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Consequences of captive-rearing and exposure to cues from potential predators on shell sizes and shapes of North American stagnicoline gastropods (family Lymnaeidae)

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Abstract: Understanding the extent to which traits that are used to delimit and diagnose species are phenotypically plastic is important for recognizing species boundaries. Shell characters have long been used for describing species of gastropods, even though these features may be influenced by environmental conditions. To determine the degree of phenotypic plasticity of two North American lymnaeid species, *Stagnicola elodes* (Say, 1821) and *Stagnicola emarginata* (Say, 1821), that occur in different habitats and differ in shell morphology, we reared individuals in captivity under similar conditions and compared shell shapes and sizes of wild-caught and captive-reared populations. We also exposed individuals of *S. elodes* to effluent from a potential predator (crayfish) to gauge the possible impact of the presence of predators on shell morphology. Although the two species remained morphologically distinct, shell shapes of captive-reared individuals of both species differ significantly from those of wild-caught individuals and show similar magnitudes of change among species. Directions of change, however, differed significantly among species. Although shell shapes of individuals of *S. elodes* that were exposed to crayfish cues were not significantly different from control snails, shell sizes of exposed snails were smaller than unexposed snails. These results suggest that exposure to predators affects growth rates of *S. elodes*. Nonetheless, given significant associations between shell shape and size that were observed in the captive-rearing and predator-exposure experiments, shell shape changes allometrically during development. These results suggest that morphological differences of other North American *Stagnicola* species reflect ecophenotypic variation, but more work is necessary to further evaluate this hypothesis.

Key words: phenotypic plasticity, allometry, geometric morphometrics, growth rates, ecophenotypes

"*The great problem in systematics of stagnicoline snails is in accurately assessing which characters are ecophenotypic and which are genetic, and of the genetic differences which are great enough to conclude that any particular population(s) is (are) distinct enough to deserve a binomial (or trinomial) name of its (their) own.*" – John B. Burch (1982)

Many organisms possess morphological, physiological, or behavioral traits that manifest differently depending on the biotic or abiotic conditions that are experienced during development, a phenomenon known as phenotypic plasticity (Via *et al.* 1995). A classic case is exhibited by the water flea *Daphnia,* which develops spines on its carapace when exposed to predators (Green 1967). If traits like these are used to delimit species, taxonomic decisions may be erroneous and recognized species may not represent truly distinct lineages. Indeed, spine and spineless forms of *Daphnia lumholtzi* (Sars, 1885) were originally described as two separate species given the distinct morphologies of the two morphs (Green 1967). Thus, recognizing the extent to which phenotypes are influenced by environmental conditions is important for interpreting species boundaries.

Gastropods exhibit phenotypic plasticity in a variety of traits (Goodfriend 1986, Bourdeau *et al.* 2015). Characters associated with shell morphology, growth, fecundity, and behavior are the ones that most commonly exhibit plasticity in aquatic gastropods and are predominantly influenced by habitat types, abiotic factors, and exposure to predators (Bourdeau *et al.* 2015). For example, two distinct morphotype of a physid species (*Physella* (= *Physa*) *acuta* (Draparnaud, 1805)) that occur in different habitat types converge upon the same shell shape when raised under similar conditions (Gustafson *et al.* 2014). Moreover, two lymnaeid species (*Radix balthica* (Linnaeus, 1758) (= *Lymnaea ovata* (Draparnaud, 1805)) and *Radix* (= *Lymnaea*) *peregra* (Draparnaud, 1805)) that exhibit distinct shell morphologies in wild populations, converge upon the same shell shape when reared under similar conditions in captivity (Wullschleger and Jokela 2002). Captive-raised populations continue to exhibit distinct growth rates and reproductive schedules and so the two species appear to be genetically distinct lineages (Wullschleger and Jokela 2002). In addition, when individuals of *R. balthica* and *P. acuta* are exposed to cues from potential predators, they become more globose in shape (Lakowitz *et al.* 2008, Brönmark *et al.* 2011, Goeppner *et al.* 2020), although responses of *R. balthica* depend on the particular predators to which individuals are exposed (Lakowitz *et al.* 2008). Also, shell features of muricids (*Nucella lamellosa* (Gmelin, 1791) and *Nucella lapillus* (Linnaeus, 1758)) that are associated with deterring predation are better developed in individuals exposed to cues from predators than in individuals that are

not exposed (e.g., apertural lips are thicker and apertural teeth are larger) (Appleton and Palmer 1988, Palmer 1990). These and numerous other studies (see Bourdeau *et al.* 2015) illustrate the plastic nature of shells of many aquatic gastropod species and stress the need to determine if and how phenotypic plasticity contributes to variation in shell morphology of species that were originally described from shell traits.

Based largely on the apparent distinctiveness of their shell morphologies, Burch recognizes 21 species of the aquatic gastropod genus *Stagnicola* Jeffries, 1830 (family Lymnaeidae) in North America (Burch and Tottenham 1980, Burch 1982, Burch 1989), a considerable reduction from the nearly 60 lineages that F.C. Baker (1911, 1928) recognizes. These species occur in a variety of aquatic habitats including ponds, rivers, and lakes, with morphologically distinct forms typically occurring in different habitat types (Burch 1989). Burch assembles these species into two main groups that are largely differentiated based on shell morphology and habitat types: the "*Stagnicola elodes* group" that includes five species with relatively elongate spires and narrow shells that occur in relatively small bodies of water and the "*Stagnicola catascopium/emarginata* group" that includes 16 species with more globose shells and compressed spires that live in rivers and lakes (Burch and Tottenham 1980, Burch 1982). Some workers have proposed that the morphological variation of all North American stagnicolines simply reflects ecophenotypic variation of a single species (e.g., Walter 1969). *Stagnicola elodes* (Say, 1821) (a member of the *Stagnicola elodes* group) and *Stagnicola emarginata* (Say, 1821) (a member of the *Stagnicola catascopium/emarginata* group), however, maintain their distinct shell morphologies in captivity even after many years of rearing under similar conditions and appear to be reproductively incompatible (Burch and Ayers 1973). Thus, although the two groups likely represent separate evolutionary units, morphological differences among species within each group could reflect ecophenotypic variation if shell morphology is influenced by environmental conditions.

Here we evaluate the role of phenotypic plasticity in determining patterns of variation in shell morphology of members of Burch's two groups of North American stagnicolines: *Stagnicola elodes* and *Stagnicola emarginata*. *Stagnicola elodes* has a shell with a relatively elongate spire, wide spaces between whorls in the spire, and narrow shell width (Burch 1982) (Fig. 1). It is distributed across North America (in the southern provinces of Canada and the northernmost states of the United States) and occurs in quiet bays or ponds, marshes, and temporary pools or streams (Burch and Tottenham 1980, Burch 1989). *Stagnicola emarginata* has a relatively globose shell with a compressed spire (Burch 1982) (Fig. 1). This species has a smaller range than *S. elodes*, is distributed primarily around the Great Lakes, and predominantly occurs on open shores of lakes (Burch and Tottenham 1980, Burch 1989).

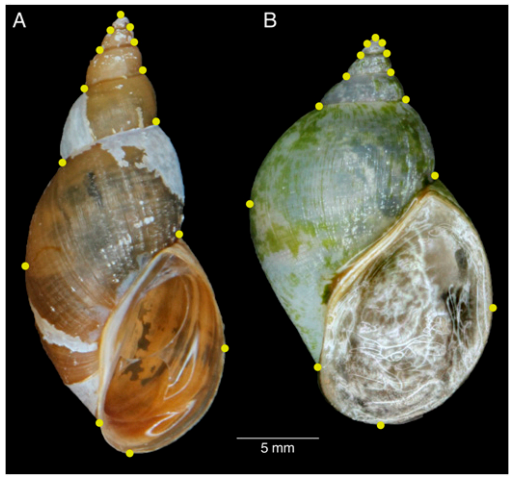

Figure 1. Photographs of specimens of (A) *Stagnicola elodes* and (B) *Stagnicola emarginata*. Locations of landmarks that were placed along the edges of shells for geometric morphometric analysis are indicated with yellow dots.

To determine if *Stagnicola elodes* and *Stagnicola emarginata* exhibit ecophenotypic variation in shell morphology, we used geometric morphometric analyses (Zelditch *et al.* 2012) of shells of snails from wild populations, snails raised in captivity, and snails exposed to cues from potential predators. We first compared shell shapes and sizes of wild-caught individuals of populations *S. elodes* and *S. emarginata* to adult progeny of these populations that were reared under similar conditions in captivity. Captive-reared individuals undoubtedly encounter very different biotic and abiotic conditions than individuals from natural populations. Hence, we predict that shell morphology of captive-reared snails will differ from shell morphology of wild-caught individuals if these species exhibit phenotypic plasticity in shell shape. To determine if exposure to predators influences the shell morphology of *S. elodes*, we established control and experimental populations of captive-raised, juvenile individuals of *S. elodes* and exposed experimental populations to effluent from crayfish. We then compared shell shapes and growth rates (i.e., shell sizes) of adult individuals from experimental and control populations.

MATERIALS AND METHODS

Specimens

We collected approximately 120 individuals of *Stagnicola elodes* and 120 individuals of *Stagnicola emarginata* at two sites at the University of Michigan Biological Station in June 2017: *S. elodes* from ephemeral ponds near Grapevine Point

at Douglas Lake and *S. emarginata* from the eastern shore of Douglas Lake at Pine Point. We preserved 40 individuals of each species in 95% ethanol and deposited them in the Mollusk Division's collections of the University of Michigan Museum of Zoology (UMMZ catalog numbers 305947–305948). We later captured images of these preserved specimens for comparison to captive-reared specimens as described below. We transferred the remaining individuals (~80 for each species) to 30-gallon aquaria at the University of Michigan Ann Arbor campus (one for each species). We maintained individuals of each species in two separate, species-specific tanks (i.e., one tank for *S. elodes* and one for *S. emarginata*).

Captive-rearing

We fed snails tropical fish flake food (*ad libitum*) several days each week. From 2017 to spring of 2018, the tanks were maintained in a room with skylights. In spring of 2018, due to the onset of renovations and construction in the building where the snails were being maintained, we transferred the snails to larger, 50-gallon aquaria in a room (in a different building) without windows and with controlled lighting with a 12-hour light/12-hour dark cycle. We added distilled water to replenish water that had evaporated approximately every two weeks and performed major water changes (i.e., removed most of the water from tanks and replaced it with conditioned tap water) when snails were transferred to new tanks and approximately six months after the transfer.

We maintained populations in tanks at relatively large sizes to promote random mating. While in captivity, *Stagnicola emarginata* individuals reproduced only during the spring of 2018 (before they were transferred to the larger aquarium and different room) and spring 2019 (when egg capsules and juvenile snails appeared in the aquarium). On the other hand, *Stagnicola elodes* produced egg cases within a few months after captive populations were established and throughout subsequent seasons. We did not maintain specific population sizes during the course of the study and except for removal of ~800 individuals from the *S. elodes* tank to perform the predator-exposure experiment (see below) did not otherwise cull individuals from tanks to achieve set densities of individuals. Although we did not perform censuses of tanks, after initial reproductive bouts, population sizes of *S. elodes* tended to be much larger (i.e., possibly hundreds to thousands of individuals at any one time) than those of *S. emarginata* throughout the course of the experiment (which likely maintained population sizes of approximately 200 to 500 individuals).

During the fall of 2018, we removed selected specimens from each tank to capture images of their shells. We chose snails that represented offspring of individuals that were initially collected from wild populations. For each specimen that we removed, we measured its shell length and width (see below), captured images of its shell (see below), marked the apex of the shell with a small spot of nail polish (to keep us from sampling it again), and returned it to its respective tank.

Exposure to predators

During April of 2019, we transferred approximately 800 juveniles (i.e., specimens with a shell length of ~1 to 2 mm) of *Stagnicola elodes* into four 50-gallon aquaria (i.e., ~200 specimens per tank). Two of the tanks held control populations; the other two held experimental populations in which water from crayfish tanks would be added to simulate the presence of potential predators (see below). We paired one of each treatment and control tanks to serve as experimental replicates and placed them at different heights on adjacent shelving units (i.e., the control tank above the experimental tank on one rack and the experimental tank above the control tank on the other). We partially filled each tank with only approximately 50 L of water (to reduce dilution effects when we added water from crayfish aquarium to the experimental tanks—see below).

We maintained four crayfish specimens (*Procambarus clarkii* (Girard, 1852)) in subdivided sections of a 10-gallon aquarium during the course of the experiment; we fed each crayfish three to four pellets of a fish food (Aquatic Arts Sinking Pellets, aquaticarts.com) several days each week (immediately following water transfers as described below). We transferred approximately 500 ml of water from the crayfish tank to each of the two aquaria holding the experimental populations several days each week (average of 3 to 4 days per week). This volume represents just 1% of the total volume of water present in the experimental tanks. We are unaware of the longevity of any chemical cues from crayfish that were transferred to experimental tanks. We replenished water that was removed from the crayfish tank with conditioned tap water. We also added 500 ml of conditioned tap water to the tanks containing control populations. We added distilled water to the crayfish tank to replenish water that had evaporated approximately every second to third week. We continued to perform these water transfers and feedings for approximately five months. In September of 2019, we preserved snails from the experimental and control tanks in 95% ethanol and deposited them in the UMMZ Mollusk Division's collections (catalog numbers 305951–305954).

Morphometric analyses

We captured images of shells with a digital camera placed on a copy stand. We captured multiple images of each shell at separate vertical focal distances of 0.5 mm—from the lower focal distance of the shell apex to the upper focal distance of the vertical peak of the body whorl with a StackShot Controller and Macro Rail (Cognisys Inc., Traverse City, Michigan, USA). We then loaded each series of images into Zerene Stacker v.1.04

(Zerene Systems LLC, Richland, Washington, USA), a program that joins photos of different focal depths to create a single image with a consistent focal depth (i.e., 'focus stacking'), and outputted the composite image for subsequent analyses.

We placed 14 landmarks on the outer edge of the shell in the composite images using tpsDig (Rohlf 2020). The landmarks occurred at the following positions: shell apex, boundaries between whorls, points on body whorl where outline tangent is parallel to coiling axis, boundary between aperture and body whorl, and the point on body whorl where outline tangent is perpendicular to coiling axis (Fig. 1). We chose these 14 landmark positions to represent fixed points that could be easily identified on images of shells of *Stagnicola* species. To better capture shape information of shells, we then placed 10 additional 'semi-landmarks' (at equal distances from each other and adjacent landmarks) between each adjacent landmark along the outline of the shell to capture the shell's curvature. We superimposed coordinates and aligned them with a Procrustes superimposition to remove non-shape information (i.e., scale, position, and orientation of images within the two-dimensional photographic plane) and semi-landmarks were slid to minimize Procrustes distances (Bookstein 1997). We superimposed configurations of landmarks and semilandmarks using the gpgagen function in geomorph, an R (R Core Team 2020) package for geometric morphometrics (Adams *et al.* 2020).

To visualize patterns of variation among shell shapes, we performed a principal component analysis and plotted results using the plotTangentSpace function in geomorph for data from both species (note: the plotTangenetSpace function was recently deprecated in geomorph and has been replaced by the gm.prcomp function). To determine if various factors exhibited an association with shell shape, we performed Procrustes ANOVA to evaluate statistical significance of their effects. For the captive-rearing experiment, we evaluated the effects of species, centroid size (see below), rearing environment (wild-caught versus captive-reared), and the interaction of these factors on shell shape of *Stagnicola elodes* and *Stagnicola emarginata*. For the predator-exposure experiment, we evaluated the effects of centroid size, predator-exposure (control versus experimental populations), and the interaction of these factors on the shape of shells of *S. elodes*; experimental group replicate was used as a random effect for the predator-exposure factor. We performed the tests using 200 permutations of residuals with the procD.lm function in geomorph to evaluate significance (Adams *et al.* 2020).

To determine if populations differ in the magnitude and/or direction of shape change across rearing environments and species, we performed a phenotypic trajectory analysis (Collyer and Adams 2013) using the trajectory.analysis function in the RRPP package (Collyer and Adams 2018). This approach determines the length, shape, and direction of phenotypic change between samples and evaluates if differences between these statistics exceed what is expected by chance through comparison to sampling distributions inferred from random permutations of data (Collyer and Adams 2013, 2018).

We measured shell lengths and widths of shells to the nearest 0.1 mm with Vernier calipers. We confirmed these measures through analyses of captured images of shells in ImageJ v.1.53a (Abràmoff *et al.* 2004) based on scale bar information that was recorded in images. We also calculated centroid sizes (i.e., the square root of the sum of squares of distances between landmark positions and the centroid position; centroid position is the average position of all landmarks), an independent measure of shell size, in geomorph through the gpagen function (Adams *et al.* 2020). To compare shell sizes of individuals in different treatments of the two experiments, we performed an ANOVA on measures of shell length, shell width, and centroid size measures among wild-caught and captive-reared individuals of both species and among control and experimental treatments from the predator-exposure experiment. We employed a Tukey test on comparisons between size metrics of control and experimental treatments from the predator-exposure experiment to determine which comparisons are significantly different. Although the three size measures are likely to be interrelated, we analyzed all three measures in case they are not.

RESULTS

Captive-rearing

We analyzed shell shapes and sizes of 37 individuals of *Stagnicola elodes* (20 wild-caught and 17 captive-reared) and 23 individuals of *Stagnicola emarginata* (13 wild-caught and 10 captive-reared). Results from Procrustes ANOVA show that shell shape is significantly associated with species ($P = 0.005$), centroid size ($P = 0.005$), and rearing condition ($P = 0.005$) as well as the interaction among species and rearing condition ($P = 0.005$) (Table 1). These factors also exhibited the largest effect sizes, with species showing the largest value (Table 1). Plotting of principal component analysis results from both species shows a clear separation of species primarily along the first principal component, with wild-caught and captive-reared individuals of the same species separating along the second principal component (Fig. 2). Trajectory analysis of shell shape of wild-caught and captive-reared specimens indicate that while *S. elodes* and *S. emarginata* did not differ in the magnitude of their shape change across wild-caught and captive-reared individuals ($P = 0.57$), they do differ significantly in the direction of change ($P = 0.005$). Plots outlining shape differences show that shells of captive-reared *S. emarginata* are narrower than shells of wild-caught individuals, while shells of captive-reared *S. elodes* are more globose than shells of wild-caught individuals (Fig. 3).

Table 1. Procrustes ANOVA results for the captive-rearing experiment. Effects from species, centroid size, rearing environment (wild-caught or captive-raised), and the interactions of these factors on shell shape of *Stagnicola elodes* and *Stagnicola emarginata*. df: degrees of freedom; R^2: coefficient of determination; F: F value; Z: effect size. Significant factors in bold.

Factor	df	R^2	F	Z	P-value
Species	1	0.742	210.3	5.65	**0.005**
Size	1	0.025	7.1	3.90	**0.005**
Rearing environment	1	0.013	3.7	2.80	**0.005**
Species x size	1	0.004	1.2	0.48	0.335
Species x rearing environment	1	0.021	6.0	3.46	**0.005**
Size x rearing environment	1	0.007	1.9	1.40	0.095
Species x size x rearing environment	1	0.004	1.0	0.42	0.320
Residuals	52	0.184			

Shells of wild-caught individuals differed significantly in size (in terms of shell length, shell width, and centroid size) from shells of captive-raised individuals for both *Stagnicola elodes* ($P < 10^{-7}$ for all metric comparisons) and *Stagnicola emarginata* (P = 0.045 for length, P = 0.003 for width, P = 0.046 for centroid size) (Fig. 4A–C). For *S. elodes*, average shell length, shell width, and centroid size of captive-reared individuals were 78-85% smaller than those of wild-caught individuals (Fig. 4A–C). On the contrary, average sizes of shells of wild-caught individuals of *S. emarginata* were 84–92% smaller than those of captive-raised ones (Fig. 4A–C).

Exposure to predator cues

We analyzed shell shapes and shell sizes of 45 individuals of *Stagnicola elodes* from the two experimental tanks that were exposed to effluent from crayfish (N = 19 and 26 from each replicate) and 38 individuals of *S. elodes* from the two control tanks (N = 20 and 18). Results from Procrustes ANOVA indicate that there was no large effect size or significant association between shell shape and predator-exposure treatment (Table 2). Centroid size (P = 0.02) and the interaction between centroid size, predator-exposure treatment (i.e., predator-exposed or control) and the random effects of replicate pairs exhibited the largest effect sizes and significant associations with shell shape (P = 0.005) (Table 2).

Results from the ANOVA that compared shell sizes of control and predator-exposed treatments reveal significant differences among samples for the size metrics ($P < 10^{-4}$ for all comparisons). Results from the Tukey test indicate that shell sizes did not differ significantly among replicates of both control and predator-exposed treatments (Fig. 4D-F). Furthermore, while centroid sizes of control and exposed treatment replicates differ significantly, shell

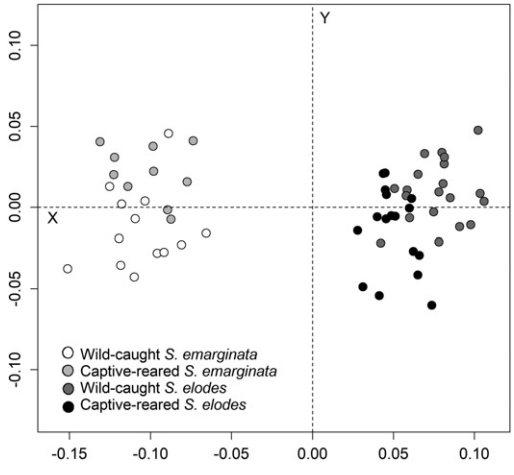

Figure 2. Principal component analysis plot of shell shape of *Stagnicola elodes* and *Stagnicola emarginata* wild-caught and captive-reared specimens (as indicated in legend). Principle component 1 (PC1) and 2 (PC2) account for 78.7% and 7.9% of the variation, respectively.

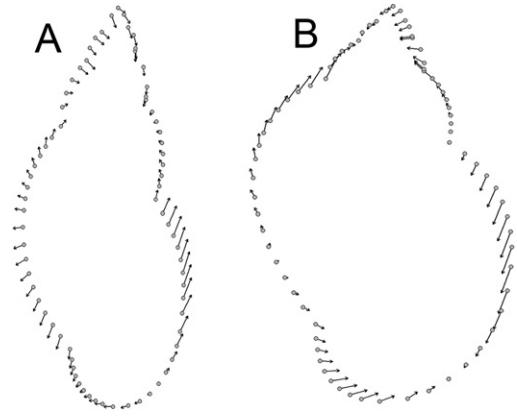

Figure 3. Plots showing average landmark shifts between wild-caught and captive-reared individuals of (A) *Stagnicola elodes* and (B) *Stagnicola emarginata*. Gray dots indicate average landmark positions of wild-caught specimens; arrows demonstrate the shifts in average landmark positions of captive-reared specimens.

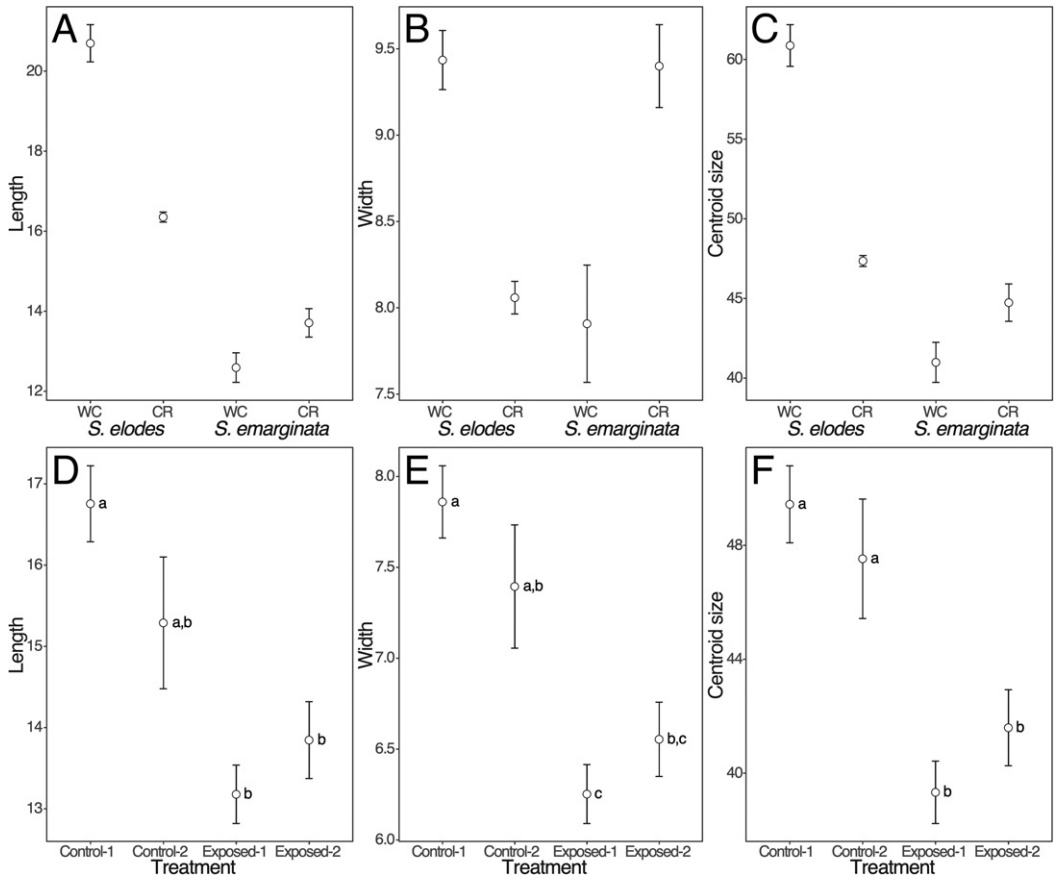

Figure 4. Comparison of shell sizes of wild-caught (WC) and captive-reared (CR) populations of *Stagnicola elodes* and *Stagnicola emarginata* (A-C) and replicate populations of control and predator-exposed treatments of *S. elodes* from the predator-exposure experiment (D-F). Empty circles indicate means; vertical lines above and below circles indicate standard errors. Lower case letters are positioned next to circles in D-F to indicate which samples are significantly different from each other based on results from a Tukey test (i.e., those with the same letter are not significantly different from each other).

length and width measures did not differ significantly among all pairwise comparisons of control and exposure treatment replicates (Fig. 4D-F). On average, shell size of specimens from experimental tanks were on average 83% smaller than those of specimens from control tanks for all three size measures.

DISCUSSION

Results from our first experiment demonstrate that captive-reared individuals of both *Stagnicola elodes* and *Stagnicola emarginata* differ significantly in shell shape to wild-caught individuals of these species from which the captive-reared populations were founded (Table 1, Fig. 2). Furthermore, although exposure to cues from crayfish does not influence shell shapes of *S. elodes*, individuals that were exposed to effluent from crayfish appear to have lower growth rates than control snails given differences in shell sizes among snails from different treatments (Fig. 4).

Shell shape changes in captive-reared stagnicolines

Individuals of *Stagnicola elodes* and *Stagnicola emarginata* that were reared in captivity differ significantly in shell shape from those from natural populations (Table 1, Fig. 2). While the magnitude of change between captive-reared and wild-caught individuals did not differ significantly among the two species, the direction of change did. These results suggest that shell morphologies of the two species exhibit similar levels of plasticity but different responses to rearing in captivity. Moreover, while shells of *S. elodes* from captive-reared populations are generally more globose than wild-caught

Table 2. Procrustes ANOVA results for the predator-exposure experiment. Effects from centroid size, predator exposure (i.e., exposure to predator cues or control), and the interactions of these factors on shell shape of *Stagnicola elodes*; experimental group replicate was used as a random effect for the predator exposure factor. df: degrees of freedom; R^2: coefficient of determination; F: F value; Z: effect size. Significant factors in bold.

Factor	df	R^2	F	Z	P-value
Size	1	0.156	15.8	4.74	**0.020**
Predator-exposure x replicate pair	2	0.036	0.6	-0.72	0.395
Size x predator-exposure x replicate pair	2	0.061	3.1	2.43	**0.005**
Residuals	81	0.124			

snails, mainly at the body whorl and aperture, shells of captive-reared *S. emarginata* are more slender, predominantly at the aperture, than wild-caught snails (see Fig. 3). Nonetheless, the two species remained morphologically distinct when reared in captivity (see Figs. 2–3). These latter results concur with earlier observations by Burch and Ayers (1973) and demonstrate that although shell shape is influenced by environmental conditions, the distinct shell shapes of *S. elodes* and *S. emarginata* are not solely an expression of ecophenotypic variation.

The observed significant association between centroid size and shape (Table 1) suggest that shells of wild-caught and captive-reared snails differ in both shape and size. For *Stagnicola elodes*, shell sizes of captive-raised snails were nearly non-overlapping with and smaller than those of wild-caught snails (Fig. 4A–C); shell shapes of captive-raised adult snails were also more globose than those of wild-caught one (Fig. 3). On the contrary, for *Stagnicola emarginata*, shells of captive-raised adult snails were larger than those of wild-caught ones (Fig. 4A–C); shell shapes of captive-raised *S. emarginata*, however, were narrower than those of wild-caught ones (Fig. 3). Given these observations, shells of both species appear to get narrower as they get larger in size.

Impact of exposure to predators on shell morphology

The factor that is most associated with phenotypic plasticity in aquatic snails is exposure to predators (Bourdeau *et al.* 2015). Responses to cues from predators typically include alterations to shell morphology that may serve to reduce predation and so provide an adaptive benefit (Bourdeau *et al.* 2015). Nonetheless, while some potential predators elicit such changes in shell morphology, others do not. For example, while shell morphology of *Radix balthica* changes when individuals are exposed to mollusc-ivorous fish (Lakowitz *et al.* 2008, Brönmark *et al.* 2011), they elicit no response when exposed to crayfish (Lakowitz *et al.* 2008). Although results for *Stagnicola elodes* are similar to those observed for *R. balthica* (in terms of the lack of change in shell shape when exposed to predators) (Table 2), given the significant differences in sizes detected for control and experimental populations (Fig. 4), individuals of *S. elodes* that are exposed to crayfish effluent exhibit slower growth rates than individuals in control conditions. Because only small volumes of water from crayfish tanks (i.e., 500 ml) were added to experimental tanks (with approximately 50 l of water), this effect is particularly striking and suggests that chemical cues from potential predators have a large influence on growth rates.

Exposure to cues from predators affects growth rates of other aquatic snail species, with increases in growth rates shown by some and decreases shown for others. For example, two physid species (*Physella acuta* and *Physella virgata* (Gould, 1855)), exhibit increased growth rates when exposed to predator cues, a strategy that presumably reduces the risk of predation by enabling individuals to more rapidly attain sizes that decrease susceptibility to predators (Crowl and Covitch 1990, Auld and Relyea 2010). On the other hand, *Physella* (= *Physa*) *heterostropha* (Say, 1817) exhibits lower growth rates when exposed to predators, a response that appears to be due to reduced access to food (i.e., snails tend to crawl out of the water and stop feeding when exposed to predators) (DeWitt 1998). Declines in growth rates may be elicited from stress responses or predation avoidance behaviors that affect energy budgets via increased energy use or fewer opportunities for acquiring resources, as implied by DeWitt (1998) for *P. heterostropha*. Although other lymnaeids are known to alter their behaviors when exposed to predators by emerging from the water (e.g., *Lymnaea stagnalis* (Linnaeus, 1758), as reported by Dalesman *et al.* 2006), we did not observe any noticeable differences in behaviors of *Stagnicola elodes* in control and experimental populations. Additional studies of growth rates of natural or experimental populations of *S. elodes* that experience different predator communities would be useful to further address this theme.

Results from analyses of shell shapes of *S. elodes* in the predator-exposure experiment show that centroid size is significantly associated with shape (Table 2). This implies, as do results from the captive-rearing experiment, that shell shape of *S. elodes* is allometric and changes during development. Our results, however, did not indicate a significant association between predator-exposure treatment (i.e., predator-exposed or control) and shell shape (Table 2). Instead, the interaction between size,

treatment, and the random effects of replicate pairs exhibited a significant association with shell shape (Table 2). Hence, while exposure to predators alone does not appear to contribute to shape differences, it influences the association between size and shape. This interaction suggests that predator-exposure effects are complex and could be affected by tank environment. Furthermore, failure to detect significant differences in shell shapes of control and predator-exposed treatments may have occurred because there was too much overlap in sizes of shells in the different treatments or because of relatively small sample sizes.

CONCLUSIONS

Our results suggest that shell shapes of *Stagnicola elodes* and *Stagnicola emarginata* are influenced by environmental conditions. Hence, the morphological variation exhibited by stagnicoline species in North America could reflect ecophenotypic variation in some cases. Clearly much more work that determines "which characters are ecophenotypic and which are genetic" (Burch 1982) is needed to address this concern. In addition, we found that while exposure to cues from crayfish does not influence the shell shape of *S. elodes*, growth rates are reduced in exposed snails. Future studies should seek to determine why and how growth rates are influenced by the presence of predators and the impacts of these changes on the fitness of individuals in natural populations.

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