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Increased Larval Density Induces Accelerated Metamorphosis Independently of Growth Rate in the Frog *Rana sphenocephala*

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ABSTRACT.—We grew larval *Rana sphenocephala* at different densities but maintained equal mean growth rates among density treatments (via equal per capita food levels) to test the hypothesis that larval density can influence metamorphic timing independently of larval growth rate. Tadpoles at high density metamorphosed earlier than tadpoles at low density despite growing at similar rates. Food reductions did not accelerate metamorphosis. These results support the hypothesis that density can be a sufficient cue to initiate metamorphosis independently of growth rate.

In amphibian larvae, metamorphic parameters affect fitness. The effects of density on metamorphosis have been demonstrated to carry over into the juvenile period with the result that larvae raised at low density generally metamorphose at larger sizes, survive better, have greater lipid stores, have higher resistance to parasites, and have increased likelihood to breed and produce larger clutch sizes (Scott, 1994; Beck and Congdon, 2000; Morey and Reznick, 2001; Scott et al., 2007). Size at metamorphosis also influences age and size at maturation (Smith, 1987; Semlitsch et al., 1988). Thus, it remains essential to clarify how environmental cues can be transduced to the endocrinological initiation of metamorphosis.

The influence of density on metamorphic size is so predictable that one can reliably create differently-sized juvenile (newly transformed) amphibians for experiments by growing larvae at varied densities, with the repeatable result that larvae at high density become small juveniles and vice versa (Scott, 1994; Morey and Reznick, 2001). In contrast, the effect of density on metamorphic timing is not so clearly understood. Several models (e.g., Wilbur and Collins, 1973; Day and Rowe, 2002) suggest that a decline in larval growth that would be associated with increased density can initiate metamorphosis. In contrast, most observations from nature and experiments indicate that slower growth that accompanies high larval density is associated with delayed metamorphosis (Newman, 1987; Scott, 1990).

Because density and growth in amphibian larvae are typically confounded in nature, we conducted an experiment wherein we varied density of larval *Rana sphenocephala* while keeping per capita growth equal. In addition, we crossed this density treatment with a growth treatment of (1) consistent growth or (2) a decline in growth. Several studies have indicated that a decline in growth that occurs during late larval development can result in a change in the timing of metamorphosis (e.g., Morey and Reznick, 2000; Ryan and Semlitsch, 2003). Our null hypothesis was that variation in density and growth regime would fail to result in variation in metamorphic size and metamorphic timing.

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MATERIALS AND METHODS

We obtained embryos of larval *R. sphenocephala* from Sullivan Supply Company (Middleboro, TN) in October 2003. At this locality and at similar latitudes (Johnson, 1992), *R. sphenocephala* breed and deposit eggs in the autumn, and tadpoles overwinter and metamorphose the following June and July. Upon hatching (October 30), tadpoles were placed into one of 72 plastic boxes (30 × 17 × 21 cm LWH) filled with 2,600 mL of reverse-osmosis (RO) water.

The boxes were divided into groups of six blocks, with 12 boxes per block. Each box was a replicate in a fully-factorial 2 × 6 design, where the factors were food reduction (yes/no) and density (1, 2, 4, 8, 10, or 20 tadpoles per box). Thus, each box in a block held 1, 2, 4, 8, 10, or 20 tadpoles, replicated twice in each block to provide a constant food treatment and a food reduction treatment. Blocks contained 90 tadpoles, for a total of 540 tadpoles. Boxes were maintained in a room with windows that provided an ambient (Minot, ND) light cycle. Temperature in the room was 20°C ± 3°C.

Every three days, tadpoles were removed from boxes, the boxes were cleaned, and refilled with 2,600 mL of fresh RO water, and tadpoles were replaced into the boxes. The total mass of tadpoles in each box was determined once per week, beginning on day 18 and ending on day 193. Mass was determined by placing all tadpoles from a box in an aquarium net, counting and recording tadpole number, blotting dry to remove excess water, and weighing the total tadpole mass to the nearest milligram using a top-loading balance.

Feedings occurred immediately after water changes (i.e., every three days). Feedings consisted of a per capita 25-mg aliquot of a finely ground 1:1 mixture of rabbit chow and fish food flakes (e.g., the one-tadpole treatment received a 25-mg aliquot, whereas the 10-tadpole treatment received a 250-mg aliquot at each feeding).

As tadpoles grew, aliquots were increased. On day 25 (November 24), aliquots were increased to 50 mg per capita, and on day 95 aliquots were increased to 75 mg per capita. Because a minimal size is required for competence to metamorphose (Wilbur and Collins, 1973), we waited until day 144 (March 22) to administer a food reduction treatment. At this date,

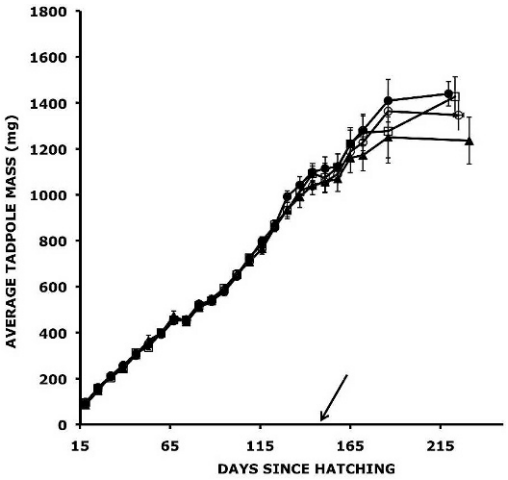

FIG. 1. Growth trajectories showing mean body size (± 1 SE) of tadpoles at density treatments. Trajectories terminate at mean metamorphic mass and date of metamorphosis. Closed triangles denote four tadpoles per box; open squares denote eight tadpoles per box; open circles denote 10 tadpoles per box; and closed circles denote 20 tadpoles per box. Arrow indicates initiation of food reductions in half the food treatments. Note the consequent increase in variance in tadpole masses in each density treatment following food reduction.

all tadpoles were prometamorphic and larger than the minimal metamorphic size of *R. sphenocephala* from wild populations (Butterfield et al., 2005). Thus, at day 144, one-half of the treatments were subjected to a food reduction that returned these treatments to a per capita 25 mg aliquot. Metamorphs (defined as forelimb emergence) were weighed, and larval period (days) was recorded. Metamorphs first appeared on day 195 (May 12), and all tadpoles had metamorphosed by day 240 (June 26). These metamorphic dates are approximately coincident with estimates from wild populations at similar latitudes (Johnson, 1992).

To confirm expected growth effects, we used a one-way MANOVA to examine the effect of density on the vector of average mass recorded each week and compared these masses at each date using a one-way ANOVA. Then, we analyzed mean metamorphic timing (in days) and mean metamorphic mass (in mg) of all transforming tadpoles with a two-way MANOVA with density and food reduction as treatment effects. Data for metamorphic timing were inversed-transformed (Alford and Harris, 1988), and data from metamorphic mass were log-transformed (Sokal and Rohlf, 1995). We used $\alpha = 0.05$ as significance criterion, and Wilks' λ was used as our multivariate test statistic.

RESULTS

Because the one-tadpole and two-tadpole density treatment groups grew at slower rates, they were removed from further analyses. The other density treatment groups (i.e., 4, 8, 10, and 20 tadpoles) grew at similar mean rates (Fig. 1). Mortality was low (<5%) and similar across all groups.

Food reduction did not result in a reduction in larval period (Table 1). However, there was a significant effect on metamorphic mass of food reduction: food-reduced groups had significantly lower metamorphic size (Fig. 2).

There was a significant difference in larval period because of variation in density: tadpoles at higher density had shorter larval periods (i.e., exhibited accelerated metamorphosis; Table 1). Density did not affect metamorphic mass (Fig. 3).

DISCUSSION

Because the causes (e.g., pond desiccation) and consequences (decreases in growth rate) of high larval density are usually confounded, our goal was to grow tadpoles living at different densities at equal mean rates to test the hypothesis that variation in density causes variation in metamorphic timing. Our results indicate that density can influence metamorphic timing in *R. sphenocephala* tadpoles. Although there was not a significant difference in body size (our intended result), metamorphic timing differed significantly between the 4-tadpole treatment and the 20-tadpole treatment, with the 20-tadpole treatment density metamorphosing earlier.

Studies on larval density have been of two kinds: density experiments (i.e., manipulations of actual number of larvae in a given volume) and desiccation experiments (i.e., manipulations of same number of larvae in declining volumes). Density experiments produced slower growth and delayed metamorphosis at smaller sizes (Morey and Reznick, 2001; Loman,

TABLE 1. Summary of multivariate and univariate analyses of the effect of treatments on log-transformed metamorphic mass (mg) and inverse-transformed larval period (days). Mean squares for metamorphic mass are $\times 10^{-2}$, mean squares for larval period are $\times 10^{-7}$. For the multivariate statistics, df = 6,400 for density and density × food, df = 2,400 for food. For univariate statistics, df = 3,201 for density and density × food, df = 1,209 for food. For metamorphic mass, error MS = 2.01×10^{-2}; for larval period, error MS = 1.27×10^{-7}.

				Univariate statistics					
	Multivariate statistics			Mass at metamorphosis			Larval period		
Source	Wilks' λ	F	P	MS	F	P	MS	F	P
Density	0.955	1.56	0.158	3.22	1.605	0.189	4.01	3.15	0.026
Food	0.824	21.28	<0.001	65.20	32.48	<0.001	4.13	3.24	0.073
Density × Food	0.970	1.03	0.404	1.60	0.796	0.497	1.03	0.812	0.488

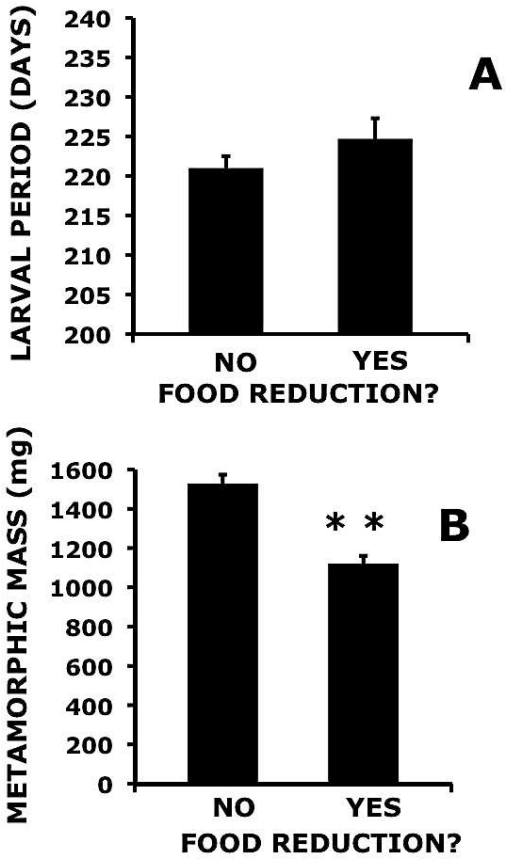

FIG. 2. Summary of responses of larval *Rana sphenocephala* to food reduction treatments. (A) Larval period in days. (B) Mass at metamorphosis. Bars represent means + 1 SE. ** $P < 0.001$.

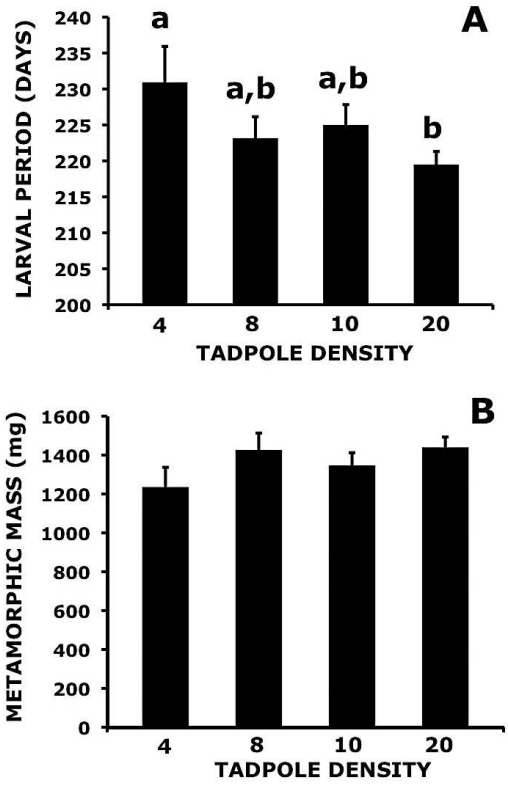

FIG. 3. Summary of responses of larval *Rana sphenocephala* to density treatments. (A) Larval period in days. (B) Mass at metamorphosis. Bars represent means + 1 SE. Letters above bars indicate treatment group means not significantly different using LSD a posteriori contrasts.

2004; Resetarits et al., 2004), whereas desiccation experiments produced accelerated metamorphosis at smaller sizes (Newman, 1988; Denver et al., 1998; Kiesecker and Skelly, 2001).

Our study was a density experiment that deviated from the studies cited above by keeping mean growth equal across density treatments. Our result of accelerated metamorphosis is more similar to desiccation experiments. What this suggests is that the retarded development in density experiments may have a nutritional basis (i.e., intraspecific competition for food prevents rapid development). In contrast, in our density experiment, larvae were freed from intraspecific competition and, thus, could accelerate development. Perhaps the reduced per capita swimming volume experienced by tadpoles at high density created similar stress conditions to those in desiccation experiments wherein food access was not restricted at the same time as water volume was declining. Thus, it is tenable that, in actual ponds, the effect of density on metamorphic development will be contingent on the nature of the larval habitat. If the pond's food resources remain sufficient to support rapid growth, then development can be accelerated to escape a drying pond. In contrast, a pond with poor growth opportunities for larvae is not likely to be able to produce any metamorphs in the case of desiccation.

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LITERATURE CITED

ALFORD, R. A., AND R. N. HARRIS. 1988. Effects of larval growth history on anuran metamorphosis. American Naturalist 131:91–106.

BECK, C. W., AND J. D. CONGDON. 2000. Effects of age and size at metamorphosis on performance and metabolic rates of Southern Toad (*Bufo terrestris*) metamorphs. Functional Ecology 14:32–38.

BUTTERFIELD, B. P., M. J. LANNOO, AND P. NANJAPPA. 2005. *Rana sphenocephala*. *In* M. J. Lannoo (ed.), Amphibian Declines: The Conservation and Status of United States Species, pp. 586–587. University of California Press, Berkeley.

DAY, T., AND L. ROWE. 2002. Developmental thresholds and the evolution of reaction norms for age and size at life-history transitions. American Naturalist 159:338–350.

DENVER, R. J., N. MIRHADI, AND M. PHILLIPS. 1998. Adaptive plasticity in amphibian metamorphosis: response of *Scaphiopus hammondii* tadpoles to habitat desiccation. Ecology 79:1859–1872.

JOHNSON, T. R. 1992. The Amphibians and Reptiles of Missouri. Missouri Department of Conservation, Jefferson City.

KIESECKER, J. M., AND D. K. SKELLY. 2001. Effects of disease and pond drying on Gray Treefrog growth, development, and survival. Ecology 82:1956–1963.

LOMAN, J. 2004. Density regulation in tadpoles of *Rana temporaria*: a full pond field experiment. Ecology 85:1611–1618.

MOREY, S., AND D. REZNICK. 2000. A comparative analysis of plasticity in larval development in three species of Spadefoot Toads. Ecology 81: 1736–1749.

———. 2001. Effects of larval density on postmetamorphic Spadefoot Toads (*Spea hammondi*). Ecology 82:510–522.

NEWMAN, R. A. 1987. Effects of density and predation on *Scaphiopus couchii* tadpoles in desert ponds. Oecologia 71:301–307.

———. 1988. Adaptive plasticity in development of *Scaphiopus couchii* tadpoles in desert ponds. Evolution 42:774–783.

RESETARITS, W. J., J. F. RIEGER, AND C. A. BINCKLEY. 2004. Threat of predation negates density effects in larval Gray Treefrogs. Oecologia 138:532–538.

RYAN, T. J., AND R. D. SEMLITSCH. 2003. Growth and expression of alternative life cycles in the salamander *Ambystoma talpoideum* (Caudata: Ambystomatidae). Biological Journal of the Linnean Society 80:639–646.

SCOTT, D. E. 1990. Effects of larval density in *Ambystoma opacum*: an experiment in large-scale field enclosures. Ecology 71:296–306.

———. 1994. The effect of larval density on adult demographic traits in *Ambystoma opacum*. Ecology 75:1383–1396.

SCOTT, D. E., E. D. CASEY, M. F. DONOVAN, AND T. K. LYNCH. 2007. Amphibian lipid levels at metamorphosis correlate to post-metamorphic terrestrial survival. Oecologia 153:521–532.

SEMLITSCH, R. D., D. G. SCOTT, AND J. H. K. PECHMANN. 1988. Time and size at metamorphosis related to adult fitness in *Ambystoma talpoideum*. Ecology 69:184–192.

SMITH, D. C. 1987. Adult recruitment in Chorus Frogs: effects of size and date at metamorphosis. Ecology 68:344–350.

SOKAL, R. R., AND F. J. ROHLF. 1995. Biometry. 3rd ed. W. H. Freeman, New York.

WILBUR, H. M., AND J. P. COLLINS. 1973. Ecological aspects of amphibian metamorphosis. Science 182:1305–1314.

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