

Early life stages are not always the most sensitive: heat stress responses in the copepod *Tigriopus californicus*

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ABSTRACT: Because of their complex life histories, different life stages of many marine invertebrates may be exposed to varying environmental challenges. Ultimately, the life stage that is least tolerant of its environment will determine the species' abundance and distribution. The intertidal copepod *Tigriopus californicus* lives in high intertidal pools along the Pacific coast of North America. Unlike most other invertebrates, the different life stages of *T. californicus* all share the same tidepool habitat. To determine physiological tolerances of various life history stages of this species, we examined responses to acute heat stress in nauplii, copepodids, and adults from 6 populations along a latitudinal gradient. Results show that early developmental stages (nauplii and copepodids) are generally more tolerant than adults. Our results contrast with the widely accepted generalization that larval forms are more sensitive to physical stressors than adults. As previously observed in adults, nauplii and copepodids from southern populations survive higher temperatures than those from northern populations. Acute heat stress was found to delay development but did not affect adult size. We hypothesize that variation in the thermal tolerance of early life stages among intertidal species reflects ecological differences in larval habitats: where larvae remain in the intertidal zone and experience the same high temperatures as adults, selection will favor high larval thermal tolerance, while in species with planktonic larvae, the buffered temperature regime of the water column might relax such selection, and thermal tolerance will be highest in the more exposed intertidal adults.

KEY WORDS: Thermal tolerance · *Tigriopus* · Larvae · Copepod · Intertidal

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INTRODUCTION

The life cycles of many marine invertebrates include developmental stages that can vary greatly in all aspects of ecology. Some life stages inhabit completely different environments, as in the case where sedentary benthic adults broadcast spawn, and the offspring undergo development as planktonic embryos and larvae (Miller et al. 2013). Given such changes in organismal ecology over a species' life cycle, various life stages may experience substantially different environmental extremes. Consequently, natural selection for tolerances to those

extremes may also vary across life stages and potentially result in stage-specific tolerances to stress. Although there have been numerous studies of the physiological tolerances to heat stress of many invertebrates, data across all life stages are available for relatively few marine species, including some mollusks (e.g. Verween et al. 2007), ascidians (Pineda et al. 2012), and arthropods (Miller et al. 2013). Most of these studies have concluded that early life stages are generally less tolerant of abiotic environmental extremes than adults of the same species. However, recent studies show that this pattern is not universal; for example, early life stages of the mollusk *Crep-*

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idula fornicata are more tolerant of heat stress than adults (Diederich & Pechenik 2013).

Because larval tolerances to thermal stress can differ from those of conspecific adults, consideration of all life stages is necessary for understanding how species persist in their local environments. For example, although geographic ranges of many species are typically attributed to adult thermal tolerances, Walther et al. (2013) recently pointed out that thermal sensitivity of larval stages can act as a physiological barrier for dispersal. They found that larvae of different gooseneck barnacle *Pollicipes elegans* populations have different thermal maximum ranges, and these reflect the environment of their respective habitats. Larvae from cooler regions north and south of the equator cannot disperse across the equator, where temperature is higher, leading to genetic differentiation of populations (Plough & Marko 2014). Similar patterns have also been found in other intertidal species such as mussels (Hilbish et al. 2000) and limpets (Koufopanou et al. 1999).

In addition to inducing mortality, heat stress may also affect other fitness parameters, such as rates of growth and larval development (Rothlisberg 1979, Preston 1985, Bryars & Havenhand 2006, Bermudes & Ritar 2008, Roberts et al. 2012). Although increasing temperature can speed up development over certain temperature ranges, higher (stressful) temperatures can also slow developmental rates (Roberts et al. 2012). Stressful temperatures can be defined as the temperature range that reduces the performance of an organism (Pörtner & Knust 2007). Reduction in performance can include slower growth and development, or it can be as severe as mortality. For meroplankton, reduced developmental rates lead to longer larval duration, which in turn increases the risk of starvation and predation (Morgan 1995). Understanding the effects of thermal stress across all life stages will allow us to better predict the fate of populations and species in a warming global climate.

The copepod *Tigriopus californicus* provides an excellent system for studying thermal adaptation. *T. californicus* has a broad geographic range extending from central Baja California, Mexico (Ganz & Burton 1995, Peterson et al. 2013), to southeastern Alaska (Dethier 1980), and several studies have revealed extensive genetic differentiation among populations across this range (Burton & Lee 1994, Edmands 2001, Willett & Ladner 2009). Since the geographic range of *T. californicus* spans a broad climatic gradient, responses to thermal stress across populations of this species have been the focus of recent research

(Edmands & Deimler 2004, Willett 2010, Kelly et al. 2012, 2013, Schoville et al. 2012, Pereira et al. 2014). These studies show a clear pattern, with adult copepods from southern populations surviving higher temperatures than adult copepods from northern populations, an observation consistent with local adaptation of populations to their respective habitats. Notably, local adaptation is most evident in population differences in survivorship following acute (short exposures of ~60 min duration) thermal stress (e.g. Willett 2010). Ecologically, this is consistent with the fact that average temperatures of tidepools in California are generally moderate, ranging from approximately 10 to 20°C, but thermal maxima can reach over 40°C occasionally in summer months (Kelly et al. 2012). Because these maximum temperatures are only achieved during relatively brief periods in the middle of hot, sunny days, natural selection will favor mechanisms for short-term tolerance to thermal extremes, and these extremes are more frequent in the southern portion of the geographic range.

To date, no studies have examined thermal stress responses among the larval stages of *T. californicus*. If pre-reproductive stages of *T. californicus* are more sensitive to heat stress than adults, the species could be more vulnerable to increasing temperature than previously predicted. However, an interesting aspect of the *T. californicus* system is that, in contrast to most other marine invertebrates with free-swimming larvae, all life stages of *T. californicus* occupy the same tidepool habitats and are subjected to the same extreme thermal regimes. Consequently, although the larvae of many marine invertebrates experience the relatively buffered thermal environment of the pelagic water column, *T. californicus* larvae do not. We hypothesize that larval thermal sensitivities will reflect the thermal environments to which they are exposed. Under this hypothesis, *T. californicus* larvae are expected to have higher thermal tolerances than larvae that develop in the water column.

In this experiment, we addressed three main questions: (1) Do different life stages of *T. californicus* (nauplius, copepodid, and adult) vary in tolerance to thermal stress? (2) Does heat tolerance in nauplii and copepodids vary among populations? (3) Does acute thermal stress have any long-term effects on larval development and adult size? In the absence of extensive migration, populations can only persist where all life stages are tolerant to local conditions; hence, understanding patterns of thermal sensitivity in different life stages and populations may provide us with insight into how *T. californicus* populations persist in stressful environments.

MATERIALS AND METHODS

Laboratory culture

Six populations of *Tigriopus californicus* were collected from high intertidal rock pools along the coast of California and Baja California (Fig. 1). Each stock population was maintained in the laboratory in 4 or more 400 ml beakers filled with 250 ml of filtered (5 μ m) seawater at 20°C with a 12 h light: 12 h dark photoperiod. Each beaker contained several hundreds to thousands of individuals, reflecting the density range observed in natural populations. Beakers containing copepods from the same population were intermixed monthly to prevent inbreeding in laboratory cultures. The copepods were fed ground *Spirulina* wafers and were acclimated to laboratory conditions for at least 1 generation (approximately 4 wk) prior to experimental treatments. Salinity was monitored with a refractometer and maintained at 35 ppt.

Heat stress experiment

All 3 life stages of *T. californicus* were obtained separately for heat stress experiments. To obtain nauplii, gravid females with mature egg sacs (indicated by orange-red color and visible naupliar eye spots under a stereomicroscope) were collected from stock populations. Egg sacs were separated from females using needles, and each clutch was placed in filtered seawater and maintained at 20°C overnight. On the following day, hatched nauplii were counted and divided equally into two treatments, control and heat stress. Except for rare undeveloped (and perhaps unfertilized) eggs, 100% hatching rates were observed in these experiments. Clutches producing less than 20 nauplii were pooled into 1 sample before separating into control and heat stress treatments, so that each control and treatment replicate had at least 10 individuals. To obtain copepodids, egg clutches were obtained the same way as the nauplii, but the larvae were kept at 20°C until the majority of each clutch metamorphosed into copepodids, approximately 8 d after hatching. The numbers of individuals from each clutch were counted, and all individuals were used in the heat stress experiment. Since all hatchlings from the same egg clutch were full-sibs, all nauplii or copepodids from the same clutch or pool of clutches were considered 1 replicate. Adult samples were collected from outbred stock populations, with each experimental replicate containing 10 individuals.

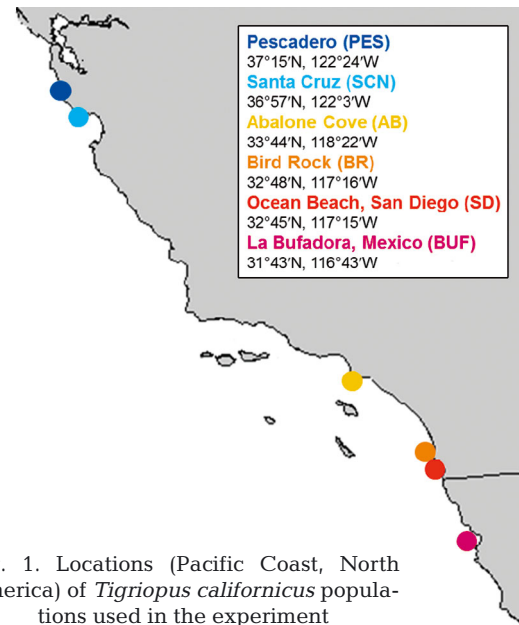


Fig. 1. Locations (Pacific Coast, North America) of *Tigriopus californicus* populations used in the experiment

To conduct heat stress experiments, copepods of the same life stage were put in 2 ml filtered (5 μ m) seawater in 15 ml Falcon tubes. Each replicate tube had at least 10 individual copepods. The number of copepods used in each life stage and treatment ranged from 10 to 299 individuals. For control treatments, copepods were maintained at 20°C with a 12 h light: 12 h dark photo period. The number of replicates used in the control treatments is shown in Table 1.

Table 1. Numbers of replicates from different copepod life stages and populations used in control treatment and average survivorship proportions \pm SE of nauplii from all populations at 20°C. p-values show the results from Wilcoxon signed-rank test of the survivorship proportions between control treatment 20°C and heat stress treatment 34°C of the same population. AB: Abalone Cove; BR: Bird Rock; BUF: La Bufadora; PES: Pescadero; SCN: Santa Cruz; SD: San Diego. n/a: not applicable; Popul.: population

Stage Popul.	No. of replicates	No. of clutches	Survivorship proportion	p
Nauplius				
PES	32	39	0.74 \pm 0.04	0.67
SCN	29	29	0.56 \pm 0.05	0.91
AB	27	34	0.76 \pm 0.12	0.21
BR	39	52	0.69 \pm 0.04	0.71
SD	38	61	0.72 \pm 0.04	0.57
BUF	28	32	0.78 \pm 0.03	0.25
Copepodid				
SD	10	10	0.93 \pm 0.03	0.65
Adult				
SD	5	n/a	1 \pm 0	n/a

From our pilot experiment, only the naupliar stage had variable survivorship. Almost all copepodids and adults survived after 3 d under the control treatment at 20°C. Therefore, we only conducted a 20°C control treatment for the naupliar stage. For short-term (acute) heat stress experiments, tubes were submerged in a water bath at temperatures ranging from 34 to 38°C for 1 h. This range of temperatures has been observed along the California coast in *T. californicus* habitats (Willett 2010, Kelly et al. 2012) and is known to cause differential mortality in adults from different populations (Willett 2010, Kelly et al. 2012, Pereira et al. 2014). The numbers of replicates for different life stages and populations are shown in the Appendix. During the experiment, the temperature in the experimental tubes increased by more than 10°C within the first 5 min and reached the target temperature within 10 min. Following stress treatments, all copepods were returned to 20°C with a 12 h light: 12 h dark photo period. Ground *Spirulina* wafer was added to each tube as food after the stress treatment. Survivors were counted 3 d after the heat stress.

To perform statistical analysis, survivorship proportions were arcsine transformed. Data were analyzed using ANOVA, with temperatures as predictor and life stage and population as covariates. Tukey-Kramer honestly significant difference tests were used as a post hoc analysis to compare the effects among different life stages and populations (analyses were carried out using JMP, v. 11; SAS Institute, Cary, NC). Copepod survivorship proportions of the control and heat stress (34°C) treatments were compared using Wilcoxon signed-rank tests.

Heat stress effects on developmental rate and adult size

San Diego (SD) nauplii from both control and heat stress treatments at 36°C were monitored after the 3 d survivorship count to test for effects of short-term heat stress on development. For each treatment sample, the date the first copepodid appeared was recorded to test for an effect of heat stress on developmental rate; because nauplii and early stage copepodids cannot be sexed, no sex-specific analysis of these results could be made. After the copepodids matured into adults, body lengths of both adult males and females were measured under a stereomicroscope. Mean body length of adult copepods ($n = 124$) from control and heat stress treatments were compared. Data on developmental time from nauplius

to copepodid and adult size were analyzed using Wilcoxon's tests (JMP, v. 11).

RESULTS

Heat tolerance across populations and life stages

Even under benign control temperatures (20°C), nauplii from all populations showed some mortality during the first 3 d after hatching. Survival rates of nauplii at 34°C were similar to those of the control samples from the same clutches (Table 1), indicating that this heat shock did not result in mortality of nauplii. In contrast, copepodids and adult copepods showed little or no mortality under control conditions and, like nauplii, experienced no mortality following exposure to mild heat stress (at 34°C) (Table 1, Fig. 2A). Although survivorship of nauplii from all 6 populations at 34 and 35°C was lower than the survivorship of copepodids and adults at the same temperature, this appears to simply reflect the mortality of nauplii under the control treatment. Survivorship of nauplii was generally higher than that of other life stages at temperatures higher than 35°C. Adults from all populations did not survive acute heat stress at 37°C (Fig. 3B). However, some nauplii and copepodids from the southern populations survived at 37°C. At 36°C, both nauplii and copepodids from the northern populations survived in higher proportions than adults of the same populations. Nauplii also generally have higher average survivorship than copepodids at the temperatures where each population was at its maximum thermal limit (37°C in southern populations and 36°C in northern populations), except for the Abalone Cove (AB) population, where average survivorship of copepodids was the highest among all life stages at 37°C (Fig. 3B). These results show that pre-reproductive stages of *Tigriopus californicus* are more heat tolerant than adults.

Significant differences in survivorship were observed between northern (Pescadero [PES], Santa Cruz [SCN]) and southern (La Bufadora [BUF], San Diego [SD], Bird Rock [BR], Abalone Cove [AB]) populations (Tables 2 & 3B). Southern populations exposed to acute heat stress at 36°C showed higher survivorship than northern populations in all 3 life stages (Fig. 3). At 37°C, difference in survivorship between northern and southern populations was the most significant in the copepodid stage. Some copepodids from southern populations survived, while copepodids from northern populations experienced 100% mortality.

Heat stress effects on developmental rate and adult size

A total of 58 clutches of SD nauplii, from control and 1 h heat stress at 36°C, were monitored to determine the effects of stress on developmental rate. Of these, 11 clutches (124 individuals) were measured

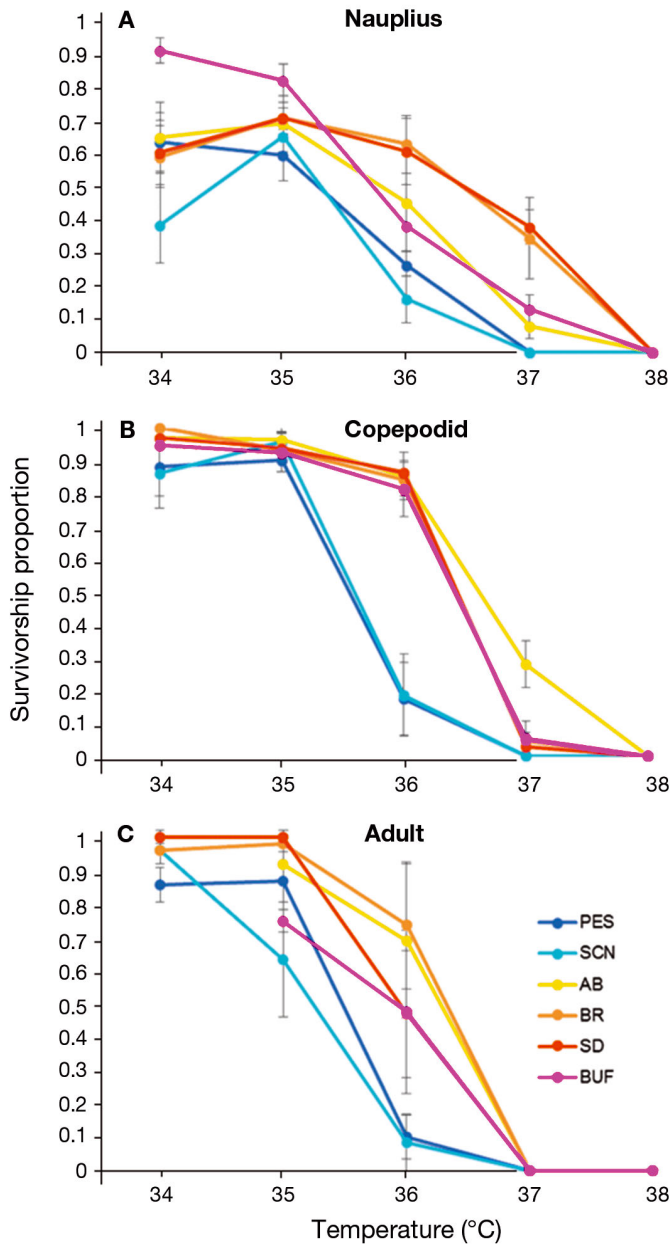


Fig. 2. Average (± 1 SE) survivorship proportions of 6 populations and 3 life stages of *Tigriopus californicus* following acute heat stress. Life stage: (A) nauplius, (B) copepodid, and (C) adult. AB: Abalone Cove; BR: Bird Rock; BUF: La Bufadora; PES: Pescadero; SCN: Santa Cruz; SD: San Diego

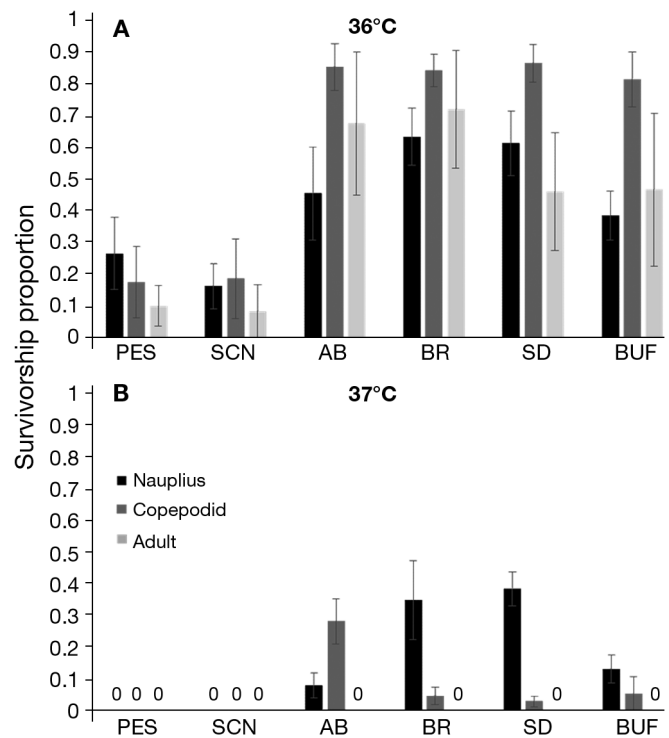


Fig. 3. Average (± 1 SE) survivorship proportions after acute heat stress of 3 *Tigriopus californicus* life stages at (A) 36 and (B) 37°C. AB: Abalone Cove; BR: Bird Rock; BUF: La Bufadora; PES: Pescadero; SCN: Santa Cruz; SD: San Diego

for adult sizes. Acute heat stress significantly affected developmental rate in *T. californicus*; nauplii that experienced acute stress took longer to develop into copepodids than nauplii that were raised at 20°C (Fig. 4, mean 8.80 d vs. mean 8.03 d, respectively; Wilcoxon's test: $Z = 3.10$, $p = 0.002$). No differences in adult size of either sex were observed between control and heat stress treatments (Wilcoxon's tests; males: $n = 74$, $Z = 0.11$, $p = 0.91$; females: $n = 50$, $Z = 0.08$, $p = 0.94$).

Table 2. ANOVA table showing the effects of temperature, life stage, and population factors on arcsine transformed survivorship proportions of *Tigriopus californicus* after acute heat stress

	df	SS	F	p
Temperature	1	58.95	393.40	<0.0001
Life stage	2	7.16	23.90	<0.0001
Population	5	9.67	12.91	<0.0001
Residual	431	64.58		
Total	439	134.98		

Table 3. Tukey-Kramer post hoc pairwise comparisons (p-values) among the effects of (A) life stages and (B) populations on survivorship proportions. AB: Abalone Cove; BR: Bird Rock; BUF: La Bufadora; PES: Pescadero; SCN: Santa Cruz; SD: San Diego

(A)	Nauplius	Copepodid				
Copepodid	<0.0001					
Adult	<0.0001	0.92				
(B)	PES	SCN	AB	BR	SD	
SCN	0.87					
AB	< 0.0049	<0.0001				
BR	<0.0001	<0.0001	0.98			
SD	<0.0001	<0.0001	0.96	1		
BUF	0.0088	0.0001	1	0.96	0.93	

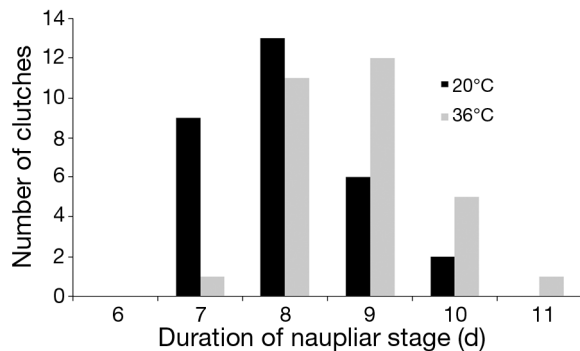


Fig. 4. Effects of acute heat stress on larval development in *Tigriopus californicus*. Frequency of the number of days the copepod clutches from the San Diego population stayed in the naupliar stage before they metamorphosed into copepods, for both nauplii that were raised at 20°C and nauplii that experienced acute heat stress at 36°C

DISCUSSION

After accounting for mortality under control laboratory conditions, we found that nauplii of the tidepool copepod *Tigriopus californicus* were generally more tolerant of acute heat stress than copepodid or adult life stages. Nauplii survived heat stress at extreme temperatures (36°C for northern populations, PES and SCN, and 37°C for southern populations, AB, BR, SD, and BUF) in greater proportions than adults. Although the statistical analysis suggests that copepodids and adults are not significantly different from each other (Table 3A), some copepodids from all 4 southern populations survived heat stress at 37°C, while the adults did not (Fig. 3B). These results indicate that both pre-reproductive stages

(nauplii and copepodids) of *T. californicus* are more heat tolerant than reproductive adults.

Our results contrast with the widely held generalization suggested by previous studies that larvae of marine invertebrates are more sensitive to environmental stresses than adults (Verween et al. 2007, Pineda et al. 2012, Miller et al. 2013). Such differences might reflect the gradient of thermal environments encountered by different life stages. Intertidal habitats are among the most physically challenging of all marine environments, and a diverse fauna has evolved adaptations to cope with rapid fluctuations in temperature, salinity, and other abiotic variables. A significant proportion of those species, however, produce planktonic larvae that develop in the water column, an environment that is substantially buffered from abiotic variation. Consequently, selection for tolerance to these stresses may be relaxed for these planktonic life stages. Miller et al. (2013) use this line of reasoning in explaining that the zoeal larval stage of a porcelain crab species (*Petrolisthes cinctipes*) has the lowest median lethal temperature (temperature which results in 50% mortality) of all life stages (i.e. zoea are the most sensitive to thermal stress); because eggs and early embryos are brooded and late larval stages recruit back to the intertidal zone, only zoea escape exposure to thermal extremes.

Although the above argument holds for a great many intertidal invertebrates, not all have planktonic development. Although all life stages of *T. californicus* are able to actively swim, genetic analyses clearly demonstrate a remarkable lack of gene flow between *T. californicus* populations inhabiting neighboring rock outcrops, indicating that dispersal in the plankton is extremely rare (Burton & Feldman 1981, Edmands 2001, Willett & Ladner 2009), and all life stages typically occur in rocky tidepools. Consequently, all life stages of *T. californicus* experience the same highly variable thermal regime and are therefore expected to be adapted to the same temperature range. In fact, we found that pre-reproductive stages of *T. californicus* are more heat tolerant than adults; this may reflect the fact that mortality in pre-reproductive stages has a stronger selective effect on the population because it results in no offspring. Therefore, in systems where all life stages occupy similar temperature ranges, pre-reproductive stages need to be at least as tolerant to abiotic stresses as adults. In this regard, the finding of high thermal tolerance in *T. californicus* larvae parallels the recent findings of Diederich & Pechenik (2013) on the brooding gas-

tropod *Crepidula fornicata*, whose embryos also experience the same thermal regime as adults.

Recent studies have revealed significant differences in thermal tolerance in adult *T. californicus* from different geographic populations. Willett (2010) and Kelly et al. (2012) showed that populations of *T. californicus* differ in lethal maximum temperatures, with southern populations consistently surviving higher acute temperature stresses than northern populations. Both studies conclude that each population is adapted to its respective local temperature range. Kelly et al. (2012) showed that temperatures in tidepools where *T. californicus* are found during spring and summer months in California range from 8 to >40°C. Results from the present study are consistent with previous studies: adults from southern populations of *T. californicus* can tolerate higher temperatures than those sampled from northern populations. Here, we found that this pattern also holds for early life stages. Both nauplii and copepodids of the southern populations can tolerate higher temperatures than the same life stages from northern populations. Again, we interpret these differences in thermal tolerance among populations of *T. californicus* as evidence of adaptation to local environments.

Since all life stages of *T. californicus* developed in a 20°C 'common garden' environment for a full generation prior to thermal stress exposure, the differences in thermal tolerance among life stages may largely reflect genetic effects. With increasingly stressful environments, the least heat-tolerant stage will determine population persistence in the future environment. Knowing the pattern of heat stress survivorship among life stages allows us to better predict how populations of *T. californicus* will fare in response to increasing temperature. For example, our results indicate that nauplii and copepodids of the southern California populations can survive at 37°C stress, but adults cannot. Frequent heat stresses above 37°C could threaten populations with extirpation because the adults do not survive and fail to reproduce. Kelly et al. (2012) suggest that populations of *T. californicus* have limited potential to adapt to increasingly stressful temperatures; consequently, local extirpation might result from increases in environmental temperature. However, Pereira et al. (2014) found that hybrid lineages between neighboring southern California populations, SD and BR, frequently have thermal tolerances exceeding either parental population. Although migration between populations is known to be very low, these results suggest a potentially important evolutionary scenario where rare dis-

persal and hybridization could result in further adaptation to environmental extremes.

As discussed above, comparisons of stress tolerance across life stages in our experiments met with one unexpected complication: nauplii (but not copepodids or adults) show significant mortality during lab rearing at the 20°C control temperature. This mortality appears to be independent of heat stress, since levels of mortality among nauplii do not increase significantly at temperatures up to 34°C. The cause of this baseline level of mortality among nauplii is unknown but is likely from both natural causes and experimental handling (required to count and transfer nauplii between culture and experimental vessels). Despite this background level of mortality, nauplii still showed higher survivorship than all adults and most copepodids under acute high temperature stress (37°C in BUF, SD, BR, AB and 36°C in SCN, PES), indicating that nauplii are, in fact, the most thermally tolerant of the 3 life stages. Survivorship of copepodids is also higher than that observed among adults of the same population, implying that adult *T. californicus* might be the most thermally sensitive life stage.

Although larvae and juveniles of *T. californicus* can survive higher temperatures than adults, acute heat stress may have other fitness consequences. Numerous studies have shown that high temperatures increase metabolic rate and lead to faster development in many marine species (Roberts et al. 2012, Runcie et al. 2012). Indeed, Egloff (1966) found that high temperature greatly accelerates larval development in *T. californicus* over the tested range of 15 to 25°C. In contrast, here we report that acute heat stress resulted in slower larval development (i.e. increased naupliar duration). This suggests that when larvae experience heat stress, they likely divert energy and resources from growth and development to stress responses, resulting in slower larval development. Prolonged larval duration as a result of acute thermal stress could be a factor affecting the population dynamics of *T. californicus*; Altermatt et al. (2012) suggested that the rapid temperature changes and increased risk of desiccation in smaller tidepools contribute to increased rates of local extinction of *T. californicus*. Our results are consistent with this suggestion, since more frequent acute heat stress in smaller pools will lead to increased mortality of all life stages and slower larval development, resulting in both slower population growth and more frequent local extinction. Given the short duration of our experimental treatment (i.e. 1 h), the observed significant increase in larval duration is remarkable.

Finally, although larval duration was increased, a single bout of acute heat stress in the naupliar stage did not have a significant effect on adult size. Given that heat stress in the natural environment probably recurs on successive days during periods of warm weather and that stressful temperatures may frequently last more than 1 h, additional testing is needed to definitively ascertain heat stress effects on adult size.

In conclusion, our results indicate that early life stages of *T. californicus* have higher thermal tolerance than adults from the same population. Although there are too few studies to make a strong generalization, we hypothesize that high thermal tolerance in larvae will be found in species where all life stages experience the same thermal environment. This is the case for brooding species, like *Crepidula fornicata* (Diederich & Pechenik 2013), and for species like *T. californicus*, where larvae develop in the same tidepools inhabited by adults. Second, as previously observed in adult *T. californicus* populations, differences in larval thermal tolerance parallel the latitudinal gradient in environmental temperature; larvae from northern populations are more sensitive to high temperatures than those from southern populations, a pattern consistent with adaptation of populations to local environment variation. Finally, acute heat stress was found to affect fitness traits other than survivorship; even a single 1 h heat stress resulted in decreased developmental rates that could ultimately translate into reduced population growth rates.

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Appendix. Numbers of replicates, clutches, and total individuals used at each heat stress treatment. AB: Abalone Cove; BR: Bird Rock; BUF: La Bufadora; PES: Pescadero; SCN: Santa Cruz; SD: San Diego

A) Nauplius															
Population	Replicates					Clutches					Individuals				
Temperature (°C):	34	35	36	37	38	34	35	36	37	38	34	35	36	37	38
PES	11	9	9	4	2	13	12	10	5	2	139	116	140	49	55
SCN	10	8	10	4	2	10	8	10	4	2	187	155	209	95	55
AB	5	6	7	8	2	7	6	8	12	2	53	72	106	110	42
BR	10	10	10	10	2	14	15	11	13	2	133	120	153	138	19
SD	10	10	10	10	2	14	18	12	18	3	126	133	140	139	30
BUF	6	6	5	9	2	8	6	7	9	2	76	85	87	115	23
B) Copepodid															
Population	Replicates				Clutches					Individuals					
Temperature (°C):	34	35	36	37	38	34	35	36	37	38	34	35	36	37	38
PES	2	4	6	6	4	2	5	8	7	4	60	63	178	104	123
SCN	2	4	8	8	3	2	4	8	8	3	112	95	231	299	89
AB	2	3	7	7	4	2	3	9	8	4	41	95	102	128	72
BR	1	3	8	8	4	2	5	9	10	6	10	42	124	129	63
SD	2	4	12	8	4	2	5	15	10	4	38	47	189	140	86
BUF	2	4	6	6	4	2	4	6	6	4	38	58	138	124	51
C) Adult															
Population	Replicates				Individuals										
Temperature (°C):	34	35	36	37	38	34	35	36	37	38					
PES	5	4	5	1	1	50	40	50	10	10					
SCN	5	5	6	1	1	50	50	60	10	10					
AB	0	3	4	1	1	0	30	40	10	10					
BR	5	5	5	1	1	50	50	50	10	10					
SD	5	5	5	1	1	50	50	50	10	10					
BUF	0	3	3	1	1	0	30	30	10	10					