Infestation of oysters and mussels by mytilicolid copepods: differences between natural coastal habitats and two offshore cultivation sites in the German Bight

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Summary

The aim of this study was to determine the macro-parasitic infestation level of oysters from the southern German Bight focussing on copepods of the genus Mytilicola. Crassostrea gigas, Ostrea edulis and Mytilus edulis were collected at five locations: three nearshore sites in the eastern Wadden Sea and two offshore cultivation sites in the German Bight. To reveal seasonal variations one sampling site was investigated in winter and summer. At the nearshore sites, Mytilicola orientalis was regularly detected in C. gigas. Prevalences ranged between 32.3% and 45.1%, intensity between 3.0 ± 0.6 and 8.2 ± 1.5. Infestation rates of C. gigas within the southern German Bight decreased from west to east: Apparently, M. orientalis has started its range extension along the German coast with gradual retardation eastwards but generally followed the invasion route of its main host, the Pacific oyster. Interestingly, we detected not only M. intestinalis but also M. orientalis as an intestinal parasite in M. edulis, which has so far not previously been described as host within this region. We conclude that M. orientalis is flexible in its host choice. Furthermore, in the eastern Wadden Sea infestation rates of oysters and mussels by copepods are similar. These results deviate from the patterns observed for the northern Wadden Sea in terms of infestation level and host specificity. No macro-parasites were found in oysters and mussels from the offshore sites. This absence can be considered as potentially beneficial for aquaculture activities in the open ocean in terms of stamina and physiological performance.

Introduction

The Pacific oyster Crassostrea gigas was introduced into Dutch tidal backwaters in the 1970s (Andrews, 1980; Chew, 1990; Rueink et al., 2005) to substitute depleted stocks of the European oyster Ostrea edulis and to reanimate declining oyster fisheries. The further invasion by C. gigas within the North Sea region developed from west to northeast along the German coast as a result of the prevailing current regime and the transport of pelagic oyster larvae (Wehrmann et al., 2000) but also through direct import for aquaculture purposes in the German Wadden Sea on the shores of the Island of Sylt. In contrast, the European oyster is considered to be extinct in German waters since it was extremely diminished by overexploitation, diseases and severe winters (Wehrmann et al., 2000). The species has not been found along the German coastline for the past half century. Populations of O. edulis still exist in the Danish Limfjord, in Norway and around Ireland but they are under threat or decline in all these regions (OSPAR, 2009). Some former Wadden Sea habitats of the European oyster and even more excessively wild mussel banks of Mytilus edulis are now more or less dominated by the invading Pacific oyster (Diederich, 2006; Schmidt et al., 2008).

Since the beginning of the 1980s Crassostrea gigas spaw was imported to Germany (Meixner, 1973; Neudecker, 1984) and environmental requirements of the species were investigated along the German coast. Various biotic and abiotic parameters affected fitness and health such as hypoxia, anoxia, symptoms of eutrophication as well as outbreaks of oyster parasites (Jackson et al., 2001): Wild and farmed oysters are subject to various diseases caused by macro- and micro-parasites such as copepods, trematodes, nematodes, polychaetes, and protozoans (e. g. Katkansky and Warner, 1968; Montes, 1990; Motes and De Paola, 1996; Aguirre-Macedo and Kennedy, 1999; Carnegie et al., 2000). Among others, two of these parasites, the intestinal mytilicolid copepods Mytilicola intestinalis and M. intestinalis are introduced species in the North Sea: M. orientalis was introduced to the southern North Sea in the 1990s together with its main host, the Pacific oyster, whereas its congener M. intestinalis was initially introduced to the North Sea in the 1930s and is a long-established common parasite of the indigenous blue mussel Mytilus edulis in this region (e.g. Dethlefsen, 1975; Lauckner, 1983; Davey, 1989; Buck et al., 2003; Thíeltges et al., 2008). The concrete effects of Mytilicola intestinalis and M. intestinalis on their specific hosts are still under discussion. Low condition indices of mussels and oysters, low ability of recover after spawning and mass mortalities along the coasts of The Netherlands and Germany have been related to high infestation rates (Katkansky et al., 1967; Korringa, 1968; Paul, 1983). Other investigations reported no effects by mytilicolid infestations (Chew et al., 1965) or only at times of unfavourable environmental conditions (Campbell, 1970; Gee et al., 1977).

In the German Wadden Sea (northern and eastern parts) high infestation rates of native mussels M. edulis by parasitic copepods of the species Mytilicola intestinalis have been observed (e.g. Buck et al., 2005; Krakau et al., 2006; Elsner et al., 2010). Interestingly, investigations on the parasite burden at offshore locations in the North Sea could not detect any macro-parasites in mussels (Buck et al., 2005; Buck, 2007; Brenner, 2010). In case of oysters, Pacific oysters in the northern Wadden Sea were not infested by M. intestinalis and the infestation of oysters by Mytilicola orientalis was remote: M. orientalis was only found with a low prevalence and intensity at one single site close to the Island of Sylt.
(Elshner et al., 2010). In the eastern part of the Wadden Sea, infestation of oysters with mytilicolid copepods has not been examined so far.

From an economic point of view, today, mussels (M. edulis and M. galloprovincialis) as well as the Pacific oyster (C. gigas) are the most important species in European shellfish production. There is also a respectable market for the European oyster O. edulis (FAO, 2009). As oysters represent high-value seafood products an aesthetic appearance of shell – especially on the half-shell market – and meat is rather important (FAO, 2011). Parasite infestations could reduce harvests and severely deplete local populations. Some macro-parasites could also evoke a deteriorated morphological appearance. Understanding the development of infestation patterns is therefore crucial for the successful site-selection in oyster cultivation. In addition to ecological and economic advantages of offshore oyster cultivation (Pogoda et al., 2011) such cultures would further benefit from an absence of parasites, as observed for blue mussels (Buck et al., 2005; Buck; 2007; Brenner, 2010).

The aim of this study was to record the presence and describe the infestation levels and host specificity of macro-parasites, particularly of the two parasitic copepod species (Mytilicola orientalis and M. intestinalis) in oysters from the eastern Wadden Sea and from two offshore cultivation sites. To demonstrate the general existence of parasite copepods and other macro-parasites in shellfish at the investigated sites, the native mussel M. edulis was also sampled. As these mussels, originating from sub- and intertidal habitats, are commonly infested by macro-parasites, M. edulis was considered to be a useful reference organism. Furthermore, one nearshore site was investigated in winter as well as in summer to reveal potential seasonal variations of the parasite burden in oysters and mussels.

Therefore, we investigated parasite infestations (i) in C. gigas and M. edulis at three nearshore wild banks located in the eastern Wadden Sea and (ii) at two offshore cultivation sites in the German Bight.

Materials and methods

Sampling sites and investigated bivalves

Examinations on macro-parasite infestations were carried out with oysters and mussels from five different sites (Fig. 1): three intertidal nearshore sites (former mussel banks which were transformed to wild oyster reefs) and two offshore sites (cultivation experiment): Site 1 Juister Watt (JW) is located in the western part of the German East Frisian Wadden Sea near the Island of Juist (53°38.5′N, 006°56.5′E). Site 2 Dornumer Nacken (DN) is located about 8 nautical miles (nmi) east of site 1, south of the western tip of the Island of Langleoog (53°41.9′N, 007°28.1′E), and site 3 Kaiserbalje (KB) another 10 nmi further to the east, southeast of Mellum island (53°38.7′N, 008°16.0′E). Oysters and mussels collected at these sites originate from bottom habitats, which fall dry several hours per day. The offshore test site Nordergründe (NG) (site 4) was located in the outer Weser estuary (53°51.0′N, 008°04.0′E). 9 nmi off the coast (offshore classification by Ryan, 2005) as part of an official testing area, which was established for the multi-use research of offshore aquaculture within offshore wind farms (Buck, 2007; Pogoda et al., 2011). The planned offshore wind farm ‘Nordergründe’ (Energiekontor, 2011) will be realized about 1 nmi off the test site. The offshore test site Butendiek (BD) (site 5) was located 15 nmi west of the North Frisian island of Sylt (54°59.1′N, 007°54.4′E), within the area of the planned wind farm ‘Butendiek’ (Buck et al., 2008). For the offshore cultivation experiments juvenile oysters were obtained from commercial hatcheries (Pogoda et al., 2011).

In total, 296 oysters (Crassostrea gigas) of 50–230 mm size were collected from the nearshore sites (Table 1). As Mytilus edulis acted only as a reference organism it was collected in lower numbers (Buck et al., 2005; Thieltges et al., 2006; Jungblut, 2011). In total, 60 mussels of 40–50 mm size were collected from the nearshore sites (Table 1). Due to the absence of the European oyster along the German coast O. edulis samples were only evaluated from the offshore cultivation experiments (Table 1). At the offshore sites juvenile oysters (C. gigas and O. edulis) were transferred to oyster lanterns for cultivation in April 2004 and 2007. At site 4 (NG) oyster lanterns were fixed to rigid steel rings welded to large offshore marker buoys, specifically constructed for offshore aquaculture research on shellfish candidates: C. gigas, O. edulis, M. edulis (Brenner et al., 2007; Pogoda et al., 2011). At site 5 (BD) oyster lanterns were fixed to steel frames attached to metal piles of a former research platform of the Federal Maritime and Hydrographic Agency. Culture plots at sites 4 and 5 were permanently submerged. After a six-month growth period in offshore waters 50 oysters of C. gigas and O. edulis (per site and species) were recollected for macro-parasite investigations in October 2004 and 2007 (Table 1). Data on macro-parasitic infestation of M. edulis from offshore site 4 (same sampling date) has been found by Brenner (2010).

Analysis of parasite infestation and condition index

Length, width and height of each animal were measured with a calliper to the nearest 0.1 mm. Oysters and mussels were then opened by cutting the adductor muscle. The soft body was separated from the shell and dried on absorbent paper for 10 s. before weighing (wet mass [WM] of meat).
Determination of mytilicolid copepods to species level was performed using detailed descriptions by Dethlefsen (1985), Gee and Davey (1986) and Elsner et al. (1998). Additional parasite species were identified following Lauckner (1980, 1983), Grzel (1985) and Watermann et al. (1990). For trematodes and shell-boring polychaetes only the prevalence was calculated using the condition index (CI) according to Davenport and Chen (1987):

\[ CI = \frac{W \times DM}{C} \times 100 \]  

\[ W = \frac{mL}{mL} \sum \]  

Digestive gland and rectum were inspected separately to detect and extract Mytilicola individuals as a whole. Adult individuals can be detected easily due to their size and bright red colour (Elsner et al., 2010). Most of the extracted copepods were stored in alcohol for detailed species determination. Some female individuals with developed nauplii in their egg sacs were reared in seawater to observe larval development. To ease investigations, parts of the soft body (digestive gland with rectum, muscle, gills, connective tissue) were squeezed separately between glass compressoria. Smaller Mytilicola individuals and further macro-parasites were then identified under the stereomicroscope. Parasite infestation was documented in terms of prevalence and mean intensity. Prevalence is defined as the percentage of infested host individuals within a certain group. Mean intensity (mi) signifies the mean number of parasites living in one host, while ni of uninfested hosts is excluded.

\[ mi = \frac{mi_i}{ni} \sum \]

\[ mI = \frac{mI}{mI} \sum \]

\[ mI_i = \text{sum of total parasites of species } i \]

\[ mI \Sigma = \text{sum of total hosts infected with parasites of species } i \]

Shells of oysters and mussels were inspected for the presence of shell-boring polychaetes. Shells were then oven dried for 48 h at 65°C and weighed (dry mass [DM]) to calculate the condition index (CI) according to Davenport and Chen (1987):

\[ CI = \frac{W \times DM}{C} \times 100 \]  

\[ D = \frac{mi}{mI} \sum \]  

\[ D_i = \text{sum of total parasites of species } i \]

\[ D \Sigma = \text{sum of total hosts infected with parasites of species } i \]

\[ D = \frac{D_{i}}{D_{\Sigma}} \sum \]

\[ D_{i} = \text{sum of total parasites of species } i \]

\[ D \Sigma = \text{sum of total hosts infected with parasites of species } i \]

Statistical analysis

Means, standard errors of the mean (mean ± SE) and confidence intervals (mean ± conf. interv.) of condition indices and intensities were calculated using MS-Excel software. Data were also tested for normality with MS-Excel software. Differences between sites were analyzed with two-tailed Mann–Whitney tests using GRAPHPAD PRISM 5.0 with significance levels of P < 0.05.

Table 1

<table>
<thead>
<tr>
<th>Site classification</th>
<th>Site name</th>
<th>Salinity range</th>
<th>Temperature range °C</th>
<th>Crassostrea gigas</th>
<th>Ostrea edulis</th>
<th>Mytilus edulis</th>
<th>Sampling time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neearshore</td>
<td>Dornumer Nacken (DN)</td>
<td>24.6–31.2</td>
<td>1.7–22.9</td>
<td>71</td>
<td>–</td>
<td>15</td>
<td>February 2011</td>
</tr>
<tr>
<td>Nearshore winter/summer</td>
<td>Kaiserbalje (KB)</td>
<td>7.8–30.8</td>
<td>1.5–23.9</td>
<td>68/70</td>
<td>–</td>
<td>15/15</td>
<td>December 2010/June 2011</td>
</tr>
<tr>
<td>Offshore</td>
<td>Nordergründe (NG)</td>
<td>23.1–33.6</td>
<td>3.1–19.6</td>
<td>50</td>
<td>50</td>
<td>15²</td>
<td>October 2007</td>
</tr>
<tr>
<td>Offshore</td>
<td>Butendiek (BD)</td>
<td>30.8–33.1</td>
<td>2.8–18.9</td>
<td>42</td>
<td>50</td>
<td>–</td>
<td>October 2004</td>
</tr>
</tbody>
</table>

1BSH (2011).
2Mussels analyzed by Brenner (2010).

Results

Individuals of Crassostrea gigas, Ostrea edulis and Mytilus edulis were examined to assess and compare their parasite burdens at nearshore and offshore sites in the German North Sea. The study focused on the dominant macro-parasites described for these species: intestinal mytilicolid copepods of the genus Mytilicola, namely the species M. intestinalis and M. orientalis.

At the nearshore sites (1–3), M. orientalis was detected in the Pacific oyster C. gigas and, interestingly, M. intestinalis as well as M. orientalis in the blue mussel M. edulis. Furthermore, another parasitic copepod of the genus Modiolicola, metacercaria of unidentified trematode species as well as unidentified nematode species and the bristle worm Polydora ciliata (Polychaeta) were detected in the Pacific oysters from the nearshore sites. In addition, Renicola roscovi (Trematoda) was detected in the blue mussel as a further macro-parasite at the nearshore sites (Table 2). No macro-parasites were detected in oysters from offshore cultivation (sites 4–5).

In the blue mussel M. edulis both parasitic copepods M. intestinalis and M. orientalis (Fig. 2) were detected at all nearshore sites. Prevalence was first calculated for infested mussels that carried only one mytilicolid species (Table 2) (Fig. 3a): For M. intestinalis prevalence ranged between 13.3% (site 2), 26.7% (site 1) and 33.3% (site 3), for M. orientalis between 20.0% (sites 2, 3) and 26.7% (site 1). As both parasite species were found in some individuals, prevalence was also calculated for mussels infected by M. intestinalis and M. orientalis: It ranged between 6.7% (sites 1, 3) and 13.3% (site 2). In total, 46.7% (site 2) to 60.0% (sites 1, 3) of blue mussels were infested by mytilicolid copepods (M. intestinalis, M. orientalis, or both species). Mean intensity ranged between one and two individuals for both species at all sites (Fig. 3b). No significant differences could be observed. For a seasonal comparison of mytilicolid infestation site 3 was investigated twice: in summer M. intestinalis showed a lower prevalence (20.0%) while M. orientalis reached a higher prevalence (33.3%) as compared to the first sampling in winter (Fig. 3a). Also deviating from the winter scheme described above, no mussels were infested by both species at the same time in summer and the total prevalence was slightly lower than in winter (53.3%). Mean intensities were one copepod per mussel for both mytilicolid species during summer, also somewhat lower than in winter (Fig. 3b).

The parasitic copepod M. orientalis occurred in C. gigas at all nearshore sites: Its prevalence ranged between 32.4% (site...
Table 2: Prevalence (P) and mean intensity (mI ± conf. Interv.) of macro-parasitic species in oysters (C. gigas and O. edulis) and mussels (M. edulis) at three intertidal nearshore sites and two offshore sites employing suspended culture systems.

<table>
<thead>
<tr>
<th>Site</th>
<th>Classification</th>
<th>Host</th>
<th>Parasites</th>
<th>Copepoda</th>
<th>Mytilicola intestinalis</th>
<th>Mytilicola orientalis</th>
</tr>
</thead>
</table>
| 1 (JW) | Nearshore | C. gigas | Copepoda | 0 | 0 | 37.9 ± 8.2
| | | | | | | ± 1.5
| | | | | | | Mytilus edulis | 26.7 ± 1.4
| | | | | | | ± 0.3 |
| 2 (DN) | Nearshore | C. gigas | Copepoda | 0 | 0 | 45.1 ± 4.6
| | | | | | | ± 0.8 |
| | | | | | | Mytilus edulis | 20 |
| | | | | | | ± 1.5 |
| | | | | | | ± 0.5 |
| 3 (KB) | Nearshore | C. gigas | Copepoda | 0 | 0 | 32.4 ± 2.9
| | | | | | | ± 0.6 |
| | | | | | | Mytilus edulis | 33.3 |
| | | | | | | ± 2.0 |
| | | | | | | ± 0.0 |
| 4 (NG) | Offshore | O. edulis | Mytilicola intestinalis | 0 | 0 | 0 | 0
| | | | | | | Mytilicola orientalis | 0 | 0 | 0 | 0
| 5 (BD) | Offshore | O. edulis | Mytilicola intestinalis | 0 | 0 | 0 | 0
| | | | | | | Mytilicola orientalis | 0 | 0 | 0 | 0

No correlation could be observed between condition index (CI) and parasite infestation (intensity) of the examined oysters (Fig. 5a–c). Non-infested Pacific oysters showed essentially the same CI at all nearshore sites (15.5 ± 4.4 at site 1, 15.7 ± 3.4 at site 2 and 16.1 ± 3.6 at site 3). The CI of infested Pacific oysters ranged between 14.9 ± 4.2 (site 1), 15.1 ± 4.0 (site 2) and 15.8 ± 3.8 (site 3) with no significant differences (P > 0.05) between the nearshore sampling sites but a weak tendency of an increasing condition from west to east. In general, mean condition values of infested oysters showed no significant differences (P > 0.05) to the CI of not infested oysters.

To allow a better comparison of oyster size data regarding parasite burden we sorted the oysters into three defined size classes: S (< 100 mm), M (100–150 mm) and L (>150 mm) (Table 3). Site 1 (JW) and site 2 (DN) show similar patterns with highest prevalence in size class M, followed by S and L. At site 3 (KB), highest prevalence was observed in size class L, followed by M and S. Intensity did not show significant differences according to host size, except size class S showed significantly higher intensity at site 1 compared to site 2 (P < 0.05) and site 3 (P < 0.005). Mussels and offshore-cultivated oysters of both species were not separated into different size classes, all individuals belonged to size-class S.

Larval development of Mytilicola orientalis

*Mytilicola orientalis* females with completely developed nauplii in their egg sacs were found during the examination of Pacific oysters from site 2. After extracting an intact *M. orientalis* individual from the rectum of the oyster, the outer membrane surrounding the egg sacs broke and swimming nauplius larvae could be observed. A total of 70 nauplii were collected and put in a beaker with artificial seawater, which was exchanged every 2 days. Larvae were maintained at 15°C and larval development could be observed successfully for 2 weeks. After passing the first developmental stages (Fig. 6a, b) most larvae died. However, at least one individual reached the infective stage (first copepodite stage). Some individuals of each stage were conserved in alcohol for subsequent examinations.

Discussion

High abundances of the Pacific oyster in the Wadden Sea raised the question, if *Crassostrea gigas* could outcompete and replace the native blue mussel *Mytilus edulis* (Diederich, 2006; Schmidt et al., 2008; Markert et al., 2010). For a successful comparison of these species it is important to distinguish between direct competition (e.g. food and space) and indirect competition, for example parasite burdens, which were in the focus of this study. Knowledge on parasite burdens in relation to their geographical variations is a relevant factor, as the absence of macro-parasites, for example at offshore locations,
is an important (market-related) quality feature in shellfish production (Buck, 2007).

The effects of the parasitic copepods *Mytilicola orientalis* and *M. intestinalis* on their specific hosts are still under discussion. Lower condition indices of infested oysters and mussels have been reported, as well as *Mytilus* extinctions in the Netherlands and Germany have been related to infestations with *M. intestinalis* (Katkansky et al., 1967; Korringa, 1968). Also, a lower ability of recovery after spawning was observed for mussels (Paul, 1983). In contrast, other investigations found no effects in case of an infestation of oysters by *M. orientalis* (Chew et al., 1965) or negative effects were detected only in times of severe environmental conditions (Campbell, 1970; Gee et al., 1977): low food supply, severe winters and high infestation rates beyond 25 copepod individuals per host. Lower amounts of parasites could not be related to host size or gonad development, while seasonal cycles and other environmental factors caused greater effects on host condition. Long-term studies suggested that mytilicolid copepods should not be classified as harmful parasites but rather live as commensals in mussels and oysters (Gee and Davey, 1986; Davey and Gee, 1988; Davey, 1989; Steele and Mulcahy, 2001). Our study did not reveal any correlation between infestation rate and condition index of oysters and mussels of the eastern Wadden Sea, which in turn supports the concept of commensalism: At the western site we found three oyster individuals infested by more than 20 mytilicolid copepods and condition indices of host animals ranged between 9 and 20, which is well within the observed range of uninfested oysters. Small and medium size classes showed highest prevalences of *M. orientalis* at sites 1 and 2. In contrast, at site 3 a constant increase of prevalence with increasing size classes was observed. Intensity decreases from west to east in every size class. Within a sampling site, intensities decrease with increasing size class. As recruitments of *Mytilicola* are local and irregular phenomena (Robledo et al., 1994), parasitization can differ over years. Aguirre-Macedo and Kennedy (1999) observed much higher prevalences and intensities in the same size classes in 1995 in the Exe estuary (Great Britain) than in 1996. However, patterns of intensity regarding the oysters’ age classes were similar for both years. Older oysters contained more parasites per individual than younger ones (Aguirre-Macedo and Kennedy, 1999), which could not be confirmed for every size class in this study.

Interestingly, recent studies suspected furthermore that heavily infested mussels could provide a competitive advantage for invasive and less infested Pacific oysters, which could enhance their successful distribution along the North Sea coast and eventually the outcompeting of the indigenous mussel (Diederich, 2005; Diederich et al., 2005; Krakau et al., 2006; Schmidt et al., 2008; Elsner et al., 2010). However, Markert et al. (2010) did not observe any suppression of *M. edulis* in the eastern Wadden Sea, even if former mussel beds had transformed to *Crassostrea* reefs. Mean abundance of *M. edulis* (individuals m$^{-2}$) was even higher within the oyster reef than in the surrounding *Mytilus* bed. The fact that mussel shell size tended to be smaller in oyster reefs was not interpreted as a disadvantage, as mussels in turn benefit from their sheltered position between robust oyster shells, protected from predation by birds (Markert et al., 2010). Additionally, our study did not reveal different infestation levels for oysters and mussels: Parasite burdens in mussels and oysters of the eastern Wadden Sea showed prevalences for *M. orientalis* in *C. gigas* between 32% and 45% at all investigated nearshore sites. Mean intensities ranged between three and eight parasites per host decreasing from west to east. Prevalences of mytilicolid copepods in *M. edulis* ranged from 32% to 45% at all investigated nearshore sites. Mean intensities ranged between three and eight parasites per host decreasing from west to east. Prevalences of mytilicolid copepods in *M. edulis* ranged from 32% to 45% at all investigated nearshore sites. Mean intensities ranged between three and eight parasites per host decreasing from west to east.

![Fig. 2](image1.jpg)

(a) *Mytilicola orientalis* female with sharp dorsal appendages, (b) *Mytilicola intestinalis* female with rounded dorsal appendages (top) and for comparison *Mytilicola orientalis* (below)

![Fig. 3](image2.jpg)

Fig. 3. Prevalence (a) and intensity (b) of mytilicolid copepods in the blue mussel *Mytilus edulis* at the nearshore sites ($n = 15$ per site and sampling). No macro-parasites were observed at offshore site NG (Brenner, 2010)
between 45% and 60% with intensities around two parasites per host. This is in substantial contrast to studies conducted in the northern Wadden Sea (Krakau et al., 2006; Thielges et al., 2006, 2008; Elsner et al., 2010), where the infestation with mytilicolid copepods in terms of prevalence and intensity was nine times higher in *M. edulis* than in *C. gigas*. Prevalence of *M. intestinalis* in *M. edulis* was around 90% and the intensity was approx. four parasites per host. In contrast, prevalence and intensity of *M. orientalis* in *C. gigas* were very low: 10% prevalence at one single site (0% at all other sites) and the intensity was two parasites per host (Elsner et al., 2010).

Furthermore, clear host specificity has been observed in the northern Wadden Sea: *M. orientalis* was only found in *C. gigas*, while *M. intestinalis* occurred only in *M. edulis* (Elsner et al., 2010). In contrast, in the eastern Wadden Sea both species, *M. intestinalis* and *M. orientalis*, were found in *M. edulis*. This is the first widespread record of *M. orientalis* in *M. edulis* in Europe. In other regions, e.g. the Pacific east coast and Sea of Japan, *M. orientalis* is known to parasitize on a variety of hosts: *C. gigas*, *Ostrea lurida*, *M. edulis*, *M. californianus*, *M. crassistera*, *Crepidula fornicata* (Katkansky et al., 1967; Grizel, 1985). In Europe, only the Pacific oyster had been infested so far. In experiments, *M. orientalis* had shown a clear preference for infecting *M. edulis* instead of the oyster *O. lurida* (Bradley and Siebert, 1978). *M. edulis* has also been the most infected host in Canadian and US waters (Odlaug, 1946; Bernard, 1969). Stock (1993) was the first scientist who found very few *M. orientalis* individuals in mussels of the East Scheldt, Netherlands. Therefore, it is not surprising that *M. orientalis* has now been detected in *M. edulis* in the North Sea, too.

The question arises, whether *M. orientalis* has only recently started to infest the indigenous blue mussel in the North Sea region? Or has it been overlooked in former studies that focused on the common parasite of the blue mussel: *M. intestinalis*?

### Table 3

<table>
<thead>
<tr>
<th>Site #</th>
<th>Size class*</th>
<th>n</th>
<th>P [%]</th>
<th>ml [ind⁻¹]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>S</td>
<td>54</td>
<td>38.9</td>
<td>9.0 ± 7.2</td>
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<tr>
<td></td>
<td>M</td>
<td>22</td>
<td>50.0</td>
<td>6.8 ± 6.3</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>11</td>
<td>9.1</td>
<td>4.0 ± 0.0</td>
</tr>
<tr>
<td>2</td>
<td>S</td>
<td>46</td>
<td>43.5</td>
<td>4.2 ± 3.8</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>13</td>
<td>76.9</td>
<td>5.4 ± 3.2</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>12</td>
<td>16.7</td>
<td>5.5 ± 0.7</td>
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<tr>
<td>3</td>
<td>S</td>
<td>50</td>
<td>28.0</td>
<td>3.3 ± 3.2</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>9</td>
<td>33.3</td>
<td>2.7 ± 2.1</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>9</td>
<td>55.6</td>
<td>2.2 ± 1.3</td>
</tr>
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</table>

Fig. 4. Prevalence (a) and intensity (b) of the mytilicolid copepod *Mytilicola orientalis* in the Pacific oyster *Crassostrea gigas* at the nearshore sites (n ≈ 70 per site and sampling). No macro-parasites were observed at the offshore sites (n ≈ 50 per site).

Fig. 5. Non-significant relation of condition index and number of parasites in the Pacific oyster *C. gigas* at nearshore sites from west to east: site 1 (a), site 2 (b) and site 3 (winter) (c) (n ≈ 70 per site and sampling). No macro-parasites were observed at the offshore sites (n ≈ 50 per site).
M. intestinalis (Buck et al., 2005; Krakau et al., 2006; Thieltges et al., 2006, 2008; Brenner, 2010)? To distinguish M. orientalis from M. intestinalis complete animals need to be examined under the stereomicroscope. If mytilicolid copepods are detected in compressed preparations, species determination is usually impossible. Squeezed individuals of M. orientalis could have been misidentified and recorded as M. intestinalis. In 2004 and 2005 no mytilicolid copepods have been found in Pacific oysters of the northern Wadden Sea and M. intestinalis was only detected in M. edulis (Krakau et al., 2006; Thieltges et al., 2006). However, oysters and mussels were always examined by compressing the soft body parts between glass plates, as described above. In British waters M. intestinalis was reported to infest C. gigas (Aguirre-Macedo and Kennedy, 1999), but soft parts were also squeezed before examination. In our study only M. orientalis was found to infest C. gigas. This parasite was mentioned first by Elsner et al. (2010) for the North Sea region. They extracted Mytilicola individuals before squeezing and found M. orientalis in C. gigas but not in M. edulis.

On the one hand, the appearance of the Pacific oyster and its parasitic copepod in the North Sea may have initiated the recent infestation of the indigenous blue mussel with the introduced parasite. On the other hand, introduced Pacific oysters might divert parasite burdens taking pressure off native shellfish species (Krakau et al., 2006). The occurrence of other parasite species in the investigated oysters and mussels support this hypothesis: C. gigas was host to at least five different species (metacercariae of trematodes were not identified to species level as well as nematodes), M. edulis hosted only three different species. This is also in contrast to findings in the northern Wadden Sea where C. gigas was host to only two parasite species, M. edulis to nine species. Hence, in our study M. edulis exhibits lower parasite richness than C. gigas and also lower than that of M. edulis from the northern Wadden Sea. But due to the fact that not all parasites could be identified to species level it can only be seen as a trend, which requires additional thorough investigations.

Aquaculture activities along the North Sea coastline form the vector of introduction of several invasive species, e.g. Pacific oysters and their subsequent biogeographic distribution. First settlements of the Pacific oyster in the western part of the German Wadden Sea have been recorded in 1996 (Wehrmann et al., 2000). The further expansion of C. gigas is apparently related to natural distribution processes via larval drift (Nehring, 2006): pelagic stages of oyster larvae are carried northeast along the German coast as a result of prevailing current systems (Wehrmann et al., 2000). Subsequently, the invasion of C. gigas shows a clear northeastward direction beginning in the Netherlands, where spat of Pacific oysters was imported for aquaculture activities from the 1960’s on (Wehrmann et al., 2000, 2009; Brandt et al., 2008; Schmidt et al., 2008). M. orientalis, a common parasite of the Pacific oyster was co-introduced to the southern North Sea in the 1990’s (Elsner et al., 2010). Observed infestation rates of M. orientalis in C. gigas decrease from west to east: Hence, M. orientalis may have started the succession along the German coast with retardation, but essentially follows the invasion pattern of its main host.

From an economic point of view the absence of macro-parasites in shellfish products is certainly favourable. Oysters are commonly eaten raw and consumers would dislike the appearance of e.g. parasitic copepods, as they are easy to recognize due to their bright red colour and size (up to 25 mm). This is an issue, as it would result in a serious decrease of the oysters’ value. In contrast, mussels are cooked before consumption and copepods loose their colour and are no longer easy to detect.

Recent studies on macro-parasite body burdens by Buck et al. (2005) and Brenner (2010) reported a zero infestation rate of blue mussels at offshore locations in the North Sea. These observations can also be confirmed in this study for oysters. No macro-parasites have been detected in the European and Pacific oysters from the offshore cultivation sites. Absence of trematodes at offshore locations can be explained by their complex life cycle: they usually infest intertidal gastropods as first intermediate hosts (e.g. Littorina littorea and Hydrobia ulva) (Lauckner, 1984; Jensen and Mouritsen, 1992; Huxham et al., 2001; Bordalo et al., 2011). Due to the absence of these exclusively coastal organisms the parasite’s life cycle cannot be completed in offshore regions (Buck et al., 2005). Mytilicolid copepods and shell-boring polychaetes (e.g. Polydora ciliata) are abundant in inshore waters (Kent, 1981; Duvey, 1989; Ambariyanto and Seed, 1991; Thieltges et al., 2006). Their short planktonic larval phase restricts successful the dispersion to coastal waters and larvae drifting away from the coast are bound to die in the absence of potential hosts (e.g. predation and starvation), which are only available at very few selected culture locations (Buck et al., 2005). An experimental study conducted in British waters also showed that young C. gigas (<25 mm) were not infested by M. intestinalis and few infestations occurred in

Fig. 6. Larval stages of Mytilicola orientalis. (a) Nauplius larvae 3 h after hatching and leaving the egg sac, (b) after 72 h. Only one individual was observed and documented to reach this stage.
individuals around 45 mm (Dare, 1981). Furthermore, some parasites are known to occur only in mature molluscs (Thieltges et al., 2006). This implies, that spat oysters even if received from coastal regions with known parasite infestation would not yet be infested when transferred to offshore cultivation sites for grow-out.

Conclusions and outlook

(1) This study emphasizes the commercial advantages of offshore shellfish cultures with regard to parasite burdens: no macro-parasites have been found in the Pacific oyster Crassostrea gigas and the European oyster Ostrea edulis at the offshore sites. Furthermore, this study shows that the infestation of nearshore oysters and mussels with the parasitic copepod Mytilicola orientalis decreases geographically from west to east and therefore, the distribution of M. orientalis follows the invasion pattern of its main host, the Pacific oyster C. gigas. With regard to the formerly existing distribution gap of the invading Pacific oyster, we assume that the co-invasion of M. orientalis along the North Sea coast is not yet completed and abundances might still increase in the northeastern region. In the eastern Wadden Sea infection levels in oysters and mussels are similar, whereas in the northern Wadden Sea the parasite burden on mussels is much higher than that on oysters. We expect that parasite setting in the northern Wadden Sea will shift to similar infection levels as observed in this study for the eastern Wadden Sea region.

(4) Mytilicola orientalis also infests the native blue mussel M. edulis in the eastern Wadden Sea. Subsequently, the former described host specificity of M. orientalis for C. gigas is no longer valid for the North Sea.

Following ICES criteria for sustainable and environmentally friendly aquaculture shellfish transfers for aquaculture purposes on regional, national and international scales should be minimized and monitored continuously. These practices fostered the invasion of hitchhiking organisms, e.g. parasitic copepods, in the past (ICES, 2010) and will increase environmental and economic problems with introduced marine species in the future (Torchin et al., 2002). Acknowledgements

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