

Federal Agency for Nature Conservation

Restoration of European oyster (*Ostrea edulis*) in the German North Sea (RESTORE preliminary investigation)

Bernadette Pogoda, Bérenger Colsoul, Tanja Hausen, Verena Merk and Corina Peter BfN-Schriften 684 2024











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Impressum

Cover picture: top row (left to right): *Ostrea edulis* spat oysters, two millimetres in size (AWI/Merk); test setup on landing gear under water (AWI/Merk); European oyster settled on shells (AWI/Colsoul); Bottom row (left to right): Divers placing European oysters in the North Sea (AWI/Mueller-Elsner); European oysters grown in field trials in an oyster basket (AWI/Merk); Measurement of European oysters (AWI/Merk)

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Summary

As hotspots of biodiversity, oyster reefs fulfil important ecological functions. Being biogenic reef builders, for many animal species they provide food, a substrate for colonisation, and a space to spawn and find shelter, thus increasing biodiversity. By filtering the water they improve water quality and enhance benthic-pelagic coupling.

European oyster (*Ostrea edulis*) and the oyster banks they form were once widespread in the North Sea. Due to overfishing until the 1920s, the species is considered functionally extinct in the German Bight. The testing and development project RESTORE, supported by the BfN with BMUV funding, is investigating methods for the restoration of the European oyster population in the German North Sea. For the purpose of sustainable population development, the project is working on technological and biological challenges and aspects of nature conservation law, and develops and trials methods for practical implementation as a part of marine nature conservation measures.

The preliminary investigation provided the legal framework for the practical testing of measures to restore European oyster in the Borkum Reef Ground Nature Conservation Area (NCA). It helped to establish a comprehensive knowledge and technology transfer network in Europe. RESTORE initiated the creation of the Native Oyster Restoration Alliance (NORA), where European projects for the restoration of the native oyster work closely together. Comprehensive field studies conducted during the preliminary investigation confirmed the fundamental suitability of European oyster for restoration in the German North Sea under the environmental conditions that prevail today. The preliminary investigation identified the specific challenge of availability of suitable seed oysters for marine conservation measures and developed suggestions for solutions. RESTORE recommended the establishment of a German seed oyster breeding programme for long-term restoration measures. This is now being implemented funded by the Federal Biodiversity Programme within the context of the PROCEED project. Through extensive laboratory and field experiments, the preliminary investigation identified suitable substrates that can be used in oyster breeding and in the practical implementation of restoration measures in the field.

In conclusion, the preliminary investigation recommended the construction and scientific investigation of a pilot oyster reef in the Borkum Reef Ground NCA. All legal, biological and technological requirements have been identified, so that this next step can be carried out as part of the RESTORE main project and subsequently evaluated by scientific monitoring.

Zusammenfassung

Austernriffe haben als Hot Spots der biologischen Vielfalt wichtige ökologische Funktionen. Als biogene Riffbildner erbringen sie wertvolle Ökosystemleistungen: Sie bieten Nahrung, Siedlungssubstrat, Laichgrund und Schutzraum für viele Tierarten und erreichen damit eine Steigerung der Biodiversität. Durch ihre Filtrationsleistung verbessern sie die Wasserqualität und steigern die benthopelagische Kopplung.

Die Europäische Auster und ihre Austernbänke waren in der Nordsee einst weit verbreitet. Durch Überfischung bis in die 1920er Jahre gilt die Art in der Deutschen Buchtheute als funktionell ausgestorben. Das vom BfN mit Mitteln des BMUV geförderte Erprobungs- und Entwicklungsvorhaben RESTORE untersucht Methoden zur Wiederherstellung der Bestände der Europäischen Auster (*Ostrea edulis*) in der deutschen Nordsee. Für einen nachhaltigen Bestandsaufbau der Art werden naturschutzrechtliche, technologische und biologische Fragestellungen bearbeitet und Methoden für die praktische Umsetzung im Rahmen von Meeresnaturschutzmaßnahmen entwickelt und erprobt.

Die Voruntersuchung liefert den rechtlichen Rahmen für die praktische Erprobung von Wiederansiedlungsmaßnahmen mit der Europäischen Auster im Naturschutzgebiet (NSG) Borkum Riffgrund. Sie leistete den Aufbau eines umfassenden Netzwerks zum Wissens- und Technologietransfer in Europa. RESTORE initiierte die Gründung der Native Oyster Restoration Alliance (NORA), in der die europäischen Projekte zur Wiederansiedlung der heimischen Austernart eng zusammenarbeiten. Die Voruntersuchung bestätigte durch umfassende Freilanduntersuchungen die grundsätzliche Eignung der Europäischen Auster für eine Wiederansiedlung in der deutschen Nordsee bei den heute vorherrschenden Umweltbedingungen. Sie identifizierte das konkrete Problem der Verfügbarkeit ausreichend geeigneter Saataustern für Meeresnaturschutzmaßnahmen und erarbeitete Lösungsvorschläge. RESTORE empfahl den Aufbau einer eigenen Saatausternzucht für langfristige Wiederansiedlungsmaßnahmen, der nun durch das Bundesprogramm Biologische Vielfalt im Rahmen des Projektes PROCEED umgesetzt wird.

Die Voruntersuchung identifizierte durch umfassende Labor- und Feldexperimente geeignete Substrate, die in der Austernzucht und bei der praktischen Umsetzung von Wiederansiedlungsmaßnahmen im Freiland eingesetzt werden können. Abschließend empfiehlt die Voruntersuchung die Errichtung und wissenschaftliche Untersuchung eines Pilotausternriffes im NSG Borkum Riffgrund. Hierfür wurden alle naturschutzrechtlichen, biologischen und technologischen Grundlagen erarbeitet, so dass dieser nächste Schritt im Rahmen des RESTORE Hauptvorhabens erfolgen und mittels wissenschaftlicher Begleitung evaluiert werden kann.

1 Introduction and Objective

As a biogenic type of reef that occurs in temperate latitudes, oyster and mussel beds are one of the most threatened habitats in the world (Airoldi, Beck 2007). European oyster beds in particular are considered to be severely endangered due to massive fishing pressure throughout Europe over recent centuries. Intact oyster beds deliver valuable ecosystem functions and services: they provide food, a substrate for settlement, shelter, and act as spawning grounds for many animal species. They are thus regarded as hotspots of biodiversity.

Oysters feed by filtering the water; in the process they improve water quality and reduce local toxic algae blooms. By ingesting and utilising planktic organisms and suspended organic matter, they increase benthic-pelagic coupling and solidify loose sediments, thereby achieving an overall increase in the value of the surrounding ecosystems (Austen 2011; Beck et al. 2011; Pogoda et al. 2019). The oyster species *Ostrea edulis*, which is native to Europe, is considered a keystone species with special ecological importance but is threatened with extinction. Accordingly, it is defined as a special priority species for the OSPAR Region II - Greater North Sea/Northeast Atlantic (OSPAR 2008) and, according to the Habitats Directive, is a component of the specially protected habitat type "reef", whose favourable conservation status must be preserved or restored (BfN 2008; Pogoda et al. 2020b).

Within the framework of the testing and development project 'RESTORE - Restoration of European oyster (*Ostrea edulis*) stocks in the German North Sea (preliminary investigation)', methods and procedures for sustainable stock restoration of European oyster in the German North Sea are being developed for the first time, as well as being implemented and tested on a pilot basis. The RESTORE project is based on the feasibility study 'Current status of European oyster (*Ostrea edulis*) and possibilities of reintroduction in the German North Sea' (Gercken, Schmidt 2014), which was compiled on behalf of the Federal Agency for Nature Conservation (BfN) and published as BfN Script 379. In this feasibility study, the authors determined on the basis of theoretical framework conditions that the prerequisites for reintroduction of European oyster in the German North Sea are in principle fulfilled. However, they recommended an in-depth preliminary investigation in order to gain insights into the practicalities of biological and technological implementation of reintroduction projects.

This is where the RESTORE preliminary investigation began: its objective was to determine the prerequisites, develop procedures and gain knowledge that would make it possible to restore stocks of European oysters in the German North Sea in a sustainable manner. As part of the preliminary investigation, important theoretical findings of the feasibility study were tested in practice, and basic scientific and technological framework conditions were defined for the next phase of implementation. The tasks of the preliminary investigation included:

- 1. reviewing the legal framework for European oyster reintroduction in the German EEZ;
- 2. determining suitable areas for reintroduction;
- 3. identifying procurement sources for suitable oysters and suitable substrate;
- **4.** investigating the biological suitability of the European oyster at potential reintroduction sites by collecting data on growth and fitness;
- 5. developing and recommending suitable technologies to be used in reintroduction.

With this project, practical measures for European oyster reintroduction in the German North Sea were tested for the first time.

The lessons learnt were processed into widely applicable recommendations defining practical implementation measures for the RESTORE main project. The complete testing and development project serves as a basis for future, long-term restoration measures and for the creation of a restoration programme for the native oyster species in the German North Sea (Fig. 1).

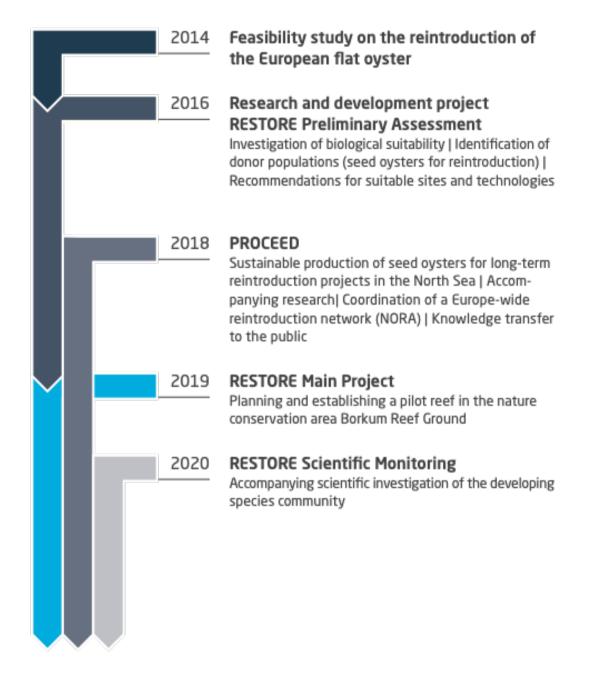


Fig. 1: German projects for European oyster reintroduction: timeline and thematic priorities of the RESTORE and PROCEED projects (AWI / Y. Nowak).

2 Ecological and nature conservation background

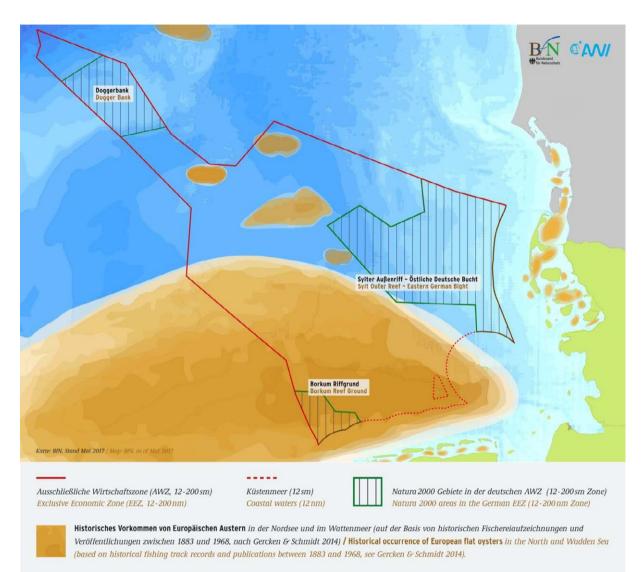
2.1 Historical distribution and reasons for the extinction of the European oyster

The European oyster is native to the German North Sea, where it has a wide distribution. Oyster beds for this species are defined as groups of five or more *O. edulis* individuals per square metre (Fig. 2). They are typically found at 0–10 metres depth, although they can occur at depths of up to 30 metres (OSPAR 2009). They provide a biogenic hard substrate on sandy sediment and thus represent a sublittoral biogenic type of reef (European Nature Information System 2019). Historic populations are documented from the German Bight for the North and East Frisian Wadden Sea, around Helgoland, and in deep sections of the southern North Sea (Fig. 3, Olsen 1883; Gercken, Schmidt 2014; Pogoda 2019).



Fig. 2: The native oyster species *Ostrea edulis* forms a three-dimensional habitat for many animal species on sublittoral soft substrates. These biogenic reefs are characterised by high biodiversity. These biogenic reefs are home to a characteristic community of high biodiversity. (Photo: AWI / S. ZankI).

From the end of the 19th century, European oyster populations suffered a dramatic decline throughout Europe. Reasons included persistent overfishing of stocks and associated habitat destruction caused by the process of harvesting the biogenic structures, the deterioration of environmental conditions due to the pollution of marine waters, and introduced pathogens (Korringa 1952; Wehrmann et al. 2000; Laing 2005; Shelmerdine, Leslie 2009; Gercken, Schmidt 2014; Pogoda 2019). In particular, the spread of the parasitic single-cell organism *Bonamia ostreae* led to high mortality rates, both in wild oyster populations and in commercial aquaculture (Lallias et al. 2008; Sas et al. 2020). Oyster populations have declined significantly over the entire area of their natural distribution – along the European Atlantic coast from



Norway to Morocco, as well as along the coasts of the Mediterranean and the Black Sea – and the species is now considered critically endangered (Airoldi, Beck 2007; Pogoda 2019).

Fig. 3: Historical distribution of the European oyster in German sea areas and North Sea coastal waters. The former offshore oyster grounds extended far west from the central German Bight. The Nature Conservation Area Borkum Reef Ground (BRG) lies within the historical distribution area and is thus a focus area for the first reintroduction measures (Figure: AWI / BfN, A. Essenberger).

In the German North Sea, the European oyster is considered as functionally extinct. However, isolated populations of this native species and the remains of formerly widespread oyster beds can still be found. They are scattered in marine waters around Great Britain and Ireland, and along the coasts of France, the Netherlands, Denmark, and Norway (OSPAR 2009; Beck et al. 2011).

2.2 Ecological significance

Due to their shell growth, oysters form biogenic reefs as defined in the Natura 2000 Interpretation Manual (European Commission DG Environment 2013). They provide ecologically valuable habitats for other animals, especially epibenthic flora and fauna, mobile invertebrates and fish (Pogoda 2019). With more than 130 associated species, they are considered hotspots of biodiversity (Smyth, Roberts 2010). As so-called 'ecosystem engineers' and 'founder species', oysters form a unique habitat. Based on the biodiversity of the historical oyster beds in the German Bight, Karl August Möbius coined the term 'biocenosis' (community) in 1877, which is now a central concept in ecology (Möbius 1877).

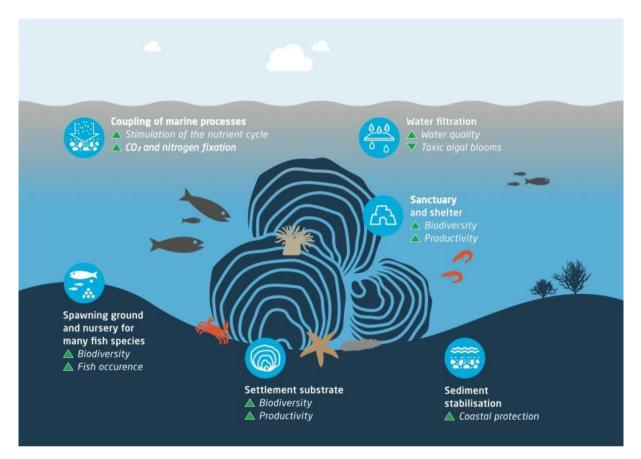


Fig. 4: Oyster reefs fulfill important ecosystem functions by providing food, space for settlement and protection, spawning grounds and water filtration and thereby improve biodiversity (Image: AWI / Y. Nowak).

The ecosystem functions and services of an oyster reef include:

- Settlement substrate for epibiotic flora and fauna
- Habitat, food, and protection for numerous invertebrate and fish species
- Spawning grounds and nursery for many fish species
- Improved water quality through high filtration performance
- Reduction of local toxic algal blooms
- Increased benthic-pelagic coupling
- Coastal protection by stabilizing sediment
- Increased nutrient absorption and carbon sequestration

Oyster reefs are among the most degraded marine habitats, not only in Europe but worldwide. It is estimated that, over the last 130 years, more than 85% of oyster reef habitats have been

destroyed worldwide (Lotze 2005; Beck et al. 2011). With the loss of reefs, the valuable ecosystem functions and services provided by intact oyster reefs are no longer available (Fig. 4, Pogoda (2019); Pogoda et al. (2019)).

2.3 Protection status and reintroduction of the European oyster

According to the EU Habitats Directive, the conservation or, if necessary, restoration of the good condition of the habitat type 'reef', including its characteristic species, is mandatory. (European Union 1992; Pogoda et al. 2020b). These natural habitats must be included in the Natura 2000 network of protected areas (Annex I of the Habitats Directive). For Germany, *Ostrea edulis* reefs are defined as biogenic reefs, which can be used to evaluate how good the conservation status of the habitat type 'reef' is (BfN 2017b). Currently, the conservation status of this habitat type is evaluated as less favourable to poor. In 2008, the European oyster was also added to the OSPAR list of endangered species and habitats (OSPAR 2008). According to OSPAR, the European oyster is considered a keystone species of particular ecological importance that is threatened with extinction. It is therefore classified as a species of special priority for the OSPAR Region II - Greater North Sea/Northeast Atlantic (OSPAR 2008). Appropriate recommendations for action for the protection of the species have been adopted (OSPAR 2013). These also apply to Germany. *O. edulis* is also on the Red List of endangered animals in Germany (BfN 2013).

The main factors that restrict natural restoration of *O. edulis* beds are intensive bottom-contact fishing in large parts of the German North Sea, a non-existent brood stock for the preservation of the population, and a lack of suitable substrate for larval settlement (Kennedy, Roberts 1999; Gercken, Schmidt 2014).

Active reintroduction measures are therefore necessary to protect the species and to restore the community with its valuable associated ecosystem functions and services (Fitzsimons et al. 2019). Many countries currently implement programmes and projects to restore endangered oyster reefs. In Europe, these are mainly located in France, Great Britain, Ireland, and the Netherlands (Pogoda et al. 2019). In the USA, the protection and strengthening of populations of endangered native oyster species has been established for a decade, and their ecosystem services are considered an important economic factor (Grabowski, Peterson 2007; Beck et al. 2011). The development of appropriate measures and their successful sustainable implementation took place in close consultation and cooperation with a number of national and international partners (Fitzsimons et al. 2019). Various reintroduction projects have also been launched in Europe. They cooperate closely within the framework of the Native Oyster Restoration Alliance (NORA), on the initiative of the BfN and the AWI (Pogoda et al. 2019).

3 Methods and Results

The preliminary investigation is divided into three stages. These include examining the current possibilities and requirements for long-term reintroduction of European oysters in the German North Sea, knowledge transfer and networking with other international reintroduction projects, as well as comprehensive preliminary studies for the specific implementation of the reintroduction as part of the RESTORE main project.

3.1 Requirements for successful reintroduction

Since the European oyster has been missing from the German North Sea for more than 50 years, as a first step it was necessary to evaluate and verify whether, from today's perspective, reintroduction is possible both from a nature conservation and ecological point of view.

3.1.1 Legal framework and Habitats Directive impact assessment

The development of a legal framework for future reintroduction measures took place at two levels, whereby findings from level I can also apply to level II:

- **A.** Level I: Examination of the legal basis for the immediate implementation of *Ostrea edulis* field experiments in the RESTORE preliminary investigation
- **B.** Level II: Examination of the legal basis for long-term reintroduction and the construction of an *Ostrea edulis* pilot reef in the RESTORE main project

General legal regulations and recommendations from international bodies

The following legal regulations and guidelines, as well as technical recommendations from international institutions and bodies, were identified for the application for permits for European oyster reintroduction projects in the German North Sea.

- Habitats Directive (HD) (92/43/EWG (European Union 1992)): The EU Directive on the Conservation of natural habitats and of wild fauna and flora serves to preserve natural habitats and wild animals and plants and, thus, the obligations entered into by EU member states in 1992 to protect biological diversity (Convention on Biological Diversity, CBD, Rio 1992). The habitat type "reefs" (code 1170) is listed as a habitat that is particularly worthy of protection, the favourable conservation status of which must be preserved or restored. The conservation status of this habitat type in Germany is currently classified as unfavourable to poor; biogenic reefs such as banks formed by Ostrea edulis are used as evaluation criteria (BfN 2017b; 2019b).
- OSPAR List of Threatened and/or Declining Species and Habitats, created by the OSPAR Convention on the Protection and Conservation of Marine Ecosystems and their Biodiversity (OSPAR 2008): According to Article 2 of Annex V of the OSPAR Convention, necessary measures and programmes to protect the seas, marine ecosystems, and biodiversity are to be applied therein (OSPAR Commission 1992). European oyster and oyster beds in general are listed as particularly worthy of protection. In addition, OSPAR Recommendation 2013/4 recommends reintroduction of European oyster in suitable marine areas (OSPAR 2013).
- Marine Strategy Framework Directive (MSFD) (European Parliament 2008): EU member states are obliged to establish good environmental status in the seas by 2020. The species *Ostrea edulis* is also listed in the relevant German draft programme of measures (BLANO

2012), although no corresponding measures have been specified for the German North Sea to date (BLANO 2016).

- "Code of Practice" (COP) of the International Council for the Exploration of the Sea (ICES 2005): Specification of instructions on how to proceed before and after the release of a species.
- Guidelines for the translocation of species within the framework of conservation measures, published by the International Union for the Conservation of Nature (IUCN/SSC 2013): instructions for all translocations of species carried out or planned within the framework of nature conservation.
- Directive 2006/88/EC of the Council of the European Union on animal health requirements for aquaculture animals and products thereof, and on the prevention and control of certain diseases in aquatic animals (European Union 2006): Based on the minimum measures of Directive 95/70/EC, the Directive specifies provisions "to ensure the necessary level of preparedness to effectively tackle the emergency situations related to one or more outbreaks of serious exotic or emerging diseases affecting aquaculture" Within this framework, projects should, "with the assistance of the competent authorities of the Member States, take more responsibility for preventing the introduction of or controlling such diseases through self-regulation and the development of 'codes of practice'". In relation to the planned reintroduction of oysters in the German North Sea, these codes of conduct and procedures were defined as part of the Berlin Oyster Recommendation (Pogoda et al. 2017; Pogoda 2019) and coordinated with other projects across Europe. For European oyster, the diseases bonamiosis and marteiliosis are of particular importance (Directive 2006/88/EC. In a decision by the EU Commission (2007/104/EC) of 15th February 2007 (European Commission 2007), areas are listed that have been approved as diseasefree areas. For reintroduction projects within the German North Sea, only animals from such designated areas should be imported in order to resettle a disease-free population of European oyster. Directive 2006/88/EC of the Council of the European Union, Part A, (European Union 2006) also stipulates this as a measure to be taken into account.
- Native Oyster Restoration Alliance (NORA): Within this European network founded in 2017, participating institutions and countries agreed on common framework conditions and recommendations for the reintroduction of European oyster in the North Sea (Pogoda et al. 2017; Pogoda et al. 2019).
- Invasive species: When translocating species there is always a risk of introducing non-native and invasive species. According to § 40 of the Federal Nature Conservation Act, the release of alien species in the wild requires the approval of the responsible authority. In the so-called mussel judgment of the Schleswig-Holstein Higher Administrative Court of December 15, 2011 (Case: 1 LB 19/10), the introduction of seed mussels (common mussel) was prohibited, among other things, in order to minimize the introduction of alien species into Wadden Sea National Park. For the field trials carried out in the RESTORE T&D Project, as well as for future projects to reintroduce European oyster, no stocking material from foreign water bodies will be used in order to rule out the risk of alien species introduction. The German projects are thus following the agreement within the European NORA network, the Berlin Oyster Recommendation, the ecological technical paper by BioConsult Schuchardt & Scholle GbR (Schuchardt et al. 2017), and the so-called mussel judgment (Oberverwaltungsgericht für das Land Schleswig-Holstein 2011; Schuchardt et al. 2017;

Pogoda et al. 2019). The reintroduction of formerly native species is also regulated in Article 22 of the Habitats Directive (92/43/EEC; European Union 1992).

 Application of mussel shells as a substrate for larval settlement: An updated protocol of the London Convention from 1996 states that the dumping of organic material of natural origin is generally permitted. However, official approval should be obtained before the material is brought in (Laing 2005; Laing et al. 2005). According to § 8 of the HoheSee-EinbrG (law prohibiting the introduction of waste and other substances and objects into the high seas), the BSH is responsible for enforcement in the German EEZ, which then consults the responsible federal and state authorities.

Level I: Examination of the legal basis for the immediate implementation of field experiments with *Ostrea edulis* within the RESTORE T&D project

The procedure for examining legal bases within the German EEZ is based on legal opinions prepared by Prof. Dr. Czybulka (University of Rostock) on the question of the necessity of an HD impact assessment as part of the present testing and development project (Czybulka, Francesconi 2016). There it is stated that "according to Article 34, Paragraph 1, Sentence 1 of the Federal Nature Conservation Act (BNatSchG), projects are to be checked for their compatibility with the conservation goals of a Natura 2000 area before they are approved or implemented, [...] if they do not directly serve the administration of the area. However, for projects that directly serve the administration of the area, which also includes the reintroduction measures of the present project, the so-called "management privilege" applies and an HD compatibility test or HD preliminary test is not required. It is assumed that the protection goals of the Natura 2000 areas will remain unchanged."

Furthermore, the legal opinion shows that for field experiments "In the wind farm [Meerwind Süd I Ost] an HD impact assessment is required because the "management privilege" according to § 34 Para. 1 S. 1 BNatSchG does not apply and – from a prognostic point of view – an impairment of the conservation goals can currently not be excluded with scientific certainty. The BSH is responsible for carrying out the impact assessment."

"If the project names the BfN as the operator in these two cases, there is only a notification obligation for the project to the BSH under maritime law, § 6 Para. 5 Sentence 1 SeeAnIV. On the other hand, an approval procedure according to Section 6 (1) SeeAnIV is required if the offshore installations are to be constructed by the AWI.

For reasons of procedural economy, the content of the documents for all [...] sub-projects should be designed in such a way that they meet the requirements of an HD impact assessment if the BSH should – contrary to expectations – classify all projects as subject to HD requirements."

For European oyster reintroduction attempts in the German North Sea, an HD compatibility test was carried out for all locations. The flowchart (Legal framework and Habitats Directive impact assessment, Fig. 5) shows the process and sequence of approvals required in advance and the involvement of various specialist authorities.

In order to define the approval process according to SeeAnIV for the intended field experiments with European oysters at the project sites, coordination took place with the BSH. A procedure for approval was mutually agreed upon. Since the present project was carried out in cooperation with the BfN, according to § 6 Para. 5 SeeAnIV, a notification of deployment and anchoring of the cages was required at the BSH. The BSH also had to have approval from the respective wind farm operator and an application for the **introduction of European oysters for field experiments** according to SeeAnIV. As part of the application, the HD report was submitted to the BSH, as well as an additional ecological article recommended by them (Schuchardt et al. 2017). Final approval was granted by the BSH after a round of consultation (Federal Environment Agency; Federal Waterways and Shipping Administration Northwest; Waterways and Shipping Authority Tönning; Federal Agency for Nature Conservation; Schleswig-Holstein Ministry of Energy Transition, Agriculture, Environment, Nature, and Digitization ME-LUND, Deutscher Fischerei-Verband, WindMW).

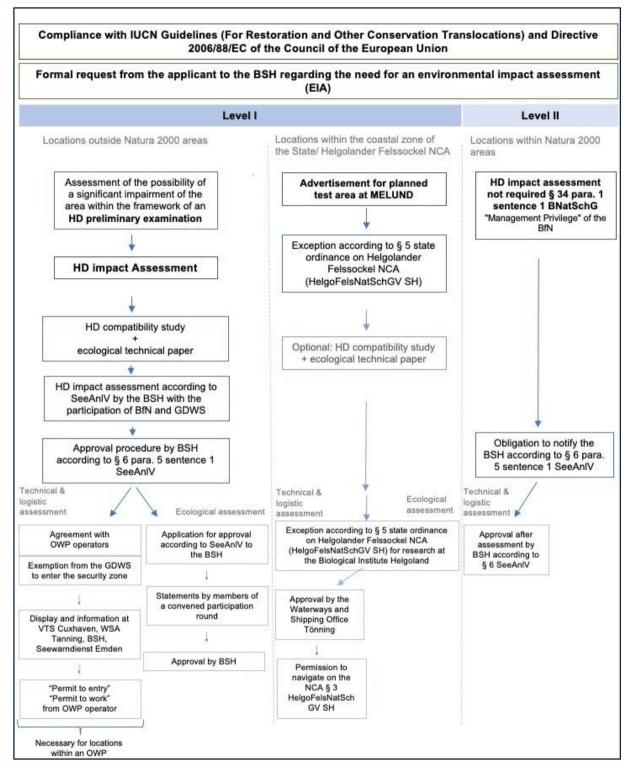


Fig. 5: Flowchart of the approval process and the involvement of the relevant competent authorities. Thematically applicable international and European guidelines take precedence. An EIA has been classified by the competent authority as a non-essential test instrument. Level I represents the permitting process outside of Natura 2000 sites (as applied for the preliminary study). Level II shows the approval process for Borkum Reef Ground Natura 2000 site (as used for the main project) (Image: AWI). As part of the preliminary investigation, the safety zone around the area of Meerwind Süd I Ost offshore wind farm (OWP) was selected as the location for field experiments (growth and health studies). The Federal Waterways and Shipping Administration (GDWS) issued the appropriate special permits.

In addition, the MELUND reported two other locations in Helgoländer Felssockel Nature Conservation Area. This is where MarGate underwater test area is located, in which field experiments (growth and health studies) were also carried out. This research work is a necessary test set-up as part of the measures taken by the Biological Institute Helgoland (state ordinance on Helgoländer Felssockel Nature Conservation Area § 5), which therefore falls under the existing exemption. A permit for navigating the area with research vessels was issued by the Waterways and Shipping Authority Tönning accordance with §3 of the regulation on navigating Helgoländer Felssockel Nature Conservation Area.

Level II: Legal framework for a long-term reintroduction measure in Borkum-Reef Ground NCA

The choice of site showed that the reintroduction of European oyster in Natura 2000 areas within the German EEZ should be planned and implemented. According to §34, Para. 1, Page 1 of the BNatSchG, the management prerogative of the BfN applies in these marine protection areas. Accordingly, no HD impact assessment is necessary for projects within these areas, which are classified by the BfN as area management (Czybulka, Francesconi 2016). Possible adverse effects on the NCA and its conservation goals are continuously checked internally by the BfN and the professional legal assessment is continuously documented. According to § 6 SeeAnIV, technical systems, such as cage frames anchored in the seabed, must be reported to the BSH and approved. Bringing in appropriate substrate for colonisation (such as mussel shells) generally falls under the Hohe-See-Einbringungsgesetz (HSEG). In the case of oyster reintroduction, however, there is no need for a permit since, according to HSEG §3 (1) sentence 2, there is no need for a permit if nature conservation measures have been carried out or ordered by the responsible authority (in this case the BfN) (Flatter 2020). Furthermore, a participatory round, as carried out in Level I (Federal Environment Agency, Federal Waterways and Shipping Administration Northwest, Federal Agency for Nature Conservation, German Fisheries Association) is to be expected to discuss and agree on the interests and safety of all those involved in the project. This should be continuously checked and ensured internally and, above all, in close consultation with the BfN to ensure that stone deployment and reef building "do not, contrary to § 30 Para. 2 BNatSchG, significantly impair other valuable and legally protected biotopes such as seagrass meadows and other marine macrophyte populations, subtidal sandbanks, silt areas with burrowing seabed megafauna, as well as species-rich gravel, coarse sand, and shell areas". In addition, it must be checked whether the stone substrate brought in for the purpose of reintroducing oysters is offshore within the meaning of § 2 (2) No. 4 SeeAnIG, according to which an alternative notification, in accordance with § 6 (5) See-AnIG, must be submitted to the BSH stating the type, purpose, and exact location of the system, and should be checked and evaluated by the BSH. Activities to reintroduce European oyster should also, as a precautionary measure, be reported to the responsible waterways and shipping administration of the GDWS and also to the Federal Office for Agriculture and Food (BLE) due to the proximity to fishing concerns (Flatter 2020).

The legal framework presented here can be used to apply for and implement further reintroduction measures for European oyster in the parts of the German Bight.

Interim conclusion:

- Appropriate national and international regulations and guidelines must be considered for the implementation of active nature conservation measures. Taking this into account, the reintroduction of European oyster is feasible from a nature conservation point of view.
- For measures at locations within the German EEZ that are not in Natura 2000 areas or have an impact on them, an HD preliminary assessment and, if necessary, an HD impact assessment must be carried out for approval by the BSH, for which an HD impact study and possibly an expert ecological report must be available.
- For locations within the coastal zone of the federal states, permission must be obtained from the responsible federal states and permission for navigation from the responsible waterways and shipping office.
- Measures at locations within a designated Natura 2000 site fall under the management prerogative of the BfN and are not subject to a Habitats Directive Impact Assessment it is a measure within the framework of site management. Nevertheless, technical installations that are anchored to the seabed must be approved by the BSH.
- The approval procedures shown here also apply to further reintroduction measures.

3.1.2 Biological framework: field trials

Suitable environmental conditions are a key requirement for a successful reintroduction of the European oyster in the German Bight. The decline of the European oyster down to its "functionally extinct" status dates back almost a century and is believed to be due to a long-term combination of stressors. Fishery reports and catch figures show massive overfishing (see Ch. 2.1). In addition, changing environmental conditions may have had an impact on the ecological success of the species in the past, or may influence the success of reintroduction in the future. Accordingly, field trials were carried out in the sublittoral of the German Bight to investigate the growth and health of European oysters under the environmental conditions prevailing today. Ecological knowledge of historical oyster reefs in the deep area of the North Sea, of their composition, structure and ecological requirements of the species are limited. The results of the field experiments presented here supplement and expand the necessary knowledge on *O. edulis* in the deep sublittoral of the German Bight and enable the development of recommendations for the implementation of long-term reintroduction measures.

Site selection and experimental procedure

Site selection was based on criteria specifically defined for the species (site selection criteria, see Ch. 3.3.1). After successful completion of the suitability test, a study area in the German North Sea was selected, where growth and health studies were carried out. The three selected sites (Fig. 3) north of the island of Helgoland, 25 nautical miles off the German coast and in the area of historical oyster distribution, the offshore oyster grounds (Olsen 1883; Boos et al. 2004; Gercken, Schmidt 2014).

Site 1 is in Meerwind South I East wind farm safety zone, at a water depth of 26 m (WF location). Locations 2 and 3 are in the Helgoländer Felssockel NCA, at a water depth of 26 m (location HD – Helgoland Deep) and 10 m (location HS – Helgoland Shallow; Fig. 6). The experiments were not exposed to any fishing risk due to a fishing ban in force at all three locations.

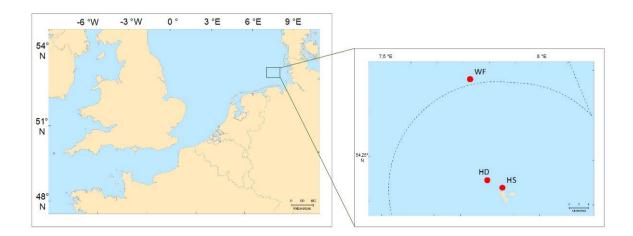


Fig. 6: Locations of the field trials to investigate the growth, health and mortality of reintroduced European oysters (in oyster cages) under today's prevailing environmental conditions (WF = Meerwind Süd I Ost wind farm safety zone, water depth 26 m; HD = Helgoländer Felssockel NCA, water depth 26 m; HS = Helgoländer Felssockel NCA, water depth 10 m; dashed line = German EEZ border).

The seabed (sediment classification) at the three sites is sandy. At site HD also isolated stones are present. Temperature, salinity, flow rate, and oxygen concentration were comparable at the three locations over the test period. Maximum water temperature was 18 °C. For water temperatures >7 °C, growth of the oysters has been proven (Ashton, Brown (2009), see Ch. 3.3.2).

At the test sites, metal cage structures were anchored to the seabed and marked with buoys on the water surface (Fig. 7). The solid metal frame allowed the attachment of up to eight oyster baskets (6 mm mesh size, 15 L volume, manufactured by SEAPA). The removal and recovery of the oysters in the oyster baskets, as well as maintenance work on the experimental setup, were carried out by research divers from the AWI Centre for Scientific Diving using AWI research vessels (Fig. 8).

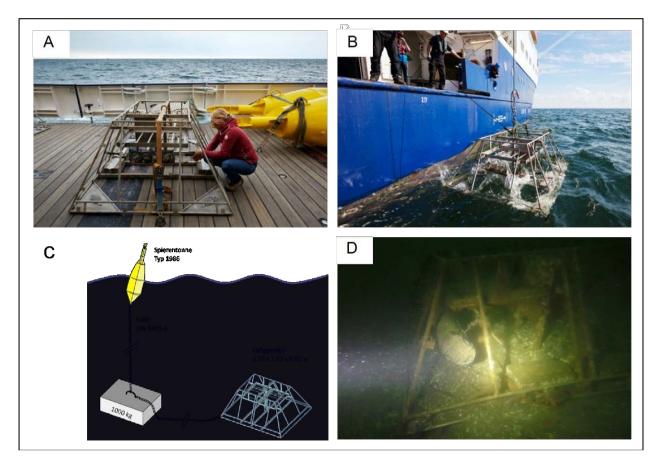


Fig. 7: Release of oyster cages on solid metal frames (so-called oyster landers): A) Preparing the metal frames and attaching oyster cages on board FS Heincke, July 2016; B) deployment of oyster landers by winch at the test site; C) Schematic diagram of the experimental setup: anchoring oyster landers on the seabed and marking them with a buoy; D) Oyster lander with oyster cages underwater at the test site (Photos: AWI / V. Merk, AWI / H. Müller-Elsner). ("Spierentonne" = Spire buoy; "Kette" = Chain; "Käfiggestell" = Cage frame)

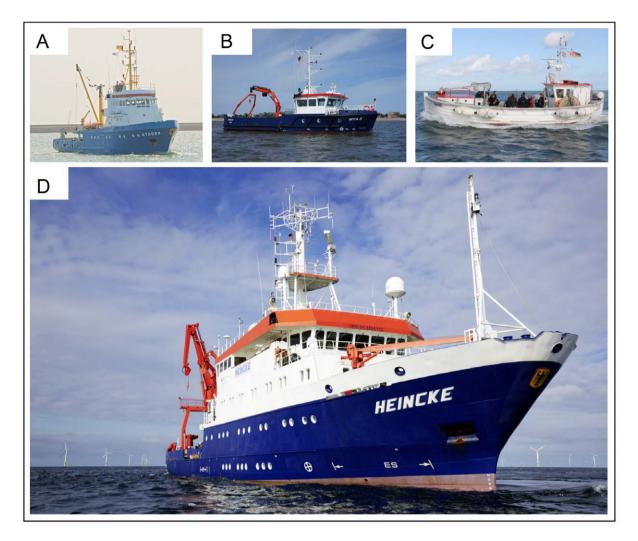


Fig. 8: Alfred Wegener Institute research vessels: **A)** RV Uthörn; **B)** RV Mya II; **C)** RV Aade; and **D)** RV Heincke (Photos: AWI / P. Flange, U. Nettelmann, F. Mehrtens, and H. Müller-Elsner).

A total of 24,000 young oysters (spat) (2 mm shell length) were deployed at the three locations between May 2017 and April 2018 and studied until May 2019 (Tab. 1). The spat originates from a breeding facility using artificial seawater to prevent the introduction of parasites, pathogens, or non-native epifauna and flora. They have been obtained with a health certificate from the French National Reference Laboratory for Mussel Diseases (GIP LABOCEA). In Europe, larger size classes are only produced in natural seawater and in the field, and their import represents a significant ecological risk due to the accompanying species that may be transported (see Ch. 3.1.1). For this reason, the import and translocation of larger juveniles or adult oysters was deliberately avoided (Pogoda et al. 2017).

Tab. 1: Schedule of the field trials: construction and maintenance of underwater structures, deployment of oysters (A), and sampling. ("Aufbau & Instandhaltung" = Construction & Maintenance; "Beprobung" = Sampling; "Gruppe" = Group)



Oyster growth and condition

Growth analysis allows the assessment of current environmental conditions (Brumbaugh et al. 2006) and provides necessary information for reintroducing a locally or functionally extinct species. Oyster growth depends on various environmental factors, such as temperature, food availability and quality, but also on the origin of the brood stock (Utting 1988; da Silva et al. 2005). Absolute oyster growth per day was determined via increase in shell length and soft body weight (Fig. 9). The young oysters examined showed steady and considerable growth (Merk et al. 2020). All groups included fast, normal, and slow growing animals, resulting in significant standard deviations in shell length, wet weight, and growth rates in individual animals. In addition, daily growth rates varied seasonally. High chlorophyll and temperature values in the summer of 2017 resulted in high oyster growth rates. A less pronounced chlorophyll maximum in the summer of 2018 led to moderate daily growth rates and thus to a lower condition index (ratio of soft body weight to shell weight). The condition index followed a natural seasonal pattern and supports the hypothesis that young European oysters invest in shell growth first (Valero 2006; Pogoda et al. 2011). Moderate growth rates (Group 3, Group 4) may be related to less than optimal feeding conditions (quality) prior to field trials. Thus far, growth studies on European oysters have mainly been carried out in coastal regions (Ivanov 1966; da Silva et al. 2005; Valero 2006). First field experiments with O. edulis at offshore locations in the German North Sea were carried out in so-called oyster lanterns near the water surface (Pogoda et al. 2011). The increase in shell length is comparable to the data collected at deeper locations as part of the present field experiments. At these deeper locations, detritus could serve as a relevant additional food source for oysters and thus compensate for seasonal fluctuations in phytoplankton concentrations (Mackinson, Daskalov 2007). Accordingly, oyster populations at deeper offshore locations may benefit from stable food availability and be more resilient to changing environmental conditions.

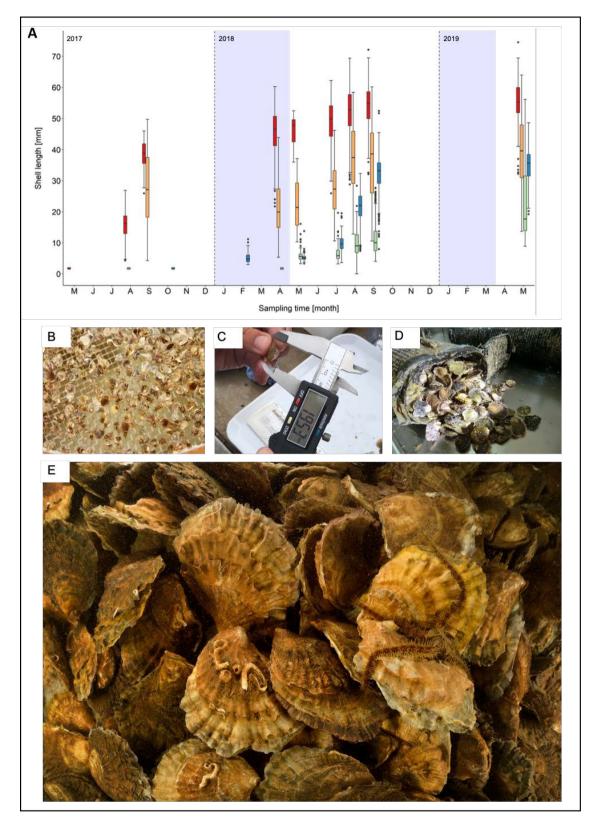


Fig. 9: Growth of reintroduced European oysters: A) Absolute shell length of oysters during field trials (24 months). In May 2017 (group 1, red), August 2017 (group 2, orange), October 2017 (group 3, blue), and May 2018 (group 4, green) each with 6000 oysters (average shell length 2 mm, N ≥ 200) (Merk et al. 2020). Periods with water temperatures <7 °C for which no growth is expected are highlighted in light blue (Pogoda et al. 2020b). B) Spats at the time of release (2 mm size). C) Oyster measurements during sampling. D) Healthy, subadult oysters in the oyster basket during sampling, September 2018. E) Adult oysters, May 2019 (Photos: AWI / V. Merk and S. Zankl).</p>

Formation of oyster aggregations (mini reefs)

During the course of the field trials, oyster shell growth resulted in the formation of aggregations of two or more oysters that were firmly grown together as a three-dimensional structure (see Fig. 10). The ability and process of reef building of the species *O. edulis* are not yet fully understood; no data exist on the structure of a pristine *O. edulis* habitat. Existing historical data on oyster reefs mainly relate to catch data and contain scattered references to "coarse oysters" and "oyster clumps" (Möbius 1877). However, this condition described by Möbius is already considered changed (degraded) since the area had already been under high fishing pressure for decades by this time. The aggregations formed in the present study from coalescing oysters, defined here as "mini reefs", correspond to the historically described "coarse oysters".

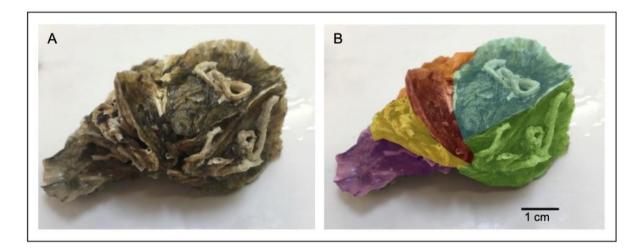


Fig. 10: During the field experiments, oyster aggregations (so-called mini-reefs) formed from 2 to 8 oysters. A) Overview of a mini-reef. B) Coloured identification of the individual oysters that have grown together to form a mini-reef (Photo: AWI / V. Merk).

The number of mini reefs increased over time, regardless of whether they were placed in mesh bags in the first few months or later in oyster cages. It was also observed that oyster clusters were formed, which were held together by epifauna such as sand mason worm Lanice conchilega or the tubeworm species Spirobranchus triqueter. These clumps could be the starting point of reef formation. Less movement of the animals potentially leads to the formation of even larger aggregations and reef structures given the appropriate density of oysters. Dead O. edulis reef structures found in the Black Sea showed occurrences of Sabellaria taurica on the shells, which may have the same function (Todorova et al. 2009). Measurements of this reef reached a height of up to 7 m, showing that O. edulis is fundamentally capable of forming substantial reefs. This is in contrast to previous assumptions (Beck et al. 2009). There is currently no explanation for the unusual size of this O. edulis habitat in the Black Sea. It is conceivable that suitable sediment types and flow patterns are necessary for this. However, the specific parameters are unknown and it remains to be seen which structures O. edulis will form in undisturbed offshore sea areas of the North Sea after large-scale reintroduction. But even the formation of smaller aggregations and reef structures shown here after just a few months has already increases the complexity, biodiversity and ecosystem services for the surrounding habitat (Merk et al. 2020; Pogoda et al. 2020c).

Oysters' mortality and reproduction

Mortality was calculated from the number of live oysters in the oyster cages. If a total count was not possible due to the high number of individuals and/or the time limit between the diving samplings, maximum sub-samples were counted. High mortality occurred in young spat oysters in particular in the first few weeks after release (Fig. 11). With the exception of low winter mortality, mortality fell to zero during the experiments. Logistical adjustments and improved oyster handling during deployment increased oyster survival. Overall oyster mortality was lower than observed in previous studies (Utting 1988; Valero 2006; Pogoda et al. 2011). However, comparability is limited due to differences in the size of spat and environmental conditions: for the first time, animals of only 2 mm in size were used and examined here. However, the small size of spat did not affect mortality (Merk et al. 2020). This finding represents an important basis for ecologically safe reintroduction measures on a large scale, as planning can be carried out accordingly with certified spat oysters from farms.

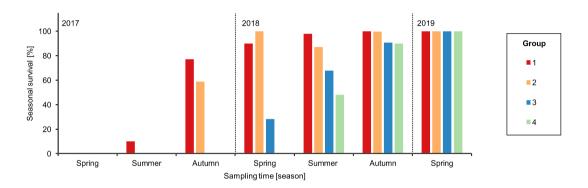


Fig. 11: Seasonal survival rate of reintroduced oysters: during field trials (24 months), May 2017 (group 1, red), August 2017 (group 2, orange), October 2017 (group 3, blue) and May 2018 (group 4, green) each with 6000 oysters (average shell length 2 mm, N ≥ 200) (Merk et al. 2020).

Highest mortality was observed in the first few weeks, but decreased in all groups over the test period. Group 1 and Group 2 achieved 100% survival in autumn 2018 and spring 2018, respectively.

In the course of the experiments, some of the oysters reached sexual maturity 9 to 14 months after being deployed in the field. Different larval stages were detected in adult female animals (Fig. 12). *O. edulis* reproduction is strongly influenced by environmental parameters such as temperature, forage availability, and composition (Berntsson et al. 1997). Since *O. edulis* is a protruding, alternating hermaphrodite (Colsoul et al. 2020a), maturation to male, sex change with maturation to female, as well as successful fertilization and spawning in some animals already took place during the field experiments (Merk et al. 2020). Given the excellent growth and condition of the oysters and the absence of macroparasites, these reproductive results complement the picture of the overall very good fitness of the reintroduced European oysters at these sublittoral offshore sites.

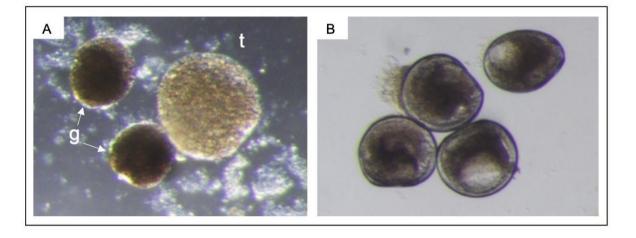


Fig. 12: Reproduction of the reintroduced oysters: larvae could be detected in 15-month-old European oysters during the field trials. A) Gastrula (g, diameter 100 μm) and trochophore (t, diameter 150 μm); B) early veliger larvae stage (diameter 150 μm) (after Merk et al. (2020)).

Health status analysis: macroparasites and pathogens

Analysis of the state of health was carried out seasonally on the sub-samples using microscopic examination (macroparasites) and genetic analysis (bonamiosis, marteiliosis). For the genetic analysis, the soft bodies of the oysters were stored at 20°C.

Oyster shells and soft bodies were examined individually for macroparasites (N~20). Organspecific crush preparations were made to examine the soft body. Genetic analyses for possible infection of *O. edulis* by *Bonamia ostrea* were carried out using the protocol developed by Carnegie et al. (2000). Genetic analyses to detect possible infection by *Marteilia refringens* were carried out according to Le Roux et al. (1999). Whole soft tissue from each individual was used for DNA extraction (Qiagen DNeasy Blood & Tissue Kit).

Neither macroparasites nor the above-mentioned pathogens were detected in any of the 100 oysters examined. Marteiliosis can be ruled out due to the high salinity in the test areas of the German North Sea (Colsoul et al. 2020a; Sas et al. 2020). The reintroduced European oysters were classified as completely healthy at the end of the field trials (after 24 months) (Merk et al. *in prep*).

Through extensive field trials, the present preliminary study was able to demonstrate the fundamental biological suitability of European oysters for reintroduction measures in the German Bight. Research results on growth, survival, and fitness show that environmental conditions in the area and the depth of the former oyster beds are very suitable for the species, even today. The additional detected reproductive activity complements these promising results regarding whether a self-sustaining population can be reintroduced in the long term. However, the cage experiments mean that the preliminary investigation cannot provide any statements on the effects of predator-prey relationships and the influence of sediment dynamics.

3.1.3 Summary

- Implementation of reintroduction projects in the German North Sea is possible from the legal and nature conservation point of view, but bound to national and international regulations and guidelines.
- Depending on the location (within the German EEZ, within the coastal zone of the federal states, within Natura 2000 protected areas), appropriate permits must be obtained and/or HD impact assessments may be necessary.
- To test biological framework conditions, test sites were set up, maintained, and sampled at water depths of 10 m and 26 m by research vessels and research divers. All locations were in non-fishing areas.
- Approximately 24,000 certified healthy spat with a shell length of 2 mm were released for the offshore field experiments. Distribution took place at four different times. The oysters were sampled regularly over a period of two years.
- Excellent growth was demonstrated for the reintroduced spat. The results show good food availability and quality for European oysters in the German North Sea.
- The condition index enables statements to be made on the general condition of the animals: as expected, the condition index of the released oysters progressed during the year and showed that the animals were in good shape overall. Some of the oysters became sexually mature in the first year in the field.
- There was no detectable infection with macroparasites or relevant pathogens. The reintroduced European oysters were classified as fully healthy at the end of the field trials.
- Survival rates have been drastically improved over the course of the trial period through improvements in logistics and handling of the oysters, and they are in line with natural rates in the field.
- Continuous formation of small reef structures was documented for the first time. Several dozen oyster aggregations (mini reefs) of two to eight individuals formed over the test period.
- During the experiment, an increase in biodiversity and the biomass of the epifauna was also determined.

3.2 Knowledge and technology transfer: cooperation with other reintroduction projects

Oyster reintroduction measures have been successfully implemented in some countries over the past two decades, especially in the USA. The formation of networks with international partners in the field of oyster reintroduction enables ongoing knowledge and technology transfer with existing research and restoration projects.

3.2.1 International cooperation

Through practical research and cooperation with international colleagues and partner institutions, similarities can be identified and valuable problem solutions developed. Existing expert knowledge is used to transfer proven methods and technologies to the situation in the German North Sea (Table 2).

USA	Oyster Restoration Program, NOAA, MD
	Hornpoint Lab Oyster Hatchery, MD
	Global Marine Team, The Nature Conservancy, RI
	Chesapeake Bay Program, The Nature Conservancy, VA
	Chincoteague Wildlife Refuge, VA
	Albemarle-Pamlico Sounds Program, The Nature Conservancy, NC
	Half Moon Reef Program, The Nature Conservancy, TX
	Washington Shellfish Initiative, WA
	Shellfish Hatchery Northwest Fisheries Science Center, NOAA, WA
Australia	South Australian Research Development Institute (SARDI), SA
	Port Stephens Fisheries Institute (PSFI), NSW
	Victorian Shellfish Hatchery Queenscliff, VIC
	Shellfish Restoration Reef Windara, SA
	Australia Oceans Program, The Nature Conservancy, VIC
New Zealand	Shellfish Production and Technology New Zealand (SPATnz)
	Cawthron Institute
	Moana New Zealand
Europe	Cooperation through the Native Oyster Restoration Alliance (NORA): For example with France (Ifremer, Novostrea Bretagne, CRC), United Kingdom (University of Portsmouth, Heriot-Watt-University Edinburgh, Natural Scotland, Rossmore Oysters Ltd., Jersey Seafarms) www.noraeurope.eu

Tab. 2: Knowledge and technology transfer with international cooperation partners.

USA

In the USA in particular, there are a large number of feasibility studies, restoration projects, and specific restoration programmes to restore and protect oyster beds. Many of these projects have been successfully implemented for years and can significantly facilitate and support reintroduction of native oyster species in the German North Sea through active knowledge transfer. Practical research in the USA initiated successful cooperation with the American nature conservation organization The Nature Conservancy (TNC), which was further developed into active knowledge and technology transfer with project locations in Virginia, Maryland, North Carolina, Louisiana, and Texas; the native oyster species *Crassostrea virginica* is being reintroduced there as part of nature conservation measures. This includes intensive exchange on planning and implementing active restoration measures and on breeding facilities specially established for oyster restoration. Another focus of cooperation with the USA are projects for native oyster species *Ostrea lurida* in Puget Sound on the US west coast. Due to species-specific, biological characteristics, experiences from these reintro-duction projects and breeding farms are particularly valuable. As a result, collaboration was established with the Washington Shellfish Initiative network, with another state-run breeding facility, and with two reintroduction projects in Puget Sound. Overall, this resulted in a valuable increase in knowledge for the planning of an oyster hatchery in Germany (Project PRO-CEED) and sound assessment of the practical implementation of oyster reintroduction in Germany (Project RESTORE).

Australia

In Australia, visits and cooperation talks took place at three mussel hatcheries and a reintroduction site: the South Australian Research Development Institute (SARDI), West Beach, Australia has various research units for mussel farming, such as the production of microalgae in outdoor shallow pool. The Port Stephens Fisheries Institute (PSFI) is not a commercial breeding facility, but a research facility that deals with optimizing cultivation of oyster larvae (genus Ostrea). Technical implementation takes place in traditional batch systems (algae cultivation, larvae housing). The Victorian Shellfish Hatchery Queenscliff is a modern hatchery specializing in Ostrea angasi. Technical implementation takes place in innovative flow systems. The operators of the facility are therefore an important partner for implementation in Germany (Project PROCEED). Ostrea angasi is cultivated very successfully there. Windara Reef, Yorke Peninsula, South Australia, was created in 2017 and is an important comparison site for Germany as sublittoral work is also carried out there with the genus Ostrea. Technological construction and biological-ecological advances provide important clues for practical implementation in Germany (https://www.youtube.com/watch?v=LAmnv1WiTWo). Successful cooperation was established with the Australia Oceans Team (TNC) for the application and further development of a diver-based technique for large-scale application of spat for subtidal reintroduction.

United Kingdom and Ireland

Intensive meetings have taken place as part of the Oceans Past Platform (EU COST Action IS1403) through a Short Term Scientific Mission (STSM) in England, Wales, Scotland, Northern Ireland, and Ireland. Furthermore, the German project was presented at the International Oyster Symposium (IOS) in Bangor, Wales, as part of a session on the subject of "Restoration", which was included in the programme of the World Oyster Society (WOS) for the first time. Together with European colleagues, content for the NORA1 workshop in Berlin was prepared. European monitoring standards are currently being developed as part of the NORA subgroup monitoring, in cooperation with the UK Network, represented by Dr. Joanne Preston and Celine Gamble. These will be published as recommendations for action for Europe, possibly as an annex to the Berlin Oyster Recommendation.

3.2.2 Native Oyster Restoration Alliance (NORA)

The resettlement of destroyed and disappeared biogenic reefs in their former distribution areas is the focus of restoration projects worldwide. In particular, the reintroduction of the native European oyster is increasingly being tested and carried out within European projects. In order to successfully interconnect these projects, there was a need for a European network to strengthen and restore native oyster species.



Fig. 13: 2017 kick-off workshop organized by BfN and AWI in Berlin: the participants (top right) presented various European projects as part of the NORA1 conference and decided to create the Native Oyster Restoration Alliance (NORA) and developed the Berlin Oyster Recommendation (Pogoda et al. 2017, 2019), (Photos: K. Wollny-Goerke).

Against this background, the Native Oyster Restoration Alliance (NORA) was founded in November 2017 as part of a kick-off workshop in Berlin, on the initiative of the BfN and the AWI. 65 colleagues from ten European countries and experts from the USA took part in this first successful workshop (NORA1) (Fig. 13). The focus was on interaction about current international reintroduction projects and discussion of potential hurdles with a corresponding need for joint action. As a result, the experts present agreed on common European reintroduction strategies and formulated the Berlin Oyster Recommendation (Pogoda et al. 2017). This document and the recommendations it contains are considered the basis for securing "best practice" methods and lasting success for European reintroduction measures (Fig. 14).



Fig. 14: Recommendations (1 to 6) of the Berlin Oyster Recommendation (Pogoda et al., 2017, 2019), which were developed as part of the NORA1 conference (Graphic: A. Essenberger).

NORA brings together reintroduction projects and associated stakeholders from over ten European countries to promote active knowledge transfer on relevant topics (biology, ecology of *O. edulis* (including pathogens such as *Bonamia ostreae*), restoration methods, oyster production, monitoring, funding opportunities, and legal framework conditions for reintroduction measures). The long-term goal of the alliance is to restore stocks of the native European oyster as a key species in the North Sea and adjacent European marine areas. Future goals are: I) further expansion of the network and extensive involvement of all interest groups associated with European oyster reintroduction; II) intensive cooperation in terms of scientific publications, regular topic-related workshops in the respective working groups, and an annual conference in one of the participating countries; III) joint development of solutions and recommendations for action for overarching problems (for example: translocation of adult wild animals; control of diseases, such as bonamiosis; sufficient spat production; expansion of research for large-scale reintroduction measures).

As part of the first NORA meeting in Berlin (NORA1), four overarching topics were identified that are of significant importance for all projects and are therefore to be continuously processed in working groups in a pan-European approach (Pogoda et al. 2020a). The various working groups also use the recommendations defined by NORA (Recommendations, Fig. 14).

The topics are

Production

One of the key processes in all European projects is the supply of healthy and suitable spat. Extensive seed oyster production is necessary for the long-term and sustainable success of European reintroduction projects, which is currently not being achieved. Accordingly, this working group deals with the development of innovative production methods, as well as with adaptation and expansion of existing production techniques and structures. In addition, standards for oyster production are being tested in order to apply them on a large scale in the future – including biosecurity aspects.

Biosecurity

Through various aspects of practical reintroduction measures, there is a risk of introducing pathogens and invasive species into reintroduction areas. In order to rule out these risks, this working group is developing specific "biosecurity guidelines" for reintroduction of European oyster in Europe. These take into account areas of production and translocation of oysters and substrate, as well as preventing further spread of the bonamiosis pathogen.

Site selection

Site selection is of immediate importance across Europe in order to carry out successful reintroduction projects. This working group deals with nature conservation conditions and interacts with political stakeholders in order to identify individual questions and problems at selected reintroduction sites. The aim is to provide up-to-date mapping of the habitat and conservation status of the European oyster at a local, regional, and Europe-wide level. On the one hand, this supports possible adjustments to applicable nature conservation regulations and, on the other hand, ecologically meaningful cross-border projects can be identified that depend on international cooperation.

Monitoring

So far, there has not been a holistic approach as to which methods are applicable, usable, and useful for investigating *O. edulis* reintroduction in Europe. Accordingly, the working group is developing joint, generally applicable monitoring methods in order to make the results of respective reintroduction measures comparable and their success measurable.

Participation in the European NORA network has continued to increase since it was founded: in 2019 a second conference was held in Edinburgh, Scotland (NORA2) with over 120 participants. Sustainable findings from the knowledge and technology transfer within NORA will also flow into the future processing of German reintroduction measures. In order to facilitate exchange between NORA members, and to further increase the visibility and exchange of content within the network, a homepage was set up (www.noraeurope.eu); this includes project descriptions, interactive maps of project locations, and a download area with currently relevant Literature and NORA documents (Fig. 15).

The NORA network was further consolidated in the two years after it was founded. Funding is initially fixed until 2021 through funding in the Federal Biological Diversity Programme (PROCEED project); this enables the coordination and organization of annual conferences.

Working Groups						
Production	Biosecurity	Site Selection	Monitoring			
1. Pro sufficient oysters for re						
suitab						
		5. Create common monitoring protocols				
		SERVE laptability of populations				

Fig. 15: Four NORA working groups (Production, Biosecurity, Site Selection, Monitoring) each process the recommendations for sustainable reintroduction of European oyster, defined by the Berlin Oyster Recommendation (Graphic based on Pogoda et al. (2020a)).

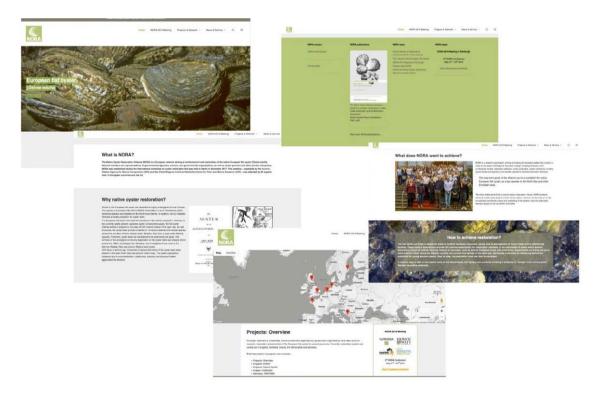


Fig. 16: Summary of the NORA website www.noraeurope.eu set up as part of international cooperation (as of July 2019).

3.2.3 Summary

- Extensive cooperation has been established at national and international level (in Europe and beyond), which is of great practical importance for implementing reintroduction measures in Germany.
- Founding the Native Oyster Restoration Alliance (NORA) was considered important in European coastal countries and received sustained support. The network is very active and growing steadily. It supports comprehensive knowledge and technology transfer between different stakeholders and countries.
- When NORA was founded in 2017, six recommendations relevant throughout Europe were defined, guaranteeing "best practice" for reintroduction of oysters: 1) production of healthy spat; 2) identification and designation of suitable reintroduction areas; 3) provision of suitable substrate for obtaining juveniles; 4) consideration of Bonamia-free areas; 5) development of common monitoring standards; and, 6) preservation of genetic diversity and population adaptability.
- Four active NORA Working Groups have been established (Production, Biosecurity, Site Selection, Monitoring) and they are actively working on important issues affecting reintroduction projects across Europe.
- The NORA website (<u>www.noraeurope.eu</u>) provides interested parties with comprehensive information on projects, project locations, and documents on European oyster reintroduction in Europe (Fig. 16).

3.3 Further studies for practical implementation of long-term reintroduction measures

To successfully reintroduce European oyster in the German North Sea in the long term, possible scenarios must be planned in advance and best procedure must be defined for the subsequent steps. This includes choice of location for reintroduction measures as well as securing a supply of young oysters and suitable substrate for settlement at the reintroduction sites.

3.3.1 Definition of Site Selection Criteria

The basic prerequisite for the implementation of large-scale reintroduction measures is exclusion of any seabed-altering activities in the reintroduction area (e.g. fishing, sand and gravel extraction, submarine cable laying). In addition, other aspects are important for successful site selection: historical distribution of the European oyster, ecological suitability of the area, as well as logistical factors and nature conservation regulations should be taken into account when recommending suitable sites for long-term reintroduction.

The relevant criteria for site selection were grouped into three categories (Clewell et al. 2000; Pogoda et al. 2020b):

- A. I) Historical distribution: evidence of historical occurrence and stocks
- B. II) Environmental conditions: adequate abiotic and biotic factors
- **C.** III) Feasibility of the measure: legal framework and logistical requirements

Comprehensive site selection criteria were defined for the target species for each of the three categories, based on the results of scientific research (Pogoda et al. 2011; Pogoda 2012; Pogoda et al. 2013), the recommendations of the feasibility study while taking into consideration

historical, ecological, biographic, and logistical parameters and the politics of nature conservation (Table 3, Pogoda et al. (2020b).

Application of Site Selection Criteria

Based on the criteria defined above, possible locations within the German Bight were examined for their suitability and selected reintroduction of European oyster.

Historical distribution of O. edulis

For reintroduction, a location must first be selected within a historically documented distribution area of the native oyster species. This is in line with the reintroduction approach defined by the International Union for Conservation of Nature (IUCN) as the intentional release of an organism into its original range from which it has disappeared (IUCN/SSC 2013). From an ecological point of view, however, this is not obligatory for the success of a measure (Gercken, Schmidt 2014).

Nature Conservation Area

A fundamental prerequisite for the suitability of corresponding marine areas is exclusion of any seabed-altering activities (e.g. fishing, sand and gravel mining). Management plans for the Nature Conservation Areas in the German EEZ (Borkum Reef Ground, Sylt Outer Reef, Dogger Bank Natura 2000 areas) currently provide for regulations for partial exclusion of fishing and for limitation of substrate degradation (BMU 2019). This reduces the pressure of use in these marine areas and enables the planning of long-term reintroduction measures.

Abiotic factors

Temperature, oxygen content, flow rate, salinity, and substrate properties are fundamental conditions on which the successful growth, survival, reproduction, recruitment, and population formation of the European oyster depend. All factors must be considered and must be within the species-specific tolerance range to define a suitable site. No preferences for specific sediment types are known for the European oyster. On this basis, no sediment type can (initially) be classified as mandatory. However, fine sand sediment type is ruled out as contraindicated, since fine particles in the water column are enriched by oysters through filtration for food intake and are excreted again through increased pseudofaeces production (Heral 1990). Food intake is correspondingly lower and pseudofaeces production costs additional energy, so the metabolic rate is not optimal (Rodhouse 1979).

Biotic factors

As the primary food source for European oysters is phytoplankton, a sufficiently high chlorophyll content is a prerequisite for successful settlement. Other important criteria are the spread of pathogens, competition for food and settlement substrate, as well as predation.

No human utilisation

Reintroduction sites should be free of contradictory uses including (but not just limited to) fishing, sand and gravel quarrying, and underwater cables (existing or planned). The lower the overall pressure of use in the selected area, the more promising is the logistical implementation and the long-term success of the reintroduction measure(s). From a logistical point of view, it also makes sense to (initially) choose a location that is not in areas with a high density of shipping traffic (traffic separation areas). All (currently) existing uses, including within marine protection areas, form corresponding logistical exclusion areas for reintroduction measures.

Depth

European oysters can be found in a water depth of 30-50 metres (OSPAR 2013; Pogoda 2019), corresponding to the maximum depth of the German North Sea of around 50 metres. Depth as a selection criterion is therefore not a biological factor here, but exclusively of logistical importance for scientific support and planned monitoring tasks.

Sediment recording

Sediments in the German Bight have not yet been mapped completely. Accordingly, locations where sediment composition has already been studied and hydrographic data are available offer a clear advantage over locations that have not yet been recorded.

Tab. 3:List of defined site selection criteria for site selection for European oyster reintroduction
measures in the German Bight (after Pogoda et al. (2020b)).

Site selection category	Site selection criteria
1. Ecological history	1. Historical distribution of O. edulis and O. edulis banks
2. Reintroduction feasibility	2. Conservation Area
	3. Exclusion of bottom-contact fishing
	4. Exclusion of bottom-contact fishing
	5. Exclusion of areas with underwater cables
	6. Absence of other contraindicated uses
	7. Depth
3. Environmental factors (abiotic)	8. Temperature
	9. Salinity
	10. Current velocity
	11. Oxygen concentration
	12. Substrate quality (Sediment type/dynamics)
4. Environmental factors (biotic)	13. Food availability (Chlorophyll concentration)
	14. Predation
	15. Competition
	16. Disease

3.3.2 Site selection for long-term reintroduction

Site selection for pilot reef construction as part of the RESTORE main project and for implementation of further long-term reintroduction measures was based on previously defined site selection criteria (Table 3), with special consideration of the criteria nature reserve (reduced pressure of use) and historical distribution (Pogoda et al. 2020b). The nature conservation criterion is fulfilled in three areas of the EEZ (Dogger Bank, Sylt Outer Reef, and Borkum Reef Ground NCAs). The Borkum Reef Ground (BRG) NCA is in the centre of the historically documented distribution of the once native oyster species (Fig. 3, Olsen 1883; Gercken, Schmidt 2014; Pogoda 2019). In the BRG NCA management plan, European oyster reintroduction to the necessary extent is given high priority (M 5.2) in the group of measures reintroduction of species or restoration of habitat types (HDHT) in their typical forms (MG 5) (BfN 2020). Fishing exclusion is currently being negotiated for BRG NCA, which will (initially) set the area apart from other areas in terms of its suitability for the planned reintroduction projects (BMU 2019). Accordingly, the BRG NCA was selected for construction of the pilot reef (RESTORE main project) and implementation of further long-term reintroduction measures. All results presented here relate to this area. Within the area, site selection was based on the previously defined site selection criteria (Table 3), with special consideration of the criteria environmental factors and utilization pressure.

Borkum Reef Ground NCA: detailed description

Geological description of the Borkum Reef Ground area

The morphological and sedimentological characteristics of the Borkum Reef Ground area have their origin in the Saale glaciation in the Pleistocene (130,000 - 200,000 BC). During this period, the southern North Sea was last covered by an inland ice sheet; through its advance and glacial movements, rock material was eroded and transported over long distances. When the ice masses melted, this material was deposited both under the glacier (ground moraine) and piled up at the edge of the ice (end moraine) (Ehlers 1994). The relief of the seabed of the German Bight was influenced and changed over time by ocean currents, rough seas, and tides. Coarse fractions of the original moraine mostly remained where they were originally deposited, which is why today's stone fields of the German Bight are considered indicators of the location of former moraines (Diesing, Schwarzer 2006b). Borkum Reef Ground can be seen as a continuation of the Saale Ice Age Oldenburg-East Frisian ground moraine (Ehlers 1994; BfN 2008). Existing reef structures there are typical of the North Sea, small-scale and patchy accumulations of stones, which occur in the form of band-like or scattered stone fields (Boedeker et al. 2006).

Parts of the reef structures in Borkum Reef Ground were examined and analysed using hydroacoustic methods (side scan sonar) (Diesing, Schwarzer 2006a). On the one hand, defined stone fields were identified in which stones are typically concentrated on coarse sediments. Density within the stone fields is relatively low, with individual stones are sometimes several metres to decametres apart. In contrast, there are large areas of the seabed that do not have any stones and in which, in addition to medium sand, mainly coarse sand and gravel occur (Figge 1981; BfN 2008). The surface sediment cover is characterised by a high degree of heterogeneity: areas with a narrow sediment change next to areas of relatively high homogeneity are characteristic. (Diesing, Schwarzer 2006a). The following is written about the persistence of sediment distribution in a study on sediment distribution in the German EEZ by Diesing and Schwarzer, 2006a:

"Investigations [...] have shown that all surface sediments show ripples induced by sea and currents and are therefore at least episodically relocated. This applies to coarse sediments as well as to fine to medium sands. In contrast, the comparison of sediment distribution patterns in the area of the former research platform "North Sea" (FPN) over several years has shown that the sediment boundaries are surprisingly stable (Werner 2004). Changes are essentially only detectable to a few metres, in a few cases decametres. Even the details of the sediment distribution pattern have been preserved in many cases."

In order to obtain a precise picture of the seabed, underwater videos recorded in the BRG NCA were examined as part of the present preliminary investigation in addition to the literature research (overview see Fig. 21, Fig. 22 and Tab. 5). For the selected BRG locations, comprehensive depth profiles and current underwater recordings were also created as part of the present preliminary investigation (HE529).

Habitat types and biotope types in Borkum Reef Ground NCA

The BRG NCA is located about 15 km north-west of the East Frisian island of Borkum and covers a total of about 625 km², mainly consisting of the habitat type (HDHT; Habitats Directive 92/43/EEC) "Sandbanks which are slightly covered by sea water all the time" (Code 1110) with scattered stone fields, defined as HDHT "reefs" (Code 1170). Typical for the hard substrate epifauna communities are: Porifera (Leucosolenia botryoides), Cnidaria (e.g., Sertularia cuppreassina, Metridium senile, Alcyonium sp.), Mollusca (e.g., Lepidochitona cinerea, Littorina littorea, Macoma balthica), Polycheata (e.g. Pomatoceros triqueter, Polydora spp., Sabellaria spinulosa), Bryozoa (e.g., Flustra sp., Membranipora membranacea, Securiflustra securifrons), Crustacea (e.g., Carcinus maenas, Homarus gammarus, Galathea spp.), Echinodermata (e.g., Asterias rubens, Echinus esculentus, Ophiothrix fragilis), and Tunicata (e.g., Ciona intestinalis) (BfN 2008; Krause et al. 2008). The stone surface of the stone fields, populated with hard substrate-typical epifauna communities in the BRG, cover a total of 23 km² (summary of the area data see Table 4). The predominant legally protected biotope type " species-rich gravel, coarse sand and shell areas (KGS)", according to § 30 Para. 2 S. 1 No. 6 BNatSchG (BfN 2017a), is evident with focal areas in the central to central-southern core area of the protected area. Due to the diverse substrate and habitat structure, as well as a characteristic, species-rich bottom fauna, Borkum Reef Ground is considered an area of high ecological importance (BfN 2008).

Tab. 4:Summary of area data and area outline of Borkum Reef Ground Marine Nature Reserve,
German North Sea (after BfN (2008)).

Biogeographic region	Atlantic
Centre point	53°52'14'' N 06°24'50'' E
Area	625 km²
''Reef'' habitat area	23 km ²
Reef proportion	4%
Water depth	18-33 m

The BRG NCA is exposed to a number of uses and pressures within the framework of marine spatial planning (Fig. 17). These include shipping lanes, submarine cables, sand and gravel extraction, fisheries, and offshore wind power. Existing uses limit the choice of location for the construction of a pilot reef and for the implementation of further, long-term reintroduction measures.

Utilisation of the seabed is of particular importance for the choice of location, especially the routes of submarine cables, some of which are in operation, approved, or planned. Various

data and power cables run through the BRG NCA. Depending on the type of cable, the operator must meet individual requirements with regard to heat generation and/or maintenance (BSH, pers. comm.). All cable types have in common that further construction work, renewal, or complete dismantling of the cables can take place in the vicinity and, thus, active reintroduction measures would be hindered, disturbed, or destroyed. Accordingly, areas that have active energy or telecommunications cables (or ones under construction) are excluded from reintroduction measures. A summary of the cables relevant to restoration projects is shown in Figure 18.

In the centre and in the southern part, reserved and priority areas for shipping run through the BRG NCA (blue areas and blue hatched areas in Fig. 18). Some of these areas are traffic separation areas and are therefore busy waterways for international maritime traffic. Although locations within these areas are not affected by activity on the seabed, they are (initially) excluded for logistical reasons and reasons of traffic safety. On the one hand, the area on which a pilot reef is to be built as part of the RESTORE main project should be accessible for regular sampling at any time and without complex logistical marking and demarcation measures. On the other hand, in the priority areas, as things stand at present, anchoring maneuvers by shipping traffic must be expected (GDWS, pers. comm.), which would impede, disrupt, or destroy active reintroduction measures. Accordingly, these areas are excluded for reintroduction measures. A summary of the traffic areas relevant to restoration projects is shown in Fig. 18.

Another limiting use is fishing activity in the protected area (Fig. 19). In the BRG NCA, there are areas that are only rarely fished. However, there is increased fishing activity in the northern areas that would impede, disrupt, or destroy active reintroduction efforts (ICES 2016). Soil-disrupting activities within the area contradict the suitability for the construction of a pilot reef and the implementation of further, long-term reintroduction measures. Future fishing exclusion in BRG NCA is currently being examined by the EU Commission as part of a delegated act based on the EU Common Fisheries Policy (CFP). Accordingly, bottom-contact fishing gear would be banned in large parts of the BRG NCA. Sand and gravel quarrying areas would also have to be ruled out due to the potentially permanent disturbance of the seabed. However, there is no ongoing or proposed extraction within the BRG NCA. Ap-proved areas for sand and gravel extraction in the German North Sea currently only exist in the Sylt Outer Reef NCA (BfN 2019a).

Overall, existing uses already severely limit the choice of location within the Borkum Reef Ground NCA.

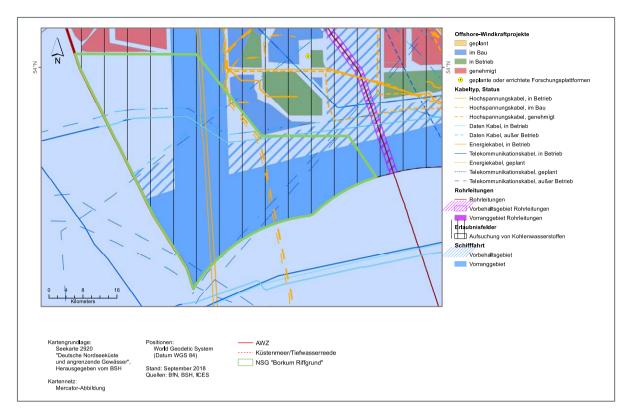


Fig. 17: Marine spatial planning for BRG NCA and adjacent sea areas: representation of selected, relevant usage groups (data: BfN 2018). (German only)

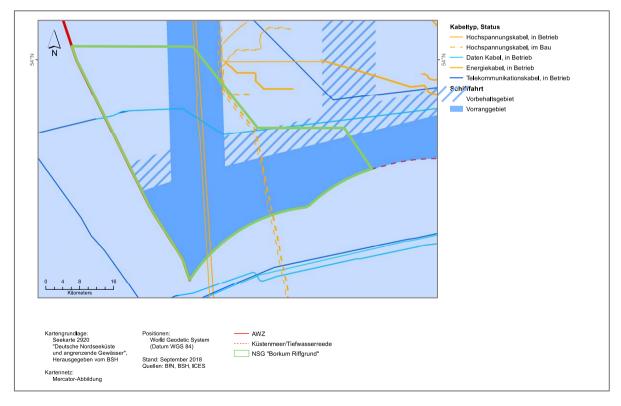
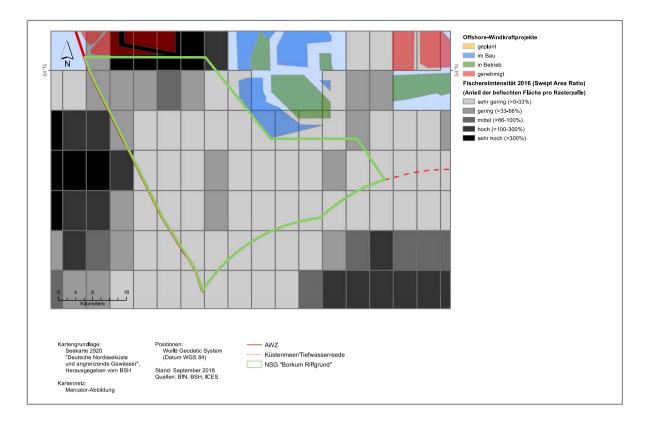


Fig. 18: Marine spatial planning for BRG NCA and adjacent sea areas: Location of the priority and reserved areas for shipping and the submarine cables that impair or exclude measures to reintroduce European oyster (data: BfN 2018). (German only)



- Fig. 19: Representation of the fishing intensity of bottom-contact fisheries (beam trawl, dredge, otter trawl, draught net) in BRG NCA in 2016 (data: BfN 2018). (German only)
- Tab. 5:List of underwater videos available and evaluated for measures to reintroduce European
oysters (AWI = Alfred-Wegener-Institute Helmholtz Centre for Polar and Marine Research;
BfN = Federal Agency for Nature Conservation; BioConsult = Bio-Consult Schuchardt &
Scholle; SaM = Senckenberg am Meer).

Year	Cruise	Ship's name	Number of transects	Source
2002	NAN BRGSK SK	Dr. Nansen	18	BfN
2012	P495 1212	Damkerort	10	BioConsult
2012	Senckenberg 34/2012	Senckenberg	20	SaM
2012	HE389	Heincke	5	SaM
2012	Senckenberg 45/2012	Senckenberg	20	SaM
2012	HE385	Heincke	8	AWI
2013	Senckenberg 05/2013	Senckenberg	24	SaM
2013	Senckenberg 36/2013	Senckenberg	27	SaM
2013	Senckenberg 40/2013	Senckenberg	14	SaM
2013	Senckenberg 45/2013	Senckenberg	7	SaM
2014	Senckenberg 02/2014	Senckenberg	7	SaM
2014	Senckenberg 05/2014	Senckenberg	8	SaM

Year	Cruise	Ship's name	Number of transects	Source
2014	Senckenberg 22/2014	Senckenberg	23	SaM
2015	GR03	Grinna	6	AWI
2015	UT03	Uthörn	1	AWI
2017	P617 MZB 0617 BRG	Damkerort	19	BioConsult
2019	HE529	Heincke	3	AWI

Description of biotic and abiotic factors

Growth, survival, reproduction, recruitment, and survival of European oysters depend on various environmental factors (Laing et al. 2005).

An important abiotic factor that controls the life cycle of the species is **water temperature**. Feeding and growth begin in spring from a temperature of 7 °C (Ashton, Brown 2009). These conditions are met in the German Bight for about eight months of the year (Pogoda et al. (2020b)) In contrast to shallower habitats near the coast (e.g. in the Wadden Sea), the water temperature and salinity in the deeper sublittoral, such as in the BRG NCA, are always within the tolerance range defined for European oysters. Maximum growth rates were measured at water temperatures of 16-18 °C (Laing 2005). Reproduction takes place at temperatures from about 15 °C onwards. Accordingly, it can be advantageous if they are resettled at shallower water depths (ca. 20 m) since these temperatures are reached there earlier in the year (Gercken, Schmidt 2014).

Hydrodynamic processes affect larval transport and food availability. Flow direction and speed are important for the spread and successful settlement of the larvae. These determine the location of further settlement and should be taken into account in long-term and largescale reintroduction measures, for example by providing settlement substrate at appropriate locations. Prevailing flow directions in the German Bight (BSH 2017) result in an expected larval drift in a north-easterly direction. With the aim of colonising larger areas within the Nature Reserve, a pilot reef should accordingly be built in the south-western area. Flow velocity is also a relevant abiotic factor for food intake. Flow velocities that are too high result in mechanical stress; oysters invest more energy in shell growth to protect themselves or are no longer able to filter food from the water stream. The condition index, which evaluates the general condition of the animals, then drops accordingly. Previous studies show that the filtration rate of oysters increases with increasing current velocity up to a maximum (Walne 1979; Ashton, Brown 2009) and that with a daily maximum tidal current of 0.45 m/s the maximum tolerance range with accordingly low growth rates was achieved (Pogoda et al. 2011). A current that is too low favours deposition of fine sediments and thus changes the subsoil. Flow rates of 0.05-0.1 m/s are therefore the necessary minimum to exclude sediment accumulation and silt formation in the area (Ashton, Brown 2009). Flow velocity in the BRG NCA is between 0.27-0.31 m/s, sufficiently high to avoid sedimentation and sufficiently low to ensure good food availability (Tab. 6, Pogoda et al. (2020b).

The average depths of the area are well suited for colonisation by the European oyster. *O. edulis* occurs in **water depths** of up to 50 m. The prevailing depths in the BRG NCA are within the tolerance range of the species of 18-33 m (Pogoda 2019). However, this poses a challenge from a logistical point of view. Other abiotic factors to consider are **sediment composition** and subsoil conditions (Pogoda et al. 2020b). There are eight different types of

sediment in the BRG NCA (Fig. 20). Areas with larger grain sizes (medium to coarse sand with/without potential KGS shares) are suitable for the construction of a pilot reef and for the implementation of further, long-term reintroduction measures. European oysters settle on various soil substrates, such as stones, gravel, and coarse or medium sand (Laing et al. 2005; Airoldi, Beck 2007; Colsoul et al. 2020b). Water turbidity at the site also depends on the grain size of the prevailing sediments and influences the growth and health of oysters (Soletchnik et al. 2007).

Tab. 6:Environmental conditions in the Borkum Reef Ground Natura 2000 area for September 2017
to September 2018 (data source Operational Circulation Model of BSH (BSHcmod), Dick et al.
2008, Dick et al. 2001 after Pogoda 2020).

Month	Temperature	Salinity	Oxygen Concentration	Current velocity	Chlorophyll concentration
	[°C]	[g/l]	[mg/l]	[m/s]	[µg/I]
Sep 17	17.63	33.54	7.38	0.31	3.06
Oct 17	15.38	34.11	7.99	0.31	1.57
Nov 17	12.14	34.34	8.59	0.31	1.11
Dec 17	8.59	34.66	9.39	0.32	0.94
Jan 18	6.49	34.29	9.88	0.32	1.17
Feb 18	5.19	33.14	10.18	0.31	2.69
Mar 18	3.15	32.74	10.80	0.32	3.92
Apr 18	4.91	32.30	10.29	0.31	1.73
May 18	8.92	32.68	9.16	0.27	1.55
Jun 18	13.07	33.72	8.28	0.24	1.57
Jul 18	14.81	33.56	7.65	0.31	2.36
Aug 18	16.75	33.07	7.23	0.31	3.13
Sep 18	17.36	33.44	7.53	0.31	2.38

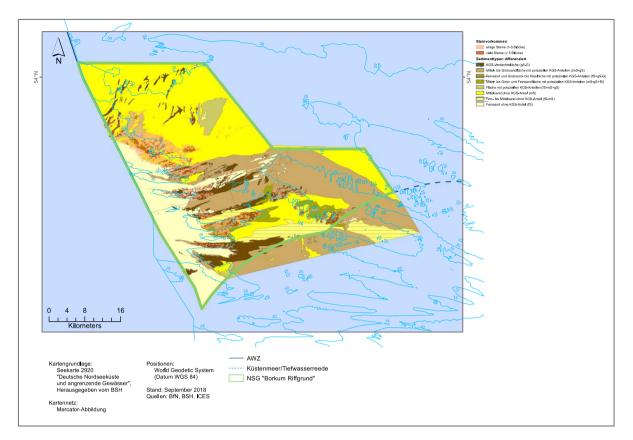


Fig. 20: Sediment distribution, rock occurrence, and bathymetry in BRG NCA (data: BfN, BSH, Geo Sea-portal). (German only)

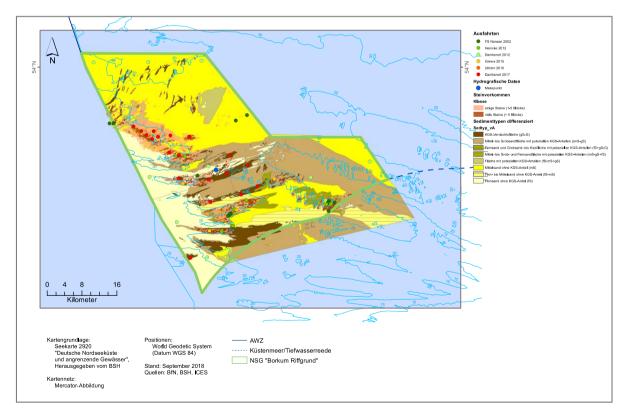


Fig. 21: Locations of the evaluated underwater videos in BRG NCA, recorded during various research trips from 2002 to 2019 (legend), and position of the measuring point for recording hydrographic parameters (data: AWI, BfN, BioConsult, BSH, GeoSea-Portal). (German only) An important biotic factor is **chlorophyll content**, which affects the availability of food for the filtering oysters and thus is the basis for healthy metabolic processes. The optimal chlorophyll concentration is 2-3 μ g/l (Rogan, Cross 1996). These conditions are met in the German Bight for around six months of the year and enable successful growth (Tab. 6).

Pathogens can attack oysters at any stage of growth and, depending on the pathogen, can cause extreme mortality rates in oyster populations. So far, no relevant pathogens (such as *Bonamia ostreae* and *Marteilia refringens*) have been documented in the BRG NCA (TSIS 2019; Merk et al. *in prep*).

Predators include starfish, such as *Asterias rubens*, and crabs, such as *Cancer pagurus* and *Homarus gammarus*. Oyster sizes with a shell diameter of up to 3 cm are particularly exposed to predation pressure due to their relatively soft shell. The composition of potential predators in the examined areas of the BRG NCA is shown in Fig. 22.

Competition for food or settlement substrate can also be important for the reintroduction of the European oyster. Naturally existing settlement substrate is already largely overgrown by sessile invertebrates. The groups of reef-typical sessile epifauna were identified by video recordings and are also filter feeders: Porifera (e.g. *Halichondria panicea*), Cnidaria (e.g. *Alcyonium digitatum, Metridium senile, Sagartia elegans*), and Bryozoa (e.g. *Flustra foliacea*) (Fritsch 2017). Due to the chlorophyll concentration (Tab. 6) a limitation of food availability cannot be assumed.

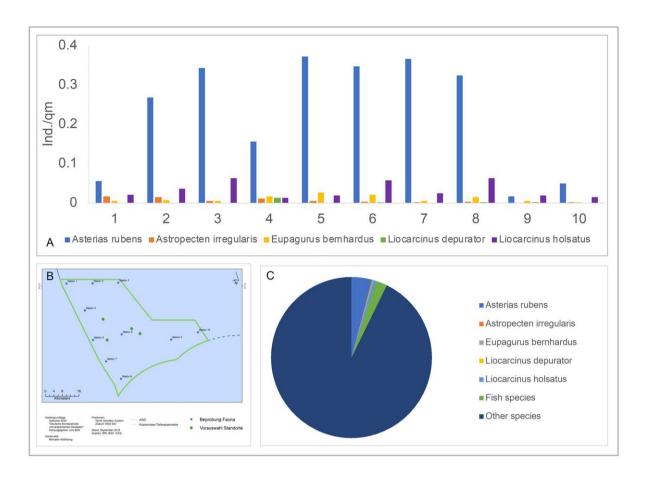


Fig. 22: Summary of fauna sampling at 10 locations in Borkum Reef Ground.
A) Station 1-10 (blue): Dredge samples Bioconsult 12/2012 (IOW et al.). Original station names: Station 1= BR-D-01, Station 2= BR-D-02, Station 3= BR-D-03, Station 4= BR-D-05, Station 6= BR-D-07, Station 6= BR-D-08, 7= BR-D-09, 8= BR-D-10, 9= BR-D-11, 10= BR-D-012). Pre-selection of locations for oyster reintroduction (green).

B) Predator distribution per station.

C) Macrofauna composition at potential reintroduction sites in Borkum Reef Ground (BRG) Natura 2000 area. Percentages of potential predators, summarized for 10 stations within the BRG. Fish species as potential larval predators:

Agonus cataphractus, Arnoglossus latern, Buglossidium luteum, Callionymus lyra, Callionymus reticulatus, Ciliata mustela, Clupea harengus, Gadus morhua, Gasterosteus aculeatus, Hyperoplus immaculatus, Limanda limanda, Liparis liparis, Merlangius merlangus, Mullus surmuletus, Myoxocephalus scorpius, Osmerus eperlanus, Pholis gunellus, Pleuronectes platessa, Pomatoschistus minutus, P. pictus, Scyliorhinus canicula, Sprattus sprattus, Syngnathus rostellatus, Trachurus trachurus, Trisopterus luscus, Andere Arten: Alcyonidium sp., Alloteuthis subulate, Aora typica, Bougainvillia sp., Chamelea gallina, Clytia hemisphaerica, Conopeum reticulum, Corophium acherusicum, Crangon allmanni, C. crangon, Echinocardium cordatum, Electra pilosa, Flustra foliacea, Hydractinia echinate, Macropodia rostrata, Mactra corallina, Melita obtusata, Membranipora membranacea, Obelia dichotoma, O. geniculate, Ophiura albida, O. ophiura, Pandalina brevirostris, Pariambus typicus, Phaxas pellucidus, Philocheras trispinosus, Phyllodoce sp., Processa parva, Sepiola atlantica, Sertularia cupressina. Abundance of all species <1 Ind m⁻².

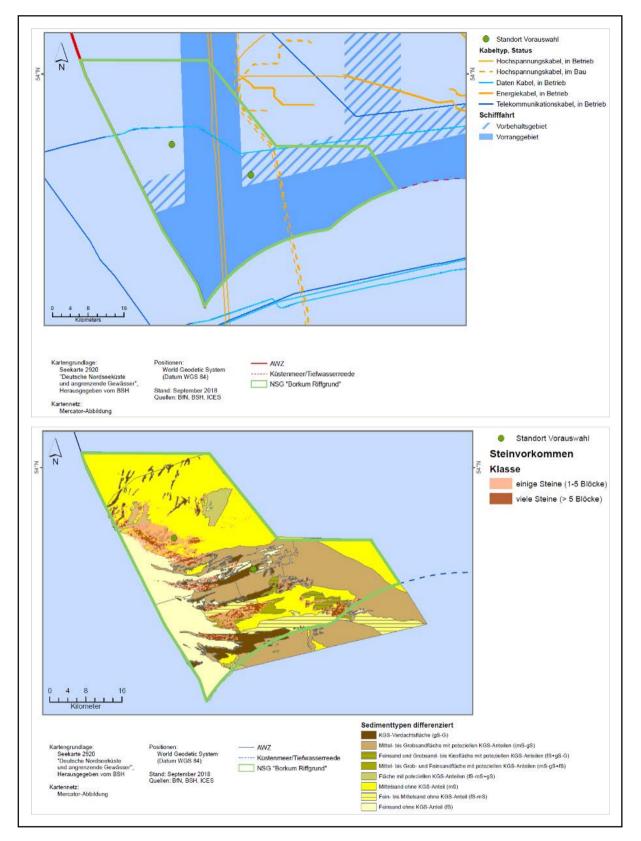


Fig. 23: Location of two suitable locations for measures to reintroduce the European oyster in Borkum Reef Ground NCA, shown on maps for marine spatial planning (top) and for sediment distribution (bottom). After considering all location factors, there are several locations with different degrees of suitability. (German only)

Interim conclusion:

- The entire BRG NCA lies within the historic distribution of European oyster (Fig. 3)
- The abiotic environmental conditions in the BRG NCA are basically suitable for reintroduction. The results of sediment mapping (Senckenberg/BSH/BfN) show that the western area north of the traffic separation scheme is particularly suitable (Fig. 23).
- From an ecological point of view (food supply, predation, competition, diseases) and due to expected larval drift (net flow direction in this area from WSW to ENE), construction of the pilot reef is recommended in the WSW area of BRG NCA. This will enable potentially high retention of the larvae in the protected area and thus the possibility of preparing and designating further areas for natural settlement in BRG NCA.
- BRG NCA maps (created in cooperation with AWI, BSH, BfN, Senckenberg am Meer, and BioConsult) integrate the historical distribution of *O. edulis*, bathymetry, sedimentology, and land use (Fig. 3, Fig. 18, Fig. 19, Fig. 20). They serve as a basis for site selection in the BRG NCA and enable the recommendation of suitable sites.
- After analysis of all existing uses in the BRG NCA and their corresponding consideration as exclusion areas, the NW area proves to be suitable.
- The prevailing depth in the BRG NCA is within the ecological tolerance range of the species and is therefore only a logistical criterion. To carry out accompanying scientific investigations and monitoring tasks, a pilot reef should be located at a depth that can be implemented technically / by diving (≤ 30 m water depth).
- The UW video recordings in this area also cover the stone fields and show diverse vegetation. Pilot reef construction may approach these areas but should not cover the natural stone deposits. The sediments northeast of the stone deposits are well suited; an impact on already existing communities on the stone fields is avoided.
- After considering all location factors, there are several locations with different degrees of suitability (Fig. 23).

3.3.3 Selection of eligible donor populations

The selection of healthy and suitable spat and the identification of suitable technologies for the practical implementation is crucial for the sustainable and long-term success of reintroduction measures. The selection and evaluation of suitable donor populations and production methods took place, on the one hand, for the field experiments of the preliminary study and, on the other hand, for the further main project and for future, long-term reintroduction measures.

Initially, hatcheries where identified where suitable oysters could be obtained. The ICES guidelines "Shellfish transfer between sites" (ICES 2011) and the Berlin Oyster Recommendation (Pogoda et al. 2019) were taken into account to rule out the introduction of non-native, potentially invasive species and pathogens. Existing recommendations from the feasibility study (breeding farms in Denmark and Sweden; Gercken, Schmidt (2014)) were also taken into account with regard to long-term, genetic variability of the population and current research results adjusted (e.g. status Bonamia certification, status breeding farms).

Consideration of pathogens and parasites

Bonamiosis and marteiliosis are currently the main causes of high mortality in European oysters in the remaining European populations (Laing et al. 2006; Gercken, Schmidt 2014; Sas et al. 2020). To date, two species of the genus Bonamia have been classified as pathogenic in Europe: *Bonamia ostreae* and *Bonamia exitiosa* (Helmer et al. 2020). Within the genus *Marteilia* only *Marteilia refringens* is known. All known pathogens of the European oyster were identified as part of an intensive literature search (scientific articles, grey literature in different languages: books, book chapters, conference papers, project reports) (Tab. 7, Colsoul et al. 2020a) and defined as exclusion criteria for the selection of breeding animals and spat. The list contains information on identification and geographic distribution, duration of infection and pathological effects of the pathogens, life cycle (if known) and detection methods. Reportable diseases and pathogens are listed with information on the relevant national reference laboratories for mussel diseases (Colsoul et al. 2020b).

New findings on the spread of the notifiable European pathogens (*B. ostreae, B. exitiosa, M. refringens, Microcytos mackini*) were continuously incorporated in order to select suitable, healthy donor populations. The information and conclusions from the feasibility study (Gercken, Schmidt 2014) were thus re-evaluated and supplemented (Colsoul et al. 2020a).

The donor populations that could be used as part of a restoration programme in the German North Sea based on this information are:

- Populations from Norway free of notifiable pathogens, subject to health certificate and quarantine.
- Populations from selected areas in Scotland free of notifiable diseases, subject to health certification and quarantine.
- Populations of selected areas in France free of notifiable pathogens, subject to health certification and quarantine.
- Populations, from Irish selective breeding at Rossmore, Port of Cork, pathogen free and resistant to bonamiosis, subject to health certificate and quarantine.

Group	Species	Effect	Distribution	Evidence
Algae	Gyrodinium aureolum	Necrosis of the central area of the digestive gland	N/A	Macroscopy
Bacteria	Nocardia crassostreae	Mortality (detection in all tissues)	Canada, Europa (Netherlands)	Histology, PCR, ISH
Bacteria	Vibrio alginolyticus	High larval mortality (up to 100%)	N/A	PCR
Bacteria	Vibrio anguillarum	Larval mortality	Europe (Spain)	PCR
Bacteria	Vibrio coralliilyticus	Larval mortality	USA, New Zeeland, Europe (France)	PCR
Bacteria	Vibrio neptunius	High larval mortality (>98%)	Europe (Spain)	PCR
Bacteria	Vibrio ostreicida	High larval mortality (86.4- 98.5%)	Europe (Spain)	PCR
Bacteria	Vibrio tubiashi	Larval mortality (deadly ex- otoxins (for larvae), bacte- rial necrosis)	USA, Europe (Spain)	PCR
Copepod	Herrmannella duggani [‡]	Gill reduction (detection in the shell cavity)	Europe (Ireland)	Macroscopy, Histology
Copepod	Mytilicola intestinalis	Minimal effects on the host (detection in the intestinal lumen)	USA, Japan, Europe	Macroscopy, Histology
Fungus	Ostracoblabe implexa	Shell anomalies	India, Canada (Nova Scotia), Europa	Macroscopy, Histology
Protozoan	Bonamia exitiosa	Mortality (detection in he- mocytes; all tissues can be affected)	Australia, New Zee- land, Tasmania, Eu- rope (Croatia, France, Italy, Portu- gal, Spain, Tunisia, UK)	Histology, PCR ISH, Electronm croscopy
Protozoan	Bonamia ostreae	Mortality (detected in he- mocytes; all tissues can be affected; larvae can be in- fected)	USA, Europe, (Bel- gium, Denmark, England, France, It- aly, Netherlands, Northern Ireland, Spain, Wales)	Histology, PCR, ISH, Elec- tron microscopy
Protozoan	Haplosporidium armoricanum	Mortality (sporadic – de- tection in connective tis- sue)	Europe (France, Netherlands, Spain)	Histology
Protozoan	Hexamita inflata	Mortality (detection in con- nective tissue)	USA, Canada, Eu- rope	Macroscopy

Tab. 7: Pathogens of European oyster O. edulis (Colsoul et al. 2020a)

Group	Species	Effect	Distribution	Evidence
Protozoan	Marteilia refringens	Mortality (extracellular parasite of digestive gland)	Europe (Albania, Croatia, France, Greece, Italy, Mo- rocco, Portugal, Spain, Sweden, Tu- nisia, UK)	Histology, PCR, ISH, Electronmi- croscopy
Protozoan	Mikrocystos ma- ckini	Mortality (intracellular par- asite in connective tissue cells)	USA, Canada (west coast)	Histology, PCR, ISH, Elec tron-micros- copy
Protozoan	Perkinsus medi- terraneus	High mortality	Spain (Balearic)	Histology, Electron- microscopy
Protozoan	Pseudoklossia (Genus)	Parasite found in the kid- neys	France	Histology, Electron- microscopy
Virus	Herpesviridae (Family)	Mortality in juveniles and young stages (sporadic - detection in connective tis- sue)	USA, Australia, New Zeeland, Europe	Histology, PCR, ISH, Elec tron-micros- copy
Virus	Papovaviridae (Family)	Evidence in connective tis- sue, gametocytes)	USA, Australia, Ko- rea, Japan, Europe (France)	Histology, Electronmi- croscopy

Consideration of genetic diversity

Maintaining a high level of genetic diversity is of fundamental importance for reintroduction measures to be successful in the long term and must be taken into account accordingly (Lallias et al. 2010). Although geographic origin is of secondary importance due to the genetic proximity of European populations, the strategy applied affects genetic diversity in long-term restoration (Gercken, Schmidt 2014).

There are three main strategies to strengthen and restore oyster stocks (Lallias et al. 2010): translocation of adults, seeding of juveniles, and release of larvae.

The second strategy is to be preferred for the reintroduction of the European oyster in the German North Sea. According to the Berlin Oyster Recommendation (Pogoda et al. 2019), translocations of mature oysters from wild stocks should not be carried out due to the increased pressure on already endangered wild populations and the risk of introducing invasive species and pathogens. However, the production and import of young spats from controlled oyster hatcheries for sowing young seed oysters is a suitable and ecologically safe way to reintroduce European oysters into the German Bight. The release of larvae, on the other hand, is more suitable for small-scale sea areas such as fjords (e.g. Norway), sea loughs (e.g. Scotland), or geographically delimited bay systems (e.g. England, France).

The production method of spat also influences genetic diversity (Lallias et al. 2010; Colsoul et al. 2020a; Pogoda et al. 2020a):

 In the case of production in oyster hatcheries (closed systems), the broo stock animals must be regularly selected in such a way that the preservation of genetic diversity is guaranteed.

- Large-scale production in "Breed Polls" (e.g. in Norway) and in "Breeding Ponds" (e.g. in Ireland) represents an alternative to breeding systems. Ideally, these techniques ensure greater genetic diversity.
- In Bonamia-free areas, the production techniques in Breed Polls and in Breeding Ponds are the preferred technologies; in areas where bonamiosis is present, use of resistant/tolerant strains from breeding ponds is also appropriate.

Identification of spat oyster producers and farms

The availability of spat is fundamental for the implementation of reintroduction measures. Hatcheries that produce European oysters are summarized in (Colsoul et al. 2020a) (Tab. 8).

From a total of 15 potential suppliers for *Ostrea edulis* in 2018, only three suppliers (Hatchery Scalpro AS, Norway; Hatchery Ostrea Aquaculture, Sweden; Ostrea Marinove SCEA, France) were able to offer spat oysters without reportable pathogens, which, as required, were kept exclusively in filtered seawater. However, in 2018 and 2019, *O. edulis* was produced on a very limited scale and with apparent technical difficulties. Overall, it was found that the successful procurement of spat in Europe is not sufficiently guaranteed and thus represents a limiting factor for future reintroduction projects.

As a specific solution strategy, planning was carried out for a German hatchery for spat oyster production, which is implemented as part of the PROCEED project. PROCEED is funded by the BfN with funds from the BMUV as part of the federal programme for biological diversity over a period of six years, starting on 1st November 2018. The first production of suitable spat for use in restoration projects was planned for 2020.

Country	Name	Production	Status	Bonamia sp. free
Canada	Dalhousie University Aquaculture Center	Hatchery	Active research	uncertain
Denmark	Dansk Skaldyrcenter	Hatchery	Active research	uncertain
Denmark	Venø Fish Farm AS	Breeding pond	Active research	uncertain
England, UK	Seasalter (Walney) Ltd	Hatchery	Active research	yes
England, UK	Seasalter Shellfish (Whitstable) Ltd	Hatchery	Inactive aquaculture	uncertain
France	CRC Bretagne Nord Shellfish Technical Centre	Hatchery	Active reintroduction	uncertain
France	Ferme Marine de l'île d'Arun EARL	Hatchery	Active Aquaculture	uncertain
France	IFREMER Argenton	Hatchery	Active research	uncertain
France	Novostrea Bretagne SAS	Hatchery	Active Aquaculture	uncertain
France	Ostrea Marinove SCEA	Hatchery	Active Aquaculture	yes

Tab. 8: European oyster *O. edulis* hatcheries (Colsoul et al. 2020a).

Country	Name	Production	Status	Bonamia sp. free
Germany	AWI Helgoland Oyster Hatchery	Hatchery	under construc- tion research	uncertain
Ireland	Atlantic Shellfish Ltd	Breeding pond	Active Aquaculture	uncertain
Ireland	Cartron Point Shellfish Ltd	Hatchery & breeding pond	Active Aquaculture	uncertain
Ireland	Tralee Bay Hatchery Co Ltd	Hatchery	Active Aquaculture	uncertain
Netherlands	NIOZ Experimental Hatchery	Hatchery	Active research	yes
Netherlands	Roem van Yerseke BV	Hatchery	Inactive Aquaculture	uncertain
Netherlands	Stichting Zeeschelp	Hatchery	Inactive research	uncertain
Norway	Bømlo Skjell AS	Breed Poll	Inactive research	yes
Norway	Scalpro AS	Hatchery	Inactive Aquaculture	yes
Norway	Storestraumen Øster AS	Breed Poll	Active Aquaculture	yes
Portugal	Marvellous Wave SA	Hatchery	Active Aquaculture	uncertain
Scottland, UK	FAI Farms Ardtoe Marine Research Facility	Hatchery	Active research	uncertain
Scottland, UK	Orkney Shellfisch Hatchery Ltd	Hatchery	Active Aquaculture	uncertain
Spain	A Ostreira SL	Hatchery	Active Aquaculture	uncertain
Spain	Centro de Cultivos Marinos de Ribadeo	Hatchery	Active Aquaculture	uncertain
Schweden	Ostrea Aquaculture	Hatchery	Active Aquaculture	yes
			•••••	

Interim conclusion:

- Donor populations for a sustainable supply of the long-term planned reintroduction measures must be free of diseases such as bonamiosis and marteiliosis.
- Translocations of adult oysters from wild stocks should not be undertaken due to the increased pressure on already vulnerable wild populations and the risk of introducing invasive species and pathogens.
- For the reintroduction of the European oyster in the North Sea, preference should be given to deploying healthy young seed oysters.
- The required availability of spat is not sufficiently guaranteed in Europe and thus represents a limiting factor for future reintroduction projects.
- In November 2018, an oyster hatchery was set up on Helgoland as part of the PROCEED project, funded by the Federal Biological Diversity Programme.

3.3.4 Selection of suitable settlement substrate

As part of sustainable reintroduction of the European oyster in the German North Sea, primarily juvenile spat oysters are initially deployed which, after successful growth, become sexually mature and reproduce after only one to two years (Merk et al. 2020). The adult females release planktonic larvae into the open water (Korringa 1952; Bayne 2017). The successful settlement of the larvae on a suitable substrate is of fundamental importance for the further reintroduction measures. Towards the end of their pelagic-planktic life phase, the free-swimming oyster larvae actively look for attractive settlement areas. If no suitable settlement substrate is available, the larvae die.

Suitable and attractive substrates must also be used in breeding operations in order to achieve optimal colonisation results and thus ensure high production success.

The selection and provision of the best possible settlement substrates is therefore of fundamental importance and was carried out with the aim of defining suitable and completely environmentally compatible substrates for both areas of application (field and hatchery). Accordingly, various natural, semi-natural, and biodegradable substrate types were examined for their suitability both in the laboratory and in the field. This examined to what extent the seabed at the selected locations must be supplemented with a suitable substrate in the long term for sustainable reintroduction of European oyster. Possible sources of supply for suitable substrate were identified and necessary pre-treatments were evaluated in order to rule out the introduction of alien organisms and pathogens.

Laboratory tests

The settlement experiments to investigate the substrate preference of *O. edulis* larvae were carried out in July 2017 in the laboratory and facilities of IFREMER in Argenton, France (Fig. 24, Colsoul et al. 2020b). First, three groups of settlement substrates were identified: 1. shells of different species; 2. inorganic substrates (rocks and mineral products); and 3. natural sediments. For each of the three groups, three to four specific substrate types were examined. (Tab. 9: Summary of selected and tested substrates for settlement behaviour of O. edulis larvae., Fig. 25).

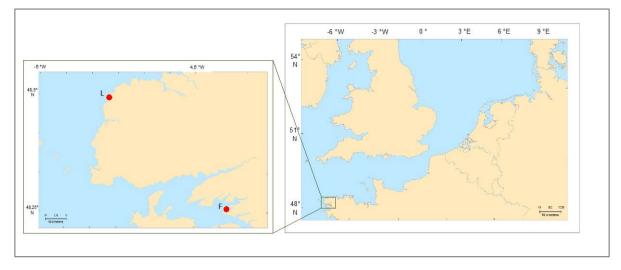


Fig. 24: Locations where experiments on the substrate preference of *O. edulis* larvae were carried out (L = laboratory studies, F = field studies).

O. edulis larvae were obtained from a commercial mussel aquaculture farm (Ferme Marine de l'île d'Arun EARL, Brittany, France) and originated from brood stock from a naturally conditioned wild population in the Bay of Brest. The experiments were carried out in test tanks with sterile seawater and the substrates to be examined (Tab. 9). The free-swimming larvae were placed in high concentrations in the experimental tanks and fed daily. The tanks were examined daily for parameters such as temperature, pH value, oxygen content, salinity, and food concentration. Settlement experiments were terminated as soon as no more swimming larvae were observed and metamorphosis was complete. Each substrate type was removed from the water, rinsed with fresh water, dried, and scored by manual counting with a dissecting scope (Fig. 26).

In July 2018, an additional settlement experiment for the settlement of sandstone reef bodies (design: Reef Design Lab[®], Australia, 3D printing technology - Boskalis Nederland BV) was carried out (for setup, see Fig. 27), (Colsoul et al. 2020b)). The design of the reefs was further developed in cooperation with Alex Goad (Reef Design Lab[®], Australia) and made available for use in the experiment. The experiments were performed with *O. edulis* larvae at the Novostrea Bretagne SAS hatchery, Sarzeau (Brittany, France). Two sandstone reefs were each placed in a 500-litre tank filled with seawater. Larvae produced in the Novostrea hatchery were then transferred to the tanks, kept in a flow-through system and fed. The concentration and size of the larvae were determined weekly via sub-samples. The abiotic parameters (see above) and food concentration in the tanks were examined daily. The settlement experiments of the larvae were terminated as soon as no more swimming larvae were observed and metamorphosis was complete. The settlement rate was evaluated by UW photo documentation. The populated reef bodies were kept for later deployment as part of reintroduction projects.

Tab. 9: Summary of selected and tested substrates for settlement behaviour of *O. edulis* larvae.

Mussel shells	Inorganic	Natural	
	substrate	sediment	
Pacific oyster	Clay	Fine gravel from a depth	
Crassosstrea gigas		of 28 m	
Common mussel	Electro mineral accretion	Coarse sand from a	
Mytilus edulis	(EMA)	depth of 27 m	
European oyster	Granite	Medium/fine sand from	
Ostrea edulis		a depth of 27.5 m	
Great scallop	Limestone		
Pecten maximus			
Field experiments			
Mussel shells	Inorganic	Plant-based	Limed surfaces
	substrate	substrate	
Pacific oyster	Clay	Common juniper	Mussel shells
Crassosstrea gigas		Juniperus communis	(C. gigas, M. edulis O. edulis)
Common mussel	Limestone	Norway spruce	Plant-based
Mytilus edulis		Picea abies	substrate
			(P. edulis, P. abies)
European oyster		Phyllostachys edulis	
Ostrea edulis			
Great scallop			

Laboratory tests

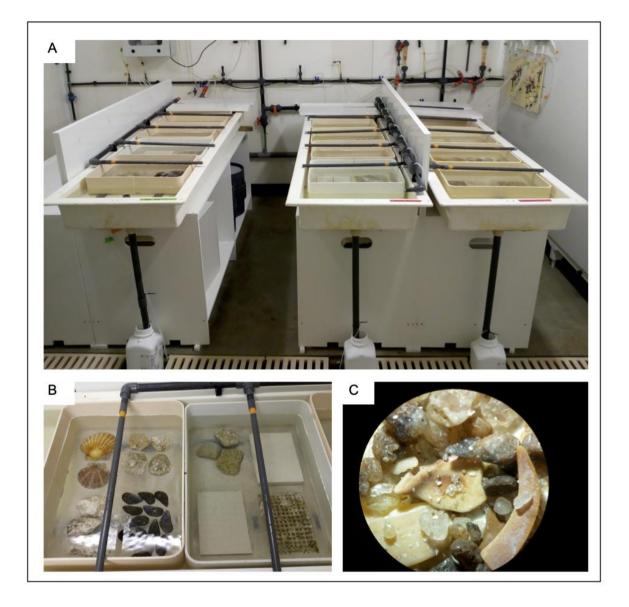


Fig. 25: A) Experiment setup in the laboratory to investigate the substrate preference of European oyster larvae. B) left: shells of the species *Pecten maximus, Ostrea edulis, Crassostrea gigas,* and *Mytilus edulis,* right: inorganic substrates such as clay and EMA grids; C) Settlement attempts on natural sediments from the German North Sea in the Borkum Reef Ground NCA (Photos: AWI / B. Colsoul).

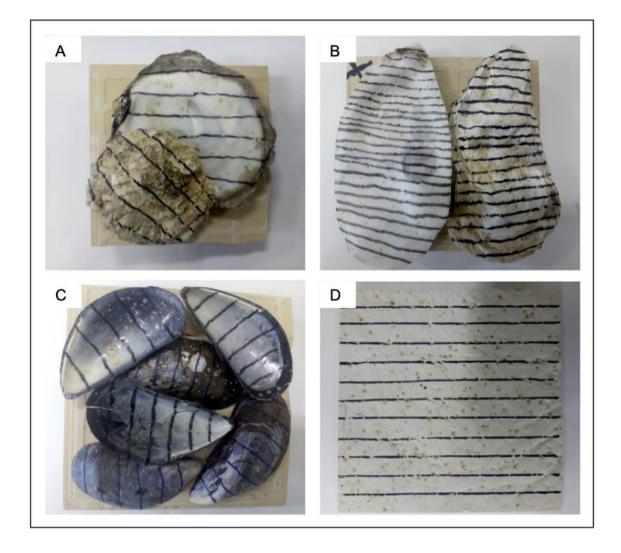


Fig. 26: Evaluation of laboratory tests: settlement of larvae on different substrates: A) European oyster shells (*O. edulis*); B) Pacific oyster shells (*C. gigas*); C) common mussel shells (*M. edulis*), and D) clay. The respective substrates were divided into equal sections for analysis in order to record the stock per area (Photos: AWI / B. Colsoul).

The sandstone reefs showed a high level of colonisation by oyster larvae on all surfaces and layers of the two reefs (Fig. 27). As already shown in the previous laboratory experiment on settling on mussel shells, *O. edulis* larvae settled increasingly on the downward-facing surfaces (reef undersides). The rate of larval settlement on the reef bodies relative to the tank walls and floors of the basin is 100%. The larvae therefore settled exclusively on the sandstone reef bodies. This result proves the suitability of this artificial but near-natural substrate type. The survival rate is also estimated at 100%.

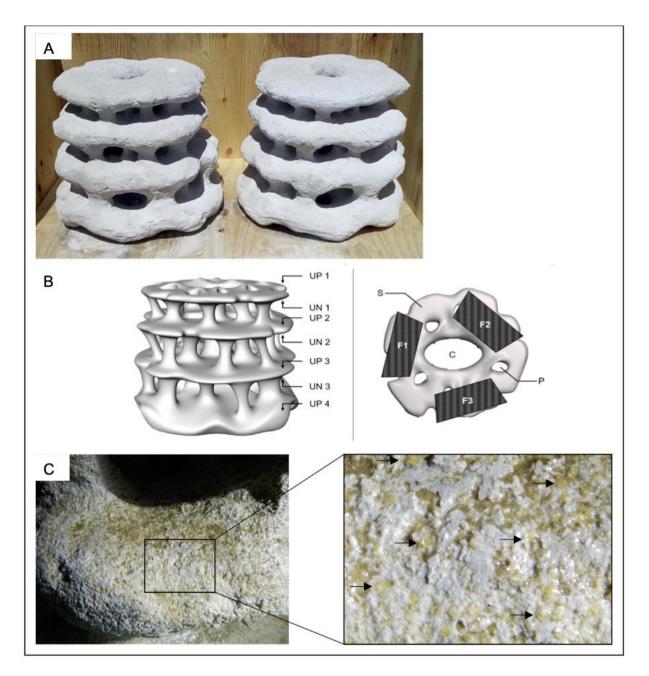


Fig. 27: Representation and colonised areas of the sandstone reef bodies tested in the laboratory experiment. A) The two sandstone reef bodies tested before the colonisation attempt in the laboratory B) Schematic representation of a reef profile with layers 1-4. UP: top; UN: underside; right: Horizontal view of a top or bottom. C: hollow centre of reef; P: hole representing the location of the pillars between layers; S: possible settlement area; F1-F3: Three photos per layer, including non-settled areas (from (Colsoul et al. 2020b), C) Overview of the colonised surface of the sandstone reefs with a detailed view of successfully settled *O. edulis* spat (see black arrows, photo: AWI / B. Colsoul).

The findings of the laboratory experiments can be implemented in seed oyster production (hatcheries) using the spat-on-shell (young oysters on shell material) or spat-on-reef (young oysters on artificial reef bodies) methods (Colsoul, Pogoda 2019; Colsoul et al. 2020b). The sandstone reef bodies are artificial but near-natural and three-dimensional substrates with limited durability. Accordingly, they can be of great importance in early reintroduction phases without permanently changing the seabed using artificial constructions.

Field investigations

The settlement experiments in the field to investigate the substrate preference of *O. edulis* larvae were carried out in cooperation with the French research institute IFREMER in the Bay of Brest (Fig. 24). There are still natural occurrences of European oysters here. The experiments started in July 2018 at the beginning of the swarming phase of *O. edulis* larvae, which was determined by an increased concentration of larvae in the water. The substrates to be examined (Tab. 9) were installed in three racks (50 cm x 50 cm) about 10 cm above the sea floor (Fig. 28). Each rack had 13 mounts for horizontal attachment of the substrates (each 9.5 cm x 9.5 cm). The respective substrates were either glued onto panels or cut into 9.5 cm x 9.5 cm pieces. Environmental parameters such as temperature, salinity, and turbidity were examined daily. The field experiment was carried out over 14 days. After that, the examined substrates were recovered, rinsed with fresh water, dried, and evaluated by manual counting by microscope.

Results of the field investigations and their applicability

In the laboratory experiment, *O. edulis* larvae preferred organic material (such as mussel shells) as well as inorganic material (such as clay and limestone) as settlement substrate. Wood and bamboo are not suitable. Compared to the laboratory tests, the highest colonisation rates were found on clay. This results in further innovative and environmentally friendly uses, such as 3D structures and reef bodies made of clay. In addition, a selection of substrates was coated with lime to test a preference for particular substrate shapes. In the field test, no difference could be found between limed panels and limed mussel shells: the shape of the limed surface seems to be negligible under certain conditions.

The findings of the field experiments are relevant for the implementation of future restoration measures to ensure successful recruitment in the event of natural larval fall (Colsoul et al. 2020b). A preference for the top or bottom of the substrate, as determined in the laboratory tests, was not observed in the field test.

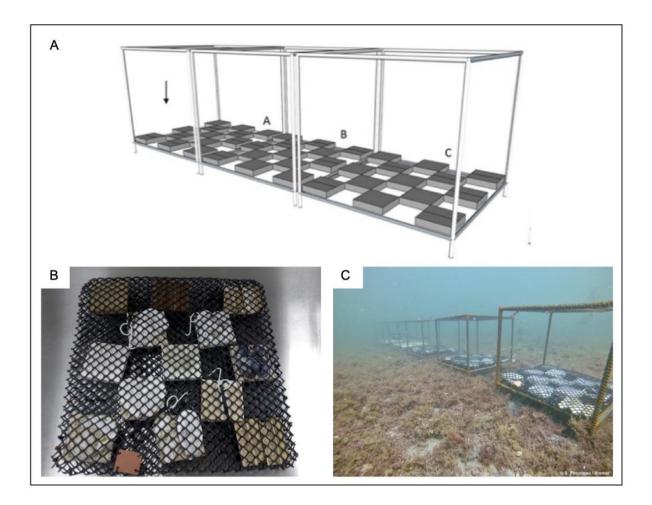


Fig. 28: Experiment planning and implementation of reintroduction experiments with Ostrea edulis larvae in the field. A) Schematic representation of the layout of the substrates tested in cages with two settled areas (top and bottom) per replica; B) Lay out of the substrates in one of the deployed nets (Photo: AWI/B. Colsoul); C) Underwater image of the experiment structures at a depth of 3 m (Photo: Ifremer / S. Pouvreau).

Interim conclusion:

- Various organic and inorganic substrates are suitable for the production of spat-on-shell and spat-on-reef in hatcheries.
- Maximum settlement rates of *O. edulis* larvae were achieved on *Mytilus edulis* and *Ostrea edulis* shells. Under laboratory conditions, the distribution of settled larvae showed that the underside of the substrate was preferred as a settlement area.
- Suitable alternatives to limited or irregularly available natural shell material are limestone and clay, for each of which a high settlement rate of *O. edulis* larvae was shown.
- Under laboratory conditions, the settlement of larvae on natural sediment was low. Here, the composition plays a decisive role in settlement success: the higher the proportion of mussel shells, the higher the settlement rate.

 Under laboratory conditions, innovative 3D sandstone reef bodies showed high settlement rates by *O. edulis* larvae. These artificial but near-natural reef bodies can be seeded in hatcheries and are characterised by limited durability. Accordingly, they can be of great importance in early reintroduction phases without permanently changing the seabed through artificial constructions.

Maximum settlement rates of *O. edulis* larvae are achieved in the field on inorganic substrates (clay and limed surfaces), with no preference for the type of limed mussel shell and location of the settlement surface (top/bottom).

3.3.5 Substrate recommendation for future pilot reef in the Borkum Reef Ground NCA

Important technological requirements for the successful reintroduction of European oysters are the provision of suitable substrate at the selected locations and also the creation of "high relief reefs" (HRR) in order to maximize settlement success (Schulte, Burke 2014; Sawusdee et al. 2015; NOAA 2018; Rodriguez-Perez et al. 2019; Colsoul et al. 2020b). The results of the settlement experiments recommend mussel shells, limestone, and sandstone reef bodies for further practical implementation (Colsoul et al. 2020b). For the construction of a pilot reef, the construction of a test area with combined suitable substrates is recommended in order to achieve the best possible subsoil for successful growth, survival, reproduction, and recruitment of *O. edulis* in the BRG NCA.

Only natural and near-natural materials should be used for this in order to make restoration sustainable and environmentally friendly. Accordingly, the following materials are suitable for preparing the seafloor: natural shell material (preferably of the *O. edulis* species), natural stones (limestone, sandstone, granite, basalt, as naturally occurring in the target areas Borkum Reef Ground NCA and Helgoland NCA, no broken granite!) and reef body made of sandstone and clay (Colsoul et al. 2020b). Since there are no or only low concentrations of larvae in the German Bight, the application of settlement substrate alone is not sufficient. Oysters (spat-on-shell, spat-on-reef) must then be placed on all areas. Spat-on-shell technology is already being used successfully in the USA and Australia (Ch. 3.2) and can be transferred to German conditions (NOAA 2018; Fitzsimons et al. 2019; Westby et al. 2019).

Here, the oyster larvae settle directly on suitable shell material (Ch. 3.3.4) in the hatchery. For reintroduction measures in the North Sea, the young spat-on-shell oysters should first be released in net bags made of biodegradable net material (jute/sisal/hemp/cotton) with appropriate mesh sizes to ensure sufficient flow rates and ensure corresponding oxygen and food supply. The young spat oysters will not so easily be drifted away using this technique because they are already firmly settled on larger shell material. Durable jute or cotton mesh bags are recommended. These sacks each hold 100-300 kg spat-on-shell. The biodegradable nets can be opened after deployment and/or gradually disintegrate. The deployment of spat-on-shell on previously deployed rocky substrate is analogous to establishing HRR. This counteracts silting up by placing the oysters on a stone base (Ch. 3.2). In this way, a higher survival rate can be achieved. The sandstone reef bodies serve to expand spatially into the water column and potentially affect local hydrodynamics. This could result in a longer retention time for the larvae in the reef area.

As part of the construction of the pilot reef, examination should be planned of the biofilm on different types of substrate (settlement plates). The biofilm composition is obviously of particular importance for successful natural larval settlement (recruitment) of *O. edulis* (Rodriguez-Perez et al. 2019).

For the introduction of stones into the BRG NCA as a substrate for the reintroduction of European oysters, the nature of the natural stone deposits and the general geology of the area were researched (support from experts from the BSH). In addition, Greenpeace provided information on the "Boulders to protect the Sylt outer reef" campaign (2008, 2011) (Greenpeace 2009). Granite stones, basalt, and limestone occur as natural rocks in the German Bight (Streif 1990) and are therefore suitable as natural settlement material or as a vertical elevation (HRR). Limestone offers the advantage that it is also a preferred settlement substrate for oyster larvae (Colsoul et al. 2020b).

3.3.6 Summary

- Site selection criteria were defined in accordance with the prevailing requirements for possible sites for long-term reintroduction measures (Pogoda et al. 2020b).
- The investigation of environmental factors and the results of growth and fitness studies of reintroduced oysters in the German Bight (Ch. 3.1.2, Merk et al. (2020)) show that the BRG NCA is suitable for the establishment of a pilot reef of European oysters. Based on the results of the settlement attempts, mussel shells, limestone and sandstone reef bodies are suitable for further practical implementation (Colsoul et al. 2020b).
- After considering these location factors, there are several locations with different levels of suitability within the BRG NCA (Fig. 23).
- For the construction of a pilot reef, the construction of a test area with combined substrates is recommended in order to achieve the best possible subsoil for successful growth, survival, reproduction, and recruitment in the BRG NCA.
- Limestone, basalt, and granite stones occur as natural rocks in the German Bight and are therefore suitable as natural settlement material or as vertical elevation (HRR).
- High settlement rates of *O. edulis* larvae have been shown for mussel shells (hatchery), limestone (hatchery and field), and sandstone reef bodies (hatchery). Correspondingly, oysters should be produced in farms as spat-on-shell and spat-on-reef.
- On reintroduction areas, the oysters (spat-on-shell) are first placed in net bags made of biodegradable net material (jute/sisal/hemp) to prevent the young oysters from drifting.

4 Final Assessment and recommendations

Based on the feasibility study 'Current status of the European oyster (*Ostrea edulis*) and options for resettlement in the German North Sea' (Gercken, Schmidt 2014), this publication presents the various aspects of the practical implementation of reintroduction. Recommendations from the feasibility study were taken up, challenges and open questions were successfully investigated in laboratory and field experiments, a review of the current literature was undertaken, and networks were established. The findings of the RESTORE preliminary investigation are ground-breaking for future reintroduction projects for the species *Ostrea edulis* in the sublittoral areas of the German Bight. The thematic blocks of this report end with interim conclusions; each chapter ends with a summary.

Finally, we present an assessment to integrate the insights gained, formulate recommendations, and provided a summary of further issues that still need to be investigated.

4.1 Implementation of restoration measures

The management plans for the protected areas of the EEZ were published immediately before the completion of this publication. The management plan for Borkum Reef Ground (BRG) NCA attributes a high priority (M 5.2) to the required level of reintroduction of the European oyster, both as part of the measures relating to the 'reintroduction of species' and those that come under 'restoration of habitat types' (LRT) in their typical forms (MG 5) (BfN 2020).

Selection of sites in the German North Sea

A comprehensive list of criteria for site selection was compiled (Tab. 3). These defined criteria were intensively analysed and investigated (Chapter 3.3.1). The results and findings of site selection inform the selection of suitable areas for future and long-term reintroduction measures in the German Bight. On the basis of the now available list of criteria, the described procedure can be applied as part of measures for the management of Natura 2000 sites in the German EEZ or used in the implementation of environmental compensation (Pogoda et al. 2020b). For the implementation of the next phase of the reintroduction of the European oyster, specific locations have been defined in the BRG NCA, where a pilot reef will be built as part of the main project (RESTORE Main). In accordance with the recommendations of the feasibility study, a status inventory of the community of species at the site was carried out in advance of the measure (Chapter 3.3.2) to record ecological changes that occur during the course of reintroduction. Potential predators were documented and the absence of relevant pathogens in that area of the German Bight was verified (Chapters 3.3.1 and 3.3.2). Extensive investigations documenting the development of the oyster reef and recording reef-associated species and their succession are carried out in parallel as part of scientific monitoring (RE-STORE SM). As recommended by the feasibility study, larva collectors are set up to record natural larval spats. They provide information on the reproductive period, reproductive success, and larval drift. In addition, recruitment can be defined as a potential indicator of successful resettlement.

Substrate for long-term resettlement

In accordance with the feasibility study's recommendations on oyster or mussel shells as a substrate base, suitable substrate types were investigated and identified in comprehensive laboratory and field experiments (Chapter 3.3.4). In the process, two areas of application were distinguished: colonisation in the hatcheries to produce seed oysters (spat-on-shell, spat-on-

reef), and natural larval spat on deployed substrate in the sea (Chapter 3.3.5). Since reintroduction measures were to take place 'on a large scale' (Gercken, Schmidt 2014), only natural and near-natural substrates were considered suitable and tested due to nature conservation aspects; this makes it possible to rule out any potential negative ecological effects that recolonisation may have. The results of the investigations show that natural shell materials (particularly from *O. edulis* and *Mytilus edulis*) are well suited to both areas of application, as were lime, clay, and sandstone reef structures. In addition to biological suitability, logistical aspects must be taken into account – such as the availability of the substrate and the necessary cleaning of the shells (for biosecurity reasons). The findings on substrate suitability presented here can be transferred to short-, medium-, and long-term projects for the reintroduction of *O. edulis* in the German North Sea, and they can also be applied in other European reintroduction projects. As substrate resources vary greatly in quantity, quality, and price; a long-term supply strategy should be developed (Colsoul et al. 2020b).

Origin of oysters for long-term reintroduction

The recommendations of the feasibility study were adapted to current developments in European hatcheries. With the establishment of the Native Oyster Restoration Alliance (NORA), the development of the Berlin Oyster Recommendation (Pogoda et al. 2017; Pogoda et al. 2019), and the establishment of the NORA Working Group on Production (Chapter 3.2.2), it became very clear that the availability of seed oysters for long-term reintroduction measures constitutes a limiting factor throughout Europe (Chapter 3.3.3). Several conferences focused on the inventory of internationally active hatcheries and potential production increases (Aquaculture Europe 2017 in Croatia, NORA1 2017 in Germany, NORA2 2019 in Scotland). Active cooperation facilitates the development of joint European strategies to address seed oysters and the suitability of brood stock populations have already been used and implemented with the establishment of a hatchery in Germany (the BPBV project PROCEED).

The ongoing inventory of pathogens is a mandatory component when selecting brood stock oysters and assessing risks of translocations (IUCN/SSC 2013). Suitable parent oysters are sourced from selected European regions to preserve biological variety (Chapter 3.3.3). The establishment of the NORA Working Group on Biosecurity (Chapter 3.2.2) has resulted in the development of appropriate Best Available Technique (BAT) and Best Environmental Practice (BEP), which are published in the NORA Biosecurity Guidelines. The production of suitable seed oysters (quality and quantity) for long-term reintroduction measures will take place in the oyster hatchery on Helgoland.

Preliminary investigations for the construction of a pilot reef

The findings gained during the field research are ground-breaking for reintroduction projects with the species *O. edulis* in sublittoral areas (Chapter 3.1.2). This applies both to the oyster reefs that have disappeared from the German EEZ and to the former Belgian and Dutch sublittoral oyster populations. It has been demonstrated that the ecological requirements of European oysters are fulfilled at these water depths: the oysters showed excellent growth, very good fitness, and low mortality rates (Merk et al. 2020). The reproductive capacity that the reintroduced oysters demonstrated during the first year proves the potential of European oysters to form intact populations in the long-term, thus restoring the oyster reef community with all its ecological functions. However, the cage experiments do not allow any conclusions to be drawn about new predator-prey relationships or the influence of sediment dynamics. These open questions, as well as the technical and biological challenges of comprehensive reintroduction measures, are investigated and tested within the framework of the main project (RESTORE M) and the scientific monitoring (RESTORE SM) with the construction of a pilot reef. After the reintroduced oysters have successfully reproduced and the first natural larvae have settled, it is the successful settlement of the larvae in particular that is a decisive criterion as to whether the population can grow after the initial settlement phase.

Overarching recommendations

Due to the insufficient and unstable availability of seed oysters and the problem of *Bonamia*infected brood stock oysters, it is essential to establish a reliable production chain for healthy seed oysters produced under controlled conditions.

According to the findings of the preliminary investigation, the use of healthy oysters from hatcheries is considered mandatory (BET & BAT) and has already been included in the Berlin Oyster Recommendation (Pogoda et al. 2017; Pogoda et al. 2019).

It is imperative that the removal of adult oysters from wild populations is avoided so as not to increase the fishing pressure on the natural populations of other regions, and to prevent the introduction of periphyton organisms, parasites, and diseases by translocation.

Restoration of European oyster populations should take place in the context of ecological restoration. Accordingly, the introduction of artificial substrate should be avoided. The use of natural and near-natural substrate, and appropriate pre-treatment (cleaning) to avoid potential negative ecological effects are expressly recommended.

The results and findings of the preliminary investigation serve as a necessary scientific basis for further practical steps in the restoration of European oyster populations in the German North Sea. They enable the direct implementation of future reintroduction measures within the context of:

- the construction of a pilot reef (main project),
- the implementation of management measures in the protected areas of the EEZ,
- the implementation of environmental compensation.

The results obtained show that the reintroduction of oysters is suitable as a measure for environmental compensation in the German Bight (BfN 2019c). The preliminary investigation therefore makes an important contribution to the complex problem of compensatory measures in German marine areas.

4.2 Further questions

In concluding the preliminary investigation, we recommend addressing a number of related and further-reaching issues that are of particular importance for the implementation of a longterm restoration programme and the large-scale restoration of native oyster populations.

Technological aspects:

- Development of a Europe-wide long-term supply strategy for healthy, certified seed oysters at the NORA level to exclude potential negative ecological effects due to translocation.
- \rightarrow To be addressed within the context of the BPBV project PROCEED
- Development of a Europe-wide long-term supply strategy for suitable substrate at the

NORA level in order to exclude potential negative ecological effects due to the introduction of artificial substrate or translocation of untreated shell substrate.

- \rightarrow To be addressed within the context of the BPBV project PROCEED
- Practical testing and development of various large-scale methods to deploy young seed oysters from breeding facilities to the prepared substrate bases. Further development and adaptation of the spat-on-shell and spat-on-reef methods.
- \rightarrow To be addressed within the context of the main project (RESTORE M) in cooperation with the German oyster hatchery (PROCEED)
- Practical testing and implementation of international monitoring standards (Baggett et al. 2014; Fitzsimons et al. 2019; Fitzsimons et al. 2020) for restored oyster reefs at deep sites in the German Bight.
- \rightarrow To be addressed within the context of the main project (RESTORE M) in cooperation with NORA partners
- Development of monitoring methods to record biodiversity and habitat structure on largescale restored sublittoral oyster reefs.

Ecological aspects:

- Recording and evaluation of effects of the natural dynamics of sediments at the restoration site. Testing and development of adapted methods for the construction of oyster reefs.
- → To be addressed within the context of the main project (RESTORE M) and the scientific monitoring (RESTORE SM) in cooperation with NORA partners.
- Recording and evaluation of the effects of new predator-prey relationships.
- \rightarrow To be addressed within the context of scientific monitoring (RESTORE SM).
- Recording of reef-associated fauna and evidence of the succession of relevant species groups. Evaluation of the restored oyster reefs in terms of their role as biodiversity hotspots.
- \rightarrow To be addressed within the context of scientific monitoring (RESTORE SM).
- Documentation of the reef structure and evaluation of the geogenic and biogenic structure of the restored oyster reefs.
- \rightarrow To be addressed within the context of scientific monitoring (RESTORE SM).

Formal aspects:

- Preparation of guidelines for the construction of oyster reefs as part of management measures or environmental compensations for the reintroduction of European oyster.
- → To be addressed within the context of the main project (RESTORE M) and the scientific monitoring (RESTORE SM).
- Development of indicators for the monitoring of success following the implementation of management measures or environmental compensation for the reintroduction of European oyster.
- Definition of the required scale of the reintroduction of the European oyster.

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Acronyms and abbreviations

Acronym/ Abbreviation	Description
AWI	Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Re- search
BfN	Federal Agency for Nature Conservation
BMU	Federal Ministry for the Environment, Nature Conservation and Nuclear Safety
BMUV	Federal Ministry for the Environment, Nature Conservation, Nuclear Safety and Consumer Protection
BNatSchG	Federal Nature Conservation Act
BPBV	Federal programme for biological diversity
BRG	Borkum Reef Ground
BSH	Federal Maritime and Hydrographic Agency of Germany
BUND	Friends of the Earth Germany
EU	European Union
EEZ	Exclusive Economic Zone
ENE	East-North-East
HD	Habitats Directive
HDHT	Habitats Directive habitat types
HRR	High-relief-reef
GDWS	Federal Waterways and Shipping Administration
GIS	Geographic Information System
IFREMER	Institut Français de Recherche pour l'Exploitation de la Mer
IUCN	International Union for the Conservation of Nature
KGS	Species-rich gravel, coarse sand and shell areas
MELUND	Schleswig-Holstein Ministry of Energy Transition, Agriculture, Environ- ment, Nature and Digitization
MSFD	Marine Strategy Framework Directive
NABU	Nature and Biodiversity Conservation Union

NCA	Nature Conservation Area
NORA	Native Oyster Restoration Alliance
OSPAR	Oslo-Paris-Convention
OWP	Offshore wind farm
PCR	Polymerase chain reaction
RESTORE M	RESTORE main project
RESTORE SM	RESTORE scientific monitoring
RK	Research cutter
RV	Research Vessel
SeeAnlV	Offshore Installations Ordinance
SL	Shell length
T&D Projects	Testing and Development projects
UW	Underwater
WP	Work package
WSV	Federal Waterways and Shipping Administration

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