

SHORTER CONTRIBUTIONS

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Developmental Plasticity and Growth Rates of Green Frog (*Rana clamitans*) Embryos and Tadpoles in Relation to a Leech (*Macrobdella decora*) Predator

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We tested for predator-induced plasticity in development and growth of embryos and young tadpoles of green frogs (*Rana clamitans*). We expected to observe developmental plasticity in response to the leech, *Macrobdella decora*, because the leech represents a high, but unpredictable, risk of mortality. Embryos raised with a leech tended to delay hatching and hatched at larger size. Young tadpoles raised with a leech grew more slowly than tadpoles raised without a leech. Such changes should enhance survival of green frogs faced with the threat of leech predation.

PREDATION can be a major source of mortality for both eggs and juvenile stages of amphibians (Duellman and Trueb, 1986; Sih and Moore, 1993; Warkentin, 1995). The presence of predators induces changes in size, color, morphology, growth rate and timing of metamorphosis (e.g., Semlitsch, 1987; Skelly and Werner, 1990; Van Buskirk and McCollum, 1999). Such predator-induced phenotypic variation is expected to evolve when the prey have reliable but nonfatal cues as to the presence of a predator, the probability of predation is high, but intermittent in time or space, and the fitness benefits of the altered phenotype, outweigh the costs (Dodson, 1989; Harvell, 1990).

Relatively few studies have examined the potential for predator-induced responses in the earliest life stages of amphibians. Embryos of the salamander, *Ambystoma barbouri*, delayed hatching and consequently hatched at larger size in the presence of predators (flatworm, *Phagocotus gracilis*, and sunfish, *Lepomis cyanellus*), which feed on hatchlings but not on embryos (Sih and Moore, 1993; Moore et al., 1996). Embryos of the arboreal red-eyed tree frog (*Agalychnis callidryas*) responded to a predatory snake by hatching immediately and falling into the water (Warkentin, 1995, 1999a,b), but these early-hatching tadpoles suffered greater mortality from predatory benthic shrimp than later hatchlings (Warkentin, 1995, 1999a). Early hatchlings also had smaller lungs, smaller, less muscular tails, and less-developed external mouthparts than older hatchlings, all of which contributed to their greater vulnerability to

shrimp (Warkentin, 1999b). These two studies of phylogenetically divergent amphibians suggest that embryonic responses to predators may be widespread in this vertebrate class.

We designed our study to investigate whether green frogs (*Rana clamitans*) showed predator-induced phenotypic variation in size at hatching and timing of hatching and whether a predator would subsequently influence the growth rate of newly hatched tadpoles. We expected to find responses in embryos and young tadpoles because we chose a predator that met the characteristics necessary for the evolution of phenotypic plasticity: the leech, *Macrobdella decora*. This leech can consume substantial proportions of frog egg clutches (Howard, 1978; GS, unpubl. data) and has been observed eating young tadpoles. Although *M. decora* can be common (Pennak, 1953; Bendell and McNicol, 1991), they are not always present in the ponds in which green frogs breed. Furthermore, some clutches are not preyed upon even when leeches are present. Thus, leeches represent a high, but unpredictable, risk to embryos and tadpoles.

MATERIALS AND METHODS

Study species.—We collected frogs and leeches from ponds near the Queen's University Biological Station (45°34'N, 76°13'W), in eastern Ontario, Canada. Green frogs breed from late May to early August in eastern Ontario. Clutches of 3500 to 4000 eggs are distributed in shallow water around the margin of permanent water bodies (Wright, 1914). After 3–5 days of development, embryos begin to secrete hatching en-

zymes and move vigorously within the egg membrane to hatch (Wright, 1914; Duellman and Trueb, 1986). Although new hatchlings are capable of swimming, they generally remain immobile.

Macrobdella decora is a large leech (up to 15 cm extended length) that is present in ponds and lakes throughout North America (Klemm, 1990). This species preys on amphibian embryos and young tadpoles and also feeds on vertebrate blood (Pennak, 1953; Howard, 1978). Frog embryos are eaten after a leech grasps the egg (or jelly surrounding the egg) with the anterior sucker, slices through the jelly and egg membrane with its teeth, and gains access to the embryo within (GS, pers. obs.). Hatchlings are eaten whole.

Experimental design and analyses.—We used six clutches of green frogs collected from two ponds between 11 June and 4 July 1996. The first two clutches were collected from a pond without *M. decora* leeches, and the remaining four clutches were collected from a pond that contained leeches. Embryos were in early developmental stages [4 cell stage (stage 4)—late cleavage (stage 9); Gosner, 1960]. Immediately following field-collection of a clutch, we separated two groups of 50 embryos from the clutch and randomly assigned embryos either to an experimental tank with a leech present or to a tank with no leech. Embryos from each group were placed in a nylon mesh bag (mesh size 1 × 1 mm, bag dimensions approximately 25 × 15 × 20 cm) suspended in each tank, which allowed us to assess the effect of leech presence without losing these embryos to leech predation.

We collected *M. decora* leeches on several occasions from a third pond. Leeches were housed individually before use in experiments. To minimize potential cues from prior feeding of leeches, the leeches were not fed before or during the experiments. Leeches used in the experiments all were relatively large (0.58–1.28g, weighed on an AE 100 Mettler balance). For each clutch, the experiment began once we placed a randomly selected leech into the “leech tank” (within 2 h of the addition of embryos).

The rectangular tanks were lined with polyethylene sheeting and were housed in an unheated, windowed laboratory. We filled tanks with 45 liters of well water 5–10 days before the addition of embryos or embryos and leeches. Because the rate of embryonic development is highly dependent on temperature (Wright, 1914; Berven et al., 1979), we measured tem-

perature in each tank daily over the duration of experiments. Tank temperatures within clutches were similar between leech treatments, ($F = 3.09$, $df_{\text{error}} = 1$, $P = 0.14$); thus, we did not use temperature as a variable in subsequent analyses.

We compared the size of hatching and timing of hatching for embryos in the presence versus absence of a leech predator. To measure hatchling size, we preserved the first 10 embryos that hatched in each tank in 10% formalin and measured the total length of each embryo (to the nearest 0.25 mm) using a Wild dissecting microscope and a stage micrometer. Lengths were measured over a two-day period approximately one month after preservation. We calculated time to hatching as the time (to the nearest 12-h period) from the beginning of the experiment to hatching, for each of the first 10 embryos to hatch. Developmental stage (Gosner, 1960) was recorded for each hatchling. Mean tank values for hatchling size and time to hatching were used as the experimental unit in all subsequent analyses.

We used ANOVA on two unreplicated randomized block designs to test for effects of a leech on either hatchling size or time to hatching, following MANOVA which considered both dependent variables simultaneously (Zar, 1984). Clutch was used as the random block term in analyses. With this experimental design, each combination of leech and clutch was represented by only a single mean value in analyses; thus, it was not possible to test for an interaction between the leech (main effect) and clutch (block) terms (Zar, 1984). However, we were interested in any between-clutch differences in response to leeches. Thus, we calculated mean hatchling size and mean time to hatching for each clutch (+ standard errors) to allow visual examination of how many clutches contributed to any significant overall effects of leeches.

We also examined whether early growth rate of tadpoles was influenced by the presence of a leech. This experiment was a continuation of our experiment examining hatching traits: we retained young tadpoles in the same tanks in which they had hatched. Hatchlings were fed boiled lettuce, which we removed and replaced with fresh lettuce on a daily basis. Repeated measurements of the same individual were not possible because handling often resulted in death of small tadpoles. Therefore, we used the mean size of the first 10 hatchlings from each tank (removed from the tank and preserved in formalin) to represent the mean size of all hatchlings at the beginning of the experiment. The growth rate experiment officially began at

TABLE 1. MANOVA AND ANOVA TESTS FOR EFFECTS OF LEECH PRESENCE AND EGG CLUTCH ON HATCHING TRAITS (HATCHLING SIZE AND TIME TO HATCHING).

Source	df	Wilks' Lambda	F	P
(A) MANOVA on hatching traits.				
Clutch	10, 8	0.0004	37.92	0.0001
Leech	2, 4	0.18	9.38	0.03
Source	df _{error}	F	P	
(B) ANOVA on hatchling size.				
Model	5	12.22	0.0074	
Clutch	5	12.65	0.0073	
Leech	1	10.06	0.025	
(C) ANOVA on time to hatching.				
Model	5	7.85	0.02	
Clutch	5	8.21	0.019	
Leech	1	6.06	0.057	

the mean hatch date of the remaining embryos (calculated after hatching was complete). Ten days after the last embryo hatched, we concluded the experiment by randomly selecting 10 hatchlings from each tank for size measurements. With this approach to determining beginning and end sizes for hatchlings, the duration of the experiment varied for each clutch. We calculated growth rate for embryos in each tank as the change in mean size divided by the duration of the experiment. We used ANOVA to examine the effects of a leech on growth rate, after blocking by clutch.

For all statistical analyses, we checked assumptions of analyses by visual examination of residual plots and used transformations as necessary.

RESULTS

Mean hatchling size and mean time to hatching were positively correlated ($r = 0.67$, $n = 12$,

$P = 0.02$), and MANOVA indicated a significant effect of leech presence on these hatching traits (Table 1A). Univariate ANOVA indicated that embryos hatched at a significantly larger size when a leech was present (average 0.4 mm larger; Table 1B). Using a similar test, we also found that hatching tended to be delayed ($P = 0.057$) when embryos were raised with a leech (approximately 0.5 day delay; Table 1C). Finally, our analyses show that clutch accounts for significant variation in hatchling size and time to hatching (Table 1).

Although there was significant variation in hatchling size and time to hatching relating to clutch and the leech treatments, all embryos appeared to hatch at the same developmental stage. We scored all embryos as hatching at Gosner stage 19 based on the relative development of external gills and tail (Gosner, 1960). However, we examined preserved specimens and thus were unable to score other definitive characteristics of developmental stage including muscular response, heart beat, and gill circulation.

Visual examination of means and standard errors associated with the hatching traits for each clutch indicate that the significant leech effect was primarily a result of the responses by the first four clutches (Table 2). In contrast, leeches had little or no effect on hatchling size or timing of hatching for embryos in clutches 5 and 6 (Table 2). These differences in response of clutches to the leech treatment do not correspond to differences among clutches in temperature of tanks; mean temperatures in tanks ranged from 15.5 to 17.7 C, with lowest mean temperatures experienced by clutch 4 (both treatments) and highest temperatures experienced by clutches 1 and 2 (both treatments).

In our growth rate experiment, hatchlings were allowed to grow for an average of 11 days

TABLE 2. AVERAGE HATCHLING SIZE (SE), HATCH TIMING (SE), AND GROWTH OF HATCHLINGS IN THE ABSENCE AND PRESENCE OF A LEECH PREDATOR FOR SIX GREEN FROG CLUTCHES. Exploratory tests on each clutch of embryos indicated a significant effect of leech presence on hatchling size ($P < 0.05$) and hatch timing ($P < 0.0002$) for clutches 1–4. Similar comparisons within clutches for growth data were not possible because only a single growth value was calculated for each clutch (see text for details).

	Clutch 1		Clutch 2		Clutch 3		Clutch 4		Clutch 5		Clutch 6	
	Leech absent	Leech present	Leech absent	Leech present	Leech absent	Leech present	Leech absent	Leech present	Leech absent	Leech present	Leech absent	Leech present
Size (mm)	4.20 (0.10)	4.65 (0.13)	5.33 (0.12)	5.80 (0.07)	5.18 (0.05)	5.63 (0.20)	4.84 (0.2)	5.65 (0.07)	5.30 (0.07)	5.43 (0.05)	4.40 (0.07)	4.38 (0.14)
Timing (# 12 h per.)	6.00 (0.00)	7.20 (0.15)	7.33 (0.17)	8.80 (0.13)	8.60 (0.07)	9.55 (0.19)	9.50 (0.27)	11.90 (0.28)	9.00 (0.00)	9.00 (0.00)	8.00 (0.00)	7.80 (0.13)
Growth (mm)	0.20	0.19	0.17	0.13	0.18	0.16	0.18	0.09	0.21	0.19	0.20	0.19

TABLE 3. ANOVA SHOWING EFFECTS OF LEECH PRESENCE AND EGG CLUTCH ON TADPOLE GROWTH RATE.

Source	df _{error}	F	P
Model	5	7.46	0.022
Clutch	5	6.4	0.031
Leech	1	12.79	0.016

with no difference in duration of the experiment between the leech and no-leech treatments (clutch as a blocking variable; $F = 0.9$, $df_{\text{error}} = 5$, $P = 0.6$). Hatchlings raised with a leech grew 35% slower than those raised without a leech (Tables 2–3).

DISCUSSION

Individuals responding to predators with altered development or morphology are thought to enjoy a fitness advantage caused by reduced risk of predation (Smith-Gill, 1983; Harvell, 1990; although for individuals in marginal populations living in heterogeneous environments, such traits can be maladaptive, Stearns, 1989). Green frog embryos raised with a leech showed altered development, that is, they hatched at larger size and tended to delay hatching. Additionally, once hatched, young tadpoles grew more slowly when a leech was present. Such predator-induced variation in phenotype was expected in green frogs because they show an unpredictable but high risk of mortality from leeches in natural situations. However, we cannot be certain which, if any, components of this correlated response are important to reducing predation risk from leeches. For example, reduced growth of young tadpoles may result from reduced feeding activity, making it more difficult for leeches to detect vibrational cues from tadpoles. Alternatively, reduced growth of tadpoles may simply be a consequence of hatching later or larger, without special significance itself.

In our study, the responses to leeches were apparent even though leeches were not allowed to feed on embryos or hatchlings. Similar experiments on wood frogs (*Rana sylvatica*) indicated that the proportion of embryos eaten by leech predators had no influence on the magnitude of response (hatchling size or time to hatching; GS, unpubl. results). These results provide evidence that embryos and young tadpoles respond to the mere presence of the predator (e.g., Petranka et al., 1987) and that responses are not based on predator-feeding cues (e.g., Crowl and Covich, 1990).

The green frog-leech interaction differs in some respects from predator-induced plastic responses of embryos that have been investigated in other studies. Previous research has shown a fitness advantage to prolonging the embryonic stage when predators do not feed on embryos (Sih and Moore, 1992; Moore et al., 1996). However, because *M. decora* eat both embryos and hatchlings of green frogs, the benefit to remaining inside the egg when a leech is present is not obvious. For at least one other amphibian (red-eyed tree frog), there are immediate direct benefits of hatching, as hatching results in a transition from a terrestrial environment (where the predator, a snake, is still feeding on unhatched eggs) to an aquatic environment (Warkentin, 1995). Such a benefit of hatching is not obvious for green frogs, because hatched individuals are in the same local environment as the predator. Indeed, the hatching process itself, which is accompanied by frequent and vigorous wiggling, may attract the leech.

Finally, not all green frog clutches contributed equally to the overall effects of leeches on hatching traits. In particular, four clutches responded to the presence of a leech and contributed to the significant leech effect; however, two clutches did not respond despite the threat that a leech would represent in a natural situation (Cory and Manion, 1953, *R. sylvatica*; Howard, 1978, *Rana catesbeiana*; GS, unpubl. results for *R. clamitans*, *R. sylvatica*, and *R. catesbeiana*). Similar variation in plastic responses has been shown in other studies (Parjeko and Dodson, 1991; Sih and Moore, 1993; Moore et al., 1996). Thompson (1991) proposed that intraspecific variability in plastic response should be maintained when the fitness consequences of plastic responses vary with the environment, whereas empirical research suggests that the match between environmental conditions and plastic response will not always be optimal (Van Buskirk and McCollum, 1999). We cannot explain why two clutches in our study showed little or no response to a leech, especially given that these clutches came from a pond where leeches occurred and the two other clutches collected from this pond showed a response. Although within-species variation in plastic development is expected, factors affecting its magnitude and its contribution to differential fitness in natural situations remain largely unknown.

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