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Invasive ecosystem engineers and parasitism: trait-mediated indirect interactions initiated by invasive oysters alter infection levels in native mussels

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Abstract

Invasive ecosystem engineers that create or modify physical habitat structure can impose strong direct and indirect impacts on native biota, e.g. by providing predation refuge or affecting prey or predator traits resulting in alterations in predation strength. In this study, we show that the habitat structure provided by invasive ecosystem engineers can also indirectly affect parasite-host interactions. Reef-building invasive Pacific oysters (*Crassostrea gigas*) are known to initiate trait-mediated indirect interactions (TMIIs) by initiating refuge seeking behaviour of native blue mussels (*Mytilus edulis*) at the bottom of the three-dimensional oyster matrix, thereby indirectly reducing crab predation on mussels. Using a replicated field experiment, in which uninfected mussels were positioned at the bottom and top of two oyster beds in the south and north of the Wadden Sea, we investigated whether the oyster matrix also induced TMIIs in regard to parasite infection levels in native mussels. After three months, mussels positioned on top of an oyster bed showed significantly higher occurrence of parasitic intestinal copepods than the ones positioned at the bottom. However, this was only clearly observed at one of the two beds and there was no difference in infection intensity depending on matrix position at both beds. For trematodes, the opposite pattern was observed, with higher occurrence in mussels positioned at the bottom of the matrix (again only clearly observed on one bed) and significantly higher infection levels at the bottom compared to the top of the matrix on both beds. This contrasting pattern most likely resulted from differences in recruitment pathways between the two parasite groups. Free-living copepod larvae recruit externally from the oyster bed, increasing the likelihood of infections for mussels positioned at the top of the oyster matrix. In contrast, trematodes recruit from inside the beds via short-lived infective stages released from their local first intermediate snail hosts, leading to a concentration of infective stages inside the matrix. The results of this study suggest a novel mechanism of how invasive species can affect parasite-host interactions in recipient ecosystems. As invasive habitat modifying organisms and parasites are omnipresent in many ecosystems, TMIIs that lead to alterations of parasite-host interactions may be a common impact of biological invasions.

Introduction

Invasive species are considered as one of the greatest threats to ecosystem biodiversity and ecological communities worldwide (Elton, 1958; Vitousek et al., 1996; Mack et al., 2000). In particular, invasive ecosystem engineers that create or modify physical habitat structure impose strong direct impacts on native biota, including effects on habitat and food availability, native species density and diversity, and changes in abiotic conditions (Jones et al., 1994, 1997b; Crooks, 2002). In addition to these direct effects, invasive ecosystem engineers may also affect other organisms in such way that it has consequences for a third species. These indirect interactions can be density-mediated (DMII's), in which the habitat modifier indirectly influences a third species by altering the density of an intermediate species (*sensu* Abrams et al., 1995). Simultaneously, habitat modifiers may affect a third species by altering the traits (e.g., behavioural, physiological, morphological, chemical) of an intermediate species (trait-mediated indirect interactions (TMII's); *sensu* Abrams et al., 1995). For instance, the availability of shelter within complex habitats created by invasive ecosystem engineers can induce prey refuge behaviour that alters predator-prey encounter rates and thereby the risk of predation (Byers et al., 2010; Pearson, 2010; Eschweiler and Christensen, 2011; Waser et al., 2015). Currently, evidence of DMII's and TMII's imposed by invasive habitat modifiers in the context of predator-prey interactions is growing and adding to the encompassing impacts of invasive species. In contrast, whether invasive ecosystem engineers can also affect other species interactions such as parasite-host relationships via indirect effects induced by their habitat modification is currently not well known.

Autogenic ecosystem engineers such as invasive oysters provide a suitable model system to study indirect effects of habitat modification on species interactions. These marine molluscs, in particular the Pacific oyster (*Crassostrea gigas*), have been introduced world-wide for aquaculture purposes (Ruesink et al., 2005). Once established in the wild after introduction, oysters create hard-substrate biogenic beds and thereby modify the environment with consequences for other organisms and species interactions (Ruesink et al., 2005). In general, oysters (native and invasive) are known to provide predation refuge for prey hiding in the biogenic matrix created by the oysters, which can have effects on predation strength (Grabowski, 2004; Hughes and Grabowski, 2006; Troost, 2010). In Europe, invasive Pacific oysters co-exist with native mussels on oyster beds (Troost, 2010; Fig. 6.1). Here, Pacific oysters are known to exert trait-mediated indirect interactions (TMIIs) by initiating refuge seeking behaviour of native blue mussels (*Mytilus edulis*). In response to predation risk, mussels actively migrate to the bottom of the three-dimensional oyster matrix which significantly reduces crab predation on mussels (Eschweiler and Christensen, 2011; Waser et al., 2015). However, this predation refuge is traded for reduced foraging success as mussel condition is decreased at the bottom of the matrix (Eschweiler and Christensen, 2011). The presence of invasive Pacific oysters is also known to affect parasite-host transmission in this invaded region by a mechanism referred to as *transmission interference* (Johnson and Thieltges, 2010; Welsh et al., 2014; Goedknegt et al., 2016). As they are unsuitable hosts, Pacific oysters significantly reduce the number of free-living trematode cercarial stages in the water column by filter feeding or trapping cercariae on the complex rough shells (Thieltges et al., 2009a; Welsh et al., 2014; Goedknegt et al., 2015). This transmission interference has been shown to reduce infection levels in native mussels (*Mytilus edulis*) placed on artificial oyster beds compared to mussels situated on bare sediment (Thieltges et al., 2009a). Hence, the presence of invasive oysters can in principle affect parasite-host interactions, but it is not known whether the TMIIs observed for predator-prey interactions

within the biogenic oyster matrix also affect parasite-host relationships. This also applies to native parasite-host interactions in which oysters serve as an alternative host for native parasites and which may also be affected via trait-mediated indirect effects induced by the oyster matrix.

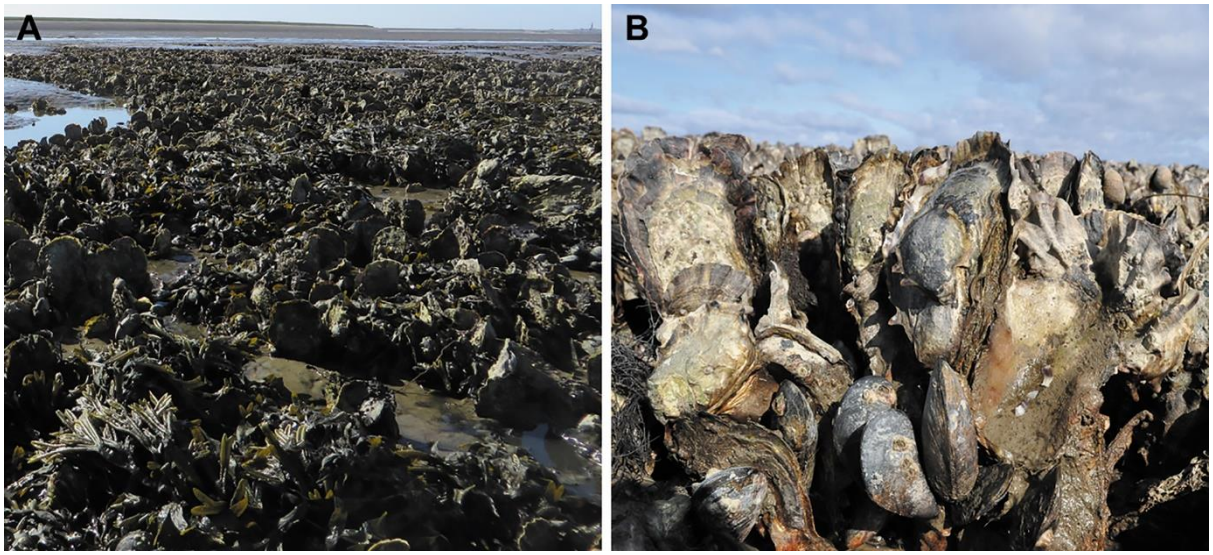

Fig. 6.1 A) A bivalve bed in the Wadden Sea and **B)** blue mussels (*Mytilus edulis*) hiding in the oyster matrix (*Crassostrea gigas*) (source: Christian Buschbaum).

In this study, we used a replicated field experiment conducted on two Pacific oyster (*C. gigas*) beds in the south and north of the European Wadden Sea to investigate whether the habitat structure provided by invasive oysters can indirectly affect parasite-host interactions in native blue mussels (*M. edulis*). In this intertidal area, two separate invasions of Pacific oysters in the north (island of Sylt, 1986; Reise, 1998) and south (island of Texel, 1983; Drinkwaard, 1999) led to the transformation of native blue mussel beds in oyster beds were both species now co-occur (Troost, 2010; Moehler et al., 2011). In the south of the Wadden Sea, both molluscs are infected with the invasive parasitic copepod *Mytilicola orientalis* (Copepoda: Mytilicolidae) that was co-introduced with the Pacific oyster and recently spilled over to blue mussels (Pogoda et al., 2012; Goedknegt et al., 2017). In addition, a previously introduced con-generic species, *M. intestinalis*, only infects native blue mussels, and although established in the entire Wadden Sea, is almost absent in the south (Goedknegt et al., 2017). Both *Mytilicola* species have a direct life cycle with a relatively long (based on studies of *M. intestinalis* 2-3 weeks; Hockley, 1951; Gee and Davey, 1986a) free-living planktonic dispersal stage after which it resides in the intestines of their hosts. Furthermore, blue mussels are infected with a range of native trematodes of which *Renicola roscovita* (Digenea: Renicolidae), is the most common species (Thieltges et al., 2006; Chapter 3). This trematode species has a complex life cycle and uses the periwinkle *Littorina littorea* living on the oyster beds as first intermediate host that is followed by a short free-living stage (<1 day; Thieltges and Rick, 2006) after which it infects blue mussels (second intermediate host) and birds (definitive host), respectively (Werding, 1969). We focussed on these parasitic copepod and trematode species and asked the following specific research questions: 1) Does the position in the oyster matrix effect parasite infection levels in blue mussels? 2) Does the effect of position in the oyster matrix on infection levels differ between parasites recruiting from outside (copepods)

and inside (trematodes) oyster beds? And 3) do the position in the matrix and parasite infection intensities affect mussel fitness (condition)?

Material and Methods

Source of uninfected mussels

In the experiment we used naturally uninfected blue mussels (*Mytilus edulis*) to investigate the effect of the position in the oyster matrix on parasite infection levels. For the southern experimental location (Texel, The Netherlands), mussels (mean ± SE; 36.2 ± 0.4 mm) were collected from groynes located on the north-west shore of the Dutch mainland (52°52'42.37" N, 4°42'25.60" E; Fig. 6.2) on 7 July 2014. For the northern location (Sylt, Germany), mussels (38.0 ± 0.5 mm) originated from groynes situated on the west coast of Sylt (54°56'45.76" N, 8°19'9.04" E; Fig. 6.2) and were collected on 6 August 2014. Previous explorations had shown that the parasites *Renicola roscovita* and *Mytilicola* spp. do not occur at these source locations (verified by dissecting 30 mussels — no infections found). Until the start of the experiment, collected mussels were maintained in 75 L flow-through tanks at 18°C under a 24-hour light cycle (12 h light and 12 h dark) and fed three times per week with fresh *Isochrysis galbana* culture, or alternatively with Phyto-Feast® when fresh culture was unavailable. In addition, any epifauna (mostly barnacles) on the uninfected mussels was carefully removed from the shells to ensure that free-living stages of *Renicola roscovita* and *Mytilicola* spp. could infect mussels without being predated or physically obstructed by epifauna during the experiment (Johnson and Thieltges, 2010).

Background infections and snail density

The experiment was conducted on Pacific oyster (*Crassostrea gigas*) beds located near two islands at both ends of the Wadden Sea: Texel (south) and Sylt (north; Fig. 6.2). Background infection levels of these beds were known from previous inspections (n = 80 on Sylt; Chapter 3) or checked by the dissection of 30 mussels in advance of the experiment (Texel) and gave assurance for successful infections in the field. Additionally, at both locations the density of the periwinkle *Littorina littorea*, the first intermediated host of trematode *R. roscovita*, was measured on top and at the bottom of the oyster matrix. We determined snail density at both locations (Texel n = 10, Sylt n = 6), by haphazardly placing a 25 x 25 cm frame on the oyster bed. Within this frame, the top of the matrix (max. 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 oyster bed top).

Experimental set-up

At both oyster beds (Fig. 6.2), we placed uninfected mussels (see section *Source of infected mussels*) at the bottom and on top of the oyster matrix. The mussels were measured to the nearest 0.01 mm with digital callipers and individually added to a mesh bag made of PE (12 x 16 cm; approximately 1 cm mesh size). Two mesh bags were then attached to a hook-shaped iron rod, and one bag was positioned at the higher end of the rod on top of the oyster matrix and the other one was positioned at the lower end of the rod on the bottom of the oyster matrix, with approximately 20 cm between both mesh bags. At each location, 40 replicates of these rods were positioned in a rectangular field of 12 m x 36 m (10 rows of 4 rods, 4 m distance among rods)

with similar oyster cover (Fig. 6.2). At termination of the experiment, mesh bags with mussels were frozen (-20 °C) for later analysis. The experiment commenced at the beginning of August 2014 and ended four months later in December. Due to bad weather conditions, part of the rods (n = 14) from Sylt could only be recovered in January 2015. To keep the infection time during the experiment at both locations as similar as possible, these 14 rods were excluded from the analysis.

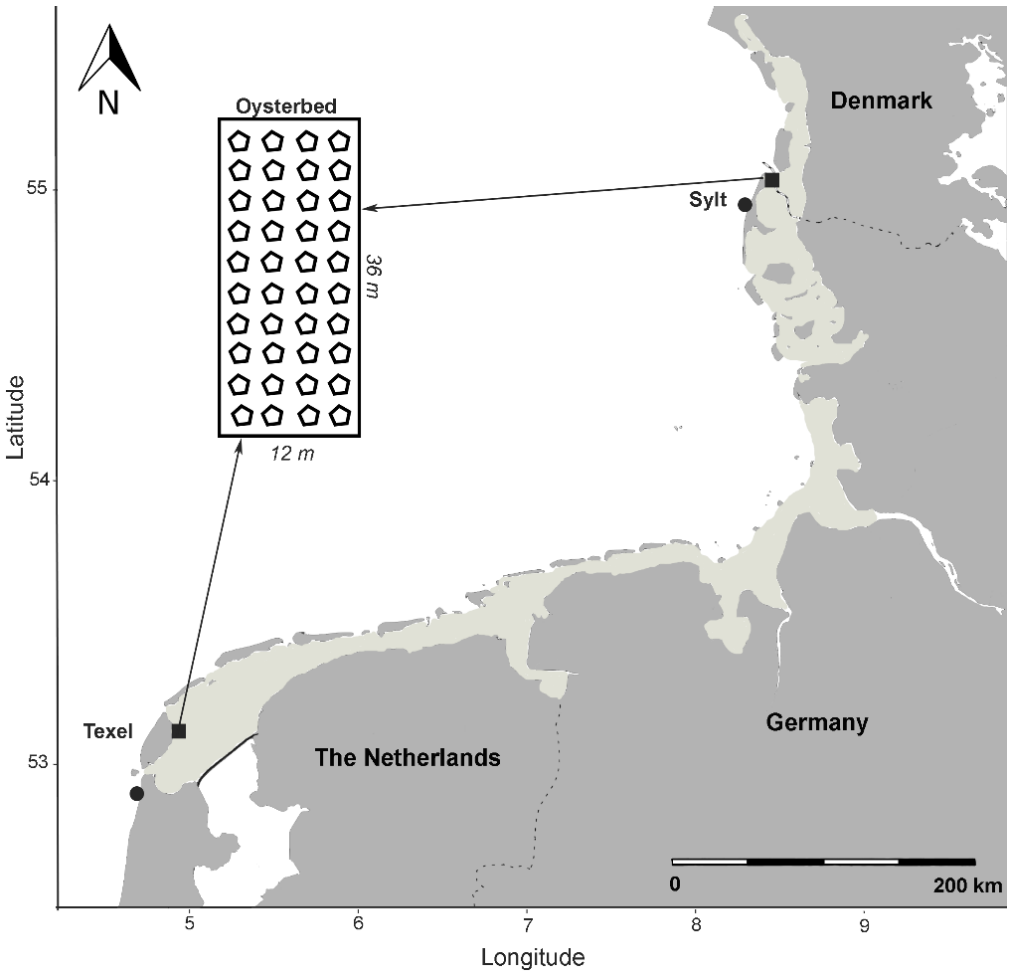

Fig. 6.2 Experimental set-up of the experiment on two oyster beds (squares), one in the south (Texel) and one in the north (Sylt) of the Wadden Sea (shaded light grey area). On each location, mussels originating from uninfected source locations (black dots) were individually added to two mesh bags attached to an iron rod that were positioned on the bottom and on the top of the oyster matrix. In total, 40 of these rods (pentagons) were placed within an area of 36 x 12 m (4 m distance among rods) on each oyster bed.

Parasite examination

Prior to dissection, we defrosted the mussels in random batches of 10 individuals. After measuring the mussel shell length with digital callipers to the nearest 0.01 mm, the mussel tissue was separated from the shell and searched for adult *Mytilicola* spp. that were retrieved from the tissue and collected in ethanol (96%). Adult *Mytilicola* individuals were later identified by using morphological characteristics described in Steuer (1902), Mori (1935), Grizel (1985) and Elsner et al. (2011). After this initial screening, the mussel tissue was compressed between glass slides

and examined under a stereo microscope (magnification 10 - 50 x) to account for all *R. roscovita* metacercariae, and larval/juvenile *Mytilicola* spp., of which the latter could not be identified on species level. As the share of unidentifiable larvae and juvenile *Mytilicola* in blue mussels was relatively large, we merged all *Mytilicola* individuals under *Mytilicola* spp.. Finally, *R. roscovita* metacercariae and larval/juvenile *Mytilicola* spp. were left in the tissue, as these were too small to be removed from the mussel flesh.

Condition of mussels

After dissecting the mussels, we separated the mussel flesh from the shell and froze (-20 °C for at least 24 hours) and freeze-dried it (48 hours) to determine mussel tissue dry weight. Condition index was determined as $CI = DW/L^3$, where DW is the tissue dry weight (mg) and L the final shell length of a mussel (cm, after Petersen et al., 2004).

Statistical analysis

At both experimental locations, background infection levels in mussels were determined for informative purposes only and hence not statistically analysed. Differences in snail density between matrix positions at each location were tested with Student's t-tests.

We used parasite data of individual mussels to model the effects of the position of mussels in the oyster matrix (bottom or top in the matrix) on the occurrence and intensity of each of the parasite species (the copepods *Mytilicola* spp. and trematode *R. roscovita*) in mussels in the statistical software package R (R Development Core Team, 2015).

In the occurrence models, we used a generalized linear mixed model (GLMM; package lme4, Bates et al., 2015) following a binomial distribution with *Mytilicola* spp. or *R. roscovita.* occurrence (presence/absence) as response variable, location and position in the matrix and the interaction between these factors as fixed factors and rod as random effect. To model differences in parasite intensities in infected mussels, we used GLMMs following a negative binomial distribution (package glmmADMB, Fournier et al., 2012) with location, matrix position, the interaction between these variables and length as fixed factors, and individual rod as random factor. Length (measured at the end of the experiment) was additionally included in the intensity models, as intensity of *R. roscovita* (Chapter 3) and *Mytilicola* spp. (Grainger, 1951; Goedknegt et al., 2017) are known to vary with mussel size. P-values of all models were obtained by comparing the full model with reduced models without the fixed effect in question by means of likelihood ratio tests following chi-square distributions.

We furthermore examined whether mussel fitness (condition) was affected by the position in the oyster matrix, the location and parasite infection levels (occurrence or intensity in two separate models). We used linear mixed models (LMM, package lme4, Bates et al., 2015), with the fitness parameter (condition index) as response variable, and position in the matrix, location and the interaction between these two variables as fixed factors. Additionally, parasite infection parameters of both parasites (occurrence or intensity), the interaction between the parasite species and their individual interactions with matrix position were included as fixed factors. Individual rod was added as a random effect in the models. Data selection for the intensity model only involved the subset of mussels that were infected with a parasite species, while the occurrence model was executed for the whole dataset.

Results

Background infection levels and snail density

Prevalences of the copepods *Mytilicola* spp. and the trematode *Renicola roscovita* were at least 50% at both experimental locations, showing evidence for well-established parasite-host relationships at these beds. Interestingly, the recently introduced copepod *Mytilicola orientalis* was only present in the south (prevalences of 50%) and barely in the north (1%) of the Wadden Sea, while its con-generic *Mytilicola intestinalis* showed the reverse spatial pattern (7% in the south, 78.8% in the north). Furthermore, while prevalence of the trematode *Renicola roscovita* were reasonably high at both locations (86.7-98.8%), the mean infection intensity (± SE) was more than three times higher in the north (181.4 ± 28.0 trematodes per infected mussel) in comparison to the south (56.6 ± 13.9). This variation in trematode intensity in infected blue mussels (*Mytilus edulis*) was also reflected in the density of the periwinkle *Littorina littorea*, the first intermediate host of the parasite, which was also about three times higher on Sylt (mean density m^{-2} ± SE; north 445.3 ± 18.8, south 145.6 ± 16.4). Furthermore, both experimental locations also varied in patterns of snail distribution within the oyster matrix structure. While for the northern location there was no significant variation in snail density with position in the oyster matrix (t-test; $p = 0.791$), in the south periwinkles were present at higher densities on top of the oysters (187.2 m^{-2} ± 24.4 SE) compared to the bottom (104.0 ± 12.2) of the oyster matrix (t-test; $t = 3.044$, $p < 0.01$).

Mussel survival, parasite infection levels and condition

At both locations, 4 of the 80 mussels did not survive the experimental period (Texel: n = 3 bottom, n = 1 top; Sylt: n = 2 both bottom and top). After four months, the surviving mussels (n = 124) were well infected with the copepod *Mytilicola* spp. (mean prevalence ± SE; 0.49 ± 0.05) and the trematode *R. roscovita* (0.89 ± 0.03). Regarding the position (top and bottom) of mussels in the oyster matrix, both parasites showed reverse patterns as *Mytilicola* spp. prevalence (statistically tested as occurrence) was higher in mussels on top of the matrix (GLMM; $\Delta_{Deviance} = 8.001$, $p < 0.05$), while for the trematode *R. roscovita* the opposite was true ($\Delta_{Deviance} = 9.617$, $p < 0.01$; Fig. 6.3). However, clear differences in prevalences with matrix position were only observed at the southern oyster bed (Texel; Fig. 6.3), resulting in a significant interaction term for *R. roscovita* ($\Delta_{Deviance} = 4.463$, $p < 0.05$) and an almost significant interaction term for *Mytilicola* spp. ($p = 0.072$, Table 6.1).

The variation in infection prevalence with mussel matrix position was also reflected in *R. roscovita* intensity, as the number of metacercariae was significantly higher in infected mussels at the bottom relative to mussels on top of the oyster matrix (GLMM; $\Delta_{Deviance} = 8.562$, $p < 0.05$; Table 6.1., Fig. 6.4). Although the interaction term was not significant (Table 6.1), the matrix position of mussels had a stronger effect at the Texel location, with mussels at the bottom of the oyster matrix experiencing about 3 times higher infection levels than mussels at the top, than at the Sylt location where mean infection levels differed only by about 10% depending on position in the matrix (Fig. 6.4). In general, intensities of *R. roscovita* were much higher at the northern location ($\Delta_{Deviance} = 91.194$, $p < 0.001$; Fig. 6.4). In contrast to trematode infections, *Mytilicola* spp. infection intensity did not differ between locations ($p > 0.05$) or depending on the position in the matrix (mean intensity ± SE; Sylt bottom 1.7 ± 0.4, top 1.8 ± 0.3; Texel bottom 1.8 ± 0.3, Texel top 1.7 ± 0.2; $p > 0.05$, Table 6.1). Finally, for both, trematodes and copepods, mussel length was not a significant predictor in the intensity models ($p > 0.05$; Table 6.1).

Overall, mussels situated on top of the oyster matrix had significantly better condition indices than mussels at the bottom of the matrix in both the occurrence and intensity models ($p < 0.01$; Fig. 6.5, Supplementary Table S6.1). Mussel condition also differed between locations, with better condition of mussels at the southern compared to the northern location ($p < 0.01$). Furthermore, in both models the difference in condition with position in the matrix was larger on Texel (south) than on Sylt (north), resulting in significant interactions with matrix position and location ($p < 0.01$). Furthermore, the condition of the mussels was not affected by the presence or intensity of the parasite species, however when mussels on top of the matrix were infected with higher intensities of *Mytilicola* spp. their condition was lower than infected mussels on the bottom of the oyster matrix, indicated by the significant interaction of matrix position with *Mytilicola* spp. intensity ($p < 0.05$; Table S6.1).

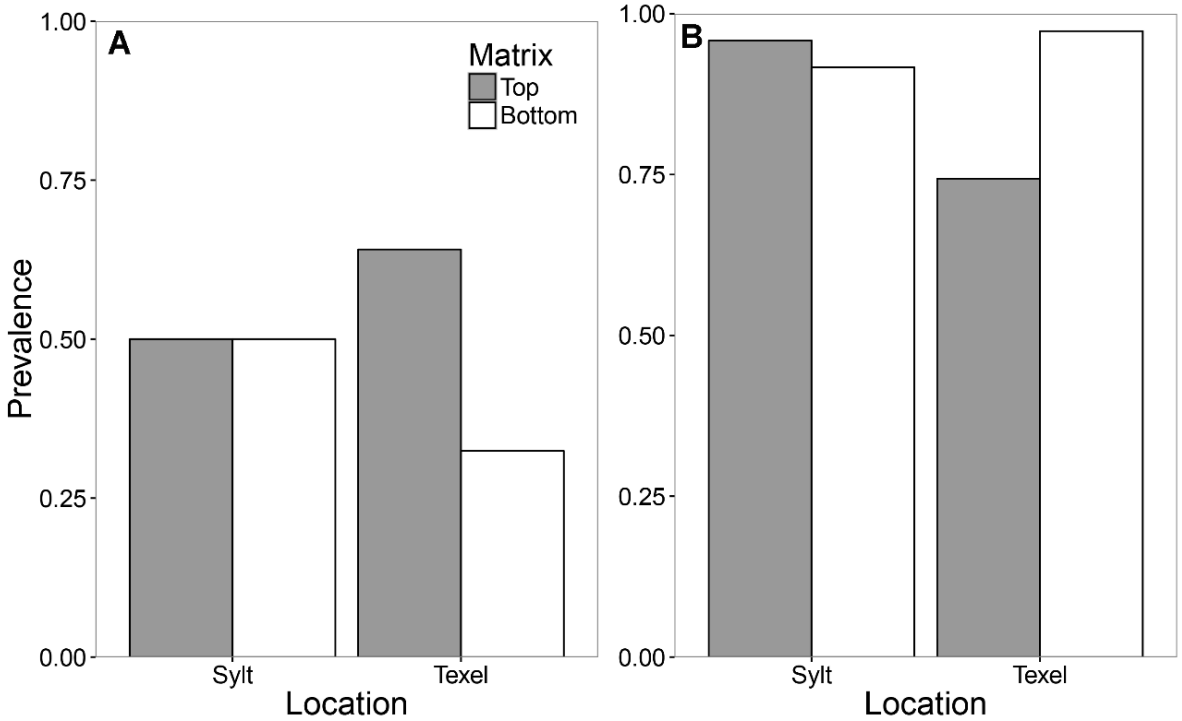

Fig. 6.3 Prevalence of **A)** the parasitic copepod *Mytilicola* spp. and **B)** the trematode *Renicola roscovita* at the top (grey) and bottom (white) of the oyster matrix.

Discussion

Invasive habitat modifiers can exert indirect effects on native species. In this study, we applied this concept for the first time in the context of parasite-host interactions and tested whether the physical structure created by invasive Pacific oysters (*Crassostrea gigas*) affected parasite infection levels in native blue mussels (*Mytilus edulis*). The results of our replicated field experiment indicate that the three-dimensional habitat created by the oysters can lead to a decrease in infections by the invasive parasitic copepods *Mytilicola* spp., but amplified infections by the native trematode *Renicola roscovita*. However, the magnitude of these effects differed between locations and the two parasites, suggesting some variability in the strength of trait-mediated effects of oysters on parasite infection levels in mussels.

Table 6.1 Results of GLMMs explaining variation in parasite occurrence and intensity in native blue mussels (*Mytilus edulis*) depending on the position (top vs. bottom) in the invasive Pacific oyster matrix (*Crassostrea gigas*), experimental location (Sylt in the north, Texel in the south of the Wadden Sea) and mussel length (for intensity only). Coefficients (Coeff.) and standard errors (SE) are shown for full models. Model selection was performed by backwards elimination of non-significant variables and values shown are from the point at which each variable was removed from the model.

Model	**Parasite species**		***Fixed effects***				***Random effects***	
		Variables	**Coeff.**	**SE**	**Δ Dev.**	**P**	**Var.**	**St. dev.**
Occurrence	*Mytilicola* spp. (n = 124)	Intercept	-0.010	0.427				
		Position in matrix	0.020	0.593	8.001	< 0.05		
		Location	0.618	0.548	3.277	0.194		
		Position * Location	-1.400	0.793	3.246	0.072		
		Rod					0.097	0.312
	R. roscovita (n = 124)	Intercept	3.174	1.154				
		Position in matrix	-0.746	1.268	9.617	< 0.01		
		Location	-2.085	1.105	6.601	< 0.05		
		Position * Location	3.288	1.716	4.463	< 0.05		
		Rod					0.181	0.426
Intensity	*Mytilicola* spp. (n = 61)	Intercept	0.086	1.494				
		Position in matrix	-0.058	0.327	0.042	0.979		
		Location	-0.013	0.300	0.055	0.973		
		Mussel length	0.011	0.031	0.124	0.725		
		Position * Location	0.085	0.416	0.041	0.839		
		Rod					0	0.002
	R. roscovita (n = 110)	Intercept	3.370	1.388				
		Position in matrix	0.250	0.305	8.562	<0.05		
		Location	-2.736	0.305	91.194	<0.001		
		Mussel length	0.023	0.405	1.472	0.435		
		Position * Location	0.494	0.029	0.610	0.225		
		Rod					0.249	0.499

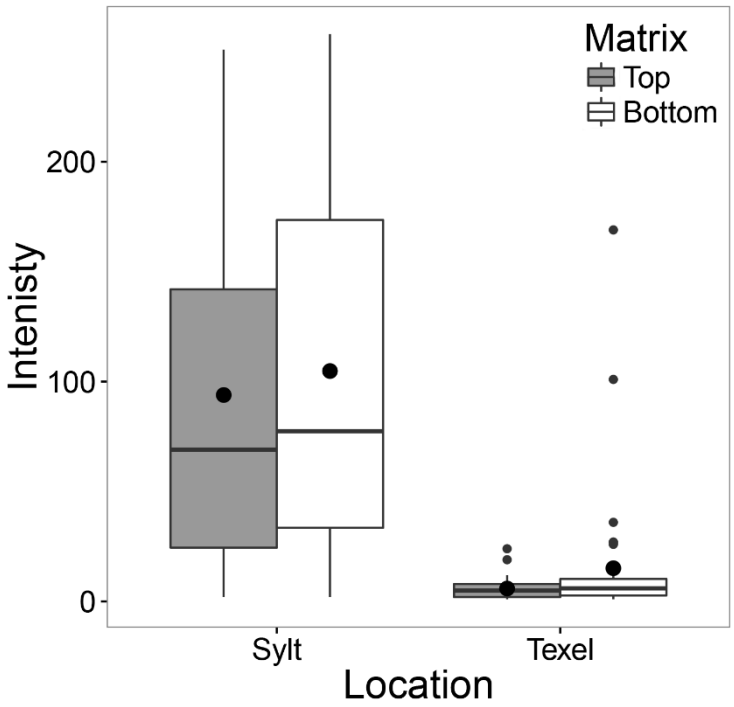

Fig. 6.4 Boxplots of the intensity of the trematode *Renicola roscovita* at the top (grey) and at the bottom (white) at the two experimental locations **A)** Sylt and **B)** Texel.

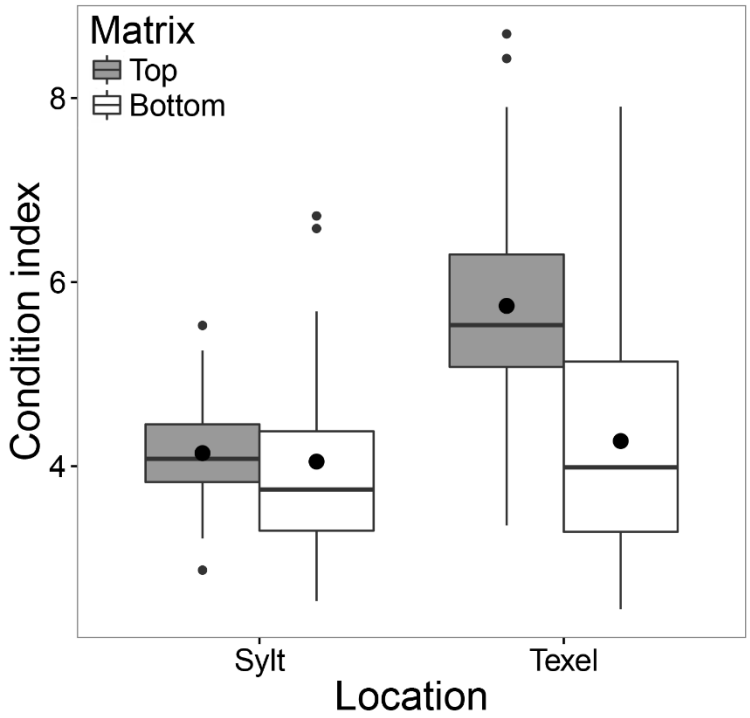

Fig. 6.5 Condition of mussels positioned on top (grey) and on the bottom (white) of the oyster matrix at both experimental locations. Black dots represent the means for each of the factor combinations.

Infection patterns of *Mytilicola* spp.

Mussels that were positioned on top of the oyster bed matrix had statistically significant higher prevalences of the parasitic copepods *Mytilicola* spp. relative to mussels situated at the bottom of the oyster bed. However, this pattern was only clearly seen at one of the two experimental locations (Texel), suggesting that the observed pattern may actually only hold true for *M. orientalis* (very rare at the northern location on Sylt), but not for *Mytilicola intestinalis* (very rare at the southern location on Texel). The revealed prevalence pattern probably relates to the direct life cycle of the parasites, which involves a free-living phase in which larvae passively distribute in the water column for 2-3 weeks (Hockley, 1951; Gee and Davey, 1986a) until it reaches its infective first copepodite stage that moves to deeper water layers by a photonegative response (Meyer and Mann, 1950; Hockley, 1951). Hence, recruitment of infective larval stages to an oyster bed originates from outside the bed and is decoupled from local production of larvae. As infective copepodids are not using chemical cues to find their hosts (Meyer and Mann, 1950; Gee and Davey, 1986b), the probability of successful infection of mussels on oyster beds is determined by the chance of encountering the hosts' field of filtration and by the strength of the host filtration current (Gee and Davey, 1986b). Therefore, it is likely that the highest concentration of *Mytilicola* spp. larvae would gather at the top of an oyster bed where they first encounter filter feeding hosts, resulting in higher prevalences of *Mytilicola* spp. in mussels positioned on top of the oyster matrix. Interestingly, a similar pattern has been observed in barnacle larvae, which, like *Mytilicola* copepodids, spend several weeks in the water column before settling on blue mussels. Densities of recently settled barnacles were 2-3 times higher on mussels placed on top of the matrix, compared to mussels situated on the bottom of the oyster bed (Buschbaum et al., 2016). For barnacles as well as *Mytilicola* spp. the recruitment of larval stages from outside the oyster bed will result in a decreased exposure of mussels at the bottom of the oyster matrix, because the

density of larvae will be diluted inside the matrix. Dilution of *Mytilicola* larvae can take place via the host competence of the Pacific oyster, which differs between both *Mytilicola* spp.. For *M. orientalis*, the invasive Pacific oyster serves as an alternative host, as this parasite-host relationship originates from the native range of both species (Mori, 1935). Infective *M. orientalis* larvae searching for hosts are likely to infect the oysters and mussels they first encounter, resulting in lower infections of mussels living deep in the oyster matrix. In contrast, the mechanism for *M. intestinalis* is different, as this parasite does not seem to infect Pacific oysters in the Wadden Sea (Goedknegt et al., 2017) and artificial infections were thus far unsuccessful (Elsner et al., 2011; pers. comm. M. Feis). This suggests that, by being an incompetent host, the oyster may interfere with the transmission of larval stages of *M. intestinalis* by acting as a decoy, thereby reducing the disease risk for mussels hiding in the matrix. In either way, competent or non-competent oysters reduce the risk of infection with *Mytilicola* spp.. for mussels positioned deep in the oyster matrix. Whether the absence of a difference in prevalence between matrix positions at the northern location, where *M. intestinalis* dominates, suggests that this effect is only relevant for *M. orientalis* remains to be investigated. In contrast to the vertical prevalence pattern, we did not observe significant differences in *Mytilicola* spp. intensity in native mussels depending on the position in the invasive oyster matrix. This suggests that encounter rates with infective larval stages may be higher at the top of the oyster bed and that infectivity is relatively constant once larval stages have made it into the matrix. It may also be that the infection levels with *Mytilicola* spp. building up during the experiment were generally too low (mean intensity < 2) to be able to detect differences in infection intensities of mussels between the two positions in the oyster matrix.

Infection patterns of *Renicola roscovita*

The trematode *Renicola roscovita* showed opposite infection patterns in the oyster matrix compared to the copepods *Mytilicola* spp., with higher infection levels (in particular intensity) in mussels positioned at the bottom than at the top of the oyster beds. This result is contradicting our expectations, as we anticipated a strong effect of the oysters' transmission interference capacity which had been previously shown in field and lab studies (Thieltges et al., 2009a; Welsh et al., 2014; Goedknegt et al., 2015) and which we expected to result in lower infection levels of mussels hiding in between the oysters. Probably the oysters still caused an interference in parasite transmission in our experiment, but this was overruled by other biological and hydrographical processes that resulted in the observed vertical distribution pattern of trematodes in the oyster matrix. In contrast to the copepods, local recruitment of larval infective stages originates from within the oyster bed, which can be ascribed to the complex life cycle of trematodes. The infection process of *R. roscovita* starts when free living stages of the parasite (cercariae) emerge from the first intermediate host, the periwinkle *Littorina littorea,* which lives inside the oyster matrix. As the free-living cercarial stage is short-lived (<1 day with the infective period being <12 hours; Thieltges and Rick, 2006) and locally produced by snails in very high numbers (Thieltges and Rick 2006; Thieltges et al., 2008), infections are expected to happen on small spatial scales in close vicinity to the first intermediated hosts. This implies that infection levels in second intermediate hosts are usually positively correlated with the density of (infected) snails (Thieltges, 2007; Thieltges and Reise, 2007). Our data show that snail density was indeed reflected in the infection intensity in mussels, as snail density and infection intensity on natural mussel beds were both about three times higher in the northern (Sylt) compared to the southern (Texel) location. However, zooming in on the oyster matrix, the vertical distribution of snails did

not relate to the trematode infection intensity in mussels. While trematode intensities were significantly higher in mussels positioned at the bottom of the oyster matrix, snail density was either higher on top of the matrix (southern location, Texel) or there was no significant difference between matrix positions (northern location, Sylt). Hence, the higher infection levels in mussels at the bottom cannot be explained by snail distribution in the matrix, although we acknowledge that in absence of data on snail infection levels we cannot exclude the possibility that infected snail aggregate at the bottom of the oyster bed. Alternatively, hydrographical rather than biological processes may explain the higher infection levels of trematodes in mussels located on the bottom of the oyster bed. Infection in marine trematodes are known to primarily occur at low tide, when large concentrations of infective stages accumulate and are trapped in small volumes of water such as tidal pools, maximizing contact with their second intermediate hosts and increasing transmission rates (Mouritsen and Jensen, 1997; Mouritsen, 2002a; Mouritsen, 2002b; Thieltges and Reise, 2007; Koprivnikar and Poulin, 2009). Similar trapping of cercariae likely occurs in the matrix of oyster beds so that mussels at the bottom are exposed to a higher density of infective stages and for a longer duration than mussels on top of the oyster bed, resulting in higher prevalences and intensities in mussels at the bottom compared to the top of the matrix. Both biological and physiological process are not mutually exclusive and future experiments will be needed to determine the relative strength of the processes responsible for the observed vertical infection pattern of the trematode *R. roscovita* in the oyster matrix. Spatial variation in the relative strength of these processes may underlie the observed difference in effect size between locations (much stronger effect of matrix position at the southern compared to the northern location) and further studies will be needed to unravel the underlying mechanisms.

Mussel condition

The predator refuge seeking behaviour of mussels in the matrix in response to predators does not only come at the cost of a higher chance on trematode infections, but also considerably lowers the availability of food particles, demonstrated by the significant lower condition of mussels positioned at the bottom of the oyster bed. This is in agreement with results of Eschweiler and Christensen (2011) who also found reduced condition of mussels at the bottom of oyster beds. Interestingly, in our study neither infection nor intensity of both *Mytilicola* spp. and *R. roscovita* significantly affected the condition of mussels, which is somewhat surprising. Both *Mytilicola* spp. have been documented to negatively impact the condition of blue mussels (e.g., Meyer and Mann, 1950; Korringa, 1952; Theisen, 1987; Chapter 7, but see Dethlefsen, 1975). Similarly, *R. roscovita* is known to cause reductions in blue mussel body condition (Stier et al., 2015). Possibly, the infection intensities in our experimental mussels were too low to detect a significant effect on mussel condition. However, in combination with position in the oyster matrix, increased *Mytilicola* spp. intensities caused a reduction in mussel condition when these were placed on top of the oysters. Finally, mussels significantly differed in condition between the two experimental locations, most likely caused by differences in environmental conditions between the northern and southern Wadden Sea.

Conclusion

In summary, our study shows that the biogenic matrix provided by invasive oysters does not only initiate trait-mediated indirect interactions (TMIIs) in the form of refuge seeking of native mussels which reduces crab predation (Eschweiler and Christensen, 2011; Waser et al., 2015),

but that these TMIIs also extend to parasite-host interactions. While the refuge seeking behaviour in the oyster matrix reduces infections with parasitic copepods to a certain extent, it comes with the cost of strongly increased exposure to trematodes. Hence, the predation (and to some extent parasitism) refuge for mussels gained from hiding in the matrix is traded for increased parasite infection levels and reduced foraging success at the bottom of the oyster bed. However, the fact that the magnitude of these effects differed between locations suggests a certain spatial variability in the strength of trait-mediated effects of oysters on parasite infection levels in mussels. Overall, the results of this study suggest a novel mechanism of how invasive ecosystem engineers can affect parasite-host interactions in recipient ecosystems. As invasive habitat modifying organisms and parasites are omnipresent in many ecosystems, TMIIs that lead to alterations of parasite-host interactions may be a common impact of biological invasions.

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Supplementary material

Table S6.1 Results of LMMs explaining variation in condition in native blue mussels (*Mytilus edulis*) depending on the position (top vs. bottom) in the invasive Pacific oyster matrix (*Crassostrea gigas*), experimental location (Sylt in the north vs Texel in the south of the Wadden Sea) and parasite infection level (occurrence and intensity for both parasite species). Coefficients and standard errors are shown for full models. Model selection was performed by backwards elimination of non-significant variables and values shown are from the point at which each variable was removed from the model.

Model	***Fixed effects***				***Random effects***	
	Variables	**Coeff.**	**SE**	**Δ Dev.**	**P**	**Var.**	**St. dev**
Occurrence (n = 124)	Intercept	4.394	0.673				
	Position in matrix	-0.172	0.868	26.905	< 0.001		
	Location	1.601	0.309	26.369	< 0.001		
	Mytilicola spp.	-0.513	0.684	0.928	0.819		
	R. roscovita	-0.271	0.652	0.917	0.821		
	Position * Location	-1.370	0.433	10.249	< 0.01		
	Mytilicola spp. * *R. roscovita*	0.602	0.724	0.735	0.391		
	Position * *Mytilicola* spp.	0.064	0.433	0.023	0.878		
	Position * *R. roscovita*	0.041	0.843	0.917	0.821		
	Rod					0.033	0.182
	Residual					1.275	1.129
Intensity (n = 52)	Intercept	4.448	0.634				
	Position in matrix	-0.303	0.969	17.34	< 0.01		
	Location	1.658	0.555	10.281	< 0.01		
	Mytilicola spp.	-0.119	0.221	4.423	0.219		
	R. roscovita	0.002	0.005	6.330	0.097		
	Position * Location	-2.047	0.793	7.492	< 0.01		
	Mytilicola spp. * *R. roscovita*	-0.003	0.003	1.271	0.260		
	Position * *Mytilicola* spp.	0.590	0.306	4.128	<0.05		
	Position * *R. roscovita*	-0.006	0.006	1.191	0.275		
	Rod					0	0
	Residual					1.137	1.066