

1 *Title page*

2 Title: Variation in developmental temperature alters adulthood plasticity of thermal
3 tolerance in *Tigriopus californicus*

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5 Running head: Development alters adulthood plasticity

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17 Keywords: phenotypic plasticity, critical thermal maximum, development, heat shock
18 protein, ATP synthesis, copepod

19

20 Summary statement:

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

24

- 25 **List of abbreviations**
- 26 Analysis of variance – ANOVA
- 27 Critical thermal maximum – CT_{max}
- 28 Electron transport system complexes I and II – CI+II
- 29 Quantitative real-time polymerase chain reaction – qRT-PCR
- 30 Temperature coefficient – Q_{10}

31 **Abstract**

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

52 **Introduction**

53 As the earth warms, organisms are increasingly impacted by the effects of high
54 environmental temperatures (e.g., Wiens, 2016; Cohen et al., 2018; Pinsky et al., 2019).
55 Indeed, the geographic range limits of many species have already shifted as a result of
56 anthropogenic climate change, and in general these shifts have been towards regions with
57 cooler temperatures (e.g., Parmesan and Yohe, 2003; Perry et al., 2005; Chen et al.,
58 2011). The extents to which these effects have occurred, and will continue to occur,
59 depend largely on the adaptive and plastic capacities of organisms to adjust key
60 physiological traits, such as thermal tolerance limits (especially in aquatic ectotherms;
61 Sunday et al., 2012; Pinsky et al., 2019), in response to increased temperatures (e.g.,
62 Crain et al., 2008; Somero, 2010; Bay et al., 2017; Kellermann and van Heerwaarden,
63 2019). In particular, given that rapid phenotypic changes are necessary due to high rates
64 of environmental change (e.g., Barrett and Hendry, 2012; Fox et al., 2019), phenotypic
65 plasticity may play a critical role in the resilience of populations and species to the effects
66 of climate change (Merilä and Hendry, 2014; Seebacher et al., 2015; Donelson et al.,
67 2019; Morley et al., 2019).

68 Phenotypic plasticity occurs across life stages and generations (e.g., Kelly et al.,
69 2011; Schulte et al., 2011; Beaman et al., 2016; Burggren, 2015). For example,
70 temperatures experienced during development or adulthood often have irreversible or
71 reversible effects on physiological traits (e.g., Schulte et al., 2011; Beaman et al., 2016),
72 and multi- or trans-generational effects of thermal variation are commonly observed (e.g.,
73 Crill et al., 1996; Massamba-N'Siala et al., 2014; Zizzari and Ellers, 2014; Donelson et
74 al., 2018). Thus, physiological phenotypes have the potential to be shaped by effects of
75 plasticity across different life stages. However, compared to the effects of adaptation on
76 phenotypic plasticity (e.g., Crispo, 2007; Hendry, 2016; Donelson et al., 2019; Kelly,
77 2019), effects of plasticity at one life stage on the expression of plasticity at another life
78 stage have received relatively little attention (Beaman et al., 2016). That said,
79 developmental conditions are known to alter the adulthood plasticity of several traits
80 (reviewed in Beaman et al., 2016). For instance, adult plasticity of swimming
81 performance and metabolic rate depends on developmental environment in mosquitofish
82 (*Gambusia holbrooki*; Seebacher et al., 2014; Seebacher and Grigaltchik, 2015). Yet,

83 despite the likely biogeographic importance of thermal tolerance limits (Sunday et al.,
84 2012), and many published examples of thermal tolerance limit plasticity in ectothermic
85 organisms as a result of developmental or adulthood temperatures (e.g., Stillman and
86 Somero, 2000; Ford and Beitinger, 2005; Fanguie et al., 2006; Angiletta, 2009; Overgaard
87 et al., 2011; Cooper et al., 2012; Tepolt and Somero, 2014; Jakobs et al., 2015; Troia et
88 al., 2015; Kingsolver et al., 2016; Pereira et al., 2017; Diamond et al., 2018; Mueller et
89 al., 2019; Yanar et al., 2019), relatively few studies have assessed the potential for
90 developmental temperatures to shape the phenotypic plasticity of upper thermal tolerance
91 in adults (although see Schaefer and Ryan, 2006; Kellermann et al., 2017; Kellermann
92 and Sgrò, 2018). Here we examine these effects, and their potential mechanistic basis in
93 populations of the intertidal copepod *Tigriopus californicus*.

94 *T. californicus* are small (~1.2 mm) harpacticoid copepods with short generation
95 times (3-4 weeks) that inhabit supralittoral tidepools along the west coast of North
96 America from Baja California, Mexico to southern Alaska, USA. Populations of this
97 species occur on rocky outcrops isolated by sandy beaches, conditions that result in very
98 low gene flow and high levels of genetic divergence among populations (Burton and Lee,
99 1994; Burton, 1997, 1998; Edmands, 2001; Peterson et al., 2013; Pereira et al., 2016;
100 Barreto et al., 2018). Although much of this divergence is likely a result of small
101 effective population sizes and genetic drift acting on selectively neutral variation,
102 signatures of directional selection have been detected across the transcriptome (Pereira et
103 al., 2016). This suggests that at least a portion of the genetic differentiation among
104 populations is likely adaptive. Moreover, several common-garden studies in laboratory-
105 raised individuals have demonstrated differences in upper and lower thermal tolerance
106 limits that are consistent with local thermal adaptation in response to the latitudinal
107 temperature gradient across the species range (Willett, 2010; Kelly et al., 2012; Wallace
108 et al., 2014; Pereira et al., 2014, 2017; Leong et al., 2018; Willett and Son, 2018; Foley et
109 al., 2019). This variation among populations has also been associated with genetically
110 based differences in the function and regulation of heat shock protein genes (Schoville et
111 al., 2012; Barreto et al., 2015; Tangwancharoen et al., 2018) and in the maintenance of
112 mitochondrial ATP synthesis rates at high temperatures (Harada et al., 2019). Few studies
113 have examined temperature-mediated phenotypic plasticity in these traits in *T.*

114 *californicus*. However, elevated developmental temperature is known to increase upper
115 thermal tolerance regardless of population (Kelly et al., 2012, 2017; Pereira et al., 2017),
116 and adult plasticity in this trait is thought to be limited (although only relatively short
117 acclimation periods have been examined [e.g., 1 d]; Pereira et al., 2017). Taken together
118 with short generation times and ease of laboratory culture, these observations make *T.*
119 *californicus* an ideal study system in which to investigate the effects of developmental
120 temperature on adulthood plasticity in an aquatic ectotherm.

121 In the current study, we use laboratory-raised *T. californicus* to test two
122 hypotheses: (1) variation in developmental temperatures changes the expression of
123 phenotypic plasticity of upper thermal tolerance in adults, and (2) the physiological
124 mechanisms involved in local thermal adaptation among populations are also involved in
125 thermal limit plasticity. First, we expand our previous study (Harada et al., 2019) that put
126 forward methods to estimate upper thermal tolerance with critical thermal maximum
127 (CT_{max}) measurements in this species. We then use this method to facilitate experiments
128 examining the effects of developmental temperature on the plasticity of this proxy for
129 upper thermal tolerance in adults of four Californian populations of *T. californicus*.
130 Finally, we assess the effects of developmental and adulthood temperatures on
131 mechanisms involved in local thermal adaptation in this species: the thermal performance
132 curve of ATP synthesis rate, and the mRNA expression levels of heat shock protein genes
133 and mitochondrial-encoded genes following acute heat stress.

134

135 **Materials and methods**

136 *Collection and culturing of copepods*

137 Adult copepods were collected from supralittoral tidepools across ten locations
138 along the west coast of North America, which spanned $\sim 21.5^\circ$ of latitude (San Roque,
139 Mexico – SR, La Bufadora, Mexico – BF, San Diego, California – SD, Bird Rock,
140 California – BR, Abalone Cove, California – AB, Estero Bay, California – EB, San
141 Simeon, California – SS, Santa Cruz, California – SC, Pescadero, California – PE, and
142 Pacific Crest, Canada – PC; Table S1; Fig. S1A,B). Collected animals were transported to
143 Scripps Institution of Oceanography (San Diego, CA) in 1 L plastic bottles containing
144 seawater obtained from the same tidepools. The collection for each location was divided

145 across several laboratory cultures, which were maintained at 20°C, 36 ppt and 12:12 h
146 photoperiod (light:dark) using filtered seawater and deionized water to adjust salinity as
147 necessary. Laboratory cultures were maintained for at least two generations (~2 months)
148 prior to experiments. During laboratory acclimations and experimental treatments,
149 copepods consumed natural algal growth within the cultures, as well as a mixture of
150 ground Spirulina (Salt Creek, Inc., South Salt Lake City, UT) and TetraMin Tropical
151 Flakes (Spectrum Brands Pet LLC, Blacksburg, VA) that was added approximately once
152 per week.

153 *Critical thermal maximum variation among populations*

154 Upper thermal tolerance was estimated by critical thermal maximum (CT_{max})
155 trials using loss of locomotor performance as the assay endpoint (as in Harada et al.,
156 2019). In brief, sixteen adult copepods of each population (8 females and 8 males for all
157 populations; divided across five trials) were transferred to 10-cm petri dishes containing
158 filtered seawater (20°C and 36 ppt) with no food overnight. In the morning, copepods
159 were individually transferred into 0.2-mL strip tubes with 100 μ L of water from the petri
160 dish. Tubes were left uncapped, and were placed in an Applied Biosystems SimpliAmp™
161 Thermal Cycler (Thermo Fisher Scientific, Waltham, MA). After 5 min at 20°C, the
162 temperature was increased using the AutoDelta function at rates of 0.1°C per 20 s from
163 20 to 32°C, and 0.1°C per min from 32°C to the temperature at which the last individual
164 in the trial lost locomotor performance. Loss of performance (i.e., knockdown) was
165 monitored by cycling 40 μ L of water in each tube with a pipettor. Typically this
166 procedure results in erratic swimming behaviour in *T. californicus*; however, at extremely
167 high temperatures, this swimming response ceases, and copepods passively sink to the
168 bottom of their tube. Endpoints were determined when an individual did not respond to
169 three sequential tests with the pipettor, and CT_{max} was recorded as the temperature at
170 which the endpoint was observed. After CT_{max} was determined, copepods were returned
171 to 10-cm petri dishes with 20°C filtered seawater (36 ppt) for recovery, and survivorship
172 assessed 8 h after the trials was >90%.

173 *Developmental and adulthood plasticity in critical thermal maximum*

174 To assess variation in the phenotypic plasticity of upper thermal tolerance in adult
175 copepods as a result of differences in developmental temperature, gravid SD and BR

176 females with mature (i.e., red) egg sacs were removed from laboratory cultures (24
177 females from 4 cultures per population). Egg sacs were dissected from the females
178 (which synchronizes hatching), placed individually in wells of 6-well plates containing
179 filtered seawater (20°C and 36 ppt), and allowed to hatch overnight. In the morning,
180 nauplii (i.e., larvae) from each egg sac were counted, and split evenly across six
181 treatments in 10-cm petri dishes (Fig. 1). Three treatments were developed at 20°C for 14
182 d, and three treatments were developed at 25°C for 10 d. Preliminary trials with the SD
183 population determined that these developmental times were those required for the
184 majority of individuals to reach adulthood (and to observe the first gravid female) at each
185 temperature, suggesting that the temperature coefficient (Q_{10}) of developmental rate
186 equals ~2 in this species. At the end of the developmental periods, the developmental
187 treatments at each temperature were transferred to one of three adult acclimations: 20°C
188 for 14 d, 25°C for 10 d, or 25°C for 14 d. These lengths of acclimations were chosen to
189 allow two weeks of acclimation at 20°C, and comparisons between equivalent
190 acclimations using either absolute time or physiologically adjusted time at 25°C
191 (assuming a continued Q_{10} of ~2 for life history traits). On the days that the adult
192 acclimations were completed, critical thermal maxima were determined as described
193 above for 16 copepods from each treatment and population. Note that individuals used in
194 the tolerance trials were transferred to fresh filtered seawater without food at their
195 acclimation conditions on the evening before the end of the acclimation treatments (i.e.,
196 the day before trials), and CT_{max} trials started from the acclimation temperatures in all
197 cases.

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

206 *Plasticity of ATP synthesis rate thermal sensitivity*

207 To examine plasticity of the thermal performance curve for ATP synthesis rate,
208 we compared two temperature treatments: 20°C for both development and adulthood
209 versus 25°C for both development and adulthood (Fig. 1). Gravid SD and BR females
210 carrying mature egg sacs (60 per population) were transferred from laboratory cultures to
211 10-cm petri dishes (6 per population) containing ~60 mL of filtered seawater (20°C and
212 36 ppt) with food. The egg sacs from the majority of these females (6-10 per plate)
213 hatched overnight. All females were removed in the morning; egg sacs that were still
214 carried by females were dissected free and returned to their respective dishes. Dissected
215 egg sacs hatched within 3 h, and once all egg sacs had hatched, three petri dishes for each
216 population were transferred to 25°C. As described above, juveniles in the 20 or 25°C
217 dishes developed for 14 or 10 d, respectively, and adult acclimations at the two
218 temperatures were also 14 or 10 d, respectively.

219 ATP synthesis rates were measured at 20, 25, 30, 33, 35 and 37°C using
220 procedures similar to those of Harada et al. (2019). On the day before the end of the adult
221 acclimation treatments, groups of 32 copepods (6 groups per population x treatment)
222 were held at their acclimation temperatures in 10-cm petri dishes with filtered seawater
223 (36 ppt) and no food overnight. In the morning, the groups of copepods were rinsed with
224 200 µL homogenization buffer (400 mM sucrose, 100 mM KCl, 70 mM HEPES, 6mM
225 EGTA, 3 mM EDTA, 1% w/v BSA, pH 7.6), which had been chilled on ice. Each group
226 was transferred to a 2-mL glass teflon homogenizer, and homogenized in 800 µL of fresh
227 buffer. Following homogenization, mitochondria were isolated by differential
228 centrifugation in 1.5 mL microcentrifuge tubes (Eppendorf, Hamburg, Germany). First,
229 the tubes were centrifuged at 4°C and 1,000 g for 5 min, and supernatants were
230 transferred to fresh tubes. Second, the new tubes were centrifuged at 4°C and 11,000 g
231 for 10 min. The resulting supernatants were discarded, and mitochondrial pellets were
232 resuspended in 205 µL assay buffer (560 mM sucrose, 100 mM KCl, 70 mM HEPES, 10
233 mM KH₂PO₄, pH 7.6). Isolated mitochondria were divided into eight 25-µL aliquots: 6
234 for synthesis reactions (1 per temperature), 1 for initial ATP concentration determination,
235 and 1 for measuring DNA content which was used to normalize ATP synthesis rate. DNA
236 content was assayed with Invitrogen™ Quant-iT™ PicoGreen™ dsDNA reagent
237 following the manufacturer's protocols (Thermo Fisher Scientific, Waltham, MA).

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

254 *Plasticity of gene expression following heat shock*

255 Variation in gene expression following heat shock was assessed for the same
256 treatments as those used to examine ATP synthesis rates: 20°C for both development and
257 adulthood, and 25°C for both development and adulthood (Fig. 1). Again, offspring from
258 60 SD and 60 BR females were divided between these treatments (repeated as described
259 above). In the evening prior to the last day of the adult acclimations, groups of 15
260 copepods (18 groups per population x treatment) were transferred to 15-mL Falcon™
261 conical tubes (Thermo Fisher Scientific, Waltham, MA) containing 10 mL of filtered
262 seawater (36 ppt) with no food at the acclimation temperature of the copepods. In the
263 morning, tubes were transferred to water baths held at 35 or 36°C for 1 h (6 per
264 population x treatment at each temperature), and then returned to the acclimation
265 temperature of the copepods for 1 h as in Barreto et al. (2015). The remaining 6 tubes
266 (per population x treatment) were handled in the same manner, but were kept at the
267 acclimation temperature of the copepods for the entire 2 h. At the end of all heat shock
268 trials, copepods were frozen at -80°C until RNA isolation.

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

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

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

308 *Statistical analyses*

309 All analyses were performed with R v3.4.0 (R Core Team, 2017) and $\alpha = 0.05$.
310 Latitudinal variation in CT_{max} across all populations failed to satisfy the assumptions for
311 parametric statistics even after log transformation. Thus, differences among populations
312 were assessed by Kruskal-Wallis analysis of variance (ANOVA) followed by Nemenyi
313 post-hoc tests. Potential effects of sex on CT_{max} were assessed by Wilcoxon rank-sum
314 tests within each population. In contrast, variation in CT_{max} associated with
315 developmental and adulthood temperatures met the assumptions for parametric tests in
316 both the SD and BR, and the SC and PE experiments. These data were assessed by
317 general linear models followed by ANOVAs with population, developmental
318 temperature, and adult acclimation treatment as factors. Post-hoc comparisons among
319 groups were performed with Tukey tests. After log transformation to meet assumptions of
320 normality and homogeneity of variances, variation in ATP synthesis rate was assessed
321 with a mixed-effect linear model followed by ANOVA with fixed effects of population,
322 acclimation treatment and assay temperature, and a random effect of mitochondrial
323 isolation. All interactions between factors were not significant in the initial model ($p \geq$
324 0.12 for all), and were removed from the final model for this test. Planned pairwise post-
325 hoc comparisons were conducted between assay temperatures within each population x
326 acclimation treatment, between acclimation treatments within each population x assay
327 temperature, and between populations within each acclimation treatment x assay
328 temperature with Student's *t* tests (84 comparisons). The resulting *p*-values were
329 corrected for multiple tests with the Benjamini-Hochberg method (Benjamini and
330 Hochberg, 1995). As an alternative method to examine variation in the thermal sensitivity

331 of ATP synthesis rate, rates were normalized to the 25°C rate within each mitochondrial
332 isolation. Variation in normalized ATP synthesis rate was assessed at assay temperatures
333 from 30 to 37°C with similar methods to those described above for the unnormalized
334 rates (although log transformation and removal of interactions among model factors were
335 not required). Finally, mRNA expression data were all log transformed to meet necessary
336 assumptions, and then differences among groups were examined by two-way ANOVAs
337 with acclimation treatment and heat shock exposure as factors followed by post-hoc
338 Tukey tests. Note comparisons of gene expression between populations were not made,
339 because the expression levels of each population were quantified relative to standard
340 curves that were population specific and for some genes the qRT-PCR primers were
341 population specific as well (Table 1). Full ANOVA tables for all models tested have been
342 uploaded to the Dryad Digital Repository (*upload will be completed and the accession*
343 *number provided should the manuscript be accepted*).

344

345 **Results**

346 *Latitudinal variation in critical thermal maximum*

347 CT_{max} demonstrated significant variation among populations ($p < 2.2 \times 10^{-16}$; Fig.
348 2), and there were no differences in this trait between females and males in the current
349 study ($p \geq 0.19$ within all populations). Overall, CT_{max} increased from northern to
350 southern populations (Fig. 2), suggesting that variation in this thermal limit generally
351 parallels previously published latitudinal variation in lethal temperatures among
352 populations (Willett, 2010; Kelly et al., 2012; Pereira et al., 2014, 2017; Leong et al.,
353 2018; Willett and Son, 2018; Foley et al., 2019). This makes CT_{max} ideal for examining
354 effects of phenotypic plasticity on upper thermal tolerance in experiments utilizing
355 designs where offspring from individual egg sacs are divided among treatments.

356 *Phenotypic plasticity of upper thermal tolerance*

357 Development at 20 or 25°C resulted in variation in CT_{max} among SD and BR
358 copepods that was significantly affected by a three-way interaction among population,
359 developmental temperature and adult acclimation treatment ($p = 0.03$). This effect was
360 resolved by post-hoc tests, although there was little evidence for differential effects
361 between the populations (Fig. 3A). For all SD and BR copepods that developed at 20°C,

362 CT_{max} values were similar regardless of adult acclimation temperature or time (range of
363 means \pm s.e.m.: 37.9 ± 0.1 to $38.3 \pm 0.1^\circ\text{C}$; $p = 0.054$ for 20°C -developed SD acclimated
364 to 20°C for 14 d versus 25°C for 10 d as adults, and $p \geq 0.22$ for all other comparisons),
365 suggesting that in 20°C -developed *T. californicus* there is no upper thermal limit
366 plasticity in adults. In contrast, relative to development at 20°C , development at 25°C
367 resulted in significant increases in CT_{max} for both SD and BR when adults were also
368 acclimated at 25°C (range of means \pm s.e.m.: 38.5 ± 0.1 to $39.1 \pm 0.1^\circ\text{C}$; $p \leq 0.04$ for all
369 comparisons within populations) and these effects were similar at 10 and 14 d of
370 acclimation ($p \geq 0.16$ between times for both populations). However, if 25°C -developed
371 SD and BR copepods were acclimated at 20°C as adults, there was a significant loss of
372 tolerance (i.e., decrease in CT_{max}) in both populations (mean \pm s.e.m.: 38.1 ± 0.1 and 37.7
373 $\pm 0.1^\circ\text{C}$ for SD and BR, respectively; $p < 0.01$ for all within population comparisons).

374 To explore these effects in populations known to be locally adapted to lower
375 temperatures than the SD and BR populations, we conducted a second experiment
376 examining plasticity of CT_{max} in the SC and PE populations. In SC and PE copepods,
377 variation in adult CT_{max} was unaffected by the three-way interaction among population,
378 developmental temperature and adult acclimation treatment ($p = 0.60$). Moreover, there
379 were no significant effects of interactions between population and either developmental
380 temperature ($p = 0.83$) or adult acclimation treatment ($p = 0.23$), or of population alone (p
381 $= 0.20$). However, the interactive effect of developmental temperature and adult
382 acclimation treatment significantly affected CT_{max} ($p = 9.9 \times 10^{-5}$). Again, post-hoc
383 comparisons resolved this effect, and suggested similar patterns of variation among
384 treatments for SC and PE as those described above for SD and BR (Fig. 3B). There was
385 no significant variation in CT_{max} as a result of adult acclimation temperature in 20°C -
386 developed copepods from either SC or PE (mean \pm s.e.m.: 36.4 ± 0.1 and $36.8 \pm 0.1^\circ\text{C}$
387 for SC, and 36.8 ± 0.1 and $37.1 \pm 0.1^\circ\text{C}$ for PE; $p \geq 0.39$ between adult temperatures for
388 both populations). In contrast, development at 25°C resulted in significantly higher CT_{max}
389 in both SC and PE copepods, but only when adults were acclimated at 25°C (mean \pm
390 s.e.m.: 38.0 ± 0.2 and $37.6 \pm 0.1^\circ\text{C}$ for SC and PE, respectively; $p \leq 0.04$ for all
391 comparisons within populations). If 25°C -developed copepods from either population
392 were acclimated to 20°C as adults, there was a significant decrease in CT_{max} (mean \pm

393 s.e.m.: 36.7 ± 0.2 and 36.7 ± 0.01 for SC and PE, respectively; $p < 0.01$ for both within
394 population comparisons).

395 *Plasticity of ATP synthesis rate*

396 Maintaining *T. californicus* at 20 or 25°C for both development and acclimation
397 as adults resulted in significant variation in the thermal performance curve for CI+II ATP
398 synthesis rates in isolated mitochondria (Table 2). Specifically, these curves were
399 affected by population ($p = 4.4 \times 10^{-5}$), development and adult acclimation temperature (p
400 $< 2.2 \times 10^{-16}$), and assay temperature ($p < 2.2 \times 10^{-16}$). In both developmental and
401 acclimation treatments, synthesis rates initially increased and then decreased with
402 increasing assay temperatures in SD and BR (Table 2), and post-hoc tests found no
403 evidence for differences between the SD and BR copepods within each treatment x assay
404 temperature combination ($q \geq 0.11$ for all). Yet, across all assay temperatures in both
405 populations, 25°C development and adult acclimation resulted in higher ATP synthesis
406 rates compared to those measured following development and acclimation at 20°C ($q \leq$
407 0.046 for all). This overall vertical shift in the thermal performance curve has the
408 potential to mask variation between these treatments in the extent to which ATP synthesis
409 rates are maintained during acute exposures to high temperatures. However, in SD
410 copepods, rates of ATP synthesis first significantly declined with temperature between
411 assay temperatures of 30 and 33°C for the 20°C treatment ($q = 7.3 \times 10^{-4}$), whereas for
412 the 25°C treatment the first decrease occurred between assay temperatures of 33 and
413 35°C ($q = 1.7 \times 10^{-3}$). This suggests that copepods developed and acclimated as adults at
414 warmer temperatures maintained synthesis rates at higher temperatures at least in this
415 population.

416 As an alternative approach to examine maintenance of ATP synthesis at high
417 temperatures, we normalized synthesis rates across temperatures to those measured at
418 25°C for each mitochondrial isolation, which allows comparisons of the proportional
419 changes in synthesis rate with assay temperature (Fig. 4). Note that this normalization
420 could also reasonably be done to the rates measured at 20°C, but the results would be
421 similar (Fig. 4). After normalization, proportional changes in rates of ATP synthesis were
422 affected by a three-way interaction among population, temperature of development and
423 adult acclimation, and assay temperature ($p = 0.02$). Post-hoc comparisons revealed

424 similar patterns of variation among assay temperatures as those detected for the
425 unnormalized rates (as would be expected), and when assayed at 37°C, 20°C-developed
426 and -acclimated SD copepods maintained higher synthesis rates than 20°C-developed and
427 -acclimated BR copepods ($q = 0.03$). Additionally, at high assay temperatures both
428 populations maintained greater rates of ATP synthesis following development and adult
429 acclimation at 25°C than following development and adult acclimation at 20°C ($q \leq 5.5 \times$
430 10^{-3} for 33 to 37°C in SD and $q = 0.02$ for 37°C in BR).

431 *Plasticity of gene expression following heat shock*

432 The mRNA expression levels of both heat shock proteins examined in the current
433 study (*hspb1* and *hsp70*) demonstrated similar effects of heat shock, and developmental
434 and adult acclimation temperature regardless of population (SD or BR; Fig. 5). In all
435 cases, gene expression was affected by a significant interaction between the heat shock
436 treatment, and the temperature of development and adult acclimation ($p = 0.04$ for *hsp70*
437 in SD, and $p \leq 5.4 \times 10^{-3}$ for all others). In general, copepods developed and acclimated
438 as adults at 25°C expressed higher levels of *hspb1* and *hsp70* than copepods developed
439 and acclimated at 20°C (particularly after heat shock), although these patterns were not
440 always resolved by post-hoc tests (Fig. 5).

441 Given the potential role of mitochondrial performance in determining upper
442 thermal tolerance (e.g., Harada et al., 2019) and a previous demonstration of decreased
443 mitochondrial-encoded mRNA levels following heat shock in *T. californicus* (Schoville
444 et al., 2012), we also examined variation in the expression of *mt-atp6*. In the current
445 study, there were interactive effects of heat shock treatment, and developmental and adult
446 acclimation temperature on the mRNA expression of *mt-atp6* in both the SD and BR
447 population ($p \leq 0.02$; Fig. 6). In SD copepods, regardless of the temperature of
448 development and adult acclimation, *mt-atp6* levels were similar in the control and 35°C
449 heat shock treatments ($p = 1.00$ for both). Within both of these treatments expression
450 levels were significantly higher in copepods developed and acclimated as adults at 25°C
451 than in those developed and acclimated at 20°C ($p < 0.001$ for both). In contrast, in SD
452 copepods that had been developed and acclimated as adults at 20°C, heat shock at 36°C
453 increased *mt-atp6* expression ($p \leq 0.02$), whereas in those that had been developed and
454 acclimated at 25°C, the same exposure decreased *mt-atp6* expression ($p < 0.001$ for both).

455 As a result, there was no effect of the temperature experienced throughout development
456 and adulthood on *mt-atp6* mRNA levels in the 36°C heat shock treatment ($p = 0.88$). In
457 BR copepods, variation in *mt-atp6* expression demonstrated somewhat different patterns
458 than those observed for SD copepods. For both developmental and adult acclimation
459 temperatures, there were trends for decreasing *mt-atp6* mRNA levels with increasing heat
460 shock temperatures, but these patterns were only resolved in post-hoc tests in BR
461 copepods developed and acclimated as adults at 25°C between the control treatment and
462 the 35 and 36°C heat shock treatments ($p < 0.001$ for both; $p \geq 0.06$ for all others).
463 However, *mt-atp6* expression was greater in BR copepods developed and acclimated as
464 adults at 25°C than at 20°C in the control treatment and in the 35 or 36°C heat shock
465 treatments ($p \leq 0.02$).

466

467 **Discussion**

468 The results presented here provide experimental support for both of our proposed
469 hypotheses. First, variation in developmental temperature resulted in differences in the
470 plasticity of upper thermal limits in adult *T. californicus*. Regardless of population, 25°C-
471 developed copepods demonstrated clear plasticity of CT_{max} between adulthood
472 temperatures of 20 and 25°C. In contrast, there was no evidence of plasticity of this trait
473 in adults that had developed at 20°C. Second, differences in developmental and adulthood
474 acclimation temperatures were associated with plastic changes in two physiological
475 mechanisms that are thought to contribute to the basis of local adaptation of upper
476 thermal tolerance in this species. Furthermore, these effects were consistent with the
477 differences in CT_{max} between these developmental and adult acclimation treatments.
478 Therefore, our data suggest that adaptive processes may have the potential to shape the
479 effects of developmental temperatures on the plasticity of thermal tolerance due to shared
480 underlying mechanisms, despite similar patterns of plasticity observed in the four locally
481 adapted populations of *T. californicus* examined in the current study.

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

483 In general, dynamic and static thermal tolerance assays (i.e., gradual ramping
484 exposures to high temperatures and abrupt exposures to a constant high temperature)
485 resolve similar patterns of variation among experimental groups or treatments (e.g., Ford

486 and Beitinger, 2005; Jørgensen et al., 2019), and our previous study suggested this was
487 also the case among three Californian populations of *T. californicus* (distributed across
488 $\sim 3^\circ$ latitude; Harada et al., 2019). In the current study, there was a clear pattern of CT_{max}
489 variation among populations that was consistent with substantial latitudinal thermal
490 adaptation of upper thermal tolerance in *T. californicus*, as has been suggested in studies
491 using static assays (Willett, 2010; Kelly et al., 2012; Pereira et al., 2014, 2017; Leong et
492 al., 2018; Willett and Son, 2018; Foley et al., 2019). Overall, CT_{max} increased from
493 northern to southern populations, although there was somewhat limited statistical
494 resolution among populations likely due to a combination of relatively small differences
495 in several comparisons and nonparametric post-hoc tests. In general, thermal tolerance
496 limits are expected to decline approximately linearly with latitude (Sunday et al., 2011),
497 whereas our results suggest this is not the case in this species (Fig. S1C). This may reflect
498 the relatively gradual latitudinal thermal gradient at higher compared to lower latitudes
499 across the species range (particularly in the summer; Fig. S1B), and consistent with this
500 possibility, both Leong et al. (2018) and Pereira et al. (2017) demonstrated approximately
501 linear changes in upper thermal tolerance with differences in habitat air temperatures
502 among *T. californicus* populations.

503 Despite this clear signature of local adaptation associated with the latitudinal
504 thermal gradient along the west coast of California, interpreting the results of the current
505 study in the context of *T. californicus* habitat temperatures is challenging. Splashpool
506 temperatures vary substantially throughout the day (e.g., Harada and Burton, 2019), and
507 the intertidal is a “mosaic” habitat in which local thermal conditions may not necessarily
508 reflect expected patterns with latitudinal variation in sea surface or air temperatures
509 (Helmuth et al., 2002, 2006; Sanford and Kelly, 2010). For instance, variation in the daily
510 timing of tidal cycles with latitude can paradoxically result in higher temperature
511 exposures at northern compared to southern latitudes (Kuo and Sanford, 2009), although
512 this is less likely to be relevant for supralittoral tidepools. There is limited published
513 temperature data for *T. californicus* tidepools; however, at least comparing the SD and
514 SC populations, overall summer temperatures tend to be warmer for the more southern
515 population (i.e., SD; Leong et al., 2018), which is consistent with the difference in CT_{max}
516 between these populations. Both average and maximum temperatures may be important

517 for local adaptation, and it is likely that for upper thermal limits maximum temperatures
518 are more relevant (e.g., Somero, 2005). Yet, with the limited available data there does not
519 appear to be a tight relationship between maximum temperatures and CT_{max} for SD and
520 SC copepods (Leong et al., 2018). In part, this may be a consequence of CT_{max} only
521 representing a proxy of the lethal thermal limit, which is typically justified as an
522 “ecological death” associated with an inability to escape predation or harmful conditions
523 due to loss of locomotor performance (e.g., Beitinger et al., 2000), and the extent to
524 which this justification is relevant for *T. californicus* is unknown. That said, there is
525 clearly local adaptation of CT_{max} in *T. californicus*, and comparing our results with those
526 of Pereira et al. (2017) suggests that there is at least reasonable concordance between
527 variation in CT_{max} and variation in lethal temperatures across populations.

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

543 One distinct result of the current study was the lack of variation in CT_{max} between
544 the sexes (see Fig S1C). There is an overall consensus that, in comparison to males,
545 female *T. californicus* are more tolerant of stressful conditions for a wide range of abiotic
546 factors, including temperature (Willett, 2010; Willett and Son, 2018; Foley et al., 2019).
547 Insufficient statistical power due to nonparametric tests, and relatively low sex-specific

548 sample sizes ($n = 8$) may explain the lack of sex effects in the current study. However,
549 two other studies have also failed to detect differences in upper thermal tolerance
550 between the sexes (Pereira et al., 2014, 2017). Regardless, CT_{max} was not statistically
551 affected by sex in our study, and as a result we did not consider variation between
552 females and males further here.

553 *Developmental temperature and adulthood plasticity of CT_{max}*

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

575 In *T. californicus*, the effects of developmental temperature on adulthood
576 plasticity of CT_{max} were relatively large, as plasticity of CT_{max} was completely absent in
577 adults after development at 20°C, whereas after development at 25°C adulthood plasticity
578 was observed in all populations. Moreover, the adulthood acclimation response ratio in

579 25°C-developed copepods (i.e., $\Delta CT_{\max} \text{ } ^\circ\text{C}^{-1}$) was typical for aquatic ectotherms (~ 0.2 ;
580 Gunderson and Stillman, 2015). Similarly, variation in developmental temperature results
581 in presence-absence differences in adulthood plasticity in *D. melanogaster* (Kellermann
582 et al., 2017), although in *Drosophila sp.* cooler developmental temperatures tend to
583 increase plasticity in adults (Kellermann et al., 2017; Kellermann and Sgrò, 2018),
584 whereas our results suggest warmer developmental temperatures increase adult plasticity
585 in *T. californicus*. The loss of adult CT_{\max} plasticity at an only moderately reduced
586 developmental temperature in *T. californicus* is potentially surprising given the
587 prevalence of at least a modest capacity for acclimation of this trait across many species
588 (e.g., Gunderson and Stillman, 2015). Acclimation to constant conditions, rather than
589 cycling thermal regimes that more closely resemble natural tidepool conditions, may
590 influence both this lack of plasticity, and the effects of developmental temperature on
591 adulthood plasticity in the current study. In addition, we examined only a relatively small
592 range of adulthood temperatures (20-25°C), which may contribute to the lack of observed
593 plasticity, and the extent to which plasticity may alter CT_{\max} in 20°C-developed copepods
594 over a wider range of adult acclimation temperatures remains an open question.

595 The short generation times of *T. californicus* and *Drosophila sp.* likely increase
596 the concordance between developmental and adulthood temperatures (particularly in the
597 habitats of *T. californicus*). This may contribute to the effects of developmental
598 temperature on adulthood plasticity of thermal tolerance in this species, because
599 developmental effects are expected to be stronger if conditions in development are
600 predictive of those experienced as adults (Cooper et al., 2010, 2012; Nettle and Bateson,
601 2015; Beaman et al., 2016). Consistent with this possibility, in the comparatively long-
602 lived zebrafish, Schaefer and Ryan (2006) observed only subtle shifts in CT_{\max} plasticity
603 in adults as a result of differences in developmental temperatures. Although the
604 interactive effect of developmental and adulthood temperatures with respect to patterns of
605 CT_{\max} plasticity was relatively strong in *T. californicus*, the maximum difference in
606 CT_{\max} among treatments was approximately 1°C. Regardless, our data demonstrate that
607 variation in developmental temperatures can have substantial effects on the adulthood
608 plasticity of upper thermal tolerance in aquatic ectotherms.

609 *Mechanisms underlying CT_{\max} plasticity and local thermal adaptation*

610 The possibility of interactions between adaptive processes and phenotypic
611 plasticity is well established (e.g., Crispo, 2007; Hendry, 2016; Donelson et al., 2019;
612 Kelly, 2019), and thus there is also the potential for local thermal adaptation to shape
613 effects of developmental temperature on the plasticity of upper thermal tolerance in
614 adults. Furthermore, if heat hardening is used as a metric of adulthood plasticity, there is
615 some evidence for adaptive variation in these effects among *Drosophila sp.* (Kellermann
616 and Sgrò, 2018). However, when this possibility is assessed with adult acclimations in
617 temperate and tropical *D. melanogaster*, developmental effects on adulthood plasticity
618 are similar among populations (Kellermann et al., 2017). Similarly, although
619 development at 20 or 25°C was associated with variation in CT_{max} plasticity in adults
620 from all of the *T. californicus* populations examined in the current study, there was no
621 variation in this effect among populations. Indeed, in 25°C-developed copepods, the
622 average CT_{max} difference between adult acclimations of 20 and 25°C were remarkably
623 similar across the four populations (SD: 1.0°C, BR: 0.8°C, SC: 1.3°C and PE 0.9°C).
624 Thus, our results suggest that effects of developmental temperature on adulthood
625 plasticity of CT_{max} have not been altered substantially by local thermal adaptation among
626 these populations of *T. californicus*. However, we also found that physiological
627 mechanisms that putatively underlie latitudinal variation in upper thermal tolerance in
628 this species (e.g., Schoville et al., 2012; Harada et al., 2019) show patterns of variation in
629 response to developmental and adulthood temperatures that parallel variation in CT_{max}.

630 Harada et al. (2019) demonstrated that, during acute exposures to elevated
631 temperatures, the temperatures at which maximal ATP synthesis rates first decline are
632 correlated with CT_{max} across the SD, AB and SC populations of *T. californicus*.
633 Consistent with this relationship, several studies have demonstrated loss of ATP synthesis
634 capacity in heart mitochondria at temperatures that are approximately equal to or are
635 immediately below the upper thermal limits in species of fishes (Iftikar and Hickey,
636 2013; Christen et al., 2018; O'Brien et al., 2018). Temperature-mediated plasticity of
637 mitochondrial functions is also often observed in ectothermic species (e.g., Guderley,
638 2004; Seebacher et al., 2010; Chung and Schulte, 2015; Chung et al., 2017a,b, 2018;
639 Bryant et al., 2018), and our data suggest that this is the case in *T. californicus*. In the SD
640 and BR populations, copepods that were developed and acclimated as adults at 25°C had

641 greater ATP synthesis rates than those that were developed and acclimated at 20°C,
642 which was consistent with higher expression levels of *mt-atp6* in these 25°C treatments
643 than these 20°C treatments under control (i.e., non-heat shocked) conditions.
644 Additionally, developmental and adult acclimation temperatures of 25°C compared to
645 20°C resulted in greater maintenance of ATP synthesis rates at high temperatures, which
646 is consistent with difference in CT_{max} between these treatments. In comparison to Harada
647 et al. (2019), the thermal performance curves observed in our study were horizontally
648 shifted to moderately lower temperatures, and were remarkably flat with maximum Q_{10}
649 values of approximately 1.4-1.5 across treatments. Although relatively thermally
650 insensitive physiological rates have been observed previously in *T. californicus* (e.g.,
651 Scheffler et al., 2019), there is clearly an unknown source of variation in these ATP
652 synthesis curves among studies. It is possible that culturing conditions could contribute to
653 this variation, as Harada et al. (2019) examined copepods taken directly from stock
654 cultures (i.e., 400-mL beakers), whereas in the current study we raised groups of
655 copepods specifically for these measurements in 10-cm petri dishes. Associated with this
656 difference, these studies likely also differ somewhat in densities of copepods, algal
657 growth and, potentially, oxygen levels under holding conditions, which have the potential
658 to result in plastic variation in mitochondrial performance between the studies.
659 Regardless, with the exception of temperature, our 20 and 25°C treatments were held
660 under equivalent conditions, and therefore the difference in high-temperature
661 maintenance of ATP synthesis rates between these treatments is likely robust to any
662 variation in thermal performance curve estimates across studies.

663 Schoville et al. (2012) examined genetically determined differences in the
664 transcriptomic response to acute heat stress between the SD and SC populations of *T.*
665 *californicus*. Both the strongest response and largest difference between the two
666 populations was the extent to which heat shock protein genes were induced following
667 heat stress. Particularly for *hspb1* and *hsp70*, heat shock protein mRNA expression was
668 increased to much higher levels in the warm-adapted SD population than in the relatively
669 cold-adapted SC population. As heat shock proteins are molecular chaperones that
670 mitigate the negative effects of high temperature due to damaged and unfolded proteins
671 (Hochachka and Somero, 2002), these transcriptomic patterns suggest that differences in

672 heat shock protein expression may contribute to the difference in upper thermal tolerance
673 between the SD and SC populations. The evidence for a correlation between large
674 inductions of heat shock protein expression and increased tolerance of high temperatures
675 is somewhat mixed among genes and species (e.g., Healy et al., 2010; Gleason and
676 Burton, 2015); however, studies in fruit flies (*D. melanogaster*) and marine snails
677 (*Chlorostoma funebris*) generally support a positive relationship between these two
678 traits (e.g., Bettencourt et al., 2008; Tomanek et al., 2008). In *T. californicus*,
679 Tangwancharoen et al. (2018) demonstrated putatively adaptive functional variation
680 associated with sequence differences among populations in both the regulatory and
681 coding regions of *hspb1*, and Barreto et al. (2015) utilized RNA interference to show that
682 knockdown of transcripts for this gene directly decreases survivorship following acute
683 thermal stress. Therefore, the increased inductions of *hspb1* and *hsp70* we observed in SD
684 and BR copepods developed and acclimated as adults at 25°C compared to those
685 developed and acclimated at 20°C are likely beneficial effects of plasticity, and are
686 consistent with CT_{max} differences between these treatments. Part of this variation in heat
687 shock protein expression may be associated with the differences in recovery temperatures
688 in our study, as each developmental and adult acclimation treatment was recovered at its
689 acclimation temperature. However, in all cases, the fold differences in expression
690 between the 20 and 25°C developmental and adulthood treatments following heat shock
691 (2.0-6.4) are greater than would be expected due to thermodynamic effects on
692 transcription rates alone (1.4-1.7 given an expected Q₁₀ of 2-3 which is consistent with
693 thermal sensitivities of transcriptional elongation rates; e.g., van Breukelen and Martin,
694 2002).

695 Taken together, our results indicate that both the extents to which ATP synthesis
696 rates are maintained and heat shock proteins are induced at high temperatures are
697 elevated in *T. californicus* that are developed and acclimated at 25°C compared to those
698 that are developed and acclimated at 20°C. The acute temperature exposures used to
699 assess these mechanisms here that matched those of previous studies in this species
700 (Schoville et al., 2012; Barreto et al., 2015; Harada et al., 2019), but these exposures were
701 notably different than the thermal ramp experienced during our CT_{max} trials, which may
702 affect comparisons among these traits (Harada and Burton, 2019). Despite this, the

703 patterns of variation in ATP synthesis rates and heat shock protein expression observed
704 here were consistent with differences in CT_{max} between the copepods that were developed
705 and acclimated as adults at 25°C and the copepods that were developed and acclimated at
706 20°C. This suggests that maintenance of ATP synthesis rates and induction of heat shock
707 proteins likely contribute to the basis for plasticity of upper thermal tolerance associated
708 with developmental and adulthood temperatures in this species. Yet, the extent to which
709 the effects of developmental temperature, specifically, on plasticity in adults can be
710 attributed to these mechanisms requires additional research, as these traits were not
711 assayed in 25°C-developed copepods that were transferred to 20°C in adulthood in the
712 current study. However, our data suggest these mechanisms may play a role in plastic
713 effects due to developmental temperatures in general.

714

715 **Conclusion**

716 The effects of environmental change on organisms ultimately depend on the
717 capacities to modulate key physiological traits to facilitate performance and persistence.
718 Phenotypic plasticity, adaptation and interactions between these two processes all play
719 important roles in these responses (e.g., Kellermann and van Heerwaarden, 2019). Here
720 we show that temperatures experienced in development also shape the adulthood
721 plasticity of upper thermal limits in the intertidal copepod *T. californicus*. These effects
722 may be particularly relevant for aquatic ectotherms as thermal tolerance limits likely
723 underlie geographic range limits in many of these species (Sunday et al., 2012; Pinsky et
724 al., 2019). Our results highlight that beneficial effects of developmental plasticity with
725 respect to environmental change have the potential to be overestimated if considered
726 without accounting for temperature variation in adulthood. Additionally, the data
727 presented here suggest that the physiological mechanisms that may underlie these effects
728 (e.g., shifts in the thermal performance curve for ATP synthesis and the regulation of heat
729 shock genes) are, at least to some extent, shared with the mechanisms associated with
730 local thermal adaptation in *T. californicus*. This mechanistic overlap indicates the
731 potential for interactions among local adaptation and plasticity at difference life stages to
732 shape variation in upper thermal tolerance in ectothermic organisms.

733

734 **Competing interests**

735 No competing interests declared

736

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740

741 **Data availability**

742 All data collected for the current study have been uploaded to the Dryad Digital

743 Repository (*upload will be completed and accession number provided should the*

744 *manuscript be accepted*).

745

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Figures

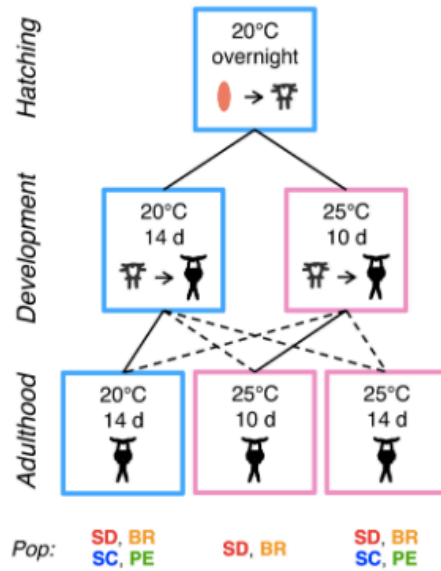


Figure 1. Flow chart of the developmental and adulthood temperature exposures for the plasticity experiments. All measurements were made at the end of the adulthood acclimations. Solid lines connect treatments (light blue boxes – 20°C; pink boxes – 25°C) with data for critical thermal maximum (CT_{max}), 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).

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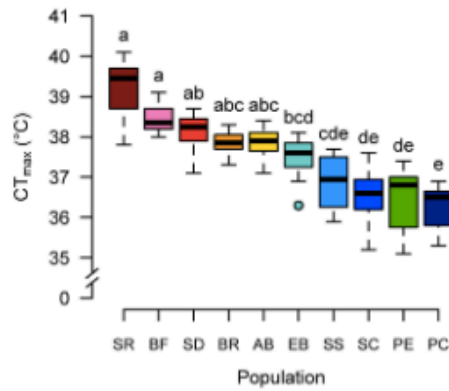


Figure 2. Variation in critical thermal maximum (CT_{max}) among populations of *T. californicus* distributed from Mexico to Canada. Populations are plotted from southernmost to northernmost (left to right): San Rogue, Mexico (SR; dark red), La Bufadora, Mexico (BF; pink), San Diego, California (SD; 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).

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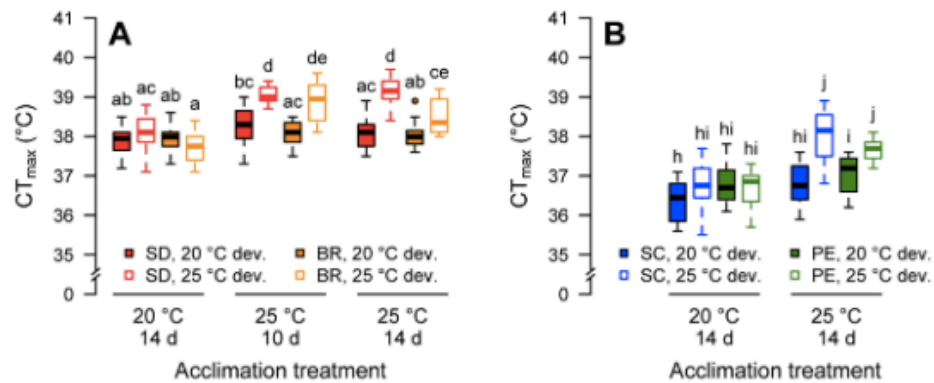


Figure 3. Phenotypic plasticity of critical thermal maximum (CT_{max}) in Californian populations of *T. californicus* as a result of temperatures experienced during development and adulthood. Panel A: San Diego (SD; red) and Bird Rock (BR; orange) copepods. Panel B: Santa Cruz (SC; blue) and Pescadero (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 $n = 15$).

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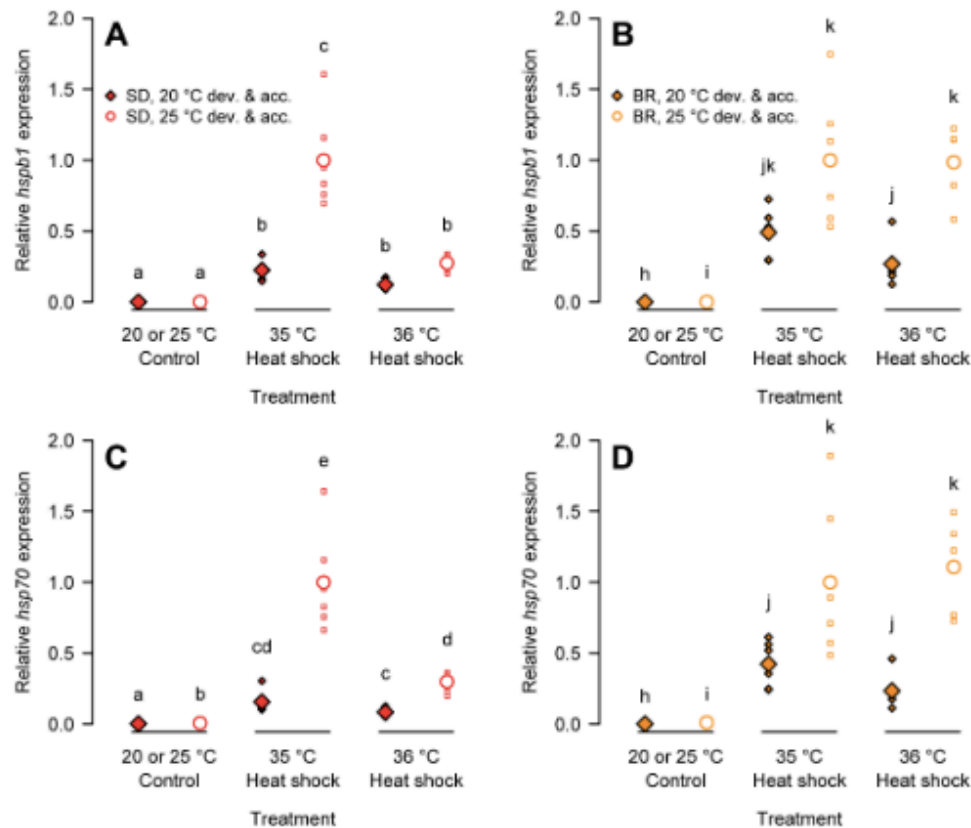


Figure 5. Variation in the induction of heat shock protein mRNA expression (A,B – *hspb1*; C,D – *hsp70*) as a result of developmental and adulthood temperatures in *T. californicus*. Panels A,C: data for the San Diego population (SD; red), and panels B,D: data for the Bird Rock population (BR; orange). 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.

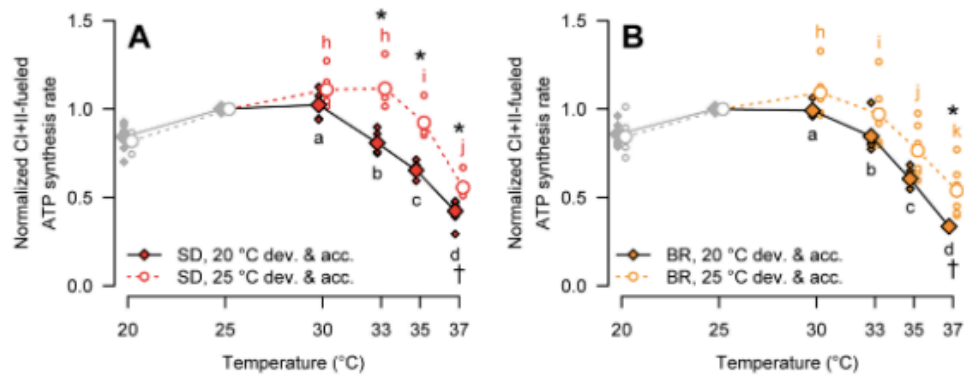


Figure 4. Proportional changes (from 25°C) in the thermal performance curves for complexes I and II (CI+II)-fueled ATP synthesis rates as a result of developmental and adulthood temperatures in *T. californicus*. Panel A: data for the San Diego population (SD; red), and panel B: data for the Bird Rock 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.

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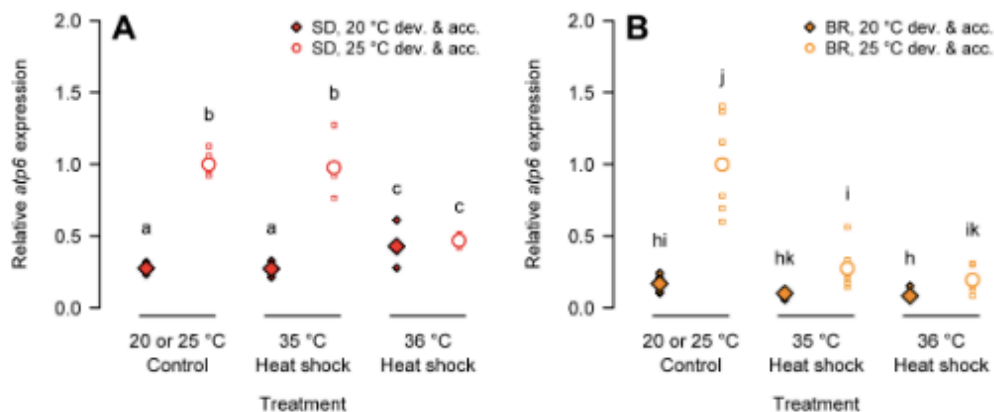


Figure 6. Variation in *mt-atp6* mRNA expression (A – SD, red; B – BR, orange) as a result of developmental and adulthood temperatures in *T. californicus*. Copepods were developed (dev.), and then acclimated as adults (acc.) at 20°C (filled diamonds; solid lines) or 25°C (open circles; dotted lines). 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.

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1128 Table 1. qRT-PCR primers.
 1129

Gene	Primer ¹	Population-specific sequence (5' to 3')	
		SD	BR
<i>hspb1</i>	F	CGATTTTCATCTGGGTCTCAA	CGATTTCCATCTGGGTCTCAA
	R	TTGAAGAACTCCTCCGCTGT	same as SD
<i>hsp70</i>	F	CTCTGTGCCGACCTTTCC	same as SD
	R	CTGGATTGATGCTCTTGTTCA	same as SD
<i>mt-atp6</i>	F	AGGACAGCCCATCTGAGG	AGGACAGCCCATCTAAGGTT
	R	CAGCCAGAGTTAAGGGACG	ACTGCCAAAGTTAATGGACGA
<i>gapdh</i>	F	CAACCACGAGCAATACGAGA	same as SD
	R	GGAGGAGGGGATGATGTTTT	same as SD

1130 ¹ F = forward; R = reverse

1131 Table 2. Developmental and adulthood plasticity in complexes I and II (CI+II)-fueled
 1132 ATP synthesis rates as a result variation in temperature.

Population	Assay temperature (°C)	CI+II ATP synthesis rate ¹ (<i>pmol min⁻¹ ng DNA⁻¹</i>)	
		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*}

1133 ¹ mean ± s.e.m.; letters indicate the results of post-hoc tests within populations and dev.
 1134 & acc. treatments; asterisks indicate differences within populations and assay
 1135 temperatures

1136 ² dev. & acc. = development and adult acclimation