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

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7

8 **ABSTRACT**

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

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

27 INTRODUCTION

28 Invasions are an increasing problem in our globalized world. Predicting, whether a particular
29 invasive species will be able to successfully spread, establish and cause problems in their
30 introduced ranges is difficult. Many traits such as abiotic tolerance, life-history strategies, and
31 behavioural traits among others (reviewed in Hayes & Barry, 2008) are known to contribute to
32 invasion success. However, finding universal rules that predict a potential invader's success is
33 hampered by the fact that each invasion event is characterized by a unique set of interactions
34 between the invader and the native community, which in turn are moderated by the
35 environmental conditions. Given its flexibility, behaviour may be especially relevant during the
36 invasion process (Carere & Gherardi, 2013; Chapple, Simmonds, & Wong, 2012; Holway &
37 Suarez, 1999) when an invading animal needs to be able to reach and then persist in its new
38 environment. However it is still unclear which behaviours might be most beneficial to invaders,
39 though trophic position (i.e. the amount of predation it will experience) is likely to be important
40 as it will determine the nature of the interactions with the resident community. Many invasive
41 animal species exhibit a suite of behavioural characteristics that have been implicated in helping
42 potential invaders disperse further (Rehage & Sih, 2004), outcompete native species (Duckworth
43 & Badyaev, 2007), and increase consumption rates (Bollache, Dick, Farnsworth, & Montgomery,
44 2008; Pintor, Sih, & Bauer, 2008). In general, many invasive species are found to show higher
45 levels of activity, aggression, exploration and/or sociability as compared to native or resident
46 species (Duckworth & Badyaev, 2007; Hudina, Hock, & žganec, 2014; Monceau, Moreau,
47 Poidatz, Bonnard, & Thiéry, 2015; Pintor et al., 2008; Rehage & Sih, 2004). These behaviours
48 are especially obvious in invasive species that lack predators in their invasive range (e.g. lionfish:
49 Green, Akins, Maljković, & Côté, 2012; cane toads: Shine, 2010). However, many invaders
50 occupy lower trophic levels and a key step to their successful invasion is likely to be avoidance of

51 consumption by predators. For these invaders, more passive behaviours may be more beneficial if
52 they help reduce invader visibility and encounter rate with predators (Briffa, Jones, & Macneil,
53 2016; De Gelder et al., 2016; Truhlar & Aldridge, 2015), however so far invader behaviour in the
54 face of predation has received considerably less attention making it unclear how important
55 behaviour in this context is for invasion success.

56 Prey behaviour is only one half of the equation as predator behaviour will also play a key
57 role in any predation event. Predators may be particularly important at stopping or limiting the
58 spread of invaders at lower trophic levels (Reusch, 1998; Salo, Nordström, Thomson, &
59 Korpimäki, 2008; Sheehy & Lawton, 2014; Smith, 2006) and the behaviour of individual
60 predators may be especially relevant as they are often able to consume multiple prey within a
61 short time. However, even within the same population not all predators can be considered
62 equivalent (Bell, Hankison, & Laskowski, 2009). There are a now several examples where
63 repeatable behavioural differences among individual predators, that is their personality or
64 behavioural type, have been shown to influence which prey an individual predator will be most
65 successful at attacking and consuming (Belgrad & Griffen, 2016; DiRienzo, Pruitt, & Hedrick,
66 2013; Pruitt, Stachowicz, & Sih, 2012; Royauté & Pruitt, 2015; Sweeney et al., 2013). In general,
67 bolder and more active predators appear to have greater success at capturing less active prey
68 (Pruitt et al., 2012; Sweeney et al., 2013). These behavioural traits may additionally be important
69 when a predator encounters a novel and (potentially) invasive prey if boldness increases a
70 predator's likelihood to encounter and consume the prey species. Thus understanding the
71 interplay between prey and individual predator behaviour should improve our predictions of the
72 success of a potential invasive prey species

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

91 Here we test how the behaviour of both the invasive *D. villosus* and a common fish
92 predator the European perch, *Perca fluviatilis*, influences the outcome of predation events
93 between these two species. To determine whether *D. villosus*' behaviour is similar to other
94 amphipod species or a potentially unique contributor to its invasion success, we compared the
95 behaviour of *D. villosus* to that of *Gammarus roeseli*. After its invasion of Europe over 150 years
96 ago, *G. roeseli* populations appear to have stabilized and it is now considered a 'naturalised'

97 species (Josens et al., 2005). We compared these two species as we expected that comparing an
98 ‘old invader’ with a newer one would help control for potential differences in other traits (e.g.
99 life-history) allowing us to better isolate the effects of behavioural differences. Additionally, *G.*
100 *roeseli* and *D. villosus* co-occurs at our study sites and are morphologically similar in size and
101 shape. Given the incredibly high densities that *D. villosus* appears to achieve in their invasive
102 range, we expected that individual-level behaviour may be less important than species-level
103 differences between the two amphipod species. However, perch are capable of consuming many
104 amphipods at a time so we expected that individual perch behaviour might be especially relevant
105 for their interactions with the amphipods. Thus, our goals were 1) to compare average behaviour
106 between the species *D. villosus* and *G. roeseli*, 2) determine whether individual perch
107 consistently differed in their own behaviour and 3) determine how the behaviour of the predator
108 and prey contributed to the outcome of predation events between individual perch and groups of
109 either single or mixed species of amphipods. Based on results found in other invasive species
110 (Duckworth & Badyaev, 2007; Hudina et al., 2014; Monceau et al., 2015; Pintor et al., 2008;
111 Rehage & Sih, 2004), we predicted that *D. villosus* would exhibit more active and exploratory
112 behaviour compared to the naturalised *G. roeseli*. We additionally predicted that individual perch
113 behaviour that were more active and bolder should be more successful at consuming the invasive
114 *D. villosus*.

115

116 **MATERIALS & METHODS**

117 *Animal collection and maintenance*

118 Amphipods were opportunistically collected from Lake Müggelsee and nearby streams around
119 Berlin, Germany in summer 2016. After collection, amphipods were housed in single species

120 aquariums at our laboratory (tank volume = 244l). Holding aquariums contained gravel, leaf litter
121 and wooden logs similar to those found at the collecting sites. Amphipods were fed daily with
122 plant-based fish food and thawed frozen bloodworms. We used adult amphipods that had been
123 acclimated to the lab for at least one week and were of similar size ($13.1 \pm 0.8\text{mm}$; mean \pm s.e.)
124 for all experiments. We could not discern between the sexes, but avoided using individuals that
125 were engaged in the clasping and mate guarding behaviour typical of individuals about to moult
126 and mate.

127 Perch were collected from Lake Müggelsee using a sink net in June 2016. All perch were ~1
128 years old and were not yet sexually dimorphic preventing us from sexing the fish. Upon capture,
129 fish were anesthetized (1ml/litre 9:1 ethanol:clove oil solution in ~5l lake water) and marked with
130 a unique combination of 3 colours of subcutaneous UV elastomer at 3 spots on their anterior side
131 to allow permanent individual identification. Fish recovered in a dark aerated bucket and all fish
132 recovered normal swimming ability within ~15 minutes. All fish survived the procedure and
133 showed no adverse effects. Perch were housed in four large (~400l) aquariums in groups and fed
134 an ad libitum diet of thawed frozen bloodworms twice daily for approximately 6 weeks prior to
135 the start of the experiments (the first foraging trials began at the end of July). We used a total of
136 24 perch for our experiments.

137 *Amphipod behavioural assays*

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

143 10cm long and 5cm diameter) similar to their holding tanks. The amphipods settled (and were not
144 fed) for 24 hours after which time we counted the number of amphipods (out of 10) engaged in
145 hiding, feeding or mating behaviour six times over the next two days (9:00, 13:00, 17:00). At the
146 beginning of an observation, we first added a small amount (~2ml) of thawed frozen bloodworms
147 to the tanks to elicit feeding behaviour. We considered amphipods to be 'hiding' when they were
148 inserted into crevices in the wooden logs and immobile; we considered amphipods to be 'feeding'
149 if they were actively consuming the bloodworms (any excess bloodworms were removed at the
150 end of the observation). Finally, we considered amphipods to be 'mating' if we saw two
151 amphipods clasping each other in the characteristic mate guarding behaviour that occurs in these
152 species. There was very little variation in the number of animals we saw engaging in each
153 behaviour over the six observations (data not shown) so to avoid repeated measures on the same
154 group of animals, we used the average proportion of individuals (out of 10) engaged in each
155 behaviour over the six observations. We then assessed amphipod exploration in a novel
156 environment by placing a single amphipod ($N = 40$ per species) into the centre of a 19 cm Petri
157 dish with 8 mm conditioned water. A grid of equally sized sections (32 sections) was drawn on
158 the bottom of the dish and we counted the number of sections crossed in a two-minute
159 observation after a one-minute acclimation. The dish was placed in a dim and sound-dampened
160 chamber to reduce outside disturbance. We observed behaviour using an overhead webcam
161 (Logitech). After behavioural observations, all amphipods were returned to their species-specific
162 holding tanks.

163 *Perch behavioural assays*

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

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

184 *Predator-prey trials*

185 To test how average amphipod species behaviour and individual perch predator behaviour
186 influenced the outcome of predation events we staged foraging trials between individual perch
187 and groups of the amphipod species. We presented individual perch ($N = 24$) with groups of
188 single (*D. villosus* or *G. roeseli*), or mixed species at four densities: 10, 20, 30 and 50 individuals

189 (mixed species trials contained 5, 10, 15 of each species, respectively; we were unable to collect
190 enough animals to perform mixed species trials at density 50). The arenas contained rocks and
191 artificial plants as potential refuges for the amphipods. Amphipods were haphazardly collected
192 from their holding tanks and were not fed 24 hours prior to the trials. We allowed the amphipods
193 to settle in the arenas for 24 hours before adding the perch (in the morning at 10:00). The perch
194 foraged for 24 hours after which time we counted the surviving amphipods. The perch were
195 starved for 24 hours prior to the foraging trial; after the foraging trial the perch were given two
196 days to recover and were fed normally (ad libitum with thawed frozen bloodworms) and then the
197 next trial began. We could perform 12 foraging trials simultaneously and each perch was used
198 once for each foraging combination (4 densities of single species trials x 2 species plus 3
199 densities of mixed species trials for a total of 11 different foraging trials per $N = 24$ perch), and
200 the trials were performed in a random order.

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

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

209 *Statistical analyses*

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

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

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

235 averaging, as the behaviours were non-normally distributed. We then tested for the correlation
236 between behaviours using a Spearman rank correlation ($N = 23$).

237 We then used generalized linear mixed models to test for the effects of amphipod species
238 and perch behaviour in the proportion of amphipods consumed by the perch. As the two perch
239 behaviours (sections swam through and latency to begin feeding) were highly correlated with
240 each other (see Results), we only included each individual's measure of latency to begin feeding
241 (sum of the latencies across the three observations) in the models as we predicted that this
242 behaviour might be more relevant to the perch's foraging behaviour (though models re-run using
243 only activity showed similar results; results not shown). Our models therefore included an
244 interaction between latency to begin feeding and amphipod species and the main effects of
245 latency to begin feeding, species, density, observation order and perch weight as fixed effects.
246 Each perch was repeatedly used for the foraging trials so perch ID was included as a random
247 effect. The two continuous fixed effects (weight and latency) were scaled to standard deviation
248 units prior to analyses to ensure model convergence. We ran separate models for the single and
249 mixed species foraging trials. We initially checked to see if consumption of either amphipod
250 species depended on the density (interaction between species and density) but this term was not
251 significant ($P > 0.15$) and not a main research questions and so we so we removed this term from
252 the models. Models were run assuming a binomial error distribution (number of animals
253 consumed/number of animals survived) and again included an observation level random effect to
254 account for over-dispersion (Harrison, 2014). By including perch behaviour in the above models
255 we were able to test for an effect of individual perch behaviour on overall consumption of each
256 species. Perch ID was included as a random effect in both models. To test the significance of
257 each fixed effect in the models, we performed log likelihood ratio tests as described above. We
258 estimated a marginal R^2 (proportion of total variance explained by the fixed effects) for each full

259 model to provide a measure of overall effect size (Nakagawa & Schielzeth, 2013). We then
260 estimated a marginal R^2 for the significant predictors alone (proportion of total variance
261 explained by a particular fixed effect). All statistics (see supplemental code online) were
262 performed in R v3.3.1 using the ‘nlme’, ‘lme4’, ‘betareg’, packages (Bates, Maechler, Bolker, &
263 Walker, 2015; Gruen, Kosmidis, & Zeileis, 2012; Pinheiro, Bates, DebRoy, & Sarkar, 2013; R
264 Core Team, 2017).

265 *Animal welfare note*

266 Lethal predation events staged between the amphipods and the perch were necessary to address
267 the primary research questions of how behavior mediates the outcome of a predation interaction.
268 To limit the number of amphipods subjected to predation we used the smallest densities possible
269 (10-50 animals) and during the trials the amphipods were provided with refuge that they could
270 use to avoid predation.

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

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

280

281 **RESULTS**

282 In a familiar environment, very few amphipods were ever observed feeding or mating
283 (proportions feeding < 0.01 ; proportions mating < 0.04) and so we focused only on differences in
284 hiding behaviour where we found that a significantly larger proportion of *D. villosus* individuals
285 hid (0.96 ± 0.01) compared to *G. roeseli* (0.60 ± 0.06 ; effect of species: *G. roeseli* = -2.19, $z = -$
286 5.25, $P < 0.001$, pseudo- $R^2 = 0.64$; Figure 1a). In a novel environment, *D. villosus* individuals
287 were significantly less active (97 ± 21.2 sections) than *G. roeseli* individuals (162.6 ± 10.9
288 sections; effect of species (*D. villosus*) = -0.51; $t = -2.44$, $P = 0.017$, pseudo- $R^2 = 0.09$; Figure
289 1b).

290 We found strong evidence that individual perch consistently differed in their behaviour.
291 Perch swam through an average of 10.96 (± 1.28) sections during the assays, but this ranged from
292 fish that never moved at all to an individual that swam through a total of 68 sections. Individual
293 perch consistently differed in their baseline activity (conditional repeatability = 0.58; $\chi^2 = 19.99$,
294 $P < 0.001$) with fish tending to decrease their activity over the three observations (effect of
295 observation = -0.12, $\chi^2 = 4.18$, $P = 0.041$); there was no effect of perch weight on activity (effect
296 of weight = 0.003, $\chi^2 = 0.51$, $P = 0.47$). Perch in general began feeding fairly quickly after the
297 startle (average latency = 125 seconds ± 15.65) though this ranged from fish that immediately
298 began to feed to individuals that did not feed at all in the observation (maximum latency of 300
299 seconds). Again individuals consistently differed in their latency to begin feeding (conditional
300 repeatability = 0.63, $\chi^2 = 26.46$, $P < 0.001$) with individuals tending to take longer to begin feed
301 over the three observations (effect of observation = 0.13, $\chi^2 = 3.74$, $P = 0.53$) and there was no
302 effect of perch weight (effect of weight = -0.0007, $\chi^2 = 0.01$, $P = 0.90$). The sums of the two

303 behaviours were also highly correlated at the individual level (Spearman correlation = 0.81, $P <$
304 0.001, $N = 23$).

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

324 **DISCUSSION**

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

335 Behaviour is a dynamic and flexible trait and thus it is predicted to play a key role in
336 determining the outcome of predation events. Contrary to our expectations, we found that
337 individual perch behaviour, at least as we measured it, did not appear to have any influence on
338 their overall consumption or their consumption of either species. Rather our results suggest that
339 prey behaviour, not predator behaviour, seems to regulate the encounter rate with predators.
340 Indeed, when the amphipods were prevented from exhibiting their natural hiding and anti-
341 predator behaviour by placing them in a barren foraging arena, all amphipods were consumed by
342 the perch regardless of species. However, once refuges were available to the amphipods we found
343 strong differences in their consumption by perch, where *D. villosus* were far less likely to be
344 consumed by perch across all densities tested. Previous work has shown that *D. villosus* will
345 actively exclude other amphipod species from potential hiding spots (De Gelder et al., 2016;
346 Truhlar & Aldridge, 2015), which is one likely explanation for the difference in consumption
347 rates between the two amphipod species. *Dikerogammarus villosus*' preference for hiding in
348 crevices likely makes it completely inaccessible to predation by fish predators. This is in contrast

349 to other studies that found that the behaviour of the predator interacted with the behaviour of the
350 prey to determine the outcome of predation events (Belgrad & Griffen, 2016; DiRienzo et al.,
351 2013; McGhee, Pintor, & Bell, 2013; Pruitt et al., 2012; Royauté & Pruitt, 2015; Sweeney et al.,
352 2013). It is important to note however that our foraging trials were conducted in relatively small
353 aquaria in the lab; perhaps predator behaviour would be more important in a larger setting where
354 predators must search for prey more extensively.

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

373 invasion by another, *Dikerogammarus* (Devin et al., 2003). Management actions that reduce the
374 likelihood or impact of *Dreissena* invasions may therefore have the added bonus of limiting
375 invasions by *Dikerogammarus*.

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

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

416 *Dikerogammarus* amphipods are one of the most successful invaders from the Ponto-
417 Caspian region. Our study adds to the growing list of traits that enhance its invasion success by
418 showing that its increased hiding behaviour likely reduces its predation exposure. We hope to
419 also highlight the need to refine predictions about the ‘best’ behavioural strategies for invaders to

420 adopt as this will depend on the functional context of the behaviour, and the trophic level of the
421 species.

422

423 **Data accessibility.** Data and R code supporting this manuscript are available online as
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575 **Table 1.** Results of general(ized) linear mixed models on the predictors of amphipod
 576 consumption by perch predators in single and mixed species trials.

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

577

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

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

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

586

587 **FIGURE CAPTIONS**

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

592

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

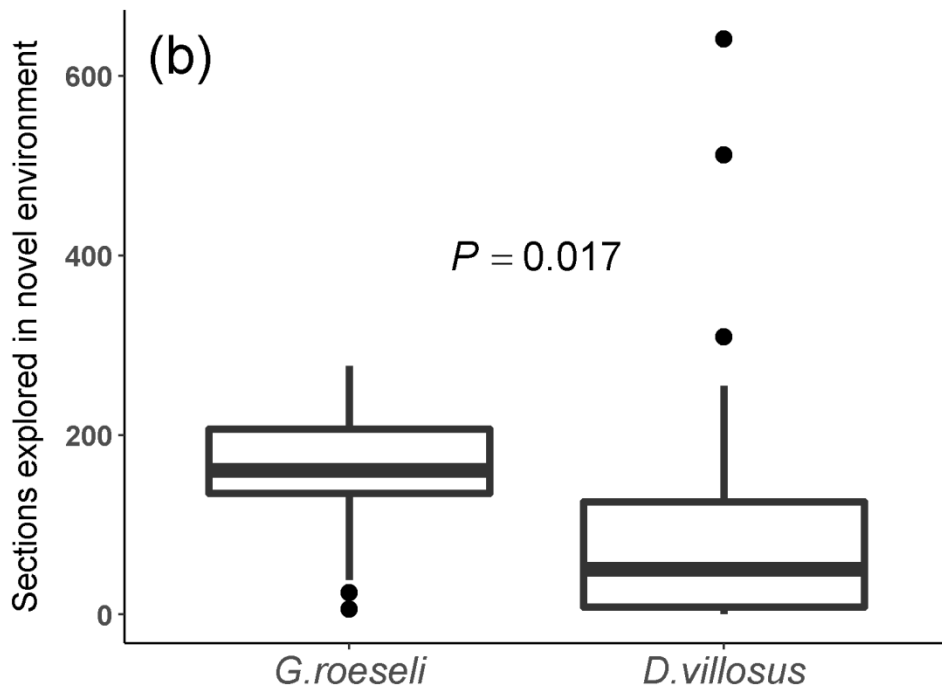
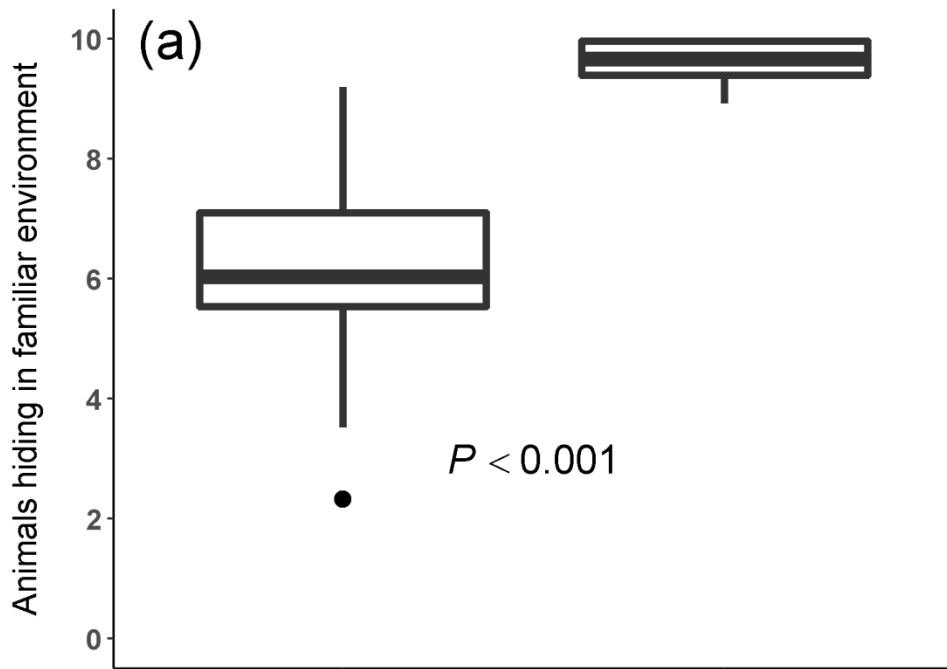
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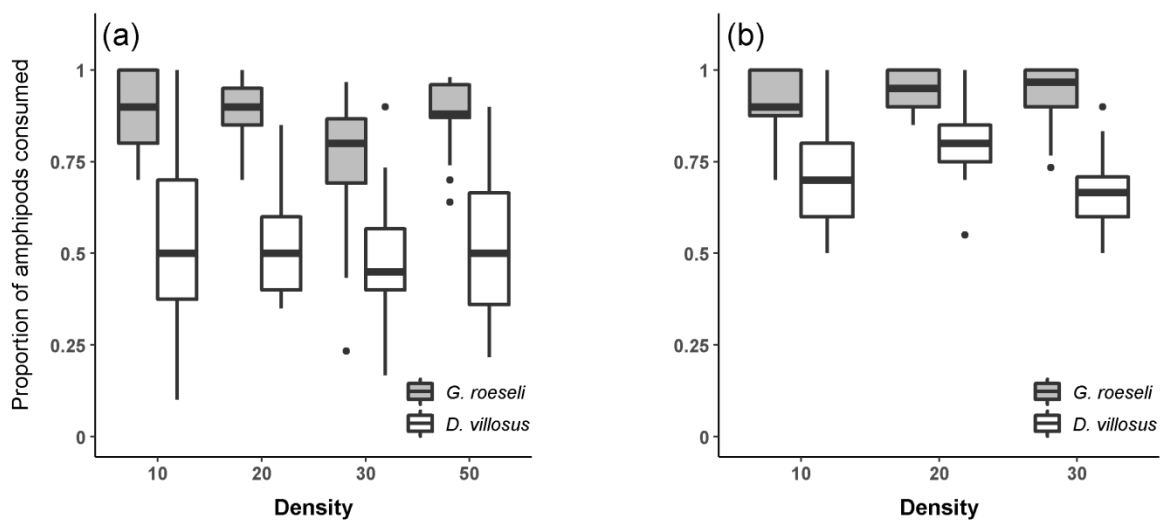
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602

603 Figure 1



604

605 Figure 2.