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Author(s): Zachary A. Cava, Amy M. McMillan, Christopher M. Pennuto, and Robert J. Warren II

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Hellbender Prey Preference Is Superseded by Native and Nonnative Prey Behavior

ZACHARY A. CAVA, AMY M. McMILLAN,¹ CHRISTOPHER M. PENNUTO, AND ROBERT J. WARREN II

Department of Biology, SUNY Buffalo State, Buffalo, New York USA

ABSTRACT.—Long-term interactions often shape predator–prey relationships in the form of a co-evolutionary “arms race.” The arrival of nonnative species may disrupt these relationships by introducing novel behaviors that shift interactions in favor of one of the participants. Here we investigated the response of an imperiled native predator, the Eastern Hellbender (*Cryptobranchus alleganiensis*), to nonnative and native crayfish prey. Crayfish constitute an important prey item for hellbenders, and in the northern portion of its range where this research was conducted, the nonnative Rusty Crayfish (*Orconectes rusticus*) has become the dominant crayfish. The objective of this study was to determine prey choice and feeding success of hellbenders presented with native (Allegheny Crayfish; *Orconectes obscurus*) and nonnative (Rusty Crayfish) crayfish prey. We tested hellbender chemoreception in discriminating between the native and nonnative prey, analyzed behavioral interactions between hellbenders and crayfish during video-recorded trials, and assessed hellbender selectivity of crayfish during overnight feeding trials. Hellbenders were able to discriminate crayfish odor from controls, showed a preference for the scent of native crayfish over nonnative crayfish, and were more likely to strike at native crayfish than at nonnative crayfish; however, more nonnative crayfish were consumed during overnight feeding trials. This discrepancy apparently resulted from differences in avoidance behavior between the prey species; native crayfish engaged more in predator-avoidance tail-flip responses and climbing retreats than the nonnatives, who tended to “stand their ground.” Accordingly, during biotic invasions, food preferences of native predators may be superseded by antipredator prey behavior.

Predator–prey dynamics are influenced by co-evolutionary history, and theory predicts that co-evolved species should possess adaptations to maximize fitness in detecting and responding to natural enemies (Darwin, 1859), often leading to an evolutionary “arms race” (Dawkins and Krebs, 1979); however, biological invasions create novel interactions among organisms that may lack a strong co-evolutionary history (Blackburn et al., 2004). Therefore, predator–prey relationships develop in which the degree of “evolutionary naïveté” between predator and prey may be a major factor determining the outcome of species introductions (Sih et al., 2010; Li et al., 2011).

There are many examples of nonnative species exploiting the naïveté of native community members. Perhaps the best-documented scenarios involve novel predators benefiting from naïve prey in invaded regions (“naïve prey hypothesis”; Cox and Lima, 2006). In such instances, prey may lack the ability to detect novel predators (Polo-Cavia et al., 2010; Gomez-Mestre and Díaz-Paniagua, 2011), or may recognize a predatory threat but exhibit an ineffective response (Macdonald and Harrington, 2003; Banks and Dickman, 2007). Furthermore, nonnative prey species can benefit from naïve native predators (“enemy release hypothesis”; Keane and Crawley, 2002). Finally, although less reported, invaders can also suffer from naïveté (“increased susceptibility hypothesis” Colautti et al., 2004; Ward-Fear et al., 2009; Sih et al., 2010; Li et al., 2011), and even highly successful invaders may be limited at local scales via top-down control from native enemies (Jones et al., 2009). When there is reciprocal naïveté between predator and prey, predicting the outcome of such novel interactions becomes especially difficult. To address this issue, Sih et al. (2010) provided a theoretical framework to account for variation in the success of nonnative predators, and such an approach may be similarly applicable for investigating how native predators interact with nonnative prey. This framework, however, requires a comprehensive understanding of the organisms involved with respect to co-evolutionary history, behavior, physiology, etc. If knowledge of one or more

species is limited, the utility of this model is diminished. Therefore, empirical studies that test interactions between novel enemies may be critical to developing a better understanding of the role of naïveté in biotic invasions.

Given that predator–prey interactions are shaped by long-term co-evolutionary history, species invasions provide opportunities to study these interactions within a novel context. Often, predator–prey research is focused on the responses of exotic prey to native predators and less is known about how native predators are affected by nonnative prey (Carlsson et al., 2009). We investigated the response of an imperiled native predator (Eastern Hellbender, *Cryptobranchus alleganiensis*) to nonnative (Rusty Crayfish, *Orconectes rusticus*) and native (Allegheny Crayfish, *Orconectes obscurus*) prey.

Hellbenders are large aquatic salamanders endemic to parts of eastern and Midwestern North America. In recent years, hellbenders have declined throughout much of their historic range (Wheeler et al., 2003; Foster et al., 2009; Burgmeier et al., 2011), and the spread of nonnative species is one factor implicated in hellbender decline (Crane and Mathis, 2011). For example, laboratory studies indicate that hellbender larvae exhibit strong antipredator responses to chemical cues derived from native fish predators, but respond weakly to stimuli from nonnative sport fish (Gall and Mathis, 2010). Whereas the potential for nonnative predators to impact *C. alleganiensis* negatively has been addressed (Gall and Mathis, 2010; Crane and Mathis, 2011), less is known about how hellbenders may react to nonnative prey.

Although hellbenders consume a variety of prey, the bulk of their adult diet is comprised of crayfish (Smith, 1907; Nickerson and Mays, 1973; Peterson et al., 1989), and in some circumstances hellbender abundance and fitness might be limited by crayfish availability (Nickerson et al., 2003, 2009; Hecht-Kardasz et al., 2012). Within the past century, native crayfish assemblages have undergone considerable changes as a result of anthropogenic introductions (Lodge et al., 2000). In North America, the Rusty Crayfish (*O. rusticus*), native to the Ohio River Drainage (Taylor, 2000), has expanded its range to encompass much of the Midwestern and northeastern United

¹Corresponding Author. E-mail: mcmillan@buffalostate.edu
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States and Ontario (Hobbs et al., 1989; Conard et al., 2016). Rusty crayfish are omnivores implicated in altering the structure and function of invaded ecosystems via displacement of native crayfish (Capelli and Munjal, 1982; Lodge and Lorman, 1987), reduction of macrophyte and macroinvertebrate abundance (Charlebois and Lamberti, 1996; Wilson et al., 2004), competition with fish for prey, and consumption of fish eggs (Morse et al., 2013).

Anecdotal evidence suggests a correlation between Rusty Crayfish establishment and hellbender decline in areas where Rusty Crayfish have spread outside their native range (Pennsylvania Natural Heritage Program [PNHP] 2011; Kobell, 2012; Spinks, 2014). Hellbenders also are declining in regions where Rusty Crayfish do not occur (e.g., Foster et al., 2009; Nickerson et al., 2009); however, this should not preclude considering this crayfish species as a potential threat, given that large-scale amphibian declines are thought to result from the interaction of multiple, local drivers, rather than broad, singular causes (Grant et al., 2016). Whereas crayfish are clearly an important food resource for hellbenders, Herman (2012) and Quinn et al. (2013) hypothesized that Rusty Crayfish may be unpalatable to hellbenders due to the crustacean's large size and aggressive nature.

We tested if hellbenders, a native predator, would struggle with or avoid Rusty Crayfish, a novel prey species not present in the drainage basin of hellbenders used in this research, and in turn whether Rusty Crayfish would respond adaptively in the presence of hellbenders, a novel predator. We first determined if hellbenders detect prey by chemoreception. We then tested whether they discriminate between native and exotic prey on the basis of scent. We analyzed prey behavioral responses to hellbender predators with the use of video-recorded interaction trials. Finally, we tested prey selectivity of hellbenders when offered both native and exotic prey.

MATERIALS AND METHODS

Husbandry.—This study was conducted at the Buffalo Zoo, in Buffalo, New York, USA. Study animals consisted of hellbenders that were reared from eggs collected in the Allegheny Drainage of New York in October 2009. Approximately 700 hellbender eggs were collected for a collaborative headstarting program between the Buffalo Zoo and the New York Department of Environmental Conservation. These eggs were collected under a single nest rock, guarded by a single resident male hellbender; however, later genetic analysis revealed multiple maternal and paternal contributions (McMillan, unpubl. data).

Hellbenders were housed in 114-L tanks ($91.4 \times 45.7 \times 30.5$ cm), with each tank containing 6–8 individuals. Chilled lake water (Lake Erie) was provided to the tanks with the use of a flow-through life support system (LSS) that included UV sterilization as well as a bead filter for mechanical and biological filtration (Aquatic Enterprises, Inc., Bridgewater, Massachusetts, USA). Water temperature varied seasonally, and during the course of this study (July–August 2015) morning water temperatures in hellbender enclosures were 17–21°C. Hellbenders were maintained on a diet of crickets (*Acheta domesticus*) earthworms (*Lumbricus terrestris*), superworms (*Zophobas morio*), and fish analog (Mazuri Fish Analog 50/10 Gel Diet, Mazuri Fresh Water Turtle Diet, calcium powder, water). Hellbenders were normally fed 4 d/wk, but had food withheld for 1 wk prior to all trials. Immediately prior to fasting, salamanders were fed ad libitum to equalize levels of satiation (Jaeger et al.,

1982). At the time of this study, hellbenders were aged ~ 5.5 yr and their mean \pm SE total length measured 39.9 ± 0.55 cm (range = 29–45 cm). All hellbenders used in this study were previously tagged with passive integrated transponder (PIT) tags.

Wild crayfish were collected using kick nets and hand collections from streams in western New York in spring 2015. Allegheny crayfish are native to New York and were collected from Cattaraugus County from streams populated by hellbenders. Rusty Crayfish are not native to New York, but have invaded the upper reaches of the Susquehanna River basin in eastern New York, where they were collected. The Susquehanna River basin, historically, was home to hellbenders, though in the northernmost reaches hellbenders are in decline (PNHP, 2011; Kobell, 2012; Spinks, 2014). Crayfish were transported to the Buffalo Zoo and decontaminated by submersion in a salt bath (50 g/L) for 5 min, then moved to 114-L tanks where they were maintained until testing.

Arena Design.—Experiments 1–3 (detection of prey scent, discrimination between native and exotic prey, and video-recorded behavior trials) were conducted with the use of a flow-through arena ($51 \times 140 \times 20$ cm) constructed from clear acrylic (thickness = 0.56 cm). The arena consisted of an “upstream” zone divided into three parallel lanes (each lane = 16×80 cm), and a “downstream” mixing zone (50×60 cm; Fig. 1). Each lane was connected to a separate 75-L head tank via 1.27 cm (outside diameter) Tygon tubing. Polyvinyl chloride ball valves affixed to head-tank outflows were used to adjust water flow through each lane, and the total flow rate for the arena was maintained at 200 ml/s (66.7 mL/s/lane). Water depth in the arena was 6.5 cm, and water exited the system through nine evenly spaced, circular openings (diameter = 1.3 cm) in the downstream panel of the arena. The turnover time was ~ 3 min.

General Testing Protocol.—All arena trials were digitally recorded with the use of a EOS 6D camera with an EF 17-40-mm f/4L lens (Canon, Inc., Tokyo, Japan) mounted on a N-2204X tripod (Sirui USA, LLC, Verona, New Jersey, USA) above the arena. Video recordings were analyzed after testing was complete. Light was provided by blue CFL bulbs during arena trials, replicating the light environment in which hellbenders were regularly fed at the zoo. The sides of the arena were covered with cardboard to prevent exposure to external visual stimuli. Arena trials lasted 10 min, and were preceded by a 5-min acclimation period. For each trial, a hellbender was selected at random from the holding tanks and transported to the test arena in an aquarium net. During acclimation, filtered lake water flowed through the arena via the “blank control” lane. The arena, head tanks, and Tygon tubing were flushed with filtered water between trials to remove residual stimuli. Scent treatments were randomly assigned to each test lane for each trial, and treatment positions were blind to the researcher until after analysis.

For trials in which hellbenders were offered live crayfish (video-recorded behavior trials, Experiment 3; overnight feeding trials, Experiment 4), crayfish were selected based on carapace length relative to hellbender length. We offered crayfish possessing carapace lengths within 5–8% of hellbender total length—a range informed by previous gut content analyses of wild hellbenders (Wiggs, 1976).

Experiment 1: Detection of Prey Scent.—The purpose of Experiment 1 was to verify that hellbenders could use chemosensory perception to detect prey. We measured hellbender response to water conditioned with fish analog, a neutral scent (parsley), and a control (filtered lake water). Fish analog was

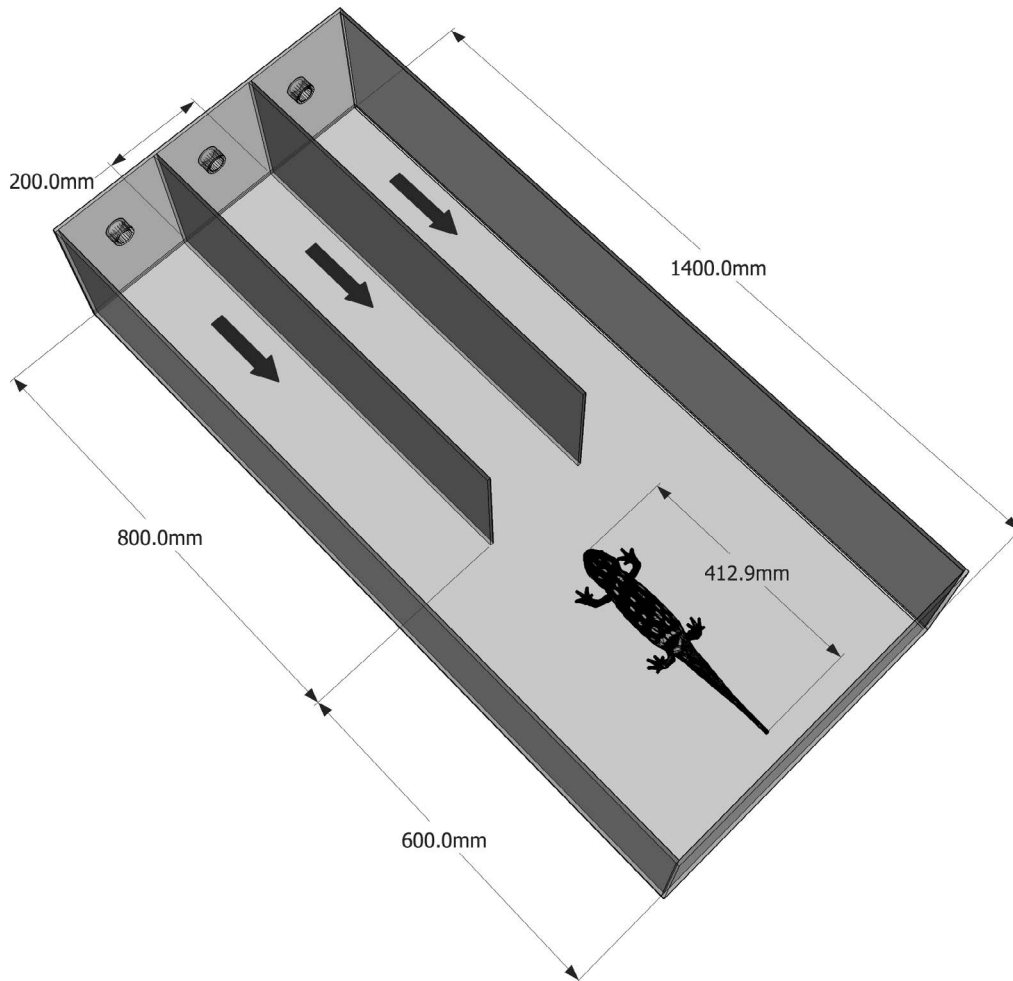


FIG. 1. Rendering of the test arena used for Experiments 1–3 (not to scale). For Experiment 1, each of the three lanes received a randomly assigned treatment (control, parsley, fish analog). During Experiment 2, the center lane was blocked, and the outer lanes received water conditioned with native (*Orconectes obscurus*) or nonnative crayfish (*O. rusticus*). In Experiment 3, all three lanes were blocked off, restricting interactions to the downstream end of the arena. Blue arrows indicate the direction of water flow; water entered the arena at the upstream end exited the arena via openings in the downstream wall (not shown).

used as the prey stimulus because it was a food to which the hellbenders were accustomed based on prior exposure, and we expected it would elicit a positive feeding response.

Hellbenders were placed in the downstream-end of the arena under a halved PVC tube placed flush against the downstream wall of the arena and allowed to acclimate for 5 min before the start of a trial. To begin each trial, the valves were opened allowing each scent to travel down its respective lane and the PVC shelter was removed. We monitored hellbender positions and recorded the number of visits to each lane and how thoroughly lanes were investigated. If a hellbender entered a lane and continued moving upstream until reaching the front wall of the arena, this was considered a complete visit. Alternatively, if a hellbender entered a lane but then exited without traveling to the end of the lane, it was considered an aborted visit. A hellbender was designated as being inside a particular treatment zone if its snout (i.e., nares) was within the zone boundary, which was delineated in black marker on the underside of the arena. A total of 30 trials were completed with 30 different hellbenders.

Experiment 2: Discrimination Between Native and Exotic Crayfish Scent.—After confirming that hellbenders could detect prey by chemoreception, we tested hellbender ($n = 30$) ability to

discriminate between native and exotic crayfish scent by exposing the salamanders to Allegheny and Rusty Crayfish-conditioned water. All but one of the hellbenders were the same as those used in Experiment 1. Trials were conducted in the same arena as Experiment 1; however, during Experiment 2 a gate was used to block the center lane of the arena for the entirety of each trial, effectively creating a Y-maze. Acclimation procedures followed those described for Experiment 1.

Crayfish-conditioned water was prepared 1 h prior to testing by separating individuals of each crayfish species into two 75-L head tanks (mean = 51.6 crayfish/tank). Each tank contained 64.4-L filtered lake water and an aerator. To standardize stimulus concentrations, total crayfish mass was kept equal between tanks (± 2 g blotted-dry weight) and the difference in crayfish number between tanks was < 5 . These trials were conducted over the course of 6 d. Because water flow from a conditioned head tank was once-through for each treatment lane, water volume decreased in each head tank over a trial duration. Water was replaced in each head tank between trials and allowed 1 h to condition with crayfish scent before commencing the next trial. Stimulus concentrations ranged from 215–231 mL water per 1 g crayfish (mean = 224 mL/g). For all trials, differences in stimulus concentrations between

crayfish treatments were < 2 mL/g. As with Experiment 1, we measured the number of visits to each lane and whether visits were complete or aborted.

Experiment 3: Video-Recorded Behavior Trials.—Interactions between hellbenders and crayfish were quantified with the same 30 hellbenders used in Experiments 1 and 2. Interaction trials consisted of a two-part series in which hellbenders were first tested using one crayfish (one native or one nonnative) and were later tested with two crayfish (one native, one nonnative) to gain some insights into prey interspecific interactions as well as hellbender response to them. These trials were restricted to the downstream mixing zone in the same arena as Experiments 1 and 2. During Experiment 3, all three lanes in the arena were gated closed and the arena received only a flow of filtered lake water from a head tank connected to the center lane.

Before the start of a trial, each hellbender was placed in the center lane of the arena and allowed to acclimate for 5 min. Following acclimation, crayfish were placed in one or both of the far-downstream corners of the arena. At the start of a trial, the center lane gate was opened, releasing the hellbender into the mixing zone of the arena. Crayfish species and placement were randomized by trial. We documented the following interactions between hellbenders and crayfish: “encounters” (hellbender and crayfish within ≤ 1 chelae length of one another; encounters were further classified as “hellbender snout contact” or “no hellbender snout contact”), “strikes” (hellbender struck at crayfish; strikes were recognized by a rapid forward or lateral movement of the snout; Elwood and Cundall, 1994), and “tail-flips” (a stereotypical crayfish escape behavior where a rapid contraction of the abdominal muscles initiates a backward swimming response; Krasne and Wine, 1984). We also reported “climbing,” which is a potential avoidance behavior exhibited by crayfish in the presence of hellbenders (Reese, 1903).

Experiment 4: Overnight Feeding Trials.—No crayfish were eaten during Experiment 3, which may reflect both the relatively short duration of trials and the novelty of the arena environment. Therefore, to assess prey selectivity by hellbenders, Experiment 4 was conducted with hellbenders in their original 114-L tanks. Hellbenders were housed individually and offered a paired native Allegheny and nonnative Rusty Crayfish of similar carapace length ($\Delta \leq 3$ mm). The crayfish were left in the hellbender enclosures overnight, and the tanks were checked the following day for missing crayfish. A total of 26 hellbenders were tested in this experiment, including 8 individuals that had not been used in any earlier trials.

DATA ANALYSIS

Experiment 1: Detection of Prey Scent.—Hellbender responses to chemosensory stimuli were analyzed with the use of generalized linear models (GLMs) in R v3.2.3 (R Core Team, 2015). These models included treatment factors (filtered lake water, parsley-conditioned water, fish analog—conditioned water), block (lane 1, 2, 3) and interaction terms (treatment \times lane). The response variables were number of visits (Poisson error distribution) and proportion of aborted visits (quasi-Poisson error distribution due to overdispersion > 1). The GLM models were fit with the use of analysis of deviance (ANODEV). ANODEV is a maximum likelihood approach used with generalized linear models where the deviance is $-2 \times$ the log-likelihood ratio, and the GLM is fit using an analysis of variance (ANOVA) model with a chi-square test. The deviance model calculates the difference between the reduced model and the full model, that includes all predictors

and is a point of comparison with models containing fewer parameters. We used a Tukey’s HSD (honest significance difference) test in the ‘multcomp’ package to determine relationships between the treatment effects when analyzing number of visits, excluding the interaction term. For the proportion of aborted visits, we calculated pairwise comparisons for proportions corrected for multiple testing in R.

Experiment 2: Discrimination Between Native and Exotic Crayfish Scent.—We also used an ANODEV model to analyze salamander response to crayfish scent, assuming a binomial error distribution. This model included treatment factors (native crayfish scent, exotic crayfish scent) and block terms (lanes 1 and 2). The response variables were number of visits and proportion of aborted visits treated as per Experiment 1.

Experiment 3: Video-Recorded Behavior Trials.—Generalized linear mixed models (GLMM) assuming binomial error distributions were used to evaluate behavioral interactions between hellbenders and crayfish during arena trials. We used the GLMM approach to account for potential autocorrelation by using “trial” as a random effect, which essentially informs the model that error is not independent by trial. The “crayfish tail-flip” model included predictor variables (crayfish species, crayfish sex, hellbender snout contact) and interaction terms (crayfish species \times hellbender snout contact). The response variable was crayfish tail flips. A “hellbender strike” model included predictor variables (crayfish species) and random effects by trial. The response variable was hellbender strikes to crayfish. GLMM models were evaluated with the Laplace approximation used in the ‘lme4’ package (Bates and Maechler, 2009). Lastly, we analyzed climbing behavior among crayfish (binomial error distribution) with the use of an ANODEV model with treatment terms consisting of crayfish species and crayfish sex. A species \times sex interaction term was included to account for an uneven representation of male and female crayfish between species.

Experiment 4: Overnight Feeding Trials.—The results of overnight feeding trials were also evaluated with the use of an ANODEV model assuming a binomial error distribution. This model evaluated crayfish eaten by hellbenders as a function of crayfish characteristics (species, sex). The sex of crayfish offered to hellbenders during overnight trials was not controlled. By chance, an even number of male and female Rusty Crayfish were offered (13 male, 13 female); however, sexes were not evenly represented for native crayfish (18 male, 8 female). Therefore, we included a species \times sex interaction term to account for the uneven representation of male and female native crayfish.

General.—We tested for collinearity among predictor variables with variance inflation tests with the use of the ‘car’ package (Fox and Weisberg, 2011). We considered coefficients with $P \leq 0.05$ as significant and coefficients with $P \leq 0.10$ as approaching significance. Models assuming Poisson or binomial error distributions with overdispersion > 2 were evaluated with “quasi” error distributions. Descriptive statistics are expressed as means \pm SE.

RESULTS

Experiment 1: Detection of Prey Scent.—Treatment had a significant effect on lane visitation frequency by hellbenders (deviance = 17.95, $df = 2$, $P < 0.001$; Table 1). In post hoc comparisons, hellbenders visited fish-analog lanes significantly more than those containing unconditioned water ($P < 0.001$) or parsley-conditioned water ($P < 0.001$; Table 1; Fig. 2A). The treatment (fish analog, parsley, or control) effect approached

TABLE 1. Results of generalized linear models testing the effects of treatment (fish analog [F], parsley [P], or control [C]), block (i.e., lane), and block \times treatment interactions on hellbender response. Results of post hoc analyses are included for treatment factors.

Response	Predictor	Deviance	df	P-value
Visits	treatment	17.948	2	<0.001
	lane	229.396	2	<0.001
	lane \times treatment	0.620	4	0.961
	treatment post hoc	<i>est.</i>		
	F : C	0.676		<0.001
	F : P	0.596		<0.001
Proportion of aborted visits	P : C	0.080		0.864
	treatment	2.220	2	0.062
	lane	1.800	2	0.103
	lane \times treatment	0.881	4	0.677
	treatment post hoc	<i>est.</i>		
	F : C	0.0002		<0.001
	F : P	0.007		0.007
P : C	0.387		0.388	

significance on the proportion of aborted visits relative to total visits (deviance = 2.22, $P = 0.062$; Table 1; Fig. 2B). Post hoc analysis indicated that fish analog-treated lanes had a lower proportion of aborted visits relative to control lanes ($P < 0.001$) and parsley-treated lanes ($P = 0.007$), but the proportion of aborted visits between parsley and control lanes was not significantly different ($P = 0.388$; Table 1; Fig. 2B).

We detected a significant block effect on lane visitation frequency in which hellbenders generally avoided the center lane of the arena. Despite this block effect, there were no significant block (i.e., lane) \times treatment interactions. Therefore, the tendency to avoid the center lane did not change the response to the different treatments (Table 1).

Experiment 2: Discrimination Between Native and Exotic Crayfish Scent.—Hellbenders made significantly more visits to lanes conditioned with native Allegheny crayfish scent compared to lanes treated with nonnative Rusty Crayfish scent (deviance = 7.34, $df = 1$, $P = 0.007$; Fig. 3A). Further, once hellbenders selected a lane with native crayfish scent, they aborted those lanes significantly less than lanes containing nonnative crayfish scent ($7.4 \pm 2.7\%$ vs. $16 \pm 3.6\%$, deviance = 6.23, $df = 1$, $P = 0.013$; Fig. 3B).

Experiment 3: Video-Recorded Behavior Trials.—The number of crayfish tail-flips observed exhibited a significant interaction effect between snout contact and crayfish species (Table 2; Fig. 4). Specifically, native crayfish demonstrated an increased number of tail-flips in response to hellbender snout contact, whereas nonnative crayfish showed no difference in tail-flips relative to snout contact. Additionally, there was a significant effect of sex on tail-flips (Table 2). Male crayfish tail-flipped twice as

TABLE 2. Results of generalized linear mixed models testing the effects of predictor variables (crayfish species, crayfish sex, hellbender snout contact) and interaction terms (crayfish species \times hellbender snout contact) on crayfish (CF) and hellbender (HB) behavior.

Response	Predictor	Coeff.	SE	P-value
CF tail-flips	CF species	1.129	0.188	<0.001
	CF sex	1.035	0.257	<0.001
	HB snout contact	-0.079	0.173	0.647
	CF species \times HB snout contact	0.843	0.224	<0.001
HB strikes	CF species	0.874	0.490	0.074

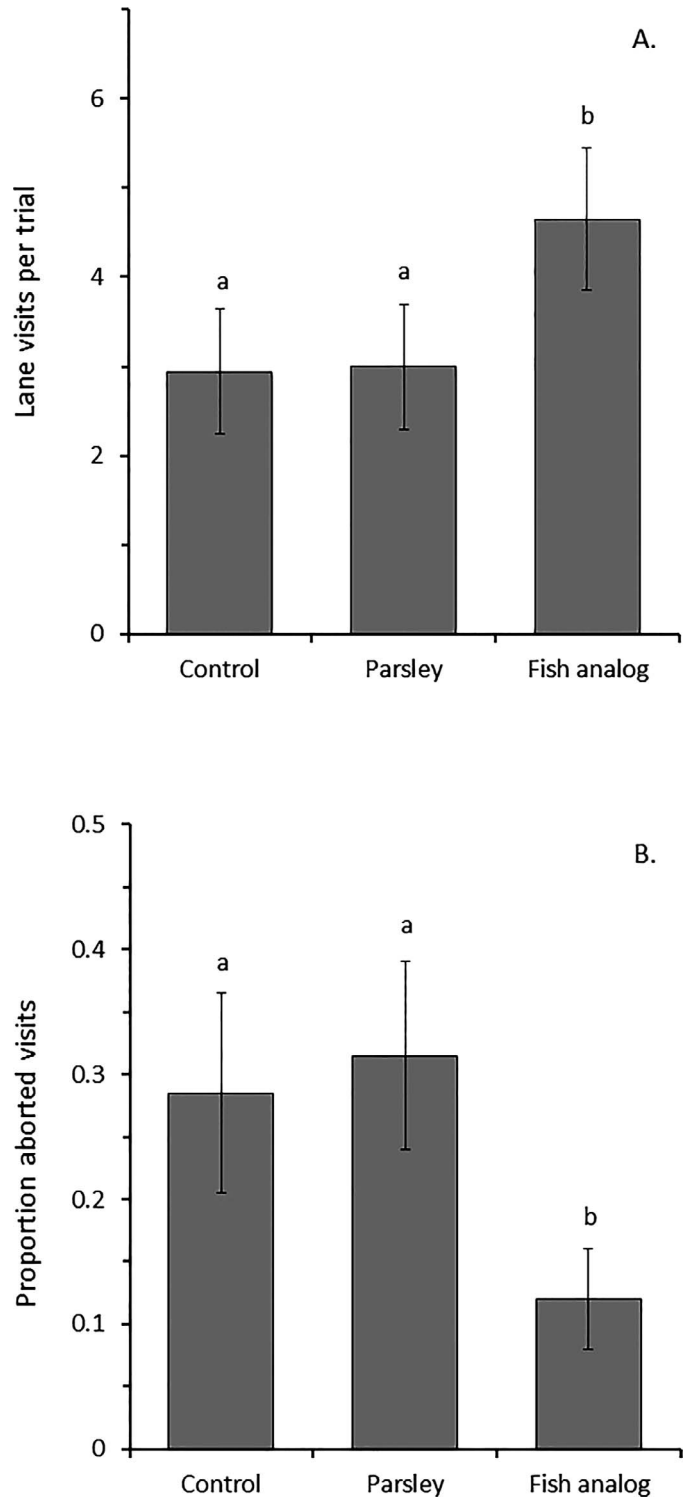


FIG. 2. (A) Mean \pm SE number of visits by treatment lane per trial (Experiment 1). Hellbenders made significantly more visits to lanes conditioned with fish analog compared to control and parsley-conditioned lanes, which were visited with similar frequency. (B) Mean \pm SE proportion of aborted visits by treatment lane per trial (Experiment 1). Hellbenders were significantly less likely to abort visits to lanes conditioned with fish analog compared to control and parsley-conditioned lanes, which were aborted with similar frequency. Different letters above error bars represent significantly different means according to post hoc analysis (Tukey's HSD: $P < 0.05$).

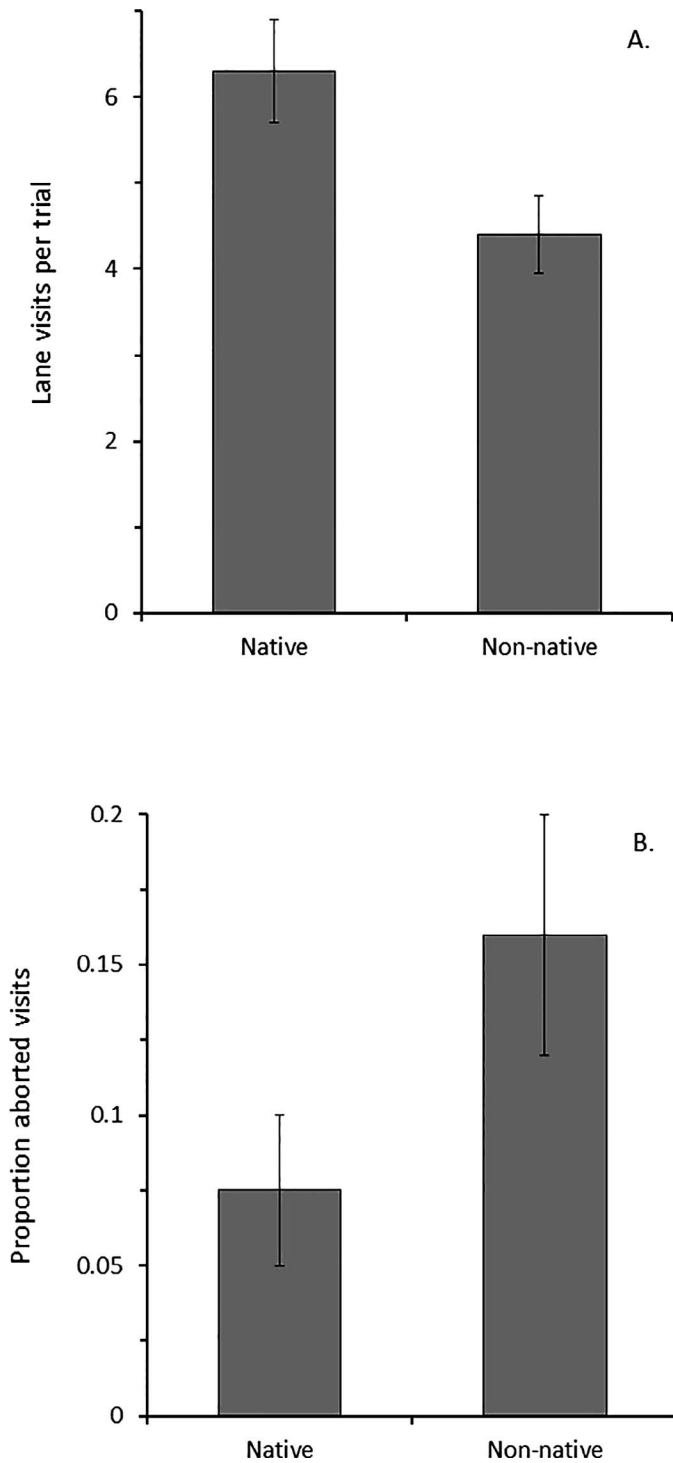


FIG. 3. (A) Mean \pm SE number of lane visits by treatment per trial (Experiment 2). (B) Mean \pm SE proportion of aborted lane visits by treatment per trial (Experiment 2).

frequently at females ($35 \pm 1.2\%$ vs. $17.9 \pm 1.4\%$) following snout contact with hellbenders.

During behavioral trials, native and nonnative crayfish exhibited a significant difference in climbing behavior in the presence of hellbenders (deviance = 25.72, $df = 1$, $P < 0.001$), with only native crayfish climbing ($36.2 \pm 7.1\%$ of native crayfish climbed vs. 0% nonnative). Further, males climbed more often than females ($25.4 \pm 0.572\%$ vs. $6.5 \pm 4.5\%$; deviance = 3.69, $df = 1$, $P = 0.055$). There was no significant

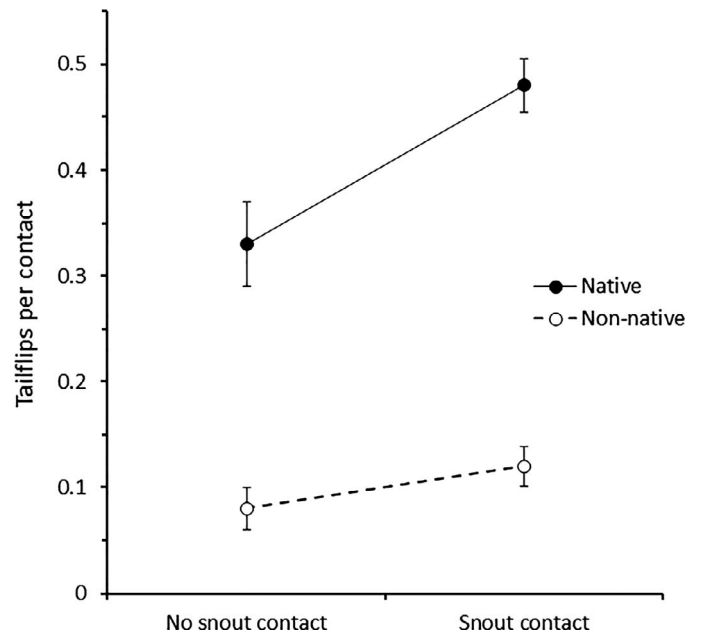


FIG. 4. Interaction plot displaying mean \pm SE number of crayfish tail-flips by species per trial, as a function of hellbender snout contact (Experiment 3). Native crayfish significantly increased tail-flips in response to snout contact by hellbenders, whereas nonnative crayfish tail-flips remained equally low regardless of hellbender snout contact ($P < 0.001$).

interaction effect between the sex and species of crayfish that climbed.

Half of the 30 hellbenders tested in Experiment 3 struck at a crayfish at least once, and a total of 26 strikes were recorded. Among those individuals that struck at crayfish, the mean number of strikes per hellbender was 1.7 ± 0.33 strikes per trial. Hellbenders were twice as likely to strike at native crayfish compared to Rusty Crayfish (1.5 strikes per 100 encounters vs. 0.7 strikes per 100 encounters), which approached statistical significance ($P = 0.074$).

Experiment 4: Overnight Feeding Trials.—Of the 26 hellbenders offered crayfish, 8 (31%) consumed ≥ 1 crayfish by the following day. Two hellbenders ate both rusty and native crayfish and six ate only Rusty Crayfish. Significantly more nonnative crayfish were consumed (31%) than native crayfish (8%) (deviance = 4.72, $df = 1$, $P = 0.029$). Additionally, more female crayfish were eaten ($n = 8/21$) than male crayfish ($n = 2/31$) (deviance = 7.66, $df = 1$, $P = 0.006$).

DISCUSSION

Our results suggest hellbenders can detect prey via chemoreception. Furthermore, hellbenders discriminated between native and exotic prey on the basis of olfactory cues. Hellbenders exhibited more interest and predatory behavior toward native crayfish, yet consumed significantly more nonnative crayfish. This disparity is best explained by differences in avoidance behavior between the prey species.

Native Allegheny crayfish exhibited a stronger tail-flipping response in the presence of hellbenders than nonnative Rusty Crayfish, suggesting that native crayfish perceived a greater degree of danger from hellbenders than nonnative crayfish (Fig. 4). Climbing, another potential hellbender avoidance behavior exhibited by crayfish (Reese, 1903), was observed among only native crayfish, providing further evidence that native crayfish

perceive hellbenders as a strong predation risk and exhibit avoidance behavior. Together, these results suggest that although hellbenders may prefer native crayfish, this preference was apparently superseded by the different behavior of prey species (e.g., Sih and Christensen, 2001).

Whereas Rusty Crayfish are nonnative in parts of the hellbender's range, the two species are naturally sympatric in parts of the Ohio River Drainage. Consequently, one might expect Rusty Crayfish to display more adaptive avoidance behaviors in the presence of hellbenders than were observed. Although interactions between hellbenders and Rusty Crayfish have not been documented in the Ohio River Drainage (Greg Lipps, Roger Thoma, pers. comm.), we suspect that hellbenders are not major predators of Rusty Crayfish in their sympatric range, because Rusty Crayfish do not treat hellbenders as a significant predation risk; there has not been strong selection for this predator archetype (*sensu* Cox and Lima, 2006). The growth and behavior of Rusty Crayfish, however, differ between native and invaded populations (Pintor and Sih, 2009), making the relationship between hellbenders and Rusty Crayfish in the Ohio River Drainage of little relevance with respect to how these species interact elsewhere.

Future studies may build upon our findings by evaluating interactions between different populations of hellbenders and crayfish. For example, to confirm that the poor avoidance behavior we observed in nonnative Rusty Crayfish was a consequence of naïveté on the part of this species, one would also need to test interactions between hellbenders and Rusty Crayfish originating from naturally sympatric populations. If native forms of the Rusty Crayfish demonstrate stronger hellbender avoidance behavior than nonnative forms, this would support the notion that the increased consumption of Rusty Crayfish reported in this study indeed reflects naïveté of the nonnative form. Alternatively, if both nonnative and native forms of Rusty Crayfish show poor hellbender avoidance behavior, hellbenders may not have exerted sufficient predation pressure to select for such traits in Rusty Crayfish within the Ohio River Drainage.

Although we detected differences in antipredator behavior between prey species, the origins of these behaviors are uncertain. Prey naïveté may result from the failure to recognize a threat from a novel predator, but naïveté can also arise when prey perceive a predator yet respond inappropriately (Cox and Lima, 2006). Additional studies that test reciprocal recognition between predator and prey (*sensu* Li et al., 2011) may provide insight into the mechanisms responsible for the differences in predator avoidance between rusty and Alleghany crayfish.

In addition to species-specific differences in hellbender avoidance, crayfish behavior also varied by sex, as incidences of tail-flipping and climbing were both significantly greater among male crayfish compared to females. The implication that male crayfish were better at evading hellbenders is consistent with the results of overnight feeding trials, in which hellbenders consumed significantly more female crayfish. The differences in avoidance behavior observed between male and female crayfish may reflect sex-specific life history traits typical of crustaceans (e.g., behavioral changes associated with the molt cycle; Stein, 1977; Lipcius and Herrnkind, 1982; Steger and Caldwell, 1983; Cromarty et al., 1991).

We demonstrate that hellbenders likely use olfactory cues during prey acquisition, and that they were attracted to native crayfish odor more than nonnative crayfish odor. Similarly, when hellbenders initiated strikes at crayfish they attempted

those strikes about twice as frequently toward native prey compared to nonnative prey. Although hellbenders may possess innate preferences toward native prey, this warrants further investigation, especially with respect to elucidating the underlying mechanisms for such preferences. Hellbenders are clearly capable of consuming nonnative Rusty Crayfish, as evidenced by their consuming this species during laboratory trials, albeit at a low rate. Therefore, prey antibehavior may trump predator preference (e.g., Li et al., 2011) in determining the outcomes of predator-prey interactions.

Predator-prey interactions play a critical role in the dynamics of natural systems (Pace et al., 1999), including biotic invasions (Mack et al., 2000). The ability of exotic taxa to invade native communities successfully is often attributed to the "ecological naïveté" of native communities relative to invaders (e.g., "enemy release hypothesis"; Keane and Crawley, 2002; "naïve prey" hypothesis; Cox and Lima, 2006); however, exotic species can likewise be subject to a novelty disadvantage (Colautti et al., 2004; Sih et al., 2010). Although the aggressive defense behavior of the nonnative Rusty Crayfish can reduce this species' susceptibility to fish predators (Garvey et al., 1994), our results suggest that those same traits could make Rusty Crayfish more vulnerable to other native predators, such as hellbenders (Cox and Lima, 2006). Therefore, we highlight the need to consider species-specific variation in the response of native predators to nonnative prey to understand the complexities of biological invasions better.

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