Experimental evidence of chemical attraction in the mutualistic zebra mussel-killer shrimp system

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

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14 **Abstract**

15 Invasion facilitation, whereby one species has a positive effect on the establishment of another

16 species, could help explain the rapid colonisation shown by some freshwater invasive species,

17 but the underlying mechanisms remain unclear. We employed two-choice test arenas to test

18 whether the presence of zebra mussel (*Dreissena polymorpha*) could facilitate the establishment

19 of the killer shrimp (*Dikerogammarus villosus*). Killer shrimp preferred to settle on mats of zebra

20 mussel, but this was unrelated to mat size, and was not different from attraction shown to

21 artificial grass, suggesting that zebra mussel primarily provides substrate and refuge to the killer

22 shrimp. Killer shrimp were strongly attracted to water scented by zebra mussel, but not to water

23 scented by fish. Chemical attraction to the zebra mussel's scent did not differ between sympatric

24 and allopatric populations of killer shrimp, suggesting that chemical attraction is not an acquired

25 or learned trait. Our study shows, for the first time, chemical attraction between two highly

26 invasive freshwater species, thereby providing a plausible mechanism for invasion facilitation.

27 This has implications for managing the spread of killer shrimp, and perhaps other freshwater

28 invasive species, because chemical attraction could significantly increase establishment success

29 in mutualistic systems. Failure to consider invasion facilitation may underestimate the risk of

30 establishment, and likely also the impact of some aquatic invaders.

32 Keywords

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

Introduction

36   The impact of biological invasions has often been examined in isolation, under the implicit

37   assumption that invaders do not interact with each other (Consuegra et al. 2011; Vanhaecke et al.

38   2015; Young et al. 2010; Young et al. 2009). However, invasion facilitation, whereby one

39   species has a positive effect on the introduction, establishment or dispersal of other invasive

40   species, is well documented, particularly in terrestrial plants and animals (Adams et al. 2003;

41   Altieri et al. 2010). For example, the presence of the European honey bee (*Apis mellifera*) has

42   increased the reproduction success of the invasive shrub *Lantana camara* in Australia (Goulson

43   & Derwent 2004), and similar positive synergies among invasive species have also been reported

44   across many taxa (Woodward et al. 1990). This led Simberloff & Von Holle (1999) to coin the

45   term 'invasional meltdown' to describe the process by which the negative impacts triggered by

46   one invasive species could be exacerbated by the interactions with other exotic species

47   (Simberloff 2006).

48   However, invasion facilitation has not received as much attention in freshwater habitats

49   as it has in terrestrial ecosystems, possibly because it is more difficult to detect (Ricciardi 2005),

50   and because it typically only benefits one species (i.e. commensalism, (Laihonen & Furman

51   1986; Ricciardi 2005). This lack of information is unfortunate because freshwater habitats,

52   particularly lakes and ponds, rank among the most threatened ecosystems in the world, and this

53   makes them particularly vulnerable to the threat of aquatic invasive species (AIS; Dudgeon et al.

54   2006). Habitat suitability models for invasive species, as well as risk maps (Crall et al. 2013;

55   Jiménez-Valverde et al. 2011), rarely take into account the fact that some invaders can

56   bioengineer their new habitat to suit their needs, or that the presence of one invasive species may

57   make the habitat more attractive to other invaders (Strayer 2012). Thus, the synergistic effects of

58   invasive species and their cumulative impacts on native fauna may be underestimated in aquatic

59   habitats if invasion facilitation exists and is not taken into account.

60   Two aquatic invaders that often occur together and may benefit from invasion facilitation

61   are the zebra mussel (*Dreissena polymorpha*) and the killer shrimp (*Dikerogammarus villosus*).

62   The two species are included in the 100 worst invasive species in Europe (www.europe-

63   aliens.org), and in the case of zebra mussel, in the world (Lowe et al. 2000). Zebra mussels form

64   dense mats on natural and artificial substrates which require expensive eradication programmes

65   (Lovell et al. 2006), and compete directly for food and space with native bivalves (Fahnenstiel et

66   al. 1995a; Fahnenstiel et al. 1995b; Johengen et al. 1995), sometimes driving them to extinction

67   (Baker & Hornbach 1997). The killer shrimp, on the other hand, has the typical profile of an

68   efficient and plastic invader (Rewicz et al. 2014; Rossano et al. 2013), being able to adapt to a

69   wide range of waters and conditions (Piscart et al. 2003). Its wide diet includes many

70   macroinvertebrates, including native gammarids, which may be displaced and driven to local

71   extinction (Dick & Platvoet 2000; Piscart et al. 2003).

72   Both species share a broad, common Ponto-Caspian geographical origin, but the extent of

73   sympatry in their native, as well as in the invaded areas, is unclear. The zebra mussel was first

74   reported in Europe during the nineteenth century, becoming highly invasive and forming large

75   populations (Son 2007). In contrast, the killer shrimp is a much more recent invader, it has only

76   been detected in Europe during the last 20 years, but has spread rapidly ever since (MacNeil et

77   al. 2010; Rewicz et al. 2014; Rewicz et al. 2017; Tricarico et al. 2010).

78   While many of the sites colonised by the killer shrimp in Europe had already established

79   populations of zebra mussel, that might be indicative of invasion facilitation (Gallardo &

80   Aldridge 2015), the killer shrimp has also invaded many areas devoid of zebra mussel (Rewicz et

81 al. 2015; van der Velde et al. 2010). Thus, whether there have been synergies in the

82 establishment of these two species is not clear (Devin et al. 2003). The zebra mussel has shown

83 mutualistic interactions with one gastropod (Ricciardi 2005; Ricciardi et al. 1997), and two

84 macrophytes (MacIsaac 1996; Skubinna et al. 1995), and can benefit the killer shrimp in various

85 ways. For example, the dense interstitial matrix formed by the shells of zebra mussels may

86 provide refuge for the killer shrimp (Ricciardi et al. 1997), allowing it to survive outside the

87 water, while the production of faeces and pseudo faeces may provide food (Gergs & Rothhaupt

88 2008a; Gergs & Rothhaupt 2008b; Ricciardi et al. 1997; Stewart et al. 1998b). Similarly, zebra

89 mussel larvae can attach to the hard chitin cover of the killer shrimp which could facilitate their

90 dispersal (Kenderov 2017; Yohannes et al. 2017).

91   Given their common geographical origin, and recent evidence suggesting that the two

92 species often occur together and might benefit each other, we hypothesized that killer shrimp

93 might be chemically attracted to the presence of zebra mussel. We further hypothesized that

94 attraction might differ depending on whether killer shrimp were found in habitats already

95 colonised by the zebra mussel, i.e. whether attraction differed between sympatric and allopatric

96 conditions.

97   Previous studies had indicated that mussel beds may provide killer shrimp with food and

98 shelter (Gergs & Rothhaupt 2008a; Gergs & Rothhaupt 2008b), but two-choice preference tests

99 yielded contradictory results (Gergs & Rothhaupt 2008a), and did not consider the effects of

100 coexistence or group dynamics on killer shrimp behaviour (Truhlar & Aldridge 2015). We

101 therefore employed an experimental approach to examine the attraction of killer shrimp to zebra

102 mussel, considering potential differences between sympatric and allopatric populations, and

103 individual versus group behaviour. In the first experiment we tested if killer shrimp had a

104   preference for high densities of zebra mussels, as one might expect from a mutualistic system. In

105   the second experiment, we tested if killer shrimp had a preference for live zebra mussels

106   compared to empty shells or an artificial substrate, and whether this was affected by group

107   behaviour, as one might expect if attraction was mostly driven by food, and not by cover; in the

108   third experiment we tested if killer shrimp could detect the presence of zebra mussel through

109   chemical cues in the water, and whether this depended on past coexistence. Ultimately, our aim

110   was to address some of the underlying mechanisms of invasion facilitation as this might help

111   design better predictive models and more effective control measures of these two aquatic

112   invaders.

113

114 **Materials and Methods**

115 *Collection and origin of samples*

116 Sympatric zebra mussel and killer shrimp were collected from Cardiff Bay (Cardiff, UK – Grid

117 reference: ST 19210 73510) in April 2016, whereas allopatric killer shrimp were collected from

118 the Upper Mother Ditch (Margam, UK, Grid reference: SS 79029 85506) in September 2016,

119 where the zebra mussel is not yet present (Figure 1). These were brought to the CSAR facilities

120 at Swansea University and maintained in 6 x 20 L tanks fed by separated recirculation

121 aquaculture systems, with a weekly replacement of c. 20% volume. Zebra mussels (~2.4 kg)

122 were fed three times per week with a 2L mixture of *Scenedesmus* sp. and *Chlorella* sp., while

123 killer shrimps (~100g) were fed three times per week with 6g of frozen bloodworms. Water

124 temperature was maintained at 15-16.5˚C. The killer shrimp used in the tests had an average size

125 of 16.8±0.9 mm and were tested at water temperature ranging between 15.4 °C and 16.2 °C.

126

127 *Experiment 1. Preference by killer shrimp of zebra mussel density*

128 To test if killer shrimp had a preferred density of zebra mussel to settle on, we employed a 3L

129 tank (L25 x H 10 x W12 cm; Figure 2A) divided into two equal sections, each with a different

130 density of zebra mussel (0, 33, 67, or 100% cover), and an acclimatisation plastic cylinder in the

131 middle. Individual killer shrimp (n = 96) were allowed to acclimatise for five minutes in the

132 cylinder, then the cylinder was lifted and the position and behaviour (swimming or hiding) of the

133 shrimp after 20 minutes was recorded. We assumed that if the shrimp was hiding it meant it had

134 found a suitable substrate, whereas if it was still swimming it meant it was still looking for a

135 refuge. We tested the killer shrimp's binary choice over six matched densities (n = 16) of zebra

136   mussel: 0-33%, 0-66%, 0-100%, 33-66%, 33-100%, and 66-100% employing a total of 96

137   specimens, and allocating the densities to the left or right sides of the test arena at random.

138

139   *Experiment 2. Preference for zebra mussel over an artificial substrate*

140   To test if the attraction of killer shrimp for zebra mussel-beds was simply related to the presence

141   of cover or to other factors (such as bio-deposited material) we compared preference for living

142   shells against either empty shells of zebra mussel or artificial grass (PE thickness 15mm) of

143   similar texture and extent of refuge . We used one killer shrimp per trial (n= 30), and then twenty

144   killer shrimp per trial (n = 80) to understand if substrate choice was affected by group dynamics.

145   The experimental protocol was the same as in Experiment 1, but in this case each side of the test

146   arena afforded 50% cover and we used a 20 L test tank (L40 x H15 x W35 cm).

147

148   *Experiment 3. Chemical attraction to zebra mussel*

149   To test if killer shrimp was chemically attracted to the scent of zebra mussel we employed a

150   simplified version of the two-choice Perspex fluviarium used by Kroon (2005) in the preference

151   of another crustacean (Figure 2B). The fluviarium consisted of an acclimatization chamber (L5 x

152   H6.5 x W5 cm) and two 0.3L choice chambers (L20 x H6.5 x W10 cm) with a total volume of

153   approximately 0.7L. We tested preferences against dechlorinated tap water (blank), water

154   scented with zebra mussel and Nile tilapia (*Oreochromis niloticus*) to control for possible

155   attraction to organic matter, as well as blank water vs blank water to control for chamber bias.

156   Individual killer shrimp were allowed to acclimatize for five minutes, the valves connected to the

157   two water inlets were opened, the gate was lifted, and the time spent in each chamber was

158   recorded for 15 minutes with a GoPro Hero camera. We compared the time spent in each arm as

159   well as the number of transitions between arms as a measure of activity. Scent drip dosage was

160   adjusted at 200 ml/min.

161   To prepare the scented water we placed either zebra mussels or fish (tilapia) in a tank

162   filled with dechlorinated water for 24 hours at a biomass of 50g/L. The fluviarium was drained,

163   cleaned with 90% ethanol and rinsed with fresh water between trials to remove potential

164   chemical cues that could affect the next experiment. We repeated the experiment with killer

165   shrimp originating from a population living in sympatry (Cardiff Bay, n = 60) or allopatry

166   (Margam, n = 60) with zebra mussels. All the zebra mussel came from Cardiff Bay.

167

168   *Statistical analysis*

169   We used R 3.3 (R Core Team, 2017) for all analysis. In experiments 1 and 2, we used a

170   generalized linear model (GLM) with a binomial log-link to test if the number of killer shrimp in

171   the scented arm differed with treatment, and we then used a two-sided binomial test to assess if

172   there was a statistically significant preference for the high or low density (Experiment 1) or

173   different substrate combinations (Experiment 2) at each binary choice comparison.   For

174   Experiment 3 (two-way choice fluviarium), we used a linear model with time spent in the

175   scented arm as the dependent variable and origin (allopatry vs sympatry) and type of scent

176   (blank, zebra mussel, tilapia) as the predictors; we then used paired t-tests to assess which type of

177   matched scent comparisons was statistically significant.

178

179   *Ethics Statement*

180   Zebra mussels and killer shrimp were collected under sampling permit CHA-01042016 from the

181   Cardiff Water Authority. All experiments were carried out in accordance with Swansea

182   University Ethical guidelines and were approved by the College of Science Ethics Committee

183   (300419/1557). Water removed from the experimental system was treated with bleach before

184   disposing, to avoid accidental dispersion of zebra mussel larvae.

185

186

187 **Results**

188 *Experiment 1. Preferred zebra mussel densities*

189 Preference for zebra mussel varied depending on the densities being compared (χ^2 = 29.09, df =

190 1, $P < 0.001$). Killer shrimp showed a clear preference for the side of the tank with zebra mussel

191 when the alternative was a bare tank bottom (Figure 3A-C; binomial proportion test: 0-33% P =

192 0.004; 0-66% $P < 0.001$; 0-100% $P < 0.001$). However, when both sides of the test arena had

193 different densities of zebra mussel, killer shrimp showed no preference (Figure 3D,F; binomial

194 proportion test 33-66%, P = 0.454; 66-100%, P =1.00) or preferred the lower density (Figure 3E;

195 33-100%, P = 0.004). After 20 minutes, the majority of killer shrimp (85/96 or 88.5%) were

196 found to be hiding, rather than swimming (binomial proportion test P <0.001) regardless of

197 treatment (χ^2 = 1.745, df = 1, P = 0.883).

198

199 *Experiment 2. Substrate preference*

200 When tested individually, killer shrimp did not prefer live zebra mussels over artificial grass

201 (Figure 4A; binomial proportion test, P = 0.584), or over empty zebra mussel shells (binomial

202 proportion test, P = 0.200; Figure 4B). The majority of individuals were found hiding (rather

203 than swimming), both when the comparison was against artificial grass (binomial proportion test,

204 83.3% $P < 0.001$) and also when there were empty shells (binomial proportion test, 76.6% P =

205 0.005).

206 However, when the experiment was repeated with 20 shrimp per trial (4 trials or 80

207 shrimp), killer shrimp strongly preferred the zebra mussel substrate over artificial grass (Figure

208 4C; binomial proportion test, P = 0.006) and also over empty shells (Figure 4D; binomial

209 proportion test, P = 0.006). As before, at the end the trials the majority of individuals were

210   hiding, both when the comparison was against artificial grass (binomial proportion test, 77.5% *P*

211   < 0.001) and also against empty shells (binomial proportion test, 72.5% *P* = 0.006).

212

213   *Experiment 3. Chemical attraction to zebra mussel*

214   No side preference was detected when killer shrimp were tested against blank water in both arms

215   of the 2-choice fluviarium, either in the sympatric (t_9 = 1.343, *P* = 0.212; Figure 5A) or allopatric

216   killer shrimp populations (t_{19} = -1.280, *P* = 0.216; Figure 5B), indicating that there was no side

217   bias. When killer shrimp were tested against water conditioned with tilapia scent, no preference

218   was observed over blank water, either in sympatry (t_{19} = 0.819, *P* = 0.423; Figure 5C) or

219   allopatry (t_{19} = -0.687, *P* = 0.500; Figure 5D). However, when killer shrimp were tested against

220   water conditioned with zebra mussel scent, there was a strong chemical attraction to the zebra

221   mussel scent, both in the sympatric (t_{27} = -2.176, *P* = 0.038; Figure 5E) and allopatric population

222   (t_{19} = -2.614, *P* = 0.017; Figure 5F). Chemical attraction for zebra mussel scent was equally

223   strong in the sympatric and allopatric populations ($F_{1,118}$ = 1.036, *P* = 0.311).

224   The analysis of activity (measured as the number of transitions between arms) indicates that

225   activity was influenced by the type of test scent (Figure 6), as killer shrimp made more changes

226   when both arms were dosed with blank water than when one arm was dosed with zebra mussel

227   scent (*P* = 0.002) or tilapia scent (*P* < 0.001). No difference in activity was observed when killer

228   shrimp were presented with the scent of zebra mussel or the tilapia scent against blank water (*P*

229   = 0.759). Overall, the sympatric population made more choices and was more active than the

230   allopatric population (*P* = 0.005).

231

232

Discussion

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

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

256   The findings of Experiment 2, where we tested the preference of killer shrimp for zebra

257   mussel over other textured substrates, are more difficult to interpret as different results were

258   obtained depending on group size. When killer shrimp were tested singly, no preference was

259   detected for live zebra mussels over empty shells or artificial grass of similar texture, suggesting

260   that substrate preference was mainly governed by the availability of refuge, which previous

261   experiments have shown confers protection from fish predators (Kinzler & Maier 2006).

262   However, when groups of twenty shrimp were tested, a strong preference for live zebra mussel

263   over other substrates was found, suggesting the existence of group behaviour that cannot solely

264   be explained by refuge availability and deserves further investigation. Killer shrimp tend to form

265   aggregations, and these are thought to be advantageous and increase the chances of successfully

266   colonising new areas (Truhlar & Aldridge 2015). However, while group behaviour can increase

267   fitness and reproductive success (Réale et al. 2007), it can also facilitate intraspecific predation

268   (cannibalism), which is frequently observed in amphipods (Dick et al. 1993; Hunte & Myers

269   1984; Ward 1985), including the killer shrimp (Dick & Platvoet 2000; Dick et al. 2002; MacNeil

270   et al. 1997). An inverse association may exist between sociability and cannibalism in amphipods

271   (Kinzler et al. 2009; Truhlar & Aldridge 2015), although this may also be influenced by

272   predation pressure (Dick et al. 1993). Cannibalism in killer shrimp does not appear to be so

273   strong as to reduce the species' sociability, possibly because cannibalism mainly targets small

274   juveniles (Kinzler & Maier 2003) which tend to be spatially segregated from larger adults that

275   could prey on them (Devin et al. 2003).

276   One novel finding of our study was the strong chemical attraction shown by killer shrimp

277   to the scent of live zebra mussels (or something associated with them), a response not seen to

278   blank water or the scent of non-predatory fish. Amphipods use chemical cues as their main form

279   of communicating between conspecifics (Thiel 2011), and also to recognize and avoid predators

280   (Wooster 1998), but chemical attraction in contexts other than conspecific recognition or prey-

281   predator interactions has, to our knowledge, not been reported before. A previous two-choice

282   study reported chemical avoidance of zebra mussel scent by killer shrimp, but this was tested

283   using water from a lake known to contain many predatory fish (Gergs & Rothhaupt 2008a).

284   Chemical detection in amphipods is mediated mainly via specific sensillae located on the

285   antennae (reviewed by Hallberg & Skog 2011) and is used in mate choice and species

286   discrimination, reducing the chances of interspecific mating between similar species (Cothran et

287   al. 2013; Dick & Elwood 1990). Chemical cues are also used by females to recognize and defend

288   their offspring against conspecifics (Mattson & Cedhagen 1989), and some amphipods can also

289   recognize alarm cues from damaged conspecifics and mount a strong freezing response as an

290   anti-predatory strategy (Sehr & Gall 2016). Killer shrimp have been reported to use chemical

291   cues to recognize and avoid potential predators such as the spiny-cheek crayfish *Orconectes*

292   *limosus* (Hesselschwerdt et al. 2009), the European bullhead *Cottus gobio* (Sornom et al. 2012)

293   and the racer goby *Babka gymnotrachelus* (Jermacz et al. 2017). Our study shows that killer

294   shrimp can also use chemical cues to find zebra mussels that provide not only cover and refuge

295   (Ricciardi et al. 1997), but also food through the production of faeces and pseudo faeces (Gergs

296   & Rothhaupt 2008a; Gergs & Rothhaupt 2008b; Ricciardi et al. 1997; Stewart et al. 1998a).

297   A preference for settling in zebra mussel beds was only manifested in our study when

298   killer shrimp were tested in groups, not when they were tested singly, which serves to highlight

299   the need to take group dynamics into account in behavioural studies of social gammarids

300   (Williams et al. 2016). The preference for zebra mussel beds shown by the killer shrimp (at least

301   when they are tested in groups), and the fact that they are strongly attracted to the zebra mussel

302 scent, suggests that this could be an example of invasion facilitation, as seen in other studies. For

303 example, positive synergies resulting in invasion facilitation have been reported for an invasive

304 algae and an invasive bryozoan (Levin et al. 2002), as well as among invasive fish parasites

305 (Hohenadler et al. 2018). Other well known examples of invasion facilitation, include the

306 bullfrog-sunfish system, where the survival of the invasive bullfrog (*Rana catesbeiana*) was

307 enhanced by the presence of the non-native bluegill sunfish (*Lepomis macrochirus*) because the

308 latter preyed on native dragonfly which in turn preyed on bullfrog tadpoles (Adams et al. 2003).

309 Similarly, predation by an invasive crab on a large native clam resulted in the spread of a smaller

310 invasive clam due to competitive release (Grosholz 2005).

311   Our study indicates that chemical attraction by the killer shrimp to the zebra mussel scent

312 was as strong under sympatric as it was under allopatric conditions, suggesting this is not a

313 recently acquired or learned trait, but rather an older behavioural adaptation. However, the

314 absence of population replication (it is very difficult to find populations of killer shrimp that do

315 not coexist with zebra mussel) makes it difficult to draw firm conclusions and would warrant

316 further studies.

317

318 **Conclusion**

319 In general, mutualist interactions are less well studied than competitive ones (Simberloff & Von

320 Holle 1999), and interactions between invasive species are less well known than those between

321 invasive and native species (Gallardo & Aldridge 2018). There is still limited knowledge on

322 positive interactions among invasive species, despite the fact that this may hold the key for more

323 effective control of new invasions. Given the strong preference for settling on zebra mussel mats,

324 and the benefits that this entails (Gergs & Rothhaupt 2008b; MacNeil et al. 2008) – including not

325   just refuge, but also benthic organic matter that can be a source of food (Ricciardi et al. 1997;

326   Stewart et al. 1998b), chemical attraction may help understand synergies between these two

327   invasive species. Chemical attraction may need to be taken into account in the study of patterns

328   of distribution of these two invasive species, since the presence of zebra mussel could make the

329   killer shrimp more likely to become established, and hence a more successful invader. For

330   example, chemical attraction to zebra mussels could help the establishment success of killer

331   shrimp by decreasing search time for food and shelter. The zebra mussel's ability to settle on a

332   wide range of substrate (Marsden & Lansky 2000) and bioengineer its own environment (Mayer

333   et al. 2001) can turn formerly unsuitable habitats into favourable locations for killer shrimp

334   settlement, expanding the potential range of suitable environments. For example, the NBN Atlas

335   (https://nbnatlas.org/), the UK's largest biodiversity database, holds 3,182 records of zebra

336   mussel but only 317 observations of killer shrimp and information on the presence of zebra

337   mussel could help make more accurate predictions of the likely spread of killer shrimp. This is of

338   particular concern when the species is a recent invader since there is typically insufficient

339   information to predict areas at risk or to guide management (Morales et al. 2017). In Great

340   Britain, the killer shrimp was first detected in 2010 (Rewicz et al. 2014) and is only present in 8

341   locations, making it difficult to predict its future dispersal (Rodríguez-Rey et al. 2019). The

342   zebra mussel, in contrast, was first detected in 1824 and is now established in 376 locations in

343   England and Wales (Aldridge et al. 2004; Rodríguez-Rey et al. 2019), potentially increasing the

344   number of favourable locations for killer shrimp many fold. Yet, current invasive species

345   prioritisation lists (Boets et al. 2014; Carboneras et al. 2018) and risk assessment guidelines (Roy

346   et al. 2018) tend to view invasive species in isolation, making no allowance for invasion

347   facilitation. Our study suggests that information on the presence of zebra mussel should be

348 incorporated into risk maps and models of killer shrimp dispersal, because ignoring chemical

349 attraction will likely underestimate the extent and consequences of invasion facilitation.

350

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356

357

358 **Author Contributions & Competing Interests**

359 CGL and SC designed the study and secured the funding. MR and EC collected the data and

360 carried out the analyses with advice from CGL. CGL and MR wrote the MS with contributions

361 from SC and DH. The authors declare no competing interests.

362

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593

594

595   **Figure legends**

596   Figure 1. Location of experimental killer shrimp populations living in sympatry (Cardiff Bay)

597   and allopatry (Upper Mother Ditch) with zebra mussel.

598

599   Figure 2. Experimental set up used to test substrate preferences of killer shrimp (A - side view; C

600   – top view) and chemical attraction to water scented by zebra mussel (B – side view; D – top

601   view).

602

603   Figure 3. Proportion of individual killer shrimp (binomial 95 CI) settling in zebra mussel beds of

604   different sizes in six binary choice tests (A-F, n = 16 shrimp/test) involving different amount of

605   zebra mussel cover (0, 33, 66, and 100% tank cover).

606

607   Figure 4. Proportion of killer shrimp (binomial 95 CI) settling in binary choice tests involving

608   different substrates (live zebra mussel, empty shells of zebra mussel, artificial grass) tested

609   singly (A-B, n = 30 shrimp/test) or in groups of 20 (C-D, n=4 tests).

610

611   Figure 5. Preference by individual killer shrimp (mean time spent, s ± 95 CI) in water

612   conditioned with different scents (blank water, n = 40; tilapia scent, n = 40; zebra mussel scent,

613   n = 40) from sympatric (A,C,E; n = 60) and allopatric (B,D,F; n = 60) populations.

614

615   Figure 6. Activity (mean number of transitions ± 95 CI) of individual killer shrimp tested in

616   water conditioned with different scents (blank water, n = 40; tilapia scent, n = 40; zebra mussel

617   scent, n = 40) from sympatric (A; n = 60) and allopatric (B; n = 60) populations.

618   .

619

620

Figure 1

Figure 1

Location of experimental killer shrimp populations living in sympatry (Cardiff Bay) and allopatry (Upper Mother Ditch) with zebra mussel.
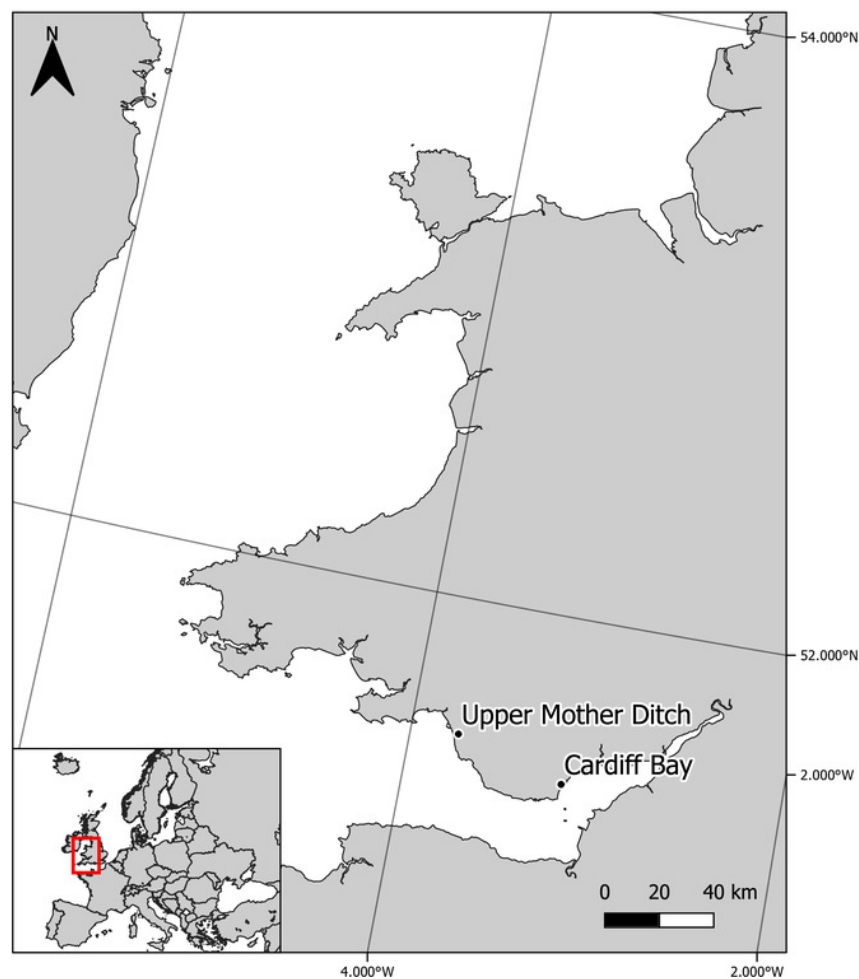
Figure 2

Figure 2

Figure 2. Experimental set up used to test substrate preferences of killer shrimp (A - side view; C – top view) and chemical attraction to water scented by zebra mussel (B – side view; D – top view).
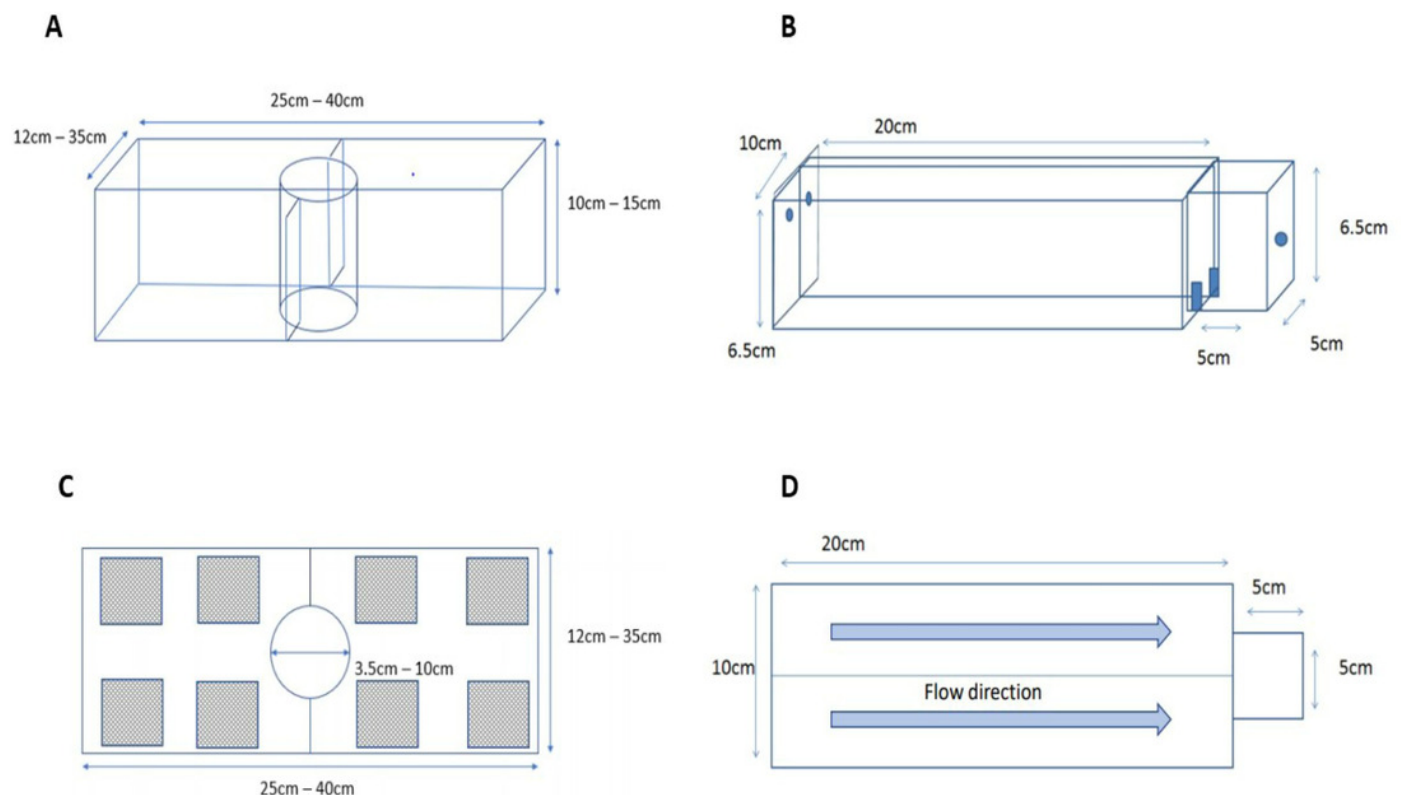
Figure 3

Figure 3

Figure 3. Proportion of individual killer shrimp (binomial 95 CI) settling in zebra mussel beds of different sizes in six binary choice tests (A-F, n = 16 shrimp/test) involving different amount of zebra mussel cover (0, 33, 66, and 100% tank cover).
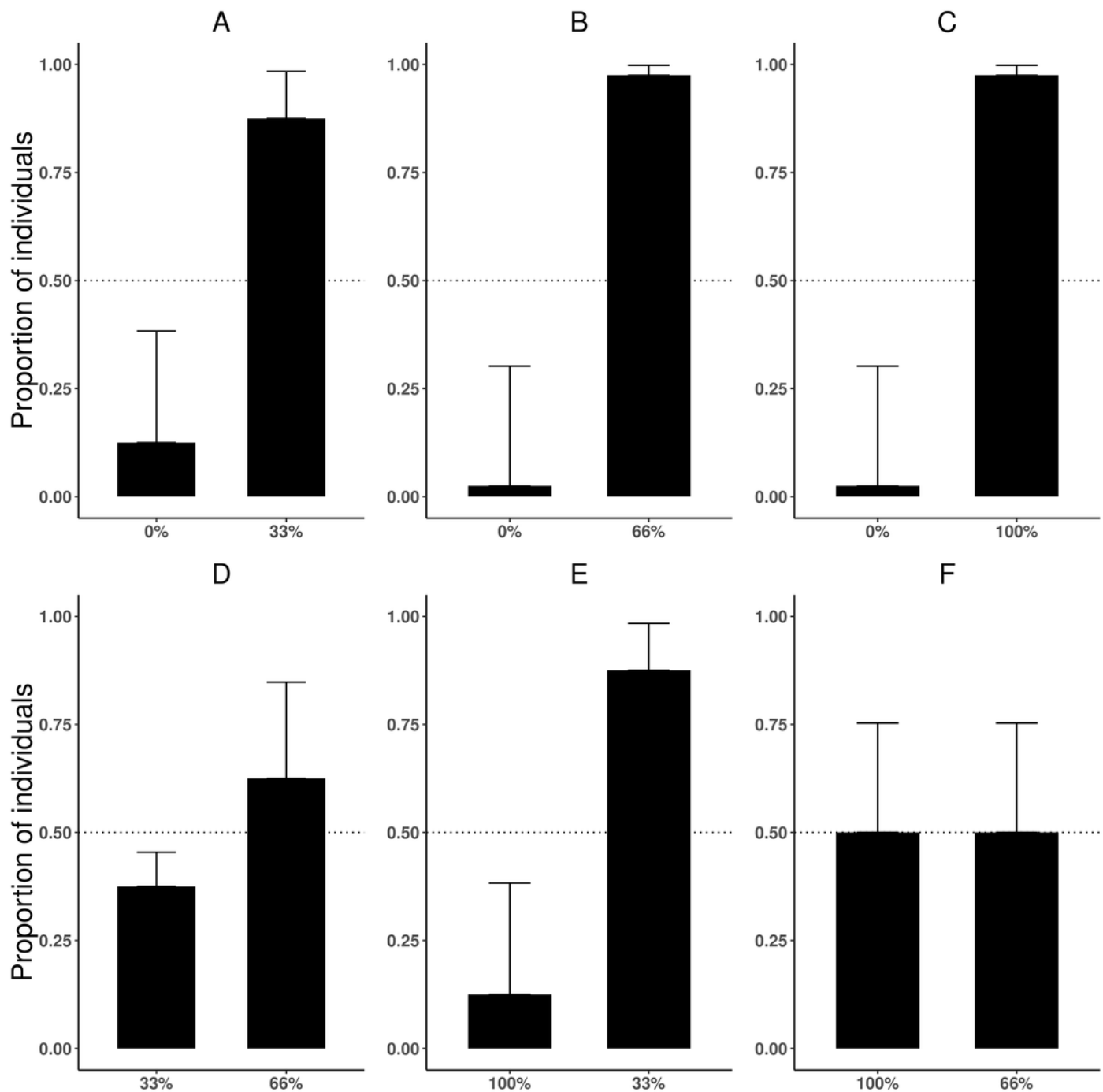
Figure 4

Figure 4.

Proportion of killer shrimp (binomial 95 CI) settling in binary choice tests involving different substrates (live zebra mussel, empty shells of zebra mussel, artificial grass) tested singly (A-B, n = 30 shrimp/test) or in groups of 20 (C-D, n=4 tests).
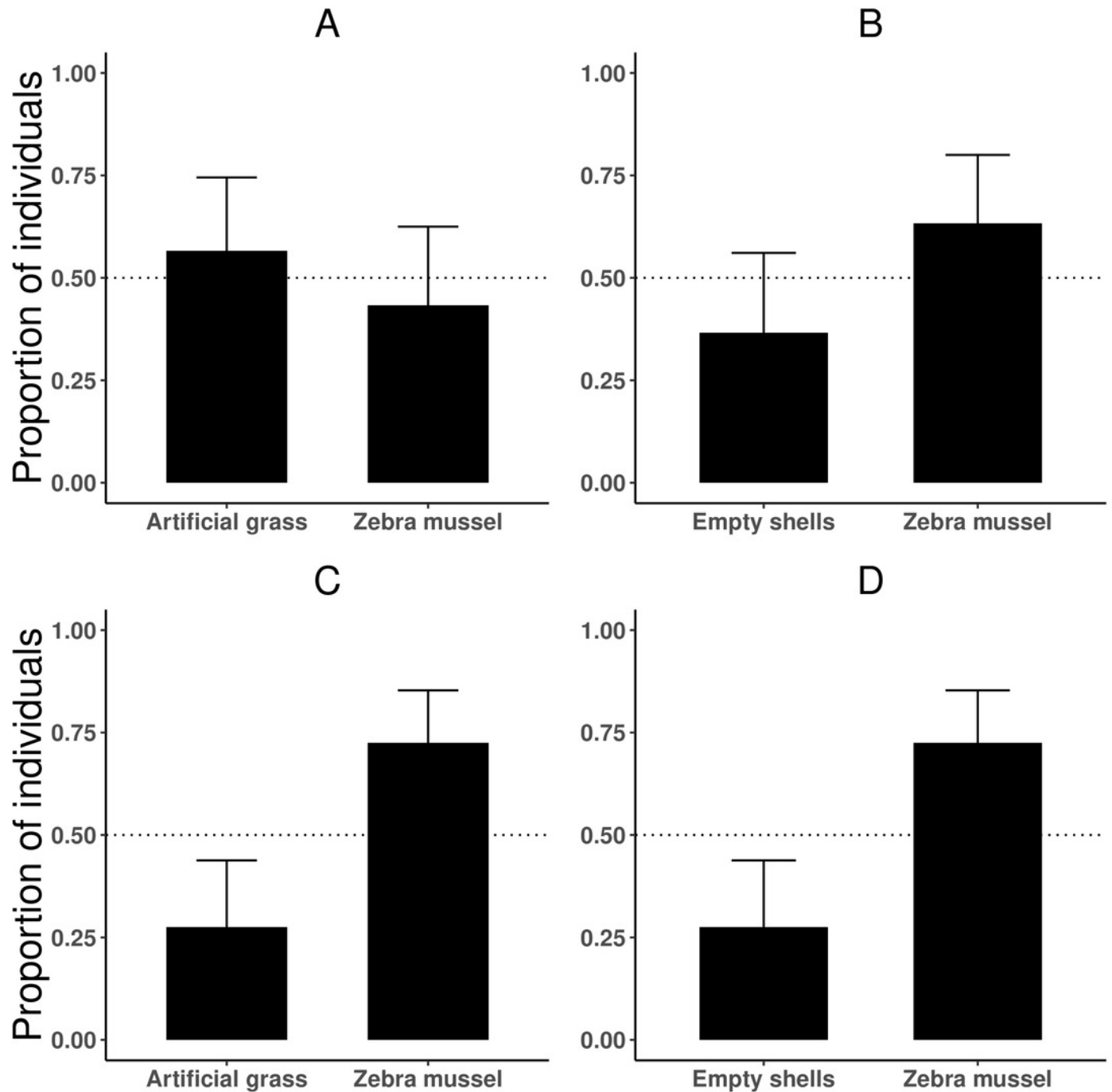
Figure 5

Figure 5

Preference by individual killer shrimp (mean time spent, s ± 95 CI) in water conditioned with different scents (blank water, n = 40; tilapia scent, n = 40; zebra mussel scent, n = 40) from sympatric (A,C,E; n = 60) and allopatric (B,D,F; n = 60) populations.
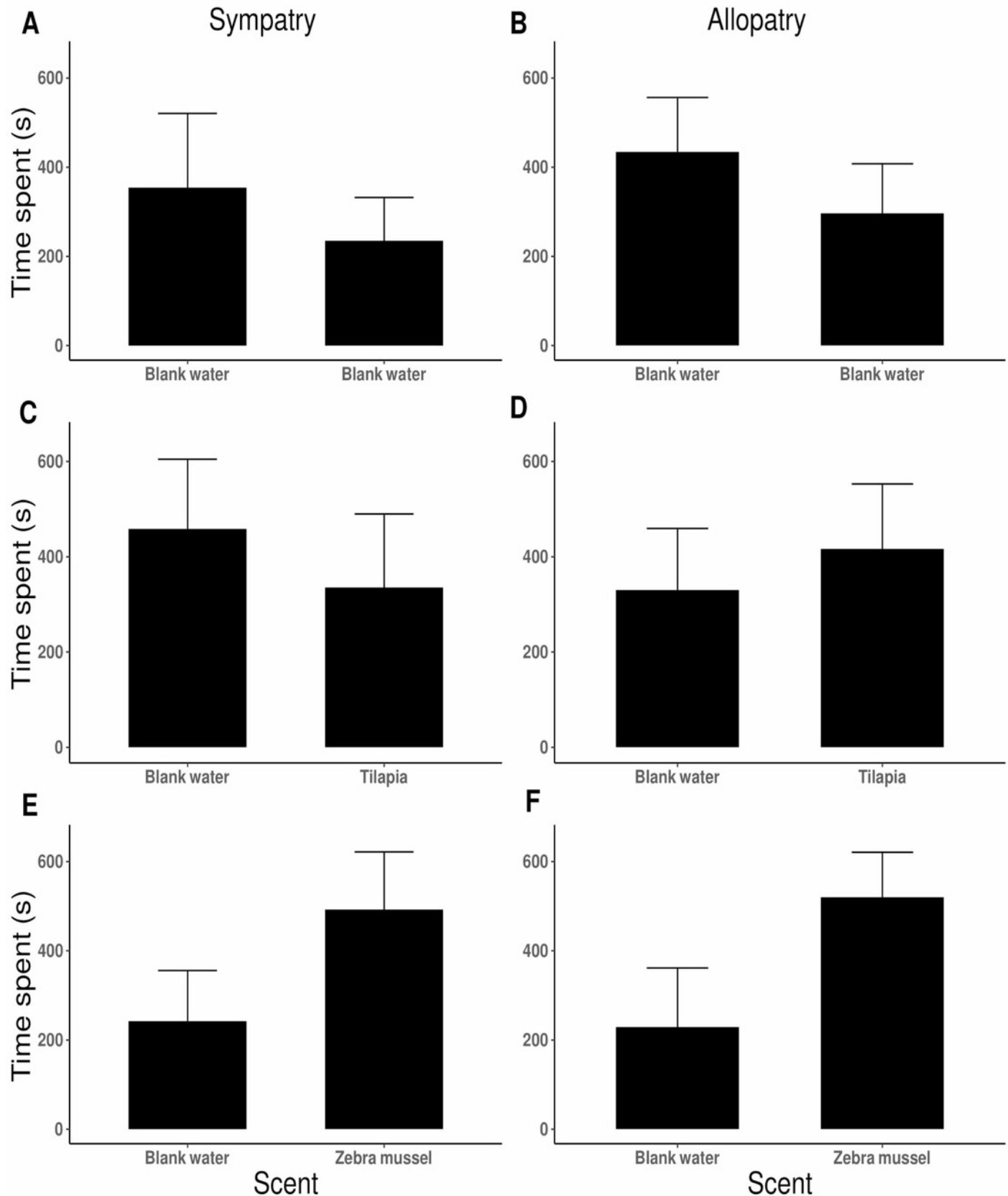
Figure 6

Figure 6

Activity (mean number of transitions ± 95 CI) of individual killer shrimp tested in water conditioned with different scents (blank water, n = 40; tilapia scent, n = 40; zebra mussel scent, n = 40) from sympatric (A; n = 60) and allopatric (B; n = 60) populations.

A Sympatry

B Allopatry

Activity (No. interactions)

Scent