Divergent Temperature Tolerances between two populations of the Splash-pool Copepod, *Tigriopus californicus*, on San Juan Island, WA

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Keywords:

Tigriopus californicus, copepod, local adaptation, Cattle Point, Rueben Tarte, thermal tolerance

Abstract –

Species living over large geographical ranges often have populations that are better adapted their local conditions (Helmuth et al. 2002). For example, populations of the splash-pool copepod, *Tigriopus californicus*, can tolerate higher temperatures as latitude decreases (Willett 2010). Studies on many organisms, including copepods, tend to look at a large geographical range and make generalizations about the thermal tolerance homogeneity of populations in specific latitude. By doing this we ignore possible effects of changing climate on some populations of copepods in areas that geographically are considered more tolerant. This study demonstrates a significant difference in the lethal temperature tolerance between two populations 30 kilometers apart on San Juan Island, WA. These sites are relatively close together compared to other studies. If we know that geographically close populations may be affected differently by climate change, then we may be more specific and cautious with our predictive modeling, not treating all populations the same.

Introduction –

Over the last 100 years, global air temperatures have risen by 0.6ºC (Root et al. 2003). Air temperatures are expected to rise another 1.4º to 5.6ºC before 2100 (Harvell et al. 2002). Water temperatures have been rising globally and the surface level of water is expected to rise 0.09 to 0.88 meters before 2100 (Harvell et al. 2002). Rising temperature can have a profound effect on organisms, harm them and push them toward the brink of extinction. It is common knowledge that some species can survive a larger temperature range and are more adaptive than other species. But some populations of some species can also be better adapted to temperature and condition changes than other populations of the same species. The mussel species *Mytilus californianus* are locally adapted to their climates up and down the west coast of the US. This difference allows some populations to be more resilient to heat stress (Helmuth et al. 2002). The splash-pool copepod *Tigriopus californicus* has populations throughout the west coast that are locally adapted to their climates. Due to their geographic isolation and their low Reynolds's number environment, they have very little gene flow between their populations. This copepod has a 4ºC high thermal tolerance range from Baja, Mexico to Oregon (Kelly et al. 2011). In these studies close proximity populations were considered to be part of one larger geographical population. If global temperatures continue to rise, we may not be able to predict which populations of certain species will be affected first and we may be slow or confused in our conservation efforts. In our study, we sought to prove that local populations on a much smaller geographical scale might have different thermal tolerances compared to each other (H_0= no difference). In this experiment we compared and found a difference in high thermal tolerance between two populations of copepods from two locations about 30 kilometers apart on San Juan Island, WA.

Methods –

Collection Methods –Copepods were collected on the northeast of the island at Rueben Tarte (48º 36' 45" N, 123º 5' 52" W) on October 21, 2011 and on the south of the island from Cattle Point on October 19, 2011 (48º 27' 7" N, 122º 57' 44" W). Sites were chosen because they have different weather, temperature and water conditions based on differences in predominant vegetation, intertidal fauna, and preliminary temperature measurements (Chris Neufeld, personal comm.). At both locations, from four or five splash pools, several cups containing copepods and from roughly the same upper intertidal zone height were collected. Later, to help us consider all copepods from one site as the same population, each cup of copepods was combined into one main bottle. For example, all cups from Cattle Point were placed into one bottle and counted as one Cattle Point population. The copepods were stored in the bottles in a 12-hour day/12-hour night incubator at 20ºC. The copepods were feed and their water in the bottles was changed with fresh, filtered seawater every week.

Lab Methods – We determined a LT_{50} (lethal temperature were 50% of the individuals will die) value for both populations. To find the general temperature range expected to contain the LT_{50} for each population, trial runs of various copepods were performed at temperatures ranging from 32ºC to 38ºC. From this a two-degree range was selected for each location. Within this range five replicates were run at every 0.2ºC. In all 275 copepods (5 replicates * 5 copepods * 11 temperatures) were tested. In order to keep our testing more consistent, we used a uniform lifecycle stage. Female copepods with egg sacks (see photos) were chosen because they are generally the same age, very easy to spot and easy to separate from the others. They were sorted out of the main cup and placed in 400 μL PCR tubes, which we then placed into a thermo-cycler (Whatman Biometra T3 Thermocycler). Trials included a two-hour ramp up from 20°C to target temperature, and one hour at the target temperature and then were returned to 20ºC. After the run, copepods were removed from the PCR tubes and placed into separate wells with filtered seawater. Copepods were then checked after 40 hours to see if they were dead or alive. (For more in depth details on methods, see Kelly, 2010.)

Analysis Methods –All analysis were performed in R ((R Development Core Team 2009). First, a simple logistic regression was made for each population and LT_{50} was calculated at each location by estimating LT_{50} from the regression model (see figure 1). Mean, standard error and a 95% confidence interval were calculated for each site (see figure 2). Secondly, a bootstrap method was used as a secondary analysis. R used our collected data to run four random copepods, as "dead" or "alive", at each temperature, 1000 times (1000 replicates * 4 copepods * 11 temperature = 44000 copepods total). This program produced a mean $LT_{50,}$ median LT_{50} and 95% confidence interval of these 1000 runs.

Results –

Mean lethal high temperature tolerance differed by about 1.2ºC with no overlap on the 95% confidence intervals between the two populations samples on San Juan Island, WA (see figure 3, table 1 and table 2).

Discussion -

Examining these two population shows that two populations approximately 30 kilometers apart can have as large a difference as two populations that are several hundred kilometers apart. Looking at our two populations, if global water temperatures were to increase just 2ºC, this may affect these populations differently. For example, say someone tested the Cattle Point copepods, which were the most thermal tolerant, and they neglected all of the other copepod populations on San Juan Island. Then if water temperatures rise in the San Juan Island area, we could lose all other populations of copepods on the island and be clueless about what went wrong with our predictions. If this type of small-scale local adaptation is more prevalent than we previously thought, we may not accurately predict what will happen to geographically close populations. Further knowledge on local adaptation can help us prevent a loss of populations that we thought were safe or would be less affected by the changing environment. More research and knowledge could lead to better and more effective conservation efforts among low gene flow populations threatened by global climate change.

Though we followed the methods set forth in another study and were careful with our data collection, we also created our own techniques. These differences in technique may affect our comparability to other studies of this type. Our thermo-cycler, although minimally, may have varied slight from actual temperature. This could affect our results when compared to another study, but would not have affected the comparative data within our study.

We also noted a trend with our copepod populations over time. As time went on in the lab, it seemed like there was adaptation in the short period of time, as their heat tolerance appeared to increase. This is reinforced by the fact that copepods from Cattle Point kept in the lab for around six weeks had a 1.02ºC (36.146 ± 0.06ºC) higher mean thermal tolerance than our data (Kim & Walls, unpublished data). For this reason we had to change our original, planned number of 275 copepods at each location. We tested only 250 copepods at Rueben Tarte because there were tests that had 100% survival at temperatures that when originally tested were almost 0% survival. We tested 350 copepods at Cattle Point because we underestimated the temperature at which they died during our preliminary tests, leading to tests at higher temperatures. In order to minimize the affect of plasticity and maintain the comparability of our populations, in most cases, both populations were run at the same temperature on the same day.

Further research is recommended in order to examine small-scale temperature tolerances and small-scale adaptation. For example, one could examine more study locations on the island and a greater number of copepods. Collection of all sites should be on the same day, along with testing all temperatures and all populations on the same day in order to minimize the affect of plasticity. Studying populations on a large and small scale may lead to creating predictive models of which populations may be most affected by climate change. Continuing research in these areas may lead to quick preventative action as our climate changes.

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Figures & Tables -

Figure 1 - Sample graph showing how LT_{50} is calculated. A line is drawn from the y-axis to the curve and then from that location another line is drawn down to the x-axis. This temperature on the x-axis becomes the LT_{50}.

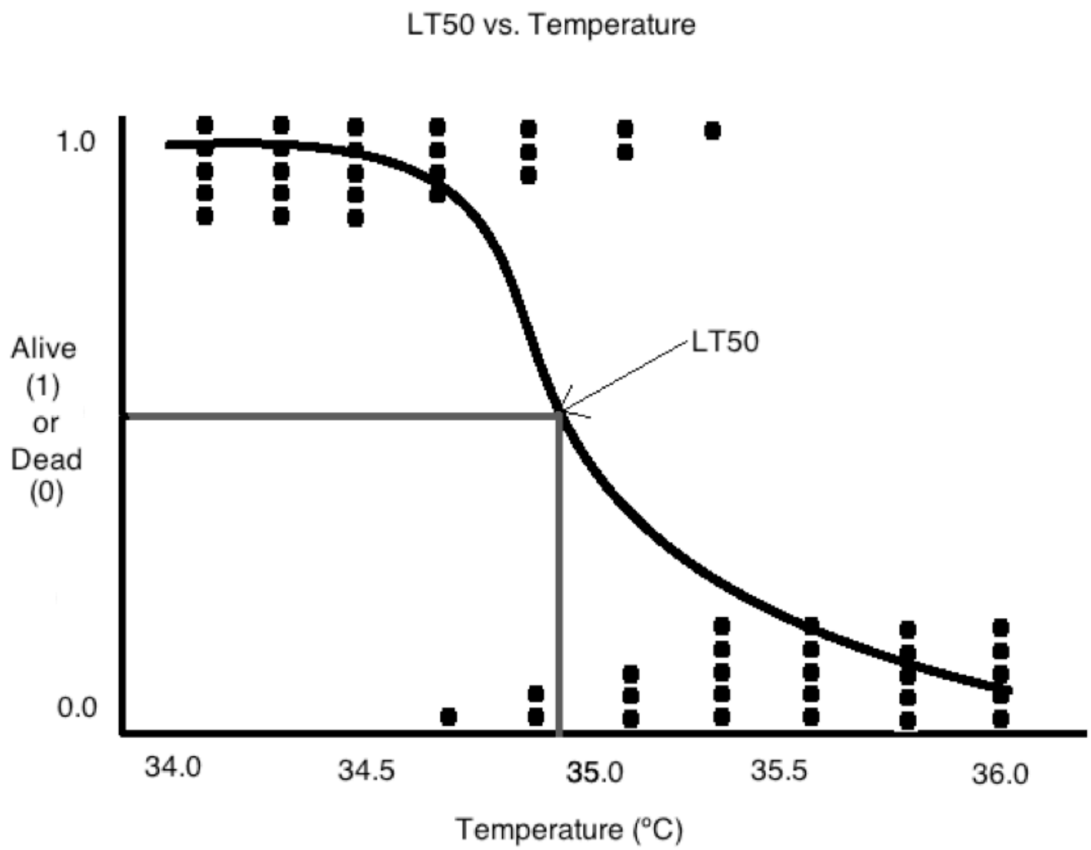
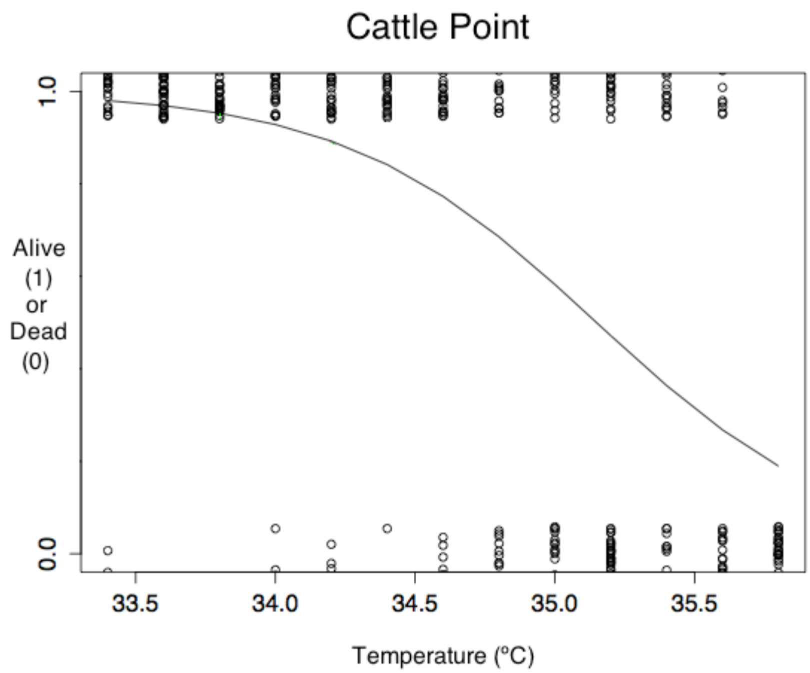


Figure 2 – Curve used to show LT_{50} (the temperature where 50% of the animals die) at Cattle Point (A) and Rueben Tarte (B). Each dot on the top and bottom of the graph show a single individual copepod. X-axis shows temperature, Y-axis shows whether a copepod was alive (1) or dead (0).

A)



B)

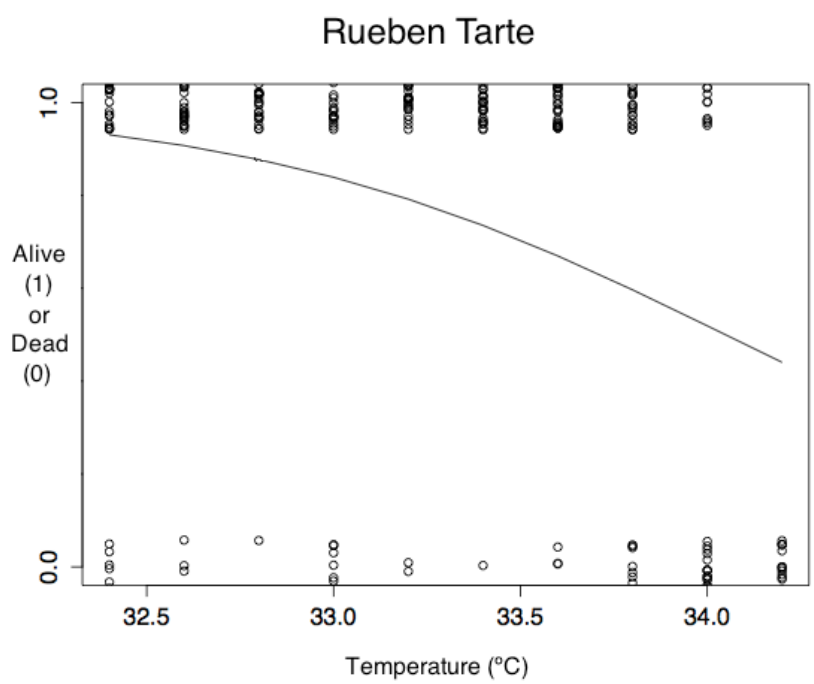


Figure 3 – Calculated LT50 values of both locations using each method (BS = Bootstrap; SLR = Single Logistic Regression). 95% confidence interval error bars added.

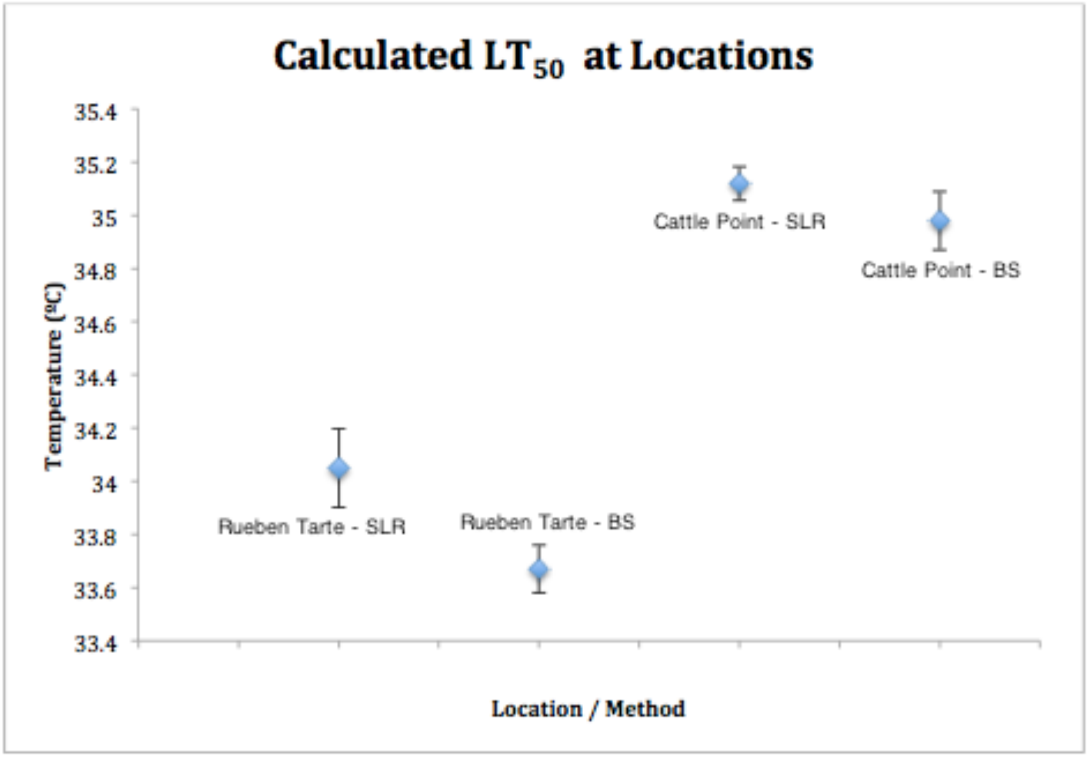


Table 1 – Summary of statistical results from Rueben Tarte population

Rueben Tarte (n=250)	
Single Logistic Regression	**"Boot Strap"**
Mean: 34.05ºC	Mean: 33.67ºC
Standard Error: ±0.148ºC	Median: 33.68ºC
95% Confidence interval: 33.76ºC and 34.34ºC	95% confidence interval: 33.58ºC to 33.74ºC

Table 2 – Summary of statistical results from Cattle Point population

Cattle Point (n=350)	
Single Logistic Regression	**"Boot Strap"**
Mean: 35.12ºC	Mean: 34.98ºC
Standard Error: ±0.064ºC	Median: 34.98ºC
95% Confidence interval: 35.02ºC to 35.27ºC	95% confidence interval: 34.87ºC to 35.12ºC

Photos – Side and top view of female copepod with egg sack. Photography credits and thanks to Gustav Paulay.

Side view:

Top view: