

# Post-Metamorphic Carry-Over Effects in a Complex Life History: Behavior and Growth at Two Life Stages in an Amphibian, *Anaxyrus fowleri*

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**Carry-over effects, which occur when an animal's early life experience has lasting effects on its later life, may be manifested in an animal's behavior, growth, and fitness, and are often overlooked as contributing factors to such aspects of animal ecology. Metamorphosis is a process that can produce such effects in animals with complex life histories, due to the significant energetic cost and physical changes undergone during this process. We explored the potential impact of tadpole density after metamorphosis in a pond-breeding amphibian, *Anaxyrus fowleri*. If larval density induces carry-over effects, then there should be a positive correlation in behavior and/or relative growth rate between pre-metamorphic tadpoles and the same animals as post-metamorphic toadlets. We raised tadpoles at six density levels, ranging from 0.08 tadpoles/L to 1.67 tadpoles/L, designed to produce variation in growth rate and toadlet size, and quantified relative activity by monitoring movement per five-second intervals in tadpoles and one-minute intervals in toadlets. Among tadpoles, activity varied with density as a quadratic curve and best predicted growth rate as a negative log<sub>e</sub>-linear function. For toadlets, their density as tadpoles did not predict relative growth rate. Alternatively, we found a positive relationship between activity level and toadlet body size and, when considered in terms of density treatments as larvae, found evidence for body size predicting opposite trends in activity between the two life stages. Our results show evidence of density-dependent carry-over effects when comparing relative growth rate, and indirectly when comparing size-dependent activity level between life stages, in this pond-breeding amphibian. Further information is needed to determine if carry-over effects impact individual fitness, which can have implications at the population level.**

A carry-over effect can be defined as an impact on an individual's performance that can be explained by its previous life history or experience (O'Connor et al., 2014). Carry-over effects may impact various traits relating to survival and fitness, such as body condition (Marra et al., 1998; Bearhop et al., 2005) and reproductive output (Olive et al., 1997; Baker et al., 2004; Catry et al., 2013), and can have lasting effects across life stages (Van Allen et al., 2010; Green and Bailey, 2015) and seasons (Norris, 2005; Harrison et al., 2011). Long term impacts on individual fitness that can result from drastic environmental changes, such as habitat loss, can occur in the form of carry-over effects (Pechenik et al., 1998; Pechenik, 2006; Harrison et al., 2011) and manifest in the next generation as maternal effects (O'Connor et al., 2014). Yet it is often difficult to amass empirical evidence for carry-over effects in wild animals since individual animals need to be tracked through successive life stages, juvenile mortality can be high in many taxa (Victor, 1986; Gosselin and Qian, 1997; Casale et al., 2015), and locating individuals over long distances or time periods can be logistically challenging.

Carry-over effects have been detected in a wide variety of taxa, including birds (Drake et al., 2013; Clausen et al., 2015), mammals (Perryman et al., 2002; Cook et al., 2004), reptiles (Goodman, 2008), fish (Kennedy et al., 2008), invertebrates (Hettinger et al., 2013; Van Allen and Rudolf, 2013), and plants (Krepkowski et al., 2013), and may be particularly strong in animals with complex life histories, such as amphibians (Earl and Semlitsch, 2013; Tarvin et al., 2015) and many marine invertebrates (Hettinger et al., 2013; Fischer and Phillips, 2014). The larvae of these organisms are exposed to very different environmental conditions than the adults, which can have lasting effects on the animals after metamorphosis (Goater, 1994; Álvarez and Nicieza,

2002; Green and Bailey, 2015). Many amphibian tadpoles can be exposed to such stressful conditions as elevated temperatures (Kern et al., 2014), low oxygen levels, or density-limited resources (Wilbur, 1977; Smith, 1983; Crespi and Denver, 2005) that are often related to the ephemeral nature of their aquatic habitat. Tadpoles trapped in shrinking aquatic habitats can become crowded to very high densities, which will negatively impact growth, size at metamorphosis, timing of metamorphosis, and foraging activity (Altwegg and Reyer, 2003; Boone, 2005; Gomez-Mestre et al., 2010). Furthermore, metamorphosis in these animals is a costly process that is frequently associated with high mortality (Wilbur, 1980; Newman, 1992) and is greatly affected by environmental conditions (Van Buskirk and Saxer, 2001; Walsh et al., 2008; Touchon et al., 2013).

All animals must manage conflicting physiological demands and, ideally, optimize trade-offs in time and resource allocation (Kozłowski, 1992; Takahashi and Pauley, 2010). Because all their reproductive effort is deferred until after metamorphosis, anuran tadpoles manage their physiological resources only between growth, development, storage, and maintenance (Fitzpatrick, 1976; Perrin and Sibly, 1993; Angilletta et al., 2003), and budget their time largely between foraging behavior and defensive behavior (Lima and Dill, 1990). Tadpoles are constrained in the extent to which they can focus on these particular activities and still be able to reach metamorphosis at the proper time and body condition (Chelgren et al., 2006; Steiner and Pfeiffer, 2007). In tadpoles, foraging vs. defensive behaviors can easily be quantified by monitoring activity level under various conditions (Anholt et al., 2000; Marshall et al., 2003) and their size immediately after metamorphosis is predicated by the conditions of their pre-metamorphic environment, particularly relative density (Scott, 1994; Pechenik et al., 1998).

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Thus an anuran tadpole's pre-metamorphic life could have a profound influence on its post-metamorphic fate, provided that its condition as a metamorph has a role in determining its subsequent success in the terrestrial landscape (Álvarez and Nicieza, 2002; Pechenik, 2006). Larger body size following metamorphosis can confer a considerable survival advantage in a variety of anurans (Chelgren et al., 2006; Cabrera-Guzmán et al., 2013; Tarvin et al., 2015). Therefore, a carry-over effect of tadpoles living at high densities may be that they are of notably smaller metamorph size and have a lower overall survivorship compared to tadpoles raised under lower densities (Yagi and Green, 2016). In contrast, smaller individuals that had suffered slow growth in early life can exhibit compensatory, or "catch-up," growth as they mature in the terrestrial environment (Boone, 2005; Tarvin et al., 2015), a phenomenon observable in other sorts of animals as well (Radder et al., 2007).

We sought to examine how larval density, used as a proxy for varying larval growth conditions (Yagi and Green, 2016), can affect individual animals with complex life histories across the pre- to post-metamorphic transition. If the effect of larval density on growth rate and activity level directly carries over after metamorphosis in animals like Fowler's Toads, *Anaxyrus fowleri*, then slow-growing or highly active larvae may remain smaller or relatively active as post-metamorphic juveniles. Alternatively, if larval density effects do not carry over after metamorphosis in these animals, then any effects density may have had on individuals before metamorphosis should disappear after metamorphosis.

## MATERIALS AND METHODS

**Study species and study site.**—The Fowler's Toad, *Anaxyrus fowleri*, is a common toad of eastern North America, noted for living in areas with sandy substrate and ranging as far north as the northern shore of Lake Erie in Ontario, Canada. In Canada, these toads exist in small, isolated populations. This study was conducted in the Thoroughfare Point Unit of the Long Point National Wildlife Area (NWA) in summer of 2014 (between 42°34'33"–42°35'3"N and 80°22'15"–80°28'24"W) in southern Ontario. This population is part of a long-term population monitoring study (Greenberg and Green, 2013; Middleton and Green, 2015), where the population estimate in 2014 was about 32 adults (unpubl. data), and natural tadpole densities are assumed to be very low (Yagi and Green, 2016). We used experimental ponds that were dug in the marshes in collaboration with the Canadian Wildlife Service in November 2012 (Yagi and Green, 2016).

**Experimental set-up.**—As described by Yagi and Green (2016), we collected egg masses from three amplexant pairs of Fowler's Toads between 24 May and 26 May 2014 and raised the larvae in floating mesh pens until they resorbed their external gills (i.e., reached stage 25; Gosner, 1960). We then mixed all tadpoles together to reduce maternal and genetic bias, and counted out groups of 100 to grow in six density treatments varied by volume, in floating mesh pens placed in a pond: 0.08 tadpoles/L, 0.13 tadpoles/L, 0.25 tadpoles/L, 0.5 tadpoles/L, 1.0 tadpoles/L, and 1.67 tadpoles/L (Yagi and Green, 2016). Each density treatment was replicated three times (i.e., in three ponds). Density treatments were designed to vary by volume in order to produce equal number of metamorphs and toadlets for further studies. The floating pens both physically protected the tadpoles from aquatic predators and ensured equal exposure of the tadpoles to any

chemical cues in the water from either predators or other tadpoles (Yagi and Green, 2016). All experiments occurring after the assignment of tadpoles to density treatments assume that the three egg masses are represented randomly in each test.

We monitored tadpole growth by photographing a sample of 20 individuals per floating pen, in a petri dish overlaid on 6 mm grid paper, repeated every 4 to 7 days through to metamorphosis. We measured total body length digitally, using the image processing software ImageJ (vers 1.46r, Schneider et al., 2012). Air and pond temperatures were measured hourly by deploying three temperature data loggers (iButton, model DS1922L; i.e., one recording ambient air and two recording water temperature) waterproofed with Plasti-dip® at each pond location (Yagi and Green, 2016). Upon reaching their developmental stage where four limbs and full tail are present (stage 42), metamorphs from each density treatment group were moved to semi-terrestrial, 2 m<sup>2</sup> fiberglass mesh enclosures with natural substrate and vegetation growth, and raised until complete tail resorption (stage 46), henceforth called toadlets. Toadlets were not fed, and instead sustained themselves on the naturally occurring insect community within the vegetated enclosures. We monitored toadlet growth every four to seven days by sampling ten individuals per group, measuring snout–vent length using dial calipers, and calculating the means. Once they had grown to >13 mm snout–vent length, toadlets were measured, weighed, and photographed for later identification. This size was chosen based on the better visibility of their dorsal spot patterns, which were used for individual identification for further recapture studies.

**Relative growth rates.**—We calculated mean relative growth rates (mm/day/mm) of tadpoles groups from each density treatment in each pond as the mean maximum growth rate (Yagi and Green, 2016) divided by the mean initial total length. For toadlets, growth rate for each density group from each pond was calculated as the difference in mean body length (i.e., snout–vent length) between the first and last mean body length measurements of each group, divided by the number of days between the two measurements. Relative growth rate was calculated by dividing this value by the mean initial toadlet body length.

**Activity levels.**—We measured activity level in 18 tadpoles, at developmental stage 39 (i.e., only hind legs present, just prior to forelimb bud formation; Gosner, 1960), from each of the six density treatment groups, for a total of 108 tadpoles (Table 1). We used six tadpoles at a time, placing each tadpole individually in one of six 20 by 20 cm Pyrex® glass dish, in ca. 2 cm of pond water, under the shade of a large umbrella. All tadpoles were filmed continuously for 60 minutes between 1200 and 1600 hrs, in their respective dishes using a digital camera (JVC, model: GZ-E300AU) mounted on a tripod. Tadpoles were only recorded on days when the weather was clear and sunny, to reduce variation in weather conditions between activity tests. Tadpole activity was scored at 5 sec intervals.

We measured activity levels in a total of 29 toadlets: seven from density levels 0.08, 0.13 and 0.25 tadpoles/L, five from density level 0.5 tadpoles/L, and three from density level 1.0 tadpoles/L, each at least 13 mm in body length to ensure both visual detection on camera and a decreased desiccation risk. Toadlets of appropriate size from the highest density level, 1.67 tadpoles/L, were not available. We placed toadlets

**Table 1.** Samples, dates, and temperatures for activity level tests of individuals as tadpoles and then as toadlets according to their densities when reared as tadpoles. Variation is represented by standard error (SE).

Rearing density (tadpoles/L)	Tadpoles			Toadlets		
	Sample size (n)	Date of test	Mean air temperature (°C)	Sample size (n)	Dates of tests	Mean air temperature (°C)
0.08	18	2 July 2014	25.75±0.36	7	30 July–5 August 2014	23.15±0.62
0.13	18	3 July 2014	21.08±0.59	7	30 July–6 August 2014	23.48±0.59
0.25	18	5 July 2014	22.25±0.35	7	2–12 August 2014	24.01±0.30
0.50	18	9 July 2014	20.71±0.42	5	9–12 August 2014	25.00±0.45
1.00	18	14 July 2014	25.79±0.49	3	16–17 August 2014	21.38±1.18
1.67	18	15 July 2014	22.08±0.18	—	—	—

individually into an arena measuring 90 cm in diameter, with ca. 2 cm of moist sand covering the bottom. All toadlet activity tests were conducted indoors, starting by 1100 hrs, and air temperature was recorded hourly using an iButton model DS1922L temperature data logger. We recorded the movements of the toadlets using an infrared, closed circuit television camera (3xLOGIC, model VX-3S-OD-I-VF, 3xLOGIC Inc.; max resolution 2048 × 1536 px) and digital video recorder (VIGIL, model MVR-4IP-2TB, 3xLOGIC Inc.). Recordings were processed with video recording software (v8.0, 3xLOGIC Inc.). The cameras were set to capture an image of the arena every 1 min. for up to 6 hrs.

The animals' activity was scored by recording "active" if the animal had moved during a 5 sec (tadpoles) or 1 min (toadlets) time period, and "inactive" if it had not. We counted the number of "active" and "inactive" time periods scored, and calculated a percentage of activity from the total number of time periods. For comparison, we then averaged individual % active scores per density treatment group and called the result "Activity Level." All toadlets were released to their natural terrestrial habitat once the activity test was complete.

**Analysis.**—Statistical analyses were conducted using appropriate packages in R v.3.3.2 (R Core Team, 2015). We tested for normality of relative growth rate, temperature, and body length data using qq-plots, Shapiro Wilk's test, Levene's test, or Bartlett's test as appropriate. We used  $\log_e$  transformed density values ( $\ln D$ ) for analysis. To assess relative growth rate in relation to  $\ln D$  in R, we used linear mixed models, using the 'lme4' package and 'lmer' function. We set 'pond' as a random effect and included mean pond temperature as an additional fixed predictor for tadpoles and mean air temperature for toadlets. To assess the effect of  $\ln D$  on activity in R, we used the binary ("active," "inactive") activity scores and conducted logistic regressions using generalized linear mixed models, with the 'glmer' function under a binomial distribution. We included body length and mean pond temperature (for tadpoles) and mean air temperature (for toadlets) as fixed predictors, while 'pond' (P) was included as a random effect variable, with "individual" (Ind) nested within it to account for repeated measures. We calculated mean temperatures for activity tests from temperatures recorded throughout the four-hour or six-hour time intervals used for tadpoles and toadlets, respectively. We employed model selection analysis (Akaike, 1987) to determine whether linear or polynomial models best fit the data. The best fit model was examined using R's 'Anova' function from the 'car' package.

To detect the presence of a carry-over effect in activity, we used tadpole Activity Level as the predictor and the corresponding toadlet Activity Level as the response in a linear regression, anticipating that a significant, positive relationship would be consistent with the presence of a carry-over effect. Similarly, to detect the presence of a carry-over effect in growth rate, we used mean tadpole growth rate as the predictor and toadlet growth rate as the response in a linear regression. As we did not have information on individual tadpole growth, we used density group means per pond to associate a tadpole growth rate measure per individual toadlet.

To evaluate the influence of body size in our carry-over effect analyses, we carried out logistic regressions using activity versus body length, separated into density groups for both life stages (tadpoles and toadlet). The significance of each regression was quantified in R using the 'Anova' function, or Wald test statistic. Differences in the Activity versus body length relationship between life stages (i.e., tadpole and toadlet) and density groups were assessed by creating a model with binary activity scores as the response variable, 'length' as a continuous predictor, and 'density' and 'life stage' as interacting categorical predictors, using R's 'glmer' function. Again, 'individual' was nested within the categorical 'pond' variable and set as a random effect term. Multiple comparisons of means via a Tukey *post hoc* test was conducted on the model using the 'glht' function from the 'multcomp' package in R.

## RESULTS

**Growth rate and body size.**—Mean relative growth rate among tadpoles ranged from  $0.048 \pm 0.009$  (SE)  $\text{day}^{-1}$  for those reared at a density of 1.67 tadpoles/L to  $0.098 \pm 0.005$  (SE)  $\text{day}^{-1}$  for those reared at a density of 0.08 tadpoles/L. Mean relative growth rate in tadpoles was negatively correlated with density ( $R^2 = 0.83$ ;  $P < 0.001$ ; Table 2; Fig. 1). Once these animals had become toadlets, however, mean relative growth rate ranged from  $0.008 \pm 0.002$  (SE)  $\text{day}^{-1}$  for those reared at a density of 1.67 tadpoles/L to  $0.016 \pm 0.001$  (SE)  $\text{day}^{-1}$  for those reared at a density of 0.25 tadpoles/L and was not correlated with density or any other physical variable (Table 2; Fig. 1). Removing density from the equation, the relative growth rates of individuals as toadlets were positively correlated with the mean relative growth rates they exhibited in tadpole groups, but the relationship was not statistically significant (Slope = 0.04,  $n = 18$ ,  $R^2 = 0.09$ ,  $P = 0.22$ ). Since trends can be observed, these non-significant results may be due to small sample size. Ponds did not differ from each

**Table 2.** Most informative models, according to Akaike's information criterion for small sample sizes (AICc), for relative growth rate as a function of  $\log_e$  transformed density, with "pond" set as a random effect, for both tadpole and toadlet life stages.  $\omega$  = AICc weight.

Stage	Model*	AICc	$\Delta$ AICc	df	$\omega$
Tadpole	$\ln D + P$	-119.60	0.00	4	0.992
	$\ln D + T_p + P$	-109.80	9.80	5	0.007
	$\ln D + \ln D^2 + P$	-105.50	14.10	5	<0.001
	$\ln D + \ln D^2 + T_p + P$	-95.30	24.30	6	<0.001
	$P$	-88.00	31.60	3	<0.001
	$T_p + P$	-78.00	41.60	4	<0.001
Toadlet	$P$	-135.50	0.00	3	0.995
	$T_a + P$	-124.50	11.00	4	0.004
	$\ln D + P$	-121.50	14.10	4	<0.001
	$\ln D + \ln D^2 + P$	-114.00	21.50	5	<0.001
	$\ln D + T_a + P$	-109.90	25.60	5	<0.001
	$\ln D + \ln D^2 + T_a + P$	-102.30	33.20	6	<0.001

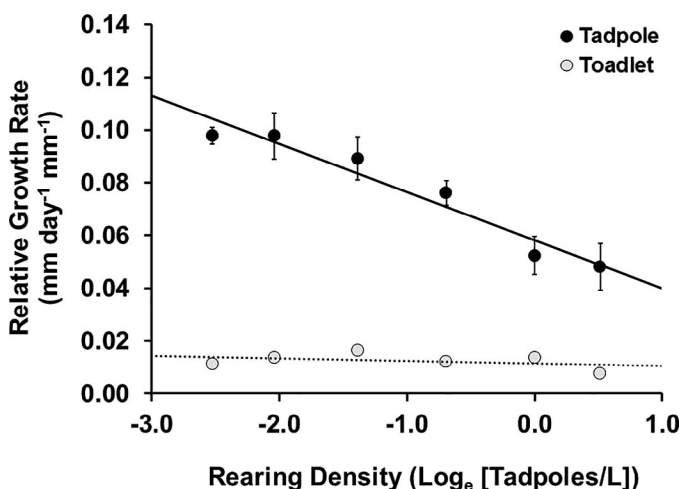
\* symbols:  $\ln D = \log_e(\text{density})$ ,  $P$  = "pond,"  $T_a$  = air temperature,  $T_p$  = pond temperature

other in mean daily temperatures over the course of the 2014 season (see Yagi and Green, 2016).

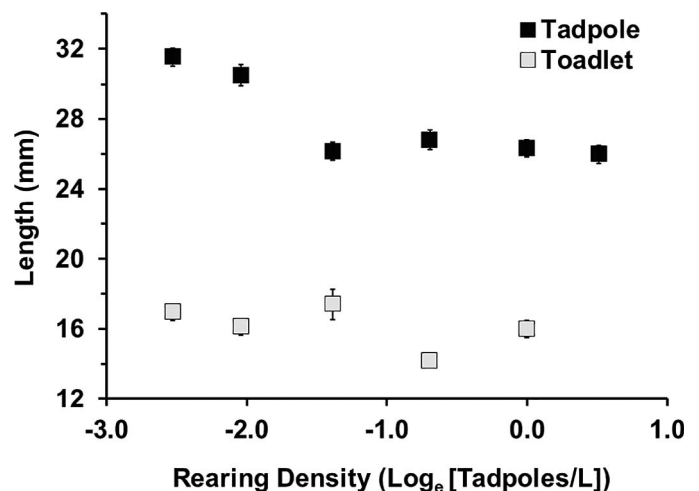
Of the individuals used for the activity tests, the mean tadpole size ranged from a maximum of  $31.6 \pm 0.51$  mm (SE) at density level 0.08 tadpoles/L, and a minimum of  $25.98 \pm 0.52$  mm (SE) at density level 1.67 tadpoles/L. The size of tadpoles from each density group used for the activity level tests negatively correlated with density (Slope =  $-1.79$ ,  $R^2 = 0.37$ ,  $F_{1,106} = 63.89$ ,  $P < 0.001$ ), but there was high degree of overlap in the size range, in the highest four density groups. Tukey *post hoc* test revealed that only density levels 0.08 and 0.13 tadpoles/L differed significantly from the rest ( $P < 0.001$ ) and were not significantly different from each other ( $P = 0.72$ ; Fig. 2). For the toadlets subjected to activity tests, the largest mean body length ranged from a maximum of  $17.40 \pm 0.87$  mm (SE) at density level 0.25 tadpoles/L to a minimum mean of  $14.18 \pm 0.33$  mm (SE) at density level 0.50 tadpoles/L. The correlation between body length and rearing density, although a negative trend, was not significant (Slope =  $-0.68$ ,  $R^2 = 0.10$ ,  $F_{1,27} = 3.05$ ,  $P = 0.09$ ; Fig. 2). However, toadlet initial mean body length (i.e., 4–7 days post emergence) ranged from  $8.81 \pm 0.43$  mm (SE) in the 1.67 tadpoles/L treatment group to  $12.52 \pm 0.52$  mm (SE) in the 0.08 tadpoles/L treatment group. There was a significant, negative,  $\log_e$ -linear relationship

between their initial body length and density (Slope =  $-1.13$ ,  $n = 18$ ,  $R^2 = 0.74$ ,  $P < 0.001$ ).

**Activity levels.**—Tadpoles and toadlets showed different patterns of activity in relation to rearing density. Tadpole mean Activity Level increased with increasing density from  $42 \pm 10\%$  (SE) at 0.08 tadpoles/L up to  $73 \pm 10\%$  (SE) at 0.25 tadpoles/L, but then decreased to  $51 \pm 8\%$  (SE) at 1.67 tadpoles/L. Mean air temperature during the tadpole activity tests was  $22.9 \pm 0.30^\circ\text{C}$  (SE), ranging from  $18.0^\circ\text{C}$  to  $29.0^\circ\text{C}$  (Table 1) and, since each test was carried out on a different day, differed significantly among density groups ( $F_{5,66} = 31.02$ ,  $P < 0.001$ ). The top four explanatory models, all of which included quadratic density coefficients ( $\ln D + \ln D^2$ ), were virtually indistinguishable in terms of information content ( $\Delta\text{AICc} \leq 1.1$ , Table 3). Accordingly, we calculated an averaged model for analysis. But since Model 2 has Models 1, 3, and 4 all nested within it, we selected it for further analysis also. Both the averaged model and Model 2 define the relationship between tadpole activity and density in terms of a quadratic curve in which activity peaks at an intermediate density level (Fig. 3). The full averaged model consisted of four coefficients, where only



**Fig. 1.** Tadpole and toadlet relative growth rates as a function of density ( $\log_e$  transformed). Tadpole data fit a negative linear relationship, whereas the toadlet data did not show any trend. Error bars indicate standard error.



**Fig. 2.** Mean body sizes for tadpoles (total length) and toadlets (snout-vent length) that were subjected to activity level trials, from each density treatment. Although there are strong negative correlations with density at both stages, there is also a high degree of size range overlap among some density groups. Error bars indicate standard error.

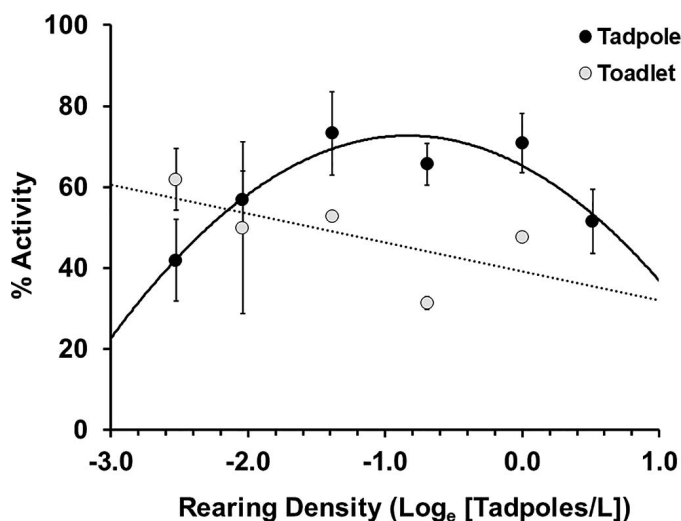
**Table 3.** Most informative models, according to Akaike's information criterion for small sample sizes (AICc), for Activity Level as a function of density, with "individual" nested within "pond" set as a random effect. Additional fixed variables include total body length, mean pond temperature (for tadpoles), and mean air temperature (for toadlets).  $\omega$  = AICc weight. Only the best six models for each stage are shown.

Stage	No.	Model*	AICc	$\Delta$ AICc	df	$\omega$
Tadpole	1	$\ln D + \ln D^2 + T_p + P_{\text{Ind}}$	45280.70	0.00	6	0.320
	2	$\ln D + \ln D^2 + L + T_p + P_{\text{Ind}}$	45280.80	0.20	7	0.300
	3	$\ln D + \ln D^2 + P_{\text{Ind}}$	45281.70	1.00	5	0.190
	4	$\ln D + \ln D^2 + L + P_{\text{Ind}}$	45281.70	1.10	6	0.190
	5	$L + P_{\text{Ind}}$	45302.30	21.70	4	<0.001
	6	$L + T_p + P_{\text{Ind}}$	45303.80	23.20	5	<0.001
Toadlet	1	$\ln D + L + T_a + P_{\text{Ind}}$	13349.90	0.00	6	0.300
	2	$L + P_{\text{Ind}}$	13350.40	0.50	4	0.230
	3	$\ln D + L + P_{\text{Ind}}$	13350.40	0.50	5	0.230
	4	$L + T_a + P_{\text{Ind}}$	13350.50	0.60	5	0.230
	5	$\ln D + T_a + P_{\text{Ind}}$	13364.80	14.90	5	<0.001
	6	$\ln D + P_{\text{Ind}}$	13367.00	17.10	4	<0.001

\* abbreviations:  $\ln D = \log_e(\text{density})$ ,  $P_{\text{Ind}}$  = individual nested within pond,  $T$  = air temperature,  $L$  = total body length

the two density terms proved to be significant ( $\ln D$ :  $Z = 4.98$ ,  $P < 0.001$ ;  $\ln D^2$ :  $Z = 5.54$ ,  $P < 0.001$ ;  $T$ :  $Z = 0.94$ ,  $P = 0.35$ ;  $L$ :  $Z = 0.70$ ,  $P = 0.49$ ), with each coefficient's relative importance ranked as:  $\ln D^2 = 1.00$ ;  $\ln D = 1.00$ ;  $T = 0.62$ ; and  $L = 0.49$ . Similarly, the only significant coefficients in Model 2 were the two density terms ( $\ln D$ :  $\chi^2 = 30.07$ ,  $df = 1$ ,  $P < 0.001$ ;  $\ln D^2$ :  $\chi^2 = 36.16$ ,  $df = 1$ ,  $P < 0.001$ ). Neither temperature,  $T$  ( $\chi^2 = 3.11$ ,  $df = 1$ ,  $P = 0.08$ ), nor body length,  $L$  ( $\chi^2 = 1.91$ ,  $df = 1$ ,  $P = 0.17$ ), were significant.

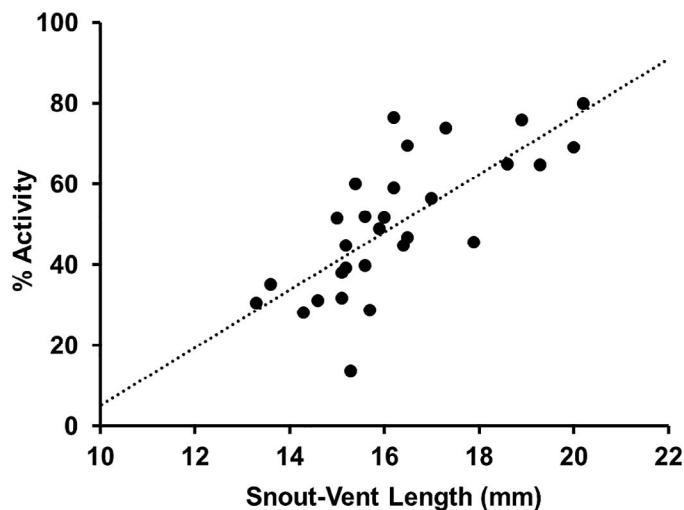
Among toadlets, Activity Levels were negatively correlated with body length when raised at higher densities (0.5 and 1.0 tadpoles/L) and positively correlated when raised at lower densities (0.08, 0.13, and 0.25 tadpoles/L), though only the result for density levels 0.25 tadpoles/L was significant (Fig. 4, Table 4). Mean air temperatures during the toadlet activity tests was  $23.9 \pm 0.17^\circ\text{C}$  (SE), ranged from  $17.0^\circ\text{C}$  to  $30.5^\circ\text{C}$  (Table 1), and did not differ significantly among density groups ( $F_{4,170} = 1.86$ ,  $P = 0.12$ ). As a result of model selection, the top four explanatory models, as observed with the tadpole data, were nearly indistinguishable in terms of information content ( $\Delta\text{AICc} \leq 0.6$ , Table 3).



**Fig. 3.** Tadpole and toadlet activity level, portrayed as a percentage of time active, shown as a function of density ( $\log_e$  transformed). The tadpole fit line conforms to a quadratic relationship whereby activity peaks at an intermediate density level. The toadlet fit line is linear and negative. Error bars show standard error.

Therefore, we calculated an averaged model for analysis. The full averaged model included the three coefficients, where only body length showed significance ( $L$ :  $Z = 4.97$ ,  $P < 0.001$ ;  $\ln D$ :  $Z = 0.78$ ,  $P = 0.43$ ;  $T$ :  $Z = 0.77$ ,  $P = 0.44$ ), and their relative importance was ranked as:  $L = 1.00$ ;  $\ln D = 0.54$ ; and  $T = 0.53$ . Since the strongest correlate with toadlet Activity was body length, we chose to run Model 2 separately, which showed a significant relationship for toadlet activity as a function of body length (Table 3;  $\chi^2 = 30.60$ ,  $df = 1$ ,  $P < 0.001$ ; Fig. 4).

The activity versus body length regressions proved to only be significant in both life stages at density level 0.25 tadpoles/L (tadpole:  $\chi^2 = 5.34$ ,  $df = 1$ ,  $P = 0.02$ ; toadlet:  $\chi^2 = 46.87$ ,  $df = 1$ ,  $P < 0.001$ ), where the log odds ratio was strongly negative for tadpoles ( $-0.23$ ) but positive for toadlets (0.33; Table 4). The interaction of these regressions between density groups proved to be significant ( $\chi^2 = 24.41$ ,  $df = 5$ ,  $P < 0.001$ ), as well as the interaction between life stages ( $\chi^2 = 20.04$ ,  $df = 1$ ,  $P < 0.001$ ). The Activity Levels of tadpoles and their Activity Levels as toadlets (Appendix 1) were negatively correlated, but the relationship was not statistically significant (Slope =  $-0.13$ ,  $n = 29$ ,  $R^2 = 0.02$ ,  $P = 0.47$ ).



**Fig. 4.** Toadlet activity, shown as a percentage of time active, displayed as a function of individual body length (snout-vent length).

**Table 4.** Relationships between activity level vs. total body length for individuals from each rearing density group as tadpoles and then as toadlets.

Rearing density (tadpoles/L)	Tadpoles				Toadlets			
	Log odds ratio	$\chi^2$	df	P	Log odds ratio	$\chi^2$	df	P
0.08	−0.04	0.39	1	0.53	0.22	4.65	1	0.03*
0.13	−0.11	3.77	1	0.05	0.30	2.91	1	0.09
0.25	−0.23	5.34	1	0.02*	0.33	46.87	1	<0.001*
0.50	−0.17	3.15	1	0.08	−0.04	0.21	1	0.65
1.00	0.16	4.68	1	0.03*	−0.15	3.00	1	0.08
1.67	0.18	4.52	1	0.03*				

\* significant at  $\alpha = 0.05$

## DISCUSSION

Our results demonstrated that density during the tadpole stage in Fowler's Toads produces some evidence of post-metamorphic carry-over effects among toadlets. Although increasing tadpole density is related to a log<sub>e</sub>-linear decline in tadpole growth rate (Yagi and Green, 2016), the relationship between toadlet relative growth rate and their density as tadpoles appears to be, more or less, a flat line (Fig. 1). This indicates that toadlets grow at the same relative rate whatever density they experienced as tadpoles. Evidently, once the density pressure imposed on them as tadpoles is released, they experience equal foraging opportunities in the terrestrial landscape (Werner and Anholt, 1993; Stamps, 2007). Since initial body size among toadlets is negatively correlated with their density during the tadpole stage, a uniform post-metamorphic growth rate should ensure that smaller toadlets will remain small relative to their larger counterparts. This could be an important carry-over effect of the animals' density as tadpoles considering the survival advantage conferred by larger body size in anurans (Chelgren et al., 2006; Cabrera-Guzmán et al., 2013; Tarvin et al., 2015). Interestingly, our results are contrary to recent findings of carry-over effects causing compensatory growth in tree frogs (Bouchard et al., 2016), although morphology and digestion were examined here, which is more specific than somatic growth rate. Body size in amphibians is a predictor of successful courtship (Houck, 1988; Mathis, 1991), female fecundity (Tejedo, 1992; Gilbert et al., 1994; Camargo et al., 2005), and overwinter survival (Reading, 2007; Scott et al., 2007).

If carry-over effects are significant drivers of adult fecundity and probability of survival, then there can be several implications, positive and negative, at the population level (Chelgren et al., 2006; Touchon et al., 2013). A carry-over effect of tadpole density might be a potential component of density-dependent regulation of population size (Green and Middleton, 2013; Green, 2015), where the ability for population size increase when at low density and decrease when at high density is intrinsic, and does not necessarily require changes in external pressures like predation. Nevertheless, body size in anuran populations may be highly variable (Galatti, 1992), and body size among adults can be negatively correlated quite strongly with their density (Denton and Beebe, 1993; Green and Middleton, 2013), indicating that the carry-over effects of larval density on growth and adult body size may be variable and intricate.

The relationship of the animals' activity level pre- and post-metamorphosis may be even more complex. In tadpoles, the level of activity appears to peak at intermediate density, possibly as a three-way trade-off between increased competitiveness for limited resources as density increases

(Skelly and Werner, 1990; Werner and Anholt, 1996), increased susceptibility to predation as activity increases (Lawler, 1989; Skelly, 1994; Anholt and Werner, 1995), and decreased resources for growth as energetic expenditure for movement increases (Scott et al., 2007). Tadpoles under stress, which may more likely occur under higher densities, may behave in a way such that smaller individuals do not have the energy reserves required to compete for resources possessed by their larger counterparts. Competition is known to induce stress responses in amphibians (Relyea, 2004; Davis and Maerz, 2009; Ledon-Rettig et al., 2009; Crespi and Warne, 2013). Among toadlets, though, the level of activity appears to be correlated predominantly with individual body size (Fig. 3), which, in turn, is negatively related to their density as tadpoles. There is thus some evidence of an indirect carry-over effect of rearing density on the toadlets' level of activity.

It is important to note that since our toadlets only came from three egg masses, which led to our relatively small toadlet sample size, the representation of carry-over effects in this population may be limited. This issue was inevitable due to the difficulty in finding egg masses in a population of low abundance and the fact that amphibian mortality over multiple life stages is naturally very high. Another potential bias of using only three egg masses is there may be some influence of maternal and genetic effects in our results. Another limitation of the tadpole activity tests was that each density level was tested on a different day. Although this could not be helped due to the effect density has on tadpole growth and development (Yagi and Green, 2016), there is no way of determining if the observed effects were caused by density or daily weather conditions. Since the weather conditions during each tadpole test were generally clear and sunny, we are confident that temperature was the best indicator of the test conditions between days. Fortunately, this was less of an issue for the toadlets since their activity test dates overlapped with individuals from different density groups.

Our study provides empirical evidence pointing to potentially significant carry-over effects after metamorphosis, both directly and indirectly stemming from larval density. The consequences of growth conditions in early life stages have many implications regarding animal interactions with the environment. For metamorphosing individuals that undergo profound niche shifts and/or changes in habitat use, any latent effects on an individual may significantly affect how they prosper in the new food web, especially, as with most anurans, if they have shifted to a different trophic level. Further investigation into carry-over effects and their impact on inter-specific interactions would be valuable for under-

standing the connections between small scale habitat conditions and broad scale ecosystem stability.

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**Appendix 1.** Summary of mean activity level, relative growth rates, body mass, and initial body length per density treatment group for tadpoles and toadlets,  $\pm$  standard error.  $N_1$  is the sample size associated with the activity tests and their mean group body mass, and  $N_2$  is the sample size associated with the relative growth rate and initial body size calculations.

Rearing density (tadpoles L <sup>-1</sup> )	Tadpoles					Toadlets					
	$N_1$	Activity level	$N_2$	Relative growth rate (day <sup>-1</sup> )	Initial body length (mm)	$N_1$	Activity level	Body mass (g)	$N_2$	Relative growth rate (day <sup>-1</sup> )	Initial body length (mm)
0.08	18	0.42 $\pm$ 0.10	20	0.098 $\pm$ 0.005	6.95 $\pm$ 0.11	7	0.62 $\pm$ 0.04	0.49 $\pm$ 0.03	10	0.011 $\pm$ 0.002	12.52 $\pm$ 0.52
0.13	18	0.57 $\pm$ 0.07	20	0.098 $\pm$ 0.009	6.61 $\pm$ 0.23	7	0.50 $\pm$ 0.08	0.41 $\pm$ 0.04	10	0.014 $\pm$ 0.000	10.84 $\pm$ 0.36
0.25	18	0.73 $\pm$ 0.10	20	0.089 $\pm$ 0.011	6.55 $\pm$ 0.19	7	0.53 $\pm$ 0.07	0.51 $\pm$ 0.08	10	0.016 $\pm$ 0.001	10.46 $\pm$ 0.22
0.50	18	0.66 $\pm$ 0.05	20	0.076 $\pm$ 0.005	6.70 $\pm$ 0.17	5	0.31 $\pm$ 0.01	0.27 $\pm$ 0.02	10	0.012 $\pm$ 0.001	9.19 $\pm$ 0.40
1.00	18	0.71 $\pm$ 0.07	20	0.052 $\pm$ 0.007	6.86 $\pm$ 0.07	3	0.48 $\pm$ 0.02	0.39 $\pm$ 0.03	10	0.014 $\pm$ 0.002	9.08 $\pm$ 0.46
1.67	18	0.51 $\pm$ 0.08	20	0.048 $\pm$ 0.009	6.74 $\pm$ 0.02	0	—	—	10	0.008 $\pm$ 0.002	8.81 $\pm$ 0.43