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Rapid metabolic compensation in response to temperature change in the intertidal copepod, *Tigriopus californicus*

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ABSTRACT

Animals living in the intertidal zone must adapt to thermal variability, including adjustments in metabolism. We examined metabolic responses to temperature in the copepod, *Tigriopus californicus*, which inhabits supratidal splash pools along the Pacific coast of North America. We maintained three populations of *T. californicus* at 20 °C, one from southern California (San Diego, “SD”) and two from Oregon (Fogarty Creek, “FCN”, Boiler Bay, “BOB”) and examined possible geographic patterns in metabolism. We measured oxygen consumption rate ($\dot{V}\text{O}_2$) at 20 °C and following 48 h (chronic) acclimation to 25, 27.5 and 30 °C. $\dot{V}\text{O}_2$ was temperature-independent, with temperature quotients (Q_{10}) values ≤ 1 in all populations, indicative of metabolic compensation. We detected no variation in $\dot{V}\text{O}_2$ or survival between populations. To explore the time course of metabolic compensation, we performed an acute acclimation experiment in which $\dot{V}\text{O}_2$ was measured at 20 °C, following immediate exposure to 25 °C, and following 2 h, 4 h and 6 h exposure to 25 °C. This acute acclimation experiment revealed that $\dot{V}\text{O}_2$ increased immediately in SD and FCN, but was no longer different than 20 °C levels by 2 h and 4 h at 25 °C, respectively. BOB showed no significant change in $\dot{V}\text{O}_2$, which may indicate complete temperature-independent metabolism or different mechanisms of compensation between populations. This study demonstrates a time course of rapid metabolic compensation in response to temperature that occurs in a small intertidal animal, and suggests intertidal invertebrates can thermally acclimate within a few hours of a significant temperature change.

1. Introduction

Temperature has a pervasive effect on physiology, as nearly all physiological processes are influenced by an animal's body temperature. A key emphasis of thermal biology is examining how animals respond to temperature changes in their environment. This focus promotes understanding of how animals live successfully in thermally variable environments, and is also useful in assessing responses to longer-term temperature alterations associated with global climate change. Intertidal habitats represent variable environments in which organisms may experience fluctuations in temperature on the order of minutes and hours (Helmuth et al., 2006b). Thus, intertidal species represent a unique opportunity to examine how organisms respond to rapid temperature changes. Physiological responses to fluctuating environments are complex and cannot be easily highlighted using studies that only focus on constant temperatures (Fuller et al., 2010; Helmuth et al., 2006a). The level of environmental variability is likely to drive how a species responds and adapts (Burggren, 2018). For instance, a species or population inhabiting a thermally variable or unstable environment may show more rapid acclimatization than a species or population from a thermally stable environment. Our understanding of such processes is limited by our rudimental understanding of how temperature varies in space and time in most environments, and how this interacts with species size, mobility, activity level, and whether the species exhibits physiological plasticity (Morash et al., 2018).

For many poikilothermic organisms, metabolic rate approximately doubles with an acute 10 °C increase in temperature, consistent with a temperature quotient, or Q_{10}, of ~2 (Scholander et al., 1953). Yet, many species, particularly those with eurythermal life histories, can show lower Q_{10} values (Scholander et al., 1953). Q_{10} values closer to 1 are indicative of temperature insensitivity, often termed ‘metabolic compensation’ (Bullock, 1955; Somero, 1969). This phenomenon of metabolic compensation has been observed in intertidal fish and invertebrates. For instance, some intertidal fish show little change in metabolism when measured immediately following an acute temperature increase (Eme and Bennett, 2009; Taylor et al., 2005). Similarly, a diverse range of intertidal invertebrates, including anemones, polychaetes, and molluscs, demonstrate temperature-independent

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metabolism across a broad range of temperatures (Branch et al., 1988; Davies, 1966; Newell and Northcroft, 1967; Newell, 1969; Sokolova and Pörtner, 2003). To our knowledge, only one study has examined metabolism in the hours immediately following a temperature change (Marshall and McQuaid, 2011). This study demonstrated a decrease, rather than an increase, in $\dot{V}O_2$ in an intertidal snail (*Echinolittorina malaccana*) during the 2 h following a rapid 10 °C temperature increase (Marshall and McQuaid, 2011). Further analysis is required to examine initial changes in metabolism in response to a temperature change before organisms achieve complete metabolic compensation.

The lack of temperature sensitivity is also evident in smaller marine organisms, such as copepods (species range from 1 to 2 mm in body length). For example, several species of Northern Atlantic intertidal copepods, which are likely to experience a more variable environment than their open ocean counterparts, show no variation in metabolic rate across a 10 °C temperature range (Gee, 1985). While some pelagic copepods also demonstrate low Q_{10} values, metabolic rates increase with temperature in many species (Castellani et al., 2005; Paffenhöfer, 2006; Teuber et al., 2013). Thus, there is a trend for metabolic compensation to be more prevalent in intertidal copepods, suggesting these organisms acclimate to local variable conditions via reduced sensitivity to changing temperatures (Démeusy, 1957). In these smaller intertidal invertebrates, the effect of thermal variability is likely to be compounded by low thermal inertia, resulting in physiological phenotypes capable of rapid acclimatization. However, the speed of acclimatization has not been thoroughly explored and the time course of metabolic compensation should be thoroughly investigated in these small intertidal animals.

Tigriopus californicus is an ideal species for examining metabolic acclimation in a small invertebrate organism that inhabits an intertidal environment. *T. californicus* is a harpacticoid copepod, approximately 1.2 mm in length, widely distributed along the Pacific coast of North America, from Baja California to southeast Alaska. The species inhabits splash pools in the supratidal zone, where tidal immersion is rare and the environment is uninhabitable to many other intertidal organisms (Kontogiannis, 1973). Temperatures in *T. californicus* splash pools can vary by as much as 17 °C over a daily cycle, with temperature changes as high as 5 °C occurring within a 30 min period (Fig. 1). *T. californicus* is a model species for population-level analyses, with extreme genetic differentiation among populations that is indicative of low gene flow and strong isolation (Alexander et al., 2014; Burton and Lee, 1994; Edmands, 2001; Edmands and Harrison, 2003; Pereira et al., 2013). With a geographical range of over 3000 km, it is possible that the species demonstrates varying metabolic responses through local adaptation. Survival assays following acute exposure to increased temperatures demonstrate that northern populations are less tolerant to heat stress than southern populations (Kelly et al., 2012; Leong et al., 2017; Pereira et al., 2013; Pereira et al., 2017; Willett, 2010), and this may reflect the higher mean temperatures in southern locations (Fig. 1). No studies have explored metabolic acclimation to temperature in this species, and whether metabolism varies between populations.

The goal of our study was to examine a time course of whole-animal metabolic responses of three populations of *T. californicus* following acclimation to various temperatures. We used two populations from Oregon and one population from southern California, each maintained under common garden conditions in the lab prior to experimentation. We first explored how oxygen consumption rate ($\dot{V}O_2$) and survival were affected by an increase in temperature (from 20 °C to 25, 27.5 and 30 °C) following a 48 h acclimation period; we consider this our ‘chronic’ temperature acclimation experiment. We hypothesized there would be limited change in $\dot{V}O_2$ with temperature, evidence of metabolic compensation in this chronic experiment. We also predicted $\dot{V}O_2$ would not differ between populations because any differences in chronic $\dot{V}O_2$ between geographic locations would be overshadowed by metabolic changes in response to thermal variability. We hypothesized that survival of the southern population would not be affected by constant acclimation to temperatures between 20 °C and 30 °C, but that northern populations would show reduced survival at 30 °C, reflecting lower thermal tolerance previously observed (Pereira et al., 2017; Willett, 2010). In our ‘acute’ temperature acclimation experiment, we measured $\dot{V}O_2$ immediately after a temperature increase (from 20 to 25 °C) and throughout the following six hours at two-hour intervals, with the aim of detecting the time course of metabolic compensation. This acute temperature experiment reflects temperature increases experienced in the field (Fig. 1). We predicted $\dot{V}O_2$ would initially increase with a temperature increase, compensation would occur within a few hours of temperature exposure, and $\dot{V}O_2$ would return to levels measured prior to the temperature increase.

2. Materials and methods

2.1. Population sampling and maintenance

T. californicus were collected from the field in Spring and Summer and housed at Oregon State University at 20 °C with a 12:12 L:D photoperiod for approximately one month before being sent to California State University San Marcos (CSUSM). The three populations studied were Boiler Bay (BOB) Oregon (44°49′48″N/124°03′30″W), and Fogarty Creek (FCN) Oregon (44°50′20″N/124°03′10″W), the two northern populations, and Ocean Beach, San Diego (SD) California (32°44′43″N/117°15′15″W), the southern population. At CSUSM, all populations were maintained under common garden conditions at 20 °C in an incubator (Thermo Scientific Precision Incubator, Marietta, OH, USA) with a 12:12 L:D photoperiod. 20 °C represents a temperature often experienced in the field by all populations, and is slightly below average summer temperature (Fig. 1). Stock populations were maintained in 35 ppt artificial seawater, created from Instant Ocean® (Blacksburg, VA, USA) and deionized water, in 250–400 ml beakers. Each beaker was fed reconstituted Spirulina powder (5 mg/ml, Bulk Supplement, Henderson, NV, USA) and live *Dunaliella salina* as needed (Carolina Biological Supply Company, Burlington, NC, USA). Partial water changes were performed once a month. Populations were maintained for at least one month prior to experimentation. The species produces offspring that reach maturity in ~14–20 d at 20 °C (Leong et al., 2017), so populations likely cycled through at least two generations since field collection.

2.2. Chronic (48 h) temperature acclimation and oxygen consumption

Oxygen consumption rate ($\dot{V}O_2$, μl h^{-1}) was measured for all populations at the maintenance temperature (20 °C), and in separate copepods following 48 h acclimation to 25, 27.5, and 30 °C. Individuals were isolated from the stock populations and placed in 200 ml of 20 °C 35 ppt artificial seawater, with spirulina and *D. salina* as described above (10–12 individuals per beaker per temperature for each population). Beakers were shifted by 2.5 °C every 48 h, so that 25 °C was reached after 96 h, 27.5 °C was reached after 144 h, and 30 °C reached after 192 h. 3–4 beakers were acclimated for each population at each temperature for 48 h before measurement. 25 °C was achieved by placing beakers in an environmental growth chamber (Chagrin Falls, OH, USA), and temperatures of 27.5 and 30 °C were achieved by placing beakers in water baths heated with an aquarium heater (Eheim Jager Thermostat heater, Deizisau, Germany). A Thermochron iButton® data logger (Model DS1922L, iButtonLink, Whitewater, WI, USA) recorded temperature every 30 min in a separate beaker with the same volume of water as the copepod beakers at all temperatures. Weekly average temperatures were used to generate a grand mean for each temperature: 20 °C = 19.8 ± 0.05 °C, 25 °C = 25.1 ± 0.07 °C, 27.5 °C = 27.5 ± 0.02 °C, and 30 °C = 29.8 ± 0.10 °C.

Once isolated individuals had acclimated at the target temperature for 48 h, $\dot{V}O_2$ was determined from the decrease in PO_2 in a closed respirometry system. Custom respirometry chambers created from borosilicate glass vials and containing a 5 mm O_2 sensor spot (Loligo Systems, Tjele, Denmark) were used as previously described (Eme et al.,

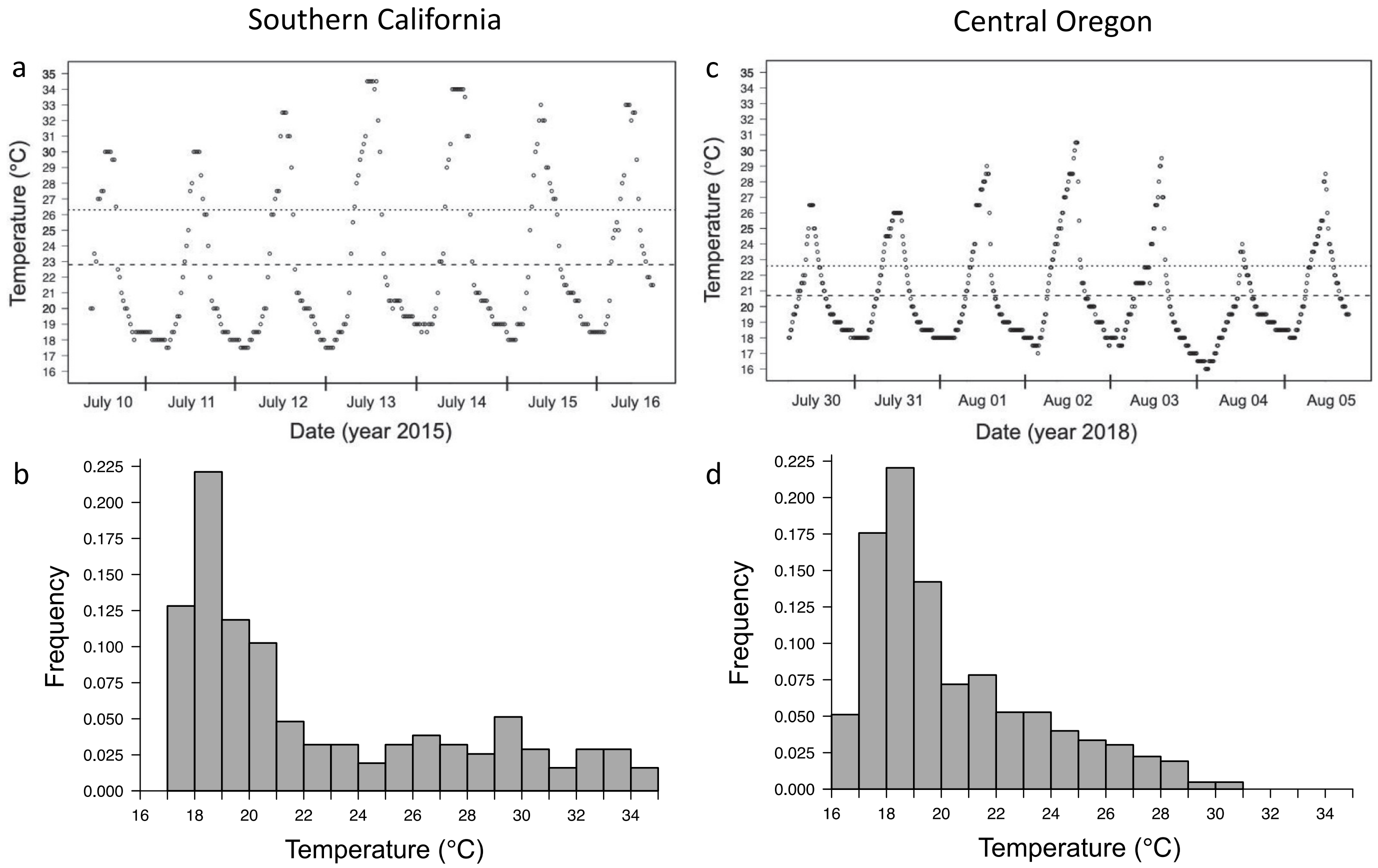

Fig. 1. Temperature recordings in high intertidal pools. a) Representative recordings and b) frequency histogram of temperature over a one-week period in summer in a southern California splash pool, and c) representative recording and d) frequency histogram of temperature in Central Oregon splash pool. Dashed line on a and b represents mean overall temperature; dotted line represents mean daytime (8 am – 8 pm) temperature. Southern California site is Ocean Beach, San Diego (32°44′43″N/117°15′15″W) and Central Oregon site is Strawberry Hill (44°15′15″N/124°06′45″W). Temperature was recorded with a Thermochron iButton® data logger (iButtonLink, Whitewater, WI, USA) affixed to the substrate with marine epoxy. Data loggers were placed approximately 25 cm below the surface in pools inhabited by copepods. Data logging intervals were 30 min in San Diego and 20 min in Strawberry Hill.

2015; Mueller et al., 2015). The final volume of the chambers with the sensor spot ranged from 500 to 600 µL. For each $\dot{V}O_2$ trial, four chambers were filled with fresh 35 ppt seawater containing no algal food at the target temperature. Individual copepods were randomly selected from the isolated beakers and placed into a chamber, vials were sealed with the caps so that no air bubbles were present in the chamber, and the lid was tightly sealed. This was repeated so that $\dot{V}O_2$ was measured in 13–16 individuals per temperature for SD, and 8–16 individuals per temperature for BOB and FCN. The respirometry system was calibrated daily, prior to experimentation, using a fresh anoxic solution of 10 mg sodium sulfite in 1 ml water (0% O_2) and air equilibrated water (100% O_2). The closed chambers were then placed into a recirculating water bath thermostated to the target temperature by a recirculating heater/chiller (Fisher Scientific, Isotemp™ 6200, Waltham, MA, USA). The sensor spot was read with a fiber optic cable (PreSens Precision Sensing, GmbH, Regensbury, Germany) connected to a Witrox 4 oxygen meter (Loligo Systems, Tjele, Denmark) and computer running Autoresp™ software (Loligo Systems, Tjele, Denmark). Average temperature of the respirometry system was held within ± 0.2 °C of the desired trial temperature.

Oxygen levels of each chamber were read every 5 min for 100–120 min. The first 40 min were used to allow copepods to reach steady state and were not included in the determination of $\dot{V}O_2$. Copepods displayed sporadic activity in the chambers throughout measurements, and thus $\dot{V}O_2$ measured represents routine $\dot{V}O_2$. $\dot{V}O_2$ was calculated by using the decrease in chamber PO_2 over time, the O_2 capacitance of the water at the relevant temperature, and the chamber volume minus the volume of the copepod (estimated from mass measurements of groups of copepods). $\dot{V}O_2$ was also measured regularly for chambers containing only seawater at each experimental temperature to control for any microorganism respiration; this value was subtracted from the $\dot{V}O_2$ values determined for copepods. No mortality occurred during $\dot{V}O_2$ trials, after which copepods were fixed in 70% EtOH and photographed (40 × -1000 × Trinocular Microscope with USB 3.0 digital camera, Am Scope, Irvine, CA, USA). Photographs were used to measure length (Amscope software) and identify sex of the individuals (Hawkins, 1962). Only adult males or females with an early stage brood of eggs were used in data analyses. Temperature sensitivity of $\dot{V}O_2$ was assessed by calculating a temperature quotient, Q_{10}, for each population between 20 °C and 30 °C by dividing $\dot{V}O_2$ at 30 °C by $\dot{V}O_2$ measured at 20 °C.

2.3. Chronic (48 h) temperature acclimation and survival

Survival was determined for each population following 48 h acclimation to each temperature. For each population, 3–5 beakers containing 10–20 adult individuals in 200 ml of 35 ppt artificial seawater were held at 20 °C, with Spirulina and *D. salina* as previously described. Survival for all beakers was recorded at 20 °C after 48 h, and following 48 h acclimation to 25, 27.5, and 30 °C, described above. Percent survival was determined for each individual beaker as the number surviving following acclimation divided by the initial number, and an average calculated for each population using beakers as replicates.

2.4. Acute (0–6 h) temperature acclimation and oxygen consumption

Acute acclimation trials were performed at 25 °C for each population. $\dot{V}\mathrm{O}_2$ of individuals maintained at 20 °C were measured as a baseline. Individuals at the 0 h acclimation time point were taken from 20 °C and placed directly into 25 °C in the mini respirometry system and $\dot{V}\mathrm{O}_2$ determined from the decrease in PO_2 measured in the chamber every 5 min for 100–120 min, as described above. Separate copepods were also isolated in beakers and moved from 20 °C to 25 °C, acclimated to 25 °C for 2, 4 or 6 h, and $\dot{V}\mathrm{O}_2$ determined during the following 100–120 min period. $\dot{V}\mathrm{O}_2$ was measured in 8–12 individuals for each population at each acclimation time point. Following the $\dot{V}\mathrm{O}_2$ trial, copepods were fixed, photographed and length and sex determined as described above.

2.5. Statistical analyses

Parametric assumptions of normality and equal variances were tested by the Anderson-Darling test and Levene's test, respectively. Differences in $\dot{V}\mathrm{O}_2$ following chronic acclimation were tested using a two-way ANCOVA to examine the effects of temperature, population, and the interaction between temperature and population. Length was used as a covariate to account for any subtle differences in length between treatments. Tukey's post hoc multiple comparison test was performed on any factors that were significant. If an interaction was significant, a Tukey's test was performed on that alone. A two-way ANOVA was used to compare survival and length between and within temperature and population groups.

Changes in $\dot{V}\mathrm{O}_2$ following an acute temperature change were examined within each population using a one-way ANCOVA, with length as a covariate, followed by Tukey's post hoc multiple comparisons test. A one-way ANOVA was used to compare length across time points within each population. All data are presented as mean ± S.E.M. and differences were accepted as statistically significant at $\alpha = 0.05$.

3. Results

3.1. Chronic (48 h) temperature acclimation and oxygen consumption

$\dot{V}\mathrm{O}_2$ ($\mu\mathrm{l\,h}^{-1}$) was significantly affected by temperature (two-way ANCOVA; $F_{3,133} = 4.0$, $P = .01$), population ($F_{2,133} = 3.7$, $P = .028$) and the interaction between temperature and population ($F_{6,133} = 2.2$, $P = .045$, Fig. 2) after controlling for length. The significant interaction between temperature and population occurred because the BOB $\dot{V}\mathrm{O}_2$ at 20 °C was greater than SD $\dot{V}\mathrm{O}_2$ measured at 25 and 30 °C and FCN $\dot{V}\mathrm{O}_2$ measured at 27.5 °C. Post hoc comparisons indicated no differences between temperatures for any population. Q_{10} values were calculated for all populations over the temperature range of 20–30 °C. All groups displayed a Q_{10} temperature coefficient of ≤ 1: SD $Q_{10} = 0.78$, FCN $Q_{10} = 1.08$, BOB $Q_{10} = 0.41$.

Table 1
Length (mm) of individuals used in oxygen consumption rate measurements from each population and at each acclimation temperature in the chronic acclimation study. Data presented as mean ± SEM. Superscript letters indicate significant differences based on a significant population*temperature interaction ($P < .05$, two-way ANOVA, Tukey post hoc comparisons).

	San Diego, CA	Fogarty Creek, OR	Boiler Bay, OR
20 °C	1.19 ± 0.03^{AB}	1.29 ± 0.02^{AB}	0.95 ± 0.10^{C}
25 °C	1.26 ± 0.03^{AB}	1.34 ± 0.02^{A}	1.20 ± 0.03^{AB}
27.5 °C	1.30 ± 0.01^{AB}	1.28 ± 0.04^{AB}	1.14 ± 0.04^{B}
30 °C	1.30 ± 0.02^{AB}	1.28 ± 0.03^{AB}	1.28 ± 0.02^{AB}

The ANCOVA indicated no relationship between length (mm) and $\dot{V}\mathrm{O}_2$ ($F_{1,133} = 0.4$, $P = .5$). There were, however, minor differences in length between temperature and population groups (two-way ANOVA; $F_{8,150} = 4.0$, $P < .001$). This interaction was due to FCN at 25 °C being significantly longer than BOB at 27.5 °C. BOB at 20 °C was also significantly shorter than all other treatments (Table 1).

3.2. Chronic (48 h) temperature acclimation and survival

Survival was significantly influenced by temperature ($F_{3,44} = 4.0$, $P = .015$), but not population ($F_{2,44} = 0.65$, $P = .53$) or the interaction between temperature and population ($F_{6,44} = 0.72$, $P = .64$). For all populations combined, survival was significantly lower at 27.5 °C compared to 20 °C, with intermediate survival at 25 and 30 °C (Fig. 3).

3.3. Acute (0–6 h) temperature acclimation and oxygen consumption

$\dot{V}\mathrm{O}_2$ was significantly different between 0 and 6 h exposures to 25 °C for the SD population (one-way ANCOVA; $F_{4,52} = 2.3$, $P = .042$) and FCN population ($F_{4,55} = 10.5$, $P < .001$) after controlling for length (Fig. 4). Tukey post hoc comparisons indicated that $\dot{V}\mathrm{O}_2$ of SD increased significantly when the individuals were immediately shifted from 20 °C to 25 °C, but returned to 20 °C levels within 2 h. This was reflected in the 20–25 °C Q_{10} calculated using average $\dot{V}\mathrm{O}_2$ values which was 4.0 at 0 h, 2.3 at 2 h, 1.7 at 4 h and 2.0 at 6 h. Likewise, individuals of FCN

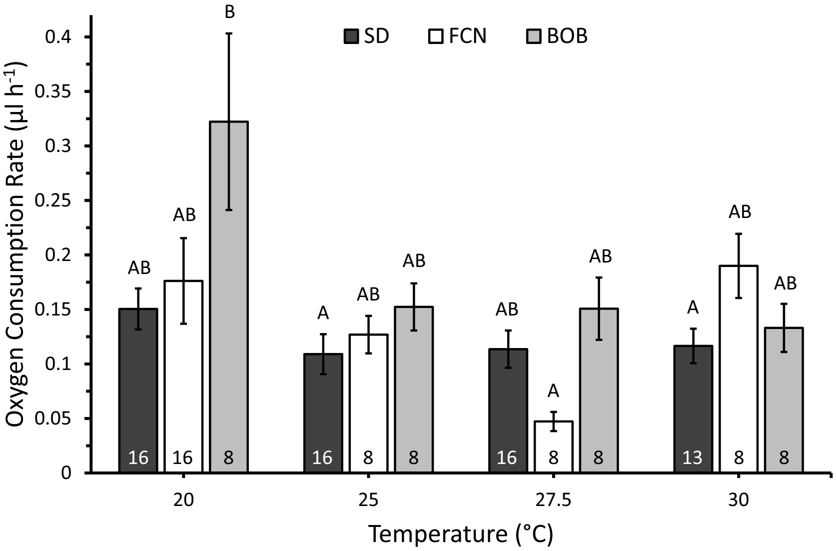

Fig. 2. Mean oxygen consumption rate ($\mu\mathrm{l\,h}^{-1}$) of three populations of *Tigriopus californicus* following chronic (48 h) acclimation to different temperatures. Populations are as follows: Ocean Beach, San Diego (SD), California; Fogarty Creek (FCN), Oregon; and Boiler Bay (BOB), Oregon. Error bars represent S.E.M. Numbers in each bar represent n. Different letters indicate significant differences ($P < .05$, 2-way ANCOVA, Tukey's post hoc comparisons).

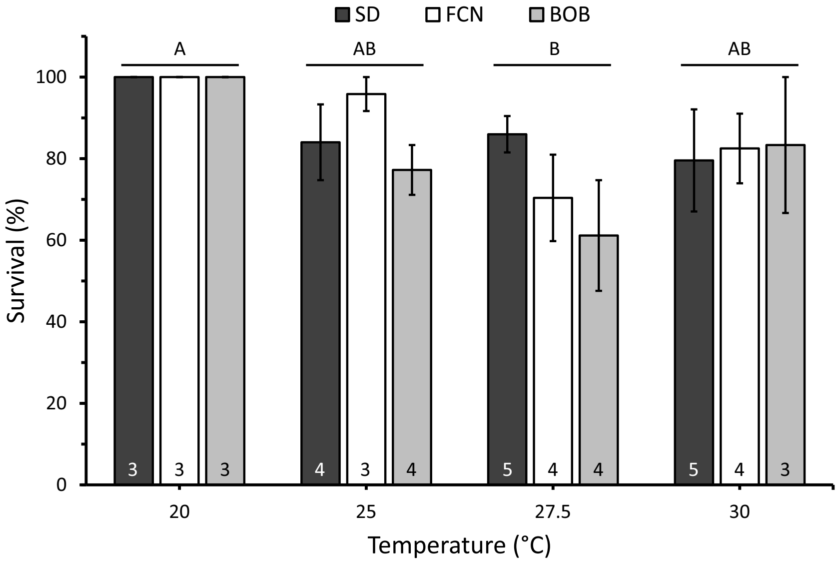

Fig. 3. Survival (%) of three *Tigriopus californicus* populations following chronic (48 h) acclimation to different temperatures. Populations are as follows: Ocean Beach, San Diego (SD), California; Fogarty Creek (FCN), Oregon; and Boiler Bay (BOB), Oregon. Error bars represent S.E.M. Numbers in each bar represent n. Different letters indicate significant differences between temperatures for populations combined ($P < .05$, 2-way ANOVA, Tukey's post hoc comparisons).

significantly increased following the shift from 20 °C to 25 °C, and $\dot{V}O_2$ remained elevated until 4 h post shift. 20–25 °C Q_{10} values for FCN were very high, and decreased across 6 h; 0 h = 20.1, 2 h = 15.4, 4 h = 8.6 and 6 h = 3.2. The BOB population did not demonstrate a significant change in $\dot{V}O_2$ when shifted from 20 °C to 25 °C, or in the 6 h following after controlling for length ($F_{4,51} = 1.1$, $P = .39$). However, the 20–25 °C Q_{10} for average $\dot{V}O_2$ was 1.9, 2.1 and 1.9 at 0, 2 and 4 h, respectively, before decreasing to 0.8 at 6 h. Length did not vary between time points for any population (one-way ANOVA; SD: $F_{4,52} = 0.7$, $P = .6$, FCN: $F_{4,55} = 2.3$, $P = .07$, BOB: $F_{4,51} = 0.6$, $P = .7$) (Table 2).

Table 2
Length (mm) of individuals used in oxygen consumption rate measurements from each population at each time point in the acute temperature change study. Data presented as mean ± SEM. Length did not differ across time points in any population ($P > .05$, one-way ANOVA).

	San Diego, CA	Fogarty Creek, OR	Boiler Bay, OR
20 °C	0.96 ± 0.03	1.02 ± 0.04	1.05 ± 0.04
25 °C – 0 h	0.97 ± 0.34	0.96 ± 0.03	1.08 ± 0.03
25 °C – 2 h	1.01 ± 0.02	1.04 ± 0.04	1.10 ± 0.02
25 °C – 4 h	0.98 ± 0.04	0.99 ± 0.02	1.09 ± 0.03
25 °C – 6 h	1.02 ± 0.04	1.09 ± 0.03	1.06 ± 0.02

4. Discussion

Temperature variability is an important environmental characteristic of intertidal zones, and successful intertidal organisms must be able to respond physiologically to temperature changes. We examined metabolism in a small, widely distributed intertidal copepod that inhabits supratidal splash pools. Metabolism was assessed in the context of short-term temperature changes and across populations that may show broad geographical differences in function. We demonstrate that *T. californicus* $\dot{V}O_2$ is not strongly influenced by temperature following chronic (48 h) acclimation, but measurement of $\dot{V}O_2$ in the hours (0–6 h) following an acute temperature change reveals rapid metabolic compensation to an increase in temperature. Our data also show that $\dot{V}O_2$ does not appear to be strongly influenced by geographic location, with only subtle population differences in acute temperature acclimation.

In support of our hypothesis, we observed that $\dot{V}O_2$ did not increase as temperature increased following chronic acclimation (Fig. 2). $\dot{V}O_2$ was predicted to be relatively unchanged with chronic acclimation due

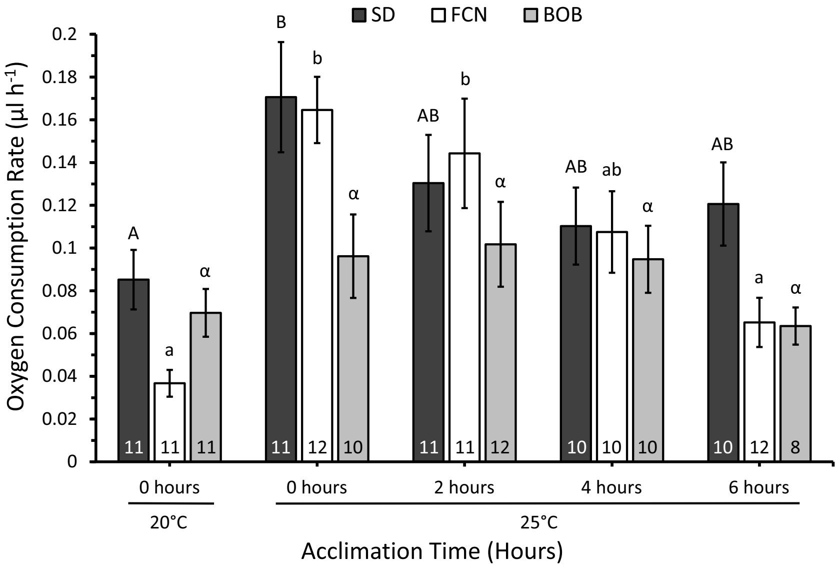

Fig. 4. Mean oxygen consumption rate (µl h^{-1}) of three populations of *Tigriopus californicus* measured at 20 °C and following 0, 2, 4 and 6 h acclimation to 25 °C. Populations are as follows: Ocean Beach, San Diego (SD), California; Fogarty Creek (FCN), Oregon; and Boiler Bay (BOB), Oregon. Error bars represent S.E.M. Numbers in each bar represent n. Different uppercase, lowercase and greek letters indicate significant differences between time points within SD, FCN, and BOB populations, respectively ($P < .05$, 1-way ANCOVA, Tukey's post hoc comparisons).

to metabolic compensation occurring within that time frame. Upon calculation of Q_{10} coefficients, we found all three populations to have a coefficient ≤ 1.1 for the temperature range of 20–30 °C. Often, poikilotherms display a $Q_{10} \geq 2$, which indicates high temperature dependence for physiological function. The low Q_{10} values of *T. californicus* represent a minor increase or decrease in $\dot{V}O_2$ in response to increased temperature. Q_{10} values of 1.0–1.2 between 10 and 20 °C in winter and 1.1–1.5 between 15 and 25 °C in summer have been shown for intertidal Atlantic copepods, *Asellopsis intermedia*, *Arenosetella tenuissima*, *Tryphoema bocqueti*, and *Paraleptastacus espinulatus* (Gee, 1985). In contrast, many pelagic copepods from arctic to tropical regions of the Atlantic, Pacific and Indian oceans are 'typical' poikilotherms with Q_{10}'s of 1.8–3.6 (Castellani et al., 2005; Ikeda et al., 2001; Paffenhöfer, 2006) and lake freshwater copepods have $Q_{10} \geq 2$ (Comita, 1968; Epp and Lewis, 1979).

Intertidal gastropods and fish also show temperature insensitivity in their metabolism compared to their subtidal counterparts. Temperate intertidal limpets (*Patella granularis* and *P. cochlear*) show reduced metabolic rate change (Q_{10} = 1.3–1.9) compared to subtidal species (*P. oculus*, Q_{10} = 2.5–3.9) over a 15 °C range (10–25 °C) (Branch et al., 1988). Tropical and cold-water intertidal gastropods also show lower Q_{10} values compared to subtidal species (Houlihan and Allan, 1982; Sokolova and Pörtner, 2003). Q_{10} values < 1.3 have been shown for intertidal common goby (*Bathygobius fuscus*), sandflat goby (*B.* spp.) and blackspot sergeant (*Abedufduf sordidus*) following an acute temperature increase from 26 °C to 32 °C (Eme and Bennett, 2009). In comparison, Q_{10} values for subtidal and reef-associated species, white-tailed humbug (*Dascyllus aruanus*) and nine-banded cardinalfish (*Apogon novemfasciatus*), exceed 2.2 (Eme and Bennett, 2009). Thus, temperature insensitivity has been demonstrated in both intertidal invertebrates and vertebrates and Q_{10} values of ~1.0 could be representative of important energy conserving physiological responses (Eme and Bennett, 2009; Gee, 1985; Marshall and McQuaid, 2011).

$\dot{V}O_2$ did not differ between populations in our chronic acclimation study, which supports our hypothesis. Previous studies indicate a possible latitudinal gradient in thermal tolerance in *T. californicus*, with southern populations of *T. californicus* demonstrating reduced sensitivity to both acute and chronic thermal exposures (Pereira et al., 2013; Pereira et al., 2017; Willett, 2010). However, the lack of population differences, and general temperature insensitivity of $\dot{V}O_2$, suggest no geographic differences in this trait (Fig. 2). While a broad latitudinal thermal cline is present in the intertidal zone down the Pacific coast, thermal patterns are variable and often unpredictable (Helmuth et al., 2006a), and are likely to affect physiological function of intertidal organisms. $\dot{V}O_2$ appears to respond more to short-term fluctuations in temperature experienced by *T. californicus* rather than to broad geographic differences.

Contrary to our prediction, we did not observe population differences in survival following chronic temperature acclimation. We also did not find a linear trend of decreasing survival as temperature increased; rather survival was significantly lower at 27.5 compared to 20 °C, but not at 25 or 30 °C (Fig. 3). Despite 30 °C only being recorded transiently in the field (Fig. 1), survival remained high (~80%) across all populations. A previous study demonstrated reduced survival in northern populations following chronic exposure to 32 °C (Willett, 2010); thus, population differences in survival may only emerge above 30 °C. High survival suggests we can likely eliminate the possibility that absence of a change in $\dot{V}O_2$ was due to higher temperatures having a detrimental effect on function in *T. californicus*. The observation that $\dot{V}O_2$ did not increase with 48 h acclimation to increased temperature does not appear to be due to a functional constraint in these animals. It is likely that *T. californicus* displays tolerance to a wide temperature range because of the high temperature variability they experience in the intertidal zone. The temperature range used in this study is within the physiological tolerance of the species and unlikely to induce thermal damage at the cellular level that would influence physiological function or population abundance (Stillman, 2002). Experiments that include exposure to temperatures on either side of the 20–30 °C range will likely clarify the extent of the physiological tolerance range of the species and if it varies geographically.

Due to the absence of increased $\dot{V}O_2$ with temperature in our chronic (48 h) acclimation study, we investigated whether we could detect changes in $\dot{V}O_2$ following a shorter acute (0–6 h) acclimation to increased temperature. Most previous studies that demonstrate no or little change in $\dot{V}O_2$ with temperature in intertidal organisms use acclimation periods ranging from 24 h to 4 d (Castellani et al., 2005; Gee, 1985; Houlihan and Allan, 1982). We found that $\dot{V}O_2$ increases immediately following a 5 °C temperature increase in two of our populations examined (Fig. 4). The SD population showed a doubling in $\dot{V}O_2$ when measured immediately at 25 °C following a shift from 20 °C. After just 2 h at 25 °C, SD population $\dot{V}O_2$ was no longer significantly higher compared to $\dot{V}O_2$ at 20 °C. The $\dot{V}O_2$ change of the FCN population was even more dramatic, increasing by 4.5 times immediately following a shift from 20 to 25 °C, returning to 20 °C levels after 4 h at 25 °C. In contrast to the SD and FCN populations, the BOB population did not show a significant change in $\dot{V}O_2$ immediately after a shift from 20 to 25 °C or 6 h following the temperature change. We know of two other studies using trout (*Onchorynchus mykiss*), freshwater crayfish (*Astacus fluviatilis*) and marine snail (*Echinolittorina malaccana*) in which $\dot{V}O_2$ was assessed in the hours immediately after a temperature change (Bullock, 1955; Marshall and McQuaid, 2011). In trout and freshwater crayfish, an increase from 5 to 15 °C resulted in an immediate increase in $\dot{V}O_2$ in both species in the first few hours after a temperature change before $\dot{V}O_2$ decreased, while a 10 °C temperature increase resulted in a decrease in $\dot{V}O_2$ in the marine snail. In contrast to *T. californicus*, $\dot{V}O_2$ of trout and crayfish did not return to those levels measured at 5 °C before the temperature change, indicating incomplete compensation (Bullock, 1955). This difference could reflect the stronger temperature change (10 °C versus 5 °C of this study) or species-specific differences that require further investigation.

Our acute acclimation study reveals subtle population differences in how $\dot{V}O_2$ responds to temperature, despite no population differences in our chronic acclimation study. Minor time course differences in acclimation result in a similar overall 48 h response. The FCN population showed the strongest acute response to a temperature increase before $\dot{V}O_2$ returned to 20 °C levels after 4 h (Fig. 4). Interestingly, the BOB population showed no significant increase in $\dot{V}O_2$. One possible explanation for this is that the change in $\dot{V}O_2$ with a temperature increase is so rapid in BOB we could not detect it in the time frame in which we measured $\dot{V}O_2$. Measurement of instantaneous $\dot{V}O_2$ may be able to detect immediate compensation (occurring in less than an hour). Alternatively, the populations may show divergent mechanisms of compensation that allow them to buffer for temperature change in different ways. For example, the initial increase in $\dot{V}O_2$ followed by rapid compensation in SD and FCN populations may occur because some expression of metabolic components (e.g. enzymes) have a time lag that results in temporary temperature dependence following by independence (Bullock, 1955). In contrast, the BOB population may utilize a number of biochemical pathways that vary in different ways with temperature, but are relatively balanced resulting in complete temperature independence (Bullock, 1955; Eme and Bennett, 2009; Newell, 1969). Another factor to consider is how thermal variability may differ between our populations; if the microhabitat of the BOB population shows the greatest variability, this population may have evolved further temperature independence. Further investigation of metabolic compensation across populations, and if mechanisms underlying compensation vary, is needed.

In conclusion, we demonstrate that rapid metabolic compensation occurs in *T. californicus* over a few hours following a temperature increase, resulting in temperature independence when metabolism is examined following chronic (48 h) acclimation. These results highlight the importance of considering the time course of metabolic measurements following a temperature change to create a complete picture of acclimation and compensation, particularly in organisms that experience high levels of natural thermal variability. Low Q_{10} values calculated for *T. californicus* may also indicate the contribution their small size (~1.2 mm) has on their ability to rapidly metabolic compensate for temperature changes. Furthermore, temperature insensitivity in $\dot{V}O_2$ following chronic temperature acclimation is consistent across populations, but there may be divergent mechanisms in how each population undertakes metabolic compensation that warrants further exploration. In accordance with its temperature insensitivity, we might predict that *T. californicus* may be able to withstand changes in its thermal environment induced by climate change. However, future studies are required to understand the limit of its rapid metabolic compensation, the underlying mechanisms, and how compensation may allow the organism to diverge and thrive in different thermal environments. Studying function in this small species could be extremely valuable for understanding mechanisms of temperature acclimatization and the impact of climate change in the intertidal zone.

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