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Density-dependent growth has ordinarily been studied in aquatic ecosystems by varying the abundance of animals in mesocosms of equal volume. Aside from the unequal sample sizes involved with using this abundance-limited method, confounding factors potentially associated with levels of social interactions may also be introduced. The alternative, volume-limited method, i.e., varying the volume while maintaining equal numbers of animals, can provide a test for the presence of potentially confounding factors. Using tadpoles of Fowler's Toad, *Anaxyrus fowleri*, we examined the effect of density on growth rate, timing of metamorphosis, and size at metamorphosis in both abundance-limited and volume-limited experiments. We found no difference in tadpole growth rate or timing of metamorphosis between these two methods, but the metamorphs emerging from abundance-limited low density treatments were significantly smaller when compared to those in volume-limited low density treatments. Because toad tadpoles may naturally form social aggregations and schools, this suggests that the actual number of animals present may be important for normal social behavior and optimizing feeding rate. If volume-limited and abundance-limited methods of manipulating density are not equivalent, treatment method may itself be a factor that can differentially affect growth variables.

CONSPECIFIC density is a fundamental factor influencing somatic growth and survival of plants and animals (Watkinson, 1980; Goater, 1994; Goldberg et al., 2001; Lorenzen and Enberg, 2002; Browne et al., 2003). At high densities, increased competition among individuals for mates and resources is usually associated with negative consequences, such as reduced growth rate (Wilbur, 1977; Sedinger et al., 2001; Lorenzen and Enberg, 2002), prolonged development rate (Warner et al., 1991), and lower survivorship (Dash and Hota, 1980; Warner et al., 1991; Browne et al., 2003). However, it is possible to have positive effects resulting from high larval densities, such that reduced survival in early life stages can increase survival in later life stages and ultimately increase recruitment (Vonesh and De la Cruz, 2002; Karraker et al., 2008). At low densities, reduced competition rates usually allow for positive responses among individuals, like faster development rate or increased somatic growth rate (Wilbur, 1977; Persson, 1986). Yet, at low densities, social interactions and mating opportunities should be less frequent or impeded altogether, which will negatively affect individual fitness and reproduction (Courchamp et al., 1999).

The phenomenon of density dependence has been examined thoroughly in aquatic organisms (Post et al., 1999; Gimnig et al., 2002; Lorenzen and Enberg, 2002; Hildrew et al., 2004), particularly in amphibians with biphasic life histories (Dash and Hota, 1980; Wilbur, 1980; Loman, 2004). The complex life history of amphibians includes a dependency on both aquatic and terrestrial habitats at different life stages. Tadpoles occupy aquatic habitats that can be unpredictable in their permanency and quality; therefore, tadpoles may experience very high local densities (Crump, 1989; Skelly, 1996; Amburgey et al., 2016). The timing of metamorphosis, a process known to incur high mortality rates (Dash and Hota, 1980), is dependent on both biotic (i.e., predators and competitors) and abiotic (e.g., pond drying) factors (Wilbur and Collins, 1973; Werner, 1986; Newman, 1998). The size at metamorphosis depends on the amount of resources tadpoles were able to store during their larval growth period (Tejedo and Reques, 1994; Newman, 1998) but is only initiated once a minimum threshold size has been reached (Wilbur and Collins, 1973). In addition, it has been shown that metamorphs incurred higher survival rates when emerging from their ponds at larger sizes, and earlier in the season (Altwegg, 2003).

The classic experimental method for examining density dependence in aquatic organisms is to raise them in aquaria or mesocosms of equal volume and to vary animal density by controlling their abundance, an *abundance-limited* method (Wilbur, 1977; Newman, 1998; McCoy and Bolker, 2008). There are numerous reasons to take this approach. For example, containers, whether mesocosms, aquaria or cattle tanks, that are all the same size and all placed in the same location can be expected to maintain closely similar conditions of water quality, temperature and community composition (Skelly and Kiesecker, 2001). Also, there has been extensive research using abundance-limited mesocosms, including examining ecological interactions affecting food web structure (Gauzens et al., 2016), measuring responses to environmental toxins (Rowe and Dunson, 1994; Boone and James, 2003; Egea-Serrano and Van Buskirk, 2016), and quantifying behavior and phenotypic plasticity in response to predator-prey interactions (Relyea, 2001, 2002, 2004; Van Buskirk, 2002). Therefore, a large body of literature exists on the success in using such methods.

Although the abundance-limited method has been used for decades to examine density-dependent effects, there are inherent issues associated with comparing animals raised in different group sizes. Aquatic animals living in high densities, like tadpoles, may exhibit social behaviors to enhance their collective survival, like forming schools or aggregations (Wilbur, 1980; Spieler and Linsenmair, 1999). There are many benefits to this behavior, such as an enhanced ability to avoid and detect predators (Watt et al., 1997; Spieler and Linsenmair, 1999; Spieler, 2003), enhanced foraging (Eterovick, 2000; Sontag et al., 2006), and thermoregulation efficiency (Brattstrom, 1962). The major cost associated with aggregation behavior is an increased degree

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of competition, such as local resource exploitation (Kuzmin, 1995; Griffiths and Foster, 1998), behavioral interference (Steinwascher, 1978), and allelopathic interference via fecal matter (Petranka, 1989; Beebee, 1991; Griffiths et al., 1991). If animals are raised in different density levels, there would be potential bias in variables such as growth rate and survival due to the supposed influence of living in a group of a particular size. Furthermore, when density is manipulated by changing the abundance of individuals, the larvae of many species of anurans may naturally aggregate to form schools (Wassersug and Hessler, 1971; Beiswenger, 1978; Griffiths and Foster, 1998) and can be expected to be able to do so effectively only when their abundance is sufficiently high. Therefore, because most mesocosm experiments are inherently abundance limited and each mesocosm is an isolated aquatic system, they may not be ideal to address all questions concerning the effects of density dependence, particularly the potential influence of social behaviors.

To test for the potential influence of social behavior on density-dependent growth and development in tadpoles, we made a comparison of responses in growth rate, timing of, and size at metamorphosis, between the abundance-limited method and an alternative method of varying density, called *volume-limited*. Using Fowler's Toad (*Anaxyrus fowleri*) tadpoles, a species from a genus known to naturally form aggregations (Wassersug, 1973; Breden et al., 1982) and typically exposed to various larval densities due to the stochastic nature of their breeding habitat (Wilbur and Collins, 1973; Smith, 1983; Semlitsch, 2002), we created a volume-limited system where the number of animals per treatment is kept constant and the size of the container is made variable. We implemented this scheme using free-floating mesh pens of various sizes deployed in the same semi-natural pond. This arrangement was designed to eliminate variance in environmental conditions between pens, including food availability, temperature, and any negative effects associated with tadpole feces (Licht, 1967; Beebee, 1991; Griffiths et al., 1991), because the mesh sides and bottom of the pens allowed particles to fall through into the open pond water. Therefore, we hypothesized that if tadpole aggregation behavior greatly influences the magnitude of their density-dependent response, then there should be differences in tadpole growth rate, timing of metamorphosis, and size at metamorphosis between abundance-limited and volume-limited trials, especially at low abundances when aggregations cannot form.

MATERIALS AND METHODS

Study species.—*Anaxyrus* (formerly *Bufo*) *fowleri,* Fowler's Toad, is a relatively small and common toad ranging widely throughout the eastern United States reaching as far north as the northern shore of Lake Erie in Ontario, Canada. In Canada, this toad is classified as Endangered both provincially and federally (COSEWIC, 2010) and is considered to be a beach-dune specialist, isolated within three remnant populations. The population we focus on has been monitored for nearly 30 years (Green, 1989), and its breeding habitat is currently being limited by the invasion of the Common Reed, *Phragmites australis* (Greenberg and Green, 2013).

Study site.—The study was conducted in the Thoroughfare Point Unit of the Long Point National Wildlife Area (NWA; between 42°34′33″–42°35′3″N and 80°22′15″–80°28′24″W)

in the Carolinian ecoregion of southern Ontario. In collaboration with the Canadian Wildlife Service, 12 ponds were dug out in the marshes, north of the beach-facing dunes in November 2012. These ponds were left to naturalize and were designed to be permanent sources of open water habitat for amphibian use. The 12 ponds, labeled 'pond 1' through 'pond 12', were situated in pairs separated by 500 m increments in a west-to-east transect bordering the edge of extensive spans of the invasive reeds. Paired ponds were placed approximately 1 m apart from each other. Individual ponds measured, on average, 13 m long, 7 m wide, and 0.9 m at the deepest point, and were lined with a mixture of organic and sandy substrate.

Although they were in early succession, we recorded the presence of emergent vegetation within and around the ponds aside from *Phragmites*. This included bladderwort, *Ultricularia* sp., milfoil, *Myriophyllum* sp., horsetail, *Equisetum* sp., Canada rush, *Juncus canadensis*, cattail, *Typha* sp., and green algae, *Spirogrya* sp. We also documented larger vegetation in the marsh area, which included dogwood shrubs, *Cornus* sp., and large cottonwood trees, *Populus* sp., but they were sparsely distributed and provided little-to-no canopy cover or shade. Other taxa documented using these ponds comprised all local amphibian species, the majority being Green Frog, *Lithobates clamitans*; Northern Leopard Frog, *Lithobates pipiens*; Bullfrog, *Lithobates catesbeianus*; and American Toad, *Anaxyrus americanus*; some reptiles, such as Snapping Turtle, *Chelydra serpentina*; Eastern Garter Snake, *Thamnophis s. sirtalis*; and Northern Watersnake, *Nerodia s. sipedon*; three fish species, Mud Minnow, *Umbra limi*; Northern Pike, *Esox lucius*; and Pumpkinseed, *Lepomis gibbosus*; and many unidentified aquatic invertebrates, including dragonfly larvae, aquatic beetles, and crayfish. Fowler's Toads were not found using the ponds in either the 2013 or 2014 breeding seasons.

Experimental set-up.—We collected egg masses from a total of four amplectant pairs of Fowler's Toads in the wild, one collected on 20 May 2013 and three collected between 24 May and 26 May 2014. We raised the larvae in floating mesh pens within 'pond 1' until they reached stage 25 of development (Gosner, 1960). All tadpoles were randomly chosen and deployed into their density trials at the same stage and time (12 June 2013 and 2 June 2014), except for the group of tadpoles deployed into 'pond 5' in 2013. Due to the relatively cool spring conditions in 2013, some toads delayed breeding up to two weeks, resulting in stage 25 tadpoles being found and deployed into their density trials 13 days later, on 25 June 2013, than the rest of the tadpoles. In 2014, the stage 25 larvae from three egg masses were mixed before being separated into their density trials.

The floating mesh pens were custom-built aquatic enclosures (John Radford, Ajax, Ontario) designed to hang from water-proofed, floating plywood and Styrofoam frames. To allow free passage of water and materials in and out of the enclosures, the sides were constructed of 0.7 mm fine nylon mesh, whereas the bottoms were made of coarser 1 mm fiberglass mesh. Each enclosure also had a 1/8-inch aluminum frame inside the mesh enclosure as a weight and a lid with 1-inch wire mesh to help protect from large predators. Eight sizes of enclosures were constructed: 1,200 L, 800 L, 400 L, 200 L, 100 L, 60 L, 30 L, and 20 L. All enclosures were 60 cm in depth and were placed in the ponds for a minimum of five days prior to initiating density treatments, to allow periphyton growth for tadpole consumption (Fig. 1).

We monitored pond temperature by deploying a total of 28 temperature data loggers (iButton, model DS1922L), water-proofed with Plastidip®, into each of two floating mesh pens in three ponds in 2013 and four ponds in 2014 for the duration of the tadpole density trials. In each pond, two loggers were set to record surface temperatures by being inserted into a small sponge to allow the logger to float with changing water levels, and fixed with tape and fishing line to the lid of two floating pens. Meanwhile two more loggers were attached to the bottom of the same two pens to record the temperatures at a depth of 60 cm.

Density of adult Fowler's Toads in this study site fluctuates annually but has ranged from 4 to 93 toads per kilometer between 1989 and 2011 (Greenberg and Green, 2013). Natural Fowler's Toad tadpole densities have not yet been estimated in Long Point. Since Fowler's Toad tadpoles tend to aggregate and their habitat is ephemeral, it would be very difficult to make any precise measurements of natural tadpole densities to compare to. Therefore, we raised tadpoles in the enclosures through to metamorphosis at a range of densities based on previous and similar studies (Wilbur, 1977; Goater, 1994; Altwegg, 2003; Relyea, 2004), while attempting to cover a broad enough range in order to detect suitable density-dependent relationships. For volume-limited experiments, all eight sizes of enclosures held 100 tadpoles each, creating initial densities of 0.08, 0.13, 0.25, 0.50, 1.00, 1.67, 3.33, and 5 tadpoles/L. Two sets of eight different sized enclosures were installed in separate ponds ('pond 4' and 'pond 5') in 2013, and three sets of eight in 2014 ('pond 4,' 'pond 5,' and 'pond 7'). We executed an abundance-limited experiment in one pond in 2013 ('pond 10') using four 216 L enclosures stocked with; 17, 28, 216, and 719 tadpoles, producing densities of 0.08, 0.13, 1.00, and 3.33 tadpoles/L, respectively. In order to determine if manipulating density by using various sized enclosures had no effect on the variance of the measured variables, we executed a *density-controlled* experiment in one pond in 2014 ('pond 6') using one set of the eight different sized enclosures. Each enclosure held a different number of tadpoles—300, 200, 100, 50, 25, 15, 8, and 5—so all eight enclosures held the same density, 0.25 tadpoles/L.

Data collection.—We measured the average total length, TL, of tadpoles, equal to the distance from the tip of the rostrum to tip of the tail (Scott, 1990; Van Buskirk 2002; Ukuwela and Ranawana, 2011), from 20 randomly chosen individuals per enclosure at the beginning of each experiment and every four to seven days thereafter over a maximum of 74 days in 2013 and 80 days in 2014. Digital photographs of the tadpoles were taken *in situ*, and the tadpoles were immediately returned to their density group afterwards. We recorded number of metamorphs emerging per treatment to estimate survival percentages. Upon the first sight of a metamorph, the pens were checked daily thereafter for new metamorphs. Initial Growth Rate, GR, of tadpoles per enclosure was quantified as TL at time t (i.e., number of days until the growth curve plateaus) minus TL at $t = 0$, divided by t. The value for t varied between ponds in 2013; 'pond 4' was 20 days, 'pond 5' was 23 days, except density level 0.08 and 0.25 tadpoles/L had a t value of 17 days, and 'pond 10' was 28 days for all density treatments. In 2014, t value was consistent at 22 days for all ponds and density treatments.

We defined size at metamorphosis, SM, as total length at stage 42 (Walsh, 2010), when the animals had four developed limbs yet the entire tail was still present. This was done to
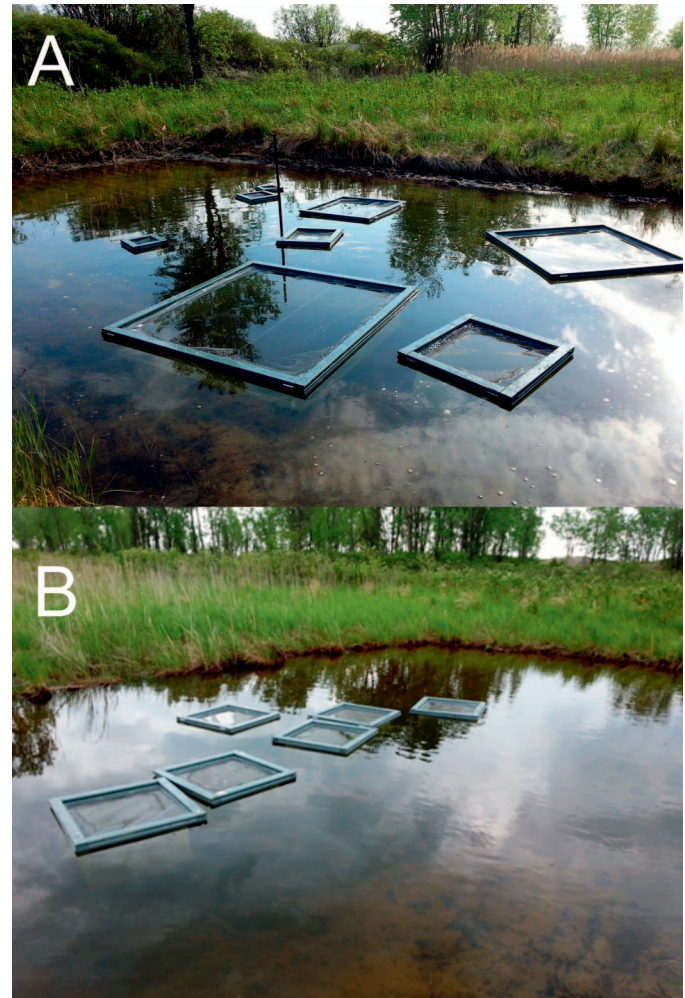

Fig. 1. Photos of ponds with tadpole density enclosures. (A) A pond with enclosures of various sizes, used for volume-limited or density-controlled treatments. (B) A pond with enclosures of the same size, used for abundance-limited treatments.

estimate size at metamorphosis while making sure to avoid complications in measuring the animals during the subsequent period of drastic physical change (i.e., tail resorption). Time to metamorphosis, TM, for each enclosure was the mean number of treatment days, t, until stage 42 metamorphs were detected. All length measurements of tadpoles were made by placing them in a petri dish underlain by 6 mm grid graph paper, photographing them, and analyzing the photographs with ImageJ (vers.1.46r, Schneider et al., 2012). All photographs were taken in the field using a small level stool and a tripod to keep the petri dish and camera level, after which the tadpoles were immediately returned to their assigned density trial.

Analysis.—We compared temperatures between ponds and years, using mean daily temperatures collected in all ponds and analyzed with a one-way analysis of variance (ANOVA). We ran the analysis under the assumption that each pond was independent from others, within and across years, and used a variable called 'pondyear' to accomplish this statistically.

Survival proportions were calculated using the number of metamorphs emerging from each density trial from each pond, divided by the initial total number. If tadpoles did not reach metamorphosis, they were not counted as survivors for

Table 1. Proportions of survival through metamorphosis of Fowler's Toad tadpoles according to density (tadpoles/L) in volume-limited, abundance-limited, and density-controlled experiments. The values for volume-limited treatments combine data from three ponds in 2014. The density-controlled experiment had all eight various-sized enclosures run at 0.25 tadpoles/L. The size of each enclosure corresponds to all density methods and is shown in volume (L).

Density (tadpoles/L)	Abundance-limited 2013 pond 10	Volume-limited 2013 pond 4	Volume-limited 2013 pond 5	Volume-limited 2014 ponds	Density-controlled 2014 pond 6	Enclosure size (L)
0.08	0.35	0.16	0.20	0.40	0.42	1200
0.13	0.21	0.26	0.58	0.46	0.41	800
0.25	—	0.09	0.39	0.39	0.40	400
0.50	—	0.15	0.09	0.49	0.48	200
1.00	0.07	—	0.52	0.37	0.72	100
1.67	—	0.04	0.19	0.31	0.47	60
3.33	0.04	—	0.08	0.09	0.63	30
5.00	—	0.02	0.05	0.08	0.00	20

their density group even if they were still alive by the end of the season. We kept calculations for the 2013 ponds separate to avoid potential biases from the late-starting tadpoles in pond 5, and we combined the data from 2014 to create mean survival proportions. Due to the lack of replication at the pond level between density methods and years, we only made simple quantitative comparisons of survival proportions.

We compared the linear relationship between initial tadpole density and GR, SM, and TM of the abundance-limited pond to the same relationships, from the two volume-limited ponds in 2013. A regression line was fit to the data from each 2013 pond separately, again allowing us to isolate any differences observed from the late-starting tadpoles in pond 5. This was accomplished using independent t-test comparisons of paired linear regressions, using the lm function and summary command in R (R Core Team, 2015). We tested the effect of the enclosures (i.e., density-controlled trials) on GR, SM, and TM using the non-parametric Mann-Whitney U test to compare between the 2014 volume-limited ponds ($n = 3$) and the density-controlled enclosures in pond 6 ($n = 8$) set at 0.25 tadpoles/L. Data from enclosures that had been damaged during the experiment were removed from the analysis. All variables were tested for normality using QQ plots and Shapiro-Wilk test, and tested for homogeneity of variance using Levene's test. All density and TM values were transformed by natural logarithm to allow for linear comparisons. All statistical tests were performed using R vers. 3.3.1 (R Core Team, 2015), with $\alpha = 0.05$.

RESULTS

All temperature data were found to have equal variance ($F = 0.92$, $P = 0.48$). In 2013, mean seasonal temperature for the ponds was 24.6°C, ranging from 19.8°C to 31.5°C, which was warmer compared to the mean seasonal temperatures in 2014, being 23.7°C, ranging from 19°C to 28.7°C. The pond temperatures were found to be different between years ($F_{1,418} = 23.4$, $P < 0.001$) but not within years (2013: $F_{2,177} = 0.07$, $P = 0.94$; 2014: $F_{3,236} = 2.55$, $P = 0.06$). In addition, aggregation behavior was never observed during our density trials; however, anecdotal observations showed tadpoles having a tendency to gather at the surface or the sides of the mesh pens, usually where the sunlight was more direct.

All GR and SM data were found to be normal with homogeneous variance. The TM data from 2013 were found to be normal once we used the natural-log transformation. A

total of ten data points were removed from the 2013 volume-limited 'pond 4' dataset due to two damaged enclosures; density levels 1.00 and 3.33 tadpoles/L, and an outlier from density level 5.00. This included two survival measures, two GR data points from density levels 1.00 and 3.33 tadpoles/L, and three data points from each of SM and TM data, at density levels 1.00, 3.33, and 5.00 tadpoles/L. Also, no data were collected from the smallest enclosure size for the density-control dataset due to high mortality rates and the low initial group size of five tadpoles.

Survival.—There was a high degree of variation in survival between density groups within volume-limited ponds. For example, the highest survival percentage came from pond 5 at 58% in the second lowest density group, 0.13 tadpoles/L, which was a 65% increase from the survival percentage in the lowest density group (Table 1). Similarly, pond 4 showed a 38% increase from 26% in density group 0.13 to 16% in group 0.08 tadpoles/L (Table 1). In both ponds, however, the highest density group, 5.00 tadpoles/L, showed the lowest level of survival at 2% in pond 4 and 5% in pond 5 (Table 1). For abundance-limited groups, the lowest density level, 0.08 tadpoles/L, had the highest survival, at 35%, which was a 67% increase from survival at 0.13 tadpoles/L. From density level 1.00 tadpoles/L decreasing to 0.13 tadpoles/L, there was a 200% increase in survival (Table 1). The highest density level had the lowest survival, at 4%, a 75% decrease from density level 1.00 tadpoles/L (Table 1). Interestingly, for the density-controlled experiment, survival percentages ranged from 40% at 0.25 tadpoles/L to 72% at 1.00 tadpoles/L, with the exception of the smallest enclosure size having zero survivors (Table 1).

Abundance-limited density.—The lowest density level (0.08 tadpoles/L) produced the fastest GR (0.76 mm/day), the largest mean SM (23.98 mm±0.48 SE), and the shortest mean TM (17 days±1.63 SE; Table 2). Density had a significant ln-linear relationship with all three variables: GR ($R^2 = 0.97$, $P = 0.01$), SM ($R^2 = 0.98$, $P = 0.008$), and TM ($R^2 = 0.93$, $P = 0.02$; Table 3).

Volume-limited density.—Overall, the lowest density level (0.08 tadpoles/L) from the volume-limited density ponds in 2013 produced the fastest mean GR (1.24 mm/day±0.03 SE), largest mean SM (29.97 mm±1.68 SE), and the shortest mean TM (17 days±0 SE; Table 2). Conversely, the highest density level from the volume-limited density ponds 5 in 2013 generated the slowest GR (0.17 mm/day, at 5 tadpoles/L; Table 2). The

Table 2. Growth rate, mean size at metamorphosis, and mean time to metamorphosis of Fowler's Toad tadpoles according to density in volume-limited, abundance-limited, and density-controlled experiments. The mean of three ponds are presented for 2014 data. All variance is measured in standard error.

	Abundance-limited	Volume-limited			Density-controlled
Density	2013 pond 10	2013 pond 4	2013 pond 5	2014 ponds	2014 pond 6
Growth rate (mm per day)					
0.08	0.76	1.21	1.27	0.68±0.03	
0.13	0.68	1.02	0.81	0.65±0.07	
0.25	—	0.90	0.73	0.59±0.09	0.64±0.02
0.50	—	0.83	0.50	0.51±0.04	
1.00	0.57	—	0.41	0.36±0.05	
1.67	—	0.68	0.42	0.32±0.06	
3.33	0.47	—	0.33	0.32±0.05	
5.00	—	0.64	0.17	0.19±0.03	
Mean size at metamorphosis (mm)					
0.08	23.98±0.48	31.65±0.71	28.28±0.45	24.41±1.28	
0.13	23.27±0.57	27.75±0.54	26.81±0.17	23.94±0.51	
0.25	—	26.17±0.69	23.97±0.29	23.30±0.98	24.04±0.12
0.50	—	25.82±0.37	24.60±0.53	22.71±0.97	
1.00	21.99±0.48	—	23.48±0.58	22.62±0.61	
1.67	—	21.39±0.24	22.01±0.20	21.54±0.77	
3.33	21.21±0.44	—	21.37±0.23	22.35±0.93	
5.00	—	—	20.31±0.50	21.53±0.59	
Mean time to metamorphosis (days)					
0.08	17±1.63	17±0.00	17±0.00	29±0.48	
0.13	18±1.29	20±0.00	19±0.46	30±1.07	
0.25	—	20±0.00	21±0.45	33±0.66	33±1.92
0.50	—	30±0.50	24±1.58	39±1.11	
1.00	33±0.63	—	29±0.69	45±1.47	
1.67	—	36±0.00	45±0.86	56±3.51	
3.33	35±0.00	—	54±0.87	64±2.23	
5.00	—	—	60±1.14	66±2.34	

highest density level from volume-limited density pond 5 in 2013 showed the smallest mean SM (20.31 mm±0.50 SE at 5 tadpoles/L; Table 2). The longest mean TM came from the highest density level in the volume-limited density ponds in 2014 (66 days±2.34 SE at 5 tadpoles/L; Table 2). Density

appeared to have a significant ln-linear relationship with all three variables in all five *pondyears* (Table 3).

Abundance-limited vs. Volume-limited.—The independent comparisons of the paired linear regressions revealed that

Table 3. The results of nine linear regressions examining the effect of density on three parameters; growth rate, GR; size at metamorphosis, SM; and time to metamorphosis, TM, under two methods of density manipulation: abundance-limited and volume-limited. Density and TM were transformed with the natural logarithm.

| Method	Model (y ~ x)	Year	Pond	Slope	R^2	n	t value	SE	P value
Abundance-limited	GR ~ Density	2013		−0.07	0.96	4	−8.27	0.01	0.014*
	SM ~ Density		pond 10	−0.71	0.98	4	−11.32	0.06	0.008**
	TM ~ Density			0.22	0.95	4	6.03	0.04	0.026*
Volume-limited	GR ~ Density	2013	pond 4	−0.13	0.87	6	−5.97	0.02	0.004**
			pond 5	−0.21	0.84	8	−6.24	0.03	0.001**
	SM ~ Density		pond 4	−2.97	0.89	5	−5.82	0.51	0.01*
			pond 5	−1.74	0.92	8	−9.17	0.19	<0.001***
	TM ~ Density		pond 4	0.26	0.91	5	6.24	0.04	0.008**
			pond 5	0.32	0.94	8	10.88	0.03	<0.001***
	GR ~ Density	2014	pond 4	−0.09	0.74	8	−4.60	0.02	0.004**
			pond 5	−0.11	0.95	8	−11.24	0.01	<0.001***
			pond 7	−0.15	0.92	8	−9.17	0.02	<0.001***
Volume-limited	SM ~ Density		pond 4	−0.58	0.54	8	−2.67	0.22	0.037*
			pond 5	−0.75	0.76	8	−4.36	0.17	0.005**
			pond 7	−0.60	0.18	8	−3.31	0.18	0.011*
	TM ~ Density		pond 4	8.52	0.95	8	11.93	0.71	<0.001***
			pond 5	11.09	0.91	8	8.56	1.30	<0.001***
			pond 7	9.76	0.91	8	8.59	1.14	<0.001***
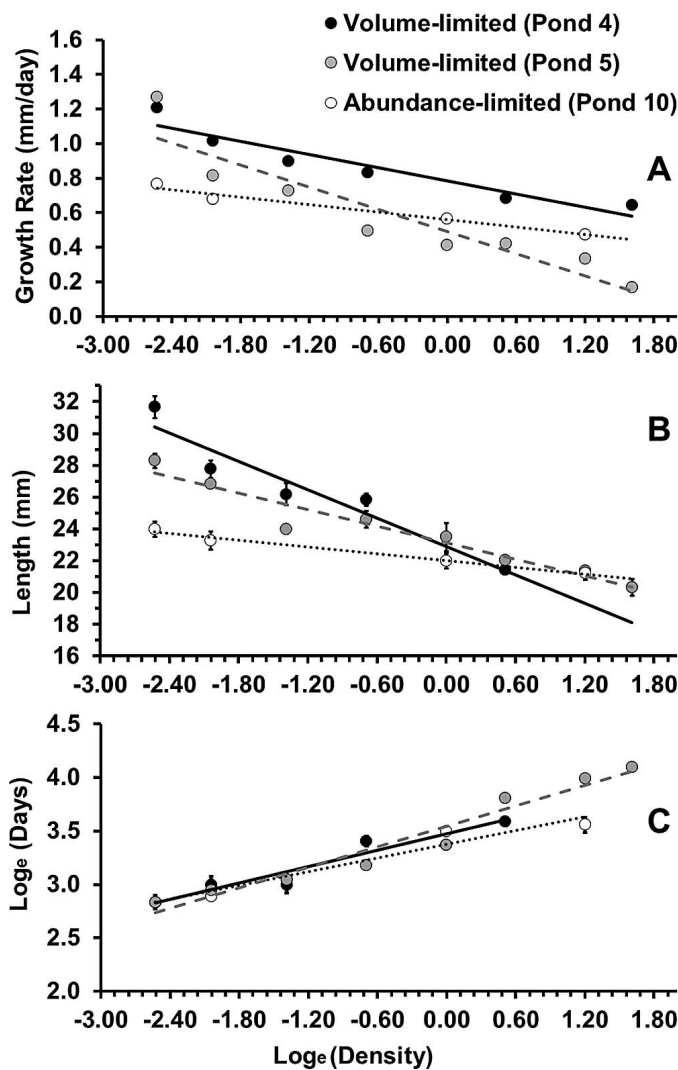
Fig. 2. Initial growth rate, GR (A), mean size at metamorphosis, SM (B), and mean days to metamorphosis, TM (C) among Fowler's Toad tadpoles raised at various densities, where density is controlled either by limiting volume or by limiting abundance, in 2013. The dotted line is the best-fit line for the abundance-limited pond, the solid line is the best fit line for volume-limited density pond 4, and the gray dashed line is for volume-limited density pond 5. Density and TM were transformed with the natural logarithm, and error bars represent standard error.

the regression slopes for the relationship between density and GR is not differ between abundance-limited pond 10 and volume-limited pond 4 ($t = -1.18$, df = 12, $P = 0.26$) but did differ with volume-limited pond 5 ($t = -3.20$, df = 12, $P =$

0.01; Fig. 2). Similarly, the same relationships were found for the TM dataset, where the slope for pond 10 did not differ from the slope for pond 4 ($t = 0.69$, df = 11, $P = 0.51$), but did differ for pond 5 ($t = 2.29$, df = 11, $P = 0.04$; Fig. 2). Interestingly, the slopes for the SM data were found to differ from pond 10 in both pond 4 ($t = -4.99$, df = 11, $P < 0.001$) and pond 5 ($t = -2.92$, df = 11, $P = 0.01$; Fig. 2).

Density control.—Tadpoles that were reared under the density-control method did not show a large difference in mean GR, SM, and TM across enclosure sizes (Table 4). These measurements produced a mean GR of 0.64 mm/day±0.02 SE, which was not significantly different from the mean GR of the volume-limited enclosures at the same density level (0.59 mm/day±0.09 SE; $U = 13$, $P = 0.65$). The mean SM for the density-control enclosures was 24.04 mm±0.12 SE, which was not significantly different from the mean SM of the volume-limited enclosures at the same density level (23.30 mm±0.98 SE; $U = 13$, $P = 0.67$). Finally, the mean TM for the density-control pens ranged from 28 to 41 days, with a mean of 33 days±1.9 SE (Table 3). This was not found to be significantly different from the mean TM for the volume-controlled pens at the same density level (33 days±0.66 SE; $U = 11.5$, $P = 0.91$).

DISCUSSION

In terms of the general effect of density on the growth rate and size at metamorphosis, our results show a clear negative trend. Looking closely at the time to metamorphosis results for the volume-limited ponds, there appears to be a threshold, where above 1.00 tadpoles/L metamorphosis is delayed substantially. When we examine the survival data closely, a similar threshold is suggested by the values for the 2013 ponds, while the 2014 ponds appear to have above 30% survival up to 1.67 tadpoles/L. In all cases, certainly the highest two density levels we tested gave results showing tadpoles most negatively impacted by density. Considering our density-controlled pond, the only survival percentage that stood out was from the 100 L enclosure. This enclosure size was used in the 1.00 tadpoles/L density level in the volume-limited ponds, suggesting that tadpoles might do better in medium to small-sized pens (i.e., 30 to 100 L) compared to larger ones.

Looking at our methods comparison, our results suggest that there may be an effect of tadpole abundance level on metamorph size, due to the differences observed in the SM vs. density regression slopes between abundance-limited and volume-limited ponds. Although we did find some differences between the two density methods for GR, these differences were only observed for pond 5, the group of

Table 4. A breakdown of the density-controlled experiment design from 2014, with summarized results. Density was kept constant across the eight various-sized enclosures at 0.25 tadpoles/L. The results were compared to those from the three volume-limited enclosures at the same density level in 2014. No data were collected from the highest density treatment due to mortality. Variance was measured as standard error.

Enclosure volume (L)	n	Growth rate (mm per day)	Size at metamorphosis (mm) ± SE	Time to metamorphosis (days)
1200	300	0.61	23.79±0.21	41
800	200	0.70	24.60±0.29	36
400	100	0.74	24.38±0.29	37
200	50	0.67	23.97±0.44	34
100	25	0.58	23.96±0.42	28
60	15	0.61	23.70±0.35	28
30	8	0.58	23.87±0.52	29
20	5	—	—	—

late-starting tadpoles. Therefore, we are not confident that these differences are due to the degree of tadpole abundance alone. Since we expected to observe greater differences in all three variables, GR, SM, and TM, our results are not completely consistent with the hypothesis.

In addition, the survival proportions showed a similar decrease with density, but the high degree of variation between density groups within a pond made it difficult to compare between ponds. After inspecting the results closely, we noticed a possible exponential decrease in survival for the abundance-limited pond, compared to a more-or-less linear relationship with the volume-limited ponds. It is possible this difference in relationship type is caused by tadpoles living in low abundance and low density; however, additional abundance-limited density trials are needed to confirm this.

In terms of social behavior, it is at lower abundances where we might expect to see a difference in density-dependent effects. We are able to see this difference more clearly when looking at the SM regression lines (Fig. 2). Here, the individuals emerging from the low-abundance and low-density groups (i.e., pond 10) emerged at much smaller sizes than their volume-limited counterparts from both ponds. This may suggest that there could be a link between small metamorph size and the lack of social interaction or aggregation ability at this abundance level, although it is important to note that we did not directly observe aggregations in our density trials. On the contrary, Griffiths and Foster (1998) discovered that bufonid tadpoles grew slower when raised in small groups under laboratory conditions compared to isolated individuals.

Tadpoles benefit from aggregations in various ways. One is the enhancement of feeding efficiency, which can occur when the substrate is stirred by small swimming motions, allowing food particles to be exposed to more individuals in the group (Beiswenger, 1972; Wilbur, 1977). Continuous feeding in tadpoles during their development can allow individuals to reach metamorphosis at a larger body size (Eterovick, 2000). It is tadpoles with larger body size who have a higher chance of reaching their post-metamorphic stage (Goater, 1994; Chelgren et al., 2006; Scott et al., 2007). However, smaller metamorph size has been also shown to induce compensatory post-metamorphic growth and high overwintering survival rates (Boone, 2005).

Another benefit of aggregation is the facilitation of creating optimal thermal conditions (Lillywhite et al., 1973; Beiswenger, 1978; Guilford, 1988; Caldwell, 1989). Aggregations of black tadpoles, like bufonids, are usually observed living as large black masses in shallow water, within which temperatures have been measured to be 2–3°C warmer than surrounding water temperature (O'Hara, 1981). It is both the coloration and behavior of such aggregations that appear to maximize their thermoregulatory ability by absorbing solar energy to heat up their microhabitat (Guilford, 1988; Caldwell, 1989). As shown by the density-controlled comparison, we did not find a difference in the measured variables and the enclosure size they were housed in. Since the enclosures were all built with the same depth, and we did not find strong differences in pond temperatures within the same year, all tadpoles likely had equal opportunities for thermoregulation. We also did not observe any noticeable differences in tadpole behavior under these controlled conditions. If tadpoles were observed basking near the surface of the water, they were doing so in all eight various-sized enclosures.

Finally, aggregations can assist individuals in reducing the impact of predation (Watt et al., 1997; Spieler, 2003). For example, tadpoles can sense chemical cues released into the water, allowing for warning signals to be detected when predators are present (Stauffer and Semlitsch, 1993; Richardson, 2006; Fraker et al., 2009). Also, when living in large groups, the impact of predation is diluted because there is equal chance for any other individual to get caught (Fraser and Keenleyside, 1995; Watt et al., 1997). A few studies have examined how predation-induced stress can cause changes in behavior and phenotypic plasticity in amphibian tadpoles (McCollum and Leimberger, 1997; Relyea and Mills, 2001; Schoeppner and Relyea, 2009; Maher et al., 2013). More importantly, a reduction in body size as a result of increased predation pressure has been documented in some studies, where the effect is caused by selection by predators favoring larger prey items (Werner, 1986; Blanckenhorn, 2000), a change in tadpole behavioral response (Laurila et al., 1997; Barry, 2014), or predation-induced stress (Relyea and Mills, 2001; Dahl et al., 2012). However, Hossie et al. (2010) reported predator-exposed tadpoles of _Lithobates pipiens_ to grow larger than their predator-exposed, stress-inhibited counterparts. Nonetheless, we postulate that when tadpoles exist in small group sizes, the effects of predation are not diluted, resulting in a reduced group metamorph size.

In general, our results showing the negative effects of increasing larval density on growth, metamorph size, days to metamorphosis, and survival, are consistent with the literature (Wilbur, 1977; Petranka, 1987; Altwegg, 2003; Relyea, 2004). It has been shown that larval density has a significant effect on growth and survival of metamorphs (Goater, 1994; Boone, 2005). Considering this, it is likely that the effects we observed in our Fowler's Toad tadpoles will carry-over into post-metamorphic life stages. Boone (2005) discovered that some frog species were able to offset small metamorph size with terrestrial growth. In addition, John-Alder et al. (1990) discovered post metamorphic impacts on body size, and subsequently locomotor ability, in individual Fowler's Toads when raised under high larval densities. If these impacts are similar to our own system, we may observe differences not only in toad growth and survival, but in dispersal ability as well.

Since the main finding of our study was that tadpoles do their best at higher abundances but at low density, these two factors must be considered together in order to properly assess the mechanisms of density-dependent growth in aquatic organisms. We recommend the use of the volume-limited density method in future studies that wish to control for potential confounding factors like social interaction, in addition to maintaining an equal sample size across treatments. We also recommend an optimal upper threshold density level for Fowler's Toad tadpoles at 1.00 tadpoles/L, and an ideal mesocosm size between 30 L and 100 L. Further experiments are needed to delve deeper into examining tadpole social behavior and its effects on individual fitness.

ACKNOWLEDGMENTS

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Appendix 1. Results for tadpole growth experiments: initial growth rate, mean size at metamorphosis, and mean time to metamorphosis of Fowler's Toad tadpoles according to specific pond and density level in volume-limited and abundance-limited experiments. Variance is represented by standard error.

Density (tadpoles per liter)	Abundance-limited			Volume-limited		
	2013 pond 10	2013 pond 4	2013 pond 5	2014 pond 4	2014 pond 5	2014 pond 7
Growth rate (mm per day)						
0.08	0.76	1.21	1.27	0.61	0.71	0.72
0.13	0.68	1.02	0.81	0.51	0.77	0.67
0.25	—	0.90	0.73	0.43	0.60	0.73
0.50	—	0.83	0.50	0.44	0.53	0.56
1.00	0.57	—	0.41	0.27	0.45	0.36
1.67	—	0.68	0.42	0.23	0.43	0.31
3.33	0.47	—	0.33	0.37	0.35	0.22
5.00	—	0.64	0.17	0.17	0.26	0.14
Mean size at metamorphosis (mm)						
0.08	23.98±0.48	31.65±0.71	28.28±0.45	26.69±0.28	23.06±0.34	22.65±0.49
0.13	23.27±0.57	27.75±0.54	26.81±0.17	24.23±0.27	24.65±0.31	22.94±0.66
0.25	—	26.17±0.69	23.97±0.29	24.22±0.23	23.93±0.25	21.15±0.58
0.50	—	25.82±0.37	24.60±0.53	23.96±0.24	22.98±0.28	20.70±0.46
1.00	21.99±0.48	—	23.48±0.58	23.56±0.23	22.82±0.24	21.49±0.69
1.67	—	21.39±0.24	22.01±0.20	22.92±0.25	21.41±0.19	20.28±0.26
3.33	21.21±0.44	—	21.37±0.23	24.22±0.17	21.40±0.34	21.44±0.49
5.00	—	—	20.31±0.50	22.99±0.51	20.97±0.31	21.62±1.37
Mean time to metamorphosis (days)						
0.08	17±1.63	17±0.00	17±0.00	30±0.43	28±0.62	30±1.10
0.13	18±1.29	20±0.00	19±0.46	31±0.46	28±0.60	31±0.38
0.25	—	20±0.00	21±0.45	34±0.56	33±0.43	32±0.39
0.50	—	30±0.50	24±1.58	40±0.50	36±0.50	40±0.78
1.00	33±0.63	—	29±0.69	45±0.67	43±0.60	48±1.66
1.67	—	36±0.00	45±0.86	50±0.60	58±0.82	61±0.62
3.33	35±0.00	—	54±0.87	60±0.00	67±0.81	66±0.83
5.00	—	—	60±1.14	65±1.12	70±0.82	63±0.79