



RECON: Reef effect structures in the North Sea, islands or connections?

Summary Report

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Summary

The RECON project was carried out from October 2015 till September 2017 and was part of the INSITE programme wherein the INfluence of man-made Structures In the Ecosystem is being investigated. The project "RECON: Reef effects of structures in the North Sea, islands or connections?" was initiated to investigate and model species patterns and inter-connectivity of reef communities on offshore structures in the North Sea.

The main research questions of the RECON project were:

1. What is the species composition of marine growth on offshore structures such as wind farms and oil and gas platforms?
2. To what extent is this composition explained by abiotic factors (e.g. depth, temperature, location, platforms age, marine growth, cleaning frequency, et cetera) and biotic factors (e.g. food availability, proximity to marine growth on other offshore structures, distance to coastal populations, et cetera)?
3. To what extent are the communities on the structures isolated from or connected to each other and how is this explained by the factors noted earlier?

Species composition on offshore structures

These questions were addressed in RECON sub-projects. Using ROV inspection videos, a total of 38 species were identified on eight Dutch and nine Danish offshore gas platforms in the southern North Sea. A significant clustering of species communities was found based on geographical location. In addition, within each geographical region, a clustering was found based on depth range, with the intertidal, bottom and middle zones splitting off separately. Future research should focus on the influence of habitat complexity, substrate orientation and inter-specific relations on species assemblages on offshore platforms. In addition, it would be interesting to know if a similar geographical pattern could be identified for species communities on ship wrecks.

Samples of marine growth were analysed to create a complete overview of the species community of oil and gas platforms and were compared with communities from wind farms and rocky reefs. It appeared that in the Dutch part of the North Sea, species composition is different on artificial compared to natural reefs. Depth, location effect and habitat type influence the species composition most strongly. The number of observed species was 138 on platforms, 95 on the wind farm and 48 on the Borkum Reef Ground rocks. Although rocky substrates hold the most species rich communities, overall biodiversity is also strongly influenced by keystone species (*Mytilus edulis*, *Psammechinus miliaris* and Tubulariidae). 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 limited overlap between communities on the relatively young rocks at the wind farm and natural reefs, while rocks around platforms and the natural reef showed more overlap. 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. Experiments wherein small-scale heterogeneity is introduced by changes to the surface of substrates, e.g. a coating with gravel and stones, are needed to verify this.

DNA barcoding and metabarcoding may in coming years develop in faster and cheaper alternatives for species identification. DNA metabarcoding couples the principles of barcoding with high-throughput parallel sequencing. In this case, species in an environmental sample can be identified by comparing obtained sequences to a standard reference library of sequences from known organisms. The RECON project aimed to further develop metabarcoding techniques for identification of species in mixed macrofauna samples taken on artificial structures in the North Sea. To attain this, samples were taken from 13 shipwrecks on a transect roughly along the Dutch coast. At each location, scuba divers recovered nets lost by fishermen from the shipwreck. Samples from salvaged nets were taken after each dive. In the lab, all fauna present on the sampled nets was collected and processed using molecular methods, where DNA was extracted, coding regions for genes 18s and 28s were amplified

and thereafter sequenced. A large number of unique sequences was found, indicating a diverse community. However, >90% of these sequences could not be assigned to a species or genus. To reach more reliable identification to species level, additional primers should be developed and open access reference datasets enlarged. No clear difference in sample-richness and species composition was found between net types. A larger species pool was discovered on beam trawl-nets compared to set nets. Using presence-absence data it is challenging to detect differences in species composition, therefore barcoding methods that include species densities should be developed.

Connectivity between offshore structures

The stepping-stone effect on marine species has been suggested in recent papers. It describes the spread of epifaunal organisms via isolated structures as stepping stones to new areas which 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 and the number of migrations per generation between the populations. *Mytilus edulis* populations were present throughout the investigated area, showing that larvae are able to reach offshore locations. Larval transport by currents has probably contributed to the initial colonisation. Direct connectivity between some locations was also shown by the particle tracking models, although this could not be validated using genetic data. Possibly the distance between the studied locations is too large for direct larval exchange. To unravel these patterns further, adding in-between locations is recommended for future research. A large genomic influence of *Mytilus galloprovincialis* was found at the study sites.

The connectivity of the small tube dwelling crustacean *Jassa herdmani* was also studied. In contrast to *M. edulis*, it can be expected for *J. herdmani* to have more pronounced population structures. *J. herdmani* has an offshore distribution, where it is bound to the natural hard-substrates bound to the seafloor. Its sedentary way of life and lack of a planktonic larval stage further reduces its ability to disperse over wider areas. The dataset produced covered 514 individuals from 17 locations, involving 42 unique haplotypes to assess connectivity. The results from the analysis show that *J. herdmani* is very differentiated in the North Sea. There is hardly any connectivity of the populations of *Jassa* amongst the different sampling locations detected in this study. Apparently, once *J. herdmani* has colonized the hard substratum, it develops a distinct population, enabled by its short life-cycle and limited dispersal capacity.

Recommendations

The results obtained in RECON should be used in future decisions on the installation of wind farms and decommissioning of oil and gas platforms. These installations harbour a high biodiversity and connect populations. Communities on scour protections are similar to natural reefs and therefore leaving in place these rocks and potentially other parts of installations should be considered in decommissioning decisions. Connectivity between installations depends on species' life cycles. Some of the connected species populations may be non-indigenous, others native. Depending on life cycles and species status (e.g. OSPAR protected or non-native), different leave-in-place options should be considered during decommissioning. These options should include leaving in place the foundations as they are, removing part of the foundations to depths with limited risk of non-indigenous colonisation, or full removal.

To enlarge the scientific base for these decisions, the following research should be carried out:

- A) Increase the spatial distribution of locations to be investigated, using identical methods as applied in RECON;
- B) Include concrete substrate as a variable of influence on species composition;
- C) Improve the connectivity work using *Mytilus edulis* by adding in-between locations to the current dataset and use single nucleotide polymorphism to obtain higher resolution data;
- D) Develop methods to attain samples from locations beyond diving depth, e.g. by a combination of remote sampling using ROVs and metabarcoding high numbers of samples.

1 Introduction

1.1 The RECON Project within the INSITE Programme

The RECON project, “Reef effects of structures in the North Sea, Islands or connections?”, was initiated to investigate and model species patterns and inter-connectivity of reef communities on offshore structures in the North Sea.

The RECON project was part of the INSITE North Sea programme wherein the INfluence of man-made Structures In the Ecosystem is being investigated. INSITE is a major industry-sponsored project with the overall aim of providing stakeholders with the independent scientific evidence-base needed to better understand the influence of man-made structures on the ecosystem of the North Sea. Its primary objectives were to help establish a) the magnitude of the effects of man-made structures compared to the spatial and temporal variability of the North Sea ecosystem, considered on different time and space scales; and b) to what extent, if any, the man-made structures in the North Sea represent a large inter-connected hard substrate system.

A number of studies were defined within the RECON project that contribute to the objectives of INSITE. First, community data from taxonomic species inventories were being collected to identify species communities in relation to the geographical location and depth of offshore structures. Second, a cost efficient method for inventory of communities on hard substrates was developed using state-of-the-art DNA metabarcoding. Third, the genetic population structures of the mussel *Mytilus edulis* and crustacean *Jassa herdmani*, abundant invertebrates with different dispersal strategies, were analysed to investigate the connectivity of populations from different platforms. The data from this study and other available data were used to model the distribution of species on offshore structures and their inter-connectedness.

The main research questions of the RECON project were:

1. What is the species composition of marine growth on offshore structures?
2. To what extent is this composition explained by abiotic factors (e.g. depth, temperature, location, platforms age, marine growth cleaning frequency, et cetera) and biotic factors (e.g. food availability, proximity to marine growth on other offshore structures, distance to coastal populations, et cetera)?
3. To what extent are the communities on the structures isolated from or connected to each other and how is this explained by the factors noted earlier?

In addition to analysing existing footage collected with remotely operated vehicles, we carried out fieldwork in a highly cost efficient manner, using fully equipped mobilized diving-vessels already present at offshore installations for regular inspection and maintenance work. ENGIE provided in kind co-funding by allowing our certified offshore diver-marine biologists to access their dive support vessels. This approach was implemented frequently and proved to be a highly successful, unique and cost-efficient method for field inventories of reef communities on offshore installations. Part of the work was financed with cooperation and co-funding from several companies. The work was carried out by junior and senior researchers under professorial supervision.

1.2 Funding & partners

RECON was supported by the INSITE Programme [Foundation Phase, 2016-2017], the Wageningen UR TripleP@Sea Innovation program under Grant KB-14-007; the Dutch Department of Economic Affairs under Grant KB-24-002-001, the Nederlandse Aardolie Maatschappij BV, Wintershall Holding GmbH, Energiebeheer Nederland B.V. and Maersk Oil Gas Denmark. Furthermore, ENGIE Exploration &

Production Nederland B.V. and the Dive the North Sea clean foundation supported the project in kind by allowing access to diving support vessels and data.

The RECON project was carried out by a research consortium of Wageningen Marine Research, Bureau Waardenburg B.V., the Royal Netherlands Institute for Sea Research, Krone-Projekte and Wageningen University Chair groups Aquatic Ecology and Water Quality Management, Marine Animal Ecology and Animal Breeding and Genetics. Furthermore, Deltares, eCOAST, Ecosub and the Royal Belgian Institute of Natural Sciences provided in kind support to the research. The RECON project was linked to the UNDINE project by sharing resources on studying connectivity of *Mytilus edulis* populations.

1.3 Scientific background

The presence of hard substrate habitats is an important factor in determining the distribution of specific species in the North Sea (Zintzen and Massin 2010). In the past, more hard substrate habitat in the North Sea was present than today. A comparison of old maps with the present day situation shows striking differences between past and present (Olsen 1883). There used to be large oyster reefs (*Ostrea edulis*), or groups of smaller reefs between the Dogger Bank and the Dutch and German coast (Olsen 1883). This species disappeared almost entirely from the North Sea due to overfishing (de Vooy et al. 2004) and failing recruitment. The oyster reefs were expectedly home to many species depending on hard substrate habitat for settlement or shelter (Houziaux et al. 2011). These natural reefs have largely disappeared but new man-made hard substrates have increased over the last century and may have partly taken over their role in the local ecosystem (Figure 1).

From research on offshore structures in the North Sea the overall conclusion can be drawn that the communities on the structure show a typical zonation (Van Buuren 1984; Lindeboom et al. 2011; Krone et al. 2013). Colonization changes with time whereby a pioneer community of hydroids and tubeworms is followed by longer living slower colonizers resulting in an increased complexity of the ecosystem (Whomersley and Picken 2003). Foundations of offshore installations are home to species such as the cold water coral *Alcyonium digitatum* (van der Stap et al. 2016) while the highly vulnerable tube worm *Sabellaria spinulosa* can build reefs around these substrates (personal observation by J.W.P. Coolen). European lobsters (*Homarus gammarus*) are also known to use offshore structures as habitat (Krone and Schröder 2011).

Artificial hard substrates can also aid in the colonisation of the far offshore North Sea by coastal species. These organisms may use the structures as 'stepping stones' to colonise the otherwise impassable sand bottomed Dutch part of the North Sea (Sheehy and Vik 2010). Depending on currents, species may distribute using offshore structures (Thorpe 2012). Many identical species are present when comparing offshore wind farms, O&G platforms or shipwrecks (Zintzen et al. 2006; Bouma and Lengkeek 2013; van der Stap et al. 2016) but it remains unknown if these structures are connected.

Most previous studies on the biodiversity and connectivity of offshore structures focused on wind farms and ship wrecks. While wind farm foundations are structurally similar to oil and gas platforms, they are relatively young when compared to oil and gas platforms that age up to 40 years in the Dutch part of the North Sea. Shipwrecks can be significantly older than offshore platforms, but lack an intertidal zone so several species present on O&G platforms are missing from shipwrecks (J.W.P. Coolen personal observation).

In the RECON project, the biodiversity and connectivity of platforms, ship wrecks, wind farms and navigational buoys, located in the southern part of the North Sea were investigated. Additional data were made available by industry and scientific partners. All contents of this report has been or will be published in scientific literature. All data generated within the RECON project will be made publicly available.

1.4 Reader's guide

In the following chapters, the separate studies that have been carried out within the RECON project are summarized and reference is made to their final products. The chapter on visual observations concerns an analysis of ROV-inspection footage to identify species assemblages on platforms at different geographical ranges and depth zones. The chapter on taxonomic identifications from field samples provides more detail on the biodiversity of fouling communities. The taxonomic identification led to the discovery of some new species for the area, and an additional chapter is devoted to these. The chapter on metabarcoding contains a summary of the work carried out so far, for which results were not available at the time of writing of this report. The next two chapters analyse the population genetics structure of two reef related species having different dispersal strategies. One chapter focuses on the blue mussel (*Mytilus edulis*), having pelagic larvae that disperse in the water column, and one chapter on a tube-dwelling amphipod (*Jassa herdmani*, scud) which produces its offspring in brood pouches. Finally, overall conclusions are drawn in relation to the research questions of the RECON project.

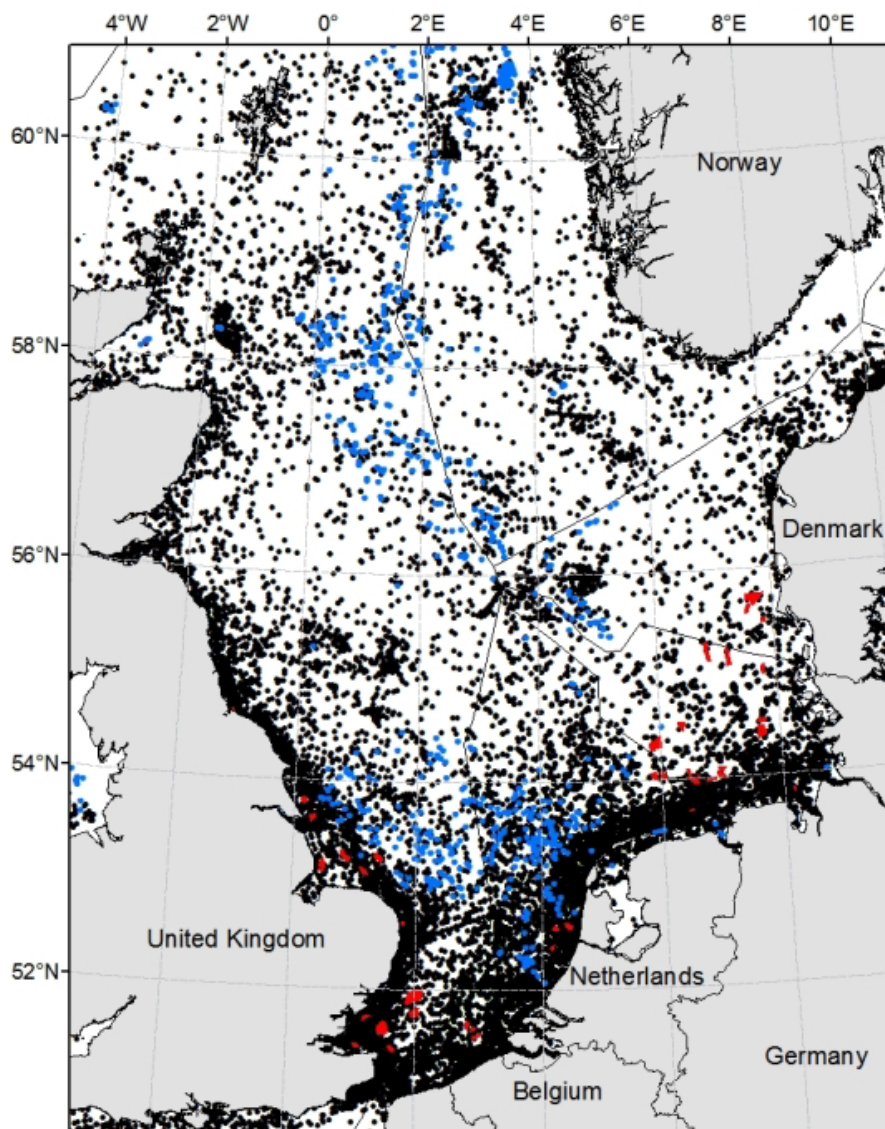


Figure 1. Overview of the North Sea showing locations of wrecks (black dots), oil and gas installations (blue dots) and wind farms (red dots).

2 Research topics

2.1 Analysis of industry provided ROV inspection footage

Summary based on manuscript in preparation to submit to a scientific journal:

Miriam Schutter, Martijn Dorenbosch, Floor Driessen, Wouter Lengkeek, Oscar G. Bos, Joop W.P. Coolen. (2017) Contribution of oil and gas platforms as artificial substrates for benthic North Sea fauna: effects of location and depth. In prep.

Introduction. An essential ingredient to model inter-connectivity is community data from species inventories. ROV inspection videos that were made for the routinely inspection of the different substructures of 17 oil and gas platforms were analysed for this. Digital footage of these inspections was made available by Petrogas E&P LLC and Maersk Oil Denmark. The inventory performed provides species data on abundant and conspicuous taxonomic groups on a large amount of locations. Approximately 3,720 and 8,520 minutes of footage were analysed for eight Dutch and nine Danish platforms, respectively (Figure 2). This resulted in 5,632 species observations, representing 38 species. These observations covered a total estimated surface area of 2726 m².

The study explored the abundance of hard substrate associated species among platforms at different geographical locations and over different depth ranges. A clustering of the hard substrate associated species community was hypothesized by geographic location of the platforms, related to differences in environmental conditions. In addition, clustering was expected based on depth range. Furthermore, differences in associated species community were expected between the steel- and concrete based platforms, as shown in earlier studies for much younger reef structures in the Kattegat (Andersson et al. 2009).

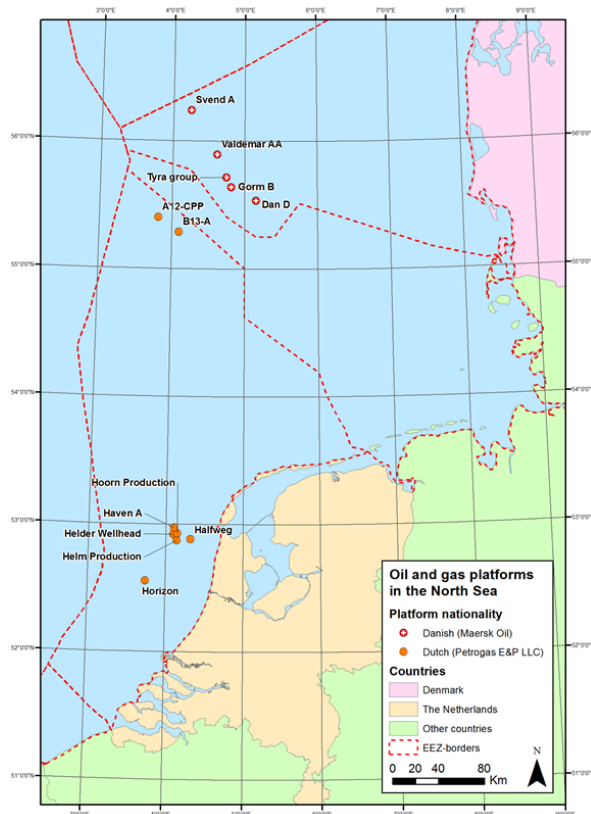


Figure 2. Geographic locations of the 17 surveyed offshore gas platforms in the southern North Sea.

Methods. Hierarchical cluster analysis was performed to identify species assemblages. As “sites” for clustering analysis, individual platforms and depth ranges (ranges of 5m each) were used. Analysis of species assemblages associated with the different platforms resulted in the distinction of three geographical-depth clusters (Figure 3): one cluster close to the Dutch shore line (southern shallow group), one cluster up north in and near the Danish EEZ in shallow waters (northern shallow group) and a cluster of northern platforms with depths >50 meter.

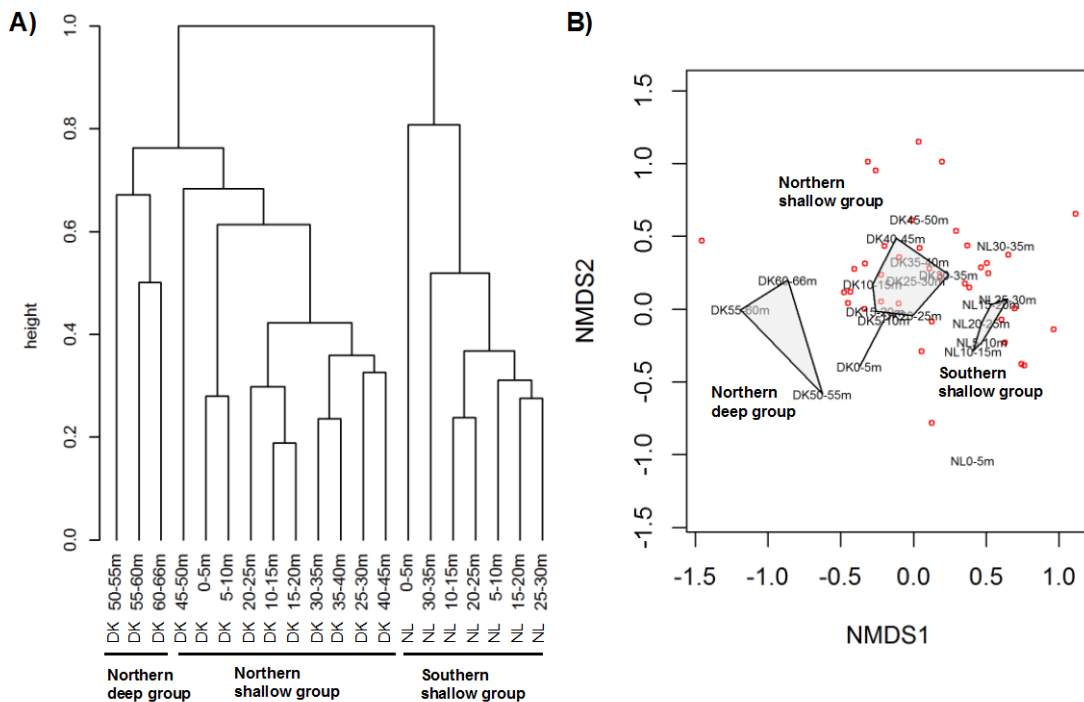


Figure 3. Hierarchical cluster and NMDS analysis results in different depth ranges: A) Dendrogram. A northern deep group, northern shallow group and southern shallow group of depth ranges are distinguished. B) Two-dimensional structure based on NMDS analysis of species assemblages in different depth zones on the 13 platforms (stress=0.09). Red dots indicate NDMS scores of different taxa whereas grey polygons connect platforms based on different depth zones as in A).

Results and discussion. The complete list of observed species is given in annex 1. A total of 38 species were observed, spread across 8 phyla. The northern geographical group had a higher total abundance of associated species (135) compared to the southern geographical group (72). Only two species were exclusively observed in the southern geographical group, whereas 14 species were exclusively observed in the northern geographical group. A total of 23 species was shared among geographical groups.

Species richness per platform ranged from 11 to 19 species. No significant differences in species richness were found between clusters, but platforms in the southern cluster had a lower mean abundance. Platforms Tyra EA and A12-CPP had the highest species richness (19 species). The concrete-dominated platform Halfweg was not an outlier in terms of species diversity, species richness and mean abundance of the associated community, when compared to the steel-dominated platforms.

The common North Sea species blue mussel *Mytilus edulis*, plumose anemone *Metridium dianthus* and common starfish *Asterias rubens* were observed on all platforms (except *A. rubens* on platform Haven A) and had a large depth range. The soft coral dead man's finger *Alcyonium digitatum* (depth range 5 - 50m) was only found in the northern geographical group. The fig sponge *Suberites ficus* (depth range 25-45m) was observed 16 times in the northern geographical group and only once in the southern geographical group on platform Helder Wellhead (Figure 4).



Blue mussel – Mytilus edulis



Plumose anemone - Metridium dianthus



Common starfish – Asterias rubens



Dead men's finger – Alcyonium digitatum



Fig sponge –Suberitus ficus

Figure 4. Examples of the blue mussel, plumose anemone, common starfish, dead men's finger and fig sponge in the North Sea (photos: Oscar Bos / WMR).

Conclusion & future research. Platforms in close proximity with similar environmental conditions also hold similar species communities. This similarity is furthermore related to depth. Future research should focus on the influence of habitat complexity, substrate orientation and inter-specific relations on species assemblages on offshore platforms. In addition, it would be interesting to know if a similar geographical pattern could be identified for species communities on ship wrecks.

2.2 Modelling species distribution patterns using samples taken on oil and gas platforms, wind farms and a natural reef.

Summary based on:

Joop W.P. Coolen, Babeth van der Weide, Joël Cuperus, Maxime Blomberg, Godfried Van Moorsel, Marco A. Faasse, Oscar G. Bos, Steven Degraer, Han J. Lindeboom (2017) *Benthic biodiversity on old platforms, young wind farms and rocky reefs*. Manuscript under peer-review.

Introduction. The introduction of artificial hard substrates in an area dominated by a sandy seabed increases habitat available to epifaunal organisms. Many biotic and abiotic variables influence the presence of epifaunal species. This sub-project aimed to evaluate the influence of depth, age, orientation, substrate type and the presence of potential keystone species *Mytilus edulis*, *Psammechinus miliaris*, *Metridium dianthus*, *Tubularia indivisa* and/or *Ectopleura larynx* on species richness and species composition.

From previous studies (van der Stap et al. 2016) it is clear that visual observations are not sufficient to create a complete overview of the species community, as each method detects species missed by other methods. Therefore additional samples of the marine growth were taken to provide data on the numbers of species, to be used in models explaining species richness patterns on reefs. Species diversity in communities present on the platforms was assessed by taxonomic assignment of specimens from samples taken from offshore structures. Five platforms, operated by ENGIE were sampled (Figure 5). The platforms were deployed in the period 1972-1999 and are supported by steel jacket foundations in 22 to 32 meters deep waters.

Methods. Samples were taken from platform foundations by a diver using a surface supplied airlift sampler, from a standardised surface. Data from platforms were combined with data from a study of the rocky reefs at the Borkum Reef grounds (BRG; Coolen et al. 2015) (Figure 6) and from the study of wind turbine foundations at the Princess Amalia Wind Farm (PAWF) (Vanagt et al. 2013; Vanagt and Faasse 2014). The complete dataset used for modelling consisted of 191 samples with 189 species and 117 taxa at a higher-than-species taxonomic level. From this dataset, species richness per sample was calculated and used to model patterns in richness over different depths, substrate types, age of installation and a selection of potential keystone species.

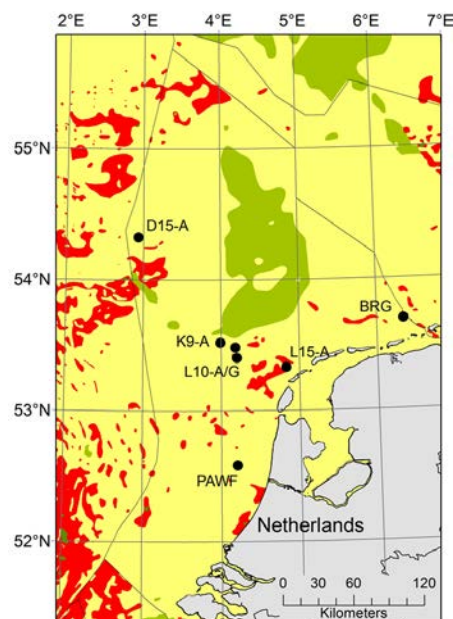


Figure 5: Habitat map of the Dutch continental shelf (black line). Red: coarse sediment; Yellow: sand; Green: muddy. Habitat data based on EMODnet data using simplified EUNIS classes (EMODnet 2015). With sample locations as black dots: BRG=Borkum Reef Grounds, PAWF = Princess Amalia Wind Farm, K9-A, L10-A/G and L15-A = oil and gas production platforms.

Results and discussion. The complete list of observed species is given in annex 2. 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. Since the number of species accumulates with increasing sample size, a predicted total species richness was assessed. The predicted number of species on platforms (177) was 25% higher than the observed number, while for the natural reefs it was 54% higher (77) and for the wind farm it was almost identical (100). This indicates that Borkum Reef Ground (BRG) would need much more samples to find most species present.

The following species were each present in at least 50% of the samples: crustaceans *Jassa herdmani*, *Stenothoe monoculoides*, and *Pisidia longicornis*, blue mussel *Mytilus edulis*, plumose anemone *Metridium dianthus*, and hairy sea-mat (a moss-animal) *Electra pilosa*. 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 found in 27% of the rock dump samples, with only 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.

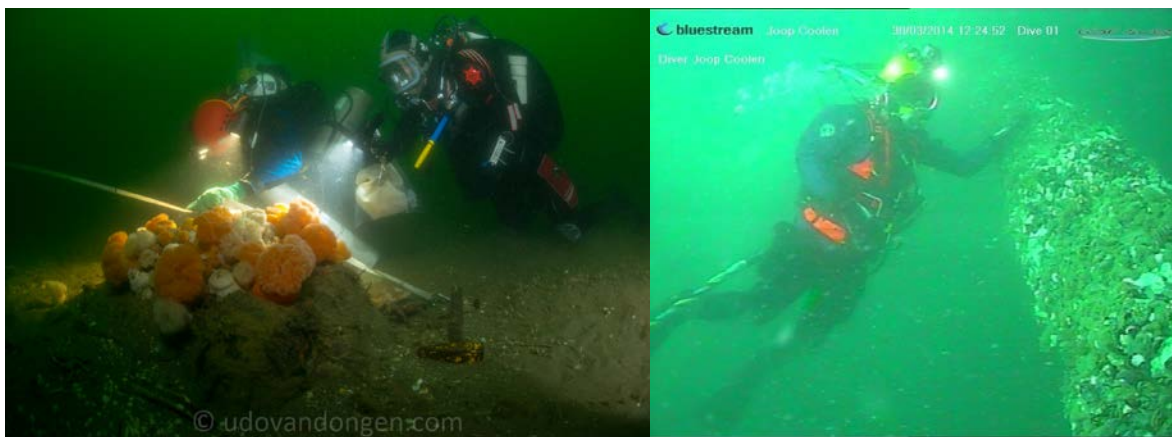


Figure 6. Sampling at the Borkum Reef Grounds (left, photo: Udo van Dongen) and at ENGIE platforms (right, photo: ENGIE).

The models showed that species richness was highest at intermediate depths (figure 7). High species richness at the bottom around the installations was caused in part by the presence of rocks, which increase heterogeneity of the substrate and thereby stimulate species richness. Species composition was found to be different between artificial and natural reefs. Depth, location effect and habitat type influence the species composition most strongly. Although rocky substrates hold the most species-rich communities, biodiversity is also strongly influenced by *Psammechinus miliaris*, *Mytilus edulis* and Tubulariidae. No significant effect of age on species richness was found, but age did have a small but significant effect on the species composition.

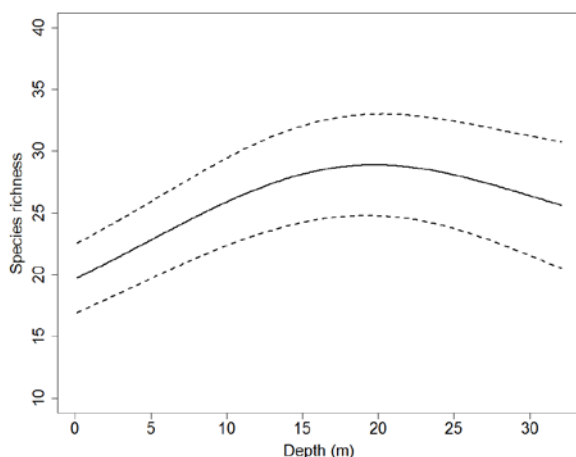


Figure 7: Modelled relation between depth and species richness (only species, no higher taxa) per sample (solid line) with standard error (dashed lines) on a typical steel structure and a mixed community of keystone species *Mytilus edulis*, *Psammechinus miliaris* and Tubulariidae.

Within the artificially created substrates, rock dump around the steel platforms showed the highest resemblance to natural rocky reefs. Shallow steel parts of the structures were the most dissimilar to natural reefs. This can be explained by a lack of intertidal zone at the natural reefs and a similar habitat structure between natural and artificially placed rocks.

Conclusion & Future research. Offshore installations are species rich. Rocky substrates hold the most species rich communities, and biodiversity is strongly influenced by depth and by keystone species (*Mytilus edulis*, *Psammechinus miliaris* and Tubulariidae). We observed that dominant species on concrete foundations are different from the dominant species on steel structures, with higher dominance of soft corals in shallow water on the concrete, even when in close proximity of concrete (personal observation J.W.P. Coolen). No detailed observations were done on concrete and to investigate this further, a comparison of steel and concrete structures of similar high age should be made.

2.3 New and rarely observed species on artificial structures in the Dutch North Sea

Summary based on:

- 1) Dias, I. M., Spierings, M., Coolen, J. W. P., Van Der Weide, B., & Cuperus, J. (2017). First record of *Syllis vittata* (Polychaeta: Syllidae) in the Dutch North Sea. *Marine Biodiversity Records*, 10(1), 16. doi:10.1186/s41200-017-0120-3
- 2) Spierings, M., Dias, I. M., Coolen, J. W. P., Van Der Weide, B., & Cuperus, J. (2017). First record of *Harmothoe aspera* (Hansen, 1879) (Polychaeta: Polynoidae) in the Dutch North Sea. *Marine Biodiversity Records*, 10(29), 4. doi:10.1186/s41200-017-0131-0
- 3) van der Weide, B.E., Coolen, J.W.P. et al. (2017) First record of *Syllis amica* on a far offshore structure in the Netherlands [working title]. Manuscript in preparation.
- 4) van der Weide, B.E., Coolen, J.W.P. et al. (2017) Records of rare and new species of Caprellidae on artificial structures in the North Sea [working title]. Manuscript in preparation.

Introduction. Artificial structures in the Dutch part of the North Sea have been ignored in monitoring programmes for a long time. Sporadically short term inventories have been performed on ship wrecks and platforms (Van Buuren 1984; Leewis and Waardenburg 1991; Lengkeek et al. 2013) or during impact monitoring of wind farms (Bouma and Lengkeek 2013; Vanagt and Faasse 2014) but large scale (bi)annual inventories are lacking. Rare species may therefore be present on these structures but changes may remain undetected due to low efforts to collect and identify macrofauna samples.

Methods. Within the RECON project an effort was made to inventory several offshore artificial structures, and several species rarely or never observed in the Netherlands were detected. These species were found in mixed macrofauna samples obtained with a surface airlift during marine growth sampling.

Results and discussion. On gas platform L15-A, situated 11 kilometres off the coast of the island of Vlieland, two specimens of *Syllis vittata* were found. This Polychaeta worm is present from British Waters to the Mediterranean Sea, Morocco and the Canary Islands and recorded from the South African coast and Indian Ocean. The finding was published in *Marine Biodiversity Records* (Dias et al. 2017) where *S. vittata* was reported for the first time in the Dutch part of the North Sea.

More recently another Syllidae species, *Syllis amica*, was found on platform D15-A. This cosmopolitan species is found in temperate and tropical seas. It is found from British Waters to the Mediterranean Sea, in the Gulf of Mexico and the Caribbean Sea. In this study *S. amica* is recorded for the first time in the Dutch part of the North Sea.

At platform L15-A two specimens of the scale worm *Harmothoe aspera* were observed. This polychaete species has been recorded in surveys from the Barents Sea to the Skagerrak, the

Mediterranean Sea, the Strait of Georgia and the Japanese sea. The recorded depth ranged from circa 48 meters to 1500 meters. This is the first report of *H. aspera* in the Dutch part of the North Sea EZ in a depth range from 15 to 20 meters.

On a number of ship wrecks in the northern part of the Dutch part of the North Sea, two rarely reported species of Caprellidae were observed. *Caprella tuberculata* had earlier been reported by Van Moorsel et al. (2014) while Van Moorsel (2003) showed presence of *Pseudoprotella phasma* on the gravelly reefs of the Cleaver Bank. Here, *Pseudoprotella phasma* was observed for the first time on artificial structures in the Dutch EEZ. *Caprella equilibra* was observed in a sample taken on a buoy near the port of Rotterdam. This will become the first record of the species for the Dutch part of the North Sea.

Conclusion & Future research. Offshore installations are clearly understudied in the southern North Sea. During the course of the RECON project, several species new to Dutch waters were found. During future analysis researchers should be aware of the potential to observe new species.

2.4 Metabarcoding of mixed macrofauna samples

Summary based on:

Glorius, S.G., F. Driessen, L.E. Becking, T. Schol, J.W.P. Coolen. Metabarcoding of marine growth sampled from fishing nets recovered from shipwrecks in the Dutch North Sea. [working title]. Manuscript in preparation.

Introduction. DNA barcoding and metabarcoding will allow for faster and cheaper species identification in coming years. DNA metabarcoding couples the principles of barcoding with high-throughput parallel sequencing. In this case, species in an environmental sample can be identified by comparing obtained sequences to a standard reference library of sequences from known organisms. The RECON project aimed to further develop metabarcoding techniques for identification of species in mixed macrofauna samples taken on artificial structures in the North Sea. Furthermore, this sub-project investigated the composition of the fouling fauna found on the remains of lost fishing nets on shipwrecks. Differences between fouling fauna on trawl nets and set nets were analysed.

Methods. Samples were taken from 13 shipwrecks on a transect roughly from south west to north east along the Dutch coast (figure 8), between 10 and 18 September 2016. At each location, scuba divers recovered nets lost by fishermen from the shipwrecks. Samples were taken from these salvaged nets after each dive. At each location, a sample from set net fishing nets and a sample from trawling fishing nets were taken. Samples were deposited in zip lock bags (220*280 mm) which were sealed, labelled and frozen in a household freezer (-20°C). After transportation, all samples were transferred to a -80°C freezer and stored until processing.

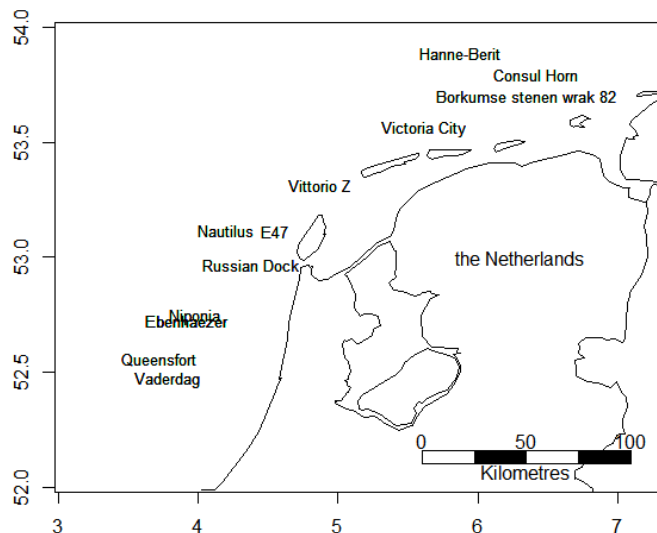


Figure 8. Sampling locations in the Dutch North Sea indicated with name of sampled shipwreck.

Molecular methods included biomass removal from the nets, grinding with an immersion blender, centrifuging to collect material for DNA extraction (Mo Bio PowerSoil DNA Isolation Kit) and sequencing using the Illumina MiSeq sequencing platform. During PCR amplification, a fragment located in the V4-section of the 18S region of the mitochondrial DNA using primers TAREuk454FWD1 and TAREukREV3 (Stoeck et al., 2010) was targeted as well as the C2-D2 region from the ribosomal 28S gene (primers by Erpenbeck et al., 2016).

After further cleaning and purification the 18s and 28s sequences were isolated and analysed separately. The primer sequences were removed from the reads, and reads that contained undetermined nucleotides as well as low quality reads were disregarded. After removal of chimera sequences the sequences were blasted against the NCBI nucleotide database. A species was assigned to sequence when the hit had an identity of 99% or higher and a length of at least 310 bp (18s) and 120 bp (28s). Unique sequences were only identified to species-level when there were no other species present. When more species but just one genus were present the genus was assigned. When several genera were present the unique sequences remained a not-identified taxonomic unit.

To test for differences between beam trawl and set nets, as well as read quality and sampling depth, richness (number of taxa in a sample) was modelled. Differences in beta diversity were investigated with species accumulation curves. Sample community composition was examined using non-metric Multidimensional scaling. Furthermore, influence of water depth, read quality, number of merged sequences and net type was tested using the function 'envfit' of the vegan package (Oksanen, 2017). All analyses were carried out in R (R Core Team, 2017).

Results

After cleaning and merging, the dataset contained 687 unique 18s sequences and 1,719 28s sequences. With these data, a total of 68 taxa were detected using 18s (34 species- and 33 genera) and 105 using 28s markers (54 species- and 51 genera). The total number of taxa detected was 158, combining 18s and 28s datasets. Additional species may have been present as >90% of the unique sequences could not be identified to species or genus level. As reference databases grow exponential over time, it is likely that, when unique sequences are blasted to reference database again in the future, additional species can be identified.

Trawl nets contained a larger number of different taxa compared to set-nets. All identified taxa that belong to the phylum Annelida were exclusively found on beam trawl nets and only using 18s gene. On the other hand, taxa that belong to the phylum Ochrophata were mainly found with 28s gene and set-nets contained the largest number of taxa within this phylum.

The statistical analysis showed that beam trawl nets held higher average species numbers for 18s (15.2) than did set nets (8.7) but this result was insignificant ($p > 0.05$) and opposite to results using 28s data (9.5 vs 13.2). Using 18s, species richness showed a significant maximum at intermediate depth ($p < 0.01$, figure 9), but 28s did not and was insignificant ($p > 0.05$). Read quality was insignificant for both 18s and 28s. The deviance explained by 18s and 28s statistical models were 33.6% and 30.5%, respectively.

nMDS ordination results showed no divergent species composition between set- and trawl nets ($p > 0.05$ for both genes). Furthermore no correlations were found between species composition and read quality and number of merged sequences. Water depth was not correlated to the species composition considering 28s while it was borderline significant for the 18s dataset ($p = 0.037$). A higher beta-diversity was found on beam trawl nets, most markedly within the 18s dataset (figure 9).

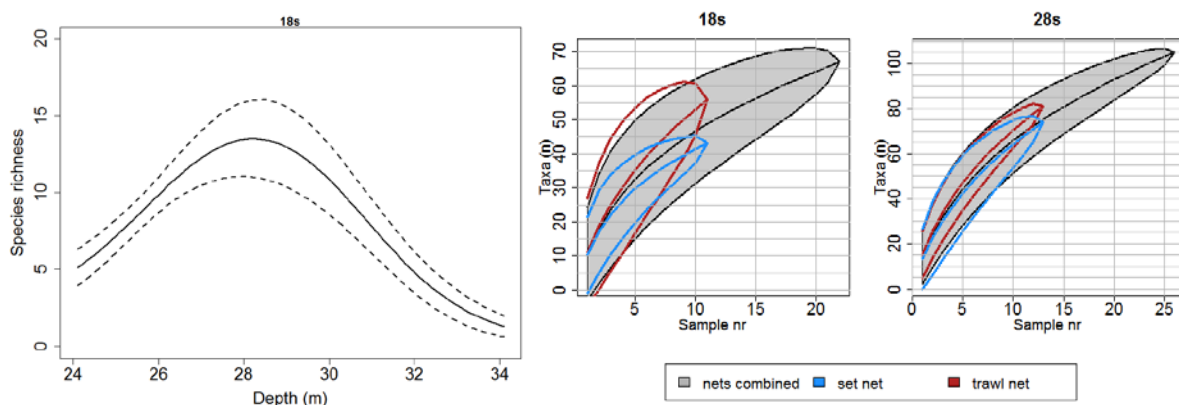


Figure 9. Typical relation between depth and species richness (left plot) using 18s marker data, $n=22$, $p < 0.01$. Species accumulation curve plots per net type and gene (right plots):

Discussion

Using metabarcoding techniques, a large number of unique sequences was found. However, $>90\%$ of these could not be linked to a taxon and, of the linked sequences many could only be linked to genus level. To become a cost effective and complete method for species detection, the metabarcoding methodology needs to be developed further. There still is a strong need to publish more barcodes from species found on artificial hard substrates in the North Sea. To reach more reliable identification to species level, additional primers should be developed and open access reference datasets enlarged.

No clear difference was found between samples from different net types considering sample-richness (alpha diversity) and species composition. A larger species pool (beta diversity) was discovered on beam trawl-nets compared to set nets. Different results between genes could be a result from the fact that each gene targets a different species pool. For example, species that belong to the phylum Annelida were only detected with the 18s gene (and found only on beam trawl nets), while the 28s gene identified much more species of the phylum Ochrophyta. Using presence-absence data it is challenging to detect differences in species composition, therefore barcoding methods that include species densities should be developed also.

2.5 Assessing installation connectivity with particle tracking models and population genetics of *Mytilus edulis*

Summary based on:

Coolen, J.W.P., P. Luttikhuisen, R. Crooijmans, A.R. Boon, H. van Pelt, L.E. Becking, F. Kleissen, D. Gerla (2017) *Marine stepping stones: Mytilus edulis population connectivity between offshore energy installations [working title]. Manuscript in preparation.*

Introduction. Man-made installations in open seas and oceans are increasingly abundant. They include wrecks, buoys, oil and gas platforms and wind turbines and provide an artificial type of hard substrate for marine organisms (figure 10). Particularly in regions where natural hard substrates are rare, they may have strong impacts on biological communities. They may form connections between hitherto unconnected habitats. We studied the possible role of man-made installations in the North Sea in facilitating genetic connectivity for the blue mussel *Mytilus edulis*.

Methods. Seven microsatellite markers and the locus Me1516 were genotyped for 1178 blue mussels *Mytilus* spp. sampled from 25 locations in the North Sea plus two reference locations (figure 10). Strong introgression was seen in the data. 51% of the mussels growing on the sampled installations were found to be hybrids between *Mytilus edulis* and *M. galloprovincialis*. 49% were *M. edulis* while no "pure" *M. galloprovincialis* were found. Only *M. edulis* were used for subsequent analyses (N = 579).

Pairwise genetic distance (F_{ST}) were calculated as measure of pairwise genetic distances. Coalescent simulations were run to estimate pairwise migration rates (Sethuraman and Hey 2016). Hydrographical model Delft3D was run to predict particle transport among installations (Ertfemeijer et al. 2009). The number of arriving particles from a source was used as pairwise distance. These three matrices were compared using a permutation correlation test based on the mantel test. Correlation between matrices was assumed significant if <5% of the permutations resulted in a higher correlation than the non-permuted correlation.

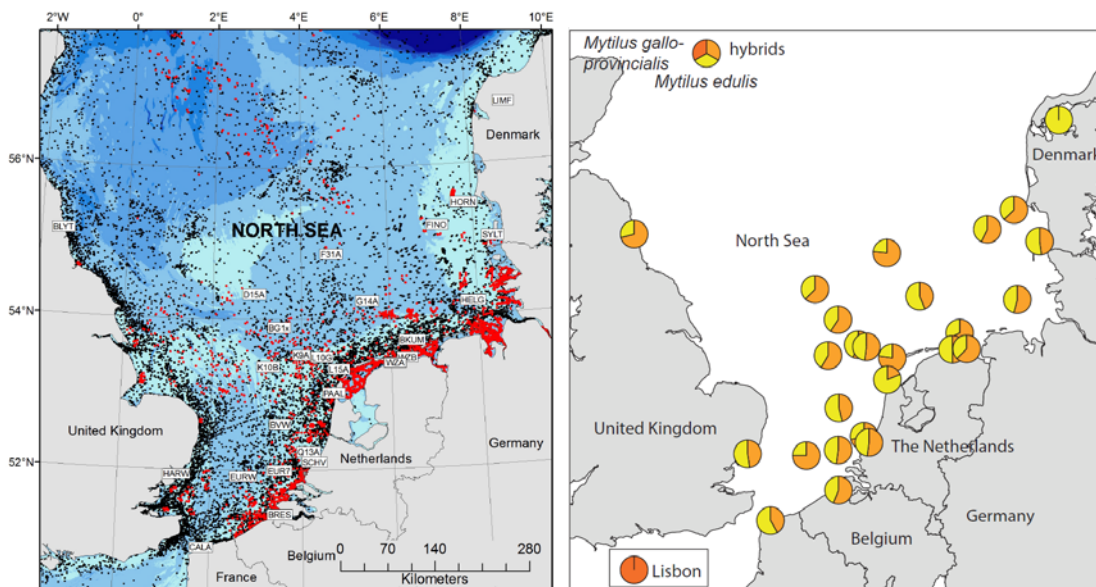


Figure 10: Left: North Sea map of all artificial reefs and sample locations. Southern North Sea, sample locations (white labels), artificial structures with surface contact (red dots) and structures without surface contact (black dots). Abbreviations of sample locations are explained in Table 1. Note no buoys are shown for British, French and Danish waters. Right: sample locations shown as pie charts indicating the amount of pure *M. edulis* and hybrids with *M. galloprovincialis*.

Results and discussion. *Mytilus edulis* populations were present throughout the investigated area, showing that larvae are able to reach offshore locations. Larval transport by currents has probably contributed to the initial colonisation. Direct connectivity between some locations was also shown by the particle tracking models, although this could not be validated using genetic data. Modelled particle transport correlated significantly with FST (Pearson correlation -0.08, $P = 0.004$) but not with the migration rates from coalescent modelling (correlation 0.06, $P = 0.13$). Expectations were that the migration rate would better correlate with the particle transport, but the opposite was found. Given the number of location-combinations with zero particle exchange, the distance between the studied locations is likely too large for direct larval exchange. Alternatively, colonisation of the locations may have been a result from rare weather events. Although mussels are present at all locations, present-day larval exchange between the studied locations seems low. This, again, may be a result from the distance between the studied locations.

Future research. In future research, the distance between sampled sub-populations should be decreased, in order to decrease the number of population combinations with zero connectivity in the modelled particle data. Furthermore, analysis of long term patterns in the particle tracking models could increase the understanding of the patterns observed in the current study. New developments in molecular techniques for genetic analysis move to single nucleotide polymorphism (SNPs), providing higher resolution genetic data. We recommend to use SNPs in future analyses.

2.6 Population genetics of *Jassa herdmani* on artificial structures in the southern North Sea

Summary based on Jak, R.G., P. Luttikhuisen, R. Crooijmans, J.W.P. Coolen (2017) Strong population differentiation in a directly developing crustacean (Jassa herdmani) inhabiting man-made offshore installations in the North Sea [working title]. Manuscript in preparation.

Introduction. *Jassa herdmani* is a small tube dwelling crustacean species reaching a maximum length of ca. 10 mm. From its tube it produces a water current to collect suspended particles for feeding. It can reach very high densities at hard substrates with reported values up to a million individuals per square meter. It usually occurs on hard substrates near the sea floor, and in the middle depth range of artificial (vertical) structures. At an average temperature of 15 °C its life span is about five to six months and females produce 4 broods with a total number of about 30 juveniles that develop in a brood pouch. The generation time is thus short and the dispersal distance is small, since no planktonic larval stage is involved. It can thus be expected that this species is more likely to build stable populations and connectivity might be limited to adjacent platforms or natural habitats.

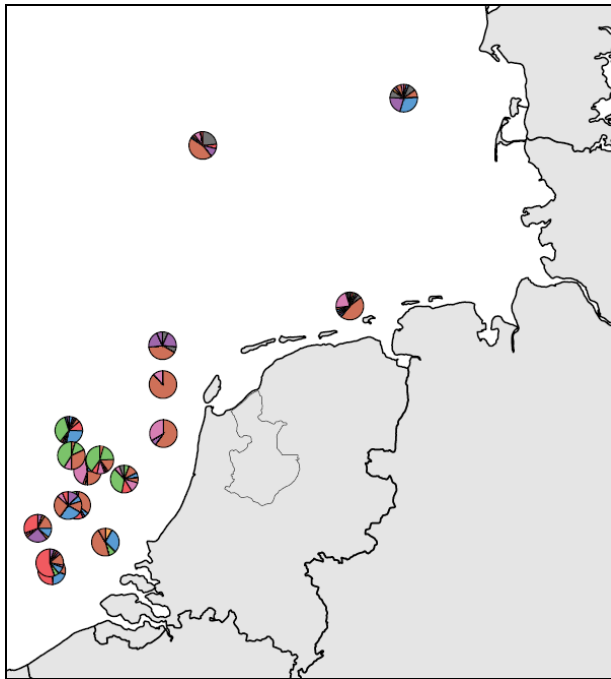


Figure 11. Spatial distribution of 42 different DNA variants (haplotypes) of 658 basepair mitochondrial sequences for *Jassa herdmani* at 17 locations in the North Sea. The genetic composition differs significantly among most sampled locations, which means that connectivity between locations is virtually non-existent.

Methods. Samples of *Jassa* from offshore platforms, ship wrecks and wind turbines were selected for the analyses of genetic data. Sampling locations covered the south eastern North Sea, i.e. the EEZ of Denmark, Germany and the Netherlands.

Results and discussion. Our genetic analyses show that genetic variation is significantly different between most of the sampled locations, which means that connectivity in the form of migration between structures is mostly absent. *Jassa herdmani* is clearly able to colonize the installations and form thriving populations on them, but afterwards seems to have limited migration to other installations, both far and near.

A portion of 658 base pairs was sequenced from the mitochondrial DNA of a total of 529 *Jassa herdmani* originating from 22 locations in the North Sea. Among these, 44 different DNA variants ('haplotypes') were detected. After selecting only locations with at least 15 successfully sequenced individuals, 17 sampling locations remained. The 514 sequenced individuals originating from these 17 locations contained a total of 42 haplotypes.

Table 1. Analysis of molecular variance for *Jassa herdmani*. Variance component of genetic variation among locations is 15.92% and significant ($P < 0.000001$).

Source of variation	Degrees of freedom	Sum of squares	Variance components	Percentage of variation
Among locations	16	155.323	0.27524	15.92
Within locations	497	722.402	1.45353	84.08
Total	513	877.726	1.72877	

The genetic composition of *Jassa herdmani* at the 17 sampling locations was significantly different (Table 1). The overall F_{ST} (a measure for population differentiation which theoretically ranges from 0 at no differentiation to 1 at complete differentiation) was 0.15921 and significantly larger than zero ($P < 0.000001$). This differentiation is not only found at the overall level but also in the majority of pairwise comparisons (see also figure 11); of all 136 pairwise comparisons only 24 were not significantly differentiated at the $P < 0.05$ level. The locations that are most likely to be connected to other locations in terms of *Jassa herdmani* migration are the shipwreck 'Vinca Gorthon', 'Rode poon', 'E22' and 'Elatma'.

These results show that the populations of *Jassa herdmani* are very differentiated in the North Sea. There is hardly any connectivity of the populations of *Jassa* amongst the different sampling locations detected in this study. Apparently, once *J. herdmani* has colonized the hard substratum, it develops a distinct population, enabled by its short life-cycle and limited dispersal capacity.

The results and discussion reported here are preliminary and will be analysed further in the near future.

3 Discussion

The broader INSITE programme of research aimed to help establish the following:

- A) The magnitude of the effects of man-made structures compared to the spatial and temporal variability of the North Sea ecosystem, considered on different time and space scales; and
- B) To what extent, if any, the man-made structures in the North Sea represent a large interconnected hard substrate system.

To gain insight in these aims, the main research questions of the RECON project were:

1. What is the species composition of marine growth on offshore structures?
2. To what extent is this composition explained by abiotic factors (e.g. depth, temperature, location, platforms age, marine growth cleaning frequency, et cetera) and biotic factors (e.g. food availability, proximity to marine growth on other offshore structures, distance to coastal populations, et cetera)?
3. To what extent are the communities on the structures isolated from or connected to each other and how is this explained by the factors noted earlier?

The species composition of marine growth on offshore structures is clearly very rich. During the course of the RECON project, over 200 hard substrate associated species were observed on the studied platforms, wind farms, wrecks and buoys.

Species composition

The composition and richness patterns on studied artificial structures are strongly influenced by geographic location, depth, and substrate type. In addition, a set of keystone species has a significant impact on other species in the community. Installations in similar hydrodynamic regions show a higher similarity when compared to objects in other regions. Furthermore, communities at similar depth and attached to similar substrates (e.g. rocks or steel) are alike. Some variables could not be assessed yet. For some variables the number of locations and therefore the variation in the explanatory variables was limiting, while for others the limited number of samples available for unravelling the relations with statistical models was the bottle neck. In general, detecting changes in species composition when various influences are present, is challenging. For a complete inventory of North Sea platforms, further monitoring of additional locations outside the current region is advised. Special attention should be payed to differences between steel and concrete foundations in comparison to natural reefs from other parts of the North Sea.

Connectivity

The connectivity analysis showed that connectivity depends on the species studied. While patterns in mussel *Mytilus edulis* population genetics shows connectivity with one method (not with a second method), analysis of this phenomena using *Jassa herdmani* samples did not. Challenges in the mussel study were likely caused by the large average distance between the studied populations. To test this, additional locations between the currently studied locations should be investigated.

4 Recommendations

The results obtained in RECON should be used in future decisions on the installation of wind farms and decommissioning of oil and gas platforms. These installations harbour a high biodiversity and connect populations. Communities on scour protections are similar to natural reefs and therefore leaving in place these rocks and potentially other parts of installations should be considered in decommissioning decisions. Connectivity between installations depends on species' life cycles. Some of the connected species populations may be non-indigenous, others native. Depending on life cycles and species status (e.g. OSPAR protected or non-native), different leave-in-place options should be considered during decommissioning. These options should include leaving in place the foundations as they are, removing part of the foundations to depths with limited risk of non-indigenous colonisation, or full removal.

To enlarge the scientific base for these decisions, the following research should be carried out:

- A. Increase the spatial distribution of locations to be investigated, using identical methods as applied in RECON;
- B. Include concrete substrate as a variable of influence on species composition;
- C. Improve the connectivity work using *Mytilus edulis* by adding in-between locations to the current datasets and use single nucleotide polymorphism to obtain higher resolution data;
- D. Develop methods to attain samples from locations beyond diving depth, e.g. by a combination of remote sampling using ROVs and metabarcoding high numbers of samples.

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7 Quality Assurance

Wageningen Marine Research utilises an ISO 9001:2008 certified quality management system (certificate number: 187378-2015-AQ-NLD-RvA). This certificate is valid until 15 September 2018. The organisation has been certified since 27 February 2001. The certification was issued by DNV Certification B.V.

Justification

Report C074/17A
Project Number: 4312100030

The scientific quality of this report has been peer reviewed by the a colleague scientist and the head of the department of Wageningen Marine Research.

Approved: Dr. D.M.E. Slijkerman
Senior Scientist

Signature:



Date: 31-01-2018

Approved: Dr.ir. T.P. Bult
Director

Signature:



Date: 31-01-2018

Annex 1 Species list chapter 2.1

Description of species community based on geographical groups and depth ranges. Listed vertically are the geographical groups that resulted from hierarchical cluster analysis and the species observed (exclusively) within each group. Species indicated with an asterisk (*) were excluded from data analysis. Listed horizontally are the depth ranges within which the mean Braun Blanquet abundance is reported (i.e. mean abundance within a depth range averaged for all platforms within a geographic group).

		0-5m	5-10m	10-15m	15-20m	20-25m	25-30m	30-35m	35-40m	40-45m	45-50m	50-55m	55-60m	60-66m
Southern group	Cnidaria													
	<i>Sagartia troglodytes</i> *	-	-	-	-	-	5.0	-	-	-	-	-	-	-
	<i>Sarsia tubulosa</i> *	-	-	-	-	-	1.0	-	-	-	-	-	-	-
	Chordata													
	<i>Callionymus lyra</i>	-	-	-	-	-	1.0	2.5	-	-	-	-	-	-
	<i>Merlangius merlangus</i>	-	-	-	-	2.0	2.0	-	-	-	-	-	-	-
Southern & northern group	<i>Myocephalus scorpius</i> *	-	-	-	-	-	1.0	-	-	-	-	-	-	-
	<i>Solea solea</i> *	-	-	-	-	-	2.0	-	-	-	-	-	-	-
	Ochrophyta													
	<i>Fucus</i> sp.	-	-	7.0	-	-	2.0	-	-	-	-	-	-	-
	Porifera													
	<i>Suberites ficus</i>	-	-	-	-	-	6.0	6.2	6.5	6.0	-	-	-	-
	Cnidaria													
	<i>Actinothoe sphyrodeta</i>	4.0	4.7	4.8	4.5	5.1	5.6	5.0	-	-	-	-	-	-
	<i>Diadumene cincta</i>	-	5.6	5.2	5.3	5.1	5.3	5.0	5.0	-	-	-	-	-
	<i>Ectopleura larynx</i>	6.6	6.2	6.6	4.5	-	2.5	4.5	6.5	6.0	-	-	5.0	3.9
	<i>Hydractinia echinata</i>	-	7.0	7.7	8.0	7.5	7.4	7.4	7.0	8.5	-	-	-	-
	<i>Metridium senile</i>	4.5	5.6	7.1	7.5	7.8	6.9	7.4	8.1	7.0	7.5	8.1	9.0	6.1
	<i>Sagartia elegans</i>	-	-	2.0	6.0	2.5	-	-	-	-	-	-	-	-
	<i>Sagartiageton undatus</i>	-	-	-	1.0	-	5.0	-	-	-	-	-	-	-
	<i>Tubularia indivisa</i>	-	3.0	7.0	4.4	5.3	5.4	4.1	6.8	3.0	6.0	-	-	-
	<i>Urticina felina</i>	2.3	2.9	2.4	5.0	-	3.6	2.0	-	-	-	-	-	-
	Echinodermata													
	<i>Asterias rubens</i>	4.2	3.5	3.1	2.9	2.1	3.4	3.4	3.0	3.3	4.3	1.0	-	2.1
	<i>Ophiotrix fragilis</i>	6.8	3.0	3.9	2.8	3.3	1.5	2.0	3.7	4.1	4.7	-	-	2.0
<i>Psammochinus miliaris</i>	3.0	4.8	5.0	-	-	-	-	-	-	-	-	-	-	
Mollusca														
<i>Alloteuthis subulata</i>	-	-	-	2.0	-	-	-	-	-	7.0	-	-	-	
<i>Mytilus edulis</i>	8.1	7.8	7.5	7.2	7.0	7.1	6.7	5.6	6.2	-	5.0	-	-	
Arthropoda														
<i>Cancer pagurus</i>	1.0	5.0	5.0	3.9	4.4	5.0	5.6	3.5	2.0	5.0	-	-	-	
<i>Carcinus maenas</i> *	-	-	-	5.0	-	5.0	-	-	-	-	-	-	-	
<i>Necora puber</i>	-	6.0	1.0	4.8	4.3	4.8	5.0	-	-	-	-	-	-	
<i>Pagurus bernhardus</i>	-	-	-	-	-	-	1.0	-	2.6	3.7	-	-	-	
Chordata														
<i>Mullus sumuletus</i>	-	-	-	-	2.0	1.6	1.0	-	1.0	-	-	-	-	
<i>Pholis gunnellus</i>	-	-	-	-	-	2.0	-	-	1.0	-	-	-	-	
<i>Trachurus trachurus</i>	-	-	-	-	3.0	2.6	2.0	2.0	-	-	-	-	-	
<i>Trisopterus luscus</i>	-	-	-	3.1	2.3	2.3	2.4	2.0	1.0	-	-	-	1.0	
Northern group	Cnidaria													
	<i>Alcyonium digitatum</i>	-	5.0	5.6	3.6	4.6	5.2	4.2	4.8	6.4	5.9	-	-	-
	<i>Cyanea lamarckii</i>	-	-	1.0	-	-	1.0	-	-	-	-	-	-	-
	Ctenophora													
	<i>Beroe gracilis</i>	1.0	1.3	1.0	1.0	1.0	-	1.0	2.0	1.0	-	-	1.0	-
	<i>Mnemiopsis leidyi</i>	2.0	1.5	1.3	1.4	1.2	1.0	1.2	1.0	1.0	-	-	-	-
	<i>Pleurobrachia pileus</i>	1.3	-	1.3	-	1.0	1.0	1.0	1.0	2.0	-	-	-	1.0
	Echinodermata													
	<i>Echinus esculentus</i>	-	-	-	-	-	6.0	5.0	5.0	5.8	-	-	-	-
	<i>Marthasterias glacialis</i>	-	-	-	-	-	-	-	-	6.3	-	-	-	-
	Mollusca													
	<i>Aeolidia papillosa</i>	-	-	5.0	5.8	4.3	6.5	-	-	5.0	-	4.0	-	-
	<i>Loligo vulgaris</i>	-	-	-	-	-	-	-	-	2.0	-	-	-	9.0
	<i>Simnia patula</i>	-	-	-	-	-	-	-	1.0	5.0	-	-	-	-
Chordata														
<i>Ctenolabrus rupestris</i>	-	-	1.0	-	-	1.0	1.0	-	-	-	-	-	-	
<i>Gadus morhua</i>	-	-	-	-	1.0	-	1.5	-	1.0	-	-	-	-	
<i>Molva molva</i>	-	-	-	-	-	-	1.0	-	-	-	-	-	-	
<i>Myocephalus scorpius</i> *	-	-	-	-	-	1.0	-	-	-	-	-	-	-	
Total number of species (excl. *):	12	16	22	20	21	28	26	18	24	8	4	3	7	

Annex 2 Species list chapter 2.2

Full species list with presence (*), absence (-) of species per reef type, non-indigenous status of each species (*=non-indigenous) and whether a species was detected using ROV analysis in chapter 2.1 (*=yes).

Species	PAWF	Platforms	BRG	Non-indigenous	ROV detected	Species	PAWF	Platforms	BRG	Non-indigenous	ROV detected
Annelida						<i>Spirobranchus triqueter</i>	*	-	-		
<i>Ctenodrilus serratus</i>	*	-	-			<i>Sthenelais boa</i>	-	*	-		
<i>Eulalia viridis</i>	*	*	*			<i>Subadyte pellucida</i>	-	*	-		
<i>Eumida sanguinea</i>	-	*	-			<i>Syllis amica</i>	-	*	-		
<i>Eunereis longissima</i>	*	*	*			<i>Syllis armillaris</i>	-	*	-		
<i>Eusyllis blomstrandii</i>	-	*	-			<i>Syllis gracilis</i>	-	*	-		
<i>Harmothoe aspera</i>	-	*	-			<i>Syllis hyalina</i>	-	*	-		
<i>Harmothoe clavigera</i>	-	*	*			<i>Syllis prolifera</i>	*	-	-		
<i>Harmothoe extenuata</i>	*	*	-			<i>Syllis vittata</i>	-	*	-		
<i>Harmothoe fernandi</i>	-	*	*			<i>Syllis vivipara</i>	-	*	-		
<i>Harmothoe impar</i>	*	*	*			Arthropoda					
<i>Harmothoe viridis</i>	-	*	-			<i>Abludomelita obtusata</i>	*	*	-		
<i>Lagis koreni</i>	*	-	-			<i>Amphibalanus improvisus</i>	*	-	-	*	
<i>Lanice conchilega</i>	*	*	-			<i>Aora gracilis</i>	-	*	*		
<i>Lepidonotus squamatus</i>	*	*	*			<i>Argissa hamatipes</i>	-	*	-		
<i>Malacoceros fuliginosus</i>	*	-	-			<i>Austrominius modestus</i>	*	-	-	*	
<i>Myrianida prolifera</i>	-	*	-			<i>Balanus balanus</i>	-	*	*		
<i>Nereis pelagica</i>	*	*	-			<i>Balanus crenatus</i>	*	*	-		
<i>Nothria conchylega</i>	-	*	-			<i>Cancer pagurus</i>	*	*	-	*	
<i>Notomastus latericeus</i>	-	*	-			<i>Caprella linearis</i>	*	*	*		
<i>Phyllodoce laminosa</i>	*	-	-			<i>Caprella mutica</i>	*	*	-	*	
<i>Phyllodoce longipes</i>	-	*	-			<i>Crassikorophium bonellii</i>	-	*	-		
<i>Phyllodoce maculata</i>	*	*	-			<i>Eualus cranchii</i>	-	*	-		
<i>Phyllodoce mucosa</i>	*	-	-			<i>Galathea intermedia</i>	-	*	-		
<i>Polydora ciliata</i>	-	*	-			<i>Gammaropsis nitida</i>	-	*	-		
<i>Proceraea cornuta</i>	-	*	*			<i>Gitana sarsi</i>	*	*	-		
<i>Proceraea prismatica</i>	-	-	*			<i>Homarus gammarus</i>	*	-	-		
<i>Psamathe fusca</i>	-	*	-			<i>Hyperia galba</i>	-	*	*		
<i>Pseudopolydora pulchra</i>	-	-	*			<i>Idotea pelagica</i>	*	*	-		
<i>Sabellaria spinulosa</i>	*	*	*			<i>Ischyrocerus anguipes</i>	-	-	*		
<i>Spirobranchus lamarcki</i>	-	*	-			<i>Jassa herdmani</i>	*	*	-		

Species	PAWF	Platforms	BRG	Non-indigenous	ROV detected	Species	PAWF	Platforms	BRG	Non-indigenous	ROV detected
<i>Jassa marmorata</i>	*	*	-			<i>Alcyonidium parasiticum</i>	*	*	*		
<i>Lepidepecreum longicornis</i>	-	-	*			<i>Arachnidium fibrosum</i>	*	*	-		
<i>Liocarcinus depurator</i>	-	*	-			<i>Aspidelectra melolontha</i>	-	*	-		
<i>Liocarcinus holsatus</i>	-	-	*			<i>Bicellariella ciliata</i>	-	*	-		
<i>Liocarcinus navigator</i>	-	-	*			<i>Bugulina turbinata</i>	-	*	-		
<i>Macropodia rostrata</i>	-	-	*			<i>Buskia nitens</i>	-	*	-		
<i>Megabalanus coccopoma</i>	*	-	-	*		<i>Callopora dumerilii</i>	*	*	-		
<i>Metopa alderi</i>	*	-	-			<i>Celleporella hyalina</i>	*	*	-		
<i>Metopa borealis</i>	-	*	-			<i>Conopeum reticulum</i>	*	*	*		
<i>Metopa bruzelii</i>	-	*	-			<i>Cribrilina punctata</i>	-	*	-		
<i>Metopa pusilla</i>	-	*	-			<i>Electra pilosa</i>	*	*	*		
<i>Microtopos maculatus</i>	-	*	-			<i>Escharella immersa</i>	-	*	-		
<i>Monocorophium acherusicum</i>	*	*	*			<i>Farrella repens</i>	*	-	-		
<i>Monocorophium insidiosum</i>	-	*	-			<i>Fenestrulina delicia</i>	*	-	-	*	
<i>Monocorophium sextonae</i>	*	*	-	*		<i>Microporella ciliata</i>	*	*	-		
<i>Necora puber</i>	*	-	-		*	<i>Schizomavella (Schizomavella) linearis</i>	*	*	-		
<i>Nototropis swammerdamei</i>	-	*	-			<i>Scruparia ambigua</i>	*	*	*		
<i>Nymphon brevistroste</i>	-	-	*			<i>Scruparia chelata</i>	-	*	-		
<i>Pandalus montagui</i>	-	-	*			<i>Smittoidea prolifica</i>	*	-	-	*	
<i>Phtisica marina</i>	*	*	-								
<i>Pilumnus hirtellus</i>	*	*	-			Chordata					
<i>Pilumnus spinifer</i>	-	*	-			<i>Diplosoma listerianum</i>	*	*	*	*	
<i>Pinnotheres pisum</i>	*	*	-			<i>Liparis liparis liparis</i>	-	-	*		
<i>Pisidia longicornis</i>	*	*	-								
<i>Semibalanus balanoides</i>	*	*	-			Cnidaria					
<i>Stenothoe marina</i>	-	*	*			<i>Actinothoe sphyrodeta</i>	-	*	-		*
<i>Stenothoe monoculoides</i>	*	*	-			<i>Alcyonium digitatum</i>	*	*	-		*
<i>Stenothoe tergestina</i>	-	*	-			<i>Calycella syringa</i>	-	*	*		
<i>Stenothoe valida</i>	*	*	-			<i>Campanularia volubilis</i>	-	-	*		
<i>Stenula solsbergi</i>	-	*	-			<i>Clytia gracilis</i>	-	*	-		
<i>Telmatogeton japonicus</i>	*	*	-	*		<i>Clytia hemisphaerica</i>	*	*	*		
<i>Tryphosa nana</i>	-	*	-			<i>Corynactis viridis</i>	-	*	-		
<i>Tryphosella sarsi</i>	-	-	*			<i>Coryne pusilla</i>	-	*	-		
<i>Verruca stroemia</i>	*	*	-			<i>Diadumene cincta</i>	*	*	-		*
						<i>Diadumene lineata</i>	-	*	-		*
Bryozoa						<i>Ectopleura larynx</i>	*	*	*		*
<i>Aetea anguina</i>	-	*	-			<i>Garveia nutans</i>	-	-	*		
<i>Alcyonidioides mytili</i>	*	*	-			<i>Gonothyraea loveni</i>	-	*	-		
<i>Alcyonidium condylocinereum</i>	*	*	-			<i>Hartlaubella gelatinosa</i>	-	*	-		
<i>Alcyonidium hydrocoailitum</i>	-	-	*			<i>Hydractinia echinata</i>	*	*	-		*
<i>Alcyonidium mamillatum</i>	*	-	-			<i>Laomedea calceolifera</i>	-	*	-		
						<i>Laomedea flexuosa</i>	*	*	-		

Species	PAWF	Platforms	BRG	Non-indigenous	ROV detected	Species	PAWF	Platforms	BRG	Non-indigenous	ROV detected
<i>Laomedea neglecta</i>	-	*	-			<i>Fabulina fabula</i>	-	*	-		
<i>Metridium dianthus</i>	*	*	*		*	<i>Heteranomia squamula</i>	*	*	-		
<i>Obelia bidentata</i>	-	*	*			<i>Hiatella arctica</i>	*	*	-		
<i>Obelia dichotoma</i>	*	*	*			<i>Hyalia vitrea</i>	-	*	-		
<i>Obelia longissima</i>	*	*	-			<i>Kellia suborbicularis</i>	-	*	-		
<i>Sagartia elegans</i>	*	*	-		*	<i>Kurtiella bidentata</i>	-	*	-		
<i>Sagartia troglodytes</i>	*	-	-		*	<i>Lepton squamosum</i>	-	*	-		
<i>Sagartiogeton undatus</i>	*	-	-		*	<i>Magallana gigas</i>	*	*	-	*	
<i>Sertularia argentea</i>	-	-	*			<i>Musculus discors</i>	-	-	*		
<i>Sertularia cupressina</i>	-	-	*			<i>Musculus subpictus</i>	-	-	*		
<i>Tubularia indivisa</i>	*	-	-		*	<i>Mytilus edulis</i>	*	*	*		*
<i>Urticina felina</i>	*	*	-		*	<i>Onchidoris bilamellata</i>	*	*	-		
Echinodermata						<i>Onchidoris muricata</i>	-	-	*		
<i>Amphipholis squamata</i>	-	*	-			<i>Ostrea edulis</i>	*	-	-		
<i>Asterias rubens</i>	*	*	-		*	<i>Sphenia binghami</i>	-	*	-		
<i>Crossaster papposus</i>	-	*	-			<i>Tergipes tergipes</i>	-	*	-		
<i>Echinocardium cordatum</i>	*	-	-			<i>Tritonia hombergii</i>	-	*	-		
<i>Ophiothrix fragilis</i>	*	*	-		*	<i>Venerupis corrugata</i>	*	-	-		
<i>Psammechinus miliaris</i>	*	*	-		*	<i>Emplectonema gracile</i>	*	-	-		
Entoprocta						Platyhelminthes					
<i>Pedicellina cernua</i>	-	-	*			<i>Leptoplana tremellaris</i>	*	-	-		
Mollusca						Porifera					
<i>Acanthodoris pilosa</i>	-	*	-			<i>Halichondria (Halichondria) panicea</i>	-	*	-		
<i>Aeolidia papillosa</i>	*	*	-		*	<i>Leucosolenia botryoides</i>	-	*	-		
<i>Brachystomia scalaris</i>	*	*	-			<i>Leucosolenia variabilis</i>	*	*	-		
<i>Catriona gymnota</i>	*	*	-			<i>Mycale (Carmia) macilenta</i>	-	*	-		
<i>Corbula gibba</i>	-	*	-			<i>Sycon ciliatum</i>	-	*	*		
<i>Crepidula fornicata</i>	*	-	*	*		Chlorophyta					
<i>Dendronotus frondosus</i>	*	*	*			<i>Blidingia minima</i>	*	-	-		
<i>Doto coronata</i>	*	-	-			<i>Prasiola stipitata</i>	*	-	-		
<i>Epitonium clathratulum</i>	*	-	-								
<i>Eubranchus exiguus</i>	-	*	-								

Annex 3 Publications based on RECON

- Coolen, J. W. P. (2017). North Sea Reefs. Benthic biodiversity of artificial and rocky reefs in the southern North Sea. PhD-thesis Wageningen University & Research, Wageningen, the Netherlands. <http://dx.doi.org/10.18174/404837>. 203 pp
- Dias, I. M., Spierings, M., Coolen, J. W. P., Van Der Weide, B., & Cuperus, J. (2017). First record of *Syllis vittata* (Polychaeta: Syllidae) in the Dutch North Sea. *Marine Biodiversity Records*, 10(1), 16. doi: 10.1186/s41200-017-0120-3
- Coolen, J. W. P., Lengkeek, W., Degraer, S., Kerckhof, F., Kirkwood, R. J., & Lindeboom, H. J. (2016). 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. *Aquatic Invasions*, 11(4), 437–449.
- Spierings, M., Dias, I. M., Coolen, J. W. P., Van Der Weide, B., & Cuperus, J. (2017). First record of *Harmothoe aspera* (Hansen, 1879) (Polychaeta: Polynoidae) in the Dutch North Sea. *Marine Biodiversity Records*, 10(29), 4. doi: 10.1186/s41200-017-0131-0

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- To conduct research with the aim of acquiring knowledge and offering advice on the sustainable management and use of marine and coastal areas.
- Wageningen Marine Research is an independent, leading scientific research institute

Wageningen Marine Research is part of the international knowledge organisation Wageningen UR (University & Research centre). Within Wageningen UR, nine specialised research institutes of the Stichting Wageningen Research Foundation have joined forces with Wageningen University to help answer the most important questions in the domain of healthy food and living environment.
