Life-history traits and shell morphology in the genus *Leptoxis* Rafinesque, 1819 (Gastropoda: Cerithioidea: Pleuroceridae)

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ABSTRACT

Life histories of the imperilled freshwater gastropod genus *Leptoxis* are poorly known, and this information is required to achieve a basic understanding of the evolution of this diverse group and to develop management strategies for species conservation and recovery. We describe egg-laying behaviours and associated traits for all 13 extant *Leptoxis* species. We also explore patterns of shell growth and assess the extent to which intraspecific shell variation is a result of phenotypic plasticity or genetic differences. Each *Leptoxis* species exhibits one of three distinct oviposition strategies: deposition of single eggs, deposition of eggs in a single line or deposition in circular clutches. Temperature cues for initiating egg laying varied from 12 to 26 °C depending on the species. There were significant differences in clutch size among species and between populations of *L. ampla* and *L. taeniata*. Furthermore, 1- and 2-year-old female *L. foremani* laid significantly fewer eggs per clutch than females 4 years or older. Finally, discrete shell morphologies that are characteristic of any given species are genetically controlled and not an eco-phenotypic response. Clutch egg laying likely represents increased parental investment compared with other behaviours and clutches may provide individual eggs protection from predation or passive dislodgement. Data from this study, including necessary conditions for successful culturing and period of oviposition for each species, can inform captive propagation efforts for imperilled *Leptoxis* species and aid in predicting how they will respond to future habitat alteration and climate change.

INTRODUCTION

The Pleuroceridae are the second most diverse freshwater gastropod family in North America (*c*.162 species) and these animals are often significant components of stream ecosystems (Richardson, Scheiring & Brown, 1988; Huryn, Koebel & Benke, 1994; Brown & Johnson, 2004; Brown, Lang & Perez, 2008). As a result of widespread human alteration of rivers, over 79% of species in the family are extinct or imperilled (Johnson *et al.*, 2013). Pleurocerids are dioecious and eggs are internally fertilized, but males lack a penis and the exact mechanism of sperm transfer has not been observed (Dazo, 1965; Strong, 2005). They reach sexual maturity after 1 or 2 years and lay eggs during species-specific periods in the spring and/or summer (Stimpson, 1864; Jones & Branson, 1964; Aldridge, 1982; Miller-Way & Way, 1989; Brown & Johnson, 2004; Johnson, 2010a, b). Pleurocerid life histories appear to vary substantially among species and these differences may have important bearing on life-history evolution and conservation. For example, life span varies from 2 years in *Leptoxis carinata* (Bruguière, 1789) to over 6 years in *Elimia* species (Stiven & Walton, 1967; Aldridge, 1982; Huryn, Koebel & Benke, 1994); eggs may be laid singly, in clutches or in lines (Winsor, 1933; Jones & Branson, 1964; Dazo, 1965), and both semelparity and iteroparity have been documented (Aldridge, 1982; Miller-Way & Way, 1989; Garner & Haggerty, 2010). However, specific life-history traits are poorly known for many species and no study has comprehensively evaluated patterns of variation across an entire pleurocerid genus.

Life-history behaviours are often phenotypic manifestations of genetic traits that can create or enhance reproductive barriers among populations (Miyatake & Shimizu, 1999; Lowry & Willis, 2010). If egg-laying behaviours such as average clutch size and period of oviposition differ among pleurocerid populations and species, then such differences may have facilitated diversification. The required substrate and flow conditions for oviposition and growth could also influence species and genus distribution patterns and how species respond to anthropogenic habitat modifications (Huryn, Benke & Ward, 1995). Furthermore, it is unknown if greater parental investment in some pleurocerid species (e.g. clutches *vs* single eggs) decreases overall fecundity. Since many pleurocerid genera—including *Leptoxis*—appear to be paraphyletic (Holznagel & Lydeard, 2000; Minton, Garner & Lydeard, 2003; Strong & Köhler,

2009), differences in egg-laying behaviours could prove to be useful for future systematic revisions.

The genus *Leptoxis* was once widespread in the southeastern United States, but 10 of 23 species [we include all species recognized by Johnson *et al.*, 2013 plus *L. subglobosa* (Say, 1825)] have gone extinct in the last 80 years, four are listed under the U.S. Endangered Species Act and most others are imperilled to some degree (Johnson *et al.*, 2013). Life-history data can aid in predicting how *Leptoxis* will react to future habitat modification (Dudgeon *et al.*, 2006; Strayer & Dudgeon, 2010). For example, females of several species begin oviposition in response to specific temperature or flow cues (Johnson, 2010a, b), and knowledge of these behaviours is fundamental for understanding potential responses to global climate change, dam operation or other factors. However, published life-history studies are only available for *L. carinata* and *L. dilatata* (Conrad, 1835) (Winsor, 1933; Aldridge, 1982; Miller-Way & Way, 1989).

Juvenile shell morphology and ontogeny is unknown for almost every *Leptoxis* species, which complicates the monitoring of recent recruitment (Cumberlandian Region Mollusk Conservation Restoration Committee, 2010; Mobile River Basin Mollusk Recovery Committee, 2010). This is of particular importance in the Mobile and Tennessee River basins, which have high pleurocerid endemism, sympatrically distributed species and juvenile morphology that can differ greatly from adult morphology (Dillon & Ahlstedt, 1997; Cumberlandian Region Mollusk Conservation Restoration Committee, 2010; Mobile River Basin Mollusk Recovery Committee, 2010). Furthermore, there has been recent debate about whether phenotypic plasticity or genetic variation is the primary cause of conchological variation of discrete characters (e.g. carinae, costae and plicae) in pleurocerids (Dillon, 2011; Whelan, Johnson & Harris, 2012a; Dillon, Jacquemin & Pyron, 2013). Rearing juveniles in homogeneous environments is an ideal method for helping to understand such conchological variation.

We document oviposition timing and behaviours of all 13 extant *Leptoxis* species. We present observational data from years of captive propagation as a part of an ongoing pleurocerid management plan (Mobile River Basin Mollusk Recovery Committee, 2010) and we describe life-history differences among *Leptoxis* species. Conchological growth is also documented to examine if *Leptoxis* morphological variation results from phenotypic plasticity. We also test three hypotheses about clutch size of clutch-laying *Leptoxis* species: (1) interspecific differences in clutch size exist; (2) there is no interpopulational effect on clutch size within species and (3) older individuals lay larger clutches. Testing these hypotheses will allow for inferences about whether life-history differences could have contributed to *Leptoxis* diversification and population dynamics.

MATERIAL AND METHODS

We collected *c*. 50 individuals from one or more populations of each extant *Leptoxis* species and transported them to a captive propagation facility at the Alabama Aquatic Biodiversity Center in Marion, Alabama (AABC; Table 1). An attempt was made to collect from type localities for species for which the locality is

Table 1. Collection localities, requisite culturing conditions to induce egg laying, egg-laying behaviour and approximate water temperature (in °C) for initiation and cessation of oviposition for species of *Leptoxis*.

Species	Collection locality	Conditions	Egg-laying behaviour	Approximate start temperature (°C)	Approximate end temperature (°C)
L. ampla	Cahaba River: 33.0791°N 87.0678°W and Little Cahaba River: 33.0537°N 86.0602°W, Alabama	Small tanks, low current	Clutch	14	27
L. taeniata	Choccolocco Creek: 33.5445°N 86.0413°W and Buxahatchee Creek: 33.0727°N 86.6775°W, Alabama	Small tanks, low current	Clutch	14	27
L. picta	Alabama River: 32.3207°N 86.8217°W, Alabama	Small tanks, low current	Clutch	20	27
L. foremani	Oostanaula River: 34.4032°N 85.0971°W, Georgia	Small tanks, low current	Clutch	12	22
L. virgata	Hiwassee River: 35.2195°N 84.5168°W, Tennessee	Large tanks, high current	Single eggs	23	27
L. umbilicata	East Fork of the Stones River: 35.8292°N 86.1784°W, Tennessee	Large tanks, high current	Single eggs	23	27
L. subglobosa	North Fork of the Holston River: 36.9534°N 81.5274°W, Virginia	Large tanks, high current	Single eggs	23	27
L. arkansensis	Spring Creek: 36.8104°N 92.1474°W, Missouri	Small tanks, low current	Clutch-dragging, deposited single eggs	13[a]	27[a]
L. praerosa	Limestone Creek: 34.6756°N 86.8789°W, Alabama	Large tanks, high current	Single eggs	26	30
L. compacta	Cahaba River: 33.1786°N 87.0175°W, Alabama	Small tanks, low current	Egg line	–	29
L. plicata	Locust Fork: 33.7245°N 86.9823°W, Alabama	Small tanks, low current	Single eggs	24	29
L. carinata	Roanoke River: 37.2334°N 80.1981°W, Virginia	Small tanks, low current	Egg line	22[a]	27[a]
L. dilatata	Indian Creek: 37.5151°N 80.7696°W, West Virginia	Small tanks, low current	Egg line	22[a]	27[a]

[a]These temperatures were measured far outside the geographic range of the respective species and should be interpreted with caution.

well-defined. However, some species such as *L. ampla* (Anthony, 1855) have broad type localities (e.g. state of Alabama). For those extirpated from their type localities, snails were collected from as near to the site as possible. We collected each species in the fall or winter of 2010 or 2011 before oviposition began, except for *L. foremani* (Lea, 1843) and *L. compacta* (Anthony, 1854). The restricted range and highly imperilled status of *L. foremani* required the use of individuals cultured at AABC. *Leptoxis compacta* was considered extinct when this study began, but it was rediscovered after the egg-laying season had begun in May 2011 and only a limited number of individuals were observed over a brief period (Whelan, Johnson & Harris, 2012b). To test for interpopulational differences in the number of eggs per clutch (i.e. clutch size), we compared individuals from two populations of *L. ampla* and *L. taeniata* (Conrad, 1834).

We followed the gastropod culturing protocol developed by Whelan, Johnson & Harris (2012a) to observe egg-laying behaviour and juvenile growth in captivity. The water supply was untreated well water that had passed through an aeration tower. All tanks were exposed to ambient temperature and natural lighting. Snails grazed on naturally-occurring algae and food was not supplemented with an artificial source. All tanks had unglazed terracotta tiles that leaned diagonally against the side of the tank for additional egg-laying substrates. For each species, we initially placed 12 randomly-selected individuals in each of three 20-l replicate tanks made of clear Plexiglas, each with a single 18.6 l/min powerhead. However, during the first season of observations (i.e. spring 2010) it became apparent that *Leptoxis* species that lay eggs singly (see Results) required a larger tank and higher flow conditions to stimulate oviposition (Table 1). For these species, all individuals (*c.* 50) were placed in a single 492-l tank with four 18.6 l/min powerheads to create higher flow, an airstone and unglazed terracotta tiles for additional egg-laying substrates. A single tank per species was used because pleurocerids often do not lay eggs in captivity if snail density is low (P.D. Johnson, unpubl.). Egg-laying behaviour of species that lay eggs in clutches did not differ qualitatively between small and large tanks. Water temperatures were measured continuously with Hobo Temp Loggers (Onset Computer Corporation) to document temperature cues for the onset of oviposition. Numerous studies examining snail behaviour and growth in captivity have shown few differences from traits observed in the wild (Dazo, 1965; Brown, 1979; Lam & Calow, 1988; Hoverman & Relyea, 2007; Lakowitz, Brönmark & Nyström, 2008; Whelan *et al.*, 2012a) and the relatively homogenous conditions in our facility are analogous to a common-garden experiment.

For clutch-laying species, the number of eggs per clutch was counted at least every 5 d, usually more frequently, throughout the oviposition period. Egg clutches were marked on the outside of each clear tank with a permanent marker to ensure that they were not counted twice. To test the hypothesis that clutch size is related to age, we counted clutch size of different age classes of *L. foremani* that were raised in separate tanks. These were different individuals than those used in the among-species comparisons described above. Age classes were captively propagated 1-, 2- and 4-year-old, and 5+ year-old wild broodstock; the exact age of the broodstock was unknown. We were unable to evaluate the relationship between body size and clutch size, because it was impossible to associate specific egg clutches with individual females, and the dependence of oviposition on high snail abundance precluded keeping individual females in separate containers. However, body size is strongly related to age in pleurocerids, and age is therefore a useful surrogate for size (Huryn, Koebel & Benke, 1994). Age-class clutch sizes were counted on a single day in early April, which is near the mid-point of *L. foremani*'s egg-laying period. Counting eggs on a single day removed confounding effects such as those caused by differences in the timing

of measurements and by temperature variation throughout the season.

To a great extent, population differences in average clutch size may reflect differences in body size rather than population-level adaptations. Therefore, average body size of the two *L. ampla* and *L. taeniata* populations was calculated for use in statistical tests of between-population clutch size differences. Measurements were made on 33 randomly-selected individuals from three populations per species collected at the river edge, quarter channel and middle channel, and included shell width (SW), aperture height (AH, both to the nearest 0.01 mm using digital callipers) and mass (nearest 0.01 g using a digital scale). Snails were air-dried for *c.*5 min before weighing. The ratio SW/AH was used as an index of size to decrease variability in measurements. After measurement, snails were returned to the river.

To account for the potential effect of phylogeny on differences in clutch size among species, we constructed a phylogeny of clutch-laying *Leptoxis* species (e.g. Felsenstein, 1985; Revell, Harmon & Collar, 2008). Only clutch-laying species were included since non clutch-laying species would have been dropped from phylogenetic signal calculations and inferring a full *Leptoxis* phylogeny with adequate outgroup sampling is outside the scope of this study. Three partial genes (COI, 16S rRNA and histone H3) were sequenced with published primers and methods (Supplementary material, Table S1; Folmer *et al.*, 1994; Palumbi, 1996; Colgan *et al.*, 1998). The phylogeny was inferred with *BEAST in BEAST v. 1.7.1 (Heled & Drummond, 2010; Drummond *et al.*, 2012) with a lognormal relaxed-clock model, a Yule prior on the species tree, and the piecewise-linear and constant-root population-size model. An arbitrary, uniform, root prior date of 25–35 Mya was used because fossil calibrations are not available for pleurocerids, but relative branch lengths, with a scale in terms of time rather than substitutions per site, were desired. *BEAST, a species-tree method, was used instead of other phylogenetic methods because divergence estimates have been shown to be more accurate using species-tree methods for species-level phylogenies (McCormack *et al.*, 2010) and it permitted us to use multiple individuals per species for phylogenetic inference. Also, an ultrametric tree was needed for downstream analyses. Blomberg's K (Blomberg, Garland & Ives, 2003) and Pagel's λ (Pagel, 1999) were calculated for log-transformed clutch size with the Phytools package (Revell, 2012) in R (R Core Development Team, 2011). These test statistics use the inferred phylogeny and a model of Brownian motion to determine if there is statistical nonindependence among species traits resulting from evolutionary relatedness.

Given a lack of phylogenetic signal in clutch size (see Results), statistically significant differences among species were tested using a nested ANOVA, with the two *L. ampla* and *L. taeniata* populations nested within species and log-transformed clutch size. Tukey-Kramer *post hoc* comparisons were then calculated. Statistically significant differences in clutch size between age classes of *L. foremani* were tested with a one-way ANOVA (log-transformed clutch size) with Tamhane's T2 *post hoc* comparisons. We tested for differences in body size and clutch size between each of the two populations of *L. ampla* and *L. taeniata* with a t-test.

We evaluated differences in shell growth among species by culturing juveniles of most species for a minimum of 160 d under the same conditions as described above. In particular, we took note of discrete conchological characters present in captively-propagated juveniles and wild-type parents of each species (e.g. carina, costae, plicae, umbilicus, aperture size and spire height) to examine the role of ecophenotypic plasticity in species-specific shell differences. If juveniles in a common-garden environment maintain the species-specific shell morphologies of their parents, then this will indicate a strong genetic component to shell shape. Shell characteristics were evaluated by eye. No *L. carinata* or *L.*

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subglobosa juveniles survived in captivity, possibly because the culture facility is south of their native range and temperatures may have exceeded their upper thermal limit. Vouchers were deposited at the National Museum of Natural History, Washington D.C. (Supplementary material, Table S2).

RESULTS

Egg-laying behaviour and clutch size

Egg-laying behaviours were invariant within species, but varied among species. For example, there were no differences in egg-laying behaviour among replicate tanks of the same species and we never observed a clutch in a tank of single egg-laying species. *Leptoxis* species laid eggs in one of three patterns: circular clutches, single eggs laid in a random pattern and linear arrangements of single eggs (Fig. 1). Eggs of all species were *c.* 0.3 mm in diameter and were laid on the walls of tanks or on terracotta tiles—usually on the underside—and sometimes on shells of other snails. Eggs were rarely deposited on the flat bottoms of tanks and all species laid eggs below the water line—except *L. foremani*, which laid eggs at, or slightly above or below, the water line. Qualitative observations in the wild indicate that eggs are laid on the undersides or vertical sides of clean, hard substrates (e.g. rocks without siltation or much vegetation). Field observations of several wild *Leptoxis* species indicate consistency in form (single, line, clutch) with captive gastropods. Eggs hatched ~14 d after oviposition for all species and a high percentage of eggs hatched (generally over 98%) regardless of oviposition pattern or species. It is unknown if unhatched eggs were unfertilized or failed to develop for other reasons.

Temperature appears to be a strong cue for oviposition, and the temperature at onset of oviposition varied widely among species, but was generally correlated with oviposition pattern (Table 1). Species that laid eggs in clutches began oviposition at cooler temperatures than most other species; *L. foremani* had the lowest onset temperature and began laying eggs in February when the water temperature reached 12 °C, but oviposition ceased temporarily when water temperature dropped <10 °C. With the exception of *L. arkansensis* (Hinkley, 1915), all other species laid eggs only at temperatures ≥22 °C, and *L. praerosa* (Say, 1821) did not begin oviposition until the temperature reached 26 °C. The temperature at onset of oviposition did not differ among populations of *L. ampla* and *L. taeniata*. After egg-laying began, all species generally continued to lay eggs for 60–90 d. With the exception of *L. foremani*, which ceased oviposition at 22 °C, the temperature at which egg-laying ceased (27–39 °C) was similar among species and showed no correlation with oviposition pattern.

Deposition of eggs in clutches

Leptoxis ampla, *L. taeniata*, *L. foremani* and *L. picta* (Conrad, 1834) all laid eggs in discrete, circular clutches, but the pattern of egg deposition varied among species. *Leptoxis ampla* and *L. taeniata* deposited eggs in concentric rings with one or two central eggs; rarely, clutches had only three eggs and were triangular
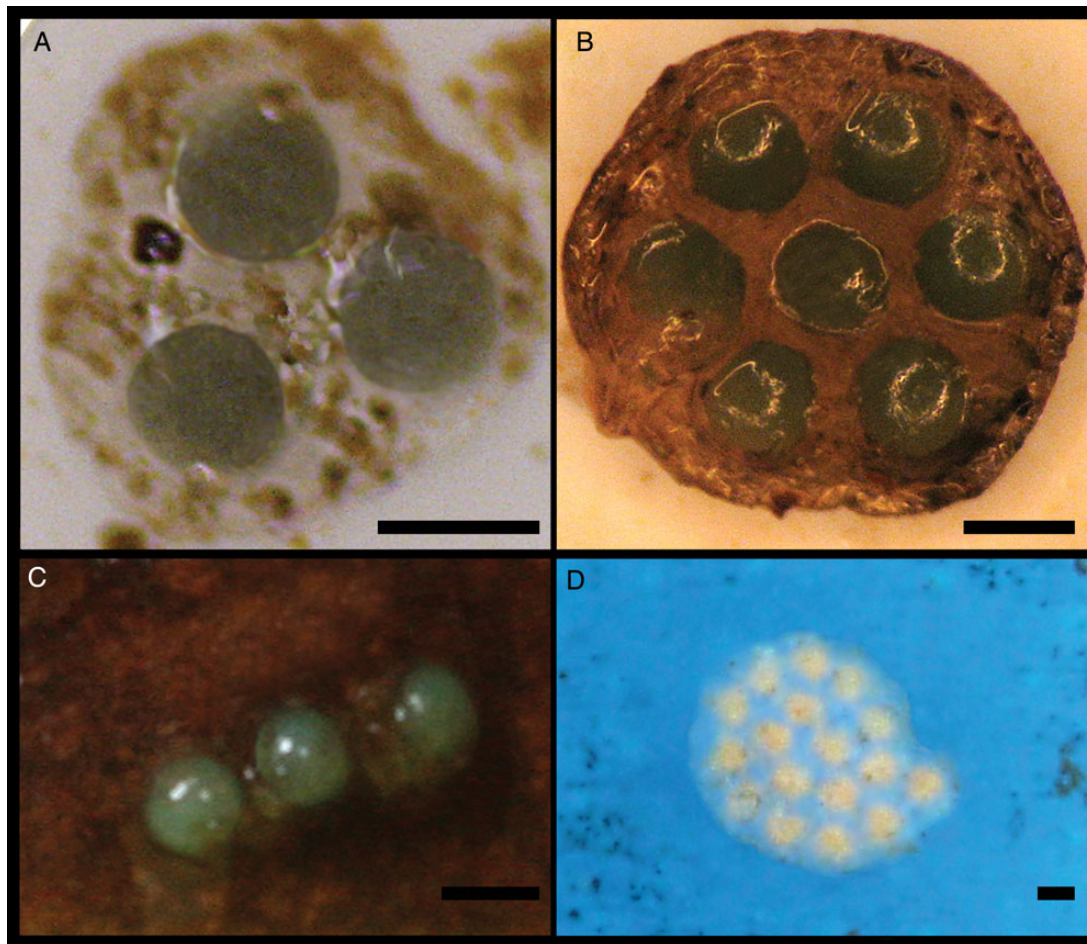

Figure 1. Egg-laying patterns of *Leptoxis*. **A.** Small *L. taeniata* egg clutch. **B.** *L. ampla* egg clutch, viewed from below, showing surface normally attached to substrate. **C.** Egg line. Single eggs (not shown) are similar to egg lines but spaced randomly. **D.** Spiral egg clutch of *L. picta*. *Leptoxis foremani* egg clutches are indistinguishable from those of *L. picta*. Scale bars = 0.3 mm.
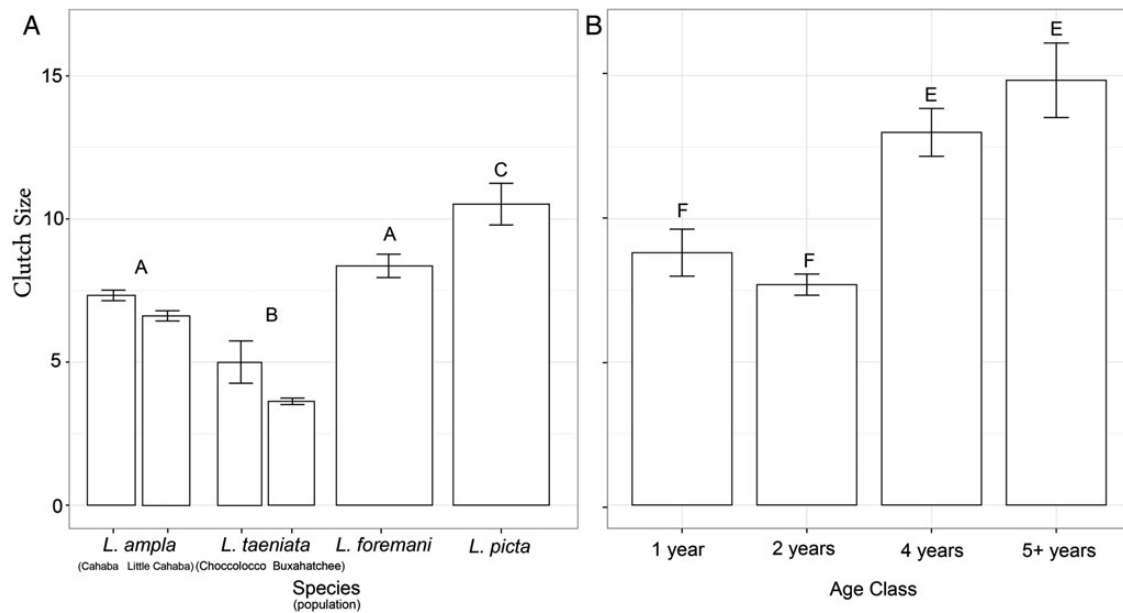
Figure 2. Bar graphs of average clutch size of *Leptoxis* species. Error bars are 95% confidence intervals. Bars with different letters above them had significantly different *post hoc* pairwise comparisons. **A.** Bar plot for clutch-laying species with *L. ampla* and *L. taeniata* subdivided by population. **B.** Bar plot for *L. foremani* age classes.

(Fig. 1A, B). *Leptoxis foremani* and *L. picta* deposited eggs in a spiral pattern (Fig. 1D). Clutches of *L. ampla* incorporated large amounts of mucus and external matter (e.g. algae or detritus) which surrounded each egg, but clutches of *L. taeniata*, *L. foremani* and *L. picta* were enveloped in mucus with little or no external matter (Fig. 1A, B, D).

All clutch-laying species were exposed to identical culture regimes and, therefore, statistical comparisons of clutch size are independent of immediate environmental variables. Both Blomberg's K and Pagel's λ failed to reject phylogenetic independence in the data ($\lambda = 0.360$, $P = 1.00$; $K = 0.059$, $P = 0.813$), so only standard statistical tests, rather than phylogenetically corrected tests, were used. Clutch size differed significantly among species (nested-ANOVA, $F_{\text{(among groups)}} = 20.1397$, 3 df, $P = 0.0477$; Fig. 2) with 26.84% of the variance explained by species. *Post hoc* comparisons showed significant differences among all species except *L. foremani* and *L. ampla*. Clutch size among different *L. foremani* age classes also differed significantly (ANOVA, $F = 48.818$, 3 df, $P < 0.001$; Fig. 2) with 42.9% of the variance explained by age class. *Post hoc* pairwise comparisons showed significantly higher clutch size for 4- and 5+ year-old individuals compared with 1 and 2 years old. The smaller average clutch size depicted for *L. foremani* in Figure 2A compared with Figure 2B results from inclusion of smaller clutches laid near the beginning and end of the egg-laying season as opposed to measurements made on a single day during the peak of oviposition.

Clutch size differed between populations of *L. ampla* and *L. taeniata*, but the magnitude of these differences was small and likely was influenced by differences in body size. Mean clutch size differences were small but significant between *L. ampla* from the Cahaba River and Little Cahaba River (mean clutch size = 7.3 eggs $\pm$ 2.2 SD, $n = 536$ clutches; 6.6 eggs $\pm$ 2.1 SD, $n = 542$ clutches, respectively; $t = -5.370$, df $= 1076$, $P < 0.001$). Shell size did not differ between these populations (mean SW/AH, Cahaba $= 1.05 \pm 0.06$ SD; Little Cahaba $= 1.03 \pm 0.07$; $t = -1.904$, 197 df, $P = 0.587$), but mass was significantly higher in the Little Cahaba River population (mean $= 0.53$ g $\pm$ 0.24 SD, Cahaba River $= 0.42$ g; $\pm$ 0.15 SD, $t = 3.771$, 197 df, $P < 0.001$). Mean clutch size differed significantly between populations of *L. taeniata* from Buxahatchee and Choccolocco

Figure 3. *Leptoxis arkansensis* female dragging a clutch of eggs behind its foot.

Creeks (mean clutch size $= 5.0$ eggs $\pm$ 1.2 SD, $n = 13$ clutches; 3.6 eggs $\pm$ 1.1, $n = 380$ clutches, respectively; $t = -4.412$, 391 df, $P < 0.001$). Shell size and mass were significantly higher in the Buxahatchee Creek population (SW/AH: $t = 12.819$, 197 df, $P < 0.001$; mass: $t = 11.748$, 197 df, $P < 0.001$). Mean SW/AH and mass were 1.18 ($\pm$ 0.06 SD) and 0.44 g ($\pm$ 0.10 SD) in snails from Buxahatchee Creek, and 1.06 ($\pm$ 0.06 SD) and 0.26 g ($\pm$ 0.12 SD) in those from Choccolocco Creek.

Random deposition of single eggs

Leptoxis praerosa, *L. umbilicata* (Wetherby, 1876), *L. virgata* (Lea, 1841) and *L. subglobosa* all laid eggs singly in an apparently random manner. *Leptoxis plicata* (Conrad, 1834) also usually laid eggs singly, but occasionally deposited two eggs in close proximity. *Leptoxis arkansensis* displayed an unusual variant of the single egg-laying strategy; females pulled a mass of eggs behind their foot as they intermittently deposited single eggs (hereafter termed clutch-dragging; Fig. 3). For all single egg-laying species

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except *L. arkansensis*, strong current, large tanks and at least 50 individuals per tank were required for captive propagation, since they would not oviposit under other culturing conditions.

Deposition of eggs in a line

Leptoxis compacta, *L. dilatata* and *L. carinata* all laid eggs in a non-random linear pattern (Fig. 1C). Individual eggs laid in a line were not bound together in a cohesive unit by mucus or other organic matter.

Shell morphology

Shell morphology of juvenile *Leptoxis* differed in several ways from adult morphology, but these differences varied among species (Figs 4–6). Adult *L. foremani* have costae (i.e. shallow ribs) and prominent striae, but these features are absent in the smallest size classes and become more conspicuous as the shell grows (Fig. 4C). Similarly, adult *L. plicata* have characteristic plicae adjacent to the suture of the body whorl, but these are indistinct or absent in juveniles (Fig. 5E). In contrast, carinae present in juvenile *L. compacta* and *L. carinata* become obsolete with age (Fig. 6A, B). Juvenile *L. praerosa* have a conspicuous

Figure 4. Growth series of clutch-laying *Leptoxis* species. **A.** *L. ampla*. **B.** *L. taeniata*. **C.** *L. foremani*. **D.** *L. picta*. Scale bar = 5 mm. Symbol ~ indicates captive-propagated individuals.

Figure 5. Growth series of single-egg laying *Leptoxis* species. **A.** *L. virgata*. **B.** *L. praerosa*. **C.** *L. subglobosa* (all wild-collected individuals). **D.** *L. umbilicata*. **E.** *L. plicata*. **F.** *L. arkansensis*. Scale bar = 5 mm. Symbol ∼ indicates captive-propagated individuals.
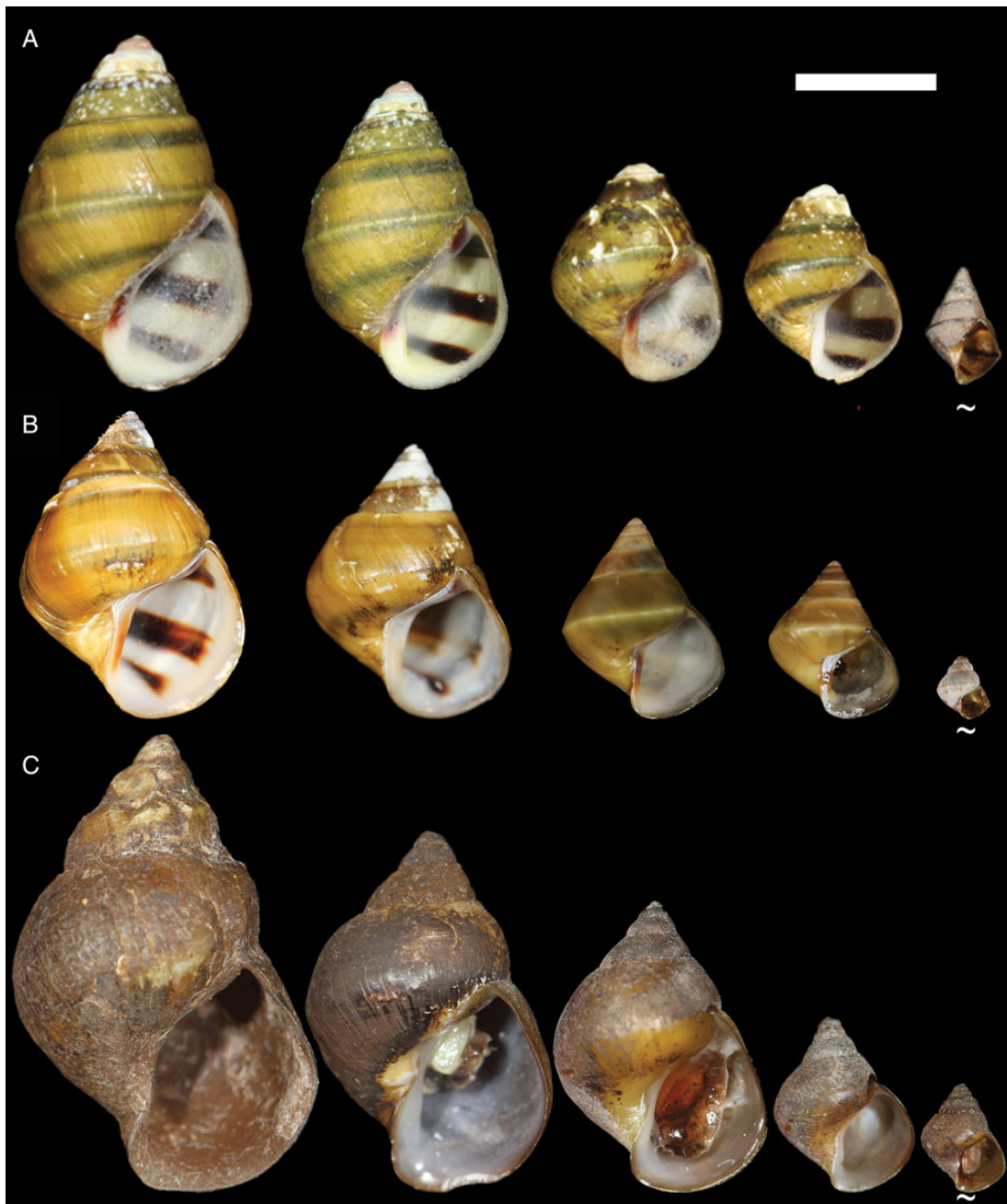
Figure 6. Conchological growth series of egg-ine laying *Leptoxis* species. **A.** *L. compacta* (adapted from Whelan, Johnson & Harris, 2012a). **B.** *L. dilatata*. **C.** *L. carinata* (all wild-collected individuals). Scale bar = 5 mm. Symbol ∼ indicates captive-propagated individuals.

umbilicus that is closed later in life, but in *L. umbilicata* the umbilicus remains open into adulthood. Coloration differed between juveniles and adults of *L. picta*, in which juveniles had distinct, interrupted colour bands that disappeared in older individuals (Fig. 4D). Juvenile *L. taeniata* also had distinct banding that was retained into adulthood. Overall, the juveniles grown in nearly identical environments in our study developed shell morphologies identical to wild-type parents, even when juvenile morphology initially differed considerably (e.g. *L. compacta*). Most snails grown in culture do not undergo erosion of upper whorls. The absence or reduction of erosion in captively-reared individuals continues after release to the wild.

DISCUSSION

The diversity of egg-laying behaviours within *Leptoxis* is notable for freshwater snails. Previous analyses of gastropod life history failed to find species-specific differences and Brown (1983) hypothesized that life-history traits may be constrained below the family level. This is clearly not the case in Pleuroceridae. However, *Leptoxis* is likely not monophyletic (Holznagel & Lydeard, 2000; Strong & Köhler, 2009), which precludes conclusions about genus-level diversity of egg-laying behaviours. There is clearly a need for comprehensive revisionary systematic studies of pleurocerids to understand *Leptoxis* diversity and life-history evolution fully.

With regard to *Leptoxis* shell morphology, phenotypic plasticity does not appear greatly to influence discrete shell characters (i.e. development of carinae, plicae, costae or the umbilicus) or periostracum colour, since juveniles of different species have different and characteristic morphologies when reared under near-identical captive conditions and develop the species-specific morphologies seen in the wild (Figs 4–6). Of note is the similarity in shell coloration of *L. taeniata* (Fig. 4B) and *L. picta* (Fig. 4D) juveniles, which may explain spurious records of *L. taeniata* from the Alabama River (Goodrich, 1922). Recent papers have proposed sweeping synonymization of pleurocerids based on untested hypotheses of 'cryptic phenotypic plasticity' (Dillon, 2011; Dillon, Jacquemin & Pyron, 2013), but our results reject phenotypic plasticity as a cause of conchological differences among currently recognized species—at least for *Leptoxis*.

Our findings can be used to inform conservation of *Leptoxis* and pleurocerids as a whole. This is the first documentation of ontogenetic variation in shell morphology for most *Leptoxis* species, and this information should be useful when monitoring recruitment of imperilled species. For example, considering that most pleurocerids in the wild undergo some degree of erosion of the apex, the absence of erosion in captively-reared individuals is a useful characteristic for monitoring reintroduced populations of imperilled species since tags are impractical for juvenile pleurocerids (Johnson, 2010a). Furthermore, when making critical habitat designations, egg-laying behaviours should be taken into consideration. Single egg-laying species seem to reproduce better in fast-flowing environments, and clutch-laying *Leptoxis* are all associated with larger rivers (Goodrich, 1921, 1922, 1940; Burch & Tottenham, 1980). Long-term survival of *Leptoxis* species will be dependent on large-river habitat preservation and/or restoration of rivers with clean, fast-flowing waters.

The initiation of egg-laying appears to be strongly influenced by temperature, and we were surprised to find that *L. praerosa* did not start laying eggs until water temperatures reached 26 °C. However, temperature results for *L. carinata* and *L. dilatata* should be viewed with caution. The culture facilities lie far south of the native range of both species and thermal stress may have caused juvenile mortality. The period of oviposition should inform monitoring techniques as disturbance during egg-laying periods is best avoided for endangered species, and checking for recruitment in the wild should be done after egg-laying has ceased. Moreover, in light of global climate change, annual temperature profiles of critical habitats should be monitored to predict temporal shifts in future egg-laying periods. Research is needed to determine the upper thermal limits of pleurocerids, but they may not be much higher than current ambient temperatures, considering the mortality of *L. carinata* juveniles. Furthermore, species that initiate egg-laying at higher temperatures (i.e. 26 °C for *L. praerosa*) may be especially susceptible to increasing thermal stress.

The greater clutch size observed in older *L. foremani* compared with younger individuals suggests higher fecundity with increasing size and/or age. This was expected and can probably be extrapolated to other *Leptoxis* species (Stearns, 2000). However, it cannot be ruled out that younger individuals could lay as many eggs in total as older individuals despite their smaller clutches. Since older individuals are larger than younger ones on average (Aldridge, 1982; Miller-Way & Way, 1989; Huryn, Koebel & Benke, 1994), body size also likely influences clutch size. The likely explanation for larger clutch size in older/larger individuals is that they allocate more resources for reproduction than younger individuals that are still building body mass. Considering that there was no significant difference in fecundity observed between 4 and 5+ year-olds, there may be an upper limit to clutch size in clutch-laying *Leptoxis* species, but there is also most likely an upper size limit of female *L. foremani*.

Among clutch-laying *Leptoxis* species, there is a clear difference in average clutch size. The differences in clutch size among species seem to correlate roughly with the average size of each species (Figs 2, 4). However, size differences are well known, inherent differences among species. Here we show that clutch-size differences are also inherent characteristics of species, but more detailed study would be necessary to determine if the underlying genetics—or other factors—that influence body size and clutch size are physically or evolutionarily linked. Clutch size was found to be statistically independent of phylogenetic history, which suggests that *Leptoxis* species may rapidly adapt clutch size to local environments to optimize clutch size (but see Revell, Harmon & Collar, 2008). For population-level comparisons of *L. ampla* and *L. taeniata*, the actual difference in clutch size ($\Delta = 0.7$ and 1.4, respectively) may not represent a biologically significant difference despite being statistically significant. Larger body size may explain the larger clutch size in the *L. taeniata* Buxahatchee Creek population, since individuals are on average larger there than at the Choccolocco Creek site. Interestingly, the larger individuals of the Cahaba River *L. ampla* population laid smaller clutches than the smaller *L. ampla* from the Little Cahaba River population.

Clutch-laying species likely expend more energy per egg since there must be costs associated with laying eggs in organized masses surrounded by mucus. The potential trade-off for this increased parental investment is lower egg mortality, since the mucus and organic matter could provide protection from predation and dislodgement from the substrate. For example, *L. ampla* clutches required considerable force to dislodge from the substrate, whereas single eggs could be detached with little effort. On the other hand, single-egg laying individuals may lay more eggs to offset the potential for greater predation/environmental risk. Observations of snails grown in captivity at AABC for conservation efforts support this hypothesis: *L. picta*—a clutch-laying species—produces *c*.10 juveniles for every individual in a tank, whereas *L. plicata*—a single-egg laying species—produces nearly 17 juveniles for every individual (P.D. Johnson, unpubl.).

A unique behaviour was clutch-dragging in *L. arkansensis*. Typically, pleurocerid eggs leave the pallial oviduct and travel down an egg groove along the right side of the female's foot before being deposited with the aid of an ovipositor (Jones & Branson, 1964). *Leptoxis arkansensis* accumulated eggs in a mass behind the foot prior to depositing them on a hard substrate. The egg-laying process was not directly observed, but multiple females were observed dragging clutches of different sizes, so this was not an aberrant behaviour of a single female. We are unaware of any other snail that has a clutch-dragging behaviour. This behaviour is only visible from the underside with the female crawling on a clear surface, and would be virtually impossible to observe in the wild. Therefore, clutch-dragging could be more common than currently known.

Both semelparity and iteroparity have been reported in *Leptoxis* and we found evidence for both lifestyles. *Leptoxis carinata* and *L. dilatata* have been reported to be semelparous biennial species that first lay eggs at 23 months (Aldridge, 1982; Miller-Way & Way, 1989). We randomly sampled *L. carinata* and *L. dilatata* from the wild and likely had both 1- and 2-year cohorts in captivity, but most adults of both species died after the egg-laying season, suggesting semelparity. Full life-cycle data are unavailable for *L. arkansensis*, but this species also had near-complete adult mortality after egg-laying and is apparently semelparous. On the other hand, it is clear that *L. foremani* reproduces in multiple years as indicated by the different age classes analysed in this study. Ongoing propagation projects at AABC have shown that *L. ampla*, *L. taeniata*, *L. picta*, *L. plicata*, *L. praerosa* and *L. virgata* reproduce for multiple years (P.D. Johnson, unpubl.). Interestingly, the three semelparous *Leptoxis* are all

from more northern latitudes than other *Leptoxis* species and semelparity has been reported in other pleurocerids from higher latitudes [e.g. *Pleurocera acuta* Rafinesque, 1831 and *Elimia livescens* (Menke, 1830); Houp, 1970; Payne, 1979]. Semelparity may be advantageous for northern species since they experience colder environments and shorter growth periods (Aldridge, 1982). A possible explanation is that a semelparous life cycle would allow for biomass accumulation in the first year for winter survival, but death occurs after resources are dedicated to reproduction rather than biomass accumulation for overwintering during the second year. Southern species are able to accumulate biomass in the warmer autumn months after reproduction and they are exposed to milder winters. This possibly makes iteroparity a more viable life-history strategy for southern species. Iteroparous *Leptoxis* may be good candidates for reintroduction efforts, since surviving adults will reproduce for multiple years, which could increase the likelihood of establishing a self-supporting population and reduce the number of yearly releases needed for establishment.

The findings of this study are a first step in understanding the life history of an ecologically important and imperilled gastropod group. Qualitative behaviours documented here (i.e. egg-laying behaviours and semelparity *vs* iteroparity) are unlikely to be different in the wild, considering the lack of variation seen within species (e.g. species characterized as laying single eggs were never observed laying a clutch). Experimental studies of predation on pleurocerid eggs by crayfish, insects and fish are needed to assess further the potential advantages and trade-offs of different egg-laying behaviours. Species differences in egg-laying behaviours and clutch size are likely evolutionarily significant (Stearns, 1977) and a comprehensive phylogeny of *Leptoxis* with adequate sampling of other pleurocerid genera will be required to understanding fully the evolution of life history in *Leptoxis* and in Pleuroceridae as a whole. Management agencies should be aware of the potential influence of anthropogenic habitat change (e.g. rising water temperatures and rapid changes in flow) and its potential to disrupt the natural egg-laying cycle of *Leptoxis* and the survivorship of offspring. We hope future studies will explore how imperilled *Leptoxis* may respond to changing environments.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *Journal of Molluscan Studies* online.

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