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[Article begins on next page]

1 **Parasites as prey: the effect of cercarial density and alternative prey on consumption of**

2 **cercariae by four non-host species**

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9 **RUNNING TITLE:**

10 Effect of cercarial density and alternative prey on the predation of cercariae

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18 **SUMMARY**

19 In parasites with complex life cycles the transmission of free-living infective stages can be

20 influenced by ambient community diversity, in particular via predation. Here, we

21 experimentally investigated whether parasite density and the presence of alternative prey can

22 alter predation rates on free-living cercarial stages of a marine trematode by several non-host

23 predators. All four predator species consumed increasing numbers of cercariae with an increase

24 in cercarial density, indicating that the removal of cercariae by predators is effective over a

25 range of natural densities as well as in the presence of alternative prey for a number of

26 predators typical of marine ecosystems. However, the relative removal rates and the effects of

27 cercarial density and alternative prey differed among predator species. In barnacles and

28 shrimps, significant interactive effects of cercarial density and alternative prey on cercarial

29 predation occurred while in oysters and crabs cercarial removal rates were unaffected by both

30 factors. As changes in cercarial densities directly translate into changes in infection levels in

31 down-stream hosts in this parasite-host system, the observed predator-specific responses

32 suggest that cercarial predation effects on disease risks will depend on the specific species

33 composition of ambient communities and not on non-host biodiversity per se.

34

35 **KEY WORDS**

36 Transmission, trematodes, cercariae, predation

37

Key findings

- With increasing cercarial density four marine predators consumed increasing numbers of cercariae.

- In barnacles & shrimps, relative removal rates were affected by alternative prey and cercarial density.

- In oysters & crabs, relative removal rates were not affected by cercarial density or alternative prey.

- Results suggest species-specific effects of predator interference with free-living infective stages

INTRODUCTION

Across the globe biodiversity is being lost at a high rate. In general, decreased biodiversity is believed to reduce ecosystem functioning and service provision (Hooper *et al.* 2005; Worm *et al.* 2006; Keesing *et al.* 2010). An important and increasingly studied additional consequence of biodiversity loss is the potential increase in the transmission of infectious diseases. The relationship between biodiversity and reduced disease transmission has been shown across a variety of ecosystems involving various pathogens, hosts and transmission pathways (Keesing *et al.* 2006; Johnson *et al.* 2015). This apparent mediation of disease risk and reduction of infection levels by ecological community diversity is explained by the so called 'dilution effect'. The term has been widely applied as a concept in terrestrial disease ecology, notably in studies on Lyme's disease and other vector-borne diseases (Keesing *et al.* 2006). Here, an increase in species diversity is said to reduce disease risk by altering the abundance of competent disease reservoirs relative to non-competent reservoir species. This in turn reduces the encounter rate between disease vectors and competent hosts, thereby reducing the number of vectors and their infection prevalence in the system (Ostfeld and Keesing, 2000; Schmidt and Ostfeld, 2001; Keesing *et al.* 2006). However, whether this effect is universal or whether the actual amplification or dilution of disease risk in a system depends on the specific species composition of reservoir hosts and vectors of that system and not on biodiversity per se is hotly debated (Randolph and Dobson, 2012; Salkeld *et al.* 2013; Lafferty and Wood, 2013; Wood and Lafferty, 2013; Johnson *et al.* 2015).

A similar 'dilution effect' as that observed in vector-borne diseases occurs in parasites with complex life cycles where the transmission of free-living infective parasite stages can be strongly influenced by changes in ambient community diversity and composition (Thieltges *et al.* 2008a; Johnson and Thieltges, 2010). Changes in species richness can interfere with the

73   transmission of infectious stages to their suitable hosts through a wider variety of mechanisms

74   than simply changing the relative abundance of competent to non-competent hosts (Orlofske *et*

75   *al.* 2012). These include predation and hyperparasitism, physical disturbances or barriers,

76   chemical disruption in the form of toxic exudates and interference by decoy and alternative

77   host organisms (Thieltges *et al.* 2008a, Johnson and Thieltges, 2010). Of these mechanisms,

78   predation on free-living stages has been particularly well studied, indicating that predators

79   often interfere with parasite transmission by removing substantial numbers of parasitic free-

80   living infectious stages from their environment, thereby reducing encounters between hosts and

81   parasites and ultimately lowering infection levels in down-stream hosts (Thieltges *et al.* 2008a;

82   Orlofske *et al.* 2012; Johnson *et al.* 2010). However, these removal rates are typically obtained

83   from experiments using specific densities of parasites (i.e. number of infectious stages) and not

84   for a range of different densities. Given that there tends to be a relationship between the

85   consumption rate of a predator and the abundance of its prey (functional response, Oaten and

86   Murdock, 1973) it may be that the strength of the observed transmission interference differs

87   across a range of parasite densities. Hence, it remains to be determined whether organisms

88   removing parasites reach a saturation point thereby impairing the transmission interference. If

89   predators were to reach saturation at high parasite densities or even reduce their consumption

90   rate due, for example, to swarming effects (i.e. where a high abundance of prey diminish

91   consumption rate through a variety of mechanisms, such as clogging of filters (Jeschke *et al.*

92   2004)) this would have important implications for the generality of observed effects of

93   transmission inference. In addition, the consumption rate of predators is also known to be

94   affected by the presence of alternative prey (Oaten and Murdoch, 1973; van Baalen *et al.*

95   2001). Under natural conditions predators have access to a range of prey species while

96   experimental set-ups typically involve a simple one predator – one prey design. The recorded

97   consumption rate of predators may therefore merely be a phenomenon observed in the lab in

98   the absence of any alternatives. Unfortunately, to date, studies on the density of infective stages

99   and the presence/absence of alternative prey mediating the rate of parasite removal by

100   predators are limited to a single system, cercarial stages of the trematode *Ribeiroia ondatrae*

101   infecting freshwater amphibians (Schotthoefer *et al.* 2007; Orlofske *et al.* 2012, 2015). This

102   clearly hinders our understanding of the generality and magnitude of the effect of predator

103   interference with parasite transmission.

104

105   In this study, we experimentally investigated the effect of parasite density and alternative prey

106   on the consumption of free-living cercarial stages of a marine trematode (*Himasthla elongata*)

107   by several non-host predators. Previous work had shown that cercariae of this species are

108   frequently consumed by a variety of predators (Welsh *et al.* 2014). The trematode species uses

109   the gastropod *Littorina littorea* as first intermediate and some bivalves (mainly mussels and

110   cockles) as second intermediate hosts and bivalve-eating birds as definitive hosts (Thieltges *et*

111   *al.* 2006). By exposing shrimps (*Crangon crangon*), crabs (*Hemigrapsus takanoi*), oysters

112   (*Crassostrea gigas*) and barnacles (*Semibalanus balanoides*), which either actively prey upon

113   motile, free living cercarial stages or passively filter them out of the water column, to several

114   ecologically relevant densities of cercariae based on calculations from literature data) in

115   presence or absence of alternative prey we aimed to quantify the effect of both factors on

116   parasite removal rates by predators. As cercarial densities directly translate into metacercarial

117   infection levels in down-stream hosts in this system (Liddell et al. in press), any changes in

118   cercarial densities due to cercarial predation can be expected to ultimately affect disease risk in

119   down-stream hosts. Hence, our experiments contribute to our still limited understanding of the

120   presence and magnitude of the effects of ambient community diversity on parasite transmission

121   interference.

122

123 MATERIALS AND METHODS

124 *Experimental organisms and alternative prey*

125 Cercariae of *Himasthla elongata* were used for the experiments. After emergence from the

126 hosts, the relatively large cercariae (body length: 605-665μm; tail length: 535-605μm; Werding

127 1969), which are visible to the naked eye, swarm actively through the water column. For the

128 experiments, cercariae were obtained from common periwinkles (*Littorina littorea*) collected in

129 the vicinity of the NIOZ Royal Netherlands Institute for Sea Research on Texel (Wadden Sea,

130 The Netherlands). Snails known to be infected from shedding trials were kept in the dark in

131 aerated flow-through aquaria and fed regularly with sea lettuce (*Ulva lactuca*) until cercariae

132 were required for experiments. Shedding of cercariae by snails was then induced by incubating

133 around 30 snails in 2.7 l of seawater at 27°C under light for 3 hours. Subsequently the

134 necessary numbers of cercariae were pipetted within 1 hour (thus the maximum age of

135 cercariae was 4 h) into pots to be administered to the appropriate containers of the experiment.

136

137 Four species with different feeding mechanisms or hunting strategies and which do not serve as

138 hosts for the trematode species were used in this study: shrimps and crabs as motile active

139 predators and oysters and barnacles as sessile filter feeders. Shrimps (*Crangon crangon*; mean

140 ± SD: 34.4 ±1.9 mm length), crabs (*Hemigrapsus takanoi;* 18.8 ± 1.5 mm carapax width),

141 barnacles (*Semibalanus balanoides*, attached to empty mussel shells; 34.5 ± 8.2 barnacles of 2-

142 3 mm diameter per shell) and oysters (*Crassostrea gigas;* 48.6 ±4.1 mm diameter) were

143 collected in the vicinity of the NIOZ in the south east Wadden Sea side of Texel (Netherlands).

144 Collected organisms were housed in aerated containers or flow through aquaria in the same

145 climate chamber at 15°C and fed regularly. Crabs were fed on a diet of oysters, mussels, fish

146 (herring) and shrimp. Shrimps were fed fish (herring) and consumed conspecifics. Oysters

147   were fed algal bivalve feed (*Isochrysis galbana*). Barnacles were collected shortly before the

148   experiment and thus did not require feeding.

149

150   The type of alternative prey items offered to predators were chosen based on knowledge on the

151   natural diets of the predators used in the experiments. The alternative prey for the crabs and

152   shrimps consisted of frozen fish (herring) which was defrosted the night before administration

153   and cut into small portions (approx. 0.96g per crab, 0.72g per shrimp) at a size that predators

154   could easily handle. The alternative prey for the oysters and barnacles consisted of highly

155   concentrated *Isochrysis galbana* algal bivalve feed (Instant Algae by Reed Mariculture Inc.

156   USA; 4.1 billion cells ml^{-1}), administered as 3-4 drops of algal feed per oyster and per unit of

157   barnacles, resulting in algal concentration inducing feeding activity in oysters and barnacles

158   based on observations in preliminary experiments. In all four predator experiments, the

159   alternative prey items added were of a significantly larger volume or quantity than the potential

160   cercarial prey to ensure that predators were offered attractive alternative choices to cercariae at

161   all cercarial densities.

162

163   *Experimental set-up*

164   Plastic containers (25 x 11 x 9.5 cm) were filled with 500 mL of seawater, constantly aerated

165   and placed on a bench in a completely randomised block design with 2 temporal blocks. The

166   room temperature was maintained at 18°C (the average summer water temperature in the study

167   area; van Aken 2008). In the case of crabs, shrimps and oysters, a single individual was placed

168   in each container and the assigned treatment administered. Barnacles were added attached to a

169   single mussel valve (34.5 ± 8.2 barnacles per container). The four species were tested in four

170   separate experiments, each using the same two-factorial block design, with cercarial density

171   (20, 60, 100 or 300 cercariae) and alternative prey (present or absent) as main factors and two

172 temporal blocks (day 1 & day 2). Each treatment combination was replicated four times in each

173 block, i.e. 8 replicates for each treatment combination in total.

174

175 Cercarial density selection was based on literature data on cercarial shedding rates of *H.*

176 *elongata* from their first intermediate host, the common periwinkle *L. littorea*, and on literature

177 data on the average abundance of periwinkles (for details see Liddell et al. in press). These

178 calculations suggested a realistic maximum shedding of about 300 cercariae in the vicinity of

179 an infected snail per tide and we thus used this as the maximum cercarial density administered.

180 As this maximum cercarial concentration is likely to be diluted in the field in the water column

181 and by intra-specific dilution in form of up-take by down-stream hosts such as mussels and

182 cockles (Thieltges and Reise, 2007, Magalhães *et al.* 2016, Mouritsen *et al.* 2003)) we used

183 several lower cercarial densities (100, 60 and 20 cercariae) to mimic various levels of cercarial

184 dilution.

185

186 Crabs, shrimps, oysters and barnacles were placed in their containers a day before the

187 experiment to acclimatise. Treatments were then administered and the experiments run for 3

188 hours. After that the organisms were removed and the contents of the containers sieved through

189 a 20 µm mesh and dyed using Rose Bengal stain (test runs had proven this method to retrieve

190 100% of cercariae). The number of parasites remaining in the sieved contents was recorded

191 using a light microscope.

192

193 *Statistics*

194 The relationship between parasite density (20, 60, 100 or 300 cercariae), the presence of

195 alternative prey (absent vs. present), and a block factor on the number of remaining parasites

196 was analyzed using a binomial Generalized Linear Model (GLM) with a log-link. Assuming a

197   so-called *linear pure death process*, which means that all removals are independent events, the

198   number of free-living cercarial stages remaining at the end of the experiment follows a

199   binomial distribution. The parameters of the distribution are given by the initial number of

200   parasites and by the probability that a parasite is still free-living at the end of the experiment.

201   This probability equals

202    $$p = e^{-\theta}$$

203   where θ is the removal rate per unit of experimental time. It is further assumed that this

204   removal rate is a function of parasite density, the presence of alternative prey, their interaction,

205   and a block effect. So

206    $$\theta = \mu + \alpha_i + \beta_j + \gamma_{ij} + \delta_k$$

207   where μ is the intercept, α is the effect of cercarial density, β of the presence of alternative

208   prey, γ their interaction, and δ the block effect. The model used the absolute number of

209   remaining parasites after the 3 hour experimental time period.

210

211   A series of GLM models from the most complex to the least complex were fitted (see Figure

212   S1). The most complex model included all explanatory variables (cercarial density, alternative

213   prey, their interaction, and a block effect) whereas the simplest model (the null model)

214   excluded all explanatory variables and only included the intercept. Testing for the best fitting

215   model by identifying significant differences between models of descending complexity was

216   carried out using the Analysis of Deviance. For example, model 1 which included all terms was

217   tested against model 2 in which the interaction was left out. The delta deviance (the difference

218   in deviance between the two models) was subsequently divided by the dispersion factor from

219   the most complete model (Δ Dev/ϕ) and compared to the delta degree of freedom Chi^2 at 0.05.

220   The dispersion factor (ϕ) was calculated by dividing the residual deviance for the most

221 complex model by the degrees of freedom. A significant difference between two models

222 reveals that the most complex model of the two is the better fit.

223

224 From the best fitting models, cercarial removal rates (per experimental runs) and cercarial

225 survival (%) were calculated. Removal rates were calculated for the 3 hour experimental period

226 and based on the estimates of the intercept for each significant factor included in the best fitting

227 model output. Cercarial survival was calculated from the estimates of the intercept for each

228 significant factor included in the best fitting model output. From these cercarial survival data,

229 the proportion cercariae removed (%) can be calculated (proportion cercariae removed= 100-

230 cercarial survival).

231

232 All analyses were carried out using R (R Development Core Team, 2013) version 3.0.2 in R

233 Studio (version 0.98.1103; RStudio, 2014).

234

235 RESULTS

236 All four predators consumed more cercariae when higher densities of cercariae were offered,

237 both when alternative prey was absent and present, i.e. the absolute removal in terms of

238 numbers of cercariae generally consumed increased with cercarial density (Figure 1). However,

239 the relative cercarial removal rates (i.e. consumption per unit time) differed among the four

240 predators depending on cercarial density and alternative prey (Table 1; see Figures S2-S). In

241 barnacles, the best fitting model included the interaction between cercarial density and

242 presence/absence of alternative prey (model 1; Table 1). This probably resulted from the fact

243 that cercarial removal rates were higher at presence than at absence of alternative prey at

244 intermediate cercarial densities while they were lower at high densities (Figure 2). In addition,

245 the best fitting model also included a temporal block effect, which resulted from overall higher

246 removal rates during the second run of the experiment (Figure 2; Table S1). Overall, the

247 survival of cercariae after removal by barnacles was between 5 and 35 % (Table S1). In

248 contrast to barnacles, none of the factors tested affected cercarial removal rates by oysters

249 (Table 1), i.e. oysters were removing cercariae at a constant rate, independent of the cercarial

250 density or the presence/absence of alternative prey. The cercarial removal rate of oysters was

251 1.01 and 36 % of cercariae survived.

252

253 For crabs, the best fitting model only included the block effect (model 7; Table 1). Cercarial

254 removal rates by crabs were slightly higher in the first (0.21) than in the second (0.14)

255 experimental run. Accordingly, cercarial survival was slightly lower in the first (81 %)

256 compared to the second run (87 %). Finally, for shrimps the best fitting model included an

257 interaction between cercarial density and presence/absence of alternative prey (Table 1). This

258 interaction was based on an almost 5-fold increase in searching rates of shrimps at the highest

259 cercarial density when alternative prey was absent (Fig. 3). Here, cercarial survival was

260 relatively low with 47%, while in all other cases cercarial survival ranged between 77 and 91 %

261 (Table S2).

262

263 DISCUSSION

264 All four predator species consumed increasing numbers of cercariae with an increase in

265 cercarial density, i.e. the absolute cercarial removal increased with cercarial density. However,

266 the relative cercarial removal rates (i.e. per unit time) and the effect of cercarial density and

267 alternative prey differed among predator species. In barnacles and shrimps, significant

268 interactive effects of cercarial density and alternative prey on cercarial consumption were

269 present while in oysters and crabs neither cercarial density nor the presence/absence of

270 alternative prey had a significant effect on cercarial removal rates by the predators.

271

The increase in the numbers of cercariae consumed by all four predator species with increasing

cercarial density can be explained in terms of the mass action principle which assumes that

predators encounter their prey randomly and that the number of encounters a predator makes is

proportional to the density of its prey (Arditi and Ginsburg, 1989). Interestingly, none of the

predators reached saturation across the range of parasite densities tested in this experiment. As

the parasite densities administered in this study were selected based on natural shedding rates

of cercariae from their host snails and therefore represent abundances of infective stages that a

predator is likely to encounter under natural conditions (see methods), the experiments suggest

that swarming effects, e.g. by clogging of filters, do not seem to occur at realistic parasite

densities in the predators tested. However, while the absolute numbers of cercariae consumed

generally increased with increasing cercarial density in all four predator species, the relative

removal rates showed different responses to cercarial density and presence/absence of

alternative prey in the four predator species. The fact that species sharing the same feeding

mechanism (active predation: crabs & shrimps vs. passive filtration: barnacles & oysters)

showed different patterns suggests that the responses are not universal or linked to specific

feeding traits but rather species specific.

In barnacles and shrimps, the best fitting models included an interaction between cercarial

density and presence/absence of alternative prey. This resulted from cercarial removal rates at

low and intermediate cercarial densities being similar or higher at presence compared to

absence of alternative prey while at the highest cercarial density removal rates they were

highest in absence of alternative prey. This was particularly the case for shrimps which showed

an almost 5-fold increase in searching rate at the highest cercarial density when alternative prey

was absent. In contrast, removal rates did not differ much between presence and absence of

296   alternative prey at lower cercarial densities. This may indicate the phenomenon of prey

297   switching (Cornell, 1976; Murdock, 1969) whereby a predator initially focuses on the most

298   abundant or easily accessible prey type in its environment (in this case the alternative prey, i.e.

299   the piece of fish or algae) and then switches to a new prey type as this becomes more abundant

300   (in this case the parasites). However, whether such prey-switching really underlies the

301   observed pattern in our experiments deserves further studies. Other work on trematodes from

302   freshwater ecosystems also found more complex relationships between cercarial consumption

303   and cercarial density, depending on both the identity of the predator (mosquitofish or damselfly

304   nymphs) as well as of the parasite species (*Echinostoma trivolvis* or *Ribeiroia ondatrae*;

305   Orlofske *et al.* 2015). Together with our study, these results suggest that the effect of cercarial

306   density on cercarial removal rates by predators actually depends on the particular parasite and

307   predator species and may be further mediated by the presence or absence of alternative prey.

308

309   In the other two cercarial predators investigated in our experiment, oysters and crabs, neither

310   cercarial density nor the presence/absence of alternative prey affected the rates with which they

311   removed cercariae. Relative removal rates were similar over the range of cercarial densities

312   administered within the two predator species and generally higher in oysters than in crabs (36%

313   and 87% cercarial survival, respectively). Oyster have previously been reported as very

314   effective predators of cercariae without serving as hosts to *H. elongata* (Thieltges *et al.* 2008a,

315   Thieltges *et al.* 2009). They are very efficient filter feeders with high pumping rates (Ren *et al.*

316   2000; Ropert and Goulletquer, 2000) and bivalves, including oysters, have generally been

317   shown to selectively consume particles of comparable size to cercariae of *H. elongata* from

318   algae mixtures (Cognie *et al.*, 2003; Barille *et al.,* 1997. Bivalves can generally show food

319   density-dependent filtering activity (Gosling, 2008) but within the realistic food levels and

320   parasite densities administered in our experiments this does not seem to occur as removal rates

321 were not affected by cercarial density or presence/absence of alternative prey. Crabs in turn

322 remove cercariae either by active predation or by uptake via their gills (without becoming

323 infected themselves; pers. observation). Given the lower removal rates in crabs, these

324 mechanisms do not seem to be as effective as in oysters, leading to lower overall cercarial

325 removal rates in crabs. However, in both cases removal rates did not differ in absence or

326 presence of alternative prey, suggesting that parasite removal is often likely to be maintained

327 even in complex communities with multiple prey species under more natural settings. Similar

328 conclusions were made by two studies on predators of the cercariae of *Ribeiroia ondatrae* in

329 freshwater systems where dragonfly and damselfly larvae, cyclopoid copepods, hydroid polyps

330 and mosquitofish continued to prey on cercariae when alternative prey was present

331 (Schotthoefer et al. 2007, Orlofske *et al.* 2012). Our study expands on these findings with

332 results from additional taxonomic groups (shrimps, crabs, barnacles, oysters) and mechanisms

333 (e.g. filter feeding bivalves) and suggests that many predator species will maintain their

334 parasite removal capabilities under more realistic multiple prey situations.

335

336 In two of the predator species investigated in our experiments, crabs and barnacles, the best

337 fitting model also included a (temporal) block effect. This resulted from significant differences

338 in the cercarial removal rates of predators between the two runs of the experiments. While

339 every effort was made to ensure that conditions remained constant in each experiment,

340 conditions may still have been experienced differently by the predators. For instance, the batch

341 of administered cercariae came from different groups of snails each day and may have been of

342 different quality in terms of motility or life span. In addition, the behaviour of predators may

343 have been affected by slight differences in ambient conditions between the different runs.

344 However, the general patterns observed were consistent between runs and by incorporating a

345 temporal block factor into the statistical models we ensured that these temporal differences

346 were taken into account when investigating the main effects.

347

348 In conclusion, the removal of cercariae by predators has been shown to be effective over a

349 range of natural cercarial densities as well as in the presence of alternative prey for a number of

350 predators typical of marine ecosystems. However, the response of removal rates of predators to

351 different cercarial densities and presence/absence of alternative prey differed among the four

352 predator species without an obvious link to specific predator traits. As changes in cercarial

353 densities directly translate into changes in infection levels in down-stream hosts in this system

354 (Liddell et al. in press), the predator-specific responses observed suggest that cercarial

355 predation effects on disease risks will depend more on the specific species composition of

356 ambient communities than on biodiversity per se. These results mirror the recent discussion

357 about the generality of dilution and related effects which suggest that the actual amplification

358 or reduction of disease risk in a system may depend more on the specific species composition

359 of ambient communities and not on biodiversity per se (Randolph and Dobson, 2012; Salkeld

360 *et al.* 2013; Lafferty and Wood, 2013; Wood and Lafferty, 2013; Johnson *et al.* 2015). Our

361 results suggest that predator specific responses to parasite density and presence/absence of

362 alternative prey add a further layer of complexity to the general interference potential of

363 predators on parasite transmission.

364

370 throughout the project. Finally, we are grateful for all the interesting and constructive

371 comments which we received from the referees during the review process.

372

373 REFERENCES

374 **Arditi, R., and Ginzburg, L. R.** (1989). Coupling in predator-prey dynamics: ratio-

375 dependence. *Journal of Theoretical Biology* **139**, 311-326.

376 **van Baalen, M., Křivan, V., van Rijn, P. C., and Sabelis, M. W.** (2001). Alternative food,

377 switching predators, and the persistence of predator-prey systems. *The American*

378 *Naturalist* **157**, 512-524.

379 **Barillé, L., Prou, J., Héral, M., and Razet, D.** (1997). Effects of high natural seston

380 concentrations on the feeding, selection, and absorption of the oyster *Crassostrea gigas*

381 (Thunberg). *Journal of Experimental Marine Biology and Ecology* **212**, 149-172.

382 **Cognie, B., Barillé, L., Massé, G., and Beninger, P. G.** (2003). Selection and processing of

383 large suspended algae in the oyster *Crassostrea gigas*. *Marine Ecology Progress Series*

384 **250**, 145-152.

385 **Cornell, H.** (1976). Search strategies and the adaptive significance of switching in some

386 general predators. *The American Naturalist* **110**, 317-320.

387 **Drent, J. and Dekker, R.** (2013). How different are subtidal *Mytilus edulis* L. communities of

388 natural mussel beds and mussel culture plots in the western Dutch Wadden Sea? *NIOZ*

389 *Report* 6

390 **Gosling, E.** (2003). *Bivalve molluscs: biology, ecology and culture*, 1st edition. John Wiley &

391 Sons.

392 **Hooper, D. U., Chapin, F. S., Ewel, J. J., Hector, A., Inchausti, P., Lavorel, S., Lawton, J.**

393 **H., Lodge, D. M., Loreau, M., Naeem, S., Schmid, B., Setälä, H., Symstad, A. J.,**

394   **Vandermeer, J. and Wardle, D. A.** (2005). Effects of biodiversity on ecosystem

395   functioning: a consensus of current knowledge. *Ecological monographs* **75**, 3-35.

396 **Jeschke, J. M., Kopp, M., and Tollrian, R.** (2004). Consumer-food systems: why Type I

397   functional responses are exclusive to filter feeders. Biological Reviews **79**, 337-349.

398 **Johnson, P. T. J., and Thieltges, D. W.** (2010). Diversity, decoys and the dilution effect: how

399   ecological communities affect disease risk. *The Journal of Experimental Biology* **213**,

400   961-970.

401 **Johnson, P. T., Dobson, A., Lafferty, K. D., Marcogliese, D. J., Memmott, J., Orlofske, S.**

402   **A., Poulin, R. and Thieltges, D. W.** (2010). When parasites become prey: ecological

403   and epidemiological significance of eating parasites. *Trends in Ecology and Evolution*

404   **25**, 362-371.

405 **Keesing, F., Holt, R. D., and Ostfeld, R. S.** (2006). Effects of species diversity on disease

406   risk. *Ecology Letters* **9**, 485-498.

407 **Keesing, F., Belden, L. K., Daszak, P., Dobson, A., Harvell, C. D., Holt, R. D., Hudson, P.,**

408   **Jolles, A., Jones, K. E., Mitchell, C. E., Myers, S. S., Bogich, T. and Ostfeld, R. S.**

409   (2010). Impacts of biodiversity on the emergence and transmission of infectious

410   diseases. *Nature* **468**, 647-652.

411 **Lafferty, K. D., and Wood, C. L.** (2013). It's a myth that protection against disease is a strong

412   and general service of biodiversity conservation: Response to Ostfeld and Keesing.

413   *Trends in Ecology and Evolution* **28**, 503-504.

414 **Liddell, C., Welsh, J., van der Meer, J., and Thieltges, D. W.** (in press) The effect of dose

415   and frequency of exposure to infectious stages on trematode infection intensity and

416   success in mussels. *Diseases of Aquatic Organisms*. https://doi.org/10.3354/dao03133.

417   **Magalhães L., Freitas R., Dairain A., and de Montaudouin X.** (2016) Can host density

418   attenuate parasitism? *Journal of the Marine Biological Association of the United*

419   *Kingdom,* In press.

420   **Mouritsen K.N., McKechnie S., Meenken S., Toynbee J.L. and Poulin R.** (2003) Spatial

421   heterogeneity in parasite loads in the New Zealand cockle: the importance of host

422   condition and density. *Journal of the Marine Biological Association of the United*

423   *Kingdom* **83**, 307-310.

424   **Nikolaev, K. E., Sukhotin, A. A., and Galaktionov, K. V.** (2006). Infection patterns in White

425   Sea blue mussels Mytilus edulis of different age and size with metacercariae of

426   *Himasthla elongata* (Echinostomatidae) and *Cercaria parvicaudata* (Renicolidae).

427   *Diseases of aquatic organisms* **71**, 51-58.

428   **Oaten, A., and Murdoch, W. W.** (1975). Functional response and stability in predator-prey

429   systems. *American Naturalist* **109**, 289-298.

430   **Orlofske, S. A., Jadin, R. C., Preston, D. L., and Johnson, P. T.** (2012). Parasite

431   transmission in complex communities: predators and alternative hosts alter pathogenic

432   infections in amphibians. *Ecology* **93**, 1247-1253.

433   **Orlofske, S. A., Jadin, R. C., and Johnson, P. T.** (2015). It's a predator–eat–parasite world:

434   how characteristics of predator, parasite and environment affect consumption.

435   *Oecologia* **178**, 537-547.

436   **Ostfeld, R. S., and Keesing, F.** (2000). Biodiversity and disease risk: the case of Lyme

437   disease. *Conservation Biology* **14**, 722-728.

438   **Randolph, S. E. and Dobson, A. D. M.** (2012). Pangloss revisited: a critique of the dilution

439   effect and the biodiversity-buffers-disease paradigm. *Parasitology* **139**, 847-863.

440   **Ren, J. S., Ross A. H., and Schiel, D. R.** (2000). Functional descriptions of feeding and

441   energetics of the Pacific oyster *Crassostrea gigas* in New Zealand. *Marine Ecology*

442   *Progress Series* **208**,119–130.

443   **Ropert M., and Goulletquer, P.** (2000). Comparative physiological energetics of two

444   suspension feeders: polychaete annelid *Lanice conchilega (*Pallas 1766) and Pacific

445   cupped oyster *Crassostrea gigas* (Thunberg 1795). *Aquaculture* **181**, 171–189.

446   doi:10.1016/S0044-8486(99)00216-

447   **Salkeld, D. J., Padgett, K. A., and Jones, J. H.** (2013). A meta-analysis suggesting that the

448   relationship between biodiversity and risk of zoonotic pathogen transmission is

449   idiosyncratic. *Ecology letters* **16**, 679-686.

450   **Schotthoefer, A. M., Labak, K. M., & Beasley, V. R.** (2007). *Ribeiroia ondatrae* cercariae

451   are consumed by aquatic invertebrate predators. Journal of Parasitology 93, 1240-1243.

452   **Schmidt, K. A., and Ostfeld, R. S.** (2001). Biodiversity and the dilution effect in disease

453   ecology. *Ecology* **82**, 609-619.

454   **Thieltges, D.W., Krakau, M., Andresen, H., Fottner, S., and Reise, K.** (2006).

455   Macroparasite community in molluscs of a tidal basin in the Wadden Sea. *Helgoland*

456   *Marine Research* **60**, 307–316

457   **Thieltges, D. W.** (2008). Effect of host size and temporal exposure on metacercarial infection

458   levels in the intertidal cockle *Cerastoderma edule*. *Journal of the Marine Biological*

459   *Association of the UK* **88**, 613-616.

460   **Thieltges, D. W., and Reise, K.** (2006). Metazoan parasites in intertidal cockles *Cerastoderma*

461   *edule* from the northern Wadden Sea. *Journal of sea research* **56**, 284-293.

462   **Thieltges, D. W., Bordalo, M. D., Caballero Hernández, A., Prinz, K., and Jensen, K. T.**

463   (2008a). Ambient fauna impairs parasite transmission in a marine parasite-host system.

464   *Parasitology* **135**, 1111-1116.

465   **Thieltges, D. W., Jensen, K. T., and Poulin, R.** (2008b). The role of biotic factors in the

466   transmission of free-living endohelminth stages. *Parasitology* **135**, 407-426.

467   **Welsh, J.E., Meer, J. van der., Brussaard, C.P.D., and Thieltges, D.W.** (2014) Inventory of

468   organisms interfering with transmission of a marine trematode. *Journal of the Marine*

469   *Biological Association UK* **94**, 697-702.

470   **Wood, C. L., and Lafferty, K. D.** (2013). Biodiversity and disease: a synthesis of ecological

471   perspectives on Lyme disease transmission. *Trends in ecology and evolution* **28**, 239-

472   247.

473   **Worm, B., Barbier, E. B., Beaumont, N., Duffy, J. E., Folke, C., Halpern, B. S., Jackson,**

474   **J. B. C., Lotze, H. K., Micheli, F., Palumbi, R., Sala, E., Selkoe, K. A., Stachowicz,**

475   **J. J. and Watson, R.** (2006). Impacts of biodiversity loss on ocean ecosystem services.

476   *Science* **314**, 787-790.

477

Tables & Figures

479

480 **Table 1:** Results of model selection procedures. From the most complete (model code 1) to the

481 least complete model (10) the degrees of freedom (df) and model deviance are given for each

482 model. Model 1 included the factors cercarial density (α), presence/absence of alternative prey

483 (β), their interaction (γ), and a block effect (δ). Model deviances of the best fitting model for

484 each species/experiment are shown in bold. The dispersion factor (ϕ) is given for the best fitting

485 model only.

486

Model Code	Model	df	Deviance			
			Barnacle	**Oyster**	**Crab**	**Shrimp**
1	$\alpha+\beta+\gamma+\delta$	55	**864.9**	1968.9	460.7	1067.8
2	$\alpha+\beta+\delta$	58	1049.1	2213.3	485.7	1627.0
3	$\alpha+\beta+\gamma$	56	1051.3	2019.6	500.3	**1085.9**
4	$\alpha+\beta$	59	1235.6	2267.4	523.0	1629.2
5	$\alpha+\delta$	59	1060.6	2325.3	485.9	1890.2
6	$\beta+\delta$	61	1111.2	2234.3	521.2	1768.9
7	δ	62	1125.8	2346.1	**521.2**	2289.8
8	α	60	1245.5	2378.4	523.1	1892.5
9	β	62	1299.6	2286.8	562.1	1779.3
10	1	63	1312.4	**2396.2**	562.9	2295.8
ϕ **best fitting model**			15.7	43.6	9.48	19.4

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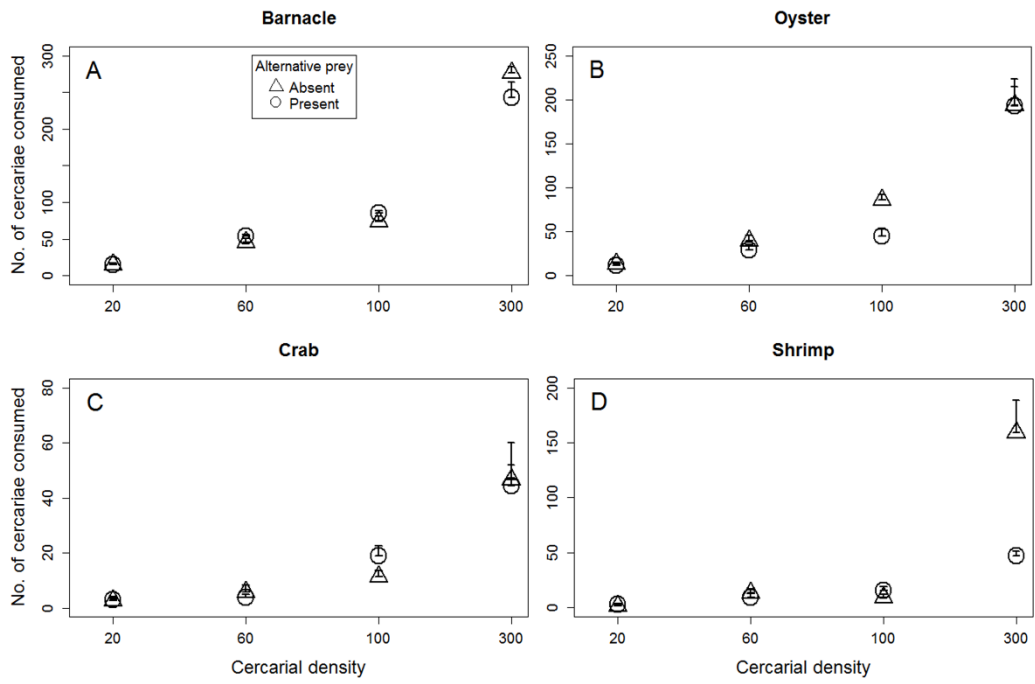
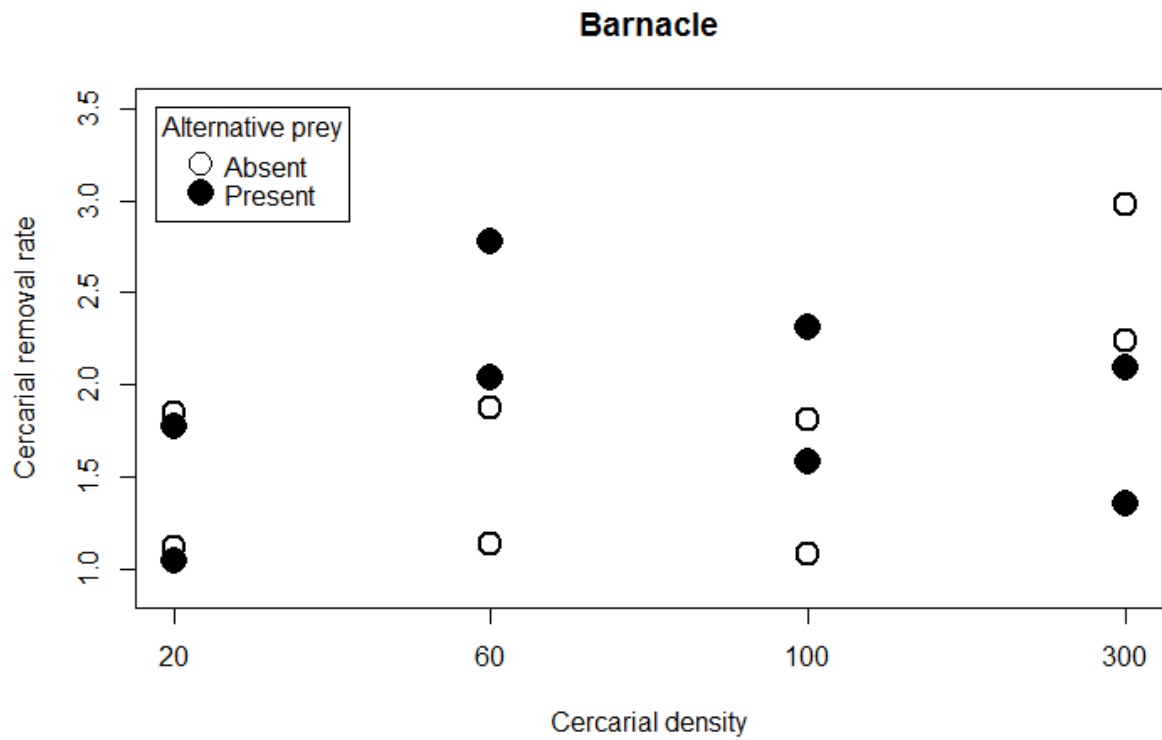**Figure 1:** Number of cercariae consumed by (A) barnacles, (B) oysters, (C) crabs , and (D) shrimps across a range of cercarial densities when an alternative food source was either absent or present. Note the different y-axes.
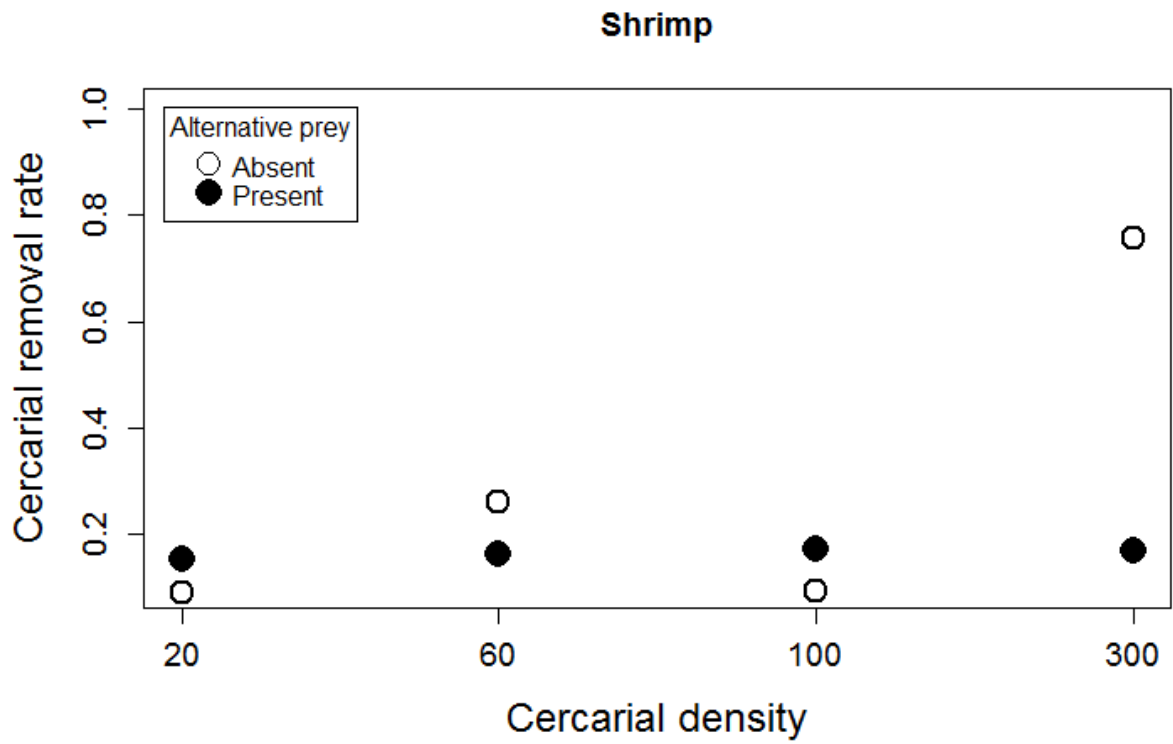
Barnacle

496

497

498   **Figure 2:** Relative cercarial removal rates (per experimental run of 3 hours) of barnacles across

499   a range of cercarial densities and when an alternative food source was either absent or present.

500   Plot based on model output and the factors contributing to the best fitting model (see Table 1).

501

502

Figure 3: Relative cercarial removal rates (per experimental run of 3 hours) of shrimps in the presence of different cercarial densities and in the presence or absence of alternative prey. Plot based on model output and the factors contributing to the best fitting model (see Table 1).