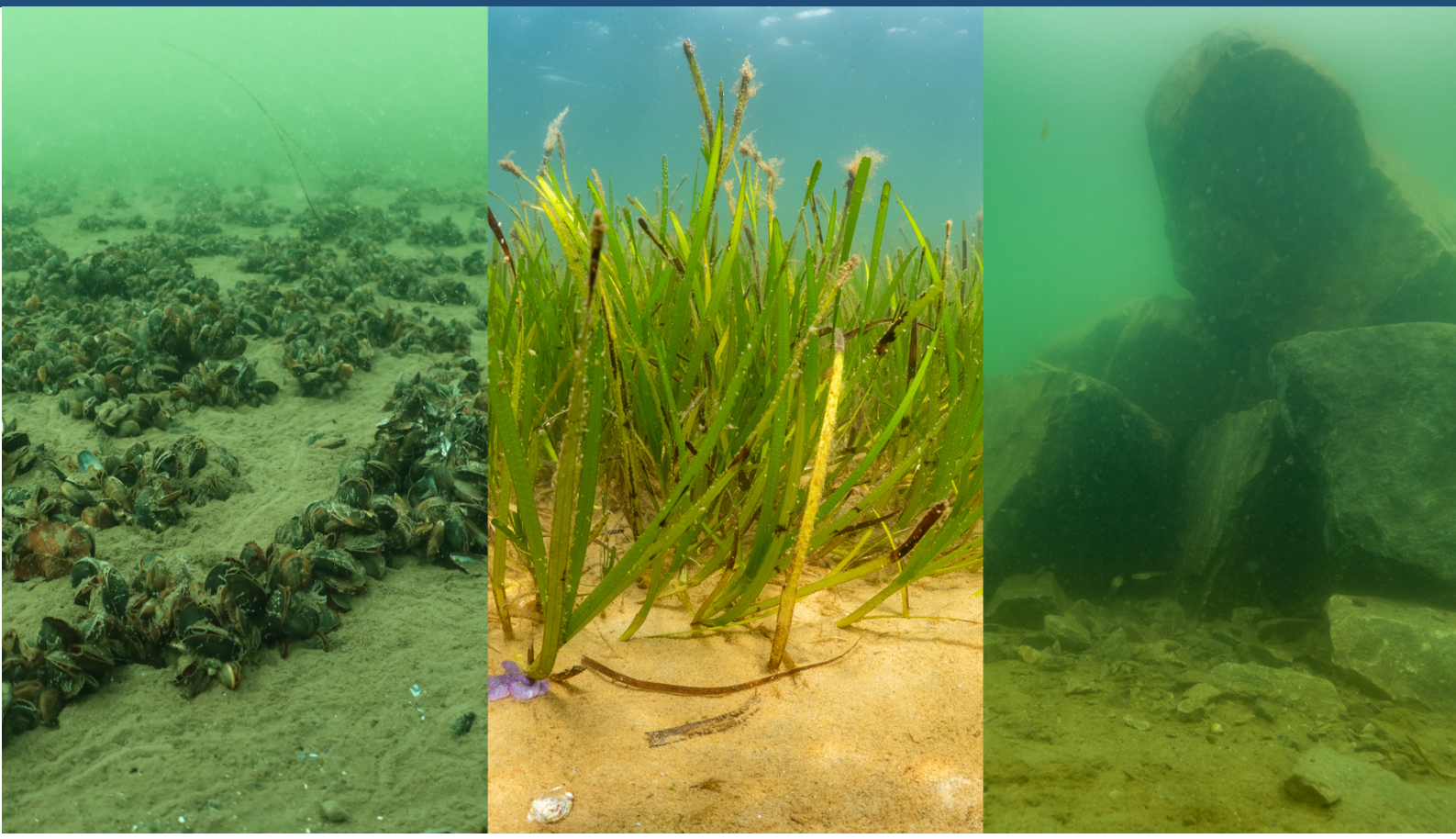
Monitoring guideline:

Ecosystem services provided by restored biogenic reefs, boulder reefs or eelgrass meadows



Center for
Marine Restoration

Colophon

Title:	Monitoring Guideline: Ecosystem Services provided by restored biogenic reefs, boulder reefs or eelgrass meadows
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Summary: This report presents a summary of key ecosystem services provided by biogenic reefs, boulder reefs, and eelgrass beds. Various monitoring methods used to record these ecosystem functions are described to align how to document the impact of the restoration effort among diverse restoration initiatives.

Content

Introduction

This guideline provides an overview of the practical considerations for developing a monitoring plan for coastal restoration projects of eelgrass beds, boulder reefs and biogenic blue mussel beds in Danish coastal waters. The first step in developing a monitoring plan for restoration of marine habitats is to clearly define the goals and objectives of the specific project. These objectives should align with the project's capacity, available resources, restoration and monitoring capabilities. This critical step should be completed during the project design phase, prior to the implementation of actual restoration activities to ensure the restoration action is appropriately planned. It allows for site selection, collection of baseline data, selection of relevant monitoring metrics, and establishment of a timeline for assessing progress.

For marine restoration efforts to be deemed successful, the project must have the ability to manipulate the ecosystem or habitat to achieve the desired outcomes, as well as evaluate whether the intervention has produced those outcomes. Data collection should follow standardized methods, be thoroughly analysed, and provide actionable insights to assess restoration success across local, regional, and ecosystem-level scales. This approach ensures that monitoring contributes not only to evaluate individual projects but also to advance the broader understanding of effective marine habitat restoration practices and be able to learn from both successes and failures.

Considerations regarding project goals and objectives

An ecosystem consists of the dynamic complex communities of plants, animals and micro-organisms interacting together with the non-living as a functional unit. The interactions within an ecosystem can be identified as different functions and services produced by each ecosystem and marine ecosystems supply numerous services daily. The services can be categorised into four overall ecosystem services as beneficial interactions to human populations:

- **Provisioning services**: any type of benefit to people that can be extracted from the marine environment e.g. food and building materials.
- **Supporting services**: the consistency of underlying natural processes e.g. photosynthesis, nutrient cycling and provisioning of habitats necessary to produce all other ecosystem services.
- **Regulating/maintaining services**: the benefit provided by ecosystem processes that moderate natural phenomena e.g. carbon storage, erosion prevention and climate regulation.
- **Cultural services**: non-material benefit contributing to the development and cultural advancement of people e.g. tourism, recreational, aesthetic, and spiritual benefits.

Tabel 1. The four main categories of ecosystem services (ES) along with their ecosystem functions are outlined. Selected ecosystem functions for the guideline are either highlighted as key ecosystem functions for the habitats; biogenic reefs, eelgrass meadows and boulder reefs and marked with an X, while minor ecosystem services offered by a habitat are denoted with an o.

Ecosystem Services (ES)	X = Key ecosystem function o = Minor ecosystem function	Biogenic Reefs	Eelgrass Meadows	Boulder Reefs
Provisioning				
Goods/products provided or produced by ecosystems	Harvest/fishing production/enhancement			
Supporting				
Services necessary for production of all other ES	Biodiversity enhancement			
	Habitat/species/population enhancement	X	X	X
	Genetic diversity			
	Ecosystem stability and improved function - food web			
Regulating/Maintaining				
Benefits from regulation of ecosystem processes	Water clarity	X	o	
	Carbon burial/immobilisation	o	o	o
	Nutrient burial/immobilisation	X	X	o
	Erosion prevention		o	X
	Sediment/substrate stability	o	o	X
Cultural				
Non-material benefits from ecosystems	Recreational visitors/citizen science etc.			
	Tourism activities and employment			
	Health and well-being			

The overall objective in marine habitat restoration projects is to restore lost or enhance degraded ecosystem functions (Table 1) to support long-term improvements of the ecosystem services by focusing on e.g.

- Biological or ecological goals: enhancing biodiversity and restoring specific habitats to support diverse marine life.
- Enhancing or stabilizing existing habitats: ensuring structural integrity and long-term ecological functionality.
- Multipurpose objectives: combining ecological outcomes with physical benefits, such as coastal protection or stabilising sediment.

The contribution of the different ecosystem services depends on a healthy environment and ensure the supporting and regulating services, and therefore these two ecosystem services are often the objectives of projects focused on eelgrass, boulder reef, or blue mussel habitat restoration.

For example, all three habitats support the ecosystem function ‘habitat enhancement’ and attract other species by providing settling ground, food or hiding refuge, which gives potential rise to more trophic levels, niche specialisation and individuals of species increasing biodiversity, improving ecosystem stability and genetic diversity. All leading to more complex food webs and supporting natural ecosystem processes such as water clarity and biogeochemical processes, which further support both cultural and provisioning services.

This guideline offers advice on monitoring ecosystem functions that contribute to supporting and regulating ecosystem services**. It emphasises monitoring key ecosystem functions in each of the three distinct habitats**, rather than attempting to cover every possible function in each habitat (Table 1). This focus should enable practitioners to develop a monitoring plan that adequately documents the attainment of the specific objectives of each marine restoration project.

Developing a monitoring plan

A marine habitat restoration project has different opportunities and constrains due to its unique objectives, partners, budgets and scale, all of which must be factored in, when developing a monitoring plan. For example, the project's progression and timeline establish the fundamental structure for the monitoring strategy. The monitoring plan must align with the project's main objectives to effectively track compliance. The budget influence the choice of monitoring design and the variety of methods employed. Additionally, the expertise and equipment accessible within the project will likely affect the final selection of monitoring techniques. The following sections will provide overall recommendations regarding monitoring design, timeline, different monitoring methods and the requirement for expertise for marine habitat restoration projects.

Monitoring design

The monitoring design should be able to evaluate the impact of the restoration effort but also provide input to a broader understanding of effective marine habitat restoration practices. To assess the impact of the habitat restoration initiatives, it is essential to compare pre- and post-restoration conditions as well as to control or reference sites that have not undergone restoration.

Baseline data

Baseline monitoring should always be included and is a key priority in terms of allocation of time and budget in any marine habitat restoration project. The monitoring data before establishment forms the baseline knowledge and play a critical role in providing information on the condition of the site prior to restoration. These data are essential for identifying trends and evaluating changes over time. Restoration monitoring activities and metrics should be designed to reflect these goals and capture the impacts of environmental changes.

Control site

Control sites, representing areas in a similar pre-restoration condition but left undisturbed, can serve as proxies if pre-restoration surveys are not feasible due to project constraints. However, control sites alone are insufficient for evaluating restoration success, as they only provide a basis for comparison, indicating whether the restored sites have changed. Incorporating data on the distance between restored areas and control or reference sites is also important, as spatial proximity can influence restoration outcomes and the detection of spillover effects.

Reference sites

Reference sites, or 'natural areas', represent the desired outcome of restoration efforts, illustrating what success should look like. However, it can be difficult to find proper reference sites due to degradation of the marine ecosystem, hence the need for restoration. Instead, comparable datasets from similar habitats or historical data can be used. Reference surveys should employ the same metrics collected at the restoration sites to enable consistent comparisons. If reference sites are not available a BACI-approach is recommended.

BACI-approach - Before-After-Control-Impact

A BACI design allows one to document differences between control areas and 'restored' areas (impact area) before and after establishment. Samples from both the control area and the impact 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 impact area before the habitat is established. Furthermore, the control area should be located at a sufficient distance so that the effects of the restored habitat do not directly affect the control area. After the habitat is established, the control area is monitored concurrently with the impact area using identical methods and collection techniques.

Sampling effort

The number of sampling events, their frequency, and the overall temporal scale and seasonal time of monitoring must be planned to balance cost-effectiveness with the ability to detect meaningful trends in restoration outcomes. For statistical considerations regarding monitoring and sampling, we refer to Foster et al. 2024[1].

Timeframe

Developing an effective monitoring plan for marine habitat restoration requires a clear timeline and consideration of monitoring methods to align with the expected ecological and physical changes.

- **Short-term** (days to weeks and up to 1yr) monitoring aims to establish a baseline for the initial condition of the restored habitat at the time of restoration or shortly thereafter and thus is linked to site selection procedures.
- **Mid-term** (months and up to five years) monitoring aims to assess colonization patterns, biodiversity recovery, and the abundance of species associated to the restored habitats, for eelgrass and biogenic reefs, survival, growth and renewal are also assessed.
- **Long-term** (>5 years), in addition to the mid-term objectives, monitoring aims to evaluate if the restored habitat becomes functionally similar in the ecosystems to a natural wild habitat and how the restored habitat integrates into the broader ecosystem, which could include its role supporting provisioning and cultural ecosystem services.

Data reporting and quality control

Standardized protocols for data collection, reporting, and quality checks are important to ensure consistency, reliability, and comparability across restoration projects, facilitating informed decision-making and adaptive management. To the extent possible, data reporting should include formats compatible to the ones used by Danmarks Miljøportal.

[1] Foster SD, Monk J, Lawrence E, Hayes KR, Hosack GR, T. Langlois, Hooper G & Przeslawski R. 2024. Statistical considerations for monitoring and sampling. In Field Manuals for Marine Sampling to Monitor Australian Waters, Version 3. Przeslawski R, Foster S (Eds). National Environmental Science Program (NESP).

Selection of monitoring methods

Marine habitat restoration projects can be designed to involve either stakeholder (ranging from citizens to consultants) initiatives or research efforts led by scientists, depending on the objectives and available resources. In both cases, monitoring plays a crucial role in ensuring the effectiveness of restoration activities and monitoring needs to be conducted by standardised methods and protocols to ensure the data collected is reliable and comparable across projects and as much as possible use methods described in the technical instructions for the Danish national marine monitoring programme (NOVANA)[2].

Regular surveys for tracking progress can be executed using various techniques, from acoustic methods covering larger areas to discrete sampling for detailed species-level data or specific processes. As a result, there is a wide array of monitoring methods, each with diverse requirements such as costs for equipment and tools, deployment options, and the expertise needed for operation and analysis. We recommend looking into the 'Field Manuals for Marine Sampling to Monitor Australian Waters'[3] providing more details for several of the different methods listed below.

Acoustic methods

Acoustic methods are often used for mapping habitat coverage and distribution. The purpose is to quantify the bottom surface area and/or percentage cover of restored habitats, and thus to determine both its initial status and subsequent evolution. Versions of side scan and multibeam sonars can now be found at lower cost. However, to produce quantitative maps sonars are both costly to acquire or contract and require a significant level of expertise and costly software to analyse. This is normally not available to non-scientists.

Side scan and multibeam sonars are active sonars (i.e. emitting and receiving acoustic signals) using transducer arrays that can be installed on a boat's hull or other platforms, such as automatic underwater vehicles (AUVs), remotely operated vehicles (ROVs) or towfish. Side scan sonars sweep the sea floor from side to side covering a relatively large area of the bottom, producing images with information on both the hardness and relief of the seafloor, but not bathymetry. Side scan sonar can be used to produce accurate large-scale maps of the seafloor, for example of shipwrecks, underwater structures and marine habitats.

Multibeam sonars send multiple sonar beams, fan-shaped from below to the sides, that are received by multiple transducers. Multibeam sonars provide information on depth and backscatter from both features in the water column (e.g. fish or gas bubbles) and bottom (e.g. rocks or sediments). Multibeam sonars allow to create both bathymetric, hardness, and roughness 2D or 3D large-scale maps of the seafloor but are often expensive.

Visual methods

Visual surveys, either video or photography, provide a complementary validation of coverage maps produced using acoustic methods but also to identify associated species. Visual methods can provide high-resolution video or photographic records of the seafloor, are cost-effective and easy to operate but cover smaller areas than acoustic methods. Furthermore, they can be used for species

[2] https://ecos.au.dk/forskningraadgivning/fagdatacentre/marint-fagdatacenter/gaeldende-tekniske-anvisninger
[3] https://marine-sampling-field-manual.github.io/

identification of both sessile and mobile fauna. The cost and availability of high-quality underwater cameras and platforms (both mobile and static setups) have improved significantly in the last decade, making them an option for non-scientific stakeholders. However, if used for quantitative mapping, visual methods require specialized knowledge and software (e.g. photogrammetry), often costly, to produce georeferenced mosaics of videos or photos.

Visual methods provide advantages to direct physical sampling (e.g. by divers), particularly coverage of a significantly larger area, are faster and easy to use, and are not necessarily more costly. However, they also have important disadvantages that can significantly restrict sampling e.g., reduced visibility and image analysis can be labour intensive and are not quantitative unless scaled (e.g. with lasers or other markers).

Discrete sampling

Discrete benthic or water samples of species or environmental parameters like oxygen, temperature, salinity, nutrient levels, chlorophyll etc. provide detailed information about the development of the number and abundance of species or the biogeochemical processes and rates by repeated sampling at representative fixed locations. Identification and quantification of species are often collected by direct sampling techniques such as diver quadrat or grabs/corers sampling, or visual sampling such as with drop down, ROV or diver cameras. Both physical or visual sampling methods can be conducted by trained non-scientists or by experts and similar for collection of physical water samples. Analysis of the biogeochemical processes in the sediment or the water-sediment interface will require experts but the collection of e.g., corers can be done by instructed non-scientists.

Sensors/loggers

Environmental indicators like chlorophyll concentration, nutrient levels, light conditions and dissolved oxygen are collected to estimate water quality and nutrient dynamics. These indicators can be monitored using various sensors or loggers, allowing collection on both larger spatial and temporal scales. Sensors can facilitate either periodic or continuous monitoring at site over extended periods, which is essential for gathering time series data. The wide array of sensors coupled with software for straightforward deployment and data processing allows users to choose cost-effective, but often expensive, and easy-to-use options making them accessible even to non-experts. Furthermore, environmental monitoring sensors and loggers typically require minimal maintenance and can offer high temporal resolution data. However, the sample space for most sensors is within a few cm from the sensor-water interface, so the spatial representation is limited to a single point in the water column. Therefore, employing additional sensors across different depths and locations can be beneficial.

A major operational consideration for fixed sensors is biofouling management. Obstruction by biofilms or sessile organisms of the sensor face will quickly degrade the signal quality to a point where data is useless (Figure 1). Antifouling coatings and brushes are variably effective for medium term (<1 month) deployments during times of heavier fouling (spring-autumn); manual cleaning is challenging to avoid. Regular calibrations with controlled materials or field samples are essential components of data quality control.

Figure 1. Barnacles and mussels fouling a sensor. Photos: Daniel Taylor.

Remote sensing by satellite or aircraft

Projects including experts can incorporate advanced tools such as remote sensing and can include e.g., hydrodynamic or habitat modelling to enhance monitoring efforts. Remote sensing, using satellites or aerial platforms, generates large-scale seafloor maps, tracks habitat or shoreline changes over time but can also be used to monitor environmental parameters like surface chlorophyll-a concentrations, turbidity, epiphytes and algal blooms. These methods provide invaluable data on temporal and spatial changes over time but can also provide data on wave energy dissipation and sediment mobility, critical indicators of coastal protection effectiveness.

How to use this guideline

In the following sections specific guidelines and recommendations for monitoring of each of the habitats ‘biogenic reefs’, ‘eelgrass beds’ or ‘boulder reefs’ are provided. In each of the sections recommended and complementary methods are listed. This approach ensures robust monitoring across a range of resource levels while maintaining flexibility and inclusivity.

Recommended methods are those that must be carried out in all monitoring efforts, regardless of whether they are led by citizen scientists or experts, as they provide the baseline data necessary for assessing restoration success.

Complementary methods offer additional insights and can be included to enhance the details and depths of the data collected. Some of the complementary methods can be carried out by non-experts but often they require involvement of experts or sophisticated equipment.

This guideline outlines various monitoring methods applicable to all habitat types or tailored to a specific habitat. It acknowledges the necessity to adapt certain methods according to the habitat surveyed and recognizes the variation in key ecosystem functions each habitat provides and the approach to monitor them. Figure 2 gives an overview designed to help readers swiftly locate information regarding habitat or methods to monitor the different key ecosystem functions.

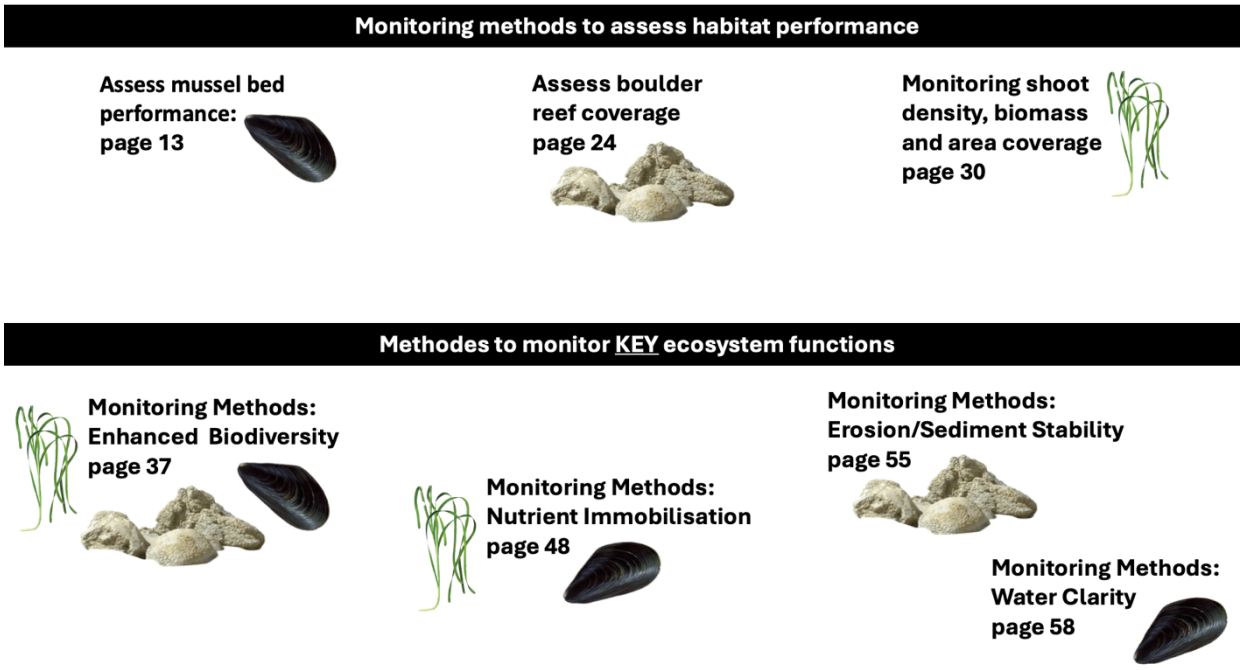

Figure 2. Overview of where information about methods to assess specific habitat performance parameters can be found and where monitoring methods for key ecosystem functions provided by the individual habitats can be found.

Data documentation, format and storage

To maintain consistent data collection, it is important to employ standardised methods and store the data in a well-organized, ideally publicly accessible database. This approach not only aids in evaluating individual projects but also enhances a wider understanding of effective marine habitat restoration strategies, allowing insights from both successes and failures. Metadata, which details the data, should be comprehensive enough to enable other researchers to locate, utilize, and integrate additional data equivalent methods. Thus, it is advised to adhere to national or international standard monitoring methods, protocols, and templates, considering the following critical elements when collecting or creating data:

- Name of the dataset or research project that produced it and a contact person for further information.
- Title, keywords or phrases of the data should be informative and describe the subject or content of the data.
- Methodology used and how the data was generated, including equipment, software used, experimental protocol etc.
- Location, where the data was collected, number of stations, replicates or other record information about its spatial coverage/distribution.
- Dates, project start and end date, specific sampling dates, time covered by the data and potential data modification date.
- Add information about if and how the data has been altered or processed, including explanation of codes, abbreviations, or variables used in the data.
- Make a note how the data is organised.

The overarching aim is to ensure the data is archived and accessible for other projects and follow-up initiatives. As a result, descriptions of the data and its methods should be replicable and comparable.

Biogenic reef

As for all types of restoration, it is also for biogenic reefs crucial to contrast conditions before and after restoration. The baseline data gathered offer essential insights into the site's state before restoration begins. Consequently, baseline data must be collected prior to starting restoration efforts and should at least include information on the presence and abundance of key species intended for restoration, alongside an evaluation of surrounding biological and environmental habitat conditions. Post-restoration monitoring should encompass short-term, mid-term, and long-term observation to assess and record the progress of ecosystem functions and services provided by the restored mussel beds as it evolves with time.

Short-term: Monitoring the restored mussel beds for a period ranging from a few months to a year post-relay will yield insights into initial mussel mortality rates and changes in their spatial distribution. The impact on local water clarity will be noticeable right after the mussels are relayed but will vary over time with changes in mussel biomass e.g., due to growth, mortality, and recruitment dynamics. The influence on biodiversity occurs over varying timescales, dependent on the life cycles and biology of related species; for instance, epifauna often settle in spring, while the restored mussel beds may immediately serve as both a food source and shelter for mobile organisms.

Mid-term: Between 1-5 years post-relay, it is expected that the mussel beds will support a greater diversity of associated infauna, epifauna, flora, and mobile species, fostering more intricate food webs and sustaining ecosystem functions such as water clarity and biogeochemical cycles.

Long-term: After five years, the mussel beds are anticipated to exhibit signs of stability and new mussel recruitment or indicate a potential need for replenishing the restored beds. In the long-term perspective, ecosystem functions should have stabilized.

Monitoring restored reef-bed performance

The restoration of biogenic reefs or more appropriately bivalve beds contribute to the ecosystem function/service species enhancement and habitat enhancement, which is the fundamental objective of biogenic reefs and habitat restoration actions. These two functions in turn support the production of other ecosystem functions and services.

Monitoring of bed performance focuses on evaluating how large, how much, and where if the mussel bed survives, it grows and renews itself, eventually becoming functionally like a natural wild bed. Monitoring covers four indictors/parameters:

- Bed coverage (recommended)
- Abundance and survival (recommended)
- Size, growth, and recruitment (complementary)
- Larvae production and maturity (complementary)

The first two indicators are essential and must be carried out in all monitoring programs. The last two indicators are complementary and provide additional information that allow a better understanding and detailed knowledge in expert lead studies.

Methods for monitoring biogenic reef-bed habitat coverage

Mussel bed coverage is an essential indicator of bed performance. The purpose is to quantify the bottom surface area and percentage cover of restored mussel beds, and thus to determine both its initial status and subsequent evolution. Monitoring methods (table 2) are acoustic (side scan sonar or multibeam sonar) or visual (drone, remotely operated vehicle (ROV), sledge, drop-down, diver cameras or visually estimated by divers). Visual methods are easy to use, fast and cover a large area. However, visual observations are limited by reduced visibility and can only measure what is observed. For detailed information for monitoring blue mussel beds, we refer to Nielsen et al. 2024 and the technical guideline TA no. M21 ´Filtrerende organismer´[4] for monitoring the marine environment, which have been prepared for the Danish Environmental Protection Agency by the Marine Data Centre.

Methods for monitoring abundance and survival

Monitoring abundance over time, as the number of individuals and biomass per m^2, of the restored species, allows to follow the evolution of total biomass, mortality and survival of a restored biogenic reef-bed or habitat. Thus, monitoring abundance is recommended to evaluate both the ecosystem function species and habitat enhancement as well as the success and performance of the restoration action. Furthermore, abundance data is essential to validate coverage maps obtained with acoustic or visual methods and to produce accurate estimates of the evolution of the actual bottom area and percentage coverage of live individuals. Samples taken for abundance can also be used to estimate growth and recruitment (see below).

Abundance can be monitored using direct sampling techniques such as diver quadrat or grabs/corers sampling, or visual sampling such as with drop down, ROV or diver cameras. Both physical and visual sampling methods can be conducted by trained non-scientists or by experts.

Frame, grab or corer sampling

Diver or grab/corer sampling are common and reliable methods that sample a defined surface area and produce absolute measurements of the number of individuals and biomass. Diver sampling allows targeted sampling of small-scale structures or features of the restored reef-bed or habitat, often an advantage in a highly patchy habitat, but has time and depth operational limitations. Sampling with grabs/corers requires higher sampling intensity, even though they are easy to operate, they are costly to acquire and require vessels equipped to operate frames that can weigh tens to over hundred kg. Physical samples need to be sorted, identified, counted and weighed.

Visual methods

Visual methods are easy to use, fast and cover a large area but can only measure what is observed. Thus, measurements of the abundance of live individuals from visual methods can have large errors and only indirectly estimate biomass from counts and size, if size-weight relationships are available.

[4] https://ecos.au.dk/fileadmin/ecos/Fagdatacentre/Marin/TA_M21_Filtrerende_organismer_ver1.pdf

Methods for monitoring of size, growth and recruitment

Monitoring of the individual size structure of a restored species over time provides complementary information other than abundance and survival to evaluate the performance and success of the restoration action. Information on growth (i.e. increase in shell dimensions and weight) can thus be obtained, evaluating how individuals perform in their new habitat from the moment of restoration. In addition, changes in size structure of a population, through length cohort analysis, can evaluate the occurrence or absence, and magnitude of natural recruitment to the restored mussel bed. This is a longer-term indicator of success and performance of the restoration of biogenic reefs/mussel beds.

Monitoring of growth and recruitment must consider its seasonal variation, which can vary by location. In Danish waters, growth in almost all bivalve species is strongly seasonal with a marked reduction or complete stop in growth over autumn and winter due to limitation in food supply, while recruitment occurs over late spring and summer. Annual growth and recruitment should thus be monitored and evaluated at the end or after the growth and recruitment seasons (Oct-Nov).

Size, growth and cohort analysis

Physical samples are collected using divers or grabs, which are then sorted, and mussels are measured onshore or on board of the sampling vessel using callipers. Alternatively, video and digital methods can be used to measure size. A minimum number of individuals (100 per sample) needs to be measured to produce an accurate measurement of the size structure of a population. Growth is the increase in shell dimensions over time. The occurrence of natural recruitment is determined by the appearance of a small cohort with sizes corresponding to that of recently settled individuals (<20 mm), and thus different that individuals introduced at the restoration action.

Recruitment and reproductive potential

Monitoring or estimating larvae production and contribution to reproductive potential provides complementary information to evaluate the long-term success and performance of the restoration action. This can be done by i) performing a desktop review of reproductive maturity and fecundity of the restored species relative to size and age and reproductive strategies. From this knowledge together with abundance and size structure of a restored mussel bed, it is then possible to estimate its spawning reproductive potential or ii) verifying and determining the gonad maturity level of individuals in the restored biogenic reef-bed and habitats over time as well as the presence of larvae in the water column. Maturation of gonads can be easily determined by dissecting individuals and visually assessing the gonads or using a microscope if needed. Quantification of gametogenesis maturity stage requires expert knowledge and expensive histological techniques. The presence of bivalve larvae can be assed from water samples or eDNA samples but requires expert and often expensive techniques and may include larvae produced elsewhere than the restored biogenic reef-bed or habitat.

*Table 2. Monitoring of ecosystem function provided by biogenic reefs: **Restored reef-bed performance**. Indicator: Evolution, stability, resilience and performance of restored biogenic habitats*

Supporting ES	Indicator	Product	Method	Scientific (S) Non- Scientific (N)	Expertise level: Specialist (S) Volunteer (V)	Recommended (R) Complementary (C)	Scale	Strength	Weakness	Processing Level Units	Timeframe Frequency	Performance Criteria
Performance of restored reef-bed	***Reef-Bed habitat coverage***	Maps or images, quantification of bivalve reefs-beds area and bottom coverage, spatial distribution, patchiness and aggregation	Side scan sonar	S	S	R	m to km	Common methods, fast acquisition and processing, large area coverage	Expert, expensive equipment and analysis, weather and depth dependent, Limits in shallow areas.	Quantitative, expert % coverage, m^2	Before restoration After restoration Sub-annually if required Annual follow up	No major reductions in area/coverage, i.e. similar distribution to wild reefs-beds.
			Multibeam	S	S	C	m to km					
			Drone camera	N	V	C	m to 100 m	Direct visual assessments, easy to use, cost effective, large area coverage	Expert and slow quantitative analysis, large volume of data, no physical sampling, limited by what can be observed on surface, small coverage on occasions. Challenges with visibility.			
			ROV camera	N	V	C	m to 100 m					
			Sledge camera	N	V	C	m to 100 m					
			Drop down camera	N	V	C	m to 100 m					
			Diver camera	N	V	C	m to 100 m	Direct visual assessments, detailed, common, targeted	Limited area, not necessarily random. Limited by visibility.			
			Diver direct visual estimation	N	V	C	M to 100 m	Direct visual assessments, detailed, common, targeted	Limited area, not necessarily random. Limited by visibility.			
	Abundance and survival	Quantification of density and biomass of live mussels, survival and mortality in the reef-bed	Diver quadrat	N	V	R	m	Direct sampling, detailed, common, targeted	Limited area, not necessarily random	Quantitative, expert Number/m^2, g/m^2, kg or tonnes, % live, % dead, mortality Qualitative, expert Number/m^2, % live, % dead, mortality	At restoration 1-3 months after Annual follow up	Stable or increase, no major reduction, i.e. like wild reefs-beds Like natural mortality
			Grab/corers/ haps	S	S	C	m	Direct sampling, reliable, common, fast	Expert. expensive equipment, limited area, non-target sampling			
			Drop down camera	N	V	C	m to 100 m	Direct visual assessments, easy to use, cost effective, large area coverage	Expert and slow for quantitative analysis, large volume of data, no physical sampling, limited by obstacles and what is observed on surface, small coverage on occasions			
			ROV camera	N	S	C	m to 100 m					
			Diver camera	N	V	C	m to 100 m	Direct visual assessments, detailed, common, targeted	Limited area, not necessarily random			
	Size, growth and recruitment	Assessment of population structure, growth and the occurrence of recruitment	Diver quadrat	S	V	C	m	Direct sampling, detailed, common, targeted	Limited area, not necessarily random	Quantitative and qualitative, expert Number of cohorts, length (mm, cm), mm/year, g/year, number spat/m^2, presence/absence of spat/new cohort	At restoration Annual follow up in spring-summer	Minimum 2 cohorts, 3 cohorts for blue mussel reef criteria Shell-length growth Presence of spat
			Grab	S	S	C	m	Direct sampling, reliable, common, fast	Expert. expensive equipment, limited area, non-target sampling			
			Desktop study on potential reproduction	S	S	C	Reef	Easy, based on previous existing knowledge of species maturity and reproduction	Limited knowledge, not same geographical locations or systems, assumes fecundity and larvae production	Quantitative, expert Larvae total		
			Water samples	S	S	C	m	Reliable, common	Expert processing and analysis, limited spatial and time resolution	Quantitative and qualitative, expert Larvae/l		
			Gonad/maturity	S	S	C	m	Reliable, easy sampling and evaluation for maturation assessment	Expert. expensive processing and analysis for quantification of gametogenesis level	Quantitative, expert maturity or spawning		

Ecosystem functions provided by biogenic reefs

Biogenic reefs contribute crucial supporting ecosystem services through the key ecosystem function 'enhanced biodiversity.' Additionally, they provide regulating and maintaining ecosystem services due to the key ecosystem functions ‘water clarity’ and ‘nutrient immobilisation’.

Ecosystem function: Biodiversity enhancement

Biodiversity represents a critical ecosystem function and increased biodiversity often constitutes a primary objective in the implementation of nature restoration projects. Species biodiversity is supported by a wide array of organisms, necessitating the employment of various sampling methods, as no single method is capable of sampling all the distinct groups of organisms comprehensively. Organisms can be categorized into three very broad groups according to their fundamental characteristics:

i) Infauna species, which live in the sediment.
ii) Sessile epifauna species and macroflora, which attach to the shells.
iii) Mobile macrofauna, which utilise the three-dimensional structure of the mussel bed for purposes such as hiding, nursery or feeding.

A range of options that may effectively sample one or more of the three organism groups are outlined (table 3). Selecting the most appropriate methods for documenting species biodiversity depends on the aim of the project and the resources and expertise available. It is therefore important to consider sampling design, timeline and sampling frequency before initiating the sampling (for more details see relevant sections above). Furthermore, it is important to consider the seasonality, as the number of species present will change over the year, thus using a recurrent sampling period to be able to compare and monitor progression over the years.

For details on specific methods for monitoring biodiversity enhancement go to page 37.

*Table 3. Monitoring of ecosystem function **Biodiversity enhancement provided by biogenic reefs**. The table highlights key parameters, data outputs, expertise levels required (volunteer or specialist), the recommended or complementary nature of methods, scale of application, timeframe, and associated strengths and weaknesses.*

Supporting ES Ecosystem Function	Indicator	Product	Method	Scientific (S) Non-scientific (N)	Expertise level: Specialist (S) Volunteer (V)	Recommended (R) Complementary (C)	Scale	Strength	Weakness	Processing Level Units	Timeframe Frequency	Performance Criteria
Biodiversity enhancement	***Species abundance, composition, richness diversity/ evenness***	**INFAUNA** Species identification and quantification of density and biomass	Sediment cores Recommended sampling time: Spring.	S/N	V/S	R	m	Reliable, common and fast. Flexibility in assessment e.g. volunteers can just do number of species or groups	Small sampling area, multiple samples, expensive equipment by boat. Expertise for fine taxonomic levels, time-consuming.	Number of species per sediment volume. List of species/groups identified. Biomass/species/groups per area sampled individuals/m^2, wet weight or dry weight g/m^2	Before restoration. 1-2 months after reef establishment Follow-up ~1 yr after reef establishment (same season). Annual thereafter.	**Short term (1-3 yrs)**: Indication of higher biodiversity and abundance on restored sites compared to control sites. **Long term (>5 yrs.)**: Statistically higher biodiversity and abundance
			Grab	S	S	C	m	Reliable, common and fast. Larger sampling volume compared to cores.	Unsuitable in areas with stones/dense mussel beds. Require larger boats and experts, expensive equipment, limited area, non-target sampling. Time-consuming post processing of samples.			
			eDNA	S	S	C	m-100m	Fast sampling, cover large areas, good snapshot of the community.	Expert, expensive analysis, quantification uncertain	Presence/absence		
		EPIFAUNA & MACROFLORA Species identification and quantification of density and biomass	Diver quadrat	N	V	R	m	Direct sampling, detailed, common	Limited area, require divers/snorkelers	Biomass/species/groups per area sampled ind/m^2 wet weight g/m^2 or or dry weight g/m^2	Before restoration. 1-2 months after reef establishment Follow-up ~1 yr after reef establishment (same season). Annual thereafter.	**Short term (1-3 yrs)**: Indication of higher biodiversity and abundance on restored sites compared to control sites. **Long term (>5 yrs.)**: Statistically higher biodiversity and abundance
			Grab	S	S	C	m	Reliable, common and fast.	Unsuitable in areas with stone/dense mussel beds. Require larger boats+experts, expensive equipment, limited area, non-target sampling. Time-consuming post processing of samples.	Number of species/m^2. List of species/groups identified. Biomass/species/groups per area sampled ind/m^2, wet weight g/m^2 or or dry weight g/m^2		
			Video transects (ROV, sledge or diver)	N	V/S	C	m-100m	Direct visual assessments, easy to use, cost effective, large area coverage	Expert quantitative analysis, no physical sampling, limited by visibility and what is observed on surface, small coverage on occasions	Number of species/m^2. List of species/groups identified. Biomass/species/groups per area surveyed ind/m^2		
			Drop down camera	N	V/S	C	m-100m					
			eDNA	S	S	C	m-100m	Fast, cover larger areas, snapshot of the community,	Expert, expensive analysis, quantification uncertain	Presence/absence		
		MOBILE FAUNA Species identification and quantification of density and biomass	Static video camera baited or unbaited, mono or stereo	S (stereo) N (mono)	S	R	m	Enables high-resolution snapshots video doc. over longer periods, monitoring species abundance and length measurements	Expert, need calibration if changes of cameras, limited coverage, especially in turbid waters.	Ind/m^2, first time species recorded, max. no.of species recorded per timeframe. For stero: length (mm) for length-freq. distribution/species.	Before restoration. 1-2 months after reef establishment Follow-up ~1 yr after reef establishment (same season). Annual thereafter	**Short term (1-3 yrs)**: Indication of higher biodiversity and abundance on restored sites compared to control sites. **Long term (>5 yrs.)**: Statistically higher biodiversity & abundance
			Video transects (ROV, sledge or diver)	N	V/S	C	m-100m	Direct visual assessments, easy to use, cost effective, large area coverage	Expert quantitative analysis, no direct sampling, limited by visibility/what is observed on surface, small coverage	Qualitative, expert Number/m^2		
			Drop nets	N	V/S	C	m	Direct sampling, detailed, common	Limited area, require divers/snorkelers	Biomass/species/groups per area ind/m^2, wet weight or dry weight g/m^2		
			Traps (fyke-nets, pots) mark and recapture	N	V	C	100m	Documentation of species day and night, flexible, species identification and length measurements,	Ethical considerations, maintenance required, risk of bycatch of birds and mammals in nets	Ind/m^2, length (mm) for length-frequency distribution/species. Catch per unit effort (CPUE,ind/h)		
			eDNA	S	S	C	m-100m	Fast, good snapshot of the community	Expert, expensive analysis, quantification uncertain	Presence/absence		

Ecosystem function: Nutrient immobilisation

Bivalve beds influence the flow of organic material in coastal ecosystems through filtration and deposition and thereby stimulating microbial processes in surrounding sediments. The capacity of bivalve populations to mediate key biogeochemical cycles, specifically those of nitrogen (N), carbon (C), and phosphorus (P), can drive systemic changes in functional regimes in coastal environments (Dame et al., 1989, Petersen et al., 2008). Accordingly, monitoring and characterising biogeochemical cycles in respect to restoration of bivalve beds supports the evaluation of bed responses to environmental change, anthropogenic pressures, as well as the effectiveness of restoration efforts on ecosystem processes (table 4).

For details on specific methods for monitoring nutrient immobilisation go to page 52.

*Table 4. Monitoring of ecosystem function provided by biogenic reefs: **nutrient immobilisation provided by biogenic reefs**. The table highlights key parameters, data outputs, expertise levels required (volunteer or specialist), the recommended or complementary nature of methods, scale of application, timeframe, and associated strengths and weaknesses.*

Regulating ES Ecosystem function	**Indicator**	**Method**	**Sub method**	**Units (output/result)**	**Expertise level: Volunteer (V) Specialist (S)**	**Recommended (R) Complementary (C)**	**Scale**	**Timeframe**	**Strength**	**Weakness**
Nutrient immobilisation	***Denitrification***	N_2/Ar	Batch/static core incubations; flow-through chambers; in-situ benthic chambers; large "whole-reef" trays/boxes	µmol N_2–N m^{-2} h^{-1}	S	R	Patch/core to section of reef	Depends on purpose. Before and after establishment. Need to correspond with biomass accretion and seasonality.	Direct, high-precision, no amendments; best overall for quantifying net N removal; captures natural conditions.	Sensitive to bubbles (photosynthesis), needs gas-tight chambers and careful flow simulation; logistically challenging for large/complex habitats.
		Isotope Pairing Technique (IPT, ^{15}N)	$^{15}NO_3^-$ additions; $^{15}NH_4^+$ additions; modified IPT for pathway partitioning	µmol N_2–N m^{-2} h^{-1}	S	C	Patch/core to section of reef	Depends on purpose. Before and after establishment. Need to correspond with biomass accretion and seasonality.	Mechanistic discrimination (denitrification vs. anammox contributions); pathway resolution.	Assumptions may be untenable in oyster habitats; typically yields lower rates than N_2:Ar in same systems; sensitive to macrofauna/bioturbation.
		Acetylene inhibition (C_2H_2 → N_2O)	C_2H_2 added to block N_2O → N_2, measure N_2O by GC	µmol N_2O–N m^{-2} h^{-1}	S	C	Patch/core to section of reef	Depends on purpose. Before and after establishment. Need to correspond with biomass accretion and seasonality.	Lower cost than other methods	Underestimates rates; can block nitrification; incomplete inhibition and poor penetration; immediately alters microbes; ignores N_2 fixation & anammox
		Molecular markers (community & genes)	nirS, norB, nosZ DNA/RNA (qPCR, RT-qPCR, meta-omics)	Gene copies / transcript abundance	S	C	Sediment samples	Depends on purpose. Before and after establishment. Need to correspond with biomass accretion and seasonality.	Mechanistic context, complementary	Not a rate; poor quantitative link to N_2 flux; cannot, alone, predict net denitrification.

Ecosystem function: Water clarity

Suspension feeding bivalves filter particles from the water column. Benthic light limitation in most coastal and estuarine waters is due to attenuation of light by dissolved and particulate matter, both organic and inorganic; light limitation in most coastal waters is typically attributed to suspended organic particles. Bivalve filtration reduce organic particle concentrations in parts of the water column, which can decrease light attenuating conditions, and is the basis of water clarification as an ecosystem function. Multiple methods can be used to monitor water clarification, and an overview of the different methods can be found in table 5.

At page 58 you can find describes of methods involved in water clarification monitoring, including examples of available tools, and notable trade-off considerations.

*Table 5. Monitoring of ecosystem function provided by biogenic reefs: **Water clarity provided by biogenic reefs**. The table highlights key parameters, data outputs, expertise levels required (volunteer or specialist), the recommended or complementary nature of methods, scale of application, timeframe, and associated strengths and weaknesses.*

Regulating ES Ecosystem function	**Indicator**	**Method**	**Sub method**	**Units (output/result)**	**Expertise level: Volunteer (V) Specialist (S)**	**Recommended (R) Complementary (C)**	**Scale**	**Timeframe**	**Strength**	**Weakness**
Water clarification	***Seston characteristics (Chlorophyll/ Phytoplankton, particles)***	Discrete water sampling	Volumetric	Inorganic/organic suspended matter dry weight, chlorophyll-a and other pigments concentrations	V/S	R	m	Weekly-Monthly, season dependent	Straightforward, can be low cost and simple, easily trainable	Moderately time consuming, low spatial and temporal resolution, fluorescence and pigment quantification require more sophisticated equipment
			Flow cytometry, particle sorting, plankton identification	Particle size spectra, particle quantitation and classification, plankton identification	S	C	m	Monthly-seasonally, season and purpose dependent	Information-rich, useful for ecological assessment and adaptive management of reef configuration	Either very time consuming or requires very specialised equipment, all methods require specialised training for interpretation
		Fixed Sensors	Single – multiple parameter	Time series of chl-a, phycoerythrin, phycocyanin, turbidity	V/S	R	m	Continuous over short-term campaign or long term	High temporal resolution: deployment, use and interpretation of time series can be straightforward and moderate expense; can be observed in 'real-time'	Limited spatial resolution without costly expansion of monitoring stations; requires specialisation for calibration and maintenance
		Synoptic Surveys, Profiling	Single – multiple parameter	Spatial characterization of phytoplankton concentrations or suspended matter in surface layers	V/S	C	m-hm	Monthly-seasonally, season and purpose dependent	2-3D coverage can describe spatial or physical phenomena, straightforward to employ after assembly	Time consuming, high calibration sequence requirements, georeferencing can be challenging, snapshot in time
		Remote sensing	Aerial or satellite observation	Time series and spatially explicit proxies (reflectance and absorption) for phytoplankton or suspended matter concentrations	S	C	m-km	Monthly-seasonally, season and purpose dependent; satellite overpass	Spatial and temporal coverage; limited equipment requirements	Typically needs local empirical relationship and suitable corrections, weather dependent, specialist interpretation, typically limited to surface layers, limited resolution
	Water movement/ Hydrodynamics	Surface currents	Drogue	Trace of surface currents over short time period	V	R	hm-km	Hour	Simple and inexpensive	Requires multiple deployments and either GPS or visual demarcation; can be influenced by wind; can be lost if not actively tracking
		Point measurement	Current meter, ADV	Time series of velocity components for a single part of the water column, turbulence (ADV)	V/S	R	cm-m	Continuous over short-term campaign or long term	Can be relatively inexpensive, provides time series of *in situ* currents	Requires multiple units to cover water column; interpretation varies by technology; ADV expensive; deployment may require expertise
		Column measurement	ADCP, profiling	Time series or intermittent profiles of water column velocity components, turbulence, stratification	S	R	m	Continuous over short-term campaign or long term	Time series of water column velocities, can characterise larger patterns	Expensive, deployment and interpretation require expertise
	Light attenuation (Optical properties)	Discrete water sampling	Volumetric	Spectrometric absorption and transmission	S	C	m	Weekly-Monthly, season dependent	Straightforward, full spectra	Expensive, time consuming, filtration requirements, low spatial and temporal resolution
		Fixed Sensors	Single – multiple parameter	Time series of PAR, beam attenuation (c m^{-1}), backscattering, transmission, attenuation coefficient (K_D)	V/S	R	m	Monthly-seasonally, season and purpose dependent	High temporal resolution: deployment, use and interpretation of time series can be straightforward and moderate expense	Limited spatial resolution without costly expansion of monitoring stations; requires specialisation for calibration and maintenance; can be very expensive
		Synoptic Surveys, Profiling	Single – multiple parameter	Spatial characterization of PAR, beam attenuation (c m^{-1}), backscattering, transmission, attenuation coefficient (K_D), Secchi disk	V/S	C	m-hm	Continuous over short-term campaign or long term	2-3D coverage can describe spatial or physical phenomena, straightforward to employ after assembly	Time consuming, high calibration sequence requirements, georeferencing can be challenging, snapshot in time; can be very expensive
		Remote sensing	Aerial or satellite observation	Time series and spatially explicit spectral reflectance and absorption of surface waters, secondary and tertiary products	S	C	m-km	Monthly-seasonally, season and purpose dependent	Spatial and temporal coverage; limited equipment requirements	Typically needs local empirical relationship and suitable corrections, weather dependent, specialist interpretation, typically limited to surface layers, limited resolution

Boulder reefs

To record and evaluate the ecological and functional evolution of boulder reef restoration projects, its core to develop clear objectives, robust performance indicators, and standardised sampling and data-management protocols, the monitoring activities should be mapped onto the six-phase 'Best practice for boulder reef restoration' (Dahl et al., 2024).

Developing an effective monitoring plan for boulder reef restoration requires a clear timeline and consideration of monitoring methods to align with the expected ecological and physical changes. Boulder reefs require extended periods (6-12 years or more) to achieve measurable success, necessitating short-, mid-, and long-term monitoring with detailed data collection approaches (Table 6).

Baseline and short-term monitoring (Year 0–1)

- Baseline surveys: conduct pre-restoration surveys to establish initial conditions, focusing on reef structure, biodiversity, and habitat functionality.
- Rapid assessment (or assessment for smaller citizen-science projects): perform visual inspections and photography within two weeks of boulder placement to identify issues like structural instability, improper placement, or sediment accumulation. Visual photography can provide a general overview of habitat conditions and functional groups but may underestimate species diversity, especially for cryptic or layered organisms.
- Implementation monitoring (use of recommended and complementary methods): evaluate the design and execution of restoration efforts, ensuring that the reef structure is stable and beginning to support colonization.

Mid-term monitoring (Years 1–5)

- Progress monitoring: assess colonization patterns, biodiversity recovery, and the abundance of species. Consider using settlement plates to track colonization dynamics. If plates are placed on an established reef, evaluate whether they reflect the 'climax community or are dominated by opportunistic pioneer species, which may influence competition dynamics and eventual community structure.
- Key metrics: monitor functional groups, habitat complexity, and competition between pioneer and K-strategy species to assess progression toward a stable and diverse community. Settlement plates can help detect early signs of community shifts or dominance by specific taxa.
- Colonization dynamics: investigate whether species colonization aligns with restoration goals and adjust management practices to promote desirable ecological outcomes.

Long-term monitoring (Years 5–12+)

- Ecosystem impact monitoring: evaluate how the restored boulder reef integrates into the broader ecosystem, including its role in enhancing biodiversity, supporting fisheries, and providing ecosystem services.
- Structural complexity and stability: measure reef rugosity and habitat persistence over time. Long-term survivorship of colonizing species and shifts in community composition should be monitored to determine if the restoration is progressing toward a stable 'climax' state.
- Adaptive management: use long-term data to refine restoration techniques, ensuring resilience to environmental disturbances such anthropogenic stressors like coastal construction or pollution.

Table 6. Monitoring timeframes, approaches, and key objectives for evaluating restoration projects using scientific/professional and citizen-science/voluntary methods.

Monitoring timeframe	Years	Scientific/Professional approach	Citizen-science/Volunteers approach
Baseline and short-term monitoring	0-1	Bathymetry mapping/new model for current speed, evaluation of construction stability, ecological evaluation Conduct precise baseline surveys focusing on bathymetry, hydrodynamic modelling, reef structure, biodiversity, and habitat functionality	Perform visual inspections and photographic documentation to identify structural stability issues or sediment accumulation
Mid-term monitoring	1-5	Assess colonization patterns, biodiversity recovery, and abundance and key metrics like habitat complexity and functional group dynamics	Use visual observations of species abundance and habitat utilization
Long-term monitoring	5-12	Evaluate reef integration into broader ecosystems, track structural complexity (e.g. functionality), and monitor community shifts (pioneer species vs K-strategy), species interaction and food webs	Track visible structural changes over time through repeated photo documentation

Methods to assess habitat coverage

Habitat coverage assessment forms a fundamental component of boulder reef restoration monitoring, providing essential data on reef structure, spatial extent, and physical characteristics that support ecosystem recovery. Remote sensing technologies including satellite imagery, aerial drone surveys, underwater ROV systems, as well as acoustic systems, and traditional diving surveys all offer valuable approaches for documenting habitat coverage, though each method provides different spatial scales, resolution levels, and accessibility for various project types. While these same survey methods can be effectively deployed for detailed species identification and abundance assessments—applications that will be described in the subsequent chapter, this section focuses specifically on their application for quantifying habitat extent, structural complexity, and spatial distribution of restored boulder reef features.

Drones, or unmanned aerial vehicles (UAVs), are increasingly effective tools for monitoring boulder reef restoration, bridging the gap between the detailed, small-scale coverage of scuba or diving surveys and the broader geographic range of satellite imagery. UAVs are equipped to collect aerial images of reef environments by flying pre-programmed flight paths, either capturing downward (nadir) or oblique-angle images that provide comprehensive spatial documentation of restoration sites. These images can be employed for mapping habitat features and extent, monitoring changes in substrate structure, documenting presence of dominant species, and tracking temporal changes in reef configuration and surrounding sediment patterns.

Modern drones are compatible with advanced sensors, including hyperspectral cameras, LiDAR, and thermal infrared systems, which provide high-resolution data tailored for detecting underwater features and distinguishing between different substrate types (Hamylton, 2017). Hyperspectral sensors enable differentiation between algae species, sediment types, and reef structures through spectral signature analysis, while LiDAR systems can penetrate shallow water to create detailed bathymetric maps of reef topography.

To optimize results and ensure data quality, flights should be conducted between 30 and 80 m altitude at 3–5 m/s during mid-morning or late afternoon when sun angles provide optimal water penetration and minimal glare, under calm conditions (wind < 5 m/s) with minimal cloud cover or haze

that could affect image quality. Water clarity significantly impacts the effectiveness of drone surveys, with optimal conditions requiring visibility >2 meters for substrate identification and >4 meters for detailed species recognition, making timing relative to tidal cycles, weather patterns, and seasonal algal blooms critical for successful data collection.

Acoustic methods, including multibeam and sidescan sonar systems, can be used as tools for mapping marine habitat coverage. Multibeam echosounders provide comprehensive seafloor coverage by emitting multiple simultaneous acoustic beams in a fan-shaped pattern, collecting both bathymetric (depth) and backscatter (acoustic intensity) data across the entire survey swath. This allows for seafloor mapping with high spatial resolution, enabling detection of habitat boundaries, structural complexity, and substrate variations. The backscatter data is particularly valuable for habitat characterization, as different seafloor types return varying acoustic signatures, hard substrates like boulder reefs typically produce high backscatter, while soft sediments generate lower returns. Sidescan sonar provides high-resolution acoustic imagery of the seafloor texture and morphology. While it does not measure depth directly, sidescan sonar allows to identify fine-scale habitat features, biological structures, and substrate patterns through detailed backscatter imagery. these acoustic survey methods are expensive to implement, requiring substantial investment in specialized equipment, vessel time, and skilled personnel. Survey planning typically involves establishing systematic survey lines with appropriate overlap to ensure complete coverage. Data processing requires expert knowledge to apply corrections for vessel motion, sound velocity variations, and geometric distortions, followed by classification of acoustic signatures into habitat types. Ground-truthing through underwater video, photography, or physical sampling validates the acoustic classifications and helps establish the relationship between acoustic signatures and actual habitat characteristics.

Ecosystem functions provided by boulder reefs

Boulder reefs contribute crucial supporting ecosystem services through the key ecosystem function 'enhanced biodiversity.' Additionally, they provide regulating and maintaining ecosystem services due to the key ecosystem function 'erosion and sediment stability.'

Ecosystem function: Biodiversity enhancement

Biodiversity recovery following boulder reef restoration is a gradual process that progresses over an extended timeframe. Initial colonization by opportunistic pioneer species often occurs within the first year. However, the development of a stable and diverse community, including the reestablishment of functional food webs, typically requires 5–12 years or longer. This progression is shaped by factors such as local environmental conditions, the proximity of source populations, and the structural complexity of the restored reef.

Restored boulder reefs not only enhance biodiversity within the site but also contribute to broader ecological functionality for example through reef effect (offering shelter and spawning habitats) and spillover effect (whereby increased biomass emigrates to adjacent areas, supporting surrounding food webs and fisheries). These effects are particularly impactful when restored reefs are situated close to natural, undisturbed reefs or other habitats (i.e. eelgrass beds or biogenic reefs), which serve as reservoirs of species and accelerate colonization. In this way, it is possible to create a connected and functional seascape, strengthening ecological resilience and supporting ecosystem services on a larger scale. This connectivity is crucial for sustaining healthy marine environments and maximizing the benefits of restoration efforts.

The succession of species on restored boulder reefs begins with the colonization by pioneer species, *i.e.*, fast-growing, opportunistic organisms. The goal is the formation of a climax community, a relatively stable and diverse assemblage of species, including K-strategy and keystone species, characterized by slower growth rates, longer lifespans, and higher competitive abilities, contribute to the reef's structural complexity and ecological stability (Taormina et al., 2020). Indeed, a climax community encompasses species across various trophic levels, including predators, herbivores, and detritivores, which together support food webs and functional diversity. Achieving a climax community can take decades, depending on factors such as environmental conditions, habitat connectivity, and species availability. The transition from pioneer species to a climax community is a crucial process for ensuring the long-term ecological success of boulder reef restoration.

Monitoring biodiversity during this process should initially focus on metrics such as habitat coverage and species abundance in the first years following restoration. These early metrics provide essential insights into colonization patterns, the establishment of pioneer species, and the development of structural complexity within the restored reef. Tracking these parameters helps assess the initial progress of the restoration and the effectiveness of habitat creation.

As restoration progresses and the ecosystem matures, later monitoring efforts should assess food web complexity and functional diversity. These advanced metrics evaluate the ecological interactions, trophic levels, and overall functionality of the reef, providing a deeper understanding of ecosystem stability and long-term success. This phased approach to monitoring ensures that restoration outcomes are thoroughly evaluated across both early and later stages of ecological recovery (Table 7).

For details on specific methods for monitoring biodiversity enhancement go to page 37.

Table 7. *Overview of ecosystem function* ***Biodiversity enhancement provided by boulder reef restoration****. The table highlights key parameters, data outputs, expertise levels required (volunteer or expert), the recommended or additional nature of methods, scale of application, timeframe, and associated strengths and weaknesses.*

Supporting ES Ecosystem function	**Indicator**	**Product**	**Method**	**Scientific (S) Non-scientific (N)**	**Expertise level: Specialist (S) Volunteer (V)**	**Recommended (R) Complementary (C)**	**Scale**	**Strength**	**Weakness**	**Processing Level Units**	**Timeframe frequency**	**Performance Criteria**
Biodiversity enhancement	***Habitat coverage***	Coverage of reef or macro species	Drone	S/N	S/V	C	ha	Rapid and cost-effective for large-scale coverage	Only surface areas, depends on weather conditions	Habitat area (m^2)	Before restoration 1-2 months after reef establishment Follow-up ~1 yr after reef establishment (same season) Annual thereafter	**Short term** (1-3 yrs): Indication of higher biodiversity and abundance on restored sites compared to control sites **Long term** (>5 yrs): Statistically higher biodiversity and abundance
		Seafloor maps	Multibeam/Side scan sonar	S	E	C	ha	Detailed, for large scale coverage	Requires expertise, time-intensive data processing, expensive	Habitat area (m^2)	Before restoration Follow-up ~1-5 yr after reef establishment	**Short term** (1-3 yrs): Indication of extent of reef **Long term** (>5 yrs): Indication of extent of reef
	Infauna/epifauna/macroflora/mobile macrofauna	Habitat coverage, species abundance/ diversity/functionality, food-web complexity	Diving (quadrat or video transects)	N	S/V	R	100 m	Rapid and cost- effective, detailed	Limited area	Qualitative/quantitative Number/m^2	Before restoration. 1-2 months after reef establishment Follow-up ~1 yr after reef establishment (same season) Annual thereafter	**Short term** (1-3 yrs): Indication of higher biodiversity and abundance on restored sites compared to control sites. **Long term** (>5 yrs): Statistically higher biodiversity and abundance
			ROV	N	S/V	C	m	Direct visual assessments, habitat-specific details	Requires expertise, time-intensive data processing, requires expertise, depends on weather conditions	Habitat area/coverage, species count (n), density/abundance of fish and pelagic/benthic megafauna, community structure	Before restoration. 1-2 months after reef establishment Follow-up ~1 yr after reef establishment (same season) Annual thereafter	**Short term** (1-3 yrs): Indication of higher biodiversity and abundance on restored sites compared to control sites. **Long term** (>5 yrs.): Higher biodiversity and abundance
			BRUVS/UBRUVS	S	S	C	m	Direct visual assessments, habitat-specific details	Expensive, time-intensive data processing	Species count (n), density/abundance of fish and pelagic/benthic megafauna, food web dynamics	Before restoration. 1-2 months after reef establishment Follow-up ~1 yr after reef establishment (same season) Annual thereafter	**Short term** (1-3 yrs): Indication of higher biodiversity and abundance on restored sites compared to control sites. **Long term** (>5 yrs.): Statistically higher biodiversity and abundance
			Drop camera	N	S/V	C	m	Easy to use, cost-effective, broad range of data	Lower taxonomic resolution, small area	Species count (n), density/abundance of fish and pelagic/benthic megafauna, community structure	Before restoration. 1-2 months after reef establishment Follow-up ~1 yr after reef establishment (same season) Annual thereafter	**Short term** (1-3 yrs): Indication of higher biodiversity and abundance on restored sites compared to control sites. **Long term** (>5 yrs): Statistically higher biodiversity and abundance
			eDNA	S	S	C	100 m	Fast sampling, detect many species including cryptic/NIS, good snapshot of the community	No abundance data, false positives, expensive processing, lacking reference for many species in database	Species count (n) of benthic (from scrapings/panels) or plankton/nekton (water sampling)	Before restoration. 1-2 months after reef establishment Follow-up ~1 yr after reef establishment (same season) Annual thereafter	**Short term** (1-3 yrs): Indication of higher biodiversity and abundance on restored sites compared to control sites. **Long term** (>5 yrs): Statistically higher biodiversity and abundance
			Settlement plates/ARMS	S	S	C	m	Long-term insights into colonization and community dynamics	Results take months to develop, time-intensive data processing	Habitat area/coverage, species count (n), abundance/coverage, community structure	Before restoration. 1-2 months after reef establishment Follow-up ~1 yr after reef establishment (same season) Annual thereafter	**Short term** (1-3 yrs): Indication of higher biodiversity and abundance on restored sites compared to control sites. **Long term** (>5 yrs): Statistically higher biodiversity and abundance

Ecosystem function: Erosion and sediment stability

Boulder reef restoration provides significant coastal protection benefits through wave energy dissipation and sediment stabilization (Bjerregaard & Grolin, 1998; Stone et al., 2005). Boulder reefs effectively attenuate wave energy through multiple mechanisms and the effectiveness of wave attenuation depends on the reef's structural complexity, geometry, and positioning relative to prevailing wave conditions, with protection benefits extending from meters to kilometres from the restoration site.

Monitoring erosion and sediment stability is essential for evaluating the effectiveness of boulder reef restoration and ensuring long-term structural integrity. The monitoring approach should combine recommended methods suitable for volunteer implementation with additional advanced techniques that provide detailed mechanistic insights for expert-led projects (Table 8).

For details on specific methods for monitoring erosion processes and sediment stability go to page 55.

Table 8. *Overview of ecosystem function* ***Erosion-sediment stability*** *provided by boulder reefs. The table highlights key parameters, data outputs, expertise levels required (volunteer or expert), the recommended or additional nature of methods, scale of application, timeframe, and associated strengths and weaknesses.*

Supporting ES Ecosystem function	**Indicator**	**Product**	**Method**	**Scientific (S) Non-scientific (N)**	**Expertise level: Specialist (S) Volunteer (V)**	**Recommended (R) Complementary (C)**	**Scale**	**Strength**	**Weakness**	**Processing Level Units**	**Timeframe frequency**	**Performance Criteria**
Erosion – sediment stability	Erosion – sediment stability	Visual surveys	Diving	N	S/V	R	100 m	Direct visual assessments, habitat-specific details	Requires training, limited area, weather dependent	Qualitative, observations on boulder stability, sediment accumulation patterns, erosion, changes in substrate composition, and overall structural integrity of reef	Before restoration 1-2 months after reef establishment Follow-up ~1 yr after reef establishment (same season) Annual thereafter. To capture detailed data: Monthly/seasonally	**Short-term** (1-3 yrs): Visible settlement and structural integrity of boulders maintained (no collapse, minimal movement). **Long-term** (>5 yrs): Reef structure remains stable, minimal erosion or displacement.
		Sediment flux rates	Sediment traps	S	S/V	C	m	Direct measurement of sediment transport	Short deployment periods, weather dependent	Quantitative, expert: $g \cdot m^{-2} \cdot day^{-1}$, sediment flux rates	Before restoration 1-2 months after reef establishment Follow-up ~1 yr after reef establishment (same season) Annual thereafter. To capture detailed data: Monthly/seasonally	**Short-term** (1-3 yrs): Sediment flux reduced vs. baseline **Long-term** (>5 yrs): Sustained reduction of sediment flux relative to baseline over 1+ years; stable or declining trend across seasons.
		Sediment structure	Sediment sampling (e.g., cores)	S/N	S/V	R	m	Long-term trends, detailed information	Time-intensive, requires expertise	Sediment size classes, porosity, porosity, bulk density	Before restoration 1-2 months after reef establishment Follow-up ~1 yr after reef establishment (same season) Annual thereafter. To capture detailed data: Monthly/seasonally	**Short-term** (1-3 yrs): Early evidence of reduced sediment resuspension and accumulation patterns indicating initial stabilization. **Long-term** (>5 yrs): Sustained changes in sediment structure showing reduced transport and accumulation patterns consistent with long-term stabilization.
		Water turbidity/Chl/nutrients	Water sampling	S/N	S/V	C	m	Real-time data collection, versatile, broad range of data	Requires maintenance, may need calibration	Quantitative, volunteer/expert: Chl-a (µg/L), nutrients (mg/L), O_2 (mg/L), turbidity	Before restoration 1-2 months after reef establishment Follow-up ~1 yr after reef establishment (same season) Annual thereafter. To capture detailed data: Monthly/seasonally	**Short-term** (1-3 yrs): Turbidity reduced relative to baseline. **Long-term** (>5 yrs): Turbidity consistently lower than baseline; nutrient levels stable or slightly reduced seasonally over multiple years.
		Flow velocity profiles, velocity data, bed shear stress, velocity profiles	Acoustic Doppler Current Profiling (ADCP)	S	S	C	m/s	Precision 3D velocity data, links erosion to hydrodynamics	Expensive, requires expertise, weather dependent	Quantitative, expert: bed shear stress (Pa), velocity profiles (m/s), flow patterns	Before restoration 1-2 months after reef establishment Follow-up ~1 yr after reef establishment (same season) Annual thereafter. To capture detailed data: Monthly/seasonally	**Short-term** (1-3 yrs): Measurable reduction in bed shear stress within 1–2 months. **Long-term** (>5 yrs): Sustained reduction in bed shear stress and stable flow patterns preventing erosion for multiple years.
		Digital elevation models	Multibeam sonar/Structure-from-motion photogrammetry	S	S	c	100 m	High-precision mapping, detailed analysis	Time consuming, requires expertise, processing intensive	Quantitative, expert: 3D models, scour pit depth (cm), area (m^2)	Before restoration 1-2 months after reef establishment Follow-up ~1 yr after reef establishment (same season) Annual thereafter. To capture detailed data: Monthly/seasonally	**Short-term** (1-3 yrs): No significant collapse detected in initial post-deployment surveys. **Long-term** (>5 yrs): Stable or minimal change in reef elevation profiles over 1+ years.
		Seafloor maps	Remote sensing – hydrodynamic models	S	S	C	ha	Broad range of data on large area	Requires expertise, time-intensive data processing	Seafloor maps, hydrodynamic models	Before restoration 1-2 months after reef establishment Follow-up ~1 yr after reef establishment (same season) Annual thereafter. To capture detailed data: Monthly/seasonally	**Short-term** (1-3 yrs): Minor detectable changes in reef area. **Long-term** (>5 yrs): Minimal erosion or sediment loss over large scale (ha) for multiple years.

Eelgrass meadows

The most essential monitoring activity after eelgrass restoration is tracking the development of the transplantation in terms of shoot density, biomass and coverage area. These parameters indicate whether the restoration is successful and provide valuable insights for future restoration projects. At the same time, increases in shoot density, biomass, and area form the basis for the ecosystem functions that develop, making them important supporting parameters. In relation to measuring associated ecosystem functions, the restoration area and its development are particularly important to monitor, as the extent of the ecosystem services provided depends on the area and biomass of eelgrass at the restoration site. Drone mapping is a precise and cost-effective tool for tracking areal development. Overview of methods for monitoring of shoot density and area can be seen in table 9 whereas detailed information can be found in the 'Praktisk guideline til ålegræsudplantning og monitering'[5], while area specific biomass sampling is described in this guidelines section 'Immobilization in the Standing Biomass'.

Seagrass beds provide a range of ecosystem functions that contribute to resilient, stable, and healthy ecosystems (Nordlund et al. 2016). Depending on the goal of the restoration project, monitoring the development of these ecosystem functions should be included to assess whether the desired aims/objectives are being achieved through the reestablishment of this habitat type. Of particular importance, seagrass beds support increased biodiversity and nutrient immobilization (Nordlund et al. 2016).

As a minimum, the monitoring plan should include monitoring of shoot densities, biomass and eelgrass area development within the restoration site. These parameters are essential to establish whether the restoration is successful and are described fully within the guideline 'Praktisk guideline til ålegræsudplantning og monitering' and this guidelines section 'Immobilization in the Standing Biomass'.

Previous studies in restored eelgrass meadows have consistently demonstrated a development of positive ecosystem functions if the habitat recovers. Accordingly, it can be assumed that the functions develop if the shoot density, biomass and area increase following transplantation. A minimal monitoring setup should cover these parameters, while measurements of specific ecosystem functions can be omitted if resources are limited. Nevertheless, information on the development of ecosystem functions can be achieved even with limited resources or minimal equipment:

In eelgrass restoration, the development of associated ecosystem functions will depend on the rate of development in shoot density, biomass and areal coverage. Consequently, eelgrass meadows and their ecosystem functions can reach a stable mature state at different time scales, depending on the local environmental conditions. Therefore, the timeframe of the monitoring activities may need to be adjusted as the restored site develops.

Depending on the ecosystem functions being monitored, different time frames for monitoring need to be considered. Some ecosystem functions develop rapidly (e.g., biodiversity), while others may first become apparent or fully developed when the meadow has achieved full coverage. Monitoring programs can be divided into baseline, short-, mid-, and long-term monitoring:

[5] https://www.marinnaturgenopretning.dk/media/72974/praktisk-guideline-til-udplantning_v2.pdf

Baseline (Pre-restoration)

- According to a Before-After-Control-Impact (BACI) sampling design, pre-restoration surveys should always be conducted. Depending on the monitored ecosystem function, this could be sediment samples, biodiversity, and water clarity.

Short-term monitoring (Year 0-1)

During the first year post-restoration, the eelgrass patches will be sparse, and the restoration area will largely remain unvegetated.

- Short-term monitoring should emphasize surveys of transplantation performance such as shoot density development.
- Initial biodiversity colonization can be monitored within sparse eelgrass patches, but care should be taken when using destructive monitoring methods (e.g., sediment cores).

Mid-term monitoring (Years 1-5)

Within 1-5 years, the eelgrass patches will likely reach densities like those of nearby natural meadows. As the restoration develops, it will expand into unvegetated areas between restored patches, increasing the overall area coverage. Depending on the restoration pattern, the area is likely not fully vegetated within 5 years, but most ecosystem functions will have developed within the vegetated patches.

- Yearly monitoring of shoot density development combined with areal development.
- Continue biodiversity monitoring.
- Monitoring of nutrient burial and immobilization

Long-term monitoring (Years 5+)

Within 5-10 years, the restored area is likely to achieve near-complete coverage. As such, most ecosystem functions are likely to be fully developed and comparable to natural reference meadows.

- Yearly monitoring of the area development. Shoot density can be preferably included, as it forms the basis of many ecosystem functions and exhibits yearly fluctuations.
- Yearly biodiversity monitoring until a fully mature (stable state) has been achieved. Afterwards, less frequent surveys (every 2-3 years) can be conducted to track long-term changes.
- Monitoring of nutrient immobilization as the meadow has fully matured.

*Table 9. Monitoring of ecosystem function provided by eelgrass: **Restored eelgrass bed performance**. The table highlights key parameters, data outputs, expertise levels required (volunteer or expert), the recommended or additional nature of methods, scale of application, timeframe, and associated strengths and weaknesses.*

Supporting ES Ecosystem function	**Indicator**	**Methode**	**Units**	**Expertise level: Specialist (S) Volunteer (V)**	**Recommended (R) Complementary (C)**	**Scale**	**Timeframe Frequency**	**Strength**	**Weakness**
Performance of restored eelgrass meadow	Shoot density	Quadrat	Shoot m-2	V	R	cm-m	Monthly. After 1 year - annually	Fast and easy	Require good visibility. In deeper waters require trained divers.
	Biomass	Quadrat	g DW m-2	V	R	cm-m	Annually	Ensures that the entire above-ground biomass is included in the sample. Sampling area large relative to cores	There is a risk of losing parts of the belowground biomass, as the sediment is not collected
		Sediment corer	g DW m-2	V	C	cm-m	Annually	Ensures that the entire below-ground biomass is included in the sample.	the aboveground biomass, as the corer potentially cut the leaves during sampling Small sampling area
	Habitat coverage (area	Drone (RGB)	m2 / ha	S	R	m-km	Before restoration Yearly hereafter	Effective mapping of large areas. Days with good weather and visibility in the water can be targeted to produce high quality outputs.	Can be expensive. Image classification requires expertise.
		Orthophoto	m2 / ha	S	C	m-km	Before restoration Yearly hereafter	Annual aerial orthophotos are freely available in Denmark. Large areal coverage.	Days with good weather or visibility are not targeted and the usability of available images can be limited. Image classification requires expertise.

Ecosystem functions provided by eelgrass beds

Eelgrass beds contribute crucial supporting ecosystem services through the key ecosystem function 'enhanced biodiversity.' Additionally, they provide regulating and maintaining ecosystem services due to the key ecosystem function ‘nutrient burial/immobilisation’.

Ecosystem function: Biodiversity enhancement

One of the most essential ecosystem functions of eelgrass is to provide habitat and nursery ground for a wide range of marine organisms. One single method would not be able to capture the wide range of organisms that live in eelgrass meadows, and several methods need to be utilized to capture the full range of biodiversity (table 10).

The flora associated with eelgrass meadows is predominantly composed of epiphytic algae that grow on the eelgrass leaves. These algae exhibit rapid life cycles characterized by pronounced boom-bust dynamics, with growth and decline occurring over short and often unpredictable timeframes. Due to the high temporal variability and stochastic nature of their blooms, consistent and reliable monitoring of epiphytic algal biodiversity presents significant methodological challenges. As a result, this guideline does not include specific protocols for monitoring the biodiversity of epiphytic algae but will focus on the biodiversity of fauna. The coverage of epiphytic algae on the eelgrass leaves should, however, be monitored as a supporting parameter that indicates the level of eutrophication in the area. This should be done in accordance with the methods described in the ‘Praktisk guideline til ålegræsudplantning og monitering’.

The fauna associated with eelgrass meadows can be broadly categorized into three groups based on key ecological characteristics, including habitat association, body size, and motility. These traits directly influence the choice of appropriate sampling methods for each group.

Infauna – Benthic macrofauna that inhabit the sediment. These species are typically small, slow-moving or sedentary, and require sediment-based sampling techniques (e.g., cores or grabs).

Epifauna – Mobile or sessile macrofauna residing on the sediment surface, within the eelgrass leaf canopy, or attached to eelgrass leaves (epifauna). This group includes species with limited or no motility, and they are typically sampled using enclosure traps (e.g. drop nets) or suction samplers.

Mobile macrofauna – Fish and large crustaceans with high motility that allow them to escape small nets. These species move freely within and beyond the eelgrass meadow and are typically surveyed using methods such as seine netting or fyke nets.

For details on specific methods for monitoring biodiversity enhancement go to page 37.

*Table 10. Monitoring of the ecosystem function **biodiversity enhancement provided by eelgrass**. The table highlights key parameters, data outputs, expertise levels required (volunteer or specialist), the recommended or complementary nature of methods, scale of application, timeframe, and associated strengths and weaknesses.*

Supporting ES Ecosystem function	**Indicator**	**Fauna group**	**Methods**	**Expertise level: Specialist (S) Volunteer (V)**	**Recommended (R) Complementary (C)**	**Units**	**Scale**	**Timeframe frequency**	**Strength**	**Weakness**
Biodiversity enhancement	Species richness, abundance, biomass, composition, diversity, evenness	Infauna	Sediment cores	S/V	R	Species list, species richness per sample, abundance ind. per m^2, biomass g AFDW m^{-2}	cm	Before restoration. 1-2 months after eelgrass restoration (aug or sep). 1 yr after restoration (same season as 2nd monitoring). Annual thereafter.	Strong scientific consensus about the method, reliable, strong area specific quantification	Small sampling area, many samples required, time consuming in the lab, requires taxonomic expert
			Grab	S	C					
			eDNA	S	C	Species list, species richness, presence/ absence data	100 m to km		Fast sampling, covers large areas to get a snapshot of community	Expensive, quantification uncertain
		Epifauna	Drop net	S	R	Species list, species richness per sample, abundance ind. per m^2, biomass g AFDW m^{-2}	m		Direct detailed area specific sampling, common methods	Requires divers, fast mobile species escape, data quality depends on the experience of the person sampling
			Shrimp net	V	R					
			Suction sampler	S	C					
			Epibenthic sledge	S	C					
			Net or plastic bag	S/V	C					
			eDNA	S	C	Species list, species richness, presence/ absence data	100 m to km		Fast sampling, covers large areas to get a snapshot of community	Expensive, quantification uncertain
		Ichthyofauna and large crustaceans	Seine net	S	R	Species list, species richness per sample, abundance ind. per m^2, biomass g WW m^{-2}	100-1000 m		Area specific sampling, efficient capture of fast swimming species, captures both demersal and pelagic fish	Can be destructive in newly planted eelgrass, time consuming in the lab, can be difficult to use in deep eelgrass meadows
			Beam- or otter-trawl	S	C					
			Fyke net	S/V	R	Species list, species richness per sample, abundance CPUE, biomass g WW CPUE			Day and night sampling, easy to deploy, effective at capturing most fish species	Not area specific, maintenance required, risk of by-catch of birds and mammals
			Gill nets	S/V	C					
			Traps	S/V	C					
			Baited or unbaited camera	S	C		m		High-resolution video over longer periods, monitoring species abundance and length measurements	Expert identification needed, calibration needed, limited coverage in turbid waters
			Visual census	S	C	Species list, species richness, abundance	10-100 m		Direct visual assessments, easy to use, cost effective, large area coverage	Expert quantitative data, no direct sampling, limited by visibility
			eDNA	S	C	Species list, species richness, presence/ absence data	100 m to km		Fast sampling, covers large areas to get a snapshot of community	Expensive, quantification uncertain

Ecosystem function: Nutrient burial and immobilisation

Eelgrass beds enhance nutrient immobilization and export through three main processes: growth dependent uptake into the biomass, burial in the sediment, and stimulation of microbial denitrification (Figure 3).

Uptake into biomass consists of two sub-processes, 1) immobilization in the standing biomass, and 2) temporary immobilization through continuous leaf production and shedding. The uptake and immobilization of nutrients in the eelgrass biomass reduces nutrient availability for epiphytes, phytoplankton and opportunistic macroalgae, supporting the development of a more stable ecosystem.

The eelgrass biomass in Denmark reaches its minimum in winter (January–February) and its maximum in late-summer/early autumn (August-October). The winter biomass represents permanent storage, while the difference between winter and summer biomass reflects temporary immobilization during the growing season. In addition, eelgrass continuously produces and sheds leaves throughout the growing season. These leaves decompose slowly, which results in temporary nutrient immobilization and reduced turnover during this period. Overall, the accumulation of biomass during the growing season, combined with continuous leaf production, results in nutrient immobilization at a time when it is most critical. Release occurs outside the growing season, when nutrient availability has less negative ecological impact.

Burial in the sediment occurs through direct deposition of dead eelgrass biomass, including roots, rhizomes, and leaf fragments. Additionally, the eelgrass rhizomes and roots stabilize the seabed, while the leaf canopy reduces currents and wave action. This leads to decreased erosion of the underlying sediment and increased accumulation of organic particles. Following eelgrass reestablishment, a new state will eventually form, where the input and export of organic material to the sediment are balanced to a new equilibrium, which is higher than in the reference condition. At that point, no further net burial takes place.

Microbial denitrification is stimulated by the presence of eelgrass, increasing the microbial conversion of nitrate to atmospheric nitrogen (N_2).

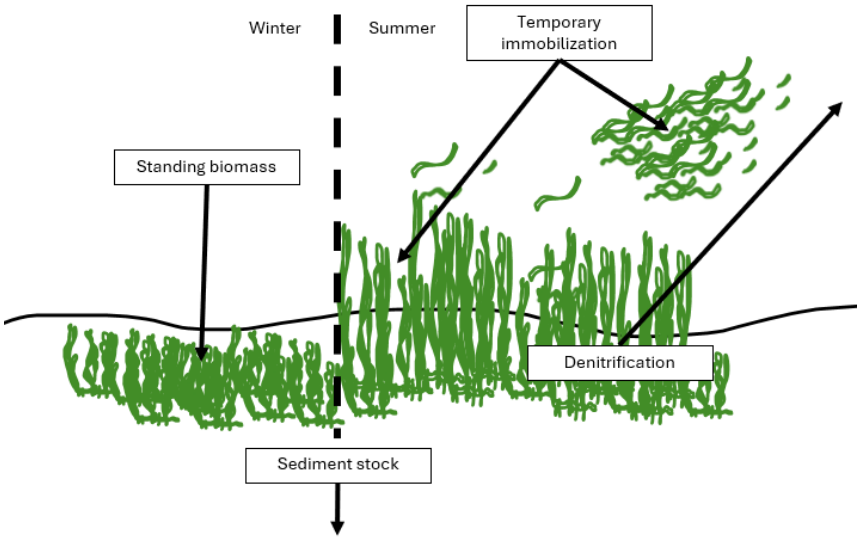

Figure 3. Conceptual diagram of the different processes governing nutrient immobilization within seagrass meadows.

The monitoring methods for quantifying nutrient burial/immobilisation can be found in table 11. For details on specific methods for monitoring nutrient burial and immobilisation go to page 48.

*Table 11. Monitoring of the ecosystem function **Immobilization and export of nutrients and carbon provided by eelgrass**. The table highlights key parameters, data outputs, expertise levels required (volunteer or specialist), the recommended or complementary nature of methods, scale of application, timeframe, and associated strengths and weaknesses.*

Supporting ES Ecosystem function	**Indicator**	**Parameter**	**Methods**	**Units**	**Expertise level: Specialist (S) Volunteer (V)**	**Recommended (R) Complementary (C)**	**Scale**	**Timeframe Frequency**	**Strength**	**Weakness**
Immobilization and export of nutrients	Immobilization in the standing biomass	N & P	Quadrat	Nitrogen content in biomass (g N m^{-2}) Phosphor content in biomass (g P m^{-2})	S	R	cm-m	Yearly or biannual after restoration	Ensures that the entire aboveground biomass is included in the sample. Sampling area large relative to cores	There is a risk of losing parts of the belowground biomass, as the sediment is not collected
		N & P	Sediment corer	Nitrogen content in biomass (g N m^{-2}) Phosphor content in biomass (g P m^{-2})	S	C	cm-m	Yearly or biannual after restoration	Ensures that the entire belowground biomass is included in the sample.	There is a risk of losing parts of the aboveground biomass, as the corer potentially cut the leaves during sampling Small sampling area
	Leaf production	N & P	Plastochrone interval	Nitrogen content in biomass (g N m^{-2}) Phosphor content in biomass (g P m^{-2})	S	R	Ind. Shoots	Require monthly sampling. Surveys conducted yearly or every second year	The optimal method for obtaining the highest number of replicates, thereby ensuring the best possible representation of the bed	Operates under the assumption that all leaves are based on the average leaf size (the third-youngest leaf), so individual leaf growth is not accounted for
	Sediment stocks	N & P	Sediment core liners	Nitrogen content in biomass (g N m^{-2}) Phosphor content in sediment (g P m^{-2})	S	R	cm-m	Before restoration Yearly hereafter	Ensures that a fixed depth can be sampled and allows for sectioning in the laboratory to analyse the content of individual layers	There is no scientific consensus on the appropriate depth for collecting sediment cores Small sampling area
	Denitrification	N	-	Denitrification rate (g N yr^{-1})	S	-	-	-		No available methods can confidently quantify the process *in situ*.
Immobilization of carbon	Immobilization in the standing biomass	C	Quadrat	Carbon content in biomass (g C m^{-2})	S	R	cm-m	Yearly (Jan-Feb)	Ensures that the entire aboveground biomass is included in the sample. Sampling area large relative to cores	There is a risk of losing parts of the belowground biomass, as the sediment is not collected
		C	Sediment corer	Carbon content in biomass (g C m^{-2})	S	C	cm-m	Yearly (Jan-Feb)	Ensures that the entire belowground biomass is included in the sample.	There is a risk of losing parts of the aboveground biomass, as the corer potentially cut the leaves during sampling Small sampling area
	Dead eelgrass biomass	C	Sediment corer	Carbon content in sediment (g C m^{-2})	S	R	cm-m	Yearly	Sampling area large relative to cores	No option to separate stocks according to depth layers

Methods for monitoring ecosystem functions

The design of a monitoring programme should ensure a minimum foundational level of monitoring even with limited resources. This minimum programme ought to include assessments of habitat performance metrics (refer to Biogenic reefs in Table 2, Boulder reefs in Table 7, and Eelgrass in Table 9), which are vital for determining restoration success. If additional resources are available, monitoring can extend to specific key ecosystem functions.

The subsequent sections provide descriptions of various monitoring methods tailored to key ecosystem functions provided by the three habitat types, recommending the inclusion of one or more such functions in the monitoring programme if resources permit. The monitoring programme can be expanded further to cover minor ecosystem functions (see Table 1), but this should only be considered after a comprehensive monitoring programme for the key functions is already in place. Prior to initiating fieldwork, a clear and robust sampling design must be developed. This design should align with the overall project objectives and consider available financial resources and the level of taxonomic expertise within the project team. Ideally, the sampling approach should follow a Before-After-Control-Impact (BACI) design.

Monitoring Biodiversity enhancement – *biogenic reefs, boulder reefs and eelgrass beds*

Area-based methods are recommended for monitoring biodiversity, as they enable direct comparisons between the spatial extent of habitat restoration and the resulting ecosystem functions. By standardizing sampling to a defined area, it becomes possible to quantitatively assess key ecological metrics such as species richness, animal abundance and biomass in the restored area. These metrics can be used to calculate essential diversity metrics such as Shannon diversity index and Pielou's evenness. Additional factors that must be considered include sampling frequency, number of replicates and area sampled. Due to the considerable effort and time required to process faunal samples, it is often necessary to prioritize either high sampling frequency or a high number of replicates, balancing these components according to the specific aims and constraints of the project.

With regards to sampling frequency, it is important to consider seasonality, as the number of species and their abundance and biomass will change over the course of the year. Thus, using recurrent sampling periods is necessary to be able to compare and monitor successional patterns over multiple years.

Identifications of species to fine taxonomic levels are time consuming and require significant expertise within the project group or economic recourses for consultants and might therefore not be possible. If resources or expertise are lacking within the project group, we recommend focusing on counting the number of species or groups (e.g., worms, bivalves, echinoderms, crustaceans) present instead. However, identifying to the species level enables the characterization of their biological traits and, consequently, the functional diversity of the species community.

A minimum of four replicate samples is recommended for each of the three faunal groups (epifauna, infauna end mobile fauna) to detect statistically meaningful patterns in biodiversity. However, for infaunal communities, it is advisable to increase the number of replicates when feasible within the project's logistical and financial constraints. This recommendation is based on two factors: (1) the relatively small area covered by each infaunal sample, and (2) the patchy, aggregated distribution patterns commonly observed among infaunal species within the sediment.

With regards to the area that needs to be sampled, it is important to acknowledge that animal abundance varies across a range of spatial scales, depending mostly on body size, and that the method for capturing them must be adapted to the targeted organisms. For example, infauna such as polychaetes often aggregate in clusters at scales of 10 cm, mobile epibenthic macrofauna such as shrimp or snails can be quantified at scales of a few meters, while fish and larger crustaceans

may be dispersed in the habitat at scales of 100 meters (Figure 4). Therefore, the sampling devices used for capturing each of the fauna groups need to be dimensioned to the scale at which the animals are present in the ecosystem. Effective sampling of fauna with varying body sizes and abundances requires a hierarchical sampling design. Large, mobile species such as fish can be sampled using gear that encloses a large area (e.g., a seine net, representing the largest quadrat in Figure 4). Within one of these large quadrats, medium-sized organisms such as shrimp and gastropods are sampled using multiple sub-quadrats (e.g., with drop nets, representing the medium sized quadrats in Figure 4). Finally, small and highly abundant invertebrates are sampled from even smaller sampling units, nested within the sub-quadrats (e.g. with cores, representing the smallest quadrant in Figure 4). This nested approach ensures adequate representation of species across different size classes and mobility levels.

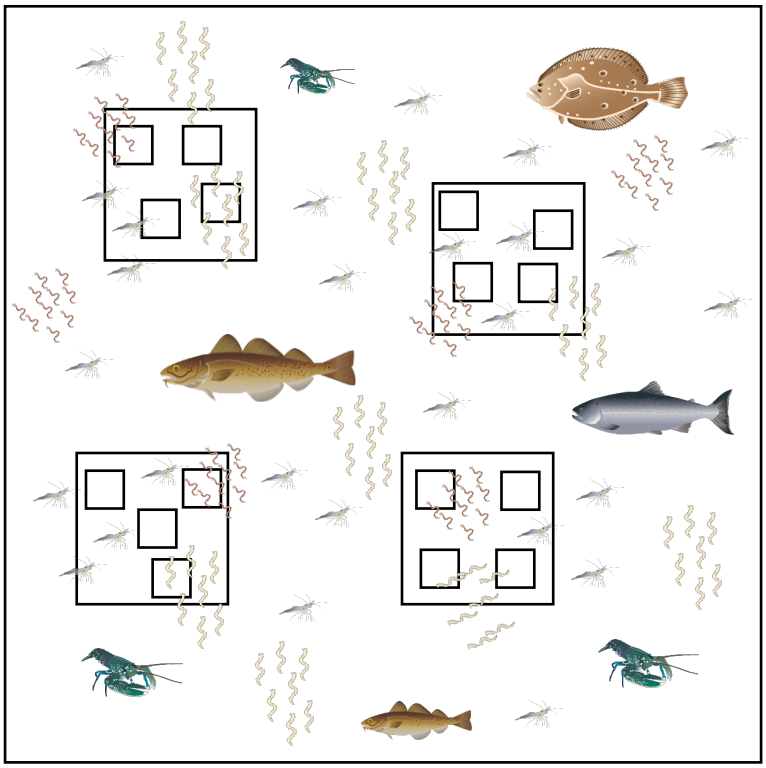

Figure 4. Graphic representation of a recommended sampling design for capturing all three faunal groups despite their differences in area specific abundance and body size. The largest quadrant represents a seine net for capturing mobile fauna. The medium sized sub-quadrats represent a drop net for capturing epifauna and the smallest quadrant represent sediment cores for capturing infauna, based on (Eleftheriou & McIntyre 2005).

The following sections provide detailed descriptions of the recommended methods for monitoring each of the three faunal groups associated with restored habitats.

Methods for sampling infauna species

A power analysis should be done to calculate the optimal number of replicates in function of budget limitations. For detailed information for collection of infauna samples we refer to the technical guideline TA no. M19 ´Blødbundsfauna´[6] for monitoring the marine environment, which have

[6] https://ecos.au.dk/fileadmin/ecos/Fagdatacentre/Marin/TA_M19_Bloedbundsfauna_ver3.pdf

been prepared for the Danish Environmental Protection Agency by the Marine Data Centre as well as the Field Manuals for Marine Sampling to Monitor Australian Waters[7].

Sediment cores – core or Haps

The advantage of using a sediment corer is to maintain the vertical structure of the sample analysed. It allows to subsample at different depths of the core to study the distribution of the species. Sediment cores (area: 0.01-0.02 m^2) can be collected by divers or operated from boats. It is recommended to take multiple randomised cores within both control and impact areas. Haps core can be used from a boat to allow infauna sampling without divers. After collection, the core samples should be sieved (e.g. 1mm sieve) and the animals preserved in 70% ethanol and later sorted and analysed in the laboratory.

Grab samples

The advantage of the grabs is that large volumes can be taken, but the integrity of the vertical structure of the sediment is not kept. Samples are usually homogenised, thus overlooking the potential depth vertical gradient of species distribution. Grabs, van Veen or box corers (area: $\geq$0.1 m^2) are operated from boats. There are different sizes, and the most common can be quite heavy and hard to operate from smaller boats. They have a larger sampling volume to sort and analyse for infauna compared to cores and therefore they might catch the distribution variability of the species in a better way than cores, and thus fewer samples might be needed. For mussel beds, the grabs are often more difficult to sample, as the grabs might slide and not penetrate the sediment. The processing of the sediment follows the same procedure as the cores.

Environmental DNA (eDNA)

Debris, mucus, faeces and other material from organisms can be detected in the environment using eDNA techniques. eDNA can be sampled at a large scale, are efficient to identify organisms at low densities and organisms that are missed with other sampling methods can often be detected with eDNA sampling. On the other hand, eDNA sampling faces challenges with quantifying species abundance and the risk for false positives and negatives due e.g., to transport of eDNA to an area or former presence of species. Furthermore, only species already in the database can be identified. The eDNA analysis require specialised equipment and experts and can therefore often be rather expensive and furthermore, it is recommended to complement with other methods for comparison. We are referring to De Brauwer et al. 2023 for more details about eDNA biomonitoring.

eDNA offers a rapid, high-throughput method for monitoring biodiversity by extracting DNA from different compartments such as water and sediment/substrate scrapings: water samples capture recent, transient or pelagic species before DNA degrades, while sediment or substrate samples provide a more persistent record of benthic communities. All tools used to collect samples must be sterilized beforehand with 10% bleach or ethanol and gloves need to be worn to ensure sterility. Using a water sampler (e.g. Niskin), water must be collected at a certain depth and approximately 0.5-1.5 L through the sterivex filter attached to the pump through silicone tubing, placing the sterivex in a graduated container to check for water volume filtered (Figure 5). Between sites, the pump should be flushed 5-6 times with local water and/or sterilize with 10% bleach or ethanol. For sediment sampling, divers or snorkelers should first position and photograph a quadrat randomly before scrape biofilm and organisms in multiple directions, and deposit material into labelled

[7] https://marine-sampling-field-manual.github.io

tubes/buckets. Water and sediment samples must be kept on ice in the field and directly frozen at -20 °C or -80 °C after filtering, and shipped to specialized laboratories for DNA extraction, sequencing and analysis. Scraping/sediment samples must be kept on ice and frozen or directly preserved in ethanol 96-99%.

Figure 5. Water pump with silicone tubing and sterivex attached.

Methods for sampling epifauna species and macroflora

Monitoring the biodiversity of sessile epifauna and macroflora species on hard substrates necessitates an adequate spatial distribution over the restored area. The recording of sessile epifauna and flora depends on whether their growth pattern is colonial or solitary. Colonial species such as sponges, bryozoans or encrusting organisms are recommended to be assessed as percent cover, while solitary species are counted as number of individuals per area. For mussel beds, potential variation in mussel densities across the mussel bed should be considered as transect methods might be better in areas with low mussel densities and discrete sampling methods with small and intense samples is better in areas with high mussel densities. Likewise, monitoring invasive species, predators or competitors might be crucial in some areas to understand the ecological dynamics and interactions in mussel bed development. Further details regarding sampling epifauna and macroflora on hard substrate can be found in the technical guideline TA no. M17 ´Fauna på kystnær hårdbund´[8] and TA no. M12 ´Makroalger på kystnær hårdbund´[9] for monitoring the marine environment.

Sampling epifauna on hard substrates cannot be directly compared to sampling epifauna in eelgrass beds as the habitat forming structures differ substantially and the organisms associated also differ in relation to e.g., motility. Not all methods applicable on hard substrates may thus be suited for sampling epifauna in eelgrass beds. Drop nets and shrimp nets are thus primarily of use for organisms with low motility in eelgrass beds and not for sessile organisms sitting on hard substrates.

Quadrat sampling by SCUBA diver

The quadrat (0.05-0.6 m^2) is randomly place by the diver at multiple locations within the restored and in control areas. All alive organisms (incl. shells for mussel beds) are collected in each quadrat sample by the diver. Each sample is sorted into species groups, and all sessile organisms are identified to the lowest taxonomic level possible and enumerated per sample. Organisms that cannot be removed without being destroyed (e.g., barnacles) should just be enumerated. For each

[8] https://ecos.au.dk/fileadmin/ecos/Fagdatacentre/Marin/TA_M17_Fauna_paa_kystnaer_haardbund_ver2.pdf
[9] https://ecos.au.dk/fileadmin/ecos/Fagdatacentre/Marin/TA_M12_Makroalger_paa_kystnaer_haardbund_ver3.pdf

sample, record the total wet weight (g) and number for each species. If bivalve species are observed measure morphometric shell length/shell height of all or minimum of 30 individuals of each species.

Video transects by SCUBA diver or Remote Operated Vehicles (ROVs)

Underwater visuals using divers (SCUBA or snorkelling), remote operated vehicles (ROVs) or towed video sledges (Figure 6) are useful to assess the abundance of epifauna and macroflora, especially in areas without strong currents or poor visibility. Transects should be pre-defined at random locations across the restored and control sites. To assess the number of species identified per surveyed area, a consistent field of view and an accurate scale are required to measure the field of view. This can be achieved by using laser pointers (sledge or ROV) or frame (diver), which must be pre-calibrated to determine both the field of view and the scale before deployment. Furthermore, it is important to keep a fixed distance typically <1 m from the seabed during the survey when visibility conditions are limited. To obtain the best conditions for analysing the videos, the transects must be surveyed at very slow speed e.g., 1-2 minutes per 10 m transect for divers or maximum speed of one knot for towed sledges and only species observed within the lasers or frame is included in the analysis. The total length of each surveyed transect is reported by either video systems or ROVs equipped with acoustic systems and GPS to plot the position or by handheld GPS/smartphone. The area covered (m^2) is calculated based on the distance travelled (e.g., start and end GPS positions or course plot/average speed) and the field of view. Key parameters should be recorded at fixed distances, keeping a defined distance from the bottom (1 m). Observations focus on substrate composition, including mud, sand/gravel, small stones, and large boulders, as well as associated flora and fauna such as seagrass, algae, fish, and invertebrates.

Water visibility may significantly affect survey effeciency, with optimal conditions requiring visibility >3 meters for species identification and >1.5 meters for basic habitat assessment, while turbid conditions may necessitate slower survey speeds (0.3-0.5 m/s) and closer proximity to substrates to maintain data quality.

Data processing requires specialized software for video analysis, with footage typically reviewed at 1-4x speed for species identification and habitat characterization, while still images extracted at regular intervals (every 10-30 seconds) enable detailed substrate analysis and percent cover calculations using point-intercept or quadrat-based methods. In very shallow reef zones, strong wave action and tight spaces between boulder structures may significantly limit ROV manoeuvrability and compromise data quality through increased turbidity, unstable camera positioning, or potential collision risks with reef structures, requiring modified survey protocols with shorter transect segments and increased use of hover stations for detailed observations. For citizen science applications, ‘mini-ROVs’ provide a lighter, more cost-effective alternative that enables volunteers trained in standard survey protocols to underwater surveys on shorter transects (20–50 m) at similar operational speeds (0.5-1 m/s). These smaller systems typically offer 2–4 hours of battery life, simplified control interfaces, and basic data logging capabilities that make them accessible to non-expert operators while still providing valuable data on species presence, habitat characteristics, and basic community structure parameters that contribute to overall restoration monitoring objectives. Furter information can be found in the Field Manuals for Marine Sampling to Monitor Australian Waters[10]

[10] https://marine-sampling-field-manual.github.io/

Figure 6. Schematic view of underwater video transects monitoring methods for blue mussel reefs. AI-generated image by OpenAI 2025.

Drop-down camera with quadrat

Sessile epifauna and macroflora can be assessed through analysis of images of quadrats taken by drop-down cameras or by divers.

During deployment, the camera is lowered to a predetermined depth and placed at a fixed distance from the seabed, then held stationary for a few minutes to prevent sediment resuspension and ensure full quadrat coverage, while georeferencing the site and recording the exact depth. Footage is analysed post-deployment to assess species presence, abundance, and behaviour, as well as habitat characteristics. It is fundamental to standardized deployment durations, angles, and site conditions (e.g., visibility and current strength) to ensure consistency and comparability across sampling events.

This method is particularly useful in areas with limited diver access, providing a cost-effective, non-invasive tool for tracking ecological recovery and habitat changes in restoration projects. However, in Danish waters, visibility can be highly variable due to suspended sediments and seasonal algal blooms, which may affect the quality of footage. Before capturing images, the camera must be positioned to cover the entire quadrat or a known area within its field of view. The sample locations should be randomly pre-assigned but the actual position (GPS coordinates) and water depth at each sampling locations should be recorded for georeferencing. At each station the drop-down camera is gently lowered until it reaches the seabed. Wait a few minutes for resuspended sediment to settle before capturing the image. Post-analysis to assess/identify species and coverage require a trained person with targeted species identification skills and can often be supported by specific software tools.

Environmental DNA (eDNA)

Details see infauna sampling

Sampling epifauna with drop net

The drop net consists of a circular rigid metal frame with a diameter of 1 meter, to which a fine mesh bag (1 mm mesh size) is securely attached. The mesh bag is fitted with a cod end that can be opened for efficient sample retrieval. This kind of sampling is particularly used in eelgrass meadows. Sampling is initiated by deploying the drop net from a boat directly onto the eelgrass meadow. A diver then collects the sample by emptying the net using a hand-held net of matching mesh size, and the collected organisms are transferred to a bucket. After three consecutive hand-net sweeps without capturing additional fauna, the drop net is visually inspected to ensure all animals have been collected and that the sample is complete. All specimens are then transferred to labelled zip-lock bags and put on ice in the field for later processing.

In the laboratory, samples are processed using the same methodology as for infauna: animals are sorted, identified to the lowest possible taxonomic level (ideally species), counted, and measured for biomass. Biomass is determined through drying and incineration to calculate ash-free dry weight.

Sampling epifauna with shrimp net

Hand-operated shrimp net sampling is suited for non-experts and volunteers in eelgrass meadows, because it does not necessarily require diving, at least not in shallow waters.

The recommended net for this method is 60 cm wide shrimp net, with a 1 mm mesh size. Sampling involves pushing the net firmly and quickly across the substrate along a transect with a pre-defined length (e.g., 6 m transects has been used in previous studies). To minimize variability due to sampling technique (e.g., speed, netting force, or accuracy), it is recommended that all netting is performed by a single person. This method effectively targets slow moving epifauna but underrepresent fast-swimming species that escape the net. All captured organisms are transferred to a bucket filled with fresh seawater and the sample can either be processed directly on shore or put on ice for later processing in the lab.

If the samples are processed on shore, all specimens need to be photographed with a ruler for scale, weighed, and identified to the lowest possible taxonomic level. Images can later be analysed using programmes, such as ImageJ software, to measure body sizes and determine biomass based on species-specific length–weight relationships.

The laboratory procedure for drop net sampling should be followed if the samples are processed in the lab.

ARMS (Autonomous Reef Monitoring Structures) and settlement plates

Autonomous Reef Monitoring Structures (ARMS) and settlement plates are valuable tools for monitoring biodiversity at boulder reef restoration sites. ARMS are internationally standardized devices deployed globally[11] designed to assess hard benthic substrates by monitoring both motile organ-

[11] https://www.oceanarms.org/deployments/search

isms inhabiting their three-dimensional structure and sessile organisms attached to the plates during deployment. ARMS perfectly mimic the complexity of boulder reef habitats, providing shelter for small invertebrates and fish while also serving as a substrate for sessile organisms. Samples from ARMS are analysed through a combination of morphological and genetic techniques, with motile organisms sorted into size fractions and sessile organisms scraped after photo-documentation. Settlement plates, while lacking the three-dimensional complexity of ARMS, are simpler to deploy and retrieve, making them accessible for use by citizen scientists or volunteers. Like ARMS, settlement plates support the integration of photography, morphological taxonomy, and possibly eDNA metabarcoding biodiversity monitoring in natural/restored areas. ARMS should be deployed for a minimum of 6-12 months to allow sufficient time for community development and colonization by cryptic organisms (Daraghmeh et al., 2024, Obst et al., 2020). For studies on temporal succession, deployments can extend over several years with regular monitoring intervals.

To ensure statistical robustness and account for spatial variability, a minimum of 3 ARMS units should be deployed as replicates at each study site, positioned at similar depths and environmental conditions to serve as biological replicates, preferably at a distance of around 10 m from each other to obtain a comprehensive representation of surrounding communities and to improve statistical power for comparative analysis (Obst et al., 2020; Pearman et al., 2020; Sembiring et al., 2023). When deployed, ARMS units can be bolted directly onto boulder reefs or positioned on 50 × 50 cm tiles and set within about 5 m of the reef (Figure 7). Upon retrieval, each plate is dismantled: sessile organisms are scraped or brushed off, while motile fauna is washed and size-fractionated through sequential sieves, then morphologically sorted for voucher specimens and preserved (typically in >90% ethanol) for downstream DNA barcoding and metabarcoding. Before imaging, all containers and tools are bleached, rinsed, and handled with gloves; plates are placed one at a time in a bleached photo tray in filtered seawater, labelled with Site-ARMS-Plate tags, and photographed, first overall, then with 4-9 overlapping close-ups per side (15-20% overlap).

High-resolution plate images are white-balanced, cropped, and resized (≈5000×5000 px at 300 dpi) before annotation in CoralNet using a standardized ARMS label set and uniform grid of points. Finally, annotated data are exported as percent-cover and point-by-point CSV files, adjusted for unavailable settlement space, and merged with metadata for comparative biodiversity analyses across sites and time. For further details see the ARMS protocols from National Museum of Natural History[12].

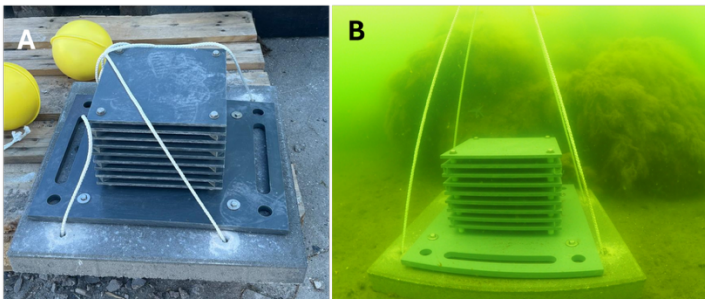

Figure 7. A) ARMS anchored on a 50 x 50 cm cement tile and submerged buoy attached. B) ARMS deployed close to restored boulder reef.

[12] https://naturalhistory.si.edu/research/global-arms-program/protocols).

Methods for sampling mobile fauna

Mussel beds, boulder reefs and eelgrass habitats can provide shelter, nursery or feeding habitat for mobile fauna such as fish, crustaceans and contribute to biodiversity and is an indicator of ecosystem health, while recordings of predators e.g., crabs, lobsters or starfish might be crucial in some areas to understand the ecological dynamics and interactions of the survival of the mussel beds. Mobile organisms can be difficult to monitor by using mobile methods, as the movements of divers or towed sledges might scare them away or the mobile is so small that they are difficult to see or catch. Therefore, it can be beneficial to use static methods to monitor mobile organisms associated to the restored habitats.

Static video camera systems - baited or unbaited

Underwater video systems are a powerful and efficient tool for monitoring mobile macrofauna if the visibility allows. Remote underwater video (RUV) can be either baited (BRUVs) or unbaited (UBRUVs). The baited systems might be an advantage in areas with low visibility. Each setup consists of a weighted frame, cameras and waterproof camera housing and for BRUVs also a bait arm and bait cage/bag (Figure 8). It is recommended to use cameras with full, high-definition resolution >1080 for better species identification, a capture rate of >30 frames per second to reduce blur from fast moving species and medium field of view to limit distortion in the image. For stereo-setup (see below), video stabilisation of the cameras must be disabled to maintain the calibration and furthermore, it is recommended to use a fixed focal length to facilitate measurements of species both close to and far from the camera setup.

The (B)RUV can be either mono-setup or stereo-setup, where the stereo-setup allows for e.g., fish lengths to be measured but requires calibration to ensure that any length measurement based in the recordings from both cameras are accurate. Pre-calibration before deployment and potentially also post-calibration after retrieving the RUVs is recommended, as any shift of the position, angle etc. of the camera during the field work will result in wrong length measurements. A calibration should include information about the distance between the base of the housings, the angle of each camera and lens distortion and each stereo-setup should be calibrated separately.

The distance between individual (B)RUVs will depend on the mobility of the species, the habitat being studied and the timeframe for the deployments but are typically >200 m to reduce the likelihood of individual animals being sampled by adjacent (B)RUV systems. The GPS coordinates at each location should be recorded. The timeframe of recordings can either be continuous for e.g., 1 h after reaching the seafloor (remember to note the time of deployment or have the correct camera time) or record in short time intervals (few minutes) e.g., every hour during daylight until retrieving. Allow an initial short adaptation time (e.g. 15 minutes) at the start of each deployment for the fauna to adapt to the presence of the (B)RUVs. Data analysis of the video footage should be processed to measure species abundance and diversity, the maximum number of individuals per species (max N) recorded per timeframe (depends on the time settings). Behavioural observations, such as species interactions, feeding habits, and territorial displays, are also documented, along with habitat features like substrate type, topography, and associated flora and fauna visible in the footage. They effectively attract a wide variety of species, increasing data richness and are suitable for deployment across a range of depths and habitats, from shallow coastal areas to deeper marine zones.

UBRUVS rely on organisms naturally passing through the camera's field of view without the influence of a bait plume, making them ideal for assessing species-habitat relationships without potential biases introduced by bait. While UBRUVS often record fewer individuals due to the absence of bait attraction, they can provide more robust data on natural species distributions and behaviours. For successful application, bait selection for baited system and deployment protocols for both type

of systems must be standardized to ensure consistency across sampling efforts. The spacing between BRUV units must account for the range of the bait plume to maintain sampling independence, while environmental factors such as water visibility and current strength should be carefully considered during study design. More information can be found in the Field Manuals for Marine Sampling to Monitor Australian Waters chapter 5[13] and 6[14].

Figure 8. UBRUVS with GoPro's camera (bottom).

Video transects by SCUBA diver or Remote Operated Vehicles (ROVs)

Details see epifauna species and macroflora sampling.

Traps (fyke-nets, pots) mark and recapture sampling

Fish and large crustaceans can be captured by deploying fyke nets or baited pots. Fyke nets can capture fish swimming from either direction toward the nets, whereas pots often are used to capture crustaceans like lobsters and crabs. The nets and pots should be deployed at random locations within the restored habitat and control sites, and it is recommended to cover both day and night sampling. Cameras can be deployed at the nets and pots to record escapees and catch successes. The fyke nets that are common to use in Denmark are DBL 80/7 models. A standard setup involves deploying four fyke nets, which are emptied and maintained every 24 or 48 hours. All fish are identified to the lowest possible taxonomic level (ideally species), counted, and measured for total length in the field before being released. Fish biomass can be estimated using species-specific length–weight relationships, which are available from FishBase.org.

Fishing can be carried out year around, but due to national regulations, there is a closed season for fyke net fishing from May 10 to July 31. However, a dispensation granted by the Danish Fisheries Agency could allow limited fyke net sampling during that period if all captured European eels (*Anguilla anguilla*) are immediately released in compliance with conservation regulations.

Ethical and animal welfare review (e.g., soak time, gentle removing of organisms from the nets and traps and storage until species identification) will be required for all methods involving fish sampling and considered for crustaceans. All mobile organisms should be released afterwards. If species identification cannot be reliably completed on site, individuals are humanely euthanized using a percussive blow to the head, in accordance with ethical guidelines from the Danish Animal Ethics

[13] https://benthic-bruvs-field-manual.github.io/
[14] https://pelagic-bruvs-field-manual.github.io/

Council and the European Food Safety Authority (EFSA). These specimens are then placed in labelled zip-lock bags and frozen for later identification in the laboratory.

Sampling using pots and nets provides catch rates (catch per unit effort, CPUE) that are related to abundance, and function as a relative abundance/density indicator. To estimate abundances (e.g. number per area) using traps, a capture-mark-recapture approach must be used (e.g. Chapman, 1951; Munch and Petersen, 1982; Schwarz and Seber, 1999).

Sampling with seine net

Fish and larger mobile crustaceans can be effectively sampled using a beach seine net. While seine net dimensions may vary, it is essential that the net can sample an area between 250 and 1000 m², to account for the relatively low fish densities typically observed in Danish coastal waters.

A recommended configuration for assessing fish biodiversity in eelgrass habitats, consists of a seine net 5 m wide and 2 m high, with a mesh size of 3.5 mm. The net is constructed with a cod end to collect the catch, a weighted lead line at the bottom to ensure contact with the seafloor (capturing benthic species), and a float line at the top to keep the net upright in the water column.

The net is towed across a standardized area of at least 5 × 50 meters (250 m²). After towing, the catch is transferred into a container filled with seawater for processing. All fish are identified to the lowest possible taxonomic level (ideally species), counted, and measured for total length in the field before being released. Fish biomass can be estimated using species-specific length–weight relationships, which are available from FishBase.org. Ethical guidelines from the Danish Animal Ethics Council and the European Food Safety Authority (EFSA) should be followed (see also section above on traps (fyke-nets, pots) sampling.

Environmental DNA (eDNA)

Details see infauna sampling.

Monitoring nutrient burial/immobilisation – *eelgrass beds and biogenic reefs*

Nutrient immobilization is an essential ecosystem function within both eelgrass beds and biogenic reefs. However, the processes governing this ecosystem function are distinctly different between the two habitats, and, as such, the recommended methods are different. Below, the recommended methods are described individually for each habitat. For an overview of the methods, refer to Table 4 (Biogenic reefs) and Table 11 (eelgrass).

This section covers the recommended methods for quantifying nutrient-related ecosystem functions within eelgrass meadows. All methods require field sampling followed by subsequent laboratory analysis. Field sampling procedures are generally simple and can be conducted with little training and equipment. Subsequent laboratory analysis requires a higher level of expertise and equipment availability. Using similar methods, carbon immobilization can likewise be quantified along with the nutrients. For specific recommendations and guidelines regarding carbon quantification, see Appendix 2.

Immobilization in the standing eelgrass biomass

The best way to quantify nutrients in standing biomass is by area-specific harvesting of eelgrass biomass from the target bed, followed by laboratory analysis. In a large-scale restoration project this can be done annually to follow the development of the area-specific biomass, but to precisely estimate the magnitude of the ecosystem function sampling should preferentially be conducted both in August to October for a maximum value and during January to February for a minimum value.

Fieldwork: Eelgrass is sampled from 10 quadrats of a defined size (e.g., 25x25 cm). Remove all living eelgrass material from the quadrat, both above (leaves) and below the sediment (rhizomes and roots). To ensure accurate division of the root zone, cut along the inside edges of the quadrat using a sharp knife (e.g., bread or insulation knife). The area is cleared using a rake or similar tool. Rinse the material on a coarse sieve to remove sand and bring it to the lab.

Samples can be stored moist and refrigerated for a few days but should be processed as soon as possible to prevent decay of the eelgrass biomass. Especially the separation of living and dead belowground biomass becomes more difficult the longer the sample is stored.

Laboratory work: In the lab, remove any dead eelgrass material, as this is accounted for in sediment pool sampling. Separate the remaining living biomass into two categories: belowground (rhizomes and roots) and aboveground (leaves). Place the material in pre-weighed aluminium trays and dry it at 60°C for at least 24 hours. Weigh the dried material to determine dry weight.

After drying, samples can be stored long-term in sealed bags, as nutrients are preserved. To measure nutrient content, homogenize and grind the plant material using a plant mill at 30/s for 30 sec. The ground biomass is then analysed for nitrogen (N) and phosphorus (P). The nutrient content can be measured using various analytical methods and laboratory instruments, depending on the availability of the laboratory used.

The relative nutrient composition (N:P:Dry weight) is used to calculate an area-specific nutrient pool (g m^{-2}) in the biomass. Winter biomass (annual minimum) represents a permanent immobilization, while the difference between winter and summer biomass represents temporary immobilization during the growing season. These measurements can be linked to spatial coverage data from drones or aerial imagery, allowing for the estimation of the area specific realized ecosystem function.

Leaf Production

Few studies have quantified total leaf production and annual productivity of eelgrass. A Danish study from 1975 estimated annual biomass production to be 2.5 times the maximum standing biomass in summer (Sand-Jensen 1975), though nutrient content was not measured.

Various methods exist for quantifying eelgrass productivity. Some focus on leaf elongation, others on internode development. Here, we recommend the plastochrone interval (P_i) method (Short & Duarte 2001), as it supports replication across multiple shoots rather than focusing on individual ones, providing an accurate representation of growth and nutrient uptake across the bed. These methods are still being refined, and recommendations may be updated.

Plastochrone interval

The plastochrone interval (P_i) is the time it takes for a new leaf to form and is applicable across seagrasses and plants. This method assumes all new leaves grow to be the same size as the average fully developed leaf (the third-youngest leaf). P_i is determined by marking the sheath of an eelgrass shoot with a needle, puncturing all existing leaves. About a month later (It's important to ensure at least one P_i has passed), new, unmarked leaves can be counted to calculate P_i (Figure 9).

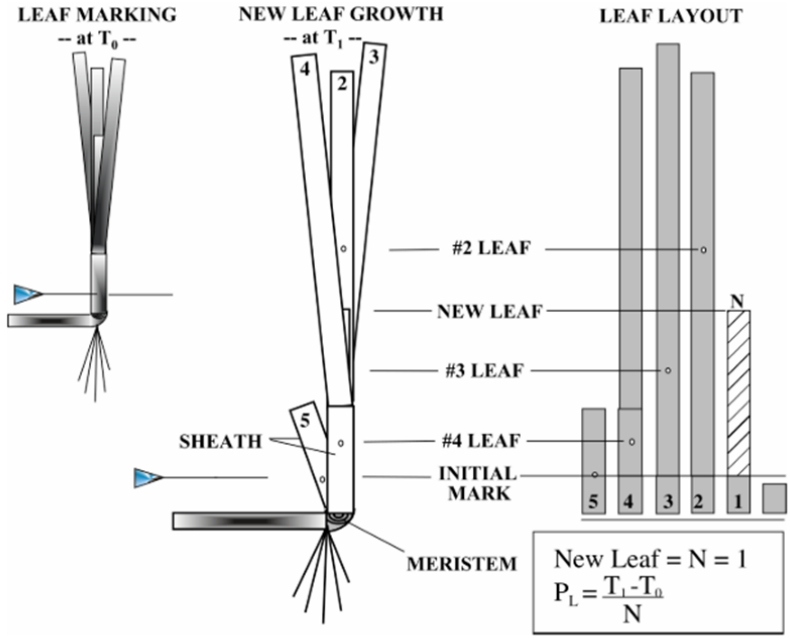

Figure 9. Diagram of P_i marking on an eelgrass shoot and identification of new leaves (Short & Duarte 2001).

Field work: Two tasks must be completed in the field: 1) marking shoots with a 21 G (0.81 mm) needle (Figure 9), and 2) determining shoot density. The number of eelgrass shoots that are marked depends on capacity and resources. However, increasing replication across the bed improves accuracy. A minimum of 10 eelgrass shoots from 3 locations is recommended. Ensure that all shoot size classes are equally represented by using frames to randomly select sampling areas. Mark the shoots with the needle (Figure 9) and mark the sampling locations for future retrieval so that the same eelgrass shoots can be found later.

Within the sampling area, shoot density is determined using randomly placed frames of appropriate size, depending on shoot density. A minimum of 10 replicates is recommended.

Allow at least one P_i to pass before harvesting the marked shoots; this varies with climate and season. In Denmark, one month is a good rule of thumb. Upon return, harvest the marked shoots and remeasure shoot density. When harvesting, ensure enough belowground material is collected—at least four internodes per eelgrass shoot.

Laboratory work: In the lab: 1) count the total number of leaves per shoot and 2) count the number of new leaves based on which leaves have puncture marks.

Divide each shoot into four fractions:

- The third-youngest leaf (from sheath upward)
- The fourth-youngest internode
- Roots from the fourth-youngest root node
- Leaf sheath

Place each fraction in pre-weighed aluminium trays and dry at 60°C for at least 24 hours. After drying, weigh to determine the dry mass. To calculate nutrient immobilization (N and P), grind the dried material in a laboratory mill and analyse the nutrient content using available laboratory methods.

Calculations: Using lab data and equations from Jacobs (1979) and Short & Duarte (2001), area-specific production can be calculated:

Jacobs 1979:

$$L_b = \frac{Leaf\ production}{shoot} = \frac{Avg.\ biomass\ of\ third\ youngest\ leaf}{P_i}$$

$$L_s = \frac{Leaf\ sheath\ production}{shoot} = \frac{Avg.\ biomass\ of\ a\ leaf\ sheath}{Avg.\ number\ of\ leaves\ per\ shoot} \cdot P_i$$

$$R_z = \frac{Rhizome\ production}{shoot} = \frac{Avg.\ biomass\ of\ fourth\ youngest\ internode}{P_i}$$

$$R_t = \frac{Root\ production}{shoot} = \frac{Avg.\ root\ biomass\ of\ fourth\ youngest\ node}{P_i}$$

Short & Duarte 2001:

$$Individual\ shoot\ production\ (g\ dw\ shoot^{-1}\ d^{-1}) = \left(\sum L_b, L_s, R_z,\ R_t\right)$$

$$Area\ specific\ production\ (g\ dw\ m^{-2}\ d^{-1}) = individual\ shoot\ production\ \cdot\ shoot\ density$$

Modified equation for calculation N/P immobilization through production, [N/P] is the relative content of N and P to dry weight, respectively:

$$[N] = \frac{g\ N}{g\ DW}$$

$$Area\ specific\ production\ (g\ N\ m^{-2}\ d^{-1}) = \left(\sum (L_b + L_s)[N], (R_z, + R_t)[N]\right) \cdot shoot\ density$$

Sediment stocks in eelgrass beds

It's recommended to take at least five sediment cores per sampling site. Site selection is crucial, as sediment pools vary with local conditions. The control site should match the restoration site in sediment type (e.g., grain size), depth, and exposure. Avoid areas with thick shell or peat layers, as these can drastically impact results and make reference and restoration sites incomparable.

Field work: Sediment cores are sampled using acrylic cylinders. The cores are pushed into the sediment and sealed with a rubber stopper to create a vacuum. The core is extracted and closed at the bottom using a secondary rubber stopper. The cores are pushed to a representative depth at which the ecosystem function is most apparent. Experience from SDU has shown that in shallow (1-2 m deep) eelgrass beds, the ecosystem function is mainly expressed in the top 10 cm of the sediment.

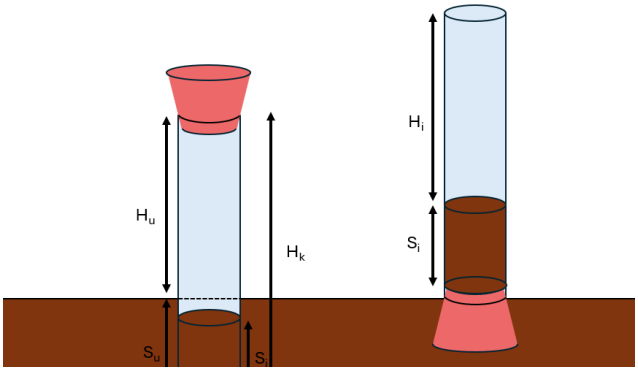

Figure 10. Measurements used in estimating the compaction factor (K_f) during sediment core extraction.

During the collection and treatment of the core, compaction may occur, especially in soft organic sediments with high water content. Therefore, for each core, a compression factor of the sediment (K_f) is calculated. K_f is calculated as the sediment height inside the core (S_i) divided by the sediment height outside the core (S_u). However, these cannot be measured directly. Instead, the full length (H_k) of the cores must be measured and the outside height of the core from the top of the sediment (H_u). Once the core is extracted, the height from the top of the sediment inside the core to the top of the core (H_i) is also measured (Figure 10). The addition of a rubber stopper at the bottom of the core increases the height of the sediment within the core. Therefore, 1-2 cm of height must be added to H_i to calculate sediment height accurately. The compression factor (K_f) can then be calculated by the formula:

$$K_f = \frac{S_i}{S_u} = \frac{H_k - H_i}{H_k - H_u}$$

The core can be stored cold before further processing in the lab. To prevent oxygen depletion, the top stopper should be removed during storage. If measuring phosphorus (P), the samples need to be processed as fast as possible (within 1-2 days).

Laboratory work: In the lab, remove surface water and, if present, large living fauna or flora. Slice cores into depth layers (e.g., 0–1 cm, 1–2 cm, 2-5 cm, 5-10 cm, etc.), adjusted for compaction (K_f). From each layer, take a subsample with a predefined volume (e.g., 5 mL), place it in a pre-weighed aluminium tray, weigh the material (wet bulk density), and dry it at 105°C for at least 24 hours, and weigh it (dry bulk density). Dry the remaining sediment similarly and mix with the density-subsample before further processing. After drying, the sediment samples can be stored dry in sealed bags as the nutrients have been fixed.

After drying, grind the sediment in a plant mill at 30/s in 30 seconds. Use the ground sediment to measure loss-on-ignition (LOI), N, P, and Fe. For LOI, burn samples at 510°C for 5 hours. N, P, and Fe are measured using standard lab techniques, depending on available instruments.

Calculations: To calculate the area-specific effect of eelgrass on the nutrient content in the sediment, we need to summarize the effect across the different sediment layers. As mentioned, the effect of eelgrass on the nutrient content in the sediment is often limited to the top 10 cm of the sediment (i.e., the rhizosphere). The area-specific stock (g N/P m^{-2}) for each layer is calculated based on nutrient content and bulk density. Here we multiply the bulk density (β), by the volume of the layer (V), the depth of the layer, and the relative nutrient content to d_w ratio [N/P], and divide it all by the area (*a*) of the core:

$$Layer = \frac{\beta \cdot V \cdot depth \cdot [N]}{a}$$

This is done for each layer, and the bare bottom (BB) layers are subtracted from the eelgrass (Zm) layers. The difference is added together for all relevant layers to sum up the full area-specific ecosystem function (Sed_{EF}):

$$Sed_{EF} = (Zm_{0-1} - BB_{0-1}) + \cdots (Zm_n - BB_n)$$

Denitrification

Eelgrass stimulates microbial conversion of nitrate to atmospheric nitrogen through denitrification, exporting nitrogen from the system (Zarnoch et al. 2017). This is enhanced by eelgrass roots introducing ammonium, labile organic material, and oxygen, which support coupled nitrification-denitrification (McGlathery et al. 2007). The complex root zone creates microhabitats with diverse biogeochemical conditions, strengthening the coupling between nitrification and denitrification and enhancing nitrogen export.

Several methods exist for measuring denitrification, including acetylene assays, isotope pairing, and N_2-argon flux. However, the complex root zone presents major measurement challenges. Reagents and isotopes cannot be distributed evenly to replicate *in-situ* conditions. Additionally, many methods require sediment cores to be brought to the lab; as such, rhizomes and roots are cut and damaged, sediments are disturbed, and oxygen, light levels, and biogeochemical conditions are altered, compromising results. Furthermore, these are rate-based methods that reflect only short-term processes. Estimating the actual ecosystem function requires extensive temporal replication.

Given these limitations, no reliable method for quantifying this process within eelgrass meadows is currently recommended. This guideline will be updated if a robust method is developed.

Methods used in biogenic reef restoration

This following text outlines current methods used to monitor N-cycling in bivalve reefs, their scientific basis, and operational considerations with relative strengths and limitations. For thorough review of existing literature on bivalve biogeochemical interactions, the reader is directed to Jackson et al., 2018, Jansen et al., 2019 and Ray and Fulweiler, 2021. For monitoring nutrients in the sediment, we are referring to the technical guideline TA no. M23 ´Næringsstoffer i sedimentet´[15]

[15] https://ecos.au.dk/fileadmin/ecos/Fagdatacentre/Marin/TA_M23_Naeringsstoffer_i_sediment_ver2_1.pdf

Denitrification

Denitrification involves the reduction of nitrate (NO_3^-) and nitrite (NO_2^-) to dinitrogen gas (N_2) and thereby removes reactive nitrogen from the aquatic ecosystem. Bivalve reefs often support elevated rates of denitrification due to the accumulation of organic matter from bivalve biodeposits and the formation of redox gradients favourable for denitrifying microbial communities. Several methodological approaches have been applied to measure denitrification in bivalve reefs, each with inherent advantages and limitations.

Accurately quantifying denitrification rates in bivalve reefs can be practically challenging and resource intensive. The process typically involves enclosing a section of the reef, its underlying sediments, and the overlying water within a chamber; or by proxy a reconstruction of this configuration in a mesocosm. Subsequently, changes in N_2 concentrations are measured over a specified duration. This can be achieved through batch incubations, where changes in N_2 concentrations are determined using regression analysis, or flow-through incubations, where differences in N_2 concentrations between inflow and outflow samples are measured. Chambers can be positioned *in situ* on the reef by hand if in a macrotidal area, by diver or by a lander (Figure 12) in subtidal conditions. While *in situ* experimentation is understood to preserve existing conditions at the site, there are obvious operational constraints to sufficient replication and sampling procedures. Automated benthic lander systems solve some of these constraints, though the complex physical structure of a reef may prove challenging for the lander's chambers to adequately seal the reef and sediment space. Samples can also be extracted from the reef to run *ex situ* experiments, though the extraction process itself can disturb biogeochemical gradients, and logistical constraints often limit replication and spatial coverage. *Ex situ* experiments should preserve the vertical structure of the sample for quantifying representative denitrification rates. Slurry methods, which involve mixing benthic samples, should be avoided as they disrupt sediment structure and natural redox gradients, potentially altering microbial community composition and denitrification potential. In habitats with ample microphytobenthos or macroalgae, light conditions can alter benthic fluxes, and differences between light and dark fluxes should be considered. The size and type of incubation chamber used should be appropriate for the complexity of the reef. Smaller chambers are easier to operate but may not capture representative parts of the system, while larger chambers can capture more realistic results in the often-heterogeneous conditions with complex communities but incur additional cost and logistical challenges. Replicating *in situ* flow conditions is also difficult, as environmental factors such as tides or wind-driven currents are subject to constant change. Water within chambers is often mixed using stir bars or impellers to simulate flow and prevent stratification. The rate of stirring requires consideration, as different speeds can influence biogeochemical exchange rates by modifying the thickness of the existing boundary layer. This all implies that a pre-experimental site investigation is prudent for appropriate experimental setup and configuration.

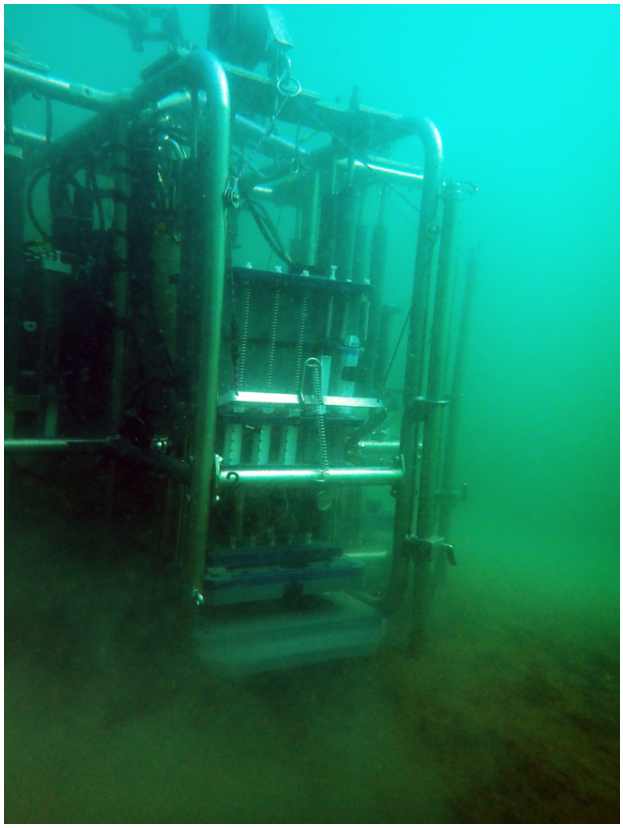

Figure 12. A University of Gothenburg in situ automated benthic lander with incubation chambers – Photo: Daniel Taylor

Several analytical methods are regularly used to quantify denitrification rates. The Acetylene Inhibition Technique involves adding acetylene (C_2H_2) to inhibit the N_2O reductase enzyme, preventing the final step of denitrification ($N_2O \rightarrow N_2$). The change in N_2O concentration is then measured via gas chromatography. While relatively simple to implement, this method often results in underestimation of denitrification due to incomplete inhibition of N_2O reductase. Acetylene can also inhibit nitrification, affecting coupled nitrification-denitrification processes, and does not account for nitrogen fixation or anammox, which contribute to the net N_2 flux. Due to these notable drawbacks, the acetylene inhibition technique is generally not recommended for quantifying denitrification in bivalve reefs. The N_2/Ar method involves measuring the change in the ratio of dissolved N_2 and Ar using a membrane inlet mass spectrometer (MIMS) (Jackson et al., 2018). This technique offers high precision and does not require headspace equilibration, providing a direct measurement of N_2 fluxes under natural conditions. Additionally, it can simultaneously measure O_2 fluxes if O_2 is not removed during analysis. However, the method is sensitive to bubble formation, which can lead to erroneous values. The potential production of nitrosonium (NO^+) during MIMS analysis can also result in higher N_2 production estimates. Despite these limitations, the N_2/Ar method is considered one of the most accurate and reliable techniques for quantifying denitrification in bivalve reefs. The Isotope Pairing Technique (IPT) involves labelling the dissolved inorganic nitrogen (DIN) pool with $^{15}NO_3^-$ or $^{15}NH_4^+$ and tracing to the N_2 pool. This method provides detailed information about the mechanistic processes contributing to net denitrification, as well as other processes, such as Dissimilatory Nitrate Reduction to Ammonium (DNRA). However, it is sensitive to the amount of label added, which can affect the environmental relevance of the measurements. IPT often results in lower denitrification rates compared to the N_2/Ar method and is sensitive to the activity of other processes, which can potentially violate methodological assumptions. While very useful for mechanistic studies, IPT may not be as reliable for quantifying net denitrification in complex bivalve reefs (Ray et al., 2021).

Erosion and sediment stability *–boulder reefs*

Boulder reef restoration provides significant coastal protection benefits through wave energy dissipation and sediment stabilization (Bjerregaard & Grolin, 1998; Stone et al., 2005). Boulder reefs effectively attenuate wave energy through multiple mechanisms and the effectiveness of wave attenuation depends on the reef's structural complexity, geometry, and positioning relative to prevailing wave conditions, with protection benefits extending from meters to kilometers from the restoration site.

Monitoring erosion and sediment stability is essential for evaluating the effectiveness of boulder reef restoration and ensuring long-term structural integrity. The monitoring approach should combine recommended methods suitable for volunteer implementation with additional advanced techniques that provide detailed mechanistic insights for expert-led projects (Table 8).

Visual methods

Visual methods (e.g., photography) can detect changes in reef structure and surrounding sediments. Establishing permanent photo points around the restoration site using fixed markers or GPS coordinates allows to document boulder stability and displacement, sediment accumulation patterns around structures, evidence of erosion features, changes in substrate composition, and overall structural integrity of reef components. This approach offers a cost-effective method for tracking large-scale changes that may not be apparent through individual observations. Photographs should be standardized, maintaining consistent angles, distances, and light conditions at each monitoring point. It is important to create a systematic numbering system for photo points and maintain detailed logs including date, time, weather conditions, and observer notes. Finally, photos and data should be stored with systematic naming conventions and develop a comprehensive photo database that allows for easy comparison over time, enabling detection of both gradual changes and sudden impacts from storm events or other disturbances.

Sediment traps

Sediment traps provide critical data on sediment flux and transport dynamics, enabling evaluation of how effectively the boulder reef mitigates coastal erosion. Install sediment traps using PVC cylinders or flat collection plates positioned downstream of boulder structures with inlets approximately 5 cm above the seabed. Deploy multiple traps per site to account for spatial variability, positioning them to capture sediment movement in prevailing current directions based on local knowledge of water flow patterns (Lund-Hansen et al., 2004). The traps should be secured to prevent displacement during deployment and marked for easy retrieval after the measurement period.

After the deployment period, retrieve traps carefully to avoid sediment loss, then dry collected material and weigh to calculate sediment flux. Record environmental conditions during deployment including wave height, current strength, and weather conditions, as these factors significantly influence sediment transport rates. It is then possible to correlate sediment flux measurements with wave and current conditions to understand the relationship between hydrodynamic forces and erosion patterns, providing valuable insights into the effectiveness of the boulder reef in reducing sediment transport and coastal erosion.

Sediment sampling - cores

Sediment cores represent a useful tool for tracking temporal changes in substrate composition, hydrodynamic conditions and the effectiveness of reef restoration measures. By extracting and analysing vertical profiles of unconsolidated material, researchers can reconstruct how the sedimentary environment responds to both natural forces and engineered interventions over time.

To implement this approach, collect simple sediment samples from representative locations around the reef, using PVC cores or cylinders. Ensure that each sampling point is logged with precise GPS coordinates to facilitate consistent resampling in future campaigns, and record field notes on the apparent proportions of mud, sand and gravel. Take standardized photographs of each core immediately following extraction to enable visual comparison across sampling periods.

Where feasible, retrieve cores in triplicate at each station to capture small-scale spatial variability and improve statistical robustness. Back in the laboratory, oven-dry samples at 60°C until they reach constant weight, then pass them through a series of standardized mesh sizes to segregate grain-size fractions. From these data calculate key sedimentary parameters such as porosity (from bulk and solid densities), bulk density (dry mass per unit volume), median grain size (D_{50}), and grain-size distribution (percentages of sand, silt and clay).

Interpreting shifts in grain-size distribution over time yields insights into local hydrodynamic energy; finer sediments generally indicate lower-energy conditions and more effective wave attenuation by boulder placements, while changes in porosity and bulk density reflect evolving substrate stability. Together, these metrics illuminate the progressive modification of the benthic habitat in response to both environmental forcing and deliberate restoration activities.

Water sampling

Water clarity and turbidity measurements indicate the amount of suspended sediment in the water column, with higher turbidity suggesting increased sediment resuspension or transport from erosional processes. Temperature measurements and basic visual observations of wave conditions, current strength, and visible sediment plumes offer contextual information about the physical forces acting on the restoration site.

Implement a simple but consistent monitoring protocol by taking measurements at the same locations and depths during each sampling event, ensuring data comparability over time. Record all environmental conditions during sampling, including weather, any unusual circumstances such as recent storms, nearby construction activity, or seasonal variations that might influence water quality parameters. Use basic Secchi disk or turbidity sensor if available, as simple visual assessments of water clarity can provide valuable trend information when conducted systematically for comparison over time.

Structure-from-motion photogrammetry and multibeam sonar

These advanced techniques generate high-resolution digital elevation models (DEMs) that enable precise quantification of morphological changes around boulder reefs with centimeter-scale accuracy. Structure-from-motion photogrammetry involves establishing ground-control markers on and between boulders using permanent benchmarks, then collecting overlapping imagery with ≥70% overlap using underwater cameras, ROV systems or drones (Ventura et al., 2022). The resulting images are processed using specialized software to create detailed DEMs that can detect subtle changes in seafloor topography, scour pit development, and sediment redistribution patterns around restored structures. Multibeam sonar applications complement photogrammetry by providing broader-scale bathymetric mapping capabilities that can detect subtidal sediment transport patterns and monitor changes in seafloor morphology over time (Ferrini & Flood, 2005). Sonar systems map larger areas more efficiently than photogrammetry and provide baseline data essential for hydrodynamic modelling efforts. Both techniques allow to compare successive surveys through GIS analysis, quantifying changes in scour pit depth and area while tracking the evolution of sediment deposits and erosional features that indicate the effectiveness of boulder reef restoration in coastal protection.

Acoustic Doppler Current Profiling (ADCP)

Acoustic Doppler Current Profiling (ADCP) offers a high-resolution view of the three-dimensional flow field around reef and boulder structures, allowing to link hydrodynamic forcing to patterns of sediment transport, erosion and habitat development over time. This represents an expert-level approach to hydrodynamic monitoring, linking detailed flow measurements directly to sediment transport processes and restoration design optimization (Kostaschuk et al., 2005). Deploy ADCP instruments 0.5-1 m above the seabed to record three-dimensional velocity profiles at 1 Hz frequency, providing detailed data on water movement and hydrodynamic conditions around boulder reef structures.

ADCP data serves multiple applications including identifying specific flow conditions that drive erosion around boulder structures, validating hydrodynamic models used for restoration design, and determining critical flow velocities for sediment entrainment. This information links observed erosion patterns with hydrodynamic forcing conditions, enabling adaptive management decisions about boulder placement, sizing, and orientation. The velocity profiles also provide essential ground-truth data for numerical models, improving predictions of restoration performance under different environmental scenarios and supporting evidence-based design modifications for future restoration projects.

Remote sensing and hydrodynamic modelling

Remote sensing using satellites or aerial platforms generates large-scale seafloor maps and tracks shoreline changes over time, providing data on wave energy dissipation patterns, sediment redistribution at landscape scales, and long-term coastal protection effectiveness. These methods enable assessment of how boulder reef restoration integrates with adjacent coastal management efforts and contributes to broader ecosystem services. Satellite imagery and aerial photography can detect changes in coastal morphology, vegetation patterns, and sediment plume distribution that indicate the far-field effects of restoration activities.

Hydrodynamic modelling complements observational data by simulating flow dynamics, sediment transport, and wave interactions with restored reefs, enabling prediction of restoration performance under different environmental scenarios. These numerical models integrate bathymetric data, wave climate information, and tidal forcing to predict how restored reefs will perform under various conditions including storm events, sea level rise, and seasonal variations.

Monitoring water clarification – *biogenic reefs and large-scale eelgrass beds*

Water movement transports particles past filtering bivalve beds; the rate of this transport is a flux of particles. The concentration of particles tends to decrease as water is transported past a bed. In eelgrass meadows, a decrease in particle concentration is noticed, because the eelgrass canopy decreases water flow thus increasing sedimentation and reducing resuspension. Furthermore, the rhizomes and roots play a role in stabilizing the sediment. However, it requires eelgrass coverage of several hectares for noticeable impact. Therefore, small-scale projects should not regard water clarity as a major ecosystem function. Appendix 1 contains methods for monitoring water clarity as provided by large-scale restoration efforts, spanning several hectares, of eelgrass beds.

For bivalve beds, the rate of particle decrease can be determined by discrete or continuous measurements of seston constituents or proxies. Therefore, characterisation of water clarification requires measurements of parameters that influence light transmission through the water column and hydrodynamics. The monitoring approach requires an assessment of resource prioritisation for capturing the desired spatial and temporal scales of water clarification. In practice, there are trade-offs due to the desired scales, resolution, expertise, equipment, and resources available.

Measurements of water clarification are always relative to the ambient conditions. This implies that reference measurements need to accompany measurements within the footprint of the biogenic reef. Evaluation of the spatial structure and extent of water clarification incorporates sampling designs that measure around and over the reef in coordination with local hydraulics. The sampling frequency of different parameters is very dependent on variability in local conditions; however, relative differences between ambient and bed conditions allow for suitable interpretation of water clarification magnitude. As water clarification is principally driven by filtration of organic particles, sampling should take place during times of the year with higher productivity (spring - early autumn), which coincides with the productive season of organisms affected by light attenuation. Assignment of qualitative or quantitative measures of this ecosystem service requires that the project proponent formulate purposes and objectives with respect to the importance of this function in the project's ecosystem. For example, if an objective is to increase water transparency in receiving seagrass habitat, the magnitude and scale of water clarification will be constrained to a relatively small area. On the other hand, if an objective is to reduce eutrophication symptoms (decreased chlorophyll-a concentrations) in the catchment, the scope of monitoring will be different.

There are two main components typically included in a monitoring program, 1) light attenuating factors, and 2) water movement. Monitoring light attenuation factors can be further divided into focusing on seston characteristics or water optical properties. The two are not mutually exclusive and indeed provide substantial overlap, but the methods, tools, and expertise involved have tended to drive divergent specialisation. The simplest monitoring approach could involve simply a relative difference between total suspended matter between a reference position and within the reef. A comprehensive program could include multiple parameters of seston constituents covering various time and spatial scales and thorough characterisation of local hydrodynamics. The following text briefly describes methods involved in water clarification monitoring, examples of available tools, and notable trade-off considerations. An overview of the different methods can be found in table 5.

Methods for monitoring seston and water optical properties

Light transmittance through the water column is influenced by several components, which are generally seston, dissolved matter, and water itself. Seston refers to suspended particles in the water column, which can be organic or inorganic, with wide ranges of sizes and properties. In Danish coastal waters, the main seston constituent is organic particles, and mostly phytoplankton. Suspended organic matter is the food source and dominant particles that are filtered by bivalves, so concentrations and concentration gradients of suspended organic matter are important to describe for water clarification as an ecosystem service. For more details we are referring to the following

technical guideline TA no. M01 ´Indsamling af vand og planktonprøver i felten´[16], TA no. M03 ´CTD måling´[17], TA no. M05 'Fluorescens'[18], TA no. M06 ´lyssvækkelse´[19] and TA no. M07 ´Klorofyl a koncentration´[20] for monitoring the marine environment, which have been prepared for the Danish Environmental Protection Agency by the Marine Data Centre.

Seston monitored by discrete water sampling

Discrete water sampling is collected as point samples and intermittently over time. Classic water sampling is performed either by pumping or capture with a sampling apparatus from a known depth. For most parameters, capture at depth is preferred as many pump types can disturb the state of the water column around the pump inlet, can modify particles (e.g. disintegration in impeller), and produces volumes typically unnecessary or untenable for analysis, however depending on flow rate of the pump. A water sampler is a simple, inexpensive device that generally operates by casting the apparatus over-board, lowering to the desired depth, triggering capture, and retrieval. Water is then analysed on board or stored in clean containers for transport back to the lab. This process is typically repeated at a fixed location for replication, and in total can take several minutes. The major advantage of discrete water samples is the capture of particles and ability for direct characterisation. The nature of fixed locations and physical capture is generally time and resource limiting due to 1) sailing time between stations, 2) physical sample storage, and 3) subsequent sample processing and analyses. This limits the ability to expand coverage while maintaining resolution in both time and space.

Parameters typically analysed in discrete water samples are generally assessed in terms of concentrations (volumetric) or by composition. Volumetric parameters include suspended matter mass, organic fraction of suspended matter, chlorophyll-a or other pigments. Suspended matter and Chlorophyll-a is determined by standard methods (for details we are referring to standard protocols like e.g. HELCOM 2017 and Walsham et al., 2022. This method is widely practiced in standard monitoring programs, and standard protocols are referenced here for specific procedures and detailed discussion of protocol considerations. Variation in some of the techniques is present, such as which solvent is used, filter maceration and centrifugation, analytical equipment, and sample conveyance. Most restoration projects could adopt suspended matter analyses and chlorophyll-a quantification with access to appropriate equipment (i.e. spectrophotometer, fluorometer) and introductory training.

Samples can also be processed to characterise the composition of seston. Traditional microscopic techniques are used for counting and identifying different phytoplankton, zooplankton, and bacterio-plankton groups. Traditional microscopic analyses require varying degrees of expertise depending on the level of identification and can be very time-consuming. Particle counters and size analysers have been used for many years and can provide size spectra to understand which fractions of particles are filtered over the reef. Flow cytometry has become an established method for characterising particle size and shape spectra. Most of these techniques are beyond the scope of monitoring in most restoration projects but are of interest to the scientific community to understand reef filtration dynamics in different conditions. Images different devices

[16] https://ecos.au.dk/fileadmin/ecos/Fagdatacentre/Marin/TA_M01_Indsamling_af_vand_og_planktonprøver_i_felten_ver1.pdf
[17] https://ecos.au.dk/fileadmin/ecos/Fagdatacentre/Marin/TA_M03_CTD_maaling_ver2.pdf
[18] https://ecos.au.dk/fileadmin/ecos/Fagdatacentre/Marin/TA_M05_Fluorescens_ver1.pdf
[19] https://ecos.au.dk/fileadmin/ecos/Fagdatacentre/Marin/TA_M06_Lyssvaekkelse_ver3.pdf
[20] https://ecos.au.dk/fileadmin/ecos/Fagdatacentre/Marin/TA_M07_Klorofyl_a_ver2.pdf

Water optical properties monitored by discrete sampling

A classical method for characterising light attenuation is the Secchi disk. This method involves lowering a white or checkered disk below the water surface and noting the depth when it is no longer visible. This is the simplest and least expensive method to qualify water clarity, though it is imprecise and subject to error due to differences in observer perception and situational aspects contributing to variable light conditions such as cloud cover, wind surface disturbance, solar angle, and vessel shadowing[21]. In absence of other methods, Secchi disk depth can still be used to characterize differences in light attenuation over the water column.

Transmittance and absorption spectra can be measured with sample water in a spectrophotometer. These spectra reveal the distinct absorption signatures of light attenuating components; for example, colored dissolved organic matter (CDOM) absorbs strongly in the ultraviolet, while phytoplankton have characteristic pigment absorption peaks, such as chlorophyll-a in the blue (~440 nm) and red (~675 nm) regions. For further information on light and optical theory in coastal and marine waters, the reader is directed to The Ocean Optics Web Book (Mobley et al., 2022[22]). Samples require pure (ultrafiltered) controls and sample filtration steps. While relatively straightforward, sample processing is time-consuming, equipment can be expensive, and interpretation requires expertise.

Water optical properties monitored by fixed sensors

Sensors can detect environmental parameters of interest and transmit a corresponding signal along a measured scale. The sample space for most sensors is within a few cm from the sensor-water interface, so the spatial representation is limited to a single point in the water column. Often it is advantageous to deploy sensors at more than one depth to characterise differences in the vertical water column.

Many sensors are available off-shelf from manufacturers and secondary suppliers with internal logging capabilities and designated software for simple deployment and post-processing steps. There are abundant resources for more do-it-yourself (DIY) oriented project proponents.

Parameters available off-shelf and relevant to water clarification:

- Physical
    - Conductivity
    - Temperature
- Optical
    - Beam attenuation
    - Backscatter
    - Absorption
    - Particle size distribution
    - Photosynthetic active radiation (PAR)
- Proxies
    - Chlorophyll-a
    - Turbidity
    - Coloured Dissolved Organic Matter (CDOM)/Fluorescent Dissolved Organic Matter (FDOM)

[21] https://www.oceanopticsbook.info/view/photometry-and-visibility/level-2/the-secchi-disk
[22] https://www.oceanopticsbook.info/view/optical-constituents-of-the-ocean/introduction-to-optical-constituents-of-the-ocean

Water Optical Properties

Most restoration projects will not require high resolution coverage for parameters like conductivity and temperature as in Danish coastal waters (microtidal) daily variability is less pronounced; often a single planar position is sufficient. Sensor models are abundant and straightforward to operate. Optical sensors (water optical properties) focus on inherent (beam attenuation, backscatter) or apparent (PAR) properties. Inherent properties are independent of solar conditions, so do not require a surface reference sensor, where monitoring apparent properties typically do require a surface reference. Backscatter will provide information on particle concentrations, which is relevant to bivalve water clarification, while beam attenuation will provide information on absolute water clarity. Backscatter sensors require considerable calibration steps and application of optical theory for interpretation. More sophisticated instrumentation can characterise absorption along with beam attenuation (i.e. Seabird ac-s) or even particle size distributions along with beam attenuation (i.e. Sequoia Scientific LISST), however these are very expensive, and use requires substantial expertise limited to scientific applications. PAR sensors at depth can be used in conjunction with reference measurements from a sensor immediately below the water surface to calculate an attenuation coefficient (K_D) that relates light attenuation to depth in the water column. Of the optical parameters, most restoration projects will limit their scope to use of PAR sensors or beam attenuation sensors. Sensors quantifying proxies to light attenuating features are widely used in water quality monitoring programs, such as chlorophyll-a fluorescence, turbidity, and coloured/fluorescent dissolved organic matter (CDOM/FDOM) and are widely accepted in reporting for both scientific and regulatory purposes. Chlorophyll-a is the most common parameter monitored relative to bivalve filtration as it represents their food source, phytoplankton. Turbidity can be difficult to interpret as it is disproportionately sensitive to reflective (inorganic) particles and requires a demanding calibration protocol using local sediments with high replication.

Dissolved Organic Matter

Coloured Dissolved Organic Matter (CDOM) and fluorescent Dissolved Organic Matter (FDOM) constitutes refractory dissolved matter that effectively absorbs light, and responsible for the brownish coloration of some rivers and lakes. Most coastal waters have nominal levels of CDOM/FDOM, however, enclosed inner brackish waters can have substantial concentrations. All these proxy sensors involve numerous assumptions, often exhibit high variability, and require periodic calibration. Their moderate price, degree of logger system integration, and interpretation may be excessive for smaller restoration projects. Selection of parameters and their associated sensors should be identified in the project's purposes and objectives and coordinated with available resources.

Seston and water optical properties monitored by synoptic surveys and profiling

Synoptic surveys and profiling collected data in line, plane or volume and intermittently over time. Integration of fixed sensors as described above in a survey system on a transecting vessel provides opportunities to map parameters over a large area in 2- or even 3-dimensions. Classical profiling of the water column with a sensor package can be a rapid method to characterise the distribution of parameters in the vertical dimension and can be repeated in a grid assignment over a larger area to similarly map parameter distributions in 2- or 3-dimensions. A common survey setup is to pump water from depth into a contained apparatus onboard a vessel (e.g. FerryBox) where the sensor package is positioned. Alternatively, sensors can be positioned aside the vessel on a rigid, depth-fixed frame with cables running to the surface onboard, or the package can be towed behind the vessel on a sledge or depressor wing. All approaches require position tracking, which should accommodate differences from sampled time delay (distance duration from inlet to sensor faces) or relative position to the GPS receiver (i.e. for towed arrays). Pumping water requires use of a pump that does not disintegrate or otherwise disturb particle masses (i.e. diaphragm or screw pump), and the pumping rate should be kept constant to coordinate vessel position.

There are numerous setup considerations, as well as potentially lengthy calibration procedures that are critical to consider for synoptic surveys. A simple setup using a packaged unit (e.g. CTD package) can be towed or fixed aside from a paddled or slow-moving vessel and requires only modest collection and processing considerations. An inline system with multiple sensor packages and pumping at variable depth requires substantially greater preparation and operational control. The reader is directed to IOCCG Protocol Volume 4 for in-depth considerations of developing and application of inline systems (Neeley et al., 2019).

Synoptic mapping exercises can provide both accurate and precise spatial information on the extent and magnitude of water clarification around a bivalve bed, useful for practitioners, scientists (filtration and environmental dynamics), and stakeholders. Mapping can also be useful to evaluate changes over time, such as reef evolution, seasonal variation, or different hydrodynamic regimes (Figure 13). As vessel speeds typically need to be slow, performing such surveys can be very time-consuming. Interpretation and post-processing of survey data may require advanced GIS or spatial statistical expertise to present accurate representations of the data.

Figure 13. Example of synoptic survey of chlorophyll-a over a suspended mussel farm on two different days. Chlorophyll concentrations are indicated in shades of green, the vessel track is indicated as dotted lines. Interpolations of chlorophyll concentrations performed within a convex hull of the survey extent (Taylor et al., 2021)

Water optical properties monitored by remote sensing

Remote sensing is collecting data in a plane and intermittently over time. Colour remote sensing from UAV, airplane, and satellites has been integral in ocean and coastal water quality and biogeochemistry observation programs. Application to bivalve filtration has been a relatively recent, though limited application due to the required spatial resolution and constrained depth for detecting relative water clarification. Among numerous parameters, chlorophyll-a and suspended matter detection have been the most applied parameters, other than physical parameters. While satellite colour data is freely available to the public via Copernicus (copernicus.eu), working with the data may require expert support. However, after suitable algorithms have been selected and a processing procedure is in place, access to maps can be streamlined for public use. A notable disadvantage of satellite colour data is that the area of interest should be cloud-free during satellite overpass. For

biogenic reefs, even in relatively shallow areas (<6m), the area of water clarification under stratified conditions may not be detectable from surface reflectance data. Use of colour remote sensing data for coastal conditions is still an active area of scientific development.

Methods for monitoring hydrodynamics

Water movement (flow) is described by the velocity and direction of water. Flow can be steady or unsteady, uniform or non-uniform, and can be directed in all three dimensions. Flow generally varies with depth generating velocity gradients in boundary layers. Flow patterns at smaller and larger scales influence nutrient transport, particle and organismal dispersion, and ecosystem dynamics. Flow in respect to water column stability is generally described as either laminar or turbulent. Laminar flow typically occurs at low velocities and is described by smooth, steady layers of water flowing in parallel and general lack of mixing. Turbulent flow is characterised by chaotic and irregular water motion, which enhances the mixing of water layers through vortex generation. At bivalve bed surfaces, which are rough, moderate changes in velocities can substantially influence the degree of mixing along the water column, implicating different particle transport dynamics in the zone where bivalves are filtering water.

Density gradients along the water column are generally generated by freshwater inputs and meteorological forcing, such as wind and solar heating. Temperature or salinity (or both) differences form density gradients that can manifest a typically thinner stable gradient layer called the pycnocline, in which separate layers of water (strata) form; this is called stratification. Stratification is an important physical feature of the water column that influences water clarification directly by limiting mixing with the whole water column and thereby can generate a defined vertical layer depleted of food particles. In addition to affecting particle distribution, different density layers provide challenges for certain measurements by optical turbulence, also known as schlieren.

Surface currents

The simplest method to observe currents is to deploy a drogue or other object that drifts along the flow. A drogue is designed to be submerged and negate wind. A floating object will follow the dominant current path but may be advanced laterally due to wind. The drifting object can be tracked by use of on-board GPS, sight-tracking and referencing, or by noting its deployment and retrieval position.

Point measurements

Several *in situ* techniques exist to record current speed and direction in a time series at a fixed point in the water column, from mechanical to acoustic. Access to instrumentation may be limited to project proponents with greater resources as instruments tend to be expensive, and interpretation can require specialised training. Several lower cost and DIY techniques have been used in bivalve bed settings to successfully track current velocities, such as tilt current meters. Tilt current meters are typically positioned on the seafloor and operate by measuring the deflection angle of a tethered, buoyant sensor as it responds to water current forces; this angle is then used to estimate flow velocity. It is relatively inexpensive and robust for long-term deployments, though less precise in turbulent or non-uniform flow conditions and requires site-specific calibration to ensure data accuracy. Single-point current meters measure water velocity at a fixed location in the water column using a mechanical rotor, acoustic pulses, or an electromagnetic sensor that responds to the flow. They are reliable, of moderate cost, and easy to deploy for point measurements, but like other point measurement devices, they inherently offer limited spatial data. Acoustic Doppler Velocimeters (ADV) measure water velocity by transmitting acoustic pulses and analysing the Doppler shift of particles in a small sampling volume. They provide high-resolution, three-dimensional velocity

data ideal for turbulence studies, though it can be sensitive to signal noise, flow disturbance from the probe, expensive, and require careful deployment and post-processing.

Column measurements

Measurement of current velocity components over the water column is typically performed with acoustic profiling, and less commonly so with a profiling single-point meter as on a tethered mooring or vehicle. Acoustic Doppler Current Profilers (ADCP) measure water velocity profiles by emitting acoustic pulses and analysing the Doppler shift from particles throughout the water column with multiple transducers. It enables high-resolution, multi-depth velocity measurements over a time series, but requires substantial power, calibration, and careful deployment and configuration considerations. In bottom or moored configurations, ADCPs can be affected by side-lobe interference in shallow or complex environments typical of restoration sites, making boundary measurements difficult to accurately capture.

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Appendix 1

Methods to monitor improved water clarity in and around large-scale (hectares) eelgrass beds, covering simple methods like Secchi disc measurements and light loggers for calculating the light attenuation coefficient (K_d). For all methods, a BACI (Before-After-Control-Impact) design is recommended. Measurements should be conducted repeatedly after establishment, as the effect is expected to increase as the eelgrass bed grows denser and expands in area.

Water Clarity measured with a Secchi Disc

This method is straightforward and offers insight into the light conditions at a specific location. It has the advantage of requiring only a Secchi disc and can therefore be carried out by anyone, including volunteers.

The method is based on a classic Secchi depth measurement. The disc is usually lowered vertically into the water until it is just barely visible, at which point the Secchi depth is recorded. However, this is often not feasible in shallow waters, where most remaining eelgrass habitats are located and where new ones can be restored as secchi depth regularly surpasses the depth. Therefore, the method is adjusted to measure horizontal visibility. This requires two divers (alternatively, a stand with the disc and one diver). One diver holds the Secchi disc just above the eelgrass canopy, so the entire disk is visible. The second diver swims away until they can no longer see the disc. Then they swim slowly back until the disc becomes visible again. The distance from the disc at the point of visibility is measured and recorded as the secchi distance.

This method provides a snapshot of visibility and is the cheapest and simplest method for assessing water clarity. However, it is somewhat imprecise and subject to visual bias—but still serves as a good indicator of general water conditions.

Water Clarity measured with Light Loggers

Stationary loggers can be used to measure the optical properties of the water. Sunlight is attenuated as it passes from air to water and diminishes with depth in the water column. This attenuation results from absorption and scattering of light by particles and the water itself. The light intensity that passes through the water column decreases according to Lambert-Beer's Law (Weinberg 1976[23]):

$$I_z = I_0 \, e^{-K_d(z)}$$

Where I_z is the light intensity at a given depth (z). I_0 is the surface light intensity and K_d is the light attenuation coefficient.

Attenuation of light—or water clarity—is expressed as the light attenuation coefficient (K_d), which can be measured directly by placing light loggers at different depths at the same location. Ideally, use PAR loggers (Photosynthetically Active Radiation). At each station, at least two loggers (preferably more) should be placed at different depths. If the depth difference between the loggers is fixed (e.g., 0.5 m), K_d can be calculated as an exponentially decreasing function as displayed by Lambert-Beer’s Law.

Due to the eelgrass leaves, measuring light directly inside the bed can be challenging, as the leaves may shade the sensors. If water depth allows, loggers should be placed above the canopy. Alternatively, in shallow areas, logger stations can be placed along the outer edge of the bed. It is

[23] Weinberg S (1976) Submarine Daylight and Ecology. Marine biology 37:291-304

essential to position the sensors so that they are not shaded by eelgrass leaves or the logger structure itself.

Using light loggers requires regular maintenance and cleaning. Sensors quickly become fouled by algae or sessile organisms such as barnacles, which can compromise data quality within a few days. Maintenance demands can be reduced by using loggers equipped with mechanical wipers that automatically clean the sensor (e.g., Odyssey Extreme PAR or MiniPAR from PME). While this does not eliminate the need for maintenance, it significantly reduces it.

As an alternative to direct light measurements, turbidity sensors can also be used to assess water clarity. There are many logger options available for measuring turbidity, but they are generally expensive and require calibration and expertise for proper interpretation. Therefore, direct light measurements and the calculation of Kd are recommended as the preferred approach for quantifying this ecosystem function.

Regulating ES Ecosystem function	**Indicator**	**Methods**	**Units**	**Expertise level: Specialist (S) Volunteer (V)**	**Recommended (R) Complementary (C)**	**Scale**	**Timeframe Frequency**	**Strength**	**Weakness**
Improved water clarity	Secchi depth	Horizontal secchi depth	m	N	E	m	Before and after restoration. Effect highest when restoration achieve full coverage	Well-known method, easy and quick to perform, inexpensive equipment.	Requires two people, result influenced by subjectivity, less precise than other available methods
	Water clarity	K_d-measurements (Loggers)	m^{-1}	E	E	m	Before and after restoration, preferably continuously over a longer temporal period before and after. Effect highest when restoration achieve full coverage	Accurate method, capable of collecting data over long time periods	Requires regular maintenance to ensure good data quality. Can be expensive.
		Turbidity loggers	NTU / FNU	E		m			Requires maintenance to ensure good data quality. Interpretation requires data calibration and a high level of expertise. Generally expensive.

Appendix 2

Like all other plants, eelgrass absorbs CO_2 and uses it to produce sugars or to build structural tissue. Parts of the aboveground—and especially the belowground—eelgrass tissue become buried within the bed and accumulate in the anoxic sediment layer, where microbial decomposition is slowed. As a result, a carbon stock develops, with decomposition occurring over many years. However, it is not only eelgrass material that can be buried—algae, fauna, and terrestrial plant material can also contribute to the carbon pool. As the bed matures, a new equilibrium is established in which carbon input and export are balanced. At this point, the pool represents a permanently increased stock relative to unvegetated areas.

From a climate perspective, the ecosystem service provided by eelgrass is defined solely as the carbon that can be directly identified as originating from eelgrass. Unidentifiable carbon is excluded from quantification, making the estimate conservative. For this reason, methods differ from those used for nutrient immobilization. Because only clearly identifiable eelgrass material is used, a reference site without vegetation is not required.

Carbon storage is measured in two pools:

Living biomass: Carbon is stored in the living eelgrass biomass. From a climate perspective, the minimum winter biomass (January–February) is used, as it represents the long-term, permanently stored pool.

Dead biomass: Clearly identifiable dead eelgrass biomass within the eelgrass bed.

Quantifying Carbon in the living Biomass

Samples are collected when eelgrass biomass is at its minimum—in January or February. The sampling method follows the same protocol as described under ‘Immobilization in Standing Biomass’ in relation to nutrient immobilization.

Carbon can be quantified in the lab using a range of methods and instruments, depending on the available equipment at the local laboratory (table 11).

Quantifying Carbon in dead biomass

Field work

To retrieve biomass buried in the sediment, a vacuum-based sediment steel corer is used (photo below)

In the field, 10 sediment core samples are taken. The corer is fully inserted, and the sediment is extracted, then sieved through a 1 mm mesh to remove sediment. Samples are transported to the laboratory and can be stored moist and refrigerated for a few days but should be processed as soon as possible to prevent decay of the eelgrass biomass. Especially the separation of living and dead belowground biomass becomes more difficult the longer the sample is stored.

Laboratory work: In the lab, living biomass is separated from dead biomass. The dead material is placed in pre-weighed aluminium trays and dried for 24 hours at 60 °C. The dry weight is then recorded. After drying, the material can be stored for an extended period before further analysis.

Carbon content can then be quantified using a selection of methods and instruments, depending on the equipment available at the local laboratory.

Center for Marin Naturgenopretning (Marin natur - Center for Marin Naturgenopretning), er et samarbejde mellem Aarhus Universitet, Institut for Ecoscience, DTU Aqua Institut for Akvatiske Ressourcer, Syddansk Universitet, Biologisk Institut, og Limfjordsrådet.

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