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Accelerated Hatching of Southern Leopard Frog (*Rana sphenocephala*) Eggs in Response to the Presence of a Crayfish (*Procambarus nigrocinctus*) Predator

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Phenotypic plasticity, such as morphological and behavioral changes in response to predators, is common in larval anurans. Less is known about inducible defenses in the embryonic stages of development. We investigated the predation risk imposed by crayfish (*Procambarus nigrocinctus*) on southern leopard frog (*Rana sphenocephala*) eggs and whether crayfish presence induces a change in the timing of hatching of *R. sphenocephala* eggs. We found that crayfish significantly reduce the hatching success of *R. sphenocephala* eggs by eating them and that eggs hatch significantly faster in the presence of crayfish than when crayfish are not present. We also found *that* the nonlethal presence of crayfish (caged with no access to eggs) induced accelerated hatching, indicating that injured conspecifics are not required to elicit the response. Reception of chemical cues produced or released by crayfish may play an important role in survival of *R. sphenocephala eggs.*

ANURAN larvae are known to alter their behavior (Skelly, 1994; Wilbur, 1997; Van Buskirk, 2001) and change their shape and color (McCollum and Leimberger, 1997; Van Buskirk et al., 1997) to increase survival in the presence of an array of predators. These plastic behavioral and developmental antipredator responses by amphibian larvae appear to be common coping mechanisms (Relyea, 2001).

Less is known about induced antipredator defenses of the embryonic stages of amphibians. Changes in the timing of the transition between egg and larval stages in the presence of predators have been documented for several species of anurans. Sih and Moore (1993) were the first to demonstrate that predation risks may affect the timing of hatching. They showed that salamander (*Ambystoma barbouri*) eggs, laid aquatically, delayed their hatching to a later time in the presence of a larval predator, the flatworm (*Phagocotus gracilis*), and in the presence of water-borne flatworm chemical cues. Later, Moore et al. (1996) found that *A. barbouri* eggs also delayed hatching in response to chemical cues from a predatory fish (*Lepomis cyanellus*).

Many more studies have documented an accelerated hatching response to a variety of egg predators. Warkentin (1995, 2000) showed that vibratory cues from the oophagous snake (*Leptodeira septentrionalis*) and the wasp (*Polybia rejecta*) would cause early hatching of well-developed red-eyed treefrog (*Agalychnis callidryas*) eggs. Similarly, Brown and Iskandar (2000) found that well-developed *Rana arathooni* embryos would hatch prematurely when physically agitated by human collectors. Both *A. callidryas* and *R. arathooni* lay their eggs terrestrially near water.

Agalychnis callidryas also hatch early in response to contact with hyphae of the pathogenic fungus (Pheaosphaeriaceae: Dothideales), possibly stimulated by oxygen depletion caused by the fungus, chemical cues released by the fungus, or cues from clutchmates that die as a result of fungal infection (Warkentin 2001). Chivers et al. (2001) found that water-borne chemical cues from egg-eating leeches accelerated hatching in the Pacific treefrog (*Hyla regilla*) and the Cascade frog (*Rana cascadae*).

Because eggs are immobile, they would appear to be the most vulnerable stage in a frog's development. Eggs are preyed on by conspecific anuran larvae (Polis, 1981; Petranka and Kennedy, 1999; Dayton and Wapo, 2002), other species of anuran larvae (Crossland, 1998), invertebrate predators (Warkentin, 1995; Richter, 2000; Chivers et al., 2001), and fish (Grubb, 1972). However, some anuran eggs are unpalatable or even toxic to some potential predators (Licht, 1968; Punzo and Lindstrom, 2001).

Southern leopard frog *(Rana sphenocephala)* eggs may be particularly apparent to predators because they are deposited as large conspicuous masses, often as aggregated clumps (Caldwell, 1986) ranging from several hundred to a few thousand per clump. The rate of egg development varies, and eggs can take more than 10 days to hatch when water temperature is low (DS, pers. obs.), thus affording predators ample time to find the eggs. Richter (2000) observed caddisflies (Trichoptera) predating *R. sphenocephala* egg masses in Mississippi and suggested

that the aggregation of egg masses may facilitate movement of predators between clumps of eggs. In this paper, we examine the effect of the crayfish (*Procambarus nigrocinctus*), a common scavenger/predator on the timing and success of hatching of *R. sphenocephala* eggs. We chose *P. nigrocinctus* because it is widespread throughout our study region and commonly coinhabits pools with *R. sphenocephala* (DS, pers. obs.).

Materials and Methods

We collected 30 crayfish, between 60 and 70 mm total length, from the Davy Crockett National Forest in eastern Texas during November and December 2001. Prior to the experiments, crayfish were housed in 3-liter tubs (19 X 9 X 33.5 cm) with aged tap water. They were each fed approximately 0.5 g of tropical fish food every three days prior to the experiments. No attempt was made to control for sex or molt status. We also collected 15 *R. sphenocephala* egg masses in varying stages of development (hetween stages 5–10; Gosner, 1960) from the Stephen F. Austin Experimental Forest in Nacogdoches County in eastern Texas on 21 December 2001.

Our experimental design consisted of' three trealmen ts: (1) a con trol group with 25 *R. sphenocephala eggs; (2)* a group with 25 *R. sphenocephala* eggs and a free roaming crayfish with access to eggs; and (3) a treatment containing 25 *R. sphenocephala eggs and a caged* (cage dimensions: 14 X 9 X 14 cm) crayfish that did not have access to the eggs. In the latter treatment, water flowed freely between the crayfish and the eggs. Caged crayfish were not fed during the experiment. Each treatment was replicated 15 times using a subset of eggs from each clutch. Each replicate was placed into a 3-liter plastic tub (19 X 9 X 33.5 cm) with two-liters of aged tap water. Temperature in the laboratory varied between approximately 10 and 13 C where the experiments were conducted.

To determine hatching success, we observed the tubs every 24 h until all normally developed emhryos had hatched, at which time the experiment was terminated. Any remaining eggs that showed no signs of development were assumed to be incapable of hatching. Crayfish were removed from the tubs, and counts were made of newly hatched tadpoles and of' eggs that failed to hatch. Crayfish in the free-roaming treatment were capable of eating eggs and hatchlings, which may have influenced our measure of hatching success. Proportional data (percent eggs that hatched) were arcsine-square-root transformed and used in an analysis of variance (ANOVA) followed by Tukey's multiple comparison test to compare egg survival between treatments.

To assess hatching timing, we observed the tubs every 24 h until we noted hatchlings from any tub iii any treatment within a replicate (full siblings), thus indicating that the egg mass was beginning to hatch. For each egg mass, the number of hatchlings present in each tub was counted 24 h after hatching began iii any treatment. The proportion hatched was calculated as a fi-action of viable eggs only; nonviable eggs were excluded from the analysis. In the free-roaming crayfish treatment, where the crayfish ate eggs, we calculated hatching rate J'rom the remaining live eggs. This method was repeated for each replicate until all 15 egg masses had hatched. The earliest clutch hatched completely in four clays, whereas the slowest took eight days to hatch from the time they were collected in the field. Proportional data (percent eggs hatched by 24 h) were arcsine-square-root transformed and used in an analysis of variance (ANOVA, randomized block design) followed by Dunnett's multiple comparison test to compare the hatching rate of the two treatments to the control. A paired *t*-test was used to compare the effect of the two predator- treatments on the hatching rate. Statistical analyses were performed using SPSS version 10.0 (SPSS Inc., 1999, Chicago, IL).

Results

Hatching success differed significantly among the three treatments (ANOVA $F_{2,42} = 43.28$, $P < 0.001$). Tukey's post hoc tests (Critical Value of Studentized Test = 3.45) revealed that tubs with free-roaming crayfish had a significantly lower hatching success (42 ± 10%, mean + SE) compared to control tubs (97 ± 2%) arid tubs with caged crayfish (98 ± 1%). In every case (n = 15), the free-roaming crayfish consumed at least a portion of the 25-egg clump, hut only three crayfish consumed all 25 eggs. The egg predation that occurred in tubs containing free-roaming crayfish was likely the primary cause of hatching failure in the rxperiment. However, crayfish also were capable of' eating hatchlings, which might have caused an overestimation of' crayfish predation on eggs. Hatching success in the majority of control tubs and caged crayfish tubs was less than 1 00%. The main cause of hatching failure appears to be unfertilized eggs, since there were no signs of development in the unhatched eggs. We found no difference in hatching success between control and caged crayfish treatments.

The timing of hatching also differed among the three treatments (ANOVA $F_{2,39} = 6.55$, $P = 0.005$). The proportion of eggs hatched in 24 h after the start of hatching was significantly lower in the control tubs (41 ± 10%) than in either the caged crayfish tubs (58 ± 7%) or the free-roaming crayfish tubs (70 ± 8%; critical value of Dunnett's $t = 2.01$). We found no difference in the timing of hatching between caged crayfish and free-roaming crayfish treatments (paired l-test: $t = -10.32$, df = 11, $P = 0.21$). It is possible that the free-roaming crayfish ate hatchlings, thereby skewing our results toward a more conservative estimate of the acceleration of hatching than actually occurred. In three instances, free-roaming crayfish ate all 25 eggs before the second day of hatching.

DISCUSSION

Our study suggests that crayfish (*P. nigrocinctus*) can significantly affect survival of *R. sphenocephala* eggs. Crayfish consumed slightly over half of the eggs in the free-roaming crayfish treatment. Predatory crayfish were observed pulling embryos from the egg capsules. The embryos were then consumed and the egg capsules discarded. Differences in the timing of hatching observed in our study clearly indicate that Ii. *sphenocephala* eggs respond to the presence of a crayfish by reducing the time to hatching. Similar responses are known from other anurans. Warkentin (1995, 2000, 2001) showed that an effective defense strategy to prevent mortality on red-eyed treefrog eggs is to hatch more quickly in the presence of predators and pathogens. This species typically lays its eggs on vegetation overhanging water. As the eggs hatch, tadpoles fall into the water where they are safe from terrestrial egg predators. Warkentin (1995) also demonstrated that the trade-off of early hatching leads to greater susceptibility of underdeveloped tadpoles to aquatic predators. More research is needed to ascertain potential costs of early batching in *R. sphenocephala.*

Prior to this research, *H. regilla* and *R. cascadae* were the only anurans that were known to accelerate the timing of hatclring in response to aquatic egg predators (Chivers et al., 2001). We present the first evidence of early hatching of ***R.*** *sphenocephala* eggs in response to a predator. We suggest that crayfish would likely have less success in predating a free-swimming tadpole than sessile eggs despite the protection afforded by the egg capsules or any toxins that *R. sphenocephala* eggs may contain. We noted that hatchling *R. sphenocephala* are generally inactive but are capable of swimming when disturbed as soon as they leave the egg capsule, which should enable them LO disperse and hide in the substrate.

Proximate mechanisms of how *R. sphenocephala* embryos detect the presence of predators are unknown. Other studies have identified tactile stimulation or agitation as mechanisms for inducing an early batching in fish (Griem and Martin, 2000) as well as anuran eggs (Warkentin, 1995, 2000; Brown and Iskandar, 2000). In our free-roaming treatment, crayfish were able to physically disturb eggs. Thus, tactile stimulation may have played a role in early hatching for this treatment. Free-roaming crayfish also consumed some of the eggs in each tub, which may have resulted in a chemical alarm stimulus that led LO accelerated hatching in the other embryos. Alarm pheromones that alert conspecifics of danger are known to accelerate hatching in ***H.*** *regilla* (Chivers et al., 2001) and are released by several species of anuran tadpoles when attacked by predators (Petranka, 1989).

However, direct mechanical stimulation and alarm pheromones cannot explain the accelerated hatching response we observed in the caged crayfish treatment. It appears that the mere presence of a crayfish can induce early hatching in ***R.*** *sphenocephala* since caged crayfish were not fed during the experiment and were not allowed to come in direct contact with the eggs. Most likely, crayfish release cues into the water that are detected by the eggs via chemoreception. Future research is needed to determine the causal mechanisms underlying the accelerated hatching phenomenon we observed.

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