

Latitudinal variation in the cold tolerance of the intertidal copepod *Tigriopus californicus*

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Blinks – NSF REU – BEACON Research Fellowship
Research Experience for Undergraduates
Summer 2012

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Keywords: *Tigriopus californicus*, local adaptation, cold tolerance, freeze tolerance, chill coma, climate change

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Abstract

Broadly distributed species may adapt to local temperature conditions such that isolated populations have different thermal tolerance ranges than the species as a whole. Therefore, to accurately predict how species' ranges will be affected by global climate change, bioclimate models would benefit from knowing the thermal tolerance of different populations within latitudinally distributed species. In this study, the intertidal copepod *Tigriopus californicus* was used as a model system to study how local adaptation influences the cold resistance of isolated populations. Among five populations spanning 18 degrees in latitude, two metrics were used to compare cold tolerance: post-freezing recovery and the temperature of chill coma onset (CT_{min}). Recovery rates following freezing were faster in copepods from colder northern latitudes. Likewise, northern populations exhibited lower chill coma onset temperatures. Importantly, both metrics showed a consistent latitudinal trend suggesting that any single metric could be used equivalently in future studies investigating latitudinal variation in cold tolerance. Our results provide evidence that populations within a single species can display strong local adaptation to spatially varying climatic conditions. Thus it would be valuable for bioclimate models to account for local adaptation when forecasting biological responses to climate change.

1. Introduction

Temperature influences the performance of ectothermic animals in many important ways, affecting their physiology, ecology, behavior, and evolution (Castañeda et al. 2005, Schmidt-Nielsen 1997). As a result, latitudinal variation in temperature can impact different populations within a broadly distributed species and lead to local adaptation to climatic conditions (Castañeda et al. 2004, Castañeda et al. 2005, Gibert et al. 2001, Kelly et al. 2011, Sisodia and Singh 2010). This potential for local adaptation suggests that isolated populations may have different ranges for thermal tolerance than the species as a whole (Kelly et al. 2011, Castañeda et al. 2004). With current forecasts for global climate change, understanding the extent to which organisms can adapt to changing temperatures is becoming increasingly relevant, and the mechanisms by which local adaptation influences the thermal physiology of a species cannot be overlooked. In addition to forecasting an overall increase in global temperatures (Folland et al. 2002),

climate change models predict widespread changes to both the maximum and minimum temperatures of several regions (Environmental Protection Agency 2012, Meehl et al. 2000). In some cases, this means that organisms will be exposed to both lower minimum and higher maximum temperatures on a seasonal basis (Cohen et al. 2012, Petoukhov and Semenov 2010). Since there may be fitness trade-offs associated with adaptive responses to extreme high and low temperatures, it is currently unclear how this will affect species ranges (Huey and Kingsolver 1993, Willett 2010). In order to assess how organisms might respond to changes in the temperature breadth of their habitat, it is necessary to examine local adaptation in both the cold and heat tolerance of a species. Although several studies have now examined the capacity of various species to locally adapt to rising temperatures (Klerks and Blaha 2009, Willet 2010), local responses to cooling temperatures have not been widely studied. By understanding the ability of a species to tolerate cold stress we can predict how changing winter minimum temperatures may act as a range-limiting factor as global climate change progresses.

In this study, we examined the cold tolerance of the intertidal harpacticoid copepod *Tigriopus californicus* to determine if populations from various latitudes exhibit differing degrees cold recovery ability. *T. californicus* makes an ideal study species because individuals have a relatively short generation time (approximately 20 days) and are easily cultured in laboratory settings (Powlik et al. 1997). In addition, the species has a broad habitat range that covers over 30 degrees of latitude from northern Mexico to southern Alaska (Ganz and Burton 1995, Dethier 1980), and neighboring populations of *T. californicus* show little gene flow (Burton et al. 1979, Burton and Feldman 1981). As a result, separate populations differentially adapt to local environmental conditions, and

evolution can occur relatively rapidly within isolated rock pools. Remarkably, some isolated populations have diverged genetically to the point where they are now reproductively incompatible (Ganz and Burton 1995). Although previous studies have shown that the heat tolerance of *T. californicus* increases with decreasing latitude (Kelly et al. 2011, Kim and Walls 2011, Willet 2010), no studies have investigated whether a similar pattern exists for this species' resistance to cold temperatures. By examining the cold resistance of a species for which a known latitudinal trend in heat tolerance exists, we can assess the capacity of these organisms to handle changes in the temperature range of their habitat. In this study we used two metrics of cold tolerance to determine if any latitudinal patterns exist in the physiological abilities for cold resilience in *T. californicus*.

One of the most common assays of cold tolerance in animals is an examination of their ability to withstand freezing conditions (Terblanche et al. 2011). *T. californicus* inhabits small upper-shore rock pools in the intertidal zone that are often isolated from the ocean for several days at a time (Burton et al. 1979, Powlik et al. 1997). As a result, these pools can experience extreme variations in temperature on both a daily and seasonal basis, and populations at northern latitudes must occasionally deal with ice forming in their pools (McAllen and Block 1997). Freeze resistant organisms can be divided into two classes: freeze tolerating species, which can survive the presence of ice crystals in their body, and freeze avoiding species, which use supercooling mechanisms to lower the freezing points of their intracellular fluids to avoid internal freezing (Schmidt-Nielsen 1997). For freeze avoiding species, the formation of internal ice crystals is lethal, and they are therefore limited to a certain range of sub-zero temperatures. Although the cryobiology of *T. californicus* has not been investigated, *Tigriopus brevicornis*, a similar

rock pool inhabitant with a wide geographic distribution in Europe, has been identified as a freeze avoiding organism. The osmoconforming nature of *T. brevicornis*, in which their internal solute concentration is equal to that of their external environment, works in conjunction with supercooling abilities to avoid any internal formation of ice (McAllen and Block 1997). Because they are members of the same genus, we assumed that *T. californicus* utilizes similar mechanisms to avoid the formation of ice crystals in their bodies. In this experiment we used freeze tolerance as a means to compare the supercooling abilities of different populations of *T. californicus* across a wide geographic range.

While freeze resistance provides a useful indication of the cyroprotection abilities of ectotherms, it is not the only ecologically relevant assay that can be used to estimate the cold tolerance of a species. In many organisms, low temperatures also induce a state of reversible dormancy several degrees above the temperature that is lethal to them (Gibert et al. 2001, Overgaard et al. 2011). This state of narcosis, defined as a chill coma, is characterized by complete immobility and a large decrease in metabolic functions (Schmidt-Nielsen 1997). Measures of the temperature at which chill-coma is entered (critical thermal minimum [CT_{min}]) are commonly used to make comparisons of cold tolerance across taxa (Hazell and Bale 2011, Macdonald et al. 2004, Ransberry et al. 2011, Terblanche et al. 2011). While several studies have demonstrated that there are interpopulational differences in the CT_{min} of terrestrial arthropods along a latitudinal scale (Castañeda et al. 2004, Castañeda et al. 2005, Gibert et al. 2001, Sisodia and Singh 2010), the chill coma characteristics of marine intertidal arthropods have yet to be thoroughly examined.

Using freeze tolerance and CT_{\min} as metrics of cold resistance, we used *T. californicus* as a model to determine if organisms widely distributed along a latitudinal gradient are adapted to regional differences in temperature. Organisms tend to develop only the level of adaptive response needed to meet an existing ecological challenge; the next level will not be developed unless changes in their environment necessitate it (Bradley 1978, Schmidt-Nielsen 1997, Slodobkin and Rapoport 1974). Given this and what we know about the greater heat tolerance of more southern populations of *T. californicus*, we hypothesized that the physiological mechanisms associated with cold resistance may exhibit a cost and would therefore be counter-selected for in populations that do not experience very low temperatures on a yearly basis. We expected to see that populations from high latitudes would demonstrate a greater cold tolerance than those from more southern localities. In other words, we predicted that the copepods would demonstrate thermal capacities that closely matched their habitat's environmental conditions.

2. Materials and Methods

2.1 Collection and maintenance of experimental copepods

T. californicus specimens were collected from five locations ranging from southern California up to Vancouver Island, BC, Canada (Table 1). Specimens were collected from 3-5 different pools within 100 m of each other at each site, after which they were placed in plastic containers and transported to the University of Washington's Friday Harbor Laboratories in Friday Harbor, WA. Five replicate cultures for each population were then established by placing 25 gravid females into plastic vials containing 100mL of seawater that had been passed through a 0.45 μ m in-line filter. Each

culture was started no more than seven days after the initial specimen collection.

Laboratory cultures were kept in an incubator maintained at 19 - 22°C with a 12-12 hour light-dark cycle. Copepod cultures were fed TetraMin Tropical Flakes fish food ad libitum, and approximately 70% (65-70mL) of the water was changed weekly. As stated above, *T. californicus* has a life cycle of approximately 20 days. In our experiments, no cultures were tested until at least 40 days after their establishment. This ensured that a minimum of two generations had grown and developed in the controlled laboratory settings, and all experimental copepods spent their entire lives in their respective culture containers. As a result, the potential confounding effects of environmental plasticity were eliminated, and differences between populations could be attributed to genetic adaptation.

Table 1. Details on the collection sites of the five *T. californicus* populations used in this study. Climate data from the most proximate weather station to each population is reported as averages from the following time periods: 1970 – 2000 for Raft Cove and Bamfield, 1998 – 2012 for Reuben Tarte, and from 1914 – 2012 for the Hopkins and Sunset Cliffs collection sites. Air temperature data for these coastal weather stations came from the National Climate Data and Information Archive from Environment Canada (http://climate.weatheroffice.gc.ca/climate_normals/index_e.html) for the two Canadian sites, and from the National Climatic Data Center (<http://www.ncdc.noaa.gov/oa/ncdc.html>) and Western Regional Climate Center (<http://www.wrcc.dri.edu/Climsum.html>) for the American localities (Willett 2010).

Locality	Latitude (°N)	Longitude (°W)	Closest weather station	Mean annual air temp. (°C)	Mean annual low air temp. (°C)	Mean annual high air temp. (°C)	Mean annual # of days at or below 0°C
Raft Cove, BC, Canada	50° 58'	128° 23'	Port Hardy, BC, Canada (50° 70' N, 127° 49' W)	8.27	0.80	17.90	56.23
Bamfield Marine Sciences Center, BC, Canada	48° 83'	125° 14'	Bamfield East, BC (48° 50' N, 127° 07' W)	9.40	1.70	18.10	52.97
Reuben Tarte, San Juan Island, WA	48° 61'	123° 10'	Friday Harbor, WA (48° 52' N, 123° 02' W)	9.72	1.89	22.06	42.40
Hopkins Marine Station, CA	36° 62'	121° 90'	Monterey NWSFO, CA (36° 36' N, 121° 51' W)	13.83	5.83	21.44	1.50
Sunset Cliffs, San Diego, CA	32° 43'	117° 15'	San Diego WSO Airport, CA (32° 71' N, 117° 15' W)	17.33	8.94	24.61	0.00

2.2 Freeze tolerance experimental protocol

To investigate if *T. californicus* showed a latitudinal trend in freeze tolerance, individuals from each population were frozen in seawater and their ability to recover from the cold shock was monitored (Figure 1). To remove the potential confounding effects of gender and life stage, only adult males were tested in these trials, and the same copepod was never used twice. The adult males were removed from their culture containers using 3mL transfer pipettes and placed into Petri dishes (60 x 10 mm) containing 30‰ salt water prepared from a mixture of RO water and Instant Ocean Mix. For this and all other copepod transfers described below, we controlled for salinity by briefly placing individuals on 0.45µm filter paper to remove excess water before moving them into the experimental dishes. Twelve adult males were tested for each of four replicate cultures for every population (i.e. 48 individuals for each collection site). Before experimentation began, individuals were observed under a dissecting microscope to confirm they were healthy and of the correct life stage. The copepods were then transferred into 0.2mL PCR tubes, with four individuals and approximately 0.110mL 30‰ seawater in each tube. These were placed into a thermocycler (Model T1 Gradient, Biometra, [Germany]) and programmed to cool down from 20°C to -3°C at a uniform rate over a 120-minute period. This slow rate of temperature decrease mimicked natural conditions and allowed the copepods to adjust to the increasing cold and avoid temperature shock.

Upon removal from the thermocycler, the adult males were transferred out of the PCR tubes using 3mL pipettes and placed in sterile Petri dishes (30 x 15 mm) sitting on top of a thermoelectric chiller/heater (Model CP-065, TE Technologies, [MI, USA]).

Each dish contained 2mL 30‰ seawater (again prepared from RO water and Instant Ocean Mix) that had been previously chilled to -3°C. The temperature of the thermoelectric chiller/heater was then decreased to -8°C and the dishes containing the adult males were left on the cold source for a 90-minute period, during which solid ice crystals formed throughout the solution. Thermal paste and the construction of Styrofoam walls around the cooling surface helped to increase the efficiency of the thermoelectric plate by improving conduction and limiting the escape of cold air. Immediately after the freezing period, the dishes were returned to their original incubator set at 19°C, where they thawed and were observed over a five-day period. We were not concerned about the temperature shock of abruptly returning the copepods to 19°C because data from local temperature loggers indicated that the temperature of the rock pools *T. californicus* inhabits can increase rapidly with incoming tides and intense sunlight (Neufeld, unpublished data). For the next five days the copepods were removed from the incubator every 24 hours, during which they were observed under a dissecting microscope and the proportion of individuals to have recovered was recorded. We assumed that only individuals who were able to propel their bodies through the water had recovered from the cold stress, and at each observation the proportion of active verses inactive individuals for each replicate was recorded. It was assumed from preliminary trials that individuals who remained immobile after the 120-hour recovery period were dead, and the proportion of individuals who survived the freezing was recorded in the final observation.

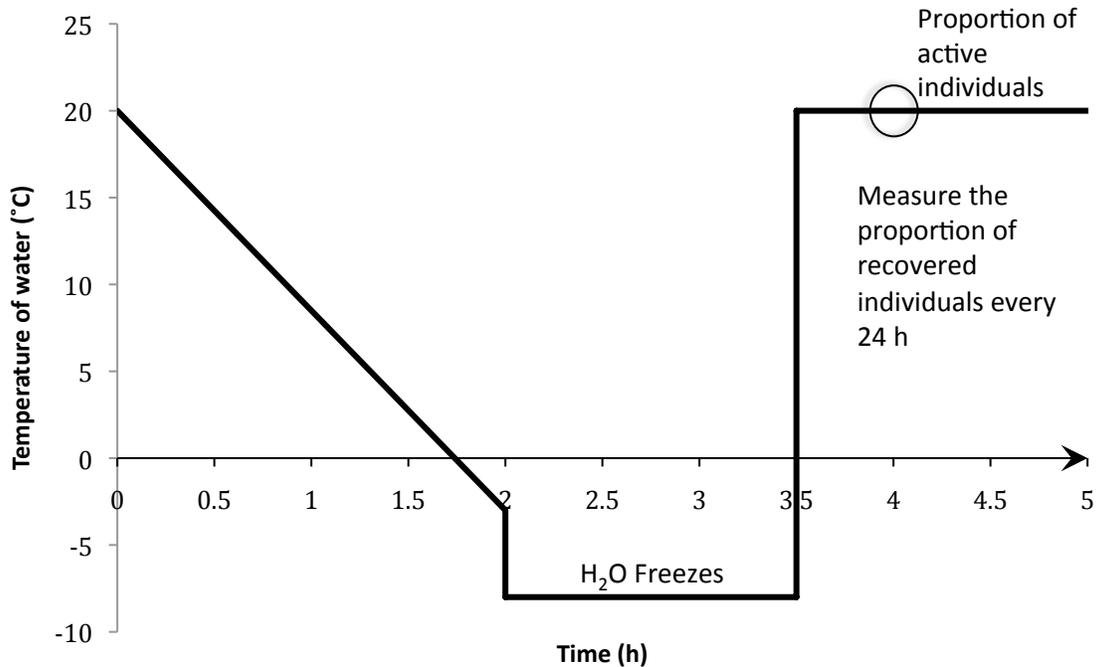


Figure 1. Experimental protocol to determine post-freezing recovery. Freeze tolerance was measured by gradually decreasing the water temperature to -3°C and then holding it at -8°C for a 90 minute period, during which freezing occurred. They were then returned to their 19°C incubator and the proportion of individuals to recover and demonstrate mobility was recorded at 24-hour observation points for five days.

2.3 Chill coma onset experimental protocol

To measure the critical thermal minima (CT_{min}) of different *T. californicus* populations, individuals were observed as they cooled down and entered into a cold-induced comatose state (Figure 2). Again, four replicate cultures from each collection site were used in this study, and only mature males were used to eliminate the possible confounding effects of life stage and gender. The experimental set-up involved the use of a TE thermoelectric chiller/heater (Model CP-065, TE Technologies, [MI, USA]). A Petri dish (30 x 10 mm) sat atop the machine, and the whole apparatus was positioned under a dissecting microscope so observations could be made throughout the experiment. Eight adult males from one replicate were pipetted into the Petri dish in one large droplet of 1.0mL 30‰ seawater. When all individuals were in place, the

thermoelectric chiller/heater was manually programmed to cool down from room temperature (ranged from 19 – 23°C) to 0°C at a rate of approximately 0.5°C per minute. Individuals were observed constantly throughout the cooling process, and the temperature at which 25 (CT_{min25}), 50 (CT_{min50}), 75 (CT_{min75}), and 100 (CT_{min100}) percent of individuals entered into a chill coma was measured using a thermocouple. Here, chill coma was defined as complete immobility, and individuals were considered to be in this state of dormancy after ten seconds without any twitching of their legs or antennae.

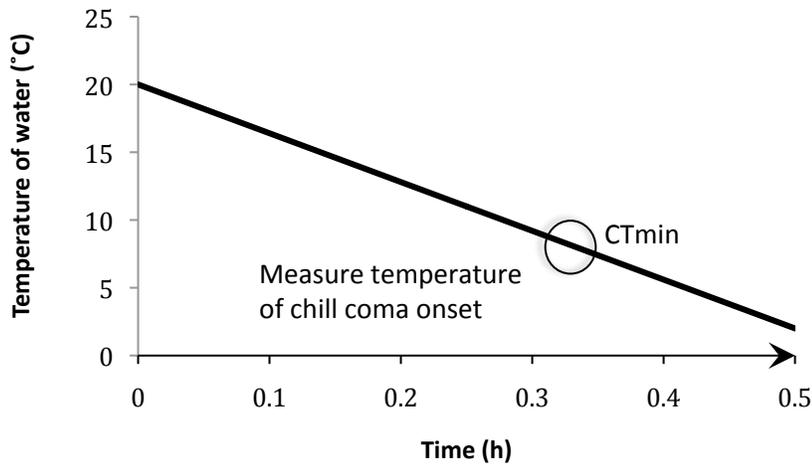


Figure 2. Protocol used to score CT_{min} temperatures. To measure these values, copepods were observed as water temperature was gradually lowered and the temperature at which they entered into a chill coma was recorded.

2.4 Statistical analysis

Three response variables within the freeze tolerance data were evaluated in our analysis: the proportion of individuals to have recovered after 24 hours, the proportion of individuals to have survived the cold stress after 120 hours, and the recovery trajectories of each population (the slope of the proportion-recovered line each replicate followed over the five-day recovery period). A one-way ANOVA was performed using population as the factor for each of these response variables to test for differences between collection sites. In addition, simple linear regressions were utilized to evaluate the relationships

between the mean annual low temperature of each population locality and these three measured variables (Table 1). To evaluate interpopulational differences in CT_{min} , a one-way ANOVA analysis was performed using CT_{min50} values. A simple linear regression was also used to test for a relationship between the CT_{min50} and mean annual low temperature of each collection site. Every one-way ANOVA analysis was accompanied by a *post hoc* Tukey-Kramer HSD test to evaluate which populations were significantly different from one another. All linear regressions were performed in RStudio v. 0.96.304, and ANOVA analyses with *post hoc* tests were performed in JMP v. 9.

3. Results

3.1 Freeze tolerance

The three northern-most populations (Raft Cove, Bamfield, and Reuben Tarte) were characterized by a high proportion of copepods recovering within the first 24 hours after the cold stress, with this number increasing only slightly over the five-day observation period. The Hopkins population had a low initial recovery rate, and the total proportion of individuals to have survived the cold stress was much lower than that of the other localities. Like the Hopkins copepods, specimens from Sunset Cliffs had very low initial recovery. However, these southern-most copepods then demonstrated a steep recovery trajectory and their final proportion of recovered individuals was very similar to those from Raft Cove, Bamfield, and Reuben Tarte (Figure 3).

Significant differences between populations were observed after 24 hours ($F_{4,15} = 131.4456$, $p < 0.001$, $R^2 = 0.972262$; Figure 4A), at which the three northern-most populations had means that significantly differed from those of two southern-most populations (Tukey-Kramer HSD, $p > 0.05$). Likewise, there were considerable

differences between populations after 120 hours ($F_{4,15} = 22.2058$, $p < 0.0001$, $R^2 = 0.855524$; Figure 4B), except here only Hopkins significantly differed from the others; Sunset Cliffs had recovered to levels statistically equivalent to those from Raft Cove, Bamfield, and Reuben Tarte (Tukey-Kramer HSD, $p > 0.05$). A comparison of each population's recovery trajectory over the five-day observation period again showed significant differences between populations ($F_{4,15} = 32.4354$, $p < 0.0001$, $R^2 = 0.896367$; Figure 4C). Only the Sunset Cliffs population had a recovery trajectory that significantly differed from the others, emphasizing that this southern-most population responded to the freeze exposure like none of the others (Tukey-Kramer HSD, $p > 0.05$).

Linear regression analysis revealed a strong relationship between the mean annual low temperature of each collection site and the proportion of individuals to have recovered from the freezing after 24 hours (slope = -0.1071 ; $p = 0.10739$, $R^2 = 0.88402$; Figure 5A), with populations from colder regions demonstrating higher proportions of recovered individuals. A strong relationship was also observed between these mean annual low temperature values and the recovery trajectory of each population (slope = 0.0009 ; $p = 0.0541$, $R^2 = 0.78593$; Figure 5C), for which copepods from warmer climates exhibited faster recovery rates. However, we found no significant relationship to exist between collection site mean annual low temperature and the proportion of individuals to have recovered after 120 hours (slope = -0.028 ; $p = 0.47099$, $R^2 = 0.18407$; Figure 5B); Sunset Cliffs had a very high survival rate compared to the Hopkins copepods.

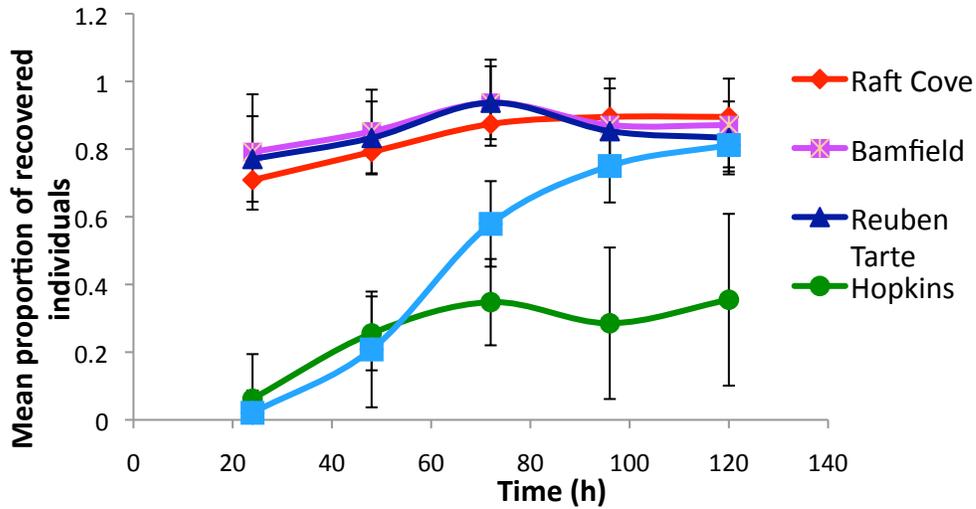


Figure 3. Mean recovery proportions for populations over a five-day period following freezing. Error bars represent 95% confidence intervals calculated using mean standard error. The legend to the right lists collection sites from north to south.

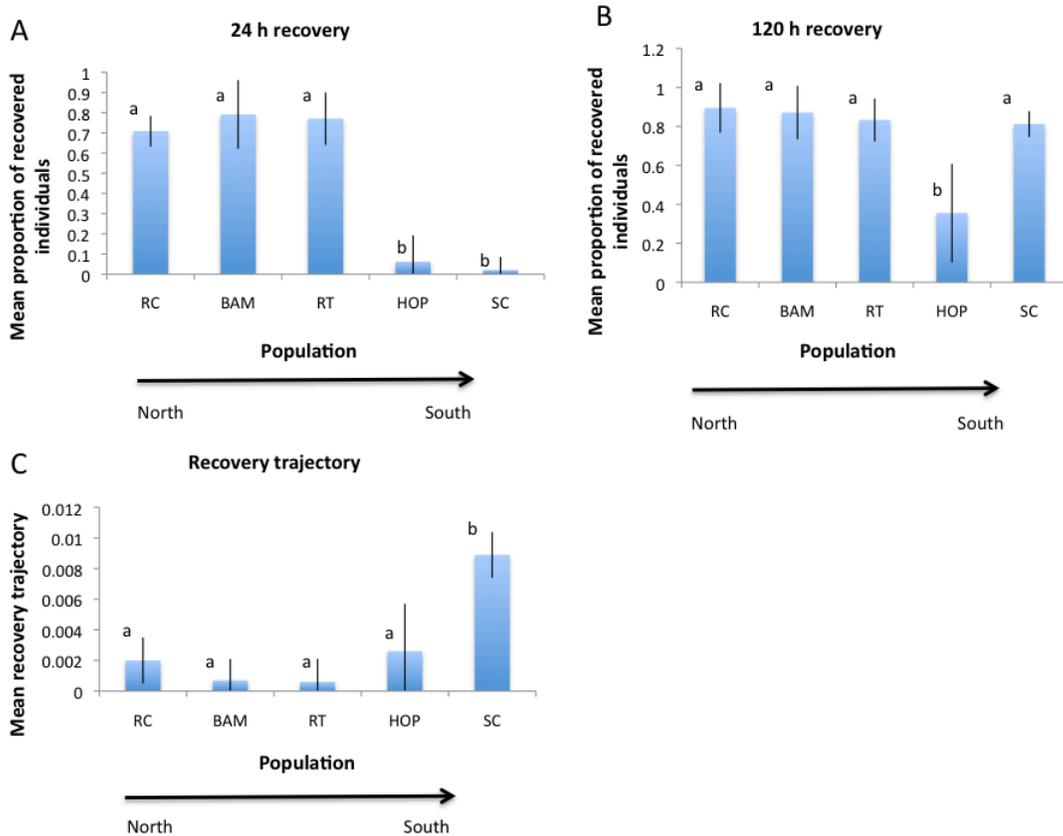


Figure 4. Freeze tolerance parameters for five populations of *T. californicus*. Populations are arranged in order of decreasing latitude, and abbreviations are as follows: RC = Raft Cove, BAM = Bamfield, RT = Reuben Tarte, HOP = Hopkins, SC = Sunset Cliffs. Error bars represent 95% confidence intervals calculated using mean standard error. Letters above bars indicate populations whose means significantly differ from each other (Tukey-Kramer HSD, $p < 0.05$). (A) The mean proportion of individuals to have recovered 24 hours after the freeze exposure, (B) mean proportion of individuals to have survived the

cold stress after a 120 hour recovery period, (C) mean recovery trajectory for each population, where higher numbers indicate a faster recovery rate.

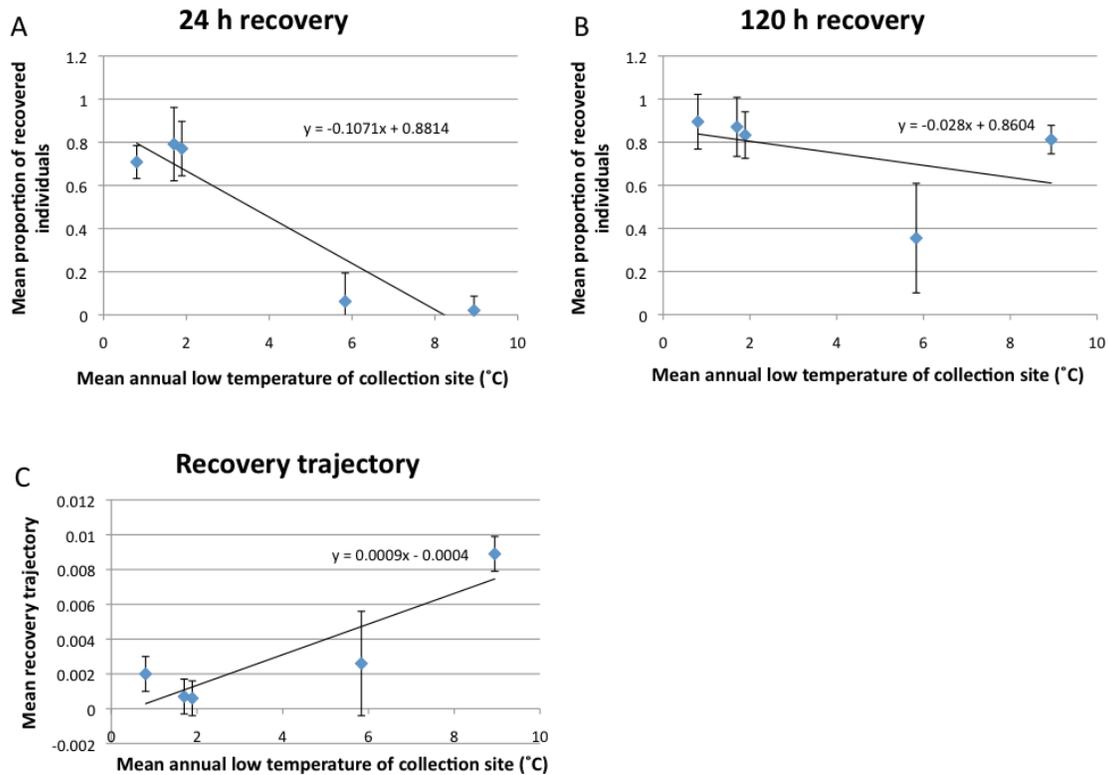


Figure 5. Regressions of freeze tolerance response variables with the mean annual low temperature of each collection site. Error bars in all graphs indicate 95% confidence intervals determined from mean standard error values. (A) Mean proportion of individuals to have recovered after 24 hours versus the mean annual low temperature of each collection site, (B) mean proportion of individuals to have recovered after 120 hours versus mean annual low temperature, (C) mean recovery trajectory versus mean annual low temperature of each collection site.

3.2 Chill coma onset

We observed significant differences between the chill coma onset temperatures of individuals from different populations at the $CT_{\min 50}$ level ($F_{4,15} = 61.7378$, $p < 0.001$, $R^2 = 0.942737$; Figure 5A). In addition, there was a significant linear relationship between locality mean annual low temperature and $CT_{\min 50}$ value (slope = 0.2963; $p = 0.00487$, $R^2 = 0.94963$; Figure 5B). Although not reported, significant differences between populations were also observed at the $CT_{\min 25}$, $CT_{\min 75}$, and $CT_{\min 100}$ levels, and similarly

strong relationships were seen between these values and locality mean annual temperature.

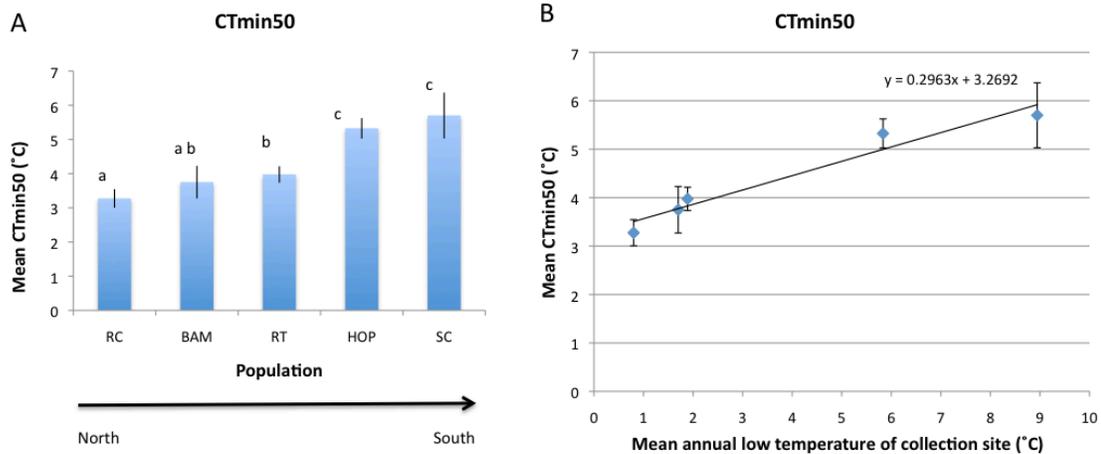


Figure 6. Parameters of chill coma analysis. (A) Mean temperature at which 50 percent of individuals from each population enter into a chill coma (CT_{min50}). Letters above bars indicate populations whose means significantly differ from one another (Tukey-Kramer HSD, $p < 0.05$). (B) A regression of average CT_{min50} values against the mean annual low temperature of each collection locality. Error bars on both graphs indicate 95% confidence intervals calculated using mean standard error values.

4. Discussion

Our results demonstrate that the cold tolerance of isolated *T. californicus* populations increases with both higher latitude and lower mean annual temperature values of collection sites. Hence, populations that experience lower temperatures in their local habitats have a greater tolerance for cold temperatures. This implies that these copepod populations have locally adapted to latitudinal differences in climate to such an extent that some populations are able to tolerate environmental conditions that others of the same species cannot. Overall, we found less variation among the three northern populations compared to the two populations from California. While this is likely due to the large distance between the Hopkins and Sunset Cliffs copepods relative to the close

proximity of the three northern localities, *T. californicus* populations north of California have been shown to be less genetically diverse than those found in the southern portion of this species' distribution (Edmands 2001, Kelly et al. 2011, Kim and Walls 2011). Since there is very little gene flow between neighboring populations, migration levels of *T. californicus* are probably low (Willet 2010). Although it is unclear how rapid evolution and environmental plasticity may help to buffer the effects of climate change in this species (Kelly et al. 2011), northern populations of *T. californicus* may be more susceptible to shifts in environmental conditions than the more genetically diverse southern populations.

Our results show that populations from different latitudes exhibit a variety of responses to being frozen. A comparison of the three northern populations (Raft Cove, Bamfield, and Reuben Tarte) with the Hopkins copepods (from central California) supports our initial hypothesis that the traits associated with cyroprotection may exhibit a cost and are therefore only selected for in populations which must deal with freezing conditions on a regular basis (Figures 3, 4, and 5). While the temperatures of the Raft Cove, Bamfield, and Reuben Tarte collection sites drop to freezing or below 42 times or more annually, this only occurs an average of 1.5 times per year in the region from which we obtained the Hopkins individuals (Table 1). Thus Hopkins copepods do not regularly encounter ice and they had a significantly higher mortality rate than the other populations, suggesting that supercooling traits have not been strongly selected for in these individuals. Even so, the fact that a small proportion of these central California individuals survived indicates that the traits necessary for avoiding the lethal formation of

internal ice crystals are present in the population, but have not been selected for in most of these copepods.

The response of the Sunset Cliffs specimens to the freeze stress was unexpected. In their natural habitat, these copepods never experience temperatures low enough to cause ice formation in their pools (Table 1). While they had the lowest recovery rate in the first 24 hours after freezing, by the end of the five-day recovery period the survival rate of the Sunset Cliffs individuals was statistically equivalent to those of the three northern populations, and much greater than those from Hopkins (Figures 3, 4, and 5). However, it took much longer for these individuals to recover compared to the northern copepods. It is therefore possible that they use completely different physiological mechanisms to cope with cold stress. For example, southern populations of *T. californicus* produce a greater abundance of heat shock proteins than do populations from colder climates (Kelly et al. 2011). Although they are traditionally only associated with heat stress, studies have shown that the production of heat shock proteins can also be triggered by low temperatures (Martinez et al. 2001, Sonna et al. 2002, Sorensen et al. 2003). It is possible that these proteins could play a role in helping organisms deal with both extreme heat and extreme cold. The response of the Sunset Cliffs copepods lends support to the theory that being able to tolerate one type of environmental stress makes an organism more adept at tolerating other stresses as well (Sunday et al. 2010); although the cellular injuries associated with heat and cold are very different, a population that has evolved to handle one type of cellular damage may be hardy enough to deal with other types of damage as well due to an overall expansion in their thermal breadth (Willet

2010). However, further molecular work is needed before definitive conclusions can be made regarding the unique freeze recovery pattern of the Sunset Cliffs population.

The results of our chill coma experiment show a significant clinal trend in which populations from higher latitudes have lower critical thermal minima (CT_{min}) values. As we expected, populations that experience colder temperatures in their natural habitats entered into a chill coma at lower temperatures than those from warmer regions. This is ecologically relevant because being in a state of narcosis makes individuals much more susceptible to predation, and interrupts activities such as feeding and reproduction (Castañeda et al 2004, Gibert et al 2001). Therefore, it is beneficial to remain active in cooling waters for a longer period of time, and the populations from the north have locally adapted to be functional in a broader range of low temperatures than those from lower latitudes.

Our study suggests that some populations have narrower thermal capacity ranges than the species as a whole. Individuals from different populations may not have the same lower lethal temperature limits, meaning that decreasing winter minimum temperatures will not affect all populations uniformly as global climate change continues (Castañeda 2004). As a result, bioclimate models that treat the environmental tolerance of a species as static across its entire habitat distribution could provide inaccurate estimations of extinction potential. Overall, our results agree with previous studies in suggesting that current models predicting biological responses to climate change be modified to account for local adaptation to both heat and cold (Kelly et al. 2011, Kim and Walls 2011). Further research on this topic may include comparing metrics of heat and cold tolerance in *T. californicus* to better understand potential costs and fitness trade-offs

associated with temperature extremes. In addition, future studies will examine the molecular physiology of different populations to determine exactly what mechanisms they may be using to deal with thermal extremes, and how these might vary in copepods from different latitudes.

Acknowledgements

The financial support of the Blinks, NSF REU, and BEACON fellowship programs is gratefully acknowledged. We would like to thank Dr. Sophie George, Dr. Billie Swalla, Dr. Adam Summers, Dr. Emily Carrington, and Jaquan Horton for their hard work in organizing and running this summer's undergraduate research program. In addition, thanks to Tiffany Kim for her hard work in our lab, and to Dr. Molly Jacobs and Dr. Ann Jane Tierney for providing comments and critique as we prepared our manuscript. Finally, we thank Friday Harbor Laboratories and the University of Washington for providing the facilities used in this study.

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