# Summary

# Samenvatting

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Human-aided introductions of species affect natural communities and ecosystems worldwide. These biological invasions can be considered to be ‘experiments in nature’ to study general ecological and evolutionary processes at large spatial and temporal scales. Studies on biological invasions have mostly been focussing on competitive or predatory interactions between invasive and resident species, and the resulting impacts on native communities. Much less known are the impacts of invasions on parasitism, the most common consumer strategy in the world. In this case, these ‘experiments in nature’ may involve the addition and loss of parasite and host species. Especially the loss of parasites in the process of an invasion has received increasing interest, as it has been hypothesized that such a loss of parasites may give invaders a potential competitive advantage over native species, contributing to their invasion success *(enemy release hypothesis*). However, recent developments in the fields of parasite ecology and invasion biology suggest additional ways of how invasive species can affect parasite-host interactions in invaded ecosystems beyond an initial parasite release. Yet, the complexity of these effects as the result of a single species invasion has not been investigated so far.

In this thesis, I addressed this knowledge gap and investigated the manifold ways in which a single invader can affect parasite-host interactions in invaded ecosystems in one of the most prominent marine invaders in the world: the Pacific oyster *Crassostrea gigas*. This invasive species is now globally distributed after initial introductions for aquaculture purposes from its native range (East Asia) to the Pacific coast of North America, followed by secondary human-aided introductions from this area to Europe and other continents. In the European Wadden Sea, the main study area of this thesis, the Pacific oyster became invasive via larval spread from oyster culture plots in the Dutch Delta and in the northern German Wadden Sea. Like in many other European coastal areas, the Pacific oyster now occurs in dense populations across the entire Wadden Sea and has transformed native blue mussel *Mytilus edulis* beds into complex oyster reef structures. In the Wadden Sea ecosystem, the Pacific oyster is associated with five invasive and native macroparasite species, providing a suitable model system to study the establishment of new and the alteration of existing parasite-host interactions as a result of species invasions.

The overall objective of my thesis was to disentangle the various roles of invasive species in affecting parasite-host interactions in invaded ecosystems and to identify the resulting ecological impacts. I specifically aimed to **I)** provide a conceptual framework for the study of parasite-host interactions as a result of marine invasions (Chapter 2), **II)** identify the multiple roles of the invasive Pacific oyster (*Crassostrea gigas*) in parasite-host interactions in the Wadden Sea ecosystem (Chapters 3-6), and **III)** determine the resulting ecological implications of changes in parasite-host interactions mediated by Pacific oysters (Chapters 7-9). The thesis ends with a **general discussion** in which I place the results in a broader ecosystem context and provide suggestions for future research (Chapter 10).

### Part I - Marine invasions and parasites: a review

In *Chapter 2*, I provided a conceptual framework of six mechanisms by which marine invasive species can affect parasite-host interactions in invaded ecosystems. To do so, I gave a comprehensive overview of what is currently known about the roles of invasive species in parasite-host interactions by reviewing conceptual developments and empirical evidence from the literature. Subsequently, I discussed the ecological and evolutionary implications for each of these mechanisms for the parasite and host species involved. The six mechanisms identified and discussed are the following:

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.

**Part II - Pacific oysters and parasite-host interactions**
In *Chapter 3*, I 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 Pacific oyster hosts across the entire Wadden Sea. In this study, I used a hierarchical field sampling design with three spatial scales to determine the spatial distribution of parasites in both host species. Furthermore, I identified the most important environmental and biological drivers of infection levels using mixed models, demonstrating that spatial infection patterns and their drivers are host and parasite specific.

In *Chapter 4*, I assessed the reliability of the morphological identification of two invasive parasitic copepods, *Mytilicola orientalis* and *Mytilicola intestinalis*, which play a potential role in three of the mechanisms identified in Chapter 2. These two parasite species have originally been described from different continents, but now co-occur in the same host species (blue mussels *M. edulis*) and at similar locations (the Wadden Sea and Dutch Delta), challenging the reliability of their identification. By using a multitude of morphological variables and multivariate statistics, I demonstrated that the invasion of both *Mytilicola* species in the Dutch Delta and Wadden Sea represents a case of 'cryptic by invasion', i.e. the two species are not reliably distinguishable by morphology in the invaded range, rendering them cryptic species (a phenomenon probably more common in species invasions than currently known). In the end of this chapter, I recommended guidelines for the identification of both parasite species when they co-occur in similar hosts and at similar locations.

In *Chapter 5,* I used the recommendations provided in Chapter 4 to identify the role of the Pacific oyster in the co-introduction and spillover of *M. orientalis* and the spillback of *M. intestinalis* to native host species, which are two of the mechanisms which were identified in Chapter 2. To this end, I used a substantial field sampling of 11 different invasive and native mollusc species across the Dutch Delta and the Wadden Sea, and found evidence for spillover of

*M. orientalis* to three native bivalve species, but no evidence for spillback of *M. intestinalis* to native mussels via invasive Pacific oyster hosts.

In *Chapter 6*, I introduced a new mechanism of how invasive ecosystem engineers, such as Pacific oysters, can affect parasite-host interactions. With a field experiment performed at both ends of the Wadden Sea, I demonstrated that invasive Pacific oysters can 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 predators by migrating to the bottom of the oyster matrix and 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 parasite infection levels. This study is the first to present evidence for an indirect modification in parasite-host interactions as a result of the physical structure of an invasive ecosystem engineer.

**Part III - Ecological implications**

In *Chapter 7*, I focused on the newly established parasite-host relationship of the invasive copepod *Mytilicola orientalis* and the native blue mussel *Mytilus edulis*. By breeding parasite larvae in the laboratory and executing controlled infections, the effects of the invasive parasite on its new blue mussel host were experimentally investigated. While the condition of the mussels was significantly reduced, mussel clearance rates and growth were not affected by the parasite. With this study, I demonstrated that controlled experimental infections with *M. orientalis* can be used to study the effects of the parasite on native and invasive host species.

In *Chapter 8,* I investigated the mechanism behind the reduced condition of native blue mussels as a result of *M. orientalis* infections (Chapter 7). I used stable isotope analyses to identify the trophic relationship of *M. orientalis* and its new native mussel host. The measured trophic enrichment of the parasite compared to its host species, indicated that the parasite is feeding on host tissue. Furthermore, with isotope mixing models I was able to show that mussel food sources (phytoplankton and microphytobenthos) could also contribute to the parasite's diet, indicating that the invasive *M. orientalis* has probably a parasitic as well as a commensalistic relationship with its new native mussel host.

*Chapter 9* explored whether the trophic enrichment found in Chapter 8 is a general pattern in parasite-host relationships. Based on a large data set compiled from the published literature on stable isotope measurements across many parasite and host taxa, a phylogenetic comparative analysis found no general pattern in trophic enrichment or depletion of parasites in nitrogen or carbon isotopes compared to their host species. This result suggests that parasite-host interactions in general may not fit well into the conservative stable isotope framework with standardized trophic fractionation factors and calls for developing an appropriate framework for parasitic trophic interactions.

**General discussion**

In *Chapter 10*, I presented an overview of the seven possible ways in which invasive species can affect parasite-host interactions in invaded ecosystems that I identified in Chapters 2 and 6. Following this, I showed that all seven mechanisms also play a role in the invasion of Pacific oysters and I discussed the results of my thesis in this respect. In addition, I provided a literature review for the mechanism of parasite release, indicating that at least five parasite species were lost during the course of the invasion which may have facilitated the initial spread of the Pacific oyster. Literature sources were also used to include microparasite species (e.g. bacteria, viruses and protozoans) into the summary of the seven mechanisms for Pacific oysters. Overall, this summary shows that the introduction of the Pacific oyster to the Wadden Sea (and elsewhere such as the Dutch Delta) has resulted in complex changes in the parasite-host interaction web in the invaded ecosystem, involving both invasive and native host and parasite species. Hence, the seven mechanisms discussed above can occur simultaneously during the invasion of a single species.

However, the large-scale field study I conducted (Chapter 3) suggests that the parasite-host interaction webs which result from these complex interactions will differ across the ecosystem depending on local environments, as strong spatial heterogeneity in the distribution and abundance of parasites infecting mussel and oyster hosts were observed across the Wadden Sea. Local environments obviously influence local parasite-host webs, resulting in a diversity of different local webs across an ecosystem. These local interaction webs can differ in the general species (parasites and host) composition and interaction architecture, but also in the strength of specific interactions between parasites and host species.

This variation in parasite-host interaction webs across the invaded ecosystem 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. Regarding local impact assessments in the Wadden Sea, parasite-host interactions involving macroparasites will mostly impact native host species (in particular blue mussels *M. edulis*), while interactions including the more virulent microparasites will mostly affect invasive Pacific oysters themselves.

While this thesis contributed to efforts to disentangle the complex effects of marine invasive species on parasite-host interactions in invaded communities, there are still many open questions for future research. In the studies presented in this thesis, I focussed particularly on the mechanisms which link invaders with native species via parasite-host interactions. However, interaction webs including these parasite-host interactions are still simplified versions of more realistic interaction webs, as other species interactions involving parasites and their hosts also exist such as parasite-parasite interactions and parasite-mediated indirect interactions. Furthermore, all these direct and indirect parasite-host interactions between invasive and native species may affect the structure and dynamics of food webs via the introduction of new nodes and links.

In conclusion, 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 invaded ecosystems. Considering the multitude of alien species which have been introduced to the Wadden Sea alone (at least 49 species), the number of biotic interactions that have been added and altered since the introduction of each of these species must be enormous, with each type of interaction resulting in ecological and evolutionary impacts for native marine communities. 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.