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POPULATION ECOLOGY – ORIGINAL RESEARCH

How invasive oysters can affect parasite infection patterns in native mussels on a large spatial scale

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Abstract

There are surprisingly few field studies on the role of invasive species on parasite infection patterns in native hosts. We investigated the role of invasive Pacific oysters (*Magallana gigas*) in determining parasite infection levels in native blue mussels (*Mytilus edulis*) in relation to other environmental and biotic factors. Using hierarchical field sampling covering three spatial scales along a large intertidal ecosystem (European Wadden Sea), we found strong spatial differences in infection levels of five parasite species associated with mussels and oysters. We applied mixed models to analyse the associations between parasite prevalence and abundance in mussels and oysters, and 12 biological and environmental factors. For each parasite–host relationship, an optimal model (either a null, one-factor or two-factor model) was selected based on AIC scores. We found that the density of invasive oysters contributed to three of the 12 models. Other biological factors such as host size (six models), and the density of target or alternative host species (five models) contributed more frequently to the best models. Furthermore, for parasite species infecting both mussels and oysters, parasite population densities were higher in native mussels, attributed to the higher densities of mussels. Our results indicate that invasive species can affect parasite infection patterns in native species in the field, but that their relative contribution may be further mediated by other biological and environmental parameters. These results stress the usefulness of large-scale field studies for detailed assessments of the mechanisms underlying the impacts of invasive species on native host communities.

Keywords Invasive species · Parasite spillover · Parasite spillback · Transmission interference · Wadden Sea

Introduction

Over the last decades, global trade and transport have expanded enormously leading to an unprecedented introduction of species to new ecosystems (Vitousek et al. 1996; Mack et al. 2000; Bax et al. 2003; Levine and D'Antonio 2003; Jackson and Grey 2013). Besides the documented direct effects on species interactions with native organisms, it is increasingly recognised that introduced species can also alter parasite–host relationships in invaded ecosystems in manifold ways. For example, with many alien organisms their native parasites can be co-introduced to recipient ecosystems (Daszak et al. 2000; Taraschewski 2006; Lymbery et al. 2014). These introduced parasites may spill over from introduced to naïve native host species (*parasite spillover*; Power and Mitchell 2004; Prenter et al. 2004; Kelly et al. 2009), which has already lead to emerging diseases and mass mortalities of native populations (Daszak et al. 2000; Goedknegt et al. 2016). Furthermore, native parasites might

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infect invasive host species in their new range which in turn may increase the disease risk for native species if the invasive hosts amplify transmission rates, resulting in increased infection levels in native host populations (*parasite spillback*; Kelly et al. 2009; Poulin et al. 2011; Telfer and Brown 2012). Alternatively, invasive host species may be non-competent hosts for native parasites and instead interfere with transmission processes by removing free-living infectious stages of native parasites from the environment (e.g., by means of predation or being dead-end hosts; *transmission interference*; Johnson and Thieltges 2010; Goedknegt et al. 2016). This can lead to a reduced disease risk for native host species, a phenomenon similar to dilution effects observed in vector-borne diseases (Keesing et al. 2006).

Due to the crucial role of invasive species in these parasite infection scenarios, the presence and abundance of an invader has the potential to affect local parasite infection levels in native hosts (Kelly et al. 2009; Poulin et al. 2011; Telfer and Brown 2012). While such effects have been studied experimentally (e.g., Kopp and Jokela 2007; Thieltges et al. 2009; Goedknegt et al. 2015), surprisingly few studies have attempted to study the effects of invasive species on infection patterns in native hosts in the field (but see Paterson et al. 2011, 2013 who used a combined approach). Parasite infection levels in native hosts are not only potentially affected by invasive species, but also influenced by many other factors which have been shown to underlie the generally high spatial heterogeneities in infection levels observed in the field (Thieltges and Reise 2007; Byers et al. 2008; Wilson et al. 2011; Galaktionov et al. 2015; Stringer and Linklater 2015). For example, the population density of native hosts often affects infection patterns across many parasite and host taxa (Arneberg et al. 1998; Galaktionov et al. 2015; Stringer and Linklater 2015; Searle et al. 2016). Other factors known to affect infection patterns include host size (Mouritsen et al. 2003; Thieltges and Reise 2007), the supply of free-living infective stages (often approximated via preceding intermediate host densities for parasites with complex life cycles; Byers et al. 2008; Wilson et al. 2011; Galaktionov et al. 2015) and environmental variables such as temperature, pH and salinity (Pietrock and Marcogliese 2003; Poulin 2006). The existence of a multitude of biological and environmental factors driving infection levels, questions the relative contribution of invasive hosts, or in other words, whether invader presence and abundance matter for infections in native hosts. Hence, field studies investigating infection patterns in native hosts in relation to the abundance of invasive species and other factors are desirable.

A suitable model system to investigate the relative importance of invasive species in determining infection levels in native hosts in the field, is the invasion of the Pacific oyster (*Magallana gigas*) along north western European coasts. This bivalve was introduced to Europe in the 1960s to replenish native oyster stocks for aquaculture purposes (Troost 2010), and today Pacific oyster populations co-occur with native blue mussels (*Mytilus edulis*) in dense bivalve beds on intertidal mudflats (Reise 1998; Troost 2010; Ruesink et al. 2005; Buschbaum et al. 2016; Reise et al. 2017). Pacific oysters co-introduced the invasive parasitic copepod *Mytilicola orientalis* that was likely co-introduced in large numbers or via multiple introductions and followed a similar invasion route as oysters (Feis 2018) and subsequently spilled over to native blue mussels (Pogoda et al. 2012; Goedknegt et al. 2017). This copepod has a direct life cycle and inhabits the intestines of its host, causing reductions in the condition of mussels (Goedknegt et al. 2018a), but not in oysters (Katkansky et al. 1967; Steele and Mulcahy 2001). A congeneric parasitic copepod species, *Mytilicola intestinalis*, has been infecting native mussels since its introduction to the region 80 years ago (Caspers 1939; Hockley 1951; Korringa 1968). While the parasite was first observed in mussels (*Mytilus galloprovincialis*) in the Mediterranean Sea (Steuer 1902), genetic studies could not confirm the Mediterranean as its native region due to low genetic diversity and a lacking population structure, and, to date, its origin is still unknown (Feis 2018). At western European coasts, the parasite does not seem to infect invasive oysters, making the Pacific oyster a potential sink for *M. intestinalis* populations (Elsner et al. 2011; Goedknegt et al. 2017). Likewise, the Pacific oyster is a not a suitable host for the native trematodes *Himasthla elongata* and *Renicola roscovita* (Thieltges et al. 2008, 2009; Welsh et al. 2014; Goedknegt et al. 2015). Instead, by filtering host-seeking trematode larvae out of the water column, the oyster interferes with the transmission between first (snails) and second intermediate hosts (several native bivalve species; Thieltges et al. 2008, 2009; Welsh et al. 2014; Goedknegt et al. 2015), preventing the parasite species to complete their life cycle in birds, the definitive host of both trematodes (gulls and waders; Stunkard 1964; Werding 1969; Lauckner 1983; Galaktionov and Bustnes 1999). Finally, for the native shell-boring polychaete *Polydora ciliata*, which infects native blue mussels (*M. edulis*) and common periwinkles (*Littorina littorea*; Buschbaum et al. 2007), invasive Pacific oysters act as a new competent host species (Thieltges et al. 2006), potentially increasing infection levels in native mussels via parasite spillback.

In this study, we analysed the relationship between the distribution and abundance of parasites in native mussels and the abundance of the invasive Pacific oyster (*M. gigas*) and other biotic and abiotic factors in the Wadden Sea, a large intertidal soft-bottom ecosystem stretching over 500 km of coastline. Using large-scale field observations we aimed to address the following questions: (1) what is the distribution and abundance of parasite species associated with parasite spillover (*M. orientalis*), spillback (*P. ciliata*) and transmission interference processes (*M. intestinalis, H. elongata, R.*

roscovita) in invasive oysters and native mussels along the entire Wadden Sea ecosystem? (2) Can the contribution of invasive oysters be unravelled among other biological and environmental factors determining infection levels in native mussels? and (3) For parasites infecting mussel and oyster hosts (*M. orientalis* and *P. ciliata*), which host species serves as the dominant host for the parasite population? By investigating the relative importance of invasive oysters for parasite infection patterns in native mussels, this study contributes to a better understanding of the role of invasive species in parasite spillover, spillback and transmission interference processes.

Materials and methods

Parasite infection patterns

Sampling on hierarchical scales

Sampling took place on eight mixed beds of invasive Pacific oysters (*M. gigas*) and native blue mussels (*M. edulis*) spread over the entire Dutch and German Wadden Sea except for the mid-German Wadden Sea, which is devoid of mussel beds (Folmer et al. 2014; see Fig. 1; Online Resource 1). Beds were selected based on geographic distribution and logistical feasibility. The following regions were sampled: West Netherlands (locations 1 and 2), East Netherlands (locations 3 and 4), South Germany (locations 5 and 6) and North Germany (locations 7 and 8). All beds were sampled in autumn 2012 (Online Resource 1) as this period is well suited for documenting infection levels of macroparasites (summer is the main period of production of trematodes (Thieltges and Rick 2006; Poulin 2006) and parasitic copepods (Grainger 1951) and of the settlement of *P. ciliata* larvae (Harms and Anger 1983)).

To demarcate a plot, a quadrant of 1 m^2 was haphazardly placed four times within each bed at low tide, at approximately similar tidal heights and with 100 m distance between plots. From each plot, 20 individuals of each bivalve species (mussels and oysters) were randomly collected for parasitological analysis. We sampled medium-to-large size classes of mussels (30–70 mm) and oysters (40–230 mm), as these size classes are regularly infected with the five parasite species (Brenner et al. 2014; Goedknegt et al. 2017). Our sampling design was hierarchical, resulting in three spatial scales of

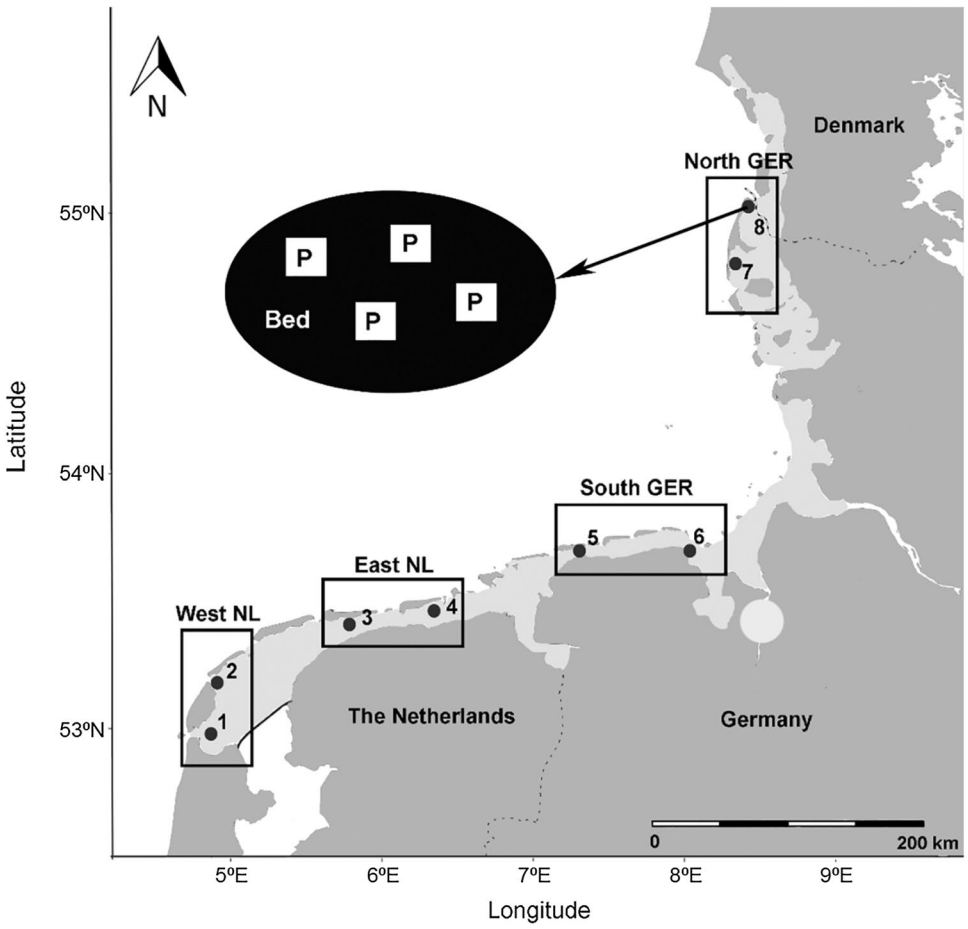

Fig. 1 Map of the eight sampling locations (mixed beds of Pacific oysters *Magallana gigas* and blue mussels *Mytilus edulis*, black dots) in four regions (black rectangles) in the Dutch (NL) and German (GER) Wadden Sea (shaded light grey area; see Online Resource 1 for coordinates and sample dates). On each bed, four plots of 1 m^2 (P; insert upper left) were haphazardly selected from which individual hosts were sampled. In each plot, two cores (not shown) were taken to determine host densities and other parameters (see text for details)

observations: region [$r = 4$], bed nested in region [$b(r) = 2$, $b_{total} = 8$] and plot nested in bed [$p(b) = 4$, $p_{total} = 32$]. In total, 640 individuals of each bivalve species were investigated for parasitic infections.

Dissection procedures for parasite screening

In the laboratory, mussel and oyster shells were opened and inspected from the inside and outside for the presence of *P. ciliata* markings as described in Catherine et al. (1990) and Ambariyanto and Seed (1991). As it was too time-consuming to crack mussel and, especially oyster shells, to find all *Polydora* individuals, we did not obtain *P. ciliata* intensities of both hosts. After shell inspections, host flesh was stored in labelled plastic bags and frozen at − 20 °C until further analysis.

We defrosted mussel and oyster flesh in batches (one species from a plot at a time, $n = 20$) and screened for the presence of endoparasites. As the mussel is host to four different endoparasite species (the copepods *M. orientalis* and *M. intestinalis*, and the trematodes *R. roscovita* and *H. elongata*; Thieltges et al. 2006; Elsner et al. 2011; Pogoda et al. 2012; Brenner et al. 2014; Goedknegt et al. 2017) and the oyster only to one (*M. orientalis*; Elsner et al. 2011; Pogoda et al. 2012; Goedknegt et al. 2017), the dissection procedures differed between the two hosts. Mussel tissue was inspected for adult copepods under a magnification glass (3–8 ×), subsequently squeezed between glass plates and scanned with a stereomicroscope (10–30 ×) for remaining copepod larvae and metacercarial stages of trematodes. For oysters, the digestive tissue was first dissected and inspected for copepods, after which remaining copepods were flushed out of the intestine with water from a squeezing bottle.

Trematode metacercaria were identified according to Werding (1969). The identification of adult *Mytilicola* was based on descriptions of Steuer (1902), Mori (1935), Ho and Kim (1992) and Elsner et al. (2011). However, as morphological species identification is not entirely reliable when both *Mytilicola* species have overlapping host ranges and distributions (Elsner et al. 2011; Goedknegt et al. 2017; Goedknegt et al. 2018b), a subset of *Mytilicola* specimens originating from blue mussels were also molecularly identified to species level to support and improve the morphological identification (see Online Resource 2).

Biological and environmental drivers of parasite infection patterns

Based on existing literature on native parasite–host relationships, we selected a total of 12 potential biological and environmental drivers of parasite infection patterns for our analyses (see Table 1 for a literature overview and Goedknegt et al. 2019 for raw data). Densities of oyster, mussel and the first intermediate snail host (i.e., mature periwinkles *L. littorea* with a shell length of > 14 mm from base to apex) for the trematodes *R. roscovita* and *H. elongata*) were obtained by taking two cores (Ø 19 cm, ± 20 cm deep) per plot. Core contents were sieved and brought to the lab where host numbers were determined. The average of the two cores was used as a measure of host density (m^{-2}) per plot. Host size was defined as the shell length (maximum anteroposterior axis) and measured with Vernier callipers to the nearest mm. To estimate densities of definitive hosts that play a role in the life cycle of trematodes, we used aerial counts (common eider *Somateria mollissima*) and high-tide roost counts (herring gull *Larus argentatus*, common gull *Larus canus*, black-headed gull *Chroicocephalus ridibundus*, oystercatcher *Haematopus ostralegus*) of long-term monitoring programmes from which we calculated the bird densities per intertidal hectare per location (see Waser 2018 and Online Resource 3 for details). Estimates of environmental data, salinity and exposure time, were obtained by means of simulation with the General Estuarine Transport Model (GETM; Burchard and Bolding 2002), which was previously used to simulate the hydrodynamics, temperature and salinity for the entire Wadden Sea (Gräwe et al. 2016). For further details regarding the simulations, we refer to Gräwe et al. (2016) and to Folmer et al. (2016) for post-processing of simulation data.

Statistical analysis

Calculations of infection measures

For each sampled plot and host species, we calculated parasite prevalence (the ratio of infected to sampled host species), intensity (the mean number of parasites per infected host), abundance (the mean number of parasites in all hosts), parasite population density m^{-2} (the product of parasite abundance and host density m^{-2}) and infected host density m^{-2} (the product of prevalence and host density m^{-2}) according to the terminology of Bush et al. (1997). For *P. ciliata* only prevalence and infected host density could be calculated due to missing intensity data. For both *Mytilicola* species, observations included morphologically as well as molecularly identified individuals, although the morphological identification error was relatively small (< 10%; see Online Resource 2). When both identification techniques disagreed on the species identity of an individual copepod, preference was given to the molecular results. Raw data of all parasite infection parameters can be found in Goedknegt et al. (2019).

Spatial infection patterns

We determined how variability in prevalence (modelled as parasite presence/absence) and abundance (numbers

Table 1 List of biological and environmental factors which were investigated as potential drivers of infection levels in the analyses

Factor	Range	Transform.	Parasite species					Hypothesis	References
			MO	MI	PC	HE	RR		
Host density (mussel)	70.5–3721.0 m^{-2}	Log	X	X	X	X	X	Positive	1–6
Host density (oyster)	0–317.4 m^{-2}	None	X	X	X	X	X	Positive (competent host) Negative (non-competent host)	1–6 7, 8
Host size (mussel)	30.0–66.0 mm	None	X	X	X	X	X	Positive	1, 3, 9–12
Host size (oyster)	40.0–228.0 mm	None	X		X			Positive	12
Salinity	22.6–31.7 psu	None	X	X	X	X	X	Positive	13, 14
Tidal exposure	0.08–0.61	None	X	X	X	X	X	Negative	15, 16
Total periwinkle density	0–317.4 m^{-2}	Log + 1			X	X	X	Positive	3
Herring gull density	701.2–6462.7 ha^{-1}	Log				X	X	Positive	4, 17, 18
Common gull density	639.9–7119.0 ha^{-1}	Log				X	X	Positive	17, 18
Black-headed gull density	911.8–5682.6 ha^{-1}	None				X	X	Positive	17, 18
Oyster catcher density	2404.2–11,377.4 ha^{-1}	Log				X	X	Positive	4
Common eider density	252.5–9581.6 ha^{-1}	Log				X	X	Positive	4, 18
Total bird density	7.8 M–29.4 M ha^{-1}	None				X	X	Positive	19

Given are the explanatory factor, its value ranges, the applied transformation, the parasite species for which the factor was included in the analyses and the directional hypothesis (positive or negative effect on the parasite) based on literature references. Abbreviations of parasite species: MO = *Mytilicola orientalis*, MI = *Mytilicola intestinalis*, PC = *Polydora ciliata*, HE = *Himasthla elongata*, RR = *Renicola roscovita*

References: (1) Gee and Davey (1986), (2) Arneberg et al. (1998), (3) Thieltges and Reise (2007), (4) Galaktionov et al. (2015), (5) Stringer and Linklater (2015), (6) Searle et al. (2016), (7) Thieltges et al. (2009), (8) Mordecai (2013), (9) Ambaryianto and Seed (1991), (10) Nikolaev et al. (2006), (11) Stier et al. (2015), (12) Goedknegt et al. (2017), (13) Pietrock and Marcogliese (2003), (14) Bolster (1954), (15) Fingerut et al. (2003), (16) Wilson et al. (2011), (17) Werding (1969), (18) Galaktionov and Bustnes (1999) and (19) Hechinger and Lafferty (2005)

of parasites in individual hosts) in mussels and oysters depended on spatial scale by using (intercept only) general linear mixed models (GLMMs) following binomial distributions for prevalence data (package lme4, Bates et al. 2015) and negative binomial distributions for abundance data (package glmmADMB; Fournier et al. 2012; Skaug et al. 2014) in the statistical software environment R (R Development Core Team 2015). We did not use intensity, as this measure of infection can only be obtained from infected hosts, which would have resulted in heavily unbalanced datasets. In the GLMMs we considered plots to be nested within beds, beds nested within region, and regions as random effects and calculated the relative variance components for each of these spatial levels. For parasites infecting both host species (*M. orientalis* and *P. ciliata*), we used similar GLMMs including host species as fixed effect and compared the results with GLMMs without this fixed term using likelihood ratio tests following Chi-square distributions. To evaluate potential co-occurrences of parasites in each host species and on the smallest spatial scale (plot level), we used pairplots and performed nMDS analyses using the vegan package (Oksanen et al. 2019).

Predictors of infection levels

Density of the invasive host (Pacific oysters), density of the native host (blue mussels), mussel host size, tidal exposure time (i.e., the mean fraction of time that the seabed is exposed to the air) and salinity (psu) were included as explanatory variables in all parasite models. We did not include temperature as the range of average summer temperatures (Jun–Sept over the years 2007–2011) in the Wadden Sea was too small to detect potential effects (16.0–16.5 °C; E. Folmer, pers. comm.). For *M. orientalis* and *P. ciliata* which also infect oysters, we additionally included oyster host size in the models. Furthermore, for *P. ciliata*, we included the density of the common periwinkle *L. littorea*, which serves as an alternative host for this parasite species. Finally, for trematodes with complex life cycles (*H. elongata* and *R. roscovita*), the density of the first intermediate host, the common periwinkle *L. littorea*, and of definitive hosts (several bird species; see Table 1) were included.

Prior to the analyses, we inspected all biological and environmental factors for skewed distributions and applied $\log_{10}$-transformations to linearise relationships when

necessary. Additionally, we examined collinearity with pair plots including Pearson correlations (Online Resource 4). We conducted a series of nested GLMMs for each parasite–host species combination, including an intercept-only model (null model), to examine the effect of biological and environmental factors on prevalence (parasite presence-absence) and abundance (number of parasites per individual host). In all models, the number of explanatory variables was kept to a minimum by including at most a single explanatory variable as fixed effect in the model. Consequently, each individual GLMM included parasite prevalence or abundance as response variable, none or one individual driver as explanatory variable and the hierarchical sampling structure as random effect. Competing models were compared based on the Akaike information criterion corrected for sample sizes (AICc) and the model with the lowest AICc score was selected as the best driver model. Then, we produced a suite of models with two fixed effects that contained the fixed effect of the top performing model plus each of the other explanatory variables in turn. Again, the best performing model was chosen based on the lowest AICc and the forward selection procedure was terminated at this point to avoid overfitting of the data. Finally, we estimated the Akaike weights of all models tested per parasite–host combination (MuMIn package; Barton 2018) to facilitate the interpretation of the AIC model comparisons.

Results

Spatial distribution of host and parasite species

Invasive Pacific oysters (mean shell length ± SE, 128.5 ± 1.5 mm) and native blue mussels (45.2 ± 0.25 mm) were present at all sampled beds in the Wadden Sea. In all beds mussel densities (mean ± SE; 1140.8 ± 121.4 m^{-2}) were higher than oyster densities (139.4 ± 11.7 m^{-2}; Online Resource 5). In addition, all targeted parasite species were found at all

Table 2 Prevalence (%), intensity and abundance (±SE) of the five parasite species in native blue mussels (*Mytilus edulis*) and invasive Pacific oysters (*Magallana gigas*) sampled at eight locations in the Wadden Sea (for map of locations see Fig. 1)

Location	Variable	*M. orientalis*		*P. ciliata*		*M. intestinalis*	*H. elongata*	*R. roscovita*
		Oysters	*Mussels*	*Oysters*	*Mussels*	*Mussels*	*Mussels*	*Mussels*
1	Prevalence	33.8 ± 5.9	58.8 ± 10.3	27.5 ± 8.3	–	8.8 ± 4.3	25.0 ± 3.5	88.8 ± 3.1
	Intensity	4.5 ± 0.9	2.7 ± 0.1	–	–	1.0 ± 0.0	1.5 ± 0.1	9.6 ± 1.8
	Abundance	1.5 ± 0.4	1.6 ± 0.4	–	–	0.1 ± 0.0	0.4 ± 0.1	8.6 ± 1.9
2	Prevalence	25.0 ± 5.4	46.3 ± 6.6	98.8 ± 1.3	0.1 ± 0.0	12.5 ± 3.2	56.3 ± 17.1	86.3 ± 7.7
	Intensity	3.4 ± 0.7	1.9 ± 0.2	–	–	1.1 ± 0.1	2.9 ± 0.8	25.9 ± 12.4
	Abundance	1.0 ± 0.4	0.9 ± 0.1	–	–	0.1 ± 0.0	1.9 ± 1.0	24.8 ± 12.3
3	Prevalence	16.3 ± 2.5	62.5 ± 6.5	83.8 ± 14.4	11.3 ± 8.0	53.8 ± 5.2	85.0 ± 15.0	100.0 ± 0.0
	Intensity	6.8 ± 1.1	4.1 ± 0.3	–	–	1.6 ± 0.2	37.3 ± 12.2	82.3 ± 20.7
	Abundance	1.1 ± 0.3	2.6 ± 0.2	–	–	0.9 ± 0.1	36.0 ± 13.2	82.3 ± 20.7
4	Prevalence	17.5 ± 10.4	73.8 ± 11.1	–	6.3 ± 2.4	27.5 ± 9.2	37.5 ± 7.8	97.5 ± 2.5
	Intensity	9.9 ± 4.3	4.7 ± 0.4	–	–	1.2 ± 0.1	1.7 ± 0.2	35.7 ± 7.9
	Abundance	1.7 ± 0.8	3.5 ± 0.5	–	–	0.4 ± 0.2	0.6 ± 0.1	35.0 ± 8.2
5	Prevalence	21.3 ± 7.5	62.5 ± 8.5	11.3 ± 2.4	6.3 ± 2.4	66.3 ± 6.6	16.3 ± 3.8	76.2 ± 6.9
	Intensity	9.8 ± 3.5	3.0 ± 0.5	–	–	2.5 ± 0.2	5.0 ± 1.8	3.9 ± 0.5
	Abundance	2.3 ± 1.0	2.0 ± 0.5	–	–	1.7 ± 0.3	0.8 ± 0.4	3.0 ± 0.4
6	Prevalence	42.5 ± 11.1	41.3 ± 7.5	32.5 ± 10.1	21.3 ± 5.9	22.5 ± 6.6	66.3 ± 9.9	93.8 ± 2.4
	Intensity	4.4 ± 0.5	1.8 ± 0.2	–	–	1.2 ± 0.1	3.2 ± 0.9	31.1 ± 2.5
	Abundance	2.0 ± 0.6	0.8 ± 0.2	–	–	0.3 ± 0.1	2.0 ± 0.4	29.2 ± 2.5
7	Prevalence	17.5 ± 11.9	60.0 ± 11.5	23.8 ± 14.6	11.3 ± 8.3	93.8 ± 2.4	100.0 ± 0.0	100.0 ± 0.00
	Intensity	4.5 ± 1.6	2.7 ± 0.4	–	–	4.0 ± 0.3	44.7 ± 6.5	325.7 ± 48.3
	Abundance	0.8 ± 0.4	1.6 ± 0.3			3.7 ± 0.3	44.7 ± 6.5	325.7 ± 48.3
8	Prevalence	0	1.3 ± 1.3	97.5 ± 1.4	2.5 ± 2.5	78.1 ± 10.3	60.7 ± 13.6	98.8 ± 0.0
	Intensity	–	1.0 ± 0.0	–	–	3.1 ± 0.2	3.39 ± 0.90	178.9 ± 29.4
	Abundance	–	0.0 ± 0.0	–	–	2.5 ± 0.4	2.4 ± 1.0	176.7 ± 29.8

The parasites play a role in parasite spillover (*Mytilicola orientalis*), spillback (*Polydora ciliata*) and transmission interference (*Mytilicola intestinalis*, *Himasthla elongata* and *Renicola roscovita*) induced by invasive oysters. Intensity data of *P. ciliata*, and prevalences of *P. ciliata* in mussels (location 1) and oysters (location 4) were not obtained

locations, although not in each host species at every single location (Table 2). Native blue mussels were infected with five parasite species (the copepods *M. orientalis* and *M. intestinalis*, the shell-boring polychaete *P. ciliata,* and trematodes *R. roscovita* and *H. elongata*) with an overall prevalence of 98.4%, while invasive Pacific oysters were only infected with the invasive *M. orientalis* and the native *P. ciliata,* with a total prevalence of 59.8%. Few parasite species tended to co-occur, as was particularly the case for the trematodes *H. elongata* and *R. roscovita* in mussels (Online Resources 6b, 7b, 8a and 8c).

Some parasite species showed a strong regional pattern in their distribution (*M. intestinalis* and *R. roscovita*, for which the abundances also highly correlated (Online Resource 8c)), while for other species (*H. elongata*; *M. orientalis* in mussels and oysters; *P. ciliata* in oysters) spatial heterogeneity was high on a more local (bed) level or even on the smallest scale within beds (*P. ciliata* in mussels) as indicated by the variance component analyses (Table 3).

Relative contribution of invasive oyster density to infection patterns in native mussels

Pacific oyster density was the factor giving the best fit for *M. intestinalis* and *R. roscovita* prevalence (Table 4) and *M. intestinalis* abundance (Table 5) in mussels. In the parasitic copepod *M. intestinalis,* prevalence and abundance were negatively affected by the density of Pacific oysters. The prevalence of the trematode *R. roscovita* in mussels increased with oyster density. Oyster density, however, did not come out in the best fitting models of the other three parasite species.

Regarding other factors driving infection levels in native mussels, host size resulted in five models as the best explanatory factor driving parasite prevalence and abundance (Tables 4, 5). Host size was an important factor determining the prevalence of the shell-boring polychaete *P. ciliata*, the prevalence and abundance of the trematode *H. elongata*, of abundance of the copepod *M. intestinalis* and of the trematode *R. roscovita*. For the two trematode species, the density of definitive hosts turned out as an additional explanatory factor of infection levels, in particular the density of common gulls (*L. canus*) for the prevalence of *R. roscovita* and the density of eider ducks (*S. mollissima*) for the prevalence of *H. elongata* (Table 4). However, this pattern was not observed when looking at trematode abundance (Table 5). Instead, the density of first intermediate host species, of the snail *L. littorea*, was identified as one of the best factors driving *H. elongata* abundances in mussels and the density of second intermediate host species (of the mussel *M. edulis*) for abundances in mussels (Table 5).

Furthermore, the prevalence of *P. ciliata* in mussels was negatively affected by the density of the common periwinkle *L. littorea,* which represents an alternative host species for this shell-boring polychaete. For *M. orientalis* in mussels, none of the prevalence and abundance models including biological and/or environmental factors was better than the null model (Tables 4, 5).

Looking at infection levels in Pacific oysters, oyster size had a positive effect on *P. ciliata* prevalence. In addition, prevalence and abundance of *M. orientalis* in oysters were affected by the environmental factors tidal exposure and salinity (Tables 4, 5).

Importance of oyster hosts for parasite species shared with mussels

Invasive oysters shared two parasite species with native mussels, the invasive copepod *M. orientalis* via spillover effects and the native polychaete *P. ciliata* via spillback processes. Interestingly, for each parasite species there was a clear co-occurrence in mussels and oyster hosts on the plot level (Online Resource 7). However, *M. orientalis* was more often present in mussel than in oyster hosts ($\Delta_{Dev.} = 130.59$, $p < 0.001$), with prevalences being, on average, twice as high in mussels (average ± SD, 50.8 ± 0.2%) compared to

Table 3 Results of the variance component analyses, with the parasite species, host species and the variance components (% variance) per spatial scale (region, bed or plot)

Parasite species	Host species	Region		Bed		Plot	
		Prev.	Abund.	Prev.	Abund.	Prev.	Abund.
Mytilicola orientalis	Oyster	18.0	16.1	67.1	75.9	14.9	8.0
	Mussel	0	0.1	94.3	97.9	5.7	2.0
Polydora ciliata	Oyster	0	–	84.3	–	15.7	–
	Mussel	0	–	3.3	–	96.7	–
Mytilicola intestinalis	Mussel	62.8	63.4	26.9	29.6	10.3	7.0
Himasthla elongata	Mussel	0	0	74.2	79.0	25.8	21.0
Renicola roscovita	Mussel	49.5	56.0	23.0	29.6	27.5	14.4

Variance components were calculated from variances of (intercept only) GLMMs using parasite prevalence (Prev., modelled as presence-absence) and abundance (Abund.) as response variable, and the nested spatial sampling structure as random effect

Table 4 Overview of the best one- and two-factor models explaining parasite prevalences of the five parasite species *Mytilicola orientalis*, *Polydora ciliata*, *Mytilicola intestinalis*, *Himasthla elongata* and *Renicola roscovita* infecting native blue mussels (*Mytilus edulis*) and the two parasite species (*M. orientalis* and *P. ciliata*) also infecting invasive Pacific oysters (*Magallana gigas*)

Parasite sp.	Host sp.	Model	Variable	Estimate (β) ± SE	AICc	ω
M. orientalis	*M. edulis*	**Null**	Intercept	− 0.226 ± 0.770	**790.9**	**0.212**
		1-Factor	Intercept	5.210 ± 4.360	791.5	0.154
			Salinity	− 0.186 ± 0.151		
		2-Factor	Intercept	4.296 ± 4.558	792.7	0.088
			Salinity	− 0.195 ± 0.155		
			Mussel density	0.408 ± 0.421		
	M. gigas	Null	Intercept	− 1.535 ± 0.770	639.6	0.012
		1-Factor	Intercept	− 2.654 ± 0.634	637.3	0.039
			Exposure	3.958 ± 1.852		
		2-Factor	Intercept	2.405 ± 1.277	**631.0**	**0.884**
			Exposure	0.484 ± 0.915		
			Salinity	− 0.184 ± 0.050		
P. ciliata	*M. edulis*	Null	Intercept	− 2.584 ± 0.320	307.1	0
		1-Factor	Intercept	− 10.721 ± 2.056	284.9	0.047
			Host size	0.168 ± 0.039		
		2-Factor	Intercept	− 8.805 ± 1.875	**279.8**	**0.634**
			Host size	0.160 ± 0.037		
			Snail density	− 0.963 ± 0.354		
	M. gigas	Null	Intercept	0.982 ± 1.143	357.4	0
		1-Factor	Intercept	− 2.167 ± 1.366	**344.5**	**0.261**
			Host size	0.024 ± 0.006		
		2-Factor	Intercept	0.530 ± 2.297	344.9	0.218
			Host size	0.023 ± 0.006		
			Exposure	− 7.851 ± 5.605		
M. intestinalis	*M. edulis*	Null	Intercept	− 0.226 ± 0.770	654.2	0.055
		1-Factor	Intercept	0.526 ± 0.822	**651.3**	**0.231**
			Oyster density	− 0.005 ± 0.002		
		2-Factor	Intercept	1.806 ± 1.192	651.4	0.220
			Oyster density	− 0.005 ± 0.002		
			Exposure	− 4.797 ± 0.345		
H. elongata	*M. edulis*	Null	Intercept	0.796 ± 0.782	643.6	0.006
		1-Factor	Intercept	− 2.171 ± 1.278	637.8	0.112
			Host size	0.066 ± 0.023		
		2-Factor	Intercept	− 9.337 ± 3.832	**636.6**	**0.204**
			Host size	0.066 ± 0.023		
			Eider duck density	2.243 ± 1.154		
R. roscovita	*M. edulis*	Null	Intercept	3.460 ± 0.763	301.6	0.023
		1-Factor	Intercept	2.413 ± 0.860	298.5	0.109
			Oyster density	0.007 ± 0.003		
		2-Factor	Intercept	− 4.944 ± 4.936	**298.2**	**0.129**
			Oyster density	0.007 ± 0.003		
			Common gull density	2.341 ± 1.571		

Best models (in bold) were selected based on their lowest AICc scores and highest Akaike's weights (ω; also in bold)

oysters (21.7 ± 0.2%). On the other hand, when oysters were infected with *M. orientalis*, overall intensities were twice as high (average ± SD, 6.2 ± 4.7) than when mussels (2.9 ± 1.2) were infected ($\Delta_{Dev.} = 78.96$, $p < 0.001$). In addition, the maximum intensity of *M. orientalis* found in oysters was 75, while in mussels a maximum of 11 copepods was found in one individual host. These contradicting patterns resulted in almost similar parasite abundances for mussel (average ± SD, 1.6 ± 1.2) and oyster (1.3 ± 1.2) hosts ($\Delta_{Dev.} = 3.12$, $p = 0.077$). However, as mussels occurred in generally higher

Table 5 Overview of the best one- and two-factor models explaining parasite abundance of the four parasite species *Mytilicola orientalis*, *Mytilicola intestinalis*, *Himasthla elongata* and *Renicola roscovita* infecting native blue mussels (*Mytilus edulis*) and the parasite species *M. orientalis* also infecting invasive Pacific oysters (*Magallana gigas*)

Parasite sp.	Host sp.	Model	Variable	Estimate (β) ± SE	AICc	ω
M. orientalis	*M. edulis*	**Null**	Intercept	−0.029 ± 0.513	**2037.3**	**0.217**
		1-Factor	Intercept	5.762 ± 4.494	2037.9	0.101
			Salinity	−0.201 ± 0.156		
		2-Factor	Intercept	5.416 ± 4.433	2039.3	0.081
			Salinity	−0.202 ± 0.153		
			Host size	−0.009 ± 0.011		
	M. gigas	Null	Intercept	−0.227 ± 0.586	1417.7	0.125
		1-Factor	Intercept	−1.510 ± 1.080	1417.8	0.123
			Exposure	4.440 ± 3.140		
		2-Factor	Intercept	6.933 ± 3.747	**1415.6**	**0.369**
			Exposure	6.415 ± 2.541		
			Salinity	−0.314 ± 0.144		
M. intestinalis	*M. edulis*	Null	Intercept	−0.544 ± 0.596	1555.4	0.018
		1-Factor	Intercept	−1.422 ± 0.712	1551.9	0.102
			Host size	0.019 ± 0.008		
		2-Factor	Intercept	−0.888 ± 0.724	**1549.1**	**0.431**
			Host size	0.018 ± 0.008		
			Oyster density	−0.004 ± 0.002		
H. elongata	*M. edulis*	Null	Intercept	0.781 ± 0.591	2877.0	0
		1-Factor	Intercept	−0.666 ± 0.812	2873.0	0
		2-Factor	Intercept	−4.802 ± 0.919	**2795.2**	**1**
			Snail density	0.814 ± 0.352		
			Host size	0.090 ± 0.010		
R. roscovita	*M. edulis*	Null	Intercept	3.687 ± 0.717	5854.7	0
		1-Factor	Intercept	1.090 ± 0.798	5828.0	0.015
			Host size	0.046 ± 0.011		
			Snail density	0.841 ± 0.323		
		2-Factor	Intercept	−3.607 ± 1.548	**5819.9**	**0.845**
			Host size	0.054 ± 0.009		
			Mussel density	1.604 ± 0.426		

Abundance data were not available for the parasite *Polydora ciliata*. Best models (in bold) were selected based on their lowest AICc scores and highest Akaike's weights (ω; also in bold)

densities than oysters (Online Resource 5), the density of infected hosts was at all locations larger in mussels than in oysters (Fig. 2a). Consequently, *M. orientalis* population densities in the Wadden Sea were much higher in native mussel compared to invasive oyster hosts (Fig. 3). More specifically, at locations where *M. orientalis* was abundant (at all locations, except for location 8), parasite population densities were 2–35 times larger in native mussels than in invasive oysters (Fig. 3).

For the native polychaete *P. ciliata,* the importance of oyster hosts for the parasite population density was less clear. Although prevalences of the polychaete were five times higher in oysters (average ± SD, 57.9 ± 0.4%) than in mussels (10.2 ± 0.11%; $\Delta_{Dev.} = 323.94$, $p < 0.001$), this difference was buffered by the high population density of mussels, resulting in a lack of an overall pattern in the density of infected hosts (Fig. 2b). At some locations the density of infected mussels was higher than infected oysters (locations 5 and 6), while at other locations this pattern was reversed (locations 2 and 8) or densities of infected hosts were similar (locations 3 and 7; Fig. 2b).

Discussion

Effects of non-native oyster density

Contrary to expectation, Pacific oyster density was not included in most of the best models explaining parasite prevalence or abundance in native mussels. Oyster density only explained prevalence and abundance of the copepod *M. intestinalis* and prevalence of the trematode *R. roscovita*. In the case of *M. intestinalis,* oyster density negatively affected the prevalence and abundance of the parasite in

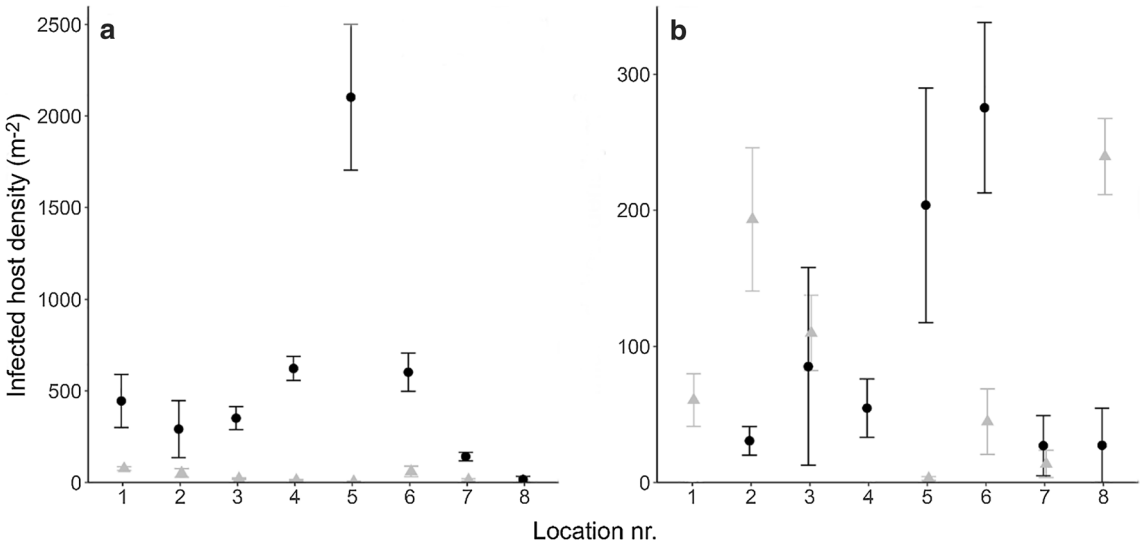

Fig. 2 Mean density m^{-2} (±SE) of infected blue mussel (*Mytilus edulis*; black dots; $N=28$ plots) and Pacific oyster (*Magallana gigas*; grey triangles; $N=28$ plots) hosts for the parasites **a** *Mytilicola orientalis* and **b** *Polydora ciliata*, the two parasite species infecting both host species. Infected host density of *P. ciliata* at location 1 (for mussels) and location 4 (for oysters) are missing because of the lack of prevalence data at these locations. Numbering of locations is as in Fig. 1

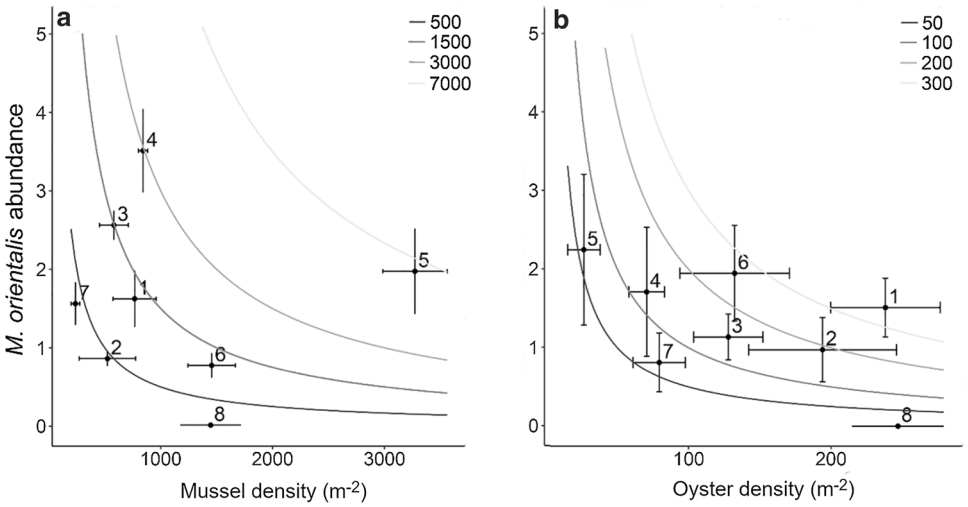

Fig. 3 Mean abundance (±SE) of the invasive parasite *Mytilicola orientalis* versus the mean density (±SE) of **a** blue mussels (*Mytilus edulis*; $N=32$ plots) and **b** Pacific oysters (*Magallana gigas*; $N=32$ plots) at each of the eight sampled bivalve beds (indicated by black numbering, numbering of locations as in Fig. 1). Grey-scaled isoclines represent parasite population densities m^{-2}

native mussels. Previous studies have not reported *M. intestinalis* in invasive oysters (Elsner et al. 2011; Goedknegt et al. 2017) and controlled infections were not successful (Elsner et al. 2011; M; Feis pers. comm.), suggesting that the Pacific oyster is not a competent host for *M. intestinalis*. Therefore, oysters may act as a sink (Elsner et al. 2011; Goedknegt et al. 2017) but the exact mechanism is yet unknown. In contrast to the negative effects on parasitic copepods, oyster density had a positive effect on *R. roscovita* prevalence in native mussels. This was not anticipated given the known negative effects of oysters on trematode infective stages via transmission interference (Thieltges et al. 2008, 2009; Goedknegt et al. 2015). The obvious explanation that oyster density could positively correlate with the densities of the first intermediate snail host of the parasite does not hold true, as exploratory investigations prior to the statistical analyses could not find any correlations between both variables. Alternatively, oysters may affect *R. roscovita* infection levels in mussels via the three-dimensional matrix structure they create. Most mussels are found deep in the oyster matrix where they gain protection from predation and detrimental barnacle epibionts (Eschweiler and Christensen 2011; Buschbaum et al. 2016). Experimental studies indicate that this position of mussels inside the matrix leads to higher prevalence and intensities of *R. roscovita* in mussels compared to conspecifics positioned on top of the matrix (Goedknegt 2017). Possibly, at the bottom of the oyster matrix, first intermediate snail hosts locally produce infective *R.*

roscovita stages which are concentrated and trapped by the oyster structure (Goedknegt 2017). With increasing oyster density, the structural complexity will also increase and likely result in the observed positive effect of oyster density on infection levels in mussels.

Effects of host size

The lack of oyster density in the best models explaining infection patterns of *M. orientalis*, *P. ciliata* and *H. elongata* in native mussels suggests that the dynamics of many parasite species are rather driven by other biotic and environmental drivers than the density of the invasive species. According to the best models identified in the GLMMs, host size was one of these factors as mussel size was an important positive driver of *P. ciliata* and *H. elongata* prevalence, and of *M. intestinalis*, *H. elongata* and *R. roscovita* abundance, which was expected according to our hypothesis. A positive relationship between host size and infection levels could reflect a relationship with host age, with older hosts accumulating more infections over time, which has been previously suggested for *P. ciliata* infecting mussels and periwinkles (Ambaryianto and Seed 1991; Warner 1997) and *H. elongata* infecting mussels (Nikolaev et al. 2006). However, the positive effect of host size does not necessarily have to be age-related but can also correspond with the larger shell surface area that is available for *P. ciliata* infection and enhanced filtration currents exerted by larger molluscs, enabling these individuals to inhale larger quantities of free-living infective stages of endoparasites resulting in higher infection levels (Nikolaev et al. 2006). Furthermore, the low number of smaller mussels with *Polydora* markings may be explained by the higher vulnerability of smaller, infected mussels to crab predation (Ambaryianto and Seed 1991) as has previously been shown for periwinkles *L. littorea* (Buschbaum et al. 2007).

Effects of alternative and obligatory host density

In addition to host size, the density of alternative hosts or obligatory hosts required to complete a life cycle, turned out to be important biological variables determining infection levels in mussels. For example, for *P. ciliata* which infects mussels and oysters, native periwinkles (*L. littorea*) are an important alternative host and therefore it is not surprising that snail density also showed to be an important factor negatively affecting *Polydora* infections in mussels. When more periwinkles are present, parasite prevalence in mussels decreases, suggesting that periwinkles are probably a more important host than native mussels. As this effect was not observed for *P. ciliata* in oysters, periwinkles are probably not dominant over the invasive host species, but more experimental work needs to be conducted to test what the exact host preference of the parasite actually is. For the trematodes *H. elongata* and *R. roscovita* with complex life cycles, densities of upstream and downstream hosts in the life cycle were identified as important determinants of infection levels in mussels. Densities of the definitive bird host, more specifically eider ducks (*S. mollissima*) and common gulls (*L. canus*), were, respectively, driving *H. elongata* and *R. roscovita* prevalences. Gull density was also found to be a driving factor of *R. roscovita* prevalence and intensity in blue mussels in the Arctic (Galaktionov et al. 2015). Furthermore, the density of the first intermediate snail host, *L. littorea*, was identified to be an important factor to determine *H. elongata* abundances in the mussel host, while for *R. roscovita* the density of the mussel host itself was positively affecting abundances of this parasite. The importance of obligatory hosts as driving factors of trematode infection levels is not surprising, as trematode species require the presence of all three hosts to complete their life cycle (Werding 1969).

Importance of environmental factors

Regarding environmental factors, tidal exposure and salinity only appeared in the best fitting model of *M. orientalis* infecting oysters. Exposure time positively affected *M. orientalis* prevalences in oysters. This was surprising, as an inverse relationship between the degree of exposure and infection rates has previously been found for *M. intestinalis* in mussels, which was attributed to the shorter submersion time of hosts in the water, limiting the time window of free-swimming infective copepodid larvae to locate and infect their host (Bolster 1954; Davey and Gee 1976). On the reasons behind the positive effect of exposure time on *M. orientalis* infection levels in oysters we can only speculate. For example, less submersion time means less exposure to currents directing the larvae away from their hosts, potentially explaining the effect found. Alternatively, mussels higher on the mudflat might be present in higher densities, presenting a source of copepodid larvae to the oysters. The negative effect of salinity confirms earlier findings from the North Pacific where higher prevalences were reported from mussels (*Mytilus trossulus*) situated in sheltered estuarine areas compared to mussels at exposed coastal shores (Goater and Weber 1996). The congeneric species *M. intestinalis* also prefers reduced salinities (Korringa 1968), but salinity was not an important driver of *M. intestinalis* prevalences and abundances in mussels, suggesting that the invasive *M. orientalis* may be more sensitive to salinity changes than *M. intestinalis*.

Potential other factors

Although we have assessed 12 biological and environmental parameters in this study, additional factors could play a role

in determining parasite infection patterns. Among these factors is local water flow velocity. For example, higher parasite prevalences were reported from mussels (*M. trossulus*) situated in sheltered estuarine areas compared to mussels at exposed coastal shores (Goater and Weber 1996). Another possible variable driving infection levels is the ambient fauna as it can play a role in transmission interference, as some species prey upon free-living stages of parasites. For example, crabs, shrimps and barnacles can reduce the number of trematode infective stages in the water column (Welsh et al. 2014). In addition, sea weeds can physically prevent parasite larvae to infect the host (Welsh et al. 2014). Finally, parasite species already infecting hosts could either prevent the establishment of novel parasite species by occupying infection space or, vice versa, make the host more susceptible to novel infections via detrimental effects on the host. The observed co-occurrence of *H. elongata* and *R. roscovita* could be an example of the latter, although the exact mechanism needs to be fully explored.

Relative importance of mussel and oyster hosts

For parasites infecting both invasive oysters and native mussels (the copepod *M. orientalis* and the polychaete *P. ciliata*) Pacific oysters were expected to be an important determinant in the distribution of both parasite populations. Indeed, each parasite species tended to co-occur in oyster and mussel hosts on the smallest spatial scale. However, in both cases, oyster density did not affect prevalence or abundance in native mussels. In addition, the calculations of parasite population densities in the two host species indicated that the oyster as host species might not be as important as previously thought.

At all locations where *M. orientalis* occurred, mean prevalences were always higher in mussels but the mean and maximum intensity was higher in oysters. The latter is likely caused by the larger digestive system of oysters, providing the intestinal parasite with ample space for multiple infections, whereas intensities in mussels are limited by mussel size (Goedknegt et al. 2017). Differences in the relative prevalence and intensity of the invasive copepod lead to almost similar abundances of *M. orientalis* in both host species. Nevertheless, when host density was taken into account, the newly acquired native mussel host appeared to carry the majority of the *M. orientalis* population in the Wadden Sea. For *P. ciliata*, the role of oysters for the total parasite population is less clear. The native shell-boring polychaete occurred in native blue mussels and invasive Pacific oysters at all sampled locations across the Wadden Sea. Since its introduction in the 1980s/1990s (Reise 1998; Drinkwaard 1999; Troost 2010), invasive oysters became an important host for this native shell-boring polychaete species with average prevalences at present being five times higher in invasive oysters compared to native mussels. However, when host density was considered, the share of infected hosts was often still higher in blue mussels relative to oysters. As the lack of a protocol limited us to acquire information on *P. ciliata* intensities, we do not know how these differences in prevalence relate to relative *P. ciliata* abundances in both host species, limiting our knowledge on host specific parasite population sizes. Therefore, whether this high competence of invasive oysters results in amplification of infection levels in native mussels (parasite spillback sensu Kelly et al. 2009) is a topic for further studies. In addition, without intensity information, the potential effects of the parasite on host populations remain to be assessed. The polychaete burrows in mollusc shells, causing reductions in the shell strength and condition (Kent 1979, 1981; Buschbaum et al. 2007) and makes infected hosts more vulnerable to crab predation (Ambaryianto and Seed 1991).

Conclusions

In this study, we have shown that invasive Pacific oysters can contribute to the distribution and abundance of parasite infections in native mussels. However, we could not identify invasive oysters as a universal driver of patterns in parasite infections of native mussels nor as the dominant host for populations of parasites infecting both native mussels and invasive oysters in the invaded region. For the two parasite species that were affected by oysters (*M. intestinalis* negatively and *R. roscovita* positively), oysters did not act as a host species, but influenced parasite populations by a more indirect way (i.e., via the filtering of infective stages or habitat effects). For the other parasite species, infections were further mediated by other biotic and environmental factors, limiting the role of oysters in determining infection levels of those parasites. This also seems to be the case in the two parasite species (*M. orientalis* and *P. ciliata*) infecting both oysters and mussels where parasite densities were mostly higher in the native mussels, suggesting a dominant role of the native species for the parasite populations of those species. The results of this case study demonstrate the usefulness of large-scale field studies in identifying the mechanisms underlying the impacts of invasive species on native parasite–host communities.

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Author contribution statement MAG, DWT and JVDM conceived and designed the study. MAG, RN, MM, CB and KMW conducted fieldwork. MAG, RN and MM performed parasite dissections. PCL conducted the molecular identification. EOF and AMW compiled data on biotic and environmental variables. MAG conducted the statistical analyses with input from JVDM. MAG and DWT wrote the manuscript with significant contributions of all other authors.

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