Research Article

Predator recognition and anti-predatory behaviour in a recent aquatic invader, the killer shrimp (*Dikerogammarus villosus*)

Matteo Rolla, Sofia Consuegra and Carlos Garcia de Leaniz*

Department of BioSciences, Centre for Sustainable Aquatic Research, Swansea University, Singleton Park, Swansea SA2 8PP, UK
Author e-mails: *matteo.rolla2014@gmail.com (MR), s.consuegra@swansea.ac.uk (SC), c.garciadeleaniz@swansea.ac.uk (CGL)*

*Corresponding author

Abstract

The killer shrimp (*Dikerogammarus villosus*) is one of the most recent and damaging aquatic invasive species in many parts of Europe, but information on how the species responds to predation pressures in recently invaded areas is very limited. We employed an open test arena to examine anti-predatory behaviour in killer shrimp exposed to either blank water or water conditioned with kairomones from the three-spined stickleback to simulate a predator threat. Killer shrimp spent much more time hiding in the presence of stickleback kairomones than when they were exposed to blank water. However, no significant difference was found in aggregation behaviour, and killer shrimp were strongly attracted to the scent of conspecifics regardless of predator threat. Given the strong selective pressures that fish predators can exert on native and invasive gammarids, our findings highlight the need to consider prey-predator interactions to better predict the dispersal and likely impact of killer shrimp into invaded ecosystems.

Key words: invasive species, anti-predatory strategy, chemical recognition, aggregation behaviour, gammarids

Introduction

From a prey-predator perspective two opposing selective forces may confront invasive species when they colonise a new area: the absence of former predators may facilitate their establishment (the enemy release hypotheses – Colautti et al. 2004), while their different appearance (the oddity effect – Almany et al. 2007) and lack of co-evolutionary history (the "naïve prey" hypothesis – Sih et al. 2010) may curtail it. Thus, whether invasive species thrive or flounder may depend on what predators they encounter, and how they respond to them. This may result in "boom and bust" cycles, reflecting prey-predator dynamics (Strayer et al. 2017). Surprisingly, very little is known about anti-predatory strategies of invasive species in novel habitats.

The killer shrimp (*Dikerogammarus villosus*) is a freshwater gammarid indigenous to the Ponto-Caspian region which has recently invaded Western Europe (Tricarico et al. 2010), and which therefore constitutes a good system to examine anti-predatory strategies in novel habitats. The species has a large size for a gammarid (1.8–30 mm; Aldridge 2015), a

flexible omnivorous diet (Mayer et al. 2008), and lives in a wide variety of freshwater and brackish habitats (Devin and Beisel 2008) where it faces many different potential predators. Despite its recent introduction, it is listed among the 100 most invasive species in Europe (DAISIE 2009) and included in the RINSE (Reducing the Impact of Non-Native Species in Europe) black list with a score of 9 out of 10 (Gallardo et al. 2016). It can displace and prey on local gammarids and reduce native biodiversity (Eckmann et al. 2008; MacNeil et al. 2013), and may already be benefitting from a boom phase in some parts of Europe, having shed some of its former parasites (Arundell et al. 2015; Grabner et al. 2015). The need for more information on this aquatic invader has been flagged as a priority (Gallardo et al. 2016; Pöckl 2009), as it is predicted that the species will cause major deleterious impacts on native fauna (MacNeil et al. 2012).

The killer shrimp displays substantial trophic plasticity in invaded habitats (Casellato et al. 2007; Platvoet et al. 2009), but information on its response to predators is limited. Gammarids are an important prey for many fishes (Mazzi and Bakker 2003; Perrot-Minnot et al. 2007) and there are reports that native brown trout and perch can feed on killer shrimp in Britain (Aldridge 2015; Madgwick and Aldridge 2011). However, knowledge on the predators of killer shrimp is mostly anecdotal and there is little information on anti-predatory behaviour of this species in newly colonized areas, which is an important aspect to consider for predicting its future spread and impact.

Hiding, aggregation and crypsis are three of the most common anti-predatory strategies in aquatic species (Keenleyside 1979), which in the case of benthic gammarids are intimately related to the nature of the substrate (Bollache et al. 2006; Holomuzki and Hoyle 1990; Jermacz et al. 2015; Kobak et al. 2014). Hiding behaviour is particularly strong in gammarids (Goedmakers 1981; Jazdzewski et al. 2004), and availability of suitable substrate to hide can be a key determinant of establishment success in invasive gammarids (Devin et al. 2003), as different species may compete for shelter. For example, De Gelder et al. (2016) reported that the killer shrimp's strong tendency to hide during daytime can displace the European native gammarid *Gammarus roeselii* from their shelters, which might put them at a higher risk of predation. Another common anti-predatory strategy is aggregation behaviour, as being part of a group can confuse predators (Krakauer 1995; Krause and Ruxton 2002) and reduce the per-capita probability of being preyed (Codella and Raffa 1995). Aggregation behaviour, however, also has costs, as it can increase resource competition and the risk of being parasitized, which may put the group at a disadvantage (Krause and Ruxton 2002).

Thus, while killer shrimp invading Europe could benefit from a boom phase caused by predator release, prey naïvety and the oddity effect might make them more vulnerable to predators in invaded habitats. To shed light

Table 1. Fish predators of the killer shrimp.

Family	Species	Type	Predator recognition	Location	Reference
Gasterosteidae	Three-spined stickleback *Gasterosteus aculeatus*	Native	Y	Upper Mother Ditch (UK)	This study
Gobiidae	Caspian big headed goby *Neogobius gorlap*	Exotic	NA	Kuibyshev Reservoir (Russia)	Semenov 2009
	Round goby *Neogobius melanostomus*	Exotic	NA	Danube River (Bulgaria)	Jurajda et al. 2013
	Monkey goby *Neogobius fluviatilis*	Exotic	NA	River Rhine (Germany)	Borcherding et al. 2013
	bighead goby *Ponticola kessleri*	Exotic	NA	River Rhine (Germany)	Borcherding et al. 2013
	Racer goby *Babka gymnotrachelus*	Exotic	Y	Laboratory (Poland)	Jermacz et al. 2017a
Percidae	Eurasian ruffe *Gymnocephalus cernua*	Native	NA	River Rhine (Netherlands)	Kelleher et al. 2000
	Zander *Sander lucioperca*	Native	NA	River Rhine (Netherlands)	Kelleher et al. 2000
	Eurasian perch *Perca fluviatilis*	Native	NA	Constance Lake (Germany, Austria, Switzerland)	Eckmann et al. 2008
	Eurasian perch *Perca fluviatilis*	Native	NA	Grafham Reservoir (UK)	Environment-Agency 2011
Salmonidae	Brown trout *Salmo trutta*	Native	NA	Grafham Reservoir (UK)	Madgwick and Aldridge 2011
	Rainbow trout *Oncorhynchus mykiss*	Exotic	NA	Grafham Reservoir (UK)	Madgwick and Aldridge 2011
Cottidae	European bullhead *Cottus gobio*	Native	Y	Laboratory (France)	Sornom et al. 2012
Lotidae	Burbot *Lota lota*	Native	NA	Constance Lake (Germany, Austria, Switzerland)	Eckmann et al. 2008
Anguillidae	European eel *Anguilla anguilla*	Native	NA	Constance Lake (Germany, Austria, Switzerland)	Eckmann et al. 2008
Cyprinidae	Common barbel *Barbus barbus*	Native	NA	Danube River (Serbia)	Djikanovic et al. 2010
Centrarchidae	Pumpkinseed *Lepomis gibbosus*	Exotic	NA	Mirgenbach Reservoir (France)	Maazouzi et al. 2011
Ictaluridae	Channel catfish *Ictalurus punctatus*	Exotic	NA	River Arno (Italy)	Haubrock et al. 2018
Siluridae	Wels catfish *Silurus glanis*	Exotic	NA	River Arno (Italy)	Haubrock et al. 2018

on this issue, we tested if killer shrimp from a recently colonized stream in Britain were able to chemically recognise the three-spined stickleback, a widespread gammarid predator (MacNeil et al. 1999; Mazzi and Bakker 2003) that had not been reported to prey on killer shrimp before (Table 1).

Materials and methods

Collection and origin of samples

Killer shrimp (average size from rostrum to urosome = 16.8 ± 0.9 mm) were collected by live trapping at a site inhabited by three-spined stickleback (Upper Mother Ditch, Margam, Wales, 51°33′19.5″N; 3°44′46.6″W) in May 2017, while three-spined stickleback (wet weight range 0.9–2.0 g) were hand-netted from an ornamental pond devoid of killer shrimp (Singleton Park, Swansea, Wales, 51°36′26.2″N; 3°58′52.4″W) in July 2017. We maintained the two species in separate 100L recirculation aquaculture systems at CSAR facilities (Swansea University) to avoid mixing their scents. Both species

were fed frozen bloodworms, the sticklebacks every day and the killer shrimp three times per week. Water temperature was maintained at 15–16.5 °C with a weekly replacement of 20% volume. Killer shrimp were first detected in Upper Mother Ditch in 2011, one year after they were detected in a nearby reservoir (Madgwick and Aldridge 2011). Using the scent of an allopatric predator population that did not prey on killer shrimp ensured that killer shrimp would not respond to diet-induced alarm cues, only to the scent of the predator (Roberts and Garcia de Leaniz 2011).

Experimental design

We set up two experiments to examine the killer shrimp's anti-predatory behaviour in relation to the presence of stickleback's kairomones (a fish predator that feeds on gammarids). In the first experiment, we compared the hiding behaviour of individual killer shrimp tested in water conditioned with stickleback kairomones compared to blank water. In the second experiment, we examined the attraction of single killer shrimp to the scent of conspecifics in an open-test arena scented with stickleback kairomones or with blank water.

Water conditioning

To obtain the kairomones used to simulate the presence of a predator, we housed 20 stickleback (biomass = 2.9 g/L) in a 10L tank of dechlorinated water for 24 hours. The conditioned water was prepared freshly the day before the experiments. No food was offered to the sticklebacks during the collection of kairomones.

Laboratory predation experiment

To ascertain whether three-spined stickleback could prey on killer shrimp (despite not being listed as a predator of killer shrimp in its native area) we housed 10 sticklebacks and 40 killer shrimp in an aquarium for 24 hours and filmed their behaviour with an underwater camera (GoPro Hero).

Experiment 1. Hiding behaviour under the threat of predation

To quantify hiding behaviour under the threat of predation we used a 2 L plastic tank (L: 20 cm, W: 10 cm, H: 10 cm) fitted with artificial grass patches (3cm^2, PE thickness 15 mm) glued to the bottom in a staggered fashion (Figure 1A), and a release cylinder (3.5 cm diameter) located in the centre of the tank. At the beginning of the experiment 250 ml of either dechlorinated water (control test) or fish-conditioned water (treatment) was added to the tank. One killer shrimp was placed inside the release cylinder and left to acclimatise for 5 min. The cylinder was then slowly lifted and the behaviour of the killer shrimp (time spent swimming or hiding in the artificial grass patches) was recorded for 10 minutes with a GoPro Hero
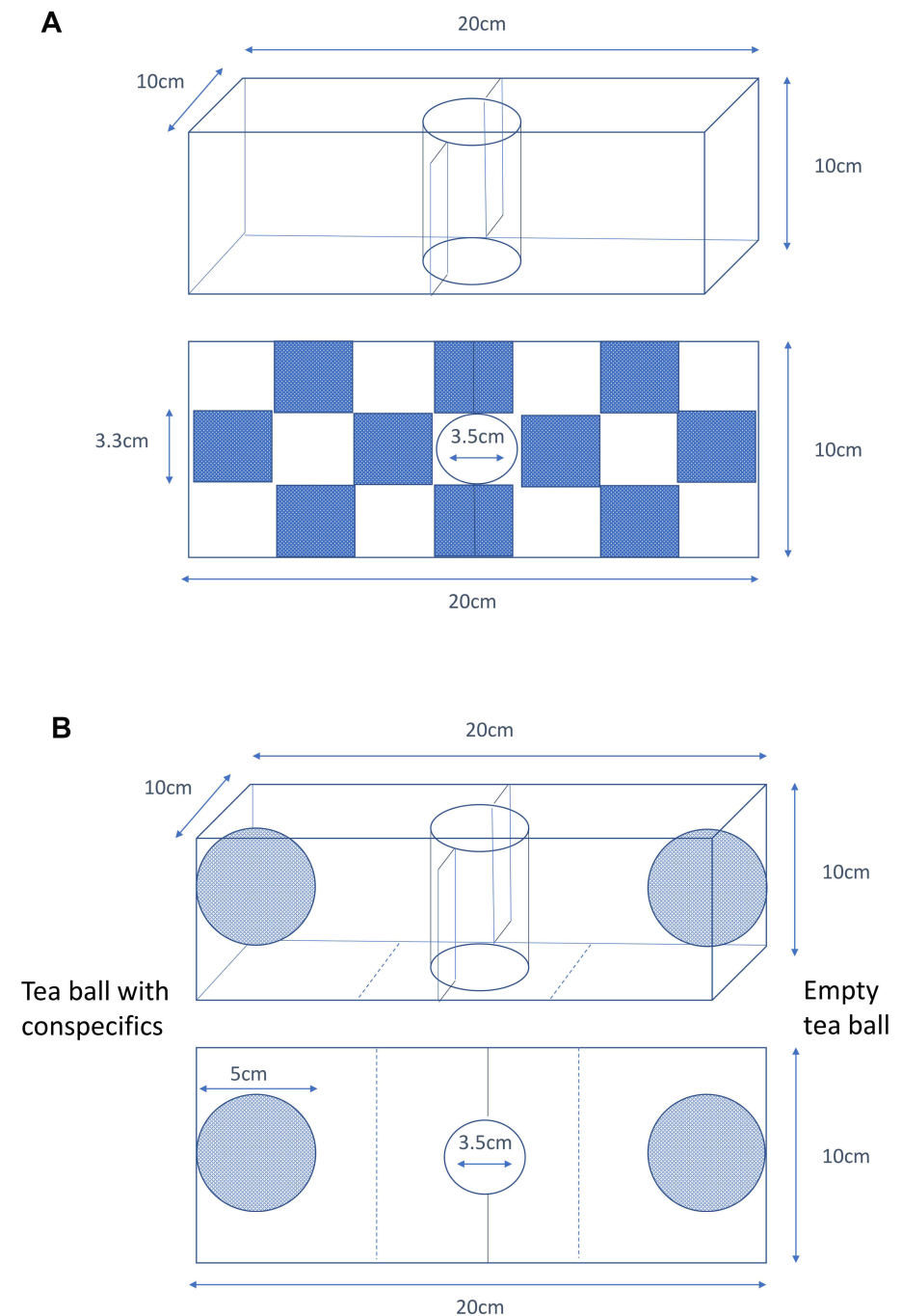
Figure 1. Experimental set up used to test (A) hiding behaviour of killer shrimp and (B) chemical attraction to conspecifics under the threat of predation from three-spined stickleback.

camera mounted above the test tank. In total, 20 mixed-sex killer shrimp were tested with water conditioned with fish kairomones and 20 with blank water, the order of which was determined at random. The time spent hiding and swimming was computed from video recordings by the lead author.

Experiment 2. Conspecific attraction

To test if killer shrimp were more attracted to conspecifics under the threat of predation, we used a tank of the same size and volume (2 L) as the one used in Experiment 1, but in this case the bottom was left bare and did not

have artificial grass patches. At the two extremes of the tank we attached two tea balls (diameter 5 cm) and drew two lines in the tank to notionally divide it into three equal sectors, two choice zones associated with the tea balls, and a middle section that served as a neutral (no choice) zone (Figure 1B). Ten killer shrimp were introduced in one of the two tea balls chosen at random, while the other one was left empty. As for experiment 1, 250 ml of fish conditioned water or dechlorinated water were added to the tank and a single individual was introduced in the acclimatization cylinder, where it was left to acclimatize for 5 min The cylinder was then removed, and the activity of the killer shrimp was recorded for 10 minutes with an overhead GoPro camera, as above. The time spent in each of the three tank zones was used to describe its behaviour: the time spent in the side containing the tea ball with conspecifics was interpreted as a measure of attraction for group protection, the time spent in the central part was interpreted as neutral behaviour, and the time spent in the side with the empty tea ball was interpreted as avoidance of conspecifics. After each trial, the tank was drained and the position of the two tea balls was alternated to control for possible external disturbances. In total, 40 killer shrimp were tested, 20 with dechlorinated water and 20 with fish scented water. The killer shrimp inside the tea ball were replaced between sessions to reduce aggressive behaviour due to confinement.

Statistical analyses

We used R 3.3 (R Core Team 2017), for analysis. For both experiments, we used a paired t-test to examine if (1) killer shrimp spent more time hiding than swimming when they were exposed to fish kairomones than when they were exposed to blank water (Experiment 1), and if (2) attraction to conspecifics was stronger when the killer shrimp were exposed to kairomones from a fish predator than when they were exposed to dechlorinated water (Experiment 2).

Ethics Statement

Experiments were carried out in accordance with Swansea University's Ethical guidelines and were approved by the Ethics Committee (070917/24, Reference Number: STU_BIOL_30638_060617140454_1). At the end of the experiments all sticklebacks were released alive at the site of capture. The killer shrimp, due to the risk they may pose for native communities, were disposed through incineration.

Results

Laboratory predation experiment

After 24 hours, sticklebacks had eaten 11 killer shrimp, resulting in a mean predation rate of 1.1 shrimp/fish/day. Underwater video filming showed two

A   B
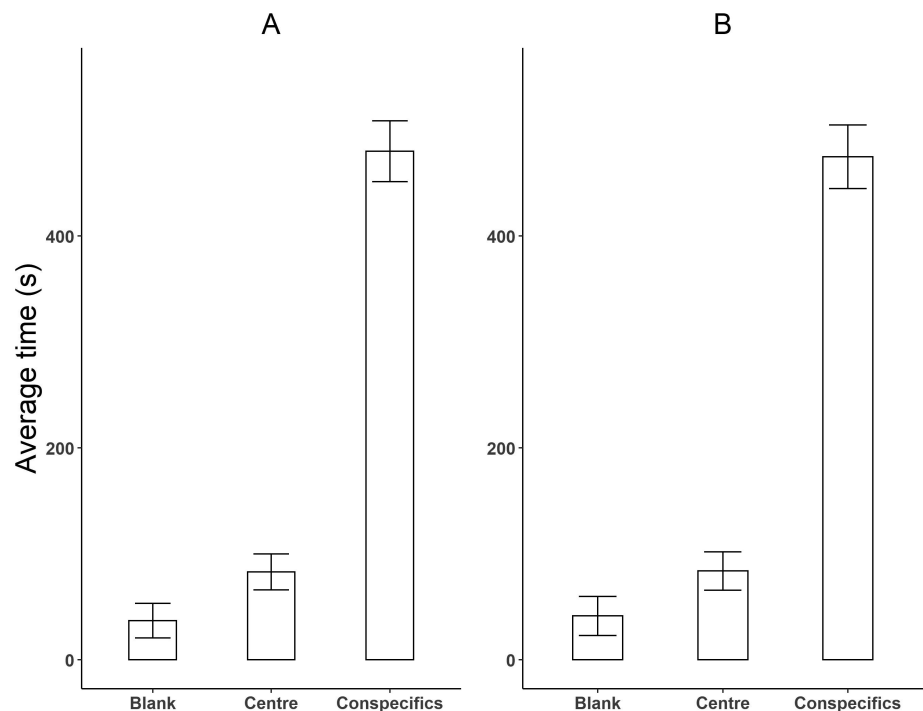
Figure 2. Hiding behaviour displaying mean time (± 95 CI) spent hiding or swimming by killer shrimp when they are exposed to (A) kairomones from a fish predator (three-spined stickleback) or (B) dechlorinated water (blank control).

sticklebacks eating killer shrimp whole, as well as one stickleback biting the legs of one shrimp and tearing it into pieces before eating it.

Experiment 1. Hiding behaviour

Killer shrimp spent significantly more time hiding when the water was conditioned with kairomones from a predatory fish (mean time ± 95CI = 543.45 ± 13.7 s) than when they were tested against blank water (mean time ± 95CI = 386.75 ± 18.5 s; behaviour × treatment interaction $F_{1,76}$ = 544.02, $P < 0.001$; Figure 2). Controls spent 50% of their time hiding and 50% swimming (t_{19} = 1.416, P = 0.173), whereas when they were exposed to fish kairomones they spent 91% of their time hiding and only 9% swimming (t_{19} = 34.789, $P < 0.001$).

Experiment 2. Attraction to conspecifics

Killer shrimp spent much more time in the side of the tank scented with conspecifics (mean time 477.5 ± 20.5 s) than in the opposite side (mean time 39.1 ± 12.2 s), but such preference was not affected by the presence of fish kairomones (t_{19} = 0.245, P = 0.808; Figure 3).

Discussion

Our study shows that killer shrimp invading a novel habitat in Britain display a strong tendency to hide when they are exposed to the chemical scent of a predatory fish (the three-spined stickleback), but not when they

A   B
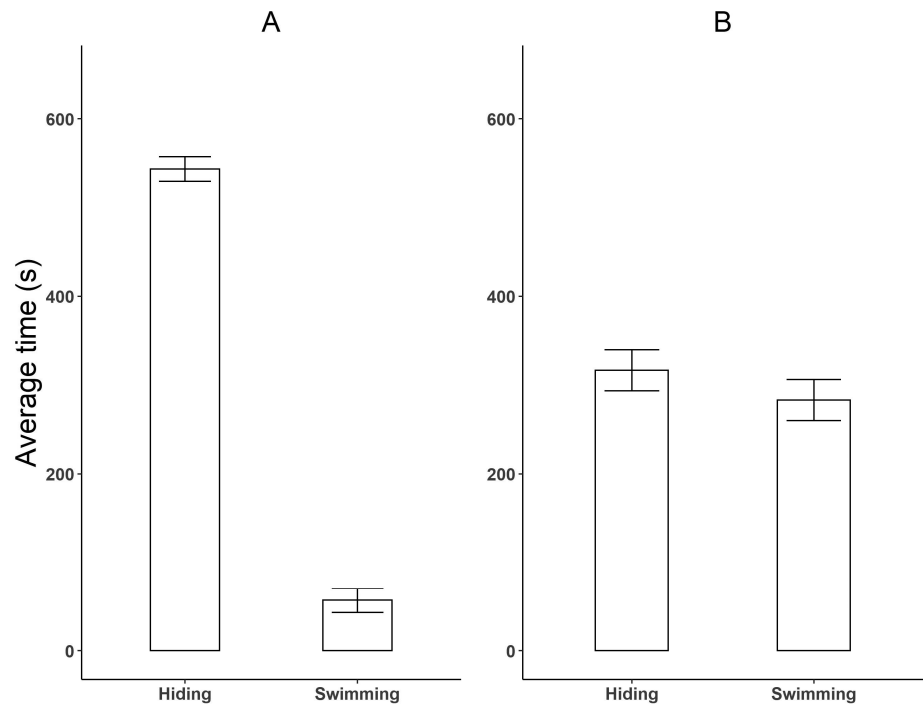
Figure 3. Aggregation behaviour showing mean time (± 95 CI) spent near conspecifics, in the centre of the tank, or away from conspecifics by killer shrimp when they are exposed to (A) kairomones from a fish predator (three-spined stickleback) or (B) dechlorinated water (blank control).

are exposed to dechlorinated water. Given that no evidence of predator avoidance was detected on the same population in relation to the scent of non-predatory Nile tilapia (Rolla et al. 2019), this suggests that chemical recognition of stickleback kairomones may constitute an adaptive trait. Previous studies have shown that killer shrimp is able to avoid both sympatric and allopatric predators (Jermacz et al. 2017a; Jermacz and Kobak 2018), which may confer some protection against predator-naivety and facilitate establishment success.

Much of our knowledge on the invasive killer shrimp outside its native range refers to its role as a predator, less information is available regarding its role as a fish prey (but see Błońska et al. 2015, 2016). This is unfortunate because predatory fish can exert strong selective pressures on gammarids (Åbjörnsson et al. 2004; Ahlgren et al. 2011; Kinzler and Maier 2006; Kotta et al. 2010; Wudkevich et al. 1997) and could play a major role in determining the killer shrimp's invasion success. The killer shrimp has been found in the diet of 17 fish species found in the introduced range (9 exotic and 8 native, Table 1), but predator recognition has only been reported for the European bullhead *Cottus gobio* (Sornom et al. 2012), the racer goby *Babka gymnotrachelus* (Jermacz et al. 2017b), the spiny-cheek crayfish *Faxonious limosus* (Hesselschwerdt et al. 2009) and the red piranha *Pygocentrus nattereri* (Jermacz et al. 2017a), therefore little is known about its antipredator behaviour. Amphipods can change their behaviour and habitat preferences when they detect chemical cues from potential predators (Baumgärtner et

al. 2003; Thiel 2010), but also from injured conspecifics (Wisenden et al. 2001; Wudkevich et al. 1997), similar to what has been observed among teleost fishes (Roberts and Garcia de Leaniz 2011) and crayfish (Hesselschwerdt et al. 2009). Kairomone detection by gammarids has been reported previously as an anti-predatory strategy (Wudkevich et al. 1997; Hesselschwerdt et al. 2009) and has been suggested recently it can faciliate killer shrimp invasions (Jermacz and Kobak 2018).

Predation by native species could reduce, or at least slow down, invasions by non-native species (Zuharah and Lester 2010, 2011) because they may not be able to recognise native predators (Sih et al. 2010), but also because their different appearance could make them easier to detect, or make them more attractive, to native predators (the "oddity prey effect" – Penry-Williams et al. 2018). For example, killer shrimp are typically larger than native freshwater gammarids (Devin et al. 2003), and this might make it easier for visual predators to detect them, although a harder exoskeleton might make it less vulnerable (Błońska et al. 2015). However, native predators may also be reluctant to feed on novel prey due to neophobia (Champneys et al. 2018), and this could result on lower predation pressure on invasive species (Crawley 1987; MacNeil et al. 2000; Trowbridge 1995; Wells and Henderson 1993). Killer shrimp could also benefit from a "shadow of safety" effect if their relative low abundance during the earlier stages of invasion deflects predation pressure to the more abundant native prey (Trillo et al. 2016). Killer shrimp can rapidly become the dominant species in invaded benthonic communities (Dick and Platvoet 2000) and can become the most abundant food resource for fish feeding on macroinvertebrates. For example, field studies have indicated that killer shrimp can replace native *Gammarus roeselii* in the diet of zoo-benthivorous fish (Eckmann et al. 2008), but other studies have suggested the opposite, and reported that native fish prefer to feed on native gammarids (Kinzler and Maier 2006; Błońska et al. 2015, 2016) . Under laboratory conditions killer shrimp tend to exhibit stronger anti-predatory behaviours than native gammarids, which may force native gammarids out of their refuges and increase their mortality due to predation (Jermacz and Kobak 2018).

Clearly, the role of predation on invasion dynamics is difficult to predict, but knowledge of the time since introduction, and of prey-predator interactions appear important in determining establishment success. This is particularly complicated in the case of the killer shrimp in Great Britain because although its arrival is very recent, it may have already learned to chemically recognise a range of novel predators during its long invasion of Europe. Killer shrimp in the British Isles are genetically similar to those in continental Europe (Rewicz et al. 2015), where the invasion started in 1992 after the opening of the Main-Danube canal (Dick and Platvoet 2000), suggesting that they are stepping stones direct descendants from the first invaders. Stepping stones strategies drive the long distance dispersal of

many species (Saura et al. 2014), and it is possible that repeated residencies in different habitats may have enabled the killer shrimp to learn to recognise different predators. Given that the three-spined stickleback is also widespread in continental Europe, our study cannot determine if the observed predator recognition was acquired in Britain, or if it represents an older behavioural legacy from previous invasions.

Two common anti-predatory strategies in amphipods are to reduce mobility and become more aggregated under the risk of predation (Åbjörnsson et al. 2000; Williams and Moore 1985; Williams et al. 2016). Results from Experiment 1 in our study indicate that killer shrimp spend more time hiding and less time swimming when they were exposed to predator kairomones, as seen in other gammarids. These findings are also in agreement with those of Sornom et al. (2012) who observed a decrease in mobility and an increase in hiding time in killer shrimp exposed to the scent of another fish predator, the European bullhead (*C. gobio*). However, our results on aggregation behaviour (Experiment 2) are more equivocal. Unlike *Gammarus pulex*, which become increasingly aggregated when exposed to stickleback kairomones (Kullmann et al. 2008), killer shrimp in our study showed the same strong preference to remain in the vicinity of conspecifics even when there was no immediate threat of predation. Exposure to bullhead kairomones also failed to elicit an increase in killer shrimp aggregation (Sornom et al. 2012), but in this case aggregation was low. Jermacz et al. (2017b) have shown that killer shrimp prefer to aggregate with conspecifics and to hide in response to predator cues, rather than aggregate, when refuges are present, and when there are no shelters and staying in a group is the only antipredator strategy possible. It is possible that aggregation behaviour in the killer shrimp depends on the availability of shelters, but also on the risk of intra-guild predation. Compared to native gammarids, killer shrimp display higher sociability and lower incidence of cannibalism (Kinzler et al. 2009; Truhlar and Aldridge 2015), which may explain their strong tendency to aggregate. Aggregation behaviour can provide not only protection from fish predators (Åbjörnsson et al. 2004), but could also facilitate dispersal, as living in a group would increase the number of founders, and propagule pressure has been found to be an important factor determining invasion success (Consuegra et al. 2011; Ricciardi et al. 2010).

Conclusions

In conclusion, prey-predator dynamics are an important, but largely neglected, determinants of invasion success and our study indicates that knowledge of anti-predatory strategies might be important for predicting dispersal pathways and risk of establishment. For example, killer shrimp might benefit from predator release during the initial stages of the invasion but may be unable to mount an efficient anti-predatory response to novel

predators due to predator-naivety and the oddity effect. Killer shrimp are dispersing at an alarmingly fast rate in Europe (DAISIE 2009; Gallardo et al. 2016), and prevention and control measures might benefit from information on prey and predators present in communities at risk. In this sense, behavioural profiling of anti-predatory strategies, using perhaps some of the simple assays shown in our study, could be incorporated into risk assessments. Knowledge of how invasive species might respond to resident predators can inform the development of more efficient management actions, as these seldom consider biotic resistance (Robinson et al. 2018, 2019). Given its strong aggregation behaviour, we also suggest that even when complete eradication is not possible, control measures that aim to reduce the density of killer shrimp might be beneficial, as a lower relative abundance and a smaller group size can make them more vulnerable to fish predators, potentially reducing their impact on native communities.

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