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Temperature and relative humidity effects on water loss and hemolymph osmolality of Littoraria angulifera (Lamarck, 1822)

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Temperature and Relative Humidity Effects on Water Loss and Hemolymph Osmolality of *Littoraria angulifera* (Lamarck, 1822)

By

Phillip J. Rose

A Thesis Presented to the Faculty of the College of Science, Mathematics and Technology in Partial Fulfillment of the Requirements for the Degree of Master of Science

In the field of Biology

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- Phillip J. Rose

Abstract

Desiccation stress is considered to be one of the more significant determining factors that influence how organisms are distributed in the marine littoral. Gastropods living above the high tide mark, referred to as eulittoral fringe gastropods are not wetted as regularly and face unpredictable and prolonged periods of emersion. Consequently, adaptations displayed by eulittoral fringe gastropods are aimed at minimizing water loss and surviving prolonged periods of desiccation stress. *Littoraria angulifera*, is a tropically distributed marine eulittoral fringe gastropod. Because *L. angulifera* spends a majority of its time emersed there was interest in studying how the weight loss and hemolymph osmolality of this species changed over a period of time in response to varying environmental conditions. Hypotheses were that weight loss and hemolymph osmolality would be dependent upon temperature and relative humidity with weight loss and hemolymph osmolality being highest at high temperatures and low relative humidities. Additionally, it was predicted that this species should also exhibit some form of regulation of either weight loss or osmolality. Specimens ranging in size from 15.24 to 28.40 mm were collected from concrete marina bulkheads in Port Isabel, Texas. Weight loss rate and hemolymph osmolality were examined at test temperatures of 15°, 25°, and 35°C and relative humidities (RH) of <5%, 33%, 53%, 75%, and >95%. Weight loss rates were tracked for 5 individuals in each temperature/RH treatment. Hemolymph osmolality of was determined at 0, 5, 10, and 15 days in each of the temperature/RH treatments. The weight loss rates were significantly affected by test temperature and relative humidity and varied significantly across each test temperature/RH combination. Hemolymph osmolality was not significantly affected by test temperature but was affected by RH. Results indicated that weight loss increased as temperatures increased and RH decreased and hemolymph osmolalities generally increasing as relative humidity decreased. Specimens did not seem to display any signs of osmoregulation, but this may have been due to an experimental shortcoming. Behavioral responses to emersion that were observed were consistent with the responses displayed by other eulittoral fringe species displaying how this species' is well adapted to life in its exacting habitat.

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I. Introduction

Project overview:

The marine littoral is considered to be an especially harsh and stressful environment (Grant and McDonald, 1979). Organisms inhabiting the marine littoral are subjected to many different forms of environmental stress, which can include salinity variation (Hicks et al., 2000; McMahon, 2003), temperature variation (Hildreth and Stickle, 1980), wave force (Trussell, 1997), and desiccation stress (Grant and McDonald, 1979; McMahon, 1988; McMahon, 1990; Hicks and McMahon, 2003; Stafford and Davies, 2004).

The high tide mark in the intertidal may act as a point where one set of limiting factors ends and another set begins. Above or below the high tide mark morphological, behavioral, and physiological adaptations are primarily responses to the absence or presence of water, respectively (McMahon, 1990; Lang et al., 1998). Accordingly, littoral gastropods can be divided into two adaptational groups. Those species living below the high tide mark, referred to as eulittoral gastropods, are immersed regularly, albeit for varying periods, which decrease with increasing height on the shore. Gastropods living above the high tide mark, referred to as eulittoral fringe gastropods are not wetted as regularly and face unpredictable and prolonged periods of emersion (McMahon, 1990). Consequently, these two groups of gastropods employ different strategies to cope with the emersion times of their respective environments (McMahon, 1988; McMahon, 1990). For example, since eulittoral gastropods are wetted and re-hydrated during each tidal cycle they rarely face desiccation stress and so display adaptations that favor maintenance of activity and foraging while emersed (McMahon, 1990). Some of these adaptations can

include evaporative cooling, elevated aerial oxygen uptake rates and lower thermal tolerance (McMahon, 1977; McMahon, 1990; McMahon, 2003). Longer periods of emergence in eulittoral fringe habitats results in greater exposure to severe desiccation stress than is experienced by eulittoral species living below the high tide mark. As a result, the adaptations displayed by eulittoral fringe gastropods are aimed at minimizing water loss and surviving prolonged periods of time without water (McMahon, 1990). Some of these adaptations include sealable opercula to prevent water loss, foot withdrawal to prevent conduction of heat from surfaces, reduction of metabolic rate, increased thermal tolerance, as well as production of a mucus holdfast to maintain position on surfaces when inactive (Garrity, 1984; McMahon, 1990; Miller, 2008). This study focuses on responses to temperature and desiccation stress during prolonged emersion in eulittoral fringe species.

The mangrove (angulate) periwinkle, *Littoraria* (formerly *Littorina*) *angulifera*, is a marine eulittoral fringe gastropod belonging to the family Littorinidae which is tropically distributed along the eastern Atlantic coast of Africa as well as in the western Atlantic throughout the Caribbean, Bermuda, Florida, and from south Texas to Brazil (Britton and Morton, 1989; Abbott and Morris, 1995; Merkt and Ellison, 1998; Tanaka and Maia, 2006; Tunnell et al., 2010). *L. angulifera* is typically found living high upon the leaves, prop-roots, stems, trunks, and branches of mangroves; principally *Rhizophora mangle* the red mangrove (Gutierrez, 1988; Kaplan, 1988; Little, 1990; Merkt and Ellison, 1998). On these mangroves, they can occur anywhere from the waterline up to seven meters above the high tide mark (Kohlmeyer and Bebout, 1986; Gutierrez, 1988; Kaplan, 1988). They migrate up and down staying just ahead of the tide (Little, 1980). In

south Texas, particularly Port Isabel and South Padre Island, *L. angulifera* is typically found clinging to man made structures such as marina bulkheads and pier pilings as *R. mangle* does not occur at this latitude due to its intolerance of even mild winter freezes (Britton and Morton, 1989; Tunnell et al., 2010). There have been some studies involving *L. angulifera*; however these studies for the most part have focused on their ecology and behavior (Kohlmeyer and Bebout, 1986; Gutierrez, 1988; Miller and Denny, 2011), shell morphological variation (Merkt and Ellison, 1998; Tanaka and Maia, 2006; Miller and Denny, 2011), genetic variation (Gaines et al., 1974), influence of size and environmental factors on radula shape (Andrade and Solferini, 2006), distribution, population dynamics and zonation patterns (Gallagher and Reid, 1979; Ortiz and Blanco, 2012), utilizing precipitins as potential diagnostic reagents (Smith, 1986), reproductive habits (Lenderking, 1954), and respiratory responses to emersion and temperature variation (Trevino, 2012). There does not seem to be much in the way of information concerning the physiological effects of desiccation on this species.

The physiological effects of desiccation on other gastropod species have been studied extensively. Desiccation stress is considered to be one of the more significant determining factors that influence how organisms are distributed in the intertidal (Gibson, 1970; Gallo, 1984; Miller et al., 2009). Locomotory behavior in mangrove littorinids was also found to be greatly influenced by physical factors associated with desiccation stress (Lee and Williams, 2002). Emson et al., (2002) demonstrated in long-term experimental studies that both water loss and increases in hemolymph concentration were greatest in the early periods of desiccation in the littorinid snail, *Cenchritis muricatus*. After the initial increase in hemolymph concentration there appeared to be evidence of

osmoregulation possibly accomplished by exchange of free water (extracorporeal) in the shell itself. Iacarella and Helmuth (2012) examined the influence microclimates within the *Spartina alterniflora* canopy on the physiological conditions of *L. irrorata* relative to thermal and desiccation stresses and how these corresponding effects might restrict this species' activity. They found that the behavioral patterns of *L. irrorata* were indeed controlled in large part by microclimates within the *S. alterniflora* canopy as well as its ability to endure desiccation stress.

Because *L. angulifera* spends a majority of its time emersed there was interest in studying how the weight loss rate and hemolymph osmolality of this species changed over a period of time in response to varying environmental conditions. It was hypothesized that weight loss rates would be dependent upon temperature and relative humidity with weight loss being greatest at high temperatures and low relative humidities. It was also hypothesized that hemolymph osmolality would also be dependent upon temperature and relative humidity with hemolymph osmolality being highest at high temperatures and low relative humidities. It is additionally predicted that this species should also exhibit some form of regulation of both weight loss and hemolymph osmolality given its prolonged exposure to these stressors in its eulittoral fringe habitat. In order address these hypotheses the following objectives were pursued:

1) to determine how different temperature and relative humidity combinations effect weight loss rates in *L. angulifera* and
2) to determine how different temperature and relative humidity combinations effect hemolymph omolalities in *L. angulifera*.

II. Methods

Collection and Maintenance of Specimens

Specimens of *L. angulifera*, ranging in size from 15.24 to 28.40 mm, were collected from concrete marina bulkheads in Port Isabel, Texas (26° 4’ N, 97° 12’ W). Specimens were transported back to the laboratory where they were kept at room temperature for one week prior to experimentation in a 10-gallon (37 liters) tank containing periphyton-covered oyster shells and approximately 11 liters of natural seawater.

Weight loss rate and hemolymph osmolality for *L. angulifera* were examined at test temperatures of 15°, 25°, and 35°C and relative humidities (RH) of <5%, 33%, 53%, 75%, and >95%. Relative humidity exposures were maintained by placing specimens in sealed desiccation chambers (7 L) using silica gel desiccant for a RH of <5%, magnesium chloride for a RH of 33%, magnesium nitrate for a RH of 53%, sodium chloride for a RH of 75%, or distilled water with a sponge placed in the bottom of the desiccation chamber to maintain the experimental RH of >95% (Byrne et al., 1998; Hicks and McMahon, 2003). Distilled water was used in the formation of all saturated salt solutions. A circular shaped screen mesh platform was used to suspend the snails over the saturated salt solutions. The screen mesh acted as a barrier to prevent the snails from crawling into the media.

Prior to being placed in the desiccation chambers, all snails were re-hydrated by immersion in 35 ‰ seawater (approximately 1,000 mOsmol kg-1). This was

accomplished by placing specimens in a covered jar filled to capacity with natural seawater (obtained from the Gulf of Mexico) for a period of 15 minutes (water temperature ≈ 25°C). The desiccation chambers, were sealed by liberally applying petroleum jelly (Equate®) to the rim of the desiccator and then placing the lid on top. The desiccation chambers were then placed inside of an environmental chamber, which was set to the test temperature of 15°C, 25°C, or 35°C. Relative humidity treatments within the desiccation chambers were monitored by means of analog hygrometers (All Living Things®) for the duration of the study.

Weight Loss

Weight loss rates were tracked for 5 individuals in each temperature/relative humidity treatment. Prior to emersion, each individual had both their initial weight as well as their shell dimensions recorded. The operculum of each snail was also lightly tapped so that the snail retracted further into the shell expelling any extra-corporal water that may have otherwise remained in the shell. After being blotted dry with absorbent tissue for the purposes of removing any adherent water, snail weights were recorded to the nearest 0.01 g (Mettler Toledo® AB204-S). Snail shell dimensions were then measured using a digital caliper (Cen-Tech ® 47256) to the nearest 0.01 mm. These dimensions included both shell length and shell width. Shell length (or height) was the maximum measurement along the central axis passing through the apex to the anterior lip of the shell and shell width was the maximum width perpendicular to the shell length distance (Chiu et al, 2002; Riascos and Guzman, 2010). After initial snail weight and snail shell measurements were made, a minute amount of a red, blue, green, yellow, or

pink nail polish was applied to the snail's shell. This was necessary so that an individual could later be identified for the purposes of tracking its weight loss over the course of the experimental period. The specimens were then reweighed to account for any weight that might have been added by the nail polish. Any weight added by the nail polish was negligible. The specimens were then placed in desiccation chambers, which in turn were placed in an environmental chamber. Individuals in each temperature/relative humidity treatment were reweighed at 5, 10, and 15 days in order to determine weight loss rates.

Hemolymph Osmolality

Hemolymph osmolality of *L. angulifera* was determined at 0, 5, 10, and 15 days in each of the temperature/relative humidity treatments described above. Hemolymph was collected from specimens of *L. angulifera* using a method similar to that of Khan et al. (1999) after they had been removed from the desiccation chambers. Each snail shell was then opened using a small vice, which was used to apply just enough pressure to crack the shell so that the soft tissue beneath could be exposed. A hypodermic needle was then used to prick the soft tissue of the snail along the top of the head. Each snail was then quickly placed in a 0.6-ml polypropylene microcentrifuge tube which, with the use of a dissecting pin, had five small holes punched in the bottom. These tubes were then placed in larger 1.5-ml polypropylene microcentrifuge tubes and these nested tubes were then spun at 6000 rpm for 5 minutes. The inner 0.6-ml tubes containing the snails were then removed, and the hemolymph that had collected in the bottom of the outer 1.5-ml tubes was collected. The hemolymph of 4 specimens was then pooled into one 1.5-ml tube using a pipette so that there was an adequate amount of hemolymph to test. Thus, the

pooled hemolymph of 4 specimens constitutes 1 sample. Two samples were measured in each temperature/relative humidity treatment combination. The tubes containing the pooled hemolymph were then centrifuged at 6,000 rpm for 5 minutes for the purposes of removing any particulate matter. The osmolality of a 20-µl sample of hemolymph was measured using a freezing point depression osmometer (Advanced® Model 3320 Micro-Osmometer, Advanced Instruments®). Preliminary data showed that hemolymph osmolalities tended to exceed the 2000 mOsm/kg capacity of the osmometer, so samples were diluted. This was accomplished by using a pipettor to add equal parts of double distilled H_20 and hemolymph to a 1.5-ml microcentrifuge tube. These diluted samples were mixed and were then centrifuged at 6,000 rpm for 5 minutes. A sample was then drawn from the 1.5-ml microcentrifuge tube and measured using a freezing point depression osmometer. In order to determine the osmolality of the diluted sample, the osmolality reading given by the osmometer was multiplied by 2.

Statistical Analyses

Mean weight loss rate for each temperature/relative humidity combination were estimated using a multiple regression model with emersion time as a repeated measure and employing a cell means parameterization. Individual size (shell length) was utilized as a covariate (Eq. 1).

$$Y_{itrd} = \mu_{rt} + \beta_{rt}d + \alpha_{rt}\,(SL - SL_o) + \gamma_{rt}d\,(SL - SL_o) + \varepsilon_{itrd}, \qquad \text{Eq. 1}$$

Where Y_{itrd} is the percentage of weight remaining, in individual *i*, at day *d*, at temperature level *t* (*t* =1 is 15°C, etc.) under relative humidity level *r* (*r* =1 is <5% RH, etc.); μ_{rt} is the average percent of weight remaining of specimens at *d* = 0 under temperature *t* and relative humidity *r*; $\beta_{rt}d$ is the average rate of percent weight loss at test temperature *t* under relative humidity *r*, when SL = SL_0 (I.E., SL_0 is the specified, 'adjust to', value of SL); α_{rt} relates average percent of weight remaining to SL, and γ, is the coefficient allowing average percent of weight remaining to relate differently to SL for different day *d* (Hicks and McMahon, 2003).

Mean hemolymph osmolality for each temperature/relative humidity combination was similarly estimated using a multiple regression model and a cell means parameterization (Eq. 2).

$$Y_{itrd} = \mu_{rt} + \beta_{rt}d + \alpha_{rt} + \gamma_{rt}d + \varepsilon_{itrd}, \qquad \text{Eq. 2}$$

Where Y_{itrd} was the hemolymph osmolality in sample *i*, at day *d*, at temperature level *t* (*t* =1 is 15°C, etc.) under relative humidity level *r* (*r* =1 is <5% RH, etc.); μ_{rt} was the average hemolymph osmolality of specimens at *d* = 0 and temperature *t* under relative humidity *r*, $\beta_{rt}d$ was the average rate of change in hemolymph osmolality at test temperature *t* under relative humidity *r* (Hicks and McMahon, 2003). Both weight loss and hemolymph osmolality models were estimated, using maximum likelihood, via the MIXED procedure in SAS® (SAS®, Cary, North Carolina)

III. Results

Weight loss

The weight loss rates of a standard 22mm SL individual were significantly affected by test temperature ($F = 3.49$, $df = 2, 270$, $P = 0.0320$) and relative humidity ($F = 23.58$, $df = 4, 270$, $P = 0.0001$) and varied significantly across each test temperature/relative humidity combination ($F = 3.25$, $df = 8, 270$, $P = 0.0015$). Across all test temperatures (15°, 25°, or 35°C) total weight loss rates (% water remaining) during emersion decreased with increasing RH. Weight loss rates were generally greater at higher temperatures than at lower temperatures (figures 1, 2, & 3). There was also a significant interaction between shell length and treatment effect ($F = 3.51$, $df = 70$, $P = 0.0002$) as well as shell length and time (*d*) ($F = 7.16$, $df = 270$, $P<0.001$). While both of these shell length interactions were significant, no clear pattern emerged relative to treatments or time.

During emersion trials, all specimens were withdrawn into their shells with the operculum closed in all temperature/relative humidity treatments, with the exception of those in the > 95% RH treatments. Those that were withdrawn into their shells were adhered by means of the mucus holdfast to the inside surfaces of the desiccation chamber, with some aggregating in a small space at the top of the chamber that forms the handle of the desiccator's lid. It is likely that the latter response was a behavior to seek a preferred microclimatic relative humidity environment. Those in the >95% RH treatment that had not withdrawn into their shells, continued to move about within the desiccation

chamber. However, they became less active over time as the end of the experimental period approached. At higher temperatures and towards the end of the experimental period, some specimens were not completely retracted into their shells and displayed little to no mobility.

At 15°C mean weight loss rates during emersion decreased with increasing relative humidity (Table 1, Figure 1). Of the five different RH treatments, only the 5% and >95% treatments were significantly different from the other four RH treatments (Scheffé test, $P<0.05$) while the 33%, 53%, and 75% RH treatments were not significantly different from each other (Table 2).

At 25°C mean weight loss rates decreased with increasing relative humidity (Table 1, Figure 5). The >5% RH treatment was significantly different from the 33% and >95% RH treatments. The 53%, and 75% RH treatments were only significantly different from that of the >95% RH treatment and the >95% treatment was significantly different from the other four RH treatments.

At 35°C mean weight loss rates decreased as relative humidity increased (Table 1, Figure 6). The >5% RH treatment was significantly different only from the >95% RH treatment and 33% was significantly different from both 75% and >95% RH treatments. The 53% treatment was only significantly different from that of the >95% RH treatment (tables 1 & 2). The >95% RH treatment was significantly different from the other four RH treatments (tables 1 & 2).

Hemolymph Osmolality

Hemolymph osmolality in *L. angulifera* was not significantly affected by test temperature ($F = 2.24$, $df = 2, 90$, $P = 0.0903$), but was affected significantly by relative humidity ($F = 17.05$, $df = 4, 90$, $P < 0.0001$). Relative humidity effects were similar across all test temperatures (15°, 25°, or 35°C) ($F = 0.34$, $df = 8, 90$, $P = 0.9327$). Osmolalities increased for the RH treatments of <5%, 33%, 53%, and 75% as time emersed increased (Figures 1, 2, & 3). The osmolalities for the >95% RH treatment decreased, as the period of time emersed increased (Figure 1, 2, & 3). The osmolality concentrations for the RH treatments of <5%, 33%, 53%, and 75% were not significantly different from each other but all were significantly different from that of the >95% RH treatment (Table 4). Behavior of specimens was as described in the weight loss study.

IV. Discussion

Weight loss

The results of this study indicate that weight loss rates in *L. angulifera* increase as temperatures increase and relative humidities decrease. Individuals generally lost weight at a constant rate over the 15 day experimental period for all temperature/RH treatments with the exception of those in the >95% RH treatment (figures 1, 2, & 3). These results are similar to the findings of desiccation studies by Iacarella and Helmuth (2011) with *L. irrorata*. The studies demonstrated that temperature and RH had a significant effect on *L. irrorata* weight loss (Iacarella and Helmuth, 2011). However, these results contrast with the results of some other studies. Sokolova and Pörtner (2001) conducted a study that sought to compare the water loss rates of low-shore and high-shore specimens of *Littorina saxatilis* from both the North and White seas. They found that water loss in *L. saxatilis*, which occurs from the upper subtidal to the supratidal splash zone, was non-linear with most water being lost within in the first 12 hours of their experiment. Britton (1992) found that *C. muricatus* lost most of its water over the first 8 days of dehydration. Similarly, Emson et al. (2002) found that water loss was greatest during the first 7 days of desiccation in their study, with the rates leveling off there after. What may account for this discrepancy is the fact that the snails in this study had their opercula lightly poked with forceps to encourage retraction into their shells, which expelled any extracorporeal water in the process. It is believed that the leveling off after seven days in the *C.*

muricatus study might have been attributed to an exchange of free extracorporeal water with in the shell (Emson et al., 2002).

The specimens within the >95% RH treatment for all test temperatures displayed an increase in weight (figures 1, 2, & 3). This was most likely due to them absorbing moisture as they moved across the surfaces within the desiccation chamber, which had water condensing on the surfaces as a result of the high humidity. Or they absorbed moisture from the water-saturated atmosphere within the desiccation chamber. This has been observed before in studies involving other gastropod species. Ejidike et al. (2004) found that the terrestrial gastropod *Archachatina marginata* displayed remarkable weight gain at lower temperature, higher humidity test treatments.

Specimens in the higher temperature lower RH treatments were withdrawn into their shells with their opercula occluding the aperture. Most were affixed to the inside surfaces of the desiccation chambers via the mucus holdfast. Both of these behaviors are examples of adaptive strategies utilized by eulittoral fringe gastropods for the purposes of dealing with periods of prolonged emersion (McMahon, 1990; McMahon, 2003). Some specimens were also observed aggregating in a small space at the top of the chamber that forms the handle of the desiccator's lid. It has been suggested that this behavior may function as a means of reducing desiccation stress (Stafford and Davies, 2004; Stafford et al., 2012; Chapperon et al., 2013; Rojas et al., 2013). It is unclear though if this behavior confers any real benefit that would aid in the reduction of desiccation stress. There are studies that have attempted to verify whether or not a relationship exists between intertidal gastropod aggregation behavior and reduction of water loss during prolonged periods of emersion (Moutinho and Alves-Costa, 2000; Stafford and Davies, 2004;

Chapperon et al., 2013; Rojas et al., 2013). It has been pointed out that a relationship between the two would seem sensible; being that the aggregation would provide a reduction in the exposed surface area to cumulative volume ratio of the snails (Stafford et al., 2012). However, many of these studies have differed with respect to conclusions drawn. For instance, Rojas et al. (2013) found that solitary individuals of *Echinolittorina peruviana* kept their opercula open for shorter periods of time than did those individuals, which were found in aggregations when exposed to increasingly drier conditions. This suggested that individuals in aggregations could sustain gaseous exchange with their environment for longer periods of time than solitary individuals in response to being emersed (Rojas et al., 2013). Additionally, Moutinho and Costa-Alves (2000) studied aggregation behavior in *Littoraria flava*, taking shell size variation into account. They found that there was an inverse relationship between the length of the snail's shell and the percentage of water lost, with the smaller snails losing a greater percentage of water than the larger snails. They also noted that snails that occupied the center of the aggregations were on average smaller than those found both on the periphery of aggregations and those isolated from aggregations. Moutinho and Costa-Alva (2000) suggested that it would make sense that smaller snails would be more susceptible to water loss due to the surface area to volume ration of their shell. From these results they proposed that the aggregations must be a behavioral response to limiting the stress induced by temperature and desiccation. Stafford & Davies (2004) findings indicated that aggregations formed by high shore littorines were not necessarily a behavioral response to desiccation stress and that these results contradicted those of similar studies. However, they did propose that the aggregations might be the result of the snails exploiting microclimates along the shore

because based on their personal observations they noticed that the aggregations were frequently associated with pits and crevices in the rocks (Stafford and Davies, 2004). It was also suggested that prevention of dislodgement by wave action, reduced desiccation stress, as well as reduced predation were all possible benefits to snails inhabiting crevices (Stafford and Davies, 2004; Stafford et al., 2012). Bates and Hicks (2005) studied microhabitat selection in two species of littoral gastropods, *Nerita versicolor* a mid-shore species and *Tectarius antonii* a high-shore species. The findings of the study demonstrated that the behavioral adaptation of crevice utilization was most likely the result of the snails trying to avoid desiccation stress rather than predation or wave dislodgment (Bates and Hicks, 2005). Therefore, a possible explanation for the observed small aggregations in the small space at the top of the desiccation chamber is that the small space may have been acting as a favorable microclimate, which the snails then sought out.

Towards the end of the experimental period at the highest temperature treatment of 35°C some specimens were not completely withdrawn into their shell and were not moving around inside the desiccation chamber. When picked up they showed little to no movement. A potential explanation for this may be that they were experiencing heat coma. When heated marine snails can enter a condition known as heat coma, which usually sets in when they are approaching their thermal tolerances limits (Hamby, 1975). Immobility, extension of the foot, and detachment from surfaces are all signs that are symptomatic of heat coma (Hamby, 1975). Some individuals did seem to exhibit these signs.

The rate of weight loss on a per day basis for *L. angulifera* was minimal relative to some of the weight loss rates observed in other species. The low-shore, mytilid bivalve *Perna perna*, at test temperatures of 15°C, 25°C, and 35°C and relative humidities of <5%, 33%, 53%, 75%, and >95%, experienced water loss rates ranging from 8.4% to 55.44% per day (Hicks and McMahon, 2003). Weight loss for *L. angulifera* under the same temperature and relative humidity treatments ranged from 0.32% to 3.34% per day. The xanthid mud crab, *Eurpanopeus depressus*, which occurs subtidally and intertidally, experienced water loss rates of up to 30% at 25°C displaying its low desiccation tolerance (Grant and McDonald, 1979). Staikou (1999) found that the polymorphic land snail *Cepaea vindobonensis* lost on average anywhere from 7% to 18% depending upon morph type and place of origin.

Hemolymph Osmolality

It was hypothesized that hemolymph osmolality would be dependent upon temperature and relative humidity with hemolymph osmolality being highest at high temperatures and low relative humidities. Additionally, it was also predicted that this species should also exhibit some form of regulation of hemolymph osmolality given its prolonged exposure to the stressors of its eulittoral fringe habitat.

The results of this study supported the hypothesis that hemolymph osmolality would be dependent upon temperature and relative humidity, with hemolymph osmolalities in *L. angulifera* generally increasing as relative humidity decreased and temperature increased (figures 3, 4, & 5). The only RH treatment that did not adhere to this trend was again the >95% RH treatment (figures 3, 4, & 5). The osmolality of the

hemolymph in the >95% RH treatments, actually became less concentrated over the 15 day experimental period (figures 3, 4, & 5). This was again probably due to the saturated atmosphere within the desiccation chamber as described for other gastropods (Ejidike et al., 2004).

Based on previous studies of other high shore littorines, it was also hypothesized that *L. angulifera* should exhibit some evidence of osmoregulation with prolonged periods of emersion. The results of this study did not support this hypothesis, showing that hemolymph osmolality in *L. angulifera* generally increased at a constant rate for all RH treatments with the exception of the >95% RH treatment which demonstrated a more or less constant decrease in hemolymph osmolality as the experiment progressed (figures 1, 2, & 3). This result was not consistent with the findings of other studies. Emson et al. (2002) reported that after an initial increase during the first week of experimentation, the hemolymph concentration of *C. muricatus* stabilized and that this was probably accomplished through an exchange with free water within the shell. *L. angulifera* on the other hand did not exhibit any obvious signs of osmoregulation (figures 1, 2, & 3). This is not to say that this species does not osmoregulate, there just was not much evidence for it here. One plausible explanation for this might lie in some slight differences with respect to the experimental procedure. Prior to experimentation specimens of *L. angulifera* had their opercula lightly poked with forceps so that they would retract into their shells with the goal of getting them to expel extracorporeal water. Emson et al. (2002) mentioned that they thought *C. muricatus* was able to accomplish osmoregulation through an exchange with the free water within the shell. It would have been difficult for *L. angulifera* to accomplish osmoregulation in this study because of the lack of free water

within the shell. In order to establish with some degree of certainty, whether or not *L. angulifera* is capable of osmoregulation one would do well to adopt the experimental procedure of Emson et al. (2002) and then just substitute *C. muricatus* for *L. angulifera*. This would allow for the elimination of any variations in experimental design.

During this study the hemolymph osmolality concentrations that were recorded for *L. angulifera* ranged from as low as 608 mOsmol kg^{-1} to as high as 3,068 mOsmol kg^{-1}. The hemolymph osmolality of 3,068 mOsmol kg^{-1} is more than double the hemolymph osmolality of 1,487 mOsmol kg^{-1} which was recorded for specimens that were sampled directly from the field. How is it that *L. angulifera* is able to tolerate these elevated hemolymph osmolalities? A possible explanation may be that *L. angulifera* has evolved an excretory system that allows them to deal with these prolonged periods of emersion. In a study which sought to examine the behavioral, physiological, and ultrastructural adaptations of *C. muricatus*, specimens were sealed in beakers at 35°C and 40% RH (Emson et al., 2002). The experimental specimens were then dissected and slides with pieces of kidney tissue were prepared and then viewed under a scanning electron microscope. The findings revealed hemolymph concentrations in excess of 2,500 mOsmol kg^{-1} d. The authors mentioned that to their knowledge there were no comparable measurements of hemolymph osmolality for other species of high-shore littorines. It was proposed that *C. muricatus* may be able to cope with these elevated hemolymph concentrations due to the structure of their excretory system, which differs from that of the general aquatic prosobranch pattern (Emson et al., 2002). *C. muricatus* lacks auricular filtration chambers and podocytes and reduced filtration happens through tubules that permeate the epicardial cells (Emson et al., 2002). This is characteristic of some

terrestrial and littoral fringe littorinids (Andrews, 1988). This is said to be indicative of an emphasis on uricotelic excretion as opposed to ammonotelic excretion as well as reducing water loss during primary urine formation (Emson et al., 2002). Uricotelic excretion is said to be ideal for animals that are small and that are often stressed by a scarcity of water (Willmer et al., 2005). In this form of excretion uric acid is produced as the nitrogenous waste product (Willmer et al., 2005). Uric acid is highly insoluble and is not toxic to tissues so it can be stored without ill effect (Willmer et al., 2005). There is also very little water loss associated with this form of excretion (Willmer et al., 2005). Furthermore, it was discovered that the nephridial gland has a reduced surface area which means that smaller amounts of primary urine are produced from which organic solutes are taken in by the gland (Taylor and Andrews, 1988). Emson et al. (2002) also found that in comparison with aquatic and mid-littoral species, *C. muricatus* contained a much greater number of excretory cells and that these cells contained one large vacuole rather than many small ones. Whether or not *L. angulifera* has these excretory system characteristics in common with *C. muricatus* is unknown, but if it does that might explain how they are able to tolerate such elevated hemolymph concentrations. In order to resolve if *L. angulifera* does indeed share the adaptive traits of the *C. muricatus* excretory system, one would have to perform a study in which one would seek to examine the ultrastructural adaptations of *L. angulifera*.

L. angulifera has evolved a suite of adaptations that allow it to withstand the prolonged periods of desiccation stress that are associated with its eulittoral fringe habitat. Behaviorally, they seek out favorable microclimates, orient themselves in such a manner that reduces the exposed surface area of the shell to the sun, foot withdrawal to

reduce heat conduction from the substratum, as well as possibly form aggregations to reduce water loss. Morphologically, they utilize the operculum to close off the aperture of the shell to trap water and to reduce evaporative water loss. Physiologically, they can enter a reduced metabolic state, high thermal tolerance so that evaporative cooling is unnecessary, and manufacture a mucus holdfast, which allows them to adhere the lip of the shell to the substratum in order to reduce the conduction of heat. This suite of adaptions is what allows L. angulifera to deal with the harsh conditions of its eulittoral fringe habitat.

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VI. Tables

Table1.Mean weight loss during emersion in % weight lost d^{-1} (±SE), shell morphometrics, and hemolymph osmolality under five relative humidity (RH) treatments for specimens of *Littoraria angulifera* at test temperatures of 15°, 25°, and 35°C.

Test Temp.	RH	Range of shell length (mm)	Range of shell width (mm)	Weight loss rates (% d^{-1} ± SE)	Hemolymph osmolality ($mOsmkg^{-1}$ d^{-1} ± SE)
15°C	<5%	21.3-26.8	11.9-14.8	-2.79 (0.242)	25.4 (15.0)
	33%	16.2-21.2	10.2-13.7	-2.03 (0.227)	20.6 (15.0)
	53%	16.1-22.4	10.8-13.6	-2.01 (0.183)	30.9 (15.0)
	75%	15.9-23.9	10.3-13.9	-1.69 (0.174)	22.2 (15.0)
	>95%	19.6-26.8	12.1-15.7	-0.32 (0.509)	-39.9 (15.0)
25°C	<5%	20.6-23.7	11.9-14.9	-2.91 (0.138)	46.3 (15.0)
	33%	19.0-22.5	12.3-13.9	-2.48 (0.047)	52.5 (15.0)
	53%	19.2-23.2	11.9-15.8	-2.56 (0.146)	43.8 (15.0)
	75%	15.2-21.7	10.1-15.0	-2.52 (0.211)	28.5 (15.0)
	>95%	20.5-26.9	12.8-16.7	0.03 (0.188)	-44.7 (15.0)
35°C	<5%	20.4-28.4	12.2-19.9	-2.89 (0.214)	51.3 (15.0)
	33%	20.1-26-5	12.1-15.4	-2.97 (0.164)	61.9 (15.0)
	53%	15.5-23.9	10.9-15.2	-2.65 (0.373)	52.9 (15.0)
	75%	17.1-24.1	11.1-15.6	-3.34 (0.073)	42.3 (15.0)
	>95%	16.9-22.7	10.5-14.8	0.41 (0.627)	-45.1 (15.0)

Table 2. The Scheffé method of pairwise differences of mean weight loss (% d^{-1}) rates among specimens of *Littoraria angulifera* in response to the test temperatures of 15°C, 25°C, and 35°C and relative humidity (RH) treatments of <5%, 33%. 53%, 75%, and >95%. The values in the table are the probabilities that the two groups that are being compared are the same.

		35°C					25°C					15°C				
		>95%	75%	53%	33%	<5%	>95%	75%	53%	33%	<5%	>95%	75%	53%	33%	<5%
15°C	<5%	<.0001	0.0328	0.7327	0.5616	0.7644	<.0001	0.3928	0.4050	0.1988	0.6623	<.0001	0.0003	0.0102	0.0228	
	33%	0.0003	<.0001	0.1661	0.0011	0.0065	<.0001	0.1196	0.0538	0.0576	0.0011	0.0024	0.2371	0.9316	1	
	53%	0.0003	<.0001	0.1299	0.0001	0.0020	<.0001	0.0697	0.0201	0.0141	0.0001	0.0021	0.2152	1		
	75%	0.0013	<.0001	0.0225	<.0001	<.0001	<.0001	0.0029	0.0002	<.0001	<.0001	0.0114	1			
	>95%	0.3609	<.0001	0.0003	<.0001	<.0001	0.5113	<.0001	<.0001	<.0001	<.0001	1				
25°C	<5%	<.0001	0.0078	0.4925	0.8229	0.9225	<.0001	0.1178	0.0775	0.0029	1					
	33%	<.0001	<.0001	0.6617	0.0048	0.0604	<.0001	0.8453	0.5977	1						
	53%	<.0001	<.0001	0.8352	0.0671	0.2015	<.0001	0.8794	1							
	75%	<.0001	0.0003	0.7753	0.0983	0.2182	<.0001	1								
	>95%	0.5599	<.0001	<.0001	<.0001	<.0001	1									
35°C	<5%	<.0001	0.0507	0.5637	0.7872	1										
	33%	<.0001	0.0404	0.4309	1											
	53%	<.0001	0.0694	1												
	75%	<.0001	1													
	>95%	1														

Table 3. The Scheffé method of pairwise differences among hemolymph osmolalities ($mOsmkg^{-1}$ d^{-1}) of pooled samples for specimens of *Littoraria angulifera*, in response to five relative humidity (RH) treatments of <5%, 33%, 53%, 75%, and >95%. Values shown in table below are the probability that the two groups that are being compared are the same.

	<5%	33%	53%	75%	>95%
<5%	1				
33%	0.7748	1			
53%	0.9005	0.8411	1		
75%	0.4173	0.2568	0.3496	1	
>95%	< 0.0001	< 0.0001	< 0.0001	< 0.0001	1

VII. Figures

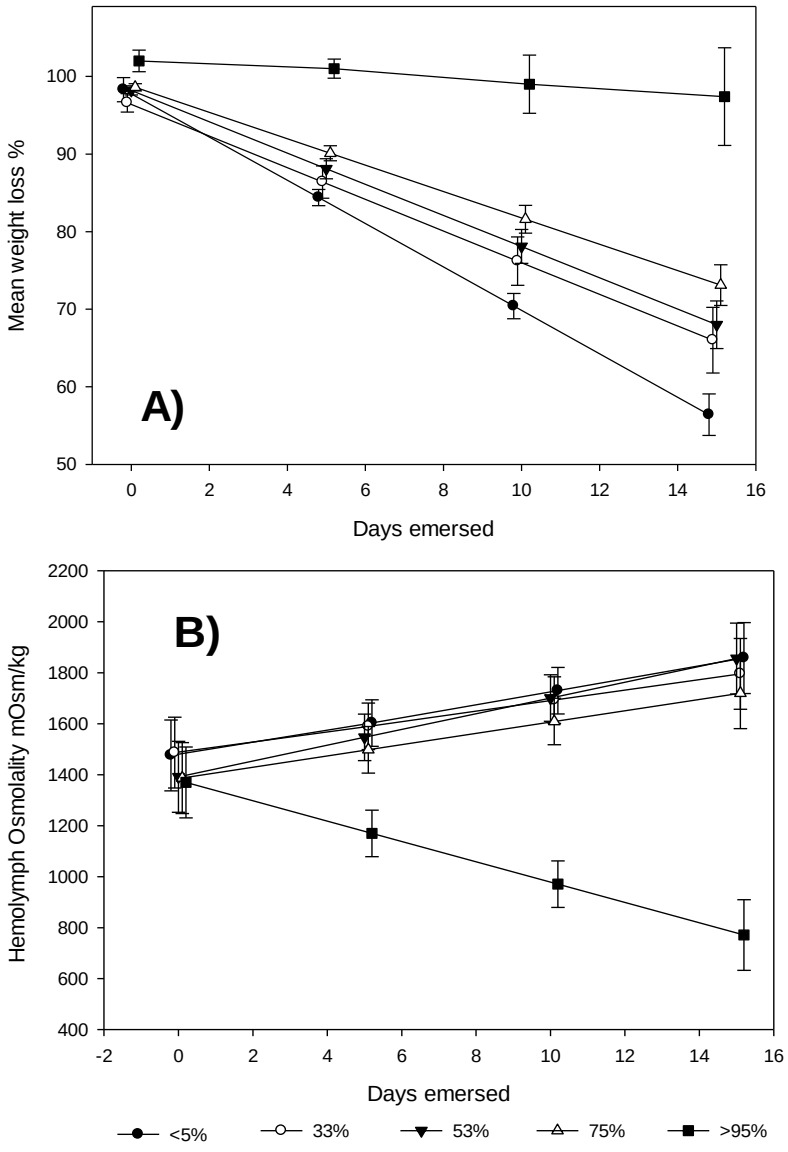

Figure 1. Percent total weight loss (A), and hemolymph osmolality (B) versus days of emersion at 15°C for specimens of *Littoraria angulifera* under <5%, 33%, 53%, 75%, or >95% relative humidity (RH). Error bars represent standard errors of percent weight loss estimates and hemolymph osmolality estimates.

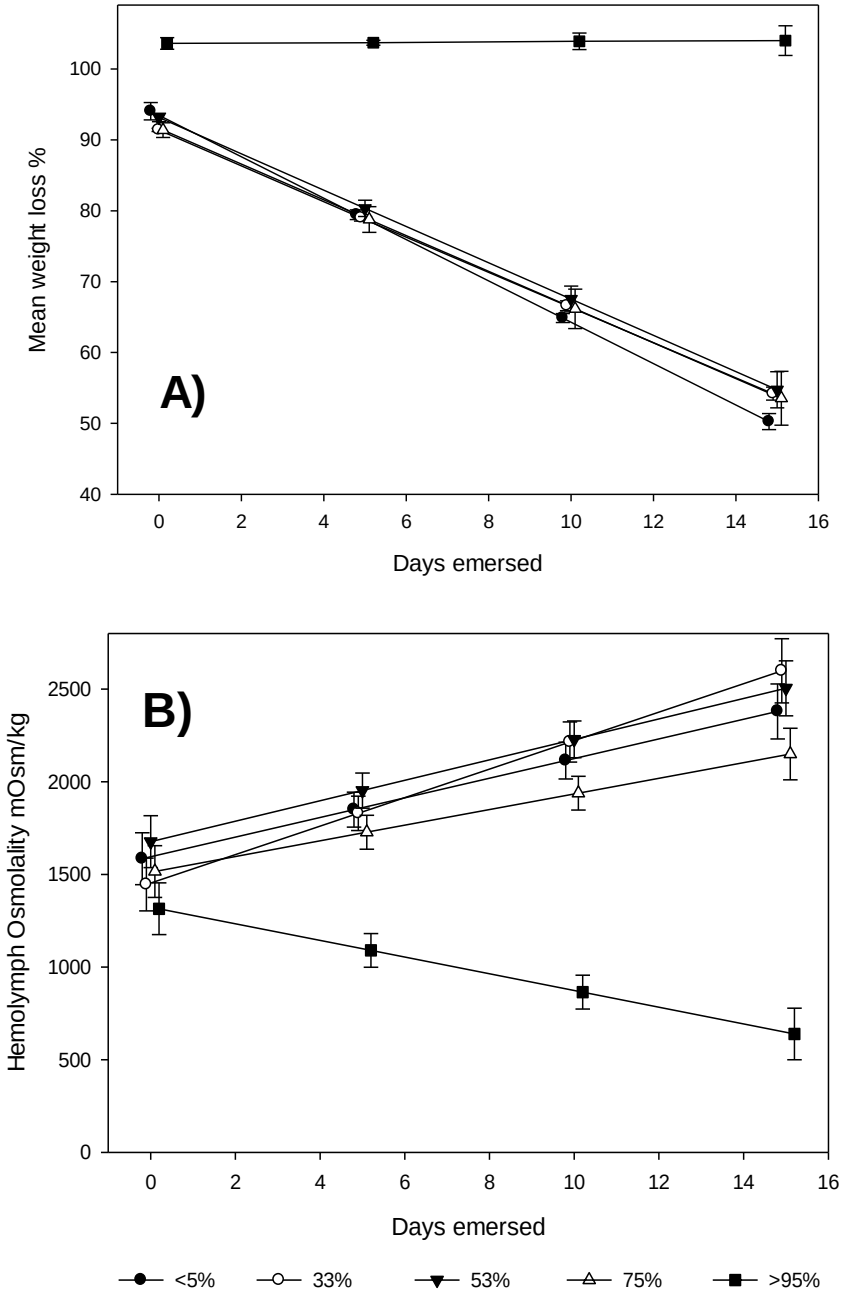

Figure 2. Percent mean weight loss (A), and hemolymph osmolality (B), versus days of emersion at 25°C for specimens of *Littoraria angulifera* under <5%, 33%, 53%, 75%, and >95% relative humidity (RH). Error bars represent standard errors of percent weight loss estimates and hemolymph osmolality estimates.

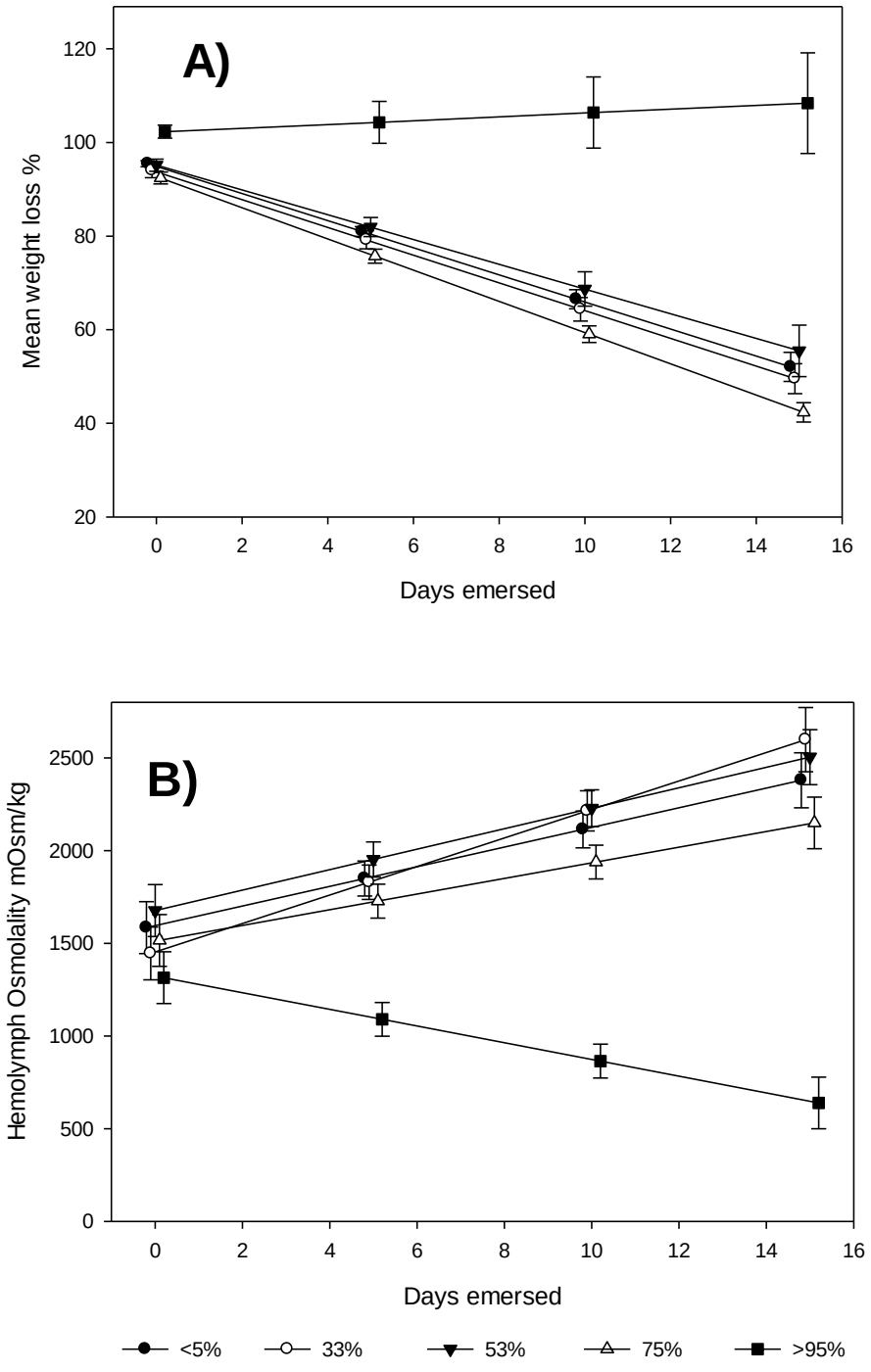

Figure 3. Percent of mean weight loss (A), and hemolymph osmolality (B) versus days of emersion at 35°C for specimens of *Littoraria angulifera* under <5%, 33%, 53%, 75%, or >95% relative humidity (RH). Errors bars represent standard errors of percent weight loss estimates and hemolymph osmolality estimates.

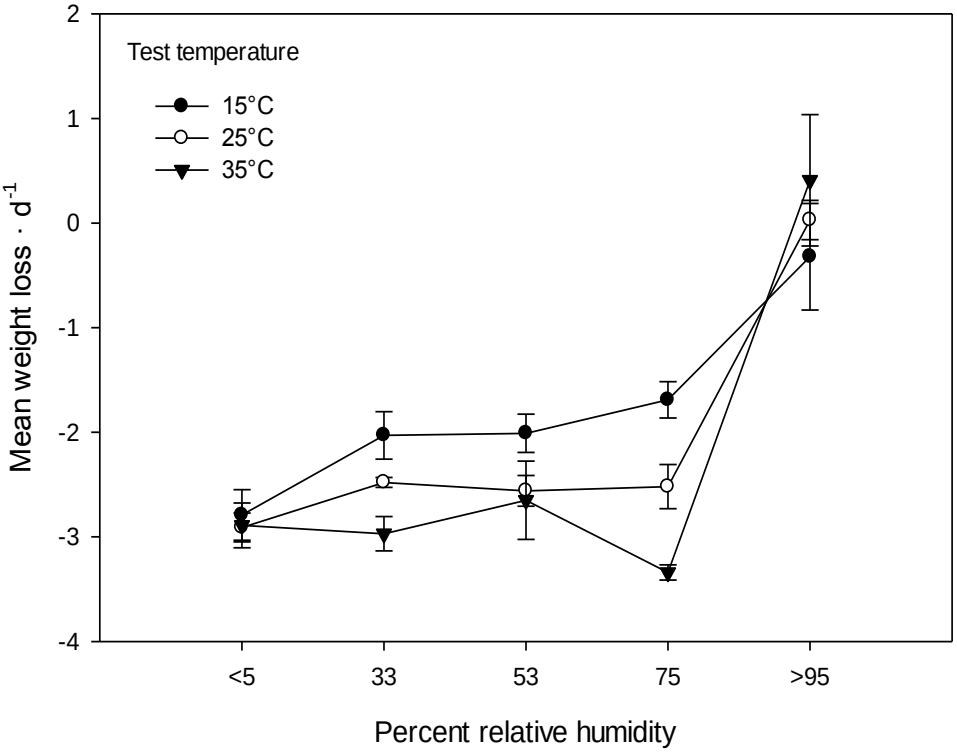

Figure 4. Rate of mean weight loss in percent total weight lost d^{-1} for standard 22 mm shell length of *Littoraria angulifera* under <5%, 33%, 53%, 75%, or >95% relative humidity at 15°, 25°, or 35°C.

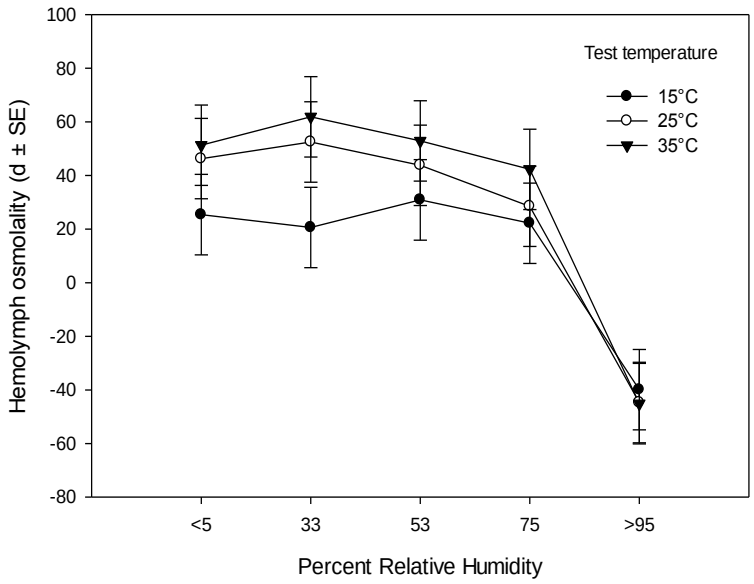

Figure 5. Mean osmolality in days for specimens of *L. angulifera* emersed under <5%, 33%, 53%, 75%, or >95% relative humidity at 15°, 25°, or 35°C. Error bars represent standard errors of mean osmolality.