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Intraspecific Density Dependence in Larval Development of the Crawfish Frog, *Lithobates areolatus*

The Crawfish Frog, *Lithobates areolatus*, is listed as state-endangered or rare in six of the 12 states within its range. Prior to 1970, *L. areolatus* were locally plentiful, but has declined markedly since that time (Minton 2001; Parris and Redmer 2005). Reasons for their decline are not well understood, but have been attributed to habitat loss, disease, introduction of predators, and failed juvenile recruitment (Palis 2009; Parris and Redmer 2005). Because of its secretive nature, we lack critical information on *L. areolatus* life-history and population demographics. A fundamental component to population stability is that recruitment equals mortality. Due to limited resources, larval-amphibian recruitment is affected by larval density (e.g., Altwegg 2003; Scott 1994). Overcrowding can delay or inhibit larval development (Adolph 1931; Morin 1986; Parris et al. 1999), resulting in increased mortality rates by predation (Caldwell et al. 1980; Travis et al. 1985), or desiccation from inadequate hydroperiods in breeding ponds (Rowe and Dunson 1995; Seigel et al. 2006). Parris and Semlitsch (1998) examined *L. areolatus* density dependence in artificial tanks and reported that interspecific competition reduced larval performance. They also examined intraspecific competition, but did not find a significant relationship between *L. areolatus* density and any of their response variables (i.e., body mass, larval-period length, survivorship; Parris and Semlitsch 1998). Therefore, the relationship between *L. areolatus* larval performance (i.e., growth and survivorship) and intraspecific larval density is not well understood. Further, the degree that density affects *L. areolatus* larval development in natural ponds is unknown. Although artificial tanks (as in Parris and Semlitsch 1998) provide insight on cause and effect relationships, field enclosures placed directly in breeding ponds include relevant environmental factors and incorporate greater realism (Semlitsch and Boone 2010; Semlitsch and Bridges 2005).

Thus, to better understand how larval-stage density affects juvenile recruitment in *L. areolatus*, we examined cohort density (i.e., intraspecific) dependence on size and time characteristics of larval development using field enclosures placed in five known crawfish frog breeding ponds, and one potential breeding pond. Our objectives were (1) to examine the extent at which metamorphosis was delayed or inhibited in high density treatments, and (2) to examine the efficacy of field enclosures as a management tool for repatriation efforts of *L. areolatus*. We hypothesized that larvae in low-density treatments would metamorphose earlier, and would be larger than high-density treatments. We also hypothesized that field enclosures would dramatically increase the survival rate in *L. areolatus* larvae, compared to natural populations, and would provide a valuable tool for managing this declining species.

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Methods.—We selected six temporary ponds on Big Oaks National Wildlife Refuge (located in southeastern Indiana, USA), for our study ponds. The ponds had similar size and depth characteristics (<0.15 ha, and <1 m deep). Five of the six ponds were known crawfish frog breeding ponds (i.e., male frogs were observed calling during previous breeding seasons). In each pond we placed two 378 L field enclosures (76.2 cm × 41.9 cm × 121.9 cm; Apogee, Dallas, Texas 75244, USA) side-by-side, in 20–30 cm of water. Field enclosures were orientated with the long end in the north–south direction. We placed 200 g (wet weight) of *Andropogon virginicus* (Broomsedge Bluestem) in each field enclosure for larval food and substrate. We collected the *A. virginicus* from a single site and then randomly placed it within each enclosure.

We collected one *L. areolatus* egg mass from a breeding pond at Big Oaks National Wildlife Refuge on 3 April 2010. To introduce larvae to the field enclosures we acclimatized them using a three-step process. First, we held the egg mass in a plastic circular pool (diameter = 1.2 m, depth = 15 cm), near the collection site from 3–9 April 2010. We did this to allow the larvae to disperse from the egg mass. Second, after the larvae dispersed (9 April 2010), we divided the larvae into six groups of approximately equal numbers of individuals and moved each section to plastic circular pools located within 5 m of each of our study ponds. The plastic pools were filled with water collected from their respective study pond. We held them in plastic pools near the study pond to allow larvae to acclimatize to the different water chemistry and to grow large enough to be held in the field enclosures. Third, on 4 May 2010, we haphazardly selected larvae with approximately the same size and vigor (i.e., the speed and amount of travel within the pools) for the field enclosures. Larvae were placed in one of two different field-enclosure treatments: low density (20 larvae), and high density (60 larvae); thus, we had 480 total larvae in our experiment.

The treatments were randomly assigned to the field enclosures with one low-density and one high-density treatment in each pond. Prior to being placed in the field enclosures, we measured the volume of each larva using water displacement, and estimated it to be negligible (mean difference <0.01 mL) between treatments and among ponds. Thus we had 12 total field enclosures that included six replicates of two treatments. One of our replicates was destroyed during rain runoff on 12 May 2010. We selected another study pond that was a known crawfish frog breeding site and added two more field enclosures with larvae on 14 May 2010. Although this replicate was initiated 10 days after our initial replicates, we followed the same protocols to introduce larvae to the treatments and therefore included it in our study. We monitored field enclosures twice weekly to remove dead larvae and to identify stages of metamorphosis. After the first frog completed metamorphosis (i.e., Gosner Stage 45 or

46; Gosner 1960), we began to monitor field enclosures daily to release frogs that completed their metamorphosis. We released frogs within 2 m of the field enclosures. We measured the mass and snout-vent length of larvae after they completely metamorphosed. We also recorded the date of complete metamorphosis. Additionally, to estimate body condition, we fit a linear regression equation between mass and snout-vent length and used the residuals.

We compared the mass, snout–vent length, date of metamorphosis of frogs, survival, and the relative body condition between the two treatments using paired 2-sample t-tests. We examined the relationship between larval mass at metamorphosis and time of metamorphosis by fitting a linear regression equation. We fit this equation at three different levels: (1) all of the combined data, (2) the pooled density estimates (low and high) for each pond (to compare a pond effect), and (3) to the pooled pond estimates for each density (to compare a density effect). We examined the regression coefficient for the slope to assess if there was a relationship between the size of the juveniles and the date they completed metamorphosis.

To examine for potential differences between ponds we measured pond chemistry and temperature weekly. We measured pond chemistry (ammonia, nitrate, nitrite, iron, dissolved oxygen, pH, sulfate, and phosphorus) using a colorimeter (LaMotte Company, Chestertown, Maryland 21620, USA). We measured pond temperature on the south side of the field enclosures. We randomized the time and the order we visited ponds. We compared pond chemistry and temperature of all the sample ponds using a single-factor analysis of variance for each metric. Additionally, we tested all metrics simultaneously using a multivariate analysis of variance (MANOVA), in which our sample ponds were the groups, and the chemistry metrics were the dependent variables.

Results.—Two hundred twenty-eight of the original 480 juvenile *L. areolatus* survived and were released from our field enclosures (overall survivorship = 48%); 85 of 120 were released from the low-density treatment, and 143 of 360 were released from the high-density treatment. The mean survival percentage in field enclosures was 68% (s = 18%) in low-density treatments and 38% (s = 29%) in high-density treatments, although this difference was not significant (t-test: P = 0.11, t = 1.89, df = 5). Two hundred fifty-two died in the field enclosures. Fifty-nine of these 252 died later in the summer after three of the six study ponds completely dried. All 59 were in high density treatments. Mean snout–vent length of larvae in the low-density treatment was 2.57 cm (s = 0.23 cm) and was 1.14 times longer (t-test: P = 0.0045, t = 4.89, df = 5) than the high density treatment ($\overline{x}$ = 2.26 cm, s = 0.22 cm). Mean mass of larvae in low-density treatments was 1.36 g (s = 0.35 g), and was 1.42 times larger (t-test: P = 0.013, t = 3.75, df = 5) than larvae in high-density treatments ($\overline{x}$ = 0.98 g, s = 0.25 g). Larvae emerged 17 days earlier (t-test: P = 0.0023, t = -5.70, df = 5) in low density treatments ($\overline{x}$ = 13 July 2010, s = 13 days) than high density treatments ($\overline{x}$ = 30 July 2010, s = 10 days). There was no difference in the body condition between treatments (t-test: P = 0.81, t = -0.25, df = 5); the mean residual distance for low-density treatments was 0.01 g (s = 0.06 g) and high-density treatments was 0.02 g (s = 0.07 g). The replicate with the earliest emergence dates, and largest juveniles for both the low- and high-density treatments was located in the one pond that had no record of crawfish frog calling.

None of the pond chemistry metrics or temperatures differed among ponds (ANOVA: P > 0.05; df = 5, 86; MANOVA: P = 0.22; approx. F = 1.24; df = 5, 65). The mean values of the pooled spatial and temporal chemistry data were: ammonia = 0.54 ppm (s = 0.66 ppm); nitrate = 0.11 ppm (s = 0.15 ppm); nitrite = 0.00 ppm (s = 0.02 ppm); iron = 1.86 ppm (s = 1.74 ppm); dissolved oxygen = 4.80 ppm (s = 2.48 ppm); pH = 5.83 (s = 0.81); sulfate = 5.04 ppm (s = 7.37 ppm); and phosphorus = 0.12 ppm (s = 0.26 ppm). The mean water temperature was 27.62°C (s = 3.28°C). There did not appear to be a strong relationship (P > 0.05) between mass-at-metamorphosis and date-of-metamorphosis at any of the three levels of data we examined (i.e., all data pooled, data pooled within each pond, data pooled within each treatment).

Discussion.—Our results suggest that *L. areolatus* larval development is affected by intraspecific density, and that these effects might have consequences for *L. areolatus* fitness. When reared in high-density treatments, larvae had smaller masses and snout–vent lengths, but did not have a significant increase in body condition (suggesting the change in size was not a trade-off from fat/lipid storage to structural growth; Perrin and Sibly 1993; Scott et al. 2007; Werner 1986). These results are consistent with patterns described for intraspecific competition in other anurans (Alford 1999). In other species, larval size at metamorphosis is positively correlated with adult size, and inversely correlated with the number of years until sexual maturity (Altwegg and Reyer 2003; Semlitsch et al. 1988; Smith 1987). If the same correlation exists in *L. areolatus*, low density ponds that produce larger juveniles may positively affect population growth because (1) frogs may reach sexual maturity faster, and (2) adults may be larger, thereby producing more eggs during reproduction (Redmer 1999). Therefore, adult lifetime fitness would be affected by larval densities; low larval densities would produce fitter adults.

In addition to size characteristics, our data suggest that high intraspecific density extends *L. areolatus* development periods in natural ponds. Extended development periods can have severe consequences for *L. areolatus* because they generally select temporary breeding ponds with abbreviated hydroperiods. Population growth and larval success depend on the appropriate larval-period length relative to the hydroperiod of the breeding pond (Semlitsch et al. 1996). Fifty-nine *L. areolatus* larvae died from desiccation in three different sample ponds after the ponds completely dried; all were in high-density treatments. Similar results may occur in natural ponds, where at some minimum hydroperiod length, there is a maximum density level, after which, increased densities will increase mortality. This is particularly important during drought years when the number of breeding ponds is reduced and higher concentrations of breeding adults use the same pond.

Increased *L. areolatus* larval-period length has been shown to be positively correlated with interspecific competition (Parris and Semlitsch 1998). Parris and Semlitsch (1998) identified the poor interspecific competitive performance of *L. areolatus* as a possible explanation for their low frequency and small population size in natural communities. Our results support the hypothesis that larval competition is affecting population size and distribution because, in addition to being poor interspecific competitors, larvae in our sample were negatively affected by intraspecific competition. Thus, competition may be limiting recruitment, and therefore population levels.

Although our data provided evidence for density-dependent effects, it is important to note that they were based on one egg mass, and therefore our sample contained little genetic variation. Differences in genetic variation are associated with differential responses by anurans to insecticide (Bridges and Semlitsch

2000; Semlitsch et al. 2000) and acid tolerance (Pierce and Sikand 1985). Likewise, increased genetic variation might facilitate differential response to overcrowding by increasing niche variation, thereby reducing resource competition (Benard and Middlemis Maher 2011). Therefore, if increased genetic variation causes differential response to intraspecific density, our results may be limited. Semlitsch and Bridges (2005) proposed a hierarchical approach, incorporating individual-level, population-level, and geographic-level genetic variation in studies on ecotoxicology. A similar study design would better describe the role of genetic variation in intraspecific competition.

Our study suggests that *L. areolatus* larvae grow and survive better when raised at low densities. However, our experimental design did not allow us to identify the critical density level at which larval development is inhibited. Further examination of a gradient of densities would better describe the relationship between density and growth, which would allow managers to maximize the number of frogs produced per unit area when using field enclosures. Additionally, examination of other environmental variables (e.g., food availability) may identify the mechanism that inhibits growth in high densities of *L. areolatus*. Our study did provide evidence that using field enclosures for repatriation may be an effective management tool for *L. areolatus* because it has the potential to dramatically increase juvenile survival when compared to survival in natural ponds (e.g., *L. areolatus* survival in a natural pond in southwestern Indiana was 0–2.3%; V. Kinney, Indiana State University, unpubl. data). However, further research comparing long-term survival of frogs raised in field enclosures to frogs raised in natural populations would better estimate the effect of repatriation on population recruitment.

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