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Thermal tolerance of *Strongylocentrotus purpuratus* early life history stages: mortality, stress-induced gene expression and biogeographic patterns

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Abstract In this study, we examined the differential thermal tolerance of *Strongylocentrotus purpuratus* early life history stages by comparing high temperature–induced mortality and the relative levels of the stress-induced gene, *hsp70*, between *S. purpuratus* embryos and larvae from adults collected throughout the species range. There was no significant difference between gastrulae and 4-arm plutei mortality from all sites examined. Furthermore, there was little variability in temperature tolerance across the biogeographic range as southern gastrulae and 4-arm plutei exhibited similar tolerances to northern individuals. Relative levels of *hsp70* mRNA expression did not differ overall between the two developmental stages at each site. Across sites, all gastrulae and 4-arm plutei exhibited maximum *hsp70* expression at approximately 25°C; however, the range of *hsp70* expression was narrower in southern individuals, suggesting they are living closer to their upper thermal limit than northern individuals.

Introduction

In marine ecosystems, temperature is known to set species' distribution limits, creating distribution boundaries on scales that range from the biogeographic (Brown et al. 1996; Pörtner 2002; Sagarin and Gaines 2002) to small scale (Helmuth and Hofmann 2001; Miller et al. 2009). For benthic invertebrates, the effects of temperature have been

studied extensively in adult forms (e.g., Stillman 2003; Somero 2005; Stillman and Tagmount 2009). Studies on earlier life history stages have often characterized them as being more vulnerable to environmental stress such as temperature and UV exposure (Adams and Shick 2001; Edmunds et al. 2001; but see Hamdoun and Epel 2007). Given that climate change models predict changes in the abiotic characteristics of oceans (IPCC 2007; Doney et al. 2009), understanding physiological tolerances of larval forms is particularly important. In addition, while oceanographic processes such as current patterns and upwelling often dictate species range boundaries for organisms with planktonic larvae (Gaylord and Gaines 2000; deRivera et al. 2007), physiological temperature tolerances of the larvae, in conjunction with oceanographic processes, strongly influence the adult biogeography (Zacherl et al. 2003; Sanford et al. 2006).

With its broad biogeographic range, extending from southern Alaska to central Baja California, Mexico, the purple sea urchin *Strongylocentrotus purpuratus* as a species experiences a wide range of environmental temperatures, ranging from 4 to 20°C (Morris et al. 1980) on average, with the potential to experience higher temperatures in shallow intertidal tide pools. *S. purpuratus* urchins have pelagic, free-swimming larvae that remain in the water column for an average of 29–86 days, depending on the latitude and water temperature (Strathmann 1978; Strathmann 1987). After this planktonic larval duration, the larvae metamorphose and settle to become benthic juveniles. It is during the pelagic duration that embryos and larvae are exposed to variable temperature conditions, moving with and potentially through water masses of different temperatures, while also experiencing daily temperature fluctuations in the surface waters. Within larval ecology, the relationship between temperature and

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larval performance has been studied extensively at the whole-organism level. Many studies have investigated the relationship between seawater temperature and larval development, correlating warmer temperatures with faster development times (Fujisawa 1989; Fujisawa and Shigei 1990; Sewell and Young 1999; Staver and Strathmann 2002). There are limits, however, when temperatures exceed physiological capabilities, and embryos and larvae are no longer able to develop and function at these extreme temperatures (Sewell and Young 1999). For example, in *S. purpuratus* specifically, Farmanfarmaian and Giese (1963) noted that embryonic offspring of central Californian adults developed normally between 13 and 20°C; however, eggs fertilized between 5 and 13°C and above 20°C developed abnormally and exposure to 25°C led to mortality shortly after exposure. As embryos and larvae approach these limits of their temperature tolerances, they exhibit a number of responses at both the molecular and whole-organism levels as a result of these temperature extremes.

At the whole-organism level in marine ectotherms, oxygen consumption, heart rate, and mortality are some of the measures that have been used as indices for thermal stress (Stillman and Somero 2000; Pörtner 2002; Stillman 2003). These indices of thermal stress, alongside molecular responses, have been widely studied in adults. The focus more recently has been on relating adult physiological performance to biogeography (Sorte and Hofmann 2004; Osovitz and Hofmann 2005; Somero 2005; see Gaston et al. 2009; Stillman and Tagmount 2009) as macrophysiological studies will provide further insight into how organisms will respond to long-term environmental changes such as climate change (Chown and Gaston 2008). Some larval studies have also explored whole-organism level thermal tolerances and development in relationship to adult habitat and biogeography (Johnson and Babcock 1994; Bingham et al. 1997). Investigating whole-organism level thermal indices, such as mortality, in early life history stages will provide more thorough profiles of marine ectotherm stress responses during their life cycles.

Larvae employ a number of molecular mechanisms to handle thermal stress. One mechanism involved in thermal tolerance is the heat-shock response (HSR). The HSR is a molecular-level gene activation event in response to physiological stresses such as high temperatures (Lindquist 1986) and is induced under thermally stressful conditions in marine organisms (Tomanek and Somero 1999; Hofmann 2005) and terrestrial invertebrates (Lindquist 1986; Krebs and Feder 1998; Goto and Kimura 1998). Of the genes that are activated during the HSR, the inducible form of *hsp70* is one that has been used extensively to quantify the HSR. *hsp70* is a 70kD family of genes that codes for heat-shock proteins (hsps), molecular chaperones that aide

in the refolding or translocation of denatured proteins allowing the cell to maintain proper functions (Lindquist 1986; Morimoto 1998). Many studies have focused their attention on inducible *hsp70* in adult organisms; however, fewer studies have been carried out with early developmental stages.

It is important to note that early embryos and larvae go through a vast number of cellular and morphological changes, often over a relatively short time (*S. purpuratus* spend just a few days transitioning from fertilized egg to pluteus larvae), potentially leaving them differentially susceptible to stress as much of their energy may be allocated to cellular divisions and rearrangements (Krebs and Feder 1998; Morimoto 1998; Hamdoun and Epel 2007). Furthermore, they may respond or manifest defense mechanisms at different capacities. Studies have found that before the gastrula stage, embryos of the Mediterranean sea urchin *Paracentrotus lividus* exposed to high temperatures produced *hsp70* transcript but at such low concentrations it was almost undetectable, and there was no discernable HSR compared to the larvae (Sconzo et al. 1986; Sconzo et al. 1995; Giudice et al. 1999). Similar results were found by Brown et al. (2004) in *Ostreola conchaphila* oyster embryos and larvae: pre-gastrula embryos lacked a measurable HSR. The absence of a measurable HSR is not surprising as, generally, embryos do not begin to transcribe their genome until they have reached the mid-blastula or gastrula stage (Wolpert 1992; Leptin 2005), transcription that includes inducible hsps. Therefore, we focused on gastrulae as the first stage of interest for this study. As it is one of the earliest points at which *hsp70* is expressed in measurable amounts (Sconzo et al. 1986; Sconzo et al. 1995; Giudice et al. 1999), gastrulae may regulate the response differently than older larvae, necessitating an embryo to larva comparison.

In the present study, we examined thermal tolerance of *S. purpuratus* larvae using two biological measures. At the whole-organism level, embryos and larvae were exposed to a gradient of increasing temperatures and mortality was assessed. At the molecular level, *hsp70* mRNA transcript was measured as it is a biological indicator of thermal stress. These two metrics were used to compare *S. purpuratus* at the gastrula and 4-arm pluteus stages to assess developmental differences in thermal tolerance and *hsp70* expression of embryos and larvae spawned from adults collected from locations across the range of *S. purpuratus*. Because the range of *S. purpuratus* is relatively large, with a potential 10–15°C difference in the temperature at which oogenesis occurs in nature between the range boundaries (National Data Buoy Center at NOAA; <http://www.ndbc.noaa.gov/>), embryos and larvae of adults from different thermal regimes may respond differently to thermal stress. Therefore, investigations of thermal tolerance of progeny

from different latitudes may elucidate the relationship between larval physiological tolerances and biogeography.

Methods

Urchin collection and larval culturing

Approximately 20 *S. purpuratus* urchins were collected from 4 sites along the west coast of North America between Oregon and Baja California, Mexico: Fogarty Creek, OR (FC; 44°51'N, 124°00'W), Santa Cruz, CA (SC; 36°53'N, 122°04'W), Santa Barbara, CA (SB; 34°14'N, 119°53'W), and Eréndira, Baja California, Mexico (ER; 31°16'N 116°23'W; Fig. 1). The monthly average temperatures range from 10 to 14°C in central Oregon, 12–15°C near Santa Cruz, 13–17°C in Santa Barbara, and 15–21°C near Eréndira (FC, SC and SB data: National Data Buoy Center at NOAA; <http://www.ndbc.noaa.gov>; ER: Lluch-Cota et al. 2000). The urchins were collected from the subtidal or very low intertidal zone during the height of the *S. purpuratus* spawning season relative to each site (ER: January; SB: February; SC: March; FC: late March–early April). Urchins were brought back to the University of California, Santa Barbara and placed in flow-through tanks set to the temperatures at which they were collected (FC: ~10°C; SC: 12–13°C; SB: ~15°C; ER: ~15°C). Urchins were held for approximately 1–4 days prior to spawning. From each sample site, 3 to 4 pairs of one male and one female were spawned, and each pair was handled separately. Urchins were shaken to induce spawning. If shaking was not sufficient, urchins were injected with ~0.5–1.0 mL of 0.5 M KCl. Females were inverted over beakers of 0.22–0.35 µm filtered sea water (FSW) into which they shed their eggs. Males were dry spawned. The sperm were removed, placed into microcentrifuge tubes, and kept cold on ice for no more than 15 min until fertilization. Eggs were fertilized with a sperm concentration of ~20 µL per 100 µL of eggs in a 1-L beaker of FSW. Fertilization success was checked within 5 min of sperm addition and only eggs with a success rate of 90% or greater were used. After fertilization, zygotes from each separate pair were washed with FSW to remove excess sperm, and each group zygotes were divided equally into 4-, 3-L culture containers at an average density of 500,000 eggs per culture. Embryos and larvae were raised at the temperatures at which the adults were collected relative to each site. Each culture was gently stirred by paddles as described in Strathmann 1987. Culture water was changed once every 24 h, any dead embryos or larvae were removed, and once late prism stage was reached (~45–75 h post-fertilization, depending on the seawater temperature), they were fed a mixed diet of algae (Reed-

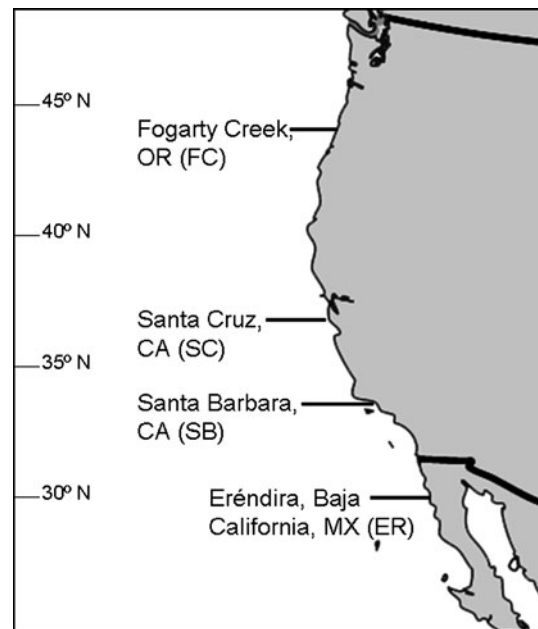


Fig. 1 *Strongylocentrotus purpuratus* collection sites: Fogarty Creek, OR (FC), Santa Cruz, CA (SC), Santa Barbara, CA (SB), Eréndira, Baja California, MX (ER)

Mariculture; Instant Algae Shellfish Diet 1800: *Isochrysis*, *Pavlova*, *Tetraselmis*, and *Thalassiosira weissflogii* at ~2 billion cells mL⁻¹) once before being sampled at the 4-arm pluteus stage (~72–114 h p.f. depending on the seawater temperature).

Larval sampling

Once cultures reached the gastrula (36–70 h p.f. depending on rearing temperature) or 4-arm pluteus stage (72–114 p.f.), gastrulae and 4-arm plutei from each male–female pair were sampled from their respective cultures and divided into 33-, 20-mL scintillation vials. To do this, gastrulae or larvae from 2 of the 3-L culture containers were combined and mixed (the remaining 2 culture containers were combined, mixed, and then individuals were split into 4-, 3-L cultures to reduce the density for the developing gastrulae that would later be sampled for 4-arm plutei). From this larger stock, 33 samples of either 26,000–32,000 gastrulae or 4,000–5,000 4-arm plutei were measured out volumetrically and concentrated down to 1 mL for each of the 33 vials. The concentrated 1 mL of either gastrulae or larvae was added to a 20-mL scintillation vial that contained 9 mL of FSW. The high densities in each scintillation vial were necessary to ensure enough material was available for the genomic analysis of *hsp70* mRNA levels, a method that is not uncommon for attaining adequate levels of genomic material for heat-shock analysis (Bédard and Brandhorst 1986; Sconzo et al. 1995; Sconzo et al. 1997; Brown et al. 2004).

Thermal tolerance trials

A temperature gradient heat block was used to conduct the thermal tolerance experiment. The gradient consisted of 11 rows of wells of different temperatures ranging from 10 to 32°C (Table 1). The following procedure was run separately for each of the 3–4 sets of fertilized eggs: three of the 20-mL vials containing gastrulae or 4-arm plutei were placed in each temperature row, representing replicates of temperature. The embryos or larvae remained at these temperatures for 1 h, at which point they were removed and scored under a light microscope. To score, the embryos or larvae were mixed by gentle stirring and 1 mL was taken from each vial and, using a densitometer and light microscopy, one hundred larvae were counted to obtain percent mortality. Swimming larvae or larvae that exhibited cilia movement were scored as alive, and those with no cilia movement were scored as dead. In order to verify that 1 h was sufficient time for a thermal challenge and to ensure that embryos or larvae scored as dead after 1 h were actually dead, a subset of gastrulae and 4-arm plutei from SB adults were exposed to a 1-h heat shock at temperatures between 15 and 32°C as described in Table 1, followed by recovery periods of 1, 2, 4, and 26 h. As mortality after each recovery period was not significantly different from the mortality observed immediately after the 1-h heat shock (data not shown), a 1-h heat shock was deemed sufficient for assessing mortality.

Total RNA extraction

After gastrulae and 4-arm plutei were counted for the mortality assessments, the FSW was filtered off for total RNA extraction. Each sample was homogenized in 500 µL of TRIzol[®] Reagent (Invitrogen, Carlsbad CA, USA) and stored at –80°C until the RNA was extracted. Total RNA was extracted following the TRIzol manufacturer's protocol with the following adjustments: After the initial TRIzol extraction, a second extraction using 100% chloroform was used. In addition to the precipitation of total RNA in the recommended volume of 100% isopropanol, a salt solution for precipitation (initial concentration: 1.2 M NaCl, 0.8 M disodium citrate) of equal volume was used. The RNA was re-suspended in nuclease-free water, and their

concentrations were measured using a Nanodrop[®] ND-1000 full-spectrum UV/vis spectrophotometer.

hsp70 analysis using qPCR

Once the concentrations of total RNA were determined, 100 ng was reverse transcribed for cDNA synthesis using oligo dT and the ImProm-II[™] Reverse Transcription System (Promega) following the manufacturer's protocol. Of the 11 heat-shock temperatures used in the thermal tolerance trials (Table 1), temperatures between 10 and 20°C were excluded for each site for *hsp70* analysis because no significant upregulation was anticipated between 10 and 20°C based on previous *hsp70* expression data in adult *S. purpuratus* (Osovitz and Hofmann 2005). Quantitative real-time PCR (qPCR) was used to determine the relative levels of *hsp70* mRNA following the method described in Osovitz and Hofmann (2005) with the following adjustments. *Elongation factor 1-α* (*efl-α*) was used as a control gene as its mRNA levels did not change significantly across temperature treatments. Primers for *efl-α* and *hsp70* were designed using Bio-Rad Beacon designer software with the published *S. purpuratus* sequences for *hsp70* (GenBank accession no. XR_026557.1) and *efl-α* (GenBank accession no. NM_001123497.1). Primers for *hsp70* were as follows: *hsp70* sense—5'-AAGATATGAGGTCCAACCCAAGAT-3'; anti-sense—5'-TGCTGAAGCACTGCTTGACA-3'. Primers for *efl-α* were as follows: *efl-α* sense—5'-CAACGAAATCGTCAGGGAGGTC-3'; anti-sense—5'-AGATTGGGATGAAGGGCACAG-3'. The concentration of the *efl-α* primer used was 0.5 µM. Briefly, quantitative real-time PCR was run in 20-µL SYBR green supermix (Bio-Rad) reactions containing 2 µL of template cDNA. The qPCRs were run with the following protocol: 1 cycle at 95°C for 3 min, 40 cycles at 58°C for 10 s and 95°C for 1 min, 1 cycle at 55°C for 1 min. Relative mRNA levels were calculated as follows. *hsp70* and *efl-α* Ct values (the value that represents the threshold cycle at which a threshold concentration of transcript is reached) were normalized to a single *S. purpuratus* larval standard run on every qPCR plate. The *hsp70* Ct values were then normalized to the corresponding *efl-α* values, resulting in the reported relative *hsp70* concentrations.

Statistical analysis

Statistical analyses were performed using JMP 7.0 (SAS). The LT₅₀, the lethal exposure temperatures at which 50% of the embryos and larvae died, was used as a measure of mortality in the thermal tolerance trials. To determine the LT₅₀, logistic regressions were calculated from the binary

Table 1 Temperatures of 1-h heat exposure (°C) for each site

Site	Temperatures of 1-h heat exposure (°C)
FC	10.5, 13.0, 15.0, 17.2, 19.3, 21.1, 23.1, 25, 26.9, 28.8, 30.8
SC	13, 15, 16.8, 18.3, 20.1, 21.1, 23.7, 25.4, 27.2, 29.1, 31.0
SB	15, 16.9, 18.7, 20.3, 21.8, 23.4, 25, 26.7, 28.4, 30.1, 31.9
ER	15, 16.9, 18.7, 20.3, 21.8, 23.4, 25, 26.7, 28.4, 30.1, 31.9

(alive or dead) mortality data from both gastrulae and 4-arm plutei at each site. For each developmental stage, the upper and lower 95% confidence intervals for the LT_{50} s were determined from the logistic regressions. Linear regressions were calculated within developmental stage to determine latitudinal trends in LT_{50} temperatures by fitting the LT_{50} data by latitude, and significant differences were indicated by non-overlapping 95% confidence intervals. A Student's *t*-test was used to determine statistical significance between gastrulae and 4-arm plutei LT_{50} s.

The *hsp70* expression data were transformed using a Box–Cox transformation to meet the assumptions for a two-way ANOVA with temperature and developmental stage as factors with male–female pair nested within temperature. Tukey–Kramer Honestly Significant Difference tests were run for post hoc analysis to characterize the *hsp70* induction profile and identify the thermal set-points for each developmental stage at each site.

Results

Thermal tolerance trials

The thermal tolerance range was determined by exposing embryos and larvae to a range of temperatures for 1 h, and thermal tolerance was measured as percent mortality. Overall, embryos and larvae exhibited an abrupt threshold in mortality, regardless of the biogeography of the adults (Fig. 2). For all sites, mortality was consistently very low until the exposure temperature reached 29–30°C, at which point mortality increased significantly and reached 100% mortality within 1–2°C of the initial increase (Fig. 2). Gastrulae and 4-arm plutei from FC and SC adults experienced 100% mortality at approximately 31°C, while gastrulae and 4-arm plutei from SB and ER adults experienced 100% mortality at approximately 32°C.

Given the sharp increase in mortality and the coarse resolution of mortality temperatures tested, the LT_{50} s were estimated from logistic regression curves. Accordingly, the LT_{50} temperatures for FC, SC, SB, and ER gastrulae were 30.3, 30.1, 30.7, and 30.9°C, respectively (Table 2). The LT_{50} temperatures for FC, SC, SB, and ER 4-arm plutei were 29.7, 30.0, 31.0, and 31.0°C, respectively (Table 2). There were no significant differences in mortality between gastrulae and 4-arm plutei for all sites (Student's *t*-test assuming unequal variances: $P = 0.9121$). A linear regression with 95% confidence intervals showed there was a relationship between LT_{50} and site for 4-arm plutei, although not significant ($r^2 = 0.7918$, $P = 0.1101$), and no relationship between LT_{50} temperature and site for gastrulae ($r^2 = 0.4692$, $P = 0.315$).

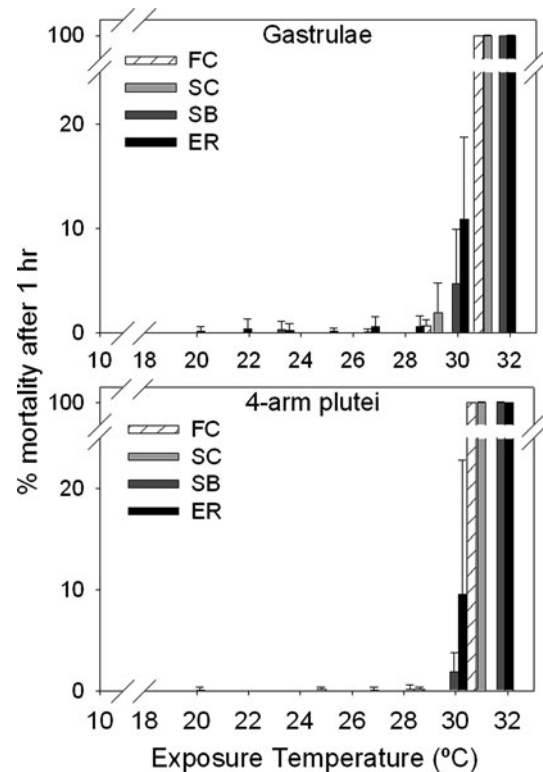


Fig. 2 Thermal tolerance plots showing percent mortality in *S. purpuratus* gastrulae and 4-arm plutei after 1-h heat shock from adults from FC (gastrulae, $n = 2$; 4-arm plutei, $n = 3$ where hereafter n represents the number of male–female pairs spawned for each batch of embryos and larvae); SC (gastrulae, $n = 3$; 4-arm plutei, $n = 3$); SB (gastrulae, $n = 3$; 4-arm plutei, $n = 4$); and ER (gastrulae, $n = 3$; 4-arm plutei, $n = 4$). Error bars indicate standard error of male–female pair replicates

Table 2 LT_{50} temperatures (°C) for *S. purpuratus* gastrulae and 4-arm plutei after a 1-h heat exposure

Stage	FC	SC	SB	ER
Gastrulae	30.3	30.1	30.7	30.9
4-arm plutei	29.7	30.0	31.0	31.0

Expression of *hsp70* mRNA

The induction profile for *hsp70* mRNA expression was similar between developmental stages and at all four sites (Fig. 3). The overall two-way ANOVA results were significant for each site ($P < 0.05$) except SB ($P = 0.1700$). However, temperature had a highly significant effect in SB samples ($P = 0.0007$; Table 3). The discrepancy may be a result of the anomalously high expression at the control temperature from possible temperature stress during sample handling. The results from the two-way ANOVA for each site showed that temperature was the only significant effect (all $P < 0.05$) except at SC where both temperature ($P < 0.0001$) and stage ($P = 0.0218$) were significant

Fig. 3 Relative *hsp70* mRNA expression levels after 1-h heat shock in *S. purpuratus* gastrulae and 4-arm plutei of adults from FC (gastrulae, $n = 2$; 4-arm plutei, $n = 3$); SC (gastrulae, $n = 3$; 4-arm plutei, $n = 3$); SB (gastrulae, $n = 3$; 4-arm plutei, $n = 4$); and ER (gastrulae, $n = 3$; 4-arm plutei, $n = 4$). Values are relative to a common *S. purpuratus* larval standard and normalized to *ef1- α* . Error bars indicate standard error of male–female pair replicates

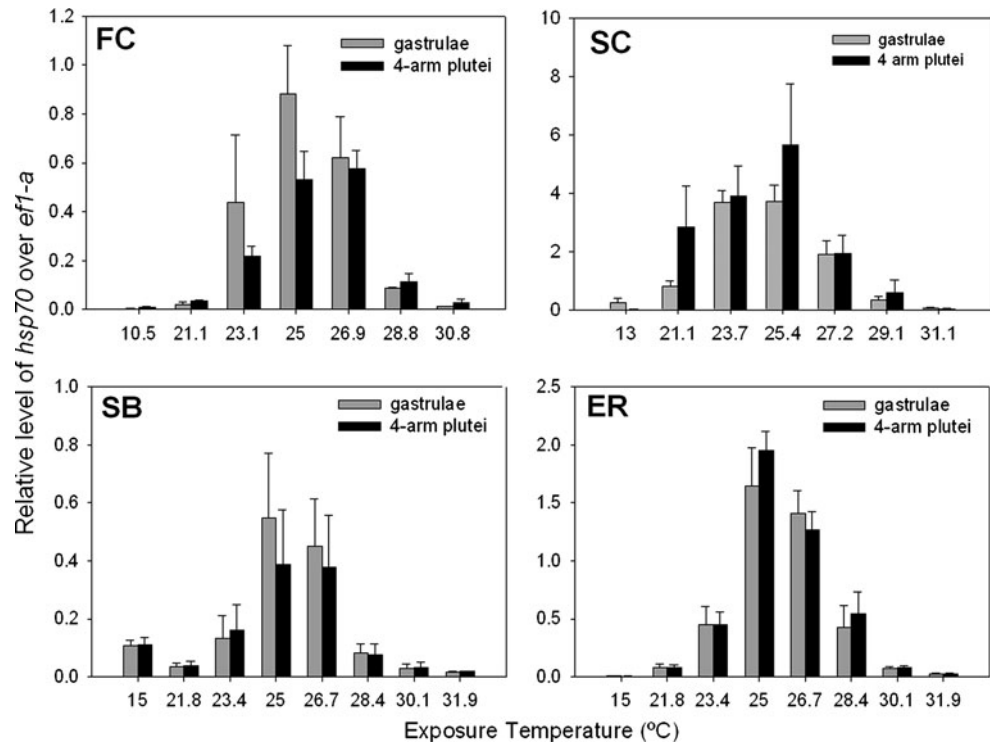


Table 3 Two-way ANOVA results testing for the effects of temperature and developmental stage on *hsp70* expression in *S. purpuratus* progeny from FC, SC, SB, and ER

	<i>df</i>	<i>F</i>	<i>p</i>
FC			
Temperature	6	41.5040	0.0001*
Stage	1	4.4644	0.0791
Mate-pair[temperature]	14	0.9890	0.5434
Temperature × stage	6	1.2608	0.3928
SC			
Temperature	6	23.4673	<0.0001*
Stage	1	6.7849	0.0218*
Mate-pair[temperature]	14	0.8158	0.6459
Temperature × stage	6	1.3802	0.2932
SB			
Temperature	7	6.8587	0.0007*
Stage	1	0.0236	0.8798
Mate-pair[temperature]	24	0.2243	0.9995
Temperature × stage	7	0.3497	0.9182
ER			
Temperature	7	12.8504	<0.0001*
Stage	1	0.2190	0.6476
Mate-pair[temperature]	24	1.6673	0.1694
Temperature × Stage	7	0.4658	0.8425

* Significant at the 0.05 level

(Table 3). However, the significance in stage at SC was solely driven by differences in expression at 13 and 21.1°C. Variability due to mate-pair was not significant at any site (all $P > 0.05$), and there were no interactions between factors at any site (all $P > 0.05$; Table 3). Significant increases in expression above the site-specific controls occurred between 21 and 30°C, with no significant variation in peak expression for each site. Thermal set-points for the initial temperature at which *hsp70* expression was significantly different from the control, T_{on} , and peak *hsp70* expression, T_{max} , are summarized in Table 4. For the FC samples, the T_{on} was 21.1°C for both developmental stages

Table 4 *hsp70* expression thermal set-points

Site	Stage	T_{on} (°C)	T_{max} (°C)
FC	Gastrulae	21.1	25
	4-arm plutei	21.1	26.9
SC	Gastrulae	21.1	23.7–25.4
	4-arm plutei	21.1	25.4
SB	Gastrulae	21.8–23.4	25–26.7
	4-arm plutei	21.8–23.4	25–26.7
ER	Gastrulae	21.8	25
	4-arm plutei	21.8	25

The initial temperature at which *hsp70* expression was significantly different from the control, T_{on} , and peak *hsp70* expression, T_{max}

(Fig. 3). Peak *hsp70* expression (T_{\max}) occurred at 25°C for gastrulae and 26.9°C for 4-arm plutei; however, the differences in T_{\max} were not significantly different (Tukey–Kramer HSD, $P = 0.9958$; Fig. 3). In the SC samples, the T_{on} occurred at 21.1°C for both stages and the T_{\max} of *hsp70* expression occurred between 23.7 and 25.4°C for gastrulae and 25.4°C for 4-arm plutei; however, differences between the T_{\max} were not significantly different (Tukey–Kramer HSD, $P = 1.0$; Fig. 3). For the SB samples, the T_{on} occurred between 21.8 and 23.4°C, while the T_{\max} occurred between 25 and 26.7°C for both gastrulae and 4-arm plutei (Fig. 3). Lastly, in the ER samples, T_{on} occurred at 21.8°C and peak *hsp70* expression occurred at 25°C for both gastrulae and 4-arm plutei (Fig. 3). Due to a shift in the Ct expression values of the internal standard between sites, comparisons in the magnitude of *hsp70* expression between sites could not be confidently made.

Comparing the range between the absolute site-specific control temperature and T_{\max} , gastrulae and 4-arm plutei from FC had a range of $\sim 15^\circ\text{C}$, SC had a range of $\sim 13^\circ\text{C}$, and SB and ER both had a range of 10°C (Fig. 4). For simplicity, data were plotted from 4-arm plutei only as there were no significant differences between the two stages in *hsp70* expression profiles.

Discussion

The aim of this study was to compare the thermal tolerance of two early life history stages of *S. purpuratus*. Additionally, we sought to examine how the latitude at which the adult females went through oogenesis affected the *hsp70* expression of the progeny. As the environmental characteristics encountered by the adults during oogenesis has the potential to affect offspring performance in most organisms (Mousseau and Fox 1998; Bacigalupe et al. 2007), including marine organisms (Li and Brawley 2004; Marshall 2008), for the present study embryos were obtained from adults that originated from various biogeographic locations throughout the recorded range of adult *S. purpuratus* (Morris et al. 1980; Tegner 2001) during the spawning season. Oogenesis in *S. purpuratus* typically occurs in autumn/winter (Gonor 1973; Pearse et al. 1986) when the temperatures range from an average of $\sim 12^\circ\text{C}$ in central Oregon (National Data Buoy Center at NOAA; <http://www.ndbc.noaa.gov/>) to an average of $\sim 19^\circ\text{C}$ near Ensenada, MX (Lluch-Cota et al. 2000). The salient findings of this study were as follows: (1) for thermal tolerance, measured as $\text{LT}_{50\text{s}}$, there were no significant latitudinal trends, (2) for the two stages tested, there was no detectable difference in response for gastrulae and larvae, (3) using the expression of *hsp70* as a bioindicator of response to acute thermal stress, embryos and larvae displayed similar

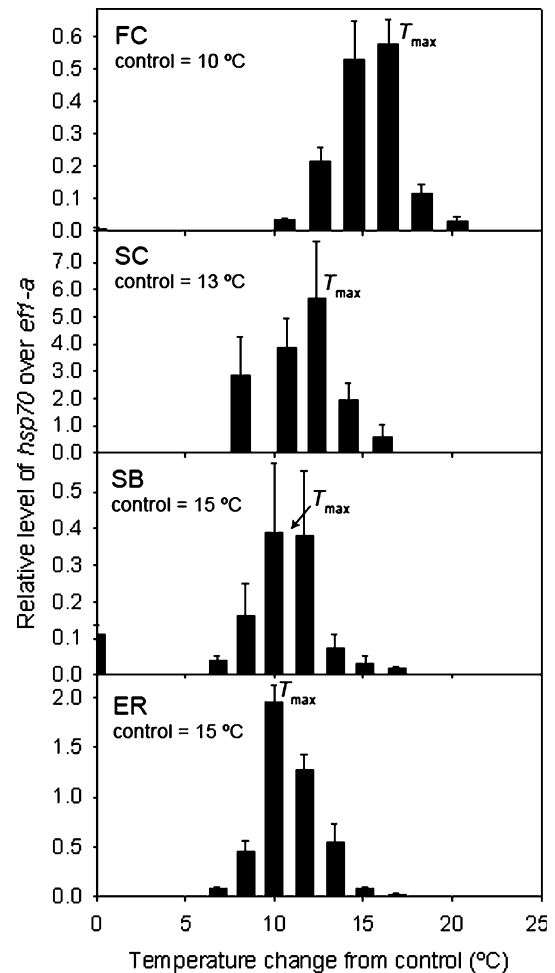


Fig. 4 Levels of *hsp70* mRNA expression in 4-arm plutei from each site relative to a common *S. purpuratus* larval standard and normalized to *efl-α*. Data were plotted from 4-arm plutei only as there were no significant differences between the gastrulae and 4-arm plutei in *hsp70* expression profiles. Values along the *x-axis* represent the change in heat exposure temperature from the control temperature used at each site

patterns of gene expression with no patterns detected across the latitudes at which adults were collected, and (4) there were subtle latitudinal trends in *hsp70* thermal set-points.

Overall, the results of this study suggest that different early developmental stages have similar whole-organism and molecular-level thermal tolerances across the range of purple urchins along the coast of the northeast Pacific.

Thermal tolerance of early life history stages: $\text{LT}_{50\text{s}}$

In this study, we observed acute shifts in survivorship where mortality increased from near zero to 100% within a few degrees. Although, not always reflecting ecological average temperatures, these LT_{50} assays were employed in

order to identify thresholds in tolerance, as has been done in numerous physiological studies (Stillman and Somero 2000; Somero 2002; Miller et al. 2009). Examinations of larval temperature tolerance have found similar abrupt increases in mortality (Brown et al. 2004; Kalosaka et al. 2009; Zippay and Hofmann 2010). For example, Boon-Niermeijer and van de Scheur (1984) exposed *Lymnaea stagnalis* snail embryos and larvae to 1-h thermal challenges, and all stages studied experienced an abrupt increase in mortality between 39 and 40°C. Sewell and Young (1999) observed abrupt decreases in survival in *Echinometra lucunter* urchin embryos and larvae exposed to 2-h thermal challenges; all stages experienced 100% survival until 38°C at which point mortality rapidly increased until ~41°C when all stages exhibited 100% mortality. The acute shift in survivorship observed in this study suggests that the embryos and larvae may have reached the critical thermal threshold for the molecular mechanisms regulating thermal tolerance; this is discussed in more detail in the following section.

The mortality results between stages did not differ significantly at any of the sites. Additionally, the LT_{50} and overall mortality profiles between stages were not significantly different, results that were similar to findings in embryos and larvae of the tropical sea urchin, *Echinometra lucunter* (Sewell and Young 1999). However, contrasting outcomes have been found in other species. Kalosaka et al. (2009) exposed 6 life history stages of the Mediterranean medfly, *Ceratitis capitata*, ranging from early embryos to adults to 1-h heat shocks at a range of temperatures and found different patterns of mortality based on life history stage. Sulkin and McKeen (1994) compared temperature-induced mortality in five zoeal stages between four congeneric *Cancer* crabs raised at 10, 15, and 20°C and found no clear pattern of mortality with temperature between the zoeal stages; different zoeal stages experienced different levels of mortality in all species investigated. The authors suggest that the differences in mortality may lead to dissimilar susceptibilities to thermal stress between developmental stages; however, it was not clear whether these unequal vulnerabilities were ecologically significant. In general, there appear to be no clear patterns in temperature-induced mortality between early life history stages across species; mortality during early development appears to be species specific, and the present data further contributes to our understanding of intraspecific temperature tolerances between early developmental stages. For the *S. purpuratus* gastrulae and 4-arm plutei investigated here, the similarity in mortality profiles suggests that the likelihood of stage-specific mortality during early development, which may affect settlement and recruitment success, is minimized.

hsp70 expression and developmental stage comparisons

Gastrulae and 4-arm plutei both upregulated *hsp70* similarly with no significant differences in expression, irrespective of the characteristic differences between these two life history stages. Gastrulae are swimming embryos that are in the process of forming the most basic structures (mouth, gut, and anus) while 4-arm plutei are feeding, swimming larvae with well-developed systems and defined structures (gastrointestinal system, early larval skeleton, etc.). Previous studies have shown that early urchin embryos do not display a heat-shock response before the gastrula stage (Sconzo et al. 1995; Giudice et al. 1999). This may, in part, be due to the fact that, for the embryo, much of the energy is expended on the proper formation of the germ layers and embryonic and larval structures. Because protein folding and molecular chaperone activities carry large energy requirements (Hochachka and Somero 2002) and because the over-expression of hsp70 can interfere with or inhibit development (Krebs and Feder 1998; Hamdoun and Epel 2007), the embryo would presumably put less energy toward mounting a defense response like the HSR and put more energy into further development than later, more developed stages. However, given the current data, this does not appear to be the case as once the embryo reached gastrula stage, it expressed *hsp70* at equal magnitudes as older, more developed plutei.

While the number of studies investigating stress-induced gene expression between early life history stages is few, the similarity in *hsp70* expression profiles between the two developmental stages studied here is similar to findings by previous studies investigating Hsp70 protein expression between early life history stages. When Botton et al. (2006) exposed *Limulus polyphemus* horseshoe crab embryos and larvae to a 3-h heat shock, both stages had Hsp70 protein present in relatively equal amounts. Additionally, Boon-Niermeijer et al. (1988) found that when *Lymnaea stagnalis* snail gastrulae and trochophore larvae were exposed to temperature stress, both stages had similar relative values of Hsp70 protein. This suggests that particular stages in early development are similar in their capacity to initiate a HSR through the upregulation of *hsp70* and translation of Hsp70 protein. The similarity in expression potentially helps ensure that the embryonic and larval stages are equally equipped to withstand temperature stress until settlement and metamorphosis.

Biogeographic comparisons: macrophysiology, larval performance, and *hsp70*

Measuring performance across large spatial scales—macrophysiology (Gaston et al. 2009)—has been used to examine response to environmental temperature in a

variety of marine invertebrates (e.g. Stillman and Somero 2000; Sorte and Hofmann 2004; Somero 2005). In this study, we measured mortality and LT_{50} temperatures as indices for thermal tolerance and found no clear latitudinal trend. The lack of a trend suggests *S. purpuratus* embryos and larvae have similar temperature tolerances at the whole-organism level throughout the species range. The number of studies comparing embryonic and larval thermal tolerances across latitude is few, and a similar study by Zippay and Hofmann (2010) on *Nucella ostrina* larvae, which develop in egg capsules attached to intertidal substrates, found contrasting results. In that study, *Nucella ostrina* egg capsules from across a large latitudinal range were collected and exposed to a range of thermal challenges in the laboratory and a strong latitudinal trend in LT_{50} temperatures was found. It is important to note, however, that the contrast in latitudinal trends between these studies may be a result of planktonic versus non-planktonic early life history stages as discussed below.

In this study, we also measured the expression of *hsp70*, a common gene in the urchin defenseome (Goldstone et al. 2006) that is rapidly upregulated in response to elevated temperature, and compared the expression in gastrulae and larvae across a large spatial scale. The *hsp70* expression profiles of both gastrulae and 4-arm plutei in this study did not differ between sites. The overall similarity in thermal set-points (i.e., T_{on} and T_{max}) across sites found in this study contrasts with a similar study by Osovitz and Hofmann (2005) that found a latitudinal trend in thermal set-points in adult *S. purpuratus*, where southern individuals exhibited a T_{max} of *hsp70* expression at a higher temperature relative to northern individuals. Interestingly, studies that have found latitudinal trends in the HSR have done so predominately in adults where the shifts in thermal set-points highlighted local acclimatization along latitudinal or vertical habitat gradients and, thus, highlighted the plasticity in their thermotolerance (Tomanek and Somero 1999; Helmuth and Hofmann 2001; Sorte and Hofmann 2004; Osovitz and Hofmann 2005; Henkel and Hofmann 2008). Additionally, the organisms examined in these studies were predominately benthic or sessile with restricted ranges of movement, conditions under which local acclimatization is more likely to occur.

Planktonic early life history stages, on the other hand, are the dispersal stage and are less likely to have experienced set temperature regimes to which they could become acclimatized to, as they are not localized to any specific area until they settle. Under these circumstances, the lack of a latitudinal trend in this study may suggest that the upregulation of *hsp70* and, consequently, the HSR, is a genetically fixed response in *S. purpuratus* early life history stages. While the thermal set-points of the HSR have been shown to typically display variable yet significant

plasticity (Barua and Heckathorn 2004), some exceptions have been found. Similar to the present study, adult *Mytilus galloprovincialis* mussels from across a large latitudinal gradient exhibited no significant variation in T_{max} after exposure to a 1-h thermal challenge, suggesting a fixed genetic response (Dutton and Hofmann 2009). Alternatively, the relatively identical T_{max} values across latitude found in this study may suggest that the early life history stages have a universal temperature tolerance as a mechanism to increase their likelihood of survival during the planktonic larval duration before settlement.

The similarity between gastrulae and 4-arm plutei in their mortality and *hsp70* expression profiles further reflects their similar timing of *hsp* gene activation and cessation, and their capacity for the HSR. Mortality did not begin to increase significantly until after *hsp70* expression reached the T_{max} value and began to cease. After the T_{max} was reached (approximately 25°C), both gastrulae and 4-arm plutei likely exhibited failure of the HSR activation machinery. The HSR is controlled by transcriptional activation via heat-shock transcription factors (HSFs) and, although this regulatory step is known to be subject to some thermal acclimation (Buckley and Hofmann 2002; Buckley and Hofmann 2004), the process likely failed at approximately 25°C in both developmental stages, suggesting that the HSR transcriptional activation machinery is not only a fixed response but is not differentially regulated during different developmental stages. Subsequently, the embryos and larvae in this study reached their critical thermal thresholds at approximately 25°C and mortality increased rapidly, a pattern typical of the HSR (Tomanek and Somero 1999; Brown et al. 2004; Kalosaka et al. 2009).

Embryos and larvae of both SB and ER exhibited a smaller range of *hsp70* expression relative to the northern sites. The range of temperatures from the control temperature (which was equal to the temperature at which the adults were collected and therefore similar to conditions the embryos and larvae would have encountered in nature) to the temperature at which *hsp70* expression ceased was smaller in the southern sites (15–32°C) than the range for the northern sites (FC: 10–31°C; SC: 13–31°C). More specifically, the range of expression between the site-specific control temperature and the T_{max} of 25°C at each site was smaller for southern individuals. These data suggest that the embryos and larvae from southern sites are closer to their upper thermal limits relative to northern individuals as there was no shift in the thermal set-points of the HSR. The narrower range in tolerance exhibited by the southern progeny would presumably make them more susceptible to increases in environmental temperatures, a similar conclusion drawn from other studies on marine ectotherms living close to their upper thermal limits (Stillman 2003; Tomanek 2008; Somero 2010).

Conclusion

In measuring thermal tolerance and *hsp70* gene expression in two early life history stages of *S. purpuratus*, the results of this study show that gastrulae and 4-arm plutei respond similarly to thermal stress. Additionally, while the temperatures at which both gastrulae and 4-arm plutei began to express maximum *hsp70* expression and experienced significant mortality were higher than their average habitat temperature, these responses help characterize the upper thermal limits of the early life history stages and provide insight into how they may respond to temperature changes in surface waters where temperatures are typically significantly warmer and where the embryos and larvae are concentrated. Investigating how marine invertebrate larvae respond to thermal stress at the whole-organism and molecular levels will assist in predicting whether and how early life history stages are likely to adjust to environmental shifts in temperature.

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