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ORGINAL PAPER

Behavioral responses of American toad and bullfrog tadpoles to the presence of cues from the invasive fish, *Gambusia affinis*

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Abstract The introduction of non-native predators is thought to have important negative effects on native prey populations. The susceptibility of native prey to non-native or introduced predators may depend on their ability to respond appropriately to the presence of these non-native predators. We conducted a laboratory based behavioral experiment to examine the response of American toad (*Bufo americanus*) and bullfrog (*Rana catesbeiana*) tadpoles to the presence of cues from the introduced mosquitofish (*Gambusia affinis*), a potential tadpole predator. Neither the American toad tadpoles nor the bullfrog tadpoles responded behaviorally to the presence of mosquitofish cues. If tadpoles are unable to respond to the presence of mosquitofish cues appropriately, then their ability to avoid predation by mosquitofish may be compromised and this may contribute to the impacts of mosquitofish on some tadpole populations.

Keywords Alien species · Behavior · *Bufo americanus* · Mosquitofish · *Rana catesbeiana* · Tadpoles

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One of the major challenges facing biodiversity is the introduction of non-native species into new regions and habitats (Soulé 1990; Vitousek et al. 1996, 1997). Non-native species are thought to be a cause of or contributor to numerous declines of various species around the world (Mack et al. 2000; Gurevitch and Padilla 2004; Clavero and García-Berthou 2005). The introduction of non-native predators in particular can have important negative effects on native prey populations (e.g., Mack et al. 2000; Kats and Ferrer 2003; Salo et al. 2007).

Prey animals often have a repertoire of antipredator responses, both morphological and behavioral, to predators. The susceptibility of native prey to non-native or introduced predators may depend on their ability to respond appropriately to the presence of these non-native predators (e.g., Hoare et al. 2007 and references therein). Aquatic prey may be particularly susceptible to introduced non-native predators because they often are naïve to novel predator "archetypes" (Cox and Lima 2006).

Mosquitofish (*Gambusia* spp.) have been introduced into ponds and lakes around the world (reviewed in Lloyd et al. 1986; Courtenay and Meffe 1989). These introductions have often had negative impacts on native fish (Schoenherr 1981; Meffe 1985; Lloyd et al. 1986; Arthington 1991; Howe et al. 1997), macroinvertebrates (Farley 1980; Bence 1988; Walton and Mulla 1991), and zooplankton (Hurlbert and Mulla 1981; Bence 1988; García-Berthou 1999; Margaritora et al. 2001; Blanco et al. 2004), with possible consequences at the ecosystem level (e.g., Hurlbert et al. 1972). In several cases, the introduction of mosquitofish has resulted in or 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. 2002a; *Taricha torosa* in California, Gamradt and Kats 1996; *Triturus alpestris* and *T. helveticus* in Europe, Denoël et al. 2005). It appears that mosquitofish can act as predators on the eggs and larvae of amphibians (Grubb 1972; Gamradt and Kats 1996; Morgan and Buttemer 1996; Webb and Joss 1997; Goodsell and Kats 1999; Komak and Crossland 2000; Pyke and White 2000). Even in its native range, *Gambusia* are important predators on tadpoles (Baber and Babbitt 2003, 2004).

Many tadpoles respond to the presence of fish predator cues by reducing activity levels (Lawler 1989; Stauffer and Semlitsch 1993; Horat and Semlitsch 1994; Eklöv and Werner 2000; Laurila 2000; Richardson 2001; Laurila et al. 2006; Parris et al. 2006) or modifying habitat or refuge use (Petranka et al. 1987; Semlitsch and Gavasso 1992; Stauffer and Semlitsch 1993; Gunzburger 2005). Given that mosquitofish are an introduced predator, it is not clear whether naïve tadpoles would respond to mosquitofish as predators or not. There are some studies that suggest that naïve tadpoles (i.e., species from outside the mosquitofish's native range) do not respond behaviorally to the cues from mosquitofish (e.g., *L. aurea*: Hamer et al. 2002b). However, Lawler et al. (1999) found that young *Rana aurora draytoni* tadpoles reduced activity in the presence of mosquitofish, and Burgett et al. (2007) found that *Rana sylvatica* reduce activity in the presence of chemical cues of mosquitofish. Thus it appears there may be a variety of responses to mosquitofish by native anuran tadpoles. We conducted a laboratory based behavioral experiment to examine the response of American toad (*Bufo americanus*) and bullfrog (*Rana catesbeiana*) tadpoles to the presence of mosquitofish cues. Southern populations of *B. americanus* and *R. catesbeiana* potentially overlap with native populations of *G. affinis*, but populations in Ohio have only overlapped with *G. affinis* recently. We are aware of no published studies that have examined the effects of mosquitofish on these two species of anurans.

Materials and methods

Our experimental set-up consisted of 37.85 l aquaria filled with aged tapwater. Each tank was divided in half, with one side containing artificial plants with gravel on the bottom creating a vegetated side and the other side containing only gravel on the bottom creating a non-vegetated side. A mesh cage (16.5 cm $W \times 12.5$ cm $L \times 13$ cm D) that contained either mosquitofish ($n = 5$) or nothing (i.e., control) was positioned in the middle of the top of each aquarium. The mosquitofish were collected >48 h before the start of the behavioral observations from a pond from which two bullfrog egg masses but no American toad egg masses were collected on the Denison University Biological Reserve (DUBR). The mosquitofish did not have access to tadpoles for at least 48 h prior to use in these experiments, thus reducing the likelihood that cues from the consumption of tadpole prey might influence the responses of the tadpoles (e.g., Chivers and Mirza 2001; Marquis et al. 2004).

American toad tadpoles with a mean mass of 0.313 ± 0.020 g (Gosner stage 26), and bullfrog tadpoles with a mean mass of 0.040 ± 0.002 g (Gosner stage 26) were used. The tadpoles used in both experiments were collected as egg masses (6 egg masses of *B. americanus* collected 20 April 2005; 4 egg masses of *R. catesbeiana* collected 17 and 19 June 2005) within 24 h of oviposition from ponds on the DUBR and allowed to hatch and develop in the laboratory prior to the experiments. The tadpoles used in these experiments were therefore naïve to all predators. Tadpoles were fed ground Purina Rabbit Chow ad libitum in large plastic containers prior to the experiments.

For each trial, one tadpole was placed in the center of an aquarium, and given 15 min to acclimate to the experimental conditions before observations were made. Observations occurred during the daytime (i.e., 1000–1500 h). Tadpoles were then observed for 15 min, recording the time the tadpole spent swimming or not swimming, and the amount of time spent on the vegetated and non-vegetated sides of the aquarium. Ten sets of observations were collected for each cue for the American Toad tadpoles (20 total observations), and thirty sets of observations for each cue were collected for Bullfrog tadpoles (60 total observations). Individual tadpoles were used only once.

We used separate analyses of variance to analyze the proportion of time tadpoles spent active and the proportion of time tadpoles spent in the vegetated side of the aquarium. Proportion data were arcsine square root transformed prior to analyses. Means are given ±1SE.

Results

The presence of mosquitofish did not have a significant effect on the time American toad tadpoles spent swimming (Fig. 1a; $F_{1,19} = 1.43$, $P = 0.25$) or the proportion of time spent in vegetation (Fig. 1b; $F_{1,19} = 0.30$, $P = 0.59$). The presence of mosquitofish also did not have a significant effect on the time bullfrog tadpoles spent swimming (Fig. 1c; $F_{1,58} = 0.56$, $P = 0.46$) or on the time bullfrog tadpoles spent in vegetation (Fig. 1d; $F_{1,58} = 0.30$, $P = 0.59$).

Discussion

Neither the American toad tadpoles nor the bullfrog tadpoles responded behaviorally to the presence of mosquitofish cues. In other experiments using similar methods and the same populations (but different years), we have shown these species of tadpoles to respond to native fish, such as bluegill (*Lepomis macrochirus*) and largemouth bass (*Micropterus salmoides*) (G.R. Smith et al. unpubl. data; see also *Rana catesbeiana*, Eklöv and Werner 2000; but see Richardson 2001; *Bufo americanus*, Richardson 2001). Thus the lack of a response to mosquitofish observed in this set of experiments is not likely due to a general lack of response to fish, but rather may reflect a lack of response to a novel predator or cue. Other species of tadpoles are known to reduce their activity or change their space use in response to the presence of introduced *Gambusia*. For example, *Litoria aurea* exposed to *Gambusia* reduced activity and avoided using the open water column compared to control tadpoles (Morgan and Buttemer 1996); however, Hamer et al. (2002b) did not observe any response of *L. aurea* tadpoles to the presence of mosquitofish. *Rana aurora* tadpoles reduce activity in the presence of *Gambusia* when at Gosner Stage 26, but show no effect on activity at Gosner Stage 33–36 (Lawler et al. 1999). It is not clear why there is such variability among species and even within species in the response to mosquitofish presence. Possible explanations include differences in experimental protocols, evolutionary history with mosquitofish or with phylogenetically related fish, or age or stage of the tadpoles. Further experiments are needed to

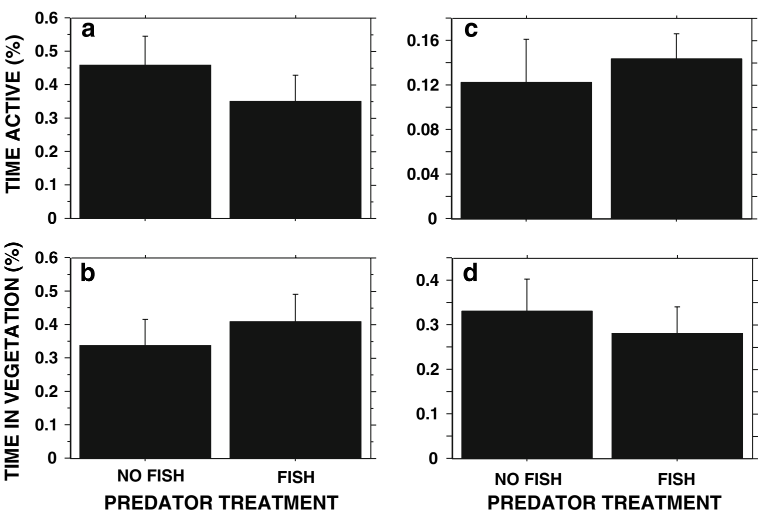

Fig. 1 The effect of the presence of mosquitofish (*Gambusia affinis*) on the time spent (**a**) active, and (**b**) on the vegetated side of the test aquarium by American toad (*Bufo americanus*) tadpoles, and on the time spent (**c**) active, and (**d**) on the vegetated side of the test aquarium by bullfrog (*Rana catesbeiana*) tadpoles. Means are given ±1SE

determine more definitively the role of each of these variables in affecting observed behavioral responses to mosquitofish.

If tadpoles are unable to respond to the presence of mosquitofish cues appropriately, then their ability to avoid predation by mosquitofish may be compromised and this may contribute to the impacts of mosquitofish on tadpole populations. For example, tailed frog tadpoles (*Ascaphus truei*) are unable to detect and respond to the presence of the predacious shorthead sculpins (*Cottus confusus*), and this lack of response may be responsible for the general lack of *A. truei* from streams where *C. confusus* are abundant (Feminella and Hawkins 1994). Tadpoles of *Rana temporaria* and *Bufo bufo* do not respond to non-native crayfish cues, although they do respond to native predators (Marquis et al. 2004; see also Mandrillon and Saglio 2005). The lack of antipredator responses to fish is widespread in species of tadpoles that typically breed in temporary ponds, possibly precluding the coexistence of these species with fish (Kats et al. 1988). Indeed, Gamradt and Kats (1996) suggested that known antipredator responses of California newts (*Taricha torosa*) to native predators, likely did not work on introduced crayfish and mosquitofish predators. Cox and Lima (2006) argued that this "naiveté" to introduced predators in aquatic prey may lead to greater negative effects of introduced predators in aquatic ecosystems compared to terrestrial ecosystems. Thus, it appears that the behavior of native prey in the presence of introduced predators needs to be considered when trying to predict the impact of non-native predator species.

There are two factors that could potentially ameliorate the impact of non-native predators on naïve tadpole prey. First, tadpoles have been shown to be able to learn to recognize cues from predators (Mirza et al. 2006), and thus may be able to respond appropriately on subsequent encounters with a novel predator. Second, tadpoles may possess alternative protections to predators, such as unpalatability. In the case of the two species we studied, they have typically been considered unpalatable to fish, but not always (see review in Gunzburger and Travis 2005). However, palatability may vary among populations and unpalatability may be relative rather than absolute (see Gunzburger and Travis 2005). For example, our observations on the palatability of the local populations of these two species of tadpoles to a native fish (*Lepomis macrochirus*) suggest that *B. americanus* is readily consumed but that *R. catesbeiana* is not. Also, it is not clear how general unpalatability is in protecting against non-native species of predator. Clearly, additional study of the interactions of native prey and non-native predators are needed to determine the potential impacts of introduced predators.

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