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Behavioral Differences Between Native and Exotic Invertebrate Prey Affect Susceptibility to Predation by a Native Amphibian Predator

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Behavioral differences between native and exotic invertebrate prey affect susceptibility to predation by a native amphibian predator

by

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ABSTRACT OF THESIS

Invasive species threaten global biodiversity via mechanisms that include altering the dynamics and structure of native food webs. Whereas much research has focused on how exotic species respond to native predators, less is known about how native predators are affected by invasive prey. Here I investigate the response of a rare and threatened native predator—the Eastern Hellbender (*Cryptobranchus alleganiensis*) to a high-profile invasive crayfish species, *Orconectes rusticus*. Hellbenders have declined throughout much of their range, and although the potential for exotic predators (i.e. sport fish) to negatively impact *C. alleganiensis* has been addressed, effects of exotic prey on hellbender populations are unknown. Crayfish are an important food resource for *C. alleganiensis*; however, some speculate the large and aggressive *O. rusticus* may be unpalatable to hellbenders in regions where these species have not historically co-occurred. The primary objective of this study was to determine how *C. alleganiensis* responds to a native prey species (*Orconectes obscurus*), relative to an exotic prey species (*O. rusticus*). Specifically, I tested to see if hellbenders discriminated between crayfish species using chemoreception, then I analyzed behavioral interactions among hellbenders and crayfish during video-recorded trials, and lastly, I assessed hellbender selectivity of crayfish prey during overnight feeding trials. *Cryptobranchus alleganiensis* generally showed a preference for the scent of native crayfish, and were more likely to strike at native crayfish. However, more invasive crayfish were consumed during overnight feeding trials. This discrepancy apparently results from differences in avoidance behavior between prey species; native crayfish (*O. obscurus*) exhibited superior avoidance abilities relative to the exotic *O. rusticus*. Thus, during biotic invasions, food preferences of native predators may be superseded by differences in antipredator behavior of prey.

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A thesis submitted in partial
fulfillment of the Master of Arts degree
in Biology at Buffalo State College

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Introduction

The spread of nonindigenous species may cause animal extinctions (Clavero & Garcia-Berthou 2005) and is a primary agent of global biotic homogenization (McKinney & Lockwood 1999). By altering the structure and dynamics of native food webs, invasive species can initiate trophic cascades (Fausch et al. 2002), which potentially simplify ecosystems, leading to reduced functionality, resilience, and stability (Olden et al. 2004). Consequently, elucidating how exotic species affect food-webs is critical to understanding the broader ecological consequences of species invasions and developing adaptive management strategies.

Studies of predator-prey relationships in invasion biology have typically focused on the responses of exotic prey to native predators, and less is known about how native predators are affected by non-indigenous prey (Carlsson et al. 2009). Of the limited data available, native predators do not show a unanimous response to exotic prey. In some instances, nonindigenous prey can reduce the fitness of native predators. For example, coastal horned lizards (*Phrynosoma coronatum*) maintained on diets of the invasive Argentine ant (*Linepithema humile*) exhibited reduced foraging rates and did not grow, whereas lizards fed native ants had growth rates similar to those measured in the field (Suarez & Case 2002). Negative impacts of exotic species on native predators have also been documented in aquatic ecosystems. For example, following the introduction of dreissenid mussels in Lakes Michigan and Huron, the growth rates of alewives (*Alosa pseudoharengus*) and lake whitefish (*Coregonus clupeaformis*) have declined significantly (Pothoven & Madenjian 2008). On the other hand, native predators can potentially benefit from introduced prey. The Lake Erie water snake (*Nerodia sipedon insularum*) is a native predator endemic to the western basin of Lake Erie. Although *N. s. insularum* was federally designated as “Threatened” in 1999, this species has apparently benefited from the introduction of round gobies (*Neogobius melanostomus*), and was de-listed in 2011 (King et al. 2006). Furthermore, there is evidence that native predators can provide resistance against biotic invasions by limiting the abundance and distribution of exotic prey (DeRivera et al. 2005; Gruner 2005). Determining the extent to which non-indigenous prey is consumed, and potentially controlled by native predators, may be important for predicting and managing species invasions. Thus, here I investigate the response of a rare and threatened native predator to exotic prey.

Compared to terrestrial ecosystems, freshwater environments may be especially susceptible to biotic invasions (Sala et al. 2000). This disparity in part reflects humans' longstanding association with water as a critical natural resource, transportation medium, and source of recreation (Lodge et al. 1998). As a result, aquatic environments are subject to high rates of human-mediated species introductions, both accidental and intentional (Sala et al. 2000; Rahel 2002). Once introduced, exotic species face fewer dispersal barriers than on land, and may spread rapidly (Lodge et al. 1998). Furthermore, high rates of endemism among some freshwater taxa, as well as widespread deterioration of freshwater habitats, puts many aquatic organisms at increased risk of extinction (Ricciardi & Rasmussen 1999; Kuhlmann & Hazelton 2007).

The Hellbender (*Cryptobranchus alleganiensis*) is a large aquatic salamander 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). The Eastern Hellbender is listed as a species of Special Concern in New York State, where it occurs in only two watersheds—the Allegheny and Susquehanna. Although declining in both systems, the status of hellbenders in the Susquehanna drainage is especially tenuous, as there have been very few documented sightings in recent years (Foster et al. 2009; Foster, unpublished data).

The spread of non-native species is a factor implicated in hellbender decline (Gall & Mathis 2010). 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 non-native sport fish (Crane & Mathis 2010). Whereas the potential for exotic predators to negatively impact *C. alleganiensis* has been addressed (Crane & Mathis 2011), little is known about how hellbenders may be affected by exotic prey, and this issue warrants investigation (Carlsson et al. 2009). Analyses of gut contents and direct observations indicate that hellbenders will consume a variety of prey; however, the bulk of hellbender diet appears to be comprised of crayfish (Smith 1907; Nickerson & Mays 1973; Peterson et al. 1989). The importance of crayfish as a prey item for hellbenders is further evidenced by qualitative data suggesting that in some circumstances hellbender abundance and fitness might be limited by crayfish availability (i.e. "bottom-up" control; Nickerson et al. 2003; Nickerson et al. 2009; Hecht-Kardasz et al. 2012). However, as primary crayfish consumers (Herman 2012), it seems equally plausible that hellbenders play an important role in keeping crayfish populations in check (i.e. "top-down" control; Keitzer 2007).

Within the past century, crayfish assemblages have undergone considerable changes as a result of anthropogenic introductions (Lodge et al. 2000). Notably, in North America the ongoing spread of the rusty crayfish (*Orconectes rusticus*) has received much attention (Lodge et al. 2000). Rusty crayfish are native to the Ohio River Drainage (Taylor 2000), but in recent decades this species has become established throughout much of the midwestern and northeastern United States, and Ontario (Hobbs et al. 1989; Conard et al. 2016). Rusty crayfish are voracious omnivores that can have profound impacts on the structure and function of invaded ecosystems by displacing native crayfish (Capelli & Munjal 1982; Lodge & Lorman 1987), reducing macrophyte and macroinvertebrate abundance (Charlebois & Lamberti 1996; Wilson et al. 2004), competing with fishes for prey, and consuming fish eggs (Morse et al. 2013).

Anecdotal evidence suggests a correlation between rusty crayfish establishment and hellbender decline in areas where *O. rusticus* has spread outside of its native range (PNHP 2011; Kobell 2012; Spinks 2014). Notably, hellbenders are also 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 *O. rusticus* 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 *C. alleganiensis*, some speculate that *O. rusticus* may be unpalatable to hellbenders due to the crustacean's large size and aggressive nature (Herman 2012; Quinn et al. 2013). However, interactions between hellbenders and rusty crayfish have not been tested empirically. Here, I investigate how *C. alleganiensis* responds to a co-occurring prey species (*Orconectes obscurus*), relative to an exotic prey species (*O. rusticus*). Specifically, in this study I sought to determine if hellbenders 1) detect prey by chemoreception, 2) discriminate between native and exotic prey on the basis of scent, 3) exhibit differences in capture/handling ability between native and exotic prey, and 4) demonstrate selectivity between native and exotic prey.

One might expect *C. alleganiensis* to show a stronger response to native prey relative to exotic prey on account of co-evolutionary history (e.g. Burghardt 1967; Cattau et al. 2010). However, the ambiguity surrounding the history between local *C. alleganiensis* and *O. rusticus* precludes considering this hypothesis alone. Whereas rusty crayfish are invasive in New York State, *O. rusticus* and *C. alleganiensis* naturally co-occur in parts of the Ohio River Drainage. Thus, hellbenders in New York may share a co-evolutionary history with rusty crayfish, and if so, these salamanders could retain traits that allow them to forage efficiently on *O. rusticus*. On the other hand, if invasive forms of *O. rusticus* differ substantially from those in the native range (Pintor & Sih 2009), local hellbenders might struggle with this prey. Furthermore, the response of *C. alleganiensis* to different prey is expected to relate, in part, to the type and specificity of prey cues hellbenders utilize, and to the ‘cue-similarity’ (*sensu* Sih et al. 2010) between native and exotic prey; to my knowledge such factors have not been investigated. Consequently, I also considered an alternate hypothesis—that hellbenders would not show a stronger response to native prey relative to exotic prey.

Methods

This study was conducted at the Buffalo Zoo, in Buffalo, New York. Research was approved under IACUCs from the Buffalo Zoo and SUNY Buffalo State (IACUC #33). Study animals consisted of hellbenders that were reared from eggs collected in the Allegheny Drainage in October 2009. Hellbenders were housed in 114-L tanks (91.4 × 45.7 × 30.5 cm), with each tank containing 6-8 individuals. Chilled lake water was provided to the tanks using 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, MA). Water temperature varied seasonally, and during the course of this study (July-August 2015) morning water temperatures in hellbender enclosures were between 17-21°C. Enclosures contained pond stone substrate, PVC and rock hides, and aerators. 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 to ensure a strong foraging response. Immediately prior to fasting, salamanders were fed *ad libitum* in order to equalize levels of satiation (Jaeger et al. 1982). At the time of the study, hellbenders were approaching sexual maturity and measured 39.94 ± 0.55 cm (mean total length ± SE; range = 29-45 cm). All of the salamanders used in this study had been previously tagged with Passive Integrated Transponder (PIT) tags, allowing us to easily identify individuals with the aid of a PIT-tag scanner.

Experiments 1-3 were conducted using a flow-through arena (51 × 140 × 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 (O.D.) Tygon® tubing. PVC 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/sec (66.7 ml/sec/lane). Water depth in the arena was 6.5 cm, and water exited the system through 9 evenly spaced, circular openings (D = 1.3 cm) in the downstream panel of the arena. The turnover time was approximately 3 min. Different color dyes were applied to each head tank to verify that water flowed through the system as desired (i.e. no backflow; mixing in downstream zone only).

To replicate the low-light environment in which hellbenders were normally fed at the zoo, during arena trials light was provided only by two blue CFL bulbs. All arena trials were digitally recorded using a Canon EOS 6D camera with a Canon EF 17-40mm f/4L lens. A Sirui N-2204X tripod was used to secure the camera in an overhead position above the arena. Before each trial, the sides of the arena were covered with cardboard to prevent exposure to external visual stimuli. All arena trials lasted 10 min, and were preceded by a 5 min acclimation period. For each trial, a hellbender was selected at random from an enclosure and transported to the test arena using an aquarium net. Each salamander was scanned with a PIT-tag reader before the start of a trial, and the associated PIT-tag code was recorded. During acclimation, filtered lake water flowed through the arena via the 'blank control' lane. The arena, head tanks, and Tygon tubing were flushed with lake water between trials to remove residual stimuli. Valve turn order, lane treatments, and head tank treatments were randomly assigned before each test.

I evaluated hellbender response to two species of crayfish: 1) the rusty crayfish (*Orconectes rusticus*), which is invasive in New York State, and 2) the native Allegheny crayfish (*Orconectes obscurus*). Relative to hellbender distribution in New York, *O. obscurus* occurs in the Allegheny and Susquehanna drainages, thus representing a potential prey item for hellbenders in both systems. In contrast, *O. rusticus* is abundant in the Susquehanna watershed, but is not yet established in the Allegheny drainage. Crayfish were collected by hand and with kick-nets between May and August of 2015 (NYSDEC scientific collection permit #1446 and invasive species permit #00-15-001). Crayfish were transported to the Buffalo Zoo and decontaminated by soaking in a salt bath (50g/L). The crayfish were fully submerged in the salt bath for 5 min, then rinsed in dechlorinated water before being moved to 114-L tanks where they were maintained until needed for testing.

Experiments 3-4 involved offering live crayfish to hellbenders. For these trials, crayfish were selected based on carapace length relative to hellbender length. I offered crayfish possessing carapace lengths within 5-8% of hellbender total length (TL)—a range informed by previous gut content analyses of hellbenders in the field (Wiggs 1976). A Vernier caliper

(Supertek) was used to measure crayfish chelae and carapace lengths to the nearest mm. Although crayfish chelae size was not controlled for, individuals with strongly disproportionate chelae (>3 mm) were excluded.

Experiment 1: Detection of Prey Scent

Salamanders use chemoreception for a variety of behaviors, including predator avoidance (McDarby et al. 1999), courtship (Houck 1986), and foraging (Placyk & Graves 2002). Hellbenders' ability to detect and respond to chemical cues of potential predators has been demonstrated in the laboratory (Gall 2008; Gall & Mathis 2010; Crane & Mathis 2011). Anecdotal observations suggest that chemical cues may play an important role in the foraging behavior of *C. alleganiensis* (Surface 1913), however effects of prey stimuli have not been evaluated experimentally.

The purpose of Exp. 1 was to verify that hellbenders could use chemosensory perception to detect prey. To do so, I measured hellbenders' response to 1) prey-conditioned water, 2) a blank control, and 3) a neutral scent. One branch of the arena was connected to a head tank filled with prey-conditioned water. Fish analog was used as the prey stimulus because it was a food item the salamanders were accustomed to, and I expected it would elicit a positive feeding response. The blank control treatment consisted of filtered lake water, and parsley-conditioned water was used as a neutral scent (i.e. something I expected hellbenders could smell, but that lacked ecological significance). The neutral scent treatment was included in order to demonstrate that investigatory behavior by hellbenders was not simply a response to any scent (vs. no scent). Mesh bags containing fresh parsley were placed in hellbender enclosures for several days prior to testing in order to familiarize the animals with this stimulus (i.e. so the parsley would not represent a “novel scent”). Scent stimuli (20 g fish analog, 20 g fresh parsley) enclosed in weighted mesh bags were placed in separate head tanks and soaked for 1 hr prior to testing. Mesh bags prevented any large particles from exiting the head tanks or clogging the outflow holes. A weighted mesh bag was also placed in the ‘blank control’ tank.

Hellbenders were placed in the downstream-end of the arena and allowed to acclimate for 5 min before the start of a trial. During acclimation all lanes were gated closed. Gates allowed the passage of filtered lake water, but prevented hellbenders from traveling up the lanes. Following acclimation, one observer (Obs. 1) gently corralled the hellbender within a half-PVC hide to face the downstream end of the arena. At the start of a trial, a second observer (Obs. 2) opened the valves on the head tanks, then removed the three gates while Obs. 1 simultaneously lifted the PVC hide. Obs. 1 was blind to treatment assignments until after video analysis was complete. A total of 30 replicate trials were completed using 30 different hellbenders.

During video analysis, Obs. 1 monitored hellbenders’ positions and recorded: 1) the number of visits to each lane, 2) time spent in each lane, 3) the first lane visited, and 4) how thoroughly lanes were investigated. If a hellbender entered a lane and continued moving “upstream” until reaching the back 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, this 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. I also marked increments of 5 cm along the long edges of the arena floor in order to measure hellbender length.

Experiment 2: Discrimination Between Native and Exotic Crayfish Scent

After confirming that hellbenders could detect prey by chemoreception, I tested hellbenders' (n = 30) ability to discriminate between native and exotic crayfish scent by exposing the salamanders to *O. obscurus*- and *O. rusticus*-conditioned water. All but one hellbender were the same as those used in Exp. 1. Trials were conducted in the same arena as Exp. 1, however during Exp. 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 Exp. 1.

Crayfish-conditioned water was prepared 1 hr prior to testing by separating individuals of each crayfish species into two 75-L head tanks ($\bar{x}$ = 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 days, during which time stimulus concentrations ranged from 215-231 ml water per 1 g crayfish ($\bar{x}$ = 224ml/g). For all trials, differences in stimulus concentrations between crayfish treatments were <2 ml/g. As with Exp. 1, I measured: 1) the number of visits to each lane, 2) time spent in each lane, 3) the first lane visited, and 4) how thoroughly lanes were investigated.

Experiment 3: Video-recorded Behavior Trials

During behavioral trials I examined interactions between hellbenders and live crayfish, using the same 30 salamanders that were tested in Exp. 2. Behavioral trials consisted of a two-part series in which hellbenders were first tested using 1 crayfish (native or invasive), and were later tested with 2 crayfish (1 native, 1 invasive). These trials were conducted in the same arena as Exp. 1-2.

During Exp. 3, all three lanes in the arena were gated closed and the arena received a continuous flow of filtered lake water from a head tank connected to the center lane.

Before the start of a trial, a hellbender was contained in the center lane of the arena and allowed to acclimate for 5 min. Following acclimation, Obs. 2 placed a crayfish in one of the far-downstream corners of the arena (L or R). A PVC hide was used to temporarily pen the crayfish in the corner. At the start of a trial, Obs. 2 lifted the PVC while Obs. 1 simultaneously lifted the center gate, releasing the hellbender from the center lane into the downstream end of the arena. Once a hellbender exited the center lane, the gate was closed behind it. If a hellbender did not readily exit the lane, Obs. 1 would gently nudge the animal to facilitate movement. Crayfish species and placement (L or R corner) were randomized by trial.

During video analysis I documented several interactions between hellbenders and crayfish, including 1) 'encounters' (hellbender and crayfish within <1 chelae length of one another; encounters were further classified as 'hellbender snout contact' or 'no hellbender snout contact'), 2) 'strikes' (hellbender struck at crayfish; 'strikes' were recognized by a rapid forward or lateral movement of the snout; Lorenz Elwood & Cundall 1994), 3) 'pinches' (crayfish pinched hellbender without provocation), 4) and 'tailflips' (stereotyped escape behavior). I also documented 5) 'climbing', which is a potential avoidance behavior exhibited by crayfish in the presence of hellbenders (Reese 1903).

Experiment 4: Overnight Feeding Trials

Although I anticipated that hellbenders would consume crayfish during Exp. 3, no crayfish were eaten. I suspected hellbenders might be more inclined to eat in their regular enclosures compared to the test arena. Thus, Exp. 4 was conducted with hellbenders in their original 114-L tanks.

Hellbenders were housed individually, and each salamander was offered a pair of crayfish consisting of 1 *O. obscurus* and 1 *O. rusticus* of similar carapace length. The crayfish were left in the hellbender enclosures overnight, and the tanks were checked the following day. A total of 26 hellbenders were tested in this experiment, including 8 individuals that had not been used in any earlier trials (Table A1).

Data Analysis

The following methodology was completed using the statistical program R (v. 3.2.3; R Core Team 2015). Hellbender responses to chemosensory stimuli (Exp. 1) were analyzed using generalized linear models (GLMs). These models included treatment factors (filtered lake water, parsley-conditioned water, fish analog-conditioned water), lane (1, 2, 3), and interaction terms (treatment × lane). Response variables included number of visits (poisson error distribution), proportion of aborted visits (quasipoisson error distribution), time spent (quasipoisson error distribution), and number of first visits (binomial error distribution), as a function of treatment and lane. The GLM models were fit using analysis of deviance (ANODEV). I used a Tukey's HSD (honest significance difference) test in the "multcomp" package to resolve relationships among most treatment effects, however, for 'proportion of aborted visits' I calculated pairwise comparisons for proportions with correction for multiple testing ('pairwise.prop.test'). Prior to executing Tukey's HSD, a likelihood-ratio test was performed to test if the interaction term significantly improved model fit. If the interaction term did not improve model fit, it was excluded from post-hoc analysis.

I used a GLM ANODEV model to analyze salamanders' response to native and exotic crayfish scent (Exp. 2), assuming a binomial error distribution. Generalized linear mixed models

(GLMM) (binomial error distributions) were used to evaluate behavioral interactions between hellbenders and crayfish during arena trials (Exp. 3). For the ‘crayfish tailflip’ model, I investigated the effects of crayfish characteristics (species, number, sex, chelae length) and hellbender contact (i.e. snout contact) on crayfish tailflip response. The ‘crayfish tailflip’ model also tested for an interaction between crayfish species × hellbender snout contact. A ‘hellbender strike’ model was used to evaluate how species and chelae length of crayfish influenced hellbender strikes. For the ‘crayfish pinch’ model, I examined how crayfish characteristics (species, number, sex, chelae length) in addition to hellbender contact (snout contact and general contact) influenced crayfish pinches to hellbenders. GLMM models were evaluated using the Laplace approximation in the “lme4” package (Bates & Maechler 2009).

I analyzed climbing behavior among crayfish (binomial error distribution) using a GLM ANODEV model with treatment terms consisting of crayfish species and crayfish sex. A species × sex interaction term was included to account for an uneven representation of male and female crayfish between species. The results of overnight feeding trials were also evaluated using a GLM ANODEV model, assuming a binomial error distribution. This model evaluated crayfish eaten by hellbenders as a function of crayfish characteristics (species, sex, chelae length), and “experience level” of both crayfish and hellbenders. For crayfish, experience was defined by whether or not an individual had prior exposure to a hellbender. For hellbenders, I measured experience as a function of the number of trials a salamander participated in with respect to native vs. invasive crayfish. 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) (Table A1). Thus, to account for the uneven representation of male and female native crayfish, I included a species × sex interaction term.

Chi-square tests were used for models with binomial or Poisson error distributions, and F-tests were used for models with quasipoisson error distributions. I used the Akaike information criterion (AIC) to inform model selection. Overdispersion (φ) was <2.5 for all GLMs except for the model evaluating 'time spent', in which $\varphi > 65$. To account for overdispersion in the 'time spent' model, a quasipoisson error distribution was used. The data analyzed with GLMMs were not overdispersed ($\varphi < 1.5$). I tested for collinearity among predictor variables using the "car" package (Fox & Weisberg 2011), and trial-level random effects were included in GLMM models to account for autocorrelation. I considered coefficients with *p*-value ≤ 0.05 as 'significant' and coefficients with *p*-value ≤ 0.10 as 'marginally significant' (*sensu* Hurlbert & Lombardi 2009).

Results

Experiment 1: Detection of Prey Scent

Treatment ('prey', 'parsley', or 'control') had a significant effect on number of visits by lane (*df* = 2, *dev.* = 17.948, *p*-value = <0.001; Fig. 2). *Cryptobranchus alleganiensis* made significantly more visits to lanes conditioned with fish analog compared to lanes with unconditioned water (*est.* = 0.676, *SE* = 0.139, *p*-value <0.001) or parsley-conditioned water (*est.* = 0.596, *SE* = 0.137, *p*-value <0.001). Mean number of visits to control and parsley treatments were similar (*est.* = 0.080, *SE* = 0.155, *p*-value = 0.864). Treatment ('prey', 'parsley', or 'control') had a marginal effect on how thoroughly lanes were investigated as a function of proportion of aborted visits relative to total visits (*df* = 2, *dev.* = 2.220, *p*-value = 0.062; Fig. 3). Specifically, post-hoc analysis indicated that fish analog-treated lanes were investigated more thoroughly (i.e. lower proportion of aborted visits; $\bar{x}$ = 0.117) relative to parsley-treated ($\bar{x}$ = 0.322, *p*-value = 0.007) and control lanes ($\bar{x}$ = 0.284, *p*-value = <0.001), and the proportion of aborted visits between parsley and control lanes was not significantly different (*p*-value = 0.388). Treatment had a marginal effect on time spent by lane (*df* = 2, *dev.* = 368.4, *p*-value = 0.090; Fig. 4). On average, *C. alleganiensis* spent the most time in lanes scented with fish analog ($\bar{x}$ = 120.6 sec.), followed by parsley-scented lanes ($\bar{x}$ = 96.2 sec.), and control lanes ($\bar{x}$ = 72.2 sec.). Post-hoc analysis revealed that *C. alleganiensis* spent significantly more time in fish analog-treated lanes compared to blank control lanes (*est.* = 0.668, *SE* = 0.259, *p*-value = 0.026), but time spent in parsley-conditioned lanes did not differ significantly from either control (*est.* = 0.309, *SE* = 0.274, *p*-value = 0.496) or fish analog treatments (*est.* = 0.359, *SE* = 0.238, *p*-value = 0.285). Treatment also had marginal influence on the first lane visited (*df* = 2, *dev.* = 4.902, *p*-value = 0.086; Fig. 5). Again, post-hoc tests show a similar “tiered response” pattern as described above for ‘time spent,’ in which *C. alleganiensis* were more likely to visit fish analog-scented lanes first compared to blank control lanes (*est.* = 1.827, *SE* = 0.680, *p*-value = 0.020), and parsley treated lanes resulted in an intermediate response that was not significantly different from control (*est.* = 0.821, *SE* = 0.652, *p*-value = 0.419) or fish analog treatments (*est.* = 1.006, *SE* = 0.634, *p*-value = 0.251).

I detected a significant block effect in which *C. alleganiensis* generally avoided the center lane of the arena. Despite this block effect, there were no significant block (i.e. lane) × treatment interactions. Thus, the tendency to avoid the center lane did not change treatment response.

Experiment 2: Discrimination Between Native and Exotic Crayfish Scent

Hellbenders made significantly more visits to lanes conditioned with native crayfish (*Orconectes obscurus*) scent compared to lanes treated with invasive crayfish (*O. rusticus*) scent (*df* = 1, *dev.* = 7.336, *p*-value = 0.007; Fig. 6). Although *C. alleganiensis* were more likely to visit *O. rusticus*-conditioned lanes first (*df* = 1, *dev.* =3.849, *p*-value = 0.050; Fig. 7), these visits were aborted more frequently than visits to *O. obscurus*-conditioned lanes (*df* = 1, *dev.* = 6.230, *p*-value = 0.013; Fig. 8). There was no significant difference in time spent by treatment (*df* = 1, *dev.* = 0.359, *p*-value = 0.549), and I found no block effect by lane (*df* = 1, *dev.* = 0.420, *p*-value = 0.517).

Experiment 3: Video-recorded Behavior Trials

There was a significant interaction effect between snout contact and crayfish species on the number of tailflips observed (*est.* = 0.843, *SE* = 0.224, *p*-value < 0.001; Fig. 9). Specifically, native crayfish demonstrated an increased number of tailflips in response to hellbender snout contact, whereas invasive crayfish showed no difference in tailflips relative to snout contact. There was a significant effect of sex on tailflips (*est.* = 1.035, *SE* = 0.257, *p*-value < 0.001; Fig. 10). Male crayfish were twice as likely to tailflip ($\bar{x}$ = 0.35, *SD* = 0.48) than females ($\bar{x}$ = 0.18, *SD* = 0.38).

Crayfish pinches to hellbenders were significantly associated with hellbender snout contact (*est.* = 1.968, *SE* = 0.610, *p*-value = 0.001; Fig. 11), and were not significantly influenced by general contact (*est.* = 12.984, *SE* = 916.436, *p*-value = 0.989), species (*est.* = -0.357, *SE* = 0.446, *p*-value = 0.424), sex (*est.* = 0.337, *SE* = 0.621, *p*-value = 0.588), chelae length (*est.* = 0.382, *SE* = 0.296, *p*-value = 0.197), or number of crayfish in the arena (*est.* = -0.227, *SE* =

0.528, *p*-value = 0.667). During behavioral trials, only native crayfish climbed (*df* = 1, *dev.* = 25.717, *p*-value < 0.001), and males climbed more often than females (*df* = 1, *dev.* = 3.688, *p*-value = 0.055). There was no significant interaction effect between the sex and species of crayfish that climbed (*df* = 1, *dev.* = 0.000, *p*-value = 0.999) (Fig. 12).

Half of the 30 hellbenders tested in Exp. 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 1.73 ± 0.33 (SE). Hellbender strikes to crayfish were significantly influenced by chelae length (*est.* = 0.604, *SE* = 0.298, *p*-value = 0.043), and to a lesser extent, species (*est.* = 0.874, *SE* = 0.490, *p*-value = 0.074). Specifically, mean chelae length of crayfish struck by hellbenders was greater than that of crayfish that were not struck (Fig. 13), and hellbenders were more likely to strike at native crayfish (Fig. 14).

Experiment 4: Overnight Feeding Trials

Of the 26 hellbenders offered crayfish, 8 (31%) had eaten ≥1 crayfish by the following day. Two hellbenders ate both rusty and native crayfish, and 6 hellbenders ate only rusty crayfish. No hellbenders ate only native crayfish. These results indicate that significantly more rusty crayfish were consumed (31%) than native crayfish (8%) (*df* = 1, *dev.* = 4.715, *p*-value = 0.030; Fig. 15). Additionally, more female crayfish were eaten (n = 8/21) than male crayfish (n = 2/31) (*df* = 1, *dev.* = 7.655, *p*-value = 0.006; Fig. 16). I found no significant interaction effect between the sex and species of crayfish consumed (*df* = 1, *dev.* = 0.491, *p*-value = 0.484). Crayfish chelae length did not affect hellbender selectivity (*df* = 1, *dev.* = 0.114, *p*-value = 0.736). Prior exposure to hellbenders did not have a significant effect on crayfish susceptibility to predation (*df* = 1, *dev.* =

0.701, *p*-value = 0.403), and prey consumption was not correlated with hellbender experience (*df* = 1, *dev.* = 0.253, *p*-value = 0.615).

Discussion

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 successfully invade native communities is often attributed to the "ecological naïveté" of native communities relative to invaders (e.g. 'enemy release hypothesis' [Keane & Crawley 2002]; 'naïve prey' hypothesis [Cox & Lima 2006]). However, exotic species can likewise be subject to a novelty *disadvantage* (Colautti et al. 2004; Sih et al. 2010). Although behavioral attributes of the invasive *O. rusticus* can reduce this species' susceptibility to fish predators (Garvey et al. 1994), my results suggest that those same traits could make rusty crayfish more vulnerable to other native predators, such as hellbenders (Cox & Lima 2006). Thus, this study highlights the need to consider species-specific variation in the response of native predators to exotic prey in order to better understand the complexities of biological invasions.

In summary, my results are consistent with the hypothesis that hellbenders can detect prey via chemoreception (Exp. 1). Furthermore, hellbenders may discriminate between native and exotic prey on the basis of olfactory cues (Exp. 2). Choice of prey scent, as well as strikes toward crayfish, however, were not predictive of selectivity by hellbenders offered live prey (Exp. 4). This disparity is best explained by differences in avoidance behavior between prey species (Exp. 3).

Hellbenders generally showed a stronger response to prey-conditioned water compared to blank control treatments and water conditioned with a neutral scent (parsley), suggesting hellbenders can detect prey by chemoreception. The ‘parsley’ and ‘control’ treatments yielded similar results for some parameters (‘total visits’ [Fig. 2] and ‘aborted visits’ [Fig. 3]). For other parameters (‘time spent’ [Fig. 4] and ‘first visits’ [Fig. 5]), hellbenders exhibited a trend toward a “tiered” response, in which fish analog-scented lanes received significantly more attention than control treatments, and parsley elicited an intermediate response that did not differ significantly from the other treatments. Although this pattern could simply represent an artifact of limited sampling, an alternate interpretation is that even ecologically “neutral” stimuli such as parsley may elicit stronger investigatory behavior relative to control treatments, which have no added stimuli (i.e. ‘scent’ = more interesting than ‘no scent’).

Hellbenders’ tendency to avoid the center lane of the arena may be attributed to the secretive nature of these animals (Smith 1907; Hillis & Bellis 1971). In the artificial arena environment, which lacked natural substrate and cover objects, hellbenders might have felt more secure moving along the periphery of the enclosure, compared to the center where they were more exposed (P. Felski, pers. comm.). These observations should be valuable in guiding future research on *C. alleganiensis* by informing experimental designs that best accommodate the unique attributes of this species.

In addition to detecting prey by chemosensory perception, our results suggest hellbenders may be able to distinguish between species of congeneric crayfish on the basis of olfactory cues alone. When given a choice between lanes conditioned with native or exotic prey (Exp. 2), hellbenders made more visits to lanes conditioned with native crayfish (Fig. 6), and aborted these visits less often (Fig. 8), suggesting hellbenders may demonstrate a preference for stimuli derived from native prey.

In light of these results, the finding that hellbenders were more likely to *first* visit lanes conditioned with exotic crayfish is ambiguous (Fig. 7). Although none of the salamanders used in this study had been offered crayfish before, native crayfish had been given to other hellbenders at the facility. Due to the flow-through system in which hellbenders were maintained, study animals might have inadvertently been exposed to native crayfish scent via the shared water supply. Excluding the possible role of innate prey preference (Burghardt 1967), indirect preconditioning to native crayfish would, by default, make exotic crayfish treatments the less familiar stimulus, potentially eliciting an initially strong investigatory response by hellbenders (Montgomery 1955; Ruggiero et al. 1979; Harris & Knowlton 2001).

Alternatively, hellbenders might not have been distinguishing between crayfish species per se, but rather were selecting treatments based on colligative properties of an olfactory cue shared by both crayfish species. Because rusty crayfish have higher metabolic rates compared to many other crayfish (Momot 1984), *O. rusticus* treatments might have provided hellbenders with a strong stimulus regardless of whether the salamanders were discriminating on the basis of general or specific cues (*sensu* Sih et al. 2010). Whereas weak stimuli tend to evoke an exploratory response, stronger stimuli can be aversive (Schneirla 1965); thus, a strong *O. rusticu*s-derived stimulus might have initially attracted hellbenders to this treatment from a distance, but upon approach the scent became overpowering, deterring further investigation.

Several instances of cannibalism were observed among crayfish during Exp. 2. Cannibalism was only documented in *O. obscurus*, and these episodes were always associated with molting. When discovered, deceased crayfish were removed and replaced with live individuals of similar size. However, newly-molted crayfish were sometimes rapidly consumed, and it is possible some of these events went unnoticed. Episodes of molting and cannibalism are problematic because these incidents may result in the release of different (and perhaps stronger) chemical cues (Adams & Moore 2003), possibly altering the scent treatment such that hellbender behavior was biased. Considering this potentially confounding issue, results from Exp. 2 should be interpreted cautiously.

An apparent preference for native crayfish, as inferred from mean number of strikes (Exp. 3; Fig. 14), is consistent with results from crayfish scent trials (Exp. 2). However, the results of overnight feeding trials (Exp. 4), in which hellbenders consumed significantly more rusty crayfish, are contradictory (Fig. 15). These seemingly paradoxical results may be reconciled by considering the behaviors of the prey species along with those of *C. alleganiensis*.

Relative to congeners, several studies have found rusty crayfish to be less vulnerable to predation on account of this species' unique morphological and behavioral traits (Capelli & Munjal 1982; Mather & Stein 1993; Garvey et al. 1994; Roth & Kitchell 2005). However, the aforementioned research has focused exclusively on fish predators, and may not apply to other major predators of crayfish, such as hellbenders. I hypothesized that the aggressive nature of *O. rusticus*—the very quality others have suggested might make this species unpalatable to hellbenders (Quinn et al. 2013)—could instead make this invader *more* susceptible to predation.

I evaluated hellbender avoidance behaviors between prey species to determine whether hellbenders' apparent selectivity for *O. rusticus* during overnight feeding trials might be explained by differences in antipredator strategies. I quantified avoidance behaviors primarily by measuring 'tailflips' (a stereotyped escape response of crustaceans; Krasne & Wine 1984). During video-recorded trials, native crayfish were more than twice as likely to tailflip in the presence of hellbenders than rusty crayfish, implying that *O. obscurus* perceived an overall greater degree of danger than *O. rusticus*. The finding that native crayfish increased tailflip behavior in response to hellbender snout contact, whereas rusty crayfish did not, serves as confirmation that native crayfish recognized hellbenders as a threat (Fig. 9). Climbing is another potential hellbender avoidance behavior exhibited by crayfish (Reese 1903). During video-recorded trials only native crayfish climbed (Fig. 12), providing further evidence that rusty crayfish demonstrated inferior predator avoidance strategies in the presence of hellbenders. Together, these results suggest that while hellbenders may possess an innate preference for native crayfish (as inferred from Exp. 2-3) this preference was apparently superseded by the different behavior of prey species (Sih & Christensen 2001).

Because native populations of both hellbenders and rusty crayfish co-occur in the Ohio River Drainage, one might expect *O. rusticus* to display more adaptive avoidance behaviors in the presence of hellbenders. Interactions between *C. alleganiensis* and *O. rusticus* have not been documented in the Ohio River Drainage (Greg Lipps; Roger Thoma, pers. comm.), but if hellbenders are not major predators of rusty crayfish there, then *O. rusticus* might not have been subject to strong selection pressures associated with this 'predator archetype' (*sensu* Cox & Lima 2006). However, considering the growth and behavior of *O. rusticus* appears to differ between native and invaded populations (Pintor & Sih 2009), the relationship between hellbenders and rusty crayfish in the Ohio River Drainage may hold little relevance with respect to how these species interact in New York State.

In addition to species-specific differences in hellbender avoidance, crayfish behavior also varied by sex, as incidences of tailflipping and climbing were both significantly greater among male crayfish compared to females (Exp. 3; Figs. 10, 12). The implication that male crayfish were better at evading hellbenders is consistent with the results of overnight feeding trials (Exp. 4), in which hellbenders consumed significantly more female crayfish (Fig. 16).

The underlying reasons for the differences in avoidance behavior I observed between male and female crayfish are uncertain, however different life history traits may play a role. This study was conducted in late summer, when male *Orconectes* transition between non-reproductive (Form II) and reproductive (Form I) forms. This change in reproductive state is accompanied by molting of the exoskeleton, prior to which calcium is absorbed, reducing the rigidity of the cuticle (Stein 1977; Schechter et al. 2008). In addition to recently-molted individuals, Stein (1977) found that male crayfish in this "premolt" condition (proecdysis) were more susceptible to predation by smallmouth bass compared to similar sized "intermolt" female crayfish. Crustaceans exhibit behavioral changes associated with the molt cycle (Steger & Caldwell 1983; Cromarty et al. 1991). Notably, Lipcius & Herrnkind (1982) reported that during proecdysis, spiny lobsters (*Panulirus argus*) were "submissive," and tailflipped frequently to avoid conspecifics. If male crayfish in the premolt condition are vulnerable to predation, a similar increased propensity to tailflip may be adaptive.

Although hellbenders were more likely to strike at crayfish with larger chelae during video-recorded trials (Exp. 3; Fig. 13), the biological significance of this finding is ambiguous, and chelae length was not predictive of crayfish consumption during overnight feeding trials (Exp. 4). It is possible that at times hellbenders perceived crayfish with larger chelae as a threat; thus some strikes interpreted as predatory might have actually been defensive in nature. However, mean chelae length of crayfish eaten by hellbenders ($\bar{x}$ = 24.3 mm) was similar to that of crayfish that were struck at ($\bar{x}$ = 25.3 mm), indicating crayfish possessing chelae in this size range were indeed recognized as prey. Perhaps crayfish with larger chelae were simply more conspicuous in the arena environment. Alternatively, hellbenders' tendency to strike at crayfish with larger chelae may reflect an optimal foraging strategy. Fish predators generally select small crayfish to reduce handling costs, and maximize digestive content (Stein & Magnuson 1976; Stein 1977). However, hellbenders may be less likely to be gape-limited, in which case chelae length would pose less of an obstacle. Additionally, the proportion of crayfish biomass available for assimilation by hellbenders is unknown, as no digestibility trials have been conducted (Dierenfeld et al. 2009). Thus, with reference to crayfish prey, optimal foraging strategies of hellbenders may differ markedly from those of other predators (i.e. fish).

Contrary to my expectations, no crayfish were eaten by hellbenders during Exp. 3. To rule out the possibility that hellbenders might simply be averse to consuming novel prey, I conducted several supplemental trials with methodology identical to that described for Exp. 3, except that salamanders were offered a variety of familiar prey items (crickets, earthworms, superworms, fish analog) instead of crayfish. Hellbenders behaved similarly during supplemental trials, and none of the familiar prey items were eaten. Reports from zoo staff indicating that hellbenders readily consumed crayfish when this prey was offered in their regular tanks, as well as observations of hellbenders feeding on standard prey once returned to their original enclosures, led me to suspect that the poor feeding response was related to the arena environment. I employed various approaches to entice hellbenders to eat, including the addition of refugia (PVC and rock hides) to the arena, and extending trial duration from 10 to 20 min., neither of which was successful.

I determined that water temperatures in the arena averaged 3.44˚C higher than water in the original enclosures. These temperatures are well within the range of temperatures experienced by hellbenders under natural conditions (Hillis & Bellis 1971; Hutchinson & Hill 1976). However, it is possible that for at least some trials, the allotted acclimation time (5 min.) was insufficient to account for the change in temperature between hellbenders' native enclosures and the test arena, in which case salamanders might have experienced stress, and been less inclined to eat as a result. Nevertheless, of all the trials conducted, only three individuals appeared visibly stressed (i.e. reduced activity, exhibited rocking/swaying behavior; Harlan & Wilkinson 1981), and 50% of hellbenders (15/30) used in Exp. 3 struck at a crayfish at least once during video-recorded trials, suggesting these animals were not overly stressed. Attempts to lower the water temperature in the arena by placing bags of ice in the head tanks were unsuccessful in eliciting a stronger feeding response.

The low numbers of crayfish consumed during overnight feeding trials (Exp. 4; Table A1) might reflect suboptimal hunger levels among hellbenders. Some populations of hellbenders apparently reduce food intake during the breeding season (Kern 1984; but see Peterson et al. 1989), presumably as resource allocation shifts in favor of reproduction. Humphries & Pauley (2005) speculated that foraging activity might vary by sex, with males devoting less time to feeding during the breeding season compared to females. In New York, hellbenders breed between August and September (Bishop 1941; Gibbs et al. 2007), and because our study was conducted in August, some of these animals might have exhibited a reduced interest in food as a consequence of reproductive development.

Aside from reproductive condition, other factors could have resulted in inadequate hunger levels among our test subjects. Hellbenders have low metabolic demands and can go without eating for extended periods of time. For example, Wiggs (1976) fasted seven hellbenders for 220 days and estimated monthly weight loss of those animals to be between 1-2%. Nickerson & Mays (1973) reported that two hellbenders appeared “vigorous” after a starvation period of ~5 months. Thus, the 1-week pre-test fasts might have been insufficient in generating a strong foraging response. This would seem to be at odds with our observations of hellbenders consuming familiar prey items after testing was completed. However, hellbenders may need to develop a stronger “hunger drive” before attempting to capture novel, and potentially threatening prey such as crayfish, compared to the familiar, and comparatively harmless prey items to which they were habituated (Young 1948). This hypothesis is consistent with the results of Stein (1977), who noted that smallmouth bass starved for 24 h would readily attack juvenile crayfish, but required longer periods of starvation (48-72 h) before attempting to capture larger crayfish.

Despite the fact that crayfish are generally considered as contributing the bulk of hellbender diet, our results suggest naive hellbenders may not initially recognize crayfish as palatable prey. The potential for hellbenders to require a “learning curve” when responding to novel prey is supported with data obtained from video-recorded trials. Of the 26 strikes made by hellbenders to crayfish, nearly half (n = 12) were “misses” (i.e. crayfish not grasped by hellbenders’ jaws). Although a similar number of strikes (n = 14) resulted in “captures” (crayfish held by hellbenders’ jaws), during video-recorded trials no captures resulted in crayfish being ingested. Our results are also at odds with data suggesting hellbenders (and other predators; Stein 1977) ingest crayfish “tail-first” (Nickerson & Mays 1973; Wiggs 1976). The majority of video-recorded strikes (62%) were directed anteriorly, with 23% and 15% of strikes being directed laterally and posteriorly, respectively. In several instances, crayfish were seized by one chela, leaving their other chela free to inflict pinches to the salamander. When pinched, a hellbender would release its hold, allowing the crayfish to escape. At other times, hellbenders released crayfish without obvious signs of provocation. The influence of learning on foraging behavior has been explored among a variety of taxa, including salamanders (Gibbons et al. 2005). It is reasonable that hellbenders must learn how to capture and handle novel prey efficiently. Perhaps the duration of the study was insufficient for most of the salamanders to acquire these skills with regard to capturing crayfish.

In conclusion, this study demonstrates that hellbenders likely use olfactory cues in prey acquisition. Although hellbenders may possess innate preferences towards certain prey types (e.g. native spp.), this warrants further investigation, especially with respect to elucidating the underlying mechanisms for such preferences. Hellbenders are clearly capable of consuming *O. rusticus*, however, and selectively consumed this species during laboratory trials. Thus, prey behavior may trump predator preference (e.g. Li et al. 2011).

Others have demonstrated that predator experience can override innate preference toward certain prey types (Darmaillacq et al. 2006), regardless of prey quality (Pekár & Cárdenas 2015). Although it was beyond the scope of this study, future research efforts incorporating a similar methodology may focus on the role of learning in *C. alleganiensis* with respect to foraging behavior. For example, if scent trials (Exp. 1) were repeated after feeding trials (Exp. 4), one might detect experience-mediated changes in predator preference. Furthermore, although I detected differences in antipredator behavior between prey species, the origins of these behaviors are uncertain. Prey naiveté may result from organisms' failure to recognize the threat of a novel predator, but also includes instances in which prey recognize a predator yet respond inappropriately (Cox & Lima 2006). Future studies that use chemical cues to test reciprocal recognition between hellbenders and their prey (*sensu* Li et al. 2011) may help elucidate the underlying mechanisms responsible for the differences in hellbender avoidance observed between *O. obscurus* and *O. rusticus*.

Field-based research is needed to determine how these results compare to the feeding habits of hellbenders under natural conditions. Furthermore, although hellbenders are not averse to consuming rusty crayfish, the fitness consequences of this prey remain unknown. In other words, when it comes to food availability during biotic invasions, native predators such as hellbenders might not always get what they want—but can they get what they need? Research efforts evaluating the assimilation efficiencies of hellbenders maintained on different dietary regimens (e.g. native vs. invasive crayfish) would help to answer such questions.

Aside from representing potential prey for *C. alleganiensis*, invasive rusty crayfish could impact hellbenders in other ways that have not been explored. For example, some speculate that *O. rusticus* might serve as a vector for pathogens such as *Batrachochytrium dendrobatidis* (P. Petokas, pers. comm.), which has decimated amphibian populations worldwide (Daszak et al. 1999) and may contribute to hellbender decline in some areas (Bodinof 2010). Salamander larvae appear to be an important food source for *O. rusticus* (Vollmer & Gall 2014), and Morse et al. (2013) found that rusty crayfish were more effective predators of substrate-nesting fish eggs compared to native *O. virilis*. At vulnerable life stages (i.e. eggs and larvae) hellbenders may also be susceptible to predation by rusty crayfish (M. Nickerson & P. Petokas, pers. comm.), but this has yet to be tested. Hellbenders, like some fish, could also be negatively impacted by indirect effects associated with *O. rusticus* invasions, such as reductions in macroinvertebrate abundance (Charlebois & Lamberti 1996; Wilson et al. 2004), and this also warrants investigation.

As biotic invasions are expected to continue, the adaptive abilities of native taxa will be tested in a variety of ways (Rahel 2002). Although much research has focused on the effects of exotic predators on native prey, species introductions also have consequences on native predators (Carlsson et al. 2009). In turn, native predators can limit biotic invasions (Reusch 1998), and the establishment of nuisance species may be facilitated by the loss of such predators (Rahel 2002).

The Eastern Hellbender is a native predator thought to play an important role in ecosystem dynamics (Humphries & Pauley 2005). Hellbenders are long-lived (Taber et al. 1975), and can attain high densities (Hillis & Bellis 1971). As crayfish form the bulk of this species' diet, hellbenders may be important in controlling crayfish populations (Keitzer 2007; Herman 2012). Considering the results of this study, it seems unlikely that hellbenders would be averse to consuming *O. rusticus* in the wild. Indeed, in June 2011, an adult hellbender captured in the Susquehanna Drainage of New York regurgitated two rusty crayfish (Peter Petokas, pers. comm.; Fig. 17). Hellbender declines have been documented across this species' range (Mayasich et al. 2003), including the Allegheny and Susquehanna River watersheds of New York (Foster et al. 2009; Foster, unpublished data). Although the impacts of rusty crayfish on native hellbender populations remain uncertain, perhaps reductions in hellbender numbers might have facilitated the spread of this invader in parts of New York.

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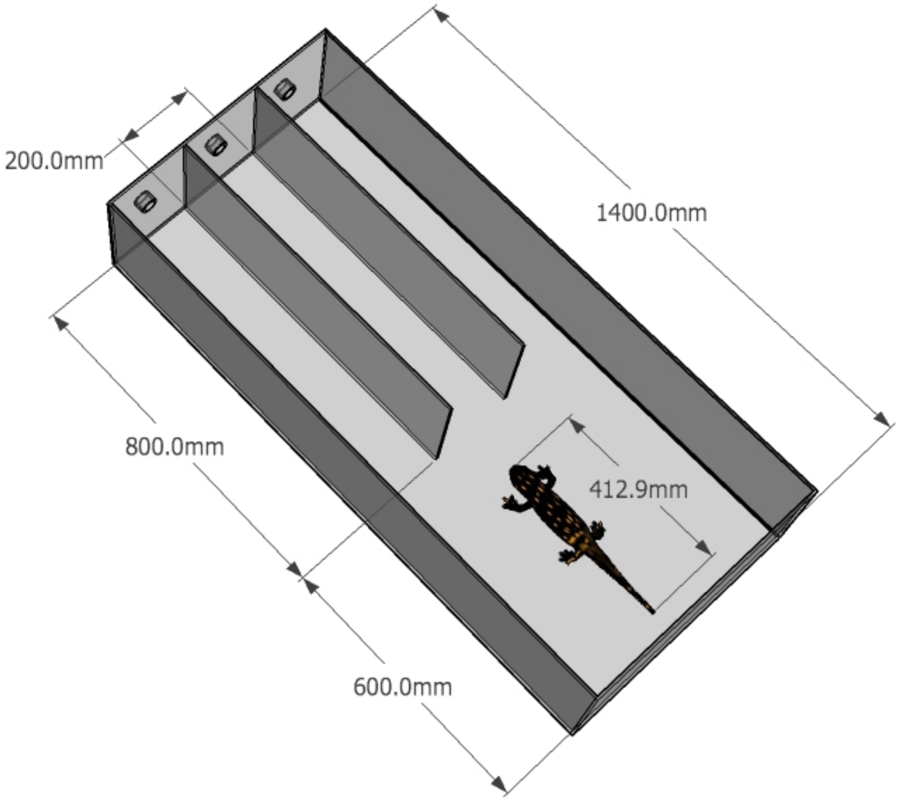

Figure 1: Rendering of the test arena used for Exp. 1-3 (not to scale). For Exp. 1, each of the three lanes received a randomly assigned treatment (control, parsley, fish analog). During Exp. 2, the center lane was blocked, and the outer lanes received native (*Orconectes obscurus*)- or invasive crayfish (*O. rusticus*)-conditioned water. In Exp. 3, all three lanes were blocked off, restricting interactions to the downstream end of the arena. Water exited the arena via openings in the downstream wall (not shown).

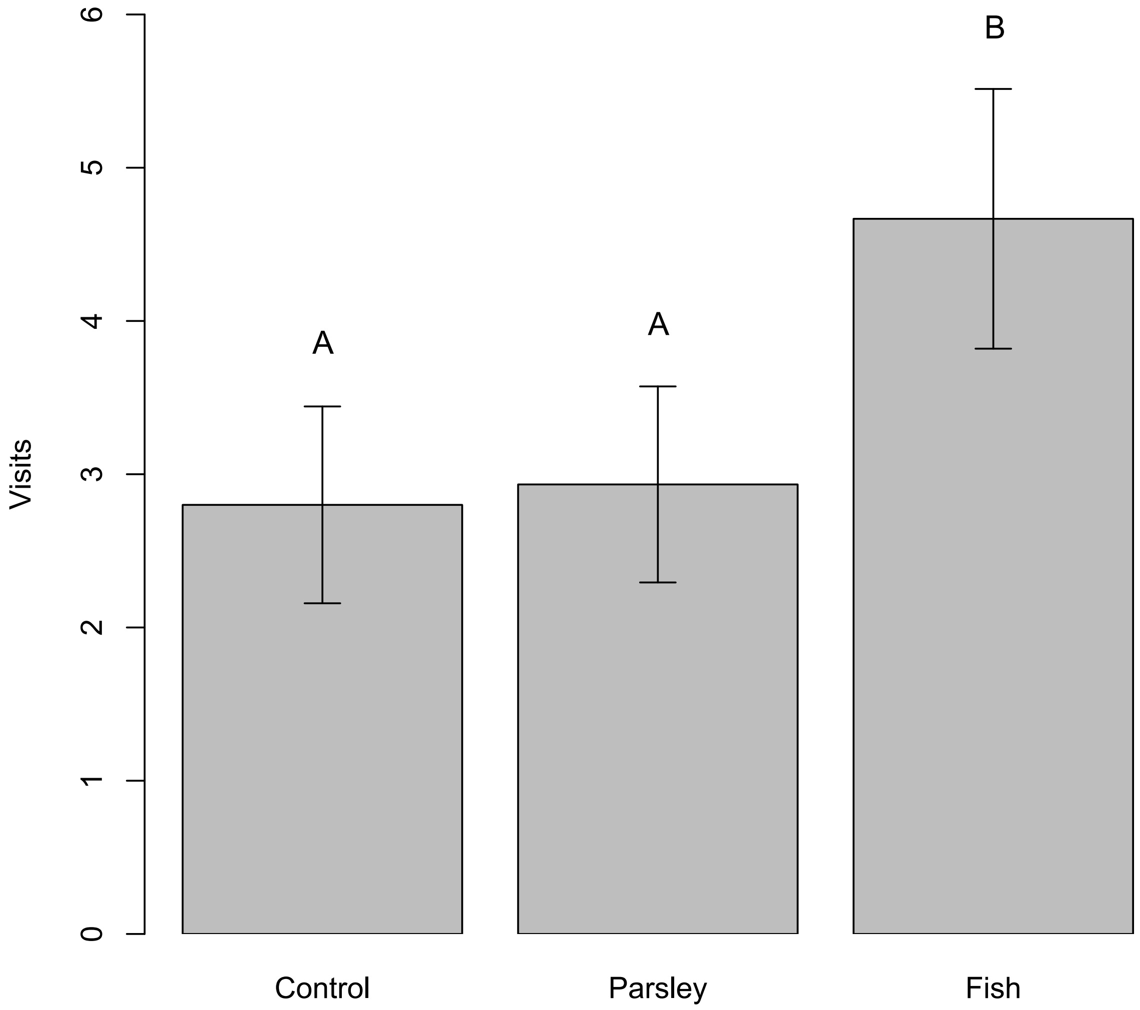

Figure 2: Mean (±1 SE) number of visits by treatment lane (Exp. 1). Hellbenders made significantly more visits to lanes conditioned with fish analog compared to control- and parsley-conditioned lanes, which were visited with similar frequency. Different letters above error bars represent significantly different means according to post-hoc analysis (Tukey’s HSD: $P < 0.05$).

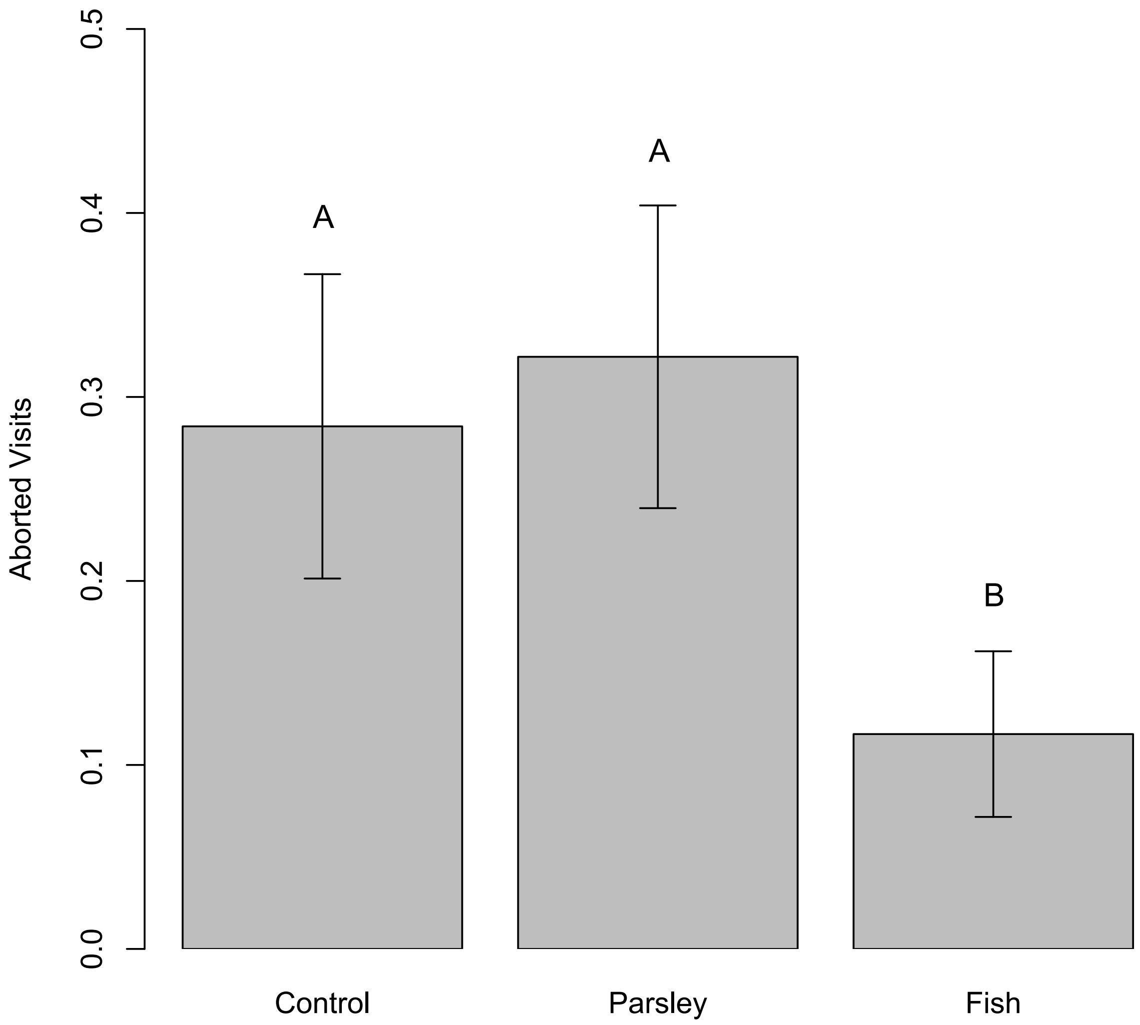

Figure 3: Mean (±1 SE) proportion of aborted visits by treatment lane (Exp. 1). Hellbenders were significantly less likely to abort visits (i.e. exit lane before reaching the upstream end) to lanes conditioned with fish analog compared to control and parsley-conditioned lanes, which were aborted with similar frequency. Different letters above error bars represent significantly different means according to post-hoc analysis (Tukey's HSD: $P < 0.05$).

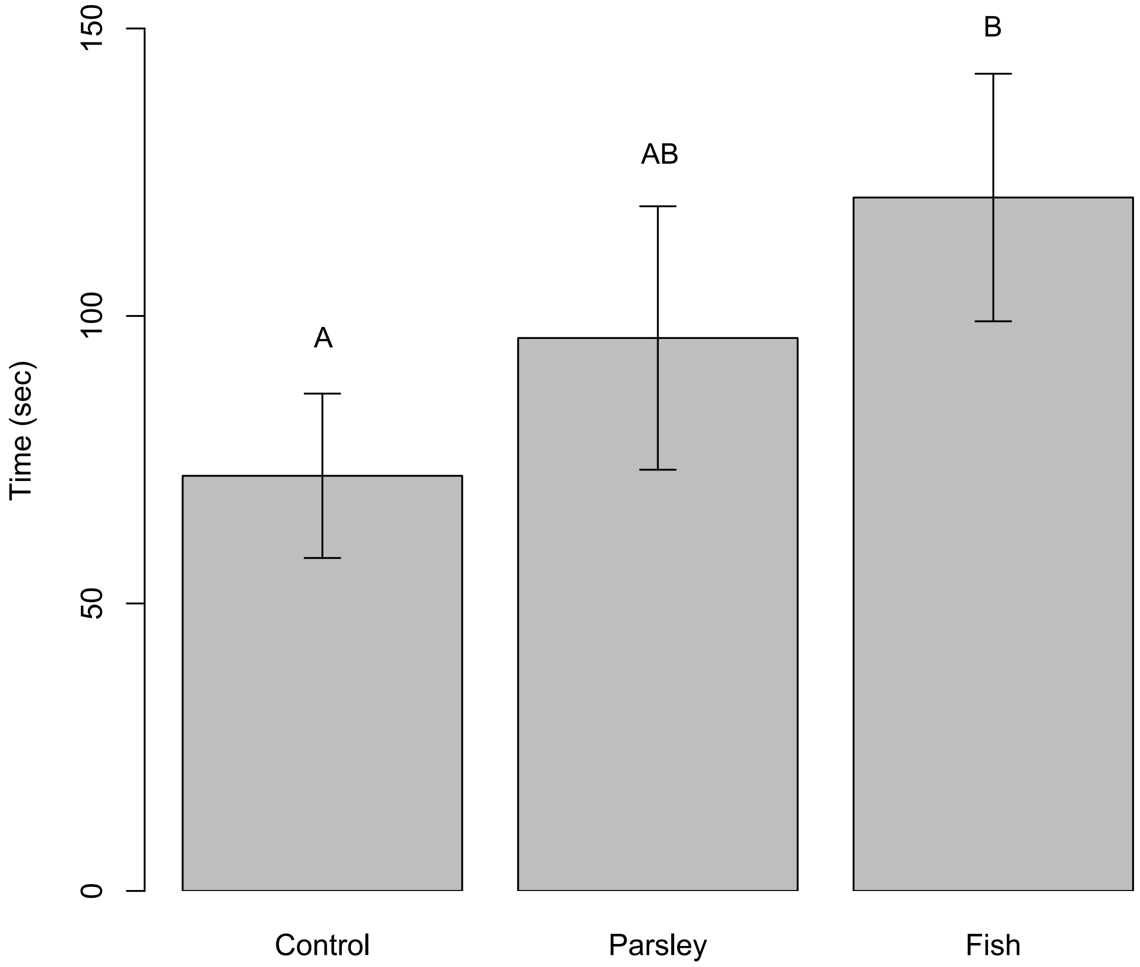

Figure 4: Mean (±1 SE) time spent in lanes by treatment (Exp. 1). Hellbenders spent significantly more time in lanes conditioned with fish analog compared to control lanes. Response to parsley-conditioned lanes was intermediate and did not differ significantly from that of control or fish analog-conditioned lanes. Different letters above error bars represent significantly different means according to post-hoc analysis (Tukey's HSD: $P < 0.05$).

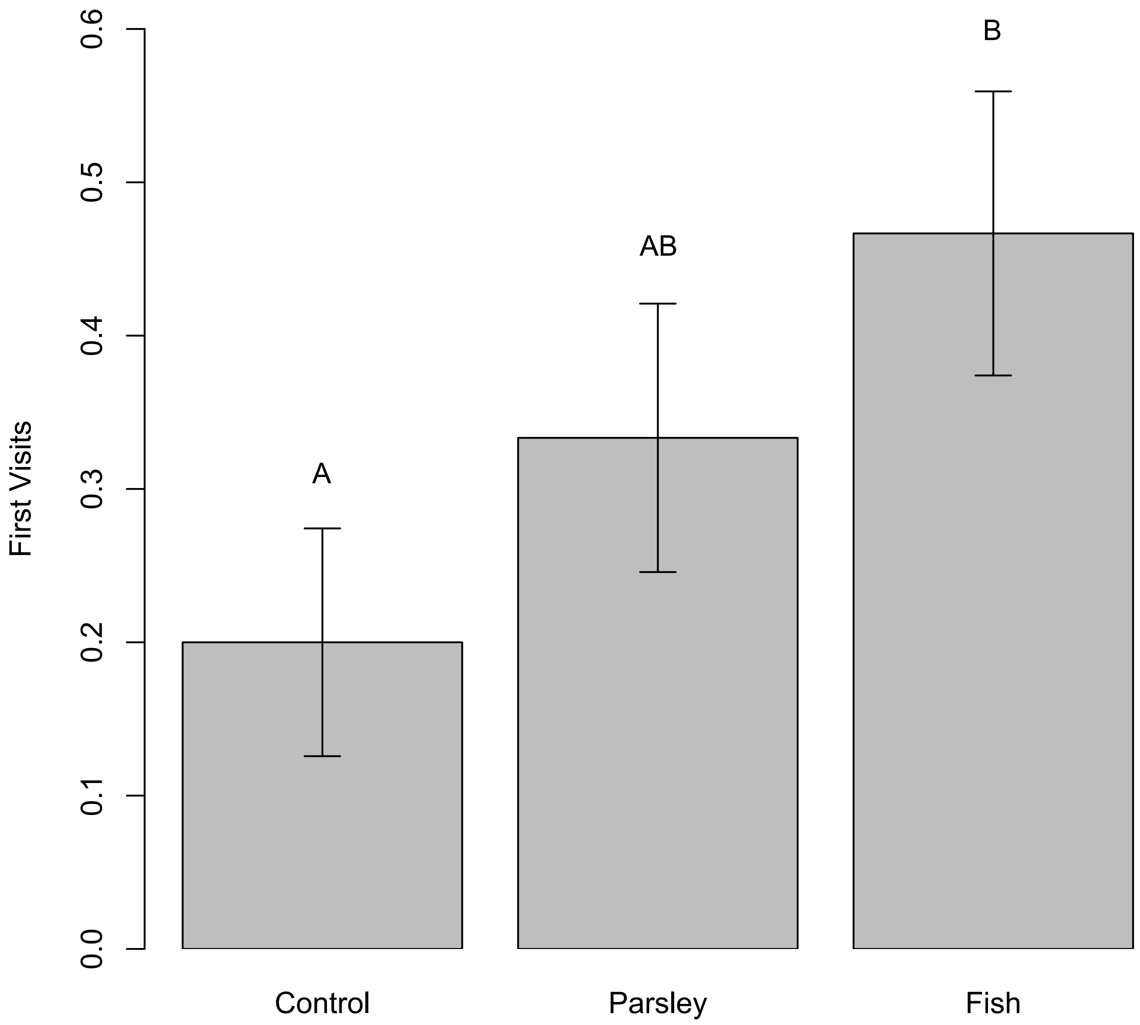

Figure 5: Mean (±1 SE) proportion of first visits by treatment lane (Exp. 1). Hellbenders were significantly more likely to first visit lanes conditioned with fish analog compared to control lanes. Response to parsley-conditioned lanes was intermediate and did not differ significantly from that of control or fish analog-conditioned lanes. Different letters above error bars represent significantly different means according to post-hoc analysis (Tukey's HSD: *P* < 0.05).

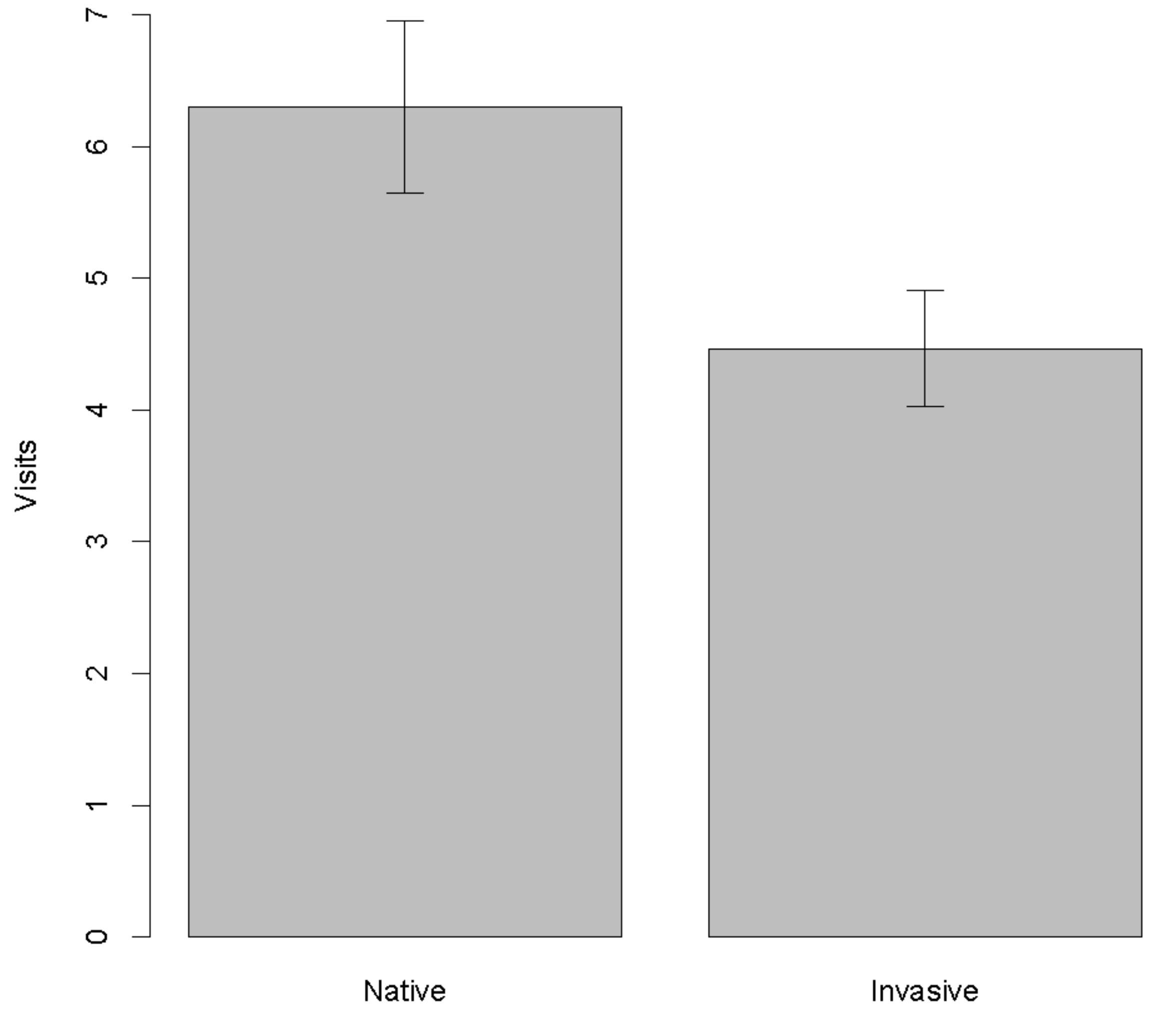

Figure 6: Mean (±1 SE) number of lane visits by treatment (Exp. 2). Hellbenders made significantly more visits to lanes conditioned with native crayfish (*Orconectes obscurus*) compared to those conditioned with invasive crayfish (*O. rusticus*) (*P* = 0.007).

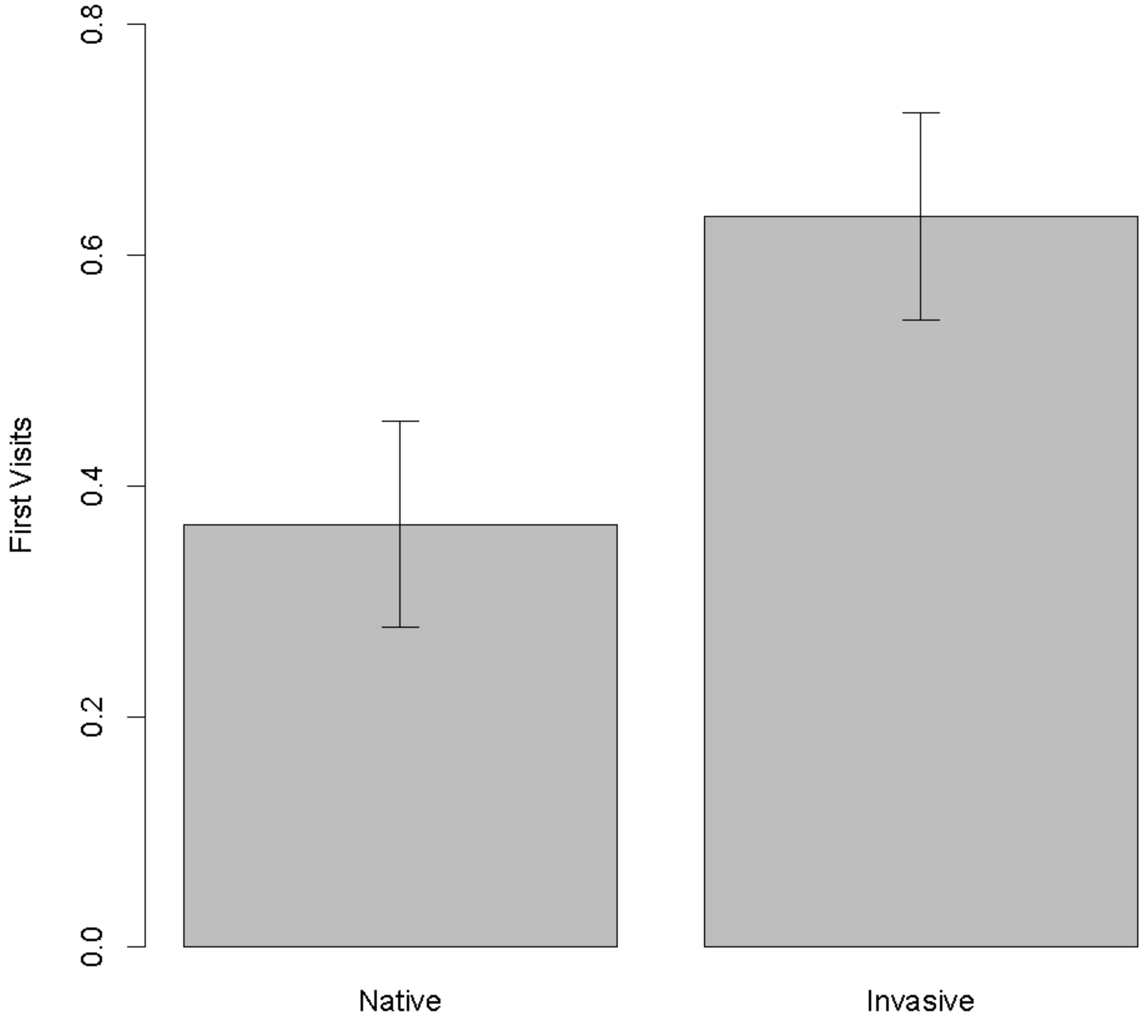

Figure 7: Mean (±1 SE) proportion of first lane visits by treatment (Exp. 2). Hellbenders were significantly more likely to first visit lanes conditioned with invasive crayfish (*Orconectes rusticus*) compared to lanes conditioned with native crayfish (*O. obscurus*) (*P* = 0.050).

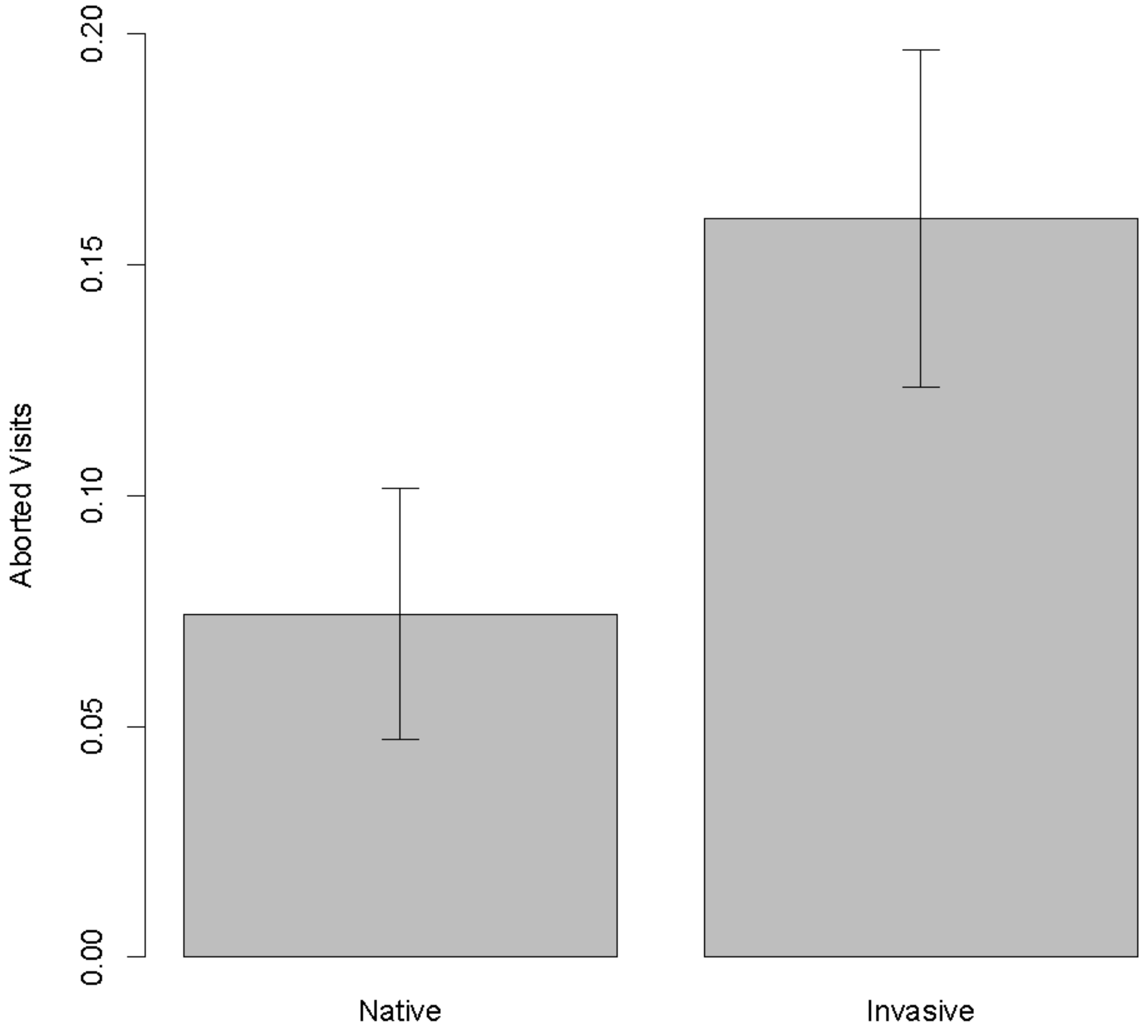

Figure 8: Mean (±1 SE) proportion of aborted lane visits by treatment (Exp. 2). Hellbenders were significantly less likely to abort visits to lanes conditioned with native crayfish (*Orconectes obscurus*) compared to lanes conditioned with invasive crayfish (*O. rusticus*) (P = 0.013).

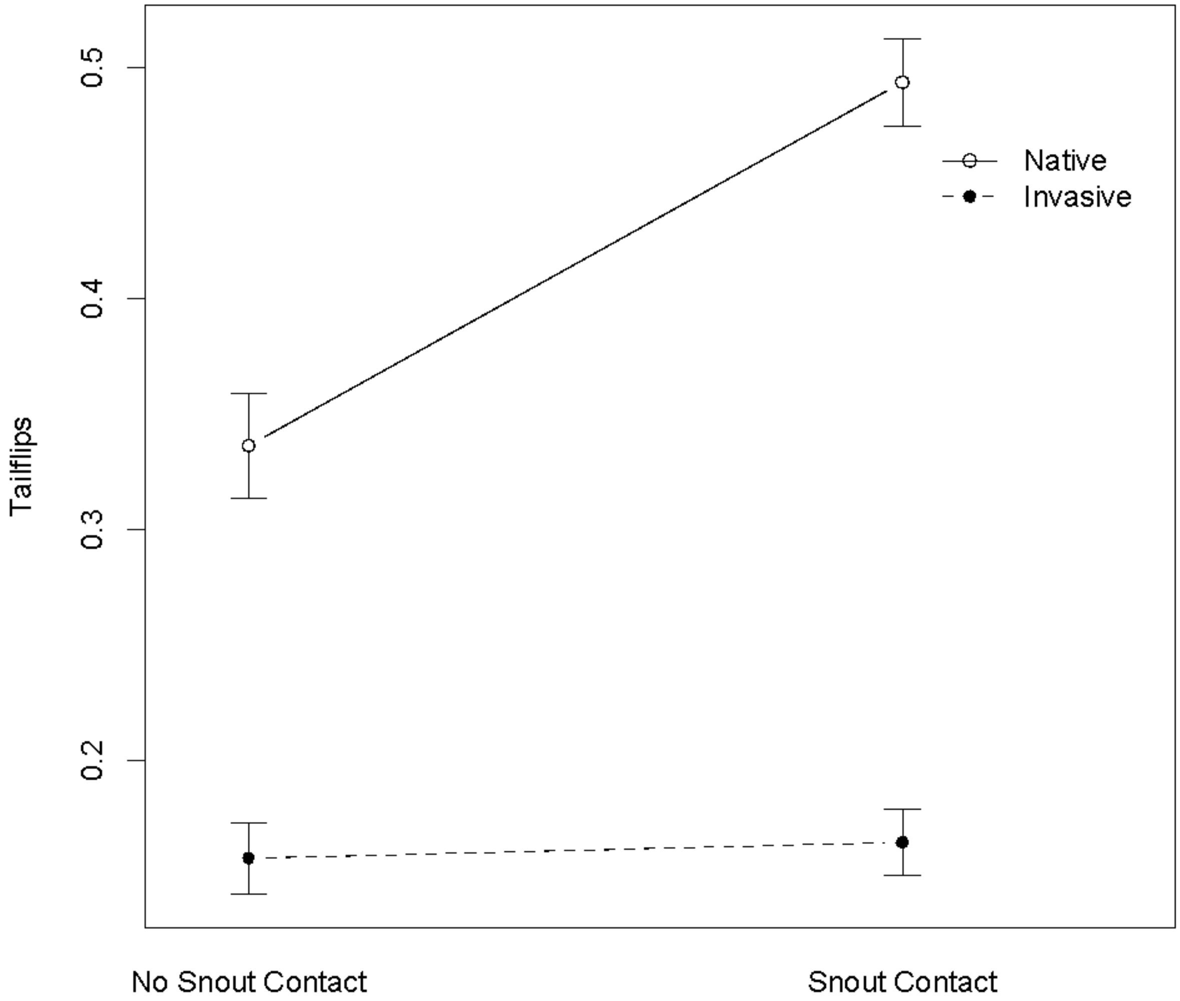

Figure 9: Interaction plot displaying mean (±1 SE) number of crayfish tailflips by species, as a function of hellbender snout contact (Exp. 3). Native crayfish significantly increased tailflips in response to snout contact by hellbenders, whereas invasive crayfish tailflips remained equally low regardless of hellbender snout contact (*P* < 0.001).

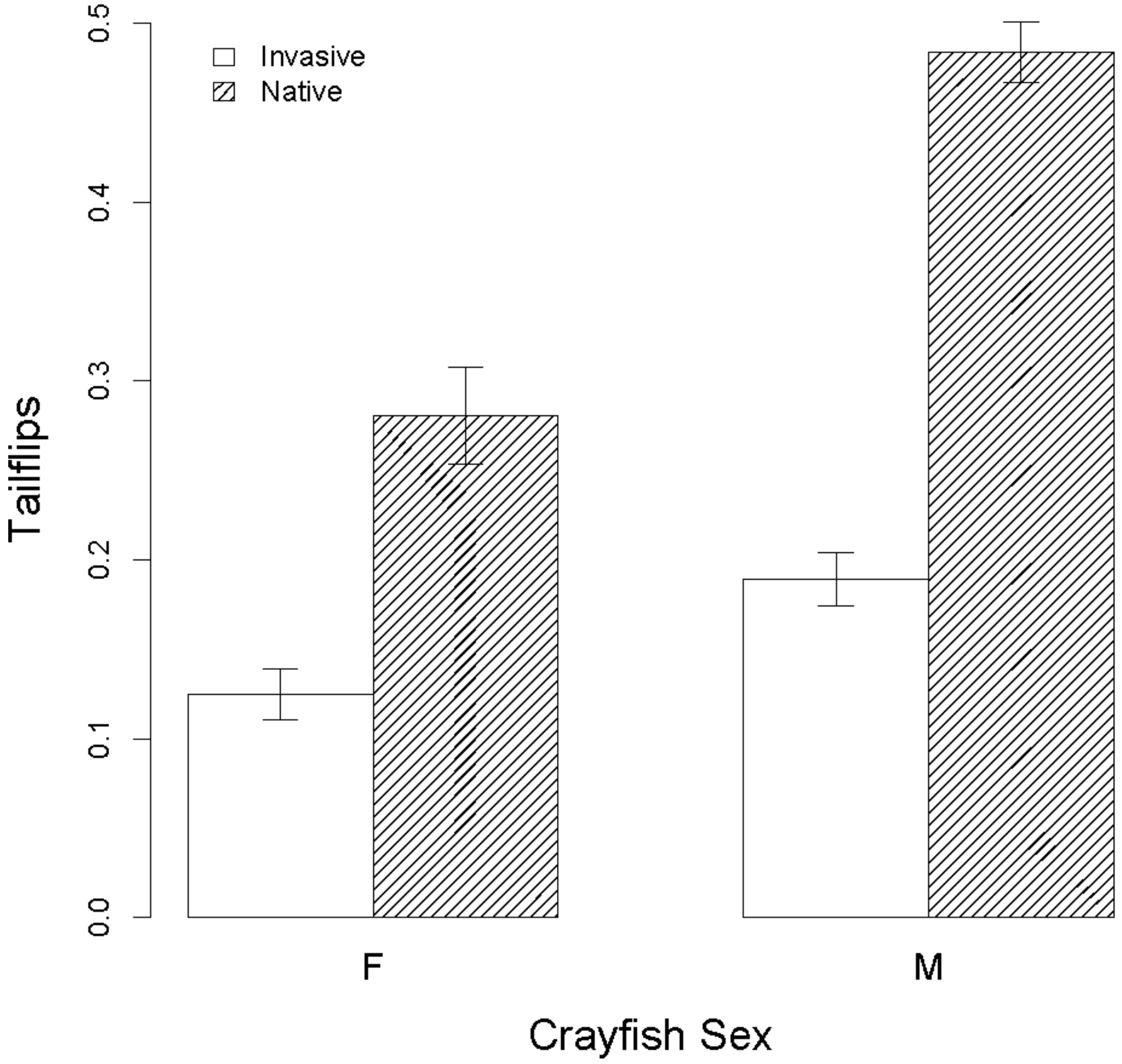

Figure 10: Mean (±1 SE) number of crayfish tailflips by sex and species (Exp. 3). Male crayfish were significantly more likely to tailflip compared to female crayfish ($P < 0.001$).

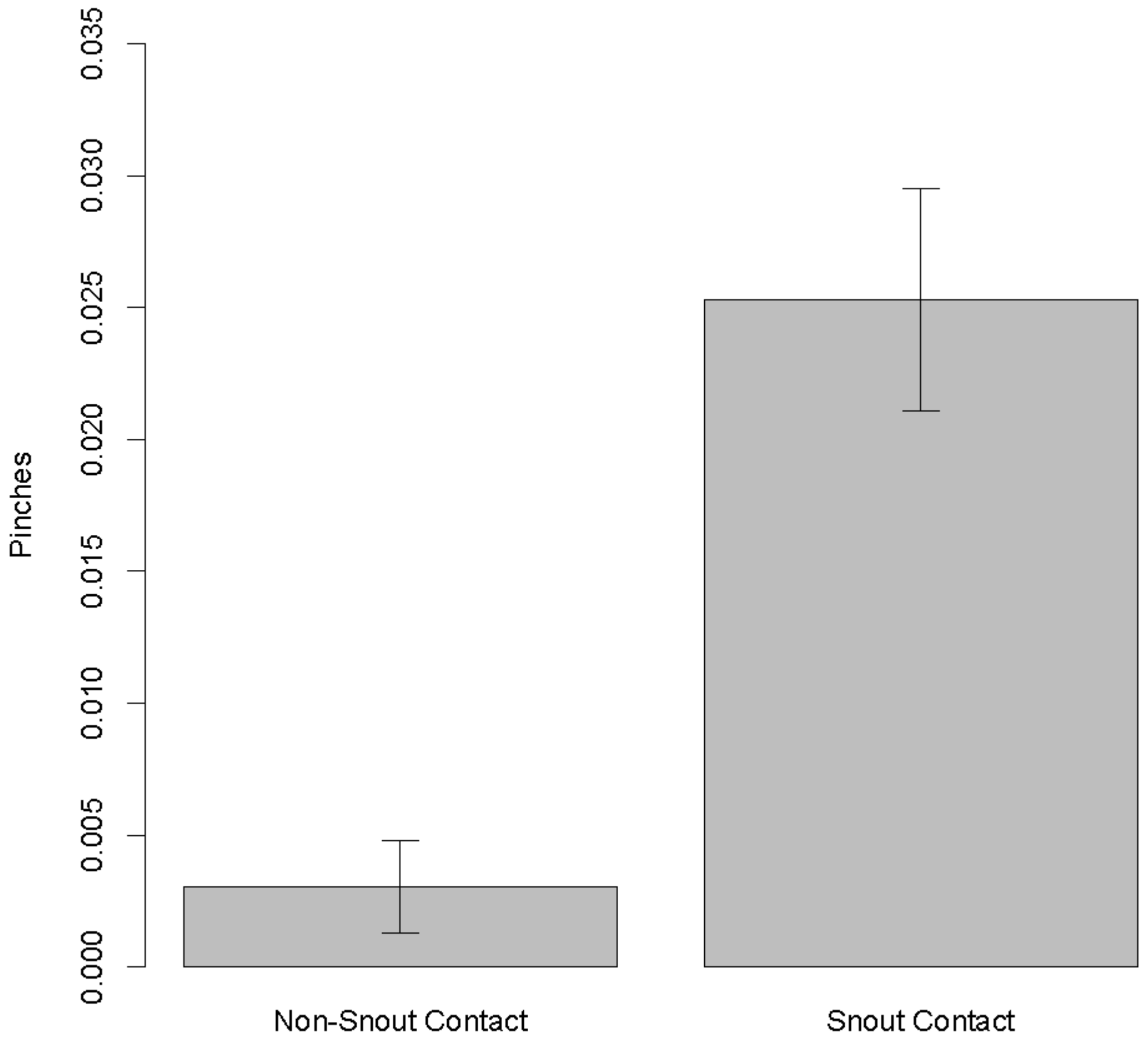

Figure 11: Mean (±1 SE) number of crayfish pinches to hellbenders as a function of contact (Exp. 3). Crayfish were significantly more likely to pinch hellbenders in response to snout contact compared to non-snout contact (*P* = 0.001).

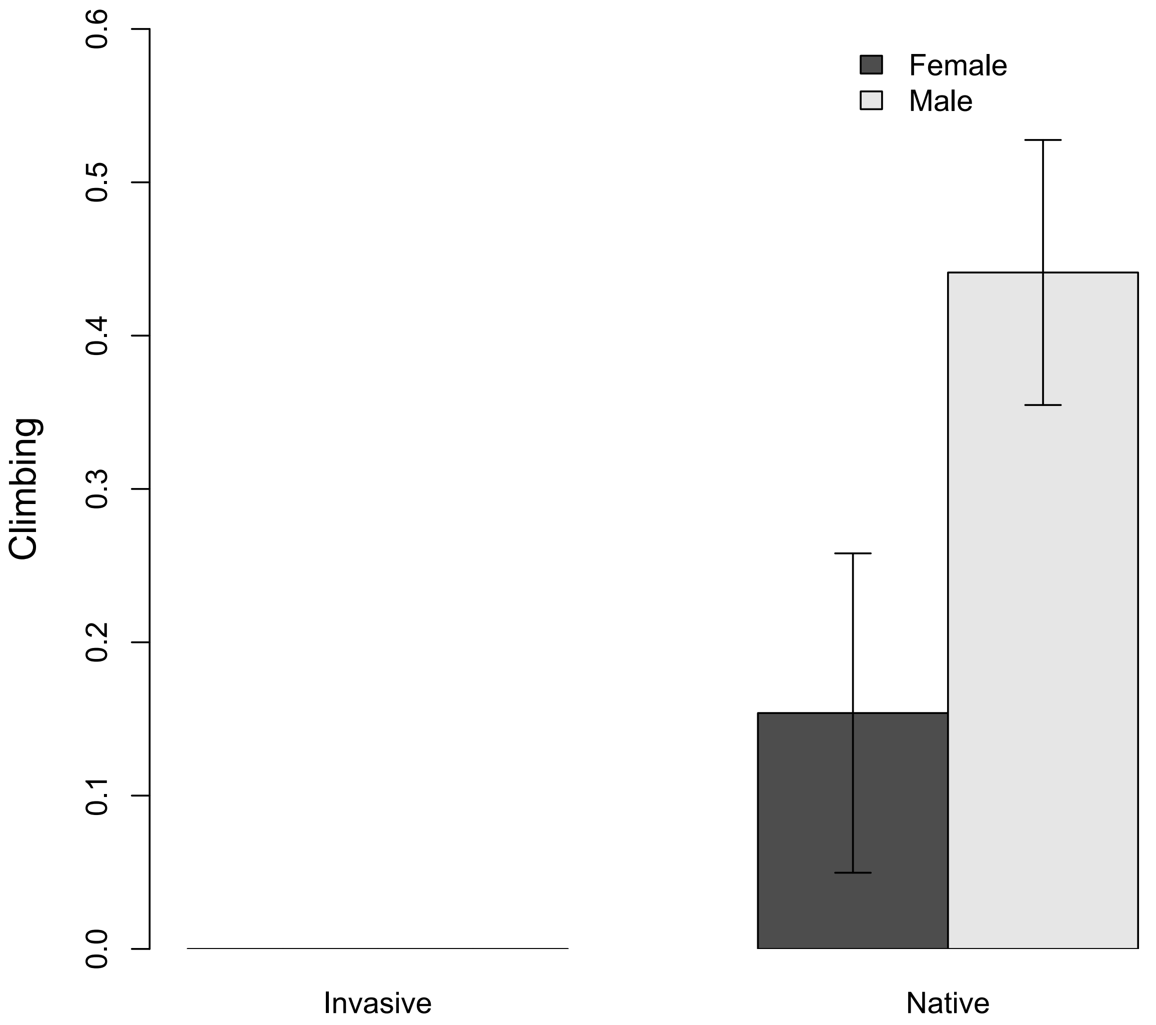

Figure 12: Mean (±1 SE) proportion of trials in which crayfish climbed, as a function of crayfish species and sex (Exp. 3). Native crayfish (*Orconectes obscurus*) were significantly more likely to climb compared to invasive crayfish (*O. rusticus*), which were never observed climbing ($P < 0.001$). Among native crayfish, males were significantly more likely to climb than females ($P < 0.055$).

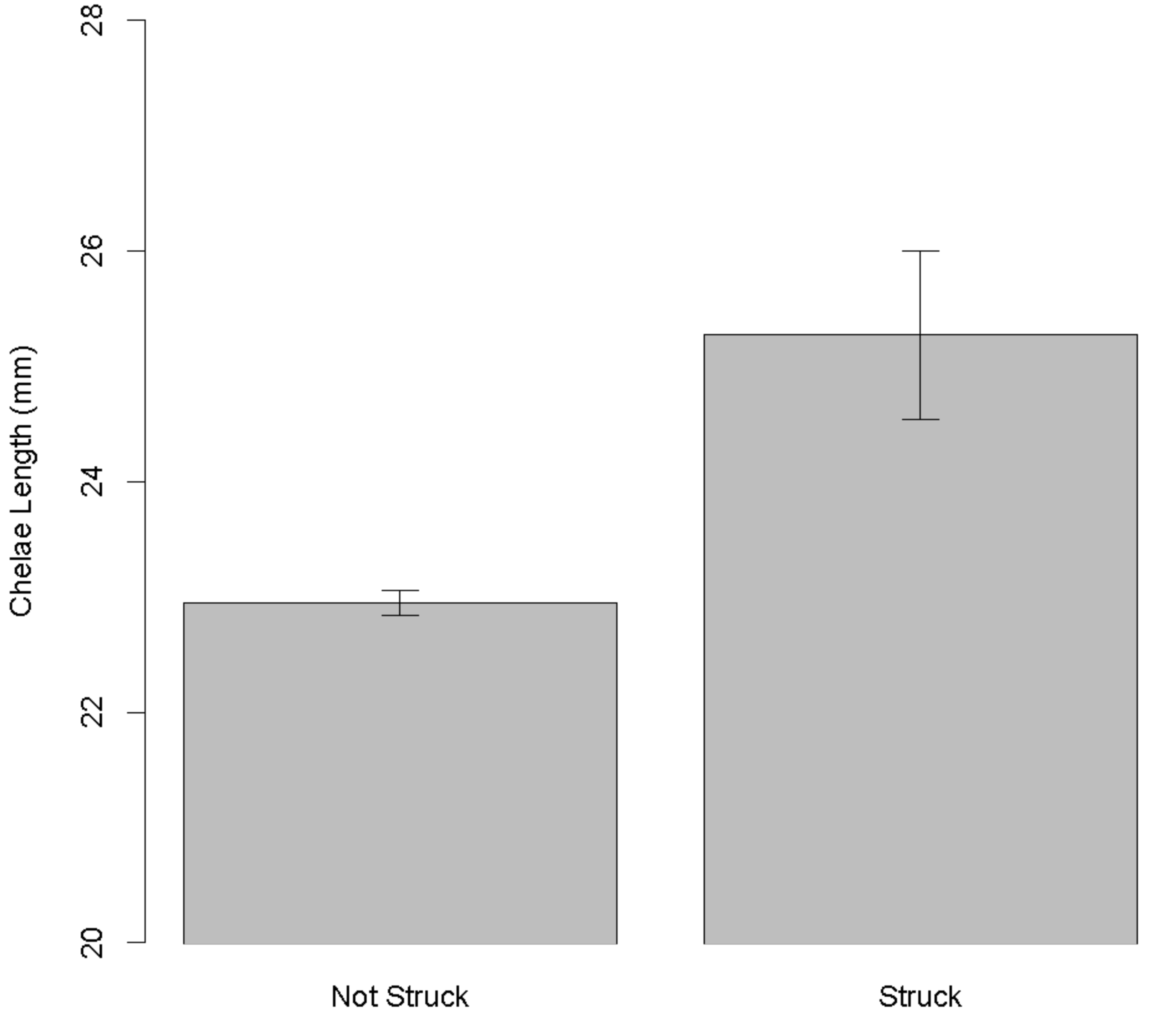

Figure 13: Mean (±1 SE) chelae length of crayfish as a function of strikes by hellbenders (Exp. 3). Chelae length was significantly greater among crayfish struck by hellbenders compared to those that were not struck (*P* = 0.043).

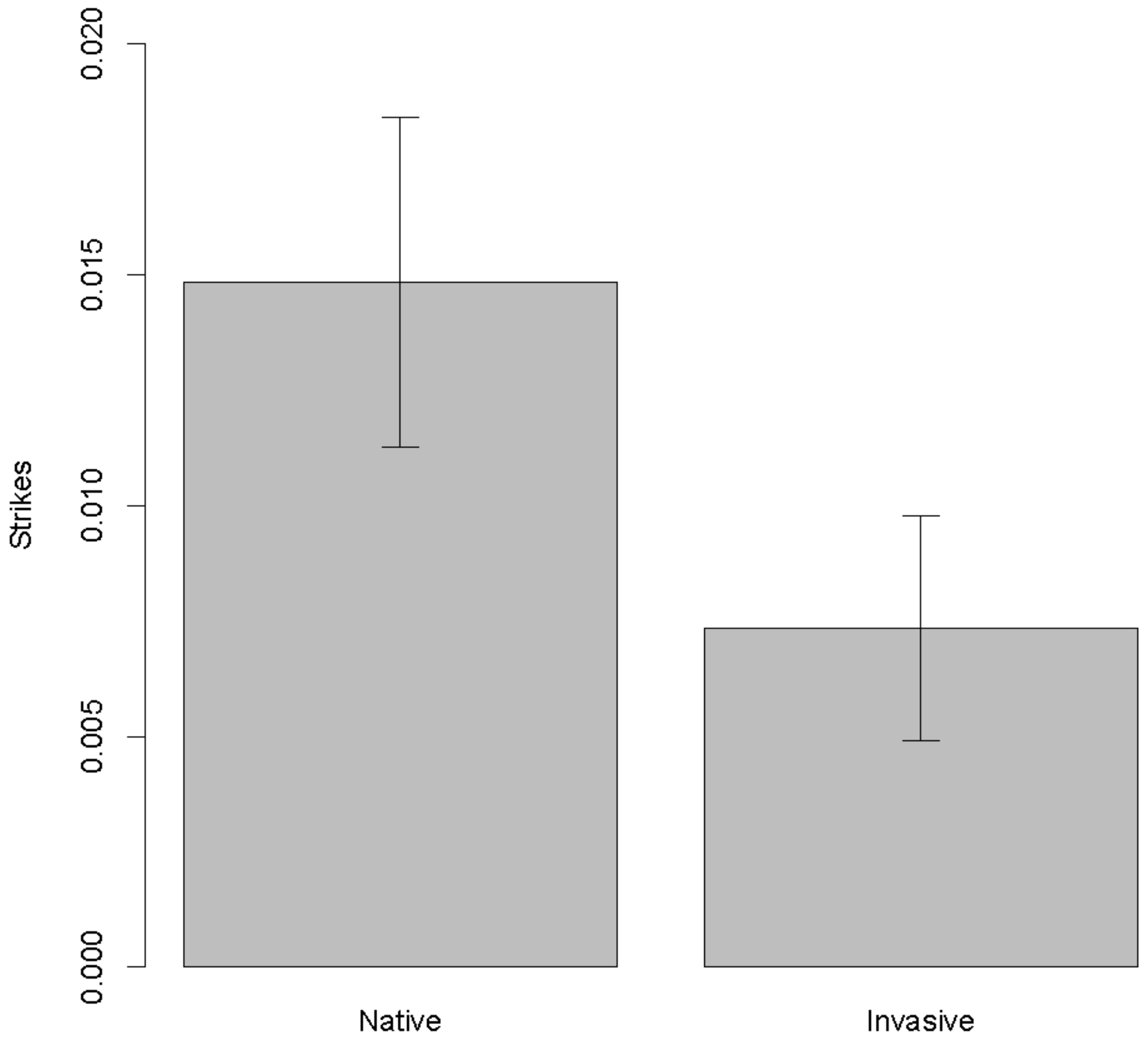

Figure 14: Mean (±1 SE) proportion of strikes by hellbenders to crayfish, as a function of crayfish species (Exp. 3). Hellbenders made significantly more strikes to native crayfish (*Orconectes obscurus*) compared to invasive crayfish (*O. rusticus*) (*P* = 0.074).

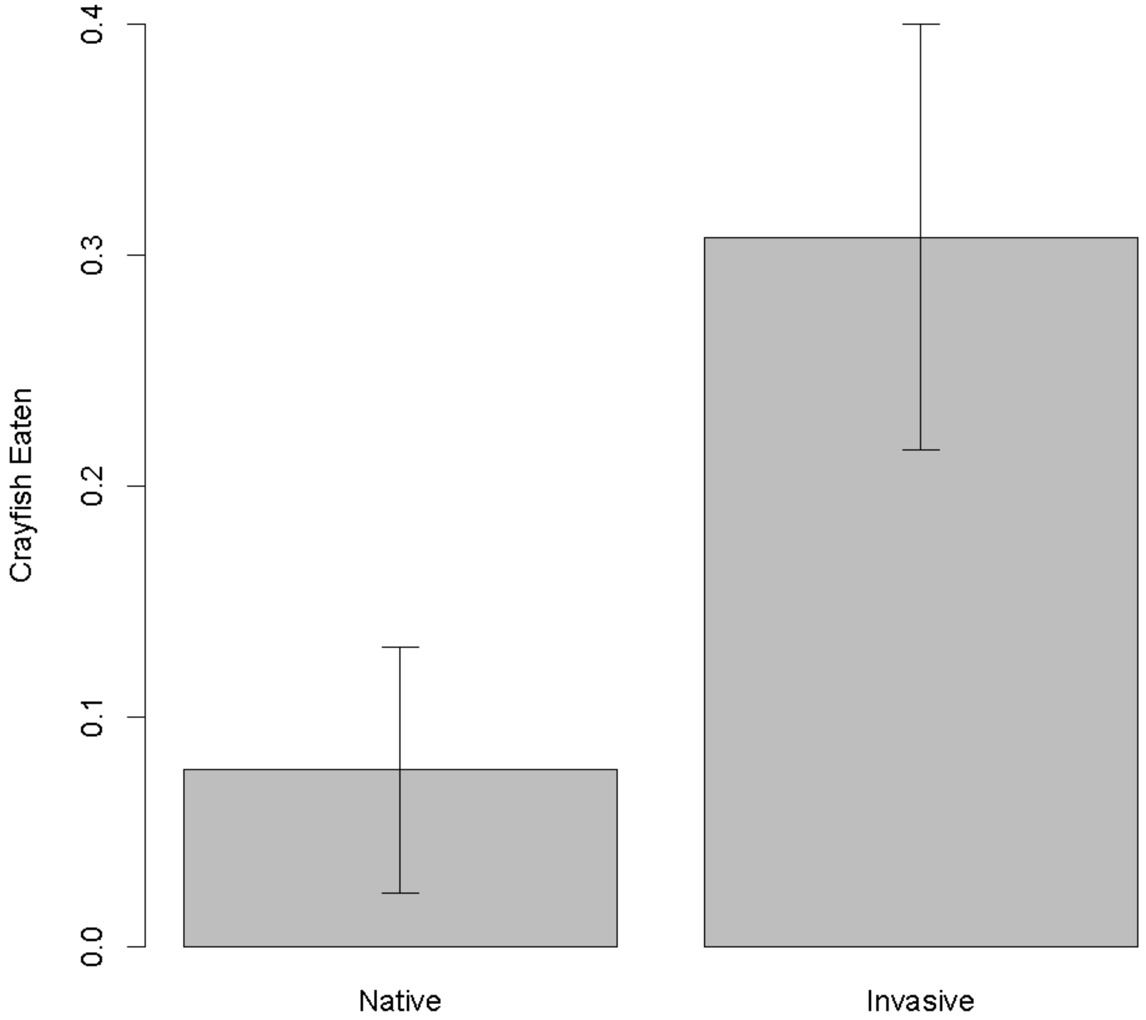

Figure 15: Mean (±1 SE) proportion of crayfish eaten by hellbenders as a function of crayfish species (Exp. 4). Hellbenders ate significantly more invasive crayfish (*Orconectes rusticus*) compared to native crayfish (*O. obscurus*) (*P* = 0.030).

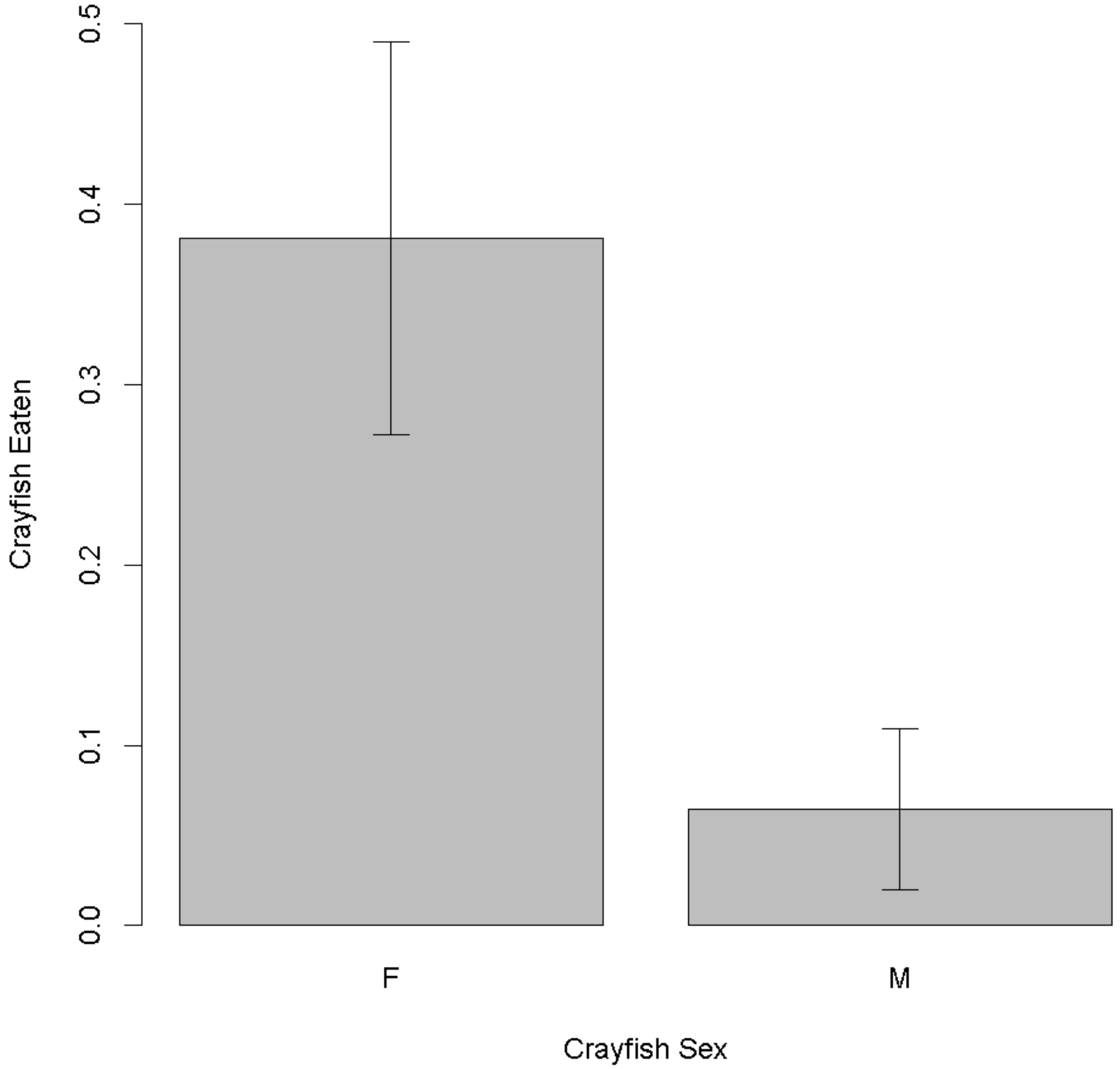

Figure 16: Mean (±1 SE) proportion of crayfish eaten by hellbenders as a function of crayfish sex (Exp. 4). Hellbenders ate significantly more female crayfish compared to male crayfish (*P* = 0.006).

Figure 17: An adult hellbender with two regurgitated *Orconectes rusticus*. This individual was captured in the Susquehanna Drainage of NYS on June 7, 2011. (Image courtesy of Peter Petokas).

Appendix

Table A1: Results of overnight feeding trials (Exp. 4). "HB" = hellbender number (n = 26). "CF" = crayfish species; "O" = native (*Orconectes obscurus*), "R" = invasive (*O. rusticus*). For crayfish with chelae of different lengths, mean chelae length was recorded. "X" indicates crayfish that were consumed. "N" = crayfish that were not exposed to hellbenders in previous trials. "HB Exp." = hellbender experience level (i.e. number of previous trials ["0", "1", or "2"] in which a hellbender was exposed to a live crayfish of a particular species). A total of 21 female and 31 male crayfish were used during overnight feeding trials. Crayfish sexes were evenly represented among *O. rusticus* (13 male, 13 female), but not *O. obscurus* (18 male, 8 female).

HB	HB Length (mm)	CF	Chelae Length (mm)	Car. Length (mm)	Sex	Eaten	Naïve to HB	HB Exp.
1 (186771)	360	O	25	29	M	X		2
		R	20	29	F	X		1
2 (184938)	420	O	30	30	M			1
		R	25	33	F	X		2
3 (205087)	430	O	22.5	30	F			2
		R	22.5	29	F	X		1
4 (187215)	390	O	18	27	F			1
		R	21	29	F	X		2
5 (185401)	430	O	22	32	F	X		0
		R	24	30	F	X		0
6 (187387)	290	O	16	25	F			0
		R	20	26	M			0
7 (185162)	360	O	21	27	M			1
		R	29.5	28	M			2
8 (186139)	370	O	25	27	M			2
		R	23.5	27	M			1
9 (188160)	370	O	17	27	F			2
		R	17.5	28	M			1
10	360	O	18	27	F			1

(187528)		R	21	27	M			2
11 (187852)	380	O	23	28	M			0
		R	21.5	30	F			0
12 (184601)	330	O	17	25	M			0
		R	19	26	M			0
13 (184735)	390	O	27	30	M			2
		R	29	29	M			1
14 (204164)	400	O	27.5	30	M			1
		R	26	30	F			2
15 (203780)	380	O	17.5	28	F			1
		R	20.5	29	F			2
16 (203193)	390	O	21.5	31	F			2
		R	31	30	M			1
17 (203257)	390	O	26	29	M			2
		R	31	31	M			1
18 (185679)	350	O	21.5	27	M			0
		R	21	27	M			0
19 (184914)	400	O	25.5	29	M			0
		R	22	31	F			0
20 (204538)	430	O	33	33	M		N	1
		R	23	32	F	X	N	2
21 (202727)	450	O	29	32	M			1
		R	28	35	F		N	2
22 (186914)	430	O	31	31	M		N	2
		R	31	32	M		N	1
23 (185452)	430	O	31	32	M			1
		R	25	31	F			2

24 (204139)	430	O	33	34	M			1
		R	25.5	33	F	X	N	2
25 (185175)	430	O	31	32	M		N	0
		R	31.5	31	M			0
26 (187098)	430	O	30.5	32	M			0
		R	35	33	M	X	N	0