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Laboratory Survivorship of Aerially Exposed Pond Snails (*Physella integra*) from Illinois

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ABSTRACT

Many aquatic snails occupy ephemeral habitats that are occasionally subjected to severe environmental conditions. To investigate the physiological capacity of these animals to resist extreme environmental changes, we aerially exposed aquatic pond snails (*Physella integra*) to temperatures of 5°C and 20°C for 6, 8, 12, 24, 36, 48, and 60 hours. Survivorship varied with temperature and exposure times. At 20°C, survivorship was 0% for snails aerially exposed for 24 hours or longer. At 5°C, 0% survivorship was attained at 60 hours exposure. Sensitivity to aerial exposure was related to shell size of individuals, with larger specimens (shell length greater than 7 mm) significantly more likely to survive exposure than smaller specimens at temperatures and intervals with incomplete mortality. These results suggest that epiphragm development and size-specific survivorship predispose these animals to rapid population recovery following severe, short-term environmental fluctuations.

INTRODUCTION

Rapid, dramatic climate change is expected by the end of the 21st century and may have a profound biological impact (reviewed by Root and Schneider, 1993). Although average global temperatures may rise as little as 1°C over the next 100 years, the most significant side effect of even the most modest change in global temperatures is increased variation in local temperatures and precipitation (Knox, 1993; Root and Schneider, 1993). This increased environmental stochasticity and its unpredictability may select strongly against organisms that cannot adapt to or avoid this rapid change (Wyman, 1991; Peters and Lovejoy, 1992; Kareiva et al., 1993).

Throughout history, of course, organisms have experienced environmental variation and basically have evolved or gone extinct in response to the most coarse-grained changes. Many taxa that survive severe environmental fluctuations possess general traits (e.g., a broad temperature tolerance) or specific adaptations (e.g., seed dormancy) (Futuyma, 1986). For example, recent extirpations of populations of the terrestrial snail *Arianta arbustorum* in Switzerland have been linked to the inability of eggs to hatch because the climate has warmed (Baur and Baur, 1993). Sympatric populations of the snail *Cepaea nemoralis*, a species with greater thermal tolerance, have not suffered the same fate. Generally, however, the ability to survive such stochastic conditions is unclear even for organisms that possess presumptive adaptations for weathering severe environmental fluctuations.

Therefore, we selected *Physella integra* (Family Physidae), a basommatophoran pulmonate snail, to investigate the extent to which such snails are able to withstand aerial exposure. This species and other species of this important North American family of aquatic snails occupy both permanent and ephemeral aquatic habitats (Clampitt, 1974; Te, 1978; Brown, 1979). This common Illinois snail is frequently found in habitats subject to drying and possesses adaptations such as greater temperature tolerance that are not present in snails occupying permanent aquatic habitats (Brown, 1979; Paukstis et al., in press). Since *P. integra* is an aquatic species, it must either possess adaptations to resist desiccation or face extirpation each time such ephemeral habitats dry. One recognizable adaptation in this species is the epiphragm, a membranous covering over the aperture formed from dried body secretions (Cheatum, 1934). An epiphragm is formed whenever the snail is exposed to air and cannot immediately return to water. The effectiveness of epiphragm formation in this species has not been investigated. Herein, we report on the abilities of Physella integra to resist desiccation when exposed to air under two temperature regimes in the laboratory.

MATERIALS AND METHODS

Physella integra were collected 6 and 11 January 1994 from a ditch near the junction of Old Poag Road and Wanda Road, SW1/4 Sec. 12, T4N, R9W, 0.3 km W of Poag, Madison County, Illinois. All experimental trials were conducted or initiated within 2 days of collection. Snails were refrigerated at 5°C prior to use. Voucher specimens are deposited in the collections of the Illinois Natural History Survey.

We gently tamped dry snails with a paper towel and randomly assigned them to experimental and control containers. Experimental and control snails were kept in open cylindrical plastic containers 21 cm in diameter and 16 cm tall. We placed experimental animals into containers so that individual snails were not in contact with each other and so that the aperture of each snail faced the bottom of the container to allow epiphragm formation. Snails remained immobile once placed into experimental containers.

Containers were then assigned to experimental treatments as follows: seven time intervals (6 hr, 8 hr, 12 hr, 24 hr, 36 hr, 48 hr, and 60 hr) completely crossed with two temperatures (5°C and 20°C). Relative humidity for the 5°C trials was 47% whereas it was 58% for the 20°C trials. Initially, we subjected two replicates of 20 snails each to each of

these 14 treatments. For both temperatures, one control replicate of 20 snails each was kept in an identical container for 60 hours, but was covered by 5 cm of water from the collecting site.

After determining survivorship among treatments for this experiment, we repeated the experiment for treatments with either few survivors or few fatalities. Thus, we gathered data in a second experiment for one other replicate for the 6 and 8 hr 20°C treatment and the 8 hr 5°C treatment, two others for the 36 and 48 hr 5°C treatment, four others for the 24 hr 5°C treatment, and five others for the 12 hr 20°C and 12 hr 5°C treatments. These additional replicates were necessary to increase sample sizes sufficiently to allow statistical comparison of shell lengths among treatments with few survivors or few fatalities, while still reducing the number of snails killed to the minimum necessary to achieve statistical relevance. In the second experiment, replicate size varied from 19 to 23 snails per container. Because survivorship did not vary between the first and second experiment, results were combined. We also maintained two control replicates with one at each temperature for 60 hours during the second experiment. In all, we used a total of 1029 individual snails in the two experiments along with 80 further individuals as controls.

At the end of each time interval, the replicates for each temperature in both experiments were covered with fresh water from the collecting site. This water was either at 5°C or 20°C whichever was appropriate to match the treatment temperature. The containers were then moved to 15°C. Mortality was assessed after 12 hr at 15°C. At this time, each snail was measured to the nearest 1 mm and preserved in 70% ethanol. Snails that did not extend the body and crawl or those that failed to retract into the shell after mechanical stimulation were considered dead.

Because sample sizes were unbalanced in the combined data for the two experiments, we used statistical methods appropriate to such an unbalanced design. We used SAS (SAS Institute 1988) to perform ANOVA with the GLM procedure, correlation analysis, and regression analysis. *G* tests were used to compare survivorship of snails among treatments (Sokal and Rohlf, 1981). The *G* test evaluates the goodness of fit of the observed data relative to the expected result. The *G* test is an appropriate statistical test to apply to mortality data (Sokal and Rohlf, 1981). Values for p were obtained by comparing the resulting *G* value to the corresponding chi-square value using SAS functions. We used the sequential Bonferroni procedure to adjust p values for multiple comparisons (Rice, 1989).

RESULTS

All four controls had 100% survivorship. However, survivorship in experimental treatments varied depending on temperature and exposure time (Table 1). Survivorship of snails at 5°C was significantly greater at 6 ($G = 10.949$, $p < 0.001$), 8 ($G = 50.689$, $p < 0.001$), 12 ($G = 111.240$, $p < 0.001$), 24 ($G = 62.407$, $p < 0.001$), 36 ($G = 10.386$, $p < 0.002$), and 48 ($G = 4.178$, $p < 0.05$) hr than that of snails at 20°C for the same time intervals. No snails survived in the 24, 36, 48, and 60 hr treatments at 20°C or in the 60 hr treatment at 5°C.

Percent survivorship at 5°C was related to the duration of aerial exposure (r = -0.94997, p < 0.0001). The relationship can be expressed as % surviving = -2.16 * exposure time in hours + 110.44 (F = 240.536, p < 0.0001). At 20°C, percent survivorship was also related to duration of aerial exposure (r = -0.69613, p < 0.0002). This relationship can be expressed as % surviving = -1.07 * exposure time in hours + 49.69 (F = 19.745, p < 0.0001). The slopes of these lines differ (F = 31.05, p < 0.0001), which was consistent with G test results. Overall, survivorship decreased faster with exposure interval at 20°C than at 5°C.

Survivorship of snails was also related to shell length, with larger specimens being more likely to survive than smaller ones in any treatment with survivorship less than 100%. Mean shell length of surviving snails was significantly greater than that of snails that died (Table 1). Mean shell lengths of snails included in each treatment were not significantly different from each other (p > 0.05 in every case), indicating that the initial assignment of snails to treatments was not a factor in the results of this experiment.

Because larger specimens were more likely to survive than smaller ones, mean shell length of snails surviving and dying was related to exposure times at 5°C. The relationship (r = 0.8668, p < 0.0001) at 5°C is Mean Shell Length Survivors = 0.09 * exposure time in hours + 5.72 (F = 46.778, p < 0.0001). Exposure time was also related to mean shell length of snails that died (r = 0.72599, p < 0.0001). The relationship can be expressed as Mean Shell Length Dead = 0.09 * exposure time in hours + 3.14 (F = 24.257, p < 0.0001). At 20°C, absence of survivors in exposure times exceeding 12 hours made correlation and regression analysis meaningless (Table 1).

DISCUSSION

Cheatum (1934) described the results of an uncontrolled experiment with *Physella integra* (incorrectly identified as *Physa sayi crassa*; see Clampitt, 1974, for corrected identification of Cheatum's snails). He found that 3 of 24 (12.5%) individuals (shell length not given) survived aerial exposure from 3 July to 10 September in a shaded outdoor enclosure that was periodically sprinkled with lake water. However, in this experiment, the snails were buried to a depth of 3 cm and were not aerially exposed.

Of course, the conditions that we subjected the snails to are much harsher than those used by Cheatum (1934), and this is reflected in the relatively short time (24 to 60 h) needed to reach 100% mortality. Even so, our data suggest that *Physella integra* from Illinois is moderately resistant to desiccation even under extreme circumstances. However, it is apparently less resistant than the gastropods placed under similar conditions by Dudgeon (1982). In his dry pan experiments conducted at 24-26°C and 60-70% relative humidity, 100% mortality took from 7 days in *Thiara scabra* to 22 days in *Sinotaia quadrata*.

We also believe that our data indicate the importance of epiphragm formation in this species. Every snail tested formed an epiphragm, with the result that the aperture was firmly glued to the plastic substratum. Once wetted, survivors rapidly became mobile.

We presume that death was due to desiccation, but we cannot eliminate the possibility that oxygen deprivation was a factor as well (e.g., Dudgeon, 1982). This species and

other pulmonates respire by taking air or water into the lung chamber in the mantle (Cheatum, 1934). Once the epiphragm is formed, aerobic respiration is probably precluded and the snails must respire anaerobically. Von Brand et al. (1950) reported that *Physa* (= *Physella*) *gyrina*, a species closely related to *P. integra*, could tolerate exposure to anoxic water through anaerobic metabolism for 6 hours at 30°C with 100% survivorship and with 3% survivorship after 16 hours at 30°C. Comparison of our results to those of Von Brand et al. (1950) suggest that the mortality we observed was due to water loss rather than to inability to respire anaerobically for a sufficient time interval to survive the treatment. This explanation is probably correct because the snails that Von Brand et al. (1950) exposed to anoxia but not to dehydration survived longer durations of exposure at higher temperatures as compared to our snails at 20°C.

Our results are important because they clearly indicate that larger individuals are more likely to survive relatively short periods of drying under harsh conditions than are smaller specimens (Table 1). These larger snails are more likely to be reproductive than are smaller individuals (Brown, 1979). Consequently, populations exposed to periodic drying may quickly recover because reproduction could recommence immediately.

The ecological relevance of our experiments is uncertain because field studies comparing size-specific effects of aerial exposure on aquatic mollusks are unavailable for any species of *Physella*. Our conclusion that larger individuals are more likely to survive short-term aerial exposure may be obviated if size-specific behavioral differences are present. For instance, small snails may be more likely or better able to seek shelter in cracks in the substrate or under cover objects than larger snails. Field studies of species that occupy ephemeral habitats would be necessary to evaluate the existence and importance of such behaviors. Regardless, our experimental results will be important in interpreting the ecological context of behavior of snails in the field.

The findings of our study are particularly relevant to the current debate over environmental scenarios expected under incipient global climate change. The epiphragm adaptation in *Physella* permits these aquatic snails to endure short periods of extreme dehydration, an environmental condition that may increase in frequency as global and local climates change (Root and Schneider, 1993). In this light, more experiments of the adaptive significance of traits linked to tolerance of environmental stochasticity would be valuable to conservation biologists.

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Table 1. Aerial exposure of a pond snail, *Physella integra*, and its relationship to shell length of surviving and dying snails.

Treatments		Snails					
exposure duration (hours)	temp (C°)	survivors mean shell length (mm)	n	fatalities mean shell length (mm)	n	Results of ANOVA *F*	*p*
6	5	6.20	40	------	0	------	------
8	5	6.89	56	5.25	4	10.42	< 0.0021
12	5	6.68	125	3.60	20	98.27	< 0.0001
24	5	7.68	75	5.73	45	75.25	< 0.0001
36	5	8.75	12	6.65	68	32.64	< 0.0001
48	5	10.00	3	6.67	78	32.79	< 0.0001
60	5	------	0	6.70	40	---	---
6	20	7.34	50	5.50	10	15.78	< 0.0002
8	20	7.81	21	6.10	40	33.52	< 0.0001
12	20	7.67	38	6.07	104	36.36	< 0.0001
24	20	------	0	6.88	40	------	------
36	20	------	0	6.20	40	------	------
48	20	------	0	6.89	80	------	------
60	20	------	0	6.33	40	------	------