General discussion

M. Anouk Goedknegt
In this thesis, biological invasions were used as ‘experiments in nature’ to study newly established parasite-host interactions and the underlying processes across spatial scales. The overall objective was to disentangle the manifold of roles of marine invasive species in parasite-host interactions and to understand the ecological and evolutionary implications for marine ecosystems.

In this chapter, the main findings of the three parts of the thesis are discussed in a broader ecological and evolutionary context, with specific emphasis on the Pacific oyster (*Crassostrea gigas*) invasion in the European Wadden Sea. First, I will discuss the manifold roles of the Pacific oyster in parasite-host interactions according to the different mechanisms of the conceptual framework provided in Part I (Chapter 2). This thesis focussed mostly on the co-introduction and spillover of an invasive parasite species (Chapters 4, 5, 7, 8), but in this chapter, I will also include additional mechanisms such as parasite release for which I provide a review of the literature. In addition, the role of the Pacific oyster in parasite-host interactions is further broadened by the inclusion of microparasites (i.e. viruses, bacteria, protozoa) using literature sources to provide a comprehensive overview of the complex ways of how a single invader can affect parasite-host interactions in an invaded ecosystem. In the second part of this chapter, I will discuss how the effect of Pacific oysters on parasite-host interactions varies along the ecosystem due to the respective environmental context in form of surrounding communities and abiotic factors. Then in the third part, I will evaluate the ecological and evolutionary impacts of the complex effects of the Pacific oyster on parasite-host interactions for the Wadden Sea ecosystem. Finally, I will end the discussion with recommendations for future research and some concluding remarks.
Marine invasions and parasites

After an alien species is introduced to a new ecosystem it can become invasive by establishing self-sustaining populations which spread in the new range (see Fig. 1.1 in Chapter 1). During this last phase in the invasion process, the invader interacts with local species via predation and competition, which increases the complexity of native interaction webs (see Fig. 1.2 in Chapter 1). In this thesis, I show that these interaction webs become even more complex by the addition and mediation of interactions between parasites and their hosts through invasive species (Fig. 10.1). Therefore, this thesis shows that the manifold of roles of invasive species in parasite-host interactions goes well beyond the initial release or reduction of parasites (enemy release hypothesis).

![Abstract illustration of a native parasite-host web](image)

**Fig. 10.1** Abstract illustration of a native parasite-host web, which involves five native host species (white semicircles) and four native parasite species (small white circles) before the arrival of an invasive species (pre-invasion). This web grows in complexity after the establishment of the invasive species (grey semicircles) and its co-introduced invasive parasites (grey circles), by increasing the number of connections between host and parasite species via the mechanisms described in Chapter 2.

In total, this thesis identified seven possible ways (see Chapters 2 and 6) in which invasive species can affect parasite-host interactions in invaded ecosystems:

1. **Parasite release or reduction**: Invasive species can leave all or some of their parasites behind in the native range, leading to potential competitive advantages of invaders over native species (enemy release hypothesis).

2. **Parasite spillback**: Invasive species can act as new competent hosts for native parasite species, potentially amplifying the native parasite population, leading to increased infection levels in native host species.

3. **Introduction of free-living stages of parasites**: Invasive species can be parasites themselves, which are introduced as free-living stages (mostly microparasites) in the introduced range, potentially infecting native host species.
4. **Parasite co-introduction with host:** Invasive parasites can be co-introduced with their invasive host to the introduced range.

5. **Co-introductions of parasites and spillover to native hosts:** Invasive parasites can be co-introduced with their invasive host to the introduced range and subsequently spill over to native host species.

6. **Interference with parasite transmission:** Invasive species can be non-competent hosts and interfere with the transmission of native parasites.

7. **Trait-mediated indirect interactions:** Invasive ecosystem engineers can exert trait-mediated indirect effects on parasite-host interactions via their complex physical structure by changing the behavior of native hosts (e.g., refuge seeking in the structure provided by an invader) which can lead to an alteration of infection levels in native hosts.

In the following, I demonstrate that these seven roles of invasive species in parasite-host interactions can occur simultaneously during a single invasion process, in this case the invasion of Pacific oysters in the European Wadden Sea.

**The Pacific oyster invasion and its effects on parasite-host interactions**

The Pacific oyster was not directly introduced from East Asia to European coastal waters, but invaded via oyster introductions from British Columbia along the North American Pacific coast where it had been introduced for oyster culture purposes before. Therefore, parasite species associated with the Pacific oyster had to pass the various barriers of the invasion process (see Fig. 1.1 in Chapter 1) at least twice, increasing the chance that parasites were lost during the two separate invasion steps. So far, a potentially resulting parasite release of the Pacific oyster in the Wadden Sea has not been investigated. However, a literature review indicates that at least five parasite species that have been described in Pacific oysters from the native range in East Asia have been lost as they have not been found in British Columbia or in the Wadden Sea (Table 10.1; Fig. 10.2): the trematode species *Gymnophalloides tokienses* and *Proctoeces* spp., the cestode *Tylosephalum* spp., the cnidarian *Eugynmanthea japonica* and the paramyxean *Marteiliodis chungmuensis*. Two of these potentially lost parasite species, the trematode *G. tokienses* and the paramyxean *M. chungmuensis*, directly impact the fitness of the Pacific oyster in its native range. Oysters heavily infected by *G. tokiensis* show depleted body reserves and reductions in growth (Hoshina and Ogino, 1951), while *M. chungmuensis* impacts the reproduction of the oyster by infecting its oocytes, resulting in abnormal development of the gonads and a disfigured appearance (Itoh et al., 2004). Hence, a release from these parasites may have resulted in a direct fitness increase of the invasive Pacific oyster predation pressure in the invaded ecosystem (Troost, 2010; Chapter 1), may have facilitated the spread of this invader.

However, despite a release from some of its own native parasites, the Pacific oyster became infected with two native parasite species once it was established in the Wadden Sea, the fungus *Ostracoblable implexa* (Thieltges et al., 2013a) and the polychaete *Polydora ciliata* (Thieltges et al., 2006; Pogoda et al., 2012; Brenner et al., 2014; Thieltges et al., 2013a; Chapter 3; Fig. 10.2). Both parasites do not directly affect the host tissue, but damage the Pacific oyster shell and weaken its structure (Catherine et al., 1990; Thieltges et al., 2013a). The fungus naturally infects European flat oysters (*Ostrea edulis*), but now found a new competent host species in the
Pacific oyster in which it is extremely prevalent in the entire Wadden Sea (Thieltges et al., 2013a). With the extinction of its native host (*O. edulis*) and adaptation of the Pacific oyster as a new host species, the fungus could remain or re-establish its presence in the Wadden Sea, illustrating the importance of the Pacific oyster invasion for the parasite population. In the Dutch Delta, European flat oysters are still naturally occurring and the presence of the invasive oyster could result in spillback to this native oyster species, potentially increasing the disease risk for the native host. Similarly, the acquisition of the native shell boring polychaete *P. ciliata* by the invasive Pacific oyster in the Wadden Sea could amplify polychaetae populations, resulting in increasing infection levels in native blue mussels (*Mytilus edulis*) and common periwinkles (*Littorina littorea*) via spillback effects. As *P. ciliata* infections are known to increase the vulnerability of mussels and periwinkles to predators (Kent, 1979, 1981; Buschbaum et al., 2007), these potential spillback effects may eventually result in an enlarged predation pressure on these native host species. The much higher prevalences of *P. ciliata* in invasive oysters compared to native mussels in the entire Wadden Sea (Chapter 3), might also suggest an ongoing host switch of the parasite, which now seems to preferably infect the Pacific oyster. As the invasive host species is currently almost predator-free in the Wadden Sea (Chapter 1), shell damage inflicted by *P. ciliata* might make the Pacific oyster more available to Wadden Sea predators, such as birds and crabs. However, the potential spillback or host switch of *P. ciliata* and its resulting effects on predation pressure on native and invasive host species remain a topic for future studies (see Box 10.1 and 10.2).

**Table 10.1** Results of a literature review on parasites of the Pacific oyster (*Crassostrea gigas*) which have probably been lost during translocations from East Asia (native range) via British Columbia (North American Pacific Coast) to the Wadden Sea (introduced range), with information on the parasite species, parasite taxa, presence in East Asia, British Columbia and the Wadden Sea, the effects of the parasite species on oyster hosts and the respective literature sources.

<table>
<thead>
<tr>
<th>Parasite species</th>
<th>Parasite taxa</th>
<th>East Asia</th>
<th>British Columbia</th>
<th>Wadden Sea</th>
<th>Direct effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Gymnophalloides tokiensis</em></td>
<td>Trematoda</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Body reserves (-) Growth (-)</td>
<td>1, 2</td>
</tr>
<tr>
<td><em>Proctoeces</em> spp.</td>
<td>Trematoda</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Unknown</td>
<td>2</td>
</tr>
<tr>
<td><em>Tylosephalum</em> spp.</td>
<td>Cestoda</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Unknown</td>
<td>3</td>
</tr>
<tr>
<td><em>Eugynmanthea japonica</em></td>
<td>Cnidaria</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Unknown</td>
<td>4</td>
</tr>
<tr>
<td><em>Marteiliodis chungmuensis</em></td>
<td>Paramyxea</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Fecundity (-)</td>
<td>5</td>
</tr>
</tbody>
</table>


Besides acquiring the two native parasites discussed above, the Pacific oyster is also infected with a wide variety of *Vibrio* bacteria which are omnipresent in the Wadden Sea (Thieltges et al., 2013a; Wendling et al., 2013; Wendling and Wegner, 2015). The origin of these (sometimes virulent) bacteria is unknown and they could be native as well as introduced as invasive infective stages, giving *Vibrio* bacteria a cryptogenic status in the Wadden Sea. *Vibrio* spp. can be found in the oyster tissue and hemolymph and are probably taken up from the environment by the oysters (Wendling and Wegner, 2015), where their presence in the water column varies seasonally with environmental temperatures (Wendling et al., 2014). Abundances of *Vibrio* spp. in oysters peak during summer (Wendling et al., 2014) and after the oysters’ main spawning period *Vibrio* infections can cause mass mortalities of Pacific oysters when
temperatures are still high (Wendling, 2013), as was observed in populations in the southern German Wadden Sea (Watermann et al., 2008b). However, under these circumstances, Pacific oysters in the Wadden Sea are known to quickly build resistance and locally adapt to virulent Vibrio strains, demonstrating fast evolutionary responses of the invasive species within a few generations (Wendling and Wegner, 2015).

Fig. 10.2 The manifold effects of the invasive Pacific oyster (Crassostrea gigas) on parasite-host interactions in the Wadden Sea (and Dutch Delta). Infections with Vibrio bacteria have not been illustrated as these parasites are from unknown origin. See text for further details. Drawings by Pieternella C. Luttikhuizen.

The Pacific oyster has not only acquired native parasites but also co-introduced several invasive parasites to the Wadden Sea. Recently, a virulent strain of oyster herpesvirus (OsHV-1 μvar) emerged in Pacific oysters in Europe (Engelsma, 2010; Lynch et al., 2012; Roque et al., 2012; Segarra et al., 2010) and appears to have originated from East-Asia (Mineur et al., 2014). So far, the virus only impacts the Pacific oyster (Fig. 10.2), causing mortalities of up to 90% among oyster larvae, spat and juveniles in the Dutch Delta (Engelsma, 2010). Recently, the virus has also been detected in juvenile oysters in the Wadden Sea (Gittenberger et al., 2015), but so far mass mortalities have not been documented in this introduced range. It will be interesting to see whether the virus will affect the population dynamics of the Pacific oysters on wild oyster beds and whether it will spillover to native host species at some point.

Such a spillover to native hosts has occurred in one of the co-introduced parasites, the parasitic copepod Mytilicola orientalis, which now infects native blue mussels (M. edulis), common cockles (Cerastoderma edule) and Baltic tellins (Macoma balthica; Chapter 5; Fig. 10.2). Prevalences in native mussels can be higher than in invasive oysters (Chapter 3), but the number of parasites in infected oysters exceeds those in mussels (Chapter 3). However, when host density
is taken into account, native mussels harbour much larger population densities of *M. orientalis* than Pacific oysters, as mussels occur in higher densities than oysters at most locations in the Wadden Sea (Chapter 3). It is still an open question whether the invasive parasite depends on both host species to maintain its population, but recent theoretical developments in parasite population modelling gives promising directions to answer this question (Box 1.2).

In this thesis, I also investigated the impact of the invasive parasitic copepod *M. orientalis* on its new native mussel host *M. edulis*. Experiments including artificial infections demonstrated that mussel condition is significantly reduced by juvenile *M. orientalis*, but that these parasite stages do not exert a negative effect on the clearance rate, growth or survival of its native host (Chapter 7). The adverse effects of the invasive copepod on the condition of its mussel host are most likely caused by the dietary needs of the parasite, as stable isotope analyses showed a trophic enrichment of the parasite compared to its host, indicating that *M. orientalis* is feeding on host tissue (Chapter 8). However, the trophic enrichment was much smaller (1.2‰) than the average trophic fractionation factor of 3.4‰ commonly used to separate trophic levels in predatory trophic interactions (Minagawa and Wada, 1984; Vander Zanden, 1997; Post, 2002). As a comparative literature-based analyses revealed (Chapter 9), this standard trophic fractionation factor may not be applicable for parasite-host relationships as neither a trophic enrichment nor a depletion of the parasite compared to its host appears to be a general pattern. Furthermore, among parasite-host relationships, nitrogen and carbon enrichment of parasites scale negatively with, respectively, the δ¹⁵N and δ¹³C isotope values of the host (Chapter 9), a pattern which was also found for nitrogen isotopes comparing the invasive parasitic copepod *M. orientalis* and its native mussel host *M. edulis* (Chapter 8). The absence of a general trophic fractionation factor and the negative scaling of parasite enrichment with host isotope values, call for a scaled rather than a fixed trophic fractionation framework, without the conservative 3.4‰ trophic fractionation factor separating trophic levels (Chapter 9). This has also consequences for the employment of isotope mixing models that are often used by biologists to reconstruct the proportions of diet sources in an organisms’ diet and require the input of correct trophic fractionation factors (Phillips et al., 2014). Therefore, we used a variety of trophic fractionation factors in these isotope mixing models, which all showed that also mussel diet sources (i.e. particulate organic matter and microphytobenthos), could make up substantial proportions of the parasites’ diet (Chapter 8). These results indicate that the invasive *M. orientalis* probably has a parasitic, but also a commensalistic relationship with its new native mussel host (Chapter 8).

Another co-introduced parasite of Pacific oysters which can potentially spill over to native species in the Wadden Sea is the bacterium *Nocardia crassostreae* (Fig. 10.2). In the summer of 2006, a mass mortality of Pacific oysters was observed in the Dutch Delta which was (partially) caused by this bacterium (Engelsma et al., 2008). Mass mortalities of Pacific oysters associated with nocardiosis have been reported earlier in Japan (Koganezawa 1975; Numachi et al., 1965) and on the west coast of North America (Sindermann and Rosenfield, 1967; Elston, 1993). The primary cause of the mass mortalities in the Dutch Delta was abiotic stress due to elevated water temperatures and low oxygen levels in summer (Engelsma et al., 2008). However, infection with the bacterium may have acted as an additional stressor facilitating the mortality of the oysters (Engelsma et al., 2008). In addition, the disease did not only affect the invasive Pacific oyster, but also the native European flat oyster (Engelsma et al., 2008), thus showing a spillover potential to native species. However, so far, *N. crassostreae* has not been detected in Pacific oysters in the Wadden Sea.
Box 10.1 Spillback of Polydora ciliata – ongoing research

The acquisition of the native shell boring polychaete Polydora ciliata by invasive Pacific oysters (Crassostrea gigas) can result in spillback effects for native blue mussels (Mytilus edulis), potentially leading to changes in predation strength on both host species. To investigate parasite spillback and estimate population sizes of the parasite in both host species, one needs to obtain information on P. ciliata intensities in host species, which is a tedious procedure requiring the cracking of host shells to accurately determine the number of worms in infected hosts. Alternatively, X-ray scans of host shells (Fig. 1) may be used to estimate the number of worms in infected shells. Results of a pilot study suggest that the percentage of damaged shell surface significantly correlates with P. ciliata intensities in oysters ($R^2 = 0.61$, $p < 0.01$) and mussels ($R^2 = 0.44$, $p < 0.001$). With such a proxy method, estimates of P. ciliata infection intensities in the field could be obtained to calculate parasite population densities in both host species according to Bush et al. (1997), by multiplying parasite abundances with host densities as has been done for the invasive parasitic copepod Mytilicola orientalis in Chapter 3. The field data used for this calculation showed that although the overall prevalence of M. orientalis was higher in native mussels, the higher intensities in invasive Pacific oysters lead to similar abundance estimates of the parasite in both host species. However, due to the much higher population densities of mussels compared to Pacific oysters, native mussels harboured most of the M. orientalis population at all investigated locations in the Wadden Sea (Chapter 3). Whether the same accounts for P. ciliata is still an open question and part of ongoing analyses.

A proxy for P. ciliata infection levels based on shell damage can also be applied to the historical shell collections of bivalves from the Wadden Sea held by the NIOZ (shellfish monitoring since the 1970s by J. Beukema and R. Dekker), in particular via the screening of mussel shells before and after the arrival of Pacific oysters. This ongoing research will allow to investigate whether the introduction of Pacific oysters has resulted in an increase in P. ciliata infections in mussels.

![Fig. 1 X-ray scans of (A) mussel and (B) oyster shells showing infections with the shell boring polychaete Polydora ciliata. White arrows indicate the presence of tubes made by the parasite.](image-url)
Box 10.2  Relative contributions of invasive and native hosts to parasite persistence - ongoing research

The invasive parasitic copepod *Mytilicola orientalis* and the native shell boring polychaete *Polydora ciliata* both infect invasive Pacific oysters (*Crassostrea gigas*) and native blue mussels (*Mytilus edulis*) but the relative contribution of the two hosts for the persistence of these parasite species in Wadden Sea remains to be determined. Recently, Fenton et al. (2015) proposed a conceptual framework for partitioning host contributions to parasite persistence in multihost communities based on key thresholds for parasite persistence. In this framework, the basic reproductive number (R\(_0\)) of the parasite for each host species first needs to be calculated via host-specific parameters such as prevalence, host density, the number of released infective stages and the transmission rate. When the calculated R\(_0\) values in both species are then plotted against each other, five regions in parameter space develop: parasite extinction, host 1 maintains the parasite population, host 2 maintains the parasite population, facultative multihost parasitism (either host can maintain the population) and obligative multihost parasitism (the parasite needs both host populations to persist; Fenton et al., 2015). Fig. 2 shows this plot for the invasive parasitic copepod *M. orientalis*, which was created by using data of the field sampling campaign described in Chapter 3 (prevalences of *M. orientalis* in mussels and oysters and host densities per plot on 7 beds (n = 28 plots) in the Wadden Sea), suggesting a case of facultative multihost parasitism where either host species can maintain the parasite population alone. This would mean that the persistence of the parasite population does no longer depend on its original host, the invasive Pacific oyster, but can also be maintained by the new native mussel hosts. However, this preliminary plot was made under the assumption that the number of released infective stages and the transmission rate are similar for each host species, and further experimental studies are needed to determine whether these assumptions are valid. In addition, similar calculations can be done for the shell boring polychaete *P. ciliata*. once data on infection levels and parasite population densities are available (see Box 10.1.).

![Fig. 2 Graph of the R\(_0\) mussel–R\(_0\) oyster parameter space for the two dominant host species (invasive Pacific oysters *Crassostrea gigas* and native blue mussels *Mytilus edulis*) of the invasive copepod *Mytilicola orientalis*. Ext. = parasite goes extinct, OMH = obligate multihost parasitism, FMH = facultative multihost parasitism.](image)
Besides serving as host for native or invasive parasites, the invasive Pacific oyster can also affect native parasite-host interactions without being a host itself by interfering with the transmission of the native trematodes *Renicola roscovita* and *Himasthla elongata*, which both have a complex life cycle (see Fig. 1.7 in Chapter 1; Fig. 10.2). In experimental settings, the Pacific oyster has been shown to act as a decoy or dead-end host for these parasite species (Thieltges et al., 2009; Welsh et al., 2014, Goedknegt et al., 2015), thereby directing infective parasite stages away from the native blue mussel host *M. edulis*. As *R. roscovita* affects the growth rate, clearance rate and condition of native blue mussels (Thieltges, 2006; Stier et al., 2015) and *H. elongata* reduces the mussels' attachment capacity by impairing its byssus-thread production (Lauckner, 1984), Pacific oysters can reduce the disease risk for native mussels by interfering with the transmission of these parasite species. However, in an observational field study, any effects of the Pacific oyster on infection levels of these two parasites were not observed (Chapter 3), suggesting that other environmental factors may often override the transmission interference effects of Pacific oysters in the field.

In addition to interfering with the transmission of native trematode species, the invasive Pacific oyster appears to also act as a sink for the previously introduced parasitic copepod *Mytilicola intestinalis*. Dissections of almost 400 oysters originating from the Wadden Sea and the Dutch Delta did not reveal any *M. intestinalis* infections (Chapter 5) and artificial infections of oysters with this invasive parasite were unsuccessful (Elsner et al., 2011; M. Feis, personal communications). In one of these experimental infection trials, 25 infective *M. intestinalis* larvae were added to a container with one Pacific oyster (40 replicates) and after 133 days only one oyster was infected with a single copepod, with almost no remaining larvae in the containers (only one individual copepod in 3 out of 40 containers; M. Feis, unpublished data). This result suggests that the Pacific oyster could act as a potential diluter of *M. intestinalis* parasites. Additional support for this hypothesis comes from field studies, where oyster density was negatively correlated with *M. intestinalis* occurrence in blue mussels across the entire Wadden Sea (Chapter 3). As experiments with artificial infections showed reductions in mussel condition with increased infection levels of *M. intestinalis* (Feis et al., 2016), Pacific oysters probably reduce the disease risk for native blue mussels by acting as a sink for the parasite species.

Finally, invasive Pacific oysters can also initiate trait-mediated indirect effects on parasite-host interactions via the habitat structure and complexity they provide. Native blue mussels use the complex structure created by the oysters as refuge from crab and bird predators by migrating to the bottom of the oyster matrix (Eschweiler and Christensen, 2011). This behavioural change induced by the oysters in presence of predators results in mussels at the bottom and top of the matrix experiencing significantly different infection levels (Chapter 6). This is the first time that a modification of parasite-host interactions as a result of trait-mediated indirect effects exerted by an invasive ecosystem engineer has been demonstrated and this mechanism may also occur in other invasive ecosystem engineers.

To conclude, the introduction of the Pacific oyster in the Wadden Sea (and Dutch Delta) has resulted in complex changes in the parasite-host interaction web in the invaded ecosystem (Fig. 10.1), involving both invasive and native host and parasite species (Fig. 10.2) which are adding to the changes in predatory and competitive interactions (Chapter 1).
Variations in parasite-host interactions across the ecosystem

Although the Pacific oyster has been identified to generally affect parasite-host interactions in the invaded ecosystem in the manifold ways discussed above, the resulting interaction webs will differ across the ecosystem depending on local environments as the results from the field study of infection levels of Pacific oysters and mussels along the Wadden Sea ecosystem suggests (Chapter 3). This study revealed strong spatial heterogeneity in the distribution and abundance of parasites which play a role in parasite spillback, parasite spillover and transmission interference across the entire Wadden Sea. This indicates that the local environment strongly influences local parasite-host webs, resulting in a diversity of different local webs across an entire ecosystem (Fig. 10.3). These local interaction webs can differ in the general species composition and interaction architecture, but also in the strength of specific interactions between parasites and hosts (Fig. 10.3).

One of the factors determining the spatial variability in parasite-host interaction webs is the presence and absence of specific parasite and host species at individual locations. For example, the invasive parasitic copepod *Mytilicola orientalis* has so far rarely been found in mussels and oysters in the north of the Wadden Sea, while it present at all southern locations in this ecosystem (Chapter 3). This means that the interactions resulting from the presence of this invasive parasite are present in parasite-host interaction webs at some locations, but not at others such as in the north of the Wadden Sea.

While the presence of links and the general architecture of parasite-host interaction webs is determined by the presence or absence of parasite and host species, the strength of specific
parasite-host interactions will be determined by the abundance of parasites at individual locations. This in turn will be the result of local biotic and abiotic factors as suggested by results presented in Chapter 3. In this study, I investigated the effect of 13 environmental drivers on parasite abundance in invasive Pacific oyster and mussels hosts. For many parasite species, the abundance of the macro-algae *Fucus vesiculosus* was particularly important, probably affecting the relative abundance of parasite species in mussel and oysters hosts via the accumulation of infective stages in the water column (Chapter 3). Also host size was a strong predictor of parasite abundances, further determining the local interaction strength of specific parasite-host interactions (Chapter 3).

Abiotic factors such as the ambient temperature can also affect the strength of parasite-host interactions. In the Wadden Sea, the range of average summer temperatures across locations was too small to be included in the analyses of Chapter 3, but for invasive species of which the invasive range stretches over multiple latitudes, temperature can be particularly important. For example, invasive species which play a role in parasite transmission interference of native parasites have been shown to exert enhanced feeding rates as a response to increasing temperatures, reducing infections in native host species to an even greater extend at elevated ambient temperatures (Goedknegt et al., 2015). At relatively warmer locations, these enhanced feeding rates will strengthen the transmission interference link between an invasive species and a native parasite, but weaken the parasitic link between the native parasite and its native host species. Furthermore, invasive parasites co-introduced with Pacific oysters and involved in spill-over events, might thrive at elevated temperatures, strengthening the links between the invasive parasite and host species. We already observed that higher temperatures during the breeding of infective *Myltilicola orientalis* larvae substantially increased larval development of the invasive parasite (Chapter 7), potentially leading to greater abundances in mussel and oyster hosts (by allowing more sequential larval production events in adults), thus further affecting the interaction strength.

Finally, the development of parasite and host pre- and post-infection traits during coevolutionary processes may determine the local interaction strength in parasite-host interaction webs (Fig. 10.4). Experimental cross-infection studies not included in this thesis (Feis et al., 2016), discovered that also these traits are subject to local environmental factors. The parasitic copepod *M. intestinalis* shows higher infectivity in native mussels and is more efficient at exploiting the host's resources in the south-western Wadden Sea than parasites originating from the northern Wadden Sea (Feis et al., 2016). In addition, southern hosts have evolved resistance to infection, whereas hosts in the north may have evolved infection tolerance (Feis et al., 2016). These results demonstrate that host-parasite coevolution depends on local environmental processes and will in turn shape the structure and interaction strength of local parasite-host interactions.

**Impact on the Wadden Sea ecosystem**

The variation in parasite-host interaction webs across the invaded ecosystem caused by different environmental processes (Fig. 10.3) will also result in different impacts on host species and local communities across the ecosystem. This compromises a general assessment of ecological and evolutionary impacts at the ecosystem scale. However, it is possible to assess the impact on smaller scales based on the condition that the local presence and abundance of parasite and host species is known.
Fig. 10.4 Graphical depiction of parasite and host pre- and post-infection traits which develop during host-parasite coevolution in the course of parasite invasion. White semi circles represent a native host species, grey small circles represent an invasive parasite species. **Pre-infection traits:** *infectivity* = the ability of the parasite to infect the host, *resistance* = the ability of the host to prevent parasitic infections. **Post-infection traits:** *virulence* = the ability of the parasite to damage the host, *tolerance* = the ability of the host to resist potential damage by the parasite.

Regarding the Wadden Sea ecosystem, the impact of many macroparasite species on native host species is known from previous investigations of native parasite-host interactions (e.g. the shell boring polychaete *Polydora ciliata* and the trematode species *Renicola roscovita* and *Himasthla elongata*) and recent experiments with invasive parasites (the parasitic copepods *Mytilicola orientalis* and *Mytilicola intestinalis*, partly Chapter 7 and Feis et al., 2016). As the effects of these macroparasite species are generally density dependent, the impacts of these macroparasites will increase when the parasites occur in higher abundances. For example, when the trematode *Renicola roscovita* occurs locally in high abundances in native blue mussels *Mytilus edulis*, the growth rate, clearance rate and condition of native blue mussels (Thieltges, 2006; Stier et al., 2015) can be expected to be negatively affected. Similarly, when the invasive copepods *Mytilicola* spp. locally occur in high abundances in native mussels, the mussel condition will be significantly reduced (Chapter 7; Feis et al., 2016). As blue mussels are ecologically and commercially important species in the Wadden Sea, these impacts of parasite infections on the host might also affect other species and general ecosystem functions and services of mussels, such as habitat and food provision for other species, the dilution of pollutants and the cleaning of the water column via filter feeding activities.

The effects of microparasite species on the other hand, are more devastating for the invasive Pacific oysters themselves. In contrast to the macroparasites discussed above, bacteria (e.g. *Vibrio* spp., *Nocardia crassostreae*) and viruses (e.g. oyster herpes virus) are more virulent, often resulting in the death of infected oysters (Engelsma 2008, 2010; Watermann et al., 2008b; see Fig. 10.4). In particular during summer, when environmental temperatures increase, microparasitic infections cause increased mortality in invasive Pacific oysters (Wendling, 2013).
Such mass mortality events may be early indications of potential boom and bust dynamics in the population growth of the Pacific oyster. Since its introduction in the Wadden Sea, a continuous population growth of Pacific oysters has been observed (Schmidt et al., 2008). However, in the Dutch Delta and the Wadden Sea, the first ‘busts’ have been reported in the form of localized mass mortalities as a result of parasite infections, in particular via infections of virulent microsparasites (i.e. *Vibrio* spp., *Nocardia crassostreae* and oyster herpesvirus; Engelsma 2008, 2010; Watermann, 2008b). However, these detrimental impacts on invasive host species also result in a strong selection pressure on Pacific oysters as observed for *Vibrio* bacteria (Wendling and Wegner, 2015). The evolutionary response of Pacific oysters to these selection pressures can be rather quick as a local adaptation to *Vibrio* strains could be observed within just a few generations (Wendling and Wegner, 2015). It will be interesting to see whether Pacific oysters will adapt to other virulent pathogens or whether oyster populations will eventually be regulated or even ‘bust’. In any case, these coevolutionary aspects indicate that the ecological impacts of the changes on parasite-host interactions imposed by Pacific oysters will not only vary spatially, but will also have temporal dynamics via the coevolution of host and parasite species.

**Outlook**

This thesis contributed to efforts to disentangle the manifold effects of marine invasive species on parasite-host interactions in invaded communities. In this thesis, I focussed particularly on the mechanisms which link invaders with native species via parasite-host interactions. However, the interaction webs in which these parasite-host interactions are included are still a simplified version of more realistic interaction webs, as other species interactions involving parasites and their hosts exist that have not been included in this thesis. In the following, I discuss several such interactions that seem promising research avenues for future studies on invasive Pacific oysters as well as for invasion ecology in general.

One of the relationships which could be added to these webs are parasite-parasite interactions, as hosts are often infected by more than one parasite species. Recently, these interspecific parasite interactions gained increasing attention in the literature (e.g. Fenton et al., 2010; Cattadori et al., 2014; Fenton et al., 2014; Woolhouse et al., 2015; Hellard et al., 2016), but so far interactions between invasive and native parasites have rarely been investigated. Numerous experimental studies (reviewed by Hellard et al., 2016) have shown that co-infecting parasites can exert strong positive or negative interactions, further affecting the interaction strength between parasite and host species, with consequences for disease dynamics (Fenton et al., 2014; Woolhouse et al., 2015). However, as experimental studies including co-infections with two different parasite species are not always possible because of ethical or logistical reasons, the interaction strength of parasite-parasite interactions can also be determined from field observational data by the use of mixed modelling (Fenton et al., 2010). This is a promising approach which will help to understand the interactions between invasive and native parasites in parasite-host webs.

A second type of interaction which could be added to parasite-host webs in the future are indirect interactions between invasive and native host species which are mediated by invasive or native parasites. When invasive and native host species compete for resources, the outcome of the competition can be altered when a parasite affects one host species, but not the other, resulting in density-mediated indirect effects (Dunn et al., 2012; Hatcher et al., 2006). In other
cases, when invasive and native species are not in direct resource competition, a shared parasite species might still mediate their interaction via apparent competition (Holt and Pickering, 1985). For example, the parasite can affect the least abundant host species, but not the other, leading to the removal of the least abundant species from the parasite-host web (Hatcher et al., 2012). Furthermore, like Pacific oysters via the biogenic matrix they provide (Chapter 6), parasites might also exert trait-mediated indirect effects on host species (Werner and Peacor, 2003) by affecting host behaviour, which results in the modification of competitive and trophic interactions between invasive and native host species (Dunn et al., 2012; Hatcher et al., 2006).

Finally, all these direct and indirect parasite-host interactions between invasive and native species may further increase the connectivity and complexity of food webs via the introduction of new nodes and links as demonstrated in Fig. 10.1. Thereby the introduction of invasive hosts and parasites to local ecosystems might affect entire communities. So far, marine food web studies which include invasive species and their co-introduced parasites to food webs are lacking (see Amundsen et al., 2013 for a freshwater food web study) and research directed to this area will provide interesting insights into the impacts of species invasion on the structure and dynamics of food webs and species interaction networks (see also Britton, 2013).

Concluding remarks

The work presented in this thesis demonstrates that only a single invasive species, exemplified by the Pacific oyster *Crassostrea gigas*, can be responsible for the establishment of various new and the modification of many existing parasite-host interactions in the introduced range (Fig. 10.1). As parasite-host interactions represent only a small share of all species interactions, which generally also involve predator-prey, competitive and commensalistic relationships, the accumulative change in biotic interactions in native communities as the result of a single species invasion is likely substantial and complex. Furthermore, as coastal ecosystems are amongst the most heavily invaded ecosystems in the world (Grosholz, 2002), the continuous introduction of alien species is rather the rule than the exception. Considering the multitude of alien species which have been introduced to the Wadden Sea alone (at least 49; Buschbaum et al., 2012), the number of biotic interactions that have been added and altered since the introduction each of these species must be enormous, with each type of interaction resulting in ecological and evolutionary impacts for native marine communities in the Wadden Sea. To unravel the complexity of these changes and the resulting impacts is a daunting task, but efforts in this direction will give significant insights into the manifold roles of parasites in marine invasions, their effects on species interaction networks and into the general functioning of marine ecosystems under an increasing pressure of species invasions.

I like to end this thesis with the notion that ecosystem change by human-aided introductions is not simply the addition of a new species to a community, but that these introductions are the start of complex and ongoing transformations of native interaction webs in invaded ecosystems. In this context, the present thesis demonstrated that the influence of invasive species on natural communities and ecosystems becomes even larger when parasite-host interactions are included in the resulting species interaction webs. Thereby the initial ecosystem change initiated by human-aided introductions is the onset of a cascade of interaction changes through entire communities and ecosystems from the smallest to the largest level, also including coevolutionary dynamics and feedbacks, thus verifying the early observations of Heraclitus that the only constant in life is change.