

# Thermal tolerance of *Crepidula fornicata* (Gastropoda) life history stages from intertidal and subtidal subpopulations

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**ABSTRACT:** The ability to withstand high summer temperatures is an extremely important determinant of species distributions in the intertidal zone; most marine organisms cannot live intertidally because of the harsh conditions experienced during aerial emersion. The members of some species, though, live both intertidally and subtidally, and their physiological tolerance to stressors may differ depending on their genetic connectivity, acclimatization, and differential postsettlement mortality. Furthermore, tolerances of organisms at different life-history stages within both habitats may differ and will play an important role in determining adult distributions. To determine the effect of habitat and life-history stage on physiological tolerance and the likely contribution of upper thermal tolerance limits to the native distribution of an important invasive species, the slippershell snail *Crepidula fornicata*, we compared the thermal tolerance of intertidal and subtidal embryos and adults in relation to conditions that they experience in the field. We found *C. fornicata* living both intertidally and subtidally in Bissel Cove, Rhode Island, where they experienced drastically different thermal conditions. Intertidal individuals achieved body temperatures as high as 42°C, more than 15°C higher than those recorded in subtidal conspecifics. However, subtidal individuals had remarkably high thermal tolerances that were nearly identical to those of intertidal conspecifics (lethal range = 33–37°C following a 3 h exposure). Furthermore, embryos were even more tolerant to high thermal stress than adults. These results are surprising: the few previous studies that have compared thermal tolerances among individuals at different life-history stages have found differences among them, and early stages are generally more sensitive than adults. Interestingly, the markedly higher temperatures that intertidal animals experienced in this study had little effect on their thermal tolerance. Intertidal (but not subtidal) slippershell snails are now living dangerously close to their upper thermal limits and will almost certainly be relegated to the subtidal as global temperatures rise.

**KEY WORDS:** Intertidal · Subtidal · *Crepidula* · Thermal stress · Physiological tolerance

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## INTRODUCTION

The intertidal zone is one of the most stressful environments on the planet. When the tide recedes, intertidal organisms must cope with rapid and dramatic changes in conditions such as temperature, food supply, predation pressure, humidity, and salinity (e.g. Davenport et al. 1980, Ellis et al. 2007, Hunt & Denny 2008, Szathmary et al. 2009, Iacarella & Helmuth

2012). Moving along the range from the subtidal up through the intertidal zone the intensity of these factors increases, and pressures selecting for traits that confer the ability to live through such changes markedly increase. It is not surprising that this gradient of physical and biological stressors at least partially controls the distribution of species in the intertidal zone, and is thought to play an important role in the evolution and ecology of many marine intertidal

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species (Connell 1972, Stillman & Somero 1996, Denny & Wethey 2001, Somero 2002, Tomanek & Helmuth 2002, Davenport & Davenport 2005).

Among all of the difficulties associated with living intertidally, temperature plays an extremely important role in determining species distributions (e.g. Somero 2002, Stillman 2002, Wethey 2002, Gilman et al. 2006, Helmuth et al. 2006). Thermal tolerances of closely related species within the intertidal zone often correlate with the magnitude of thermal stress that organisms of those species experience, due to their vertical position on the shoreline (Tomanek & Somero 1999, Stillman & Somero 2000, Stillman 2002, Stenseng et al. 2005). For example, Stillman & Somero (1996) have shown that porcelain crabs living high in the intertidal zone can maintain higher levels of aerial oxygen consumption, have little whole-body lactate accumulation, and have higher thermal tolerances when compared to low-intertidal congeners. Intertidal species require specialized adaptations such as high physiological tolerance to thermal stress, because although they are physiologically capable of living subtidally, they often do not live there due to intense predation and competition (e.g. Connell 1972, Perez et al. 2009, Bourdeau 2011). Similarly, organisms that can compete subtidally are unlikely to be found in the intertidal zone because it is such a harsh environment.

Though most marine animals can be found either intertidally or subtidally, some species live both intertidally and subtidally (e.g. Dame 1972, Fletcher 1984, Saier 2002, Schaffmeister et al. 2006). Though researchers have investigated phenotypic differences among conspecifics living at different tidal heights within the intertidal zone, relatively few studies have investigated the adaptations of single-species populations that live both intertidally and subtidally. Due to differential mortality, acclimatization, or genetic differentiation, we might expect to see differences among intertidal and subtidal subpopulations, especially when observing physiological tolerances to stressors. In fact, in the few studies available, when intertidal and subtidal conspecifics have been compared, behavioral, physiological, and morphological differences among the 2 groups have commonly been found (e.g. Sharp et al. 1994, Bingham et al. 1997, Altieri 2006, Weihe & Abele 2008). However, though there is increasing evidence for local adaptation in marine invertebrates across a wide range of spatial scales (reviewed by Sanford & Kelly 2011), phenotypic differences among many populations do not exist (e.g. Kuo & Sanford 2009, Sanford & Worth 2010) due to extensive genetic mixing and

high degrees of plasticity (reviewed in Sanford & Kelly 2011).

Studies that assess the physiological tolerances of marine species and those that compare tolerances among conspecifics from different populations generally focus on a single life history stage. When multiple life history stages have been compared, differences in physiological tolerances to various stressors have often been found, with early life history stages often more vulnerable to stressors than adults (e.g. Crisp & Ritz 1967, Gosselin & Chia 1995, Qiu et al. 2002, Freitas et al. 2010). Since early life history stages (embryos, hatchlings, juveniles) are often found in the same habitat as adults, the ability of all life history stages to endure physiological stressors is important for an organisms' ability to survive in a particular habitat like the intertidal zone.

The gastropod *Crepidula fornicata* is one species with conspicuous intertidal and subtidal populations; in New England their populations extend from at least 5 m below low tide up into the low/mid intertidal zone (Lindsey et al. 2006, C. M. Diederich pers. obs.). Though *C. fornicata* is widely studied and often collected both intertidally (e.g. Thielges et al. 2003, Diederich et al. 2011) and subtidally (e.g. Le Cam & Viard 2011, Hoch & Cahill 2012), the upper thermal tolerance of this species and the degree to which its members are adapted to live in either zone has not been reported. The effects of temperature on members of this species may be particularly important since individuals of *C. fornicata* are sessile as adults—and thus cannot rely on behavioral adaptations to avoid thermal stress—and brood developing embryos in the mantle cavity for several weeks (Conklin 1897), exposing those embryos to the same temperature fluctuations experienced by the adults.

Investigating the thermal conditions that *Crepidula fornicata* experience and their tolerances to these conditions in various life-history stages allows us to explore the degree to which the environment shapes the phenotypic variation in a population. Furthermore, understanding the thermal physiology of intertidal and subtidal *C. fornicata* is important because this species is not only a key member of its native communities in the western Atlantic, but is also an important invasive species in many areas—most notably France and the UK (Blanchard 1997). With global temperatures rising, learning how close these organisms are living to their thermal maximum and how much variation exists in upper thermal tolerance within the population is important for predicting future changes in distribution in response climate change.

In this study, we first aimed to document the subtidal-to-intertidal distribution of *Crepidula fornicata* in a Rhode Island population where it is extremely abundant. In order to learn just how different the conditions are that intertidal and subtidal animals face, we then estimated the temperatures of intertidal and subtidal animals using biomimetic temperature loggers during the summer months in that population. Finally, to determine the effect of environment on thermal tolerance and the role of temperature in controlling the upper distribution of this species, we investigated the tolerances of field-acclimatized embryos and adults as well as laboratory-reared *C. fornicata* juveniles to thermal stress.

## MATERIALS AND METHODS

### Population characteristics

All experiments were performed on individuals from a population in Bissel Cove near Wickford, Rhode Island, USA. *Crepidula fornicata* adults are very abundant in the tidal channel leading from the cove to Narragansett Bay, and in the associated intertidal and subtidal areas adjacent to the cove. Surveys of intertidal and subtidal subpopulations were performed in September 2011 by running horizontal transects at different tidal heights (+0.6 m, +0.4 m, +0.2 m and -1.0 m mean low lower water). Hereafter, any mention of tidal height or water level is made in reference to the mean low lower water (MLLW) mark. Five square plots (each 1 m<sup>2</sup>) were placed along the transect every 2 m, and all *C. fornicata* in each plot were counted. Average emersion time was determined over the months of June, July and August 2010 and 2011 using verified water level data from a nearby National Data Buoy Center buoy (operated by the National Oceanic and Atmospheric Administration) located at Quonset Point, Rhode Island (Station QPTR1-8454049). During Hurricane Irene on 27 and 28 August 2011, the predicted water levels were used in computing average emersion times, as the actual water levels on those days were drastically different than those predicted.

### Biomimetic temperature loggers

To determine the thermal environment that intertidal and subtidal *Crepidula fornicata* experience, we deployed biomimetic temperature logging devices (hereafter termed 'robosnails') intertidally and subti-

dally from June to August 2011. Similar devices have previously been successfully constructed and deployed in the field to record approximate tissue temperatures of other mollusks (e.g. Helmuth & Hofmann 2001, Lima & Wetthey 2009, Seabra et al. 2011), though this is the first time such a device has been used for *C. fornicata*. A Thermochron® iButton® (Part # DS1921G – Maxim Integrated Products) that was set to record temperature every 15 min was placed into an empty *C. fornicata* shell and the shell was subsequently filled with 3M Scotchcast™ 2130 Flame Retardant Compound, which hardens after mixing and has been shown to mimic tissue temperature very well (Lima & Wetthey 2009). We further confirmed the usefulness of the Scotchcast™ compound as a suitable substance for accurate estimates of tissue temperatures by mimicking intertidal conditions in the laboratory and heating (Philips™ 90W heat lamp) adjacent robosnails and live animals in air (live animal vs. robosnail,  $r = 0.961$ ). Due to the size of the loggers, only larger adult *C. fornicata* shells could be used for robosnails, but we found that tissue temperatures (sampled in the field) of animals within a stack of *C. fornicata* varied by only ~1.1°C (range 0.6–2.0°C). The robosnail was attached to a small rock with a thin layer of the Scotchcast™ compound. Robosnails were deployed among living *C. fornicata* in the intertidal zone at +0.4 m and subtidally at -1.0 m MLLW and were swapped out with new robosnails approximately every 15 d due to their finite recording capabilities. The intertidal zone in Bissel Cove is composed mainly of rocks and empty *C. fornicata* shells that do not provide shade or refuge from conditions during aerial exposure, and thus robosnails were placed in areas that experienced conditions that were identical to living *C. fornicata*. Temperature loggers were excised from used robosnails and temperature data was downloaded using OneWire-Viewer software (Maxim Integrated Products).

### Thermal stress in adults

Adult *Crepidula fornicata* were collected at Bissel Cove, RI from intertidal (approximately +0.4 m MLLW) and subtidal (-1.0 m MLLW) habitats in June and August 2010 and August 2011. This species is sedentary, and forms stacks of several members (usually 2–10) presumably to ensure reproductive success. Stacks are composed of small, newly metamorphosed males on top and successively larger, older animals on the bottom. *C. fornicata* is a protandrous hermaphrodite; the lowest member

of a stack is always female with transitional individuals and smaller males higher in the stack (Coe 1936). Top members of each stack were removed so that only the bottom-most member of the stack remained attached to the substrate (empty *C. fornicata* shell or rock); thus all adults collected were female (size range of 20. to 41.4 mm, longest shell dimension). Adults were transferred to the laboratory and all thermal stress experiments were performed within 48 h of collection, to avoid letting the animals acclimate to laboratory conditions (e.g. Widdows & Bayne 1971, McMahon & Payne 1980). Animals were measured and separated into size classes, then distributed into treatments prior to thermal stress to ensure homogeneity of size among replicates and treatments. Seawater was filtered to 1  $\mu\text{m}$  and heated to the desired temperature (measured with Control Company Traceable® waterproof digital thermometers) in 5 l plastic aquaria, and placed in incubators (Percival, Model #I-30BL) to maintain constant temperature throughout the experiments. After seawater in the incubators remained at the desired temperatures for ~1 h, animals were placed into the heated seawater where they remained for 3 h (5 replicates of at least 8 animals for each treatment). A temperature of 23°C was chosen for the control stress (and recovery) temperature because that was the typical subtidal temperature recorded in the field during the time of the experiments, while stress temperatures of 32 to 37°C were chosen because pilot experiments revealed these temperatures (for a 3 h duration) to be realistic on a particularly hot summer day.

Animals were thermally stressed in water, not air, to ensure that their tissues reached the desired temperature and to avoid the additional stress of desiccation. Water temperatures were monitored throughout the experiments and they did not fluctuate more than 0.4°C. At the conclusion of the 3 h thermal stress period, animals were transferred to individual glass dishes with ~75 ml phytoplankton suspension per dish (equal parts *Isochrysis galbana* clone T-ISO and *Dunaliella tertiolecta* clone DUN) at 23°C. Phytoplankton suspensions were changed once after 24 h. Mortality was determined 24 and 48 h after the end of the thermal stress by touching a probe to the head of the animal and monitoring muscular response. At 24 h after the thermal stress, animals that could not easily be pried from the substrate were scored as alive. At 48 h after the thermal stress all animals were pried off their substrate and prodded for muscular response.

### Collecting and maintaining animals and thermal stress in juveniles

In order to obtain juveniles for studies of variability in thermal tolerance, adult *Crepidula fornicata* were collected from Bissel Cove, Rhode Island (see above) in June, July and August 2010 and 2011. Animals were maintained at 23°C in glass aquaria of aerated seawater (30 psu). Adults were fed phytoplankton suspensions composed of a mixture of T-ISO and DUN once or twice daily and water was changed every other day. Larvae released by adults were collected on 120  $\mu\text{m}$  mesh filters, rinsed with seawater, and transferred to glass aquaria in 0.45  $\mu\text{m}$ -filtered seawater. Larvae were fed T-ISO at  $\sim 18 \times 10^4$  cells  $\text{ml}^{-1}$  (Pechenik & Lima 1984, Pechenik et al. 2002) with water changed every other day. When larvae reached ~900  $\mu\text{m}$  they were exposed to 20 mM excess KCl in seawater for 6 h to induce metamorphosis (Pechenik & Heyman 1987, Pechenik & Gee 1993), which does not affect juvenile growth or survival (Eyster & Pechenik 1988).

Juveniles were then maintained in glass aquaria of aerated seawater (changed daily), and fed T-ISO suspensions twice daily until their shell lengths (longest dimension) reached an average size of ~2.5 mm (reared for ~10 d after metamorphosis). Thus, juveniles from both intertidal and subtidal mothers spent at least 3 wk exposed to identical laboratory conditions before experimentation and were presumed to be fully acclimated to laboratory conditions by then (Bayne 1976, Newell & Kofoed 1977). Thermal tolerance experiments were then performed identical to adults (see above), except that juveniles were placed in 6-well plastic tissue culture plates (1 juvenile per well), and the plates were submerged in the temperature-controlled seawater for the duration of the experiment. After the end of the thermal stress, juveniles were removed from the incubators and the water in each well was replaced with 23°C water containing T-ISO. Phytoplankton suspensions were replaced once after 24 h. Mortality was determined at 24 and 48 h after thermal stress by observing pedal and head movement, monitoring contraction into the shell when stimulated with a stream of water, and observing heart beat (visible through the shell at this stage of development).

### Thermal stress in embryos

To determine the effect of environment and life history stage on thermal tolerance, experiments were

performed on embryos collected from intertidal and subtidal *Crepidula fornicata* adults from Bissel Cove, Rhode Island. Adults were gently pried from their substrate and egg masses were carefully removed. The color of the egg mass is a good indicator of the stage of development of the embryos contained within; only dark-grey, late-stage egg masses that had been exposed to the typical environmental conditions for an extended period of time (weeks; Conklin 1897) were used for these experiments. To confirm that all embryos were at the same stage of development before experimentation, egg masses were dissected in the laboratory and only late-stage, shelled veliger larvae of approximately 300  $\mu\text{m}$  (longest shell length) were used. Since embryos that are prematurely removed from the protective care of their mothers die soon after removal (Conklin 1897, C. M. Diederich pers. obs.), they were subsequently placed in vials containing 0.22  $\mu\text{m}$  filtered seawater with 50  $\text{mg l}^{-1}$  streptomycin sulfate and 40  $\text{mg l}^{-1}$  penicillin G (Henry et al. 2006). This antibiotic-treated seawater allows for survival to maturity and was used for all experiments containing embryos.

Thermal tolerance experiments were performed on all embryos within 24 h of collection to avoid laboratory acclimation. Prior to thermal stress, egg masses were sliced open and embryos were emptied into a small glass dish. They were mixed thoroughly and randomly portioned into replicates (25–30 embryos per replicate, 5 replicates per treatment). Embryos were pipetted into small glass dishes containing water at the desired temperatures and maintained at that temperature for 3 h. At the end of the thermal stress, embryos were removed from the heated water and transferred to water at 23°C. Water was changed after 24 h. Mortality was monitored by observing swimming and muscle movement at 24 and 48 h after the end of the stress.

### Data analysis

Densities of *Crepidula fornicata* at different tidal heights were compared using an ANOVA with a Bonferroni multiple comparisons test to determine specific differences among snail densities at each tidal height.

In order to meet the assumptions of the statistical tests, all percent survival data were arcsine transformed before specific comparisons were made (GraphPad Prism Software v. 4.03). In previous studies, the amount of time between the end of the stress and the measurement of mortality is highly variable

(e.g. Sanders et al. 1991, Tomanek & Somero 1999, Hammond & Hofmann 2010, Sorte et al. 2010, Zippay & Hofmann 2010). Furthermore, in our pilot studies many animals that survived the first 24 h after the stress ended died in the following 24 h. Therefore, we checked mortality of all individuals at both 24 and 48 h after the end of the thermal stress. For each thermal tolerance experiment, differences in mortality between 24 and 48 h after the end of the thermal stress were compared using paired *t*-tests matched for each replicate. In animals that were kept in the laboratory for longer than 48 h, no additional mortality was observed.

To determine if embryos were more susceptible than adults to death from thermal stress, comparisons were made between the survival of these 2 life history stages using 2-way ANOVAs with temperature and life-history stage as independent variables. Comparisons were only made between embryos and adults collected during the same time of year (to control for seasonal effects on thermal tolerance) and that originated from the same tidal height (to control for the effect that environment may have on thermal tolerance). Experiments performed on intertidal individuals in August 2010 and August 2011 and subtidal individuals in August 2010 fit the requirements of this statistical test.

To determine the effect of tidal height on thermal tolerance, comparisons were made between the survival of individuals using 2-way ANOVAs with temperature and tidal height as independent variables. Comparisons were only made between animals at a particular life-history stage (embryo or adult; to control for potential effects of life history stage on thermal tolerance) collected during the same time of year (to control for seasonal effects on thermal tolerance). Individual thermal tolerance comparisons between intertidal and subtidal animals at a single temperature and recovery time were determined after ANOVA using Bonferroni multiple comparisons tests ( $\alpha < 0.05$ ). Experiments performed on adults in June and August 2010 and embryos in August 2010 and June 2011 fit the requirements of this statistical test.

For thermal tolerance experiments with laboratory-acclimated juveniles, all larvae and juveniles were treated identically before and after thermal stress (see above). This allowed us to pool the results from the 3 experiments in which juveniles came from intertidal mothers, and pool the results of the 3 experiments in which juveniles came from subtidal mothers, to determine if there were differences in thermal tolerance between intertidal and subtidal organisms. Comparisons were made between inter-

tidal and subtidal juveniles using a 2-way ANOVA with temperature and mother's habitat (i.e. intertidal or subtidal population) as independent variables.

## RESULTS

### Population characteristics

Individuals of *Crepidula fornicata* were abundant both intertidally and subtidally in Bissel Cove, Rhode Island (Fig. 1). Densities (snails  $m^{-2}$ ) varied significantly along the vertical gradient (1-way ANOVA,  $F = 11.55$ ,  $p = 0.0003$ ), but densities in the low intertidal (+0.2 m) and subtidal (-1.0 m) were not significantly different (Bonferroni multiple comparison test,  $p > 0.05$ ). At this location, the average high tide mark in the summers of 2010 and 2011 was +1.38 m. Thus, since *C. fornicata* populations become less dense at approximately +0.4 m and are absent above +0.6 m, they occupy the mid and low intertidal zone and can be found continuously into the subtidal zone (Fig. 1).

*Crepidula fornicata* occupying the intertidal zone experienced different degrees of aerial exposure depending on their location within the intertidal zone (Fig. 1). Though on average animals at +0.4 m spent 3 h 39 min per tidal cycle (single low tide) exposed to

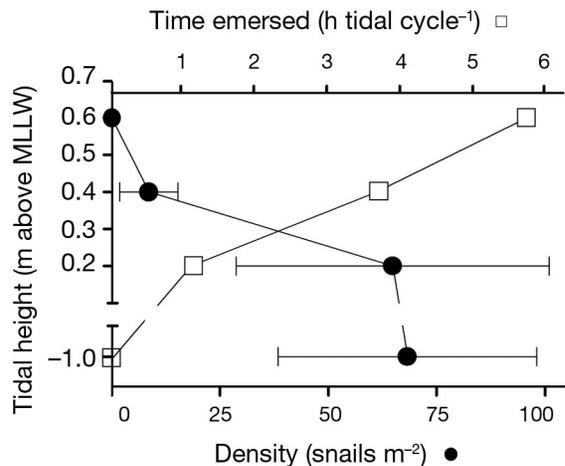


Fig. 1. *Crepidula fornicata*. Field distribution and average emersion times at different tidal heights in Bissel Cove, Rhode Island. Filled circles = mean ( $\pm$ SD) of 5 quadrats along a horizontal transect at each tidal height. Open squares = the average time an individual at a particular tidal height was exposed to air during 1 tidal cycle from June to August 2010 and 2011. A tidal height of 0 m is defined as the mean low lower water mark (MLLW) as determined by the Quonset Point, RI, buoy operated by the NOAA National Data Buoy Center

air (Fig. 1), they were exposed for as long as 5 h 41 min during spring tides, but were often not exposed to air during neap tides. Similarly, the range of exposure time for animals at +0.2 m was 0 to 4 h 27 min per tidal cycle in the summer months of 2010 and 2011. Since tides are semidiurnal at this location, intertidal *C. fornicata* at the higher end of their vertical range (+0.4 m) spent on average 30.4% of the time exposed to the air during the summers of 2010 and 2011, while subtidal animals (-1.0 m) were never exposed to air (Fig. 1).

### Thermal environment

Intertidal and subtidal animals experienced drastically different thermal environments in the summer months of 2011 (Figs. 2 & 3). The highest temperature that subtidal animals experienced was 26.5°C, while intertidal animals experienced temperatures more than 15°C warmer, reaching a maximum of 42°C in July 2011 (Fig. 3). Intertidal animals did spend most of the time within the range of temperatures experienced by subtidal conspecifics (81.3%), but when they experienced temperatures outside of this range they tended to be warmer (12.0%) rather than cooler (6.7%) (Fig. 3). The temperature changes that intertidal *Crepidula fornicata* experienced were also often very rapid (Fig. 2), with tissues warming as quickly as 0.4°C  $min^{-1}$  and cooling as quickly as 0.5°C  $min^{-1}$ .

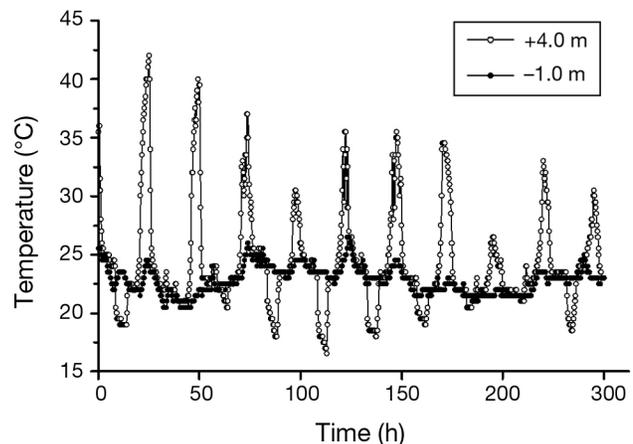


Fig. 2. Typical thermal environment of intertidal and subtidal *Crepidula fornicata* in Bissel Cove, RI. Temperatures were recorded from biomimetic robosnails placed among living *C. fornicata* in the intertidal zone (+0.4 m mean low lower water [MLLW], open circles) and the subtidal zone (-1.0 m MLLW, filled circles) in June, July and August 2011. Data are from a representative 10 d time period in July 2011; data points are 15 min apart

When intertidal *Crepidula fornicata* experienced high temperatures in the field, they often did so for extended periods of time (Fig. 4). On clear days when low tides occurred during the early afternoon, *C. fornicata* experienced temperatures above 30°C for as long as 5 h 15 min. On one occasion the intertidal robosnail recorded temperatures at or above 37°C for 3 continuous hours (Fig. 4) (temperatures above 30°C for 3 straight hours occurred 22 times), which is the amount of time we chose to thermally stress animals in the laboratory (Figs. 5–7).

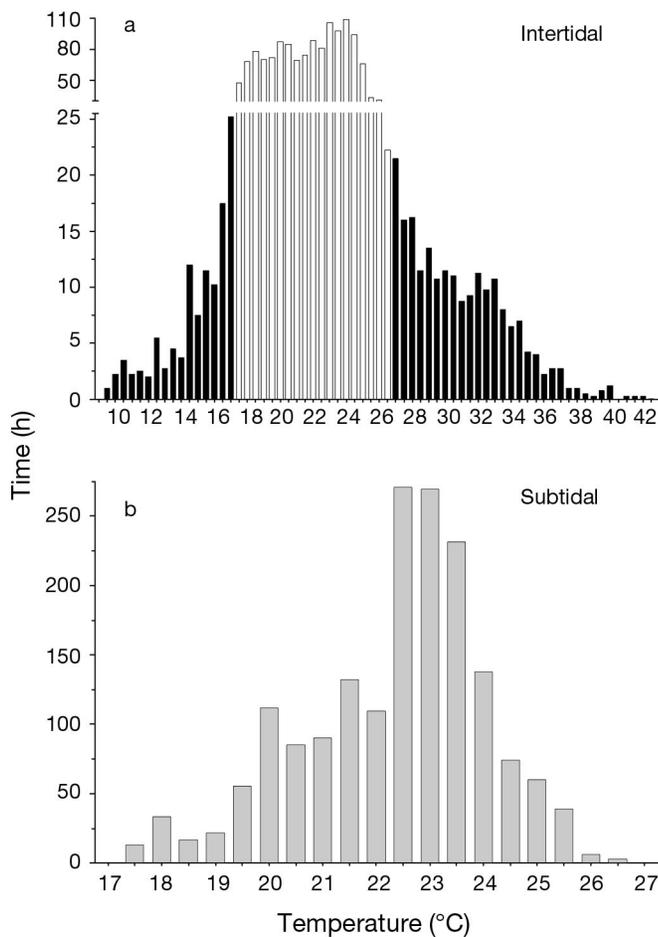


Fig. 3. Temperatures experienced by intertidal and subtidal *Crepidula fornicata* in the summer of 2011. Continuous recordings from biomimetic robosnails placed among living *C. fornicata* were made in June, July, and August 2011. Recordings were taken every 15 min; therefore, cumulative time was estimated by multiplying the number of recordings at a particular temperature by 15 min. (a) Recordings taken at +0.4 m mean low lower water (MLLW). Black bars = temperatures experienced by intertidal robosnails that were outside the range of temperatures experienced by subtidal robosnails; white bars = within the range of subtidal robosnails. (b) Recordings taken at -1.0 m MLLW

## Thermal tolerance

When thermally stressed in the laboratory, intertidal and subtidal embryos did not differ in upper thermal tolerance (Fig. 5, Table 1). Some embryos died after being stressed for 3 h at temperatures as low as 33°C in June 2011 (Fig. 5b), and nearly all (Fig. 5a) or all (Fig. 5c) embryos died following a single 3 h exposure to 37°C. Adults collected intertidally and subtidally also did not differ in thermal tolerance in June 2010 (Fig. 6a, Table 1), but intertidal adults were significantly more tolerant of high thermal stress in August 2010 (Fig. 6b, Table 1).

Life history stage had a significant effect on thermal tolerance, as embryos were more tolerant of the thermal stress than adults were (Figs. 5 & 6, Table 2). In August 2010 all embryos collected from intertidal and subtidal adults survived a 35°C stress (3 h) (Fig 5a) while some adults died following thermal stresses of 34 or 35°C (Fig 5b). Similarly, in August 2011 nearly all embryos from intertidal adults survived a thermal stress of 35°C (Fig 5c) while nearly all intertidal adults died after experiencing the same stress (Fig. 6c). However, though thermal tolerances were significantly different between life history stages (Table 2), absolute tolerances differed only slightly, as nearly all embryos died after being

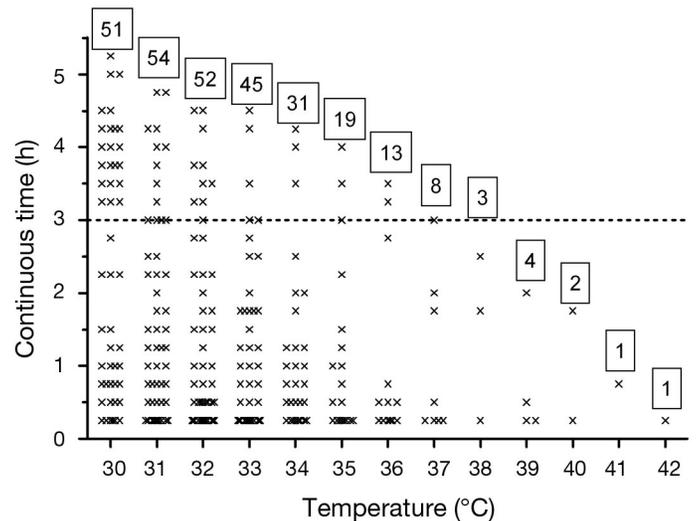


Fig. 4. Thermally challenging events in the intertidal zone (+0.4 m mean low lower water [MLLW]) at Bissel Cove in Narragansett Bay, RI. Boxed numbers = number of times intertidal robosnails remained at or above the given temperature from June to August 2011. Symbols = amount of time spent at or above the given temperature on each occasion. Horizontal dashed line = amount of time we chose to thermally stress animals in laboratory experiments

exposed to thermal stresses only 1°C higher than those experienced by adults (Figs. 5 & 6).

We could detect no differences in traits responsible for upper thermal tolerance between intertidal and subtidal animals: laboratory-reared, fully-acclimated (23°C) juveniles originating from intertidal and sub-

tidal mothers had nearly identical upper thermal limits (Fig. 7, Table 3). Variation in upper thermal limits did exist among individuals, but only over a small range of temperatures: in our study every juvenile survived a 3 h stress at 32°C, but nearly all died following a 3 h stress at 35°C (Fig. 7).

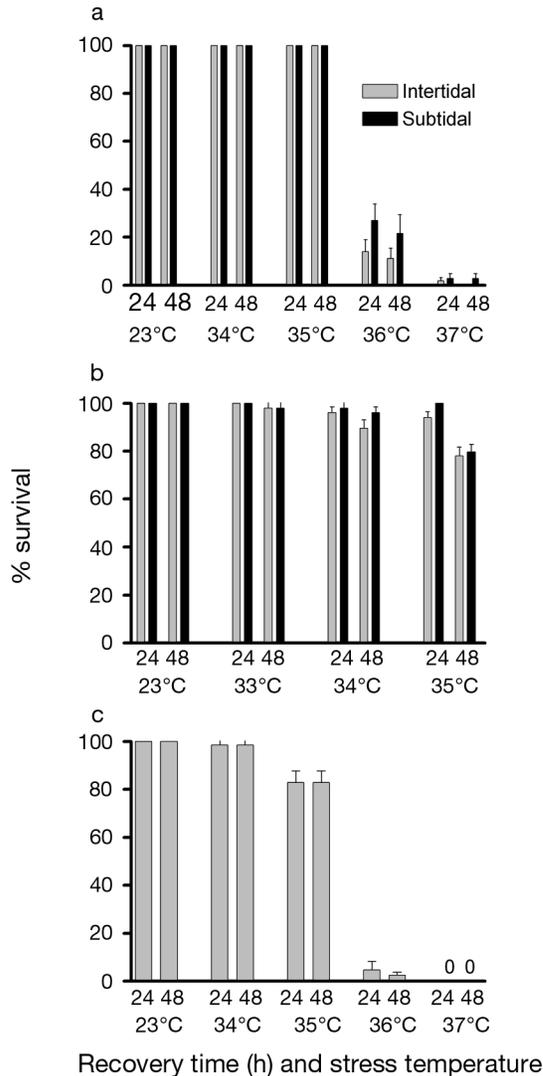


Fig. 5. *Crepidula fornicata*. Thermal tolerance of intertidal (grey bars) and subtidal (black bars) embryos collected from Bissel Cove, RI in (a) August 2010, (b) June 2011, and (c) August 2011. Embryos were stressed for 3 h at the indicated temperature and mortality was assessed 24 and 48 h later. Bars = mean (+SD) of 5 replicates with 25–30 embryos per replicate. Survival was significantly different (paired *t*-tests) between 24 and 48 h of recovery time in (a,  $p = 0.0070$ ) and (b,  $p < 0.0001$ ) but not (c,  $p = 0.324$ ). No significant differences between survival of intertidal and subtidal embryos at a particular temperature and recovery time were found (Bonferroni multiple comparisons, all  $p > 0.05$ ). Detailed statistical analyses regarding the effects of temperature, tidal height, and life history stage can be found in Tables 1 & 2

Table 1. Two-way ANOVA determining the effects of temperature (Temp) and tidal height (TH) on survival (after 48 h of recovery time) of field-collected *Crepidula fornicata*. **Bold:** significant at  $p < 0.01$

Source	df	MS	<i>F</i>	<i>p</i>	cf. Fig.
<b>Aug 2010 – Embryos</b>					
Temp	4	5.700	411.0	<b>&lt;0.0001</b>	5a
TH	1	0.02928	2.111	0.1540	
Temp × TH	4	0.01126	0.8120	0.5240	
Residual	40	0.01387			
<b>Jun 2011 – Embryos</b>					
Temp	3	0.4410	28.39	<b>&lt;0.0001</b>	5b
TH	1	0.02031	1.308	0.2613	
Temp × TH	3	0.01550	0.9981	0.4063	
Residual	32	0.01553			
<b>Jun 2010 – Adults</b>					
Temp	3	1.744	67.23	<b>&lt;0.0001</b>	6a
TH	1	0.04967	1.914	0.1761	
Temp × TH	3	0.03618	1.395	0.2623	
Residual	32	0.02594			
<b>Aug 2010 – Adults</b>					
Temp	3	1.380	40.33	<b>&lt;0.0001</b>	6b
TH	1	0.3542	10.36	<b>&lt;0.0001</b>	
Temp × TH	3	0.4536	13.26	<b>0.0030</b>	
Residual	32	0.03421			

Table 2. Two-way ANOVA determining the effects of temperature (Temp) and life history stage (LHS) on survival (after 48 h of recovery time) of field-collected *Crepidula fornicata*. **Bold:** significant at  $p < 0.01$

Source	df	MS	<i>F</i>	<i>p</i>	cf. Fig.
<b>Aug 2010 – Intertidal</b>					
Temp	2	0.0817	5.762	<b>0.0090</b>	5a & 6b
LHS	1	0.2118	17.93	<b>0.0007</b>	
Temp × LHS	2	0.0817	5.762	<b>0.0090</b>	
Residual	24	0.0142			
<b>Aug 2010 – Subtidal</b>					
Temp	2	1.102	35.08	<b>&lt;0.0001</b>	5a & 6b
LHS	1	1.317	41.91	<b>&lt;0.0001</b>	
Temp × LHS	2	1.102	35.08	<b>&lt;0.0001</b>	
Residual	24	0.0314			
<b>Aug 2011 – Intertidal</b>					
Temp	3	5.364	314.9	<b>&lt;0.0001</b>	5c & 6c
LHS	1	134.7	7.57	<b>&lt;0.0001</b>	
Temp × LHS	3	0.9449	55.47	<b>&lt;0.0001</b>	
Residual	32	0.0170			

## DISCUSSION

In Narragansett Bay, Rhode Island, *Crepidula fornicata* is common both intertidally and subtidally (Fig. 1). This is not surprising, as they have long been

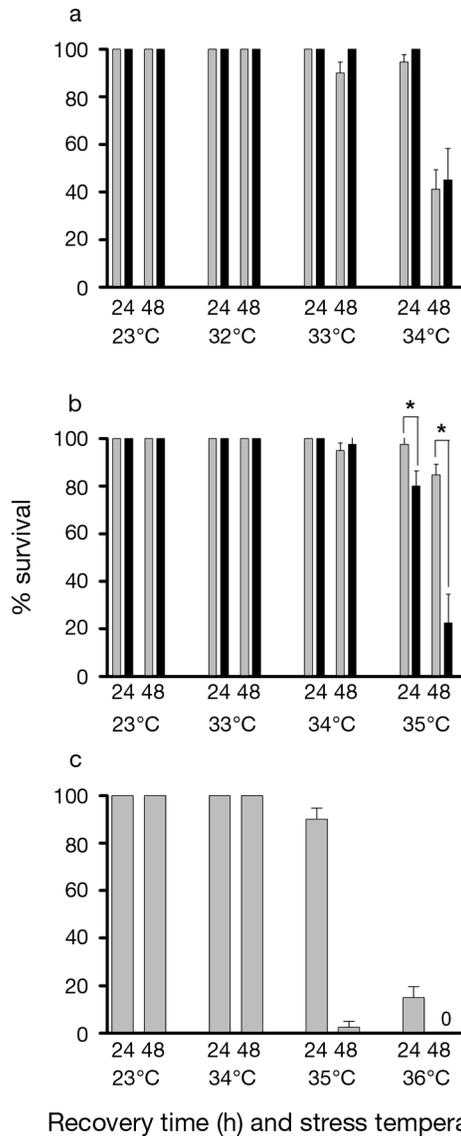


Fig. 6. *Crepidula fornicata*. Thermal tolerance of intertidal (grey bars) and subtidal (black bars) adults collected from Bissett Cove, RI, in (a) June 2010, (b) August 2010, and (c) August 2011. Adults were stressed for 3 h at the indicated temperature and mortality was assessed 24 and 48 h later. Bars = mean ( $\pm$ SD) of 5 replicates with 8 animals per replicate. Survival was significantly different (paired *t*-tests) between 24 and 48 h of recovery time in all experiments: (a,  $p = 0.0005$ ); (b,  $p = 0.0009$ ); (c,  $p = 0.0043$ ). Asterisks = significant differences between survival of intertidal and subtidal adults at a particular temperature and recovery time (Bonferroni multiple comparisons,  $p < 0.001$ ). Detailed statistical analysis regarding the effects of temperature, tidal height, and life history stage can be found in Tables 1 & 2

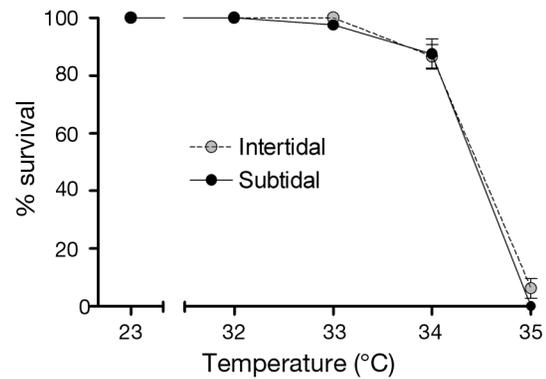


Fig. 7. *Crepidula fornicata*. Variation in thermal tolerance between laboratory-reared juveniles originating from 3 sets each of intertidal (grey circles, dashed line) and subtidal (black circles, solid line) parents. All juveniles ( $\sim 2.5$  mm shell length) were reared from hatching in the laboratory at 23°C for at least 3 wk before being subjected to a 3 h thermal stress at the indicated temperature. Data points = mean ( $\pm$ SD) survival after 48 h of recovery time. Sample sizes of data points: 23°C,  $N = 15$ ; 32°C,  $N = 5$ ; 33°C,  $N = 15$ ; 34°C,  $N = 15$ ; 35°C,  $N = 10$  replicates with 8 juveniles per replicate. Thermal tolerances of juveniles from intertidal and subtidal mothers were not significantly different ( $p = 0.4027$ , see 2-way ANOVA results in Table 3)

collected from both habitats in both their native range (e.g. Diederich et al. 2011, Hoch & Cahill 2012) and their invasive range (Thieltges et al. 2003, Viard et al. 2006). However, though their distribution has been anecdotally described by some (e.g. Collin 2001), this is the first time that the distribution of a single population of *C. fornicata* has been formally recorded in both the intertidal and subtidal zones. Although *C. fornicata* is not alone in straddling the low tide mark (e.g. Dame 1972, Palmer 1980, Fletcher 1984, Jensen & Armstrong 1991, Bingham et al. 1997, Saier 2002, Altieri 2006, Schaffmeister et al. 2006), their considerable abundance in both zones is surprising: unlike many intertidal species that are overwhelmed by subtidal predators or subtidal competitors (Stephenson & Stephenson 1949, Paine 1974, Perez et al. 2009), populations of *C. fornicata* do very well subtidally, directly adjacent to thriving populations living in the physically harsh intertidal zone.

Among the challenges associated with living in the intertidal zone (e.g. exposure to periodic desiccation, salinity stress, and starvation), thermal stress can be particularly harsh, as temperature changes upon emersion can be both large and extremely rapid (e.g. Helmuth & Hofmann 2001, Wethey 2002, Lima & Wethey 2009, Szathmary et al. 2009). Similarly, our biomimetic robosnails recorded considerable temperature increases in the intertidal zone upon emer-

Table 3. Two-way ANOVA determining the effects of temperature (Temp) and mother's environment (mother's tidal height, MTH) on survival of laboratory-acclimated juvenile *Crepidula fornicata*. **Bold:** significant at  $p < 0.01$

Source	df	MS	F	p	cf. Fig.
Temp	4	8.654	284.85	<b>&lt;0.0001</b>	7
MTH	1	0.02144	0.71	0.4027	
Temp × MTH	4	0.03213	1.06	0.3811	
Residual	110	0.03038			

sion, with temperatures reaching 15.5°C higher than those of subtidal robosnails in the summer of 2011 (Figs. 2 & 3) and increasing as rapidly as 6°C in 15 min (Fig. 2). In addition to experiencing rapid temperature changes, *C. fornicata* robosnails also remained at high temperatures for many hours at a time (Fig. 4). For example, robosnails reached 35°C (8.5°C higher than subtidal animals ever reached) 19 different times in 3 months during our study, and they remained at or above that temperature for up to 4 h at a time (Fig. 4). These temperature recordings highlight one of the major difficulties in living intertidally: most organisms that normally live subtidally and experience only a relatively small range of temperatures that change very slowly (seasonally) may not be able to cope with the large temperature changes that occur so quickly in the intertidal zone.

Individuals of *Crepidula fornicata*, like many intertidal organisms, are sessile as adults (Conklin 1898) and cannot rely on behavioral adaptations (e.g. movement subtidally or into shaded, damp crevices) to reduce high thermal stress during low tide. Thus, these organisms must rely entirely on high physiological tolerance to high temperatures in order to occupy the intertidal zone. In some species, intertidal organisms may have higher thermal tolerance limits than subtidal organisms due to environmentally induced plasticity (e.g. via the heat-shock response, Feder & Hofmann 1999) upon experiencing high temperatures in the field. Indeed, tolerances to environmental stressors across the intertidal–subtidal boundary have been shown to be inducible in other organisms including mussels (Altieri 2006), and the upper thermal limits of many organisms are effected by prior acclimation temperatures (e.g. Cuculescu et al. 1998, Beitinger & Bennett 2000, Stillman & Somero 2000). Additionally, selective post-settlement mortality of individuals with low thermal tolerance in the intertidal zone can result in phenotypic differences among subpopulations, even when those subpopulations are extensively mixed. Schmidt & Rand

(1999) for example, have shown that barnacles occupying different microhabitats in the intertidal zone undergo post-settlement mortality that favors different genotypes in different microhabitats. Though newly-settled barnacles are genetically homogeneous across microhabitats, this differential mortality would be expected to yield adult subpopulations with different tolerances to intertidal stressors (e.g. temperature and desiccation) (Schmidt & Rand 2001). Finally, local adaptation, even among species with long-lived larvae, is surprisingly prevalent in marine invertebrates (reviewed by Sanford & Kelly 2011). Fine-scale population structure has since been found in species with long-lived larvae (Hoffman et al. 2012) and even between intertidal and subtidal conspecifics with differing stress responses (Weihe & Abele 2008, de Aranzamendi et al. 2008, but see Hoffman et al. 2010). In these cases, intertidal organisms would be expected to have higher average thermal tolerance limits when compared with subtidal conspecifics that do not experience such high temperatures; but that is not what we found for this population of *C. fornicata*.

In our experiments, the upper thermal tolerance limits of intertidal and subtidal *Crepidula fornicata* were nearly identical (Figs. 5 & 6). On only one occasion in our study were intertidal individuals more thermally tolerant than subtidal individuals, and that was only by about 1°C (Fig. 6b). However, even in that experiment, subtidal animals had remarkably high thermal tolerances considering the relatively low temperatures that they consistently experience in the field. Furthermore, the upper thermal tolerance limits of juvenile *C. fornicata* reared entirely in the laboratory from larvae obtained from intertidal and subtidal parents had identical thermal tolerance limits (Fig. 7), indicating that there are no phenotypic differences in thermal tolerance between individuals in these 2 subpopulations.

Though many species' upper thermal tolerance limits have been shown to be positively correlated with the temperatures that they experience, on both local and more broad geographic scales (e.g. Sharp et al. 1994, Bingham et al. 1997, Kuo & Sanford 2009), this is not always the case. For example, though Kuo & Sanford (2009) found differences in thermal tolerance among *Nucella canaliculata* from different populations over a broad geographic range (100s of km), many populations within their study had nearly identical upper thermal limits, despite differences in mid-day exposure time (and presumably temperature). In our study, subtidal *Crepidula fornicata* may have obtained relatively high upper thermal limits by

'seeding' (i.e. genetic mixing) from warm-adapted intertidal populations (Somero 2010). *C. fornicata* have long-lived, highly dispersive larvae which may allow for extensive genetic mixing of intertidal and subtidal subpopulations. If there are no costs to maintaining this high thermal tolerance in the subtidal zone, extensive mixing could yield similar physiological tolerances among these subpopulations.

In addition, warm-adapted subtidal *Crepidula fornicata* may be the source from which intertidal populations arise. This species has lived for millions of years in southern climates (Hoagland 1977) and living intertidally is apparently derived within the genus (Hoagland 1977). Collin (2001) has shown that clades of *C. fornicata* in the western Atlantic (sampled from New Brunswick to Florida) were not based on geography (little population structure exists). She also noted that *C. fornicata* does not form intertidal populations in southern habitats (Collin 2001). Thus, subtidal *C. fornicata* may have obtained their relatively high thermal tolerance limits from warm-adapted ancestors or southern populations, and intertidal organisms may be part of a sink population that are living under suboptimal conditions. Indeed, in our study, individuals of *C. fornicata* from intertidal populations died following thermal stresses that they occasionally experience in the field, and so they may not be optimally adapted to intertidal conditions. However, we cannot rule out the possibility that there may still be slight differences in thermal tolerance among members of intertidal and subtidal subpopulations. When stressing the animals in our experiments, we chose controlled and relevant stress temperatures and durations, but did not mimic intertidal conditions completely. Further investigation including, for example, twice daily exposures to high temperature for several days, might produce slight differences in stress tolerance between animals of the 2 subpopulations.

Most previous studies on thermal tolerance of marine organisms have been conducted with a single life-history stage. However, since adults of *Crepidula fornicata* brood embryos for several weeks (Conklin 1897) (exposing embryos to about the same environmental conditions experienced by adults), the ability of this species to live intertidally is also contingent upon high thermal tolerance limits for all of its life history stages. Indeed, we found that both intertidal and subtidal *C. fornicata* embryos were at least as tolerant of thermal stress as were adults (Fig. 5). This is not the case for many species, as early life history stages are often less tolerant of many environmental stressors than later stages of development (Crisp &

Ritz 1967, Kinne 1970, Gosselin & Chia 1995, Freitas et al. 2010, but see Miller et al. 2013). For example, Gosselin & Chia (1995) found that nearly all new hatchlings—but no adults—of *Nucella emarginata* died from an aerial thermal stress of 30°C for 8 h. Thus, species distributions may in some cases be determined (and limited) by the sensitivity of early life history stages, or gametes, to environmental stressors that adults can tolerate (Andronikov 1975). In the future, multiple life-history stages should be assessed when determining the tolerance of a species to physiological stressors in order to develop a more complete picture of the overall capacity of a species to withstand such stressors. The remarkable ability of *C. fornicata* embryos to withstand temperatures as high as adults can tolerate has apparently allowed these organisms to occupy a substantial portion of the intertidal zone, and to take advantage of a habitat that is too harsh for most other marine species to occupy.

Though members of *Crepidula fornicata* now successfully occupy the intertidal zone in New England, our data suggest that these intertidal populations may be vulnerable to climate change in the coming years. Somero (2010) suggests that though many intertidal organisms have higher thermal tolerances than subtidal organisms have, they are also living close to their upper thermal limits and are thus likely to be the 'losers' as climates warm. For example, Tomanek & Somero (1999) found that although snails of the genus *Tegula* (now *Chlorostoma*) living in the intertidal zone were able to survive much higher temperatures (37°C for 2.5 h) than their subtidal congeners (30°C for 2.5 h), the intertidal individuals experienced field temperatures closer to their thermal maximum (max. temp of 33°C intertidally as opposed to 24°C subtidally). In our experiments, some adult *C. fornicata* died after a single 3 h thermal stress at 34°C in the laboratory, a stress level that intertidal robosnails recorded many times in our study. It is possible that robosnail recordings could slightly overestimate tissue temperatures of animals in the field, because *C. fornicata* may lift up from their substrate and evaporatively cool their tissues. However, this behavior has been shown to cool tissues by only 2°C in limpets (*Cellana grata*) and it was effective for less than 2 h (Williams et al. 2005). Thus, the temperatures that killed intertidal *C. fornicata* in the laboratory were very close to temperatures that they experience in the field; subtidal animals, on the other hand, did not experience temperatures within 7°C of temperatures that kill them. Furthermore, the one occasion on which intertidal organisms had a

slightly higher thermal tolerance than subtidal organisms in our study (Fig. 6b) may have been the result of high mortality of less-tolerant intertidal organisms in the months preceding the study (though differential acclimatization is also possible). These data indicate that intertidal—but not subtidal—*C. fornicata* in our study area are living close to their upper thermal limit. As the anticipated climate change further warms coastal habitats, *C. fornicata* will likely be relegated to the subtidal, where, unlike intertidal specialists, they are not outcompeted or heavily preyed upon. Their disappearance from the intertidal could have interesting community-wide effects, as they presently compete with other intertidal suspension feeders for food (e.g. mussels and oysters, Lesser et al. 1992, Decottignies et al. 2007); are eaten by many other animals including the predatory gastropod *Urosalpinx cinerea*, a number of crab species (*Dyspanopeus sayi*, *Cancer borealis*, *Pagurus longicarpus*, and *Hemigrapsus sanguineus*), and the sea star *Asterias forbesi* (Hoagland 1974, Pratt 1974, Lindsey et al. 2006, Pechenik et al. 2010); and are a host for the ectoparasitic gastropod *Boonea seminuda* (Boss & Merrill 1965, C. M. Diederich pers. obs.) and the boring sponge *Cliona celata* (Hoagland 1974, LeCam & Viard 2011). In addition, their shells provide homes for numerous polychaetes and other invertebrates such as juveniles of the invasive crab, *Hemigrapsus sanguineus* (C. M. Diederich pers. obs.) and hermit crabs (Williams & McDermott 2004) while creating biogenic structures for organisms such as xanthid crabs (Lindsey et al. 2006).

Though we now know that individuals of *Crepidula fornicata* may be killed by summer temperatures presently characterizing the intertidal zone in New England, the specific cause of thermal death in this species is not clear. Somero (2002) reviews many possible 'weak links' in physiological systems that cause thermal death, including failure of organ systems (especially the heart), action potential generation, mitochondrial respiration, membrane integrity, the heat shock response, and physical stability of enzymes. No doubt the failures of these systems act on different time scales. The fact that in all but one of our experiments (Fig. 5c) most animals survived for at least 24 h after the thermal stress ended indicates that some of the faster acting mechanisms for death (e.g. heart failure, nervous system failure) are probably not the cause of death for *C. fornicata*. It would be interesting to learn the specific cause(s) of death in this and other intertidal species, as the cause of death from high temperatures would be the most likely target of selection for animals living close to their upper

thermal limits; the ability of these physiological systems to change will be important in determining the fate of these species in the face of global climate change (Somero 2010).

In our experiments, the fact that few individuals died within the first 24 h after the thermal stress was applied underscores the importance of monitoring organisms for an extended period of time in experiments assessing tolerance to environmental stressors. Though stressors may be applied to organisms in the laboratory depending on specific ecological conditions that those organisms are likely to encounter, the time selected to observe organisms for mortality after the stress ends varies widely (e.g. immediately; Zippay & Hofmann 2010, after 6 h recovery; Kenny 1969, after 12 h recovery; Singletary 1971, after 24 h recovery; Sanders et al. 1991, after 48 h recovery; Sorte et al. 2010, after 30 d recovery; Coles & Jokiel 1978). Our data suggest that monitoring organisms for less than 24 h may produce misleading results.

In summary, *Crepidula fornicata* can be found in considerable numbers both intertidally and subtidally in New England; these populations experience drastically different thermal environments. However, the temperature differences that they face are not reflected in their physiological tolerance to thermal stress, as both embryos and adults sampled from intertidal and subtidal subpopulations had nearly identical thermal tolerances, as did juveniles reared in the laboratory from intertidal and subtidal mothers. Though they are a major member of both the intertidal and subtidal zones in the temperate Atlantic, intertidal *C. fornicata* are now living very close to their thermal maximum and will likely be lost from the intertidal zone in the coming years as summer maximum air temperatures increase. However, compared with most marine species, *C. fornicata* is relatively eurythermal, a factor that could have contributed to their substantial success as an invader in Europe and elsewhere (Blanchard 1997) and that should allow them to persist subtidally in the face of global climate change.

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## LITERATURE CITED

- Altieri AH (2006) Inducible variation in hypoxia tolerance across the intertidal–subtidal distribution of the blue mussel *Mytilus edulis*. *Mar Ecol Prog Ser* 325:295–300
- Andronikov VB (1975) Heat resistance of gametes of marine invertebrates in relation to temperature conditions under which species exist. *Mar Biol* 30:1–11
- Bayne BL (1976) *Marine mussels*. Cambridge University Press, London
- Beitinger TL, Bennett WA (2000) Quantification of the role of acclimation temperature in temperature tolerance of fishes. *Environ Biol Fishes* 58:277–288
- Bingham BL, Bacigalupi M, Johnson LG (1997) Temperature adaptations of embryos from intertidal and subtidal sand dollars (*Dendraster excentricus*, Eschscholtz). *Northwest Sci* 71:108–114
- Blanchard M (1997) Spread of the slipper limpet *Crepidula fornicata* (L. 1758) in Europe. Current state and consequences. *Sci Mar* 61:109–118
- Boss KJ, Merrill AS (1965) Degree of host specificity in two species of *Odostomia* (Pyramidellidae: Gastropoda). *J Molluscan Stud* 36:349–355
- Bourdeau PE (2011) Constitutive and inducible defensive traits in co-occurring marine snails distributed across a vertical rocky intertidal gradient. *Funct Ecol* 25:177–185
- Coe WR (1936) Sexual phases in *Crepidula*. *J Exp Zool* 72:455–477
- Coles SL, Jokiel PL (1978) Synergistic effects of temperature, salinity and light on hermatypic coral *Montipora verrucosa*. *Mar Biol* 49:187–195
- Collin R (2001) The effects of mode of development on phylogeography and population structure of North Atlantic *Crepidula* (Gastropoda: Calyptraeidae). *Mol Ecol* 10:2249–2262
- Conklin EG (1897) The embryology of *Crepidula*, a contribution to the cell lineage and early development of some marine gasteropods. *J Morphol* 13:1–226
- Conklin EG (1898) Environmental and sexual dimorphism in *Crepidula*. *Proc Acad Nat Sci Philadelphia* 50:435–444
- Connell JH (1972) Community interactions on marine rocky intertidal shores. *Annu Rev Ecol Syst* 3:169–192
- Crisp DJ, Ritz DA (1967) Changes in temperature tolerance of *Balanus balanoides* during its life-cycle. *Helgol Wiss Meeresunters* 15:98–115
- Cuculescu M, Hyde D, Bowler K (1998) Thermal tolerance of two species of marine crab, *Cancer pagurus* and *Carcinus maenas*. *J Therm Biol* 23:107–110
- Dame RF (1972) Comparison of various allometric relationships in intertidal and subtidal American oysters. *Fish Bull* 70:1121–1126
- Davenport J, Davenport JL (2005) Effects of shore height, wave exposure and geographical distance on thermal niche width of intertidal fauna. *Mar Ecol Prog Ser* 292:41–50
- Davenport J, Busschots PMCF, Cawthorne DF (1980) Influence of salinity on behavior and oxygen uptake of the hermit crab *Pagurus bernhardus* L. *J Mar Biol Assoc UK* 60:127–134
- de Aranzamendi MC, Sahade R, Tatian M, Chiappero MB (2008) Genetic differentiation between morphotypes in the Antarctic limpet *Nacella concinna* as revealed by inter-simple sequence repeat markers. *Mar Biol* 154:875–885
- Decottignies P, Beninger PG, Rince Y, Riera P (2007) Trophic interactions between two introduced suspension-feeders, *Crepidula fornicata* and *Crassostrea gigas*, are influenced by seasonal effects and qualitative selection capacity. *J Exp Mar Biol Ecol* 342:231–241
- Denny MW, Wetthey DS (2001) Physical processes that generate patterns in marine communities. In: Bertness MD, Gaines SD, Hay ME (eds) *Marine community ecology*. Sinauer Associates, Sunderland, MA
- Diederich CM, Jarrett JN, Chaparro OR, Segura CJ, Arelano SM, Pechenik JA (2011) Low salinity stress experienced by larvae does not affect post-metamorphic growth or survival in three calyptraeid gastropods. *J Exp Mar Biol Ecol* 397:94–105
- Ellis JC, Shulman MJ, Wood M, Witman JD, Lozyniak S (2007) Regulation of intertidal food webs by avian predators on New England rocky shores. *Ecology* 88:853–863
- Eyster LS, Pechenik JA (1988) Comparison of growth, respiration, and feeding of juvenile *Crepidula fornicata* (L) following natural or KCl-triggered metamorphosis. *J Exp Mar Biol Ecol* 118:269–279
- Feder ME, Hofmann GE (1999) Heat-shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology. *Annu Rev Physiol* 61:243–282
- Fletcher WJ (1984) Variability in the reproductive effort of the limpet, *Cellana tramoserica*. *Oecologia* 61:259–264
- Freitas V, Cardoso JFMF, Lika K, Peck MA, Campos J, Kooijman SALM, van der Veer HW (2010) Temperature tolerance and energetics: a dynamic energy budget-based comparison of North Atlantic marine species. *Philos Trans R Soc Lond B* 365:3553–3565
- Gilman SE, Wetthey DS, Helmuth B (2006) Variation in the sensitivity of organismal body temperature to climate change over local and geographic scales. *Proc Natl Acad Sci USA* 103:9560–9565
- Gosselin LA, Chia FS (1995) Characterizing temperate rocky shores from the perspective of an early juvenile snail—the main threats to survival of newly-hatched *Nucella emarginata*. *Mar Biol* 122:625–635
- Hammond L, Hofmann G (2010) Thermal tolerance of *Strongylocentrotus purpuratus* early life history stages: mortality, stress-induced gene expression and biogeographic patterns. *Mar Biol* 157:2677–2687
- Helmuth BST, Hofmann GE (2001) Microhabitats, thermal heterogeneity, and patterns of physiological stress in the rocky intertidal zone. *Biol Bull (Woods Hole)* 201:374–384
- Helmuth B, Broitman BR, Blanchette CA, Gilman S and others (2006) Mosaic patterns of thermal stress in the rocky intertidal zone: implications for climate change. *Ecol Monogr* 76:461–479
- Henry JQ, Perry KJ, Martindale MQ (2006) Cell specification and the role of the polar lobe in the gastropod mollusc *Crepidula fornicata*. *Dev Biol* 297:295–307
- Hoagland KE (1974) Biological and physical causes of mortality in New England *Crepidula* species. *Am Malacol Bull* 39:14–17
- Hoagland KE (1977) Systematic review of fossil and recent *Crepidula* and discussion of evolution of Calyptraeidae. *Malacologia* 16:353–420
- Hoch J, Cahill A (2012) Variation in size at sex-change among natural populations of the protandrous hermaphrodite, *Crepidula fornicata* (Gastropoda, Calyptraeidae). *Mar Biol* 159:897–905
- Hoffman JI, Peck LS, Hillyard G, Zieritz A, Clark MS (2010) No evidence for genetic differentiation between Antarctic

- tic limpet *Nacella concinna* morphotypes. *Mar Biol* 157: 765–778
- Hoffman JI, Clarke A, Clark MS, Fretwell P, Peck LS (2012) Unexpected fine-scale population structure in a broadcast-spawning Antarctic marine mollusc. *PLoS ONE* 7:e32415
- Hunt LJH, Denny MW (2008) Desiccation protection and disruption: a trade-off for an intertidal marine alga. *J Phycol* 44:1164–1170
- Iacarella JC, Helmuth B (2012) Body temperature and desiccation constrain the activity of *Littoraria irrorata* within the *Spartina alterniflora* canopy. *J Therm Biol* 37:15–22
- Jensen GC, Armstrong DA (1991) Intertidal zonation among congeners: factors regulating distribution of porcelain crabs *Petrolisthes* spp. (Anomura: Porcellanidae). *Mar Ecol Prog Ser* 73:47–60
- Kenny R (1969) Temperature tolerance of polychaete worms *Diopatra cuprea* and *Clymenella torquata*. *Mar Biol* 4: 219–223
- Kinne O (1970) Temperature: animals: invertebrates. In: Kinne O (ed) *Marine ecology*, Vol 1: Environmental factors. Wiley, London, p 407–514
- Kuo ESL, Sanford E (2009) Geographic variation in the upper thermal limits of an intertidal snail: implications for climate envelope models. *Mar Ecol Prog Ser* 388: 137–146
- Le Cam S, Viard F (2011) Infestation of the invasive mollusc *Crepidula fornicata* by the native shell borer *Cliona celata*: a case of high parasite load without detrimental effects. *Biol Invasions* 13:1087–1098
- Lesser MP, Shumway SE, Cucci T, Smith J (1992) Impact of fouling organisms on mussel rope culture: interspecific competition for food among suspension-feeding invertebrates. *J Exp Mar Biol Ecol* 165:91–102
- Lima FP, Wethey DS (2009) Robolimpets: measuring intertidal body temperatures using biomimetic loggers. *Limnol Oceanogr Methods* 7:347–353
- Lindsey EL, Altieri AH, Witman JD (2006) Influence of biogenic habitat on the recruitment and distribution of a subtidal xanthid crab. *Mar Ecol Prog Ser* 306:223–231
- McMahon RF, Payne BS (1980) Variation of thermal tolerance limits in populations of *Physa virgata* Gould (Mollusca, Pulmonata). *Am Midl Nat* 103:218–230
- Miller NA, Paganini AW, Stillman JH (2013) Differential thermal tolerance and energetic trajectories during ontogeny in porcelain crabs, genus *Petrolisthes*. *J Therm Biol* 38:79–85
- Newell RC, Kofoed LH (1977) Adjustment of the components of energy balance in the gastropod *Crepidula fornicata* in response to thermal acclimation. *Mar Biol* 44: 275–286
- Paine RT (1974) Intertidal community structure: experimental studies on relationship between a dominant competitor and its principal predator. *Oecologia* 15:93–120
- Palmer MA (1980) Variation in life-history patterns between intertidal and subtidal populations of the meiobenthic copepod *Microarthridion littorale*. *Mar Biol* 60:159–165
- Pechenik JA, Gee CC (1993) Onset of metamorphic competence in larvae of the gastropod *Crepidula fornicata* (L.), judged by a natural and an artificial cue. *J Exp Mar Biol Ecol* 167:59–72
- Pechenik JA, Heyman WD (1987) Using KCl to determine size at competence for larvae of the marine gastropod *Crepidula fornicata* (L.). *J Exp Mar Biol Ecol* 112:27–38
- Pechenik JA, Lima GM (1984) Relationship between growth, differentiation, and length of larval life for individually reared larvae of the marine gastropod, *Crepidula fornicata*. *Biol Bull (Woods Hole)* 166:537–549
- Pechenik JA, Jarrett JN, Rooney J (2002) Relationships between larval nutritional experience, larval growth rates, juvenile growth rates, and juvenile feeding rates in the prosobranch gastropod *Crepidula fornicata*. *J Exp Mar Biol Ecol* 280:63–78
- Pechenik JA, Ambrogio OV, Untersee S (2010) Predation on juveniles of *Crepidula fornicata* by two crustaceans and two gastropods. *J Exp Mar Biol Ecol* 384:91–98
- Perez KO, Carlson RL, Shulman MJ, Ellis JC (2009) Why are intertidal snails rare in the subtidal? Predation, growth and the vertical distribution of *Littorina littorea* (L.) in the Gulf of Maine. *J Exp Mar Biol Ecol* 369:79–86
- Pratt DM (1974) Behavioral defenses of *Crepidula fornicata* against attack by *Urosalpinx cinerea*. *Mar Biol* 27:47–49
- Qiu JW, Tremblay R, Bourget E (2002) Ontogenetic changes in hyposaline tolerance in the mussels *Mytilus edulis* and *M. trossulus*: implications for distribution. *Mar Ecol Prog Ser* 228:143–152
- Saier B (2002) Subtidal and intertidal mussel beds (*Mytilus edulis* L.) in the Wadden Sea: diversity differences of associated epifauna. *Helgol Mar Res* 56:44–50
- Sanders BM, Hope C, Pascoe VM, Martin LS (1991) Characterization of the stress protein response in two species of *Collisella* limpets with different temperature tolerances. *Physiol Zool* 64:1471–1489
- Sanford E, Kelly MW (2011) Local adaptation in marine invertebrates. *Annu Rev Mar Sci* 3:509–535
- Sanford E, Worth DJ (2010) Local adaptation along a continuous coastline: prey recruitment drives differentiation in a predatory snail. *Ecology* 91:891–901
- Schaffmeister BE, Hiddink JG, Wolff WJ (2006) Habitat use of shrimps in the intertidal and shallow subtidal seagrass beds of the tropical Banc d'Arguin, Mauritania. *J Sea Res* 55:230–243
- Schmidt PS, Rand DM (1999) Intertidal microhabitat and selection at Mpi: interlocus contrasts in the northern acorn barnacle, *Semibalanus balanoides*. *Evolution* 53: 135–146
- Schmidt PS, Rand DM (2001) Adaptive maintenance of genetic polymorphism in an intertidal barnacle: habitat- and life-stage-specific survivorship of Mpi genotypes. *Evolution* 55:1336–1344
- Seabra R, Wethey DS, Santos AM, Lima FP (2011) Side matters: microhabitat influence on intertidal heat stress over a large geographical scale. *J Exp Mar Biol Ecol* 400: 200–208
- Sharp VA, Miller D, Bythell JC, Brown BE (1994) Expression of low molecular weight Hsp 70 related polypeptides from the symbiotic sea anemone *Anemonia viridis* Forskall in response to heat shock. *J Exp Mar Biol Ecol* 179:179–193
- Singletary RL (1971) Thermal tolerance of ten shallow-water Ophiuroids in Biscayne Bay, Florida. *Bull Mar Sci* 21: 938–943
- Somero GN (2002) Thermal physiology of intertidal animals: optima, limits, and adaptive plasticity. *Am Zool* 41: 1591–1592
- Somero GN (2010) The physiology of climate change: how potentials for acclimatization and genetic adaptation will determine 'winners' and 'losers'. *J Exp Biol* 213:912–920
- Sorte CJB, Williams SL, Zerebecki RA (2010) Ocean warming increases threat of invasive species in a marine fouling community. *Ecology* 91:2198–2204

- Stenseng E, Braby CE, Somero GN (2005) Evolutionary and acclimation-induced variation in the thermal limits of heart function in congeneric marine snails (Genus *Tegula*): implications for vertical zonation. *Biol Bull (Woods Hole)* 208:138–144
- Stephenson TA, Stephenson A (1949) The universal features of zonation between tide-marks on rocky coasts. *J Ecol* 37:289–305
- Stillman JH (2002) Causes and consequences of thermal tolerance limits in rocky intertidal porcelain crabs, genus *Petrolisthes*. *Integr Comp Biol* 42:790–796
- Stillman J, Somero G (1996) Adaptation to temperature stress and aerial exposure in congeneric species of intertidal porcelain crabs (genus *Petrolisthes*): correlation of physiology, biochemistry and morphology with vertical distribution. *J Exp Biol* 199:1845–1855
- Stillman JH, Somero GN (2000) A comparative analysis of the upper thermal tolerance limits of eastern Pacific porcelain crabs, genus *Petrolisthes*: influences of latitude, vertical zonation, acclimation, and phylogeny. *Physiol Biochem Zool* 73:200–208
- Szathmary PL, Helmuth B, Wethey DS (2009) Climate change in the rocky intertidal zone: predicting and measuring the body temperature of a keystone predator. *Mar Ecol Prog Ser* 374:43–56
- Thieltges DW, Strasser M, Reise K (2003) The American slipper limpet *Crepidula fornicata* (L.) in the northern Wadden Sea 70 years after its introduction. *Helgol Mar Res* 57:27–33
- Tomanek L, Helmuth B (2002) Physiological ecology of rocky intertidal organisms: a synergy of concepts. *Integr Comp Biol* 42:771–775
- Tomanek L, Somero GN (1999) Evolutionary and acclimation-induced variation in the heat-shock responses of congeneric marine snails (genus *Tegula*) from different thermal habitats: implications for limits of thermotolerance and biogeography. *J Exp Biol* 202:2925–2936
- Viard F, Ellien C, Dupont L (2006) Dispersal ability and invasion success of *Crepidula fornicata* in a single gulf: insights from genetic markers and larval-dispersal model. *Helgol Mar Res* 60:144–152
- Weihe E, Abele D (2008) Differences in the physiological response of inter- and subtidal Antarctic limpets *Nacella concinna* to aerial exposure. *Aquat Biol* 4:155–166
- Wethey DS (2002) Biogeography, competition, and microclimate: the barnacle *Chthamalus fragilis* in New England. *Integr Comp Biol* 42:872–880
- Widdows J, Bayne BL (1971) Temperature acclimation of *Mytilus edulis* with reference to its energy budget. *J Mar Biol Assoc UK* 51:827–843
- Williams JD, McDermott JJ (2004) Hermit crab biocoenoses: a worldwide review of the diversity and natural history of hermit crab associates. *J Exp Mar Biol Ecol* 305:1–128
- Williams GA, De Pirro M, Leung KMY, Morritt D (2005) Physiological responses to heat stress on a tropical shore: the benefits of mushrooming behaviour in the limpet *Cellana grata*. *Mar Ecol Prog Ser* 292:213–224
- Zippay ML, Hofmann GE (2010) Physiological tolerances across latitudes: thermal sensitivity of larval marine snails (*Nucella* spp.). *Mar Biol* 157:707–714

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