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Effects of mosquitofish (*Gambusia affinis*) cues on wood frog (*Lithobates sylvaticus*) tadpole activity

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Abstract. We examined the changes in activity of wood frog (*Lithobates sylvaticus*) tadpoles exposed to combinations of visual, chemical, and mechanical cues of the invasive mosquitofish (*Gambusia affinis*). We also examined whether the responses of the tadpoles to the predator cues were influenced by the short-term accumulation of chemical cues in the experimental container. In our experiment, the activity of wood frog (*L. sylvaticus*) tadpoles was not affected by the presence of various cues from mosquitofish. Our experiment demonstrated that the repeated use of trial water can influence the activity level of tadpoles, regardless of the predator cue treatment used. Tadpoles in the first trial tended to be less active than tadpoles in subsequent trials. This effect does not appear to be mediated by the accumulation of predator cues since there was no significant interaction term. Our results suggest that short-term accumulation of predator chemical cues do not affect the behavior of wood frog tadpoles: however, our results suggest that the repeated use of the same water in consecutive trials may affect tadpole behavior, perhaps through the accumulation of conspecific chemical cues.

Keywords. activity, behavior, conspecific cues, *Gambusia affinis*, *Lithobates sylvaticus*, predator cues.

INTRODUCTION

Non-native species are among the many threats to amphibian populations around the world, and in particular, the introduction of non-native fish frequently results in declines of native amphibians (see Kiesecker, 2003). Such non-native predators frequently have greater effects on native prey than native predators do because the prey are naïve to the predator and thus may not respond to them as a predator (Banks and Dickman, 2007; Salo et al., 2007).

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One of the most widespread introduced fish are the mosquitofish (*Gambusia affinis* and *Gambusia holbrooki*, biology and ecology reviewed in Pyke, 2005, 2008). Mosquitofish are known predators of amphibians, both within their native range (Baber and Babbitt, 2003; Stanback, 2010) and outside their native range (e.g., Komak and Crossland, 2000; Gregoire and Gunzburger, 2008; Segev et al., 2009). Previous studies have shown that some species of tadpoles show anti-predator behaviors, such as reducing their activity or changing their space use, in response to the presence of mosquitofish or mosquitofish cues (e.g., Lawler et al. 1999; Burgett et al. 2007; Smith et al. 2009, 2010, 2011), but other species do not (e.g., Hamer et al. 2002; Smith et al. 2008, 2009).

We examined the changes in activity of wood frog (*Lithobates sylvaticus*) tadpoles exposed to combinations of visual, chemical, and mechanical cues of mosquitofish to determine which cues, if any, elicited a change in behavior. We predicted that (1) tadpoles would have reduced activity in the presence of a mosquitofish (based on a previous study of wood frogs by Burgett et al., 2007), and that (2) tadpoles would reduce their activity in the presence of an uncaged predator compared to with a caged predator (i.e., greater reduction of activity with greater risk – a threat-sensitive response; Helfman, 1989; Stankowich and Blumstein, 2005). In addition, we examined whether the responses of the tadpoles to the predator cues were influenced by the short-term accumulation of chemical cues, both from predators and conspecifics, in the experimental container. We predicted that responses would be greater in later trials than in earlier trials in the same container.

MATERIAL AND METHODS

We collected *L. sylvaticus* egg masses (N = 8) from a pond on the Denison University Biological Reserve, Granville, Licking Co., Ohio on 13 March 2010. This pond occasionally has mosquitofish in years in which it retains water year-round, but not during 2010. Eggs were allowed to hatch in the laboratory. Tadpoles from multiple egg masses (3-4) were maintained in mixed groups in large plastic containers for two weeks and fed ad libitum until used in the experiment. Mosquitofish used in this experiment were collected from another pond on the Denison University Biological Reserve.

We began the experiment when tadpoles had reached Gosner Stage 26 (Gosner, 1960; mean mass = 0.023 g). We used female mosquitofish (total length = 4.5-5 cm) that had not been fed for at least 24 h. We established three treatments: a control treatment with a tadpole and an empty cage (i.e., no predator cues), a caged predator treatment with a tadpole and one caged mosquitofish (i.e., visual and chemical predator cues), and an uncaged predator treatment with a tadpole, an uncaged mosquitofish, and an empty cage (i.e., visual, chemical, and physical predator cues). Cages were 17 cm length × 12 cm width × 13.5 cm height and made of fine mesh (1 mm) netting (measured light transmittance ≈ 100%). After allowing the tadpole to acclimate for five minutes, we recorded its activity as either swimming or non-swimming every 60 seconds for 15 minutes. At the end of each trial, we immediately replaced the tadpole and repeated the procedure. After three trials, we replaced the water with aged tap-water and the mosquitofish. We did not use any tadpole more than once. Each treatment was replicated 11 times.

We used a two-way ANOVA to assess treatment effects on the number of times the tadpoles were observed swimming, and the effect of trial number (i.e., did the accumulation of cues affect activity?). Preliminary analyses indicated no statistical difference in the 2nd or 3rd trials ($P > 0.05$), so we pooled these trials for the analyses.

RESULTS

Predator cue treatment had no effect on swimming activity of the wood frog tadpoles (Table 1; $F_{2,27} = 0.15$, $P = 0.86$). Tadpoles in the first trial were less active than tadpoles in the second or third trial (Table 1; $F_{1,27} = 4.47$, $P = 0.044$). The interaction between predator cue treatment and trial number was not signficant (Table 1; $F_{2,27} = 0.34$, $P = 0.71$).

Table 1. Effect of predator treatment and experimental trial on the proportion of observations during which wood frog (*Lithobates sylvaticus*) tadpoles were swimming. Means are given ± 1 S.E.

Trial Number	Predator Treatment		
	Control	Caged Predator	Uncaged Predator
First	0.24 ± 0.12	0.23 ± 0.19	0.17 ± 0.08
Other	0.42 ± 0.13	0.37 ± 0.11	0.52 ± 0.14

DISCUSSION

In our experiment, the activity of wood frog (*L. sylvaticus*) tadpoles was not affected by the presence of cues from mosquitofish, whether those cues came from caged or uncaged fish. This result is not consistent with another study on the effects of mosquitofish on the activity of wood frog tadpoles. Burgett et al. (2007) found that activity in wood frog tadpoles was lower in the presence of chemical cues from mosquitofish. However, the amount of chemical cue used in Burgett et al. (2007) was probably much higher than the cues used in our experiment. Burgett et al. (2007) used chemical cues from several mosquitofish accumulated over a much longer period of time in their experiment than we used in our experiment. However, their experiment did not include visual or physical cues. An additional possible explanation might be that there is genetic variation in the ability to respond to mosquitofish cues in this Wood Frog population and the variation in response ability may reflect such genetic variability. Indeed, Smith et al. (2010) found variation in behavioral responses to mosquitofish cues among sibships of green frog tadpoles (*L. clamitans*) with some sibships showing changes in activity levels and others not.

The lack of a response to mosquitofish cues by the wood frog tadpoles is not unique among ranids, a group in which responses to mosquitofish are variable among species and even within species. For example, Burgett et al. (2007) found that *L. sylvaticus* tadpoles decrease activity when exposed to cues from mosquitofish, as did *L. clamitans* tadpoles (Smith et al., 2010, in press), whereas tadpoles of *L. catesbeianus* did not respond to the presence of mosquitofish (Smith et al. 2008). Lawler et al. (1999) found that younger tadpoles (Gosner Stage 26) of *Rana draytonii* reduced activity levels in the presence of mosquitofish, but older tadpoles (Gosner Stage 33-36) showed no reduction in activity. Variation in antipredator responses among species is not entirely unexpected since mosquitofish are not native and thus responses may depend on the ability of each species to generalize their anti-predator response between native and non-native predator species.

Our experiment demonstrated that the repeated use of trial water can influence the activity level of tadpoles, regardless of the predator cue treatment used. Tadpoles in the first trial tended to be less active than tadpoles in subsequent trials. This effect does not appear to be mediated by the accumulation of predator cues since there was no significant interaction term. It may be that the accumulation of conspecific cues, in the absence of consumption cues (no tadpoles were consumed by the mosquitofish in the trials), may induce greater activity. Indeed, previous studies have shown higher levels of activity in the presence of higher densities of conspecifics in tadpoles (e.g., Golden et al., 2001; Relyea, 2002; Awan and Smith, 2007b; Smith and Awan, 2009). perhaps reflecting an increase in foraging activity in the presence of competitors or due to a perceived reduction of predation risk in the presence of conspecifics. However, Awan and Smith (2007a) found no change in activity level in wood frog tadpoles with changes in tadpole density (see also Golden et al., 2000 for *Xenopus laevis*). Thus it may be the increased activity of the wood frog tadpoles in our experiment is the result of accumulated conspecific cues, but additional work is needed to more fully explain the results.

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