

VARIATION IN NATIVE CRAYFISH AGONISTIC RESPONSE TO THE INVASION OF THE RUSTY CRAYFISH *ORCONECTES RUSTICUS* (GIRARD, 1852)

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ABSTRACT

The consequences of biological invasions warrant continued research on the mechanisms underlying the spread of exotic species, yet interspecific behavioral interactions are largely overlooked as a contributing factor or consequence of introductions of exotic species. Our study evaluates how native crayfish species, *Cambarus bartonii* (Fabricius, 1798) and *Orconectes propinquus* (Girard, 1852), respond behaviorally to the invasion of the rusty crayfish, *Orconectes rusticus* (Girard, 1852) in the Upper Susquehanna River Basin, NY, USA using experiments in a semi-natural setting. We found significant effects of species, sex, year, novelty to invasion, and abiotic environment on the proportion of time native crayfish species exhibited agonistic behavior toward invading rusty crayfish. *Cambarus bartonii* females were generally more aggressive than males, particularly in year two, but the opposite was true for *O. propinquus* as males were more aggressive than females. Behavioral variation in recipient crayfish communities is a potential mechanism influencing the spread of introduced crayfishes although future study is necessary to assess the relative extent of such influence. The mechanisms driving the spread of invasive species are clearly complex and interactive, but the development of effective management plans is limited by the completeness of our understanding, which thus far has been lacking a substantial ethological component.

KEY WORDS: behavior, biotic resistance, *Cambarus bartonii*, ethology, generalized linear mixed-effects models (GLMM), invasive species, *Orconectes propinquus*

DOI: 10.1163/1937240X-00002404

INTRODUCTION

The negative impacts of exotic species have been extensively studied across many different taxa (Strayer, 2010; Simberloff et al., 2013), and the spread of invasive species has contributed to the breakdown of community structure (Richter-Boix et al., 2013), competitive exclusion of native species (Clavero and García-Berthou, 2005), alterations in nutrient composition (Lovett et al., 2006), and habitat degradation (Martin et al., 2010). With increasing awareness of these consequences, particularly the high economic costs of invasive species management (Pimentel et al., 2000), identification and evaluation of the forces influencing the spread of exotic species has become increasingly important.

Both biotic and abiotic factors have been proposed as mechanisms driving distribution patterns of invasive species, particularly in regards to how such factors are altered by anthropogenic activities. For example, man-made impoundments, facilitate the spread of invasive species, serving as stepping-stone habitats between natural bodies of freshwater (Johnson et al., 2008). Human demographic parameters, such as national wealth and population density, are positively associated with areas of high exotic species richness in Europe (Pyšek et al., 2010). Spatial and temporal heterogeneity of environmental and habitat factors, likely influenced by anthropogenic factors, facilitate the spread of exotic species (Meekins and McCarthy, 2001; Urban et al., 2008; Platvoet et al., 2009). Aside from anthropogenic

causes, life history characteristics and genetic factors of the exotic species also contribute to invasiveness (Poulin et al., 2005; Coutts et al., 2011). Yet the behavioral interactions between native and invasive congeners, a potential mechanism influencing the spread of invasive species, remains largely unexplored.

Behavioral mechanisms are often overlooked within the field of invasion biology as influential components involved in the spread of exotic species, and when considered usually focus on characteristics of the invader (Pintor et al., 2008; Llewelyn et al., 2010; Cote et al., 2011; Brodin and Drotz, 2014) or the evolutionary responses of native species (e.g., Freeman and Byers, 2008). The outcome of interspecific behavioral interactions, however, influence populations based on the cumulative effect of competition on individuals, particularly in regards to food acquisition, shelter acquisition, and predator avoidance. For example, Wrobel et al. (1980) suggested that aggressiveness and foraging superiority were associated with body mass in competing salamander species. Pintor et al. (2009) showed that exotic signal crayfish (*Pacifastacus leniusculus* (Dana, 1852)) were able to attain higher than expected population densities due to the coupling of aggressive behaviors and enhanced foraging activity (i.e. behavioral syndrome). Gherardi and Daniels (2004) observed in a laboratory experiment that the invasive crayfish *Procambarus clarkii* (Girard, 1852) excluded native crayfishes from presented shelters. Furthermore, Polo-Cavia

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et al. (2009) showed that behavioral asymmetries in predator avoidance contributed to the competitive advantage of an introduced turtle species relative to its native congeners. These examples showcase how invasive species influence population sustainability through multiple pathways, including reproductive potential, intensity of competition, and predation pressures. Thus, whether a native population can persist when faced with an invader likely depends, at least partially, on its behavioral response to the invasive species. Heterogeneity in the behavioral responses of native species may provide insight into the dynamic and heterogeneous nature of the dispersal patterns of invasive species (Hardin, 1960; Suarez et al., 2001). Unfortunately, few studies have assessed the heterogeneity of behavioral responses of native species to invasive species, despite prompting from researchers within the field of invasion biology (e.g., Holway and Suarez, 1999).

One of the major aquatic invasive species in North America is the rusty crayfish *Orconectes rusticus* (Girard, 1852). The rusty crayfish is native to the Ohio River Basin, USA within the states of Ohio, Indiana, Kentucky, and the southeast corner of Michigan, but since the late 1960s the rusty crayfish has spread across the contiguous United States following anthropogenic introductions. The broad scale consequences of rusty crayfish invasion are well documented, including displacement of native congeners (Taylor and Redmer, 1996), loss of macrophyte biomass and richness (Lodge and Lorman, 1987), alteration of snail communities (Kreps et al., 2012), and predation of fish eggs (Morse et al., 2013). The success of the rusty crayfish is attributed to its high dispersal rate (Wilson et al., 2004; Jansen et al., 2009), broad habitat niche (Freeman et al., 2010), and large chelae, a structural feature beneficial during agonistic encounters (Hill and Lodge, 1999).

The crayfish assemblage within the Upper Susquehanna River Basin (USRB), New York, USA is an ideal system in which to evaluate the potential behavioral heterogeneity of native crayfishes to the invasion of the rusty crayfish. It is hypothesized that the rusty crayfish was originally introduced into the system in Otsego Lake, with subsequent introductions in other locations producing a spatially discontinuous distribution (Kuhlmann and Hazelton, 2007). The rate of spread of the rusty crayfish within the range of the USRB varies spatially, possibly due to the influence of native crayfishes. Nevertheless, the role of the behavior of native crayfish on rusty crayfish invasion rate and native population displacement is unclear.

The overarching question we addressed in this study was how native crayfishes respond agonistically to the invasion of the rusty crayfish. Our study examined crayfish behavior in semi-natural conditions within a river system to determine whether the behavior of native crayfish varies in the presence of rusty crayfish, a pre-requisite for behavior to be a mechanism contributing to the heterogeneous spread of the rusty crayfish. Specifically, we assessed how the behavioral interactions of native and rusty crayfishes are influenced by the type of species and sex of the interacting individuals. We hypothesized species level differences between the two native species investigated, *Cambarus bartonii* (Fabricius, 1798) and *Orconectes propinquus* (Girard,

1852). *Orconectes propinquus* has greater niche overlap and shares more recent ancestry with the rusty crayfish than *C. bartonii* does, and *O. propinquus* and *O. rusticus* can produce viable hybrid offspring in the wild (Hamr and Berrill, 1985; Perry et al., 2001). We did not anticipate sex-based differences, as the sexual dimorphism in chelae size occurs only during the breeding season (Stein, 1976) and we conducted our study prior to this period. We also evaluated if behavior varies between years or sites. Although habitat structure and food resources are correlated with behavioral responses during agonistic encounters in crayfishes (Bergman and Moore, 2003), we did not predict significant differences in crayfish behavior between our study sites, as habitat characteristics were highly similar across sites. Lastly, we evaluated if naïve native crayfish respond differently to the rusty crayfish than experienced native crayfish.

METHODS

Study Site

We conducted 104 behavioral trials at seven stream sites (five different streams) within the Upper Susquehanna River Basin (USRB), NY, USA, in June and July 2011 and 2012 (Fig. 1). All study sites were located in streams within the USRB. The basin comprises an area of 12,804 km² (11,707 km² in New York and 1098 km² in Pennsylvania) for which its major tributaries branching off the Susquehanna River include Chenango, Tioughnioga, and Unadilla. Otsego Lake in Cooperstown, NY is the source of the Susquehanna River and matrix of the subbasin is dominated by agriculture and five major population centers (Binghamton, Johnson City, Endicott, Cortland, and Oneonta). Although the Susquehanna River is classified as an 8th-order stream, all of our study sites were on 2nd- and 3rd-order tributaries of the Susquehanna River, were wadeable across their widths, and had similar abiotic environmental characteristics (see Results). Stream sites were selected from previously surveyed locations (Kuhlmann and Hazelton, 2007; M. L. Kuhlmann, unpublished data) based on the presence of both the rusty crayfish (*Orconectes rusticus*) and at least one of the two native crayfish species (*Cambarus bartonii* and *Orconectes propinquus*), with the exception of two uninhabited sites. Low abundance of native crayfish in the summer of 2012 at sites where both native and invasive species were previously present led to the inclusion of two additional sites where the rusty crayfish was absent to evaluate how novelty to an invader influences behavior (Sites 24 and 52 in Table 1). On each day the two uninhabited sites were used, rusty crayfish were temporarily brought from, and returned to, Charlotte Creek at the Pine Lake Environmental Campus, Hartwick College, Oneonta, NY, USA (Fig. 1). Prior to conducting any behavior trials, transported rusty crayfish were given a 30-minute acclimation period in individual containers of water obtained from the new site. None of the rusty crayfish we transported escaped into previously uninhabited sites during our study.

Experimental Setup

Behavioral trials were conducted in a rectangular arena constructed using 2.54 cm PVC pipe, plastic mesh netting, and a 1 × 1 m detachable Plexiglass (Ridout Plastics, San Diego, CA, USA) bottom. We haphazardly selected arena locations by tossing a weighted marker from the stream bank until it landed in a stream section with a depth between 20 and 60 cm and of sufficient clarity to permit video observation. No trials were conducted within 10 m of any previous trial. We obtained the substrate for each trial from a location directly adjacent to each arena. To ensure substrate quantities and composition were consistent across trials, a bottomless five-gallon bucket was inserted 12 cm into the stream bottom and all substrate within the bucket was collected.

We captured adult crayfish using kick nets within a 10 m radius of the arena and matched crayfish individuals by size (carapace length difference < 10%; Rahm et al., 2005) and sex, confounding factors that have been shown to influence behavior during agonistic encounters in crayfishes (Rabeni, 1985; Pavey and Fielder, 1996; Figler et al., 2005). Each individual was uniquely marked on the carapace with Wite-out (BIC,

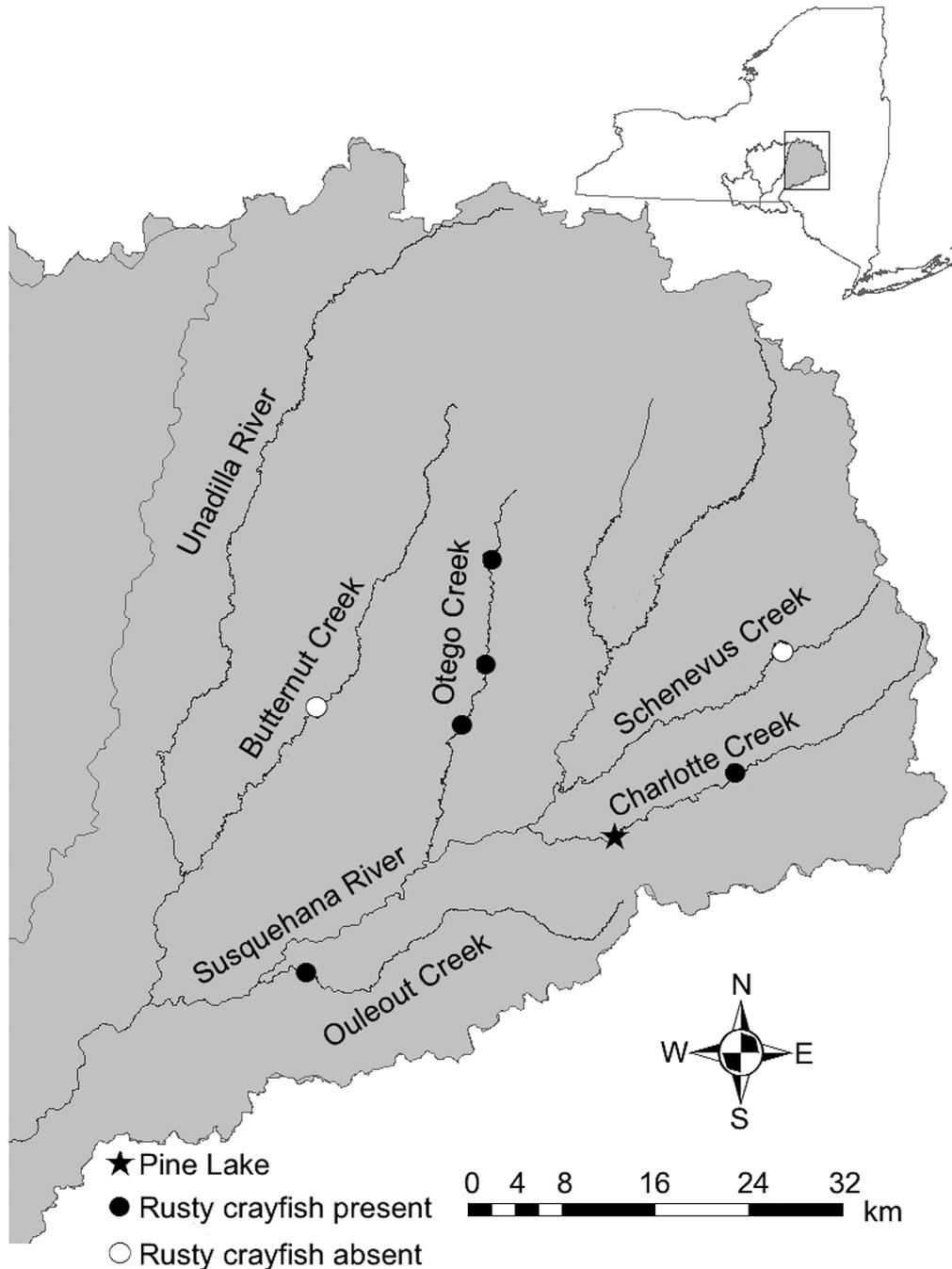


Fig. 1. Study sites in the Upper Susquehanna River Basin, NY, USA. The rusty crayfish (*Orconectes rusticus*) was absent from two of the seven sites (white circles), thus behavioral trials between native and rusty crayfish were done using rusty crayfish temporarily transported from Charlotte Creek at Pine Lake, Hartwick College, NY, USA (black star).

Shelton, CT, USA) for ease of identification during video recording and to prevent repeated use if recaptured. We released a native individual in the center of the arena and provided a two-minute acclimation period before a rusty crayfish was introduced. A 15-minute trial was video recorded by an observer standing downstream of the arena. We ensured that individuals could perceive the presence of the other, without forcing an interaction, by placing the rusty crayfish two body lengths in front of the native crayfish (Bergman and Moore, 2003). Crayfish were removed from the arena after each trial and a walking leg was collected from each individual as a genetic sample for use in another study. The missing walking leg also served as a

more long-term identifier, preventing us from reusing the same individuals in future trials, as Wite-out markings disappear after a few days.

Video Analysis

Agonistic behavior was defined as the display of meral spread, wrestling, or pinching behaviors (as described in Bergman and Moore, 2003) by the native crayfishes and directed towards the rusty crayfish. Meral spread behavior occurs when a native crayfish extended and raised its claws towards a conspecific within one body length away, wrestling behavior when a native crayfish used its claws to actively push or wrestle with the rusty crayfish, and pinching behavior when a native crayfish used its claws

Table 1. Number of behavioral trials conducted for each species of native crayfish by sex and site. ^aUninvaded site (rusty crayfish, *Orconectes rusticus*, absent). ^bNumber of trials conducted in year 1 and year 2 separated by a comma.

| Species | Sex | Site 18 | Site 24 ^a | Site 52 ^a | Site 72 | Site 82 | Site 83 | Site 84 | Total |
|----------------------|---------------------|---------|----------------------|----------------------|---------|---------|---------|---------|-------|
| <i>C. bartonii</i> | Male ^b | 7, 1 | 1, 0 | 0, 0 | 5, 17 | 1, 1 | 0, 3 | 0, 2 | 38 |
| | Female ^b | 5, 1 | 1, 0 | 0, 0 | 3, 7 | 2, 0 | 0, 1 | 0, 0 | 20 |
| <i>O. propinquus</i> | Male | 3 | 3 | 10 | 0 | 0 | 7 | 2 | 25 |
| | Female | 2 | 0 | 13 | 0 | 1 | 3 | 2 | 21 |

to grab or pinch an opponent. If none of the aforementioned behaviors were observed, but crayfish were in contact, the behavior was classified as an interaction and not included in the calculated proportion of time native crayfish exhibited agonistic behavior.

Abiotic Environment

Although the influence of environmental factors on crayfish behavioral responses to invasion was not of primary interest for our study (evaluated by Davis and Huber, 2007), we measured six abiotic variables (proportion cobble and boulder, flow velocity, dissolved oxygen, water temperature, pH, and water depth) to ensure similar environments across our seven study sites. To quantify substrate composition, each size category was weighed after being separated using sieves (64 and 4 mm; larger than 64 mm was cobble and boulder, between 4 and 64 mm was pebble, and smaller than 4 mm was granule, sand, and silt). Proportion cobble and boulder was used as a substrate composition metric because it was the most reliable wet-weight measurement and the most variable between trials. Flow velocity was measured using a Flow Probe (Model FP101, Global Water, Gold River, CA, USA), water temperature and pH using a Waterproof Palm pH Meter (Model PH220A, Extech Instruments, Waltham, MA, USA), and dissolved oxygen using an ExStik DO600 (Extech Instruments).

We used linear discriminant analysis (LDA) to summarize the abiotic environment within each arena. Such dimensionality reduction was necessary for downstream analyses (i.e., for model convergence). Unlike principal component analysis (PCA), which generates its axes based on similarities, LDA builds its axes based on differences and thus is a more conservative approach. For example, we generated one axis that maximized the difference in abiotic environment between invaded and uninvaded sites, while maintaining all of the variation in the abiotic environment in a single axis.

Statistical Analyses

We used generalized linear mixed-effects models (GLMMs) to determine whether species, sex, year, and site category (invaded or uninvaded) influenced agonistic behavior in native crayfish. The fixed effects (i.e. predictor variables) in our models were species, sex, year, site category, and abiotic environment, whereas the random effect was the specific site. We coded the fixed effects as dummy variables: species (*C. bartonii* = 0; *O. propinquus* = 1), sex (male = 0; female = 1), year (2011 = 0; 2012 = 1), and site category (uninvaded = 0; invaded = 1), except the abiotic environment, which was the continuous, numerical value generated by the LDA. We assigned a binomial distribution for the response variable, the number of seconds exhibiting agonistic behavior with the number of seconds not displaying agonistic behavior. Only the following models could be compared using Akaike Information Criteria with correction (AIC_c) due to the sampling design restrictions (Table 1): (1) no interactions between fixed effects, (2) species and sex interaction, (3) sex and year interaction, (4) sex by site category interaction, (5) site category by abiotic environment interaction, (6) species by abiotic environment interaction, (7) sex by abiotic environment interaction, and (8) year by abiotic environment interaction (Table 2). The model with the lowest AIC_c value and delta AIC_c > 2 was interpreted as the best model given our dataset.

RESULTS

Environmental Summary Statistics

The substrate at all sites was predominantly cobble and small boulder (72.5–100%), flow velocity was <0.5 m/s, dissolved oxygen ranged from 5.96 to 12.26 mg/l, water temperature

ranged from 20.1 to 27.3°C, pH ranged from 6.99 to 8.49, and water depth ranged from 20 to 56 cm.

The abiotic variables were transformed to meet the assumptions of the LDA: a square root transformation was used for proportion cobble and boulder, a log plus one transformation for flow velocity, and a log transformation for the remaining variables. The standardized coefficients of the LDA for each abiotic variable were as follows: proportion cobble and boulder 2.19, flow velocity -0.41, dissolved oxygen 0.26, water temperature -0.49, pH 0.91, and water depth -0.74.

Behavior Summary Statistics

Although we size-matched individual crayfish within a 10% carapace length difference, a commonly used standard in studies of crayfish behavior (Schroeder and Huber, 2001; Bergman and Moore, 2003; Zulantz et al., 2008; Hudina et al., 2015), our actual percentage size differences ranged from 0–8.3%, with a mean of 2.9% (0–2.2 mm with a mean of 0.806 mm). In 15 out of 104 trials (14.4%), the native crayfishes never encountered the rusty crayfish, whereas in the remaining 89 trials encounters were relatively short but highly variable in duration (15.5 ± 31.8 seconds). The average total time engaged in agonistic behavior during the 15-minute trials was 57 ± 89 seconds, which typically included multiple independent encounters (4.4 ± 4.7 encounters per trial).

Full Model

The model with a species and sex interaction term with site as the random effect (Table 2) was the best model of the eight models compared (delta AIC_c of 7610.9). Species, sex, year, site category, abiotic environment, and the interaction between species and sex were all significant predictors of the number of seconds native crayfishes exhibited agonistic behavior toward rusty crayfish, species being the best predictor and abiotic environment being the worst predictor among the variables examined.

Based on the dummy coding we used for the fixed effects, the standardized coefficients were interpreted as follows (Table 2): positive values for the species variable indicated that *O. propinquus* were more likely to exhibit agonistic behavior longer than *C. bartonii*, positive values for the sex variable indicated that females were more likely to exhibit agonistic behavior than males, negative values for the year variable indicated that native crayfish were more likely to exhibit agonistic behavior in 2011 than in 2012, and positive values for the site variable indicated that native crayfish in invaded sites were more likely to exhibit agonistic behavior than crayfish in uninvaded sites. The best model showed that

Table 2. Generalized linear mixed-effects model comparison for agonistic behavior of native crayfishes given the fixed effects Species, Sex, Year, SiteCat (site category, i.e., whether or not the site has been invaded by rusty crayfish), LD (abiotic environment axis generated by an LDA maximizing the difference between invaded and uninvaded sites), and the random effect, site location (ranked from lowest to highest AIC_c value). ^aAll full models contain all variables (species, sex, year, site category, and LD), they differ based on the interaction allowed. ^bLDy = abiotic environment axis generated by an LDA maximizing the difference between the first and second year. Only parameter values for models within a delta AIC_c value of less than 10 (relative to the best model) are shown.

| Model | AIC _c | df | Parameter | Estimate | SE | P |
|--|------------------|----|---------------|----------|------|--------|
| Full models^a | | | | | | |
| Species × Sex | 7610.9 | 8 | Species | 2.09 | 0.06 | <0.001 |
| | | | Sex | 0.81 | 0.04 | <0.001 |
| | | | Year | -0.57 | 0.05 | <0.001 |
| | | | SiteCat | 0.85 | 0.22 | <0.001 |
| | | | LD | -0.16 | 0.02 | <0.001 |
| | | | Species × Sex | -1.95 | 0.06 | <0.001 |
| Sex × LD | 8358.6 | 8 | | | | |
| Sex × SiteCat | 8550.5 | 8 | | | | |
| Species × LD | 8561.2 | 8 | | | | |
| Year × LD | 8630.5 | 8 | | | | |
| Sex × Year | 8645.7 | 8 | | | | |
| SiteCat × LD | 8699.3 | 8 | | | | |
| No interactions | 8729.3 | 7 | | | | |
| <i>Cambarus bartonii</i> models | | | | | | |
| Sex × Year + LDy ^b | 2855.1 | 6 | Sex | 0.23 | 0.05 | <0.001 |
| | | | Year | -1.38 | 0.08 | <0.001 |
| | | | LDy | 0.21 | 0.02 | <0.001 |
| | | | Sex × Year | 1.18 | 0.08 | <0.001 |
| Year + Sex × LDy | 2894.8 | 6 | | | | |
| Sex × Year | 2930.7 | 5 | | | | |
| Sex + Year × LDy | 2998.1 | 6 | | | | |
| Sex + Year + LDy | 3049.7 | 5 | | | | |
| Sex × LDy | 3060.0 | 5 | | | | |
| Sex + Year | 3128.8 | 4 | | | | |
| Sex + LDy | 3191.4 | 4 | | | | |
| Sex | 3193.4 | 3 | | | | |
| Year × LDy | 3331.8 | 5 | | | | |
| Year + LDy | 3402.6 | 4 | | | | |
| LDy | 3435.6 | 3 | | | | |
| Year | 3472.1 | 3 | | | | |
| <i>Orconectes propinquus</i> models | | | | | | |
| Sex + SiteCat × LD | 4319.8 | 6 | Sex | -1.07 | 0.04 | <0.001 |
| | | | SiteCat | 1.42 | 0.62 | 0.022 |
| | | | LD | -0.35 | 0.03 | <0.001 |
| | | | SiteCat × LD | 0.68 | 0.05 | <0.001 |
| Sex × SiteCat | 4484.1 | 5 | | | | |
| Sex × SiteCat + LD | 4485.0 | 6 | | | | |
| SiteCat + Sex × LD | 4518.8 | 6 | | | | |
| Sex × LD | 4519.3 | 5 | | | | |
| Sex + SiteCat | 4529.5 | 4 | | | | |
| Sex | 4529.8 | 3 | | | | |
| Sex + Sitecat + LD | 4531.3 | 5 | | | | |
| Sex + LD | 4531.6 | 4 | | | | |
| SiteCat × LD | 4932.7 | 5 | | | | |
| LD | 5237.5 | 3 | | | | |
| SiteCat + LD | 5238.6 | 4 | | | | |
| SiteCat | 5284.1 | 3 | | | | |

O. propinquus males were most likely to exhibit agonistic behavior during trials in invaded sites (Fig. 2); however, the significant interaction between species and sex in this model inhibits the independent interpretation of any of the fixed variable coefficients.

Individual Species Models

We conducted two follow-up analyses, one for each species, to explore the effects of year and site category on crayfish behavior. Due to the significant species-by-sex interaction observed in the full model we ensured that sex was included

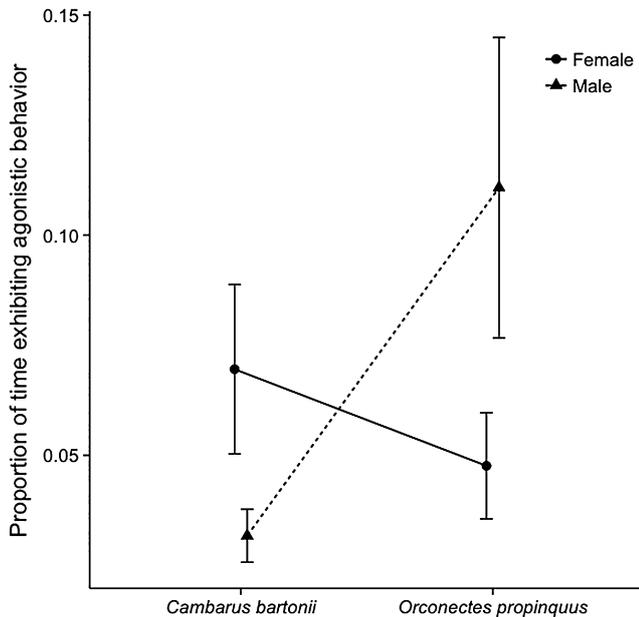


Fig. 2. Mean proportion of time native crayfish species, *Cambarus bartonii* and *Orconectes propinquus*, exhibited agonistic behavior toward the invasive rusty crayfish, *Orconectes rusticus*, during 15-minute behavioral trials in 2011 and 2012 at sites previously invaded by the rusty crayfish. Solid triangles with dotted lines represent male-male trials and solid circles with solid lines represent female-female trials. Standard error bars are shown around the means.

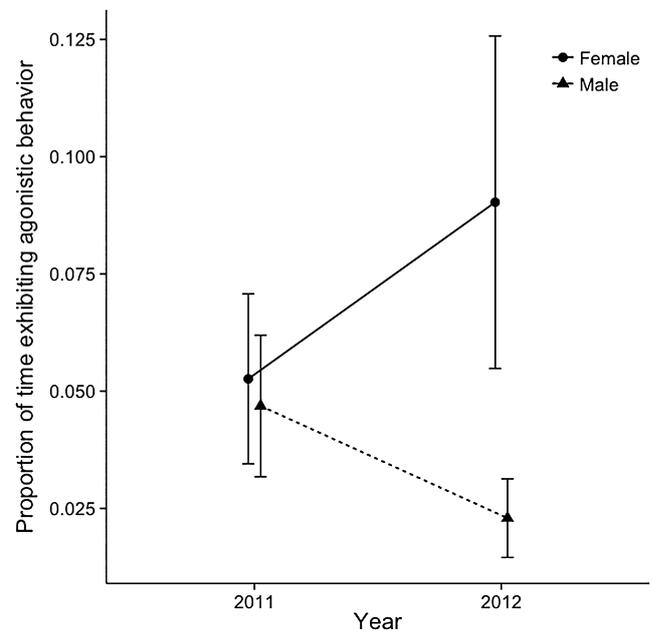


Fig. 3. Mean proportion of time native crayfish, *Cambarus bartonii*, exhibited agonistic behavior toward invasive rusty crayfish, *Orconectes rusticus*, during 15-minute behavioral trials in 2011 and 2012. Solid triangles with dotted lines represent male-male trials and solid circles with solid lines represent female-female trials. Standard error bars are shown around the means.

in both analyses. Our sampling design prevented analyses of all variables separately for each species (Table 1), but for *C. bartonii* the predictor variables accessed included sex, year, and abiotic environment and for *O. propinquus* we included sex, site category, and abiotic environment. Because site category was not relevant to the *C. bartonii* analysis we conducted a new LDA to reduce the dimensionality of the abiotic variables that maximized the difference in abiotic environment between the first and second year. A separate LDA that maximized differences between site categories was used for the analysis of the *O. propinquus* data (see Methods).

Of the 13 models compared, the best model for *C. bartonii* behavior included sex, year, and abiotic environment, as well as a sex-by-year interaction, for which year was the strongest predictor (Table 2). *Cambarus bartonii* males and females spent approximately the same amount of time exhibiting agonistic behavior toward invasive rusty crayfish in 2011, but in 2012 females spent substantially more time exhibiting agonistic behavior than males (Fig. 3). Of the additional 13 models compared, the best model for *O. propinquus* behavior included sex, site category, abiotic environment, as well as a site category by abiotic environment interaction for which site category was the strongest (but insignificant) predictor (Table 2). Naïve *O. propinquus* males and females spent approximately the same amount of time in agonistic interactions with rusty crayfish, but in systems with experienced native crayfish, males spent more time in agonistic interactions than females (Fig. 4).

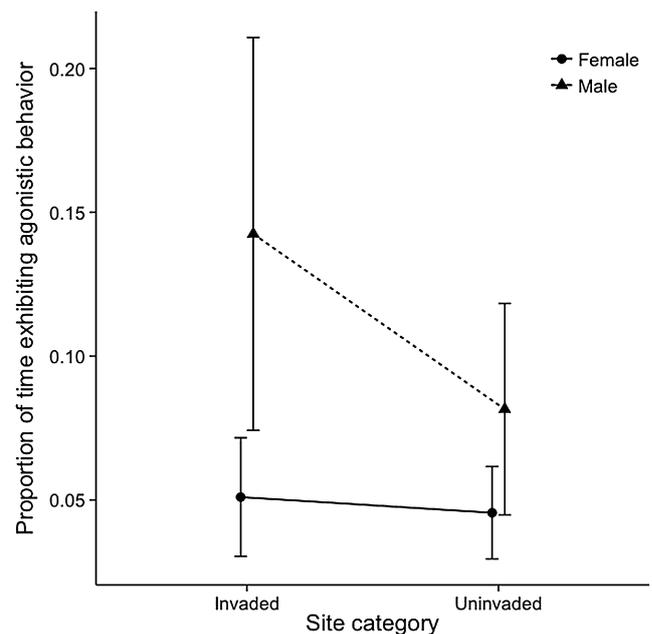


Fig. 4. Mean proportion of time native crayfish, *Orconectes propinquus*, exhibited agonistic behavior toward invasive rusty crayfish, *Orconectes rusticus*, during 15-minute behavioral trials in previously invaded and uninvaded sites. Rusty crayfish were temporarily brought to uninvaded sites from Charlotte Creek (solid star in Fig. 1) for trials. Solid triangles with dotted lines represent male-male trials and solid circles with solid lines represent female-female trials. Standard error bars are shown around the means.

DISCUSSION

Our results show that the native crayfishes vary in their behavioral response to the invasive rusty crayfish. We observed species-level variation as predicted; however, the sex of the dyads influenced whether native *Cambarus bartonii* or *Orconectes propinquus* displayed more agonistic behavior toward invasive rusty crayfish. *Cambarus bartonii* individuals were more aggressive during female-female encounters, but *O. propinquus* individuals were more aggressive in male-male encounters. Contrary to our predictions, the year in which the behavior trial took place influenced the behavioral response of native crayfish, but the site category or the novelty of native crayfish to rusty crayfish did not.

Evaluation of Hypotheses

We found a significant effect of species on the proportion of time crayfish individuals exhibited agonistic behavior toward rusty crayfish in our best generalized linear mixed-effect (full) model, although a significant interaction between species and sex inhibits the isolated interpretation of the species coefficient. Several previous laboratory experiments support our findings, including the behavioral variation between crayfish species belonging to the same family relative to those of different families (Gherardi and Daniels, 2004), and dominance of invasive crayfish species over native species (Söderbäck, 1994). Contrary to our findings, Vorburger and Ribí (1999) showed that neither *Pacifastacus leniusculus* (an introduced species) nor *Austroptamobius torrentium* (Schrank, 1803) (a native species) was inherently dominant during interspecific encounters, but due to the size-dependent nature of interactions the larger of the two species (*P. leniusculus*) was generally favored.

We also found a significant effect of sex on the behavioral response of native crayfishes to the invasive crayfish in all models compared, but again the significant interaction between species and sex prevents the direct interpretation of the parameter coefficient for sex. Evaluating each species independently, *C. bartonii* females spent a greater proportion of time exhibiting agonistic behavior during trials than males, whereas the opposite was true for *O. propinquus*. While our study and others (e.g., Bruski and Dunham, 1987), found behavioral differences between male and female crayfish such differences do not exist across all crayfish species (Figler et al., 1999), limiting the utility of sex as a predictor variable for the influence of invasive crayfishes on the behavior of native crayfishes. We hypothesize that male *O. propinquus* may be more aggressive toward male rusty crayfish because of competition for females, since the two species can hybridize, although we would expect this effect to be weaker outside of the breeding season.

We observed temporal variation in the behavioral response of *C. bartonii* toward rusty crayfish during our two-year study. Females were more aggressive in 2012 than in 2011, whereas males were less aggressive in 2012. To the best of our knowledge, our study was the first to explicitly look for temporal variation in crayfish agonistic behavior across years, but other studies have shown temporal variation in habitat use (e.g., Flinders and Magoulick, 2007) and predator avoidance behavior (e.g., Pecor and Hazlett, 2003). Nevertheless, both studies concluded that there could be a

number of factors driving temporal patterns, such as crayfish size and habituation, neither of which are likely responsible for our observations because of our study design. Temporal variation has been observed in crayfishes across seasons, where male crayfish were more aggressive during the mating season, which may improve mating success of males and contribute to better mate choice in females who observe male-male interactions (Aquiloni et al., 2008). Our year-to-year behavioral differences are nevertheless not likely, however, due to a result of the influence of breeding modified behaviors, as our study was conducted during the nonbreeding season in both years. Further study is necessary to discriminate between the many potential mechanisms driving temporal variation not just in agonistic behavior, but also in habitat use and predator avoidance behavior (Pecor and Hazlett, 2003; Flinders and Magoulick, 2007).

Lastly, we did not observe a significant effect of site (invaded vs. uninvaded) on *O. propinquus* behavior toward rusty crayfish. Our findings are counterintuitive, as previous studies have shown that past experience influences outcomes of future agonistic encounters in crayfish (e.g. Rubenstein and Hazlett, 1974). If invaded sites contained native individuals that had engaged in a previous encounter with a rusty crayfish, we would have expected the behavior of *O. propinquus* to be different between invaded and uninvaded sites, but site category (i.e. naïve or experienced native crayfish) did not have a significant effect on behavior, although the significant interaction between site category and the abiotic environment make interpretation of either main effect difficult. Although all the sites in our study were surveyed every few years, some every year (M. L. Kuhlmann, unpublished data), it is possible that rusty crayfish had been introduced to what we considered uninvaded sites, but did not persist, permitting previous, but undetected interactions between *O. propinquus* and rusty crayfish individuals. Alternatively, past experience with invasive crayfish may not be the primary mechanism contributing to the behavioral differences observed in previous studies, as our study suggests other factors (e.g. species, sex, and year) have a significant effect on crayfish behavior.

Implications

Our study extends what previous studies have shown regarding patterns of crayfish behavior by better mimicking natural conditions. Past studies have shown differences in crayfish behavior regarding species and sex-level differences, but ours extends such knowledge beyond static laboratory environments to a semi-natural, flowing stream system. Furthermore, we have developed an experimental protocol that takes crayfish behavioral studies out of the laboratory and into the field, while still controlling for possible confounding factors. Bergman and Moore (2003) created a useful method for observing natural crayfish behaviors, but were unable to identify the sex of interacting individuals or control for confounding variables, which inhibit assessment of cause-and-effect relationships. The method we used is a middle ground between laboratory studies, which may not reflect characteristic behaviors, and field studies, which are limited in their ability to test causal relationships because of confounding variables. While our study minimized size differences between paired individuals to better explore the influence of

species, sex, year, and novelty to invasion on behavior, the methodology could be modified to explore agonistic behaviors between individuals of different sizes in a semi-natural setting, as such contests are fundamentally different than the size-matched scenario we used in this study.

Holway and Suarez (1999) emphasized the importance of behavioral studies in the context of invasion biology, yet behavioral responses of native species to invasion have largely been overlooked as a component influencing the spread of invasive species. While our study cannot explicitly validate that variation in the behavioral response of native crayfishes has contributed to the heterogeneous spread of the rusty crayfish, we were able to show that behavioral variation to an invasive species exists within native communities. The influence of species and sex on behavior suggests that species composition and demographic characteristics within populations of native crayfish species may influence the biotic resistance of communities to invasion by other crayfish species. We could use this knowledge in future studies to look for relationships between native community composition and crayfish invasion patterns and better assess behavior as a mechanism driving the spread of invasive species.

There are undoubtedly multiple factors involved in the spread of invasive species and our awareness and understanding of these mechanisms is vital for the development of successful control strategies following the introduction of such invasive species. While we encourage studies like ours that explore factors influencing the success of invasions, prevention is still the most effective way to inhibit the spread of invasive species. Multiple anthropogenic introductions by anglers is likely the primary cause of the current distribution of the rusty crayfish across North America (Lodge et al., 2000; Puth and Allen, 2005; DiStefano et al., 2009), and enactment of fishing regulations prohibiting the transport of live crayfish between water bodies is an effective first step to minimize introductions (Dresser and Swanson, 2013).

ACKNOWLEDGEMENTS

We would like to thank our three undergraduate field assistants (Emileigh Johnston, Kylie Hool, and Lena Hunt), who volunteered to travel over 700 miles and battle leech-infested waters for the sake of science. We would also like to acknowledge Richard Gary and Maribeth Rubenstein as well as Hartwick College's Pine Lake Environmental Campus for providing affordable housing accommodations during the field season. A special thank you to the Humboldt Mfg. Co. for generously donating three sieves needed to quantify substrate composition. We would also like to acknowledge the two anonymous reviewers and faculty from the Ecology and Evolutionary Biology Department of the University of Tennessee who provided valuable suggestions to the manuscript. This research was funded by the Marion Whitney Summer Scholarship and the Graduate Student Research and Creative Endeavors Grant through Central Michigan University.

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RECEIVED: 2 July 2015.

ACCEPTED: 10 December 2015.

AVAILABLE ONLINE: 2 February 2016.