Parasite patterns in invasive oysters and native mussel hosts – Are parasite spillover, spillback and transmission interference the underlying processes?


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Abstract

Species invasions do not only impact native ecosystems by competition and predation, but can also alter parasite-host interactions. Alien species can co-introduce parasites that spill over to native host species (parasite spillover), be competent hosts for native parasites amplifying parasite populations (parasite spillback) or interfere with native parasite transmission as non-hosts (transmission interference), affecting the disease risk for native host species. In this study, we investigated the effects of the invasion of Pacific oysters (Crassostrea gigas) on the distribution and abundance of parasites in native mussel (Mytilus edulis) and invasive oyster hosts across a large coastal ecosystem, the European Wadden Sea. Using a hierarchical sampling design with three spatial scales (region, mussel/oyster bed, plot) we found strong differences in infection levels of five invasive or native macroparasite species associated with spillover, spillback and transmission interference across the Wadden Sea, suggesting the associated impacts on native hosts to spatially vary accordingly. The variability in parasite occurrence and abundance differed among spatial scales and was generally parasite and host specific, indicating different underlying processes. This was supported by mixed effects models which identified different biological and environmental drivers of parasite occurrence and abundance among parasite and host species, with only host length, mussel abundance and macroalgal Fucus vesiculosus cover on beds contributing to several of the best models. Interestingly, oyster density was only included in 2 out of 12 models suggesting only a minor effect of invasive oysters on current infection patterns. Our study shows that patterns and processes of parasite spillover, spillback and transmission interference as the result of human-mediated host introductions depend on spatial scale, and the parasite and host species involved, which has implications for the assessment of the associated impacts on invaded ecosystems.
Introduction

Over the last decades, global trade and connectivity 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). Besides the well documented impacts on competition with and predation on native species, it is increasingly recognised that species introductions can also alter parasite-host interactions in invaded ecosystems in manifold ways. For example, with many introduced 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), potentially leading to emerging diseases (Daszak et al., 2000) and/or mass mortalities of native populations (reviewed by Goedknegt et al., 2016). In turn, native parasites might infect introduced host species in their new range which may alter the disease risk for native species if competent introduced 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, introduced 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 (Keising et al., 2006).

While these invasion-driven alterations of parasite-host interactions have recently been well conceptualised, studies investigating the effects of host invasions on the actual distribution and abundance of invasive and native parasites in invaded ecosystems are still rare. parasite spillover, spillback and transmission interference can simultaneously occur in a single invasion process (Goedknegt et al., 2016), but their effects may spatially vary causing geographical heterogeneity in parasite distributions with important implications in regard to their impact on native ecosystems. While such spatial heterogeneities have been frequently observed in native parasite-host relationships (Thieltges and Reise, 2007; Wilson et al., 2011; Byers et al., 2008, Galaktionov et al., 2015; Stringer and Linklater, 2015), similar studies on infection patterns resulting from host and parasite introductions are largely missing. A broad range of spatial variability in infections can be captured by assessments on large, nested spatial scales, as previously shown for epidemiological and ecological studies (i.e. Jackson et al., 2006; Werneck et al., 2007; Byers et al., 2008). Such hierarchical sampling schemes allow to identify and compare spatial patterns of infections in introduced and native hosts on a variety of spatial scales, which is pivotal information for the assessment of the associated impacts on invaded ecosystems.

Large-scale hierarchical sampling schemes also allow to investigate the processes driving patterns in the distribution and abundance of parasites in introduced and native hosts, as drivers of parasite infection levels usually operate simultaneously at several spatial levels (Werneck et al., 2007). From existing studies on native parasite-host relationships and on parasite spillover, spillback and transmission interference several potential drivers of infection patterns can be identified. Host competence is the driving factor of parasite spillover, spillback and transmission interference (Kelly et al., 2009; Poulin, 2011; Telfer and Brown, 2012) and the availability of (non) competent hosts can be expected to determine infection levels of native and invasive parasite species. In addition to host competence, host population densities affect parasite infection levels across a wide range of parasite and host taxa (Arneberg, 1998; Thieltges and Reise, 2007; Galaktionov et al., 2015; Stringer and Linklater, 2015; Searle et al., 2016), and for parasite
Spatial patterns and processes of parasite infections

dynamics may be as important as host competence (Paterson et al., 2011; Searle et al., 2016). In addition, host density can be used to estimate parasite densities (i.e. numbers of individuals per unit surface; Bush et al., 1997), allowing to evaluate the share of two competent host species in the total parasite population (e.g. in spillover and spillback scenarios). Other factors driving the parasite distribution and abundance in introduced and native hosts may be host size (Mouritsen et al., 2003; Thieltges and Reise, 2007), the supply of free-living infective larvae (often approximated via upstream host densities for parasites with complex life cycles; Byers et al., 2008; Wilson et al., 2011; Galaktionov et al., 2015), the presence of other species interfering with parasite transmission in the vicinity (e.g. macroalgae laying on top of the bivalves; Johnson and Thieltges, 2010; Welsh et al., 2014), and environmental variables such as temperature, salinity and tidal exposure (Fingerut et al., 2003; Pietrock and Marcogliese, 2003; Poulin, 2006 b). Of all these potential drivers of patterns in parasite distribution and abundance, the influence of biological factors may be more local, while environmental drivers have been suggested to act on larger regional scales (Thieltges, 2009b; Byers et al., 2008).

A good model system to study patterns and processes of parasite spillover, spillback and transmission interference as the result of human-mediated host introductions is the invasion of the Pacific oyster (Crassostrea 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 now oyster populations co-occur with native blue mussels (Mytilus edulis) in dense bivalve beds situated on intertidal mudflats (Reise, 1998; Troost, 2010; Ruesink et al., 2005; Buschbaum et al., 2016). Pacific oysters co-introduced the invasive parasitic copepod Mytilicola orientalis that 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 (Chapter 7), but no effects in oysters (Katkansky et al., 1967; Steele and Mulcahy, 2001). A congeneric parasitic copepod species, *M. intestinalis*, has been infecting native mussels since its introduction to the region 80 years ago (Caspers, 1939; Hockley, 1951; Korringa, 1968), but 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 competent host for the native trematodes Himasthla elongata and Renicola ros covita (Thieltges et al., 2008b; Welsh et al., 2014; Goedknegt et al., 2015). Instead, the oyster interferes with the transmission between the first intermediate host (snails) to its second intermediate host (bivalve), preventing the parasite species to complete their life cycle in birds, the definitive host of both trematodes (Thieltges et al., 2008b, 2009a; Welsh et al., 2014; Goedknegt et al., 2015). Finally, for the shell boring polychaete Polydora ciliata, which infects native blue mussels (*M. edulis*) and common periwinkles (*Littorina littorea*), invasive Pacific oysters act as a new competent host species (Thieltges et al., 2006), potentially increasing infection levels in native mussels (parasite spillback). The occurrence of invasive oysters across large spatial scales, their sessile life style in distinct habitat patches and the shared parasites with native mussels, make this invasion an ideal system to study the spatial variability and drivers of parasite infections as a result of a species introduction on large and multiple spatial scales.

In this study, we aim to investigate the effect of the invasion of Pacific oysters (*C. gigas*) on the distribution and abundance of parasites in native and invasive hosts across a large coastal ecosystem as a result of parasite spillover, spillback and transmission interference processes. We conducted our study in the European Wadden Sea, a temperate coastal ecosystem with extensive tidal flats stretching over 500 km along the Dutch, German and Danish coasts (CWSS, 2008). Here, Pacific oysters were introduced to the southern part in the 1980s (Drinkward, 1999; Troost, 2010) and to the northern part in the 1990s (Reise, 1998; Moehler et al., 2011) and now co-occur.
with native blue mussels (*M. edulis*) on epibenthic mixed mussel and oyster beds (Reise, 1998; Troost, 2010). Using a hierarchical sampling of invasive oysters and native mussels at three spatial scales (region, mussel/oyster beds, plots) and a set of 13 potential biological and environmental drivers we aimed to address the following questions: 1) Can spatial patterns be detected in the spatial distribution and abundance of parasites associated with spillover, spillback and transmission interference in invasive oysters and native mussel hosts across the ecosystem? 2) Which spatial scales are most important in explaining variation in parasite infection levels and does their relevance differ between invasive oysters and native mussel hosts? And 3) what are the most important biological and environmental drivers of infection levels across the ecosystem and do they differ between invasive oyster and native mussel hosts? Our study contributes to a better understanding of the patterns and processes of parasite spillover, spillback and transmission interference as the result of human-mediated host introductions and points to the relevance of spatial scales in assessing the manifold impacts of invasive species on native ecosystems.

**Material and Methods**

**Parasite infection patterns**

*Sampling on hierarchical scales*

Sampling took place on eight mixed invasive Pacific oyster (*Crassostrea gigas*) and native blue mussel (*Mytilus edulis*) beds spread over the Dutch and German Wadden Sea (Fig. 3.1). These beds were selected based on geographic distribution and logistic feasibility. We sampled throughout the entire Wadden Sea, except for the mid-German Wadden Sea, which is devoid of mussel beds (Folmer et al., 2014). The following regions were sampled: West-Netherlands (1. Balgzand and 2. Texel), East-Netherlands (3. Ameland and 4. Schiermonnikoog), South-Germany (5. Norddeich and 6. Hornumersiel) and North-Germany (7. Puan Klent and 8. Königshafen; Fig. 3.1). Beds were sampled between mid-September and late-October 2012 (Supplementary Table S3.1) as this period is well suited for documenting infection levels of macroparasites (after summer, the main period of production of trematodes (Thieltges and Rick, 2006; Poulin, 2006b), parasitic copepods (Grainger, 1951) and settlement of *Polydora ciliata* larvae (Harms and Anger, 1983)).

Per bed, a plot of 1 m² was haphazardly placed four times within the bed. From each plot, 20 blue mussels and 20 Pacific oyster hosts were randomly collected and analysed for the presence of parasites (see section *Dissection for parasites*). We sampled medium to large size classes of mussels (30-60 mm) and oysters (80-160 mm), as these size classes are regularly infected with the five parasite species (Brenner et al., 2014; Goedknegt et al., 2017). However, at some beds these size classes were not available resulting in the collection of relatively larger or smaller specimens. Our sampling design was hierarchical resulting in three spatial scales of 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). As 20 mussels and oysters were collected from each plot (see above) the total number of host individuals sampled was 640 per host species.

*Dissections for parasites*

In the laboratory, mussel and oyster shells were opened and inspected from the in- and outside for the presence of *P. ciliata* markings (e.g. blistering, tubes and holes as described in Catherine et al., 1990; Ambariyanto and Seed, 1991). Due to time constraints, it was not possible to
determine the number of *P. ciliata* in the shells, limiting observations to occurrences (present/not present) of this shell boring parasite species. After shell inspections, the meat was separated from the shells and stored in labelled plastic bags and frozen until further analysis.

Fig. 3.1 Map of the eight sampling locations (mixed Pacific oyster *Crassostrea gigas* and blue mussel *Mytilus edulis* beds, black dots) in four regions (black rectangles) in the Dutch (NL) and German (GER) Wadden Sea (shaded light grey area; see Supplementary Table S3.1 for coordinates and sample dates). On each bed, four plots of 1 m² (p; insert upper left) were haphazardly selected from which individual hosts were sampled. In addition in each plot, two cores (not shown) were taken to determine host densities and other parameters (see text for details).

Three months later we defrosted mussel and oyster flesh in batches (one species from a plot at a time, n = 20) and screened for the presence of parasites. As the mussel is host to four different endoparasite species (*Mytilicola orientalis*, *M. intestinalis*, *Renicola roscovita* and *Himasthla 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 procedure differed between the two hosts. In mussels, we first searched the tissue for the presence of *Mytilicola* individuals under a magnifying glass (magnification 3-8×) and extracted the copepods found. The tissue was then squeezed between two glass slides and examined under a stereo microscope (magnification 10-30×) to check for remaining copepods. At the same time we screened the mussel tissue for the presence of the trematode species *Himasthla elongata* and *Renicola roscovita* and counted all parasites detected. For the oysters, the procedure was slightly different as the meat of the oyster was too
voluminous and leathery to be squeezed between glass plates. Therefore, after the first dissection
of the digestive tissue, we flushed the intestine with water from a squeezing bottle to find
previously undetected copepods. Between the tissue parts, the red colour of the parasites could
easily be observed. Using a stereo microscope (magnification 10-30×), we counted all juvenile
and adult copepods and identified the adults for sex and species based on descriptions of Steuer
(1902), Mori (1935), Ho and Kim (1992), and Elsner et al. (2011). The copepods were stored in
1.5 mL Eppendorf tubes with 96% denatured ethanol.

Identification of Mytilicola spp.
Although morphological descriptions of Mytilicola spp. in their native range are available (Steuer,
1902; Mori, 1935), species identification based on morphology is not entirely reliable when both
Mytilicola species have overlapping host ranges and distributions (Elsner et al., 2011; Goedknegt
et al., 2017). As both Mytilicola species co-occur only in blue mussels in the Wadden Sea (Pogoda
et al., 2012; Goedknegt et al., 2017; Chapter 4), a subset of Mytilicola specimens originating from
blue mussels were also molecularly identified to species level to support and improve the
morphological identification for both sexes. Molecular identification was done with specimens
from one of the two beds of each region: Texel (Netherlands-West), Ameland (Netherlands-East),
Hornummersiel (Germany-South) and Puan Klent (Germany-North) (locations 2, 3, 6 and 7 in Fig.
3.1 and Supplementary table S3.1). In total, 875 Mytilicola individuals originating from 194 native
blue mussels (M. edulis) spread over the four locations were investigated (location 2: n = 39,
location 3: n = 72, location 6: n = 39, location 7: n = 44). These individual copepods were stored
in separate wells of 96-well plates containing 96% denatured ethanol. DNA extraction was
conducted at Baseclear B.V. (Leiden, Netherlands). Here, a portion of the cytochrome-c-oxidase I
gene was amplified and sequenced using Sanger sequencing as described in Goedknegt et al.
(2017). The DNA sequences were aligned manually in BioEdit 7.2.5 (Hall, 1999) and assigned to
either M. intestinalis or M. orientalis. The latter could be done by eye, because the interspecific
sequence differences for the locus are much larger than the intraspecific variation (see Elsner et
al., 2011; Goedknegt et al., 2017).

Biological and environmental drivers of parasite occurrence and abundance
Based on existing literature on native parasite-host relationships and on parasite spillover,
spillback and transmission interference, we selected 14 potential biological and environmental
drivers of parasite occurrence and abundance for our analyses (Table 3.1). For Mytilicola spp. at
least six (for M. orientalis seven) biological and environmental drivers were selected (Table 3.1).
Models of the shell boring polychaete P. ciliata, included furthermore the density of periwinkles
(alternative host; Table 3.1). Finally, for trematodes with complex life cycles (H. elongata and R.
rosocovita) information on first intermediate and definitive hosts was included, resulting in a total
of 13 potential drivers for these parasite species (Table 3.1). Although temperature is well known
to affect infection levels (Pietrock and Marcoglise, 2003; Poulin, 2006b), the range of average
summer temperatures (jun-sept over the years 2007-2011) in the Wadden Sea is too small to
detect potential effects (16.0-16.5 °C; E. Folmer, unpublished data) and therefore we did not
include temperature as driver in the analyses.
Table 3.1 List of biological and environmental factors which were investigated as potential drivers of infection levels in the analyses. Given are the value ranges, the applied transformation, the parasite species for which the driver was included in the analyses and the directional hypothesis (positive or negative effect) based on literature references.

<table>
<thead>
<tr>
<th>Drivers</th>
<th>Range</th>
<th>Transform.</th>
<th>Parasite sp.</th>
<th>Hypothesis</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Host density (mussel)</td>
<td>70.5 - 3721.0 m⁻²</td>
<td>Log</td>
<td>All</td>
<td>Positive</td>
<td>1-6</td>
</tr>
<tr>
<td>Host density (oyster)</td>
<td>0 - 317.4 m⁻²</td>
<td>none</td>
<td>All</td>
<td>Positive (competent)</td>
<td>1-6</td>
</tr>
<tr>
<td>Host size (mussel)</td>
<td>30.0 - 66.0 mm</td>
<td>none</td>
<td>All</td>
<td>Positive</td>
<td>1, 3,</td>
</tr>
<tr>
<td>Host size (oyster)</td>
<td>40.0 – 228.0 mm</td>
<td>none</td>
<td>M. orientalis P. ciliata</td>
<td>Positive</td>
<td>12</td>
</tr>
<tr>
<td>Fucus abundance</td>
<td>0 - 7900.43 g m⁻²</td>
<td>Log + 1</td>
<td>All</td>
<td>Negative</td>
<td>13, 14</td>
</tr>
<tr>
<td>Salinity</td>
<td>22.6 - 31.7 psu</td>
<td>None</td>
<td>All</td>
<td>Positive</td>
<td>15, 16</td>
</tr>
<tr>
<td>Tidal exposure</td>
<td>0.08 - 0.61</td>
<td>None</td>
<td>All</td>
<td>Negative</td>
<td>17, 18</td>
</tr>
<tr>
<td>Total periwinkle density</td>
<td>0 - 317.4 m⁻²</td>
<td>Log + 1</td>
<td>Trematodes P. ciliata</td>
<td>Positive</td>
<td>3</td>
</tr>
<tr>
<td>Herring gull density</td>
<td>701.2 - 6462.7 ha⁻¹</td>
<td>Log</td>
<td>Trematodes</td>
<td>Positive</td>
<td>4, 20, 21</td>
</tr>
<tr>
<td>Common gull density</td>
<td>639.9 - 7119.0 ha⁻¹</td>
<td>Log</td>
<td>Trematodes</td>
<td>Positive</td>
<td>20, 21</td>
</tr>
<tr>
<td>Black-headed gull density</td>
<td>911.8 - 5682.6 ha⁻¹</td>
<td>none</td>
<td>Trematodes</td>
<td>Positive</td>
<td>20, 21</td>
</tr>
<tr>
<td>Oyster catcher density</td>
<td>2404.2 - 11377.4 ha⁻¹</td>
<td>Log</td>
<td>Trematodes</td>
<td>Positive</td>
<td>4</td>
</tr>
<tr>
<td>Common eider density</td>
<td>252.5 – 9581.6 ha⁻¹</td>
<td>Log</td>
<td>Trematodes</td>
<td>Positive</td>
<td>4, 21</td>
</tr>
<tr>
<td>Total bird density</td>
<td>7772270 - 29396434 ha⁻¹</td>
<td>none</td>
<td>Trematodes</td>
<td>Positive</td>
<td>22</td>
</tr>
</tbody>
</table>

Host densities

We gathered information on invasive oyster, native mussel and periwinkle densities during the hierarchical sampling campaign (see section Host densities). Per plot (four plots per bed) two cores (Ø 19 cm, ± 20 cm deep) were taken and sieved and the contents were brought to the lab. Here, we determined oyster and mussel densities per core (all sizes), which were averaged per plot to obtain mean host densities per m².

Oyster and mussel host size

Prior to the dissections of the hosts gathered in the field (see section Dissections for parasites), we measured the maximum shell length of the mussel and oyster hosts with vernier callipers to the nearest mm.

Supply of free-living stages

Polydora ciliata and Mytilicola spp. have a direct life cycle with a free-living phase with larvae spending multiple days in the water column prior to infection of their host species (Grainger, 1951; Anger et al., 1986). Estimates of the supply of free-living stages are therefore difficult to obtain in the field and not included in the analyses of these parasite species. However, for trematodes with complex life cycles (H. elongata and R. roscovita), the supply of infective stages can be approximated with densities of the first intermediate molluscan and definitive bird hosts due to the local production and short lifespan of infective stages (Thieltges and Reise, 2007; Byers et al., 2008; Wilson et al., 2011; Galaktionov et al., 2015).
First intermediate host density - periwinkles Littorina littorea

We obtained densities of the first intermediate host, the common periwinkle Littorina littorea, from plot cores in a similar way as for host densities as described in section Host densities. Only mature periwinkles (> 14 mm) were included in the analyses, as trematodes infect only the reproductive tissue of their molluscan host (Werding, 1969; Lauckner, 1983).

Definitive host density - birds

Gulls and waders serve as definitive hosts for the trematodes R. roscovita and H. elongata (Stunkard, 1964; Werding, 1969; Lauckner, 1983; Galaktionov and Bustness, 1999). In the present study, we included the molluscivorous and benthivorous species herring gull (Larus argentatus), common gull (Larus canus), black-headed gull (Chroicocephalus ridibundus), oyster catcher (Haematopus ostralegus) and common eider (Somateria mollissima) associated with mixed mussel and oyster beds in the Wadden Sea (Waser et al., 2016) as potential drivers of infection levels in second intermediate mussel hosts. For our data composition, we used aerial counts (common eider) and high-tide roots counts (all other investigated species) for which we calculated the bird densities per intertidal hectare per location. See supplementary Appendix S1 for more information on these estimates.

Macroalgal biomass

We included the biomass of macroalga as a driver, because of its potential transmission interference capacities demonstrated in experimental settings (Johnson and Thieltges, 2010; Welsh et al., 2014). During the hierarchical sampling campaign, we collected the (by far) most abundant macroalga Fucus vesiculosus from individual cores (see sections Sampling on hierarchical scales and Host densities), weighted the biomass of the algae to the nearest 0.1 gram (wet weight) and averaged the values over the two cores to obtain estimates of Fucus biomass per plot (1 m²).

Salinity and exposure time

Salinity and exposure time data were obtained by means of simulation with the General Estuarine Transport Model (GETM; Burchard and Bolding, 2002). Within the scope of the PACE project, GETM was used to simulate the hydrodynamics, temperature and salinity for the entire Wadden Sea on a resolution of 200 x 200 m (Gräwe et al., 2016). The model output was used to obtain estimates of mean exposure time (i.e. the mean fraction of time that the seabed is exposed to the air) and salinity (PSU) over the period 2009–2011 (Folmer et al., 2016). For further details regarding the simulations we refer to Gräwe et al. (2016) and to Folmer et al. (2016) for postprocessing of simulation data.

Statistical analysis

Calculations of infection measures

For each sampled plot, we calculated parasite prevalences (the ratio of infected to sampled host species), intensities (the average number of parasites per infected host), abundances (the average number of parasites in all (infected and uninfected) hosts and population densities (the product of parasite abundance and host density) according to Bush et al. (1997). For Mytilicola spp.
Spatial patterns and processes of parasite infections

observations included morphologically as well as molecularly identified individuals, as the morphological identification error was relatively small (< 10%, see section Spillover of the invasive parasitic copepod Mytilicola orientalis).

**Parasite infection patterns**

We determined how variability in parasite occurrence (present/not present) and abundance (not available for *P. ciliata* see section Dissections for parasites) in native blue mussels and invasive Pacific oysters depends on the spatial scale. Because of the hierarchical design we used GLMMs for binomial (package lme4, Bates et al., 2015) and negative binomial data (package glmmADMB, Fournier et al., 2012, Skaug et al., 2014) in the statistical software environment R (R Development Core Team 2015). 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 *M. orientalis* and *P. ciliata*, which infect both invasive Pacific oysters and native blue mussels, we used similar mixed effects models including host species as fixed effect and compared these models with GLMMs without this fixed term with likelihood ratio tests following chi-square distributions.

**Drivers of parasite occurrence and abundance**

Prior to the analyses, we inspected all biological and environmental drivers for normality with histograms, boxplots and qqplots, and applied log-transformations when necessary (see Supplementary Table 3.1 for an overview of transformations). Additionally, we examined collinearity with pairplots including Pearson correlations (see Supplementary Fig. S1). We conducted a series of nested generalized linear models (GLMMs with binomial and negative binomial distributions) for each parasite/host species combination, including an intercept only model (null model), to examine the effect of biological and environmental factors on parasite occurrences (present/not present; for all parasite species) and abundances (all species except for *P. ciliata*) in native blue mussels and invasive Pacific oysters. Initially, the number of explanatory variables was kept to a minimum by including at most a single driver as fixed effect in the model. Consequently, each individual GLMM included parasite occurrence or abundance as response variable, none or one individual driver as explanatory variable and the hierarchical sampling structure as random effect (see section Calculations of infections measures). For each parasite species and response variable, 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 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 (Zuur et al., 2009).

**Results**

**Spatial infection patterns**

While native blue mussels (*Mytilus edulis*; mean shell length ± SE 45.2 ± 0.25 mm) were infected with five parasite species (the invasive parasitic copepods *Mytilicola orientalis* and *M. intestinalis*, the native shell boring polychaete *Polydora ciliata*, and the native trematodes *Renicola roscovita*.
and Himasthla elongata) with an overall prevalence of 98.4%, invasive Pacific oysters (Crassostrea gigas; 128.5 ± 1.5 mm) were only infected with the invasive M. orientalis and native P. ciliata, with a total prevalence of 59.8%. All parasites were found at all locations, except for M. orientalis in Pacific oysters at location 8 (Fig. 3.2). The most commonly found parasite was the trematode R. roscovita with an average prevalence of 98.8%.

In general, we found strong differences in infection levels of the five invasive or native macroparasite species associated with spillover, spillback and transmission interference across the Wadden Sea. Likewise, the variability in parasite occurrence and abundance differed among spatial scales and was generally parasite and host specific. Mixed effects models identified different biological and environmental drivers of parasite occurrence and abundance among parasite and host species. Hence, in the following we report the results separately for each parasite species.

**Spillover of the invasive parasitic copepod Mytilicola orientalis**
The molecular identification of 875 adult Mytilicola individuals from 4 beds indicated that that 91.3% (43/495) of the males and 89.2% (41/380) of the females were correctly identified by morphology (average identification error 9.7%). Due to this relatively minor identification error we decided to use the full dataset of morphologically identified copepods for further analyses.

Infections of the invasive parasitic copepod Mytilicola orientalis were found in native mussels and invasive oysters spread over the entire Wadden Sea, with exception of the extreme northern German Wadden Sea (location 8), where only one infected mussel (prevalence 1.3%; intensity of 1) was found and no infected oysters (Fig. 3.2, 3.3). In general, the invasive copepod occurred more in mussel than in oyster hosts (LRT; ΔDeviance = 130.59, p < 0.001; Fig. 3.2) and prevalences were overall twice as high in the native mussel (average ± SD, 50.8 ± 0.2%) compared to the invasive oyster (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 (LRT; ΔDeviance = 78.96, p < 0.001; Fig. 3.3). These contradicting patterns resulted in almost similar parasite abundances for mussel (average ± SD, 1.6 ± 1.2) and oyster (1.3 ± 1.2) hosts (LRT; ΔDeviance = 3.12, p = 0.077), however when host density was taken into account M. orientalis population densities were dominated by native blue mussels (Fig. 3.4A). More specifically, at locations were 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. 3.4A, B). Furthermore, looking closer at the distribution of M. orientalis population densities in individual host species, parasite densities in oyster hosts varied predominately because of variations in host density (Fig. 3.4B), while in mussels parasite abundance was more important in determining M. orientalis population densities (Fig. 3.4A). Finally, the level of individual beds explained the most spatial variation in M. orientalis occurrence and abundance in both mussels and oysters (Fig. 3.5).

Occurrences of the invasive copepod in native mussels and invasive oysters were best explained by the abundance of the macroalga Fucus vesiculosus, and the environmental factors salinity and exposure time. While Fucus biomass had positive effects on occurrences of M. orientalis in mussels (β = 0.384, SE = 0.228), the effect was negative for oyster hosts (β = -0.367, SE = 0.139). Model performance improved when salinity (β = -0.277, SE = 0.174) and exposure time (β = 2.257, SE = 1.021) were included in the mussel and oyster models, respectively (Table 3.2). Furthermore, none of the one or two factor models performed better than the null model
Fig. 3.2 Average prevalence (± SE) of the five parasite species in blue mussel (*Mytilus edulis*) and Pacific oyster (*Crassostrea gigas*) hosts at the eight sampled bivalve beds. *Prevalence of *Polydora ciliata* was not available for mussels at location 1 and oysters at location 4.
explaining *M. orientalis* abundances in blue mussels (Table 3.2). However, for oyster hosts, *M. orientalis* abundances were best explained by the two factor model including exposure time ($\beta = 6.415, \text{SE} = 2.541$) and salinity ($\beta = -0.314, \text{SE} = 0.144$).

**Fig. 3.3** Boxplots of intensities of the four intestinal parasite species in blue mussel (*Mytilus edulis*) and Pacific oyster (*Crassostrea gigas*) hosts at the eight locations for which intensities were available (not available for *Polydora ciliata*). The black dots inside the boxes indicate mean intensities, the black dots outside the boxes indicate outliers.

**Spillback of the native parasite *Polydora ciliata***
The shell boring polychaete *Polydora ciliata* was present at all eight beds sampled, with plot prevalences ranging from 0-35% in native blue mussels and 0-100% in invasive Pacific oysters (Fig. 3.2). Average prevalences differed between host species (LRT: $\Delta\text{Deviance} = 323.94, p < 0.001$) and were more than five times higher in the invasive than in the native host (average prevalence
Spatial patterns and processes of parasite infections

in oysters (± SD) 57.9 ± 0.4%, in mussels 10.2 ± 0.11%). The spatial scale explaining *Polydora* occurrences differed between host species; for oysters spatial variety was highest at the scale of individual mussel beds, while for mussels spatial variety was highest at plot level (Fig. 3.5).

The most important driver of *P. ciliata* occurrence was host length for both blue mussel ($\beta = 0.168, \text{SE} = 0.039$) and Pacific oyster hosts ($\beta = 0.024, \text{SE} = 0.06$). For mussels, model performance further increased by including *Fucus* biomass in the model, which had a negative effect on *Polydora* occurrence ($\beta = -0.546, \text{SE} = 0.203$). For oysters, the best two-factor model did not perform better than the one-factor model including host length (Table 3.2).

![Graphs showing parasite abundance vs host density](image)

**Fig. 3.4** Mean parasite abundance (± SE) versus mean host species density (± SE), with isoclines representing parasite population densities per m$^2$ at each of the eight sampled beds (see section *Sampling on hierarchical scales* and Fig. 3.1). **A)** Invasive *Mytilicola orientalis* in native blue mussels (*Mytilus edulis*) and **B)** in invasive Pacific oysters (*Crassostrea gigas*), **C)** previously established *Mytilicola intestinalis* in mussels, **D)** native *Himasthla elongata* in mussels and **E)** native *Renicola roscovita* in mussels. Abundances were not available for *Polydora ciliata*. 
Fig. 3.5 Variance components for parasite occurrence (present/not present) in A) mussels and B) oysters, and for parasite abundance in C) mussels and D) oysters, calculated from individual GLMMs for each parasite species infecting blue mussels. Abundances were not available for *Polydora ciliata*.

**Transmission interference of the invasive copepod *Mytilicola intestinalis***

The previously introduced copepod *Mytilicola intestinalis* was only found in native mussel hosts, but at all locations in the Wadden Sea (Fig. 3.2, 3.3) with an average prevalence (± SD) of 45.4% (± 0.3%) and an intensity of 2.0 (± 1.1). In contrast to *M. orientalis*, infection levels of *M. intestinalis* were highest in the northern German Wadden Sea with prevalences (78-94%) and intensities (3.1-4.0) ranging above the total averages (Fig. 3.2, 3.3). In the western Dutch region the opposite was true, with relatively lower prevalences (9-13%) and intensities (1.0-1.1). Furthermore,
Spatial patterns and processes of parasite infections

region was the most important spatial scale in explaining variation in *M. intestinalis* occurrence and abundance in mussels (Fig. 3.5).

Occurrences of the copepod in blue mussels were best explained by *Fucus* abundance ($\beta = 0.428$, SE = 0.162) and oyster density ($\beta = -0.005$, SE = 0.002) in the two factor model (Table 3.2). In contrast, *Fucus* abundance ($\beta = 0.456$, SE = 0.082) and host length ($\beta = 0.019$, SE = 0.008) were the most important drivers of *M. intestinalis* abundances (Table 3.2).

Table 3.2 Overview of the best models explaining parasite occurrence and abundance of the five invasive and native parasite species in native blue mussels (*Mytilus edulis*) and invasive Pacific oysters (*Crassostrea gigas*). Abundance models were not performed for the polychaete *Polydora ciliata* as information on the total number of polychaetes was lacking (see section Dissections for parasites). (+) and (-) denote significantly positive and negative effects of a factor, respectively.

<table>
<thead>
<tr>
<th>Parasite species</th>
<th>Host species</th>
<th>Parasite occurrence Model</th>
<th>AICc</th>
<th>Parasite abundance Model</th>
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<td>(-) Salinity</td>
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<tr>
<td></td>
<td></td>
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<td>(-) Salinity + <em>Fucus</em> biomass (+)</td>
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<td>Null</td>
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<td></td>
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<td>(+) Exposure</td>
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</tr>
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<td></td>
<td></td>
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<td>(+) Exposure + Salinity (-)</td>
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<td></td>
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<td>(+) Host length</td>
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<td></td>
<td></td>
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<td></td>
<td></td>
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<td>(+) Host length + Mussel density (+)</td>
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</table>

Transmission interference of the native trematode *Himasthla elongata*

The trematode *Himasthla elongata* was omnipresent in mussels in the entire Wadden Sea (Fig. 3.2, 3.3), with prevalences ranging between 16-100% and an average intensity ($\pm$ SD) of 12.5 ($\pm$ 19.0). *H. elongata* abundances in mussels were highest at locations 3 and 7, resulting in the largest parasite population densities on these beds (Fig. 3.4D). Furthermore, individual beds explained most of the spatial variation in parasite infections and abundances, followed by plots nested on mussel beds (Fig. 3.5).
Both occurrences and abundances of the trematode species were positively affected by host length (occurrence $\beta = 0.068$, SE = 0.023; abundance: $\beta = 0.090$, SE = 0.01) and the addition of *Fucus* abundance in the two factor model (occurrence $\beta = 0.869$, SE = 0.395; abundance $\beta = 0.667$, SE = 0.067). For both response variables, models including two driving factors performed best (Table 3.2).

**Transmission interference of the native trematode *Renicola roscovita***

Of all parasite species, the native trematode *Renicola roscovita* was observed in native mussels in the highest prevalences (average ± SD, 98.8% ± 0.0%; Fig. 3.2) and intensities (86.6 ± 113.2; Fig. 3.3). Like *M. intestinalis*, intensities varied between both ends of the Wadden Sea, with extremely high intensities in the northern German region (178.9-325.70) and low intensities in the western Dutch Wadden Sea (9.6-25.9; Fig. 3.3). The extremely high infection intensities were increasing *R. roscovita* abundances in mussels and this resulted in exceptionally high and low *R. roscovita* population densities in the northern German and western Dutch Wadden Sea, respectively (Fig. 3.4E). Furthermore, the largest spatial scale (region) explained most of the variation in *R. roscovita* infections and abundances (Fig. 3.5).

Occurrences of the trematode were best explained by oyster density ($\beta = 0.007$, SE = 0.003), and the density of common gulls ($\beta = 2.340$, SE = 0.571), both factors positively affecting infection levels. However, *R. roscovita* abundances in blue mussels were merely driven by host length ($\beta = 0.054$, SE = 0.009) and mussel density ($\beta = 1.605$, SE = 0.426). Again, models including two explanatory variables were the best performing models (Table 3.2).

**Discussion**

**General patterns**

Our hierarchical sampling (region/beds/plots) of native mussels (*Mytilus edulis*) and invasive Pacific oysters (*Crassostrea gigas*) on eight mixed mussel/oyster beds in the Wadden Sea, revealed strong heterogeneity in the spatial distribution of infection levels of all five parasite species associated with spillover, spillback and transmission interference across the ecosystem. In general, the variability in parasite occurrence and abundance differed among spatial scales and was generally parasite and host specific. The regional variability was high for the previously introduced *Mytilicola intestinalis* and native trematode *Renicola roscovita*, related to the relatively high and low prevalences and intensities found in the northern German and western Dutch Wadden Sea, respectively. In contrast, variability was highest on individual bivalve beds for the invasive copepod *Mytilicola orientalis* and the native trematode *Himasthla elongata*. For the native *Polydora ciliata*, the most important spatial scale was host specific, with highest variation on individual beds for oysters and highest variation among plots nested within beds for native mussels. These contrasting patterns suggest differences in the underlying processes among parasite species and below we discuss this separately for each species.

**Patterns of *Mytilicola orientalis* infections**

Spillover of the invasive parasitic copepod *Mytilicola orientalis* from Pacific oysters to native blue mussels (*Mytilus edulis*) occurred almost in the entire Wadden Sea at all spatial scales investigated. However, we observed relatively low infection levels of *M. orientalis* in northern populations of blue mussels, and no infections at all in Pacific oysters at the northernmost
location (see also Elsner et al., 2011; Goedknegt et al., 2017). Whether this results from a different susceptibility of the genetically distinct northern Pacific oyster populations (Moehler et al., 2011) or from a later invasion of *M. orientalis* remains a topic for further studies. At all locations where *M. orientalis* occurred, prevalences were always higher in mussels but intensities higher in oysters. The latter is likely caused by the relative size of both bivalve species. Oysters are larger and exhibit stronger filtration currents than mussels (Troost, 2010), increasing the chance to become infected with multiple infective larvae. In addition, the digestive system is enlarged in 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. This suggests that the population dynamics of *M. orientalis* may now mainly be driven by native mussels and not by invasive oysters, but what exactly the effects of both host species on the parasite's population dynamics are remain a topic for future studies. However, these results show that calculations of parasite densities can be useful in multihost communities (i.e. in spillover and spillback scenarios) to evaluate the contribution of different host species to parasite population densities.

Although host size and density may explain the observed differences in infection patterns between mussel and oyster hosts, both factors did not significantly contribute to the best models in our analyses of drivers of parasite occurrence and abundance across the ecosystem. In contrast, the abundance of the macroalga *Fucus vesiculosus* was the most important predictor of *M. orientalis* occurrences in both mussel and oysters, albeit with opposite effects for both host species (positive in mussels and negative in oysters). The latter we had hypothesized as macroalgae are known to exhibit transmission interference capacities in small-scale laboratory studies (Welsh et al., 2014). Here, trematode cercariae become entangled in the seaweed, reducing the numbers of free swimming cercariae when the algae were removed from the aquaria. That we observed a positive effect of *Fucus* biomass on *M. orientalis* occurrence and abundance in mussels was surprising. Such a positive effect may be explained by the life cycle of *M. orientalis* that involves a free-living larval stage of 2-3 weeks in the water column (based on observations of *M. intestinalis*; Hockley, 1951; Gee and Davey, 1986a), which suggests that recruitment to mussels and oysters occurs from outside the beds. When algal density is high, eggs and larvae of the invasive parasitic copepod might be trapped by the algae, causing aggregations of larvae on top of bivalve beds, ultimately leading to high abundances of infective stages infecting mussel hosts. Although both explanations for negative and positive effects of *Fucus* abundance might be valid, it does not explain the opposing effects in both host species for which we do not have an explanation.

In addition to *Fucus* abundance, environmental variables were important in determining *M. orientalis* infection levels in mussel and oysters. The negative effect of salinity confirms earlier findings from the North Pacific where higher prevalences were reported from mussels (*Mytilus trossulus*) situated in estuarine areas compared to mussels at coastal shores (Goater and Weeber, 1996). The congeneric species *M. intestinalis* also prefers reduced salinities (Korringa, 1968), but salinity was not an important driver of *M. intestinalis* occurrences and abundances in mussels, suggesting that the invasive *M. orientalis* may be more sensitive to salinity changes than *M. intestinalis*. Furthermore, exposure time positively affected *M. orientalis* occurrence and abundance 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
dedicated 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 found effect.

**Patterns of *Polydora ciliata* infections**
The native shell boring polychaete *Polydora ciliata* 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 being five times as high compared to native mussels. As time 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) or whether this is the beginning of a host switch of the parasitic polychaete is a topic for further studies.

Although not the entire range of mussel and oyster sizes were sampled in our study, host size was an important driver of *P. ciliata* infection levels in both mussel and oyster hosts. A positive relationship between host size and infection levels could reflect a relationship with host age, with older hosts accumulating more infections over time as has been previously suggested for native mussels *M. edulis* and periwinkles *L. littorea* (Ambaryianto and Seed, 1991; Warner, 1997). However, the shell surface area that is available for infection is also larger for larger hosts, increasing infection space for the polychaete. Furthermore, the low number of smaller mussels with *Polydora* markings can be explained by the higher vulnerability of smaller, infected mussels to crab consumption (Ambaryianto and Seed, 1991) as has previously also been shown for periwinkles (Buschbaum et al., 2007). Similar reasoning does not apply for Pacific oysters, as adult individuals of this invasive bivalve are rarely consumed by crabs and birds (Troost, 2010). Apart from host size, abundance of the macroalga *F. vesiculosus* was also an important driver for this parasite, negatively affecting occurrences of the native polychaete in blue mussels. Like *M. orientalis*, *P. ciliata* has a free-living larval stage which spends at least several days in the water column (Anger, 1986) so that recruitment to beds originates from the wider surroundings. Therefore, as hypothesized, the seaweed can act as a transmission interfering organism, preventing free-swimming infective larvae from infecting native mussels by physical obstruction (Johnson and Thieltges, 2010).

**Patterns of *Mytilicola intestinalis* infections**
The invasive parasitic copepod *M. intestinalis* was only found in mussels, but present in the entire Wadden Sea. Infection levels were lower in the western part relative to the northern German Wadden Sea (see also Goedknegt et al., 2017), while the parasite invaded both ends of the Wadden Sea at a similar time (1970s; reviewed by Feis et al., 2016). Possibly, the high infection levels in the north might be due to the absence of *M. orientalis* in this region. However, the potential competition between both parasite species is currently under investigation (C. Buschbaum, pers. comm.).

Similar to *M. orientalis*, the abundance of the macroalga *F. vesiculosus* positively affected the occurrence and abundance of *M. intestinalis* in mussels. The mechanism for both congeneric
species is probably similar, in which free-living eggs and larvae can be trapped by the seaweed, causing aggregations of larvae on top of bivalve beds and resulting in high infection levels in mussels (as discussed above for *M. orientalis* infections in mussels). Furthermore, mussel size positively influenced *M. intestinalis* infections as previously found in other studies (Gee and Davey 1986b; Goedknegt et al., 2017), which is probably age-related or due to larger filtration currents as discussed above for *M. orientalis* and *P. ciliata*. Finally, we observed the effect of transmission interference by invasive Pacific oysters for this parasite species. This was expected as *M. intestinalis* has so far not been found in the invasive bivalve species (Elsner et al., 2011; Goedknegt et al., 2017) and artificial infections were thus far unsuccessful (Elsner et al., 2011; M. Feis, unpublished results), suggesting that the Pacific oyster is not a competent host for *M. intestinalis* (Elsner et al., 2011; Goedknegt et al., 2017). This resulted in a greater dilution capacity when Pacific oysters occur in high density populations, thereby confirming earlier suggestions that the invasive species acts as a sink for *M. intestinalis* (Elsner et al., 2011; Goedknegt et al., 2017).

**Patterns of trematode infections**

The native trematodes *Himasthla elongata* and *Renicola roscovita* were omnipresent in native blue mussels throughout the entire Wadden Sea. While regional patterns were not observed in the distribution of *H. elongata*, the trematode *R. roscovita* showed strong regional patterns in prevalences and intensities similar to those of *M. intestinalis*.

For both trematode species host length was one of the most important factors positively affecting infection levels in mussels, which corresponds with earlier observations of these parasite species (Nikolaev et al., 2006; Thieltges and Reise, 2007; Stier et al., 2015). Like in *Mytilicola* and *P. ciliata*, this effect could be age related. However, among mussels of the same age class, larger mussels can be infected with higher trematode intensities (Nikolaev et al., 2006). This is attributed to the enhanced filtration currents exerted by larger molluscs, enabling these individuals to inhale larger quantities of free-living infective larvae resulting in higher infection levels (Nikolaev et al., 2006).

Similar to other parasite species in our study, both the occurrence and the abundance of *H. elongata* infections in mussels were positively affected by the abundance of the macroalga *F. vesiculosus*. As discussed above, this can be explained by the life cycle of the trematode that includes a free-living stage with cercariae spending a limited amount of hours (10–12 h; de Montaudouin et al., 1998) in the water column before infecting their host. Instead of drifting away with the currents during this time, cercariae may be trapped between the algae as discussed above.

The occurrence and the abundance of the trematode *R. roscovita* were further positively affected by densities of the definitive bird host (the common gull *Larus canus*) and second intermediate mussel host, respectively. Gull density was also a driving factor of *R. roscovita* infection levels in blue mussels in the Arctic (Galaktionov et al., 2015). That densities of the second intermediate mussel host further increased *R. roscovita* abundances in mussels was also expected, as it is easier for parasites to locate hosts when they occur in higher numbers (Arneberg et al., 1998; Stringer and Linklater, 2015). Finally, we found a positive effect of invasive Pacific oyster density on the occurrence of *R. roscovita* in mussels, instead of the anticipated negative effect via transmission interference for which support was found in an earlier experimental study (Goedknegt et al., 2015). The obvious possibility that oyster density positively correlated with the densities of first intermediate snail hosts of the parasite, thereby indirectly stimulating infection levels in mussels, does not hold true, as exploratory investigations prior to the statistical analyses
indicated that this was not the case. Alternatively, oysters may affect *R. roscoyvita* 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. roscoyvita* in mussels compared to conspecifics positioned on top of the matrix. Possibly, at the bottom of the oyster matrix, first intermediate snail hosts locally produce infective *R. roscoyvita* stages which are concentrated and trapped by the oyster structure (Chapter 6). 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.

**Conclusions**

The strong differences in infection levels of the five invasive and native macroparasite species associated with spillover, spillback and transmission interference across the Wadden Sea, suggest that the associated impacts on native hosts spatially vary accordingly. All five parasite species investigated in our study exert negative effects on mussels. Both *Mytilicola* species as well as the two trematodes species are known to decrease the condition and growth of native mussels (Lauckner, 1983; Thieltges, 2006; Stier et al., 2015; Chapter 7). In addition, *H. elongata* infections reduce the mussels’ attachment capacity as they preferentially infect the mussels which compromises byssus production and attachment to substrate (Lauckner, 1984). *P. ciliata* infections are known to weaken the shell strength of mussels, increasing predation pressure by crabs (Ambaryanto and Seed, 1991). Given the spatial distribution of parasites across the ecosystem, mussels can be relatively relieved from impacts of some parasites in specific regions (e.g. low infection levels of *M. intestinalis* and *R. roscoyvita* in the western Wadden Sea), while in others the impact will differ depending on specific beds (e.g. high infection intensity of *H. elongata* only in 2 out of the 8 beds). Hence, nested sampling designs on large spatial scales across entire affected ecosystems are a valuable approach to identify distributional patterns and potential impacts on native species in parasites associated with spillover, spillback and transmission interference in the course of a host invasion.

The generally high variability in parasite occurrence and abundance among spatial scales and the different drivers among parasite and hosts species identified in our mixed models suggest that the specific underlying processes are parasite and host species specific. Interestingly, oyster density was only included in 2 out of the 12 models (negative effect on *M. intestinalis* occurrence and positive effect on *R. roscoyvita* occurrence). This suggests that the dynamics of many parasite species are rather driven by other biotic and environmental drivers than oyster density. For example, in the case of spillover, oysters have been instrumental in co-introducing *M. orientalis*, but parasite population densities are now much higher in native mussels which likely decouples the parasite dynamics from oyster dynamics. In other cases, oysters seem to exert unexpected effects on native parasites as observed in *R. roscoyvita* which may be amplified in occurrence in mussel hosts by the matrix provided by the oysters. These observations suggest that patterns and processes of parasite spillover, spillback and transmission interference as the result of human-mediated host introductions depend on spatial scale and the parasite and host species involved and call for more studies to evaluate whether this observation is universal.
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**Supplementary material**

*Table S3.1* Information on sampling locations, including number and name of the sampled mixed mussel/oyster beds, the region (NL: Netherlands; GER: Germany), sampling dates and coordinates (for map see Fig. 3.1).

<table>
<thead>
<tr>
<th>Bed</th>
<th>Region</th>
<th>Date</th>
<th>Coordinates</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Balgzand</td>
<td>18/9/2012</td>
<td>52°55.830’N 004°54.153’E</td>
</tr>
<tr>
<td>2.</td>
<td>Texel</td>
<td>17/10/2012</td>
<td>53°08.852’N 004°54.183’E</td>
</tr>
<tr>
<td>3.</td>
<td>Ameland</td>
<td>12/10/2012</td>
<td>53°25.900’N 005°43.375’E</td>
</tr>
<tr>
<td>5.</td>
<td>Norddeich</td>
<td>26/10/2012</td>
<td>53°40.941’N 007°16.400’E</td>
</tr>
<tr>
<td>6.</td>
<td>Hornumersiel</td>
<td>25/10/2012</td>
<td>53°41.663’N 008°01.922’E</td>
</tr>
<tr>
<td>7.</td>
<td>Puan Klent</td>
<td>28/9/2012</td>
<td>54°47.475’N 008°18.405’E</td>
</tr>
<tr>
<td>8.</td>
<td>Königshafen</td>
<td>26/9/2012</td>
<td>55°01.747’N 008°26.048’E</td>
</tr>
</tbody>
</table>
Fig. S3.1 Pairplot for all biological and environmental drivers (see Table 3.1). The lower diagonal elements contain the (absolute) correlations.