecological importance in intertidal gastropods (Genus *Tegula*). Integrative and Comparative Biology **42**(4): 797-807.

Tomanek, L. and E. Sanford. 2003. Heat-shock protein 70 (Hsp70) as a biochemical stress indicator: an experimental field test in two congeneric intertidal gastropods (Genus: Tegula). Biological Bulletin **205**: 276-284.

Trowbridge, C.D. 1998. Stenophagous, herbivorous, sea slugs attack desiccation-prone, green algal hosts (*Codium* spp.): indirect evidence of prey-stress models (PSMs)? Journal of Experimental Marine Biology and Ecology **230**(1): 31-53.

Tsuchiya, M. 1983. Mass mortality in a population of the mussel *Mytilus edulis* L. caused by high temperature on rocky shores. Journal of Experimental Marine Biology and Ecology **66**(2): 101-111.

Viarengo, A., L. Canesi, M. Pertica, D.R. Livingstone, and M. Orunesu. 1991. Age-related lipid peroxidation in the digestive gland of mussels: the role of the antioxidant defence systems. Experientia **47**: 454-457.

Videla, L.A., V. Fernandez, and A. Valenzuela. 1987. Age-dependent changes in rat liver lipid peroxidation and glutathione content induced by acute ethanol ingestion. Cell Biochemistry and Function **5**(4): 273-280.

Walther, G.-R., E. Post, P. Convey, A. Menzel, C. Parmesan, T.J.C. Beebee, J.M. Fromentin, O. Hoegh-Guldberg, and F. Bairlein. 2002. Ecological responses to recent climate change. Nature **416**: 389-395.

Wang, Y., A.B. Salmon, and L.G. Harshman. 2001. A cost of reproduction: oxidative stress susceptibility is associated with increased egg production in *Drosophila melanogaster*. Experimental Gerontology **36**: 1349-1359.

Webb, M.A.H., J.P. Van Eenennaam, S.I. Doroshov, and G.P. Moberg. 1999. Preliminary observations on the effects of holding temperature on reproductive performance of female white sturgeon, *Acipenser transmontanus* Richardson.

Welshmeyer, N.A. 1994. Fluorometric analysis of chlorophyll-*a* in the presence of chlorophyll-*b* and pheopigments. Limnology and Oceanography **39**: 1985-1992.

Widdows, J., B.L. Bayne, D.R. Livingstone, R.I.E. Newell, and P. Donkin. 1979. Physiological and biochemical responses of bivalve molluscs exposed to air. Comparative Biochemistry and Physiology **62**A: 301-308.

Williams, G.C. 1966. Natural selection, the cost of reproduction and a refinement of Lack's principle. The American Naturalist **100**: 687-690.

Wingfield, J.C. and R.M. Sapolsky. 2003. Reproduction and resistance to stress: when and how. Journal of Neuroendocrinology **15**: 711-724.

Woodall, A.A., G. Britton, and M.J. Jackson. 1996. Dietary supplementation with carotenoids: effects on α-tocopherol levels and susceptibility of tissues to oxidative stress. British Journal of Nutrition **76**: 307-317.

APPENDICES

APPENDIX A. Supplementary Materials, Chapter 4

Fig. A1. Representative colors of mussel gonadal tissue for field color scoring system.

Fig. A2. Photograph of male mussel from the high edge of the mussel bed exhibiting bright orange gonadal tissue. Photo courtesy of J. Lubchenco.

APPENDIX B. Supplementary Materials, Chapter 5

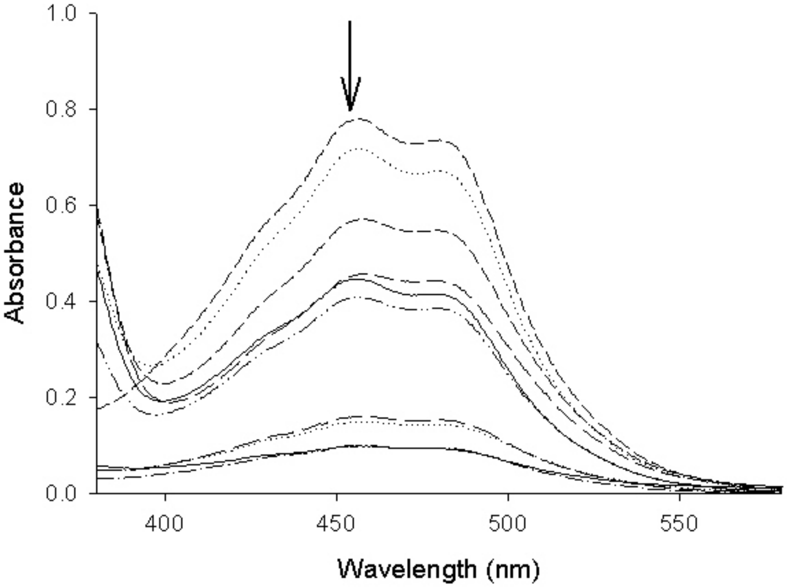

Fig. B1. Representative spectra of gonadal tissue from individual mussels. Arrow indicates wavelength (452 nm) at which analyses were conducted.

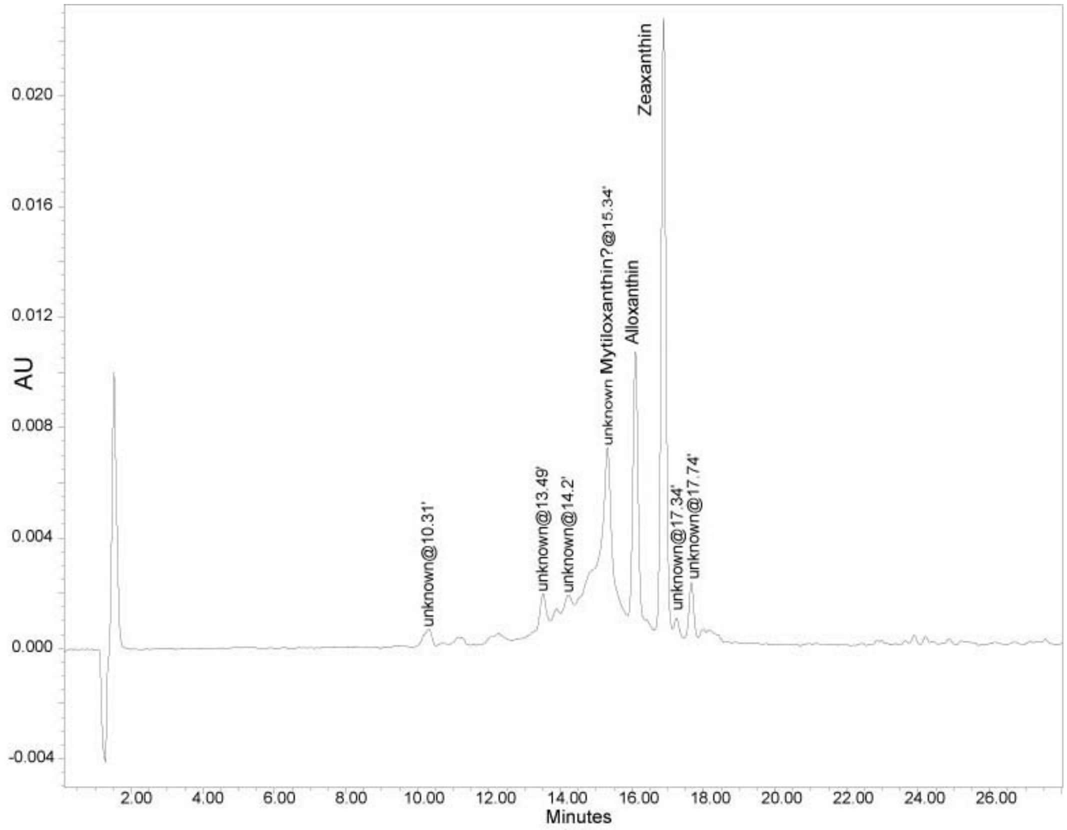

Fig. B2. Example of chromatogram from HPLC analyses of mussel gonadal tissue.