Copeia 2011, No. 1, 5–8

Ontogenetic Shift in Response to Amphibian Alarm Cues by Banded Sculpins (*Cottus carolinae*)

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We exposed two size classes of Banded Sculpins, *Cottus carolinae*, to alarm cue secretions from an adult Hellbender (*Cryptobranchus alleganiensis*) or to a blank control and examined foraging responses. Banded Sculpins exhibited size-dependent responses, with small individuals exhibiting avoidance behavior and large individuals exhibiting increased foraging behavior. These data suggest that Banded Sculpins undergo an ontogenetic shift in response to Hellbender alarm cues that corresponds to changes in predation risk and foraging profitability.

MANY species of fishes and amphibians possess chemical alarm signals that are released into the surrounding water when certain cells in the epidermis are damaged by a predatory attack (Chivers and Smith, 1998). In many cases, conspecifics and some sympatric heterospecifics have adopted these cues as indication of an immediate predatory threat (Smith, 1977, 1992; Mathis and Smith, 1993a, 1993b). Among the amphibians that have been shown to possess damaged–released alarm signals are some caudates (Lutterschmidt et al., 1994; Marvin and Hutchison, 1995; Chivers et al., 1996a; Woody and Mathis, 1998) and numerous anurans (reviewed in Summey and Mathis, 1998; Gonzalo et al., 2007; Hagman, 2008). Recognition of alarm cues by prey typically results in predator avoidance or antipredator responses such as decreased activity, increased refuge use, and shoaling (reviewed in Chivers and Smith, 1998).

In some cases, predators have also evolved to respond to prey alarm cues. For example, predatory fishes and dytiscid diving beetles have been shown to be attracted to minnow alarm cues (Mathis et al., 1995; Wisenden and Thiel, 2002) and to increase foraging strikes when the alarm cue is released (Wisenden and Thiel, 2002). Attraction of predators to prey alarm cues likely increases their probability of obtaining a meal by indicating the presence of additional prey (for gregarious prey species), providing them with an opportunity to steal the injured prey from the first predator, or by allowing them to eat the initial predator (Chivers et al., 1996b).

Whether individuals respond to chemical stimuli from heterospecifics with an antipredator response or a foraging response can be influenced by their developmental stage, especially for species in size-structured populations where ontogenetic changes are characterized by niche shifts that include changes in diet and habitat use (Olson, 1996; Post, 2003; Amundsen et al., 2004) and in vulnerability to predation (Anders and Bronmark, 2000). For example, small Ringed Salamander larvae, *Ambystoma annulatum*, show fright response to chemical stimuli from predatory newts, *Notophthalmus viridescens*, but larger larvae do not; small larvae are more vulnerable to predation from newts than large larvae (Mathis et al., 2003). This ontogenetic difference in response to alarm cues can be even more dramatic when the role of predator and prey switch so that an individual may be prey of a particular species when young (=small), but prey upon the same species when older (=larger). Size-based ontogenetic shifts in response to conspecific and heterospecific fish alarm cues have been documented in several species (Brown et al., 2001, 2002; Marcus and Brown, 2003; Harvey and Brown, 2004). For example, small Pumpkinseed Sunfish (*Lepomis gibbosus*) respond to conspecific alarm cues with antipredator behavior, while sub-adults respond with foraging activity (Marcus and Brown, 2003). This shift allows individuals to take advantage of trade-offs between foraging and predation risk, maximizing their life-time probability of survival and subsequent reproductive success.

Two species that are sympatric and potentially swap roles as predator and prey due to large ontogentic changes in body size are Hellbenders (*Cryptobranchus alleganiensis*), a permanently aquatic salamander, and Banded Sculpins (*Cottus carolinae*). Hellbenders reach adult body sizes of over 50 cm (Nickerson and Mays, 1973) and Banded Sculpins can grow to over 17 cm in length (Pfleiger, 1997). Therefore, large sculpins could potentially consume small Hellbenders, but small sculpins could be prey for large Hellbenders. We tested the hypothesis that body size influences response of sculpins to Hellbender alarm cues. Hellbenders produce a milky secretion that appears to function as a conspecific alarm cue (Crane and Mathis, unpubl. data) and stimulates foraging by some predatory fishes (Gall and Mathis, 2010). Following exposure to Hellbender alarm secretions, we predicted that small sculpins should show antipredator behavior and large sculpins should exhibit either no change in behavior or feeding responses.

MATERIALS AND METHODS

Collection of Hellbender secretions.—Secretions were collected from a wild-caught Ozark Hellbender (*C. a. bishopi*; snout–vent length = 30.5 cm) on 9 July 2007. The Hellbender was placed in a plastic container (38 cm × 25 cm, 23 cm depth) with 4.0 L of river water and was agitated by being restrained in a net until the milky secretion was produced (approximately 1 min). The agitation process was continued until a sufficient volume of secretion was present in the container; the entire process took less than 5 min. Although it would have been ideal to have collected secretions from more than one individual, after consulting with the state of Missouri's herpetologist, we decided to minimize disturbance to the species due to conservation concerns (the Ozark Hellbender is state endangered and is a candidate species for listing as federally endangered). Hellbender secretions that were collected from other individuals using an identical procedure have been shown to be effective at eliciting responses

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Submitted: 21 December 2009. Accepted: 1 September 2010. Associate Editor: J. W. Snodgrass.

DOI: 10.1643/CE-09-229

from conspecifics and heterospecifics (Gall and Mathis, 2010; Crane and Mathis, unpubl. data). The secretion was placed in plastic containers, stored on ice, and taken to the Missouri Department of Conservation's Shepherd of the Hills Fish Hatchery (SHFH) in Taney County, MO. The secretion solution was then transferred to 50-ml centrifuge tubes (Fisher Scientific, Hampton, NH) in 30-ml aliquots and frozen; dechlorinated tap water was also frozen in the same manner to serve as a control stimulus.

Collection of sculpins.—Banded Sculpins were collected on 14 June 2007 by backpack electro-fishing from the North Fork of the White River (North Fork) and were transferred immediately to SHFH. Sculpins were collected from a location where Hellbenders are still abundant and reproductive activity is relatively common (due to conservation concerns the specific locality is withheld). The sculpins fell into two naturally occurring size classes, that we assigned as either small (7.6–9.6 cm; $n = 15$) or large (12.1–16.4 cm; $n = 13$). They were segregated by size and placed into separate 76-L aquaria (73 cm L × 29 cm W × 45 cm D) supplied with a constant inflow of well water (17°C); water continually drained from the aquaria (a ''flow-through'' system), so there was no filtration. Each aquarium contained a layer of gravel and was lined with black plastic on all sides. Sculpins were fed hatchery-collected Rainbow Trout (*Oncorhynchus mykiss*; mean TL = 3.0 cm, range: 2.8–3.2 cm) *ad libitum*.

Testing protocol.—Individual sculpins were exposed to one of two treatments: a dechlorinated water blank (control) or milky-white secretion from an agitated Hellbender. Testing tanks were 76-L aquaria (73 cm × 29 cm, 45 cm depth) lined with black plastic on three sides to minimize visual differences between tanks. The front wall of the tank was left clear for observations. Tanks had natural rock substrate and flow-through well water (13.3 L/min). Individual test fish were placed into test tanks and acclimated for 48 hrs prior to testing. Two small Rainbow Trout (prey, 2.8–3.2 cm) were added to the tank at the same time as the test fish to ensure the predator would feed in the confines of the test tank. A sculpin was eliminated from testing if it failed to eat both initial prey. Immediately prior to testing, the flow-through water system was turned off and the test stimulus was thawed in a warm water bath. At the start of a trial, the lid covering the tank was removed, and a clear plastic barrier was placed in front of the focal test fish, separating the tank into two halves with the test fish at the back of the tank. Ten prey (mean TL = 3.0 cm, range: 2.8–3.2 cm) were introduced into the front of the tank with a dip net. A randomly chosen test solution (30 ml of blank or Hellbender secretion) was poured into the back of the tank. After 30 sec, the barrier was removed and the following quantitative response variables were recorded during the 20-min observation period: (1) latency to first approach (approach = tip of snout within 2 cm of prey); (2) latency to first strike (strike = rapid open and close of mouth within 1 cm of a prey fish); (3) number of approaches; and (4) number of strikes. When testing was complete, all fish were measured and placed in a holding aquarium separate from untested fish until testing was complete. All sculpins were returned to the river of origin at the completion of testing. Individual fish were tested only once.

Effect of secretions on prey (trout) behavior.—Because the Hellbender secretion may have altered the behavior of the trout prey, the prey's behavior was assessed by comparing the activity of prey exposed to dechlorinated water blank (control) and Hellbender secretion ($n = 10$ groups of 5 prey for each treatment). Groups of prey fish (juvenile Rainbow Trout) were tested in the same testing tanks as used in the other trials. To assess activity of prey fishes, we drew a grid across the front of the testing tank to indicate the top, middle, and bottom thirds of the tank and the left and right sides. A randomly chosen stimulus solution (blank versus Hellbender secretion) was poured into the test tank in the same manner as in the other trials, and the number of lines crossed by a randomly chosen focal fish was recorded during a three-minute observation period. A focal prey fish was chosen by selecting the fish inside or closest to the top right grid quadrant. After each trial the clockwise adjacent quadrant was used to select the next focal fish. Activity was measured as number of lines crossed.

Statistical analysis.—We conducted a MANOVA with treatment (Hellbender secretion or control) and body size (large or small) as the two factors because of possible correlation between the response variables. To gain a more detailed picture of the responses of sculpins to the treatments, we then performed a two-way ANOVA for each individual response variable with body size and treatment as the two factors. Assumptions of normality and homoscedasticity were met by these data.

RESULTS

The results of the MANOVA indicate there is no significant main effect of treatment ($P = 0.99$) or size ($P = 0.67$), but suggests a tendency toward a treatment by size interaction ($P = 0.085$). Results of the individual two-way ANOVAs also yield no significant main effects of treatment or size for any response variable (all $P > 0.13$). However, there was a significant interaction effect between treatment and size for latency to strike ($F = 4.96$; $P = 0.036$; Fig. 1B) and number of strikes ($F = 4.85$; $P = 0.038$; Fig. 1C), and the interaction between treatment and size was marginally significant for latency to approach ($F = 3.90$; $P = 0.061$; Fig 1A). For each of these response variables, the foraging of small sculpins was inhibited in the presence of Hellbender secretion, whereas large sculpins were stimulated to forage. There was no significant interaction between treatment and size for number of approaches ($F = 1.29$; $P = 0.268$). There was no difference in the number of lines crossed between prey fishes in the control (mean = 24.6; SE = 5.6) and Hellbender secretion (mean = 29.7; SE = 4.9) treatments (*t*-test; $t = -0.68$; $P = 0.503$).

DISCUSSION

Our results indicate that Banded Sculpins exhibit an ontogenetic shift in their response to chemical alarm cues from a predator, the Hellbender. This is the first demonstration of such a shift involving a fish and amphibian. Reduced activity is a common antipredator response of sculpins (Chivers et al., 2001), and small sculpins in this study reduced activity and decreased foraging activity in the presence of the Hellbender secretions. In contrast, large sculpins responded to Hellbender secretions with a foraging response, as evident by reduced latencies to approach and strike prey and increased number of prey strikes. The differential responses of large and small Banded Sculpins

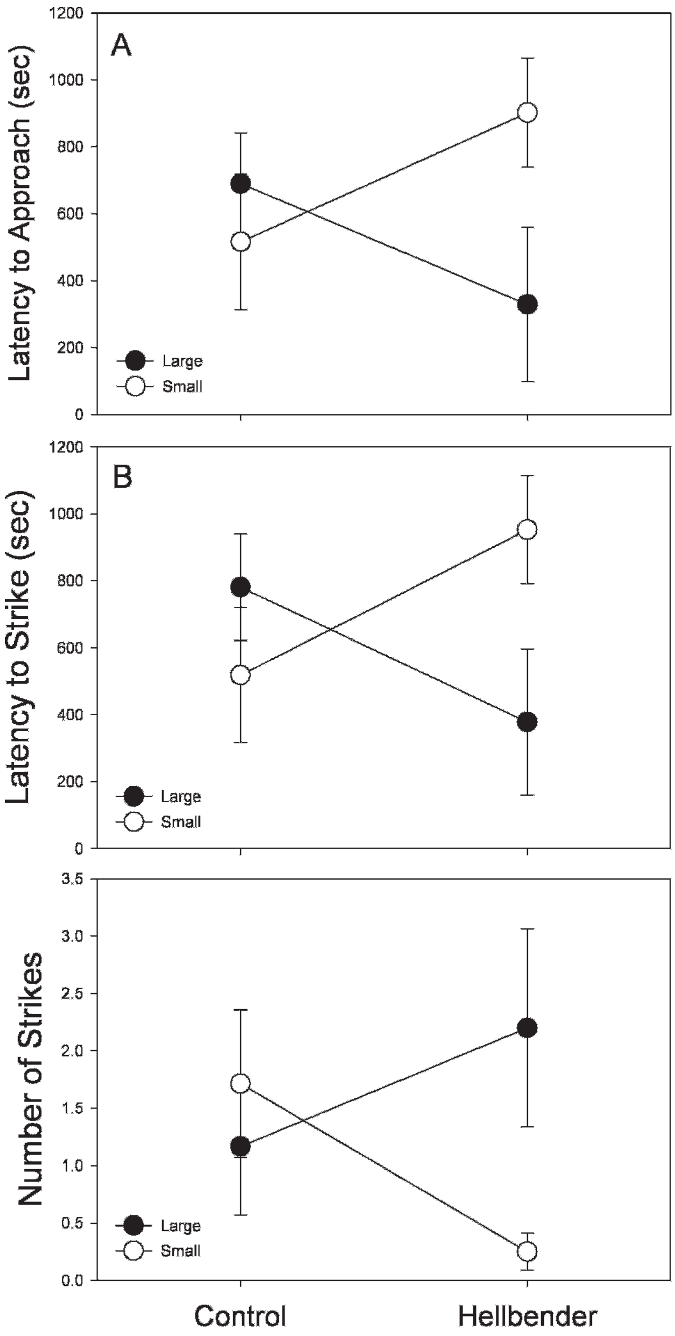

Fig. 1. Mean (± SE) latency to first approach (A), latency to strike (B), and number of strikes (C) for large and small sculpin exposed to dechlorinated water (control) and Hellbender secretions. Interaction effects: $P = 0.061$, 0.036, 0.038, respectively. Connecting lines are included to indicate trends.

to the same Hellbender secretion indicate a threat-sensitive antipredator behavioral response by these fishes (Helfman, 1989).

Sculpins and Hellbenders occupy the same benthic region in Missouri streams, and both are (or historically were) exceedingly abundant in this microhabitat (Nickerson and Mays, 1973; Cooper, 1975; pers. obs.). Although the primary prey of Hellbenders is crayfish, they are opportunistic ambush predators that consume small fishes including sculpins (Nickerson and Mays, 1973; pers. obs.); we have collected a single ''small'' sculpin from the stomach contents of an adult Hellbender. Because Hellbenders are sit-and-wait predators, the antipredator response of small sculpins to Hellbender alarm cues would likely confer survival benefits by decreasing their probability of encountering a Hellbender and being consumed.

Although no sculpin is large enough to consume an adult Hellbender, some sculpins are large enough to be predators of small Hellbenders and particularly Hellbender larvae. Due to their limited gape, small sculpins are less likely than large sculpins to be significant predators of Hellbender eggs and larvae. Field data from an extensive study (Cooper, 1975) of sculpin stomach contents support the hypothesis that small sculpins are not likely to be significant predators of Hellbenders. Cooper (1975) examined the stomach contents of 521 ''small'' (2.6–10.0 cm) Mottled Sculpins (*Cottus bairdi*) and 18 ''small/medium'' (4.0–12.6 cm) Banded Sculpins (*Cottus carolinae*) from the North Fork River between July 1970 and May 1971. Although the sampling location and time frame included a large Hellbender population where extensive reproductive activity occurred, she found no evidence of consumption of Hellbender eggs or larvae.

As sculpins grow, responding to Hellbender alarm cues with antipredator behavior may no longer confer significant survival benefits. Hellbenders are gape-limited predators, so large sculpins may be relatively safe from predation by Hellbenders. The presence of a Hellbender (as indicated by the secretions), might indicate an opportunity for large sculpins to consume Hellbender eggs, larvae, or juveniles. Attraction of some predators to alarm cues from Ostariophysan fishes was demonstrated by Mathis et al. (1995) and Chivers et al. (1996b). Attraction to prey alarm chemicals increases the predator's probability of locating and capturing injured prey or locating nearby conspecifics, particularly if prey are gregarious as occurs for at least young Hellbender larvae (Smith, 1912). Although we collected secretions from an adult Hellbender, larval Hellbenders also can produce the alarm secretion, beginning at least 25 weeks post-hatching (Gall et al., 2010). To large sculpins, the presence of the alarm cue may indicate specific foraging opportunities on the smallest Hellbender age class, especially if the risk of predation by nearby adult Hellbenders is small.

Similar size-based ontogenetic shifts in response to chemical alarm cues have been shown for other species of fishes. Brown et al. (2001) demonstrated that Largemouth Bass (*Micropterus salmoides*) exhibit an ontogenetic shift in antipredator response to Finescale Dace (*Phoxinus neogaeus*) alarm cues associated with the switch from invertebrate prey to piscivory. In this study, small bass (<50 mm) exhibited a significant fright reaction to the alarm cue whereas large bass (>50 mm) exhibited a foraging response. Similar responses to both conspecific and heterospecific alarm cues have been documented in Yellow Perch (*Perca flavescens*) and Pumpkinseed Sunfish (Marcus and Brown, 2003; Harvey and Brown, 2004).

The responses of Banded Sculpins to Hellbender alarm cues indicate a size-based ontogenetic shift, with small sculpins exhibiting antipredator behavior and large sculpins exhibiting a foraging response. Brown et al. (2001) hypothesized that the trade-offs and eventual switch between antipredator behavior and foraging responses are regulated by a variety of proximate sources such as predation pressure, habitat structure, and hunger level. In this system, the timing of the behavioral shift and the specific factors that regulate it require further investigation.

ACKNOWLEDGMENTS

The Missouri Department of Conservation provided valuable assistance during this study. We especially thank J. Briggler, M. Siepker, and J. Civiello for helpful comments and technical aid throughout this study. We thank E. Gall for help with data collection and laboratory assistance. The Utah State University herpetology group provided valuable comments. This research was conducted under Missouri State University's animal use protocol 2007H and was supported by the Missouri Department of Conservation and the Missouri State University Biology Department and Graduate College.

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