

**Variation in thermal tolerance of *Tigriopus californicus* among isolated populations
and between life stages**

Tiffany Kim^{1,2}, Caroline Walls^{1,3}

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¹Friday Harbor Laboratories, University of Washington, Friday Harbor, WA 98250

²Dept. of Environmental Sciences, Northwestern University, Evanston, IL 60208

³Dept. of Environmental Policy & Culture, Northwestern University, Evanston, IL 60208

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Contact Information:

Tiffany Kim

7539 Jackwood St.

Houston, TX 77074

tk12@u.northwestern.edu

ABSTRACT

Climate change will alter life on Earth, but the specific impact on any given species is less certain. Most current forecasting models lump all members of a species under one climatic envelope, thus ignoring the possibility of local adaptation among isolated populations of a wide-ranging species to result in differing climatic needs for each population compared to the species as a whole. To provide experimental evidence of the importance of local adaptation, we test each life stage of seven populations of copepod *Tigriopus californicus* ranging fifteen degrees in latitude, expecting populations and possibly life stages to differ in thermal tolerance. Our results show populations living in cooler climates generally have a lower thermal tolerance, and also juveniles exhibit higher thermal tolerances than adults, which could affect how a population responds to changing conditions. Thus, it is important for models to consider the roles of adaptation and evolution in species survival.

INTRODUCTION

It is clear the profound rate of extinction expected within the near future (Thomas *et al.*, 2004) will only worsen if global temperatures rise the predicted 1.1-6.4 °C within the century (IPCC, 2007). However, the response of any particular species is much less certain, and conservation efforts are limited by physical and monetary resources as well as time; thus, more accurate methods to estimate a species' ability to survive or adapt in the near future will allow for the most efficient use of those valuable resources.

In most climate change models, an entire species falls under a homogenous bioclimatic envelope based on its current geographic range, and that envelope is used to predict how abiotic changes in environment will affect the species' range in the future

(Jeschke & Strayer, 2008). However, the usefulness of such a one-dimensional model has been repeatedly questioned (Pearson & Dawson, 2003), and there is some evidence that it may in fact be inadequate for many species (Sax *et al.*, 2007). A key limitation of such a model is that it fails to consider the roles of adaptation and evolution, which are important to a species' chances of survival (Chown *et al.* 2010, Hoffmann & Sgrò, 2011). One consequence is that the bioclimatic model assumes all individuals of a broadly distributed species have a wide range of habitable climates and are probably thus less vulnerable to climate change (Araujo *et al.*, 2005). However, it does not take into account the possibility of local adaptation creating distinct populations that have different tolerances than the species as a whole (Kelly *et al.*, 2011), and failing to do so could result in less accurate forecasting.

In this study, we examine thermal tolerance in tidepool copepod *Tigriopus californicus* to determine the extent to which tolerance may vary between populations of different local climates in a single species. *T. californicus* is an ideal model because it has a short generation time (20-30 days) (Powlik *et al.*, 1997) and is found in a broad geographic range covering at least 30 degrees in latitude, from Baja California, Mexico (Ganz & Burton, 1995) to Alaska (Dethier, 1980). We can also be certain that the tested populations are fairly isolated, since the copepod lives in the high intertidal zone and is unable to move over a centimeter on dry ground, and gene flow between populations appears highly restricted (Burton *et al.*, 1979). Assuming latitude is a valid proxy for temperature, we expect thermal tolerance of *Tigriopus* to decrease with latitude because the more southern populations will be better adapted to higher temperatures.

A similar study was recently conducted using *T. californicus*, but it did not test any populations north of Oregon at 44° 50' N, and it only used adult males (Kelly *et al.*, 2011). In addition to testing populations from various latitudes, we also decided to investigate potential variation in thermal tolerance between life stages, which has been observed in *Drosophila buzzatii* (Krebs & Loeschcke, 1995). Such variability may influence a population's rate of adaptation, especially in organisms for which one life stage is more important than another for population growth. Here *Tigriopus* is again particularly useful because (juveniles) are easily distinguished from adults not only by size (Powlik *et al.*, 1997) but also the fact that all males in a mated pair are known to be fully mature, as are all females with external egg sacs (Burton, 1985).

METHODS

T. californicus was collected from populations along the North American west coast from south California to Canada. We tested populations from the seven following sites ranging 15 degrees in latitude (from north to south): Bamfield, Canada (48.83°N), Cattle Point, WA (48.45°N), Boardman, OR (45.84°N), North Salmon Creek Beach, CA (38.36°N), Luff Beach, Hearst Park, CA (35.64°N), Montana de Oro State Park, CA (35.26°N), Laguna Beach, CA (33.53°N). All populations were kept in bottles at room temperature for a few generations to control for phenotypic plasticity.

Methods we used to heat shock the copepods were heavily borrowed from Morgan Kelly's experiment (2011). Right before heating, we transferred copepods into 400 µL PCR tubes to be put into a thermocycler, keeping the water level below the top of the heating blocks to ensure a uniform temperature throughout. Each tube contained either two copepodites, two mated pairs, or two females with egg sacs. One replicate

consisted of three tubes, one of each life stage (i.e. 8 individuals). To test a single population's thermal tolerance at a certain temperature (e.g. 36.2°C), we placed three replicates (i.e. 24 individuals) into the thermocycler programmed to spend two hours ramping up from 20°C to the target temperature and one hour sitting at that temperature. The copepods were then transferred from the PCR tubes into well trays and incubated at 20°C for at least forty hours. In this way, copepods that were fatally injured but not yet dead would not be miscounted as survivors, and copepods that were in a heat-induced coma and appeared dead had time to revive. Only after the waiting period did we count the number of dead and live copepods under a dissecting microscope. No surviving copepods were reused to test another temperature.

Each population was tested at 0.2°C intervals from the temperature at which 100% of individuals from that population survived to the temperature at which 100% died. We then used a logistic regression to be able to estimate the lethal temperature at which mortality rate reached 50% (LT_{50}) (example in fig. 1). LT_{50} was calculated for each life stage of each replicate, and this data was analyzed with a two-way ANOVA (fig. 3). We also performed a bootstrap analysis on the raw data (ignoring previously identified replicates), obtaining the mean LT_{50} of each population by subsampling 18 out of the 24 individuals tested at each temperature 1000 times (fig. 2). LT_{50} estimations were performed in RStudio v. 0.94.110, and ANOVA analyses were performed in SigmaPlot v. 11.0. The mean annual temperature of each site was found on Weatherbase. Where data from our tested site was unavailable, we used data from the nearest available site, none of which were farther than 11 km from our tested sites.

RESULTS

Among the seven sites tested, the order of the sites from south to north was approximate to their order by decreasing mean annual temperature; the only exception was North Salmon Creek, which, though approximately 3° farther north, had a higher mean temperature than Montana de Oro and Luff Beach. Overall, the bootstrapped data shows *T. californicus* from warmer climates have higher thermal tolerances (LT₅₀) than those of colder climates (fig. 2), although not in an entirely linear fashion. The Bamfield and Cattle Point populations in particular have higher LT₅₀'s than would be expected from the mean temperature of those sites.

In the mean LT₅₀ of the three replicates per life stage per population, an ANOVA (with normality test failed) shows both population ($F = 65.1, p < 0.001$) and life stage ($F = 20.245, p < 0.001$) as significant sources of variation in thermal tolerance. However, *post hoc* pairwise multiple comparison tests (Tukey tests) show the thermal tolerance of only Laguna Beach is significantly higher ($p < 0.05$) than all other populations. Tukey tests also show the thermal tolerance of the “copepodite” group is significantly higher ($p < 0.05$) than the other life stages in four out of seven populations (fig. 3).

DISCUSSION

The trend in increasing thermal tolerance with increasing mean site temperature suggests *Tigriopus californicus* is capable of adapting to local environments to such an extent that some populations are unable to tolerate conditions that others of the same species can. Although latitude did not correspond exactly with mean annual temperature, the fairly linear trend we obtained (fig. 2) indicates that it is probably temperature and not geographic location of the site that is affecting thermal tolerance. The exceptions to this

may be Bamfield and Cattle Point; all other populations were collected along coastline facing open ocean, whereas Bamfield and Cattle Point are both located among the San Juan Islands, where there may be some feature of the channels (e.g. runoff from the islands increasing available nutrients) that have resulted in *Tigriopus* populations being slightly more resilient than expected. Despite this small variation, fig. 3 clearly shows less differentiation among thermal tolerance of northern populations than among those of populations from California. A recent study of the phylogeography of *Tigriopus californicus* suggests this is likely the result of significantly less genetic variation among populations north of California than among populations from northern California to Mexico (Edmands, 2001). Both that study and our results also corroborate Kelly's research (2011), which demonstrates a noticeably smaller difference in thermal tolerance between north California and Oregon populations than between Mexico and southern California populations.

The experiment also demonstrated significantly higher thermal tolerance in juveniles compared to adults in over half the populations tested. It has been suggested that this pattern, at least in *Drosophila*, may be a result of the juvenile's high stress sensitivity making it especially dependent on heat shock proteins for survival, despite the costs associated with expressing these proteins (Sørensen *et al.*, 2003). Since copepodites need no parental care, the costs of having a higher thermal tolerance may also be outweighed by the benefit of having that extra buffer to continue the population even if, for example, most adults are killed off in a heat wave.

One factor that may have skewed data was the tendency for dead copepods to fragment or be eaten by live copepods. After realizing and testing this, we were

convinced to count missing copepods as dead, but we cannot rule out the cause of some missing copepods to be human error in transferring to and from the thermocycler.

Another possible source of error is in the algal growth that occurred in some but not all populations. This may have given certain populations an advantage in increased food supply or disadvantage in harboring other detrimental organisms. However, we believe our data was not appreciably affected by either of these variables. We should also note that although the females in mated pairs are technically copepodites, male *Tigriopus* preferentially attach to female copepodites nearest maturity (Burton, 1985); thus, the smaller, free-swimming copepodites collected were most likely younger overall than females in mated pairs.

Our results imply that different populations of the same species may be capable of adapting to local conditions to such an extent that the same change in environment will not affect all populations uniformly. They also highlight the physiological differences between life stages in organisms with complex life cycles, which can affect the way an entire population is able to respond to environmental changes. If these implications are true, current bioclimate models may not be enough in forecasting a species' response to climate change. More accurate models taking into account local adaptation and differences between life stages would help in directing limited time and resources towards conservation efforts that will benefit the species or populations most urgently at risk of extinction. Further research may include comparing cold tolerance at these sites to better understand any costs or trade-offs of thermal tolerance and experiments in phenotypic plasticity, since plasticity may affect rates of adaptation.

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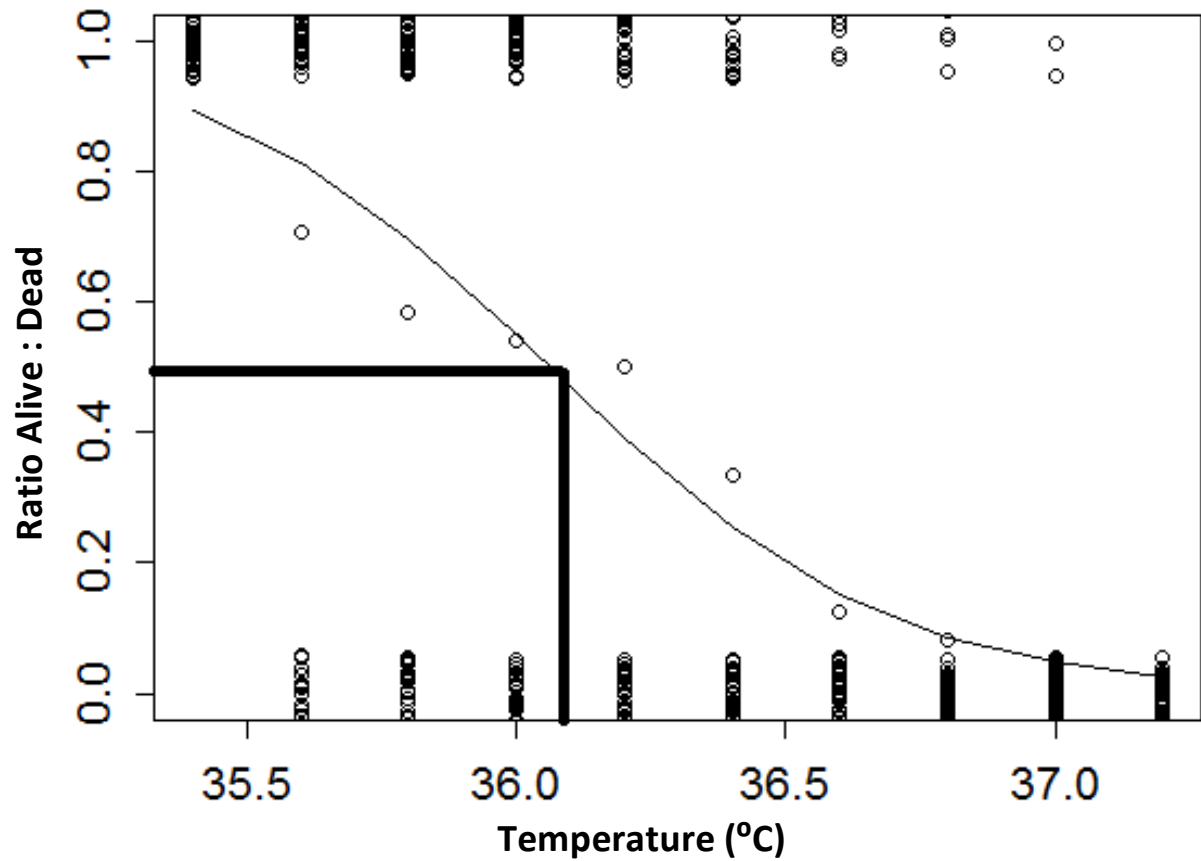


Figure 1. Logistic regression of Alive:Dead ratio of three replicates of *T. californicus* after one hour exposure to given temperature. Each replicate consisted of eight individuals taken from the Boardman (45.84° N) population. From this regression the lethal temperature at which mortality rates are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

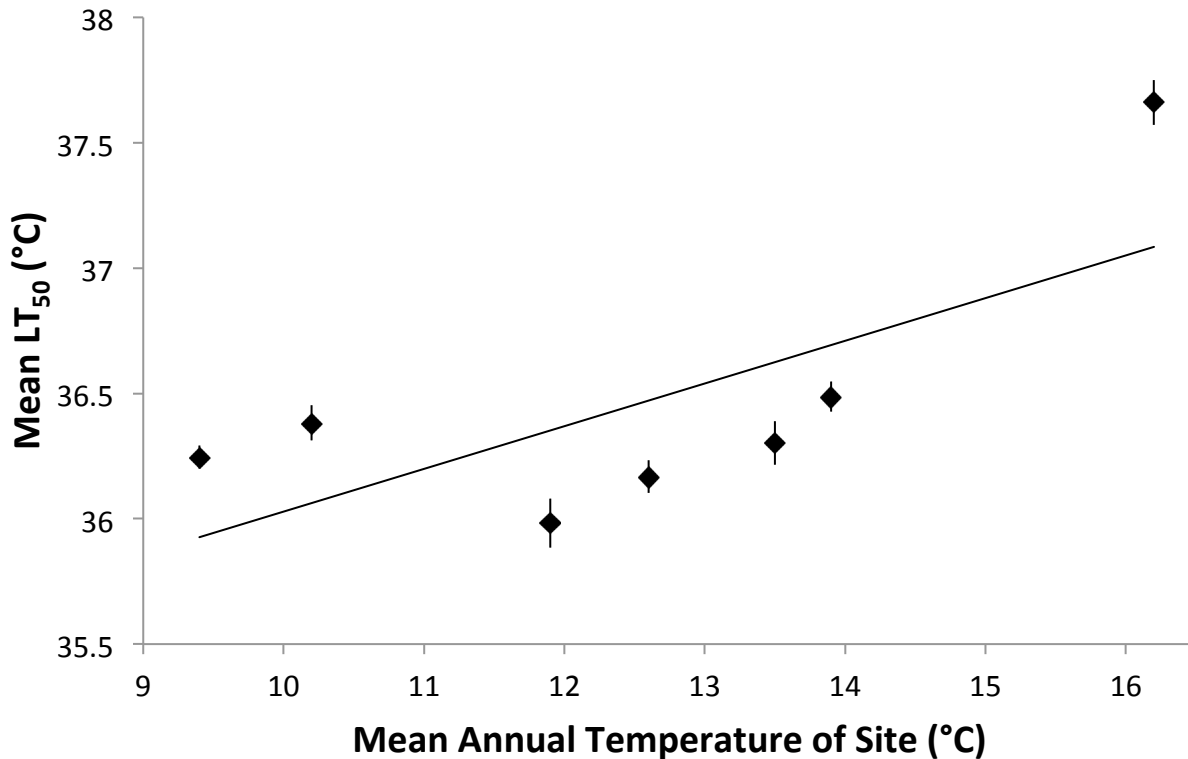


Figure 2. Lethal temperatures at which mortality rates are 50% (LT₅₀) +/- s.e. of laboratory reared *Tigriopus californicus* originally from seven sites along the North American west coast. Each data point represents the mean LT₅₀ of 1000 bootstrapped subsamples of 18/24 individuals. A positive trend is shown between a site's mean temperature and thermal tolerance (LT₅₀) ($R^2 = 0.51$). Sample sites (north to south): Bamfield, Canada (9.4°C); Cattle Point, WA (10.2°C); Boardman, OR (11.9°C); North Salmon Creek Beach, CA (13.9 °C); Luff Beach, Hearst Park, CA (12.6°C); Montana de Oro State Park, CA (13.5°C); Laguna Beach, CA (16.2°C).

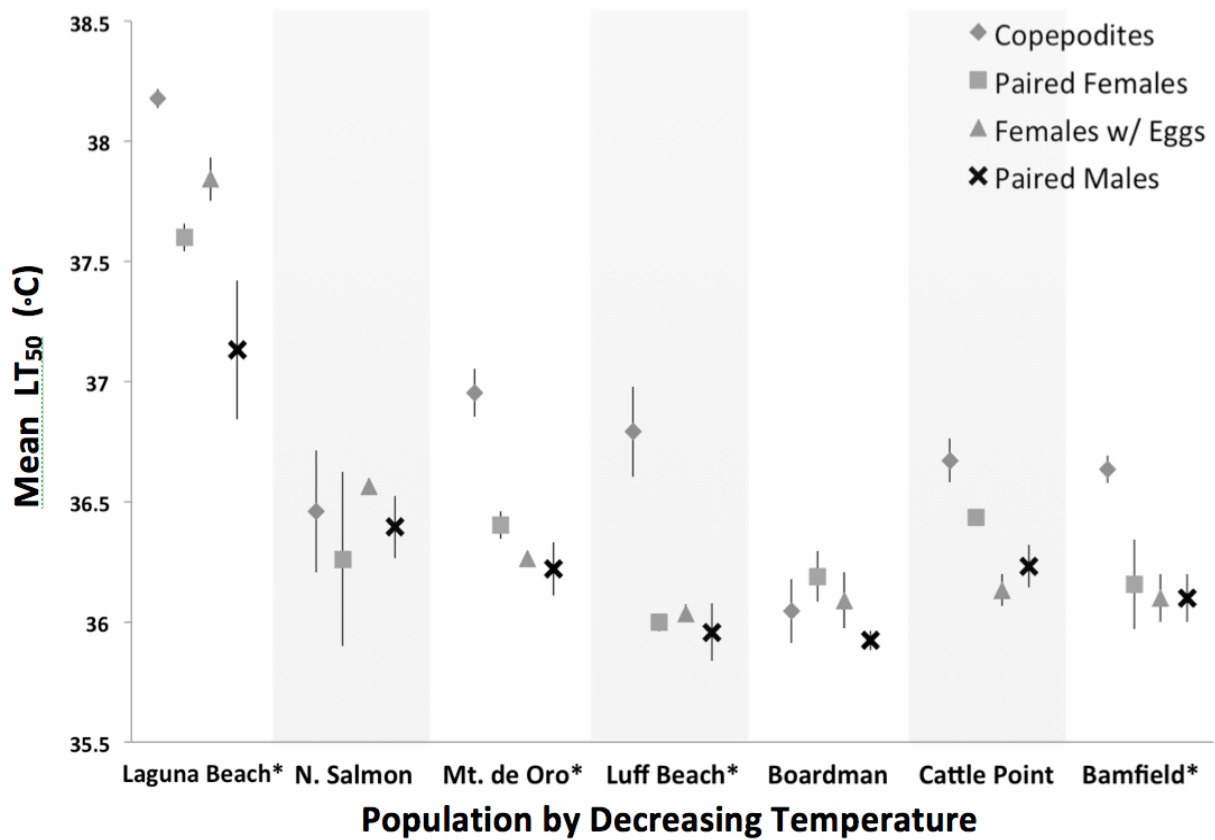


Figure 3. Lethal temperatures at which mortality rates are 50% (LT_{50}) \pm s.e. for copepod *Tigriopus californicus* from each of the sampled sites ($n=7$). Each data point represents the mean LT_{50} of three replicates consisting of two individuals (i.e. 6 individuals total). Diamonds represent copepodites (juveniles), squares represent females in a mated pair, triangles represent mature females with egg sacs, and crosses represent mature males in a mated pair. There is a significant difference between LT_{50} of Laguna Beach and every other population (ANOVA: $p < 0.05$). Asterisks denote populations for which there is a significant difference between LT_{50} of copepodites and all other life stages (ANOVA: $p < 0.05$).

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