



Plasticity of foot muscle and cardiac thermal limits in the limpet *Lottia limatula* from locations with differing temperatures

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ABSTRACT: Species distributions are shifting in response to increased habitat temperatures as a result of ongoing climate change. Understanding variation in physiological plasticity among species and populations is important for predicting these distribution shifts. Interspecific variation in intertidal ectotherms' short-term thermal plasticity has been well established. However, intraspecific variation among populations from differing thermal habitats remains a question pertinent to understanding the effects of climate change on species' ranges. In this study, we explored upper thermal tolerance limits and plasticity of those limits using a foot muscle metric and 2 cardiac metrics (Arrhenius breakpoint temperature, ABT, and flatline temperature, FLT) in adult file limpets *Lottia limatula*. Limpets were collected from thermally different coastal and inland-estuarine habitats and held for 2 wk at 13, 17 or 21°C prior to thermal performance assays. Compared to limpets from the warm estuary site, limpets from the cold outer coast site had similar foot muscle critical thermal maxima (CT_{max} ; 35.2 vs. 35.6°C) but lower cardiac thermal tolerances (ABT: 30.5 vs. 35.1°C). Limpets from the cold coast site had higher acclimation responses in foot muscle CT_{max} (0.22°C per 1°C rise in acclimation) than those of the warm estuary site (0.07°C per 1°C rise in acclimation), but lower acclimation responses in cardiac thermal tolerance (ABT: -0.85°C per 1°C rise in acclimation) than those of the estuary site (ABT: 0.10°C per 1°C rise in acclimation). Since outer coast populations had lower cardiac plasticity and higher mortalities in the warm acclimation, we predict *L. limatula* from colder habitats will be more susceptible to rising temperatures. Our findings illustrate the importance of population-specific variation in short-term thermal plasticity when considering the effects of climate change on ectotherms.

KEY WORDS: Intertidal · Ectotherm · Thermal tolerance · Plasticity · Acclimation · Ecophysiology

1. INTRODUCTION

Since the effects of temperature are pervasive on many levels of biological organization (Somero 1997), thermal gradients are strong determinants of species distributions (Badgley & Fox 2000, Lo Presti &

Oberprieler 2009). In intertidal ectotherms, thermal tolerances are thought to be adapted to their maximal habitat temperatures (Tomanek & Somero 1999, Morley et al. 2009, Zippay & Hofmann 2010). Upper thermal tolerance limits have been shown to generally decrease with latitude; i.e. species living closer

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to the equator are more capable of withstanding heat (Sunday et al. 2011). However, macrophysiological patterns do not occur latitudinally in intertidal zone species that experience mosaic patterns of habitat temperature maxima due to local topography and timing of tides (Helmuth et al. 2006, Kuo & Sanford 2009). Interspecific variation of upper thermal tolerance and plasticity has been well documented in intertidal ectotherms, but we lack a clear consensus on general patterns of intraspecific variation. In copepods, crabs, and snails, populations of one species from warmer and drier climates have been shown to have higher thermal tolerance (Willett 2010, Madeira et al. 2012, Gleason & Burton 2015). However, not all species show correlated environmental and physiological variation; for example, the intertidal snails *Littorina keenae* and *Crepidula fornicata* show weak to no evidence of latitudinal cline in thermal tolerance (Lee & Boulding 2010, Diederich & Pechenik 2013). The degree to which adaptation over local habitat temperature gradients reflects macrophysiological patterns is important to understand in the context of predicting responses to environmental change.

In cases where species have small thermal safety margins (i.e. thermal tolerance limits are close to maximal habitat temperatures), the plasticity of thermal tolerance is a secondary important determinant of response to habitat warming (Gunderson & Stillman 2015). In environments of rising temperatures, thermal plasticity is a strategy to cope with increased temperatures; as an organism acclimates to a higher temperature, its upper thermal tolerances can increase in response (Pörtner et al. 2006). For example, species can undergo heat-hardening transiently adjusting their upper thermal limits through heat-shock responses (Dahlhoff & Somero 1993, Hamdoun et al. 2003). Several hypotheses serve to explain macrophysiological variation in plasticity of thermal tolerance. The latitudinal hypothesis predicts that species living at higher latitudes have higher potentials of thermal plasticity in order to withstand seasonality and fluctuating temperatures (Janzen 1967, Levins 1968, Gabriel & Lynch 1992, Bozinovic et al. 2011). The Brattstrom hypothesis predicts that widespread species with larger geographic ranges experience broader thermal environments, and thus have higher thermal tolerance plasticity (Brattstrom 1968, Spicer & Gaston 2009). The trade-off hypothesis posits that species of higher thermal tolerances have smaller thermal safety margins and exhibit lower potentials of thermal plasticity (Tomanek & Somero 1999, Tomanek & Helmuth 2002, Stillman 2003, Sten-

seng et al. 2005, Gunderson & Stillman 2015, Armstrong et al. 2019). This suggests that species or populations with the highest upper thermal tolerances are most vulnerable to extirpation as a result of climate change (Stillman 2003). However, not all comparative studies have yielded support for these hypotheses. Calosi et al. (2008) found thermal tolerance to be positively correlated with tolerance plasticity in *Deronectes* diving beetles, and Simon et al. (2015) found no relationship between upper thermal tolerance and plasticity. The degree of thermal plasticity has been shown to depend on factors like thermoregulatory behavior, life stage, and evolutionary trajectory, further complicating our understanding of macrophysiological patterns (Marais et al. 2009, Bozinovic et al. 2011, Sheldon & Tewksbury 2014, Gunderson & Stillman 2015).

This study focused on the thermal limits and plasticity of those limits in the file limpet *Lottia limatula* (Carpenter, 1864) across thermally variable sites in northern California. *L. limatula* ranges from central Oregon to Baja California, where they inhabit the mid to low intertidal zones and graze microscopic algae (Lindberg 1981). The population is generally continuous in southern California, but north of Santa Cruz, *L. limatula* are only found in locations where water temperatures are warmer (Test 1945). For example, an isolated population, likely recruited from a southern population during a Southern Oscillation event (El Niño), resides within the inner Tomales Bay (Jacobs et al. 2004). This population was first named *Lottia morchii* by Dall (Dall, 1878). Like other intertidal limpets, range limits of *L. limatula* are in part constrained by stressful thermal environments (Miller et al. 2009, Fenberg & Rivadeneira 2011).

The goals of this study were to explore variation in thermal physiology in *L. limatula* collected from sites where the species is likely to have experienced long-term differences in habitat temperatures. We hypothesized that upper thermal tolerances of *L. limatula* populations are positively correlated with their corresponding maximal habitat temperatures. Since there is a cost to maintaining plasticity for higher thermal tolerances (Kingsolver & Huey 1998, Ernande & Dieckmann 2004), selection can favor higher upper thermal tolerances and lower plasticity in cases of evolution to extreme heat (Chevin et al. 2010). A previous study on *Tegula* spp., a similar intertidal gastropod, found a negative relationship between upper thermal tolerance and its plasticity (Stenseng et al. 2005). Accordingly, we hypothesized that limpets with higher upper thermal tolerances have reduced plasticity of heat tolerance.

2. MATERIALS AND METHODS

2.1. Site selection

Elkhorn Slough (36° 80' 86" N, 121° 78' 89" W), Inner Tomales Bay (38° 13' 33" N, 122° 89' 41" W), and San Francisco Bay (SF Bay; 37° 89' 28" N, 122° 44' 77" W) were chosen as the 3 collection sites (Fig. 1). Sites were chosen to best represent the range of environmental conditions to which *Lottia limatula* are exposed in northern California. Inner Tomales Bay (Marin County, California) is a small, shallow bay that experiences heavy seasonal temperature swings. This area also receives relatively warm freshwater influxes, creating a strong thermal and saline gradient between the inner and outer bay. Inner Tomales Bay and SF Bay were selected as warmer inland estuary sites, whereas Elkhorn Slough (Moss Landing, California) is closest to the outer coast and most exposed to the cold, marine regime.

2.2. Site temperature

Sea surface temperature (SST) and air temperature data (1 Jun 2016–30 Nov 2016) were downloaded from NOAA's National Estuarine Research Reserve System (NERRS) (<http://cdmo.baruch.sc.edu/>) and Coastal Observations and Monitoring Science (COMS) (<http://coastalobservations.sfsu.edu>), respectively. Tomales Bay SST data were collected and provided by

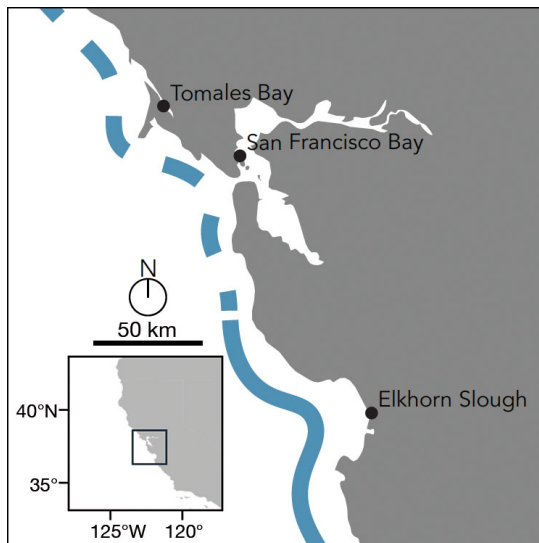


Fig. 1. Collection sites of limpets in northern California. Blue line: range of *Lottia limatula*; the population is generally continuous in southern California, but north of Santa Cruz, *L. limatula* are only found in locations where water temperatures are warmer (Test 1945)

the Grosholz Lab at the University of California at Davis. Temperature data points were collected every 15 min and used as raw data for the following calculations. The 6 highest daily temperature maximums were used to represent the maximum for the summer (1 Jun 2016–31 Aug 2016) and fall (1 Sep 2016–30 Nov 2016) seasons. Seasonal variances were calculated by averaging the squared differences from the daily mean temperature. Annual variance was calculated from the year prior to limpet collection (15 Nov 2015–15 Nov 2016).

2.3. Limpet collection and housing

Adult *L. limatula* were sampled between mid-November and early December 2016 at mid-intertidal zones (~0.6 m above mean lower low water) where they were most abundant. Limpets ($n = 66$) were collected from each site (permit: CA DFW SCP-13357) and transported to the laboratory, where they were equally divided among flow-through seawater aquaria of 3 acclimation temperatures: 13 ± 1 , 17 ± 1 , and $21 \pm 1^\circ\text{C}$ and held for 14–16 d, which has been demonstrated as being long enough for limpets to achieve steady-state plasticity in physiological phenotypes (Bjelde et al. 2015, Khlebovich 2017). The first acclimation temperature, 13°C , was based on the mean SST of the week of collection (15 Nov–22 Nov 2016; Elkhorn Slough: 13.5°C ; SF Bay: 15.3°C ; Tomales Bay: 12.2°C). The 17°C acclimation represented maximal 2016 summer SST of inland estuary sites (1 Jun–31 Aug 2016; Elkhorn Slough: 14.9°C ; SF Bay: 17.0°C ; Tomales Bay: 17.0°C), and the 21°C acclimation represented future summer SST of those sites based on IPCC's RCP8.5 Scenario 2100 forecast ($+4^\circ\text{C}$) (IPCC 2014). Limpets from all 3 sites were kept in the same tank for each acclimation temperature, separated by site with enclosures, and provided algae-covered cobbles from their collection locations for food. Limpets were monitored daily and dead limpets were removed promptly. Salinity was maintained at 29.1 ± 1.6 ppt.

L. limatula non-lethal upper thermal tolerance limits were evaluated using 3 metrics. These were foot muscle critical thermal maximum (CT_{max}) and 2 metrics of cardiac thermal tolerance: Arrhenius break temperature (ABT) and flatline temperature (FLT).

2.4. Foot muscle CT_{max}

Foot muscle CT_{max} was defined as the temperature at which the organism loses its neuromuscular ability

to grasp a vertical surface. Fallen limpets are more susceptible to predation or being washed away by waves, which have negative fitness consequences (Marshall et al. 2015); thus, this is an ecologically important metric. Other studies on mollusks and limpets have referred to the temperature of neuromuscular failure or detachment from substratum as the onset of heat coma temperature (HCT) or non-lethal thermal tolerance (Clarke et al. 2000, Sokolova & Pörtner 2003, Denny et al. 2006, Marshall et al. 2015, Miller et al. 2015, Chapperon et al. 2016). Limpets are known to use adhesive mucus to hold onto the substrate, but this strategy is primarily used during emersion in air (Smith 2002). Since limpets mostly use suction to grip onto surfaces just after moving (Smith et al. 1999), we were not concerned with mucus influencing our foot muscle CT_{max} data. Foot muscle CT_{max} was determined in limpets from Tomales Bay and Elkhorn Slough. We were unable to sample foot muscle CT_{max} for SF Bay limpets due to experimental limitations. Limpets were gently attached pointing downward on the vertical walls of 150 ml glass beakers filled with 100 ml of air-saturated seawater; beakers were temperature-controlled in a recirculating water bath. Limpets rested at their acclimation temperatures for 30 min before the $+4^{\circ}\text{C h}^{-1}$ heat ramp commenced. Consistent with similar studies, the heat ramp rate was chosen based on a realistic environmental rate (Tomanek & Somero 1999, Stenseng et al. 2005). Temperature inside the water bath was monitored using iButton thermochron dataloggers (Maxim Integrated™). To determine foot muscle CT_{max} , the time when limpets lost grip and fell off the vertical wall was matched to the temperature at that time recorded by the nearest iButton.

2.5. Cardiac thermal tolerance (ABT and FLT)

Two cardiac thermal tolerance metrics were assessed: ABT and FLT. ABT was defined as the temperature at which heart rate sharply declined (Dahlhoff & Somero 1993). We measured ABT to compare with other studies on cardiac thermal tolerances of mollusk and limpets (Stenseng et al. 2005, Bjelde et al. 2015, Drake et al. 2017). Since gastropods can survive well past their ABT, we recorded FLT, the temperature at which limpet hearts stopped beating (Stenseng et al. 2005, Polgar et al. 2015). Heart beats of limpets not used for foot CT_{max} determination were monitored as described previously (Bjelde & Todgham 2013, Bjelde et al. 2015). Between 18 and 24 h before heart rate monitoring, 2 holes, 2 mm in diameter, were drilled

into both sides of the limpet shell apex. Limpets were immediately returned to their original tanks for recovery. An hour before the heat ramp, 2 electrodes, made with 40 gauge ceramic-coated copper wire, were inserted in the holes to surround either side of the limpet heart. Electrodes were firmly glued in place on the shell with cyanoacrylate glue (Bob Smith Industries). Limpets were individually placed in glass Petri dishes fitted in wells of an aluminum block circulated with a temperature-controlled programmable water bath set to the acclimation temperature (Lauda, Lauda-Königshofen). Glass dishes were filled with 25 ml of seawater to completely immerse limpets. Impedance between the 2 electrodes was converted into voltage by UFI 2991 Impedance Converters and recorded with a PowerLab (ADInstruments) using LabChart 5 software (ADInstruments). Limpets rested for 30 min at their acclimation temperatures before $+4^{\circ}\text{C h}^{-1}$ heat ramps commenced. Heat ramps ran until limpet hearts stopped beating for 15 min. Heart rates (beats min^{-1}) and temperatures were averaged over consecutive 30 s intervals.

To measure ABT, Arrhenius plots were created by plotting the natural log of heart rates versus inverse temperatures (1000 K^{-1}). Robust linear regressions were fitted to the data before and after the sharpest inflection in the slope of heart rate using the 'robust-base' package (Maechler et al. 2019) in R v.3.2.4 (R Core Team 2016). The ABT was defined as the temperature at which the 2 regression lines intersect (Figs. S1 to S3 in the Supplement at www.int-res.com/articles/suppl/b028p113_supp.pdf). FLT was determined as the temperature at which the limpet heart stopped beating.

Once the heat ramps were completed, limpets were removed from their shells and dried for 48 h at room temperature. Dry body mass and shell mass (epibiota were removed) were determined to the nearest 0.01 g on an analytical balance (Mettler-Toledo).

2.6. Statistical analyses

We used a multi-model inference approach to estimate the proportion of limpet deaths during acclimation treatments and explore which variables most related to mortality (Zuur et al. 2009, Symonds & Moussalli 2011). In the 'nlme' package (Pinheiro et al. 2019), a full logistic regression model was run with binary outcome data (death:1, alive:0) as our response variable, and the following predictor variables as fixed effects: site, acclimation, and their interaction. Assumptions of normality, no multicollinearity, and

homogeneity of variances were met. A series of models representing all possible combinations of predictor variables was generated and subsequently compared to the full model using Akaike's Information Criterion (AIC), which is a measure of fit with a penalty on overfitting. Weights based on AIC were assigned to each model. A weighted averaged model was calculated from the set of top-ranked models, whose cumulative AIC weights were ≤ 0.95 , by averaging the parameter estimates in the 'MuMIN' package (Barton 2019).

The 2 hypotheses — limpet CT_{max} is positively correlated with their maximum habitat temperatures and limpets with higher CT_{max} have reduced plasticity — were separately evaluated for each metric (foot muscle CT_{max} , ABT, and FLT). A multi-model inference approach, similar to the one described before, was used to predict means of each acclimation-site group and explore which variables most related to CT_{max} . Generalized least squares models were run with CT_{max} as the response variable and the following predictor variables as fixed effects: site, acclimation temperature treatment, dry body weight, and their interactions. Number of ABTs was included as an additional predictor variable for ABT and FLT analyses because *Lottia* sp. have been previously documented to have multiple ABTs, which could result from active depression of heart rate in order to conserve energy during heat stress (Bjelde et al. 2015, Bjelde & Todgham 2013). Since we recorded limpets with only 1 or 2 ABTs, proportions of limpets with 2 ABTs were analyzed with a separate binomial logistic regression analysis. Assumptions of normality and homogeneity of variances were met using variance structures fixed along dry weight (variance of CT_{max} was proportional to dry weight). A linear regression between shell weight and dry body weight revealed that they were strongly collinear (Fig. 2). Thus, shell weight was dropped from our analysis, and dry body weight was chosen as the better variable representing limpet body mass. A weighted averaged model was calculated from the set of top-ranked models (cumulative sum ≤ 0.95) using AIC_{cr} , a version of AIC for small sample sizes. Site coefficients of the weighted averaged models were compared to explore the effects of site on CT_{max} . Coefficients of the interactions between site and acclimation were compared to explore differences of plasticity among sites.

Elkhorn Slough and SF Bay limpets tended to be larger than Tomales Bay limpets (Fig. S4 in the Supplement). Linear regressions were performed between dry body weight and CT_{max} metrics to determine if there was a general trend between body size and thermal tolerance for *L. limatula*. Dry body

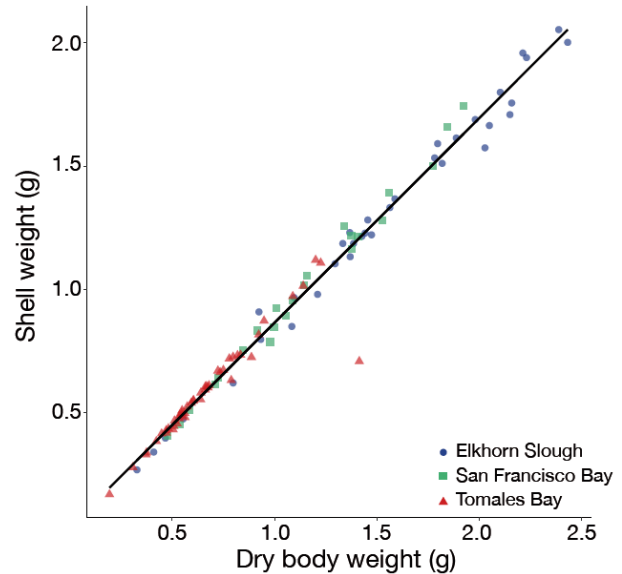


Fig. 2. Linear regression between shell weight and dry body weight of *Lottia limatula* (slope = 0.83, $F_{1,103} = 5141$, $p < 0.001$, $r^2 = 0.98$)

weight and site were found to be confounding variables for ABT and FLT. In order to evaluate the effects of site on ABT and FLT more confidently, the multi-model inference analyses were performed again on a subset of limpets with dry body weights between 1.0 and 2.0 g. This additional multi-model inference analysis used linear regressions with no variance structure and did not include dry body weight as a predictor variable.

Plasticity of limpets was additionally compared using the acclimation response ratio (ARR), the slope of the line describing the CT_{max} change based on the acclimation temperature difference (Claussen 1977, Gunderson & Stillman 2015). An ARR of 1 means that limpets increased their CT_{max} by 1°C for every 1°C rise in acclimation temperature. For each site and CT_{max} metric group, ARR was defined as the slope of the linear regression of all CT_{max} points collected between 13 and 21°C acclimation temperatures. Likelihood-ratio tests comparing the goodness-of-fit between the null and alternative models were used to further evaluate the hypothesis that limpets of higher CT_{max} have reduced plasticity (see Methods in the Supplement).

3. RESULTS

3.1. Site temperature

Compared to the other 2 sites, the Elkhorn Slough site had lower SST (18.4°C) and air temperature

Table 1. Maximum summer (1 Jun–31 Aug 2016) and fall (1 Sep–30 Nov 2016) air and sea surface temperatures (SST) at the 3 *Lottia limatula* collection sites. Air and SSTs were averaged daily; variances of each season were calculated by averaging the squared differences from the mean; annual variance was calculated from the year prior to limpet collection (15 Nov 2015–15 Nov 2016). SF Bay: San Francisco Bay; ND: no data

Site	SST					Air				
	Summer max. (°C)	Summer variance (°C ²)	Fall max. (°C)	Fall variance (°C ²)	Annual variance (°C ²)	Summer max. (°C)	Summer variance (°C ²)	Fall max. (°C)	Fall variance (°C ²)	Annual variance (°C ²)
Elkhorn Slough	18.4	0.5	19.1	0.8	0.9	22.5	1.1	31.0	5.0	5.7
SF Bay	19.6	0.3	19.2	0.8	4.4	28.6	2.1	32.3	7.8	8.1
Tomaes Bay	22.5	0.2	19.1	3.4	4.4	ND	ND	ND	ND	ND

(22.5°C) maxima for summer (Table 1). Of the 3 sites, Tomales Bay had the highest summer maxima for SST (22.5°C; Table 1). Tiburon, California of SF Bay, which latitudinally lies between Tomales Bay and Elkhorn Slough, had a moderate climate and summer SST maxima (19.6°C). Inner Tomales Bay SST had the greatest variability in temperature (annual variance: 4.4°C²; fall variance: 3.4°C²), Tiburon SST had moderate variability (annual variance: 4.4°C²; fall variance: 0.8°C²), and Elkhorn Slough SST had the least variability (annual variance: 0.9°C²; fall variance: 0.8°C²).

3.2. Mortality

Mortality, or the binomial probability of death, in limpets from all sites and at all acclimation conditions was best explained by 2 model predictions that had $\Delta\text{AIC} < 2$. The top-ranked model included acclimation and site as predictor variables while the second-best model included acclimation, site, and their interaction as predictor variables ($\Delta\text{AIC} = 1.8$). Averaged, this model (Table 2, $\Delta\text{AIC} = 0.5$) was strongly influenced

Table 2. Full averaged-models results of binomial *Lottia limatula* mortality data, with 13°C acclimation (Acc), Elkhorn Slough site, and their interactions as the intercept. SF Bay: San Francisco Bay. Statistically significant variables are in **bold text**

Variable	Estimate	SE	z-value	p-value
Intercept	-2.21	0.89	2.48	<0.05
17°C Acc	1.27	0.94	1.34	0.18
21°C Acc	2.86	1.09	2.63	<0.01
SF Bay	-0.35	1.06	0.33	0.74
Tomales	-0.99	1.40	0.70	0.48
17°C Acc × SF Bay	-0.38	0.99	0.39	0.70
21°C Acc × SF Bay	-0.69	1.31	0.53	0.60
17°C Acc × Tomales	-0.85	1.59	0.53	0.59
21°C Acc × Tomales	-0.98	1.69	0.58	0.56

by the 21°C acclimation condition. At 21°C, mortality was significantly higher than at the 2 other acclimation temperatures, with Elkhorn Slough limpets experiencing the largest increase in mortality (Fig. 3). Overall, Tomales Bay had the lowest mortality rates across acclimation conditions (Tomales Bay mortality: 0.11; SF Bay: 0.21; Elkhorn Slough: 0.35). Site effects were not statistically significant in the averaged model; however, their inclusion in the 2 separate top model predictions is important to note for future studies.

3.3. CT_{max} of foot muscle

Mean foot muscle CT_{max} ranged from 35 to 37.5°C across locations and acclimation conditions, with highest values occurring under warmer acclimation conditions (Fig. 4A). The foot muscle CT_{max} of Tomales Bay

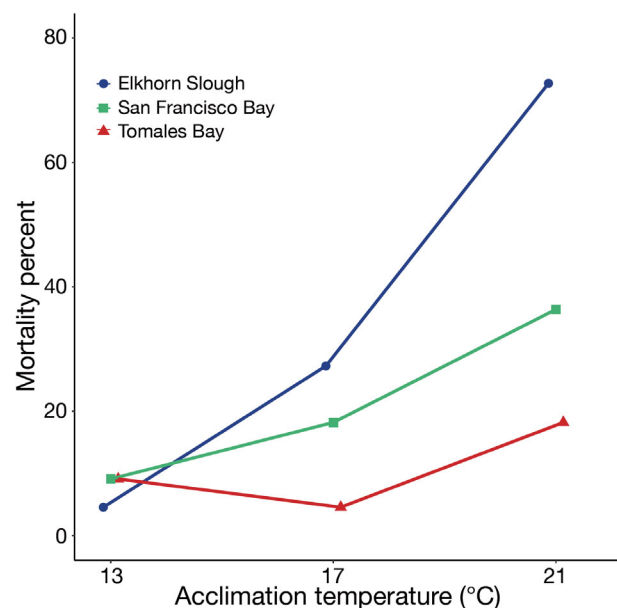


Fig. 3. Limpets from different collection sites varied in their survival across acclimation temperatures

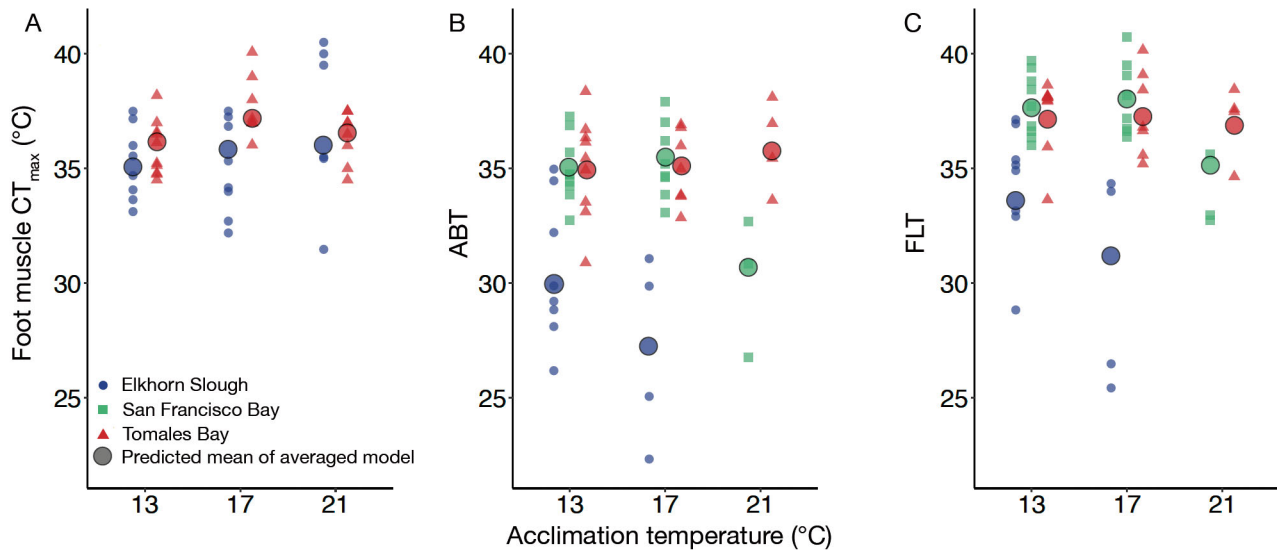


Fig. 4. (A) Foot muscle critical thermal maxima (CT_{max}), (B) Cardiac Arrhenius breakpoint temperature (ABT), and (C) cardiac flatline temperature (FLT) of *Lottia limatula* subjected to heat ramps of $+4^{\circ}\text{C h}^{-1}$. Smaller symbols: individual limpets; larger circles: averaged models' predicted means of treatment groups

limpets was 2.7°C higher than Elkhorn Slough limpets in 17°C -acclimated specimens (Fig. 4A); at 13 and 21°C acclimation temperatures there were no differences between populations. Variance of Tomales Bay limpets' foot muscle CT_{max} remained consistently low across the 3 acclimation temperatures, while the variance of Elkhorn Slough foot muscle CT_{max} increased with acclimation temperature (Fig. 4A). Since the predictor variables of acclimation, site, and their interaction did not have statistically significant estimates in the averaged model (Table 3A), they did not have any effect on foot muscle CT_{max} . Since site did not have an effect on foot muscle CT_{max} , these results did not support the hypothesis that limpet foot muscle CT_{max} is positively correlated with their maximum habitat temperatures. There was no relationship between foot muscle CT_{max} and dry body weight (Fig. 5A).

Tomales Bay limpets had lower foot muscle CT_{max} plasticity compared to Elkhorn Slough limpets (ARR: 0.07 ± 0.08 vs. $0.22 \pm 0.16^{\circ}\text{C}$ per 1°C rise in acclimation, respectively). We expected an interaction between site and acclimation temperature on foot muscle CT_{max} in the statistical model. However, none of the predictor variables had statistically significant effects (Table 3A), indicating that foot muscle CT_{max} plasticity was not statistically different between Tomales Bay and Elkhorn Slough limpets. These results also did not support the hypothesis that limpets with higher foot muscle CT_{max} have lower plasticity (Table 2A); likelihood-ratio test results also supported these findings (see supplemental Results and Tables S1 & S2A in the Supplement).

3.4. ABT

Mean cardiac ABTs ranged from 27 to 36°C in limpets from across acclimation temperatures and collection sites (Fig. 4B). Limpets from Elkhorn Slough had 5 and 8°C lower ABTs than limpets from the other 2 sites at 13 and 17°C acclimation temperatures, respectively (Fig. 4B). Acclimation to 21°C reduced ABT of SF Bay limpets by about 5°C , whereas ABT of Tomales Bay limpets was unaffected and remained close to values at 13 and 17°C acclimation temperatures (Fig. 4B). The averaged model predicted similar mean results to the observed arithmetic means (on average the absolute difference was only 0.24°C) for all 3 acclimation temperatures (Fig. 4B). The averaged model showed that site had strong positive effects on ABT, and confirmed that the higher ABTs in Tomales Bay and SF Bay limpets were statistically significant (Table 3B). Since both Tomales Bay and SF Bay sites had higher summer maximum temperatures than Elkhorn Slough, these results partially supported our first hypothesis that limpet ABTs are positively correlated with their maximum habitat temperatures. Based on their high p-values, number of ABTs, dry weight, and their interactions had little relation to ABT (Table 3B). A binomial logistic regression revealed that the proportion of limpets with 2 ABTs did not have a statistically significant relationship ($p > 0.05$) with site, acclimation treatment, or their interactions (Fig. S5 in the Supplement).

Table 3. Full averaged-models results of 3 metrics of *Lottia limatula* heat tolerance. (A) Foot muscle CT_{max}: 13°C acclimation (Acc), the Elkhorn Slough site, and their interactions as the intercept; (B) Arrhenius breakpoint temperature (ABT): 13°C acclimation, Elkhorn Slough site, 1 ABT, their interactions with other variables, and 21°C Acc × SF Bay as the intercept (there were no 21°C-acclimated Elkhorn Slough limpets sampled for ABT); (C) flatline temperature (FLT): 13°C acclimation, Elkhorn Slough site, 1 ABT, their interactions with other variables, and 21°C Acc × SF Bay as the intercept (there were no 21°C-acclimated Elkhorn Slough limpets sampled for FLT). Statistically significant predictor variable estimates are in **bold** text

Variable	Estimate	SE	z-value	p-value
(A) Foot muscle CT_{max}				
Intercept	34.39	1.16	28.97	<0.001
17°C Acc	2.20	1.55	1.39	0.16
21°C Acc	-0.26	1.09	0.23	0.81
Dry weight	0.78	0.84	0.90	0.36
Tomales	2.46	2.06	1.18	0.24
17°C Acc × dry weight	-1.58	1.17	1.32	0.19
21°C Acc × dry weight	0.93	1.01	0.89	0.37
Tomales × dry weight	-2.13	2.06	1.02	0.30
(B) ABT				
Intercept	26.59	2.31	11.34	<0.001
17°C Acc	-0.85	4.40	0.19	0.84
21°C Acc	3.18	8.05	0.39	0.69
Dry weight	2.89	2.35	1.22	0.22
SF Bay	8.18	3.60	2.26	0.02
Tomales	7.12	2.32	3.01	0.003
17°C Acc × dry weight	-1.92	2.70	0.71	0.48
21°C Acc × dry weight	-0.39	6.16	0.06	0.95
17°C Acc × SF Bay	2.69	2.59	0.99	0.32
21°C Acc × Tomales	6.60	4.41	1.58	0.14
17°C Acc × Tomales	2.04	2.91	0.69	0.49
ABT Number	-0.68	1.31	0.52	0.60
17°C Acc × ABT number	0.90	1.72	0.52	0.60
21°C Acc × ABT number	-1.48	4.3	0.34	0.74
Dry weight × SF Bay	-1.95	3.11	0.62	0.53
Dry weight × Tomales	-0.86	2.31	0.37	0.72
(C) FLT				
Intercept	32.57	1.53	20.90	<0.001
SF Bay	5.03	1.75	2.83	<0.01
Tomales	3.68	1.71	2.12	0.03
17°C Acc	-2.24	2.80	0.80	0.42
21°C Acc	1.43	5.00	0.28	0.78
Dry weight	0.94	1.34	0.70	0.48
17°C Acc × SF Bay	2.59	2.82	0.91	0.36
21°C Acc × Tomales	2.52	2.79	0.90	0.37
17°C Acc × Tomales	2.48	2.72	0.90	0.37
ABT number	-0.32	1.10	0.29	0.77
17°C Acc × ABT number	0.27	0.96	0.28	0.78
21°C Acc × ABT number	-0.62	2.66	0.23	0.82
17°C Acc × dry weight	-0.39	1.30	0.28	0.78
21°C Acc × dry weight	-1.00	3.53	0.28	0.78
Dry weight × SF Bay	-0.39	1.22	0.32	0.75
Dry weight × Tomales	0.57	1.66	0.34	0.74
ABT number × SF Bay	-0.21	1.01	0.21	0.83
ABT number × Tomales	-0.20	0.91	0.22	0.83

Though there was a general statistically significant decline in ABT with increasing dry weight (Fig. 5B; $F_{1,50} = 4.62$, $p < 0.05$, $r^2 = 0.08$), the averaged model for limpets between 1.0 and 2.0 g confirmed that Elkhorn Slough limpets had lower ABTs despite differences in mass among sites (Table S3A in the Supplement). ABT plasticity was highest in Tomales Bay limpets (ARR: $0.10 \pm 0.14^\circ\text{C}$ per 1°C rise in acclimation), and was negative in limpets from SF Bay ($-0.44 \pm 0.17^\circ\text{C}$ per 1°C rise in acclimation) and Elkhorn Slough ($-0.85 \pm 0.52^\circ\text{C}$ per 1°C rise in acclimation), suggesting that the warmer acclimation temperatures were beyond the thermal optima for those limpets. The full averaged model showed that the interaction between acclimation temperature and site did not have a statistically significant effect on ABT (Table 3B); however, likelihood-ratio tests showed that the ABT acclimation responses in limpets from Tomales Bay and SF Bay were statistically significantly more positive than in limpets from Elkhorn Slough (Table S2B). Since Tomales Bay limpets had the highest ABT and the largest positive ABT acclimation response, these results contradicted our second hypothesis that limpets with higher ABT have lower plasticity.

3.5. FLT

Mean FLTs were generally higher than ABTs, ranging from 30 to 38°C in limpets from across acclimation temperatures and collection sites (Fig. 4C). The same pattern across acclimation temperatures and collection sites was observed for FLT as was observed in ABT; limpets from Elkhorn Slough had 3 and 8°C lower FLTs than the other 2 sites at 13 and 17°C acclimation temperatures, respectively (Fig. 4C). Acclimation to 21°C reduced the FLT of limpets from SF Bay by 4°C, but did not affect the FLT of limpets from Tomales Bay (Fig. 4C). Similar to ABT results, the averaged model fit predicted similar FLT means to the observed arithmetic means (on average the absolute difference was only 0.42°C) for all 3 acclimations (Fig. 4C). There was a strong effect of collection site on FLTs in the averaged model (Table 3C), confirming that FLTs of Tomales Bay and SF Bay limpets were statistically higher than that of Elkhorn Slough limpets. Since both Tomales Bay and SF Bay sites had higher summer maximums than the Elkhorn Slough site, these results partially support our first hypothesis that limpet FLTs are positively correlated with their maximum habitat temperatures. The number of ABTs, dry weight, and their interactions had little relation to FLT (Table 3C).

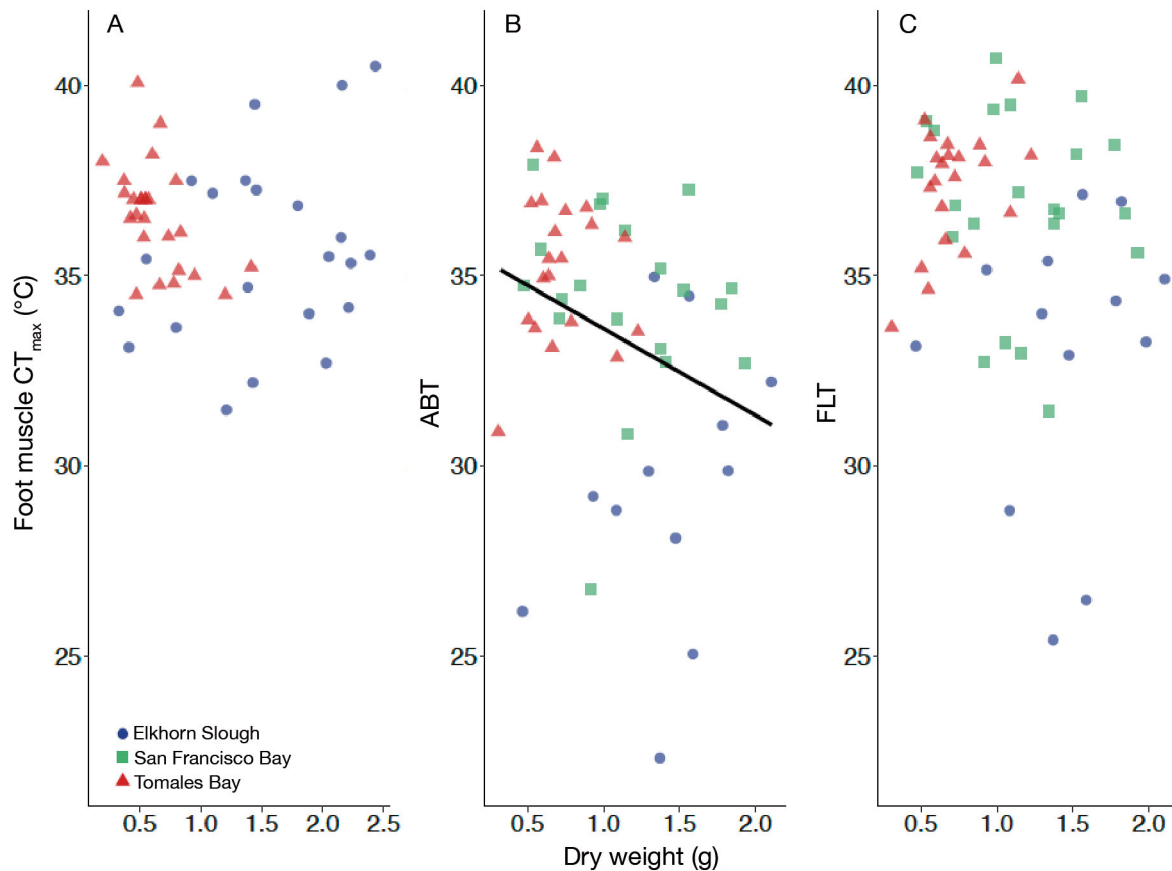


Fig. 5. (A) Foot muscle critical thermal maxima (CT_{max}), (B) Cardiac Arrhenius breakpoint temperature (ABT), and (C) cardiac flatline temperature (FLT) of *Lottia limatula* plotted against dry body weight. Only the linear regression between ABT and dry weight was statistically significant ($F_{1,50} = 4.62$, $p < 0.05$, $r^2 = 0.08$)

There was no statistically significant trend between FLT and dry weight (Fig. 5C). An averaged model restricted to limpets of 1.0–2.0 g body mass revealed that limpets from Elkhorn Slough had lower FLTs despite differences in mass among sites (Table S3B).

Plasticity of FLTs followed a similar pattern to plasticity of ABTs; limpets from Tomales Bay had the highest positive FLT plasticity ($-0.03 \pm 0.12^\circ\text{C}$ per 1°C rise in acclimation), and FLT plasticity was negative in limpets from SF Bay and Elkhorn Slough (-0.50 ± 0.13 and $-1.03 \pm 0.49^\circ\text{C}$ per 1°C rise in acclimation, respectively), following what was observed for ABT. The averaged model indicated that site, but not acclimation temperature, had a positive effect on FLT plasticity (Table 3C), and likelihood-ratio tests showed that the FLT acclimation responses in limpets from Tomales Bay and SF Bay were statistically significantly more positive than in limpets from Elkhorn Slough (Table S2C).

4. DISCUSSION

This study compared the plasticity and absolute levels of upper thermal tolerance of foot muscle and cardiac function in the limpet *Lottia limatula* from sites with different thermal histories. Mortality that occurred during thermal acclimation varied in limpets from across collection locations, as did absolute levels of thermal tolerance. Relationships of thermal tolerance plasticity and thermal habitat varied across collection sites.

4.1. Upper thermal tolerance differences reflect thermal habitat across collection sites

Cardiac ABT and FLT values supported the hypothesis that *L. limatula* from warmer sites have higher CT_{max} ; limpets from the warmer sites (Tomales Bay, SF Bay) had higher ABTs and FLTs across acclima-

tion temperatures compared to limpets from the cooler site (Elkhorn Slough). As limpets from Tomales Bay experience higher maximal habitat temperatures than those from SF Bay, we expected the ABT and FLT Tomales Bay limpets to be higher than that of SF Bay limpets, but that difference was only observed at the highest acclimation temperature (21°C). The correspondence of species' thermal tolerances with maximal temperatures experienced in their environments has been well documented in many other studies on marine taxa (Stillman & Somero 1996, Tomanek & Somero 1999, Tomanek & Helmuth 2002). Miller et al. (2015) showed that *Lottia* spp. foot muscle CT_{max} was positively correlated with intertidal zone height. For example, *L. digitalis* lives higher in the intertidal zone than *L. limatula* (Lindberg 1981), and has an ABT 5°C higher than that of *L. limatula* (Bjelde & Todgham 2013).

Lower ABT and FLT values of Elkhorn Slough limpets may be attributed to a combination of site and their larger mean body size (Fig. S4). While the site and dry weight variables were confounding, Elkhorn Slough limpets had lower cardiac CT_{max} values than Tomales Bay limpets and SF Bay limpets at the same mass (Fig. 5). Furthermore, dry weight did not show significant effects on cardiac CT_{max} in the averaged models for all limpets or for the subset of limpets within 1.0–2.0 g. These results suggest that site likely has a greater impact on cardiac CT_{max} than body size.

4.2. No evidence for adaptive plasticity

There was no evidence that increased acclimation temperature resulted in increased temperature tolerance for foot or heart function, indicating that *L. limatula* are unlikely to benefit from plasticity to reduce the impact of increased habitat temperature. Increasing acclimation temperature from 13 to 17°C had a large negative impact on heat tolerance in the least heat tolerant limpets (those collected from Elkhorn Slough), whereas limpets from SF Bay had a similar decline in tolerance between 17 and 21°C, suggesting that those limpets were at temperatures warmer than their optimal range. These negative ARR and high cardiac variances of Elkhorn Slough and SF Bay limpets are indicative of physiologically damaging acclimation conditions and insufficient means to sustain responses to stress (Gundersen & Stillman 2015), which is supported by the mortality of *L. limatula* observed during exposure to elevated temperatures (Fig. 3). A similar result was found in a study on the cardiac plasticity of the water diving

beetle *Deronectes* spp. (Calosi et al. 2008, Overgaard et al. 2011).

While limpets from Tomales Bay (which had the high cardiac ABT and FLT values) did not suffer a decline in heat tolerance with increasing acclimation temperature, they also did not have the capacity to increase their thermal tolerance. Perhaps Tomales Bay limpets, which experience a relatively variable thermal environment seasonally and annually during their 6–10 yr lifespan (estimated from Dawson et al. 2014), can endure fluctuating temperatures and maintain their high thermal tolerance, either through genetic differentiation or field acclimatization. Future genetic work is needed to confirm that these 3 populations (Tomales Bay, SF Bay, Elkhorn Slough) are reproductively isolated populations, which would expand the inferences regarding local adaptation in these limpets.

4.3. Differences in heat tolerance of foot and heart muscle differed among sites

We observed similar levels of heat tolerance between foot and cardiac muscle (i.e. FLT) in limpets from Tomales Bay, whereas foot function was much more heat tolerant than cardiac function in limpets from Elkhorn Slough, as the foot CT_{max} did not decline with increasing acclimation temperature in contrast to cardiac FLT. It is possible that the differences between foot and cardiac thermal tolerance lie in physiological differences between these muscles. The limpet foot is a complicated muscular structure which is not as functionally reliant on aerobic metabolism as cardiac muscle. The limpet foot mechanism uses energetically economic smooth muscles to lock into the 'catch' state, which clamps the foot into suction with the substrate (Frescura & Hodgson 1990, Smith 1991, Galler et al. 2010). Compared to cardiac tissue, limpet foot muscles have low mitochondrial density and high anaerobic capacities (Marshall & McQuaid 1989, Morley et al. 2009, Suda et al. 2015). If limpets from Elkhorn Slough were in a physiologically compromised state at warmer acclimation temperatures, they may have lacked adequate energy to maintain the aerobic demands of cardiac tissue, whereas the low energetic demands of catch smooth muscle could have been better maintained. It is also possible that the larger mean body sizes of Elkhorn Slough limpets (Fig. S4) could have contributed to increased strain on the cardiac pump, though we do not have any evidence to test that hypothesis. If physiological energetics play a role in maintaining ther-

mal tolerance of tissues, we would expect energetic state (e.g. adenylate charge) to differ between foot and cardiac muscle differently between limpets from the Tomales Bay and Elkhorn Slough. It would be interesting to know if limpets from those 2 sites differ in other energetic traits (e.g. enzyme levels, maximal performance levels, growth rates, fecundity).

4.4. Implications for limpets in nature

We compared thermal tolerances of limpets acclimated to summer maximal average habitat temperatures (17–18°C across sites) in order to make environmentally relevant inferences about the thermal ecology of limpets at present during the warmest seasons. We used low-resolution thermal records to infer habitat temperature, but those data are not likely entirely accurate measures of the thermal microhabitats of *L. limatula* across their spatial distribution range. The intertidal zone experiences complex spatiotemporal thermal patterns that are considerably influenced by even fine-scale features such as substratum angle and slope aspect (Helmuth & Hofmann 2001). The stable temperature, submerged acclimation conditions commonly used in thermal acclimation studies, including ours, do not accurately represent the environmental complexity of the intertidal zone, specifically the interaction of tide and weather that leads to high variability in temperature, salinity, desiccation, and oxygen.

Since acclimation responses of *L. limatula* thermal tolerance traits were in contrast to expectations, such as what has been described for intertidal crabs and snails (Stillman 2003, Stenseng et al. 2005, Bjelde & Todgham 2013), it would be worth investigating levels and plasticity of thermal performance traits at a lower range of acclimation temperatures, as well as acclimation to variable temperatures. Under more naturalistic acclimation conditions, physiological shifts may vary (Paganini et al. 2014, Gunderson et al. 2016). *L. digitalis* acclimated under repeated emersion increase their heat tolerances 4.5°C more than submerged limpets (Drake et al. 2017), and *L. limatula* in their native habitat may be able to increase their heat tolerance beyond what we report. Intertidal zone organisms adapted to fluctuating thermal environments may be under physiological stress during acclimation to sustained high temperatures (Dowd et al. 2015), which could explain the high mortality of Elkhorn Slough limpets in the 21°C acclimation even though 21°C is not near their thermal tolerance limits. Constant immersion may have resulted

in lower thermal tolerances (ABT and FLT) during acute heat exposure in Tomales Bay and SF Bay limpets. In a similar study on *L. digitalis*, Bjelde & Todgham (2013) found that emersed limpets on average had ABTs 3°C higher than immersed limpets. Though not exactly similar to natural conditions, our acclimation conditions at least represent average habitat conditions and allow our results to be comparable to related studies with similar acclimation conditions (Stillman 2003, Stenseng et al. 2005, Bjelde & Todgham 2013, Armstrong et al. 2019).

5. CONCLUSIONS

We have demonstrated that intraspecific variation in thermal physiology exists in individuals from locations with different thermal conditions. Differences in heat tolerance and plasticity could be due to acclimatization (i.e. plasticity), local adaptation, or both. Understanding the mechanisms by which *L. limatula* from Tomales Bay achieve higher heat tolerance than limpets from other sites may provide novel inferences of the potential for populations of intertidal zone organisms to respond to climate change.

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