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

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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 of sea stars and mussels to environmental stress 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 high-edge cages had higher levels of total Hsp70 and exhibited spawning activity earlier in the summer than mussels in the low-edge cages. Sea stars suffered high mortality in the high edge, and low-edge sea stars lost weight but showed no differences in Hsp70 production. These results suggest that stress in the intertidal zone affected the mobile predator more than its sessile prey, which is consistent with predictions of consumer stress models.

Keywords Heat shock proteins · *Mytilus californianus* · *Pisaster ochraceus*

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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 top predators are more affected by environmental stress than their 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 predators may not rely primarily on physiological defense mechanisms to increase chances of survival because they can typically move to avoid 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. 2002). Alternatively, 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 and can move to more moderate conditions (Menge and Olson 1990; Menge et al. 2002). In this scenario, prey would invest energy in defense against stress at a cost of increased susceptibility to predation. Few empirical tests of environmental stress models have been performed in the field, but evidence supports both predator (e.g. Menge 1978a, b; McClanahan 1992; Menge et al. 2002) 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 ideal for investigating environmental stress gradients and responses of predators and prey. The intertidal zone can be a harsh environment, exposing organisms 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 from calm to severely wave-swept. Upper distributional limits in the rocky intertidal zone are thought to be determined by desiccation and temperature stress (Connell 1972), whereas lower limits are frequently set by species interactions, including predation pressure (Connell 1961; Paine 1974; Lubchenco and Menge 1978). Mussels are the competitive dominants for space on many temperate rocky shores throughout the world (Seed 1976; Seed and Suchanek 1992), including the west coast of North America. Also on the west coast, the sea star *Pisaster ochraceus* is a keystone predator; in its absence, the mussel *Mytilus californianus* colonizes and dominates the low-intertidal zone, thereby decreasing species diversity (Paine 1966, 1974; Menge et al. 1994). Mussels typically span the entire vertical stress gradient, from the low intertidal (low-stress environment) to the high intertidal (high-stress environment) zones, whereas sea stars are generally limited to the low zone. The vertical range of mussels and sea stars thus overlaps only at the lower edge of the mussel bed, raising the question of why sea stars do not forage higher on the shore. A few recent studies have documented the 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 the ecology and physiology of these invertebrates 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 thermal 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; consequently, under conditions of thermal stress, their synthesis may interfere with the production of more critical proteins (Helmuth and Hofmann 2001), potentially reducing the scope for growth (Roberts et al. 1997). Constitutive (Hsc75; 75 kDa) and inducible (Hsp72; 72 kDa) isoforms of Hsp70 can be examined to determine both background levels of Hsp and levels of Hsp induced upon exposure to thermal stress (Hofmann and Somero 1995; Helmuth and Hofmann 2001; Halpin et al. 2002). Hsp70 can respond to both acute, short-term stress (Tomanek and Sanford 2003) and chronic, long-term stress (Helmuth and Hofmann 2001), and the level of total Hsp70 produced appears to be the best predictor of thermotolerance (Sorte and Hofmann 2005).

Reproduction can also be examined as an indicator of sublethal stress (Michalek-Wagner and Willis 2001; Schreck et al. 2001). The process of reproduction shows the effects of stress earlier than many other biological processes. Repeated, acute stress can decrease the 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 the reduced survival of gametes through embryogenesis, which causes a decline in the ecological fitness of individuals in the population (Bayne 1972). However, little is known about the effects of physiological repair mechanisms on reproduction.

The goal of this study was to test the effects of stress on the ecology and physiology of two important players in the intertidal zone, the predatory sea star *P. ochraceus* and its principal prey, the mussel *M. californianus*.

Materials and methods

Experimental design and set-up

A field experiment was initiated at Strawberry Hill (44°25′N, 124°12′W) on the central Oregon coast in April 2003. The effects of stress on mussels and sea stars were tested by transplanting individuals of both species into cages at both the high (more stressful environment) and low

(less stressful environment) edges of an intertidal mussel bed. The effects of food availability on sea star growth and stress responses were also examined by establishing cages in both edges with and without a mussel food source. Cages ($n = 4$ per treatment) were therefore established in cleared plots at both the low and high edges with the following three treatments:

(1) sea stars and no mussel food source;
(2) sea stars with mussel food source;
(3) mussels and no sea star predators.

All mussels used in the experiment were collected from Bob Creek, Oregon (44°24′N, 124°11′W), which is approximately 1 km south of Strawberry Hill, on 13 April 2003. To select individuals that had been previously exposed to average levels of thermal and desiccation stress, we haphazardly collected mussels from the vertical middle of the mussel bed (not the high or low edges). After collection, each mussel (initial size approx. 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). Prior to transplantation, mussels were held in ambient seawater tanks overnight at Oregon State University's Hatfield Marine Science Center in Newport, Oregon. On 14 April 2003 at Strawberry Hill, mussels were placed into stainless-steel mesh cages (38 × 38 × 15 cm) that were fastened to the rock using lag screws screwed into wall anchors placed in holes drilled in the rock. Fifty mussels were placed into each mussel cage of treatments (2) and (3); these mussels were held in place with plastic vexar mesh fastened to the rock for 4 weeks to allow time for byssal thread reattachment to the substrate (Menge et al. 2004).

On 19 May 2003, the plastic mesh was removed, and three weighed and measured sea stars were added to each sea star cage of treatments (1) and (2). Sea star average initial wet mass [± standard error (SE)] per cage was 319.6 ± 10.4 g, and average standard length (madreporite to the tip of the opposite arm; SE) was 12.0 ± 0.2 cm. All cages had plastic vexar mesh lids to prevent sea stars from escaping and to provide similar thermal environments to all treatments. This design was likely to have moderated thermal 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 the thermal environment as perceived by mussels, we quantified the temperature within cages. Temperature logger-containing mussel mimics ("Robomussels") were created by embedding TidbiT temperature loggers (Onset Computer, 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 min for the entire experiment.

Field sampling protocol

Cages were sampled every 4 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 (2) (+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 treatment (3) (−sea stars, +mussels) cages, measured, then dissected. 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 Hsp analyses. Each month, all of the sea stars were removed from the sea star cages [treatments (1) and (2)] and individually weighed in the field. A small sample of tube-foot tissue was removed from each individual and flash-frozen in liquid nitrogen for transport to the −80°C freezer in Corvallis. The sea stars were placed back into their cages immediately after sampling.

Histological processing and analyses

In the laboratory, 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 (Leica DMLS; Leica Microsystems, Bannockburn, IL). The sex of each individual was identified, and the stage of maturity was categorized as follows:

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 follicles;
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 in follicles;
8. post-spermiation—completely spawned-out, all follicles empty.

Stages of maturity 1–4 are only found in immature, juvenile mussels that have never spawned. Consequently, only stages 5–8 were documented in individuals in this study, indicating that all mussels were of adult size.

Reproductive potential was quantified for females only because male tissue was composed of masses of follicles that were variable in size, maturity, and cell density. For each female, the number of vitellogenic oocytes within a field-of-view at 400× magnification was counted for ten randomized areas of gonadal tissue per slide (one slide per mussel), and an average "reproductive potential" was calculated from these areas.

Heat shock protein analyses in mussels

Mussel gill tissues were homogenized in a Tris-sodium dodecyl sulfate (SDS) buffer [50 m*M* Tris-HCl pH 6.8, 4% SDS, 1 m*M* ethylenediaminetetraacetic acid (EDTA), 1 m*M* phenylmethanesulphonylfluoride (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 according to Halpin et al. (2004). The supernatant was decanted, stored at −80°C, and used for subsequent analyses. The protein concentrations of the samples were determined using modified Bradford protein assays according to the manufacturer's instructions (Coomassie Plus-200; Pierce, Rockford, IL). A 10-µg aliquot of protein from each sample was loaded onto 7.5% SDS-polyacrylamide gels (VWR: 53225-106) adjacent to 10 µg of Hsc70 protein positive control (SPP-751; StressGen, Victoria, B.C., Canada) and a Kaleidoscope pre-stained molecular weight marker (161-0324; BioRad, Hercules, CA). Bands were resolved using electrophoresis (running buffer: 25 m*M* Tris, 192 m*M* glycine, 0.1% SDS) at 125 V for 100 min. Gels were transferred electrophoretically in buffer (25 m*M* Tris, 192 m*M* glycine, 20% methanol) onto polyvinylidene difluoride (PVDF) membranes (IPVH00010; Fisher Scientific, Waltham, MA) at 100 V for 60 min.

Western blotting was performed on the PVDF membranes according to Tomanek and Sanford (2003). The membranes were incubated at a 1:2500 dilution with monoclonal rat antibody (IgG) against Hsp70 (MA3-001; Affinity Bioreagent, Golden, CO). After washing, the membranes were incubated with rabbit-anti-rat bridging antibody (AI-4000; Vector Laboratories, Burlingame, CA) at a dilution of 1:2000. After more washing, they were incubated with horseradish-peroxidase protein A solution (BioRad) at a dilution of 1:5000. Bands were visualized by first developing the membranes using a chemiluminescence detection method (SuperSignal; Pierce) and then exposing the blots to X-ray film. Induced (72 kDa) and constitutive (75 kDa) bands of Hsp70 were quantified from the developed film with densitometry and ImageQuaNT software (Molecular Dynamics, Sunnyvale, CA). Sample band intensities were separated by band intensities of the Hsc70 positive controls for each gel in order to allow comparisons between gels [see S1a of the Electronic Supplementary Material (ESM) for an image of a Western blot].

Heat shock protein analyses in sea stars

Sea star tube-foot 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; SPA-757, Stressgen) at a 1:1,000 dilution and secondary antibody (ECL anti-rabbit IgG, HRP-conjugated; NA934V, Amersham Biosciences, Pittsburgh, PA) at 1:5000 were used for the 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 S1b in ESM for an image of a Western blot).

Statistical analyses

Average monthly high temperatures (aerial) were calculated from "Robomussel" temperature data.

Experimental data were analyzed with JMP ver. 6.0 (SAS, Cary, NC). Repeated-measures multivariate analysis of variance (RM-MANOVA) tests were used to examine responses across the four sampling periods. For mussels, edge (low vs. high) was used as the explanatory variable, and Hsp70 (total, induced, and constitutive), stage of maturity, and reproductive potential were separately analyzed as

response variables. Mussel growth data were analyzed only for the initial and final sampling time points. Data were examined visually 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, variance was not normally distributed for total and induced Hsp70 on certain sampling time points. To test the relationship between reproductive potential and total Hsp70, we performed a regression on ln-transformed values pooled across time points.

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 high-edge cages (see below), responses of the sea stars in terms of weight and total Hsp70 production could only be analyzed for low-edge sea stars, 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 the analyses. Sea star mortality (proportion dead) data 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 we therefore analyzed untransformed values.

Results

"Robomussel" temperatures

Average monthly high temperatures (aerial) were higher at the high edge of the mussel bed than at the low edge (Fig. 1), confirming our inference that thermal stress increases with tidal height and that this gradient persists through time. At low tide, monthly high aerial temperatures were 4–8°C higher at the high edge than the low edge of the mussel bed (Fig. 1).

Mussel responses to environmental stress

Although mussel growth increased over time ($F_{1,6} = 18.41$, $p = 0.005$), growth did not differ between edges (see Table 1 for all statistical results; $F_{1,6} = 0.61$, $p = 0.46$), likely due to the relatively short time span of the experiment and, therefore, little mussel growth overall. Total Hsp70 production for mussels was higher in the high edge than in the low edge (Fig. 2; $F_{1,6} = 5.72$, $p = 0.05$) 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. 2; $F_{1,6} = 3.34$, $p = 0.11$),

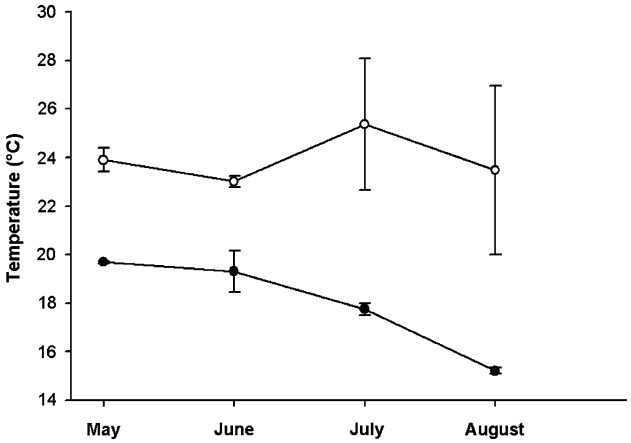

Fig. 1 Average monthly high temperatures for "Robomussels" deployed in cages in the low edge (*filled circle*) and high edge (*open circle*) of the intertidal mussel (*Mytilus californianus*) bed at Strawberry Hill, OR, USA from May to August 2003 (mean ± SE)

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 in the high-edge mussels as in the low-edge mussels (Fig. 2; $F_{1,6} = 4.09$, $p = 0.09$), but production fluctuated with time ($F_{3,4} = 15.31$, $p = 0.01$).

Stage of reproductive maturity was higher in high-edge than low-edge mussels (Fig. 3a; $F_{1,6} = 15.55$, $p = 0.008$), reflecting more spawning activity in the high edge. Stage of maturity increased marginally over the course of the summer ($F_{3,4} = 5.24$, $p = 0.07$), suggesting increased spawning activity over time at both edges. Reproductive potential was higher in low-edge than in high-edge mussels (Fig. 3b; $F_{1,5} = 13.78$, $p = 0.01$), indicating a higher number of gametes available for spawning. Overall, total Hsp70 varied inversely with reproductive potential, suggesting a possible trade-off between Hsp70 production and reproduction ($R^2 = 0.16$, $F_{1,29} = 5.33$, $p = 0.03$), although the low R^2 value indicates that this was not a strong relationship.

Sea star responses to environmental stress

Sea stars at the high edge of the mussel bed suffered higher mortality than sea stars at the low edge ($F_{1,10} = 22.37$, $p = 0.0008$), and mortality increased with time ($F_{3,8} = 5.60$, $p = 0.02$). Food availability (±mussels) had no effect on mortality ($F_{1,10} = 0.54$, $p = 0.48$). Due to the high mortality in high-edge sea stars, suggesting a severe effect of thermal stress, the high-edge treatments were omitted from subsequent analyses. By the final sampling date, mortality was 100% in high-edge sea stars from both treatments, and mortality was 55.5 ± 29.4% (±SE) in low-edge treatment (1) (−mussel) sea stars and 66.7 ±19.2% in the low-edge treatment (2) (+mussel) sea stars.

Table 1 Results of repeated-measures multivariate analysis of variance tests for mussels (*Mytilus californianus*) and sea stars (*Pisaster ochraceus*)

Organism	Response variable	Parameter	*df*	*F*	*p*
Mytilus californianus	Growth[a]	Edge	1.6	0.61	0.46
		Time[a]	1.6	18.41	0.005[a]
		Time × edge	1.6	0.61	0.46
	Total Hsp70[a] (ln-transformed)	Edge[a]	1.6	5.72	0.05[a]
		Time	3.4	1.98	0.26
		Time × edge	3.4	2.91	0.16
	Induced Hsp70[a] (ln-transformed)	Edge	1.6	3.34	0.11
		Time	3.4	0.62	0.64
		Time × edge	3.4	2.15	0.24
	Constitutive Hsp70[a] (ln-transformed)	Edge	1.6	4.09	0.09
		Time[a]	3.4	15.31	0.01[a]
		Time × edge	3.4	0.49	0.71
	Stage of maturity[a] (ln-transformed)	Edge[a]	1.6	15.55	0.008[a]
		Time	3.4	5.24	0.07
		Time × edge	3.4	1.58	0.33
	Reproductive potential[a] (ln-transformed)	Edge[a]	1.5	13.78	0.01[a]
		Time	3.3	1.78	0.32
		Time × edge	3.3	2.79	0.21
Pisaster ochraceus	Mortality[a] (arcsin-square root transformed)	Edge[a]	1.10	22.37	0.0008[a]
		Treatment	1.10	0.54	0.48
		Treatment × edge	1.10	0.80	0.39
		Time[a]	3.8	5.60	0.02[a]
		Time × edge	3.8	0.62	0.62
		Time × treatment	3.8	0.70	0.58
		Time × treatment × edge	3.8	0.63	0.62
	Weight[a]	Treatment[a]	1.3	14.74	0.03[a]
		Time[a]	2.2	24.41	0.04[a]
		Time × treatment	2.2	5.05	0.16
	Total Hsp70[a] (ln-transformed)	Treatment	1.3	0.37	0.58
		Time	3.1	1.91	0.48
		Time × treatment	3.1	5.39	0.30

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

[a] Parameters and values with $p \leq 0.05$

Low-edge sea stars also appeared to be affected by stress. Because high winds affected 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 (2); Fig. 4a; $F_{1,3} = 14.74$, $p = 0.03$], indicating 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, therefore, reduced feeding activity. Regardless of food availability, total Hsp70 production was the same in low-edge sea stars (Fig. 4b; $F_{1,3} = 0.37$, $p = 0.58$) and did not change with 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 exposure to physiological stress than their sessile prey (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, as all sea stars, including those with a mussel food source, lost weight throughout the experiment. While mussels exhibited physiological signs of

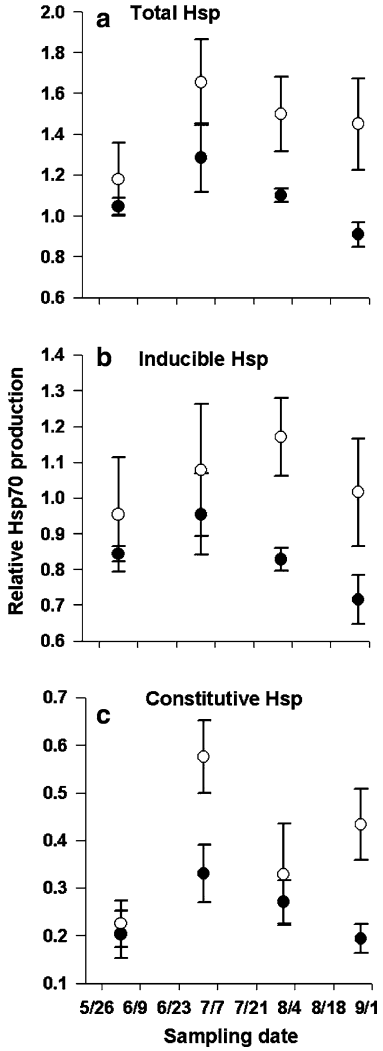

Fig. 2 Average heat shock protein production in mussels in the low-edge (*filled circle*) and high-edge (*open circle*) treatments during June–August sampling. **a** Total Hsp70 levels, **b** inducible Hsp70 levels, **c** constitutive Hsp70 levels as quantified relative to Hsc70 standard (mean ± SE)

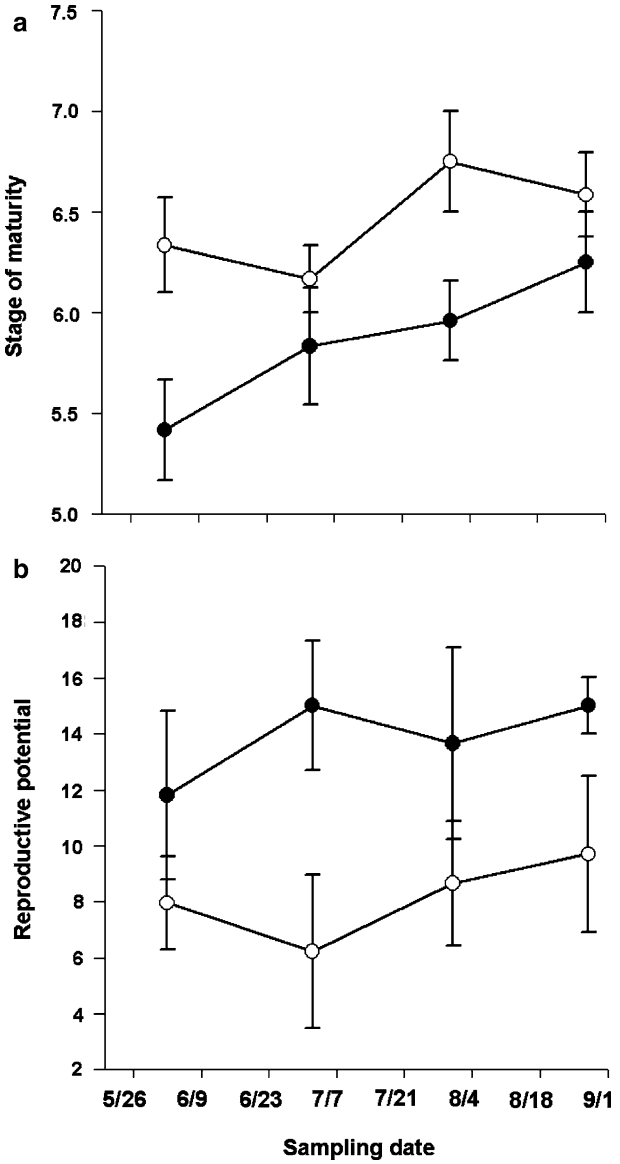

Fig. 3 Stage of maturity (**a**) and reproductive potential (**b**) of mussels at the low (*filled circle*) and high (*open circle*) edges during June–August sampling (mean ± SE). Stage of maturity increases from five (ripe) to eight (spawned-out) as spawning occurs. Reproductive potential is the number of vitellogenic oocytes within field-of-view at 400× magnification

sublethal stress, no heat-related mortality was documented, as no gaping shells were present in cages without predators (L. Petes, personal observation). A comparison of all our results suggests that both sea stars and mussels respond negatively to thermal stress and that sea stars are more negatively affected than are mussels.

The high mortality of sea stars compared to the low mortality of mussels indicates that—under the constraints of the experimental conditions—the 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, tidepools, etc.) or retreating to the lower shore. In contrast to the sea star results, 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 Sanford 2003). This suggests that some mobile animals in the intertidal zone do use Hsp to defend themselves from physiological damage. The high mortality of sea stars observed in our experiments may therefore indicate that sea stars do not effectively use Hsp as a defense to combat tissue-damaging thermal stress. Consistent with this hypothesis, even the few surviving high-edge treatment sea stars that were available for tissue sampling showed no increase in Hsp production (data not shown). Sea stars likely invest a majority of their

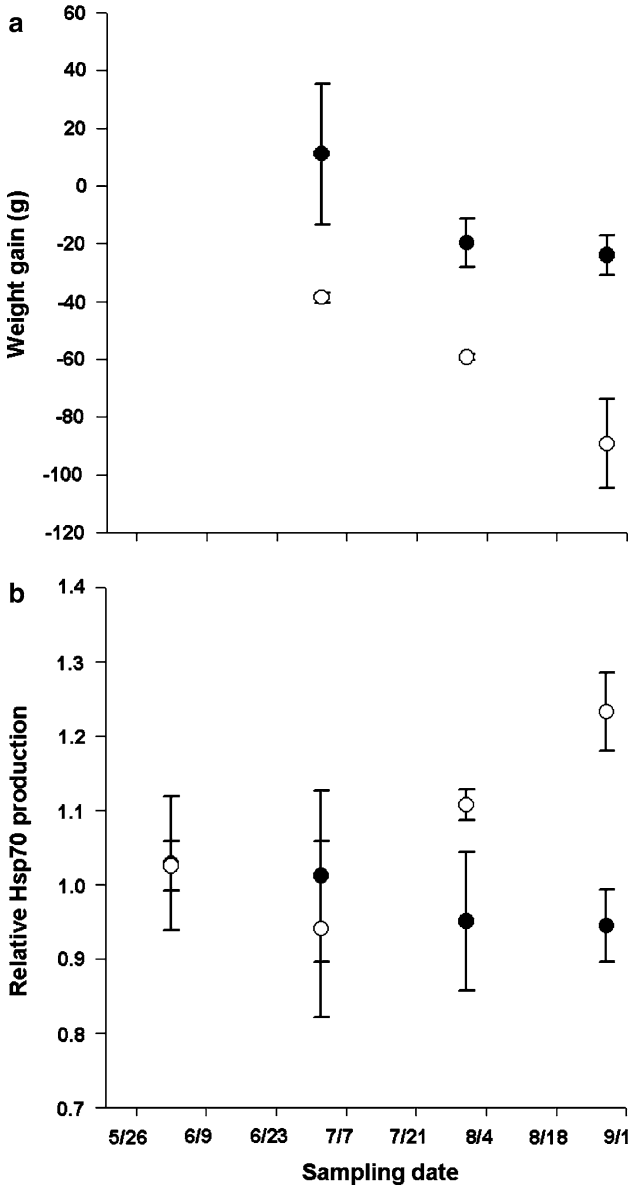

Fig. 4 Weight gain (**a**) and relative Hsp70 (**b**) production of low-edge sea stars (*Pisaster ochraceus*) with mussel food source (*filled circle*) and without mussel food source (*open circle*) during June–August sampling (mean ± SE)

energy under stressful conditions into behaviorally avoiding stress by moving to spatial refuges.

Mussels, as sessile organisms, cannot move to shelter and have to rely instead on defenses against stress to prevent physiological damage or mortality from occurring. Mussels increased their production of Hsp as a plastic response to stress. As also shown by Halpin et al. (2004), mussels increased Hsp production when transplanted to the high edge. While the production of inducible and constitutive Hsp70 did not differ 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 may thus respond "inducibly," contrary to its conventional definition. Total Hsp70, perhaps a more reliable metric of thermal tolerance (Sorte and Hofmann 2005), was higher in mussels transplanted to the high edge.

It is important to note that Hsp was quantified monthly, and if sampling had been conducted more frequently, Hsp could have varied more than we observed. However, while some evidence (e.g. Tomanek and Sanford 2003) indicates that Hsp can respond to temperature on a timescale of days, other studies on *Mytilus californianus* indicate that sampling on timescales of weeks (e.g. Roberts et al. 1997) or months (e.g. Halpin et al. 2004) can also provide insight into sublethal stress. Further, as noted by Helmuth and Hofmann (2001), Hsp production is regulated by exposure to both acute, short-term stress during a single tide as well as chronic, long-term stress due to repeated exposure at low tide. High-edge mussels in our study likely responded at both of these temporal scales, and total Hsp70 was consistently higher in the high-edge mussels on all collection dates, suggesting that these mussels are frequently exposed to stressful aerial conditions. Future studies should be conducted at several different temporal resolutions to better elucidate the complex relationship between aerial temperature and Hsp production. In addition, similar studies could be performed in geographical locations that are warmer or cooler than the Oregon intertidal zone to determine how adaptation to local thermal environments affects the magnitude of the heat shock response. Because thermal history helps determine the magnitude, duration, and induction temperature of the heat shock response (e.g. Helmuth and Hofmann 2001; Halpin et al. 2004; Sorte and Hofmann 2005), animals in warmer regions may be better adapted to hot temperatures and may not upregulate Hsp production until temperatures reach extreme, "stressful" levels.

The timing of reproduction and reproductive potential were strongly influenced by stress. Mussels transplanted to the high edge showed increased spawning activity and a decrease in the number of ripe gametes in their tissue (lower reproductive potential). Many organisms exhibit accelerated maturity (e.g. Dethier et al. 2005) or spawning in response to stress (Schreck et al. 2001; Philippart et al. 2003; Petes et al. 2007). When this occurs, gametes that are spawned may not be mature and viable (Schreck et al. 2001), may be of lower quality (Marshall and Keough 2007), 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 have reported that organisms eliciting a heat shock response exhibit reduced fecundity. We found a negative correlation between Hsp production and reproductive potential in mussels. The production of Hsp is costly to the organism, 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 especially vulnerable to climate change because organisms are exposed to both terrestrial and marine conditions (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. The results of this study indicate that sessile organisms in the intertidal zone can survive exposure to physiological stress. However, sessile organisms living at their tolerance limit may exhibit mortality if defense mechanisms are insufficient for coping with unpredictable, acute (e.g. Petes et al. 2007) or chronic thermal stress. In addition, under climate change scenarios, it is possible that low-edge mussels will be at a disadvantage relative to their sea star predators, as 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 sublethal stress from high aerial temperatures, feeding activity could be reduced, potentially decreasing the interaction strength between sea stars and mussels.

Overall, it seems likely that major shifts in intertidal community structure may result from climate change, 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 changes 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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