Guidelines for the Establishment of Blue Mussel Beds and Follow-up Monitoring

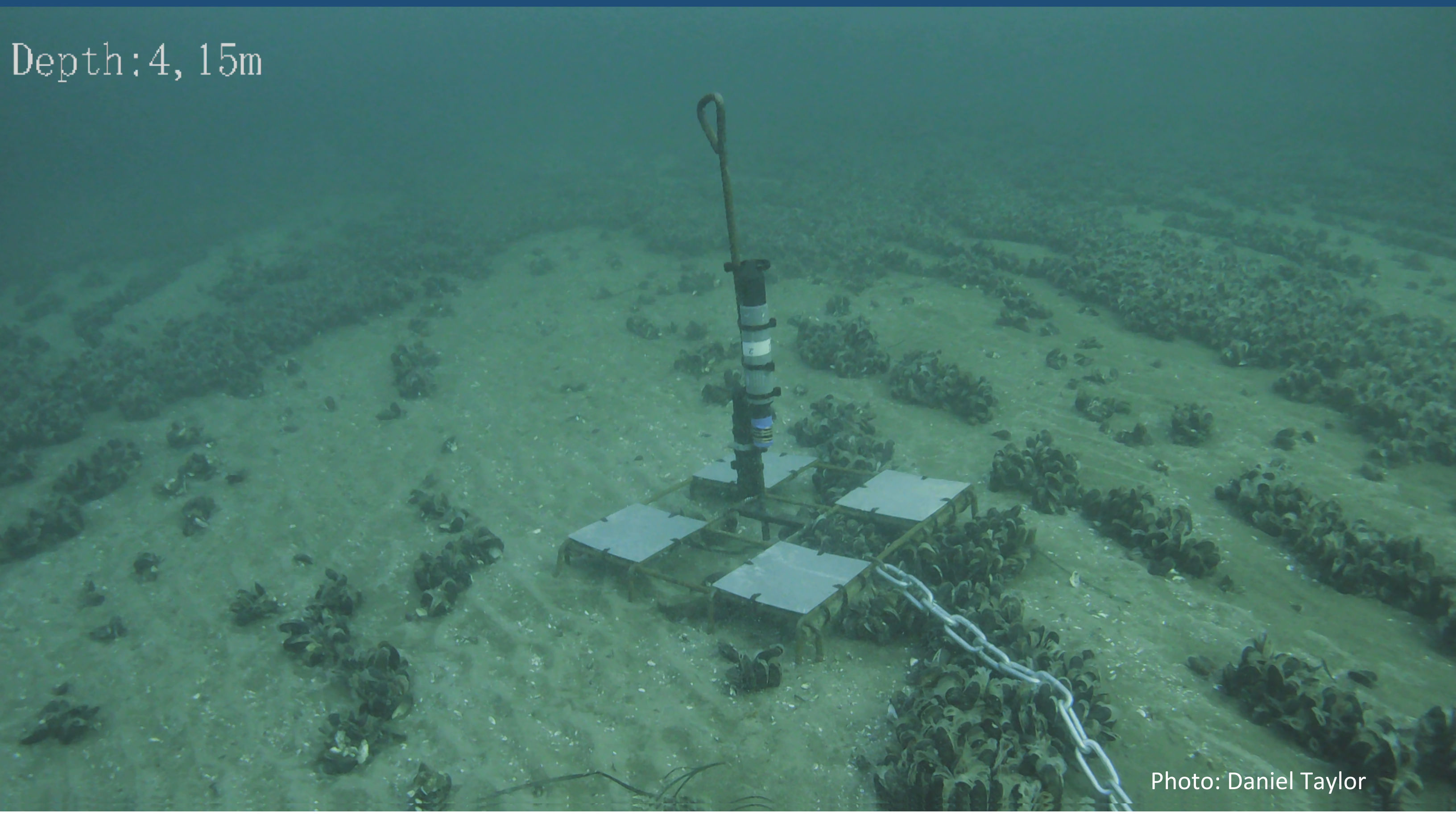

Photo: Daniel Taylor

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Title: Guidelines for the Establishment of Blue Mussel Beds and Follow-up Monitoring

Authors: Pernille Nielsen, Daniel Taylor, Jens Kjerulf Petersen, Pedro Freitas, Timi L. Banke, Louise C. Flensborg, Isabelle Johansson, Camille Saurel

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Quality control: Peter A.U. Stæhr, Aarhus University

Torben Bramming Jørgensen, Limfjord Council, Aalborg Municipality

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Summary: This report provides an overview of the practical implementation of habitat restoration of blue mussel beds in Danish coastal waters. This is a process that involves pre-planning, project design and defining objectives, preparation for the establishment of blue mussel beds, execution, and monitoring both before and after establishment. This report provides an overview of relevant rules, permits and authorities on the restoration of biogenic reefs, as well as specific recommendations on seed collection, design and establishment of blue mussel beds, and monitoring of reef development.

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Introduction

Biogenic reefs confer a wide range of ecosystem services to the surrounding environment and are considered important habitats, described by the EU Habitats Directive and specifically designated in the EU Biodiversity Strategy. Biogenic reefs can be formed by several different organisms, with relevant Danish species including blue mussels, horse mussels, and European flat oysters. Since the beginning of the 20th century, the distribution of biogenic reefs has reduced, attributed to poor environmental conditions at the seabed, such as hypoxia and high organic sediment content, fishing, and other exploitation activities of marine resources. Therefore, in some areas, there may be a need for restoration of lost biogenic habitats.

The guidelines presented in this document cover the establishment of biogenic reefs in the form of blue mussel (*Mytilus edulis*) beds. Establishing reefs of other bivalve species, such as horse mussels or European flat oysters, require their own specific methods, some like those presented here, others divergent and deserve separate guidance. This guide is structured as a step-by-step process covering project planning, methods to collect mussel spat, to relay beds, and follow-up monitoring programmes. It is an accompanying document to the 'Biogenic reefs – site selection guideline for blue mussel bed establishment' prepared by the Center for Marine Nature Restoration, which reviews the criteria that can be used to select suitable areas for the establishment of blue mussel beds. In addition to selecting a suitable area, the restoration project should identify a clear purpose and prioritisation of objectives. The purpose can, for example, be the establishment of biogenic habitat, improved nutrient cycling, water quality, or sediment stabilisation.

Once a suitable area has been identified, implementation can proceed, including a project plan and design, the acquisition of blue mussel seed for relaying and then a specific relay strategy of the mussels adapted to the selected area. Technical and practical measures for establishing restoration beds or biogenic habitat largely follow the same procedures used in the establishment of bottom culture plots. Bottom culture plots consist of mussel spat relayed for growth in suitable areas and subsequent harvest by fishing the plots. While culture plots are designed for maximum biomass yield with minimal fishing pressure and the delivery of high-quality mussels for the market, the configuration of restored or newly established beds as nature improvement can vary depending on the purpose of establishing the bed and local conditions. Therefore, an established mussel bed will suit a specific purpose in the selected area, affecting both the practical implementation and the subsequent monitoring, which are adapted and designed for the specific project. Blue mussel beds can be established for several purposes. For historical habitat restoration, it is generally a requirement for reasonable documentation of mussel beds having previously existed in the selected area, (Concepts in relation to marine nature restoration, Petersen et al. 2024). However, it can be difficult to document where precisely mussel beds have historically existed because historically mapping of blue mussel beds has often been inadequate in inner Danish waters, but also due to the naturally dynamic distribution of mussel beds over time. Alternatively, mussel beds can be relayed as part of nature-based solutions, as part of a marine mitigation measure, or in relation to nature-inclusive design in connection with offshore constructions (Petersen et al. 2024).

Definition of Blue Mussel Reefs

In relation to the Habitat Directive's habitat type "biogenic reefs," there is a Danish definition of blue mussel beds. The definition considers that blue mussel beds are formed by living blue mussels that develop dynamically over time and are not stable as stone reefs. Additionally, the definition considers that blue mussel beds in inner Danish waters (excluding the Wadden Sea) are characterised by conditions with low tidal influence, which can affect the development and stability of the beds.

The Danish definition is as follows: Biogenic reefs of blue mussels are continuous areas of at least 2500 m^2 with an average coverage of blue mussels of at least 30% and the presence of at least three cohorts of blue mussels (Dahl & Petersen 2018).

General Project Planning for the Establishment of Blue Mussel Beds

The establishment of blue mussel beds involves four different overarching phases (see figure 1). Phase 1 concerns feasibility and pre-planning. Phase 2 covers the design of the project and the definition of general objectives. Phase 3 includes the practical preparations before the establishment of the mussel bed, while Phase 4 implements the establishment of the mussel bed. Additionally, there will be a follow-up phase (Phase 5) that involves monitoring the effects of mussel bed establishment and evolution of the bed. Each of the phases will include different sub-elements, which are discussed in the following sections.

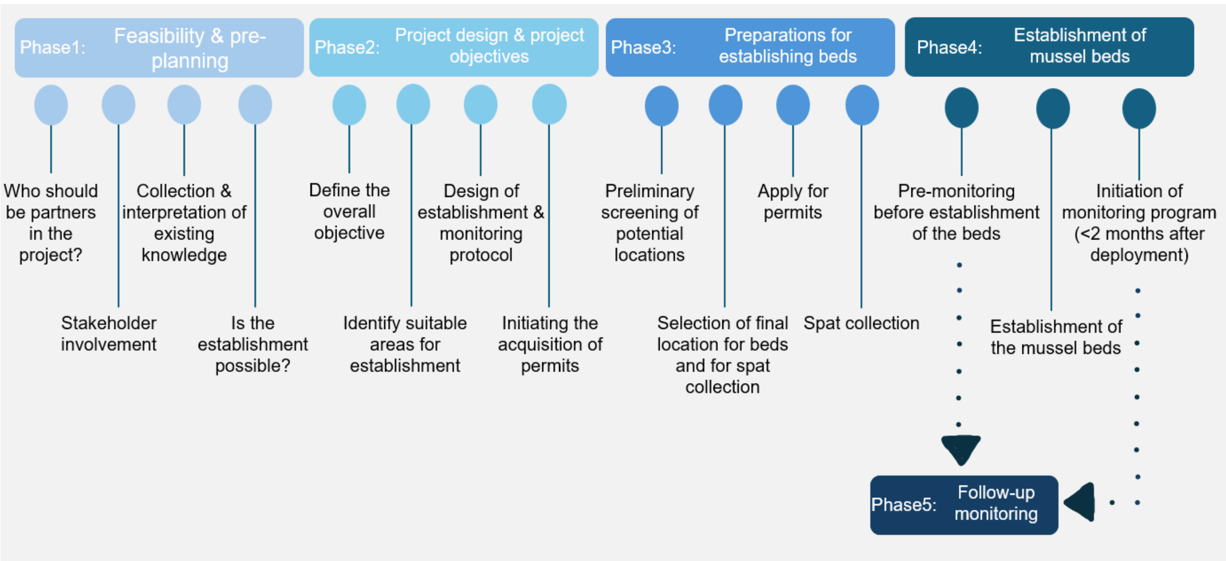

Figure 1. *Generalised but typical timeline divided into phases for the establishment of blue mussel beds in Danish waters.*

Phase 1 - Feasibility and Pre-Planning

To carry out a project involving the establishment of blue mussel beds, it is important that the project is properly scoped both in terms of the financial resources available and in terms of including the relevant partners in the project. This ensures a solid foundation for the successful completion of the project, through appropriate definition and achievement of the project's specific objectives by the end of the project.

Public Participation

The participation of stakeholders can significantly impact the implementation of projects focused on nature restoration and nature-improvement activities. Local residents, associations, and fishing communities can contribute in various ways, such as providing useful knowledge about historical presence of beds, suitable locations, designs and purposes, and bringing valuable skills into the project. Stakeholder participation often promotes implementation and ensures broad support for the establishment of blue mussel beds and subsequent protection of the beds. Typical stakeholders include local authorities, commercial ports, the fishing industry, recreational fishers, sport divers, leisure sailors, and local nature and outdoor organisations. Stakeholder participation can occur through active participation in restoration actions, public meetings, or service in an advisory group consisting of stakeholders who have the greatest interest in following and contributing to the project through its entire duration.

Communication

It is also important to inform the public about the project to raise awareness of its background and purpose, as well as the project's progress. Marine nature restoration takes place at sea and underwater, which makes it not immediately visible and challenging to present. Communication activities can include public meetings where relevant knowledge can be gathered, and discussion about the project's implementation can take place, as well as consultation with authorities about local management plans. In addition to physical meetings, various platforms can be used for knowledge sharing and project dissemination:

- Website with general information about the project, pictures, news, publication of meeting minutes, etc.
- Social media platforms
- Videos on YouTube
- Features in local media, such as focus pieces and interviews
- Physical newsletters for local distribution

Communication is important before, during, and at the conclusion of the project and should therefore be ongoing to keep the public updated on the project's progress and maintain engagement.

Gathering Existing Knowledge

The central elements in selecting suitable areas include a historical analysis of blue mussel bed presence, an analysis of environmental conditions, an analysis of current sea use including potential conflicts with other uses, and nature and marine area management.

Historical Analysis

Documentation of historical blue mussel presence in each area is important if the project is to be categorised as nature restoration. It is also important to characterize existing environmental conditions for areas where blue mussels have historically been found before the final selection of the site, as environmental conditions may have changed. Even if the precise historical locations of blue mussel beds in the area are not known or the area is no longer suitable, it may still be possible to establish blue mussel beds as nature-improving measures in the vicinity of historical positions.

Natura 2000 Areas

In Natura 2000 areas, it will be a sufficient argument if the habitat type "biogenic reefs" is designated, and the reefs are designated as blue mussel reefs. For some Natura 2000 areas, documentation of blue mussel presence dates back several years, and not all locations have been verified according to the definition of “biogenic reefs” of blue mussels. If biogenic reefs are not a designated habitat type, it will often be necessary in relation to the permit procedure to be able to document that the establishment of blue mussel beds will not compromise other habitat types in the area and demonstrate that environmental conditions will promote the natural occurrence of blue mussel beds in the area. Environmental assessments in accordance with Natura 2000 areas may require a proper impact assessment of the establishment in relation to the designation of the area to be carried out. The geographical location of Natura 2000 areas and their designation basis according to both the habitat and bird protection directives can be found on the Danish Environmental Protection Agency's website: https://mst.dk/natur-vand/natur/natura-2000/.

It is also important that the establishment of blue mussel beds does not result in the establishment of other species that are not native to the area, and there should be documentation indicating the need for the restoration of blue mussel beds in the area.

Environmental Conditions

Criteria for suitable environmental conditions for the establishment of blue mussel beds include water depth, water salinity, seabed conditions, and oxygen levels, and are further described in the ‘Biogenic reefs – site selection guideline for blue mussel bed establishment’ (Nielsen et al. 2024). Additionally, the slope of the seabed may be significant to suitability, where steep slopes should preferably be avoided to promote bed stability and integrity.

Sea Use

Establishing blue mussel beds will, under most circumstances, not conflict with other uses of the sea, but there will nevertheless be a wide range of activities that can affect the choice of suitable locations. Such uses include fishing with bottom trawling gear, dumping of dredged material, mineral extraction, placement of submarine cables, or areas designated for port expansion. Despite plans for sea use, it cannot be ruled out that multiple activities, including the establishment of blue mussel beds, may occur simultaneously.

Denmark's Marine Spatial Plan (https://havplan.dk/da/om-havplanen/data-og-gis) provides an overview of both planned usage zones and the background data. There is no unified portal with all relevant data, and the marine map of Denmark is not always updated depending on the data sources. Other relevant sources for maritime territory use include the Coastal Atlas (https://kyst.dk/klimatilpasning/vaerktoejer/kystatlas), environmental GIS (https://mst.dk/erhverv/tilskud-miljoeviden-og-data/data-og-databaser/miljoegis-data-om-natur-og-miljoe-paa-webkort), or the Environmental Data portal (https://miljoedata.miljorportal.dk), while areas of interest for various recreational activities are mapped in Kaae et al. (2018).

Cultural Resources

Special conservation considerations apply to seabed cultural resources, where there may be potential effects to the marine cultural heritage, although it is considered unlikely in the establishment of mussel beds. This will be clarified in consultation with the authorities' processing of the application. More information can be found on the website of the Danish Agency for Culture and Palaces (https://slks.dk/fortidsminder/marin).

Phase 2 - Project Design and Establishment of Overall Purpose

In projects that involve the establishment of blue mussel beds, it is important to define the specific purpose of the establishment, such as whether it involves nature restoration or nature improvement measures, as the purpose is relevant for management considerations. However, the fundamental purpose of establishing permanent blue mussel beds will always be to create important habitats for a key species characterised as an ecosystem engineer, an organism that provides habitat and ecosystem functions supporting other organisms and processes. Blue mussel beds confer a wide range of ecosystem services that may be specific to the given area. Since the effect or extent of the ecosystem services provided will depend to some extent on the size, design, and location of the beds as well as local environmental conditions, it is important that the project has defined and established clear and specific objectives. The purposes for establishing blue mussel beds can, for example, include:

- Re-establishing biogenic reefs in Natura 2000 areas to achieve a favourable conservation status for the habitat type.
- Creating habitats and foraging grounds for fish and juvenile fish such as eels.
- Increasing the food source for mussel-eating (protected) bird species.
- Strengthening the marine food web in general and thus increasing the robustness of the local ecosystem.
- Improving water quality by increasing clarity of the water above and around the mussel beds, for example, in relation to benthic vegetation.
- Binding nutrients during the productive period.
- Strengthening blue corridors for the spread of organisms between areas.

An adjustment of purpose during the planning phase may be necessary based on the information and data collected about the site and any previous presence of blue mussel beds in the area. However, by the end of phases 1 and 2 (see figure 1), the final objective(s) must be established, as this is crucial for the effective implementation of phases 3 and 4.

Phase 3 - Preparations for Establishing the Mussel Bed

After selecting a general area, field pre-surveys must be conducted to help identify the specific project area for establishing the mussel bed and thereby the area to specify in the application procedure. This area needs a precise delimitation with specific GPS coordinates. Field pre-surveys can also document existing animals and vegetation in the area before establishing the mussel bed. Epifaunal animals and vegetation, including any current occurrences of blue mussels, can be documented with various equipment, such as video sleds or bottom samples. Sampling in connection with the pre-surveys must be conducted so that the effects of the established blue mussel beds can be assessed. This means that a nearby control area with conditions similar to the establishment areas must be included, and methods must be used that will also be employed in subsequent sampling after establishment to document the effects of the established mussel beds. The scope of a pre-survey programme will vary depending on specific purposes for the establishment, the size of the established beds, regulatory requirements (e.g., when placed in Natura 2000 areas), and the project's objectives. A more detailed review of monitoring programmes and methods is outlined in the monitoring guidelines.

Obtaining Permits

Two types of permits are necessary to establish blue mussel beds: one for the production or collection of seeding material if the project aims to produce or cultivate mussel larvae or spat, and another permit for the establishment of the bed(s). Unless seeds are produced in a hatchery (see section below) or seed is purchased from a local mussel farmer, a permit is required to either collect mussel larvae in the water column or harvest mussels from either wild populations or culture plots. This requires a licence to fish mussels, which only commercial fishing vessels can obtain. The Danish Fisheries Agency is the main authority responsible for processing both types of permits, but presently there is uncertainty in this regard (see below).

Permit for Suspended Seed Collection

If the project involves production of blue mussels, a permit for a mussel farm is required if not already obtained. Permits for the establishment of cultivation facilities are administered under the Fisheries Act and the Fisheries Agency is the regulating agency. Specifically, the administration follows regulation on the cultivation of mussels and oysters in the water column[1]. In the application process, the Fisheries Agency conducts consultations with several parties defined by the Agency, including relevant public authorities, fishing associations, and environmental organisations. There are certain formal barriers to the establishment of mussel farms, including protected areas that ensure that fish passage from rivers to the sea. Protected areas can be found here: https://fiskeristyrelsen.dk/lyst-og-fritidsfiskeri/fredningsbaelter. These areas often do not have practical significance for a permit because they usually cover only shallower water depths than those relevant for mussel cultivation. It is possible to obtain a cultivation permit in a Natura 2000 area, but this will require an impact assessment. Restrictions related to location in relation to navigation channels, dumping grounds, other nature and environmental considerations, etc., will be part of the specific case processing and emerge through consultations. Acknowledging and avoiding these potential conflict areas in the application will facilitate the process.

Currently, as of mid-2024, there is a moratorium on applications for new permits for cultivation. The moratorium has been in effect since June 2022. The Ministry of Food, Agriculture, and Fisheries and the Fisheries Agency are currently working on drafting new regulations for permitting of blue mussel cultivation in the water column, which are expected to come into effect when the moratorium is lifted. Therefore, in 2024, it is not possible to establish blue mussel beds based on suspended mussel farms unless they were established before the moratorium. If the mussel farm is for scientific purposes (non-commercial facilities), applications can be made regardless of the moratorium.

[1] https://www.retsinformation.dk/eli/lta/2021/1456

Permit for Establishing Beds

In Denmark, so fare the establishment of mussel beds has mainly been in the form of culture plots with the purpose of subsequently fishing the beds once the mussels have reached a size suitable for consumption. For these types of beds, the Fisheries Agency is the primary regulatory authority. Some blue mussel beds that have been established as part of nature restoration projects, such as the 'Sund Vejle Fjord', have also received cultivation permits through applications to the Fisheries Agency. Culture plots, that is, plots where seeding material comes from suspended mussel farms and is relayed with the intention of subsequent fishing when the mussels have reached consumption size, are subject to the moratorium on applications as mentioned above.

However, it is unclear whether the establishment of blue mussel beds that are not to be fished and considered permanent should be managed by the Fisheries Agency. It is not clear who is the primary authority in this regard. The relay of hard substrate such as blue mussel shells can also be considered a 'establishment of a facility' under Section 16a of the Coastal Protection Act. According to the Danish Coastal Authority, administratively, shells do not differ from stones (typically as attachment sites for macroalgae) or other hard surfaces intended to form the basis for reef habitats. Therefore, the Danish Coastal Authority should handle an application, or at least be consulted. Furthermore, since live mussels also contain nutrients such as nitrogen and phosphorus, there could potentially be objections to the establishment of mussel beds in areas that do not meet the requirements for good ecological status according to the EU Water Framework Directive, because the relay would import nutrients into the area unless the mussels were cultivated in the same water body. This would need to be assessed by the Environmental Protection Agency, but likely as part of the consultation process. Overall, there is currently some uncertainty and significant challenges in obtaining permits to collect mussel larvae in the water column and establish mussel beds in the relevant coastal areas.

In cases where it is deemed necessary to introduce blue mussels from other areas, international regulations and biosafety measures regarding animal husbandry, genetic diversity, and veterinary hygiene should be followed (e.g., Sas et al., 2019), to minimise the risk of spreading pathogens between different water bodies. The introduction of new pathogens into a water body can have dire ecological consequences and should be avoided based on the precautionary principle until potential pathogen risk assessments and appropriate screening techniques are developed.

Another aspect that can be considered in preparation prior to implementation is how established blue mussel beds should be protected against impacts from human activities that could jeopardize the persistence of the beds. Such typical activities include fishing with bottom contact gear, dumping, extraction of raw materials, installation of wastewater outfalls, and infrastructure development. Mapping such activities should be part of the mapping of suitable areas (see Nielsen et al. 2024) but can also be included as proposals for conservation measures once the beds are established. Protecting mussel beds after their establishment may potentially require an extensive regulatory process if the area is not already protected.

Phase 4 - Establishing the Mussel Bed

The establishment of mussel beds occurs when the mussels are relayed on the sea floor. The specific procedure for relay will depend on the type of mussels being used (see section below), but there are some recommended guidelines:

- The mussels should be relayed on the same day they are harvested or fished.
- Relaying should not be done during periods with high water temperature or in the middle of summer, as this often leads to increased mortality.
- Mussels should be relayed in a single layer and not in piles. Mussels relayed in a single layer will subsequently self-organise into a heterogeneous distribution, while mussels relayed in piles/thick layers can cause mussels underneath to die, thereby destabilising the overlying mussels.
- Relay should not affect a selected control area.

About a month after the establishment of the mussel bed, a first monitoring effort is recommended to document the development of the bed over time. This will constitute the ‘baseline’ values and are fundamental to document the development of the bed. If monitoring is carried out immediately after the relay, the mussels will not have had time to ‘self-organise’ and thus develop their typical heterogeneous distribution. Subsequently, follow-up monitoring should be conducted at regular intervals, both to document the development of the mussel bed over time and to document any other project objectives in terms of desired ecosystem services (see monitoring section for details). The following sections will describe the different sub-phases of establishing blue mussel beds.

Different Methods for Collecting Blue Mussels for Bed Establishment

Understanding the life cycle of the blue mussel (Figure 2) is important for successful seed collection. In Denmark, blue mussels typically spawn eggs and sperm in the spring, after which the larval stage lasts two to four weeks before they are ready to settle on the seabed or other hard substrate. This means that mussel larvae for relay on the seabed are typically available from September to December of the same year after months of growth following settlement. According to the Danish definition of a biogenic reef consisting of blue mussels, there should be three cohorts (age groups) of mussels, potentially requiring mussels to be relayed over three years. This is done with the assumption that there will be no natural recruitment to the mussel bed in the following years. However, natural recruitment is expected to occur, which will eventually lead the established mussel bed to develop into a biogenic reef by definition. Blue mussels reach sexual maturity within about a year and, therefore are capable of reproducing, while the relay of mussel beds can facilitate the recruitment of mussels in the area (Commito et al. 2014).

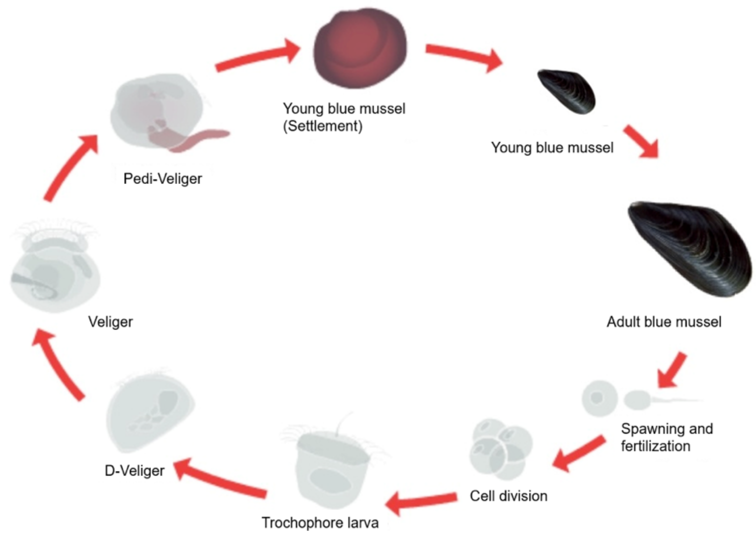

Figure 2. *Blue mussel life stages from the spawning of eggs and sperm by adult mussels in spring, through the various larval stages lasting 2-4 weeks, until the larvae are ready to settle. © DTU Aqua.*

There are two main methods for obtaining blue mussels for the establishment of mussel beds: 1) collection of mussels from wild stocks or natural recruitment or 2) hatchery-produced mussel spat. Large-scale hatchery production of blue mussels is very costly compared to collecting spat from wild stocks and is currently not practiced in Denmark. The collection of blue mussels from natural stocks can be done by collecting mussel larvae in the water column or from mussels that have settled on the seabed. Although it is also possible to collect blue mussels from rocks, pylons, or other hard substrates, this is relatively cumbersome, time consuming and often does not provide sufficient biomass for the establishment of mussel beds. However, it may be possible to obtain enough blue mussels from larger offshore infrastructures (wind turbines, etc.), but this would require special agreements with the owners of the specific infrastructure to gain access to collection. Another possibility to obtain mussel spat from wild stocks could potentially be from fish farms, where mussel larvae settle on fish cages, and the mussel spat is removed to ensure flow through the cages. Coordination with fish farmers and the timing of cage cleaning is necessary. Finally, it may be possible to purchase mussels from local mussel fishers or farmers. An overview of the advantages and disadvantages of each method are presented in Table 1.

Regardless of the method, it is advisable to consult mussel farmers or sea garden associations, as they can contribute knowledge and experience to the project. The following sections will describe the different methods for collecting blue mussel spat. It is recommended that mussels are collected from the local area and not imported from other water bodies that do not have a natural connection, such as importing blue mussels from the Limfjorden to the fjords along the east coast of Jutland. This will protect the genetic composition and diversity of local populations and thus take advantage of their adaptation to local conditions.

Hatchery-produced Blue Mussel Seed

In the future, mussel larvae from hatcheries may be an alternative to seed from wild populations. However, production costs remain very high (430 euros/kg Kamermans et al. 2013), even though a low-tech hatchery protocol, where costs can be reduced, is described by Saurel et al. (2022). The hatchery production of blue mussel larvae could advantageously be used in situations where local populations are particularly small and where larval production is too low to support suspended seed collection. Therefore, the method can help to maintain local genetic diversity with high biosecurity, as hatchery conditions significantly reduce the risk of transferring potential pathogens during deployment.

Fished Blue Mussel Seed

Generally, the recommendation is not to use fished blue mussels for nature projects, however some coastal Danish water bodies experience annual recurring and prolonged oxygen depletion events, which can result in significant mussel mortality. In such cases, fished blue mussels seed can be used in habitat restoration projects, if mussel seed beds are thinned and fished mussel seeds are deployed in another area, which can benefit both the donor and deployment areas (Maar et al., 2021). Fishing for blue mussel seeds from wild populations requires coordination with at least one mussel fisher holding a valid licence or with the fishing industry, for example, through the Mussel Industry Association.

***Table 1**. Overview of the advantages and disadvantages of the different methods of collecting blue mussels for relay.*

	Hatchery-produced	Fished	Collection in the water column	Other methods
Description	Purchased from hatchery (currently not available)	Purchased by mussel fishers who fish the mussel seed locally	Local seed can be purchased by mussel farmers or by the project that establishes seed collectors. Alternatively, buy local seed from farmers.	Collection from rocks, infrastructure, or other hard substrates
Costs (kr / ton)	3,200,000 (Kamermans et al. 2013)	900-1000 (personal communication 2024)	1,800-3,000 (personal communication 2024). Does not include farm establishment costs.	Unknown
Benefits	Local mussels can be used for seed production. High biosecurity and low risk of pathogen transmission.	Recommended only if the mussels are harvested from an area affected by annual hypoxia events.	The seeds are of the same size (single cohort) and easily accessible in Denmark.	Potential beneficial use of a nuisance fouling community.
Disadvantages	The costs are very high.	Potential risk of introducing pathogens or impacts to genetic diversity.	Potential risk of introducing pathogens or impacts to genetic diversity if the seeds are not produced locally. Possibly requiring permission to establish lines for seed collection.	Potential difficulty and time-consuming process with the risk of not being able to collect sufficient biomass.

Mussel Seed Collected in the Water Column

Collecting mussel seeds in the water column requires the installation of spat collectors (Figure 3) or the purchase of mussel seeds from a local mussel farmer. Depending on the need for mussel seed for deployment, a few lines or a larger facility with multiple lines can be established. The collection of mussel seeds is simple to scale within the footprint of a mussel farm.

The area selected for establishing lines must first be marked with clear yellow navigation markers in the form of either end- or corner markers (respectively, for establishing a single line or multiple lines). A facility consists of a series of horizontal mainlines, consisting of 14 or 16 mm thick, hard-twisted ropes typically 100-200 m long. Two anchors, one at each end, must be used for each mainline (Figure 4). The anchors can be screw-anchors screwed into the sea floor or various types of anchors. The key is that the anchors do not move, thereby slackening the lines, leading to entanglement or drifting away, posing a risk to maritime traffic and the integrity of the mussels. The mainlines are kept afloat by buoys of 9 or 17 litres in a neutral colour to minimise the visual impact. When setting out the spat collectors (see below), it is recommended to use approximately 40-50 buoys per line if the line is 100 m long. As mussels grow and weigh down the lines, additional buoys are continuously added. In addition to buoys, concrete blocks are also used to stabilise the lines in the water column. The blocks are made of concrete poured into buckets and weigh 25-35 kg each. Approximately 20 concrete blocks are used per 100m line. For securing both buoys and concrete blocks, it is recommended to use 6 or 8 mm rope. The installation of mainlines can be done year-round, but it is recommended to establish them before spring to be prepared for deployment of spat collectors.

Spat collectors are typically 5 cm wide nylon-woven webbing that hangs from the mainlines. it is recommended to hang the spat collectors in loops with a 40 cm between loops and 60 cm to the next tie-up on the mainline (Figure 4) and hanging them from the surface and a few metres down into the water, depending on depth of the water, but always at least 1 m above the bottom. In April-May, when the water temperature is approximately 10°C, the wild mussel populations spawn, and spat collectors are deployed during this period, for mussel larvae to settle in May-June. Throughout the summer, and until the mussels are harvested, additional buoys are continuously added to maintain buoyancy.

It is recommended to use a boat capable of lifting the mainlines out of the water with, for example, a crane equipped with a winch or line hauler. The boat should be able to move along the lines, providing good working conditions for adding spat collectors, concrete blocks, and buoys to the mainline, or when mussels are harvested (Figure 3).

***Figure 3**. Typical configuration of a longline mussel farm. Top: Aerial view of the mussel farm. Middle: Spat collectors with mussels underwater. Bottom: Harvesting mussels with a boat. Photo by Daniel Taylor.*

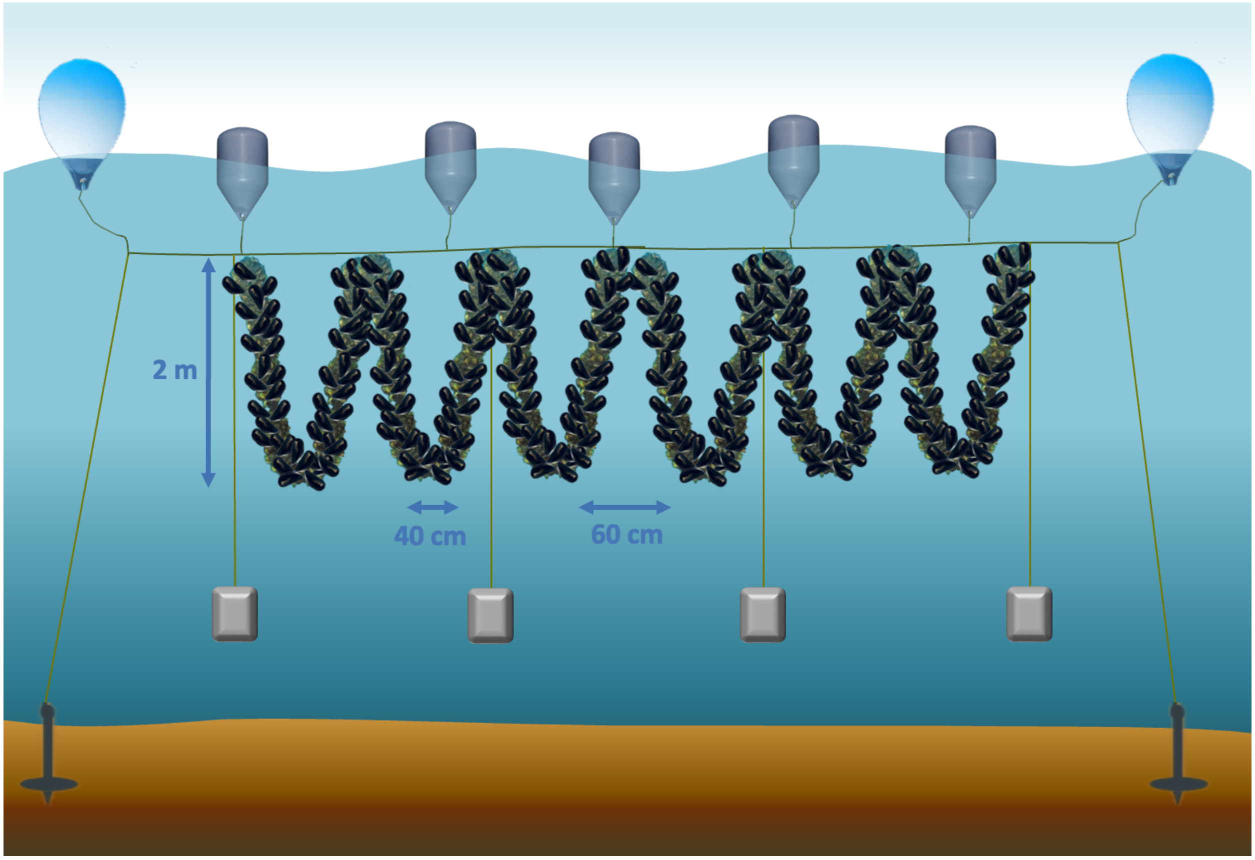

Figure 4. *Schematic drawing of the setup of a longline to collect mussel larvae in the water column. © DTU Aqua*

Design and Establishment of Blue Mussel Beds

The design of mussel beds influences the survival and living conditions for mussels in aggregate, for example, by minimising competition for space and food within the mussel bed and between beds, minimising predation impacts, and supporting recruitment. The size of the bed, size of the mussels deployed, mussel densities, deployment patterns, and potentially additional attachment materials are crucial elements to consider for successful establishment and continuity.

Site-specific conditions play a significant role in the establishment of mussel beds and their viable self-sustenance (Fraschetti et al., 2021). Therefore, before and during the establishment of blue mussel beds, it is important to identify potential risk factors affecting mussel survival, such as sediment smothering, predators, or wave exposure (de Paoli et al., 2015). The origin of the mussels (water column, bottom, or hatchery), their age, size, shell thickness, and byssus formation will influence the mussels' ability to endure adverse factors such as wave exposure, predators, and the relaying process. Additionally, ecosystem-scale factors such as seston composition and the presence of wild mussel populations will also affect the evolution of the new bed and future recruitment. Refer to 'Biogenic reefs – site selection guideline for blue mussel bed establishment' (Nielsen et al., 2023) for more details on the criteria that should be considered.

The Shape and Distribution of Mussel Beds

The orientation, distribution, and densities of the relayed mussel beds will depend on local biological and physical conditions (Commito et al., 2016; van de Koppel et al., 2008), but also depends on the density of the mussels (Bertolini et al., 2017). For example, blue mussel beds will self-organise into different patterns on different scales, creating a three-dimensional structure across the sea floor. Pattern formation can regulate and respond to resilience, recruitment, growth, and survival of the beds (e.g., Svane and Ompi, 1993; Commito and Rusignuoo, 2000; Crawford et al., 2006). Therefore, the spatial relay pattern and the density of mussels should support the probability that established mussel beds are long-lasting and therefore contribute to the success of restoration and nature-enhancing activities (Bertolini et al., 2019).

Blue mussel beds have a varying spatial and often clumped distribution as mussels try to optimise their position in relation to food availability, predation pressure, and attachment to other mussels or hard materials to reduce the risk of detachment from wave exposure or currents (de Jager et al., 2011; van der Koppel et al., 2012). Beds are often structured as clumps arranged in long bands on large areas of bare sediment (Figure 5) that supports the longevity of beds (de Paoli et al., 2017). Relay density and relay pattern are a balance between having enough individuals to form clumps and bands, but without internal competition for space and food among the mussels. In addition to density, the size of blue mussels also affects their ability to clump together, since smaller blue mussels tend to aggregate more and benefit from refuge formation that reduces predation.

Figure 5*. A relayed blue mussel bed, where the mussels have self-organised into clumps with bare sediment in between. Photo by Timi L. Banke, SundVejleFjord.*

It is recommended that when relaying blue mussels, consideration is given to their ability to self-organise within the blue mussel beds. Mussels should therefore be relayed in bands with 10-20 m in-between and not at too high densities (see below). Similarly, it is recommended that when multiple mussel beds are relayed within the same area, they are placed with >100 m between the beds to reduce the risk of food depletion between the blue mussel beds.

Use of Attachment Material

Habitat-forming species like blue mussels often rely on the recruitment of new mussel larvae to develop and maintain their aggregate structure, for example, in response to deterioration after storms or predation (e.g., Paoli et al., 2017; van den Bogaart et al., 2023b). Here, both mussel density, spatial distribution, and complexity of structure play a crucial role in structural formation of aggregates (Bertolini et al., 2019). Attachment substrates are deployable materials that can facilitate mussel attachment and bed structural formation but can at the same time reduce the stability of the bed, for example, by making it more sensitive to wave exposure. There are various materials that can be used as substrates for mussel beds. The most common materials are: 1) shells, 2) various biodegradable materials such as coconut nets, nets/mats, spat collectors, or 3) wooden structures. The facilitating effects of attachment substrates on the survival and longevity of mussel beds will vary and depend on local conditions. Attachment material can be a good idea but is usually not necessary and will typically be used if the initial relays have failed. Alternatively, mussel shells can be relayed as a natural substrate to promote natural recruitment, however, there is no guarantee that settlement will occur. In connection with restoration projects or nature-enhancing measures, it is recommended that if attachment material is included, only natural materials from the sea (e.g., shells) or biodegradable materials are used. If non-degradable materials are to be used, it should be considered whether they can be completely excluded or replaced with natural/biodegradable materials. If this is not possible, it will complicate the permitting process and may prolong approval. Including attachment material will increase costs and should be used in situations where the natural substrate presents limitations, such as particularly muddy sediment that does not support the formation of robust mussel beds.

Biomass Densities of Relayed Blue Mussels

The density of mussels (kg/m^2 or number/m^2) is crucial for their ability to self-aggregate and therefore also for their growth and survival (e.g., Gascoigne et al., 2005; Capelle et al., 2014; Liu et al., 2014; Bertolini et al., 2019). Targeted relaying density should balance between enough mussels for adequate self-aggregation and formation of stable structures without significant internal competition between the mussels (Bertolini et al., 2020). In Holland, on tidal flats, the density of relayed mussels typically ranges from 1 to 2.5 kg/m^2 (van den Bogaart et al., 2024). Generally, densities of 2.5-3.5 kg/m^2 are recommended in previous studies with mussel size of 1-3 cm (Spencer 2002; Dolmer et al., 2012). The latest Danish studies that involved the relay of mussels in culture plots have been carried out in connection with the KulturMus project (GUDP 2020-2024), where both wild-caught mussel spat and spat collected in the water column were relayed with mussel densities of 2.7 kg/m^2 and 5 kg/m^2. The results suggest that both wild-caught mussel spat and spat collected in the water column had good survival rates at the chosen densities, with a significant increase in harvestable density to 6.5-13 kg/m^2.

International and Danish studies consistently indicate that relayed densities of 2-3 kg/m^2 support growth and stability. A portion of relayed mussels will inevitably be lost to burial in sediments, mortality due to relay-induced stress, or inefficiently distributed such that relayed densities are less than targeted densities. Therefore, it is recommended that mussels are relayed with an initial density of around 5 kg/m^2 to counteract loss of mussel biomass immediately after the relay.

The Size of Blue Mussels for Relay

Several studies have been conducted on the establishment of mussel beds in both tidal areas and areas with permanent water cover, using both mussel spat and adult mussels, from both fished populations and suspended seed collectors (e.g., Kristensen and Lassen, 1997; Kamermans et al., 2002; Capelle et al., 2016a; Schotanus et al., 2020). Both size and age have been found to affect the survival and growth of relayed mussels (e.g., Capelle et al., 2016b, Alder et al., 2020; van den Boogart et al., 2023b). Mussel spats are 0.5-1-year-old mussels with a shell length of 1-3 cm, while adult mussels are older and typically have a shell length >4 cm. Studies have noted a greater loss of small mussels (<3 cm) immediately after relay and at higher densities compared to relay of mussels with larger sizes (Capelle et al., 2016b), which was due to handling and stress caused by fishing or harvesting, detachment from clumps, food competition during aggregation (van de Koppel et al., 2005; Okamura, 1986; Saurel et al. 2013), and increased vulnerability to predation (O'Neill et al., 1983; Murray et al., 2007; van den Bogaart et al., 2023a) affecting smaller mussels more than larger ones. Other factors also play a role; as transport time and season have temperature and air-exposure stressors and relay method may cause physical stress. On the contrary, there may be reasons to prefer smaller over larger mussels when establishing mussel beds given potential lower costs, time savings, or availability. To increase survival probability and successful establishment, it is recommended to use mussels with a shell length >3 cm for relay.

Relaying Methods

Mussels are relayed from a vessel to the project area at sea. Relaying can be done by simply pushing them in bulk overboard a barge or by other means. Special vessels with hold pump-out capabilities can be advantageously used, where mussels are pumped out from the side of the vessel or via conveyor belts, which can result in higher relay efficiency, as well as a more uniform distribution of mussels with the desired density. In Denmark, the tradition is to relay the mussels for bottom culture in strips (Figure 6) while, for example, in the United Kingdom, they are relayed in circular patterns (Saurel et al., 2013). For relay of mussels on a smaller scale, the mussels can be scooped out from containers from smaller boats, or by cutting a hole in prefilled big bags while hanging over the side of the boat. The latter method often results in mussels falling mainly to the bottom in a single pile, where divers are subsequently used to distribute the mussels in the desired density, which often results in a very uneven distribution.

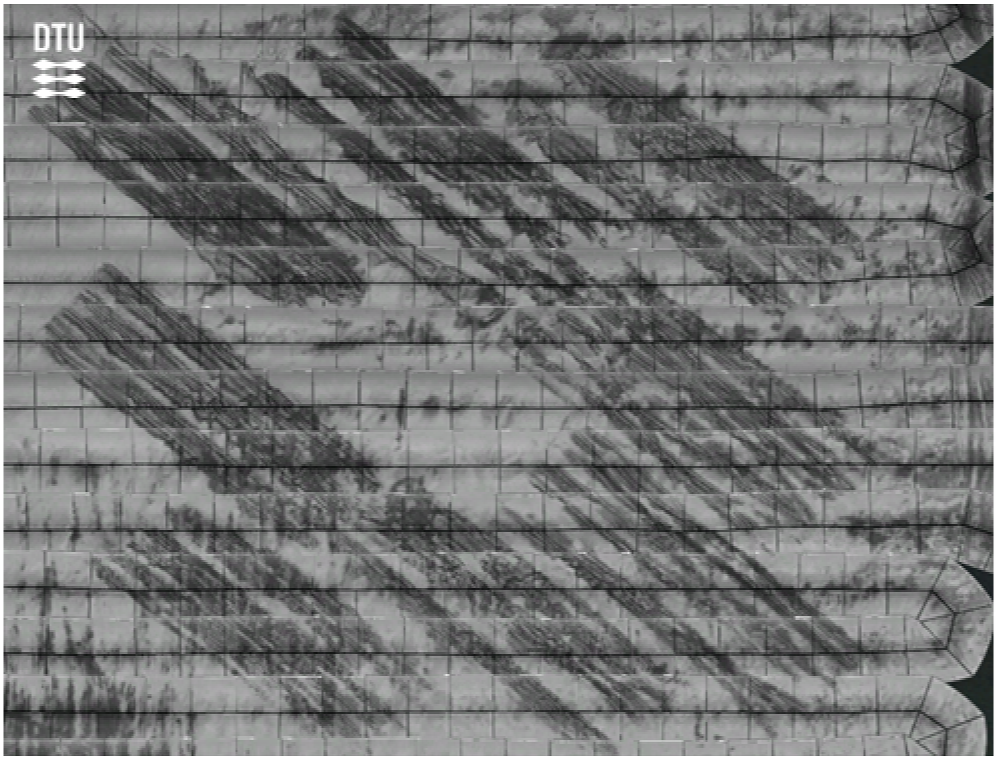

Figure 6*. Sidescan sonar image of blue mussel beds replayed with a ship. The relay process involved pumping the blue mussels from each side of the ship with spacing between the strips. © DTU Aqua*

What should a Monitoring Programme Include?

This chapter addresses pre- and post-monitoring during the establishment of the mussel beds. For monitoring related to site selection, refer to 'Biogenic reefs – site selection guideline for blue mussel bed establishment' (Nielsen et al. 2024). To document whether the ecological objectives of the establishment of the blue mussel bed have been achieved, monitoring is required. A monitoring programme should provide basic information on how the established bed develops over time and help provide experiences that can be transferred to other projects.

A monitoring programme has two basic components: pre- and post-monitoring of the area where the mussel bed is deployed and monitoring of a control area. It is recommended to start monitoring the selected project area for mussel bed establishment approximately one year before actual establishment to document, for example, hydrological and ecological conditions in the area before establishment. The monitoring data before establishment form the baseline, which is used to evaluate and quantify the environmental effects and ecosystem services provided by the established mussel bed. After relaying the mussels, it is recommended to sample in a selected control area. A control area is an area that is representative or has the same conditions (e.g., sand or mud substrate), but is not affected by the relayed mussel bed; that is, an area where conditions remain unchanged by the actions of the project. If it is not possible to conduct a baseline survey (pre-monitoring) before the establishment of the mussel bed, the comparison between the establishment area and the control area is crucial to document the effects of the relayed mussel bed and whether the project objectives are achieved.

What Type of Data?

The content of a monitoring programme should naturally reflect the specific objectives, and to assess the achievement of the desired effects. They must be translated into concrete criteria that are measurable, along with corresponding or supporting metrics that should be included in the monitoring protocol. There are different purposes for monitoring: 1) basic documentation of the establishment, 2) the temporal development/stability of the beds themselves, and 3) evaluation of whether the desired ecosystem services are achieved (Table 2). It is recommended that any restoration project involving biogenic reefs at least documents the bed establishment, but ideally also the temporal development of the mussel bed. Documenting the desired ecosystem services can often be challenging within projects with a short lifespan (2-3 years), long-term monitoring can be adopted in follow-up projects.

Table 2*. Overall division of monitoring into the three main phases (Basic documentation, Temporal development, and ecosystem services) and the basic information to be monitored during the establishment of blue mussel beds.*

<table>
<tr><th colspan="4">Basic documentation</th></tr>
<tr><th colspan="2">Area (m^2)</th><th colspan="2">Biomass density (kg/m^2, $number/m^2$)</th></tr>
<tr><td colspan="2">Measurement of the affected seabed area. Can be measured as two separate measurements: 1) the project's footprint (constructed beds including the gaps between beds). 2) The specific area covered by the beds.</td><td colspan="2">Blue mussels often have a clustered distribution. Samples should be collected across the beds (possibly along transects) to ensure that the data are sufficient to document year-to-year variation.</td></tr>
<tr><td colspan="2">Sampling frequency: The first sampling is conducted 1-3 months after the establishment of the beds and then annually. Additional sampling may be considered after disturbances, e.g., storm events.</td><td colspan="2">Sampling frequency: The first sampling should be conducted 1-3 months after the beds are established and then annually.</td></tr>
<tr><td colspan="2">Evaluation criterion: The bed area should remain stable or increase with time.</td><td colspan="2">Evaluation criterion: Stable or increasing density (kg/m^2)</td></tr>
<tr><th colspan="4">Temporal development</th></tr>
<tr><th colspan="2">Size distribution (number of age classes)</th><th colspan="2">Coverage rate (% mussel cover)</th></tr>
<tr><td colspan="2">Collecting multiple samples across the beds (possibly along transects). For each sample, the shell length of a minimum of 100 mussels is measured.</td><td colspan="2">Sidescan or multibeam sonar monitoring is preferred in shallow areas (<10 m). Alternatively, video transects across the reefs can be used.</td></tr>
<tr><td colspan="2">Sampling frequency: The first sampling is conducted approximately half a year after the establishment of the beds. Subsequently, annually in late summer/autumn.</td><td colspan="2">Sampling frequency: The coverage rate should be measured within 1-3 months after deployment and then annually, at the same time of the year.</td></tr>
<tr><td colspan="2">Evaluation criterion: Over time, presence of at least two age classes/occurrence of smaller mussels</td><td colspan="2">Evaluation criterion: Stable/increasing coverage rate. No major overall reductions.</td></tr>
<tr><th colspan="4">Ecosystem Services</th></tr>
<tr><th>Biodiversity:
Epifauna & Flora</th><th>Biodiversity:
Infauna</th><th>Light Conditions</th><th>Benthic Vegetation</th></tr>
<tr><td>Documentation of attached macroalgae and fauna and other organisms on the bed as well as mobile organisms (e.g., fish and crustaceans) that utilise the bed.</td><td>Documentation of organisms living in sediment, on and around the bed.</td><td>The filtration by blue mussels reduces the concentration of microalgae, which improves the light conditions for benthic vegetation (e.g., eelgrass and macroalgae), if light limits photosynthesis and growth.</td><td>If the purpose of establishing mussel beds is to promote conditions for benthic vegetation (e.g., eelgrass), changes in the quality (density and coverage) of eelgrass beds can be monitored.</td></tr>
</table>

How Should Data be Collected?

Once the purpose of the monitoring is determined, the next step in the process is to determine which specific methods will be used, how the surveys should be conducted, and for how long the monitoring should last. It is recommended that monitoring of the effects of establishing biogenic reefs use a BACI design (Before-After-Control-Impact). A BACI design allows one to document differences between control areas and project areas before and after establishment (Figure 7). Samples from both the control area and the project area should be taken simultaneously. It is important that the control area has similar habitat characteristics, such as sediment, water depth, salinity, and exposure, as the reef area before the reef is established. Furthermore, the control area should be located at a sufficient distance so that the effects of the reef do not directly affect the control area, for example, by increasing sedimentation or migration of mobile organisms. After the mussel bed is established, the control area is monitored concurrently with the reef area using identical methods and collection techniques.

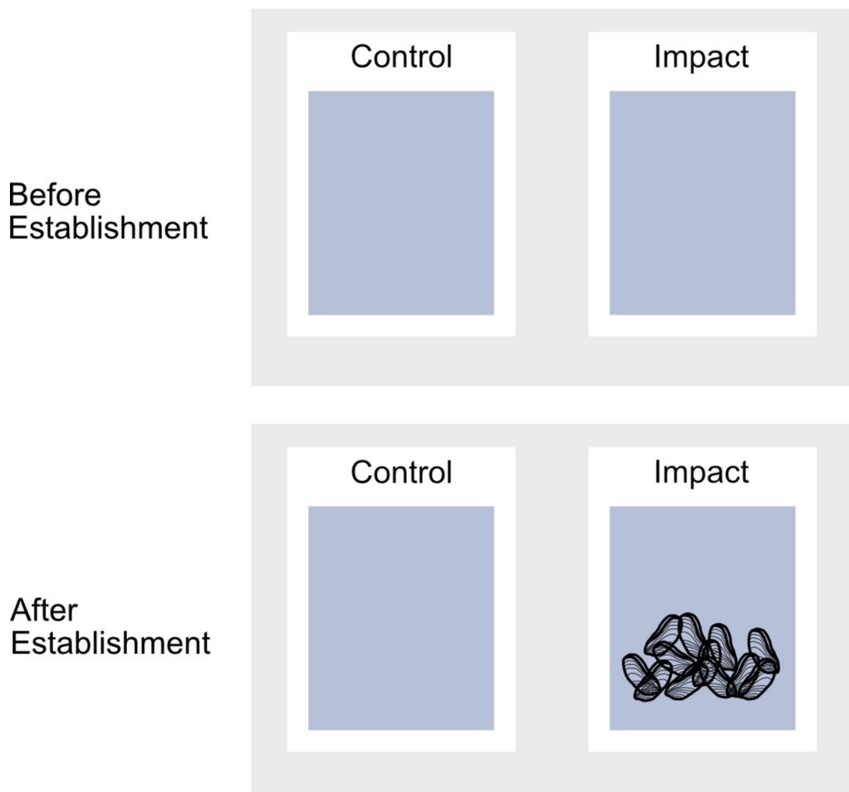

Figure 7*. Schematic overview of a Before-After-Control-Impact design (BACI), where the 'impact area' is the area where the blue mussel bed is established. © DTU Aqua*

Sampling Techniques

There are various techniques for collecting samples. The techniques can be systematic via transects or random, either as random sampling or stratified random sampling.

Random Sampling: To eliminate bias when collecting samples, the samples must be independent of each other. One method to achieve independent sampling is to divide the area where samples are to be taken into smaller sub-areas. Each sub-area is assigned a number, and then numbers are selected, for example, using a random number generator or otherwise selected randomly. Samples are then collected only in the sub-areas chosen by the number generator (Figure 8A). While random sampling eliminates the sampler's bias, it may inadvertently introduce other forms of bias, such as spatial orientation.

Stratified Random Sampling: This method is often used if the examined area is not homogeneous and can be divided into zones, for example, by different sediment conditions or differences in water depth. The area is divided into zones, and within each zone, random sampling stations are selected as in random sampling (Figure 8B).

Systematic Sampling: This method is often used in environmental gradients (e.g., distance to another habitat type such as eelgrass beds or stone reefs) or to measure changes in species composition. A transect is placed along the gradient and squares are placed at regular intervals (Figure 8C), or broad transects can be used, where occurrences of specific species are recorded at a specified distance on each side along the entire transect.

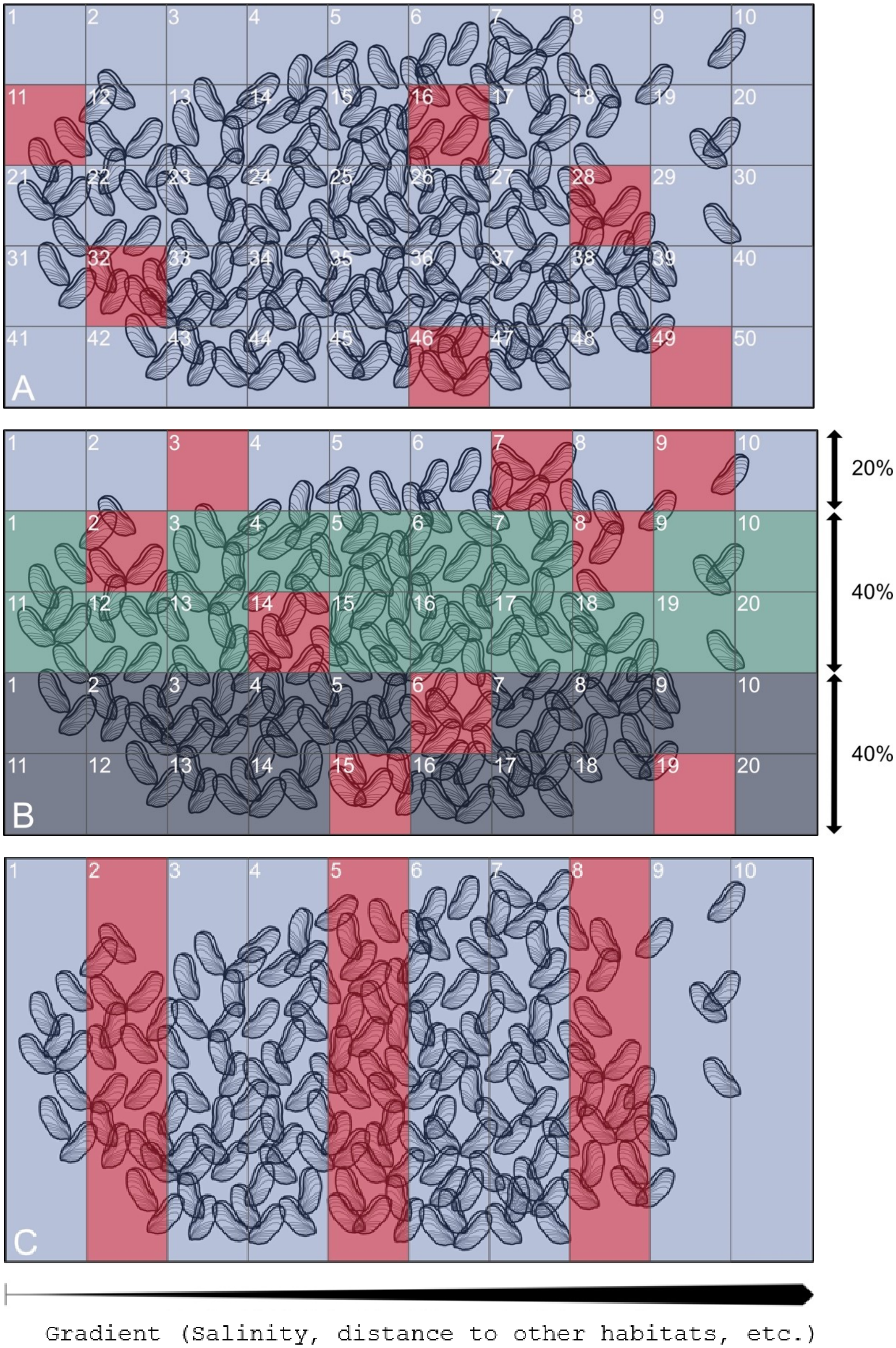

Figure 8. *Schematic drawing of the different sampling techniques A: Random sampling B: Stratified random sampling and C: Systematic sampling*

How Many and What Size Samples Should be Collected?

Because each establishment project has a different scope, sets of methods, environmental conditions, and purposes, the number of samples to be taken per site for each criterion (see Table 2) will vary for each project. Additionally, monitoring costs can vary greatly, especially when using advanced methods and/or when large areas need to be assessed. The number of repeated sample collections should also be considered. However, there must be enough data to make a robust assessment, which requires sufficient replicates (repeated measurements) and account for the fact that mussel beds are dynamic over time. Blue mussel beds are known to be stable for only a few years, and their spatial structure and age distribution change over time; their sudden disappearance has been observed for centuries (Field, 1922). Therefore, it is essential to document the development of established reefs over time in relation to what characterises growth and stability.

Practical Considerations

The selection of which methods to use in monitoring depends not only on the project budget, but also largely on: 1) the existing expertise and tools available to the project; 2) the parameters to be investigated; 3) the frequency of data collection; and 4) the accuracy and precision desired for sample and measurement collection. Therefore, it is important from the beginning to establish what is necessary and what is extra/supplementary data.

Considerations Regarding Boats and Equipment

Mussel beds are typically established in areas with depths of water greater than three metres. This reduces the effects of potential ice scouring and wave exposure but also ensures sufficient draught for the relay vessel. Monitoring boats must, at a minimum, be able to transport a crew of two people with all necessary equipment for a day's tasks and collected samples safely. Larger boats are limited by draught and must operate from a harbour or slipway if they are on a trailer. Smaller boats can potentially be launched from the beach or from smaller slipways. In the cases where only water samples are collected, dinghies or kayaks can be used. Heavier sampling or the use of monitoring equipment (e.g., video/camera sleds, underwater robots, or grabs) requires more space and stability, as well as engine power and winches. Depending on the tasks to be performed and crew composition, other considerations may include access to power (12VDC or 220VAC), computers, GPS, anchoring capability, hydraulic systems, crane/boom/A-frame, tables and rinse pumps for sorting, or diving capabilities (e.g., ladders, tank storage). Additionally, shelter from wind and weather, as well as access to toilet facilities, should also be considered.

In the following sections, common methods and parameters are reviewed, which are relevant to be included in a monitoring programme for the establishment of blue mussel beds, as well as any supplementary environmental parameters. A comprehensive overview of the parameters and methods is found in Table 3. The final section provides a brief overview of methods that can help document biodiversity ecosystem services.

Methods for Monitoring Coverage, Footprint Area, and Bed Area

Monitoring the development of established mussel beds over time can include a full mapping of the establishment area, selected sub-areas, or point-based sampling. The method used largely depends on access to boats, equipment, qualified labour, and the project budget. In general, the areal monitoring of blue mussel beds can be determined in two different ways: 1) the 'footprint area' of the mussel bed, i.e., the total area of the mussel bed and sediment areas between the mussel beds, or 2) the area of the blue mussel bed itself, that is, only the areas where blue mussels are present. The areas can be measured in square metres (m^2) and the percentage coverage of mussels in the areas.

There are several different methods to determine the footprint, habitat area, and coverage of mussel beds.

Monitoring with Cameras

Mapping mussel beds with cameras is a standard method that can cover a large area in a relatively short time. Digital cameras now allow for high-quality image and video recording, which can be used to estimate the biomass distribution of blue mussels. If calibrated, cameras can be used for the overall identification of sediment types and to identify and quantify other organisms associated with the mussel bed. Video sleds can be used, which are dragged over the seabed or maintained at a controlled height (e.g., with a winch and tail) over the mussel bed. Surveys using a fixed height above the mussel bed can be challenged by swells as it affects the height. It is recommended to conduct video surveys in calm weather conditions and using a camera that can withstand impacts from collisions with, for example, stones. Alternatively, drop-down cameras can be used at selected positions on the bed.

Divers can also perform image/video monitoring by swimming along a marked transect or a visual monitoring of coverage. The transect can be relayed as a sinking line from the boat at known coordinates. Alternatively, marking buoys can be used if visibility is sufficient. The diver should maintain a relatively fixed distance from the bottom and a fixed camera angle to ensure consistency in the analysis of videos or still images. Regardless of the camera method used, lighting conditions can be a challenge, as there are often poor lighting conditions in the inner Danish waters, as well as reduced light at deeper depths. This can be partially remedied by using lamps, but turbid water significantly reduces image quality and often makes videos difficult to analyse. Therefore, it is generally recommended to perform camera surveys when visibility is greater than two metres.

Drones

An alternative to divers is the use of remotely operated underwater vehicles (ROVs) or autonomous underwater vehicles (AUVs). AUVs are a relatively new technology and are relatively expensive to acquire and is likely in exceedance of the project’s budget unless the project already has access to an AUV. However, there are a variety of ROVs in different sizes and price ranges. All models include a camera and propulsion with steering capabilities to operate the ROV from the surface. They are typically light and relatively easy to use, while the more advanced models can also collect water samples or objects, can mount various sensors, or have sonar-based GPS positioning capabilities. Battery life can be a limiting factor for larger surveys, especially in colder weather.

Monitoring blue mussel beds can also be carried out with flying drones (Unoccupied Aerial Vehicles, UAVs). UAVs can be used to map coastal habitats such as eelgrass beds and mussel beds (Thomasberger and Nielsen, 2023). With sufficient visibility, surveying from the air can rapidly map mussel beds at water depths less than ten metres over a relatively large area. However, Danish coastal areas often have turbid water due to either high phytoplankton concentrations or resuspension, which limits both the number of days when aerial mapping can be used, and the monitoring depth is often less than three metres. Repeated mapping of the establishment area (including before establishment) can help document the development of the established mussel bed over time and any other effects in the area. Some UAVs can be equipped with specialised cameras or sampling equipment that can collect small samples from the mussel bed (Espriella et al., 2023).

Side-Scan and Multibeam Sonar

Side-scan sonar (SSS) is a technology that uses acoustic sensors to 'map' the seabed. Modern SSS units are relatively inexpensive, provide good resolution, and provide visual identification of various structures on soft sediments. SSS images combined with GPS positions/navigation routes can be georeferenced and overlaid on other maps. When SSS images are compared with video monitoring transects, diver transects, or drone recordings, they can be used to validate mussel beds versus the background (sediments, rocks, macroalgae, etc.), as well as to estimate coverage, spatial organisation, and the area of the mussel bed. Acoustic surveys with multibeam sonar, for example, are also possible but are often more difficult to interpret, significantly more expensive to acquire, and typically require greater water depth than SSS.

Monitoring of Blue Mussel Bed Growth and Stability

The collection of blue mussels from the established bed can be done by hand collection by diving or snorkelling, or from boats using grab or core samplers. The clustered distribution within mussel beds and potential orientation in strips can make it difficult to collect representative samples from the surface in areas >4 m deep and without visibility to the bottom, while diver-based collection is better for extracting representative samples. When sampling with a grab or corer, multiple samples can typically be collected in a shorter time, thereby covering larger parts of the bed during monitoring, while diver collection is limited by the total bottom time for the diver, which varies depending on depth, dive times, and number of dives per day. Diver collection involves using a frame (i.e., a square or circle with known dimensions). The frame is placed at a randomly selected sampling site, then blue mussels and any other material within the frame are collected in a net bag and transported to the surface. The size of the frame affects both the collection time and subsequent analyses (Pringle, 1984), which is why it is generally recommended to use frames <0.5 m^2. A camera can optionally be mounted on the frame so that a picture of the frame's contents can be taken after the frame is placed and before material collection begins.

Density of Blue Mussels

The density of blue mussels is measured per unit area as either the number of mussels (eg, individuals/m2) or biomass (kg/m^2), and samples can be collected, for example, by diving or grab. Due to the self-aggregation behaviour of blue mussel beds and their heterogeneous distribution, mussels will distribute unevenly, causing the coverage rate to vary. Additionally, growth can differ along the edges and in the middle of the clusters (Saurel et al. 2013). This means that it is often necessary to collect a large number of replicates (>10 samples) to estimate the density of blue mussels in the different areas of the mussel bed and potentially calculate the total biomass of the bed.

Size Distribution of Blue Mussels

An assessment of the size distribution of blue mussels provides information on the composition in terms of age, size, and number of each size. The number of cohorts (age groups) in the bed is important in relation to examining whether the established beds fully establish into a biogenic reef (three cohorts according to the Danish definition). It is also interesting in terms of whether there is recruitment of new mussels, as three cohorts are associated with the mussel bed's longevity (Johansson et al. 2024). Samples are taken randomly over the mussel bed or alternatively in the middle and edge of mussel clusters to investigate whether there are differences in size distribution depending on the position in the mussel bed. Various morphometric measurements (shell length, width, height) can be taken of the sampled blue mussels; only the length is used to determine the number of cohorts.

Measurements can be taken either onboard the boat or on land using callipers or a ruler. It is recommended to measure at least 30 mussels and preferably 100 mussels per sample. The measured mussels can optionally be returned to the mussel bed. For each station, all length measurements are compiled into a length frequency diagram (shell length on the x-axis and frequency (%) on the y-axis) and the average length (± standard deviation) is calculated for each station. If there are no differences in size distribution between the stations/station types (e.g., edge, middle, or cluster), a common size distribution diagram and an average length are reported. If there are significant differences in the average length, each station type's average length and possibly size distribution are reported. Annual monitoring is recommended to follow the growth and survival of different cohorts.

Survival and Growth

The growth rate of blue mussels provides an indication of whether the mussels are thriving and whether the bed is in good growth. Negligible or very low growth rates can be due to several factors related to conditions at the location or deployment design. If blue mussel spat consisting of only one cohort is deployed, it is straightforward to monitor the growth of the cohort and survival over time. Samples from size distribution can be used to estimate growth rate (preferably 100 mussels per replicate); which is expressed in mm/year, while survival rate is measured as changes in mussel density (number/m^2) over time.

Larval Release and Recruitment

Supplementary studies can include observations of mussel larvae during the spawning season (primarily March-April) around the established mussel bed as a potential indicator of recruitment to the mussel bed. Various methods can be used to monitor mussel larvae or settlement.

1) Placement of spat collectors in the water column above the mussel bed, which protects against predators. A study in Limfjorden showed that although mussel larvae were observed in the water above wild mussel beds, only a few larvae settled on spat collectors placed about 50 cm above the mussel beds (Saurel et al. 2021).
2) The collection of water samples with a 40 µm plankton net over and around the established blue mussel bed can be used to calculate the number of blue mussel larvae in the water column. However, this method is time-consuming and it can be difficult to distinguish blue mussel larvae from other mussel larvae.

3) eDNA analyses can be used to identify whether the correct mussel larva species is in the water at a given time, however, these analyses may be limited to laboratories with access to specialized equipment and require advanced interpretation.

Regardless of the collection method, it is not necessarily certain that the mussel larvae collected in water samples will settle on the established blue mussel bed. Therefore, recruitment is better characterized by collecting samples from the blue mussel bed to determine the size distribution (see above). Based on the size distribution, it can be relatively easily identified whether there has been subsequent recruitment on the established mussel bed. Supplementary genetic analysis can be performed on newly recruited and deployed blue mussels to determine if the deployed blue mussels have a significantly different genetic composition, which is unlikely if the deployed blue mussels are collected in the local area.

Environmental Parameters

The growth and stability of mussel beds are related to the physiology of mussels, the interactions between mussels, and the interactions of the mussel bed with the surrounding environment. There are several environmental factors, such as water temperature and oxygen concentration, that impact mussel survival. Including environmental parameters in a monitoring programme can help assess the stability of the established mussel bed or help explain why parts of the mussel bed perform better or worse than others. In the following sections, various methods are discussed that can be used to collect data on some of the most relevant environmental parameters in relation to the growth and survival of mussels.

Water Samples

Collecting water samples to determine parameters such as temperature, salinity, and phytoplankton concentration is usually done with a water sampler (e.g., Niskin, Ruttner, or Van Dorn), which allows the collection of water samples at desired depths. Mussel beds are located at the bottom, so water samples should be collected 0.5-1 m above the bottom (Muschenheim and Newell, 1992), while samples can also be taken at other water depths depending on the purpose of the sampling. Be careful not to disturb the sediment during sampling and resuspend sediments. A sufficient number of replicates should be taken, typically a minimum of three at each location (e.g., depth, station, area, etc.).

Many of the environmental parameters (temperature, oxygen, salinity, and phytoplankton) can also be collected/measured with sensors. Monitoring can be done either by 1) slowly lowering one or more instruments, depending on the sensor configuration, through the water to obtain a depth profile showing changes through the water column, or 2) placing sensors with a built-in data logger at a specific depth at a given location for a period of time. For long-term monitoring, there may be a need for a power supply or surface contact for wireless data transmission, as well as mooring. Regular maintenance and cleaning of biofouling from instruments are often required.

Hydrodynamic Conditions

Hydrodynamic conditions have a significant impact on the growth and stability of blue mussel beds and can be characterised by several methods. The simplest method is to use a neutral buoyant sea anchor placed at a fixed distance from the surface and equipped with a small buoy. The start and end positions are recorded, and then the direction and current speed can be calculated. Current meters can also be used to record current speed and direction over time at a specific depth. More complex and expensive instruments, such as Acoustic Doppler Current Profilers (ADCP), can measure current speeds and directions simultaneously at multiple depths, allowing analysis of the water column and properties such as turbulence and density stratification. Alternatively, inexpensive measuring instruments, such as tilt-current meters, can be used to continuously measure the near-bottom current speed and direction.

Coverage by Sediment

Coverage or smothering of mussels by sediment with a high content of organic material can negatively affect the growth and survival of mussel beds. Large volumes of fine sediments can move across the seabed, potentially covering large sections of mussel beds, which can cause increased mortality (Cottrell et al. 2016; Hutchison et al. 2016). The organic content of the sediment (loss of ignition; LoI) and grain size can be determined in the laboratory by collecting sediment samples with, for example, cores, haps, or grabs collected from a boat or by diver, while sedimentation rates are determined with sediment traps. Sedimentation rates are useful for understanding sediment transport processes but are time-consuming and difficult to collect, and measurements tend to vary with season and weather. Resuspension events can suddenly cover sections or whole mussel beds, however, due to their intermittency and associated poor weather conditions, it is difficult to measure resuspension. Visual observation of the mussel beds is recommended with a video sled, a diver transect, an ROV, or a dropdown camera, which can provide qualitative knowledge about the degree of coverage over the mussel bed(Figure 9). Coverage can be indicated as a percentage in each area and assessed as partially to completely covered. Other observations can be beneficially included, such as white patches (sulphur bacteria mats) formed by sulphur bacteria (e.g. *Beggiatoa* spp.) on dead blue mussels (Figure 9).

Figure 9. *Established blue mussel beds in Limfjorden.* ***Top left****: The edge of a mussel bed, where coverage is often observed.* ***Top right****: Partially covered blue mussels, where the siphons are above the surface, but large parts of the blue mussels are covered.* ***Bottom left****: Blue mussels almost completely covered, where only the edges of the mussels are exposed.* ***Bottom right****: Dead blue mussels with sulphur bacteria mats (sulphur bacteria) on top of the sediment (Photo by Daniel Taylor).*

Monitoring of Ecosystem Services Provided by the Mussel Bed

The establishment of blue mussel beds is motivated by the potential to create a biogenic reef and thereby achieve associated ecosystem functions and services such as increased biodiversity and support for stable marine food webs, improved water quality in the form of clearer water that benefits benthic vegetation, nutrient binding, or the establishment of corridors for the transfer of organisms between areas. To document these ecosystem services, a monitoring programme must be established to quantify the ecosystem services delivered by the mussel bed. The monitoring programme should therefore be based on site and species-specific success criteria and objectives for the specific project.

Monitoring of Biodiversity Effects

The establishment of blue mussel beds will have an impact on both animals that live in the bottom (infauna) and animals and macroalgae that live on (attached macrofauna and flora) or between blue mussels (epifauna and mobile macrofauna). Different methods are used to document the effects on the abundance and diversity of associated organisms (see Table 4). For **infauna**, samples can be collected using grabs or cores. Variation over time in infaunal composition can be expected as sediment conditions may change. Samples can also be collected for determining sediment type. Infaunal samples should be examined for species identification, as well as biomass and dry weight determination, so that temporal variations in species diversity, density, and biomass can be monitored. **Epifauna, attached macrofauna and flora** can be documented by visual diver inspection, video monitoring in a small area, or by collecting samples with grabs, for example. **Mobile macrofauna** such as crabs, starfish, lobsters, sea snails, and fish can be monitored with fixed underwater video cameras, as camera sleds or ROVs often scare the animals away. Fixed underwater video cameras can be used with or without bait and by filming at set time periods, for example, two minute recordings three times a day during daylight hours. Trained scuba or free divers can alternatively be used to estimate species richness and density, but there is also a risk of scaring away mobile animals by the diver. For crustaceans (e.g., lobsters and crabs), traps can be used.

eDNA monitoring can also be used for the identification of species of both infauna, epifauna, attached macrofauna and flora, and mobile organisms. Regardless of the method used, it is recommended that all samples are collected at the same time of year, as seasonal variations often occur and should include monitoring before the establishment of the mussel bed and post-monitoring.

Special attention should be given to observing invasive species and, if present, recording their density and biomass.

Overview tables of different sub-elements in the monitoring programme

Monitoring programme to document the stability and temporal development of established mussel beds (recommended and supplementary)

Recommended investigations	Methods	Unit	Frequency	Performance Criteria
Coverage rate, footprint area, and area of blue mussel beds	Camera Diver Drones, ROV etc. Sidescan sonar	% m^2	Pre-establishment. Immediately after establishment. Annual follow-up monitoring.	Stable or increasing area/coverage rate. No major reductions.
Density of blue mussels	Frame Grab	Kg/m^2 $Individuals/m^2$	1-3 months after establishment Annual follow-up monitoring.	Stable or increasing densities (kg/m2)
Size distribution of blue mussels	Frame Grab	Number of cohorts Mean length (cm)	1-3 months after establishment. Annual follow-up monitoring.	Over time, there are a minimum of 2 cohorts/presence of smaller mussels.
Survival and growth	Frame Grab	$Number/m^2$ mm/year	1-3 months after establishment. Follow-up annual monitoring	No major long-term reductions in $number/m^2$. Shell-length growth
Supplementary investigations	**Methods**	**Unit**	**Frequency**	**Performance Criteria**
Larval release and recruitment	Spat collectors Water samples eDNA	$Larvae/m^2$ Larvae/L	Spring after establishment. Annual follow-up monitoring.	Observations of bottom-settled mussel larvae. Occurrence of larvae in bottom water.
Supplementary environmental parameters	**Methods**	**Parameter**	**Frequency**	**Performance Criteria**
Water samples	Water sampler Sensors	Temperature, Chl-a, salinity, oxygen	Fixed frequency over the years Continuous for shorter or longer periods	Within the tolerance levels of blue mussels (see Nielsen et al. 2024)
Hydrodynamic conditions	Mooring anchor Flow meters ADCP, etc.	Flow velocity, flow direction, turbulence	Fixed frequency throughout the year. Continuous for periods of time.	Within the tolerance levels of blue mussels (see Nielsen et al. 2024)
Sediment coverage	Grab, grab sampler, core sampler Visual inspection using diver video or camera	Loss of Ignition (LoI) Grain size Sediment coverage (%)	Annually or as needed	Within the tolerance levels of blue mussels (see Nielsen et al. 2024)

Table 3. *Overview of the different parameters that should be included as a minimum in a monitoring programme for the establishment of blue mussel beds, as well as any supplementary parameters.*

Monitoring of ecosystem services

Biodiversity	Methods	Parameter	Frequency
Infauna	Grab, Haps eDNA	Species composition, species richness, biomass, and dry weight determinations	Pre-establishment. Immediately after establishment. Annual follow-up monitoring.
Epifauna and attached macrofauna and flora	Grab, Grab sampling, visual inspection using diver- or video transects. eDNA	Species composition, species richness, densities	Pre-establishment. Immediately after establishment. Annual follow-up monitoring.
Mobile macrofauna	Fixed-mounted video camera with or without bait. Traps eDNA	Species composition, species richness, densities	Pre-establishment. Immediately after establishment. Annual follow-up monitoring.
Invasive species	Frames, visual inspection, or fixed-mounted camera. eDNA	Species composition, species richness, densities	Pre-establishment. Immediately after establishment. Annual follow-up monitoring.

***Table 4**. Overview of different parameters that can be included in a monitoring programme to document biodiversity ecosystem services during the establishment of blue mussel beds.*

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The main purpose of the center is to promote knowledge-based implementation of marine habitat restoration, aiming to enhance the resilience of marine ecosystems, ecological balance, and a wide range of ecosystem services in Danish waters.