Responses of grey treefrog and American toad tadpoles to the presence of cues from multiple predators

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Prey may often need to confront and integrate cues from multiple predators simultaneously. We examined the effects of the cues of two potential predators, mosquitofish and odonates, individually and in combination, on the behaviour of two species of anuran tadpoles, grey treefrog (Hyla versicolor) and American toad (Bufo americanus). Mosquitofish cues alone reduced the activity of tadpoles of Hyla versicolor, but had no effect on activity of the tadpoles of Bufo americanus. Odonate cues had no independent effects on the behaviour of B. americanus or H. versicolor. The behaviour of neither species was affected differently by the simultaneous exposure to mosquitofish and odonate cues compared to the independent effects of each predator cue. Habitat use was not affected by any cues or combination of cues in either species. Our results suggest that grey treefrog tadpoles and American toad tadpoles do not respond to the combination of cues from multiple predators any differently than would be expected from their exposure to each cue independently. Our results also demonstrate that the behavioural response of tadpoles to predator cues can be variable among species of prey, as well as among species of predator.

Key words: activity, antipredator response, Bufo americanus, Hyla versicolor; predator cues

INTRODUCTION

Many prey respond behaviourally to single predators, and in many cases prey can discriminate and react appropriately to different types of predators and respond in relation to the perceived threat (i.e. a threat-sensitive response; Helfman, 1989, and see review and analysis in Stankovich & Blumstein, 2005). However, when confronted by the simultaneous presence of multiple predators, prey must try to respond to potentially conflicting cues. This conflict can frequently result in greater predation mortality in the presence of two or more species of predators than would be expected from each predator in isolation (e.g. Harvey et al., 2004), but not always (e.g. if predators interact or interfere with each other; Vance-Chalcraft & Soluk, 2005). In particular, if different predators induce different or conflicting responses, there could be “risk-enhancement” (Sih et al., 1998). In many cases, the presence of multiple predators can have complex effects on community dynamics or structure (e.g. Peckarsky & McIntosh, 1998; Schmitz et al., 2004; Borkhataria et al., 2006).

Tadpoles have frequently been used to examine the responses of prey to predators (e.g. Marquis et al., 2004; Laurila et al., 2006; Smith et al., 2008a,b; and references therein). More recently, some studies have examined the effect of simultaneous exposure of tadpoles to multiple predators. Eklöv (2000) found that small Rana catesbeiana tadpoles responded to the combined presence of Anax and bluegill (Lepomis macrochirus) in the same way they responded to the Anax-only treatment. The tadpoles of Rana sylvatica can discriminate and respond to different predator species, and when confronted with simultaneous cues from different predators will respond to the more “dangerous” predator (Relyea, 2003). Teplitzky et al. (2004) found that tadpoles of Rana dalmatina and Rana ridibunda exhibit morphological responses appropriate to the more dangerous sit-and-wait odonate predator than the active forager/pursuer fish predator when exposed to the predators’ cues simultaneously. Thus it appears that a general rule for tadpoles is to respond to the more dangerous of the predators they simultaneously confront. We examined the effects of the cues of two potential predators, mosquitofish (Gambusia affinis) and odonates (family Aeschnidae), individually and in combination, on the behaviour of two species of anuran tadpoles, grey treefrog (Hyla versicolor) and American toad (Bufo americanus).

MATERIALS AND METHODS

Several egg masses of each of the species of anurans used in this study were collected from small ponds on the Denison University Biological Reserve located in Licking Co., Ohio, USA (40°5'N, 82°31'W) throughout the spring and summer of 2006, and incubated in aged tapwater at 17–19 °C in the laboratory. Upon hatching, tadpoles were maintained in large plastic containers and fed ground Purina Rabbit Chow ad libitum until they were introduced into the experiments. Each species was maintained separately. We used tadpoles in the experiments once they were free-swimming and feeding (stage 26; Gosner, 1960). Grey treefrog tadpoles had a mean mass of 0.028±0.001 g and American toad tadpoles a mean mass of 0.018±0.001 g at the time of the experiment.

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We conducted experimental trials in four 37.85 litre aquaria (50 cm L × 25 cm W × 30 cm H). In one half of each experimental aquarium we placed plastic aquarium plants (approximately 50% of water column occupied by plants), whereas the other half of the aquarium had no plants. The orientation of the vegetated side of the aquarium was alternated between aquaria to reduce potential effects of any uncontrolled gradients (e.g. light, temperature). In each experimental aquarium, we also placed a small cage (17 cm L × 12 cm W × 13.5 cm H; approximately 7% of the aquarium volume) made of fine mesh netting in the middle of the long axis of the aquarium (i.e. on the border of the vegetated and non-vegetated halves of the aquarium). Predators were housed in these cages during experimental trials, and the mesh was such that visual (measured light transmittance approximately 100%) and chemical cues from the predators could be available to the tadpoles. The treatments were: six mosquitofish (fish), three odonates (odonate), six mosquitofish and three odonates (fish+odonate), and nothing (control). Mosquitofish were collected from Spring Peeper Pond on the DUBR and odonate nymphs were purchased from a commercial supplier. Predators did not have access to tadpoles or other food for at least 48 h prior to use in the experiments, thus reducing the likelihood that cues from the consumption of prey might influence the response of the tadpoles (e.g. Chivers & Mirza, 2001; Marquis et al., 2004). Two trials were run simultaneously (in two separate aquaria). All four predator treatments were represented in consecutive pairs of trials. Twelve trials were performed per treatment for the grey treefrog tadpoles. Fifty trials were performed on American toad tadpoles: 13 control, 12 with fish, 12 with odonates, and 13 fish+odonate.

For each trial, one tadpole was placed in the experimental aquarium for fifteen minutes before observations were made for acclimation to the experimental conditions (see Mathis & Vincent, 2000; Orizaola & Braña, 2003; Pearl et al., 2003 for use of similar or shorter acclimation periods). The tadpoles were then observed for fifteen minutes, recording the time spent swimming or not swimming and the time spent on the open water side and the vegetated side of the aquarium.

We used one-way ANOVAs with cue treatment as the independent variable on arcsine-square root transformed proportion data. Means are given ±1 SE.

RESULTS

Grey treefrog. The activity level of grey treefrog tadpoles was significantly affected by cue treatment (Fig. 1A; \(F_{3,44}=5.05, P=0.0043\)). In general, tadpoles exposed to cue treatments including mosquitofish were less active than tadpoles in the control and odonate-only treatments (Fig. 1A). The use of the vegetated side of the aquarium by grey treefrog tadpoles was not affected by cue treatment (Fig. 1B; \(F_{3,44}=1.70, P=0.18\)).
American toad. Cue treatments had no effect on the activity level of American toad tadpoles (Fig. 1C; $F_{3,46}=0.134, P=0.94$). Cue treatments also had no significant effect on the use of the vegetated side of the aquarium (Fig. 1D; $F_{3,46}=2.23; P=0.097$).

**DISCUSSION**

Many prey respond to the cues from potential predators, and such an ability probably helps the individual avoid being eaten. The response of the individual to the presence of predator cues is often threat-sensitive (Helfman, 1989). Such threat-sensitive responses are common in amphibian larvae (e.g. Anholt et al., 2000; Mathis & Vincent, 2000; Relyea, 2001b, 2004). Far less is known about how amphibian larvae respond to simultaneous exposure to more than one predator cue.

Our results for both grey treefrog tadpoles and American toad tadpoles suggest that the presence of simultaneous cues from both mosquitofish and odonates did not affect their activity levels compared to each cue independently (e.g. for grey treefrog tadpoles, activity in the control and odonate treatments were similar and both higher than the fish and fish+odonate treatments, which were themselves similar to each other). Previous results on the effects of simultaneous exposure to predator cues on tadpole activity have similarly found that the response to more than one predator cue does not differ from the response to either one of the cues’ independent effects (e.g. Eklöv, 2000), and the response that is manifested is the response observed toward the predator that presents the greatest risk (e.g. Relyea, 2003; Teplitsky et al., 2004).

Applying these previous results to our observations, we might conclude that mosquitofish present a greater risk to grey treefrogs than do odonates. Grey treefrog tadpoles reduced activity in the presence of mosquitofish (alone and with odonates equally), but did not alter their activity in the presence of odonates alone. In addition, grey treefrog tadpoles did not alter their habitat use in the presence of any cues. Mosquitofish are known to be effective predators on tadpoles, both within their native range (Walls et al., 2002; Baber & Babitt, 2003, 2004; Gunzburger & Travis, 2004) and outside their native range (Morgan & Buttemer, 1996; Webb & Joss, 1997; Goodsell & Kats, 1999; Komak & Crossland, 2000; Pyke & White, 2000). Fish in general can have a large negative impact on *Hyla* tadpoles (e.g. Semlitsch & Gibbons, 1988; Smith et al., 1999; Rieger et al., 2004). However, it is not clear whether mosquitofish, or fish in general, are always better predators on hyld tadpoles. Odonates are known to be predators on hylids (e.g. McCollum & Van Buskirk, 1996). In addition, Gunzburger & Travis (2004) found that aeshnid and libellulid nymphs were more effective predators on *H. cinerea* tadpoles than were mosquitofish (*G. holbrooki*) and flier sunfish (*Centrarchus macropterus*), but not bass (*Micropterus salmoides*). In addition, Gunzburger & Travis (2005) found that mortality of *H. cinerea* tadpoles exposed to bass and odonate nymphs separately was similar. Therefore, while our results are consistent with the type of behavioural response expected if mosquitofish were a greater predation risk than odonates, additional experiments on actual predation risk are necessary to confirm this.

Neither predator cue affected the activity or habitat use of American toad tadpoles. Fish cues are often found to reduce activity in tadpoles of *Bufo* (*B. americanus*, Relyea, 2001a; Richardson, 2001; *B. woodhousei*, Lawler, 1989). However, a previous study on the effects of mosquitofish cues on the behaviour of *B. americanus* tadpoles also found no effect on tadpole behaviour (Smith et al., 2008a). Reduction in activity in the presence of odonates is a common response in other *Bufo* and populations of *B. americanus* (Richardson, 2001; Anholt et al., 1996; Skelly & Werner, 1990; Petranka & Hayes, 1998; Relyea, 2001a; *B. arenarum*, Perotti et al., 2006). It is not clear why the *B. americanus* tadpoles in our study did not respond to either mosquitofish or odonate cues. The lack of response to the predator cues by the American toad tadpoles may reflect the fact that the predators had not recently fed, and thus there may have been fewer or different cues available to the tadpoles (e.g. Chivers & Mirza, 2001; Marquis et al., 2004). However, this seems unlikely given 1) the response of *H. versicolor* tadpoles to the same set of cues, and 2) the fact that tadpoles of *B. americanus* from this same population, and using similar experimental procedures, responded to cues from bluegill sunfish *Lepomis macrochirus* (Smith et al., 2008b; Smith & Aw, 2009) and dytiscid beetle larvae (Smith & Aw, 2009). Thus, the lack of response in this study indicates the tadpoles may not recognize these species as predators, rather than a general lack of response to predator cues.

Our results on the effects of mosquitofish on tadpole behaviour allow us to evaluate the potential for native amphibians to respond to an invasive fish, such as mosquitofish. The failure of individuals to respond to cues from non-native predators might limit their ability to avoid predation. Cox & Lima (2006) argued that “naïveté” to non-native predators in aquatic prey may result in more negative effects of introduced predators in aquatic ecosystems relative to terrestrial ecosystems. Given that mosquitofish cues alone reduced the activity of tadpoles of *Hyla versicolor*, but had no effect on activity of the tadpoles of *Bufo americanus*, we might predict greater potential for mosquitofish to affect American toad populations than grey treefrog populations, at least if we assume the reduction of activity of tadpoles is important in reducing mortality from mosquitofish and there are no other defences (e.g. it appears that American toad tadpoles in this population are readily consumed by fish; G.R. Smith, pers. obs.). Reduced activity has been shown to reduce predation risk in the presence of fish (Lawler, 1989; Eklöv & Werner, 2000) and other predators (e.g. Chovanec, 1992; Kruuk & Gilchrist, 1997; Relyea, 2001b; McIntyre et al., 2004). Previous research suggests that other species of tadpoles also vary in their behavioural response to mosquitofish. For example, the presence of mosquitofish cues reduces the activity of tadpoles of *Litoria aurea* (Morgan & Buttemer, 1996; but see Hamer et al., 2002), *Rana aurora* tadpoles (at Gosner stage 25 but not 33–36; Lawler et al., 1999), and *R. sylvatica* (Burgett et al., 2007). However, mosquitofish cues had no effect on
the behaviour of *Rana catesbeiana* and *B. americanus* (Smith et al., 2008a). *Rana capito* tadpoles increased their use of refugia in the presence of mosquitofish (*G. holbrooki*); however, *Rana sphenocephala* tadpoles did not change their use of refugia in the presence of mosquitofish (Gregoire & Gunzburger, 2008), and nor did the tadpoles of *H. squirella* (Walls et al., 2002). Thus, a better understanding of the ability of tadpoles to respond behaviourally to potential non-native predators will require species-specific, and perhaps population-specific, evaluation.

In conclusion, our results suggest that grey treefrog tadpoles and American toad tadpoles do not respond to the combination of cues from multiple predators any differently than would be expected from their exposure to each cue independently (i.e. there was no evidence of a synergistic or even additive interaction between predator cues). Our results also demonstrate that the behavioural response of tadpoles to predator cues can be variable among species of prey, as well as among species of predator. In particular, the response to cues from a non-native predator can vary among species, suggesting the potential for differential effects of non-native predators on potential prey species that may have consequences for community dynamics.

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