UC San Diego

UC San Diego Previously Published Works

Title

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

Permalink

<https://escholarship.org/uc/item/5186d975>

Authors

Tangwanchaoen, S
Burton, RS

Publication Date

2014-12-15

DOI

10.3354/meps11013

Peer reviewed

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

Sumaetee Tangwanchaoen*

Email: stangwan@ucsd.edu

Ronald S. Burton

Email: rburton@ucsd.edu

Address

Marine Biology Research Division

Scripps Institution of Oceanography

University of California, San Diego

9500 Gilman Drive

La Jolla, CA 92093-0202

U.S.A

19

20 **Abstract**

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

39

40

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, such as 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 is 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 *Crepidula 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 most often 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 southeast 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, Schoville et al. 2012, Kelly et al. 2013, Pereira et al. 2013). These studies show a clear pattern with adult copepods from southern populations surviving higher temperatures than do adult copepods from northern populations, an observation consistent with local adaption 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 tide pools in California are generally moderate, ranging from approximately 10 – 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 with marine invertebrates with free-swimming larvae, all life stages of *T. californicus* occupy the same tide

pool habitats and experience 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? and (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.

123

124 **Materials and Methods**

125

126 **Laboratory culture**

127 Six populations of *Tigriopus californicus* were collected from high intertidal rock pools along
128 the coast of California and Baja California (Figure 1). Each stock population was maintained in
129 the laboratory in 4 or more 400 ml beakers filled with 250 ml of filtered sea water (5 μ m) at 20
130 °C with a 12-hour light-dark photoperiod. Each beaker contained several hundreds to thousands
131 of individuals, reflecting the density range observed in natural populations. Beakers containing
132 animals from the same population same population were intermixed monthly to prevent
133 inbreeding in laboratory cultures. The animals were fed ground *Spirulina* wafers and were
134 acclimated to laboratory conditions for at least one generation (approximately four weeks) prior
135 to experimental treatments. Salinity was monitored with a refractometer and maintained at 35
136 ppt.

137

138 **Heat stress experiment**

139 All three life stages of *T. californicus* were obtained separately for heat stress experiments. To
140 obtain nauplii, gravid females with mature egg sacs (indicated by orange-red color and visible
141 naupliar eye spots under a stereomicroscope) were collected from stock populations. Egg sacs
142 were separated from females using needles and each clutch was placed in filtered seawater and
143 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 one sample before separating into control and heat stress treatment 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 eight days 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 one replicate. Adult samples were collected from outbred stock populations with each experimental replicate containing 10 individuals.

To conduct heat stress experiments, copepods of the same life stage were put in 2 ml filtered 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, animals were maintained at 20° C with a 12-hour light-dark period. The number of replicates used in the control treatments is shown in Table 1. From our pilot experiment, only the naupliar stage had variable survivorship. Almost all copepodids and adults survived after 3 days under the control treatment at 20 °C. Therefore, we only conducted 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 one hour. 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. 2013). The numbers of replicates for different life stages and populations are shown in supplement Table S1. During the experiment, the temperature in the experimental tubes increased by more than 10 °C within first five minutes and reached the target temperature within 10 minutes. Following stress treatments, all animals were returned to 20° C with a 12-hour light-dark period. Ground *Spirulina* wafer was added to each tube as food after the stress treatment. Survivors were counted three days after the heat stress.

To perform statistical analysis, survivorship proportions were arcsine transformed. Data were analyzed using Analysis of Variance (ANOVA) with temperatures as predictor and life stage and population as covariates. Tukey-Kramer Honest Significant Difference (HSD) tests were used as a post-hoc analysis to compare the effects among different life stages and populations. Copepod survivorship proportions of the control and heat stress (34 °C) treatments were compared using Wilcoxon signed-rank test.

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 three day 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.

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 three days after hatching; survival rates of nauplii (from the same clutches) at 34°C were similar to those of the control samples (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, Figure 2A). Although survivorship of nauplii from all six populations at 34 °C 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 other life stages at temperatures higher than 35 °C. Adults from all populations did not survive acute heat stress at 37 °C (Figure 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 whose average survivorship of copepodids was the highest among all life stages at 37 °C (Figure 3B). These results show that pre-reproductive stages of *T. californicus* are more heat tolerant than adults.

Significant differences in survivorship were observed between northern (Pescadero, PES and Santa Cruz, SCN) and southern (La Bufadora Mexico, BUF; San Diego, SD; Bird Rock, BR and Abalone Cove, AB) populations (Table 2, Table 3B). Southern populations exposed to acute heat stress at 36 °C showed higher survivorship than northern populations in all three life stages (Figure 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 one hour heat stress at 36 °C, were monitored to determine the effects of stress on developmental rate. Of these, 11 clutches (124 individuals) were measured 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 (Figure 4, mean 8.80 days vs. mean 8.03 days 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: $n = 50$, $Z = 0.08$, $p = 0.94$).

Discussion

After accounting for mortality under control laboratory conditions, we found that nauplii of the tidepool copepod *T. californicus* were generally more tolerant of acute heat stress than copepodid or adult life stages. Nauplii survived heat stress at severe temperatures (36 °C for northern populations, PES and SCN, and 37 °C for southern populations, AB, BR, SD and BUF) in greater proportions than did adults. Although the statistical analysis suggests that copepodid and adult 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 (Figure 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 that larvae of marine invertebrates are more sensitive to environmental stresses than adults. Several previous studies have suggested that larval stages of marine species are typically more sensitive to heat stress 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 the fact that the zoeal larval stage of a porcelain crab species (*Petrolisthes cinctipes*) has the lowest LT50 (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, 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 and Pechenik (2013) on the brooding gastropod *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) have shown 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) have shown that temperatures in tide pools where *T. californicus* during spring and summer months in California range from 8 °C to more than 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 response among life stages allows us to better predict how populations of *T. californicus* 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 dispersal 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 due to 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, and AB and 36 °C in SCN and PES), indicating that nauplii are, in fact, the most thermally tolerant of the three 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 15-25 °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 hour), 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 one hour, 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 temperature 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 one-hour heat stress resulted in decreased developmental rates that could ultimately translate into reduced population growth rates.

Acknowledgements

We thank four anonymous reviewers who provided valuable suggestions and comments on the manuscript. Felipe Barreto, Ricardo Pereira, and Lani Gleason collected the *T. californicus* populations used in this experiment. Eric Allen, Martin Tresguerres, Felipe Barreto, Ricardo Pereira and Lani Gleason provided helpful comments on earlier versions of the manuscript. This research as supported in part by NSF (DEB 1051057 to RSB) and the Thai government for stipend and tuition support to ST.

364

365

366 **Literature cited**

367 Altermatt F, Bieger A, Morgan SG (2012) Habitat characteristics and metapopulation dynamics
368 of the copepod *Tigriopus californicus*. Marine Ecology Progress Series 468:85

369 Bermudes M, Ritar AJ (2008) Response of early stage spiny lobster *Jasus edwardsii* phyllosoma
370 larvae to changes in temperature and photoperiod. Aquaculture 281:63-69

371 Bryars SR, Havenhand JN (2006) Effects of constant and varying temperatures on the
372 development of blue swimmer crab (*Portunus pelagicus*) larvae: Laboratory observations
373 and field predictions for temperate coastal waters. Journal of Experimental Marine
374 Biology and Ecology 329:218-229

375 Burton RS, Feldman MW (1981) Population genetics of *Tigriopus californicus*. II.
376 Differentiation among neighboring populations. Evolution:1192-1205

377 Burton RS, Lee B-N (1994) Nuclear and mitochondrial gene genealogies and allozyme
378 polymorphism across a major phylogeographic break in the copepod *Tigriopus*
379 *californicus*. Proceedings of the National Academy of Sciences USA 91:5197-5201

380 Dethier MN (1980) Tidepools as refuges: Predation and the limits of the harpacticoid copepod
381 *Tigriopus californicus* (Baker). Journal of Experimental Marine Biology and Ecology
382 42:99-111

383 Diederich CM, Pechenik JA (2013) Thermal tolerance of *Crepidula fornicata* (Gastropoda) life
384 history stages from intertidal and subtidal subpopulations. Marine Ecology Progress
385 Series 486:173-187

386 Edmands S (2001) Phylogeography of the intertidal copepod *Tigriopus californicus* reveals
387 substantially reduced population differentiation at northern latitudes. Molecular Ecology
388 10:1743-1750

389 Edmands S, Deimler JK (2004) Local adaptation, intrinsic coadaptation and the effects of
390 environmental stress on interpopulation hybrids in the copepod *Tigriopus californicus*.
391 Journal of Experimental Marine Biology and Ecology 303:183-196

392 Egloff DA (1966) Ecological aspects of sex ratio and reproduction in experimental and field
393 populations of the marine copepod *Tigriopus californicus*. Stanford University

394 Ganz H, Burton R (1995) Genetic differentiation and reproductive incompatibility among Baja
395 California populations of the copepod *Tigriopus californicus*. Marine Biology 123:821-
396 827

- 397 Hilbish TJ, Mullinax A, Dolven SI, Meyer A, Koehn RK, Rawson PD (2000) Origin of the
398 antitropical distribution pattern in marine mussels (*Mytilus* spp.): routes and timing of
399 transequatorial migration. *Marine Biology* 136:69-77
- 400 JMP[®], Version 11. 2013. SAS Institute Inc., Cary, NC, 1989-2007.
- 401 Kelly MW, Grosberg RK, Sanford E (2013) Trade-offs, geography, and limits to thermal
402 adaptation in a tide pool copepod. *The American Naturalist* 181:846-854
- 403 Kelly MW, Sanford E, Grosberg RK (2012) Limited potential for adaptation to climate change in
404 a broadly distributed marine crustacean. *Proceedings of the Royal Society B-Biological*
405 *Sciences* 279:349-356
- 406 Koufopanou V, Reid DG, Ridgway SA, Thomas RH (1999) A molecular phylogeny of the
407 patellid limpets (Gastropoda: Patellidae) and its implications for the origins of their
408 antitropical distribution. *Molecular Phylogenetics and Evolution* 11:138-156
- 409 Miller N, Paganini A, Stillman J (2013) Differential thermal tolerance and energetic trajectories
410 during ontogeny in porcelain crabs, genus *Petrolisthes*. *J Therm Biol* 38:79-85
- 411 Morgan S (1995) Life and death in the plankton: larval mortality and adaptation. *Ecology of*
412 *marine invertebrate larvae*:279-321
- 413 Pereira RJ, Barreto FS, Burton RS (2014) Ecological novelty by hybridization: experimental
414 evidence for increased thermal tolerance by transgressive segregation in *Tigriopus*
415 *californicus*. *Evolution* 68:204-215
- 416 Peterson DL, Kubow KB, Connolly MJ, Kaplan LR, Wetkowski MM, Leong W, Phillips BC,
417 Edmands S (2013) Reproductive and phylogenetic divergence of tidepool copepod
418 populations across a narrow geographical boundary in Baja California. *Journal of*
419 *Biogeography* 40:1664-1675
- 420 Pineda MC, McQuaid CD, Turon X, López-Legentil S, Ordóñez V, Rius M (2012) Tough adults,
421 frail babies: an analysis of stress sensitivity across early life-history stages of widely
422 introduced marine invertebrates. *PLoS One* 7:e46672
- 423 Plough LV, Marko PB (2014) Characterization of microsatellite loci and repeat density in the
424 gooseneck barnacle, *Pollicipes elegans*, using next generation sequencing. *Journal of*
425 *Heredity* 105:136-142
- 426 Preston N (1985) The effects of temperature and salinity on survival and growth of larval
427 *Penaeus plebejus*, *Metapenaeus macleayi* and *M. bennettiae*. *National Prawn Seminar* 2,
428 *Cleveland*
- 429 Pörtner HO, Knust R (2007) Climate change affects marine fishes through the oxygen limitation
430 of thermal tolerance. *Science* 315:95-97

- Roberts SD, Dixon CD, Andreacchio L (2012) Temperature dependent larval duration and survival of the western king prawn, *Penaeus (Melicertus) latisulcatus* Kishinouye, from Spencer Gulf, South Australia. *Journal of Experimental Marine Biology and Ecology* 411:14-22
- Rothlisberg PC (1979) Combined effects of temperature and salinity on the survival and growth of the larvae of *Pandalus jordani* (Decapoda: Pandalidae). *Marine Biology* 54:125-134
- Runcie DE, Garfield DA, Babbitt CC, Wygoda JA, Mukherjee S, Wray GA (2012) Genetics of gene expression responses to temperature stress in a sea urchin gene network. *Molecular Ecology* 21:4547-4562
- Schoville SD, Barreto FS, Moy GW, Wolff A, Burton RS (2012) Investigating the molecular basis of local adaptation to thermal stress: population differences in gene expression across the transcriptome of the copepod *Tigriopus californicus*. *BMC Evolutionary Biology* 12
- Verween A, Vincx M, Degraer S (2007) The effect of temperature and salinity on the survival of *Mytilopsis leucophaeata* larvae (Mollusca, Bivalvia): The search for environmental limits. *Journal of Experimental Marine Biology and Ecology* 348:111-120
- Walther K, Crickenberger SE, Marchant S, Marko PB, Moran AL (2013) Thermal tolerance of larvae of *Pollicipes elegans*, a marine species with an antitropical distribution. *Marine Biology* 160:2723-2732
- Willett CS (2010) Potential fitness trade-offs for thermal tolerance in the intertidal copepod *Tigriopus californicus*. *Evolution* 64:2521-2534
- Willett CS, Ladner JT (2009) Investigations of fine-scale phylogeography in *Tigriopus californicus* reveal historical patterns of population divergence. *BMC Evolutionary Biology* 9

Figure legends

Figure 1: Map showing locations of *T. californicus* populations used in the experiment:

Pescadero (PES: 37°15N 122°24W), Santa Cruz (SCN: 36°57N 122°3W), Abalone Cove (AB: 33°44N 118°22W) Bird Rock (BR: 32°48N 117°16W) San Diego (SD: 32°45 N 117°15 W) and La Bufadora (BUF: 31°43N 116°43W).

Figure 2: Average survivorship proportions of six populations and three life stages of *T. californicus* following acute heat stress: Graphs show survivorship with ± 1 standard error after acute heat stress of different life stages: nauplius (A), copepodid (B), and adult (C).

Figure 3: Survivorship in three life stages: Graphs show average survivorship proportions ± 1 standard error after acute heat stress of *T. californicus* at 36 °C (A) and 37 °C (B).

Figure 4: The effects of acute heat stress on larval development in *T. californicus*: The histograms show frequency of the number of days the copepods from San Diego population stayed in the naupliar stage before they metamorphosed into copepodids for both nauplii that were raised at 20 °C and nauplii that experienced acute heat stress at 36 °C.

Table 1: Numbers of replicates from different 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.

Stage	Population	Number of replicates	Number of clutches	Proportion survivorship	Wilcoxon test p value
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

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

	Degrees of freedom	Sum of squares	F ratio	p value
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		

496

497

498 **Table 3: p value results from Tukey-Kramer post hoc pairwise comparisons among the**
 499 **effects of life stages (A) and populations (B) on survivorship proportions**

500 A

	nauplius	copepodid	adult
nauplius			
copepodid	<0.0001		
adult	<0.0001	0.92	

501

502 B

	PES	SCN	AB	BR	SD	BUF
PES						
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	

503

Figure 1

Figure 2

Figure 3

513

514

515 Figure 4