

INVASIVE RUSTY CRAYFISH (*FAXONIUS RUSTICUS*) CAN SERVE AS PREY OF EASTERN HELLBENDERS (*CRYPTOBRANCHUS ALLEGANIENSIS ALLEGANIENSIS*)

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Abstract.—Invasive Rusty Crayfish (*Faxonius rusticus*) are large and aggressive, allowing them to outcompete native competitors and avoid predation by fish. Invasive Rusty Crayfish have been hypothesized to negatively impact Eastern Hellbenders (*Cryptobranchus a. alleganiensis*), a crayfish dietary specialist, through dietary exclusion, as well as habitat displacement from shelter rocks. We quantified hellbender and crayfish behavior in simulated streams to investigate if invasive Rusty Crayfish are consumed by hellbenders, displace hellbenders from shelter rocks, affect hellbender activity, or respond differently than native crayfish to hellbenders. Hellbenders consumed both Rusty and native crayfish. No evidence of displacement of hellbenders from shelter rocks by crayfish was observed. Hellbender total exposure time (TET), a proxy for activity, was not affected by crayfish species or density. There was no difference between native and Rusty Crayfish in frequency of defensive or retreat responses towards hellbenders. These results suggest invasive Rusty Crayfish can serve as prey for eastern Hellbenders and do not displace hellbenders from shelter rocks.

Key Words.—competition; Giant Salamander; invasive species; Pennsylvania; predation; Susquehanna River Drainage

INTRODUCTION

Invasive species have been identified as a primary threat to imperiled species in freshwater ecosystems (Dextrase and Mandrak 2006). Invasive crayfish can impact a wide variety of organisms (e.g., algae, macrophytes, macroinvertebrates, fish, amphibians) because crayfish play key ecological roles in freshwater ecosystems (Holdich 1988; Lodge et al. 2000; Twardochleb et al. 2013). Rusty Crayfish (*Faxonius* [=*Orconectes*] *rusticus*), a species native to the lower Ohio River drainage within Ohio, Indiana, and Kentucky of the USA, has been introduced and become a prolific invader in many areas (Momot 1992; Bouchard et al. 2007; Taylor et al. 2007; Olden et al. 2009; Lieb et al. 2011). Invasive Rusty Crayfish have been associated with declines and loss of native species including macrophytes, macroinvertebrates (including other crayfish), and fish (Capelli 1982; Taylor and Redmer 1996; Wilson et al. 2004; McCarthy et al. 2006; Olden et al. 2006). Rusty Crayfish are thought to be less susceptible than native prey to predation as a result of their large body size, aggressive nature, and large chelae

(Olsen et al. 1991; Mather and Stein 1993; Garvey et al. 1994; Hill and Lodge 1999; Roth and Kitchell 2005). This potential preclusion of Rusty Crayfish as prey is of particular concern for imperiled predators with diets primarily comprised of crayfish, such as Hellbenders (*Cryptobranchus alleganiensis*).

Hellbenders are large (≤ 74 cm total length), nocturnal, aquatic salamanders that typically inhabit cool, high quality streams within the eastern United States (Nickerson and Mays 1973; Petranka 1998). Crayfish comprise the greatest proportion of the prey of adult hellbenders (Nickerson and Mays 1973; Peterson et al. 1989; Petranka 1998; Philips and Humphries 2005). Hellbender population declines and extirpations have been reported throughout their range (Williams et al. 1981; Briggler et al. 2007; Pitt et al. 2017). It has been hypothesized that invasive Rusty Crayfish are a factor in the decline of certain hellbender populations due to their aggressive nature precluding them as suitable prey and allowing them to displace hellbenders from cover rocks, coupled with the decline they cause in native crayfish prey (Briggler et al. 2007; Quinn et al. 2013). Yet, the native ranges of the Eastern Hellbender subspecies (*C. a.*

alleganiensis) and Rusty Crayfish overlap in portions of the lower Ohio River drainage in Indiana, Kentucky, and Ohio, indicating a coevolutionary history (Nickerson and Mays 1973; Taylor et al. 2007). In a stream in Indiana where both Rusty Crayfish and hellbenders are native and sympatric, the crayfish exhibit predator-avoidance behavior when exposed to hellbender kairomones (Kenison et al. 2018); these results suggest these species have a predator-prey relationship in their native range. Invasive Rusty Crayfish, however, may grow larger and behave differently than Rusty Crayfish in their native range, and thus may alter interspecific interactions (Pintor and Sih 2009). Cava et al. (2018) found that captive-raised Eastern Hellbenders responded to chemical cues of invasive Rusty Crayfish and consumed them, but data are lacking as to what relationships occur between wild (as opposed to captive-raised) hellbenders and invasive Rusty Crayfish, particularly from the Susquehanna River drainage where invasive Rusty Crayfish have been hypothesized as a factor involved in hellbender decline (Quinn et al. 2013; Michelle Herman, unpubl. report).

Populations of Eastern Hellbenders have declined and become extirpated in portions of the Susquehanna River drainage in the USA (Gates et al. 1985; Quinn et al. 2013; Pitt et al. 2017). Conservation designations for this species within native range states encompassing the Susquehanna River drainage include Endangered in Maryland (Maryland Department of Natural Resources 2016) and a Species of Greatest Conservation Need in both Pennsylvania and New York (New York Department of Environmental Conservation 2015; Pennsylvania Game Commission and Pennsylvania Fish and Boat Commission 2015). Invasive Rusty Crayfish have expanded their range within this drainage, been implicated in the decline and extirpation of native crayfish, and typically become the dominant crayfish species within invaded streams (Bouchard et al. 2007; Kuhlman and Hazelton 2007; Kilian et al. 2010; Lieb et al. 2011). Rusty Crayfish are thus likely to become the predominant crayfish available as potential prey to Eastern Hellbenders in the Susquehanna River drainage. Additionally, because Rusty Crayfish and hellbenders both use large rocks as cover (Prins 1968; Nickerson and Mays 1973; Petranka 1998), Rusty Crayfish, especially at high densities, may also compete with and displace hellbenders from cover rocks.

Our goal was to evaluate several of the potential implications of Rusty Crayfish invasion on adult Eastern Hellbenders. We did this by conducting an *ex situ* study examining behavior of hellbenders, native crayfish, and Rusty Crayfish collected from Susquehanna River tributaries and housed in raceways set up to mimic a typical hellbender stream. We examined behavior of hellbenders and crayfish to determine if: (1) Rusty

Crayfish were unsuitable prey for larger (subadult to adult) hellbenders due to their larger size and aggressive nature compared to native crayfish; (2) Rusty Crayfish displace hellbenders from cover rocks; and (3) Rusty Crayfish affect hellbender activity.

MATERIALS AND METHODS

Housing, animals, and adjustment to captivity.

We set up two 8.4×0.8 m indoor raceways lined with natural cobble substrate. We placed four 0.6×0.6 m ceramic tiles per raceway on top of substrate to serve as cover objects for hellbenders. We fitted the internal side of raceway walls with aluminum flashing to prevent crayfish from escaping. We filled raceways to approximately 0.6 m depth with reverse-osmosis water reconstituted to match water quality parameters (e.g., conductivity, pH, dissolved oxygen) of the source-stream from which hellbenders were subsequently collected. A pumping system simulated stream flow. We maintained water temperature by ambient conditions (18° – 20° C) encompassing a similar temperature range to the source stream of hellbenders. Raceways were subject to natural light cycles via large windows. We monitored water quality and maintained it via biweekly 30% water changes, as per Ettling et al. (2013).

We collected four Eastern Hellbenders (29.7–45.0 cm total length [TL]) in May 2016 from a tributary of the Susquehanna River in eastern Pennsylvania (exact locality not reported due to conservation concerns) and placed two hellbenders in each raceway. Based on size, the hellbenders used in our study likely consisted of one subadult individual (i.e., not yet sexually mature based on size at maturity estimates for Pennsylvania specimens in Hulse et al. 2001) and three adults. Our sample size was limited due to the imperiled status of hellbenders, a common limitation in conservation-driven studies of hellbenders and other imperiled species (Davies and Gray 2015; Garamszegi 2016; Settle et al. 2018). When introduced into captivity, hellbenders typically undergo an adjustment period during which individuals minimize feeding and body mass reduces temporarily before rebounding (i.e., acclimation period; Ettling et al. 2013). Prior to the initiation of crayfish trials, we allowed hellbenders to adjust to captivity and monitored their mass as an index of adjustment. Hellbenders ate *ad libitum* from a selection of prey organisms (i.e., declawed native crayfish, fish, earthworms) that were subjected to species-specific decontamination protocols (e.g., saline treatments) prior to deposition into raceways per the protocol of Ettling et al. (2013). After 10 weeks, hellbenders returned to their capture mass, suggesting adjustment had occurred.

We collected invasive Rusty Crayfish and native Spiny-cheek Crayfish (*Faxonius limosus*) and native

TABLE 1. Hellbender-crayfish behavioral trial dates and crayfish treatments. Crayfish included native crayfish and invasive Rusty Crayfish (*Faxonius rusticus*).

Trial	Dates	Raceway 1 Treatment (crayfish/m ²)	Raceway 2 Treatment (crayfish/m ²)
1	30 July to 3 August	Low Density Rusty Crayfish (2.61/m ²)	Native Crayfish (2.61/m ²)
2	10–14 August	Native Crayfish (2.61/m ²)	Low Density Rusty Crayfish (2.61/m ²)
3	18–22 August	High Density Rusty Crayfish (4.6/m ²)	High Density Rusty Crayfish (4.6/m ²)

Common Crayfish (*Cambarus bartonii bartonii*) from middle-order tributaries of the Susquehanna River in July and August 2016. We subjected crayfish to 50 ppt saline treatments for 5 min to remove pathogens, then rinsed with clean water for 5 min following the protocol of Ettling et al. (2013). Crayfish were maintained in single-species colonies in aquaria with oxygenated, reconstituted reverse osmosis water (maintained through daily water changes), locked mesh lids (to prevent escapes), cobble substrate, and cover rocks for 3–7 d prior to trials. We fed crayfish washed vegetables *ad libitum*.

Study trials.—We conducted three trials during which varying densities of native or Rusty Crayfish were placed into raceways with hellbenders (Table 1). Trials, each lasting five nights, were conducted between 30 July and 22 August 2016 following the adjustment of hellbenders to captivity. Treatments consisted of low density native crayfish (control), low density Rusty Crayfish, and high density Rusty Crayfish (Table 1). We selected a density of 2.62 crayfish/m² for low density and 4.6 crayfish/m² for high density based on our field observations and an approximate median of published data (e.g., Patrick 1996; Taylor and Redmer 1996; Kuhlman and Hazelton 2007; Kilian and Cicatto 2014). Because native crayfish occur in much lower densities than invasive Rusty Crayfish (Lieb et al. 2011), we did not conduct trials with high densities of native crayfish. The native crayfish treatment in trial 1 consisted entirely of Spiny-cheek Crayfish; however, during trial 2 we used both native Spiny-cheek Crayfish (n = 7) and native Common Crayfish (n = 11) due to limited availability of Spiny-cheek Crayfish. Each crayfish treatment was composed of a variety of size classes (range 1.1–18.6 g mass) in a distribution and frequency representing those in the collection stream. An *a priori* test revealed no significant difference in mean mass of crayfish among treatments ($F_{2,131} = 0.808, P = 0.447$). Crayfish sex ratio was approximately 1:1. Before each trial, we blotted dry, weighed, and marked each crayfish by branding a dot pattern into the carapace with a soldering iron (Abrahamson 1965). We weighed hellbenders before and after each trial.

We recorded hellbender and crayfish behaviors with GoPro Hero 4 Camcorders (GoPro Inc., San Mateo, California, USA) with waterproof housings and extended battery packs. For nocturnal observations, we deployed three camcorders, set to night mode, in

each raceway in designated locations, each covering a field of view approximating one-third of the raceway. Raceway lighting was fitted with two layers of 3 mm thickness Rosco Supergel #27: Medium Red-light filter gel (Rosco Laboratories, Stamford, Connecticut, USA), which filters out about 96% of all wavelengths besides red light. Red light causes little or no disturbance to hellbenders (Nickerson 1977) and crayfish (Musil et al. 2010) and provided sufficient lighting for nocturnal recording. Prior to the trials, we conducted nocturnal observations with red lights on and off to determine qualitatively that neither hellbender nor crayfish behaviors were altered. We recorded video nightly from approximately 2200 (time at which hellbenders typically became active) to 0300, when camera memory reached capacity and/or batteries died. At the conclusion of each trial, we collected, counted, weighed, and identified all remaining crayfish. At the end of the study, we released hellbenders into their native stream at the exact location from which they were collected.

Behavioral data collection.—We collected approximately 300 h of nocturnal footage. Video footage was analyzed in approximately 18-min increments by the same person in a quiet room with no distractions and 10% of the footage was re-watched for quality control. We quantified the total exposure time (TET) of hellbenders (i.e., time spent partially or fully outside of their cover), and the time spent engaged in walking, swimming, and immobile (no locomotion) behaviors while exposed. We quantified defense and retreat behavioral responses of crayfish to pursuit/attempted predation by hellbenders, and unprovoked aggressive behaviors of crayfish towards hellbenders. Defense behavior consisted of antagonistic actions towards hellbenders and included crayfish facing the hellbender and raising and/or contacting the hellbender with chelae. Retreat behavior consisted of crayfish backing/walking away or conducting a swimming retreat in response to a hellbender. We scanned all video for evidence of hellbender displacement from cover objects by crayfish and noted the location of hellbenders each morning.

Data analysis.—We performed all statistical analyses in R version 4.0.3 (R Core Team 2020, with α of 0.05. All analyses were performed with the stats package (R Core Team 2020) unless otherwise indicated. Preliminary analyses indicated that mass

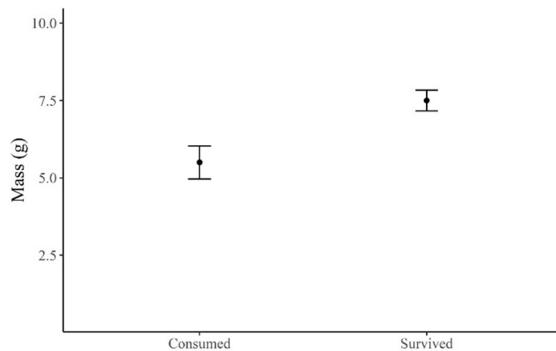


FIGURE 1. Mean mass (g) of crayfish (inclusive of all crayfish species used in trials) consumed ($n = 37$) and those that survived ($n = 101$) trials with Eastern Hellbenders (*Cryptobranchus a. alleganiensis*). Error bars represent ± 1 standard error from mean mass.

data for hellbenders and crayfish were normally distributed and had equal variances, and thus we used parametric tests to evaluate these data. We implemented independent-sample t -tests to test for differences in: (1) the mean mass of crayfish that were consumed and those that survived trials; (2) the mean mass of crayfish consumed by larger and smaller hellbenders; and (3) the mean mass of consumed Rusty and native crayfish. We compared hellbender mass before and after each trial using paired sample t -tests to determine if hellbender mass significantly changed. To test if hellbender mass change differed among crayfish treatments, we analyzed data with a Repeated Measures ANOVA using the car package (Fox and Weissburg 2011).

Preliminary analyses revealed a non-normal distribution in hellbender data for TET and time spent conducting walking, immobile, and swimming behaviors. Thus, these data were log-transformed (which made data fit a normal distribution) prior to further analysis. We used the lme4 package (Bates et al. 2015) to perform a Linear Mixed Effect Analysis of the relationship between hellbender TET and crayfish treatments. Mixed effect models include both fixed effects and random effects and can thus be used to resolve non-independencies while still allowing for the use of full data sets (Harrison et al. 2018). We included crayfish treatment as a fixed effect and intercepts for camera and trial as random effects. We found no obvious deviations from homoscedasticity or normality based on visual inspection of the residual plots. We compared

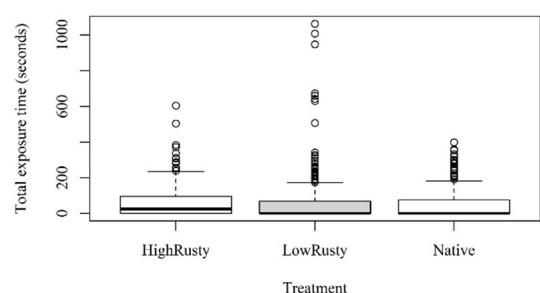


FIGURE 2. Total exposure time (seconds) of Eastern Hellbenders (*Cryptobranchus a. alleganiensis*) subjected to three crayfish treatments: low density native crayfish, low density Rusty Crayfish (*Faxonius rusticus*), and high-density Rusty Crayfish. The band inside each box represents the median. The bottom and top of each box represent the first and third quartiles. The ends of the whiskers are the minimum and maximum excluding outliers. Open circles are major outliers.

the full model to a null model using a Likelihood Ratio Test. We used Chi-squared tests of independence with Yates' Correction to determine if the proportion of time spent swimming, walking, and immobile by hellbenders was dependent on crayfish treatments. We used Fisher's Exact tests to test for differences in the response (defense or retreat behavior) of native crayfish and Rusty Crayfish to hellbender approach/attempted predation.

RESULTS

Hellbenders in the native crayfish treatment consumed 14 of the 36 crayfish available in trials 1 and 2 combined. Hellbenders in the low-density Rusty Crayfish treatment ate nine of the 36 crayfish available in trials 1 and 2 combined. Hellbenders in the high-density Rusty Crayfish treatments ate 10 of the 62 crayfish available in trial 3. Mean mass (\pm standard deviation) of crayfish consumed by hellbenders (5.50 ± 2.69 g) was significantly less than surviving crayfish ($= 7.50 \pm 3.38$ g; $t = -3.920$, $df = 136$, $P < 0.001$; Fig. 1). There was no significant difference in the mean mass of native ($= 5.10 \pm 2.85$ g) or Rusty Crayfish ($= 5.70 \pm 3.20$ g) consumed by hellbenders ($t = 0.476$, $df = 35$, $P = 0.637$). There was no significant difference in hellbender mass before and after each crayfish treatment (Table 2) or among treatments ($F_{2,6} = 1.678$, $P = 0.281$).

TET of hellbenders was not significantly affected by crayfish treatment ($\chi^2 = 1.847$, $df = 2$, $P = 0.397$; Fig. 2). Proportion of time spent by hellbenders engaging in

TABLE 2. Mean mass of four Eastern Hellbenders (*Cryptobranchus a. alleganiensis*) before and after crayfish treatments and mean change in mass. No significant differences between mean pre-and post-treatment mass were observed (Paired Sample t -tests).

Crayfish Treatment	Pre (g)	Post (g)	Change (g)	t	df	P
Native Crayfish	285.1	285.2	0.1	-0.115	3	0.915
Rusty Crayfish (Low Density)	286.1	288.6	2.5	-1.333	3	0.275
Rusty Crayfish (High Density)	288.1	286.8	-1.3	1.218	3	0.310

TABLE 3. Proportion of time spent by hellbenders engaging in swimming, walking, and immobile behaviors among crayfish treatments. Behavior was dependent on crayfish treatment (Chi-square test of independence with Yates's Correction). Crayfish included native crayfish and invasive Rusty Crayfish (*Faxonius rusticus*). The abbreviations are PTSS = percentage of time spent swimming, PTSW = percentage of time spent walking, and PTSI = percentage of time spent immobile.

Crayfish Treatment	PTSS	PTSW	PTSI	Statistical results
Native Crayfish	63%	16%	21%	$\chi^2 = 4,007$, df = 2, $P < 0.001$
Rusty Crayfish (Low Density)	31%	15%	54%	
Rusty Crayfish (Low Density)	31%	15%	54%	$\chi^2 = 2,707$, df = 2, $P < 0.001$
Rusty Crayfish (High Density)	56%	17%	27%	

swimming, walking, and immobile between treatments of native crayfish and a low density of Rusty Crayfish was dependent on crayfish treatment, with hellbenders spending a significantly greater proportion of time swimming in the native crayfish treatment and immobile in the low density Rusty Crayfish treatment ($\chi^2 = 4007$, df = 2, $P < 0.001$; Table 3). The proportion of time spent by hellbenders engaging in these behaviors was also dependent on treatment for low density and high density Rusty Crayfish treatments, with hellbenders spending a significantly greater proportion of time immobile in the low density treatment and swimming in the high density treatment ($\chi^2 = 2707$, df = 2, $P < 0.001$; Table 3). The video captured 70 responses of crayfish towards hellbenders (Table 4). We found no significant differences in the proportion of defense versus retreat behaviors between native and low density Rusty Crayfish treatments (Fisher's Exact Test, $P = 1.00$), or between low and high density Rusty Crayfish treatments (Fisher's Exact Test, $P = 1.00$; Table 4). No evidence of unstimulated aggression by crayfish towards hellbenders, or evidence of displacement of hellbenders from cover by crayfish was observed in any of the video. Additionally, hellbenders exhibited site fidelity throughout the trials to the cover objects they selected (slate tiles) when brought into captivity, while both Rusty Crayfish and native crayfish typically were observed to seek refuge either within the interstitial spaces of cobble substrate or under tiles unoccupied by hellbenders.

DISCUSSION

Our study is the first to examine interspecific interactions between wild-captured Eastern Hellbenders and crayfish species found within the Susquehanna River watershed, an area in which Rusty Crayfish have been hypothesized as contributing to Eastern Hellbender decline (Quinn et al. 2013; Michelle Herman, unpubl. report). Hellbenders consumed both native and Rusty Crayfish, suggesting that Rusty Crayfish can serve as prey for Eastern Hellbenders ≥ 29.7 cm TL. Furthermore, Rusty and native crayfish did not differ in behavioral response (defense vs. retreat) to

hellbenders. Our results are consistent with those of Cava et al. (2018) who found that captive-reared Eastern Hellbenders consumed Rusty Crayfish. Our results do not support previous hypotheses (e.g., Quinn et al. 2013; Michelle Herman, unpubl. report) that Rusty Crayfish defense behavior would preclude them from hellbender diet. Additionally, the lack of significant differences in hellbender mass before and after treatments, or among crayfish treatments suggests that at least in the short-term, different species and abundance of crayfish prey, as long as prey is available, do not affect Eastern Hellbender mass due to differences in feeding or stress (Ettling et al. 2013). We acknowledge that our sample sizes were limited, however, due to our focus on an imperiled species, and while our study is important to move knowledge about this imperiled species forward (Davies and Gray 2015), larger sample sizes, while not practical for our study, may alter statistical interpretations.

Eastern Hellbenders consumed significantly smaller crayfish on average, but some larger-sized native and Rusty Crayfish were consumed (e.g., one native and three Rusty Crayfish >10 g in mass) suggesting larger individuals, regardless of species, are not excluded from hellbender diet. Rusty Crayfish may avoid predatory fish by having larger chelae than native crayfish and by assuming a raised-claw defensive posture to deter fish predators attacking from above (Garvey et al. 1994; Roth and Kitchell 2005); however, adult hellbenders typically ambush prey from a sit and wait foraging position in the benthos (Nickerson and Mays 1973; Nickerson and Krysko 2003). Therefore, raised-claw defensive postures used by Rusty Crayfish might be less effective in deterring hellbenders than predatory fish because of differences in foraging techniques. In terms

TABLE 4. Number of defensive and retreat response behaviors of crayfish towards Eastern Hellbenders (*Cryptobranchus a. alleganiensis*).

Crayfish	Defense	Retreat
Native Crayfish	3	10
Rusty Crayfish (Low-Density)	5	19
Rusty Crayfish (High-Density)	8	25

of prey recognition, genomic evidence suggests that Eastern Hellbenders colonized the Susquehanna River drainage relatively recently in geologic history, likely dispersing from populations in the Ohio River drainage (Routeman et al. 1994; Sabatino and Routeman 2009) where Eastern Hellbenders share a coevolutionary history with native populations of Rusty Crayfish (Nickerson and Mays 1973; Taylor et al. 2007; Kenison et al. 2018). Eastern Hellbenders in the Susquehanna River drainage may thus recognize Rusty Crayfish as prey due to a coevolutionary history between ancestral populations of these two species in the Ohio River drainage. Rusty Crayfish often appeared to move to a section of the raceway in which hellbenders were not active or retreated under cover when hellbenders were walking or swimming, suggesting predator avoidance.

Eastern Hellbenders displayed no alteration in TET among crayfish treatments. Eastern Hellbenders exhibited strong site fidelity throughout the study, and we observed no displacement from cover by native crayfish or Rusty Crayfish at high or low densities. These results suggest that Rusty Crayfish do not displace larger hellbenders from cover, although future studies should examine if juvenile hellbenders may be displaced from cover by Rusty Crayfish, especially where natural cover may be limited. The proportion of time spent by Eastern Hellbenders engaging in swimming, walking, and immobile behaviors was dependent on crayfish treatment among trials, with hellbenders spending a greater proportion of time swimming during native crayfish treatments and a greater proportion of time immobile with treatments consisting of a low density of Rusty Crayfish. Although the factor(s) contributing to this behavioral discrepancy are unclear, we speculate it might be due to the hellbenders in our study being naïve to Rusty Crayfish prior to the trials, as hellbenders can distinguish between the scents of native and non-native crayfish species (Cava et al. 2018) and might have altered behavior in response to this novel prey source. Hellbenders spent similar proportions of time engaging in behaviors with native crayfish treatments and a high density of Rusty Crayfish. Most likely, this is the result of a seasonal influence in our dataset. Trial 3 (i.e., high density of Rusty Crayfish in both raceways) was conducted in mid-late August, near the fall breeding season for Eastern Hellbenders during which they are most active outside of cover (Nickerson and Mays 1973; Hulse et al. 2001).

Although invasive Rusty Crayfish have been hypothesized as a factor involved in the decline of some hellbender populations (Quinn et al. 2013; Michelle Herman, unpubl. report), our results suggest that subadult/adult Eastern Hellbenders are able to consume invasive Rusty Crayfish and are not displaced by Rusty Crayfish. Rusty Crayfish could potentially

negatively affect smaller size classes of Eastern Hellbenders, though, by food resource limitation (although juvenile hellbenders may rely less on crayfish as a food resource than adults and typically consume smaller macroinvertebrates such as aquatic insects; Pitt and Nickerson 2006; Hecht et al. 2017) or impact hellbenders by other (non-dietary) means. For instance, it has been hypothesized that invasive Rusty Crayfish may be predators of hellbender larvae and eggs (Quinn et al. 2013; Cava et al. 2018), though they would have to successfully avoid nest guarding adult male hellbenders to access larvae and eggs. Additionally, crayfish have been identified as vectors of amphibian disease, such as the amphibian chytrid fungus (*Batrachochytrium dendrobatidis*; McMahon et al. 2013), and thus non-native crayfish might facilitate the spread of amphibian diseases to hellbender populations. These potential mechanisms remain unexplored, thus future research could examine them. In terms of the impacts of direct interactions between Rusty Crayfish and subadult/adult Eastern Hellbenders, this study provides important data regarding hellbender conservation in that it suggests invasive Rusty Crayfish readily serve as prey.

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