North Sea Reefs

Benthic biodiversity of artificial and rocky reefs in the southern North Sea

Joop W.P. Coolen

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Abstract

The objective of the research presented in this thesis was to understand the patterns of benthic biodiversity on reefs in the North Sea. To gain this understanding, I studied which species are present on natural and artificial reefs, what environmental and biotic variables influence the presence and absence of a selection of these species and one of the possible pathways by which these species may colonise the reefs studied; the stepping stone effect. The first goal was to increase the available knowledge on which species are present at reefs. The second goal was to understand the patterns observed in the variation of species at these reefs. The third goal was to evaluate whether *Mytilus edulis* utilises offshore artificial structures as stepping stone to colonise very far offshore locations. The final goal was to assess the impact artificial reefs have on the benthic biodiversity of the North Sea. To attain these goals, natural and artificial reefs were sampled using diver operated airlift samplers and box corers. To evaluate the effects generalised linear and additive models were created. When available, additional data from other sources were also used.

The following conclusions are drawn: *Lanice conchilega* is an ecosystem engineer creating intermediate sand-reef systems. When rocky reefs are present on a sandy bottom, local biodiversity is doubled. Distributions of the native *Caprella linearis* and the invasive *Caprella mutica* showed a significant difference, demonstrating that *C. linearis'* habitat preference does not fully overlap with that of *C. mutica*. Thus, the native and alien Caprellids are likely to be able to co-exist in the North Sea. *M. edulis* presence increases habitat heterogeneity, which increases species richness. *Mytilus edulis* uses offshore structures as stepping stones to colonise locations in the North Sea that cannot be reached in a single generation.

Depth, location effect and habitat type influence the species composition on North Sea reefs most strongly. The relation between depth and species richness on artificial reefs is non-linear, with a maximum at intermediate depths. Although substrates with mixed surface orientation (i.a. rocks) hold the most species rich communities, biodiversity is also strongly influenced by *M. edulis* and *Psammechinus miliaris*. When artificial reefs are to be colonised by communities that are similar to natural reefs, their structures should resemble natural reefs as much as possible.

Chapter 1:

INTRODUCTION

Chapter 1: Introduction

Reefs are among the most species rich habitats in the world (Reaka-kudla 1997). Reefs in temperate waters are home to a large amount of benthic species, including many long lived species (Zintzen et al. 2007; Sheehan et al. 2013; Hill et al. 2014). They provide attachment surface for epifouling organisms (Inger et al. 2009; Walles et al. 2016), increase available hiding spaces for mobile organisms, impacting predator-prey relations (Johnson et al. 1998; Grabowski and Powers 2004) and further increase habitat heterogeneity by providing colonisation surface in orientations other than the horizontal provided by sand bottoms (Knott et al. 2004; Perkol-Finkel and Benayahu 2004; Moura et al. 2008). Reefs that are present in temperate sandy bottom environments, can more than double local biodiversity and increase the local biomass up to a hundred fold (Zintzen 2007). In Europe, natural reefs are regarded as important for biodiversity conservation and the European Union has defined natural reefs as protected habitats in its Habitat Directive (European Commission 1992).

Reefs can be classified as being of biogenic, geogenic, or anthropogenic origin. Biogenic reefs are *'essentially in place calcareous deposits created by sessile organisms'* (Riding 2002). This defines reefs as non-motile substrates built by bioconstructing organisms. These reefs are made by a wide variety of species, among which are calcareous algae, corals, sabellariids, serpulids, oysters, mussels, barnacles, bryozoans and sponges (Cocito 2004). Other substrates of biological origin may also function as reefs, for example peat layers (also called moorlog) originating from the period when sea levels were lower (Whitehead and Goodchild 1909; Godwin 1943). These are not defined anywhere as biogenic reefs, but, if still present, could probably host a community of species similar to other organic reefs (Lindeboom et al. 2011).

Geogenic reefs are rocky reefs that can take the form of coastal rocky outcrops, boulders or gravel beds. They are all composed of hard compact substrata of non-biogenic origin, including soft rocks such as chalk (European Commission 2013).

Anthropogenic reefs (henceforth: artificial reefs) are defined by Guerin et al. (2007), as '*a submerged structure, placed either deliberately or accidentally, which functions as a habitat for marine biota*'. Such artificial reefs include coastal defences, harbour pilings and pontoons, shipwrecks, buoys, offshore wind turbine foundations, oil and gas platform foundations, artificial reef balls, and any other unnatural hard surfaced structure present in water.

North Sea reefs

In this thesis, I focus on rocky and artificial reefs in the temperate waters of the North Sea. Much of the North Sea bottom is covered with mobile sediments such as mud and sand, and only about 20% is covered in coarse sands, gravels and rocks (EMODnet 2015; Figure 1). The North Sea is a coastal sea, largely enclosed by land. It is connected to the north east Atlantic

Ocean via the English Channel and between Scotland and Norway. The North Sea covers approximately 575,300 km². Temperatures in North Sea range between 2–8 °C in winter and 12–21 °C in coastal waters in the summer (Otto et al. 1990). There is a strong nutrient gradient in the North Sea, especially at locations with high river discharge, waters can be highly turbid (Brockmann et al. 1990). Salinity is also influenced by inflow of these rivers, and a gradient is present between the North Sea coasts and waters near the Atlantic Ocean (Otto et al. 1990). Water flow in the North Sea is largely driven by tidal currents entering from the Atlantic Ocean and the direction of these currents is counter clockwise (Thorpe 2012).

Many artificial reefs are present in the North Sea. About 27,000 wrecks locations are known to hydrographic services around the North Sea (Leewis et al. 2000; Lettens 2015). These shipwrecks are scattered throughout the North Sea, but the highest concentrations are found near the shipping routes in the coastal waters of the southern North Sea (Figure 2).

Furthermore, about 1,500 offshore oil and gas structures are present. Approximately 50% of these installations are sub-sea, meaning they are placed on the seabed without water surface contact, while the others have structures reaching to the water surface. The latter category can either be a fixed structure, e.g. a steel jacket or gravity based concrete foundation, or a floating installation (OSPAR Commission 2015). These installations are clustered in the centre of the southern North Sea and between Scotland, Norway and Denmark in the northern part (Figure 3). The oldest North Sea oil and gas platforms date from the early 1960s, when production started in Scottish waters (Shepherd 2015). In the '70s the first platforms were placed on the Dutch continental shelf (EBN 2014). Platforms in the North Sea are placed in water depths up to 378 m, although depths up to 850 m can be found in Norwegian waters north east of the Shetland Islands, just outside of the North Sea (OSPAR Commission 2015).

Currently, approximately 2,400 wind turbines have been installed in the North Sea (Figure 4; EWEA 2016), but every year hundreds of new wind turbines are placed so these numbers increase fast. Wind turbine foundations can consist of gravity based concrete or steel jackets, but most foundations are constructed using a steel monopile (EWEA 2016).

Ecology of North Sea artificial reefs
The primary ecological effect of placing artificial reefs in the North Sea, is the addition of hard substrate to a mostly sandy bottomed environment (Gallaway et al. 1981; Langhamer et al. 2009; Krone et al. 2013b; De Mesel et al. 2015). This hard substrate provides new habitat to species that are unable to establish populations on sand bottoms (Inger et al. 2009). Since rocks are placed around many installations to prevent scouring of the sand around the foundation, additional hard substrates become available at the bottom. These
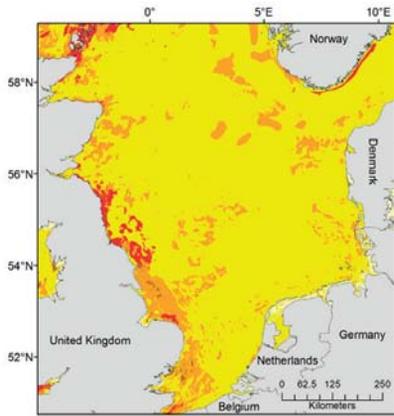
Figure 1: Sediments of the North Sea. Yellow: sand and mud, brown: mixed; orange: coarse (including gravels); red: rocks (EMODnet 2015).
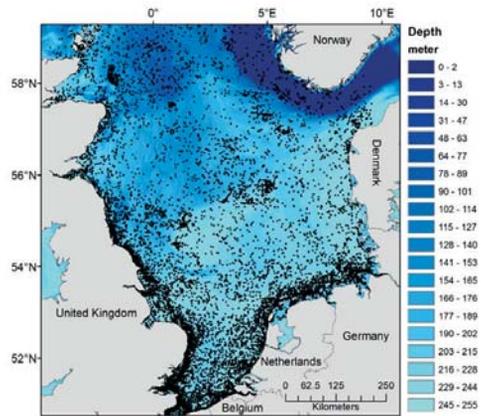
Figure 2: Shipwrecks North Sea (black dots) (Lettens 2015).
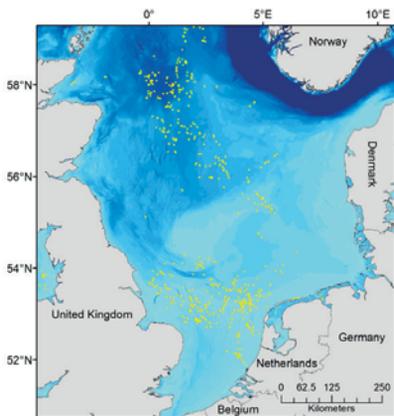
Figure 3: Offshore oil and gas platforms North Sea (yellow dots; OSPAR Commission 2015).
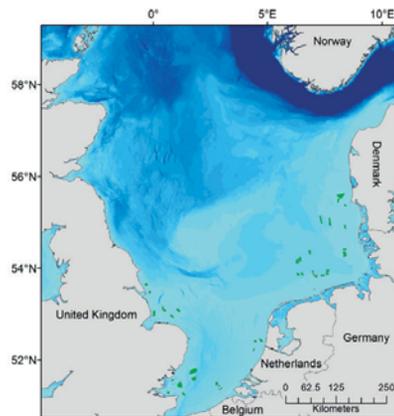
Figure 4: Wind farms (green). Offshore wind dataset compiled by J.T. van der Wal (July 2016) based on a.o. the WindSpeed project and drawing from several outside sources including OSPAR , 4C Offshore and national authorities from Norway, Denmark, Germany, Belgium, the United Kingdom, and the Netherlands.

rocks, called "rock dump" in the oil and gas industry and "scour protection" in the wind industry, further increase habitat heterogeneity and availability of hard substrate (Petersen and Malm 2006).

Once an artificial reef has been placed, it will be colonised by a community of species that normally resides on rocky reefs. This community includes epifouling species, which are directly dependent on the hard substrate, and associated mobile species, which depend on the epifouling species (Lindeboom et al. 2011; Krone et al. 2013a; De Mesel et al. 2015). On water column penetrating artificial reefs, a clear vertical zonation can be seen in this community. Near the bottom, anemones and soft corals are dominant, followed by a mix of hydroids and amphipods in the centre of the water column. The shallow part of the construction is often colonised primarily by mussels, barnacles and algae (Whomersley and Picken 2003; Bouma and Lengkeek 2013; Krone et al. 2013b; De Mesel et al. 2015).

The placement of offshore installations such as wind farms and oil and gas platforms in the North Sea, creates hard substrate intertidal zones in environments that normally lack these. Since there is a decrease in nutrient availability from coast to offshore in the southern North Sea (Brockmann et al. 1990), the communities settling in these intertidal zones may differ from intertidal zones at the coasts. This may explain why intertidal zones in wind farms, for example, have been found to host a large amount of non-indigenous species (De Mesel et al. 2015). With the increasing amount of installations in the North Sea, the artificial reefs with intertidal zones are suspected of providing pathways for these non-indigenous species to colonise areas normally out of reach (Lindeboom et al. 2015; De Mesel et al. 2015). Especially when installations are placed at the boundaries of the dispersal potential of species, they may facilitate the distribution to areas that are otherwise out of reach (Adams et al. 2014).

Community patterns
Artificial reefs increase the available habitat and distribution potential of native and non-native species. In addition to habitat availability at different water depths, many variables are known to influence the ability of benthic species to colonise an area (Reiss et al. 2014). Species composition on artificial reefs may vary with age (Whomersley and Picken 2003), material of which the reef is constructed (Andersson et al. 2009), surface orientation (Perkol-Finkel and Benayahu 2004), salinity (Zintzen et al. 2007), sea water temperature (Southward et al. 1995), food availability (Page and Hubbard 1987), wave action (Burt et al. 2010), water current speed or direction (Thorpe 2012; Macleod 2013), light (Hardy 1981) and the proximity of other reefs providing a source of larvae (Thorpe 2012).

To understand the impact these variables have, they are often studied in isolation from the others. Often these effects can be collinear (Graham 2003). For example, light attenuates with depth (Hardy 1981), and both salinity and food availability can change with distance

from river outflow (Brockmann et al. 1990; Otto et al. 1990). With the rise of computing power and an increase in the resolution of environmental data, modelling techniques are available today that allow us to evaluate the combined effects of such variables (Gayer et al. 2006; Brown et al. 2011; Reiss et al. 2014).

Therefore, one of the aims of this thesis is to evaluate the effect of these variables on the presence of species and on the structure of their communities. I aim to understand the patterns in these communities by using multilinear modelling techniques.

Stepping stones
When offshore installations are placed at the boundaries of larval distribution potential, they may function as stepping stones to increase the spread of certain species. This stepping stone effect of offshore installations has been suggested in many recent publications, warning for expansion of native and non-native species previously unable to colonise offshore areas (Langhamer 2012; Mineur et al. 2012; Thorpe 2012; Gittenberger et al. 2013; Miller et al. 2013; Ros et al. 2013; Krone et al. 2013b; Adams et al. 2014; Coates et al. 2014; Friedlander et al. 2014; Lindeboom et al. 2015; De Mesel et al. 2015).

Thorpe (2012) showed that part of the offshore oil and gas platforms in the North Sea may function as a large interconnected reef. In his calculations, the isolated installations are connected via pelagic larvae drifting with the currents from one platform to the next. Whether a larva actually reaches a suitable location, depends strongly on the currents, which are driven by wind and tides. Thorpe concludes that about 60% of the platforms in the UK sector of the southern North Sea are interconnected.

The stepping stone effect has been described and tested with field data in naturally occurring populations (Treml et al. 2008; Crandall et al. 2012), but to my knowledge, no literature existed that shows that offshore installations function in a similar manner. Therefore, I aim to evaluate the stepping stone effect on offshore installations in the North Sea, validating particle tracking models with population genetic data from mussel populations on artificial reefs.

Ecological value of artificial reefs
With their high numbers of species, the ecological importance of rocky reefs is high, and they are protected under the Habitat Directive of the EU (European Commission 1992). But what is the ecological value of the thousands of artificial reefs in the North Sea? To what extent do the species communities overlap between rocky and artificial reefs in the North Sea? The North Sea bottom is mostly covered with sandy sediments. Hard substrates of natural origin are rare here. Species present on artificial reefs, therefore, could be seen as an unnatural addition to the benthic ecosystem. But are they unnatural?

To evaluate the significance of artificial reef communities, one should put the current presence of reefs in the North Sea, in a historical context. Therefore, I start this thesis with a history of natural reefs in the North Sea.

Artificial reef communities have been compared to natural reefs extensively in many seas around the world (Smith and Rule 2002; Badalamenti et al. 2002; Knott et al. 2004; Perkol-Finkel et al. 2006; Wilhelmsson and Malm 2008; Fauvelot et al. 2009). In almost every study, artificial reefs differ from natural reefs, caused by structural differences (Perkol-Finkel et al. 2006) or by the relatively young age of artificial reefs when compared to natural ones (Perkol-Finkel et al. 2005). The most striking difference between rocky reefs and offshore platforms and wind farms in the Netherlands, is the presence of an intertidal zone on the artificial reefs. With platforms over 40 years old, I expected that some parts of the installations may (start to) resemble rocky reefs. I hypothesised that shallow parts of the artificial structures would differ significantly from subtidal rocky reef communities, while the deeper steel parts and rocks around the foundation would resemble the rocky reefs present in the Netherlands. Therefore, to evaluate the value of artificial reefs, I compare the species communities on artificial reefs to those on rocky reefs.

A history of southern North Sea reefs
In the past there was more hard substrate in the North Sea than there is today. Old maps show striking differences between the past and present. The Piscatorial Atlas for the North Sea (Olsen 1883), for example, contains maps that show large oyster reefs, gravel fields and moorlog banks (Figure 5) at locations where they are currently lacking (Figure 6).

Oysters
From historic reports we learn that at least until the beginning of the 20th century, oyster reefs (*Ostrea edulis*) were present south of the Dogger Bank (Olsen 1883; Anonymous 1886; Lübbert 1906). Oysters were also found on every part of the British coast from Shetland to the Channel Isles (Jeffreys 1862), along the Belgian coast and at offshore locations (Hostyn 1982; Degrendele et al. 2008; Rappé 2008; Houziaux et al. 2011), in the Wadden Sea and Dutch Zuiderzee (now Ijsselmeer; MacKenzie and Burrell 1997; Berghahn and Ruth 2005; de Vooys 2010), and around Helgoland (Möbius 1877; Caspers 1950; Berghahn and Ruth 2005). Basically, oysters were present along all coasts of the southern North Sea (Korringa 1952a).

Mobius (1877) described the largest offshore oyster bed in the North Sea as beginning in a narrow strip south east of Helgoland, from where it stretched to a 15 to 22 kilometre wide strip very far to the west. The two oyster beds depicted by Olsen (1883) have a combined surface area of 27.700 km². The location of the larger of the two is similar to that described by Mobius but the bed is much wider than 15-22 km. Lübbert (1906) mapped an oyster reef south of the Dogger Bank, similar to Olsen (1883) but stretching more to the north-east

(Berghahn and Ruth 2005). Based on the surface area covered by oyster beds, the spawning stock of oysters must have been in the order of billions of individuals in the open North Sea (Berghahn and Ruth 2005).
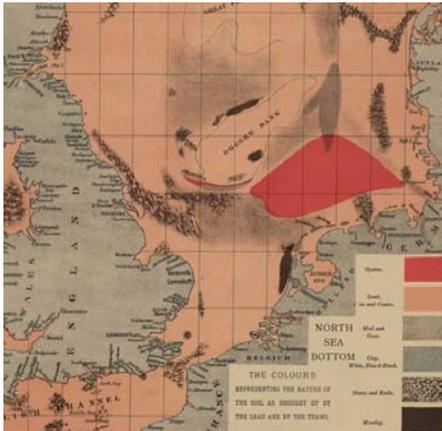
Figure 5: Fragment a map (Olsen 1883) showing the North Sea bottom habitats. Red: oysters; black: moorlog; coarse looking: stones and rocks; light coloured pinkish: sand bottom.
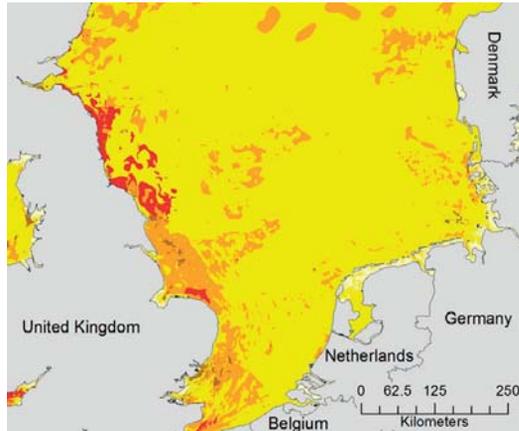
Figure 6: Modern habitat map. Yellow: sand and mud, brown: mixed; orange: coarse (including gravels); red: rocks (EMODnet 2015).

Oysters have been fished in the North Sea region already since Roman times (MacKenzie and Burrell 1997; Berghahn and Ruth 2005). The oldest records of trawl fisheries in Britain date back to 1376 (Berghahn and Ruth 2005). At first these fisheries were close to the coast, but with the advance of fishing techniques, fishermen went farther offshore (de Vooys 2001; Berghahn and Ruth 2005; Roberts 2007). Already in the 1870's British fishermen were fishing the oyster beds far offshore (de Vooys 2001). Mobius (1877) accounts that Dutch and German fishermen fishing on the oyster beds south of the Dogger bank caught up to 1,000 oysters in a single haul. Dutch fishers caught between 11 and 18 million oyster in the North Sea in 1889 (de Vooys 2001; Berghahn and Ruth 2005). At the turn of the century, German fishermen landed more than 2 million oysters annually (de Vooys 2001; Berghahn and Ruth 2005).

Forbes and Hanley (1853) provide oyster consumption statistics for London. Their informants estimated that about 120.000 to 130.000 bushels of oysters were consumed every year in London. Bushels contained between 8 and 21 gallons (Collard 1902). Jeffreys (1862) also gives consumption statistics on oysters in London and Paris: '*in London alone about 700 millions of oysters are annually consumed, and that in the provinces there is equal voracity and constant crying out for more. The consumption in*

Paris in 1861 reached 132 millions'. These figures seem extremely high, but in those times oysters were very cheap and consumed in large amounts by the poor (Vallely 2010). When these figures are compared to the FAO current global production statistics on *Ostrea edulis* (FAO 2014), it is clear that the amount of oysters in the North Sea was enormous: the average annual global production of *Ostrea edulis* in the period 2001-2011 was only 4.6 kilotons. A ton of oysters contains between 1800 and 3000 individuals (Hostyn 1982), so a high estimate of current production gives about 14 million edible oysters produced globally each year.

Together with the introduction of steam engines on fishing vessels, the coastal oyster beds declined rapidly, although effects of natural variation on this cannot be excluded (Berghahn and Ruth 2005). Sowerby (1854) already warned that natural oyster beds should be *'guarded by stringent rules, to prevent its exhaustion by eager fishermen'*. Jeffreys (1862) describes the decline of the oyster in the North Sea (then called German Ocean): '*The German Ocean has been so long the fishing-pond of Europe, that its supplies are beginning to fail us'*. It is clear from these accounts that by the time Olsen (1883) created his atlas, the oyster stock in the North Sea had already declined and that the North Sea may have contained much more natural oyster beds than drawn on his maps.

Caspers (1950) described the decline of German oyster beds around Helgoland in the period 1872 – 1885. He showed yearly landings of oysters, compared to the days at sea. In the first years the number of days at sea increased while the number of landed oysters increases in a similar way. But by 1878 the number of landed oysters per day at sea per ship, halved and stayed at this level, while the number of fishing days decreased until 1886 when there were only two ships left of the 56 there were in 1876. Caspers noted that at that moment, the fishing of oysters is not seen as beneficial. He attributes the decline of the oyster beds to overfishing, as even after a decline of the beds, the fishing continued and the stock was not allowed a chance at revival (Caspers 1950).

Oyster stocks in the North Sea were simply too small after the overfishing in the 19th century to recruit successfully (Gross and Smyth 1946). In present day, natural oyster beds have disappeared almost completely from the southern North Sea (Dalido 1948; Korringa 1952a), due to overfishing (Korringa 1946a; Korringa 1952a; de Vooys et al. 2004; Fraussen and Wera 2010), the resulting failing recruitment (Korringa 1946b; Caspers 1950), strong winters and disease (de Vooys 2001).

Tesch (1910) provides an overview of the fauna on the Oyster Grounds south of the Dogger Bank. Oysters were found here, mainly at locations where the silt layer was thin, in clusters of 4-5 together. The oysters were overgrown with *Alcyonium digitatum* and hydroid species. Species living between these were *Galathea intermedia*, *Janira maculosa*,

Nephrops norvegicus, *Tritinia hombergi*, *Tritonia plebeja*, *Archidoris tuberculata*, *Coryphella rufibranchialis*, *Policera quadralineata*. In and on the bottom the following species were found: *Astropecten irregularis*, *Parechinus miliaris*, *Brissopsis squamatus*, *Aphrodite aculeata*, *Cyprina islandica*, *Cardium echinatum*, *Neptunea antiqua*.

Moorlog

When fishermen fished the Dogger Bank for the first time, it seemed to be covered with masses of peat, known to the fishermen as moorlog (Reid 1913). The peat deposits were not only found at the Dogger Bank (Whitehead and Goodchild 1909; Stather 1912; Reid 1913), but also on the Oyster Grounds (Stather 1912), on the coasts of the Netherlands (Olsen 1883; Whitehead and Goodchild 1909), in a broken line on the British coast southward from Cromer and northward on the continental shores as far as Helgoland (Whitehead and Goodchild 1909). At coastal locations, peat layers were also found deeper in the sediment, covered by silt or layers of clay (Godwin 1945). The peat deposits were not only dredged up by fishermen, but also washed up abundantly on the shores of the coasts of Denmark and the Netherlands (Reid 1913), as is still the case (but probably less abundant) in the Netherlands (my personal observations) today.

On the Dogger Bank, the pieces dredged up were up to 2 meters long and 44 centimetres thick (Clark 1936). The moorlog pieces most likely were torn from larger beds by the trawling fishing vessels (Reid 1913). Large moorlog pieces were dredged up very frequently (Stather 1912). It was so abundant that trawl fishermen considered it a great annoyance and when caught they broke it up in pieces before discarding it (Whitehead and Goodchild 1909; Reid 1913). Because moorlog was found in such large amounts in such a wide area on the Dogger Bank, the area must have been covered with a layer of moorlog (Reid 1913). The texture of the moorlog was variable, from a mossy deposit to a fine hard mass of wood-like texture (Clark 1936). Moorlog was often found with shelly clay attached to it. Stather (1912) concluded that the moorlog covered the older clay layers present in the bottom and that these combined materials covered large areas in the southern North Sea.

Reid (1913) reports that he found recently dead *Barnea parva* in the moorlog. Whitehead and Goodchild (1909) discovered live specimens of *Barnea parva* in the moorlog. This indicates that at that time, there was a stable peat layer present, which implies that the studied moorlog pieces were part of a larger structure.

Rocks and gravels

In the Dutch part of the North Sea large boulders were found dating from the period before the North Sea was formed, when glaciers covered part of the land and river landscape was present where the North Sea currently is (Laban 1999; Houziaux et al. 2011). Stones were

found in irregular concentrations in several areas in the North Sea, differing in size from small pebbles to several hundred kilogrammes in weight, all of them rounded (Tesch 1910).

On the higher grounds of the Dogger Bank and on the Cleaver Bank, patches of pebbles were found. The depth at which the gravel occurred on the Dogger Bank varied from 20 to 46 m. The gravel in patches found on the higher grounds of the Dogger Bank was coarser than on the deeper parts. This was most likely caused by water movements washing out the finer sands of higher grounds in times when the sea level was lower and wind swept tides had more influence on the sediment (Veenstra 1965). Stones in the area south west of the Dogger Bank were so abundant they were a great nuisance to trawling fishermen (Tesch 1910). Reid (1913) described many 'erratic blocks' scattering the Dogger Bank and reports that these blocks damaged the trawls. The blocks were taken on board and thrown back in the sea in deeper waters (Reid 1913). Further rocky reefs were found on the western part of the Dogger Bank, between 1° and 2° east and 54° and 55° north in depths between 21 and 38 meters. These stones were sized between small pebbles and medium large boulders (Tesch 1915). Boulder clays (clay containing rocks) were also found on the Dogger Bank, Cleaver Bank and the area east of the Outer Silver Pit (Pratje 1951; Veenstra 1965).

Further rocky reefs were present near Texel, north of Terschelling and north of Borkum (Tesch 1910; Tesch 1915; Erdbrink 1950; Pratje 1951; Laban 1999). The Texel Rough also contained boulder clay (Veenstra 1969). Erdbrink (1950) reported rocky reefs from the Belgian Noord Hinder, east of the Dutch Sealand islands and east of Rotterdam. The gravel beds at the Noord Hinder were also reported by Gilson (1900) and confirmed by Houziaux (2011).

Tesch (1910) already concluded that the community in gravel fields was more diverse than that on other substrates. He attributes this to the complexity of epifauna species attached to the stones, creating additional habitat for other, mobile species. He also reports an overview of the fauna on the 'Groote Visserschbank' (Great Fisher Bank, north of the Dogger Bank): incredible masses of bryozoa were present, among which *Flustra securifrons*, *Alcyonidium gelatinosum*, *Alcyonidium hirsutum*, which were attached to all possible hard objects present. He reports an *Alcyonidium* colony with a length of over one foot to be attached to a single *Mactra* shell. The fauna in this area was richer than on the Oyster Grounds. On gravels from the Hinder Banks, only the upper part of large pebbles was overgrown with organisms, indicating that the pebbles were not moved by the currents (Veenstra 1969). On the Dogger Bank, gravel coarser than 8 mm was frequently covered only on the topside with organisms: bryozoans, serpulids and hydrozoans. This proves that these pebbles were not moved by currents (Veenstra 1969).

The stones from the Texel Rough have partly been brought ashore by fishermen (Lindeboom et al. 2008b). However, many are still present in the area near Texel (Montfrans et al. 1988;

van Moorsel 1994; personal observation J.W.P. Coolen), on the Cleaver Bank (Montfrans et al. 1988; van Moorsel 1994) and near Borkum (Bos and Paijmans 2012).

Thesis outline

It is clear that, before the onset of large scale fisheries, natural reefs were abundantly present in British, Belgian, Dutch and German waters. When I started, some of the reefs had been poorly studied, or even were presumed lost (Texel rough; Lindeboom et al. 2008). Therefore, in **Chapter 2** of this thesis, we investigated the benthic presence and biodiversity of rocky reefs in the area north of the islands Schiermonnikoog and Borkum; the Borkum Reef Grounds. Results of this study are later used in modelling species distributions in **Chapter 4.2** and in a comparison between natural rocky and artificial reefs in **Chapter 6**.

Companies operating offshore oil and gas platforms, regularly perform inspections of the foundations of these installations, using remotely underwater vehicles (ROVs) equipped with camera's. Although these images are not created for biological inventory, some of the species present are visible in the images. Therefore we started our inventory of benthic communities on the offshore platforms (i.e. artificial reefs), with analysing ROV footage from five gas platforms, described in **Chapter 3**.

In **Chapter 4**, I first describe the first record of a hard coral observed on a ship wreck on the Dogger Bank. It includes suggestions of the origin of the corals. Then I describe a distribution model I created for two species that are closely related, but which occupy different habitats.

To further investigate the origin of species present on artificial reefs in the North Sea, we studied the population genetic structure of the blue mussel *M. edulis* on coastal and offshore locations. The results strongly support that *M. edulis* utilises offshore intertidal installations as stepping stones to expand its distribution in the North Sea (**Chapter 5**).

In **Chapter 6**, I combine data obtained from oil and gas platforms with data from wind farms surveys and the Borkum Reef Grounds study reported in **Chapter 2**, to evaluate in how far North Sea natural and artificial reefs are different.

Chapter 7 holds the synthesis of all these investigations.

Chapter 2:

REEFS, SAND AND REEF-LIKE SAND:
A COMPARISON OF THE BENTHIC BIODIVERSITY OF HABITATS IN THE DUTCH BORKUM REEF GROUNDS

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Abstract

Reefs play an important role in the distribution of species associated with hard substrates and are of value for biodiversity conservation. High densities of the habitat building annelid *Lanice conchilega* also increase local biodiversity. This study describes the benthic biodiversity of a rocky reef and its surrounding sand bottom with dense *L. conchilega* beds in the Borkum Reef Grounds, north of the island of Schiermonnikoog in the Dutch North Sea. A side-scan sonar survey revealed distinct seabed areas with high acoustic reflections, indicating the presence of hard substrate on the sandy seafloor. To ground truth the side-scan sonar data and make an inventory of the biodiversity of the observed habitats, a multi-method sampling approach (box corer, scuba airlift sampler and visual transects, drop down camera) was used. This revealed (1) rocky reefs: a combination of gravel, stones and rocks; (2) individual rocks in a sandy environment; (3) sand with dense *L. conchilega* beds (>1,500 ind.m^{-2}) and (4) sand bottom habitat. A total of 193 taxa was found with many unique species per habitat. Species richness was significantly higher on sand when compared to the rocky reef (NB-GLM; p=0.006), caused by the presence of dense *L. conchilega* beds (Poisson GLM; p<0.001). Including dense *L. conchilega* beds as an additional habitat showed these held a higher species richness than the rocky reefs (NB-GLM; p=0.002), while sand without dense *L. conchilega* beds did not (NB-GLM; p=0.14). Since the rocky reefs were present on a sandy bottom, the local biodiversity more than doubled with the presence of rocky reefs. The nMDS plot clearly separated the sand and rocky reef communities and also showed a clustering of dense *L. conchilega* beds within the sand samples. Each method detected unique species, demonstrating the value of a multi-method approach compared to e.g. box coring alone. This study identified several species previously unknown to the Borkum Reef Grounds area. The total area of rocky reefs in the southern part of the Dutch Borkum Reef area is estimated to be 9.8 km^2 and of *L. conchilega* beds with densities >1,500 ind.m^{-2} to be 74 km^2. Further research should focus on the possible function of *L. conchilega* as an ecosystem engineer creating intermediate sand-reef systems. For mapping these *L. conchilega* beds, we advise using side-scan sonar imaging combined with ground truthing by drop down cameras.

Introduction

Natural reefs in temperate waters are important to local biodiversity and can host long-lived species (Sheehan et al. 2013; Fariñas-Franco et al. 2014). Such reefs can be of abiotic origin, such as gravel fields, boulder clay or rocky outcrops, or biogenic origin, formed by organisms such as shellfish (e.g. *Modiolus modiolus*), corals (e.g. *Lophelia pertusa*) or annelids (e.g. *Sabellaria spinulosa*) (Davis 2009; Fariñas-Franco et al. 2014). Around the British Isles, reefs are extensively present but they are rare in the south eastern part of the North Sea, where they are only found on the Hinder Banks, the Cleaver Bank and the Texel Rough (Veenstra 1969), and the Borkum Reef Grounds (Dörjes 1977). Further east, reefs are present around Helgoland (de Kluijver 1991).

Maps by Olsen (1883) show a vast area of reefs present in the southern North Sea. *Ostrea edulis* reefs were found south of the Dogger Bank, in the Wadden Sea, the Dutch 'Zuiderzee' and around Helgoland (Jeffreys 1862; Möbius 1877; Olsen 1883; Caspers 1950; Korringa 1952b). The *O. edulis* reefs have since disappeared from the Dutch continental shelf (Beck et al. 2011). Olsen (1883) also mapped 'Stones and Rocks' on the Cleaver Bank, Dogger Bank and the Borkum Reef grounds, and locations with 'Moorlog', a peat deposit, west of the Dutch coast and on the Dogger Bank.

These reefs may have played an important role in the distribution across the North Sea of species associated with hard substrates. It is therefore of importance to document the remaining reefs as they form the last remnants of a once more extensive reef community in the North Sea. Furthermore, the fauna associated with these reefs is of importance for biodiversity conservation. In Europe, reefs are protected under the EU Habitats Directive, which defines reefs as "*either biogenic concretions or of geogenic origin. They are hard compact substrata on solid and soft bottoms, which arise from the sea floor in the sublittoral and littoral zone.*" (habitat code 1170; European Commission, 2013, 1992).

Rabaut *et al.* (2009) concluded that high densities of the tube-dwelling annelid *Lanice conchilega* also meets the criteria to be defined as reefs, following the 'reefiness' evaluation of *Sabellaria spinulosa* by Hendrick and Foster-Smith (2006). Furthermore, *L. conchilega* influences benthic biodiversity by modifying the available habitat and creating attachment surfaces for other species (Rabaut et al. 2007). It increases the species richness in low structured sand habitats (Zühlke 2001). Dense *L. conchilega* beds were previously observed in the Borkum Reef Ground area (Lindeboom et al. 2008a) and may be relevant for the evaluation of the area for protection under the EU Habitats Directive.

Limited information on the biodiversity of reefs in the southern North Sea is available, and most of it is published in reports and other non-peer reviewed publications. Houziaux *et al.* (2008) made an inventory of the rocky reefs on the Belgian Hinder Banks, and van Moorsel

(1994) and Schrieken *et al.* (2013) investigated the Cleaver Bank. The fauna of the German part of the Borkum Reef Grounds was studied by Dörjes (1977), who focussed on reef species, but did not record many common bryozoan and hydrozoan species. The Dutch part was previously investigated by Bergman *et al.* (1991, 1992), who focussed on soft-sediment species.

The presence of the Borkum Reef Grounds was already documented in the 18th century by Guitet (1710), who noted the 'Borkomer Riff' north of the island of Rottum. Further accounts of the presence of the reefs are found throughout the years (Olsen 1883; Dörjes 1977) but the reported area occupied by them varies broadly and often lacks mention of reefs west of the German-Dutch border (in Dutch waters). Therefore, the extent of the reefs in the Dutch part of the Borkum Reef Grounds remained uncertain.

The aim of this paper is to provide an inventory of the benthic fauna of the Dutch part of the Borkum Reef Grounds and to map the presence of rocky reefs in the area in order to evaluate the need to protect the reefs under the EU Habitats Directive. We report on the locations of gravel fields and large rocks (henceforth named 'rocky reefs'), the surrounding sandy habitats (sand) and *L. conchilega* beds which had densities of >1,500 ind.m^{-2}. Furthermore, a species list and a comparison between the different communities is given.

Materials and methods

Study area
The Borkum Reef Grounds are situated in the North Sea north of the Wadden Sea island of Schiermonnikoog (Figure 1). The area is characterised by water depths between 10 and 40 m, maximum currents between 0.4 and 1.0 ms^{-1} and water temperatures varying between 3 and 19°C (Joschko et al. 2008). The seafloor has previously been described as a 'rough ground', containing coarse sand, gravel and stone fields (Olsen 1883; Tesch 1910). This study focuses on the Dutch part of Borkum Reef Grounds which has an area of approximately 600 km^2.

Side-scan sonar survey
From 17 to 22 September 2009, a side-scan sonar survey was conducted following methods modified from Lafferty (2006) using a Klein 3900 500/950kHz side-scan sonar set at 500kHz frequency. The sonar fish was towed at a speed of 4.0 to 4.6 ms^{-1} along parallel northwest – southeast transects 1,500 m apart, covering a strip of 100 m width. Positioning was performed with a C-NAV GPS. Acoustic units identified on the side-scan sonar data were characterised as sand bottom, gravel and stone fields and crust-like structures, or single larger rocks (>30 cm). Extra transects were conducted where hard substrate appeared to be present. During acquisition, data quality was assessed continuously by a side-scan sonar

technician. In total, approximately 63 km^2 of side-scan sonar data were obtained, covering approximately 7.4% of the surveyed area.

Sediment and biodiversity survey

From 12 to 17 August 2013, the side-scan sonar survey was ground truthed. Due to safety limitations on scuba diving (depths <30 m), a main method for the ground truthing, only the southern part of the area was studied. Selection of sample locations (Figure 1) was based on the acoustic units identified by the 2009 survey, realising a maximum spread over the area with representative sampling of the expected sand and rocky reef habitats (gravel and rock).

To characterise the sediment type, sediment sub-samples were obtained from a Van Veen grab and box corer samples, by sub-sampling with a 5.5 cm^2 x 11 cm deep corer. Further sediment cores were obtained by scuba divers sampling directly from the seafloor. Divers also recorded the dimensions of observed large rocks. All sediment samples were freeze-dried for 96 h. Sub-samples of the sediments (0.5-5 ml) were dry sieved over a 2-mm mesh sieve after which grain size and silt content were determined using a Coulter LS 13 320 particle analyser and auto-sampler. In total, 20 sediment samples were analysed.

The fauna in the sand and on the rocky reefs was inventoried using a combination of four methods:

1. The sand and gravel fields were sampled using a box corer (0.076 m^2). Samples with less than 15 cm penetration depth were rejected and the seafloor was resampled. Before processing on board a photo was taken of each sample. From each sample, the top 2.5 cm layer was removed and stored for later processing, the remaining fraction was sieved over a 1 mm mesh sieve. The residue was then transferred to a plastic storage container. Epifauna were relaxed in an oversaturated seawater menthol solution for a minimum of 2 hours. All samples were finally fixed in a borax-buffered formaldehyde-seawater solution (6%). In total, eight box corer samples were taken (seven in sand and one from the rocky reef, containing gravel).
2. Epifauna on large rocks were sampled using a scuba diver operated, airlift sampler. Although airlift samplers are not routinely employed for benthic inventories in the Netherlands, they have been used successfully in a number of studies in temperate waters (e.g. Barnett and Hardy, 1967; Benson, 1989). The airlift used in this study resembled that of Chess (1978) and was constructed of 50 mm PVC tubing ending in a net of 500 μm mesh positioned in a downward, vertical angle, from the tube end. It was fed by compressed air from a cylinder attached to the divers harness. Samples were collected by placing a metal frame (0.050 m^2) on a rock and removing all growth in the frame with a putty knife attached to the airlift. During removal the sample was sucked into the airlift and deposited in the net. The net was replaced between samples

and sealed with a lid. All sampling activities were filmed by a camera system (GoPro HERO3 Black with 2 Metalsub VL1242 LED video lights) mounted on the diver's head. In total 11 airlift samples were taken from nine rocks (with duplicates at two rocks). Following collection, airlift samples were processed in the same manner as the box corer samples.

3. Demersal megafauna were surveyed by scuba divers using line transect observations. Methods by Holt and Sanderson (2001) were adjusted to local circumstances, i.e. a decreased pole length of 2 m operated by a single diver, as low visibility and strong currents were expected. A shot-line with buoy was anchored at the diving location, and a 50 m tape was reeled out from the anchor in a random direction. A diver followed the tape while recording all fish and benthos >1 cm in size located within the 2 m guide pole, providing a 100 m^2 transect.

4. Images of the seabed were recorded with a drop-down camera system. This system used a stereo Prosilica GE4900C, machine-vision camera, with a 14 mm lens, recording 5 frames per second at a resolution of 11 megapixels, covering an area of approximately 0.25 m^2 per frame. In a calm sea, the camera hovered at a fixed height above the sea bed. In higher waves, the 'stamping technique' was used where the camera goes down to the seabed and up, moving at low horizontal speed. To obtain an area of >10 m^2 at each location, a minimum of 5 minutes of recording was required. Images were collected at a total of 14 locations.

Processing of samples and images

For processing in the lab, the samples taken from the top 2.5 cm layer of the box corer were sieved over a 500 μm mesh sieve and then treated as the other samples. Prior to identification, fauna present in the box corer and airlift samples were sorted into major taxonomic groups. Individuals were counted and identified to the lowest possible taxon (but Nemertea and Phoronida were identified only to phylum level) using a stereo microscope (Zeiss SteREO Discovery.V8 with a Achromat S 0.63x FWD 107 mm objective) and a Zeiss AXXIO Scope.A1. Identification was aided by the World Register of Marine Species (2014) as taxonomic reference.

Using the scuba diver video images and a scale visible on the sampling frame, percentage cover in the airlift samples was estimated for species with mixed or colonial growth, such as hydroids, bryozoans and tunicates. For box corer samples, percentage cover was estimated from the photos taken before sample processing. Species with high abundance and high percentage cover were classed using percentage cover. To enable comparison between box corer and airlift samples, all counts and percentages were categorised into the one scale of values (Table 1). For abundance classes, individuals per standardised sample size (0.05m^2) were used. For countable taxa with a cover of >5%, the cover percentage was estimated.

Although mostly published in reports only, this method is commonly used for studies of reef biodiversity in the Netherlands (e.g. Leewis et al., 2000).

From each location, 30 non-overlapping images with at least 0.25 m² of seabed visible and in focus were selected. Species were identified to the lowest possible taxon and counted using ImageJ software (National Institutes of Health 2013).

Table 1. Category values used for statistical analysis of box corer and airlift samples: count classes were based on individuals per 0.05 m².

Class	Value
1 individual	1
2-5 individuals	2
6-50 individuals	3
>50 individuals, <5% cover	4
5-15% cover	5
16-25% cover	6
26-50% cover	7
21-75% cover	8
76-100% cover	9

Statistical analysis

The total species list (see results; Table 2) was based on the raw data on all observed taxa from all methods combined. During statistical analysis, only the data from box corer and scuba airlift samples were used for comparison between the sand and rocky reef habitats. Box corer data from station 'reef 3' were removed from the analysis as this was the only sample taken at a gravel field. Within the remaining data, observations of juvenile or damaged specimens identified to the genus level or higher were either combined with a species in the same taxon or omitted when more than one species was present in the taxon. Only juvenile and damaged taxa in samples with no species in the same taxon were left at the higher level. Data from the scuba line transect sampling were not used for statistical analyses due to the low number of observations on the rocky reefs (only three transects). Drop-down camera data were not used for statistical analyses, since taxonomic determination was often not possible to the species level.

Data were analysed using R version 3.1.1 (R Core Team 2014a) and RStudio version 0.98.976 (RStudio 2014a). Indices of species richness (number of taxa per sample), Shannon-Wiener diversity index (Shannon 1948) and Pielou's evenness (Pielou 1966) were calculated per sample using the categorised data from airlift and box corer samples (without 'reef 3'). During data exploration, *L. conchilega* beds with densities of >1,500 ind.m⁻² were recognised as a potential third habitat type, following Rabaut *et al.* (2009) who used this amount to

indicate a high concentration of *L. conchilega*. Therefore, this habitat type was included in the statistical analysis and compared with the rocky reefs and the sand with lower *L. conchilega* densities.

The effect of habitat types on the species richness of the samples was tested using Generalised Linear Models (GLMs). Two GLMs were constructed with data from box coring and airlift samples, using the negative binomial distribution (NB-GLM; glm.nb function from the MASS package ;Venables and Ripley, 2002). The first NB-GLM described the difference between the rocky reef and the sand habitat (including all *L. conchilega* densities) and the second NB-GLM described the difference between all three habitat types. NB-GLM was used as normal and Poisson distributions resulted in overdispersed residuals (Zuur et al. 2013). The effect of dense *L. conchilega* beds within the sand habitat was modelled with a GLM using the Poisson distribution. Independence, distribution and homogeneity of the residuals of all models were inspected for patterns with plots of the residuals against the fitted values and boxplots to test if the model assumptions were met, following Zuur *et al.* (2013).

Multivariate differences between the communities in the different habitats were visualised by non-metric multidimensional scaling (nMDS; Kruskal, 1964) based on the Bray–Curtis similarity measure (Bray and Curtis 1957) using untransformed category data (as per Table 1). Significance of differences was tested by permutational multivariate analysis of variance (PERMANOVA; Anderson, 2001), using the adonis function in the package 'vegan' (Oksanen et al. 2008). To detect the species responsible for the differences in communities, a SIMPER analysis (simper function in the vegan package; Oksanen et al., 2008) was performed on the sand vs rocky reef samples and the sand vs *L. conchilega* samples.

Results

The ground truthing of the side-scan sonar revealed three different habitat types, based on the data from all methods. The following habitat types were defined: (1) sand bottom, (2) rocky reefs (gravel with stones and rocks, or individual rocks on sand) and (3) *Lanice conchilega* beds with densities of >1,500 ind.m^{-2} (Figure 1 and 2).

At locations 'reef 1' and 'reef 2' the presence of isolated rocks of approximately 1 – 1.5 m maximum length was confirmed by the scuba divers, and at 'reef 3' a gravel and stone field (stones sized 20 - 40 cm) was found (Figure 1). The total area of rocky reefs was estimated to cover 9.8 km^2. *L. conchilega* beds with densities >1,500 ind.m^{-2} were estimated to cover 74 km^2 (Figure 1). For this estimation the assumption was made that the highly reflective seafloor in the dark grey area of figure 1 represented rocky reefs and that all other highly reflective locations represented *L. conchilega* beds with densities >1,500 ind.m^{-2} (Figure 3). The average median grain size in the sand habitat was 248 ± 20 μm (95% confidence interval

around the mean, n=21) and in the area around the rocky reefs 445± 498 μm (n=2). For grain size measurements, no distinction was made between high and low *L. conchilega* densities.

A total of 193 taxa was observed in all the techniques combined: the visual surveys by the divers, the drop-down camera images, the total of eight box corer samples and 11 airlift samples (Table 2). Of the taxa, 73% (n=141) were identified to the species level and 9% (n=17) to the genus level: 18% (n=35) could not be identified to the genus level. Overall, annelids (54 taxa) and arthropods (53 taxa) represented the most abundant species groups. Molluscs were represented by 28 taxa, cnidarians by 23, chordates by 14, echinoderms by eight, bryozoans by seven, sponges by two and the remaining taxa by only one taxon per group. Of the total taxa, 120 were found in the sand and 137 on the rocky reefs (including the gravel sample which was omitted from the statistical analysis).
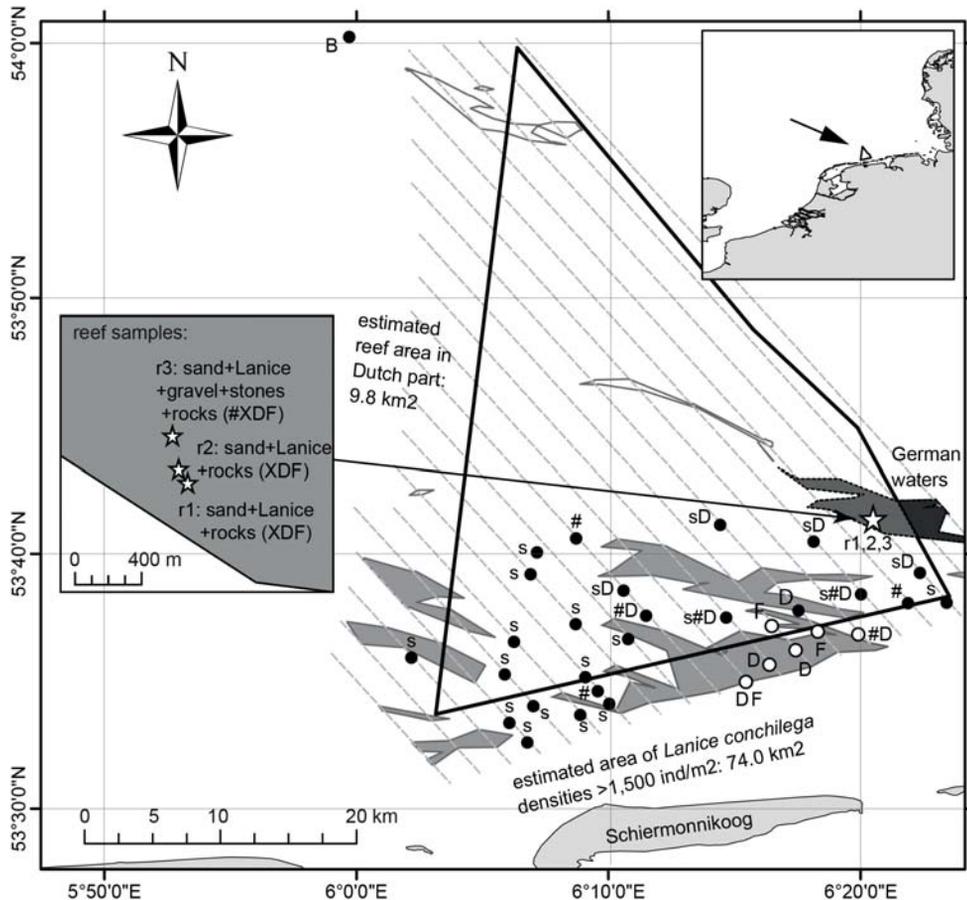
Figure 1. Study area. Map showing distribution of rocky reefs (gravel, stones, rocks), sand (black dots) and dense *Lanice conchilega* beds (>1500 ind.m^{-2}, white dots). #=Box corer, X= scuba airlift sampler, F= scuba visual transect, D=drop-down camera observation. Grey: areas with high acoustic reflections, as interpolated from the side-scan sonar survey (dashed lines) in 2009. The dark grey area (9.8 km^2) was estimated to consist of hard substrate (including confirmed rocks, stars) and the light grey areas (74 km^2) of dense *L. conchilega* beds. The white areas marked in the northern part were not ground truthed. Thick line: Dutch part of the Borkum Reef Grounds as mapped in national policy documents. B: Location studied by Bergman *et al.* (1992).
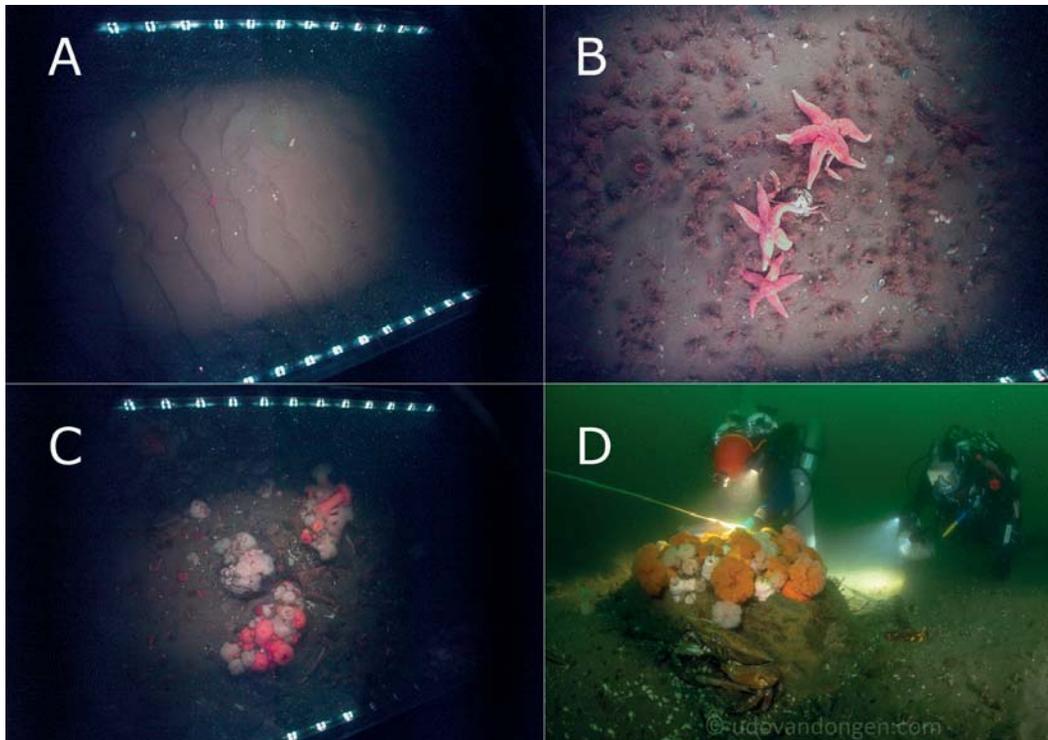
Figure 2. Photos of (A), sandy habitat (drop-down camera image) (B) sandy habitat with *Lanice conchilega*, (C) gravel and stones (D) scuba divers sampling a rock on a sandy bottom.

The SIMPER analysis showed that most taxa responsible for the dissimilarity between habitats, were present in the *L. conchilega* habitat and missing from either sand or rocky reef (Table 3). The most abundant species in the sand habitat were *Urothoe poseidonis*, *Scoloplos armiger* and *Spiophanes bombyx*, but these were more abundant in the dense *L. conchilega* beds. The abundant species *Owenia fusiformis* was only present in the samples from the dense *L. conchilega* beds. On part of the rocky reefs, *Diplosoma listerianum* was found in extreme abundance, while it was absent in sand and dense *L. conchilega* beds. *Electra pilosa* and *Ophiura albida* were very abundant on rocky reefs and also present in the *L. conchilega* samples.

With a total of 357 individuals (4,728 ind.m^{-2}), *L. conchilega* covered almost 100% of the single gravel sample. Other abundant taxa in this sample were the annelid *Scoloplos agg.* (2,079 ind.m^{-2}), juvenile echinoderms of the class Ophiuroidea (1,351 ind.m^{-2}), the mollusc *Abra alba* (1,311 ind.m^{-2}), capitellid annelids (1,298 ind.m^{-2}) and juvenile *Ophelia spp*. (1,166 ind.m^{-2}).

Species richness (S; number of taxa), was found to be significantly higher (NB-GLM; p=0.006) in the sand habitat (including *L. conchilega* beds with densities >1,500 ind.m^{-2}) when compared to rocky reefs. When including dense *L. conchilega* beds as a habitat in the

model, this resulted in in a significantly higher S for *L. conchilega* habitat when compared to rocky reefs (NB-GLM; p=0.002) but not higher for sand vs. rocky reefs (NB-GLM; p=0.14). The presence of dense *L. conchilega* beds within the sand habitat resulted in a significantly higher S (Poisson GLM; p<0.001).

The nMDS plot on species abundance in different samples (stress=0.056) clearly separated the sand, *L. conchilega* and rocky reef habitat communities (Figure 5), as confirmed by the PERMANOVA (p=0.001 based on 999 permutations).
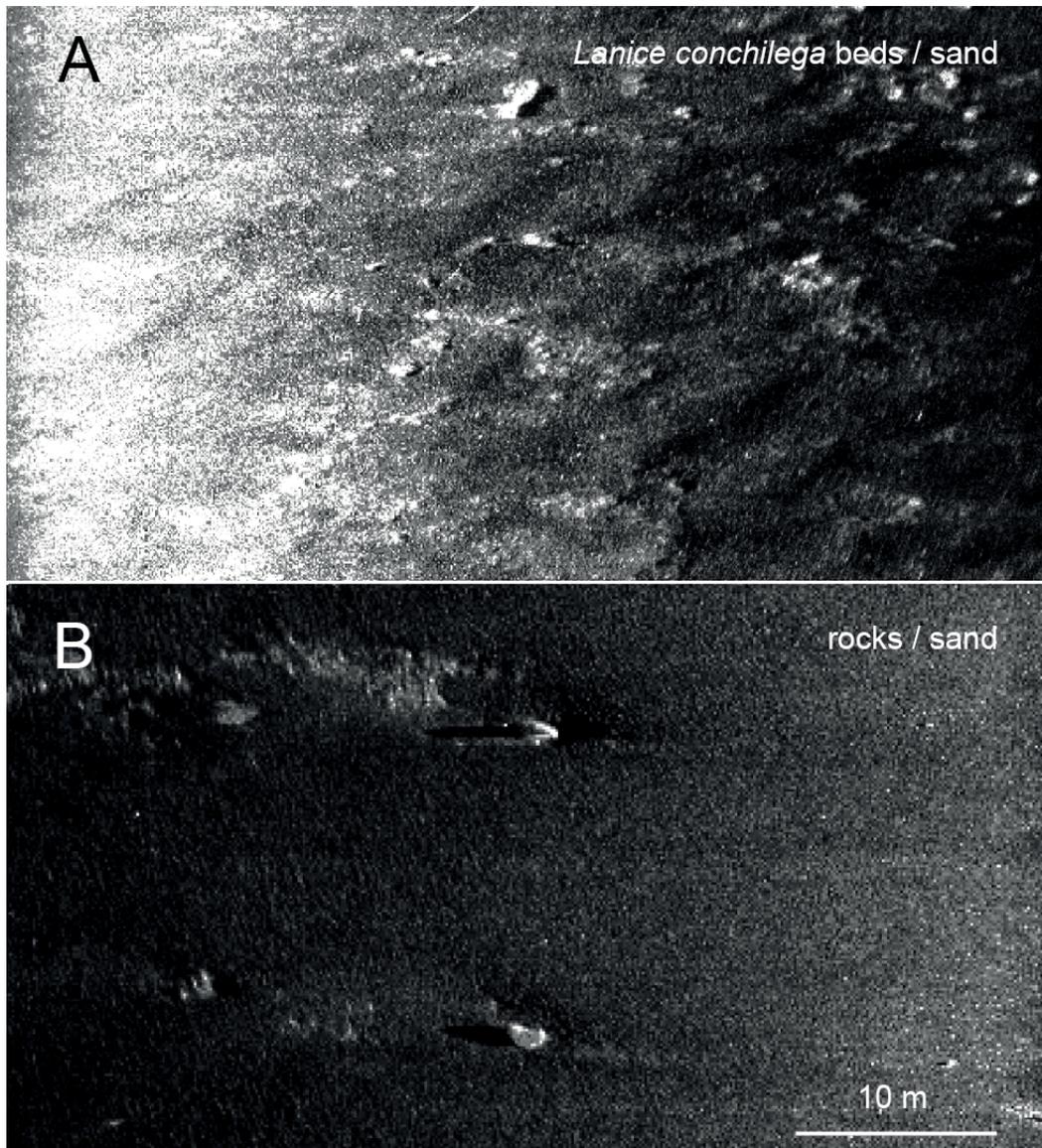
Figure 3. Side scan sonar images of (A) sand habitat with dense *Lanice conchilega* beds and (B) sand habitat with isolated rocks of approximately 1 – 1.5 m maximum length.

Table 2. Taxa found on the rocky reef (R; including gravel) or in the sand bottom (S), resulting from all sampling methods. Dense *Lanice conchilega* beds were included in S since no discrimination between the habitats was made during field observations by scuba divers and photo-analysis. Open circles indicate taxa found in dense *Lanice conchilega* beds in box corer samples.

	R	S		R	S
Porifera			*Gattyana cirrhosa*	●	o
Calcarea			*Goniadella bobretzkii*	●	o
Sycon ciliatum	●	-	*Harmothoe clavigera*	●	-
Demospongiae	-	-	Harmothoe fernandi	●	-
Halichondria panicea	●	●	*Harmothoe impar*	●	-
Cnidaria			*Harmothoe spp.*	●	-
Hydrozoa			*Heteromastus filiformis*	-	o
Bougainvilliidae	●	-	*Lagis koreni*	●	o
Calycella syringa	●	-	Lanice conchilega	●	o
Campanularia volubilis	●	-	*Lepidonotus squamatus*	●	-
Campanulariidae	●	o	*Magelona filiformis*	-	o
Clytia hemisphaerica	●	o	*Magelona johnstoni*	-	o
Ectopleura larynx	●	o	*Magelona mirabilis*	-	o
Eudendrium spp.	●	-	Malmgreniella arenicolae	-	o
Garveia nutans	●	-	*Mediomastus fragilis*	-	●
Hydractinia echinata	●	-	*Nephtys assimilis*	-	●
Leptothecata spp.	-	●	*Nephtys caeca*	-	o
Obelia bidentata	●	o	*Nephtys cirrosa*	●	o
Obelia dichotoma	●	-	Nephtys hombergii	-	o
Obelia spp.	●	o	Nereididae	●	o
Sertularia argentea	●	-	*Ophelia borealis*	●	o
Sertularia cupressina	●	-	*Owenia fusiformis*	●	o
Sertularia spp.	●	-	*Pectinaria spp.*	●	o
Tubularia indivisa	-	o	Pholoe baltica	●	-
Tubulariidae	●	o	*Phyllodoce groenlandica*	●	o
Anthozoa			*Phyllodoce mucosa*	-	o
Alcyonium digitatum	●	-	*Phyllodoce spp.*	-	●
Cerianthus lloydii	-	o	*Pisione remota*	●	-
Metridium senile	●	-	Poecilochaetus serpens	●	o
Sagartia elegans	●	●	*Polydora spp.*	●	-
Sagartia troglodytes	-	o	Polynoidae	●	o
Nemertea	-	o	*Proceraea cornuta*	●	-
Annelida			*Proceraea prismatica*	●	o
Polychaeta			Protodorvillea kefersteini	●	-
Aonides paucibranchiata	●	o	*Pseudopolydora pulchra*	●	o
Aricidea minuta	-	●	*Sabellaria spinulosa*	●	-
Capitellidae	●	o	*Scolelepis bonnieri*	-	●
Chaetozone setosa	-	o	*Scolelepis squamata*	-	o
Eteone cf. longa	●	o	Scoloplos agg.	●	o
Eulalia viridis	●	-	*Spio spp.*	●	o
Eumida bahusiensis	●	o	*Spio goniocephala*	●	o
Eunereis longissima	●	o	Spionida	●	o
Exogone naidina	-	o	*Spiophanes bombyx*	●	o

	R	S
Syllis spp.	●	●
Clitellata		
Oligochaeta	-	o
Entoprocta		
Pedicellina cernua	●	-
Arthropoda		
Maxillopoda		
Balanomorpha	●	-
Balanus balanus	●	-
Sessilia	●	-
Malacostraca		
Amphilochus neapolitanus	●	-
Amphipoda	●	-
Aora gracilis	●	-
Bathyporeia elegans	-	●
Bathyporeia guilliamsoniana	-	●
Bathyporeia pelagica	-	●
Brachyura	●	●
Cancer pagurus	●	-
Caprella linearis	●	-
Caprellidae	-	o
Carcinus maenas	●	●
Corophium multisetosum	-	o
Crangon crangon	●	o
Cumacea	-	●
Decapoda	●	o
Ericthonius spp.	●	-
Gammarus spp.	●	o
Hyperia galba	●	-
Ischyrocerus anguipes	●	-
Isopoda	●	●
Jassa spp.	●	●
Lepidepecreum longicornis	●	-
Leucothoe lilljeborgi	-	o
Liocarcinus holsatus	●	o
Liocarcinus navigator	●	o
Lysianassidae	●	-
Macropodia rostrata	●	-
Megaluropus agilis	-	●
Microprotopus maculatus	●	o
Monocorophium acherusicum	●	-
Mysis spp.	●	●
Nototropis swammerdamei	-	o
Pagurus bernhardus	●	●
Palaemonidae	●	●
Pandalus montagui	●	-
Pariambus typicus	●	o

	R	S
Perioculodes longimanus	-	o
Pestarella tyrrhena	●	-
Phtisica marina	-	o
Polybiidae	-	o
Pontocrates altamarinus	-	●
Portunidae	●	-
Pseudocuma longicorne	-	o
Stenothoe marina	●	o
Synchelidium maculatum	-	●
Thalassinidae	-	o
Tryphosella sarsi	●	-
Upogebia spp.	●	o
Urothoe poseidonis	-	o
Pycnogonida		
Nymphon brevirostre	●	-
Mollusca		
Gastropoda	●	-
Aeolidiidae	●	-
Alvania lactea	●	-
Crepidula fornicata	●	-
Dendronotus frondosus	●	-
Onchidoris muricata	●	-
Bivalvia	●	o
Abra alba	●	o
Cardiidae	-	o
Donax vittatus	-	●
Ensis directus	●	o
Euheterodonta	●	-
Kurtiella bidentata	●	-
Lutraria spp.	●	-
Macoma balthica	-	●
Mactra stultorum	-	●
Musculus discors	●	-
Musculus subpictus	●	-
Mytilus edulis	●	●
Mytilidae	●	-
Phaxas pellucidus	●	-
Spisula elliptica	●	o
Spisula solida	-	o
Striarca lactea	●	-
Tellimya ferruginosa	●	o
Tellina fabula	-	o
Tellina tenuis	-	o
Thracia phaseolina	●	-
Bryozoa		
Gymnolaemata		
Alcyonidium hydrocoalitum	●	-

2

	R	S
Alcyonidium parasiticum	●	-
Alcyonidium spp.	●	-
Conopeum reticulum	●	-
Electra pilosa	●	o
Schizomavella linearis	●	-
Scruparia ambigua	●	-
Echinodermata	-	●
Asteroidea	●	-
Asterias rubens	●	●
Astropecten irregularis	-	●
Ophiuroidea		
Ophiura albida	●	o
Ophiura ophiura	-	●
Echinoidea	-	o
Echinocardium cordatum	●	o
Psammechinus miliaris	-	●
Chordata		
Ascidiacea		
Diplosoma listerianum	●	-
Actinopterygii		
Agonus cataphractus	●	●
Callionymus lyra	-	●
Callionymidae	●	●
Ctenolabrus rupestris	●	-
Eutrigla gurnardus	-	●
Liparis liparis liparis	●	-
Microstomus kitt	●	-
Pleuronectes platessa	-	●
Pomatoschistus spp.	●	●
Solea solea	-	●
Syngnathus acus	-	●
Trachurus trachurus	-	●
Leptocardii		
Branchiostoma lanceolatum	●	o
Ciliophora	●	-
Phoronida	●	o

Table 3. Results from the SIMPER analysis in alphabetic order. Mean densities (ind.m^{-2} ± 95% confidence interval around the mean) or average percentage cover for all taxa contributing >1.5% to the dissimilarity between habitats sand (S), *L. conchilega* beds with densities >1,500 ind.m^{-2} (L) and rocky reefs (R) are given.

Taxa	S	L	R
Campanulariidae	2.5±2.8%	5±0%	1.4±2.6%
Clytia hemisphaerica	0	3.3±3.3%	2.7±3.0%
Conopeum reticulum	0	0	2.3±3.0%
Diplosoma listerianum	0	0	107,000±149,247
Ectopleura larynx	0	3.3±3.3%	1.70%
Electra pilosa	0	1.7±3.3%	22.7±43.9%
Eumida bahusiensis	0	150±167	0
Magelona johnstoni	162±122	265±241	0
Nephtys hombergii	23±31	22±9	0
Obelia bidentata	0	3.3±3.3%	0.9±2.3%
Ophelia borealis	36±63	57±87	0
Ophiura albida	0	238±285	22±39
Owenia fusiformis	0	755±760	0
Scoloplos armiger	232±351	1,298±680	0
Spiophanes bombyx	179±112	2,530±2,649	0
Tellimya ferruginosa	76±51	88±92	0
Tubularia indivisa	0	3.3±3.3%	0
Urothoe poseidonis	380±421	517±108	0
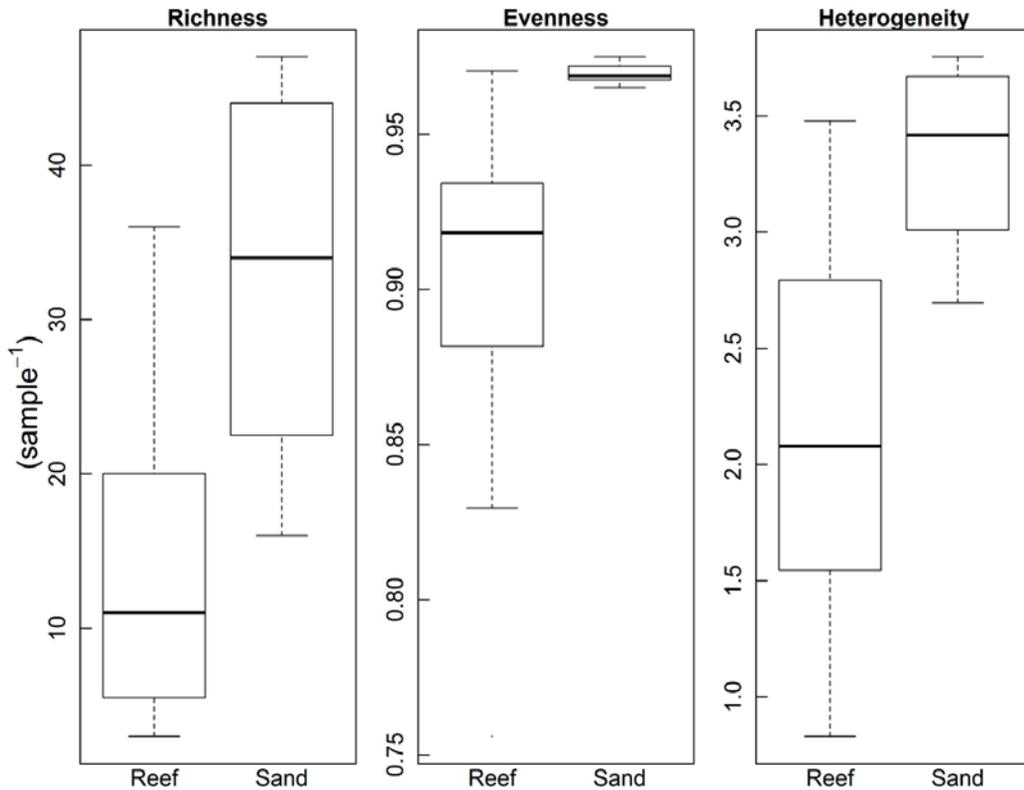
Figure 4. Median species richness, Pielou's evenness and Shannon-Wiener diversity index per sample at two different habitats (Rocky reef; reef; n=11 and sand including *Lanice conchilega* beds with density >1,500 ind.m^{-2}; n=7).
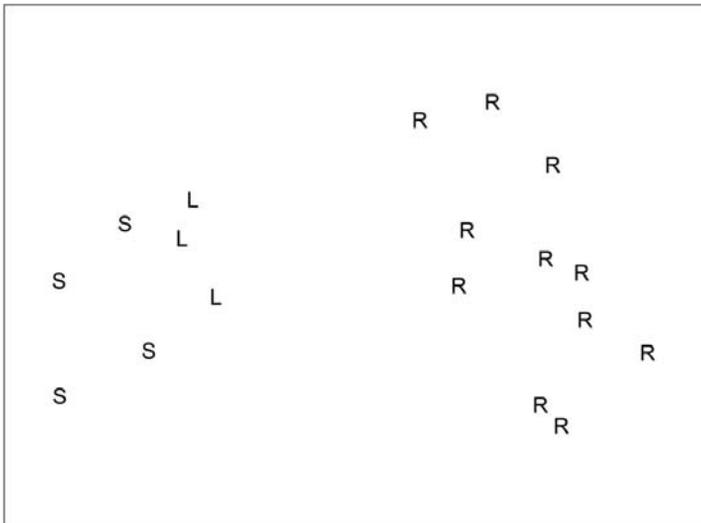
Figure 5. nMDS plot (stress=0.056) on species communities in samples from rocky reefs (R; n=11), sand with *L. conchilega* densities <1,500 ind.m^{-2} (S; n=4) and sand with *L. conchilega* density >1,500 ind.m^{-2} (L; n=3), based on Bray–Curtis similarity measure calculated with categorised data. Only samples from the box corer and airlift sampler were used, the single box corer, gravel sample was removed before analysis.

Discussion

The ground truthing revealed that many locations classified as highly reflective in the side-scan sonar analysis, consisted of dense *L. conchilega* beds. Degraer *et al.* (2008) studied *L. conchilega* formations in Belgian waters and observed that dense *L. conchilega* beds coincided with high reflectivity of side scan sonar imagery. Here, we confirm this finding and advise to use side-scan sonar analysis for future mapping of *L. conchilega* formations. To validate the side scan sonar interpretation, drop-down camera registration seems most cost effective, since *L. conchilega* beds can be very patchy and can cover extensive areas, as was also observed by Degraer *et al.* (2008). Our results are also consistent with a study on reefs built by the annelid *Sabellaria spinulosa* in a wind farm site in the North Sea. Using side scan sonar and ground truthing with photo analysis, Pearce *et al.* (2014) showed areas of high reflectivity contained high densities of *Sabellaria spinulosa*.

From the modelling and SIMPER results it was clear that part of the high overlap between the sand and rocky reef habitat was caused by the prevalence of *L. conchilega* in many of the sand samples. This contrasts with the notion that rocky reefs act as biodiversity hot-spots (Roberts et al. 2002), which implies that rocky reefs should have a higher biodiversity than sand communities. *L. conchilega* is a bioengineer, constructing tubes with coarse grained sand, which increases local biodiversity (Zühlke et al. 1998; Rabaut et al. 2007). However, the nMDS showed no mixing of samples from dense *L. conchilega* beds with the rocky reef samples. We argue that *L. conchilega* creates an intermediate sand-reef system available for species present on sand and rocky reefs. For some species *L. conchilega* may also have a corridor function, connecting rocky reefs for species not able to migrate across large sand patches. This should be addressed in future research as this effect could play an important role for biodiversity conservation. Rabaut *et al.* (2009) even argue that dense *L. conchilega* beds should classify as biogenic reefs for the EU Habitats Directive (European Commission 1992). Our findings confirm the arguments of Rabaut *et al.* (2009), since many species found on the rocky reefs were also present in the dense *L. conchilega* beds. However, these results should be confirmed with studies based on a larger amount of samples from a larger geographical area.

Evaluation of the total taxa found with all methods showed that the rocky reef and sand community were well separated with an overlap in species composition of 32%. Sand and rocky reef each held a similar amount of total taxa, with 63% of the total taxa found in sand and 71% on rocky reefs. Similar results were reported in comparisons between shipwrecks and surrounding sand communities in Belgium where an even lower overlap of 9.6% was found (Zintzen 2007). Overall, local biodiversity was enhanced by the presence of the shipwrecks on a sandy bottom. In the present study, the mixed gravel sample contained the largest number of taxa of all samples taken, supporting the concept that a mosaic of substrates maximises species diversity.

The lower diversity in the rocky reef community (excluding the gravel sample) was caused by the dominance of a small number of species. In samples dominated by the anemone *Metridium senile*, only a small number of other taxa was found. Other authors have also observed the richness around this species to be lower than communities dominated by *L. conchilega* (de Kluijver and Leewis 1994) or *Ectopleura larynx* (Zintzen et al., 2007). Most likely this is caused by the deterring characteristics of anemones (Kaplan 1984), i.e. nematocyst discharging when touched by other species.

The community of the Dutch Borkum Reef Grounds was clearly more diverse than previously reported (Bergman *et al.*, 1991, 1992). Examples of reef species were *Alcyonium digitatum*, *M. senile*, *Diplosoma listerianum* and all gastropods found (Aeolidiidae, *Alvania lactea*, *Crepidula fornicata, Dendronotus frondosus, Onchidoris muricata*). All these taxa were observed only on the rocky reef. *A. digitatum* has been reported previously as present on reefs in the North Sea (de Kluijver 1991; de Kluijver 1993; Kühne and Rachor 1996; Schrieken et al. 2013) and in the English Channel (Sheehan et al. 2013). *M. senile* was also observed on reefs in Denmark (Riis and Dolmer 2003) and in the Southern North Sea (de Kluijver 1991; Kühne and Rachor 1996). The invasive tunicate *D. listerianum* has been reported from wrecks in the North Sea (Leewis et al. 2000; Schrieken et al. 2013) and on a reef in Western Scotland (Hall-Spencer and Moore 2000). Other species typically observed on reefs in the Southern North Sea were *Crepidula fornicata* (Korringa 1952b; Franke and Gutow 2004), *Dendronotus frondosus* (de Kluijver 1991; Kühne and Rachor 1996), *Onchidoris muricata* (Caspers 1950), *Conopeum reticulum* (de Kluijver 1991; Kühne and Rachor 1996), *Electra pilosa* (de Kluijver 1991; Kühne and Rachor 1996; Schrieken et al. 2013), and *Schizomavella linearis* (de Kluijver 1991). *Alcyonidium parasiticum* was reported from reefs in the Northern North Sea as was *Scruparia ambigua* (de Kluijver 1993).

Chaetozone setosa, *Magelona johnstoni* and *Urothoe poseidonis* were present in all sand samples, and *Nephtys hombergii* and *Angulus fabula* were found in five out of seven sand samples. These species have all been previously reported from sandy North Sea bottoms (Veenstra 1965; Creutzberg 1986; Bergman and Hup 1992; Heip and Craeymeersch 1995; Wieking and Kröncke 2001; Reiss et al. 2010) while *C. setosa and A. fabula* have also been observed in reef areas (Kühne and Rachor 1996). Several of the species (e.g. *Spiophanes bombyx, Nephtys hombergii* and *Magelona* sp.) found to be positively associated with dense *L. conchilega* beds in offshore Belgian waters were also abundant in the dense *L. conchilega* beds of the Borkum Reef Grounds (Rabaut et al. 2007).

This study applied a multi-method approach, including visual sampling by scuba divers and drop-down camera photography, to create a thorough overview of the species richness in the area. The combination of visual observations with box corer sampling and airlift sampling led to a taxonomic list that was more comprehensive than could have been attained using

only a selection of these techniques. Each technique detected unique species. If only a box corer had been used for the species inventory, 61% of the current taxa would have been detected, but 79% of the rocky reef taxa would have been missed. Box coring techniques are unsuitable for sampling large rocks, as the rocks either do not fit in the box corer or jam the closing mechanism. The airlift and box corer together, sampled 90% of the taxa found in this study. The addition of diver observations or drop-down camera photo-analysis to this approach added 8 and 4% of the taxa, respectively.

In summary, this study identified species previously unknown to the Dutch Borkum Reef Grounds, showed the uniqueness of three distinct habitats within the Grounds and also demonstrated that a multi-method approach gives a comprehensive overview. For future studies on temperate reefs and their surrounding substrates we recommend that where possible a similar combination of box coring, airlift sampling and diver observations be adopted. Further research should focus on the possible function of *L. conchilega* as an ecosystem engineer creating intermediate sand-reef systems that may serve a corridor function for specific rocky reef species. To map *L. conchilega* beds, we recommend the use of side-scan sonar imaging combined with ground truthing by drop-down camera.

As a final comment, the area of rocky reefs present in the Dutch Borkum Reef Grounds, estimated to be 9.8 km^2, is highly unique and qualifies for protection under the EU Habitats Directive. The biodiversity values of other reefs in the Dutch North Sea (e.g. the Texel Rough) also needs to be investigated.

Acknowledgements

Chapter 3:

MARINE FOULING ASSEMBLAGES ON OFFSHORE GAS PLATFORMS IN THE SOUTHERN NORTH SEA: EFFECTS OF DEPTH AND DISTANCE FROM SHORE ON BIODIVERSITY

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Abstract

Offshore platforms are known to act as artificial reefs, though there is on-going debate on whether this effect is beneficial or harmful for the life in the surrounding marine environment. Knowing what species exist on and around the offshore platforms and what environmental variables influence this species assemblage is crucial for a better understanding of the impact of offshore platforms on marine life. Information on this is limited for offshore platforms in the southern North Sea. This study aims to fill this gap in our knowledge and to determine how the composition and the abundance of species assemblages changes with depth and along a distance-from-shore gradient. The species assemblages on five offshore gas platforms in the southern North Sea have been inventoried using Remotely Operated Vehicles inspection footage. A total of 30 taxa were identified. A Generalised Additive Model of the species richness showed a significant non-linear relation with water depth (p=0.001): from a low richness in shallow waters it increases with depth until 15-20 m, after which richness decreases again. Using PERMANOVA, water depth (p≤0.001), community age (p≤0.001) and the interaction between distance from shore and community age (p≤0.001) showed a significant effect on the species assemblages. Future research should focus on the effect additional environmental variables have on the species assemblages.

Introduction

Offshore constructions have been known to act as artificial reefs (Forteath et al. 1982; Van Buuren 1984; Lewbel et al. 1987; Ponti 2002; Whomersley and Picken 2003; Yan et al. 2006; Guerin 2009). Foundations of wind farms (Lindeboom et al. 2011; Lindeboom et al. 2015; De Mesel et al. 2015), oil and gas production platforms (Roberts 2002) and other energy structures (Langhamer et al. 2009) add hard substrata to the marine environment, supporting a great diversity of marine life by offering habitat for algae (Butler and Connolly 1999; Whomersley and Picken 2003; Bouma and Lengkeek 2013), fish (Caselle et al. 2002; Jørgensen et al. 2002; Wilhelmsson et al. 2006; Langhamer O, Wilhelmsson D 2009) and invertebrates (Butler and Connolly 1996; Whomersley and Picken 2003; Page et al. 2006; Langhamer O, Wilhelmsson D 2009; Bergmark and Jørgensen 2014). There is on-going debate on whether this effect is beneficial for the life in the surrounding marine environment, and whether or not oil and gas platform foundations should be removed after decommissioning or left in place as artificial reefs, also known as 'Rigs-to-Reefs' (Picken and McIntyre 1989; Picken et al. 2000; Macreadie et al. 2011; Jørgensen 2012a). Knowledge of the effects of these artificial reefs on marine life is of significant importance for understanding the effect of the presence of several thousand offshore energy structures such as wind turbines (Lindeboom et al. 2011; EWEA 2015a) and their subsequent removal at the end of production life (Fowler et al. 2014; Smyth et al. 2015).

Fouling assemblages on offshore platforms have been inventoried in different areas such as the Beibu Gulf in China (Yan et al. 2006), the Gulf of Mexico (Lewbel et al. 1987; Sammarco et al. 2004; Sammarco et al. 2014), off the Californian coast (Page et al. 1999; Caselle et al. 2002; Helvey 2002; Bram et al. 2005; Goddard and Love 2010; Claisse et al. 2014), the southern Arabian Gulf (Stachowitsch et al. 2002), the Mediterranean (Ponti 2002) and the Celtic Sea (Southgate and Myers 1985). Research has also been conducted on marine fouling on offshore platforms in several areas of the northern and central North Sea (Forteath et al. 1982; Roberts 2002; Whomersley and Picken 2003; Gass and Roberts 2006). Previous research on the effects of offshore oil and gas structures in the North Sea focussed on marine mammals (Russell et al. 2014), fish (Mathers et al. 1992; Cripps and Aabel 2002; Soldal et al. 2002; Jørgensen et al. 2002; Lokkeborg et al. 2002), algae (Hardy 1981; Moss et al. 1981), corals (Roberts 2002; Gass and Roberts 2006; Bergmark and Jørgensen 2014) and invertebrate assemblages (Hardy 1981; Forteath et al. 1982; Olsgard and Gray 1995; Cripps and Aabel 2002; Whomersley and Picken 2003; Guerin 2009).

Several abiotic factors have been proposed to explain the species composition of the marine fouling on offshore platforms, such as water temperature (Lewbel et al. 1987), platform age (Sammarco et al. 2004), depth (George and Thomas 1979; Lewbel et al. 1987; Whomersley and Picken 2003; Yan et al. 2006) and distance from shore (Yan and Yan 2003). The effect of depth on marine fouling has been reported from wind farms (Kerckhof et al. 2010; Lindeboom

et al. 2011; Krone et al. 2013b) and offshore platforms (Van Buuren 1984) in the southern North Sea. However, all the available data on invertebrate assemblages on installations in temperate waters were generated in the northern North Sea or from near shore installations that were constructed <10 years before investigation. The southern North Sea has a strong near- to offshore gradient in environmental variables, such as food availability (Jickells 1998), which is absent in the northern parts. Furthermore, large differences in water depth, temperature and salinity exist between the northern and southern parts of the North Sea (Otto et al. 1990). Whomersley (2010; (Whomersley and Picken 2003)) showed that even after 11 years, fouling assemblages still changed in composition. With offshore platforms in the southern North Sea now reaching ages of >40 years, an opportunity presents itself to compare installations of old and young ages and at different locations with different environmental circumstances. This will give insight in the long term effects of proposed developments, such as the short term installation of thousands of offshore wind turbines (EWEA 2015a) and is much needed information to aid in evaluating the impact of future Rigs-to-Reefs programmes: to reef or not to reef (Macreadie et al. 2012; Jørgensen 2012b; Martin 2012; Fowler et al. 2014; Fowler et al. 2015a)?

This study aims to determine how the composition of species assemblages (including epifauna, fish and mobile benthic fauna on and in the visible vicinity of the installation) changes with depth and along a distance-from-shore gradient. The species assemblage on five offshore gas platforms in the southern North Sea was inventoried using inspection footage from Remotely Operated Video robots (ROVs).

Material and methods

Study sites
We selected five offshore gas platforms (coded P1 to P5) in the southern North Sea along a gradient of increasing distance from shore, with P1 situated 48 km offshore, and P5 at 177 km offshore (Table 1; Figure 1). The platforms are situated in water depths between 27 and 43 meters, surface water temperatures varying between 4 and 18°C throughout the year (Rijkswaterstaat 2014), on a seafloor composed primarily of sand. The year of structure installation of the structures varies between 1972 and 2009. They are all operated by ENGIE E&P Nederland B.V. and have a steel jacket foundation constructed of 4 to 10 legs with cathodic protection by anodes. Each leg within a jacket provides between ~500 and ~800 m^2 of surface area available for marine growth, depending on water depth.

Table 1. Platform locations, distance from shore, maximum observed depths on the footage, year of installation of the platform, community age (years since last cleaning activities) at the time the video was recorded, for depths < 10 m >.

Platform name (codes)	Coordinates (WGS84)	Distance from shore (km)	Maximum depth of video footage (m)	Year of installation	Community age > 10 m (years)	Community age <10 m (years)
L10-AD (P1)	53°24'12''N, 04°12'03''E	48	27.4	1972	39	5
K9ab-B (P2)	53°33'04''N, 03°46'47''E	80	34.5	1999	12	7
K2b-A (P3)	53°56'55''N, 03°39'44''E	114	42.8	2005	7	7
E17a-A (P4)	54°05'53''N, 03°21'36''E	140	42.5	2009	3	3
D15-A (P5)	54°19'29''N, 02°56'05''E	177	40.0	1999	13	13
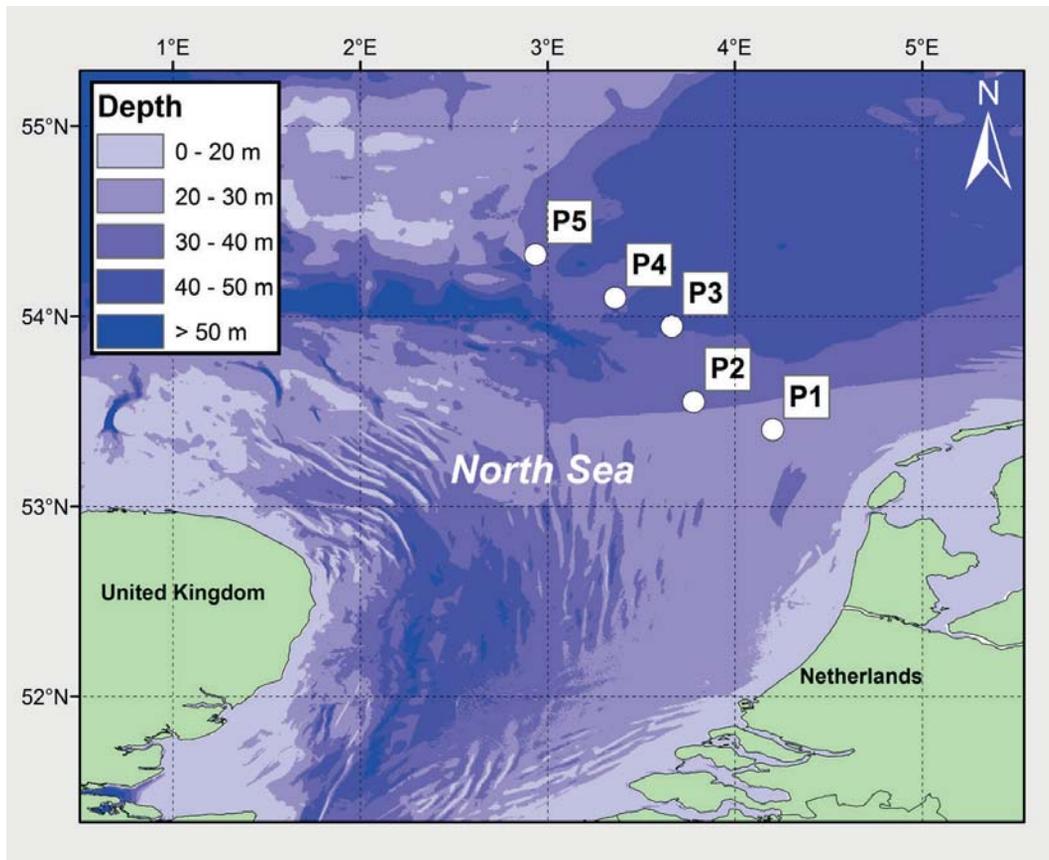
Figure 1. Locations of investigated platforms. Studied locations of five gas platforms in the southern North Sea (○) with bathymetry.

Sampling and analysis

Offshore operators regularly perform technical underwater inspections of the structures, resulting in a large amount of digital video footage, made available to us for analysis. The footage provided consisted of close visual inspection (CVI), showing details of specific parts, e.g. conductors or caissons, and general visual inspection (GVI), giving a complete and systematic overview of each leg of the underwater structure from two approximately perpendicular angles.

For an overview of all species present at each platform, both CVI and GVI footage was used. For the systematic analysis of distance from shore and depth effects, only GVI footage was used. Footage was viewed using VLC mediaplayer version 2.0.5 (VideoLAN 2013). To train the observer, all GVI footage was viewed and scored twice during the systematic analysis of species abundance, retaining only data from the second viewing for statistical analysis. Species abundance was estimated from footage while playing, as video stills were often blurry given the low video resolution and fast movements of the camera.

To create replicate samples from identical depths within each platform, all legs from every platform were divided into 5 m depth bands, resulting in a total of 215 unique platform*leg*depth combinations (henceforth named samples). The precise amount of inspected surface area was unknown and we estimate that the area viewed per sample was between 8 and 10 m², assuming that ~50% of the leg was showing in the video and that all legs were the same diameter. Depth and time of recording were visible on the video. To correct for tidal differences, depths were converted to Amsterdam Ordnance Datum using data from Waterbase (Rijkswaterstaat 2014).

Due to the different growth forms, high densities of organisms and limited video resolution, it was not always possible to count individuals for every taxon. Therefore species abundance per sample was assigned a value using an adapted version of the Braun-Blanquet scale, following Leewis *et al* (2000 (Leewis et al. 2000); Table 2) and Coolen *et al* (2015 (Coolen et al. 2015a)). This 1 – 9 scale allows for a quantitative registration of colonial species and individuals while scaling down bias caused by counting problems from a combination of high densities of individuals with blurry video images. All observed organisms were identified at the lowest taxonomic rank possible. The World Register of Marine Species (WoRMS Editorial Board 2015) was used as standard for taxonomical nomenclature.

Table 2. Different classes with corresponding analysis value

Class	Analysis value
1 individual	1
2 – 5 individuals	2
6 – 50 individuals	3
>50 individuals, <5% cover	4
5 – 15% cover	5
16 – 25% cover	6
26 – 50% cover	7
51 – 75% cover	8
76 – 100% cover	9

Given the low image resolution, blurry video caused by fast camera movement and movement of the organisms, smaller specimens are likely to remain undetected in a sample. Therefore the probability to observe an individual was quantified by defining a detectability score for each taxon. Taxa were scored based on their mobility (1: very mobile, e.g. benthopelagic fish – 5: sessile, e.g. anemones) and individual adult or colonial size (1: small, 3-5 cm – 5: large, >30 cm). By multiplying these scores, taxa were separated in groups of low detectability (scoring 1 - 6) and high detectability (scoring 7 - 25). The assumption was made that taxa with high detectability were truly absent when not observed. In high quality footage the size of the smallest organisms or colonies registered was approximately 3 cm, whereas in low quality footage this was approximately 5 cm.

Data on explanatory variables were collected on year of installation of each platform, age of the community at each depth band (the structures are cleaned between 0 and approximately 10 m depth on a regular basis depending on hard marine growth presence), available video length, quality of the footage and distance from the nearest coast. The quality of the footage was scored in consultation between two authors, on a scale of 1 (low quality) – 10 (excellent quality).

Datasets created in this study are publicly accessible through Dryad (van der Stap et al. 2015). For the statistical analyses, R: A language and Environment for Statistical Computing, version 3.0.2. (R Core Team 2014b) and RStudio version 0.98.994 (RStudio 2014a) were used. Taxa with low detectability or with a single observation were removed from the dataset. Species richness (number of species; S) per sample was used to construct a univariate model explaining its relation with the explanatory variables. The collected data were explored following the protocol described by Zuur *et al.* (2010; (Zuur et al. 2010)). To identify outliers, collinearity, relationships and interactions, species richness and all independent variables were plotted using Cleveland dotplots (Cleveland 1985), boxplots, pairplots and multi-panel scatterplots (xyplot function in lattice package; (Sarkar 2008)). Since non-linear patterns in

the relation between species richness and depth were observed, a Generalised Additive Model (GAM; gam function in the mgcv package; (Wood 2011)) was constructed. Backward selection using Akaike Information Criteria (Akaike 1973) was performed to exclude variables and optimise the GAM. This optimised model was validated by plotting residuals versus fitted values and versus all variables included and excluded during the model selection. PERMANOVA (adonis function in package vegan; (Oksanen et al. 2008)) was used to test the significance of the effect depth, community age and distance from shore had on the species assemblages. Also, PERMANOVA was used to examine whether quality of the videos, video length, platform age and community age had a significant effect on the observed species assembly.

Results

Species inventory
Approximately 550 minutes of footage were analysed for the five platforms and a total of 30 taxa were identified (Table 3). Nine taxa were observed on all platforms, while four taxa were found on one platform only. After removal of taxa with a single observation or low detectability, 11 out of 30 taxa remained for the statistical analysis.

Species abundance estimation
Platforms P3, P4 and P5 were fully covered with marine fouling at all depths, but the composition and abundance of the marine fouling varied over depth and along the distance-from-shore gradient. Several legs on P1 and P2 up to a depth of 10 m were not fully covered. Tables 4-8 show averaged abundance estimations for the 11 high detectable species in each depth band on platforms P1 – P5, based on the Braun-Blanquet values. *Metridium senile* was the dominant species in depth range 25 – 45 m on all platforms, except on P4. In the depth range 0 – 20 m, *Mytilus edulis* was often present, especially on P1 and P2. However, it was almost completely absent from P3 and P5, platforms located further offshore. On P4 *M. edulis* was present up to a depth of 15 m. Rhodophyta were found on all platforms, between 0 – 5 m, while on P5 they were present up to 10 m. *Alcyonium digitatum* was not observed on P1 and P2, but increased along the distance-from-shore gradient at P3, P4 and P5. Porifera species were not observed on P1 and P2, but were observed on the other platforms, although in low abundance. Abundance of *Cancer pagurus*, *Asterias rubens*, *Ophiothrix fragilis*, *Necora puber* and *Psammechinus miliaris* decreased along the distance-from-shore gradient, and these species were very rare on P5.

Table 3. All observed taxa (●) per platform with detectability scores (≤6 = low, >6 high).

Taxon	Platform					
	P1	P2	P3	P4	P5	Score
Rhodophyta*	●	●	●	●	●	20
Porifera	●	●	●	●	●	15
Hydrozoa**			●	●	●	5
Ectopleura larynx	●	●	●	●	●	5
Tubularia indivisa*	●	●	●		●	-
Anthozoa						
Diadumene cincta*	●				●	-
Metridium senile	●	●	●	●	●	20
Sagartia elegans*			●			-
Hexacorallia	●	●	●	●	●	15
Alcyonium digitatum			●	●	●	20
Annelida						
Serpulidae	●	●	●	●	●	5
Arthropoda						
Cancer pagurus	●	●	●	●	●	20
Necora puber	●	●	●	●	●	12
Paguridae*			●	●	●	-
Amphipoda	●	●	●	●	●	5
Mollusca						
Mytilus edulis	●	●	●	●	●	15
Echinodermata						
Asterias rubens	●	●	●	●	●	12
Ophiothrix fragilis	●	●		●	●	8
Psammechinus miliaris	●	●	●	●	●	8
Pisces**		●	●	●	●	3
Agonus cataphractus*	●			●		-
Ctenolabrus rupestris*	●			●	●	-
Cottidae	●					-
Gadidae		●	●	●	●	4
Gadus morhua				●		5
Labridae	●					4
Mugilidae*	●				●	-
Trisopterus luscus	●	●		●		4
Perciformes*	●			●	●	-
Pleuronectidae*			●	●	●	-

* Taxa not observed on GVI footage.
** Observation of unidentified Hydrozoa and Pisces.

The observed species richness (S) categorised in depth bands and platforms is shown in Figure 2. Model selection for the GAM resulted in the inclusion of depth, the interaction between distance from shore and the community age and video length as explanatory variables, which explained 42% of the deviance. S increased significantly with increasing community age and video length (p<0.001). With increasing distance from shore, S decreased significantly although this effect interacted with the age of the community (p<0.001). Depth showed a non-linear significant relationship with species richness (p=0.001). Species richness initially increased with depth, but then decreased again after 15-20 m (Figure 3).

PERMANOVA showed that depth, community age, quality of the footage and the interaction effect between distance and community age have a significant effect (p≤0.001) on the species assemblages found on offshore platforms (Table 9). Total video length varied per platform, but PERMANOVA showed this had no significant effect.
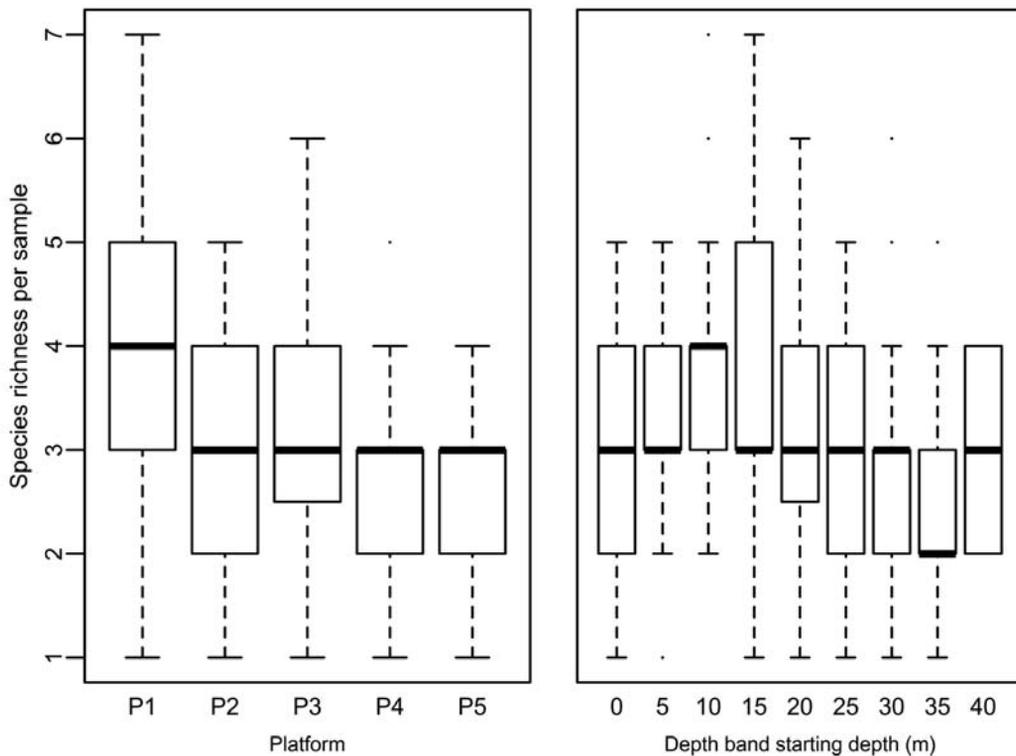
Figure 2. Species richness per platform and depth band. Boxplot showing the number of species per sample (n=215), per platform with all depths combined (left image) and per depth-band with all platforms (n=5) combined.
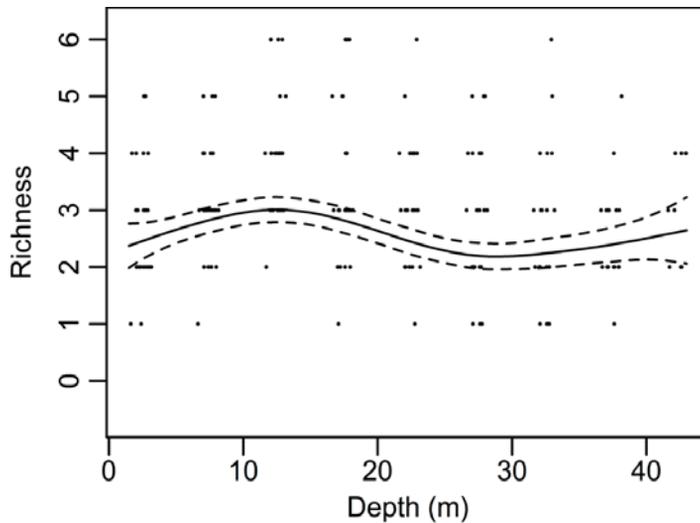
Figure 3. Modelled relation of species richness (S) with depth. Plot of the fitted Generalised Additive Model smoother showing the significant non-linear effect of depth (p=0.001) on the species richness (S) on all platforms.

Discussion

The present study provides insight in the composition of the species assemblages found on five offshore gas platforms in the southern North Sea. Our results show that a variety of marine species are found on these offshore gas platforms. Many thousands of artificial reef structures are present in the North Sea in the form of shipwrecks, wind farms and oil and gas platforms (OSPAR Commission 2013; Coolen et al. 2015b). Furthermore, thousands of wind turbine foundations will be installed in the North Sea in the near future (EWEA 2015a). Before the onset of industrial fisheries, large areas of the southern North Sea bottom were covered with natural reefs, many of which are now lost (Olsen 1883; Coolen et al. 2015a). Artificial reefs in this area might compensate for the loss of this habitat. In previous studies, 90% of the species present on artificial hard substrata in the southern North Sea were shown to be absent in the soft bottomed surroundings (Zintzen 2007). The presence of an artificial object will therefore have a very strong effect on the local biodiversity, almost doubling it (Lengkeek et al. 2013). However, it remains unclear to what extent the species assemblages present on artificial reefs resemble those of natural reefs. Published species observations as presented in the current study and many others, are needed to evaluate the ecological value of current and future artificial reefs.

Trends in community composition

Although similarities in species assemblages are found between offshore platforms, there are also striking differences. Especially the abundance of *M. edulis* on P4 differs from the other far offshore platforms, P3 and P5. With 3 years community age at all depths, P4 is young compared to the minimum age of 7 and 13 years for P3 and P5, respectively. This suggests

that *M. edulis* is an early colonizer of offshore platforms, which is confirmed by wind farm colonisation studies in the southern North Sea where *M. edulis* was dominant in the first years after construction (Lindeboom et al. 2011; Krone et al. 2013b). *M. edulis* growth rate is dependent on food availability (Page and Hubbard 1987), explaining the high abundance in the depth range 0 – 20 m on platforms closer to shore, where food concentrations are higher (Tett et al. 1993).

Alcyonium digitatum was not observed on P1 and P2, but was observed on the other platforms. Abundance of *A. digitatum* correlated positively with distance from shore, in line with the pattern found on shipwrecks in the Belgian part of the North Sea, where *A. digitatum* was only found on wrecks far offshore and never close to shore (Zintzen and Massin 2010). This may be explained by a water temperature or food availability gradient, since both decrease with distance from shore in the southern North Sea (Tett et al. 1993).

Species with a low detectability score were omitted from the data. Using these adjusted data in the GAM, the species richness was highest on P1 and decreased with increasing distance from shore. However, this effect interacted with the community ages, which varied between 3 and 39 years. A similar significant interaction was found in the PERMANOVA results. Platforms are cleaned regularly between the water surface and approximately 10 m water depth, depending on the abundance of hard marine growth (e.g. mussels and barnacles). Platforms closer to shore were cleaned more recently than those further offshore. Cleaning effectively resets community succession every few years, keeping it in a continuous young stage, affecting the species composition. This may explain the significant effect of the age:distance interaction, obscuring the distance from shore effect communicated by other authors (Lewbel et al. 1987; Yan et al. 2006). The operator of the platforms informed us that platforms close to shore indeed are cleaned more often than locations far offshore.

The lower richness in shallow parts, as shown by the GAM, can also be explained by the impact of higher wave action near the surface, which is known to decrease species richness (England et al. 2008). In the deeper parts of the platform the richness was also significantly lower than at intermediate depths, caused by the dominance of a limited amount of taxa. Anemones such as *M. senile* are known to deter other organisms (Kaplan 1984), explaining the lower species richness around these species. A similar effect was observed on rocky reefs in the Netherlands (Coolen et al. 2015a). Both the wave disturbance and deterring effect of *M. senile* are in line with the intermediate disturbance hypothesis, which states that biodiversity is highest at intermediate disturbance rates and smaller at high and low rates (Svensson et al. 2007). It is suggested that at low rates of disturbance, strong competitors exclude competitively inferior species, whereas at high rates of disturbance, recruitment cannot balance the high rates of mortality, and slow recruiting species disappear from the

community. This effect was most prominent on P5, where the deeper parts of the platform were dominated by *M. senile* and *Alcyonium digitatum*.

Our results are in line with research on offshore platforms in other waters, where depth was also found to have a significant influence on species composition of the marine fouling (Lewbel et al. 1987; Yan et al. 2006). However, depth, community age, quality of the footage and the age:distance from shore interaction only explained 47% of the variance in the PERMANOVA and 42% of the deviance in the GAM. The amount of unexplained variation indicates that other environmental variables, such as salinity, water temperature, water currents, food supply, light penetration, silt content and the position on the leg (interior/ exterior) in relation to the direction of the current may also play a role (Judge and Craig 1997; Guerin 2009).

Evaluation of ROV footage used for species identification
Data used in this study were collected from images collected for technical inspection. The use of such images is a time and cost effective method to gain insight in the organisms present on offshore platforms. It allows for the inventory of large species present on vast amounts of surface area, in all depths, which can be challenging using other methods such as diving surveys (Coolen et al. 2015a). Many locations can be investigated and if needed, several years are available for time series analysis (Whomersley and Picken 2003). Furthermore, identifications are easily confirmed by peers, increasing the quality of the data.

However, ROV inspection footage is created to obtain an overview of the technical integrity of the installation, not for biological study. As such, the quality was often insufficient to identify taxa to species level. Furthermore, video footage will only show the organisms on top of the fouling layer, missing species in the deeper layers. Therefore, the number of taxa identified in this study is an underestimation of the true number of species present. For a thorough overview of the species present, a combination of methods such as destructive sampling for small organisms and *in situ* observations for rare, fast moving or inconspicuous organisms should be applied, as shown on rocky reefs in the southern North Sea (Coolen et al. 2015a).

Conclusion
Using ROV footage, a total of 30 taxa were identified in the species assemblages on five offshore gas platforms in the southern North Sea. Species richness initially increased with depth, but decreased after 15-20 m. Species richness decreased significantly with increasing distance from shore; although, this effect may be obscured by the younger community age in <10 m depths on platforms closer to shore resulting from the regular cleaning of these platforms. Not all variability was explained by depth and the distance from shore effect, indicating that other environmental variables also play a role. Further research with

higher quality images, *in situ* observations and sampling of the marine fouling is needed to understand what other environmental variables influence the species assemblages on offshore platforms.

Acknowledgements

Table 4. Averaged categorised abundance of the 11 high detectable taxa, with 95% confidence interval around the mean, per depth band on platform P1.

Platform P1						
Taxa	**Depth band**					
	0 – 5	**5 – 10**	**10 – 15**	**15 – 20**	**20 – 25**	**25 – 30**
Rhodophyta	3 ± 1.70	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
Porifera	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
Metridium senile	0 ± 0.00	0 ± 0.00	6 ± 0.56	8 ± 0.40	9 ± 0.28	8 ± 0.58
Hexacorallia	4 ± 2.29	6 ± 1.11	6 ± 0.82	3 ± 1.75	2 ± 1.73	3 ± 1.81
Alcyonium digitatum	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
Cancer pagurus	0 ± 0.00	0 ± 0.58	1 ± 0.96	2 ± 0.51	2 ± 0.79	2 ± 0.79
Necora puber	0 ± 0.00	0 ± 0.00	0 ± 0.00	1 ± 0.84	1 ± 0.90	2 ± 0.51
Mytilus edulis	4 ± 2.91	6 ± 1.95	8 ± 0.51	7 ± 0.40	4 ± 1.98	0 ± 0.00
Asterias rubens	1 ± 1.08	3 ± 1.20	3 ± 1.43	2 ± 0.94	0 ± 0.49	1 ± 0.96
Ophiothrix fragilis	0 ± 0.00	2 ± 1.73	3 ± 1.54	4 ± 1.26	1 ± 1.64	0 ± 0.00
Psammechinus miliaris	0 ± 0.84	0 ± 0.00	1 ± 1.28	0 ± 0.00	0 ± 0.00	0 ± 0.00

The rounded abundance values are based on Table 2.

3

Table 5. Averaged categorised abundance of the 11 high detectable taxa, with 95% confidence interval around the mean, per depth band on platform P2.

Platform P2							
Taxa	**Depth band**						
	0 – 5	**5 – 10**	**10 – 15**	**15 – 20**	**20 – 25**	**25 – 30**	**30 - 35**
Rhodophyta	3 ± 3.14	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
Porifera	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
Metridium senile	2 ± 1.96	3 ± 2.17	5 ± 0.49	7 ± 0.94	8 ± 0.57	9 ± 0.00	9 ± 0.00
Hexacorallia	5 ± 0.00	7 ± 0.49	7 ± 0.49	6 ± 4.00	6 ± 1.13	4 ± 2.65	0 ± 0.00
Alcyonium digitatum	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
Cancer pagurus	0 ± 0.00	0 ± 0.49	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.49	0 ± 0.00
Necora puber	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.49	0 ± 0.49	0 ± 0.57
Mytilus edulis	7 ± 0.49	7 ± 1.27	7 ± 0.98	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
Asterias rubens	5 ± 0.00	5 ± 0.49	4 ± 0.80	3 ± 0.94	2 ± 1.79	0 ± 0.49	0 ± 0.00
Ophiothrix fragilis	0 ± 0.00	0 ± 0.00	0 ± 0.00	1 ± 2.45	0 ± 0.00	0 ± 0.00	0 ± 0.00
Psammechinus miliaris	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00

The rounded abundance values are based on Table 2.

Table 6. Averaged categorised abundance of the 11 high detectable taxa, with 95% confidence interval around the mean, per depth band on platform P3.

Taxa	Platform P3								
	Depth band								
	0 – 5	5 – 10	10 – 15	15 – 20	20 – 25	25 – 30	30 – 35	35 – 40	40 - 45
Rhodophyta	2 ± 2.32	5 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 3.54
Porifera	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 1.39	0 ± 0.00	0 ± 0.00	0 ± 0.00
Metridium senile	7 ± 2.02	2 ± 0.94	4 ± 1.23	5 ± 0.49	5 ± 0.57	6 ± 0.00	6 ± 0.00	6 ± 0.00	6 ± 0.00
Hexacorallia	0 ± 0.49	3 ± 1.23	4 ± 0.94	6 ± 2.93	5 ± 0.57	6 ± 1.96	3 ± 1.47	3 ± 1.96	2 ± 0.00
Alcyonium digitatum	4 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 2.02	1 ± 2.26	1 ± 1.86	1 ± 0.00	2 ± 2.17
Cancer pagurus	2 ± 0.00	0 ± 0.00	0 ± 0.49	0 ± 0.49	0 ± 0.57	0 ± 0.49	0 ± 0.57	1 ± 0.49	0 ± 0.49
Necora puber	1 ± 0.49	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.49	0 ± 0.00	0 ± 0.94	1 ± 0.49	0 ± 1.39
Mytilus edulis	0 ± 0.00	4 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
Asterias rubens	0 ± 1.39	1 ± 0.00	3 ± 0.00	3 ± 0.94	2 ± 0.94	2 ± 0.00	1 ± 0.49	1 ± 0.00	0 ± 0.00
Ophiothrix fragilis	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
Psammechinus miliaris	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00

The rounded abundance values are based on Table 2.

Table 7. Averaged categorised abundance of the 11 high detectable taxa, with 95% confidence interval around the mean, per depth band on platform P4.

Taxa	Platform P4								
	Depth band								
	0 – 5	5 – 10	10 – 15	15 – 20	20 – 25	25 – 30	30 – 35	35 – 40	40 - 45
Rhodophyta	0 ± 1.42	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	1 ± 0.00	2 ± 0.00	1 ± 0.00	0 ± 0.00
Porifera	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	1 ± 0.00	1 ± 0.00	1 ± 0.98	1 ± 3.15	0 ± 0.00
Metridium senile	5 ± 1.11	5 ± 0.94	6 ± 1.21	7 ± 1.04	6 ± 0.82	4 ± 1.34	2 ± 1.28	5 ± 0.63	5 ± 1.44
Hexacorallia	5 ± 0.00	5 ± 1.55	2 ± 1.04	1 ± 1.89	1 ± 2.10	3 ± 2.03	5 ± 1.83	6 ± 0.98	4 ± 0.00
Alcyonium digitatum	1 ± 0.00	1 ± 0.00	1 ± 0.00	3 ± 0.00	5 ± 0.00	4 ± 1.55	2 ± 1.96	3 ± 1.65	2 ± 0.48
Cancer pagurus	0 ± 0.00	0 ± 0.00	0 ± 0.47	0 ± 0.22	1 ± 0.58	0 ± 0.48	0 ± 0.66	0 ± 0.67	0 ± 0.39
Necora puber	0 ± 0.24	0 ± 0.24	0 ± 0.24	0 ± 0.24	0 ± 0.00	0 ± 0.24	0 ± 0.32	0 ± 0.00	0 ± 0.00
Mytilus edulis	0 ± 0.36	0 ± 2.16	0 ± 1.71	0 ± 0.00	0 ± 0.00	2 ± 0.00	2 ± 0.00	2 ± 0.00	1 ± 0.00
Asterias rubens	2 ± 0.52	2 ± 0.86	2 ± 0.24	1 ± 0.73	1 ± 0.36	1 ± 0.90	0 ± 0.91	1 ± 0.73	1 ± 0.39
Ophiothrix fragilis	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
Psammechinus miliaris	0 ± 0.84	0 ± 0.00	0 ± 1.28	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00

The rounded abundance values are based on Table 2.

Table 8. Averaged categorised abundance of the 11 high detectable taxa, with 95% confidence interval around the mean, per depth band on platform P5.

	Platform P5							
Taxa	**Depth band**							
	0 – 5	**5 – 10**	**10 – 15**	**15 – 20**	**20 – 25**	**25 – 30**	**30 – 35**	**35 – 40**
Rhodophyta	4 ± 1.90	1 ± 1.44	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
Porifera	0 ± 0.00	1 ± 1.57	2 ± 1.92	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
Metridium senile	0 ± 0.78	5 ± 0.78	6 ± 0.78	8 ± 0.48	9 ± 0.00	9 ± 0.00	9 ± 0.00	9 ± 0.39
Hexacorallia	9 ± 0.00	8 ± 0.48	8 ± 0.39	7 ± 0.62	4 ± 1.96	2 ± 1.92	0 ± 0.00	4 ± 1.82
Alcyonium digitatum	0 ± 0.00	5 ± 0.00	5 ± 0.39	3 ± 2.74	3 ± 2.40	1 ± 1.57	3 ± 2.11	5 ± 0.00
Cancer pagurus	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.39	0 ± 0.00
Necora puber	0 ±±0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
Mytilus edulis	0 ± 0.78	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
Asterias rubens	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
Ophiothrix fragilis	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
Psammechinus miliaris	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00

The rounded abundance values are based on Table 2.

Table 9. PERMANOVA on variables that influence species assemblages significantly.

Source	d.f.	SS	MS	F	R^2	P
Depth	1	11.031	11.0311	113.085	0.28374	0.001
Age	1	2.518	2.5179	25.812	0.06477	0.001
Quality	1	1.080	1.0801	11.073	0.02778	0.001
Age:Distance	1	3.763	3.7629	38.576	0.09679	0.001
Residuals	210	20.485	0.0975		0.52692	
Total	214	38.877			1.00000	

D.f. = degrees of freedom. SS = sum of squares. MS = mean of squares. Age:Distance tests the effect of the interaction between these two variables. Age refers to the age of the community.

Chapter 4:

ARTIFICIAL REEFS INFLUENCE SPECIES DISTRIBUTION

4.1: FIRST RECORD OF *CARYOPHYLLIA SMITHII* IN THE CENTRAL SOUTHERN NORTH SEA:
ARTIFICIAL REEFS AFFECT RANGE EXTENSIONS OF SESSILE BENTHIC SPECIES

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Abstract

In the North Sea, observations of the solitary coral *Caryophyllia smithii* are mostly limited to the north east coast of the United Kingdom including the Shetland and Orkney islands. To date, *C. smithii* has not been reported from far offshore locations in the North Sea south of 57.7°N. Distribution of this species appears limited by the restricted availability of natural hard substrates in the central southern North Sea. There are, however, many artificial hard substrates in this area in the form of shipwrecks and offshore oil and gas installations. These may provide stepping stones for *C. smithii* to expand its distribution. Here we report the first sighting of *C. smithii* in the central southern North Sea on an unidentified wreck on the Dogger Bank. This is the first offshore observation of any hard coral in the central southern North Sea.

Introduction

In the North Sea, observations of *Caryophyllia smithii* (Stokes & Broderip, 1828) are mostly limited to the north east coast of the United Kingdom including the Shetland and Orkney islands (Wilson 1975; de Kluijver 1993; UK National Biodiversity Network: Joint Nature Conservation Committee 2010a; UK National Biodiversity Network: Marine Biological Association 2010a; UK National Biodiversity Network: Marine Conservation Society 2010; UK National Biodiversity Network: Porcupine Marine Natural History Society 2010; UK National Biodiversity Network: Scottish Natural Heritage 2010; VLIZ Belgian Marine Species Consortium 2010; UK National Biodiversity Network: Joint Nature Conservation Committee 2010b; UK National Biodiversity Network: Marine Biological Association 2010b). *C. smithii* has also been reported from offshore installations in the northern North Sea (Guerin 2009). Further to the east it occurs on the south western coast of Norway (UK National Biodiversity Network: Joint Nature Conservation Committee 2010b; Natural History Museum University of Oslo: Norwegian Biodiversity Information Centre 2015). South of the North Sea it has been reported at coastal locations with the most eastern observations on wrecks in the English Channel, near Eastbourne (UK National Biodiversity Network: Marine Conservation Society 2010).

The life cycle of *C. smithii* includes a larval planktotrophic stage with a duration of 8 to 10 weeks (Tranter et al. 1982). During this time, the released larvae float freely in the water column and are transported in the direction of net water movement, which is driven by tidal currents and wind (Holmedal and Wang 2015). These residual currents in the North Sea range between 0.02 m.s^{-1} and 0.08 m.s^{-1} (Prandle 1984; Thorpe 2012). The potential for distribution is high for species with a long planktonic larval stage (Kinlan et al. 2005), since the dispersal distance is correlated to the duration of the pelagic stage (Shanks 2009). However, *C. smithii* needs hard substrata to attach to at the end of its planktonic stage (Best 1968; Wilson 1976), for example in the form of exposed rocks (Hiscock and Howlett 1976). The bottom of the central southern North Sea is mostly composed of muddy and fine sand (Duineveld et al. 1991) which is unsuitable for attachment by *C. smithi* larvae. To distribute itself to central southern North Sea locations, *C. smithii* would need locations with alternative hard substrates present on the sand bottom. These are provided by artificial structures, as shown by Guerin (2009) who observed *C. smithii* at several offshore oil production platforms in the northern North Sea. Species with planktonic stages like *C. smithii* may use these hard substrata as stepping stones, whereby the next generation continues the expansion, depending on current direction, mixing of the water column and larval behaviour.

To date, the coral has not been reported from far offshore locations in the North Sea south of 57.7°N. Here we report the first sighting of *C. smithii* on a shipwreck on the Dogger Bank, central southern North Sea.

Materials and methods

From 4 to 13 September 2014 a group of scuba divers visited 14 wrecks in the Dutch and British part of the southern North Sea (Table 1). During this expedition a wreck of an unknown steam ship on the Dogger Bank was visited. The wreck was found on side scan sonar images during an archaeological assessment of geophysical data acquired for the Environmental Impact Assessment of the Forewind Creyke Beck wind farm development (Wessex Archaeology 2013) with number WA70500. The wreck is located at 55.037°N 1.703°E, approximately 200 km east from Newcastle upon Tyne, on a sandy bottom in a maximum water depth of 33 meters.

The scuba divers performed a visual biodiversity inventory for a wide range of species and collected specimens for later identification, as described in Schrieken *et al.* (2013). A total of 2 dives were carried out on wreck WA70500 on the 10th of September 2014: one during daylight and one at night, to a maximum depth of 32 meters for 50 and 41 minutes, respectively.

Table 1. Names, numbers or descriptions and positions (WGS84) of the wrecks visited during the diving expedition.

Wreck	Lat	Long
Vinca Gorthon	52.7°N	4.2°E
Maasburg	52.9°N	4.5°E
Vittorio Z	53.3°N	4.8°E
Unidentified	53.8°N	5.2°E
Healdton	54.0°N	5.1°E
59695	54.5°N	2.8°E
Ocean Prince	54.4°N	2.6°E
WA70502	54.7°N	2.1°E
WA70500	55.0°N	1.7°E
WA70501	54.8°N	1.6°E
6884	54.2°N	1.2°E
Britta	53.9°N	3.1°E
Russian submarine	53.0°N	3.2°E
Vaderdag	52.4°N	3.7°E

Inventory of previous reports

A literature search was performed to assess the known distribution of *C. smithii* in the North Sea between 50.5° and 61° north, 3.5° west and 10.5° east, ignoring locations on the west of the British Isles. Descriptive locations without coordinates were given an estimated coordinate in the approximate centre of the described location. In total 429 records were

found in the North Sea and 469 in the English Channel, the oldest account dating back to 1828. Records with a coordinate precision of less than 10,000 m were excluded. All remaining records are included in figure 1.
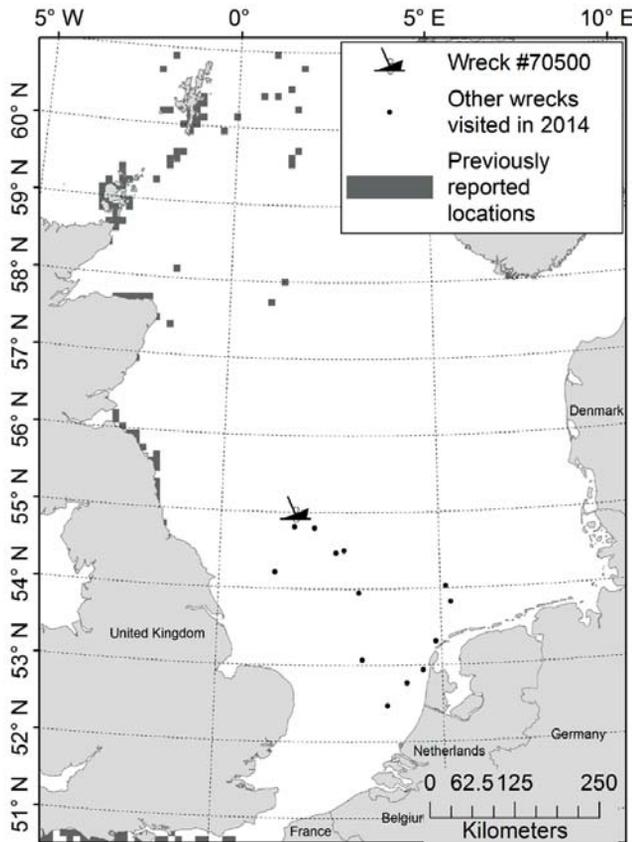
Figure 1. Locations of previously reported (as compiled from literature) and new sightings of *Caryophyllia smithii* (Stokes & Broderip, 1828) on the WA70500 wreck. Reports west of 3.5°W and west of the British Isles were excluded.

Results

During the dives on the WA70500 shipwreck 2 pseudocolonies (with 2 and 3 individuals per pseudocolony) and 2 solitary individuals were observed. The corals were all fixed to different vertical steel or stone parts of the wreck, with varying distances between them. Both photographic and video equipment was used to document live specimens in their natural habitat. One specimen was collected for confirmation in the IMARES Wageningen UR benthic laboratory and stored in a borax buffered formaldehyde solution (4%) in the IMARES Wageningen UR reference collection (number 2014IRC050001). For identification the works by Manuel (1988) and Cornelius *et al.* (1995) were used. The specimen was confirmed to have a solid and imperforate substance of corallum, with pali present and tentacles terminating in knobs (Figure 2).

Figure 2. In situ top view photograph of *Caryophyllia smithii* (Stokes & Broderip, 1828) on wreck WA70500. The terminal knobs, which are an important diagnostic feature, are clearly visible.

Discussion

The observation of *C. smithii* on the Dogger Bank shows the distribution of this coral is wider than previously reported. Furthermore, this is the first report of any hard coral in the central southern North Sea as all previous offshore observations of hard coral were from northern North Sea locations (Wilson 1979; Bell and Smith 1999; Roberts 2002; Gass and Roberts 2006; Guerin 2009). Several other observations of *C. smithii* have been reported from shipwrecks in British waters (Hiscock 1981; UK National Biodiversity Network: Marine Conservation Society 2010; Hiscock et al. 2010), demonstrating that the species favours steel substrata. With its bright colours, *C. smithii* is a conspicuous species, easy to find for the trained observer. The untrained observer, however, may mistake it for a species belonging

to other Hexacorallia groups, such as the Actiniaria, resulting in an underestimation of the number of earlier identifications.

Hiscock & Howlett (1976) report low abundance of *C. smithii* at locations with low tidal current strength and high sedimentation. On the Dogger Bank the currents can be weak, increasing sedimentation (Kröncke and Knust 1995). This would decrease the colonisation potential of *C. smithii* on the Dogger Bank. Densities reported under ideal circumstances by Hiscock & Howlett (1976) are up to 482 individuals/m^2, a number far exceeding the few individuals we have observed.

From all current and previous observations, it is clear that *C. smithii* is able to colonise artificial reefs in temperate waters. Even where natural reefs are lacking, the coral is able to expand its distribution by using the wrecks scattered on the sea bottom as stepping stones. With a planktonic stage of 8 to 10 weeks (Tranter et al. 1982), *C. smithii* can easily disperse over large distances. Prandle (1984) observed the maximum residual currents in the North Sea to be less than 0.06 m.s^{-1} while Thorpe (2012a) communicated average flows between 0.02 m.s^{-1} and 0.08 m.s^{-1}. Using these extremes in an extremely simplified estimate combining them with a pelagic stage of 8-10 weeks, *C. smithii* larvae may travel between 96 and 480 km. Since the offshore currents in the area of interest are parallel to the British coast (Thorpe 2012), a source population of the specimens on wreck WA70500 should be sought in a northerly direction. The nearest known observation of *C. smithii* in that direction is over 320 km away at the Andrew oil platform (Guerin 2009). Although this is within the potential dispersal range of *C. smithii* larvae, Shanks (2009) has shown that dispersal models tend to overestimate the dispersal distance of species with a long planktonic larval stage. Therefore, it seems likely that the observed specimens originated from a population located between the WA70500 wreck and the Andrew platform. This source is likely to be one of the many platforms and shipwrecks present in the central North Sea. Over 27,000 wrecks are present in the area we had specified for our inventory of earlier observations of *C. smithii* (Lettens 2015) and the potential for further distribution is large. In the near future, the development of large offshore wind farms throughout the North Sea, may further facilitate the stepping stone effect for species like *C. smithii*. However, since the WA70500 ship wreck was identified as a steam ship, it is very likely that this wreck is much older than the expected life span for any planned or constructed wind farm. While *C. smithii* does inhabit offshore installations in the northern North Sea (Guerin 2009), it is unknown if the species will also colonise the future offshore structures on the Dogger Bank. Since we found the species on several locations on the wreck, we expect the find was not incidental and that it is possible that *C. smithii* inhabits other shipwrecks in this area as well. However, *C. smithii* was not encountered on the 2 nearby wrecks we investigated on the Dogger Bank. The exact distribution of this species remains unclear.

Conclusion

We observed several live specimens of *C. smithii* on a unidentified shipwreck on the Dogger Bank. This is the first offshore observation of any hard coral in the central southern North Sea. The find of this coral suggests that species with long pelagic larval stages, are capable of extending their range using artificial structures such as wrecks, oil and gas platforms and wind farms as stepping stones. With the many shipwrecks present in these waters, we expect future investigations of shipwrecks in the Dogger Bank area will show that the distribution of this species includes the central southern North Sea.

Acknowledgements

4.2: DISTRIBUTION OF THE INVASIVE *CAPRELLA MUTICA* SCHURIN, 1935 AND NATIVE *CAPRELLA LINEARIS* (LINNAEUS, 1767) ON ARTIFICIAL HARD SUBSTRATES IN THE NORTH SEA: SEPARATION BY HABITAT

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Abstract

Studying offshore natural and artificial hard substrates in the southern North Sea (51°N-57°N/1°W-9°E), the invasive introduced Japanese skeleton shrimp *Caprella mutica* Schurin, 1935 was found to co-exist with the native *Caprella linearis* (Linnaeus, 1767) only on near-shore locations that had an intertidal zone (e.g., wind farm foundations). In contrast, on far offshore and strictly subtidal locations, such as shipwrecks and rocky reefs, only *C. linearis* was found. Based on these exploratory observations, we hypothesised that artificial structures that are only subtidal are inhabited exclusively by *C. linearis*, and never by *C. mutica*. To test this hypothesis and understand factors driving each species' habitat preferences, habitat suitability models were constructed using generalised additive models, based on samples collected in 2013-2015 from offshore gas platforms, buoys, shipwrecks, and rocky reefs and combined with data from other published and unpublished surveys (2001-2014). The models showed that the presence of *C. mutica* is explained by the availability of intertidal and floating hard substrates, suspended particulate matter density (SPM), mean annual sea surface temperature, salinity, and current velocity. The *C. linearis* model included subtidal hard substrates, SPM, salinity, temperature, and current velocity. The modelled distributions showed a significant difference, demonstrating that *C. linearis*' habitat preference does not fully overlap with that of *C. mutica*. Thus, the native and alien *Caprella* species are likely to be able to co-exist in the North Sea.

Introduction

Non-indigenous invasive species can threaten native species and cause extinctions (Simberloff 2010). In the marine environment, no extinctions have been directly linked to invasions, but invasive species may still drive native species to potentially less preferred habitats (Hill and Lodge 1999). This distribution shift can prevent extinction of the native species (Gurevitch and Padilla 2004) but alters the local species community.

Fast-growing introduced species are likely to establish viable populations after introduction (Sakai et al. 2001). The invasive Japanese skeleton shrimp *Caprella mutica* Schurin, 1935, a caprellid amphipod, has fast reproductive capabilities (Cook et al. 2007b; Shucksmith et al. 2009). *Caprella mutica* females can release their first brood within 24-26 days after they themselves hatch, allowing a rapid expansion once introduced (Cook et al. 2007b). In its native range in north-east Asia (Schurin 1935; Ashton 2006), it is found associated with macro-algae in shallow water (Fedotov 1991; Ashton et al. 2007) and on aquaculture structures (Kawashima et al. 1999). *Caprella mutica* was first recorded in Europe in the Netherlands in 1994, and only 14 years later it had been identified on all coasts along the North Sea, English Channel, and Celtic Sea (Platvoet et al. 1995; Cook et al. 2007a). Using mitochondrial DNA, Ashton et al. (2008) noted that *C. mutica* may have been initially introduced to European waters in Scotland and Norway, with secondary dispersal from there. Such secondary dispersal is most likely aided by flotsam and human activities, e.g., ship traffic and aquaculture (Ashton 2006). This amphipod is known to cling to ship hulls, which may be an important factor for longer range transportation (Cook et al. 2007a; Frey et al. 2009; Adams et al. 2014; Zabin et al. 2014). *Caprella mutica* is able to survive 20 days without food, ensuring a good survival capacity for long transportation periods (Cook et al. 2007b).

Several caprellids are native to North Sea waters (Guerra-García 2014). *Caprella linearis* (Linnaeus, 1767) is one of the most common species (Bate and Westwood 1868; Hoek 1889; Stock 1955; Guerra-García 2014). Similar to *C. mutica*, this species associates with fouling organisms on hard substrates (Guerra-García 2002; Page et al. 2006; Strong et al. 2009; Coolen et al. 2015a). For example, *C. linearis* is often found clinging to the surfaces of the sponge *Halichondria panicea* (Peattie and Hoare 1981), algae (Bate and Westwood 1868), and to biogenic reefs (Drent and Dekker 2013). Anecdotally, *C. mutica* and *C. linearis* have been observed independently on natural rocky intertidal locations (Guerra-García 2002; Wasson et al. 2005) and sympatrically on artificial structures (Shucksmith 2007; Macleod 2013). *Caprella mutica* is present in shallow water, at depths <17 m (Fedotov 1991; Vanagt and Faasse 2014), while *C. linearis* is reported from both shallow and deeper waters (Moen and Svensen 2004; Cook et al. 2007a). Thus, the native and invasive species may co-occur and perhaps compete for space and food in shallow water locations.

Both *C. mutica* and *C. linearis* are observed on anthropogenic structures (Ashelby 2005; Buschbaum and Gutow 2005; Page et al. 2006; Cook et al. 2007a; Bouma and Lengkeek 2013; Macleod 2013; Nall et al. 2015). In the North Sea, large numbers of artificial structures are present, all providing potential habitat for both species. Several authors have suggested that offshore anthropogenic structures can function as stepping stones for invasive species (Mineur et al. 2012; Adams et al. 2014; De Mesel et al. 2015). Stepping stones provide habitat in an environment normally unsuitable for survival (Mac Arthur and Wilson 1967), facilitating species to spread faster to potential recipient locations than they otherwise could. Adams et al. (2014) suggests *C. mutica* may be transported by ships between the coast and offshore structures, which they then colonise. From there, the invasive species may spread even further.

Shipwrecks (strictly subtidal) are common artificial structures in the North Sea (Coolen et al. 2015b). Many other man-made structures are also present in the North Sea, including buoys, wind farms and offshore oil and gas (O&G) installations. One difference between most shipwrecks, and the other structures is that the others penetrate the surface, providing surface/ inter-tidal habitats. All these artificial structures represent potential habitat for *C. mutica* and *C. linearis*. In previous studies of fouling diversity on artificial structures in the North Sea, only *C. linearis* was recorded from shipwrecks (Zintzen and Massin 2010; Lengkeek et al. 2013) while both species were recorded from turbine foundations of wind farms in Dutch waters (Bouma and Lengkeek 2013). This suggested that *C. mutica* may be excluded from artificial structures that are strictly subtidal whereas these could be colonised by *C. linearis*. To test this hypothesis, we reviewed available sampling data and conducted additional sampling programs of fouling communities in the North Sea. With advances in remote sensing and modelling techniques (Gayer et al. 2006; Brown et al. 2011), it was possible to obtain datasets with sufficiently fine resolution to model the combined effects of a range of variables on the presence of species (Reiss et al. 2014). In this study, we investigated the distribution of *C. mutica* and *C. linearis* in the offshore southern and central North Sea using habitat suitability models based on generalised additive models (GAM) to determine if the habitats occupied differed.

Material and Methods

Study area
The North Sea (surface area of 575,300 km^2) is a coastal sea in the north east Atlantic Ocean. It is largely surrounded by land with tidal water entering from the Atlantic Ocean via the English Channel to the south and between the United Kingdom and Norway to the north (southern and central North Sea: Figure 1). Water circulation in the North Sea is driven counter-clockwise by tides and wind. Sea surface temperatures vary between 2–8°C in winter and 12–21°C in coastal waters during summer (Otto et al. 1990; Rijkswaterstaat

2015). The input of nutrients from rivers in the surrounding countries has a strong influence on turbidity in coastal zones (Brockmann et al. 1990 and see Figure 2).

The seafloor of the North Sea largely consists of sandy and muddy (mobile) sediments, interrupted by zones of coarse material, mainly gravel and rocks (Coolen et al. 2015a). The area covered with coarse substrates is about 100,000 km^2 (estimated from EMODnet 2015 data).

Many artificial structures are present in the North Sea. In Belgian, Dutch and German North Sea waters alone there are 4,700 buoys and other navigational aids (Afdeling kust - Flemish Ministry of Mobility and Public Works - Agentschap Maritieme Dienstverlening en Kust 2015; Bundesamt für Seeschifffahrt und Hydrographie [BSH] 2015; Ministry of Infrastructure and Environment - Directorate-General Rijkswaterstaat 2015). Many more are present in British, Danish and Norwegian waters. There are at least 27,000 shipwrecks (Coolen et al. 2015b), 1,397 O&G production installations (OSPAR Commission 2013), and about 1,575 wind turbines (estimated from EWEA 2015) in the North Sea (Figure 3).

4
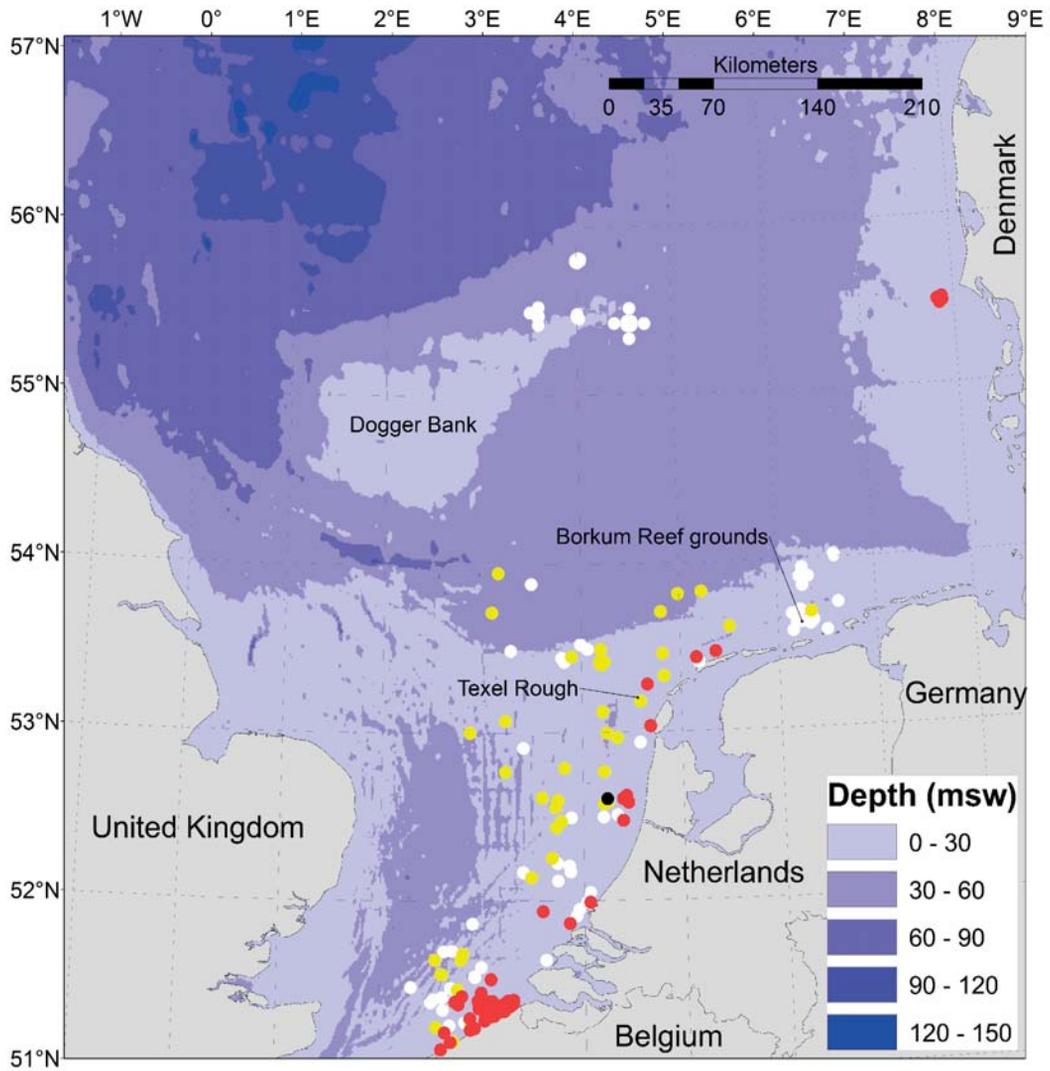
Figure 1. Caprellid distributions in the southern and central North Sea. Locations with non-native *Caprella mutica* only: red dots, with native *Caprella linearis* only: yellow dots, with both species: black dots, with neither species: white dots.
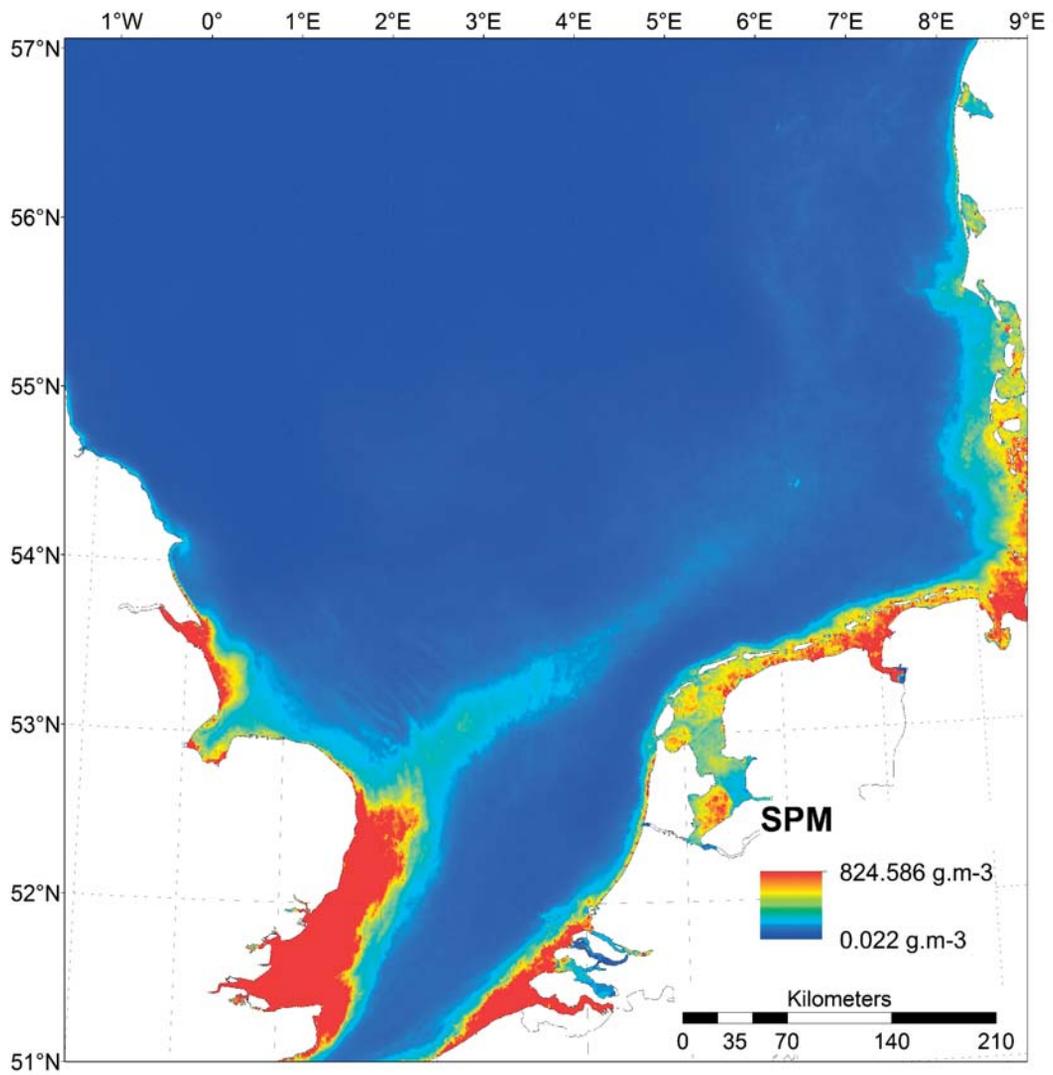
**Figure 2.** Mean annual sea surface suspended particulate matter concentration (SPM) in g.m^{-3}.
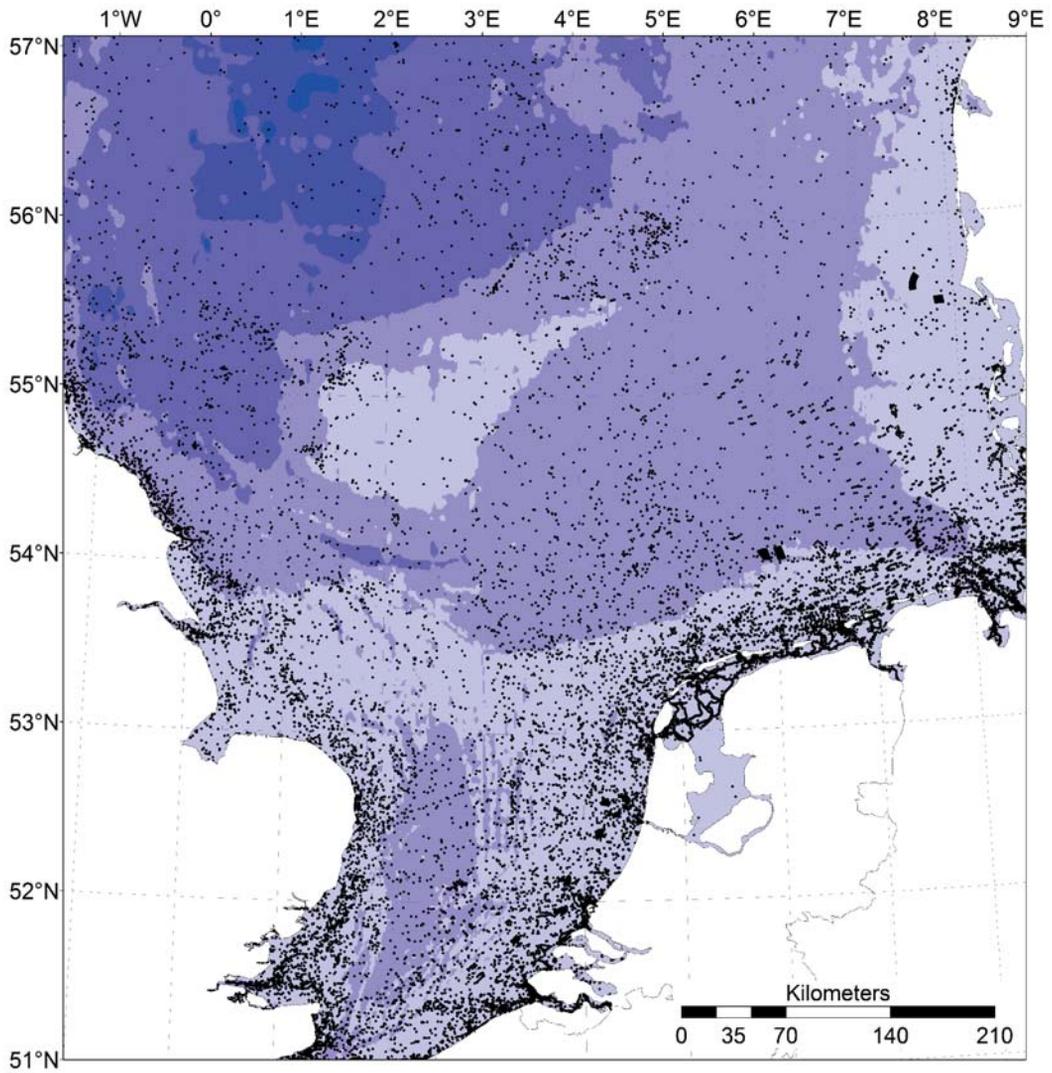
Figure 3. Map with locations of all artificial structures (black dots) used for modelling. Depth contours as in Figure 1.

Acquisition of samples

We collected 150 samples from 64 unique locations in the Dutch and British Exclusive Economic Zones of the North Sea. Samples were extracted from different sources, between 2013 and 2015.

- Mixed macrofauna samples were obtained from offshore gas production installations, shipwrecks, the Borkum Reef Grounds, and Texel Rough. We used surface supplied diving equipment (platforms) and scuba equipment (all other objects). We collected samples using an airlift sampler fed by surface supplied air or by air from side-mounted scuba tanks. A 500 cm^2 frame was attached to the sampled surface and the surface scraped clean using a putty knife. The suction part of the airlift was held close to the putty knife to collect the scraped specimens, which were deposited in a 500 μm mesh size net. The methods used are described in more detail in Coolen et al. (2015a);
- In addition to airlift sampling, we actively searched for caprellids on additional shipwrecks during wreck inventories in 2013-2015. During these dives we searched for caprellids for at least 15 minutes, or until at least 25 specimens were collected from different surfaces. Caprellids are easily spotted under water due to their elongated bodies protruding from the surfaces they cling to. We followed methods also applied in Coolen et al. (2015b). About 50 dives (including airlift sampling) were made in water depths between 20 and 45 meters between 2013 and 2015;
- During the diving expeditions described above, we did not survey every location visited, but inspected ghost fishing nets removed from the shipwrecks by other scuba divers for presence of caprellids at some locations;
- During inspection, repair and maintenance (IRM) work by SSE divers on offshore gas installations, caprellids clinging to divers suits were collected opportunistically from the suits after the IRM divers submerged. Both species tend to cling to clothes and working gear when removed from the substrate (pers. obs. J.W.P. Coolen);
- During IRM work on offshore buoys in Dutch waters, qualitative macrofauna samples were taken from the marine growth left on deck after cleaning of the buoys. At all buoy locations, about 1 litre of mixed macrofauna with mussels was collected by the IRM vessel crew and frozen in a household freezer; and
- Eight macrofauna samples were obtained from a gravel reef and the surrounding sandy sediments in the Borkum Reef Grounds using a box-corer (0.076 m^2). Only samples with >15 cm penetration depth were retained. Detailed methods are described in Coolen et al. (2015a).

All samples were stored in a borax-buffered formaldehyde-seawater solution (6%) except the buoy samples, which were frozen in a -20° C freezer. All observed caprellids were identified to the lowest taxonomic level possible, using the World Register of Marine Species (WoRMS

Editorial Board 2015) as a standard for taxonomical nomenclature. For identification, keys by Stock (1955), Larsen (1998) and Guerra-García (2014) were consulted.

Additional presence-absence data

Further data with presence-absence observations of *C. mutica* and *C. linearis* were acquired from published and unpublished sources. In order to ensure that only true absence data were included, confirmation that both species were sought was asked from the people involved in creating the data. If such confirmation was not possible, the species was excluded from the model data for that specific dataset (NA in Table S1). If the identification of both species was unsure, the full dataset was excluded. The following sources were included in the analysis:

- Macrofauna surveys at wind farms: Dutch - Egmond aan Zee (Bouma and Lengkeek 2013) and Princess Amalia Wind Farm (Vanagt and Faasse 2014), Danish - Horns Rev 1 (Leonhard and Frederiksen 2006), German - Alpha Ventus and FINO 1 research platform (Krone et al. 2013b; Gutow et al. 2014) and Belgian – C-Power and Belwind (De Mesel et al. 2015);
- Invasive species surveys around the Shetland Islands (unpublished data supplied by Shucksmith and Shelmerdine, NAFC Marine Centre, 2015);
- Surveys of wrecks in Belgian waters (Zintzen and Massin 2010);
- Post drill surveys near the German A6-A installation on the Dogger Bank (Glorius et al. 2014a) and the German L1-2 installation in the Borkum Reef Ground area (Glorius et al. 2012);
- Baseline survey for a proposed pipeline between the German A6-A installation and Danish Ravn platform (Glorius et al. 2014b) and a proposed exploration well in the German B11-5 block on the Dogger Bank (Glorius et al. 2013); and
- Buoy surveys in Belgian waters between 1998 and 2014 (Kerckhof, unpublished data).

A dataset of all locations is provided as online supplement and the data obtained from newly sampled locations were deposited in the Dryad Digital Repository (Coolen et al. 2016a).

Model creation

The predictor variables used to model the presence and absence of *C. mutica* and *C. linearis* (Table 1) were obtained from various sources and included mean annual sea surface temperature (MASST), salinity (MASSS), current velocity (MASSCV), suspended particulate matter (SPM) and three types of hard substrates; subtidal (SHS), intertidal (IHS) and floating hard substrate (FHS). The categorical hard substrates location data were converted to presence (1) and absence (0) raster data (presence-absence, PA). Combination into a single factor variable was not possible since offshore installations placed on the sea bottom contain both subtidal and intertidal substrates. Sea bottom depth was initially considered

as a possible predictor variable but was not included in the models because the available data only included bottom depth and did not reflect the true local depth, e.g. on intertidal or floating locations.

Data for all species were combined into a single dataset (Table S1). Within this dataset, all observations were converted to PA data to compensate for differences in sampling methods. Using ArcGIS 10.2.1.3497 for Desktop (ESRI 2015, Redlands, CA), both species presence-absence and predictor variable data were fitted to 1 km square grid cells extending between 50°N and 61°N, and 4°W and 9°E. A subset of unique locations from an area outside the dataset was excluded at this stage but used for later validation of the optimised models. This subset comprised the data from the buoy and wind farm surveys in Belgium and the September 2015 wreck survey. Every grid cell containing an observation of presence or absence of either *C. mutica* or *C. linearis* was exported, including the value of each predictor variable per cell. This resulted in a training dataset with PA observations of 160 locations for *C. mutica* and of 137 locations for *C. linearis*.

Table 1: List of variables used in the habitat suitability models for *Caprella mutica* and *Caprella linearis* with description of the variable, units used, study period, and data source.

Variable	Description	Units	Study period	Source
MASST	Mean annual sea surface temperature	°C	2002-2010	MARSPEC (Sbrocco and Barber 2013)
MASSS	mean annual sea surface salinity		1955–2010	MARSPEC (Sbrocco and Barber 2013)
MASSCV	Mean annual sea surface current velocity	$m.s^{-1}$	2009	Copernicus EU project (MYOCEAN 2015a)
SPM	Mean annual density of suspended particulate matter	$g.m^{-3}$	2014- 2015	Copernicus EU project (MYOCEAN 2015b)
SHS	Subtidal hard substrates: shipwrecks, coarse sediments and reefs, wind farms, O&G platforms	Presence / absence	Unknown/ various	OSPAR (2013), wrecksite.eu (Lettens 2015), (BSH; 2015), (EMODnet) Seabed Habitats project (EMODnet 2015)
IHS	Intertidal hard substrates: wind farms, O&G platforms, coastal natural hard substrates	Presence / absence	Unknown/ various	OSPAR (2013) and the EMODnet Seabed Habitats project (EMODnet 2015)
FHS	Floating hard substrates: buoys	Presence / absence	2015	BSH (2015), Rijkswaterstaat (2015) Flemish Ministry of Mobility and Public Works (2015)

For analysis, R version 3.2.0 (R Core Team 2015a) and RStudio version 0.98.1103 (RStudio 2014b) were used. Data were explored following methods described in Zuur et al. (2010). Using Cleveland dotplots, boxplots, pairplots, Pearson correlation coefficients, variance inflation factors, and multi-panel scatterplots (Cleveland 1985; Sarkar 2008; Dormann et

al. 2013) the presence of outliers, multi-collinearity, relationships and interactions were analysed for combinations of all caprellid observations and predictor variables.

Species distribution often shows a non-linear relation with continuous abiotic data such as temperature (Austin 2007). Therefore, a GAM was created for both species, using the gam function in the mgcv package (Wood 2011). The data were modelled using the Bernoulli distribution with logit link function. GAMs are prone to overfitting (Wood and Augustin 2002); therefore, the number of knots for the smoothers for the continuous variables was limited to four. All continuous variables were initially included as smoothers and the need for smoothing evaluated during model optimisation. The initial full model took the following form [s(*) indicates smoothed variables]:

Presence of species ~ IHS + SHS + FHS + s(MASST) + s(MASSS) + s(MASSCV) + s(SPM)

To exclude predictor variables and optimise the models, backward selection using Akaike Information Criteria (AIC; Akaike 1973) was combined with ecological evaluation of the influence of included and excluded effects. The optimal models were validated to verify the underlying assumptions. Model residuals were plotted against fitted values to analyse homogeneity of variance and against all covariates used during model selection to assess model fit. The part of the data that was excluded at the beginning of the analysis contained 69 locations for *C. mutica* and 63 for *C. linearis*. This dataset was used to validate the predictions of the models using a generalised linear model (GLM). The relation between *C. mutica* and *C. linearis* was then modelled using a GLM and *C. mutica* presence was added to the validated *C. linearis* model to evaluate this relation in combination with the predictor values, following Ros et al. (2015).

With the resulting models and the full dataset of predictor variables, a presence absence prediction raster was calculated using the predict.gam function (Wood 2011). To test for differences between the distributions of *C. mutica* and *C. linearis*, the presence absence predictions of both species were compared using a Students T-Test for paired samples. The resulting presence absence prediction grids for both species were visualised using ArcGIS.

Results

Obtained data

From 289 possible locations *C. mutica* was present 74 times and *C. linearis* 41 times. Within these locations, *C. mutica* and *C. linearis* only co-existed in two wind farm locations near the Dutch coast (Table S1; Figure 1).

Caprella mutica was only present on floating objects or intertidal offshore structures. *C. mutica* was never observed on any of the subtidal shipwrecks or rocky reef locations. Most *C. linearis* observations were from offshore locations with intertidal or subtidal hard substrates with a few observations on floating hard substrates. On most wrecks, *C. linearis* was present as well as on most O&G installations, and the rocky reefs of the Borkum Reef grounds, and the Texel Rough. *Caprella mutica* and *C. linearis* only co-occurred at intertidal locations. Neither species was observed on any of the sandy sediment locations.

Model selection & visualisation
During model selection for *C. mutica*, all predictor variables from the initial full model were included in the final model. All the continuous data (temperature, salinity, current velocity and SPM) were left in the model as non-linear terms (Figure 4). The resulting model to predict the presence and absence of *C. mutica* in the North Sea was:

> *C.mutica presence ~ -7.3401 + 1.6232 * IHS + 1.7425 * SHS + 1.8128 * FHS + s(MASST) + s(MASSS) + s(MASSCV) + s(SPM)*

Within this model, significant effects were found for temperature (p= 0.043), salinity (p= 0.015), current velocity (p= 0.014) and SPM (p= 0.013), with SPM showing a positive effect in most of the data range. Effects of floating (p= 0.059, intertidal (p= 0.141) and subtidal hard substrates (p= 0.059) were non-significant but had explanatory value and were therefore kept in the model. The deviance explained by the model was 57.2% with an adjusted r^2 of 0.517.

During model selection for *C. linearis,* floating hard substrates were removed since inclusion of this variable resulted in a strong negative effect, and AIC evaluation forced subsequent removal of subtidal hard substrates from the model. The removal of floating hard substrates (keeping subtidal hard substrates) made more ecological sense. After removal of floating hard substrates, the AIC score for the model with subtidal hard substrates was lower than without. Intertidal hard substrates was removed as this had a low explanatory value resulting in a lower AIC for the model without intertidal hard substrates. The smoothers for both salinity and SPM were linear (edf=1). Temperature and current velocity were included as non-linear terms (Figure 5). The resulting model to predict the presence and absence of *C. linearis* was:

> *C.linearis presence ~ -66.6783 + 1.3725 * SHS + 1.9492 * MASSS - 0.4642 * SPM + s(MASST) + s(MASSCV)*

Significant effects were found for subtidal hard substrates (p= 0.036), temperature (p <0.001), salinity (p= 0.008) and SPM (p= 0.020). Contrary to *C. mutica*, SPM had a negative

effect on the presence of *C. linearis*. Current velocity was non-significant (p= 0.231) but did explain part of the deviance and so was kept in the model. The deviance explained by the model was 49.9% with an adjusted r² of 0.529.

The predicted distributions differed significantly (t-test, t= 27.875, p <0.001). The relation between *C. mutica* and *C. linearis* was non-significant (GLM, p=0.529) and inclusion of *C. mutica* presence in the *C. linearis* model resulted in a higher AIC than the validated model without *C. mutica* as well as a non-significant effect for *C. mutica* (p=0.111).

The training set for the *C. mutica* model included data from the northern North Sea but the *C. linearis* set did not. Furthermore, no other data were obtained from the North Sea area north of the Dogger Bank. Therefore the model validation and visualisation were limited to the southern and central North Sea (51°N-57°N/1°W-9°E). Validation showed the model for *C. mutica* explained 37.5% of the deviance of the validation dataset (GLM, p= 0.007) and for *C. linearis* it explained 20.3% of the deviance (GLM, p= 0.005). This is 66% and 40% of the deviance explained by the main models, respectively. The model visualisations showed *C. mutica* was highly suited to nearshore waters and partially suited to artificial structures that were floating or had intertidal surfaces in more offshore waters (Figure 6). *Caprella linearis* was highly suited to both nearshore to offshore waters and was suited to locations with completely subtidal reefs (Figure 7).
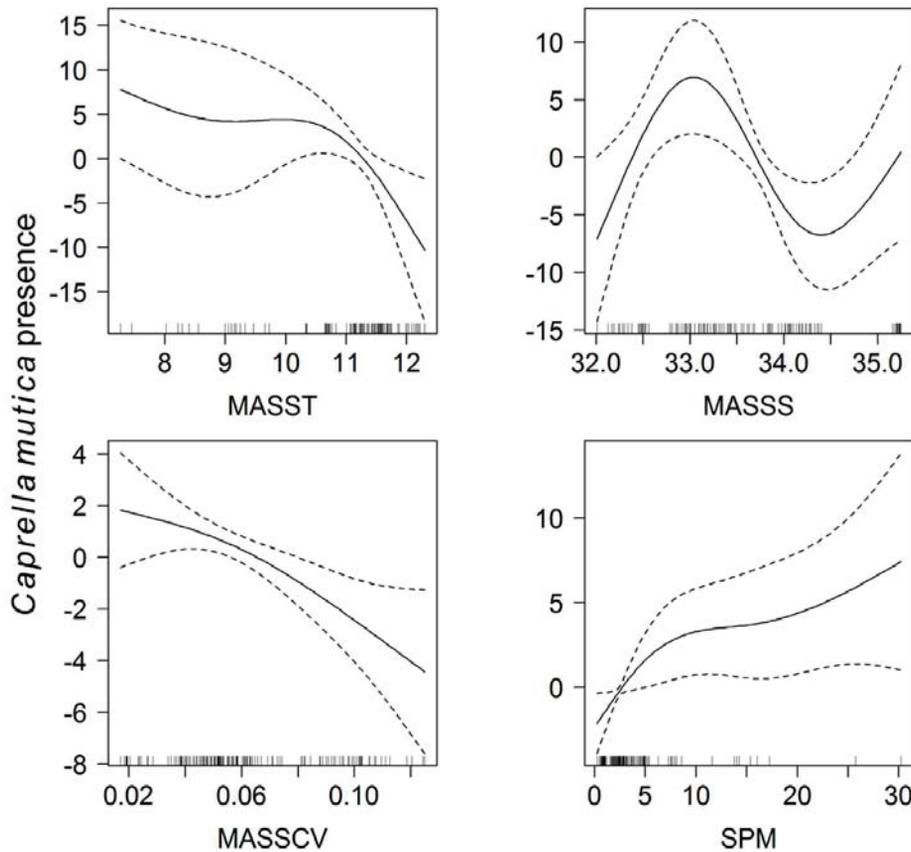
Figure 4. Predicted relations between environmental variables and probability of *Caprella mutica* presence for: mean annual sea surface temperature (MASST; °C), mean annual sea surface salinity (MASSS), mean annual sea surface current velocity (MASSCV; m.s^{-1}), and mean annual density of suspended particulate matter (SPM; g.m^{-3}). Dashed lines show 95% confidence interval, short vertical lines on the x axis show the density of samples in the model.
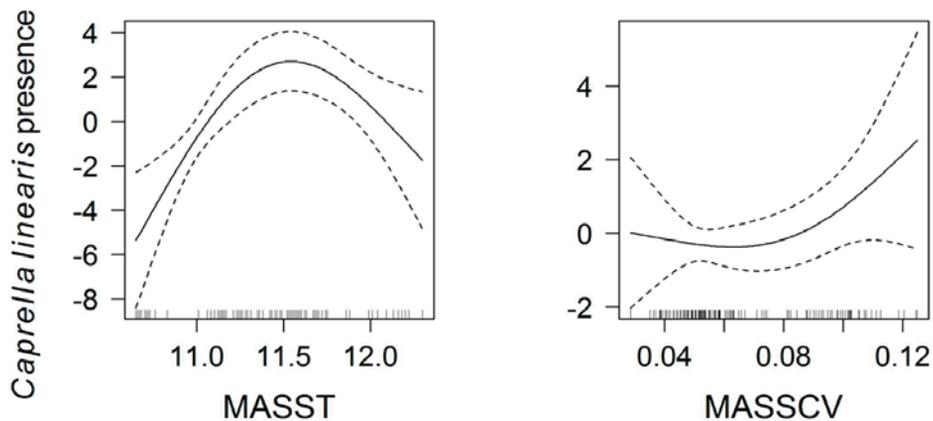
Figure 5. Predicted relations between environmental variables and probability of *Caprella linearis* presence for: mean annual sea surface temperature (MASST; °C), and mean annual sea surface current velocity (MASSCV; m.s^{-1}). Dashed lines show 95% confidence interval, short vertical lines on the x axis show the density of samples in the model.
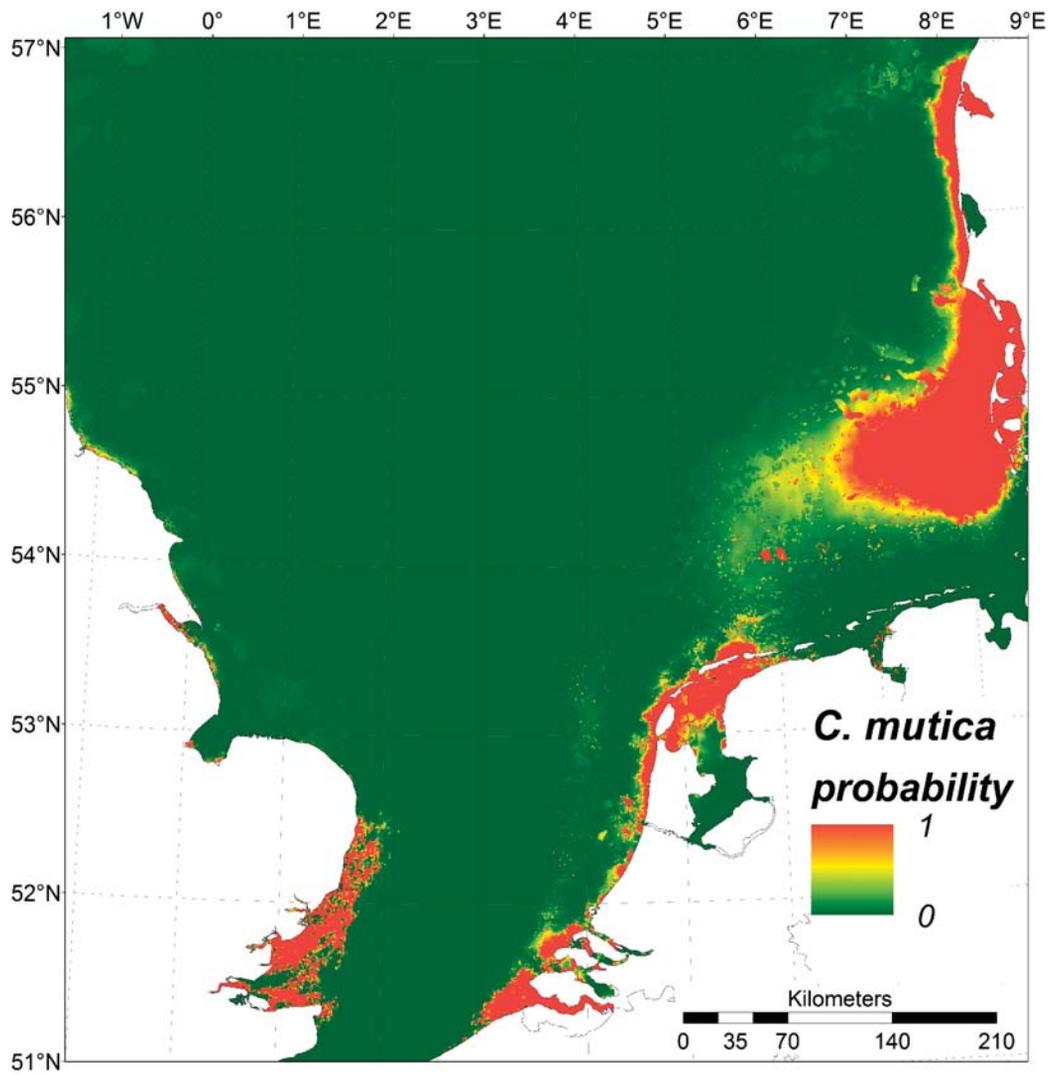
Figure 6. Predicted habitat suitability *Caprella mutica* (red=high and green=low).
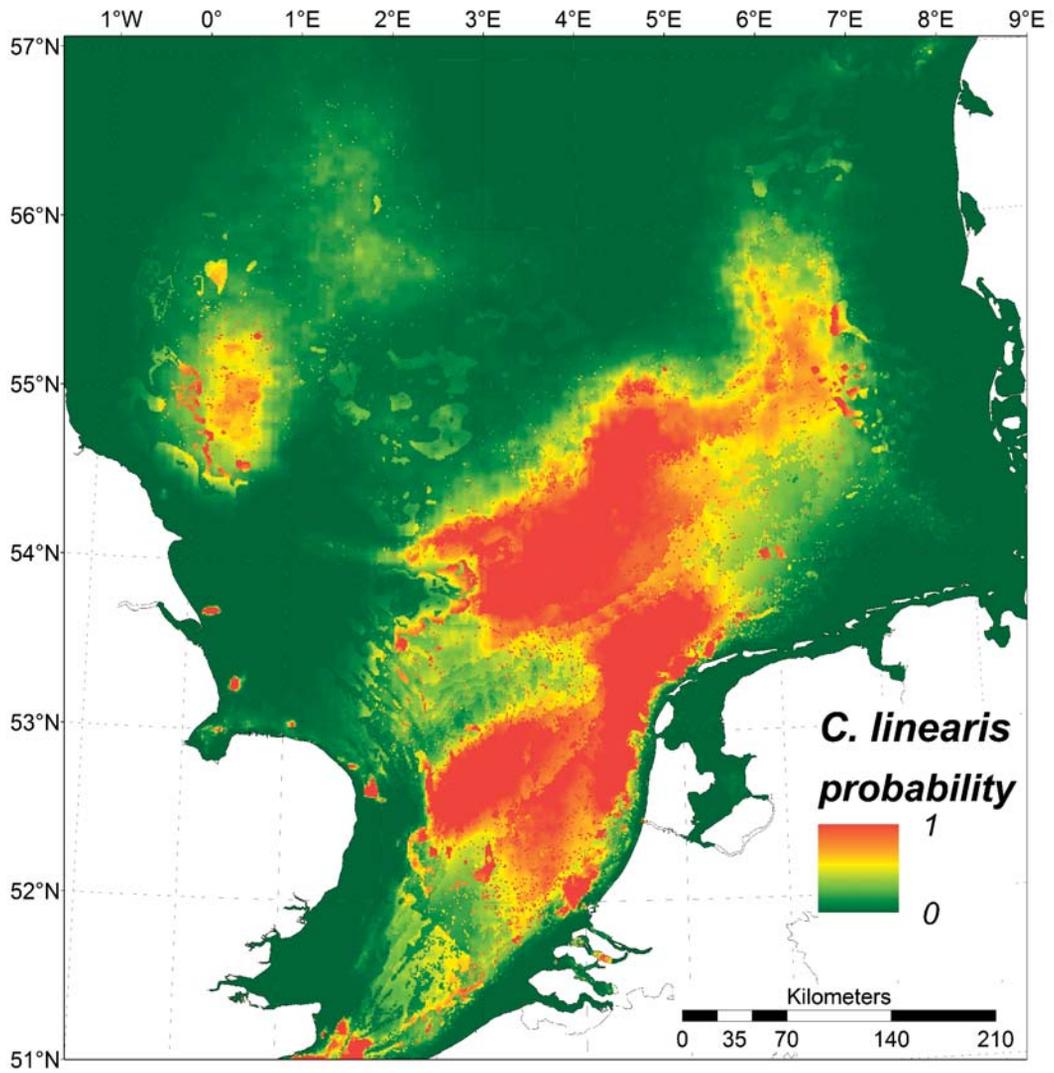
Figure 7. Predicted habitat suitability *Caprella linearis* (red=high and green=low).

Discussion

The modelled distributions of the alien, invasive, *C. mutica* and native *C. linearis* showed a significant difference. *Caprella mutica* occurred more frequently nearshore whereas *C. linearis* occurred more frequently offshore. Suspended particulate matter (SPM) density, which includes detritus, the main food source for both species (Guerra-García and Tierno de Figueroa 2009), had a contrasting effect on the species. SPM had a significant positive effect on the presence of *C. mutica* and a negative effect on the presence of *C. linearis*. This suggests that *C. linearis* prefers habitats with lower levels of SPM, such as waters that are far offshore (Brockmann et al. 1990; Jickells 1998).

There was a clear negative non-linear relation between current velocity and presence of *C. mutica*. Macleod (2013) also demonstrated the negative impact of high currents on *C. mutica*. Current velocity was included in the model for *C. linearis*, but there was no significant effect. This is in line with findings by Macleod (2013) who attributes this to the smaller body size of *C. linearis*, mean adult length 5.12 mm, compared to *C. mutica*, mean adult length 11.39 mm (Shucksmith et al. 2009). However, intertidal sites are more exposed to other hydrodynamic factors such as wave induced currents, resulting in higher disturbance than is present at subtidal sites (England et al. 2008). Guerra-García (2001) observed that caprellid species on wave-exposed sites were larger than those on sheltered sites. These findings contradict with our results and should be explored in future models, by including, for example, mean wave agitation as an additional factor (e.g., Dutertre et al. 2013).

Both caprellid species showed a significant non-linear relation with mean annual sea surface temperature, with an optimum between 11 and 12°C for *C. linearis* and an optimum at lower temperatures, <11°C, for *C. mutica*. Boos (2009) reported a significant increase of *C. mutica* mortality when experimentally kept at continuous temperatures >20°C. *Caprella mutica* may have a preference for the lower range of average temperatures in the North Sea which may explain the limited reports of *C. mutica* presence from southern Europe (Cook et al. 2007a but see Almón et al. 2014), where mean annual sea surface temperatures are between 19 and 21°C, with over 25°C for summer averages (Skliris et al. 2012). *Caprella linearis* is also known from northern waters with lower temperatures than observed here (Larsen 1998). Shucksmith et al. (2009) suggest temperature as well as salinity play a role in the co-existence of both species. Salinity had a small but significant effect on *C. linearis*, while *C. mutica* showed a non-linear relation that may partly be explained by the lack of data from the central to northern North Sea, where the salinity is between 34.5 and 35. The model visualisation (Figure 6) was limited to the area with salinity <34.5.

Notable was *C. mutica*'s association with shallow water objects, which meant they had a high potential to encounter rafts, such as macroalgae. Both *C. mutica* and *C. linearis* are known to use such rafts (Thiel and Gutow 2005). Rafting may have aided in the fast dispersal

of *C. mutica* in European waters. Buschbaum and Gutow (2005) propose that *C. mutica* may have colonised Helgoland using rafts, and Ashton (2006) showed this species' ability to use drifting algae for dispersal over distances >5 km. *Caprella linearis* may have colonised intertidal locations in a similar manner. However, to colonise strictly subtidal reefs, rafting does not help since drifting objects are unlikely to reach a sub-tidal-only location. The colonisation of these locations may have been aided by objects transported by currents near the bottom, such as free rolling sponges that have been observed in the southern North Sea (pers. obs. J.W.P. Coolen). A combined preference for shallow waters and high SPM density, explains why *C. mutica* was absent from intertidal hard substrate that was far offshore while *C. linearis* was present there. In the current study *C. mutica* was never observed at depths >17 meters. *Caprella mutica* is known from depths between 0 and 17 m and *C. linearis* between 0 and 65 m (Fedotov 1991; Moen and Svensen 2004; Vanagt and Faasse 2014). Our observations confirm that *C. linearis* is able to occupy deeper locations, such as wrecks, which are outside *C. mutica*'s preferred depth range. Offshore hard substrates are scarce in the North Sea. Therefore, artificial hard substrates likely play an important role in the distribution of *C. linearis* in offshore waters.

Conflicting with the absence of *C. mutica* from deep subtidal hard substrates was that our models showed that *C. mutica* presence was partly explained by the presence of subtidal hard substrates. This resulted because near-shore installations were modelled as being both intertidal and subtidal habitats. Our models had a high 'predictor to response variable' ratio, which may cause overfitting (Babyak 2004) and decrease model accuracy (Vittinghoff and Mcculloch 2006; Wisz et al. 2008). However, the models were validated with an independent dataset that demonstrated their ability to predict the presence of the modelled species. The deviance explained by these predictions was limited to 37.5% for *C. mutica* and 20.3% for *C. linearis*. To improve these percentages more data is needed and additional explanatory variables such as mean wave agitation need to be explored. Further model improvement could include using not only mean annual values for predictor variables but also annual maxima or minima, for example for sea surface temperatures. We limited the prediction area to the southern and central North Sea since only a limited amount of data were available for northern parts. Extrapolations to other areas should be validated using additional survey data.

Habitat selection is dependent on competition between species and competition between species is habitat dependent (Morris 2003): strong competitors in one habitat may be less competitive in another habitat. This was shown by Ros et al. (2015) for the relationship between other native and non-native caprellids around the Iberian Peninsula. There, the non-native species was dominant in more southern waters that were warmer and saltier. In the present study, no data on *C. linearis* distribution prior to and immediately after the introduction of *C. mutica* to the North Sea were available. We were therefore unable to

draw conclusions about if competition from *C. mutica* had displaced *C. linearis* from former habitats. In any case, hard deep water habitats, including artificial structures, represent refuges for *C. linearis*, where populations can survive away from possible competition from *C. mutica*.

Acknowledgments

Chapter 5:

MARINE STEPPING STONES: WATER FLOW DRIVES *MYTILUS EDULIS* POPULATION CONNECTIVITY BETWEEN OFFSHORE ENERGY INSTALLATIONS

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Abstract

The stepping-stone effect on marine species has been suggested in many recent papers, describing that epifaunal organisms may use isolated structures as stepping stones to spread to new areas that are too distant to reach in a single generation. With thousands of artificial hard substrate structures present in the North Sea, we hypothesise that most of these structures are directly or indirectly connected by water currents and that offshore energy installations in the North Sea act as a large interconnected reef for species with a relatively short pelagic larval stage. If they do, the population genetic structure should follow a pattern that can be predicted by particle tracking models (PTM; hydrodynamic distance). To test this, we analysed the population genetic structure of the mollusc *Mytilus edulis*, based on microsatellite markers, and tested whether hydrodynamic distance between the *M. edulis* sample locations explained the genetic variation between the samples. Our results strongly support that the marine stepping stone effect is present on offshore energy installations in the North Sea. The marine stepping stone effect is important for the distribution of *M. edulis* and it may influence the distribution of other species with similar strategies in a similar way. The presence of offshore energy installations and other objects is the only way in which species such as *M. edulis* are able to survive at deeper locations. Stepping stones may increase the speed at which future invasions take place, making new habitats at isolated locations available for non-indigenous species in shorter time periods than they would take without marine stepping stones. However, other factors such as hull fouling and ballast water exchange also strongly affect distribution of non-indigenous species.

Introduction

The stepping stone effect on marine species has been suggested in many recent papers, describing that epifaunal organisms may use isolated structures as stepping stones to spread to new areas (Langhamer 2012; Mineur et al. 2012; Thorpe 2012; Gittenberger et al. 2013; Miller et al. 2013; Ros et al. 2013; Krone et al. 2013b; Adams et al. 2014; Coates et al. 2014; Friedlander et al. 2014; Lindeboom et al. 2015; De Mesel et al. 2015; Coolen et al. 2015b). This stepping stone effect was first described in detail by Mac Arthur & Wilson (1967), to explain the distribution of organisms among islands in the Pacific Ocean. To migrate to new habitats with suitable habitat that are too distant to reach in a single generation, species may use locations in between as stepping stones to reach the new habitat in the next generation.

In recent years, the impact of the stepping stone effect was often mentioned during assessment of the environmental effects of offshore wind farms (see e.g. Leonhard 2006; Royal HaskoningDHV 2014), but it was already a concern of environmental assessment agencies in the '80s, during permitting procedures for offshore oil and gas production installations in the Dutch North Sea (personal communication Norbert Dankers, retired member of the Dutch EIA committee).

The marine stepping stone effect caused by offshore installations has been modelled extensively (Thorpe 2012; Adams et al. 2014). Offshore oil and gas installations in the North Sea, for example, were modelled as forming a large interconnected reef, given assumed circumstances such as larval stage duration and buoyancy (Thorpe 2012). For species with pelagic larvae, dispersal patterns will follow the direction of currents. Some sub-populations will then be connected by currents while others are not, or are connected only one way, whereby the next generation will only exist on downstream locations (Thorpe 2012). Adams et al. (2014) showed that offshore energy installations indeed provide habitat for larvae that would otherwise have perished offshore. Furthermore, they showed that many installations provide both origin and destination sub-populations for these species, increasing dispersal potential. The marine stepping stone effect has been described repeatedly using models, but to our knowledge, no empirical evidence exists for the use of offshore energy devices as stepping stones by native or non-native species.

Fowler et al. (2014) also call for more insight in the influence artificial structures have on the spread of invasive species. In particular structures connecting the sea bed with the water surface introduce an intertidal zone that is not present in most offshore waters. These intertidal zones may function as stepping stones for non-native species (De Mesel et al. 2015). Whether offshore objects function as stepping stone, depends on the life history traits of the species concerned. Some species utilise floating objects as rafts (Thiel and Gutow 2005) and therefore may not need fixed stepping stones to distribute to new locations (Coolen et al. 2016b). Other species migrate as adults over distances that far exceed distances between

stepping stones (e.g. the edible crab *Cancer pagurus* ; Hunter et al. 2013) and therefore do not need locations as stepping stones. The distance between proximate objects in the North Sea varies between a few hundred meters and tens of km (Figure 1). Stepping stones are probably of use only for epifouling species that distribute over distances within and beyond this range.

The stepping stone effect may be most important for species with a preference for rare offshore habitats, such as the intertidal zone. *Mytilus edulis* is common on offshore installations (Page and Hubbard 1987; Joschko et al. 2008; Lindeboom et al. 2011; De Mesel et al. 2015; van der Stap et al. 2016). In the coastal North Sea, recruitment of *M. edulis* is limited by the availability of hard substrates rather than larval dispersal (Joschko et al. 2008). *Mytilus edulis* densities on artificial structures show a vertical pattern, with highest abundance in shallow waters down to 20 m (Joschko et al. 2008; De Mesel et al. 2015; van der Stap et al. 2016). Structures without an intertidal zone, such as shipwrecks, show lower *M. edulis* abundance than intertidal structures (personal observations J.W.P. Coolen). *Mytilus edulis* is controlled by predation by *Asterias rubens* in subtidal areas (Saier 2001), explaining its low abundance on shipwrecks. Langhamer (2010) suggests that *A. rubens* is sensitive to wave exposure, resulting in a higher survival of *M. edulis* on the shallow parts of installations. Its limitation to intertidal hard substrates, makes *M. edulis* a good candidate to investigate general patterns in the connectivity of offshore installations.

Mytilus edulis has a pelagic larval stage of 16 to 70 days, depending on environmental variables such as temperature, after which the larvae metamorphose to the pediveliger stage during which it is capable of settling on suitable substrates (Bayne 1965; Filgueira et al. 2014). In the southern North Sea, highest concentrations of *M. edulis* larvae are observed between March and July (personal communication Pauline Kamermans). Coolen et al. (2015b) suggested that species with a pelagic larval stage of up to 10 weeks, are unlikely to colonise far offshore objects in the North Sea without another intertidal object with a sub-population located upstream. Thorpe (2012) showed that the time it takes for water masses to flow from one oil and gas platform to the next, can be as low as 60 hours in the southern North Sea. With pelagic larval durations of at least 16 days, a direct connection between closest platforms seems unlikely, since the larvae have not reached the pediveliger when arriving at the next downstream location. However, many platforms and other shallow objects such as wind farms and navigational buoys exist in the North Sea (Coolen et al. 2016b; Figure 1), making it likely that larvae do reach a suitable artificial substrate further down-stream at the time the pediveliger stage begins.

Mytilus edulis abundance can increase rapidly on anthropogenic structures, such as offshore wind turbine foundations (Bouma and Lengkeek 2013; Krone et al. 2013b; De Mesel et al. 2015). Krone et al. (2013) reported over 400,000 *M. edulis* specimens on a single installation

and suggested that a similar amount is produced locally and lost to the surrounding sea bottom every year. They suggest this will lead to an increase in shell debris and therefore increase the coarse fraction of the bottom sediment. This may lead to additional reefs outside the area covered by the foundations and rock dump around it, leading to increased larvae release by epifouling organisms and higher filtration of the water (Krone et al. 2013b). Clumps of *M. edulis* falling off also lead to increased food availability to predators such as *Cancer pagurus* (Langhamer and Wilhelmsson 2009).

The population genetic structure of the genus *Mytilus* has been studied with a broad range of morphological and genetic markers and over a large geographic range, including the North Sea (e.g. Gosling 1992; Heath et al. 1995; Rawson et al. 2001; Steinert et al. 2012).

Around the North Sea region three species of *Mytilus* are found which hybridize where they meet, and introgression of alleles radiating from these hybrid zones is common (Roux et al. 2014). *Mytilus galloprovincialis* is found in the south with a hybrid zone with *M. edulis* along the English Channel coasts of France and southern England (Bierne et al. 2003). *Mytilus trossulus* inhabits the Baltic Sea and hybridizes with *M. edulis* in the Kattegat and Danish Straits area (Riginos and Cunningham 2005). Coastal North Sea blue mussels are pure *M. edulis* (Bierne et al. 2003), although low levels of *M. galloprovincialis*-alleles may be encountered when sampling large numbers (Luttikhuizen et al. 2002). At the intraspecific level, both significant differentiation between populations and homogeneity among populations of *M. edulis* has been reported for various parts of its distribution range (Penney and Hart 1999; Riginos and Henzler 2008; Silva and Skibinski 2009; Smietanka et al. 2014). Population differentiation among the artificial hard substrates of the North Sea has never been studied. Any differentiation in *M. edulis* genetics between these objects must have arisen after the origin of the objects (i.e., less than 40 years ago).

The combination of shallow water preference, high abundance at offshore installations, a pelagic larval stage, easy collection and identification at sea and availability of genetic markers make *M. edulis* an ideal model organism to study the stepping stone effect in marine organisms.

Yund et al. (2015) showed that the connectivity of *M. edulis* sub-populations can be interrupted by currents, reducing gene flow between locations. We assume that the population genetic structure of *M. edulis* in the North Sea is largely influenced by the degree of isolation between the sub-populations on installations, leading to isolation by distance (Wright 1943; Kimura and Weiss 1964). This is expected when gene flow is limited by dispersal distance, leading to a stepping-stone population model whereby neighbouring locations are much more likely to exchange migrants (Kimura and Weiss 1964). In marine environments, the degree of isolation is mostly influenced by a combination of geographic

distances, current direction and current speed (White et al. 2010; Thorpe 2012), although marked exceptions are also known (e.g. Galindo et al. 2010). Since currents in the North Sea are bi-directional, connectivity between locations depends on whether stepping stones are located downstream from each other, or in a perpendicular direction from each other (Thorpe 2012). Therefore, we assume that the genetic connectivity of structures in the North Sea can be explained by a two dimensional stepping stone model with a different larval exchange rate between most combinations of locations (see Figure 2 for an extremely simplified example). We hypothesise that most of these structures are directly or indirectly connected in this manner and that offshore energy installations in the North Sea act as a large interconnected reef. If they do, the population genetic structure should follow a pattern that can be predicted by particle tracking models (PTM) that calculate the hydrodynamic distance between locations. To test this, we analysed the population genetic structure of *M. edulis*, based on microsatellite markers and tested whether hydrodynamic distance between the same locations contributes to the genetic differentiation between the locations.
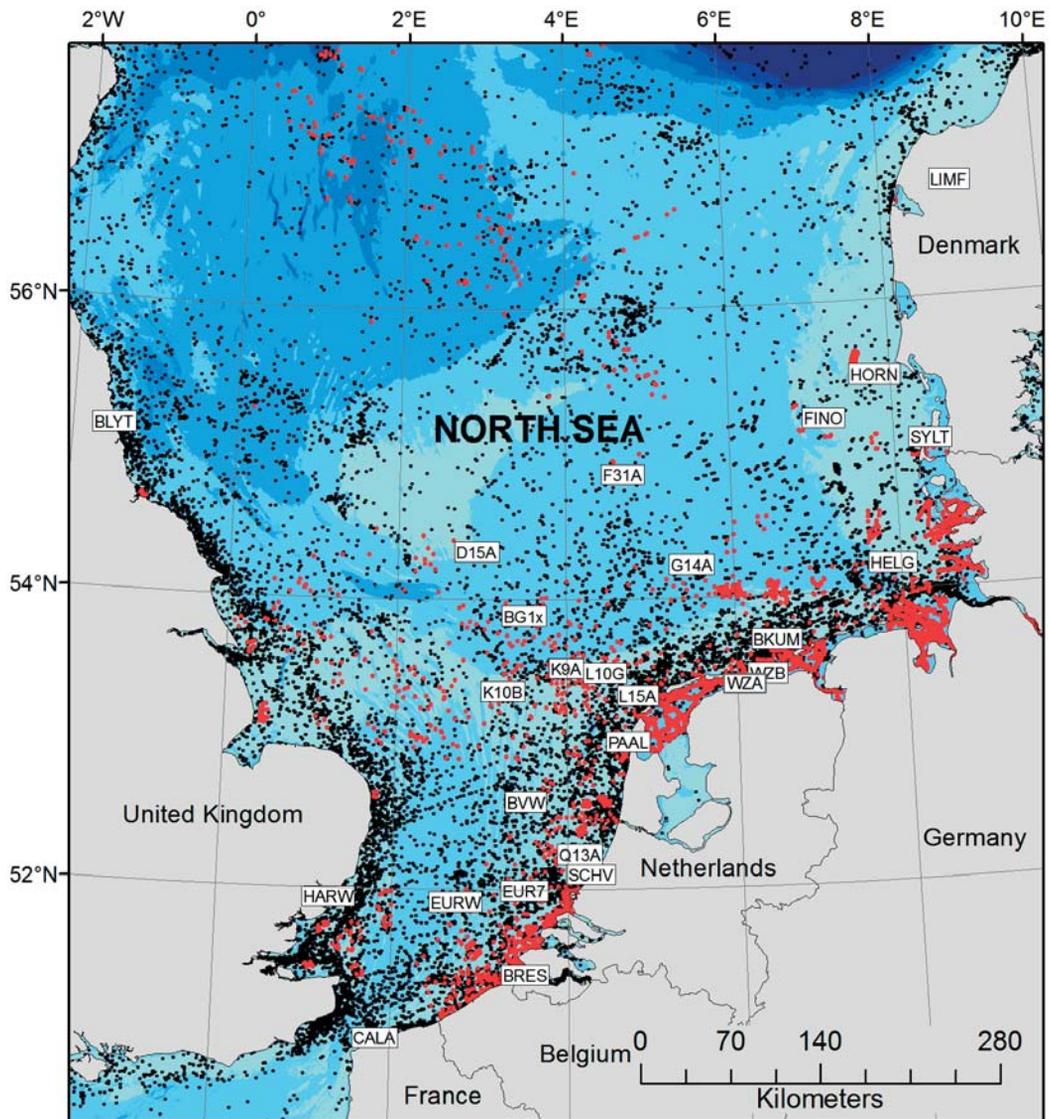
Figure 1: North Sea map of all artificial reefs and sample locations. Southern North Sea, sample locations (white labels), artificial structures with surface contact (red dots) and structures without surface contact (black dots). Abbreviations of sample locations are explained in Table 1. Note no buoys are shown for British, French and Danish waters.
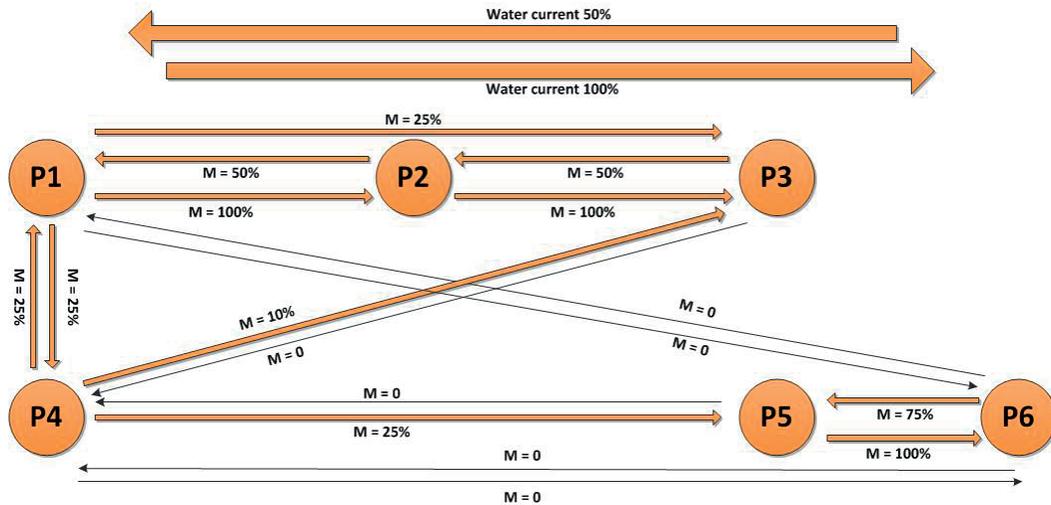
Figure 2. Schematic stepping stone model offshore connectivity. Simplified stepping stone model representing the variation in connectivity between offshore installations in the North Sea. Migration (M) between sub-populations (P1 to P6) represented as percentage of maximum. Sub-populations close to each other in the direction of the current are highly connected (e.g. P1 and P2), while sub-populations far from each other and perpendicular to the current are connected only unidirectionally (e.g. P3 and P4), or not at all (P1 and P6). Sub-populations very far from each other are only connected indirectly via sub-populations at intermediate distance (P4 to P6 via P5). Connections between some locations (e.g. P1-P5, P2-P4, etc.) were left out for further simplification.

Material and Methods

Study area

The North Sea is located in the north east Atlantic and is largely enclosed by land. Tidal water enters the North Sea in the south through the English Channel and from the north between Scotland and Norway. This results in a counter-clockwise circulation. The average water depth is 74 m (Otto et al. 1990). The North Sea bottom largely consists of sandy and muddy sediments, interrupted by areas with coarse substrates such as gravel and rocks (Coolen et al. 2015a). Many anthropogenic structures provide artificial hard substrates in the North Sea. There are >27,000 shipwrecks, 1,397 offshore oil and gas production installations, ~1,500 wind turbines and a large (but not exactly known to us) number of navigational buoys (Coolen et al. 2016b).

Mytilus edulis population genetics

Samples were collected at 27 locations (henceforth named sub-populations) between April 2014 and January 2016: from coastal sub-populations during low tide, by commercial and scientific divers from oil and gas platforms and wind farm foundations and during inspection, repair and maintenance work on buoys and offshore installations from structures lifted out of the water (Table 1). Sampling depth varied between 0 and 27 meters. Two likely outgroup sub-populations were included; from a harbour in Lisbon, Portugal and from mussel longlines

in the Limfjorden in Denmark. Geographic distances between sub-populations (excluding outgroups) varied from 17 km (Scheveningen and Q13-A) to 1,105 km (Blyth and Sylt) with an average of 348 km.

Table 1. Samples sub-populations
From left to right: Abbreviated name of sample locations, full name of sample locations; sample date, structure type, sample size (number of specimens), sample depth (m) and position in WGS1984.

Name	Full name	Date	Structure	Size	Depth	Lat.	Lon.
BG1x	BG 1	11 March 2015	Buoy	44	0	53.8833	3.4983
BKUM	Borkum Riffgat	29 June 2014	Wind farm	25	4	53.6900	6.4800
BLYT	Blythe	14 November 2014	Pier	48	0	55.1258	-1.4983
BRES	Breskens	20 August 2014	Breakwater	48	0	51.4068	3.5121
BVW	BV W	30 September 2014	Buoy	48	0	52.6007	3.5170
CALA	Calais	20 August 2014	Pier	24	0	50.9661	1.8433
D15A	D15-A	3 October 2015	O&G platform	48	7	54.3247	2.9346
EUR7	EURO 7	15 September 2014	Buoy	48	0	51.9900	3.5031
EURW	Euro W	24 July 2014	Buoy	48	0	51.9095	2.7232
F31A	F3-1A	1 September 2014	O&G platform	47	5	54.8520	4.6949
FINO	FINO 3	23 September 2015	Research platform	28	4	55.1950	7.1583
G14A	G14-A	16 July 2014	O&G platform	48	13	54.2241	5.4986
HARW	Harwich	15 November 2015	Pebble beach	42	0	51.9348	1.2813
HELG	Helgoland	15 January 2016	Harbour	48	0	54.1760	7.8945
HORN	Horns Rev	10 June 2015	Wind farm	67	0	55.4789	7.8110
K10B	K10-B	1 October 2014	O&G platform	48	27	53.3626	3.2539
K9A	K9-A	27 August 2014	O&G platform	48	0	53.5202	3.9925
L10G	L10-G	08 June 2014	O&G platform	48	10	53.4904	4.1952
L15A	L15-A	5 June 2014	O&G platform	48	6	53.3295	4.8302
LIMF	Limfjorden	16 June 2014	Longlines	48	2	56.7830	8.9110
LISB	Lisbon	14 February 2015	Harbour	37	0	38.7635	-9.0926
PAAL	Texel	29 June 2014	Breakwater	48	0	53.0118	4.7083
Q13A	Q13-A	28 May 2014	O&G platform	48	0 to 7	52.1911	4.1361
SCHV	Scheveningen	8 July 2014	Breakwater	48	0	52.0987	4.2582
SYLT	Sylt	3 June 2014	Breakwater	48	0	55.0216	8.4403
WZA	Wadden Sea A	30 April 2014	Intertidal mussel bed	24	0	53.4521	6.3042
WZB	Wadden Sea B	6 May 2014	Subtidal mussel bed	24	2	53.4600	6.3583

At least 50 specimens were sampled randomly from every sub-population and stored in -20°C or on ethanol 70% for transport to the lab. There all samples were cleaned of most marine growth and placed at -80°C for long-term storage.

Marker choice

Both nuclear and mitochondrial DNA sequences have been used to identify phylogeographic patterns of genetic diversity in the genus *Mytilus*. In contrast to these markers, the use of microsatellite markers results in a higher degree of exclusive alleles, which is important to estimate the degree of isolation of subpopulations (Oliveira et al. 2006). So far, twenty microsatellites have been developed, which consistently and reliable amplify for *M. edulis* (Lallias et al. 2009; Ye et al. 2014). A limitation of the use of microsatellite markers might be the unusual high frequency of null alleles found in marine bivalves and as a consequence a higher level of heterozygote deficiency (Bierne et al. 2003; Carlsson 2008; Lemer et al. 2011). These high levels are likely caused by high nucleotide diversity in the noncoding regions as a consequence of large effective mussel populations size (Bierne et al. 2003). Since correction methods are available for the analysis of datasets with high null allele frequencies (Chapuis and Estoup 2007; Panova et al. 2008), microsatellites markers developed for marine bivalves can be used to provide data for phylogeographic studies.

Molecular methods

From each sample, between 24 and 48 specimens were selected randomly and genomic DNA was isolated from the abductor muscles by using the 96 well genomic DNA extraction kit according to the manufacturer's protocol of FAVORGEN Biotech Corp. DNA concentration was measured with the Nanodrop and diluted to 10 ng/µl. Two multiplex sets of 4 markers (set1: Med367, Med379, Med722 and Med733; set2: Med737, Med740, Med747 and Me15/16) where markers were used individually in the PCR, pooled per set and analysed on the ABI3730 DNA Analyser. All markers are microsatellite markers described in Lallias et al. (2009) except Me15/16, this marker amplifies a part of the VD1 gene which discriminates between *M. edulis*, *M. galloprovincialis* an *M. trossulus* (Inoue et al. 1995). Marker information, PCR conditions and multiplex conditions are indicated in Table 2. The GeneScan 500 LIZ marker was used as internal marker. Allele calling was performed in Genemapper v3.7 (Applied Biosystems 2004).

Modelling mussel larvae transport

The transport of the mussel larvae in the North Sea was modelled using two Delft3D software modules: FLOW and PART (Deltares 2016a; Deltares 2016b). The PART module is able to simulate mid-field water quality and particle tracking, based on a hydrodynamic forcing output from the other Delft3D module, FLOW.

Table 2: Marker characteristics
From left to right: Name of markers used; fluorescent dye; annealing temperature (°C); allele size range in base pairs; PCR dilution in multiplex set; reference of marker used.

Marker	Dye	Temp	Size range	Dilution	Reference
Set 1					
Med 367	VIC	58	190-290	250x	Lallias et al. (2009)
Med379	NED	60	140-230	125x	Lallias et al. (2009)
Med722	PET	55	150-270	125x	Lallias et al. (2009)
Med733	FAM	58	140-270	83.3x	Lallias et al. (2009)
Set 2					
med 737	VIC	58	130-280	250x	Lallias et al. (2009)
Med 740	FAM	55	150-280	125x	Lallias et al. (2009)
Med 747	NED	55	100-320	125x	Lallias et al. (2009)
Me15/16	PET	55	120-200	83.3x	Inoue et al. (1995)

Hydrodynamical model

Delft3D-FLOW solves the unsteady shallow-water equations in two or three dimensions. The large number of processes included in this module means that Delft3D-FLOW can be applied to a wide range of river, estuarine, coastal and marine situations (Lesser et al. 2004). The model incorporates a large number of processes, such as wind shear, wave forces, tidal forces, density-driven flows, stratification, atmospheric pressure changes, air temperature and the exposure and inundation of intertidal flats. Flow equations were solved on a curvilinear grid consisting of 8710 computational elements (Roelvink et al. 2001). The vertical resolution of the model was 10 water layers using a sigma-coordinated approach (i.e. proportional to water depth; Stelling and van Kester 1994). Hydrodynamic transport was computed using detailed bathymetry and open boundary forcing based on tidal constituents. The model was forced using meteorological data from the High Resolution Limited Area Model (KNMI 2015), which comprised 2 horizontal wind velocity components (at 10 m above mean sea level) and other atmospheric variables such as air pressure and temperature, archived every 6 h. The freshwater discharges from 18 rivers were included in the model; 7 of these discharges varied temporally (daily averages) and 11 were constant (based on long-term averages). This model is described in more detail in Erftemeijer et al. (2009).

Two grid lay-outs covering the southern North Sea (including the Wadden Sea) were used in this study: a moderately fine grid (ZUNOGROF) and a domain decomposition model grid (ZUNO-DD) with a much higher grid resolution in the Dutch coastal zone and the Wadden Sea (Figure 3).

Particle transport model

Particle tracking models (PTM) are often used in environmental modelling (e.g. North et al. 2008; Broekhuizen et al. 2011; Postma et al. 2013). Here, the Delft3D-PART module was used

to calculate the larval transport across the southern North Sea. Delft3D-PART is a random walk particle-tracking model, based on the principle that movement of dissolved substances in water can be described by a limited but potentially large number of discrete particles that are subject to advection due to currents and by horizontal and vertical dispersion. The movement of the particles in the model consists of two steps: advection, which is driven by the FLOW results, and dispersion, which is a stochastic random walk process. In addition, the horizontal and vertical movement of the particles can be adjusted to account for swimming preferences or changes in buoyancy. Particle tracking allows water quality processes to be described in a detailed spatial pattern, resolving sub-grid concentration distributions. Delft3D-PART is shown to be locally mass conservative (Postma et al. 2013).

Modelling set up

FLOW calculated the hydrodynamic conditions for the southern North Sea for two consecutive years, 2004 and 2005. These years have been chosen because they represent average climatic forcing conditions in northwest Europe. Such conditions importantly determine the geographic destination of particles such as larvae, superimposed on the regular transport conditions in the southern North Sea. For the PART model, a relatively simple set up was used to simulate the mussel larval transport. Each location acted as an origin point of larvae and the density of the larvae throughout the consecutive months at the other locations (destinations) was calculated. Larval release was started at day 59 (1 March) of each year, from the top layer in the model. It was assumed that the larvae are largely passive and that they are transported only by advection and general dispersion. They were also considered neutrally buoyant. Each origin released one million particles within a day, after which no further release of particles occurred. The program calculated the horizontal transport and vertical dispersion to lower layers with a time step of 30 minutes for 70 days. Although mortality of larvae is very high, we did not include such a process (via e.g. a decay formulae) in the PART model. The purpose of the study was to assess the chances of arrival of larvae at a specific destination, which was assumed to be proportional to the average concentration of larvae at a specific destination. These average concentrations were converted to a distance matrix (hydrodynamic distance) by inversing the maximum total arrived particles from each origin-destination combination. In case no particles arrived, the value for the combination was set to the minimum non-zero amount of arriving particles from all other combinations, to prevent infinitely large distances.
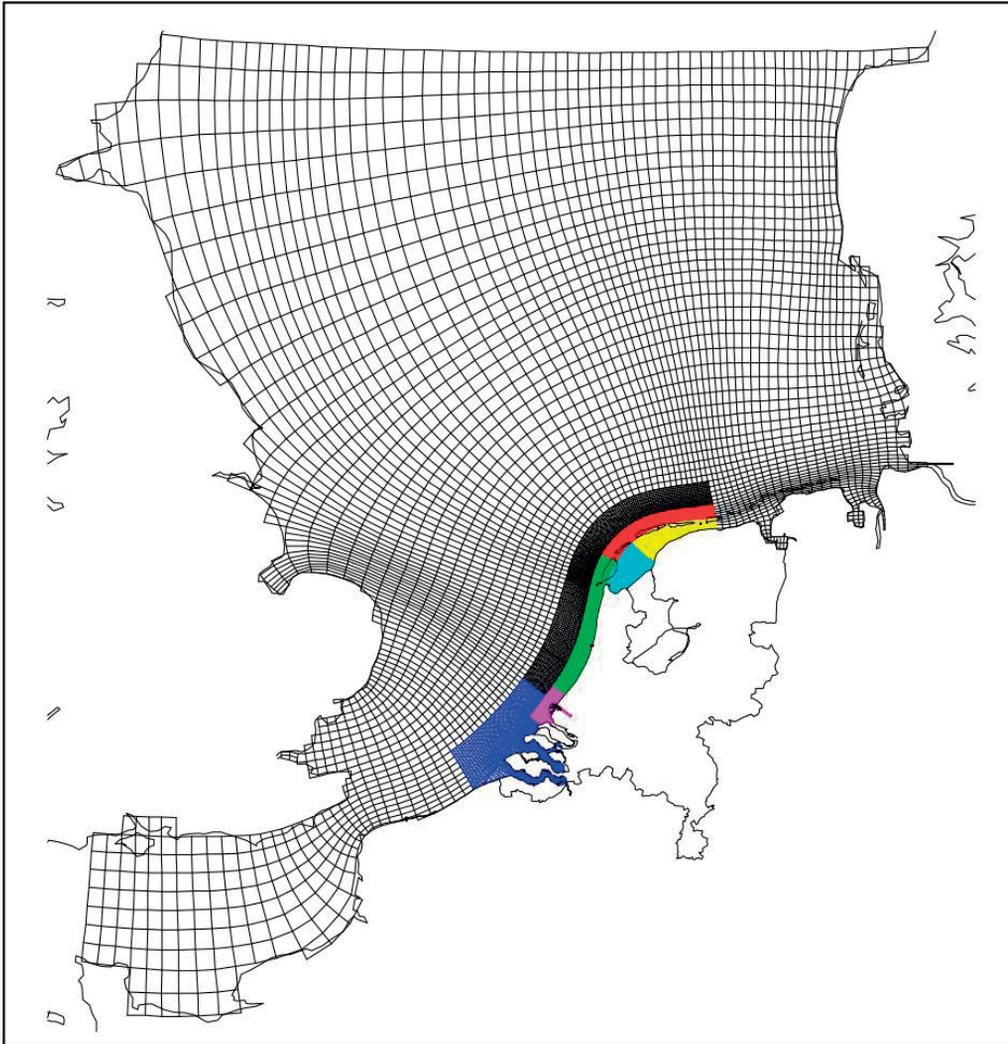
Figure 3: Particle tracking grid. Overview of the ZUNOGROF grid covering the southern North Sea (white cells), and the high resolution ZUNO DD grid in the Dutch coastal and inshore areas (coloured cells).

Data analysis

Because alleles of *Mytilus galloprovincialis* and *M. trossulus* were observed in our data at the species diagnostic locus Me1516, we estimated the hybrid index for each individual using a maximum likelihood approach that estimates the proportion of alleles inherited from one of two hybridizing species (Buerkle 2005; Buerkle and Lexer 2008). This approach was applied as implemented in the R package Introgress (Gompert and Alex Buerkle 2010). The samples from Lisbon and Limfjorden were used as parental reference samples and locus Med379 was not taken into account because of high levels of missing data for several samples. All individuals identified as non-pure *M. edulis* (h<1.0) were omitted from subsequent analyses. This was necessary to exclude possible selectively non-neutral influences on connectivity inferences.

Population-level analyses were performed using GenAlEx 6.5 (Peakall and Smouse 2006; Peakall and Smouse 2012), R (R Core Team 2015b) and Rstudio (RStudio Team 2015). Observed and expected heterogeneity were calculated with the adegenet package in R (Jombart 2008).

To visualise patterns in the pairwise F_{ST} and pairwise hydrodynamic distance, MDS ordinations (10,000 permutations and 500 tries) were created using the metaMDS function from the R vegan package (Oksanen et al. 2008) and plotted. Smoothed surfaces of the distance to the nearest coastline were added to the MDS plots with the ordisurf function (vegan package; Oksanen et al. 2008).

Our hypothesis was that the two dimensional stepping stone model, calculated with the PTM, explains variation in the genetic population differentiation. To test this, a mantel test (Mantel 1967) of the genetic differentiation matrix versus the hydrodynamic distance matrix was performed using the mantel function from the ecodist package (Goslee and Urban 2007) with 1,000,000 permutations. Explanatory values of distance to the nearest coastline and sampling depth for F_{ST} patterns, were tested with PERMANOVA using the adonis function (vegan package; Oksanen et al. 2008) with distance to coast and sample depth as predictor variables (10,000 permutations).

Results

In total, 1178 specimens of 27 locations, further called sub-populations were genotyped. Sample size varied between 24 and 67 specimens (Table 1). The number of alleles per locus varied between 3 and 69 (Table 3) with between 6 and 55% null-alleles. The resulting dataset had 23.14% null alleles. These high numbers of null alleles are in agreement with earlier published data on microsatellite analysis in marine bivalves and gastropods (Bierne et al. 2003; Carlsson 2008; Panova et al. 2008; Lemer et al. 2011).

Results from the species diagnostic locus Me1516 indicated the presence of non-pure *M. edulis* genetic material among our samples; a single *M. trossulus*-type allele (126 bp) was observed in a heterozygote from Scheveningen as well as a large number of heterozygotes and homozygotes for the *M. galloprovincialis*-type allele (126 bp; Figure 4). Because introgression in *Mytilus* spp. is pervasive, this single locus is not sufficient for detecting hybrid status of individuals, but a hybrid index is more informative. Using Lisbon (N=37) and Limfjorden samples (N=48) as reference samples in the hybrid analysis, a wide range of hybrid indices was detected among the remaining individuals (Figure 5). Omitting all non-pure *M. edulis* individuals resulted in a remaining total of 579 genotyped specimens for connectivity analysis.

Table 3. Information per locus.
From left to right: Locus name; number of alleles observed in full dataset; fraction of null alleles; observed heterozygosity (Hardy Weinberg equilibrium); expected heterozygosity; significance of difference between observed and expected heterozygosity.

Locus	n alleles	Null alleles	Obs. het.	Exp. het	P value
Med367	52	0.0662	0.7500	0.9091	<0.001
Med379	45	0.5543	0.5829	0.9475	<0.001
Med722	53	0.1316	0.5982	0.9343	<0.001
Med733	37	0.0611	0.5461	0.8686	<0.001
Me15-16	3	0.2742	0.0854	0.1563	<0.001
Med737	61	0.0671	0.8153	0.9548	<0.001
Med740	61	0.3183	0.6040	0.9594	<0.001
Med747	69	0.4100	0.5986	0.9714	<0.001
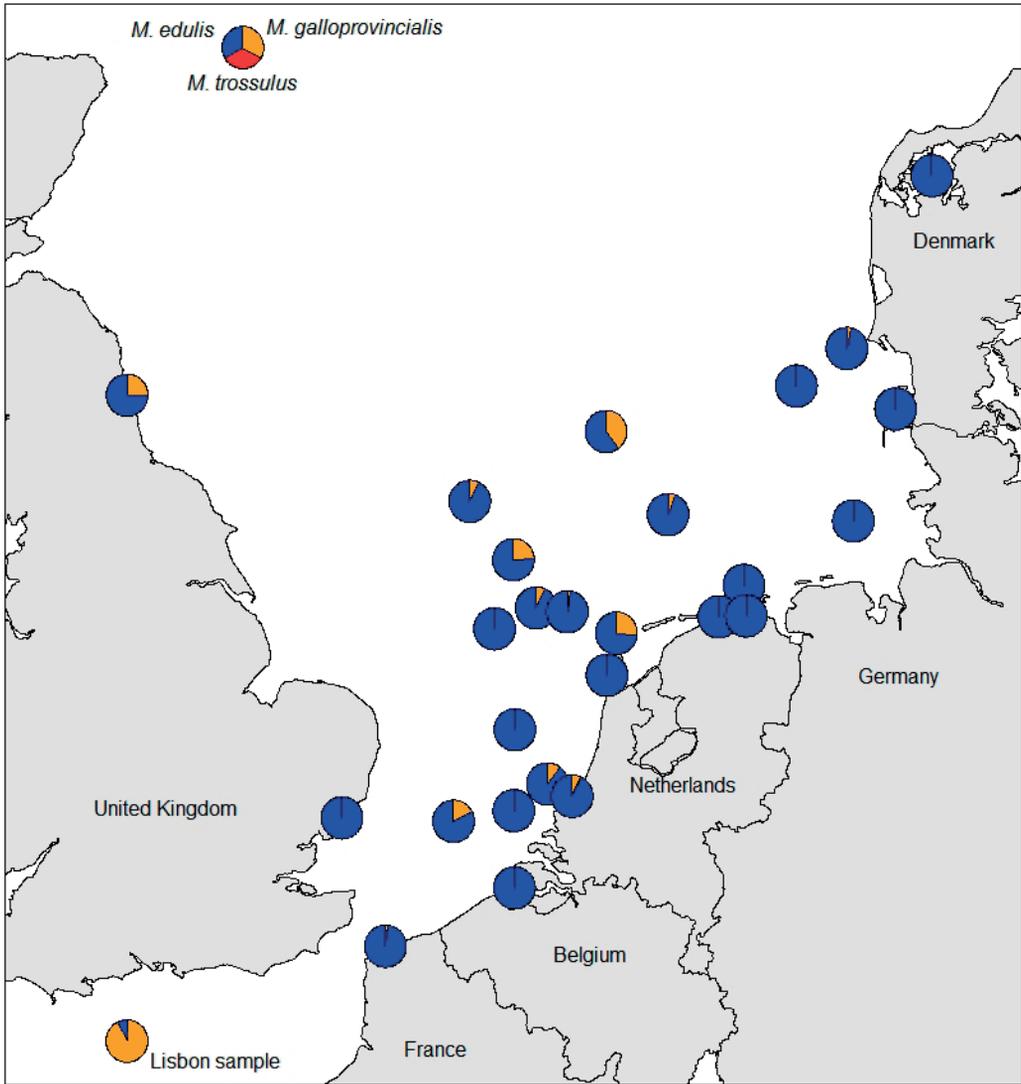
Figure 4: Distribution of species-diagnostic alleles at locus Me1516. Pie chart diagrams showing spatial distribution of species-diagnostic alleles at nuclear DNA locus Me1516. Legend (top) indicates *Mytilus edulis*-type allele (180 basepairs), *M. galloprovincialis*-type allele (126 bp) and *M. trossulus*-type allele (170 bp). *Mytilus trossulus* not visible in sample pie charts due to low abundance. Pie chart in bottom left originates from sample taken in Lisbon, Portugal.
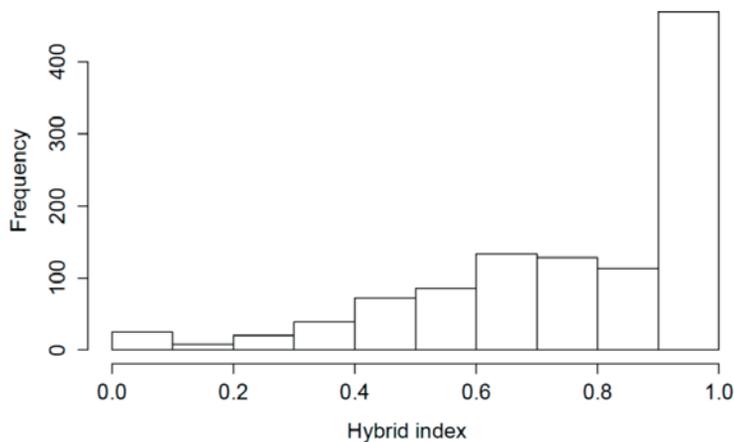
Figure 5: Frequency distribution of hybrid index. Histogram of hybrid index variation found among samples of *Mytilus* spp. In the North Sea region (N = 1093 individuals). Individuals with H-index = 1 may be considered as pure *M. edulis* as compared to reference sample from Limfjorden, Denmark; those with H-index = 0 as pure *M. galloprovincialis* compared to Lisbon, Portugal. A range of hybrid genomic backgrounds was detected, with a majority pure *M. edulis* (53%) and, among hybrids, a bias towards stronger introgression from *M. edulis*.

Genetic connectivity

Pairwise F_{ST} (Table 4) ranged between 0 and 0.16 with an average of 0.06. Average pairwise F_{ST} at locations EURW, F3-1A, Horns Rev, L15-A and Sylt were the highest with values >0.07. With values of <0.05, Calais, EUR7, FINO and Scheveningen had the lowest average pairwise F_{ST}.

Patterns in the MDS plots (figure 6) could not be fitted with a significant distance to coast surface plot (p=0.6, deviance explained 0.002%). The distance to coast effect was also tested in PERMANOVA which resulted in a non-significant effect for distance to coast depending on model order and on combinations with and without sampling depth.

Particle tracking model

Wind farm Horns Rev included two samples from separate wind turbine foundations but the PTM combined these in a single grid cell (Table 5). Data from these samples were therefore combined for further analysis. The distance matrix of origin-destination combinations was visualised by MDS plot with added surface plot for the distance to coast effect (p<0.001; deviance explained 94%; Figure 6). This showed a clear pattern from coastal sub-populations to farther offshore. Blyth was left out of the MDS plot since zero particles from other locations arrived here, resulting in a clustering of all other locations extremely far away from Blyth.

Particle tracking model vs genetic results

The mantel test showed a significant effect of pairwise predicted hydrodynamic connectivity on the pairwise F_{ST} results (p<0.012) with a mantel coefficient of 0.19. The effective travel distance for particles predicts part of the variation in F_{ST}. Location combinations without particle exchange, have a higher F_{ST} when compared to combinations with exchange (Figure 7), which is in line with the mantel test.
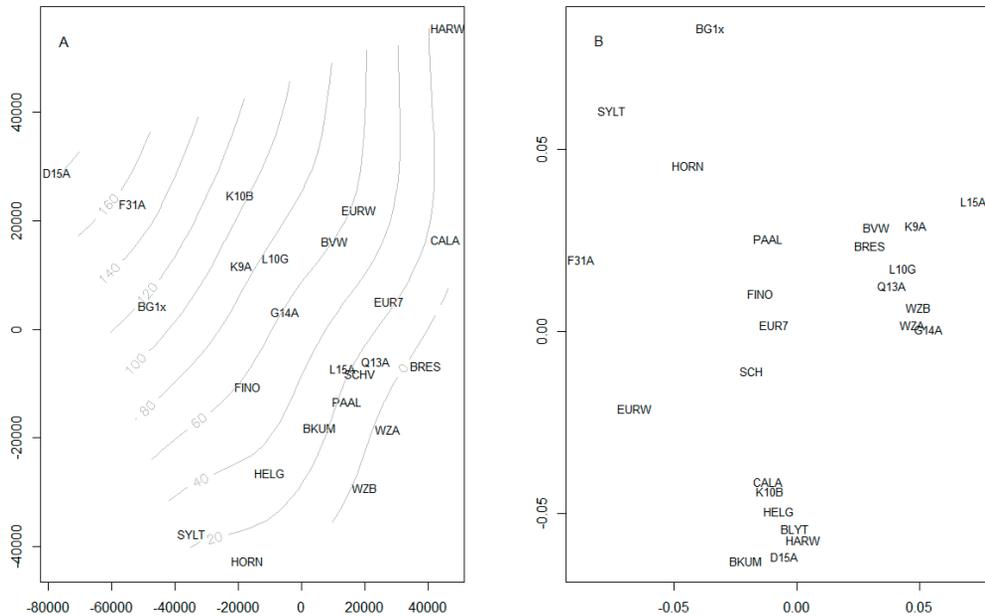
Figure 6: Multidimensional scaling plots of particle tracking and F_{ST}. A: MDS plot (stress = 0.06) of the particle tracking model results (excluding Blyth) converted to distance matrix, with surface plots of the effect of distance to coast in km (p<0.001; deviance explained 94%). B: MDS plot (stress 0.10) of the pairwise F_{ST} matrix. Text code legend see Table 1. Sub-populations shown in black, surface plot in grey.
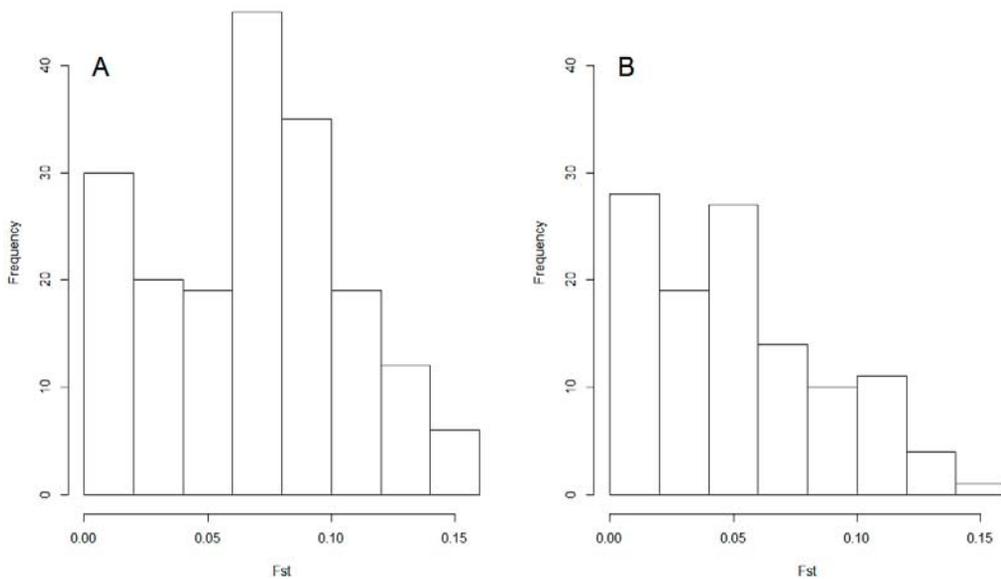
Figure 7: Histograms of pairwise F_{ST} frequencies. A: Histogram of pairwise F_{ST} frequencies of location combinations that exchange **no** particles in the particle tracking models (PTM). B: Histogram of pairwise F_{ST} frequencies of location combinations that exchange particles in the PTM.

Discussion

Our results strongly support the hypothesis that the marine stepping stone effect is present on offshore energy installations as well as other structures. Based on the particle tracking models, several offshore installations are directly connected to others but not connected directly to coastal locations: e.g. F3-1a to K9-A, K10-B to L10-G. Some locations are connected only indirectly via intermediate stepping stone locations: e.g. F3-1a with the coastal Breskens via L10-G. The modelled route travelled by particles between structures significantly explains the variation found in the genetic population structure of *M. edulis*. With a pelagic larval stage of up to 70 days and a current regime that isolates near-shore from offshore locations, *M. edulis* on offshore installations can only have colonised the locations from origin sub-populations that were also offshore. Furthermore, even though our analysis probably included specimens of several different year classes, which have likely colonised the structures under different hydrodynamic regimes, we nevertheless show a strong correlation between genetic and hydrodynamic distance.

Treml et al. (2008), who modelled connectivity of coral populations in the tropical Pacific, found similar results as us. They showed that connectivity not only depended on geographic distance, but also on the direction of currents and on the availability of stepping stones. Population genetic patterns observed in tropical neritid snails depended on intermediate stepping stones and rare long range dispersal events (Crandall et al. 2012). Crandall et al. (2012) also note that there may also be an influence of extremely long range dispersal events, but suggest that this occurs on the millennium scale, which is not relevant for installations with ages <40 years. Rare changes in wind directions also occur in the North Sea (Lapworth and McGregor 2008), making connections possible between locations that are normally isolated from each other and pushing larvae further to the west than is typical. This may underlie part of the unexplained variation in our data.

The intertidal *M. edulis* requires shallow hard substrate to survive since it is limited by predation in deeper waters (Langhamer 2010). By adding many intertidal surfaces to the North Sea, the manmade structures provide habitat for shallow water species such as *M. edulis*. Without these shallow hard substrates present, these species would be unlikely to survive in offshore North Sea waters. The increased distribution of large amounts of *M. edulis* was nick-named the 'Mytilusation' of the North Sea by Krone et al (2013). They showed that large amounts of *M. edulis* shells are exported from the intertidal parts of the installation to the surrounding bottom, and suggest this may change the local habitat structure. In samples taken on rock dump around offshore oil platforms, Coolen et al. (2017b; Chapter 6) also observed more *M. edulis* specimens than in samples from a natural rocky reef without surface reaching man-made structures nearby. They suggest that *M. edulis* and associated fauna fall off the structures and change the local benthic species composition.

Marine stepping stones also affect other species such as caprellids, although this depends on distribution strategies (Coolen et al. 2016b). For example, species that also utilise floating objects as rafts to expand their distribution, may be much less dependent on stepping stones than species with a pelagic larval stage.

The stepping stone effect was often mentioned in policy related publications, warning for a faster distribution of non-indigenous species (IPIECA 2010; Macreadie et al. 2011; Adams et al. 2014). Here, we used an indigenous species as model organism to prove the concept of marine stepping stones. It has been shown that non-indigenous species are present at offshore energy installations (De Mesel et al. 2015; Simons et al. 2016; Coolen et al. 2016b), and that they can be the most frequent group in the intertidal zone (De Mesel et al. 2015). It is likely that some of these non-indigenous species also utilise offshore energy installations and other structures as stepping stones. This will depend on reproduction and distribution strategies and seems most important for epifauna species with pelagic larval stages that last several weeks, e.g. the oyster *Ostrea edulis* which is pelagic 10-30 days (Berghahn and Ruth 2005) or the limpet *Crepidula fornicata* (Shanks 2009) with a pelagic stage of 2-3 weeks. Other species, such as the bryozoan *Celleporella hyalina* (Goldson et al. 2001), are pelagic for only a few hours and are unlikely to reach the next location downstream. Species with very long pelagic larval stages are capable of travelling large distances and may not need stepping stones to colonise distant locations (Reisser et al. 2014). This may be the case for e.g. the starfish *Asterias rubens*, which has a pelagic stage of 90 days (Barker and Nichols 1983) or *Sabellaria alveolata*, with a pelagic stage up to eight months (Wilson 1968). Some organisms, such as the amphipod *Caprella mutica* and the polychaete *Harmothoe* spp. also utilise floating objects such as ship hulls or debris (Gollash and Riemann-Zürneck 1996; Thiel and Gutow 2005), and some are transported by ballast water (Drake and Lodge 2004), enabling them to colonise distant locations. This may be the most important factor driving the invasion by some non-native species (Coolen et al. 2016b).

Considering the young age of the artificial substrates we studied, and hence also of the populations of blue mussels living on them, the level of population genetic differentiation between many of the samples is remarkably strong. Population genetic differentiation takes a number of generations in the order of the effective population size (the number of reproducing individuals in a random mating population) to develop into its final, equilibrium state. We therefore suspect that effective population sizes of *Mytilus* spp. on these man-made structures in the North Sea are relatively small. Nevertheless, the observed levels of differentiation may not yet have reached their equilibrium values and it would be advisable to carry out additional analyses that do not assume equilibrium, such as coalescent analysis of isolation-with-migration (Hey and Nielsen 2007; Sethuraman and Hey 2016). With such analyses, bidirectional migration rates can be estimated in conjunction with the timing of

population subdivision, allowing for a more detailed assessment of the stepping stone effect demonstrated here.

The long-term effects of these stepping stones remain unclear. They may increase the speed at which (future) invasions take place, making new habitats at isolated locations available for non-indigenous species in shorter time periods than they would take without marine stepping stones. However, other factors such as hull fouling and ballast water exchange also strongly affect distribution of non-indigenous species. Marine stepping stones are also important for the offshore distribution of native species, e.g. as suggested for *Caryophyllia smithii* (Coolen et al. 2015b). Most species present on offshore artificial reefs in the North Sea are native (De Mesel et al. 2015; Coolen personal observation) and some even survive on these locations outside the range of their invasive relatives (Coolen et al. 2016b). Whether species (native and non-native species alike) profit from stepping stones, depends on their life-history traits. Species with long dispersal ranges (e.g. >1,000 km) may not need stepping stones, while species that only distribute for a few km, may be unable to use stepping stones that are too far apart. For newly built installations, effects need to be placed in a the context of tens of thousands of other artificial objects already present, some of which have been there for >100 years (Coolen et al. 2015b). Additional installations only make a difference if they are placed at the borders of the current distribution potential of species. For example, when placed between reefs that are currently not connected but become connected after the new installation is placed.

The marine stepping stone effect is important for the distribution of *Mytilus edulis* and it may influence the distribution of other species with similar strategies in a similar way. The presence of offshore energy installations and other objects is the only way species such as *M. edulis* are able to survive at deeper offshore locations.

Acknowledgments

Table 4. Pairwise F_ST. Pairwise F_{ST} for all origin-destination combinations of sub-populations.

	BG1x	BKUM	BLYT	BRES	BVW	CALA	D15A	EUR7	EURW	F31A	FINO	G14A	HARW	HELG	HORN	K10B	K9A	L10G	L15A	PAAL	Q13A	SCH	SYLT	WZA
BKUM	0.16																							
BLYT	0.14	0																						
BRES	0.077	0.097	0.08																					
BVW	0.072	0.107	0.084	0																				
CALA	0.113	0	0	0.071	0.07																			
D15A	0.15	0.008	0	0.084	0.09	0.005																		
EUR7	0.075	0.037	0.031	0.03	0.038	0.029	0.035																	
EURW	0.088	0.046	0.057	0.103	0.109	0.042	0.063	0.044																
F31A	0.036	0.12	0.116	0.093	0.096	0.082	0.125	0.08	0.055															
FINO	0.034	0.055	0.049	0.021	0.025	0.028	0.044	0.01	0.035	0.035														
G14A	0.114	0.095	0.069	0.009	0.015	0.073	0.079	0.041	0.116	0.128	0.051													
HARW	0.134	0	0	0.085	0.083	0.002	0.013	0.042	0.066	0.103	0.052	0.075												
HELG	0.125	0.007	0	0.072	0.077	0	0	0.029	0.051	0.098	0.036	0.072	0.005											
HORN	0.047	0.095	0.098	0.069	0.074	0.082	0.111	0.03	0.036	0.064	0.028	0.097	0.108	0.091										
K10B	0.117	0.013	0.01	0.068	0.069	0	0.011	0.041	0.05	0.081	0.03	0.07	0.008	0.006	0.092									
K9A	0.108	0.092	0.084	0.021	0.02	0.068	0.089	0.038	0.117	0.125	0.047	0.022	0.082	0.085	0.082	0.073								
L10G	0.097	0.1	0.08	0.009	0.006	0.076	0.082	0.033	0.115	0.122	0.034	0.006	0.079	0.075	0.086	0.068	0.01							
L15A	0.123	0.109	0.093	0.045	0.048	0.088	0.107	0.055	0.134	0.149	0.075	0.035	0.087	0.096	0.105	0.097	0.013	0.035						
PAAL	0.063	0.064	0.061	0.042	0.044	0.051	0.076	0.018	0.051	0.099	0.017	0.055	0.076	0.066	0.017	0.067	0.041	0.046	0.057					
Q13A	0.088	0.087	0.08	0	0.007	0.059	0.085	0.04	0.114	0.098	0.036	0.01	0.077	0.076	0.084	0.062	0.016	0.012	0.037	0.051				
SCH	0.057	0.016	0.019	0.044	0.053	0.001	0.031	0.007	0.021	0.061	0.008	0.058	0.029	0.018	0.031	0.023	0.056	0.059	0.066	0.014	0.046			
SYLT	0.079	0.134	0.141	0.085	0.103	0.115	0.129	0.059	0.071	0.073	0.027	0.141	0.143	0.109	0.044	0.112	0.118	0.105	0.153	0.072	0.118	0.069		
WZA	0.122	0.086	0.06	0.002	0.007	0.064	0.072	0.029	0.119	0.14	0.043	0	0.072	0.066	0.091	0.065	0.018	0.009	0.032	0.046	0.003	0.045	0.127	
WZB	0.107	0.095	0.074	0	0	0.066	0.081	0.047	0.125	0.11	0.04	0.003	0.072	0.065	0.103	0.062	0.023	0.006	0.045	0.064	0.003	0.059	0.132	0.006

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Table 5. Pairwise particle arrivals; Total arriving particles, pairwise combinations of all locations in both directions

	BG1x	BKUM	BLYT	BRES	BVW	CALA	D15A	EUR7	EURW	F31A	FINO	G14A	HARW	HELG	HORN	K10B	K9A	L10G	L15A	LIMF	LISB	PAAL	Q13A	SCHV	SYLT	WZA	WZB
BG1x	-	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	1.64998	0.00256	0.34048	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
BKUM	0.00000	-	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.02722	0.00000	0.00002	2.98911	0.00591	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
BLYT	0.00000	0.00000	-	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
BRES	0.00000	0.00000	0.00000	-	0.00000	0.00000	0.00000	0.31183	0.00054	0.00000	0.00000	0.00073	0.00000	0.00000	0.00000	0.00000	0.00000	0.00006	0.03215	0.00000	0.00000	0.02764	0.96046	0.18393	0.00000	0.00000	0.00000
BVW	0.00000	0.00006	0.00000	0.00000	-	0.00000	0.00000	0.00212	0.00000	0.00000	0.00000	0.65889	0.00000	0.00000	0.00000	0.00000	0.00020	0.04135	0.53143	0.00000	0.00000	0.01969	0.03350	0.00072	0.00000	0.00785	0.01220
CALA	0.00000	0.20374	0.00000	0.28248	0.00004	-	0.00000	0.60271	0.08981	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
D15A	0.00035	0.00000	0.00000	0.00000	0.00000	0.00000	-	0.00000	0.00000	0.67355	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00156
EUR7	0.00000	0.01060	0.00000	0.16461	0.00000	0.00000	0.00000	-	0.00000	0.00000	0.00000	0.09126	0.00000	0.00000	0.00000	0.00000	0.00000	0.00134	2.03278	0.00000	0.00000	1.90475	8.83463	3.29385	0.00000	0.00012	0.00000
EURW	0.00000	0.00000	0.00000	0.00000	6.42049	0.00000	0.00000	2.13840	-	0.00000	0.00000	0.03047	0.00000	0.00000	0.00000	0.00000	0.00000	0.06545	0.94831	0.00000	0.00000	0.01271	0.00570	0.00029	0.00000	0.00000	0.00000
F31A	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	-	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00381	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
FINO	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	-	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
G14A	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.59023	-	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
HARW	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00006	0.00000	0.00000	0.00000	-	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
HELG	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.53847	0.00000	0.00000	-	1.49818	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.07020	0.00000	0.00000
HORN	0.00000	0.00196	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00037	0.00000	0.00000	-	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
K10B	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00314	0.00270	0.27075	0.00000	0.00000	0.00000	-	0.58461	0.69424	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
K9A	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00260	0.08849	1.39962	0.00000	0.00065	0.00000	0.00000	-	4.96122	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
L10G	0.00000	0.00001	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00018	0.14231	0.92690	0.00000	0.00207	0.00000	0.00000	1.51639	-	0.00000	0.00000	0.00000	0.00074	0.00000	0.00000	0.00000	0.00000	0.00000
L15A	0.00000	4.37763	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.04326	0.03773	0.00000	0.14377	0.00000	0.00000	0.00000	0.00000	-	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	3.20499	2.23325
LIMF	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	-	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
LISB	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	-	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
PAAL	0.00000	2.92663	0.00000	0.00000	0.00000	0.00000	0.00000	0.00001	0.00000	0.00000	0.02111	0.08584	0.00000	0.04194	0.00000	0.00000	0.00000	0.00003	4.93725	0.00000	0.00000	-	4.53012	0.00000	0.00000	1.46618	0.00000
Q13A	0.00000	0.44771	0.00000	0.00000	0.00000	0.00000	0.00000	0.00001	0.00000	0.00000	0.00009	0.28072	0.00000	0.00000	0.00000	0.00000	0.00000	0.01370	3.74662	0.00000	0.00000	4.53012	-	4.50240	0.00000	0.04280	0.06165
SCHV	0.00000	0.86907	0.00000	0.00000	0.00000	0.00000	0.00000	0.00016	0.00000	0.00000	0.00022	0.21019	0.00000	0.00004	0.00000	0.00000	0.00000	0.00262	3.96498	0.00000	0.00000	5.69139	7.61096	-	0.00000	0.11624	0.11913
SYLT	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.03126	0.00000	0.00000	0.00000	1.37214	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	-	0.00000	0.00000
WZA	0.00000	1.45874	0.00000	80162	0.00000	0.00000	0.00000	0.11334	0.00021	0.00000	0.00000	0.00052	0.00000	0.00003	0.00000	0.00000	0.00000	0.00005	0.02094	0.00000	0.00000	0.01670	0.49728	0.11308	0.00000	-	615.73
WZB	0.00000	1.74085	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00558	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	1920	-

Chapter 6:

BENTHIC BIODIVERSITY ON OLD PLATFORMS, YOUNG WIND FARMS AND ROCKY REEFS

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Abstract

The introduction of artificial hard substrates in an area dominated by a sandy seabed increases habitat available to epifouling organisms. Many biotic and abiotic variables influence the presence of epifouling species. Samples were taken on old offshore oil and gas platforms, and data were compared with a young wind farm and a natural reef on the Dutch Continental Shelf, in order to evaluate the influence of depth, age, orientation, substrate type and the presence of potential keystone species *Asterias rubens*, *Mytilus edulis*, *Psammechinus miliaris* and *Metridium dianthus* on species richness and species composition. Species richness varied strongly between natural and artificial reefs. The number of observed species was 127 on platforms, 90 on the wind farm and 50 on the Borkum Reef Ground rocks. The predicted number of species on platforms (173) was 36% higher than the observed number, while for the natural reefs it was 56% higher (78) and for the wind farm it was almost identical (93). Depth (p<0.001), presence of *M. edulis* (p<0.001), *P. miliaris* (p=0.005) and orientation (p=0.004) all had a significant effect on species richness. Multivariate analysis showed a large overlap in communities on steel and rock, between different surface orientations and between the wind farm and platforms. There was no overlap between communities on the relatively young rocks at the wind farm and natural reefs, but rocks around platforms and the natural reef showed overlap. Depth differences, location effects and substrate type influenced the species composition most strongly. Substrates with mixed surface orientation (i.a. rocks) hold the most species-rich communities. When an artificial reef is supposed to be colonised by communities similar to those on a natural reef, its structure should resemble a natural reef as much as possible.

Introduction

The introduction of artificial hard substrates in areas dominated by a sandy seabed increases habitat available to epifouling organisms (Gill 2005; Wilson and Elliott 2009; Langhamer 2012). As a result of the increasing number of offshore energy devices throughout our seas and oceans, the amount of available artificial hard substrate increases steadily. The European Wind Energy Association estimates that the North Sea will hold up to 62 GW of offshore wind energy installations in 2030 (EWEA 2015b). Assuming 5 MW turbines, this sums up to 12,400 turbine foundations, all providing artificial hard substrates to epifouling organisms.

Oil and gas companies have been installing platforms in the North Sea since the 1960s (Shepherd 2015) and expanded to the Dutch Continental Shelf (DCS) from the early '70s onwards (EBN 2014). The oldest North Sea offshore wind farm was constructed in 2002 (Leonhard and Christensen 2006). Therefore, offshore wind development is still a young industry. Most oil and gas platforms in the North Sea were constructed using steel jacket foundations, which structurally resemble offshore wind turbine foundations, offering similar substrate to fouling organisms. It is therefore expected that offshore wind farms and oil and gas platforms house similar benthic communities if all other variables are constant. However, other variables often differ between locations. For example, fouling communities on offshore installations evolve over time with dominance changing between species (Whomersley and Picken 2003). Furthermore, species richness may increase with installation age (van der Stap et al. 2016). Age differences between offshore wind farms and oil and gas installations offer an opportunity to study the effect of substrate aging on epifouling communities.

While in recent years the number of offshore wind farms in the North Sea has been increasing, a large-scale removal of offshore oil and gas installations has set in. Many oil and gas platforms are reaching their financial or technical end-of-life and will be decommissioned over the next years (Royal Academy of Engineering 2013). To prevent high removal costs, alternative uses are proposed (Day and Gusmitta 2016), including so called Rigs-To-Reefs (RTR) schemes, in which platform foundations may be left at sea as artificial reefs, either in place, toppled over, or after relocation (Picken and McIntyre 1989; Macreadie et al. 2012). Similar proposals (named Renewables-To-Reefs; RTR) may be expected once offshore wind farms reach their end-of-life (Smyth et al. 2015; Fowler et al. 2015b). To evaluate the ecological potential of both RTR types, more insight is needed into the current state of the epifouling communities present. Nowadays, natural reefs are present in the North Sea in the form of biogenic reefs, e.g. *M. edulis* beds in the Wadden Sea, but offshore in the Netherlands they are composed solely of geogenic reefs, such as rocky and gravel reefs (Coolen et al. 2016b). Since these natural reefs are protected under the European Habitat Directive (European Commission 1992), benthic communities present on rocky reefs may be of use to assess whether species on artificial reefs are desirable. This insight

could then be used in the evaluation of future RTR proposals or in impact assessments for new installations. To evaluate the added value, if any, of these structures to the North Sea ecosystem structure and more importantly, functioning, the following questions should be answered: what organisms have colonised these artificial structures, what characteristics of these structures influence the colonisation, what are the habitat preferences of the species currently found on these structures, are the communities found similar to those on natural reefs and are some habitats in particular suitable for non-native species?

In recent years, there is an increasing call from environmental organisations and national governments to use construction methods and materials that consider benefits for nature (Waterman 2010; van Slobbe et al. 2013). Examples are the large-scale 'Building with Nature' projects including coastal protection with a mega sand nourishment using natural processes to transport sand (Stive et al. 2013), and ecosystem-based sand extraction management (de Jong et al. 2016). Recently, van Duren et al. (2016) proposed to improve the effect offshore installations have on benthic biodiversity. One way to do this may be by modifying the available substrate structure to attract species communities that are similar to those on natural reefs. To do this, further insight in the variables influencing species presence on artificial and natural reefs is needed.

Many variables influence the presence of a species, as is known for soft bottom benthos (Reiss et al. 2014), but even for closely related taxa on artificial reefs they are not equally relevant for each species (Coolen et al. 2016b). Depth, for example, is widely known to influence the vertical distribution of species on artificial reefs; some species are present only on deeper reefs (Coolen et al. 2016b). Furthermore, the highest species richness can be found at intermediate depths, e.g. around 15 meters on gas platforms with a maximum depth of 30 meters (van der Stap et al. 2016). Similar patterns have been observed on coral reefs (Cornell and Karlson 2000) and for bryozoans in the North Atlantic (Clarke and Lidgard 2000). Substrate orientation (horizontal vs. vertical) also has a significant effect on species richness and composition (Knott et al. 2004). Furthermore, species richness increases with age of artificial structures (van der Stap et al. 2016). Comparisons between natural and artificial reefs in tropical waters have demonstrated that differences between these reefs diminish with age (Perkol-Finkel et al. 2005). The location of the community under study can greatly influence the presence of species, since turbidity, maximum depth, water temperature and salinity vary among locations (Coolen et al. 2016b).

Structural differences between reefs also explain part of the variation in the associated community (Coolen et al. 2015a). Straight steel surfaces are different from rocky reefs, with a more complex surface area including holes and small-scale variation in surface orientation. Therefore, it is hypothesised that species richness of benthic communities is higher on rock than on smooth steel surfaces.

Relations among species also influence species composition. Keystone species are defined as species that have disproportionally large effects on their environment (Paine 1969). Several types of keystone species have been defined, e.g. by Mills et al. (1993), and several species present on artificial reefs may fit these types. Species such as the starfish *Asterias rubens* predating on the mussel *M. edulis* (Saier 2001) could fulfil a role as keystone predator. In low numbers, such predators can increase local species richness by creating patchiness in the fauna cover, allowing new species to colonise the substrate (Menge and Sutherland 1967). On their turn, mussels increase habitat heterogeneity resulting in increased species richness (Drent and Dekker 2013; De Mesel et al. 2015). *Mytilus edulis* could therefore be considered a keystone modifier. *Asterias rubens* may also have an indirect negative effect on species richness when consuming high numbers of *M. edulis*. At intermediate levels of non-human disturbance biodiversity is known to be highest (Townsend et al. 1997; Lenz et al. 2004; Svensson et al. 2007). We expect that species richness on reefs in the North Sea is influenced in a similar manner by the presence of *Psammechinus miliaris*, an omnivorous sea urchin that removes marine growth from artificial structures (personal observations J.W.P. Coolen, G. van Moorsel, Hughes 2006), potentially functioning as a keystone predator.

Metridium dianthus is known to smother and thereby kill other species (Kaplan 1984; Nelson and Craig 2011). Coolen et al. (2015) noticed that samples dominated by *M. dianthus* had less species than samples without this sea anemone. Whomersley and Picken (2003) reported *M. dianthus* to first colonise deeper parts of oil and gas platforms and then expand its cover to shallower parts, meanwhile outcompeting hydroids and soft corals. Therefore it is hypothesised that *M. dianthus* has a negative influence on species richness through space monopolisation, functioning as a keystone modifier.

The effects of most of these variables have already been evaluated to some extent in previous research. However, these assessments only included a small amount of variables, e.g. distance to coast, age and depth on platforms (van der Stap et al. 2016) or orientation on concrete artificial reefs in southern Portugal (Moura et al. 2008). Therefore we aimed to assess the combined effect of these variables. Using samples taken on old offshore oil and gas platforms (22-40 years of age), a young wind farm (5 years) and a natural reef on the Dutch continental shelf, we evaluated the effect of depth, age, orientation, substrate type and the presence/absence of suspected keystone species on species richness and composition.

Methods

Study area
Samples were taken on natural and artificial reefs on the Dutch continental shelf (DCS) in the North Sea. Most of the DCS seabed consists of fine mud and fine to coarse sand (European Biodiversity data centre (BDC) 2016), interrupted by isolated gravel beds on the Cleaver Bank, Texel Rough, Dogger Bank and Borkum Reef Grounds (Duineveld et al. 1991; EMODnet 2015; Coolen et al. 2016b; Figure 1).

Many artificial structures are present on the DCS: 10,000 wreck locations (Leewis et al. 2000; Lettens 2015), although only 1,000 of these are known as large objects (probably >10 m length; van der Weide 2008). Furthermore, 160 oil and gas production installations (Noordzeeloket 2016), 289 wind turbine foundations (4C Offshore 2016) and hundreds of buoys (Ministry of Infrastructure and Environment - Directorate-General Rijkswaterstaat 2015) are present offshore on the DCS (Figure 2).

Platform data
Four oil and gas production platforms, operated by ENGIE Exploration & Production Nederland B.V. (ENGIE) were sampled in 2014 (Figure 1). The platforms were deployed in the period 1972-1992 and are supported by steel jacket foundations with cathodic protection with anodes. The seabed surrounding these installations is between 22 and 32 meters deep (Table 1) and has been covered with rocks of between 5 and 50 cm, to prevent scouring of the sediment around the legs (rock dump, also known as scour protection).

Sampling methods
Platform samples were taken by a diver using a surface supplied airlift sampler constructed of 50 and 75 mm standard PVC pipes. A 0.05 m^2 metal frame was attached to the structure using magnets and all fauna within the frame was scraped off using a putty knife. Removed fauna was then sucked up by the airlift sampler and collected in a net with a 0.5 mm mesh size. In between sampling, nets were replaced and sealed with a PVC screw lid. Three replicate samples were taken at five-meter depth intervals. When rock dump or gravel was present at the seabed around the installations, approximately 0.05 m^2 gravel or rocks was collected by hand from a metal frame and placed in zip lock bags.

Samples were pre-processed on board. Samples were removed from the nets using running seawater, placed in 2.5 litre containers and covered with fresh seawater. An oversaturated menthol solution was added and the samples were stored at 4°C for a minimum of five hours. Next borax-buffered formaldehyde was added together with fresh seawater to reach a final concentration of 6% formaldehyde. Within seven days after sampling, all samples

were rinsed to minimise formaldehyde residue and placed on ethanol 70% with 3% glycerol for long-term storage.

All platform samples were sorted into taxonomic groups. All taxa present in each sample were then identified to the lowest taxonomic level possible, preferably species. The World Register of Marine Species (WoRMS Editorial Board 2016) was used as a reference of taxonomic nomenclature.
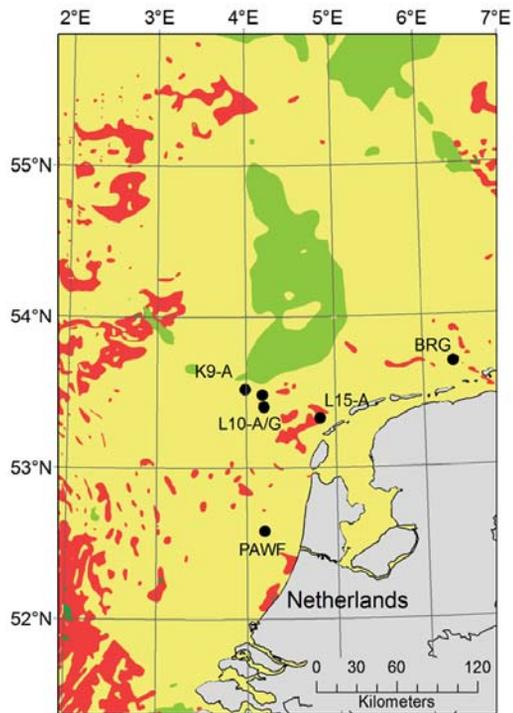
Figure 1: Habitat map of the Dutch continental shelf (black line). Red: coarse sediment; Yellow: sand; Green: muddy. Habitat data based on EMODnet data using simplified EUNIS classes (EMODnet 2015; European Biodiversity data centre (BDC) 2016). With sample locations as black dots: BRG=Borkum Reef Grounds, PAWF = Princess Amalia Wind Farm, K9-A, L10-A/G and L15-A = oil and gas production platforms.
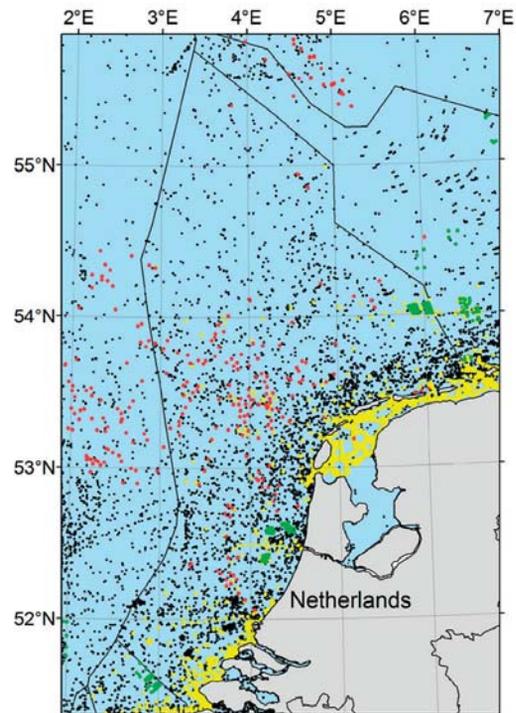
Figure 2: Shipwrecks (black dots), platforms (red), wind farms (green), buoys (yellow) present on Dutch continental shelf (outlined with black line).

Table 1. Locations of samples with maximum and minimum sampling depth, maximum age, location coordinates (decimal degrees, WGS1984) and substrate type sampled. Coordinates of PAWF are average of all samples.

Location	Max depth	Min Depth	Max age	Coordinates N	Coordinates E	Substrate sampled
L10-A	25	0	40	54.85	4.73	Steel/rock
L10-G	26	0	30	53.49	4.20	Steel/rock
L15-A	22	22	22	53.33	4.83	Rock
K9-A	32	0	27	53.52	3.99	Steel/rock
PAWF	25	0	5	52.60	4.20	Steel/rock
BRG	28	28	-	53.69	6.34	Rock

Additional data

For comparison of platform data with natural reefs, unpublished raw data were acquired from the study of the benthic diversity of the rocky reefs at the Borkum Reef grounds (BRG; Coolen et al. 2015). For a comparison with younger installations, raw data from the study of wind turbine foundations at the Princess Amalia Wind Farm (PAWF) were used (Vanagt et al. 2013; Vanagt and Faasse 2014). The BRG were sampled using identical equipment and methods as used in our platform study. At the PAWF, 0.056 m^2 samples were taken using a putty knife and collected in 0.25 mm mesh size nets. Taxonomy of the BRG and PAWF data sets was synchronised with the platform data using the Worms AphiaID service (WoRMS 2015) and combined with the platform data in a single dataset.

For each sample, location name, depth (m), substrate type (steel/rock), orientation of the substrate (horizontal/vertical/mixed) and age (years; only artificial structures) were registered (Table 1). Age was considered to be either the number of years since construction, or when cleaning of the construction had taken place after construction: the number of years since colonisation of the substrate. Offshore operators regularly remove epifouling to reduce wave load on the structure, depending on thickness of encrusting organisms and mussel thickness. The platforms under study here had been cleaned in different years to <10 m depth (personal communication E. Schmidt, ENGIE).

Data analysis

Data for all species were combined into a single dataset. Samples of the L15-A platform were only taken on rock dump at a depth of 22 m. All observations were converted to presence - absence data to compensate for differences in sampling methods and data formats among projects. All taxa above species level were removed from the data set, as well as algae and copepods, since these were not registered in some studies. For data analysis, R version 3.3.0 (R Core Team 2016) and RStudio version 0.99.486 (RStudio Team 2015) were used. Both univariate and multivariate analyses were performed.

Univariate analysis

For the univariate analysis, species richness (S; number of unique species) was calculated for each sample and used as response variable. The protocol by Zuur et al. (2010) was used for data exploration. The presence of outliers, multi-collinearity, and relations between species richness, other biotic, and abiotic variables was assessed using boxplots, Cleveland dotplots (Cleveland 1985), pairplots, Pearson correlation coefficients, variance inflation factors, and multi-panel scatterplots from the lattice package (Sarkar 2008). Univariate models were prepared using selections of the dataset, depending on the hypotheses to be tested.

Depth is known to have a non-linear relation with species richness (van der Stap et al. 2016). To allow for easy calculation of such relations, all models were constructed using Generalised Additive Models (GAM). As species richness only results in positive numbers and our exploratory model residuals using a Poisson distribution were overdispersed, a negative binomial distribution with log link were used, applying the gam function from the mgcv package (Wood 2011). Random location effects were included in the GAM to remove dependency among samples, viz. multiple samples taken at a single platform. Depth was included as a smoothed non-linear variable and location was used as a smoothed random effect. The maximum number of knots of the depth smoother was set to four to reduce overfitting of the smoother and to allow for easier visual interpretation.

Backward model selection using Akaike Information Criterion (AIC, Akaike 1973) was applied to the full models and in every selection step, the least significant variable was removed, after which the AIC was calculated for the new model and compared to the AIC of the previous model. Then the model with the lowest AIC was chosen for the next step, unless AIC between models differed <2, then the simpler model was used for the next step. These steps were repeated until all variables in the model were significant.

The final models were validated to assess if underlying assumptions of homogeneity of variance and normality of the residuals were met. Model residuals were plotted against all variables in and outside the model as well as fitted values to assess model fit.

The first model was based on the complete dataset. Since age is unknown for the natural reefs, this variable was excluded. The model included all other measured variables and all species of interest (*A. rubens*, *P. miliaris*, *M. dianthus*, and *M. edulis*), resulting in the following form:

$ln(S) = \alpha + f(depth_i) + \beta_1 * orientation_i + \beta_2 * substrate_i + \beta_3 * M.dianthus_i + \beta_4 * M.edulis_i + \beta_5 * P.miliaris_i + \beta_6 * A.rubens_i + \varepsilon_i$

Where S is the species richness for sample *i*. Term f(depth) is a smoothing function for depth (m), orientation is horizontal, vertical or mixed (for small rocks with all orientations present), substrate is rock or steel, species are included as present or absent. The residuals ε_i were assumed to be normally distributed with a mean of 0 and variance of σ.

The second model was created to assess the effect of age, by using only data for which age was known: samples taken from steel at the platforms and the PAWF. This model took the form shown above, with the addition of age as variable.

The total predicted number of species per structure type (platforms, PAWF, BRG) was estimated with the specpool function (vegan package, Oksanen et al. 2008) using the Chao estimate (Chao 1987). Species accumulation curves were created using the specaccum function (vegan package).

Multivariate analysis

For the multivariate analysis, the raw dataset with species presence-absence per sample were used. A dissimilarity matrix (Jaccard dissimilarity index; Jaccard 1901) was created by non-metric multidimensional scaling (MDS) using the function metaMDS from the vegan package (Oksanen et al. 2008) with a minimum of 100 tries. The number of dimensions needed for the MDS was assessed using scree plots (Cattell 1966). The goodness of fit of the MDS was evaluated using a Shepard plot (Shepard et al. 1972). In plots of the dissimilarity matrix, clustering of samples according to location group, substrate type and orientation were visualised using the ordiellipse function (vegan package).

A PERMANOVA (100,000 permutations, method Jaccard; Anderson 2001) was applied to assess the effect of the same variables as used for the univariate analysis, including location as additional factor variable, using the adonis function from the vegan package (Oksanen et al. 2008). Using a MDS plot, the multivariate spread among groups (structure type) was assessed, since PERMANOVA is sensitive to differences in multivariate spread (Anderson 2005).

Results

The complete dataset used for modelling consisted of 169 samples with a total of 179 unique species, from 6 locations (Table 2). The data used to model the effect of age contained 136 samples (all samples excluding BRG), with 129 species, from 5 locations. In total, 127 species (n=66 samples) were observed on platforms, 90 species (n=92) on the foundations located in the PAWF, and 50 species (n=11) on the BRG. Species richness varied strongly between locations and surface orientations (Figure 3). The total predicted species richness for platforms (S=173) was almost double that of the PAWF (S=93) and more than double that of the BRG (S=78; Table 2, Figure 4).

Table 2. Observed and predicted species richness for the three structure types, with minimum number of species per sample (S_{min}), maximum (S_{max}), average (S_{mean}) with standard deviation (S_{SD}), number of samples (n), total species richness (S_{total}), and predicted species richness (Chao) with standard error ($Chao_{SE}$).

Type	S_{min}	S_{max}	S_{mean}	S_{SD}	n	S_{total}	Chao	$Chao_{SE}$
Oil and gas platforms	6	39	16.5	7.9	66	127	172.6	20.6
Princess Amalia Wind Farm (PAWF)	2	29	16.4	6.3	92	90	93.3	3.5
Borkum Reef Grounds (BRG)	2	26	10.2	8.7	11	50	77.9	14.8

The number of species was higher on oil and gas platforms than on the offshore wind farm PAWF, but the average richness per sample was almost identical (Table 2). In these samples, the following species were each present in at least 50% of the samples: *Jassa herdmani*, *M. edulis*, *M. dianthus*, *Stenothoe monoculoides*, *Electra pilosa*, and *Pisidia longicornis*. Of these common steel species, *M. dianthus* and *E. pilosa* were also present in >50% of the samples of BRG rocks and rock dump around artificial structures, whereas *J. herdmani* and *S. monoculoides* were common on rock dump, but never observed in BRG rock samples. *M. edulis* was only found in 27% of the rock dump samples, with a single observation on the BRG rocks. *P. longicornis* was found in 41% of the rock dump samples but never on rocks of the BRG.
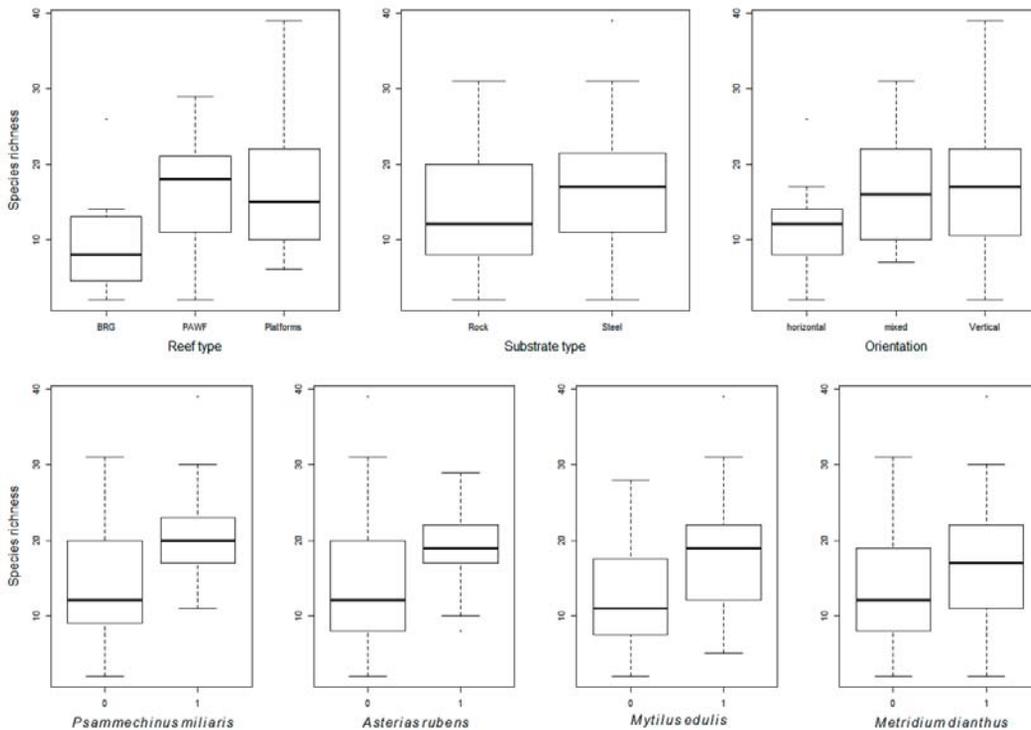
Figure 3: Boxplots of observed species richness (169 samples; 179 species). Samples grouped per reef type, substrate type, orientation and by absence(0)/presence (1) of key species. BRG = Borkum Reef grounds, PAWF = Princess Amalia Wind Farm.
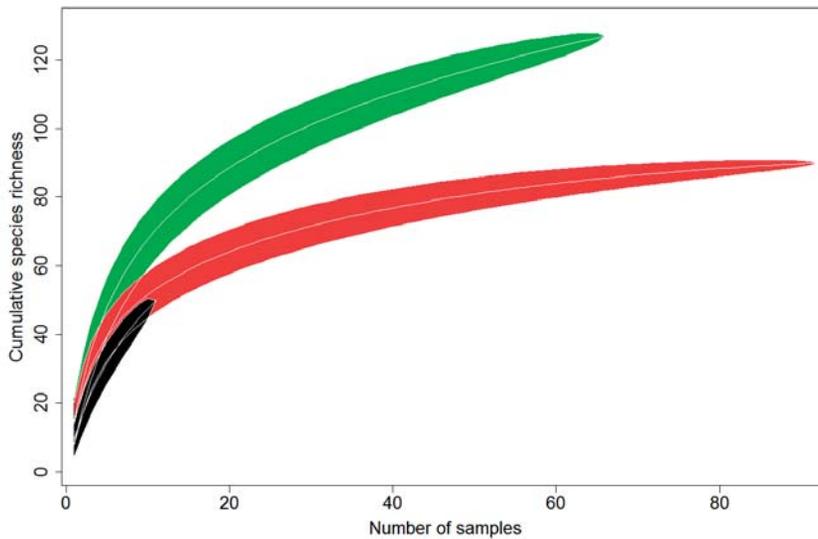
Figure 4: Species accumulation curves for the Borkum Reef Grounds (black), platforms (green) and the PAWF (red), with confidence intervals.

Univariate analysis

After model selection with the complete dataset, depth (p<0.001), presence of *M. edulis* (p<0.001), *P. miliaris* (p=0.005) and orientation (p=0.004) were included in the GAM. Depth was included as a smoothing term, other variables as factor terms. Deviance explained by the model was 36.6%. The final model took the following form, with a base model using factors orientation = horizontal, *M. edulis* = absent and *P. miliaris* = absent.

$$ln(S) = 2.23 + f(Depth) + 0.42*Orientation_{mixed} + 0.11*Orientation_{vertical} + 0.36*M.edulis + 0.21*P.miliaris$$

The non-linear relation between depth and species richness for a typical steel structure is visualised in Figure 5. In general, the model shows the highest species richness on substrates of mixed orientation (always rocky substrates), with presence of *P. miliaris* and *M. edulis*. Model validation showed homogeneity and normal distribution of the residuals, with no strong unwanted patterns.

After model selection using only samples taken on steel, depth (p<0.001), presence of *M. edulis* (p<0.001), *P. miliaris* (p=0.014) and surface orientation (p=0.047) were included in the GAM. Model validation showed homogeneity and normal distribution of the residuals, with no strong unwanted patterns. Age was removed in the last step of model selection using AIC as it explained only 0.3% of the data and had a non-significant effect (p>0.5). Within the model that included age, species richness increased with $e^{0.001}$ with each year, lower than any of the other variables in the model, even for the maximum age of 40 years. Since age was removed from the model, the final model is not represented here.
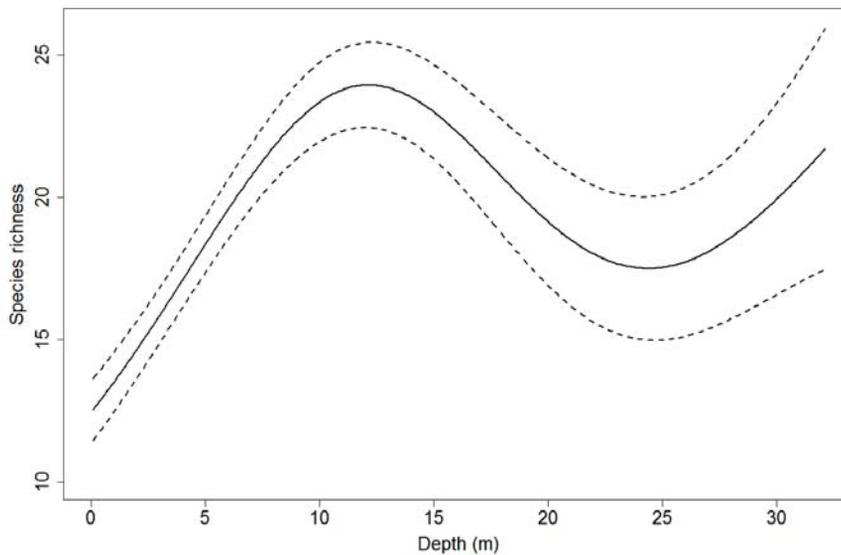
Figure 5: Modelled relation between depth and species richness per sample (solid line) with standard error (dashed lines). Factor variables in the model set at steel substrates in vertical orientation, and presence of *Mytilus edulis* and *Psammechinus miliaris*.

Multivariate analysis

The scree plot indicated two as optimal number of dimensions for the MDS. The MDS had a non-metric fit (R^2) of 0.965 with stress 0.188. The visualisation showed a large overlap in steel and rock samples, between the different orientations and between the PAWF and platforms. PAWF and BRG did not overlap, but platforms and BRG overlapped in the MDS (Figure 6).

All terms included in the PERMANOVA had a significant effect (p<0.001) on community composition, with the exception of orientation (P=0.004; Table 3). Location explained most of the variation in the dataset (R^2 = 0.15) while depth R^2 was 0.05. R^2 of the other variables varied between 0.01 and 0.03. The total R^2 of the formula was 0.30.

To assess the effect of age, another PERMANOVA was performed. All terms included in the PERMANOVA had a significant effect on the community composition (Table 4). Location, again, explained most of the variation in the dataset (R^2=0.14, p<0.001) while R^2 for depth and age was 0.06 (p<0.001) and 0.02 (p=0.003), respectively. R^2 of the other variables varied between 0.01 and 0.03 with p values all smaller than 0.02. The total R^2 of the PERMANOVA formula was 0.31.
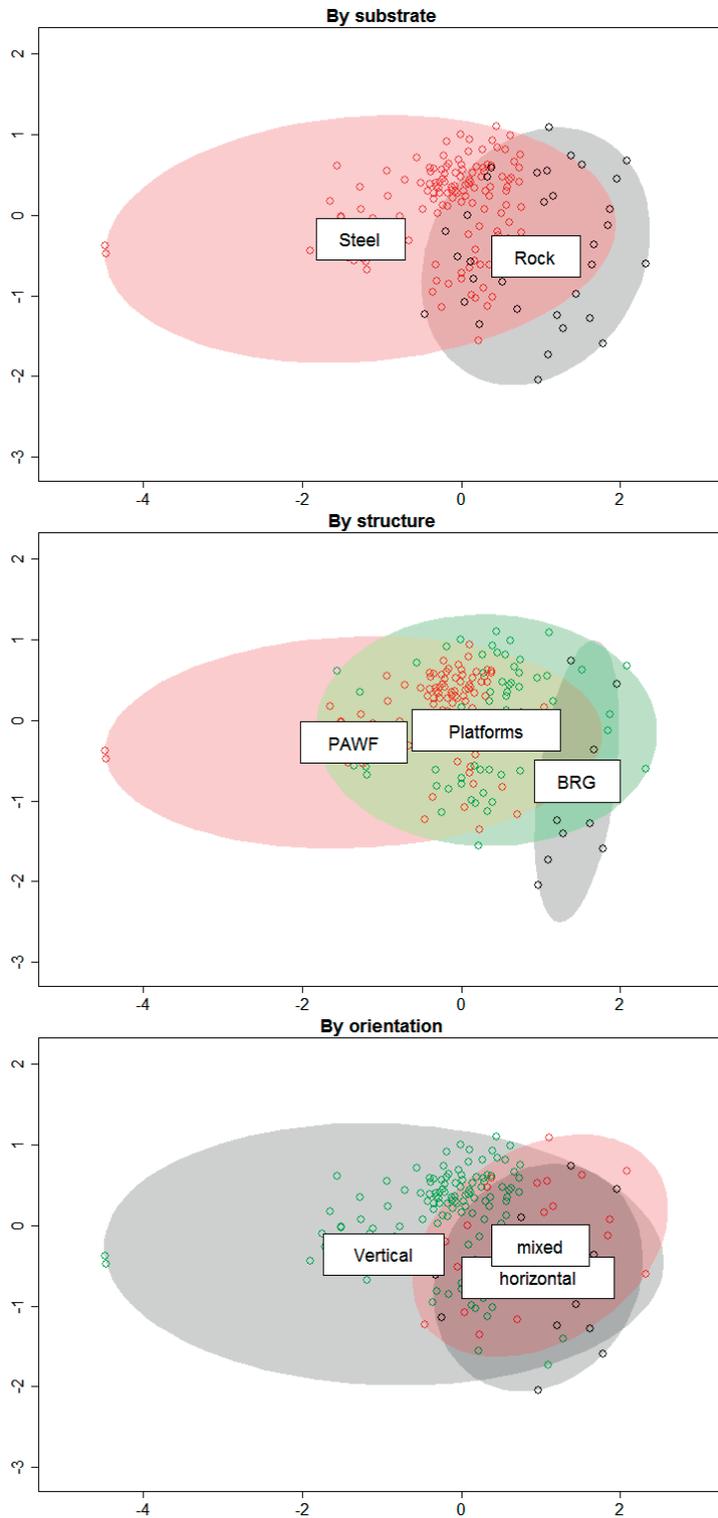
Figure 6: MDS plots of all reef data, showing variables per sample: top = by substrate type (red: steel, black: rock); middle = by reef type (Black: BRG = Borkum Reef Grounds; Red: PAWF = Princess Amalia Wind Farm, green: platforms); bottom = by surface orientation (green: vertical, red: mixed, black: horizontal). Stress = 0.188. Pair of points on the far left originate from the intertidal zone in PAWF and hold only 2 and 3 species.

Table 3. PERMANOVA all data, based on Jaccard distance measure.

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)
Location	5	8.928	1.7856	6.7596	0.15073	1.00E-05
Depth	1	3.204	3.2042	12.1299	0.0541	1.00E-05
Substrate	1	1.676	1.6761	6.345	0.0283	1.00E-05
Mytilus edulis	1	0.758	0.7585	2.8712	0.01281	0.00012
Metridium dianthus	1	1.019	1.0188	3.8569	0.0172	1.00E-05
Psammechinus miliaris	1	0.956	0.9563	3.6204	0.01615	2.00E-05
Asterias rubens	1	0.932	0.9323	3.5295	0.01574	2.00E-05
Orientation	1	0.548	0.5477	2.0734	0.00925	0.00426
Residuals	156	41.208	0.2642		0.69573	
Total	168	59.23			1	

Table 4. PERMANOVA results steel data only, based on Jaccard distance measure.

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)
Location	3	6.265	2.08832	8.5411	0.14057	1.00E-05
Depth	1	2.851	2.85051	11.6585	0.06396	1.00E-05
Age	1	0.717	0.71668	2.9312	0.01608	0.00036
Mytilus edulis	1	0.744	0.74369	3.0417	0.01669	0.00035
Metridium dianthus	1	1.182	1.18161	4.8327	0.02651	1.00E-05
Psammechinus miliaris	1	1.047	1.04721	4.283	0.0235	1.00E-05
Orientation	1	0.465	0.46513	1.9024	0.01044	0.01756
Asterias rubens	1	0.736	0.73612	3.0107	0.01652	0.00036
Residuals	125	30.563	0.2445		0.68574	
Total	135	44.569			1	

Discussion

Depth

The depth distribution of species richness clearly follows a non-linear pattern with maximum species richness at intermediate depths between 5 and 15 m (Figure 5). Richness also increases slightly in deeper waters, with a larger confidence interval. At these depths, most data come from rocks, which are exclusively present in deeper waters. No rocks could be sampled at depths <20 m, since we only investigated deeper offshore waters. Similar patterns have been observed on other reefs, both temperate and tropical (Clarke and Lidgard 2000; Cornell and Karlson 2000; van der Stap et al. 2016). Intermediate depth richness maxima are also known from Californian soft-bottom temperate waters (Bergen

et al. 2001). Depth, together with location effects, had the highest explanatory value in our multivariate analysis. Therefore, these are the most important variables to determine presence of species on these reefs.

Orientation

Species richness per sample was higher on vertical surfaces than on horizontal ones. Moura et al. (2008) showed differences within surface orientation, with shallow horizontal structures being more species rich than deep horizontal ones. Moura et al. (2008) attribute this difference to variation in sedimentation. There may be a similar effect present on the reefs investigated in this study. Zintzen et al. (2006) investigated differences in community structure on horizontal and vertical surfaces on two shipwrecks in the Belgian part of the North Sea. They observed that soft-sediment species were more common on horizontal surfaces and also attribute this to the higher sedimentation rate on horizontal surfaces. However, they failed to find significant differences in species richness between orientations. Wendt et al. (1989) investigated shipwrecks of different ages in South Carolina and Georgia (US) and also compared orientation. They found significantly higher biomass and number of species on vertical surfaces. Knott et al. (2004) report differences in faunal composition between horizontal and vertical surfaces and suggest surface orientation is more important than whether a reef is natural or artificial. Our multivariate analysis showed that both orientation and substrate type accounted for a significant amount of the variation in species composition. Furthermore, our univariate model selection included orientation in the final model, while excluding substrate type. Likely there is collinearity between orientation and substrates, since mixed orientation is present exclusively with rocks. This may explain the significant effect in our multivariate analysis, while excluding substrate type from the univariate models.

Age and location

The models did not show a positive effect of age on species richness. Although the platforms were all older than the PAWF foundations and total observed and predicted richness on these platforms were higher, the average richness per sample was similar between platforms and the PAWF. It is likely that the higher total richness is in part caused by the large spatial distribution of the platforms when compared to the PAWF. This spatial distribution, with both coastal and far offshore structures, introduces variation in environmental factors, such as salinity, temperature, origin of larvae and food availability (e.g. Coolen et al. 2016) which were not included for in our models. The low number of locations without variation within them for most of these variables limited us to include additional variables. Following the rule of thumb of ~10 observation per variable, we would have needed many more locations (Vittinghoff and Mcculloch 2006; Wisz et al. 2008). The multivariate analyses showed a small but significant effect of age on species composition, accounting for 1.6 % (p<0.001) of the variation. Our results are in line with findings by Wendt et al. (1989), who

found no significant differences in species richness among shipwrecks of different ages. Their subjects, however, differed <6.5 years in age. We did find a small significant effect on species composition using much more difference in age (up to 38 years). Possibly effects of the difference in age are only detectable on very long timescales. Whomersley and Picken (2003) studied marine growth patterns on four offshore production platforms over a period of 11 years and observed large variations in percentage cover in the dominant species. This large variation may result in non-significant results when trying to detect trends. In studies on much older artificial reefs in tropical waters, >100 year old artificial reefs resembled natural adjacent reefs more than the younger ones (Perkol-Finkel et al. 2005). This supports our suspicion that detecting age effects may only be possible on reefs much older than the average offshore installation in the North Sea.

Key species

Some species are common on both natural and artificial reefs. *Metridium dianthus* and *Electra pilosa* dominate on steel, rock dump and BRG rocks. The cosmopolitan *E. pilosa* (Nikulina et al. 2007) is one of the few species that was present on both substrate types. *Electra pilosa* is a common epibenthic species in the southern North Sea (Jennings et al. 1999). It is often observed on artificial structures such as shipwrecks (Schrieken et al. 2013; Faasse et al. 2016) and oil platforms (Forteath et al. 1984; Beukhof et al. 2016). Species that are very common on steel but rare on the natural rocks are *Jassa herdmani*, *Mytilus edulis* and *Stenothoe monoculoides*. These are all species that mostly occur at shallow or intermediate depths and are uncommon at the seabed around the artificial structures. Given that they are rare in rock samples, it is likely that these species are only present offshore due to artificial structures providing habitat. Their presence at the rock dump around platforms is probably caused by *M. edulis* clumps falling off the structure (Krone et al. 2013b).

Asterias rubens is known to feed carnivorously (Barker and Nichols 1983) and we expected this to have an influence on species richness. However, no significant relation between the presence of *A. rubens* and species richness was detected. We found a small species composition effect in the PERMANOVA, although this explained only 1.2% of the variation ($p < 0.001$). *P. miliaris* did have a significant positive effect on species richness. Contrary to *A. rubens*, this species utilises a wide variety of food sources (Hughes 2006; Kelly et al. 2013). We included the species in our models as we observed large densities of *P. miliaris* completely clearing patches of steel-substrate epifouling (personal observations J.W.P. Coolen). This was also seen on a ship wreck in the North Sea that sank two years before being studied (van Moorsel et al. 1989). Most likely, grazing by *P. miliaris* increases heterogeneity in the local fouling community by creating new settling opportunities for additional species. This is known as the intermediate disturbance theory (IDT; Connell 1978). IDT is know from rainforests and coral reefs, grazing by cattle on grasslands (Yuan et al. 2016), wave disturbance on shallow marine sites (England et al. 2008) and was suggested by van der Stap

et al. (2016) as the main explanation for higher species richness at intermediate depths in offshore platforms, where both wave disturbance and dominance of *M. dianthus* was low.

Although *M. dianthus* has been suggested to have a strong negative effect on species richness (Coolen et al. 2015a; van der Stap et al. 2016), this is not apparent here. The species does have some effect on the community, shown by the significant PERMANOVA results, but its presence only explains between 1 and 3% of the variation in the data. It should be noted that abundance data were converted to presence-absence data. A high abundance of *M. dianthus* might have a negative effect on other species. To investigate this, modelling the effects of the predictor species used here should be repeated using an abundance dataset.

Presence of *M. edulis* had a significant positive effect on species richness in our univariate models. The effect of mussels, increasing local variation in surface orientation, is very similar to that of rocky reefs. *M. edulis* also provides secondary hard substrates, which augments biodiversity (Gutierrez et al. 2003; Norling and Kautsky 2008). Likewise, presence of *M. edulis* increases habitat heterogeneity in the Wadden Sea and thereby positively influences species richness and composition (Drent and Dekker 2013).

Intertidal zones
Several physical differences between steel and BRG rocks can be observed. The penetration of the water surface is very likely to be the most 'unnatural' feature of such installations. The structures offer intertidal, clear-water habitat that is normally not available offshore, nor on coastal locations on the DCS, since Dutch coastal intertidal locations are predominantly turbid (Coolen et al. 2016b). Non-native species are common in the offshore intertidal (De Mesel et al. 2015), as well as native species that would be uncommon offshore without these structures (Coolen et al. 2017a; Chapter 5). To prevent this effect installations would have to be built without intertidal zones, which is not feasible. For future RTR projects, it is advisable to remove the intertidal zone from abandoned installations to reduce the presence of intertidal species at offshore locations.

Rock vs steel
Substrate type had a significant effect on species composition, but species richness did not differ significantly between rocks and steel. This may be caused by collinearity between substrate type and surface orientation. Complexity of surfaces is positively related to species richness, since it increases the amount of surface available (Johnson et al. 2003), and the available surface is more heterogeneous in orientation (Johnson et al. 2003; Kostylev et al. 2005). Since mixed orientations were only present on rocky substrates, orientation may be much more important on the scale of our samples than substrate type.

Do the species communities on the natural substrates resemble those on artificial ones? Rocks and steel are not fully separated in the MDS. Figure 6, however, shows that there is no overlap between the samples of BRG rock and steel. There is overlap between rocks around platforms and from BRG (not between BRG and PAWF) but steel structures are separated from natural rocks. Thus, rocks from natural reefs differ from steel structures. In most studies, communities on artificial and natural reefs are shown to differ significantly (Page et al. 2007; Wilhelmsson and Malm 2008). However, very old (>100 year) tropical artificial reefs may resemble natural reefs (Perkol-Finkel et al. 2005). Furthermore, Perkol-Finkel et al. (2006) also hypothesise artificial reefs to only resemble natural reefs if they are structurally similar. Our findings support this hypothesis; communities on rocks placed artificially around the offshore platforms, did overlap strongly with natural rocks from the BRG. These rocks are structurally very similar (personal observation J.W.P. Coolen), but differ strongly from straight, water column penetrating steel structures. Following Perkol-Finkel et al. (2006), significant differences will probably always be found between irregular rocky reefs and flat steel reefs, regardless of study method or structure age.

When artificial structures are to be colonised by a species community similar to that on natural reefs (see e.g. van Duren et al. [2016]), changes should be made to current designs. Rock dump around the offshore platforms supported a species community most similar to natural rocks. These rocks were structurally almost identical to the BRG rocks. This is the most likely explanation for their similar species community. Steel parts of the structures were significantly different from the BRG rocks.

Changing the substrate material, e.g. steel to concrete, may increase similarity to natural reefs. The colonising community of steel and concrete foundations can be significantly different (Andersson et al. 2009), and on the DCS, we observed that dominant species on concrete foundations are different from the dominant species on steel structures, even when in close proximity of concrete (personal observation J.W.P. Coolen). To investigate this further, a comparison of steel, concrete and natural reefs should be made.

Increasing the complexity of the substrate, e.g. by introducing crevices of different sizes, or by coating structures with rocky substrate, may be a feasible way to mimic a natural reef structure. This has been done earlier on Dutch water works, where small gravels combined with polyurethane were attached to concrete blocks placed on dikes (Lock et al. 2009; Dekker et al. 2014). This material, however, has been evaluated only on the short term and was only compared to basalt and asphalt alternatives, not steel, nor natural reefs. Offshore steel structures coated with gravel or rock based substances should be followed in time to compare the development of the community to uncoated steel structures and natural reefs.

Conclusion

In the Dutch part of the North Sea, species composition is different on artificial and natural reefs. Depth, location effect and habitat type influence the species composition most strongly. Although substrates with mixed surface orientation (i.a. rocks) hold the most species rich communities, biodiversity is also strongly influenced by *Mytilus edulis* and *Psammechinus miliaris*. When artificial reefs are to be colonised by communities that are similar to natural reefs, their structures should resemble natural reefs as much as possible. To investigate the potential of solutions to increase the similarity between artificial and natural reefs, we suggest experiments with changes to the surface of the substrates by introducing small-scale heterogeneity, e.g. a coating with gravel and stones.

Acknowledgments

Chapter 7:

SYNTHESIS

The objective of the research presented in this thesis was to understand the patterns of benthic biodiversity on reefs in the North Sea. To gain this understanding, I studied which species are present on natural and artificial reefs, what environmental and biotic variables influence the presence and absence of a selection of these species, how total species richness is influenced by these variables and one of the possible pathways by which these species may colonise the reefs studied; the marine stepping stone effect.

The first goal was to increase the available knowledge on which species are present at reefs. I started with investigating the epifouling organisms present at a rocky reef near the islands of Schiermonnikoog and Borkum: the Borkum Reef Grounds (chapter 2). Then I investigated the epifouling present on offshore oil and gas platforms by analysing video footage taken during routine inspections (chapter 3) and by taking samples from the fouling communities on the foundations and from the rock dump around these installations (chapters 4-6). I increased the available data further by taking samples on shipwrecks, from navigational buoys and from coastal reefs, such as rocks, harbours and coastal defence structures.

The second goal was to understand the patterns observed in the variation of species at these reefs. These patterns can be influenced by many variables, including depth, temperature, salinity, food availability, reef type, community age, presence of predators or prey, et cetera (chapters 3, 4, 6). For every sample taken on the reefs, I collected this information where possible and was able to evaluate the effect of these variables on the presence of species in the samples taken.

The third goal was to test a concept that has become accepted as true in most environmental assessment documents, but, to my knowledge, was never proven by actual collection of field data at sea: the stepping stone effect from the introduction or artificial structures in a sandy bottom ecosystem. Organisms are suspected to use artificial installations as stepping stones to colonise locations normally not reachable in a single generation. This is known as the stepping stone hypothesis. This theory states that larvae are limited in their migration range and that some areas are out of their reach in a single generation. To counter this limitation, species use locations at intermediate distance that are within their reach to settle and reproduce, so their offspring can, perhaps via other intermediate locations, eventually colonise far away installations or natural populations. The intermediate locations are the stepping stones the species needs to reach the far end of the migration route (Chapter 5).

The final goal was to assess the impact artificial reefs have on the benthic biodiversity of the North Sea. To evaluate this impact, I compared the species communities on artificial reefs to those on natural reefs. For this I used data collected from the field during the work for this thesis, as well as data from earlier investigations of natural and artificial reefs. I hypothesised that in the lower part of the water column artificial hard substrate communities in the Dutch

North Sea are identical to natural hard substrate communities. If this hypothesis was true, the impact of artificial reefs from the deeper parts of the reefs would be limited. I also hypothesised that the shallow subtidal and tidal parts of artificial structure would differ significantly from natural reefs. This then would have a significant impact on the benthic biodiversity of the North Sea, since it changes the local composition of benthic species.

In the following synthesis I will elaborate on the findings on the second, third and final goals.

Understanding community patterns

The variation observed in species communities is influenced by a range of variables, impacting the species in different ways. To understand this complexity of effects, I used multiple linear and additive modelling. This allowed me to isolate the effects of each variable and evaluate its influence on the species richness or presence of specific species. These effects can be non-linear, e.g. when there is an intermediate maximum. This intermediate maximum, for example, was present in the vertical zonation on platforms. The relation between depth and species richness (number of species per sample) always resulted in a maximum at intermediate depths, e.g. between 5 and 15 m, with lower richness in the shallow and deep parts of the reef (Figure 1; chapters 2 and 6).
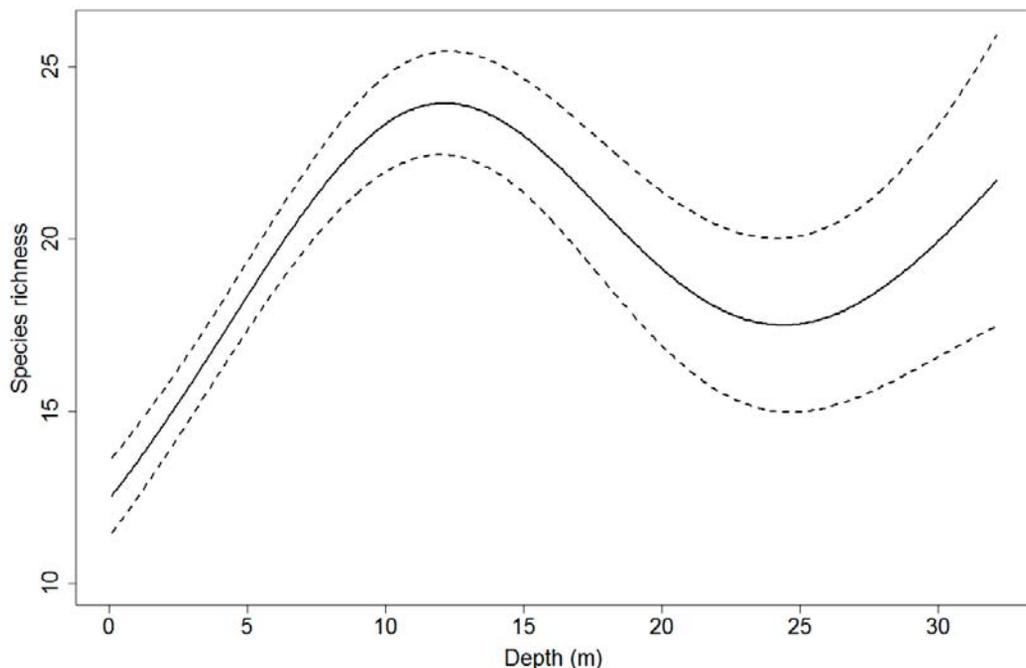
Figure 1: Typical relation between depth and species richness per sample (solid line) with standard error (dashed lines), on steel artificial reefs (chapter 6).

This pattern can be explained by variables acting together on the different species present. In the shallow parts of the structures, wave action may disturb the settled species too much, causing a lower richness than in the intermediate depths (England et al. 2008). However, *Mytilus* edulis concentrations are highest between 0 and 20 meters, stimulating a high species richness (chapter 6) when moving away from the intertidal zone. On the deep side, *Metridium dianthus* is often the dominant species. I observed that with dominance of this species, richness often is very low (Chapters 2 and 3) and the species is known to deter and smother organisms (Kaplan 1984; Nelson and Craig 2011). At intermediate depth, wave disturbance is lower and *M. dianthus* is not that abundant, while *M. edulis* still is. The disturbance therefore is intermediate, causing higher species richness. Although, using presence-absence data, I had to exclude *M. dianthus* from our species richness model as it did not explain enough of the variation in species richness (chapter 6), I still expect this species to have a strong effect on richness. Most likely, this will become apparent when we include abundance data of predictor species in future models. The higher richness at moderately disturbed depths is known in ecological theory as the intermediate disturbance hypothesis (Townsend et al. 1997). It has many examples, e.g. from cattle foraging on grasslands (Yuan et al. 2016), macroinvertebrates in streams (Townsend et al. 1997) and marine phytoplankton (Sommer 1995). Intermediate disturbance may also explain the effect of the urchin *Psammechinus miliaris* on species richness. I included this species in a model to evaluate effects on natural and artificial reef communities. Although I observed the species to forage quite intensely on epifouling during my dives, the impact of the species on the richness per sample was positive (Chapter 6). I hypothesise that this species increases heterogeneity in epifouling cover by grazing away the dominant species, creating open patches that can then again be colonised by fresh species. This will increase local species richness.

Mytilus edulis is limited to shallow parts as its main predator, *Asterias rubens*, prefers to feed on subtidal mussels (Saier 2001), perhaps limited by high wave action in shallow parts. *Mytilus edulis* is known to increase habitat heterogeneity, increasing settlement opportunities for other species and thereby increasing species richness (Chapter 6; Drent and Dekker 2013; De Mesel et al. 2015). This is known as facilitation (Bruno et al. 2003). I included *M. edulis* in a model to describe the species richness on natural and artificial reefs (Chapter 6) and concluded that *M. edulis* indeed had a significant positive effect on species richness.

From literature I knew that there is a strong zonation in the epifouling communities on renewable energy installations and oil and gas platforms. In all North Sea surrounding countries, the shallow part is dominated by the blue mussel, *M. edulis*. Often, this is then followed by a zone with a mixture of hydroids (e.g. Tubulariidae), often overgrown with Gammaridae (e.g. *Jassa herdmani*). In deeper zones anemones like *M. dianthus* and the

soft coral *Alcyonium digitatum* then take over (Whomersley and Picken 2003; Leonhard and Christensen 2006; Langhamer 2010; Krone 2012; Bouma and Lengkeek 2013; De Mesel et al. 2015). The patterns I observed on offshore oil and gas platforms are the same as previously reported.

Artificial reefs as stepping stones for dispersal
The species present on offshore reefs can colonise these in different manners. Some species, e.g. *Homarus gammarus*, are capable of travelling long distances and can simply walk to a newly built reef (Krone and Schröder 2011). Other species can use floating objects as rafts to travel long distances and colonise objects the raft comes in contact with (Thiel and Gutow 2005). I suggested this as one of the methods by which *Caprella* species colonise offshore installations (Chapter 4.2). Many species distribute as pelagic larvae, drifting with currents until a suitable location is reached. The specimens of *C. smithii* I observed on a wreck on the Dogger Bank, may have originated from other wrecks upstream (Chapter 4.1). Similar suggestions have been made by others, e.g. for non-native species (De Mesel et al. 2015) that were observed on offshore wind farms. These organisms are suspected to use artificial installations as stepping stones to colonise locations normally not reachable in a single generation. This is known as the stepping stone hypothesis, originally drafted to describe connections between island populations (Kimura and Weiss 1964; Mac Arthur and Wilson 1967). This effect is known for marine snails living around islands connected by ocean currents (Crandall et al. 2012). Thorpe (2012) modelled the stepping stone potential of offshore oil and gas platforms in the North Sea and concluded that about 60% of the installations in the southern North Sea were connected by currents that transport larvae of fouling species.

I decided to test Thorpe's conclusion using a species that is abundant in the intertidal and subtidal zones of offshore installations, but rare on reefs that are exclusively subtidal. *Mytilus edulis* has a pelagic larval stage, but is very rare on shipwrecks (personal observation; Chapter 6). Furthermore it is abundant on most offshore installations (Joschko et al. 2008; Langhamer 2010; Bouma and Lengkeek 2013; De Mesel et al. 2015), making it an ideal model organism to test the stepping stone hypothesis.

I collected mussels from locations onshore and offshore. Using population genetic techniques and particle tracking models, we showed that the pattern resulting from the models, significantly explained the genetic patterns found in the mussels. The amount of particles arriving when travelling between connected locations, expressed as distance by taking the inverse, significantly predicted the genetic variation (F_{ST}) observed in the *M. edulis* sub-populations. Location combinations without particle exchange had a higher F_{ST} than combinations with particle exchange (Figure 2).
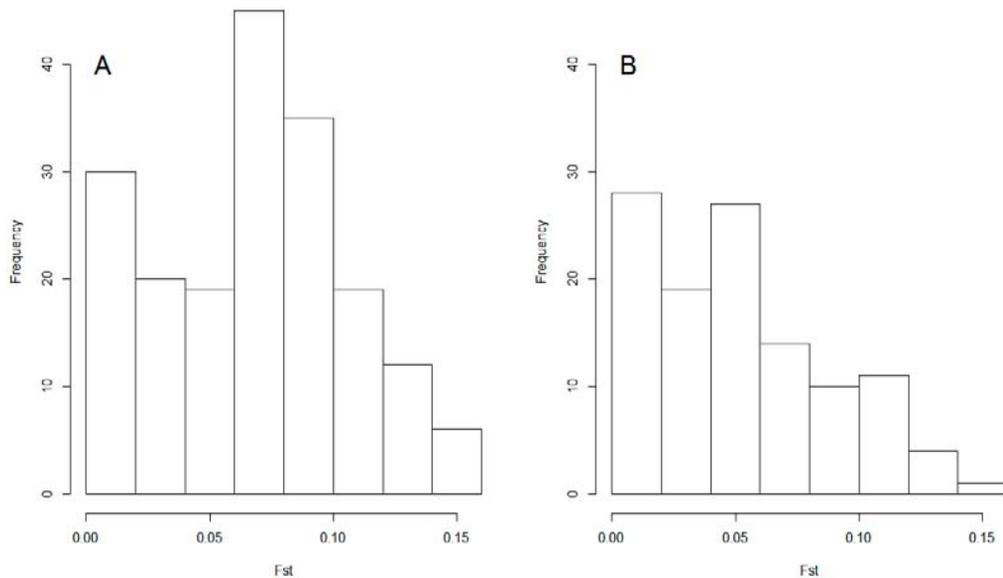
Figure 2: Histograms of F_{ST} frequencies. A: Histogram of pairwise F_{ST} frequencies of location combinations that exchange **no** particles in the particle tracking models (PTM; Chapter 5). B: Histogram of pairwise F_{ST} frequencies of location combinations that exchange particles in the PTM.

This evidence strongly suggests that *M. edulis* indeed uses offshore installations as stepping stones. It may be the only pathway available for species with short larval dispersal capacity, and a preference for intertidal zones, to colonise far offshore structures. Many of the species present on the platforms I investigated (chapter 6), disperse as pelagic larvae (MarLIN 2006) and may therefore have originated from upstream offshore installations. The presence of these installations is therefore important for these species. Without the availability of these settlement opportunities, the larvae would likely have perished in the otherwise unsuitable offshore North Sea.

How natural are artificial reefs?

I assessed the impact of the presence of artificial reefs in the North Sea on a local scale, by comparing communities on artificial reefs with communities on natural, rocky reefs (Chapter 6). For this, I used our Borkum Reef Grounds rock data (Chapter 2) to compare with our platform data and with data from the Princess Amalia Wind Farm (Vanagt and Faasse 2014).
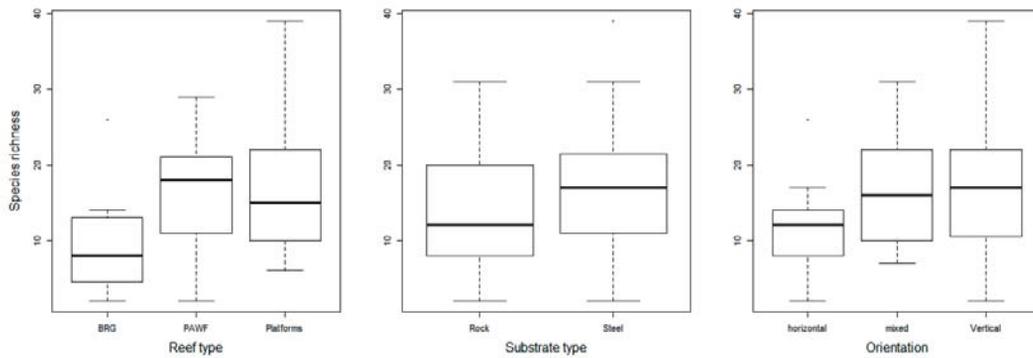
Figure 3: Left: Species richness in samples obtained from the Borkum Reef Grounds (BRG), Princess Amalia Wind Farm (PAWF; rocks & steel) and platforms (rocks & steel). Middle: Species richness per substrate type: Rock and steel. Right: species richness in different surface orientations (Chapter 6).

Rocky reefs have an increased surface area and create crevices that increase the available habitat for hard substrate associated species (Johnson et al. 2003; Kostylev et al. 2005). Rocks also have a different structure when compared to steel. This structural difference between natural and artificial reefs was already known to cause differences in species communities from tropical areas (Perkol-Finkel et al. 2006) and in the Baltic Sea, Wilhelmsson and Malm (2008) showed that reference boulders held more species than a nearby wind farm with steel foundations. We, however, did not find a difference between species richness on rocks and steel. There was a difference between different orientations of the substrate, which is related to substrate type, since rocks can have all combinations of orientations on a very small scale.

The species composition on artificial and natural reefs did differ significantly. Some species present on steel substrates, can also be found on the natural reefs. For these species, the presence of artificial reefs increases the connectivity between natural sub-populations. Using artificial reefs as stepping stones, species may be able to migrate between these natural reefs. This may have been impossible without these artificial reefs, as most natural reefs originally present in the Dutch part of the North Sea have now disappeared (Chapter 1).

However, most species found on artificial reefs, were not observed on the offshore natural reefs studied. Some features of platforms and wind farms are very different from natural reefs. The most striking difference is the presence of an intertidal zone in an offshore environment. There is no naturally occurring equivalent in the Dutch North Sea. Water bodies offshore are different from coastal waters in availability of food, water temperature and salinity (Chapter 4.2). These offshore intertidal zones may therefore attract different species than present on coastal intertidal zones. From wind farms we know that these offshore intertidal zones can

harbour a high amount of non-indigenous species when compared to the deeper parts of the structure (De Mesel et al. 2015).

The rocks that are deposited on the bottom around installations (rock dump or scour protection) were found to be most similar to natural rocky reefs when comparing the community structure. Although only 19 out of a total of 123 species were shared between these types of rocks, the multidimensional scaling placed the rocks around platforms in a cluster with the Borkum Reef Ground Rocks. Furthermore, samples taken on steel surfaces close to the bottom, are also placed close to the rock dump and close to the Borkum Reef Ground samples (Figure 4). This shows that there is a high degree of similarity between deep steel and natural rocky reef communities when compared to shallow/intertidal steel.

In general, epifouling communities on steel structure, differ significantly from those on natural reefs. The difference is strongest between intertidal steel and natural reefs. The difference is weak between deep steel and natural reefs and very small between rock dump and natural reefs.
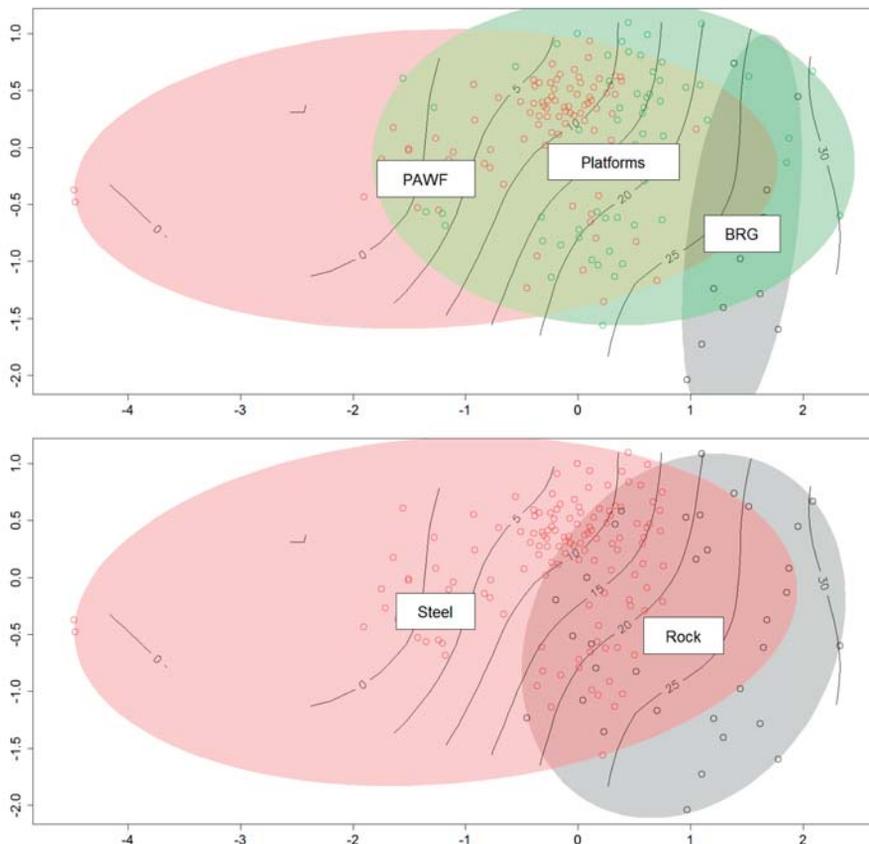
Figure 4: MDS plots of reef data from the Borkum Reef Grounds (BRG), Princess Amalia Wind Farm (PAWF) and oil and gas platforms. Depth gradients are shown in black lines. Top: ellipses drawn around structure type (red: PAWF; green: platforms; black: BRG); bottom: ellipses drawn around habitat type (black: rock; red: steel).

Implications for offshore installations

The work carried out for this thesis was set against a background of large changes in the offshore industry. Firstly, end of life is coming for many oil and gas platforms, due to low oil prices, dwindling production and calls for renewable energy sources. Secondly, new offshore wind farms and other renewable energy devices are placed in our waters every year. Lastly, new initiatives such as offshore fish farming, sea weed farming and shell fish culture present themselves on the horizon. In some outlooks these activities are combined and envisioned to go hand in hand with nature protection and recovery, e.g. in the form of multifunctional used platforms and building with nature. All these activities have a large effect in common: they change the availability of artificial hard substrates in an offshore environment. What could be the implications of the findings in this thesis for these developments in the North Sea?

Many of the species present on artificial reef, live exclusively on and around hard substrates. The presence of hard substrates on a soft bottom strongly increases local biodiversity (chapter 2). When installations are removed, epifouling species will also be removed and will not return on the sandy seabed, decreasing local biodiversity.

Prevent intertidal zones where possible

Some of the species present on offshore installations would never be present without intertidal zones, a habitat that does not naturally belong to most of the offshore North Sea. I showed that the shallow parts of these structures host species communities that are not like natural subtidal offshore reefs (chapter 6). If platform foundations are left in place after their productive live has ended, one should aim to mimic natural (historic) hard substrate communities. Before the onset of industrial fisheries, the bottom of the southern North Sea was covered with enormous reefs composed of rocks and oysters (Chapter 1). Both these original and current natural offshore reefs were completely subtidal. The shallow intertidal zones on platform foundations left in place should therefore be removed to prevent intertidal species from settling. This can be attained by toppling the foundations in place so all of it is permanently under water, or cutting them at a certain depth (e.g. 10 meters or deeper following safety regulations) and removing everything above that. Whether the communities become more like natural rocky reef communities, should then be monitored following the years after removal of the intertidal zone. For new developments, intertidal zones often cannot be prevented but one could aim to minimise the available substrate and try to prevent long term settlement of intertidal species by cleaning the shallow substrates regularly.

Increase rocky substrate

The communities on the rock dump surrounding offshore installations are more like natural rocky reefs than the steel foundations are. Since rocky reefs are a protected habitat in the

European Habitat Directive (H1170), the presence of this rock dump can be considered a positive effect. As mentioned in the introduction, subtidal natural hard substrate was abundant in the North Sea in the past, e.g. as oyster reefs and moorlog. To stimulate this further, the addition of more rock dump to increase the area covered around new installations and future repurposed platforms should be considered. This will stimulate the development of communities and adds rare species to the local biodiversity. In addition to attraction of rare species, rock dump may also serve as a stepping stone for these species, connecting isolated populations of species in the North Sea.

The structural difference between steel and rock is the most likely cause of the differences in the epifouling communities present. To improve the ecological quality of offshore installations, alternatives to steel should be considered. Although I have not investigated the communities on concrete foundations, I did observe that the dominant species are different among steel and concrete. Possibly, concrete offers more small scale heterogeneity, stimulating different species to settle. This should be investigated more thoroughly in future research, by including concrete foundations of old platforms in the analysis performed in chapter 6.

Recommendations for future research
In the models presented in this thesis, much variation in species richness and species composition remained unexplained. In chapter 4.2 I showed that variables such as water temperature, salinity, current velocity and suspended particulate matter influence the presence of *Caprella* spp. These variables were lacking in the models describing variation in species richness in chapter 6, since I felt the number of locations we had available was too low to include variables that have no variation within a location. To investigate the influence of these variables and include them in models to further understand the patterns in species communities, future research should include more locations, adding enough variation in location specific abiotic variables. Doing this we may be able to increase our insight in indirect location effects and reduce the unexplained variation. Future research should therefore focus on unravelling in how far sea water temperature, salinity, current velocity, suspended matter concentration and other abiotic variables, influence species richness on artificial reefs in the North Sea. This may be of use to predict settlement of species on new installations where no data is currently available, e.g. for the impact assessment of future wind farms at locations like the Dogger Bank (Figure 5).

Furthermore, I showed that species within the communities studied in this thesis, can have a strong effect on one another and can significantly influence species richness and community structure (chapter 6). Possibly, some of them function as keystone species (Paine 1969). Different types of keystone species have been defined, e.g. by Mills et al. (1993), and the species that remained included in my model of the species richness on reefs, do fit these

types. I found a highly significant positive effect of *M. edulis* on species richness. This mussel alters the available habitat by increasing habitat heterogeneity and could therefore be considered a keystone modifier. The sea urchin *Psammechinus miliaris* forages on a wide range of prey and creates patches in dominant species cover. Perhaps this species functions as a keystone predator. Whether these species (and others) do function as keystone species and are therefore highly important for the local species community, should be studied more thoroughly. Species with potential to influence community structure should be quantified in a systematic manner. One of the challenges here is that some of these species cannot easily be counted as they are colonial and often form dense three dimensional structures (e.g. *Ectopleura larynx*). For these species quantification should take the form of weight, area covered or volume, e.g. by using the SACFOR abundance scales (Hiscock 1996). Species that warrant further investigation to improve the understanding of keystone species on North Sea reefs include *M. edulis*, *M. dianthus*, *P. miliaris*, *Tubulariidae* species (e.g. *Tubularia indivisa* and *Ectopleura larynx*) and *E. pilosa*. To fully unravel the importance of some of these species as keystone species, experiments with removal or exclusion of the potential modifiers and predators may be needed.

Some questions on the stepping stone effect (chapter 5) remain unanswered: in how far are the northern and southern North Sea connected through stepping stones? Using models based on oil and gas platforms alone, Thorpe (2012) showed that north and south are isolated. But many other structures are present in the area from north to south. The most abundant are the -exclusively subtidal- shipwrecks which may influence the distribution of subtidal species (Chapter 4.1). In the coming years, new wind farms will be constructed far offshore on the Dogger Bank (Forewind 2015), which will add intertidal zones to an area where they are lacking at the moment (Figure 5). This will most likely increase the connectivity between north and south for intertidal species. To investigate this, additional genetic data on *M. edulis* should be obtained from these waters.

Our analysis was based on genetic dissimilarity and supported the idea that *M. edulis* migrates between offshore installations and uses them as stepping stones to colonise far offshore platforms. To further verify this concept, a coalescent analysis of isolation-with-migration should be carried out (discussion Chapter 5). Due to problems with hybridisation in the samples, we were unable to carry out such an analysis during my thesis work. This analysis may answer the question in how far *M. edulis* migrates between the investigated locations.

Our work uncovered the population genetic patterns for a single species. Other species, e.g. with other life histories or dispersal mechanisms should be investigated to further understand the connectivity of offshore artificial reefs.
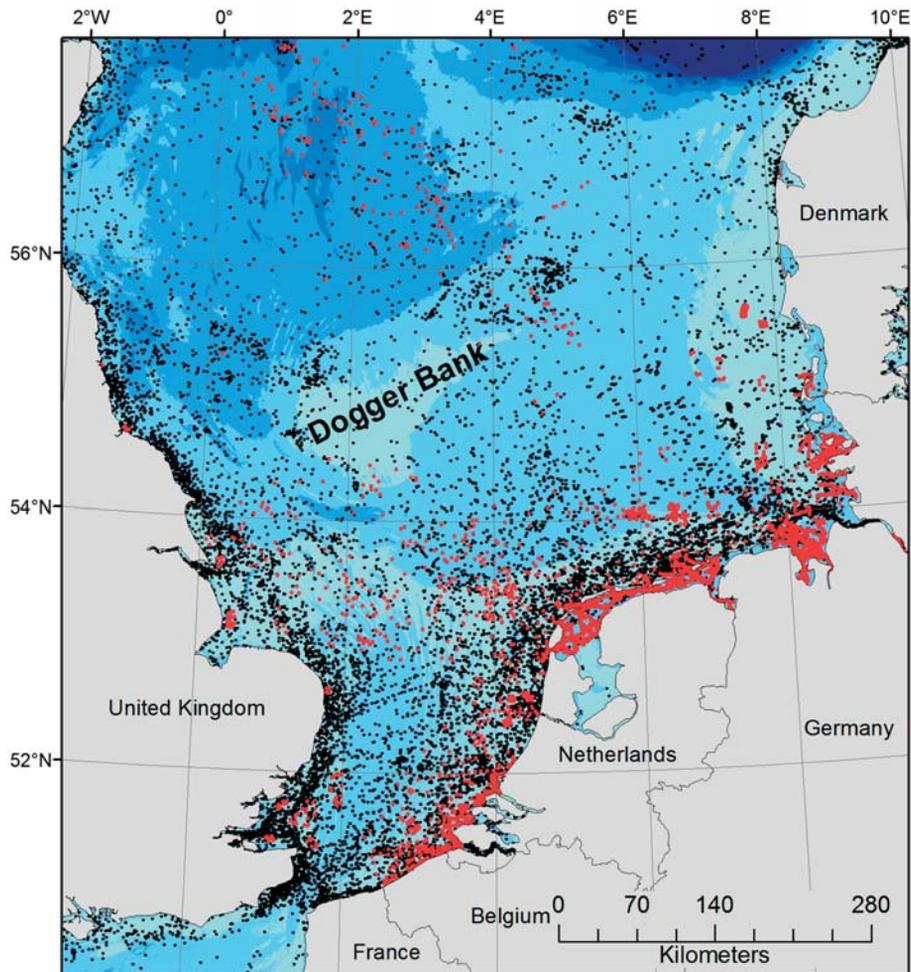
Figure 5: North Sea map of all artificial reefs. North Sea with depth (blue layer, darker = deeper), artificial structures with surface contact (red dots) and structures without surface contact (black dots). Reef locations sourced from various databases (Afdeling kust - Flemish Ministry of Mobility and Public Works - Agentschap Maritieme Dienstverlening en Kust 2015; Bundesamt für Seeschifffahrt und Hydrographie [BSH] 2015; Lettens 2015; Ministry of Infrastructure and Environment - Directorate-General Rijkswaterstaat 2015; OSPAR Commission 2015).

Species investigated could include *Cancer pagurus*, a species that can migrate >200 km (Hunter et al. 2013), making it likely that offshore installations do not play a stepping stone role for them. Species with life histories different from *M. edulis* could include *Jassa herdmani* or *Caprella mutica*. Both species do not have a pelagic larval stage and have a short generation time. This is likely to present a different pattern compared to *M. edulis.* Since non-native species are often described as a negative result of the stepping stone effect, such species should also be included. *Caprella mutica* makes a good candidate, although the species is rarely observed far offshore (chapter 4.2).

In short, future research should focus on answering the following questions:

- In how far do sea water temperature, salinity, current velocity, suspended matter concentration and other abiotic variables, influence species richness on artificial reefs in the North Sea?
- Which species on offshore reefs function as keystone species and how do these species influence the species community?
- In how far are the northern and southern North Sea connected through intertidal stepping stones? How will this change with the construction of new wind farms and other installations?
- How does the presence of artificial reefs in the North Sea increase the distribution of invasive species?

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Summary

Reefs are among the most species rich habitats in the world. Reefs in temperate waters are home to a large amount of benthic species, including many long lived species. Reefs that are present in temperate sandy bottomed environments, can more than double local biodiversity and increase the local biomass up to a hundred fold. In Europe, reefs are regarded as important for biodiversity conservation and the European Union has defined reefs as protected habitats in its Habitat Directive. Much of the North Sea bottom is covered with mobile sediments such as mud and sand, and only about 20% is covered in coarse sands, gravels and rocks.

Many artificial reefs are present in the North Sea. About 27,000 wrecks locations are known to hydrographic services around the North Sea, 1,500 offshore oil and gas structures are present. The oldest North Sea oil and gas platforms date from the early 1960s. In the '70s the first platforms were placed on the Dutch continental shelf. Approximately 2,400 wind turbines have been installed in the North Sea, but every year hundreds of new wind turbines are placed so these numbers increase fast. The primary ecological effect of placing artificial reefs in the North Sea, is the addition of hard substrate to a mostly sandy bottomed environment. This hard substrate provides new habitat to species that are unable to establish populations on sand bottoms. The presence of these species at different locations, is influenced by biotic and abiotic variables. One of the aims of this thesis is to evaluate the effect of these variables on the presence of species and on the structure of their communities.

When offshore installations are placed at the boundaries of larval distribution potential, they may function as stepping stones to increase the spread of certain species. I aim to evaluate the stepping stone effect on offshore installations in the North Sea, validating particle tracking models with population genetic data from mussel populations on artificial reefs.

With their high numbers of species, the ecological importance of rocky reefs is high. But what is the ecological value of the thousands of artificial reefs in the North Sea? To what extent do the species communities between rocky and artificial reefs in the North Sea overlap? The North Sea bottom is mostly covered with sandy sediments. Hard substrates of natural origin are rare here. Species present on artificial reefs, therefore, could be seen as an unnatural addition to the benthic ecosystem. But are they unnatural? To evaluate the value of artificial reefs, I compare the species communities on artificial reefs to those on rocky reefs.

In chapter 2, we investigated a natural reef in the North Sea. Reefs play an important role in the distribution of species associated with hard substrates and are of value for biodiversity conservation. High densities of the habitat building annelid *Lanice conchilega* also increase

local biodiversity. This study describes the benthic biodiversity of a rocky reef and its surrounding sand bottom with dense *L. conchilega* beds in the Borkum Reef Grounds, north of the island of Schiermonnikoog in the Dutch North Sea. A side-scan sonar survey revealed distinct seabed areas with high acoustic reflections, indicating the presence of hard substrate on the sandy seafloor. To ground truth the side-scan sonar data and make an inventory of the biodiversity of the observed habitats, a multi-method sampling approach (box corer, scuba airlift sampler and visual transects, drop down camera) was used. This revealed (1) rocky reefs: a combination of gravel, stones and rocks; (2) individual rocks in a sandy environment; (3) sand with dense *L. conchilega* beds (>1,500 ind.m^{-2}) and (4) sand bottom habitat. A total of 193 taxa was found with many unique species per habitat. Species richness was significantly higher on sand when compared to the rocky reef (NB-GLM; p=0.006), caused by the presence of dense *L. conchilega* beds (Poisson GLM; p<0.001). Including dense *L. conchilega* beds as an additional habitat showed these held a higher species richness than the rocky reefs (NB-GLM; p=0.002), while sand without dense *L. conchilega* beds did not (NB-GLM; p=0.14). Since the rocky reefs were present on a sandy bottom, the local biodiversity more than doubled with the presence of rocky reefs. The nMDS plot clearly separated the sand and rocky reef communities and also showed a clustering of dense *L. conchilega* beds within the sand samples. Each method detected unique species, demonstrating the value of a multi-method approach compared to e.g. box coring alone. This study identified several species previously unknown to the Borkum Reefs Grounds area. The total area of rocky reefs in the southern part of the Dutch Borkum Reef area is estimated to be 9.8 km^2 and of *L. conchilega* beds with densities >1,500 ind.m^{-2} to be 74 km^2. Further research should focus on the possible function of *L. conchilega* as an ecosystem engineer creating intermediate sand-reef systems. For mapping these *L. conchilega* beds, we advise using side-scan sonar imaging combined with ground truthing by drop down cameras.

Chapter 3 Investigates epifouling biodiversity on offshore platforms using video data obtained by remotely operated vehicles. Offshore platforms are known to act as artificial reefs, though there is on-going debate on whether this effect is beneficial or harmful for the life in the surrounding marine environment. Knowing what species exist on and around the offshore platforms and what environmental variables influence this species assemblage is crucial for a better understanding of the impact of offshore platforms on marine life. Information on this is limited for offshore platforms in the southern North Sea. This study aims to fill this gap in our knowledge and to determine how the composition and the abundance of species assemblages changes with depth and along a distance-from-shore gradient. The species assemblages on five offshore gas platforms in the southern North Sea have been inventoried using Remotely Operated Vehicles inspection footage. A total of 30 taxa were identified. A Generalised Additive Model of the species richness showed a significant non-linear relation with water depth (p=0.001): from a low richness in shallow waters it increases with depth until 15-20 m, after which richness decreases again. Using PERMANOVA, water depth

(p≤0.001), community age (p≤0.001) and the interaction between distance from shore and community age (p≤0.001) showed a significant effect on the species assemblages. Future research should focus on the effect additional environmental variables have on the species assemblages.

Chapter 4.1 describes an observation of a solitary hard coral on the Dogger Bank. In the North Sea, observations of the solitary coral *Caryophyllia smithii* are mostly limited to the north east coast of the United Kingdom including the Shetland and Orkney islands. To date, C. smithii has not been reported from far offshore locations in the North Sea south of 57.7°N. Distribution of this species appears limited by the restricted availability of natural hard substrates in the central southern North Sea. There are, however, many artificial hard substrates in this area in the form of shipwrecks and offshore oil and gas installations. These may provide stepping stones for *C. smithii* to expand its distribution. Here we report the first sighting of *C. smithii* in the central southern North Sea on an unidentified wreck on the Dogger Bank. This is the first offshore observation of any hard coral in the central southern North Sea.

Chapter 4.2 compared habitat suitability of an invasive and a native species on artificial reefs. Studying offshore natural and artificial hard substrates in the southern North Sea (51°N-57°N/1°W-9°E), the invasive introduced Japanese skeleton shrimp *Caprella mutica* Schurin, 1935 was found to co-exist with the native *Caprella linearis* (Linnaeus, 1767) only on near-shore locations that had an intertidal zone (e.g., wind farm foundations). In contrast, on far offshore and strictly subtidal locations, such as shipwrecks and rocky reefs, only *C. linearis* was found. Based on these exploratory observations, we hypothesised that artificial structures that are only subtidal are inhabited exclusively by *C. linearis*, and never by *C. mutica*. To test this hypothesis and understand factors driving each species' habitat preferences, habitat suitability models were constructed using generalised additive models, based on samples collected in 2013-2015 from offshore gas platforms, buoys, shipwrecks, and rocky reefs and combined with data from other published and unpublished surveys (2001-2014). The models showed that the presence of *C. mutica* is explained by the availability of intertidal and floating hard substrates, suspended particulate matter density (SPM), mean annual sea surface temperature, salinity, and current velocity. The *C. linearis* model included subtidal hard substrates, SPM, salinity, temperature, and current velocity. The modelled distributions showed a significant difference, demonstrating that *C. linearis'* habitat preference does not fully overlap with that of *C. mutica*. Thus, the native and alien *Caprella* species are likely to be able to co-exist in the North Sea.

Chapter 5 shows that the blue mussel probably uses offshore installations as stepping stones to distribute itself to far offshore locations. The stepping-stone effect on marine species has been suggested in many recent papers, describing that epifaunal organisms may use isolated

structures as stepping stones to spread to new areas that are too distant to reach in a single generation. With thousands of artificial hard substrate structures present in the North Sea, we hypothesise that most of these structures are directly or indirectly connected by water currents and that offshore energy installations in the North Sea act as a large interconnected reef for species with a relatively short pelagic larval stage. If they do, the population genetic structure should follow a pattern that can be predicted by particle tracking models (PTM; hydrodynamic distance). To test this, we analysed the population genetic structure of the mollusc *Mytilus edulis*, based on microsatellite markers, and tested whether hydrodynamic distance between the same locations explained the genetic variation between the samples. Our results strongly support that the marine stepping stone effect is present on offshore energy installations in the North Sea. Stepping stones may increase the speed at which future invasions take place, making new habitats at isolated locations available for non-indigenous species in shorter time periods than they would take without marine stepping stones. However, other factors such as hull fouling and ballast water exchange also strongly affect distribution of non-indigenous species. The marine stepping stone effect is important for the distribution of *M. edulis* and it may influence the distribution of other species with similar strategies in a similar way. The presence of offshore energy installations and other objects is the only way in which species such as *M. edulis* are able to survive at locations with deep bottoms.

Chapter 6 explains community patterns observed on artificial and natural reefs. The introduction of artificial hard substrates in an area dominated by a sandy seabed increases habitat available to epifouling organisms. Many biotic and abiotic variables influence the presence of epifouling species. Samples were taken on old offshore oil and gas platforms, and data were compared with a young wind farm and a natural reef on the Dutch Continental Shelf, in order to evaluate the influence of depth, age, orientation, substrate type and the presence of potential keystone species *Asterias rubens*, *M. edulis*, *Psammechinus miliaris* and *Metridium dianthus* on species richness and species composition. Species richness varied strongly between natural and artificial reefs. The number of observed species was 127 on platforms, 90 on the wind farm and 50 on the Borkum Reef Ground rocks. The predicted number of species on platforms (173) was much higher than the observed number, while for platforms it was almost identical (93). Depth ($p<0.001$), presence of *M. edulis* ($p<0.001$), *P. miliaris* ($p=0.005$) and orientation ($p=0.004$) all had a significant effect on species richness. Multivariate analysis showed a large overlap in communities on steel and rock, between different surface orientations and between the wind farm and platforms. There was a lack of overlap between communities on the rocks at the wind farm and natural reefs, but rocks around platforms and the natural reef showed overlap. Depth differences, location effects and substrate type influenced the species composition most strongly. Substrates with mixed surface orientation (i.a. rocks) hold the most species-rich communities. When an artificial

reef is supposed to be colonised by communities similar to those on a natural reef, its structure should resemble a natural reef as much as possible.

Chapter 7 evaluates the findings in this thesis and addresses the implications for offshore installations: Many of the species present on artificial reefs, live exclusively on and around hard substrates. The presence of hard substrates on a soft bottom, highly increases local biodiversity. When installations are removed, epifouling species will also be removed and will not return on the sandy seabed, decreasing local biodiversity. Some of the species present on offshore installations would never be present without intertidal zones, a habitat that does not belong in most of the offshore North Sea. Shallow parts of these structures host species communities that are not like natural subtidal offshore reefs. If platform foundations are left in place after their productive life has ended, one should aim to mimic natural (historic) hard substrate communities. The shallow intertidal zones should therefore be removed to prevent intertidal species from settling. This can be attained by toppling the foundations in place so all of it is permanently under water, or cutting them at a certain depth (e.g. 10 meters) and removing everything above that. Whether the communities become more like natural rocky reef communities, should then be monitored following the years after removal of the intertidal zone. For new developments, intertidal zones often cannot be prevented but one could aim to minimise the available substrate and try to prevent long term settlement of intertidal species by cleaning the shallow substrates regularly. The communities on the rock dump surrounding offshore installations are more like natural rocky reefs than the steel foundations are. Since rocky reefs are a protected habitat in the European Habitat Directive (H1170), the presence of this rock dump can be considered a positive effect. To stimulate this further, the addition of more rock dump to increase the area covered around new installations and future repurposed platforms should be considered. This will stimulate the development of communities and add rare species to the local biodiversity. In addition to attraction of rare species, rock dump may also serve as a stepping stone for these species, connecting isolated populations of species in the North Sea.

The structural difference between steel and rock, is the most likely cause of the differences in the epifouling communities present. To improve the ecological quality of offshore installations, alternatives to steel should be considered. Although I have not investigated the communities on concrete foundations, I did observe that the dominant species are different among steel and concrete. Possibly, concrete offers more small scale heterogeneity, stimulating different species to settle. This should be investigated more thoroughly in future research, by including concrete foundations of old platforms in the analysis performed in this thesis.

Future research should focus on answering the following questions:

- Which species on offshore reefs function as keystone species and how do these species influence the species community?
- In how far are the northern and southern North Sea connected through intertidal stepping stones? How will this change with the construction of new wind farms and other installations?
- How does the presence of artificial reefs in the North Sea increase the distribution of invasive species?

Samenvatting

Riffen behoren tot de meest soortenrijke habitattypen in de wereld. In gematigde wateren zijn riffen een thuis voor grote aantallen bodemsoorten, inclusief langlevende soorten. Wanneer riffen aanwezig zijn op zandige bodems, kan de lokale biodiversiteit meer dan verdubbeld worden en kan de biomassa verhonderdvoudigen. In Europa worden riffen beschouwd als belangrijk voor biodiversiteitsbehoud en de Europese Unie heeft riffen aangewezen als beschermd habitattype in haar Habitatrichtlijn. Het merendeel van de Noordzeebodem is bedekt met mobiele sedimenten zoals zand en slechts 20% is bedekt met stevige en harde substraten (riffen) zoals grindhoudend zand, grindbanken, stenen en keien.

In de Noordzee is een grote hoeveelheid kunstriffen aanwezig. In de archieven van hydrografische diensten en wrakduikers, zijn ongeveer 27.000 wraklocaties te vinden. Er zijn 1.500 offshore olie- en gasplatforms aanwezig. De oudste platforms dateren uit de jaren '60 in Schotland. In Nederland werden in de 70er jaren de eerste installaties gebouwd. Verder zijn er ongeveer 2.400 wind turbines aanwezig in de Noordzee, maar dit aantal stijgt jaarlijks door nieuwbouw van honderden windturbines. Het belangrijkste ecologische effect van het plaatsen van deze kustriffen in de Noordzee, is de toevoeging van kunstmatig hard substraat aan een zandige omgeving. Dit harde substraat creëert nieuw habitat voor soorten die niet op zandige bodems kunnen leven. De aanwezigheid van deze soorten op verschillende locaties, wordt beïnvloed door omgevingsverschillen en biologische variabelen. In dit proefschrift onderzoek ik de samenstelling van soortgemeenschappen op harde substraten. Eén van de doelen is het beter begrijpen van de invloed van deze variabelen op de aanwezigheid van soorten op riffen en op hun samenstelling.

Wanneer offshore bouwwerken geplaatst worden op de grenzen van de verspreidingspotentie van pelagische larven, kunnen zij functioneren als stapstenen waardoor de verspreiding van deze soorten wordt vergroot. Mijn doel is om dit zogenaamde stapsteeneffect te toetsen, door modellen die de verspreiding van deeltjes in de Noordzee beschrijven, te vergelijken met populatie genetische data van mosselen op kunstriffen.

Met hun hoge aantallen soorten, is de ecologische waarde van rotsige riffen hoog. Maar wat is de ecologische waarde van de duizenden kunstriffen in de Noordzee? In hoeverre zijn de soortgemeenschappen op rotsige riffen en kunstriffen gelijk? De Noordzeebodem is vooral bedekt met zandige sedimenten. In tegenstelling tot het verleden zijn harde substraten van natuurlijke afkomst nu zeldzaam, zeker in het Nederlandse deel van de Noordzee. Soorten op kunstriffen zouden daarom gezien kunnen worden als een onnatuurlijke toevoeging aan het bodemecosysteem. Maar zijn ze werkelijk zo onnatuurlijk? Om dit te onderzoeken, vergelijk ik de soortgemeenschappen op kunstriffen met die op natuurlijke rotsige riffen.

In hoofdstuk 2 onderzochten we een natuurlijk rif in de Noordzee: De Borkumse Stenen. Dit rif ligt ten noorden van Schiermonnikoog in een waterdiepte van 28 meter. We brachten de aanwezigheid van afwijkende bodembedekking in kaart met een side scan sonar survey. De verwachting was dat deze afwijkende bedekking vooral veroorzaakt werd door rots of grindbedekking. Om dit te bevestigen onderzochten we de bodem verder door o.a. monsters te nemen met een box corer en met duikers die airlift-monsters namen. Rond het rif troffen we hoge aantallen schelpkokerwormen *Lanice conchilega* in de zandbodem aan (>1.5000 ind. per m^2). Het is bekend dat deze soort de lokale biodiversiteit kan verhogen. Verder troffen we een rotsig rif aan dat bestond uit een combinatie van grind, stenen en keien op een kleibodem. In totaal vonden we 193 taxa. De soortenrijkdom op de zandbodem was significant hoger dan op de stenen, dit werd veroorzaakt door de hoge concentraties schelpkokerwormen. Bij een vergelijking tussen zand, schelpkokerwormvelden en stenen, was dit verschil tussen zand en stenen niet meer aanwezig, maar bleken de schelpkokerwormvelden wel rijker te zijn dan stenen. Doordat de riffen op een zandige bodem lagen, bleek de lokale biodiversiteit meer dan verdubbeld te worden. De gemeenschappen op het zand, de schelpkokerwormvelden en de stenen verschilden significant van elkaar. De schelpkokerwormvelden bleken in samenstelling tussen het zand en de stenen in te liggen. Zij zijn een soort tussenvorm van riffen en zand, waarschijnlijk doordat zij zand vastleggen in kleine harde structuren. Tijdens dit onderzoek werden meerdere soorten gevonden die voorheen niet eerder bij de Borkumse Stenen werden aangetroffen. Het rifgebied bedekt naar schatting 9,8 km^2, terwijl de schelpkokerwormvelden ongeveer 74 km^2 bedekken.

Hoofdstuk 3 beschrijft een onderzoek naar de biodiversiteit van de aangroei op offshore platforms met gebruik van video-opnamen gemaakt door op afstand bestuurbare onderzeeërs (remotely operated vehicles; ROVs). Om het begrip van de invloed van deze offshore platforms op het leven in zee te vergroten, is het noodzakelijk om informatie te verzamelen over de aanwezigheid van soorten op deze platforms. ROV-beelden van vijf offshore platforms in de Nederlandse Noordzee werden onderzocht. In totaal werden 30 taxa aangetroffen. De modelanalyse van de soortenrijkdom op de platforms liet zien dat er een significante invloed was van diepte, waarbij de soortenrijkdom eerst toenam met diepte maar na een optimum rond de 15 meter weer afnam. Verder werden er significante effecten gevonden van de leeftijd van de gemeenschap en de afstand tot de kust. Waarschijnlijk heeft het regelmatig verwijderen van de aangroei op locaties die dicht bij de kust staan, een invloed op de soortsamenstelling, omdat deze hierbij telkens verjongd wordt.

In hoofdstuk 4.1 beschrijf ik de ontdekking van het eierdopkoraal *Caryophyllia smithii* op een scheepswrak op de Doggersbank. Dit harde koraal was voorheen alleen bekend van de noordelijke Noordzee rond de Shetland en Orkney eilanden. Het werd niet eerder aangetroffen in de centrale zuidelijke Noordzee. Uit de literatuur blijkt dat de soort zich beperkt tot natuurlijke en kunstmatige harde substraten. Tijdens een wrakduikexpeditie

in 2014 troffen wij de soort aan op een oud scheepswrak. Gezien de larvale verspreiding van deze soort en de richting van de stroming in het gebied, is het waarschijnlijk dat deze exemplaren het wrak gekoloniseerd hebben als larven die afkomstig waren van een noordelijk gelegen ander wrak.

In hoofdstuk 4.2 onderzoek ik de geschiktheid van harde substraten in de Noordzee voor één invasieve en één inheemse bewoner van kunstriffen. Tijdens eerder werk viel ons op dat de invasieve Japanse spookkreeft *Caprella mutica* algemeen aanwezig was op harde substraten aan de kust, maar afwezig op de scheepswrakken die wij bezochten. Op deze wrakken was juist het inheemse wandelend geraamte *Caprella linearis* aanwezig, terwijl deze op kusten zelden werd aangetroffen. Dit vormde de aanleiding om op alle wrakken en platforms die ik bezocht, spookkreeften te verzamelen. Dit kon ik aanvullen met monsters van kustlocaties, scheepvaartboeien en windturbines op zee. Met deze monsters waren we in staat om habitat-geschiktheids-modellen te maken om te toetsen of het wandelend geraamte exclusief voorkwam op locaties zonder getijdenzone. De modellen lieten zien dat het voorkomen van de Japanse spookkreeft werd voorspeld door de aanwezigheid van drijvende objecten (boeien) en installaties met een getijdenzone. Verder bleek de concentratie deeltjes in het water, temperatuur, zoutgehalte en stroomsnelheid van belang voor beide soorten. Voor het wandelend geraamte waren juist sub-getijde locaties van belang. De voorspelde verspreiding van beide soorten verschilde significant. De inheemse soort kan waarschijnlijk op diepe locaties zonder getijdenzone overleven buiten het bereik van de invasieve soort.

Hoofdstuk 5 beschrijft hoe mosselen *Mytilus edulis* waarschijnlijk gebruikmaken van offshore installaties (windturbines, platforms, boeien) bij hun verspreiding naar locaties verder op zee. Het stapsteeneffect is breeduit beschreven in de literatuur. Deze hypothese stelt dat de bouw van installaties op de grenzen van de verspreidingsmogelijkheden van larven van hard substraat soorten, een verdere verspreiding van deze soorten naar normaal onbereikbaar gebied faciliteert. De nieuwe bouwwerken fungeren als stapstenen voor volgende generaties van de soort. Om dit te onderzoeken verzamelde ik mosselmonsters van locaties aan de kust en offshore bouwwerken (platforms, windmolens, boeien). Met gebruik van moleculaire markers brachten we de populatiegenetica van de soort in kaart. Als het stapsteeneffect aanwezig is, zouden patronen in deze data, verspeld kunnen worden met stromingsmodellen. Om dit te toetsen maakten we gebruik van deeltjesverspreidingsmodellen (particle-tracking models) waarmee de verspreiding van kunstmatige mossellarven in een computermodel werd gesimuleerd. De uitkomsten van dit model werden vergeleken met de gegevens over de populatie genetische verspreiding van de mosselen. Uit deze vergelijking bleek dat een deel van de variatie in de genetische data werd verklaard door de gemodelleerde afstand. Het is daarmee zeer waarschijnlijk dat mosselen inderdaad gebruikmaken van offshore installaties om zich verder te verspreiden op de Noordzee dan van nature mogelijk is. Het

stapsteeneffect is daarmee belangrijk voor de aanwezigheid van de mossel in gebieden ver op zee. Andere soorten (zowel inheems als uitheems) met een vergelijkbare levenscyclus, gebruiken de installaties mogelijk op dezelfde manier.

Hoofdstuk 6 probeert de patronen in de aangroei op offshore platforms, windmolens en de riffen van de Borkumse Stenen te verklaren. De aanwezigheid van soorten is afhankelijk van een samenspel van omgevingsinvloeden en de aanwezigheid van sleutelsoorten. Ik onderzocht de invloed van deze variabelen door de soortsamenstelling en soortenrijkdom in monsters van platforms te vergelijken met een deel van de gegevens uit hoofdstuk 2 en bestaande gegevens uit onderzoek in een windpark. Hierbij werd het belang van diepte, leeftijd van het substraat, oriëntatie van het substraat (horizontaal, verticaal, beiden), substraat type (steen of staal) en de aanwezigheid van mogelijke sleutelsoorten (gewone zeester *Asterias rubens*, mosselen, gewone zeeappel *Psammechinus miliaris* en de zeeanjelier *Metridium dianthus*) geëvalueerd. In totaal troffen we 127 soorten aan op de platforms, 90 op de windturbines en 50 op de stenen van de Borkumse Stenen. Het voorspelde aantal soorten was het hoogst voor platforms (173) terwijl de bemonstering van de windmolens bijna compleet leek te zijn met een voorspeld aantal van 93 soorten. De statistische analyse liet zien dat diepte, oriëntatie en de aanwezigheid van mosselen en zeeappels een significant effect hadden op de soortenrijkdom. Net als in hoofdstuk 3 was de soortenrijkdom het hoogst op gemiddelde diepten. Zowel mosselen als zeeappels hadden een positief effect op de soortenrijkdom. De samenstelling van de soortgemeenschappen liet een grote overeenkomst zien tussen zowel staal als steen en tussen de windturbines en de platforms. De natuurlijke stenen van de Borkumse Stenen overlapten echter niet met de steenbestortingen rond windturbines, terwijl ze wel overlapten met de stenen rond platforms. De hoogste soortenrijkdom werd gevonden op stenen, waarschijnlijk omdat hier verschillende oriëntaties aanwezig zijn in een klein oppervlak. Om de soortenrijkdom van stalen substraten meer op die van stenen te laten lijken, zou overwogen kunnen worden om het staal te bekleden met rotsachtig substraat.

In hoofdstuk 7 van dit proefschrift bespreek ik de uitkomsten van de voorgaande hoofdstukken in samenhang met elkaar en hun mogelijke belang voor de ontwikkelingen in de offshore industrie. Veel van de soorten die op de onderzochte installaties leven, zijn exclusief aanwezig op hard substraat. Door de aanwezigheid van kunstriffen op de zandige Noordzeebodem, neemt de lokale biodiversiteit toe. Wanneer bouwwerken aan het eind van hun levensduur verwijderd worden, zullen de soorten ook verdwijnen en zal de biodiversiteit weer afnemen omdat deze soorten niet op het zand kunnen overleven. Een deel van deze soorten zou zonder getijdenzone niet ver op zee aanwezig zijn. De ondiepe delen van installaties herbergen een soortgemeenschap die sterk verschilt van de gemeenschappen op diepe natuurlijke riffen. Indien delen van installaties achterblijven na verwijdering, zou men als doel moeten nemen om deze kunstriffen zoveel mogelijk te laten lijken op natuurlijke

offshore riffen. Daarom zou het deel aan het wateroppervlak verwijderd moeten worden om te voorkomen dat soorten uit de getijdenzone zich vestigen. Dit zou kunnen worden bereikt door installaties op de bodem te leggen waardoor alles onder water komt te liggen, of door het afsnijden van de installaties ruim onder het wateroppervlak, bijvoorbeeld op 10 meter diepte. Of de gemeenschappen daarna gaan lijken op natuurlijke riffen zou in opvolgende jaren onderzocht moeten worden. Het voorkomen van getijdenzones bij nieuw te bouwen installaties is meestal niet mogelijk. Om te voorkomen dat ongewenste soorten zich in deze zone vestigen zou men in het ondiepe deel regelmatig de aangroei moeten verwijderen. De soortsamenstelling op stenen op de bodem rond platforms lijkt meer op natuurlijke rotsige riffen dan dat de aangroei op staal daar op lijkt. Aangezien rotsige riffen een beschermd habitattype zijn in de Europese habitatrichtlijn (H1170), kunnen de zogenaamde 'rock dumps' gezien worden als een positief effect. Om dit effect te vergroten kan men meer rock dump aanbrengen rond bestaande en nieuwe installaties. Dit zal de lokale biodiversiteit stimuleren en de kans op zeldzame soorten vergroten.

Het structurele verschil tussen staal en steen, is de meest waarschijnlijke oorzaak van de verschillen in aangroeigemeenschappen tussen installaties en natuurlijke riffen. Om de ecologische kwaliteit van offshore installaties te verbeteren, zouden alternatieve bouwmaterialen overwogen kunnen worden. Alhoewel ik geen onderzoek gedaan heb naar de aangroei op betonnen structuren, heb ik wel de mogelijkheid gehad om één van beton gemaakt platform te bekijken op video. Dit leek veel te verschillen van een nabijgelegen stalen platform. Wellicht geeft de grove structuur van beton een substraat dat meer lijkt op dat van natuurlijk steen. Dit zou nader onderzocht moeten worden in een vergelijking tussen stalen en betonnen platforms.

Toekomstig onderzoek zou zich moeten richten op de volgende vragen:

• Welke soorten op kunstriffen fungeren als sleutelsoorten en hoe beïnvloeden zij de soortgemeenschap?
• In hoeverre zijn de noordelijke en zuidelijke Noordzee verbonden via stapstenen? Hoe zal dit veranderen door de aanleg van nieuwe windparken en andere installaties?
• Op welke manier vergroot de aanwezigheid van kunstriffen in de Noordzee de verspreidingsmogelijkheden van invasieve soorten?

Appendix

H 4.2 Supplement S1

Table S1. Full dataset of presence (1) and absence (0) of *Caprella* mutica (CM) and *Caprella* linearis (CL), including all locations used in the model and validation datasets. Sorted by latitude. Geographic coordinates in decimal degrees WGS84. NA: No data available.

All data included here are part of the following publication:

Distribution of the invasive Caprella mutica Schurin, 1935 and native Caprella linearis (Linnaeus, 1767) on artificial hard substrates in the North Sea: separation by habitat

Journal: Aquatic Invasions

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Location type	Year	Latitude	Longitude	CL	CM	Source
Buoy	2007	51.117	2.600	0	1	Cook et al (2007a)
Buoy	2015	51.160	2.692	0	1	Kerckhof unpublished data
Buoy	2013	51.163	2.717	1	0	Kerckhof unpublished data
Buoy	2006	51.217	2.633	0	1	Cook et al (2007a)
Buoy	2005	51.233	2.883	0	1	Cook et al (2007a)
Buoy	2010	51.242	2.922	0	1	Kerckhof unpublished data
Buoy	2014	51.242	2.915	0	1	Kerckhof unpublished data
Buoy	2011	51.243	2.925	0	1	Kerckhof unpublished data
Buoy	2014	51.247	2.922	0	1	Kerckhof unpublished data
Buoy	2014	51.248	2.905	0	1	Kerckhof unpublished data
Wreck	2002-2005	51.249	2.550	1	0	Zintzen and Massin (2010)
Buoy	2012	51.250	2.908	0	1	Kerckhof unpublished data
Wreck	2005	51.259	2.681	0	0	Zintzen and Massin (2010)
Buoy	2015	51.262	2.890	1	0	Kerckhof unpublished data
Wreck	2004	51.278	2.830	0	0	Zintzen and Massin (2010)
Buoy	2008	51.289	3.023	0	1	Kerckhof unpublished data

Location type	Year	Latitude	Longitude	CL	CM	Source
Buoy	2014	51.301	2.880	0	1	Kerckhof unpublished data
Buoy	2011	51.313	3.083	0	1	Kerckhof unpublished data
Buoy	2014	51.313	3.027	0	1	Kerckhof unpublished data
Buoy	1998	51.317	3.117	0	1	Cook et al (2007a)
Buoy	2003	51.317	3.117	0	1	Cook et al (2007a)
Buoy	2010	51.317	3.102	0	1	Kerckhof unpublished data
Buoy	2010	51.325	3.127	0	1	Kerckhof unpublished data
Buoy	2009	51.345	3.213	0	1	Kerckhof unpublished data
Buoy	2010	51.347	3.183	0	1	Kerckhof unpublished data
Buoy	2012	51.347	3.217	0	1	Kerckhof unpublished data
Buoy	2013	51.352	2.615	0	0	Kerckhof unpublished data
Buoy	2009	51.353	3.207	0	1	Kerckhof unpublished data
Buoy	2009	51.357	3.190	0	1	Kerckhof unpublished data
Buoy	2013	51.358	3.188	0	1	Kerckhof unpublished data
Buoy	2010	51.359	3.204	0	1	Kerckhof unpublished data
Buoy	2004	51.359	3.043	0	1	Kerckhof unpublished data
Buoy	2009	51.360	2.967	0	1	Kerckhof unpublished data
Buoy	2011	51.360	3.183	0	1	Kerckhof unpublished data
Buoy	2003	51.367	3.167	0	1	Cook et al (2007a)
Experimental frame	2007	51.367	3.117	0	1	Cook et al (2007a)
Buoy	2010	51.373	3.117	0	1	Kerckhof unpublished data
Buoy	2010	51.375	3.178	0	1	Kerckhof unpublished data
Buoy	2011	51.375	3.088	0	1	Kerckhof unpublished data
Buoy	2003	51.376	3.272	0	1	Kerckhof unpublished data
Buoy	1998	51.383	2.967	0	1	Cook et al (2007a)
Buoy	2005	51.383	2.767	0	1	Cook et al (2007a)
Buoy	2006	51.383	3.083	0	1	Cook et al (2007a)
Buoy	2014	51.385	3.200	0	1	Kerckhof unpublished data
Buoy	2007	51.386	3.002	0	1	Kerckhof unpublished data
Buoy	2008	51.387	3.248	0	1	Kerckhof unpublished data
Buoy	1998	51.391	3.304	0	1	Cook et al (2007a)
Buoy	2011	51.395	3.098	0	1	Kerckhof unpublished data
Wreck	2003-2005	51.396	2.497	0	0	Zintzen and Massin (2010)
Buoy	2006	51.400	2.733	0	1	Cook et al (2007a)
Buoy	2010	51.403	3.098	0	1	Kerckhof unpublished data
Buoy	2008	51.405	3.048	0	1	Kerckhof unpublished data

Location type	Year	Latitude	Longitude	CL	CM	Source
Buoy	2014	51.407	3.248	0	1	Kerckhof unpublished data
Buoy	2014	51.407	2.957	0	1	Kerckhof unpublished data
Wreck	2005	51.411	2.729	0	0	Zintzen and Massin (2010)
Gravel	2014	51.414	2.527	0	0	Kerckhof unpublished data
Wreck	2005	51.425	2.606	0	0	Zintzen and Massin (2010)
Buoy	2007	51.433	2.802	0	1	Cook et al (2007a)
Buoy	2007	51.455	2.989	0	1	Cook et al (2007a)
Buoy	2013	51.473	2.758	1	0	Kerckhof unpublished data
Wreck	2005	51.482	2.689	0	0	Zintzen and Massin (2010)
Wreck	2005	51.486	2.305	0	0	Zintzen and Massin (2010)
Buoy	2012	51.534	3.085	0	1	Kerckhof unpublished data
Wind farm	2008	51.547	2.928	0	0	Kerckhof unpublished data
Wind farm	2009	51.550	2.923	0	0	Kerckhof unpublished data
Buoy	2007	51.563	2.607	0	0	Kerckhof unpublished data
Buoy	2013	51.563	2.597	0	0	Kerckhof unpublished data
Artificial reef	2014	51.607	2.990	0	0	Kerckhof unpublished data
Buoy	2015	51.648	3.623	0	0	This study
Wreck	2001-2005	51.650	2.538	1	0	Zintzen and Massin (2010)
Wind farm	2010	51.652	2.792	1	0	Kerckhof unpublished data
Wind farm	2011	51.653	2.787	0	0	Kerckhof unpublished data
Wind farm	2012	51.685	2.812	1	0	Kerckhof unpublished data
Wreck	2005	51.699	2.622	0	0	Zintzen and Massin (2010)
Artificial reef	2014	51.702	2.705	0	0	Kerckhof unpublished data
Buoy	2015	51.865	2.903	0	0	This study
Buoy	2014	51.866	3.857	0	1	This study
Buoy	2015	51.909	3.924	0	0	This study
Buoy	2014	51.938	3.594	0	1	This study
Buoy	2015	51.948	3.950	0	0	This study
Buoy	2015	51.990	4.028	0	0	This study
Buoy	2015	51.991	4.060	0	1	This study
Buoy	2015	52.048	4.060	0	0	This study
Buoy	2015	52.119	3.744	0	0	This study
Wreck	2013	52.139	3.485	1	0	This study
Buoy	2015	52.170	3.403	0	0	This study
Buoy	2014	52.172	3.869	0	0	This study
Buoy	2015	52.208	3.857	0	0	This study

Location type	Year	Latitude	Longitude	CL	CM	Source
Buoy	2014	52.223	3.741	0	0	This study
Wreck	2013	52.255	3.689	1	0	This study
Wreck	2013	52.437	3.733	1	0	This study
Wreck	2015	52.471	3.783	0	NA	This study
Buoy	2015	52.474	4.397	0	1	This study
Buoy	2015	52.494	3.881	0	0	This study
Buoy	2015	52.496	4.200	0	0	This study
Buoy	2015	52.509	4.339	0	0	This study
Wreck	2013	52.556	3.723	1	0	This study
Wind farm	2013	52.574	4.211	1	0	Vanagt and Faasse (2014)
Wind farm	2013	52.581	4.217	1	0	Vanagt and Faasse (2014)
Wind farm	2011	52.582	4.449	1	1	Bouma and Lengkeek (2013)
Wind farm	2013	52.587	4.246	1	0	Vanagt and Faasse (2014)
Wreck	2013	52.598	3.751	1	0	This study
Wind farm	2013	52.605	4.241	1	1	Vanagt and Faasse (2014)
Wind farm	2011	52.605	4.397	0	1	Bouma and Lengkeek (2013)
Wreck	2013	52.613	3.593	1	0	This study
Wind farm	2011	52.623	4.431	0	1	Bouma and Lengkeek (2013)
Buoy	2014	52.766	4.216	0	0	This study
Wreck	2015	52.766	4.213	0	0	This study
Wreck	2013	52.767	3.229	1	0	This study
Wreck	2013	52.788	3.818	1	0	This study
Buoy	2015	52.909	3.409	0	0	This study
Wreck	2014	52.938	4.578	0	0	This study
Wreck	2013	52.966	4.352	1	0	This study
Wreck	2013	52.995	4.247	1	0	This study
Wreck	2013	53.000	2.876	1	0	This study
Buoy	2005	53.033	4.683	0	1	Cook et al (2007a)
Wreck	2013	53.071	3.232	1	0	This study
Wreck	2015	53.121	4.207	1	0	This study
Rocky reef	2013	53.180	4.589	1	0	This study
Buoy	2014	53.282	4.660	0	1	This study
O&G installation	2014	53.330	4.830	1	0	This study
O&G installation	2014	53.400	4.200	1	0	This study
O&G installation	2014	53.403	4.201	1	0	This study
Buoy	2014	53.410	5.189	0	0	This study

Location type	Year	Latitude	Longitude	CL	CM	Source
O&G installation	2014	53.417	4.183	1	0	This study
O&G installation	2014	53.417	4.233	1	0	This study
Buoy	2014	53.419	3.826	0	0	This study
Buoy	2014	53.436	5.162	0	1	This study
Wreck	2013	53.442	3.799	0	0	This study
O&G installation	2014	53.450	3.900	1	0	This study
Wreck	2015	53.463	4.819	1	0	This study
Buoy	2014	53.469	5.359	0	1	This study
Wreck	2013	53.487	3.287	0	0	This study
O&G installation	2014	53.490	4.195	1	0	This study
Buoy	2015	53.495	4.062	0	0	This study
O&G installation	2014	53.520	3.992	0	0	This study
Buoy	2014	53.573	6.501	0	0	This study
Lanice conchilega reef	2013	53.575	6.154	0	0	Coolen et al. (2015a)
Sand	2013	53.614	6.332	0	0	Coolen et al. (2015a)
Wreck	2015	53.615	5.507	1	0	This study
Lanice conchilega reef	2013	53.626	6.244	0	0	Coolen et al. (2015a)
Sand	2013	53.626	6.192	0	0	Coolen et al. (2015a)
Sand	2013	53.634	6.365	0	0	Coolen et al. (2015a)
Sand	2013	53.640	6.334	0	0	Coolen et al. (2015a)
Lanice conchilega reef	2013	53.677	6.145	0	0	Coolen et al. (2015a)
Rocky reef	2013	53.685	6.344	0	0	Coolen et al. (2015a)
Rocky reef	2013	53.686	6.343	1	0	Coolen et al. (2015a)
Rocky reef	2013	53.688	6.343	0	0	Coolen et al. (2015a)
Buoy	2014	53.697	6.230	0	0	This study
Wreck	2015	53.711	4.810	1	0	This study
Buoy	2014	53.715	3.097	1	0	This study
Buoy	2014	53.736	6.620	0	0	This study
Wreck	2013	53.815	4.987	1	0	This study
Wreck	2014	53.826	5.227	1	0	This study
Sand	2011	53.841	6.259	0	0	Glorius et al. (2012)
Buoy	2015	53.883	3.498	0	0	This study
Sand	2011	53.886	6.286	0	0	Glorius et al. (2012)
Sand	2011	53.886	6.259	0	0	Glorius et al. (2012)

Location type	Year	Latitude	Longitude	CL	CM	Source
Sand	2011	53.891	6.273	0	0	Glorius et al. (2012)
Sand	2011	53.891	6.246	0	0	Glorius et al. (2012)
Sand	2011	53.891	6.259	0	0	Glorius et al. (2012)
Sand	2011	53.893	6.259	0	0	Glorius et al. (2012)
Gravel	2011	53.895	6.320	0	0	Glorius et al. (2012)
Gravel	2011	53.895	6.290	0	0	Glorius et al. (2012)
Sand	2011	53.895	6.263	0	0	Glorius et al. (2012)
Gravel	2011	53.895	6.275	0	0	Glorius et al. (2012)
Sand	2011	53.895	6.229	0	0	Glorius et al. (2012)
Gravel	2011	53.895	6.267	0	0	Glorius et al. (2012)
Sand	2011	53.895	6.259	0	0	Glorius et al. (2012)
Sand	2011	53.895	6.252	0	0	Glorius et al. (2012)
Sand	2011	53.895	6.244	0	0	Glorius et al. (2012)
Sand	2011	53.895	6.256	0	0	Glorius et al. (2012)
Gravel	2011	53.897	6.259	0	0	Glorius et al. (2012)
Gravel	2011	53.900	6.273	0	0	Glorius et al. (2012)
Gravel	2011	53.900	6.259	0	0	Glorius et al. (2012)
Sand	2011	53.900	6.246	0	0	Glorius et al. (2012)
Gravel	2011	53.904	6.286	0	0	Glorius et al. (2012)
Gravel	2011	53.904	6.259	0	0	Glorius et al. (2012)
Wreck	2014	53.949	3.162	1	0	This study
Gravel	2011	53.949	6.259	0	0	Glorius et al. (2012)
Wind farm	2012	54.008	6.594	0	0	AWI unpublished data
Research platform	2006	54.014	6.588	0	0	AWI unpublished data
Sand	2012	55.333	4.551	0	0	Glorius et al. (2013)
Sand	2012	55.410	4.576	0	0	Glorius et al. (2013)
Sand	2012	55.411	4.531	0	0	Glorius et al. (2013)
Sand	2012	55.414	4.554	0	0	Glorius et al. (2013)
Sand	2012	55.417	4.565	0	0	Glorius et al. (2013)
Sand	2012	55.417	4.543	0	0	Glorius et al. (2013)
Sand	2012	55.420	4.559	0	0	Glorius et al. (2013)
Sand	2012	55.420	4.548	0	0	Glorius et al. (2013)
Sand	2014	55.420	3.593	0	0	Glorius et al. (2014a)
Sand	2012	55.421	4.712	0	0	Glorius et al. (2013)
Sand	2012	55.422	4.557	0	0	Glorius et al. (2013)
Sand	2012	55.422	4.551	0	0	Glorius et al. (2013)

Location type	Year	Latitude	Longitude	CL	CM	Source
Sand	2012	55.422	4.556	0	0	Glorius et al. (2013)
Sand	2012	55.422	4.553	0	0	Glorius et al. (2013)
Sand	2012	55.423	4.570	0	0	Glorius et al. (2013)
Sand	2012	55.423	4.554	0	0	Glorius et al. (2013)
Sand	2012	55.423	4.538	0	0	Glorius et al. (2013)
Sand	2012	55.424	4.556	0	0	Glorius et al. (2013)
Sand	2012	55.424	4.553	0	0	Glorius et al. (2013)
Sand	2012	55.425	4.557	0	0	Glorius et al. (2013)
Sand	2012	55.425	4.551	0	0	Glorius et al. (2013)
Sand	2012	55.425	4.396	0	0	Glorius et al. (2013)
Sand	2012	55.426	4.560	0	0	Glorius et al. (2013)
Sand	2012	55.426	4.549	0	0	Glorius et al. (2013)
Sand	2012	55.429	4.566	0	0	Glorius et al. (2013)
Sand	2012	55.430	4.543	0	0	Glorius et al. (2013)
Sand	2012	55.432	4.554	0	0	Glorius et al. (2013)
Sand	2012	55.436	4.577	0	0	Glorius et al. (2013)
Sand	2012	55.436	4.532	0	0	Glorius et al. (2013)
Sand	2014	55.455	4.021	0	0	Glorius et al. (2014a)
Sand	2014	55.464	4.005	0	0	Glorius et al. (2014a)
Sand	2014	55.465	3.594	0	0	Glorius et al. (2014a)
Wind farm	2005	55.469	7.849	NA	1	Leonhard and Frederiksen (2006)
Sand	2014	55.470	4.001	0	0	Glorius et al. (2014a)
Sand	2014	55.471	3.585	0	0	Glorius et al. (2014a)
Sand	2014	55.472	3.595	0	0	Glorius et al. (2014a)
Sand	2014	55.472	3.591	0	0	Glorius et al. (2014a)
Sand	2014	55.472	3.594	0	0	Glorius et al. (2014a)
Sand	2014	55.472	3.592	0	0	Glorius et al. (2014a)
Sand	2014	55.472	3.594	0	0	Glorius et al. (2014a)
Sand	2014	55.472	3.594	0	0	Glorius et al. (2014a)
Sand	2014	55.472	4.003	0	0	Glorius et al. (2014a)
Sand	2014	55.473	4.003	0	0	Glorius et al. (2014a)
Sand	2014	55.473	3.594	0	0	Glorius et al. (2014a)
Sand	2014	55.473	3.593	0	0	Glorius et al. (2014a)
Sand	2014	55.473	3.592	0	0	Glorius et al. (2014a)
Sand	2014	55.473	3.594	0	0	Glorius et al. (2014a)
Sand	2014	55.474	3.596	0	0	Glorius et al. (2014a)

Location type	Year	Latitude	Longitude	CL	CM	Source
Sand	2014	55.474	3.591	0	0	Glorius et al. (2014a)
Sand	2014	55.474	3.581	0	0	Glorius et al. (2014a)
Sand	2014	55.475	4.002	0	0	Glorius et al. (2014a)
Sand	2014	55.475	3.585	0	0	Glorius et al. (2014a)
Sand	2014	55.481	4.006	0	0	Glorius et al. (2014a)
Sand	2014	55.481	3.581	0	0	Glorius et al. (2014a)
Wind farm	2005	55.484	7.846	NA	1	Leonhard and Frederiksen (2006)
Wind farm	2005	55.484	7.881	NA	1	Leonhard and Frederiksen (2006)
Wind farm	2005	55.493	7.825	NA	1	Leonhard and Frederiksen (2006)
Sand	2014	55.494	3.505	0	0	Glorius et al. (2014a)
Wind farm	2005	55.499	7.876	NA	1	Leonhard and Frederiksen (2006)
Wind farm	2005	55.505	7.876	NA	1	Leonhard and Frederiksen (2006)
Sand	2012	55.513	4.558	0	0	Glorius et al. (2013)
Sand	2014	55.525	3.594	0	0	Glorius et al. (2014a)
Sand	2014	55.789	4.013	0	0	Glorius et al. (2014b)
Sand	2014	55.794	3.996	0	0	Glorius et al. (2014b)
Sand	2014	55.794	4.026	0	0	Glorius et al. (2014b)
Sand	2014	55.794	3.997	0	0	Glorius et al. (2014b)
Sand	2014	55.795	4.001	0	0	Glorius et al. (2014b)
Sand	2014	55.796	4.005	0	0	Glorius et al. (2014b)
Sand	2014	55.797	4.004	0	0	Glorius et al. (2014b)
Sand	2014	55.797	4.003	0	0	Glorius et al. (2014b)
Sand	2014	55.798	4.007	0	0	Glorius et al. (2014b)
Sand	2014	55.799	4.039	0	0	Glorius et al. (2014b)
Sand	2014	55.799	4.011	0	0	Glorius et al. (2014b)
Sand	2014	55.800	4.014	0	0	Glorius et al. (2014b)
Sand	2014	55.801	4.018	0	0	Glorius et al. (2014b)
Sand	2014	55.802	4.017	0	0	Glorius et al. (2014b)
Sand	2014	55.802	4.016	0	0	Glorius et al. (2014b)
Sand	2014	55.803	4.021	0	0	Glorius et al. (2014b)
Sand	2014	55.804	4.024	0	0	Glorius et al. (2014b)
Sand	2014	55.804	3.995	0	0	Glorius et al. (2014b)
Sand	2014	55.805	4.027	0	0	Glorius et al. (2014b)
Sand	2014	55.806	4.031	0	0	Glorius et al. (2014b)
Sand	2014	55.807	4.031	0	0	Glorius et al. (2014b)
Sand	2014	55.807	4.030	0	0	Glorius et al. (2014b)

Location type	Year	Latitude	Longitude	CL	CM	Source
Sand	2014	55.808	4.034	0	0	Glorius et al. (2014b)
Sand	2014	55.809	4.037	0	0	Glorius et al. (2014b)
Sand	2014	55.809	4.009	0	0	Glorius et al. (2014b)
Sand	2014	55.810	4.041	0	0	Glorius et al. (2014b)
Sand	2014	55.814	4.022	0	0	Glorius et al. (2014b)
Marina	2012	59.539	-1.603	NA	0	NAFC 2015 (unpublished data)
Marina	2013	59.885	-1.285	NA	0	NAFC 2015 (unpublished data)
Settling plates	2013	60.081	-1.332	NA	1	NAFC 2015 (unpublished data)
Dive site	2014	60.134	-1.285	NA	1	NAFC 2015 (unpublished data)
Marina	2012-2013	60.137	-1.267	NA	1	NAFC 2015 (unpublished data)
Pier legs	2014	60.154	-1.142	NA	1	NAFC 2015 (unpublished data)
Settling plates	2014	60.169	-1.161	NA	0	NAFC 2015 (unpublished data)
Marina	2012	60.186	-1.438	NA	0	NAFC 2015 (unpublished data)
Marina	2012	60.226	-1.562	NA	0	NAFC 2015 (unpublished data)
Intertidal beach	2012	60.379	-1.387	NA	0	NAFC 2015 (unpublished data)
Marina	2013	60.392	-1.366	NA	0	NAFC 2015 (unpublished data)
Dive site	2014	60.397	-1.381	NA	0	NAFC 2015 (unpublished data)
Intertidal beach	2014	60.400	-1.120	NA	0	NAFC 2015 (unpublished data)
Marina	2012	60.423	-0.761	NA	1	NAFC 2015 (unpublished data)
Ferry terminal	2012	60.488	-1.149	NA	0	NAFC 2015 (unpublished data)
Marina	2013	60.497	-1.044	NA	0	NAFC 2015 (unpublished data)
Marina	2013	60.541	-1.349	NA	0	NAFC 2015 (unpublished data)
Marina	2012	60.702	-1.005	NA	1	NAFC 2015 (unpublished data)

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After sampling, I always arrived at the lab with large containers with epifouling species. I started with limited experience in identifying these species. I had to learn to identify most of them and luckily I had two experienced colleagues to teach me the inner workings of a benthic lab. Joël Couperus and Babeth van der Weide spent a lot of time helping me out at first, even though my research project initially lacked funding to cover their direct hours target. After a while the sample processing operation became more efficient and at some point even funded by the oil and gas industry, so we could really start working on this project as a team. I am very grateful for all the time Joël and Babeth spent on my samples and for the advice they gave during all the years we worked together. Furthermore, I thank many other colleagues and others who contributed to the PhD-thesis process: Piet Wim van Leeuwen, Edwin Foekema, Klaas Kaag, Erik Meesters, Santiago Alvarez Fernandez, Arno Kangeri, Maarten de Jong, Klaus Luke, Andre Meiboom, Andrea Sneekes, Erika Koelemij, Martijn Spierings, Inês Dias from Wageningen Marine Research, Ilse de Mesel from RBINS, Floor Driessen from Bureau Waardenburg, Roland Krone from Krone-projekte and Rob Dekker from NIOZ.

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At sea, I spent most of the time on or below deck, since the time spent under water regularly only takes up less than 10% of each day. During my trips with Duik de Noordzee schoon, sailing with the Cdt. Fourcault, much fun was shared with the royal underwater photography team, which consists of Cor Kuyvenhoven and Udo van Dongen, who taught me about 'Brabants eten' (which was unknown to me even though I was born and raised in Brabant), and 'Lekker met je handen werken'. After ten days at sea, anything becomes funny with these guys. The Duik de Noordzee Team consists of many, many volunteers, who all contribute in different ways, but some people contributed significantly to my work by making

At least 50% of the work carried out in the lab, sorting and identifying specimens in my mixed samples, was done by students from universities (including Hogescholen) in the Netherlands. They not only performed practical work, helping me obtain more data, but also inspired me with their questions and comments on my own work. I thank all of them and hope they can start carreers just as interesting as mine after they graduate: Tristan da Graça, Tim van der Stap, Jasmijn Noorlander, Esther Beukhof, Jerry Lust, Mark van Oorschot, Floor Driessen, Lana Allertz, Caroline Schlepper, Jorien van Schie, Douwe Boerstra, Youri van der Meer, Elsa Poorter, Maxime Blomberg.

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The SENSE Research School declares that **Mr Joop Coolen** has successfully fulfilled all requirements of the Educational PhD Programme of SENSE with a work load of 52.9 EC, including the following activities:

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o Environmental research in context (2014)
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Other PhD and Advanced MSc Courses

o Basic statistics, Wageningen University (2013)
o Introduction to R for statistical analysis, Wageningen University (2013)
o Multivariate methods for ecologists, Swedish University of Agricultural Sciences (2014)
o Writing a scientific article, VU University (2014)
o Data management planning, Wageningen University (2014)
o Data exploration, regression, GLM and GAM, Highland Statistics Limited (2014)
o Introduction to mixed modelling and GLMM - Bayesian version, Highland Statistics Limited (2015)
o Introduction to GAM and GAMM, Highland Statistics Limited (2015)
o Introduction to zero inflated GLM and GLMM, Highland Statistics Limited (2015)
o Introduction to using GIS in marine biology, GIS in Ecology (2015)
o Introduction to species distribution modelling in the marine environment, GIS in Ecology (2015)

External training

o Commercial diver training, Dutch Navy (2012)

Management and Didactic Skills Training

o Supervising ten BSc students for theses and internships at Wageningen Marine Research (2014-2016)
o Supervising three MSc students for theses and internships at Wageningen Marine Research (2014-2016)
o Project manager for Wageningen Marine Research research projects (2015-2016)

(Selection of) Oral Presentations

o *Introduction to PhD work.* Working Group on Marine Benthal and Renewal Energy Developments (WGMBRED) meeting, 21-25 April 2015, Oban, United Kingdom
o *Biodiversity of Offshore Platforms.* Rigs-2-Reefs meeting, 18 June 2015, The Hague, the Netherlands
o *Biodiversity North Sea artificial reefs.* Noordzeedagen, 01-02 October 2015, Vlissingen, the Netherlands

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