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1 **Introduced marine ecosystem engineer indirectly affects parasitism in native mussel**
2 **hosts**

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22 **ABSTRACT**

23 The alteration of habitat structure by introduced ecosystem engineers imposes direct impacts
24 on native biota but can also exert trait-mediated indirect effects (TMIEs). In this study, we
25 show that the habitat structure provided by invasive Pacific oysters (*Crassostrea gigas*) can
26 also indirectly affect parasitism in blue mussels (*Mytilus edulis*). We conducted a three-month
27 field experiment, in which uninfected mussels were positioned at the bottom and top of two
28 intertidal oyster reefs in the Wadden Sea. On one reef, we detected a significantly higher
29 prevalence of parasitic copepods (*Mytilicola* spp.) in mussels positioned on top of oysters than
30 in mussels at the bottom, but no difference in infection intensity. For trematodes (*Renicola*
31 *roscovita*), a different pattern was observed, with higher prevalence (one reef) and significantly
32 higher infection intensities (both reefs) in mussels positioned at the bottom of the oyster reef.
33 We suggest that the contrasting pattern results from differences in parasite lifecycles.
34 *Mytilicola* spp. larvae spend 2-3 wks in the water column before infecting their hosts and,
35 therefore, mussels positioned at the top are exposed to higher numbers of planktonic larvae
36 than mussels at the bottom. In contrast, infective trematode larvae spend less than 12 h in the
37 water column and primarily infect mussels during low tide, which may explain higher
38 prevalence and intensity of *R. roscovita* in mussels near the bottom of the oyster reef. Our
39 results demonstrate that indirect effects leading to alterations of parasite-host interactions may
40 be a more common but hitherto rarely considered impact of biological invasions.

41

42 **KEYWORDS**

43 Trait-mediated indirect effects, invasive species, ecosystem engineer, parasite-host interaction,
44 *Renicola roscovita*, *Mytilicola*, *Crassostrea gigas*, *Mytilus edulis*

45

46 **INTRODUCTION**

47 Introduced species are considered as one of the greatest threats to ecosystem biodiversity and
48 ecological communities worldwide (Elton 1958; Vitousek et al. 1996; Mack et al. 2000). In
49 particular, invasive ecosystem engineers that create or modify physical habitat structure impose
50 strong direct impacts on native biota, including effects on habitat and food availability, native
51 species density and diversity, and changes in abiotic conditions (Jones et al. 1994, 1997;
52 Crooks 2002). In addition to these direct effects, invasive ecosystem engineers may also affect
53 other organisms in such ways that it has consequences for a third species. These indirect effects
54 can be density-mediated, in which the habitat modifier indirectly influences a third species by
55 altering the density of an intermediate species (density mediated indirect effects (DMIEs);
56 *sensu* Abrams 1995). For example, in north western America, the complex stem structure of
57 invasive spotted knapweed (*Centaurea maculosa*) serves as new substrate for native spiders in
58 grass land habitats, increasing densities of spider webs that, in turn, negatively affect densities
59 of their insect prey (Pearson 2010). Simultaneously, habitat modifiers may affect a third
60 species by altering the traits (e.g., behavioural, physiological, morphological, chemical) of an
61 intermediate species (trait-mediated indirect effects (TMIEs); *sensu* Abrams 1995). For
62 instance, in southeastern Australia, an ecosystem engineering species of invasive alga
63 (*Caulerpa taxifolia*) is known to indirectly facilitate community diversity by modifying the
64 burying behaviour of the clam *Anadara trapezia*, a native ecosystem engineer (Gribben et al.
65 2009). In *Caulerpa*-invaded habitats, clams could not burrow themselves completely, thereby
66 providing rare hard substrate for colonizing species, resulting in an increase in abundance and
67 richness of epibiont species compared to un-vegetated sediments (Gribben et al. 2009). In
68 addition, the availability of shelter within complex habitats created by invasive ecosystem
69 engineers, can induce prey refuge behaviour that alters predator-prey encounter rates and
70 thereby the risk of predation (Byers 2010; Pearson 2010; Eschweiler and Christensen 2011;

71 Waser et al. 2015). Currently, evidence of DMIEs and TMIEs on predator-prey and competitive
72 interactions imposed by invasive habitat modifiers is growing and helps to understand the full
73 impacts of invasive species.

74 Other types of biological interactions that can be modified by invasive ecosystem
75 engineers are less well-known. These also include parasite-host interactions, which can be
76 altered in many different ways. For example, when invasive ecosystem engineers are
77 introduced to new ecosystems, they can co-introduce parasites that can spill over to native
78 species (Li et al. 2014; Goedknegt et al. 2017). Furthermore, invasive species are known to
79 interfere with native parasite transmission cycles by predating on free-living infective stages
80 or limiting their dispersal by providing physical obstructions (Bartoli and Boudouresque 1997;
81 Thieltges et al. 2009; Welsh et al. 2014; Goedknegt et al. 2015) Much less is known on indirect
82 effects that invasive ecosystem engineers can exert on parasite-host interactions. Most evidence
83 on TMIEs on parasite-host interactions originates from native species in freshwater systems,
84 where native predators affect traits of their prey, which in turn can determine how surviving
85 prey can interact with parasites. For example, fish shoaling in response to predators can
86 facilitate parasite transmission between individual fish, resulting in higher infection levels at
87 locations with higher predation pressure (Stephenson et al. 2015). Similarly, predator-
88 avoidance strategies of migrating water fleas can result in higher exposure to parasitic spores
89 and, consequently, higher infection levels (Decaestecker et al. 2002). However, to our
90 knowledge, no studies on altered parasite-host interactions as an indirect result of habitat
91 modification by invasive ecosystem engineers exist.

92 Ecosystem engineers such as invasive oysters provide a suitable model system to study
93 indirect effects of habitat modification on parasite-host interactions. These marine molluscs, in
94 particular the Pacific oyster (*Crassostrea gigas* also known as *Magallana gigas*), have been
95 introduced world-wide for aquaculture purposes (Ruesink et al. 2005). Once established in the

96 wild after introduction, oysters create hard-substrate biogenic reefs and thereby modify the
97 environment with consequences for other organisms and species interactions (Ruesink et al.
98 2005). In general, oysters (native and invasive) are known to provide predation refuge for prey
99 hiding in the biogenic matrix created by the oysters, which can have indirect effects on
100 predation strength (Grabowski 2004; Hughes and Grabowski 2006; Troost 2010). In Europe,
101 invasive Pacific oysters co-exist with native mussels (*Mytilus edulis*) in dense aggregations
102 termed oyssel reefs (Reise et al. 2017a; Troost 2010; Fig. 1). Mussel larvae use oysters as hard
103 substrate for their settlement and can be found on top of the oyster reef, but also between the
104 oysters. Moreover, mussels make use of the shelter provided by the three-dimensional matrix
105 of oysters in order to escape predators. In response to predation risk, mussels actively migrate
106 to the bottom of the oyster matrix which significantly reduces mussel predation by crabs
107 (Eschweiler and Christensen 2011; Waser et al. 2015). Hence, Pacific oysters exert TMIEs by
108 creating complex habitats that initiate refuge seeking behaviour of mussels. However, this
109 predation refuge is traded off against reduced foraging success suggested by decreased mussel
110 condition at the bottom of the matrix (Eschweiler and Christensen 2011). The presence of
111 invasive Pacific oysters is known to affect parasite infections in mussels by a mechanism
112 referred to as *transmission interference* (Johnson and Thieltges 2010; Welsh et al. 2014;
113 Goedknegt et al. 2016). As unsuitable hosts for trematodes, Pacific oysters significantly reduce
114 the number of free-living trematode larval stages (cercariae) in the water column by filter
115 feeding or trapping larvae on the complex rough shells (Thieltges et al. 2009; Welsh et al. 2014;
116 Goedknegt et al. 2015). This transmission interference has been shown to reduce infection
117 levels in native mussels placed on artificial oyster reefs compared to mussels situated on bare
118 sediment (Thieltges et al. 2009). Consequently, mussels may experience reduced trematode
119 infection levels when they hide at the bottom of the oyster matrix.

120 Hence, the presence of invasive oysters can affect parasite-host interactions on local
121 scales, but it is not known whether the TMIEs observed for predator-prey interactions within
122 the biogenic oyster matrix also affect parasite-host relationships on individual oyssel reefs.
123 This also applies to native parasite-host interactions in which oysters serve as an alternative
124 host for native parasites and which may also be affected via TMIEs induced by the oyster
125 matrix. To investigate these potential indirect effects, we used a replicated large-scale field
126 experiment conducted on two oyssel reefs in the south and north of the European Wadden Sea
127 to investigate whether the habitat structure provided by invasive Pacific oysters can indirectly
128 affect parasitism in native blue mussels. In the intertidal area of the Wadden Sea, two separate
129 invasions of Pacific oysters in the north (island of Sylt, 1986; Reise 1998, Reise et al. 2017a)
130 and south (island of Texel, 1983; Drinkwaard 1999) led to the transformation of native blue
131 mussel beds into oyssel reefs were both species now co-occur (Troost 2010; Moehler et al.
132 2011; Reise et al. 2017b). In the south of the Wadden Sea, both molluscs are infected with the
133 invasive parasitic copepod *Mytilicola orientalis* (Copepoda: Mytilicolidae) which was co-
134 introduced with the Pacific oyster and recently spilled over to blue mussels (Pogoda et al. 2012;
135 Goedknegt et al. 2017). In addition, a previously introduced con-generic species, *Mytilicola*
136 *intestinalis*, only infects native blue mussels, and although established in the entire Wadden
137 Sea, is almost absent in the south (Goedknegt et al. 2019). Both *Mytilicola* species have a direct
138 life cycle with a free-living planktonic dispersal stage, which spends about 2-3 weeks in the
139 water column before it resides in the intestines of its bivalve host (based on studies of *M.*
140 *intestinalis*; Hockley 1951; Gee and Davey 1986a). Furthermore, blue mussels are infected
141 with a range of native trematodes of which *Renicola roscovita* (Digenea: Renicolidae) is the
142 most common species (Thieltges 2006; Goedknegt et al. 2019). This trematode species has a
143 complex life cycle and uses the periwinkle *Littorina littorea* as first intermediate host, which
144 achieves much higher densities on oyster reefs than on surrounding sand flats. From its

145 gastropod host a short-lived free-living stage (< 1 day; Thieltges and Rick 2006) of *R. roscovita*
146 emerges which subsequently infects blue mussels as second intermediate host. Birds serve as
147 definitive host for this parasite (Werding 1969).

148 We focussed on these parasitic copepod and trematode species to investigate whether
149 the refuge seeking behaviour of native blue mussels initiated by oysters can exert TMIEs on
150 parasites by asking the following specific research questions: 1) Does the refuge seeking
151 behaviour of blue mussels and transmission interference by oysters affect parasite infection
152 levels in blue mussels? 2) Does the resulting effect on parasite infection levels differ between
153 parasites with direct (copepods) and complex (trematodes) life cycles? And 3) do the position
154 in the matrix and parasite infection intensities affect mussel condition?

155

156 MATERIAL AND METHODS

157 Source of uninfected mussels

158 We used naturally uninfected blue mussels (*M. edulis*) to investigate the effect of the position
159 in the oyster matrix on parasite infection levels. For the southern experimental location (Texel,
160 The Netherlands), mussels (mean ± SE; 36.2 ± 0.4 mm) were collected from groynes located
161 on the north-west shore of the Dutch mainland (52°52'42.37" N, 4°42'25.60" E; Fig. 2) on 7
162 July 2014. For the northern location (Sylt, Germany), mussels (38.0 ± 0.5 mm) originated from
163 groynes situated on the west coast of Sylt (54°56'45.76" N, 8°19'9.04" E; Fig. 2) and were
164 collected on 6 August 2014. Previous explorations had shown that the parasites *R. roscovita*
165 and *Mytilicola* spp. seldom occur at these exposed source locations (confirmed by the
166 dissection of 30 mussels at each source location; no infections found). Collected mussels were
167 maintained in 75 L flow-through tanks at 18°C under a 24-hour light cycle (12 h light and 12
168 h dark) and fed three times per week with fresh *Isochrysis galbana* culture, or alternatively

169 with Phyto-Feast® when fresh culture was unavailable. In addition, any epifauna (mostly
170 barnacles) was carefully removed from the mussel shells to ensure that free-living stages of *R.*
171 *roscovita* and *Mytilicola* spp. could infect mussels without being predated or physically
172 obstructed by epifauna during the experiment (Johnson and Thieltges 2010).

173

174 **Experimental locations**

175 The experiment was conducted on Pacific oyster (*C. gigas*) reefs located near two islands at
176 both ends of the Wadden Sea: Texel (south; reef area of about 12 ha, 53.0646 °N, 4.5434 °E)
177 and Sylt (north; reef area of 6 ha, 55.0175 °N, 8.2605 °E Fig. 2). Background infection levels
178 on these reefs have been acquired from previous morphological (and for *Mytilicola* spp.
179 additional molecular) inspections (Goedknegt et al. 2019; Online Resource 1). Importantly, at
180 the time of investigation the recently introduced copepod *M. orientalis* was pre-dominantly
181 present in the southern (prevalence of 46.3%) and barely in the northern Wadden Sea (1.3%),
182 while its con-generic *M. intestinalis* showed the reverse spatial pattern (12.5% in the south,
183 78.1% in the north; Goedknegt et al. 2019; Online Resource 1). For the trematode *R. roscovita*
184 (prevalences: 86.3% in south and 98.8% in the north) infection intensity (± SE) is more than
185 seven times higher in the north (178.9 ± 29.4 trematodes per infected mussel) in comparison
186 to the south (25.9 ± 12.4; Goedknegt et al. 2019; Online Resource 1).

187

188 **Vertical distribution of snails**

189 To acquire knowledge on the vertical distribution of the first intermediate host of the trematode
190 *R. roscovita*, the density of common periwinkles *L. littorea* was measured at low tide on top
191 and at the bottom of the oyster matrix. We determined snail density by haphazardly placing a
192 25 x 25 cm frame on the oyssel reef (Texel n = 10, Sylt n = 6). Within this frame, the top of
193 the matrix (upper 10 cm) was first visually inspected for snails which were counted and

removed. Subsequently, all oysters and mussels were detached from the area within the frame and the number of snails was counted that were found between the bivalves at the bottom of the matrix (approximately 10-20 cm depth from top of oyssel reef).

Experimental set-up

At both experimental locations, we placed uninfected mussels at the bottom and on top of the oyssel reef. Mussels were measured to the nearest 0.01 mm with digital calipers and placed individually into mesh bags made of PE (12 x 16 cm; 1 cm mesh size). Two mesh bags were then attached to an iron rod, with one bag positioned at the higher end of the rod on top of the oyster matrix and the other bag positioned at the lower end of the rod on the bottom of the oyster matrix (after carefully removing and replacing the matrix), with approximately 20 cm between both mesh bags (Fig. 2). At each location, 40 replicates of these rods were positioned in a rectangular field of 12 m × 36 m (10 rows of 4 rods, 4 m distance among rods) with similar oyster cover (Fig. 2). At termination of the experiment, dead mussels were counted and mesh bags with live mussels were frozen (-20 °C) until later examination. The experiment commenced at the beginning of August 2014 and ended four months later in December. Due to bad weather conditions, some of the rods (n = 14) from Sylt could not be recovered until January 2015. To keep the infection time during the experiment at both locations as similar as possible, these 14 rods were excluded from the statistical analysis.

Parasite examination

Prior to dissection, we defrosted the mussels in random batches of 10 individuals. After measuring the mussel shell length with digital calipers to the nearest 0.01 mm, the mussel tissue was separated from the shell and searched for adult *Mytilicola* spp. that were retrieved from

218 the tissue and collected in ethanol (96%). Adult *Mytilicola* individuals were later identified by
219 using morphological characteristics described in Goedknegt et al. (2018). After this initial
220 screening, the mussel tissue was compressed between glass slides and examined under a stereo
221 microscope (magnification 10 - 50×) to account for all *R. roscovita* metacercariae, and
222 larval/juvenile *Mytilicola* spp., of which the latter could not always be identified on species
223 level. As the share of unidentifiable larvae and juvenile *Mytilicola* in blue mussels was
224 relatively large, we merged all *Mytilicola* individuals under *Mytilicola* spp. Finally, *R.*
225 *roscovita* metacercariae and larval/juvenile *Mytilicola* spp. were left in the tissue, as these were
226 too small to be removed from the mussel flesh.

227

228 **Condition of mussels**

229 After dissecting the mussels, the separated mussel flesh was frozen (-20 °C for at least 24
230 hours) and freeze-dried (48 hours) to determine mussel tissue dry weight. We determined the
231 condition index of mussels in a similar way as Eschweiler and Christensen (2011), who tested
232 the effect of oyster TMIEs on predator-prey interactions, and used the formula $CI = DW/L^3$,
233 where DW is the tissue dry weight (mg) and L the final shell length of a mussel (cm, after
234 Petersen et al. 2004).

235

236 **Statistical analysis**

237 All statistical analyses were conducted in the statistical software environment R (R
238 Development Core Team 2015). Differences in snail density between matrix positions (bottom
239 or top) at each location (Texel and Sylt) were tested with Student's t-tests. Regarding the
240 experiment, we used parasite data of individual mussels to model the effects of the position of
241 mussels in the oyster matrix on the prevalence (the ratio of infected to sampled host species)

242 and infection intensity (the mean number of parasites per infected host) of each of the parasite
243 species (the copepods *Mytilicola* spp. and trematode *R. roscovita*) in mussels. Prevalence
244 characterized as parasite presence/absence in individual mussels) was modelled by using a
245 generalized linear mixed model (GLMM; package lme4, Bates et al. 2015) following a
246 binomial distribution, while for infection intensity (i.e. the number of parasites in infected
247 mussels) a GLMM following a negative binomial distribution was used (package
248 glmmADMB, Fournier et al. 2012). In both models, mussel position in the matrix, location and
249 their interaction were regarded as fixed effects, and rod as random effect. Length (measured at
250 the end of the experiment) was additionally included as a covariate in the infection intensity
251 models, as *R. roscovita* intensity (Goedknegt et al. 2019) and *Mytilicola* spp. intensity
252 (Grainger 1951; Goedknegt et al. 2017) are known to vary with mussel size.

253 We furthermore examined whether mussel condition as a decisive component of fitness
254 was affected by the position in the oyster matrix and parasite infection levels (prevalence or
255 infection intensity). We used linear mixed models (LMM, package lme4, Bates et al. 2015),
256 with the mussel condition as response variable. Position in the matrix, location, parasite
257 infection parameters of both parasites (prevalence or infection intensity), and interactions (see
258 Table 2) were included as fixed factors. Rod was again added as a random effect to the model.

259 P-values of all models were obtained by comparing the full model with reduced models
260 without the fixed effect in question by means of likelihood ratio tests following Chi square
261 distributions. P-values < 0.05 were considered as being significant.
262

263 **RESULTS**

264 **Vertical distribution of snails**

265 Both experimental locations varied in patterns of snail distribution within the oyster matrix
266 structure. While for the northern location there was no significant variation in snail density

267 with position in the oyster matrix (mean ± SE; top 440.00 individuals m^{-2} ± 29.1, bottom 450.67
268 ind. m^{-2} ± 26.3; t-test, $p = 0.791$), periwinkles were present at higher densities on top of the
269 oysters (187.2 ind. m^{-2} ± 24.4 SE) compared to the bottom (104.0 ind. m^{-2} ± 12.2) of the oyster
270 matrix in the south (t-test, $t = 3.044$, $p < 0.01$).

271

272 **Mussel survival**

273 At both locations survival of mussels was high. In total 4 of the 80 mussels did not survive the
274 experimental period at each site (Texel: n = 3 bottom, n = 1 top; Sylt: n = 2 both bottom and
275 top).

276

277 **Mussel infections**

278 After four months, the surviving mussels were collected (n = 124 remaining, after removing
279 the rods that were retrieved after the storm and all the dead mussels). These mussels harbored
280 frequent infections with the copepod *Mytilicola* spp. (mean prevalence of both locations ± SE;
281 49.3% ± 0.7%) and the trematode *R. roscovita* (89.6% ± 4.1).

282 On average, *Mytilicola spp*. infection rates of mussels at the bottom of the matrix were lower
283 ($\Delta_{Deviance} = 7.920$, $p < 0.05$; Table 1) than mussels placed on top of the matrix. However, this
284 pattern was only observed at the southern oyster bed, leading to an almost significant
285 interaction term in the prevalence model of *Mytilicola* spp. (location * matrix interaction
286 $\Delta_{Deviance} = 3.182$, $p = 0.074$, Fig. 3). For *Renicola roscovita* infections the infection patterns
287 were reversed, with significantly more infections at the bottom of the matrix. Again, this
288 pattern was mainly driven by infections in the southern oyster bed, resulting in a significant
289 interaction between matrix position and location ($\Delta_{Deviance} = 4.458$, $p < 0.05$; Table 1).

290 The variation in infection prevalence with mussel position was also reflected in *R.*
291 *roscovita* intensity, as the number of metacercariae was significantly higher in infected mussels
292 at the bottom relative to mussels positioned on top of the oyster matrix ($\Delta_{Deviance} = 6.708$, $p <$
293 0.05; Table 1). However, this statistical result was driven by the two southern mussels with
294 relatively high infection intensities compared to the population background (Fig. 4). Such
295 values are well within the range of observed infection intensities (Fig. 4) and since there were
296 no biological or methodological reasons to remove these mussels, the analysis is based on the
297 complete data set including those two specimens. Although the interaction term was not
298 significant ($\Delta_{Deviance} = 1.676$, $p = 0.196$), the matrix position of mussels had a stronger effect at
299 the southern location (Fig. 4), with several mussels at the bottom of the oyster matrix
300 experiencing substantially higher infection levels (max to mean ratio of 11) than mussels at the
301 top (max to mean ratio of 4). At the northern location mean infection levels where much higher
302 but differed only by about 10% between matrix positions (max to mean ratio of 2 for both
303 matrix positions; Fig. 4). In contrast to trematode infections, *Mytilicola* spp. intensity in
304 mussels was independent from the position in the matrix and did not differ between locations
305 (Table 1). Finally, for both trematodes and copepods, mussel length was not a significant
306 predictor in the infection intensity models ($p > 0.05$).

307

308 **Mussel condition**

309 Overall, mussels situated on top of the oyster matrix had significantly better condition
310 indices than mussels at the bottom of the matrix in both the prevalence ($\Delta_{Deviance} = 26.863$, $p <$
311 0.001; and infection intensity models ($\Delta_{Deviance} = 17.967$, $p < 0.01$; Fig. 5). Mussel condition
312 also differed between locations, with better condition of mussels at the southern compared to
313 the northern location ($p < 0.01$ for both models; Fig. 5). Furthermore, in both models the

314 difference in condition with position in the matrix was larger at the southern location than at
315 northern location, resulting in significant interactions with matrix position and location ($p <$
316 0.01 for both models).The condition of the mussels was not affected by infection by either
317 parasite. However, there was a significant interaction of mussel position with *Mytilicola* spp.
318 intensity ($\Delta_{Deviance} = 3.998$, $p < 0.05$; Table 2). While condition increased with higher *Mytilicola*
319 spp. intensity for mussels positioned at the bottom of the matrix, the condition of mussels
320 placed on top of the oyster matrix tended to decrease with *Mytilicola* spp. intensity (Online
321 Resource 2).
322
323 **DISCUSSION**
324 In this study, we observed that an invasive ecosystem engineer can indirectly affect parasite-
325 host interactions if the host adjusts its predator-avoidance behaviour to move deeper into the
326 oyster reef structure. Our field experiment demonstrated that blue mussels at the top of an
327 oyssel reef can experience different parasite infection levels than mussels situated at the
328 bottom. Thus, mussels that hide to deeper layers in the oyster matrix to escape from predators
329 (i.e. birds and crabs) also affect their parasite load. That predator presence can lead to
330 behavioural changes of hosts which in turn result in alterations of parasite-host interactions has
331 previously been documented in freshwater systems. Most of these studies observed that
332 predator-avoidance behaviour leads to an increase in parasite infection levels. For example,
333 female guppies that exert a higher tendency to shoal as a response to predators are infected
334 with more directly transmitted monogenean parasites compared to male guppies which do not
335 shoal under high predation pressure (Stephenson et al. 2015). Therefore, the degree of exposure
336 to infected fish determined by sex-specific differences in shoaling behaviour is driving parasite
337 infection levels in guppies. Interestingly, our study showed that the effect of escaping from
338 predators on parasite infections can vary between different ecological conditions and can even

339 lead to opposing patterns for different parasite groups. While infections by the parasitic
340 copepod *Mytilicola spp*. decreased, trematode infections by *R. roscovita* were amplified. These
341 results can be explained in the context of differential parasite exposure relating to inter-specific
342 differences in parasite life cycles.

343

344 **Infection patterns of *Mytilicola* spp.**

345 Mussels that were positioned on top of the oyster matrix had significantly higher prevalences
346 of the parasitic copepods *Mytilicola* spp. relative to mussels situated at the bottom of the oyssel
347 reef. However, this pattern was only clearly detected at the southern location, suggesting that
348 the observed patterns depended on the encountered ecological ramifications. For *Mytilicola*
349 spp. infections this may reflect differences between the parasite species: *M. orientalis* was very
350 rare at the northern location Sylt, whereas it was the dominant species at the southern location
351 where *M. intestinalis* was very rare. It was already shown that even within *Mytilicola* species
352 the interaction mechanisms with the host can differ between both locations (Feis et al. 2016,
353 2018), suggesting that larger differences could be expected between the species.
354 At the southern location, the observed pattern probably relates to the direct life cycle of the
355 parasites, which involves a free-living phase in which larvae passively distribute in the water
356 column for 2-3 weeks (Hockley 1951; Gee and Davey 1986a) until they reach their infective
357 first copepodid stage that moves to deeper water layers by a photonegative response (Meyer
358 and Mann 1950; Hockley 1951). Hence, production of larvae within the reef is decoupled from
359 recruitment of infective larval stages, because earlier life stages leave the oyssel reef and new
360 infections originate from outside the reef. As infective copepodids are mainly using the hosts'
361 field of filtration and the strength of the host filtration current as settlement cues (Gee and
362 Davey 1986b), the probability of successful infection of mussels on oyssel reefs is determined
363 by the chance of encountering hosts. Therefore, it is likely that the highest concentration of

364 *Mytilicola* spp. larvae would gather at the top of an oyssel reef where they first encounter filter
365 feeding hosts, resulting in higher prevalences of *Mytilicola* spp. in mussels positioned on top
366 of the oyster matrix. Interestingly, a similar pattern has been observed in barnacle larvae,
367 which, like *Mytilicola* copepodids, spend several weeks in the water column before settling on
368 blue mussels. Densities of recently settled barnacles were 2-3 times higher on mussels placed
369 on top of the matrix, compared to mussels situated on the bottom of the oyssel reef (Buschbaum
370 et al. 2016). For barnacles as well as *Mytilicola* spp., the recruitment of larval stages from the
371 water column will result in a decreased exposure of mussels at the bottom of the oyster matrix.
372 Reduced exposure might only be a consequence of the physical structure of the reef but may
373 also be further reduced due to dilution inside the matrix. Dilution of *Mytilicola* larvae can take
374 place via the host competence of the Pacific oyster, which differs between both species of
375 *Mytilicola*. For *M. orientalis*, the invasive Pacific oyster serves as the principle host, as this
376 parasite-host relationship originates from the native range of both species (Mori 1935).
377 Infective *M. orientalis* larvae searching for hosts are likely to infect the oysters and mussels
378 they first encounter, resulting in lower infections of mussels living deep in the oyster matrix.
379 In contrast, the mechanism for *M. intestinalis* is different, as this parasite does not seem to
380 infect Pacific oysters in the Wadden Sea (Goedknegt et al. 2017) and artificial infections were
381 thus far unsuccessful (Elsner et al. 2011; pers. comm. M. Feis). This suggests that, by being an
382 incompetent host, the oyster may interfere with the transmission of larval stages of *M.*
383 *intestinalis* by acting as a decoy, still filtering *M. intestinalis* larvae out of the water but
384 remaining uninfected, thereby reducing the disease risk for mussels hiding in the matrix. In
385 either way, competent or non-competent oysters reduce the risk of infection with *Mytilicola*
386 spp. for mussels positioned deep in the oyster matrix. Whether the absence of a matrix position
387 effect at the northern location, where *M. intestinalis* dominates, suggests that this effect is only
388 relevant for *M. orientalis* or rather depends on other characteristics differentiating both sites

389 remains to be investigated. In contrast to the vertical prevalence pattern, we did not observe
390 significant differences in *Mytilicola* spp. intensity in native mussels depending on the position
391 in the invasive oyster matrix. This suggests that encounter rates with infective larval stages
392 may be higher at the top of the oyssel reef leading to a more even spread of infections, while
393 larval stages inside the matrix have a reduced encounter rate leading to more aggregated
394 distributions. Alternatively, the comparatively low infection levels with *Mytilicola* spp.
395 building up during the experiment (mean infection intensity < 2) led to low statistical power to
396 detect differences in infection intensities of mussels between the two positions in the oyster
397 matrix.
398
399 **Infection patterns of *R. roscovita***
400 The trematode *R. roscovita* showed different infection patterns in the oyster matrix compared
401 to the copepods *Mytilicola* spp., with higher infection levels (in particular infection intensity)
402 in mussels positioned at the bottom of oyssel reefs at the southern site. This result is
403 contradicting our expectations, as we anticipated a strong effect of the oysters' transmission
404 interference capacity which had been previously shown in field and laboratory studies
405 (Thieltges et al. 2009; Welsh et al. 2014; Goedknegt et al. 2015) and which we expected to
406 result in lower infection levels of mussels hiding in between the oysters. Probably the oysters
407 still interfered in parasite transmission in our experiment, but this was overruled by other
408 biological (e.g. down-ward orientated host-searching behaviour of *Renicola* spp. cercaria,
409 Nicolaev et al., 2017), and hydrographical processes (e.g. differences current flow on top and
410 between the oysters) that resulted in the observed vertical distribution pattern of trematodes in
411 the oyster matrix. In contrast to the copepods, local recruitment of trematode larval infective
412 stages mainly originates from within the oyssel reef, which can be ascribed to the complex life
413 cycle of trematodes. The infection process of *R. roscovita* starts when free-living stages of the

414 parasite (trematode cercariae) emerge from the first intermediate host, the periwinkle *L.*
415 *littorea.* This host attains highest densities in oyster reefs in comparison to other intertidal
416 habitats. As the free-living cercarial stage is short-lived (<1 day and the infective period being
417 <12 hours; Thieltges and Rick 2006) and locally produced by snails in very high numbers
418 (Thieltges and Rick 2006; Thieltges et al. 2009), infections are expected to occur on small
419 spatial scales in close vicinity to the first intermediate hosts. This explains why infection levels
420 in second intermediate trematode hosts are usually positively correlated with the local density
421 of (infected) snails (Thieltges 2007; Thieltges and Reise 2007). Our data show that snail density
422 was indeed reflected in the infection intensity in mussels, as snail density and infection
423 intensity on natural oyssel reefs were both about three times higher in the northern (Sylt)
424 compared to the southern (Texel) location. However, zooming in on the oyster matrix, the
425 vertical distribution of snails did not relate to the trematode infection intensity in mussels.
426 While trematode intensities were significantly higher in mussels positioned at the bottom of
427 the oyster matrix, snail density was either higher on top of the matrix (southern location, Texel)
428 or there was no significant difference between matrix positions (northern location, Sylt). It
429 must be noted though, that we have only investigated the snail distribution in the oyster matrix
430 at low tide, and high tide investigations might challenge these results. Without data on snail
431 infection levels we can, however, not exclude the possibility that also infected snails aggregate
432 at the bottom of the oyssel reef, e.g., as a result of parasite manipulation of the behaviour of
433 infected snails (Curtis et al., 1987).

434 Alternatively, hydrographical rather than biological processes may explain the higher
435 infection levels of trematodes in mussels located on the bottom of the oyssel reef. Infection by
436 marine trematodes are known to primarily occur at low tide, when large concentrations of
437 infective stages accumulate and are trapped in small volumes of water such as tidal pools,
438 maximizing contact with their second intermediate hosts and increasing transmission rates

439 (Mouritsen and Jensen 1997; Mouritsen 2002a, b; Thieltges and Reise 2007; Koprivnikar and
440 Poulin 2009). Similar trapping of cercariae likely occurs in the matrix of oyssel reefs so that
441 mussels at the bottom are exposed to a higher density of infective stages and for a longer
442 duration than mussels on top of the oyssel reef, resulting in higher infection levels in mussels
443 at the bottom compared to the top of the matrix. Both biological and hydrographical processes
444 are not mutually exclusive and future experiments will be needed to determine the relative
445 strength of the processes responsible for the observed vertical infection pattern of the trematode
446 *R. roscovita* in the oyster matrix. Spatial variation in the relative strength of these processes
447 may underlie the observed difference in effect size between locations. Obviously spatial
448 heterogeneity is much higher in the southern oyssel reef, especially in the bottom of the reef
449 where particularly high infection intensities could be observed. This could suggest that
450 infective cercarial stages were trapped in only a few pockets of the oyster matrix in the south.
451 In the north, the much higher supply of cercaria might have masked such spatial heterogeneity
452 and reef position effects in a density-dependent fashion. However, further studies will be
453 needed to unravel the exact underlying mechanisms.

454

455 **Mussel condition**

456 Dwelling in deeper layers of the oyster matrix near the bottom does not only increase the risk
457 of becoming infected by trematodes, but also considerably lowers the availability of food
458 particles, demonstrated by the significant lower condition of mussels positioned at the bottom
459 of the oyssel reef. This is in agreement with results of Eschweiler and Christensen (2011) who
460 also found reduced condition of mussels at the bottom of oyssel reefs. We did not find a
461 significant effect of *R. roscovita* intensity on condition index. This is surprising as *R. roscovita*
462 is known to cause reductions in blue mussel condition (Stier et al. 2015). Possibly, the duration
463 of our experiment was too short to detect a significant effect on the condition of the mussels.

464 For *Mytilicola* spp. we did not detect an overall effect of infection intensity on mussel condition
465 index, but we detected a significant interaction of infection intensity with mussel position in
466 the matrix. While mussel condition tended to increase with *Mytilicola* spp. intensity when
467 mussels were placed at the bottom of the matrix, there was a slight decrease in infection
468 intensity when mussels were placed on top of the oyster reef. Based on the fact that the found
469 patterns is only driven by three points (two points at an infection intensity of 4 and one point
470 at an infection intensity of 5) and low number of mussels with a high intensity of infection by
471 copepods (see Online Resource 2), we are uncertain whether the found interaction pattern is
472 actually real. Therefore, we consider this result as a statistical artefact and elude any biological
473 significance. Finally, mussels significantly differed in condition between the two experimental
474 locations, most likely caused by differences in environmental conditions and production
475 between the northern and southern Wadden Sea.

476

477 **CONCLUSIONS**

478 In summary, our study shows that the biogenic matrix provided by invasive oysters does not
479 only initiate TMIEs in the form of refuge seeking of native mussels which reduces crab
480 predation and detrimental barnacle overgrowth (Eschweiler and Christensen 2011; Waser et al.
481 2015; Buschbaum et al. 2016), but that these indirect effects also extend to parasites: refuge
482 seeking behaviour in the oyster matrix reduces infections with parasitic copepods to a certain
483 extent, but it comes at the cost of an increased exposure to trematodes. These indirect effects
484 of the invasive oysters are the net result of complex abiotic and biotic mechanisms related to
485 the position of mussels in the oyster matrix as discussed above and thus apply to the scale of
486 oyssel reefs. On larger spatial and temporal scales, oysters may also cause direct effects on
487 parasites by transmission interference or parasite spillover which may ultimately affect
488 background infection levels in the ecosystem. Hence, the net effect of invasive oysters on the

489 ecosystem level may differ from their net effect on the scale of oyssel reefs. However, the
490 magnitude of ecosystem-wide as well as ecosystem specific impacts remains to be investigated.

491 Overall, the results of our study suggest that invasive ecosystem engineers can
492 exert TMIEs on parasites, which is a novel mechanism of how invasive species can affect
493 recipient ecosystems. Given the increasing evidence that ecosystem engineers in general can
494 exert manifold indirect effects on species interactions and food webs (White and O'Donnell
495 2010; Sanders et al. 2014; Wetzel et al. 2016; Griffen et al. 2017; Mourant et al. 2017), it seems
496 highly likely that many of these indirect effects will also trigger TMIEs (and also DMIEs) on
497 parasites if they include changes in the behaviour or density of species that serve as hosts for
498 parasites. Hence, indirect effects of invasive ecosystem engineers on parasite-host interactions
499 in recipient ecosystems may be much more common than realized today and they possibly add
500 significantly to the known diversity of impacts of invasive ecosystems engineers (Crooks 2002;
501 Guy-Haim et al. 2018). Further research into the diversity and magnitude as well as the
502 underlying mechanisms of indirect effects of invasive ecosystem engineers on parasitism is
503 needed, and we hope that our study will spark further interest in this direction.

504

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512

513 **DATA ACCESSIBILITY**

514 Data will be archived at https://data.4tu.nl/repository/.

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681 **Table 1** Results of GLMMs explaining variation in parasite prevalence and intensity in native
682 blue mussels (*Mytilus edulis*) depending location, (southern vs. northern Wadden Sea), the
683 position (top vs. bottom) in the oyster matrix and mussel length (for intensity only).
684 Coefficients (Coeff.), standard errors (SE), odd ratios (OR) with lower (LL) and upper (UL)
685 95% confidence intervals of fixed effects, and variance (Var.) and standard deviations (SD) of
686 random effects (rod) are shown for full models.

Model	Parasite species	*Fixed effects*			*Odd ratio*			*Random effects*	
		Variable	**Coeff.**	**SE**	**OR**	**LL**	**UL**	**Var.**	**SD**
Prevalence	*Mytilicola* spp. (n = 124)	Intercept	-0.007	0.427	0.993	0.430	2.293		
		Position (Bottom)	0.007	0.592	1.007	0.316	3.215		
		Location (South)	0.617	0.560	1.853	0.619	5.550		
		Position (Bottom) * Location (South)	-1.138	0.795	0.251	0.053	1.192		
		Rod	-	-	-	-	-	0.183	0.428
	R. roscovita (n = 124)	Intercept	3.136	1.022	23.000	3.106	170.309		
		Position (Bottom)	-0.738	1.261	0.478	0.040	5.658		
		Location (South)	-2.071	1.085	0.126	0.015	1.058		
		Position (Bottom) * Location (South)	3.256	1.659	25.956	1.005	670.058		
		Rod	-	-	-	-	-	0	0
Infection Intensity	*Mytilicola* spp. (n = 61)	Intercept	0.086	1.494	-	-	-		
		Position (Bottom)	-0.058	0.327	-	-	-		
		Location (South)	-0.013	0.300	-	-	-		
		Position (Bottom) * Location (South)	0.085	0.416	-	-	-		
		Mussel length	0.011	0.031	-	-	-		
		Rod	-	-	-	-	-	0	0.001
	R. roscovita (n = 110)	Intercept	3.875	1.433	-	-	-		
		Position (Bottom)	0.154	0.304	-	-	-		
		Location (South)	-2.836	0.333	-	-	-		
		Position (Bottom) * Location (South)	0.524	0.403	-	-	-		
		Mussel length	0.014	0.030	-	-	-		
		Rod	-	-	-	-	-	0.281	0.530

687 **Table 2** Results of LMMs explaining variation in condition in native blue mussels (*Mytilus*
688 *edulis*) depending on the position (top vs. bottom) in the oyster matrix, experimental location
689 (north vs south) and parasite infection level (prevalence or intensity for both parasite species
690 *Mytilicola spp.* and *Renicola roscovita*). Coefficients (Coeff.) and standard errors (SE) are
691 shown for fixed effects, and variance (Var.) and standard deviations (SD) of random effects
692 (rod) are shown for full models.

Model	***Fixed effects***			***Random effects***	
	Variables	**Coeff.**	**SE**	**Var.**	**St. dev**
Prevalence (n = 124)	Intercept	4.422	0.676		
	Position in matrix (Bottom)	-0.186	0.866		
	Location (South)	1.609	0.311		
	Mytilicola spp. presence	-0.544	0.686		
	R. roscovita presence	-0.307	0.653		
	Position (Bottom) * Location (South)	-1.373	0.437		
	Mytilicola spp. presence * *R. roscovita* presence	0.622	0.726		
	Position (Bottom) * *Mytilicola* spp. presence	0.063	0.433		
	Position (Bottom) * *R. roscovita* presence	0.054	0.838		
	Rod			0	0
	Residual			1.308	1.144
Intensity (n = 52)	Intercept	4.335	0.629		
	Position in matrix (Bottom)	-0.152	0.923		
	Location (South)	1.803	0.552		
	Mytilicola spp. presence	-0.131	0.220		
	R. roscovita presence	0.003	0.005		
	Position (Bottom) * Location (South)	-2.089	0.745		
	Mytilicola spp. presence * *R. roscovita* presence	-0.003	0.003		
	Position (Bottom) * *Mytilicola* spp. presence	0.562	0.301		
	Position (Bottom) * *R. roscovita* presence	-0.006	0.005		
	Rod			0.219	0.468
	Residual			0.913	0.955

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FIGURES

Fig. 1 a) An oyssel reef in the Wadden Sea with b) blue mussels (*Mytilus edulis*) hiding in the oyster matrix (*Crassostrea gigas*) indicated by the white circle and arrow.

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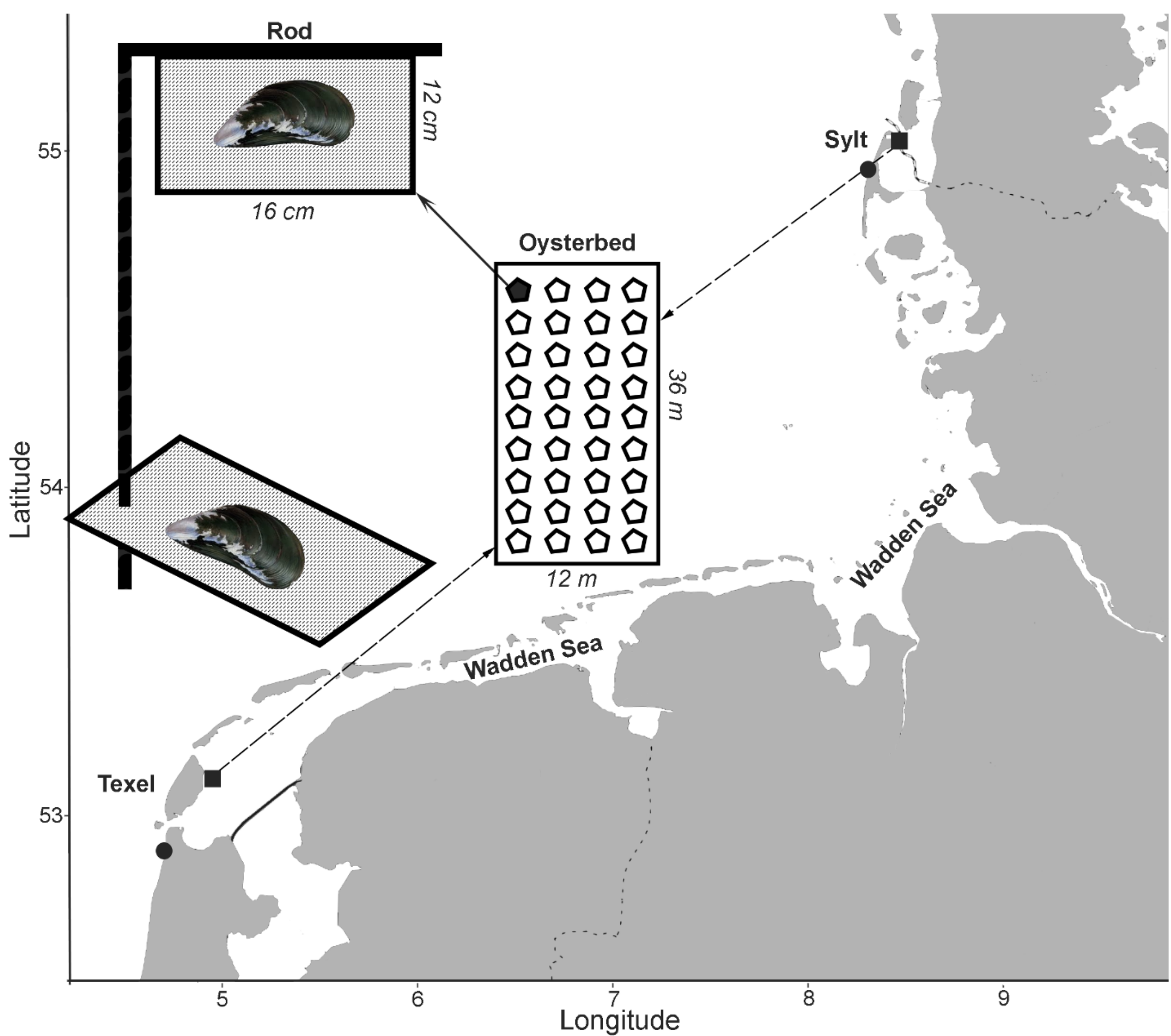

724 **Fig. 2** Experimental set-up of the experiment on two oyssel reefs (squares), one in the south
725 (Texel) and one in the north (Sylt) of the Wadden Sea. On each location, mussels originating
726 from uninfected source locations (black dots) were individually added to two mesh bags
727 attached to an iron rod that were positioned on the bottom and on the top of the oyster matrix.
728 In total, 40 of these rods (pentagons) were placed within an area of 36 x 12 m (4 m distance
729 among rods) on each oyssel reef.

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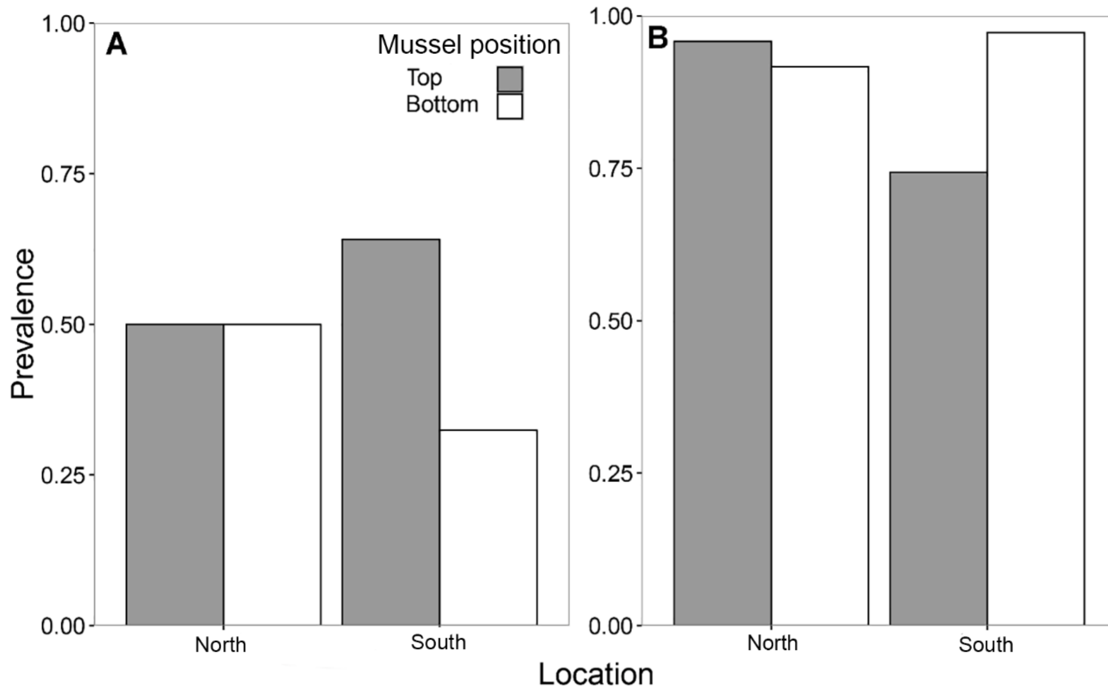

741 **Fig. 3** Prevalence of A) the parasitic copepod *Mytilicola* spp. and B) the trematode *Renicola*
742 *roscovita* at the top and bottom of the oyster matrix at both experimental locations.

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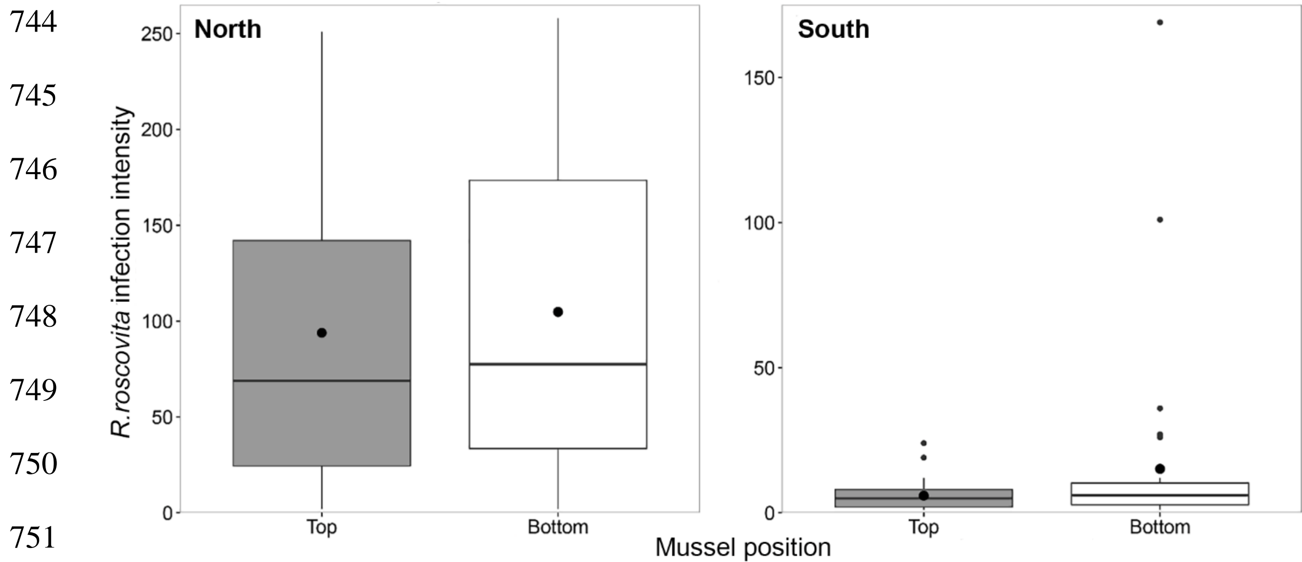

752 **Fig. 4** Mean infection intensity (± SE) of the trematode *Renicola roscovita* at the top and
753 bottom of the oyster matrix at the northern and southern location. The boxes represent the
754 interquartile range, the whiskers denote the lowest and highest values within the 1.5
755 interquartile range, the black line in each box denotes the median, the large black dots represent
756 the mean condition indices of each group and the smaller dots outside the boxes are outliers.
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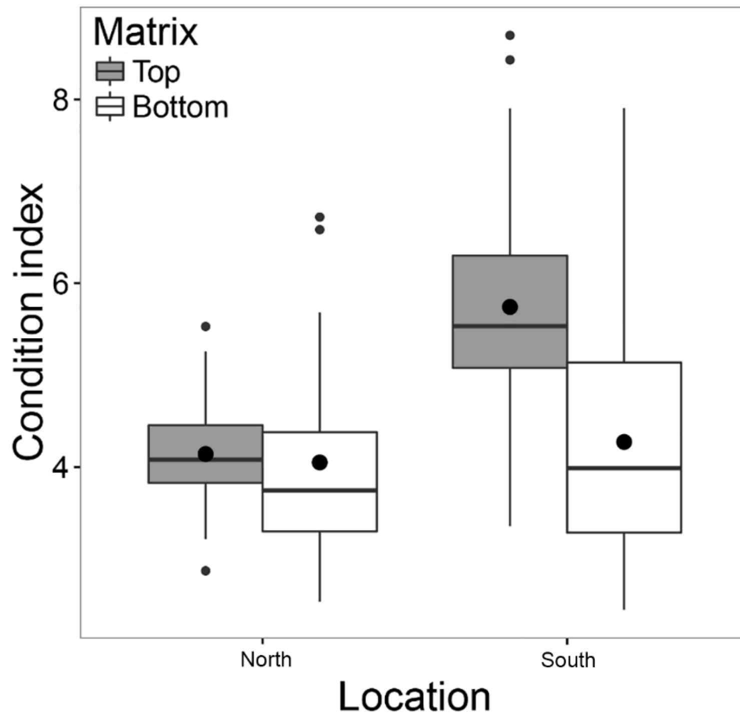

769 **Fig. 5** Condition of mussels positioned on top (grey) and on the bottom (white) of the oyster
770 matrix at the northern and southern location. The boxes represent the interquartile range, the
771 whiskers denote the lowest and highest values within the 1.5 interquartile range, the black line
772 in each box denotes the median, the large black dots represent the mean condition indices of
773 each group and the smaller dots outside the boxes are outliers. Note the truncated y-axis.