Experimental evidence of invasion facilitation in the zebra mussel-killer shrimp system

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Abstract

Invasion facilitation, whereby one species has a positive effect on the establishment of another species, could help explain the rapid colonisation shown by some freshwater invasive species, but the underlying mechanisms remain unclear. We employed two-choice test arenas to test whether the presence of zebra mussel (*Dreissena polymorpha*) could facilitate the establishment of the killer shrimp (*Dikerogammarus villosus*). Killer shrimp preferred to settle on mats of zebra mussel, but this was unrelated to mat size, and was not different from attraction shown to artificial grass, suggesting that zebra mussel primarily provides substrate and refuge to the killer shrimp. Killer shrimp were strongly attracted to water scented by zebra mussel, but not to water scented by fish. Chemical attraction to the zebra mussel's scent did not differ between sympatric and allopatric populations of killer shrimp, suggesting that chemical facilitation is not an acquired or learned trait. Our results have implications for managing the spread of killer shrimp, and perhaps other freshwater invasive species, because invasion facilitation could significantly increase establishment success. Failure to consider invasion facilitation may underestimate the risk of establishment and likely impact of some aquatic invaders.

Keywords

Invasive species, Synergy, Chemical recognition, Zebra mussel, Killer shrimp

Introduction

The impact of biological invasions has often been examined in isolation, under the implicit assumption that invaders do not interact with each other (Consuegra et al. 2011; Vanhaecke et al. 2015; Young et al. 2010; Young et al. 2009). However, invasion facilitation, whereby one species has a positive effect on the introduction, establishment or dispersal of other invasive species, is well documented, particularly in terrestrial plants and animals (Adams et al. 2003; Altieri et al. 2010). For example, the presence of the European honey bee (*Apis mellifera*) has increased the reproduction success of the invasive shrub *Lantana camara* in Australia (Goulson & Derwent 2004), and similar positive synergies among invasive species have also been reported across many taxa (Woodward et al. 1990). This led Simberloff & Von Holle (1999) to coin the term ‘invasional meltdown’ to describe the process by which the negative impacts triggered by one invasive species could be exacerbated by the interactions with other exotic species (Simberloff 2006).

However, invasion facilitation has not received as much attention in freshwater habitats as it has in terrestrial ecosystems, possibly because it is more difficult to detect (Ricciardi 2005), and because it typically only benefits one species (i.e. commensalism, (Laihonen & Furman 1986; Ricciardi 2005). This lack of information is unfortunate because freshwater habitats, particularly lakes and ponds, rank among the most threatened ecosystems in the world, and this makes them particularly vulnerable to the threat of aquatic invasive species (AIS; (Dudgeon et al. 2006). Habitat suitability models for invasive species, as well as risk maps (Crall et al. 2013; Jiménez-Valverde et al. 2011), rarely take into account the fact that some invaders can bioengineer their new habitat to suit their needs, or that the presence of one invasive species may make the habitat more attractive to other invaders (Strayer 2012). Thus, the synergistic effects of

invasive species and their cumulative impacts on native fauna may be underestimated in aquatic habitats if invasion facilitation exists and is not taken into account.

Two aquatic invaders that often occur together and may benefit from invasion facilitation are the zebra mussel (*Dreissena polymorpha*) and the killer shrimp (*Dikerogammarus villosus*). The two species are included in the 100 worst invasive species in Europe (www.europe-alien.org), and in the case of zebra mussel, in the world (Lowe et al. 2000). Zebra mussels form dense mats on natural and artificial substrates which require expensive eradication programmes (Lovell et al. 2006), and compete directly for food and space with native bivalves (Fahnenstiel et al. 1995a; Fahnenstiel et al. 1995b; Johengen et al. 1995), sometimes driving them to extinction (Baker & Hornbach 1997). The killer shrimp, on the other hand, has the typical profile of an efficient and plastic invader (Rewicz et al. 2014), being able to adapt to a wide range of waters and conditions (Piscart et al. 2003). Its wide diet includes many macroinvertebrates, including native gammarids, which may be displaced and driven to local extinction (Dick & Platvoet 2000; Piscart et al. 2003).

Both species share a broad, common Ponto-Caspian geographical origin, but the extent of sympatry in their native, as well as in the invaded areas, is unclear. The zebra mussel was first reported in Europe during the nineteenth century, becoming highly invasive and forming large populations (Son 2007). In contrast, the killer shrimp is a much more recent invader, it has only been detected in Europe during the last 20 years, but has spread rapidly ever since (MacNeil et al. 2010; Rewicz et al. 2014; Rewicz et al. 2017; Tricarico et al. 2010).

While many of the sites colonised by the killer shrimp in Europe had already established populations of zebra mussel, that might be indicative of invasion facilitation (Gallardo & Aldridge 2015), the killer shrimp has also invaded many areas devoid of zebra mussel ((Rewicz

et al. 2015; van der Velde et al. 2010). Thus, whether there have been synergies in the establishment of these two species is not clear (Devin et al. 2003). The zebra mussel has shown mutualistic interactions with one gastropod (Ricciardi 2005; Ricciardi et al. 1997), and two macrophytes (MacIsaac 1996; Skubinna et al. 1995), and can benefit the killer shrimp in various ways. For example, the dense interstitial matrix formed by the shells of zebra mussels provides refuge for the killer shrimp (Ricciardi et al. 1997), allowing it to survive outside the water, while the production of faeces and pseudo faeces provides food (Gergs & Rothhaupt 2008a; Gergs & Rothhaupt 2008b; Ricciardi et al. 1997; Stewart et al. 1998b). Similarly, zebra mussel larvae can attach to the hard chitin cover of the killer shrimp which could facilitate their dispersal (Kenderov 2017; Yohannes et al. 2017).

Given their common geographical origin, and recent evidence suggesting that the two species often occur together and might benefit each other, we hypothesized that killer shrimp might be chemically attracted to the presence of zebra mussel. We further hypothesized that attraction might differ depending on whether killer shrimp were found in habitats already colonised by the zebra mussel, i.e. whether attraction differed between sympatric and allopatric conditions. Ultimately, our aim was to address some of the underlying mechanisms of invasion facilitation as this might help design better predictive models and more effective control measures of these two aquatic invaders.

Materials and Methods

Collection and origin of samples

Sympatric zebra mussel and killer shrimp were collected from Cardiff Bay (Cardiff, UK – Grid reference: ST 19210 73510) in April 2016, whereas allopatric killer shrimp were collected from the Upper Mother Ditch (Margam, UK, Grid reference: SS 79029 85506) in September 2016, where the zebra mussel is not yet present (Figure 1). These were brought to the CSAR facilities at Swansea University and maintained in 6 x 20 L tanks fed by separated recirculation aquaculture systems, with a weekly replacement of c. 20% volume. Zebra mussels (~2.4 kg) were fed three times per week with a 2L mixture of *Scenedesmus* sp. and *Chlorella* sp., while killer shrimps (~100g) were fed three times per week with 6g of frozen bloodworms. Water temperature was maintained at 15-16.5°C.

Experimental design

We set up three experiments to test possible synergies between the two species. In the first experiment we tested if killer shrimp had a preference for particular densities of zebra mussel; in the second experiment we tested if killer shrimp had a preference for zebra mussel cover compared to an artificial substrate; in the third experiment we tested if killer shrimp could detect the presence of zebra mussel through chemical cues in the water. The killer shrimp used in the tests had an average size of 16.8±0.9 mm and the water temperature during tests ranged between 15.4 °C and 16.2 °C.

Experiment 1. Choice of zebra mussel density

To test if killer shrimp had a preferred density of zebra mussel to settle on, we employed a 3L tank (L25 x H 10 x W12 cm; Figure 2a) divided into two equal sections, each with a different density of zebra mussel (0, 33, 67, or 100% cover), and an acclimatisation plastic cylinder in the middle. Individual killer shrimp ($n = 96$) were allowed to acclimatise for five minutes in the cylinder, then the cylinder was lifted and the position and behaviour (swimming or hiding) of the shrimp after 20 minutes was recorded. We assumed that if the shrimp was hiding it meant it had found a suitable substrate, whereas if it was still swimming it meant it was still looking for a refuge. We tested the killer shrimp's binary choice over six matched densities ($n = 16$) of zebra mussel: 0-33%, 0-66%, 0-100%, 33-66%, 33-100%, and 66-100% employing a total of 96 specimens, and allocating the densities to the left or right sides of the test arena at random.

Experiment 2. Preference for zebra mussel over an artificial substrate

To test if the attraction of killer shrimp for zebra mussel-beds was simply related to the presence of cover or to other factors (such as bio-deposited material) we compared preference for living shells against either empty shells of zebra mussel or artificial grass (PE thickness 15mm) of similar texture and extent of refuge. We used one killer shrimp per trial ($n = 30$), and then twenty killer shrimp per trial ($n = 80$) to understand if substrate choice was affected by group dynamics. The experimental protocol was the same as in Experiment 1, but in this case each side of the test arena afforded 50% cover and we used a 20 L test tank (L40 x H15 x W35 cm).

Experiment 3. Chemical attraction to zebra mussel

To test if killer shrimp could chemically recognise the scent of zebra mussel we employed a simplified version of the two-choice Perspex fluvium used by Kroon (2005) in the preference of another crustacean (Figure 2b). The fluvium consisted of an acclimatization chamber (L5 x H6.5 x W5 cm) and two 0.3L choice chambers (L20 x H6.5 x W10 cm) with a total volume of approximately 0.7L. We tested preferences against dechlorinated tap water (blank), water scented with zebra mussel and Nile tilapia (*Oreochromis niloticus*) to control for possible attraction to organic matter, as well as blank water vs blank water to control for chamber bias. Killer shrimp were allowed to acclimatize for five minutes, the valves connected to the two water inlets were opened, the gate was lifted, and the time spent in each chamber was recorded for 15 minutes with a GoPro Hero camera. We compared the time spent in each arm as well as the number of transitions between arms as a measure of activity. Scent drip dosage was adjusted at 200 ml/min.

To prepare the scented water we placed either zebra mussels or fish (tilapia) in a tank filled with dechlorinated water for 24 hours at a biomass of 50g/L. The fluvium was drained, cleaned with 90% ethanol and rinsed with fresh water between trials to remove potential chemical cues that could affect the next experiment. We repeated the experiment with killer shrimp originating from a population living in sympatry (Cardiff Bay, n = 60) or allopatry (Margam, n = 60) with zebra mussels. All the zebra mussel came from Cardiff Bay.

Statistical analysis

We used R 3.3 (R Core Team, 2017) for all analysis. In experiments 1 and 2, we used a generalized linear model (GLM) with a binomial log-link to test if the number of killer shrimp in

the scented arm differed with treatment, and we then used a two-sided binomial test to assess if there was a statistically significant preference for the high or low density (Exp 1) or different substrate combinations (Exp 2) at each binary choice comparison. For experiment 3 (two-way choice fluvium), we used a linear model with time spent in the scented arm as the dependent variable and origin (allopatry vs sympatry) and type of scent (blank, zebra mussel, tilapia) as the predictors; we then used paired t-tests to assess which type of matched scent comparisons was statistically significant.

Ethics Statement

All experiments were carried out in accordance with Swansea University Ethical guidelines and were approved by the Ethics Committee (300419/1557). Water removed from the system was treated with bleach before disposing, to avoid accidental dispersion of zebra mussel larvae.

Results

Experiment 1. Zebra mussel density preference

Preference for zebra mussel varied depending on the densities being compared ($\chi^2 = 29.09$, $df = 1$, $P < 0.001$; Figure 3). Killer shrimp showed a clear preference for the side of the tank with zebra mussel when the alternative was a bare tank bottom (binomial proportion test: 0-33% $P = 0.004$; 0-66% $P < 0.001$; 0-100% $P < 0.001$). However, when both sides of the test arena had different densities of zebra mussel there was no preference (binomial proportion test 33-66%, $P = 0.454$; 66-100%, $P = 1.00$) or preferred the lower density (33-100%, $P = 0.004$). After 20 minutes, the majority of killer shrimp (85/96 or 88.5%) were found to be hiding, rather than swimming (binomial proportion test $P < 0.001$) regardless of treatment ($\chi^2 = 1.745$, $df = 1$, $P = 0.883$).

Experiment 2. Substrate preference

When tested individually, killer shrimp did not prefer live zebra mussels over artificial grass (binomial proportion test, $P = 0.584$), or over empty zebra mussel shells (binomial proportion test, $P = 0.200$, Figure 4a). The majority of individuals were found hiding (rather than swimming), both when the comparison was against artificial grass (binomial proportion test, 83.3% $P < 0.001$) and also when there were empty shells (binomial proportion test, 76.6% $P = 0.005$).

When the experiments were repeated with 20 shrimps per trial (4 trials or 80 shrimp), killer shrimp strongly preferred the zebra mussel substrate over artificial grass (binomial proportion test, $P = 0.006$) and also over empty shells (binomial proportion test, $P = 0.006$; Figure 4b). As before, at the end the trials the majority of individuals were hiding, both when the

comparison was against artificial grass (binomial proportion test, 77.5% $P < 0.001$) and also against empty shells (binomial proportion test, 72.5% $P = 0.006$).

Experiment 3. Chemical attraction to zebra mussel

No side preference was detected when killer shrimp were tested against blank water in both arms of the 2-choice fluvium, either in the sympatric ($t_9 = 1.343$, $P = 0.212$; Figure 5a) or allopatric killer shrimp populations ($t_{19} = -1.280$, $P = 0.216$; Figure 5b), indicating that there was no side bias. When killer shrimp were tested against water conditioned with tilapia scent, no preference was observed over blank water, either in sympatry ($t_{19} = 0.819$, $P = 0.423$; Figure 5c) or allopatry ($t_{19} = -0.687$, $P = 0.500$; Figure 5d). However, when killer shrimp were tested against water conditioned with zebra mussel scent, there was a strong chemical attraction to the zebra mussel scent, both in the sympatric ($t_{27} = -2.176$, $P = 0.038$; Figure 5e) and allopatric population ($t_{19} = -2.614$, $P = 0.017$; Figure 5f). Chemical attraction for zebra mussel scent was equally strong in the sympatric and allopatric populations ($F_{1,118} = 1.036$, $P = 0.311$).

The analysis of activity (measured as the number of transitions between arms) indicates that activity was influenced by the type of test scent (Figure 6), as killer shrimp made more changes when both arms were dosed with blank water than when one arm was dosed with zebra mussel scent (test, $P = 0.002$) or tilapia scent ($P < 0.001$). No difference in activity was observed between the zebra mussel scent and the tilapia scent ($P = 0.759$). Overall, the sympatric population made more choices and was more active than the allopatric population ($P = 0.005$).

Discussion

Our study provides experimental insights into the underlying reasons for the joint occurrence of zebra mussel and the killer shrimp, two of the world's worst aquatic invaders (Lowe et al. 2000). We found that killer shrimp showed a strong tendency for hiding in zebra mussel beds, and were also chemically attracted to the scent of zebra mussels, which may facilitate their invasion.

In our experiments, killer shrimp consistently avoided the empty side of the tank (substrate coverage 0%) that did not afford any refuge, and generally preferred to settle on zebra mussel beds, even when tested with blank water and without any threat of predation. The strong preference for a substrate that offers refuge is in agreement with observations under natural conditions, where the species is typically found living among gravel, cobbles and boulders, and absent in places where there is silt or substrates that do not afford refuge (Boets et al. 2010; MacNeil et al. 2010). While juvenile killer shrimp may also be found living among macrophytes (Devin et al. 2003), as happens for juveniles of several other predatory amphipods (Berezina 2007), adults tend to prefer hard substrates of large grain size, including cobble and roots (Devin et al. 2003), pebbles (Van Riel et al. 2009), fissured stones (Kley et al. 2009), and coarse gravel (Boets et al. 2010). Kobak et al. (2015) have suggested that the substrate preference of the killer shrimp is size dependent and determined by the interstitial spaces of the substrate, as this influences ease of movement and the ability to find refuge. The species appears to choose fissures that closely match its body size (Platvoet et al. 2009), which might explain why they prefer larger substrates as they become older. In this sense, zebra mussel beds provide an ideal refuge for juveniles and adults alike, because as the mussels grow the interstitial spaces also become larger.

The findings of Experiment 2, where we tested the preference of killer shrimp for zebra mussel over other textured substrates, are more difficult to interpret as different results were obtained depending on group size. When killer shrimp were tested singly, no preference was detected for live zebra mussels over empty shells or artificial grass of similar texture, suggesting that substrate preference was mainly governed by the availability of refuge, which previous experiments have shown confers protection from fish predators (Kinzler & Maier 2006). However, when groups of twenty shrimp were tested, a strong preference for live zebra mussel over other substrates was found, suggesting the existence of group behaviour (sociability) that cannot solely be explained by refuge availability and deserves further investigation. Killer shrimp tend to form aggregations, and these are thought to be advantageous and increase the chances of successfully colonising new areas (Truhlar & Aldridge 2015). However, while sociability within a group can increase fitness and reproductive success (Réale et al. 2007), it can also facilitate intraspecific predation (cannibalism), which is frequently observed in amphipods (Dick et al. 1993; Hunte & Myers 1984; Ward 1985), including the killer shrimp (Dick & Platvoet 2000; Dick et al. 2002; MacNeil et al. 1997). An inverse association may exist between sociability and cannibalism in amphipods (Kinzler et al. 2009; Truhlar & Aldridge 2015), although this may also be influenced by predation pressure (Dick et al. 1993). Cannibalism in killer shrimp does not appear to be so strong as to reduce the species' sociability, possibly because cannibalism mainly targets small juveniles (Kinzler & Maier 2003) which tend to be spatially segregated from larger adults that could prey on them (Devin et al. 2003).

One novel finding of our study was the strong chemical attraction shown by killer shrimp to the scent of live zebra mussels (or something associated with them), a response not seen to blank water or the scent of non-predatory fish. Amphipods use chemical cues as their main form

of communicating between conspecifics (Thiel 2011), and also to recognize and avoid predators (Wooster 1998), but chemical attraction to other species has, to our knowledge, not been reported before. Chemical detection in amphipods is mediated mainly via specific sensillae located on the antennae (Hallberg & Skog 2011) and is used in mate choice and species discrimination, reducing the chances of interspecific mating between similar species (Dick and Elwood 1990 (Cothran et al. 2013; Dick & Elwood 1990). Chemical cues are also used by females to recognize and defend their offspring against conspecifics (Mattson & Cedhagen 1989), and some amphipods can also recognize alarm cues from damaged conspecifics and mount a strong freezing response as an anti-predatory strategy (Sehr & Gall 2016). Killer shrimp have been reported to use chemical cues to recognize and avoid potential predators such as the spiny-cheek crayfish *Orconectes limosus* (Hesselschwerdt et al. 2009), the European bullhead *Cottus gobio* (Sornom et al. 2012) and the racer goby *Babka gymnotrachelus* (Jermacz et al. 2017), and our study shows they can also use chemical cues to find zebra mussels that provide not only cover and refuge, as seen in our study and other studies (Ricciardi et al. 1997), but also food through the production of faeces and pseudo faeces (Gergs & Rothhaupt 2008a; Gergs & Rothhaupt 2008b; Ricciardi et al. 1997; Stewart et al. 1998a).

The preference for zebra mussel beds shown by the killer shrimp (at least when they are tested in groups), and the fact that they are strongly attracted to the zebra mussel scent, suggests this could be an example of invasion facilitation, as seen in other studies. For example, positive synergies resulting in invasion facilitation have been reported for an invasive algae and an invasive bryozoan (Levin et al. 2002), as well as among invasive fish parasites (Hohenadler et al. 2018). Other well known examples of invasion facilitation, include the bullfrog-sunfish system, where the survival of the invasive bullfrog (*Rana catesbeiana*) was enhanced by the presence of

the non-native bluegill sunfish (*Lepomis macrochirus*) because the latter preyed on native dragonfly which in turn preyed on bullfrog tadpoles (Adams et al. 2003). Similarly, predation by an invasive crab on a large native clam resulted in the spread of a smaller invasive clam due to competitive release (Grosholz 2005). Our study indicates that chemical attraction by the killer shrimp to the zebra mussel scent was as strong under sympatric as it was under allopatric conditions, suggesting this is not a recently acquired or learned trait, but rather an older behavioural adaptation. However, the absence of population replication (it is very difficult to find populations of killer shrimp that do not coexist with zebra mussel) makes it difficult to draw firm conclusions and would warrant further studies.

In general, mutualist interactions are less well studied than competitive ones (Simberloff & Von Holle 1999), and interactions between invasive species are less well known than those between invasive and native species (Gallardo & Aldridge 2018). There is still limited knowledge on positive interactions among invasive species, despite the fact that this may hold the key for more effective control of new invasions. Given the strong preference for settling on zebra mussel mats, and the benefits that this entails (Gergs & Rothhaupt 2008b; MacNeil et al. 2008) – including not just refuge, but also benthic organic matter that can be a source of food (Ricciardi et al. 1997; Stewart et al. 1998b), chemical attraction may help understand synergies between these two invasive species. Chemical attraction may need to be taken into account in the study of patterns of distribution of these two invasive species, since the presence of zebra mussel could make the killer shrimp more likely to become established, and hence a more successful invader. The zebra mussel's ability to settle on a wide range of substrate (Marsden & Lansky 2000) and bioengineer its own environment (Mayer et al. 2001) can turn formerly unsuitable habitats into favourable locations for killer shrimp settlement, expanding the potential range of

suitable environments. For example, the NBN Atlas (<https://nbnatlas.org/>), the UK's largest biodiversity database, holds 3,182 records of zebra mussel but only 317 observations of killer shrimp and information on the presence of zebra mussel could help make more accurate predictions of the likely spread of killer shrimp. This is of particular concern when the species is a recent invader since there is typically insufficient information to predict areas at risk or to guide management (Morales et al. 2017). In Great Britain, the killer shrimp was first detected in 2010 (Rewicz et al. 2014) and is only present in 8 locations, making it difficult to predict its future dispersal (Rodriguez-Rey et al., under review). The zebra mussel, in contrast, was first detected in 1824 and is now established in 376 locations in England and Wales (Aldridge et al. 2004), Rodriguez-Rey et al., under review), potentially increasing the number of favourable locations for killer shrimp many fold. Yet, current invasive species prioritisation lists (Boets et al. 2014; Carboneras et al. 2018) and risk assessment guidelines (Roy et al. 2018) tend to view invasive species in isolation, making no allowance for invasion facilitation. Our study suggests that information on the presence of zebra mussel should be incorporated into risk maps and models of killer shrimp dispersal, because ignoring chemical attraction will likely underestimate the extent and consequences of invasion facilitation.

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Author Contributions & Competing Interests

CGL and SC designed the study and secured the funding. MR and EC collected the data and carried out the analyses with advice from CGL. MR and CGL wrote the MS with contributions from SC and DH. The authors declare no competing interests.

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Figure legends

Figure 1. Map showing the sampling point of (A) sympatric (Cardiff Bay) and (B) allopatric (Upper Mother Ditch) populations of zebra mussel and killer shrimp.

Figure 2. Experimental set up used to test (A) substrate preferences of killer shrimp, and (B) chemical attraction to water scented by zebra mussel

Figure 3. Proportion of killer shrimp (binomial 95 CI) settling in zebra mussel beds of different sizes in binary choice tests involving different amount of zebra mussel cover (0, 33, 66, and 100% tank cover).

Figure 4. Proportion of killer shrimp (binomial 95 CI) settling in binary choice tests involving different substrates (live zebra mussel, empty shells of zebra mussel, artificial grass)

Figure 5. Preference by killer shrimp (mean time spent, $s \pm 95$ CI) in water conditioned with different scents (blank water, tilapia scent, zebra mussel scent).

Figure 6. Activity (mean number of transitions ± 95 CI) of killer shrimp tested in water conditioned with different scents (blank water, tilapia scent, zebra mussel scent).

2A

2B

A

B

C

D

A – Sympatry

B – Allopatry

Sympatry

Allopatry