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Comparative freeze tolerance and physiological adaptations of three species of vertically distributed rocky intertidal gastropods from southeast Alaska

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ABSTRACT

Tidal emersion temperatures are ameliorated on the coast of the continental United States because of the timing of low spring tides but spring low tides occur during the day in the summer and during the night in the winter in the inside passage north of Seattle. Extreme low air temperatures during the emersion of intertidal organisms at the northern intertidal sites render them vulnerable to freezing. To quantify the effects of freezing air temperatures on the supercooling point and freeze tolerance of *Littorina sitkana* from the upper intertidal, *Nucella lima* from the mid-intertidal, and *Nucella lamellosa* from the low intertidal, ambient temperatures were monitored with Hobo probes in two transects encompassing their intertidal distribution. To simulate the range of their emersion time the 2 and 5 h supercooling and freeze tolerance of *N. lamellosa*, 5 h supercooling and freeze tolerance of *N. lima*, and 5 and 10 h freeze tolerance of *L. sitkana* were determined. Seasonal variability in the degree of hydration and free amino acid concentrations of these species was also determined. The number of days when emersion temperature fell below 0 °C increased with intertidal height as did the number of hours per day when the emersion temperature was <0 °C. The freeze tolerance temperature of these species increased directly with their intertidal distribution in 5 hour emersion exposures and did not change with emersion temperature duration typical of their intertidal range. *L. sitkana* and *N. lima* were more tolerant of freezing in the winter than in the summer but there was no seasonal difference in the freeze tolerance of *N. lamellosa*. The supercooling point of the species varied directly with their intertidal distribution, did not vary seasonally and reduced the period of time during emersion when they were subject to freezing. Free amino acids contributed to the increased freeze tolerance of *L. sitkana* and *N. lima* in the winter. Although the total free amino acid pool of *N. lamellosa* was significantly higher by 30.6% in the winter than the summer, no single free amino acid was higher. There were significantly higher concentrations of total FAA (54.6% increase), taurine (22%) and glycine (2150%) in *N. lima* in the winter. There were significant increases of total FAA (27.6% increase), taurine and glycine in the winter compared to the summer in *L. sitkana*. These FAA are important compatible colligative osmolytes which enhance winter freeze tolerance.

1. Introduction

The interaction of climate and the timing of low tides in the rocky intertidal zone of the west coast of North America create a complex mosaic of thermal environments during emersion that is more thermally stressful at northern locations than at southern sites (Helmuth et al., 2002). Although seasonal variation in seawater temperature is small, tidal amplitude is large and exposure to air temperatures which deviate from seawater temperature may be significant in both the summer and winter (Helmuth et al., 2006). Air temperatures during tidal emersion are ameliorated on the coast of the continental United States because low spring tides occur at night during the summer and in the day during the winter. In contrast, spring low tides occur during the day in the summer and during the night in the winter in the inside passage north of Seattle. Air temperatures in the mid intertidal zone fell as low as −12.79 °C in February 2008 at Bridget Cove just north of Sunshine Cove, Alaska (Stickle et al., 2010). More extreme air temperatures during the emersion of intertidal organisms at the northern intertidal sites render them more vulnerable to emersion temperature stress (Helmuth et al., 2002, 2006).

The severity and duration of temperature stress on intertidal organisms is dependent on the air–water temperature differential and the intertidal distribution of the species. Intertidal invertebrates living in temperate regions of the world are generally freeze tolerant. Osmotic and mechanical damage to their tissues is limited as a result

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of freezing of the extracellular fluid that draws water out of the intracellular fluid compartment (Murphy, 1983; Loomis, 1995). The intertidal height distribution of the species and pattern of tidal cycles determines the duration of exposure to emersion events (Murphy, 1983; Stickle et al., 2010). Adaptations to freezing include supercooling, the synthesis of proteinaceous or the use of bacterial ice nucleators, the synthesis of cryoprotectants and behavioral avoidance through migration to a lower position on the shore, into crevices, into sediment at the base of rocks, or under rocks (Loomis, 1995; Sinclair et al., 2004; Stickle et al., 2010).

Gastropods are important members of rocky intertidal communities. Cold hardiness of rocky intertidal molluscs has been thoroughly reviewed by Ansart and Vernon (2003). All species studied are freeze tolerant; the upper intertidal salt marsh pulmonate snail, *Melampus bidentatus* and the mid-tidal *Nucella lima* exhibit a supercooling point for freeze avoidance which lowers the freezing point of the snail below that of the osmolality of the extracellular fluid compartment during the initial aerial exposure (Hayes and Loomis, 1885; Hilbish, 1981; Loomis, 1985; Madison et al., 1991 and Stickle et al., 2010). Because the duration of aerial emersion increases with intertidal height, the duration of freeze exposure should be considerably shorter during emersion for the mid-tidal *N. lima* (Stickle et al., 2010) than the upper tidal *M. bidentatus* (Loomis, 1995). The time elapsed as a result of snails not freezing because of their supercooling point should occupy a higher percentage of the emersion time of *N. lima* compared with *M. bidentatus*.

In order to assess the effects of freezing air temperatures on the supercooling point and freeze tolerance of *Littorina sitkana* from the upper intertidal through the upper half of the mid-intertidal mussel zone, the mid-intertidal zone *N. lima*, and the below zero to shallow subtidal *Nucella lamellosa* ambient temperatures were monitored in two transects of Hobo temperature probes encompassing the snail's intertidal distribution. In order to simulate the range of emersion time 2 and 5 h supercooling point and freeze tolerance of *N. lamellosa*, 5 h supercooling point and freeze tolerance of *N. lima*, and 5 and 10 h supercooling point of *L. sitkana* were determined. Finally, seasonal variability in the degree of hydration and free amino acid concentrations of *L. sitkana*, *N. lima* and N. lamellose were determined. A more detailed study of the freeze tolerance on *N. lima* is published by Stickle et al. (2010) but comparative data are presented here for understanding freeze tolerance of gastropods as a function of their rocky intertidal distribution. Experiments were designed to test the null hypothesis that there is no seasonal thermal regime or intertidal height effect on the supercooling point, freeze tolerance, degree of hydration, and free amino acid composition of *L. sitkana*, *N. lima*. and *N. lamellosa*.

2. Methods

Two vertical transects of ProV2 Hobo temperature probes covered with protective sleeves were deployed at Bridget Cove which is 1.1 km north of Sunshine Cove (latitude 58°30.8′ N; longitude 134°55.8′ W) along Lynn Canal, AK as described in Stickle et al. (2010). The two transects were separated by 40 m; transect 1 had a shallower slope than at transect 2. Probes were deployed at mean high high water (+5.0 m), the upper (+2.5 m) and lower (1.5 m) edges of the mid intertidal range of *N. lima*, and the zero tide level (0 m). One additional probe was deployed in the middle of the high intertidal zone (+3.5 m) and two probes were partially buried at the lower edge of the mid intertidal range (1.5 m). One of the buried probes was inserted into tightly packed particulate material (transect 1) and one was placed in loosely packed particulate material (transect 2). Sunshine (Ravioli) and Bridget Coves are salinity stressed during the summer due to meltwater from the Eagle and Herbert rivers (Stickle and Denoux, 1976).

Four aspects of the temperature records were extracted from the data set as described by Stickle et al. (2010). The degree hours when the ambient air temperature was below each species supercooling point was determined. This value was determined by multiplying the difference between the negative temperature value and the winter supercooling point of the species every time it occurred times 5 min per recording plus 2.5 min before and after the recording when the probe temperatures fell below the supercooling point. The degree hours of emersion below the supercooling point were calculated by multiplying the hours of emersion below the supercooling point times the recorded temperature below the supercooling point and expressed as °h.

Freeze tolerance of the three species of gastropods was compared for snails collected on July 19, 2007, and January 7, and June 6–7, 2008. The freeze tolerance of 20 *L. sitkana* per treatment from the high intertidal zone was determined by exposing separate groups of snails to five temperatures below freezing for both 5 and 10 h of emersion. *N. lima* (15 snails per treatment) collected from the mid-intertidal zone were exposed to five temperatures below freezing for 5 h emersion periods and 12 *N. lamellosa* from the below the zero tidal level were exposed to five temperatures below freezing for both 2 and 5 h of emersion. Comparisons could then be made of seasonal variation in freeze tolerance of each species over a common 5 h emersion and also on the effects of season and duration of exposure indicative of that species position in the intertidal zone. The freeze tolerance and super cooling points of each species was determined by procedures described in Stickle et al. (2010). Snail supercooling points were determined for the July 19, January 7, and June 6–7, 2008 collections of *N. lima* and *N. lamellosa* by measuring individual supercooling temperatures. The supercooling point of *L. sitkana* was only determined in the February 2, 2009 collection.

In order to determine free amino acid concentrations it was necessary to determine percent water values for summer and winter collections as detailed in Stickle et al. (2010). *N. lima* and *N. lamellosa* were dissected into gonad-digestive gland, remaining visceral mass, and foot components. Only the foot was sampled in this study. All soft tissues of *L. sitkana* were used for wet weight and free amino acid determination. Percent tissue water values were then determined, and after arc-sin transformation, collection date data were compared with unpaired Students t-tests.

Wet tissue samples stored at −80 °C at LSU were homogenized and analyzed for free amino acid concentrations as detailed in Stickle et al. (2010). Every sample was analyzed in duplicate and the usual sample size was 4–5.

Free amino acid data expressed as mmol kg wet weight^{-1} were analyzed with two-way ANOVA with season and free amino acids being the main effects and an interaction term was also calculated. The Bonferroni post hoc test was also used to test for significant differences between individual free amino acid concentrations as a function of season.

3. Results

All three species of gastropods were emersed for a significant number of days when air temperature was below 0 °C during our study (Fig. 1A). *N. lamellosa* was collected about a meter below the 0 tide probes in the winter. The average number of days when the air temperature fell below 0 °C for the zero tide probes in the two transects was 32. *N. lima* was exposed to air temperatures <0 °C for an average of 45 days at the lower end of its intertidal range (+1.5 m) and 95 days at the upper end (+2.5 m). The probe which had tightly packed sediment around it at the +1.5 m level was exposed to temperatures <0 °C on 9 days of emersion while the loosely packed probe was exposed to emersion temperatures <0 °C for 88 days. *L. sitkana* was exposed to 45 days of air temperatures below 0 °C at the lower end of its vertical distribution (+2.5 m) and 82 days at the mid upper intertidal zone (+3.25 m). The temperature recordings at the mean high high water level (+5 m) was only below 0 °C f or 53 days due to snow cover which insulated the probe for a number of days.

The number of hours per day on days when air temperature fell below 0 °C to which *N. lamellosa* at the 0 tide level were exposed to freezing air temperatures averaged 2.41 h (Fig. 1B). The probe which