



Symposium Article

Latitudinal Clines in Temperature and Salinity Tolerance in Tidepool Copepods

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Abstract

Local adaptation has been understudied in marine systems, but might be expected to be pronounced in the tidepool copepod *Tigriopus californicus*, which has a broad geographic range and extremely restricted dispersal. Tolerance to temperature and salinity was assessed in 14 populations over a 20° latitudinal range. Adaptive differentiation to temperature and salinity was found at scales as low as 5.6 km. Latitudinal clines were significant, with northern populations being more tolerant of low salinity and less tolerant of high temperature and high salinity. Both temperature and salinity tolerance were more closely associated with long-term thermal maxima than with long-term precipitation data. Hyperthermal and hyposmotic tolerance were inversely correlated, a pattern that could potentially slow adaptation to future conditions. Together, these studies of intraspecific geographic patterns in resistance to multiple stressors are important in predicting how environmental change may effect range shifts and local extinctions.

Subject area: Molecular adaptation and selection

Key words: local adaptation, salinity stress, thermal stress, *Tigriopus californicus*

Introduction

Local adaptation is the evolution of traits optimized for their local environment, such that resident genotypes have higher fitness in their local habitats than do genotypes derived from other habitats (Williams 1966; Kawecki and Ebert 2004). The pattern and process of local adaptation have been studied extensively in terrestrial and aquatic habitats (e.g., DeMeester 1996; Hufford and Mazer 2003). Considerably less attention has been given to local adaptation in marine habitats, in part due to the long-standing belief that gene flow in the ocean tends to be too extensive for adaptive differences between populations to accumulate. This paradigm has gradually changed over recent decades due largely to molecular data showing population genetic structure even in species with high dispersal potential (e.g., Edmands et al. 1996; Sotka et al. 2004, Palumbi 2004). The realization that marine populations are

often less connected than previously thought, combined with evidence that coastal habitats are less homogenous than previously thought (e.g., Menge et al. 1997; Feely et al. 2008), has led to increased interest in local adaptation in marine organisms. Recent studies (e.g., Sotka 2005; Conover et al. 2006; Marshall et al. 2010; Sanford and Kelly 2011; Gleason and Burton 2016) have shown adaptive divergence among populations for a variety of marine taxa, including those with planktonic dispersal, suggesting that local selective forces can be strong enough to counteract ongoing gene flow.

Better understanding of local adaptation is critical for effective management of marine environments, including the design of marine reserve networks and optimal strategies for stock enhancement and aquaculture. Information on adaptive divergence is particularly crucial for predicting impacts of climate change and improving climate

change models, which often assume that all populations of a species have the same range of tolerance (Ikeda et al. 2016). Such an assumption may underestimate extinction risk if individual populations have a smaller tolerance range than the species as a whole (Sanford and Kelly 2011). Incorporating information on intraspecific genetic and phenotypic variation can also greatly improve our ability to predict range shifts induced by environmental change (Ikeda et al. 2016; Marcer et al. 2016). Predictions often focus on alterations in individual environmental factors, yet the complexity of global climate change necessitates better understanding of how organisms respond to multiple stressors (Boyd et al. 2015; Deutsch et al. 2015).

The rocky intertidal is arguably “one of the most stressful habitats on earth” (Miller et al. 2009), making it a particularly interesting place to study adaptive differentiation. Due to steep environmental gradients (temperature, salinity and desiccation stress, for example) intertidal organisms frequently live at or near their physiological limit (e.g., Foster 1971; Boese et al. 2005; Dong et al. 2011). Indeed, environmentally induced latitudinal range shifts in some intertidal species (Barry et al. 1995; Sagarin et al. 1999; Jones et al. 2009, 2010) are several times greater than those reported from terrestrial habitats (Parmesan and Yohe 2003; Somero 2012, Pinsky et al. 2013).

The tidepool copepod *Tigriopus californicus* lives in the particularly harsh habitat of supralittoral splash pools, where it can experience temperatures greater than 40 °C and salinities as high as 139 ppt (Powlick 1999; Kelly et al. 2012). This species has a number of advantages for studying local adaptation to environmental stress. Unlike many marine organisms, *T. californicus* is easily raised in the laboratory over many generations (e.g., Pritchard et al. 2013), allowing genetic adaptation to be distinguished from plastic acclimation (e.g., Sun et al. 2014). Several factors may favor adaptive differentiation in this species. First, decades of work reveal extremely restricted gene flow, with fixed genetic differences between populations on rocky outcrops separated by even a few kilometers of sandy beach (e.g., Burton et al. 1979; Edmands 2001; Willett and Ladner 2009; Peterson et al. 2013). Second, the species extends from Alaska (Dethier 1980) to central Baja California (Ganz and Burton 1995), spanning a broad range of physical factors such as temperature, salinity, oxygen concentration, pH and chemical contamination. Third, the species has no dormant or resting stages (Edmands and Deimler 2004), thereby forcing adaptation to the extreme physical conditions of its splash pool habitat.

Previous work on *T. californicus* has indeed shown local adaptation to high temperature, with higher tolerance at lower latitudes (Willett 2010). Kelly et al. (2012) found a similar cline in upper thermal tolerance, and used a selection experiment to demonstrate limited potential for the evolution of increased tolerance. Geographic patterns in salinity tolerance have received much less attention in this and other species. In this study we assessed tolerance to both temperature and salinity, factors that frequently co-vary, across a broad geographic sample of *T. californicus* populations.

Materials and Methods

Population survey of thermal and salinity tolerance

T. californicus were collected at 14 locations: 1) Wizard, BC, Canada (48°51'28"N, 125°09'38"W); 2) Friday Harbor Labs, WA, USA (48°32'46"N, 123°0'40"W); 3) Sunset Beach, WA, USA (48°30'14"N, 122°41'25"W); 4) Strawberry Hill, OR, USA

(44°15'16"N, 124°6'45"W); 5) Santa Cruz, CA, USA (36°56'58"N, 122°2'48"W); 6) Catalina Island, CA, USA (33°26'48"N, 118°29'6"W); 7) Abalone Cove, CA, USA (33°44'15"N, 118°22'30"W); 8) Royal Palms, CA, USA (33°42'58"N, 118°19'11"W); 9) La Jolla, CA, USA (32°50'26"N, 117°16'56"W); 10) San Diego, CA, USA (32°44'47"N, 117°15'18"W); 11) Punta Morro, BCN, Mexico (31°51'41"N, 116°40'7"W); 12) Los Morros Colorados, BCN, Mexico (29°22'59"N, 115°W); 13) El Cuervito, BCN, Mexico (29°18'50"N, 114°54'35"W); 14) Playa Altamira, BCN, Mexico (28°31'45", 114°5'16"W).

Before tolerance assays began, animals were maintained in the laboratory for at least two generations in incubators held at 20 °C with a 12-h light, 12-h dark cycle. All tolerance assays were done on adult males, as males have been found to be more sensitive than females to multiple environmental stressors (Willett 2010; Kelly et al. 2012; Foley 2017). Further, female stress tolerance may fluctuate due to maternal transfer during egg development (Raisuddin et al. 2007). Maternal transfer can increase a female's susceptibility to stress following the release of eggs or increase their tolerance as they pass on a portion of a chemical stressor to the egg sac relieving their own body burden.

Median lethal temperature (LT₅₀) was assayed in a thermal cycler (no heated lid) with one copepod plus 200 µl 3x filtered seawater in each tube of an 8-well strip tube. The thermocycler was programmed to ramp up from 20 °C to the target temperature in 10 increments over 2h, then hold for 1h at the target temperature and then hold at 20 °C for 2 h. Animals were then scored as dead if they did not respond to gentle stimulus with a probe. Assays were repeated at increments of 1 °C until complete mortality occurred. For assays of median lethal salinity (LS₅₀) solutions were made using distilled water and Instant Ocean (Spectrum Brands). Seven concentrations were used to measure low salinity tolerance (1–25 ppt, at 5 ppt increments) and eight concentrations were used to measure high salinity tolerance (100–135 ppt, at 5 ppt increments), using a salinity meter with appropriate range. For each concentration, one adult male plus 200 µl instant ocean solution was placed in each tube of an 8-well strip tube and held at 20 °C for 1 h. Copepods were again scored as dead if they did not respond to gentle stimulus with a probe. Sets of 8 males were tested at increasingly stressful concentrations until complete mortality occurred. LC₅₀ and LS₅₀ were calculated using the package MASS in R (R Core Team 2014) to fit curves and estimate median lethality, SE and 95% CI.

Relationships among tolerance measurements (high temperature, high salinity and low salinity), latitude and climate variables were tested in R using both linear and quadratic regression. For the nearest weather station to each collection site, which includes sites in Canada, the United States and Mexico, climate data for average maximum monthly temperature and average maximum monthly precipitation were obtained from WolframAlpha (www.wolframalpha.com) for dates spanning 1954 to 2017.

Temperature monitoring in the field

Temperature monitoring was conducted at three locations used in the population survey above: Santa Cruz (SC), Catalina Island (CAT) and San Diego (SD). Monitoring was done during the summer of 2015, from June to August. Temperature measurements were made using iButton thermometers (Maxim Integrated, US), which were waterproofed using Plasti-Dip (Plasti Dip International, US). This method of waterproofing has been proven to have minimal influence on temperature readings (Roznik and Alford 2012). The iButtons are

reported to have temperature measurement resolution of 0.5 °C with an accuracy ± 1 °C between -40 °C and $+85$ °C (Maxim Integrated, US). To confirm accuracy, monitoring trials were done in temperature controlled environments: 10 °C, 15 °C, 20 °C, and 27 °C. The accuracy was confirmed to be within the range of ± 1 °C. iButtons were originally placed in 3 separate locations at each sampling site, with the exception of Catalina Island where only 2 pools inhabited by copepods were found. However, loggers from only 2 pools per site were successfully recovered for the full duration of the monitoring period. Chosen pools were either permanent or semi-permanent (present for most of the year) and continually inhabited by *T. californicus* based on previous observations and confirmed during each iButton deployment event. The iButtons were fastened to the deepest part of the tide pool by marine epoxy (Pettit Marine Paint, US). SC tidepool #1 was approximately $0.3 \times 0.3 \times 0.5$ m and tidepool #2 was approximately $1.0 \times 0.5 \times 0.5$ m. CAT tidepool #1 was approximately $0.2 \times 0.6 \times 0.1$ m and tidepool #2 was approximately $1.6 \times 0.4 \times 0.1$ m. SD tidepool #1 was approximately $5.0 \times 4.0 \times 0.5$ m and tidepool #2 was approximately $2.0 \times 0.33 \times 0.7$ m. These are the approximate dimensions during a single sampling event. However, the volume of tidepools can rapidly change depending on a number of factors, such as whether measurements are made during high or low tide where the level of inundation and evaporation are potentially very different and result in different tidepool volumes. The tidepools at SC and SD are qualitatively lighter in color, and are made of sandstone. Catalina tidepools are made of a darker volcanic rock, basalt.

Air temperature was obtained from the National Oceanographic and Atmospheric Administration's (NOAA) National Climatic Data Center (www.ncdc.noaa.gov). Temperature stations closest to our sites with the highest coverage of data available were selected. The climate stations used were: 1) Santa Cruz (GHCND:USC00047916; $36^{\circ} 59' 25.7994''$ N, $121^{\circ} 59' 27.96''$ W), which is 6.75 km from the sampling site, 2) San Diego (GHCND:USC00047741; $32^{\circ} 46' 1.92''$ N, $117^{\circ} 13' 32.88''$ W), 3.57 km from the sampling site and 3) Catalina Island (GHCND:USW00023191; $33^{\circ} 24' 18''$ N, $118^{\circ} 24' 56.9874''$ W), which is 7.91 km from the sampling site. A nested two-way ANOVA was used to test for significant differences between sites and between air and water temperatures. Likewise, differences between individual pools at each site were analyzed with a nested two-way ANOVA.

Average rate of temperature increase during the monitoring period was calculated as the temperature difference between the daily minimum and the daily maximum temperatures, divided by the number of hours between the daily minimum and the daily maximum temperatures. The smallest monitored pool by volume was selected for rate analysis to illustrate the upper bounds of thermal stress these populations potentially experience. Due to several hours throughout the early morning and afternoon sharing the same temperature, the hour closest to the temperature change was selected as the boundary of the rate measurement. A one-way ANOVA was used to determine significant differences in rates and post hoc Bonferroni adjusted *t*-tests were used to determine significant differences between sites.

Salinity monitoring in the field

At the Catalina (CAT) site, salinity was measured in 3 pools (2 of which were inhabited by *T. californicus*) between June and August 2014. Measurements were made using a hand-held refractometer in the bottom center of each pool. The maximal readout for the refractometer (Vee Gee Scientific, model A366ATC) was 100 ppt.

Results

Relationships among tolerance, latitude and climate

Tolerance to high temperature, high salinity and low salinity was measured in *T. californicus* sampled from fourteen locations on the west coast of North America, after a minimum of two generations under common garden conditions (Figure 1). For all three stressors, divergent levels of median lethality were found between populations separated by as little as 5.6 km, as determined by non-overlapping confidence intervals, a conservative metric of differences in median lethality (Wheeler et al. 2006). Median lethality levels in the 14 populations were highly divergent, spanning >4 °C for high temperature, >30 ppt for high salinity and >14 ppt for low salinity. Stress resistance was significantly correlated with latitude, with northern populations showing lower tolerance to high temperature (Figure 1a), lower tolerance to high salinity (Figure 1b) and higher tolerance to low salinity (Figure 1c). All three tolerance measurements were more closely associated with average maximum monthly temperature than with average maximum monthly precipitation. This relationship was significant for high temperature tolerance (Figure 1d) but marginally non-significant for high salinity tolerance (Figure 1e) and low salinity tolerance (Figure 1f). Tolerance to high temperature and low salinity were positively correlated (linear $R^2 = 0.486$, $P = 0.004$), indicating a tradeoff in tolerance. No significant correlations were found between tolerance of high temperature and high salinity (linear $R^2 = 0.147$, $P = 0.176$) or high and low salinity (linear $R^2 = 0.013$, $P = 0.696$). Average maximum monthly temperature was negatively associated with latitude (quadratic $R^2 = 0.913$, $P = 1.44e-06$) while average maximum monthly precipitation showed a positive but marginally non-significant association with latitude (linear $R^2 = 0.523$, $P = 0.053$). The relationship between average maximum monthly temperature and average maximum monthly precipitation was negative but non-significant (linear $R^2 = 0.219$, $P = 0.092$).

Field measurements of temperature and salinity

During summer 2015, tidepool water temperatures were monitored at SC, CAT and SD, and air temperatures were obtained from the nearest NOAA (U.S.) climate station (Figure 2). Daily maximum tidepool temperatures, assessed by two-way repeated measures ANOVA, show significant differences among the three sites ($F_{2,365} = 319.29$, $P < 0.001$) as well as between pools within sites ($F = 29.53_{2,365}$, $P < 0.001$). Daily average warming rate was assessed for the smaller pool at each location (pool 1 at SC and CAT, pool 2 at SD) because small pools are expected to generate greater warming stress, and *T. californicus* are known to have extensive gene flow among pools within rock outcrops (Burton and Swisher 1984) so effects of selection in one pool can be propagated to other local pools. One-way ANOVA showed significant differences in warming rate among locations ($F_{2,185} = 39.98$, $P < 0.001$). Post hoc Bonferroni pair-wise comparisons showed that all rates are significantly different from each other ($P < 0.001$), with the highest warming rate in the geographically intermediate location CAT, followed by southern location SD, followed by northern location SC. Air temperature was compared to tidepool temperature, using the larger tidepool monitored at each site because the larger pool was expected to be more buffered against temperature swings, making this a more conservative approach to testing differences between air and tidepool temperature. Paired, two-tailed *t*-tests of maximum daily temperatures showed highly significant differences at the SC ($t_{61} = -12.90$, $P < 0.001$), CAT ($t_{61} = 14.60$, $P < 0.001$), and SD ($t_{61} = 13.81$, $P < 0.001$) locations. At the southern locations CAT and SD, maximum daily tidepool

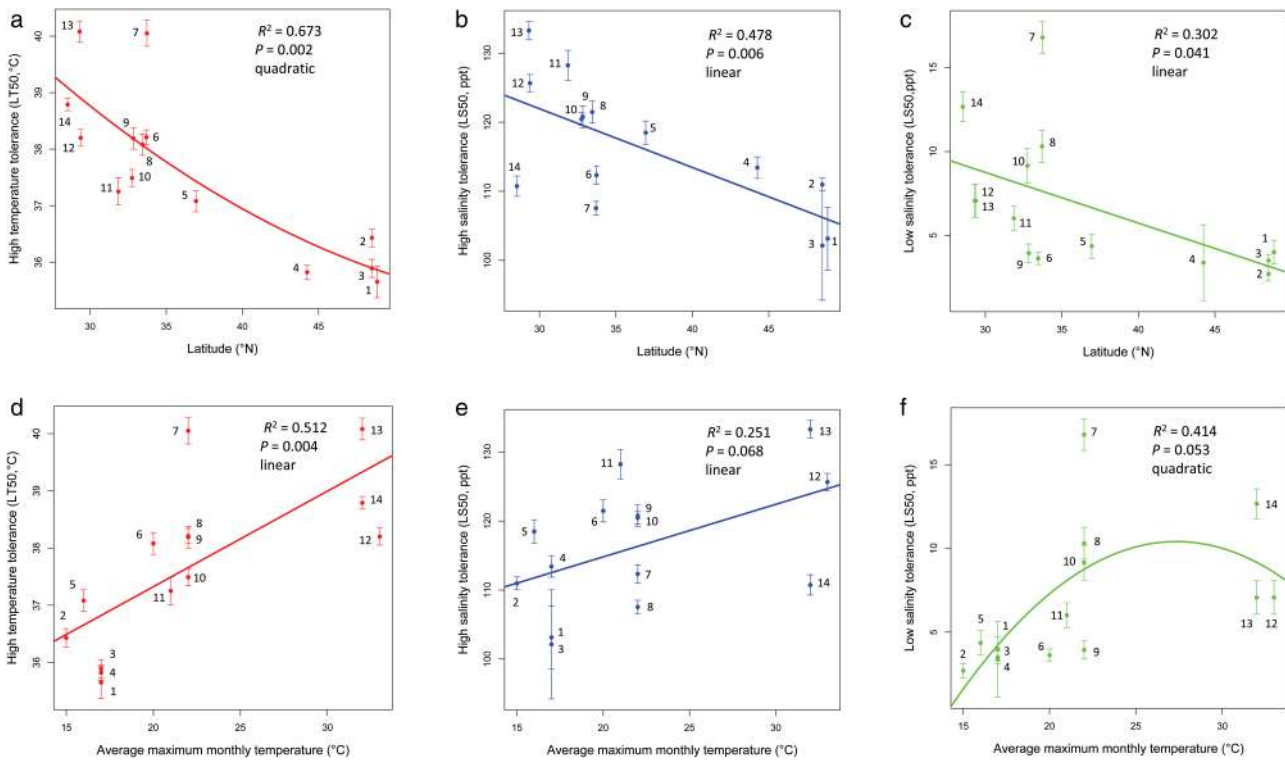


Figure 1. Regressions of median lethality on latitude (a–c) and climate data for average maximum monthly temperature (d–f). Tolerance assays were done on adult males exposed to 3 stressors: 1 h at high temperature (a,d), 24 h at high salinity (b,e) and 24 h at low salinity (c,f). Assays were done in the following 14 populations: 1) Wizard (BC, Canada), 2) Friday Harbor Labs (WA), 3) Sunset Beach (WA), 4) Strawberry Hills (OR), 5) Santa Cruz (CA), 6) Catalina Island (CA), 7) Abalone Cove (CA), 8) Royal Palms (CA), 9) La Jolla (CA), 10) San Diego (CA), 11) Punta Morro (Mexico), 12) Los Morros Colorados (Mexico), 13) El Cuervito (Mexico), 14) Playa Altamira (Mexico). Error bars are ± 1 SE.

temperature was generally greater than the corresponding air temperature. However at the northern site SC, tidepool temperature was generally lower than the air temperature (Figure 2).

Salinity measurements (Figure 3) for one location (CAT) over summer 2014 showed highly variable values of ~ 35 to 100 ppt (the measurement limit of the refractometer) in the smaller pool (tidepool 1) and fairly stable values of ~ 35 to 50 ppt in the larger pool (tidepool 2). The steep drop in salinity in later June / early July in tidepool 1 is likely due to an exceptionally high tide that refilled pools.

Discussion

Geographic scale of adaptive differentiation

Our geographic survey of temperature and salinity tolerance showed adaptive differentiation between populations separated by as little as 5.6 km. This distance is certainly smaller than the most commonly reported scale of divergence for marine invertebrates, which is estimated at hundreds of kilometers (Sanford and Kelly 2011). However, this fine-scaled adaptive divergence is certainly not unprecedented, as other intertidal invertebrates have been reported to show adaptive differentiation over latitudinal distances on the scale of kilometers (e.g., Kirby et al. 1994) and over vertical intertidal gradients on the scale of meters (e.g., Schmidt et al. 2000; Schmidt & Rand 2001, Hays 2007). Such spatially restricted adaptation in *T. californicus* is somewhat surprising. On the one hand, local adaptation should be favored by the lack of a dispersive planktonic stage, extensive evidence for genetic subdivision (e.g., Burton et al. 1979; Edmands 2001; Willett and Ladner 2009; Peterson et al. 2013) as

well as the fine-grained mosaic of stressors among rocky intertidal locations. Divergent mitonuclear interactions in isolated populations may further facilitate local adaptation, since, for example, particular mitonuclear combinations have been shown to increase heat tolerance (Pereira et al. 2014; Hill 2015). However, on the other hand, local adaptation may be thwarted by spatial and temporal variation in physical stresses among tidepools within a rock outcrop, since these pools have been shown to be well connected by gene flow over time periods from weeks to months (Burton and Swisher 1984). Our results, as well as previous evidence for local thermal adaptation (Willett 2010; Kelly et al. 2012; Pereira et al. 2017), suggest that these fluctuations in physical stresses among pools within outcrops are outweighed by the effects of divergent selection among outcrops.

Geographic clines

Results show significant clines over 20 degrees of latitude for both temperature and salinity tolerance, with southern populations having greater resistance to high temperature and high salinity, and northern populations having greater resistance to low salinity. Latitudinal clines in upper thermal limit have been found in other marine invertebrates, including intertidal species (e.g., Stillman and Somero 2000; Jansen et al. 2007; Zippay and Hofmann 2010). Reports of latitudinal clines in the salinity tolerance of intertidal organisms are less common. Work by Pennings et al. (2003) did assess salinity tolerance in salt marsh plants that occur in the intertidal, and found that in two of three species southern plants had greater salinity tolerance than their northern conspecifics. Geographic clines in salinity tolerance may be enhanced in species like *T. californicus* living in

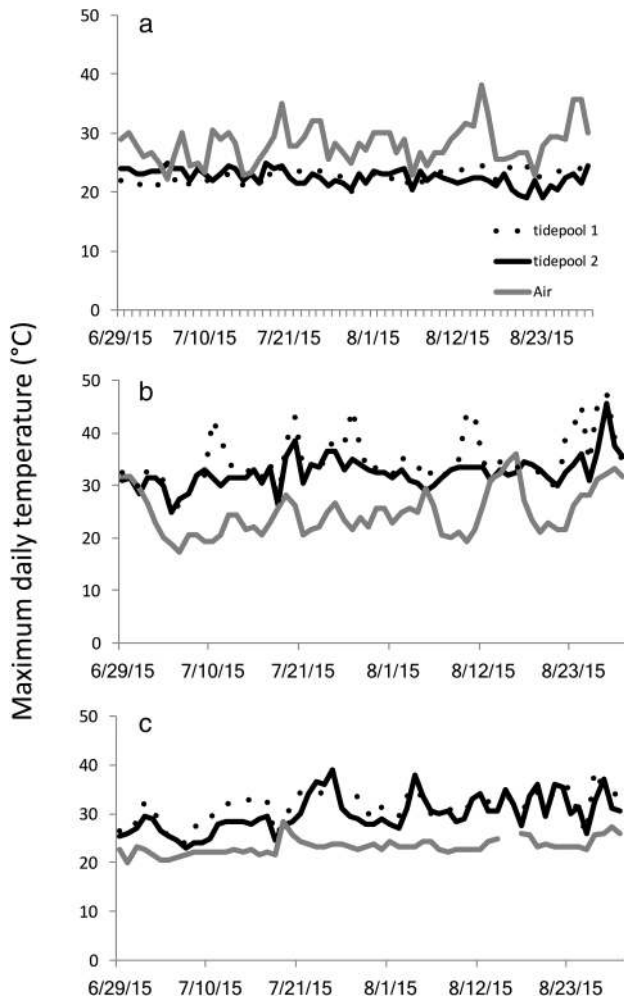


Figure 2. Temporal variation in daily maximum air and tidepool water temperature at three locations: Santa Cruz (a), Catalina Island (b) and San Diego (c). Air temperature was taken from the nearest NOAA climate station and water temperature was collected using iButton temperature loggers in tidepools inhabited by *T. californicus*.

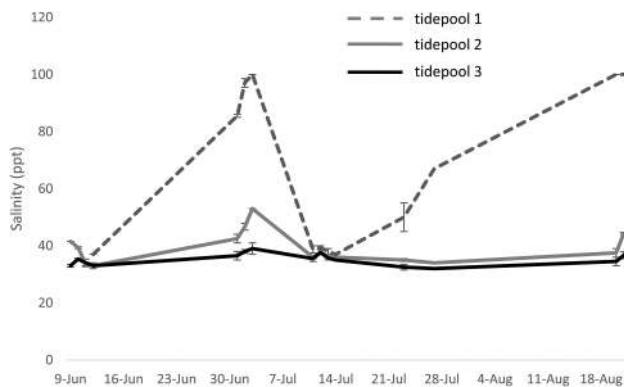


Figure 3. Salinity measurements for June to August 2014 in 3 tidepools at Catalina Island, CA. Tidepools 1 and 2 were inhabited by *T. californicus* whereas tidepool 3, approximately one meter away, was not. Error bars are standard error of the mean.

supralittoral pools subject to extreme salinity events. Despite the significant overall latitudinal trends, the patterns in Figure 1 reveal a complex geographic mosaic. Stress levels at proximal locations can

vary due to a range of factors such as coastline orientation, wave energy, rock type and pool depth. Further, maximal intertidal temperatures do not follow a simple north–south cline due to geographic variation in tidal cycles. Indeed, maximal thermal stress may be higher in parts of Washington and Oregon, where the lowest summer tides occur in the middle of the day, than in parts of central and southern California, where the lowest summer tides occur during the night (e.g., Helmuth et al. 2002, 2006). As discussed by Helmuth et al. (2006), the mosaic effect of tidal emersion timing may vary with tidal height and may be somewhat less important for supralittoral organisms (such as *T. californicus*) that are not subject to full tidal immersion.

Stress tolerance versus environmental conditions

Our field measurements, combined with previous work in *T. californicus*, suggest that environmental conditions in tidepools do occasionally reach lethal levels. For two of the three locations where tidepool temperature was monitored (CAT and SD), temperature exceeded the median lethality level for the population during the short 2-month measurement period. For salinity monitoring, which was restricted to a single population, recorded values did not reach either the high or low salinity levels causing median lethality. However, a previous study (Powlick 1999) reported salinities in *T. californicus* pools ranging from <1 to 139 ppt, values that exceed the median lethality levels for all the populations we tested.

Interestingly, both thermal and salinity tolerance were better explained by long-term temperature data than by long-term precipitation data. This may be because evaporation plays a particularly important role in driving salinity of supralittoral pools. It should be noted that our long-term environmental data came from terrestrial weather stations which are a crude proxy for intertidal conditions. Previous work shows that both satellite derived sea surface temperatures and terrestrial weather station air temperatures are poor predictors of thermal profiles in the intertidal (Lathlean et al. 2011). While conditions in the supralittoral zone may be more heavily influenced by terrestrial regimes (e.g., Helmuth et al. 2006), our results show that terrestrial weather stations still do not predict tidepool conditions. Specifically, our data showed that summer tidepool temperatures at all three sites were consistently different from air temperatures taken from the nearest NOAA station, with pool temperatures warmer than air at two sites and colder than air at the third site. This pattern may shift between seasons, due to seasonal variation in wave energy combined with geographic variation in the timing of tides (e.g., Helmuth et al. 2002, 2006).

Trait correlations and tradeoffs

Results showed a negative correlation between high temperature tolerance and low salinity tolerance, a pattern that can be inferred to have a genetic basis since the populations were raised in a common garden. This pattern likely results from correlated environmental selection pressures, such as increased evaporation with warmer temperatures and/or a negative association between temperature and precipitation (e.g., DeFrenne et al. 2013). The extent to which these correlated traits can respond to environmental change depends on their specific genetic basis (Blows and Hoffmann 2005; Walsh and Blows 2009; Saltz et al. 2017). It may be that high temperature tolerance and low salinity tolerance are underlain by different loci. In this case, the negative association between tolerance traits would be eroded by changes in correlated selection pressures and by gene flow followed by recombination. On the other hand, if the same loci underlie temperature and salinity tolerance, then the observed pattern would be an example

of antagonistic pleiotropy. A genetic tradeoff such as this would be expected to slow the progress of adaptation (Walsh and Blows 2009) and limit geographic distributions (Angert et al. 2008). This might be a particular impediment to adaptation in the face of global change, where simultaneous high temperatures and low salinities could become increasingly common (Fischer and Knutti 2015).

Previous work on the same species (Kelly et al. 2016) found a tradeoff between hyperthermal and hyposmotic stress. However, in this case the tradeoff was physiological (meaning that simultaneous exposure to two stressors reduces tolerance to one or both stressors) rather than genetic (meaning that shared genetic architecture prevents two traits from being optimized by natural selection). This physiological tradeoff could be driven by competing energetic demands as well as by high temperatures increasing membrane permeability (Hochachka and Somero 2002) and thereby reducing the efficiency of ion regulation.

Conclusions

Understanding how populations differ in their adaptive tolerance to environmental stress is critical to predicting how global change will impact local extinctions and range shifts. The tidepool copepod *T. californicus* inhabits supralittoral rock pools where environmental stressors are extreme and not easily predicted from more abundant terrestrial data. Results showed adaptive differentiation to temperature and salinity over scales as low as 5.6 km. Temperature and salinity tolerance showed latitudinal clines, with a significant correlation between high temperature and low salinity tolerance. If these traits are underlain by shared loci this compromise that may slow adaptation to future climates projected to be both hotter and wetter.

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