Response of native and introduced fishes to presumed antipredator secretions of Ozark hellbenders (*Cryptobranchus alleganiensis bishopi*)

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Summary

Like many amphibian populations around the globe, populations of hellbenders (*Cryptobranchus alleganiensis*) have declined substantially in the past three decades. The cause(s) of the decline are unknown, but one hypothesis is that predation pressure by non-native fishes has played a role. Hellbenders produce a milky, frothy secretion when stressed, and this secretion is assumed to serve an antipredator function. In this study, we tested whether the presence of the secretion would deter foraging activity by native and introduced fishes. We found little evidence to support an antipredator function for the secretion, at least at the relatively low concentrations that we used in our study. Two species, non-native brown trout (*Salmo trutta*) and native walleye (*Sander vitreus*), were stimulated to approach prey by the presence of the hellbender secretions. In a palatability experiment, rainbow trout rejected food pellets soaked in hellbender secretion more often than control pellets whereas brown trout did not. Although our data indicate that the presence of the hellbender secretion does not deter fish predators from approaching hellbenders, it is possible that the strong concentration that would be experienced during an actual predation event, particularly on larger individuals, might serve a deterrent function.

Keywords: amphibian decline, *Cryptobranchus alleganiensis*, foraging, hellbender, introduced trout, predator-prey, stress secretion.

Introduction

The apparent global decline of amphibian populations has revealed a host of anthropogenic factors that are negatively impacting amphibian communities

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at an alarming rate. The variables that may be contributing to the decline of amphibian populations include increased sedimentation and eutrophication, Ultraviolet-B (UV-B) radiation exposure (but see Palen et al., 2002), agricultural pollution (i.e., pesticides and fertilizers), livestock waste runoff, disease, parasites, climate shifts, and introduced or invasive species (Stebbins & Cohen, 1995; Semlitsch, 2003; Mendelson et al., 2006; Sodhi et al., 2008).

With respect to introduced species, nonnative predatory fishes have been linked to population declines for a number of species. Trout (Family Salmonidae), in particular, have been introduced around the world (MacCrimmon, 1971) and have been shown to have strong negative effects on some amphibian populations. For example, introduction of trout into fishless mountain lakes in the western United States has led to population extinctions of the yellow-legged frog (*Rana muscosa*) throughout much of its range (Knapp & Matthews, 2000; Vredenburg, 2004; Finlay & Vredenburg, 2007).

In the Ozark Mountains of Missouri, rainbow and brown trout have been extensively introduced for recreational fishing. Rainbow trout were introduced to Missouri in 1882 and were naturally reproducing by 1887 (Maynard, 1887). Brown trout were introduced around 1890 and were haphazardly stocked until 1936; fingerlings were again introduced in 1966 and stocked until 1980 (Alsup, 2005). Stocking of adult (20–30.5 cm in length) brown trout began in 1980 and resulted in the introduction of almost 400 000 fish by 1999 (Alsup, 2005). Approximately 1.8 million brown and rainbow trout are currently stocked in Missouri's trout waters each year (MDC, 2003). The streams and rivers that make trout stocking and propagation in Missouri so desirable are also the principle habitat of the hellbender (*Cryptobranchus alleganiensis*).

The hellbender is a giant salamander native to the continental United States. At a maximum length of more than 70 cm, it is North America's largest salamander. This fully aquatic species lacks gills and relies on cold spring-fed waters with high dissolved oxygen concentrations for cutaneous gas exchange. Of the two sub-species, the eastern hellbender (*C. a. alleganiensis*) has a larger geographic distribution, ranging north from Georgia to southern New York and west to Missouri. The Ozark hellbender (*C. a. bishopi*) is geographically limited to southern Missouri and northern Arkansas. Populations of both eastern and Ozark hellbenders in Missouri declined by almost 80% between the early 1980s and late 1990s (Wheeler et al., 2003). A shift in age structure was also observed for all populations, with samples from the late 1990s yielding significantly fewer young individuals (Wheeler et al., 2003). This decline in recruitment could indicate reproductive failure, high egg mortality, or low survivorship of larvae. Because of this decline, both the eastern hellbender (*C. a. alleganiensis*) and Ozark hellbender (*C. a. bishopi*) are listed as state endangered in Missouri (MDC, 2006), and the Ozark hellbender is a candidate for federal listing (USFWS, 2007).

Because of their large size, adult hellbenders probably have few predators (Nickerson & Mays, 1973). In addition, when stressed or captured, metamorphosed hellbenders often produce a milky secretion that is distasteful (bitter) when applied to the tongue (Brodie, 1971; Gall et al., 2010), and may be unpalatable to predators. Empirical observations by Nickerson & Mays (1973) indicate the secretion is at least distressful to some predators. The authors placed several live channel catfish (*Ictalurus punctatus*) in a cooler that contained live hellbenders. The fish thrashed violently, but stopped when placed in fresh water. Similar negative responses were elicited from other fishes including *Noturus albater*, *Cottus carolinae*, *C. bairdii* and *Ambloplites rupestris*. Unlike adult hellbenders, larval and juvenile hellbenders are probably highly vulnerable to predation due to their small size and slow developmental rate (Nickerson & Mays, 1973). It is unknown exactly when the ability to produce secretion develops in hellbenders. During our laboratory observations, young hellbender larvae (0–9 weeks post hatching) did not appear to produce the secretion (Gall et al., 2010), and may be incapable of doing so. However, 25-week-old larvae produced copious amounts of a milky secretion that was similar in appearance and tasted similar to the adult secretion (Gall et al., 2010). Hellbenders, including larvae, typically produce the secretion soon after being handled or stressed (Nickerson & Mays, 1973; Gall et al., 2010), and it is likely that they would also produce the secretion during an actual predation event.

Of the potential antipredator mechanisms exhibited by amphibians, noxious skin secretions are considered the most effective at repelling potential predators (Brodie, 1977; Brodie et al., 1979). These secretions typically function to glue the predator to itself or other objects, irritate the predator's senses, make the prey distasteful, or poison and kill the predator (Brodie, 1977; Evans & Brodie, 1994). Noxious skin secretions are particularly well developed in caudate amphibians and the antipredator function of these secretions is well studied in this group (Brodie, 1968, 1977; Brodie et al., 1974, 1979, 1984). Because hellbenders are completely aquatic, fishes are probably the most important predators of hellbenders and their larvae; however, the influence of secretions on predator-prey interactions between hellbenders and fishes has not been studied.

The hypothesis that predation from introduced fishes has played a role in hellbender population declines is of particular interest due to the rapid increase in stocking of trout. Trout have now been introduced into all functional hellbender habitats in Missouri (reviewed in Alsup, 2005), and may have increased predation pressure on larval, juvenile or adult hellbenders. Trout may be particularly detrimental to Missouri's hellbenders because there are no native trout in Missouri, and hellbenders do not appear to have evolved behavioural defences against these predators (Gall & Mathis, 2010). However, amphibians that co-exist with fish predators often are unpalatable to fishes (e.g., Hero et al., 2001), and hellbenders produce a white secretion when agitated that may be unpalatable to some predators (Nickerson & Mays, 1973; Gall et al., 2010). In this study, we tested the hypothesis that the hellbender secretion functions to deter fish predation by testing whether hellbender secretions influence foraging behaviour of native and introduced fishes and whether the hellbender secretion is unpalatable to introduced trout.

Materials and methods

Collection of secretions

Secretions were collected from four wild-caught adult Ozark hellbenders (mean snout-vent length (SVL) ± SE $= 27.6 \pm 1.4$ cm) in June and July 2007. Each hellbender was placed in a plastic container ($38 \times 25 \times 23$ cm depth) with 4.0 l of river water. The hellbender was agitated by being restrained in a net until the milky secretion was produced (approx. 1 min). This process was repeated until a copious quantity of secretion was present in the container (up to four times); the entire collection process usually lasted only a few minutes. The secretion was collected in plastic containers, stored on ice, and taken to the Missouri Department of Conservation's Shepherd of the Hills Fish Hatchery (SHFH) in Branson, MO. The secretion solution was then transferred to 50-ml plastic centrifuge tubes in 30-ml aliquots and frozen at $-6°$C: dechlorinated tap water was also frozen in the same manner to serve as a control stimulus. Approximately 4 l of secretion was left in the plastic containers and frozen for use in the second experiment. Freezing is a common technique utilized to ensure test organisms are subjected to chemical cues of similar concentration and stability (e.g., Marvin & Hutchison, 1995; Chivers et al., 2002). Frozen hellbender secretion can elicit fright responses in larval hellbenders two years after collection and freezing (Crane & Mathis, 2010).

Collection of test fishes

We tested four species of predatory native fishes (Walleye, *Sander vitreus*; Spotted Bass, *Micropterus punctulatus*; Ozark Bass, *Ambloplites constellatus*; and Smallmouth Bass, *Micropterus dolomieu*), two species of predatory nonnative fishes (Rainbow Trout, *Oncorhynchus mykiss*; and Brown Trout, *Salmo trutta*), and one species of nonpredatory fish (Redhorse, *Moxostoma* sp.) (Table 1). In addition, hatchery-reared and wild-caught rainbow and brown trout were tested. We use 'predatory' to describe species that are known to consume vertebrate prey. Each species was assigned to a native or non-native category according to Pflieger (1997) based on their historical distribution in Missouri (Table 1). Most test fishes were collected in June 2007 from the North Fork of the White River (North Fork) and were transferred immediately to SHFH. Walleye were rare during sampling of the

Table 1. Species of fishes tested, including assignment to predator/non-predator and native/non-native categories, and range and mean length for tested fishes.

Species	Predator/ non-predator	Native/ non-native	Fish length (cm)		
			Min	Max	Mean ± SE
Redhorse spp. (*Moxostoma* spp.)	Non-predator	Native	22.9	39	34.9 ± 0.93
Rainbow trout (*Oncorhynchus mykiss*), hatchery	Predator	Non-native	19.2	29.2	23.9 ± 0.36
Rainbow trout (*Oncorhynchus mykiss*), wild	Predator	Non-native	25.2	36.4	30.4 ± 0.74
Brown trout (*Salmo trutta*), hatchery	Predator	Non-native	21.7	29	24.6 ± 0.38
Brown trout (*Salmo trutta*), wild	Predator	Non-native	24.6	42	33.5 ± 0.62
Smallmouth bass (*Micropterus dolomieu*)	Predator	Native	15.6	35	29.4 ± 0.78
Ozark bass (*Ambloplites constellatus*)	Predator	Native	16.9	24.6	20.0 ± 0.36
Spotted bass (*Micropterus punctulatus*)	Predator	Native	11.5	34	27.8 ± 0.85
Walleye (*Sander vitreus*)	Predator	Native	17.5	25.6	20.8 ± 0.28

North Fork, and so were collected from Bull Shoals Lake near Branson, Missouri. All wild-caught fishes were collected by boat electro-fishing. For the non-native species (Rainbow and Brown Trout), we also tested responses of individuals that were reared at the SHFH.

All fishes were placed into an open-air outdoor raceway (15.2 × 1.5 × 0.5 m D) that was supplied with a constant inflow of well water and water from Table Rock Lake; water continually drained from the raceway (a 'flow-through' system), so there was no filtration. Hatchery-reared rainbow and brown trout were maintained in the same raceway as the wild-caught fishes separated by a wire screen. Water temperatures ranged from 11 to 12°C. Fishes were fed hatchery-collected rainbow trout (mean total length (TL) = 4.8 cm, range = 4.3–5.2 cm) and floating trout pellets ad libitum; sinking pellets were provided for nonpredatory redhorse.

Experiment 1: fish foraging behaviour

The goal of this experiment was to determine what impact the hellbender secretion has on the foraging behaviour of native and introduced fishes. Possible responses are that presence of the secretion: (1) stimulates foraging behaviour; (2) depresses foraging behaviour; or (3) is neutral with respect to foraging behaviour.

Individual predatory fishes ($N = 10$–17; Table 2) were exposed to one of two treatments: (1) a dechlorinated water blank (control); or (2) milky-white secretion from agitated hellbenders. Testing tanks were 76-l aquaria (73 × 29 × 45 cm D) lined with black plastic on three sides to reduce external visual stressors and to minimize visual inconsistencies among 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 fishes were placed into test tanks and acclimated for 48 h 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 predatory fish 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. Secretions from a single hellbender were used for all trials of a single fish species; secretion from different hellbenders was qualitatively similar in appearance at the time of collection (white foam abundant on the surface and milky white

Table 2. Species of fishes tested in Experiment 1 (fish foraging behaviour), including response variables (No. = number; Lat = latency; App = approach), treatment, sample size, median, Mann–Whitney test statistic (W) and corresponding p-value.

Species	Response variable	Treatment	N	Median	W	p-value
Redhorse (*Moxostoma* sp.)	No. pellets eaten	Control	9	19	65	0.157
		Hellbender	7	20		
Rainbow trout (*Oncorhynchus mykiss*), hatchery	Lat to App	Control	17	65	319	0.48
		Hellbender	17	62		
	Lat to Strike	Control	17	120	299	0.973
		Hellbender	17	130		
	No. App	Control	17	6	287	0.729
		Hellbender	17	7		
	No. Strikes	Control	17	5	299	0.972
		Hellbender	17	5		
Rainbow trout (*Oncorhynchus mykiss*), wild	Lat to App	Control	13	1028	180	0.5
		Hellbender	16	1051		
	Lat to Strike	Control	13	1200	182	0.405
		Hellbender	16	1200		
	No. App	Control	13	1	210	0.513
		Hellbender	16	0.5		
	No. Strikes	Control	13	0	209	0.386
		Hellbender	16	0		
Brown trout (*Salmo trutta*), hatchery	Lat to App	Control	13	1200	236	0.007*
		Hellbender	14	85		
	Lat to Strike	Control	13	1200	182	1
		Hellbender	14	1200		
	No. App	Control	13	0	145	0.061*
		Hellbender	14	1		
	No. Strikes	Control	13	0	182	1
		Hellbender	14	0		
Brown trout (*Salmo trutta*), wild	Lat to App	Control	17	262	319	0.48
		Hellbender	17	99		
	Lat to Strike	Control	17	1200	299	0.973
		Hellbender	17	1200		
	No. App	Control	17	1	287	0.729
		Hellbender	17	2		
	No. Strikes	Control	17	0	299	0.972
		Hellbender	17	0		

coloured water), and two different secretions utilized by (Crane & Mathis, 2010) elicited quantitatively similar fright responses in larval hellbenders. At the start of a trial, the lid covering the tank was removed, and a clear plastic

Table 2. (Continued.)

Species	Response variable	Treatment	*N*	Median	*W*	*p*-value
Smallmouth bass (*Micropterus dolomieu*)	Lat to App	Control	13	209	168	0.356
		Hellbender	15	307		
	Lat to Strike	Control	13	1200	205	0.403
		Hellbender	15	1200		
	No. App	Control	13	3	174	0.5
		Hellbender	15	4		
	No. Strikes	Control	13	0	175	0.495
		Hellbender	15	0		
Ozark bass (*Ambloplites constellatus*)	Lat to App	Control	14	381	228	0.443
		Hellbender	15	224		
	Lat to Strike	Control	14	652	226	0.508
		Hellbender	15	534		
	No. App	Control	14	1	191	0.393
		Hellbender	15	2		
	No. Strikes	Control	14	1.5	207	0.91
		Hellbender	15	1		
Spotted bass (*Micropterus punctulatus*)	Lat to App	Control	14	110	186	0.626
		Hellbender	13	99		
	Lat to Strike	Control	14	240	174	0.28
		Hellbender	13	915		
	No. App	Control	14	5	201	0.826
		Hellbender	13	5		
	No. Strikes	Control	14	2	211	0.481
		Hellbender	13	1		
Walleye (*Sander vitreus*)	Lat to App	Control	12	1200	177	0.009*
		Hellbender	10	374		
	Lat to Strike	Control	12	1200	149	0.346
		Hellbender	10	1200		
	No. App	Control	12	0	102	0.014*
		Hellbender	10	2		
	No. Strikes	Control	12	0	128	0.392
		Hellbender	10	0		

*$p < 0.05$, **$0.1 > p > 0.05$. Response variables ‘Latency to Approach’ and ‘Latency to Strike’ are in s.

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. Five prey (mean TL = 4.8 cm, range = 4.3–5.2 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 s 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. These response variables are similar to those recorded in other studies of fish feeding behaviour (e.g., Wahl & Stein, 1988; Eklov & Hamrin, 1989; Harvey & Brown, 2004). When testing was complete, all fish were measured and placed in a holding raceway separate from untested fishes. Individual fishes were tested only once.

Because brown trout were extremely agitated by the introduction of the clear barrier, it was not used in tests of this species. At the beginning of a trial with brown trout, the test stimulus was poured into the back of the tank. After 30 s, the front of the tank's lid was raised, and five prey, which had been placed in a 1-l beaker with approximately 200 ml of well water, were gently poured into the front of the tank. For this species, observations were completed behind a black plastic blind 0.5 m from the front of the test tank. Data were recorded as for the other species.

The current population status of Missouri's hellbenders precludes testing the effectiveness of hellbender secretion directly. Thus, for the purposes of our experiment, we used an alternative prey to test whether the secretion influences foraging behaviour of predatory fishes. Testing the efficacy of noxious secretions with an alternative prey has been demonstrated to be an effective way to evaluate a stimulus' influence on a predators foraging behaviour. For example, Gamberale-Stille & Guilford (2004) and Skelhorn & Rowe (2006), used chick crumbs coated in quinine to examine the influence of sampling behaviour of domestic chicks (*Gallus gallus domesticus*) on the evolution of aposematism. Skelhorn & Rowe (2009) used mealworms (*Tenebrio molitor*) to test the role of bitter-tasting compounds as an antipredator defense against birds. Hopkins & Migabo (2010) fed an agar and bloodworm (*Lumbriculus variegates*) mix to crayfish (genus *Cambarus*) to test the antipredator effectiveness of *Ambystoma macrodactylum* skin secretion. Although the secretions we presented to predatory fishes in this study are not secreted by the rainbow trout prey, any change in predator foraging behaviour may be directly attributable to the presence of secretion relative to the control conditions.

We compared the responses of each species to the blank and hellbender treatment using Mann–Whitney U-tests ($\alpha = 0.05$). Due to the large number of species tested and differing baseline activity levels, we do not attempt to examine foraging patterns between fish species.

Because the hellbender secretion may have altered the behaviour of the prey, the prey's behaviour was assessed by comparing the activity of prey exposed to a dechlorinated water (blank) control and hellbender secretion ($N = 10$ groups of 5 prey for each treatment). Groups of prey fishes (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 3-min observation period. A focal 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, and analyzed with a t-test ($\alpha = 0.05$).

Experiment 2: secretion palatability

Feeding larval or juvenile hellbenders to potential predators is unethical due to their current population status, so we tested the palatability of the hellbender secretion to a non-predatory fish and two predatory fish by soaking food pellets in the secretion. Only the hatchery-reared fishes (brown and rainbow trout) and the non-predator (Redhorse) were appropriate for this study because the other fishes would not readily consume food pellets in captivity.

Individual Redhorse were transferred to empty testing tanks, and given 100 sinking pellets (Silver Cup 1/8 inch sinking trout pellets; Nelson & Sons, Murray, UT, USA). Testing occurred after a 21-day acclimation period. One week prior to testing, the Redhorse were fed 20 sinking trout pellets. Five min before testing, 20 sinking pellets were soaked for 5 min in either dechlorinated water blank (control) or hellbender secretion. Excess liquid was drained, soaked pellets were placed at the front of each test tank and observations were made for 1 h. None of the fish consumed any pellets during these initial observations, so the pellets were left in the tank and checked the following morning. The number of pellets consumed during the night was recorded.

At the conclusion of testing the foraging behaviour of rainbow and brown trout in the presence of hellbender secretion (Experiment 1), all remaining prey were removed from test tanks and the predatory fish were acclimated for 24 h. Prior to testing, Silver Cup floating trout pellets (1/8 inch or 0.31 cm; Nelson & Sons) were soaked in 2 l of hellbender secretion or 2 l of dechlorinated water blank (control) for 1 h. At the completion of soaking, pellets were removed from the treatment solution, transferred to plastic containers and frozen at −6°C.

Immediately prior to testing, the inflow of well water was shut off and treatment pellets were thawed by placing the containers in a warm-water bath. To ensure that the test fish in the secretion-soaked pellet treatment had no prior experience with hellbender secretion during previous trials, each trout was assigned the opposite treatment it had been assigned during the live-prey foraging study (Experiment 1). Ten pellets from the appropriate treatment were introduced into the middle of the test tank. We recorded the number of pellets consumed and the number of pellets expelled from the mouth during a 5-min observation period. Individual fishes were tested only once. Data for each species were analyzed using Kruskal–Wallis tests ($\alpha = 0.05$).

Results

Experiment 1

The behaviour of two species appeared to be stimulated by the presence of the hellbender secretion. Walleye approached prey significantly faster and more often in the hellbender treatment compared to control ($p < 0.01$, Figure 1 and $p < 0.01$, Figure 3, respectively; Table 2). Similarly, hatchery-collected brown trout also approached prey faster and more often in the presence of hellbender secretion compared to control ($p < 0.01$, Figure 1 and $p = 0.06$, Figure 3, respectively; Table 2).

Smallmouth bass, spotted bass, Ozark bass, wild-caught rainbow trout, hatchery-collected rainbow trout, and wild-collected brown trout were neither stimulated nor inhibited to forage in the presence of the hellbender secretion (Figures 1–4; Table 2).

There was no difference in the number of lines crossed between prey in the control (mean number of lines crossed (LC) ± SE $= 24.6 \pm 5.6$) and

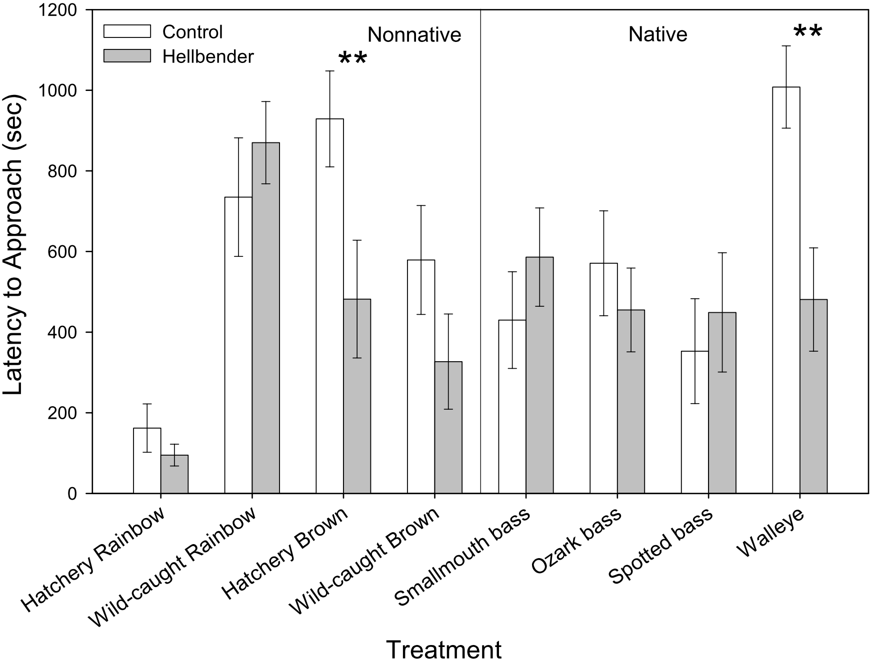

Figure 1. Mean (± SE) latency to first approach for 8 predatory fish species exposed to dechlorinated water (control) and hellbender secretions. $^{**}p < 0.05$, all others $p > 0.10$.

hellbender secretion (mean LC $= 29.7 \pm 4.9$) treatments (t-test; $t = -0.68$, $p = 0.503$).

Experiment 2

Rainbow trout consumed significantly more control pellets than hellbender pellets ($H = 5.2$, $p = 0.02$; Figure 5); and expelled more hellbender pellets than control pellets ($H = 5.6$, $p = 0.02$, Figure 5). In contrast, brown trout did not differ between hellbender and control treatments in terms of either number of pellets consumed ($H = 0.17$, $p = 0.68$, Figure 5) or expelled ($H = 2.8$, $p = 0.10$; Figure 5).

The presence of hellbender secretions did not affect the number of pellets consumed by redhorse (control: mean ± SE $= 13.1 \pm 3.28$, $N = 9$; hellbender: 17.1 ± 2.86, $N = 7$, $p = 0.16$; Table 2).

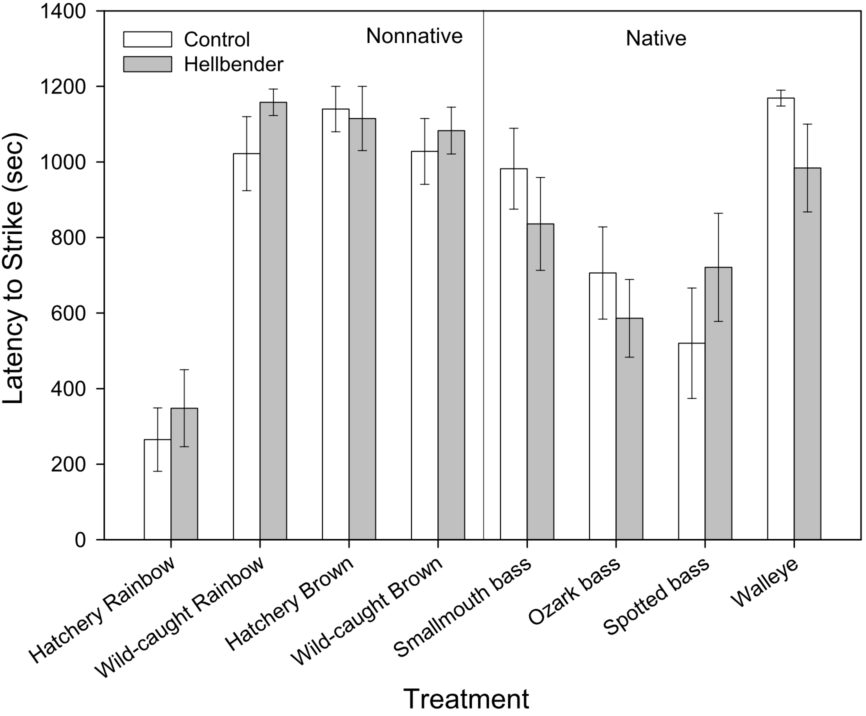

Figure 2. Mean (± SE) latency to first strike for 8 predatory fish species exposed to dechlorinated water (control) and hellbender secretions. All $p > 0.10$.

Discussion

Although the hellbender secretion is commonly assumed to have an antipredator function (Brodie, 1971; Nickerson & Mays, 1973), this assumption has not been well tested. In our study, most native species that we tested neither increased nor decreased foraging in the presence of the hellbender secretion. One native species, the walleye, however, approached prey faster and more often in the presence of hellbender secretions, which is contrary to the prediction of an antipredator function. Walleye are primarily nocturnal predators (Kelso, 1978) and so share activity periods with hellbenders. However, opportunities for interactions between hellbenders and walleye may be limited because hellbenders tend to occupy riffle areas in the streams (Nickerson & Mays, 1973), and walleye tend to prefer deeper pools (Pflieger, 1997). At the time of the study, density of walleye in the North Fork River appeared to be low (2 captures in two all-day sampling events).

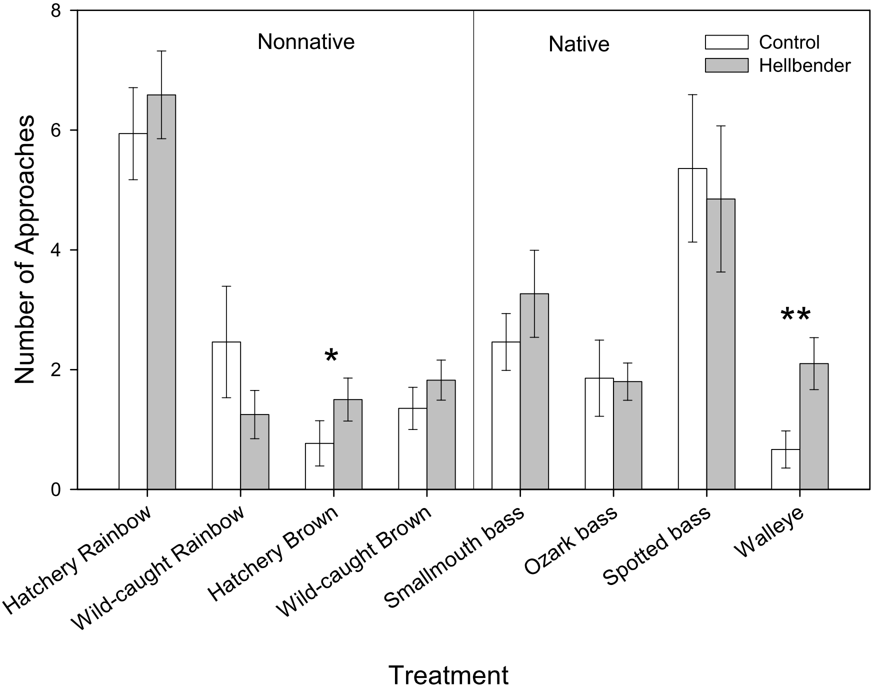

Figure 3. Mean (± SE) number of approaches in 20 min for 8 predatory fish species exposed to dechlorinated water (control) and hellbender secretions. $^{**}p < 0.05$, $^{*}p < 0.10$; all others $p > 0.10$.

For the two non-native trout species that we tested, rainbow trout gave neutral responses and brown trout were stimulated to approach prey in the presence of the hellbender secretions. Attraction to prey is the first step in a successful predation event (Lima & Dill, 1990). Although 'approach' did not always lead to predation attempts by hatchery-reared brown trout in the confined areas of the testing tanks, increased levels of approach would likely lead to increased predation in natural habitats. Both brown trout and hellbenders are primarily nocturnal (Elliot, 1973; Nickerson & Mays, 1973; Young, 1999), so there is greater potential for interactions to occur between these two species.

It is not clear whether the walleye and brown trout were attracted by the chemicals associated with the hellbender secretion specifically or if they were simply responding to the presence of a novel scent. The hypothesis that brown trout recognized hellbender scent, per se, seems unlikely because the strongest response was by hatchery-collected trout with no experience

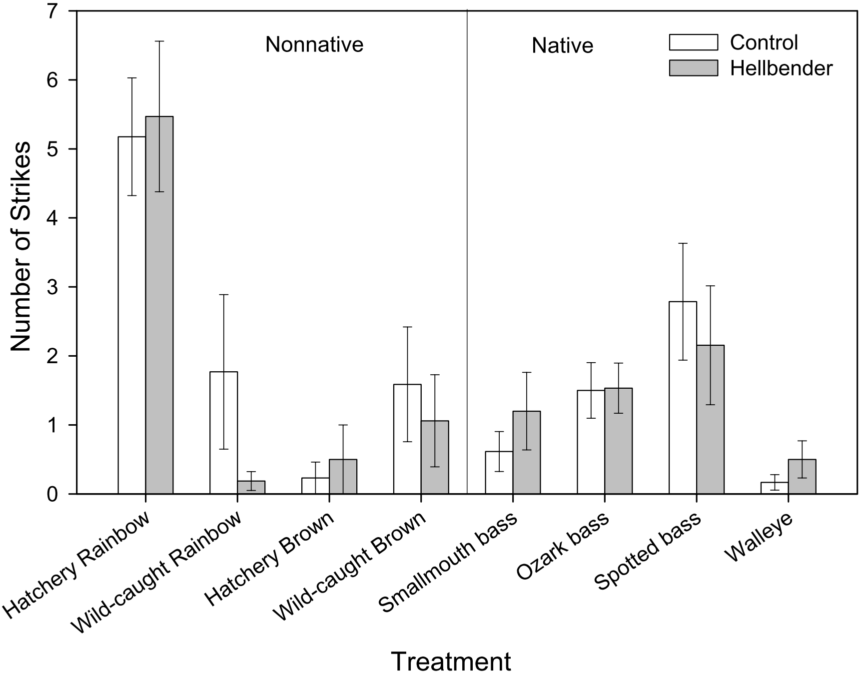

Figure 4. Mean (± SE) number of strikes in 20 min for eight predatory fish species exposed to dechlorinated water (control) and hellbender secretions. All $p > 0.10$.

with hellbenders (data for wild-caught trout showed only a non-significant positive trend). Trout are known to use chemical cues to detect prey (e.g., Olsén et al., 1986; Bres, 1989), and a new odour may indicate a potential new food resource. Alternatively, these species may have an innate attraction to general stimuli associated with amphibians. Whether the attraction to the secretions is a specific response to the hellbender secretion or a more general response, attraction should increase detection of hellbenders and, thus, likely lead to increased predation.

We tested the foraging behaviour of potential predators, yet prey behaviour during a predation sequence can be equally important in determining the outcome of a predation event (Lima & Dill, 1990). Gall & Mathis (2010) exposed larval hellbenders to chemical stimuli from native and non-native predatory fishes and found that chemical cues from native predatory fishes elicit predator avoidance behaviours that should decrease their predation risk to those predators. Although walleye in this study approached prey faster when exposed to hellbender secretion, hellbenders coevolved with wall-

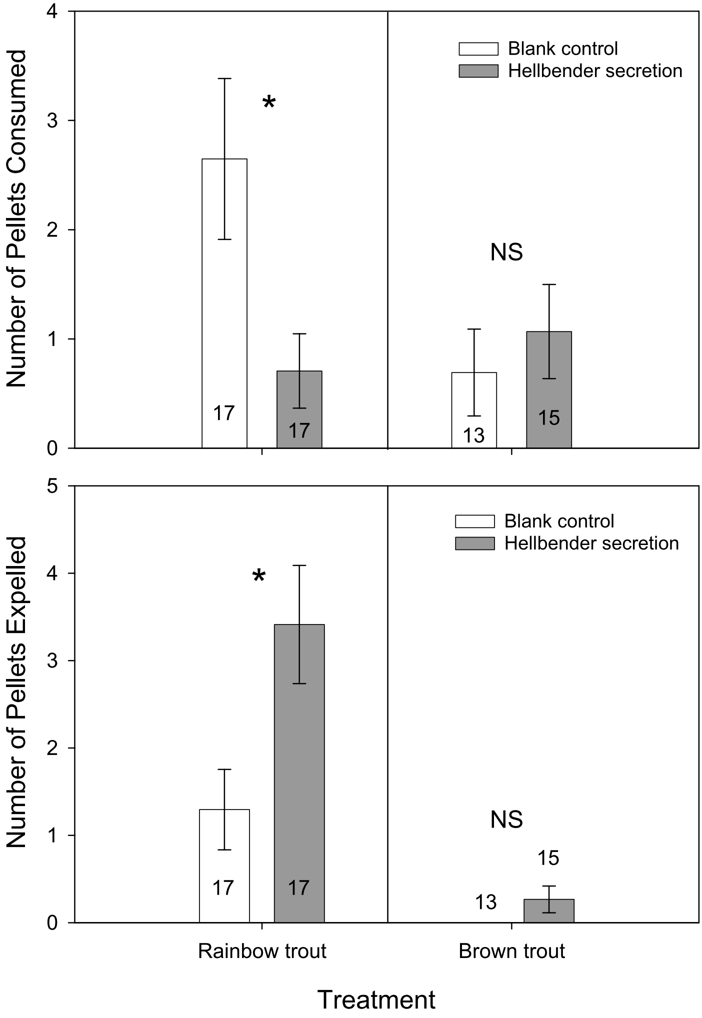

Figure 5. Mean (± SE) number of pellets consumed (a) and number of pellets expelled from the mouth (b) by rainbow and brown trout in blank control (white bars) and hellbender secretion (gray bars) treatments. Number on bar is N. Consumed: $^*p = 0.022$; NS, $p = 0.677$; Expelled: $^*p = 0.018$; NS, $p = 0.094$.

eye and react to walleye chemical cues with predator avoidance behaviour, likely decreasing their probability of consumption by this species. On the other hand, hellbenders did not exhibit a strong predator avoidance response to stimuli from non-native brown trout (hatchery-reared and wild-caught) which may make them vulnerable to predation by this introduced species.

Interestingly, neither wild-caught nor hatchery-collected rainbow trout altered their foraging behaviour in the presence of hellbender secretions. The reason for the difference between the response of rainbow and brown trout is not known. Both species eat a variable diet that can include amphibians. Unlike brown trout, benthic feeding by rainbow trout typically occurs diurnally (Sánchez-Vázquez & Tabata, 1998), so they may rely more heavily on visual cues than on chemical cues for benthic feeding.

Although hellbender secretion may alter the foraging behaviour of potential predators, if the secretion is unpalatable when ingested the threat of predation may be minimal. In experiment two, we assessed the relative palatability of the secretion to introduced trout. Rainbow trout and brown trout differed in their response to pellets soaked with hellbender secretion versus the control-soaked pellets. Rainbow trout responded by expelling more pellets and consuming fewer pellets when they were soaked in hellbender secretion than in the control condition. These data indicate that rainbow trout found the hellbender secretion to be distasteful. However, brown trout did not distinguish between the two types of pellets and consumed slightly more pellets soaked in the hellbender secretion than in the control stimulus. Although these data suggest brown trout may not find hellbender secretion to be unpalatable, additional testing is required to accurately determine palatability of the secretion to this species. It is unknown why Redhorse did not avoid hellbender secretion soaked pellets. Because Redhorse were reluctant to feed in the test tanks, the concentration of secretion in the pellets may have become diluted between the initiation of testing and foraging and may account for the lack of avoidance in this species. Alternatively, the difference in pellet soak time can also not be ruled out; however, pellets were ‘swollen’ with secretion after only a few minute of soaking.

Introduced trout have been shown to negatively impact amphibian populations on multiple continents, and have been documented to reduce populations of riverine amphibians. Gillespie (2001) showed that brown trout preyed upon lotic and lentic tadpole species, while native stream fishes preyed only on the lentic species. The dramatic decline in lotic anuran populations in Australia is likely attributable to trout introductions. Although most studies of effects of trout on amphibian populations have been studies of anurans (see references in Kats & Ferrer, 2003), predation by trout has also been shown to influence salamander populations. In New England streams, larval salamander densities were higher above waterfalls where native brook trout were absent than in areas containing trout; artificial addition of brook trout to these areas reduced salamander densities after just one year (Barr & Babbitt, 2007). In several areas across Europe, the presence of introduced fishes, including trout, caused the local extinction of paedomorphic newt populations where fishes were introduced (Denoel et al., 2005).

The results of this study indicate that two species of fishes, including non-native brown trout, respond to hellbender secretions with increased foraging activity, which could lead to increased predation of hellbender eggs, larvae, or juveniles. These data are inconsistent with the hypothesis that the secretion serves an antipredator function with respect to fish predation, at least at the concentration used in our study. The concentration of our stimulus was likely weak (after dilution in the test chamber) compared to the intensity that a predator might experience during an actual attack; secretion would be expelled in a predator's oral cavity while subduing a hellbender in its mouth. In an actual predatory attack, an antipredator function might be more readily apparent. Nickerson & Mays (1973) exposed catfish (*Ictalurus punctatus*) to a strong solution of secretions from numerous hellbenders in a small container and observed negative effects. Nevertheless, we conclude that the presence of the hellbender secretion in the water does not deter predators from approach, and may be attractive to some species. The concentrations that we used may approximate that which would be experienced as the secretion becomes diluted in a natural stream habitat. Moreover, small individuals might be consumed whole by large fish predators before they could produce large quantities of the secretion. The combination of increased foraging behaviour by brown trout when exposed to hellbender stimuli and the lack of antipredator response exhibited by larval hellbenders to brown trout stimuli (Gall & Mathis, 2010), indicates that hellbenders may be particularly vulnerable to predation by this species.

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