Habitat preference of *Cipangopaludina chinensis*

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Abstract

Cipangopaludina chinensis, a viviparid snail, is believed to be an invasive species in the Land o' Lakes, WI area. Studying habitat preference of *C. chinensis* is important, as introduction of a non-native species can alter habitats, communities, and interspecific interactions. I characterized *C. chinensis* habitat selection behavior by examining colonization (by unit surface area) of sand, muck, cobble, and macrophyte habitats. I expected to find that only baby snails would colonize macrophyte shoots and that cobble would be preferred over all other substrates due to its periphyton growth. Three habitat selection experiments were conducted using baby and adults *C. chinensis* individuals. Neither baby nor adult snails colonized macrophyte shoots. χ^2 analyses of pooled hour 7 experimental data indicated that density distributions of babies and adults were significantly different from each other ($\chi^2 = 23.01$, df = 3, $p < 0.001$) and that neither baby ($\chi^2 = 13.84$, df = 3, $p < 0.01$) nor adult ($\chi^2 = 9.42$, df = 3, $p < 0.05$) snail distributions were equal based on surface area. Examination of differences between χ^2 observed and expected snail counts for each substrate indicated that babies colonized muck and adults colonized cobble at higher densities than predicted by an equal distribution by surface area model. These results suggest that small snails may avoid predation by hiding in muck substrates during youth. When the snails possess a sturdier, adult shell that protects against predation, they then may be more likely to graze on periphyton-rich cobble substrates.

Introduction

The Chinese Mystery Snail (*Cipangopaludina chinensis*) is a viviparid snail commonly found throughout Asia. This freshwater gastropod is non-native to the Land o' Lakes, WI area; however, *C. chinensis* has been discovered in several locations on the University of Notre Dame Environmental Research Center property in Land o' Lakes. Although these prosobranch snails have not been thoroughly characterized, it is believed that they are an invasive species. It has been suggested that *C. chinensis* may displace native snails, but this relationship has not yet been documented. There is very little research on the basic life history characteristics of *C. chinensis*. One of the most basic and important life history characteristics is the habitat preference of a species. Habitat preference will dictate where a species is found and what other species it will interact with. Introduction of non-native snail species can alter habitats and aquatic communities, thus it is important to characterize interaction between the snail species and the habitats it prefers (Bertness 1984).

Previously, characterization of invertebrate habitat preference has shown species-specific responses with respect to habitat choice (Cyr and Downing 1988, Kershner and Lodge 1990). Previous studies conducted on other macroinvertebrates have indicated that substrate association may depend on surface area, architectural complexity of substrates, degree of refuge from predation, and food quality and availability (Kershner and Lodge 1990).

Architectural complexity influences colonization; however, species-specific morphology and behavior seem to dictate these habitat choices (Kershner and Lodge 1990).

In examining habitat choices of a snail species, Brown and Lodge (1993) note that it is important to consider the substrate surface area that the snails use. Rather than examining densities based on a per unit bottom area basis (Figure 1a), it is much more informative to examine densities based on surface area available for snail colonization (Figure 1b). Brown and Lodge (1993) stress the importance of creating a null surface area model hypothesis in which it is expected that snails will be distributed equally on a per unit usable surface area basis across all examined habitats. Deviation from this equal distribution provides insight into which substrates are preferred in colonization. Whereas it was once suggested that macrophytes were the preferred substrate for snails, it is now believed that macrophytes are utilized much less when examined from a per unit surface area perspective.

Creating a proper null surface area model includes understanding how much surface area is available for colonization. Kershner and Lodge (1990) explain that snail foot size is an important factor in determining what substrates a snail can colonize. *C. chinensis* adults have a large foot size of several centimeters, whereas babies tend to have a foot size of 1.5 cm or less. Adhering to a macrophyte stem, such as that of *Potamogeton amplifolius* (stem diameter < 0.75 cm), may not be practical or even possible for adults; however, babies with small foot size may readily colonize the macrophyte. Generating an accurate null surface area model for *C. chinensis* involves understanding which substrates are available to snails of all size classes within the species—this may require creating two or more separate models depending on snail size classes.

Macrophytes have been characterized as a food-poor substrate for snails, especially when compared to lake-bottom substrates (Brönmark 1990, Lewis 2001). It is more common that snails graze on periphyton, algae, and aquatic detrital matter instead of feeding on living macrophyte tissue (Reavell 1980, Brönmark 1990).

The goal of this project is to characterize the habitat preference of *C. chinensis*. If *C. chinensis* is provided the choice of several different substrates, I expect that the gastropods will preferentially colonize cobble, sand, muck, and macrophyte substrates respectively. Brown and Lodge (1993) expect this preference because, unlike most macrophytes, cobble and sand are available for periphyton growth throughout the growing season. I also expect that distribution of baby snails will differ from that of adult snails. I expect baby snails to colonize macrophytes due to small foot size whereas I expect that adults will be unable to colonize the macrophyte shoots. The results from this experiment will help us understand what habitats *C. chinensis* will likely utilize in nature. This information will help us better understand what other organisms *C. chinensis* will interact with and what the potential impacts of *C. chinensis* invasions may be.

Materials & Methods

Arena setup

In laboratory experiments, I tested habitat and substrate selection of *C. chinensis*. Each experimental arena was a large (130-cm diam, 24 cm deep) plastic wading pool and was placed outside under partial shade behind the UNDERC Wet Lab. Each arena was equally divided into four wedge-shaped quadrants, providing snails a choice of four different habitats: sand (~2-cm deep); sand covered by a cobble layer (7.5-cm diam; range, 4.0-12.1); sand with shoots from submersed *P. amplifolius* macrophytes; and muck (~1-cm covering 1 cm of sand). Each arena was filled with water from Tenderfoot Lake.

Substrate collection

In each arena, I placed an initial 1-cm spread of sand from the Old Gravel Pit upon which I constructed each habitat. Sand used as substrate (top 5 cm collected from 0.5 m deep water) was collected from the northeast shore of Tenderfoot Lake and strained to separate out cobble and larger pebbles. Cobble (from 0.5 m deep) was also collected from Tenderfoot and was handled so as not to disturb its natural periphyton growth. *P. amplifolius* macrophytes (from 1 to 1.5 m deep) were collected from the northeast shore of Tenderfoot and searched for native snails, which were removed. Muck was collected from the north shore of Tenderfoot and strained before use.

Surface area calculation

Sand and muck surface area was calculated as a planar surface in which each habitat comprised one-fourth of the total arena bottom area. The wading pool walls were sometimes colonized, so surface area was calculated as a cylinder with no top or bottom. Wall area represented the 17.5 cm of wall that was above the substrates and below the water surface. Cobble surface area was calculated according to Brown and Lodge (1993), in which each rock was treated as if it were spherical with half of its surface area available for snail colonization. Macrophyte surface area was estimated using wet weight-surface area regressions developed by applying geometric surface area formulae to measurements of macrophyte segments (Cattaneo and Carginan 1983).

I collected 22 samples of *P. amplifolius* macrophytes of various weights (range, 1.62-14.44 g) in buckets of water. Each shoot was blotted gently to remove excess water and then weighed to determine the wet weight. Stem segment length was measured to the millimeter using a ruler. Stem diameter was also measured with a ruler; however, certain stem segments varied in diameter, so the surface area of these segments was calculated separately before the surface area of all the plant's segments were added together. For all macrophytes, the stem of the plant was assumed to be cylindrical in surface area calculations. I measured the length and maximum width of each leaf and calculated the leaf surface area geometrically by assuming each leaf was elliptical in shape. I conducted a regression of surface area (SA, cm^2) on macrophyte wet weight (W, g), which was $SA = 212.0W + 107.4$ ($R^2 = 0.876$, $n = 22$ shoots, Figure 2).

For each arena, I used approximately 100 g of *P. amplifolius* macrophyte. When the surface area of the 100 g was added to the surface area of the sand it was anchored in, the macrophyte habitat represented a total surface area of 24625.7 cm^2. I anchored each shoot in the sand using small pebbles, which were then covered by sand. Each macrophyte habitat was constructed such that its macrophyte density was approximately 300 g m^{-2} (wet wt). These macrophyte densities are well within the range of Vilas County, WI lakes (10-2,900 g m^{-2}) measured by Lodge et al. (1989).

Snail collection and rearing

Approximately 250 adult snails were collected in late May, 2007 from Wildcat Lake and the downstream area of Brown Creek and another 300 were collected on June 28, 2007. Throughout the summer, these snails produced approximately 400 babies. Of the snails that were captured and raised, 300 adults and 180 baby snails were used for all of my experiments. Snails were housed in two large cylindrical tanks in the UNDERC Wet Lab. All snails in the facility were cared for by members of the University of Notre Dame Lodge lab.

Snails used in experiments were starved for at least 12 hours before use.

No adult snails were used in more than one experiment; however, 180 baby snails were used twice. All babies were labeled by painting their shells with bright pink or magenta nail polish to make them more visible and easier to count. Brown and Lodge (1993) spray painted adult snails, noting no deaths or sublethal effects; however, when I tried this technique on the baby snails, approximately 80% of baby snails died due to spray paint covering their opercula and drying them shut. This high mortality necessitated that experiments involving baby snails were conducted with 6 instead of 9 pool replicates.

Experimental Procedure

Two experiments were conducted in which only adult snails and only baby snails were used. For each experiment, several pools were used to increase the number of snails that could be used, while keeping the snail densities within experimental pools within natural densities. I conducted 9 replicates of 20 adults per pool, 6 replicates of 30 babies per pool, and 6 replicates of pools each containing 20 adults and 30 babies. Adult experiments were conducted on June 1, babies on July 19, and both together on July 20, 2007.

Each experimental run lasted seven hours to observe snail presence on the various substrates over time. Surveys of how many snails were located on each substrate were taken each hour. To begin each experiment, snails were placed with their opercula facing upward. Snails were determined to have moved if they turned over. Individuals that remained motionless were not included in data sets until they turned over.

Statistical Analysis

All statistical analyses were conducted using SYSTAT 12.0 (Systat Software, Inc.; San José, CA) and Excel 2007 (Microsoft Corp.; Redmond, WA). An OLS regression of macrophyte wet weight on surface area was conducted to ensure macrophyte surface area was approximately equal across all experimental arenas.

Separate χ^2 tests were conducted to determine if equal proportions of snails (based on substrate surface areas listed in Table 1) were found on each substrate. χ^2 tests were conducted on pooled data from the last hour of experiments. Seventh hour data from all three experiments were pooled by experiment according to age class (adult or baby). Totals of final number of snails per substrate were designated as observed values (O_i) in the χ^2 formulae. Expected values were calculated using the following formula:

$$E_i = \left(\frac{\text{SA of Substrate}}{\text{Total SA of All Substrates}}\right)(\text{Total Active Snails in Arenas for 7th Hour})$$

The wall substrate was not included in calculations (Table 2) and all values for surface areas are found in the surface area null model column of Table 1. I also conducted χ^2 analyses comparing the distributions of baby versus adult *C. chinensis*.

Results

Macrophyte wet weight-surface area relationship

I conducted an OLS regression of *P. amplifolius* macrophyte surface area on shoot wet weight. The regression indicated a positive linear relationship between surface area (SA, cm^2) and wet weight (W, g). For *P. amplifolius* macrophytes on the east shore of Tenderfoot, I found that $SA = 212.0W + 107.4$ ($R^2 = 0.876$, $n = 22$ shoots, Figure 2). This equation can be used to facilitate constructing many macrophyte habitats of approximately the same surface area.

Characterization of C. chinensis distributions

A preliminary analysis of collected data showed that snail densities increased in certain habitats over time. Average snail counts were highest for these specific habitats at the last hour of my experiment (Figures 3-5), so I used data from hour 7 for all χ^2 analyses.

Adult C. chinensis habitat distribution vs. equal distribution model

Adult snail distributions at hour 7 (Figure 3) were significantly different from what would be predicted by an equal distribution model such as the null surface area model hypothesis (Table 3). For adults, the equal distribution model hypothesis was rejected, as χ^2 analysis indicated a significant difference ($p < 0.05$). The individual χ^2 value for the cobble substrate listed in Table 2b indicates that the summed χ^2 value is driven most by the high number of adult snails in cobble.

Baby C. chinensis habitat distribution vs. equal distribution model

Baby snail distributions at hour 7 (Figure 4) were significantly different from the distribution predicted by the null surface area model hypothesis (Table 3). For babies, the equal distribution model hypothesis was rejected, as χ^2 analysis indicated a significant difference ($p < 0.01$). The individual χ^2 value for the muck substrate listed in Table 4a indicates that the summed χ^2 value is driven most by the high number of baby snails in muck.

Baby vs. adult distributions

Distributions of babies and adults at hour 7 were significantly different from each other (Table 3). χ^2 analysis indicated a significant difference between the two age classes ($p < 0.01$). The individual χ^2 value for the muck substrate listed in Table 4b indicates that the summed χ^2 value is driven most by the high number of baby snails on muck compared to lower numbers of adults on muck substrates.

Discussion

Null surface area model hypothesis

Understanding the habitat selection behavior of a snail species requires an examination of how it utilizes the substrate surface area available to it (Brown and Lodge 1993). Designing a null surface area model was the first step to understanding distributions of *C. chinensis*. During experiments I noted that only 2 of 180 baby snails and 1 of 300 adult snails actually utilized macrophyte shoot surface area as a substrate. It appeared that neither baby nor adult snails colonized this substrate, possibly due to the naturally large foot size of the species. I therefore simplified my null model to take into account only the surface area of the sand used to anchor the macrophytes (Table 1). Similarly, snail colonization of arena walls was negligible given the surface area of the substrate and was eliminated from χ^2 analyses such that it did not have an undue effect on count values expected for each substrate (Table 2). Ultimately, the null model I created for *C. chinensis* represented sand, muck, and macrophyte surface area with the arena bottom area that each of the three substrates covered (Table 1). Cobble remained the only substrate which varied in surface area from the arena bottom area it covered.

Interpreting adult snail distributions

I conducted a χ^2 analysis and determined that adult snails distributions do not follow the equal distribution model ($p < 0.05$). Results displayed in Table 2b show that high counts of adults existed on cobble at hour 7. This high count was a primary influence on the χ^2 value. This data suggests that adult snails prefer grazing on cobble habitats.

Reavell (1980) and Brönmark (1990) documented the snail diet as dominated by periphyton and algae grazing. Shells of *C. chinensis* babies are much more easily crushed by predators than the strong shells of adults (T. Campbell, personal communication). Periphyton and algae, rich in nutrients not found in muck, would provide snails with good nourishment. As adult *C. chinensis* individuals are much more able to resist and survive predator attack due to a strong shell, it is possible that they are willing to risk predator attack in order to feed better.

Interpreting baby snail distributions

By χ^2 analysis, I determined that baby snails do not follow an equal distribution model for their distributions ($p < 0.01$). Table 4a indicates that the high counts of babies observed in muck substrate at hour 7 influenced the χ^2 value. Therefore, it is likely that baby snails select muck as a habitat for growth.

Bourne (1993) states that a snail's size and the substrate it colonizes affects its antipredatory behavior. *C. chinensis* produce more offspring in mucky environments (M. Mohrman, personal communication), thus it is likely there is something about this environment that makes it beneficial for the baby snail. It is possible that muck may provide an ample amount of nutrients necessary for survival through youth and yet still provide refuge from predators (B. Peters, personal communication). Unlike a pure sand substrate, baby snails are able to slightly burrow into mucky substrates, which would make them accessible to predators.

Interpreting differences between baby and adult snails

I analyzed differences in distribution between baby and adult snails by using a χ^2 analysis. The distributions of the two age classes were significantly different from each other ($p < 0.001$). Table 4b indicates the high influence of babies located in muck in hour 7 on the final χ^2 value. This data suggests that babies and adults use different habitat selection methods.

Based on analysis of the baby experiments and adult experiments, it is possible that baby snails bide their time in low-nutrient muck as an anti-predatory measure until adulthood. Once adulthood is reached, it is possible that the snails' strong shells protect them against predators, which must crush the snail's shell in order to consume the gastropod. This would allow the adult snails to take the risk of no refuge from predators in exchange for better feeding.

Future study

These habitat selection experiments definitely helped to characterize surface area usage by *C. chinensis*. I suggest conducting further study of substrate selection by the snail. I believe that density distributions across substrates will be better understood with many replicates of this experiment. I would suggest using 30 adult snails in every arena instead of 20 to strike a balance between collecting enough data to see trends while not overcrowding snails beyond reasonable densities. Many replicates of these experiments would help better characterize adult substrate choice, providing more data with which to test the null surface model hypothesis.

Many freshwater organisms, including viviparid snails, possess predator-induced defenses triggered by water-borne cues (Prezant et al. 2006). Such cues could influence habitat preference to favor habitats that would best provide refuge from predation. It is important to understand these responses because they influence the real-world distribution of organisms. As *C. chinensis* rarely colonized *P. amplifolius* macrophyte shoots when not under predation, it would be interesting to see if *C. chinensis* attempts to use the shoots as refugia with predatory cues present in the water.

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Tables

Table 1. Estimate absolute area (cm^2) and percentage bottom area for four habitats available to snails in habitat selection experiments.

	Arena bottom coverage		**Surface area null model**	
	(cm^2)	**(%)**	**(cm^2)**	**(%)**
Muck	3318.3	25	3318.3	22.59
Sand	3318.3	25	3318.3	22.59
Cobble	3318.3	25	4734.3	32.23
Macrophytes	3318.3	25	3318.3[†]	22.59[†]
Arena Wall	(7147)[‡]	0[‡]	(7147)[‡]	0[‡]
Total	13273.2	100	14689.2	100

[†] Total macrophyte habitat surface area was 24625.7 cm^2; however, as snails opted to colonize the sand used to anchor the macrophyte habitat rather than the macrophyte shoots themselves, arena bottom area was used in the surface area null model so as not to give it undue weight in χ^2 calculations.

[‡] Arena wall substrate was not included in null model calculations. The substrate's negligible snail colonization and high surface area gave it undue weight in χ^2 calculations (Table 2).

Table 2. Elimination of wall substrate in χ^2 calculations. (a) The arena wall substrate was not included in any χ^2 calculations because it unduly inflated the χ^2 value. The substrate had high surface area and was rarely colonized. (b) Without the wall substrate, the χ^2 calculation is reasonable.

a) Adults vs. Equal Distribution Model with Wall Substrate

	O_i	E_i	$\frac{(O_i - E_i)^2}{E_i}$
Muck	33	27.35	1.17
Cobble	75	39.03	33.16
Macro	33	27.35	1.17
Sand	33	27.35	1.17
Wall	6	58.91	47.53

χ^2 = **84.18**

b) Adults vs. Equal Distribution Model without Wall Substrate

	O_i	E_i	$\frac{(O_i \quad E_i)^2}{E_i}$
Muck	33.00	39.31	1.01
Cobble	75.00	56.08	6.38
Macro	33.00	39.31	1.01
Sand	33.00	39.31	1.01

χ^2 = **9.42**

Table 3. χ^2 calculation results. Tests were conducted between experiments and the equal distribution model and between different experimental groups.

Experiment	χ^2	**df**	***p*-value**
Adults vs. Equal Distribution Model	9.42	3	$p < 0.05$
Babies vs. Equal Distribution Model	13.84	3	$p < 0.01$
Adults vs. Babies	23.01	3	$p < 0.001$

Table 4. χ^2 analysis broken down by substrate. **(a)** Highest influence on the χ^2 value for babies compared to the equal distribution model came from the difference in observed and expected values for muck substrate. **(b)** Highest influence on difference between babies and adults came from the muck substrate. Observed values were the pooled values for babies in all substrates at hour 7 of the experiment. Expected values were calculated by multiplying the proportion of large snails in each substrate multiplied by the total number of baby snails active at hour 7.

(a) Babies vs. Equal Distribution Model

	O_i	E_i	$\frac{(O_i - E_i)^2}{E_i}$
Muck	48.00	31.40	8.78
Cobble	45.00	44.80	0.00
Macro	20.00	31.40	4.14
Sand	26.00	31.40	0.93

$\chi^2 = \mathbf{13.84}$

(b) Babies vs. Adults

	O_i	E_i	$\frac{(O_i - E_i)^2}{E_i}$
Muck	48	26.36	17.76
Cobble	45	59.91	3.71
Macro	20	26.36	1.54
Sand	26	26.36	0.00

$\chi^2 = \mathbf{23.01}$

Figures

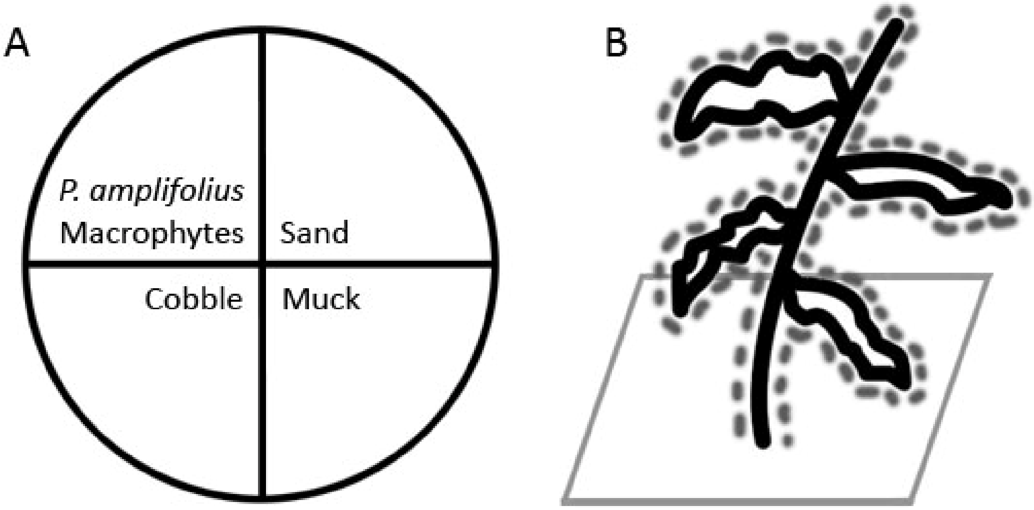

Figure 1. Null surface area model setup. (a) Division of pool into four wedges of equal arena bottom area. (b) Arena bottom area represented by gray box; substrate surface area represented by area enclosed by dotted lines and gray box. Note that in experiment, substrate surface area for macrophytes was actually represented by arena bottom area (Table 1).

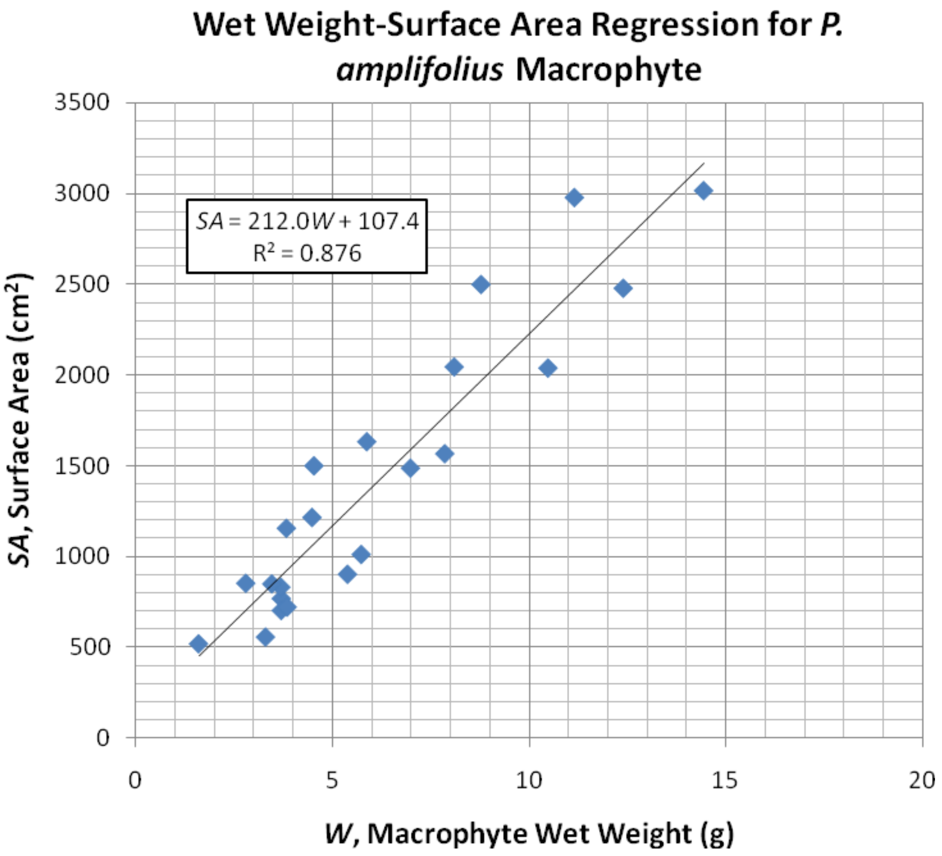

Figure 2. Wet weight-surface area regression for *P. amplifolius* macrophyte. OLS regression analysis indicated a positive linear relationship between shoot wet weight and surface area.

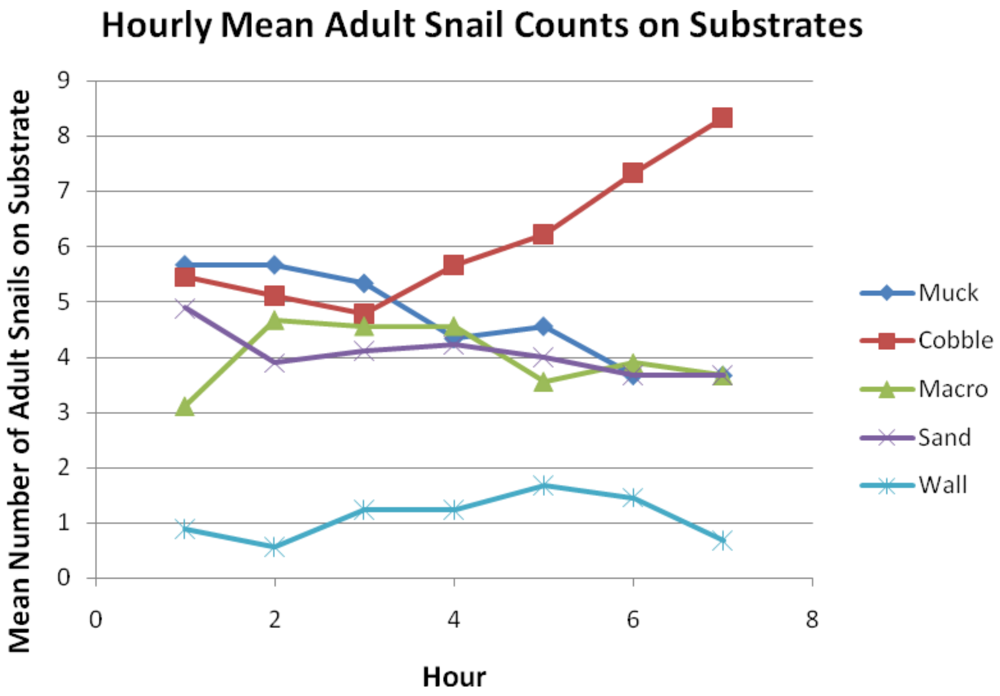

Figure 3. Hourly mean adult snail counts on substrates. Mean snail numbers across all arenas (*n* = 9) suggest snail movement toward cobble over time.

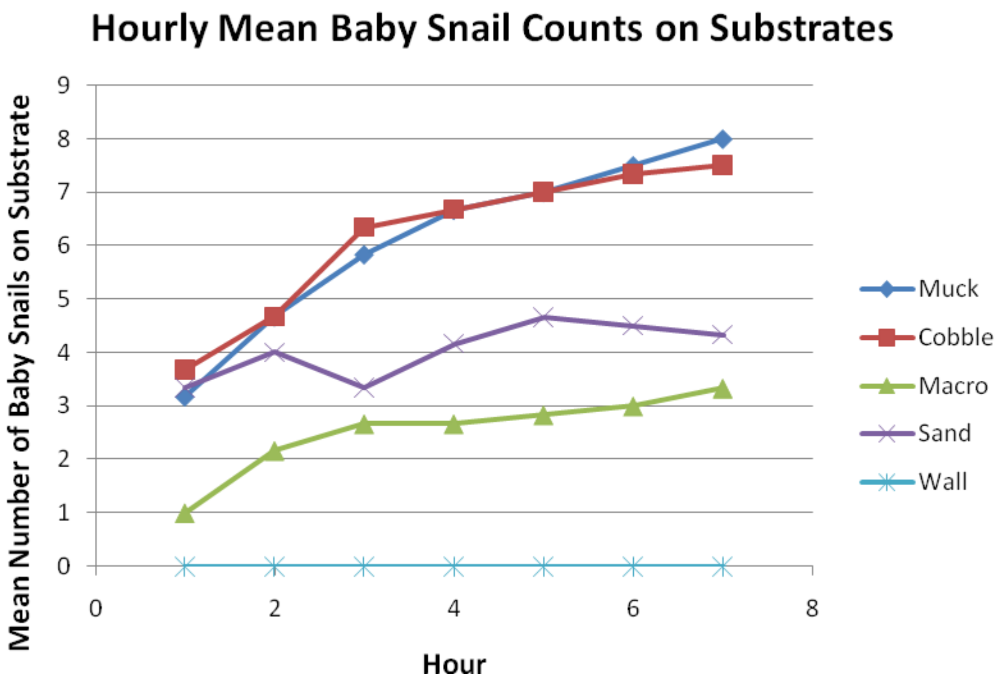

Figure 4. Hourly mean baby snail counts on substrates. Mean snail numbers across all arenas (*n* = 6) suggest baby snail movement toward muck and cobble over time.

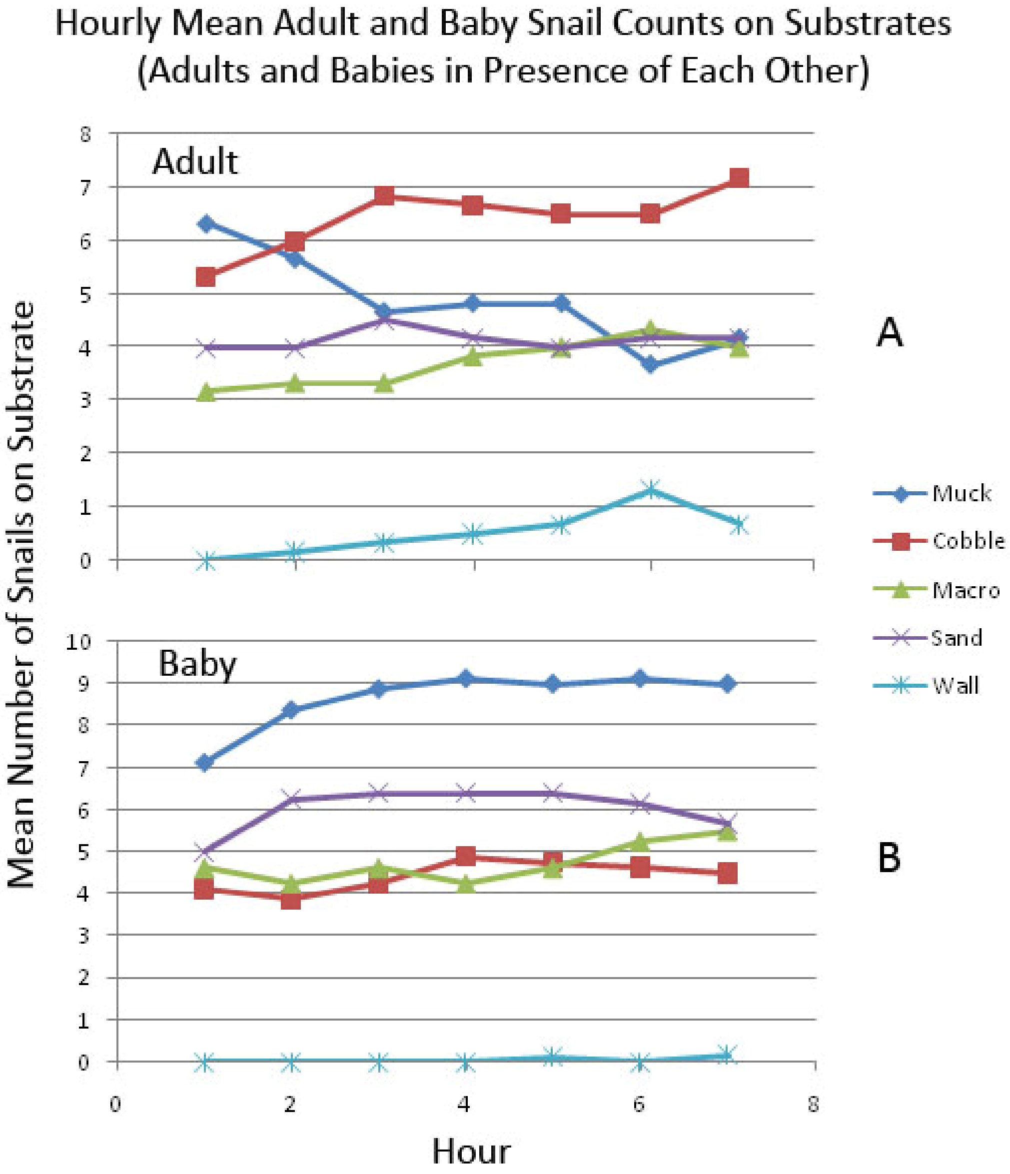

Figure 5. Hourly mean adult and baby snail counts on substrates (adults and babies in presence of each other). Mean snail counts across all arenas (*n* = 6) suggest **(a)** adults move toward cobble and **(b)** babies move toward muck when each age class is in the presence of the other.