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Research Article

Potential impacts of invasive crayfish on native crayfish: insights from laboratory experiments

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Abstract

Despite the extreme diversity of crayfish in the Southeastern United States, many taxa are threatened with extinction due to invasive counterparts. Invasive crayfish alter invaded habitats causing community and population level impacts due to competition, predation and transmission of diseases. We experimentally evaluated the potential impacts of invasive red swamp crayfish (*Procambarus clarkii*) and virile crayfish (*Orconectes virilis*) on native Piedmont crayfish (*Cambarus* sp. C) using size-matched pairs. We tested 1) species-specific aggressive behavior with aggression assays, and 2) survival in sympatry with survival analysis. Both invasive crayfish species tended to express higher levels of aggression compared to the native counterpart. However, we failed to detect any statistically significant differences. During survival experiments, the proportional survival of native *Cambarus* sp. C in sympatry with invasive *O. virilis* was significantly lower than that of invasive *O. virilis*. However, we did not find a significant difference in the proportional survival between invasive *P. clarkii* and native *Cambarus* sp. C. These experiments demonstrate inconsistent and limited negative impacts of two invasive crayfish species on a native species, when size-matched. Therefore, in order to make novel and effective decisions in managing these invasive species, it is critical to understand species-specific invasion dynamics and size-dependent potential impacts of invasive crayfish on native taxa.

Key words: alien species, invasive species impact, invasive species management, survival experiments, aggression assays

Introduction

North America is the global hotspot for freshwater crayfish (Crustacea, Decapoda, Astacida) diversity (Richman et al. 2015) with more than 400 species described, which is approximately 80% of the global total (Taylor et al. 2007; Richman et al. 2015). The greatest diversity of native North American crayfish is within the Southeastern United States (Richman et al. 2015), which includes many species with very restricted ranges (Taylor et al. 1996; Taylor et al. 2007). Nearly 50% of these native crayfish are threatened with extinction (Richman et al. 2015). Major threats include habitat loss, pollution and invasive species impacts. These impacts are exaggerated by restricted ranges of the majority of these native crayfish species (Lodge et al. 2000; Taylor et al. 2007; Richman et al. 2015).

Crayfish have been identified as one of the major aquatic invasive taxa in both Europe (Lodge et al. 2000; Gherardi and Acquistapace 2007) and North America (Taylor et al. 1996; Taylor et al. 2007). Taylor et al. (2007) identified non-native crayfish as the major threat to the existence of native crayfishes. Despite the substantial diversity of native crayfish in the United States, 34 crayfish species are considered non-native (United States Geological Survey 2015), primarily North American crayfish introduced outside of their native range (Hobbs et al. 1989; Rebrina et al. 2015). Some of these are invasive, causing serious ecological and economical problems (Hobbs et al. 1989; Lodge et al. 2000; Taylor et al. 2007). Many native crayfish stocks have experienced significant population declines and local extirpations upon introduction of non-native crayfish (Westman et al. 2002; Lodge et al. 2000). Invasive crayfish may prey upon native species (Dunn et al. 2009); replace native crayfish due to competitive superiority for limited food and shelter (Hill and Lodge 1994; Holdich and Domaniewski 1995; Hill and Lodge 1999); and may also indirectly cause substantial community-level impacts on native crayfish by reducing aquatic macrophytes and macroinvertebrates (Hill and Lodge 1995; Gherardi and Acquistapace 2007). Invasive North American signal crayfish *Pacifastacus leniusculus* (Dana, 1852) have also caused a significant decline of native European crayfish mainly due to spread of crayfish plague (Alderman 1993) leading to the complete extirpation of native crayfish populations (Vorburger and Ribi 1999). In some other cases, invasive crayfish hybridized with native congeners (Berrill and Arsenault 1985).

Often the degree of impact of a given invasive species depends on many biological and ecological factors including interacting species, the ecological system they occupy, and the complexity of species interactions (Simberloff and Von Holle 1999; Didham et al. 2005; Rodriguez 2006; Henkanaththegedara and Stockwell 2013, 2014; Reid and Nocera 2015). This may lead to a range of possible outcomes from rapid replacement of native species (Vorburger and Ribi 1999) to co-existence of natives with invasive species without apparent negative impacts (Henkanaththegedara and Stockwell 2013, 2014). Therefore it is important to have some insights into potential impacts of invasive species, either before invasion occurs or before the invasive species spreads widely. A better understanding of these potential impacts may help conservation practitioners to implement novel management and control strategies and allocate limited resources to priorities (Henkanaththegedara and Stockwell 2014).

Our study focused on crayfish in Virginia. McGregor (2002) listed 28 crayfish species from Virginia including three non-native species, i.e. red swamp crayfish *Procambarus clarkii* (Girard, 1852), virile crayfish *Orconectes virilis* (Hagen, 1870) and rusty crayfish *O. rusticus* (Girard, 1852). The known range of invasive crayfish in Virginia is restricted (McGregor 2002); however, there is a risk that these invasives may spread and pose a significant threat to native crayfish. The Central Piedmont region of Virginia harbors many native species of crayfish including several species with very restricted ranges and federal/state protection (Virginia Department of Game and Inland Fisheries 2015). We attempted to simulate a "first contact" scenario between native and invasive crayfish species and experimentally evaluated the potential impacts of invasive red swamp crayfish (*P. clarkii*) and virile crayfish (*O. virilis*) on native species using Piedmont crayfish *Cambarus* sp. C (an undescribed widely distributed species, Cooper 2001; Loughman 2013). We tested species-specific aggressive behavior with aggression assays (Vorburger and Ribi 1999) because aggression levels may vary between species which could result in an array of outcomes from dominance of native species to replacement of natives by invasive species. We also assessed survival of crayfish in sympatry with survival analysis (Henkanaththegedara and Stockwell 2013) to understand how the interactions between native and invasive crayfish would affect their survival.

Methods

Collection, acclimation and preparation of crayfish

Invasive virile crayfish (*O. virilis*) were collected from Sleepy Creek (13.8 °C) in Morgan County, West Virginia using seines. Invasive red swamp crayfish (*P. clarkii*) were collected from Guion Pond (15.3 °C) at Sweet Brier College in Amherst County, Virginia using dip nets. The native Piedmont crayfish (*Cambarus* sp. C) were collected from a first order tributary of Buffalo Creek (14.1 °C) in Prince Edward County, Virginia using dip nets (Figure 1). All crayfish were collected between March and May 2015 and kept with stream water in coolers during transportation. Subsequently, crayfish were acclimated to laboratory conditions at room temperature (21 °C) with treated tap water in 37.85 L glass aquaria. Aquarium bottoms were covered with a layer of pea gravel and the water depth was maintained at 10 cm. Up to eight crayfish were housed in each aquarium with eight 7.5 cm long pieces of PVC as shelter. Crayfish were fed once a week (one pellet per crayfish) with pelleted crayfish food. Crayfish were weighed (to the nearest 0.1 g) using a digital scale, and measured (carapace length, claw length and palm width to the nearest 0.1 mm) using dial calipers. All claw measurements were taken from right chelae. Subsequently, crayfish were transferred to 4 L plastic experimental units attached to a flow-through tank system (Aquarius Fish Systems™) at room temperature at least a week before experiments. We used only intermolt individuals for these experiments.

Aggression assays

Invasive and native crayfish were paired based on carapace length (N = 16 pairs), to avoid any size-dependent dominance behavior, and subsequently introduced to plastic experimental arenas (36 cm × 30 cm × 14 cm with rounded corners) after a 48 hour starvation period (Guiaus and Dunham 1999).

Figure 1. Native and invasive crayfish utilized for current experiments. (a) Native Piedmont crayfish *Cambarus* sp. C, invasive (b) virile crayfish *Orconectes virilis*, and (c) red swamp crayfish *Procambarus clarkii*. Photos © Sujan M. Henkanaththegedara.

Experimental arenas were covered with a thin layer of pea gravel and filled up to 5 cm with treated water at room temperature. One crayfish was directly introduced to the experimental arena and the other kept in a 1L glass beaker (we alternated invasives and natives) placed inside the experimental arena and acclimated for 20 minutes, thus allowing visual interactions. Upon completion of the acclimation period, crayfish were allowed to interact with each other and individual crayfish were monitored by two observers for 10 minutes, each focusing on one individual crayfish, recording the behavior every 10 seconds (Pintor et al. 2008) according to the scoring system developed by Karavanich and Atema (1998; Table 1).

Upon completion of aggression assays, individual overall aggression scores were generated by multiplying behavior-specific aggression score by the number of events followed by summing all eight behavior-specific aggression scores. The mean differences of overall aggression scores between native and invasive crayfish were statistically compared using Wilcoxon rank sum test (R Development Core Team 2010).

Survival in sympatry

Upon completion of aggression trials, each crayfish pair (N = 16 pairs) was transferred to 8L plastic experimental units attached to a flow-through tank system (Aquarius Fish Systems™) to test survival in sympatry at room temperature (21 °C). Each unit contained one 7.5 cm long piece of PVC for shelter. Crayfish were fed with pelleted crayfish food and the survival of individual crayfish was monitored every 8 hours for up to 120 hours. Time to death (TTD) for each crayfish was estimated upon completion of the survival experiments. Package *survival* in R Statistical Software Program (R Development Core Team 2010) was utilized to analyze crayfish survival (Therneau and Lumley 2009). This package uses the *surv( )* function to simultaneously evaluate time to death and the censoring information (0=live; 1=dead; Maindonald and Braun 2010). Survival functions were estimated with Kaplan-Meier survival estimate (*survfit f*unction) using TTD data. Hazard functions for treatment groups were tested using Cox proportional hazards model (*coxph* function).

Table 1. Scoring system developed by Karavanich and Atema (1998) to study crustacean aggressive behavior.

Score	Behavior	Definition
-2	Fleeing	Walking away (rapidly), tail flip
-1	Avoidance	Walking away (slowly), turning away
0	Separate	At least one body length apart or not facing each other
1	Initiation (no contact)	Facing, approaching, turning towards opponent
2	Threat display (no contact)	High in legs, claws open, meral spread, claws forward, antenna point
3	Physical contact (no claw grasping)	Antenna touching, or whipping, claw touching, claw tapping, claw pushing
4	Physical contact (claw grasping)	Claws used to grasp opponents claws (claw lock)
5	Unrestrained use of claws	Snapping, ripping, swimming while in claw lock, grasping legs or rostrum

Table 2. Average size differences (mm ± SE) between invasive (*O. virilis* or *P.clarkii*) and native (*Cambarus* sp. C) crayfish utilized in the experiment (N = 16 pairs).

Morphometric measurement (mm)	Invasive species	Native species	p - value
	Orconectes virilis	*Cambarus* sp. C	
Carapace length	28.58 (± 1.20)	26.58 (± 1.20)	0.235
Palm width	7.24 (± 0.41)	6.22 (± 0.38)	< 0.05
Claw length	17.82 (± 1.38)	15.49 (± 0.90)	0.086
Palm width / claw length	0.43 (± 0.07)	0.40 (± 0.01)	0.468
Claw length / carapace length	0.62 (± 0.04)	0.58 (± 0.02)	< 0.05
	Procambarus clarkii	*Cambarus* sp. C	
Carapace length	30.39 (± 1.00)	27.31 (± 1.09)	< 0.05
Palm width	3.97 (± 0.30)	6.41 (± 0.58)	< 0.01
Claw length	14.98 (± 1.01)	15.16 (± 1.19)	0.955
Palm width / claw length	0.26 (± 0.01)	0.42 (± 0.01)	< 0.001
Claw length / carapace length	0.48 (± 0.02)	0.54 (± 0.02)	0.102

Results

Invasive *O. virilis* used for experiments were not significantly larger than paired native *Cambarus* sp. C in carapace length (W = 160, p = 0.235) and claw length (W = 174, p = 0.086). However, *O. virilis* palm width was larger than that of *Cambarus* sp. C (W = 181.5, p < 0.05). *Orconectes virilis* had relatively longer claws with respect to carapace length than native *Cambarus* sp. C (W = 184, p < 0.05). Invasive *P. clarkii* specimens were slightly larger than paired native *Cambarus* sp. C in carapace length (W = 180.5, p < 0.05), but there was no significant difference in claw length between two groups (W = 126, p = 0.955). Additionally, *P. clarkii* had significantly narrower palms compared to native *Cambarus* sp. C (W = 53, p < 0.05) making native *Cambarus* sp. C palms significantly wider with respect to claw length (W = 0, p < 0.001). However, there was no difference in claw length with respect to carapace length between two groups (W = 84, p = 0.102). These results suggest similar claw morphologies between invasive *O. virilis* and native *Cambarus* sp. C, but invasive *P. clarkii* has uniquely narrow claws compared to *Orconectes* and *Cambarus* taxa (Table 2; Figure 1).

Aggression assays

Although the median aggression score for invasive *O. virilis* (63.0) was greater than that of native *Cambarus* sp. C (39.5), the difference was not statistically significant (W = 168.5; p = 0.132; Figure 2A). The same trend was observed for the other pairing, where the median aggression score for invasive *P. clarkii* (31.5) was slightly greater than that of native *Cambarus* sp. C (24.0) making the difference statistically non-significant (W = 148; p = 0.462; Figure 2B). We also noted a general positive trend between carapace length and overall aggression score for both invasive and native crayfish whereby larger individuals displayed more aggressive behavior.

Survival in sympatry

The proportional survival of native *Cambarus* sp. C (44%) in sympatry with invasive *O. virilis* (88%) was significantly lower than that of invasive *O. virilis* (X^2 = 7.18; d.f. = 1; P < 0.01; Figure 3A). However, we did not find a significant difference in the proportional survival between invasive *P. clarkii* (62%)

and native *Cambarus* sp. C (81%; X^2 = 1.51; d.f. = 1; P = 0.218); Figure 3B). When mortality was associated with a given native-invasive crayfish pair, the dead specimen was always partially or completely (except the exoskeleton) consumed by the live specimen within an 8 hour period.

Discussion

This study provides limited evidence for potential negative impacts of invasive crayfish, *O. virilis* and *P. clarkii*, on size-matched native Piedmont crayfish *Cambarus* sp. C. Although, we did not detect any significant differences in aggression levels between invasive and native crayfish, the survival of native *Cambarus* sp. C in sympatry with invasive *O. virilis* was low suggesting a threat to the existence of native species. Interestingly, the negative impacts of the two invasive crayfish species tested against native *Cambarus* sp. C were not consistent.

Interspecific aggression may have a direct influence on the fitness of organisms by limiting access to food, shelter or other scarce resources, and may lead to replacements of less-aggressive species by highly aggressive species (Heller 1971; Moore 1978). Although higher for invasive crayfish, the size-matched aggression levels observed in this study were not significantly different between native and invasive species. Similar results were reported by Vorburger and Ribi (1999), where size-matched pairs of invasive *P. leniusculus* and native *Austropotamobius torrentium* (Schrank, 1803) showed no significant differences in aggression levels. However, they found significantly greater aggression in large-bodied crayfish matched with smaller individuals regardless of their native or invasive status (Vorburger and Ribi 1999). In contrast, other studies found that invasive crayfish were more aggressive than similar-sized native species (Capelli and Munjal 1982; Gherardi and Daniels 2004). Nevertheless, the degree of threat may vary between invasive species based on aggression levels, and aggressive interactions between native and invasive taxa may result in an array of outcomes depending on the interacting species. For example, size-dependent aggression-induced species interactions (i.e. interspecific competition for food and shelter) between native and invasive crayfish in invaded systems may be complicated due to fast growth rates of invasive taxa (Dunn 2012). Although we did not detect differences of aggression levels between native and invasive crayfish when size-matched, the faster growth rate and overall larger size of invasive *O. virilis* and *P. clarkii* may lead them to outcompete the native taxa in the long run (Hobbs et al. 1989).

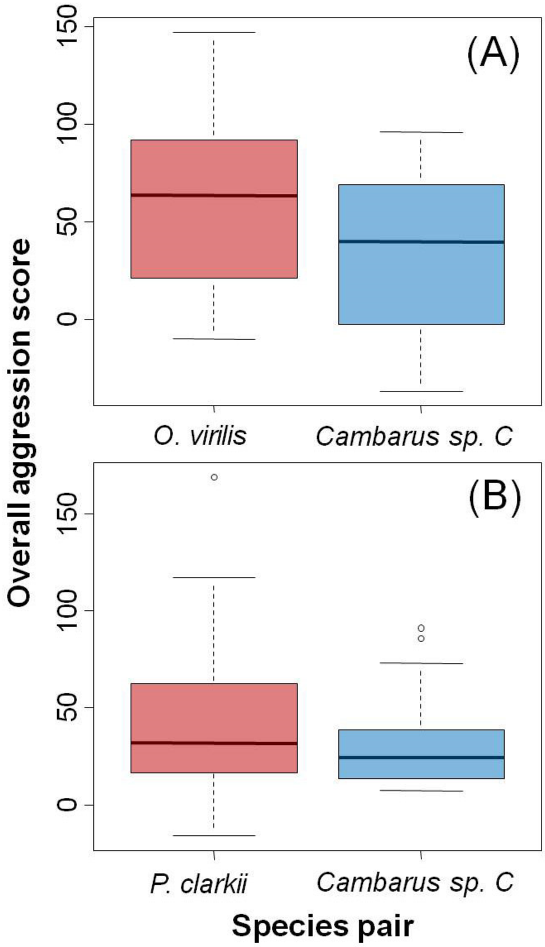

Figure 2. Overall median aggression scores for invasive and native crayfish species (N = 16 pairs). Although median aggression scores for invasive *Orconectes virilis* (A) and *Procambarus clarkii* (B) were higher compared to native *Cambarus* sp. C, the differences were not statistically significant. Blue boxes indicate native species while red boxes show invasive species. Thick horizontal bars in the middle of the box show the median value. Upper limit of the box shows 75th percentile and the lower limit shows the 25th percentile of the data.

Crayfish utilize their claws (i.e. chelipeds) for courtship and mating, prey capture, burrowing and offensive or defensive behavior when competing for resources (Holdich and Reeve 1988). It appears that there is a correlation between crayfish claw morphology and interspecific aggression levels (Larson and Olden 2010; also see Table 2 and Figure 1). Crayfish with wide claws (*O. virilis* and *Cambarus* sp. C) had higher aggression levels compared to taxon with narrow claws (*P. clarkii*). In fact, we observed a higher degree of potential threat from the wide-clawed *O. virilis* to native *Cambarus* sp. C compared to mixed results from the narrow-clawed *P. clarkii*.

Furthermore, native *Cambarus* sp. C. achieved greater survival than *P. clarkii* in sympatry. Although there is no experimental evidence, several cases suggest that larger claw size of invasive crayfish may have contributed to replacement of native counterparts (Garvey and Stein 1993; Klocker and Strayer 2004). This phenomenon warrants further investigation which may be useful in predicting effects of a given non-native crayfish species.

The survival experiments under sympatry of native crayfish with invasive taxa generated inconsistent results. Although our survival experiment did not contain an allopatric control due to limited number of specimens available, previous observations support 100% survival of all three crayfish species in same experimental tanks for six weeks under allopatric conditions (Henkanaththegedara unpublished data). In contrast, in the current study, there was a strong negative effect on native *Cambarus* sp. C in sympatry with invasive *O. virilis*, with nearly a 60% reduction in the survival of native crayfish compared with only 10% mortality of the invasive taxa over about five days. However, we failed to detect statistically significant differences in mortality rates between invasive *P. clarkii* and native *Cambarus* sp. C. Surprisingly, native *Cambarus* sp. C had relatively higher survival rates (~80%) compared to sympatric *P. clarkii* (~60%) suggesting a potential negative impact of the native crayfish species on survival of invasive taxa. This may be explained by relatively higher long-term survival of native *Cambarus* sp. C with much wider claws compared to similar-sized invasive *P. clarkii* with much narrower claws (Garvey and Stein 1993; Klocker and Strayer 2004).

Our attempt to simulate a "first contact" scenario between native and invasive crayfish species with aggression assays have some limitations such as reduced complexity and limited space of experimental arenas, and short duration of the experiments, and we acknowledge that *in-situ* responses of such interactions may be different and much more complex (Bergman and Moore 2003). Additionally, we agree that our experiments are not perfect due to lack of controls: we decided to maximize replication at the expense of exclusion of controls due to limited numbers of crayfish specimens available. However, we suggest that our results provide some important insights into understanding interactions between native and invasive crayfish during an invasion event. These results collectively suggest that invasive crayfish impacts on native crayfish are not identical. Moreover, impact may be a result of an interplay between species-specific biological factors of interacting species (e.g. relative body size, aggression levels, growth rates), influence of other organisms (e.g. prey, predators, competitors, disease-causing agents, parasites), as well as physico-chemical properties of the invaded system (e.g. distribution and availability of shelter, flow rates, pollutants).

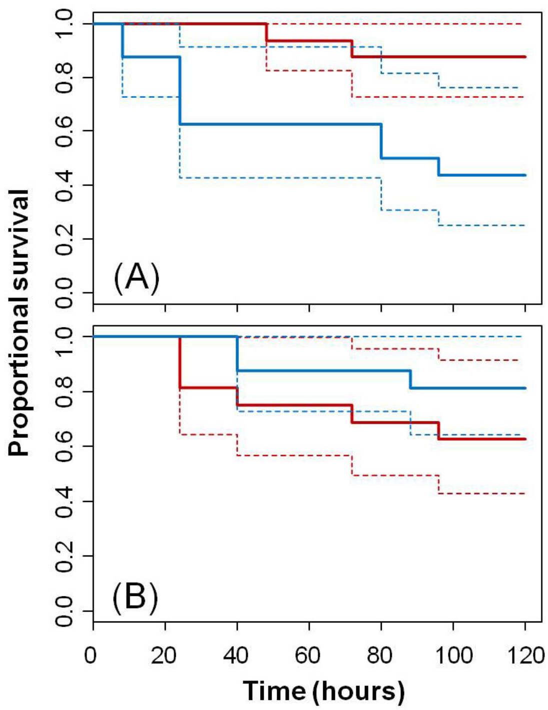

Figure 3. The proportional survival of native *Cambarus* sp. C in sympatry with invasive *Orconectes virilis* (A) and *Procambarus clarkii* (B). Red lines indicate invasive crayfish species and blue lines indicate native *Cambarus* sp. C. The overall survival of native *Cambarus* sp. C significantly dropped by 56% compared to a 12% drop of invasive *Orconectes virilis* over a 120-hour period (A). However, the overall survival of native *Cambarus* sp. C and invasive *Procambarus clarkii* dropped only by 19% and 38% respectively, during the same time period without any significant differences of survival rates between two species (B). Solid lines indicate Kaplan-Meier proportional survival function, and dashed lines indicate 95% confidence intervals.

Crayfish taxonomists describe several new species of crayfish every year, mainly from Southeastern United States, often with very restricted ranges (Taylor et al. 2007; Richman et al. 2015). At the same time, some invasive crayfish species (e.g. Rusty crayfish, *Orconectes rusticus*) expand their non-native range at an alarming rate, potentially risking the existence of those native crayfish, especially range- restricted species (Lodge et al. 2000; Taylor et al. 2007). In many Wisconsin lakes, invasive *O. rusticus* have replaced native *O. virilis* (Hill and Lodge 1994, 1995, 1999), and in Sleepy Creek in Morgan County, West Virginia, native Allegheny crayfish (*Orconectes obscurus*) have been replaced by invasive *O. virilis* (Loughman and Welsh 2013).

Therefore it is clear that the same crayfish species may play the role of either "dominant invasive" or "inferior native" depending on the scenario. In some other cases, the native crayfish may be resilient enough to defend against an invasion. It is therefore critical to understand species-specific and size-dependent potential impacts of invasive crayfish on native taxa to understand invasion dynamics, and similar experiments with various size classes of invasive and native crayfish would be useful in this respect. In the light of this more complete information, conservation practitioners may be able to make novel and effective decisions for controlling invasive species to minimize their harmful effects on native species.

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