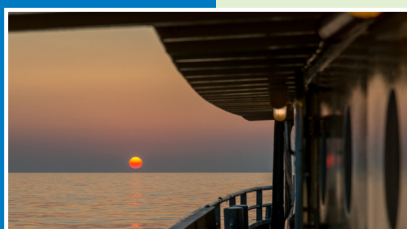


WWF & ARK Nature Borkum Reef Ground oyster pilot

Active restoration of native oysters in the North Sea
Monitoring september 2020



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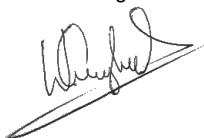
Bureau Waardenburg
Ecologie & Landschap

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Preface

As a result of overfishing, habitat destruction and diseases, shellfish reefs, once occupying about 30% of the Dutch part of the North Sea, almost entirely disappeared.

Since natural recovery seems impossible within decades, World Wide Fund for Nature (WWF) and ARK Nature, in collaboration with Bureau Waardenburg, Wageningen Marine Research and Sas Consultancy, have been working in recent years on recovery opportunities for shellfish reefs in the Dutch coastal zone (Voordelta) and off shore (Borkum Reef Ground, Gemini wind farm and on wreck M435). Within the Borkum Reef area in 2018 the first attempt to actively restore shellfish reefs in deeper parts of the North Sea was conducted. Since then the reef ground has been monitored on a yearly basis. In September 2020 the third expedition to Borkum Reef Ground was organized for studying the current state of the oysters and the progress of the reef bed formation.

This study was initiated and commissioned by WWF Netherlands. We would like to express our special thanks to Get Wet Maritime (Ben Stiefelhagen), the crew of the MS Tender and other crewmembers, Melchior Stiefelhagen, Maarten Slooves and Klaudie Bartelink, WWF representatives Brenda van Doorn-Deden and Emilie Reuchlin-Hugenholtz and our Bureau Waardenburg colleagues Udo van Dongen, Wouter Lengkeek, Karin Didderen and WMR colleague Pauline Kamermans.



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1 Introduction



Photo: Udo van Dongen



1.1 General background

From historical documentation, we know that epibenthic shellfish reefs, consisting mainly of flat oysters (*Ostrea edulis*), once occupied about 30% of the Dutch part of the North Sea seafloor (e.g. Olsen 1883). Recently, scientist and practitioners throughout Europe have been focusing on the endangered status of *O. edulis* habitats, and there is scope for restoration (Airoldi and Beck, 2007; Farinas-Franko *et al.* 2018; Gercken and Schmidt, 2014; Sawusdee *et al.*, 2015; Smaal *et al.*, 2015; Smyth *et al.*, 2018). Moreover, *O. edulis* reefs are now identified as a priority marine habitat for protection in European MPAs (OSPAR agreement 2008-6, OSPAR Commission, 2011).

In the Netherlands feasibility of the recovery of epibenthic shellfish reefs is estimated as feasible (Smaal *et al.*, 2015). The time for restoration of epibenthic shellfish reefs is right and shellfish reef restoration in the North Sea area is now supported by current Dutch and EU government policy, among others through the Marine Framework Directive, for the Dutch North Sea area implemented by the Marine Strategy policy paper, part 3 (Marine Strategy, 2015).

Based on the first findings of natural flat oyster reefs (Christianen *et al.*, 2018; van der Have *et al.*, 2016) and experiences with epibenthic shellfish reef restoration in the Voordelta, knowledge is being developed for near shore flat oyster reefs (Sas *et al.*, 2017; 2018, Christianen *et al.*, 2018).

Connecting projects to this research are the Revifes and Ecofriend research programs. In Revifes, at the Borkum Reef Ground site, research is being done on ecosystem services of naturally occurring reef systems such as stones and *Lanice* (sand mason worm) reefs. The focus of Ecofriend is on reproduction of oysters. To this end, samples were taken and analyzed on site in July 2020.

1.2 WWF & ARK Borkum Reef Ground oyster pilot

The Borkum Reef Ground pilot is the first example of restoration of shellfish reefs in deeper parts of the North Sea. As a first pilot location for offshore flat oyster restoration efforts, the Borkum Reef Ground area was selected, because of the natural presence of hard substrate. Didden *et al.* (2019) described the results of the first year of monitoring including detailed information on the pilot design and two field reports of two field trips - installation and first monitoring. This report is the follow-up and contains the results of the third field trip in September 2020.

1.3 Objectives

The pilot project, initiated in May 2018, focused on the possibilities for restoration of shellfish reefs in the deeper parts of the North Sea. Main objectives of the project were:

- starting shellfish reefs in deeper parts of the North Sea;
- investigate the key factors for success and failure for active restoration of structure-forming shellfish reefs in deeper parts of the North Sea.

1.4 Reading this field report

This report contains results of monitoring the pilot 28 months after deployment. This third monitoring campaign took place 22-23 September 2020.

2 Methods



Photo: Udo van Dongen



Table 2.1 Overview of all intended monitoring activities. This table is derived from Didden et al., 2019 where all specific research questions are further elaborated. The numbers in the first column correspond with the paragraphs (§) in Chapter 3 where these activities are addressed.

| § | Monitoring activities | Trip 5: Sept 2020 | Research questions | Common | Diagnostic | Ecosystem |
|-----|--|----------------------|-----------------------|--------|------------|-----------|
| | Dropcam survey | | 1-3, 6, 10 | X | | X |
| | Temperature measurements | | 8 | | X | X |
| 3.2 | Oyster measurements: | X | 3-10 | X | | |
| | Wet weight measurement | X | | X | | |
| | Length measurement | X | | X | | |
| | Condition assessment | | | | X | |
| | Gonad development | | | | X | |
| | DW determining | | | | X | |
| | <i>Presence of Bonamia</i> | | | | X | |
| 3.1 | Visual observation of survival | X | 1, 4 | X | | |
| 3.3 | Visual observation of present life forms | X | 1, 2, 5, 6 | X | X | X |
| 3.4 | Visual observation of rack & 3D structure damage | X | 9 - 11 | | | |
| 3.5 | Visual observation of biofouling and predators | X | 9 - 11 | | X | X |
| | Visual observation of spat settlement | X | 3, 4, 5, 6, 7 | X | | |
| 3.6 | Visual observation of oyster bed development | X | 1, 2 | X | | |
| 10 | Larvae sampling & counting | | 3, 8-10 | | X | |
| 11 | Spat collector | | 4, 5, 6, 7 | X | | |

2.1 Preparations

Mobilisation

The offshore research team and their equipment were mobilized on September 21st at the Lauwersoog harbour to spend two days at sea onboard of the MS Tender. The research period for the Borkum Reef oyster project was 22 - 23 September. A RHIB was used to bring the divers from the main ship (MS Tender) to the study area (Figure 2.1&Figure 2.2).

Briefing and safety

Onboard of the MS Tender a full briefing was held with the entire research team. In this briefing, everyone was updated on the project goals and instructed on their specific task during diving operations and on-board operations. A full safety briefing and Last-Minute Risk Analysis were also conducted. It was checked that all safety measures (e.g. decompression chamber, medical equipment, communications equipment, safety diver) was operational and had a designated operator.

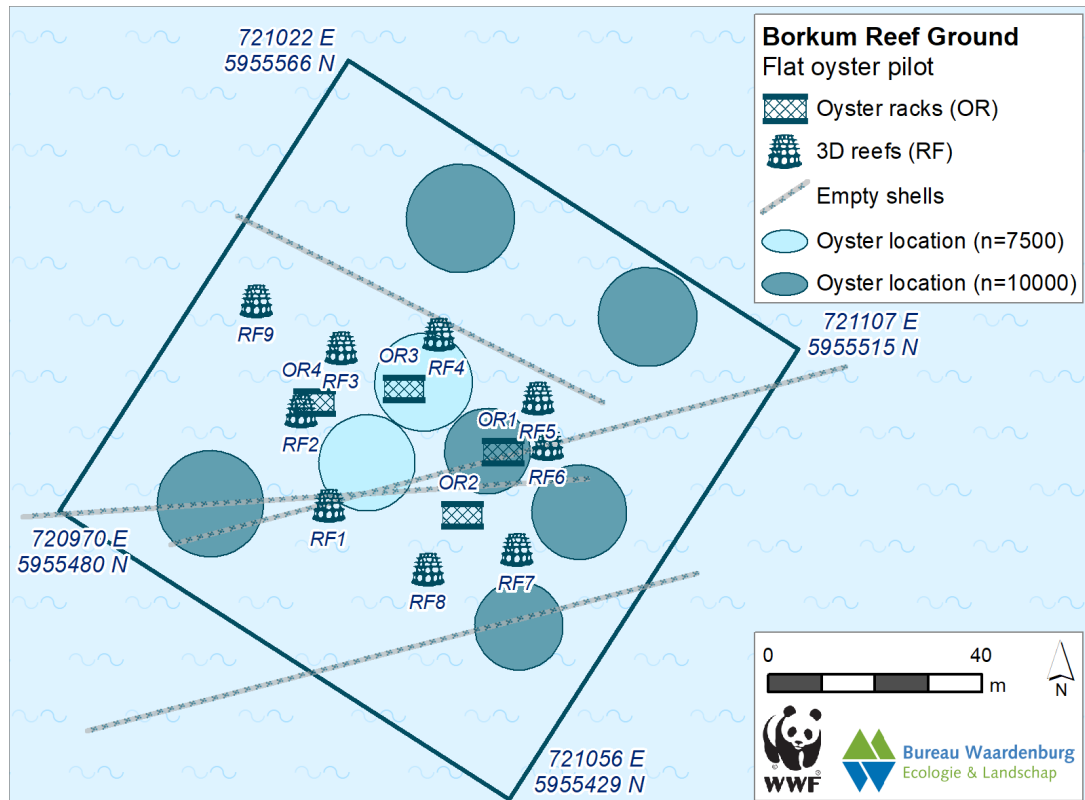


Figure 2.1 Pilot lay out, 9 3D reefs, 4 monitoring racks and 8 patches of oysters were installed on 24 and 25 May 2018.

Diving

Three research dives were made in optimal diving weather conditions on 22 and 23 September 2020. The slack water was short because of spring tide (23-09-2020). Dive times were 48, 52 and 51 min respectively. Diving depth was 26,4 meters. A nitrox

breathing gas and the NOAA diving tables were used. Dive times were all well within the no-decompression limits.

Near bottom the current was quite strong (approaching 0,5 knots) and visibility ranged between 0,5 and 2 meters.



Figure 2.2 Divers making ready for their dive to lift baskets, on board of the RHIB.

2.2 Activities

The monitoring activities of this expedition included:

- 1. Research racks (2 and 4): retrieval of baskets** with oysters for wet weight and length measurements and spat settlement;
- 2. Dive transects (UVS)** for visual observations of oyster density and survival; present life forms / biodiversity; rack & 3D structure condition; marine growth and predators; recruitment / spat settlement; oyster bed development.
- 3. Replacement of tools:** acoustic release as aid for future positioning and a new CTD logger.

Top priority was tracking down rack number 4 (Figure 2.3), because this was the only rack that had not been monitored yet since out placement in May 2018.

Other priority during diving was finding recruits on the seabed during the observations of the transects, as no other (remote sensing) technique is really suitable for finding recruits.



Figure 2.3 Oyster baskets from rack 4 on deck.

2.3 Research racks

Acoustic release mechanism

In September 2020, one rack was equipped with acoustic releases (rack 1). Unfortunately, the acoustic release did not work, due to low battery. Therefore, we sailed over the coordinates of rack 4 and deployed a small anchor with a buoy. Thereafter, two divers located two racks (4 and 2) and three of the artificial structures and connected them with a guideline for further orientation. The divers attached a new and clean acoustic release (Frequency 100.1) to rack 4.

Retrieving oyster baskets

Each research rack contained four oyster baskets. The racks can be opened under water. The baskets were retrieved by two divers: packed in a large net and sent to the surface by means of a lifting bag. The net with baskets was then picked up by a small RHIB and transported to the main vessel after the divers finished their dive.

Temperature data

No temperature loggers were retrieved, as rack 3 could not be located. A new logger (CTD) was placed at rack 2 on the central pole.

Oyster survival, length and wet weight

At installation in 2018, each research rack contained 4 baskets with 40 oysters (Table 2.2). Oyster baskets contained different subgroups including “holding tower”, “small” and “large”. (Holding tower: in two baskets per rack the oysters are placed in specific small mounting, enabling monitoring of identified individuals).

Table 2.2 Number of oysters per research rack and basket at time of installation.

| Rack number | Basket number | Number of oysters | Comments | Min shell width (mm) | Max shell width (mm) |
|-------------|---------------|-------------------|---------------|----------------------|----------------------|
| 1 | 28 | 40 | small | 52.1 | 88.5 |
| Position C3 | 8 | 40 | holding tower | 59.8 | 88.2 |
| | 10 | 40 | holding tower | 62.9 | 92.8 |
| | 17 | 40 | large | 68.6 | 111.1 |
| 2 | 21 | 40 | small | 40.0 | 76.3 |
| Position C4 | 11 | 40 | holding tower | 64.2 | 95.4 |
| | 6 | 40 | holding tower | 64.5 | 93.0 |
| | 16 | 40 | large | 53.5 | 98.0 |
| 3 | 23 | 40 | small | 47.9 | 76.7 |
| Position C2 | 3 | 40 | holding tower | 62.4 | 93.7 |
| | 12 | 40 | holding tower | 56.5 | 85.9 |
| | 25 | 40 | large | 71.1 | 106.1 |
| 4 | 1 | 40 | holding tower | 59.3 | 92.0 |
| Position C1 | 5 | 40 | holding tower | 64.5 | 87.2 |
| | 24 | 40 | small | 45.6 | 75.0 |
| | 32 | 40 | large | 73.2 | 106.1 |

On September 22th, 2020, research racks 2 and 4 were localised and oyster baskets taken out and brought up to the vessel. All oysters from each rack were handled. As rack 2 was already studied in July 2018, and dead animals were removed from the set-up, the number of oysters per basket was lower than the initial 40 as placed in the racks back in May 2018. Per basket the live and dead oysters were separated, oysters were numbered, biodiversity attached was registered, pictures were made of the retrieval and the percentage survival was established (Figure 2.4). All dead oysters within the baskets of rack 2 were collected and deployed in basket number 16 in order to serve as potential substrate for oyster larvae in the future.



Figure 2.4 Left: Studying biodiversity attached to the baskets and oyster shells. Right: Measuring length, weighing oysters after removal of the attached species.

The live oysters were cleaned from marine growth, weighed (wet weight in gram), measured (shell width in mm) and replaced in their original baskets. Values for wet weight and shell width were compared with initial values obtained in May 2018 and values obtained in September 2020.



Figure 2.5 Upper: Alive (numbers 1-17) and dead flat oysters of basket 21 (rack 2). After photographing, dead oysters were removed and collected in basket 16 (rack 2). Lower: Alive and dead flat oysters (unsorted) of basket 1 (Holding tower) (rack 4). After photographing, dead oysters were removed, alive oysters were cleaned, measured and weighted.

Recruitment

No new recruits had settled on live adult oysters. During the three dives no recruits were found on the seabed either. In contrast to earlier expeditions this time local circumstances did not allow to reserve an explicit diving time frame for finding recruits. The search for recruits this year was limited to the search patterns for the racks as illustrated in Figure 2.6.

2.4 Dive Transects UVS

Visual observation measurements were performed on dive 2 and 3 on 22 September. The blue lines in Figure 2.6 depict the different transects that were studied in the pilot area, each dive with two divers doing different measurement per transect.

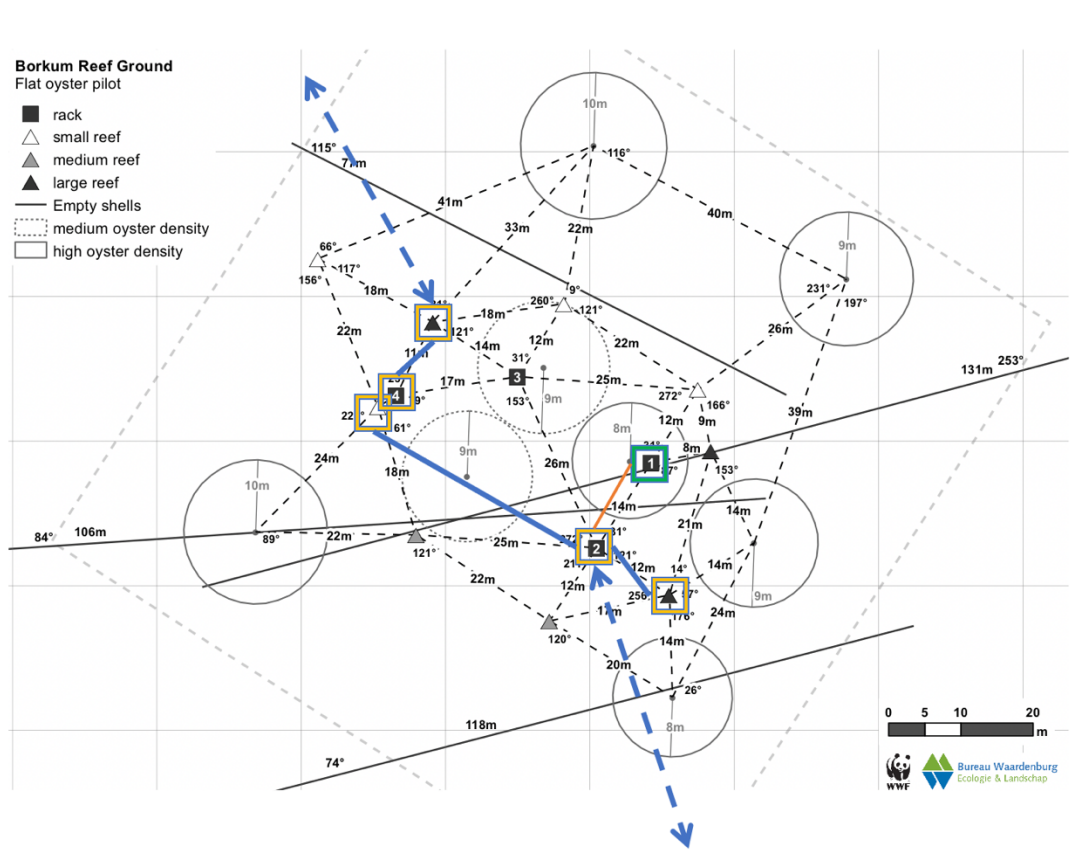


Figure 2.6 Different dive transects (blue lines) followed in the pilot area on 22 September 2020. Blue lines depict the set-out guideline (transect line) along which the UVS were performed and photos were taken. Orange squares are researched objects. Blue dotted lines depict enter and exit points of the divers. The Orange line depicts an additional transect on dive 3 to retrieve the old acoustic release.

During dives the following data were retrieved:

- Pilot: Visual observation position and condition of infrastructure, including research racks, 3D-reefs and loose shell material;
- Oysters: Visual observations survival, density and growth of adult oysters, presence of recruits, 5 individuals were additionally collected;
- Risks: Visual observations for predators and other risks to oysters;
- Biodiversity: Visual observations of biodiversity on seabed and 3D reef structures.

Oyster and predator density transects were carried out by following a transect line including a width of 2 meter for visual census. Each 5 meter (translating to 10 m²) the number of oysters and predators was recorded. Oysters were classed as dead or alive based on visibly wide-open shells when oysters are dead and visible filtering activity from live oysters.

Biodiversity on reef structures was quantified by using a 20x30cm quadrant randomly placed horizontally and vertically on the structures. In each quadrant, all visible species were recorded and named (to the highest taxonomic level of which our taxonomic specialist diver could be certain under water) and their density counted or estimated. This was repeated 8 times.

Additional species on the structures that were not present within the quadrants were also documented. Total biodiversity on the reefs was observed and recorded, in part by photo and video analysis, densities were not quantified. Furthermore, biodiversity found on the seabed along the transect lines was also registered. The length of transect lines was this year shorter in comparison to earlier years. On board, the search for additional fouling species was restricted to one expert, whereas in earlier expeditions at least 2 man focused on this aspect of the expedition.

3 Results



Photo: Udo van Dongen

3.1 Oyster survival

Table 3.1 Flat oyster survival rates in research racks (24 months for rack 2 July 2018 – Sep 20, 28 months for rack for rack 4 May 2018 – Sep 20).

| Rack | Basket | Oyster size | Number at start (May 2018) | Total abundant (Sept 2020) | Alive | Dead | Survival % Sept 2020 |
|------|--------|---------------|----------------------------|----------------------------|-------|------|----------------------|
| 2 | 6 | small | 40 | 21 | 16 | 5 | 76 |
| 2 | 11 | holding tower | 40 | 16 | 13 | 3 | 81 |
| 2 | 16 | holding tower | 40 | 10 | 0 | 10 | 0 |
| 2 | 21 | large | 40 | 31 | 17 | 14 | 55 |
| 4 | 1 | small | 40 | 40 | 28 | 12 | 70 |
| 4 | 5 | holding tower | 40 | 40 | 27 | 13 | 68 |
| 4 | 24 | holding tower | 40 | 40 | 24 | 16 | 60 |
| 4 | 32 | large | 40 | 40 | 29 | 11 | 73 |

Oyster survival in racks

Flat oyster survival in rack 4 varied from 60 – 73 % after 28 months (Table 3.1). Minimum survival rate (60 and 68%) was observed for oysters that were placed in the holding towers (basket 5 and 24). This is similar to the observations in 2019, where the survival rates in baskets with holding towers were lower (Didderen *et al.* 2019). It can be assumed that the size of the oysters became too large for the holding towers, which highly likely confined them and therefore hampered them in their feeding / breathing behavior and caused mortality. The holding towers were during this expedition removed from the racks.

Rack 2 was surveyed earlier (July 2018), since all dead oysters were removed back then, the oysters left over were less than the initial 40 per basket (starting back in May 2018). In addition, in July 2018 oyster were partially harvested for fertility and condition index research. Survival of the remaining oysters varied from 0 – 81% after 28 months. In 2020 no oysters were collected for measuring the condition index. Like concluded in 2019 (Didderen *et al.*) survival of large oysters in both racks 2 and 4 was highest.



Figure 3.1 Adult Flat oysters providing habitat for epifauna species groups (Anthozoa, Hydrozoa, Bryozoa) in sandy substrate with *Laniche conchilega*.

3.2 Oyster width and wet weight

Oyster width and wet weight

In September 2020 in total 154 oysters originating from the oyster racks 2 and 4 were measured and weighted.

Flat oyster width in racks varied in September 2020 from 61 – 109 mm, with an average of 84 mm, the range in May 2018 was 40-106 mm (Table 3.2). In September 2020 the wet weight varied from 40-352 g, with an average of 156 g. In May 2018 wet weight varied from 10-287 g. Wet weight measurements are hard to compare since weighing onboard will be influenced by swell and wave-action, and the fouling of other organisms to the oyster shells. The values are indicative.

Table 3.2 Flat oyster length and wet weight in research racks.

| Rack | Basket | May 2018 Width (mm) MIN | May 2018 Width (mm) MAX | May 2018 Width (mm) AVG | July 2018 Width (mm) MIN | July 2018 Width (mm) MAX | July 2018 Width (mm) AVG | Sep 2020 Width (mm) MIN | Sep 2020 Width (mm) MAX | Sep 2020 Width (mm) AVG | Sept 2020 #oysters alive |
|----------------|--------|-------------------------------|-------------------------------|-------------------------------|--------------------------------|--------------------------------|--------------------------------|-------------------------------|-------------------------------|-------------------------------|--------------------------------|
| 2 | 21 | 40 | 76 | 60 | 47 | 82 | 65 | 61 | 90 | 84 | 16 |
| 2 | 11 | 64 | 95 | 78 | 72 | 89 | 80 | 80 | 92* | 87* | 13 |
| 2 | 6 | 65 | 93 | 78 | 68 | 95 | 81 | x | x | x | 0 |
| 2 | 16 | 54 | 98 | 79 | 61 | 104 | 84 | 76 | 89* | 82* | 17 |
| 4 | 1 | 59 | 92 | 79 | | | | 65 | 92 | 78 | 28 |
| 4 | 5 | 65 | 87 | 77 | | | | 63** | 97 | 81 | 27 |
| 4 | 24 | 46 | 75 | 60 | | | | 44** | 88 | 69 | 24 |
| 4 | 32 | 73 | 106 | 87 | | | | 79 | 109 | 93 | 29 |
| Total Sep 2020 | | | | | | | | 61 | 109 | 81 | 154 |

| Rack | Basket | May 2018 weight (g) MIN | May 2018 weight (g) MAX | May 2018 weight (g) AVG | July 2018 weight (g) MIN | July 2018 weight (g) MAX | July 2018 weight (g) AVG | Sep 2020 Wet weight (g) MIN | Sep 2020 Wet weight (g) MAX | Sep 2020 Wet weight (g) AVG | Sept 2020 #oysters alive |
|----------------|--------|-------------------------------|-------------------------------|-------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|
| 2 | 21 | 10 | 148 | 36 | 29 | 63 | 46 | 154 | 265 | 193 | 16 |
| 2 | 11 | 45 | 106 | 78 | 65 | 135 | 94 | 74 | 352 | 212 | 13 |
| 2 | 6 | 41 | 130 | 82 | 65 | 125 | 94 | x | x | x | 0 |
| 2 | 16 | 22 | 212 | 100 | 44 | 237 | 129 | 55 | 230* | 128 | 17 |
| 4 | 1 | 37 | 112 | 67 | | | | 44 | 172 | 113 | 28 |
| 4 | 5 | 26 | 116 | 67 | | | | 50 | 147 | 107 | 27 |
| 4 | 24 | 9 | 56 | 29 | | | | 40 | 160 | 107 | 24 |
| 4 | 32 | 32 | 287 | 147 | | | | 88 | 342 | 230 | 29 |
| Total Sep 2020 | | | | | | | | 40 | 352 | 156 | 154 |

* In July 2018 rack 2 has been lifted, oysters were measured and some were collected for Bonamia and condition research.

** In September 2020 the smallest oysters seem smaller than initially measured in 2018, probably a result of measurement inaccuracy.

Oysters of the racks 2 and 4 were analyzed and compared to initial values (May 2018) (Figure 3.2). The average width of the oysters in rack 2 (mean \pm SD) in 2020 increased around 10 mm in comparison to the initial average size (mean \pm SD) in this rack. In rack 4 (mean \pm SD) the average increase in size is around 5 mm when oysters of 2020 are compared to the initial sizes in 2018 (mean \pm SD).

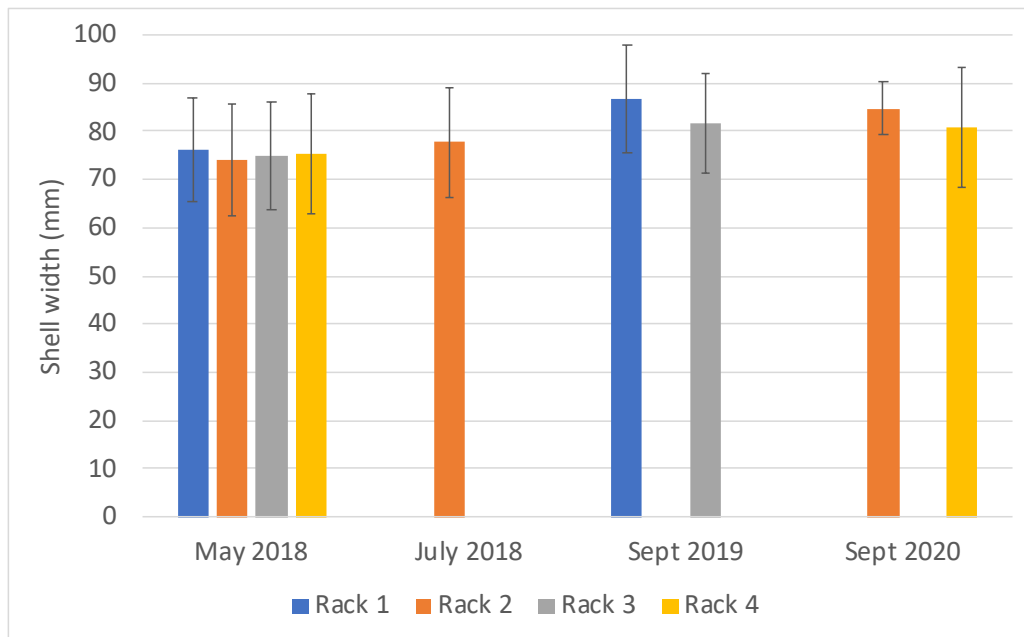


Figure 3.2 Shell width in mm in initial subsamples (May 2018), rack 2 (July 2018), rack 1 and 3 (September 2019) and rack 2 and 4 (September 2020).

3.3 Biodiversity

Seabed

The seabed consisted of sandy substrate, with patches of gravel, shell substrate, *Lanice* congregations and oysters. A total of 29 species (16 unique species for this monitoring) was observed on the sand (Table 3.3, Figure 3.4 and Appendix B).

Reefs, racks and flat oysters

Table 3.3 presents data from 8 quadrants in which biodiversity is quantified on the artificial 3D-reef structures (19 species). Substantial biodiversity was also observed on the baskets and flat oysters in the baskets, flat oysters on the seabed, 3D-reefs and research racks (Table 3.3, Appendix B). A total of 68 species were observed on these structures. Noteworthy were the substantial amounts of mobile species such as goldsinny wrasse, butterflyfish (Figure 3.2) and edible crab that were attracted by the reefs, and young recruits of the cold-water coral dead man's finger (Figure 3.4, Figure 3.5).



Figure 3.3 Species found in research racks: *Ctenolabrus rupestris* (Gold sinny wrasse) (left) and *Pholis gunnellus* (butterfish) (right).

Table 3.3 Biodiversity and density of species in the pilot area presented as mean densities per square meter. Data retrieved from 8 quadrants of 20x30 cm.

| Species % | Common name | Mean coverage % |
|-------------------------------|-----------------------|----------------------|
| <i>Porifera sp.</i> | | 0,70 |
| <i>Electra pilosa</i> | | 0,20 |
| <i>Escharoides coccinea</i> | | 1,45 |
| <i>Obelia sp.</i> | | 0,45 |
| <i>Jassa</i> | | 2,67 |
| <i>Diplosoma sp.</i> | | 1,58 |
| Species n | | Mean coverage per m2 |
| <i>Alcyonium digitatum</i> | Dead mans finger | 0,12 |
| <i>Diadumene cincta</i> | | 0,06 |
| <i>Sagartia elegans</i> | Elegant anemone | 0,30 |
| <i>Sagartia troglodytes</i> | Mud sagartia | 0,42 |
| <i>Metridium dianthus</i> | Plumose anemone | 0,18 |
| <i>Halecium halecinum</i> | Herring-bone hydroid | 0,08 |
| <i>Polycera quadrilineata</i> | Four-striped polycera | 0,60 |
| <i>Macropodia sp.</i> | | 0,12 |
| <i>Necora puber</i> | Velvet crab | 0,06 |
| <i>Cancer pagurus</i> | Edible crab | 0,06 |
| <i>Asterias rubens</i> | Common sea star | 0,06 |
| <i>Pholis gunnelus</i> | Rock gunnel | 0,06 |
| <i>Ctenolabrus rupestris</i> | Gold sinny wrasse | 0,09 |

A total of **84 species**, mainly mollusc and arthropods, were observed by the divers in the pilot area (Figure 3.4, Figure 3.5, Appendix B, combining seabed and reef, rack, flat oyster data). Traces (empty shells, nudibranch eggs, bones, dead animals) of 8 additional species were observed by the divers, these are not included in the species list. In 2019, only 42 species were reported. In September 2020 biodiversity was mainly studied on hard substrates (3D reef structures, species attached to flat oyster), while there was a focus on biodiversity measurements on the seabed in 2019. A total species list of the monitoring surveys (2019 and 2020) is presented in Appendix B.

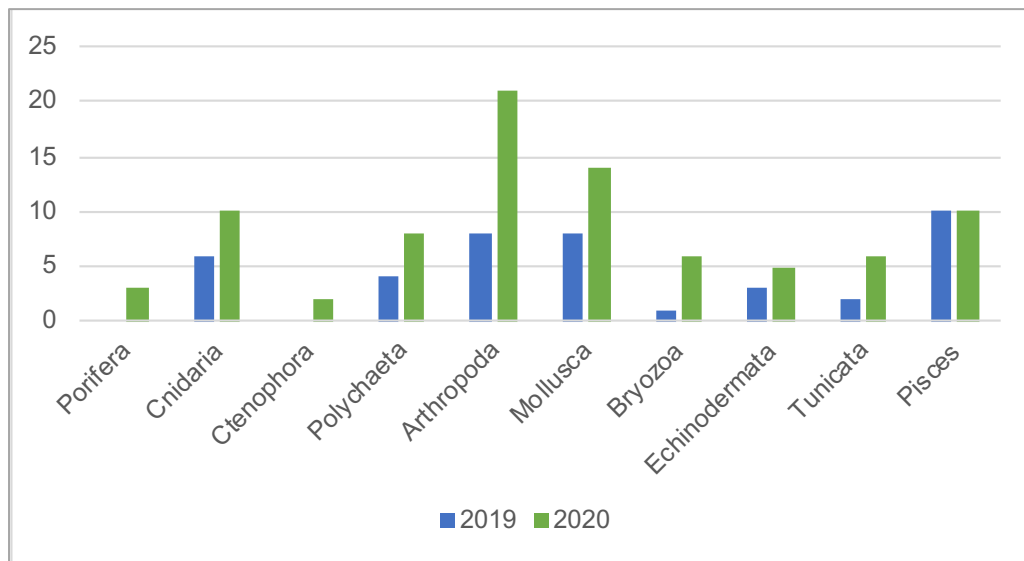


Figure 3.4 Number of species per phylum found in monitoring surveys (September 2019, September 2020) in the pilot area.

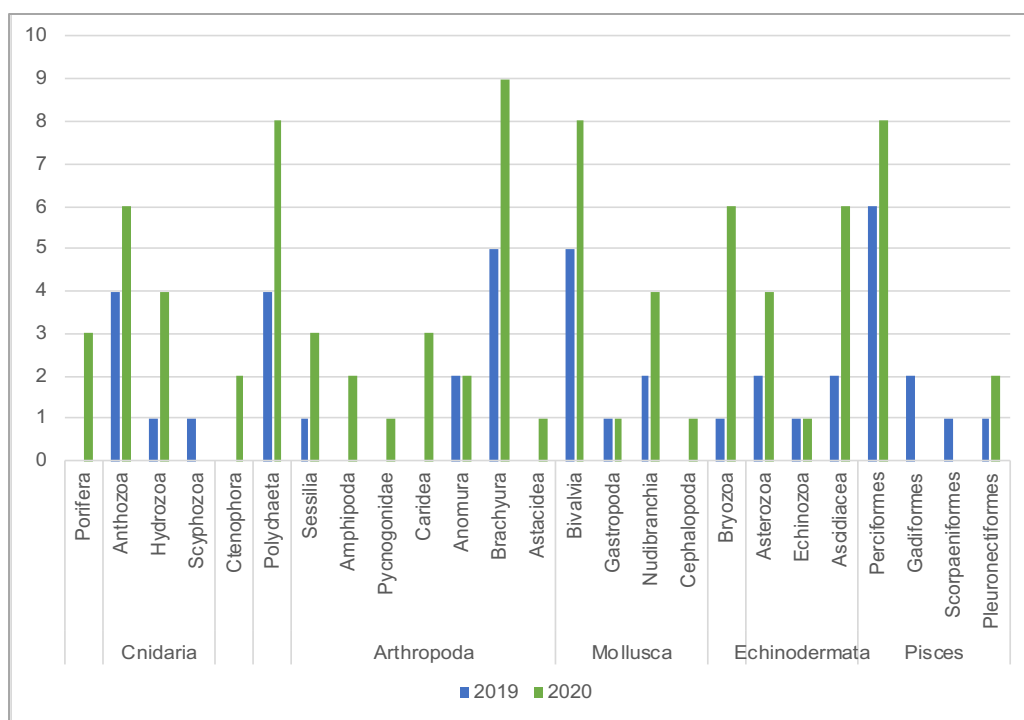


Figure 3.5 Number of species per group (per phylum) found in monitoring surveys (September 2019, September 2020) in the pilot area.

Oysters

Although no quantitative data of epifauna growing on the oysters were collected, divers noted a large biodiversity on and underneath oysters. This included eggs of the Atlantic bobtail (*Sepiola atlantica*) (Figure 3.6, left), nudibranchs with eggs, different species of sea anemones, bryozoans and hydrozoans, and the tube worm (*Pomatoceros triqueter*). Furthermore, crab and fish species such as the goldsinny wrasse and goby species hiding underneath oysters and in the reef structures were observed (Figure 3.6, right).



Figure 3.6 Left: Eggs of the Atlantic bobtail *Sepiola atlantica* attached to a Flat oyster (basket 6, rack 2).
Right: 3D Reef structure small 'Jan Willem' with representatives of different taxa groups such as soft coral, anemones, hydrozoans, bryozoans, starfish, crustaceans, tunicates and fish.

3.4 Performance of research rack and 3D-reef structure

Inspection by divers in September 2020 showed structures, both racks and 3D reefs (Jan Willem & Alex) standing upright and intact (Figure 2.3, Figure 3.6, Figure 3.7). Like reported before (Didderen et al., 2019) 3D reef Emilie had toppled sideways, but was intact.

Over the years the 3D structures are developing a small hard substrate community similar to adjacent rocks. Sessile animals like sponges, bryozoa, hydroids, anemones and soft coral (table 3.3) are now fully covering the structure. Attracting mobile species like fishes, squids, sea slugs, starfish and crabs for feeding, reproduction and shelter. For example, the dead mans finger (soft coral) was previously smaller and not on every 3D structure.



Figure 3.7 Another intact reef structure with high biodiversity attracted, large reef 'Alex'.

3.5 Biofouling and predators

Fouling organisms

The reefs were colonised by a diverse epifaunal community, which consisted of benthic and mobile species. Both research racks and reefs were greatly covered in encrusting sessile organisms. This fouling of the research racks could have been unbeneficial to the condition of the oysters in the baskets, but the baskets were not overgrown to a state in which suffocating would be a risk. During inspection of the oysters in the research racks several species were observed to be growing on the oysters, such as blue mussel, hydroid species and the tubeworm *Spirobranchus triqueter*.

Predators

Common starfish (*Asterias rubens*) and edible crab are known predators, especially for young oysters, and were observed in the pilot area (Appendix B). High survival percentage of adult oysters, however, suggests that this is no direct threat to the adult oysters.

3.6 Oyster recruitment and oyster bed development.

Adult oysters are present on the seabed, growing, as observed by their growth edge, and healthy. Within a transect of 1,5 meters wide and 50 meters long 8 alive and 5 dead oysters were observed. This transect is depicted in figure 2.6. The observed density in this transect is lower than was observed in transects during the monitoring in 2019. Outside of this transect, live oysters were also observed on the seabed, but these were not quantified. Recruitment on the seabed was observed in the pilot area only in 2019. Randomly, 5 alive adult Flat oysters were collected from the seabed, their size and weight were determined (Table 3.4). They will be used for further research (isotope analysis) and stored by -20C in the ReViFES project.

Table 3.4 *Random measurement (length and weight) of 5 adult alive Flat oysters collected from the seabed (26,4 meters depth).*

| | gram | mm |
|----------|------|----|
| Oyster 1 | 347 | 94 |
| Oyster 2 | 271 | 87 |
| Oyster 3 | 243 | 93 |
| Oyster 4 | 290 | 94 |
| Oyster 5 | 232 | 92 |



Additional field observation

Whilst drifting away from the reef during the end of the dive to the south east, patches with higher densities of oyster (3 or 4 together) were observed outside the original placement fields. See also Figure 2.6, the dotted blue line in South East direction.

4 Discussion



Conclusion

The 2020 expedition to the Borkum Reef ground was aimed at monitoring the survival of the oysters as they were deployed in May 2018. Adding to observations in 2019, in 2020 results are positive: adult oysters placed in the research racks survived and increased in size over the 28 months since deployment in 2018. At the same time oysters on the seabed are still present and surviving.

Observed density of oysters on the seabed was fairly low in 2020's quantitative transect, but based on 1 transect, it cannot be concluded if this is indicative for a decreasing overall density or caused by local differences in density within the pilot area.

In 2020 no new recruits are identified, not in the research racks, nor during the study of the seabed. The observed biodiversity increased over the last year, indicating the presence of the oysters serve as an attractive hotspot for various organisms.

Also the 3D reefs develop successfully with more species attracting and obvious growth of the animals inhabiting the artificial structures.

In conclusion, the Borkum Reef Ground oyster reef, that was actively restored in 2018, shows survival, growth, reproduction and recruitment and serves as a reef: a hotspot for biodiversity, with species such as *Ctenolabrus rupestris* (Gold sinny wrasse), *Sepiola atlantica* (Atlantic bobtail), and *Polycera quadrilineata* (four striped polycera).

4.1 Lessons learned

Oyster pilot

The main lessons learned in 2020 are:

- It is possible to introduce installations and flat oysters on the sea floor at a deep (23 meters at low tide) offshore location and retrieve them at a later point in time (28 months) for monitoring purposes.
- The placement of 5500 kg, or 80.000 specimen, adult flat oysters on the sea floor has so far led to the result of flat oyster specimen being alive and showing growth. These results, 28 months after installation, although largely based on qualitative data, are promising.
- On the seabed, alive oysters are still present. Observed densities, both in 2019 and 2020, seem lower than can be expected from the initial placed density. The decrease of the density may be the result of oysters being displaced and moved outside the pilot area due to stormy conditions and are now distributed over a larger area than the initial pilot area.

Monitoring techniques

Earlier lessons learned during monitoring were confirmed in the 2020 fieldtrip:

- Divers can effectively monitor several aspects of the oyster bed in two days' time.
- Using a rehoistable research rack is a good way to get quantitative results of oyster parameters like survival, growth and presence of recruits.

4.2 Recommendations

These positive and promising results encourage further monitoring of the pilot and its long-term effects. In subsequent monitoring expeditions, the following monitoring is recommended:

- Survival and growth of adult oysters
- Monitoring of larval presence
- Density development of adult oysters on the seabed
- Epifaunal assemblages directly related to the oysters
- Biodiversity development on the seabed, also compared to a reference area
- Searching for evidence of adult oysters being moved out of the initial pilot area

Initiate a spat on shell experiment offshore

Offshore conditions are suitable to support flat oysters and the 2019 study showed that the oysters reproduce (Didderen *et al.* 2019). The density of the oysters on the sea bed seems to be decreasing, possibly due to dispersal during storms. The critical density essential for further fertilisation and growth of the reef may be under pressure. A new boost to the pilot area can be the introduction of spat on shell. The method is a cost-effective way to support the reef to become a self-sustaining population. To keep the spat on shell within the pilot area it is essential to have them settle on larger substrates which will be able to withstand currents due to stormy weather.

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Appendix A

Textbox: Objectives of the Borkum Reef Ground oyster pilot

(Source: Reuchlin-Hugenholtz, 2018)

The following overall objectives are formulated for this project:

1. Kick start shellfish beds in deeper parts of the North Sea;
2. Get insight in the key success and failure factors for active restoration of structure-forming shellfish beds in deeper parts of the North Sea;

More specifically, the 2018 pilot project at the Borkum Reef Ground aims at:

- A. Developing a methodology for construction and restoration of structure-forming shellfish beds of mussels and flat oysters.
- B. Construction of a pilot flat oyster bed at deeper water in the North Sea at the Borkum Reef Ground area, by placement of:
 - live flat oysters (originating from Norway) at the pilot area of 100x100m.
 - research racks with flat oysters to study survival, growth and reproduction.
 - 3D artificial reefs to study facilitation of oyster bed restoration (incl. elevation).
 - shells (empty) in the surroundings of the live oysters to function as hard substrate for larvae settlement.
- C. Learning from the pilot project by studying the following research questions in a field (and laboratory) monitoring programme:
 - a. What is the mortality rate of introduced oysters, and what is the cause?
 - b. Can the introduced oyster population survive and reproduce, and if (not), why (not) (long-term objective)?
 - c. Can the introduced oyster population reproduce: i.e. produce gonads, resulting in larvae in the water column, resulting in recruitment on substrates?
 - d. Is biodiversity enhanced in the vicinity of the pilot area, through the formation of a natural reef?
 - e. Did oysters in the pilot die, and if so, why?
 - f. What are the critical success factors for the pilot project?
 - g. What are the critical fail factors for the pilot project?
 - h. Is biodiversity enhanced in the vicinity of the pilot area?

All activities in the pilot project are closely monitored, to determine success and failure factors and based on these factors to determine and describe a successful methodology for restoration of flat oyster beds in the North Sea.

Appendix B

Total species list obtained during diving operations in September 2019 & September 2020. In 2020, species observed on the seabed and on structures (Flat oysters in the baskets, Flat oysters on the seabed, 3D-reefs and research racks) are marked.

| Total species list | | 2019 | 2020 | |
|------------------------------|--------------------------|------|--------|-------|
| Scientific name | Common name | | Seabed | Reefs |
| <i>Porifera sp.</i> | | | 1 | X |
| <i>Suberites ficus</i> | Fig sponge | | 1 | X |
| <i>Sycon sp.</i> | | | 1 | X |
| <i>Diadumene cincta</i> | Orange anemone | | 1 | X |
| <i>Metridium senile</i> | Plumose anemone | 1 | 1 | X |
| <i>Sagartia elegans</i> | Elegant anemone | | 1 | X |
| <i>Sagartia troglodytes</i> | Mud sagartia | 1 | 1 | X |
| <i>Sagartiogeton undatus</i> | Small snakelocks anemone | 1 | 1 | X |
| <i>Alcyonium digitatum</i> | Dead mans finger | 1 | 1 | X |
| <i>Hydrozoa sp.</i> | Hydrozoans | 1 | 1 | X |
| <i>Obelia sp.</i> | | | 1 | X |
| <i>Plumulariidae</i> | | | 1 | X |
| <i>Halecium halecinum</i> | Herring-bone hydroid | | 1 | X |
| <i>Chrysaora hysoscella</i> | Compass jellyfish | 1 | | |
| <i>Beroe gracilis</i> | Melon jellyfish | | 1 | X |
| <i>Mnemiopsis leidyi</i> | American comb jelly | | 1 | X |
| <i>Hediste diversicolor</i> | Ragworm | | 1 | X |
| <i>Laniche conchilega</i> | Sand mason worm | 1 | 1 | X |
| <i>Lepidonotus sp.</i> | | | 1 | X |
| <i>Nephtys</i> | | | 1 | X |
| <i>Nereididae</i> | | | 1 | X |
| <i>Pomatoceros triqueter</i> | Tube worm | | 1 | X |
| <i>Sabellaria spinulosa</i> | Ross worm | 1 | 1 | X |

| Total species list | | 2019 | 2020 | |
|--------------------------------|----------------------------|------|--------|-------|
| Scientific name | Common name | | Seabed | Reefs |
| <i>Spionidae</i> | Spionid worms | 1 | X | |
| <i>Spirorbidae</i> | | | 1 | X |
| <i>Spirobranchus triqueter</i> | Keelworm | 1 | X | |
| <i>Balanus crenatus</i> | | | 1 | X |
| <i>Verruca stroemia</i> | | | 1 | X |
| <i>Sessilia</i> | Barnacles | 1 | 1 | X |
| <i>Corophidae</i> | Amphipods | | 1 | X |
| <i>Jassa sp.</i> | Tube building amphipods | | 1 | X |
| <i>Pycnogonidae</i> | Sea spider | | 1 | X |
| <i>Caridae sp.</i> | Shrimp | | 1 | X |
| <i>Crangon sp.</i> | Brown shrimp | | 1 | X |
| <i>Pandalus sp.</i> | Northern shrimp | | 1 | X |
| <i>Pagurus bernhardus</i> | Common hermit crab | 1 | 1 | X |
| <i>Pisidia longicornis</i> | Long-clawed porcelain crab | 1 | 1 | X |
| <i>Ebalia sp.</i> | | | 1 | X |
| <i>Macropodia sp.</i> | Long-legged spider crab | 1 | 1 | X |
| <i>Inachidae</i> | Spider crabs | 1 | X | |
| <i>Liocarcinus holsatus</i> | Flying crab | 1 | 1 | X |
| <i>Liocarcinus navigator</i> | Arch-fronted swimming crab | | 1 | X |
| <i>Liocarcinus sp. juv.</i> | Swimming crab juvenile | 1 | 1 | X |
| <i>Necora puber</i> | Velvet swimming crab | | 1 | X |
| <i>Carcinus maenas</i> | Shore crab | | 1 | X |
| <i>Pilumnus hirtellus</i> | Hairy crab | | 1 | X |
| <i>Cancer pagurus</i> | Edible crab | 1 | 1 | X |
| <i>Homarus gammarus</i> | European lobster | | 1 | X |
| <i>Bivalvia</i> | Bivalves | 1 | 1 | X |
| <i>Aequipecten opercularis</i> | Queen scallop | 1 | X | |

| Total species list | | 2019 | 2020 | |
|--------------------------------|-----------------------|------|--------|-------|
| Scientific name | Common name | | Seabed | Reefs |
| <i>Ensis sp.</i> | Razor shell | 1 | 1 X | |
| <i>Heteranomia squamula</i> | Prickly jingle | | 1 | X |
| <i>Kellia suborbicularis</i> | | | 1 | X |
| <i>Modiolus modiolus</i> | Northern horse mussel | | 1 | X |
| <i>Mytilus edulis</i> | Blue mussel | 1 | 1 | X |
| <i>Ostrea edulis</i> | European flat oyster | 1 | 1 X | X |
| <i>Magallana gigas</i> | Pacific oyster | | 1 | X |
| <i>Nassarius sp.</i> | | | 1 X | |
| <i>Trivia</i> | Cowrie | 1 | | X |
| <i>Acanthodoris pilosa</i> | | | 1 | X |
| <i>Onchidoris bilamellata</i> | Rough-mantled doris | | 1 | X |
| <i>Polycera quadrilineata</i> | Four-striped polycera | 1 | 1 | X |
| <i>Aeolidia papillosa</i> | Common grey sea slug | 1 | X | |
| <i>Tritonia plebeia</i> | | | 1 | X |
| <i>Sepiola atlantica</i> | Atlantic bobtail | | 1 X | X |
| <i>Alcyonidium sp.</i> | | | 1 | X |
| <i>Bryozoa</i> | Moss animals | 1 | 1 | X |
| <i>Bugula sp.</i> | | | 1 | X |
| <i>Electra pilosa</i> | Sea mat | | 1 | X |
| <i>Escharoides coccinea</i> | | | 1 | X |
| <i>Scrupocellaria scruposa</i> | | | 1 | X |
| <i>Amphipholis squamata</i> | Brooding brittle star | | 1 | X |
| <i>Asterias rubens</i> | Common sea star | 1 | 1 | X |
| <i>Astropecten irregularis</i> | Sand sea star | 1 | X | |
| <i>Ophiotrix fragilis</i> | Common brittle star | | 1 | X |
| <i>Ophiuroidea sp.</i> | Brittle stars | | 1 X | X |
| <i>Psammechinus miliaris</i> | Green sea urchin | 1 | 1 | X |

| Total species list | | 2019 | 2020 | | |
|---------------------------------|---------------------|------|--------|-------|---|
| Scientific name | Common name | | Seabed | Reefs | |
| <i>Ascidacea</i> | Sea squirts | 1 | 1 | X | X |
| <i>Botrylloides sp.</i> | | | 1 | | X |
| <i>Ciona intestinalis</i> | | | 1 | | X |
| <i>Didemnum sp.</i> | | | 1 | | X |
| <i>Diplosoma listerianum</i> | | 1 | 1 | | X |
| <i>Styela clava</i> | Leathery sea squirt | | 1 | | X |
| <i>Ammodytes tobianus</i> | Small sandeel | | 1 | X | |
| <i>Aphia minuta</i> | Transparent goby | | 1 | X | |
| <i>Gobiidae</i> | Gobies | 1 | 1 | X | |
| <i>Pomatoschistus minutus</i> | Sandgoby | | 1 | X | |
| <i>Parablennius gattorugine</i> | Tompot blenny | 1 | 1 | | X |
| <i>Ctenolabrus rupestris</i> | Gold sinny wrasse | 1 | 1 | | X |
| <i>Callionymus lyra</i> | Common dragonet | 1 | | X | |
| <i>Mullus surmuletus</i> | Striped red mullet | 1 | 1 | X | |
| <i>Pholis gunnellus</i> | Rock gunnel | 1 | 1 | X | X |
| <i>Raniceps raninus</i> | Tadpole fish | 1 | | | X |
| <i>Ciliata mustela</i> | Fivebeard rockling | 1 | | | X |
| <i>Agonus cataphractus</i> | Hooknose | 1 | | X | |
| <i>Limanda limanda</i> | Common dab | 1 | 1 | X | |
| <i>Microstomus kitt</i> | Lemon sole | | 1 | X | |



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