MECHANISMS OF RUSTY CRAYFISH INVASION
IN THE UPPER SUSQUEHANNA RIVER BASIN

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ABSTRACT

MECHANISMS OF RUSTY CRAYFISH INVASION
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by Cassie Marie Dresser

The consequences of exotic species introductions are well understood, yet considerably less is known about the mechanisms driving invasions. For popular bait species, such as the rusty crayfish (*Orconectes rusticus*) the rapid spread and resulting patchy distribution across the Unites States has been attributed to multiple anthropogenic introductions and variation in biotic resistance of native communities. Previous survey data showed a similar distribution of rusty crayfish at a smaller scale, within the Upper Susquehanna River Basin (USRB). Two possible explanations for the patchy distribution pattern were explored; variation in native crayfish behavioral defense against invading rusty crayfish and multiple anthropogenic introductions of rusty crayfish. Behavioral trials between native crayfish species (*Cambarus bartonii* and *Orconectes propinquus*) and rusty crayfish were conducted in temporary arenas positioned in streams within the USRB. Native crayfish behavioral defense varied based on species and sex. Generally, *C. bartonii* females were more aggressive than *O. propinquus* females, but *O. propinquus* males were more aggressive than *C. bartonii* males. The results suggest that native species composition, as well as the demographic parameters of existing populations, such as sex ratio may contribute to the biotic resistance of communities to invasion. Amplified fragment length polymorphisms were used to evaluate the presence of multiple genetically distinct populations of rusty crayfish in the USRB, which due to their recent introduction would indicate that multiple anthropogenic introductions had occurred. Crayfish from a total of seven sites above and below a 50 km distribution gap observed in 1999 were sampled in 2011. Only one

genetic population of rusty crayfish was detected with low genetic differences between sample locations, but with private alleles in each site. The genetic similarity between sample sites on either side of the historic distribution gap and the prevalence of private alleles at all sample sites suggests that at least two introductions have occurred from the same source population. Understanding the number of sources from which anthropogenic introductions occurs facilitates our ability to prevent future introductions and initiate appropriate management regimes and regulations. Currently, no post-invasion control strategies have effectively and consistently eradicated rusty crayfish, thus the enactment of legal policies is often recommended to prevent anthropogenic introductions of invasive species. The effectiveness of proactive state legislation policies in deterring rusty crayfish colonization was evaluated and network-based spatial analysis was used to predict the spread of rusty crayfish into previously uninvaded states. Regulations that did not explicitly prohibit transport, applied to specific drainages, or prohibited only rusty crayfish were not effective. However, preemptive legislation was effective in reducing the likelihood of invasion, if regulations prohibited the transport of all live crayfish species between water bodies, as only 1 state which passed such legislation prior to invasion by rusty crayfish was subsequently colonized ($S = 12$, $p = 0.031$). Five states are likely to be invaded by rusty crayfish via range expansion across interstate drainages, and dispersal rates suggest that all of these states could be colonized within 10 years. While it is unlikely that regulations will prevent dispersal-based invasion across state lines through shared drainages, preemptive legislation can effectively retard the range expansion of rusty crayfish.

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Abstract

The consequences of exotic species introductions are well understood, yet considerably

less is known about the mechanisms driving invasions, particularly with regards to the ability of

native species to resist invasion. Our study was the first field study to directly evaluate

behavioral responses of native crayfish to invasive crayfish as a contributing mechanism for the

patchy distribution of invasive crayfish. We conducted behavioral trials between native crayfish

species (*Camabrus bartonii* and *Orconectes propinquus*) and invasive rusty crayfish (*Orconectes

rusticus*) within temporary arenas positioned in streams within the Upper Susquehanna River

Basin (New York, USA). We found that native crayfish behavioral defense against rusty

crayfish differed based on species and sex. Generally, *C. bartonii* females were more aggressive

than *O. propinquus* females, but *O. propinquus* males were more aggressive than *C. bartonii*

males. Our results suggest that native species composition, as well as the demographic

parameters of existing populations, such as sex ratio may contribute to the biotic resistance of

communities to invasion.

Introduction

The negative impacts of exotic species have been extensively studied across many

different taxa (Strayer 2010; Simberloff et al. 2013) and have contributed to the breakdown of

community structure (Richter-Boix et al. 2012), 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). Although the consequences of exotic species introductions are

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well understood, the ability of managers to develop effective invasive species control strategies is limited by our understanding of the mechanisms driving exotic species introductions and spread. In particular, behavioral mechanisms are often overlooked as influential components in invasive species distribution patterns. Studies that address behavioral aspects largely focus on characteristics of the invading species (Hazlett et al. 2003; Sih et al. 2004; Harrington et al. 2009) while few studies consider the immediate behavioral responses exhibited by native species, although long-term evolutionary responses by native species have been explored (Monello et al. 2006; Freeman and Byers 2008). Immediate behavioral responses by native species may contribute to the biotic resistance of an existing community by preventing the successful establishment of invasive species through competitive exclusion (Britton 2012). Insight into the behavior of native species may help us understand invasion patterns, particularly jump dispersal invasions, which produce a discontinuous patchy distribution rather than the continuous invasion front assumed by diffusion models of spread (Holway and Suarez 1999; Suarez et al. 2001; Rödder 2009).

The introduction of the rusty crayfish (*Orconectes rusticus*) to the Upper Susquehanna River Basin (USRB), New York, USA, is representative of a jump dispersal invasion. Rusty crayfish were likely introduced in the Susquehanna River headwaters in Otsego Lake in the early 1970s and can now be found as far southwest as Bainbridge (approximately 90 km downstream), but are still absent in intermediate river sections within the drainage (Kuhlmann and Hazelton 2007). The rusty crayfish is a successful invader because it has a high dispersal rate (Wilson et al. 2004; Jansen et al. 2009), is a habitat generalist (Freeman et al. 2010), and is a dominant competitor with large chelae (Hill and Lodge 1999). However, to the best of our knowledge no studies have examined the behavioral response of native crayfish species to invading rusty

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crayfish, which may contribute to the patchy distribution pattern displayed by rusty crayfish. Previous studies on crustaceans have uncovered behavioral differences based on species (*Orconectes virilis* and *Orconectes rusticus*, Bergman and Moore 2003), sex (*Homarus americanus*, Peeke et al. 1998), size (*Gonodactylus viridis*, Caldwell and Dingle 1979), and environmental conditions (*Orconectes virilis* and *Orconectes rusticus*, Bergman and Moore 2003), which suggest that the response of native crayfish to rusty crayfish may be highly variable. Bergman and Moore (2003) found that shelter habitat influenced the duration of crayfish fights, as closely size matched individuals typically had longer bouts and could be accommodated by the same sized shelter. Stream flow velocity can also influence agonistic behavior as the ability of individual crayfish to maneuver across flow velocities varies by species (Maude and Williams 1983). Additionally, water depth may alter agonistic behavior in crayfish due to varying predation risk, particularly by terrestrial predators in shallow water (Englund and Krupa 2008). Lastly, water chemistry and temperature are known to influence crayfish metabolic activity and growth (Mundahl and Benton 1990) and may impact competitive interactions between crayfish.

Multiple anthropogenic introductions of rusty crayfish in the USRB may have contributed to initial distribution patterns of rusty crayfish (Kuhlmann and Hazelton 2007), but native crayfish behavior may promote the persistence of rusty crayfish patchy distribution patterns. Our objective was to determine if native crayfish behavioral defense against rusty crayfish varied based on species (*Cambarus bartonii* and *Orconectes propinquus*), sex, or abiotic environment.

Methods

Study Site

Seventy-nine behavioral trials were conducted across seven stream sites (five different streams) within the Upper Susquehanna River Basin, New York, USA, in July of 2012 (Figure 1). The substrate among sites was predominantly cobble and small boulder (72.5% – 100%), flow velocity was <0.5 m/s, dissolved oxygen ranged from 5.96 mg/L to 12.26 mg/L, water temperature ranged from 20.1°C to 27.3°C, pH ranged from 6.99 to 8.49, and water depth ranged from 20 cm to 56 cm. Native crayfish behavioral responses to the invasive *Orconectes rusticus* (rusty crayfish) were examined for two different native crayfish species, *Cambarus bartonii* and *Orconectes propinquus*, which included 33 (9 female and 24 male) and 46 (21 female and 25 male) trials, respectively. Stream sites were selected from previously surveyed locations (Kuhlmann and Hazelton 2007; ML Kuhlmann, unpublished data) based on the presence of both rusty crayfish and at least one of the two native crayfish species. Due to low native crayfish abundances in the summer of 2012 at sites where both native and rusty crayfish were previously present, we included two additional sites (Figure 1) where rusty crayfish were not yet present (but present at nearby downstream sites) and native crayfish were in high abundance. On each day that these sites were used, rusty crayfish were brought from, and returned to, Charlotte Creek at the Pine Lake Environmental Campus, Hartwick College, Oneonta, NY.
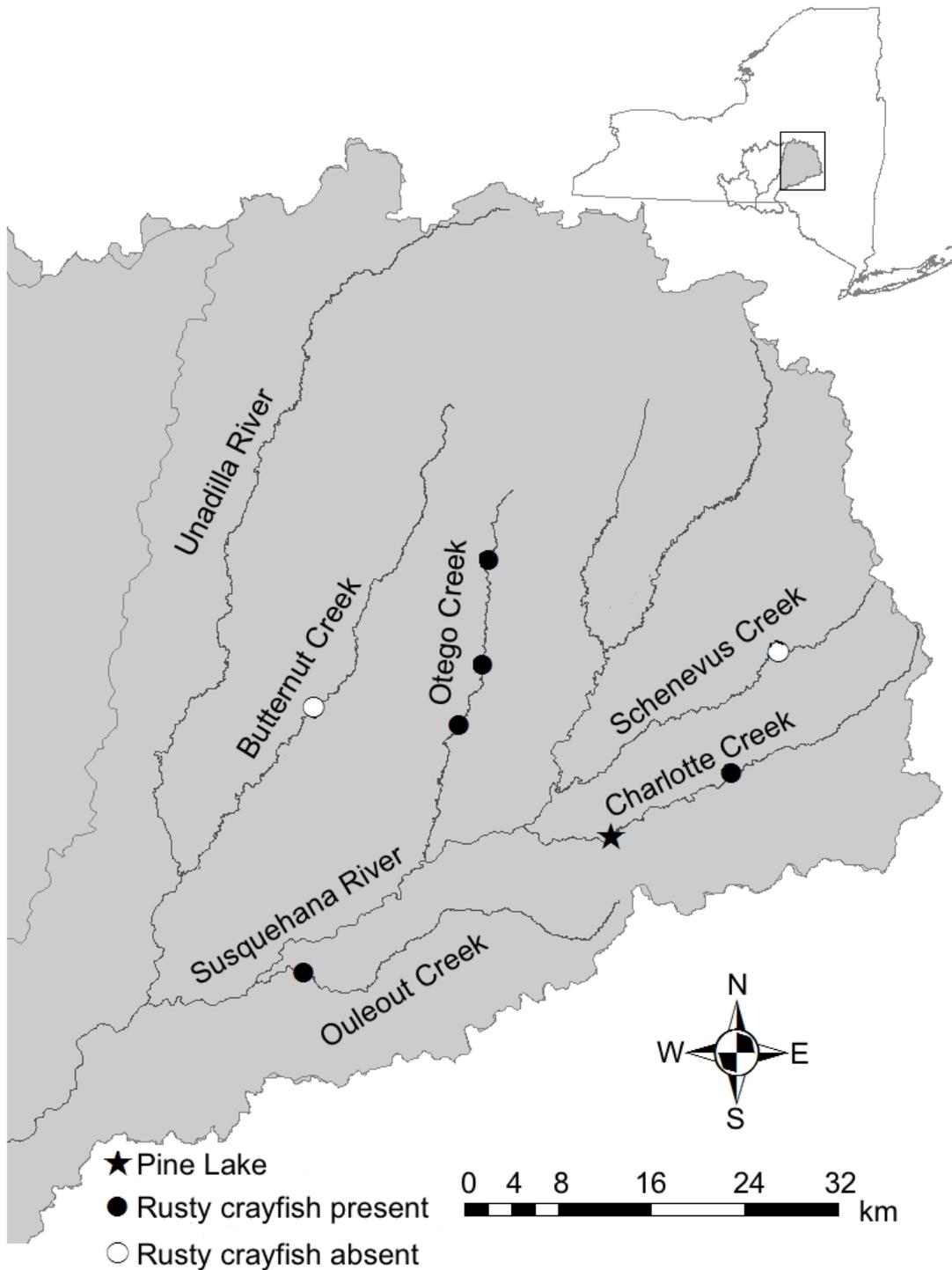
Figure 1. Behavioral trial study sites in the Upper Susquehanna River Basin, NY. Rusty crayfish were absent from two of the seven sites (white circles), thus behavioral trials between native and rusty crayfish were done using rusty crayfish temporarily transported from Charlotte Creek at Pine Lake, Hartwick College, NY (black star).

Experimental setup

Behavioral trials were conducted in a rectangular box arena constructed using 2.54 cm PVC pipe and plastic mesh netting and a 1 x 1 m detachable Plexiglass (Ridout Plastics, San Diego, California, USA) bottom. Arena locations were haphazardly selected 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 allow for video observation. If multiple behavioral trials were conducted at a site, arena locations were positioned 10 m upstream of the previous trial. Substrate for each behavioral trial was obtained from a location directly adjacent to the arena. To ensure consistency between trials, a bottomless five-gallon bucket was inserted 12 cm into the stream bottom and all substrate within the bucket was collected. Boulders along the circumference of the bucket were included if more than half of the boulder fell within the target area. Three substrate categories (gravel, cobble, and boulder) were separated using two sieves, 64 mm and 4 mm; larger than 64 mm was boulder, smaller than 64 mm but larger than 4 mm was cobble, and smaller than 4 mm was gravel. Each size category was weighed in order to quantify substrate composition and then remixed before adding to the arena.

Crayfish were captured using kicknets at each site and kept in individual containers to permit matching by size and sex. Each native crayfish (either *C. bartonii* or *O. propinquus*) was matched with a rusty crayfish of the same sex and size (carapace length difference < 10 %) for each trial (Rahm et al. 2005). Each crayfish was uniquely marked on the carapace with Wite-out (BIC, Shelton, Connecticut, USA) for ease of identification during video recording and to prevent repeated use if recaptured. The native crayfish was released in the center of the arena and given a two-minute acclimation period before the rusty crayfish was introduced. A 15 min trial was video recorded by an observer standing downstream of the arena. We ensured that

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crayfish could perceive the presence of each other, without forcing an interaction, by placing the rusty crayfish two body lengths in front of the native crayfish (Bergman and Moore 2003).

After each trial, the GPS coordinates (GeoExplorer3, Trimble Navigation Limited, Sunnyvale, CA), flow velocity (Flow Probe Model FP101, Global Water, Gold River, CA), water temperature, pH (Waterproof Palm pH Meter Model PH220A, Extech Instruments, Waltham, MA), dissolved oxygen (ExStik DO600, Extech Instruments, Waltham, MA), and water depth were recorded. Crayfish were removed from the arena and a walking leg was collected as a genetic sample from all three species for use in another study. The missing walking leg also served as an additional identification mark to prevent us from reusing crayfish, since Wite-out markings disappear after several days. The arena frame was then detached from the bottom and the crayfish were released in the body of water from which they came.

Video Analysis

Behaviors displayed by native crayfish were classified using a modified ethogram from Bergman and Moore (2003). Three agonistic behaviors were classified in order of increasing intensity level: meral spread, wrestling, and pinching. Meral spread behavior was defined as wide, raised claws directed towards the rusty crayfish within one body length away. Wrestling behavior was defined as the use of claws to actively push or wrestle with the rusty crayfish, but claws were not used to pinch. If the claws were used to pinch or grab the rusty crayfish the behavior was classified as pinching. If the native crayfish was in contact with the rusty crayfish, but not displaying any of the three agonistic behaviors, the behavior was classified as an interaction. For statistical analyses, the proportion of time native crayfish exhibited agonistic behavior (sum total of meral spread, wrestling, and pinching behavior) of the total trial time was calculated.

We also calculated the proportion of rusty crayfish retreats relative to the total number of encounters during a trial that resulted in one of the two crayfish retreating. A rusty crayfish retreat was classified based on the occurrence of a walk retreat, when the rusty crayfish walked backwards away from the native crayfish during an encounter or a swim retreat, when the rusty crayfish used a tail flip maneuver to quickly escape from the native crayfish.

Statistical Analyses

Several statistics were used to compare crayfish size, encounter lengths, and rusty crayfish retreats and are presented as the mean ± one standard deviation unless otherwise indicated. Two-sample t-tests were used to evaluate the possibility of size-based sexual dimorphism for each species and paired t-tests were used to confirm that size matched opponents did not differ significantly by size. Additional summary statistics were calculated to compare the encounter lengths and the amount of time native crayfish exhibited the individual behaviors: meral spread, wrestling, and pinching. Lastly, two-tailed binomial tests were used to determine if the percentage of rusty crayfish retreats from opponents (*C. bartonii* females, *C. bartonii* males, *O. propinquus* females, and *O. propinquus* males) differed from random chance.

Behavioral differences between male and female crayfish of each species and between the two native species of the same sex were evaluated by comparing the proportion of time each exhibited agonistic behavior using nonparametric Mann-Whitney U tests. We used a Bonferroni corrected $\alpha = 0.025$ because multiple comparisons were made, first comparing species of the same sex and then comparing sexes of the same species.

To test for a possible relationship between the abiotic environment and the proportion of time native crayfish exhibited agonistic behavior, nonparametric Spearman rank correlations were performed. First, principal components analysis was used to generate a single synthetic

variable from the six abiotic variables measured: proportion boulder, flow velocity, dissolved

oxygen, water temperature, pH, and water depth. Then the principal components that explained

the most variation in the dataset (PC1 and PC2; Table 1) were correlated with the proportion of

time native crayfish exhibited agonistic behavior towards rusty crayfish. We ran correlations

between both axes (PC1 and PC2) and several subsets of the behavioral data, including, *C.

bartonii* females, *C. bartonii* males, *O. propinquus* females, and *O. propinquus* males.

Table 1. Variable loadings for the first three principal
components (PC1, PC2, and PC3).

	Loading		
Variable	PC1 (0.293)	PC2 (0.233)	PC3 (0.176)
Proportion boulder	0.183	0.147	0.820
Flow velocity	-0.293	-0.604	-0.201
Dissolved oxygen	-0.255	-0.293	0.434
Water temperature	-0.645	0.275	0.190
pH	0.574	-0.418	0.138
Water depth	0.263	0.527	-0.209

Values in the parentheses represent the proportion of variation in
the dataset explained by each individual principal component.
The cumulative proportion of variation explained by the three
principal components was 0.702.

Results

The mean carapace length of *C. bartonii* females (30.5 ± 4.5 mm) used in this study was

similar to the mean carapace length of *C. bartonii* males (30.7 ± 4.8 mm; 2-sample t-test, $T = -$

0.11, $P = 0.910$; Table 2). There was a size difference between male (27.2 ± 1.9 mm) and female

(25.9 ± 2.3 mm) *O. propinquus*, (2-sample t-test, $T = -2.05$, $P = 0.047$; Table 2). There was no

difference in the sizes of paired crayfish (native and rusty crayfish) used in the trials (Table 2).

Table 2. Mean carapace length (± SD) of native (*Cambarus bartonii* or *Orconectes propinquus*) and invasive (*Orconectes rusticus*) crayfish with t-test results of trial pairings (native vs. invasive crayfish size) and native crayfish sexes (male vs. female sizes for each species; shown in grey).

Female trial pairings		Male trial pairings	
C. bartonii	*O. rusticus*	*C. bartonii*	*O. rusticus*
30 ± 4.5 mm	30.6 ± 3.3 mm	30.7 ± 4.8 mm	30.4 ± 4.6 mm
Paired t-test, $T = -0.22$, $P = 0.833$		Paired t-test, $T = 1.11$, $P = 0.278$	
C. bartonii female vs. *C. bartonii* male, 2-sample t-test, $T = -0.11$, $P = 0.910$			
O. propinquus	*O. rusticus*	*O. propinquus*	*O. rusticus*
25.9 ± 2.3 mm	26.0 ± 2.3 mm	27.2 ± 1.9 mm	27.3 ± 1.9 mm
Paired t-test, $T = -0.59$, $P = 0.560$		Paired t-test, $T = -0.74$, $P = 0.467$	
O. propinquus female vs. *O. propinquus* male, 2-sample t-test, $T = -2.05$, $P = 0.047$			

In 10 out of 79 trials, the native crayfish never encountered the rusty crayfish. On average

the crayfish only interacted 6% ± 1.1% of the time during the 15 min behavioral trial. In general,

encounters were relatively short (15.5 ± 31.8 seconds) although multiple encounters occurred in

68% of trials with an average of 4.4 ± 4.7 encounters per trial. Native crayfish exhibited meral

spread behavior in 49% of the trials, wrestling behavior in 47% of the trials, and pinching

behavior in 28% of the trials.

In encounters that resulted in a retreat by either crayfish, rusty crayfish retreated from *O.*

propinquus females (6.7 % of encounters), significantly less often than expected ($S = 1$, $P =$

0.001). The percentage of rusty crayfish retreats from *C. bartonii* females, *C. bartonii* males, and *O. propinquus* males was not significantly different from random chance ($S = 6$, $P = 0.508$; $S = 9$, $P = 0.146$; $S = 7$, $P = 0.359$ respectively). Rusty crayfish retreated from *C. bartonii* females 66.7 % of the time, from *C. bartonii* males 75.0 % of the time, and *O. propinquus* males 36.8 % of the time.

      C. bartonii and *O. propinquus* showed significant differences in the median proportion of time they exhibited agonistic behavior (Figure 2). *Cambarus bartonii* females displayed agonistic behaviors more than *O. propinquus* females ($W = 207.0$, $P = 0.001$), but *O. propinquus* males exhibited agonistic behaviors with rusty crayfish more often than *C. bartonii* males ($W = 417.5$, $P < 0.001$). There were also significant differences in agonistic behavior between male and female native crayfish (Figure 2). *Cambarus bartonii* females exhibited agonistic behavior more than males during encounters with rusty crayfish ($W = 218.5$, $P = 0.006$). However, *O. propinquus* males exhibited agonistic behavior more than females ($W = 305.0$, $P < 0.001$).
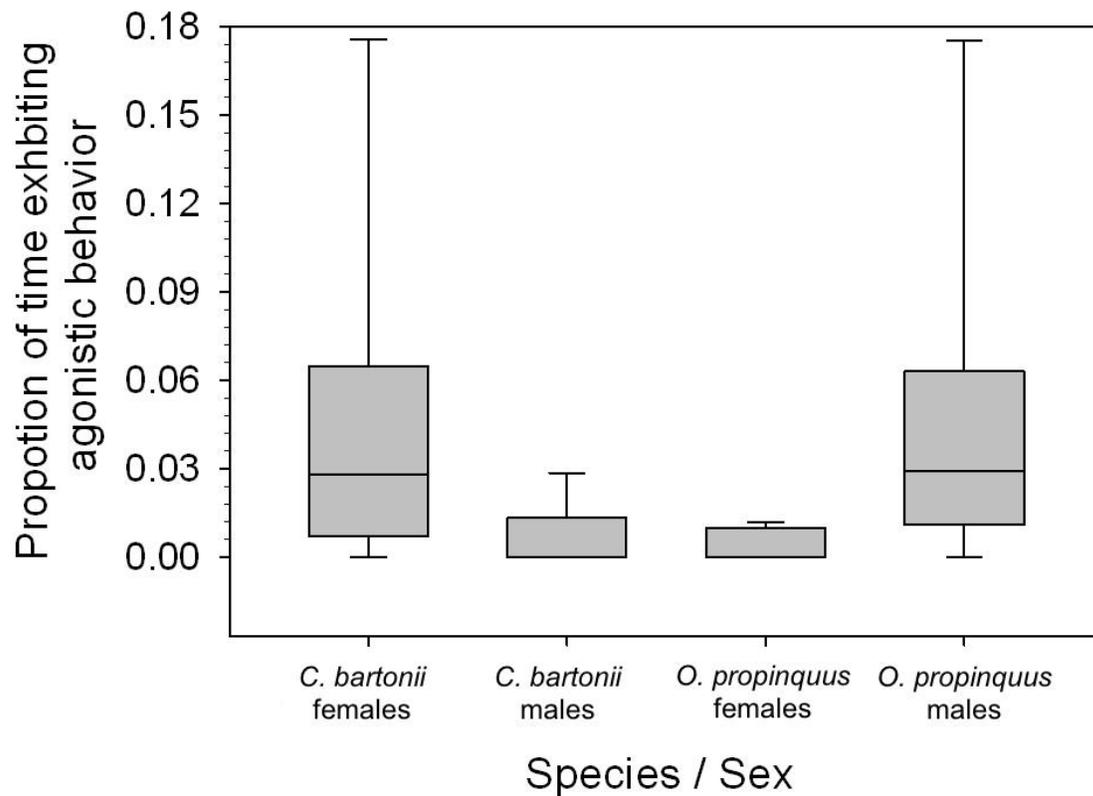
Figure 2. Median proportion of time during crayfish behavioral trials where native individuals (*Cambarus bartonii* and *Orconectes propinquus*) exhibited agonistic behavior toward rusty crayfish (*Orconectes rusticus*). Each trial consisted of a 15 minute observational period between one native crayfish and one invasive crayfish (*Orconectes rusticus)* of the same size and sex within an arena anchored in a natural stream (sites shown in Figure 1). The sample sizes for each of the species / sex categories shown along the x-axis include, 9 *C. bartonii* females, 24 *C. bartonii* males, 21 *O. propinquus* females, and 25 *O. propinquus* males. The box plot shows the median value (middle horizontal line), upper and lower quartiles (upper and lower limits of the box) and standard errors (range bars extending beyond the box).

The first principal component, composed of six abiotic factors (proportion boulder, flow velocity, dissolved oxygen, water temperature, pH, and water depth) explained 29.3% of the variation in the data set, while the second component explained 23.3% of the variation (Table 1). Female *C. bartonii*, female *O. propinquus*, male *C. bartonii*, and male *O. propinquus* behaviors were not significantly correlated with PC1 (respectively: $r_s = 0.100$, $P = 0.798$; $r_s = 0.034$, $P = 0.884$; $r_s = 0.0043$, $P = 0.843$; $r_s = 0.331$, $P = 0.106$), nor were behaviors correlated with PC2 (respectively: $r_s = 0.617$, $P = 0.077$; $r_s = 0.181$, $P = 0.432$; $r_s = -0.019$, $P = 0.930$; $r_s = 0.068$, $P = 0.748$).

Discussion

The mechanisms contributing to the patchy distribution pattern of the invasive rusty crayfish in the Upper Susquehanna River Basin are poorly understood. Previous studies have evaluated the characteristics of rusty crayfish, including behavioral characteristics that provide the species with a competitive advantage when introduced into novel environments (Larson and Magoulick 2009; Pintor et al. 2009; Pintor and Sih 2009). Our study is one of the few that consider how characteristics of existing crayfish communities contribute to the ability of the rusty crayfish to successfully invade (Capelli and Magnuson 1983; Olden et al. 2011) and is the first to directly measure agonistic behaviors by native crayfish in the field.

We found significant differences in native crayfish behavioral defense against rusty crayfish based on species and sex. *Orconectes propinquus* were more likely to retreat than *C. bartonii* when confronted by a rusty crayfish. Although crayfish were size and sex matched for each behavioral trial, the average size of *O. propinquus* in the local population was less than either *O. rusticus* or *C. bartonii* (ML Kuhlmann, unpublished data). Because past experience

influences outcomes of future bouts (Rubenstein and Hazlett 1974) and larger crayfish are usually dominant over smaller crayfish (Pavey and Fielder 2009), *O. propinquus* individuals may have been pre-disposed to retreat as they were more likely to have encountered and lost to a larger crayfish (either *C. bartonii* or *O. rusticus*) than *C. bartonii* individuals. Often, male crayfish are more aggressive than females (Bruski and Dunham 1987), but our results show this generality may vary by species, as *C. bartonii* females were more aggressive than males. Male crayfish may be more aggressive during the mating season as female crayfish can obtain information important for mate selection by observing male vs. male interactions (Aquiloni et al. 2008). However, the sex differences we observed are unlikely due to behavioral modification during the mating season, as only Form II (non-reproductive) males were used in behavioral trials and two trials where mating behavior occurred between males (flipping opponents and pinning down chelipeds) were excluded from our analyses.

Differences in agonistic behavior between species have been previously described for same-species encounters in the laboratory (Hudina and Hock 2012), but our study shows that interspecific encounters of native and rusty crayfish differ by species in natural environments. Since the level of aggression displayed by native crayfish towards rusty crayfish differs by species, the species composition of an invaded community may influence the ability of rusty crayfish to successfully colonize and spread. Based on our findings, future study is necessary to empirically test whether native crayfish species composition is a mechanism driving the patchy distribution pattern of rusty crayfish not only in the Upper Susquehanna River Basin, but also across the contiguous United States (USGS; http://nas.er.usgs.gov:80/queries/collectioninfo.aspx?SpeciesID=214; last date accessed 02/06/2013).

Lastly, we did not observe enough evidence to suggest that variation in abiotic environment is correlated with native crayfish agonistic behaviors. However, the possibility of such a relationship cannot be dismissed entirely because our study area was restricted to a portion of a drainage basin and only six abiotic variables were measured.

In this study, we provide a behavioral perspective for understanding the jump dispersal invasion pattern of rusty crayfish, a common distribution pattern for many invasive species. Our results highlight the complexity of crayfish behavior, as agonistic behavior varies by species and sex. Consequently, native crayfish species composition and demographic characteristics within populations, such as sex ratio may influence the biotic resistance of communities to rusty crayfish invasions. Although species interactions between native and invasive individuals may reduce the establishment of individual invaders, inter-species interactions may not completely repel invasions (Levine et al. 2004). Future studies are necessary to determine if this observation, from exotic plant invasions, exists in crayfish communities. Another mechanism that has been suggested as the driver of current rusty crayfish distribution patterns is the occurrence of multiple anthropogenic introductions by anglers using crayfish as live bait (Lodge et al. 2000; Puth and Allen 2005; DiStefano et al. 2009), but this mechanism has yet to be supported empirically. Until the mechanisms driving exotic crayfish species introductions are better understood, the success of invasive species management plans are limited to proactive policies, such as the enactment of fishing regulations prohibiting the transport of live crayfish between water bodies (Dresser and Swanson 2013).

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MANUSCRIPT II

GENETIC EVALUATION OF AN ANTHROPOGENIC INTRODUCTION OF RUSTY
CRAYFISH (*ORCONECTES RUSTICUS*)

Abstract

The spread of exotic species is often attributed to anthropogenic introductions,
particularly for bait species, such as the rusty crayfish (*Orconectes rusticus*). We used amplified
fragment length polymorphisms to determine if multiple genetic populations were present in the
Upper Susquehanna River Basin, NY, USA, which due to their recent introduction would
suggest that multiple anthropogenic introductions have occurred. In 2011, we sampled crayfish
from a total of seven sites above and below a 50 km distribution gap observed in 1999. We found
only one genetic population of rusty crayfish with low genetic differences between sample sites,
but with private alleles at each. The genetic similarity between sample sites on either side of the
historic distribution gap and the prevalence of private alleles at all sample sites suggests that at
least two introductions have occurred from the same source population. Understanding the
number of sources from which anthropogenic introductions occurs facilitates our ability to
prevent future introductions and initiate appropriate management regimes and regulations.

Introduction

The successes of invasive species control strategies rely on the extent of knowledge
available to managers. Typically, the consequences of invasive species (Lodge 1993; Simberloff
et al. 2013) and the qualities of the invasive species and the invaded ecosystem (Strayer 2010)
are among the first elements to be examined. Unfortunately, this information is obtained
subsequent to exotic species establishment, which makes information pertaining to the

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mechanisms of introduction and spread more difficult to obtain. The spread of many invasive species deviate from the continuous range expansion assumed by the standard reaction-diffusion model (wavefront dispersal), resulting in an underestimated of the rate and extent of spread (Buchan & Padilla 1999). Rather, several invasions exhibit discontinuous range expansion known as jump dispersal (Holway & Suarez 1999; Suarez *et al.* 2001; Rödder 2009). Jump dispersal distribution patterns are often attributed to multiple anthropogenic introductions, but can be cryptic when invasive species presence is detected long after introduction (Geller *et al.* 1997). Ultimately, the absence of survey data during the initial colonization phase of an invasive species limits our ability to determine if a patchy distribution is the result of multiple introductions or wavefront dispersal and subsequent site abandonment.

The spread of rusty crayfish (*Orconectes rusticus*) through the Upper Susquehanna River Basin (USRB), NY, USA has demonstrated the patchy distribution pattern characteristic of jump dispersal invasion. Although the precise location and timing of rusty crayfish introduction into the basin is unknown, suggestions have been made that rusty crayfish were first introduced to Otsego Lake at the headwaters of the Susquehanna River, in the early 1970s (Kuhlmann & Hazelton 2007). Furthermore, survey data regarding rusty crayfish distribution is available for the USRB from 1999 thru 2010 (Fig. 1; M. Kuhlmann, unpublished data). The survey data indicated that although rusty crayfish have spread across the basin, they were still absent from some intermediate river sections.
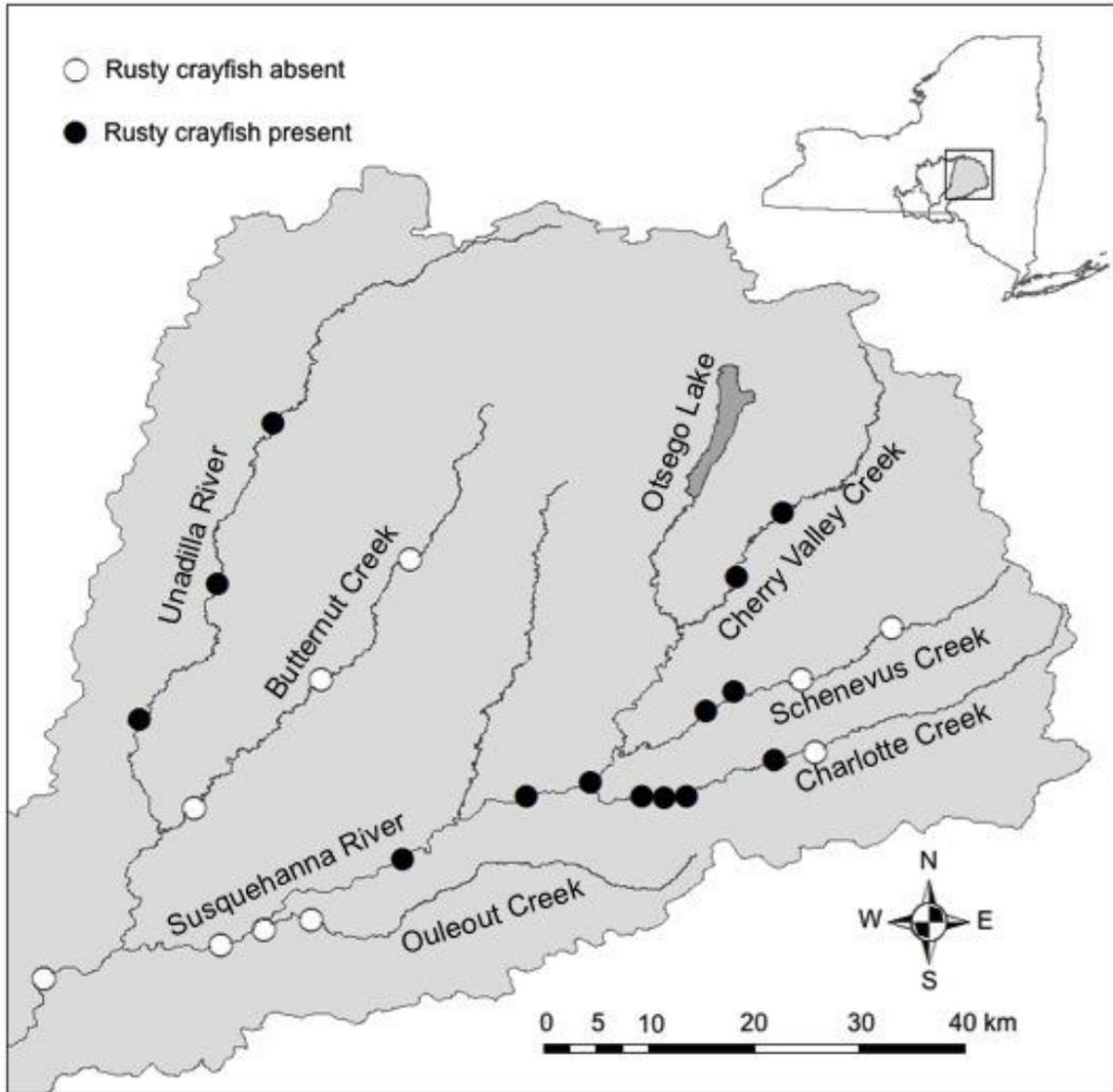
Figure 1. Rusty crayfish (*Orconectes rusticus*) distribution data within the Upper Susquehanna River Basin, NY in 1999 (M. Kuhlmann, unpublished data). Each circle represents a survey site where crayfish were collected using kick-net and hand netting sampling techniques between June and August. White circles represent sites where rusty crayfish were absent and black circles represent sites where rusty crayfish were present. Otsego Lake was the suggested introduction point in the 1970s.

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The successful invasion of rusty crayfish in the USRB is likely due to their life history characteristics (e.g., high dispersal rate, Wilson *et al.* 2004; high abundance, Jansen *et al.* 2009; habitat generalists, Freeman *et al.* 2010) and ecosystem characteristics (e.g., abiotic environment, Capelli & Magnuson 1983; species composition, Olden *et al.* 2011). Rusty crayfish are also a popular bait species among anglers, which has prompted the suggestion that multiple anthropogenic introductions have contributed to crayfish invasion patterns (Ludwig & Leitch 1996; DiStefano *et al.* 2009), but no studies have provided empirical evidence that multiple introductions have occurred or contributed to the rapid spread of crayfish within a river basin.

The objective of our study was to test the hypothesis that multiple anthropogenic introductions of rusty crayfish from different sources have occurred in the Upper Susquehanna River Basin. We used amplified fragment length polymorphisms (AFLPs) to determine the number of genetic populations and the population structure of rusty crayfish within the USRB basin, for which we have survey data suggesting multiple introductions (Fig. 1). The presence of multiple genetic clusters (i.e., populations) and high genetic diversity between sample sites would indicate that multiple anthropogenic introductions have occurred from different source populations. The presence of a single genetic cluster and low genetic diversity between sample sites would indicate that either one anthropogenic introduction with subsequent dispersal and site abandonment had occurred or multiple anthropogenic introductions from the same source population produced the distribution gap observed in 1999 (Fig. 1).

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Methods

Sampling

We collected a total of 210 rusty crayfish from seven stream sites, including five tributary sites and two sites on the Susquehanna River in the northern portion of the Upper Susquehanna River Basin, NY, USA (Fig. 2). Sites were selected based on known crayfish distribution obtained from previous survey data (M. Kuhlmann, unpublished data). As part of our sampling design, we selected sites upstream and downstream of the distribution gaps revealed by the 1999 survey data. All samples were collected in the summer of 2011 between June 17th and July 13th. Crayfish were sampled using kick-nets and a walking leg was collected, dried, and stored in a manila envelope until extraction.
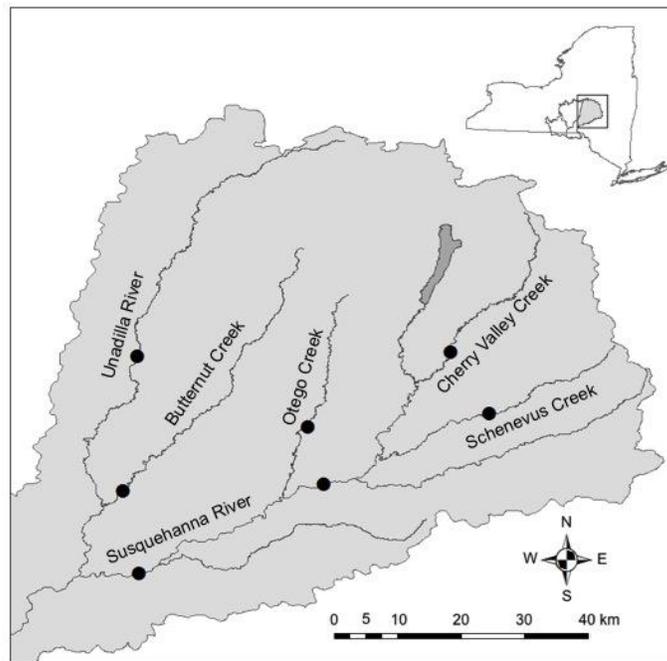
Figure 2. Seven rusty crayfish (*Orconectes rusticus*) sample sites in the Upper Susquehanna River Basin, NY. A walking leg was collected as a genetic sample for 30 individuals from each site.

DNA extraction and AFLP amplification

We extracted DNA from the walking legs using DNeasy tissue kits (QIAGEN Inc., Valencia, CA). We modified the AFLP Plant Mapping Protocol (Life Technologies Corp., Carlsbad, CA) by performing 30 PCR cycles rather than 20 cycles during the final selective amplification step of the protocol. The template DNA was digested with MseI and EcoRI enzymes and the associated adaptor pairs were annealed by incubating the solution for two hours at 37°C. The restriction-ligation samples were then diluted with 189 uL of $TE_{0.1}$ buffer to obtain the appropriate concentration for subsequent PCR. The preselective amplification thermal cycler parameters included two minutes at 72°C, then 30 cycles of 20 seconds at 94°C, 30 seconds at 56°C, and two minutes at 72°C, followed by 30 minutes at 60°C. Preselective amplification products were diluted with 190 uL of $TE_{0.1}$ buffer prior to the subsequent PCR. We used one primer pair combination for selective amplification, *Mse*I-CAC and a fluorescently labeled *Eco*RI-TAG (5~HEX). The thermal cycler parameters for selective amplification were the same as preselective amplification except a touch-down PCR was used for the first 10 cycles (66°C − 56°C with one degree drop each cycle), followed by 20 cycles using a 56°C annealing temperature. The fluorescently labeled amplified fragments were visualized using an ABI Prism 3130 Genetic Analyzer (Life Technologies Inc., Foster City, CA) and analyzed with GeneMapper 4.0 software (Life Technologies Inc., Foster City, CA). We included bands (fragments) between 50 and 600 bp. We also repeated the process with four individuals, to ensure our initial results could be reproduced and to calculate the genotyping error rate.

Genetic Analyses

We used the Bayesian analysis program STUCTURE V.2.2.3 (Pritchard *et al.* 2000) to construct populations based on genotypic similarities. In STRUCTURE V.2.2.3 genotypes themselves are classified as unknown rather than designating loci as haploid and the second allele as unknown, which is a method used in other programs (Bonin *et al.* 2007). We were not able to use *a priori* locations, as the additional algorithm used in STRUCTURE to accommodate binary data is not included in *a priori* analyses. We assumed the admixture model and performed five iterations for each value of K (ranging from one to seven), with a burn-in of 50,000 steps and MCMC length of 100,000 steps. We plotted the log-likelihood scores for each K (Evanno *et al.* 2005) to determine the most likely number of genetic clusters (or populations) in the dataset using STRUCTUE HARVESTER (Earl & vonHoldt 2012).

For each sample site, we performed an analysis of molecular variance (AMOVA) in GENALEX 6.5 (Peakall & Smouse 2012) to calculate pairwise genetic differences (ϕ_{PT}) and we calculated pairwise Nei's genetic distances. We also tested for allele homoplasy using a Spearman Rank Correlation between band frequency and fragment size for all sample sites. Lastly, we tested for genetic isolation by distance using a Mantel test in GENALEX, which looks for a correlation between the Nei's genetic distance matrix and the geographic distance matrix based on 999 random permutations of the data.

Results

The single selective primer pair produced 190 bands, with an average of 3.19 $\pm$ 2.77 ($\pm$ SD) bands per individual. We successfully amplified DNA from 198 individuals; 30 individuals from Schenevus Creek, 29 from the downstream Susquehanna River site, 28 from the upstream Susquehanna River site, 30 from Cherry Valley Creek, 27 from Butternut Creek, 27 from

Unadilla River, and 27 from Otego Creek. The average percentage of private bands across

sample sites was 14.8 % (55 out of 190 bands); 9.8 % (4 out of 41 bands) for Schenevus Creek,

11.9 % (10 out of 84 bands) for the lower Susquehanna River, 14.8 % (9 out of 61 bands) for the

upper Susquehanna River, 12.5 % (3 out of 24 bands) for Cherry Valley Creek, 17.9 % (5 out of

28 bands) for Butternut Creek, 24.1 % (19 out of 79 bands) for Unadilla River, and 12.8 % (5 out

of 39 bands) for Otego Creek. The calculated genotyping error rate (0.095) fell below the

recommended maximum allowable error rate of 0.1. We did find a negative correlation between

the size of the amplified fragment and the frequency of occurrence ($r_s = -0.445$, $P < 0.001$),

suggesting the presence of allele homoplasy in the dataset.

Estimated likelihood of K and Evanno's K indicated that the most likely number of

populations was two ($LnP(K_1) = -2677.74$, $\Delta K_1 = NA$; $LnP(K_2) = -2535.68$, $\Delta K2 = 132.25$;

$LnP(K_3) = -2649.96$, $\Delta K_3 = 1.66$; $LnP(K_4) = -2837.54$, $\Delta K_4 = 4.84$; $LnP(K_5) = -2748.54$, ΔK_5

$= 1.44$; $LnP(K_6) = -2710.64$, $\Delta K_6 = 0.95$; $LnP(K_7) = -2706.00$, $\Delta K_7 = NA$); however, since the

Evanno method cannot evaluate the likelihood of $K = 1$ and based on the $K = 2$ Q-plot outputted

from STRUCTURE (Fig. 3) we concluded that the most likely number of genetic clusters was

one. Additionally, 53.5 % of all individuals were admixed; 63.3 % for Schenevus Creek, 62.1 %

for the lower Susquehanna River, 57.1 % for the upper Susquehanna River, 26.7 % for Cherry

Valley Creek, 51.9 % for Butternut Creek, 77.8 % for Unadilla River, and 37.0 % for Otego

Creek. The overall genetic differentiation among sample sites was low for both measures of

genetic distance, ϕ_{PT} and Nei's genetic distance (Table 1). Pairwise values of ϕ_{PT} ranged from 0

to 0.069 with a global ϕ_{PT} of 0.016 ($P = 0.001$) and Nei's genetic distance values ranged from 0

to 0.001 . The Mantel test indicated that genetic differences are unlikely a result of isolation by geographic distance ($r = 0.007$, $P = 0.378$).
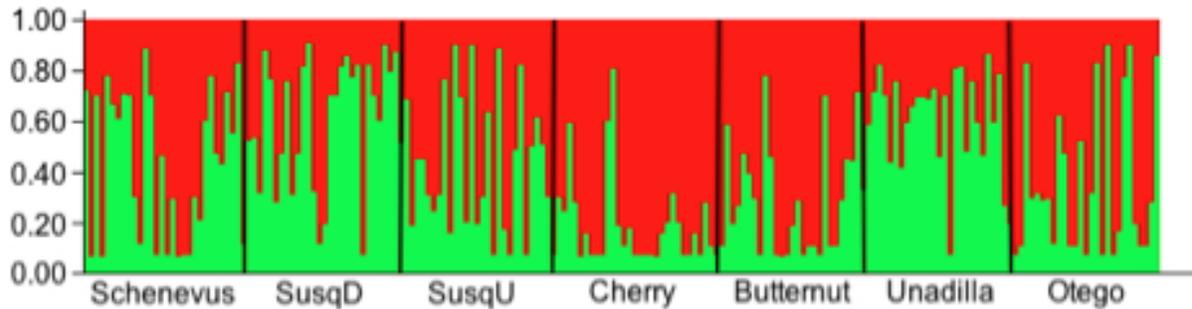
Figure 3. K =2 Q-plot of rusty crayfish (*Orconectes rusticus*) genetic population clusters assigned by program STRUCTURE from the Upper Susquehanna River Basin, NY. Each vertical bar represents an individual crayfish partitioned into K = 2 segments. The proportion of each color per individual signifies the probability that the individual belongs to each of the two populations. The abbreviation SusqD and SusqU represent the downstream and upstream sample sites on the Susquehanna River (left and right site respectively shown in Figure 2).

Discussion

In this study, we tested the hypothesis that multiple anthropogenic introductions of rusty crayfish have occurred in the Upper Susquehanna River Basin. Previous survey data illustrated a jump dispersal distribution pattern (Fig. 1; M. Kuhlmann, unpublished data); however, the mechanisms contributing to the distribution were unclear. Jump dispersal can result from unusual dispersal opportunities, such as flooding, but can also result from multiple anthropogenic introductions (MacIsaac *et al.* 2001). Using genetic techniques we were able to distinguish between three possible explanations for the patchy distribution of rusty crayfish, multiple introductions from multiple source populations, one introduction followed by dispersal and site abandonment, or multiple introductions from the same source population.

Table 1. Pairwise comparisons of Nei's genetic distance (above the diagonal) and ϕ_{PT} values (below the diagonal) for rusty crayfish (*Orconectes rusticus*) from seven sample sites in the Upper Susquehanna River Basin, NY, USA

	Schenevus	Susque-hanna (Down)	Susque-hanna (Up)	Cherry Valley	Butternut	Unadilla	Otego
Schenevus	----	0.000	0.000	0.001	0.000	0.000	0.000
Susque-hanna (Down)	0.009	----	0.000	0.001	0.000	0.000	0.000
Susque-hanna (Up)	0.017	0.003	----	0.000	0.000	0.001	0.000
Cherry Valley	0.067*	0.040*	0.019	----	0.000	0.001	0.001
Butternut	0.010	0.010	0.004	0.038	----	0.000	0.000
Unadilla	0.009	0.000	0.007	0.048*	0.007	----	0.000
Otego	0.000	0.011	0.010	0.069*	0.010	0.010	----

*Significant ϕ_{PT} values based on a Bonferroni corrected $\alpha = 0.0023$

We were able to rule out the possibility that multiple introductions from multiple source populations occurred, due to the presence of a single genetic cluster and low genetic differentiation between sample sites. Although ΔK suggested that the most likely number of genetic clusters was two, this is biologically unlikely given the geographic scattering of individuals within each cluster and the high proportion of admixed individuals. If even low levels of population structure were present, our sample size (30 individuals per site) and the number of informative loci (190) used were adequate to detect population structure (Bonin *et al.* 2007). However, the suitability of these standards may vary by species (Cavers *et al.* 2005) and our genetic distance measures should be interpreted cautiously as they may be biased downwardly due to allele homoplasy. The presence of a single genetic cluster is unlikely a result of homogenization between multiple introductions from different source populations that have mixed via dispersal. The river distance between our two farthest sites (Cherry Valley Creek and Unadilla Creek) is approximately 130 km and with an average dispersal rate of 5 km/yr (Dresser & Swanson 2013) it would take 26 years for at least one migrant to disperse from the Cherry Valley Creek site to the Unadilla Creek site. Even the distance between the two closest sites where rusty crayfish were present in 1999 is still about 50 km and would take crayfish about 10 years to disperse between (unidirectional). Since our genetic samples were collected 12 years after the initial survey it is unlikely that the effective number of migrants needed for homogenization of populations actually dispersed.

We also suggest that the distribution gap present in 1999 is unlikely a result of site abandonment by rusty crayfish following a single introduction event, as more recent survey data show high abundances of rusty crayfish at sites within the distribution gap present in 1999 (M. Kuhlmann, unpublished data). Furthermore, the reasons rusty crayfish would temporarily

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abandon specific stream sections are unclear. Rusty crayfish are classified as habitat generalists and can be found in a wide variety of habitats across the country, thus it is unlikely that small stream sections within the same river basin would be inhabitable for rusty crayfish (Capelli & Magnuson 1983). Additionally, if rusty crayfish spread along a wavefront from the proposed introduction point at Otsego Lake, we would expect to see fewer private alleles (bands) at sites closest to Otsego Lake since those sites represent the founding population and should contain all of the alleles present in the subsequent downstream sites. However, the frequency of private alleles is relatively high for all sample sites regardless of geographic location. Furthermore, there was no correlation between pairwise genetic distance and geographic distance matrices supporting the likelihood that multiple introductions occurred rather than wavefront dispersal from a single introduction point (Wilson *et al.* 1999).

The presence of a single genetic cluster, low genetic differentiation between sites, high prevalence of private alleles, no isolation by distance, and 1999 survey data showing a patchy distribution of rusty crayfish, suggests that multiple anthropogenic introductions have occurred from a single source population. The amount of admixture seen in the Q-plot outputted by STRUCTURE could be indicative of a mixed source population; over half of the samples show a mixture of genotypes from two populations. It is unlikely that this arose from the mixing of individuals post-introduction from two genetic populations because of the distances and completeness across all of our sample locations. Rather, the source of rusty crayfish introductions into the USRB may have been comprised of two populations. For example, if a regional bait shop were the source and the bait shop purchased crayfish from different providers or a single provider who used two different populations; the bait shop would have a mixed population of crayfish. The use of multiple providers by bait shops is not unusual, as multiple

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bait shops in Missouri reported using a combination of methods, including ordering crayfish from out of state, catching crayfish locally, and farming their own bait in private ponds (DiStefano *et al.* 2009). The high frequency of unique alleles we observed has also been observed and attributed to multiple introductions in other systems, such as round goby introductions in North America (Brown & Stepien 2008). We attributed the lack of isolation by distance to the occurrence of multiple introductions, which was also an interpretation made by Wilson *et al.* (1999) to explain the spread of quagga mussels.

Multiple anthropogenic introduction events present a challenge to managers striving to not only contain the range expansion of aquatic invasive species, but to predict when and where the species will distribute through connected waterways. The presence of a single source population contributing to introductions within the Upper Susquehanna River Basin can improve the ability of managers to prevent further introductions of rusty crayfish, as limited resources can be allocated more efficiently at the single source location. Educational outreach programs and more stringent policies regarding bait transfer can be implemented to minimize future introductions (DiStefano *et al.* 2009, Dresser & Swanson 2013). Invasive species spread resulting from multiple introductions from multiple sources may present more challenging issues to overcome, but statewide policies prohibiting the transport of all live crayfish between water bodies would significantly retard the anthropogenic spread of rusty crayfish (Dresser & Swanson 2013). Preemptive legislation would likely reduce the spread of other invasive species which have multiple source populations, such as the introduction of *Anoles* lizards in Florida and the Dominican Republic (Kolbe *et al.* 2007), Eurasian annual grass in the western USA (Novak & Sforza 2008), and swamp eels in the southeast USA (Collins *et al.* 2002).

Genetic analyses can be a valuable tool for understanding the mechanisms of biological invasions, particularly as it pertains to multiple anthropogenic introductions (Darling & Folino-Rorem 2009; LaRue et al. 2011). Although identification of source populations may be retroactive for communities that have already been invaded, such as the invasion of rusty crayfish in the Upper Susquehanna River Basin, measures taken to prevent additional introductions from a source can protect the remaining uninvaded communities.

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MANUSCRIPT III

PREEMPTIVE LEGISLATION INHIBITS THE ANTHROPOGENIC SPREAD OF AN AQUATIC INVASIVE SPECIES, THE RUSTY CRAYFISH (*ORCONECTES RUSTICUS*)

Abstract

The enactment of legal policies is often recommended to prevent anthropogenic introductions of invasive species. In this paper, we evaluated the effectiveness of proactive state legislative policies in deterring colonization by rusty crayfish (*Orconectes rusticus*) and the expected spread into previously uninvaded states using network-based spatial analysis. We found that the presence of regulations was positively associated with the presence of rusty crayfish ($p = 0.027$), but often regulations were put into effect subsequent to the invasion. Regulations that did not explicitly prohibit transport, applied to specific drainages, or prohibited only rusty crayfish were not effective. However, preemptive legislation was effective in reducing the likelihood of invasion, if regulations prohibited the transport of all live crayfish species between water bodies, as only 1 state which passed such legislation prior to invasion by rusty crayfish was subsequently colonized ($S = 12$, $p = 0.031$). Five states are likely to be invaded by rusty crayfish via range expansion across interstate drainages, and dispersal rates suggest that all of these states could be colonized within 10 years. While it is unlikely that regulations will prevent dispersal-based invasion across state lines through shared drainages, preemptive legislation can significantly reduce anthropogenic spread of aquatic invasive species between drainages and effectively retard the expansion of rusty crayfish. Our results suggest that the most effective form of legislation is one that does not require individuals to identify species, thus we recommend states enact policies that explicitly prohibit the transport of all live crayfish between water bodies.

Introduction

Anthropogenic introductions of non-native species, especially those used as live bait, such as crayfish have led to the establishment of invasive populations, which negatively impact existing communities. For example, the introduction of *Procambarus clarkii* has contributed to habitat degradation and biodiversity loss in the northeast United States (Antonelli et al. 1999) and in 25 European countries (Gherardi 2006). Legislative policies have been enacted to prevent anthropogenic introductions of exotic species; however policy enactment is still lacking for many invasive species (Fowler et al. 2007). The development of legal policies is an attractive management strategy as the cost associated with development and passage of such regulations is comparatively less expensive than having to react to, and manage, invasions after they occur (Keller et al. 2008). Enactment of legislative policies also improves our ability to standardize policies across ecologically relevant units and increase public awareness regarding the risks associated with anthropogenic introductions of exotic species. Unfortunately, as with any management strategy, there are limitations to legal policies. The timing of policy enactment is critical in regards to effectiveness, as policies that are enacted reactively are less effective than proactive policies (Peters and Lodge 2009). The degree of effectiveness is also dependent on cooperation across political boundaries because range expansion of invasive species is constrained by ecological boundaries rather than political boundaries. Furthermore, legislative policies may be more effective for species inhabiting terrestrial ecosystems than aquatic ecosystems, as species tend to spread more quickly in aquatic environments (Kinlan and Hastings 2005). Thus, proactive policy enactment is even more critical for exotic aquatic species.

One aquatic species that has been introduced outside of its native range and is now considered invasive throughout most of the United States is the rusty crayfish (*Orconectes rusticus*). Since the late 1960s, the distribution of rusty crayfish has rapidly spread beyond the Ohio River Basin (Fig. 1), displacing native crayfish species (Taylor and Redmer 1996) through hybridization (Perry et al. 2001b) and resource competition (Hill and Lodge 1999). The invasive ability of the rusty crayfish stems from their high genetic diversity (Yue et al. 2010), high abundance (Jansen et al. 2009), high dispersal rate (Wilson et al. 2004), and their role as habitat and resource generalists (Freeman et al. 2010).   In addition to the displacement of native crayfish species, rusty crayfish prey on fish eggs (Horns and Magnuson 1981) and contribute more strongly to the decline of game fish than native crayfish species due to their high abundance, large chelae, and aggressive nature (Roth and Kitchell 2005).
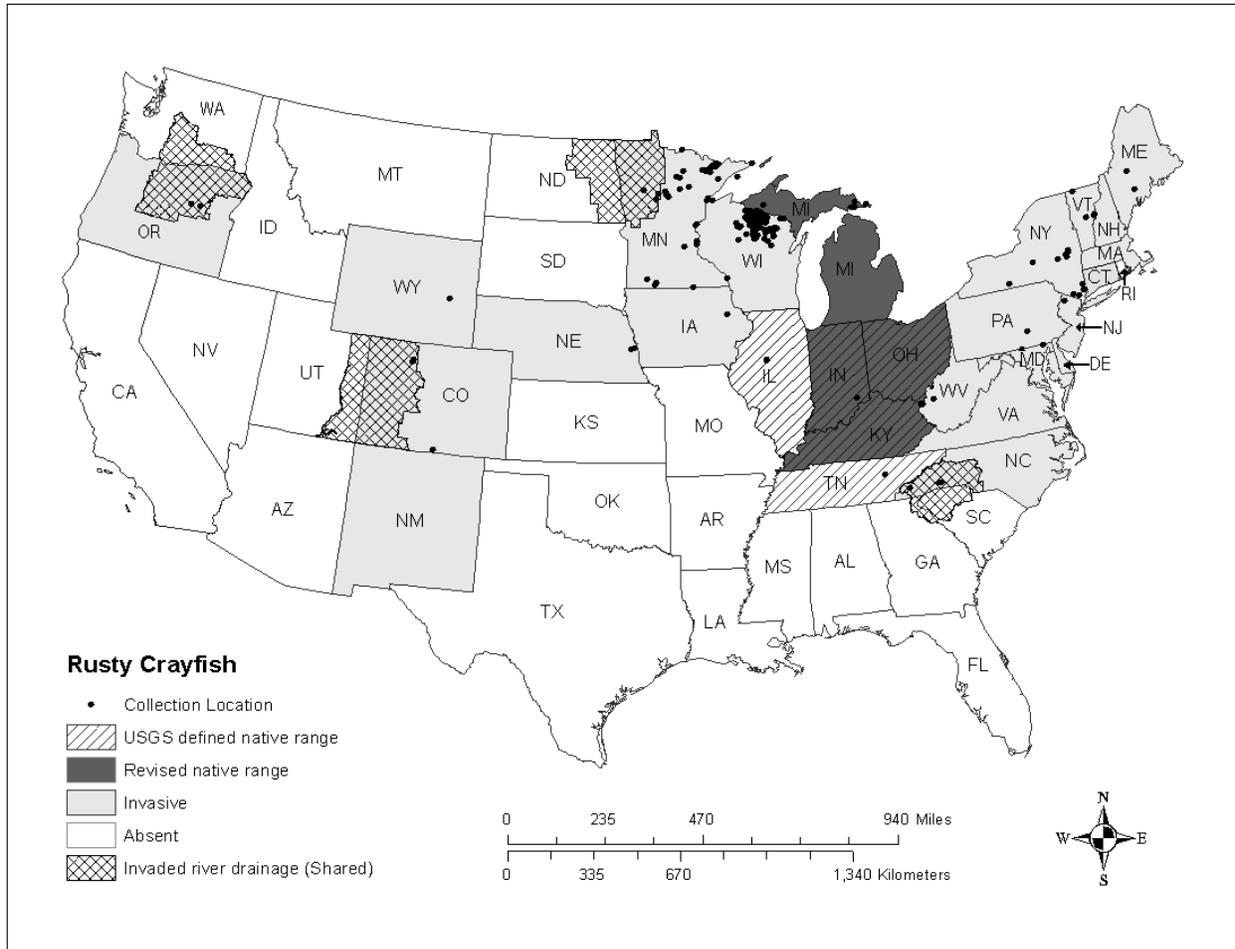
Figure 1. Distribution of rusty crayfish (*Orconectes rusticus*) within the contiguous United States by state. The United States Geological Survey (USGS) defines the native range of rusty crayfish to be IN, IL, KY, OH, and TN (diagonal lines); however, state and university crayfish researchers argue that the native range should be revised to IN, MI, OH, and KY (dark gray). Rusty crayfish are considered non-native in all remaining states; both invaded (light gray) and not invaded states (white). Four invaded river drainages are shared with five uninvaded states (cross-hatching). River distances from known collection points (black dots) to uninvaded state lines (black lines) along with known dispersal rates were used to estimate the time of rusty crayfish arrival into a previously uninvaded state through dispersal-based range expansion of antropogenically-introduced populations.

The suspected primary cause of rusty crayfish introduction is the transfer of live crayfish, for use as bait, between water bodies by anglers (Lodge et al. 2000; DiStefano et al. 2009). Repeated human introductions over time could exacerbate the rate of spread and increase the difficulty of controlling this invasive species. Several studies have recommended the enactment of proactive regulations to stop anthropogenic introductions (Puth and Allen 2005; Taylor et al. 2007; DiStefano et al. 2009), but the intention of our study was to quantitatively evaluate the effectiveness of regulations to determine if regulation enactment is an effective strategy to control invasive species. While previous studies have evaluated legislative policies in specific regions within the United States (DiStefano et al. 2009, Peters and Lodge 2009), our review is a nationwide evaluation of the effectiveness of state legislative policies in deterring invasion by rusty crayfish. The three objectives of this study were to test for an association between regulations and rusty crayfish, evaluate the effectiveness of regulations, and estimate rusty crayfish arrival time in states sharing a drainage with a previously invaded state.

Methods

Data Collection

We obtained copies of fishing regulations from all states within the contiguous United States (US), through fishing regulation booklets and administrative codes. Fishing regulation booklets are published annually or biannually and summarize fishing regulations, including newly enacted policies relevant to anglers and the general public; the administrative codes include formal details and histories associated with each regulation and are available through each state's government website. We requested past and present fishing regulations from each state and obtained at least the current (2010-2011) regulations from all states within the

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contiguous US. We used each state's administrative code to obtain the date of enactment and follow changes made to each regulation. We defined the presence of a regulation to prevent anthropogenic introductions of rusty crayfish based on the following criteria; (1) policy explicitly prohibits transport of all live crayfish between bodies of water or (2) policy specifically prohibits the transport of rusty crayfish. For example, regulations were considered absent if only the purchase or sale of crayfish was prohibited, but transport was permitted. Regulations were considered present if transport of live crayfish was prohibited, but the transport of dead crayfish was permitted.

Rusty crayfish collection locations throughout the contiguous US were obtained from the United States Geological Survey (USGS; http://nas.er.usgs.gov:80/queries/collectioninfo.aspx?SpeciesID=214; last date accessed 11/30/2011), which incorporated all published or reported collection locations from 1961 through 2011. We supplemented USGS collection data with data obtained from personal communications with crayfish researchers and employees from the various natural resource departments in each state, some of whom had not reported their collections to the USGS. Each USGS specimen has a corresponding specimen number, which is associated with a state, county, river or lake name, drainage name, and hydrological unit code (HUC) number. The scale at which drainages are defined corresponds to the number of units in the hydrological unit code, where more digits represent a finer scale. For example, a HUC 8 is an 8-digit hydrological unit code, which partitions a HUC 4 drainage into finer categories. The maximum number of HUC digits, or the finest scale at which a drainage can be defined is HUC 12. However, we used a broader scale HUC (HUC 8) to define a single drainage in our study as some collection locations only provided an 8 digit HUC.

We also conducted a literature search to estimate the dispersal rate of rusty crayfish using Web of Science and Google Scholar with the search terms "crayfish dispersal" and "dispersal rate of crayfish". Should regulations be effective in preventing anthropogenic introductions of rusty crayfish among drainages, proactive measures should be taken in currently uninvaded states. However, regulations cannot prevent movement of crayfish through shared stream connections between states. In the case where populations are established in interstate drainages we determined which states are likely to be invaded through dispersal-based range expansion and when the invasion is expected to occur. Few estimates of rusty crayfish dispersal rates are available in the literature and all reflect dispersal within lake systems (Perry et al. 2001a; Wilson et al. 2004). However, rusty crayfish are more likely to invade new states through stream systems, thus we have included dispersal rates of other crayfish species through stream systems (Table 1). Dispersal rates varied between upstream and downstream movement, so we used a conservative (slowest rate) and an aggressive (fastest rate) estimate of crayfish dispersal capabilities (0.68 km/yr and 24.4 km/yr, respectively) to incorporate the variation in crayfish dispersal rate present in the literature.

Table 1. Dispersal rates of crayfish species in either a lake or river system.

Crayfish Species	Water Body Type	Flow Direction	Dispersal Rate (km/yr)	Reference
Orconectes rusticus	Lake		0.68	Wilson et al. 2004
Orconectes rusticus	Lake		0.7	Perry et al. 2001a
Pacifastacus leniusculus	River	Downstream	18.0-24.4	Hudina et al. 2009
Orconectes limosus	River	Upstream	2.5	Hudina et al. 2009
Pacifastacus leniusculus	River	Downstream	2.8	Bernardo et al. 2011
Procambarus clarkii	River	Not specified	2.0	Bernardo et al. 2011

Data Analysis

Our first objective was to test for an association between regulations and rusty crayfish. States where rusty crayfish are considered native and states outside of the contiguous US (Alaska and Hawaii) were excluded from this analysis. Due to the discrepancy between the native range proposed by the USGS (Ohio, OH, Kentucky, KY, Indiana, IN, Tennessee, TN, and Illinois, IL) and the range accepted by researchers of invasive crayfish (Taylor 2000; DiStefano et al. 2009; Peters and Lodge 2009) (OH, KY, IN, and Michigan, MI) we conducted two analyses, one for each native range (USGS native range and revised native range). Each state was placed into one of four categories, (1) both regulations and rusty crayfish are present, (2) regulations are present and rusty crayfish are absent, (3) regulations are absent and rusty crayfish are present, and (4) both regulations and rusty crayfish are absent. If regulations were present prior to invasion by

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rusty crayfish states were placed in the first category, but if regulations were passed subsequent to rusty crayfish presence states were placed into the third category. We used a Fisher's exact test in program R to test for a significant association between regulations and rusty crayfish.

Our second objective was to evaluate the effectiveness of regulations against the spread of rusty crayfish. Wisconsin was the first state to enact regulations against the use of live crayfish bait in 1983; however, rusty crayfish were discovered prior to policy enactment, thus the first proactive regulation was not enacted until 1986 by Utah. At the time of the initial enactment of the first proactive state regulation, rusty crayfish had not invaded 35 of the 43 contiguous and non-native states, which reflects both of the proposed native ranges. Between 1986 and 2011, 13 states were invaded, yielding a 37.1% (13 of 35 states) invasion probability. We used a binomial test in R to determine if the number of states with proactive regulations and were subsequently invaded was less than expected, based on the invasion probability of 37.1%.

Our third objective was to estimate the time required for rusty crayfish to invade states sharing a drainage with a previously invaded state. A drainage was identified based on its unique HUC 8 number as defined by the USGS and a drainage was classified as invaded if at least one rusty crayfish collection point was located within the drainage. Shared invaded drainages were identified by overlaying the three data layers, state boundaries, drainage boundaries, and USGS rusty crayfish collection locations in ArcMap 10 (ESRI, Redlands, CA). After states at risk for dispersal-based invasion across state borders were identified, ArcGIS Network Analyst was used to conduct a network-based spatial analysis, which calculated the river distance between the known collection point in the previously invaded state and the uninvaded state border. The river distances were divided by the conservative and aggressive dispersal rates of crayfish obtained from the literature to estimate the amount of time required for

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rusty crayfish to arrive into the previously uninvaded state. The date associated with the rusty

crayfish collection point was used to estimate when rusty crayfish would arrive in the uninvaded

state.

Results

Since the first documented discovery of rusty crayfish outside of their native range in

1967, rusty crayfish have invaded approximately 50% of states in the contiguous US, depending

on the native range used (21 out of 43 states based on the USGS native range and 22 out of 44

states based on the revised native range; Fig. 1). Despite the rapid range expansion of rusty

crayfish and their known detrimental effects on ecological communities, approximately 37% of

states in the contiguous US, where rusty crayfish are considered non-native, still have not

enacted regulations (16 out of 43 based on the USGS native range and 16 out of 44 based on the

revised native range; Fig. 2).
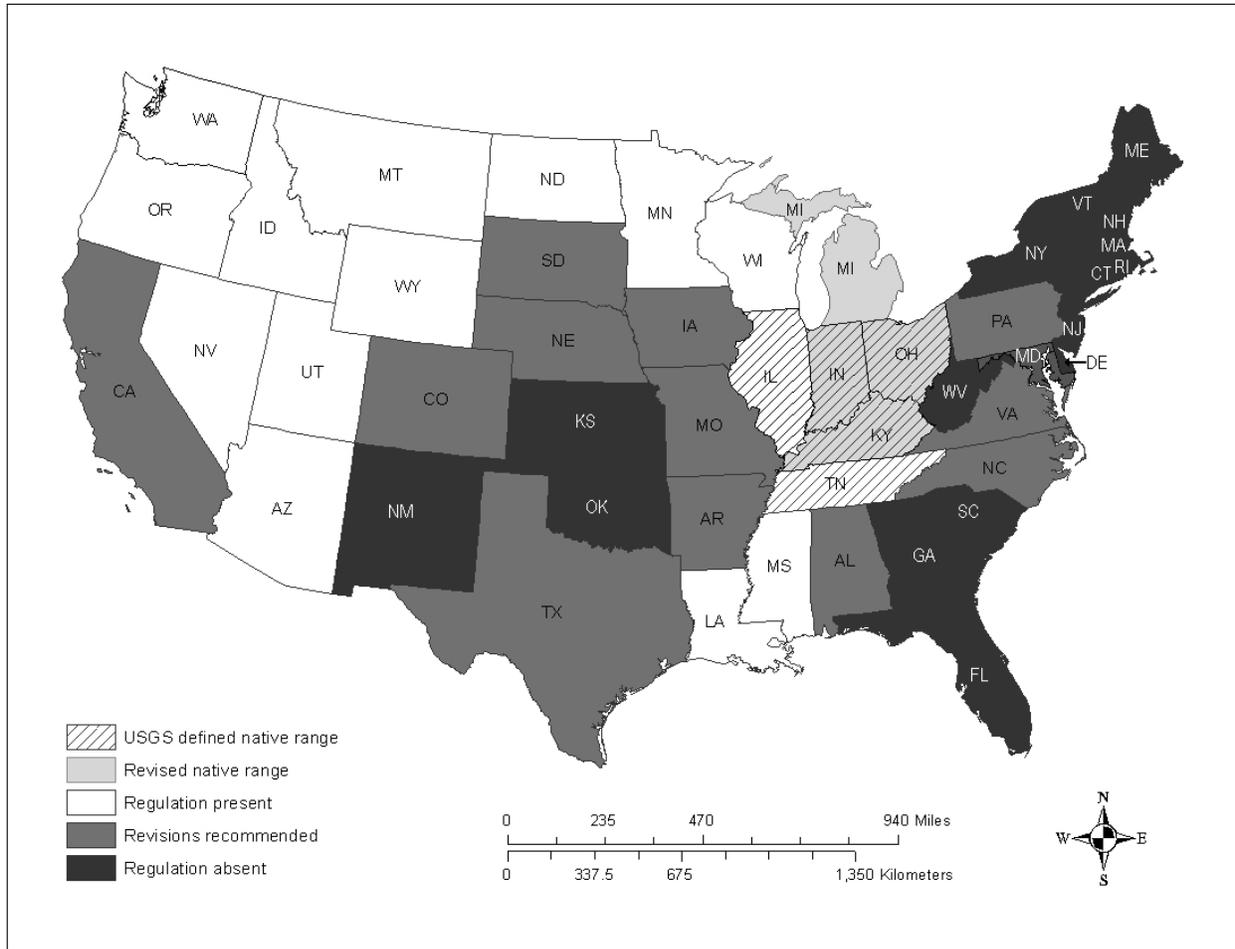
Figure 2. Presence of regulations to stop anthropogenic introductions of non-native crayfish species by state. States were labeled post-hoc as regulation present (white) if phrasing clearly prohibits transport of all crayfish species, revisions recommended (medium gray) if current phrasing requires species identification knowledge or applies only to a subset of drainages, and regulation absent (dark gray) if none exist. The United States Geological Survey (USGS) defines the native range of rusty crayfish (*Orconectes rusticus*) as IN, IL, KY, OH, and TN (diagonal lines); however, state and university crayfish researchers argue that the native range should be revised as IN, MI, OH, and KY (light gray).

At the time of our analysis in 2011, we found a significant positive relationship between the presence of regulations and the presence of rusty crayfish using both proposed native ranges. Excluding states where rusty crayfish are considered native by the USGS and the non-contiguous states, 4 states had proactive regulations and rusty crayfish, 12 states had proactive regulations and no rusty crayfish, 17 states had no regulations or reactive regulations and rusty crayfish, and 10 states had neither regulations nor rusty crayfish ($p = 0.027$, Fisher's exact test). Excluding states where rusty crayfish are considered native by crayfish experts and the non-contiguous states, 4 states had proactive regulations and rusty crayfish, 12 states had proactive regulations and no rusty crayfish, 18 states had no regulations or reactive regulations and rusty crayfish, and 10 states had neither regulations nor rusty crayfish ($p = 0.027$, Fisher's exact test).

In our analysis of the impact of proactive regulations on the likelihood of invasion, we found that the number of invaded states was not significantly lower than expected using either proposed native ranges ($S = 16$, $p = 0.233$, Binomial test). Following the enactment of the first proactive regulation in 1986, 16 states had proactive regulations; however, 4 states were invaded, which was not significantly lower than the expected number of state invasions of 5.94 (5.94 of 16 states or 37.1%). However, when the presence of regulations was redefined to exclude state regulations that required species identification knowledge, the number of invaded states was significantly lower than expected using both proposed native ranges (USGS native range, $S = 12$, $p = 0.031$, Binomial test; Revised native range, $S = 13$, $p = 0.021$, Binomial test).

Three states with proactive legislation (North Dakota, ND, Utah, UT, and Washington, WA) and two states without legislation (South Carolina, SC and Georgia, GA) would likely be invaded by rusty crayfish, regardless of regulations, due to range expansion through shared stream connections (Fig. 1). Of these five states, three states may have already been invaded

based on our estimated arrival times. South Carolina would likely be invaded between 2001 and 2078 through a shared drainage with North Carolina, Georgia would likely be invaded between 2003 and 2077 also through a shared drainage with North Carolina, and North Dakota between 1995 and 2160 through a shared drainage with Minnesota. Two states with shared drainages likely remain uninvaded, even if rusty crayfish continuously move at the maximum dispersal rate; Utah is expected to be invaded between 2021 and 2424 and Washington is expected to be invaded between 2017 and 2438 through a shared drainage with Oregon.

Discussion

Our results show that proactive regulations successfully impeded the spread of rusty crayfish at the national level. This is especially relevant based on the mechanisms of rusty crayfish spread and the consequences of establishment (Lodge et al. 2000; Taylor et al. 2007; DiStefano et al. 2009). However, our results have two caveats, legislation is only effective if transport of all crayfish is prohibited and it only reflects the prevention of anthropogenic introductions rather than dispersal-based range expansion through shared stream connections. Dispersal-based range expansion is likely restricted to within a drainage, given the poor dispersal ability of crayfish across terrestrial habitats (Claussen et al. 2000). Rusty crayfish invasion, via range expansion from an anthropogenic introduction point, threatens five states (GA, ND, SC, UT, and WA) because they share drainages with previously invaded states. While our analyses indicated rusty crayfish arrival as early as 1995, based on a dispersal rate of 24.4 km/yr, new research indicates that daily movement of *Orconectes hylas* can be as fast as 0.25 km/day (Westhoff 2011), thus rusty crayfish arrival could be much earlier than predicted. Several of these states have proactive regulations; however, these policies may require more rigorous

enforcement, greater public awareness, and improved cooperation within and between states in order to contain the probable invasion to a single drainage. For those states that have yet to implement regulations, our findings strongly support the proactive enactment of regulations to control the spread of rusty crayfish.   Alternative to the enactment of regulations, post-invasion strategies, such as trapping adult crayfish and increasing fish predation, can reduce rusty crayfish abundance, but do not completely eradicate rusty crayfish after introduction, although these strategies have only been evaluated in lake systems (Hein et al. 2006). Thus, enactment of proactive regulations banning the transportation of all crayfish currently appears to be the most effective strategy to control the spread of rusty crayfish.

We have shown that enactment of regulations at the state level is effective at preventing the anthropogenic spread of rusty crayfish, but we believe that state regulations could be more effective if neighboring states mutually agreed upon regulations. DiStefano et al. (2009) mentioned that one of the bait shops sampled in 2004 indicated that they expected a shipment of *Orconectes rusticus* from a wholesaler in Wisconsin, despite regulations prohibiting transport of live rusty crayfish within the state of Wisconsin, regulations do not restrict movement of crayfish out of the state. Although the shipment was never confirmed, the lack of communication and cooperation between states is clear. We encourage all states to prohibit the transport and sale of all live crayfish between water bodies. Not only would prohibiting the transport of all live crayfish stop the anthropogenic spread of rusty crayfish, but it would also act as a preventative measure against the spread of other invasive crayfish species. Another benefit of these regulations is that they do not require anglers or bait shop owners to be competent in distinguishing between native versus invasive crayfish species, knowledge that is lacking among bait shop owners in particular. A survey conducted in Missouri in 2007, indicated that 87% of

the surveyed bait shops admitted that they did not know what species they sold, and 97% of bait shop managers were unable to name the crayfish species they sold (DiStefano et al. 2009). The inability of bait shops to identify the species they sell likely extends to the anglers that use the live crayfish bait. By enacting regulations that apply to all crayfish species, bait shop owners and anglers do not have to be able to identify the crayfish species they are using. Lastly, regulations banning the transport of all live crayfish would still allow anglers to catch and use crayfish as live bait within the same body of water and allow anglers to transport dead bait; minimizing the necessary alterations in angler behavior, while preventing non-native introductions across drainages.

Our study was the first nationwide evaluation of state regulations in regards to preventing the spread of the invasive rusty crayfish; however, these data are presented with the caveat that collection locations reflect the search and reporting effort made by each state to the USGS, which may vary between states. We attempted to minimize this bias by supplementing USGS data with data obtained from personal communications with natural resource department representatives and crayfish experts from each state. Additionally, our ability to assign an accurate enactment date for state regulations was dependent on the availability of old fishing regulations, documentation of wording changes in the administrative codes, and cooperation of state legislative representatives. Despite these limitations, slight deviations in enactment dates or initial rusty crayfish collection dates are unlikely to change the qualitative results of this study as incorporation of newly acquired data from experts rarely changed the category to which the state was originally assigned. Furthermore, this study focuses on the presence or absence of state regulations in fishing regulation handbooks and the administrative code; however, enforcement

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and public awareness are also likely important factors in the effectiveness of regulations and should be considered in future research.

In this study, we have provided quantitative evidence that legislative regulations are effective in stopping the spread of an invasive aquatic invertebrate, which we hope will inspire policy makers to instate proactive regulations to stop the anthropogenic spread of rusty crayfish in the US. While we focused on the effectiveness of regulations in preventing the spread of rusty crayfish, proactive regulations are likely to be effective in preventing the spread of other aquatic invasive species, particularly several other crayfish invaders that are having negative effects in the US and Canada. Although the effectiveness of regulations has not been quantified in Europe, a few examples suggest that regulations are an effective management strategy within and between countries as well. Such as in Northern Ireland, where stringent legislation was passed prior to 2003 banning imports of crayfish and as of 2011 still do not have any non-indigenous crayfish species. The majority of eradication and control strategies for invasive species are reactive and often ineffective because they are implemented after non-native species have already begun to spread (Gherardi and Angiolini 2004). While, regulations can be implemented at any time during the invasion process, and may slow down the invasion, our results show that proactive regulations can prevent the initial invasion. The one caveat though, is that the regulations need to be worded broadly enough, such that species identification is not required of the general public. Given this, we recommend that all states enact or revise their current policies to prohibit the transport of all live crayfish species.

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