Large Differences over Small Distances: Plasticity in the Shells of *Elimia potosiensis* (Gastropoda: Pleuroceridae)

Russell L. Minton (Corresponding author)

Department of Biology, University of Louisiana at Monroe

700 University Avenue, Monroe, Louisiana 71209-0520, USA

Tel: 1-318-342-1795   E-mail: minton@ulm.edu

Elise M. Lewis & Bryan Netherland

Department of Biology, University of Louisiana at Monroe

700 University Avenue, Monroe, Louisiana 71209-0520, USA

David M. Hayes

Department of Biological Sciences, Eastern Kentucky University

521 Lancaster Avenue, Richmond, Kentucky 40475, USA

Abstract

Phenotypic plasticity in freshwater mollusks is a well-known phenomenon, occurring in both bivalves and gastropods. Most work on freshwater snail plasticity has focused either on presence/absence of a single factor, or has looked at responses to environmental gradients over large geographical scales. Using the pleurocerid snail *Elimia potosiensis* from a spring and creek in Arkansas, we show a plastic environmental response at a scale thousands of times smaller than was previously known. Shells of *E. potosiensis* exhibit plasticity consist with that seen in other pleurocerids over the course of hundreds of meters. Possible explanations for this small-scale plasticity are offered.

Keywords: Phenotypic plasticity, Freshwater, Snails, Geometric morphometrics, Pleurocerids

1. Introduction

Phenotypic plasticity in freshwater mollusk shells is a well-documented phenomenon, occurring in both bivalves (Utterback, 1916; Watters, 1994) and gastropods (Adams, 1900; DeWitt, 1998; Minton et al., 2008). In both groups, shell shape and sculpture tend to exhibit a unidirectional transition from thinner, less inflated, smoother shells in shallow headwater reaches to more inflated, thicker, sculptured shells in deeper main stem areas (Wilson and Clark, 1914; Dussart, 1987). This phenotypic gradient in single river systems is anecdotally known as Ortmann's law of stream position (Ortmann, 1920), though similar findings were reported decades earlier (e.g. Sell, 1908; Buchner, 1910; Haas and Schwarz, 1913). Historically, many species have been described based on this now well-documented pattern of variation in shell form. For example, variations in sculpture led authors to describe 13 species of *Io*, all now synonymized as the monotypic *I. fluvialis* (Tryon, 1873; Graf, 2001). Similar over-description was seen in the unionid bivalve *Fusconaia flava* in the upper Midwestern United States (Graf, 1998). Adams (1900; 1915) was the first to suggest Ortmann's patterns in freshwater snails with his work on *Io*. In freshwater snails, these changes are often accompanied by a change in spire height and aperture size; high, narrow spires and small apertures may reduce predation in headwaters (Krist, 2002), while low spires and large apertures may reduce dislodging and damage during tumbling (Haase, 2003). Minton et al. (2008) were the first to quantify this variation in freshwater snails using geometric morphometrics to show both the degree of variation and rate of change (shape difference per river kilometer) in the pleurocerid *Lithasia geniculata* from the Duck River, Tennessee, USA.

Ortmann and others' pattern of shell plasticity has been attributed to individual mollusks responding to an environmental gradient from upstream to downstream (Ortmann, 1920), similar to that described by the river continuum concept (RCC; Vannote et al., 1980). Within the RCC framework, rivers evolve in a unidirectional fashion such that river mileage can be used as a surrogate to coarsely represent environmental conditions along

the gradient. While the applicability of RCC to all lotic systems has been debated, it provides a simple and reasonable context within which to explore the range of phenotypic plasticity exhibited by freshwater mussels and snails.

In the aforementioned and other studies dealing with plasticity in freshwater mollusks, most research has focused on two scales: binary presence/absence studies of a given factor (e.g. predator [DeWitt, 1998]; parasite [Krist, 2000]); or change occurring in large rivers covering hundreds of river kilometers (Adams, 1915; Graf, 1998; Minton et al., 2008). For snails, no studies have examined Ortmann's law in small streams. Our objectives were to examine to what degree shell shape changed, if at all, in these short reaches of aquatic habitat, and to see if the rates of shape change as measured over water distance in these two reaches were similar. We also were interested in comparing any shape changes to those seen previously in large rivers.

For this research we focused on two populations of the pleurocerid *Elimia potosiensis* in Arkansas. *Elimia potosiensis* (Lea, 1841) is a morphologically diverse and widespread species endemic to the Interior Highlands (Gordon, 1980; 1982) of Arkansas and Missouri (Figure 1). It is the only member of the genus occurring in western drainages of the Mississippi River, with western limits in Oklahoma and Kansas and possibly south to Louisiana (Burch and Tottenham, 1980; Graf, 2001). The species shows variation in shell shape and sculpture, as three subspecies of *E. potosiensis* (*crandalli*, *ozarkensis*, and *potosiensis*) have been described as narrow-range ecophenotypes (Goodrich, 1939; Jones and Branson, 1964) across its range, while a fourth, *E. p. plebius*, is the most widespread.

We followed Minton et al (2008) in assessing these phenotypic changes using geometric morphometrics (GM). Shell variation is traditionally quantified through straight-line shell measurements and ratios to distinguish between individuals and populations at the species level (Heller et al., 2005; Tanaka and Maia, 2006). Recently, GM have been employed in examinations of snail shells, both to provide direct size-free analyses of shell shape and to answer broader evolutionary questions (Pfenninger and Magnin, 2001; Hollander et al., 2006; Hayes et al., 2007). GM have the advantage over traditional point-to-point measurements or ratios of such measurements (Smith and Patton, 1988) in being free of effects due to size, position, rotation, and scale (Rohlf and Marcus, 1993). GM also captures the geometric relationships among physical features in ways that traditional morphometrics (Reyment, 1991) do not.

In order to further our knowledge regarding possible phenotypic gradients in freshwater snails, we asked three questions regarding morphological variation in *E. potosiensis*. First, does the species exhibit the same type of phenotypic change as seen in other pleurocerids (*Lithasia* [Minton et al., 2008] and *Io* [Adams, 1915]) in smaller aquatic systems? Second, do shells in the spring and the tributary change at the same rate, measured as shape change per unit river distance across their separate systems? Finally, if change in *E. potosiensis* is present, how does it compare with that seen in larger river examples?

2. Materials and Methods

Analyses used 500 individual *E. potosiensis* shells from both an unnamed spring and unnamed tributary to Walnut Creek, west of Crystal Springs, Arkansas (Figure 2). The unnamed spring emerges from a poorly delineated head and flows 150 m into the unnamed tributary. The tributary emerges at a poorly defined head, is roughly 300 m long and drains into Walnut Creek. Shells used included 175 from the spring run and the remainder from the tributary. Individuals were collected at 25 m intervals along each system; the spring was sampled (seven total) from the head until it joined the tributary, while the tributary was sampled (13 samples) from its head until it drained into Walnut Creek. The largest 25 snails were collected to reduce potential shape changes due to allometry, and all shells had intact apices.

Shells were digitally photographed using a Nikon CoolPix 4500 and dissecting microscope. Care was taken to align the plane of the aperture parallel to the work surface. Images were brought into MakeFan6 (Sheets, 2003) where they were overlaid with a series of 30 evenly spaced lines. This 'comb' was anchored at two points, one at the apex of the shell and the other at the anterior-most point of the aperture. Landmarks were digitized in tpsDig2 (Rohlf, 2008) by placing one each at the two anchor points, as well as at the points where the 'comb' lines crossed the periphery of the shell, providing an outline of the shell based on 58 points (Figure 3). This method has been used successfully in capturing curve shape in a variety of applications (e.g. Del Papa and Perez, 2007; López-Sauceda and Aragón, 2008). Shells from the spring and tributary were analyzed separately. Landmarks were subjected to a standard generalized Procrustes alignment to remove differences between specimens based on scale, rotation, and location. The alignment procedure iteratively estimated a mean form and aligned all specimens on it. Once aligned, the landmarks were treated as points in the statistical analyses carried out in linear tangent space to the underlying curved shape space.

Shell shape correlation to an upstream-downstream gradient in accordance with RCC as well as effects due to allometry and water chemistry were analyzed using tpsRegr (Rohlf, 2005) and MorphoJ (Klingenberg, 2008). Potential allometric effects were tested for by regressing shape against centroid size in each system. Changes along the spring and tributary were each assessed separately by regressing shell shape against distance from the system's mouth. For the spring, where it emptied into the tributary was used; for the tributary, the mouth was where it emptied into Walnut Creek. Statistical significance was measured using the Wilks' lambda test at a significance level of P=0.05. To compare the rates of shell shape change between the spring and tributary, a MANCOVA was performed in tpsRegr to compare the slopes of the two regression equations for the two systems. Differences in the slopes would indicate that shells in each location changed differently over the same linear distance. We additionally regressed Procrustes distance between samples with physical distance between samples. We predicted that shells from one sample would be more similar to shells from adjacent samples than they would be to shells taken from samples farther away. Combined with the regression of shape versus distance, we predicted this data would support that any shape changes were occurring in a unidirectional fashion. Finally, we regressed shell shape against four water chemistry variables (pH, conductivity [μS], temperature [°C], and dissolved oxygen [mg/L]) to determine their effects on overall shell phenotype (Figure 4). Measurements were not taken at the springhead due to the shallowness of the water and intrusion of vegetation.

3. Results

Regression of shell shape to centroid size indicated that tributary shells showed significant allometry (F=2.464, df=112, 207, P<0.01), while spring shells did not (P=0.056). Although significant, allometry accounted for less than 1% of the total variation seen in tributary shells. Regression of shell shape to distance from the system mouth showed a significant relationship in both the spring (F=1.837, df=112, 62, P<0.01; Figure 5) and the tributary (F=3.135, df=112, 207, P<0.01; Figure 6). Shells further from the mouth upstream were longer (apex to anterior aperture) and thinner than shells taken from downstream. Position within the system accounted for 13.1% and 2.2% of the total shell shape variation in the spring and tributary respectively. The rates of shape change in the two systems differed significantly (F=3.1117, df=112, 380, P<0.01), with spring shells changing more over the same distance than tributary shells. Regression of Procrustes distances between samples to physical distance between samples showed significant correlation in both the spring and tributary snails (figure 7), suggesting a continuous and sequential shift in morphology from upstream to downstream. Spring snails changed shape at a faster rate as indicated by the steeper slope. Spring snail shape was significantly correlated with pH (F=3.449, df=112, 62, P<0.01) and temperature (F=2.730, df=112, 62, P<0.01), but not conductivity (P=0.413) nor dissolved oxygen (P=0.111). Tributary snail shape, however, was significantly correlated with conductivity (F=2.081, df=112, 207, P<0.01), temperature (F=2.795, df=112, 207,P<0.01), and dissolved oxygen (F=1.584, df=112, 207, P<0.01), but not with pH (P=0.401).

4. Discussion

Phenotypic plasticity as a result of ecological and environmental interactions is ubiquitous in nature (Fordyce, 2006). In freshwater organisms, plasticity gradients in response to the environment have been shown across unrelated taxa (mollusks, references herein; fish, Langerhans, 2008; crayfish, Mead, 2008; turtles, Rivera, 2008). *Elimia potosiensis* is the latest freshwater mollusk to exhibit variation consistent with Ortmann's "law," joining the handful of pleurocerid snails where this pattern has been quantified (Adams, 1915; Minton et al., 2007; 2008). Shells of *E. potosiensis* were narrower and longer in upstream areas, while downstream shells were shorter and wider. This variation was readily apparent in shells taken from the spring run, while those from the tributary exhibited the same variation at a reduced level. The pattern of plasticity observed in *E. potosiensis* was significantly correlated to river position in a manner consist with the expectations of the RCC, much like that seen previously in *Lithasia*. Shell shape in *E. potosiensis* was also significantly correlated with water chemistry parameters in a manner seen in other species (Dillon, 1980).

Until now, the phenotypic gradients seen in freshwater mollusks have been studied on the order of hundreds of river miles. Our results indicated that the same gradients occur on a much finer scale, and are detectable over extremely small distances. Both *E. potosiensis* and *L. geniculata* (Minton et al., 2008) show similar phenotypic plasticity, in that upstream shells tend to be narrower and longer than downstream shells, and in how much plasticity is accounted for by river position (~13% in *E. potosiensis*, ~14% in *Lithasia*). However, *E. potosiensis* exhibit this change, both visibly and statistically, on a scale 4000x smaller (~100 m vs. ~400 river km) than *L. geniculata*. We find it interesting that so much change can occur over such a small distance, and that the environment of short aquatic runs can produce phenotypic effects of this degree. The significant differences seen in the amount of change between the spring and tributary accentuate the fact that plasticity needs to be examined not only across large drainages, but also in the smaller components of lotic systems. Our data also suggest that

the general physical patterns predicted by RCC may be present in small systems as well. However, the difference in significant correlations between water chemistry measures and shell shape between the spring and tributary highlights the need to better understand microhabitat structure within and throughout the systems.

Minton et al. (2008) suggested the relationship between shell form and river position was indicative of a single genetic lineage exhibiting phenotypic responses to an environmental gradient, rather than random chance or strong local pressure producing a string of discontinuous genotypes each with their own form. We concur that random chance is unlikely, given the strong relationship seen herein with *Elimia*, combined with that seen in other pleurocerids, freshwater snails and freshwater mussels. However, we have yet to discount the prospect of locally adapted genotypes producing an apparent phenotypic progression. Populations of freshwater snail species often show significant genetic structuring (e.g. Carini and Hughes, 2006; Liu and Hershler, 2009), though this concept is currently unknown in *E. potosiensis*. There is also evidence of size sorting in *E. potosiensis* in single rivers (Gore, 1983). For now, then, we presume that the populations of *E. potosiensis* studied represent a single genetic entity responding to its environment in the upstream to downstream manner described by Ortmann and others. Exploration of this issue using microsatellites or other population markers combined with manipulation of environmental factors is planned for the future.

Shell plasticity occurs across freshwater snails in many different forms, including sexual dimorphism, lotic versus lentic environments, and response to environmental cues (Langerhans and DeWitt, 2002; Holomuzki and Biggs, 2006; Jakubic, 2006). This study adds a new level of scale to the detectable limits of this plasticity and shows it potential effects on examinations of shell morphology within a species. Future studies of shell plasticity, especially those looking at continual responses across ecological and environmental gradients should take care to examine patterns at all possible geographical scales so as to better understand the nature of phenotypic variation in freshwater snails.

Acknowledgements

References

Adams, C.C. (1900). Variation in *Io*. *Proceedings of the American Association for the Advancement of Science*, 49, 208-225.

Adams, C.C. (1915). The variations and ecological distribution of the snails of the genus *Io*. *Memoirs of the National Academy of Science*, 12, 1-92.

Buchner, O. (1910). Ueber individuel Formverschiedenhieten bei Anodonten. Jahreshelfte des Vereins für Väterlandischer naturkunde in Wätemburg. *Fünfundsechziegester Jahrgang*, 56, 60-223.

Burch, J.B., & Tottenham, J.L. (1980). North American freshwater snails: species list, ranges, and illustrations. *Walkerana*, 1, 81-215.

Carini, G. & Hughes, J.M. (2006). Subdivided population structure and phylogeography of an endangered freshwater snail, *Notopala sublineata* (Conrad, 1850) (Gastropoda: Viviparidae), in Western Queensland, Australia. *Biological Journal of the Linnean Society*, 88, 1-16.

Del Papa, M.C., & Perez, S.I. (2007). The influence of artificial cranial vault deformation on the expression of cranial nonmetric traits: its importance in the study of evolutionary relationships. *American Journal of Physical Anthropology*, 134, 251-262.

DeWitt, T.J. (1998). Costs and limits of phenotypic plasticity: tests with predator-induced morphology and life history in a freshwater snail. *Journal of Evolutionary Biology*, 11, 465-480.

Dussart, G.B.J. (1987). Effects of water flow on the detachment of some aquatic pulmonate gastropods. *American Malacological Bulletin*, 5, 65-72.

Fordyce, J.A. (2006). The evolutionary consequences of ecological interactions mediated through phenotypic plasticity. *Journal of Experimental Biology*, 209, 2377-2383.

Goodrich, C. (1939). Pleuroceridae of the Mississippi River basin exclusive of the Ohio River. *Occasional Papers of the Museum of Zoology, University of Michigan*, 406, 1-4.

Gordon, M.E. (1980). Recent Mollusca of Arkansas with annotations to systematics and zoogeography. *Proceedings of the Arkansas Academy of Science*, 34, 58-62.

Gordon, M.E. (1982). Mollusca of the White River, Arkansas and Missouri. *Southwestern Naturalist*, 27, 347-352.

Graf, D.L. (1998). Freshwater pearly mussels: pigtoes and Ortmann's law. *American Conchologist*, 26, 20-21.

Graf, D.L. (2001). The cleansing of the Augean stables, or a lexicon of the nominal species of the Pleuroceridae (Gastropoda: Prosobranchia) of recent North America, north of Mexico. *Walkerana*, 12, 1-124.

Haas, F. & Schwarz, E. (1913). Die Unioniden des Gebietes zwischen Mainz und deutschen Donau in tiergeographischer Hinsicht. *Abhandlugen der Mathematisch-Physikalischen Classe der Königlich Bayerischen Akademie der Wissenschaften*, 26, 1-34.

Haase, M. (2003). Clinal variation in shell morphology of the freshwater gastropod *Pomatopyrgus antipodarum* along two hill-country streams in New Zealand. *Journal of the Royal Society of New Zealand*, 33, 549-560.

Hayes, D.M., Minton, R.L., & Perez, K.E. (2007). *Elimia comalensis* (Gastropda: Pleuroceridae) from the Edwards Plateau, Texas: multiple unrecognized endemics or native exotic? *American Midland Naturalist*, 158, 97-112.

Heller, J., Mordan, P., Ben-Ami, F., & Sivan, N. (2005). Conchometrics, systematics and distribution of *Melanopsis* (Mollusca: Gastropoda) in the Levant. *Zoological Journal of the Linnean Society*, 144, 229-260.

Hollander, J., Adams, D.C., & Johannesson, K. (2006). Evolution of adaptation through allometric shifts in a marine snail. *Evolution*, 60, 2490-2497.

Holomuzki, J.R. & Biggs, B.J.F. (2006). Habitat-specific variation and performance trade-offs in shell armature of New Zealand mudsnails. *Ecology*, 87, 1038-1047.

Jakubic, B. (2006). Reproductive pattern of *Viviparus viviparus* (Linnaeus, 1758) (Gastropoda: Viviparidae) from littoral aggregations in a through-flow reservoir (central Poland). *Polish Journal of Ecology*, 54, 39-55.

Jones, W.C., & Branson, B.A. (1964). The radula, genital system, and external morphology in *Mudalia potosiensis* (Lea, 1841) (Gastropoda: Prosobranchiata: Pleuroceridae) with life history notes. *Transactions of the American Microscopical Society*, 83, 41-62.

Klingenberg, C.P. (2008). MorphoJ [Online]. Faculty of Life Sciences, University of Manchester, UK. Available: http://www.flywings.org.uk/MorphoJ_page.htm.

Krist, A.C. (2000). Effects of the digenean parasite *Proterometra macrostoma* on host morphology in the freshwater snail *Elimia livescens*. *Journal of Parasitology*, 86, 262-267

Krist, A.C. (2002). Crayfish induce a defensive shell shape in a freshwater snail. *Invertebrate Zoology*, 121, 235-242.

Langerhans, R.B. (2008). Predictability of phenotypic differentiation across flow regimes in fishes. *Integrative and Comparative Biology*, 48, 750-768.

Langerhans, R.B. & DeWitt, T.J. (2002). Plasticity constrained: over-generalized induction cues cause maladaptive phenotypes. *Evolutionary Ecology Research*, 4, 857-870.

Liu, H.-P. & Hershler, R. (2009). Genetic diversity and population structure of the threatened Bliss Rapids snail (*Taylorconcha serpenticola*). *Freshwater Biology*, 54, 1285-1299.

López-Sauceda, J., & Aragón, J.L. (2008). Eutacticity in sea urchin evolution. *Bulletin of Mathematical Biology*, 70, 625-634.

Mead, K.S. (2008). Do antennule and aesthetasc structure in the crayfish *Orconectes virilis* correlate with flow habitat? *Integrative and Comparative Biology*, 48, 823-833.

Minton, R.L., Reese, S.A., Swanger, K., Perez, K.E., & Hayes, D.M. (2007). Changes in shell morphology of *Elimia comalensis* (Gastropoda: Pleuroceridae) from the Edwards Plateau, Texas. *Southwestern Naturalist*, 52, 475-481.

Minton, R.L., Norwood, A.P., & Hayes, D.M. (2008). Quantifying phenotypic gradients in freshwater snails: a case study in *Lithasia* (Gastropoda: Pleuroceridae). *Hydrobiologia*, 605, 173-182.

Ortmann, A.E. (1920). Correlation of shape and station in freshwater mussels (naiades). *Proceedings of the American Philosophical Society*, 59, 269-312.

Pfenninger, M., & Magnin, F. (2001). Phenotypic evolution and hidden speciation in *Candidula unifasciata* ssp. (Helicellinae, Gastropoda) inferred by 16S variation and quantitative shell traits. *Molecular Ecology*, 10,

2541-2554.

Reyment, R. (1991). *Multidimensional Paleobiology*. New York: Pergamon Press.

Rivera, G. (2008). Ecomorphological variation in shell shape of the freshwater turtle *Pseudemys concinna* inhabiting different aquatic flow regimes. *Integrative and Comparative Biology*, 48, 769-787.

Rohlf, F.J. (2005-2008). tpsDig2, tpsRegr [Online]. Available: http://www.life.bio.sunysb.edu/morph.

Rohlf, F.J., & Marcus, L.F. (1993). A revolution in morphometrics. *Trends in Ecology and Evolution*, 8, 129-132.

Sell, H. (1908). Biologische Beobachtungen an Najades. *Archiv für Hydrobiologie und Planktonkunde*, 1908, 179-188.

Sheets, H.D. (2003). MakeFan6 [Online]. Available: http://www3.canisius.edu/~sheets.

Smith, M.F., & Patton, J.L. (1988). Subspecies of pocket gophers: causal bases for geographic differentiation in *Thomomys bottae*. *Systematic Zoology*, 37, 163-178.

Tanaka, M.O., & Maia, R.C. (2006). Shell morphological variation of *Littorina angulifera* among and within mangroves in NE Brazil. *Hydrobiologia*, 599, 193-202.

Tryon, G.W. (1873). Land and freshwater shells of North America. Part IV. Strepomatidae. *Smithsonian Miscellaneous Collections*, 253, 1-435.

Utterback, W.I. (1916). The Naiades of Missouri. Reprinted from *American Midland Naturalist*.

Vannote, R.L., Minshall, G.W., Cummins, K.W., Sedell, J.R., & Cushing, C.E. (1980). The river continuum concept. *Canadian Journal of Fisheries and Aquatic Sciences*, 37, 130-137.

Watters, G.T. (1994). Form and function of unionoidean shell sculpture and shape (Bivalvia). *American Malacological Bulletin*, 11, 1-20.

Wilson, C.B., & Clark, H.W. (1914). The mussels of the Cumberland River and its tributaries. *United States Bureau of Fisheries Documents*, 781, 1-63.
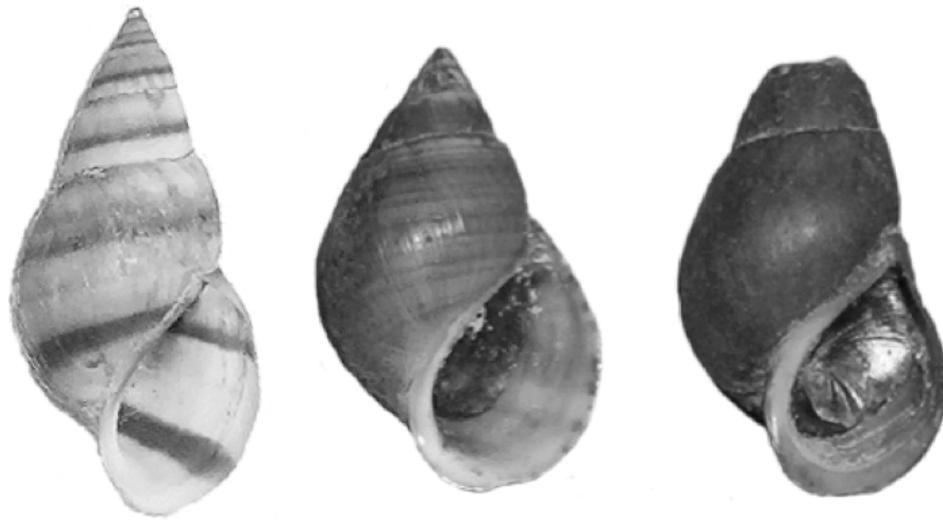
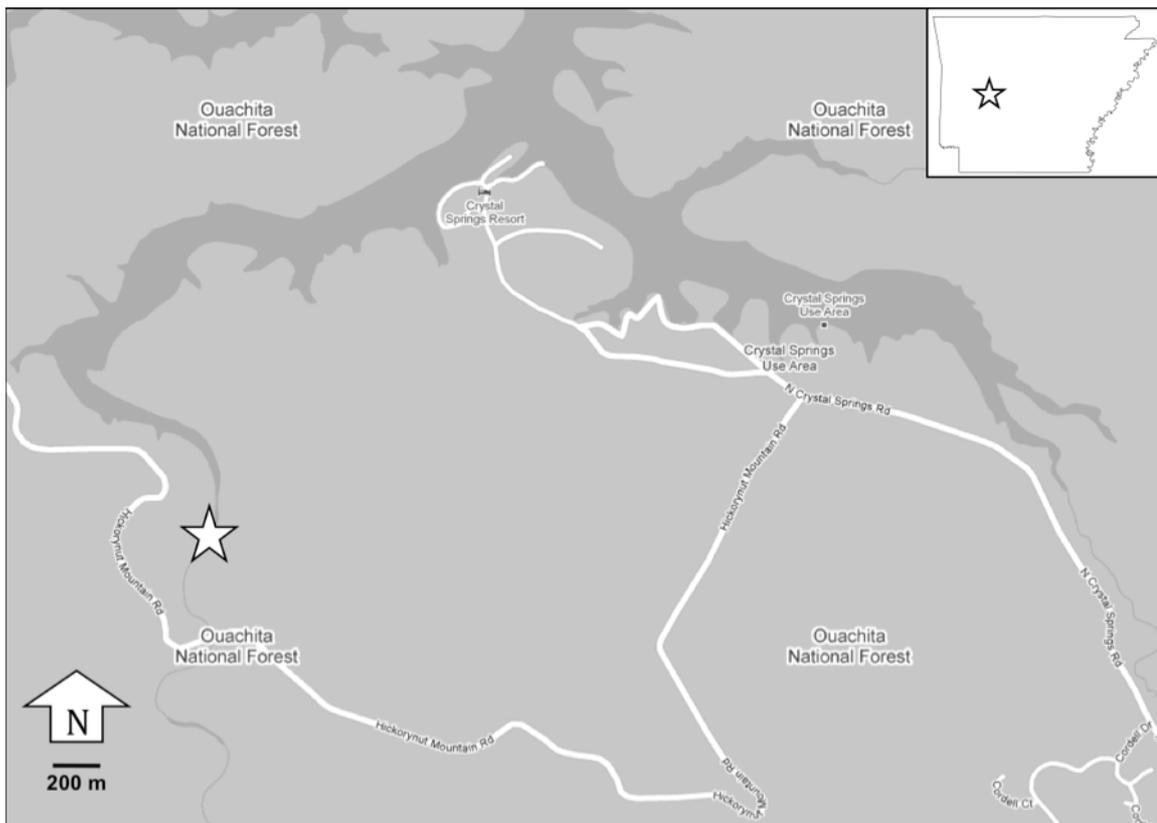Figure 1. Morphological variation in *Elimia potosiensis* from Arkansas

Figure 2. Stars indicate the location of the collection site in Arkansas (inset) and relative to Crystal Springs, AR
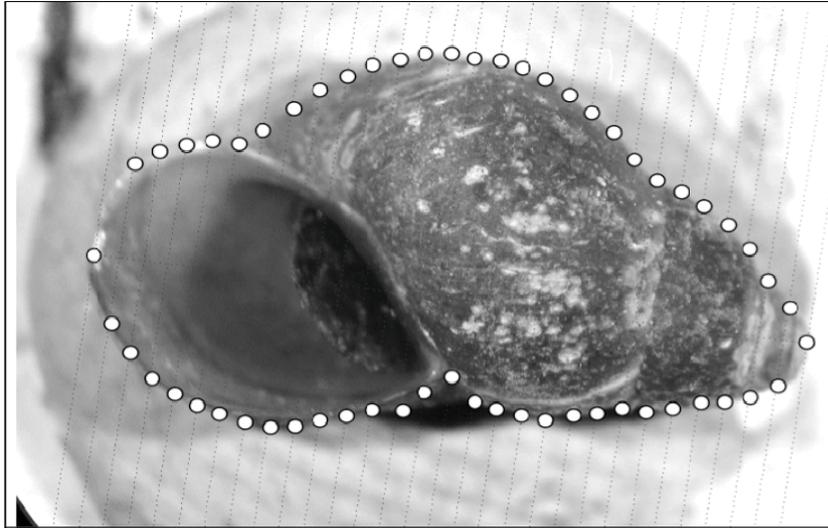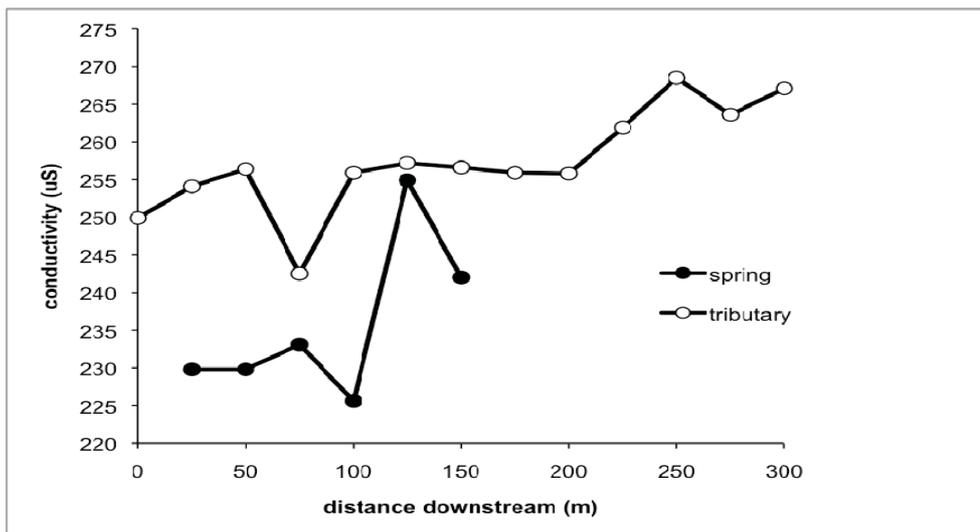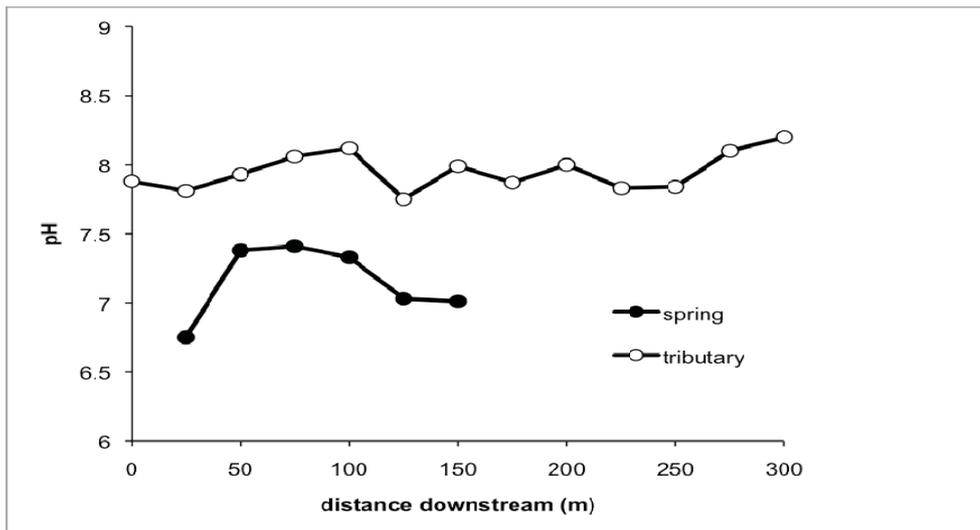
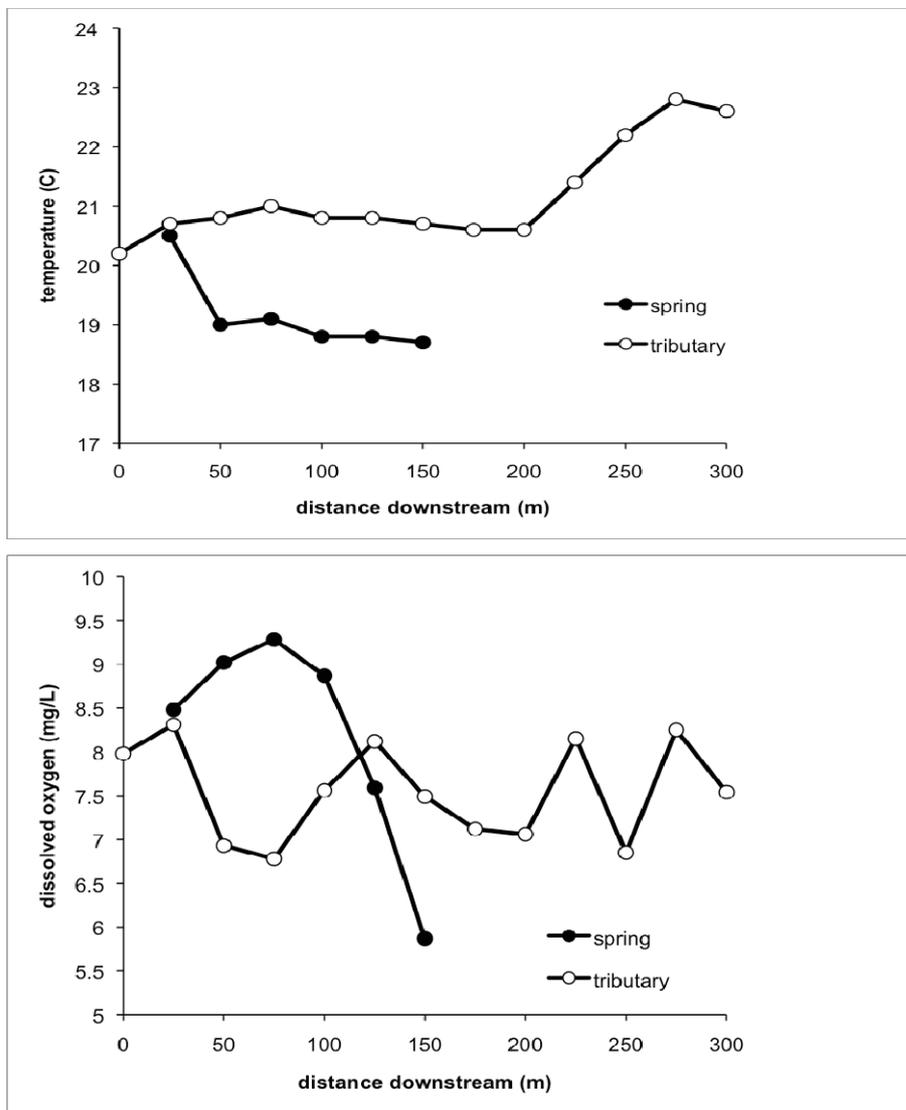Figure 3. Shell of *Elimia potosiensis* overlaid with landmark comb

Figure 4. Environmental variables for the spring and tributary

Figure 5. Mean outlines of spring shells taken at 0 m (spring head; grey line with open circles) and at 125 m (confluence with tributary; black line with closed circles) aligned by centroid

Figure 6. Mean outlines of tributary shells taken at 0 m (tributary head; grey line with open circles) and at 300 m (confluence with Walnut Creek; black line with closed circles) aligned by centroid
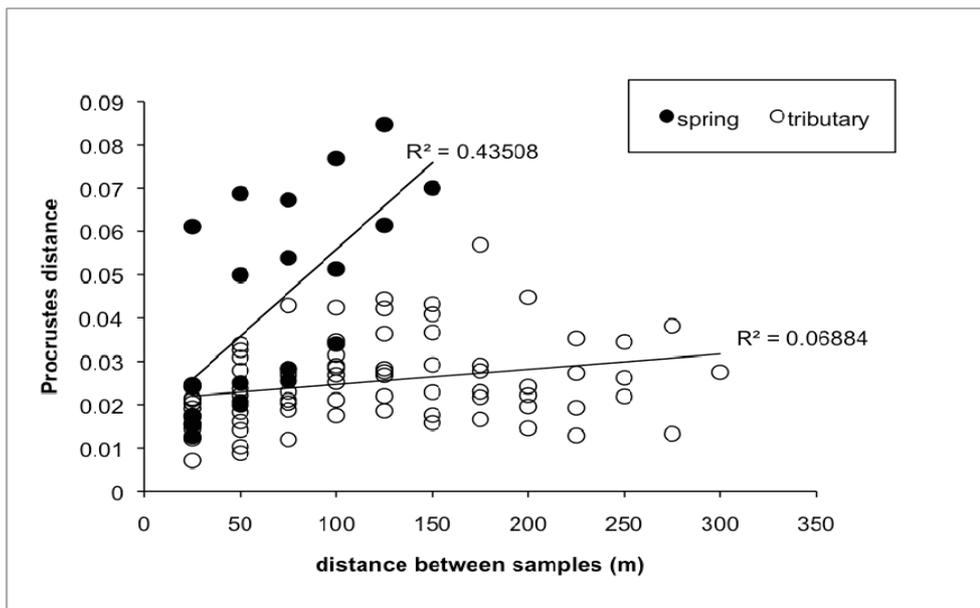

Figure 7. Graph of Procrustes distance versus geographic distance for spring and tributary samples. Snails from sites near one another tended to have more similar shapes than those from sites farther away, suggesting a sequential shift in morphology. Both trends were significant (P<0.01), though spring snails changed at a faster rate (difference per distance) than tributary snails