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## Density-Dependent Fitness Attributes and Carry-Over Effects in Crawfish Frogs (*Rana areolata*), a Species of Conservation Concern

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**Fitness attributes acquired in aquatic habitats by amphibians exhibiting complex life histories have been shown to cascade through terrestrial juveniles into adulthood, a phenomenon termed carry-over effects. We explored density-dependent fitness attributes and carry-over effects in Crawfish Frogs (*Rana areolata*) using a set of field enclosure experiments and a series of field data. Using field enclosures, we hypothesized that 1) at high densities, intraspecific competition would produce smaller Crawfish Frog juveniles that took longer to metamorphose; 2) at high densities, interspecific competition would also produce smaller Crawfish Frog juveniles that took longer to metamorphose; and 3) vertebrate (ambystomatid salamander) predation on Crawfish Frog larvae would reduce survivorship, but by releasing competition pressure would produce relatively larger tadpoles that metamorphosed earlier. Further, we hypothesized 4) that these enclosure results would apply to field data, and that fitness attributes in newly metamorphosed Crawfish Frogs would carry over to first-time breeding adults. Our results confirmed all four hypotheses. Specifically, in Crawfish Frogs, at high densities, both intra- and interspecific competition reduced size (length and mass) at metamorphosis (hypotheses 1 and 2), and predation reduced survivorship and increased size at metamorphosis (hypothesis 3). Finally, we observed density-dependent fitness effects on newly metamorphosed Crawfish Frog juvenile size (length and mass), and carry-over effects from the larval stage on juvenile survival, adult size, and breeding adult numbers (hypothesis 4). In the absence of predators, high densities of intra- and interspecific competitors had no effect on Crawfish Frog larval survivorship. We also present suggestive evidence for compensatory effects. We discuss the potential mechanisms underlying the patterns of these interactions, as well as the role of these relationships in informing management guidelines intended to ensure the future of this species of conservation concern.**

**I**N North America north of Mexico, about two-thirds of the roughly 356 recognized species of amphibians exhibit some form of complex life history, characterized by aquatic eggs and larvae, and, following an abrupt metamorphosis, terrestrial juveniles and adults (Lannoo, 2005; Green et al., 2014; AmphibiaWeb, 2018). In amphibians, fitness attributes (*sensu* Hunt and Hodgson, 2010) acquired by aquatic larvae have been shown to cascade through terrestrial juveniles to influence adult phenotypes (Semlitsch et al., 1988; Beck and Congdon, 2000; Morey and Reznick, 2001), a phenomenon called carry-over effects (Van Allen et al., 2010; Harrison et al., 2011; Earl and Semlitsch, 2013). Berven (1990) described carry-over effects on amphibian survival and fitness as follows. The negative effects of egg or tadpole density on time of metamorphosis and metamorphic size carry over into juvenile success; larger juveniles and those metamorphosing early enjoy higher survival, earlier age at first reproduction, and are larger as adults. Larger adult size subsequently affects fitness by increasing the numbers and size of eggs produced by females (Berven, 1990). Carry-over effects can also influence post-metamorphic survival (Goater, 1994; Beck and Congdon, 2000; Chelgren et al., 2006), activity patterns (Yagi and Green, 2018), and locomotion (John-Alder and Morin, 1990; Álvarez and Nicieza, 2002; Cabrera-Guzmán et al., 2013).

Wilbur (1997) summarized the effects of this density dependence in aquatic amphibian larvae by noting that size at metamorphosis is an exponentially decreasing function of initial density of the population. He observed that at low density, many individuals metamorphose at a large size,

while at high densities, most individuals metamorphose at what may be the minimum metamorphic size threshold. Further, he suggested that survival is affected, because as density increases, reduced growth leads to a decreasing probability that a small, less-developed animal will achieve the minimum metamorphic size before the pond dries. Subsequent studies have generally confirmed these effects of larval density on size at and timing to metamorphosis (Semlitsch and Caldwell, 1982; Smith, 1983; Altwegg, 2003; Williams et al., 2012).

Crawfish Frogs (*Rana areolata*) are large-bodied ranids that breed in the early spring and whose tadpoles metamorphose by mid- to late-summer (Thompson, 1915; Gloyd, 1928; Cagle, 1942). They are considered a species of conservation concern in each of the 13 states they inhabit (Lannoo et al., 2017; Lannoo and Stiles, 2020). As the conservation status of *R. areolata* becomes more tenuous (of the 243 counties known to have been inhabited across their historic range, today Crawfish Frog populations remain in 159 counties, a 35% decline in occupancy; Lannoo et al., 2017), there is an urgency to understand the population dynamics of this species in order to enact management practices that will prevent further extirpations of these highly specialized animals.

Towards this end, we conducted a set of field enclosure experiments and collected a series of field data to test four hypotheses. We predicted that:

- 1) at high densities, intraspecific competition would produce smaller Crawfish Frog juveniles that took longer to metamorphose and had lower larval survivor-

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ship (e.g., Wilbur, 1977a, 1977b; Semlitsch and Caldwell, 1982; Berven, 1990);

- 2) at high densities, interspecific competition with first-year Southern Leopard Frog (*R. sphenoccephala*) tadpoles and second-year Green Frog (*R. clamitans*) would mimic the effects of intraspecific competition (e.g., Alford and Wilbur, 1985; Wilbur and Alford, 1985; Werner, 1992);
- 3) vertebrate (ambystomatid salamander) predation on Crawfish Frog larvae would lower survivorship compared to high-density controls, but by releasing competition pressure would produce relatively larger tadpoles that metamorphose early (e.g., Wilbur et al., 1983; Sredl and Collins, 1992; Relyea and Rosenberger, 2018); and
- 4) not only would these experimental results apply to our field data, but we would find evidence of density-dependent carry-over effects resulting from high breeding female, egg, and larval densities in first-time breeding adults (e.g., Semlitsch et al., 1988; Berven, 1990; Van Allen et al., 2010).

## MATERIALS AND METHODS

**Study site.**—Our study site was located on the 729-hectare Hillenbrand Fish and Wildlife Area-West (HFWA-W), approximately 5 km south of Jasonville, in Greene County, Indiana. Prior to EuroAmerican settlement, this region was eastern deciduous forest punctuated by pocket prairies (Transeau, 1935). With settlement, this landscape was converted to agriculture then farmed for over a century before being surface mined for coal from 1976–1982. Post-mining, the soil was re-contoured and seeded to herbaceous vegetation in accordance with the federal Surface Mining Control and Reclamation Act (SMCRA; P.L. 95-87, enacted on August 3, 1977). The state purchased this property in 1988, and since then the Indiana Department of Natural Resources' (IDNR) Division of Fish and Wildlife has managed this land as native prairie (Lannoo et al., 2009; Terrell et al., 2014).

Most of our Crawfish Frog demographic data came from a previously unnamed wetland we called Nate's Pond. This wetland was formed unintentionally by soil slumping due to uneven overburden compaction during the reclamation process. Nate's Pond functions as a semi-permanent wetland, holding water throughout the year in most years, but it dries during droughts. The only time we observed it dry completely when Crawfish Frogs were present was during the 2012 drought, when it dried on 30 May and eliminated that year's cohort of tadpoles.

**Study species.**—Crawfish Frogs have long been considered the most secretive ranid species in North America (Wright and Myers, 1927) because they occupy abandoned crayfish burrows and typically never range far from them, except to breed (Heemeyer and Lannoo, 2012; Heemeyer et al., 2012). Early field biologists observed that Crawfish Frogs bred in the spring and metamorphosed in July and August, suggesting a larval period of about 90 days (Thompson, 1915; Wright and Meyers, 1927; Gloyd, 1928; Wright and Wright, 1933; Cagle, 1942).

At our study site, peak Crawfish Frog breeding ranged from 14 March to 4 April, but was generally centered around 1 April (Lannoo et al., 2017). Larval period length varied from 71 to 116 days (Table 1). This large variation meant peak

metamorphosis occurred during a six-week period ranging from the middle of June through late July.

Nearby, in southern Illinois, the majority of breeding Crawfish Frogs in one study population were either three or four years old, and none of the 59 breeding adults was more than five years old (Redmer, 2000). Male Crawfish Frogs first breed at two years old, females at three (Redmer, 2000). Over the eight years of our study, we processed 1,102 breeding Crawfish Frog adults consisting of 729 males, 371 females, and 2 unsexed frogs. Confirming Redmer's (2000) results, in our study roughly two-thirds of all Crawfish Frog males first bred in their second year, with the remainder breeding in their third year. Further, only one-third of all Crawfish Frog females first bred in their second year, with the remaining two-thirds breeding in their third year (Lannoo and Stiles, 2020). Breeding adults averaged 92 mm (males) and 97 mm (females) in length (SVL, snout-vent length), and 93 g (males) and 82 g (females). The longest frog we captured was a gravid female at 120 mm (183 g, unknown age). The heaviest frog we captured was a gravid female at 193 g (116 mm, unknown age). The smallest breeding male frog was 61 mm and 33 g (two years old); the smallest breeding female was 71 mm and 42 g gravid (unknown age). Survivorship varied by life history stage, as follows: eggs, ~98%; larvae, ~1%; juveniles, ~3%; adults, ~70% (Lannoo and Stiles, 2020). From these data, it is clear that the highest mortality in Crawfish Frogs occurs during the larval and juvenile stages.

Size at metamorphosis varied. Wright and Wright (1933) offer that the average size of newly metamorphosed juveniles is ~30 mm. In our study, in every year except 2014, juveniles were larger than this (Table 1). However, in 2013, a population of Crawfish Frogs on private property, 5 km from our study site, which bred at the same time as frogs in our population, produced juveniles in shallow prairie swale wetlands that metamorphosed a full month earlier and nearly a centimeter shorter (25 mm SVL;  $n = 51$ ; Lannoo and Stiles, 2020) than juveniles at Nate's Pond.

At our study site, Crawfish Frog tadpoles develop syntopically with three other ranid species—Green Frogs, American Bullfrogs (*R. catesbeiana*), and Southern Leopard Frogs. Green Frogs and Southern Leopard Frogs are the most abundant of the four ranids (Lannoo et al., 2009), and their tadpoles likely compete with developing Crawfish Frog tadpoles (Terrell et al., unpubl.). At HFWA-W, Green Frogs and Bullfrogs breed during the summer; their tadpoles overwinter and complete metamorphosis the following summer (Minton, 1972). Southern Leopard Frogs may breed in the fall or spring (Lannoo, 2005; Stiles and Lannoo, 2015), but in 2011, when we conducted our enclosure study, adults bred only in the spring, syntopically with Crawfish Frogs. Due to these natural history differences, at the beginning of the enclosure study, second-year Green Frog tadpoles were substantially larger (2.0–2.5 cm SVL) than either recently hatched Crawfish Frog tadpoles or Southern Leopard Frog tadpoles, which were similarly sized (~0.5 cm SVL).

**Enclosure studies.**—To test the effects of high density (both intraspecific and interspecific) and vertebrate predation on the growth and survivorship of Crawfish Frog tadpoles, we used 379 L aquatic mesh enclosures (Reptariums®, Apogee, Dallas, TX; filled to ~340 L). On 27 April 2011 (day 0), we placed 15 enclosures in the shallows of Erosion Control Pond

**Table 1.** Crawfish Frog field data (collected at Hillenbrand Fish and Wildlife Area-West, Greene County, Indiana) showing effects of larval density on juvenile size, time to metamorphosis, survivorship, and breeding adult size.

(A)		Initial breeding			
Cohort year	# Females breeding	Females avg SVL (mm)	Est. # eggs laid	Survivorship to metamorphosis (%)	
2009	31	100±2	196,478	0.1	
2010	22	100±3	136,380	1.5	
2011	32	97±3	185,504	1.7	
2012	8	102±3	52,194	0	
2013	52	88±2	211,828	0.004	
2014	37	93±2	183,889	0.5	
(B)		Juveniles produced			
Cohort year	# Juveniles produced	Avg SVL (mm)	Avg mass (g)	Days to metamorphosis	Survivorship to breeding (%)
2009	286	34±0.2	4.6±0.07	90±0.5	2.8
2010	2,103	33±0.1	3.4±0.03	82±0.3	4.1
2011	3,122	31±0.1	2.5±0.02	97±0.5	2.6
2012	0	—	—	—	—
2013	8	33±1.7	2.9±0.70	116±8.6	0
2014	844	30±0.2	2.5±0.04	71±0.3	2.5
(C)		Adults produced			
Cohort year	# Adults (male/female)	Avg SVL (mm)	Avg mass (g)		
2009	5/3	91±3.5	73±16.7		
2010	50/37	86±1.4	71±3.9		
2011	51/31	79±1.9	57±4.6		
2012	—	—	—		
2013	0	—	—		
2014	16/5	90±2.4	80±6.9		

at HFWA-W. We separated tadpoles into five treatments, each with three replicates, as follows: 1) 30 Crawfish Frogs (CF; density of 0.09 tadpoles/L); 2) 90 CF (0.27 tadpoles/L); 3) 45 CF and 45 Leopard Frogs (0.13 tadpoles/L/species); 4) 45 CF and 20 Green Frogs (0.13 CF tadpoles/L; 0.06 GF tadpoles/L); and 5) 90 CF and 5 Small-mouthed Salamander larvae (0.27 CF tadpoles/L; 0.02 SS larvae/L). The enclosures were placed linearly along the bank, blocked by replicates containing treatments. Each replicate had one enclosure of each treatment randomly assigned at the start of the experiment within the block. Each enclosure was situated such that a minimum 7.5 cm gap was present between the water surface inside the enclosure and the mesh top, to ensure tadpoles had access to atmospheric oxygen. To accomplish this, enclosures were slid down-bank as this wetland dried, and up-bank following heavy rains. Enclosure placements were never adjusted more than a meter up or down bank, and never more often than once a week.

For the density treatments, we obtained Crawfish Frog and Southern Leopard Frog tadpoles from Cattail Pond. The wetland is a population sink. Each year of our study, from 2009–2016, between 20 and 40 Crawfish Frogs bred in this wetland, but the egg masses deposited (all of which were viable) produced few Crawfish Frog juveniles (46 total over eight years), which in turn generated no breeding adults. We collected tadpoles of both species from egg masses laid on the same night. We trapped Green Frog tadpoles at the same

HFWA-W wetland (Erosion Control) where we conducted the treatments. Erosion Control Pond was a recently constructed (2008), permanent wetland naturally colonized by Southern Leopard Frogs, Green Frogs, and Bullfrogs in 2009, and Crawfish Frogs in 2010 (Lannoo et al., 2009; Lannoo and Stiles, 2020). The western end of this wetland is deep, and always held water, while the eastern end, where we placed our enclosures, was shallow (<0.5 m) and would often dry by late summer. Before placing tadpoles in enclosures, we reared Crawfish Frog and Southern Leopard Frog tadpoles in small, floating aquaria until the animals were free swimming. We fed these tadpoles frozen, chopped spinach *ad libitum* prior to assigning them to treatments. For the predation treatment, we trapped or seined HFWA-W wetlands for Small-mouthed Salamander (*Ambystoma texanum*) larvae.

We supplied each enclosure with 454 g per week of frozen, chopped spinach, a rate V.C.K.T. considered, based on her undergraduate experiences with mesocosms, sufficient for growth but creating competition. (Indeed, we found that, typically, throughout most of the experiment, spinach would persist from one feeding to the next in the low-density intraspecific treatment but be fully consumed in the high-density treatment.) We examined each enclosure daily for metamorphosed frogs and salamanders, as well as for any predators such as odonate naiads that might have negotiated the mesh or the zippered openings (we never found any). As we collected metamorphosing juveniles, we measured their



SVL (mm) and mass (g), and provided an identification mark (toe-clip) prior to release near their natal wetland. We ended the experiment after all the tadpoles had metamorphosed.

**Field studies.**—From 2009 through 2016, we set up a drift fence/pitfall trap array around Nate's Pond (details in Kinney, 2011; Lannoo et al., 2017), which allowed us to trap breeding Crawfish Frog adults and newly metamorphosed juveniles. Given the low trespass rate of breeding adults (<1%; Kinney, 2011), and the small size and restricted locomotor capacity of newly metamorphosed juveniles, we consider our drift fence data to be a census rather than a sampling of this population (Alford et al., 2001; Lannoo et al., 2017). We weighed and measured each captured adult and juvenile, except in 2013, where the large number of juveniles and time constraints forced us to process every tenth animal. We inserted a passive integrated transponder (PIT) tag into each captured Crawfish Frog adult, which allowed us to identify individuals. We toe-clipped juveniles to indicate year and pond, which allowed us to identify cohorts. We considered the number of days to metamorphosis to be from the date of peak breeding to the date tadpoles completed metamorphosis (Stage 46; Gosner, 1960). We calculated estimated number of eggs laid using the following regression equation (Kinney, 2011), based on data presented in Redmer (2000):

$$\begin{aligned} \text{Total \# of eggs deposited} \\ = \# \text{ of breeding females} \times (-10,974.3 + 172.4 \times \text{mean SVL}). \end{aligned}$$

When assessing adult size across cohorts, we only used data from 2-year-old, first-time breeding animals (to avoid confounding our results by mixing size data from these animals and larger 3-year-old animals). When assessing juvenile survivorship to first reproduction, we used data from all known first-time breeding adults produced by a cohort. These animals included both 2- and 3-year-old first-time breeders, plus any adults collected subsequently (one female bred at Nate's Pond every year of our study [from 2009–2016], which made her at least ten years old; Lannoo et al., 2017; Lannoo and Stiles, 2020). Because we terminated our study in 2016, in order to estimate juvenile survivorship for the 2014 cohort, we calculated the ratio of 2-year-old breeders to all other adults collected for the 2009, 2010, and 2011 cohorts (0.99:1.00), then used this ratio to estimate survivorship from metamorphosis to first reproduction in the 2014 cohort.

**Statistics.**—In our enclosure studies, to determine whether competition and predation affected survival, length, mass, and days to metamorphosis, we compared treatments using one-way analysis of variance (ANOVA) and *post hoc* Tukey's honestly significant difference tests in Program R version 3.3.0 (R Core Team, 2013). We transformed survivorship data using an arcsine function to meet the parametric assumption of a normally distributed sample. For these results, we report means with 95% confidence intervals and statistical significance at  $P < 0.05$ .

Our field data, representing only four breeding years (cohorts; see below) of useable data, but encompassing 12 variables (Table 1), presented the statistical challenge of a small sample size relative to the number of variables being considered. We eliminated potential explanatory variables based on correlations with other variables (e.g., number of

**Table 2.** Linear regression models constructed in program R assessing fitness and recruitment. Data are from Nate's Pond cohorts 2009, 2010, 2011, 2014. Abbreviations: #Eggs = estimated number of eggs deposited; Cohort size = number of juveniles; Days = days to metamorphosis; and Survival = survivorship from metamorphosis to first breeding. \*  $P < 0.05$ .

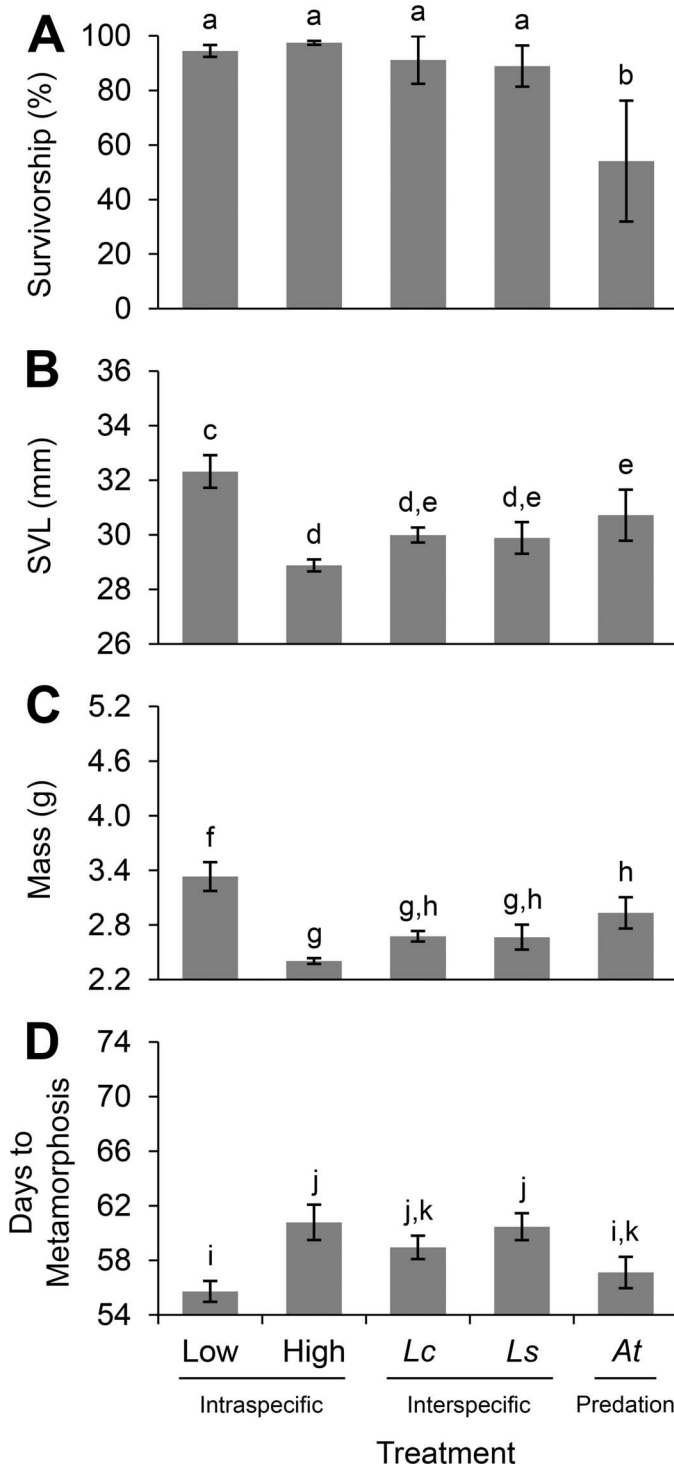
(A) Direct density-dependent effects			
Response	Explanatory	$R^2$	$P$ -value
Cohort size	#Eggs	0.14	0.624
Juvenile SVL	#Eggs	0.03	0.836
	Cohort size	0.09	0.694
Juvenile mass	#Eggs	0.01	0.891
	Cohort size	0.36	0.402
Days	#Eggs	0.06	0.749
	Cohort size	0.24	0.513
(B) Carry-over effects			
Response	Explanatory	$R^2$	$P$ -value
Survival	Cohort size	0.04	0.794
	Juvenile SVL	0.27	0.485
Adult SVL	Cohort size	0.94	0.028*
	Juvenile SVL	0.09	0.698
Adult mass	Cohort size	0.70	0.165
	Juvenile SVL	0.00	1.000
Adult number	Cohort size	0.86	0.074
	Juvenile SVL	0.01	0.906

females breeding and female size was positively correlated with estimated eggs deposited, and juvenile mass was positively correlated with juvenile SVL). We chose candidate models *a priori* based on knowledge acquired from our eight years of field data collected on Crawfish Frogs (as recommended by Burnham and Anderson, 2010).

We divided our analysis into direct density effects (effects of larval factors on newly metamorphosed juvenile characteristics) and carry-over effects (effects of larval factors on older juvenile and breeding adult characteristics; Table 2). To assess direct density effects, we used the explanatory variables of estimated number of eggs laid (a proxy for early density) and cohort size (juvenile number, a proxy for late density), on juvenile SVL, juvenile mass, and days to metamorphosis (Table 2A). To address carry-over effects, we used the explanatory variables of cohort size and juvenile SVL on the response variables of survival to first breeding, adult SVL, adult mass, and adult number (Table 2B). We constructed simple linear regression models in Program R. As indicated above, we excluded data from two years with low juvenile numbers (2012,  $n = 0$ ; 2013,  $n = 8$ ), and as a result of small sample sizes, we only included one predictor variable ( $df = 2$ ) in each model. We present  $R^2$  values (i.e., the variation in the dependent variable that can be explained by the explanatory variable) and  $P$ -values (with significance indicated by  $P < 0.05$ ). To compare direct density effects with carry-over effects, we used the common explanatory variable of cohort size.

## RESULTS

**High intraspecific density effects.**—In our enclosure studies, the high-density treatment among Crawfish Frog tadpoles decreased growth, increased the number of days to meta-



**Fig. 1.** Results of enclosure experiments to determine effects of intraspecific competition, interspecific competition, and predation treatments on larval survivorship, size (SVL and mass), and time to metamorphosis. In the two Intraspecific treatments, Low = 30 Crawfish Frog tadpoles/enclosure (0.09 tadpoles/L) and High = 90 Crawfish Frog tadpoles/enclosure (0.27 tadpoles/L). In the two Interspecific treatments, Lc = Green Frog tadpoles and Ls = Southern Leopard Frog tadpoles. In these treatments, in each enclosure we placed 45 Crawfish Frog and 45 Leopard Frog tadpoles (0.13 tadpoles/L/species), and 45 Crawfish Frog (0.13 tadpoles/L) and 20 Green Frog tadpoles (0.06 tadpoles/L), respectively. In the Predation treatment (At), we placed 90 Crawfish Frog tadpoles (0.27 tadpoles/L) and 5 Small-mouthed Salamander larvae (0.02 larvae/L) per enclosure. Lowercase letters above each column reflect statistical significance using ANOVA. Columns with different letters are statistically significant ( $P < 0.05$ ).

morphosis, and had no effect on larval survivorship (Fig. 1A–D). Newly metamorphosed juveniles from the high-density tadpole treatment (0.27 tadpoles/L) were on average smaller and weighed less than juveniles from the low-density tadpole treatment (0.09 tadpoles/L; ANOVA: SVL,  $F_{4,10} = 18.73$ ,  $P < 0.001$ ; mass,  $F_{4,10} = 30.90$ ,  $P < 0.001$ ; Fig. 1B, C). Further, tadpoles raised at the higher density required an average of five days longer to reach metamorphosis than tadpoles raised at the lower density ( $F_{4,10} = 17.27$ ,  $P < 0.001$ ; Fig. 1D). Larval survivorship was unaffected by high intraspecific competition; there was no statistical difference in survivorship between tadpoles raised at high and low densities ( $F_{4,10} = 7.35$ ,  $P = 0.01$ ; Tukey's HSD:  $P = 0.94$ ; Fig. 1A).

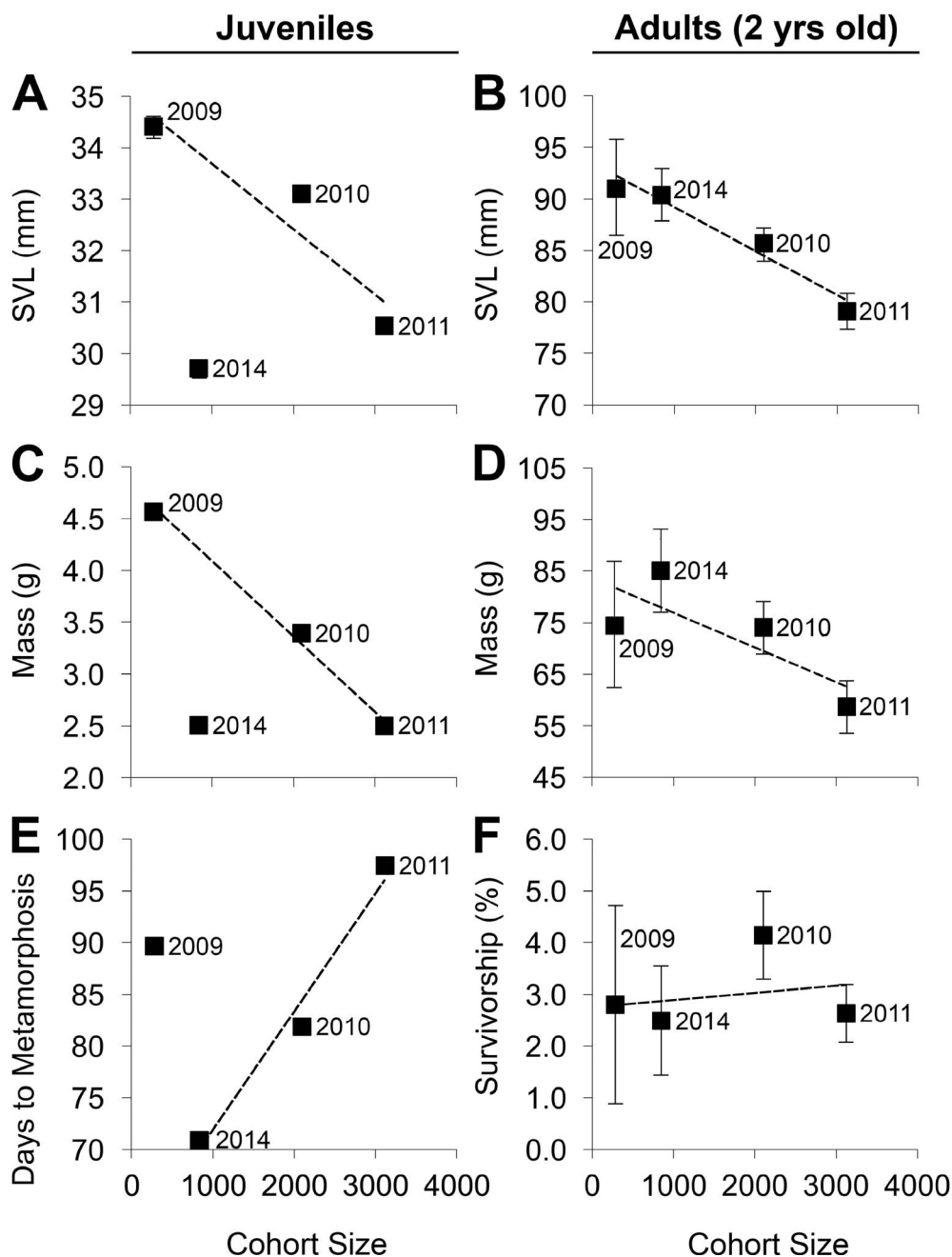
**High interspecific density effects.**—In our enclosures, Crawfish Frogs reared with Green Frogs and Southern Leopard Frogs were smaller (ANOVA: SVL,  $F_{4,10} = 18.73$ ,  $P < 0.001$ ; weight,  $F_{4,10} = 30.90$ ,  $P < 0.001$ ) and required more time to reach metamorphosis ( $F_{4,10} = 17.27$ ,  $P < 0.001$ ) than Crawfish Frogs reared alone at the low density (Fig. 1B–D). Crawfish Frogs reared with Green Frogs were a similar size and weight at metamorphosis as those reared with Southern Leopard Frogs. We found no statistically significant differences across interspecific competition treatments in Crawfish Frog growth (Tukey's HSD: SVL,  $P = 1.00$ ; mass,  $P = 1.00$ ) and time to metamorphosis ( $P = 0.31$ ; Fig. 1). Larval survivorship was high in Crawfish Frogs reared at high densities with both Green Frogs and Southern Leopard Frogs, with no statistically significant species effect (Tukey's HSD:  $P = 0.95$ ; Fig. 1A).

**Predation effect.**—In our enclosure study, predation by Small-mouthed Salamanders significantly reduced survivorship of Crawfish Frog tadpoles (ANOVA:  $F_{4,10} = 7.35$ ,  $P < 0.01$ ; Fig. 1A). However, Crawfish Frog tadpoles reared with salamanders produced larger juveniles that weighed more than juveniles raised in the high-density intraspecific treatment (SVL,  $F_{4,10} = 18.73$ ,  $P < 0.001$ ; mass,  $F_{4,10} = 30.90$ ,  $P < 0.001$ ). These juveniles were, however, smaller and weighed less than in the low-density intraspecific treatment (Fig. 1B, C). Crawfish Frog tadpoles reared with salamanders metamorphosed significantly faster than tadpoles reared in either the high-density intraspecific treatment or co-reared with Southern Leopard Frogs ( $F_{4,10} = 17.27$ ,  $P < 0.001$ ; Fig. 1D).

**Field data: number, size, and survivorship.**—The number of breeding females at Nate's Pond varied from 8 to 52 (Table 1A). On average, the largest females bred in 2012, the smallest in 2013. We estimate the highest number of eggs was laid in 2013, the lowest in 2012. Survivorship to metamorphosis (larval survivorship) ranged from 1.7% of estimated eggs laid to 0 (100% mortality).

The number of newly metamorphosed Crawfish Frog juveniles at Nate's Pond varied from 286 to 3,122 (Table 1B). Juveniles were longest and heaviest in 2009, and shortest and lightest in 2014. The number of days required for metamorphosis was fewest in 2014 and greatest in 2013. As indicated above, we excluded cohorts with low juvenile numbers (2012,  $n = 0$ ; 2013,  $n = 8$ ) in our analyses.

The number of breeding adult Crawfish Frogs produced by each cohort also varied, from 8 (2.8% survivorship) to 87 (4.1%; Table 1B, C). Based on the ratio of 2-year-old breeders to all other adults, we estimate an additional 21 frogs from the 2014 cohort (making a total of 42 frogs) would have bred



**Fig. 2.** Field data showing effects of juvenile density on juvenile body size (A, C) and time to metamorphosis (E), and breeding adult size (B, D) and survivorship (F). Lines reflect regression calculations. While suggestive, none of these trends is significant except for the adult SVL data shown in B.

if our study continued beyond 2016. This estimate increases the survivorship of the 2014 cohort from 2.5% to 5.0%. The size of breeding adults produced also varied by cohort. The 2009 cohort produced the longest adults, while 2014 produced the heaviest adults (Table 1C). The 2011 cohort produced both the shortest and the lightest adults.

**Field data: direct density dependence and carry-over effects.**—From a statistical perspective, there were no significant effects of estimated number of eggs laid or cohort size on juvenile SVL and mass, or days to metamorphosis (Table 2A). Similarly, with one exception (the inverse effects of cohort size on adult SVL;  $R^2 = 0.94$ ,  $df = 0.94$ ,  $P = 0.03$ ), there were no significant effects of cohort size and juvenile SVL on survival to first breeding, adult SVL or mass, or adult number (Table 2B). A visualization of these data, however, suggests that the relationships between cohort size and juvenile and

adult characteristics are stronger than the power of our statistical tests to detect them (Fig. 2). In particular, in 2009, 2010, and 2011, but not 2014, cohort size had a clear negative effect on juvenile length (Fig. 2A) and mass (Fig. 2C). In 2010, 2011, and 2014, but not 2009, cohort size had a clear positive effect on days to metamorphosis (Fig. 2E). These relationships between cohort size and body size carried over to adult length (Fig. 2B) and mass (Fig. 2D). There was no correlation between cohort size and survivorship from metamorphosis to first breeding ( $R^2 = 0.04$ ,  $df = 2$ ,  $P = 0.79$ ; Fig. 2F).

## DISCUSSION

Using cage experiments, we tested three hypotheses addressing the effects of high-density intra- and interspecific competition, and predation, on Crawfish Frog larvae. Using



field data, we then tested a fourth hypothesis on cohort size on newly metamorphosed juvenile size, survivorship, and length of larval period (density-dependent effects). As well, we assessed the effects on adult breeding size and survivorship (carry-over effects). We consider these hypotheses, below.

**Density dependence and predation.**—We hypothesized the high intraspecific density treatment would produce smaller Crawfish Frog juveniles that took longer to metamorphose. Further, we hypothesized high interspecific density would have the same negative effect on Crawfish Frog larvae as high intraspecific density. Our results generally confirmed these hypotheses—high intra- and interspecific densities slowed larval growth and increased time to metamorphosis (Fig. 1B–D). Neither the high intraspecific or interspecific density treatments had an effect on Crawfish Frog larval survivorship (Fig. 1A). Our results echoed (Williams et al., 2012) and confirmed for Crawfish Frogs the effects of high larval densities on juvenile size and time to metamorphosis (Semlitsch and Caldwell, 1982; Smith, 1983; Altwegg, 2003). These results may have differed if we had introduced Crawfish Frog eggs into enclosures containing overwintered Green Frog tadpoles. Sours and Petranks (2007) have shown that ranids will prey on newly laid anuran eggs, and reduce survivorship to hatching.

We further hypothesized that ambystomatid salamander predation on Crawfish Frog larvae would be severe (e.g., Sredl and Collins, 1992; Relyea and Hoverman, 2003), but releasing competition pressure would produce larger larvae that metamorphosed early (Relyea and Rosenberger, 2018), which our data confirmed (Fig. 1B, C). However, the magnitude of this effect was less than observed in other studies (see below).

Importantly, we observed similar density effects in our field data. Cohort size negatively influenced newly metamorphosed Crawfish Frog juvenile length and mass (Fig. 2A, C), and positively influenced time to metamorphosis (Fig. 2E). (Outliers from 2014 [Fig. 2A, C] influenced our statistical results [Table 2]. Our suspicion is that food [algae] levels were reduced in 2014 compared to other years, but we did not measure this variable, and therefore can only speculate.) Conversely, cohort size positively influenced time to metamorphosis (in 2010, 2011, and 2014; Fig. 2E), consistent with other field studies (Semlitsch et al., 1988; Berven, 1990; Wilbur, 1997). (The 2009 cohort took much longer to metamorphose—potentially the result of lower than average water temperatures [once again, a variable we did not track].)

**Carry-over and compensatory effects.**—Our field data suggested both carry-over and compensatory effects from the larval to the juvenile and adult life history stages (Fig. 2). In particular, cohort size negatively affected juvenile size (in three years of our study), which in turn affected adult size and number (Fig. 2B, D). These suggested carry-over effects on Crawfish Frogs generally agree with results from studies on a range of other species (*A. talpoideum* [Semlitsch et al., 1988]; *R. sylvatica* [Berven, 1990]; *A. opacum* [Scott, 1994]; *Bufo terrestris* [Beck and Congdon, 2000]).

What is perhaps most interesting about these data, however, is that the 2014 outliers we observed in data detailing the effects of cohort size on *juvenile* SVL and mass (Fig. 2A, C) are no longer outliers when considering the effect

cohort size has on *2-year-old breeding adult* SVL and mass (Fig. 2B, D); they have been compensated for, similar to the findings of Boone (2005). As further evidence for compensatory effect, we note the slopes describing the relationship between cohort size and 2-year-old breeding adult SVLs and mass (Fig. 2B, D) are shallower than the slopes describing the relationship between cohort size and newly metamorphosed juvenile SVL and mass (Fig. 2A, C), suggesting that the high and low extremes seen in juvenile size metrics were moderated in first-time breeding adults.

**How should we be assessing larval density?**—Our regression models suggest that cohort size (a proxy for final density) correlated better with juvenile SVL and mass, and days to metamorphosis than did the estimated number of eggs laid (a proxy for initial density; Table 2A). As most aquatic amphibian biologists know, an accurate assessment of larval density is difficult to obtain, or even estimate. While the general trend is for seasonal and semi-permanent wetlands to fill with snowmelt and rain water in the early spring, then gradually dry as the warm season progresses, this is far from predictable on a day-to-day, or year-to-year basis (Semlitsch et al., 1996). Late-spring thunderstorms can refill drying basins, and in a wet spring following a dry winter, there may be more water present at the time of metamorphosis than there was three months earlier when eggs were laid (Euliss and Mushet, 1996). In general, drawdowns concentrate animals and increase density, while heavy predation reduces density (Wilbur et al., 1983; Sredl and Collins, 1992; Relyea and Rosenberger, 2018).

Moreover, most density estimates fail to consider animal size. It is possible to fit 1,000 newly hatched Crawfish Frog tadpoles in a liter of water, but impossible to fit 1,000 pre-metamorphic tadpoles in the same volume. Rather than number of animals per volume of water, a more accurate assessment of density effects might compare volumes of animals per volume of water. All of these uncertainties are in play when considering density-dependent effects on the animals experiencing them.

Despite Wilbur's (1997) observation that size at metamorphosis is an exponentially decreasing function of *initial density* of the population, field studies of density-dependent effects in amphibians with complex life histories have used juvenile number (*final density*) as the proxy for density (Semlitsch et al., 1988). In fact, during years with wetland drawdowns and low predation, it is likely that the time immediately prior to metamorphosis is the time of highest tadpole density. But this may not always be true, and other proxies for density, such as number of breeding females and estimated number of eggs deposited—which assess densities earlier in the larval period—might serve just as well (and be consistent with Wilbur, 1997).

**How to define metamorphosis?**—There is one final consideration regarding the timing of metamorphosis that has never been fully addressed, which certainly contributes noise to data such as ours. If metamorphosis is defined not when animals actually metamorphose, but when juveniles emerge and appear in drift fence buckets (technically, post-metamorphic dispersal), then metamorphosis as defined in studies such as ours (e.g., Semlitsch et al., 1988) is not some continuous, normally distributed function related to the completion of larval development, but rather the saltatory



response of post-metamorphic animals to periodic nighttime rains—an environmental condition permitting distant dispersal across terrestrial landscapes hostile to animals prone to desiccation (Kinney, 2011; Lannoo et al., 2017). True metamorphosis likely occurs days, if not longer, before metamorphosis is signified by dispersing juveniles appearing in pitfall traps.

**Conservation implications.**—Our experimental data confirm the negative effects of high larval density on juvenile body size and time to metamorphosis in Crawfish Frogs (Williams et al., 2012; Fig. 1). Our field data are consistent with this relationship, and further show that the negative effects of high larval densities on juvenile body size carry over to influence breeding adult body size, although we also show evidence for offsetting compensatory effects (Fig. 2). With these facts in mind, we revisit the findings of Berven (1990). Given that larger adult size positively affects fitness by increasing the numbers of eggs produced by females, our data then suggest that the second consequence of increased adult fitness—that is the consequence of having increased numbers of eggs and therefore larvae—becomes diminished fitness, as density-dependent factors act on this increased number of larvae by making each animal smaller. These small larvae then produce small juveniles, which in turn produce smaller adults, which have comparatively reduced fitness. Following this logic, one consequence of increased adult fitness is, eventually, reduced adult fitness. We suppose by reverse logic the converse is also true—that smaller adults produce fewer eggs that produce larvae that, by not being constrained by density, grow bigger and produce juveniles that go on to produce larger adults that have increased fitness.

The question for our Crawfish Frog population, and pond-breeding amphibian populations in general, then becomes, which situation is better for population persistence: large numbers of small adults, or small numbers of large adults? Typically, amphibian population assessments are made based on body counts (e.g., Pechmann et al., 1991; Green, 2003), suggesting that population size is more important than body size when considering population viability. But, from the standpoint of reproductive potential, might a decline in population size be compensated for by an increase in the body size of the remaining individuals? The data we present here suggest they can, up to a point. In 2013, 52 small (88 mm SVL) Crawfish Frog females at Nate's Pond produced an estimated 211,828 eggs, while in 2009 only 31 large (100 mm SVL) females produced an estimated 196,478 eggs (Table 1A); that is, a reduction of 40% in the number of females breeding (31 vs. 52) produced a reduction of only 7% in the estimated number of eggs deposited (196,478 vs. 211,828). It is beyond the scope of this paper to formally consider this question, but if due to larval density-dependent factors there is an inverse relationship between adult size and adult number, a population decline may become irreversible when the increased reproductive potential of the remaining large adults is insufficient to overcome the reduced reproductive potential caused by the diminished population size.

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