Variation in developmental temperature alters adulthood plasticity of thermal tolerance in *Tigriopus californicus*

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Summary statement:
Developmental temperatures affect thermal limit plasticity in adults of a marine ectotherm, and changes in these limits are paralleled by differences in ATP synthesis rate and heat shock protein expression.

List of abbreviations

Analysis of variance – ANOVA

Critical thermal maximum – CT_{max}

Electron transport system complexes I and II – CI+II

Quantitative real-time polymerase chain reaction – qRT-PCR

Temperature coefficient – Q_{10}

Abstract

In response to environmental change, organisms rely on both genetic adaptation and phenotypic plasticity to adjust key traits that are necessary for survival and reproduction. Given the accelerating rate of climate change, plasticity may be particularly important. For organisms in warming aquatic habitats, upper thermal tolerance is likely to be a key trait, and many organisms express plasticity in this trait in response to developmental or adulthood temperatures. Although plasticity at one life stage may influence plasticity at another life stage, relatively little is known about this possibility for thermal tolerance. Here we used locally adapted populations of the copepod *Tigriopus californicus* to investigate these potential effects in an intertidal ectotherm. We found that low latitude populations had greater critical thermal maxima (CT_{max}) than high latitude populations, and variation in developmental temperature altered CT_{max} plasticity in adults. After development at 25°C, CT_{max} was plastic in adults, whereas no adulthood plasticity in this trait was observed after development at 20°C. This pattern was identical across four populations, suggesting that local thermal adaptation has not shaped this effect among these populations. Differences in the capacities to maintain ATP synthesis rates and to induce heat shock proteins at high temperatures, two likely mechanisms of local adaptation in this species, were consistent with changes in CT_{max} due to phenotypic plasticity, which suggests that there is likely mechanistic overlap between the effects of plasticity and adaptation. Together, these results indicate that developmental effects may have substantial impacts on upper thermal tolerance plasticity in adult ectotherms.

Introduction

As the earth warms, organisms are increasingly impacted by the effects of high environmental temperatures (e.g., Wiens, 2016; Cohen et al., 2018; Pinsky et al., 2019). Indeed, the geographic range limits of many species have already shifted as a result of anthropogenic climate change, and in general these shifts have been towards regions with cooler temperatures (e.g., Parmesan and Yohe, 2003; Perry et al., 2005; Chen et al., 2011). The extents to which these effects have occurred, and will continue to occur, depend largely on the adaptive and plastic capacities of organisms to adjust key physiological traits, such as thermal tolerance limits (especially in aquatic ectotherms; Sunday et al., 2012; Pinsky et al., 2019), in response to increased temperatures (e.g., Crain et al., 2008; Somero, 2010; Bay et al., 2017; Kellermann and van Heerwaarden, 2019). In particular, given that rapid phenotypic changes are necessary due to high rates of environmental change (e.g., Barrett and Hendry, 2012; Fox et al., 2019), phenotypic plasticity may play a critical role in the resilience of populations and species to the effects of climate change (Merilä and Hendry, 2014; Seebacher et al., 2015; Donelson et al., 2019; Morley et al., 2019).

Phenotypic plasticity occurs across life stages and generations (e.g., Kelly et al., 2011; Schulte et al., 2011; Beaman et al., 2016; Burggren, 2015). For example, temperatures experienced during development or adulthood often have irreversible or reversible effects on physiological traits (e.g., Schulte et al., 2011; Beaman et al., 2016), and multi- or trans-generational effects of thermal variation are commonly observed (e.g., Crill et al., 1996; Massamba-N'Siala et al., 2014; Zizzari and Ellers, 2014; Donelson et al., 2018). Thus, physiological phenotypes have the potential to be shaped by effects of plasticity across different life stages. However, compared to the effects of adaptation on phenotypic plasticity (e.g., Crispo, 2007; Hendry, 2016; Donelson et al., 2019; Kelly, 2019), effects of plasticity at one life stage on the expression of plasticity at another life stage have received relatively little attention (Beaman et al., 2016). That said, developmental conditions are known to alter the adulthood plasticity of several traits (reviewed in Beaman et al., 2016). For instance, adult plasticity of swimming performance and metabolic rate depends on developmental environment in mosquitofish (*Gambusia holbrooki*; Seebacher et al., 2014; Seebacher and Grigaltchik, 2015). Yet, despite the likely biogeographic importance of thermal tolerance limits (Sunday et al., 2012), and many published examples of thermal tolerance limit plasticity in ectothermic organisms as a result of developmental or adulthood temperatures (e.g., Stillman and Somero, 2000; Ford and Beitinger, 2005; Fangue et al., 2006; Angiletta, 2009; Overgaard et al., 2011; Cooper et al., 2012; Tepolt and Somero, 2014; Jakobs et al.,

2015; Troia et al., 2015; Kingsolver et al., 2016; Pereira et al., 2017; Diamond et al., 2018; Mueller et al., 2019; Yanar et al., 2019), relatively few studies have assessed the potential for developmental temperatures to shape the phenotypic plasticity of upper thermal tolerance in adults (although see Schaefer and Ryan, 2006; Kellermann et al., 2017; Kellermann and Sgrò, 2018). Here we examine these effects, and their potential mechanistic basis in populations of the intertidal copepod *Tigriopus californicus*.

T. californicus are small (~1.2 mm) harpacticoid copepods with short generation times (3-4 weeks) that inhabit supralittoral tidepools along the west coast of North America from Baja California, Mexico to southern Alaska, USA. Populations of this species occur on rocky outcrops isolated by sandy beaches, conditions that result in very low gene flow and high levels of genetic divergence among populations (Burton and Lee, 1994; Burton, 1997, 1998; Edmands, 2001; Peterson et al., 2013; Pereira et al., 2016; Barreto et al., 2018). Although much of this divergence is likely a result of small effective population sizes and genetic drift acting on selectively neutral variation, signatures of directional selection have been detected across the transcriptome (Pereira et al., 2016). This suggests that at least a portion of the genetic differentiation among populations is likely adaptive. Moreover, several common-garden studies in laboratory-raised individuals have demonstrated differences in upper and lower thermal tolerance limits that are consistent with local thermal adaptation in response to the latitudinal temperature gradient across the species range (Willett, 2010; Kelly et al., 2012; Wallace et al., 2014; Pereira et al., 2014, 2017; Leong et al., 2018; Willett and Son, 2018; Foley et al., 2019). This variation among populations has also been associated with genetically based differences in the function and regulation of heat shock protein genes (Schoville et al., 2012; Barreto et al., 2015; Tangwancharoen et al., 2018) and in the maintenance of mitochondrial ATP synthesis rates at high temperatures (Harada et al., 2019). Few studies have examined temperature-mediated phenotypic plasticity in these traits in *T. californicus*. However, elevated developmental temperature is known to increase upper thermal tolerance regardless of population (Kelly et al., 2012, 2017; Pereira et al., 2017), and adult plasticity in this trait is thought to be limited (although only relatively short acclimation periods have been examined [e.g., 1 d]; Pereira et al., 2017). Taken together with short generation times and ease of laboratory culture, these observations make *T. calfornicus* an ideal study system in which to investigate the effects of developmental temperature on adulthood plasticity in an aquatic ectotherm.

In the current study, we use laboratory-raised *T. californicus* to test two hypotheses: (1) variation in developmental temperatures changes the expression of phenotypic plasticity

of upper thermal tolerance in adults, and (2) the physiological mechanisms involved in local thermal adaptation among populations are also involved in thermal limit plasticity. First, we expand our previous study (Harada et al., 2019) that put forward methods to estimate upper thermal tolerance with critical thermal maximum (CT_{max}) measurements in this species. We then use this method to facilitate experiments examining the effects of developmental temperature on the plasticity of this proxy for upper thermal tolerance in adults of four Californian populations of *T. californicus*. Finally, we assess the effects of developmental and adulthood temperatures on mechanisms involved in local thermal adaptation in this species: the thermal performance curve of ATP synthesis rate, and the mRNA expression levels of heat shock protein genes and mitochondrial-encoded genes following acute heat stress.

Materials and methods

Collection and culturing of copepods

Adult copepods were collected from supralittoral tidepools across ten locations along the west coast of North America, which spanned ~21.5° of latitude (San Roque, Mexico – SR, La Bufadora, Mexico – BF, San Diego, California – SD, Bird Rock, California – BR, Abalone Cove, California – AB, Estero Bay, California – EB, San Simeon, California – SS, Santa Cruz, California – SC, Pescadero, California – PE, and Pacific Crest, Canada – PC; Table S1; Fig. S1A,B). Collected animals were transported to Scripps Institution of Oceanography (San Diego, CA) in 1 L plastic bottles containing seawater obtained from the same tidepools. The collection for each location was divided across several laboratory cultures, which were maintained at 20°C, 36 ppt and 12:12 h photoperiod (light:dark) using filtered seawater and deionized water to adjust salinity as necessary. Laboratory cultures were maintained for at least two generations (~2 months) prior to experiments. During laboratory acclimations and experimental treatments, copepods consumed natural algal growth within the cultures, as well as a mixture of ground Spirulina (Salt Creek, Inc., South Salt Lake City, UT) and TetraMin Tropical Flakes (Spectrum Brands Pet LLC, Blacksburg, VA) that was added approximately once per week.

Critical thermal maximum variation among populations

Upper thermal tolerance was estimated by critical thermal maximum (CT_{max}) trials using loss of locomotor performance as the assay endpoint (as in Harada et al., 2019). In brief, sixteen adult copepods of each population (8 females and 8 males for all populations; divided across five trials) were transferred to 10-cm petri dishes containing filtered seawater

(20°C and 36 ppt) with no food overnight. In the morning, copepods were individually transferred into 0.2-mL strip tubes with 100 µL of water from the petri dish. Tubes were left uncapped, and were placed in an Applied Biosystems SimpliAmp™ Thermal Cycler (Thermo Fisher Scientific, Waltham, MA). After 5 min at 20°C, the temperature was increased using the AutoDelta function at rates of 0.1°C per 20 s from 20 to 32°C, and 0.1°C per min from 32°C to the temperature at which the last individual in the trial lost locomotor performance. Loss of performance (i.e., knockdown) was monitored by cycling 40 µL of water in each tube with a pipettor. Typically this procedure results in erratic swimming behaviour in *T. californicus*; however, at extremely high temperatures, this swimming response ceases, and copepods passively sink to the bottom of their tube. Endpoints were determined when an individual did not respond to three sequential tests with the pipettor, and CT_{max} was recorded as the temperature at which the endpoint was observed. After CT_{max} was determined, copepods were returned to 10-cm petri dishes with 20°C filtered seawater (36 ppt) for recovery, and survivorship assessed 8 h after the trials was >90%.

Developmental and adulthood plasticity in critical thermal maximum

To assess variation in the phenotypic plasticity of upper thermal tolerance in adult copepods as a result of differences in developmental temperature, gravid SD and BR females with mature (i.e., red) egg sacs were removed from laboratory cultures (24 females from 4 cultures per population). Egg sacs were dissected from the females (which synchronizes hatching), placed individually in wells of 6-well plates containing filtered seawater (20°C and 36 ppt), and allowed to hatch overnight. In the morning, nauplii (i.e., larvae) from each egg sac were counted, and split evenly across six treatments in 10-cm petri dishes (Fig. 1). Three treatments were developed at 20°C for 14 d, and three treatments were developed at 25°C for 10 d. Preliminary trials with the SD population determined that these developmental times were those required for the majority of individuals to reach adulthood (and to observe the first gravid female) at each temperature, suggesting that the temperature coefficient (Q_{10}) of developmental rate equals ~2 in this species. At the end of the developmental periods, the developmental treatments at each temperature were transferred to one of three adult acclimations: 20°C for 14 d, 25°C for 10 d, or 25°C for 14 d. These lengths of acclimations were chosen to allow two weeks of acclimation at 20°C, and comparisons between equivalent acclimations using either absolute time or physiologically adjusted time at 25°C (assuming a continued Q_{10} of ~2 for life history traits). On the days that the adult acclimations were completed, critical thermal maxima were determined as described above for 16 copepods

from each treatment and population. Note that individuals used in the tolerance trials were transferred to fresh filtered seawater without food at their acclimation conditions on the evening before the end of the acclimation treatments (i.e., the day before trials), and CT_{max} trials started from the acclimation temperatures in all cases.

To examine the potential for local thermal adaptation of the effects of developmental temperature on adult thermal tolerance plasticity, we performed a second experiment beginning with gravid females from the SC and PE populations. This experiment was conducted as described above for the SD and BR populations; however, the 25°C 10 d adult acclimation treatments were excluded, meaning egg sacs for each population were split across four treatments in total (Fig. 1). Again, CT_{max} was determined for 16 copepods for all treatments except the PE 25°C development and 25°C adulthood treatment for which n = 15.

Plasticity of ATP synthesis rate thermal sensitivity

To examine plasticity of the thermal performance curve for ATP synthesis rate, we compared two temperature treatments: 20°C for both development and adulthood versus 25°C for both development and adulthood (Fig. 1). Gravid SD and BR females carrying mature egg sacs (60 per population) were transferred from laboratory cultures to 10-cm petri dishes (6 per population) containing ~60 mL of filtered seawater (20°C and 36 ppt) with food. The egg sacs from the majority of these females (6-10 per plate) hatched overnight. All females were removed in the morning; egg sacs that were still carried by females were dissected free and returned to their respective dishes. Dissected egg sacs hatched within 3 h, and once all egg sacs had hatched, three petri dishes for each population were transferred to 25°C. As described above, juveniles in the 20 or 25°C dishes developed for 14 or 10 d, respectively, and adult acclimations at the two temperatures were also 14 or 10 d, respectively.

ATP synthesis rates were measured at 20, 25, 30, 33, 35 and 37°C using procedures similar to those of Harada et al. (2019). On the day before the end of the adult acclimation treatments, groups of 32 copepods (6 groups per population x treatment) were held at their acclimation temperatures in 10-cm petri dishes with filtered seawater (36 ppt) and no food overnight. In the morning, the groups of copepods were rinsed with 200 µL homogenization buffer (400 mM sucrose, 100 mM KCl, 70 mM HEPES, 6mM EGTA, 3 mM EDTA, 1% w/v BSA, pH 7.6), which had been chilled on ice. Each group was transferred to a 2-mL glass teflon homogenizer, and homogenized in 800 µL of fresh buffer. Following homogenization, mitochondria were isolated by differential centrifugation in 1.5 mL microcentrifuge tubes (Eppendorf, Hamburg, Germany). First, the tubes were centrifuged at 4°C and 1,000 g for 5

min, and supernatants were transferred to fresh tubes. Second, the new tubes were centrifuged at 4°C and 11,000 g for 10 min. The resulting supernatants were discarded, and mitochondrial pellets were resuspended in 205 µL assay buffer (560 mM sucrose, 100 mM KCl, 70 mM HEPES, 10 mM KH_2PO_4, pH 7.6). Isolated mitochondria were divided into eight 25-µL aliquots: 6 for synthesis reactions (1 per temperature), 1 for initial ATP concentration determination, and 1 for measuring DNA content which was used to normalize ATP synthesis rate. DNA content was assayed with Invitrogen™ Quant-iT™ PicoGreen™ dsDNA reagent following the manufacturer's protocols (Thermo Fisher Scientific, Waltham, MA).

ATP synthesis reactions were conducted in 0.2-µL strip tubes, and were initiated by adding 5 µL of a saturating substrate cocktail (final assay substrate concentrations 5 mM pyruvate, 2 mM malate, 10 mM succinate and 1 mM ADP in assay buffer), resulting in electron donation to both complex I and complex II (CI+II) of the electron transport system. Following substrate addition, tubes were immediately transferred to an Applied Biosystems SimpliAmp™ Thermal Cycler and incubated at the desired assay temperatures for 10 min. At the end of the reactions, 25 µL of each assay was added to 25 µL of CellTiter-Glo (Promega, Madison, WI), which stops ATP synthesis, and is used for ATP quantification. To determine initial ATP concentrations in the assays, one aliquot of each mitochondrial isolation was added to CellTiter-Glo immediately following substrate addition. All assays were held in the dark at room temperature for 10 min after addition of CellTiter-Glo, and then luminescence was determined with a Fluoroskan Ascent® FL (Thermo Fisher Scientific, Waltham, MA). ATP concentrations were calculated by comparison to a prepared standard curve (5 nM to 10 µM in assay buffer), and synthesis rates at each temperature were determined by subtracting the initial ATP concentration from the final ATP concentrations at each temperature for each mitochondrial isolation.

Plasticity of gene expression following heat shock

Variation in gene expression following heat shock was assessed for the same treatments as those used to examine ATP synthesis rates: 20°C for both development and adulthood, and 25°C for both development and adulthood (Fig. 1). Again, offspring from 60 SD and 60 BR females were divided between these treatments (repeated as described above). In the evening prior to the last day of the adult acclimations, groups of 15 copepods (18 groups per population x treatment) were transferred to 15-mL Falcon™ conical tubes (Thermo Fisher Scientific, Waltham, MA) containing 10 mL of filtered seawater (36 ppt) with no food at the acclimation temperature of the copepods. In the morning, tubes were transferred to water baths held at 35 or 36°C for 1 h (6 per population x treatment at each

temperature), and then returned to the acclimation temperature of the copepods for 1 h as in Barreto et al. (2015). The remaining 6 tubes (per population x treatment) were handled in the same manner, but were kept at the acclimation temperature of the copepods for the entire 2 h. At the end of all heat shock trials, copepods were frozen at -80°C until RNA isolation.

Briefly, RNA was isolated using TRI Reagent® (Sigma-Aldrich, Inc., St. Louis, MO) with half-volume reactions according to the manufacturer's instructions. RNA pellets were resuspended in 12 µL of Invitrogen™ UltraPure™ DNase/RNase-Free Distilled Water (Thermo Fisher Scientific, Waltham, MA) and were incubated at 56°C for 5 min. Isolations were treated with DNase using Invitrogen™ TURBO DNA-*free*™ Kits (Thermo Fisher Scientific, Waltham, MA) following the supplied protocols, and RNA concentration was determined with a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA). RNA integrity was confirmed by gel electrophoresis using two high-concentration samples. 100-150 ng of total RNA was used to synthesize cDNA for each sample with Applied Biosystems™ High-capacity RNA-to-cDNA™ Kits (Thermo Fisher Scientific, Waltham, MA) as instructed by the manufacturer, and the resulting cDNA samples were normalized to 2 ng input RNA μL^{-1}.

The mRNA expression levels of heat shock protein beta 1 (*hspb1*), heat shock protein 70 (*hsp70*), mitochondrial-encoded ATP synthase membrane subunit 6 (*mt-atp6*) and glyceraldehyde 3-phosphate dehydrogenase (*gapdh*) were assessed by quantitative real-time polymerase chain reaction (qRT-PCR). Primers for *hspb1*, *hsp70* and *gapdh* for the SD population were obtained from Barreto et al. (2015). If necessary due to single nucleotide polymorphisms between the populations, equivalent primers were designed for the BR population using a population-specific reference genome (Barreto et al., 2018). Primers for *mt-atp6* for each population were designed using population-specific mitochondrial genomes (DQ913891; Burton et al., 2007; Barreto et al., 2018). All primer sequences are listed in Table 1. 15 µL qRT-PCR reactions were prepared in duplicate with 4 µL cDNA, 5 pmol of each primer, and 7.5 µL iTaq Universal SYBR Green Supermix (Bio-Rad Laboratories, Inc., Hercules, CA). All reactions were conducted in an AriaMx Real-time PCR System (Agilent Technologies, Inc., Santa Clara, CA) with the following protocol: 95°C for 2 min, then 95°C for 10 s followed by 58°C for 30 s for 40 cycles. The presence of a single amplicon was confirmed by a melting curve analysis after each reaction. Samples for each population were quantified relative to population-specific 5-point standard curves that were included on all reaction plates, and were prepared by serial dilution (1X to 1/625X) of a high-concentration heat shock sample from each population. Transcript levels of *hspb1*, *hsp70* and *mt-atp6* were

then expressed relative to those of *gapdh*, which has been confirmed to be an appropriate housekeeping gene for heat shock studies in *T. californicus* (Schoville et al., 2012; Barreto et al., 2015; Harada and Burton, 2019). Final qRT-PCR sample sizes for the majority of our treatments and genes were n = 6; however, for some groups n = 4 or 5 due to a combination of insufficient RNA for cDNA synthesis, failed reactions as a result of extremely low *hspb1* expression levels under control conditions, or insufficient cDNA to assess all genes (one instance resulting in no estimate for *mt-atp6*). As a result, the final sample sizes for all qRT-PCR data are presented in detail in Table S2.

Statistical analyses

All analyses were performed with R v3.4.0 (R Core Team, 2017) and $\alpha = 0.05$. Latitudinal variation in CT_{max} across all populations failed to satisfy the assumptions for parametric statistics even after log transformation. Thus, differences among populations were assessed by Kruskal-Wallis analysis of variance (ANOVA) followed by Nemenyi post-hoc tests. Potential effects of sex on CT_{max} were assessed by Wilcoxon rank-sum tests within each population. In contrast, variation in CT_{max} associated with developmental and adulthood temperatures met the assumptions for parametric tests in both the SD and BR, and the SC and PE experiments. These data were assessed by general linear models followed by ANOVAs with population, developmental temperature, and adult acclimation treatment as factors. Post-hoc comparisons among groups were performed with Tukey tests. After log transformation to meet assumptions of normality and homogeneity of variances, variation in ATP synthesis rate was assessed with a mixed-effect linear model followed by ANOVA with fixed effects of population, acclimation treatment and assay temperature, and a random effect of mitochondrial isolation. All interactions between factors were not significant in the initial model ($p \geq 0.12$ for all), and were removed from the final model for this test. Planned pairwise post-hoc comparisons were conducted between assay temperatures within each population x acclimation treatment, between acclimation treatments within each population x assay temperature, and between populations within each acclimation treatment x assay temperature with Student's *t* tests (84 comparisons). The resulting *p*-values were corrected for multiple tests with the Benjamini-Hochberg method (Benjamini and Hochberg, 1995). As an alternative method to examine variation in the thermal sensitivity of ATP synthesis rate, rates were normalized to the 25°C rate within each mitochondrial isolation. Variation in normalized ATP synthesis rate was assessed at assay temperatures from 30 to 37°C with similar methods to those described above for the unnormalized rates (although log transformation and removal of interactions among model factors were not required). Finally,

mRNA expression data were all log transformed to meet necessary assumptions, and then differences among groups were examined by two-way ANOVAs with acclimation treatment and heat shock exposure as factors followed by post-hoc Tukey tests. Note comparisons of gene expression between populations were not made, because the expression levels of each population were quantified relative to standard curves that were population specific and for some genes the qRT-PCR primers were population specific as well (Table 1). Full ANOVA tables for all models tested have been uploaded to the Dryad Digital Repository (*upload will be completed and the accession number provided should the manuscript be accepted*).

Results

Latitudinal variation in critical thermal maximum

CT_{max} demonstrated significant variation among populations ($p < 2.2 \times 10^{-16}$; Fig. 2), and there were no differences in this trait between females and males in the current study ($p \geq 0.19$ within all populations). Overall, CT_{max} increased from northern to southern populations (Fig. 2), suggesting that variation in this thermal limit generally parallels previously published latitudinal variation in lethal temperatures among populations (Willett, 2010; Kelly et al., 2012; Pereira et al., 2014, 2017; Leong et al., 2018; Willett and Son, 2018; Foley et al., 2019). This makes CT_{max} ideal for examining effects of phenotypic plasticity on upper thermal tolerance in experiments utilizing designs where offspring from individual egg sacs are divided among treatments.

Phenotypic plasticity of upper thermal tolerance

Development at 20 or 25°C resulted in variation in CT_{max} among SD and BR copepods that was significantly affected by a three-way interaction among population, developmental temperature and adult acclimation treatment ($p = 0.03$). This effect was resolved by post-hoc tests, although there was little evidence for differential effects between the populations (Fig. 3A). For all SD and BR copepods that developed at 20°C, CT_{max} values were similar regardless of adult acclimation temperature or time (range of means ± s.e.m.: 37.9 ± 0.1 to 38.3 ± 0.1°C; $p = 0.054$ for 20°C-developed SD acclimated to 20°C for 14 d versus 25°C for 10 d as adults, and $p \geq 0.22$ for all other comparisons), suggesting that in 20°C-developed *T. californicus* there is no upper thermal limit plasticity in adults. In contrast, relative to development at 20°C, development at 25°C resulted in significant increases in CT_{max} for both SD and BR when adults were also acclimated at 25°C (range of means ± s.e.m.: 38.5 ± 0.1 to 39.1 ± 0.1°C; $p \leq 0.04$ for all comparisons within populations) and these effects were similar at 10 and 14 d of acclimation ($p \geq 0.16$ between times for both

populations). However, if 25°C-developed SD and BR copepods were acclimated at 20°C as adults, there was a significant loss of tolerance (i.e., decrease in CT_{max}) in both populations (mean ± s.e.m.: 38.1 ± 0.1 and 37.7 ± 0.1°C for SD and BR, respectively; $p < 0.01$ for all within population comparisons).

To explore these effects in populations known to be locally adapted to lower temperatures than the SD and BR populations, we conducted a second experiment examining plasticity of CT_{max} in the SC and PE populations. In SC and PE copepods, variation in adult CT_{max} was unaffected by the three-way interaction among population, developmental temperature and adult acclimation treatment ($p = 0.60$). Moreover, there were no significant effects of interactions between population and either developmental temperature ($p = 0.83$) or adult acclimation treatment ($p = 0.23$), or of population alone ($p = 0.20$). However, the interactive effect of developmental temperature and adult acclimation treatment significantly affected CT_{max} ($p = 9.9 \times 10^{-5}$). Again, post-hoc comparisons resolved this effect, and suggested similar patterns of variation among treatments for SC and PE as those described above for SD and BR (Fig. 3B). There was no significant variation in CT_{max} as a result of adult acclimation temperature in 20°C-developed copepods from either SC or PE (mean ± s.e.m.: 36.4 ± 0.1 and 36.8 ± 0.1°C for SC, and 36.8 ± 0.1 and 37.1 ± 0.1°C for PE; $p \geq 0.39$ between adult temperatures for both populations). In contrast, development at 25°C resulted in significantly higher CT_{max} in both SC and PE copepods, but only when adults were acclimated at 25°C (mean ± s.e.m.: 38.0 ± 0.2 and 37.6 ± 0.1°C for SC and PE, respectively; $p \leq 0.04$ for all comparisons within populations). If 25°C-developed copepods from either population were acclimated to 20°C as adults, there was a significant decrease in CT_{max} (mean ± s.e.m.: 36.7 ± 0.2 and 36.7 ± 0.01 for SC and PE, respectively; $p < 0.01$ for both within population comparisons).

Plasticity of ATP synthesis rate

Maintaining *T. californicus* at 20 or 25°C for both development and acclimation as adults resulted in significant variation in the thermal performance curve for CI+II ATP synthesis rates in isolated mitochondria (Table 2). Specifically, these curves were affected by population ($p = 4.4 \times 10^{-5}$), development and adult acclimation temperature ($p < 2.2 \times 10^{-16}$), and assay temperature ($p < 2.2 \times 10^{-16}$). In both developmental and acclimation treatments, synthesis rates initially increased and then decreased with increasing assay temperatures in SD and BR (Table 2), and post-hoc tests found no evidence for differences between the SD and BR copepods within each treatment x assay temperature combination ($q \geq 0.11$ for all). Yet, across all assay temperatures in both populations, 25°C development and adult

acclimation resulted in higher ATP synthesis rates compared to those measured following development and acclimation at 20°C ($q \leq 0.046$ for all). This overall vertical shift in the thermal performance curve has the potential to mask variation between these treatments in the extent to which ATP synthesis rates are maintained during acute exposures to high temperatures. However, in SD copepods, rates of ATP synthesis first significantly declined with temperature between assay temperatures of 30 and 33°C for the 20°C treatment ($q = 7.3 \times 10^{-4}$), whereas for the 25°C treatment the first decrease occurred between assay temperatures of 33 and 35°C ($q = 1.7 \times 10^{-3}$). This suggests that copepods developed and acclimated as adults at warmer temperatures maintained synthesis rates at higher temperatures at least in this population.

As an alternative approach to examine maintenance of ATP synthesis at high temperatures, we normalized synthesis rates across temperatures to those measured at 25°C for each mitochondrial isolation, which allows comparisons of the proportional changes in synthesis rate with assay temperature (Fig. 4). Note that this normalization could also reasonably be done to the rates measured at 20°C, but the results would be similar (Fig. 4). After normalization, proportional changes in rates of ATP synthesis were affected by a three-way interaction among population, temperature of development and adult acclimation, and assay temperature ($p = 0.02$). Post-hoc comparisons revealed similar patterns of variation among assay temperatures as those detected for the unnormalized rates (as would be expected), and when assayed at 37°C, 20°C-developed and -acclimated SD copepods maintained higher synthesis rates than 20°C-developed and -acclimated BR copepods ($q = 0.03$). Additionally, at high assay temperatures both populations maintained greater rates of ATP synthesis following development and adult acclimation at 25°C than following development and adult acclimation at 20°C ($q \leq 5.5 \times 10^{-3}$ for 33 to 37°C in SD and $q = 0.02$ for 37°C in BR).

Plasticity of gene expression following heat shock

The mRNA expression levels of both heat shock proteins examined in the current study (*hspb1* and *hsp70*) demonstrated similar effects of heat shock, and developmental and adult acclimation temperature regardless of population (SD or BR; Fig. 5). In all cases, gene expression was affected by a significant interaction between the heat shock treatment, and the temperature of development and adult acclimation ($p = 0.04$ for *hsp70* in SD, and $p \leq 5.4 \times 10^{-3}$ for all others). In general, copepods developed and acclimated as adults at 25°C expressed higher levels of *hspb1* and *hsp70* than copepods developed and acclimated at 20°C

(particularly after heat shock), although these patterns were not always resolved by post-hoc tests (Fig. 5).

Given the potential role of mitochondrial performance in determining upper thermal tolerance (e.g., Harada et al., 2019) and a previous demonstration of decreased mitochondrial-encoded mRNA levels following heat shock in *T. californicus* (Schoville et al., 2012), we also examined variation in the expression of *mt-atp6*. In the current study, there were interactive effects of heat shock treatment, and developmental and adult acclimation temperature on the mRNA expression of *mt-atp6* in both the SD and BR population ($p \leq 0.02$; Fig. 6). In SD copepods, regardless of the temperature of development and adult acclimation, *mt-atp6* levels were similar in the control and 35°C heat shock treatments ($p = 1.00$ for both). Within both of these treatments expression levels were significantly higher in copepods developed and acclimated as adults at 25°C than in those developed and acclimated at 20°C ($p < 0.001$ for both). In contrast, in SD copepods that had been developed and acclimated as adults at 20°C, heat shock at 36°C increased *mt-atp6* expression ($p \leq 0.02$), whereas in those that had been developed and acclimated at 25°C, the same exposure decreased *mt-atp6* expression ($p < 0.001$ for both). As a result, there was no effect of the temperature experienced throughout development and adulthood on *mt-atp6* mRNA levels in the 36°C heat shock treatment ($p = 0.88$). In BR copepods, variation in *mt-atp6* expression demonstrated somewhat different patterns than those observed for SD copepods. For both developmental and adult acclimation temperatures, there were trends for decreasing *mt-atp6* mRNA levels with increasing heat shock temperatures, but these patterns were only resolved in post-hoc tests in BR copepods developed and acclimated as adults at 25°C between the control treatment and the 35 and 36°C heat shock treatments ($p < 0.001$ for both; $p \geq 0.06$ for all others). However, *mt-atp6* expression was greater in BR copepods developed and acclimated as adults at 25°C than at 20°C in the control treatment and in the 35 or 36°C heat shock treatments ($p \leq 0.02$).

Discussion

The results presented here provide experimental support for both of our proposed hypotheses. First, variation in developmental temperature resulted in differences in the plasticity of upper thermal limits in adult *T. californicus*. Regardless of population, 25°C-developed copepods demonstrated clear plasticity of CT_{max} between adulthood temperatures of 20 and 25°C. In contrast, there was no evidence of plasticity of this trait in adults that had developed at 20°C. Second, differences in developmental and adulthood acclimation

temperatures were associated with plastic changes in two physiological mechanisms that are thought to contribute to the basis of local adaptation of upper thermal tolerance in this species. Furthermore, these effects were consistent with the differences in CT_{max} between these developmental and adult acclimation treatments. Therefore, our data suggest that adaptive processes may have the potential to shape the effects of developmental temperatures on the plasticity of thermal tolerance due to shared underlying mechanisms, despite similar patterns of plasticity observed in the four locally adapted populations of *T. californicus* examined in the current study.

Inter-population variation in CT_{max} is consistent with local thermal adaptation

In general, dynamic and static thermal tolerance assays (i.e., gradual ramping exposures to high temperatures and abrupt exposures to a constant high temperature) resolve similar patterns of variation among experimental groups or treatments (e.g., Ford and Beitinger, 2005; Jørgensen et al., 2019), and our previous study suggested this was also the case among three Californian populations of *T. californicus* (distributed across ~3° latitude; Harada et al., 2019). In the current study, there was a clear pattern of CT_{max} variation among populations that was consistent with substantial latitudinal thermal adaptation of upper thermal tolerance in *T. californicus*, as has been suggested in studies using static assays (Willett, 2010; Kelly et al., 2012; Pereira et al., 2014, 2017; Leong et al., 2018; Willett and Son, 2018; Foley et al., 2019). Overall, CT_{max} increased from northern to southern populations, although there was somewhat limited statistical resolution among populations likely due to a combination of relatively small differences in several comparisons and nonparametric post-hoc tests. In general, thermal tolerance limits are expected to decline approximately linearly with latitude (Sunday et al., 2011), whereas our results suggest this is not the case in this species (Fig. S1C). This may reflect the relatively gradual latitudinal thermal gradient at higher compared to lower latitudes across the species range (particularly in the summer; Fig. S1B), and consistent with this possibility, both Leong et al. (2018) and Pereira et al. (2017) demonstrated approximately linear changes in upper thermal tolerance with differences in habitat air temperatures among *T. californicus* populations.

Despite this clear signature of local adaptation associated with the latitudinal thermal gradient along the west coast of California, interpreting the results of the current study in the context of *T. californicus* habitat temperatures is challenging. Splashpool temperatures vary substantially throughout the day (e.g., Harada and Burton, 2019), and the intertidal is a "mosaic" habitat in which local thermal conditions may not necessarily reflect expected patterns with latitudinal variation in sea surface or air temperatures (Helmuth et al., 2002,

2006; Sanford and Kelly, 2010). For instance, variation in the daily timing of tidal cycles with latitude can paradoxically result in higher temperature exposures at northern compared to southern latitudes (Kuo and Sanford, 2009), although this is less likely to be relevant for supralittoral tidepools. There is limited published temperature data for *T. californicus* tidepools; however, at least comparing the SD and SC populations, overall summer temperatures tend to be warmer for the more southern population (i.e., SD; Leong et al., 2018), which is consistent with the difference in CT_{max} between these populations. Both average and maximum temperatures may be important for local adaptation, and it is likely that for upper thermal limits maximum temperatures are more relevant (e.g., Somero, 2005). Yet, with the limited available data there does not appear to be a tight relationship between maximum temperatures and CT_{max} for SD and SC copepods (Leong et al., 2018). In part, this may be a consequence of CT_{max} only representing a proxy of the lethal thermal limit, which is typically justified as an "ecological death" associated with an inability to escape predation or harmful conditions due to loss of locomotor performance (e.g., Beitinger et al., 2000), and the extent to which this justification is relevant for *T. californicus* is unknown. That said, there is clearly local adaptation of CT_{max} in *T. californicus*, and comparing our results with those of Pereira et al. (2017) suggests that there is at least reasonable concordance between variation in CT_{max} and variation in lethal temperatures across populations.

Several other factors may influence comparisons of CT_{max} and splashpool temperatures, and additional temperature recordings will be necessary to examine this relationship in a comprehensive manner. For our data, these comparisons are likely dependent on the acclimation temperatures in the current study. 20 and 25°C are not atypical average weekly or monthly summer tidepool temperatures for the SD and SC populations (e.g., Leong et al., 2018), but it is unclear if average conditions control field acclimatization (e.g., Fangue et al., 2011), and variable or cycling temperatures may alter acclimation responses compared to constant conditions (e.g., Paaijmans et al., 2013). Moreover, the thermal ramping rates in our CT_{max} trials are likely faster than natural rates of temperature increase in *T. californicus* tidepools in most cases, which may indicate that our CT_{max} values underestimate upper thermal tolerance under habitat conditions (Harada and Burton, 2019). Taken together, results in *T. californicus* to date consistently suggest that latitudinal temperature variation plays an influential role in inter-population variation in upper thermal tolerance, but the roles of local-scale differences in temperature and of habitat variability in determining upper thermal limits are yet to be fully resolved.

One distinct result of the current study was the lack of variation in CT_{max} between the sexes (see Fig S1C). There is an overall consensus that, in comparison to males, female *T. californicus* are more tolerant of stressful conditions for a wide range of abiotic factors, including temperature (Willett, 2010; Willett and Son, 2018; Foley et al., 2019). Insufficient statistical power due to nonparametric tests, and relatively low sex-specific sample sizes (n = 8) may explain the lack of sex effects in the current study. However, two other studies have also failed to detect differences in upper thermal tolerance between the sexes (Pereira et al., 2014, 2017). Regardless, CT_{max} was not statistically affected by sex in our study, and as a result we did not consider variation between females and males further here.

Developmental temperature and adulthood plasticity of CT_{max}

Across the SD, BR, SC and PE populations of *T. californicus*, we consistently observed variation in CT_{max} plasticity in adults as a result of temperatures experienced during development. After development at 25°C, CT_{max} was higher in copepods acclimated to 25°C in adulthood than in copepods acclimated to 20°C, whereas 20°C-developed copepods displayed no difference in upper thermal limits between the adult acclimation treatments. These patterns could be the result of differences in reversible adult plasticity due to developmental temperatures, or of temperature-dependent reversibility of developmental plasticity, but in either case this phenotypic variation is consistent with an interactive effect between developmental and adulthood temperatures. To our knowledge, this is the first demonstration of interactive effects between temperatures in development and in later stages of life on thermal limit plasticity in a marine ectotherm. Alternatively, these patterns could be potential consequences of differences in developmental survival between 20 and 25°C as we did not directly monitor survivorship in this study; however, Pereira et al. (2017) and Harada et al. (2019) observed little evidence of differential survival at these temperatures across most *T. californicus* populations. Furthermore, previous studies have also detected interactive effects of developmental and adulthood temperatures on upper thermal tolerance in zebrafish (*Danio rerio*; Schaefer and Ryan, 2006) and fruit flies (*Drosophila sp.*; Kellermann et al., 2017; Kellermann and Sgrò, 2018), which in combination with the results of the current study suggest there is mounting evidence that these effects may be common for this trait.

In *T. californicus*, the effects of developmental temperature on adulthood plasticity of CT_{max} were relatively large, as plasticity of CT_{max} was completely absent in adults after development at 20°C, whereas after development at 25°C adulthood plasticity was observed in all populations. Moreover, the adulthood acclimation response ratio in 25°C-developed copepods (i.e., ΔCT_{max} $°C^{-1}$) was typical for aquatic ectotherms (~0.2; Gunderson and

Stillman, 2015). Similarly, variation in developmental temperature results in presence-absence differences in adulthood plasticity in *D. melanogaster* (Kellermann et al., 2017), although in *Drosophila sp.* cooler developmental temperatures tend to increase plasticity in adults (Kellermann et al., 2017; Kellermann and Sgrò, 2018), whereas our results suggest warmer developmental temperatures increase adult plasticity in *T. californicus*. The loss of adult CT_{max} plasticity at an only moderately reduced developmental temperature in *T. californicus* is potentially surprising given the prevalence of at least a modest capacity for acclimation of this trait across many species (e.g., Gunderson and Stillman, 2015). Acclimation to constant conditions, rather than cycling thermal regimes that more closely resemble natural tidepool conditions, may influence both this lack of plasticity, and the effects of developmental temperature on adulthood plasticity in the current study. In addition, we examined only a relatively small range of adulthood temperatures (20-25°C), which may contribute to the lack of observed plasticity, and the extent to which plasticity may alter CT_{max} in 20°C-developed copepods over a wider range of adult acclimation temperatures remains an open question.

The short generation times of *T. californicus* and *Drosophila sp.* likely increase the concordance between developmental and adulthood temperatures (particularly in the habitats of *T. californicus*). This may contribute to the effects of developmental temperature on adulthood plasticity of thermal tolerance in this species, because developmental effects are expected to be stronger if conditions in development are predictive of those experienced as adults (Cooper et al., 2010, 2012; Nettle and Bateson, 2015; Beaman et al., 2016). Consistent with this possibility, in the comparatively long-lived zebrafish, Schaefer and Ryan (2006) observed only subtle shifts in CT_{max} plasticity in adults as a result of differences in developmental temperatures. Although the interactive effect of developmental and adulthood temperatures with respect to patterns of CT_{max} plasticity was relatively strong in *T. californicus*, the maximum difference in CT_{max} among treatments was approximately 1°C. Regardless, our data demonstrate that variation in developmental temperatures can have substantial effects on the adulthood plasticity of upper thermal tolerance in aquatic ectotherms.

Mechanisms underlying CT_{max} plasticity and local thermal adaptation

The possibility of interactions between adaptive processes and phenotypic plasticity is well established (e.g., Crispo, 2007; Hendry, 2016; Donelson et al., 2019; Kelly, 2019), and thus there is also the potential for local thermal adaptation to shape effects of developmental temperature on the plasticity of upper thermal tolerance in adults. Furthermore, if heat

hardening is used as a metric of adulthood plasticity, there is some evidence for adaptive variation in these effects among *Drosophila sp.* (Kellermann and Sgrò, 2018). However, when this possibility is assessed with adult acclimations in temperate and tropical *D. melanogaster*, developmental effects on adulthood plasticity are similar among populations (Kellermann et al., 2017). Similarly, although development at 20 or 25°C was associated with variation in CT_{max} plasticity in adults from all of the *T. californicus* populations examined in the current study, there was no variation in this effect among populations. Indeed, in 25°C-developed copepods, the average CT_{max} difference between adult acclimations of 20 and 25°C were remarkably similar across the four populations (SD: 1.0°C, BR: 0.8°C, SC: 1.3°C and PE 0.9°C). Thus, our results suggest that effects of developmental temperature on adulthood plasticity of CT_{max} have not been altered substantially by local thermal adaptation among these populations of *T. californicus*. However, we also found that physiological mechanisms that putatively underlie latitudinal variation in upper thermal tolerance in this species (e.g., Schoville et al., 2012; Harada et al., 2019) show patterns of variation in response to developmental and adulthood temperatures that parallel variation in CT_{max}.

Harada et al. (2019) demonstrated that, during acute exposures to elevated temperatures, the temperatures at which maximal ATP synthesis rates first decline are correlated with CT_{max} across the SD, AB and SC populations of *T. californicus*. Consistent with this relationship, several studies have demonstrated loss of ATP synthesis capacity in heart mitochondria at temperatures that are approximately equal to or are immediately below the upper thermal limits in species of fishes (Iftikar and Hickey, 2013; Christen et al., 2018; O'Brien et al., 2018). Temperature-mediated plasticity of mitochondrial functions is also often observed in ectothermic species (e.g., Guderley, 2004; Seebacher et al., 2010; Chung and Schulte, 2015; Chung et al., 2017a,b, 2018; Bryant et al., 2018), and our data suggest that this is the case in *T. californicus*. In the SD and BR populations, copepods that were developed and acclimated as adults at 25°C had greater ATP synthesis rates than those that were developed and acclimated at 20°C, which was consistent with higher expression levels of *mt-atp6* in these 25°C treatments than these 20°C treatments under control (i.e., non-heat shocked) conditions. Additionally, developmental and adult acclimation temperatures of 25°C compared to 20°C resulted in greater maintenance of ATP synthesis rates at high temperatures, which is consistent with difference in CT_{max} between these treatments. In comparison to Harada et al. (2019), the thermal performance curves observed in our study were horizontally shifted to moderately lower temperatures, and were remarkably flat with maximum Q_{10} values of approximately 1.4-1.5 across treatments. Although relatively

thermally insensitive physiological rates have been observed previously in *T. californicus* (e.g., Scheffler et al., 2019), there is clearly an unknown source of variation in these ATP synthesis curves among studies. It is possible that culturing conditions could contribute to this variation, as Harada et al. (2019) examined copepods taken directly from stock cultures (i.e., 400-mL beakers), whereas in the current study we raised groups of copepods specifically for these measurements in 10-cm petri dishes. Associated with this difference, these studies likely also differ somewhat in densities of copepods, algal growth and, potentially, oxygen levels under holding conditions, which have the potential to result in plastic variation in mitochondrial performance between the studies. Regardless, with the exception of temperature, our 20 and 25°C treatments were held under equivalent conditions, and therefore the difference in high-temperature maintenance of ATP synthesis rates between these treatments is likely robust to any variation in thermal performance curve estimates across studies.

Schoville et al. (2012) examined genetically determined differences in the transcriptomic response to acute heat stress between the SD and SC populations of *T. californicus*. Both the strongest response and largest difference between the two populations was the extent to which heat shock protein genes were induced following heat stress. Particularly for *hspb1* and *hsp70*, heat shock protein mRNA expression was increased to much higher levels in the warm-adapted SD population than in the relatively cold-adapted SC population. As heat shock proteins are molecular chaperones that mitigate the negative effects of high temperature due to damaged and unfolded proteins (Hochachka and Somero, 2002), these transcriptomic patterns suggest that differences in heat shock protein expression may contribute to the difference in upper thermal tolerance between the SD and SC populations. The evidence for a correlation between large inductions of heat shock protein expression and increased tolerance of high temperatures is somewhat mixed among genes and species (e.g., Healy et al., 2010; Gleason and Burton, 2015); however, studies in fruit flies (*D. melanogaster*) and marine snails (*Chlorostoma funebralis*) generally support a positive relationship between these two traits (e.g., Bettencourt et al., 2008; Tomanek et al., 2008). In *T. californicus*, Tangwancharoen et al. (2018) demonstrated putatively adaptive functional variation associated with sequence differences among populations in both the regulatory and coding regions of *hspb1*, and Barreto et al. (2015) utilized RNA interference to show that knockdown of transcripts for this gene directly decreases survivorship following acute thermal stress. Therefore, the increased inductions of *hspb1* and *hsp70* we observed in SD and BR copepods developed and acclimated as adults at 25°C compared to those developed

and acclimated at 20°C are likely beneficial effects of plasticity, and are consistent with CT_{max} differences between these treatments. Part of this variation in heat shock protein expression may be associated with the differences in recovery temperatures in our study, as each developmental and adult acclimation treatment was recovered at its acclimation temperature. However, in all cases, the fold differences in expression between the 20 and 25°C developmental and adulthood treatments following heat shock (2.0-6.4) are greater than would be expected due to thermodynamic effects on transcription rates alone (1.4-1.7 given an expected Q_{10} of 2-3 which is consistent with thermal sensitivities of transcriptional elongation rates; e.g., van Breukelen and Martin, 2002).

Taken together, our results indicate that both the extents to which ATP synthesis rates are maintained and heat shock proteins are induced at high temperatures are elevated in *T. californicus* that are developed and acclimated at 25°C compared to those that are developed and acclimated at 20°C. The acute temperature exposures used to assess these mechanisms here that matched those of previous studies in this species (Schoville et al., 2012; Barreto et al., 2015; Harada et al., 2019), but these exposures were notably different than the thermal ramp experienced during our CT_{max} trials, which may affect comparisons among these traits (Harada and Burton, 2019). Despite this, the patterns of variation in ATP synthesis rates and heat shock protein expression observed here were consistent with differences in CT_{max} between the copepods that were developed and acclimated as adults at 25°C and the copepods that were developed and acclimated at 20°C. This suggests that maintenance of ATP synthesis rates and induction of heat shock proteins likely contribute to the basis for plasticity of upper thermal tolerance associated with developmental and adulthood temperatures in this species. Yet, the extent to which the effects of developmental temperature, specifically, on plasticity in adults can be attributed to these mechanisms requires additional research, as these traits were not assayed in 25°C-developed copepods that were transferred to 20°C in adulthood in the current study. However, our data suggest these mechanisms may play a role in plastic effects due to developmental temperatures in general.

Conclusion

The effects of environmental change on organisms ultimately depend on the capacities to modulate key physiological traits to facilitate performance and persistence. Phenotypic plasticity, adaptation and interactions between these two processes all play important roles in these responses (e.g., Kellermann and van Heerwaarden, 2019). Here we show that temperatures experienced in development also shape the adulthood plasticity of

upper thermal limits in the intertidal copepod *T. californicus*. These effects may be particularly relevant for aquatic ectotherms as thermal tolerance limits likely underlie geographic range limits in many of these species (Sunday et al., 2012; Pinsky et al., 2019). Our results highlight that beneficial effects of developmental plasticity with respect to environmental change have the potential to be overestimated if considered without accounting for temperature variation in adulthood. Additionally, the data presented here suggest that the physiological mechanisms that may underlie these effects (e.g., shifts in the thermal performance curve for ATP synthesis and the regulation of heat shock genes) are, at least to some extent, shared with the mechanisms associated with local thermal adaptation in *T. californicus*. This mechanistic overlap indicates the potential for interactions among local adaptation and plasticity at difference life stages to shape variation in upper thermal tolerance in ectothermic organisms.

Competing interests

No competing interests declared

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Data availability

All data collected for the current study have been uploaded to the Dryad Digital Repository (*upload will be completed and accession number provided should the manuscript be accepted*).

References

Barreto, F. S., Schoville, S. D. and Burton, R. S. (2015). Reverse genetics in the tide pool: knock‐down of target gene expression via RNA interference in the copepod *Tigriopus californicus*. *Mol. Ecol. Resour.* **15**, 868-879.

Barreto, F. S., Watson, E. T., Lima, T. G., Willett, C. S., Edmands, S., Li, W. and Burton, R.S. (2018). Genomic signatures of mitonuclear coevolution across populations of *Tigriopus californicus*. *Nat. Ecol. Evol.* **2**, 1250.

Barrett, R. D. H. and Hendry, A. P. (2012). Evolutionary rescue under environmental change. In *Behavioural Responses to a Changing World: Mechanisms and Consequences* (ed. U. Candolin and B. B. M. Wong), pp. 216–228. Oxford: Oxford University Press.

Bay, R. A., Rose, N., Barrett, R., Bernatchez, L., Ghalambor, C. K., Lasky, J. R., Brem, R.B., Palumbi, S. R. and Ralph, P. (2017). Predicting responses to contemporary environmental change using evolutionary response architectures. *Am. Nat.* **189**, 463-473.

Beaman, J. E., White, C. R. and Seebacher, F. (2016). Evolution of plasticity: mechanistic link between development and reversible acclimation. *Trends Ecol. Evol.* **31**, 237-249.

Beitinger, T. L., Bennett, W. A. and McCauley, R. W. (2000). Temperature tolerances of North American freshwater fishes exposed to dynamic changes in temperature. *Environ. Biol. Fishes.* **58**, 237-275.

Benjamini, Y. and Hochberg, Y. (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. R. Stat. Soc. Series B.* **57**, 289-300.

Bettencourt, B. R., Hogan, C. C., Nimali, M. and Drohan, B. W. (2008). Inducible and constitutive heat shock gene expression responds to modification of Hsp70 copy number in *Drosophila melanogaster* but does not compensate for loss of thermotolerance in Hsp70 null flies. *BMC Biol.* **6**, 5.

Bryant, H. J., Chung, D. J. and Schulte, P. M. (2018). Subspecies differences in thermal acclimation of mitochondrial function and the role of uncoupling proteins in killifish. *J. Exp. Biol.* **221**, jeb186320.

Burggren, W. W. (2015). Dynamics of epigenetic phenomena: intergenerational and intragenerational phenotype ‘washout’. *J. Exp. Biol.* **218**, 80-87.

Burton, R. S. (1997). Genetic evidence for long term persistence of marine invertebrate populations in an ephemeral environment. *Evolution.* **51**, 993-998.

Burton, R. S. (1998). Intraspecific phylogeography across the Point Conception biogeographic boundary. *Evolution*. **52**, 734-745.

Burton, R. S., Byrne, R. J. and Rawson, P. D. (2007). Three divergent mitochondrial genomes from California populations of the copepod *Tigriopus californicus*. *Gene*. **403**, 53-59.

Burton, R. S. and Lee, B. N. (1994). Nuclear and mitochondrial gene genealogies and allozyme polymorphism across a major phylogeographic break in the copepod *Tigriopus californicus*. *Proc. Natl. Acad. Sci. USA.* **91**, 5197-5201.

Chen, I. C., Hill, J. K., Ohlemüller, R., Roy, D. B. and Thomas, C. D. (2011). Rapid range shifts of species associated with high levels of climate warming. *Science.* **333**, 1024-1026.

Chung, D. J., Bryant, H. J. and Schulte, P. M. (2017a). Thermal acclimation and subspecies-specific effects on heart and brain mitochondrial performance in a eurythermal teleost (*Fundulus heteroclitus*). *J. Exp. Biol.* **220**, 1459-1471.

Chung, D. J., Morrison, P. R., Bryant, H. J., Jung, E., Brauner, C. J. and Schulte, P. M. (2017b). Intraspecific variation and plasticity in mitochondrial oxygen binding affinity as a response to environmental temperature. *Sci. Rep.* **7**, 16238.

Chung, D. J. and Schulte, P. M. (2015). Mechanisms and costs of mitochondrial thermal acclimation in a eurythermal killifish (*Fundulus heteroclitus*). *J. Exp. Biol.* **218**, 1621-1631.

Chung, D. J., Sparagna, G. C., Chicco, A. J. and Schulte, P. M. (2018). Patterns of mitochondrial membrane remodeling parallel functional adaptations to thermal stress. *J. Exp. Biol.* **221**, jeb174458.

Cohen, J. M., Lajeunesse, M. J. and Rohr, J. R. (2018). A global synthesis of animal phenological responses to climate change. *Nat. Clim. Change.* **8**, 224-228.

Cooper, B. S., Czarnoleski, M. and Angilletta Jr, M. J. (2010). Acclimation of thermal physiology in natural populations of *Drosophila melanogaster*: a test of an optimality model. *J. Evol. Biol.* **23**, 2346-2355.

Cooper, B. S., Hammad, L. A., Fisher, N. P., Karty, J. A. and Montooth, K. L. (2012). In a variable thermal environment selection favors greater plasticity of cell membranes in *Drosophila melanogaster*. *Evolution.* **66**, 1976-1984.

Cooper, B. S., Tharp II, J. M., Jernberg, I. I. and Angilletta Jr, M. J. (2012). Developmental plasticity of thermal tolerances in temperate and subtropical populations of *Drosophila melanogaster*. *J. Therm. Biol.* **37**, 211-216.

Crain, C. M., Kroeker, K. and Halpern, B. S. (2008). Interactive and cumulative effects of multiple human stressors in marine systems. *Ecol. Lett.* **11**, 1304-1315.

Crill, W. D., Huey, R. B. and Gilchrist, G. W. (1996). Within- and between- generation effects of temperature on the morphology and physiology of *Drosophila melanogaster*. *Evolution*. **50**, 1205-1218.

Crispo, E. (2007). The Baldwin effect and genetic assimilation: revisiting two mechanisms of evolutionary change mediated by phenotypic plasticity. *Evolution.* **61**, 2469-2479.

Christen, F., Desrosiers, V., Dupont-Cyr, B. A., Vandenberg, G. W., Le François, N. R., Tardif, J. C., Dufresne, F., Lamarre, S. G. and Blier, P. U. (2018). Thermal tolerance and

thermal sensitivity of heart mitochondria: mitochondrial integrity and ROS production. *Free Radic. Biol. Med.* **116**, 11-18.

Diamond, S. E., Chick, L. D., Perez, A., Strickler, S. A. and Martin, R. A. (2018). Evolution of thermal tolerance and its fitness consequences: parallel and non-parallel responses to urban heat islands across three cities. *Proc. R. Soc. B.* **285**, 20180036.

Donelson, J. M., Salinas, S., Munday, P. L. and Shama, L. N. (2018). Transgenerational plasticity and climate change experiments: where do we go from here?. *Glob. Change Biol.* **24**, 13-34.

Donelson, J. M., Sunday, J. M., Figueira, W. F., Gaitán-Espitia, J. D., Hobday, A. J., Johnson, C. R., Leis, J. M., Ling, S. D., Marshall, D., Pandolfi, J. M. and Pecl, G. (2019). Understanding interactions between plasticity, adaptation and range shifts in response to marine environmental change. *Philos. Trans. R. Soc. B.* **374**, 20180186.

Edmands, S. (2001). Phylogeography of the intertidal copepod *Tigriopus californicus* reveals substantially reduced population differentiation at northern latitudes. *Mol. Ecol.* **10**, 1743-1750.

Fangue, N. A., Osborne, E. J., Todgham, A. E. and Schulte, P. M. (2011). The onset temperature of the heat-shock response and whole-organism thermal tolerance are tightly correlated in both laboratory-acclimated and field-acclimatized tidepool sculpins (*Oligocottus maculosus*). *Physiol. Biochem. Zool.* **84**, 341-352.

Foley, H. B., Sun, P. Y., Ramirez, R., So, B. K., Venkataraman, Y. R., Nixon, E. N., Davies, K. J. and Edmands, S. (2019). Sex-specific stress tolerance, proteolysis, and lifespan in the invertebrate *Tigriopus californicus*. *Exp. Gerontol*. **119**, 146-156.

Ford, T. and Beitinger, T. L. (2005). Temperature tolerance in the goldfish, *Carassius auratus*. *J. Therm. Biol.* **30**, 147-152.

Fox, R. J., Donelson, J. M., Schunter, C., Ravasi, T. and Gaitán-Espitia, J. D. (2019). Beyond buying time: the role of plasticity in phenotypic adaptation to rapid environmental change. *Philos. Trans. R. Soc. B.* **374**, 20180174.

Gleason, L. U. and Burton, R. S. (2015). RNA- seq reveals regional differences in transcriptome response to heat stress in the marine snail *Chlorostoma funebralis*. *Mol. Ecol.* **24**, 610-627.

Guderley, H. (2004). Metabolic responses to low temperature in fish muscle. *Biol. Rev.* **79**, 409-427.

Gunderson, A. R. and Stillman, J. H. (2015). Plasticity in thermal tolerance has limited potential to buffer ectotherms from global warming. *Proc. R. Soc. B.* **282**, 20150401.

Harada, A. E. and Burton, R. S. (2019). Ecologically relevant temperature ramping rates enhance the protective heat shock response in an intertidal ectotherm. *Physiol. Biochem. Zool.* **92**, 152-162.

Harada, A. E., Healy, T. M. and Burton, R.S. (2019). Variation in thermal tolerance and its relationship to mitochondrial function across populations of *Tigriopus californicus*. *Front. Physiol.* **10**, 213.

Healy, T. M., Tymchuk, W. E., Osborne, E. J. and Schulte, P. M. (2010). Heat shock response of killifish (*Fundulus heteroclitus*): candidate gene and heterologous microarray approaches. *Physiol. Genomics.* **41**, 171-184.

Helmuth, B., Broitman, B. R., Blanchette, C. A., Gilman, S., Halpin, P., Harley, C. D., O'Donnell, M. J., Hofmann, G. E., Menge, B. and Strickland, D. (2006). Mosaic patterns of thermal stress in the rocky intertidal zone: implications for climate change. *Ecol. Monogr.* **76**, 461-479.

Helmuth, B., Harley, C. D., Halpin, P. M., O'Donnell, M., Hofmann, G. E. and Blanchette, C. A. (2002). Climate change and latitudinal patterns of intertidal thermal stress. *Science*. **298**, 1015-1017.

Hendry, A.P. (2015). Key questions on the role of phenotypic plasticity in eco-evolutionary dynamics. *J. Hered.* **107**, 25-41.

Hochachka, P. W. and Somero, G. N. (2002). *Biochemical Adaptation: Mechanism and Process in Physiological Evolution.* Oxford, New York: Oxford University Press.

Iftikar, F. I. and Hickey, A. J. (2013). Do mitochondria limit hot fish hearts? Understanding the role of mitochondrial function with heat stress in *Notolabrus celidotus*. *PLoS One*. **8**, e64120.

Jakobs, R., Gariepy, T. D. and Sinclair, B. J. (2015). Adult plasticity of cold tolerance in a continental-temperate population of *Drosophila suzukii*. *J. Insect Physiol.* **79**, 1-9.

Jørgensen, L .B., Malte, H. and Overgaard, J. (2019). How to assess *Drosophila* heat tolerance: unifying static and dynamic tolerance assays to predict heat distribution limits. *Funct. Ecol.* **33**, 629-642.

Kellermann, V. and van Heerwaarden, B. (2019). Terrestrial insects and climate change: adaptive responses in key traits. *Physiol. Entomol.* **44**, 99-115.

Kellermann, V., van Heerwaarden, B. and Sgrò, C. M. (2017). How important is thermal history? Evidence for lasting effects of developmental temperature on upper thermal limits in *Drosophila melanogaster*. *Proc. R. Soc. B.* **284**, 20170447.

Kellermann, V. and Sgrò, C. M. (2018). Evidence for lower plasticity in CT_{MAX} at warmer developmental temperatures. *J. Evol. Biol.* **31**, 1300-1312.

Kelly, M. (2019). Adaptation to climate change through genetic accommodation and assimilation of plastic phenotypes. *Philos. Trans. R. Soc. B.* **374**, 20180176.

Kelly, M. W., Pankey, M. S., DeBiasse, M. B. and Plachetzki, D. C. (2017). Adaptation to heat stress reduces phenotypic and transcriptional plasticity in a marine copepod. *Funct. Ecol.* **31**, 398-406.

Kelly, M. W., Sanford, E. and Grosberg, R. K. (2012). Limited potential for adaptation to climate change in a broadly distributed marine crustacean. *Proc. R. Soc. B.* **279**, 349-356.

Kelly, S. A., Panhuis, T. M. and Stoehr, A. M. (2011). Phenotypic plasticity: molecular mechanisms and adaptive significance. *Compr. Physiol.* **2**, 1417-1439.

Kingsolver, J. G., MacLean, H. J., Goddin, S. B. and Augustine, K. E. (2016). Plasticity of upper thermal limits to acute and chronic temperature variation in *Manduca sexta* larvae. *J. Exp. Biol.* **219**, 1290-1294.

Kuo, E. S. and Sanford, E. (2009). Geographic variation in the upper thermal limits of an intertidal snail: implications for climate envelope models. *Mar. Ecol. Prog. Ser.* **388**, 137-146.

Leong, W., Sun, P. Y. and Edmands, S. (2017). Latitudinal clines in temperature and salinity tolerance in tidepool copepods. *J. Hered.* **109**, 71-77.

Massamba-N'Siala, G., Prevedelli, D. and Simonini, R. (2014). Trans-generational plasticity in physiological thermal tolerance is modulated by maternal pre-reproductive environment in the polychaete *Ophryotrocha labronica*. *J. Exp. Biol.* **217**, 2004-2012.

Merilä, J. and Hendry, A. P. (2014). Climate change, adaptation, and phenotypic plasticity: the problem and the evidence. *Evol. Appl.* **7**, 1-14.

Morley, S. A., Peck, L. S., Sunday, J. M., Heiser, S. and Bates, A. E. (2019). Physiological acclimation and persistence of ectothermic species under extreme heat events. *Glob. Ecol. Biogeogr.* **28**, 1018-1037.

Mueller, C. A., Bucsky, J., Korito, L. and Manzanares, S. (2019). Immediate and persistent effects of temperature on oxygen consumption and thermal tolerance in embryos and larvae of the Baja California chorus frog, *Pseudacris hypochondriaca*. *Front. Physiol.* **10**, 754.

Nettle, D. and Bateson, M. (2015). Adaptive developmental plasticity: what is it, how can we recognize it and when can it evolve?. *Proc. R. Soc. B.* **282**, 20151005.

O'Brien, K. M., Rix, A. S., Egginton, S., Farrell, A. P., Crockett, E. L., Schlauch, K., Woolsey, R., Hoffman, M. and Merriman, S. (2018). Cardiac mitochondrial metabolism may contribute to differences in thermal tolerance of red-and white-blooded Antarctic notothenioid fishes. *J. Exp. Biol.* **221**, jeb177816.

Overgaard, J., Kristensen, T. N., Mitchell, K. A. and Hoffmann, A. A. (2011). Thermal tolerance in widespread and tropical *Drosophila* species: does phenotypic plasticity increase with latitude?. *Am. Nat.* **178**, S80-S96.

Paaijmans, K. P., Heinig, R. L., Seliga, R. A., Blanford, J. I., Blanford, S., Murdock, C. C. and Thomas, M. B. (2013). Temperature variation makes ectotherms more sensitive to climate change. *Glob. Change Biol.* **19**, 2373-2380.

Parmesan, C. and Yohe, G. (2003). A globally coherent fingerprint of climate change impacts across natural systems. *Nature*. **421**, 37-42.

Pereira, R. J., Barreto, F. S. and Burton, R. S. (2014). Ecological novelty by hybridization: experimental evidence for increased thermal tolerance by transgressive segregation in *Tigriopus californicus*. *Evolution*. **68**, 204-215.

Pereira, R. J., Barreto, F. S., Pierce, N. T., Carneiro, M. and Burton, R. S. (2016). Transcriptome‐wide patterns of divergence during allopatric evolution. *Mol. Ecol.* **25**, 1478-1493.

Pereira, R. J., Sasaki, M. C. and Burton, R. S. (2017). Adaptation to a latitudinal thermal gradient within a widespread copepod species: the contributions of genetic divergence and phenotypic plasticity. *Proc. R. Soc. B.* **284**, 20170236.

Peterson, D. L., Kubow, K. B., Connolly, M. J., Kaplan, L. R., Wetkowski, M. M., Legon, W., Phillips, B. C. and Edmands, S. (2013). Reproductive and phylogenetic divergence of tidepool copepod populations across a narrow geographical boundary in Baja California. *J. Biogeogr.* **40**, 1664-1675.

Perry, A. L., Low, P. J., Ellis, J. R. and Reynolds, J. D. (2005). Climate change and distribution shifts in marine fishes. *Science*. **308**, 1912-1915.

Pinsky, M. L., Eikeset, A. M., McCauley, D. J., Payne, J. L. and Sunday, J. M. (2019). Greater vulnerability to warming of marine versus terrestrial ectotherms. *Nature*. **569**, 108-111.

R Core Team (2017) *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. *https://www.R-project.org/*

Sanford, E. and Kelly, M. W. (2011). Local adaptation in marine invertebrates. *Annu. Rev. Mar. Sci.* **3**, 509-535.

Schaefer, J. and Ryan, A. (2006). Developmental plasticity in the thermal tolerance of zebrafish *Danio rerio*. *J. Fish Biol.* **69**, 722-734.

Scheffler, M. L., Barreto, F. S. and Mueller, C. A. (2019). Rapid metabolic compensation in response to temperature change in the intertidal copepod, *Tigriopus californicus*. *Comp. Biochem. Physiol. A.* **230**, 131-137.

Schoville, S. D., Barreto, F. S., Moy, G. W., Wolff, A. and Burton, R. S. (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 Evol. Biol.* **12**, 170.

Schulte, P. M., Healy, T. M. and Fangue, N. A. (2011). Thermal performance curves, phenotypic plasticity, and the time scales of temperature exposure. *Integr. Comp. Biol.* **51**, 691-702.

Seebacher, F., Beaman, J. and Little, A. G. (2014). Regulation of thermal acclimation varies between generations of the short-lived mosquitofish that developed in different environmental conditions. *Funct. Ecol.* **28**, 137-148.

Seebacher, F., Brand, M. D., Else, P. L., Guderley, H., Hulbert, A. J. and Moyes, C. D. (2010). Plasticity of oxidative metabolism in variable climates: molecular mechanisms. *Physiol. Biochem. Zool.* **83**, 721-732.

Seebacher, F. and Grigaltchik, V. S. (2015). Developmental thermal plasticity of prey modifies the impact of predation. *J. Exp. Biol.* **218**, 1402-1409.

Seebacher, F., White, C. R. and Franklin, C. E. (2015). Physiological plasticity increases resilience of ectothermic animals to climate change. *Nat. Clim. Change.* **5,** 61-66.

Somero, G. N. (2005). Linking biogeography to physiology: evolutionary and acclimatory adjustments of thermal limits. *Front. Zool.* **2**, 1.

Somero, G.N. (2010). The physiology of climate change: how potentials for acclimatization and genetic adaptation will determine 'winners' and 'losers'. *J. Exp. Biol.* **213**, 912-920.

Stillman, J. H. and Somero, G. N. (2000). A comparative analysis of the upper thermal tolerance limits of eastern Pacific porcelain crabs, genus *Petrolisthes*: influences of latitude, vertical zonation, acclimation, and phylogeny. *Physiol. Biochem. Zool.* **73**, 200-208.

Sunday, J. M., Bates, A. E. and Dulvy, N. K. (2011). Global analysis of thermal tolerance and latitude in ectotherms. *Proc. R. Soc. B.* **278**, 1823-1830.

Sunday, J. M., Bates, A. E. and Dulvy, N. K. (2012). Thermal tolerance and the global redistribution of animals. *Nat. Clim. Change.* **2**, 686-690.

Tangwancharoen, S., Moy, G. W. and Burton, R. S. (2018). Multiple modes of adaptation: regulatory and structural evolution in a small heat shock protein gene. *Mol. Biol. Evol.* **35**, 2110-2119.

Tepolt, C. K. and Somero, G. N. (2014). Master of all trades: thermal acclimation and adaptation of cardiac function in a broadly distributed marine invasive species, the European green crab, *Carcinus maenas*. *J. Exp. Biol.* **217**, 1129-1138.

Tomanek, L. (2008). The importance of physiological limits in determining biogeographical range shifts due to global climate change: the heat-shock response. *Physiol Biochem. Zool.* **81**, 709-717.

Troia, M. J., Whitney, J. E. and Gido, K. B. (2015). Thermal performance of larval longfin dace (*Agosia chrysogaster*), with implications for climate change. *Environ. Biol. Fishes*. **98**, 395-404.

van Breukelen, F. and Martin, S. L. (2002). Reversible depression of transcription during hibernation. *J. Comp. Physiol. B.* **172**, 355-361.

Wallace, G. T., Kim, T. L. and Neufeld, C. J. (2014). Interpopulational variation in the cold tolerance of a broadly distributed marine copepod. *Conserv. Physiol.* **2**, cou041.

Wiens, J. J. (2016). Climate-related local extinctions are already widespread among plant and animal species. *PLoS Biol.* **14**, e2001104.

Willett, C. S. (2010). Potential fitness trade-offs for thermal tolerance in the intertidal copepod *Tigriopus californicus*. *Evolution*. **64**, 2521-2534.

Willett, C. S. and Son, C. (2018). The evolution of the thermal niche across locally adapted populations of the copepod *Tigriopus californicus*. *Bull. South Calif. Acad. Sci.* **117**, 150-157.

Yanar, M., Erdoğan, E. and Kumlu, M. (2019). Thermal tolerance of thirteen popular ornamental fish Species. *Aquaculture*, **501**, 382-386.

Zizzari, Z. V. and Ellers, J. (2014). Rapid shift in thermal resistance between generations through maternal heat exposure. *Oikos*. **123**, 1365-1370.

Figures

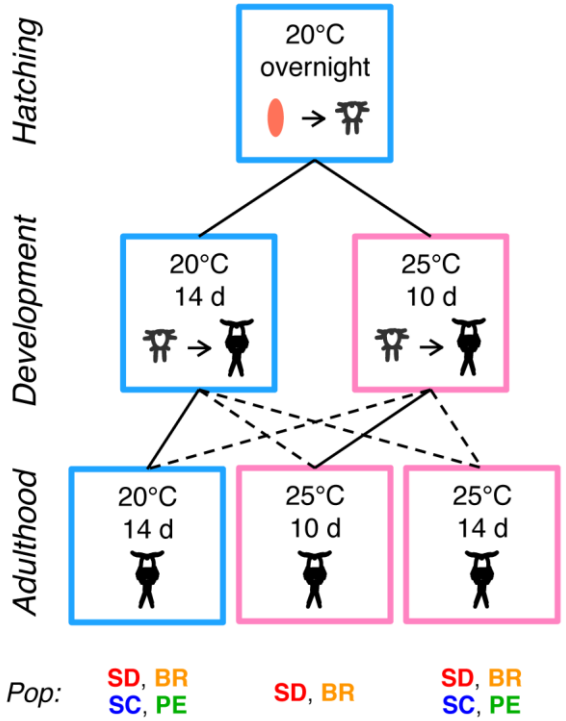


Figure 1. Flow chart of the developmental and adulthood temperature exposures for the plasticity experiments. All measurements were made at the end of the adulthood acclimations. Solid lines connect treatments (light blue boxes – 20°C; pink boxes – 25°C) with data for critical thermal maximum (CT_{max}), ATP synthesis rates and mRNA expression levels. Dashed lines connect boxes for treatments with data for only CT_{max}. Populations used for each treatment are shown below the adulthood boxes (San Diego, California – SD, red; Bird Rock, California – BR, orange; Santa Cruz, California – SC, blue; Pescadero, California – PE, green).

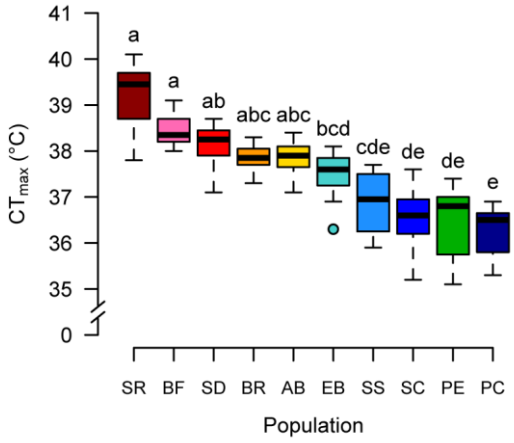


Figure 2. Variation in critical thermal maximum (CT_{max}) among populations of *T. californicus* distributed from Mexico to Canada. Populations are plotted from southernmost to northernmost (left to right): San Rogue, Mexico (SR; dark red), La Bufadora, Mexico (BF; pink), San Diego, California (SD; red), Bird Rock, California (BR; orange), Abalone Cove, California (AB; yellow), Estero Bay, California (EB; teal), San Simeon, California (SS; light blue), Santa Cruz, California (SC; blue), Pescadero, California (PE; green) and Pacific Crest, Canada (PC; dark blue). Data are displayed as standard box and whisker plots, and lower case letters indicate the results of post-hoc comparisons among populations (n = 16 for all populations).

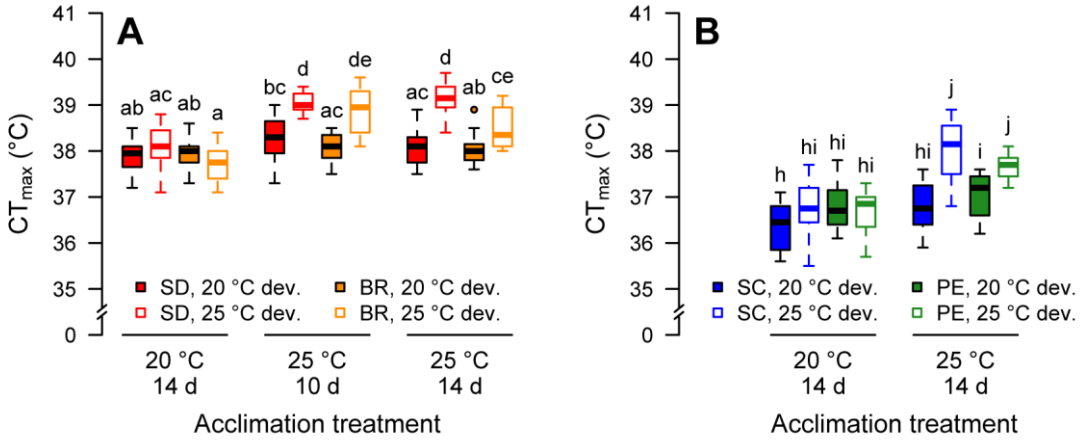


Figure 3. Phenotypic plasticity of critical thermal maximum (CT_{max}) in Californian populations of *T. californicus* as a result of temperatures experienced during development and adulthood. Panel A: San Diego (SD; red) and Bird Rock (BR; orange) copepods. Panel B: Santa Cruz (SC; blue) and Pescadero (PE; green) copepods. Data are displayed as standard box and whisker plots (20°C development – filled boxes; 25°C development – open boxes), and lower case letters indicate the results of post-hoc comparisons among treatments within each panel (n = 16 for all groups except 25°C-developed and 25°C-acclimated PE for which n = 15).

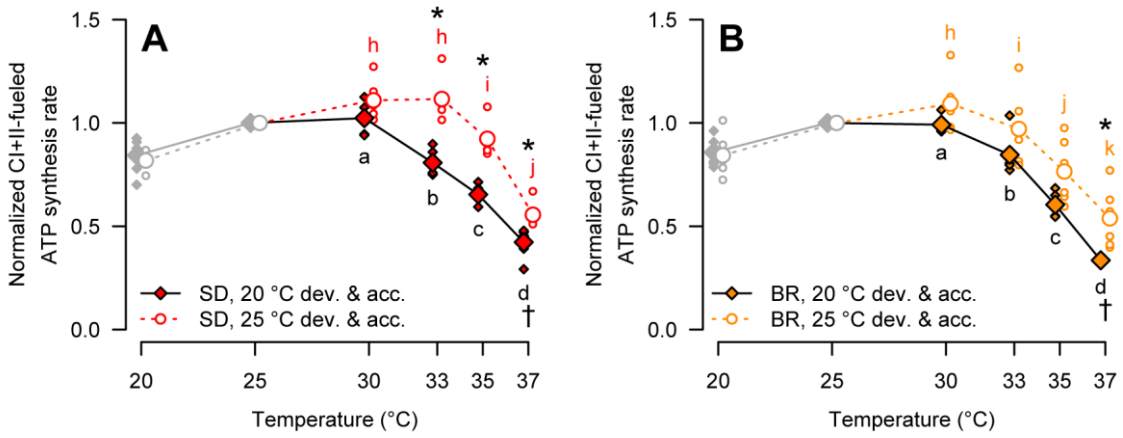


Figure 4. Proportional changes (from 25°C) in the thermal performance curves for complexes I and II (CI+II)-fueled ATP synthesis rates as a result of developmental and adulthood temperatures in *T. californicus*. Panel A: data for the San Diego population (SD; red), and panel B: data for the Bird Rock population (BR; orange). Copepods were developed (dev.), and then acclimated as adults (acc.) at 20°C (filled diamonds; solid lines) or 25°C (open circles; dotted lines). Small symbols display individual data points (n = 6 per population and dev. & acc. treatment), and large symbols display mean values for each group. Grey symbols show data that were not assessed statistically after normalization. Lower case letters indicate the results of post-hoc comparisons among assay temperatures within each dev. & acc. treatment for each panel, asterisks indicate differences between the dev. & acc. treatments within assay temperatures for each population, and daggers indicate differences between populations for specific assays temperatures within each dev. & acc. treatment.

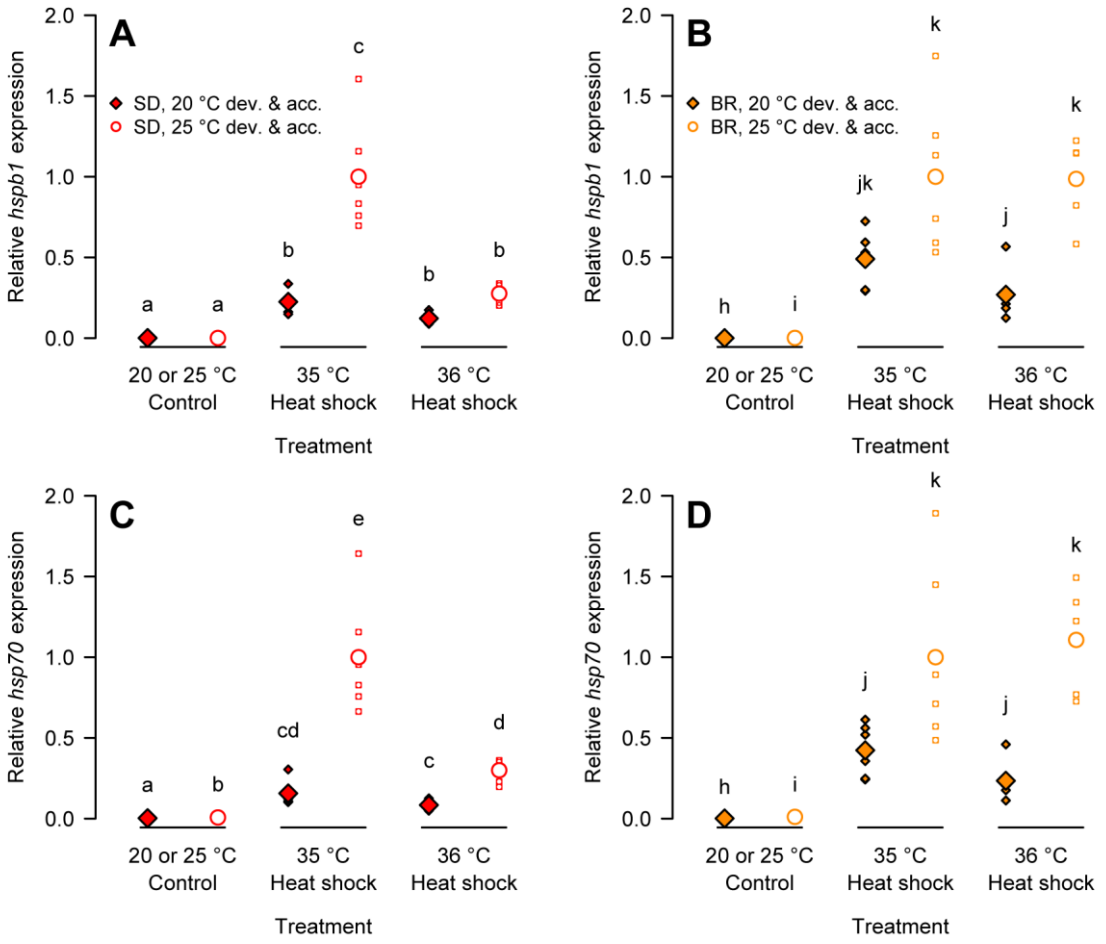


Figure 5. Variation in the induction of heat shock protein mRNA expression (A,B – *hspb1*; C,D – *hsp70*) as a result of developmental and adulthood temperatures in *T. californicus*. Panels A,C: data for the San Diego population (SD; red), and panels B,D: data for the Bird Rock population (BR; orange). Copepods were developed (dev.), and then acclimated as adults (acc.) at 20°C (filled diamonds; solid lines) or 25°C (open circles; dotted lines). Expression levels were quantified relative to those of the housekeeping gene *gapdh*, and are displayed normalized to the mean expression level of the 35°C heat shock treatment for the 25°C dev. & acc. copepods. Small symbols display individual data points (n = 5 or 6 for all treatments except the 36°C heat shock treatment for the SD 20°C dev. & acc. copepods for which n = 4; see Table S2 for details), large symbols display mean values for each treatment, and lower case letters indicate the results of post-hoc comparisons among treatments within each panel.

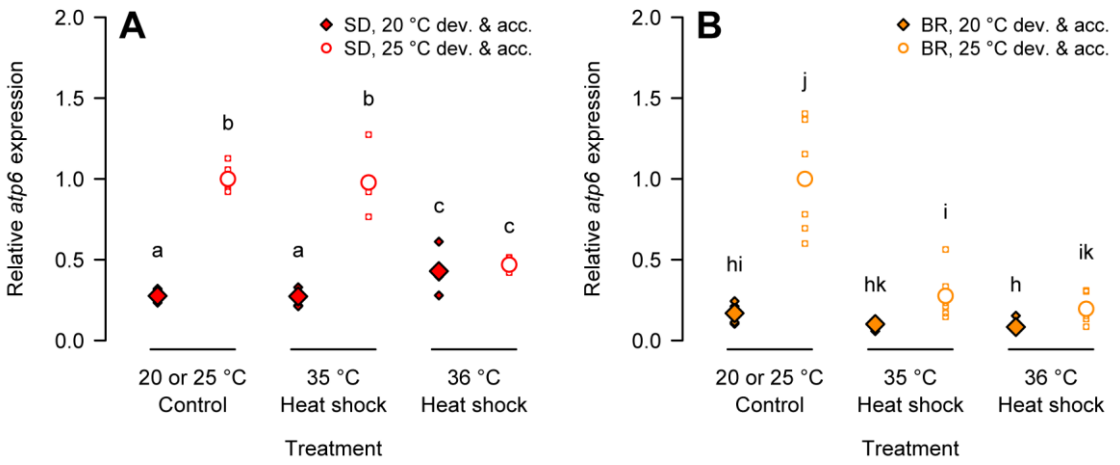


Figure 6. Variation in *mt-atp6* mRNA expression (A – SD, red; B – BR, orange) as a result of developmental and adulthood temperatures in *T. californicus*. Copepods were developed (dev.), and then acclimated as adults (acc.) at 20°C (filled diamonds; solid lines) or 25°C (open circles; dotted lines). Expression levels were quantified relative to those of the housekeeping gene *gapdh*, and are displayed normalized to the mean expression level of the control treatment for the 25°C dev. & acc. copepods. Small symbols display individual data points (n = 5 or 6 for all treatments except the 36°C heat shock treatment for the SD 20°C dev. & acc. copepods for which n = 4; see Table S2 for details), large symbols display mean values for each treatment, and lower case letters indicate the results of post-hoc comparisons among treatments within each panel.

Table 1. qRT-PCR primers.

Gene	Primer[1]	Population-specific sequence (5′ to 3′)	
		SD	BR
hspb1	F	CGATTTTCATCTGGGTCTCAA	CGATTTCCATCTGGGTCTCAA
	R	TTGAAGAACTCCTCCGCTGT	same as SD
hsp70	F	CTCTGTGCCGACCTTTTCC	same as SD
	R	CTGGATTGATGCTCTTGTTCA	same as SD
mt-atp6	F	AGGACAGCCCATCTGAGG	AGGACAGCCCATCTAAGGTT
	R	CAGCCAGAGTTAAGGGACG	ACTGCCAAAGTTAATGGACGA
gapdh	F	CAACCACGAGCAATACGAGA	same as SD
	R	GGAGGAGGGGATGATGTTTT	same as SD

[1] F = forward; R = reverse

Table 2. Developmental and adulthood plasticity in complexes I and II (CI+II)-fueled ATP synthesis rates as a result variation in temperature.

Population	Assay temperature *(°C)*	CI+II ATP synthesis rate[1] *(pmol min^{-1} ng DNA^{-1})*	
		20°C dev. & acc.[2]	25°C dev. & acc.[2]
San Diego, California (SD)	20	1.48 ± 0.20^{a}	2.58 ± 0.35^{h}*
	25	1.74 ± 0.21^{b}	3.13 ± 0.39^{i}*
	30	1.78 ± 0.22^{b}	3.43 ± 0.37^{j}*
	33	1.42 ± 0.20^{a}	3.47 ± 0.41^{j}*
	35	1.14 ± 0.14^{c}	2.88 ± 0.35^{i}*
	37	0.74 ± 0.11^{d}	1.75 ± 0.25^{k}*
Bird Rock, California (BR)	20	1.34 ± 0.06^{a}	2.13 ± 0.26^{hi}*
	25	1.58 ± 0.11^{b}	2.54 ± 0.34^{ij}*
	30	1.56 ± 0.11^{b}	2.80 ± 0.41^{j}*
	33	1.32 ± 0.07^{a}	2.47 ± 0.36^{i}*
	35	0.95 ± 0.07^{c}	1.97 ± 0.32^{h}*
	37	0.53 ± 0.03^{d}	1.38 ± 0.24^{k}*

[1] mean ± s.e.m.; letters indicate the results of post-hoc tests within populations and dev. & acc. treatments; asterisks indicate differences within populations and assay temperatures

[2] dev. & acc. = development and adult acclimation

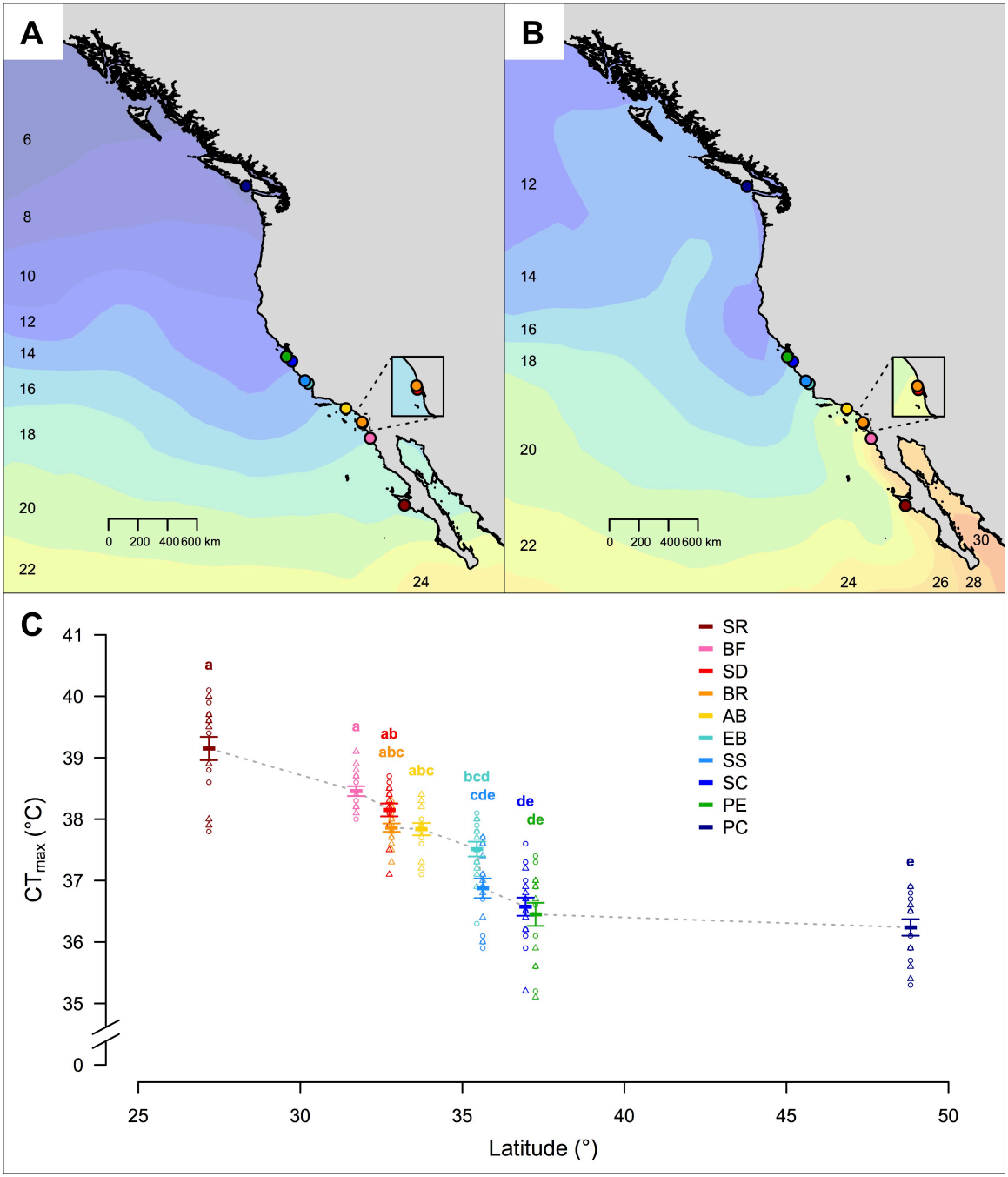


Figure S1. Population locations, sea surface temperatures (A: winter – January 2018; B: summer – July 2018), and latitudinal variation in critical thermal maximum (CT_{max}; C). San Rogue (SR; dark red), La Bufadora (BF; pink), San Diego (SD; red), Bird Rock (BR; orange), Abalone Cove (AB; yellow), Estero Bay (EB; teal), San Simeon (SS; light blue), Santa Cruz (SC; blue), Pescadero (PE; green) and Pacific Crest (PC; dark blue). Dashes and error bars display mean ± s.e.m, dotted grey line segments connect population means, small symbols show individual data points (females – circles; males – triangles), and lower case letters indicate the results of post-hoc comparisons among populations (n = 16 for all populations). Sea surface temperatures obtained from *http://www.cpc.ncep.noaa.gov/products/GIS/GIS_DATA/sst_oiv2/index.php*.

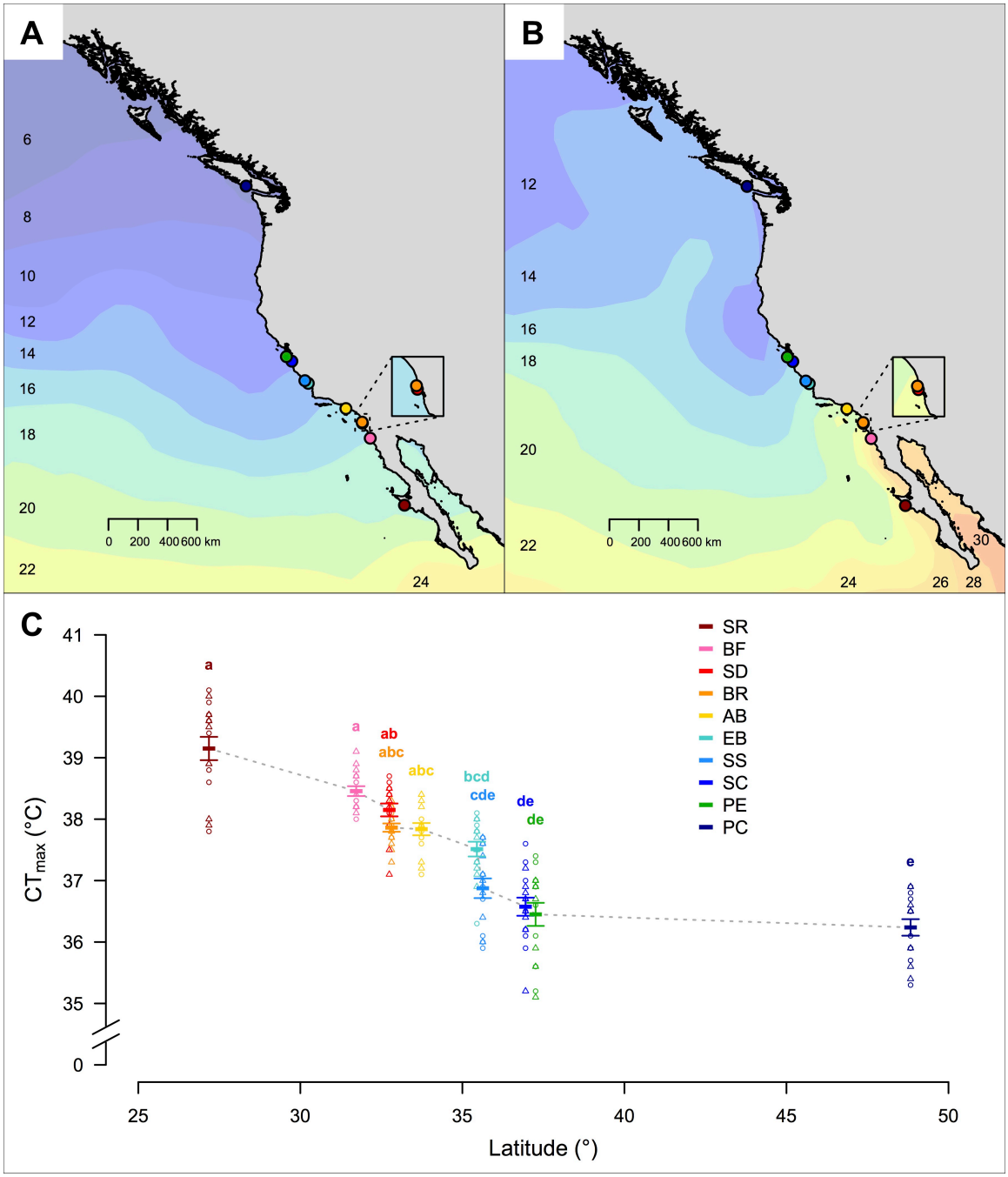
Table S1. Geographic coordinates for *T. californicus* collection locations.

Population	Country, State or Province	Latitude	Longitude
San Rogue (**SR**)	Mexico, Baja California Sur	27.179937	-114.397685
La Bufadora (**BF**)	Mexico, Baja California	31.723725	-116.721815
San Diego (**SD**)	USA, California	32.744828	-117.255264
Bird Rock (**BR**)	USA, California	32.814202	-117.273379
Abalone Cove (**AB**)	USA, California	33.736857	-118.373817
Estero Bay (**EB**)	USA, California	35.447477	-120.931204
San Simeon (**SS**)	USA, California	35.629308	-121.160777
Santa Cruz (**SC**)	USA, California	36.949433	-122.046358
Pescadero (**PE**)	USA, California	37.259631	-122.414116
Pacific Crest (**PC**)	Canada, British Columbia	48.829868	-125.151587

Table S2. qRT-PCR sample sizes for each gene, population and treatment.

Population	Gene	dev. & acc.[1] treatment	Heat shock treatment sample size		
			Control	35°C	36°C
San Diego, California (SD)	*hspb1*	20°C	5	6	4
		25°C	5	6	5
	hsp70	20°C	6	6	4
		25°C	6	6	5
	mt-atp6	20°C	5	6	4
		25°C	6	6	5
	gapdh	20°C	6	6	4
		25°C	6	6	5
Bird Rock, California (BR)	*hspb1*	20°C	6	6	5
		25°C	6	6	6
	hsp70	20°C	6	6	5
		25°C	6	6	6
	mt-atp6	20°C	6	6	5
		25°C	6	6	6
	gapdh	20°C	6	6	5
		25°C	6	6	6

[1] dev. & acc. = developmental and adult acclimation



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		25°C	5	6	5
	hsp70	20°C	6	6	4
		25°C	6	6	5
	mt-atp6	20°C	5	6	4
		25°C	6	6	5
	gapdh	20°C	6	6	4
		25°C	6	6	5
Bird Rock, California (BR)	*hspb1*	20°C	6	6	5
		25°C	6	6	6
	hsp70	20°C	6	6	5
		25°C	6	6	6
	mt-atp6	20°C	6	6	5
		25°C	6	6	6
	gapdh	20°C	6	6	5
		25°C	6	6	6

[1] dev. & acc. = developmental and adult acclimation