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INTERTIDAL MUSSELS EXHIBIT ENERGETIC TRADE-OFFS BETWEEN REPRODUCTION AND STRESS RESISTANCE

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Abstract. Life-history theory suggests that trade-offs exist between fitness components, with organisms balancing investment in reproduction against survival and future reproduction. This study examined the influence of stress on physiological trade-offs in the dominant rocky intertidal mussel *Mytilus californianus* on the central Oregon coast, USA. The intertidal zone is a highly heterogeneous thermal environment that could lead to intrapopulation variation in stress responses. Stress 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. Reproduction and carotenoid content were compared in mussels from the low and high vertical edges of the mussel bed. High-edge mussels invested less relative energy in reproduction and also spawned all of their gametes in the early summer, whereas low-edge mussels continuously spawned small batches of gametes throughout the year. High-edge mussels accumulated high concentrations of carotenoid pigments into their gonadal tissues, potentially to protect gametes from damaging oxidative stress experienced during aerial exposure. A reciprocal transplant experiment revealed plastic responses in growth and reproduction to increased stress. In contrast, carotenoid content did not increase in response to stress, suggesting that carotenoids may not change rapidly or may not be easily lost or gained. Our results indicate that mussels exhibit physiological trade-offs and, under increased stress predicted from climate change scenarios, may allocate energy away from reproduction toward costly physiological defenses.

Key words: *carotenoid; energy trade-offs;* Mytilus californianus; *Oregon coast, USA; oxidative stress; reproduction; rocky intertidal zone.*

Introduction

Life-history theory indicates that physiological trade-offs exist in all organisms when resources are limited, because each individual has a certain amount of energy available to maintain physiological processes, such as growth, reproduction, metabolism, and immune function (e.g., Roff 1992, Stearns 1992). Brett (1958:81) defined a physiological stress state as being induced "by any environmental factor which extends the normal adaptive responses of an animal, or which disturbs the normal functioning to such an extent that the chances of survival are significantly reduced." Here we define stress as any environmental factor (e.g., temperature, desiccation) leading to a physiological stress state. When a stress state occurs, organisms must reallocate energy away from these processes toward defense and repair mechanisms in order to survive. The cost of reproduction is the most prominent life-history trade-off, because costs are paid in terms of survival and future reproduction (Stearns 1992). Reproduction is energetically expensive (Williams 1966), and reproductive processes may therefore be compromised under stressful conditions in an attempt to devote more energy toward 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. 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 Hörak 1996), amphibians and reptiles (e.g., Moore and Jessop 2003), and mammals (e.g., Moburg 1991). While there have been studies investigating the effects of stress on the physiology of marine invertebrates (e.g., Helmuth and Hofmann 2001, Sukhotin et al. 2002), the manner in which these consequences translate into life-history trade-offs remains poorly understood.

All organisms living in the intertidal 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 (e.g., Menge and Sutherland 1987) increases along a vertical gradient in the rocky intertidal zone, with relatively low stress in the low intertidal zone and relatively high stress in the high intertidal zone, as organisms in the high intertidal zone are exposed to aerial conditions for a longer period of time at low tide (Connell 1972). Stress in the high intertidal zone is the result of both decreased food

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availability and increased exposure to aerial temperature and desiccation at low tide. Intertidal organisms living at their upper limit exhibit physiological consequences of exposure to stress, such as reduced growth (Menge et al. 2002), increased heat shock protein production (Halpin et al. 2004), and mortality (Tsuchiya 1983, Petes et al. 2007). Extreme low tides ("spring" tides) occur on the Oregon coast, USA, during daylight hours in the spring and summer months and can therefore lead to high thermal and desiccation stress (Helmuth et al. 2002). In all organisms, harmful reactive oxygen species (ROS) are generated as by-products of normal food oxidation and metabolism (Livingstone 2003). Aerial exposure in the intertidal zone can cause oxidative stress, because oxygen metabolism increases under high temperature and desiccation, leading to increased production of ROS (Livingstone 2003), which can cause damage to DNA, proteins, carbohydrates, and lipids (e.g., Di Mascio et al. 1991). However, the effects of oxidative stress are relatively unstudied in intertidal systems.

Mussels are the dominant competitors for space in many temperate rocky intertidal systems around the world (e.g., Paine 1966, 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 abiotic environment, as this area is exposed to air for relatively short periods of time at low tide, whereas the upper edge is an area of relatively high stress due to potentially long periods of aerial exposure (Seed and Suchanek 1992, Davenport and Davenport 2005). Because they are sedentary, 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), 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) because of 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 their reproduction (Sastry 1966, 1979).

A previous study conducted on the central Oregon coast (L. E. Petes, B. A. Menge, F. Chan, and M. A. H. Webb, *unpublished manuscript*) discovered that a majority of *M. californianus* in the stressful high edge of the mussel bed had brightly pigmented orange gonadal tissue, possibly due to the presence of carotenoid pigments. Carotenoid pigments are known for their critical role in defense against oxidative stress, because they 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). All 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 are known to affect the concentration of carotenoids present in mussel gonads (Scheer 1940, Campbell 1969, 1970), but the effects of stress on carotenoid content in intertidal mussels are relatively unknown. In addition to carotenoids, other low-molecular-mass scavengers and specific antioxidant enzymes, including superoxide dismutase (SOD), defend organisms from free radicals (Livingstone 2003). Superoxide dismutase scavenges superoxide anion radicals ($O_2^{\cdot-}$) and converts them into hydrogen peroxide (H_2O_2; Halliwell and Gutteridge 1999).

Petes et al. (L. E. Petes, B. A. Menge, F. Chan, and M. A. H. Webb, *unpublished manuscript*) 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. They 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. While high concentrations of carotenoid pigments had been previously documented in female mussels (Campbell 1969, 1970), orange tissue in males had never been seen before. The question remains whether orange coloration in males is due to the presence of carotenoids and whether this pattern is consistent across spawning seasonality. It also has yet to be determined whether these sessile animals exhibit plastic physiological trade-offs when transplanted into a high-stress environment, investing less relative energy into growth and reproduction and more energy into defense.

The purpose of this study was to evaluate the manner in which stress affects physiological trade-offs between defense and reproduction in intertidal mussels. The following hypotheses were tested in the Oregon intertidal zone: (1) Mussels in high-stress environments and mussels with lower food availability (fewer resources) invest relatively less energy into reproduction. (2) Mussels in high-stress environments and mussels with higher food availability (more resources) invest relatively more energy into stress resistance (carotenoids and SOD). (3) Mussel growth, reproduction, and carotenoid content respond plastically to stress.

Materials and Methods

To determine whether life-history trade-offs occur in mussels and to evaluate how reproduction and stress

resistance vary with stress and availability of resources (food), a variety of both environmental and biological parameters were quantified.

Field sites for surveys of reproduction and carotenoid content

Two capes on the central Oregon coast have differing oceanographic conditions and coastal shelf topography that affect primary productivity, leading to differential food abundance and juvenile recruitment success for intertidal organisms (e.g., Menge et al. 2002, 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), whereas Cape Foulweather (~80 km north) has relatively low phytoplankton abundance (e.g., Menge et al. 2002, 2004). Stress (temperature), food (chlorophyll *a*), carotenoid pigments, and mussel reproduction were investigated at two sites: Fogarty Creek (FC; 44°50′24″ N, 124°3′36″ W) on Cape Foulweather and Strawberry Hill (SH; 44°15′0″ N, 124°7′12″ W) on Cape Perpetua. These two intertidal sites have similar wave exposure and topography.

Temperature recording

To measure seasonality of temperature throughout the year, one TidbiT temperature logger (Onset Computer, Pocahassett, Massachusetts, USA) was deployed in the middle of the mussel bed (mid-zone) at both FC and SH. These loggers recorded continuous temperature every 2 min from 1 May 2005 to 31 July 2006. Two breaks in continuous recording occurred at SH between 27 May and 24 June and between 18 September and 17 October 2005 due to equipment failure. Thermal data during these two time periods were therefore unavailable for SH. While these unmodified loggers do not accurately measure organismal temperature (Fitzhenry et al. 2004), seasonal patterns of aerial and aquatic temperature can be determined.

To quantify the differences in mussel body temperature between edges of the mussel bed, two "Robomussels" (mussel mimics consisting of a TidbiT logger embedded within epoxy and resin; see Helmuth and Hofmann 2001) were deployed each at the low and high edges of the mussel bed on 15 November 2005 at FC. These temperature loggers accurately simulate mussel body temperature to within 2°C (Fitzhenry et al. 2004). The loggers approximated body temperature (underwater or when exposed to air depending on the tidal cycle) every 10 min for 225 d until 27 June 2006. The battery of one logger in the low edge failed on 27 March 2006, and data after this date were therefore only available from one low-edge logger.

Chlorophyll a *measurements*

To quantify the food source for mussels, phytoplankton concentration was estimated using 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 250-mL bottle samples ($n = 3$) of water were taken from shore, and an aliquot of 50 mL from each sample was filtered in the field through combusted Whatman GF/F glass fiber filters. Chlorophyll *a* concentration of samples was determined using a Turner Designs TD-700 fluorometer (Turner Designs, Sunnyvale, California, USA) according to Welshmeyer (1994). The fluorometer was calibrated with a chl *a* standard from Sigma Chemical Company (St. Louis, Missouri, USA).

Field surveys

To evaluate how reproductive condition varied between sexes, edges, and sites through time, mussels were collected every other month at SH and FC from May 2005 to July 2006. Mussels 4–7 cm in length were collected from the high (approximately +2 m mean lower low water [MLLW]) and low (approximately +1 m MLLW) edges of the mussel bed (n = 15–50 per collection from each edge) and were dissected in the field. To determine whether gonadal color is an accurate measure of carotenoid content, tissue color was scored visually on a 1–3 system with 1 = white, 2 = peach, and 3 = orange. To assess sex and stage of maturity using microscopy, gonadal tissue was removed and fixed in 10% formalin in seawater for histological processing.

Histological analyses of sex and stage of maturity

To evaluate 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 (Leica DMLS, Leica Microsystems, Bannockburn, Illinois, USA) at 100–400×. Sex (male or female) was identified on each slide, and stage of maturity was assigned based on the categories listed in Table 1. Stages of maturity from 1 to 4 are only found in juvenile mussels that have never spawned, and all mussels collected in this study were adults (>4 cm in length) in stages 5–8. Mussels can progress from stage 5 (ripe) through stage 8 by spawning, then back through stages 7 and 6 by maturation to stage 5 again. It is impossible to distinguish mussels that have partially spawned (stage 6) or mostly spawned (stage 7) from mussels that are maturing again after spawning within a given time point, and therefore stages 6 and 7 could be the result of one of two alternatives (initiated spawning or maturing after spawning). However, by examining stages of maturity within the population over an annual cycle, spawning peaks can be identified.

To determine whether stage of maturity represents loss of gametes through spawning, oocyte counts were performed on female slides from the July and November 2005 collections. Five haphazard oocyte counts were performed in a 0.0676-mm^2 area and averaged for each slide. Male slides would have been too difficult to

TABLE 1. Stages of reproductive maturity in female and male mussels (*Mytilus californianus*) assessed from histological slides of gonadal tissue.

Stage	Females	Males
1	immature (no gametes)	immature (no gametes)
2	pre-vitellogenic: only oogonia present; no evidence of prior spawning	pre-meiotic: only spermatogonia present; no evidence of prior spawning
3	early vitellogenesis: few yolk platelets in primary oocytes; no evidence of prior spawning	early meiosis: spermatogonia and primary spermatocytes; no evidence of prior spawning
4	mid-vitellogenesis: more yolk platelets accumulated, germinal vesicle central; no evidence of prior spawning	mid-meiosis: spermatogonia, primary and secondary spermatocytes, spermatids; no evidence of prior spawning
5	ripe: oocytes full of yolk, germinal vesicle migrating toward the animal pole	ripe: all of the above plus spermatozoa
6	a) early ovulation: some spawning activity indicated by presence of empty follicles b) returning to ripe through vitellogenesis after spawning	a) early spermiation: some spawning activity, some spermatozoa missing b) returning to ripe through meiosis after spawning
7	a) mid-ovulation: few ripe oocytes, many empty follicles present from previous spawning b) initiating vitellogenesis again after spawning	a) late spermiation: mostly spawned, few spermatozoa remaining b) initiating meiotic divisions again after spawning
8	post-ovulation: spawned-out, all follicles empty	post-spermiation: spawned-out, gonadal tissue empty of ripe gametes

quantify given the variable shapes and orientations of cells.

Gonadosomatic index (GSI)

To determine how stress influences relative energy allocation toward reproduction, beginning in September 2005, gonadal and somatic tissues were excised from the mussels, and wet tissues were weighed prior to gonadal tissue fixation to develop a gonadosomatic index (GSI; e.g., Roff 1992):

$$\text{GSI} = \frac{\text{gonadal tissue mass}}{\text{gonadal} + \text{somatic tissue mass}}.$$

The relationship between wet and dry GSI has been calculated for this mussel species and is strongly linear (L. E. Petes, *unpublished data*). In addition, drying gonads would have damaged tissue for histological preservation.

During every other collection (July 2005, November 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 and the other half was flash-frozen in liquid nitrogen for transport back to a −80°C freezer in Corvallis, Oregon, USA, 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 assays (described in *Superoxide dismutase assays*).

Carotenoid pigment analyses

To determine whether tissue color is representative of carotenoid content and to investigate how carotenoid content varies with sex and stress, gonadal tissues (0.04–0.20 g) were thawed on ice and subsequently homogenized in 2 mL 90% high-pressure liquid chromatography (HPLC)-grade acetone. The volume of acetone was then brought up to 4 mL. Samples were extracted at −20°C for 3 h prior to reading pigment levels. Tubes were centrifuged at 5000 rpm (4193 gravities [*g*] for 15-cm rotor radius) for 5 min. 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, Maryland, USA). Spectral scans were performed from 380 to 580 nm (see Appendix A for representative spectra). Carotenoid levels were corrected by tissue mass, and the maximum absorbance for each sample was selected for analyses.

To identify 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 both spectrophotometry and HPLC. Tissue samples were divided in half, such that one-half was analyzed using spectrophotometry and the second half was homogenized as described for spectrophotometry and stored at −20°C overnight for pigment extraction prior to HPLC. Samples were analyzed with reversed-phase HPLC using a Waters Alliance 2690 separation module (Waters Corporation, Milford, Massachusetts, USA) equipped with an Allsphere C8 3-μm column with a C8 Allsphere guard column (Alltech, Deerfield, Illinois, USA). Solvent A consisted of 75% 0.5 mol/L 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 ~25 min using the following gradient from 100%

A to 100% B at a flow rate of 1 mL/min ([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 from previous runs on the same machine in addition to published retention times and spectra (Jeffrey et al. 1997) that had used the same solvent mixtures.

Superoxide dismutase assays

To determine how stress affects antioxidant enzyme activity, mussel gonadal tissues were thawed and homogenized in a Tris-HCl buffer (0.25 mol/L sucrose, 10 mmol/L Tris, 1mmol/L EDTA, pH 7.5) at a mass : volume ratio of 1:4. Samples were cold-centrifuged at 10 000 *g* for 15 min at 4°C. The supernatant was decanted, stored at −80°C, 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, Illinois, USA). Superoxide dismutase (EC 1.15.1.1) levels were assayed using a kit (S311-10) from Dojindo Molecular Technologies (Gaithersburg, Maryland, USA) according to the 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 (Sigma-Aldrich S2515, St. Louis, Missouri, 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 whether mussel growth, reproduction, and carotenoid content respond plastically to stress, a field transplant experiment was conducted at SH in summer 2004. Mussels 5–8 cm in length were haphazardly collected from the low and high edges of the mussel bed on 9 April 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 (in millimeters per day) was calculated as

$$\text{growth rate} = \frac{\text{new shell growth/initial length}}{\text{no. days since beginning of experiment}}.$$

Mussels were transplanted into plots of 40 individuals in three transplant treatments: (1) within-edge transplants, wherein mussels were transplanted within an edge, low to low ("LL") edge and high to high ("HH") edge; (2) between-edge transplants, wherein mussels were transplanted between edges, low to high ("LH") edge and high to low ("HL") edge; or (3) in situ, in which mussels were marked and not transplanted to control for potential effects of transplantation stress. There were $n = 4$ replicates per treatment in each edge for a total of 24 experimental plots. Mussels were placed ventral-side down and covered with plastic mesh for byssal thread attachment according to Menge et al. (2004), and the mesh was removed after six weeks.

Three mussels were removed from each plot monthly from May to August 2004. These mussels were measured for shell growth and were subsequently dissected. Gonadosomatic index 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

All analyses were performed using JMP 6.0 (SAS Institute, Cary, North Carolina, USA) statistical software. Field surveys were analyzed using four-factor analysis of variance (ANOVA), with month, edge, site, sex, and all interactions as explanatory variables, and GSI and carotenoid content (from spectrophotometry) as separate response variables. 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 honestly significant difference (HSD) tests based on least square means estimated from main effects or interaction terms at $P < 0.05$. For stage of maturity, due to the ordinal nature of the data, ordinal logistic regression was performed with the same explanatory variables as for the four-factor ANOVA.

Several regression analyses were performed to determine whether linear relationships existed in the data. The relationship between stage of maturity and number of oocytes was examined for data pooled across the two time points and both edges. The relationship between color (assessed visually) 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 examined to determine whether mussels that are "spawned-out" have lower GSI than those that are ripe. A regression was also performed separately for females and males to determine the relationship between stage of maturity and carotenoid content.

The HPLC data were analyzed using two-factor ANOVA with sex and edge as explanatory variables and each of the three major carotenoid pigments as separate response variables. The linear relationship between absorbance from spectrophotometry and relative carotenoid values from HPLC was examined for each pigment.

The SOD data were analyzed with one-way ANOVA using edge as the explanatory variable and SOD and carotenoid content as separate response variables. The linear relationship between SOD and carotenoids was then examined.

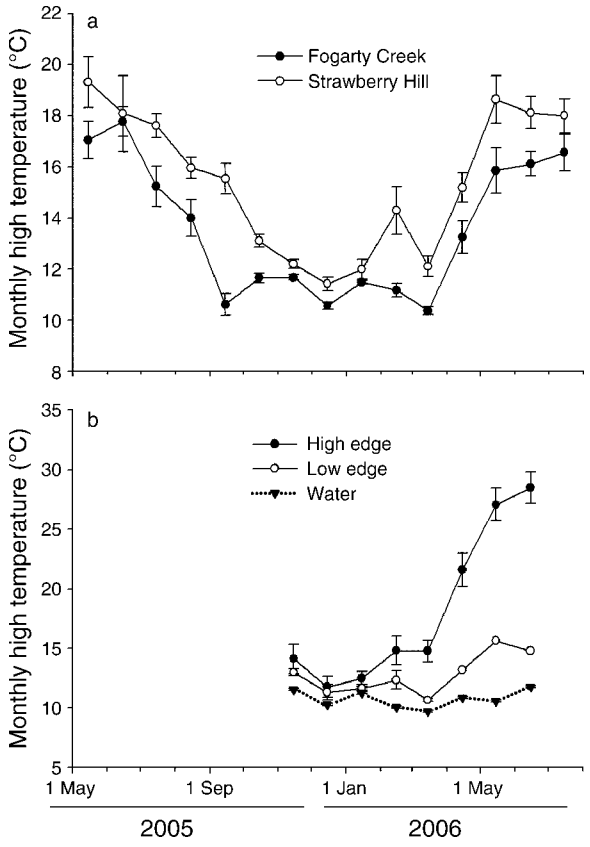

FIG. 1. (a) Monthly high temperature (mean ± SE) in the mid-zone at Fogarty Creek and Strawberry Hill on the Oregon, USA, coast based on daily high temperatures. Error bars are standard errors of daily high temperatures. (b) Monthly high temperature (mean ± SE) at the high and low edges of the mussel bed based on means of Robomussel daily high temperatures. Aerial temperatures (solid lines) are compared to water temperatures (dotted line). Error bars are standard error of two temperature loggers. See *Materials and methods: Temperature recording* for a description of the Robomussel.

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-related mortality); and (3) within the high edge across all treatments and time points. While we acknowledge that stage of maturity data are more appropriately treated as ordinal, given that the experimental unit (one plot) was an average of stages of several individuals, stage data were treated as continuous for the purpose of these analyses. Growth rate, GSI, stage of maturity, and carotenoid pigments were examined as separate response variables. No growth rate or GSI information was collected in May, and this time point was therefore omitted for these two parameters. For these parameters in analysis 2, data were analyzed for the second time point (June) as an ANOVA.

Because strong sex-specific differences in carotenoid content were discovered, females and males were also examined separately by averaging carotenoid levels of the zero to three individuals of each sex per plot. An RM-MANOVA analysis was not possible with the resulting degrees of freedom, and May and June were therefore analyzed separately for sex, treatment, and sex × treatment interactions. While we realize 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 mean high temperatures were highest in the late spring to early summer (May–June 2005) at both FC and SH due to seasonal changes in ambient temperature and tidal cycle (Fig. 1a). Temperatures decreased and reached a minimum in December 2005 before steadily increasing to reach a second maximum in May 2006. Both sites followed this same pattern through time, although SH temperatures were higher on average than FC temperatures. Mean monthly high mussel body temperatures (approximated by Robomussels) were up to twice as high at the high edge than at the low edge of the mussel bed (Fig. 1b), and daily temperatures at the high edge of the mussel bed reached 30°C 27 times from 15 April to 27 June 2006, compared to only one 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 (17–24 June 2006). A previous study at SH documented that costly heat shock protein production is induced in mussels at temperatures above 30°C (Halpin et al. 2004). In addition, Oregon mussels produce the highest heat shock protein levels of mussels on the entire West Coast from Baja California through British Columbia (Sagarin and Somero 2006), corresponding with the timing of peak summer daytime emersion in Oregon (Helmuth et al. 2002). Our data therefore indicate that high-edge mussels are frequently exposed to thermal stress.

Chlorophyll a

The chl *a* concentration was higher on average during the summer at SH than FC (Appendix B), consistent with previous studies in this area (e.g., Menge et al. 2002, 2004).

Stress and food effects on energy allocation toward reproduction: field surveys

Several two-way and three-way interactions were significant with time (month; Appendices C and D).

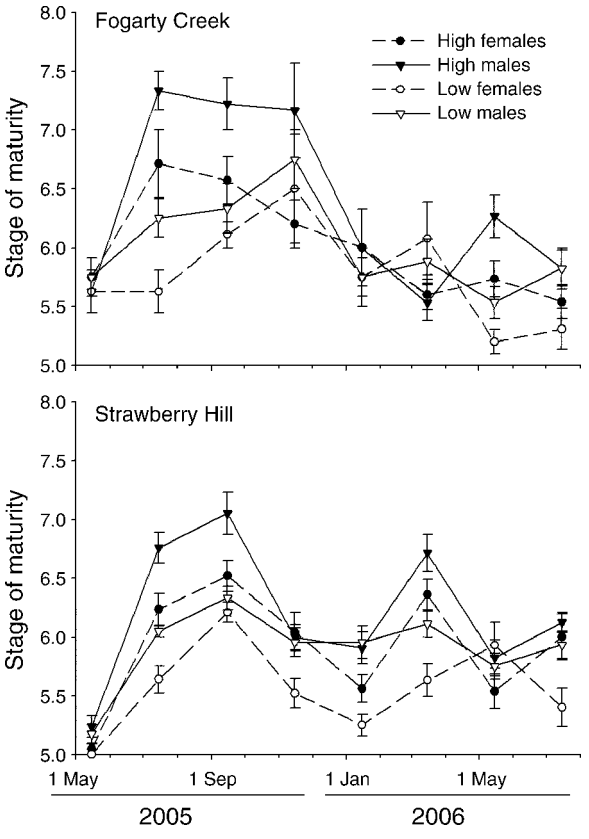

FIG. 2. Stage of maturity (mean ± SE) of mussels (*Mytilus californianus*) collected from high and low edges of the mussel beds at Fogarty Creek and Strawberry Hill in May 2005–July 2006. Values along the *y*-axis correspond to the stages in Table 1.

For our purposes, we will focus on the interactions that appeared the most biologically relevant.

Stage of maturity varied between both sites and edges over time (Fig. 2). Spawning events occurred during different times at SH than at FC, revealing a relationship between site and spawning time (Wald's $\chi^2 = 29.40$, $P = 0.0001$; Fig. 2, Appendix C). Overall, spawning occurred asynchronously between the high-edge and the low-edge mussels (Wald's $\chi^2 = 22.76$, $P = 0.002$). High-edge mussels at SH appeared to show two spawning pulses, one in July–September 2005 and one in March 2006, whereas high-edge mussels at FC only spawned during the first pulse and remained spawned-out for a longer period of time, potentially indicating failure to generate new gametes (Wald's $\chi^2 = 18.16$, $P = 0.001$). Average number of oocytes decreased with stage of maturity ($F_{1,30} = 21.68$, $P < 0.0001$, $R^2 = 0.42$), showing that increasing stage of maturity likely represents loss of gametes with spawning (average oocyte count = 20.80 − 2.311(stage of maturity)).

GSI fluctuated with time and varied between sites and edges (Fig. 3). Low-edge GSI decreased between September (at FC) or November (at SH) 2005 and March 2006, then increased through July 2006. In contrast, high-edge GSI increased between September (at SH) or November (at FC) 2005 and January 2006, then decreased at SH or fluctuated with no clear pattern at FC ($F_{5,240} = 4.59$, $P = 0.0005$; Fig. 3, Appendix D). GSI was higher overall at SH than at FC ($F_{1,240} = 21.13$, $P < 0.0001$), indicating more relative energy allocation toward reproduction at this site. GSI was higher overall in the low-edge mussels than in the high-edge mussels ($F_{1,240} = 65.55$, $P < 0.0001$), suggesting that mussels in lower-stress environments dedicate more relative energy toward reproduction. GSI was lower in females than in males ($F_{1,240} = 20.00$, $P < 0.0001$). 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 stage of maturity. 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.

Stress and food effects on energy allocation toward stress resistance: field surveys

Carotenoid content (based on spectrophotometric quantification) varied between edges (stress), sites (food availability), and sexes over time (Appendix D). The

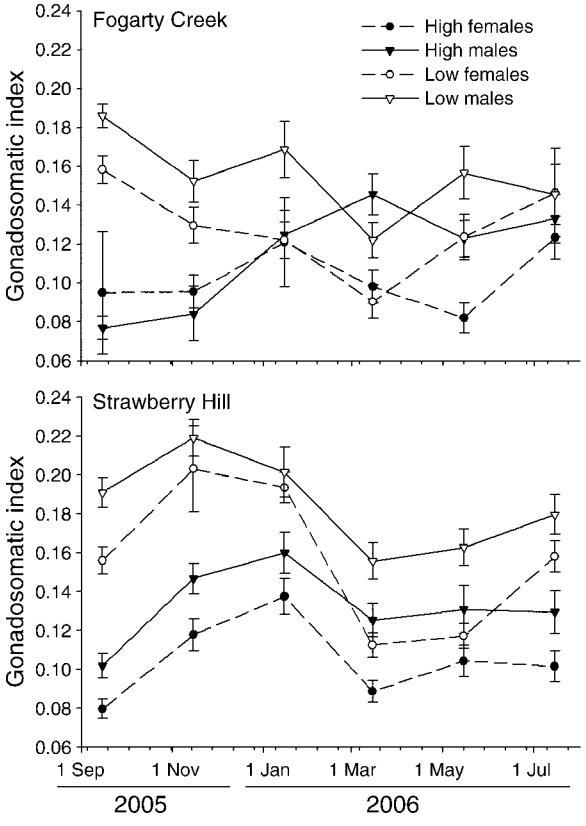

FIG. 3. Gonadosomatic index (GSI; mean ± SE) of mussels collected from high and low edges of the mussel beds at Fogarty Creek and Strawberry Hill in September 2005–July 2006. Gonadosomatic index = (gonadal tissue mass)/(gonadal + somatic tissue mass).

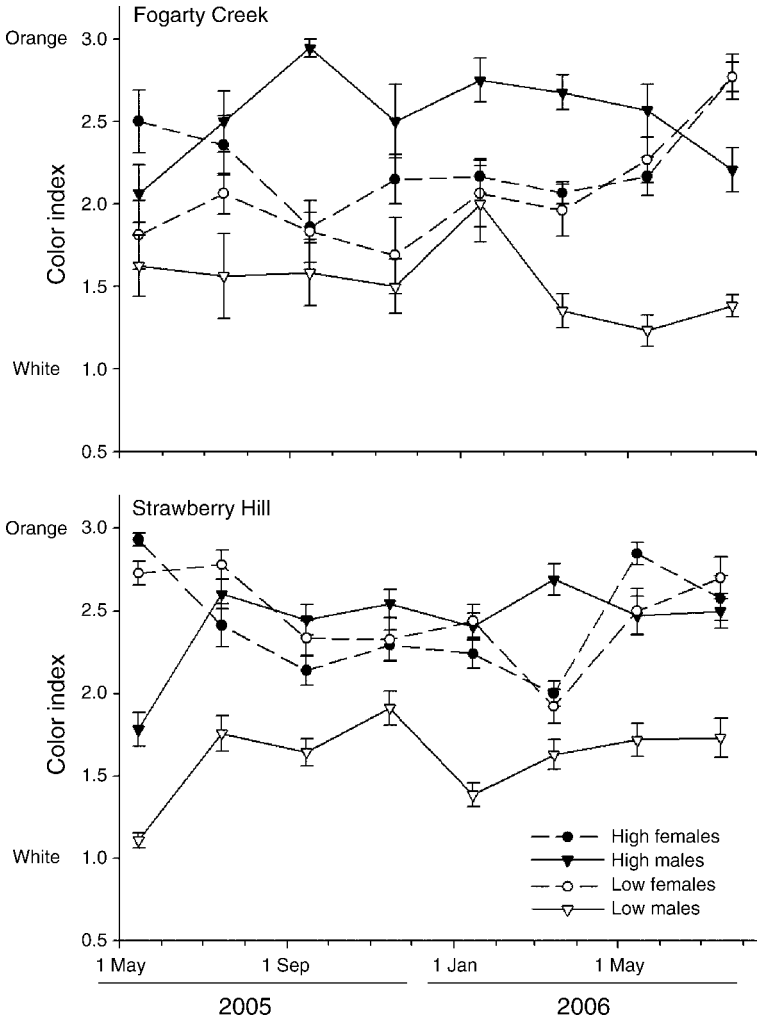

FIG. 4. Color index (1, white; 2, peach; 3, orange; mean ± SE) of mussels collected from high and low edges of the mussel beds at Fogarty Creek and Strawberry Hill in May 2005–July 2006. Colors were scored visually in the field prior to confirmation of carotenoid content using spectrophotometry.

same general patterns were also seen with the field tissue color-scoring system (Fig. 4). Overall, the dominant carotenoid patterns were as follows: (1) Carotenoid content was higher in high-edge males than females from both edges, which had higher levels than low-edge males ($F_{1,160} = 48.23$, $P < 0.0001$; Fig. 5, Appendix D). (2) Patterns of carotenoids varied through time and between sites ($F_{3,160} = 3.45$, $P = 0.02$), with carotenoid content higher overall at SH than at FC ($F_{1,160} = 11.11$, $P = 0.001$). (3) Judging from sum of squares (Appendix D), edge (stress) appeared to have the greatest overall effect on carotenoid content. Carotenoid content (pooled across time) increased with field color score as assessed visually ($F_{1,190} = 328.91$, $P < 0.0001$, $R^2 = 0.63$), indicating that the field scoring system may be representative of carotenoid content (ln(carotenoid content) = 0.06 + 1.60[ln(color index score)]). However, with untransformed data, there was a funnel shape to the relationship of color vs. carotenoid content, suggesting that the category "orange" (scored as 3 in the field) obscured a broader range of carotenoid pigment concentrations than did "white" and "peach."

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 ($F_{1,94} = 11.33$, $P = 0.001$), indicating that female mussels with fewer ripe oocytes 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 stage of maturity ($F_{1,94} = 7.40$, $P = 0.008$), showing that as loss of sperm occurred, carotenoid concentration 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

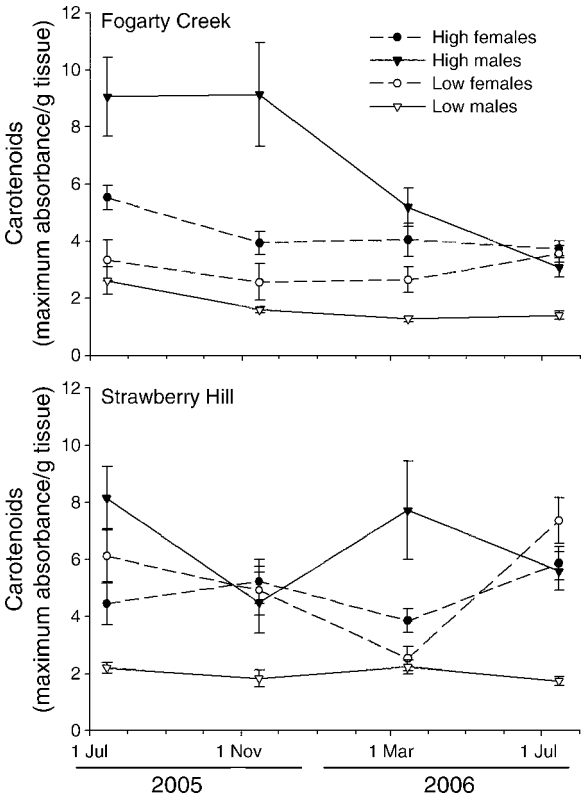

FIG. 5. Carotenoid pigment content (maximum absorbance between 380 and 580 nm corrected by tissue mass; mean ± SE) of mussels collected from high and low edges of the mussel beds at Fogarty Creek and Strawberry Hill in July 2005–July 2006.

gonadal tissue, carotenoid content evidently 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 oxidative damage (e.g., Blount et al. 2002, Lamare and Hoffman 2004).

HPLC revealed three major carotenoid pigments in mussel gonads (see Appendix E for an example of a chromatogram), and these three pigments comprised the major peaks in all samples examined. The presence of a zeaxanthin-like pigment was identified with comparison to retention times and spectra from published studies that used the same solvents and gradient (Jeffrey et al. 1997) and a previous run using a zeaxanthin standard and the same solvents, gradient, and column. An alloxanthin-like pigment was tentatively identified based on similar retention times and spectra from published studies (Jeffrey et al. 1997) and previous samples thought to contain this pigment that had been run using the same solvents, gradient, and column, but no standard was available. The third carotenoid is unknown, but based on evidence from other studies of mussel carotenoids (e.g., Scheer 1940, Campbell 1970), could potentially be mytiloxanthin. Few differences were found in each of the three pigments between sexes and edges, likely due to high variability in relative values (Fig. 6, Appendix F). However, there was relatively less of the unknown carotenoid in males than females ($F_{1,20} = 6.66$, $P = 0.02$), and the alloxanthin-like pigment was marginally lower in low-edge males than females and high-edge males ($F_{1,20} = 3.65$, $P = 0.07$). Absorbance from spectrophotometry varied linearly with the zeaxanthin-like pigment from HPLC ($F_{1,22} = 19.15$, $P = 0.0002$, $R^2 = 0.47$), as well as with the alloxanthin-like pigment ($F_{1,22} = 18.37$, $P = 0.0003$, $R^2 = 0.46$) and the unknown carotenoid ($F_{1,22} = 12.18$, $P = 0.002$, $R^2 = 0.36$). Therefore, total carotenoid content (maximum absorbance from spectrophotometry) appears to reflect the three major carotenoids found using HPLC.

Activity of SOD did not differ between males from high and low edges of the mussel bed ($F_{1,12} = 1.25$, $P = 0.28$). Although carotenoid content was higher in high-edge males than low-edge males ($F_{1,12} = 21.66$, $P = 0.0006$), carotenoid content and SOD activity were unrelated within individuals ($F_{1,12} = 0.66$, $P = 0.43$, $R^2 = 0.05$).

Plasticity of growth, reproduction, and carotenoid content to stress: field transplant experiment

Growth.—In the in situ plots, mussels grew faster at the low edge than the high edge ($F_{1,6} = 12.72$, $P = 0.01$; Fig. 7a, Appendix G), but growth rate did not vary over time ($F_{2,5} = 0.04$, $P = 0.96$). Between all 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 no differences occurred among treatments ($df = 2$, $t = 1.13$, $P = 0.35$). Within the high edge, growth rate did not differ between treatments ($F_{2,5} = 1.57$, $P = 0.30$) or across time ($F_{2,4} = 0.93$, $P = 0.47$).

Stage of maturity.—In the in situ plots, stage of maturity was higher at the high edge than at the low edge

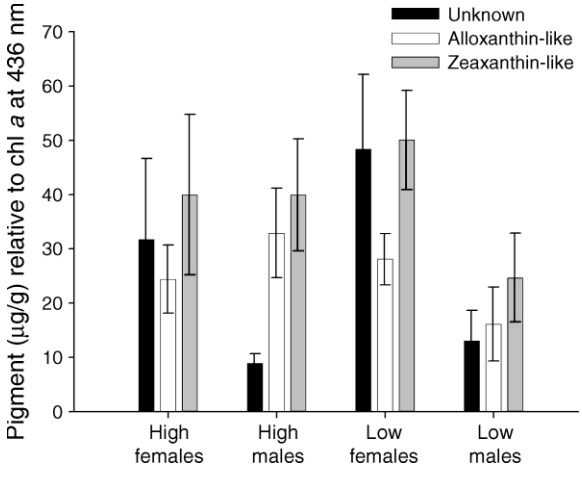

FIG. 6. Values (mean ± SE) for each of the three major carotenoid pigments (unknown, alloxanthin-like, zeaxanthin-like) of mussels collected from high and low edges of the mussel beds at Strawberry Hill in July 2006 relative to chlorophyll *a* (chl *a*) at 436 nm.

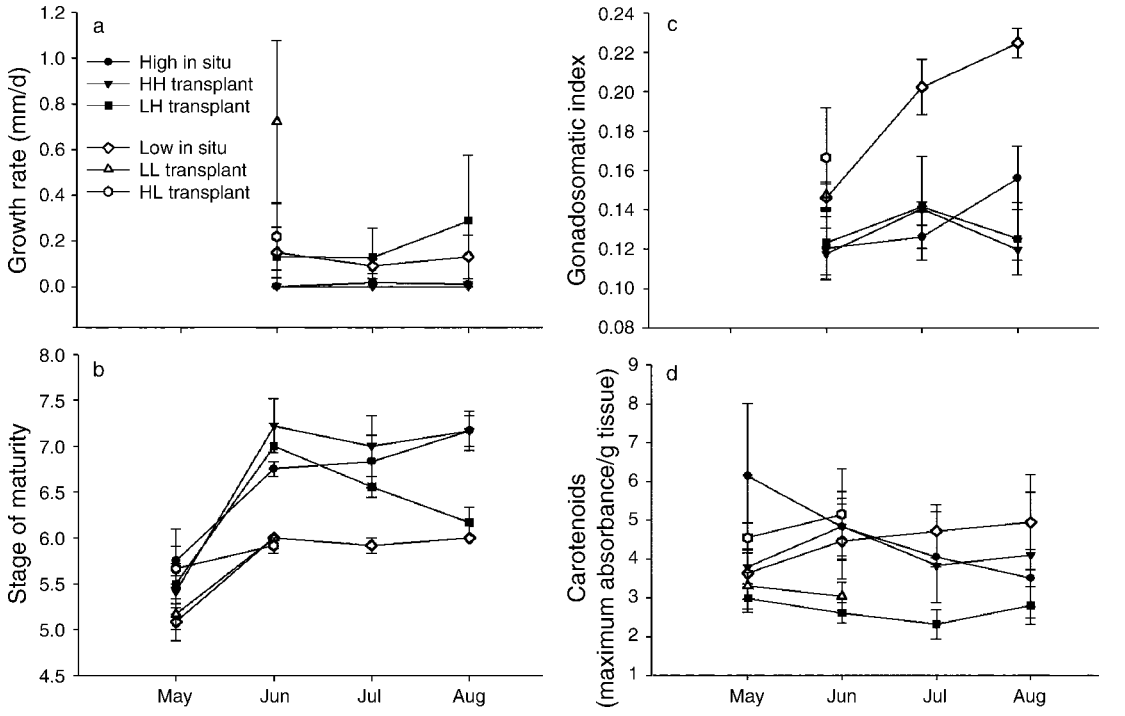

FIG. 7. (a) Growth rate, (b) stage of maturity, (c) gonadosomatic index (GSI), and (d) carotenoid content (all values mean ± SE) of mussels from the transplant experiment conducted between May and August 2004. Carotenoid content is maximum absorbance between 380 and 580 nm corrected by tissue mass. Abbreviations are: HH, high to high; LH, low to high; LL, low to low; and HL, high to low.

($F_{1,6} = 52.86$, $P = 0.0003$; Fig. 7b, Appendix G), indicating more spawning activity in the high edge. In addition, stage of maturity increased over time ($F_{3,4} = 128.70$, $P = 0.0002$) at both edges as spawning occurred. 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) 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 ($F_{1,15} = 9.27$, $P = 0.008$). 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.—In the in situ plots, GSI was higher in low-edge than high-edge mussels ($F_{1,6} = 13.66$, $P = 0.01$; Fig. 7c, Appendix G), indicating higher relative energy allocation toward reproduction in low-edge mussels. GSI increased over time in low-edge mussels ($F_{2,5} = 15.78$, $P = 0.007$). In the transplant experiments 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 ($F_{4,8} = 0.94$, $P = 0.46$).

Carotenoid content.—In the in situ plots, carotenoid content was the same in mussels from both edges ($F_{1,6} = 0.01$, $P = 0.94$; Fig. 7d, Appendix G) and did not change with time ($F_{3,4} = 0.23$, $P = 0.87$). In May and June for all treatments, carotenoid content was higher in the in situ high-edge treatments than other treatments ($F_{2,15} = 4.68$, $P = 0.03$) but did not vary with time ($F_{1,15} = 0.33$, $P = 0.58$). At the high edge, carotenoid content did not vary between treatments ($F_{2,5} = 1.41$, $P = 0.33$) 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, for all treatments in May, females overall had higher carotenoid content than males ($F_{1,32} = 71.83$, $P < 0.0001$), and HL and in situ high-edge treatments had higher levels than LL ($F_{5,32} = 4.71$, $P = 0.003$), suggesting that mussels did not lose pigments rapidly when transplanted into the low edge. In addition, in situ females from both edges, HL, and HH females had the highest carotenoid content, and in situ low-edge, LL, and LH males had the lowest ($F_{5,32} = 2.48$, $P = 0.05$). The same pattern held in June, with females having higher carotenoid content than males ($F_{1,20} = 6.77$, $P = 0.02$) and the in situ high-edge treatment having higher content than the LH treatment ($F_{5,20} = 3.41$, $P = 0.02$), suggesting that pigments were not accumulated after transplantation to the high edge.

DISCUSSION

Energy trade-offs exist in all organisms, and in the face of stress, growth and reproduction can be compromised to conserve energy for costly physiological

defenses that increase chances of survival (Stearns 1992). The results of this study indicate that as in birds (e.g., Ots and Hörak 1996), fish (e.g., Schreck et al. 2001), and reptiles and amphibians (e.g., Moore and Jessop 2003), this is likely to be the case for intertidal mussels. Our results suggest that mussels allocate proportionally less energy toward growth and reproduction under stress and invest more energy in the inefficient (Fox and Hopkins 1966) and energetically expensive (Brush 1990, Hill 1996) process of carotenoid pigment assimilation. The patterns of carotenoid content in the intertidal zone show that, as in birds (Blount et al. 2002), sea urchins (Lamare and Hoffman 2004), and other organisms, mussels may utilize carotenoid pigments to protect their gametes from oxidative damage. To our knowledge, this study is also the most comprehensive investigation of the reproductive ecology and physiology of intertidal mussels conducted to date. Our analyses reveal that the effects of stress are complex, varying with time of year, food availability, and tidal height. Below we discuss these points and place the results in the context of both oxidative stress and implications for mussels under predicted climate change scenarios.

Stress and food effects on energy allocation toward reproduction

This study suggests that the effects of stress on reproduction are complex and strong. Mussels at the high edge of the mussel bed showed accelerated timing of spawning when compared to low-edge mussels. Thermal stress can lead to spawning (e.g., Schreck et al. 2001, Petes et al. 2007), and mussels experiencing food shortage cannot maintain ripe gametes (Bayne and Thompson 1970). Given that the less-stressed mussels in the low edge released gametes more gradually, it is possible that mussels at the high edge do not have enough energy to maintain ripe gametes in their tissues and therefore release their gametes over a shorter period of time and earlier in the season. In addition, mussels at the high edge remained spawned-out for a longer period of time than mussels at the low edge, potentially indicating that these mussels do not have enough energy to regenerate mature gametes quickly after spawning.

The interaction between food availability and temperature can play a critical role in the timing and success of reproduction. Gonadal growth in bivalves often co-occurs with increasing temperature and peak phytoplankton production in the summer, and spawning often follows this growth period (Sastry 1966). The SH site had higher chl *a* levels, and GSI was higher in mussels at SH than those at FC, indicating that these animals were dedicating relatively more energy toward reproduction. The SH mussels spawned earlier in the summer than mussels at FC, which could be due either to more energy stores or potentially a response to higher thermal stress. In addition, mussels at SH exhibited two pulses in spawning activity throughout one annual cycle, whereas FC mussels only had one, suggesting that mussels at SH had more energy available for reproduction. GSI was higher in low-edge mussels than high-edge mussels, indicating that mussels at the low edge have relatively 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 success of larvae. The highest intertidal temperatures were recorded in May and June, and a majority of spawning activity in the high-edge mussels occurred during this time period. Seasonally intermittent upwelling leading to offshore transport of propagules typically begins in mid-April and intensifies in July off the Oregon coast (Menge et al. 2004). Peak recruitment of mussel juveniles normally occurs after a planktonic larval duration of 9–45 d (Becker et al. 2007) 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). Mussels at the high edge spawned out all of their gametes (mass spawning) during the summer, rather than only a fraction, as in low-edge mussels ("dribble-spawning"), perhaps suggesting 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 larval survival and successful return of propagules back onshore rely on the timing of oceanographic processes. Recent anomalies in oceanic circulation off the Oregon coast have exacerbated the relatively unpredictable (e.g., Menge et al. 2004) duration, timing, and frequency of seasonal upwelling events (Barth et al. 2007). Given these uncertainties, the strategy of consistent low levels of "dribble spawning" exhibited by low-edge mussels throughout the year may be more advantageous in this system.

Stress and food effects on energy allocation toward stress resistance

The combination of food availability and physical stress affected carotenoid content in mussels. Carotenoid content was higher in mussels at SH than in those at FC, which is consistent with patterns of higher chl *a* levels at Cape Perpetua than at Cape Foulweather (Menge et al. 2002, 2004, Leslie et al. 2005; Appendix B). 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 incorporating 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* fluctuated with time in this study, carotenoid content tended to remain relatively constant, indicating that mussels likely maintain carotenoids in their tissues. Carotenoids are not easily lost from mussel tissues except upon starvation (Scheer 1940).

Differences in food availability between the high and low edges of the mussel bed may have affected carotenoid content. Mussels can 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 for longer periods of time than high-edge mussels (e.g., 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 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 content varied with sex, edge (stress), and stage of maturity. Carotenoid content was always lowest in low-edge males, whereas high-edge males had either higher or the same concentrations 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. The major carotenoid pigments found in gonadal tissues of *Mytilus californianus* were a zeaxanthin-like pigment, an alloxanthin-like pigment, and an unknown pigment (possibly mytiloxanthin), which are consistent with findings from previous research on mussel carotenoids (Campbell 1970). Accumulation of zeaxanthin was previously considered unique to females (Scheer 1940), but the finding that the zeaxanthin-like pigment occurred equally in male and female mussels indicates that this is not a true secondary sex characteristic. Selective accumulation of this pigment may occur to protect gametes from oxidative damage, particularly given that spring and summer low tides occur during daylight hours in Oregon, in contrast to the nighttime timing of spring and summer low tides in southern California (e.g., Helmuth et al. 2002). Zeaxanthin has high antioxidant activity and quenches singlet oxygen radicals twice as effectively as β-carotene (Miki 1991, Woodall et al. 1996). In plants, zeaxanthin prevents accumulation of ROS that lead to lipid peroxidation and protects thylakoid membrane lipids from damaging heat stress (Havaux and Niyogi 1999), and in the human retina, high concentrations of zeaxanthin lead to decreased risk of macular degeneration and cataracts (Moeller et al. 2000).

Maintaining high concentrations of carotenoid pigments in gonadal tissue may be an adaptive response to protect both adults and gametes from oxidative damage. Reactive oxygen species can be produced as a result of thermal stress experienced during aerial exposure in intertidal organisms (Abele et al. 1998). Carotenoid pigments could serve an important role in protecting gametes from UV damage after release by the adults, particularly for females, which produce buoyant, lipid-rich oocytes. Embryos resulting from sea urchin gonads with high carotenoid concentrations show lower sensitivity to UV radiation than embryos fertilized from females with low gonadal carotenoid levels (Lamare and Hoffman 2004). In contrast, accumulation of carotenoid pigments in high-edge males likely functions primarily for adult protection and, in part, to prevent peroxidation from occurring in the lipid-rich storage tissue that surrounds their non-pigmented gametes. One could therefore predict that males would show plasticity in carotenoid accumulation, with high-edge males accumulating costly pigments and low-edge males remaining relatively free of pigments, whereas females must all maintain pigments for incorporation into their oocytes. Reactive oxygen species can also be generated as by-products of reproductive effort. Oocyte maturation in crayfish (Liñán-Cabello et al. 2004) and breeding in birds (Alonso-Alvarez et al. 2004) lead to increased metabolic activity and production of ROS, and oxidative stress occurs as a consequence of reduced energy available for antioxidant defenses. It is possible that increased spawning activity in high-edge mussels of both sexes could lead to the generation of ROS and increased susceptibility to oxidative stress, and thus carotenoids may be needed to counteract the resulting oxidative damage.

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). Decreased antioxidant enzyme activity in older mussels makes them more susceptible to oxidative stress than younger individuals (Viarengo et al. 1991), which is consistent with the free radical theories of aging. Growth of mussels is slower in the high edge of the mussel bed than the low edge, as shown from the transplant experiment. Mussels collected in the field survey from the low and high edges were of the same size class, and it is very likely that the mussels from the high edge were older than the mussels from the low edge. This could indicate that the older mussels in the high edge 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 mussel carotenoid content decreases with age, suggesting that this possibility is unlikely. Alternatively, older mussels

may continuously assimilate carotenoid pigments to compensate for decreased metabolic rate and antioxidant enzyme performance.

The finding that SOD activity was not different between mussels from the low and high edges of the mussel bed was surprising but consistent with previous studies (e.g., Livingstone et al. 1990, Viarengo et al. 1991, Sukhotin et al. 2002). There are several possible explanations for this. First, gonadal tissue may not be the most enzymatically active tissue in mussels and therefore may not have shown increased enzyme activity, but gonadal tissue is known to have high lipid peroxidizing enzyme activity (Musgrave et al. 1987), indicating that this possibility is unlikely. Second, carotenoids in high-edge mussels may be so effective at scavenging free radicals that SOD is not used as a major defense in this system.

Plasticity of growth, reproduction, and carotenoid content to stress

Loss of low-edge treatments due to predation prevented direct comparison of increased-stress (LH transplant) treatments with decreased-stress (HL transplant) treatments after the second sampling date. However, the available data revealed that mussel growth and reproduction respond plastically to stress. The high-stress environment at the high edge of the mussel bed (higher temperatures, lower food availability) led to decreased growth rates, reduced relative energy allocation toward reproduction (lower GSI), and increased spawning activity. Mussels transplanted into the high edge (LH treatment) exhibited reduced relative energy allocation toward reproduction and increased spawning. Prior to predator-induced mortality, mussels transplanted into the low edge (HL treatment) invested more relative energy in reproduction, maintained their gametes (did not spawn), and grew faster than mussels at the high edge. These findings indicate that mussels exhibit a plastic response to stress, as there were strong effects of edge but no differences between treatments within an edge.

There were few differences in carotenoid content in the transplant experiment. This is likely due in part to the strong sex-specific differences in carotenoid content, which were 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. However, carotenoids are not readily lost from mussel tissues (Scheer 1940). There could also 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 carotenoid content in high-edge mussels and the lack of temporal fluctuation in carotenoids with changing phytoplankton abundance, it is possible that the phytoplankton food resources supplying carotenoids are not limiting in the environment of the highly productive central Oregon coast (e.g., Menge et al. 2004). However, while these pigments may be abundant, accumulation of carotenoids may be costly, which could help to explain why low-edge males do not incorporate high levels of carotenoids into their gonads. Metabolic transformations of carotenoid pigments from the diet can be inefficient (Fox and Hopkins 1966) and energetically expensive (Brush 1990, Hill 1996).

Conclusions

Intertidal mussels in this study exhibited energetic trade-offs under stress, as energy toward growth and reproduction was reduced and energy toward physiological defenses was increased. 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 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. Early spawning and energy allocation away from reproduction toward costly physiological defenses 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. 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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Literature Cited

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.

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

Barth, J. A., B. A. Menge, J. Lubchenco, F. Chan, J. M. Bane, A. R. Kirincich, M. A. McManus, K. J. Nielsen, S. D. Pierce, 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: 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.

Becker, B. J., L. A. Levin, F. J. Fodrie, and P. A. McMillan. 2007. Complex larval connectivity patterns among marine invertebrate populations. Proceedings of the National Academy of Sciences (USA) 104:3267–3272.

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.

Brett, J. R. 1958. Implications and assessments of environmental stress. Pages 69–83 *in* P. Larkin, editor. The investigation of fish-power problems. H. R. MacMillan lectures in fisheries. University of British Columbia, Vancouver, British Columbia, Canada.

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

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.

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.

Connell, J. H. 1972. Community interactions on marine rocky intertidal shores. Annual Review of Ecology and Systematics 3:169–172.

Connolly, S. R., B. A. Menge, and J. Roughgarden. 2001. A latitudinal gradient in recruitment of intertidal invertebrates in the northeast Pacific Ocean. Ecology 82:1799–1813.

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.

Di Mascio, P., M. E. Murphy, and H. Sies. 1991. Antioxidant defense systems: the role of carotenoids, tocopherols, and thiols. American Journal of Clinical Nutrition 53:194S–200S.

Fitzhenry, T., P. M. Halpin, and B. Helmuth. 2004. Testing the effects of wave exposure, site, and behavior on intertidal mussel body temperatures: applications and limits of temperature logger design. Marine Biology 145:339–349.

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

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

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.

Harman, D. 1981. The aging process. Proceedings of the National Academy of Sciences (USA) 78:7124–7128.

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., C. D. G. Harley, P. M. 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.

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.

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

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.

Jeffrey, S. W., R. F. C. Mantoura, and S. W. Wright, editors. 1997. Phytoplankton pigments in oceanography. UNESCO, Paris, France.

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.

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, F. Chan, J. Lubchenco, and B. A. Menge. 2005. Barnacle reproductive hotspots linked to nearshore ocean conditions. Proceedings of the National Academy of Sciences (USA) 102:10534–10539.

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 49:363–375.

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.

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

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 4:415–424.

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

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. J. Kronberg, editors. Earth system responses to global change. Academic Press, New York, New York, USA.

Luna, L. G. 1968. Manual of histological staining methods of the Armed Forces Institute of Pathology. McGraw-Hill, New York, New York, USA.

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., 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 74:663–684.

Menge, B. A., E. Sanford, B. A. Daley, T. L. Freidenburg, G. Hudson, and J. Lubchenco. 2002. 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., and J. P. Sutherland. 1987. Community regulation: variation in disturbance, competition, and predation in relation to environmental stress and recruitment. American Naturalist 130:730–757.

Miki, W. 1991. Biological functions and activities of animal carotenoids. Pure and Applied Chemistry 63:141–146.

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

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 52:609–612.

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. Hörak. 1996. Great tits *Parus major* trade health for reproduction. Biological Sciences 263:1443–1447.

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

Petes, L. E., B. A. Menge, and G. D. Murphy. 2007. Environmental stress decreases survival, growth, and reproduction in New Zealand mussels. Journal of Experimental Marine Biology and Ecology 351:83–91.

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 48:2171–2185.

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

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 and Hall, New York, New York, USA.

Sagarin, R. D., and G. N. Somero. 2006. Complex patterns of expression of heat-shock protein 70 across the northern biogeographical ranges of the intertidal mussel *Mytilus californianus* and snail *Nucella ostrina*. Journal of Biogeography 33:622–630.

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. Volume 5. Academic Press, New York, 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.

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., 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, Amsterdam, The Netherlands.

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

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.

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 66:101–111.

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 5:273–280.

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

Williams, G. C. 1966. Natural selection, the cost of reproduction and a refinement of Lack's principle. 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.

APPENDIX A

Representative spectra of gonadal tissue from individual mussels (*Ecological Archives* M078-015-A1).

APPENDIX B

Chlorophyll *a* levels at Fogarty Creek and Strawberry Hill in 2005 and 2006 (*Ecological Archives* M078-015-A2).

APPENDIX C

Results of ordinal logistic regression Wald tests on data subset from field surveys examining effects of month, site, edge, sex, and all possible interactions on stage of maturity (*Ecological Archives* M078-015-A3).

APPENDIX D

Results of four-factor ANOVA on a data subset from field surveys examining effects of month, site, edge, sex, and all possible interactions on gonadosomatic index and carotenoid content (*Ecological Archives* M078-015-A4).

APPENDIX E

Example of chromatogram from high-pressure liquid chromatography (HPLC) analyses of mussel gonadal tissue (*Ecological Archives* M078-015-A5).

APPENDIX F

Results of two-factor ANOVA testing the effects of sex, edge, and the sex × edge interaction on three major carotenoid pigments (*Ecological Archives* M078-015-A6).

APPENDIX G

Results of repeated-measures MANOVA tests for growth rate, stage of maturity, gonadosomatic index, and carotenoids in the field transplant experiment (*Ecological Archives* M078-015-A7).