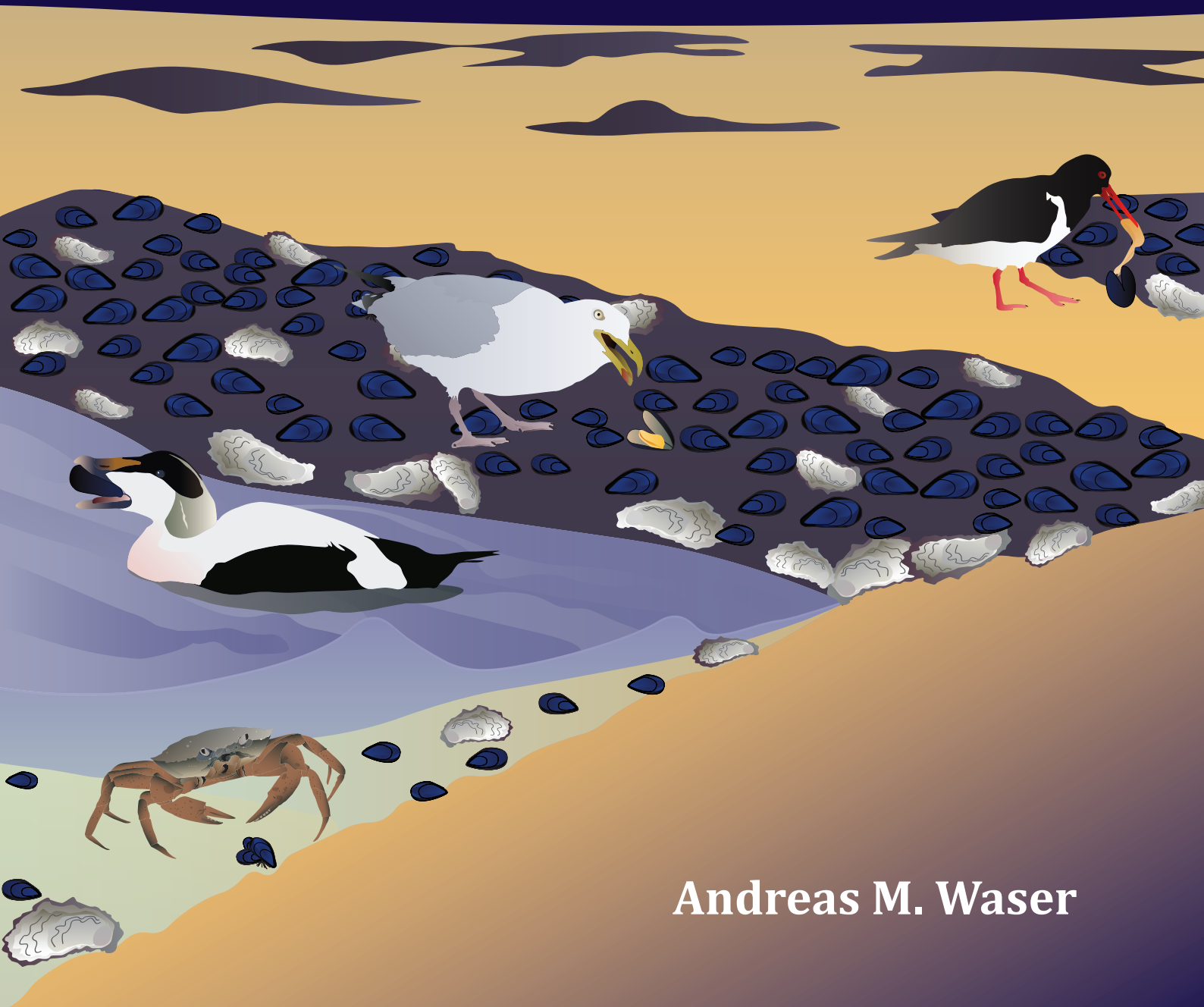# Predation on intertidal mussels

Influence of biotic factors
on the survival of epibenthic bivalve beds

Andreas M. Waser

Predation on intertidal mussels

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Predation on intertidal mussels

Influence of biotic factors on the survival of epibenthic bivalve beds

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door

Andreas Manfred Waser

geboren te Engelskirchen, Duitsland

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Contents

1 | General introduction

Andreas M. Waser

Coastal areas are amongst the most productive ecosystems in the world. They provide diverse habitats (e.g., open waters, subtidal gullies, and intertidal flats) that support a variety of different bird species and large numbers of aquatic secondary consumers such as shrimps, crabs and fishes (Pihl & Rosenberg 1982, Zwarts & Wanink 1993, van de Kam et al. 2004). Important components of these coastal areas are habitats rich in three-dimensional structure. These structures often provide key services through nutrient cycling, processing pollutants, and stabilizing land in the face of changing sea levels by trapping sediments and buffering land from storms. Moreover, they are very diverse habitats that provide substratum, shelter or food for many associated organisms, such as various fish and invertebrates. These structures are widely distributed and can be found throughout the globe. Typical examples for complex structures in tropical waters are coral reefs and mangrove forests, and vegetated habitats (seagrasses, salt marshes) or aggregations of reef building filter feeders (tube worms, mussels, oysters) for temperate systems.

Habitat-forming species in the Wadden Sea

In the course of human settlement and intensified urbanization coastal areas often have experienced profound ecosystem changes (Jackson et al. 2001, Lotze et al. 2005; 2006, Airoldi & Beck 2007). Particularly in temperate coastal areas, anthropogenic stressors, including habitat destruction and overexploitation, caused severe changes and led to declines of many coastal species (Wolff 2000a, Jackson et al. 2001, Lotze et al. 2006). One of these anthropogenically influenced coastal areas is the European Wadden Sea (Lotze et al. 2006). It is the largest temperate coastal ecosystem worldwide, bordering the Danish, Dutch and German North Sea coast. Over the last centuries, this area experienced intense human impact that caused dramatic losses of large predators and habitat-forming species (Reise 1982, Reise et al. 1989, Wolff 2000a;b, Lotze et al. 2005). Historically, several complex three-dimensional structures were common throughout the Wadden Sea. These were beds of blue mussels (*Mytilus edulis*) in the lower intertidal to upper subtidal zone, inter- and subtidal seagrass meadows (*Zostera marina*) and beds of European flat oysters (*Ostrea edulis*) and reefs of colonial tube worms (*Sabellaria spinulosa*) in the shallow subtidal and along deep channels (Riesen & Reise 1982, Reise 1982, Reise & Schubert 1987, Reise et al. 1989, Figure 1.1). These structures diversified the Wadden Sea landscape and provided diverse habitats for a variety of species depending on hard substratum, protection or food supply. During the late 19th and early 20th century, most of these habitat-building species were heavily exploited or destroyed directly or indirectly by fisheries and eventually disappeared (Reise 1982, Reise & Schubert 1987, Reise et al. 1989). After the disappearance of the *Ostrea-* and *Sabellaria-*reefs, mussel beds expanded both in the intertidal and subtidal down to 20 m depth and became the last complex-habitat left in many parts of the Wadden Sea (Riesen & Reise 1982, Reise 1982, Reise et al. 1989). Mussels remained the only common habitat-forming species in the Wadden Sea for many decades, until the non-native Pacific oyster (*Crassostrea gigas*) was introduced into the area. In the 1970s, the Pacific oyster was repeatedly imported for aquaculture purposes and soon after feral oyster populations established in the Wadden Sea. In the late 1990s and early 2000s, *C. gigas* proliferated extensively and became a common habitat-structure throughout the entire Wadden Sea (Reise 1998, Wehrmann et al. 2000, Troost 2010, Figure 1.1, Box 1.2).

Mussel fisheries in the Wadden Sea

In the 1950s, commercial mussel culture and mussel fisheries were introduced and proliferated throughout the Dutch and German Wadden Sea (Dijkema 1997, Seaman & Ruth 1997). Mussel beds were intensively harvested, and seed mussels (2–3 cm in shell length) of intertidal beds were fished for relaying to subtidal culture plots (Dijkema 1997, Seaman & Ruth 1997). These
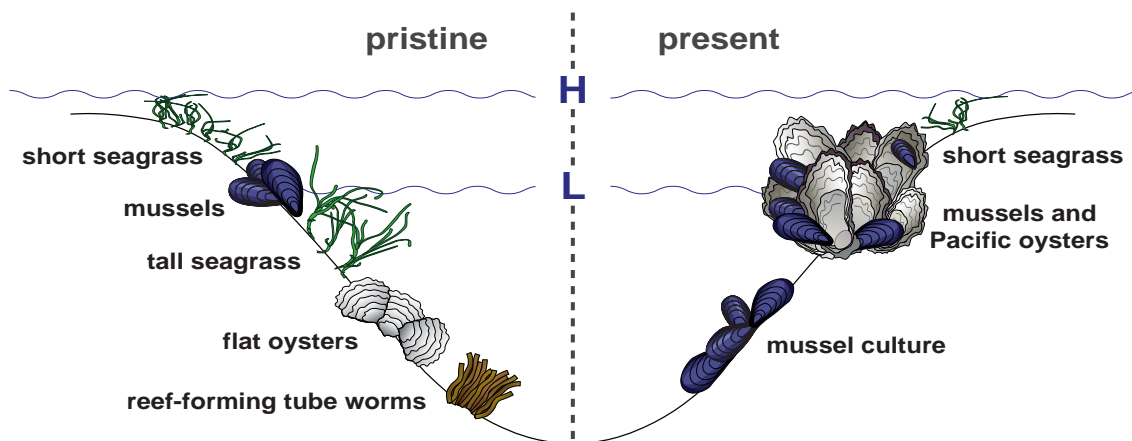
Figure 1.1: Schematic cross section through a tidal area including common habitat-forming structures in the pristine (left) and the present Wadden Sea (right). In the pristine Wadden Sea, common habitat-forming structures were short seagrasses: intertidal *Zostera noltii* and *Z. marina*, mussels: *Mytilus edulis*, tall seagrass: subtidal *Z. marina*, flat oysters: *Ostrea edulis* and reef-forming tube worms: *Sabellaria spinulosa*. Over the years, most of these structures disappeared and in the present day only mussels and short seagrass remain. A novel habitat structure is formed by the recently introduced Pacific oyster (*Crassostrea gigas*), which occupies similar intertidal habitats as the mussel. H and L indicate high and low tide level, respectively. Scheme after Reise (2005).

fisheries flourished for several decades and spatfall regularly replenished the inter- and subtidal mussel stocks (Beukema et al. 2015). In absence of recruitment events, however, essential rejuvenation of the mussel population failed and mussel beds were at risk to decrease in area. For instance, recruitment failures in Lower Saxony contributed to sharp declines in mussel bed area during the late 1980s and early 1990s (Obert & Michaelis 1991, Herlyn & Millat 2000). In the Dutch Wadden Sea, several successive years with low recruitment and ongoing fisheries resulted in the loss of nearly all intertidal mussel beds in the early 1990s (Dankers et al. 2001, Ens 2006, Figure 1.2). The disappearance of the beds and the consequential food shortages for molluscivorous birds (Oystercatcher and Eider; Beukema 1993, Beukema & Cadée 1996, Camphuysen et al. 1996; 2002, Smit et al. 1998) gave rise to intense public and political concern. In order to promote the recovery of intertidal mussel beds in the Dutch Wadden Sea, fishing quotas were introduced and some areas were closed for fisheries in 1993. Thereafter, bed area slowly increased in some areas (Dankers et al. 2001), but remained fairly low until good spatfall occurred in the early 2000s (Ens et al. 2004). Since then intertidal beds approximated a surface area of around 2000 hectares (Figure 1.2). However, mussel bed area in the western Dutch Wadden Sea remained low (Folmer et al. 2014) and many beds experienced important changes through the invasion of the Pacific oyster (*Crassostrea gigas*). After the introduction of *C. gigas* into the Wadden Sea, many intertidal mussel beds transformed into mixed bivalve beds or even into oyster dominated beds (Figure 1.2, Box 1.2).

Measures to increase surface area of intertidal mussel beds

Although the mussel bed area has recovered, bed area was still below the desired aim of an surface area of 2000–4000 hectares (e.g., CBS et al. 2017), which was based on aerial pictures taken from mussel beds in the late 1960s and 1970s (Dijkema et al. 1989, Dijkema 1991). In order to further increase the area of intertidal mussel beds, restoration measures were considered (Eriksson et al. 2010). However, the restoration of mussel beds is complicated and the creation of artificial mussel beds often proved unsuccessful (Ens & Alting 1996, Capelle et al. 2014, Dankers & Fey-Hofstede 2015, de Paoli et al. 2015). Many artificial mussel beds disappeared shortly after they had been created. The low survival of newly settled beds is also known
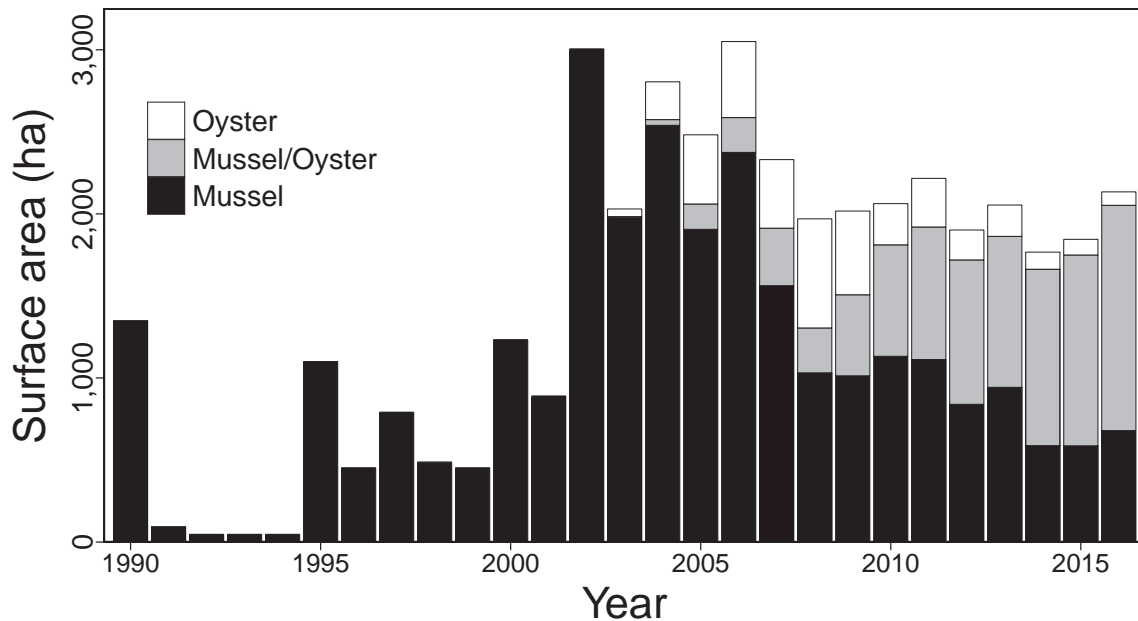
Figure 1.2: Development of the surface area (ha) of intertidal bivalves beds in the Dutch Wadden Sea between 1990 and 2016. For the period 1990–1994, mussel bed area was determined based on estimates of annual mussel stocks on intertidal flats during spring (see Bult et al. 2004, for detailed information on intertidal mussel stocks in the Dutch Wadden Sea). Surveys for the distribution and extent of intertidal bivalve beds in the period 1995–2016 were carried out each year during spring. Based on mussel and oyster coverage, beds were classified as either mussel bed (mussel > 5%; oyster < 5% cover), oyster bed (mussel < 5%; oyster > 5% cover) or mixed bed (mussel > 5%; oyster > 5% cover). Data: 1990–1994 from Ens et al. (2004); 1995–2016 from van den Ende et al. (2016b).

from naturally occurring mussel beds (Dankers et al. 2004). Mussel beds frequently disappear a few months after they are established, due to natural causes, such as storms, ice scouring or predation (Nehls & Thiel 1993, Zwarts & Ens 1999, Strasser et al. 2001). In order to increase the chances of successful mussel bed restoration, it is essential to gain more insights in the various environmental and ecological processes affecting the survival of mussel beds. The work presented in this thesis formed part of the 'Mosselwad' project, which was launched in 2010 to increase knowledge on several factors that play an important role in the survival and the stability of mussel beds. In this thesis I focus on crucial biotic factors that act upon the survival of intertidal mussel beds. In particular, I will focus on the predation on the intertidal mussels and the impact of the recent introduction of the Pacific oyster into the Wadden Sea.

Predation on intertidal mussels

In the Wadden Sea, mussels of various sizes (see Figure B1.2 in Box 1.1) are subject to predation by a suite of predators, including many invertebrates, fish and birds. Shortly after settlement, young mussels face predation particularly by shrimps (*Crangon crangon*), juvenile shore crabs (*Carcinus maenas*) and bottom living fish species (Reise 1977, van der Veer et al. 1998). Mussels in subtidal areas are preyed upon by *C. maenas*, starfish (*Asterias rubens*) and the Common Eider (*Somateria mollissima*) (Kamermans et al. 2009, Cervencl et al. 2015). In this thesis, I focus on the predation on intertidal mussels that have overcome the period of post-settlement predation (~ 5 mm in shell length). Therefore, predators preying solely on subtidal mussels (starfish) are not considered in this thesis.

Intertidal mussel beds provide food for several shorebird species, including molluscivorous Oystercatcher (*Haematopus ostralegus*), Red Knot (*Calidris canutus*), and Herring Gull (*Larus argentatus*) that feed on the mussels during low tide (Zwarts & Drent 1981, van de Kam et al.

2004). When the beds are submerged during high tide, intertidal mussels are also subject to predation by the shore crab and Common Eider (Dare et al. 1983, Nehls et al. 1997). Of these predators, Common Eider and Oystercatcher consume preferably larger mussels, whereas shore crabs, Herring Gull and Red Knot prey upon smaller sized specimen. Except for the Red Knot, which prefers thin shelled molluscs, like *Macoma balthica*, and only occasionally feeds on mussels (Zwarts & Blomert 1992, Piersma et al. 1993), bivalve eating birds are important mussel predators that can have substantial impact on intertidal mussel beds (Zwarts & Drent 1981, Goss-Custard et al. 1982, Nehls et al. 1997, Zwarts & Ens 1999). Regarding *C. maenas* little is known about its potential impact on intertidal mussel beds (but see McGrorty et al. 1990, Nehls et al. 1997). However, this species is a voracious predator, with a preference for molluscan prey (Ropes 1968, Elner 1981, Raffaelli et al. 1989). It has increased considerably in the Dutch Wadden Sea during the last 20 years, as revealed by annual sampling in the tidal channels (Tulp et al. 2012). Therefore, *C. maenas* is expected to have noticeable impacts on intertidal mussel beds (e.g., de Paoli et al. 2015). Assuming that the predation on post-settling mussels by juvenile shore crabs may play important roles in the rejuvenation of the intertidal mussel population and hence the bivalve bed persistence, the predation by juvenile crabs on mussels is also briefly discussed, although not of primary importance in this thesis.

Predation pressure on a given intertidal mussel bed, i.e. the amount of mussels taken by the different predators, depends on the one hand on predator specific energy requirements that determine their food demands and on the other hand on local predator abundances. Birds can be found foraging on mussel beds all year round and often peak in numbers during autumn and winter (Goss-Custard et al. 1982, Zwarts et al. 1996, Nehls et al. 1997). Shore crabs, in contrast, avoid intertidal areas during winter, spending cold periods in deeper waters (Naylor 1962, Thiel & Dernedde 1994). With increasing water temperatures in spring, crabs remigrate to shallower waters and exploit intertidal areas during high tide periods. Aside from regulating the crabs seasonal migration patterns, water temperature also acts on the activity of the crabs. As shore crabs are ectothermic animals, low temperatures result in reduced activity and suppressed feeding of *C. maenas* (Ropes 1968, Dries & Adelung 1982). Another factor potentially influencing feeding rates of shore crabs is the infection with parasites. Parasites are increasingly recognized for the important roles they play in natural food webs (Wood et al. 2007, Lafferty et al. 2008) and several studies have shown that parasites can have significant effects on the feeding rates of crustacean hosts (Dick et al. 2010, Haddaway et al. 2012, Toscano et al. 2014). For example, acanthocephalan infection resulted in an increased feeding of up to 30% in the gammarid hosts (Dick et al. 2010), while infection with rhizocephalan parasites caused a reduction in feeding rates of up to 75% in brachyuran crabs (Toscano et al. 2014). A wide range of parasites is known to infest *C. maenas* (Torchin et al. 2001, Zetlmeisl et al. 2011), but the extent and effect of parasite infection in this species in the Dutch Wadden Sea is largely unknown.

Finally, predation on mussels might be also influenced by the introduction of the Pacific oyster into the Wadden Sea and the accompanied change in habitat complexity of many mussel beds (Box 1.2). Previous work suggests that mussel beds showing high occurrences of Pacific oysters are less attractive for species preying on mussels. For instance, mussel-feeding birds may be negatively affected by the invasion of the oysters (Scheiffarth et al. 2007, Markert et al. 2013), since mussels may exhibit a reduced body condition resulting in a reduced prey profitability for the birds. Moreover, the increase in habitat complexity may additionally hamper access to the mussels (Eschweiler & Christensen 2011).

Study outline

This thesis is concerned with the predation on intertidal mussel beds in the Dutch Wadden Sea. More specifically, this thesis focuses on the impact of shore crabs and mussel-feeding birds on the stability of these biogenic structures. Attention will also be given to the recent introduction of the Pacific oyster, which led to a transformation of many mussel beds and is assumed to affect the predation on mussels considerably. In **Chapter 2**, we set out to explore the potential differences in waterbird distributions between different regions of the Wadden Sea. Specifically, waterbird numbers for the period 1999–2013 are compared in relation to the surface area of several foraging habitats among the tidal basins of the Dutch and German Wadden Sea. The habitat areas were characterized by data on abiotic characteristics (tidal exposure and sediment structure) and on distributions of epibenthic bivalve beds. Linear regressions are used to explore bird-habitat associations, where the regression coefficients reflect bird densities in the various habitats. We further use the model residuals to compare shorebird densities among the different Wadden Sea tidal basins corrected for the area of the different foraging habitats. In the subsequent chapters, we zoom in on the Dutch Wadden Sea. **Chapter 3** describes the fate of bivalve beds within the Dutch Wadden Sea for the period 1999–2013. Bed survival is analysed in relation to several covariates such as orbital speed, inundation time, bed size and bed type. In this respect, attention is also given to the recently introduced Pacific oyster. This species has invaded many intertidal mussel beds, which often led to the transformation into oyster dominated bivalve beds. The effect of oysters on the species community was furthermore explored in **Chapter 4** and **Chapter 5**. Whereas **Chapter 4** focusses on the impact of the oysters on the coastal bird fauna, **Chapter 5** explores methods to quantify the abundance of the shore crab (*Carcinus maenas*) on epibenthic bivalve beds with varying degrees of Pacific oyster occurrence. The studies described in these two chapters were based on surveys of several intertidal bivalve beds throughout the Dutch Wadden Sea and therefore also give valuable information on areawide abundances of mussel predators (Oystercatcher, Eider, Herring Gull and the shore crab). In **Chapter 6** we focus on the impact of oysters on the survival of different sized mussels while being exposed to shore crab predation. Mussel survival is documented in short-term experiments in presence and absence of Pacific oysters. In **Chapter 7** and **Chapter 8**, the potential importance of parasitism in relation to predation on mussels is explored. In these two chapters, we close knowledge gaps in parasite prevalences in brachyuran crabs in the Dutch Wadden Sea. **Chapter 7** describes an extensive field survey for the rhizocephalan parasite *Sacculina carcini* infecting shore crabs (*Carcinus maenas*) throughout the Dutch Wadden Sea. Specifically, the distribution of *C. maenas* infected with *S. carcini* is investigated at 12 locations and in 3 adjacent habitats (intertidal mussel beds, intertidal bare sand flats and subtidal gullies) along a tidal elevation gradient in the Dutch Wadden Sea. In **Chapter 8** we concentrated our sampling activities on the Western Dutch Wadden Sea and compared macroparasite richness, prevalence, and intensity among three brachyuran crab species. Next to *C. maenas*, the two invasive crabs *Hemigrapsus sanguineus* and *H. takanoi* were also screened for potential parasite infection. **Chapter 9** synthesizes the main findings and implications of this thesis in relation to existing literature. This chapter further illustrates the extent of predation pressure on mussels exerted by the different mussel-predators and provides ideas for future restoration measures.

Box 1.1   Biology of the blue mussel (*Mytilus edulis*)

The blue mussel (*Mytilus edulis*) is a sessile epifaunal bivalve, that attaches itself to hard surfaces using strong thread-like structures called byssal threads. Like most other bivalves, it is a suspension feeder that actively filters the surrounding water (e.g., Riisgård et al. 2011). It is widely distributed in the northern hemisphere, occurring in European waters from Spitsbergen to western France, and on the North American Atlantic coast from the Canadian Maritimes southward to North Carolina (Gosling 2015, and references therein). It occupies a broad variety of habitats, extending from high intertidal to subtidal regions, from fully marine to estuarine conditions as low as as 4–5 psu and from sheltered to extremely wave-exposed shores (e.g., Gosling 2015).
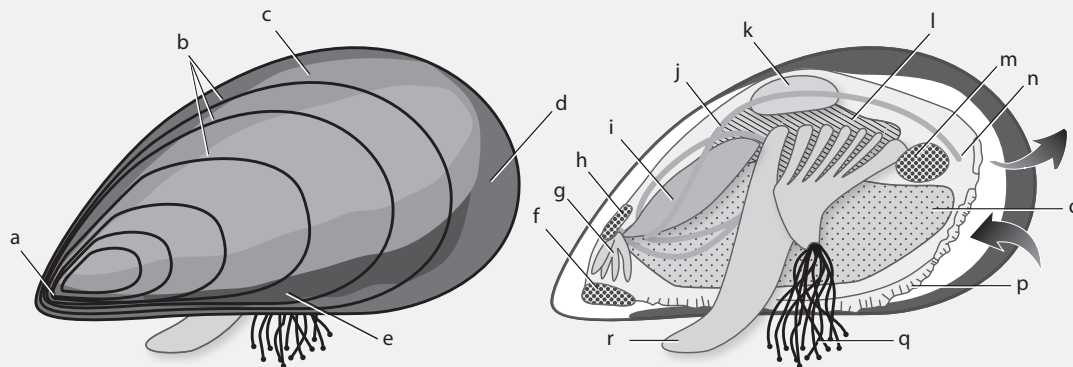
Figure B1.1: Scheme of the external and internal features of the blue mussel *(Mytilus edulis)*; a: anterior, b: concentric rings, c: dorsal, d: posterior, e: ventral, f: anterior adductor muscle, g: labial palps, h: anterior retractor muscle, i: stomach, j: intestine, k: heart, l: digestive gland, m: posterior adductor muscle, n: anus, o: gills, p: mantle edge, q: byssal threads, r: foot. Arrows indicate direction of water flow. Modified from Dankers & Fey-Hofstede (2015).

Morphology

The two shell valves are similar in size, and are roughly triangular in shape, elongating with rounded edges (Figure B1.1). The shell is smooth with a sculpturing of fine concentric growth rings. At the posterior of the animal are the inhalant and exhalant openings. Incoming water is filtered by the gills (ctenidium), which also function as respiratory organs. During the filtration process, lateral mantle cilia create a current, the latero-frontal cilia collect and the frontal cilia transport the captured particles towards the palps, where food particles are ingested. Faeces, together with rejected particles (pseudofaeces), are ejected through the exhalant opening.

   M. edulis is semi-sessile and is able to reposition itself with the help of a muscular foot. The foot possesses glands that are able to secrete byssal threads. These byssal threads emerge through the ventral part of the shell and serve as mooring lines for attachment of the mussel to the substratum. They are composed of four distinct regions: root, stem, thread and plaque. The root is embedded in the muscular tissue at the base of the foot. The stem divides into several sections that each merge into a separate thread. Each thread, in turn, ends in a plaque, that attaches to the substratum. For details on the composition of the byssus and the procedure of byssal attachment see Carrington et al. (2015).

Box 1.1

Life cycle

Like most marine invertebrates, *M. edulis* has planktonic larvae, that hatch from small eggs with little yolk. The larval phase of the life cycle of *M. edulis* (Figure B1.2) is generally comparatively short and lasts about 3–5 weeks (Bayne 1976, De Vooys 1999). Mussels are dioecious, and once gonads are ripe (in females typically orange; in males creamy-white) eggs and sperms are released into the water column for fertilization. Spawning occurs between spring and autumn (Bayne 1976, Pulfrich 1996, De Vooys 1999). Eggs are produced in huge quantities (up to 8 10^6 eggs per female), and develop rapidly after fertilisation. A few hours after fertilisation, a single egg has divided multiple times into a ball of cells that begins to swim once cilia appear. About 24 hours after fertilization, a ciliated trochophore stage is reached. At this stage the larvae are still reliant on the yolk for nutrients. Within a few days, trochophore larvae develop into veliger larvae. The veliger larvae possess a velum, a circular lobe of tissue bearing a ring of cilia, which serves as a swimming and feeding organ. After a few weeks, the larvae develop into pediveliger larvae (development of a foot) and are ready for settlement and subsequent metamorphosis. When a swimming pediveliger encounters a surface, the velum is retracted and the specific surface is explored by means of the foot.
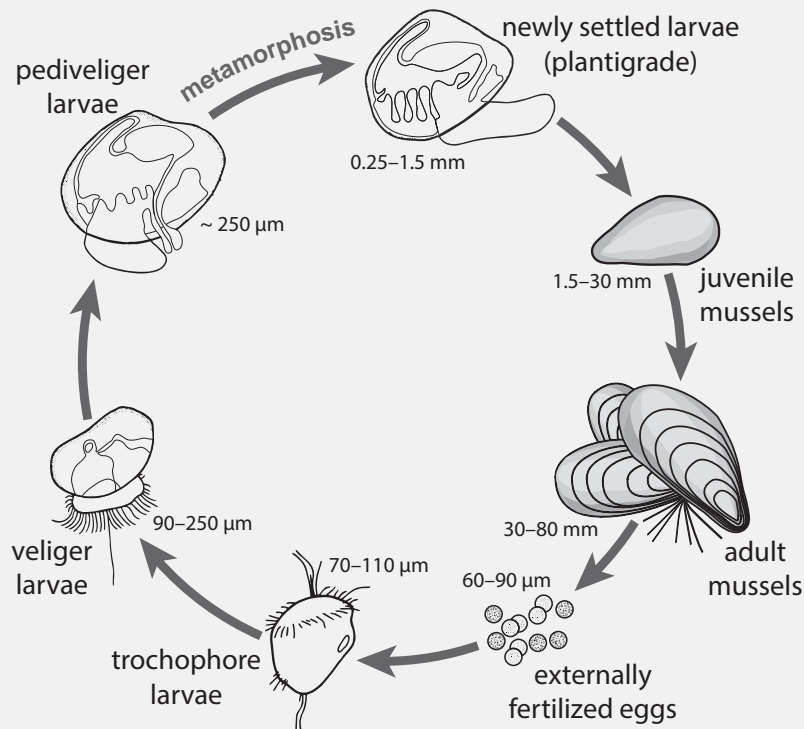
Figure B1.2: Generalized life cycle of the blue mussel *(Mytilus edulis)*. Modified from Stewart (1994).

Once a suitable substrate has been located, the larva stops crawling and begins attaching itself by means of byssal threads and metamorphoses into the juvenile form, now called a post-settled larva or plantigrade (Bayne 1964; 1976). During this metamorphosis, the larva loses its velum and develops gills. Settlement occurs on a wide variety of substrates, such as rocks, filamentous macroalgae, protruding tubes of *Lanice conchilega*, or onto shells of other bivalves including adult mussels (Pulfrich 1996, Callaway 2003, wa Kangeri et al. 2014). This addition of young individuals into an existing population surviving to a practical moment in time is called recruitment, determined days to months after settlement (Seed & Suchanek 1992).

Box 1.2   The Pacific oyster in the Wadden Sea

The Pacific oyster (*Crassostrea gigas*) is a large (up to 30 cm in shell length) epifaunal bivalve, that is permanently attached to hard surfaces. It is a suspension feeder, filtering large amounts of planktonic organisms and detritus from the surrounding water. The Pacific oyster originates from coastal areas of the north-western Pacific and the Sea of Japan (Troost 2010) and nowadays has successfully invaded all temperate coastal ecosystems around the world (Ruesink et al. 2005). It is an estuarine species, generally attached to firm bottom substrates, rocks, debris and shells from the lower intertidal to subtidal zones. It is able to reproduce in salinities of 14–32 psu and in temperatures of 20–35 °C (Korringa 1976, Quayle 1988, Mann et al. 1991). Due to aquaculture purposes, *C. gigas* was deliberately introduced to several locations along the European North Sea coast during the 1960s and 1970s in the belief that water temperatures were too cold to allow proliferation of the oysters (Troost 2010). This assumption proved to be wrong and feral Pacific oyster populations established along much of the European shoreline (Reise 1998, Drinkwaard 1999, Wehrmann et al. 2000, Troost 2010, Wrange et al. 2010, Lejart & Hily 2011, Herbert et al. 2016). In Europe today, *C. gigas* can be found in the Mediterranean and along the North Atlantic coast, including the British Isles, up to Scandinavia.

Like *M. edulis*, the Pacific oyster has planktonic larvae. It has a high reproductive output and produces up to 200 10^6 eggs per female (Kang et al. 2003). In the northern hemisphere gametes are mainly released into the water in July to early September, when water temperatures are highest. After a pelagic phase of about 3–4 weeks (Figure B1.3), the larvae settle onto hard surface, such as rock or bivalve shells, and their lower cupped valve becomes cemented to the substratum.
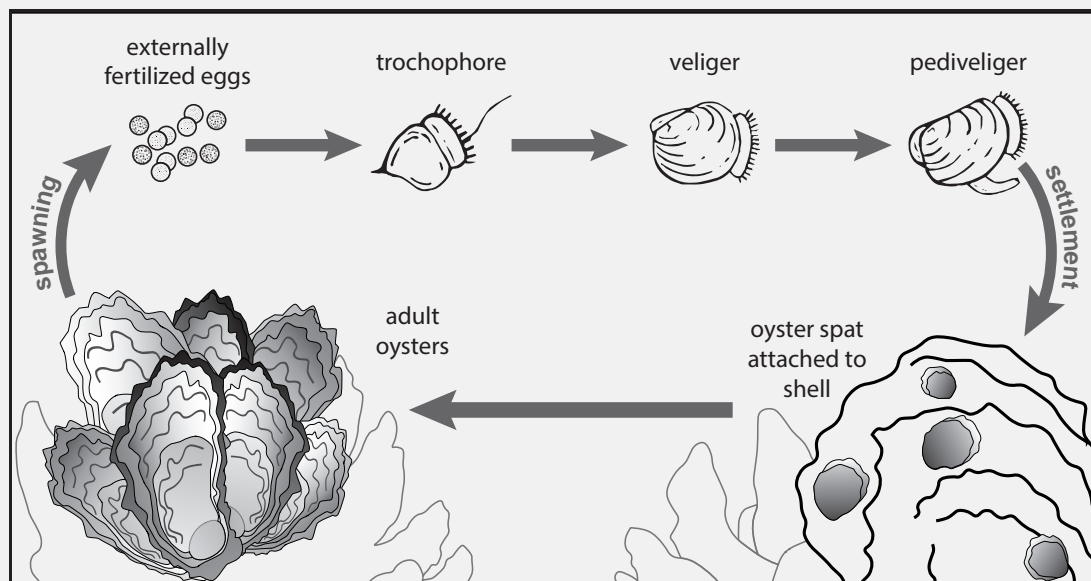
Figure B1.3: Life cycle of the Pacific oyster (*Crassostrea gigas*). Adults release gametes into the water column where fertilisation takes place. Fertilised eggs develop via the trochophore stage and the veliger stage into the pediveliger larvae within 3–4 weeks. Pediveliger larvae settle on suitable hard substrata and metamorphose into benthic juvenile stages. The oyster spat grows and after a period of 1–2 years they become mature and start reproducing themselves. Modified from Goldsborough & Meritt (2001).

Box 1.2

The creation of novel habitats in the Wadden Sea

Feral Pacific oysters were first recorded in the Wadden Sea near the Dutch island Texel in 1983 (Fey et al. 2010, Troost 2010). Since then, *C. gigas* has proliferated all over the Wadden Sea (Reise 1998, Wehrmann et al. 2000, Troost 2010). The colonization of tidal flats generally starts with few oyster larvae settling on pieces of hard substratum (shell fragments) or on mussel beds (Reise 1998, Diederich 2005, Troost 2010). Continuous oyster settlement eventually leads to increased oyster densities. High oyster densities, in turn, facilitate settlement of new oyster cohorts, as *C. gigas* larvae preferably settle on conspecifics (Diederich 2005). When oysters settle onto mussel beds, the beds may ultimately transform into an oyster dominated habitat (Figure B1.4), raising conservation concerns over competition with the native mussels.
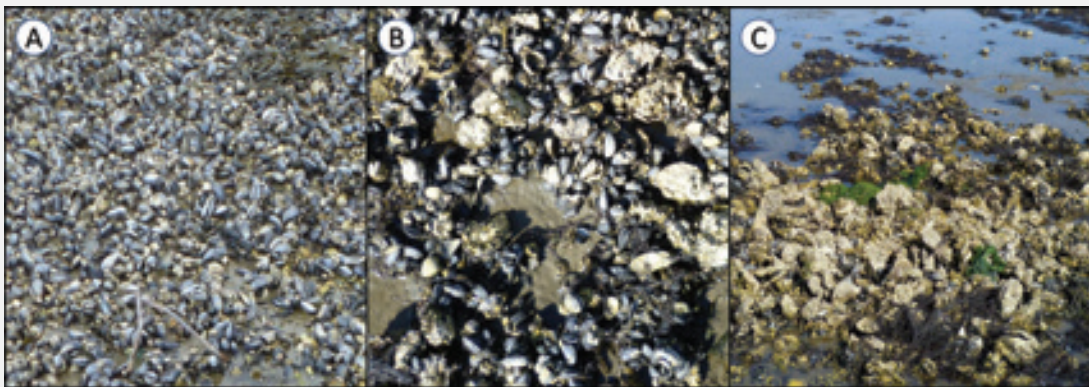
Figure B1.4: Different types of epibenthic intertidal bivalve beds in the Wadden Sea. A) A bed dominated by blue mussels (*Mytilus edulis*). B) A mixture of *M. edulis* and the non-native Pacific oyster (*Crassostrea gigas*). C) An oyster dominated bed.

Although mussels and oysters similarly provide hard substrata for sessile species (Kochmann et al. 2008), they differ in their size, three-dimensional structure, heterogeneity and formed micro-habitats (Gutierrez et al. 2003). As Pacific oysters reach maximum sizes that are up to 4-times larger than of native mussels, habitats formed by the invader show a high habitat heterogeneity and provide ample surface area for attachment and crevices for refuge of other organisms. Since both species also differ in their attachment mechanisms, aggregations of multiple specimens differ considerably in structural complexity. Mussels are adhered to the substratum via temporary byssus threads (Bell & Gosline 1996) and the continuous process of generating new threads leads to flexible and dynamic meshworks of individual mussels (van de Koppel et al. 2005). In contrast, Pacific oysters remain permanently attached to each other via an organic-inorganic adhesive (Burkett et al. 2010) and continuous larval settlement onto conspecifics leads to the creation of rigid and persisting structures (Walles et al. 2015a).

2 | Waterbird and habitat distributions in a major coastal wetland: revelation of regional differences in the Wadden Sea

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Abstract

The Wadden Sea is one of the world's largest intertidal wetlands bordering the coasts of the Netherlands, Germany and Denmark. It is a very productive ecosystem and supports large numbers of waterbirds. It is also exposed to numerous anthropogenic pressures. Several studies show the impact of intense shellfish fisheries on waterbirds in the Dutch Wadden Sea and some claim that these fisheries caused the ecosystem to collapse. However, few efforts were made to compare the ecosystem's state and functioning to other Wadden Sea regions where fishery was less intensive. Here, we investigated the numbers of 21 waterbird species across the Dutch and German Wadden Sea in relation to surface areas of six specific foraging habitats: epibenthic bivalve beds, four bare intertidal habitats differing in tidal exposure and sediment structure and the subtidal. We used linear regressions to explore the relationships between bird numbers at high tide roosts and surface areas of available foraging habitats in the vicinity of the roosts. Most species were positively correlated with bivalve beds and intertidal areas with low tidal exposure (below 28%) and rather coarse sandy sediment (median grain size > 138.5 µm). By inspecting the regression residuals, we identified higher bird abundances in the western Dutch Wadden Sea and in the south of Schleswig-Holstein, while lower abundances were found in the eastern Dutch Wadden Sea, in Lower Saxony and the north of Schleswig-Holstein. Interestingly, these patterns were similar for birds with contrasting prey preferences. These results are hard to reconcile with the suggested ecosystem collapse of the heavily exploited Dutch Wadden Sea. The observed regional differences in bird abundance may be related to the abundance of Peregrine Falcons, human disturbance and properties of the landscape. However, alternative explanations cannot be ruled out and further research is needed to identify the involved drivers.

Introduction

Coastal ecosystems are highly productive systems that support large numbers of aquatic secondary consumers such as shrimps, crabs and fishes and coastal birds (Pihl & Rosenberg 1982, Zwarts & Wanink 1993, van de Kam et al. 2004). Throughout history, coastal areas have been focal points of human settlement and in the course of intensified urbanization these areas often have suffered from biodiversity loss leading to dramatic degradation of food-web complexity and ecosystem services (Lotze et al. 2005; 2006, Airoldi & Beck 2007). Anthropogenic stressors affecting coastal areas include transformation of natural areas by large-scale hydraulic engineering (e.g., diking and land reclamation), pollution with nutrients and chemicals, intense exploitation of marine life (e.g., towed bottom fishing) and the introduction of non-native species (Wolff 2000a, Cloern 2001, Jackson et al. 2001, Lotze et al. 2006, Airoldi & Beck 2007, Katsanevakis et al. 2014).

The Wadden Sea is one of the world's largest coherent systems of intertidal sand and mud flats bordering the Danish, Dutch and German North Sea coast. It is extremely productive and serves as an important nursery area for many fish species and is major feeding area for birds (e.g., Zijlstra 1983, Zwarts & Wanink 1993, van de Kam et al. 2004). Both breeding and migratory populations of waterbirds depend on the intertidal prey. The latter, including many waterfowl and shorebird species, breed mainly in the High Arctic and visit the Wadden Sea as a fuelling and stopover site during long distant migrations or as a wintering site.

The Wadden Sea is also subject to considerable human pressures and is among the most anthropogenically influenced and degraded coastal ecosystems worldwide (Lotze et al. 2006). Today, conservation and management efforts of the different Wadden Sea regions (The Netherlands, three federal states of Germany (Lower Saxony, Hamburg and Schleswig-Holstein) and Denmark) are coordinated by the Trilateral Wadden Sea Cooperation (TWSC) with the support of the Common Wadden Sea Secretariat (CWSS). Under the implementation of the Trilateral Monitoring and Assessment Programme (TMAP), regional differences of a suite of different properties between the five Wadden Sea regions are summarised in regularly appearing so-called Quality Status Reports (Marencic & de Vlas 2009). Besides these reports, only few scientific studies have attempted to address the regional differences of the Wadden Sea (e.g., Swennen et al. 1989, Dijkema 1991, van Roomen et al. 2012, Folmer et al. 2014). Instead, many studies focussed on areas where research stations happened to be located (e.g., Philippart et al. 2007, Eriksson et al. 2010, Baird 2012, Schückel et al. 2015). One of the areas often considered in scientific studies is the Western Dutch Wadden Sea. This area has been subject to multiple pressures such as extensive changes of the hydrodynamics through the construction of the Afsluitdijk (Den Hartog & Polderman 1975), large scale changes in eutrophication (Philippart et al. 2007) and extensive mechanical shellfish fishery (Piersma et al. 2001, Ens 2006). Eriksson et al. (2010) claimed that these pressures caused the ecosystem to collapse towards a turbid state with low occurrence of seagrass meadows and reefs of benthic filter feeders and that large-scale restorations were required to restore the system. However, these claims have not been adequately substantiated and comparisons to other Wadden Sea regions that differ in the extent of human impact have not been made.

Recently, there have been efforts to investigate various characteristics of the entire Wadden Sea ecosystem on the level of tidal basins (van Beusekom et al. 2012, van Roomen et al. 2012, Folmer et al. 2014; 2016). Tidal basins are natural morphological subunits of the Wadden Sea that share hydrodynamic and trophic properties. Such ecosystem scale investigations are useful from scientific perspectives and may provide important information for management.

We surmise that an ecosystem collapse should be reflected in the waterbird community. The quality of a coastal area for waterbirds depends on its feeding conditions which depend on the density and availability of invertebrate prey (Zwarts & Blomert 1992, Goss-Custard et al. 2002, van de Kam et al. 2004, Folmer et al. 2010). The invertebrate benthos community strongly

depends on habitat properties such as exposure time and sediment grain size (Compton et al. 2009, Kraan et al. 2010). In addition, waterbird occurrence and foraging success also directly relates to habitat characteristics such as exposure time and sediment grain size (Quammen 1982, Goss-Custard & Yates 1992, Mouritsen & Jensen 1992, Yates et al. 1993). Since the total amount of prey per tidal basin depends on the areas of the different habitats, the areas can be considered as proxies for carrying capacity.

Here, we explore the variation in bird numbers in relation to characteristics of foraging areas at the scale of tidal basins within the Wadden Sea. As detailed information on the distribution of invertebrate prey organisms is not available for the entire study area and only restricted to the Dutch Wadden Sea (Compton et al. 2013), we used detailed information on abiotic characteristics of the Wadden Sea to classify the area of each tidal basin into five different foraging habitats: the subtidal and four intertidal habitats differing in tidal exposure and sediment structure. Moreover, to determine the importance of foraging habitat connected with benthic assemblages of complex physical structure, we make use of data on the distribution of epibenthic bivalve beds consisting of blue mussels (*Mytilus edulis*) and non-native Pacific oysters (*Crassostrea gigas*). These epibenthic structures provide a habitat for many benthic and epibenhtic species (e.g., Buschbaum et al. 2009) and attract numerous birds that feed on bivalves and the associated benthos (Zwarts & Drent 1981, Goss-Custard et al. 1982, van de Kam et al. 2004, chapter 4: Waser et al. 2016a). The aim of our study is to look for signs of ecosystem collapse by analysing relationships between habitat and abundance of 21 different waterbird species in 35 connected tidal basins in the Dutch and German Wadden Sea. We used linear regressions to estimate the associations for the 21 bird species. The regression coefficients estimate bird densities in the various habitats. The residuals of the final models, are used to identify tidal basin specific differences in bird numbers in relation to the area of foraging habitat.

Material and Methods

Study region and tidal basins

The Wadden Sea is a shallow tidal wetland located in the south eastern part of the North Sea bordering the coastal mainland of Denmark, Germany, and the Netherlands (Figure 2.1). It is one of the world's largest coherent systems of intertidal sand and mud flats, comprising an intertidal area of about 4500 km^2 (ca. 8000 km^2 total area). The area contains coastal waters, intertidal sandbanks, mudflats, shallow subtidal flats, drainage gullies and deeper inlets and channels. Apart from the central Wadden Sea, barrier islands are found throughout the entire area. Tidal amplitudes range between 1.5 and 3.0 m in the north-eastern and south-western Wadden Sea and exceed 3.0 m in the central part. Based on various shared morphological, hydrodynamic, and trophic properties (van Beusekom et al. 2012), the area can be divided into a total of 39 tidal basins, which are delineated by the mainland, barrier islands, tidal divides and are connected to the North Sea via tidal inlets.

Bird feeding habitats

We used three different data sets to characterize the different tidal basins into six habitat types: bivalve beds (B), high coarse-grained intertidal (HC), high fine-grained intertidal (HF), low coarse-grained intertidal (LC), low fine-grained intertidal (LF) and subtidal areas (S). The first data set consists of annual bivalve bed distributions throughout 36 tidal basins (TB 4–39) in the German (Lower Saxony, Hamburg, Schleswig-Holstein) and Dutch Wadden Sea (Figure 2.1) for the period 1999–2013. For convenience, the small Hamburg National Park (137 km^2) will be considered together with the Lower Saxonian Wadden Sea as region 'Lower Saxony'. The Danish
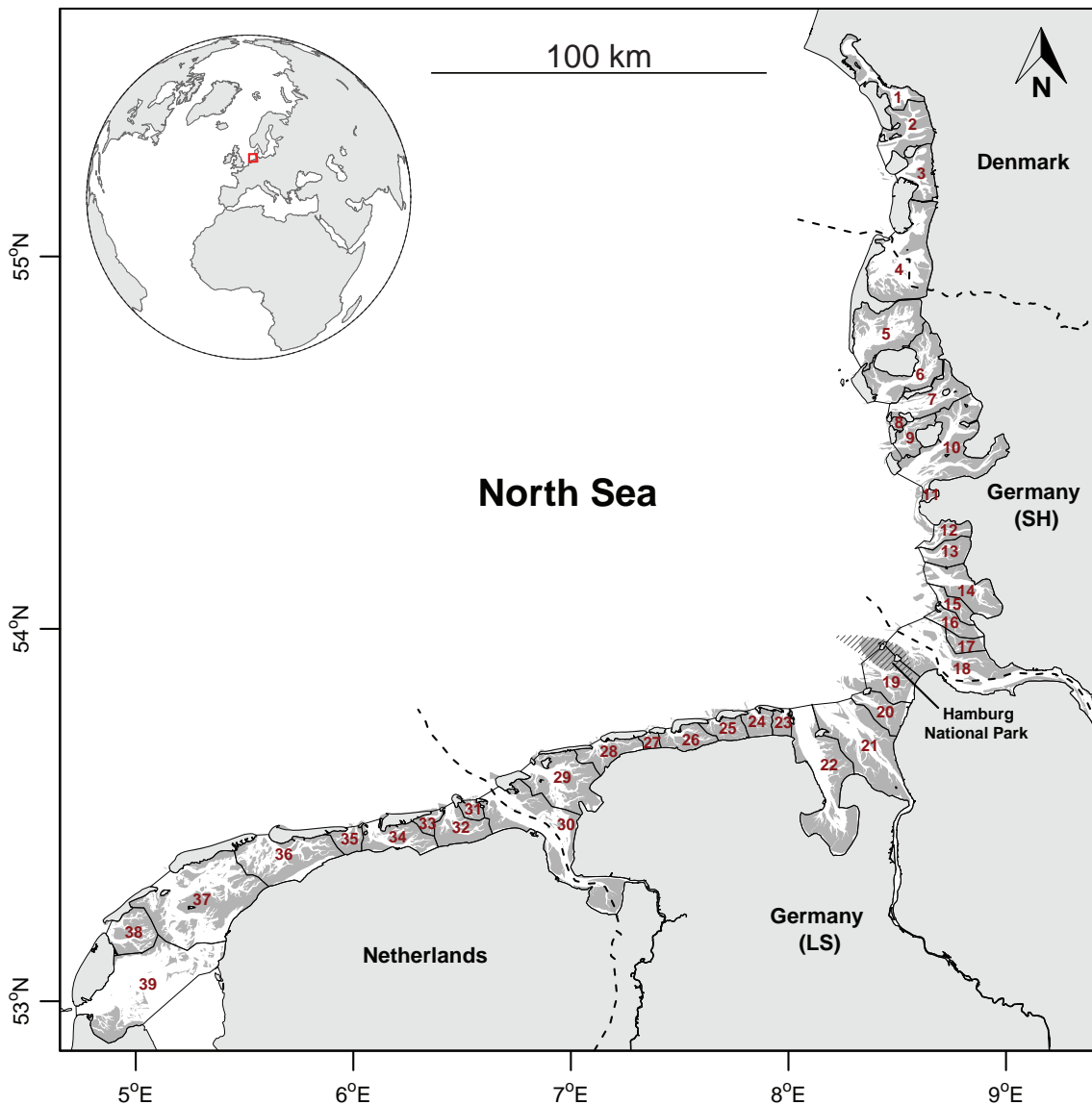
Figure 2.1: Map of the Wadden sea, including different regions (the Netherlands, Lower Saxony (LS), Hamburg, Schleswig-Holstein (SH) and Denmark) and tidal basins (number code). White areas: subtidal; dark grey areas: intertidal flats exposed during low tide; light grey: land; dashed lines: borders between the different Wadden Sea regions.

Wadden Sea (TB 1–3 and the northern half of TB 4) was not included in this study because bivalve beds in Denmark were mapped following a different survey protocol and surveys were only conducted every two years till monitoring was stopped in 2008. Therefore, our study focussed on the tidal basins 4–39, at which TB 4 comprised beds only from its southern part. Due to practical reasons for allocating bird numbers to tidal basins (see section counts and population numbers of waterbirds), we merged the basins 8 and 9 (Figure 2.1) so that in total 35 basins were considered in the present investigation.

In Germany and the Netherlands, the contours of bivalve beds were determined by a combination of aerial surveys and photographs, and by walking along the bed edges with a hand-held GPS following a common definition of a bivalve bed (de Vlas et al. 2005). Aggregations of epibenthic bivalves are considered as bivalve bed if the percentage cover by bivalves equals or exceeds 5%. In the Netherlands (TB 30–39) intertidal bivalve beds were monitored by Wageningen Marine Research (WMR, formerly IMARES) and MarinX; in Lower Saxony (TB 18–30) by the National Park Authority Wadden Sea Lower Saxony (NLPV); in Schleswig-Holstein

(TB 4–18) by BioConsult SH on behalf of the Schleswig-Holstein Agency for Coastal Defence, National Park and Marine Conservation, National Park Authority (LKN SH). More insight and region specific survey details of the bivalve monitoring can be found in Folmer et al. (2014).

The other two datasets used to characterize the tidal basins were raster layers of the exposure time (i.e., the mean fraction of time that the seabed is exposed to the air) and the median grain size for the entire study area with a resolution of 200 × 200 m. Data on the exposure time were simulated with the General Estuarine Transport Model (Burchard & Bolding 2002), which is designed for coastal ocean simulations with drying and flooding of intertidal flats. A bathymetry with resolution 200 × 200 m for the entire Wadden Sea was used as a basis for the simulation of the exposure time over the period 2009–2011 (see Folmer et al. 2016, Gräwe et al. 2016, for a detailed description). Sediment median grain size (mgs, μm) data covering the Dutch and German Wadden Sea, which was compiled from various sources within the AufMod project, were provided by the German Federal Maritime and Hydrographic Agency (Bundesamt für Seeschifffahrt und Hydrographie; BSH).

We used the annual data on bivalve bed distributions and computed the bivalve bed area per tidal basin by summing the areas of the separate bivalve bed polygons intersecting with the tidal basins. Data of the exposure time was used to split the areas that were not classified as bivalve bed into subtidal (< 1% tidal exposure) and intertidal areas (> 1% tidal exposure). We used the median exposure time (28%) of the entire German and Dutch Wadden Sea to split the intertidal area into equally sized lower intertidal (< 28%) and upper intertidal (>= 28%) classes. Both the lower and higher intertidal were furthermore divided into fine- and coarse-grained areas. This division was based on the median of the mgs data (138.5 μm) in the Dutch and German Wadden Sea. All cells below this median were classified as fine-grained and the ones above as coarse-grained. Thus, the fine-grained areas were composed of fine sediments: silt (4–62.5 μm) and very fine sand (62.5–125 μm), and the coarse-grained areas consisted of fine sand (125–250 μm) and medium sand (250–500 μm).

Counts and population numbers of waterbirds

Waterbirds (gulls: Laridae, waders: Charadrii and waterfowl: Anatidae) were counted on high tide roosts adjacent to intertidal flats and Common Eider (*Somateria mollissima*) was counted from aeroplane. The high tide roost counts are coordinated by the Joint Monitoring of Migratory Birds (JMMB) project of the Trilateral Monitoring and Assessment Program (TMAP; see van Roomen et al. 2012, Blew et al. 2016, for details) and are organized in a dataset dating back to the season 1987/1988. The aerial surveys for Common Eider are organised on a regional level. In the Netherlands (TB 30–39), aerial counts are organised by Rijkswaterstaat (RWS) and in some years additional counts were performed by WMR. The aerial counts in Lower Saxony (TB 18–30) are organised by NLPV and in Schleswig-Holstein (TB 4–18) by LKN SH. In Denmark (TB 1–4), aerial counts were carried out by the Danish Centre for Environment and Energy (DCE, formerly NERI). In all regions, aerial winter counts were consistently performed from 1992 onwards. In this study, we only focussed on bird numbers for the period 1999/2000–2013/2014 (hereafter period 1999–2013) to determine average bird numbers per tidal basin, since a continuous data set for epibenthic bivalve beds is only available for the years 1999–2013. We focussed on 21 species that primarily feed on prey sources within intertidal flats. Following Blew et al. (2016), the bird species were grouped into four different feeding guilds; molluscivorous: species predominantly feeding on bivalves, polychaetivorous: species that preferably feed on worms, benthivorous: species that opportunistically feed on various benthic macroinvertebrates and piscivorous: species which diet includes a high portion of fish (Figure 2.3).

Data resulting from three types of counts were used: 1) simultaneous total counts of all waterbird species at all high-tide roosts along the Wadden Sea (two counts per year took place on a trilateral level, and up to three additional counts on regional level), 2) frequent counts (at least once a month) of all waterbird species in a selection of high-tide roosts (see Laursen et al. 2010, for detailed methodology), 3) aerial winter counts of Common Eiders (Laursen et al. 2008, Cervencl et al. 2015).

Based on the assumption that birds that are counted on roosts during high tide forage on the nearest emerging tidal flats during low tide, we matched the numbers counted at the roosts with the nearest tidal basin (van Roomen et al. 2012). When a roosting area was located at the border of two tidal basins, bird numbers were divided equally between the two tidal basins. This procedure was used for all tidal basins except basins 8 and 9. Since the allocation of roosting areas to the small tidal basin 8 was impractical, it was merged with basin 9. We calculated the seasonal average of the period July–June population sizes per tidal basin based on monthly counts. The use of 12 months in these seasonal indices adds robustness to the index and combines several functional periods (migration, wintering, and moult) for the same species. Not all roosting areas were monitored monthly and therefore missing counts were imputed with UINDEX (Bell 1995), on the basis of site, month and year factors estimated from the non-missing counts (Underhill & Prys-Jones 1994).

For Common Eider, aerial counts during winter were used to determine the population numbers of the different tidal basins. Each year, at least one aerial count per Wadden Sea region was conducted in January or February (Figure S2.1). In the Netherlands, aerial counts are conducted during high tide using a high-winged plane flown along predefined north-south oriented transects covering the entire area of the Dutch Wadden Sea and the adjacent North Sea coastal zone (see Cervencl et al. 2015, for detailed methods). The German counts (Lower Saxony and Schleswig-Holstein) are performed during low tide, when Eiders are concentrated in a few tidal creeks, following the edges of the tidal channels throughout the entire German Wadden Sea. In Denmark, groups of Eiders are counted during high tide following a consistent flight route (e.g., Laursen et al. 2008). For each group of Common Eider recorded in the different areas, the geographical location as well as the number of individuals was determined. Based on the geographical locations, flocks of Eiders were allocated to the different tidal basins in order to arrive at a total number of individuals per basin. To combine counts of Eiders with the high tide roost counts, aerial counts of a certain year were allocated to the preceding season. For example, aerial counts in January or February 2011 were assigned to 2010 (2010/2011).

Data analysis

We calculated the average surface area of each habitat per tidal basin from the 15-year data series. For the different bird species, we calculated averages and trends in numbers in the entire Wadden Sea and per tidal basin. We analysed the relationships between the number of birds per species and the surface area of the different habitat types per tidal basin with linear regression models. To avoid possible spurious correlations, we only included habitats which are known to be used for foraging by a given species as predictors in the regression models. For instance, only the surface area of the subtidal was included as predictors for species that are known to forage in subtidal areas (i.e., Common Eider, European Herring Gull (*Larus argentatus*), Great Cormorant (*Phalacrocorax carbo*), Black-headed Gull (*Larus ridibundus*) and Common Gull (*Larus canus*); Leopold et al. 1998, Kubetzki & Garthe 2003, Cervencl et al. 2015). All regression models were forced through the origin by fixing the intercept to zero so that the regression coefficients can be interpreted as the average densities (number of birds per hectare) per habitat type. As densities of organisms can only be greater or equal to zero, we did not accept negative regression coefficients. Therefore, we first estimated the initial (full) model based on the possible habitat types for each bird species. Next, we reduced the full model by omitting predictors with

negative coefficients (effectively setting the density of a species in a given habitat to zero). If more than one of the predictors had negatives coefficients, they were omitted in order of increasing P-values. The resulting model only contained predictors with positive coefficients and is labelled 'plausible model'. The plausible model was further reduced on the basis of statistical criteria. Particularly, we selected the final model, from all possible plausible models, on the basis of minimization of the Akaike's information criterion (AIC). To compare bird abundances in the different habitats among the different tidal basins, we inspected the residuals of all final models. We used standardized residuals (residuals divided by their standard deviation), which allow direct comparison between the different bird species. To help visualize the non-linear patterns of the residuals across the tidal basins, local regression smoothers (LOESS with local polynomial weighted fitting) were used. All statistical analyses were performed using the R platform (R Development Core Team 2015).
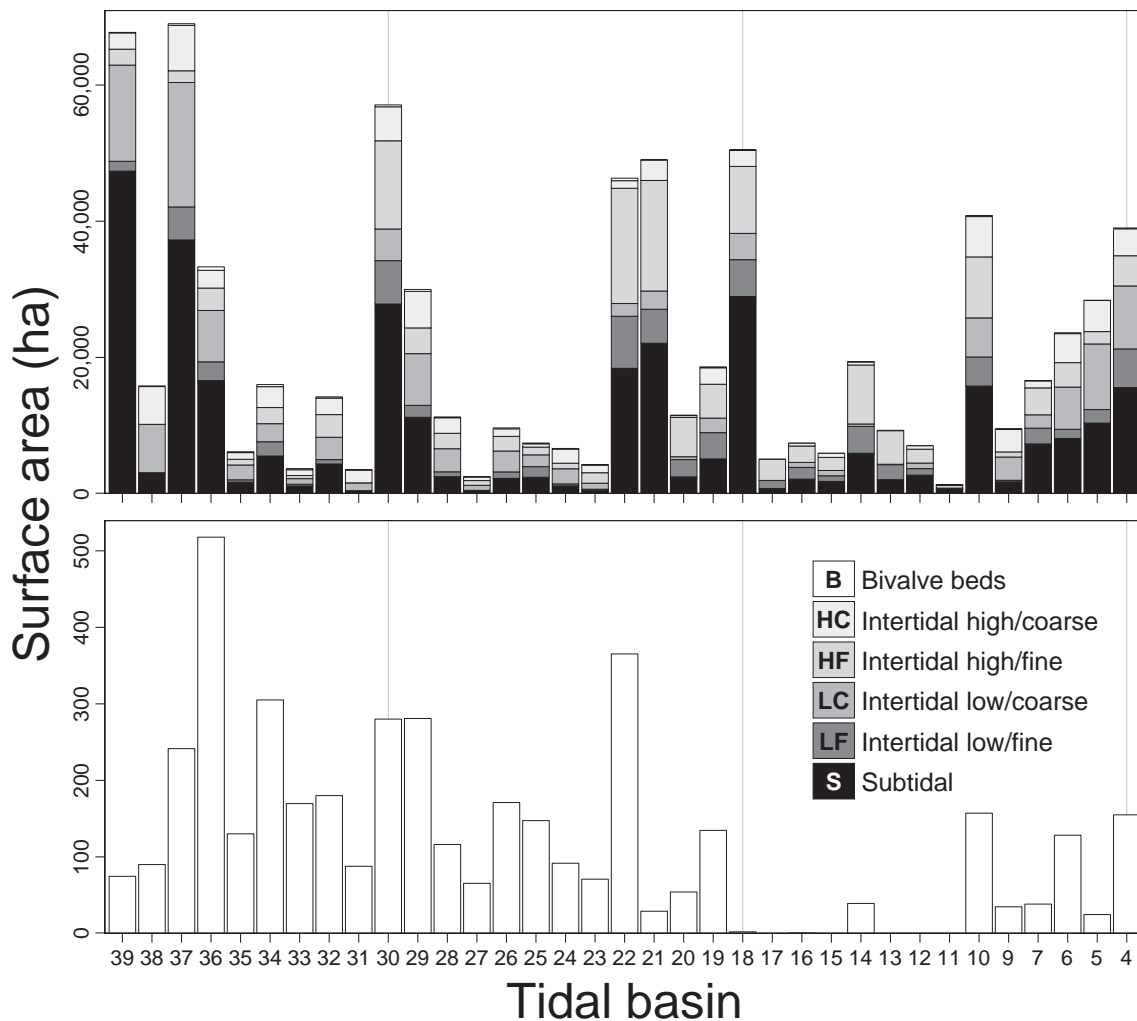
Figure 2.2: Average surface areas (ha) of the six habitats in tidal basins 4–39. The upper panel represents the area of all habitats in each tidal basin and the lower panel presents the area of bivalve beds. The tidal basins are aligned from south-west to north-east, starting with tidal basin 39 in the western Dutch Wadden Sea. Vertical lines indicate borders between the different regions. Note that tidal basin 9 is the merger of basins 8 and 9.

Results

Surface area of bird feeding habitats

The tidal basins differed in total surface area and in their habitat composition (Figure 2.2). The largest basins (39, 37, 30, 22, 21 and 18) hold the biggest fractions of subtidal area of about 50% or more, while in the smaller basins the fractions of intertidal areas are largest. The area of the different intertidal habitats showed a high variability among the different tidal basins. Whereas the intertidal of most tidal basins showed high fractions of coarse-grained sediments, in the central Wadden Sea (TB 11–22), where barrier islands are absent, the intertidal area was dominated by habitats of fine-grained sediment (Figure S2.2). It should be noted that the area of the different intertidal habitats may vary slightly between years due to variation in the area of bivalve beds. Bivalve beds occurred in most tidal basins for the entire period. However, they were virtually absent in the tidal basins 11–18 (Figure 2.2). Detailed insight into the 15 year (1999–2013) time series of the different habitats for all tidal basins is presented in the supplementary material (Figures S2.3–S2.8).
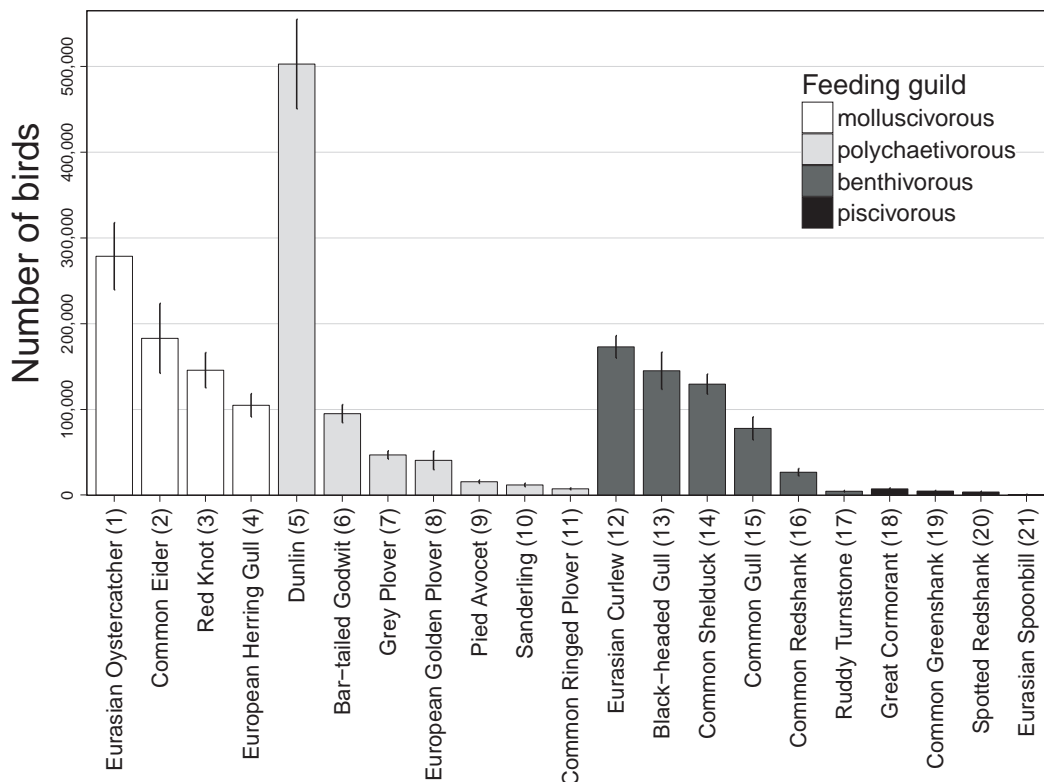
Figure 2.3: Averages (± SD) of the seasonal population sizes of the 21 investigated bird species in the entire Wadden Sea for the period 1999–2013. The species were classified into four different feeding guilds (after Blew et al. 2016). Numbers in parentheses facilitate species identification in Figure S2.30 in the supplementary material.

Numbers of waterbirds

Figure 2.3 presents the average population sizes and Figure 2.4 shows the demographic trends. The population sizes of most species were stable or only showed marginal changes (average annual rate of change < 2%) over the period 1999–2013. Considerable declines (rate of annual population decline) were found for the Spotted Redshank (*Tringa erythropus*; -4.6%), Eurasian Oystercatcher (*Haematopus ostralegus*; -3.1%), Common Eider (*Somateria mollissima*; -2.8%),
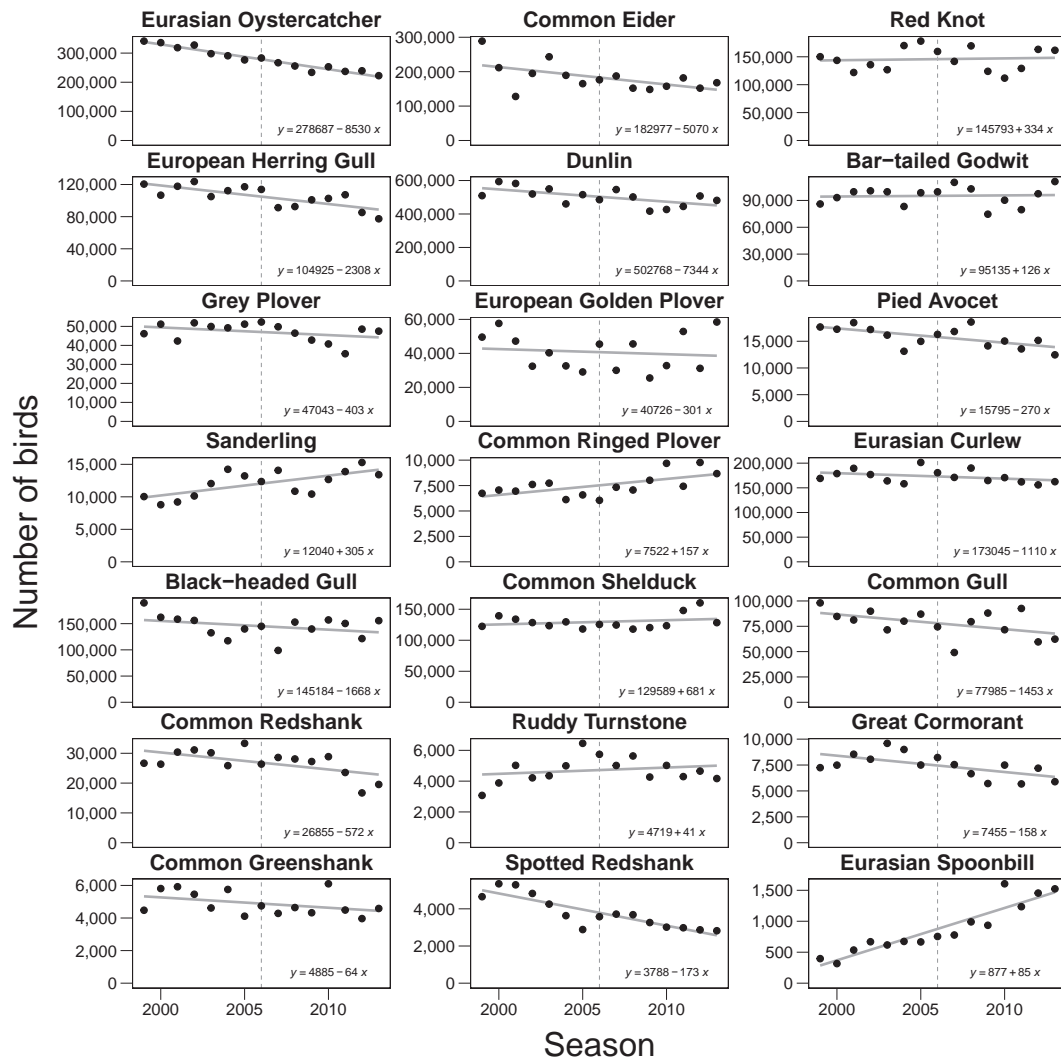
Figure 2.4: Seasonal population sizes of 21 bird species in the entire Wadden Sea for the period 1999–2013. Regression equations indicate average population sizes (dashed vertical lines: adjusted intercepts) and demographic trends over the 15-year period.

Herring Gull (*Larus argentatus*; -2.2%), Common Redshank (*Tringa totanus*; -2.1%) and Great Cormorant (*Phalacrocorax carbo*; -2.1%) (Figure 2.4). Considerable increases (rate of annual population growth) were found for species with relatively small populations (Eurasian Spoonbill (*Platalea leucorodia*; 9.6%), Sanderling (*Calidris alba*; 2.5%) and Ringed Plover (*Charadrius hiaticula*; 2.1%); Figure 2.4). Details on bird numbers in the different tidal basins for the period 1999–2013 are provided in the supplementary material (Figures S2.9–S2.29).

Bird density-habitat models

Overall, the area-based regression models had a high predictive power and all R^2 values were equal to or greater than 0.55 (Table 2.1). The lowest R^2 values were obtained for species with small population sizes, i.e. Sanderling (0.55), Ringed Plover (0.55) and Spoonbill (0.57). The highest R^2 values were obtained for the common species Eurasian Oystercatcher (0.89), Black-headed Gull (0.89) and Eurasian Curlew (*Numenius arquata*) (0.87). For most species, two different habitats were explaining the numbers of birds in the final models (Table 2.1). The numbers of Bar-tailed Godwits (*Limosa lapponica*) and Eurasian Spoonbills were explained by only one habitat type and numbers of Eurasian Oystercatcher, Black-headed Gull (*Larus*

ridibundus) and Common Shelduck (*Tadorna tadorna*) were associated with three different habitat types. Most of the investigated species (16 out of 21) were positively associated with the lower/coarse-grained intertidal (LC) and about half of the species (13 out of 21) were related to epibenthic bivalve beds (B) (Table 2.2). The other intertidal habitats: HC, HF and LF were less associated with the birds with only 6, 2 and 3 species, respectively being correlated to the different habitats. Of the five species where we also considered the subtidal as a potential feeding habitat, Common Eider, Great Cormorant and Black-headed Gull were associated with the surface area of this habitat (Table 2.2).

The model residuals (i.e., the difference between observed and predicted bird abundance) revealed clear patterns. In the western Dutch Wadden Sea (TB 36–39) and in Dithmarschen in the south of Schleswig-Holstein (TB 10–17) the majority of the residuals were positive, indicating that the bird abundances in these basins were higher than would be expected on the basis of the distribution of habitat only (Figure 2.5). In contrast, the basins in the eastern Dutch Wadden Sea (TB 30–32), Lower Saxony (TB 19–30) and North Frisia (TB 4–6), had many negative residuals and thus were lower than the model predictions (Figure 2.5). These patterns were not only apparent when considering all investigated bird species together, but also when the specific feeding guilds were considered separately (Figure 2.5).

Table 2.1: Linear regression models for the different bird species. Asterisks indicate species where the subtidal is considered as a feeding habitat.

Species	Model	Habitat	Estimate	SE	t	P	R^2	AIC
Eurasian Oystercatcher	full	B	27.44	4.97	5.52	< 0.001	0.90	670.1
(*Haematopus ostralegus*)		HC	0.55	0.45	1.22	0.233		
		HF	0.18	0.28	0.64	0.525		
		LC	0.47	0.24	1.98	0.057		
		LF	-0.02	0.63	-0.03	0.973		
	plausible	B	27.41	4.81	5.70	< 0.001	0.90	668.1
		HC	0.56	0.44	1.26	0.216		
		HF	0.17	0.12	1.44	0.161		
		LC	0.47	0.21	2.27	0.030		
	final	B	28.77	4.73	6.08	< 0.001	0.89	667.9
		HF	0.23	0.11	2.03	0.051		
		LC	0.67	0.13	5.25	< 0.001		
Common Eider *	full	B	-0.26	3.69	-0.07	0.945	0.91	648.5
(*Somateria mollissima*)		HC	-1.09	0.40	-2.73	0.011		
		HF	0.09	0.24	0.37	0.718		
		LC	1.41	0.31	4.50	< 0.001		
		LF	-0.15	0.46	-0.34	0.739		
		S	0.11	0.09	1.26	0.217		
	plausible/final	LC	0.84	0.15	5.46	< 0.001	0.86	654.2
		S	0.14	0.06	2.31	0.028		
Red Knot	full	B	0.86	4.70	0.18	0.856	0.78	666.2
(*Calidris canutus*)		HC	1.12	0.43	2.60	0.014		
		HF	-0.76	0.27	-2.82	0.008		
		LC	0.17	0.23	0.78	0.444		
		LF	1.54	0.59	2.59	0.015		
	plausible	B	1.20	5.20	0.23	0.819	0.72	672.4
		HC	0.74	0.45	1.63	0.113		
		LF	0.02	0.28	0.09	0.931		
		LC	0.51	0.21	2.38	0.024		
	final	HC	0.80	0.39	2.04	0.049	0.72	668.5
		LC	0.51	0.21	2.49	0.018		
European Herring Gull *	full	B	11.25	2.38	4.73	< 0.001	0.86	617.8
(*Larus argentatus*)		HC	-0.14	0.26	-0.53	0.602		
		HF	-0.19	0.16	-1.21	0.235		
		LC	0.32	0.20	1.57	0.127		
		LF	0.25	0.29	0.86	0.396		
		S	0.04	0.06	0.70	0.490		
	plausible	B	9.47	2.04	4.65	< 0.001	0.85	616.4
		LC	0.37	0.09	3.93	< 0.001		
		S	0.004	0.03	0.10	0.918		
	final	B	9.49	1.99	4.76	< 0.001	0.85	614.4

Table 2.1 Continued.

Species	Model	Habitat	Estimate	SE	t	P	R^2	AIC
		LC	0.38	0.06	6.25	< 0.001		
Dunlin (*Calidris alpina*)	full	B	59.39	11.85	5.01	< 0.001	0.85	730.9
		HC	1.30	1.08	1.20	0.240		
		HF	-0.94	0.68	-1.39	0.174		
		LC	0.46	0.57	0.80	0.428		
		LF	2.25	1.50	1.50	0.143		
	plausible	B	59.82	12.02	4.98	< 0.001	0.84	731.1
		HC	0.82	1.04	0.79	0.436		
		LC	0.87	0.49	1.77	0.087		
		LF	0.37	0.66	0.56	0.577		
	final	B	66.54	10.18	6.54	< 0.001	0.84	728.5
		LC	1.24	0.31	4.03	< 0.001		
Bar-tailed Godwit (*Limosa lapponica*)	full	B	4.47	5.01	0.89	0.380	0.69	670.7
		HC	0.49	0.46	1.07	0.292		
		HF	-0.52	0.29	-1.83	0.077		
		LC	0.48	0.24	2.00	0.054		
		LF	0.62	0.63	0.99	0.332		
	plausible	B	1.87	4.92	0.38	0.707	0.64	672.8
		HC	0.05	0.44	0.12	0.908		
		LC	0.71	0.22	3.27	0.003		
	final	LC	0.77	0.10	7.67	< 0.001	0.63	669.0
Grey Plover (*Pluvialis squatarola*)	full	B	6.98	1.38	5.04	< 0.001	0.79	580.6
		HC	0.31	0.13	2.44	0.021		
		HF	-0.02	0.08	-0.23	0.823		
		LC	-0.04	0.07	-0.61	0.547		
		LF	-0.06	0.18	-0.34	0.737		
	plausible/final	B	6.29	1.26	4.98	< 0.001	0.77	576.8
		HC	0.21	0.07	2.86	0.007		
European Golden Plover (*Pluvialis apricaria*)	full	B	3.47	1.73	2.00	0.054	0.66	596.3
		HC	-0.10	0.16	-0.66	0.515		
		HF	-0.11	0.10	-1.09	0.286		
		LC	0.15	0.08	1.75	0.090		
		LF	0.34	0.22	1.53	0.136		
	plausible	B	3.06	1.68	1.82	0.079	0.63	594.9
		LC	0.14	0.05	2.88	0.007		
		LF	0.09	0.09	1.03	0.310		
	final	B	3.86	1.49	2.59	0.014	0.62	594.0
		LC	0.15	0.05	3.39	0.002		
Pied Avocet (*Recurvirostra avosetta*)	full	B	3.05	0.71	4.28	< 0.001	0.74	534.2
		HC	-0.11	0.07	-1.66	0.107		
		HF	0.04	0.04	1.07	0.292		
		LC	0.04	0.03	1.28	0.211		
		LF	0.03	0.09	0.38	0.705		
	plausible	B	2.76	0.71	3.88	< 0.001	0.72	535.3
		HF	0.02	0.04	0.56	0.577		
		LC	0.0002	0.02	0.01	0.991		
		LF	0.06	0.09	0.67	0.511		
	final	B	2.70	0.63	4.27	< 0.001	0.72	531.8
		LF	0.10	0.04	2.91	0.007		
Sanderling (*Calidris alba*)	full	B	0.67	0.59	1.13	0.266	0.59	521.2
		HC	0.09	0.05	1.64	0.112		
		HF	-0.04	0.03	-1.10	0.280		
		LC	0.02	0.03	0.76	0.452		
		LF	0.03	0.07	0.46	0.650		
	plausible	B	0.42	0.56	0.75	0.457	0.55	520.3
		HC	0.05	0.05	1.06	0.296		
		LC	0.04	0.02	1.54	0.135		
	final	HC	0.07	0.05	1.48	0.148	0.55	518.9
		LC	0.04	0.02	1.62	0.116		
Common Ringed Plover (*Charadrius hiaticula*)	full	B	0.28	0.32	0.86	0.398	0.56	478.8
		HC	0.03	0.03	0.99	0.332		
		HF	-0.001	0.02	-0.04	0.966		
		LC	-0.004	0.02	-0.25	0.801		
		LF	0.04	0.04	1.08	0.287		
	plausible	B	0.27	0.32	0.86	0.397	0.56	476.8
		HC	0.02	0.02	1.25	0.221		

Table 2.1 Continued.

Species	Model	Habitat	Estimate	SE	t	P	R^2	AIC
		HF	0.002	0.02	0.11	0.915		
		LF	0.04	0.04	1.11	0.275		
	final	HC	0.03	0.02	1.89	0.068	0.55	473.7
		LF	0.05	0.02	3.03	0.005		
Eurasian Curlew (*Numenius arquata*)	full	B	27.51	4.04	6.80	< 0.001	0.88	655.6
		HC	-0.34	0.37	-0.92	0.367		
		HF	0.19	0.23	0.81	0.423		
		LC	0.66	0.19	3.41	0.002		
		LF	-0.46	0.51	-0.90	0.377		
	plausible/final	B	25.65	3.38	7.58	< 0.001	0.87	651.3
		LC	0.47	0.10	4.61	< 0.001		
Black-headed Gull * (*Larus ridibundus*)	full	B	11.18	2.85	3.92	< 0.001	0.90	630.5
		HC	0.47	0.31	1.52	0.140		
		HF	-0.07	0.19	-0.38	0.704		
		LC	0.04	0.24	0.15	0.883		
		LF	-0.09	0.35	-0.26	0.797		
		S	0.18	0.07	2.61	0.014		
	plausible	B	10.08	2.57	3.92	< 0.001	0.90	627.6
		HC	0.30	0.23	1.29	0.206		
		LC	0.19	0.15	1.28	0.211		
		S	0.13	0.04	3.18	0.004		
	final	B	10.01	2.60	3.85	< 0.001	0.89	627.4
		HC	0.49	0.18	2.82	0.008		
		S	0.16	0.03	5.15	< 0.001		
Common Shelduck (*Tadorna tadorna*)	full	B	14.57	3.48	4.19	< 0.001	0.81	645.1
		HC	-0.40	0.32	-1.24	0.223		
		HF	-0.04	0.20	-0.21	0.834		
		LC	0.26	0.17	1.55	0.131		
		LF	0.72	0.44	1.64	0.111		
	plausible/final	B	13.37	3.36	3.98	< 0.001	0.79	643.3
		LC	0.13	0.10	1.38	0.177		
		LF	0.57	0.18	3.10	0.004		
Common Gull * (*Larus canus*)	full	B	11.00	2.12	5.19	< 0.001	0.85	609.7
		HC	-0.11	0.23	-0.48	0.638		
		HF	-0.04	0.14	-0.31	0.756		
		LC	0.19	0.18	1.06	0.296		
		LF	-0.17	0.26	-0.64	0.528		
		S	0.08	0.05	1.59	0.124		
	plausible	B	8.56	1.83	4.68	< 0.001	0.82	608.8
		LC	0.22	0.08	2.58	0.015		
		S	0.03	0.03	1.08	0.286		
	final	B	8.76	1.82	4.81	< 0.001	0.82	608.1
		LC	0.29	0.06	5.22	< 0.001		
Common Redshank (*Tringa totanus*)	full	B	5.33	0.95	5.60	< 0.001	0.81	554.4
		HC	-0.25	0.09	-2.90	0.007		
		HF	0.03	0.05	0.52	0.608		
		LC	0.21	0.05	4.71	< 0.001		
		LF	-0.11	0.12	-0.88	0.386		
	plausible/final	B	3.87	0.92	4.22	< 0.001	0.74	559.9
		LC	0.10	0.03	3.76	< 0.001		
Ruddy Turnstone (*Arenaria interpres*)	full	B	0.77	0.19	4.17	< 0.001	0.75	440.0
		HC	-0.03	0.02	-1.90	0.067		
		HF	-0.001	0.01	-0.09	0.930		
		LC	0.03	0.01	3.52	0.001		
		LF	-0.004	0.02	-0.17	0.867		
	plausible/final	B	0.58	0.16	3.54	0.001	0.70	439.8
		LC	0.02	0.005	3.69	< 0.001		
Great Cormorant * (*Phalacrocorax carbo*)	full	B	0.23	0.18	1.29	0.206	0.92	437.7
		HC	-0.04	0.02	-2.01	0.054		
		HF	-0.002	0.01	-0.19	0.853		
		LC	0.03	0.02	2.24	0.033		
		LF	-0.04	0.02	-1.62	0.117		
		S	0.02	0.004	4.96	< 0.001		
	plausible/final	LC	0.02	0.01	2.71	0.011	0.84	453.5
		S	0.01	0.003	4.30	< 0.001		

Table 2.1 Continued.

Species	Model	Habitat	Estimate	SE	t	P	R^2	AIC
Common Greenshank (*Tringa nebularia*)	full	B	0.55	0.12	4.55	< 0.001	0.82	409.3
		HC	0.01	0.01	1.21	0.237		
		HF	-0.01	0.01	-1.38	0.178		
		LC	0.001	0.01	0.14	0.894		
		LF	0.03	0.02	1.72	0.096		
	plausible	B	0.55	0.12	4.52	< 0.001	0.81	409.4
		HC	0.01	0.01	0.80	0.428		
		LC	0.005	0.005	0.99	0.330		
		LF	0.01	0.01	1.08	0.290		
	final	B	0.65	0.10	6.18	< 0.001	0.79	408.0
		LC	0.01	0.003	2.93	0.006		
Spotted Redshank (*Tringa erythropus*)	full	B	-0.01	0.17	-0.04	0.970	0.64	435.5
		HC	0.02	0.02	1.39	0.174		
		HF	0.01	0.01	0.60	0.553		
		LC	-0.01	0.01	-0.96	0.344		
		LF	0.03	0.02	1.27	0.214		
	plausible	HC	0.01	0.01	1.12	0.273	0.63	432.5
		HF	0.01	0.01	1.32	0.196		
		LF	0.02	0.02	0.95	0.352		
	final	HC	0.01	0.01	1.85	0.074	0.62	431.5
		HF	0.02	0.004	4.59	< 0.001		
Eurasian Spoonbill (*Platalea leucorodia*)	full	B	0.14	0.04	3.08	0.004	0.74	339.4
		HC	-0.01	0.004	-3.17	0.004		
		HF	0.01	0.003	2.31	0.028		
		LC	0.01	0.002	6.07	< 0.001		
		LF	-0.02	0.01	-2.83	0.008		
	plausible	B	0.05	0.05	1.20	0.237	0.59	350.0
		LC	0.01	0.001	4.24	< 0.001		
	final	LC	0.01	0.001	6.68	< 0.001	0.57	349.5

Table 2.2: Coefficients of the final linear regression models. Each coefficient represents bird abundance for a specific habitat and is expressed as individuals per hectare (ha). Asterisks indicate species where the subtidal is considered as a feeding habitat.

| Feeding guild	Species	B	HC	HF	LC	LF	S
molluscivorous	Eurasian Oystercatcher	28.77		0.23	0.67		
	Common Eider *				0.84		0.14
	Red Knot		0.80		0.51		
	European Herring Gull *	9.49			0.38		
polychaetivorous	Dunlin	66.54			1.24		
	Bar-tailed Godwit				0.77		
	Grey Plover	6.29	0.21				
	European Golden Plover	3.86			0.15		
	Pied Avocet	2.70				0.10	
	Sanderling		0.07		0.04		
	Common Ringed Plover		0.03			0.05	
benthivorous	Eurasian Curlew	25.65			0.47		
	Black-headed Gull *	10.01	0.49				0.16
	Common Shelduck	13.37			0.13	0.57	
	Common Gull *	8.76			0.29		
	Common Redshank	3.87			0.10		
	Ruddy Turnstone	0.58			0.02		
piscivorous	Great Cormorant *				0.02		0.01
	Common Greenshank	0.65			0.01		
	Spotted Redshank		0.01	0.02			
	Eurasian Spoonbill				0.01		
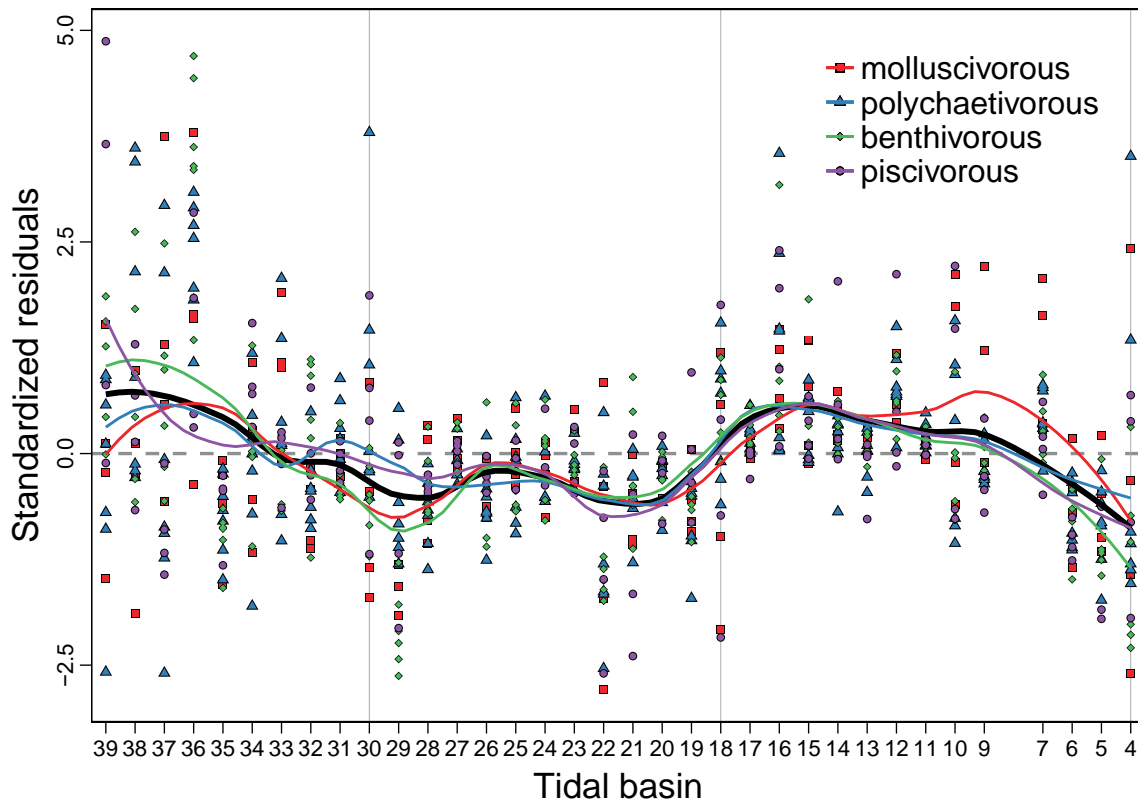
Figure 2.5: Standardized residuals of the final regression models for the 21 bird species across tidal basins 4–39. The tidal basins are aligned from south-west to north-east, starting with the westernmost tidal basin 39 (Marsdiep) in the Dutch Wadden Sea. Note that tidal basin 9 is a merger of basins 8 and 9. Vertical lines indicate borders between the different regions. The horizontal dashed line at 0 provides a common reference to more easily distinguish between positive and negative residuals. Positive residuals indicate that observed abundances are higher than predicted, and negative values indicate that observed abundances are lower than predicted. Non-linear local regression smoothers (LOESS; span = 0.3) are added as a visual aid (solid black line: all species together; coloured lines: species classified into feeding guilds). For feeding guild specific residuals see Figure S2.30 in the supplementary material.

Discussion

Bird-habitat associations

Our study shows that area-based models can be used to effectively predict the large scale distributions of waterbirds at different habitat types across the Wadden Sea tidal basins. In general, model performance was high. Even for models with the lowest predictive power, more than 50% of the variation in bird numbers could be explained by the surface area of the different habitat types. Overall, the models revealed a maximum of three and for most species two different habitat types that were explaining the bird numbers. The bird-habitat associations obtained by our modelling approach were generally in agreement with previous observations (Goss-Custard & Yates 1992, Yates et al. 1993, Granadeiro et al. 2007, chapter 4: Waser et al. 2016a). One of the habitats that was essential for many different bird species, was the habitat of epibenthic bivalve beds. Despite being the habitat covering the lowest surface area by far (accounting not more than 5% of the intertidal area; Folmer et al. 2014), more than half (13 out of 21) of the tested bird species were significantly correlated to this habitat type. This finding is remarkable and corroborates previous observations that these beds provide important and stable feeding areas for many waterbirds (van de Kam et al. 2004, chapter 4: Waser et al. 2016a). In line with field observations (chapter 4: Waser et al. 2016a), the models revealed much

higher bird densities on bivalve beds compared to densities on bare intertidal flats. However, the densities obtained by the regression models generally exceeded the densities observed in the field (Markert et al. 2013, chapter 4: Waser et al. 2016a). A possible reason for these differences in bird density might be that the characteristics (e.g., hydrodynamic and sedimentological) that promote bivalve beds in certain basins may equally support other benthic prey species that in turn attract several bird species. Moreover, differences in bird density might be caused by the attraction of the birds to a much larger area than the bivalve bed-boundaries itself. Both mussels and oysters are known for shaping the environment far beyond their own bed-boundaries in terms of sediment and benthos composition (Zwarts et al. 2004, van der Zee et al. 2012, Walles et al. 2015b). Hence, areas nearby bivalve beds are very productive and may offer improved feeding conditions for a bulk of bird species which would explain high bird numbers in tidal basins that are rich in bivalve bed area. These bivalve induced large-scale effects may also explain significant relationships between bivalve beds and bird species that typically do not forage on the beds itself. For instance, we found a significant correlation between bivalve bed area and numbers of the Pied Avocet (*Recurvirostra avosetta*). This species typically does not frequent epibenthic bivalve beds (chapter 4: Waser et al. 2016a) and preferably forages on worms in muddy sediments (Moreira 1995). It is possible that areas rich in bivalve beds may provide optimal feeding conditions for the Avocet due to promoted sedimentation caused by epibenthic bivalves (Zwarts et al. 2004, van der Zee et al. 2012, Walles et al. 2015b).

We found that 16 out of 21 species were linked to the low coarse-grained intertidal. In contrast, the other intertidal habitats were associated with only a few bird species (high coarse-grained intertidal: 5, high fine-grained intertidal: 2, low fine-grained intertidal: 3 species). This suggests that the low coarse-grained intertidal is generally very productive, rich in macro-benthic prey and provides suitable foraging grounds for the majority of the bird species in the Wadden Sea. Indeed, the highest biomass values of macrobenthos in coastal systems are found at lower intertidal levels between mean tide level (MTL) and halfway between MTL and mean low water (MLW) level (e.g., Beukema 2002). Moreover, in many European coastal areas the majority of bird species were observed preferably on lower intertidal flats (Yates et al. 1993, Brinkman & Ens 1998, Granadeiro et al. 2004, Ens et al. 2005). For several species such as Red Knot (*Calidris canutus*), Bar-tailed Godwit (*Limosa lapponica*), Eurasian Oystercatcher (*Haematopus ostralegus*), Common Greenshank (*Tringa nebularia*), Common Ringed Plover (*Charadrius hiaticula*), Ruddy Turnstone (*Arenaria interpres*) and Sanderling (*Calidris alba*), the preference for rather coarse and sandy sediments, obtained by our regression models, was in agreement with previous studies focussing on the low tide distributions of waterbirds in several European coastal areas (Yates et al. 1993, Brinkman & Ens 1998, Granadeiro et al. 2004, Ens et al. 2005, Granadeiro et al. 2007). In contrast to our model predictions, however, the same studies found that Dunlin (*Calidris alpina*), Common Redshank (*Tringa totanus*), Grey Plover (*Pluvialis squatarola*) and Eurasian Curlew (*Numenius arquata*) showed preferences for fine-grained, rather muddy sediments. The discrepancy between our study and the other studies in preferences for sediment structure of the four bird species might be caused by local differences in habitat preference of the benthic prey organisms. However, as benthic prey organisms show fairly consistent distribution patterns across European coastal areas (Compton et al. 2009) it is unlikely that differences in habitat preferences of the birds are caused by deviant habitat preferences of the Wadden Sea benthos. An alternative explanation are possible imprecisions in defining surface areas of the four bare intertidal habitats. Possible imprecisions in the classification of the intertidal feeding habitats might be either caused by interpolation- and data errors in the raster layers of the abiotic data (tidal exposure, sediment structure) or by incorrect threshold values for characterizing the surface area of the different habitat types. Since both data sets, tidal exposure and sediment structure, were validated with extensive sets of observations, they are reliable and representative for the actual conditions found in the Wadden Sea. However, it is possible that the resolution (200 × 200 m) of the raster sets was too coarse to accurately depict potential

local small scale differences in the abiotic data. Hence, imprecisions in the classification of the intertidal feeding habitats might be caused by the use of slightly inaccurate median values of the abiotic variables.

Subtidal areas are used by only a few of the investigated species, such as diving birds (Great Cormorant, Common Eider) and opportunistic foragers (Herring Gull, Common Gull, Black-headed Gull). In our regression models, we found that numbers of Great Cormorant, Common Eider and Black-headed Gull were significantly correlated to the subtidal surface area, whereas numbers of Herring Gull and Common Gull were not linked to subtidal area. These findings are in agreement with the known habitat preferences of the five investigated species. While the diving birds are specialised in preying on subtidal prey (Cormorant: fish, Eider: primarily bivalves; Leopold et al. 1998, Cervencl et al. 2015), the opportunistic gulls may exploit subtidal areas occasionally by either foraging directly from the water surface or scavenging discards from fishing vessels. The three gull species, however, differ somewhat in their foraging strategies and use of foraging habitats (Kubetzki & Garthe 2003). The Herring Gull forages primarily in intertidal habitats and uses subtidal areas only sporadically, whereas Common Gull and Black-headed Gull are more generalist predators that exploit various different habitats more equally (Kubetzki & Garthe 2003, Schwemmer & Garthe 2008).

Regional differences in bird abundance

We found strikingly similar patterns in the residuals between species which differ in diet and population trends. While parts of the southern Wadden Sea (Lower Saxonian and the most eastern Dutch Wadden Sea, TB 19–32) and of North Frisia (TB 4–6) showed relatively low bird abundances, bird abundances in Dithmarschen (TB 10–17) and the western Dutch Wadden Sea (TB 36–39) were relatively high. These regional differences, particularly the relatively high numbers in the Dutch Wadden Sea are remarkable. Particularly, this part of the Wadden Sea, in contrast to the other Wadden Sea regions, was subject to intense mechanical shellfish fisheries (Dankers et al. 2001, Ens et al. 2004, Nehls et al. 2009a) that caused important changes of the ecosystem. In the early 1990s overfishing in combination with low recruitment led to the disappearance of almost all intertidal bivalve beds (at that time solely composed of *M. edulis*) in the Dutch Wadden Sea. The disappearance of these beds, which remained virtually absent for several years and only slowly recovered (Dankers et al. 2001, Ens et al. 2004), in combination with mechanized cockle fishery caused severe food shortages for the molluscivorous Eider and Oystercatcher (Ens 2006). Piersma et al. (2001) argued that sediment disturbance caused by mechanical cockle dredging led to declines in bivalve settlement success (e.g., in cockles and Baltic tellins) which in turn is assumed to reduce quality in foraging habitat for the Red Knot (van Gils et al. 2006). Eriksson et al. (2010) even claimed that due to the fishing induced sediment disturbance, the ecosystem had collapsed and that large-scale restoration projects were required to restore ecosystem health. However, our results are hard to reconcile with the suggested ecosystem collapse of the heavily exploited Dutch Wadden Sea. The bird abundances observed in our study do not correlate with fishing intensity, as bird abundance in the Dutch Wadden Sea was high and for the most part of the German Wadden Sea low, despite lower fishery impact. In addition, one would expect considerable differences in bird abundance between the different feeding guilds, as fisheries mainly affected species preying on bivalves. Our results however, indicate no considerable difference between abundances of the different feeding guilds throughout the Wadden Sea tidal basins.

How can the differences in bird abundance between different parts of the Wadden Sea be explained? It is often difficult to identify causalities of the phenomena observed in natural systems due to the large number of factors involved. Several factors to explain differences in bird numbers between regions were discussed by van Roomen et al. (2012). Their study focussed on a comparison of long-term trends of waterbirds within different regions and tidal basins of the

Wadden Sea between 1991 and 2009. They found for example, increases in polychaete specialists in the Netherlands, whereas other investigated populations were decreasing throughout the German and Dutch Wadden Sea. Next to the impact of shellfish fisheries, the authors deemed climate change (temperature increase), eutrophication, invasive species and increases of bird of prey as the most important factors potentially linked to differences in long-term trends. In addition, Laursen et al. (2010) point out that negative trends dominate particularly in the central Wadden Sea, which has large tidal amplitudes and is devoid of barrier islands, and hypothesize that changes in storm regime could affect the sediment composition primarily in the central Wadden Sea which would have negative effects on bird numbers. However, van Roomen et al. (2012) conclude that most of these factors such as climate change, eutrophication, fisheries and invasive species cannot or only partly explain the observed long-term trends. Regarding bird abundance, these factors are also unlikely to be responsible for the observed differences in abundance between the different parts of the Wadden Sea. For instance, no direct relation is found between bird abundance and mean (winter-) temperature, as we observed high abundances in Dithmarschen (TB 10–17), which is several degrees colder than in the Dutch Wadden Sea. Moreover, high chlorophyll a values are found in the eastern Dutch Wadden Sea and in Lower Saxony (van Beusekom et al. 2009), where bird densities are relatively low.

The Central Wadden sea differs considerably from other regions of the Wadden Sea in that it contains relatively fine sediments. Our results of observed bird abundances, however, do not fully agree with the proposed hypothesis of Laursen et al. (2010) that the sediment composition in the central Wadden Sea would be unfavourable for many waterbird species. While we observed rather low bird abundances in basins 18–22, the other basins of the central Wadden Sea (TB 12–17) showed relatively high bird abundances.

During the last few decades, the Wadden Sea has been invaded by dozens of exotic species (Buschbaum et al. 2012) of which a few have severe impacts on the Wadden Sea ecosystem. One of the most conspicuous non-native species that has changed the ecosystem considerably is the Pacific oyster (*Crassostrea gigas*). For instance, it has invaded many mussel beds in the Wadden Sea (Troost 2010), which decreased the attractiveness of these beds as feeding habitat for birds feeding on mussels (chapter 4: Waser et al. 2016a). It would have been interesting to investigate the habitat use of birds within the entire Wadden Sea also in relation to the spatial distribution of this invader, but regional differences in the classification of bivalve beds into "mussel beds" and "oyster beds" prevented this. However, oysters do not seem to affect the abundance of species not feeding on mussels (chapter 4: Waser et al. 2016a). As abundances generally showed similar patterns between the different bird species it seems unlikely that invaders, as the example of the Pacific oyster shown here, will play an important role in shaping large scale distributions of waterbirds in the Wadden Sea.

Finally, regional differences in bird numbers may be related to the avoidance of humans and/or avian predators. Among several birds of prey species that occur in the Wadden Sea, the most lethal predator is the Peregrine Falcon (*Falco peregrinus*). During the last few decades, populations of the Peregrine Falcon in Scandinavia and Germany expanded westwards (Ratcliffe 1993), resulting in population increases in the Wadden Sea (van den Hout 2009, Duijns 2014). Peregrine Falcons may not only kill birds but also, and more importantly, may cause behavioural responses that result in birds avoiding areas where falcons are present (Cresswell 2008, Cresswell et al. 2010). Although no comparable data of Peregrine Falcon abundance for the entire Wadden Sea exist, predation pressure due to Peregrine Falcons is expected to be higher in Germany, since the number of breeding pairs is higher in the German Wadden Sea compared to the other regions (van Roomen et al. 2012). Concerning the Netherlands, the abundance of Peregrine Falcons is about 5 times higher in the eastern Dutch Wadden Sea (seasonal mean 1998/99–2012/13 = 0.35 birds per 10 km^2) compared to the western part (seasonal mean 1999/2000–2012/2013 = 0.08 birds per 10 km^2) (Duijns 2014). In addition, differences in the landscape might play a role in explaining our observed patterns in bird

abundance. Peregrine Falcons often make use of the vegetation or other structures to conceal themselves while approaching prey (Bijlsma 1990, Cresswell & Whitfield 1994). For this reason, hunting success of the predators is higher in vegetated habitats, such as salt marshes, compared to structureless habitats and generally declines with distance to the habitat structures (Cresswell 1994, Ydenberg et al. 2002, Pomeroy 2006). Across the Wadden Sea, there are large differences in the landscapes. On the one hand, tidal basins differ in their width, which may influence predation risk as distances to habitat structures differ. On the other hand, the habitat itself differs considerably within the Wadden Sea. While in Lower Saxony and Schleswig-Holstein about half of high tide roosts are classified as salt marsh, in the Netherlands salt marshes only account for 25% of the high tide roosts (Koffijberg et al. 2003). Although these figures only represent the fraction of roosts that are considered as salt marsh and therefore does not relate to the salt marsh area, it still gives a general idea about the habitat differences in the Wadden Sea. In addition, also anthropogenic disturbances might cause differences in regional bird abundance. While the number of roosts influenced by recreational activities, farming, military use and civil air do not considerably differ between the regions, the fraction of roosts associated to hunting is comparatively higher in Lower Saxony than in Schleswig-Holstein and the Netherlands (Koffijberg et al. 2003). It seems worthwhile to further explore the possibility that the lower bird abundances in the Wadden Sea of Lower Saxony that we observed are related to the abundance of Peregrine Falcons, human disturbance and its landscape properties.

In conclusion, the integration of several different monitoring and modelling data proved to be useful in modelling the large scale habitat distributions of waterbirds in the international Wadden Sea. In general, the predicted distributions obtained by our models showed a fair degree of agreement with low-tide distributions assessed at much smaller scale at several European coasts. Studying species distributions and their habitat preferences at large spatial scales is of special interest for managers and conservationist in order to compare the state and functioning of ecosystems. We discovered that waterbird abundances showed pronounced differences between different Wadden Sea regions. The large scale distributions of the waterbirds are hard to reconcile with the suggested ecosystem collapse of the Dutch Wadden Sea. However, the causalities for the observed differences in bird abundance are still not well understood. Further research is needed to identify the driving forces behind these differences in bird abundance.

Acknowledgements
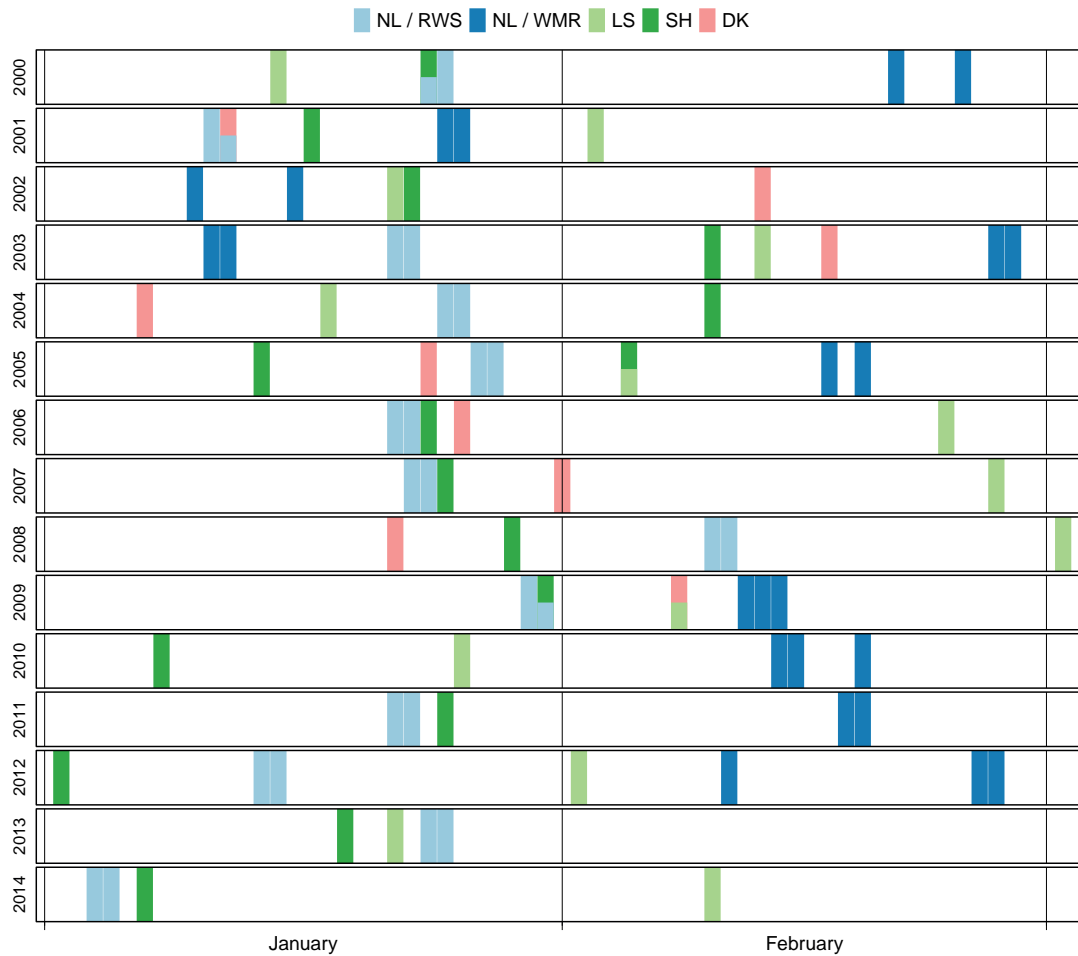
Supplementary material

Figure S2.1: Overview of the aerial winter counts of Common Eider performed in the different parts of the Wadden Sea for the seasons 1999/00–2013/14. In the Netherlands (tidal basins (TB) 30–39), the counts are organised by Rijkswaterstaat (RWS) and in some years additional counts were performed by Wageningen Marine Research (WMR, formerly IMARES). The counts are conducted during high tide using a high-winged plane flown along predefined north-south oriented transects on two (consecutive) days covering the entire area of the Dutch Wadden Sea and the adjacent North Sea coastal zone. The aerial counts in Lower Saxony (TB 18–30) are organised by the National Park Authority Wadden Sea Lower Saxony (NLPV) and in Schleswig-Holstein (TB 4–18) by the Schleswig-Holstein Agency for Coastal Defence, National Park and Marine Conservation, National Park Authority (LKN SH). The German counts are performed during low tide, when Eiders are concentrated in a few tidal creeks, following the edges of the tidal channels throughout the entire German Wadden Sea. In Denmark (TB 1–4), aerial counts are performed during high tide and are organized by the Danish Centre for Environment and Energy (DCE, formerly NERI). For each group of Common Eider recorded in the different areas, the geographical location as well as the number of individuals was determined. Based on the geographical locations, flocks of Eiders were allocated to the different tidal basins in order to arrive at a total number of individuals per basin.
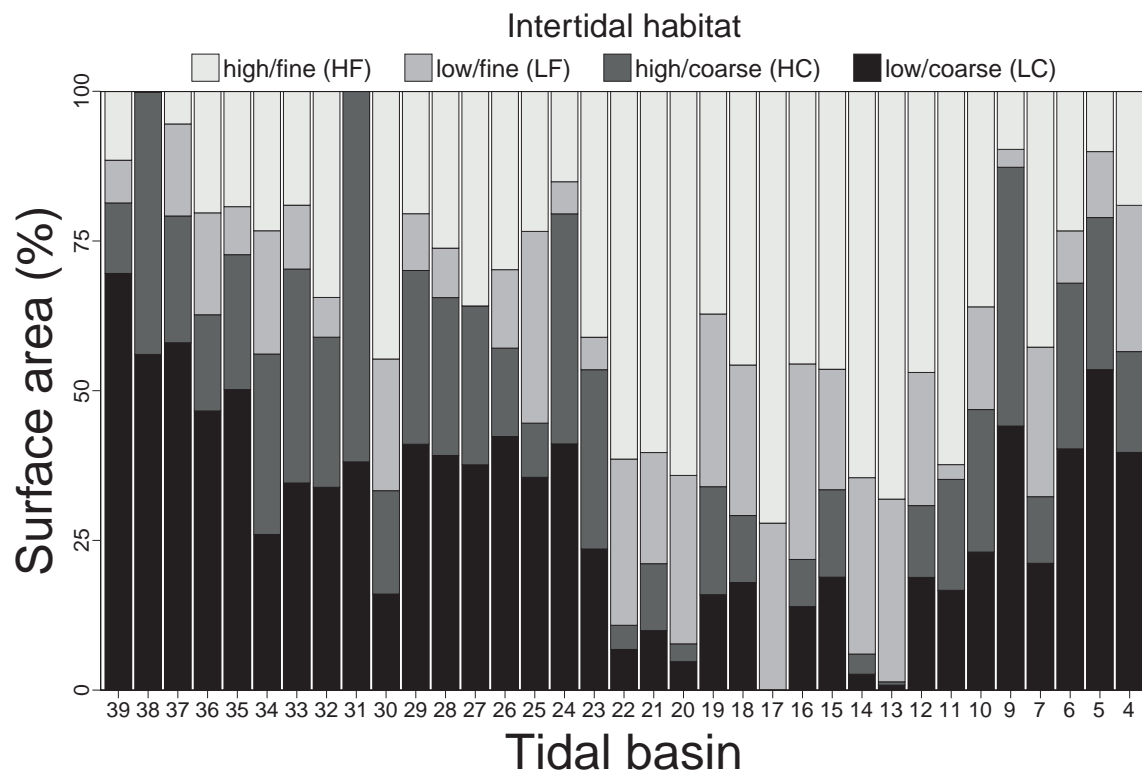
Figure S2.2: Fractions of intertidal surface area (%) of the four bare intertidal habitats for Wadden Sea tidal basins 4–39. The tidal basins are aligned from south-west to north-east, starting with tidal basin 39 in the western Dutch Wadden Sea. Note that tidal basin 9 is a merger of basins 8 and 9.
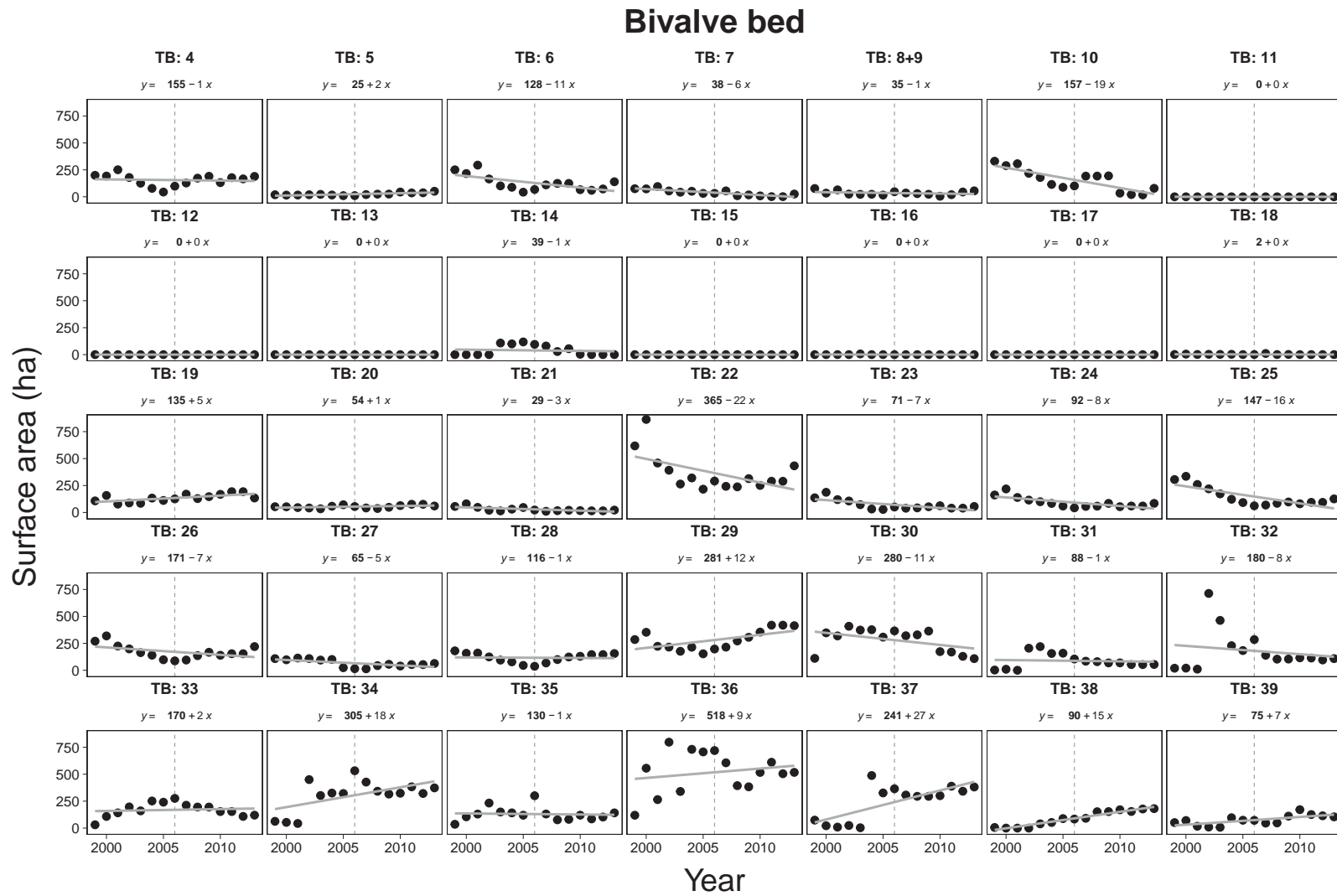
Bivalve bed

Figure S2.3: Surface area (ha) of bivalve beds (B) from the different tidal basins (TB) of the Wadden Sea for the period 1999-2013.
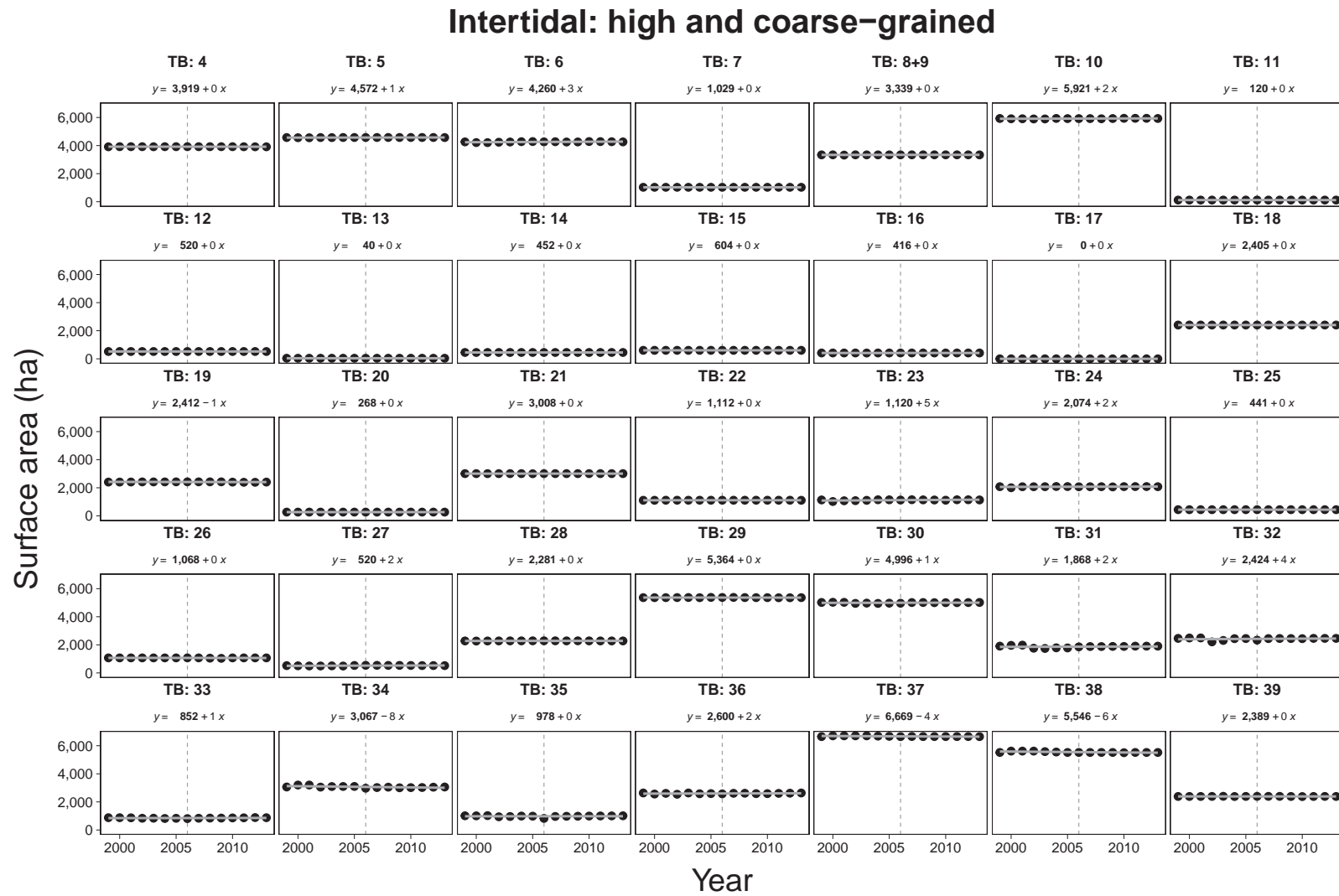
Intertidal: high and coarse−grained

Figure S2.4: Surface area (ha) of the high and coarse-grained intertidal (HC) from the different tidal basins (TB) of the Wadden Sea for the period 1999-2013.
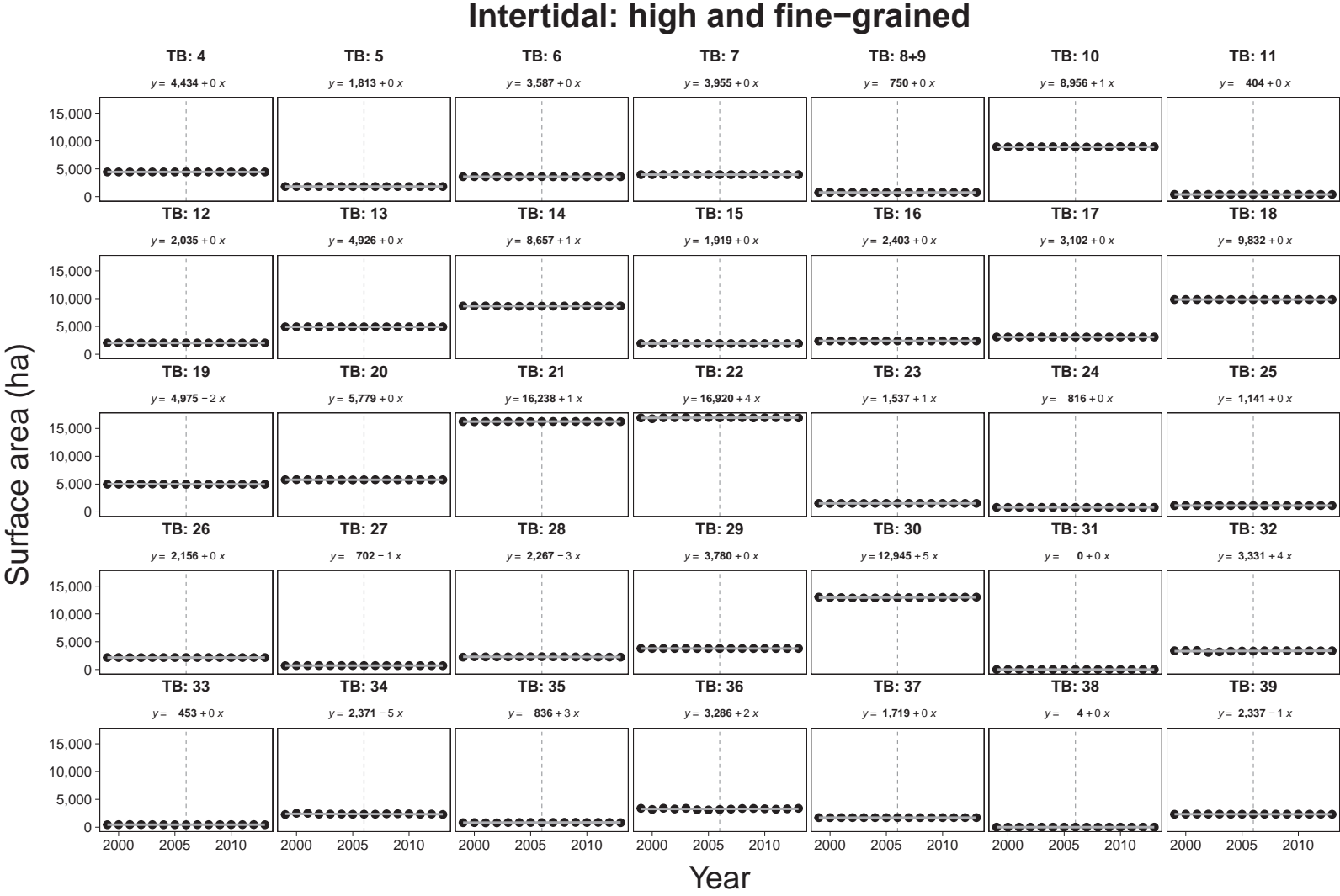
Intertidal: high and fine−grained

Figure S2.5: Surface area (ha) of the high and fine-grained intertidal (HF) from the different tidal basins (TB) of the Wadden Sea for the period 1999-2013.

Intertidal: low and coarse−grained

Figure S2.6: Surface area (ha) of the low and coarse-grained intertidal (LC) from the different tidal basins (TB) of the Wadden Sea for the period 1999-2013.
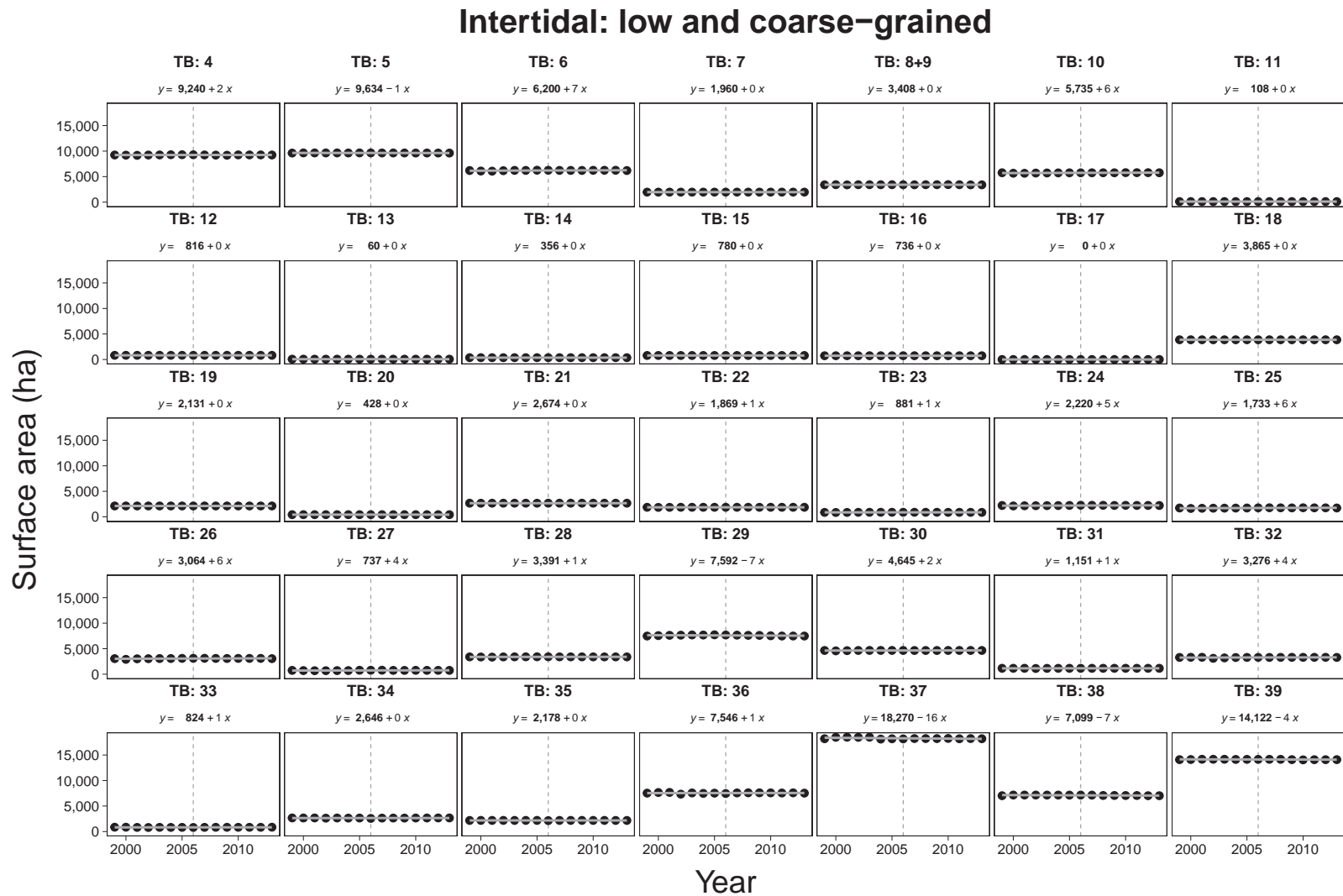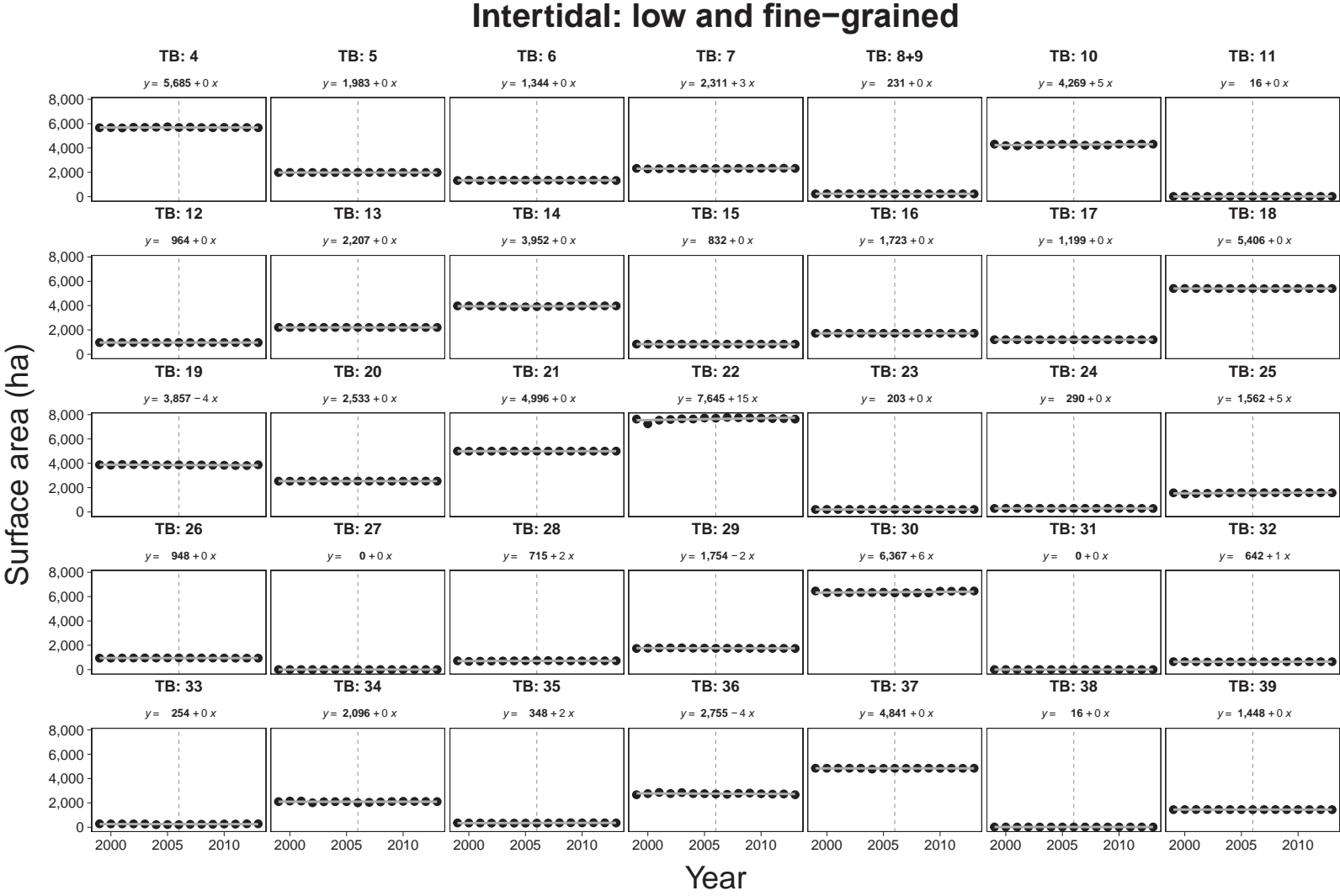
Figure S2.7: Surface area (ha) of the low and fine-grained intertidal (LF) from the different tidal basins (TB) of the Wadden Sea for the period 1999-2013.
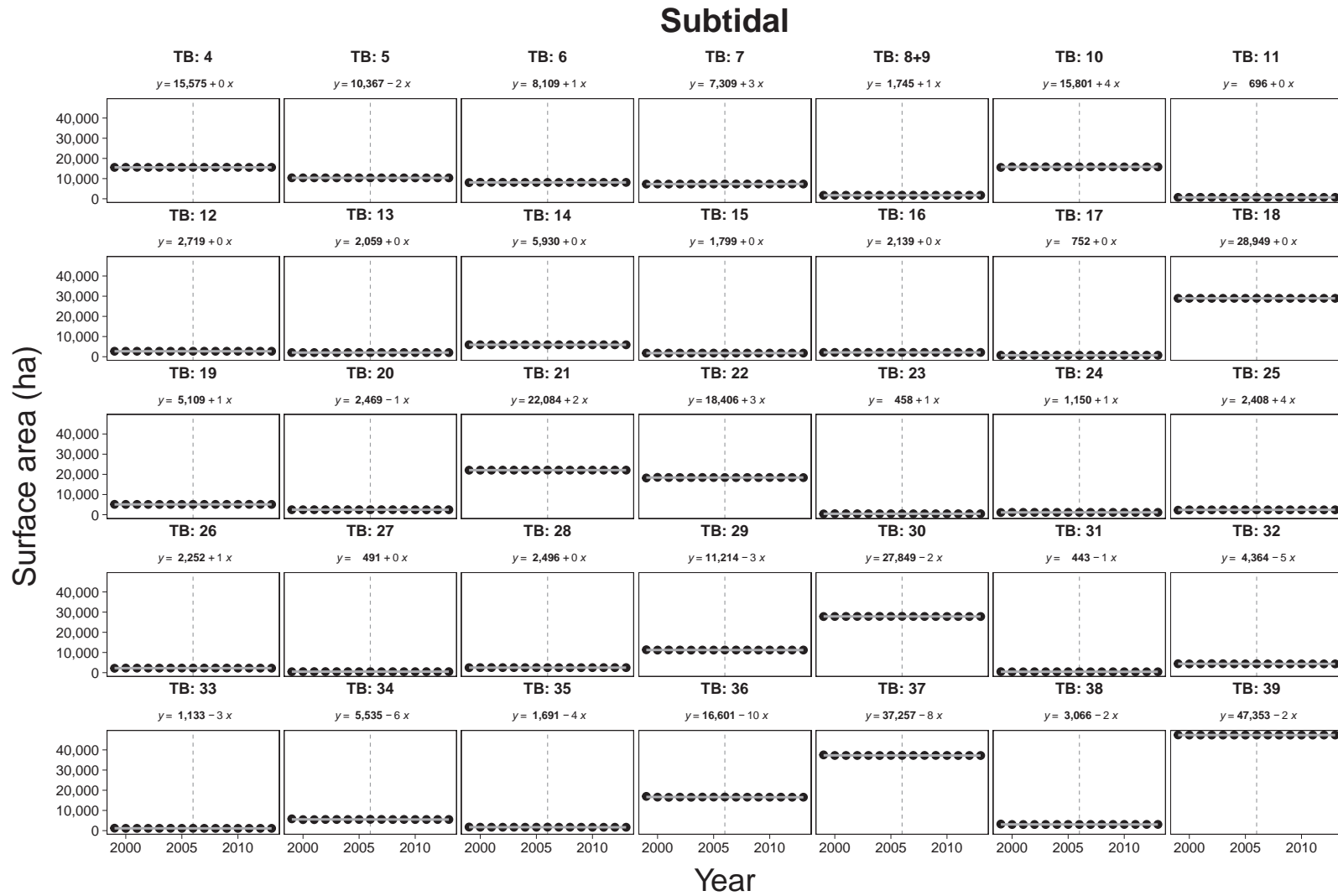
Subtidal

Figure S2.8: Surface area (ha) of the subtidal (S) from the different tidal basins (TB) of the Wadden Sea for the period 1999-2013.

Eurasian Oystercatcher
(molluscivorous)

TB: 1	TB: 2	TB: 3	TB: 4	TB: 5	TB: 6	TB: 7	TB: 8+9
$y = 4957 - 98\,x$	$y = 7839 - 430\,x$	$y = 3617 - 136\,x$	$y = 7382 - 269\,x$	$y = 6251 - 31\,x$	$y = 9244 - 101\,x$	$y = 9783 - 171\,x$	$y = 7263 - 320\,x$

TB: 10	TB: 11	TB: 12	TB: 13	TB: 14	TB: 15	TB: 16	TB: 17
$y = 15692 - 639\,x$	$y = 805 - 20\,x$	$y = 4728 - 290\,x$	$y = 2271 - 63\,x$	$y = 4889 - 163\,x$	$y = 3464 - 131\,x$	$y = 4911 - 187\,x$	$y = 541 + 10\,x$

TB: 18	TB: 19	TB: 20	TB: 21	TB: 22	TB: 23	TB: 24	TB: 25
$y = 8394 - 149\,x$	$y = 3595 - 119\,x$	$y = 1517 - 36\,x$	$y = 5146 - 83\,x$	$y = 8442 - 173\,x$	$y = 3810 - 6\,x$	$y = 4735 - 206\,x$	$y = 7330 - 44\,x$

TB: 26	TB: 27	TB: 28	TB: 29	TB: 30	TB: 31	TB: 32	TB: 33
$y = 7314 - 288\,x$	$y = 3828 - 226\,x$	$y = 7192 - 367\,x$	$y = 9370 - 333\,x$	$y = 16596 - 307\,x$	$y = 3309 - 90\,x$	$y = 7634 - 216\,x$	$y = 8630 - 494\,x$

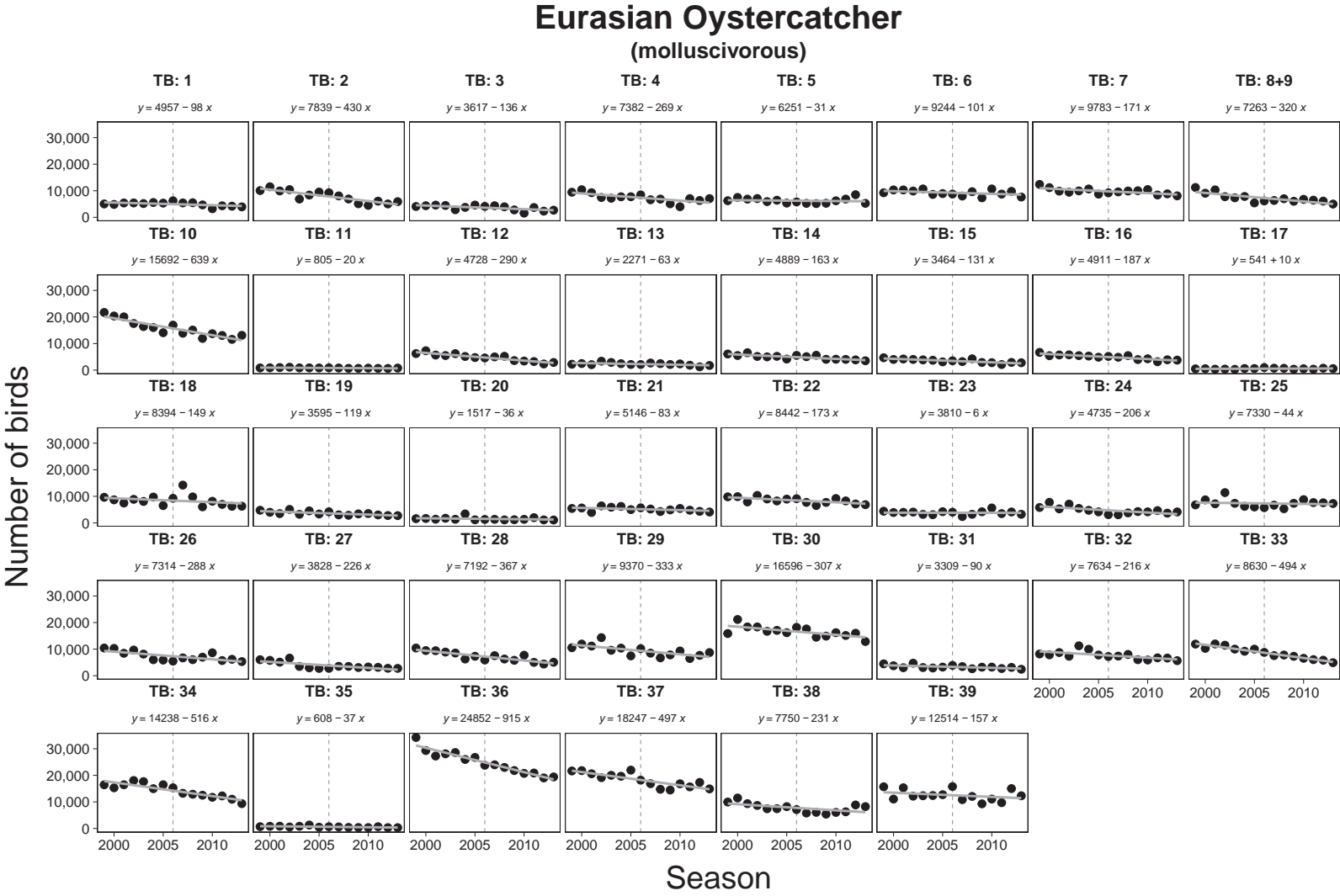| TB: 34	TB: 35	TB: 36	TB: 37	TB: 38	TB: 39		
$y = 14238 - 516\,x$	$y = 608 - 37\,x$	$y = 24852 - 915\,x$	$y = 18247 - 497\,x$	$y = 7750 - 231\,x$	$y = 12514 - 157\,x$		

Number of birds

Season

Figure S2.9: Numbers of Eurasian Oystercatcher (*Haematopus ostralegus*) from the different tidal basins (TB) of the Wadden Sea for the period 1999-2013.
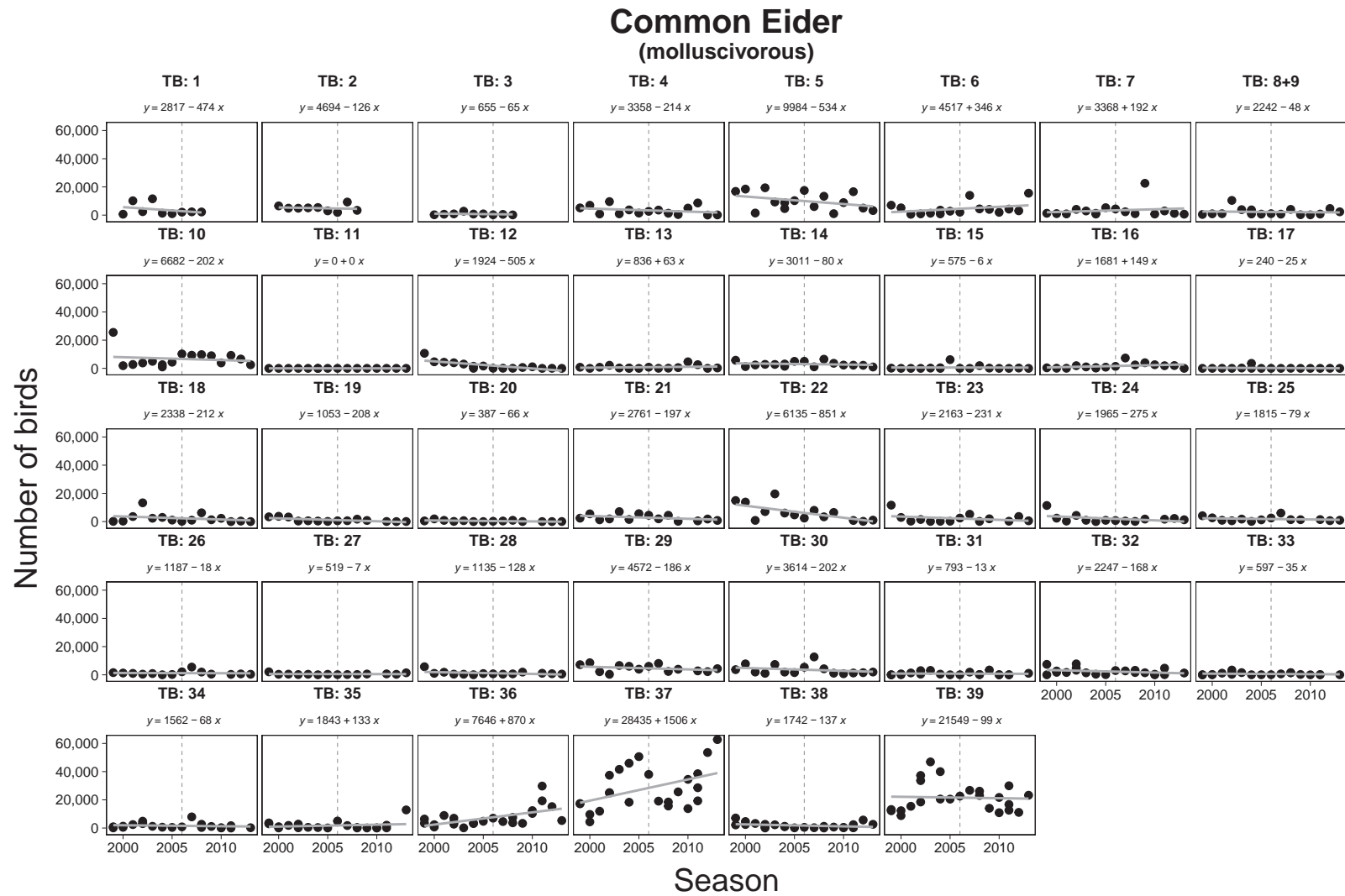
Common Eider
(molluscivorous)

Figure S2.10: Numbers of Common Eider (*Somateria mollissima*) from the different tidal basins (TB) of the Wadden Sea for the period 1999-2013.
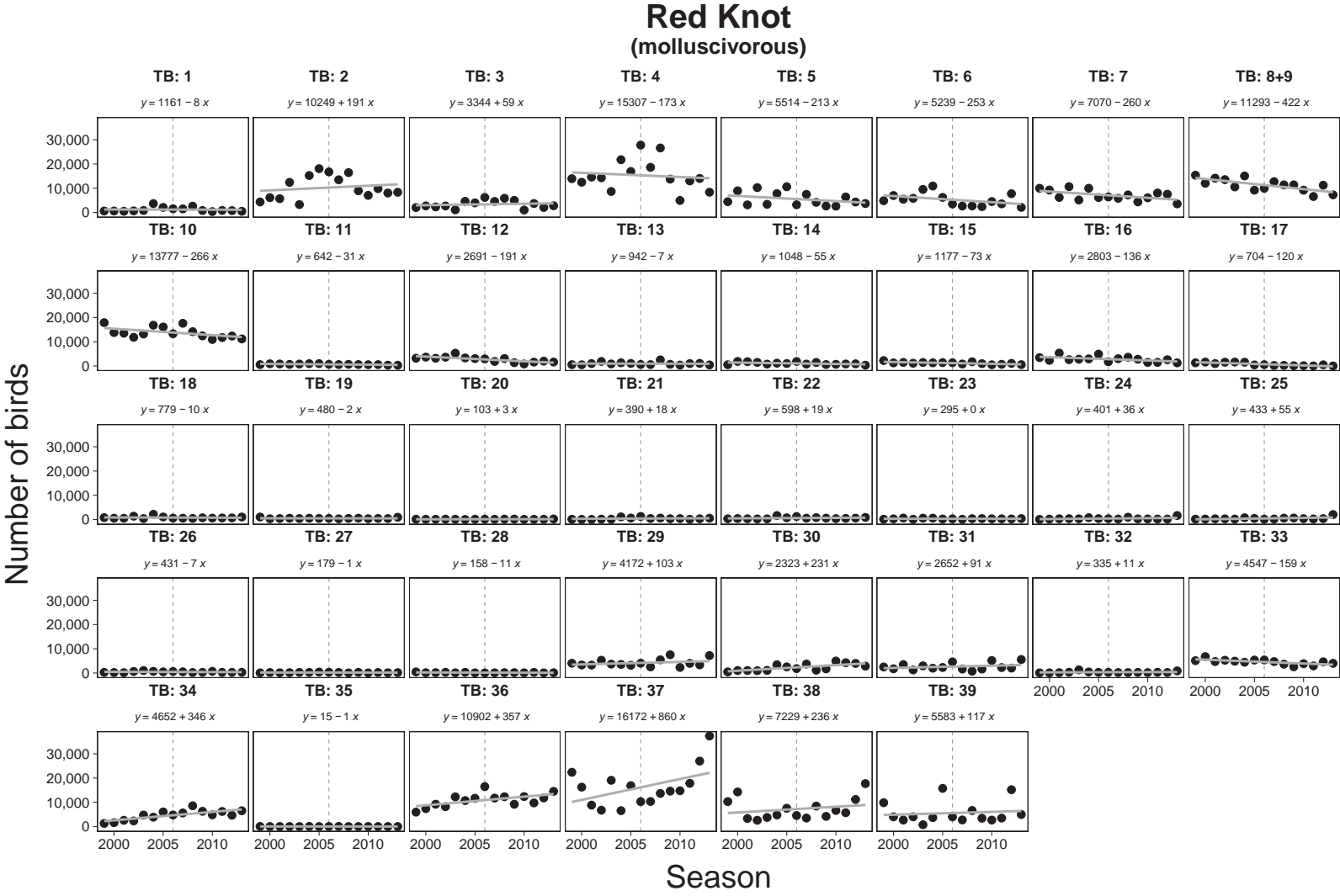
Red Knot
(molluscivorous)

TB: 1 $y = 1161 - 8\,x$
TB: 2 $y = 10249 + 191\,x$
TB: 3 $y = 3344 + 59\,x$
TB: 4 $y = 15307 - 173\,x$
TB: 5 $y = 5514 - 213\,x$
TB: 6 $y = 5239 - 253\,x$
TB: 7 $y = 7070 - 260\,x$
TB: 8+9 $y = 11293 - 422\,x$

TB: 10 $y = 13777 - 266\,x$
TB: 11 $y = 642 - 31\,x$
TB: 12 $y = 2691 - 191\,x$
TB: 13 $y = 942 - 7\,x$
TB: 14 $y = 1048 - 55\,x$
TB: 15 $y = 1177 - 73\,x$
TB: 16 $y = 2803 - 136\,x$
TB: 17 $y = 704 - 120\,x$

TB: 18 $y = 779 - 10\,x$
TB: 19 $y = 480 - 2\,x$
TB: 20 $y = 103 + 3\,x$
TB: 21 $y = 390 + 18\,x$
TB: 22 $y = 598 + 19\,x$
TB: 23 $y = 295 + 0\,x$
TB: 24 $y = 401 + 36\,x$
TB: 25 $y = 433 + 55\,x$

TB: 26 $y = 431 - 7\,x$
TB: 27 $y = 179 - 1\,x$
TB: 28 $y = 158 - 11\,x$
TB: 29 $y = 4172 + 103\,x$
TB: 30 $y = 2323 + 231\,x$
TB: 31 $y = 2652 + 91\,x$
TB: 32 $y = 335 + 11\,x$
TB: 33 $y = 4547 - 159\,x$

TB: 34 $y = 4652 + 346\,x$
TB: 35 $y = 15 - 1\,x$
TB: 36 $y = 10902 + 357\,x$
TB: 37 $y = 16172 + 860\,x$
TB: 38 $y = 7229 + 236\,x$
TB: 39 $y = 5583 + 117\,x$

Number of birds — Season

Figure S2.11: Numbers of Red Knot (*Calidris canutus*) from the different tidal basins (TB) of the Wadden Sea for the period 1999-2013.

European Herring Gull
(molluscivorous)
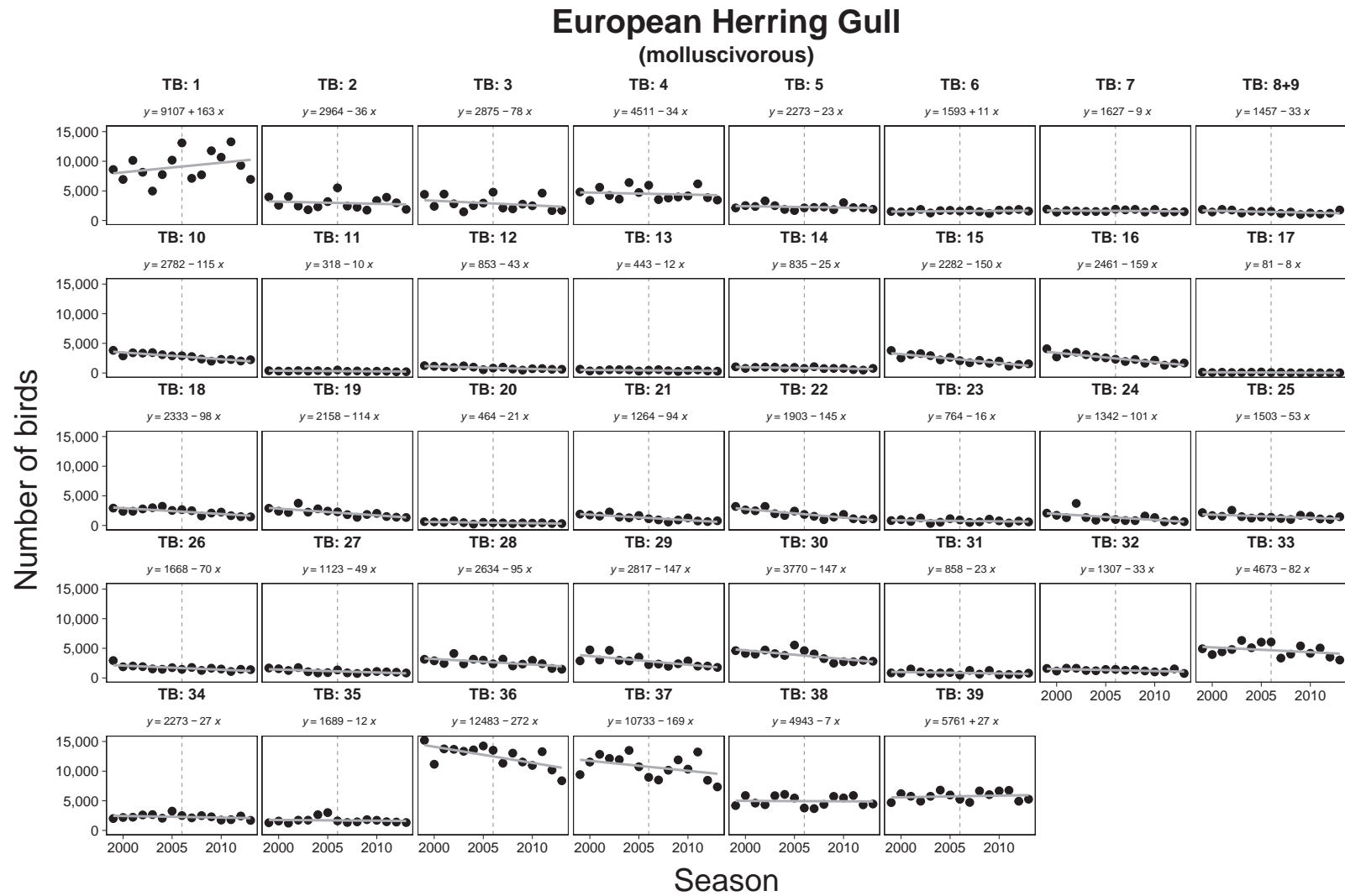
Figure S2.12: Numbers of European Herring Gull (*Larus argentatus*) from the different tidal basins (TB) of the Wadden Sea for the period 1999-2013.
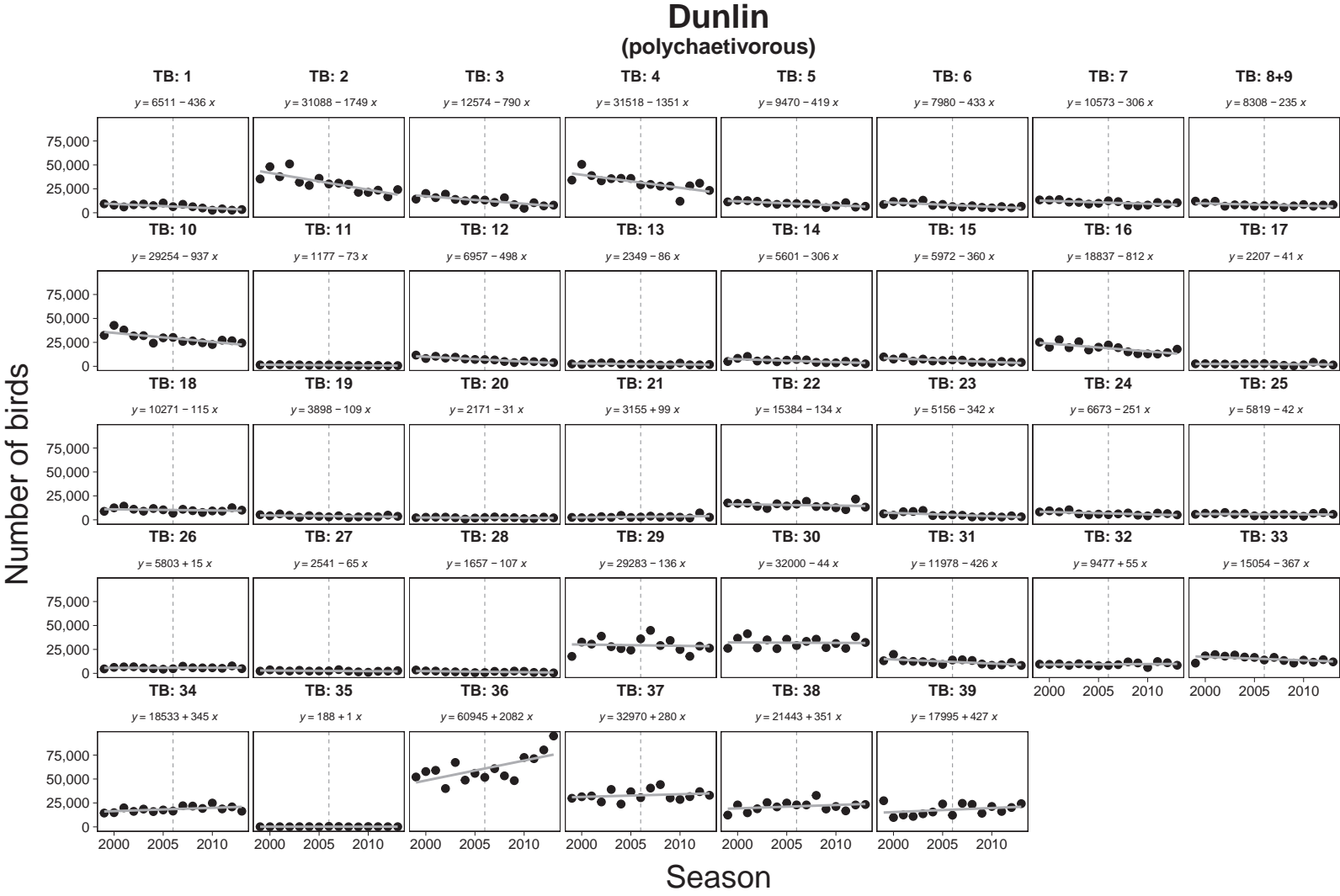
Figure S2.13: Numbers of Dunlin (*Calidris alpina*) from the different tidal basins (TB) of the Wadden Sea for the period 1999-2013.
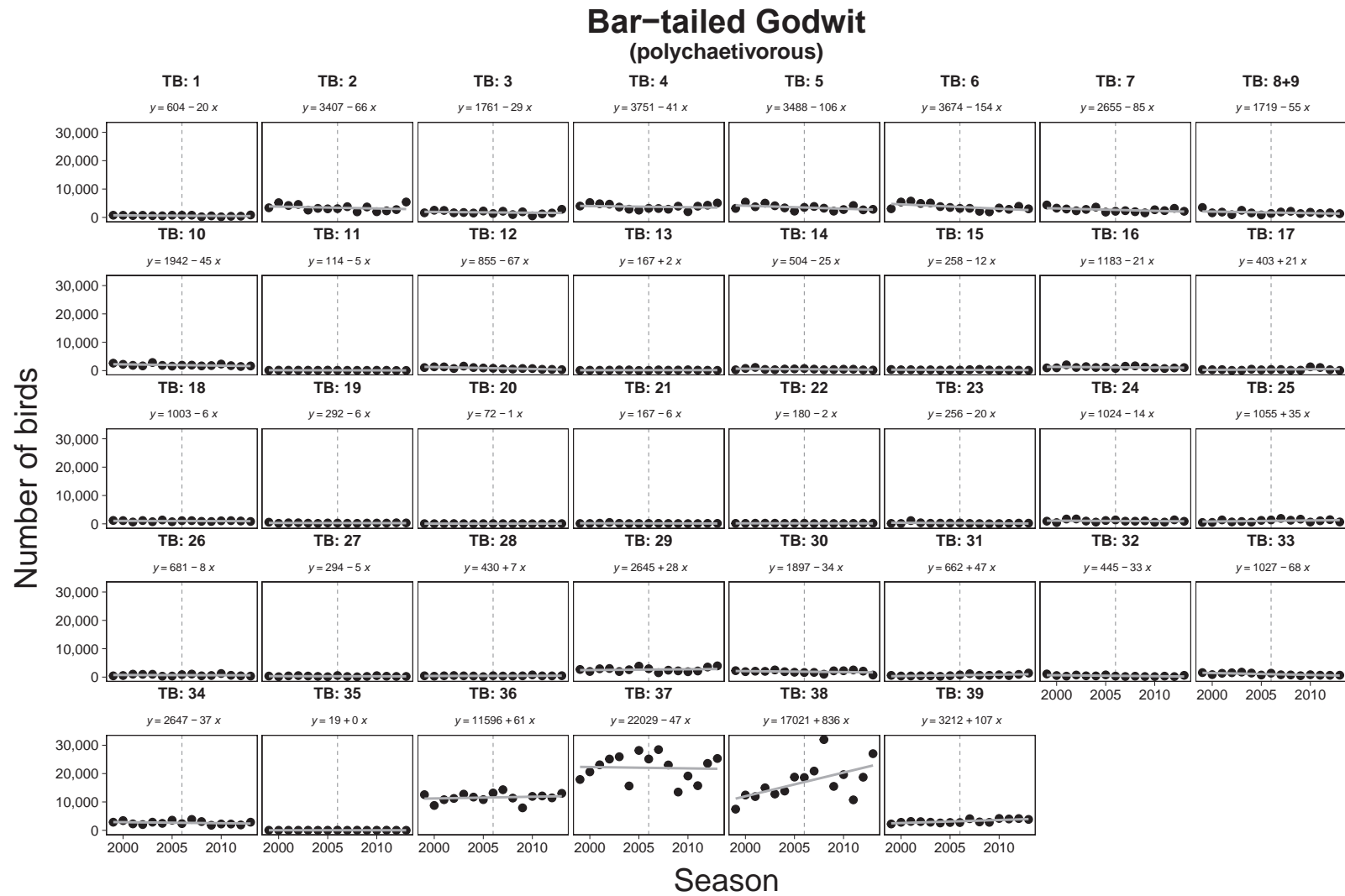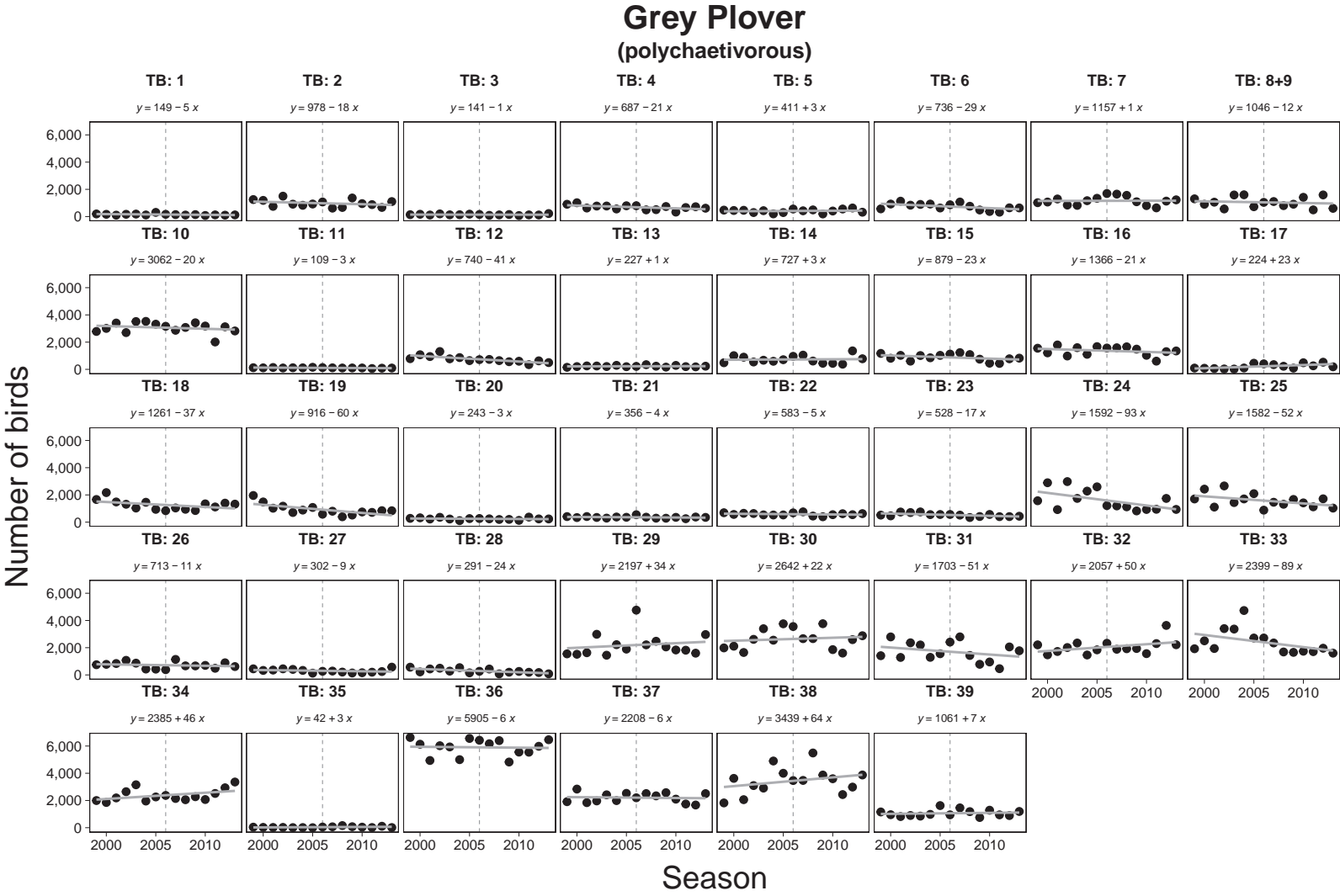
Bar−tailed Godwit
(polychaetivorous)

Figure S2.14: Numbers of Bar-tailed Godwit (*Limosa lapponica*) from the different tidal basins (TB) of the Wadden Sea for the period 1999-2013.

Grey Plover
(polychaetivorous)

Figure S2.15: Numbers of Grey Plover (*Pluvialis squatarola*) from the different tidal basins (TB) of the Wadden Sea for the period 1999-2013.

European Golden Plover
(polychaetivorous)
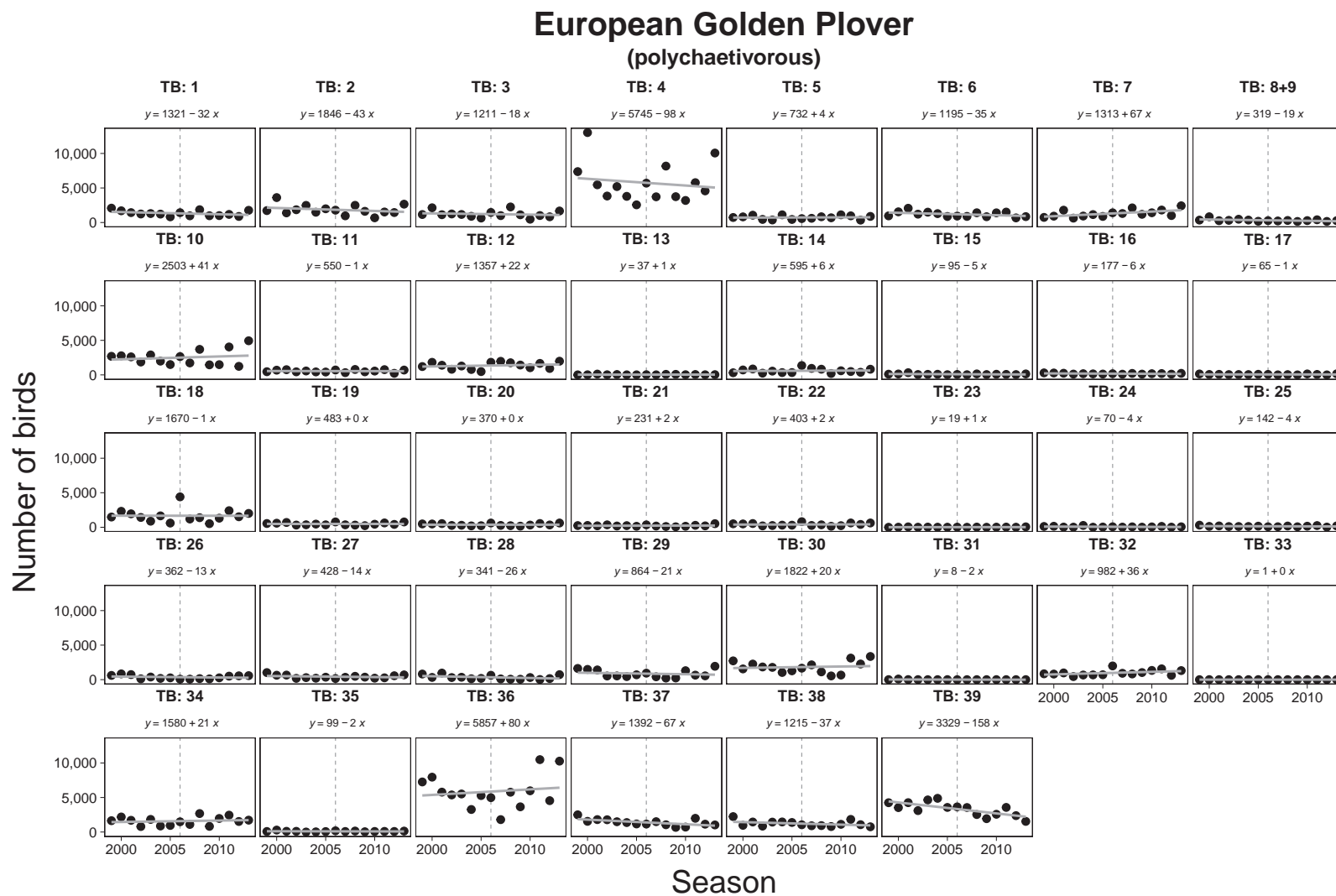
Figure S2.16: Numbers of European Golden Plover (*Pluvialis apricaria*) from the different tidal basins (TB) of the Wadden Sea for the period 1999-2013.
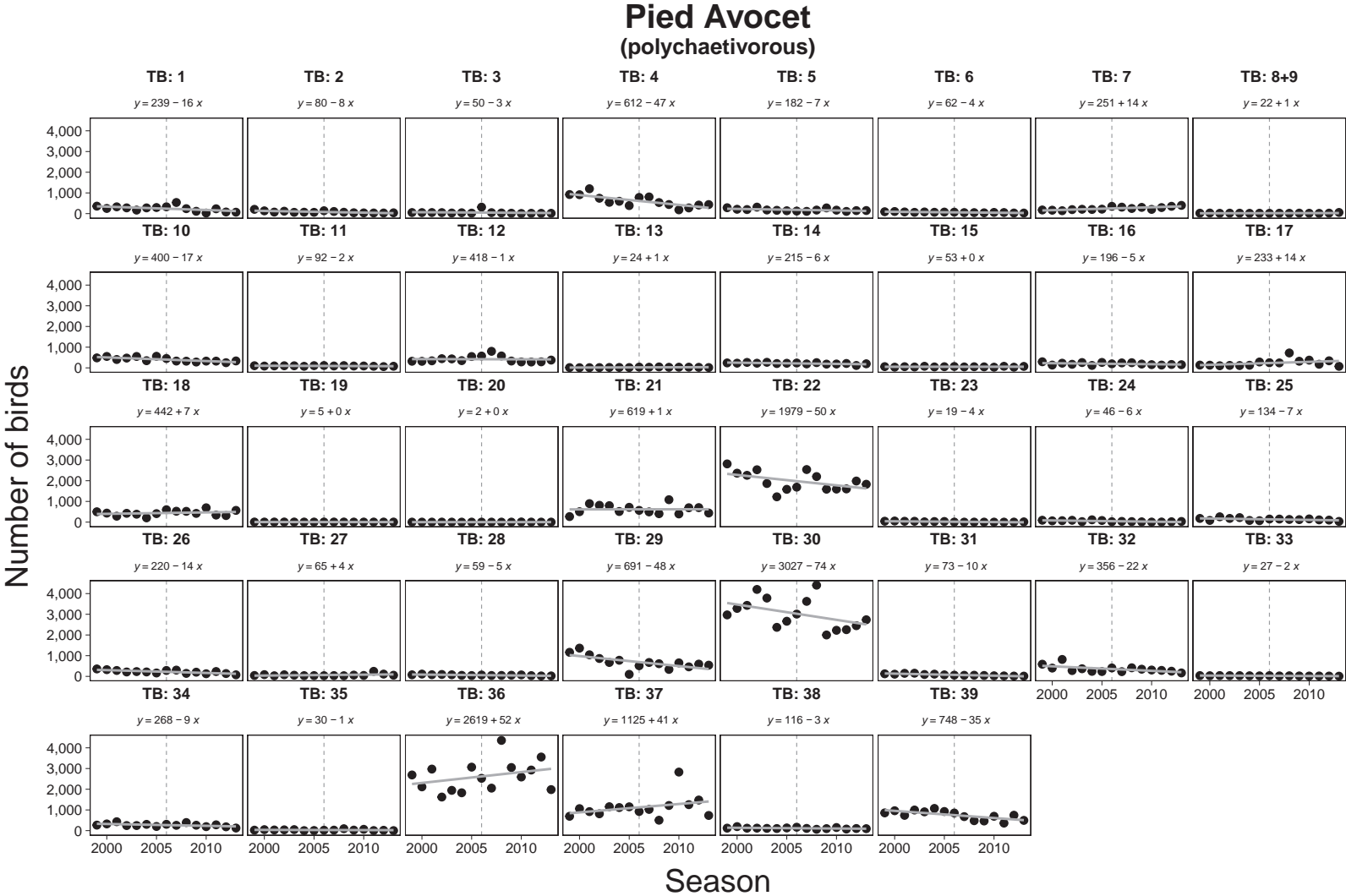
Figure S2.17: Numbers of Pied Avocet (*Recurvirostra avosetta*) from the different tidal basins (TB) of the Wadden Sea for the period 1999-2013.

Sanderling
(polychaetivorous)
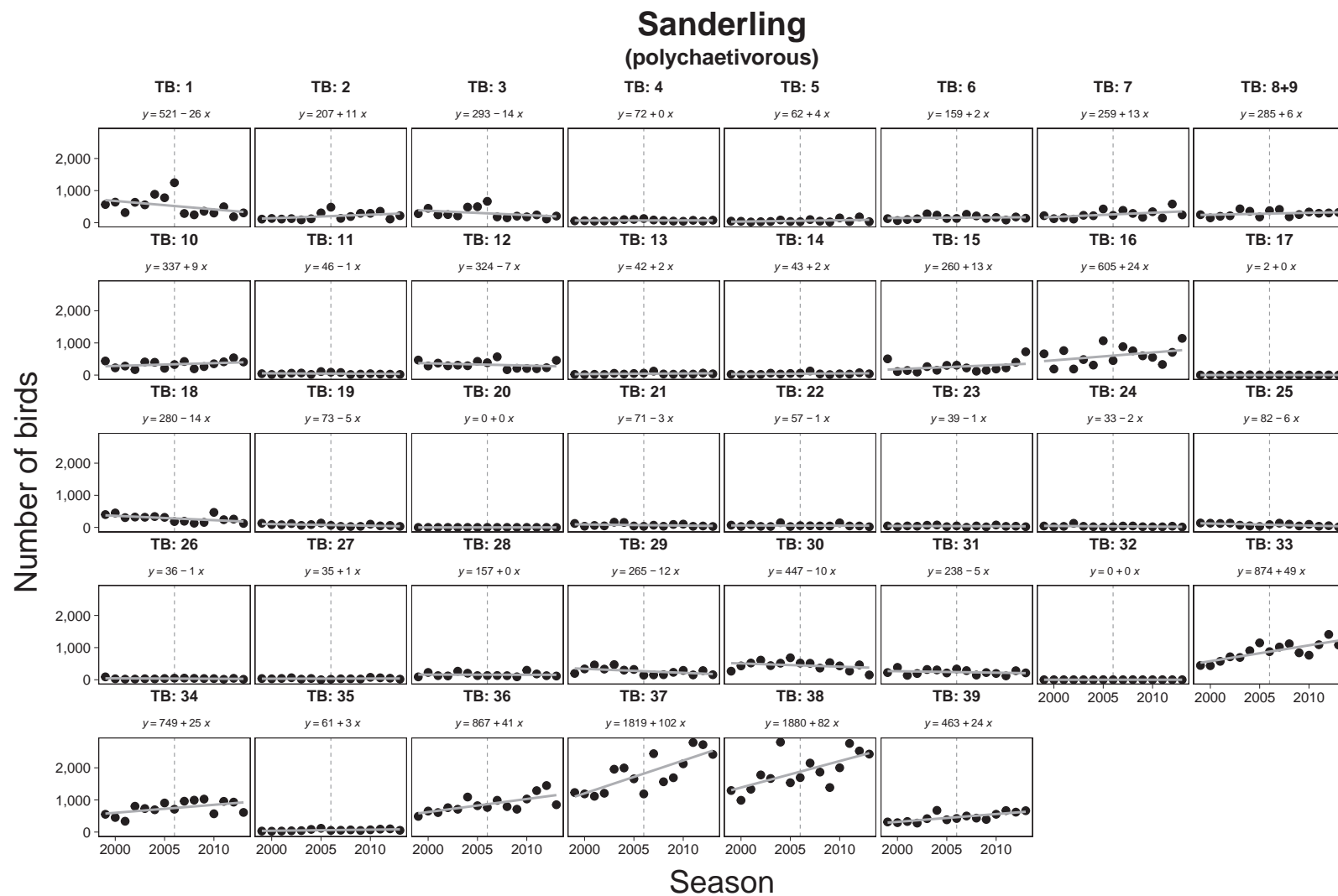
Figure S2.18: Numbers of Sanderling (*Calidris alba*) from the different tidal basins (TB) of the Wadden Sea for the period 1999-2013.

Common Ringed Plover
(polychaetivorous)
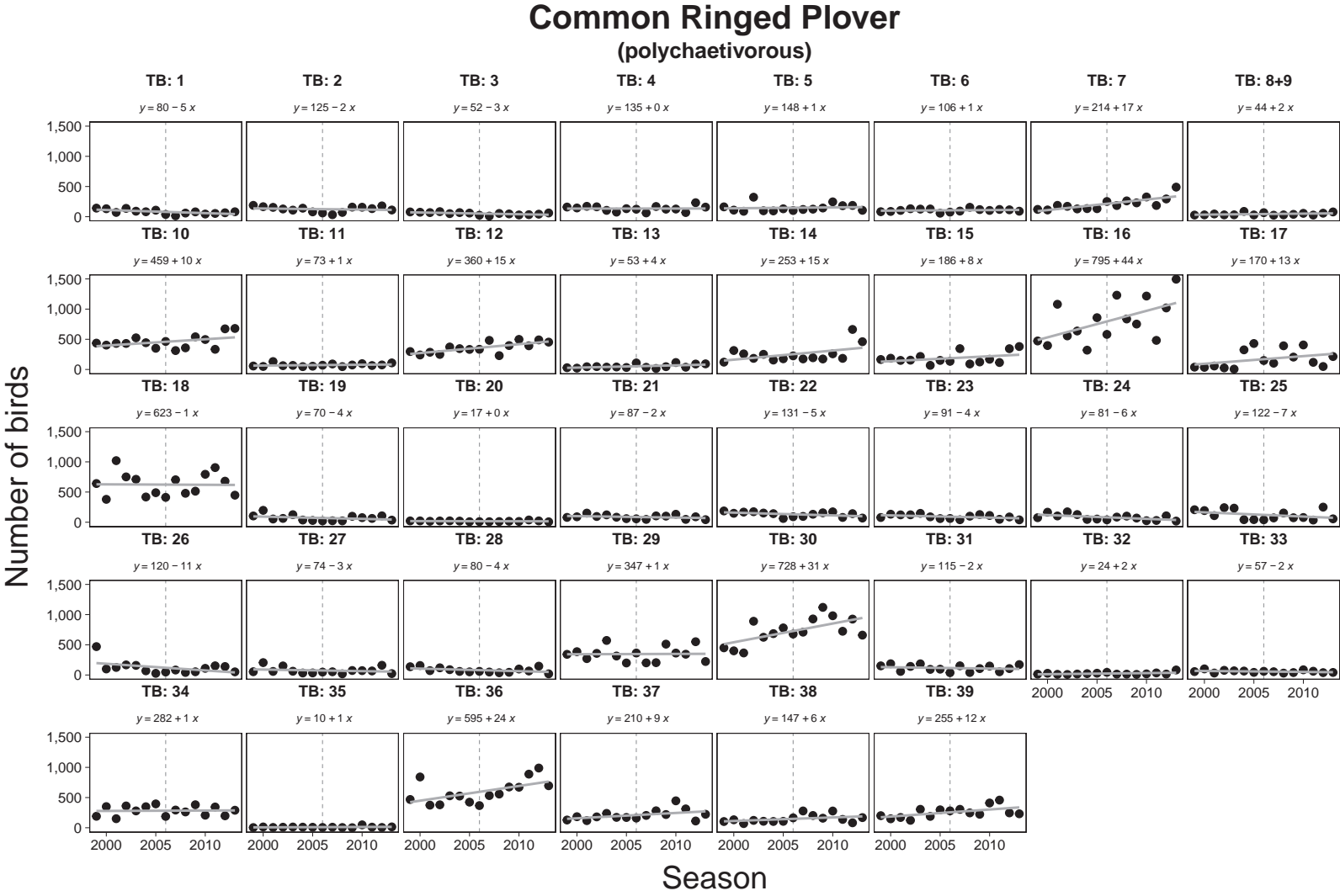
Figure S2.19: Numbers of Common Ringed Plover (*Charadrius hiaticula*) from the different tidal basins (TB) of the Wadden Sea for the period 1999-2013.

Eurasian Curlew
(benthivorous)
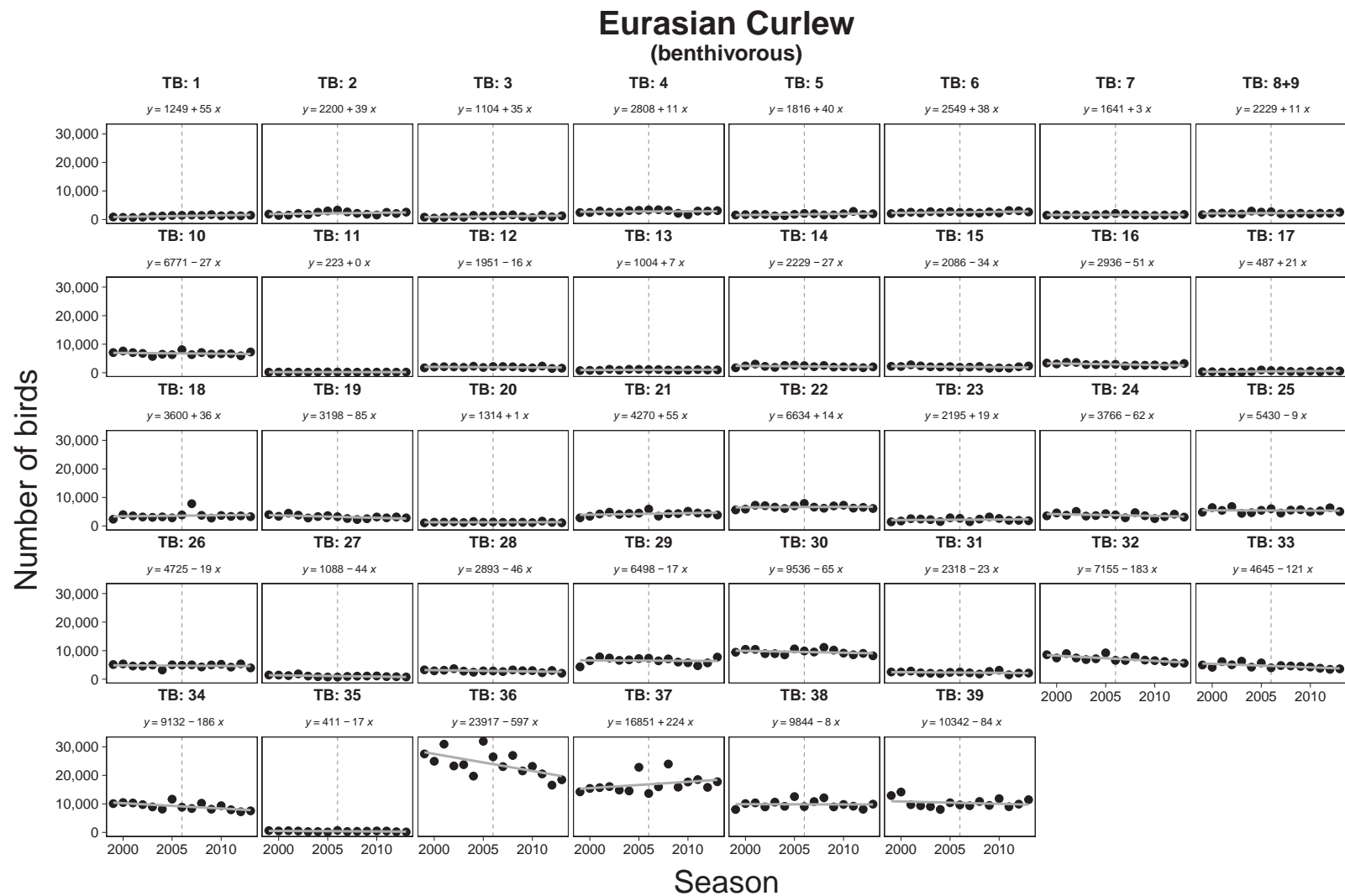
Figure S2.20: Numbers of Eurasian Curlew (*Numenius arquata*) from the different tidal basins (TB) of the Wadden Sea for the period 1999-2013.

Black−headed Gull
(benthivorous)
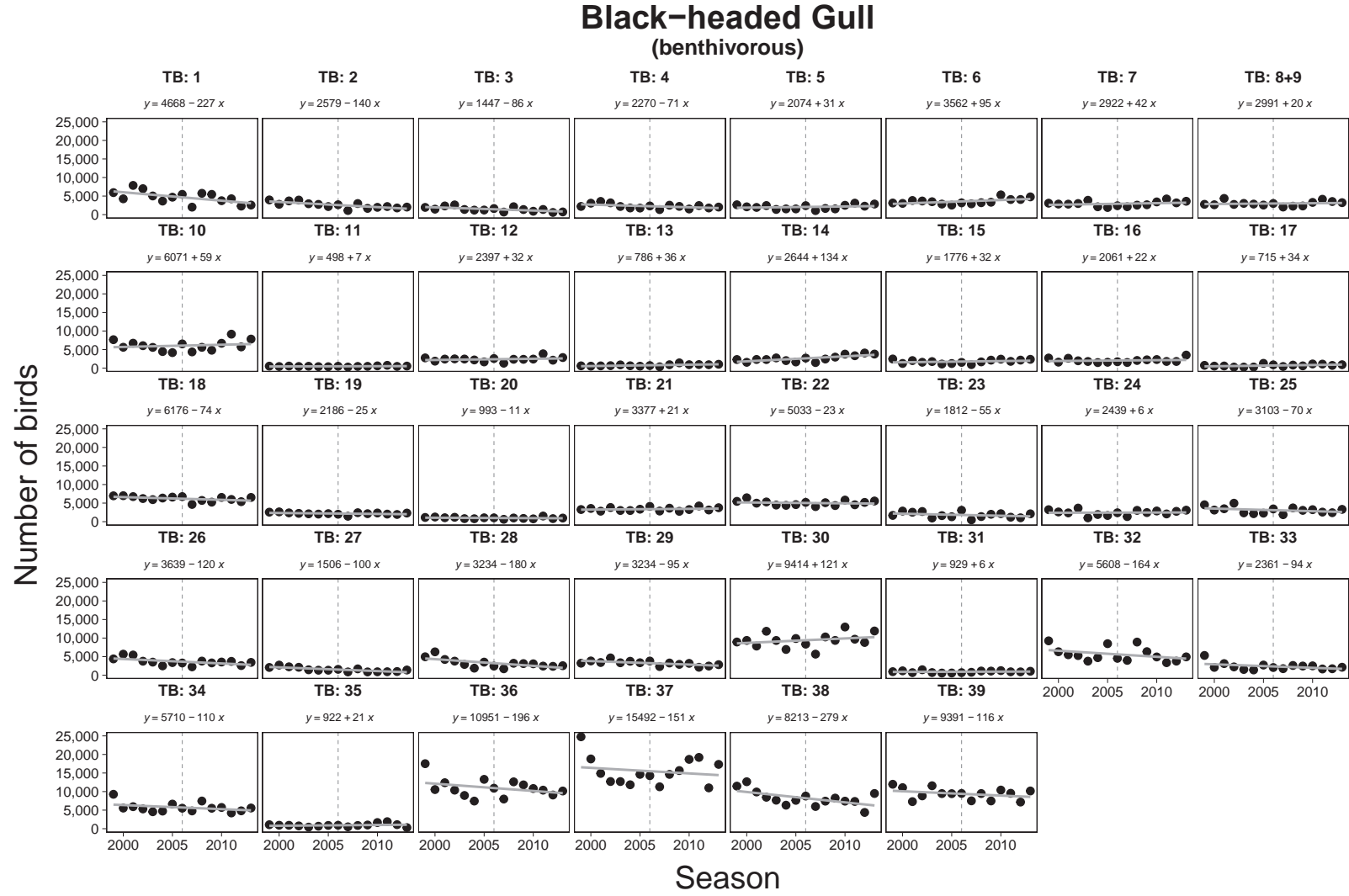
Figure S2.21: Numbers of Black-headed Gull (*Larus ridibundus*) from the different tidal basins (TB) of the Wadden Sea for the period 1999-2013.
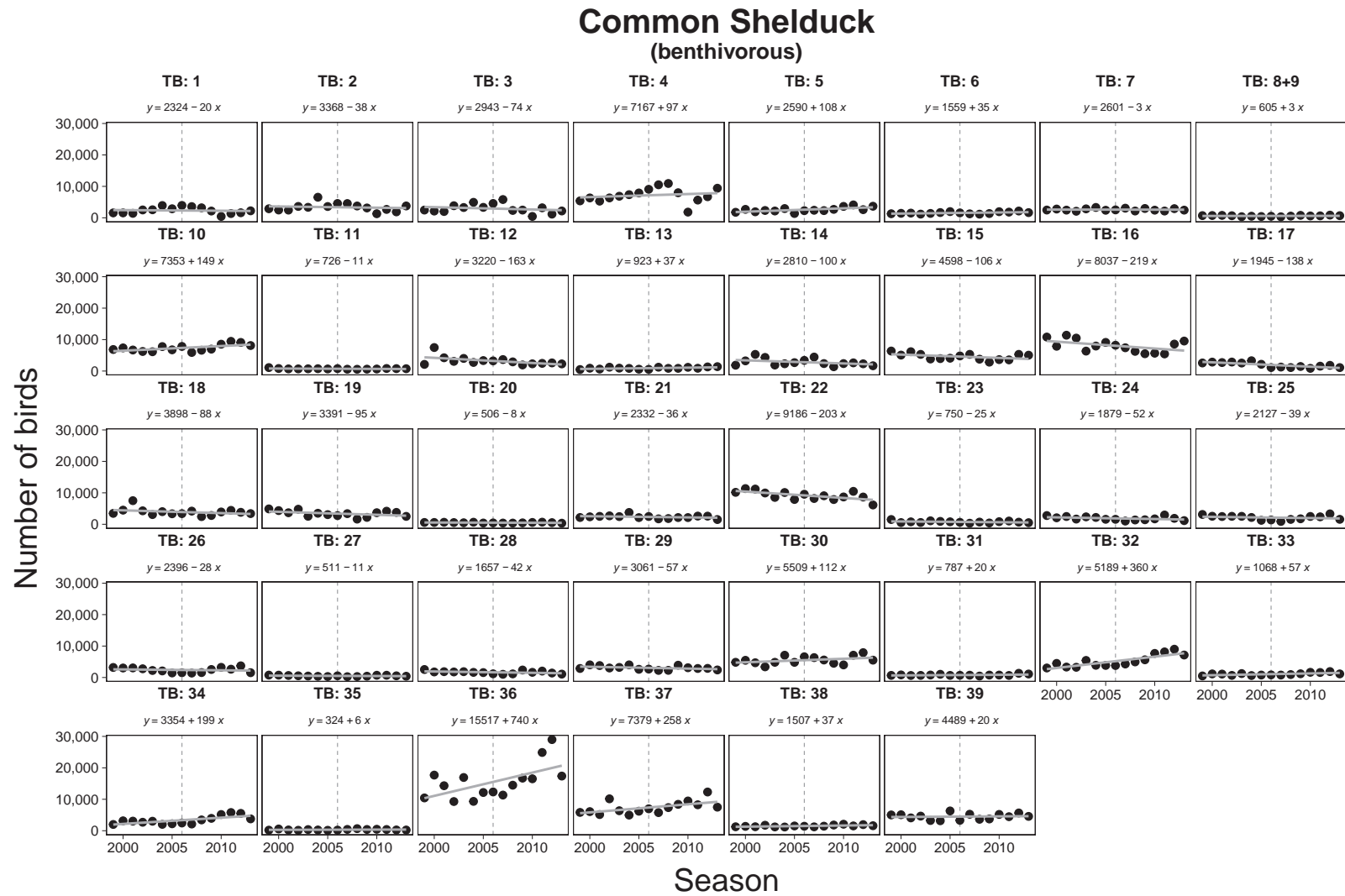
Common Shelduck
(benthivorous)

Figure S2.22: Numbers of Common Shelduck (*Tadorna tadorna*) from the different tidal basins (TB) of the Wadden Sea for the period 1999-2013
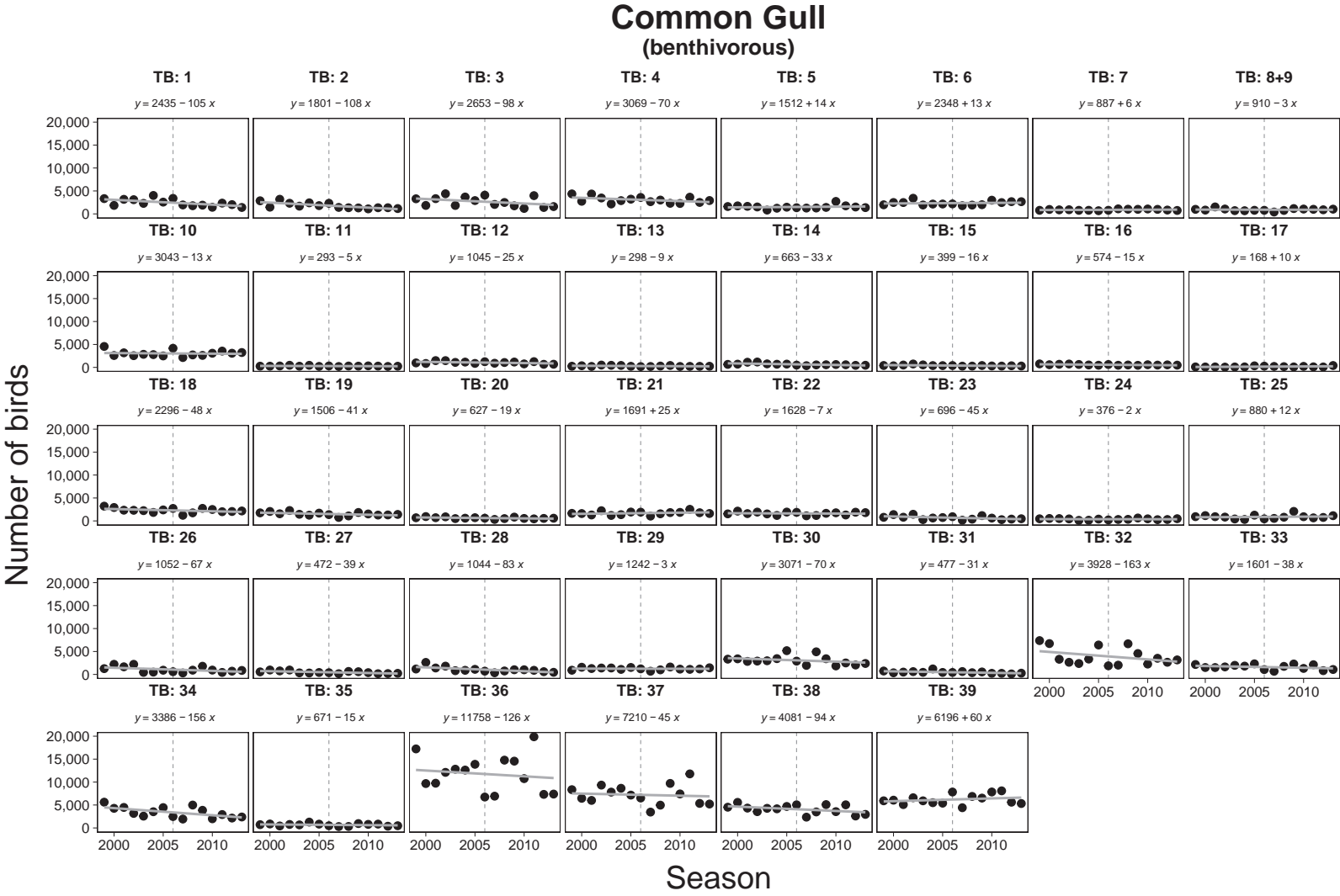
Common Gull
(benthivorous)

Figure S2.23: Numbers of Common Gull (*Larus canus*) from the different tidal basins (TB) of the Wadden Sea for the period 1999-2013.
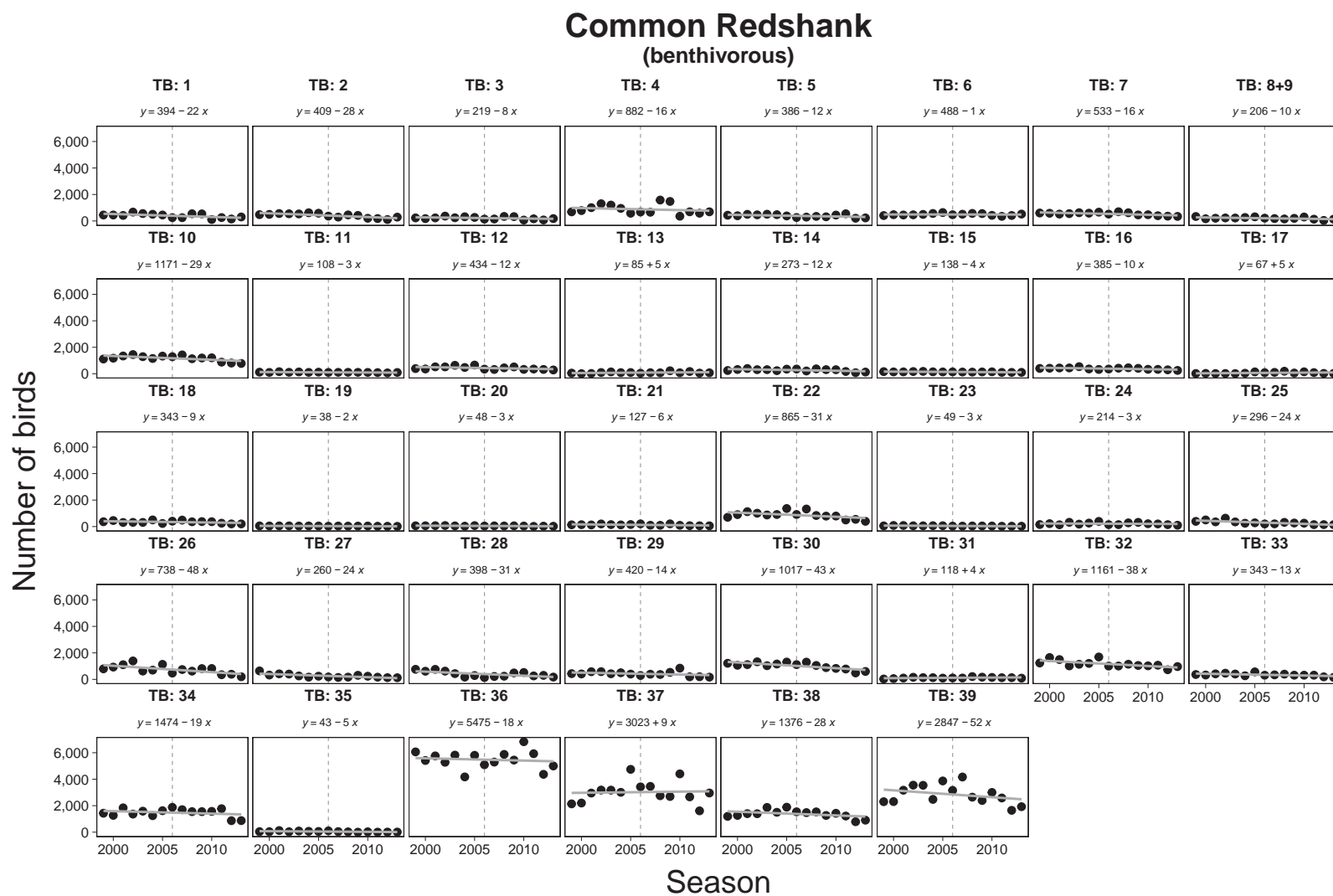
Common Redshank
(benthivorous)

Figure S2.24: Numbers of Common Redshank (*Tringa totanus*) from the different tidal basins (TB) of the Wadden Sea for the period 1999-2013.
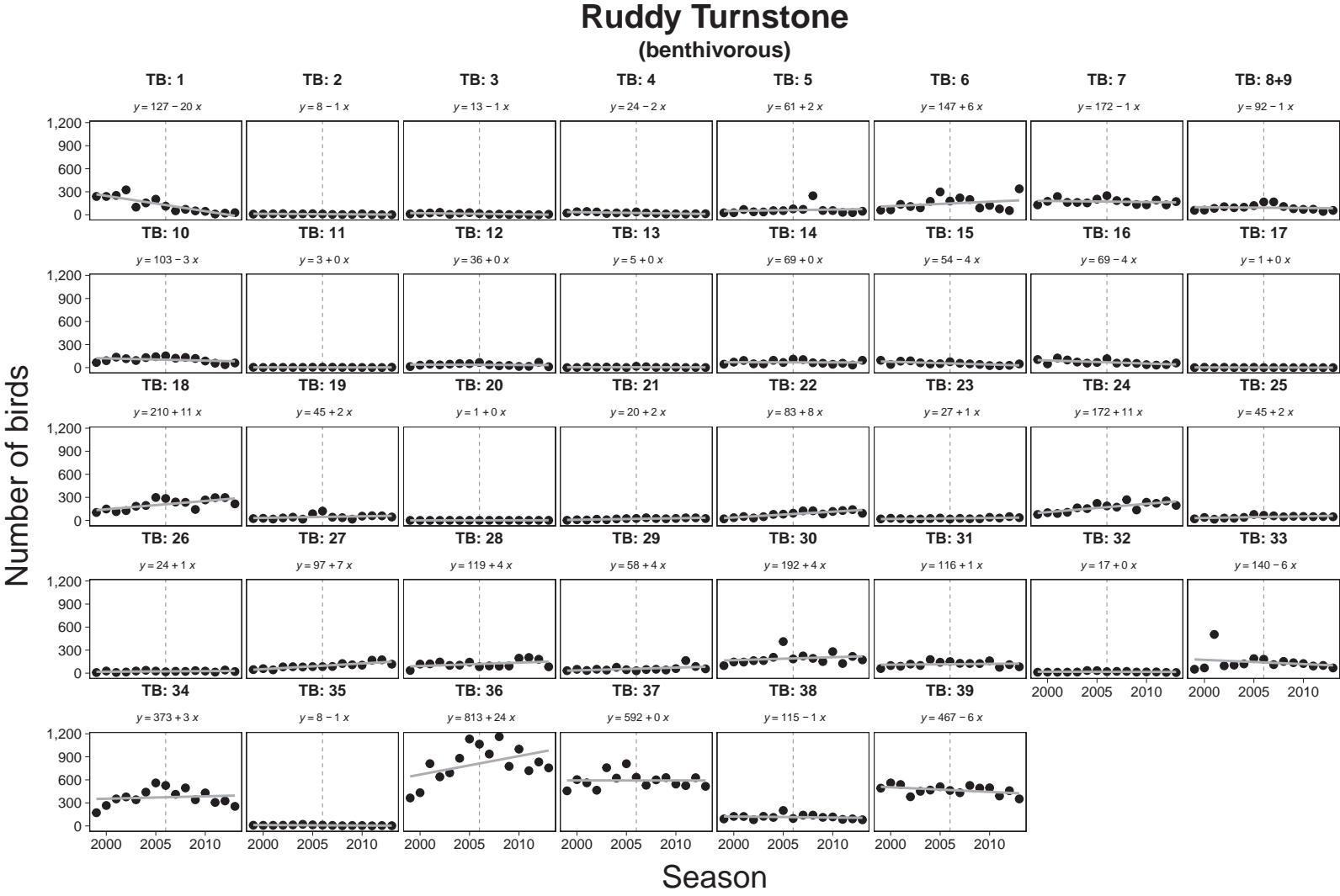
Figure S2.25: Numbers of Ruddy Turnstone (*Arenaria interpres*) from the different tidal basins (TB) of the Wadden Sea for the period 1999-2013.
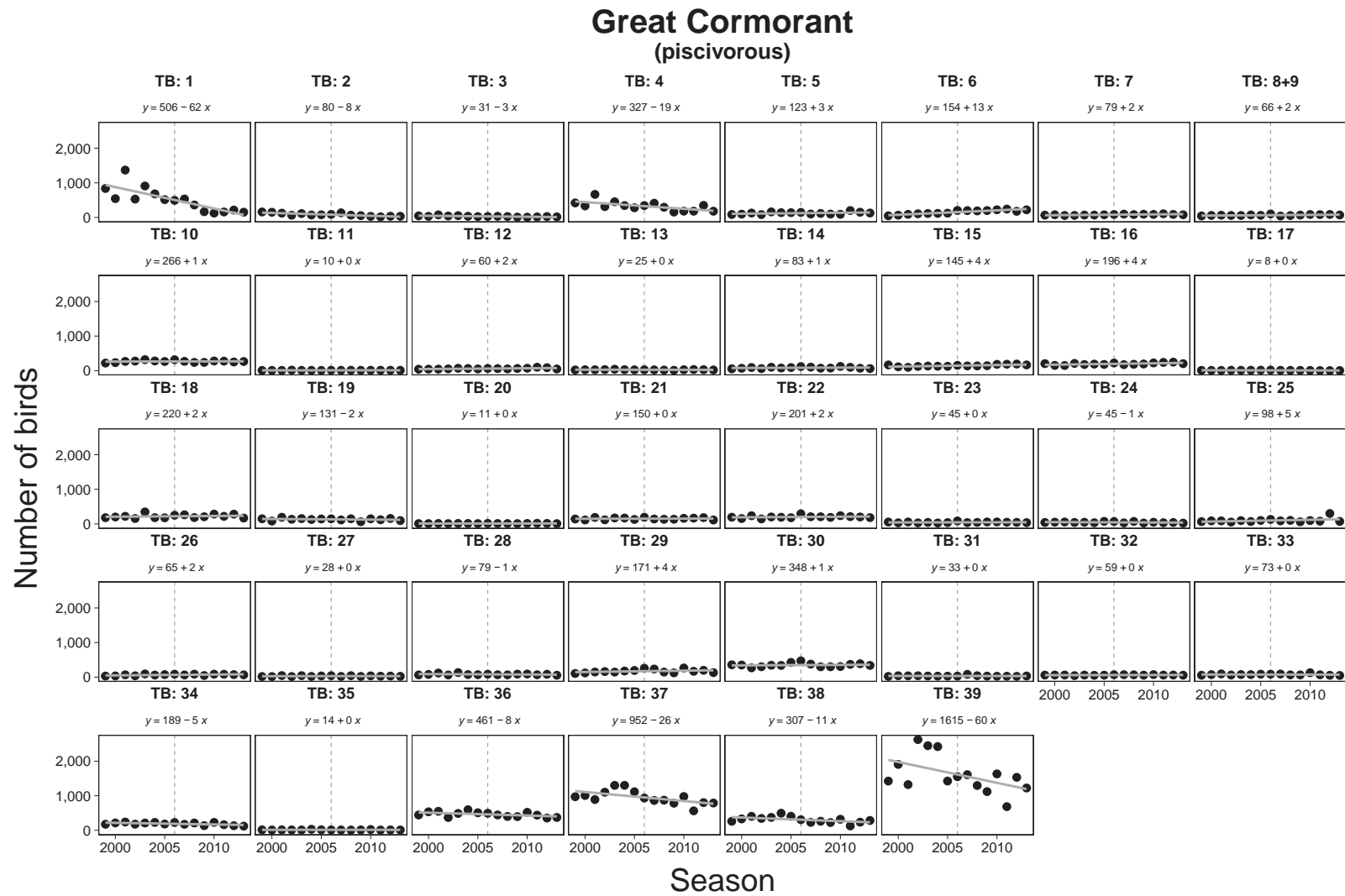
Great Cormorant
(piscivorous)

Figure S2.26: Numbers of Great Cormorant (*Phalacrocorax carbo*) from the different tidal basins (TB) of the Wadden Sea for the period 1999-2013.
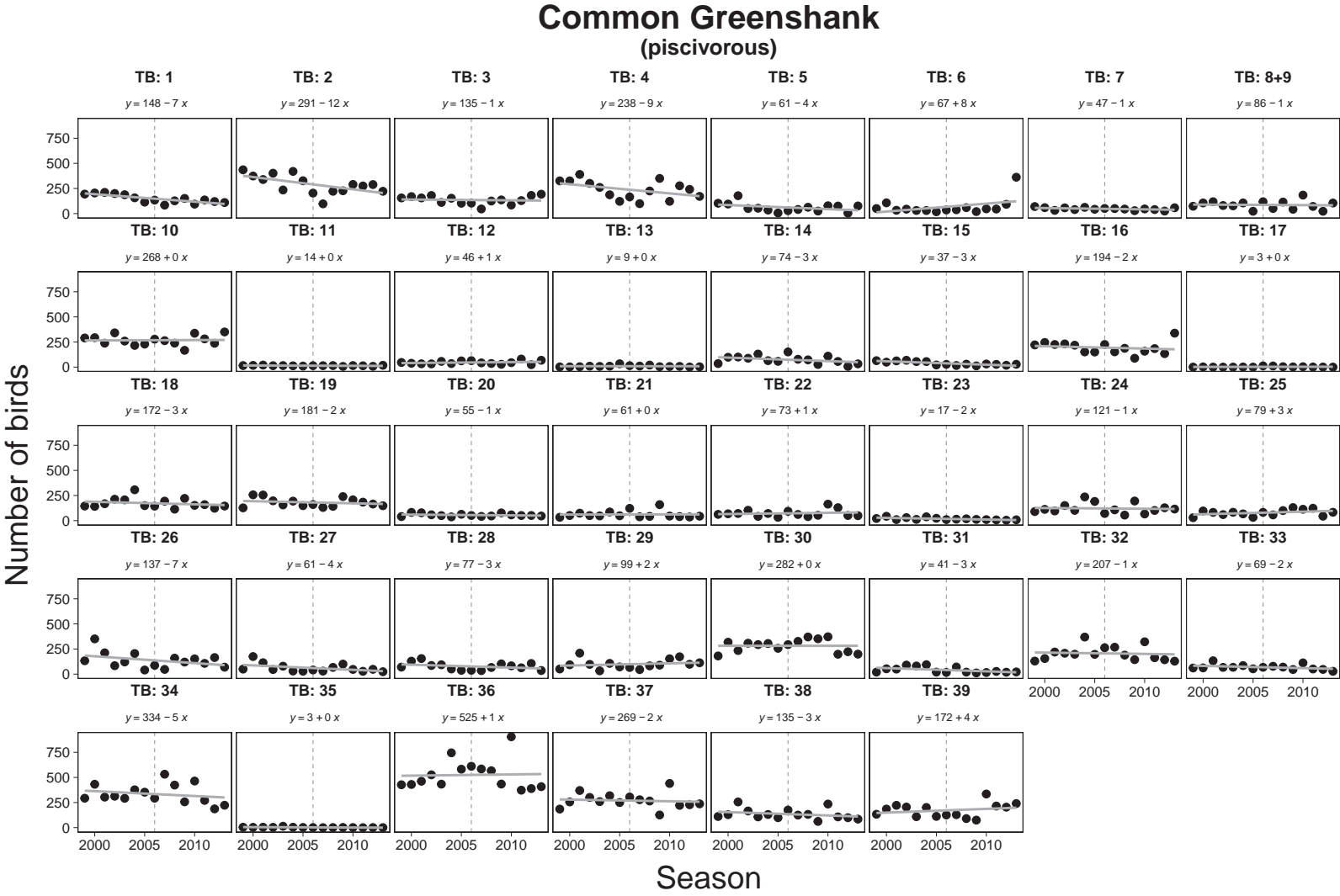
Common Greenshank
(piscivorous)

Figure S2.27: Numbers of Common Greenshank (*Tringa nebularia*) from the different tidal basins (TB) of the Wadden Sea for the period 1999-2013.
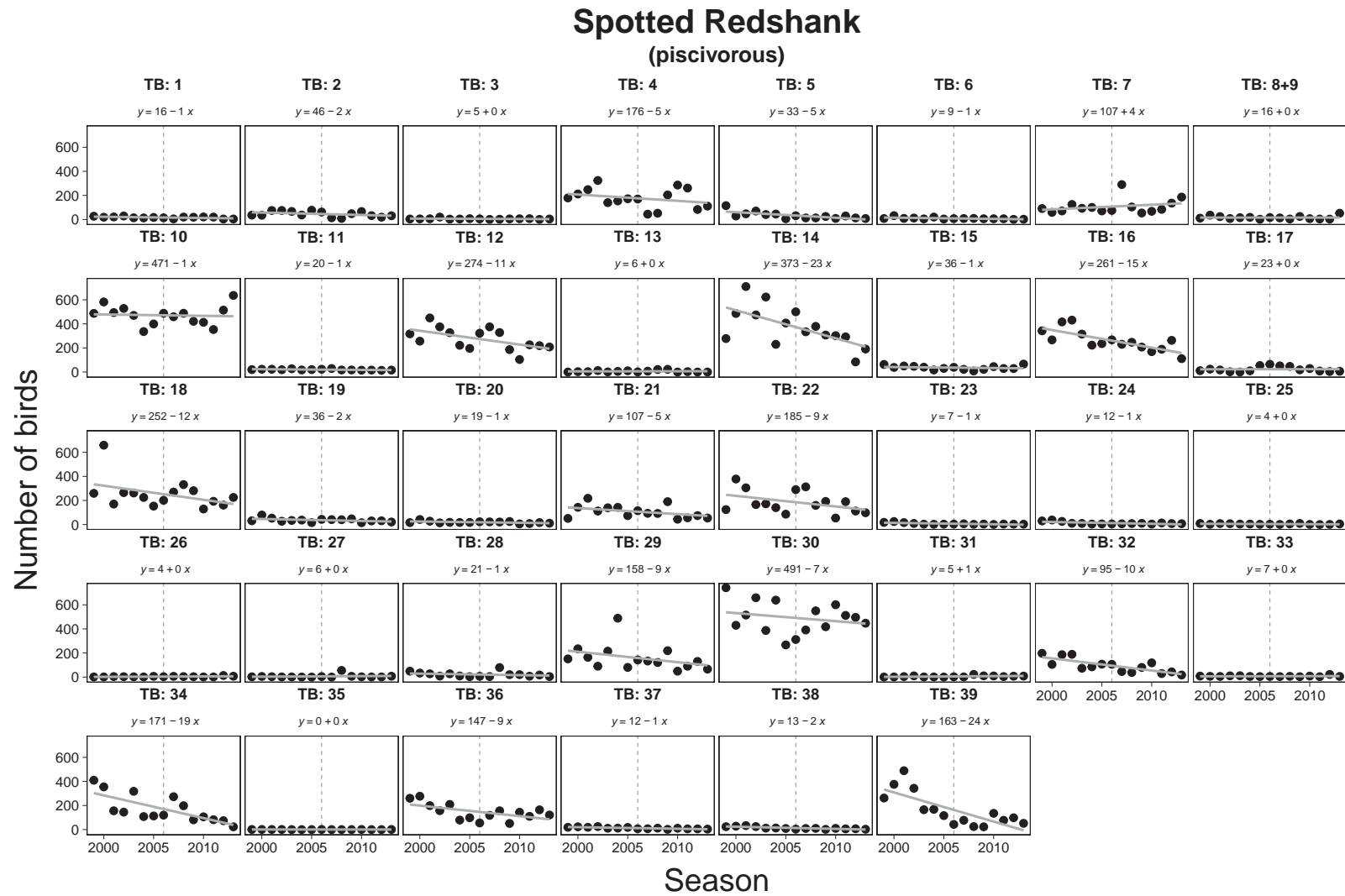
Spotted Redshank

(piscivorous)

Figure S2.28: Numbers of Spotted Redshank (*Tringa erythropus*) from the different tidal basins (TB) of the Wadden Sea for the period 1999-2013.
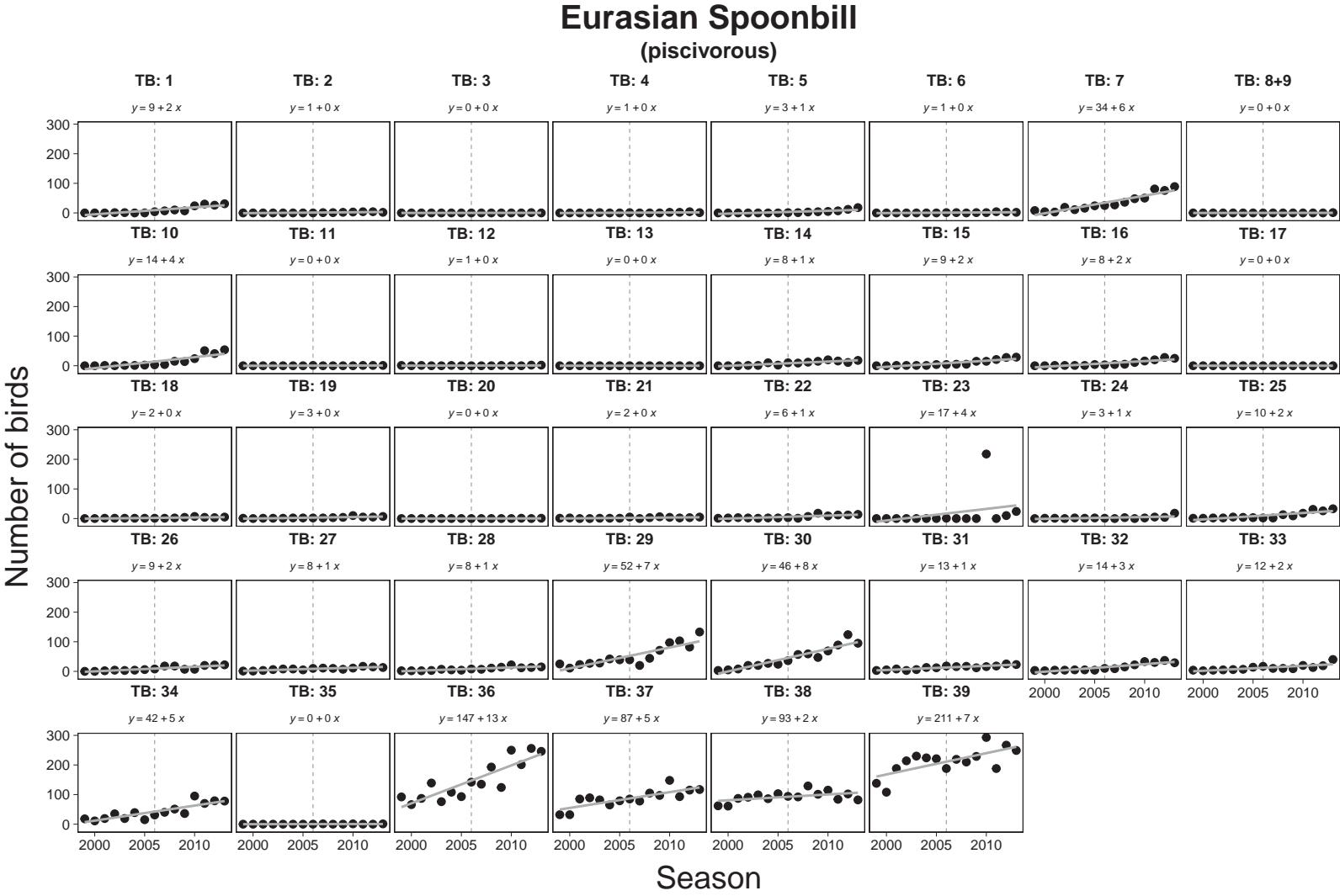
Figure S2.29: Numbers of Eurasian Spoonbill (*Platalea leucorodia*) from the different tidal basins (TB) of the Wadden Sea for the period 1999-2013.
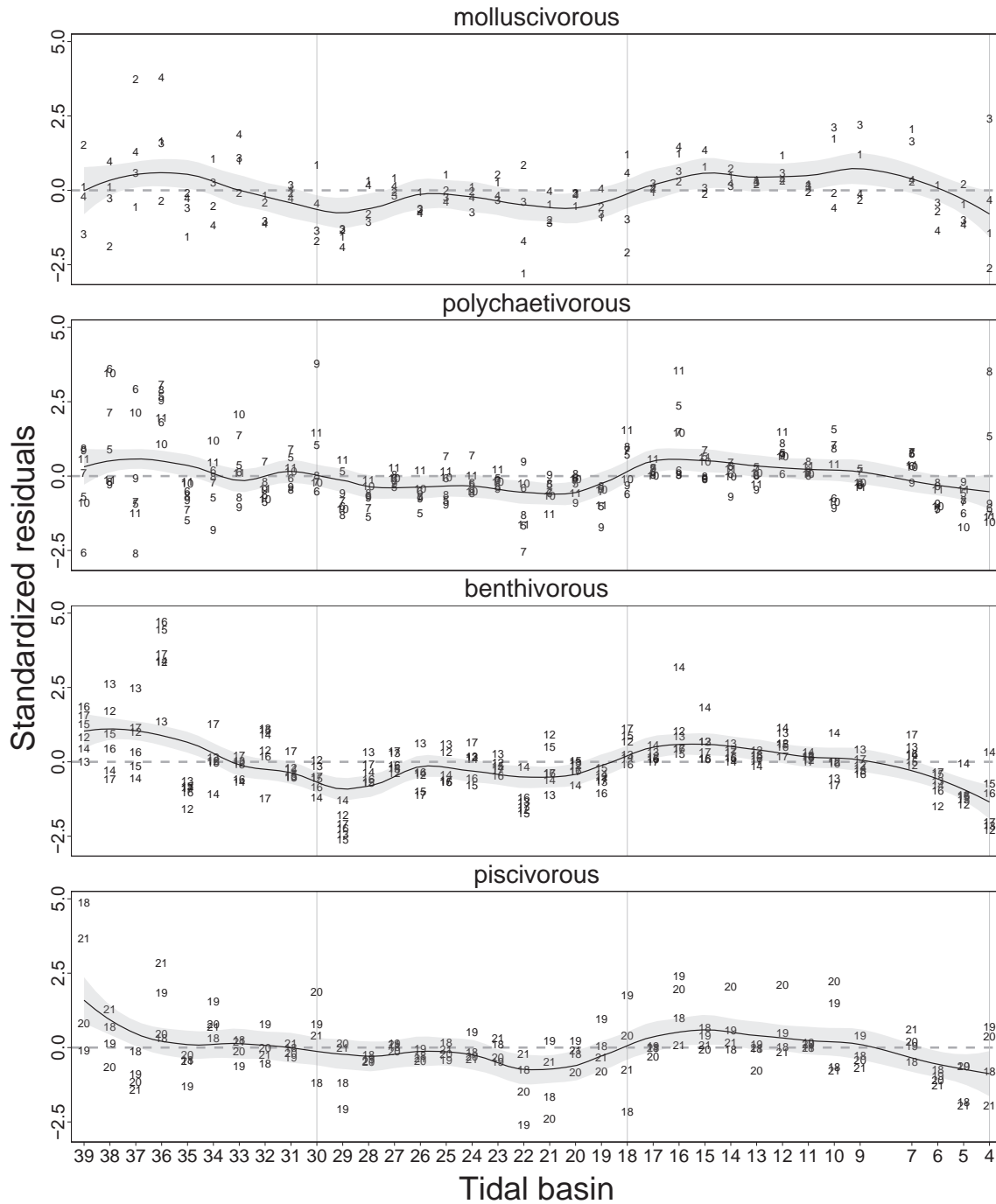
Figure S2.30: Standardized residuals (a residual divided by its standard deviation) of the final models (bird numbers versus habitat area) of the investigated bird species across the Wadden Sea (Tidal basins 4-39) divided into the different feeding groups. For visualisation purposes, the tidal basins are west-east aligned, starting with the westernmost tidal basin 39 (Marsdiep) in the Dutch Wadden Sea. Vertical lines indicate borders between the different regions. The horizontal dashed lines at 0 provide common references to more easily distinguish between positive and negative residuals. Positive residuals indicate higher bird abundances in a particular tidal basin compared to the expected value from the model estimate, while negative values indicate lower abundances than the model estimate. Note that tidal basin 9 is a merger of basins 8 and 9. For species identification see number coding used in Figure 2.3. A LOESS smoother (solid black line, span = 0.3) with 95% confidence intervals (grey shading) is added for interpretation aid.

3 | The birth, growth and death of intertidal soft-sediment bivalve beds: No evidence for ecosystem collapse in the Dutch Wadden Sea

Jaap van der Meer, Norbert Dankers, Bruno J. Ens, Marnix van Stralen, Karin Troost and Andreas M. Waser

Abstract

Recruitment and fate of all 1436 mussel and oyster beds of the Dutch Wadden Sea were studied for the period 1999-2013. Cox's proportional hazard rate model with fixed-time covariates such as orbital speed, inundation time and bed size and type, showed that large, shallow lying beds that experience a low orbital speed, live longer. Yet the most striking result was that mixed beds do have a much lower hazard rate than pure oyster or mussel beds. Simulation studies, using the observed recruitment series, which was very variable, and the estimated baseline survival curve, showed large variability and strong serial correlation in total bed area, implying that the present area, though it is lower than before, does not point to a systematic deviation from the pre-1990 situation, i.e. the situation before intensive fisheries and the disappearance of most beds around 1990. Claims that we have witnessed ecosystem collapse as a result of the fisheries and that bivalve bed recovery is impossible without restoration efforts are premature and not supported by our analysis. On the contrary, the observed high survival rate of mixed beds and the expectation that mixed beds will predominate in the near future, can easily result in much larger future bed coverage than what has ever been measured before.

Introduction

Birth and death processes are at the core of population ecology and demography. Obviously, most attention has been paid to the birth and death rates of individual organisms. Sometimes, however, it might be more convenient to work at a higher level of biological organization (Boughton & Malvadkar 2002). Students of colonial insects such as ants and termites, often focus on the birth and death of colonies. The life time of an ant colony may coincide with that of the queen, in which case one might argue that work is still done at the level of the individual organism. But, as queen supersedure within a colony occurs in many species, such is not necessarily true. In plant ecology, Watt's seminal paper on the dynamics of plant communities, resulted in the idea of gap-phase dynamics, which still plays a central role in forest ecology (Watt 1947, Gratzer et al. 2004, Hunter et al. 2015). In forests, gaps appear as a result of, for example, storms or of the fall of a single canopy individual. The regeneration rate of the gap is then related to the size of the gap and characteristics of the surroundings (van der Maarel 1996). Similar thoughts developed in coastal ecology, where hard substrata are discontinuous pieces of habitat surrounded by sand and mud. These hard substrata can almost entirely be covered by epifaunal invertebrates such as sponges, tunicates and mussels. Clearings are regularly made either by physical forces or by predators. The recolonization of such clearing will then, just as in the case of forest gaps, depend upon the size of the clearing and the characteristics of the surroundings (Connell & Keough 1985). Eventually these ideas evolved in a more general theory of patch dynamics, where the birth, growth or shrinkage, and death of patches rather than of individual organisms is the central issue (Levin & Paine 1974, Levin et al. 1993, Pickett & White 1985). Metapopulation ecology, introduced by Hanski, makes a further step by using entire populations, connected into a meta-population, as the main unit of interest (Hanski & Gilpin 1991). That is, the birth and death of connected populations are studied.

It is interesting to note that the choice of Watt was not so much based on theoretical, but merely on practical considerations. He acknowledges that 'the ultimate parts of the community are the individual plants', but adds 'but a description of it in terms of the characters of these units . . . is impracticable . . . ' (Watt 1947). In fact, the same argument is used by students of colonial insects, subtidal epifauna and island populations. It is much easier and less data demanding to describe the fate of patches, communities or populations than that of of individual organisms.

Our interest is in the dynamics of intertidal bed-forming bivalve populations living on the soft-sediments of the Dutch Wadden Sea, such as the blue mussel (*Mytilus edulis*) and the recently introduced Pacific oyster (*Crassostrea gigas*). These intertidal bivalve beds are the habitat of many benthic and epibenhtic species and attract numerous birds and fishes, for which these beds provide a rich food source (Goss-Custard et al. 1982). As such they have a high conservation value, and conservationists were worried to see that in the early 1990s, in a period with low spatfall and ongoing fisheries, hardly any intertidal bed was left (Herlyn & Millat 2000, Ens 2006). Since then these beds have partly recovered (Dankers et al. 2001), but doubts remain to what level compared to the pristine situation (Dankers et al. 2001). It has even been claimed that due to sediment disturbance by mechanical fishing activities, the Wadden Sea has undergone drastic changes and collapsed into an alternative stable state, where recovery of bivalve beds is basically impossible without large-scale restoration projects (Eriksson et al. 2010). Recently, quite some effort has been put in initiating artificial restoration projects, but without much success (van der Heide et al. 2014, de Paoli et al. 2015).

The aim of our study is to investigate if the disappearance of intertidal mussel beds at the end of the 1980s has indeed caused the Wadden Sea ecosystem to collapse into an alternative stable state. To this end, we will estimate present recruitment and survival rates of intertidal bivalve beds, predict population size and fluctuations therein, and compare that with available recent and historical data, using the bivalve bed as the study unit, instead of the individual organisms. It must be noted though that in one respect, the system cannot return to its original

state, due to the invasion of the Pacific oyster (Troost 2010). Although the Pacific oyster was introduced in the Dutch Wadden Sea already around 1978, it did not really spread until the late 1990s, i.e. well after the disappearance of the intertidal mussel beds (Reise 1998, Diederich 2005, Nehls et al. 2006, Troost 2010). Whereas in the past bivalve beds consisted entirely of mussels, nowadays bivalve beds may consist entirely of mussels or oysters, or a mixture of these two species. Apart from studying present-day recruitment and survival of bivalve beds in general, we will also estimate these parameters for different types of bivalve bed.

Finally, we will show that there is no evidence at all for an ecosystem collapse and that present recruitment and survival rates of mussel-oyster beds are sufficient to achieve and maintain historic population levels. Due to the presence of the invading Pacific oyster, we even predict an increase in bed area in the long run.

Materials and methods

Definition of a mussel bed

In 2002, Dutch, German and Danish scientists agreed upon a common definition of littoral mussel beds to facilitate trilateral comparisons (CWSS 2002, Herlyn 2005, Nehls et al. 2009b). A mussel bed was defined as a benthic community structured by blue mussels that may consist of an irregular collection of more or less protruding smaller patches, separated by open spaces. Patches should be larger than 1 m in diameter and within 25 m distance from each other. A collection of humps smaller than 1 m in diameter should have an areal coverage of more than 5% in order to be grouped within one patch (Figure 3.1). Following the expansion of the Pacific oyster into many mussel beds of the Wadden Sea the original definition gained a more general character and is now used to define different types of bivalve beds with varying amounts of mussels and oysters. In the Netherlands, beds are defined as pure mussel beds if the cover of Pacific oysters is less than 5% and as pure oyster beds if the cover of mussels is below 5%. In all other cases, beds are denoted as mixed beds.
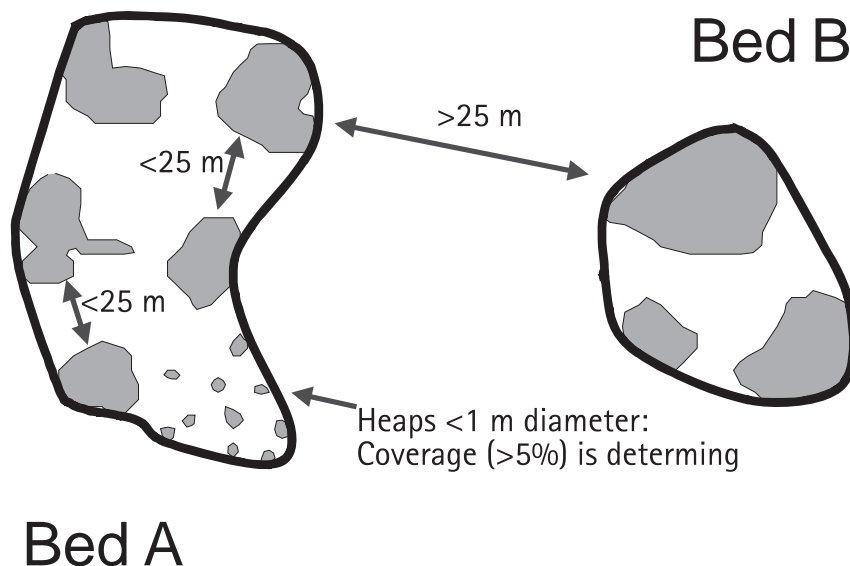

Figure 3.1: Visual representation of the demarcation of mussel beds (adapted from Nehls et al. 2009b).

We follow this definition, but since it does not say anything about the time component, we added the rule that patches from different years that overlap in space belong to the same bed. If two patches from the same year spatially overlap with the same bed in the previous year or in the following year, then these two patches are considered to belong to the same bed, even if

they are further away from each other than 25 meter (Figure 3.2). Within this definition, beds can neither split nor merge. They can only be born, survive or die. The definition requires an iterative procedure to uniquely classify all patches in beds.
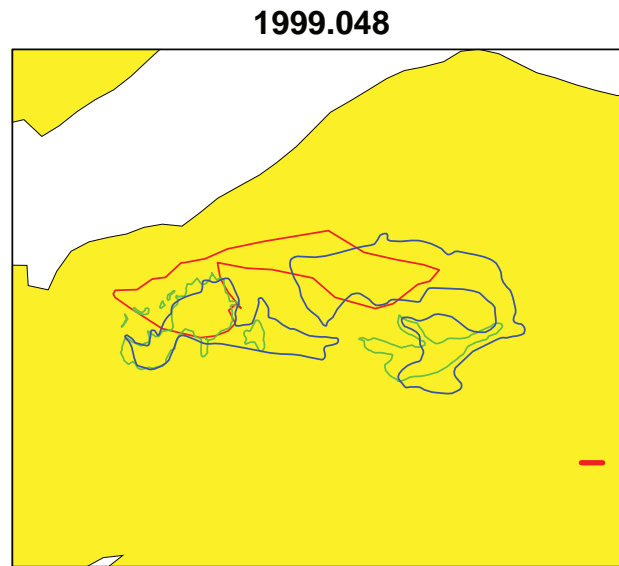
1999.048

Figure 3.2: Changes over time of mussel bed 1999.048. Each colour represents a different year. Note that some green patches are only grouped within the same bed, as they overlap with the same blue bed, that in itself consists of patches that only form one bed because they overlap with the same red bed. The thick red line indicates a distance of 50 m.

Field data

Each spring Wageningen Marine Research (formerly IMARES) and the private company MarinX map all the littoral mussel and oyster beds in the Dutch Wadden Sea. During low tide, researchers walk around the beds to demarcate the contours and their tracks are recorded by GPS and imported into GIS. Characteristics of the beds, such as percentage cover of mussels and oysters, are recorded. Bed locations are determined by a previous inspection of the area from an aeroplane flying at an altitude of 500 m. Due to time constraints not all locations can be visited each year. For unvisited locations, use may be made of the data set from the previous year or the year after. For example, if in year 2008 a 2-year old bed is found at a particular location which was not recorded in 2007, the 2007 data set is updated to include that bed. Bed distribution data for the period 1999–2013 are used. Details of the sampling procedure are in Steenbergen et al. (2006).

Survival analysis

Mussels and oysters spawn in spring and early summer and larval settlement occurs a few weeks after spawning. These beds become visible to the human eye in autumn. The survey is in spring and the recruitment of beds thus occurs when the bed is about half a year old. Beds are then followed until their disappearance ('death') or until the end of the study period, which was 2013. The bed lifetime data might thus be left censored, when recruitment occurred before the start of the study period in 1999, right censored, when the beds were still alive in 2013, or interval censored, when they are simultaneously left and right censored (Table 3.1). The statistical analysis should consider this censoring and therefore a survival analysis using Cox's proportional hazards model with fixed-time qualitative and quantitative covariates was performed (Klein & Moeschberger 2003). The qualitative covariate indicates the overall bed type, and each bed was classified as either a mussel bed, an oyster bed or a mixed bed. Beds are

only classified as mussel beds when they were categorized as such for all years in which they existed. The same holds for oyster beds. All other cases are classified as mixed beds. So, for example, a bed that experiences a single transition from a mussel bed to an oyster bed only, is classified as a mixed bed in the survival analysis. The quantitative covariates (longitude, log bed size, depth, orbital speed, inundation time) are also considered as fixed-time covariates, which means that they are assumed not to change over the study period.

Table 3.1: Censoring. For those beds 'born' in 1999 or still alive in 2013, the exact lifetime is not known. One can only say that they 'lived' for at least a specified number of years, i.e. 4+ means a life time of at least 4 years.

	1999	2000	2001	2002	2003	...	2011	2012	2013
1999	1+	2+	3+	4+	5+		13+	14+	15+
2000		1	2	3	4	...	12	13	14+
2001			1	2	3	...	11	12	13+
2002				1	2	...	10	11	12+
2003					1	...	9	10	11+
...									
2011							1	2	3+
2012								1	2+
2013									1+

Simulation model

We developed a very simple simulation model of bed dynamics, where in each year of the simulation one number is randomly selected from the observed 14-year series (2000-2013) of recruitment area data. These data are visualized in Figure 3.5. The sampled number is the initial size of a simulated cohort. For each cohort and each subsequent year, the fraction of the initial recruitment area that had survived was simply taken from the overall survival curve, which is visualized in Figure 3.4 by the black line. So the stochasticity in the predicted series is entirely determined by the recruitment, and the predicted variability should therefore be considered as a minimum. In practice variability in survival will also contribute. The population was simulated for a period of 1000 years.

Software

All analyses were performed using the R platform (R Development Core Team 2015). We used the packages sp, maptools, rgeos, rgdal, raster, and spdep for spatial analysis and the package survival for survival analysis. Scripts are available from the corresponding author.

Results

The classification of all observed or interpolated bed contours over the period 1999–2013 resulted in a total of 1436 beds, each year of their existence categorized as either a mussel bed, an oyster bed or a mixed one (Table 3.2). A plot of the surface area of each bed in a specific year against the area of the same bed in the following year revealed no clear pattern of growth or shrinkage (Figure 3.3). Most beds only show relatively small changes in area, either a slight growth or a slight shrinkage. Apparently, bed growth cannot be described by a simple model, comparable to say the Bertalanffy growth model, which for many species adequately describes the growth of the individual organism.

Table 3.2: The original data can be summarized by a 1436 by 15 matrix where each element contains the type of bed; M is mussel, C is oyster, B is mixed, and 0 indicates that the bed has not yet been established or has disappeared.

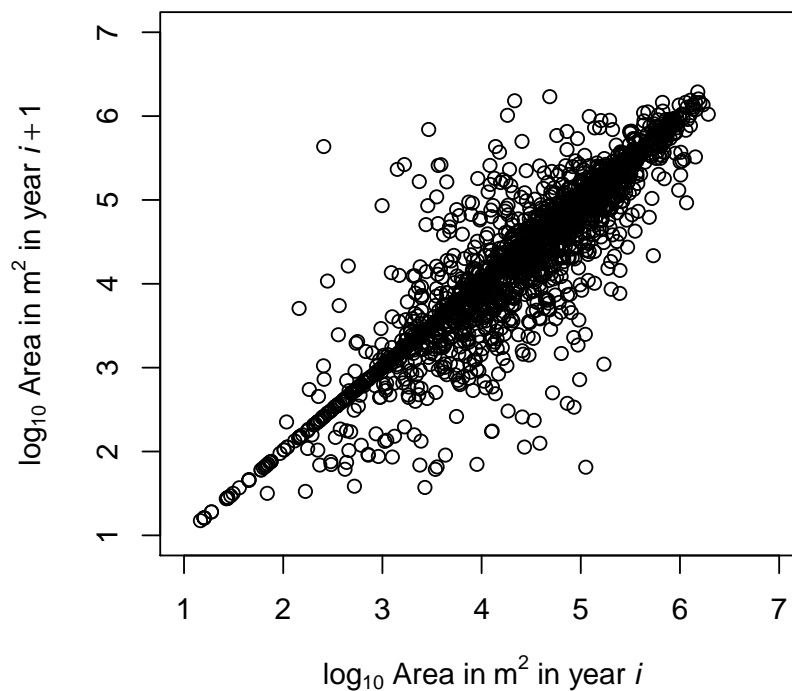|	**1999**	**2000**	**2001**	**2002**	**2003**	**2004**	**...**	**2010**	**2011**	**2012**	**2013**
1999.001	M	0	0	0	0	0	...	0	0	0	0
1999.002	M	M	M	B	B	C	...	B	C	B	B
1999.004	M	M	M	M	0	0	...	0	0	0	0
1999.007	M	0	0	0	0	0	...	0	0	0	0
1999.008	M	0	0	0	0	0	...	0	0	0	0
1999.009	M	0	0	0	0	0	...	0	0	0	0
.	.	.	.	.	.	.	...	.	.	.	.

Figure 3.3: The area of individual beds in two succeeding years. Points above the diagonal line point to growth and those below the line to shrinkage of beds.

The fate of all beds, in terms of their year of birth and year of death is summarized in Table 3.3. The first striking result is that more than half of the beds, i.e. 751 out of 1436, are on the main diagonal, which tells that these beds are born and die in the same year. Another 232 and 121 die in the following two years. This result is confirmed by estimating the overall survival curve, or the curve for each overall bed type separately (Figure 3.4). The survival curves, which show the probability of being alive versus age, drop down quickly in the first few years. Yet, after an age of about 4 to 5 years the survival functions go down very slowly, which implies that the hazard rate (the chance of disappearing) becomes very low. The baseline survival function of Cox's proportional hazard model confirms this result. The inclusion of various standardized covariates (which means that their mean is zero and standard deviation one) in Cox's proportional hazard model significantly improved the fit (Table 3.4). The tabulated coefficients show the effect of the various covariates. For example, for those beds that are one standard deviation larger than the mean bed size, the hazard rate is only 63% of the average hazard rate (Table 3.4). Beds that have a one standard deviation higher inundation time, do have a 15% higher hazard rate (Table 3.4). The effect of orbital speed is significant, but small (Table 3.4). To summarize, large, shallow lying beds that experience a low orbital speed, do live longer.

Table 3.3: Year of 'birth' (rows) versus year of 'death' (columns).

	99	00	01	02	03	04	05	06	07	08	09	10	11	12	13
1999	55	34	9	12	5	2	0	0	2	0	1	0	1	1	23
2000	0	60	9	10	3	1	1	0	1	1	2	0	2	2	9
2001	0	0	15	12	2	1	0	1	1	1	0	0	0	1	2
2002	0	0	0	138	35	16	12	15	3	4	3	0	2	0	35
2003	0	0	0	0	42	3	3	0	2	0	0	0	0	0	8
2004	0	0	0	0	0	99	23	24	2	7	5	0	1	5	37
2005	0	0	0	0	0	0	62	13	1	1	1	0	2	1	15
2006	0	0	0	0	0	0	0	74	21	8	9	1	3	3	8
2007	0	0	0	0	0	0	0	0	43	13	8	1	2	3	2
2008	0	0	0	0	0	0	0	0	0	9	8	2	6	2	15
2009	0	0	0	0	0	0	0	0	0	0	29	8	6	8	13
2010	0	0	0	0	0	0	0	0	0	0	0	44	22	14	19
2011	0	0	0	0	0	0	0	0	0	0	0	0	22	1	18
2012	0	0	0	0	0	0	0	0	0	0	0	0	0	8	30
2013	0	0	0	0	0	0	0	0	0	0	0	0	0	0	51
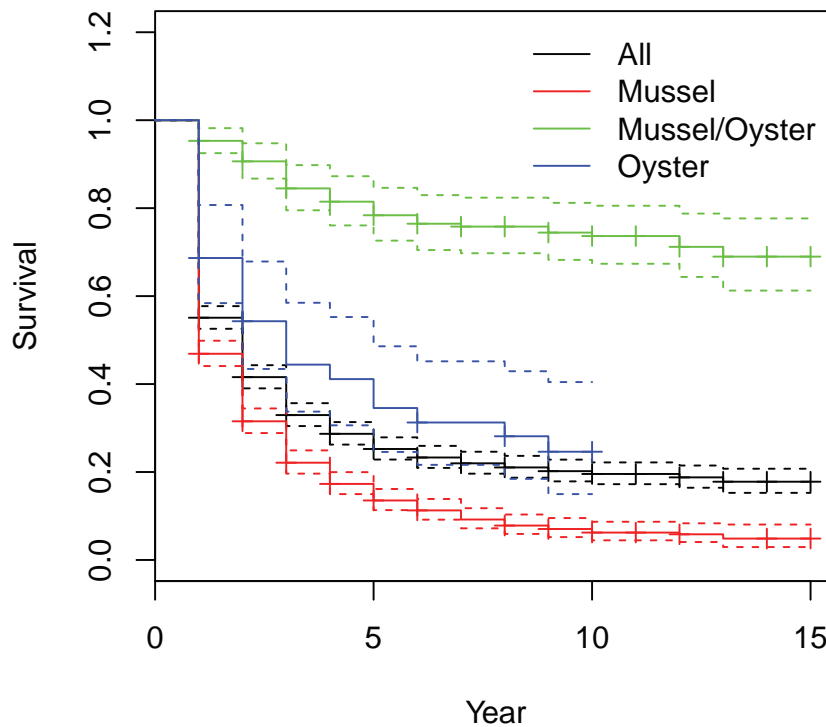
Figure 3.4: Survival curves of all beds together, and of the different bed types separately; mussel beds, mixed beds and oyster beds. Dashed lines show average ± SEs.

The most remarkable result is that mixed beds do have a much lower hazard rate and thus a much higher survival than pure mussel beds, the hazard rate is only 12% of that of pure mussel beds (Table 3.4, Figure 3.4). Oyster beds take an intermediate position. But, recall that what is called a mixed bed in this analysis did not necessarily started as a mixed bed. In theory such result could have been due to the fact that beds that are initially single-species beds, simply have a higher chance of becoming a mixed bed once they live longer. The causation of the 'mixed-beds live longer' observation, is then in the opposite direction. Long-lived beds have a higher chance of becoming mixed. Yet, an additional analysis where annual transitions are considered separately for the different bed types, does indeed confirm the idea that mixed beds do have a higher survival chance. Looking at beds of all ages, mixed beds disappeared in 64 out

Table 3.4: Results of Cox proportional hazards model.

	β	$\exp(\beta)$	$se(\beta)$	z	p
Longitude	0.43	1.04	0.035	1.25	0.21
Bed size	-0.46	0.63	0.033	-14.01	< 0.001
Depth	-0.05	0.95	0.042	-1.24	0.21
Orbital speed	0.09	1.09	0.037	2.39	0.017
Inundation	0.14	1.15	0.047	2.98	0.003
Mixed	-2.17	0.12	0.153	-14.17	< 0.001
Oyster	-0.70	0.50	0.165	-4.21	< 0.001

Table 3.5: Transition matrix showing annual transitions from (rows) to (columns) a specific bed type, all 1436 beds by 14 transitions per bed.

	None	**Mussel**	**Mixed**	**Oyster**
None	14855	1055	44	52
Mussel	1122	1718	31	4
Mixed	64	111	494	51
Oyster	105	10	18	370

Table 3.6: As Table 3.5, but None now stands for no bed or for a bed younger than 4 years old. Mussel, Mixed and Oyster refer to beds older than 3 years.

	None	**Mussel**	**Mixed**	**Oyster**
None	18289	115	19	12
Mussel	242	591	22	3
Mixed	32	80	406	33
Oyster	58	1	11	190

of 720 cases, which is 9% (Table 3.5). Oyster beds in 105 out of 503 (21%) and mussel beds even in 39%, i.e. in 1122 out of 2875 cases (Table 3.5). Restricting the analysis to beds older than 3 years yields similar disappearance rates of 6%, 22% and 28%, respectively (Table 3.6). So, the differences are large, but not as large as the proportional hazards model indicates.

The expected total bed area follows from multiplying the sum of all the survival function elements (black line in Figure 3.4) times the annual average of the sum of all 'newborn' bed areas (Figure 3.5). As the survival function could only be estimated up to the age of 15 years old, the function was extrapolated beyond that age by assuming a further drop of 0.01 per year, which is in line with the decrease in the surviving fraction after about 5 years (Figure 3.4). The oldest age becomes then 27 years, after which age all beds are assumed to have disappeared. The sum of the survival function is then 5.62 (note that this is also the expected lifespan of a bed) and the average total area of newborn beds is 3.17 km^2. So the expected total bed area is 17.8 km^2. The simulations showed that the variability around this expectation is large and has a high serial correlation (Figure 3.6). This is of course due to the large variability in recruitment and the average lifetime of a bed being much larger than one year. For these simulations we have used the average survival curve. The expected lifetime of mixed beds is estimated at more than 31 years, which is more than 5 times higher than the overall expected lifetime. So if we would have taken the survival curve for mixed beds (the green line in Figure 3.4) the expected total bed area would also have been 5 times larger. On the other hand, if only pure mussel beds are considered, the expected total bed area would have been smaller.
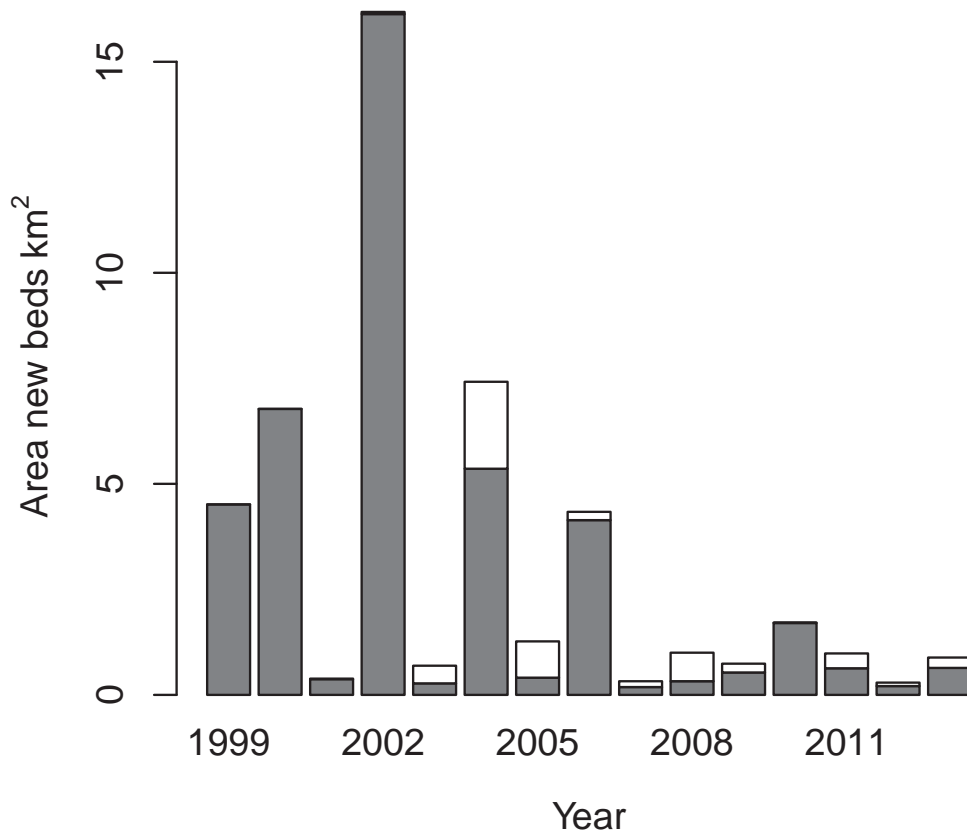
Figure 3.5: Surface area of new beds in spring versus year. Grey indicates the area of pure mussel beds.

Discussion

Using Cox's proportional hazard model with fixed-time covariates implies that some simplifying assumptions were made. First, the assumption that the hazard rates of two beds are proportional means that the relative risk of disappearing of one bed in comparison to that of another bed does not change with age or time. So the model is not able to accurately describe the possible case that, say, mussel beds will only have a higher risk compared to oyster beds when they are both young, but the same risk when they are both old. Further inspection of the separate survival (see, for example, Figure 3.4) and hazard curves for the various groups showed, however, that the assumption of a proportional hazard rate is quite reasonable. For the quantitative covariates, the data were split into two groups (above and below the median value) to simplify the inspection. Second, applying fixed-time covariates ignored possible changes over time in the value of the covariates. For example, initial bed area was used to describe bed size, but bed size may change. But changes over time were relatively small compared to differences among beds. For depth, orbital velocity, and inundation, only data from a single survey were used. Annual data are not available. But most likely these variables will not change much over time, e.g., shallow areas generally remained shallow over the entire study period (Elias et al. 2012). For the qualitative covariate bed type, annual data were available. Looking at all annual transitions yielded a result very similar to that of Cox's model, namely that mixed beds do have a much lower hazard rate than pure mussel beds, and they also have a lower death risk than oyster beds. It is easy to understand that oyster and mussel beds differ in hazard rate, since they do have a totally different physical structure. Mussel beds consist of a dynamic meshwork of individual mussels attached to each other via temporary byssus threads, lacking a permanent anchorage in the substrate. Van de Koppel and co-workers emphasize that this flexibility induces spatial self-organization, thereby increasing resilience
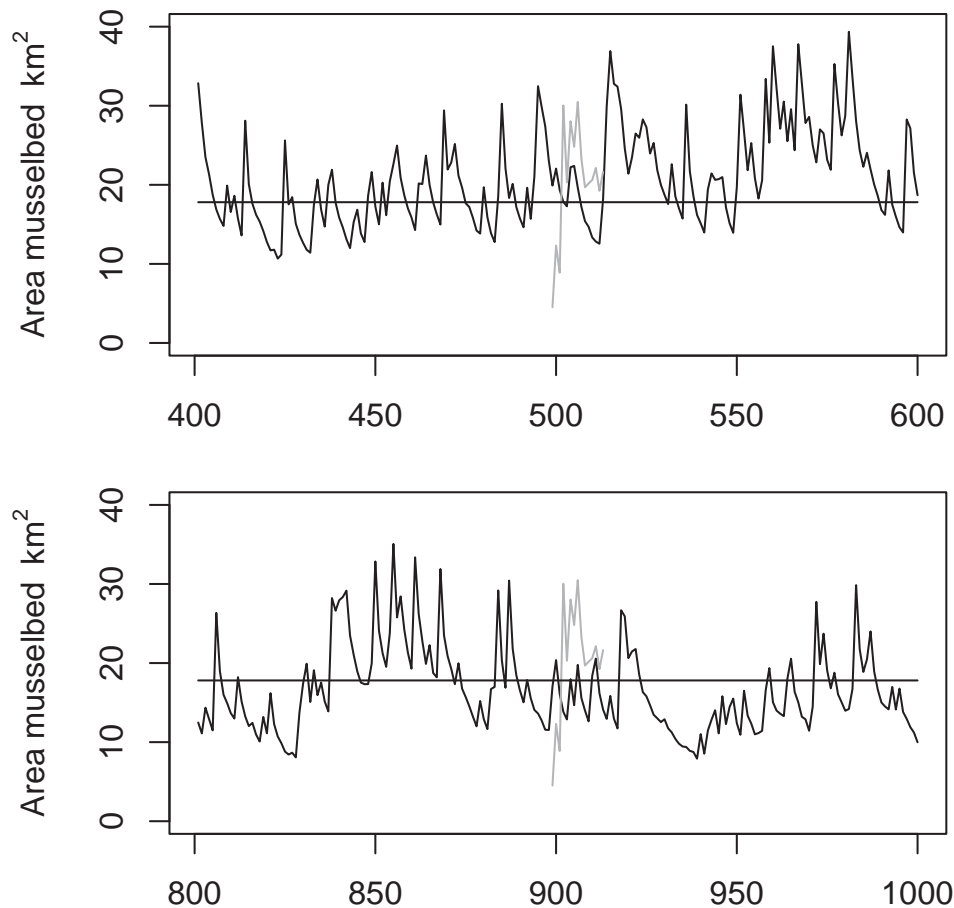
Figure 3.6: Simulated total surface area of all mussel beds for two arbitrary periods. Grey line shows the true total bed area over the study period 1999–2013.

against disturbance (van de Koppel et al. 2005). Nonetheless, mussel beds are vulnerable to storms and ice scouring (Nehls & Thiel 1993, Donker et al. 2015). In contrast, Pacific oysters are permanently attached to each other via an organic-inorganic adhesive. Dead oysters remain attached to each other and oyster larvae preferentially settle on conspecifics, creating rigid and persistent structures (Walles et al. 2015a). Furthermore, there is also a difference in risk of predation. All size classes of mussels on an intertidal mussel bed are subject to predation by a suite of predators, most notably shore crabs and shellfish-eating birds (Zwarts & Drent 1981, Smallegange & van der Meer 2003, van de Kam et al. 2004). In contrast, Pacific oysters are only subject to predation when small (Dare et al. 1983, Mascaró & Seed 2001a, Markert et al. 2013, Walles et al. 2015a). It is not immediately clear, however, why mixed beds are better survivors than oyster beds, instead of having a survival in between that of pure oyster and pure mussel beds. Pacific oysters cannot grow when tidal emersion exceeds 33% per tide (Walles et al. 2016), whereas intertidal mussel beds regularly occurred in areas with an emersion time exceeding 40% (Brinkman et al. 2002). So we can expect differences between the different types of bed in the abiotic environment where they occur and this may impact survival. However, by including covariates such as inundation time and orbital speed, the model already corrected for much of the differences in abiotic environment. Similarly, bed size is also considered simultaneously in the model. Perhaps the better survival of mixed beds relates to the fact that oyster beds may suffer from burial by sedimentation (Walles et al. 2016). In a mixed bed, the mussels may crawl on top of the sediment, thereby retaining the integrity of the bed. Whatever the explanation, on the basis of the high survival of mixed beds, we expect them to become the dominant type of bed in the future. In 2015, mixed beds already predominated in the Wadden Sea, comprising 1152 ha (62%) of a total of 1846 ha bivalve beds (van den Ende et al. 2016a).

We set out to study survival and recruitment of bivalve beds to investigate the claim that the ecosystem had collapsed after overfishing in the late 1980s. We assume that ecosystem collapse implies that nowadays bed areas are much lower and bed dynamics very different from the past. Thus, we must compare our results to historical information.

The simulation study showed that the large observed variability in annual recruitment of new beds produces strong serial correlation, where periods of several decades of bed area far above or far below average bed area are no exception. Even in the well-studied Dutch Wadden Sea, data before 1990 of total bed area are uncertain. Historic data spanning several decades are entirely lacking. In an extensive overview of all data reported in governmental reports, Dankers et al. (Dankers et al. 2003) conclude that in the 1970s and 1980s between 10 and 56 km^2 of bed area was present. A slightly different approach by the same authors arrived at a somewhat smaller, but still large interval of 17–48 km^2 (Dankers et al. 2003). The indicated uncertainty is not so much due to interannual variability, but merely to the use of different methods, such as aerial or ground sampling, and different ways of demarcating beds. Our approach yielded a range of 10–30 km^2, which is entirely due to interannual variability. Other long-term studies also point to a huge variability and a very right-skewed distribution of mussel recruitment among years. For example Beukema's 1970–2008 series at Balgzand, a tidal flat in the westernmost Dutch Wadden Sea, only contained four years with good mussel recruitment (Beukema et al. 2010). A recent study by Beukema confirms these results (Beukema et al. 2015). This is a general phenomenon for most bivalve species in soft-sediment habitats. The same Beukema series revealed six outstanding recruitment years for the Baltic tellin *Limecola balthica*, and also only four years for the cockle *Cerastoderma edule*. In the Wash, spatfall of cockles and mussels was poor in most years between 1990 and 1999. Significant recruitment that increased the shellfish stock occurred in only 14% and 19% of the study years for mussels and cockles, respectively (Dare et al. 2004). Thus, as already indicated by the simulation study, full recovery of bivalve bed area after the disappearance in the early 1990s to pre-1990 levels, might take some time. And if one also considers the uncertainty about the pre-1990 area, it seems premature to assume ecosystem collapse and invest effort in costly large-scale restoration programs.
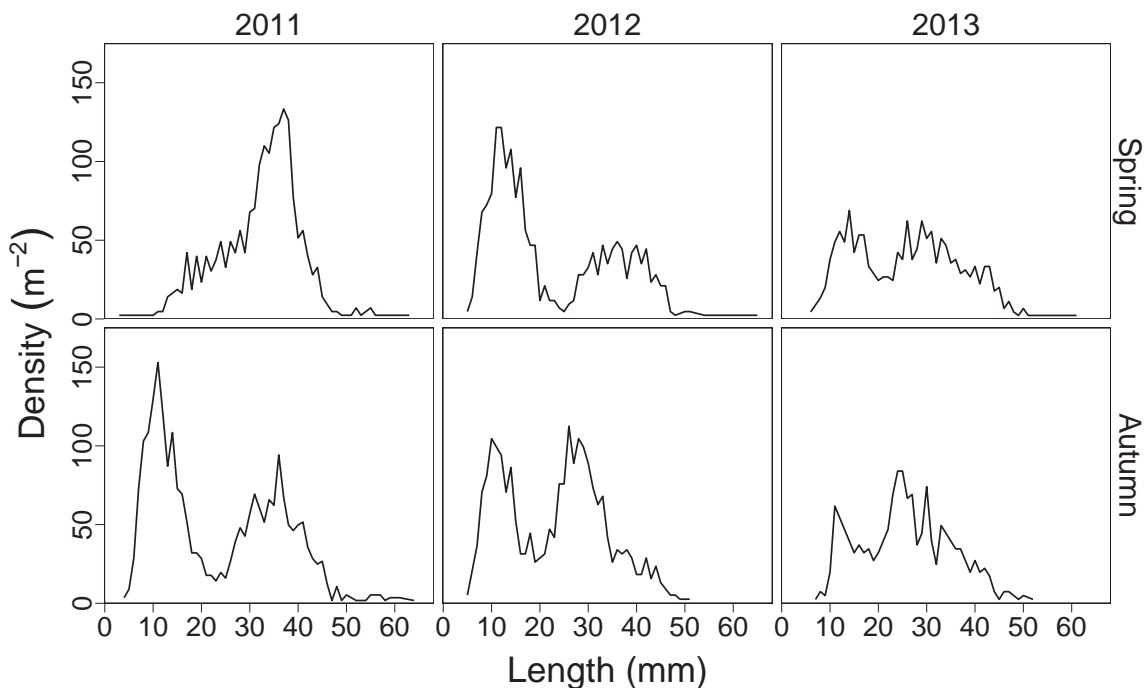

Figure 3.7: Size-frequency plots of mussels on a bed at Balgzand (W015), showing that beds are constantly renewed by the disappearance of old cohorts and the renewal with recruits (unpublished data from Andreas Waser, see Chapter 4: Waser et al. (2016a) for the precise location of the bed).

The much higher survival probability of oyster beds and particularly mixed beds than that of pure mussel beds, even suggests that total bed area may increase to much higher levels compared to the pre-1990 situation. Further simulation studies (not presented here) arrived at an area close to 100 km^2 when all beds become mixed ones, but density-dependent processes will probably prevent that such large values will be reached (Folmer et al. 2014). Nevertheless, our analysis points to larger bed areas in the near future than presently observed.

Using beds as the unit of observation, and estimating their recruitment and survival, provided some insight in the underlying processes and relevant environmental factors that govern total bed area. Though of interest to managers and conservationists, it does not tell much about the fate, in terms of recruitment and survival, of individual mussels and oysters. Beds are continuously replenished with new cohorts (Figure 3.7), and we have not investigated the link between bed survival and recruitment and survival of individual animals. So we do not know, for example, whether most recruitment in terms of individuals, occurs in existing beds or in newly formed beds. It also cannot be ruled out that stable beds with low hazard rates are linked with relatively low survival of individuals, but high recruitment of new cohorts. Perhaps beds that are constantly renewed are more stable than beds that lack regular replenishment. The link between these two levels of biological organization, the individual bivalve and the bed, is an interesting topic for future studies.

Acknowledgments

Box 3.1   Recruitment, growth and survival of mussels

When studying the progression of specific bivalve beds it is also of interest to consider the fate of individual mussels. Information on recruitment, survival and growth of the mussels allows to get more insight into the link between bed survival and the fate of individual mussels as well as on the predator-prey relationships, and secondary production of the mussels.
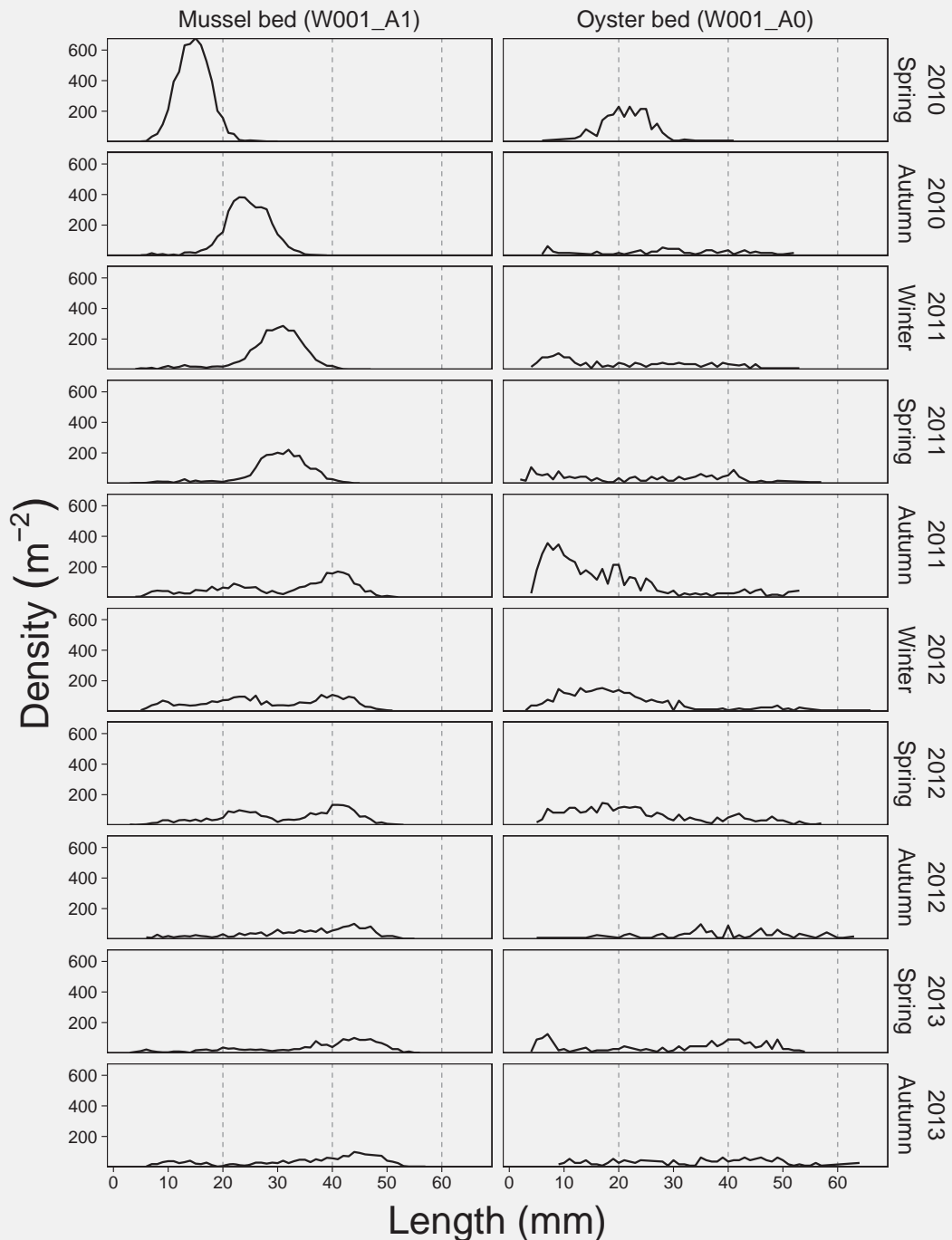

Figure B3.1: Size-frequency distributions over time for the period 2010–2013 of mussels at two bivalve beds differing in their composition of bivalve species. While bed W001_A1 consists almost entirely of mussels, bed W001_A0 is dominated by many large oysters. Dashed vertical lines at every 20 mm are added as a visual aid. Detailed analysis of cohorts on the mussel bed W001_A1 is given in Figure B3.2.

Box 3.1

As part of long-term investigations of several epibenthic bivalve beds (see Chapter 4: Waser et al. 2016a, for an overview map and sampling details), mussels were repeatedly sampled in order to investigate several parameters (i.e., birth, growth and death) of the different cohorts within the mussel population on a given bivalve bed. In bivalves, concentric growth rings on the shell surface, which are usually produced annually during the winter period of ceased shell growth, are commonly used to age and assign the individuals to a specific cohort. In mussels, however, age determination via growth lines is complicated, since growth rings are either absent or difficult to discern from irregular occurring disturbance rings (storms, dredging, predation) (reviewed in Richardson 2001).

Length frequency distributions of mussels

Alternatively, the separation of distinct cohorts can be achieved with the help of length frequency distributions, in which mussels can be statistically assigned to a specific cohort on the basis of their length (Bhattacharya 1967, Wanink & Zwarts 1993). Figure B3.1 shows the length frequency distributions of mussels sampled at two different bivalve beds sampled in 2010–2013. Both beds are located at the northern tip of Texel and differ in their composition of bivalve species. While bed W001_A0 is characterized by a high biomass of Pacific oysters, bed W001_A1 consists almost entirely of mussel recruitment from the year 2009.

This analysis requires that the number of individuals sampled is big enough and reflects the size structure of the actual population. Where recruitment is seasonal and variability in individual growth rates is low, individual year classes can be identified as distinct modes and can be followed over time, as in the case for bed W001_A1 (Figure B3.2). In contrast, the bed rich in oysters (W001_A0) showed a variability in mussel distribution between the different sample dates (Figure B3.1). This variability was observed on all investigated oyster dominated beds, presumably caused by high fluctuations in individual growth rates or size specific predation (see e.g., Chapter 6: Waser et al. 2015), and prevents detailed analysis of mussel cohorts.

Von Bertalanffy growth

The classification of mussels into specific age classes can be used to assess the cohort specific survival rates (Figure B3.3) and to estimate the mussel growth over time (Figure B3.4). In bivalves, the Von Bertalanffy growth equation is commonly used to describe individual growth. However, the original equation does not take seasonal variations in growth rate into account. A popular modification considering fluctuations in seasonal growth is Somers' Von Bertalanffy growth model (Somers 1988, García-Berthou et al. 2012). This growth model is described as

$$L(t) = L_\infty(1 - \exp(-K(t - t_0) - S(t) + S(t_0))),$$
$$\text{with} \quad S(t) = (CK/2\pi)\sin(2\pi(t - t_S)),$$
$$\text{so} \quad S(t_0) = (CK/2\pi)\sin(2\pi(t_0 - t_S)),$$

where $L(t)$ is the expected length at time t; L_∞ is the asymptotic length; K is the exponential rate to approach the asymptotic length; t_0 is the theoretical time at which the average length would be zero; C modulates the amplitude of the growth oscillations (i.e., $C = 0$: no seasonal oscillation; $C = 1$: stopped growth e.g. in winter); t_s is the time between time 0 and the start of the convex portion of the first sinusoidal growth oscillation (i.e., the inflection point).
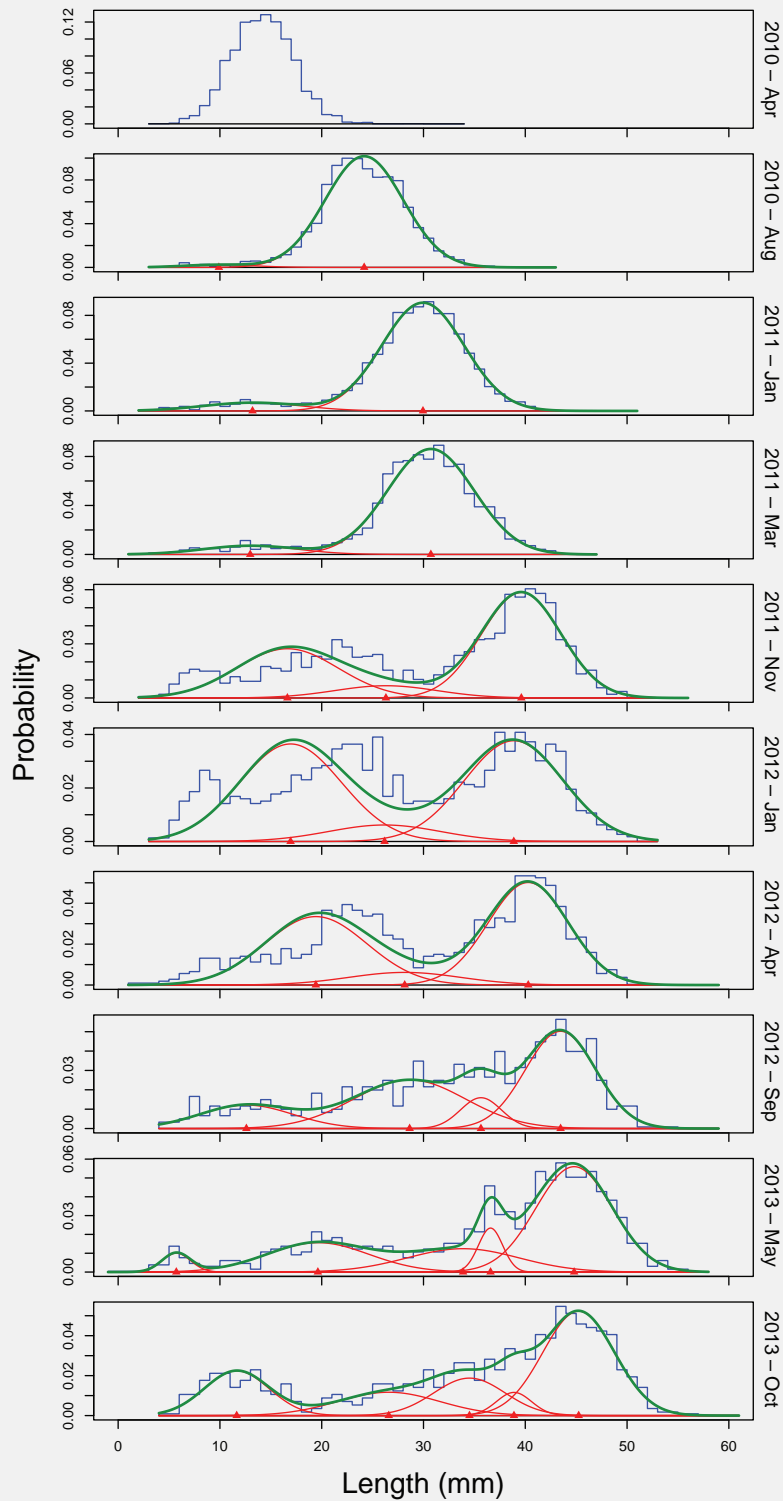
Figure B3.2: Mixture distributions of mussels at bed W001_A1 sampled in 2010–2013. Note that on the first sampling date only a single cohort (mean length = 14.16 mm) was present. Length-frequency distributions were analysed using the R platform (R Development Core Team 2015), supplemented by the the R package mixdist (Macdonald & Du 2015). This analysis uses a maximum likelihood method to estimate proportions of mussels associated to the specific cohorts. Figures B3.3 and B3.4 were used as guidance to reduce inaccuracies between sampling dates (i.e., shrinkage in body size or growth in population size of the specific cohorts). Blue lines: length-frequency histograms; red lines: cohort specific frequency distributions with triangles indicating mean cohort lengths; green lines: sum of all cohort specific frequency distributions.
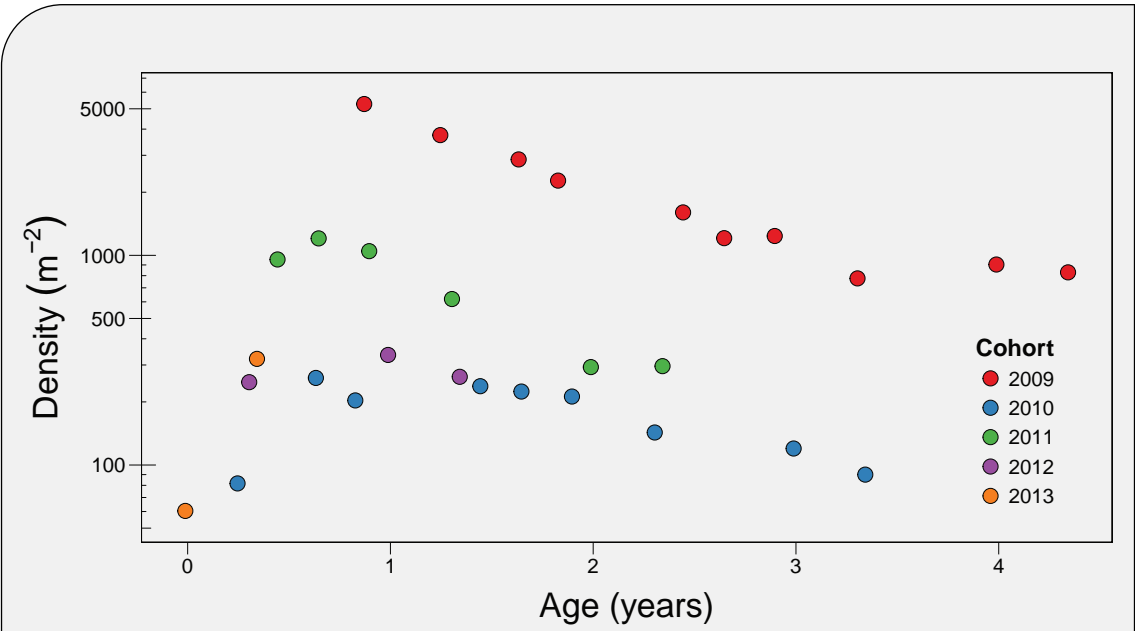
Box 3.1

Figure B3.3: Catch curves of different mussel cohorts on bivalve bed W001_A1 sampled in 2010–2013. It is assumed that new recruitment stages are born each year in May.
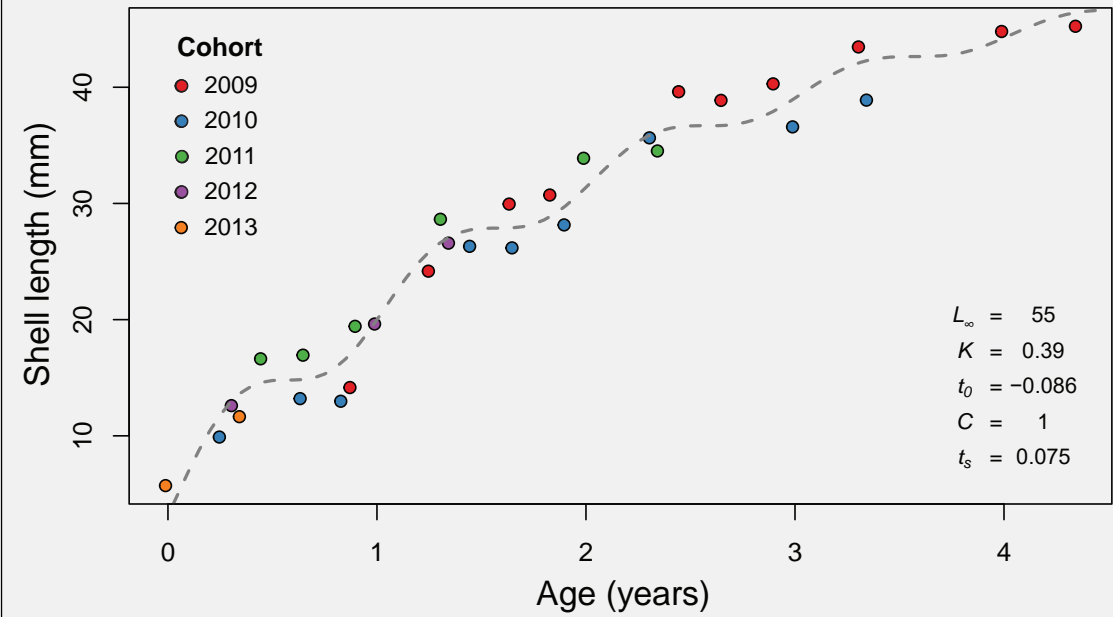

Figure B3.4: Somers' Von Bertalanffy growth for mussels on the mussel bed W001_A1 in 2010–2013. It is assumed that new recruitment stages are born each year in May.

Secondary production

Finally, cohort specific information resulting from the length frequency analysis in combination with the relationships between size and flesh weight can be used to estimate secondary production of the different mussel cohorts. Secondary production can be estimated by adding either the growth increments (increment-summation) or the weight losses (removal-summation) caused by size-dependent mortality (Crisp 1984). Both methods provide identical results (see van der Meer et al. 2013). In Table B3.1, production of the 2009 cohort in the period 2010–2013 is estimated following the removal-summation method. This method considers both the matter that leaves the cohort by mortality, and the difference between the total biomass of the cohort at the end of the observation period and at the start of the period.

Table B3.1: Production calculations for the 2009 cohort of *Mytilus edulis* on the bivalve bed W001_A1. Samples were taken solely from mussel covered patches. For the estimation of secondary production on the entire bed area (including bare patches) the production on mussel covered patches was multiplied by the fraction of mussel cover of the entire bed area. All mass indications refer to ash free dry mass (AFDM) measurements.

Date	Density mussel patch (m^{-2})	Mass (g)	Mass gain (g)	Mean density (m^{-2})	Production mussel patch (g m^{-2})	Mussel cover (%)	Production total bed (kg ha^{-1})
2010–Apr	5269	0.027					
2010–Aug	3745	0.117	0.091	4507	409.3	8.25	337.5
2011–Jan	2867	0.079	-0.038	3306	-126.5	8.49	-107.4
2011–Mar	2272	0.088	0.009	2570	23.5	10.26	24.1
2011–Nov	1602	0.181	0.092	1937	178.7	12.40	221.6
2012–Jan	1208	0.210	0.030	1405	41.9	12.21	51.1
2012–Apr	1237	0.198	-0.012	1222	-14.8	16.78	-24.8
2012–Sep	776	0.323	0.125	1007	125.5	17.76	222.9
2013–May	904	0.295	-0.028	840	-23.5	15.00	-35.3
2013–Oct	830	0.506	0.211	867	182.5	16.01	292.1
Sum					796.6		982.0

Concluding remarks

Studying a mussel population on a given bivalve bed over a longer time period may yield in specific information on the different mussel cohorts (i.e., age specific length and abundance), which were classified with the help of length frequency distribution analysis. Cohort specific information, in turn, can be used to estimate the secondary production of the different mussel year classes. This approach requires, however, that recruitment is regular, a low variability in individual growth rates, and little overlap of year classes. For some bivalve beds, these requirements could not be met and the classification into cohorts was not possible. That was particularly the case for beds with high portions of Pacific oysters. These beds showed large inconsistencies in mussel length between the different sample dates, preventing that cohorts can be accurately followed in time.

4 | Impact on bird fauna of a non-native oyster expanding into blue mussel beds in the Dutch Wadden Sea

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Abstract

Intertidal mussel beds are important for intertidal ecosystems, because they feature a high taxonomic diversity and abundance of benthic organisms and are important foraging grounds for many avian species. After the introduction of the Pacific oyster (*Crassostrea gigas*) into the European Wadden Sea, many mussel beds developed into oyster dominated bivalve beds. Despite the fact that oysters have been colonizing many European intertidal areas for about two decades, their impact on the ecosystem is still poorly understood. Here, we investigated the impact of oysters on the condition of mussels and on the spatial distribution of birds on 18 bivalve beds with different grades of oyster occurrence throughout the Dutch Wadden Sea. Moreover, in comparing bird densities on bivalve beds with densities expected on the total intertidal area, we could detect which species exhibit a preference for the structured habitat. Overall, 50 different bird species were observed on the beds, of which about half regularly frequent intertidal flats. Most of these species showed a preference for bivalve beds. The condition of mussels decreased with the oyster dominance, whereas the majority of bird species was not affected by the oyster occurrence. However, three of the four species that were negatively affected depend on intertidal mussels as food source. Even though the Pacific oyster is a non-native species, attempts to fight it may do more harm to avian biodiversity than good.

Introduction

Shallow intertidal systems are characterized by their high primary productivity and great abundance of benthic primary consumers, including many mollusc, polychaete, and crustacean species. Consequently, these ecosystems are important nursery areas for aquatic secondary consumers such as shrimps, crabs and fishes and are major feeding grounds for many water-bird species that benefit from the high productivity (Pihl & Rosenberg 1982, Zwarts & Wanink 1993, van de Kam et al. 2004). The highest productivity is often found in habitats rich in three-dimensional structure, and one of these complex habitats in shallow intertidal systems is created by blue mussels (*Mytilus edulis*), which aggregate with conspecifics and accordingly form mussel beds. Although mussel beds only account for up to 5% of the intertidal area (Folmer et al. 2014), they represent an important feature of the intertidal ecosystem by providing hard substrate and increasing habitat complexity, reducing hydrodynamics, and modifying the sediment by depositing large amounts of pseudo-feces and other fine particles (Gutierrez et al. 2003). Many studies have shown that these beds have an important effect on the benthic community (Asmus 1987, Dittmann 1990, Markert et al. 2009) and that the beds themselves are important foraging grounds for avian consumers (Zwarts & Drent 1981, Goss-Custard et al. 1982, Nehls et al. 1997, van de Kam et al. 2004).

In the past, intertidal mussel beds were severely overfished on several occasions, such as in the Wash, UK (Atkinson et al. 2003) and in the Dutch Wadden Sea (Ens 2006). The overfishing is in line with the general observation that the degradation of coastal ecosystems is most often due to human exploitation (Lotze et al. 2006). However, more recently, many intertidal systems in Europe are experiencing drastic changes resulting from the invasion of the Pacific oyster (*Crassostrea gigas*). The introduction of *C. gigas*, native to marine waters of Japan and South-east Asia, led to a transformation of many intertidal mussel beds into mixed bivalve beds or even into oyster reefs (Nehls et al. 2009b, Fey et al. 2010, Troost 2010). As a result, bivalve beds in an increasing number of European intertidal areas consist solely of mixed mussel and oyster populations (Nehls et al. 2009b). While both species similarly provide hard substrate for sessile species (Kochmann et al. 2008), differences between mussels and oysters arise in the three-dimensional structure, heterogeneity and formed micro-habitats, due to the spatial arrangement of shells and individual shell traits (surface area and shell texture) (Gutierrez et al. 2003).

Furthermore, the bed morphology differs between both species, due to different attachment mechanisms. Mussels are adhered to the substratum by a byssus, an assemblage of numerous extracellular, collagenous fibers ending in an adhesive plaque that attaches to the substrate (Bell & Gosline 1996). Byssal threads are temporary features, which generally exhibit longevities of around 8 weeks (Bell & Gosline 1996, Moeser & Carrington 2006). The continuous process of generating new threads leads to flexible and dynamic meshworks of individual mussels (van de Koppel et al. 2005). Lacking a permanent anchorage in the substrate, mussel beds are further subject to a dynamic large scale distribution, being particularly vulnerable to storms and ice scouring (Nehls & Thiel 1993, Büttger et al. 2011, Donker et al. 2015). In contrast, oysters attach themselves permanently by generating an organic-inorganic adhesive (Burkett et al. 2010). Even after the death of individuals, oyster shells often remain anchored in the sediment. The complex of dead and alive oysters serves as settling ground for oyster larvae, which preferably settle on conspecifics (Diederich 2005). In the long run, the process of multiple settlement leads to the creation of rigid and persisting structures (Reise & van Beusekom 2008, Walles et al. 2015a). For many macroinvertebrate species, the complex structures formed by these two bivalves are likely to provide different resources in terms of nesting sites, shelter from predators and feeding opportunities, thus leading to differences in the species community (Markert et al. 2009). Moreover, the conversion of mussel beds into oyster dominated beds may ultimately lead to a change of the food web structure (Baird 2012) as well as of the feeding opportunities for

secondary consumers (Eschweiler & Christensen 2011, Chapter 6: Waser et al. 2015).

Intertidal mussel beds are valued and protected because of their contribution to biodiversity, especially avian biodiversity, so it is important to know how the spread of Pacific oysters will affect avian biodiversity. It has been suggested, that molluscivorous species, like the Eurasian Oystercatcher (*Haematopus ostralegus*) and the Common Eider (*Somateria mollissima*) may be particularly negatively affected by the invasion of the oysters (Scheiffarth et al. 2007, Markert et al. 2013), since mussels as their preferred prey are in direct competition with the oysters and therefore may exhibit a reduced body condition (Troost 2009) resulting in a reduced prey profitability for the birds. A consolidation of oysters may additionally hamper access to the mussels. Other waterbird species commonly present on mussel beds, like for example the Eurasian Curlew (*Numenius arquata*), the Common Redshank (*Tringa totanus*), or the Black-headed Gull (*Larus ridibundus*) feed on the associated fauna (Ens & Alting 1996, Nehls et al. 1997, Goss-Custard et al. 2006, Folmer et al. 2010) and might be little affected by the habitat change (Scheiffarth et al. 2007, Markert et al. 2013). Earlier investigations however, compared the bird abundance of the present oyster-transformed beds with historic abundance data on pure mussel beds. Furthermore, these observations were limited in terms of investigated bird species and study sites, only focusing on one locality with a very low number of focal species (Markert et al. 2013) making it difficult to draw general conclusions.

In this study, we investigated the spatial distribution of waterbirds on bivalve beds with different grades of Pacific oyster occurrence. To do so, we studied 18 bivalve beds in the Dutch Wadden Sea in terms of bed properties and linked these to the appearance and number of different bird species between the years 2010 and 2013. In order to ascertain to what extent the different species show a preference for bivalve beds to habitats of less structural complexity, we furthermore used counts of birds during high tide on high tide roosts in the vicinity of tidal flats. Assuming that all birds counted during high tide on a roost will be distributed across the emerging tidal flats closest to that specific roost, it is possible to estimate the mean abundance of the different species during low tide.

The sampling design, together with data on numbers of birds during high tide at high tide roosts allowed us to investigate two main research questions: (a) Which bird species prefer bivalve beds as a low-tide feeding habitat? (b) What is the impact of the composition of the bivalve bed (i.e., the predominance of Pacific oysters) on the avian community?

Materials and Methods

Properties of bivalve beds

The 18 investigated bivalve beds were located throughout the Dutch part of the Wadden Sea (Figure 4.1). In this area, three different types of intertidal bivalve beds can be distinguished: mussel dominated beds, where oysters are absent or occur only in very low numbers; beds with a balanced proportion of mussels and oysters and beds where oysters dominate in terms of biomass (van Stralen et al. 2012). In order to identify the proportion between both mussels and oysters, the study sites were mapped and different mussel bed properties were measured twice a year, in spring and autumn, between 2010 and 2013. Firstly, the contours of each bed were determined by walking around the bed with a hand-held GPS device following a common definition of a mussel bed (de Vlas et al. 2005). The contours gave on the one hand the spatial extent (area) of the beds and on the other hand, contours were used to delimit and create a set of multiple random sampling points. All created sample points were visited. Those points that were covered by mussels or oysters were sampled for epibenthos with a rectangular frame of 0.0225 m^{-2} (15 × 15 cm). The samples were sieved (1 mm square meshes) in the field and subsequently sorted for mussels and oysters. These were counted and sized individually using
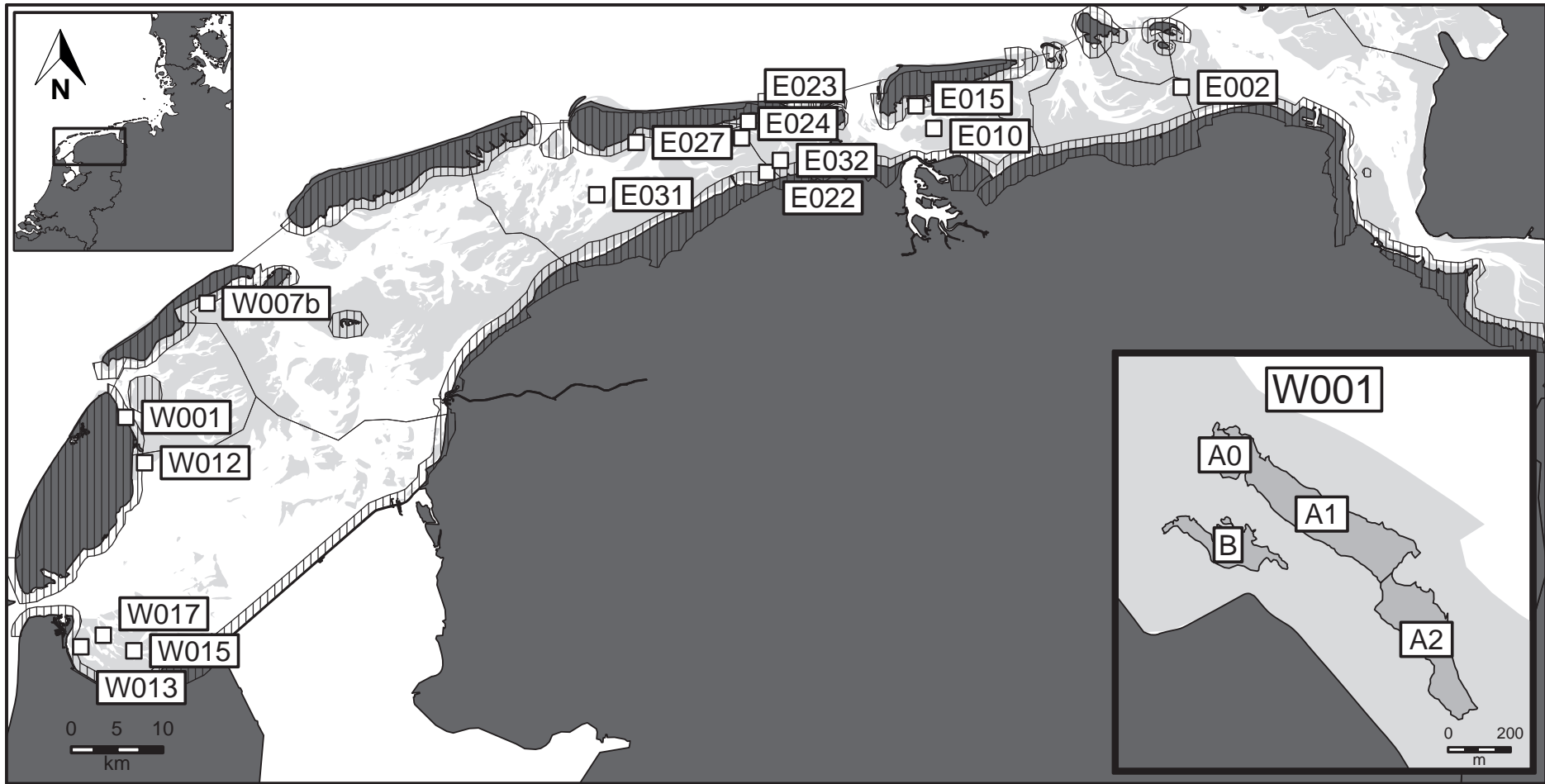
Figure 4.1: Overview of the different tidal basins in the Dutch Wadden Sea. Borders of the tidal basins are represented by thin black lines. Locations of the investigated bivalve beds are indicated by white squares. Beds in the western Dutch Wadden Sea are indicated by a 'W' and accordingly an 'E' in a label represents a bivalve bed in the eastern part of the Dutch Wadden Sea. White areas represent water, light grey areas indicate intertidal flats exposed during low tide, intermediate grey indicates bivalve beds and land is represented by dark grey. High-tide roosts of the different bird species are indicated by shaded areas. Bed W001 was subdivided into 4 different sectors which are shown in the detailed map on the bottom right.

Table 4.1: Overview of some characteristics of the investigated bivalve beds. For each bed, the area on which birds were counted, densities and biomass of oysters and mussels and the fraction of the total bivalve biomass contributed by Pacific oysters are given. Values given are mean ±SE.

Bivalve bed	Area (ha)	Mussel density (n m^{-2})	Oyster density (n m^{-2})	Mussel biomass (kg m^{-2})	Oyster biomass (kg m^{-2})	Fraction of oysters of the biomass (%)
W001_A0	1.2	2223 ± 457	326 ± 56	1.67 ± 0.25	2.24 ± 0.34	57.34
W001_A1	6.8	2770 ± 434	25 ± 11	2.07 ± 0.28	0.02 ± 0.02	1.06
W001_A2	3.8	1626 ± 494	5 ± 5	0.78 ± 0.24	0.02 ± 0.02	2.32
W001_B	1.2	1222 ± 305	50 ± 20	1.05 ± 0.31	0.02 ± 0.01	2.16
W012	2.8	1444 ± 206	571 ± 134	2.26 ± 0.25	2.14 ± 0.22	48.70
W013	17.8	1237 ± 98	73 ± 32	1.34 ± 0.18	0.07 ± 0.02	4.73
W017	6.3	11381 ± 230	116 ± 80	2.15 ± 0.22	0.05 ± 0.02	2.40
W015	3.9	1798 ± 108	533 ± 242	1.15 ± 0.14	0.41 ± 0.04	26.43
W007b	9.6	1525 ± 160	622 ± 78	2.47 ± 0.27	4.13 ± 0.77	62.53
E031	11.0	1668 ± 97	113 ± 30	2.92 ± 0.28	0.19 ± 0.04	6.07
E027	17.3	546 ± 74	220 ± 67	1.16 ± 0.16	1.30 ± 0.13	52.95
E024	70.1	7080 ± 1363	0 ± 0	2.54 ± 0.47	0 ± 0	0
E023	56.4	697 ± 317	2 ± 2	0.53 ± 0.14	0.01 ± 0.01	1.28
E022	34.5	2049 ± 321	37 ± 14	1.57 ± 0.15	0.02 ± 0.01	1.16
E032	30.1	4138 ± 364	1 ± 1	2.11 ± 0.01	0.0002 ± 0.0002	0.01
E015	17.5	815 ± 104	220 ± 76	1.29 ± 0.16	0.29 ± 0.07	18.15
E010	66.8	1290 ± 149	419 ± 188	1.96 ± 0.28	0.65 ± 0.10	24.87
E002	3.7	3505 ± 897	54 ± 51	3.96 ± 0.83	0.02 ± 0.02	0.51

digital callipers, to the nearest 0.01 mm, in the laboratory. In order to estimate the ratio between mussel and oyster biomass, the individual shell length (L) of both mussels and oysters was converted into a volumetric length (V), representing biomass, by a fixed dimensionless shape coefficient (δM): $V = (\delta M \times L)^3$. The shape coefficient is a parameter that relates the real length with the structural length in the context of the dynamic energy budget (DEB) theory (Kooijman 2010) and is well established for oysters (0.175; van der Veer et al. 2006), as well as for mussels (0.297; Saraiva et al. 2011). Based on the assumption, that the density of bivalve flesh approaches the one of water, the volumetric length was further converted into a measure of biomass (wet weight in kg). The precise body condition of mussels was estimated by measuring the ash-free dry mass of the soft tissue ($AFDM_{flesh}$). To do this, mussels were sorted to discrete shell length classes (every 2.5 ± 0.5 mm beginning with a length of 5 mm, e.g. 5, 7.5, 10, etc.), the soft parts of a random sample of individuals from each length class (max. 15 individuals) was pooled and dried to constant weight, weighed, incinerated and weighed again to obtain by subtraction the $AFDM_{flesh}$.

Moreover, the tidal elevation of the bivalve beds (m below mean tide level, MTL) was obtained based on the bivalve bed contours and a bathymetric grid (20 × 20 m) of the Dutch Wadden Sea provided by Rijkswaterstaat (Dutch Ministry of Infrastructure and Environment; 'vaklodingen'; http://opendap.deltares.nl). For each bivalve bed, the mean tidal elevation of all grid-points overlapping with the bed contours was calculated.

Bird abundance on bivalve beds

All bird species within the contours of the bivalve beds were counted. Counts were usually performed in intervals covering about half of a tidal cycle (from high tide, over outgoing tide to low tide, or from low tide over incoming tide to high tide). Due to logistical reasons or bad weather such interval counts were not always possible. In these cases, one low tide count for a recently exposed bivalve bed was performed. The counts were repeated several times and performed throughout the entire year (Table S4.1). In order to obtain seasonal trends of bird numbers throughout the year sinusoidal functions (e.g., Cardoso et al. 2007) were applied to each separate species on all counts made in the four different years of the study period. The overall function was $y = a + b \times \sin((x - c)/365 \times 2\Pi)$, in which a, b, and c are parameters for the average, the amplitude and the reference day where the number equals the average, y is the predicted number of birds and x is the Julian day, ranging from 1 to 365. In some cases, bird numbers were low and thus resulting in the sinus function predicting negative values. All of these negatively predicted numbers were set to 0. All sinusoidal functions were fitted using R v3.2.1 (R Development Core Team 2015), with parameters estimated using the Levenberg-Marquardt algorithm implemented in the function nlsLM from the R package minpack.lm (Elzhov et al. 2015). An overview of bird numbers on bivalve beds and the seasonal trends for the species common on intertidal flats is provided in the supplementary material (Figures S4.1–S4.24).

Bird abundance on tidal flats

Counts of waterbirds on high-tide roosts adjacent to intertidal flats between July 2010 and June 2014 in combination with the extent of intertidal area were used to estimate average abundance on the intertidal flats (Figure 4.1). Three types of counts were used: 1) simultaneous total counts covering all high-tide roosts, excluding the roosts along the North Sea shoreline, of all waterbird species (two counts a year were organized on a trilateral level (the Netherlands, Germany and Denmark), and up to three additional counts on regional level), 2) frequent counts (at least once a month) of all waterbird species in a selection of counting units (see van Roomen et al. 2005, for a detailed description of the high tide roost counts), 3) dedicated aerial counts of Common Eiders in the Dutch Wadden Sea (Cervencl et al. 2015). Bird numbers were investigated per tidal

basin by allocating counting units to the nearest tidal basin (van Roomen et al. 2012). When a counting unit was located at the border of two tidal basins bird numbers were divided equally between the two different tidal basins. The estimates on abundance are based on monthly averages. Accounting for missing counts is done with UINDEX (Bell 1995), on the basis of site, month and year factors estimated from the non-missing counts (Underhill & Prys-Jones 1994). The seasonal index, which is the mean of the monthly averages of the four seasons (2010/11–2013/14) was used for further calculations. For Common Eider only, aerial counts between August 2010 and January 2014 were used to calculate an overall seasonal index per tidal basin. The counts were conducted by the Institute for Marine Resources and Ecosystem Studies (IMARES) or by Rijkswaterstaat (RWS) during high tide using a high-winged plane flown along predefined north-south oriented transects covering the entire area of the Dutch Wadden Sea and the adjacent North Sea coastal zone (Table S4.2). For each group of Common Eider, the geographical location as well as the number of individuals was determined (see Cervencl et al. 2015, for detailed methods). For further analysis, only groups of Eiders above the intertidal of the Wadden Sea were included. The flocks of Eiders were further grouped to the different tidal basins in order to gain a total number of birds per basin. Per tidal basin, the seasonal trend of Eider numbers was calculated by the sinus function: $y = a + b \times \sin((x - c)/365 \times 2\Pi)$, in which a, b, and c are parameters, y is the number of eiders per tidal basin and x is the Julian day, ranging from 1 to 365. In some cases, Eider numbers were low and thus resulting in negative predictions. All of these negatively predicted numbers were set to 0 (supplementary appendix, Figure S4.25).

Data analysis

The relationship between body condition and length of mussels was analysed with linear regressions on a log-log scale. To compare $AFDM_{flesh}$ between differently sized mussels, we extracted the residual of the different size classes from the linear fits, which reflects the relative $AFDM_{flesh}$. For representation purposes, we back transformed these residuals into ratios representing the observed body composition relative to the expected value for that length class.

A bootstrap approach was used to estimate relationships between mean abundance on intertidal flats and mean abundance on bivalve beds for the different bird species. 1000 bootstrap samples were taken both from the 10 different tidal basins (e.g., a sample of 10 with replacement) and from the 18 different bivalve beds. Each tidal basin bootstrap sample was summarized by the mean index density, which is given by $\sum 10\, Y / \sum 10\, A$, where Y is the seasonal index and A the area. The bivalve bed bootstrap sample was similarly summarized: $\sum 18\, X / \sum 18\, A$, where X is the seasonal mean of bird numbers and A the area.

The relationships between both Pacific oyster occurrence and the ratio of observed and predicted mussel biomass and oyster occurrence and tidal elevation as well as the effect of oyster occurrence on the abundance of the different avian consumers were tested using Spearman's rank correlations.

Moreover, Spearman's rank correlations were used to explore the relationship between the ratios of observed and predicted mussel biomass and the abundance of birds preying on mussels. All statistical analyses were performed using R v3.2.1 (R Development Core Team 2015).

Results

The 18 investigated bivalve beds differed both in their size and composition of bivalve species (Table 4.1). Eleven beds comprised of very few or mainly small individuals of Pacific oysters, so that the fraction of the total bivalve biomass constituted by the oysters was negligible for most
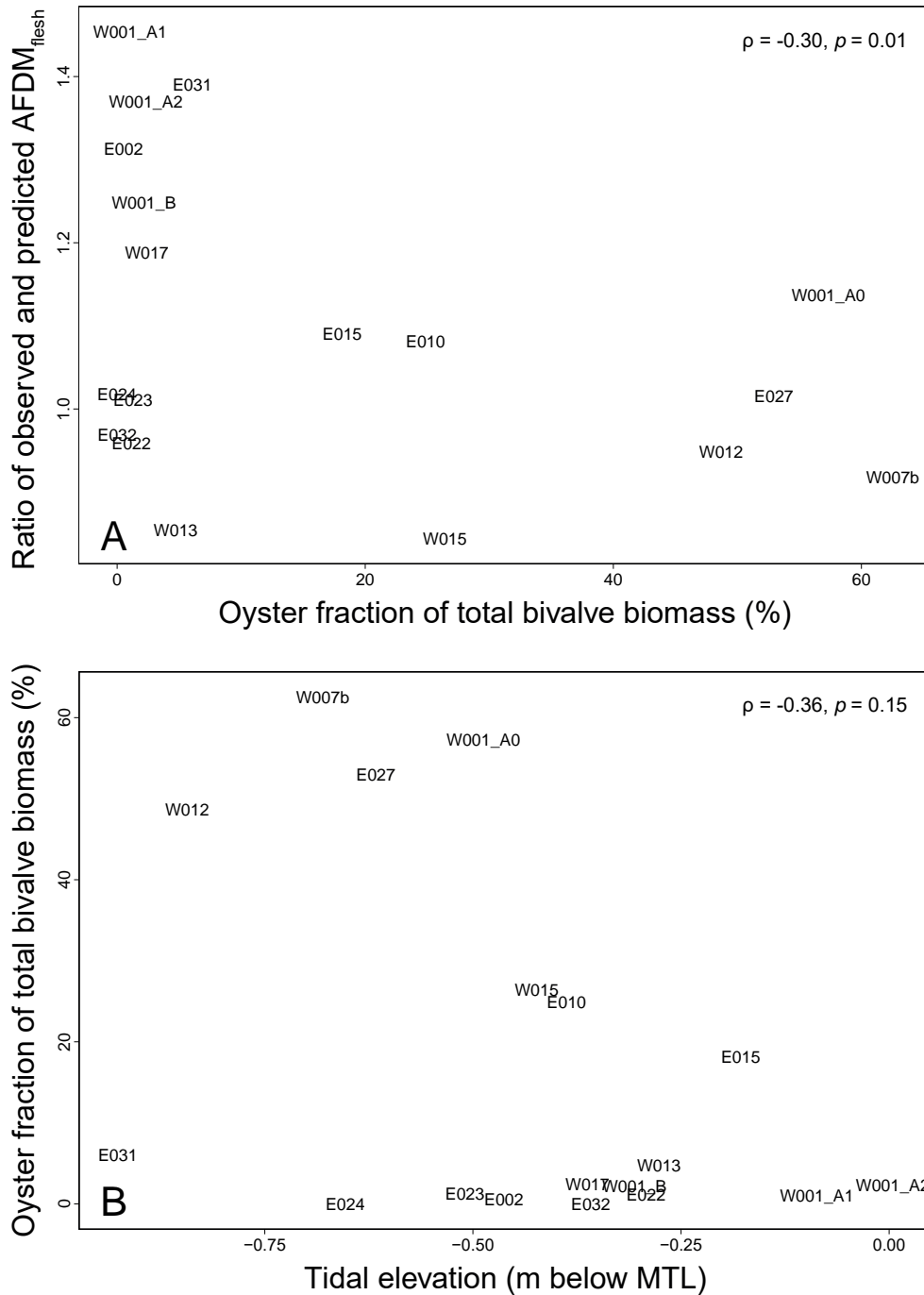
Figure 4.2: A) The ratio of observed and predicted mean ash-free drymass of musselflesh ($AFDM_{flesh}$) depending on the oyster fraction of the total bivalve biomass (%) and B) the oyster fraction of the total bivalve biomass (%) depending on the tidal elevation (m below mean tide level, MTL). The coding of the two graphs indicates mean values of the respective bivalve beds.

of these beds and overall did not exceed fractions of 6%. Three beds (W015, E015 and E010) showed intermediate amounts of oyster biomass, resulting in oyster fractions of 18–26% of the total bivalve biomass and four beds (W001_A0, W012, W007b and E027) contained considerable amounts of oyster biomass, resulting in fractions of 49–63% of the total amount of bivalve biomass. A comparison between all beds showed that the body condition of mussels was negatively correlated with the oyster dominance (Spearman correlation, S = 81,131, ρ = -0.3, p = 0.009, Figure 4.2A) and that oysters occurred primarily in lower elevated parts of the intertidal (S = 1314, ρ = -0.36, p = 0.147, Figure 4.2B).

Table 4.2: Results of the bird counts on bivalve beds. For all bird species that were observed on the beds, the number of beds on which a species was observed, the mean appearance and abundance based on all counts and an indication whether a specie was considered to be common on intertidal flats are given. Values given are mean ± SE.

Common name	Scientific name	Number of beds the species was observed on	Mean appearance (%)	Mean abundance (n ha^{-1})	Intertidal species
Great Crested Grebe	*Podiceps cristatus*	7	6.2 ± 2.4	0.012 ± 0.004	
Great Cormorant	*Phalacrocorax carbo*	14	20.0 ± 4.2	0.084 ± 0.050	
Little Egret	*Egretta garzetta*	9	7.9 ± 2.7	0.009 ± 0.005	X
Grey Heron	*Ardea cinerea*	3	1.2 ± 0.7	0.001 ± 0.001	
Eurasian Spoonbill	*Platella leucorodia*	17	37.0 ± 4.9	0.126 ± 0.031	X
Greylag Goose	*Anser anser*	1	0.8 ± 0.8	0.001 ± 0.001	
Dark-bellied Brent Goose	*Branta bernicla*	17	18.6 ± 3.2	0.280 ± 0.107	
Common Shelduck	*Tadorna tadorna*	16	45.2 ± 6.7	1.206 ± 0.383	X
Eurasian Wigeon	*Anas penelope*	12	12.2 ± 2.7	0.493 ± 0.221	
Gadwall	*Anas strepera*	1	0.9 ± 0.9	0.003 ± 0.003	
Common Teal	*Anas crecca*	3	0.9 ± 0.5	0.003 ± 0.002	
Mallard	*Anas platyrhynchos*	14	29.8 ± 5.7	0.332 ± 0.136	X
Northern Pintail	*Anas acuta*	9	7.2 ± 2.1	0.088 ± 0.044	X
Northern Shoveler	*Anas clypeata*	3	1.8 ± 1.2	0.006 ± 0.004	
Common Eider	*Somateria mollissima*	18	73.2 ± 5.5	3.039 ± 1.099	X
Common Goldeneye	*Bucephala clangula*	1	0.8 ± 0.8	0.004 ± 0.002	
Red-breasted Merganser	*Mergus serrator*	10	10.3 ± 3.4	0.047 ± 0.021	
Eurasian Sparrowhawk	*Accipiter nisus*	1	0.6 ± 0.6	0.001 ± 0.001	
Peregrine Falcon	*Falco peregrinus*	10	6.4 ± 1.9	0.004 ± 0.001	
Eurasian Oystercatcher	*Haematopus ostralegus*	18	98.6 ± 1.0	10.702 ± 2.270	X
Common Ringed Plover	*Charadrius hiaticula*	6	5.2 ± 2.2	0.009 ± 0.006	X
European Golden Plover	*Pluvialis apricaria*	9	12.8 ± 4.5	3.064 ± 2.326	X
Grey Plover	*Pluvialis squatarola*	17	40.5 ± 6.1	0.252 ± 0.078	X
Northern Lapwing	*Vanellus vanellus*	1	0.4 ± 0.4	0.001 ± 0.001	
Red Knot	*Calidris canutus*	14	23.2 ± 4.9	1.659 ± 0.871	X
Sanderling	*Calidris alba*	4	1.6 ± 0.8	0.001 ± 0.001	X
Dunlin	*Calidris alpina*	16	40.4 ± 6.9	1.984 ± 0.733	X
Common Snipe	*Gallinago gallinago*	1	0.4 ± 0.4	0.0002 ± 0.0002	
Black-tailed Godwit	*Limosa limosa*	5	2.9 ± 1.2	0.008 ± 0.004	
Bar-tailed Godwit	*Limosa lapponica*	18	54.4 ± 4.3	0.839 ± 0.181	X
Eurasian Whimbrel	*Numenius phaeopus*	15	19.6 ± 3.5	0.133 ± 0.078	X
Eurasian Curlew	*Numenius arquata*	18	99.1 ± 0.6	6.228 ± 1.410	X
Spotted Redshank	*Tringa erythropus*	9	6.9 ± 2.4	0.022 ± 0.015	X
Common Redshank	*Tringa totanus*	18	65.9 ± 6.5	1.748 ± 0.514	X
Common Greenshank	*Tringa nebularia*	18	36.5 ± 4.5	0.194 ± 0.059	X
Ruddy Turnstone	*Arenaria interpres*	18	55.8 ± 4.8	0.371 ± 0.109	X
Parasitic Jaeger	*Stercorarius parasiticus*	1	0.6 ± 0.6	0.00001 ± 0.00001	
Little Gull	*Larus minutus*	1	0.8 ± 0.8	0.002 ± 0.002	
Black-headed Gull	*Larus ridibundus*	18	80.0 ± 2.7	2.928 ± 0.462	X
Common Gull	*Larus canus*	18	72.3 ± 3.1	0.954 ± 0.280	X
Lesser Black-backed Gull	*Larus fuscus*	14	21.0 ± 4.9	0.159 ± 0.128	
Herring Gull	*Larus argentatus*	18	89.9 ± 2.8	2.331 ± 0.483	X
Great Black-backed Gull	*Larus marinus*	15	17.6 ± 3.0	0.013 ± 0.005	X
Sandwich Tern	*Sterna sandvicensis*	5	2.4 ± 1.0	0.014 ± 0.012	
Common Tern	*Sterna hirundo*	6	5.1 ± 2.1	0.006 ± 0.004	
Little Tern	*Sterna albifrons*	4	2.0 ± 0.9	0.006 ± 0.005	
Black Tern	*Chlidonias niger*	1	0.8 ± 0.8	0.0003 ± 0.0003	
Carrion Crow	*Corvus corone corone*	8	17.9 ± 7.7	0.044 ± 0.021	
Hooded Crow	*Corvus corone cornix*	2	1.2 ± 0.9	0.003 ± 0.003	
Snow Bunting	*Plectrophenax nivalis*	1	0.4 ± 0.4	0.0001 ± 0.0001	

During the entire study period a total of 50 bird species was observed on the different bivalve beds (Table 4.2). Not all species observed on the beds use intertidal habitats regularly and typically do not forage on these habitats at all or only in very low numbers. Hence, only species commonly foraging in intertidal habitats were considered for detailed analyses of the habitat use (Table 4.2). Of the species that predominantly forage in the intertidal, most of them were also present on intertidal bivalve beds (Table 4.3; Figure 4.3). Only two species, the Sanderling (*Calidris alba*) and the Common Ringed Plover (*Charadrius hiaticula*), were almost completely absent on bivalve beds and showed an averseness for this habitat. For both species, the comparison of the bootstrap samples indicated that the abundances were in all cases higher on the intertidal than on bivalve beds (Table 4.3; Figure 4.3). Species showing a more or less balanced distribution between bivalve beds and the intertidal were the Great Black-backed Gull (*Larus marinus*), the Mallard (*Anas platyrhynchos*) and the Northern Pintail (*Anas acuta*), where 35–70% of the bootstrap samples had a higher abundance on the bivalve beds. All other species were found with higher abundances on bivalve beds (82–100%, Table 4.3; Figure 4.3),

of which the Little Egret (*Egretta garzetta*) showed the highest preference for bivalve beds with an abundance 47 times higher than on bare intertidal flats (Table 4.3). Other species showing a relatively high preference for bivalve beds were: Common Eider (*Somateria mollissima*), Common Greenshank (*Tringa nebularia*), Eurasian Whimbrel (*Numenius phaeopus*), Common Redshank (*Tringa totanus*), Ruddy Turnstone (*Arenaria interpres*), Eurasian Spoonbill (*Platalea leucorodia*), and Herring Gull (*Larus argentatus*). These species were, in descending order, between 20 and 11 times more abundant on bivalve beds than on bare intertidal flats (Table 4.3). The most abundant bird on the bivalve beds, the Eurasian Oystercatcher (*Haematopus ostralegus*), also had a high preference for these beds (bed preference of 9.3, Table 4.3).

In four species, bed composition significantly affected their abundance: the Eurasian Oystercatcher, the Common Gull (*Larus canus*), the Red Knot (*Calidris canutus*) and the Dunlin (*Calidris alpina*). The abundance of these four species was negatively correlated with the fraction of oysters in the total bivalve biomass (Spearman correlation, all p < 0.03, Figure 4.4). All other species showed no significant response to the dominance of oysters (Figure 4.4). Focusing on the species preying on mussels, a significant relationship between bird abundance and prey quality (ratio of observed and predicted $AFDM_{flesh}$) was only observed for the Common Eider (Spearman correlation, S = 378, ρ = 0.61, p = 0.007, Figure 4.5). In contrast, the abundances of the Oystercatcher, the Red Knot and the Herring Gull were not correlated with prey quality (Spearman correlation, all p > 0.05, Figure 4.5).

Discussion

Epibenthic bivalve aggregations are important structures in shallow intertidal soft-bottom environments. They often feature a higher or different taxonomic diversity and abundance of organisms than surrounding bare flats (Asmus 1987, Buschbaum et al. 2009, Markert et al. 2009) and therefore serve as important foraging grounds for many bird species. Indeed, the majority (18 out of 24) of the investigated bird species showed a preference for these biogenic structures. One might argue that we overestimated the number of bird species preferring bivalve beds, because we used bird numbers at high-tide roosts to estimate the abundance on intertidal flats at low tide, instead of direct counts. However, since low tide feeding densities on bare tidal flats on the basis of low tide counts were generally similar to our calculated densities (Folmer et al. 2010, van den Hout & Piersma 2013), we are confident that our procedure did not misjudge the low tide feeding densities leading to a reliable estimate of bird species preferring bivalve beds.

The introduction of the Pacific oyster has led to significant changes of the intertidal systems of the Dutch Wadden Sea. At present, about 50% of the intertidal mussel beds in the Dutch Wadden Sea contain many oysters and are either a mix of oysters and mussels or dominated by oysters (van Stralen et al. 2012). In line with this, about 40% (7 out of 18) of the beds investigated in our study contained a considerable amount of oyster biomass. The impacts of the Pacific oyster introduction are manifold, comprising both positive and negative effects. Positive effects of the introduction include numerous important ecosystem services, such as improvement of the water quality, seashore stabilization, carbon burial and habitat provision for other organisms (Grabowski et al. 2012, Katsanevakis et al. 2014). In contrast, the occupancy of the same habitats as native *M. edulis* leads to competition for space and food between the two species. We found that the body condition of mussels generally decreased with increasing oyster dominance. However, some beds featured a low mussel body condition despite oysters being absent or only present in low numbers. Since oysters are intolerant to short inundation times resulting in the failure to grow and persist in higher elevated intertidal areas (Rodriguez et al. 2014, Walles et al. 2016), mussels dominate these high areas. The short inundation times that the mussels face in the high intertidal may result in low body conditions (Goss-Custard et al. 1993).
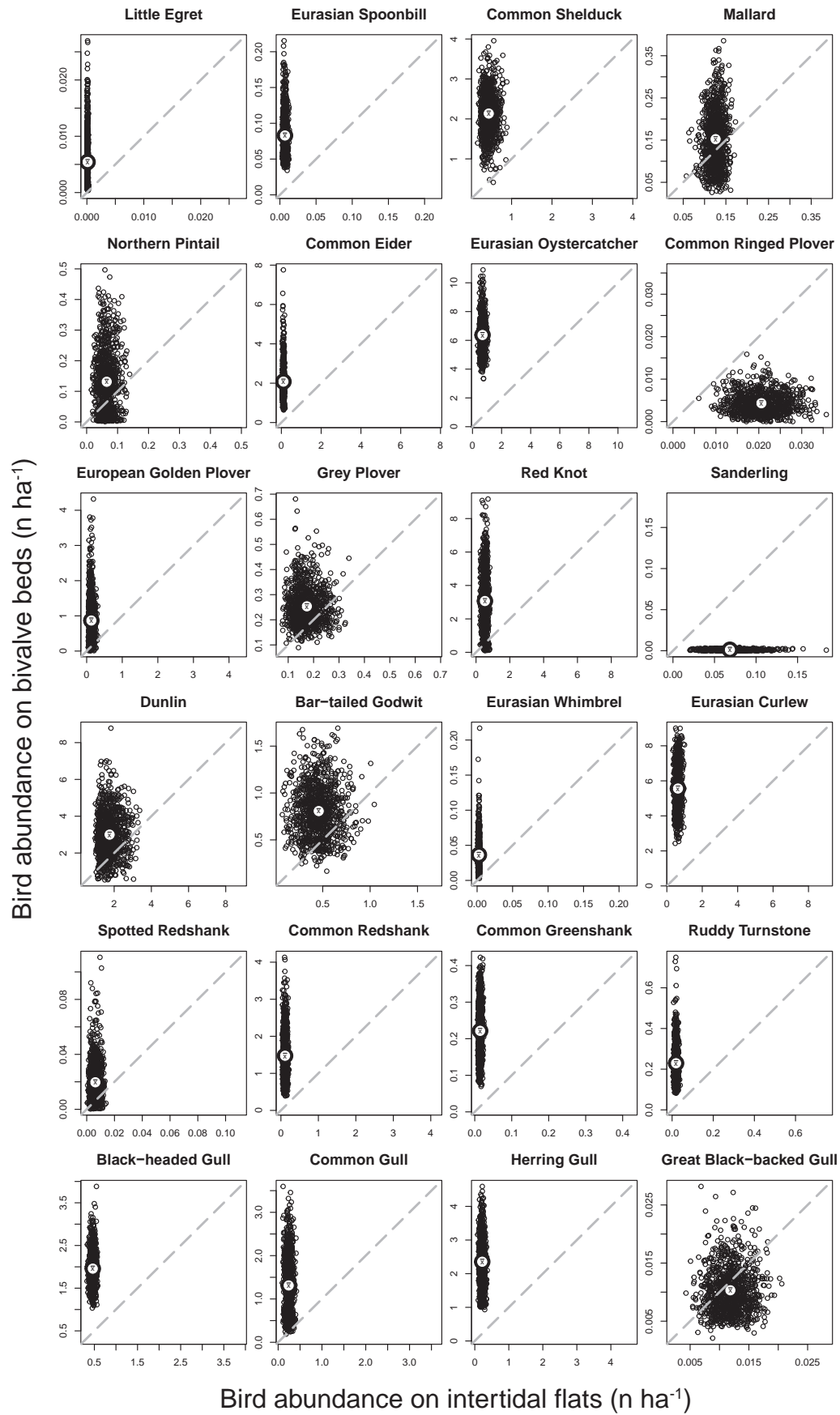
Figure 4.3: Results of the bootstrapping of bird abundances. The bird abundance on bivalve beds (n ha^{-1})was plotted against the bird abundance intertidal flats (n ha^{-1}). The grey dashed line represents the $x = y$ line. The mean of all bootstrap samples per species is indicated by an encircled $\bar{x}$.

Table 4.3: Results of the bootstrap calculations of bird abundance on the intertidal and on bivalve beds. For each species considered to be common on intertidal flats the mean of the total population in the Dutch Wadden Sea (2011–2014), abundances on bare intertidal flats and on bivalve beds, the portion of all bootstrap observations exhibiting a higher abundance on bivalve beds and a comparison between bare intertidal- and bivalve bed abundance (preference factor) are given. The asterisk indicates part of the population of Common Eider using the intertidal during high tide. Values are given ± SE

| Species	Population total (mean high water counts)	Abundance intertidal (n ha^{-1})	Abundance bivalve bed (n ha^{-1})	Fraction bird abundance higher on mussel beds (%)	Preference factor for bivalve beds
Little Egret	15 ± 3	0.0001 ± 0.000002	0.005 ± 0.0001	100	46.7
Common Eider	13037 ± 587 *	0.103 ± 0.001	2.080 ± 0.026	100	20.1
Common Greenshank	1948 ± 478	0.015 ± 0.0001	0.221 ± 0.002	100	15.2
Eurasian Whimbrel	337 ± 104	0.003 ± 0.00002	0.036 ± 0.001	99.9	14.5
Common Redshank	14787 ± 1807	0.111 ± 0.001	1.475 ± 0.020	100	13.3
Ruddy Turnstone	2557 ± 160	0.019 ± 0.0002	0.229 ± 0.003	100	11.9
Eurasian Spoonbill	935 ± 173	0.007 ± 0.0001	0.083 ± 0.001	100	11.8
Herring Gull	29077 ± 1705	0.218 ± 0.001	2.355 ± 0.022	100	10.8
Eurasian Oystercatcher	91766 ± 6171	0.688 ± 0.003	6.371 ± 0.036	100	9.3
Eurasian Curlew	83688 ± 5466	0.627 ± 0.003	5.560 ± 0.038	100	8.9
European Golden Plover	17682 ± 2316	0.132 ± 0.002	0.868 ± 0.019	96	6.6
Red Knot	70549 ± 6686	0.526 ± 0.003	3.068 ± 0.054	92.8	5.8
Common Gull	32080 ± 3566	0.240 ± 0.002	1.312 ± 0.021	99.5	5.5
Common Shelduck	58643 ± 5727	0.434 ± 0.004	2.134± 0.017	99.9	4.9
Black-headed Gull	62483 ± 9379	0.463 ± 0.001	1.958 ± 0.013	100	4.2
Spotted Redshank	835 ± 145	0.006 ± 0.0001	0.020 ± 0.0004	82.3	3.2
Northern Pintail	8408 ± 1225	0.065 ± 0.001	0.131 ± 0.003	70.5	2
Bar-tailed Godwit	62027 ± 5675	0.457 ± 0.005	0.807 ± 0.009	86.5	1.8
Dunlin	231404 ± 16122	1.754 ± 0.015	2.999 ± 0.038	83.7	1.7
Grey Plover	22343 ± 2250	0.173 ± 0.001	0.254 ± 0.002	82.9	1.5
Mallard	17004 ± 1798	0.126 ± 0.001	0.152 ± 0.002	62.7	1.2
Great Black-backed Gull	1577 ± 168	0.012 ± 0.0001	0.010 ± 0.0001	34.6	0.9
Common Ringed Plover	2776 ± 599	0.021 ± 0.0001	0.004 ± 0.0001	0	0.2
Sanderling	9079 ± 702	0.069 ± 0.001	0.001 ± 0.00002	0	0
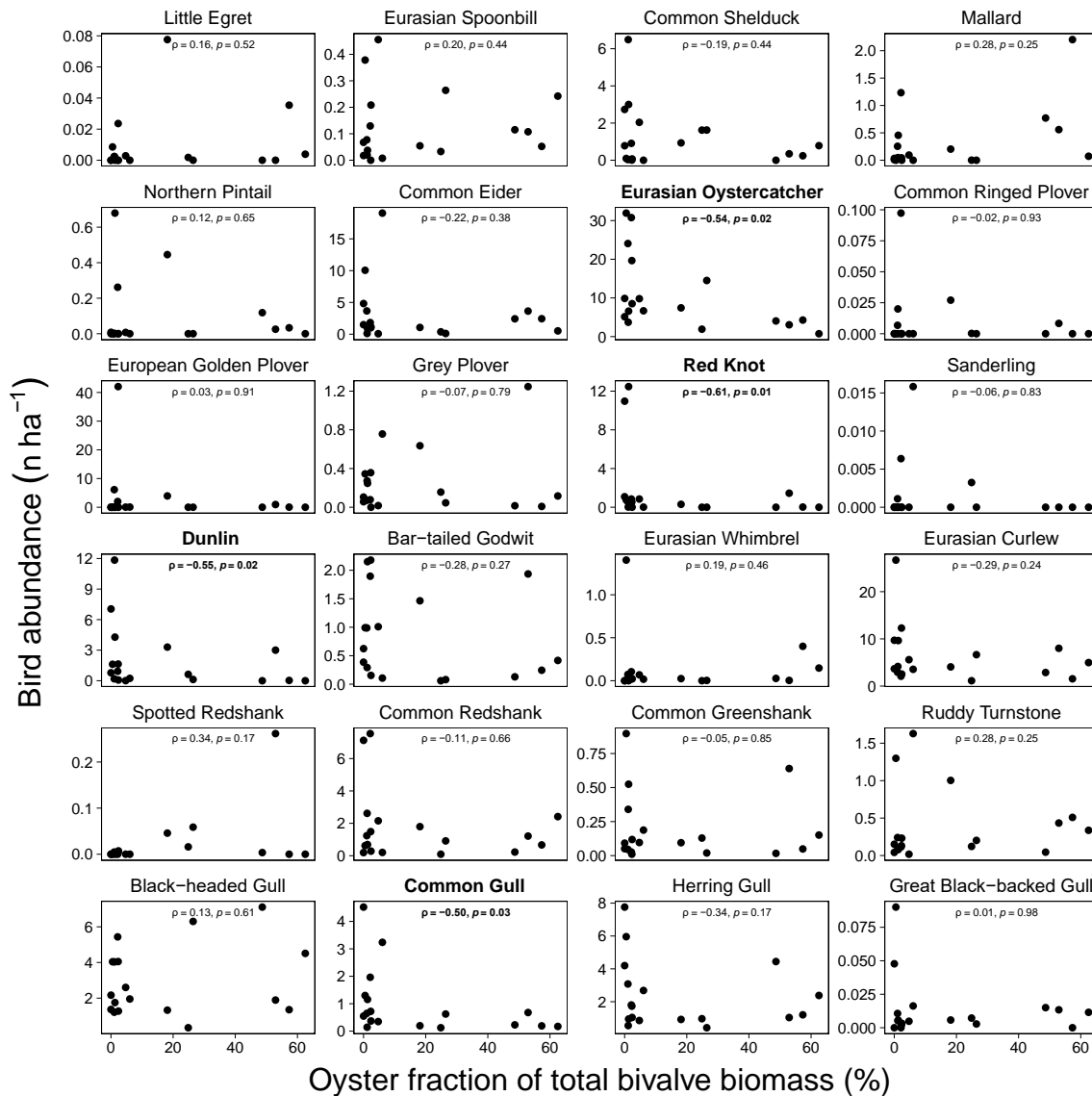
Figure 4.4: Relationship between the fraction of oysters on total bivalve biomass and the abundance of the different bird species (n ha^{-1}). Species showing a significant relationship between their abundance and the oyster occurrence are highlighted in bold font.

Yet, our study revealed that the majority of the bird species making use of mussel beds show no clear signs of being affected in terms of feeding density by the changes caused by the oyster introduction. We found no evidence for positive impacts. Negative impacts, indicated by a reduction in abundance, were only evident for four species. These negative impacts are particularly expected for species preying on mussels themselves (Scheiffarth et al. 2007).

Four bird species recorded in our study feed on the mussels: Red Knot, Oystercatcher, Herring Gull and Common Eider. These species may experience changes through the appearance of the invader both in the profitability (Troost 2009, Markert et al. 2009, this study) as well as in the accessibility of the prey (Eschweiler & Christensen 2011, Chapter 6: Waser et al. 2015). Therefore, as prey accessibility and prey profitability are important factors determining the fraction of harvestable food for wading birds (Zwarts & Wanink 1993), we expect these species to face a reduction in harvestable prey items leading them to avoid those beds where oysters are prevalent. This was observed for the Oystercatcher and Red Knot, but not for Common Eider and Herring Gull. How can this be explained?
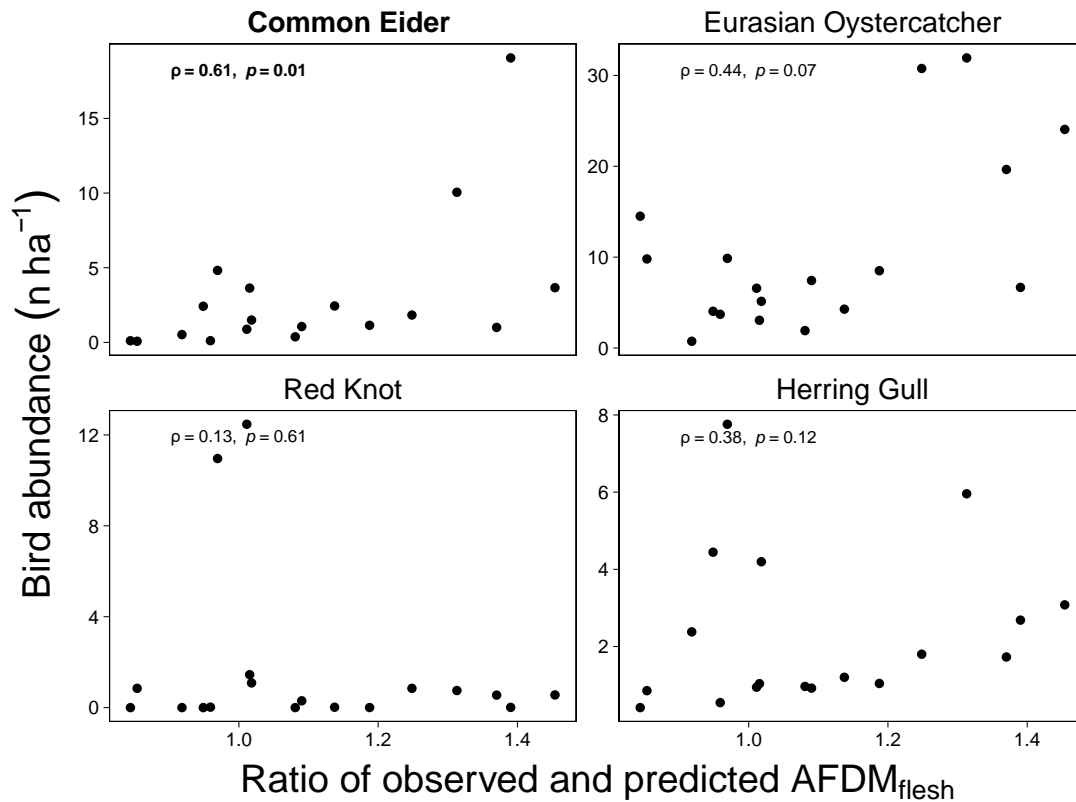
Figure 4.5: Relationship between the body condition of mussels and the abundance of molluscivorous birds. Plotted are the bird abundance of the four bird species (n ha^{-1}) against the relative mean ash-free dry mass of mussels (AFDM$_{flesh}$). Species showing a significant relationship between their abundance and the mussel condition are highlighted in bold font.

For Oystercatchers, our finding corroborates an earlier study based on observations on one single location before and after oyster invasion that Oystercatchers appear in much lower densities on oyster dominated beds, even though this species is actually able to feed on small-sized oysters (Markert et al. 2013). Although expected, we are not aware of previous reports that Red Knots might be negatively affected by an increased appearance of Pacific oysters. The Knot swallows bivalves whole and is only able to feed on mussels with a length below 20 mm (Zwarts & Blomert 1992). Hence, it only feeds on recently established mussel beds with a preponderance of small mussels. Old mussel beds with many large mussels are not attractive, even in the absence of Pacific oysters, explaining the great range in feeding densities observed on bivalve beds with no or few Pacific oysters.

The reason that densities of Herring Gulls did not decrease with the occurrence of Pacific oysters may be due to the fact that they may not forage exclusively on mussels when they frequent bivalve beds, but may feed on a broad range of resources (Camphuysen 2013). Although mussels represent an important part of their diet, this applies primarily to mussel spat, not older than 1 year and not exceeding lengths of 20 mm (Camphuysen 2013).

Since this size range of mussels appears hidden amongst bigger conspecifics in older beds, Herring Gulls typically prey on the mussel spat on young beds only. On older established bivalve beds, Herring Gulls use other resources, such as shore crabs, which are not heavily influenced by the oyster dominance (Markert et al. 2013).

A similar explanation may apply to the Common Eider, as it also feeds on shore crabs (Cramp & Simmons 1977). In addition, it should be remarked that although Eiders do feed in the intertidal during the period that it is covered with water, they show a strong preference for subtidal areas with high quality food, i.e. molluscs with a high flesh/shell ratio (Nehls 2001, Cervencl et al. 2015). This preference also showed up in a strong correlation between Eider

abundance and prey quality. Despite the fact that mussel condition was negatively correlated with presence of oysters, we did not find a negative correlation between Eider abundance and presence of oysters, as already mentioned. This may be due to Eiders feeding on shore crabs instead of mussels on oyster beds, as well as avoiding mussel beds high in the intertidal zone, where no oysters occur, but mussels are of poor quality.

Our study corroborated the prediction of Scheiffarth et al. (2007) that bird species feeding on associated fauna would be little affected by the invasion of Pacific oysters into intertidal mussel beds, except for the Dunlin and the Common Gull, which were clearly negatively affected by an increase in the presence of oysters. The decreasing numbers of Common Gulls might be directly coupled with the decrease of Oystercatchers. Oystercatchers feeding on mussels need to open the shells in order to swallow the soft flesh of the prey (Goss-Custard & Durell 1988), which requires relatively long handling times. As a result, the birds are sensitive to interference and allowing other individuals to steal prey (kleptoparasitism) (Ens & Goss-Custard 1984, Wood et al. 2015). Within avian assemblages, gulls are known to steal food items from several wading bird species (Amat & Aguilera 1990, Ens et al. 1990, Wood et al. 2015). The study of Zwarts & Drent (1981) found that Common Gulls present on mussel beds depended almost entirely on stealing mussels from Oystercatchers, suggesting that the density of Common Gulls reflects the Oystercatcher density. In our study, the densities of Common Gulls and Oystercatchers were indeed correlated with each other (Spearman correlation, S = 446, ρ = 0.54, p = 0.021).

In contrast, the Dunlin does not feed on bivalve covered patches at all, but forages on worms in the open areas in between. While in mussel dominated beds mussels establish hummocks rising above the immediate surrounding, in oyster dominated beds, the between-patch areas often silt up to mud-hummocks higher than the bivalves themselves. This characteristic may decrease their attractiveness as feeding areas for the Dunlin.

Our study demonstrates that mussel beds are very important for avian biodiversity and that the colonization of these beds by Pacific oysters does not improve avian biodiversity, but only has negative impacts, most clearly for three species: Oystercatcher, Common Gull and Knot. One might consider removing the oysters in the hope that the vacant space would be taken by mussels, restoring good feeding opportunities for the affected bird species. However, a complete removal of oyster beds is challenging, since aggregations of oysters are firmly anchored into the sediment and dredging only removes peripheral oysters (Wijsman et al. 2008). After dredging, buried parts of the oyster complex remain in the sediment, providing ideal settling grounds for oyster spat, in the long run leading to a recolonization by Pacific oysters (Wijsman et al. 2008). Moreover, the removal of the oysters has clear negative impacts on the birds, as most species avoid the oyster fished areas (Wijsman et al. 2008) and this is in line with our finding that for the majority of species preferring bivalve beds, high densities of Pacific oysters may not decrease the attractiveness of the bivalve bed as a feeding area. Hence, fishing away the oyster beds would be detrimental for the birds.

As attempts to fight the Pacific oyster, by e.g. fishing away the oysters, will negatively affect the birds using bivalve beds as foraging areas, other measures are needed to mitigate the invasion of the Pacific oyster into intertidal areas. Moreover, the possible effects on avian diversity should be considered when managing the commercial exploitation of Pacific oysters. In the Netherlands it is currently discussed whether fishery of Pacific oysters should be allowed. Hand picking of oysters is the only feasible option on wild beds. Our study shows that it is impossible to visit oyster beds without disturbing birds, but that the number of birds that are disturbed can be minimized by restricting fishery to beds with the highest densities of Pacific oysters.

Acknowledgements

Supplementary material

Table S4.1: The number of counting days of birds on the studied intertidal bivalve beds in the Dutch Wadden Sea for the study years.

Bivalve bed	2010	2011	2012	2013	Total
W001_A0	9	6	3	7	25
W001_A1	10	6	3	7	26
W001_A2	10	2			12
W001_B	9	6	3	6	24
W012		5	3	5	13
W013		4	4	5	13
W017			3	4	7
W015		4	4	5	13
W007b		4	2	1	7
E031		4	5	1	10
E027		5	4	4	13
E024				5	5
E023			1	4	5
E022	1	3	6	3	13
E032			1	8	9
E015	1	5	7	6	19
E010	2	2	4	2	10
E002		5	4	3	12

Table S4.2: Overview of the aerial counts of Common Eider in the Dutch Wadden Sea performed by IMARES and Rijkswaterstaat (RWS).

Season	Date	Institution
	14.8.2010 [w]	IMARES
	15/16/19.11.2010	IMARES
	10/11/12.12.2010	IMARES
2010/2011	22/23.1.2011	RWS
	18/19.2.2011	IMARES
	11/12.3.2011	IMARES
	8/9.4.2011	IMARES
	7/8.8.2011	IMARES
	14/15.1.2012	RWS
2011/2012	11.2.2012 [w]	IMARES
	26/27.2.2012	IMARES
	17.3.2012 [w]	IMARES
2012/2013	24/25.1.2013 [w]	RWS
2013/2014	15/16.11.2013	RWS
	4/5.1.2014	RWS

[w] Counts only covered the western part of the Dutch Wadden Sea (holding about 90% of the total Dutch Eider population)

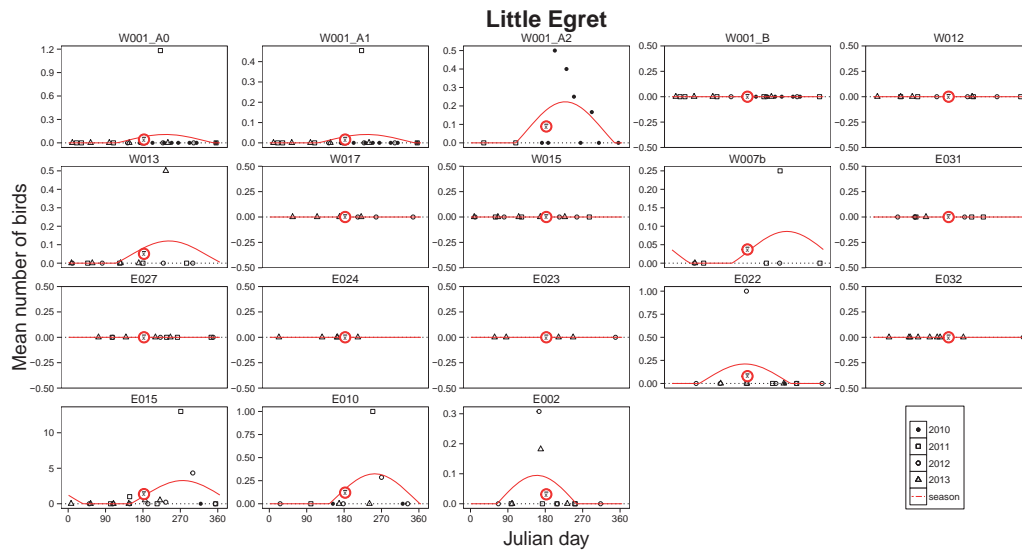**Little Egret**

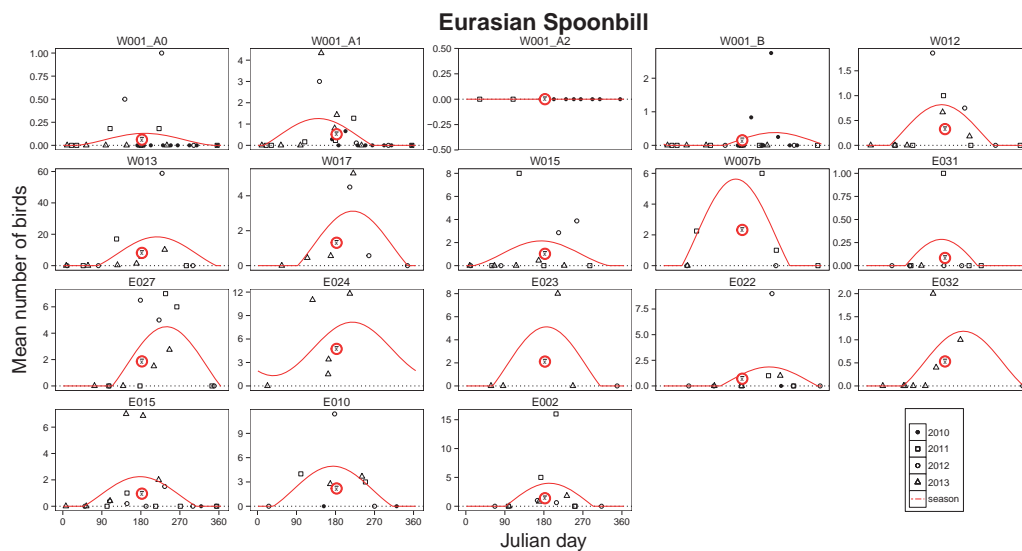**Figure S4.1:** Numbers of the Little Egret (*Egretta garzetta*) on bivalve beds.

Eurasian Spoonbill

Figure S4.2: Numbers of the Eurasian Spoonbill (*Platalea leucorodia*) on bivalve beds.
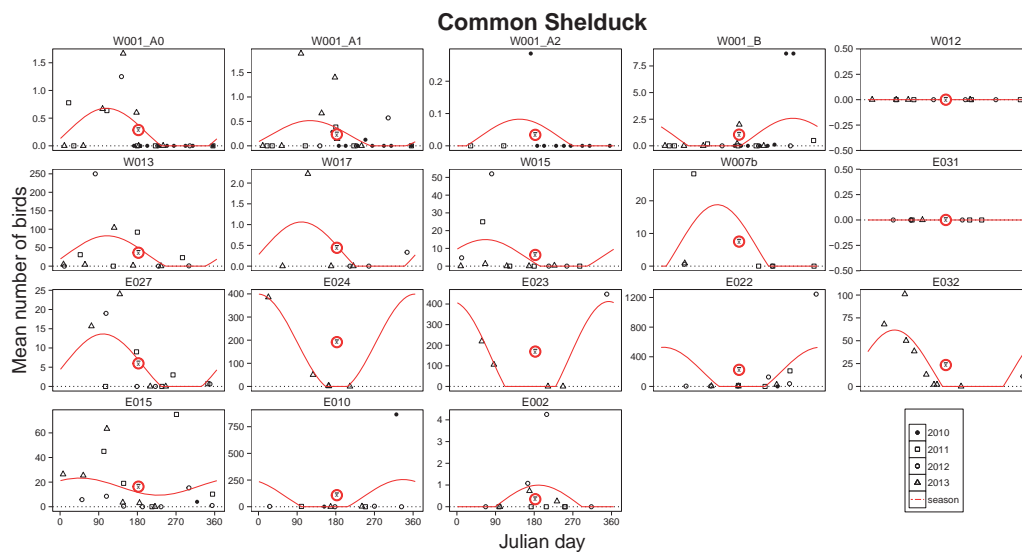
Common Shelduck

Figure S4.3: Numbers of the Common Shelduck (*Tadorna tadorna*) on bivalve beds.
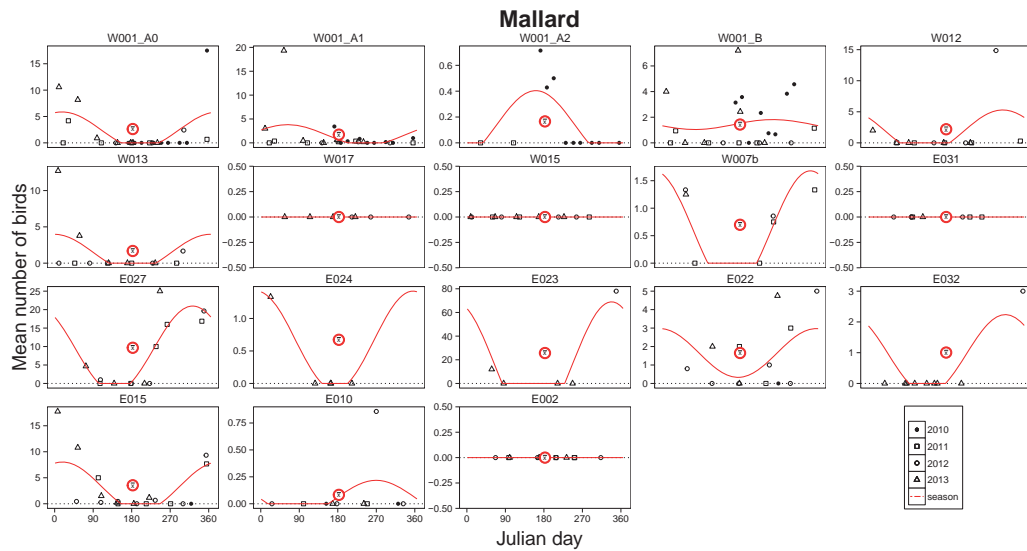
Mallard

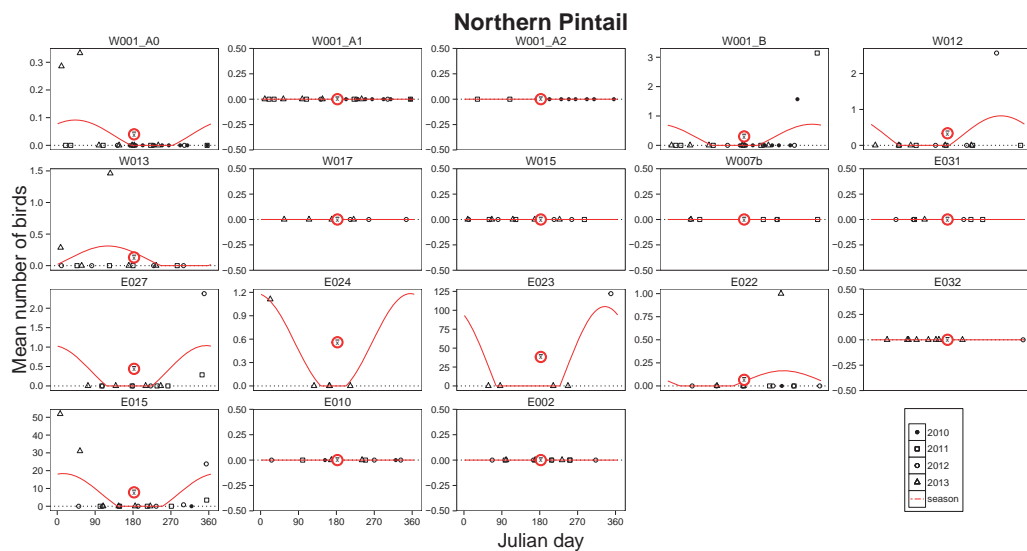**Figure S4.4:** Numbers of the Mallard (*Anas platyrhynchos*) on bivalve beds.

Northern Pintail

Figure S4.5: Numbers of the Northern Pintail (*Anas acuta*) on bivalve beds.
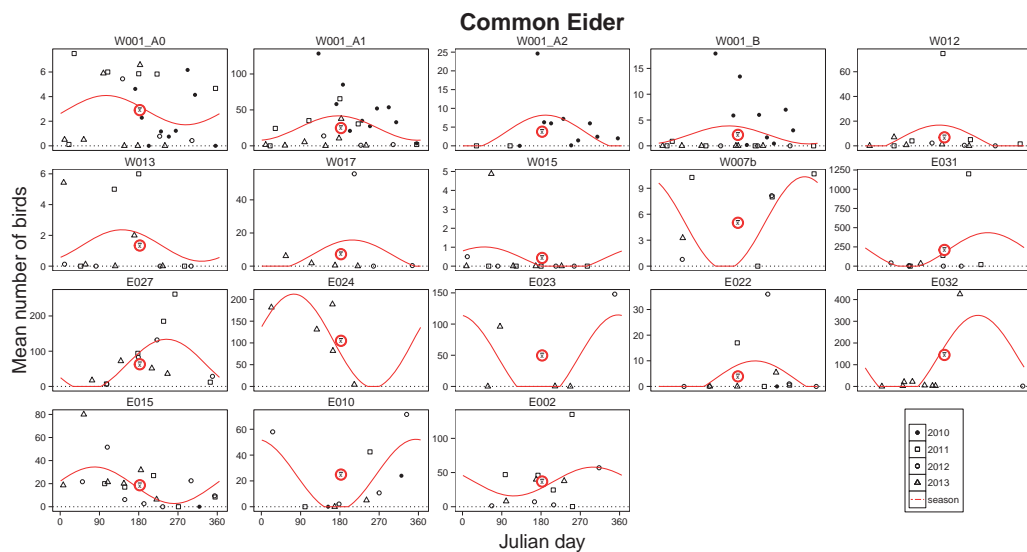
Common Eider

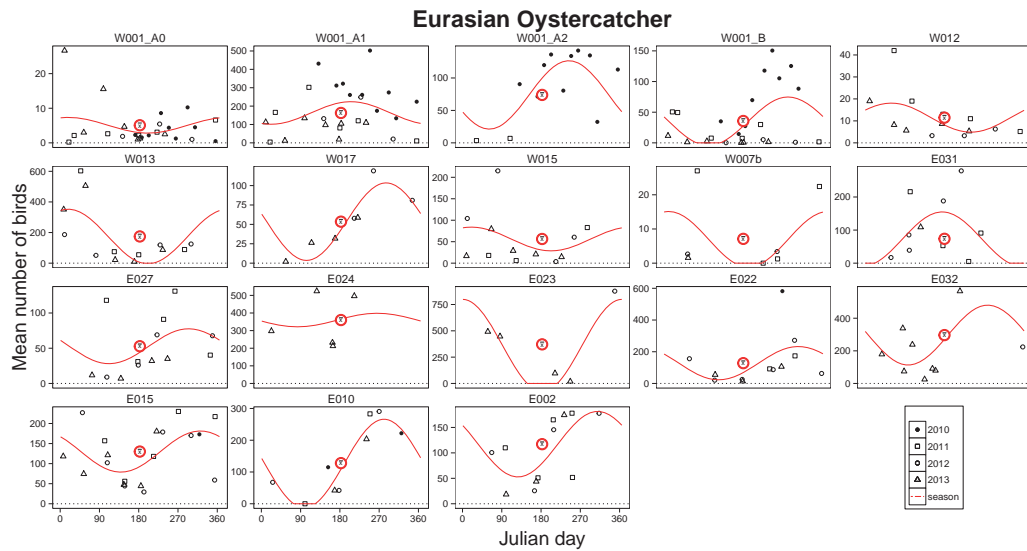**Figure S4.6:** Numbers of the Common Eider (*Somateria mollissima*) on bivalve beds.

Eurasian Oystercatcher

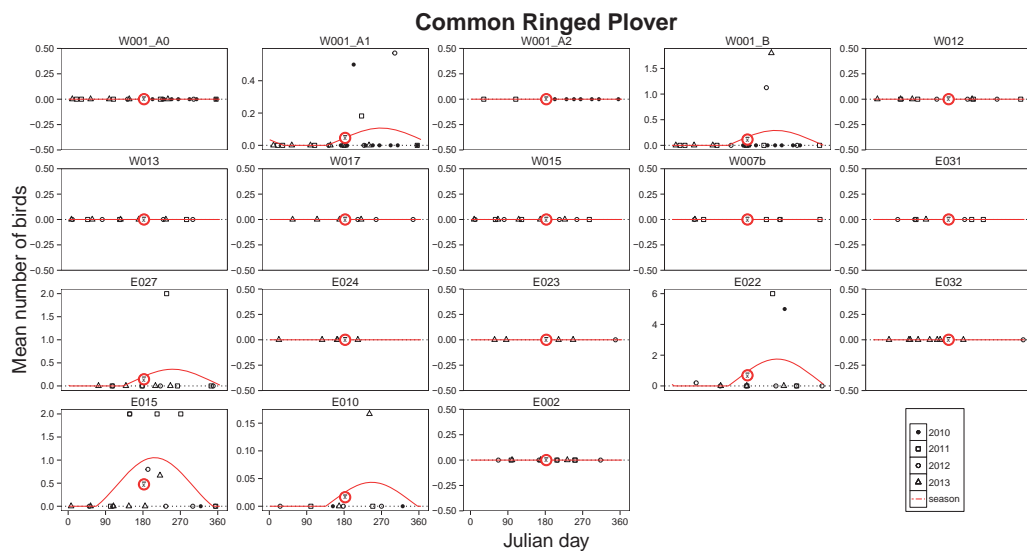**Figure S4.7:** Numbers of the Eurasian Oystercatcher (*Haematopus ostralegus*) on bivalve beds.

Common Ringed Plover

Figure S4.8: Numbers of the Common Ringed Plover (*Charadrius hiaticula*) on bivalve beds.

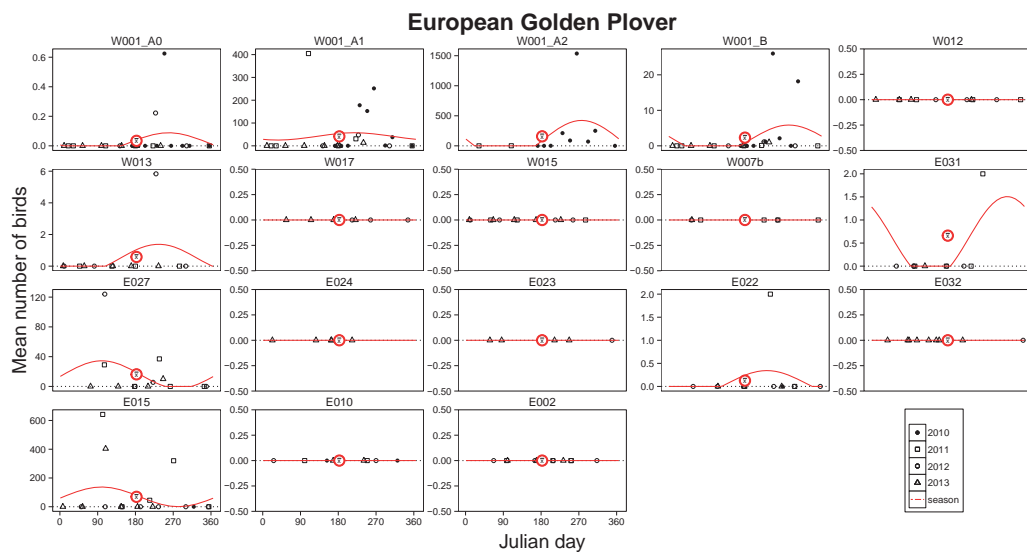**European Golden Plover**

Figure S4.9: Numbers of the European Golden Plover (*Pluvialis apricaria*) on bivalve beds.
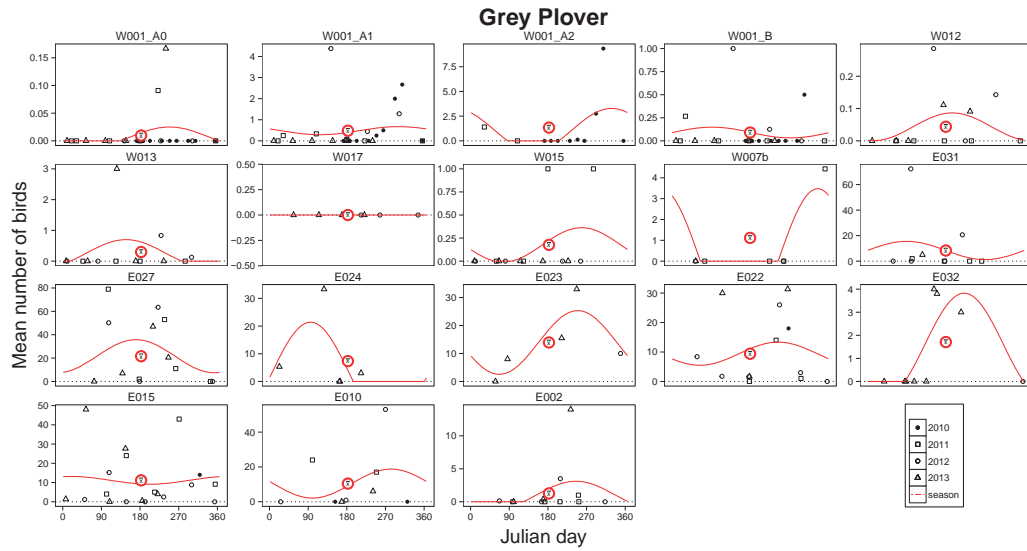
Grey Plover

Figure S4.10: Numbers of the Grey Plover (*Pluvialis squatarola*) on bivalve beds.

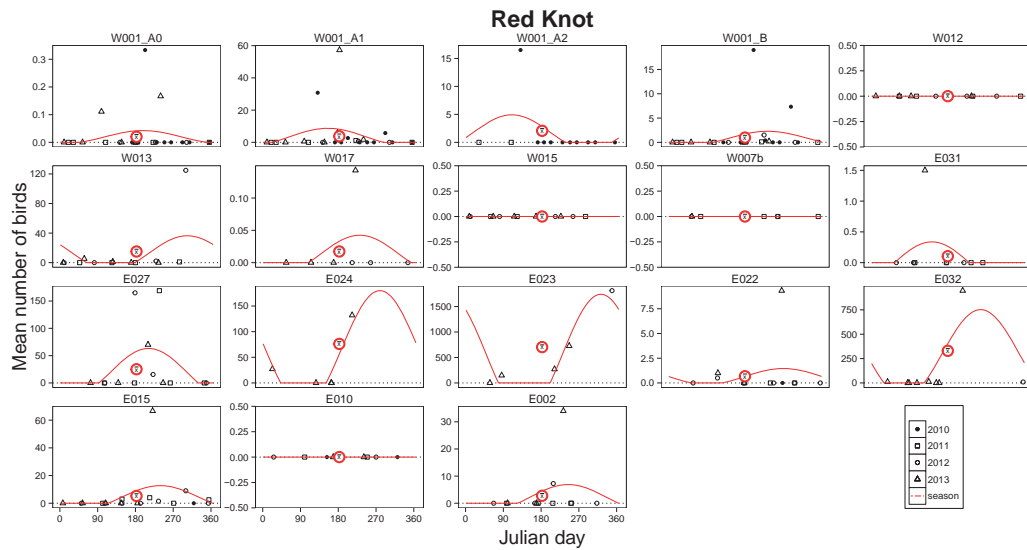**Red Knot**

Figure S4.11: Numbers of the Red Knot (*Calidris canutus*) on bivalve beds.
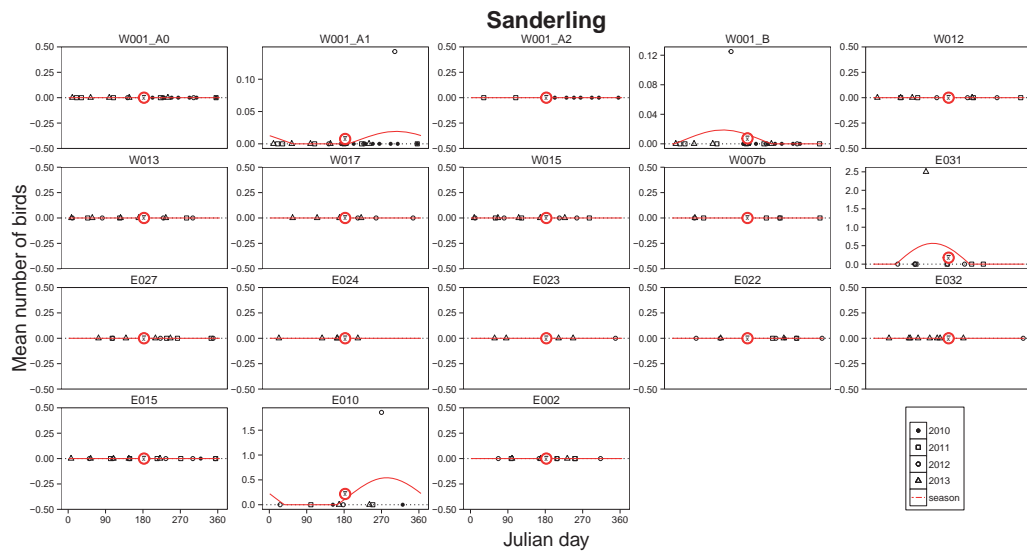
Sanderling

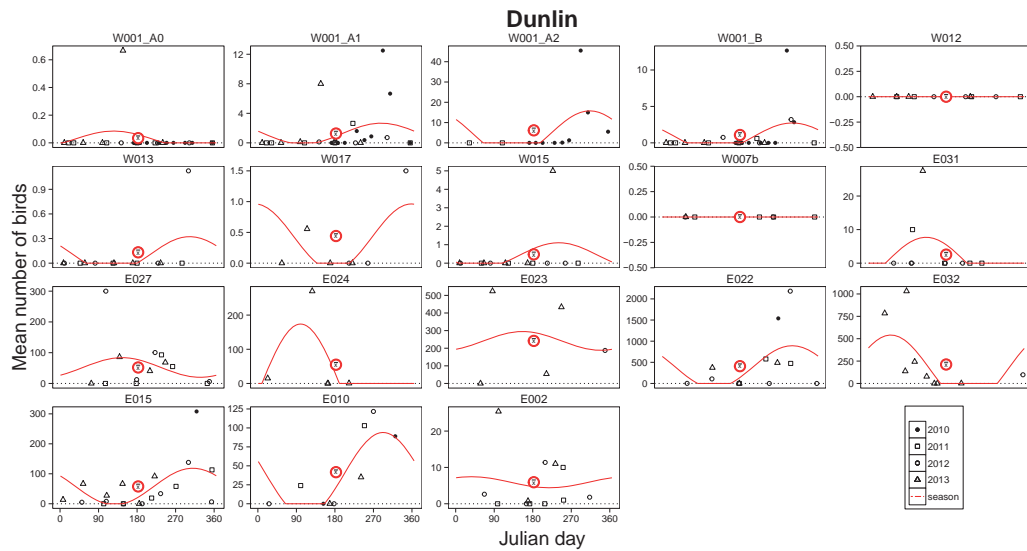**Figure S4.12:** Numbers of the Sanderling (*Calidris alba*) on bivalve beds.

Figure S4.13: Numbers of the Dunlin (*Calidris alpina*) on bivalve beds.
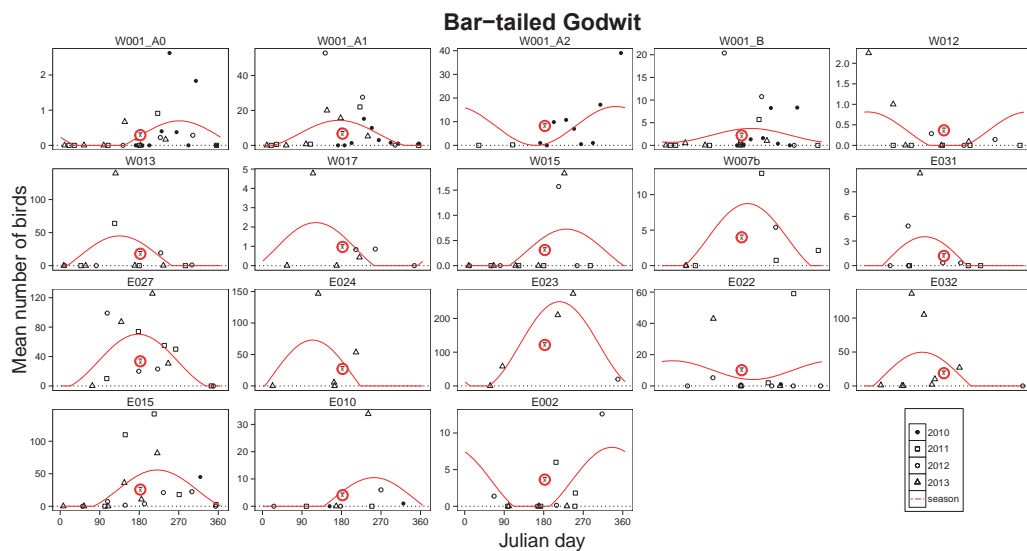
Figure S4.14: Numbers of the Bar-tailed Godwit (*Limosa lapponica*) on bivalve beds.
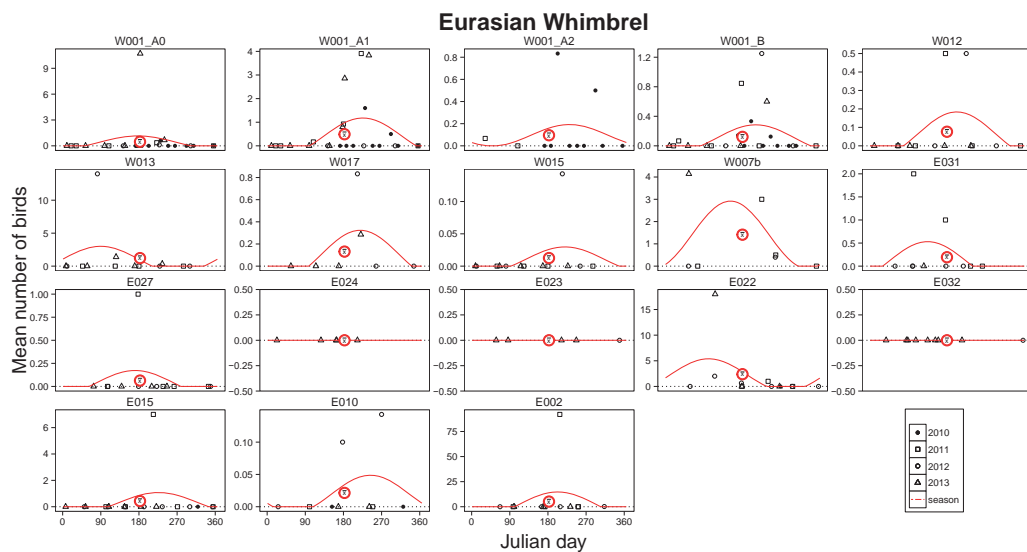
Figure S4.15: Numbers of the Eurasian Whimbrel (*Numenius phaeopus*) on bivalve beds.
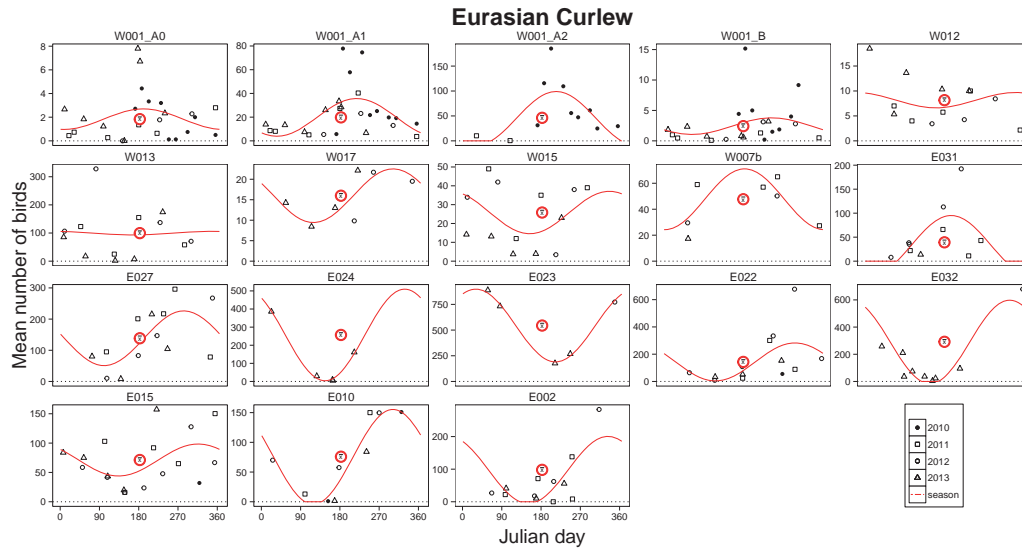
Figure S4.16: Numbers of the Eurasian Curlew (*Numenius arquata*) on bivalve beds.
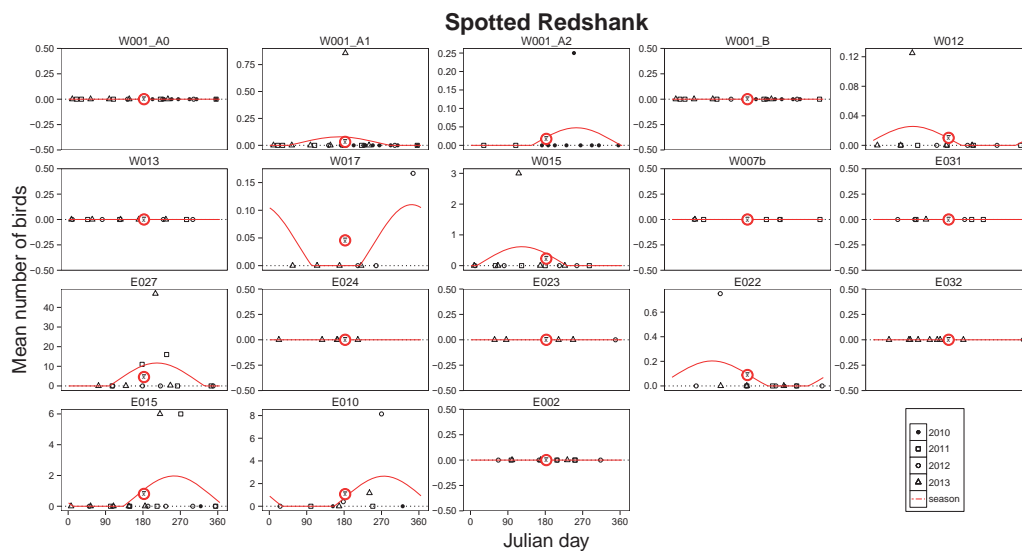
Figure S4.17: Numbers of the Spotted Redshank (*Tringa erythropus*) on bivalve beds.
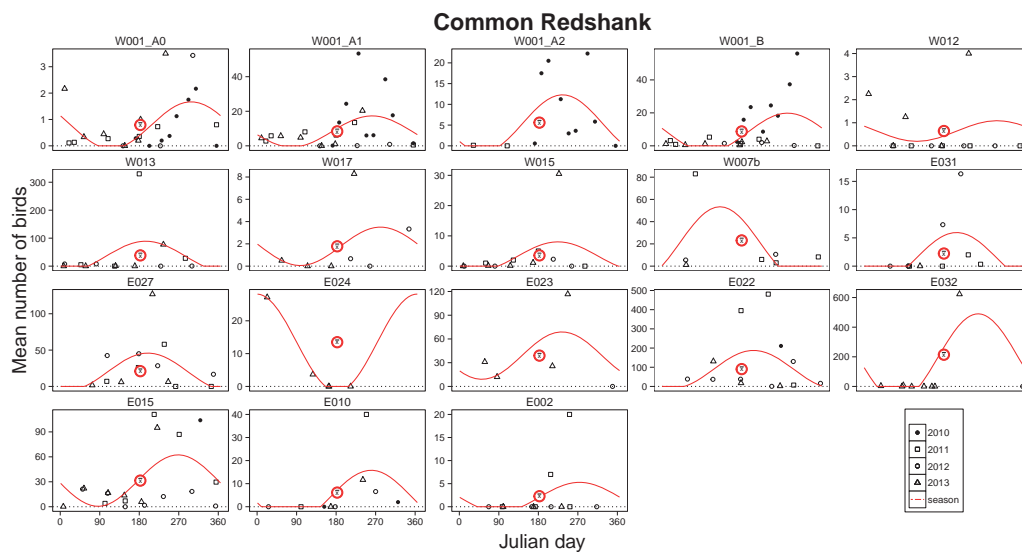
Figure S4.18: Numbers of the Common Redshank (*Tringa totanus*) on bivalve beds.
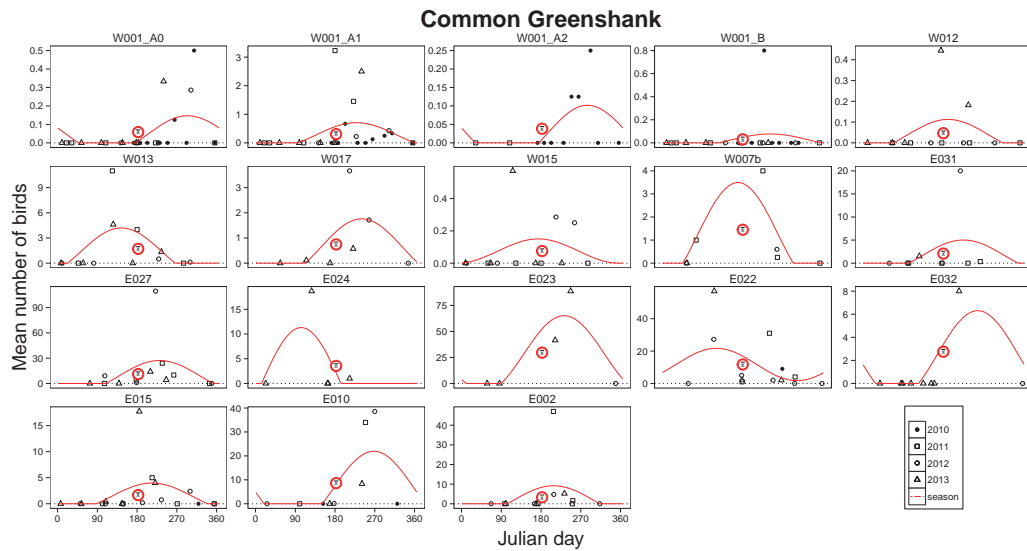
Figure S4.19: Numbers of the Common Greenshank (*Tringa nebularia*) on bivalve beds.
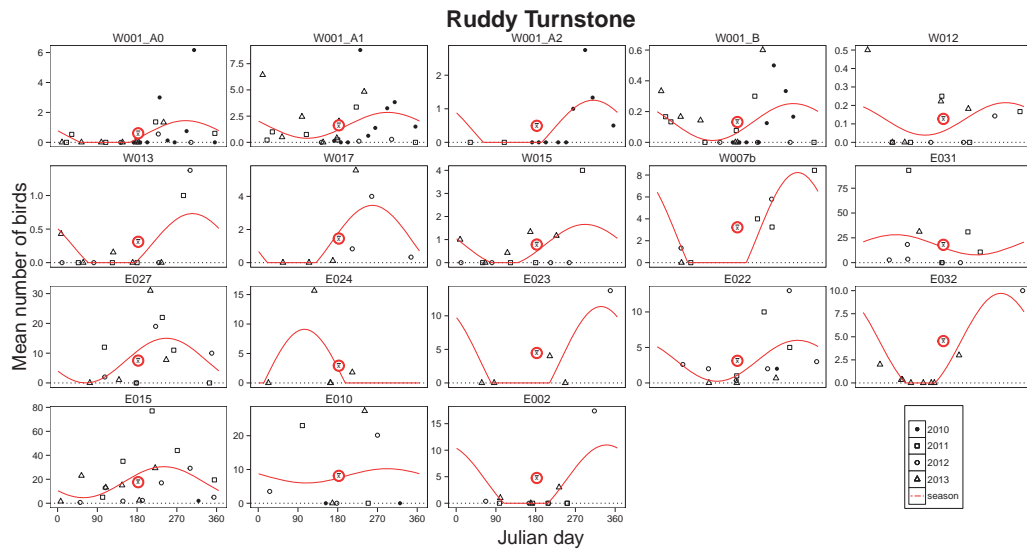
Figure S4.20: Numbers of the Ruddy Turnstone (*Arenaria interpres*) on bivalve beds.
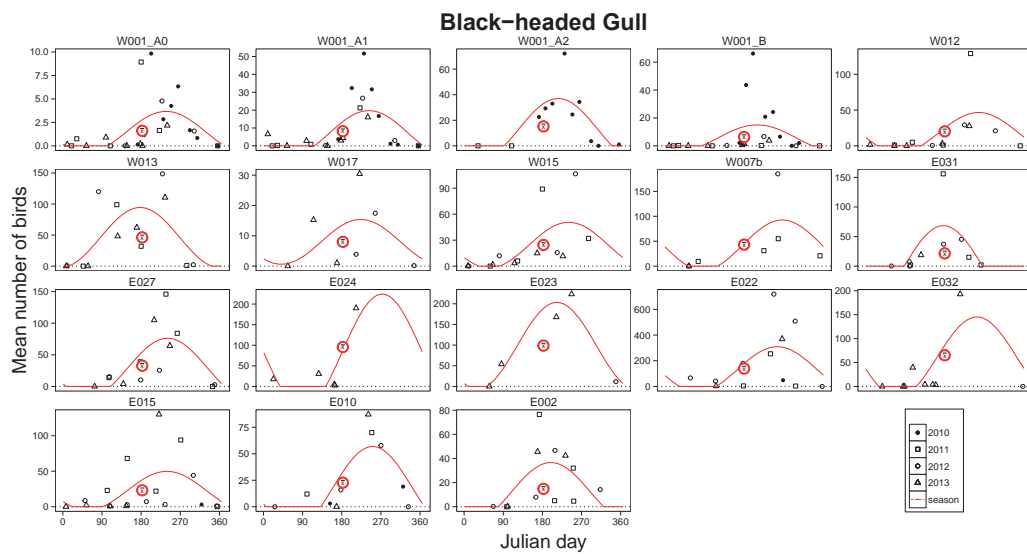
Figure S4.21: Numbers of the Black-headed Gull (*Larus ridibundus*) on bivalve beds.
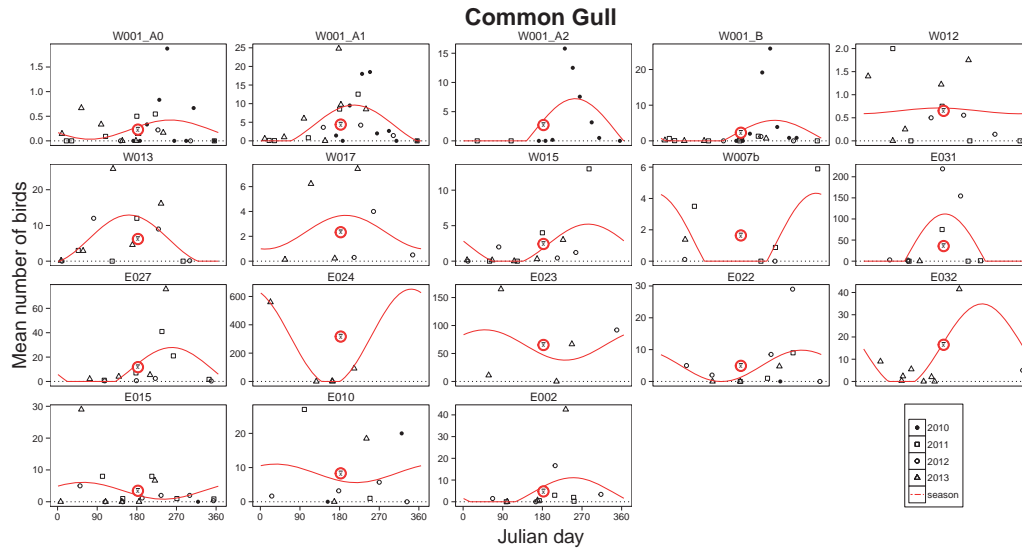
Figure S4.22: Numbers of the Common Gull (*Larus canus*) on bivalve beds.
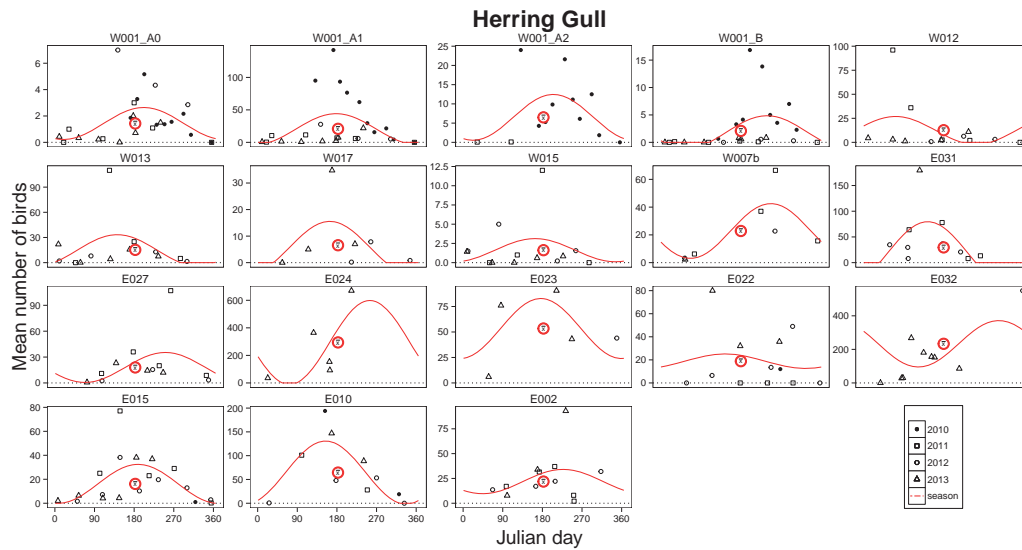
Figure S4.23: Numbers of the Herring Gull (*Larus argentatus*) on bivalve beds.
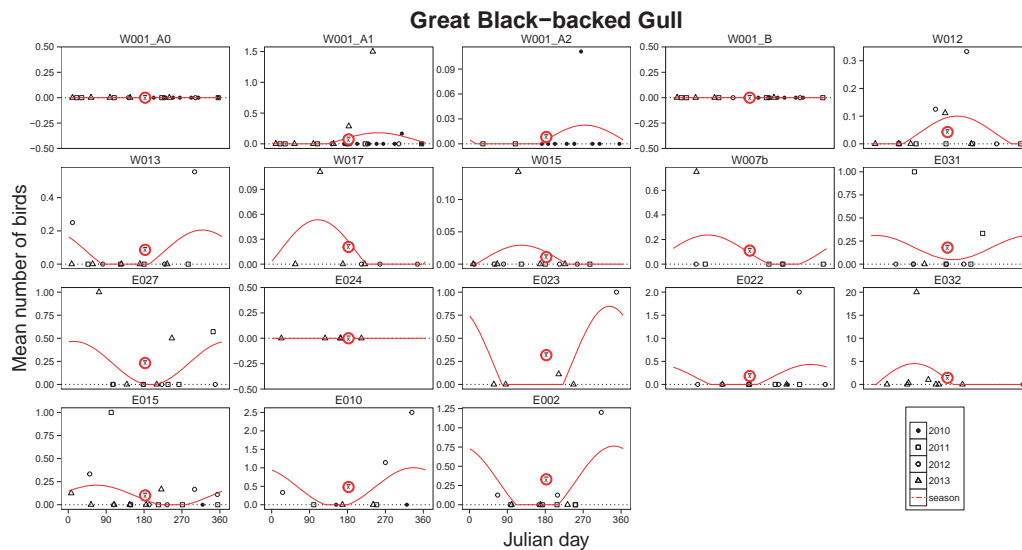
Figure S4.24: Numbers of the Great Black-backed Gull (*Larus marinus*) on bivalve beds.
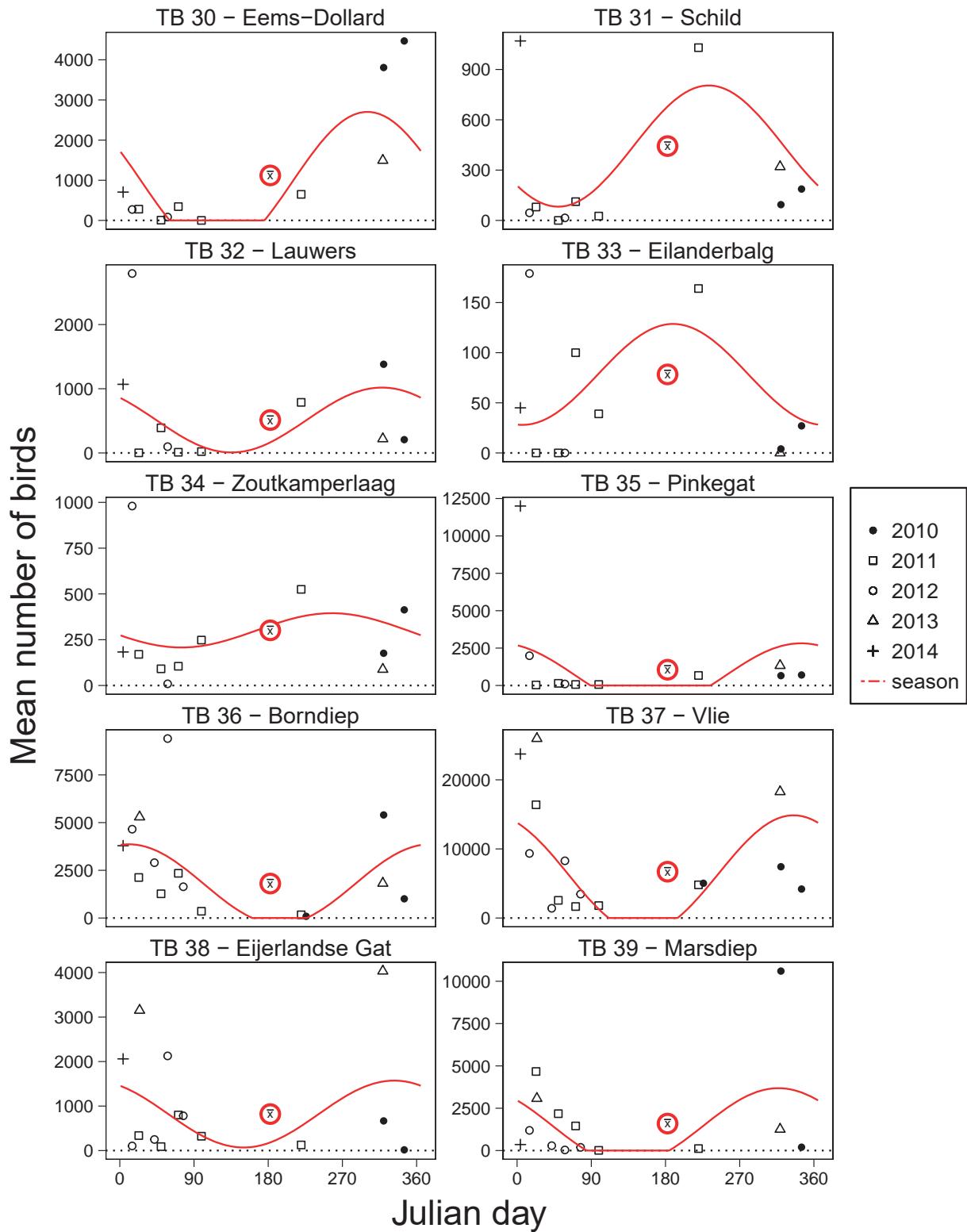
Figure S4.25: Numbers of Common Eider (*Somateria molissima*) above the intertidal.

5 | Quantifying tidal movements of the shore crab *Carcinus maenas* on to complex epibenthic bivalve habitats

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Abstract

Many subtidal predators undertake regular tidal migrations into intertidal areas in order to access abundant prey. One of the most productive habitats in soft bottom intertidal systems is formed by beds of epibenthic bivalves such as blue mussels (*Mytilus edulis*) and Pacific oysters (*Crassostrea gigas*). In the Dutch Wadden Sea, these bivalves might face substantial predation pressure by the shore crab (*Carcinus maenas*), which increased considerably in numbers during the last 20 years. However, the quantification of this species on bivalve beds is challenging, since most methods common for quantifying animal abundance in marine habitats cannot be used. This study investigated the potential of two methods to quantify the abundance of *C. maenas* on 14 epibenthic bivalve beds across the Dutch Wadden Sea. The use of the number of crabs migrating from subtidal towards intertidal areas as a proxy of abundance on bivalve beds yielded unreliable results. In contrast, crabs caught with traps on the beds were correlated with the abundance assessed on the surrounding bare flats by beam trawl and therefore provided usable results. The estimates, however, were only reliable for crabs exceeding 35 mm in carapace width (CW). The application of these estimates indicated that crab abundances on bivalve beds were influenced by the biogenic structure. Beds dominated by oysters attracted many large crabs (> 50 mm CW), whereas abundances of medium-sized crabs (35–50 mm CW) showed no relationship to the oyster occurrence. The combination of traps and trawls is capable of quantifying crab abundance on bivalve beds, which offers the possibility to study biotic processes such as predator-prey interactions in these complex structures in more detail.

Introduction

Shallow intertidal zones are very productive areas and feature a great abundance of benthic primary consumers, including many mollusc, polychaete, and crustacean species. With rising tide, many aquatic mobile secondary consumers such as fishes and decapods migrate from the subtidal zone into these productive areas to access abundant prey (Rilov & Schiel 2006, Jones & Shulman 2008, Silva et al. 2014). The highest productivity is often found in habitats rich in three-dimensional structure, and one of these complex habitats in soft bottom intertidal systems is created by epibenthic bivalves such as blue mussels (*Mytilus edulis* L., 1758) and Pacific oysters (*Crassostrea gigas* Thunberg, 1793), which aggregate and accordingly form bivalve beds. These beds represent important features of the intertidal ecosystem by providing hard substrate, increasing habitat complexity, reducing hydrodynamics, and modifying the sediment by depositing large amounts of pseudo-feces and other fine particles (Gutierrez et al. 2003, van der Zee et al. 2012).

The Pacific oyster is native to coastal waters of the north-western Pacific Ocean and nowadays has successfully invaded all temperate coastal ecosystems around the world (Ruesink et al. 2005). After the introduction of *C. gigas* into the European Wadden Sea in the 1970s (Troost 2010), many pure mussel beds developed into mixed bivalve beds or even into oyster-dominated beds since the late 1990s (Nehls et al. 2009b, Troost 2010). Mussels and oysters similarly provide hard substrata for sessile species (Kochmann et al. 2008), but differ in their size, three-dimensional structure, heterogeneity, and formed micro-habitats (Gutierrez et al. 2003). Due to newly constructed biogenic reef structures, formed by the large-sized oysters, bivalve beds increased in habitat heterogeneity and in the amount of surface area for attachment and crevices for refuge of other organisms. Since both species also differ in their attachment mechanisms, aggregations of multiple specimens differ considerably in structural complexity. Mussels are adhered to the substratum via temporary byssus threads (Bell & Gosline 1996), and the continuous process of generating new threads leads to flexible and dynamic meshworks of individual mussels (van de Koppel et al. 2005). In contrast, Pacific oysters remain permanently attached to each other via an organic-inorganic adhesive (Burkett et al. 2010) and continuous larval settlement onto conspecifics leads to the creation of rigid and persisting structures (Walles et al. 2015a). Consequently, the complex structures formed by these two bivalve species are likely to provide different resources in terms of nesting sites, shelter from predators, and feeding opportunities, thus potentially leading to differences in the species community (Markert et al. 2009). Moreover, the conversion of mussel beds into oyster-dominated beds may ultimately lead to a change of feeding opportunities for predators (Eschweiler & Christensen 2011, Chapter 6: Waser et al. 2015, Chapter 4: Waser et al. 2016a).

Crabs are among the most prominent predators that undertake tidal migrations to forage in intertidal areas during flood tides (Hamilton 1976, Hill et al. 1982, Holsman et al. 2006, Silva et al. 2014). These tidal migrations are also typical for the common shore crab (*Carcinus maenas* L., 1758) (e.g., Hunter & Naylor 1993, Silva et al. 2014), one of the most conspicuous and ecologically important benthic predators in many intertidal marine and estuarine environments around the world. It is native to coasts of Europe and North Africa and has successfully invaded many coastal areas worldwide (Carlton & Cohen 2003). While juvenile crabs remain in the high intertidal zone, with particularly high densities reported from complex biogenic structures like bivalve beds and seagrass meadows (Klein Breteler 1976b, Reise 1985, Thiel & Dernedde 1994, Moksnes 2002), adults tend to perform vertical tidal migrations, foraging in the intertidal during high tide and withdrawing to the subtidal zone during low tide (Crothers 1968, Hunter & Naylor 1993, Warman et al. 1993). Shore crabs are opportunistic feeders, with a preference for molluscan prey (Ropes 1968, Elner 1981, Raffaelli et al. 1989), and are capable of having drastic impacts on the stocks of commercial bivalve species (Ropes 1968, Walton et al. 2002, Murray et al. 2007). They generally forage on

young bivalves up to shell lengths of about 3 cm with a preference for thinner-shelled species (Dare et al. 1983, Mascaró & Seed 2001a, Miron et al. 2005, Pickering & Quijón 2011). Although multiple prey choice experiments indicated that Pacific oysters are less preferred prey of shore crabs (Dare et al. 1983, Mascaró & Seed 2001a), field observations suggest that predation by *C. maenas* might have crucial effects on the survival of juvenile oysters (Walne & Davies 1977, Dare et al. 1983, Ruesink 2007, Kochmann & Crowe 2014).

In the Dutch Wadden Sea, annual sampling in the tidal channels revealed that the shore crab population increased considerably in the last 20 years (Tulp et al. 2012) and is therefore expected to have noticeable impacts on the different bivalve populations. However, little is known of the potential impact of *C. maenas* on epibenthic bivalve populations. Earlier studies assumed a considerable impact on the recruitment of *M. edulis* (McGrorty et al. 1990) and claimed a minor importance on adult mussels (Nehls et al. 1997). These argumentations are, however, purely speculative, in the absence of reliable estimates of the abundance of adult shore crabs on intertidal bivalve beds, due to the lack of an accurate and cost-efficient method to quantify the abundance of adult crabs present at high tide.

Moreover, little is known to what extent *C. maenas* responds to the change in habitat complexity due to the invasion of the Pacific oyster. Earlier studies which investigated the distribution of juvenile crabs in the Wadden Sea during low tide found no clear pattern in habitat preference. While Kochmann et al. (2008) report a preference for pure mussel habitats compared to mixed (mussel/oyster) and pure oyster habitats in young crabs of 5–10 mm CW in autumn and no preference in these crabs in the spring thereafter, Markert et al. (2009) found a much higher abundance of crabs in oyster-dominated areas compared to mussel-rich sites. However, to our knowledge, no previous study focused on adult crabs, which are main bivalve predators, within the structures of the two bivalve species.

In the present study, we quantified the tidal migration of adult *C. maenas* on to bivalve beds differing in the bivalve composition (i.e., mussel dominated, oyster dominated, or balanced). To this end, we sampled crabs at 14 locations spread across the Dutch Wadden by using beam trawls and baited crab traps. We tested two different approaches to derive a quantitative estimate of crab abundance on bivalve beds: (1) beam trawling in subtidal gullies and on bare intertidal flats to assess the number of crabs migrating from the subtidal towards the intertidal and (2) combining crab traps placed on bivalve beds with absolute abundance estimates by beam trawling on bare flats adjacent to the bivalve beds. To investigate the differences in shore crab abundance among the different bivalve beds, we further tested to what extent crab abundance is influenced by prey density (juvenile bivalves) and by the predominance of Pacific oysters.

Our survey addresses the following questions: (1) How can the abundance of mobile *C. maenas* on bivalve beds at high tide be quantified? (2) What is the impact of the composition of the bivalve bed (the predominance of Pacific oysters or the density of bivalve recruits) on baited trap arrays and crab abundance?

Material and Methods

Study area

The Wadden Sea is a shallow sea located in the south-eastern part of the North Sea bordering the coastal mainland of Denmark, Germany, and the Netherlands. It is one of the world's largest coherent systems of intertidal sand and mud flats. The Dutch part of the Wadden Sea comprises an area of about 2500 km^2 and contains coastal waters, intertidal sand- banks, mudflats, shallow subtidal flats, drainage gullies, and deeper inlets and channels. Tidal amplitudes gradually increase from about 1.5 m in the west to 3 m in the east. Up to 5% of the intertidal area is covered by epibenthic bivalve beds (Folmer et al. 2014), of which three different types can be

distinguished: mussel-dominated beds, where oysters are absent or occur only in very low numbers; beds with a balanced proportion of mussels and oysters; and beds where oysters dominate in terms of biomass (van Stralen et al. 2012, chapter 4: Waser et al. 2016a).

Properties of bivalve beds

Overall, 14 locations spread across the Dutch part of the Wadden Sea were investigated in terms of shore crab abundance on bivalve beds (Figure 5.1). The bivalve beds were monitored as part of a long-term investigation focusing on epibenthic bivalves and its potential predators (chapter 4: Waser et al. 2016a). Locations were selected in such way that they varied according to multiple characteristics (Table S5.1): distance to the shore, age (indication for amount of bivalve recruitment), and bivalve composition (ratio between oysters and mussels). Each bivalve bed was surveyed twice a year, in spring and autumn. For this study, we selected surveys performed shortly (up to about 1–2 months) before crabs were sampled at the same locations.

Firstly, the contours of each bed were determined by walking around the bed with a hand-held GPS device following a common definition of a mussel bed (de Vlas et al. 2005). The contours were used to delimit and create a set of multiple random sampling points. All created sample points were visited, and points covered by epibenthic bivalves (mussels/oysters) were further sampled for benthos using a rectangular frame of a 0.0225 m^{-2} (15 × 15 cm) surface. The samples were sieved (1 mm square meshes) in the field and sorted for mussels, oysters, and other bivalve species, which were subsequently counted and sized individually by means of digital callipers to the nearest 0.01 mm. All bivalves smaller than 3 cm were considered as potential prey for shore crabs and were summed to determine the overall bivalve recruit density during spring/early summer for the different locations. It has to be noted that the chosen size threshold of 3 cm for juvenile bivalves is a rough approximation, and for some smaller species (i.e., *Cerastoderma edule*, *Macoma balthica*) also, adult individuals might be included. However, since adult individuals of these species occur in very low numbers, the proportions of adults in the recruit (< 3 cm) densities are negligible.

In order to estimate the ratio between mussel and oyster biomass, the individual shell length (L) of both species was converted into a volumetric length (V), representing biomass, by a fixed dimensionless shape coefficient (δ_M): $V = (\delta_M \times L)^3$. The shape coefficient is a parameter that relates the real length with the structural length in the context of the dynamic energy budget (DEB) theory (Kooijman 2010) and is well established for Pacific oysters (0.175, van der Veer et al. 2006), as well as for mussels (0.297, Saraiva et al. 2011).

Shore crab sampling and estimation of crab abundance

Conventional methods such as visual estimation methods or direct trawling on the bivalve beds were considered unsuitable for this study because of the turbidity of mixed estuarine water resulting in low visibility (e.g., Philippart et al. 2013) and in order to prevent persisting damage to either the habitat, the community, or the sampling gear. Alternatively, we tested two other approaches to quantify the amount of *C. maenas* that use bivalve beds as foraging habitat during high tide: (1) beam trawling in the subtidal during high and low tides and during high tide on bare intertidal flats in order to estimate the number of crabs migrating towards the intertidal and (2) baited trap arrays on bivalve beds in combination with beam trawl sampling along the edges of the beds on the surrounding bare flats.

The shore crab sampling was executed in May/June of the years 2012 and 2013 (Chapter 7: Waser et al. 2016b), except for one location (E002) which was investigated in September 2011 (Table 5.1). For logistical reasons, all sampling activities were performed during daytime. According to the study of Hunter & Naylor (1993), the numbers of migrating crabs do not significantly differ between daytime and nighttime. In general, each location (Figure 5.1) was
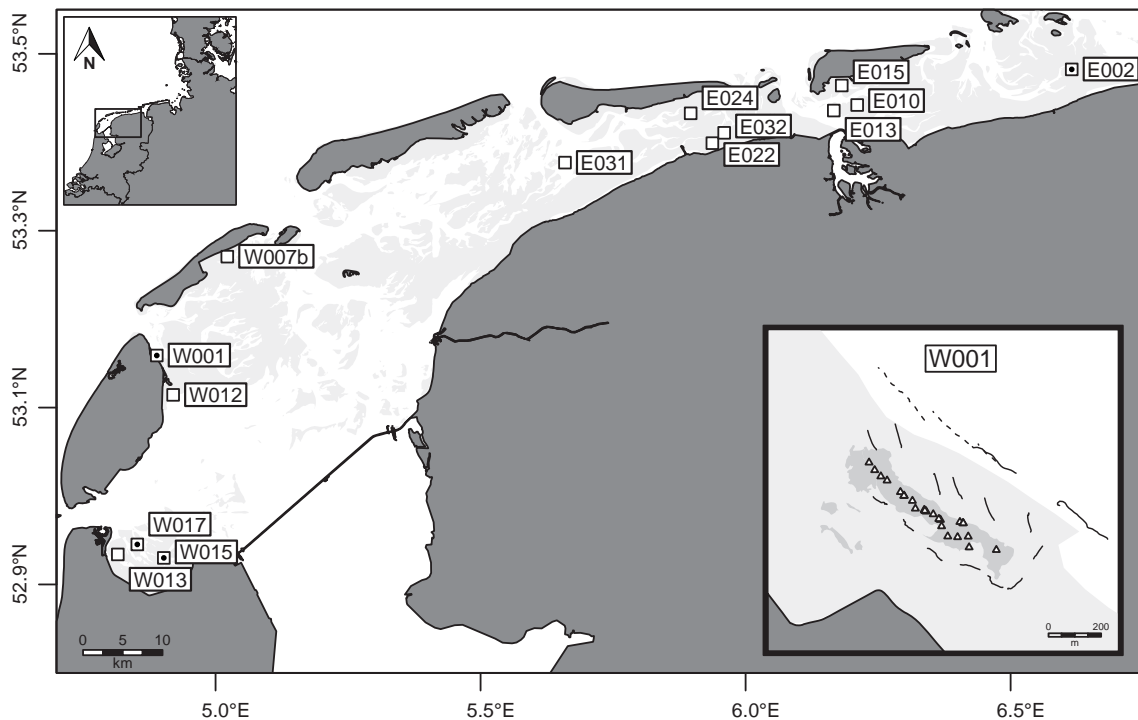
Figure 5.1: Sampling locations (white squares) in the Dutch Wadden Sea. Locations where CPUE of traps was compared between intertidal bare flats and bivalve beds are indicated by black circles inside the white squares (for numbers of samples, see Table 5.1). White areas: subtidal; light grey areas: intertidal flats exposed during low tide; intermediate grey (in inset): bivalve beds; dark grey: land. Inset: specific sampling design of one site. White triangles: positions of traps; lines: hauls taken by beam trawl (dashed lines: hauls at low tide; solid lines: hauls at high tide).

characterized by an intertidal bivalve bed surrounded by intertidal bare mud flats and subtidal areas (Figure S5.1). However, not all locations were suitable for sampling crabs in the subtidal, since gullies or channels which allowed beam trawling by boat were situated too far from the respective beds. Hence, at these locations (E024 and E002), only intertidal sampling was carried out. Further, two bivalve beds (E022 and E032) were located in the vicinity of the same gully, and therefore, the sampling in the gully was used for both locations (Table 5.1). Shore crabs in the subtidal were caught around low and high tides (± 1.5 h). In general, sampling was done for both tidal levels with a 2 m beam trawl (mesh size of 5.5 mm; one tickler chain) towed by a rubber dinghy. In a few cases (9 out of 73 hauls), sampling in the deep subtidal areas (> 5 m water depth) was carried out with a 3 m beam trawl (mesh size of 10 mm; one tickler chain) towed by RV "Navicula" (Table 5.1). Since the study focused on the migrating part of the population and thus the larger individuals, the differences between the different mesh sizes in catching efficiency of the smallest crabs (< 10 mm) could be ignored. Crabs on the intertidal mud flats were collected around high tide (± 1.5 h) by a 2 m beam trawl (mesh size of 5.5 mm; one tickler chain) towed by a rubber dinghy along the edges of the different bivalve beds (Figure 5.1, inset). The depth at high tide on the intertidal flats between the different locations ranged from 0.5 to 1.5 m. The location and exact distance of each haul were assessed using a hand-held GPS receiver. All catches were sorted immediately, and the numbers caught were converted into numbers per hectare (10,000 m^2).

Table 5.1: Overview of the location codes used in Figure 5.1 as well as sampling dates and the number of samples being taken per different sample method. Numbers in parenthesis show the number of hauls taken by 3 m beam trawl. Locations in the western Dutch Wadden Sea are indicated by a 'W' and accordingly, a location in the eastern part of the Dutch Wadden Sea is coded by an 'E'.

Location code	Date	No. of hauls subtidal at low tide (n_L)	No. of hauls subtidal at high tide (n_H)	No. of hauls intertidal at high tide (n_I)	No. of traps bivalve bed at high tide (n_B)	No. of traps: comparison intertidal and bivalve beds [c]
W013	29.5.2012	2	3(2)	10	18	
W017	30.5.2012	3(3)	3(3)	9	18	2 × 15
W015	4.6.2012	3	3	10	17	2 × 9
W001	7.6.2012 / 8.6.2012	3	2(1)	13	30	2 × 10
W012	5.6.2012	3	3	10	18	
W007b	6.6.2012 / 7.6.2012	3	3	7	19	
E031	12.6.2013	3	4	5	16	
E022	11.6.2013	4[a]	4[a]	10	10	
E032	11.6.2013	4[a]	4[a]	7	10	
E024	13.6.2013	NA[b]	NA[b]	9	18	
E013	18.6.2013	4	4	5	8	
E015	19.6.2013	4	4	10	16	
E010	17.6.2013	4	4	10	17	
E002	8.9.2011	NA[b]	NA[b]	9	8	2 × 8

[a] Same adjacent gully
[b] No subtidal sampling; gullies/channels located too far from the respective beds
[c] For dates and details of the arrangements of crab traps, see section "Estimation of shore crab abundance"

The relative abundance of shore crabs on bivalve beds and the surrounding flats was determined by trapping crabs with baited commercial plastic crayfish traps (61 cm long × 31.5 cm wide × 25 cm high; mesh 10 mm × 40 mm) with inverted entry cones at both ends. The traps were scattered across the area during low tide and anchored into the substratum. The traps were baited with several (4–7) frozen juvenile (< 7 cm) herring (*Clupea harengus*), set out overnight, and were emptied after about 18 h (ca. 1.5 high-tide periods). Although this method is limited to catching active, foraging crabs and is biased towards catching larger individuals (Williams & Hill 1982, Miller 1990), the catch per unit effort (CPUE) from traps can provide a proximate estimate of proportional abundance of crabs among different locations.

Immediately after collection, shore crabs were sized according to carapace width (CW, the maximum distance between the two prominent lateral spines) with electronic callipers to the nearest 0.01 mm and assigned to one of three size classes: small (CW < 35 mm), medium (CW 35–50 mm), or big (CW > 50 mm). The classification into size classes was based on (1) the migration behaviour: small shore crabs (< 35 mm CW) are mostly juveniles and burrow on the tidal flats during low tide (Hunter & Naylor 1993) and (2) size preference of mussels: crabs smaller than 50 mm CW hardly prey on mussels bigger than 1 cm in shell length (Elner & Hughes 1978, Smallegange & van der Meer 2003, Chapter 6: Waser et al. 2015). Moreover, it has to be noted that in the Wadden Sea, *C. maenas* typically reaches a maximum size of about 75 mm CW, but specimens larger than 65 mm are scarce (Klein Breteler 1976a, Wolf 1998, Chapter 7: Waser et al. 2016b). Therefore, the majority of crabs in the largest size class were between 50 and 65 mm CW.

Tidal migration as proxy for abundance on bivalve beds

The relationship between the numbers of crabs during high and low tides can be described as $A_S L_S = A_S H_S + A_I H_I + A_B H_B$, where A stands for surface area and L and H for crab abundance, in terms of numbers per surface area, at low tide and at high tide, respectively. The indices S, I, and B refer, respectively, to the subtidal, the bare intertidal, and the bivalve beds. The mean abundance of crabs migrating to the intertidal (M_S) is expressed as the difference in crab abundance in the subtidal between low and high tides: $M_S = L_S - H_S$. Accordingly, the abundance of crabs on bivalve beds at high tide based on tidal migration can be calculated as follows: $H_B = \dfrac{A_S M_S - A_I H_I}{A_B}$.

The surface area of each bivalve bed (A_B) was obtained by determining the bed contours via GPS (see section "Properties of bivalve beds"). The area of the bare intertidal (A_I) and the subtidal (A_S) was obtained by dividing the area encircling the contours of the specific bivalve beds by a distance of 500 m, approximating the suggested distance of tidal crab migrations (Dare & Edwards 1981, Holsman et al. 2006), into subtidal and intertidal sections. The partitioning into sub- and intertidal sections was based on bathymetric data (grid of 20 × 20 m) of the Dutch Wadden Sea provided by Rijkswaterstaat (Dutch Ministry of Infrastructure and Environment; "vaklodingen", http://opendap.deltares.nl) together with information on local tidal amplitude (M2 tidal constituent, about 50% of the tidal amplitude, Duran-Matute et al. 2014). All grid points whose sum of bathymetric data and M2 tidal constituent was below 0 were defined as subtidal and points with a positive sum as intertidal. Adjacent intertidal and subtidal grid points were respectively converted into polygons, allowing us to define the sub- and intertidal area per location (Figure S5.1).

Proportionality between catches of trawls on intertidal flats and traps on bivalve beds

In the second method, abundance of shore crabs on bivalve beds is estimated by relating absolute shore crab abundance on intertidal bare flats in close proximity to the bivalve beds to the relative abundance of crabs on the beds assessed by crab traps (CPUE). To determine the relationship in catches between crab traps on bivalve beds (R_B) and the crab density on the bare intertidal bare flats (H_I) adjacent to bivalve beds, general linear models (GLM) were applied. To normalize the data, abundances were transformed to log (value + 1).

In order to test to what extent the relative crab abundance on bivalve beds (R_B) differs from the relative abundance on intertidal bare flats (R_I), crab traps were deployed simultaneously in these two habitats on four locations: W017, W015, W001, and E002 (Figure 5.1). While locations W001 and E002 were sampled in September 2011 with 10 and 8 traps, respectively, aligned on a straight transect (W001: 400 m; E002 1000 m) per habitat, W015 was sampled in June 2012 and W017 in July 2012. In total, 9 traps per habitat were aligned along a 120 m long transect at location W015, and at W017, 15 traps were randomly scattered at each habitat.

Data analysis

For all analyses, relative and absolute shore crab abundances were subdivided into three classes based on life stage (small juveniles, medium-sized adults, and big-sized adults). Furthermore, the sum of all size classes (total catch) was included in plots.

Differences in relative abundance, the CPUE of crab traps, between bivalve beds (R_B) and intertidal bare flats (R_I) at four different locations, were tested with a MANOVA. Data were log (value + 1) transformed, to normalize the data.

The most trustworthy estimate of crab abundance on bivalve beds (H_B) was used to test the effects of prey density (juvenile bivalves) and occurrence of Pacific oysters on the estimated crab abundance using Spearman's rank correlations. To exclude any variation based on season, location E002 (sampled in autumn 2011) was omitted for these analyses. As the main interest was the comparison among the crab abundance and bivalve bed parameters, locations from both years (2012 and 2013) were included in the analyses, despite the possibility that the difference in sampling year could confound location effects.

All statistical analyses were performed using R v3.2.1 (R Development Core Team 2015). For spatial data handling and production of the map we used the R packages sp (Pebesma & Bivand 2015), rgeos (Bivand & Rundel 2015), rgdal (Bivand et al. 2015), maptools (Bivand & Lewin-Koh 2015) and raster (Hijmans 2015). For plotting, the package ggplot2 (Wickham 2009) was used.

Results

Tidal migration as proxy for abundance on bivalve beds

Considering all individuals of all life stages, the number of crabs found on the intertidal bare flats was generally higher than the number of crabs estimated to migrate from the subtidal towards the intertidal (Figure 5.2). This finding was mainly driven by the small crabs (< 35 mm CW), which were numerous on the intertidal flats during high tide and rare in the subtidal. Hence, the number of crabs smaller than 35 mm CW migrating from the subtidal towards the intertidal was small (Figure 5.2). For the other two size classes (medium and big), the number of crabs migrating from the subtidal towards the intertidal was higher than the number of crabs on bare intertidal flats at about half of the studied locations (Figure 5.2). The fact that in most cases, the number on the intertidal ($A_I H_I$) was higher than the number of migrating crabs ($A_S M_S$) results
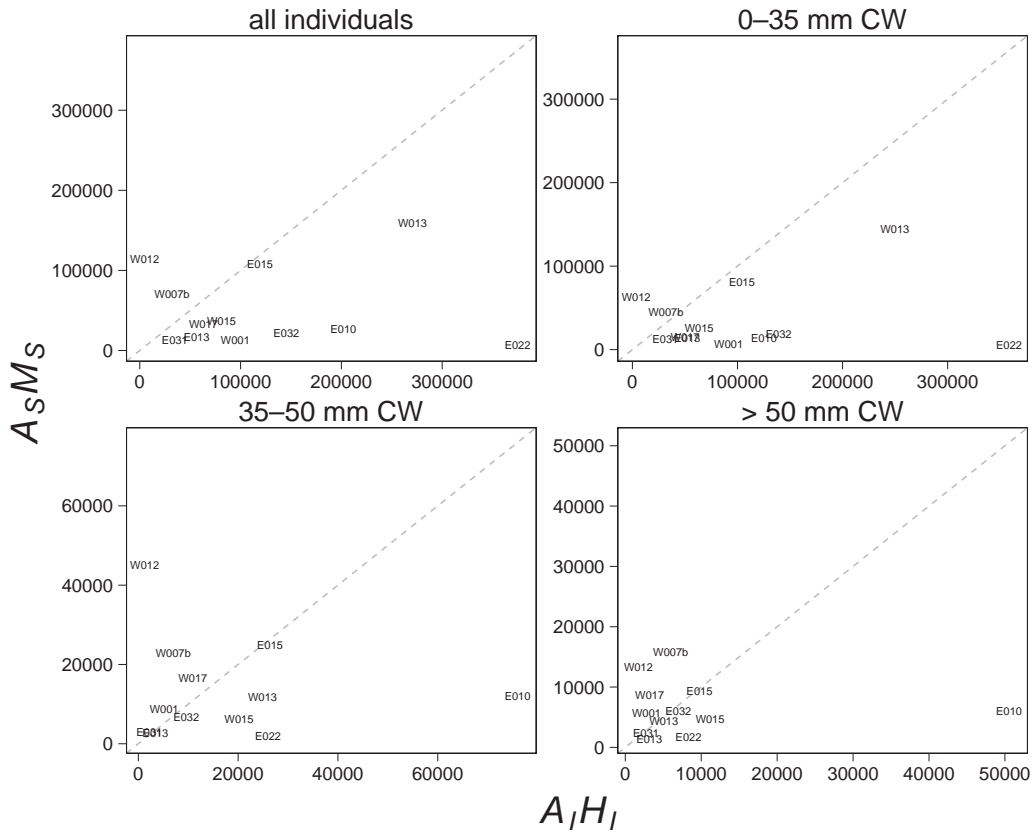
Figure 5.2: Comparison of the mean number of different crab sizes on the intertidal bare flats (A_IH_I) with the estimated number of crabs migrating from the subtidal towards the intertidal (A_SM_S). The grey dashed line represents the $x = y$ line.

in negative estimates of H_B (Figure 5.3). Thus, as this approach tends to predict negative values, it was not used to estimate crab abundance on bivalve beds.

Proportionality between catches of trawls on intertidal flats and traps on bivalve beds

The number of crabs caught on the bivalve beds by crab traps (R_B) showed a clear relationship with the crab density assessed by beam trawling on the intertidal flats (H_I) for medium-sized individuals (35–50 mm CW, GLM: $F_{1,12} = 18.78$, $R^2 = 0.61$, p = < 0.001, Figure 5.4) and for big individuals (> 50 mm CW, GLM: $F_{1,12} = 36$, $R^2 = 0.75$, p = < 0.001, Figure 5.4). The relationships are described by the equations: $y = 1.1 + 1.16x$ for medium crabs and $y = 0.02 + 2.03x$ for big crabs, respectively, where y is the is the log abundance on intertidal bare flats (H_I) and x is the log CPUE on bivalve beds (R_B). For the smallest crabs, the number of crabs caught on the beds showed no correlation with the crabs caught on bare intertidal flats at all (GLM: $F_{1,12}$ = 0.004, $R^2 = 0.0004$, p = 0.948, Figure 5.4). Small crabs were almost absent in the traps on the beds, but found in high numbers on the intertidal flats. Due to the discrepancy in the catch of the small crabs, the total number of crabs caught on the bivalve beds also did not show a correlation with the total crab density on intertidal flats (GLM: $F_{1,12} = 0.564$, $R^2 = 0.045$, p = 0.467, Figure 5.4). Comparisons of catch rates of crab traps on bivalve beds (R_B) and on intertidal bare flats (R_I) indicate that CPUE of the traps per size category (small, medium, and big) did not differ between the two habitats (MANOVA: Wilks' lambda = 0.16, df = 3,1, p = 0.496, Figure 5.5).
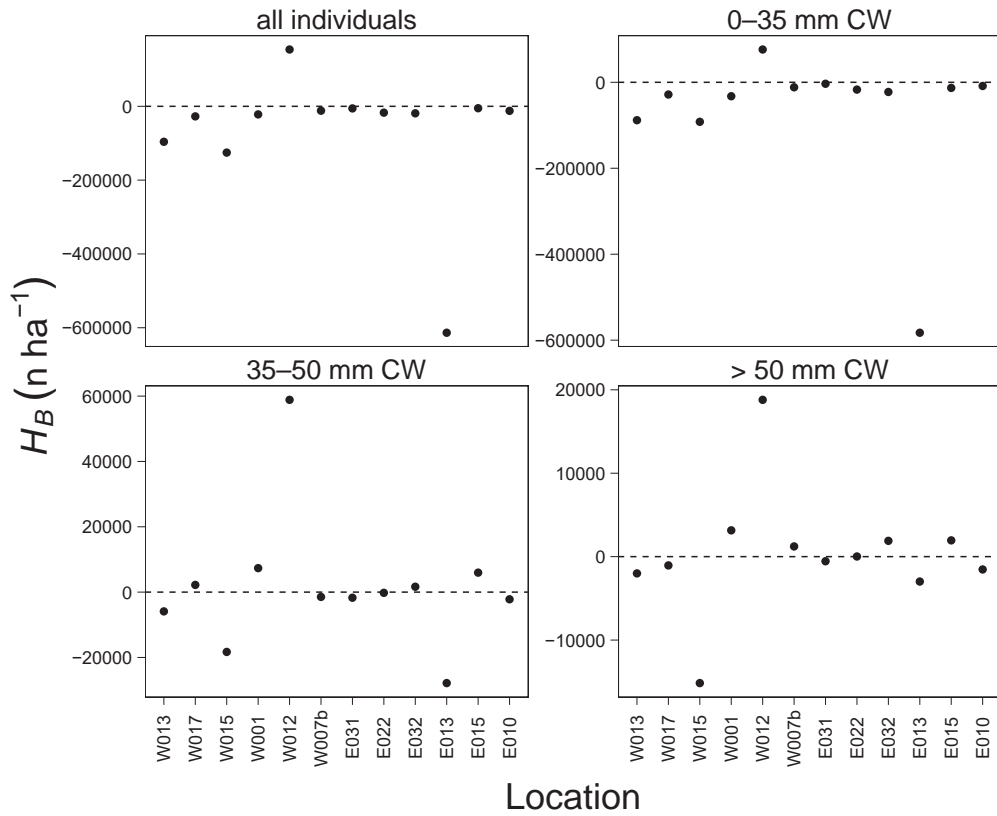
Figure 5.3: Estimated density (n ha^{-1}) of different crab sizes on the investigated bivalve beds (H_B). The horizontal dashed line represents a crab density of 0.
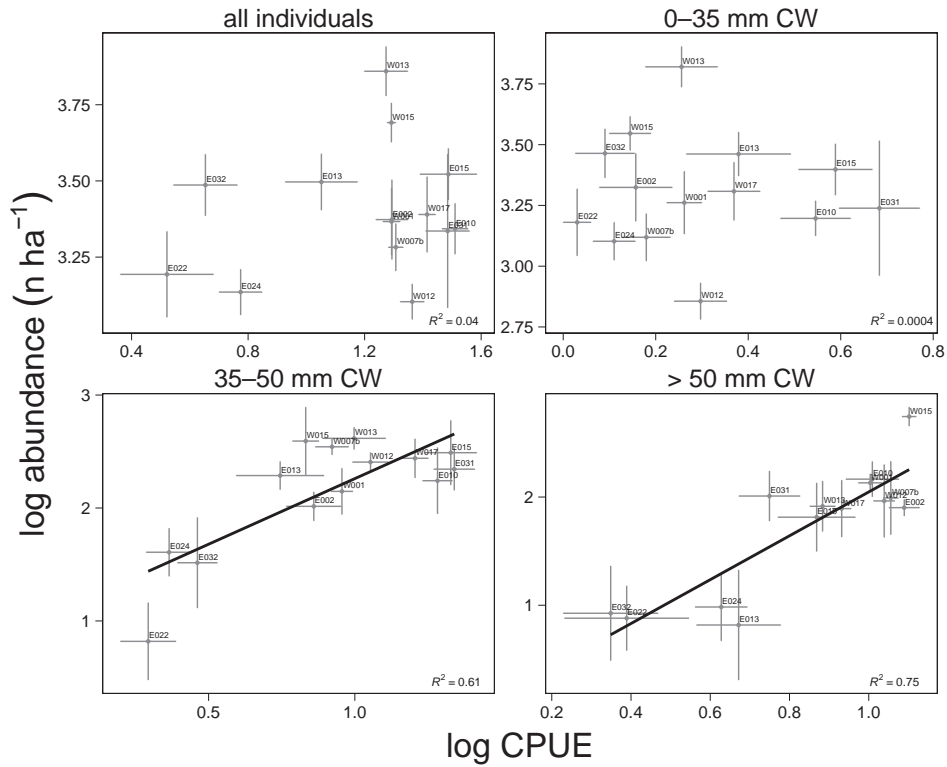
Figure 5.4: Relationship between the number of the different crab sizes caught in the traps (R_B, log CPUE ± SE) on bivalve beds and the density on the intertidal bare flats sampled by beam trawl (H_I, log n ha^{-1} ± SE). The relationships are described by the equations: $y = 1.1 + 1.16x$ for crabs of 35–50 mm CW and $y = 0.02 + 2.03x$ for crabs bigger than 50 mm CW, respectively.
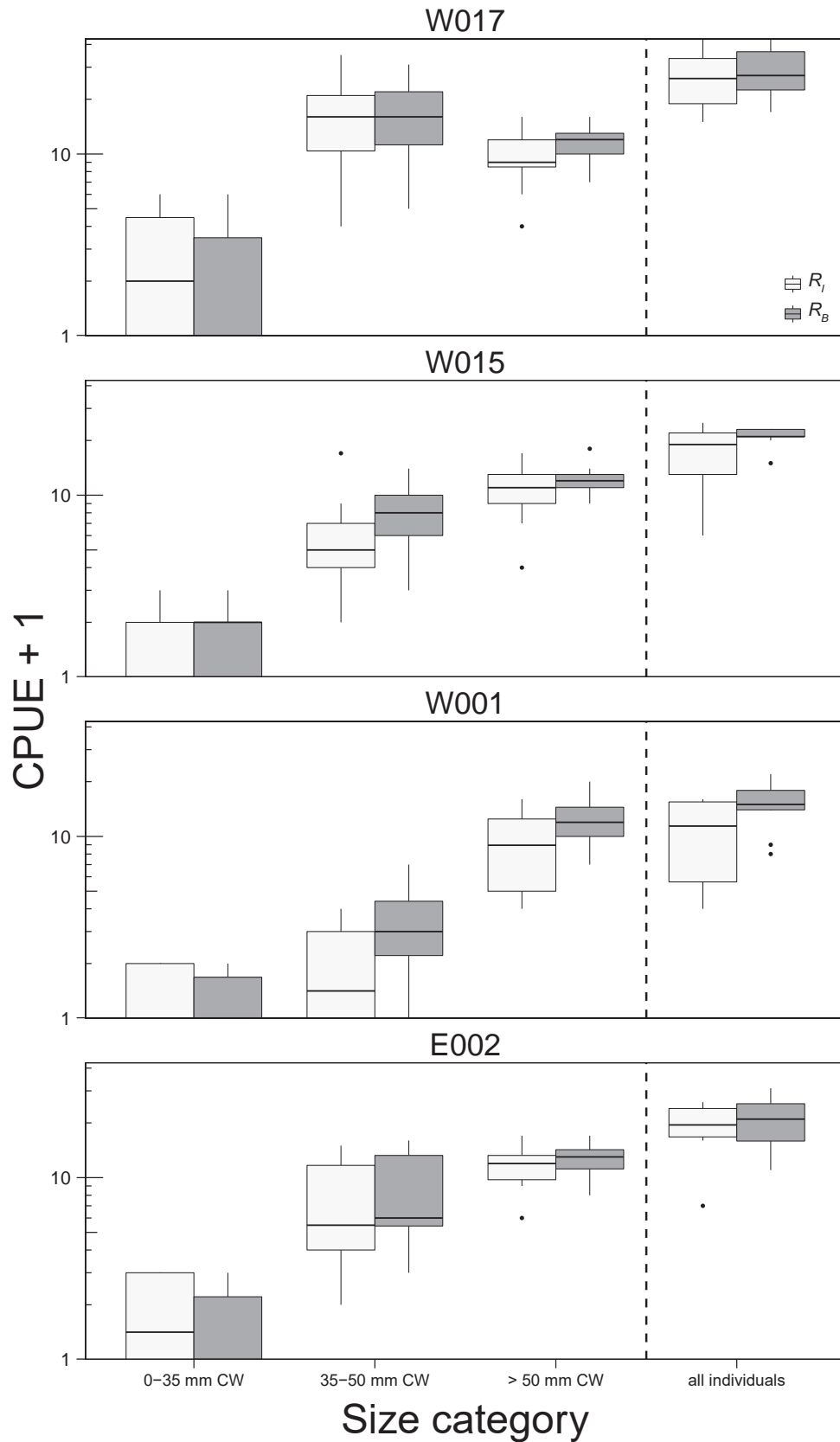
Figure 5.5: Comparison of relative abundance between crabs of different size classes on intertidal bare flats (R_I) and crabs on bivalve beds (R_B) caught with crab traps (CPUE). Box and whisker plots indicate the median (horizontal line inside the box), interquartile range (box), range (whiskers) and outliers (small dots).
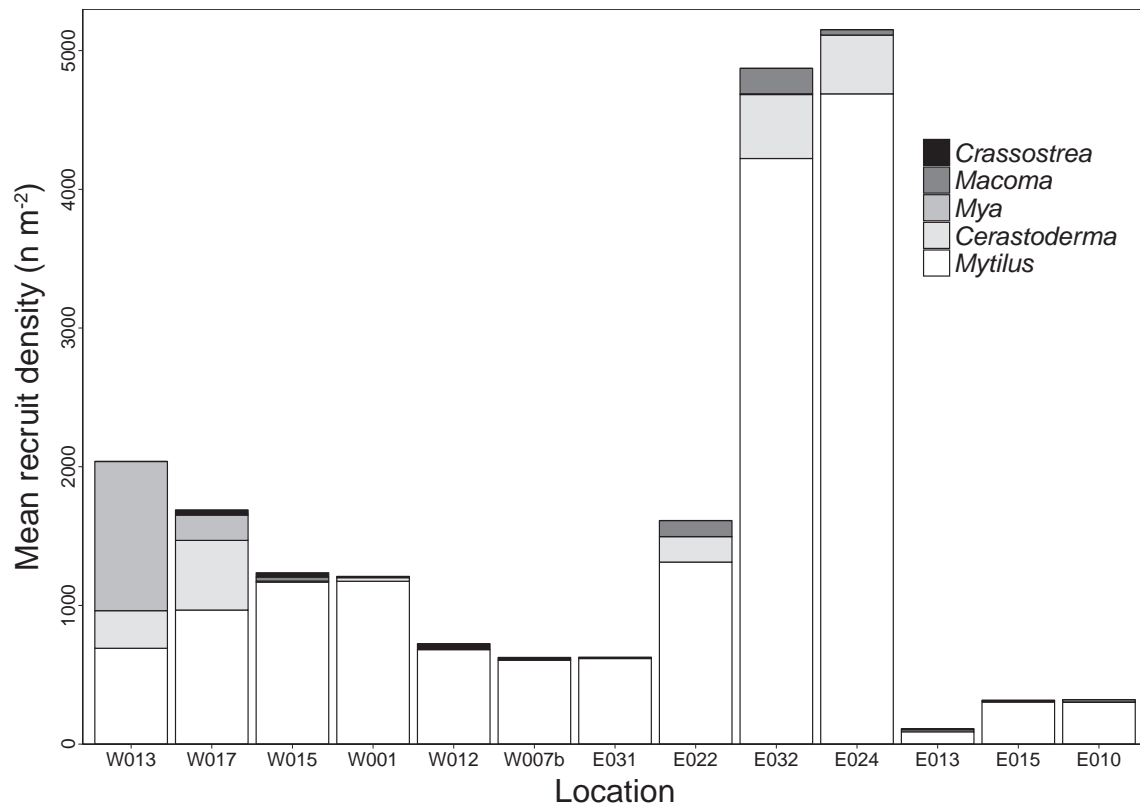
Figure 5.6: Mean density (n m^{-2}) of juveniles (individuals smaller than 3 cm) of the different bivalve species (*Crassostrea gigas, Macoma balthica, Mya arenaria, Cerastoderma edule, Mytilus edulis*) on bivalve-covered patches at 13 different bivalve beds in spring/early summer.
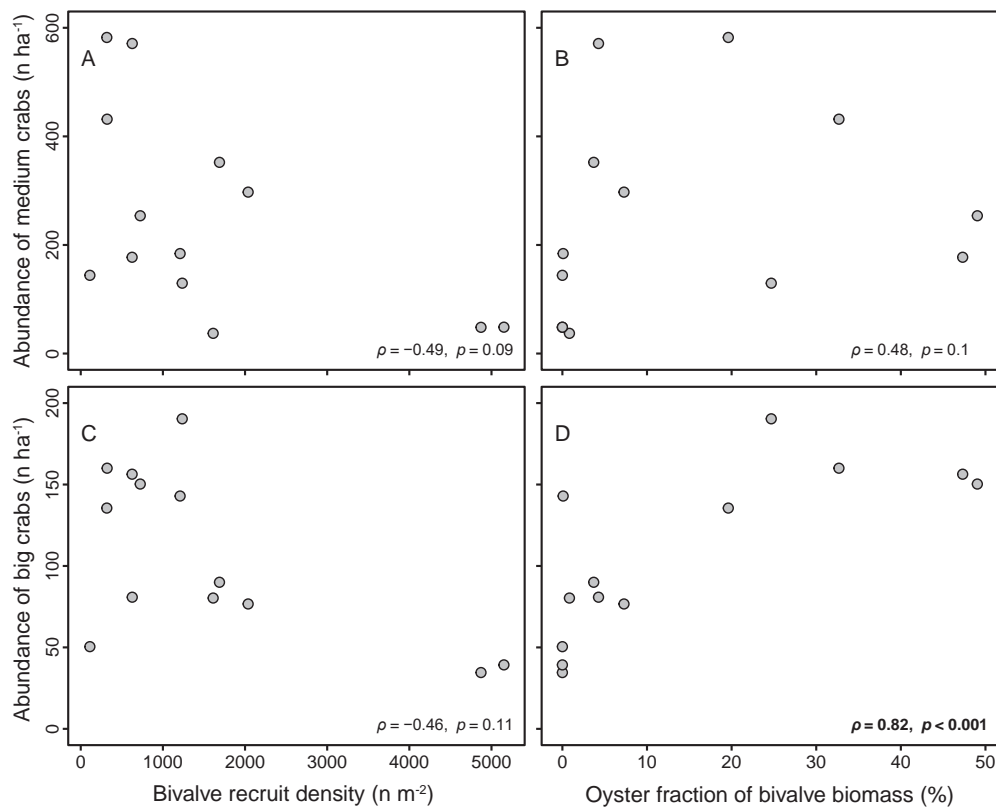
Figure 5.7: Crab abundance (n ha^{-1}) on bivalve beds of A, B medium-sized (35–50 mm CW) and C, D big-sized *Carcinus maenas* (> 50 mm CW) depending on A, C density of juvenile bivalves (< 3 cm) on bivalve covered patches (n m^{-2}) and B, D the fraction of Pacific oysters of the bivalve biomass (%).

Crab abundance in relation to bivalve bed properties

Based on the CPUE data on bivalve beds for the different locations and the linear relationships between relative and absolute crab abundance listed above, we can now estimate the densities of medium (35–50 mm CW) and big (> 50 mm CW) crabs on the beds. We found the abundance on bivalve beds of medium-sized crabs (mean: 251 n ha^{-1}; range: 40–580 n ha^{-1}) to be more than twice as high as the abundance of big crabs (mean: 107 n ha^{-1}; range: 35–190 n ha^{-1}). With these estimates, it is possible to investigate to what extent the density of bivalve recruits and the predominance of the Pacific oyster are related to the crab abundance. Overall, we found recruitment of five different bivalve species on the beds, with juveniles of *M. edulis* being the most abundant (Figure 5.6). Although, *C. gigas* was present on most of the beds, individuals smaller than 3 cm of shell length were only found in very low numbers throughout all locations (Figure 5.6). The bivalve recruit density showed no correlation with the abundance of both crab sizes (medium crabs: Spearman correlation, S = 542, ρ = -0.49, p = 0.09, Figure 5.7A; big crabs: Spearman correlation, S = 532, ρ = -0.46, p = 0.11, Figure 5.7C). While there was no significant effect of Pacific oyster predominance on the abundance of medium crabs (Spearman correlation, S = 191, ρ = 0.48, p = 0.1, Figure 5.7B), the abundance of big *C. maenas* was significantly correlated with the oyster occurrence (Spearman correlation, S = 66, ρ = 0.82, p < 0.001, Figure 5.7D).

Discussion

In this study, we investigated the tidal movements of adult shore crabs over epibenthic bivalve beds. To that extent, we explored the potential of two different methods for estimating the crab abundance on the beds during high tide. The first method, using crab migration as a proxy of abundance on bivalve beds, is based on the assumption that the vast majority of individuals are concentrated in the subtidal during low tide and parts of the population migrate to the intertidal with rising tide (Silva et al. 2014). Accordingly, differences in density between the two tidal levels in the subtidal zone should represent the fraction of individuals migrating to the intertidal and hence yield in an indirect estimate of species abundance for the intertidal at high tide. In our study, the estimated number of crabs emigrating from the subtidal towards the intertidal was in most cases lower than the estimated number of crabs in the intertidal at high tide. This resulted in negative estimates for the abundance on bivalve beds. One of the reasons for these negative abundance estimates is the behaviour of juvenile crabs, which do not show tidal migration behaviour and remain in the high intertidal zone (Crothers 1968, Hunter & Naylor 1993, Warman et al. 1993). However, negative abundances were also observed for adult crabs. A possible explanation for the false estimation of the abundance of adult crabs could be the classification of the intertidal area into subtidal and bare intertidal areas surrounding the bivalve beds, which was based on the distance that shore crabs can cover during tidal migrations.

Very little is known about this migration distance, and for *C. maenas*, only the study of Dare & Edwards (1981) investigated the distance that crabs migrate during a single tide, by suggesting maximum migration distances of about 400m in the Menai Strait (North Wales, UK). Moreover, Holsman et al. (2006) report tidal migration distances of up to 600 m into intertidal flats within a single tide for radio-tagged Dungeness crabs (*Cancer magister*) in Willapa Bay (WA,USA). Further studies are needed to clarify whether these observed crab migration distances can be adopted for *C. maenas* in the Wadden Sea. However, based on the limited knowledge, we have chosen a maximum migration distance of 500 m as radius around the contours of the different bivalve beds to define subtidal and bare intertidal areas. With this chosen radius, most locations possessed a larger intertidal area compared to the subtidal, which resulted in higher values of $A_I H_I$ compared to $A_S M_S$, resulting in negative values for the crab abundance on bivalve beds. With larger migration distances (i.e., 1000 m or more) used as radius around the bivalve beds, the proportion subtidal/intertidal would have increased in favour for the subtidal

at most of the studied locations, which would have resulted in less negative estimates for crab abundance on bivalve beds. Furthermore, the timing of the fishing might be very crucial for detecting migrating crabs. In order to sample crabs at multiple stations, we trawled for up to 3 h (ca. 1.5 h before and after the exact tide level) per bivalve bed location. This time frame may have been too wide, such that crabs may not have yet arrived or have already left the gullies at the time of sampling. In addition, due to logistic reasons, it was not always possible to sample crabs during high tide simultaneously in the subtidal and intertidal, which might also have influenced the results.

In the second method, a combination of baited crab traps and intertidal beam trawling during high tide was used to convert the relative abundance obtained on the beds (R_B) into an absolute estimate (H_B). In general, this method provided trustworthy estimates of crab abundance on bivalve beds, but the outcomes varied with crab size. While for adult crabs (size: medium and big), the numbers of individuals caught on the different beds were correlated to the abundance assessed on the adjacent bare flats, small crabs showed no correlation between the trap and the trawl samples. The strong mismatch in the small crabs resulted from the low catches of the traps on the beds. Yet, the evidence acquired with sampling during low tide indicates that the abundance of small crabs is higher on bivalve beds than on bare sand flats (Klein Breteler 1976b, Thiel & Dernedde 1994, Moksnes 2002), suggesting that our method applied may not be suitable to sample small crabs in this habitat. Generally, catches of crab species in baited traps are biased towards larger individuals (Williams & Hill 1982, Miller 1990). It is possible that the small crabs either avoided entering the traps due to the presence of bigger conspecifics, which are superior competitors (Smallegange & van der Meer 2006, Fletcher & Hardege 2009), or the small crabs might have entered the traps, but escaped before traps were retrieved. In order to detect the exact mechanisms and the behaviour of small crabs in relation to traps, further studies are needed, such as detailed video observations of crabs attracted to traps. However, edited traps, where the entry size was reduced using cable ties, preventing larger crabs to enter, also barely caught any crabs smaller than 35 mm CW (Waser, unpublished data), suggesting that the crabs escaped before trap retrieval. Regardless of the exact mechanisms, the combined use of baited traps and beam trawl is only beneficial for estimating abundances of *C. maenas* larger than 35 mm CW. However, other methods such as sampling with sediment cores at low tide are commonly used for abundance estimates of juvenile crabs on structural complex habitats (e.g., Klein Breteler 1976b). Since these crabs do not migrate between the tides (e.g., Hunter & Naylor 1993), abundances of these juveniles measured at low tide also apply for high tide at the same location.

As both sampling methods were applied in two different habitats, i.e., bare intertidal flats and bivalve beds, it is also of interest to ascertain to what extent trap catches differ between the two habitats. Although we expected considerable higher crab numbers in traps placed on bivalve beds, due to a higher productivity, we found no significant difference in crab catches between traps placed on bivalve beds and intertidal bare flats. This observation might be based on either a reduced catch of traps placed on the beds and on the other hand increased trap catches on bare flats. Possible reasons for reduced catches of traps are that crabs might have stopped entering the traps after a while, either because traps became too crowded (saturation effect; Miller 1990), making it likely to prevent more crabs from entering the traps, or attraction to traps might have been reduced (Miller 1990), since bait fish was devoured by already caught crabs. In contrast, traps might additionally attract crabs through the provision of shelter. It is likely that the effects of shelter provision are more important in habitats of low structural complexity, such as bare intertidal flats. Moreover, it is possible that traps placed on bare flats also attracted and caught some crabs that initially were migrating towards the bivalve beds.

With the combination of traps and beam trawl, we estimated an average abundance of about 360 n ha^{-1} for adult shore crabs (250 and 110 n ha^{-1} for medium and big crabs, respectively) on epibenthic bivalve beds in the Dutch Wadden Sea. This abundance estimate is more or less in agreement with the findings of a study that investigated shore crab abundance at various different habitats in the Northern Wadden Sea (Scherer & Reise 1981). Although Scherer & Reise (1981) did not explicitly sample *C. maenas* on mussel beds, they assumed a crab abundance of about 1500 n ha^{-1} on intertidal mussel beds. The difference in crab abundance between the two studies is mainly based on different size spectra used to derive the estimates of crab abundance. While our study focused on crabs larger than 35 mm CW, Scherer & Reise (1981) also considered smaller-sized crabs with minimum CW of 15 mm.

Shore crabs are opportunistic feeders, with a preference for molluscs (Ropes 1968, Elner 1981, Raffaelli et al. 1989). Furthermore, they are known to primarily feed on the most abundant prey species (Scherer & Reise 1981). On all studied bivalve beds, the species with the highest abundance of individuals vulnerable to crab predation (< 3 cm shell length) was *M. edulis.* Except for the two beds (W013 and W017), small individuals of other bivalves were scarce. Although some beds showed a high density and biomass of the Pacific oyster (Table S5.1), densities of small individuals of *C. gigas* (< 3 cm shell length) were low at all studied locations. That indicates that for the crabs sampled in our study, recruitment stages of *C. gigas* are of minor importance. The estimates of crab abundance on bivalve beds given above allowed us to assess general predation rates on intertidal mussels. Smallegange (2007) investigated the consumption rates of satiated shore crabs feeding on *M. edulis* in laboratory experiments, which indicated that medium crabs consumed on average about three mussels of 18 mm length (CW ~ 35 mm: 2 mussels; CW ~ 45 mm: 4 mussels) and big crabs (CW ~ 55 mm) foraged on about 4.5 mussels within a period of 6 h. For practical reasons, we considered the foraging period of 6 h, used in the experiments of Smallegange (2007), to approximate the inundation time of bivalve beds during a single high tide. Considering that crabs solely forage on mussels, *C. maenas* reaches daily predation rates of about 2500 mussels (medium crabs 750 mussels within 6 h; big crabs 500 mussels/6 h) per 1 ha of bivalve bed. As shore crabs occur on intertidal flats for approximately 180 days a year (May–October), spending cold periods in deeper waters (Naylor 1962, Thiel & Dernedde 1994), annual predation rates of shore crabs amount to 450,000 mussels (270,000 and 180,000 mussels for medium and big crabs, respectively) per 1 ha of bivalve bed.

Furthermore, we expected the abundance of crabs to increase with prey density (juvenile bivalves), but abundances of both medium and big crabs were not significantly positively correlated with the bivalve recruit density. If anything, the correlation was negative. How can we explain the absence or even a negative relationship between crabs and bivalve recruitment? Perhaps, the bivalve recruit densities assessed prior to the shore crab sampling decreased substantially between the two sampling occasions, due to either mortality (predation) or growth, leading to the observed patterns between bivalve recruitment and shore crabs. Moreover, the two beds with the highest density of small bivalves (~ 5000 n m^{-2}) showed very low crab abundances, which also considerably affected the observed relationship between crab abundance and bivalve recruit density. In general, the success of bivalve recruitment is strongly related to predator abundance (e.g., Beukema & Dekker 2014), suggesting that recruit density is particularly high at locations where (crab) predation is low.

Although differences in habitat complexity between oyster- and mussel-dominated beds were not quantified explicitly in the present study, a much higher habitat complexity in oyster-rich beds seems likely, since oysters are multiple times larger than mussels. In terms of crab abundance, earlier studies report mixed results concerning the habitat preferences of juvenile *C. maenas* in oyster and mussel structures (Kochmann et al. 2008, Markert et al. 2009) so that it is difficult to judge whether juvenile crabs show a preference for oyster-dominated bivalve structures. We found that beds with high oyster occurrences favour the abundance of larger crabs, while the abundance of medium-sized crabs seems to be unrelated to the oysters. The

increase in interstitial space, attributed to the increase of oyster dominance, may offer suitable refuges and attract also adult crabs. As big *C. maenas* are superior in competing for resources compared to smaller conspecifics (Smallegange & van der Meer 2006, Fletcher & Hardege 2009), high densities of large crabs would presumably prevent smaller-sized individuals of finding shelter in the interstitial space and might explain why smaller-sized crabs do not occur in high numbers at exactly the same locations. Likewise, previous studies found dominant crab species to be present in high densities in habitats of high complexity whereas species being weaker competitors were found avoiding those areas occupied by dominant crabs (Lohrer et al. 2000, Holsman et al. 2006).

In conclusion, we could show that the combination of baited traps and beam trawling is a suitable method to estimate the abundance of shore crabs larger than 35 mm in CW on epibenthic bivalve beds in soft bottom intertidal systems. The method developed in this study provides one possible solution for future monitoring of shore crab populations on epibenthic bivalve beds. It also offers the possibility to study biotic processes such as predator-prey interactions in these complex structures in more detail. While the focus was the shore crab on intertidal bivalve beds, there are important implications for surveys of other species (e.g., other crab species or demersal fish species) and of other intertidal habitats (e.g., rocky intertidal and intertidal seagrass beds). Different species and different habitats may require an adjusted set of sampling gears to adequately survey the populations in question.

Acknowledgements

Supplementary material

Figure S5.1: Partitioning of the tidal area surrounding the investigated bivalve beds. The tidal area surrounding the beds by a distance of 500 m was divided into sub- and intertidal parts based on bathymetry and local tidal amplitude. Black denotes bivalve beds (A_B) and tidal sections are indicated by dark grey (intertidal; A_I) and light grey (subtidal; A_S).

Table S5.1: Overview of the properties of the investigated bivalve beds including bed age, bed area and distance to shore. Shown are also mean densities and mean biomass of oysters and mussels and the fraction of the total bivalve biomass contributed by Pacific oysters for the period 2011–2013.

Bed	Age[a]	Area (ha)	Distance to shore (km)	Mussel density (n m^{-2})	Oyster density (n m^{-2})	Mussel biomass (kg m^{-2})	Oyster biomass (kg m^{-2})	Fraction of oysters of the biomass (%)
W013	2008	17.8	1.7	1237	73	1.34	0.07	4.73
W017	2008	6.3	5	1381	116	2.15	0.05	2.4
W015	2003	3.9	3.7	1798	533	1.15	0.41	26.43
W001	2009	6.8	0.3	2770	25	2.07	0.02	1.06
W012	2005	2.8	2.1	1444	571	2.26	2.14	48.7
W007b	2003	9.6	0.7	1525	622	2.47	4.13	62.53
E031	2001	11	5	1668	113	2.92	0.19	6.07
E022	2002	34.5	0.8	2049	37	1.57	0.02	1.16
E032	2011	30.1	1.7	4138	1	2.11	0.0002	0.01
E024	2012	70.1	1.5	7080	0	2.54	0	0
E013	2010	1.2	2.5	1207	1	1.1	0.0002	0.02
E015	2001	17.5	0.7	815	220	1.29	0.29	18.15
E010	2006	66.8	3.2	1290	419	1.96	0.65	24.87
E002	2009	3.7	4.1	3505	54	3.96	0.02	0.51

[a] Indicates the year at which beds developed into approximately the same surface area as sampled in 2012 and 2013. Some beds may contain parts that are much older

Box 5.1 Assessing crab abundance during low tide

Juvenile shore crabs (*Carcinus maenas*) are important predators of juvenile bivalves (e.g., Scherer & Reise 1981, Reise 1985). As juvenile crabs find refuge in the complex structures formed by mussels (*Mytilus edulis*) and oysters (*Crassostrea gigas*), crabs occur in high densities at these bivalve structures (Klein Breteler 1976b, Reise 1985, Thiel & Dernedde 1994, Moksnes 2002) and presumably exert high predation pressures on the juvenile mussels (McGrorty et al. 1990). In order to access whether the bivalve composition (indication of habitat complexity, see Chapter 5) affects the abundance of juvenile crabs, the abundance of crabs during low tide was assessed on several epibenthic bivalve beds in spring and autumn of the years 2012 and 2013. At each bivalve bed about 15–30 samples per sampling date, randomly distributed throughout the bed contours, were taken with a rectangular frame of 15 × 15 cm on bivalve covered patches. All parts of bivalve shells (dead and alive) and other organisms within the sample frame were removed and sieved (1 mm square meshes). Thereafter, samples were sorted and crabs were identified to taxonomic level, counted, and sized according to carapace width (CW).

Crabs occurring on intertidal bivalve beds in the Wadden Sea

Next to *C. maenas*, two invasive crabs, the Asian shore crab (*Hemigrapsus sanguineus*) and the brush-clawed shore crab (*Hemigrapsus takanoi*), can be found within the structures of intertidal bivalve beds in the Wadden Sea. Both *Hemigrapsus* species are native to the northwestern Pacific (Epifanio 2013, Markert et al. 2014) and nowadays have successfully invaded many European coasts, ranging from the Bay of Biscay to the North Sea (Dauvin et al. 2009, Markert et al. 2014). *Hemigrapsus* spp. were first discovered in the Dutch Wadden Sea in 2004 (*H. sanguineus*) and 2006 (*H. takanoi*), respectively (Gittenberger et al. 2010). They largely occupy the same habitats as native *C. maenas* (see also Chapter 8: Goedknegt et al. 2017).

Size distributions of crabs found on intertidal bivalve beds

Except of a few individuals of *C. maenas* that had a CW of up to 61 mm, the majority of the crabs on bivalve beds during low tide were well below 20 mm in CW (Table B5.1). For *C. maenas* sizes below 20 mm in CW correspond to juvenile stages only (Chapter 5). As *Hemigrapsus* spp. are smaller than *C. maenas* (maximum size: ~30 mm CW; Dauvin 2009, Landschoff et al. 2013, Gothland et al. 2013; 2014, van den Brink & Hutting 2017) and reach maturity at sizes of 10–15 mm CW (Noél et al. 1997, Dauvin 2009, Gothland et al. 2013; 2014), both juvenile and adult life stages of these crabs were equally present on the beds during low tide.

Low tide crab abundances on intertidal bivalve beds

On all beds, we found *C. maenas* to be the dominant crab species with an average density of 60 and 165 m^{-2} in spring and autumn, respectively. In contrast, *Hemigrapsus* spp. were found in average densities of about 25 m^{-2} (Figure B5.1).

The abundance of *C. maenas* and *Hemigrapsus* spp. did not seem to be related to habitat complexity caused by differing bivalve compositions (i.e., mussel dominated, oyster dominated or balanced). Other studies in the Waddden Sea that sampled crabs (particularly juvenile *C. maenas*) during low tide at different bivalve structures, also reported mixed results concerning habitat preferences of shore crabs (Kochmann et al. 2008, Markert et al. 2009).

Box 5.1

Table B5.1: Overview of the crab sizes sampled during low tide for spring and autumn in 2012 and 2013. Given are mean values ± SD and the maximum observation of carapace width (mm) for *Carcinus maenas* and *Hemigrapsus* spp. (*H. sanguineus* and *H. takanoi*). Cases where no crabs were found are denoted by n/a.

| | Spring | | | | Autumn | | | |
| | *Carcinus maenas* | | *Hemigrapsus* spp. | | *Carcinus maenas* | | *Hemigrapsus* spp. | |
Bed	mean	max	mean	max	mean	max	mean	max
W013	7.3 ± 5.9	60.7	n/a	n/a	6.6 ± 2.5	13.8	8.5 ± 2.9	14.0
W017	6.0 ± 1.9	12.2	5.4 ± 3.0	13.5	8.6 ± 2.8	17.5	7.1 ± 4.9	17.0
W015	6.8 ± 2.1	11.8	8.4 ± 3.7	13.3	8.4 ± 3.1	21.1	10.8 ± 5.2	16.5
W001_A0	7.8 ± 2.6	17.2	15.6 ± 4.9	22.2	9.6 ± 3.5	18.4	6.3 ± 1.1	7.1
W001_A1	8.9 ± 2.6	13.9	7.3 ± 3.0	13.5	7.9 ± 2.4	14.5	5.4 ± 2.3	10.0
W012	11.3 ± 7.1	43.4	13.0 ± 6.7	31.7	9.6 ± 5.5	40.3	8.5 ± 5.3	22.9
E031	6.6 ± 2.5	15.0	6.5 ± 3.1	13.9	6.7 ± 2.6	18.2	8.1 ± 5.1	17.1
E027	8.2 ± 7.0	43.8	5.5 ± 3.6	14.2	8.4 ± 3.4	23.2	4.9 ± 3.2	13.1
E022	13.3 ± 10.8	37.9	5.0 ± 2.0	8.2	5.6 ± 1.5	7.6	n/a	n/a
E032	4.3 ± 0.9	5.6	3.4 ± 0.1	3.4	6.3 ± 2.1	11.9	2.9	2.9
E024	4.2 ± 1.2	8.7	3.4 ± 0.8	5.0	6.0 ± 2.2	12.3	3.5 ± 0.8	4.6
E013	6.2	6.2	5.0 ± 1.6	10.1	6.0 ± 3.2	18.6	4.1 ± 2.0	10.6
E015	7.7 ± 2.9	18.3	5.9 ± 2.9	13.3	8.3 ± 3.8	29.2	6.7 ± 4.2	15.9
E010	7.4 ± 2.6	12.3	6.1 ± 2.6	14.4	9.1 ± 6.5	50.9	4.8 ± 3.1	14.1
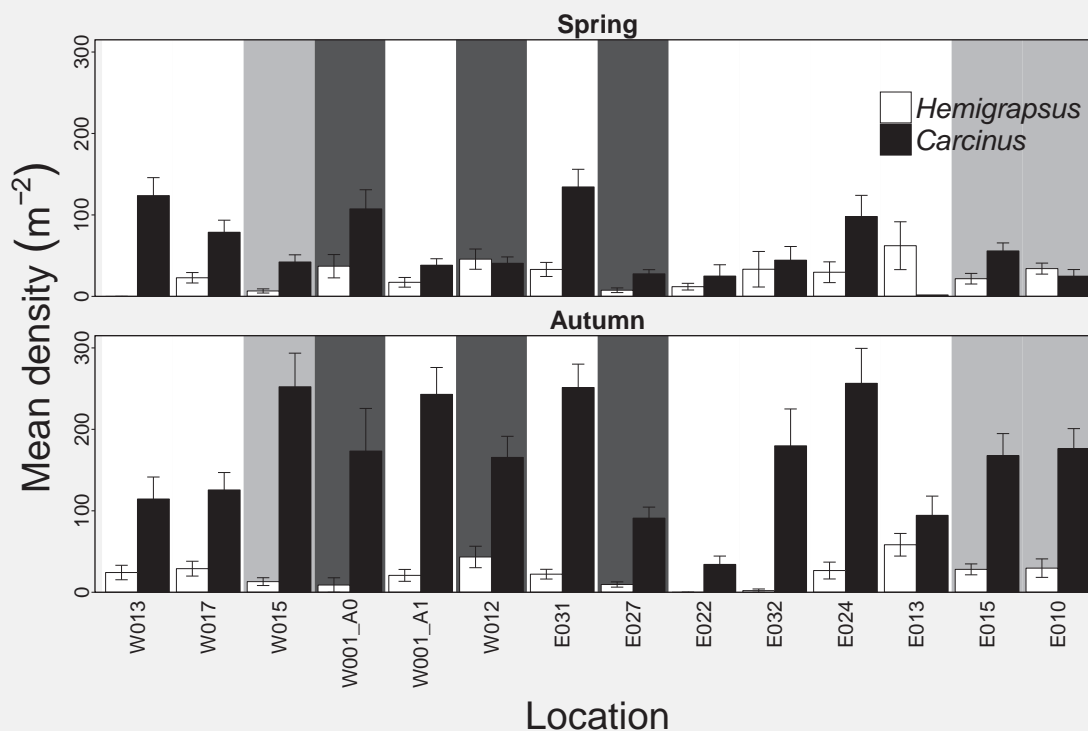
Figure B5.1: Average abundances (m^{-2}) ± SE of the native *Carcinus maenas* and invasive *Hemigrapsus* spp. (*H. sanguineus* and *H. takanoi*) sampled at low tide on several epibentic bivalve beds throughout the Dutch Wadden Sea in spring and autumn of the years 2012 and 2013. The type of bivalve bed is indicated by the background colour (white: mussel; light grey: balanced; dark grey: oyster). For maps showing the different sampling locations see Figures 4.1 and 5.1.

Box 5.2   Seasonal occurrence of *Carcinus maenas*

The estimates of shore crab abundances on bivalve beds (Chapter 5: Waser et al. 2018), that were used to estimate predation pressure on intertidal mussels, were based on non-repeated sampling activities in early summer on the respective bivalve beds. In order to more accurately assess predation rates of *C. maenas* throughout the year, detailed information on the seasonal occurrence of this species on intertidal mussel beds is needed. Shore crabs are known to leave the shallow intertidal, migrating into deeper waters, during autumn when water temperatures decrease and return with increasing water temperatures in spring (Naylor 1962, Thiel & Dernedde 1994). In order to study the phenology of *C. maenas* on bivalve beds in detail, crabs were repeatedly sampled on the bivalve bed W001_A1 at the northern tip of Texel in the period 2011–2013. As these observations were made at only one location within a relatively limited time period, additional information on shore crab phenology in the Western Dutch Wadden Sea originating from two NIOZ long-term monitoring programmes (kom-fyke programme and high-water sampling programme) was also considered.
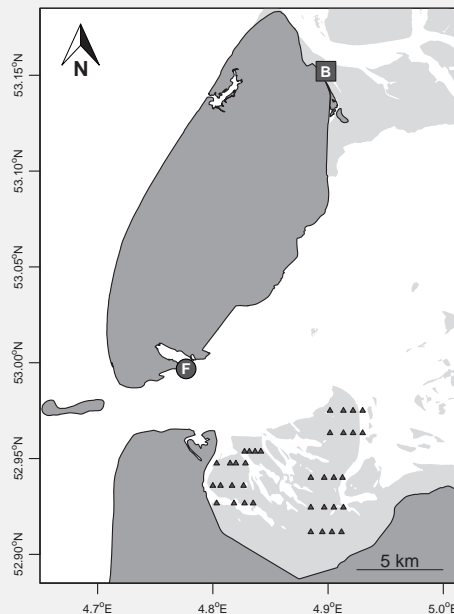
Figure B5.2: Sampling locations of *Carcinus maenas* in the western Dutch Wadden Sea. Grey square (B): bivalve bed W001_A1; grey circle (F): NIOZ kom-fyke; grey triangles: high-water sampling stations.

Methods

Occurrence on bivalve beds

Shore crabs on the bivalve bed W001_A1 at de Cocksdorp (Figure B5.2) were repeatedly sampled in 2011–2013 with baited crayfish traps (see Chapter 5 and Chapter 7 for a detailed method description). In these three years, a total of 238 traps were employed between March and September. The traps were scattered across the bed during low tide, baited with frozen juvenile (< 7 cm) herring (*Clupea harengus*), and anchored into the substrate. After about 18–24 h, traps were emptied and crabs were counted, sized according to carapace width (CW), and assigned to one of three size classes: small (CW: < 35 mm), intermediate (CW: 35–50 mm) or big (CW: > 50 mm). Note that this method is less suitable for catching small crabs (< 35 mm CW; Chapter 5). As a result, small crabs were only caught infrequently in the course of the investigation and catches of this size class were not considered for an analyses of seasonal crab occurrence.

Box 5.2

Temporal occurrence on intertidal flats

Crabs at Balgzand, a 50 km^2 tidal-flat area in the western Dutch Wadden Sea (Figure B5.2), were monitored as part of the NIOZ Balgzand high-water programme (e.g., van der Veer et al. 2010), which has been initiated in the mid-1970s. Sampling generally started in February–April and continued at frequent intervals (2 to 4 weeks) until about October–November. A total of 15 years (ranging from 1976 to 2014) was considered for the present investigation of seasonal crab occurrence. In total, 36 stations (9 transects each with 4 fixed stations) on the intertidal flats (Figure B5.2) were sampled during daytime around high tide (± 1.5 h) with a 1.9 m beam trawl (one tickler chain; mesh size 5 × 5 mm) towed by a rubber dinghy. Haul lengths were assessed by means of a meter wheel equipped to the trawl. The catch was sorted and crabs were sized according to CW and assigned to either of the three size classes: small, intermediate or big. Numbers of crabs of the different size classes were counted and crab densities at the different sample stations were assessed. Subsequently, the arithmetic means of all 36 stations sampled during a survey were calculated for the three size classes as well as for the total crab abundance.

Annual differences in shore crab phenology

Since 1960, the abundance of fish and epibenthic macroinvertebrates is monitored daily by means of a passive fishing kom-fyke located at the southern tip of Texel, the westernmost island of the Wadden Sea (Figure B5.2). This trap is a combination of a pound net and a fyke with a 200 m leader running from above high water into the subtidal where two end chambers catch and retain fish and other epibenthic species. The stretched mesh-size of both the leader and the two chambers is 20 mm (see van der Veer et al. 1992, van der Meer et al. 1995, for more details). Apart from winter and summer, where fishing paused in order to avoid possible damage by ice floes or clogging by drifting material (e.g., macroalgae, jellyfish), the kom-fyke was usually emptied daily in spring (March–June) and autumn (September–November). In a few cases (e.g., due to low animal abundance), the fyke was emptied irregularly and fishing periods may surpassed a period of 24 h. For the present analysis covering the years 1960–2015, only catches with a maximum fishing period of 48 h were considered, since longer periods may have resulted in losses due to decay or consumption. Catches were sorted immediately, identified to species level and individuals of each species were counted. Note that sizes of shore crabs are not measured in this monitoring programme and therefore, no discriminations are made between juvenile and adult specimen.

Data analysis

In order to obtain seasonal trends of shore crabs throughout the year, sinusoidal functions were applied to the different datasets used. The overall function was $y = a + b \times \sin((x-c)/365 \times 2\Pi)$, in which a, b, and c are parameters for the average, the amplitude and the reference day where the number equals the average, y is the predicted number/abundance of crabs and x is the Julian day, ranging from 1 to 365. On the bivalve bed W001_A1, numbers of the crabs caught with baited traps were low and thus resulting in the sinus function predicting negative values. All of these negatively predicted values were set to 0. All sinusoidal functions were fitted using the R platform (R Development Core Team 2015), with parameters estimated using the Levenberg-Marquardt algorithm implemented in the function nlsLM from the R package minpack.lm (Elzhov et al. 2015).
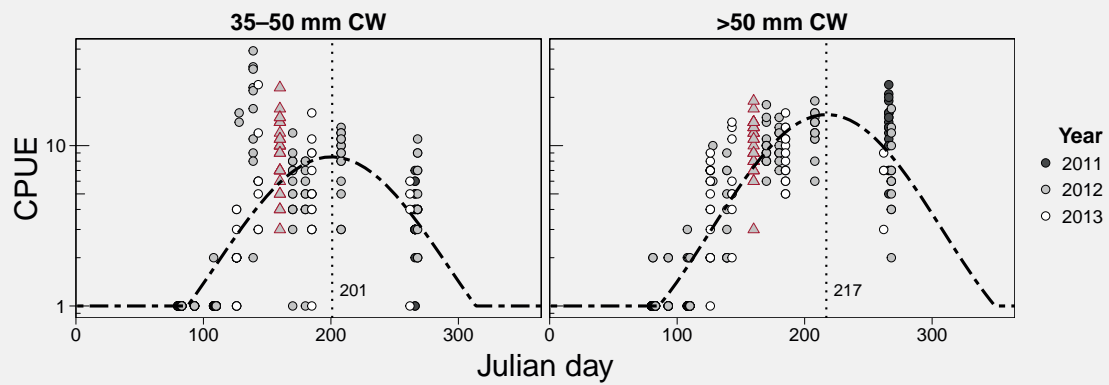
Figure B5.3: Seasonal patterns of *Carciuns maenas* caught with baited traps (CPUE; on a log-scale) on the bivalve bed W001_A1 for the period 2011–2013. Note that all values were increased by 1. Black dashed lines represent the seasonal trends of crabs caught on the bivalve bed. The dotted vertical line together with the number inside the plot indicate the Julian day at which the seasonal trend peaks. Red framed triangles indicate catches that were used to calculate crab abundance on the bivalve bed in June 2012 (see Chapter 5).

Figure B5.4: Seasonal average abundances (ha^{-1}; on a log-scale) of *Carcinus maenas* at intertidal flats of Balgzand during high tide for the period 1976–2014. Note that all values were increased by 1. Black dashed lines represent the seasonal trends of crabs on the intertidal flats. The dotted vertical line together with the day-number inside the plot indicate the Julian day with the seasonal trend peak.

Box 5.2

Results and Discussion

In combining observations of shore crab abundance from multiple years, a general pattern of seasonal occurrence emerged: crab numbers increased around April, peaked between end of July and beginning of August and decreased about in October. This seasonal pattern corroborates earlier observations (e.g., Naylor 1962, Thiel & Dernedde 1994) and was consistent for all investigated life stages of *C. maenas* on the bivalve bed (Figure B5.3) as well as on intertidal flats at Balgzand (Figure B5.4). However, the 56–year time series from the NIOZ fyke revealed that this general seasonal pattern may not apply to the phenology of shore crabs in each and every year. In fact, the phenology of *C. maenas* is highly variable and, apart from the seasonal pattern described above, may show constant population sizes of shore crabs with only slight seasonal differences or drastic seasonal differences with crab numbers peaking either early or late in the course of the year (Figure B5.5). This variability in shore crab phenology between the years might further explain the remarkably high numbers of intermediate sized crabs (35–50 mm CW) caught on the bivalve bed during spring and early summer (Figure B5.3). Fyke catches of 2012–2013 indeed suggest a high presence of *C. maenas* in the Wadden Sea already during spring. However, the fact that we did not observe high numbers of large crabs on the bivalve bed during spring may point to other factors being responsible for the high catches of crabs 35–50 mm CW early in the year. Moreover, the lower abundance of intermediate crabs compared to large ones observed on the bed (Figure B5.3) contradicts the general picture derived from crabs sampled by beam trawl at the intertidal flats during high tide (Figure B5.4).

An alternative explanation for high numbers of intermediate crabs caught with traps particularly in spring might be that catches of intermediate crabs are influenced by the abundance of large specimen (> 50 mm CW). The catches of crabs on several bivalve beds described in Chapter 5 suggest that high numbers of big crabs caught with the traps may hamper smaller specimen from entering the traps. Likewise, the occurrence of large crabs may also influence trap catches of intermediate crabs in the course of the season, resulting in reduced catches from about June onwards when numerous large crabs are present on the bed. The avoidance of traps occupied with many large crabs would then also be responsible for a reduction in amplitude of the seasonal trend.

In conclusion, crabs in the Wadden Sea in general and specifically on bivalve beds show a distinct seasonal pattern with numbers commonly peaking between end of July and beginning of August. However, the baited traps used in the study seem only to be suitable for sampling crabs of a carapace width of 50 mm and larger. Smaller crabs are likely to be underrepresented in the catches in periods when big crabs are abundant.

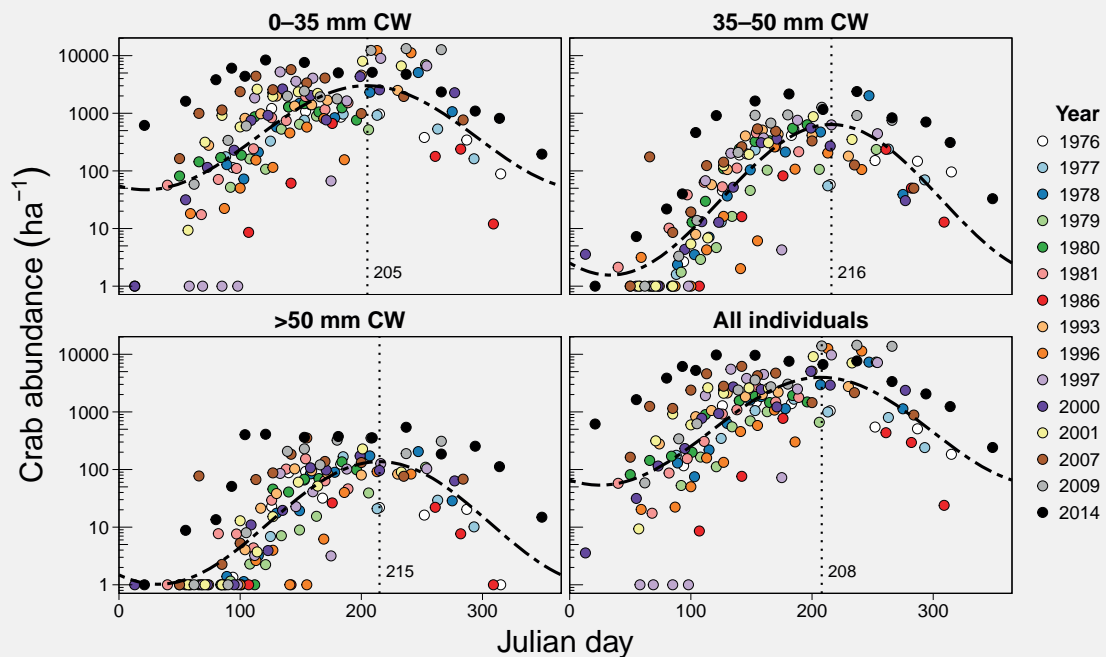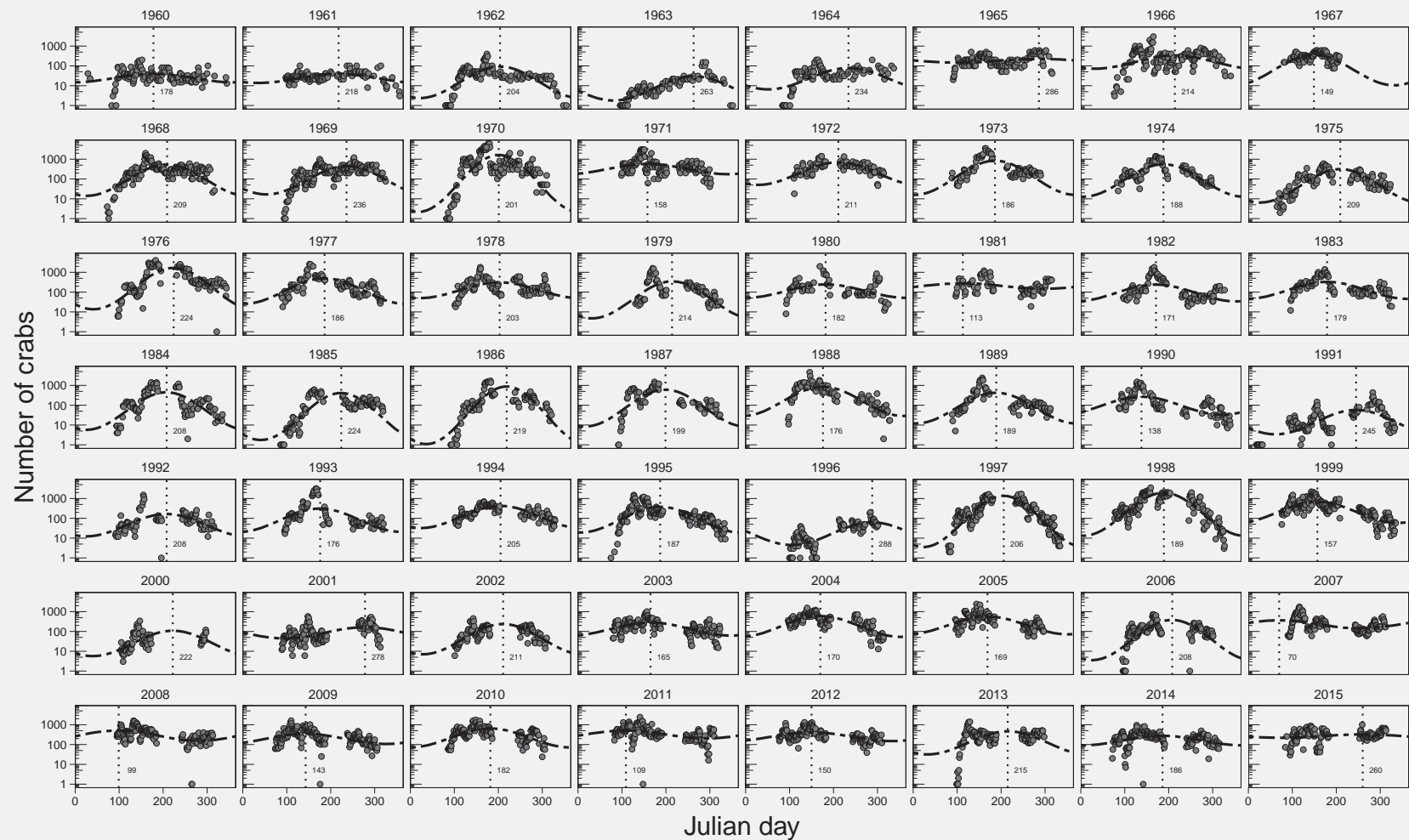**Figure B5.5:** Seasonal patterns of *Carciuns maenas* (log-scale) caught in the Nioz fyke for the period 1960–2015. Note that all values were increased by 1. Black dashed lines represent the seasonal trends of caught crabs. The dotted vertical line together with the number inside the plot indicate the Julian day with the seasonal trend peak.

6 | Indirect effects of invasive species affecting the population structure of an ecosystem engineer

Andreas M. Waser, Wouter Splinter and Jaap van der Meer

Abstract

Species invasion is of increasing concern as non-native species often have negative impacts on ecosystems that they were introduced to. Invaders negatively affect the abundance of native species due to direct interactions like predation and competition. Additionally, invaders may benefit native biota by imposing indirect effects on resident species interactions. Invaders indirectly affect resident species via both density-mediated indirect interactions (DMIIs) and trait-mediated indirect interactions (TMIIs). Previous studies on these different indirect interactions have largely examined the effects on structuring ecological systems, with paying little attention to the role of body size. Here, we experimentally demonstrate that an invasive habitat modifier of European coastal waters, the Pacific oyster (*Crassostrea gigas*), alters the population structure of native mussels (*Mytilus edulis*) by modifying the size specific predator-prey interaction between the mussels and the shore crab (*Carcinus maenas*). In laboratory split-plot experiments, the presence of Pacific oysters reduced the mortality of unconditioned mussels as well as mussels that were acclimatized in presence of predatory cues, while being exposed to predation by crabs of two different size classes. The reduction in mortality was size-dependent both in terms of the predators and the prey. The presence of oysters notably reduced mussel mortality in presence of small crabs, while the mortality rate in presence of big crabs was less affected. Mussels that benefited the most by the presence of oysters were those of recruitment stages, smaller than 20 mm in shell length. Our results suggest that oysters cause a strong shift in the population structure of *M. edulis*, reducing particularly the mortality of smaller sized mussels.

Introduction

The invasion by non-indigenous species is an increasing ecological, conservational and economic problem in ecosystems around the world (Vitousek et al. 1996, Wilcove et al. 1998, Mack et al. 2000, Olden et al. 2004). The introduction of non-indigenous species may affect entire species communities and ecosystems by imposing new species interactions and altering existing ones (Sax et al. 2005). In the past, most studies on species invasions focused on various negative effects on ecosystems, which all potentially result in a reduction of species diversity. These include: outcompeting of native species, hybridization with native species, increased transmission of pests and diseases and drastic changes in the new habitats and environments (Vitousek et al. 1996, Ruiz et al. 1997, Mack et al. 2000, Crooks 2002, Grosholz 2002). However, the effects of non-native species are not always negative. For example, ecosystems that suffered high (human-based) disturbance in the past, may benefit from the arrival of newcomers that fill vacant ecological niches (Donlan et al. 2006, Griffiths et al. 2010). Moreover, native species may profit from a new partner in trophical or non-trophical species interactions. Thus, invaders may function as additional food source (Bulleri et al. 2006, Carlsson et al. 2009) or may provide protection to native biota (Wonham et al. 2005, Severns & Warren 2008). In addition, there may be more subtle effects caused by indirect interactions between invaders and native species.

Indirect interactions occur when one species influences a second via its interactions with a third species. These interactions can affect ecological communities either by altering densities due to direct consumption or competition (density-mediated indirect interactions (DMIIs)) or by changing behavioural, morphological or physiological traits (trait-mediated indirect interactions (TMIIs)) (Werner & Peacor 2003, Schmitz et al. 2004, Preisser et al. 2005). Research on DMIIs and TMIIs has focused on top-down trophic pathways where interaction cascades are initiated by predators, transmitted by consumers, and received by lower trophic levels, usually primary producers. However, indirect interactions are not limited to top-down trophic pathways. In theory, any organism at any taxonomic level can act as an initiator, a transmitter, or a receiver species. For example, habitat modifying species cause DMIIs by increasing habitat complexity which provides refuge for other species and may influence predator-prey encounter rates (Grabowski 2004, Pearson 2010). Further, habitat modifiers may also impose TMIIs on predator-prey interactions that result in changes of morphological and behavioural traits which may affect predation rates (Griffen & Byers 2006, Byers et al. 2010, Pearson 2010).

Most studies dealing with TMIIs implicitly assumed that all individuals within a population display trait changes of the same order of magnitude. However, a few studies showed that strength of TMIIs within populations varies according to individual behaviour (Griffen et al. 2012) and body size (Rudolf 2006; 2008; 2012, Preisser & Orrock 2012, Krenek & Rudolf 2014). Body size often represents the state of ontogenetic development of individuals (de Roos & Persson 2013) and is affected by changes in resource and habitat use. These changes are referred to as ontogenetic niche shifts (Werner & Gilliam 1984) and may cause alterations of the intensity of species interactions. Hence, TMIIs occurring within size-structured populations may strongly vary with the size of individuals in both predator and habitat modifying species. Ultimately, variations in trait changes between different states of ontogenetic development within a population may result in shifts in population structure. This may also affect the interactions with other species.

Study System

The Pacific oyster *Crassostrea gigas*, an invasive habitat-modifier, originates from marine waters of Japan and Southeast Asia and nowadays has successfully invaded all temperate coastal ecosystems around the world (Ruesink et al. 2005). In Europe, oysters occupy the same habitats as native blue mussels *Mytilus edulis*. On intertidal soft bottom habitats as the Wadden Sea, *M.*
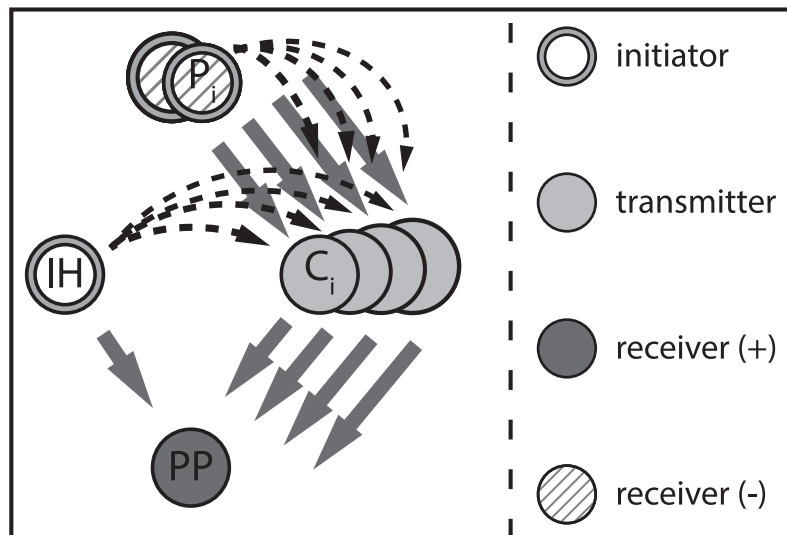
Figure 6.1: The indirect interaction pathways within a simplified food web composed of four species: phytoplankton, a primary producer (PP), the blue mussel, a consumer species (C), that feeds on PP, the Pacific oyster, an invasive habitat modifier (IH) and the shore crab, a predator (P), which predates on C but not on IH. Grey solid lines represent trophic interactions and black dashed lines indicate effects on traits of the species to which the arrow is pointing. Different life stages C_i of the structured population C may each transmit the indirect effects that are solely initiated by IH as well as indirect effects arising simultaneously from IH and P with different orders of magnitude.

edulis aggregates and forms dense beds, which provide refuge and suitable habitat for an array of associated organisms (e.g., Tsuchiya & Nishihira 1985; 1986). By shaping their environment mussel beds function as ecosystem engineers (Jones et al. 1994, Buschbaum & Saier 2001, Crooks 2002, Gutierrez et al. 2003). They may form very stable and long-lived structures, if losses from factors such as hydrodynamic forces, storm events or predation are balanced by recruitment (Nehls & Thiel 1993, Nehls & Ketzenberg 2002). In the 1990s and the 2000s, natural losses and anthropogenic induced losses (e.g., extensive fishing) could not be balanced by recruitment. Several successive years of poor recruitment resulted in a decline in mussel bed area in many parts of the Wadden Sea (Beukema & Cadée 1996, Nehls et al. 2006). At the same time Pacific oyster bed area increased. However, these trends do not appear to be causally linked but rather both are the effect of global change (Nehls et al. 2006). Field observations indicate that *M. edulis* can coexist alongside *C. gigas* (Troost 2010, and references therein). Moreover, increasing oyster biomass results in an increased number of *M. edulis* at the expense of individual body condition (Troost 2009). While a decrease in condition indicates direct negative effects on mussels imposed by oysters through food limitation, an increase in mussel numbers might indicate the existence of indirect effects. For example, the presence of oysters may provide shelter from predation and environmental extremes resulting in a reduced mortality of juvenile and adult *M. edulis* within oyster/mussel patches compared to pure mussel patches (Markert et al. 2009). Indirect effects might be crucial for the persistence of mussels especially within areas where recruitment is generally low or during periods of low recruitment. Eschweiler & Christensen (2011) showed that mussels, cohabiting with oysters, experience a trade-off between survival and food supply. This study demonstrated that mussels tend to seek shelter from predation at the costs of growth and condition in presence of the shore crab *Carcinus maenas*, an abundant and widely spread omnivore along European coasts.

The chance of finding shelter for mussels in oyster patches is size dependent. Since the interstices on oyster shells and crevices between small oysters favour mussels of smaller sizes (A. M. Waser, personal observation) the order of magnitude of the TMIIs induced by the oysters may highly vary with mussel size. When mussels are exposed to predation, oyster initiated TMIIs

may lower mortality rates in smaller sized mussels. Furthermore, direct encounters of mussels and crabs evoke additional, crab initiated, TMIIs that provoke mussels to migrate deeper into the oyster matrix in favour of finding shelter, which may lead to an even stronger decrease in size specific mussel mortality (Figure 6.1).

In the present study, we conducted an experiment to examine the indirect effects of oysters, as well as crabs on the mortality of different life stages of *M. edulis*. We hypothesize that the impact of oyster triggered TMIIs on mussel mortality is higher in combination with crab triggered TMIIs compared to oyster triggered TMIIs alone. Therefore, we designed treatments, in which mussels either experienced TMIIs initiated by oysters and crabs simultaneously or were unaffected by predator TMIIs. These different treatment modifications were executed during the acclimatization phase of the mussels that were either given to experimental units together with or without any oysters. The mussels were exposed to predation by a single individual of *C. maenas*. Prey selection of shore crabs is driven by the maximization of energy intake, as well as by the risk of damaging claws (Elner & Hughes 1978, Smallegange & van der Meer 2003), therefore a crab of a certain size will tend to forage on mussel sizes which fit its specific needs best. Consequently, predator size strongly affects the predation pressure of different sized mussels. In order to examine this effect, two different size classes of crabs were used on a range of mussel sizes.

Materials and Methods

We experimentally simulated the short-term survival of different sized *M. edulis* in relation to the presence of two sizes of the predatory *C. maenas* depending on either presence or absence of the invasive *C. gigas*. Furthermore, it was tested if predatory cues could stimulate hiding behaviour of mussels, thus enhancing mussel survival. All specimens for this experiment were collected from different places along the westernmost Wadden Sea island, Texel, the Netherlands. Pacific oysters were collected from a wild oyster population on the east coast of Texel. After collection, biofouling was removed from oysters. *M. edulis* were scraped from ballast piers on the west coast of Texel and were cleaned of any attached fouling organisms before the length (ML, maximum length of the shell) of each individual was measured to the nearest 0.01 mm using electronic callipers. Mussels were then assigned to one of four size classes: 6.00–8.99 mm, 12.00–14.99 mm, 18.00–20.99 mm and 24.00–26.99 mm in ML, further on referred to as size class categories 6, 12, 18, and 24. Shore crabs were gathered from the NIOZ harbour by deploying small baited traps (18 L volume). Following their capture, each crab was sized according to carapace width (CW, the maximum distance between the two prominent lateral spines) using electronic callipers and assigned to one of two size classes: small (45.00–49.99 mm CW) and big (60.00–64.99 mm CW). Only undamaged male crabs were used in the experiments in order to reduce variability associated with morphology and sex. Oysters and mussels were kept separately in large basins with continuously running seawater, while crabs were placed individually in plastic aquaria (19 × 12 × 13 cm) that were submerged in a large basin. All basins had a temperature of 15 °C at a 12 h light:12 h dark cycle. Mussels and oysters were fed daily with concentrated algal feed (Instant Algae Shellfish Diet 1800, Reed Mariculture, Campbell, CA, USA). Crabs were fed open mussels regularly, but were starved for one week and then fed two days prior to the experiments to standardize their hunger levels. After each experiment crabs were kept in the laboratory for another ten days to make sure they were not in proecdysis. Thereafter, crabs were released.

The experimental design as well as the analysis followed a split plot design approach, where all four mussel size classes were placed together in small aquaria (plots) to which eight different treatments were applied (Figure 6.2A), due to the combination of the three different among-plot factors, each with two levels (acclimatization type: control and induced clumping, crab size: small and big crabs, and oyster presence: presence and absence of oysters). Each of the

treatments was replicated 10 times. Due to the complexity and size of the experimental design it was not possible to conduct all trials simultaneously. Consequently, the experiment was divided into four separate experimental periods, each run with 20 plots. Each of the experimental periods, including preparations, took approximately one week. The experiment started in mid- September 2012 and lasted in total six weeks. In the first two periods the trials of the acclimatization control were conducted and trials of the induced clumping treatments were performed in period three and four. At each period the experimental combinations of small crab without oysters, small crab with oysters, big crab without oysters and big crab with oysters were replicated five times. The plots of each of the experimental periods were randomly assigned to a flow through system which provided all of the aquaria with an equal water inflow (about 2–3 L/h) and aeration by membrane pump. Both, the acclimatization of the mussels as well as the experiments were conducted at same conditions as previously described with experiments being carried out in the light period.

Mussels of varying sizes were put together into all experimental plastic aquaria (32.5 × 17.5 × 18.5 cm), with either only a layer of sand and small shell material as substrate or next to sand and shell debris also oysters were offered, in order to allow the mussels to either form mussel patches or to seek shelter in the oyster matrix. A total of 95 mussels, which corresponds to a density of 1670 individuals m^{-2}, was introduced per aquarium. The chosen density reflects the abundance of *Mytilus* found in pure mussel beds as well as in mussel-oyster beds in the Wadden Sea ranging between 1000 and 3000 individuals m^{-2} (Büttger et al. 2008, Buschbaum et al. 2009, Markert et al. 2009). In years with high recruitment densities may reach up to 6000 individuals m^{-2} (Büttger et al. 2008). In order to approximately match the total biomass of the different size classes within each aquarium, the amount of mussels given to all aquaria differed between the size classes. The numbers of the different size classes were determined based on ash free dry mass from mussels taken from various mussel beds across the Dutch Wadden Sea in spring and autumn of the years 2010–2012 (A. M. Waser et al., unpublished data). The size classes 6, 12, 18 and 24 comprised, in descending order, of 50, 25, 13 and 7 individuals of mussels, resulting in densities of 880, 440, 230 and 125 individuals m^{-2}. However, equal biomasses were not exactly matched, since the biomass of the smallest size class (6) amounted only about a fourth of the biomass of the other three size classes. Using different numbers of mussel size classes could potentially lead to disproportionate effects on the percentaged mortality between the different sizes. For example, one consumed mussel of the biggest size class has a much bigger impact on the percentaged mortality than one mussel of the smallest size class. But in order to match energy requirements a crab needs to consume much higher numbers of small mussels compared to bigger ones.

The total volume of oysters in the experimental aquaria varied between 0.5 and 0.55 L. After oysters were loosely placed in the oyster (+) treatment aquaria, mussels were added to all aquaria and were given 3 days to acclimatize and either hide in the interspaces of the oyster matrix or form aggregates with conspecifics.

Two different treatments of mussel acclimatization were applied, without and with crab and injured conspecifics being present during the acclimatization phase of the mussels. The latter should stimulate clumping behaviour of the mussels and therefore create a more comparable setting to field conditions. Crabs that were not involved in the actual feeding trials were placed into rectangular cages (fish net breeder, 16.7 × 12.3 × 13.4 cm) covered with nylon mesh (1 mm), which were mounted on the upper side of the plastic aquaria. This design allowed the scent of predators and damaged conspecifics to reach the mussels at the bottom, but prevented the predators from consuming or damaging the experimental mussels. The crabs in the cages were provided with a continuous amount of fresh mussels. As a result of the feeding process, there were plenty of damaged mussels in these cages. After mussels were acclimatized for three days, crabs were placed into the aquaria and were given a foraging period of six hours, mimicking tidal submersion. Each crab was only used once in the experiment. After the six hours of
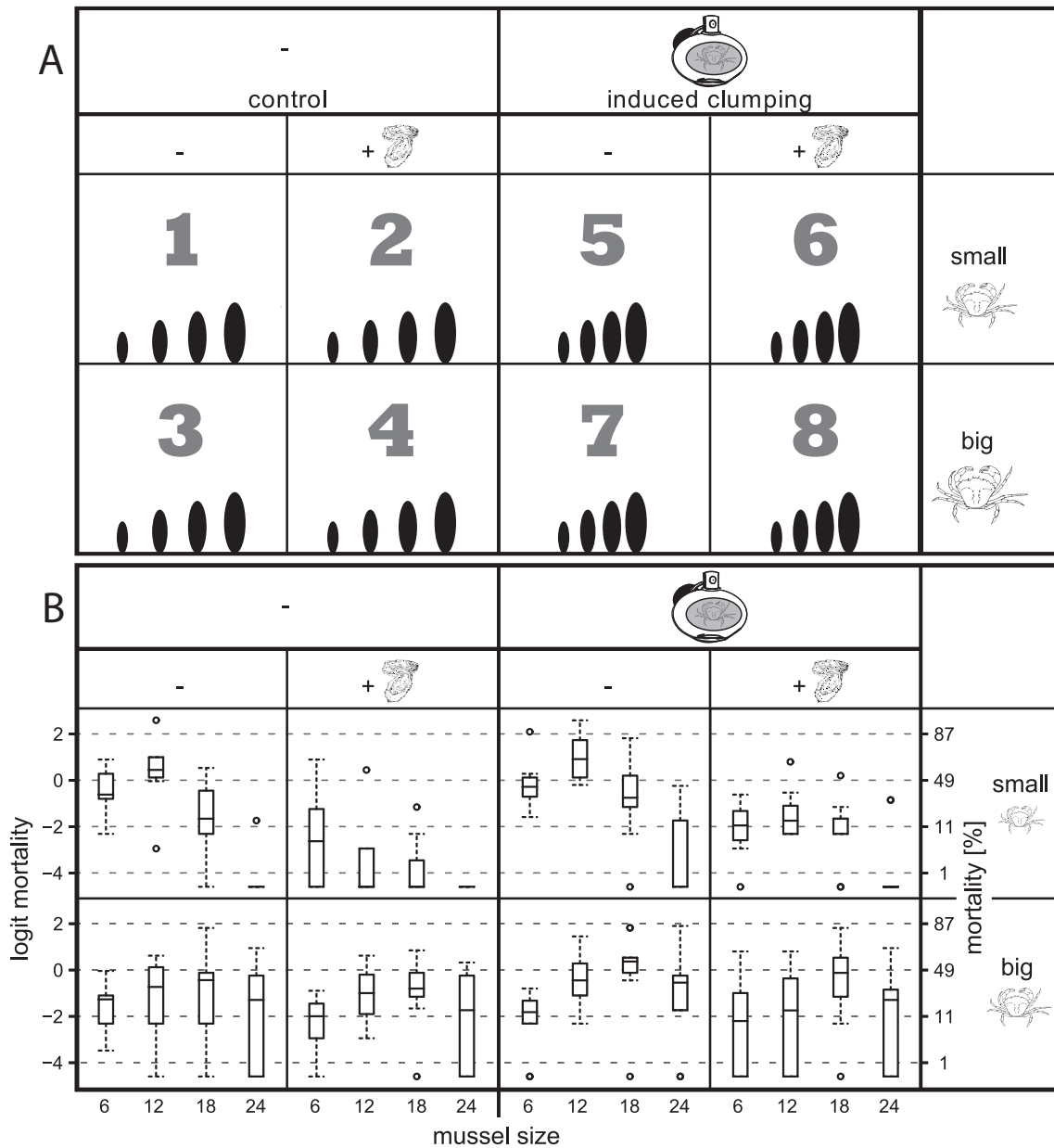
Figure 6.2: Scheme of the experimental design (A). Displayed are the separate plots (aquaria) with the different sized mussels (within plot) and different among plot combinations (grey numbering; acclimatisation type, crab size and oyster presence). Box plot of the logit mortality of the 4 different mussel size classes under different treatments (A). Box and whisker plots give the median (horizontal line inside the box), interquartile range (box), and outliers (small dots). Grey dashed lines to support orientation.

predation, crabs were removed and aquaria checked for surviving mussels. The fraction of consumed mussels per size class was logit-transformed to normalize the data using the formula:
$y = \ln(\frac{c + 0.01}{1 - (c + 0.01)})$; with c as the fraction of consumed mussels per size class.

After experimentation, two crabs were found to be in proecdysis and one died and therefore all trials with these crabs were excluded from analysis. All three crabs originated from the small size class, at which two were supposed to prey in presence of oysters and one in oyster absence, resulting in an unbalanced experimental design. To cope with the unbalance a split plot ANOVA with sums of squares Type III was applied. This type tests for the presence of a main effect after the other main effect and interaction and is therefore valid in the presence of significant interactions. However, the results need to be interpreted with caution (in the presence of interactions, main effects are not easy to interpret). The error structure of the ANOVA is defined in the nested error term, which is the biggest plot size (aquarium) in relation to the within factor (mussel size). Effects were considered to be statistically significant if p < 0.05. Statistical analysis was performed using R v3.02 (R Development Core Team 2015) supplemented by the package ez (Lawrence 2013).

Results

Due to the complexity of the experimental design, we will first focus on the among plot variability without regard to within plot variability (i.e., coupled to mussel size) (Table 6.1). Our analysis indicates that the presence of oysters significantly affected the mortality of mussels, but that the effect varied according to crab size (C:Oy interaction; F = 6.14, p = 0.016). For all levels of the other two factors (Ac and C), oyster presence reduced the amount of predated individuals (Figure 6.3). Yet, the magnitude of reduction in mussel mortality due to oysters was higher in the presence of small crabs. In addition to the impact on the among plot variability, oyster presence also had significant effect on the within plot variability. The survival of the different mussel sizes with respect to predator size and oyster presence/absence differed significantly from each other (C:Oy:M interaction; F = 3.84, p = 0.011). Small crabs predominantly foraged on smaller mussels of category 6 and particularly 12 (Figure 6.2B). The biggest size class of mussels (24) was only chosen as prey by a few small crabs resulting in low or no mortality at all in the 4 different treatments (Figure 6.2B). Conversely, the presence of oysters reduced mussel mortality in the three smaller mussel size classes (6, 12 and 18) dramatically. At acclimatization control, mortality of size classes 6 and 12 accounted for 40% and 55% in absence of oysters, respectively, and could be reduced to 6% and 2% in presence of oysters. Mortality in the size class 18 was reduced from 15% in absence of oysters to 1% in the presence of oysters. In trials where clumping was induced, the mussel mortality tended to be higher compared to the mortality in control treatments. However, crabs preferred the same mussel sizes as in the acclimatization control. There was also a dramatic decrease in mortality ranging from 30–70% to 10–15% in mortality of size classes 6, 12 and 18 due to the presence of oysters (Figures 6.2B, 6.4).

Big crabs were capable of foraging on all four mussel sizes offered, but preferably consumed mussels of size classes 12 and 18. The introduction of oysters had a lower effect on the survival of the mussels exposed to predation by big crabs compared to small crabs. In trials with control acclimatization the effect of oysters was minimal, with size classes 12 and 18 being unaffected and only a slight reduction in mortality of size classes 6 and 24 (Figure 6.4). The effect of oysters tended to be higher in trials with induced clumping so that consumption rates of size classes 12, 18 and 24, that amounted to 25–50% in absence of oysters, could be reduced to 10–35% when oysters were present. The survival of size class 6 showed little effect.

The chemical cues of crabs and injured mussels during the acclimatization period visually induced a denser clumping of the conspecific mussels. However, the cues did not improve the survival of mussels. Moreover, the consumption of the mussels was slightly higher in the

Figure 6.3: Mean logit mortality (± SE of parameter estimation) of all mussels per individual treatment. White symbols indicate the trials of the acclimatization control and grey symbols the ones for the induced clumping trials.

Figure 6.4: Mean mortality in presence of oysters in relation to mean mortality in absence of oysters per individual treatment. The numbers within the circles represent the different mussel size classes. Black solid line represents $y = x$ line; circles on or close to the solid line indicate no or minor differences in mortality related to oyster presence. Circles underneath the line point to a higher mortality in the absence of oysters compared to oysters being present. The higher the distance of a circle to the line, the greater the difference in mussel survival between the two levels of oyster presence. The dashed line indicates a drop of the mortality by half of its original value with oysters being absent. All mortality rates below the dashed line could be reduced to 50% or more.

Table 6.1: ANOVA sums of squares Type III under a split plot design for the response variable logit mortality.

Factor	df	SS	MS	*F*	*p*
Among plots					
Acclimatization (Ac)	1	25.66	25.66	4.31	0.042
Crab size (C)	1	30.22	30.22	5.08	0.027
Oyster presence (Oy)	1	108.59	108.59	18.25	<0.001
Ac:C	1	8.28	8.28	1.39	0.242
Ac:Oy	1	0.07	0.07	0.01	0.916
C:Oy	1	36.57	36.57	6.14	0.016
Ac:C:Oy	1	8.52	8.52	1.43	0.235
Residual	69	410.60	5.95		
Within plots					
Mussel size (M)	3	149.19	49.73	35.63	<0.001
Ac:M	3	9.70	3.23	2.32	0.077
C:M	3	112.76	37.58	26.93	<0.001
Oy:M	3	19.87	6.62	4.74	0.003
Ac:C:M	3	4.91	1.64	1.17	0.321
Ac:Oy:M	3	1.09	0.36	0.26	0.854
C:Oy:M	3	16.06	5.35	3.84	0.011
Ac:C:Oy:M	3	6.81	2.27	1.63	0.184
Residual	207	288.93	1.40		

treatments with induced clumping compared to control acclimatization. In addition, there was no significant interaction on the type of acclimatization and the different size classes of the mussels on mussel survival (Table 6.1).

Discussion

Habitat forming species can affect species survival either purely by their physical structure (i.e., reducing predator-prey encounter rates) (DMIIs) and/or by affecting species behaviour (TMIIs). Here we demonstrate that the Pacific oyster reduces overall mussel mortality by means of crab predation. This result agrees with previous findings, indicating that habitat complexity caused by oysters can reduce bivalve mortality by altering predator-prey interactions (Hughes & Grabowski 2006). Furthermore, studies on other habitat forming species suggest that effects of habitat modifiers on other species may change with ontogeny. For example, seagrass facilitates bivalve recruitment by providing a surface for colonization and/or a refuge from predation (Orth et al. 1984, Williams & Heck 2001), while Gribben & Wright (2014) found no mediating effect of seagrass structure on predation risk of adult clams. Moreover, seagrass negatively affects adult clam survival since seagrass provokes a shallower burial depths of clams that increases both predation and non- predation mortality (Gribben & Wright 2014). In our study, we found the reduction in mortality of mussel recruitment to be size specific. With the introduction of the Pacific oyster, mussel mortality was particularly reduced in sizes smaller than 20 mm in shell length. These results suggest that, besides the change of general predator-prey encounter rates, also other mechanisms may affect the survival of mussels in presence of oysters. When different sized mussels were placed next to oyster patches in a laboratory setup, mussels were found to migrate towards the oysters and were found to be on average smaller in the interspaces (17.35 ± 4.46 mm) than on the edges of oysters (19.09 ± 4.07 mm) (A. M. Waser, unpublished data). The size specific differences in hiding behaviour are due to the fact that

spatial refuges arising from oyster presence are mostly small and provide refuge for smaller individuals of mussels. Furthermore, smaller mussels are more active which would facilitate locating most suitable refuge.

To our knowledge, our study is the first to clearly show a relationship between habitat complexity and size specific mortality. In top-down trophic pathways the importance of body size in the strength of TMIIs is already well-known (Rudolf 2006; 2008; 2012, Preisser & Orrock 2012, Krenek & Rudolf 2014). Yet, the findings of these studies found mixed support for size specific TMIIs. While a meta-analysis investigating the role of size range across different species did not find a clear relationship between TMIIs and prey survival (Preisser & Orrock 2012), a study of an interaction of two predatory hemipteran and one copepod species indicated a clear relationship between size ratio of multiple predators and the strength of TMIIs (Krenek & Rudolf 2014).

In our study, we only focused on the importance of oyster appearance on the survival of mussels up to 27 mm in shell length. In the Wadden Sea adult *M. edulis* readily reaches lengths up to 65 mm (e.g., Buschbaum et al. 2009). To what extent the oysters affect the survival of the larger individuals of *M. edulis* is unknown. However, we believe that oyster induced habitat complexity has no impact on the survival of the very large mussels in terms of predation by shore crabs, since crabs tend to avoid bigger mussels to either achieve higher net intake rates and to prevent themselves from claw damage (Smallegange & van der Meer 2003). Even largest size classes of crabs (carapace width of 70–75 mm) prefer relatively small mussels of around 22.5 mm in shell length (Elner & Hughes 1978). Predators that preferably prey on larger mussels are several bird species (Oystercatcher, Herring Gull and Common Eider). Field observations suggest that oyster presence decreases predation rates of the birds, causing a reduction in mussel mortality (Scheiffarth et al. 2007).

We identified not only differences based on size in the transmitting species, the mussels, but also in the receiving species, the crabs. In our trials, oysters had a higher impact on the predation efficiency of small crabs compared to big crabs. In presence of oysters, small crabs were less successful in preying on mussels compared to trials where no oysters were present, whilst predation of big crabs was less influenced by oyster presence. These observations contradict previous studies, which found complex substrates to influence predation success of bigger predators more than of small predators, because prey in crevices within complex substrates are still accessible to small sized predators (Clemente et al. 2012, Toscano & Griffen 2013). The contrariety of observations could be based on the experimental setup where oysters were not fixed in the substrate matrix. This allowed, other than under natural conditions, crabs to manipulate the matrix and to move single oysters in order to gain access to hidden mussels. This may explain the lack of effect of oysters on the predation success of big crabs as their increased strength would enable them to move oysters and increase access to more mussels.

We hypothesized that the settling behaviour of mussels differs between scenarios where only TMIIs triggered by oysters were active and scenarios where TMIIs simultaneously triggered by crabs and by oysters were affecting the behaviour of mussels. Simultaneous TMIIs triggered by crabs and by oysters should cause mussels to migrate away from the outer edges of the oyster matrix towards deep interspaces of the oyster matrix resulting in a lower mortality. Unexpectedly, we found that the addition of crab triggered TMIIs did not reduce the mortality of mussels. Actually, we observed the opposite trend of higher mortality in mussels where TMIIs additionally were induced by crabs. On the basis of our experimental design, having all plots of the acclimatization control conducted in the first two blocks and the plots of the induced clumping in the last two, our experiment has limitations about interpreting the survival of mussels in terms of the factor acclimatization treatment. As this factor is confounded with time, we cannot rule out the effect of time related changes, such as the condition of animals, as a reason for differences in mortality. Since we experienced some logistical problems during the experiment (essential material for the induced clumping treatments was not available in the

beginning of the experiment), we were forced to conduct the clumping treatments in different blocks. A factor that might have influenced the survival of mussels is that the mussels used in the experiments were taken from environments where they were constantly exposed to crabs and also other predators. They might have habituated to the presence of predators and may be adapted through alterations in their morphology (significantly thicker shell density and adductor muscle diameter). Adaptive modifications of induced aggregation behaviour and attachment in mussels in response to predators can weaken over time in favour of more passive anti-predator response by altering shell density and adductor muscle diameter (Christensen et al. 2012). Also, the cues produced by one crab might have been not strong enough to induce anti-predator behaviour. It was found that anti-predator behaviour is highly affected by the body mass of predators, which can be either achieved of one really big individual or by plenty of smaller ones (Hill & Weissburg 2013).

The state of ontogenetic development plays a crucial role on the strength of indirect effects. The effects of TMIIs therefore vary strongly within size structured populations. This is particularly vital when evaluating the consequences of environmental changes caused by the introduction of new species to ecological systems. So far, effects of invaders on different life stages of the native fauna has received little attention. Sized structured variation in strength of TMIIs induced by invaders may ultimately cause shifts in the population structure of native species.

Acknowledgements

7 | Tidal elevation and parasitism: patterns of infection by the rhizocephalan parasite *Sacculina carcini* in shore crabs *Carcinus maenas*

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Abstract

While the distinct zonation patterns of benthic organisms along intertidal elevation gradients have been extensively documented, relatively little is known about the impact that tidal elevation has on the distribution and abundance of marine parasites that are common in intertidal ecosystems. In this study, we investigated the distribution of shore crabs *Carcinus maenas* infected with the rhizocephalan parasite *Sacculina carcini* at 12 locations and in 3 adjacent habitats (intertidal mussel beds, intertidal bare sand flats and subtidal gullies) along a tidal elevation gradient in the Dutch Wadden Sea. Our sampling revealed that of the 27629 crabs investigated, most infected crabs were found in the subtidal gullies and almost none on intertidal bare sand flats or mussel beds at all of the 12 locations. This probably resulted from a parasite-induced manipulation of infected crabs to behave like egg-bearing females which migrate towards deeper waters, as the same pattern was observed in the distribution of non-infected ovigerous females. The prevalence of both infected crabs and ovigerous females in the gullies was significantly correlated with water depth, and both tended to increase (albeit not significantly) with increasing salinity. As water depth and salinity are expected to affect larval survival of both parasites and crabs, this suggests that the migration into subtidal habitats may result in favourable conditions for reproduction and dispersal. By using a replicated and nested sampling design as well as a large sample size, our study significantly increases the limited understanding of parasite distributions along tidal elevation gradients.

Introduction

The distinct zonation patterns of benthic organisms along intertidal elevation gradients have fascinated marine biologists for a long time, and the effect of tidal elevation on the distribution and abundance of marine organisms has been extensively documented (e.g., Lewis 1964, Benson 2002, Bertness et al. 2013). In contrast, little is known about the relationship between tidal elevation gradients and the distribution and abundance of marine parasites, which are common in intertidal ecosystems (Mouritsen & Poulin 2002, Torchin & Høeg 2008). Parasites may be affected by tidal elevation gradients in several ways. First, as parasites depend on the presence of their hosts, they will inevitably follow the distribution of their hosts along elevation gradients and thus only occur where their host is present. Second, parasites may show distinct distribution patterns within the tidal range occupied by a host species if the exposure of hosts to parasites varies within this range. This could result from changing abiotic and biotic factors known to affect parasite transmission along tidal elevation gradients (for reviews of potential factors see Pietrock & Marcogliese 2003, Thieltges et al. 2008b). In addition, in the case of parasites with complex life cycles, it could result from distributional patterns of intermediate hosts up-stream in a parasite's life cycle, as those are known to be strong drivers of infection patterns (Hechinger & Lafferty 2005, Thieltges & Reise 2007). Finally, parasites may show a distinct distribution along tidal elevation gradients due to parasite-induced behavioural changes of their hosts. For example, snails serving as first intermediate hosts for trematodes have been shown to move higher up the shore when infected, presumably to increase transmission to the down-stream hosts in their life cycles (Curtis 1987, McCarthy et al. 2000). Apart from trematodes, however, distributional patterns along tidal elevation gradients have been rarely studied for most intertidal parasite-host systems.

This is also the case for common shore crabs *Carcinus maenas* (L., 1758) infected with the rhizocephalan barnacle *Sacculina carcini* Thompson, 1836. Shore crabs are native to European and North African shores and have been introduced to many coastal areas worldwide (Carlton & Cohen 2003). They occur in two colour morphs, which are associated with the crab's moulting stage; green crabs are those which have recently moulted, and red crabs have undergone a prolonged duration of intermoult (Crothers 1968, McGaw & Naylor 1992, Reid et al. 1997, Styrishave et al. 2004). In intertidal sedimentary ecosystems like the European Wadden Sea, shore crabs occur in both subtidal and intertidal habitats, with particularly high densities reported from biogenic structures like mussel beds and seagrass meadows (Klein Breteler 1976b, Reise 1985, Thiel & Dernedde 1994).

Shore crabs are infected by a range of parasites (Torchin et al. 2001), with the rhizocephalan *S. carcini* being the most conspicuous one (Høeg 1995, Høeg & Lützen 1995). Shore crabs and many other portunid crab species (see Øksnebjerg 2000, for host range of *S. carcini*) become infected with this parasite when cyprid larvae of *S. carcini* settle on the crab cuticle, penetrate into the hemocoel and develop an internal root-like network, the interna, throughout the tissue of the crab. After a duration of 1 to 3 years, *S. carcini* matures, and part of the interna ruptures the crab's abdominal exoskeleton and forms a distinct sac-like structure underneath the pleon of infected crabs (Lützen 1984). This structure is called the externa and contains the reproductive organs of the parasite (Høeg & Lützen 1995). The parasite infects and castrates both sexes of *C. maenas* (Høeg 1995). Moreover, male crabs become to a certain extent morphologically feminized, induced by effects of the parasite on the hormonal system of the host, involving an enlargement of the pleon and a reduction in cheliped size (Rubiliani et al. 1980, Høeg 1995, Kristensen et al. 2012). The morphological feminisation occurs stepwise with every moulting event of the infected host, while *S. carcini* remains internal (Høeg 1995). Once the externa emerges and crabs become externally infected, moulting ceases (parasitic anecdysis; O'Brien & Van Wyk 1985). These morphological changes are also accompanied by behavioural changes. Infected individuals exhibit brood mimicry by carrying the externa in

the same place where egg-bearing females keep their eggs (underneath the pleon) and by behaving like ovigerous females, for instance grooming the parasite externa (Høeg & Lützen 1995). In addition, it has been proposed that infected crabs migrate towards deeper waters, thus copying the behaviour of ovigerous females (Rasmussen 1959, Rainbow et al. 1979, Lützen 1984). However, replicated studies at several locations along a tidal elevation gradient or a direct comparison of distributional patterns of parasitized crabs and ovigerous females along such a gradient are lacking.

In this study, we investigated the distribution of shore crabs infected with *S. carcini* along a tidal elevation gradient and compared it with the distribution of ovigerous females at identical locations. To do so, we sampled crabs at 12 locations in the Dutch Wadden Sea from 3 adjacent habitats: intertidal mussel beds, intertidal bare sand and subtidal gullies. This sampling design together with data on water depth and salinity allowed us to investigate 3 main research questions: (1) Do the levels of infection with *S. carcini* differ between the 3 habitats and does this co-vary with patterns observed for ovigerous females? (2) To what extent are water depth and salinity drivers of infection levels with *S. carcini* and the occurrence of ovigerous females? (3) Do infection levels differ among sexes or colour morphs? By using a replicated and nested sampling design, as well as a large sample size, our study significantly increases the limited understanding of parasite distributions along tidal elevation gradients.

Materials and Methods

Sampling of crabs

Carcinus maenas were sampled in early summer 2012 and 2013. In 2012, 6 locations were sampled in the western part of the Dutch Wadden Sea (Weeks 22 and 23) and in 2013, 6 locations in the eastern part (Weeks 24 and 25; Figure 7.1 and Table 7.1). At each location, 3 adjacent habitat types were sampled: intertidal mussel bed, bare intertidal sand flat and subtidal gully. On mussel beds, crabs were caught with baited, funnel-shaped, plastic crayfish traps (61 cm long × 31.5 cm wide × 25 cm high) with inverted entry cones at both ends. The traps were baited with several (4–7) frozen juvenile (< 7 cm) herring *Clupea harengus* and were set at low tide and emptied after high tide periods. Intertidal mudflats adjacent to mussel beds were investigated at high tide (water depth 0.5–1.5 m) using a 2 m beam trawl (mesh size 5.5 mm) towed by a small motorboat. With the same gear, the gully closest to each mussel bed was sampled during high and low tide. However, in a few cases (9 out of 73 hauls) fishing in the deep subtidal (> 5 m water depth) was carried out with a 3 m beam trawl (mesh size 10 mm) towed by a larger research vessel (RV 'Navicula' of NIOZ Royal Netherlands Institute for Sea Research). For 2 mussel beds (E022 and E032), the closest gully to the respective mussel bed was the same for both beds (Table 7.1). The location and exact distance of each haul was determined using a Global Positioning System (GPS) receiver.

For all crabs, carapace width (CW), which is the maximum distance between the two prominent lateral spines, was measured with digital callipers to the nearest 0.01 mm. Their colour morph (white, yellow or green-coloured abdomen was recorded as green and a predominantly orange or red abdomen as red) and sex were determined by visual inspection. Although parasitized males resemble females in appearance (feminisation), both sexes, even if parasitized, can be clearly distinguished from each other (for photographs see Kristensen et al. 2012). In addition, the ventral abdomen was inspected to identify ovigerous females as well as specimens infected with *Sacculina carcini* (clearly visible externa).
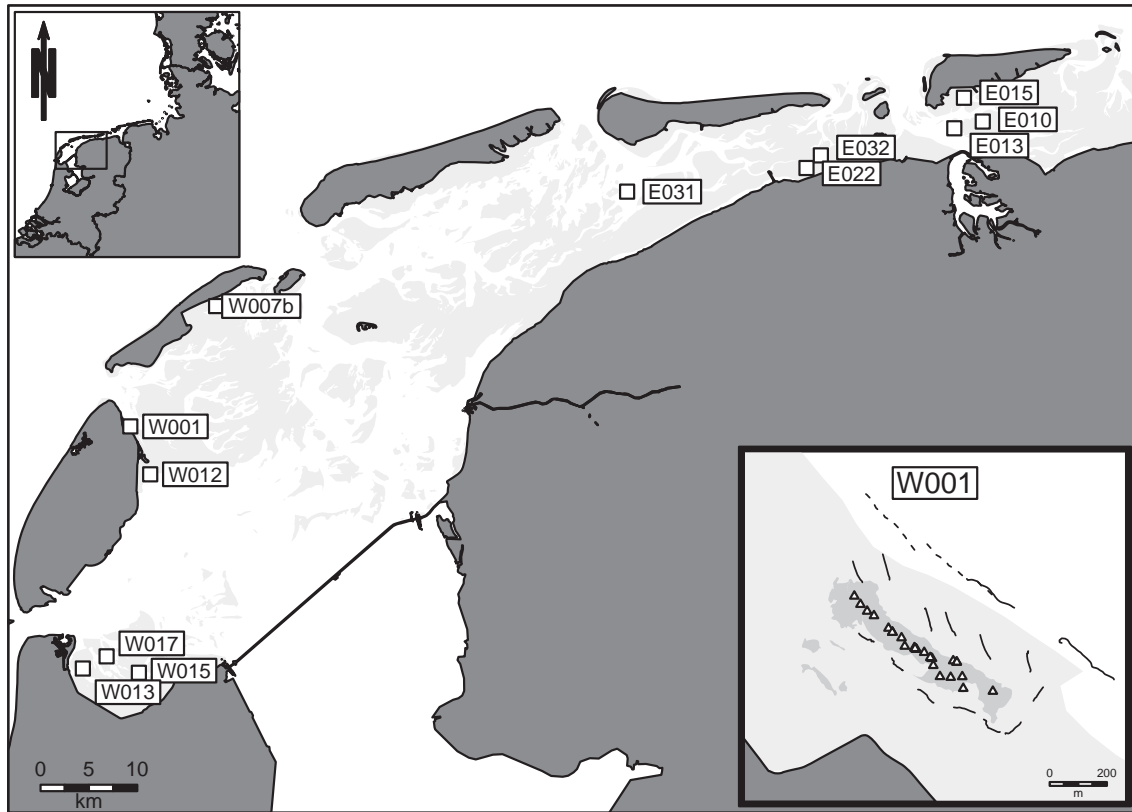
Figure 7.1: Sampling locations in the Dutch Wadden Sea. At each location (white squares), intertidal mussel beds and adjacent intertidal bare sand flats and subtidal gullies were sampled. For location names, geographic coordinates and numbers of crabs sampled see Table 7.1. White areas: subtidal; light grey areas: intertidal flats exposed during low tide; intermediate grey (in inset): mussel beds; dark grey: land. Inset: Specific sampling design of one site. White triangles: positions of traps; lines: hauls taken by beam trawl (dashed lines: hauls at low tide; solid lines: hauls at high tide).

Table 7.1: Locations and location codes (W: west; E: east) used in Figure 7.1 as well as coordinates and dates at which shore crabs *Carcinus maenas* were collected in the Dutch Wadden Sea in 2012 (west) and 2013 (east). For each location, the number of traps set on mussel beds as well as the number of hauls on intertidal bare sand flats and in subtidal gullies are given. Numbers in parentheses show the total number of crabs investigated per habitat. For details of sampling design see 'Materials and methods: Sampling of crabs'.

Code	Location	Coordinates	Date	Mussel bed Trap (n)	Intertidal flat Haul (n)	Subtidal gully Haul (n)
W013	Kuitje	52°56' 2.22"N, 4°48' 56.14"E	29 May	18 (398)	10 (1383)	5 (3346)
W017	Napoleondam	52°56' 42.94"N, 4°51' 8.56"E	30 May	18 (467)	9 (700)	6 (4824)
W015	Amsteldiep	52°55' 48.07"N, 4°54' 8.57"E	4 Jun	17 (319)	10 (858)	6 (690)
W001	De Cocksdorp	53°9' 31.97"N, 4°53' 20.8"E	7 & 8 Jun	30 (593)	13 (659)	5 (613)
W012	Krassekeet	53°6' 51.77"N, 4°55' 11.85"E	5 Jun	18 (427)	10 (190)	6 (371)
W007b	Vlieland	53°16' 14.45"N, 5°1' 19.64"E	6 & 7 Jun	19 (379)	7 (248)	6 (401)
E031	Kromme Balg	53°22' 37.16"N, 5°39' 33.29"E	12 Jun	16 (583)	5 (514)	7 (186)
E022	Ternaard south	53°23' 56.33"N, 5°56' 14.33"E	11 Jun	10 (55)	10 (844)	8 (1622)[a]
E032	Ternaard north	53°24' 38.45"N, 5°57' 34.47"E	11 Jun	10 (51)	7 (556)	8 (1622)[a]
E013	Roode Hoofd	53°26' 9.10"N, 6°9' 59.02"E	18 Jun	8 (110)	5 (263)	8 (756)
E015	Schiermonnikoog	53°27' 51.16"N, 6°10' 53.07"E	19 Jun	16 (611)	10 (570)	8 (1675)
E010	Brakzand	53°26' 31.67"N, 6°12' 37.91"E	17 Jun	17 (571)	10 (504)	8 (1292)

[a] Same adjacent gully

Crab density, water depth and salinity in subtidal gullies

Density of crabs was calculated using the known haul length and net width of the beam trawls. Data on the water depth of subtidal gullies (m below mean tide level, MTL) were generated based on the tracks of the hauls and a bathymetric grid (20 × 20 m) of the Dutch Wadden Sea provided by Rijkswaterstaat (Dutch Ministry of Infrastructure and Environment; 'vaklodingen'; http://opendap.deltares.nl). Salinities (psu) in the Wadden Sea are substantially influenced by high amounts of freshwater discharges of several outlet sluices and thus subject to seasonal and tidal fluctuations of up to 6 psu (van Aken 2008a), making point measurements during sampling unreliable. To obtain more accurate measures of local salinities, we used model output from a salinity model with a grid of 250 × 250 m, simulating salinities over a period of 2 years (2009–2010; Duran-Matute et al. 2014). For each gully, the mean water depth and salinity of all grid-points overlapping with the tracks of the hauls was calculated.

Data analysis

The total number of crabs caught was used to plot the size frequency distributions of infected and uninfected crabs (both for males and females separately) as well as that of ovigerous females. Following this, the proportions of infected crabs and ovigerous females of the total sample of crabs per habitat and location were calculated (hereafter called prevalence). As the prevalences in the subtidal gullies did not differ between the high tide and low tide samples (Student's t-test, *S. carcini*: t = -0.96, p = 0.35; ovigerous females: t = -0.21, p = 0.84), data from both tidal periods were summed for further analysis. Statistical differences in prevalence of infections and ovigerous females among the 3 habitats were tested using generalized linear mixed models (GLMM) with a binomial distribution (preliminary exploration did not show overdispersion). The model included habitat as a fixed factor and location as a random factor. As the main interest was the comparison among the three habitats, locations from both years were included in the analyses, despite the possibility that the difference in sampling year could confound location effects. For the two locations that shared the same adjacent subtidal gully (E022 and E032; Table 7.1, Figure 7.1), the value of this gully was used for both locations, considering this mild pseudoreplication to be unproblematic for the question at hand.

The effect of water depth, salinity and crab density on the prevalence of infected crabs and ovigerous females was tested using generalised linear models (GLM) with a quasi-binomial error distribution (to correct for overdispersion observed in preliminary explorations). For these analyses only crabs from the 11 subtidal gullies were used (locations E022 and E032 shared the same gully).

Finally, statistical differences between the different colour morphs and sexes in parasite infection status of the crabs caught in the subtidal gullies was tested using likelihood ratio G-tests. Comparisons were based on the totals of subtidal crabs (N = 15086, n = 10 locations: same gully for E022 and E032; no parasitized crabs at W015).

All statistical analyses were performed using R v3.2.1 (R Development Core Team 2015) supplemented by the package lme4 (Bates et al. 2015). For spatial data handling and production of the map, we used the R packages sp (Pebesma & Bivand 2015), rgeos (Bivand & Rundel 2015), rgdal (Bivand et al. 2015), maptools (Bivand & Lewin-Koh 2015) and raster (Hijmans 2015). For plotting, the package ggplot2 (Wickham 2009) was used.
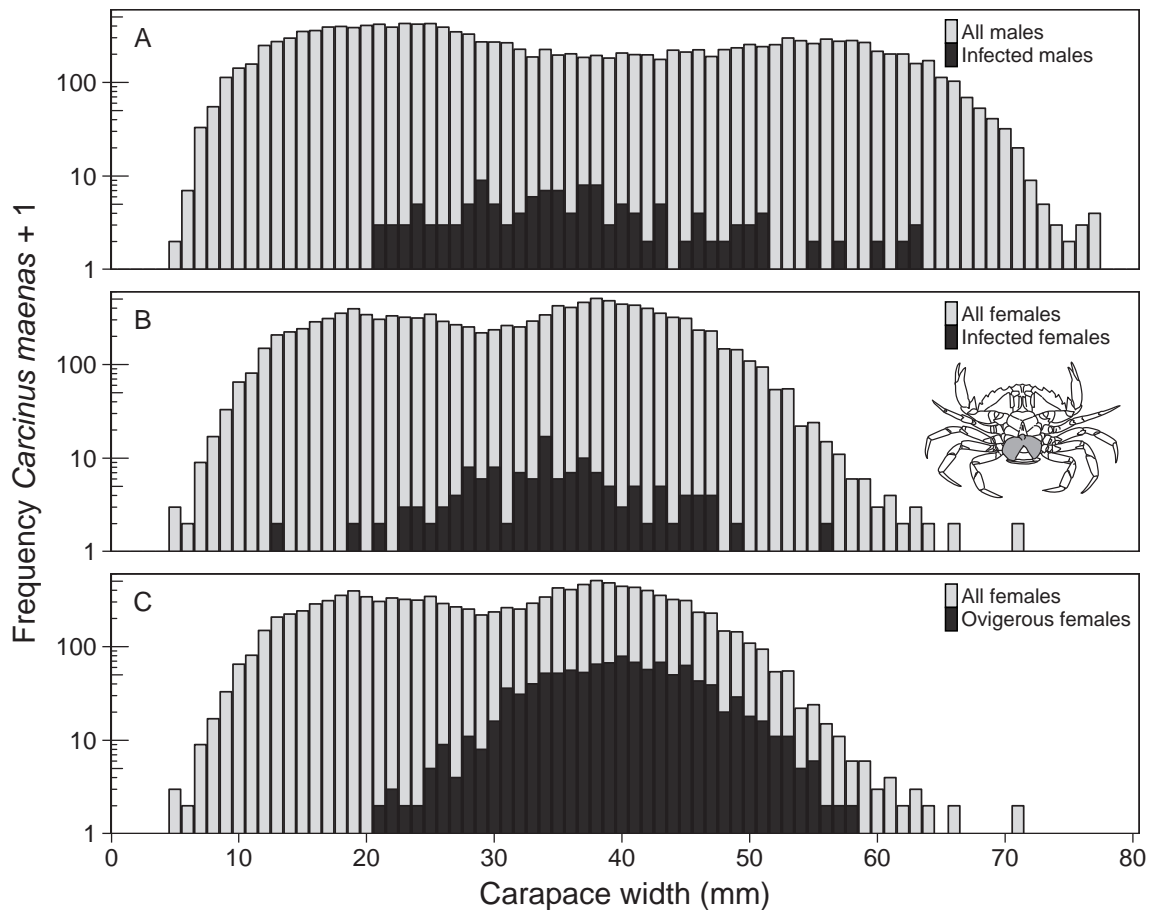
Figure 7.2: Size frequency distributions of *Carcinus maenas* (on a log-scale) summed for all locations and habitats: (A) male crabs infected with *Sacculina carcini* in comparison to all male crabs caught; (B) female crabs infected with *S. carcini* and the total of female crabs caught; and (C) ovigerous female crabs compared to all female crabs caught. All *Carcinus maenas* frequency values were increased by 1 in order to make frequencies of 1 visible.

Results

Of the 27629 crabs investigated at the 12 locations, 217 carried an externa of the parasite *Sacculina carcini*. The size of these infected crabs ranged from 12.7 to 62.8 mm CW (Figure 7.2A, B), and the size frequency distribution of infected crabs reflected more or less the one of uninfected crabs (Figure 7.2A, B). In contrast, ovigerous females were generally larger (20.5–57.8 mm CW; Figure 7.2C). In general, the prevalence of infected crabs differed among the three habitats (GLMM, likelihood ratio test, $\chi^2 = 119.3$, p < 0.001; Table 7.2, Figure 7.3A): these crabs showed highest prevalence (up to 2.8%) and occurred at all locations but one (W015) in the subtidal gullies, where in total 206 infected crabs were found. Infections at the two intertidal habitats only occurred at a few locations with low prevalence (below 0.5%; Figure 7.3A). The distribution of ovigerous females also significantly differed among habitats (GLMM, likelihood ratio test, $\chi^2 = 619.94$, p < 0.001; Table 7.2, Figure 7.3B). Egg-bearing females occurred with higher prevalence and at all but one (W015) location in the subtidal gullies, while they only occurred at a few locations and with very low prevalence on intertidal sand flats and mussel beds (Figure 7.3B). In general, the size frequency distribution of crabs was similar in the intertidal and the subtidal, and similar sizes of crabs were infected in both habitats (Figure S7.2).
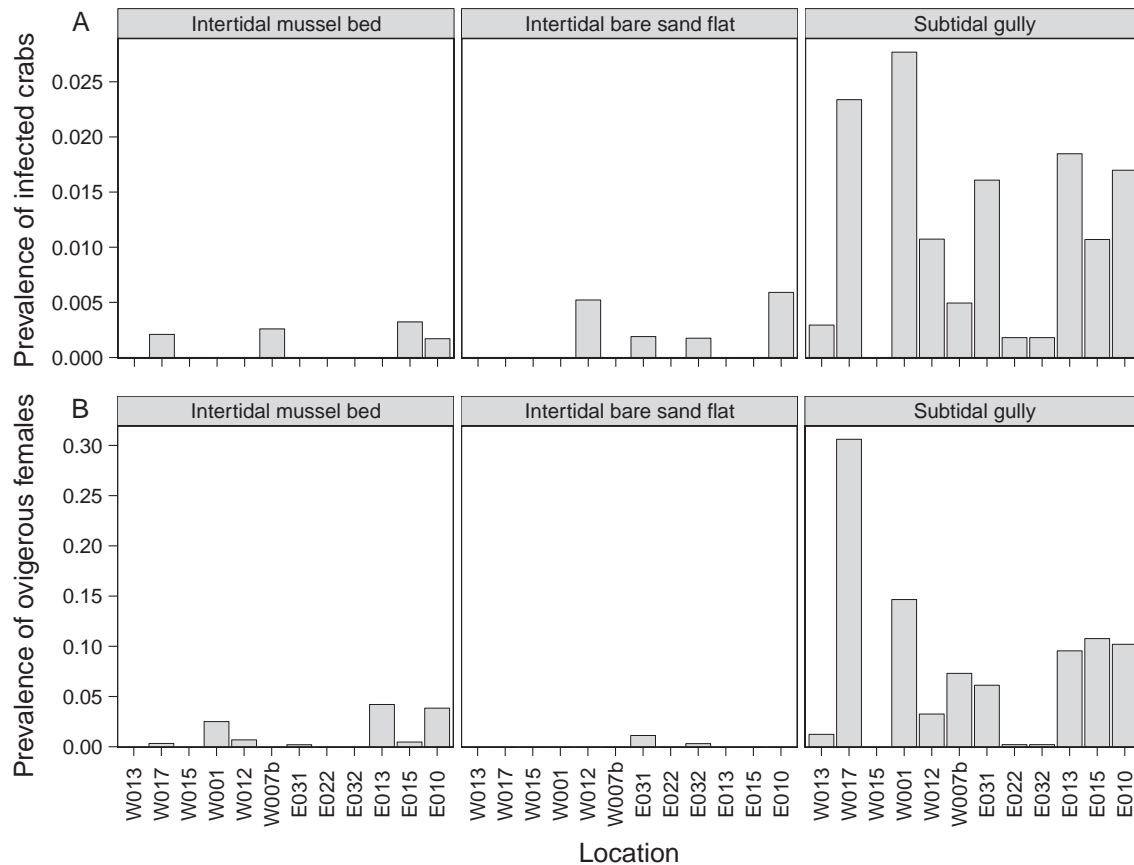
Figure 7.3: Prevalence of (A) shore crabs infected with *Sacculina carcini* and (B) ovigerous female crabs in 3 habitats at 12 locations in the Dutch Wadden Sea. For location codes and sample sizes see Table 7.1. Note that mussel beds E022 and E032 shared the same subtidal gully and hence the same value.

Both prevalence of infected crabs and prevalence of ovigerous females differed among the subtidal gullies (Table 7.2, Figure 7.3), and both were positively correlated with each other (Pearson correlation; r = 0.77, p < 0.01; see Figure S7.1). This covariance was likely the result of a similar effect of the same abiotic factors on parasite and ovigerous female prevalence. Water depth explained prevalence of infected crabs and ovigerous females the best; prevalence of both generally increased with water depth (GLM with binomial distribution and quasi-binomial error fix, because of overdispersion; Table 7.3, Figure 7.4). Moreover, both infected crabs and ovigerous females tended to occur more frequently at locations with higher salinity, although this was not or only marginally significant (Figure 7.4; Pearson correlations, infected crabs: r = 0.57, p = 0.07; ovigerous females: r = 0.42, p = 0.2, see Figure S7.1), although salinity was dropped in the model selection procedures and was not included in the final model (Table 7.3).

Finally, infection status of the crabs caught in subtidal gullies significantly differed between the different colour morphs and sexes (G-test: G = 440.35, df = 3, p < 0.001; Table 7.4). In males only 1.15% (0.11% green and 1.04% red) of the population was found to be externally infected. The non-infected males accounted for 98.85% (90.66% green and 8.19% red) (G-test: G = 374.96, df = 1, p < 0.001; Table 7.4). In contrast, in the females the proportion of individuals of the green morph was much less. Only 44.42% of the females were found to be green-coloured and non-infected, while 0.18% were externally infected green females. Most of the red females, 53.93% of the total female population, carried no externa of *S. carcini* and 1.47% of the females were red and externally infected (G-test: G= 58.253, df = 1, p < 0.001; Table 7.4).

Table 7.2: Results of general mixed effects model (binomial error structure) analysing the prevalences of infected crabs and ovigerous females in different habitats. Table shows predictor estimates, standard errors (SE), Wald statistic (*z*) and p-values. Model included habitat as a fixed factor and location as a random factor. The habitat intertidal mussel bed is included in the intercept. Alpha was set at 0.05.

Predictors	Estimate	SE	*z*	p
Infected crabs				
Intercept	-7.37	0.537	-13.714	< 0.001
Bare sand flat	0.01	0.609	0.025	0.98
Subtidal gully	2.50	0.463	5.403	< 0.001
Ovigerous females				
Intercept	-5.73	0.527	-10.87	< 0.001
Bare sand flat	-1.88	0.545	-3.46	< 0.001
Subtidal gully	2.52	0.219	11.53	< 0.001

There was an overall significance of habitat for prevalences of infected crabs (likelihood ratio test statistic = 119.3, df = 2, p < 0.001) and ovigerous females between habitats (likelihood ratio test statistic = 619.94, df = 2, p < 0.001).

Table 7.3: General linear models (quasibinomial error structure) of prevalences of infected crabs and ovigerous females. Predictors in the full models are depth (m below mean tide level), density of crabs (ind. m^{-2}) and salinity (psu). The final model was obtained by model selection: all predictors with the highest p-values were deleted in a stepwise, backward selection procedure. Alpha was set at 0.05. Predictor estimates, standard errors (SE), *t*, p and R-squared values and dispersion parameters (φ) are shown.

Model	Predictors	Estimate	SE	*t*	p	R^2	φ
Infected crabs							
Full	Intercept	-8.77	3.993	-2.197	0.064	0.64	6.47
	Depth	0.17	0.107	1.586	0.157		
	Density	-0.19	0.59	-0.315	0.762		
	Salinity	0.16	0.137	1.157	0.285		
Final	Intercept	-5.39	0.482	-11.187	< 0.001	0.45	7.11
	Depth	0.26	0.096	2.704	< 0.05		
Ovigerous females							
Full	Intercept	-5.98	5.588	-1.07	0.32	0.7	37.11
	Depth	0.36	0.155	2.309	0.054		
	Density	-0.31	0.806	-0.389	0.709		
	Salinity	0.12	0.193	0.608	0.562		
Final	Intercept	-3.82	0.684	-5.579	< 0.001	0.63	34.96
	Depth	0.46	0.126	3.652	< 0.01		

Table 7.4: Percentage of non-infected and infected shore crabs (visible externa) belonging to either the green- or the red-coloured morph for male and female *Carcinus maenas* in the subtidal gullies. Data are from 10 locations (same gully for E022 and E032; no parasitized crabs at W015).

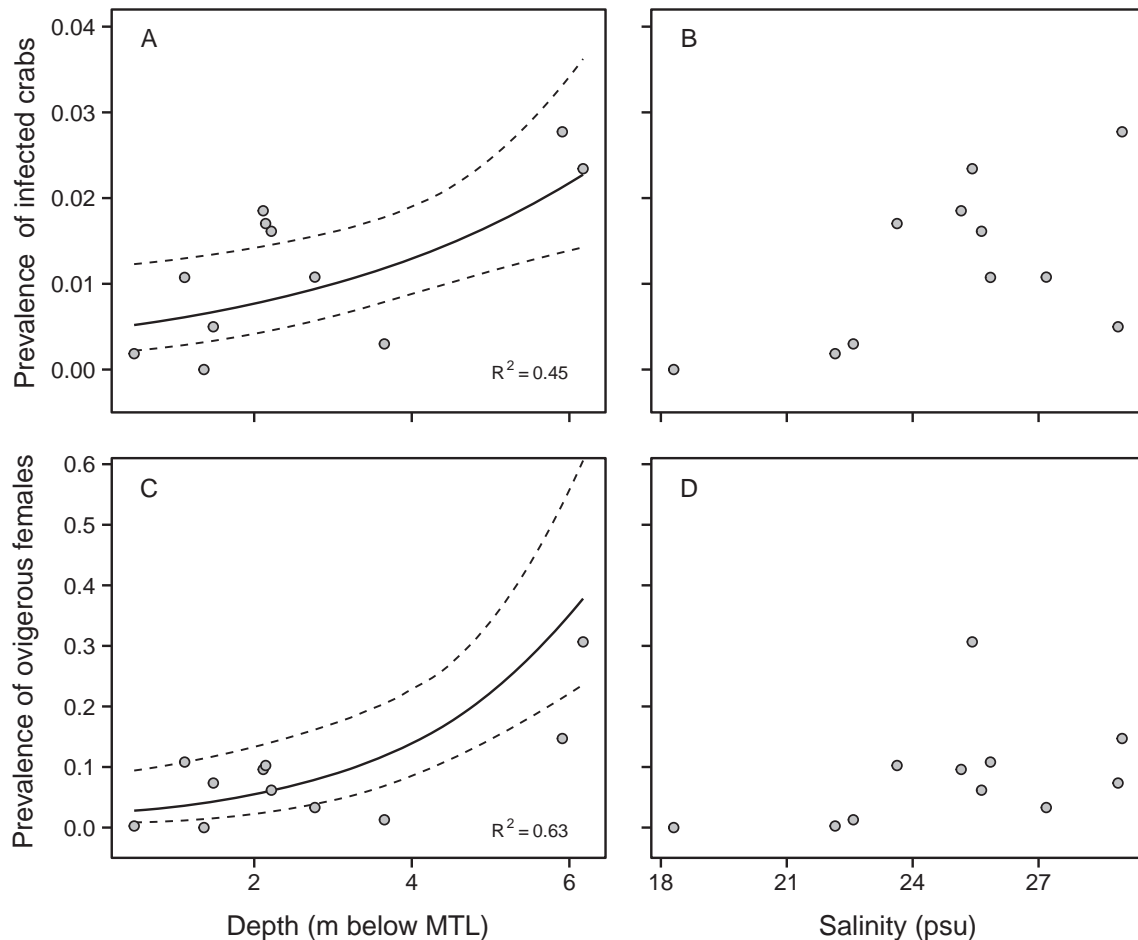|	Males (N = 8566)		Females (N = 6520)	
Colour morph	Non-infected	Infected	Non-infected	Infected
Green	90.66	0.11	44.42	0.18
Red	8.19	1.04	53.93	1.47

Figure 7.4: Prevalence of (A,B) shore crabs infected with *Sacculina carcini*, and (C,D) ovigerous female crabs in subtidal gullies depending on (A,C) water depth (m below mean tide level, MTL) and (B,D) salinity (psu). Plots show observed values (dots) and fitted values of the final generalised linear models (GLMs) (black line) with 95% confidence intervals (dashed lines).

Discussion

Our study reveals that most infected crabs were found in the subtidal gullies and almost none on intertidal bare sand flats or mussel beds at all of the 12 locations. This pattern was also observed in rhizocephalans infecting hermit crabs along the shore of Okinawa Island, Ryukyu Archipelago, southwestern Japan (R. Yoshida pers. comm.) and corroborates earlier notes in the literature based on very limited observations that infected *Carcinus maenas* migrate towards deeper waters (Rasmussen 1959, Rainbow et al. 1979, Lützen 1984). We observed a similar pattern in the distribution of ovigerous females, which also predominantly occurred in the subtidal habitat. The migration towards the subtidal is thought to result from parasite-mediated behavioural changes in infected crabs which lead to typical behaviour patterns of ovigerous females, even in the case of infected males, ultimately resulting in the observed distribution pattern (brood mimicry; Høeg & Lützen 1995). However, an alternative explanation for the dominant occurrence in deeper waters may be a higher exposure of crabs to infective propagules in subtidal compared to intertidal habitats. As infected crabs accumulate in the subtidal gullies, the release of nauplius larvae will also predominantly take place here, suggesting a higher infection risk in the subtidal habitat that could lead to a positive feedback loop. However, the development from the nauplius to the infective cypris larval stage takes about 4–5 days (at 12–18°C; Høeg & Lützen 1995); thus, the site of larval release and the site of actual infection are

most likely disconnected due to some dispersal of the infective stages, especially in turbulent and current rich tidal ecosystems like the Wadden Sea. This suggests that crabs may become infected either in the intertidal and then migrate towards deeper waters or, alternatively, they become infected in the subtidal and never migrate into the intertidal. The actual causality will only be ascertained experimentally, for example by releasing marked infected crabs in the intertidal and recapturing them over a tidal elevation gradient.

Both the prevalence of infected crabs and of ovigerous females significantly increased with water depth. Furthermore, there was an indication (marginally significant or non-significant) of an increase in prevalence of infected crabs with salinity. Hence, both abiotic factors are probably relevant for reproduction, and the migration towards the subtidal gullies may ultimately be related to the conditions faced by the larval stages of the crab and the parasite. Deep and saline water may provide favourable physiological conditions for the larval stages of both the host and the parasite. Indeed, mortality of larval stages of crabs and rhizocephalan barnacles is known to increase with decreasing salinity (Thresher 1996, Anger et al. 1998, Kashenko & Korn 2002, Anger 2003). In addition, larval release and subsequent dispersal may be more successful in deeper waters than in the intertidal, where larval stages most likely will face higher risks of heat stress in the warmer months (Thresher 1996). Regardless of the exact mechanisms, water depth and salinity seem to explain at least in part the observed differences in prevalence among the 12 locations investigated in the Wadden Sea. They may also underlie the differences in prevalence of *Sacculina carcini* infection compared to other locations where the parasite has been studied. In general, prevalences of *S. carcini* infection are known to vary widely with prevalence of over 50% observed at some localities along the native range of the shore crabs (Bourdon 1960, Minchin 1997, Torchin et al. 2001, J. T. Høeg pers. comm.). However, more detailed studies will be necessary to unravel the underlying mechanisms of the large-scale distribution of the parasite.

In general, mature *S. carcini* were mainly observed in crabs between 20 and 45 mm CW in both sexes. In male crabs, however, some infected individuals reached 60 mm in CW. Ovigerous females were also absent in the very small-sized females, with 20 mm CW as the minimum size for egg-bearing females. In contrast to parasitized female crabs, ovigerous females were also numerous in sizes beyond 45 mm CW, reaching a maximum of about 60 mm CW. Similar patterns have been observed in previous studies (e.g., Lützen 1984, Dittmann & Villbrandt 1999, Costa et al. 2013) and result from the biology of crabs and parasites. Infection with *S. carcini* preferentially occurs on recently moulted crabs compared to crabs in intermoult stages (Glenner & Werner 1998). As smaller crabs have higher moulting rates than larger crabs, they face a higher infection risk. Moreover, as moulting ceases in infected crabs with an externa (O'Brien & Van Wyk 1985), they do not increase in size, causing the observed size distribution. Crabs below 20 mm CW, i.e. crabs of 1 year or younger (Klein Breteler 1975b), barely featured a mature externa of *S. carcini*, which can be attributed to the maturation period of the parasite which at minimum takes about 1 year (Lützen 1984). Even in the very small infected crabs, measuring only a few mm in CW, an externa generally does not appear before crabs reach a CW of around 20 mm (e.g., Lützen 1984, Costa et al. 2013, J. T. Høeg pers. comm.).

Hence, as barely any crabs below 20 mm CW were infected with *S. carcini*, the calculated prevalence values for the different habitats depend to a certain extent on the size range used for calculations. On intertidal bare sand flats, for example, the inclusion of the very abundant juvenile ~ one-year-old crabs (< 20 mm CW) in the prevalence estimates leads to an underestimation in prevalence of infected crabs. If only the potentially infected size range was used, prevalences would be slightly higher, and comparisons with other studies need to take the actual size range of the respective samples into consideration. The actual size range sampled in our study also differed slightly between habitats, as baited traps are known to catch preferentially larger crabs (Williams & Hill 1982, Smith et al. 2004) due to larger individuals being more aggressive, thereby restricting the likelihood of smaller animals entering the trap.

Hence, our prevalence values calculated for mussel beds may be relatively higher compared to intertidal sand and subtidal gullies. However, as there were only very few crabs infected with *S. carcini* on intertidal mussel beds and at intertidal sand flats (11 out of 217 infected crabs), some imprecision in the actual prevalence values among habitats does not affect the overall distributional pattern observed. Another potential problem of using baited crab traps may be a difference in catch efficiency between infected and non-infected crabs, for example caused by lower feeding activity of infected crabs. However, experiments with *S. carcini* infected and non-infected shore crabs did not find any differences in prey consumption rates (Larsen et al. 2013), so that this is unlikely to confound the method. Baited crab traps may also catch less ovigerous females due to the bias towards catching preferentially bigger and male individuals, suggesting the absence of ovigerous females on mussel beds to be an sampling artefact. However, our trawling (where this potential sampling bias is absent) in the intertidal revealed that ovigerous females are generally scarce on sandy flats. In addition, our long-term experience in the field and previous qualitative literature reports (Rasmussen 1959, Rainbow et al. 1979, Lützen 1984) also indicate that ovigerous females and infected crabs are rarely found on mussel beds and in the intertidal in general. This suggests that the pattern of a predominantly subtidal occurrence of ovigerous females and infected crabs is unlikely to be an artefact of our sampling design.

Finally, while the prevalence of *S. carcini* did not differ between sexes, the majority of the infected crabs were of the red colour morph. This dominance of the red colour morph in infected crabs probably results from the fact that moulting ceases after the emergence of the externa in infected crabs (parasitic anecdysis; O'Brien & Van Wyk 1985), with infected crabs remaining at this intermoult stage as long as they carry the externa (Andrieux 1968). A prolonged duration of intermoult stages has generally been associated with a red carapace colour, while recently moulted crabs show a green carapace (Crothers 1968, McGaw & Naylor 1992, Reid et al. 1997, Styrishave et al. 2004). The exact mechanism for this colour change is not known, but it has been suggested that it is related to a denaturation of pigments in the carapace (Reid et al. 1997). The lack of a difference in infection levels between males and females contrasts with previous findings. For example, Costa et al. (2013) found significantly higher prevalence of *S. carcini* in females than in males at some but not all sampling events at the Mondego estuary in Portugal. However, Lützen (1984) found generally lower infection levels in female than in male crabs in the Isefjord in Denmark, and Werner (2001) found similar infection levels of both sexes on the west coast of Sweden. Whether these differences are related to some underlying mechanism or just result from spurious correlations remains to be investigated.

In conclusion, *S. carcini* shows a distinct distributional pattern in the intertidal ecosystem of the Wadden Sea, with most infected crabs occurring in the subtidal and probably driven by water depth and salinity. This indicates that other parasite groups besides the well-documented effects of trematodes are able to affect the distributional patterns of their hosts along tidal elevation gradients. Hence, parasite-mediated distributional patterns may be much more common in marine systems than currently known. More well-replicated studies at multiple locations and use of a nested sampling design including tidal elevation would be valuable to further assess the effect of tidal elevation gradients on the distribution and abundance of other marine parasites.

Acknowledgements

Supplementary material

Figure S7.1: Pearson correlations between the various variables obtained from samples from the subtidal gullies (n = 11 locations: same gully for E022 and E032): prevalence of the rhizocephalan parasite *Sacculina carcini* (Sacculina), prevalence of ovigerous females (Ov- females), density of crabs (Density), water depth (Depth) and salinity (Salinity).
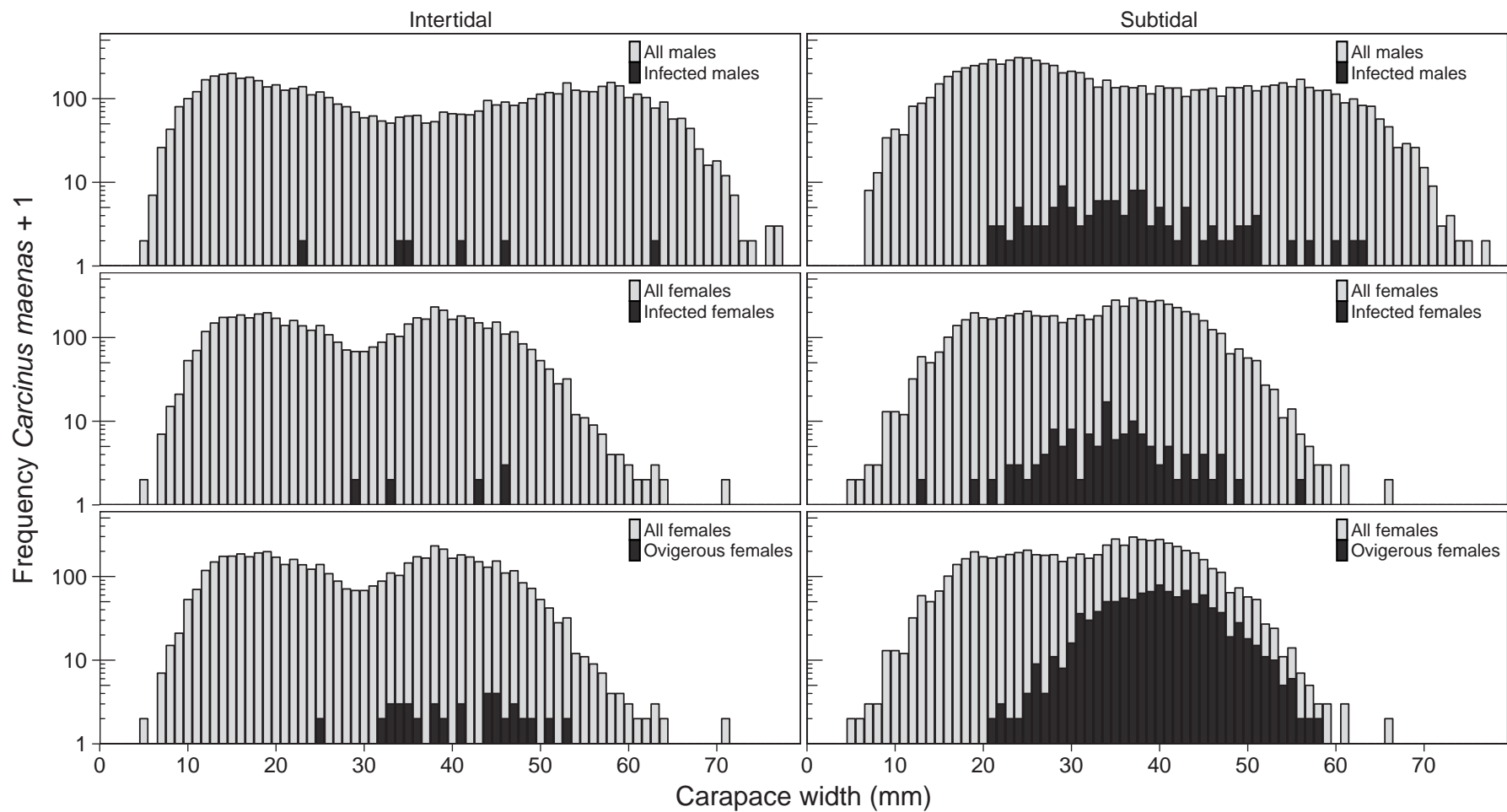
Figure S7.2: Size frequency distributions of *Carcinus maenas* (on a log-scale) in intertidal (left) and subtidal (right) habitats summed for all locations: male crabs infected with *Sacculina carcini* in comparison to all male crabs caught in the intertidal (top left) and in the subtidal (top right); female crabs infected with *S. carcini* and the total of caught female crabs in the intertidal (middle left) and in the subtidal (middle right); and ovigerous female crabs compared to all female crabs caught in the intertidal (bottom left) and in the subtidal (bottom right). All *Carcinus maenas* frequency values were increased by 1 in order to make frequencies of 1 visible.

8 | Cross-species comparison of parasite richness, prevalence and intensity in a native compared to two invasive brachyuran crabs

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Abstract

An introduced species' invasion success may be facilitated by the release of natural enemies, like parasites, which may provide an invader with a competitive advantage over native species (enemy release hypothesis). Lower parasite infection levels in introduced versus native populations have been well documented. However, any potential competitive advantage will depend on whether native competitors exhibit higher parasite loads than introduced hosts and whether native hosts suffer more (e.g., reduced reproduction or growth) from parasite infections than introduced hosts. In this study, we compared macroparasite richness, prevalence, and intensity in sympatric populations of one native and two introduced brachyuran crab hosts in the centre of their European range. While the native green crab *Carcinus maenas* (Linnaeus, 1758) hosted three parasite groups (acanthocephalans, microphallid trematodes, rhizocephalans), the two invasive crab species (*Hemigrapsus sanguineus* (De Haan, 1835) and *H. takanoi* Asakura and Watanabe, 2005 were only infected with acanthocephalans. All acanthocephalans were molecularly identified (COI) as the native *Profilicollis botulus* (Van Cleave, 1916). Prevalence and intensities of *P. botulus* were generally lower in the introduced than in the native crabs. Metacercariae of microphallid trematodes were only found in the native *C. maenas*, with mean infection levels of 100–300 metacercariae per host, depending on geographical location. Likewise, the castrating rhizocephalan barnacle *Sacculina carcini* Thompson, 1836 was only found in *C. maenas* at a few locations with low prevalences (< 3%). This first study on infection levels in invasive *Hemigrapsus* species in Europe indicates that these invasive crabs indeed experience lower infection levels than their native competitor *C. maenas*. Future experiments are needed to investigate whether this difference in infection levels leads to a competitive advantage for the invasive crab species.

Introduction

One of the mechanisms potentially facilitating the invasion success of introduced species is the release from natural enemies during the process of translocation (*enemy release hypothesis*; Elton 1958, Keane & Crawley 2002). During translocation, various barriers can reduce the number of predators and parasites that are co-introduced to the species' new range (Keane & Crawley 2002, Torchin et al. 2003, Colautti et al. 2004). This reduction in or release from enemies can result in direct fitness benefits for introduced populations when a species is negatively affected by the lost enemies in its native region (*regulatory release*; Colautti et al. 2004). In addition, a reduced set of enemies in the introduced range may release physiological resources otherwise invested in defence mechanisms (e.g., immune system) leading to increased fitness of the introduced host (*compensatory release*; Colautti et al. 2004). The two types of release are not mutually exclusive and may lead to a competitive advantage for introduced species over native species (Keane & Crawley 2002, Grosholz & Ruiz 2003, Mitchell & Power 2003, Torchin et al. 2003, Parker et al. 2013).

Regarding parasites, a general reduction of parasite burdens in introduced hosts has been well documented and seems to be particularly strong in aquatic ecosystems (Torchin et al. 2003, Torchin & Lafferty 2009, Blakeslee et al. 2013). A review by Torchin et al. (2003) showed that parasite richness (number of species) and prevalence (proportion of hosts infected) are, on average, 2–3 times lower in hosts in their introduced compared to their native range. In general, the level of parasite reduction seems to differ among parasite groups. For example, in marine ecosystems, rhizocephalan parasites seem to be regularly lost during the process of introduction, while other parasite groups, like cestodes, are usually lost at a lower frequency (Blakeslee et al. 2013). It is important to note that the parasite richness of introduced hosts often consists of co-introduced parasites, but also of native or previously established parasites that have been acquired by the introduced species in the invasive range (Torchin & Mitchell 2004). This parasite acquisition may ultimately amplify the population size of these parasites and increase parasite loads in native hosts (*parasite spillback*; Kelly et al. 2009). Regardless of the parasite origin and level of reduction, the generality of the observed patterns suggests that many introduced hosts may have a competitive advantage over native species due to regulatory and compensatory release. However, a potential competitive advantage will depend on whether native competitors actually exhibit higher parasite loads than introduced hosts and whether native hosts suffer more (e.g., reduced reproduction or growth; Calvo-Ugarteburu & McQuaid 1998a;b, Byers 2000, Bachelet et al. 2004) from parasite infections than introduced hosts (Torchin & Mitchell 2004, Hatcher et al. 2006, Torchin & Lafferty 2009, Dunn et al. 2012). Studies comparing local infection levels between competing native and introduced hosts (community studies or cross-species comparisons, *sensu* Colautti et al. 2004, Torchin & Mitchell 2004) suggest that parasite richness, prevalence, and abundance are indeed often higher in native compared to introduced host species (Georgiev et al. 2007, Dang et al. 2009, Roche et al. 2010, Gendron et al. 2012). However, for most introduced host species, such cross-species comparisons between introduced and native competitors are lacking. This is also true for brachyuran crab species, some of which have been globally introduced into coastal waters and have been studied with respect to parasite release. The most prominent case is the European green crab *Carcinus maenas* (Linnaeus, 1758), which has been introduced to North America, Australia, Tasmania and parts of Japan and South Africa (Carlton & Cohen 2003). In a seminal study, Torchin et al. (2001) investigated infection levels in global *C. maenas* populations and found that crabs in native populations generally harboured more parasite species and showed higher infection levels than populations in areas where the crab species had been introduced. However, how this general parasite release of introduced *C. maenas* compares to parasite infection levels in native competitors has not been investigated to date. Another invasive crab species, the Asian shore crab *Hemigrapsus sanguineus* (De Haan, 1835), has been introduced from the North-West Pacific (with a native

range from Russia along the coasts of Japan, Korea and China up to Hong-Kong; Epifanio 2013) to the North Atlantic coasts of North America (Williams & Mcdermott 1990, Epifanio 2013) and Europe (Dauvin et al. 2009). In North America, only three parasite species have been found in introduced populations of *H. sanguineus* (Torchin et al. 2001, Blakeslee et al. 2009, Kroft & Blakeslee 2016), while the crab species is infected with nine parasite species in native locations (reviewed in Blakeslee et al. 2009, McDermott 2011). Furthermore, infection intensities were much lower in populations in the introduced compared to the native range (Blakeslee et al. 2009, McDermott 2011). In comparison to other crab species at the Atlantic coast of North America, there was no significant difference in parasite richness and prevalence between the invasive *H. sanguineus* and two native crab species (Kroft & Blakeslee 2016), but compared to another invader, *C. maenas*, richness and prevalence were relatively lower in *H. sanguineus* (Blakeslee et al. 2009). However, whether *H. sanguineus* also shows enemy reduction in Europe is presently unknown. A third species, the brush-clawed shore crab *Hemigrapsus takanoi* Asakura and Watanabe, 2005, has been introduced to Europe from the same region as *H. sanguineus* and now occupies the same range in Europe as its congener (northern Spain to Sweden Dauvin et al. 2009, NORSAS 2012, Markert et al. 2014). As *H. takanoi* was only recently identified as a pseudocryptic sibling species of *H. penicillatus* (Takano et al. 1997, Asakura & Watanabe 2005), literature records on parasite infections from native and introduced populations do not exist.

This study conducted a cross-species comparison of macroparasite infection levels in the two Hemigrapsus species (*H. sanguineus* and *H. takanoi*) introduced to Europe, with infection levels in their main native competitor (*C. maenas*). By sampling sympatric populations of the three species in the Dutch Wadden Sea, located in the centre of the invasive European range of the two species of *Hemigrapsus*, the study aimed to answer two main questions: 1) Is there evidence for parasite reduction in European populations of the two introduced *Hemigrapsus* species?; and 2) how do parasite richness and infection levels of the introduced crabs compare with those levels of their main native competitor *C. maenas*? As *Hemigrapus* spp. is currently expanding its range, and negative impacts of invasion have been documented in the US where it is also invasive (e.g., Lohrer & Whitlatch 2002, Tyrrell et al. 2006, Brousseau et al. 2014), this first investigation on parasite infections in introduced *Hemigrapsus* species in Europe contributes to the understanding of the magnitude and relevance of parasite release for native and introduced host populations.

Material and Methods

Sampling and dissection

Sampling of crabs was carried out between May and September 2012 at ten locations around the island of Texel in the southern Wadden Sea in the Netherlands (Figure 8.1, Table 8.1). In the intertidal zone, three habitats (dykes reinforced with rocks, epibenthic bivalve beds composed of invasive Pacific oysters (*Crassostrea gigas*) and native blue mussels (*Mytilus edulis*), and sandy tidal flats) were sampled at low tide by collecting crabs (> 1 cm carapace width) by hand and by setting crab traps which were retrieved the following low tide. Previous studies indicated that crabs can be collected without a size class bias by these methods (Landschoff et al. 2013). In the subtidal zone, a single location was sampled by collecting crabs caught in a kom-fyke net used by the NIOZ Royal Netherlands Institute for Sea Research for long-term monitoring of fish and macroinvertebrates (Campos et al. 2010, van der Veer et al. 2015). Sample sizes depended on local abundances of crabs and generally differed among locations and the three crab species (Table 8.1).

Figure 8.1: Sampling locations of crabs (1–10) around the island of Texel in the southern Wadden Sea in the Netherlands (black dot in the small insert left top corner) as well as sampling location of gull colony on Texel (G) from which additional acanthocephalans were sourced from gulls for molecular identification.

After collection, all crabs were brought to the laboratory and stored frozen at -18 °C for later dissections. The dissection protocol for crabs was similar as the one described by Torchin et al. (2001). Prior to dissection, sex was determined for each crab, identified to species level, and carapace width (CW in mm) measured between the fifth spines on the dorsal side of the carapace. Before removing the carapace, crabs were checked for infection by the rhizocephalan *Sacculina carcini* Thompson 1836 (visible externa). As early infections without a visible externa could not be detected with this approach, our estimates of rhizocephalan infection levels are conservative. The carapace was then opened, the internal carapace tissue carefully removed and squeezed between two large glass plates and examined under a stereomicroscope. All macroparasites found were identified and counted. Acanthocephalans found in *Carcinus maenas* and the two *Hemigrapsus* species were carefully removed from the tissue and stored in pure ethanol for molecular analysis.

Molecular identification

To identify potentially introduced acanthocephalans, a sub-set of acanthocephalans found in the two invasive crab species (*Hemigrapsus takanoi* n = 14, *Hemigrapsus sanguineus* n = 10) and of the ones found in *C. maenas* (n = 17; one acanthocephalan per individual crab) were molecularly identified (Supplementary information Table 8.2). To compare the data from larval stages collected from crab hosts with adult stages from local definitive hosts, we also added two adult parasites that were retrieved from two Herring Gull (*Larus argentatus*; Pontoppidan, 1763) chicks from a breeding colony on Texel (e.g., Camphuysen 2013) (Figure 8.1; see Supplementary information for the dissection protocol).

Parasite genomic DNA was extracted using the GenElute Mammalian Genomic DNA Miniprep Kit (Sigma) according to the manufacturer's instructions. DNA concentrations were determined using spectrophotometry (ND-1000, NanoDrop Technologies). New primers were

Table 8.1: Habitat, sampling dates and sample sizes of native (*Carcinus maenas*) and invasive (*Hemigrapsus* spp.) crabs collected in the Wadden Sea around the island of Texel.

Location	Habitat	Sampling dates	*C. maenas* Females	Males	*H. sanguineus* Females	Males	*H. takanoi* Females	Males
1	Dyke	18, 19 Jun	10	56	40	11	7	27
2	Mussel/oyster bed	19, 29 Jun; 16 Aug	38	94	3	0	0	3
3	Mussel/oyster bed	14 Aug	4	22	17	1	5	19
4	Dyke	4 Jun; 2 Jul	7	5	28	24	5	26
5	Dyke	30 May; 1, 5, 12 Jun; 5 Jul	21	16	29	16	5	23
6	Mussel/oyster bed	24 May; 5 Sep	62	22	10	2	5	15
7	Dyke	7, 20, 21 Jun	14	23	25	12	8	33
8	Channel	20 Jun	5	31	0	0	0	0
9	Sand flat	18 Jun	4	14	0	0	0	0
10	Sand flat	18 Jun	3	4	0	0	0	0
Total			**168**	**287**	**152**	**66**	**35**	**146**

designed based on an alignment of COI sequences for the acanthocephalans *Profilicollis botulus* (Van Cleave, 1916) and *Polymorphus minutus* (Zeder, 1800) (Genbank accession numbers EF467862 and EF467865, respectively). With the help of the primers AcaCOf (TGATATATGTTTT GGTTAGGTTRTGAA) and AcaCOr (CACCYCCTGTAGGATCAAAA), a portion of the cytochrome-c-oxidase I (COI) gene was amplified in a total volume of 50 µl containing 1× PCR buffer, 0.25 mM of each dNTP, 1 µM of each primer and 1 unit Biotherm+ DNA polymerase, using 2 µl undiluted DNA extract. Initial denaturation was performed at 94 °C for 2 min., followed by 35 cycles of denaturation for 30 s at 94 °C, annealing for 30 s at 55 °C and extension for 1 min. at 72 °C, with a final extension step of 72 °C for 10 min. Sequencing of the PCR products was carried out at Macrogen, Korea. Sequences were aligned manually in BioEdit 7.2.5 (Hall 1999) (Hall 1999) and compared to published acanthocephalan COI sequences. Genetic distances were estimated with MEGA 6 (Tamura et al. 2013), and minimum spanning networks among all haplotypes detected was constructed using the R package Pegas version 0.8-2 (Paradis 2010).

Phylogenetic trees were constructed in MEGA 7 (Kumar et al. 2016) by adding as outgroup two acanthocephalan sequences from different species (EF467865 from *P. minutus* and KF835320 from *Profilicollis altmani* (Perry, 1942) Van Cleave, 1947). A condensed Maximum Parsimony Tree was produced by using ten random addition trees and 500 bootstrap replicates. For the Maximum Likelihood Tree the best nucleotide substitution model was selected to be HKY+G (Hasegawa-Kishino-Yano with gamma distribution) based on both the AIC (Akaike Information Criterion) and the BIC (Bayesian Information Criterion) criteria, and 500 bootstrap replicates were run.

Statistical analyses

For each location and crab species, prevalence (proportion of infected crabs) and mean intensities (no. of parasites per infected crab) were calculated. Differences in prevalence between species or between locations were tested with likelihood-ratio tests (G-tests). Differences in intensity of acanthocephalans among the three hosts and the sampling sites were tested with general linear models (GLM) with intensities (log-transformed) as response variable and location and crab species as fixed factors. These analyses included all locations at which the three crabs co-occurred. Differences in intensities of trematodes in *C. maenas* among locations were tested with a GLM with intensity as response variable and location as fixed factor. Test assumptions were verified by inspecting residual plots. Relationships between intensity and host size as well as between prevalence and mean intensity per location among the three crab species were tested with Spearman correlations. All analyses were performed using the statistical software R v3.2.1. (R Development Core Team 2015).

Results

We sampled 854 crabs from ten locations: 455 were *Carcinus maenas*, 218 were *Hemigrapsus sanguineus*, and 181 were *Hemigrapsus takanoi* (Table 8.1). Although not quantified, *C. maenas* seemed more abundant on mussel/oyster beds than *Hemigrapsus* spp., while along dykes it was the opposite. *Hemigrapsus* spp. were absent at the subtidal location (location 8) and the two sandflat locations (locations 9 and 10).
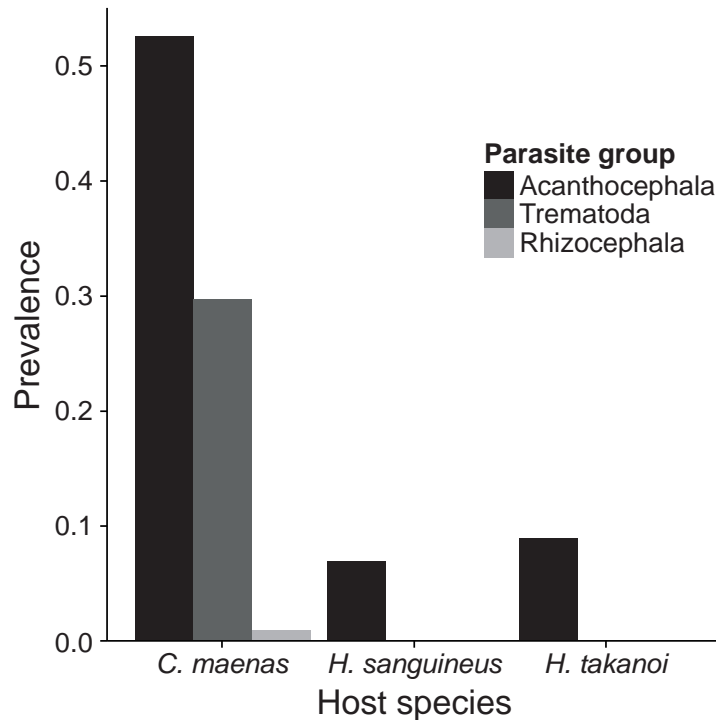

Figure 8.2: Overall prevalence of the three parasite groups (Acanthocephala, Trematoda, Rhizocephala) found in the three crab host species *Carcinus maenas* (n = 10 sampling locations), *Hemigrapsus sanguineus* and *Hemigrapsus takanoi* (both n = 7 sampling locations).

Carcinus maenas was infected by three parasite taxa: acanthocephalans, trematodes, and rhizocephalans (Figure 8.2). In contrast, the *Hemigrapsus* species were only infected by acanthocephalans. In these two invasive crab species, prevalence was generally lower than in the native *C. maenas* (G-test, G = 218.68, p < 0.001). All acanthocephalans (cystacanth stage) molecularly identified (sequences deposited at Genbank, accession numbers KX279893-KX279935) from the two *Hemigrapsus* species, *C. maenas*, and Herring Gull chicks were *Profilicollis botulus* (Van Cleave, 1916) (García-Varela & Pérez-Ponce de León 2008). The maximum p-distance among any two sequences was 0.0243, while the smallest distance to any sequence in Genbank except *P. botulus* was p = 0.1739 to *Profilicollis altmani* (Perry, 1942) Van Cleave, 1947 (KF835320) (see also Figures S8.1 and S8.2). The trematodes found in *C. maenas* were metacercarial stages of microphallids, with a mix of two probably native species, *Maritrema subdolum* Jägerskiöld, 1909 and *Microphallus claviformis* (Brandes, 1888), based on previous investigations in the study region (Thieltges et al. 2008a). However, more detailed molecular analyses are pending.

Within individual sampling locations, acanthocephalan prevalence was significantly higher in native *C. maenas* than in the two invasive *Hemigrapsus* species in all but one location (Figure 8.3A; location 2: G-test, G = 3.050, p = 0.218, for all others p < 0.01). Within species, prevalence differed among the sampling locations in *C. maenas* (G-test, G = 39.271, p < 0.001) but not in *H. sanguineus* (p = 0.088) and *H. takanoi* (p = 0.107). Intensity of acanthocephalan infection
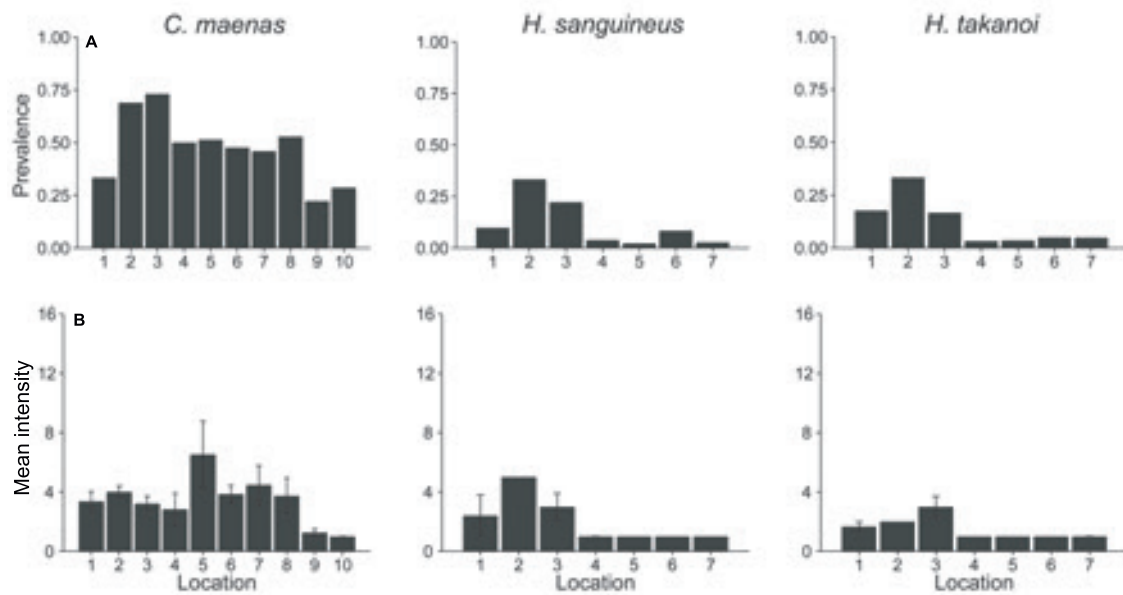
Figure 8.3: A) Prevalence and B) mean intensity (± SE) of acanthocephalan infections in the three crab host species at the sampling locations. For sample size per location see Table 8.1. Note that both *Hemigrapsus* species were only present at locations 1–7.
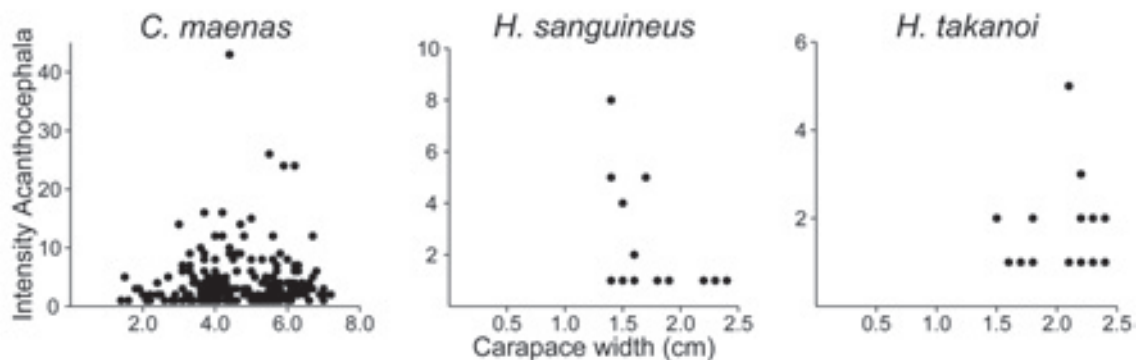

Figure 8.4: Intensity of acanthocephalans in infected individuals of the three host crab species (*Carcinus maenas*; n = 239, *Hemigrapsus sanguineus*; n = 15, *Hemigrapsus takanoi*; n = 16) depending on host size (carapace width). Note the different axes scales.

significantly differed between species, with highest infection levels in *C. maenas* (Figure 8.3B; GLM, $F_{2,242}$ = 6.172, p < 0.01). Although mean intensities tended to differ among locations (Figure 8.3B), this was not statistically significant (GLM, $F_{6,236}$ = 0.793, p = 0.570). There was also no statistically significant interaction between location and crab species (GLM, $F_{12,224}$ = 0.626, p = 0.819). Intensity of acanthocephalan infections in individuals of the three host crab species did not significantly increase with host size (Figure 4, Spearman correlation, all p > 0.170). Both females and males of the three crab species were infected, and the size of infected individuals ranged between 14–72 mm CW for *C. maenas* and between 14–25 mm CW and 15–25 mm CW for *H. sanguineus* and *H. takanoi*, respectively (Figure S8.3). Mean prevalence per location was positively correlated between *H. sanguineus* and *H. takanoi* (Spearman correlation, Spearman's ρ = 0.86, p = 0.014) but not between *C. maenas* and *H. sanguineus* (Spearman's ρ = 0.36, p = 0.432) or *H. takanoi* (Spearman's ρ = 0.11, p = 0.819; Figure S8.4). Similarly, mean intensity at the different locations was not correlated between *C. maenas* and *H. sanguineus* (Spearman's ρ = -0.20, p = 0.672) nor between *C. maenas* and *H. takanoi* (Spearman's ρ = -0.32, p = 0.491), but positively correlated between the two *Hemigrapsus* species (Spearman's ρ = 0.96, p < 0.001;

Figure S8.5).

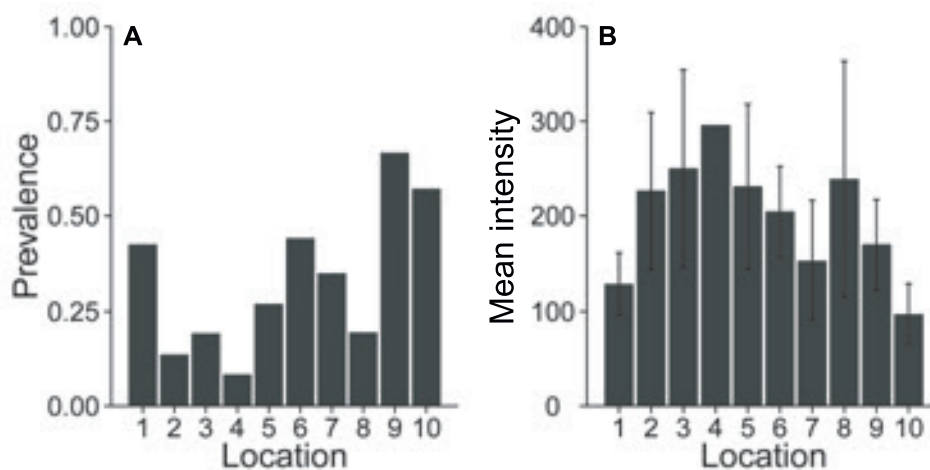**Figure 8.5:** A) Prevalence and B) mean intensity (± SE) of trematode infections in *Carcinus maenas* (n = 135) at the 10 sampling locations. For sample size per location see Table 8.1.
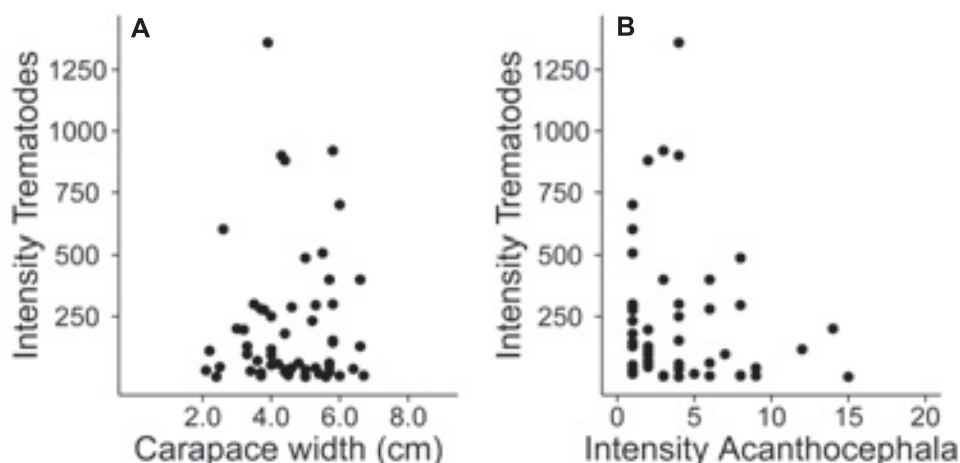
Figure 8.6: A) Intensity of trematodes in infected *Carcinus maenas* crabs depending on host size (n = 135) and B) intensity of acanthocephalan infections (n = 57).

Prevalence of trematode infection in the native *C. maenas* varied significantly among locations (G-test, G = 51.501, p < 0.001), with the two sand flat habitats (locations 9 and 10) showing highest prevalences (Figure 8.5A). In contrast, intensities did not differ significantly between the ten locations (Figure 8.5B; GLM, $F_{9,125}$ = 0.480, p = 0.886). Crabs were, on average, infected with 100–300 metacercariae of microphallid trematodes depending on location (Figure 8.5B), and individual crabs were infected with up to 1,400 metacercariae. Like acanthocephalan infections, trematodes infected both sexes of *C. maenas* and the size of infected individuals (14–67 mm CW) was similar to that of uninfected crabs (13–75 mm CW, Figure S8.6). Mean intensity in infected crabs did not significantly correlate with crab size (Spearman's correlation, Spearman's ρ = 0.15, p = 0.080, Figure 8.6A). Moreover, the intensity of trematodes was independent of acanthocephalan intensity (Spearman's ρ = -0.12, p = 0.364; Figure 8.6B). Prevalences of infections of *C. maenas* with the rhizocephalan *S. carcini* (based on visible externa) were generally very low (< 3%) and only occurred at four of the ten locations (Figure S8.7).

Discussion

In this study, we compared parasite richness, prevalence, and intensities in sympatric populations of a native and two introduced brachyuran crab host species in the centre of their European range. While the native green crab *Carcinus maenas* hosted three parasite groups (acanthocephalans, microphallid trematodes, rhizocephalans), the two invaders (*Hemigrapsus sanguineus* and *H. takanoi*) were only infected with one group (acanthocephalans). All acanthocephalans were identified as *Profilicollis botulus*.

In this first study on parasite richness in the two invasive crab species in Europe, we found fewer parasite species compared to findings from invasive *H. sanguineus* populations in North America, where the crabs are infected with three parasite species (an unidentified nematode, a microphallid trematode, and an acanthocephalan – most likely *P. botulus*; Torchin et al. 2001, Blakeslee et al. 2009, Kroft & Blakeslee 2016). In contrast to invasive populations in America and Europe, *H. sanguineus* is infected by at least nine microsporidian, rhizocephalan, or trematode parasite species in its native range (Blakeslee et al. 2009, McDermott 2011). Hence, *H. sanguineus* seems to have a reduced set of parasites in its introduced range in Europe (parasite reduction), similar to observations in North America and corresponding with findings in introduced populations of other crab species like *C. maenas* (Torchin et al. 2001). The other introduced crab species, *H. takanoi*, was also only infected with acanthocephalans. As *H. takanoi* was only recently identified as a sibling species of *Hemigrapsus penicillatus* (De Haan, 1835) (Asakura & Watanabe 2005), no literature records on parasite infections from native populations exist. However, for the sibling species *H. penicillatus*, at least eight parasite species have been reported from its native range, with many of the species also infecting *H. sanguineus* (McDermott 2011). This suggests that parasite escape is also likely for the invasive populations of *H. takanoi* in Europe. However, data on infection levels within the native range of this species will be needed for a final assessment of the existence of parasite release.

While the two introduced crab species, *H. takanoi* and *H. sanguineus*, have escaped their native parasites, they have recently acquired an acanthocephalan parasite species in their introduced European range. Our molecular analyses indicated that all acanthocephalans belonged to the same species (*P. botulus*), which has never been recorded in the native range of *Hemigrapsus* spp. (McDermott 2011) and, therefore, was unlikely to be co-introduced by the invasive crabs. However, whether *P. botulus* is native in Europe, a recent invader from North America, or native to both regions is difficult to ascertain.

The acanthocephalan *P. botulus* has been recorded extensively in the northeast Atlantic, but has also been found in the northwest Atlantic (Van Cleave 1916) and the northeast Pacific (Ching 1989). Our own sequences show that all sequences known to date, which originate from Herring Gulls (Wadden Sea area), three crab species (Wadden Sea area), and two waterfowl species (Mallard *Anas platyrhynchos* Linnaeus, 1758 from Pacific North America and Common Eider *Somateria mollissima* Linnaeus, 1758 from Denmark), all group together in one haplotype network (Figure S8.1). Phylogenetic analyses with an outgroup also demonstrate that all our sequences belong to the same cluster and that there is no support for separate clades within the *P. botulus* sequences (Figure S8.2). We can therefore be confident that all acanthocephalans encountered belong to the same species. However, we cannot be certain that the *P. botulus* we identified from the Wadden Sea is native to the area. Alternatively, *P. botulus* is native to North America and may have recently been introduced from there to the northeast Atlantic and our study area. In addition, it is also possible that the species has a wide natural distribution without population differentiation, perhaps as a result of natural dispersal vectors such as migratory birds or widely distributed species such as Herring Gull. A more detailed phylogeographic study is needed to distinguish among these possibilities. The level of variability we observed is rather high (Figure S8.1), which may be interpreted in favour of the parasite being native to the study area. Hence, we tentatively assume that *P. botulus* is most likely native in the study region.

Introduced and native crabs differed in infection levels of the acanthocephalans, with generally lower prevalences and intensities in the introduced than in the native crabs. It is unlikely that this is only due to the size difference among the crab species, as crab size was not a significant predictor of infection intensity, suggesting that other factors are more important in determining the differences in infection levels between native and invasive crabs. Given that prevalence in both *Hemigrapsus* species was strongly correlated and that this was not the case between *Hemigrapsus* species and *C. maenas*, the underlying mechanisms may be the same for the two *Hemigrapsus* species. Besides size, host age may explain differences in infection intensity between invasive and native hosts, as host age is usually correlated with the actual parasite exposure over time, suggesting higher intensities in older crabs. Based on published maximum carapace width (75 mm CW; Klein Breteler 1976a, Wolf 1998, Chapter 7: Waser et al. 2016b) and ages (Dries & Adelung 1982, Lützen 1984) of native *C. maenas* in Europe, the 20–70 mm CW of sampled shore crabs corresponded with ages between 2–4 years. Similarly, based upon studies in the European introduced range, the 15 to 25 mm CW of both invasive *Hemigrapsus* crabs corresponded with an age of 2–3 years (Dauvin 2009, Gothland et al. 2014). Hence, the native green crab *C. maenas* sampled was probably slightly older and had the potential to acquire more parasite infections over time. Therefore, age may be one contributor to the differences in infection intensity between native and invasive crabs. Similarly, it may be that the actual exposure (sensu Combes 2001) to infective stages shed by bird definitive hosts into the environment differs between the native *C. maenas* and the two invasive *Hemigrapsus* species. While *C. maenas* occupies both subtidal and intertidal zones and regularly migrates between the two zones (e.g., Silva et al. 2014), *Hemigrapsus* spp. are often found in between boulders and rocks higher in the intertidal zone (Lohrer et al. 2000 and references therein, Dauvin 2009), which may result in a different likelihood of parasite encounters for invasive crabs. However, in our study, crabs were collected at locations where all species occurred in close sympatry (i.e., on oyster beds and dykes); hence differences in tidal exposure cannot explain differences in infection levels. Nevertheless, the microhabitat use of invasive and native crabs within locations may differ. Due to their small size, both invasive crab species can be expected to hide deeper in mussel and oyster beds or within the boulders and pebbles at the bottom of dykes, potentially reducing exposure to infective stages of acanthocephalans. Physical structures and ambient organisms have been shown in other studies to reduce parasite transmission as a result of interference, predation, or other means (Thieltges et al. 2008b, Johnson & Thieltges 2010), and deserve further experimental study in our system. Alternatively, the lower acanthocephalan prevalences and intensities in invasive compared to native crabs, may result from the fact that both *Hemigrapsus* species do not share an evolutionary history with *P. botulus*, which our evidence suggests is native in the study area. Consequently, the parasite may show a preference for the native crab species, resulting in higher parasite prevalences, intensities, and abundances compared to invasive crabs as observed in other species (Georgiev et al. 2007, Dang et al. 2009, Roche et al. 2010, Gendron et al. 2012). However, given the passive transmission process from *P. botulus* eggs to crabs, this does not seem very likely. The eggs of the acanthocephalan are released via bird faeces into the water column and infection occurs via accidental ingestion of eggs by crabs (Thompson 1985), making the potential for parasite preferences in determining infection levels in crab species rather small. The potential mechanisms discussed above are not mutually exclusive and further experiments and analyses are needed to disentangle the underlying mechanisms of differential infection levels in native and invasive crabs.

Surprisingly, metacercariae of microphallid trematodes were only found in the native *C. maenas* but not in the two invasive crab species, while invasive populations of *H. sanguineus* and *C. maenas* in North America each harbour a microphallid trematode species: *Gynaecotyla adunca* (Linton, 1905) in *H. sanguineus*; and *Microphallus similis* (Jägerskiöld, 1900) Nichol, 1906 in *C. maenas* (Blakeslee et al. 2009, Kroft & Blakeslee 2016). Also in its native range, *H. sanguineus* is commonly infected with several species of trematodes (Blakeslee et al. 2009,

McDermott 2011). This suggests that invasive populations of *Hemigrapsus* in Europe may not serve as suitable hosts for local trematode parasites, although the crabs are, in principle, suitable hosts for trematodes as indicated by infections in their native range and in North America. This absence of trematode infections in *Hemigrapsus* species may again relate to differences in parasite exposure and/or host susceptibility and further experiments will be needed to clarify this. In contrast to *Hemigrapsus*, individuals of *C. maenas* were, on average, infected with 100–300 metacercariae per host at the various locations. Such high infection levels have been previously reported from the wider study region (Thieltges et al. 2008a, Zetlmeisl et al. 2011) and from other native populations in Europe (Zetlmeisl et al. 2011).

In addition to trematodes, visible externa of the rhizocephalan barnacle *Sacculina carcini* were also only observed in *C. maenas*, at a few locations with low prevalence (< 3%), and never in either of the two *Hemigrapsus* species. Such low prevalences in this range have previously been reported from the wider study region (Zetlmeisl et al. 2011, Chapter 7: Waser et al. 2016b). In its native range, *H. sanguineus* is infected by three species of rhizocephalans (reviewed by McDermott 2011), indicating a parasite escape of this group of parasites in European populations of the species. Such a complete loss of rhizocephalan parasites in the course of introductions seems to be a general pattern in marine invasions (Blakeslee et al. 2013).

The observed reduced set of parasites infecting the two invasive *Hemigrapsus* species in the centre of their European range suggests the potential for a competitive advantage of the invasive crabs over the native *C. maenas*. Theoretically, invasive species that escaped their parasites might invest physical resources on host fitness parameters (e.g., reproduction and growth) that might otherwise be spent on immune responses to parasites, enhancing the competitiveness of invasive species (Calvo-Ugarteburu & McQuaid 1998a;b, Byers 2000, Bachelet et al. 2004). However, it is unclear whether the impact of the various native parasite species on the native *C. maenas* is strong enough to mediate competition with the invasive *Hemigrapsus* spp. Castrating parasites like *S. carcini* can substantially reduce the testes weight of green crabs (Zetlmeisl et al. 2011), and the loss of these parasites has been associated with faster growth, greater longevity, and/or greater biomass of invasive green crab populations (Torchin et al. 2001). Nevertheless, the low prevalences with *S. carcini* in our and wider study regions (North Sea and Wadden Sea; Zetlmeisl et al. 2011, Chapter 7: Waser et al. 2016b) suggest that very few individuals are affected by rhizocephalan castration, which is unlikely to translate into sweeping population level effects. Furthermore, a study on the effects of trematode and acanthocephalan infections on the reproduction index of native *C. maenas* could not find any negative effect of the parasites on crab testes weight (Zetlmeisl et al. 2011). In addition, in introduced populations of *C. maenas*, Blakeslee et al. (2015) did not find strong effects of trematode infections on the physiology or behaviour of infected crabs; however, crabs may respond differently in the native range and this remains to be studied. The effects of *P. botulus* infections on the two invasive *Hemigrapsus* species have not been tested, and experiments are needed to investigate whether the observed lower parasite load of invasive crabs compared to the native green crab actually leads to a competitive advantage. Although evidence for effects of the acanthocephalan on native and invasive crabs is lacking, the addition of both invasive *Hemigrapsus* crab species to the host range of *P. botulus* in Europe might have pronounced effects on native birds, the definitive host of the parasite. An increase in the number of competent intermediate host species potentially leads to an amplification of the population size of *P. botulus* in crabs, ultimately resulting in an increase in acanthocephalan infections in bird species that have brachyuran crabs in their diet (parasite spillback). Birds are known to suffer from *P. botulus* infections (e.g., mass mortalities reported for Eider ducks (*S. mollissima*) in Europe and the US, reviewed in Garden et al. 1964) and therefore the inclusion of *Hemigrapsus* spp. in *P. botulus*' host range has the potential to impact higher trophic levels via these parasite spillback effects.

In conclusion, this first study on parasite infection levels in invasive *Hemigrapsus sanguineus* and *H. takanoi* in Europe indicates parasite reduction/escape and lower infection prevalences and intensities in the two invasive crabs compared to their native competitor, the green crab. Although this suggests a potential competitive advantage for invasive crabs, there is limited evidence to date that the fitness of native *C. maenas* is compromised by native parasites. Hence, whether a competitive advantage due to parasite mediated competition for invasive crabs actually exists in these invader-native pairings is questionable and deserves further experimental study. Such community studies or cross-species comparisons are a valuable approach in understanding the actual relevance of enemy release for local communities of native and invasive competitors.

Acknowledgements

Supplementary material

Dissection protocol of Herring Gull *Larus argentatus* chicks for Acanthocephala parasites

Two dead *L. argentatus* chicks belonging to marked nests were collected during the late breeding season (June-July) of 2011 from two different areas within their breeding colony (53°01'N, 04°43'E, Kelderhuispolder, Texel western Wadden Sea, The Netherlands, Figure 8.1). The chicks had been ringed for identification purposes as part of a large monitoring project (e.g., Camphuysen 2013) and their last recorded age was 10 and 25 days old, respectively. Sex of the chicks could not be determined at this stage. Both animals were frozen at -80 °C until later processing. Prior dissections the two chicks were left to thaw overnight at room temperature (~20 °C). Using a surgical scalpel an incision in the abdomen running from above the keel to the height of the cloaca was made exposing the breast muscles. Cutting transversally through each side of the ribs with scissors, the ribcage was lifted to expose the internal organs. The intestines were clipped with scissors at the highest point possible, removed from the animal and immediately dropped in 90% ethanol until further processing. To check for parasites, the intestines were taken out of the ethanol containers and cut into smaller segments to fit in a petri dish under the microscope. Using a surgical scalpel, the segments of the intestine were cut open exposing their content and their lining. Each segment and their content was examined through the microscope and all particles that resembled a parasite were removed with tweezers. Particles determined as parasites were deposited in a glass vial with 90% ethanol after a preliminary morphological identification.

Table 8.2: Sources of acanthocephalans for the molecular identification, indicating location, host species, host sex and host size (carapace width for crabs). For locations see Figure 8.1; F = female; M = male; n/a = not applicable.

No.	Location	Host species	Host sex	Host size (cm)	Genbank accession no.
1	4	*Hemigrapsus sanguineus*	F	1.4	KX279895
2	5	*H. takanoi*	M	2.1	KX279905
3	6	*Carcinus maenas*	M	5.6	KX279919
4	6	*C. maenas*	F	5	KX279920
5	6	*H. takanoi*	M	1.6	KX279906
6	6	*H. takanoi*	M	1.7	KX279907
7	6	*H. sanguineus*	F	2.4	KX279896
8	5	*C. maenas*	F	4.7	KX279921
9	5	*C. maenas*	F	4	KX279922
10	1	*C. maenas*	M	5.6	KX279923
11	1	*C. maenas*	M	3.7	KX279924
12	1	*H. takanoi*	M	2.2	KX279908
13	1	*H. sanguineus*	M	1.6	KX279897
14	9	*C. maenas*	M	6	KX279925
15	9	*C. maenas*	M	3.2	KX279926
16	1	*C. maenas*	M	2.2	KX279927
17	1	*C. maenas*	M	4.6	KX279928
18	1	*H. takanoi*	M	2.4	KX279909
19	1	*H. takanoi*	M	2.2	KX279910
20	1	*C. maenas*	M	5.7	KX279929
21	1	*H. sanguineus*	F	1.8	KX279898
22	1	*H. takanoi*	M	2.4	KX279911
23	1	*H. sanguineus*	F	1.4	KX279899
24	1	*H. takanoi*	M	2.3	KX279912
25	1	*H. sanguineus*	F	1.9	KX279900
26	10	*C. maenas*	M	6.6	KX279930
27	2	*C. maenas*	M	5.5	KX279931
28	2	*C. maenas*	M	3.6	KX279932
29	2	*C. maenas*	F	3.3	KX279933
30	4	*H. takanoi*	M	2.3	KX279913
31	3	*H. sanguineus*	F	1.5	KX279901
32	3	*C. maenas*	M	5.7	KX279934
33	3	*H. takanoi*	M	1.8	KX279914
34	3	*C. maenas*	F	4.3	KX279935
35	3	*H. takanoi*	M	2.1	KX279915
36	3	*H. takanoi*	M	2.2	KX279916
37	3	*H. sanguineus*	F	1.7	KX279902
38	6	*H. sanguineus*	F	1.5	KX279903
39	6	*H. takanoi*	M	1.8	KX279917
40	2	*H. takanoi*	M	1.5	KX279918
41	2	*H. sanguineus*	F	1.4	KX279904
42	G	*Larus argentatus*	n/a	n/a	KX279893
43	G	*L. argentatus*	n/a	n/a	KX279894
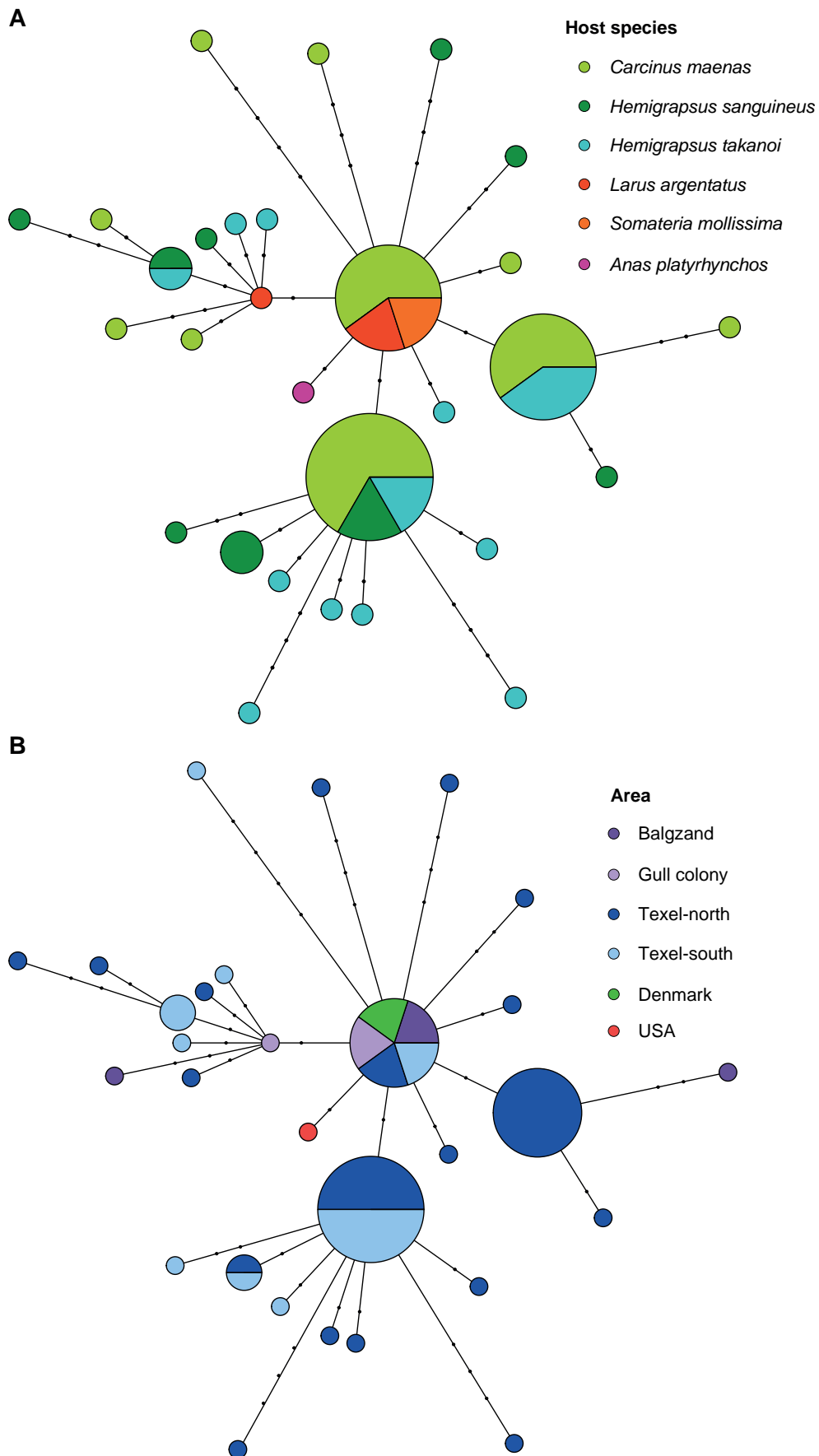
Figure S8.1: Minimum spanning network among partial cytochrome-c-oxidase I haplotypes of *Profilicollis botulus* in different A) host species and B) sampling areas.
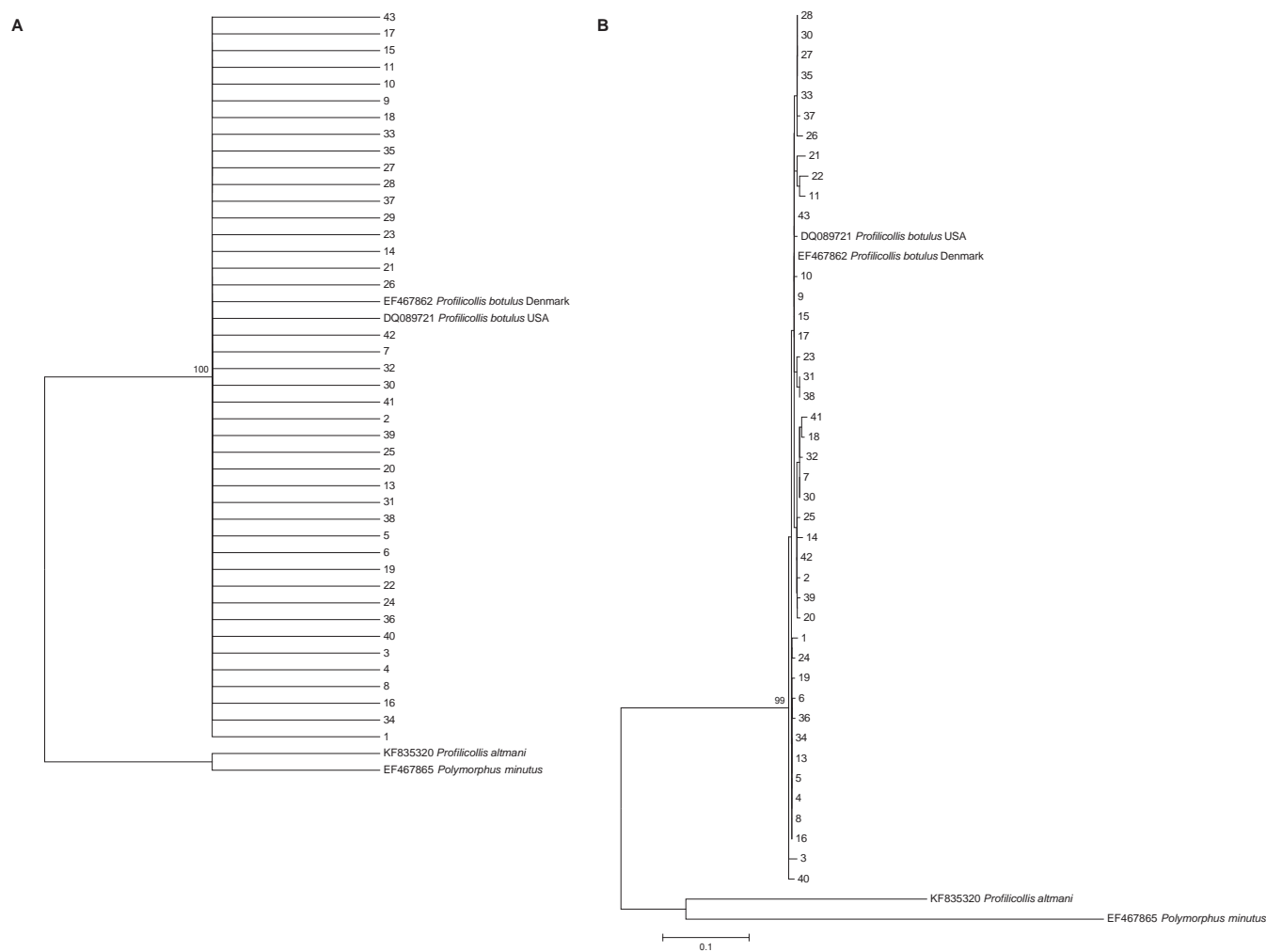
Figure S8.2: Rooted phylogenetic trees of *Profilicollis botulus* based on partial COI sequences. A) Maximum parsimony tree; B) Maximum likelihood tree. Values on trees are bootstrap values from 500 bootstrap replicates; values below 80% are not shown.
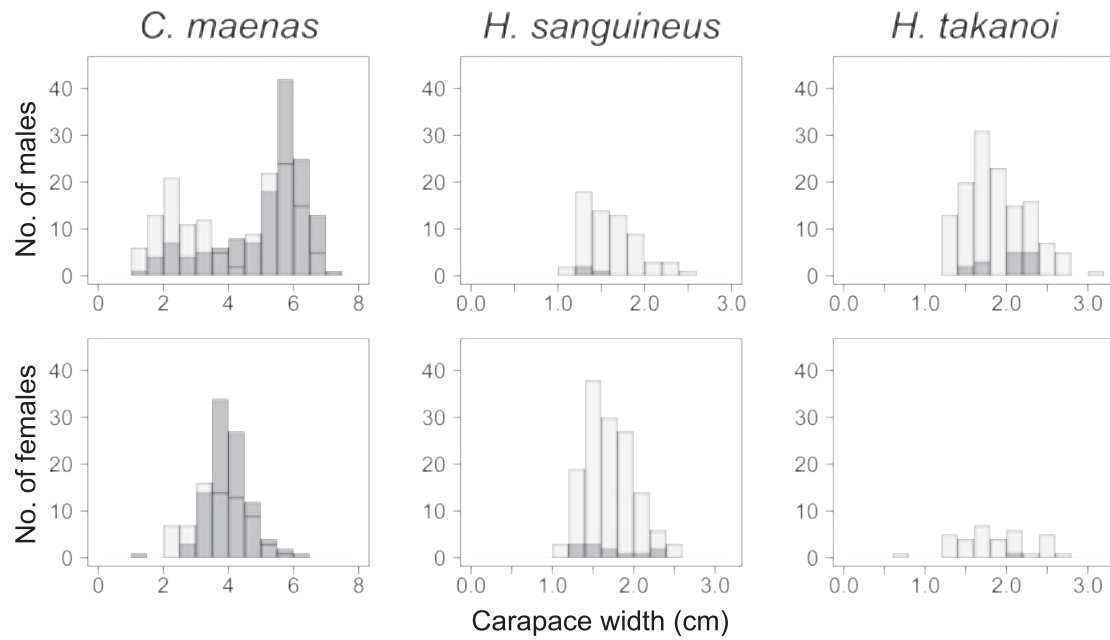
Figure S8.3: Frequency distribution of crab host sizes of the three crab species (*Carcinus maenas, Hemigrapsus sanguineus, Hemigrapsus takanoi*) separated into males (above) and females (below), with dark grey bars indicating crabs infected with acanthocephalans and in a transparent light grey layer on top the numbers of uninfected crabs. Both uninfected and infected crabs had sometimes overlapping sizes, resulting in intermediate grey bars. For sample sizes see Table 8.1.
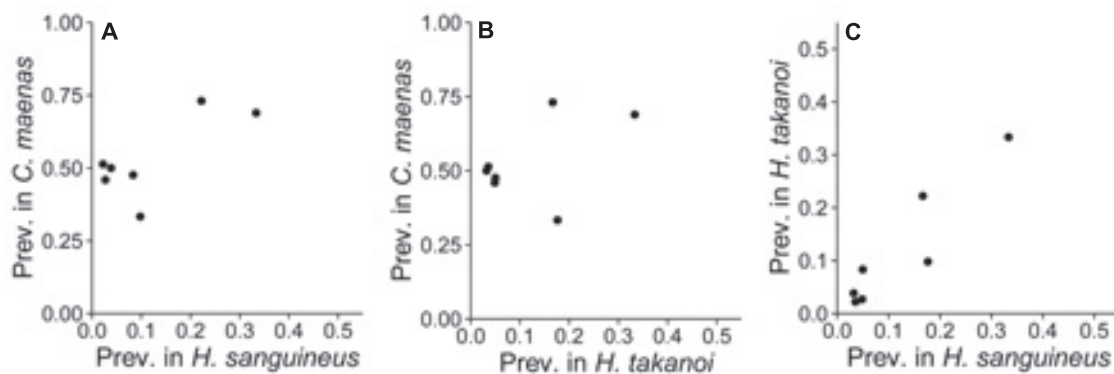
Figure S8.4: Correlations of prevalences of acanthocephalan infections at 7 sampling locations between A) *Carcinus maenas* and *Hemigrapsus sanguineus*, B) *C. maenas* and *Hemigrapsus takanoi*, and C) *H. takanoi* and *H. sanguineus*. For sample sizes see Table 8.1.
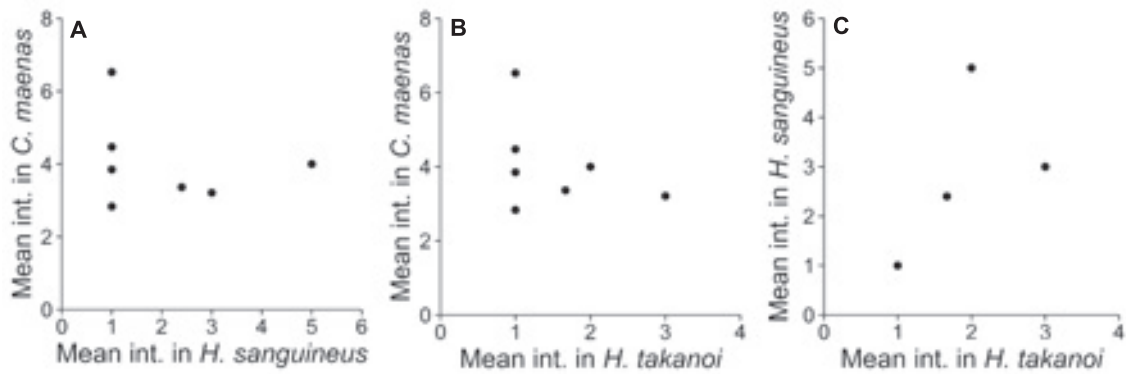
Figure S8.5: Correlations of mean intensities of acanthocephalan infections at 7 sampling locations between A) *Carcinus maenas* and *Hemigrapsus sanguineus*, B) *C. maenas* and *Hemigrapsus takanoi*, and C) *H. takanoi* and *H. sanguineus*. In this figure four locations had a mean intensity of 1 for both introduced crab species.
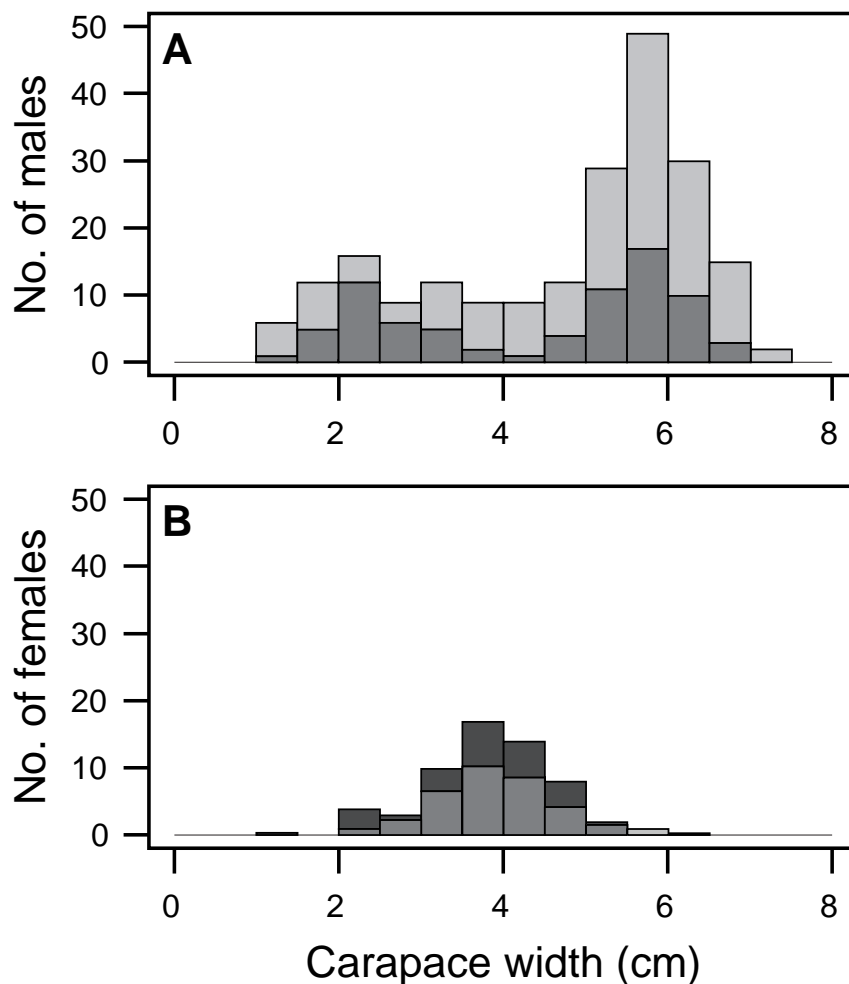
Figure S8.6: Frequency distribution of *Carcinus maenas* carapace sizes separated into males (A) and females (B), with dark grey bars indicating crabs infected with trematodes and in a transparent light grey layer on top the numbers of uninfected crabs. Both uninfected and infected crabs had sometimes overlapping sizes, resulting in intermediate grey bars. For sample sizes see Table 8.1.
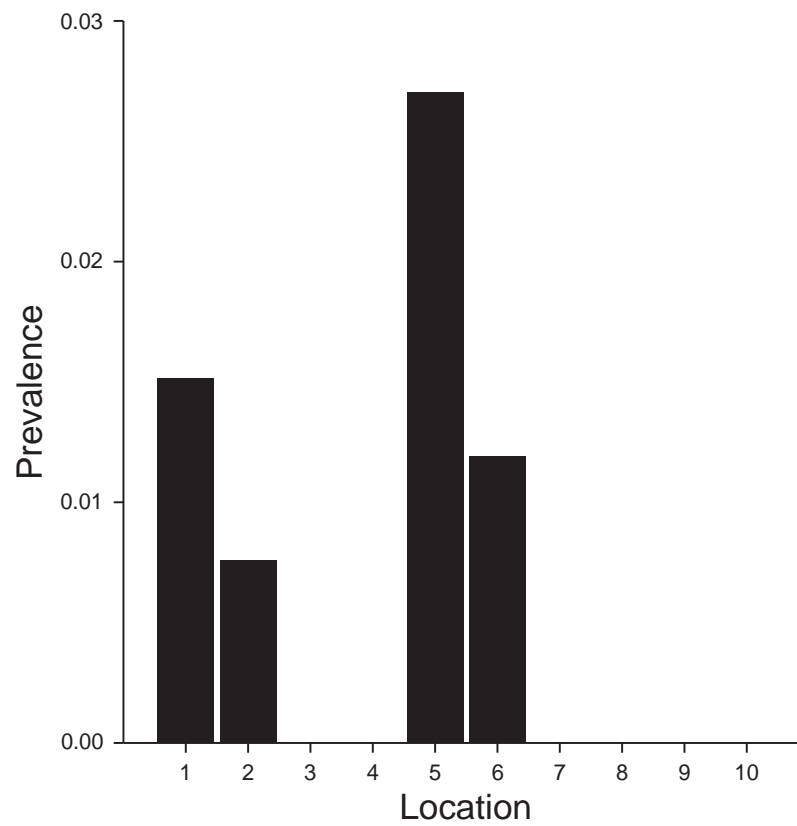
Figure S8.7: Prevalence of the rhizocephalan *Sacculina carcini* in *Carcinus maenas* crab hosts at the 10 sampling locations. For sample sizes see Table 8.1.

9 | General discussion

Andreas M. Waser

The work presented in this thesis was part of two major research projects ('Mosselwad' and 'Waddensleutels'), exploring the need and the possibilities for mussel bed restoration in the Dutch Wadden Sea. The aim of the work was to investigate how predation on intertidal mussels affects the survival of littoral beds. The findings of this thesis supplement previous work that focussed on other aspects affecting the stability of intertidal mussel beds in the Dutch Wadden Sea (Donker 2015, de Paoli 2017). In this final Chapter, the main results and conclusions are summarized and their implications for mussel bed restoration measures are discussed.

Regional differences in predation pressure on intertidal mussels

Mussel beds are a common habitat feature throughout the Wadden Sea (Dijkema et al. 1989, Dijkema 1991, Folmer et al. 2014). Areas with low exposure to tidal currents and waves offer the most favourable conditions for littoral mussel beds and show highest mussel bed coverages in the Wadden Sea (Folmer et al. 2014). The coverage of mussel beds in the different Wadden Sea regions is subject to considerable year-to-year variations, which cannot entirely be explained by environmental conditions such as cold winters and storms (Folmer et al. 2014). Amongst other things, predation pressure on intertidal mussels is one of the potential factors affecting the short-term dynamics of mussel beds.

Predation pressure on a given mussel bed largely depends on the local predator abundances. All size classes of mussels on intertidal mussels beds are subject to predation by a suite of predators, most notably shore crabs and shellfish-eating birds (Zwarts & Drent 1981, Smallegange & van der Meer 2003, van de Kam et al. 2004). A comparison of waterbird abundances corrected for the surface area of specific foraging habitats among the tidal basins of the Dutch and German Wadden Sea, revealed pronounced differences in bird density between the different Wadden Sea regions (**Chapter 2**). These patterns were also manifested in the shellfish-eating birds, showing high densities in the western Dutch Wadden Sea and in the south of Schleswig-Holstein and lower densities in the eastern Dutch Wadden Sea, in Lower Saxony and in the north of Schleswig-Holstein. These area corrected bird abundances only allow a general comparison of predation pressure on the benthos among the different Wadden Sea areas. A thorough analysis of predation pressure, requires comprehensive information on prey distribution and prey quality. Extensive sampling of the macrobenthos is only performed in the Dutch Wadden Sea, such as a grid-point survey designed for the macrobenthic species on the tidal flats (Compton et al. 2013) and surveys focussing on commercially important bivalves (e.g., mussels, cockles; van den Ende et al. 2016b, van Asch et al. 2016). In Germany, no such area-wide surveys exist, which hampers efforts to compare the predation pressure between the different Wadden Sea regions.

Furthermore, for an overall comparison of predation pressure on mussels at intertidal bivalve beds in the different Wadden Sea regions, the abundance of other mussel predators (i.e. shore crabs) should also be considered. However, current monitoring programmes that survey annual abundances of fish and epibenthic macroinvertebrates (e.g., crabs, shrimp) throughout the Wadden Sea (Demersal Fish Survey (DFS) in the Netherlands and Demersal Young Fish Survey (DYFS) in Germany) are hardly capable of identifying differences of shore crab abundance among different intertidal areas. This is mainly because these surveys are restricted to tidal channels and gullies deeper than 2 m (e.g., Tulp et al. 2012; 2016) and presumably can only give inadequate information of the intertidal crab population. Moreover, the surveys in the Dutch and German Wadden Sea differ slightly in their sampling methodology. While in the Dutch DFS beam trawls are equipped with a tickler chain and therefore can catch epibenthic organisms more efficiently, trawls in the German DYFS programme do without extra chains (e.g., Tulp et al. 2016). Up to date differences in catching efficiency between the two beam trawl types are not well known, making comparisons of the two surveys complicated (Tulp et al. 2016). Focussing only on the Dutch DFS, however, no explicit differences in shore crab abundance

in subtidal areas are apparent between the western and eastern Dutch Wadden Sea (Tulp et al. 2012). Assuming that no substantial differences in the relation of population sizes of intertidal and subtidal habitats between both regions of the Dutch Wadden Sea exist, densities of shore crabs on intertidal flats should also be similar between the two areas.

Focussing on the Dutch Wadden Sea, the previously described higher abundances of shellfish-eating birds in the western Dutch Wadden Sea add to the hydrodynamical conditions that are less favourable for mussel beds in the western Dutch Wadden Sea (see Donker 2015, for details). Unsurprisingly, the surface area of intertidal flats occupied by epibenthic bivalve beds in the western Dutch Wadden Sea is relatively low compared to the eastern Dutch Wadden Sea (Folmer et al. 2014, Donker 2015).
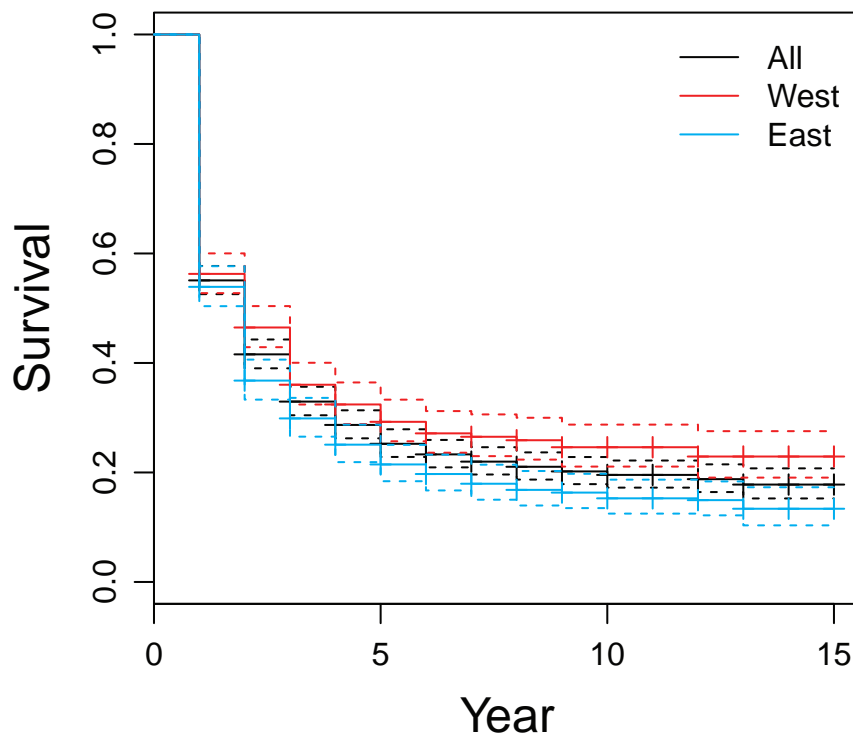
Figure 9.1: Comparison of survival curves of bivalve beds in the eastern and western Dutch Wadden Sea for the period 1999–2013. Analysis similar as described in Chapter 3. Dashed lines show average ± SEs.

This low bivalve bed area might be a result of low establishment or high loss rates of the bivalve beds. A survival analysis similar to the one described in **Chapter 3**, however, could not verify high loss rates in the west and instead indicated a slightly higher bivalve bed survival in the western Dutch Wadden Sea compared to the eastern Dutch Wadden Sea (van der Meer et al. unpublished data, Figure 9.1). This analysis, however, only considers beds that already survived the first year. Hence, less suitable conditions during the earlier establishment phase of bivalve beds, such as hydrodynamics (Donker 2015) or predation on post settlers (by e.g. shrimps and juvenile shore crabs; Reise 1985, Andresen 2013, Beukema & Dekker 2014), might be the primary reason for the sparse occurrence of bivalve beds in the western Dutch Wadden Sea.

Importance of parasitism in intertidal shore crab populations

The comparably high survival of established bivalve beds in the western Dutch Wadden Sea contradicts the observations of adverse hydrodynamical conditions and of high predation pressures exerted by shellfish-eating birds. This suggests that also other factors might be important with respect to the persistence of intertidal bivalve beds. Besides the numerical abundances, also the food demands of predators are important in governing the predation

pressure on a given prey item. A factor that can have crucial impacts on the feeding rates of crustacean predators is the infestation with parasites (Dick et al. 2010, Haddaway et al. 2012, Toscano et al. 2014). For example, acanthocephalan infection resulted in an increased feeding of up to 30% in the gammarid hosts (Dick et al. 2010), while infection with rhizocephalan parasites caused a reduction in feeding rates of up to 75% in brachyuran crabs (Toscano et al. 2014). In the Dutch Wadden Sea, the shore crab *Carcinus maenas*, one of the most notable predators on intertidal mussels, was found to be infested with four parasite species of three different taxonomic groups (acanthocephalans: *Profilicollis botulus*; microphallid trematodes: *Maritrema subdolum* and *Microphallus claviformis*; rhizocephalans: *Sacculina carcini*). While a considerable portion of *C. maenas* was found to be infested with acanthocephalans (~50%) and trematodes (~30%), prevalences of crabs infected with *S. carcini* were low (**Chapter 7** and **Chapter 8**). However, to what extent infection rates with these parasites differ between the western and eastern part of the Dutch Wadden Sea is still uncertain. Only the infection with the rhizocephalan *S. carcini* was assessed in both areas, indicating no substantial differences between west and east but an increased infection rate in subtidal areas (**Chapter 7**). The low infection rates with rhizocephalans and trematodes in the area corroborate studies on the feeding behaviour of *C. maenas* showing that rhizocephalan- and trematode infection both have little effects on the crabs feeding ecology (Larsen et al. 2013, Blakeslee et al. 2015). In contrast, little is known on the impact of acanthocephalans, and the fact that about half of the investigated shore crabs were found to be infected with these parasites shows the urgent need for further studies to clarify the effects of acanthocephalan infection on the feeding ecology of *C. maenas*.

Pacific oysters and their impact on bed survival

Another possible reason for differences in bivalve bed survival between the western and eastern Dutch Wadden Sea could be based on considerable regional differences in bivalve bed composition. After the collapse of intertidal mussel beds in the early 1990s (Figure 1.2), the recolonization of the intertidal by mussels differed between the western and eastern part of the Dutch Wadden Sea. While many intertidal areas of the eastern Dutch Wadden Sea readily got recolonized by mussels (e.g., Dankers et al. 2001), re-establishment of mussel beds in the western Dutch Wadden Sea remained very low. Yet, many intertidal areas of the western Dutch Wadden Sea, formerly occupied by mussels, were colonized by Pacific oysters, which settled on shell debris of the former mussel beds. Consequently, bivalve beds differed in their composition between the western and eastern Dutch Wadden Sea, with a low fraction of pure mussel beds in the west and high portions of this bed type in the east (van Stralen et al. 2012, Figure 9.2). Interestingly, pure mussel beds exhibit a lower persistence compared to beds occupied by Pacific oysters (Reise et al. 2017b, **Chapter 3**).

The higher survival of beds rich in Pacific oysters is due to the permanent anchorage of the oysters, resulting in rigid and persistent structures (Walles et al. 2015a). In contrast, mussels are temporarily attached via byssus threads and are vulnerable to harsh environmental conditions, such as storms and ice scouring (Nehls & Thiel 1993, Strasser et al. 2001, Donker 2015). Furthermore, *C. gigas* are less prone to predation compared to mussels, as primarily small oysters are preyed upon and larger specimens are only taken sporadically (Dare et al. 1983, Mascaró & Seed 2001a, Cadée 2001; 2008b;a, Markert et al. 2013, Weerman et al. 2014).

Initial concerns that Pacific oysters may outcompete the native mussels (e.g., Troost 2010) seem not to come true, as both bivalve species are found to coexist at many locations throughout the Wadden Sea (Markert et al. 2013, Reise et al. 2017b;a, Figure 1.2) and elsewhere in Europe (Groslier 2014, Holm et al. 2015; 2016, Norling et al. 2015, Herbert et al. 2016). Moreover, mussels may take advantage of the biocenosis with the alien oyster by settling in the inter-spaces between adult oysters (Buschbaum et al. 2016, Reise et al. 2017b) and thereby gaining shelter from harsh environmental conditions and from predation. For example, mussels cohabiting with oysters are

less prone to predation by shore crabs (Eschweiler & Christensen 2011, **Chapter 6**). Moreover, bivalve beds rich in Pacific oysters are less attractive foraging grounds for shellfish-eating birds (Markert et al. 2013, **Chapter 4**), due to reductions in the accessibility (**Chapter 6**) and body condition (**Chapter 4**) of the mussels.
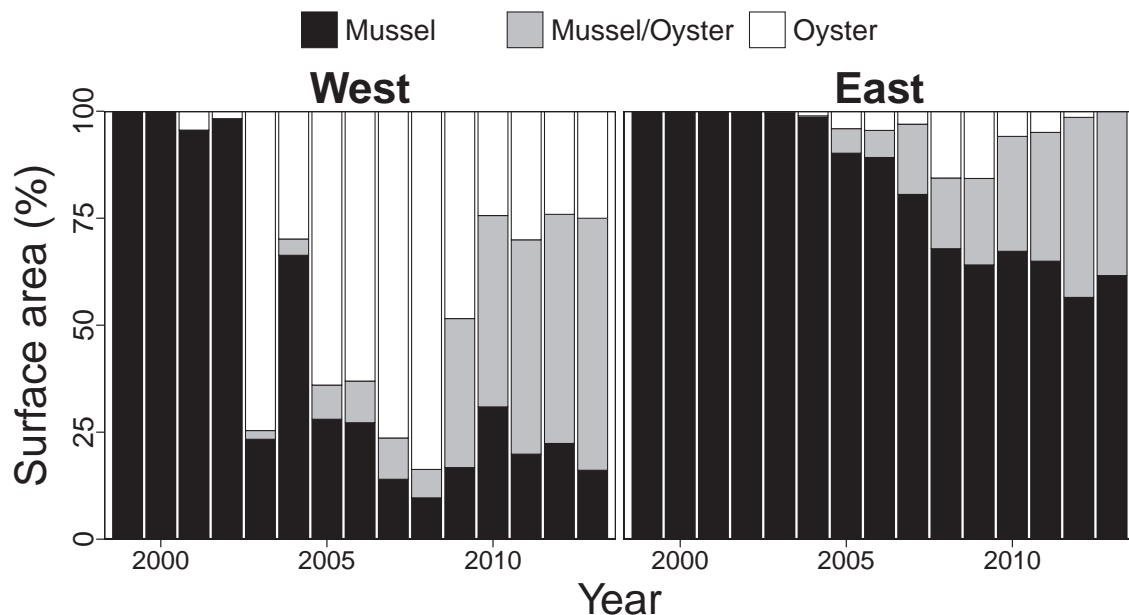

Figure 9.2: Surface area (%) of the different types of bivalve beds in the western and eastern Dutch Wadden Sea in the period 1999–2013. Plots based on surface area (ha) data from CBS et al. (2017).

Consequently, differences in persistence between beds differing in bivalve composition were also apparent in the comprehensive surveys of a selection of bivalve beds throughout the Dutch Wadden Sea in the years 2010–2013. These surveys were initially designed to follow the fate of newly established beds. But low recruitment resulted in only one new bed (W001_A1) in the entire Dutch Wadden Sea, so that the surveys were conducted mostly on perennial beds. The surveys showed that beds with many Pacific oysters remained fairly consistent in their areal extent over a period of three years, corroborating the findings of the long-term study of intertidal beds in the entire Dutch Wadden Sea for the period 1999-2013 (**Chapter 3**). In contrast, pure mussel beds, commonly showed a gradual decrease in bed area and/or a thinning in mussel coverage throughout the study period. This gradual decrease in mussel area, is to a certain extend based on the intense predation pressure on the mussels. For example, on the bivalve bed W001_A1, the average annual predation pressure on the macrobenthos exerted by mussel-eating birds in the period 2010–2013 accounted for about 680 kg AFDM per hectare bivalve bed (Table 9.1) and exceeded the secondary production of the dominating mussel cohort from 2009 (average annual production ca. 250 kg AFDM ha^{-1}, Table B3.1). Due to this high predation pressure, the mussel population is subject to drastic depletion and density decreases over time, if no substantial new spatfall occurs (Figure B3.1, Figure B3.2). In contrast, beds rich in Pacific oysters, show much lower abundances of shellfish-eating birds (**Chapter 4**), resulting in a reduced depletion of the mussels.

Crab predation and its potential impact on mussel recruitment

Hence, replenishment of the mussel population by regular recruitment events is of particular importance for pure mussel beds in order to balance the depletion of older specimens and to persist over long time periods. However, mussel recruitment on the intertidal beds often is insufficient to compensate the losses and rejuvenate the existing mussel population (see

Table 9.1: Estimated average predation pressure during summer (May–October) and winter (November–April) on macrobenthos by molluscivorous birds on the bivalve bed W001_A1 for the period 2010–2013. All mass indications refer to ash free dry mass (AFDM) measurements.

Season	Species	Mass (g ; g AFDM [a])	Daily consumption (g d^{-1})	Density (n ha^{-1})	Consumption Daily (kg ha^{-1} d^{-1})	Consumption Total (kg ha^{-1})
Summer	Oystercatcher	544 [b]; 184	43 [e]	29.55 [h]	1.27	231.1
	Eider	2162 [c]; 730	143 [f]	4.91 [i]	0.7	127.4
	Herring Gull	944 [d]; 319	66 [g]	4.83 [j]	0.32	58.2
	Sum					416.7
Winter	Oystercatcher	593 [b]; 200	52 [e]	18.2 [h]	0.95	172.9
	Eider	2162 [c]; 730	177 [f]	2.36 [i]	0.42	76.4
	Herring Gull	944 [d]; 319	66 [g]	1.26 [j]	0.08	14.6
	Sum					263.9
Total sum						680.6

[a] mass conversion: $y = 0.3378x$, with y = ash free dry mass (AFDM) and x = wet mass (WM); after Horn & de la Vega (2016)
[b] seasonal data in the Wadden Sea from Fig. 15 in Zwarts et al. (1996b)
[c] data: shot birds in the Wadden Sea from Chapter 6 of Kats (2007); no seasonal specific information available
[d] after Appendix 10 from Camphuysen (2013); no seasonal specific information available
[e] after Hilgerloh (1997)
[f] based on monthly data from Nehls (1995)
[g] daily requirements: (1462.5 kJ, Camphuysen 2013); conversion energy to biomass: (22 kJ per g AFDM, Zwarts et al. 1996a)
[h] data: seasonal trend of bird numbers on bed W001_A1 (6.8 ha) in Figure S4.7
[i] data: seasonal trend of bird numbers on bed W001_A1 (6.8 ha) in Figure S4.6
[j] data: seasonal trend of bird numbers on bed W001_A1 (6.8 ha) in Figure S4.23

Table 9.2: Estimated predation pressure during summer (May–October) on macrobenthos by shore crabs of varying sizes on the bivalve bed W001_A1. Abundance estimates are based on sampling in 2012–2013 and 2012 for juveniles and adults, respectively. All consumption figures refer to 15 °C, which is the mean sea surface temperature (SST) for the period May–October in the western Dutch Wadden sea (van Aken 2008b). Consumption at 15 °C was calculated based on life-stage dependent feeding rates measured at slightly higher temperatures (17 °C and 20 °C) and a Q10 value of 2.11 (*C. maenas* for 15–22 °C; Robertson et al. 2002). All mass indications refer to ash free dry mass (AFDM) measurements.

Size (mm CW)	Mass (g)	Daily consumption (g d^{-1})	Density (n ha^{-1})	Consumption (kg ha^{-1} d^{-1})	Consumption (kg ha^{-1})
Small (8.5)	0.03 [a]	0.005 [c]	168000 [e]	0.76	138.6
Medium (42.5)	3.09 [b]	0.124 [d]	184.14 [f]	0.02	4.2
Big (57.5)	7.92 [b]	0.411 [d]	142.94 [f]	0.06	10.7
Sum					153.5

[a] crabs < 20 mm CW: $\log y = -1.2557 + 2.8573 \log x$, with y = ash free dry mass (AFDM, mg) and x = carapace width (mm) after Klein Breteler (1975a)
[b] crabs > 20 mm CW: $\log y = -1.57709 + 3.112 \log x$, with y = ash free dry mass (AFDM, mg) and x = carapace width (mm) after Afman (1980)
[c] juveniles (< 20 mm CW) at 20 °C;: $\log y = -0.2152 + 0.7383 \log x$, with y = daily consumption (mg) and x = ash free dry mass (AFDM, mg) after Klein Breteler (1975a)
[d] adult males (> 20 mm CW) at 17 °C: $y = 17.56 + 4.88x$, where y = feeding rate in kJ and x = carapace width in cm after Elner (1980); conversion energy to biomass (22 kJ per g AFDM) after Zwarts et al. (1996a)
[e] data: ~ 140 crabs m[-2] on bivalve covered patches (Figure B5.1); bivalve cover ~ 12% = 168000 crabs per ha bivalve bed
[f] abundance calculations after Chapter 5

Table 9.3: Comparison of predation pressure on intertidal mussels at bed W001_A1 exerted by shellfish-eating shorebirds and by shore crabs. Given are the predators annual consumption, prey size range and the daily intake rate. It has to be noted that for the birds daily intake rates were estimated on the basis of an entire year and for shore crabs only for the period May–October.

Predator	Annual consumption (kg AFDM ha^{-1})	Prey size (mm)	Prey mass (g AFDM [e])	Daily intake rate (m^{-2} d^{-1}) min	max
Oystercatcher	404	25–45 [a]	0.0693–0.3976	0.28	1.6
Eider	203.8	25–60 [a]	0.0693–0.9347	0.06	0.81
Herring Gull	72.8	10–20 [b]	0.0046–0.0357	0.59	4.56
Small crab	138.6	1–5 [c]	0.00002–0.0012	63.46	3807.69
Medium crab	4.2	5–15 [d]	0.0012–0.0152	0.15	1.92
Big crab	10.7	15–25 [d]	0.0152–0.0693	0.08	0.39

[a] (Bult et al. 2004) [b] (Camphuysen 2013) [c] (Mascaró & Seed 2001b) [d] (Mascaró & Seed 2001a)
[e] length-biomass relationship of juveniles (1–4 mm): $W = 18.8L^{2.6}$, where W = weight AFDM in µg and L = shell length in mm, after Riisgård et al. (1980); mussels (> 5 mm) at W001_A1 Waser unpubl.: $\log y = -5.313166 + 2.971523 \log x$, where y = mass AFDM (g); x = length (mm)

Box 3.1, Figure B3.1). Such low recruitment is often caused by high predation rates on the recently settled bivalves (Reise 1985, Andresen 2013, van der Heide et al. 2014). In the Wadden Sea, the main predators of post-settled bivalves are shrimps and juvenile crabs (Reise 1985, Andresen 2013, Beukema & Dekker 2014). Predation by shrimps on the macrobenthos can be substantial, causing high predation pressure on post-settling bivalves (e.g., Beukema & Dekker 2005, Andresen & van der Meer 2010, Jung et al. 2017). However, the extent of shrimp predation on juvenile mussels settling on the investigated bivalve beds is unknown, as densities of this mobile predator on the beds could not be quantified. In contrast, densities of juvenile shore crabs on the bivalve beds could be estimated from general bivalve surveys during low tide (Box 5.1). A rough estimate of potential predation pressure on mussels exerted by juvenile shore crabs (~ 8.5 mm CW) during summer (May–October) at bed W001_A1 is given in Table 9.2. Although these figures reflect a simplified picture of the abundance of juvenile crabs, as they are only based on four sampling occasions in spring and autumn of the years 2012 and 2013 (see Box 5.1), these figures still illustrate the considerable impact juvenile crabs may have on post-settling mussels with an estimated consumption of about 140 kg AFDM (Table 9.2) per hectare bivalve bed (this amounts to a daily intake rate of up 3808 mussels (size of 1 mm) per m^2; Table 9.3) in the period May–October. A thorough analysis of predation impact and hence density of juvenile crabs, however, would require more frequent sampling in order to capture differences in crab density and size distribution throughout the season (Klein Breteler 1976b, Beukema 1991, Moksnes 2002, Box 5.2, Figure B5.4). Another uncertainty is in the size preferences of these juvenile crabs. Although a few studies investigated size preferences of juvenile shore crabs preying upon mussels (Mascaró & Seed 2001b, Breen & Metaxas 2008, Morton & Harper 2008), none of these studies adequately investigated the size preferences of crabs as small as 8.5 mm CW. Hence, the prey range used for this crab size is ambiguous and could only be guessed from prey preferences of slightly bigger crabs (15–25 mm CW; Mascaró & Seed 2001b). A further factor, complicating the assessment of the impact of juveniles crabs on small mussels is in the difficulty of estimating ash free dry weight of small mussels. These very small mussels are only present on intertidal flats for a rather short time interval and are easily overlooked when sampling larger mussels, so that the very small individuals sampled on the bivalve beds are underrepresented (Figure B3.1, Figure B3.2). Since small mussels have a higher flesh to shell ratio, and hence, a higher body mass index (BMI) than bigger specimens (Rob Dekker pers. communication, own observation), extrapolation of the length-weight relationship of bigger mussels results in underestimation of the biomass of the very small mussels. To overcome this

problem, length-weight relationships focussing on these small mussels were taken from the literature (Riisgård et al. 1980). However, the estimated amount of mussels with a length of 1 mm eaten per in a m^2 bivalve bed per day (3808; Table 9.3) seems rather high, suggesting an underestimation of mussel biomass as a potential source of error.

Juvenile mussels may further be subject to predation by two introduced small crab species: the Asian shore crab (*Hemigrapsus sanguineus*) and the brush-clawed shore crab (*Hemigrapsus takanoi*), which both occupy similar habitats as *C. maenas* (Box 5.1, **Chapter 8**). Studies on the feeding ecology on these crabs are scarce, with only a few conducted with *H. sanguineus* on the US East Coast (e.g., Brousseau et al. 2001, Lohrer & Whitlatch 2002, Griffen et al. 2015). These studies showed that *H. sanguineus* readily feeds on juvenile *M. edulis*. Further studies are needed to determine size-specific energy demands and prey-size preferences of both *Hemigrapsus* species. Based on low tide sampling in 2012 and 2013, densities of about 20 *Hemigrapsus* spp. (majority: *H. takanoi*) per m^2 bivalve bed could be determined for bed W001_A1. Assuming that these crabs have similar energetic requirements as juvenile *C. maenas*, *Hemigrapsus* spp. exerts predation pressures of about 20 kg AFDM ha^{-1} on the macrobenthos during summer time.

Concerning the predation pressure on mussels exerted by adult shore crabs, crab abundance on the bivalve beds was assessed indirectly by a combination of baited trap arrays on the beds with beam trawling along the bed contours (see **Chapter 5**). These efforts were made in June and it must be further tested if the attained relationship between trap catches on the beds and crab abundance on the surrounding bare flats can also be used to convert trap catches made earlier or later in the season. Repeated deployment of crab traps on the bivalve bed W001_A1 indicated that the rate of medium sized crabs caught in the traps changed throughout the season (see Box 5.2, Figure B5.3), suggesting that the relationship of the two methods attained in **Chapter 5** is less suited to estimate crab abundance throughout the year. Therefore, the estimate of crab abundance used to calculate the predation pressure on the mussels was solely based on one sampling event from early summer (**Chapter 5**). It is assumed that the resulting density estimates reliably reflect average crab densities during summer periods, so that the estimated predation pressures of 15 kg AFDM (4.2 and 10.7 kg AFDM ha^{-1} for medium (42.5 mm CW) and big crabs (57.5 mm CW), respectively, Table 9.2) per hectare bivalve bed should be more or less representative. Crabs are known to select mussel sizes that pose the lowest risks for claw damage (Elner & Hughes 1978, Smallegange & van der Meer 2003). Hence, adult crabs preferably prey on juvenile mussels, not exceeding shell lengths of 25 mm, that can easily be crushed with their claws. The daily intake rates during summer achieved on bed W001_A1 by adult crabs amount up to 1.9 and 0.4 mussels per m^2 for medium and big crabs, respectively (Table 9.3). The figures illustrate that crab predation in combination with the predation exerted by Herring Gulls (4.6 m^{-2}d^{-1}, Table 9.3), which similarly prey on juvenile mussels, may play important roles in the survival of young mussels of 0–2 years of age. Older mussels, well above shell lengths of 25 mm are preyed upon by Oystercatcher and Common Eider. As these larger mussels have higher biomasses, the maximum daily intake rates of the birds are with 1.6 (Oystercatcher) and 0.8 (Eider) (Table 9.3) lower than the rates achieved of the Gulls and crabs on the smaller mussels.

In order to be able to quantify differences in predation pressure among bivalve beds differing in the bivalve composition, detailed information on the mussel population as well as on the predators are required. In this thesis, first attempts towards a quantification of the predation pressure on mussels on different types of bivalve beds have been performed. Several important drawbacks became apparent, which make the comparison of predation pressure among different types of bivalve beds extremely difficult. Besides the difficulties in estimating the predation pressure on mussels by shore crabs, further restrictions were observed concerning the classification of mussels into different age cohorts. Particularly on oyster dominated beds, mussels showed a high variability in shell length, which made it impossible to follow cohorts through time (Box 3.1). Even for pure mussel beds, the assignment into cohorts was not without difficulty, as the recruitment of mussels showed irregular patterns. For example, it seems that in

the year 2011 several spawning events occurred on the bivalve bed W001_A1, as more than one new size mode emerged (see Figure B3.2).

Implications for mussel bed restoration

In the past, many attempts to restore intertidal mussel beds proved unsuccessful (Capelle et al. 2014, Dankers & Fey-Hofstede 2015, de Paoli et al. 2015). These restoration efforts often comprised the relaying of fished subtidal mussels in varying densities on the intertidal flats. In most cases these man-made mussel beds disappeared shortly after they had been created. The low success rate of this procedure is attributed to the subtidal origin of the mussels, as these mussels are barely capable to withstand the harsh conditions in intertidal areas (hydrodynamics, predators; de Paoli 2017). Earlier work showed that bed survival can be increased severalfold, when instead of subtidal mussels, intertidal mussels are used for the creation of beds (Donker 2015, de Paoli 2017). Yet, destroying natural intertidal mussel beds to restore mussel beds elsewhere on the intertidal is not a viable option.

An alternative to transplanting mussels is the enhancement of larval settlement onto the intertidal flats. Analogous to the restoration of oyster reefs (e.g., Nestlerode et al. 2007, George et al. 2015), the provision of settlement structures may facilitate mussel recruitment by significantly reducing predation on post-settling mussels. In the Dutch Wadden Sea, recent mussel bed restoration efforts focussed on the viability of Biodegradable Elements for Starting Ecosystems (BESE). These structures are made of starch from potato waste and offer settlement surface and predation refuge for settling mussel larvae. These structures are assumed to entirely decompose, leaving behind a functional mussel bed with plentiful adult mussels. However, it is not known how long it will take for the structures to decompose. On the basis of assumptions, not measurements, it is estimated that the structures will survive at most 10 years until fully decomposed. Furthermore, it remains to be seen how mussels settling on these structures will perform in the long run and whether these structures indeed are suitable alternatives in restoring mussel beds.

An alternative to the use of the plastic-like BESE structures may be offered by Pacific oysters inhabiting the Wadden Sea. This thesis demonstrates that mixtures of mussels and Pacific oysters show a much higher survival in comparison to pure mussel beds (see also Reise et al. 2017b). Consequently, employment of Pacific oysters in restoration measures should enhance the likelihood that man-made structures persist over long time periods. In the Dutch Wadden Sea, there are no experiences with man-made aggregations of both Pacific oysters and mussels. However, such constructions proved to be successful in the Northern Wadden Sea near the island Sylt, where six 10 × 10 m squares of a mixture of mussels and Pacific oysters, constructed in summer 2008, persisted over several years and remained fairly stable with only minor losses in surface area (Reise et al. 2017a). It seems likely that created structures of mixed oysters and mussels would also persist over long time periods in the Dutch Wadden Sea. However, future work is needed to find out the best options for creating such mixed bivalve beds. To avoid fishing up intertidal beds consisting of established Pacific oysters, alternatives would be to use oysters of subtidal origin or the use of oyster shells to create artificial reefs (e.g., Walles et al. 2016).

From an ecological perspective the term 'mussel bed' seems largely outdated, as many beds in the Dutch Wadden Sea nowadays are a mixture of mussels and Pacific oysters. Pure mussels beds seem to be restricted to higher parts in the tidal zone (see Figure 4.2). It is uncertain if these pure beds will persist in the future, as recent monitoring data indicate the proliferation of oysters and the change into mixed beds is still ongoing (Figure 1.2). As Pacific oysters became established in the Wadden Sea and are now ineradicable, mixed beds of oysters and mussels should be accepted as a historical contingency (Reise et al. 2017b) and should be seen as a vital addition to the habitat complexes within the Wadden Sea (e.g., Ssymank & Dankers 1996).

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Summary
Samenvatting
Zusammenfassung

Summary

Coastal areas are amongst the most productive ecosystems in the world. Important components of these coastal areas are reef-forming bivalves such as mussels and oysters, as they have important facilitating effects on many associated organisms through the provision of substratum, shelter or food. In the pristine Wadden Sea, intertidal beds of blue mussels (*Mytilus edulis*) and subtidal reefs of European flat oysters (*Ostrea edulis*) were common throughout the area and diversified the seascape. Extensive exploitation in the 19th century, however, led to the disappearance of the flat oyster from the Wadden Sea. As a consequence, mussels gained in importance as human food source, ultimately resulting in the introduction of commercial mussel cultures in the 1950s. Mussel beds were intensively harvested, and juvenile mussels were fished from intertidal beds for relaying to subtidal culture plots. In the early 1990s, several successive years with low recruitment and ongoing fisheries resulted in the loss of nearly all intertidal mussel beds in the Dutch Wadden Sea. Fishing restrictions subsequently allowed for the recovery of the intertidal beds. However, recovery was lower than hoped and a new potential threat emerged from the invasion of the Pacific oyster (*Crassostrea gigas*). Since the early 2000s, the alien oyster settled to an increasing degree on mussel beds, which often resulted in transformations into oyster dominated reefs, raising conservation concerns over competition with the native mussels. In order to further promote the recovery of intertidal mussel beds, restoration measures were considered. Earlier experiences indicated the difficulty of the mussel bed restoration, as most man-made beds disappeared shortly after they had been created.

In order to increase the success of mussel bed restoration measures, detailed insights in the various environmental and ecological processes affecting the survival of mussel beds were needed. The work presented in this thesis formed part of the 'Mosselwad' project, which was launched in 2010 to increase knowledge on several factors that play important roles in the survival and the stability of mussel beds. This thesis aims to investigate crucial biotic factors that act upon the survival of intertidal mussel beds. In particular, this thesis is concerned with the predation by shore crabs and mussel-feeding birds on the intertidal mussels and the impact of the recent introduction of the Pacific oyster into the Wadden Sea.

As a measure of predation pressure on mussels as well as other macrobenthic organisms among different Wadden Sea regions, waterbird distributions in relation to the extent of potential foraging habitats were explored (**Chapter 2**). Specifically, numbers of 21 waterbird species for the period 1999–2013 were investigated in relation to the surface area of six different habitats among the tidal basins of the Dutch and German Wadden Sea. The habitat areas were characterized by data on abiotic characteristics (tidal exposure and sediment structure) and on distributions of epibenthic bivalve beds. Linear regressions were used to explore bird-habitat associations, where the regression coefficients reflect bird densities in the various habitats. Most species were positively correlated with bivalve beds and intertidal areas with low tidal exposure (below 28%) and rather coarse sandy sediment (median grain size > 138.5 μm). By inspecting the regression residuals, we identified higher bird abundances of all investigated feeding guilds in the western Dutch Wadden Sea and in the south of Schleswig-Holstein, while lower abundances were found in the eastern Dutch Wadden Sea, in Lower Saxony and the north of Schleswig-Holstein. The observed regional differences in bird abundance may be related to the abundance of Peregrine Falcons, human disturbance and properties of the landscape. However, alternative explanations cannot be ruled out and further research is needed to identify the involved drivers.

Chapter 3 describes the fate of bivalve beds within the Dutch Wadden Sea for the period 1999–2013. Bed survival is analysed in relation to several covariates such orbital speed, inundation time, bed size, and bed type with respect to the bivalve composition (mussel dominated, dominated by Pacific oysters, or mixtures of both bivalve species). In general, beds were found to have a high survival when large, lying shallow, and experiencing low orbital speed. The highest effect on bed survival, however, was due to composition of bivalve species. Mixed beds had a much lower hazard rate than pure oyster or mussel beds.

In **Chapter 4**, the impact of Pacific oysters on the condition of mussels and on the spatial distribution of birds on 18 bivalve beds with different grades of oyster occurrence throughout the Dutch Wadden Sea was explored. Moreover, in comparing bird densities on bivalve beds with densities expected on the total intertidal area, species exhibiting a preference for the structured habitat could be detected. Overall, 50 different bird species were observed on the beds, of which about half regularly frequent intertidal flats. Most of these species showed a preference for bivalve beds. The condition of mussels decreased with oyster dominance, whereas the majority of bird species was not affected by oyster occurrence. Four species were found to be negatively affected by oyster occurrence. Three of these species (Oystercatcher, Red Knot, and Common Gull) depend on intertidal mussels as food source, while the Dunlin primarily uses other food sources.

Estimating shore crab (*Carcinus maenas*) abundance on bivalve beds is challenging, since most methods common for quantifying animal abundance in marine habitats cannot be used. As abundance estimates are needed for the quantification of crab predation on mussels, the potential of two methods to quantify crab abundance on 14 epibenthic bivalve beds across the Dutch Wadden Sea was explored in **Chapter 5**. The use of the number of crabs migrating from subtidal towards intertidal areas as a proxy of abundance on bivalve beds yielded unreliable results. In contrast, crabs caught with traps on the beds were correlated with the abundance assessed on the surrounding bare flats by beam trawl and therefore provided usable results. The estimates, however, were only reliable for crabs exceeding 35 mm in carapace width (CW). The application of these estimates indicated that crab abundances on bivalve beds were influenced by the biogenic structure. Beds dominated by oysters attracted many large crabs (> 50 mm CW), whereas abundances of medium-sized crabs (35–50 mm CW) showed no relationship to the oyster occurrence.

In **Chapter 6**, the impact of Pacific oysters on the survival of different sized mussels, while being exposed to shore crab predation, was experimentally explored. Mussel survival was documented in laboratory short-term experiments. A split-plot design was followed, with differences in the three among-plot factors (acclimatization type: control and induced clumping; crab size: small (45–50 mm CW) and big crabs (60–65 mm CW); and oyster presence: presence and absence of oysters) and within-plot differences in the survival of four different mussel size classes (6, 12, 18, and 24 mm shell length). The presence of Pacific oysters reduced the mortality of unconditioned mussels as well as mussels that were acclimatized in presence of predatory cues, while being exposed to predation by crabs of two different size classes. The reduction in mortality was size-dependent both in terms of the predators and the prey. The presence of oysters notably reduced mussel mortality in presence of small crabs, while the mortality rate in presence of big crabs was less affected. Mussels that benefited the most by the presence of oysters were those of recruitment stages, smaller than 20 mm in shell length.

In **Chapter 7** and **Chapter 8**, the potential importance of parasitism in relation to predation on mussels is explored. These two chapters focus on parasite infections in brachyuran crabs in the Dutch Wadden Sea. **Chapter 7** describes an extensive field survey throughout the Dutch Wadden Sea for the rhizocephalan parasite *Sacculina carcini* infecting shore crabs. The distribution of shore crabs infected with the rhizocephalan parasite was investigated at 12 locations and in 3 adjacent habitats (intertidal mussel beds, intertidal bare sand flats and subtidal gullies) along a tidal elevation gradient. The sampling revealed that of the 27629

crabs investigated, most infected crabs were found in the subtidal gullies and almost none on intertidal bare sand flats or mussel beds at all of the 12 locations. This probably resulted from a parasite-induced manipulation of infected crabs to behave like egg-bearing females which migrate towards deeper waters, as the same pattern was observed in the distribution of non-infected ovigerous females. The prevalence of both infected crabs and ovigerous females in the gullies was significantly correlated with water depth, and both tended to increase (albeit not significantly) with increasing salinity. As water depth and salinity are expected to affect larval survival of both parasites and crabs, this suggests that the migration into subtidal habitats may result in favourable conditions for reproduction and dispersal.

In **Chapter 8**, the macroparasite richness, prevalence, and intensity among three brachyuran crab species were explored in the Western Dutch Wadden Sea. Next to the native *C. maenas*, the two invasive crabs *Hemigrapsus sanguineus* and *H. takanoi* were screened for potential parasite infection. While native shore crabs hosted three parasite groups (acanthocephalans, microphallid trematodes, rhizocephalans), the two invasive *Hemigrapsus* species were only infected with acanthocephalans. All acanthocephalans were molecularly identified (COI) as the native *Profilicollis botulus*. Prevalence and intensities of *P. botulus* were generally lower in the introduced than in the native crabs. Metacercariae of microphallid trematodes were only found in the native *C. maenas*, with mean infection levels of 100–300 metacercariae per host. Likewise, the castrating rhizocephalan *S. carcini* was only found in *C. maenas* at a few locations with low prevalences (< 3%). This first study on infection levels in invasive *Hemigrapsus* species in Europe indicates that these invasive crabs indeed experience lower infection levels than their native competitor *C. maenas*.

The integration of the information gained in this thesis indicated that the predation of mussel-eating birds and shore crabs is able to significantly affect the survival of intertidal bivalve beds (**Chapter 9**). High waterbird abundances may strongly reduce mussel biomass by preying primarily on larger mussels, whereas shore crabs of various life stages may play important roles in the survival of post-settling mussels. The juvenile mussels are of particular importance for mussel beds in order to persist over long time periods, as they rejuvenate the mussel population and compensate the losses (e.g., by bird predation) of older specimens. The introduction of Pacific oyster on the mussel beds, led to substantial decreases in the predation pressure exerted by mussel-eating birds and also resulted in reduced predation by shore crabs. This may have contributed to the overall observation that beds colonised by Pacific oysters show a higher survival than pure mussel beds. Consequently, employment of Pacific oysters in restoration measures could be a viable option to increase the likelihood that man-made bivalve beds persist over long time periods. As Pacific oysters became established in the Wadden Sea and are now ineradicable, mixed beds of oysters and mussels should be accepted as a historical contingency and should be seen as a vital addition to the habitat complexes within the Wadden Sea.

Samenvatting

Kustgebieden behoren tot de meest productieve gebieden van de wereld. Rif- of bankvormende tweekleppige schelpdieren zoals oesters en mossels vormen een belangrijk onderdeel van kustgebieden. Veel andere soorten profiteren van de aanwezigheid van deze banken en riffen, omdat ze er voedsel of beschutting vinden. Oorspronkelijk waren droogvallende banken van de mossel *Mytilus edulis* en riffen van de platte oester *Ostrea edulis* algemeen in de Waddenzee. Door hevige exploitatie in de 19e eeuw zijn de platte oesters verdwenen. Hierdoor verschoof de aandacht naar de mosselvisserij en dit leidde in de jaren 1950 tot het ontstaan van een commerciële mosselkweek. Mosselzaad werd intensief bevist en verplaatst naar dieper gelegen mosselpercelen. In het begin van de jaren 1990 bleef nieuwe aanwas van mosselzaad uit, maar ging de zaadvisserij door, met als gevolg dat bijna alle droogvallende mosselbanken uit de Nederlandse Waddenzee verdwenen. Visserijverboden werden ingesteld en dit leidde tot de terugkeer van de banken. Het herstel was echter langzamer dan gehoopt en een nieuwe dreiging verscheen in de vorm van de geïntroduceerde Japanse oester *Crassostrea gigas*. Sinds het begin van de jaren 2000, bezette deze nieuwkomer in toenemende mate de mosselbanken. De overgang van mosselbank naar oesterrif baarde de natuurbescherming de nodige zorg. Herstelmaatregelen werden overwogen. Eerdere ervaringen met het kunstmatig aanleggen van mosselbanken waren echter niet erg goed, omdat veel banken al snel na de aanleg verdwenen.

Om het herstel succesvoller te laten verlopen was meer inzicht nodig in de milieuomstandigheden en ecologische processen die de overleving van mosselbanken beïnvloeden. Het in dit proefschrift gepresenteerde werk is onderdeel van het 'Mosselwad' project, dat in 2010 gestart is om meer te weten te komen van de factoren die een rol spelen bij de overleving en stabiliteit van mosselbanken. Dit proefschrift onderzoekt de biotische factoren die van belang zijn bij de overleving van de banken. Met name richt dit proefschrift zich op de predatie door strandkrabben *Carcinus maenas* en vogels op mossels en op de rol van de recent geïntroduceerde Japanse oester.

De relatie tussen het aantal vogels en de oppervlakte aan geschikt foerageergebied werd bestudeerd om iets te kunnen zeggen over de predatiedruk op mossels en andere bodemdieren (**Hoofdstuk 2**). Dat wil zeggen, voor 21 wadvogelsoorten werden de in elk van de kombergingen van de Nederlandse en Duitse Waddenzee gedurende de periode 1999–2013 aanwezige aantallen gerelateerd aan de oppervlakte van zes verschillende habitattypen. De habitattypen werden geclassificeerd op basis van abiotische eigenschappen (overspoelingduur en sedimentsamenstelling) en de aanwezigheid van schelpdierbanken of riffen. De vogel-habitat associatie werd beschreven met behulp van lineaire regressiemodellen, waarbij de regressiecoëfficiënten de vogeldichtheden in de verschillende habitattypen weergeven. De meeste vogelsoorten waren positief gecorreleerd met schelpdierbanken en met de laaggelegen gebieden (minder dan 28% van de tijd droogvallend) en wat grover sediment (mediane korreldiameter groter dan 138,5 μm). De residuen van het model lieten zien dat er meer vogels dan het model voorspelde zaten in de westelijke Nederlandse Waddenzee en het zuidelijk gedeelte van Sleeswijk-Holstein en minder in oostelijk Nederland, Nedersaksen en het noordelijk deel van Sleeswijk-Holstein. Deze regionale verschillen in de aantallen vogels zouden te maken kunnen hebben met de aantallen slechtvalken, verstoring door de mens of het type landschap. Andere verklaringen kunnen niet uitgesloten woorden en verder onderzoek is nodig.

Hoofdstuk 3 beschrijft het lot van de schelpdierbanken in de Nederlandse Waddenzee voor de periode 1999–2013. De overleving van de banken is gerelateerd aan variabelen als orbitaalsnelheid, overspoelingduur, bankgrootte en type bank in termen van

soortensamenstelling (gedomineerd door mossels, door oesters of een mengsel). Grote, laag in het getijgebied gelegen banken waar de orbitaalsnelheid laag is, bleken de beste overleving te kennen. Het grootste effect op de overleving had echter de soortensamenstelling. Gemengde banken hadden een veel lagere kans om te verdwijnen dan pure mossel- of oesterbanken.

In **Hoofdstuk 4** word het effect van de Japanse oester op de conditie van de mossel en op de aanwezigheid van vogels bestudeerd. 18 banken verspreid over de hele Nederlandse Waddenzee, elk met zijn eigen hoeveelheid oesters, werden onderzocht. Voor 50 verschillende vogelsoorten werd het aantal individuen op de banken vergeleken met het verwachte aantal enkel op basis van oppervlakte. De meeste soorten bleken een voorkeur te hebben voor schelpdierbanken. De conditie van de mossels was slechter als er veel oesters waren, maar de meeste vogels leken geen last te hebben van de oesters. Slechts vier soorten kwamen minder algemeen voor in door oesters gedomineerde banken. Drie daarvan, de scholekster, kanoet en stormmeeuw, eten ook daadwerkelijk de nodige mossels, maar de bonte strandloper eet ander soort voedsel.

Het schatten van de aantallen strandkrabben op een schelpdierbank is erg lastig en de standaardtechnieken om zeedierenaantallen te bepalen kunnen niet gebruikt worden. Omdat aantalsschattingen wel nodig zijn om iets te kunnen zeggen over de predatiedruk, zijn in **Hoofdstuk 5** twee nieuwe methoden uitgeprobeerd, op 14 verspreid over de Nederlandse Waddenzee gelegen banken. Het indirect schatten van de hoeveelheid krabben die elk getij migreren van de dieper gelegen geulen naar de banken bleek niet te werken. Het aantal in fuiken gevangen krabben bleek wel te correleren met het aantal met een net gevangen krabben in de gebieden rond de banken en lijken dus bruikbare resultaten op te leveren. Maar het werkte alleen voor de grotere krabben (met een schild breder dan 35 mm). Het bleek dat banken met veel oesters vooral de grootste krabben aantrekken (schild groter dan 50 mm). Middelgrote krabben (35–50 mm groot schild) lieten geen relatie zien tussen aantallen en oestervoorkomen.

In **Hoofdstuk 6** wordt een kortdurend laboratoriumexperiment beschreven, waarin de rol van oesters onderzocht wordt op hoeveel mossels krabbenpredatie ontlopen. Een zogeheten 'split-plot' opzet werd gevolgd. De plots verschilden op drie manieren: al of niet geacclimatiseerd aan predatie, kleine (45–50 mm groot schild) of grote (60–65 mm) krabben aanwezig en oesters aan- of afwezig. Binnen elke plot werd gekeken naar de overleving van vier verschillende grootteklassen mossels (6, 12, 18 en 24 mm schelplengte). De aanwezigheid van oesters zorgde voor een lagere sterfte. Het waren vooral de kleinste drie mosselgroottes die het meest profiteerden. Ook was het effect van de oesteraanwezigheid sterker wanneer de mossels belaagd werden door de kleine krabben.

In **Hoofdstuk 7** en **Hoofdstuk 8** werd de rol van parasieten in de predatie op mossels onderzocht. **Hoofdstuk 7** beschrijft de resultaten van een Nederlandse Waddenzee-brede inventarisatie van het krabbenzakje *Sacculina carcini*, een parasitaire rankpootkreeft. Op 12 locaties werd in drie habitattypen (droogvallende mosselbanken, droogvallende wadvlakten en geulen) gekeken hoeveel strandkrabben geïnfecteerd waren. In totaal zijn 27629 krabben onderzocht en bijna op alle 12 plekken werden de geïnfecteerde krabben alleen in de geulen gevonden, en vooral in geulen met relatief zout water. Het lijkt er op dat de parasieten er voor zorgen dat de krabben zich gaan gedragen als eierdragende vrouwtjes, die zich immers ook in de geulen terugtrekken. Geulen met zout water zijn blijkbaar niet alleen een geschikt habitat voor krabbenlarven, maar ook voor krabbenparasieten. **Hoofdstuk 8** onderzocht in drie krabbensoorten de soortenrijkdom, de prevalentie (welke fractie van de krabben is geïnfecteerd) en de intensiteit (hoeveel parasieten herbergt een krab gemiddeld) van macroparasieten. Naast de strandkrab, werden ook twee invasieve krabbensoorten onderzocht, de blaasjeskrab *Hemigrapsus sanguineus* en de penseelkrab *H. takanoi*. De inheemse strandkrab herbergde drie groepen parasieten (haakwormen, zuigwormen en parasitaire rankpootkreeften), maar de beide invasieve soorten waren alleen geïnfecteerd met de haakwormsoort *Profilicollis botulus*. Ook de prevalentie en intensiteit van deze parasiet waren lager bij de invasieve krabben.

Hoofdstuk 9 integreert alle verkregen informatie en laat zien dat predatie door vogels en krabben een belangrijke rol speelt in het al of niet overleven van droogvallende schelpdierbanken. De vogels kunnen de totale biomassa van een bank sterk verlagen, omdat ze vooral op de grote mosselen foerageren. De krabben eten minder, maar zullen het aantal mossels veel meer omlaag brengen omdat zij vooral het jonge broed opeten. Zonder een regelmatige aanwas van jong broed, zal een bestaande bank zich niet kunnen vernieuwen en geen lang leven beschoren zijn. De introductie van de Japanse oester heeft geleid tot een sterke afname in de predatie niet allen door vogels, maar ook door krabben. Dit zal er mede voor gezorgd hebben dat mosselbanken die gekoloniseerd zijn door oesters een hogere overleving lieten zien dan pure mosselbanken. Bij restauratiemaatregelen zouden oesters dus goed gebruikt kunnen worden om de overleving van de aangelegde banken te verhogen. Japanse oesters hebben zich gevestigd in de Waddenzee, zijn niet meer weg te krijgen en kunnen dus beter niet alleen geaccepteerd worden, maar zelfs gezien worden als een belangrijke toevoeging aan de bestaande habitattypen in de Waddenzee.

Zusammenfassung

Küstengebiete zählen zu den produktivsten Ökosystemen der Welt. Wichtige Habitatstrukturen innerhalb dieser Gebiete entstehen durch bank- oder riffbildende Muschelarten wie Miesmuscheln und Austern. Diese Riffbildner begünstigen viele assoziierte Organismen durch die Bereitstellung von Lebensraum, Schutz oder Nahrung. Ursprünglich waren eulitorale Miesmuschelbänke (*Mytilus edulis*) und sublitorale Bänke der Europäischen Auster (*Ostrea edulis*) im gesamten Wattenmeer weit verbreitet. Intensiver Raubbau im 19. Jahrhundert führte jedoch zum Verschwinden der Europäischen Auster. Daraufhin gewann die Miesmuschelfischerei im Wattenmeer zunehmend an Bedeutung und führte so in den 1950er Jahren letztlich zur Einführung von kommerziellen Muschelkulturen. Saatmuscheln von eulitoralen Muschelbänken wurden intensiv befischt und als Besatz für die sublitoralen Kulturflächen verwendet. In den frühen 1990er Jahren führten mehrere aufeinanderfolgende Jahre mit geringen Brutfällen und fortdauernder Befischung zum Verschwinden fast aller eulitoralen Muschelbänke im niederländischen Wattenmeer. Darauffolgende Fangbeschränkungen ermöglichten eine leichte Bestandserholung der Muschelbänke. Einige Gebiete erholten sich jedoch nur schwer und eine neue potenzielle Bedrohung entstand durch die Einwanderung der Pazifischen Auster (*Crassostrea gigas*). Seit Anfang der 2000er Jahre siedelte sich die gebietsfremde Auster zunehmend auf Miesmuschelbänken an, was oftmals die Umwandlung zu Austern dominierten Riffen zur Folge hatte. Dies führte zu Befürchtungen, dass die eingewanderte Auster die einheimische Miesmuschel verdrängen könnte.

Um die Bestände von eulitoralen Miesmuschelbänken zu erhöhen, wurden Wiederansiedlungsmaßnahmen in Betracht gezogen. Frühe Versuche, Muschelbänke künstlich anzulegen, blieben meist ohne Erfolg, da die meisten Bänke bereits kurz nach ihrer Anlegung wieder verschwunden waren. Genauere Erkenntnisse über die Entwicklung von Muschelbänken in Bezug zu verschiedenen abiotischen und biotischen Prozessen wurden benötigt, um die Erfolgsaussichten der Wiederansiedlungsmaßnahmen von Miesmuschelbänken zu verbessern. Die dieser Doktorarbeit zugrundeliegenden Untersuchungen waren Teil des Projekts 'Mosselwad', das 2010 startete um mehr über die Faktoren, die für die Stabilität von Muschelbänken bedeutsam sind, in Erfahrung zu bringen. Der Fokus dieser Arbeit lag auf den biotischen Faktoren, die das Überleben von eulitoralen Muschelbänken entscheidend beeinflussen können. Im Besonderen wurden in dieser Arbeit der Fraßdruck von Strandkrabben (*Carcinus maenas*) und Wattvögeln auf eulitorale Miesmuscheln untersucht. Darüber hinaus wurden die Auswirkungen der Einwanderung der Pazifischen Auster auf Miesmuscheln im niederländischen Wattenmeer ermittelt.

Als Maßstab für den Fraßdruck auf Miesmuscheln sowie deren Begleitfauna in den verschiedenen Wattenmeerregionen wurden Wattvogelverteilungen in Bezug auf die Oberfläche von potentiellen Nahrungsgebieten untersucht (**Kapitel 2**). Im Besonderen wurden die Anzahlen von 21 Vogelarten für den Zeitraum 1999–2013 in Bezug auf die Fläche von sechs verschiedenen Habitattypen innerhalb der Gezeitenbecken des niederländischen und deutschen Wattenmeeres untersucht. Die Flächen der verschiedenen Habitate wurden anhand von abiotischen Eigenschaften (Trockenfalldauer und Sedimentstruktur) und der Ausbreitung von Muschelbänken ermittelt. Lineare Regressionen wurden verwendet, um den Zusammenhang zwischen Vogelanzahlen und Habitatflächen zu ermitteln, wobei die Regressionskoeffizienten die Vogeldichten in den verschiedenen Habitaten darstellen. Die meisten Arten waren positiv sowohl mit der Fläche von Muschelbänken als auch mit den Wattflächen, welche durch eine niedrige Trockenfalldauer (unter 28%) und grobkörnigem Sediment (über 138,5 μm) charakterisiert sind,

korreliert. Ferner konnten höhere Vogeldichten im westlichen niederländischen Wattenmeer und im Süden Schleswig-Holsteins identifiziert werden, während im östlichen niederländischen Wattenmeer, in Niedersachsen und im Norden Schleswig-Holsteins niedrigere Dichten gefunden wurden. Diese regionalen Unterschiede in den Vogeldichten könnten mit dem Auftreten von Wanderfalken, menschlichen Störungen sowie Landschaftseigenschaften zusammenhängen. Allerdings sind andere Faktoren nicht auszuschließen und weitere Untersuchungen sind erforderlich, um die beteiligten Faktoren zu identifizieren.

Kapitel 3 beschreibt die Entwicklung von Muschelbänken im niederländischen Wattenmeer für den Zeitraum 1999–2013. Das Überleben der Bänke hing von verschiedenen Faktoren wie Orbitalgeschwindigkeit, Überschwemmungszeit, Bankgröße und deren Artenzusammensetzung (dominiert von Miesmuscheln, Austern oder eine Mischung beider Arten) ab. Große Bänke, die im flachen Gezeitenbereich lagen, wo die Orbitalgeschwindigkeit niedrig war, wiesen eine hohe Stabilität auf. Der größte Effekt auf das Überleben der Bänke jedoch war auf die Artenzusammensetzung zurückzuführen. Bänke, die gleichermaßen aus Miesmuscheln und Austern bestanden, hatten eine viel geringere Chance zu verschwinden als reine Austern- oder Miesmuschelbänke.

In **Kapitel 4** wurde der Einfluss der Pazifischen Auster sowohl auf den Zustand der Miesmuscheln als auch auf die räumliche Verbreitung von Wattvögeln in insgesamt 18 in unterschiedlichem Grad von Pazifischen Austern besiedelten Muschelbänken im niederländischen Wattenmeer untersucht. Insgesamt 50 verschiedene Vogelarten konnten im Bereich der Muschelbänke beobachtet werden, von denen rund die Hälfte die Strukturen regelmäßig nutzte. Die Kondition der Miesmuscheln wurde mit zunehmender Dominanz der Auster negativ beeinflusst, während für die meisten Vogelarten keine unmittelbaren Effekte festzustellen waren. Negative Folgen durch zunehmende Austerndominanz ließen sich dennoch für vier Arten ermitteln, wobei für Austernfischer, Knutt und Sturmmöwe Miesmuscheln eine bedeutende Nahrungsquelle darstellen. Der Alpenstrandläufer, hingegen, bevorzugt in erster Linie andere Nahrungsquellen.

Die Abschätzung von Dichten der Strandkrabbe auf Muschelbänken ist kompliziert, da die meisten gängigen Methoden zur Quantifizierung von Abundanzen in marinen Lebensräumen nicht angewendet werden können. Um den Fraßdruck durch Krabben auf Miesmuscheln abschätzen zu können, wurden in **Kapitel 5** zwei verschiedene Methoden zur Quantifizierung der Krabbendichten auf 14 eulitoralen Muschelbänken im niederländischen Wattenmeer ausgetestet. Die Anzahlen von Krabben, die mit der Flut von den Prielen zu den Wattflächen wandern, waren als Indikatoren für die Krabbendichten auf den Bänken ungeeignet. Demgegenüber korrelierten die mit Fallen auf den Bänken gefangenen Krabben mit den mittels Baumkurren bestimmten Krabbendichten auf den umgebenden Wattflächen. Mittels dieser Korrelation konnten die Dichten auf Muschelbänken für Krabben ab einer Carapaxbreite (CB) von 35 mm ermittelt werden. Ferner konnte gezeigt werden, dass Austern dominierte Bänke bevorzugte Habitate von großen Krabben (über 50 mm CB) waren, wohingegen die Dichten von mittelgroßen Krabben (35–50 mm CB) nicht vom Austernvorkommen beeinflusst wurden.

In **Kapitel 6** wird ein Laborexperiment beschrieben, in dem die Rolle der Pazifischen Auster auf das Überleben von unterschiedlich großen Miesmuscheln in Abhängigkeit von Prädation durch Strandkrabben untersucht wurde. Das Überleben der Muscheln wurde anhand eines Split-Plot-Designs untersucht, wobei das Überleben von vier verschiedenen Muschelgrößenklassen (6, 12, 18 und 24 mm Schalenlänge) hinsichtlich drei unterschiedlicher Faktoren (akklimatisiert in An- oder Abwesenheit von Krabben, Prädation durch kleine (45–50 mm CB) oder große (60–65 mm CB) Krabben und An- oder Abwesenheit von Austern) untersucht wurde. Die Anwesenheit von Austern führte zu einer geringeren Mortalität der Miesmuscheln. Es waren hauptsächlich die drei kleinsten Größenklassen, die am meisten von der Anwesenheit der Auster profitieren konnten. Die Wirkung der Austern war zudem um ein vielfaches größer, wenn kleine Krabben anwesend waren.

In den **Kapiteln 7** und **8** wurde der potenzielle Einfluss von Parasiteninfektionen bei Krabbenartigen (Brachyura) auf die Prädation von Miesmuscheln untersucht. **Kapitel 7** beschreibt eine umfangreiche Untersuchung im gesamten niederländischen Wattenmeer über den Befall von Strandkrabben mit dem parasitären Wurzelkrebs *Sacculina carcini*. Die Verteilung der mit diesem Parasiten befallenen Strandkrabben wurde an 12 Standorten in insgesamt drei aneinandergrenzenden Habitaten (eulitorale Muschelbänke, sowie Sandflächen und sublitorale Priele) untersucht. Insgesamt wurden von den untersuchten 27629 Krabben die meisten parasitierten Krabben bei fast allen 12 Untersuchungsstandorten in den Prielen gefunden. Die Infektionsrate in tiefen Prielen mit hohem Salzgehalt war besonders hoch. Es scheint, dass die Parasiten die Krabben dazu bringen, sich wie Eier-tragende Weibchen zu verhalten, welche sich ebenfalls vorwiegend in den tieferen Prielen aufhalten. Da Wassertiefe und Salzgehalt das Überleben sowohl der Parasiten- als auch Krabbenlarven beeinflussen, deutet dies darauf hin, dass die Migration in sublitorale Bereiche zu günstigen Bedingungen für die Reproduktion und Ausbreitung führt.

In **Kapitel 8** wurden Infektionsraten mit Makroparasiten bei drei verschiedener Krabben-arten im westlichen niederländischen Wattenmeer untersucht. Neben *C. maenas* wurden die beiden aus dem Pazifikraum eingewanderten Krabben *Hemigrapsus sanguineus* und *H. takanoi* auf mögliche Parasiteninfektion untersucht. Während bei einheimischen Strand-krabben drei Parasitengruppen (Kratzwürmer (Acanthocephala), Saugwürmer (Trematoda) und Wurzelkrebse (Rhizocephala)) nachgewiesen werden konnten, waren die beiden eingewanderten *Hemigrapsus*-Arten nur mit Kratzwürmern der Art *Profilicollis botulus* infiziert. Prävalenz und Intensitäten von *P. botulus* bei den beiden Neozoen waren im Vergleich zu einheimischen *C. maenas* im allgemeinen niedriger. Diese erste Untersuchung zu Befallsraten der gebietsfremden *Hemigrapsus*-Arten in Europa deutet darauf hin, dass diese eingewanderten Krabben tatsächlich niedrigere Infektionsraten als ihre einheimischen Konkurrenten *C. maenas* aufweisen.

Die in dieser Arbeit gewonnenen Informationen konnten aufzeigen, dass der von muschel-fressenden Vögeln und Strandkrabben ausgehende Fraßdruck das Überleben von eulitoralen Muschelbänken erheblich beeinträchtigen kann (**Kapitel 9**). Hohe Dichten an Wattvögeln können die Miesmuschelbiomasse stark reduzieren, da sie sich vorwiegend von größeren Muscheln ernähren. Im Gegensatz dazu fressen Strandkrabben weniger Biomasse, erbeuten aber mehr Individuen durch Prädation von vor allem kleineren, jungen Miesmuscheln. Die Dezimierung dieser Jungmuscheln kann sich negativ auf die Verjüngung der Muschelpopulation auswirken und so im erheblichem Maße das Fortbestehen von Miesmuschelbänken über lange Zeiträume beeinflussen. Die Einwanderung der pazifischen Auster und die darauffolgende Transformation der Muschelbänke führte zu beträchtlichen Abnahmen des Fraßdrucks auf Miesmuscheln, da diese neue Lebensgemeinschaft sowohl die Prädation durch Vögel als auch Strandkrabben vermindert. Dies kann zur allgemeinen Beobachtung beigetragen haben, dass die von Pazifischen Austern besiedelten Bänke ein höheres Überleben aufweisen als reine Miesmuschelbänke. Folglich könnte der Einsatz von Pazifischen Austern bei Wiederansiedlungs-maßnahmen eine vielversprechende Option sein, um die Lebensdauer künstlich angelegter Muschelbänke deutlich zu erhöhen. Da sich die Pazifische Auster im Wattenmeer etabliert hat und ihre Ausrottung nahezu unmöglich ist, sollten gemischte Bänke, bestehend aus Austern und Miesmuscheln, nicht nur akzeptiert, sondern auch als positive Ergänzung zu den bestehenden Lebensräumen im Wattenmeer angesehen werden.

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List of publications

Refereed journals

Waser, A. M., R. Dekker, J. IJ. Witte, N. McSweeney, B. J. Ens, J. van der Meer (2018). Quantifying Tidal Movements of the Shore Crab Carcinus maenas on to Complex Epibenthic Bivalve Habitats. Estuaries and Coasts 41:507–520.

Goedknegt, M. A., J. Havermans, **A. M. Waser**, P. C. Luttikhuizen, E. Velilla, C. J. Camphuysen, J. van der Meer, D. W. Thieltges (2017). Cross-species comparison of parasite richness, prevalence, and intensity in a native compared to two invasive brachyuran crabs. Aquatic Invasions 12:201–212.

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Submitted manuscripts

Waser, A. M., E. O. Folmer, G. Scheiffarth, H. Büttger, K. Troost, J. Blew, R. Kleefstra, K. Günther, B. Hälterlein, J. Ludwig, G. Millat, T. Bregnballe, J. Van der Meer, B. J. Ens. Waterbird and habitat distributions in a major coastal wetland: revelation of regional differences in the Wadden Sea.

van der Meer, J., N. Dankers, B. J. Ens, M. van Stralen, K. Troost, **A. M. Waser**. The birth, growth and death of intertidal soft-sediment bivalve beds: No evidence for ecosystem collapse in the Dutch Wadden Sea.

Non-refereed journals and reports

Ens, B. J., **A. M. Waser**, S. Deuzeman, A. K. wa Kangeri, E. van Winden, J. Postma, P. de Boer, J. van der Meer (2016). Onderzoek naar de relatie tussen de samenstelling van schelpdier-banken en de benutting door vogels in de Waddenzee - advies ten behoeve van ontwikkeling beleidskader voor het handmatig rapen van Japanse oesters. Sovon-rapport 2016/17. Sovon Vogelonderzoek Nederland, Nijmegen.