Defence is the best offence: invasive prey behaviour is more important than native predator behaviour

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Defence is the best offense: invasive prey behaviour more important than native predator behaviour

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ABSTRACT

Finding universal rules that predict the success of potential invaders is difficult given the breadth of interactions that occur among the invader and the species in its introduced range. Among animal species, behavioural traits may play an especially relevant role in mediating these interactions. Whereas the predatory behaviour of invasive predators is especially well documented, less is known about how behaviour may mediate the success in invasive prey species. Here we test how the behaviour of both an invasive prey species, the amphipod *Dikerogammarus villosus*, and a common fish predator, European perch *Perca fluviatilis*, affect the outcome of predation events. Invasive *D. villosus* exhibited significantly greater sheltering and lower exploratory behaviour compared to a naturalised amphipod *Gammarus roeseli*. This increased sheltering behaviour in the invasive amphipod appears to have a major functional consequence as this species was far less likely to be predated by the perch compared to the naturalised amphipod. Contrary to our predictions, the behaviour of the individual perch had no influence on amphipod consumption of either species, suggesting that amphipod behaviour was the key determinant of the success of a predation event. Our results highlight the importance of prey behaviour during predation events and emphasize that consideration of anti-predator behaviour in potentially invasive prey species may help improve predictions of invasion success.

Keywords: *Dikerogammarus villosus*, foraging, *Gammarus roeseli*, invasion syndrome, predator-prey interactions,

INTRODUCTION

Invasions are an increasing problem in our globalized world. Predicting, whether a particular invasive species will be able to successfully spread, establish and cause problems in their introduced ranges is difficult. Many traits such as abiotic tolerance, life-history strategies, and behavioural traits among others (reviewed in Hayes & Barry, 2008) are known to contribute to invasion success. However, finding universal rules that predict a potential invader's success is hampered by the fact that each invasion event is characterized by a unique set of interactions between the invader and the native community, which in turn are moderated by the environmental conditions. Given its flexibility, behaviour may be especially relevant during the invasion process (Carere & Gherardi, 2013; Chapple, Simmonds, & Wong, 2012; Holway & Suarez, 1999) when an invading animal needs to be able to reach and then persist in its new environment. However it is still unclear which behaviours might be most beneficial to invaders, though trophic position (i.e. the amount of predation it will experience) is likely to be important as it will determine the nature of the interactions with the resident community. Many invasive animal species exhibit a suite of behavioural characteristics that have been implicated in helping potential invaders disperse further (Rehage & Sih, 2004), outcompete native species (Duckworth & Badyaev, 2007), and increase consumption rates (Bollache, Dick, Farnsworth, & Montgomery, 2008; Pintor, Sih, & Bauer, 2008). In general, many invasive species are found to show higher levels of activity, aggression, exploration and/or sociability as compared to native or resident species (Duckworth & Badyaev, 2007; Hudina, Hock, & žganec, 2014; Monceau, Moreau, Poidatz, Bonnard, & Thiéry, 2015; Pintor et al., 2008; Rehage & Sih, 2004). These behaviours are especially obvious in invasive species that lack predators in their invasive range (e.g. lionfish: Green, Akins, Maljković, & Côté, 2012; cane toads: Shine, 2010). However, many invaders occupy lower trophic levels and a key step to their successful invasion is likely to be avoidance of consumption by predators. For these invaders, more passive behaviours may be more beneficial if they help reduce invader visibility and encounter rate with predators (Briffa, Jones, & Macneil, 2016; De Gelder et al., 2016; Truhlar & Aldridge, 2015), however so far invader behaviour in the face of predation has received considerably less attention making it unclear how important behaviour in this context is for invasion success.

Prey behaviour is only one half of the equation as predator behaviour will also play a key role in any predation event. Predators may be particularly important at stopping or limiting the spread of invaders at lower trophic levels (Reusch, 1998; Salo, Nordström, Thomson, & Korpimäki, 2008; Sheehy & Lawton, 2014; Smith, 2006) and the behaviour of individual predators may be especially relevant as they are often able to consume multiple prey within a short time. However, even within the same population not all predators can be considered equivalent (Bell, Hankison, & Laskowski, 2009). There are a now several examples where repeatable behavioural differences among individual predators, that is their personality or behavioural type, have been shown to influence which prey an individual predator will be most successful at attacking and consuming (Belgrad & Griffen, 2016; DiRienzo, Pruitt, & Hedrick, 2013; Pruitt, Stachowicz, & Sih, 2012; Royauté & Pruitt, 2015; Sweeney et al., 2013). In general, bolder and more active predators appear to have greater success at capturing less active prey (Pruitt et al., 2012; Sweeney et al., 2013). These behavioural traits may additionally be important when a predator encounters a novel and (potentially) invasive prey if boldness increases a predator’s likelihood to encounter and consume the prey species. Thus understanding the interplay between prey and individual predator behaviour should improve our predictions of the success of a potential invasive prey species

The freshwater amphipod, *Dikerogammarus villosus*, native to the Ponto-Caspian region, is now rapidly invading throughout Western Europe (Bij de Vaate, Jazdzewski, Ketelaars, Gollasch, & Van der Velde, 2002). In Germany, this species began invading relatively recently with the opening of the Main-Danube canal in 1992 (Rewicz, Grabowski, MacNeil, & Bacela-Spychalska, 2014). *Dikerogammarus villosus* exhibits a suite of traits that appear to make it an ideal invader (reviewed in Rewicz et al., 2014). For example, it exhibits high tolerance to a broad range of abiotic conditions (Bruijs, Kelleher, Van der Velde, & De Vaate, 2001; Wijnhoven, Van Riel, & Van der Velde, 2003), has a high reproductive capacity (Devin, Piscart, Beisel, & Moreteau, 2004; Pöckl, 2009) and fast growth rate (Piscart, Devin, Beisel, & Moreteau, 2003). Importantly, its behaviour also plays a key role in its success. *Dikerogammarus villosus* is highly aggressive and exerts significant predation pressure on lower trophic levels allowing it to out-compete resident amphipod species (Bollache et al., 2008; Dick & Platvoet, 2000; MacNeil & Platvoet, 2005) and leading to decreases in local biodiversity (Van Riel et al., 2006). These behaviours suggest that, at least in a competitive context, *D. villosus* exhibits the increased aggression and activity characteristic of many invasive species. However, less is known about how this amphipod behaves when instead, it is the target of predation (but see Briffa et al., 2016; De Gelder et al., 2016; Truhlar & Aldridge, 2015) where active and aggressive behaviour may actually increase the amphipod's visibility and encounter rates with its own predators.

Here we test how the behaviour of both the invasive *D. villosus* and a common fish predator the European perch, *Perca fluviatilis,* influences the outcome of predation events between these two species. To determine whether *D. villosus'* behaviour is similar to other amphipod species or a potentially unique contributor to its invasion success, we compared the behaviour of *D. villosus* to that of *Gammarus roeseli*. After its invasion of Europe over 150 years ago, *G. roeseli* populations appear to have stabilized and it is now considered a 'naturalised' species (Josens et al., 2005). We compared these two species as we expected that comparing an ‘old invader’ with a newer one would help control for potential differences in other traits (e.g. life-history) allowing us to better isolate the effects of behavioural differences. Additionally, *G. roeseli* and *D. villosus* co-occurs at our study sites and are morphologically similar in size and shape. Given the incredibly high densities that *D. villosus* appears to achieve in their invasive range, we expected that individual-level behaviour may be less important than species-level differences between the two amphipod species. However, perch are capable of consuming many amphipods at a time so we expected that individual perch behaviour might be especially relevant for their interactions with the amphipods.   Thus, our goals were 1) to compare average behaviour between the species *D. villosus* and *G. roeseli*, 2) determine whether individual perch consistently differed in their own behaviour and 3) determine how the behaviour of the predator and prey contributed to the outcome of predation events between individual perch and groups of either single or mixed species of amphipods. Based on results found in other invasive species (Duckworth & Badyaev, 2007; Hudina et al., 2014; Monceau et al., 2015; Pintor et al., 2008; Rehage & Sih, 2004), we predicted that *D. villosus* would exhibit more active and exploratory behaviour compared to the naturalised *G. roeseli*. We additionally predicted that individual perch behaviour that were more active and bolder should be more successful at consuming the invasive *D. villosus*.

MATERIALS & METHODS

Animal collection and maintenance

Amphipods were opportunistically collected from Lake Müggelsee and nearby streams around Berlin, Germany in summer 2016. After collection, amphipods were housed in single species aquariums at our laboratory (tank volume = 244l). Holding aquariums contained gravel, leaf litter and wooden logs similar to those found at the collecting sites. Amphipods were fed daily with plant-based fish food and thawed frozen bloodworms. We used adult amphipods that had been acclimated to the lab for at least one week and were of similar size (13.1 ± 0.8mm; mean ± s.e.) for all experiments. We could not discern between the sexes, but avoided using individuals that were engaged in the clasping and mate guarding behaviour typical of individuals about to moult and mate.

Perch were collected from Lake Müggelsee using a sink net in June 2016. All perch were ~1 years old and were not yet sexually dimorphic preventing us from sexing the fish. Upon capture, fish were anesthetized (1ml/litre 9:1 ethanol:clove oil solution in ~5l lake water) and marked with a unique combination of 3 colours of subcutaneous UV elastomer at 3 spots on their anterior side to allow permanent individual identification. Fish recovered in a dark aerated bucket and all fish recovered normal swimming ability within ~15 minutes. All fish survived the procedure and showed no adverse effects. Perch were housed in four large (~400l) aquariums in groups and fed an ab libitum diet of thawed frozen bloodworms twice daily for approximately 6 weeks prior to the start of the experiments (the first foraging trials began at the end of July). We used a total of 24 perch for our experiments.

Amphipod behavioural assays

We assessed two behaviours that we predicted could be relevant both for the amphipods' invasion ability and their ability to avoid or escape predation: hiding and activity behaviour in a familiar environment and exploration in a novel environment. To measure behaviour in a familiar environment, we placed groups (N = 10 individuals per aquarium; 10 aquaria per species) of a single species in small (tank volume = 3 l) aquaria each containing a single wooden log (roughly

10cm long and 5cm diameter) similar to their holding tanks. The amphipods settled (and were not fed) for 24 hours after which time we counted the number of amphipods (out of 10) engaged in hiding, feeding or mating behaviour six times over the next two days (9:00, 13:00, 17:00). At the beginning of an observation, we first added a small amount (~2ml) of thawed frozen bloodworms to the tanks to elicit feeding behaviour. We considered amphipods to be ‘hiding’ when they were inserted into crevices in the wooden logs and immobile; we considered amphipods to be ‘feeding’ if they were actively consuming the bloodworms (any excess bloodworms were removed at the end of the observation). Finally, we considered amphipods to be ‘mating’ if we saw two amphipods clasping each other in the characteristic mate guarding behaviour that occurs in these species. There was very little variation in the number of animals we saw engaging in each behaviour over the six observations (data not shown) so to avoid repeated measures on the same group of animals, we used the average proportion of individuals (out of 10) engaged in each behaviour over the six observations. We then assessed amphipod exploration in a novel environment by placing a single amphipod (N = 40 per species) into the centre of a 19 cm Petri dish with 8 mm conditioned water. A grid of equally sized sections (32 sections) was drawn on the bottom of the dish and we counted the number of sections crossed in a two-minute observation after a one-minute acclimation. The dish was placed in a dim and sound-dampened chamber to reduce outside disturbance. We observed behaviour using an overhead webcam (Logitech). After behavioural observations, all amphipods were returned to their species-specific holding tanks.

Perch behavioural assays

All perch were individually tested three times in two separate behavioural assays designed to measure the repeatability of baseline activity and latency to begin feeding after a risky stimulus.

Perch were transferred to individual observation arenas (tank volume = 38 l) and allowed to acclimate in these arenas for three days prior to the start of observations. Each arena contained gravel and two black plastic plants for cover. The sides of the arenas were blinded to prevent disturbance and all perch behaviour was observed using overhead webcams (Logitech). Fish were starved for 24 hours prior to observation to standardise hunger levels. We observed the swimming behaviour of the perch for 10 minutes to estimate their baseline activity. We superimposed a grid of equally sized sections on the arena (each grid-square was approximately 1 body length, ~15cm) and counted the number of sections crossed in 10 minutes. After 10 minutes, we exposed the perch to a risky stimulus by approaching the tank and lifting the front blind on the arena. In all cases, this caused the fish to hide under the black plastic plants available in the arena. We then added ~5ml of thawed frozen bloodworms to the front centre of the tank (nearest the lifted blind). We replaced the blind and measured how long until the perch emerged from the plants and began feeding to a maximum of five minutes. Fish were fed as usual upon completion of the trial. These trials were repeated every other day for a total of three trials. We had 24 observation arenas allowing us to observe all 24 fish simultaneously; however the camera malfunctioned on one arena preventing us from assessing the behaviour on one perch resulting in a sample size of *N* = 23 for the perch behavioural trials. After the behavioural trials, perch were replaced back in their group housing tanks.

Predator-prey trials

To test how average amphipod species behaviour and individual perch predator behaviour influenced the outcome of predation events we staged foraging trials between individual perch and groups of the amphipod species. We presented individual perch (*N* = 24) with groups of single (*D. villosus* or *G. roeseli*), or mixed species at four densities: 10, 20, 30 and 50 individuals (mixed species trials contained 5, 10, 15 of each species, respectively; we were unable to collect enough animals to perform mixed species trials at density 50). The arenas contained rocks and artificial plants as potential refuges for the amphipods. Amphipods were haphazardly collected from their holding tanks and were not fed 24 hours prior to the trials. We allowed the amphipods to settle in the arenas for 24 hours before adding the perch (in the morning at 10:00). The perch foraged for 24 hours after which time we counted the surviving amphipods. The perch were starved for 24 hours prior to the foraging trial; after the foraging trial the perch were given two days to recover and were fed normally (ad libitum with thawed frozen bloodworms) and then the next trial began. We could perform 12 foraging trials simultaneously and each perch was used once for each foraging combination (4 densities of single species trials x 2 species plus 3 densities of mixed species trials for a total of 11 different foraging trials per *N* = 24 perch), and the trials were performed in a random order.

We additionally tested for differences in absolute preference between the amphipod species by the perch in a barren foraging arena, thus preventing the amphipods from exhibiting their natural hiding/anti-predator behaviour. We used a subset of 8 perch and each perch performed two trials, one with 15 *D. villosus* and one with 15 *G. roeseli*, in random order.

As we expected that fish size might influence their behaviour or predatory styles, we additionally measured each perch’s wet mass (to the nearest g) after completion of the trials. As abiotic conditions likely influence the behaviour of these ectothermic animals, all experiments were conducted in a climate chamber with a constant temperature (17°C) and light cycle (12:12 L:D).

Statistical analyses

We used generalized linear models to test for species differences in amphipod behaviour. We ran separate models for each behaviour (average proportion of individuals hiding, feeding or mating

(*N* = 10 data points per species) in a familiar environment or sections swam through in a novel environment (*N* = 40 data points per species) and included species as a fixed effect. Preliminary analyses indicated that the errors of our behavioural variables were non-normally distributed and best fit a Poisson error distribution for sections swam through and a binomial error distribution for the proportion variables (i.e. number of animals hiding/number of animals not hiding). As over-dispersion can be problem with these types of error distributions (i.e. Poisson, binomial), we corrected for this by including an observation level random effect (Harrison, 2014). We estimated a 'pseudo-R^2' value for each model as '1 – (residual deviance/null deviance)'. Similar to R^2 for general linear models, this statistic offers a unit-less measure of how much variance is explained by the model (Faraway, 2016).

We used general linear mixed models to test for the repeatability of perch behaviour. We ran a separate model for each behaviour (sections swam through and latency to begin feeding), included perch body weight and observation (1-3) as fixed effects and perch ID as a random effect. We estimated the conditional repeatability of each behaviour as the proportion of variance attributable to perch ID compared to the total variance from these models (after controlling for the fixed effects). We tested for significance of the perch ID random effect by estimating a χ^2 statistic as twice the difference of the log likelihoods of a model containing the random effect to one without, with the degrees of freedom being equal to the number of extra parameters being estimated in the more complex model (i.e. log likelihood ratio test on nested models). Both sections swam through by the perch and the latency to begin feeding after a risk stimulus met assumptions of normality after a log transformation. We then tested whether these two behavioural traits were correlated at the individual level. To do this, we summed each behaviour for each individual over the three observations. We summed the behaviours, as opposed to averaging, as the behaviours were non-normally distributed. We then tested for the correlation between behaviours using a Spearman rank correlation ($N = 23$).

We then used generalized linear mixed models to test for the effects of amphipod species and perch behaviour in the proportion of amphipods consumed by the perch. As the two perch behaviours (sections swam through and latency to begin feeding) were highly correlated with each other (see Results), we only included each individual's measure of latency to begin feeding (sum of the latencies across the three observations) in the models as we predicted that this behaviour might be more relevant to the perch's foraging behaviour (though models re-run using only activity showed similar results; results not shown). Our models therefore included an interaction between latency to begin feeding and amphipod species and the main effects of latency to begin feeding, species, density, observation order and perch weight as fixed effects. Each perch was repeatedly used for the foraging trials so perch ID was included as a random effect. The two continuous fixed effects (weight and latency) were scaled to standard deviation units prior to analyses to ensure model convergence. We ran separate models for the single and mixed species foraging trials. We initially checked to see if consumption of either amphipod species depended on the density (interaction between species and density) but this term was not significant ($P > 0.15$) and not a main research questions and so we so we removed this term from the models. Models were run assuming a binomial error distribution (number of animals consumed/number of animals survived) and again included an observation level random effect to account for over-dispersion (Harrison, 2014). By including perch behaviour in the above models we were able to test for an effect of individual perch behaviour on overall consumption of each species. Perch ID was included as a random effect in both models. To test the significance of each fixed effect in the models, we performed log likelihood ratio tests as described above. We estimated a marginal R^2 (proportion of total variance explained by the fixed effects) for each full model to provide a measure of overall effect size (Nakagawa & Schielzeth, 2013). We then estimated a marginal R^2 for the significant predictors alone (proportion of total variance explained by a particular fixed effect). All statistics (see supplemental code online) were performed in R v3.3.1 using the ‘nlme’,‘lme4’, ‘betareg’, packages (Bates, Maechler, Bolker, & Walker, 2015; Gruen, Kosmidis, & Zeileis, 2012; Pinheiro, Bates, DebRoy, & Sarkar, 2013; R Core Team, 2017).

Animal welfare note

Lethal predation events staged between the amphipods and the perch were necessary to address the primary research questions of how behavior mediates the outcome of a predation interaction. To limit the number of amphipods subjected to predation we used the smallest densities possible (10-50 animals) and during the trials the amphipods were provided with refuge that they could use to avoid predation.

Perch behavioral and foraging assays were non-invasive and occurred in tanks designed to minimize outside disturbance and stress. It was necessary to permanently mark the fish with subcutaneous elastomer to ensure individual identification; however this was done under anesthesia and all animals recovered and there was no evidence of any adverse effects on the perch at any time during the experiment (there was no mortality or sign of stress or disease). After completion of the experiments, all perch were released at the point of their capture as per animal care regulations.

All experiments comply with local and German law and were approved by Berlin’s Landesamt für Gesundheit und Soziales (LaGeSo protocol G-0115/14).

RESULTS

In a familiar environment, very few amphipods were ever observed feeding or mating (proportions feeding < 0.01; proportions mating < 0.04) and so we focused only on differences in hiding behaviour where we found that a significantly larger proportion of *D. villosus* individuals hid (0.96 ± 0.01) compared to *G. roeseli* (0.60 ± 0.06; effect of species: *G. roeseli* = -2.19, $z = -5.25$, $P < 0.001$, pseudo-$R^2 = 0.64$; Figure 1a). In a novel environment, *D. villosus* individuals were significantly less active (97 ± 21.2 sections) than *G. roeseli* individuals (162.6 ± 10.9 sections; effect of species (*D. villosus*) = -0.51; $t = -2.44$, $P = 0.017$, pseudo-$R^2 = 0.09$; Figure 1b).

We found strong evidence that individual perch consistently differed in their behaviour. Perch swam through an average of 10.96 (± 1.28) sections during the assays, but this ranged from fish that never moved at all to an individual that swam through a total of 68 sections. Individual perch consistently differed in their baseline activity (conditional repeatability = 0.58; $\chi^2 = 19.99$, $P < 0.001$) with fish tending to decrease their activity over the three observations (effect of observation = -0.12, $\chi^2 = 4.18$, $P = 0.041$); there was no effect of perch weight on activity (effect of weight = 0.003, $\chi^2 = 0.51$, $P = 0.47$). Perch in general began feeding fairly quickly after the startle (average latency = 125 seconds ±15.65) though this ranged from fish that immediately began to feed to individuals that did not feed at all in the observation (maximum latency of 300 seconds). Again individuals consistently differed in their latency to begin feeding (conditional repeatability = 0.63, $\chi^2 = 26.46$, $P < 0.001$) with individuals tending to take longer to begin feed over the three observations (effect of observation = 0.13, $\chi^2 = 3.74$, $P = 0.53$) and there was no effect of perch weight (effect of weight = -0.0007, $\chi^2 = 0.01$, $P = 0.90$). The sums of the two behaviours were also highly correlated at the individual level (Spearman correlation = 0.81, $P < 0.001$, $N = 23$).

There were strong differences in predation on each of the amphipod species. Importantly, these differences appear to be driven by amphipod anti-predator behaviour as when the foraging trials were performed in a barren arena lacking any refuge, all amphipods were consumed by the perch regardless of species. However, when the foraging arenas contained refuges for the amphipods we found that significantly fewer *D. villosus* were consumed compared to *G. roeseli* in both single and mixed species trials (Table 1, Figure 2). In the single species trials, there was no effect of perch behaviour on consumption of either species (interaction between perch latency and amphipod species, Table 1) or overall consumption (main effect of perch latency, Table 1). There was additionally no effect of density, observation order or perch weight on consumption (Table 1). The fixed effects in the full model explained a total of 20% of the total variation in consumption (marginal R^2), with the majority of this being explained by the effect of species (R^2 of species = 0.19). In the mixed species trials, the largest effect was that of amphipod species but there was also a significant effect of density driven by a slight decrease in consumption on both species at density = 30; again there was no effect of the interaction between perch behaviour and species, observation order, perch weight or perch behaviour on its own. As in the single species trials, the fixed effects in the mixed species trials explained 19% of the total variation (marginal R^2) with species being the strongest predictor (R^2 of species = 0.18). Against our predictions, individual perch behaviour (latency to begin feeding after a startle) did not predict the perch's amphipod consumption in either the single or mixed species trials (Table 1).

DISCUSSION

Our experiments demonstrate strong differences in behaviour between the invasive amphipod *D. villosus* and its naturalised counterpart *G. roeseli*. *D. villosus* were significantly less active in a novel environment and spent more time hiding in a familiar environment. We also show that *D. villosus* is far less likely to be consumed by a common fish predator and that this reduction in predation pressure appears to be a result of the amphipod's behaviour and not due to differences in predator behaviour or preferences. Altogether our results demonstrate that prey avoidance behaviour drives the outcome of the predation event at least between perch and these two amphipod species. We suggest that when considering potential invaders that occupy lower trophic levels, a better understanding of their anti-predator behaviour may help improve our predictions about their potential invasion success.

Behaviour is a dynamic and flexible trait and thus it is predicted to play a key role in determining the outcome of predation events. Contrary to our expectations, we found that individual perch behaviour, at least as we measured it, did not appear to have any influence on their overall consumption or their consumption of either species. Rather our results suggest that prey behaviour, not predator behaviour, seems to regulate the encounter rate with predators. Indeed, when the amphipods were prevented from exhibiting their natural hiding and anti-predator behaviour by placing them in a barren foraging arena, all amphipods were consumed by the perch regardless of species. However, once refuges were available to the amphipods we found strong differences in their consumption by perch, where *D. villosus* were far less likely to be consumed by perch across all densities tested. Previous work has shown that *D. villosus* will actively exclude other amphipod species from potential hiding spots (De Gelder et al., 2016; Truhlar & Aldridge, 2015), which is one likely explanation for the difference in consumption rates between the two amphipod species. *Dikerogammarus villosus'* preference for hiding in crevices likely makes it completely inaccessible to predation by fish predators. This is in contrast to other studies that found that the behaviour of the predator interacted with the behaviour of the prey to determine the outcome of predation events (Belgrad & Griffen, 2016; DiRienzo et al., 2013; McGhee, Pintor, & Bell, 2013; Pruitt et al., 2012; Royauté & Pruitt, 2015; Sweeney et al., 2013). It is important to note however that our foraging trials were conducted in relatively small aquaria in the lab; perhaps predator behaviour would be more important in a larger setting where predators must search for prey more extensively.

Behaviour has the potential to play a role in a potential invader's success along many steps in the invasion process from transport to establishment to spread. The low activity and high sheltering behaviour of *D. villosus* likely contributes to its invasion success by increasing its chances of passive transport. As compared to many other invaders that show active dispersal (e.g. cane toads Brown, Phillips, & Shine, 2014; mosquitofish Cote, Fogarty, Weinersmith, Brodin, & Sih, 2010), it seems more likely that *D. villosus* is spread through more passive means (Truhlar & Aldridge, 2015) such as transport in zebra mussels attached to boats (Bacela-Spychalska, Grabowski, Rewicz, Konopacka, & Wattier, 2013) or ballast water (Bruijs et al., 2001). This kind of dispersal may be especially problematic to contain due to its stochastic nature unlike tracking the edge of an invasion front that is being pushed forward by movement of the animals themselves. And in addition to increasing opportunities for *D. villosus* to 'stow away' and be transported to new locations, our results show its increased sheltering behaviour may then help reduce exposure to pelagic fish predators once it arrives, increasing their likelihood of establishing in the new environment. *D. villosus* prefers to hide itself in stony and cobbled substrates, especially those covered with another highly invasive species, *Dreissena spp.* zebra mussels (Devin, Piscart, Beisel, & Moreteau, 2003; Hesselschwerdt, Necker, & Wantzen, 2008). Its close association with *Dreissena* also suggests the possibility of an 'invasional meltdown' (Simberloff & Von Holle, 1999) whereby the invasion of one species, *Dreissena*, encourages invasion by another, *Dikerogammarus* (Devin et al., 2003).   Management actions that reduce the likelihood or impact of *Dreissena* invasions may therefore have the added bonus of limiting invasions by *Dikerogammarus.*

Our results on the behaviour of the invader *D. villosus* also provide new insight into which types of behaviours might be most useful for potential invaders. There is good evidence that many invasive species exhibit greater aggressive, exploratory and/or active behaviour compared to resident species (Cote et al., 2010; Duckworth & Badyaev, 2007; Pintor et al., 2008; Rehage & Sih, 2004). However, our results add to a literature that suggests that this is not universally the case, especially when behaviour is considered outside of a competitive context. Previous work on invasive amphipods, including *D. villosus*, suggests that they might exhibit more passive behaviour (De Gelder et al., 2016; Truhlar & Aldridge, 2015) at least in an anti-predator context. Our results now add to this by showing that these behavioural differences may contribute to the amphipods' success by reducing its risk of predation. Thus considering an invader's potential predation risk and the functional context of its behaviour may help refine predictions about their likely behavioural strategies: aggressive behaviours are likely advantageous during intra-guild competition (e.g. Dick & Platvoet, 2000), but passive behaviours likely reduce risk under the threat of predation (e.g. Pennuto & Keppler, 2008). Indeed, the ability of *D. villosus* to exhibit highly aggressive and active behaviour in the face of competition, coupled with its high sheltering behaviour in the face of predation add to the growing list of traits that may make this the 'perfect' invader (Rewicz et al., 2014). Future work investigating behavioural strategies in invasive species across a range of trophic positions may help build a more general framework for when and why certain behaviours will be most beneficial for invasion success.

Dikerogammarus villosus is a relatively recent invader to Germany; while its exact date of arrival in the Berlin area is apparently not known, it was first recorded in Germany in 1992 with the opening of Main-Danube canal (Bij de Vaate et al., 2002; Rewicz et al., 2014) suggesting it likely arrived in Berlin sometime after that. One explanation for the increased hiding behaviour in this invader is that in the 25 years since its invasion into Germany, evolutionary processes have selected against increased activity and exploration meaning the animals we measured now might not represent the behavioural variation in the first invaders. However Truhlar and Aldridge (2015) found no differences in behaviour in *D. villosus* from edge and core populations in the UK; both populations exhibited high sheltering behaviour, suggesting that there was not a selective disadvantage to increased sheltering in older populations. (Josens et al., 2005) If predation pressure was strongly selecting for sheltering and anti-predator behaviour, it is then surprising that this species does not show as strong sheltering behaviour as *D. villosus*, given that it has been present in Germany for far longer. It appears that this extreme anti-predator and sheltering behaviour is quite characteristic of D. villosus and may be contributing to its ability to surpass the former invader *G. roeseli*. It may be that this difference in behaviour has driven the eventual 'naturalization' of *G. roeseli* in Europe. Josens et al. (2005)describes naturalised species as "former exotic species [that have been] well established for a long period and [are now] in balance with [their] environment". Based on our sampling, it also appears that there might be habitat segregation between the two species so *G. roeseli* may instead be avoiding areas of high predation altogether.

Dikerogammarus amphipods are one of the most successful invaders from the Ponto-Caspian region. Our study adds to the growing list of traits that enhance its invasion success by showing that its increased hiding behaviour likely reduces its predation exposure. We hope to also highlight the need to refine predictions about the 'best' behavioural strategies for invaders to adopt as this will depend on the functional context of the behaviour, and the trophic level of the species.

Data accessibility. Data and R code supporting this manuscript are available online as supplemental material. .

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Author contributions. KLL conceived of the study, GJM collected the data, KLL analyzed the data and both authors wrote the manuscript.

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Table 1. Results of general(ized) linear mixed models on the predictors of amphipod consumption by perch predators in single and mixed species trials.

Effect	**Estimate (± s.e.)**	**z-value**	**χ^2**	**p-value (χ^2)**
Single species: consumption (marginal $R^2 = 0.20$)				
Intercept	1.59 (± 0.60)			
Species:Latency	-0.009 (± 0.12)	-0.08	0.006	0.94
Species[†]: D. villosus	-1.88 (± 0.12)	-15.36	153.52	**<0.001**
Density[¥]: 20	0.08 (± 0.27)	0.30	4.22	0.24
30	-0.19 (± 0.46)	-0.41		
50	-0.21 (± 0.29)	-0.73		
Observation order	0.09 (± 0.10)	0.91	0.82	0.36
Weight	0.11 (± 0.08)	1.25	1.51	0.22
Latency	0.05 (± 0.09)	0.61	0.37	0.54
Mixed species: consumption (marginal $R^2 = 0.19$)				
Intercept	1.66 (± 0.73)			
Species:Latency	-0.03 (± 0.14)	-0.29	0.08	0.78
Species[†]: D. villosus	-1.71 (± 0.13)	-12.63	110.4	**<0.001**
Density[¥]: 20	0.78 (± 0.32)	2.38	11.50	**0.003**
30	0.61 (± 0.57)	1.07		
Observation order	0.11 (± 0.09)	1.26	1.56	0.21
Weight	-0.02 (± 0.07)	-0.38	0.15	0.70
Latency	-0.04 (± 0.07)	-0.53	0.28	0.60

Significance of fixed effects was tested using a log-likelihood ratio test (χ^2 statistic) on nested models (see methods); significant effects are bolded. All models additionally included perch ID as a random effect to account for the multiple observation of each individual perch.

[†] Baseline (intercept) level was taken as the consumption rate of *G. roeseli*. This therefore reflects the difference in consumption of D. villosus as compared to *G. roeseli*.

[¥] Baseline (intercept) level was taken as density of 10 amphipods. These estimates therefore reflect the difference in consumption of amphipods at densities 20, 30 and 50 as compared to at density 10.

FIGURE CAPTIONS

Figure 1. Number of each species of amphipod that were observed hiding (out of 10) in a familiar environment (a) and the number of sections crossed in a novel environment (b). The dark centre line shows the median, the boxes the upper and lower quartiles and the whiskers are the quartile plus 1.5 the interquartile range. Data outside this range are represented by dots.

Figure 2. Proportion of each species of amphipod consumed by perch at different densities in either single species trials (a) or mixed species trials (b). The dark centre line shows the median, the boxes the upper and lower quartiles and the whiskers are the quartile plus 1.5 the interquartile range. Data outside this range are represented by dots.

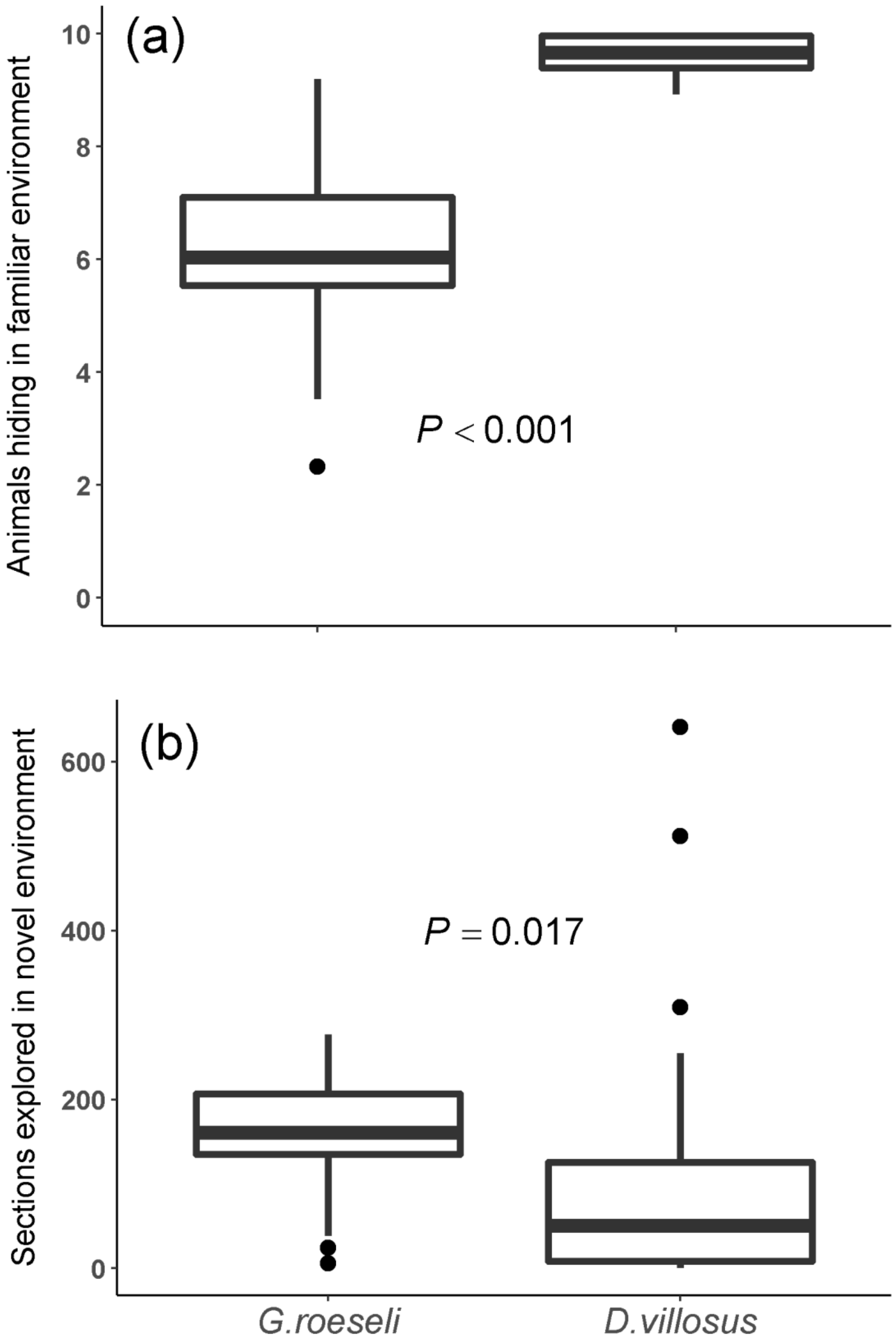
(a)
Animals hiding in familiar environment
0
2
4
6
8
10
$P < 0.001$
(b)
Sections explored in novel environment
0
200
400
600
$P = 0.017$
G.roeseli
D.villosus

Figure 1

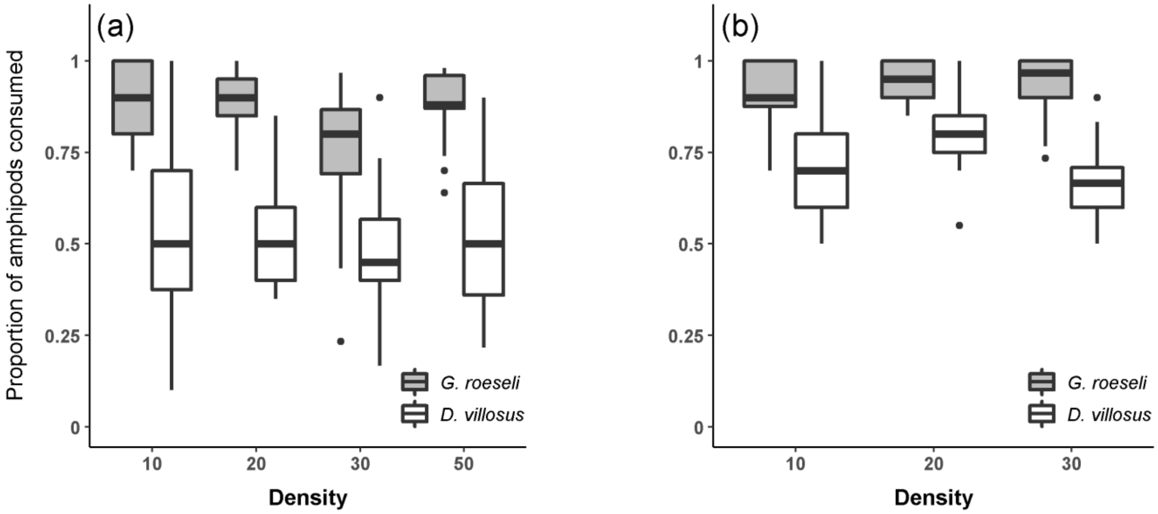
(a)
(b)
Proportion of amphipods consumed
1
0.75
0.5
0.25
0
10
20
30
50
Density
G. roeseli
D. villosus

Figure 2.