

## HATCHING PLASTICITY OF WOOD FROG (*RANA SYLVATICA*) EGGS IN RESPONSE TO MOSQUITOFISH (*GAMBUSIA AFFINIS*) CUES

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**Abstract.**—We conducted a laboratory experiment to examine how the presence of Mosquitofish (*Gambusia affinis*), a potential amphibian egg predator, affected the timing of hatching, hatching success, and hatchling size in the Wood Frog, *Rana sylvatica*. Our experimental design also allowed us to compare and isolate the influence of physical access of the fish and chemical cues of the fish on hatching. Embryos in no barrier treatments hatched significantly faster than embryos in the barrier treatments, which hatched significantly earlier than embryos in the control treatments. The final mass of hatchlings did not differ among treatments. The proportion of eggs hatching also did not differ among treatments. Our results suggest that Wood Frogs can accelerate hatching in the presence of cues from Mosquitofish. Chemical cues alone were sufficient to accelerate hatching (in the barrier treatment), but the presence of both physical and chemical cues (i.e., no barrier treatment) increased the effect of Mosquitofish presence.

**Key Words.**—Competitor cues; embryos; *Gambusia affinis*; Mosquitofish; predator cues; *Rana sylvatica*; time to hatching; Wood Frog

### INTRODUCTION

In organisms with complex life cycles, the timing of transitions between life stages often depends, either proximately or ultimately, on avoiding increasing risks in one life stage by moving to the next stage (e.g., Wilbur and Collins 1973). Recently, the transition from egg/embryo to hatchling/tadpole in anurans has been recognized as having a surprising amount of plasticity. For example, several species of anurans have been shown to accelerate hatching in the presence of cues from egg predators (e.g., Johnson et al. 2003; Saenz et al. 2003; Capellán and Nicieza 2007; Gomez-Mestre and Warkentin 2007; Ireland et al. 2007), and several species have been shown to delay hatching in the presence of cues from tadpole or larval predators (Laurila et al. 2002; Schalk et al. 2002; Ireland et al. 2007). Such responses can reduce the risks to which embryos and tadpoles are exposed. Much of the work examining embryonic responses to predator cues has focused on native predators. However, non-native predators are a major threat to amphibian populations (e.g., Alford and Richards 1999; Kiesecker 2003). Therefore, evaluating the response of native amphibians to non-native predators may help understand the potential for invasive species to impact amphibian populations.

Mosquitofish (*Gambusia* spp.) have been introduced around the world (reviewed in Lloyd et al. 1986; Courtenay and Meffe 1989) and can act as predators on the eggs and larvae of amphibians (e.g., Komak and Crossland 2000; Baber and Babbitt 2003, 2004; Zeiber et al. 2008). Mosquitofish introductions appear to have contributed to the decline or disappearance of some amphibians (e.g., *Litoria aurea* in Australia: Pyke and

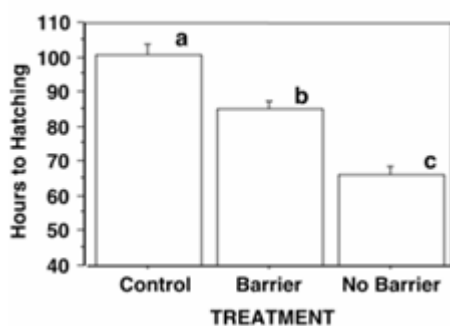
White 1996, 2001; White and Pyke 1996; Lewis and Goldingay 1999; Mahoney 1999; but see Hamer et al. 2002; *Taricha torosa* in North America, Gamradt and Kats 1996; *Triturus alpestris* and *T. helveticus* in Europe, Denoël et al. 2005).

We conducted a laboratory experiment to examine how the presence of the non-native Mosquitofish (*Gambusia affinis*), a potential amphibian egg predator, affected the timing of hatching, hatching success, and hatchling size in native Wood Frogs, *Rana sylvatica*. Our experimental design also allowed us to compare the relative influence of physical and chemical cues of the fish on hatching.

### MATERIALS AND METHODS

We collected four egg masses of *Rana sylvatica* from a local pond in Granville, Ohio, USA within 12 h of oviposition. Egg masses were maintained in a single container in the laboratory until used in the experiment (< 24 h after collection). We collected *Gambusia affinis* from the same local pond as the Wood Frog egg masses and within 24 h of the collection of the Wood Frog egg masses.

Our experiment had three treatments (barrier, no barrier, and control) replicated eight times. The barrier treatment consisted of a plastic container (15 cm x 30 cm x 10 cm; 2 L of aged [48 h] tap water) divided in half with fiberglass window screening attached to the walls with silicone, with eggs on one side and a single Mosquitofish on the other side. The no barrier treatment consisted of a plastic container with a Mosquitofish and an egg cluster, but with no fiberglass window screen barrier. The control treatment consisted of one egg



**FIGURE 1.** Effect of Mosquitofish treatments (control = no Mosquitofish; barrier = chemical cues only; no barrier = chemical and physical cues) on the time to hatching in Wood Frog (*Rana sylvatica*) embryos. Means include + 1 S.E. Means with different letters were significantly different (Fisher's PLSD,  $P < 0.0001$  in all three cases).

cluster and no Mosquitofish. For both the no barrier and control treatments, we attached a piece of fiberglass screen the same size as the barrier in the barrier treatment with silicone to the bottom of the container to control for any leaching of chemicals from the screen or the silicone. Each egg cluster contained 25 eggs and we haphazardly assigned them to each treatment. Each egg mass was used equally among treatments (unfortunately clusters were not identified by clutch upon data collection). After placing egg clusters and, where applicable, Mosquitofish into the containers, we recorded the number of hatchlings once every 24 hours for a week. At the end of one week, we removed hatchlings from the containers, dried them with paper towels, and weighed them to obtain an average hatchling mass per container. We did not observe any evidence of consumption of eggs, embryos, or hatchlings (i.e., no embryos or hatchlings disappeared over the course of the experiment). The laboratory in which the experiments were conducted remained at a constant controlled temperature ( $\sim 19 - 20$  °C) throughout the experiment and on a natural photoperiod using natural light.

We used a MANOVA to examine the effects of predator cue treatment on time to hatching, hatchling mass, and proportion of eggs hatching (arcsine-square root transformed to meet the assumptions of parametric tests; see Sokal and Rohlf 1995). We followed up the significant MANOVA with univariate ANOVAs. We used post-hoc Fisher's Protected Least Squares Difference to examine differences among the treatments for significant ANOVAs (Sokal and Rohlf 1995). For all tests,  $\alpha = 0.05$ .

## RESULTS

There was a significant treatment effect (Wilks'  $\lambda$ :  $F_{6,38} = 10.6$ ,  $P < 0.0001$ ). Embryos in no barrier treatments hatched significantly faster than embryos in the barrier treatments, which hatched significantly faster

than embryos in the control treatments ( $F_{2,21} = 58.7$ ,  $P < 0.0001$ ; Fig. 1). The final mass of hatchlings did not differ among treatments (control:  $0.144 \pm 0.004$  mg; barrier:  $0.139 \pm 0.006$  mg; no barrier:  $0.131 \pm 0.003$  mg;  $F_{2,21} = 1.92$ ,  $P = 0.17$ ). The proportion of eggs hatching also did not differ among treatments (control:  $0.945 \pm 0.015$ ; barrier:  $0.960 \pm 0.015$ ; no barrier:  $0.935 \pm 0.013$ ;  $F_{2,21} = 0.83$ ,  $P = 0.45$ ).

## DISCUSSION

Our results suggest that Wood Frogs are able to accelerate hatching in the presence of cues from Mosquitofish. In other anurans, egg predators often induce early hatching (e.g., Capellán and Nicieza 2007; Gomez-Mestre and Warkentin 2007; Ireland et al. 2007) and tadpole predators often induce delayed hatching (e.g., Laurila et al. 2002; Schalk et al. 2002; Ireland et al. 2007). Our results therefore suggest that Wood Frogs respond as if Mosquitofish were egg predators. Mosquitofish are known to be predators on anuran eggs and tadpoles (e.g., Komak and Crossland 2000; Baber and Babbitt 2003, 2004; Zeiber et al. 2008). However, it may be Mosquitofish are a greater risk for embryos than for tadpoles. Indeed, Mosquitofish can cause mortality in tadpoles but often through an accumulation of injuries rather than complete immediate consumption (Komak and Crossland 2000; Baber and Babbitt 2004; Zeiber et al. 2008).

Our results contrast with previous experiments examining the effects of predator or predator-related cues on embryos of Wood Frogs. Previous studies have failed to find any changes in hatching time in response to a variety of predators or predator-related cues (e.g., odonates, Anderson and Petranka 2003; crushed conspecific eggs or tadpoles, Touchon et al. 2006; odonates, Mosquitofish, congeneric tadpoles, Dibble et al. 2009). Some of the differences may arise from differences in experimental designs and methods. For example, using Wood Frogs and Mosquitofish from the same pond as we did (although in a different year), Dibble et al. (2009) found no effect of Mosquitofish cues on hatching in Wood Frog embryos. However, they used water from a Mosquitofish holding aquarium to provide chemical cues only (i.e., the fish were not in the experimental containers). Touchon et al. (2006) used crushed tadpoles to simulate conspecific alarm cues, as opposed to cues of the predator itself. Anderson and Petranka (2003) did add a predator directly to their experimental containers; however their predator, an odonate nymph, likely would produce different physical and chemical cues than a fish. Thus, the difference in results among these experiments and ours may reflect how the "predator" cue was created, as well as the predators used as the source of the cues. In addition, there appear to be differences among siblings of *R.*

*sylvatica* in time of hatching (but not in responses to cues; Dibble et al. in press); thus, variation among experiments may reflect genetic variation in the egg masses used. Our results suggest that extrapolating results too generally may limit our understanding of how various cues affect anuran embryos. In other words, simply because some cues or experimental designs do not induce early hatching does not mean that other cues or species of predators will not.

Our experimental design allowed us to determine that chemical cues alone (i.e., the conditions in the barrier treatment) were sufficient to accelerate hatching, but also that the presence of both physical and chemical cues (i.e., no barrier treatment) increased the effect of Mosquitofish presence. In addition, or alternately, vibrational cues from the swimming Mosquitofish may be influential in inducing early hatching. The vibrations propagated in the water might act as a cue to the presence of a potential predator and thus stimulate early hatching. The importance of vibrational cues to the induction of early hatching has been demonstrated in Red-eyed Treefrogs, *Agalychnis callidryas* (e.g., Warkentin et al. 2007; Caldwell et al. 2009). Visual cues might also be contributing to the induced response. While relatively little is known about the visual capabilities of anuran embryos, it seems unlikely that this can explain the induced responses since the visual system is not well developed early in development (e.g., Currie and Cowan 1975; Niazi and Niazi 1990; Schlosser and Roth 1997; Schlosser 2008). It thus appears that Wood Frog embryos may be able to assess the level of threat and exhibit a threat-sensitive response similar to those exhibited by tadpoles and other organisms (Stankowich and Blumstein 2005). However, further experiments are necessary to determine the specific cue or cues responsible for inducing early hatching in Wood Frogs.

Our observation that Mosquitofish induce early hatching but have no effect on hatchling mass after a week may arise for several reasons. It may be that early hatching embryos accelerated their growth so they hatched at the same size as the later hatching embryos. Alternatively, and perhaps more likely, the early hatching embryos may have hatched at a smaller size, but were able to catch up to the later hatching embryos by the end of the experiment (see Vonesh 2005; Vonesh and Bolker 2005). We do not have the data to differentiate between these two alternatives because we chose not to weigh hatchlings until the end of the experiment to minimize the disturbance to the eggs.

Differences in hatching time induced by predator cues, such as those observed here, may have consequences for later life stages, but the results are equivocal as to whether these consequences are substantial and long-lived. For example, while differences in hatching time of Common Frogs, *Rana temporaria*, did not affect

metamorphic traits, they did affect juvenile morphometric traits but had no effect on juvenile performance (Capellán and Nicieza 2007). Buckley et al. (2005) found that Coquí frogs, *Eleutherodactylus coqui*, that hatched earlier due to disturbance of their egg mass did not perform as well as froglets that hatched normally and were lighter, thus suggesting a potential cost for early hatching. In addition, escaping egg predators via early hatching may result in higher predation risk for hatchlings due to higher vulnerability to hatchling predators (e.g., Warkentin 1995, 1999; Gomez-Mestre and Warkentin 2007). However, in some cases predator induced hatching does not affect subsequent performance and survivorship is actually higher in early hatching individuals than in late hatching individuals, although they were smaller at metamorphosis which may have negative consequences for survivorship (*Hyperolius spinigularis*, Vonesh and Bolker 2005). Thus it is unclear what might be the long term consequences of accelerated hatching in Wood Frogs in response to the presence of the invasive predator, Mosquitofish.

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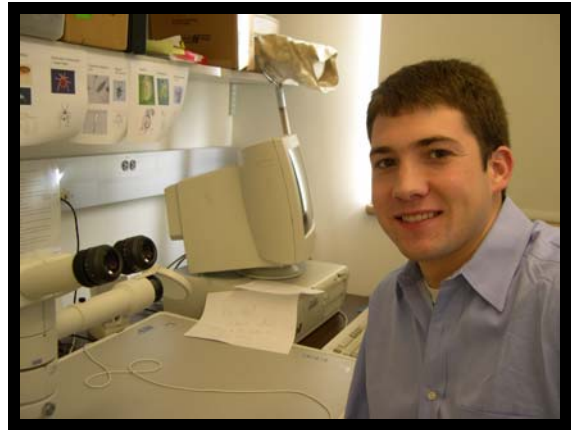
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