

Local temperature adaptation among populations of *Tigriopus californicus* along the North American west coast

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Abstract

Many current models predicting the effects of climate change assume species are homogenous across geographic distribution and life stage. However local adaptations may result in variable impacts of climate change across isolated populations. Furthermore, individuals of different life stages may exhibit different tolerances to variations in climatic conditions. We use the widely distributed tide pool copepod *Tigriopus californicus* as a model organism to test differences in thermal tolerance across populations and life stage of broadly distributed species. We tested the thermal tolerance of seven populations (spanning 15° in latitude) from the northern extent of the species range. We found that thermal tolerance differed among populations and life stage. However the degree of differentiation between populations was less pronounced in these northern populations than previously observed in southern populations. This is likely due to significantly less interpopulation mtDNA differentiation in the northern extent of the range than in the southern.

Introduction

Anthropogenic climate change is expected to result in a 1.1°-6.4° C rise in global temperatures in the coming century (IPCC 2007). This raises questions about the ability of many species to tolerate temperature changes in their native home ranges (Root *et al.* 2003; Thomas *et al.* 2004). Resources available to devote to conservation efforts are ultimately limited, thus we should direct them toward regions or species that could stand to benefit most. The use of models to predict biological responses to climate change has

become an important tool in determining where to focus efforts. Many current models assume species are genetically and phenotypically constant over space and time; however studies indicate local adaptations may impact predicted effects of climate change (Pearson and Dawson 2003; Jeschke and Strayer 2008; Hoffmann and Sgro 2011). Some current models also suggest broadly distributed species may be better suited to adapt to climate change than species with more narrow distributions. However these models assume all individuals of a given species possess uniform tolerance to environmental factors found throughout the species range (Thomas *et al.* 2004; Araújo *et al.* 2005). Local populations may be ill-equipped to tolerate variations in temperature, despite species-wide tolerance. Models that ignore population level adaptations may underestimate extinction risk if populations possess localized tolerances (Harte *et al.* 2004; Atkins and Travis 2010).

In this study we test the hypothesis that individual populations of a widely distributed species vary in maximum thermal tolerance. It is impractical to perform such an experiment on large, long-lived species. Thus we use the tide pool copepod *T. californicus* as a model organism to inform how species that reside in highly fragmented habitats may be impacted by climate change. *T. californicus* is a species of zooplankton that grows up to 1mm in length, with generation times between 21 and 30 days (Powlik *et al.* 1997). The habitat range of *T. californicus* spans from Baja California, Mexico (27°N) to southeast Alaska, USA (57°N) (Dethier 1980; Ganz and Burton 1995). *T. californicus* reside in high intertidal pools with very limited mobility, so individual populations remain highly fragmented (Brown 1991; Burton and Lee 1994). *T. californicus* are known to have extremely high levels of interpopulation mtDNA

differentiation (up to 23%), which may influence the level of local adaptations (Edmands 2001).

Accurate models must include individuals of every life stage to estimate how an entire population will be effected by climate change. Other species are known to vary in thermal tolerance by life stage (Krebs and Loeschcke 1995). In this experiment we test the thermal tolerance of individuals from every life stage for each population to determine if a difference exists.

Populations of *T. californicus* from lower latitudes generally have greater thermal tolerances than populations from higher latitudes. (Kelly *et al.* 2011). Previous research has examined mature male copepods from populations spanning from Punta Prieta, MX (27° N) to Fogarty Creek, OR (44°N). Our research extends the range of populations northward and expands to include all life stages.

Methods

We obtained samples of *T. californicus* from seven different populations along the Pacific Coast of North America, spanning 15° in latitude. From south to north, the populations sampled came from: Laguna Beach, CA (33.53°N), Montana de Oro, CA (35.26°N), Luff Beach, Hearst Park, CA (35.64°N), North Salmon Creek, CA (38.36°N), Boardman, Oregon (45.84°N), Cattle Point, WA (48.45°N), and Bamfield, Canada (48.83°N). To control for phenotypic plasticity, the samples were placed in an incubator and raised at a constant temperature of 20°C for at least four generations before thermal tolerance experiments commenced. The populations were kept in separate bottles filled

with filtered sea water. Every 1-2 weeks we changed the water in the bottles and added aquarium fish food.

We used a Biometra T3 Thermocycler to heat shock individuals from each population sample. We tested temperatures that ranged from the highest temperature samples could tolerate with 100% survival to the lowest temperature that resulted in 100% mortality, with temperatures spaced 0.2°C apart. These ranges differed for each population. We tested 3 replicates from each population at each temperature within the range of 100% survival to 100% mortality. Replicates consisted of two individuals from each life stage: nauplii (larvae), copepodites (juveniles), mated pairs (recorded individually as “paired males” and “paired females”), and females with egg sacs. Individuals from each life stage were placed in separate 400 µL thin-walled polymerase chain reaction (PCR) tubes within the thermocycler. We used a 2 hour ramp up from 20°C to the target temperature of each test and then maintained the target temperature for 1 hour.

After the heat shock we transferred copepods to separate well trays with filtered sea water to ensure they received adequate oxygen, and then replaced them in the 20°C incubator for 40 hours before observing samples under a dissecting microscope to determine mortality. These heat shock procedures follow previous established methods for testing thermal tolerance (Kelly *et al.* 2011). Mortality was determined by complete immobility of individuals and, in some cases, partial or complete fragmentation of corpses. We also observed cannibalism, which resulted in the complete absence of some bodies after the 40 hour incubation period. Pilot studies determined that cannibalism

only occurred after individuals had already deceased, and not as a result of predation, therefore we confidently recorded missing individuals as deceased from heat stress.

We used the data collected to estimate the lethal temperature of 50% of each population (LT_{50}). This was done by plotting the proportion of surviving individuals against temperature and fitting a logistic regression to determine a point estimate for 50% mortality (See Figure 1 for example of LT_{50} graph). We calculated estimates for the LT_{50} of each population and each life stage within each population. We eliminated nauplii from the analysis because mortality rates were highly variable and appeared to be closely correlated with the timing of feeding rather than temperature exposure.

Our sample size for each population was relatively small ($n=3$ replicates) so we also utilized bootstrapping techniques to evaluate the LT_{50} s of each population. For each population we took the mean of 1000 LT_{50} s calculated from random samples with replacement of 18 out of the 24 individuals tested at each temperature.

Since latitude is simply a proxy for local environmental conditions, it may be more informative to look at the relationship between LT_{50} and mean annual temperature. We do not have temperature data for each study site, but we have mean annual temperature records of nearby sites obtained from weatherbase.com. Temperature estimates are from sites no more than seven miles away from each study site.

Results

The results of the bootstrap-derived LT_{50} 's for each population plotted against mean annual temperature estimates for each site can be found in Figure 2. Fitting a linear

regression to this data shows a positive correlation between LT_{50} and mean annual temperature with an $R^2 = 0.5052$ for the regression line.

A one-way ANOVA test of the LT_{50} 's derived from non-bootstrapped raw data for each population found a statistically significant difference between populations ($F_{4,10} = 54.128$; $P < 0.001$). The southern-most population, Laguna Beach, is significantly different from all other populations ($P < 0.001$). None of the remaining populations are significantly different from each other ($P > 0.05$).

The estimates of LT_{50} for the four life stages analyzed from each population (copepodite, paired male, paired female, female with egg sac) can be found in Figure 3. A two-way ANOVA test found a statistically significant interaction between population and life stage ($F_{18,56} = 2.262$; $P = 0.010$). The test also found life stage to be statistically significant ($F_{3,56} = 20.245$; $P < 0.001$). Copepodites from the Laguna Beach, Montana de Oro, Luff Beach, and Bamfield samples were found to be significantly different from at least 2 out of the 3 other life stages ($P < 0.05$), and in each case copepodites had a greater thermal tolerance than all other life stages. With one exception (Laguna Beach- Females with egg sacs vs. Paired Males), no other life stage was significantly different from any other life stage in any population. It should be noted that the data failed the Shapiro-Wilk normality test ($P = 0.05$), however the two-way ANOVA test is robust to such shortcomings (Box 1954).

Discussion

The hypothesis that individual populations of a broadly distributed species vary in their maximum thermal tolerance is supported by our data. The southern-most

population, Laguna Beach, has a significantly greater thermal tolerance than all populations north of it, and the bootstrapped analysis shows a strong trend of increasing LT_{50} with increasing mean annual temperature. While the LT_{50} 's of the six sites north of Laguna Beach were not significantly different, this could be due to a couple of factors. First, the sample size was small ($n=3$ replicates); with a larger sample size the observed differences may become statistically significant. Second, mtDNA analyses of *T. californicus* populations show interpopulation differentiation to be five times greater in southern populations (between Baja California, MX and central California) than in northern populations (between northern California and Alaska) (Edmands 2001). This is hypothesized to be a result of postglacial contractions and expansions that depleted genetic differentiation in northern populations, and longer generation times that occur under cooler conditions (Edmands 2001; Vittor 1971). This helps explain the more significant difference previously found in southern populations (Kelly *et al.* 2011).

Our analysis also found a significant interaction between life stage and thermal tolerance, and between population and life stage. This indicates the relationship between thermal tolerance and life stage is population-dependent. In four out of the seven populations copepodites had a significantly greater thermal tolerance than at least 2 out of 3 other life stages. In five out of the seven populations copepodites exhibited the highest thermal tolerance of all life stages. The reason behind the greater thermal tolerance of copepodites is not known, but two possible explanations come to mind. First, copepodites have not yet reached reproductive maturity; in contrast, paired males and females with egg sacs have reached reproductive maturity and paired females are frequently very close (just one molt away) (Burton 1985). There is a potential cost to

maintaining a high thermal tolerance (Willett 2010); therefore it may be advantageous for copepodites to invest in a greater tolerance that is reduced as they reach reproductive maturity. During our study, we observed numerous occasions where females with egg sacs died of heat stress, while their eggs survived to hatch viable nauplii during the 40 hour post-heat-stress incubation period. This anecdotal evidence supports the finding that younger copepods have a greater thermal tolerance and indicates that a mature female's offspring will still survive an extreme heat event even if her tolerance for such events has decreased with age. Another possible explanation is that phenotypic plasticity allows copepodites to achieve a greater thermal tolerance. Mature males raised under different thermal conditions (constant 19°C vs. 19°C for 18 h/28°C for 6 h cycle) exhibit different maximum thermal tolerances (Kelly *et al.* 2011). It could be that copepodites maintain a greater maximum thermal tolerance because they have not yet reached the point of maturity at which their maximum thermal tolerance is fixed.

These results contribute to a growing understanding of interpopulation differentiation and the extent of local adaptations. They also reveal that local adaptations can be expressed differently in each life stage. Accurate models predicting the effects of climate change must acknowledge that species are not homogenous in space, time, or life stage.

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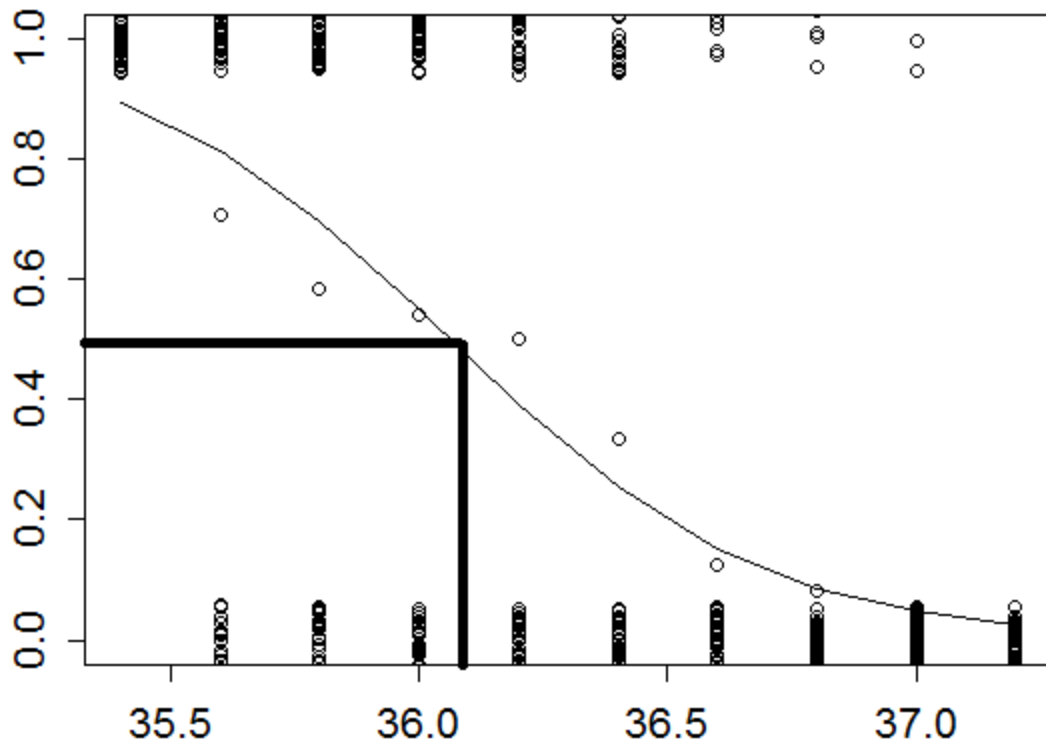


Figure 1: Logistic regression of Alive: Dead ratio of three replicates of *T. californicus* after one hour exposure to given temperature. Each replicate consisted of eight individuals taken from the Boardman (45.84° N) population. From this regression the lethal temperature at which mortality rates are 50% (LT₅₀) is estimated to be 36.1° C. Such estimates for LT₅₀ were estimated for each of the five populations tested, and can be found plotted in Figure 2.

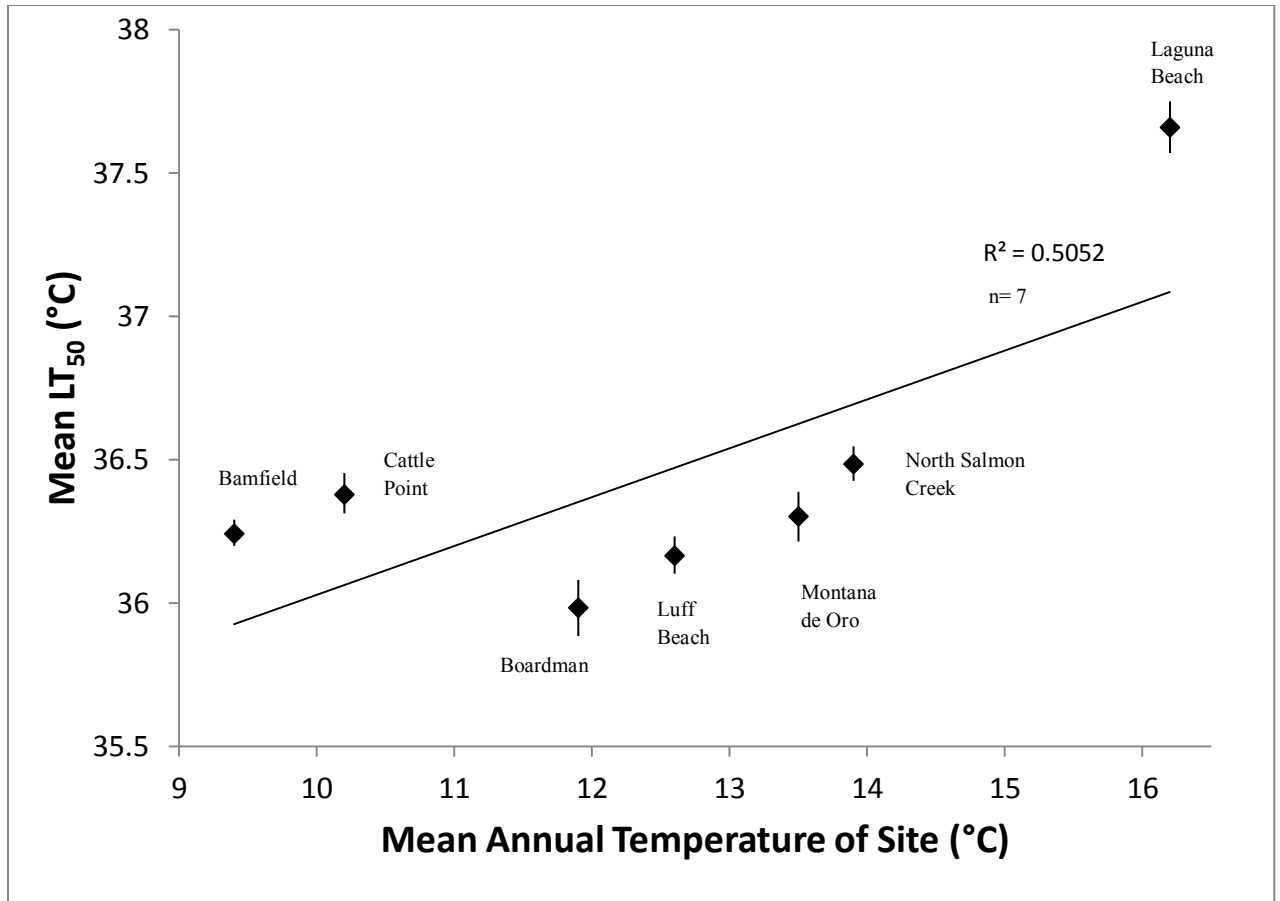


Figure 2: Y-Axis: Mean LT₅₀ (°C) +/- 95% C.I. of n = 1000 bootstrapped samples of raw data for seven populations of *T. californicus*. Raw data obtained from 3 replicates containing 2 individuals from each life stage (copepodite, mated pairs, females with egg sacs). Replicates were tested every 0.2°C at temperatures within the range of 100% survival and 100% mortality. Bootstrapped samples pulled 18 out of 24 individuals tested at each temperature from each population. Populations sampled: Laguna Beach, CA (33.53°N), Montana de Oro, CA (35.26°N), Luff Beach, Hearst Park, CA (35.64°N), North Salmon Creek, CA (38.36°N), Boardman, Oregon (45.84°N), Cattle Point, WA (48.45°N), and Bamfield, Canada (48.83°N).

X-Axis: Estimates of mean annual temperature of study sites. Estimates obtained from weatherbase.com records of mean annual temperature of sites no more than seven miles away from each population site.

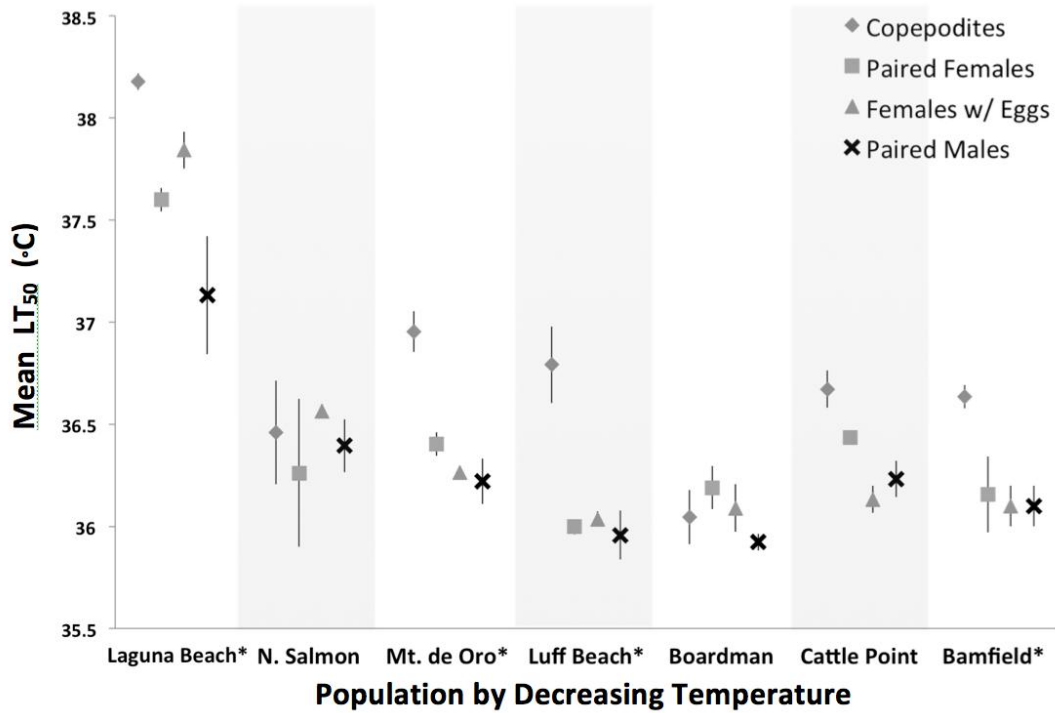


Figure 3. Lethal temperatures at which mortality rates are 50% (LT₅₀) +/- s.e. for copepod *Tigriopus californicus* from each of the sampled sites (Laguna Beach, CA (33.53°N), Montana de Oro, CA (35.26°N), Luff Beach, Hearst Park, CA (35.64°N), North Salmon Creek, CA (38.36°N), Boardman, Oregon (45.84°N), Cattle Point, WA (48.45°N), and Bamfield, Canada (48.83°N)). Each data point represents the mean LT₅₀ of three replicates per life stage. Each replicate consisted of 2 individuals tested every 0.2°C at temperatures within the range of 100% survival and 100% mortality. Diamonds represent copepodites (juveniles), squares represent females in a mated pair, triangles represent mature females with egg sacs, and crosses represent mature males in a mated pair. Asterisks mark populations for which there is a significant difference between LT₅₀ of copepodites and at least two other life stages (p < 0.05).