

Desiccation tolerance and lifting behavior in *Crepidula fornicata* (Gastropoda)

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ABSTRACT: Desiccation stress is a major limiting factor for many organisms in the intertidal zone. Sessile mollusks often reduce water loss by isolating themselves from the external environment upon aerial exposure, which can result in other stressful conditions within their sealed mantle cavity. Interestingly, some intertidal animals willfully expose their tissues to the air when emersed ('gaping,' 'mushrooming,' or 'lifting' behavior). This basic behavior—and the desiccation tolerance—of the cosmopolitan gastropod *Crepidula fornicata* have not been thoroughly investigated. We found that individuals of *C. fornicata* almost always have their shells lifted off of the substrate when submerged, but that they also perform this lifting behavior periodically when exposed to air. The lifting behavior was costly, however, as air-exposed juveniles that were free to lift lost significantly more water than those that were artificially clamped to the substrate. We found no measurable difference in desiccation tolerance between intertidal and subtidal adults, which all survived aerial exposures of up to 10 h. In contrast, most small juvenile *C. fornicata* died following only 2 to 6 h of exposure, after losing between 38.5 and 75.5% of their tissue water. Because juveniles lost water so quickly, desiccation stress may be an important limiting factor for *C. fornicata*, unless juveniles can move to wet areas when exposed to air until they are large enough to withstand longer periods of aerial exposure.

KEY WORDS: Desiccation · Lifting behavior · Gaping · Intertidal · *Crepidula*

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INTRODUCTION

The intertidal zone is characterized by a suite of environmental stressors that make life there difficult for most marine organisms. Although multiple environmental factors may act in concert to stress intertidal organisms (Firth & Williams 2009, Williams et al. 2011, Iacarella & Helmuth 2012), the ranges of some intertidal species can be limited by the overwhelming negative effects of individual stressors (Kensler 1967, McQuaid 1982, Wetthey 1983, Somero 2010). In

particular, desiccation stress is widely reported as a major limiting factor for many marine organisms in the intertidal zone (Kensler 1967, Sutherland 1970, Branch 1975, McQuaid 1982, Jenewein & Gosselin 2013). When soft-bodied marine organisms are exposed to the air, they can lose tissue water rapidly (Branch 1975, McQuaid 1982); the percentage of tissue water that organisms can lose before they die is species-specific, but typically ranges from 10 to 70% in mollusks (Lent 1968, Davies 1969, Branch 1975, Marshall & McQuaid 1992, Williams & Morritt 1995,

Sokolova & Pörtner 2001, Hicks & McMahon 2003). Within a species, small individuals (e.g. juveniles) lose tissue water more rapidly than adults, due to their higher surface-to-volume ratio, and thus juveniles are often especially susceptible to desiccation stress (Davies 1969, Branch 1975, McQuaid 1982, Williams et al. 2005, Jenewein & Gosselin 2013).

Many organisms have behavioral adaptations that are critical for surviving daily bouts of aerial exposure. Sessile or very slow-moving organisms must prevent exposing their tissues to the air to avoid losing water. Bivalve mollusks do this by withdrawing their tissues into their shells and tightly closing their shell valves (e.g. McMahon 1988). Some gastropods (limpets) may tightly clamp to the substrate in order to achieve similar isolation (e.g. Wolcott 1973), while other gastropods may completely withdraw into their shell and use their operculum for isolation from the external environment (McMahon 1990).

Although the ability to seal off from the outside environment may reduce water loss, the isolated chamber of bivalves and gastropods (the mantle cavity) may itself become a stressful environment, as oxygen is depleted and metabolic end products accumulate (Williams et al. 2005, Chaparro et al. 2009, 2011). When exposed to the air, some animals do avoid aerial exposure of their tissues for extended periods of time (McMahon 1990) while other mollusks perform behaviors that periodically expose their tissues to the air: 'gaping' in bivalves (Lent 1968, Boyden 1972, Byrne & McMahon 1994, Nicastro et al. 2010) and 'mushrooming' or shell 'lifting' in limpets where the foot remains attached to the substrate (Wolcott 1973, Garrity 1984, Williams & Morrill 1995, Williams et al. 2005). Although such exposure to air should aerate the mantle cavity and may provide other benefits like evaporative cooling of tissues (Williams et al. 2005, Nicastro et al. 2012), it will also increase tissue water loss.

The gastropod *Crepidula fornicata* is an important member of both intertidal and subtidal communities along the east coast of North America (Diederich & Pechenik 2013). Juvenile *C. fornicata* are mobile (Coe 1936), but because adults are sedentary and fertilization is internal, members of this species live attached to one another in stacks to ensure reproductive success (Richard et al. 2006). Native to the western Atlantic, *C. fornicata* has become an extremely successful invasive species, especially along the coastlines of France and the UK (Blanchard 1997). Although this invasion has spurred a considerable amount of research on *C. fornicata*, little is known about many basic aspects of its biology and ecology.

In particular, the lifting behavior of this species has never been studied, and work on the role of desiccation stress in controlling the intertidal distribution of this species is limited (but see Hoagland 1984).

In this study, we first aimed to document the proportion of time that *C. fornicata* individuals spent with their shells lifted above the substrate compared with the amount of time they spent with their shells clamped against the substrate, while submerged and while exposed to the air. To investigate the degree to which desiccation stress limits the intertidal distribution of this species, we also determined the desiccation tolerance of *C. fornicata* adults and juveniles. Additionally, we measured the amount of water loss necessary to kill *C. fornicata* juveniles. We also compared water loss in organisms that were free to lift their shells above the substrate or which were artificially clamped to the substrate, to determine the extent to which lifting behavior increases water loss during aerial exposure in this species.

MATERIALS AND METHODS

Collection of animals and field measurements

All field observations and all animal collections for laboratory experiments were made at Bisset Cove, in Narragansett Bay, Rhode Island, USA. At this site, *Crepidula fornicata* is abundant both intertidally and subtidally (Diederich & Pechenik 2013). In the intertidal zone, it was possible to get very close to individuals of *C. fornicata* without disturbing them (see Fig. 2). Adults (N = ~250) were collected at approximately +0.4 m MLLW (mean lower low water, intertidal) and -1.0 m MLLW (subtidal) for desiccation tolerance experiments and to obtain offspring. At this location and tidal height, *C. fornicata* may spend up to ~6 h exposed to the air per tidal cycle (Diederich & Pechenik 2013).

Maintenance of laboratory animals

Desiccation tolerance experiments and laboratory lifting behavior observations with intertidal and subtidal *C. fornicata* adults were performed within 48 h of collection, to avoid acclimation to laboratory conditions (Rising & Armitage 1969, Widdows & Bayne 1971). Otherwise, adults were maintained in 3 l glass aquaria with 0.45 μm filtered seawater at ~20°C and a salinity of 30. Aquaria were constantly aerated and water was changed every other day. Adults were fed

phytoplankton suspensions composed of a mixture of *Isochrysis galbana* (clone T-ISO) and *Dunaliella tertiolecta* (clone DUN) twice daily until larvae were released. Larvae hatched from several intertidal and subtidal mothers within 1 d of each other. Those larvae were collected on 120 μm mesh filters, rinsed with seawater, and maintained in 3 l glass aquaria filled with 0.45 μm filtered seawater. Larvae from intertidal and subtidal mothers were subsequently mixed to avoid any genetic or maternal effects on subsequent results. Larvae were fed daily with T-ISO at approximately 18×10^4 cells ml^{-1} (Pechenik & Lima 1984, Pechenik et al. 2002). Water was changed every other day until larvae were approximately 900 μm , at which point they were exposed to seawater with 20 mM excess KCl for 6 h to induce metamorphosis (Pechenik & Heyman 1987, Pechenik & Gee 1993). This method for the induction of metamorphosis does not affect juvenile survival or growth (Eyster & Pechenik 1988). After metamorphosis, juveniles were maintained just as adults were, except that they were initially fed only T-ISO for approximately 2 wk before switching to a diet of T-ISO and DUN.

Desiccation tolerance

Freshly collected intertidal and subtidal adults (see above) were brought into the laboratory, and the top-most members of each stack were removed so that each individual was alone on its substrate (small rock or empty shell); these individuals were not damaged during this process. Adults used for these experiments ranged in shell length from 25.3 to 43.2 mm (intertidal) and 24.4 to 44.0 mm (subtidal). For each treatment, 12 to 15 intertidal and subtidal adults each were taken out of their aquaria and were placed into a Percival model I-41VL temperature and humidity controlled incubator at 26°C and 75% relative humidity (RH), conditions similar to those found at the field site. Different groups (treatments) of animals were removed from the incubator after 1, 2, 3, 4, 5, 6, and 10 h of desiccation stress. Animals were immediately returned to aquaria filled with aerated, 0.45 μm filtered seawater at ~20°C and a salinity of 30 and fed a mixture of T-ISO and DUN (see above). Mortality was assessed 48 h after the end of the desiccation stress by prying animals from their substrate and testing muscular response by prodding with a small probe.

Glass containers (275 ml) were used for desiccation stress experiments involving lab-reared juveniles. In these sealed containers, specific RH levels in the air can be achieved using saturated solutions of various

ions (Winston & Bates 1960). The bottom ~2 cm of each glass container was filled with a saturated solution of a particular ion. Solutions were made according to Winston & Bates (1960) by dissolving solids of each ion into heated distilled water and then adding additional solid as the solution cools, until solid crystals remained undissolved in each container. In this way, at 20°C, RH levels of approximately 100%, 85%, 75%, and 32% were achieved using distilled water alone, and saturated solutions of KCl, NaCl, and CaCl_2 , respectively. Humidity levels were confirmed using a handheld digital psychrometer that also measures temperature; temperatures remained within 0.5°C of 20°C for the duration of each experiment. At the time of desiccation stress, 5 or 6 lab-reared juveniles per replicate (3–6 replicates per humidity level) were placed on plastic Petri dishes (diameter 40 mm) and quickly suspended over each solution in the sealed containers, so that they remained exposed to each humidity level for the duration of the stress period (1–6 h). Juveniles ranged in size from 1.3 to 4.6 mm, and average sizes of juveniles in each treatment were not statistically different (1-way ANOVA, $F_{31} = 1.265$, $p = 0.17$). After the end of the desiccation stress, juveniles were removed from the containers and placed in individual glass dishes with ~75 ml phytoplankton suspension per dish (equal parts T-ISO and DUN) at 20°C. Water was changed after 24 h and mortality was assessed 48 h after the end of the stress by observing pedal and head movements and monitoring contraction into the shell when stimulated with a probe (Diederich & Pechenik 2013).

For comparison, although juveniles and adults were exposed to 75% RH, juveniles were exposed at 20°C while adults were exposed at 26°C. Because temperature has an effect on the absolute amount of water vapor in the air, adults were actually exposed to drier air and thus to more stressful conditions than were juveniles.

Impact of water loss

To determine the amount of water loss necessary to kill *C. fornicata* juveniles (2.7–5.3 mm shell length), individuals were desiccated (see above) in 32 to 100% RH at 20°C for up to 5 h. Before desiccation, the shell of each juvenile was blotted dry before the juvenile was weighed in a Mettler Toledo model AL104 balance. The juveniles were then desiccated and weighed again following desiccation; they were subsequently transferred to individual dishes con-

taining 0.45 μm filtered seawater and mortality was assessed 18 h later. Immediately after mortality was assessed, all juveniles were dried at 50°C in a VWR model 133OG drying oven for 48 h to determine final dry weight. Percent water loss (WL) was determined following the approach of Sokolova & Pörtner (2001):

$$\%WL = [(W_i - W_e)/(W_i - W_d)] \times 100 \quad (1)$$

where W_i is the initial weight, W_e is the weight after the desiccation period, and W_d is the final dry weight. Although some of the organisms died during the 18 h recovery period, their tissues were still intact before determination of final dry weight.

Lifting behavior

Behavioral observations of intertidal individuals were made in the laboratory within 48 h of collection. All animals were individually observed in a 1.1 l clear plastic aquarium that was initially filled with 0.45 μm filtered seawater at a salinity of 30 and temperature of ~20°C. Before observations were made, individuals were transferred from their aquaria to the observation tank and allowed to acclimate in the observation tank for 10 min. They were then observed for 15 min (while submerged); the times of all lifting or clamping movements were recorded. Following the 15 min submerged observation period, water was slowly drained from the bottom of the observation tank to simulate the outgoing tide. Once the tank was empty, the lifting and clamping behaviors of individuals were observed for an additional 15 min while animals were exposed to the air. Twenty-five individuals were observed, after which the percentage of time spent lifted while individuals were both submerged and exposed to the air was calculated.

Effect of lifting on water loss

To determine the degree to which lifting behavior affects tissue water loss, real-time water loss was measured in *C. fornicata* juveniles that were free to move or were artificially clamped to the substrate. Juveniles (2.8–5.5 mm shell length) were placed on pre-weighed glass slides and were either allowed to move or were artificially clamped to the substrate with an elastic band. Each animal was then placed in a Mettler Toledo model AL104 balance at 20°C and approximately 75% RH. Weight (loss) was recorded every 30 s for 10 min, after which juveniles were removed from the balance and placed in individual

aquaria filled with 0.45 μm filtered seawater. After approximately 6 h recovery time, the experiment was repeated but animals that were free to move were now artificially clamped and vice versa. Percent water loss was computed using Eq. (1).

Statistical analysis

All statistical analyses were done in GraphPad Prism v.4.03. To meet the assumption of the statistical tests, all percentage data were arcsine transformed before comparisons were made. For the behavioral assay, the percentage of time that animals spent lifted while submerged versus exposed to the air was compared using a 2-tailed, paired *t*-test.

The effects of exposure time and RH on *C. fornicata* juvenile survival were compared using a 2-way ANOVA; because no adults died following even a 10 h exposure to 75% RH, desiccation tolerance of intertidal and subtidal adults could not be statistically compared. The percentage of water that organisms lost and the resulting outcome of that water loss (dead or alive) was analyzed using logistic regression.

The percentage of water lost from animals that were free to move and those that were artificially clamped to the substrate was compared using a repeated-measures, 2-way ANOVA with time and condition (free to move or clamped) as factors.

RESULTS

Desiccation tolerance and impact of water loss

When exposed to air in the laboratory at 32% RH, almost half of the juvenile *Crepidula fornicata* died after only 3 h of desiccation stress (Fig. 1). The duration of aerial exposure (2-way ANOVA, $F_{5,72} = 67.80$, $p < 0.0001$) and the RH that juvenile *C. fornicata* were exposed to (2-way ANOVA, $F_{3,72} = 79.61$, $p < 0.0001$) had a significant effect on their mortality, and the interaction between those 2 variables was also significant (2-way ANOVA, $F_{15,72} = 15.65$, $p < 0.0001$; Fig. 1). Although all juveniles exposed to 75% RH for 6 h died, none of the adults, either intertidal or subtidal, died when exposed to the same stress, even after 10 h of exposure at a higher temperature (Fig. 1 inset). Additionally, the relationship between juvenile water loss when exposed to the air and their subsequent survival was significant (Fig. 1; logistic regression, max-rescaled $r^2 = 0.77$, Wald $\chi^2 = 27.06$,

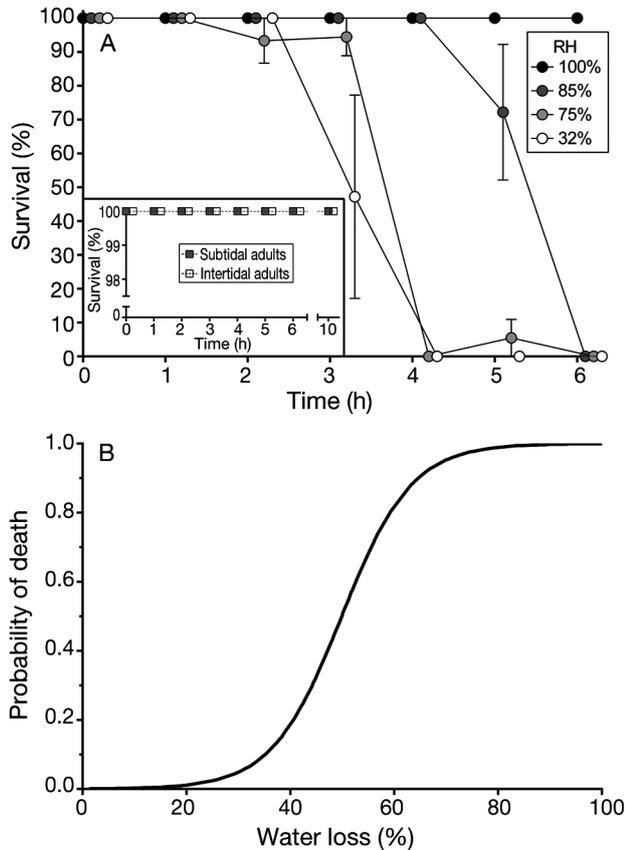


Fig. 1. Desiccation tolerance of juvenile *Crepidula fornicata*. (A) Individuals (1.3–4.6 mm shell length) were exposed to air only once, for 0 to 6 h at the indicated relative humidity (RH) level at 20°C (5–6 ind. replicate⁻¹, 3–6 replicates treatment⁻¹, error bars are SEM). Inset shows tolerance of intertidal and subtidal adults (12–15 adults treatment⁻¹). (B) Amount of water loss required to kill juvenile *C. fornicata*; individuals (N = 133) were subjected to 32 to 100% RH at 20°C for up to 5 h; the resulting curve was generated from logistic regression

C. fornicata juveniles losing between 38.5 and 75.5% of their tissue water varied among individuals.

Lifting behavior and its effect on water loss

When observed in the intertidal zone in the field for at least several minutes, individuals periodically lifted their shells above the substrate and then clamped themselves to the substrate (Fig. 2). During 15 min observation sessions in the laboratory, individuals rarely moved (rather, they stayed lifted) while submerged (Fig. 3), allowing them to keep their tissues continuously exposed to the water for long periods of time (Fig. 3). When exposed to the air, on average their shells were lifted off of the substrate—which exposed their tissues to the air—for approximately 40% of the exposure time, which was significantly less time than when submerged (Fig. 3, $t_{48} = 4.55$, $p < 0.0001$). While in the air, all juveniles lost water over time (Fig. 3, 2-way ANOVA, $F = 2.14$, $p = 0.0036$). However, individuals that were free to

$p < 0.0001$) When out of the water, all *C. fornicata* juveniles that lost less than 38.5% of their tissue water survived. Conversely, all juveniles that lost more than 75.5% of their tissue water died; the fate of

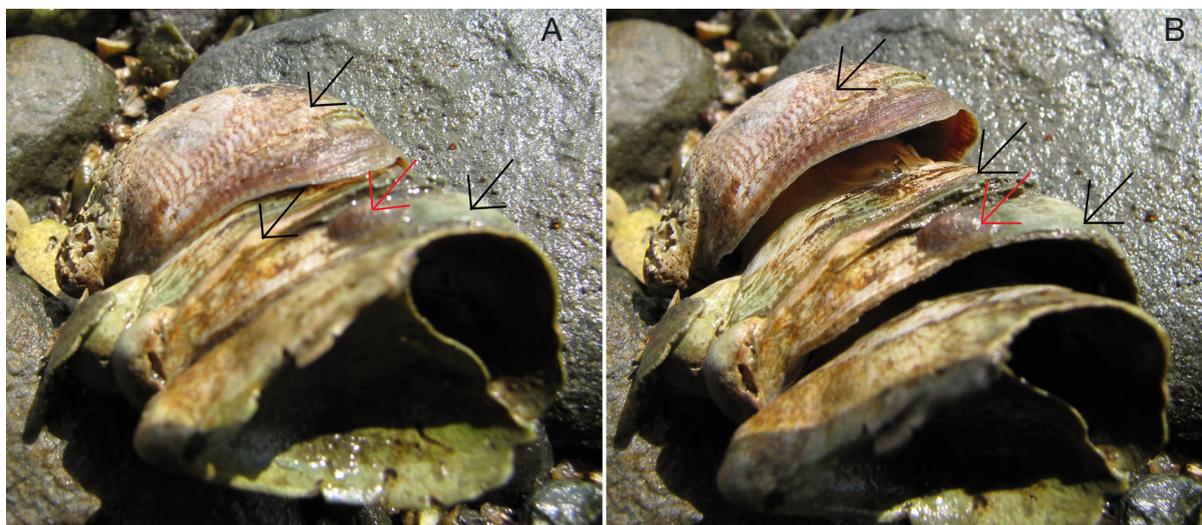


Fig. 2. Lifting behavior in *Crepidula fornicata*. (A) A stack of 3 adults (black arrows, bottom-most adult is on an empty shell) and 1 small juvenile (red arrow) all clamped to the substrate. (B) Seconds later, the top-most and bottom-most members of the stack have lifted off of the substrate

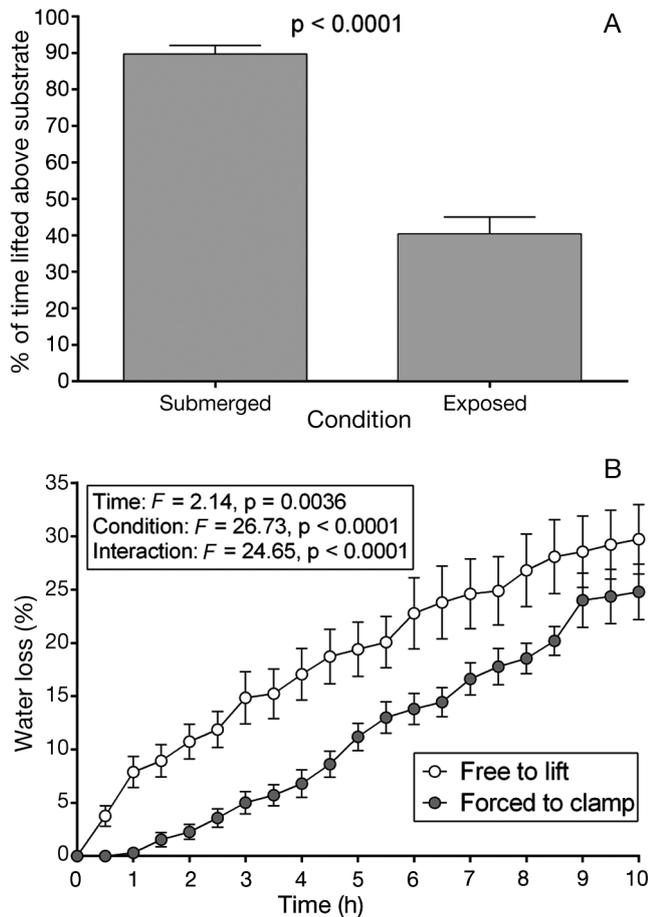


Fig. 3. (A) Lifting behavior of intertidal *Crepidula fornicata* ($N = 25$; error bars are SEM) when submerged or exposed to the air; results of paired t -tests are above the bars. (B) Water loss of juveniles when organisms were exposed to the air while they were either free to lift off of the substrate (white circles) or forced to clamp to the substrate (gray circles); results of 2-way ANOVA are presented on the graph

lift off of the substrate lost significantly more water than those that were forcibly clamped to the substrate (Fig. 3, 2-way ANOVA, $F = 26.73$, $p < 0.0001$), and the interaction between the 2 factors was also significant (2-way ANOVA, $F = 24.65$, $p < 0.0001$).

DISCUSSION

At Bissel Cove in Narragansett Bay, Rhode Island, intertidal individuals of *Crepidula fornicata* lifted their shells above the substrate frequently when exposed to the air. In fact, at any given time, approximately half of the organisms we observed were lifted above the substrate during any observation time (not shown). When submerged, individuals of *C. fornicata* almost always had their shells lifted off of

the substrate, presumably for feeding and respiration. However, when exposed to air during 15 min observational periods in the laboratory, individuals of *C. fornicata* spent only about half of their time clamped to the substrate. While some high intertidal and subtidal mollusks seal themselves off from the external environment for extended periods of time upon emersion (Nicchitta & Ellington 1983, McMahon 1990), other intertidal mollusks perform behaviors similar to those that we observed for *C. fornicata* (Lent 1968, Boyden 1972, Widdows et al. 1979, Garrity 1984, Widdows & Shick 1985, Byrne & McMahon 1994, Dowd & Somero 2013).

This lifting behavior has not previously been explored in *C. fornicata*, which is surprising because individuals of this species can be found intertidally in both their native (Diederich et al. 2011, Hoch & Cahill 2012, Diederich & Pechenik 2013) and invasive (Thieltges et al. 2003, Viard et al. 2006) ranges. Additionally, before our study, little work had been done concerning the contribution of this behavior to water loss and the tolerance of *C. fornicata* to desiccation stress. Hoagland (1984) found that large individuals of *C. fornicata* were able to survive in air for much longer than juveniles. In our study, we also found that, at relatively benign temperatures and humidity levels, adult *C. fornicata* survived longer than juveniles, suggesting a potential size escape from desiccation-induced mortality. Size escape from desiccation stress has been found in other mollusks (Davies 1969, Branch 1975, McQuaid 1982, Williams et al. 2005, Jenewein & Gosselin 2013), and is not surprising as larger animals have more water to lose and a lower surface area to volume ratio. A loss of approximately 40% of total water killed some juveniles in our study, and no juveniles were able to lose more than 75% of their total water without dying. This amount of water loss corresponded to approximately 23 to 39% total weight loss, which agrees with the results of Hoagland (1984), who found that small individuals of *C. fornicata* could only tolerate a weight loss from desiccation of approximately 30%. The amount of water loss that intertidal mollusks can tolerate is highly variable, with some species able to tolerate over 80% water loss (Wolcott 1973, Price 1980). More commonly, however, individuals tend to die after losing 10 to 70% of their tissue water (Lent 1968, Davies 1969, Branch 1975, Marshall & McQuaid 1992, Williams & Morrill 1995, Sokolova & Pörtner 2001, Hicks & McMahon 2003).

Since we found that lifting behavior during aerial exposure increased rates of water loss, we believe there could be an adaptive benefit to such a behav-

ior, or else we would expect the animals to completely isolate their tissues from the air for the duration of the exposure. Although lifting behavior may help to cool tissues through evaporation when air temperatures are high in the intertidal zone (Williams et al. 2005, Nicastro et al. 2012), we have observed individuals of *C. fornicata* with their shells lifted above the substrate over a wide range of temperatures (not shown). We suggest that individuals of *C. fornicata* perform this behavior for respiratory purposes. *C. fornicata* are able to efficiently utilize oxygen in the air: aerial respiration rate was approximately 70% of the submerged rate over the temperature range 5 to 25°C (Newell & Kofoed 1977). Thus, lifting off of the substrate for short periods of time should allow these organisms to release built up CO₂ and replenish oxygen. Several other intertidal mollusks apparently also perform this behavior for respiratory purposes (e.g. Lent 1969, Boyden 1972, Widdows et al. 1979, Dowd & Somero 2013). Their ability to maintain aerobic metabolism in air and control their water loss with periodic clamping and lifting are important factors that may in part be responsible for the broad vertical distribution of this species; characterization of aerial respiration as it relates to the lifting behavior exhibited by *C. fornicata* should be explored in future studies.

Some juveniles in our study died after an aerial exposure of only 2 to 3 h, which is a realistic amount of aerial exposure time in nature (Diederich & Pechenik 2013). Thus, desiccation alone may be important for controlling the upper distribution of this species. However, the short time that individuals of *C. fornicata* are susceptible to desiccation stress (as young juveniles) is also the time when they are mobile. This mobility could allow small *C. fornicata* to position themselves in moist areas to avoid desiccation stress when exposed to the air. Most *C. fornicata* can be found living upon one another in stacks of up to 15 members (Coe 1936). Smaller individuals often position themselves at the shell margin of larger females, probably for mating purposes (Coe 1936). This positioning of small individuals may have an additional benefit, as lifting by females when exposed to air often results in the release of a small volume of fluid that was retained in the female mantle cavity (*C. Diederich pers. obs.*). Small juveniles at the shell margin can thus be wetted by this mantle cavity fluid, possibly allowing them to tolerate aerial exposure until they are large enough to be highly tolerant of desiccation stress.

As individuals of *C. fornicata* grow larger, their upper distribution is more likely to be determined by

factors other than resistance to desiccation. Most notably, both high (Diederich & Pechenik 2013) and low (Thieltges et al. 2004) temperatures have been reported as possible determinants of range limits in *C. fornicata*. Additionally, individuals of this species need to spend enough time submerged to collect an adequate amount of food by suspension feeding. As is true for many other species, it may be a combination of many stresses associated with intertidal life, including desiccation, that limits the upper intertidal distribution of this species (e.g. Firth & Williams 2009, Williams et al. 2011, Iacarella & Helmuth 2012). Even so, our study highlights the importance of desiccation stress and lifting behavior in the life history of this species. Individuals cannot tolerate long periods of aerial exposure when they are very small, so *C. fornicata* juveniles probably have to avoid desiccation for at least the first few days or weeks after metamorphosis.

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