

# Growing Safe: Acute Size Escape from Desiccation in Juvenile *Crepidatella peruviana* (Mollusca: Gastropoda)

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**Abstract.** Desiccation is an important limiting factor in the intertidal zone. Generally decreasing seaward, desiccation stress can also be alleviated in wet microhabitats. Juvenile snails are generally more susceptible to desiccation than adults, and, for some species, juveniles must therefore hide in microhabitats to survive emersion. The transition from hiding in safe microhabitats to being able to survive fully exposed for the duration of low tide is not well documented. In this study, we investigated the influence of size on desiccation tolerance in juveniles of the calyptraeid gastropod *Crepidatella peruviana* to determine the size at which they can first survive exposure to air. Juveniles 2–13 mm long were exposed to 75% or 100% relative humidity for 0.5–6.5 hours. Juveniles smaller than 5 mm in shell length did not survive emersion at 75% relative humidity for even 0.5 hours; surprisingly, most also perished after short exposures to air at 100% relative humidity, suggesting that something other than desiccation stress may also be at play. In marked contrast, 82% of juveniles larger than 6 mm in shell length survived exposure to 75% relative humidity for the full 6.5 hours. In a field survey, no juveniles smaller than 9 mm were found on exposed rock but rather were found only in wet microhabitats. We suggest that the clearly defined size escape from desiccation may reflect a change in gill functioning or a newfound ability to retain water more effectively within the mantle cavity at low tide.

Received 15 June 2017; Accepted 10 October 2017; Published online 26 December 2017.

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*Abbreviations:* LD<sub>50</sub>, median lethal dose; RH, relative humidity; VPD, vapor pressure deficit.

## Introduction

The intertidal zone is stratified by a number of biotic and abiotic stresses along the gradient from land to sea. Biotic stresses, such as predation and competition, generally increase seaward, while physical stresses, such as desiccation and thermal stress, generally increase landward. These stresses act in concert to determine species boundaries in this strongly zoned habitat (Connell, 1961a, b; Paine, 1969).

Desiccation is a particularly powerful environmental factor that plays an important role in regulating the distribution of organisms in the intertidal zone (Kensler, 1967; Foster, 1971; Wolcott, 1973; Jenewein and Gosselin, 2013). When exposed to air, intertidal organisms rapidly lose tissue water through evaporation (Branch, 1975; McQuaid, 1982), and this can ultimately result in death. Although the magnitude of desiccation stress generally increases landward, it can vary substantially between adjacent microhabitats, and organisms frequently take advantage of concentrated bits of moisture to survive, such as under algal canopies (Brawley and Johnson, 1991) or oyster shells (Grant and McDonald, 1979) or within crevices (Kensler, 1967).

Many molluscs can also limit water loss by closing themselves off from the environment. Bivalves can tightly close the two valves of their shells (McMahon, 1988), limpets can clamp themselves to the substrate (Wolcott, 1973), and other gastropods can seal their shell closed with the operculum (McMahon, 1990). However, this isolation response also restricts access to oxygen by cutting off exposure to air. Thus, when clamped, molluscs first deplete the oxygen stored in their mantle cavity water and then must either switch to anaerobic respiration (Segura *et al.*, 2015, 2016) or periodically open their shells to exchange oxygen and carbon dioxide *via* aerobic res-

piration, despite the water loss that accompanies such behavior (Diederich *et al.*, 2015).

The impacts of stresses such as desiccation vary with life-history stage. For many intertidal invertebrates, smaller juveniles are more susceptible to desiccation because they have not yet developed the fully functional defenses of adult animals, such as thick protective shells and carapaces (Gosselin and Qian, 1997; Hunt and Scheibling, 1997), and thus lose tissue water more rapidly than adults (Branch, 1975; McQuaid, 1982). For example, newly hatched juveniles of the marine snail *Nucella emarginata* (Deshayes, 1839) must hide in protective microhabitats to avoid predation and desiccation; otherwise, mortality levels reach 99%–100% within 4–5 h of emersion, compared to only 1.7%–36% in nearby protected regions (Gosselin and Chia, 1995). Young juveniles of that species do not develop the ability to withstand direct exposure to desiccation for the duration of low tide until they reach shell lengths of 3–6.5 mm (Gosselin, 1997).

*Crepidatella peruviana* (Lamarck, 1822) (formerly named *Crepidatella fecunda* (Gallardo, 1979)) (Veliz *et al.*, 2012) is a brooding and protandric South American gastropod. A very abundant member of the subtidal and intertidal zones in some areas, up to 1.5 m above mean lower low water (Pechenik *et al.*, 2017), *C. peruviana* ranges from Lima, Peru, in the north (Collin, 2003) to Quitalco Fjord in Chilean Patagonia in the south (Gallardo and Penchaszadeh, 2001). Females brood their egg capsules until larvae hatch, at shell lengths of about 330  $\mu\text{m}$ . The larvae then grow and develop in the water column for about 2 wk until they become competent to metamorphose, at shell lengths of approximately 650  $\mu\text{m}$  (Chaparro *et al.*, 2005). Juveniles recruit both subtidally and intertidally, mature into males at around 13 mm in shell length (Chaparro *et al.*, 2001), and transform to females as they grow larger. Early and advanced larvae have been identified in tide pools during low tide (Montory *et al.*, 2016), implying that recruitment might also occur in those areas. As in many calyptraeid gastropods, the early stages of development (juveniles and young males) are mobile, while adult females are stationary (Conklin, 1897; Chaparro *et al.*, 1998, 2001). Thus, juveniles and young males have the ability to use locomotion as a means to avoid physical stress, an option largely unavailable to females and older males of this and related species.

Diederich *et al.* (2015) studied the desiccation tolerance of the calyptraeid gastropod *Crepidula fornicata* (Linnaeus, 1758) in New England and found that no adults died after even 10 h of desiccation stress at a relative humidity (RH) of 75%. In contrast, most of the juveniles of that species that were tested (1.3–4.6 mm in shell length) died after only 2–6 h of exposure to RHs of 85% and lower. Thus, *C. fornicata* seems to exhibit a transition from being motile and susceptible to desiccation to being immobile and relatively immune. However, the sizes at which individuals first become relatively tolerant to desiccation stress have not been determined for any members of this family. To what extent does size play a role in desiccation re-

sistance for juvenile calyptraeids, and is the transition gradual with increased size, or is it abrupt?

In this study, we sought to determine the relationship between juvenile size and desiccation tolerance in the Chilean calyptraeid gastropod *C. peruviana*. We exposed juveniles from 2–13 mm in shell length to air at 75% and 100% RH for up to 6.5 h to determine how juvenile tolerance to desiccation varied with juvenile size. In addition, we surveyed the size distribution of juveniles in field intertidal microhabitats that differed in humidity levels to determine whether desiccation tolerance is reflected in the microhabitats occupied by these juvenile snails.

## Materials and Methods

### *Animal collection and maintenance*

Juvenile *Crepidatella peruviana* were collected from Puerto Montt, Chile (41°29'28.1" S, 72°53'47.7" W) in January, May, and June 2013; January and December 2014; and February 2015. Juveniles were then brought to the Laboratorio Costero de Recursos Acuáticos Calfuco (Calfuco Coastal Laboratory of Aquatic Resources) of the Universidad Austral de Chile. Juveniles were maintained in a large bucket filled with locally collected seawater at 12–14 °C that was aerated continuously with a bubbler and changed daily. Some of the juveniles positioned themselves near the waterline. The unicellular alga *Isochrysis galbana* was occasionally added to maintain high food concentrations. Juveniles were generally kept in the lab for 2–3 d before experiments, except for experiment 1 (see Experimental design, below), when they were kept in the lab for 2–10 d before trials.

### *Desiccation experiments*

Fifteen jars (600 ml) were modified so that a small plastic dish (5.5-cm diameter) could be hung inside the top third of the jar, forming a swing above the fluid at the bottom of the jar. The swing was small enough in diameter to ensure sufficient room for air circulation. Ten of the jars were filled with about 200 ml of a 100% saturated solution of noniodized table salt to approximate an environment of 75% RH (Winston and Bates, 1960). This method maintains an RH of 75.5% that deviates by no more than 1% RH over the temperature range of 2–50 °C (Winston and Bates, 1960). The other 5 jars were reserved as controls and filled with 200 ml of distilled water to create an environment of 100% RH. The water or salt solutions filled approximately the bottom 2 cm of the jar such that the swing hovered above but was never in contact with the water. Juveniles were subjected to different RHs in air using these jars, as previously described by Diederich *et al.* (2015). Spot checks with a handheld psychrometer in this previous study confirmed that this technique maintained the desired humidity levels (Diederich *et al.*, 2015). All weight measurements were

made with a Denver Instruments (Bohemia, NY) AA-20 ODS balance to the nearest 0.1 mg.

At the beginning of the experiment, each juvenile was removed from the seawater in which it was kept, and its longest shell length was measured with digital calipers to the nearest 0.1 mm. The juvenile was then placed on a piece of preweighed foil, blotted dry, and weighed to the nearest 0.1 mg. Next, each juvenile (still on the piece of preweighed foil) was transferred to the swing suspended above the solution at the bottom of a jar, and the jar was then tightly closed. Exposure to ambient air was minimized as much as possible. Only one juvenile was placed in each jar, and each juvenile was used in only one experiment; no juveniles were ever reused in subsequent experiments. Once closed, the jars were gently swirled once to circulate air and ensure that the RH reached the desired level. When the predetermined exposure time concluded, the juvenile's final wet weight was recorded.

During recovery, each juvenile was placed in a separate compartment of a plastic tray that was then submerged in seawater. The plastic trays had mesh-covered openings to allow water to circulate through each chamber. Seawater was bubbled, and the phytoplankton *I. galbana* was added to ensure sufficient oxygen and food availability for the recovering juveniles. Mortality was assessed after 24 h. Juveniles were determined to be dead when they exhibited no pedal or head movements, were not attached to the substrate, and did not contract into the shell when stimulated with a probe (Diederich and Pechenik, 2013; Diederich *et al.*, 2015). A total of 677 juveniles of various sizes were used in our experiments.

### Experimental design

Eight experiments were conducted, each with a distinct size range of juveniles that were desiccated for different amounts of time in different trials (Table 1). Seven of the experiments (experiments 1–6, 8) were conducted at 22 °C; in one additional experiment (experiment 7), juveniles were exposed to air at 3 different temperatures (19, 22, and 25 °C). Each trial consisted of 6–10 juveniles exposed to 75% RH and 5 juveniles exposed to 100% RH. Since the 75% RH treatment had higher mortality, we exposed more individuals to 75% RH to increase our chances of having at least one survivor in each treatment. Juveniles were exposed for predetermined periods of 0.5–6.5 h. Summer daily maximum air temperature in a shaded area of the collection site at Puerto Montt was  $18 \pm 1.8$  °C (mean  $\pm$  SD) in 2002–2003 (K. Pashcke, Instituto de Acuicultura, Universidad Austral de Chile, unpubl. data), so 22 °C was determined to be a reasonable temperature for desiccation exposure, considering that temperatures would be higher in the sun. Nevertheless, to test for the effect of temperature on the desiccation tolerance of small juveniles, juveniles were desiccated at 19, 22, or 25 °C in experiment 7. These exposures correspond to vapor pressure deficits (VPD) of 0.548, 0.659, and 0.790 kPa for temperatures of 19, 22, and 25 °C

at 75% RH, respectively. VPD is a measure of the amount of water vapor that can be added to a given volume of air, has a value of 0 kPa for 100% RH, and was calculated from air temperature and RH after Jenewein and Gosselin (2013).

Because many small juveniles died after exposure to 100% RH (the initial control treatment), additional control trials were added (experiment 8) to determine the source of this mortality. In this experiment, a small drop of seawater was pipetted onto the top of each juvenile's shell, so that juveniles were sitting in a small drop of seawater surrounded by air at 100% RH. This enabled us to determine whether lab procedures were causing the experimental mortality we observed in 100% RH or whether very small juveniles simply cannot live without being in seawater.

### Field observations

A pilot field experiment was conducted in December 2015 to collect some information on the distribution of juvenile *C. peruviana* in microhabitats. In this pilot study, 83 juvenile *C. peruviana* smaller than 10 mm in shell length were haphazardly sampled at low tide (0.6 m above the lowest astronomical tide) from the intertidal area of Pelluco Beach, Puerto Montt. We searched for juveniles in tide pools, from the margins of adult female shells (sometimes under or surrounded by seaweeds but often on exposed rocks) and directly on exposed rocks. In the lab, juveniles were photographed using a stereomicroscope and a reference ruler. Juvenile shell lengths were obtained using ImageJ (Schneider *et al.*, 2012).

### Statistical analyses

All statistical analyses were conducted using R (R Core Team, 2015). We first used logistic regressions to analyze the factors determining the mortality of juvenile *C. peruviana* exposed to air. Since weight (*i.e.*, water) loss was an important driver of mortality, we then used multiple regression to determine the major determinants of how much water weight juveniles lost during the experimental exposures.

The effects of desiccation intensity (measured as percent water lost), juvenile size (measured as shell length), and RH (75% or 100%) on mortality (alive or dead) were analyzed using logistic regression. Percent water loss was strongly correlated with shell length, so two logistic regressions were run: one with percent water lost and humidity as predictors and another with shell length and humidity as predictors. These tests were run only on data from experiments conducted at 22 °C since experiments conducted at 25 °C or 19 °C resulted in either complete survival or complete mortality. Median lethal dose (LD<sub>50</sub>) values were calculated to determine the shell length and percent water loss associated with 50% mortality at 75% and 100% RH. These values were calculated from the logistic regressions using R package MASS (Venables and Ripley, 2002).

**Table 1**

*Summary of all experiments conducted with juveniles of Crepipatella peruviana*

Experiment	Time desiccated (h)	Temperature (°C)	Sample size 75% RH (100% RH)	Mean length ± SD (mm)	Percent survival 75% RH (100% RH)
1	1.5	22	10 (5)	11.52 ± 0.76	100 (100)
	2	22	10 (5)	11.31 ± 1.12	100 (100)
	2.5	22	10 (5)	11.56 ± 0.97	80 (100)
	3	22	10 (5)	11.40 ± 0.98	100 (100)
	3.5	22	10 (5)	11.28 ± 0.68	100 (100)
	4	22	10 (5)	10.58 ± 0.63	100 (100)
	5	22	10 (5)	9.59 ± 0.60	80 (100)
2	6	22	10 (5)	10.43 ± 1.02	100 (100)
	1.5	22	7 (5)	6.39 ± 0.73	100 (100)
	2.5	22	7 (5)	6.65 ± 1.03	71 (100)
	3.5	22	6 (5)	6.41 ± 0.74	83 (100)
	4.5	22	7 (5)	6.81 ± 0.65	86 (100)
	5.5	22	6 (5)	7.02 ± 0.53	83 (80)
3	6.5	22	7 (5)	7.56 ± 0.57	57 (100)
	1.5	22	7 (5)	6.39 ± 0.73	100 (100)
	2.5	22	7 (5)	6.65 ± 1.03	71 (100)
	3.5	22	6 (5)	6.41 ± 0.74	83 (100)
	4.5	22	7 (5)	6.81 ± 0.65	86 (100)
4	5.5	22	6 (5)	7.02 ± 0.53	83 (100)
	6.5	22	7 (5)	7.56 ± 0.57	57 (100)
	1.5	22	9 (5)	3.84 ± 0.92	0 (0)
	2.5	22	10 (5)	3.58 ± 0.67	0 (40)
	3.5	22	10 (5)	3.56 ± 0.97	0 (0)
5	4.5	22	9 (5)	2.45 ± 0.32	0 (0)
	5.5	22	9 (5)	2.16 ± 0.35	0 (0)
	6.5	22	10 (5)	2.75 ± 0.87	0 (0)
	0.5	22	10 (5)	4.23 ± 0.80	0 (100)
	1	22	10 (5)	3.71 ± 0.58	0 (100)
6	0.5	22	10 (5)	2.92 ± 0.42	0 (20)
	1.5	22	10 (5)	2.90 ± 0.44	0 (0)
	2.5	22	10 (5)	2.96 ± 0.45	0 (0)
	3.5	22	10 (5)	3.08 ± 0.33	0 (0)
	4.5	22	10 (5)	3.17 ± 0.48	0 (0)
	5.5	22	10 (5)	3.44 ± 0.29	0 (0)
	6.5	22	10 (5)	3.28 ± 0.29	0 (0)
7	0.5	25	10 (5)	4.19 ± 1.15	0 (0)
	0.5	22	10 (5)	5.04 ± 1.30	0 (0)
	0.5	19	10 (5)	3.57 ± 1.21	0 (100)
	1	22	10 (5)	4.50 ± 0.88	0 (0)
	1	19	10 (5)	5.18 ± 1.23	0 (100)
	2	25	10 (5)	4.49 ± 0.68	0 (0)
	2	22	10 (5)	4.49 ± 0.87	0 (0)
	2	19	10 (5)	4.31 ± 1.24	0 (80)
	3	19	10 (5)	5.65 ± 0.65	0 (60)
	4	25	10 (5)	4.25 ± 0.68	0 (0)
	4	19	10 (5)	4.01 ± 1.35	0 (40)
	5	19	10 (5)	4.55 ± 1.30	0 (0)
	6	19	10 (5)	5.11 ± 1.00	0 (80)
8	0.5	22	(5)	3.97 ± 0.29	(100)
	1.5	22	(5)	3.46 ± 0.24	(100)
	2.5	22	(5)	3.69 ± 0.49	(100)
	3.5	22	(5)	3.85 ± 0.27	(100)
	4.5	22	(5)	3.77 ± 0.44	(100)
	5.5	22	(5)	3.48 ± 0.36	(100)
	6.5	22	(5)	3.79 ± 0.40	(100)

In the columns for sample size and percent survival, data for the control juveniles exposed to 100% relative humidity (RH) are enclosed in parentheses. Experiment 8 included only control juveniles that were sitting in a small drop of water for the duration of the experiment, to test for possible sources of mortality in the control treatment. Note that mean shell lengths of individuals tested in experiments 4–7 are generally lower than that of those individuals tested in the first three experiments.

The effects of experimental duration, humidity, and juvenile shell length and all their interactions on percent water loss were analyzed with multiple linear regression, after percent water loss was arcsine square root transformed to make the data resemble a normal distribution. This test was run only on data from experiments conducted at 22 °C; separate tests were conducted on data from juveniles that survived and those that did not survive desiccation. The Akaike information criterion (AIC) was used to select the best model.

A similar multiple regression was conducted on data from experiment 7, in which juveniles were desiccated at one of three different temperatures. For this regression, the factors of experimental duration, humidity, shell length, and temperature and all of their three-way interactions were included initially, and the best reduced model was then selected with AIC. This test was also run separately on data from juveniles that survived and those that did not survive desiccation. However, since surviving juveniles from that experiment had all been subjected to 19 °C and 100% RH, the regression predicting percent weight loss by surviving juveniles from this experiment could only be used to test the effects of shell length, time, and their interaction.

For the field survey data, shell lengths of juveniles collected near adult females or in tide pools were compared with a *t* test after using the Shapiro-Wilk test to verify that the data were normally distributed.

## Results

### Mortality

The extent of mortality following air exposure was related to the duration of air exposure, RH, percentage weight loss, and air temperature. At 22 °C, almost all juveniles died within 24 h after being exposed to air if they either (a) lost >25% of their initial wet weight or (b) had shell lengths of <5 mm (Fig. 1; Table 1, experiments 4–8). At that same temperature, about 27% of the small juveniles (<5 mm) survived when exposed to 100% RH for less than 2.5 h, but no juveniles <5 mm in shell length survived exposure to 75% RH, even for as short as 0.5 h (Fig. 1; Table 1, experiments 4–8). In the temperature stress experiment (experiment 7) conducted on small juveniles 2–7 mm long, juveniles only survived after being exposed to air at the lowest temperature (19 °C) and at 100% RH (Fig. 2; Table 1). All juveniles survived exposure to 19 °C and 100% RH for 1 h, and most juveniles as small as 5 mm survived even 6 h of exposure to these conditions (Fig. 2; Table 1). However, all juveniles in experiment 7 perished following exposure to 19 °C and 75% RH, as did all small juveniles exposed at 22 and 25 °C to air at both 75% and 100% RH, even for as little as 0.5 h (Fig. 2C; Table 1). At 22 °C, the LD<sub>50</sub> for juvenile length was 6.30 mm at 75% RH and 4.76 mm at 100% RH (Fig. 3A). Likewise, the LD<sub>50</sub> for percent weight lost was 22.1% at 75% RH and 21.8% at 100% RH (Fig. 3B).

Juveniles were significantly more likely to die if they were smaller (logistic regression,  $P < 0.0001$ ; Table 2) and had lost more weight during desiccation (logistic regression,  $P < 0.0001$ ; Table 2; Fig. 3). Humidity mediated the effect of weight loss on mortality, such that small juveniles with a high percent weight loss had a small chance of surviving at 100% RH, while none survived at 75% RH (Fig. 1B).

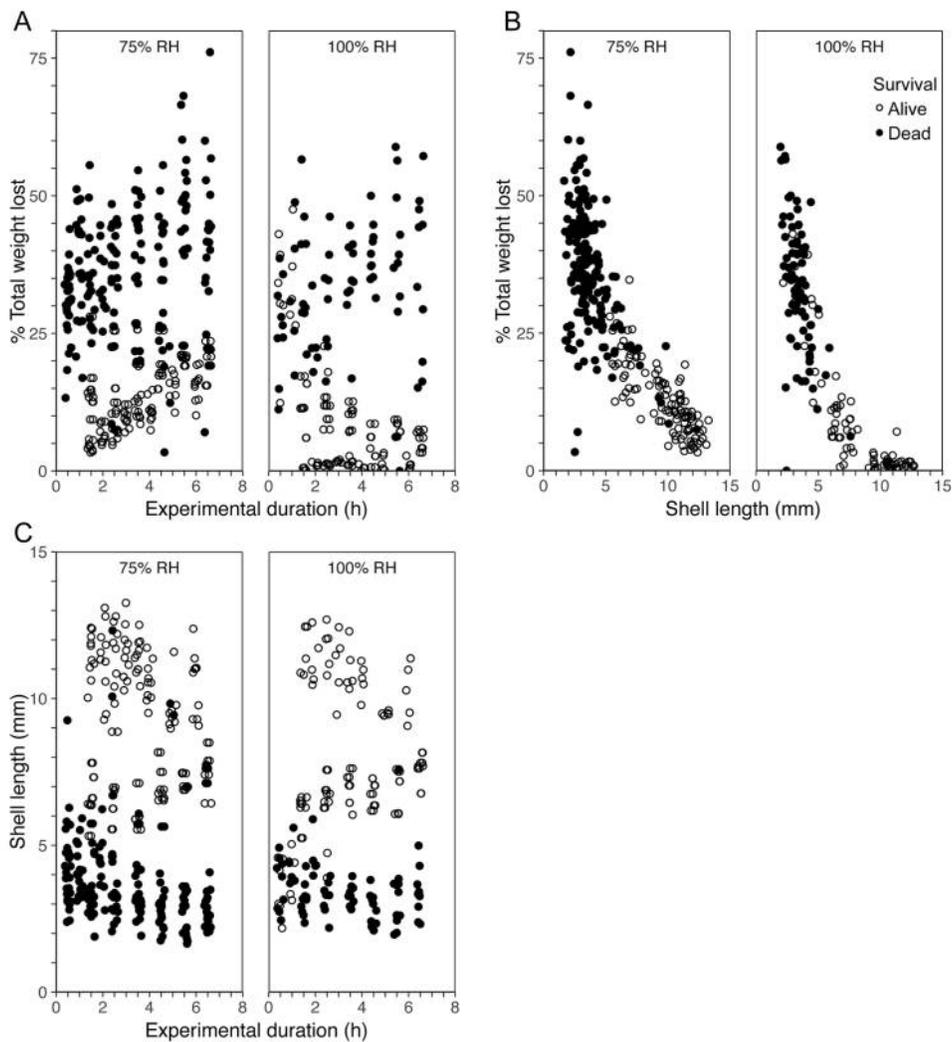
Additional control trials were conducted to narrow down potential sources of mortality for small juveniles subjected to the 100% RH treatment. When juveniles in air at 100% RH were treated exactly the same way as in other experiments—except that they sat in a small drop of seawater for the duration of the exposure to air—they all survived, even after spending 6.5 h in the experimental containers (Table 1, experiment 8).

### Weight loss

Humidity, shell length, and experimental duration and all their 2-way interactions had significant effects on the amount of weight lost by juvenile *C. peruviana* that survived the experiment (multiple regression,  $P < 0.05$ ,  $R^2 = 0.8382$ ; Table 3). Juveniles lost proportionally more weight if they were smaller, exposed to their experimental conditions for a longer time, and exposed to air at 75% RH rather than 100% RH. Juvenile size had a stronger influence on weight loss at 75% RH than at 100% RH; thus, smaller juveniles lost proportionally even more weight relative to larger juveniles at the lower humidity. Looking at juveniles >5 mm long, it is apparent that juvenile size had a greater impact on percent weight loss for juveniles exposed to air at 75% than for those exposed at 100% RH (Fig. 1B). Surviving juveniles exposed to 100% RH for less time actually appeared to have lost more weight than those exposed for more time (Fig. 1A). This was likely due to the unbalanced experimental design in which smaller juveniles were more heavily represented in the shorter experiments, since they were dying much sooner (Fig. 1C), and smaller juveniles lost proportionally more of their initial weight (Fig. 1B).

For *C. peruviana* juveniles that did not survive the 24 h following their experimental exposure, the relationships between weight loss and humidity, shell length, and experimental duration were much weaker (multiple regression,  $P < 0.05$ ,  $R^2 = 0.3121$ ; Table 3). Again, juveniles lost significantly more weight if they were smaller, exposed for a longer time, or exposed to lower humidity; and shell length had a larger influence on the amount of weight lost at the lower humidity than at the higher humidity (Fig. 1).

In the temperature experiment (experiment 7), at 19 °C and 100% RH (the only conditions the small juveniles from this experiment survived) surviving juveniles lost significantly more weight if they were smaller or exposed to air for more time, and the effect of size on weight lost was greater at longer exposure times (multiple regression,  $P < 0.05$ ,  $R^2 = 0.7239$ ; Table 4; Fig. 2). However, across all conditions in experi-



**Figure 1.** Survival and weight lost by *Crepipatella peruviana* juveniles of differing shell lengths after exposure to air at 22 °C for 0.5–6.5 h at 75% or 100% relative humidity (RH). Plots depict survival as a function of (A) experimental duration and percent weight lost, (B) shell length and percent weight lost, and (C) experimental duration and shell length (*i.e.*, experimental design, since both variables were fixed). Each point represents data from 1 juvenile, with open circles representing alive individuals and closed circles representing dead individuals. Mortality was assessed after 24 h of recovery.

ment 7, juveniles that did not survive the treatments lost significantly more weight at the lower temperatures and lower humidity if they had smaller shells or if they had been exposed for a longer time (multiple regression,  $P < 0.05$ ,  $R^2 = 0.4976$ ; Table 4; Fig. 2).

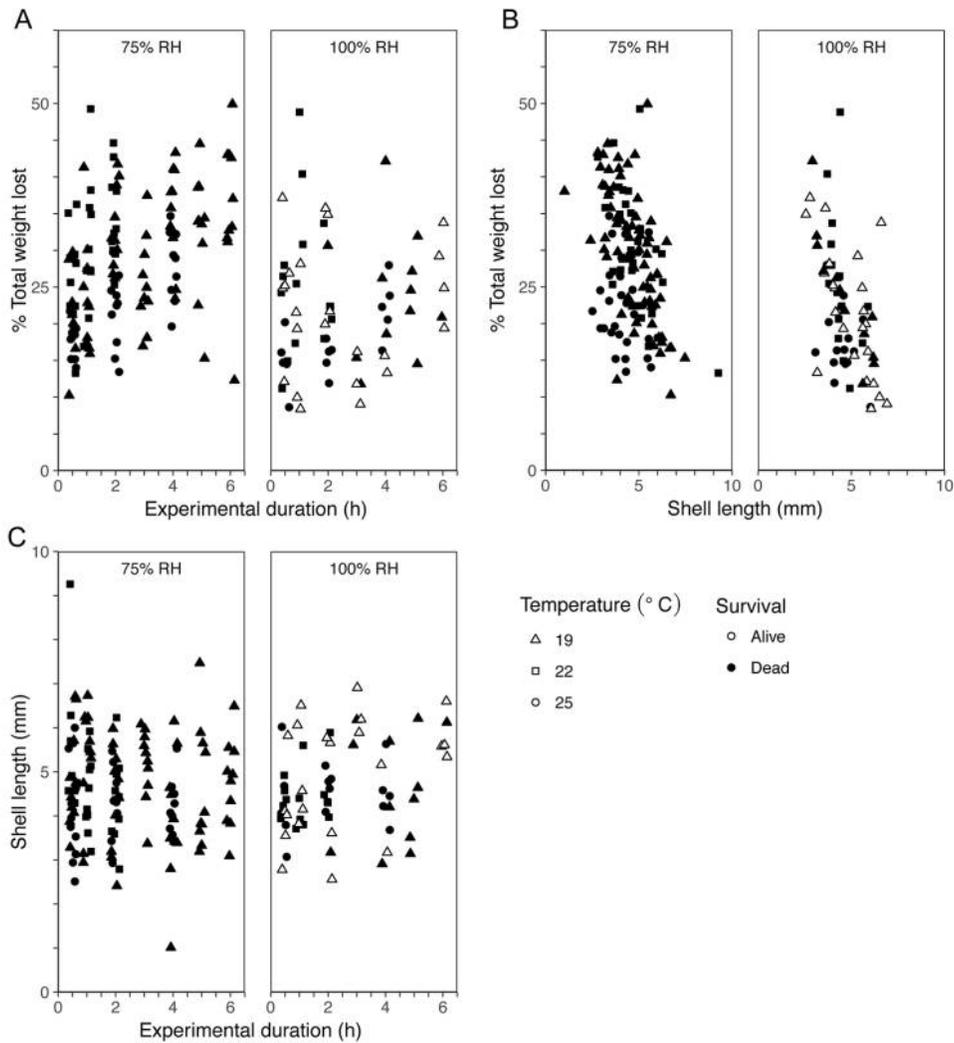
#### Field observations

No juveniles <10 mm in shell length were found on bare rock in the field survey, but 47 individuals were collected on or near adult females, and 36 were collected in tide pools. Juveniles collected in tide pools were significantly larger than those collected near adult females ( $t$  test,  $t_{81} = 9.697$ ,  $P < 0.0001$ ; Fig. 4). However, these size differences should be in-

terpreted cautiously because the smaller juveniles may have simply been more difficult to find within the tide pools.

#### Discussion

This study demonstrates that small juveniles of *Crepipatella peruviana* are extremely vulnerable to air exposure and desiccation until they reach shell lengths of about 6 mm. The size-related change in susceptibility was remarkably well defined: no juveniles under 5 mm in shell length survived exposure to air at 75% RH, even when exposures were as short as 30 min, whereas 82% of juveniles that exceeded 6 mm in shell length survived exposure to 75% RH for the full 6.5 h. The impact of air exposure was also affected dramatically by expo-

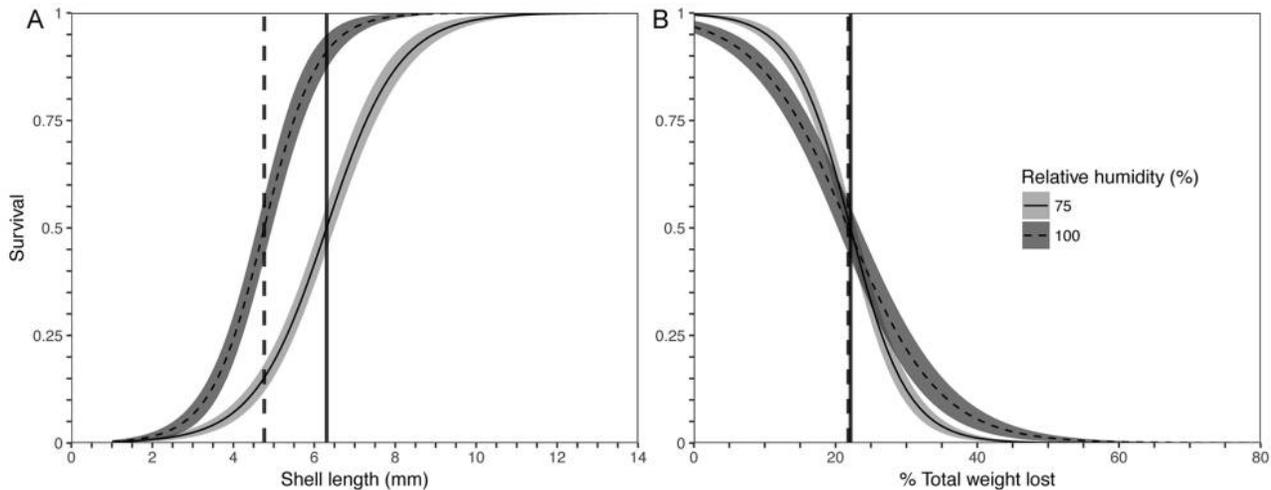


**Figure 2.** Effect of temperature (19, 22, or 25 °C) on survival and weight lost by *Crepipatella peruviana* juveniles of differing shell lengths after exposure to air for 0.5–6.5 h at 75% or 100% relative humidity (RH). Plots depict survival as a function of (A) experimental duration and percent weight lost, (B) shell length and percent weight lost, and (C) experimental duration and shell length (*i.e.*, experimental design, since both variables were fixed). Each point represents 1 juvenile, and mortality was assessed after 24 h of recovery.

sure temperature and the RH. For small individuals (<4 mm; see Table 1) exposed to air at 100% RH, we saw better survival at 19 °C than at 22 °C or 25 °C.

These results support the idea of a substantial ecological (and perhaps physiological) transition in the juvenile phase of *C. peruviana*, similar to that proposed by Gosselin (1997) for juveniles of the intertidal gastropod *Nucella emarginata*. Those juvenile snails also underwent pronounced changes in vulnerability to desiccation, as well as in shell coloration, distribution in microhabitats, and susceptibility to predators over the 3–8-mm size range. However, the size-related ecological transition of *N. emarginata* juveniles was not as distinct as we discovered here for *C. peruviana*. While the shift in desiccation tolerance of *N. emarginata* occurred over a range of sizes from 3.1 to 6.5 mm (Gosselin, 1997), the transition that

we found for *C. peruviana* was much more abrupt, occurring as juveniles grew to shell lengths between 5 and 6 mm. It was such a sharp transition that shell length was clearly the best predictor of susceptibility in our logistic regressions—far better than the amount of time that juveniles were exposed to low humidity or the amount of weight they lost during exposure. Note that juvenile *N. emarginata* were much more tolerant of desiccation than *C. peruviana*; even hatchlings of *N. emarginata* with shells as small as 1–1.5 mm in length could survive exposures of more than 2 h at 77%–86% RH. One important distinction between these two gastropod species is that the shell of the limpet-shaped *C. peruviana* covers only the upper surface of the animal, whereas *N. emarginata* is completely enclosed within its shell except for a small opening that can be sealed with the operculum.



**Figure 3.** Predicted logistic survival curves with standard error (shading) from logistic regression models of juvenile *Crepipatella peruviana* survival predicted by (A) humidity and shell length and (B) humidity and weight lost. Vertical lines indicate the median lethal dose ( $LD_{50}$ ) values at each humidity level.

Our field observations support the idea that small (<6 mm) juveniles of *C. peruviana* are especially vulnerable to aerial exposure. Small juveniles at our field study site were only found in microhabitats in which they would be exposed to seawater, such as in tide pools and adjacent to the shell margins of adult females, from which escaping water frequently bubbles (O. Chaparro, pers. obs.). Adults, on the other hand, live in stacks of up to five individuals and are frequently found in exposed microhabitats (O. Chaparro, pers. obs.). This is similar to what Gosselin and Chia (1995) found for *N. emarginata*; almost all juveniles of that species were found in structurally complex microhabitats, whereas adults were usually fully exposed to air during low tide.

The standard explanation for increased desiccation tolerance with increased size is that the surface area : volume ratio decreases with increasing size and thus slows the rate of water loss, which depends on surface area (Davies, 1969; Vermeij, 1972). However, the remarkably pronounced size-related shift in desiccation tolerance that we found for *C. peruviana* suggests a different mechanism for this species, something that could act without the gradual transition characteristic of the

surface area argument. The juveniles of this species may undergo an abrupt behavioral, morphological, or physiological change when they reach shell lengths of 5–6 mm that gives them the ability to withstand exposure to air for the duration of low tide. Jenewein and Gosselin (2013) found a similar ontogenetic shift in desiccation tolerance for juvenile mussels (*Mytilus trossulus*): juveniles became largely resistant to desiccation stress once they reached a shell length of 3 mm.

Our additional control trials provide evidence for some possible mechanisms that may help explain this abrupt transition in desiccation tolerance for *C. peruviana*. Interestingly, most of the small (<5 mm) control juveniles in our study died following exposure to air at 100% RH for as little as 30 min. Further experiments demonstrated that it was not the handling stress of the experimental procedure that was killing them, since similarly sized juveniles exposed to the same conditions while sitting in a very small drop of seawater did not die, even after a 6.5-h exposure. Emergence for 6.5 h is longer than would occur naturally in the intertidal zone, even during the most extreme low tides. The upper vertical range limit of *C. peruviana* is about 1.5 m above mean lower low tide

**Table 2**

Results from logistic regression predicting mortality, based on experiments conducted at 22 °C

Model	Source	Estimate	SE	Z-value	P-value
Length	<b>Humidity</b>	<b>2.0935</b>	<b>0.4290</b>	<b>4.880</b>	<b>&lt;0.0001</b>
	<b>Length</b>	<b>3.2513</b>	<b>0.3646</b>	<b>8.919</b>	<b>&lt;0.0001</b>
	Humidity × length	1.1165	0.7295	1.530	0.1259
Weight lost	Humidity	0.1697	0.3213	0.528	0.5974
	<b>Weight lost</b>	<b>−3.6787</b>	<b>0.4127</b>	<b>−8.914</b>	<b>&lt;0.0001</b>
	<b>Humidity × weight lost</b>	<b>1.3687</b>	<b>0.5080</b>	<b>2.694</b>	<b>0.0071</b>

Bold entries indicate  $P < 0.05$ .

**Table 3**

Results from multiple regressions predicting the percent weight lost by juveniles during desiccation experiments

Mortality	Source	Estimate	SE	t-value	P-value
Alive <sup>a</sup>	<b>Length</b>	<b>-0.1343</b>	<b>0.0071</b>	<b>-18.890</b>	<b>&lt;0.0001</b>
	<b>Humidity</b>	<b>0.1208</b>	<b>0.0119</b>	<b>10.173</b>	<b>&lt;0.0001</b>
	<b>Time</b>	<b>-0.0640</b>	<b>0.0069</b>	<b>-9.321</b>	<b>&lt;0.0001</b>
	<b>Length × humidity</b>	<b>0.0485</b>	<b>0.0093</b>	<b>5.241</b>	<b>&lt;0.0001</b>
	<b>Humidity × time</b>	<b>0.0827</b>	<b>0.0088</b>	<b>9.358</b>	<b>&lt;0.0001</b>
	<b>Length : time</b>	<b>0.0291</b>	<b>0.0060</b>	<b>4.854</b>	<b>&lt;0.0001</b>
Dead <sup>b</sup>	<b>Length</b>	<b>-0.2150</b>	<b>0.0418</b>	<b>-5.141</b>	<b>&lt;0.0001</b>
	<b>Humidity</b>	<b>0.1252</b>	<b>0.0377</b>	<b>3.321</b>	<b>0.0010</b>
	<b>Time</b>	<b>0.0068</b>	<b>0.0035</b>	<b>1.977</b>	<b>0.0491</b>
	Humidity × length	0.0861	0.0442	1.949	0.0523

Tests were conducted separately on juveniles that were alive and those that were dead 24 h later. Bold entries indicate  $P < 0.05$ .<sup>a</sup>  $R^2 = 0.8382$ .<sup>b</sup>  $R^2 = 0.2976$ .

(Pechenik *et al.*, 2017), which corresponds to a maximum emersion time of 4.25 h per tidal cycle at this site during the summer months. It seems that very small juveniles of this species (shell lengths <5 mm) must be in physical contact with at least a small amount of seawater to survive. Small juveniles of *C. peruviana* may have trouble respiring in air and may need to be in nearly constant contact with at least a small amount of seawater for sufficient respiration to occur. As discussed below, this intolerance of small juveniles to emersion could reflect a shift in behavior, gill structure, or gill function.

Many intertidal gastropods, including the related species *Crepidula fornicata*, lift their shells to aerobically respire during emersion, despite the increased water loss caused by this behavior (McMahon, 1988; Diederich, 2015). When exposed

to air, young juveniles of *C. fornicata* (Diederich, 2015) and large juveniles of *C. peruviana* (S. Bashevkin, pers. obs.) do not remain clamped to the substrate but frequently lift their shell above the substrate or move around. This lifting behavior releases all fluid stored in their mantle cavity, creating a little puddle beneath the snail. A series of studies (Houlihan, 1979; Houlihan *et al.*, 1981; Houlihan and Innes, 1982) found that respiration rates for eight species of gilled gastropods were up to twice as high when mantle cavity fluid was present than when it was absent; in contrast, two species of lunged gastropods experienced no reduction in respiration rate in the absence of mantle cavity fluid. The importance of mantle cavity fluid for effective respiration in gilled species suggests that the mantle cavity fluid may play an important role

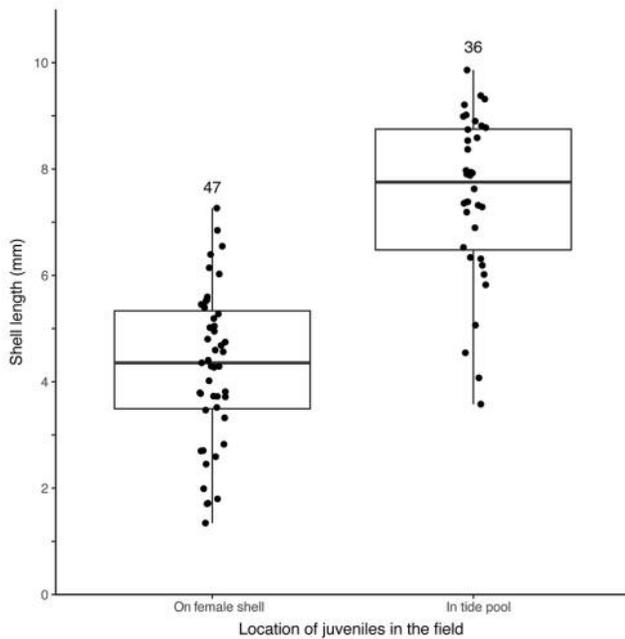
**Table 4**

Results from multiple regression analyses predicting percent weight lost by juveniles during experiment 7, when juveniles were exposed to air at 19, 22, or 25 °C

Mortality	Source	Estimate	SE	t-value	P-value
Alive <sup>a</sup>	<b>Length</b>	<b>-0.3766</b>	<b>0.0499</b>	<b>-7.544</b>	<b>&lt;0.0001</b>
	<b>Time</b>	<b>0.0322</b>	<b>0.0073</b>	<b>4.417</b>	<b>0.0003</b>
	<b>Length × time</b>	<b>0.0962</b>	<b>0.0196</b>	<b>4.898</b>	<b>&lt;0.0001</b>
Dead <sup>b</sup>	<b>Length</b>	<b>-0.7824</b>	<b>0.1693</b>	<b>-4.622</b>	<b>&lt;0.0001</b>
	<b>Humidity</b>	<b>-0.0771</b>	<b>0.0313</b>	<b>-2.462</b>	<b>0.0149</b>
	Time	-0.0365	0.0308	-1.186	0.2373
	Temperature	-0.0055	0.0054	-1.011	0.3135
	<b>Length × humidity</b>	<b>-0.1049</b>	<b>0.0428</b>	<b>-2.455</b>	<b>0.0152</b>
	<b>Length × time</b>	<b>0.0225</b>	<b>0.0096</b>	<b>2.338</b>	<b>0.0206</b>
	<b>Length × temperature</b>	<b>0.0299</b>	<b>0.0076</b>	<b>3.937</b>	<b>0.0001</b>
	Humidity × time	-0.0134	0.0080	-1.671	0.0967
	<b>Time × temperature</b>	<b>0.0030</b>	<b>0.0015</b>	<b>1.985</b>	<b>0.0488</b>

Tests were conducted separately on juveniles that were alive and those that were dead 24 h later. Only juveniles at 19 °C survived these experiments, so the “alive” model includes only data from experiments at 19 °C, while the “dead” model includes data from experiments at all temperatures. Bold entries indicate  $P < 0.05$ .

<sup>a</sup>  $R^2 = 0.7239$ .<sup>b</sup>  $R^2 = 0.4906$ .



**Figure 4.** Relationship between shell sizes of juvenile *Crepipatella peruviana* and their proximity to adult female shells. Juveniles smaller than 10 mm in shell length were surveyed at Puerto Montt, Chile, during a 0.6-m low tide in December 2015. No juveniles were found on bare rock. Numbers above the boxplots indicate sample size. The difference in shell lengths for animals from the two types of habitat was statistically significant ( $t$  test,  $t_{81} = 9.697$ ,  $P < 0.0001$ ).

in supporting the gills during aerial exposure; when this fluid is removed, the gill may collapse, reducing the surface area exposed for gas exchange (Houlihan, 1979; Houlihan *et al.*, 1981).

Our data suggest, then, that when juvenile *C. peruviana* reach shell lengths of 5–6 mm, (1) the gills are now strong enough to resist collapsing in air, (2) the gills are large enough that they can still effectively exchange gases despite collapsing, or (3) the larger juveniles have now developed the ability to effectively retain fluid within the mantle cavity during aerial exposure. Any of these options could potentially explain the distinct size-related shift in desiccation and air tolerance at 5–6 mm in shell length. A metabolic explanation of the pronounced size escape from desiccation susceptibility in this species is further bolstered by the fact that 66% of small juveniles survived exposures to 100% RH at the lowest temperature tested (19 °C), compared to only 47% surviving for juveniles in the same size range tested at 22 °C and no juveniles surviving at 25 °C. The lower temperature would reduce metabolic demand (Newell and Kofoed, 1977) and thus dampen the metabolic imbalance caused by reduced respiration in air related to a lack of seawater in the mantle cavity. Indeed, low temperatures have been shown to reduce the impact of other stressors in some gastropods (*e.g.*, salinity) (Berry and Hunt, 1980; Przeslawski *et al.*, 2005; Bashevkin and Pechenik, 2015). Future studies are needed to test these hypotheses and

document the mechanism for the markedly well-defined size escape from desiccation in early juveniles of *C. peruviana*.

The complete intolerance of small juveniles of *C. peruviana* to even short periods of exposure to air illustrates the importance of microhabitat selection for these snails at or after metamorphosis. In the related species *C. fornicata*, early postsettlement mortality, most likely driven by intertidal physical stresses, is very high and may drive adult distributions in the intertidal zone (Bohn *et al.*, 2013). Our study suggests that small juveniles of *C. peruviana* must find a suitably wet habitat or they are highly likely to perish after a single low tide.

The pronounced size-related escape from desiccation that we have documented here for *C. peruviana* juveniles has implications for ontogenetic niche differentiation in habitat selection. While small juveniles of this species are extremely constrained in the habitats they may occupy, larger juveniles and adults with high desiccation tolerance are relatively free to occupy either exposed or sheltered regions. However, adult females are sedentary in this protandrous hermaphrodite (Chaparro *et al.*, 2001). Thus, young juveniles should be restricted to sheltered areas by their intolerance of desiccation and air exposure despite their ability to move around, as seen for the mussel *Mytilus trossulus* (Jenewein and Gosselin, 2013) and the snail *Nucella emarginata* (Gosselin and Chia, 1995). On the other hand, older juveniles and males should have greater freedom of movement in the field, being constrained neither by desiccation intolerance nor by locomotive inhibition. The microhabitat distribution of adult and juvenile *C. peruviana* should be examined in future comprehensive field studies.

In summary, this study with juveniles of *C. peruviana* exposed to air at different RHs and temperatures documents a pronounced size-related transition from complete intolerance of even surprisingly limited periods of water loss at a fairly high RH (75%) to almost complete invulnerability to moderate desiccation. Small juveniles of this species must be in contact with seawater to survive so much as 30 min of aerial exposure even at an RH of 100%, but cooler temperatures may reduce mortality when juveniles settle on bare surfaces. These findings suggest that a physiological or morphological change in aerial respiratory abilities may be responsible for the conspicuous change in desiccation tolerance that takes place when juveniles reach shell lengths between 5 and 6 mm. This interpretation is compatible with our pilot field study data. Further studies are needed to determine the specific mechanisms responsible for this transition.

### Acknowledgments

Funding for this project was provided by the Sandler International Research Fund at Tufts University. SMB was funded by the National Defense Science and Engineering Graduate Fellowship during preparation of this manuscript. Also we thank the Fondo Nacional de Investigación Científica y Tec-

nológica (FONDECYT-Chile) for help through grant FONDECYT 1141052 to ORC. Special thanks to Casey Diederich for advice and insight during this study. Thanks also to L. P. Miller and an anonymous reviewer for helpful comments and suggestions.

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