EUROPEAN NATIVE OYSTER HABITAT RESTORATION MONITORING HANDBOOK

NOVEMBER 2021

Editors: Philine zu Ermgassen, Oscar Bos, Alison Debney, Celine Gamble, Azra Glover, Bernadette Pogoda, Stéphane Pouvreau, William Sanderson, David Smyth, Joanne Preston.

NATIVE OYSTER NETWORK
UK & IRELAND

NORA

ACKNOWLEDGEMENTS

This publication has received funding from the LIFE Programme of the European Union. This publication reflects the author's view only. The European Commission is not responsible for any use that may be made of the information it contains.

We thank the authors of the "Restoration Guidelines for Shellfish Reefs" for provision of the handbook design template.

ISBN: 978-0-900881-89-3

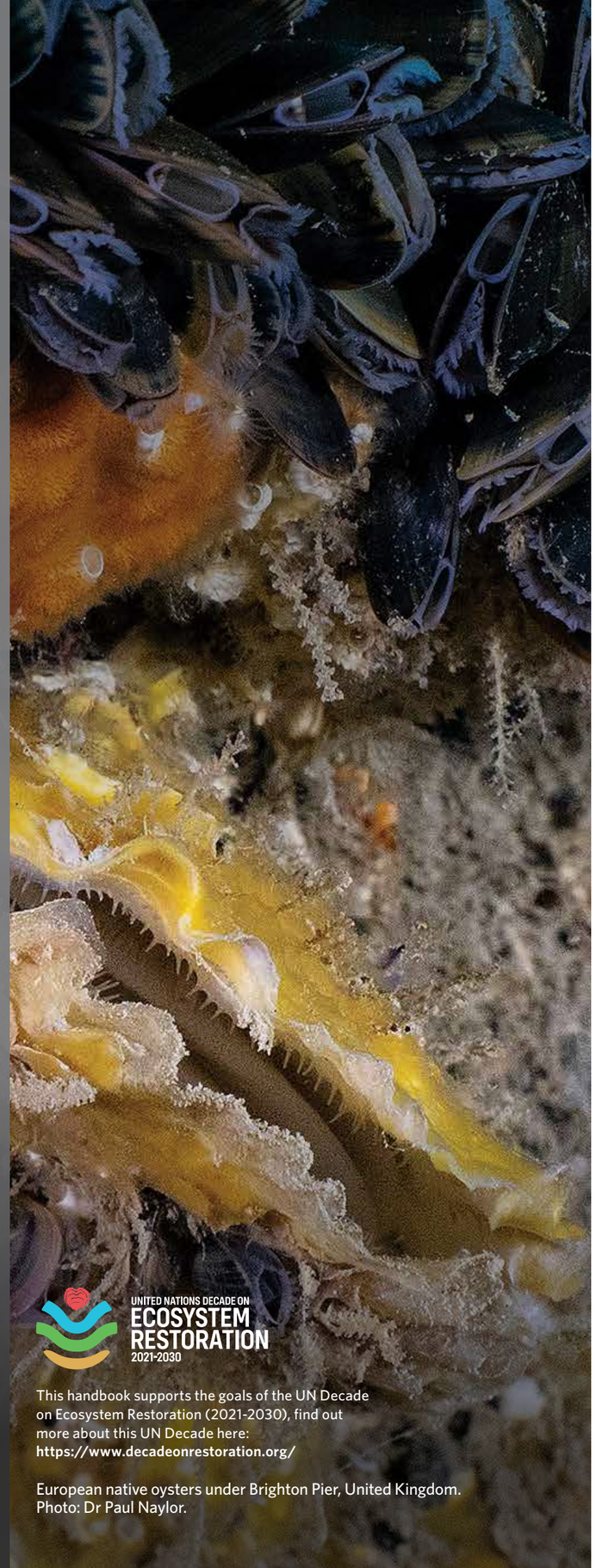
The Native Oyster Network – UK & Ireland was co-founded by the Zoological Society of London and the University of Portsmouth, with funding from the John Ellerman Foundation.

The Native Oyster Restoration Alliance (NORA) is funded in the framework of the project PROCEED by the Federal Agency for Nature Conservation with resources from the Federal Ministry for the Environment, Nature Conservation and Nuclear Safety. PROCEED is realised within the Federal Programme for Biological Diversity ("Bundesprogramm Biologische Vielfalt").

Suggested citation for publication:
zu Ermgassen, P.S.E., Bos, O., Debney, A., Gamble, C., Glover, A., Pogoda, B., Pouvreau, S., Sanderson, W., Smyth, D. and Preston, J. (eds) (2021). *European Native Oyster Habitat Restoration Monitoring Handbook*. The Zoological Society of London, UK., London, UK.

Suggested citation for chapters (for example):
Preston *et al.* (2021). Monitoring European Native Oyster Restoration Projects: An introduction. In: *European Native Oyster Habitat Restoration Monitoring Handbook* (eds. zu Ermgassen, P.S.E., Bos, O., Debney, A., Gamble, C., Glover, A., Pogoda, B., Pouvreau, S., Sanderson, W., Smyth, D. and Preston, J). The Zoological Society of London, UK., London, UK.

MIX Paper from responsible sources FSC® C022913



UNITED NATIONS DECADE ON ECOSYSTEM RESTORATION 2021-2030

This handbook supports the goals of the UN Decade on Ecosystem Restoration (2021-2030), find out more about this UN Decade here: **https://www.decadeonrestoration.org/**

European native oysters under Brighton Pier, United Kingdom. Photo: Dr Paul Naylor.

EDITORS

EDITORS

Philine zu Ermgassen, Native Oyster Restoration Alliance, Berlin, Germany. Email: secretariat@noraeurope.eu

Oscar Bos, Wageningen Marine Research, Den Helder, The Netherlands. Email: Oscar.bos@wur.nl

Alison Debney, Zoological Society of London, Regent's Park, London, NW1 4RY, UK. Email: Alison.Debney@zsl.org

Celine Gamble, Zoological Society of London, Regent's Park, London, NW1 4RY, UK. Email: Celine.Gamble@zsl.org

Azra Glover, Zoological Society of London, Regent's Park, London, NW1 4RY, UK. Email: Azra.Glover@zsl.org

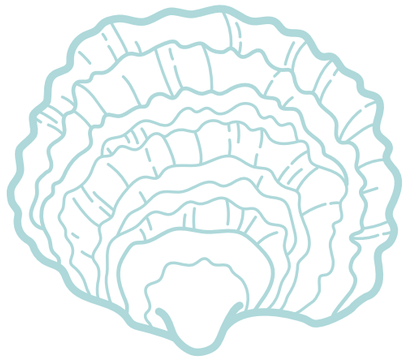
Bernadette Pogoda, Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research, Bremerhaven/Helgoland/Sylt, Germany. Email: bernadette.pogoda@awi.de

Stéphane Pouvreau, Ifremer, Laboratory of Environmental Marine Sciences (UMR 6539 LEMAR), 29280 Plouzané, France. Email: Stephane.Pouvreau@ifremer.fr

William Sanderson, Heriot-Watt University, Edinburgh Campus, Riccarton, EH14 4AS, UK. Email: w.g.sanderson@hw.ac.uk

David Smyth, Shellfish Centre, Bangor University Wales, LL59 5AB, UK. Email: d.smyth@bangor.ac.uk

Joanne Preston, Institute of Marine Sciences, University of Portsmouth, Ferry Road, Eastney, PO4 9LY, UK. Email: Joanne.Preston@port.ac.uk



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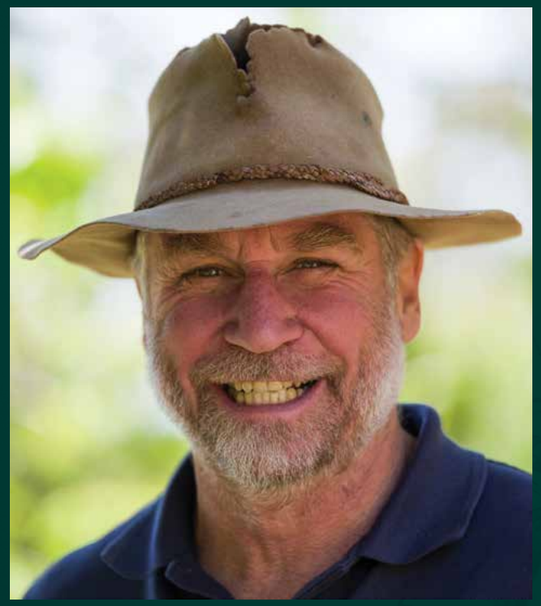
MONITORING WORKSHOP AND CONTRIBUTING AUTHORS

In December 2019, the Native Oyster Network and NORA co-hosted a 'European Native Oyster Restoration Monitoring Workshop' at the University of Portsmouth, during which these monitoring metrics were developed.

The following list of participants includes workshop attendees and authors who have contributed to this publication: Jenna L. Alexander, Isabelle Arzul, Liz Ashton, Oscar Bos, Cass Bromley, Tom Cameron, Mark Chatting, Trish Daly, Alison Debney, Monica Fabra, Celine Gamble, Azra Glover, Jacob Kean-Hammerson, Tanja Hausen, Chris Hauton, Maria Hayden-Hughes, Luke Helmer, Zoë Holbrook, Patrick Joyce, Hannah Lee, Alice Lown, Sharon Lynch, Verena Merk, Charlie Mountain, Bernadette Pogoda, Stéphane Pouvreau, Joanne Preston, Chris Ranger, Simon Reeves, Ana Rodriguez-Perez, William Sanderson, Eric Scott Harris, David Smyth, Oliver Tully, Emma Ward, Rob Whiteley, Ben Wray, Philine zu Ermgassen.

Foreword
by Boze Hancock, PhD.

Senior Marine Habitat Restoration Scientist, Global Protect Oceans Team at The Nature Conservancy.

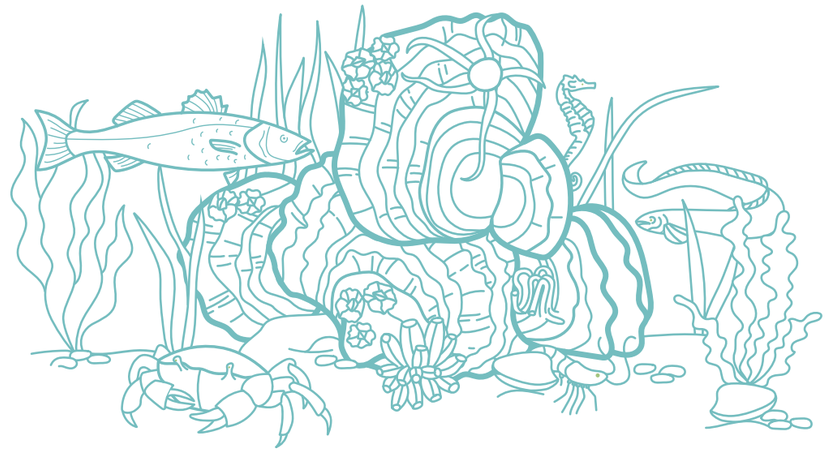


In this, the UN Decade on Ecosystem Restoration, it is recognised that restoring the function and productivity of our planets ecosystems is critical and must be scaled up as quickly as possible. Oyster reefs and beds have historically been a critical ecosystem in the world's bays, estuaries and shallow seas. The filter feeding of oysters collects the material and nutrients suspended in the water and makes them available to fuel the biodiversity these habitats are known for, provide fish for our fisheries, and mitigate the nutrient pollution that creates marine dead zones. The structure provided by oyster habitats also helps stabilise sediments, moderate wave energy and support a vast number of reef associated species. Despite their importance, oyster reefs and beds are also the world's most impacted marine ecosystem; native oysters and the habitat they create have been virtually destroyed in most regions of the world. The valuable benefits provided by oyster habitats have driven a huge interest in their restoration.

In a young and rapidly growing field such as oyster habitat restoration it is critical that the restoration community can compare techniques and assess both success and failures to progress and scale the work. This requires monitoring and gathering data that are sufficiently similar to allow comparison. However, no two sites are identical, and local conditions demand constant adaptation. It is both challenging and critical to describe monitoring protocols and metrics that provide the flexibility to accommodate the vastly different conditions faced by restoration projects, from the intertidal zones of estuaries to the deep, high energy areas of the North Sea. This manual builds on earlier work and provides valuable guidelines tailored to the European context. We look forward to these guidelines being applied to help scale the critical work of restoring the oyster reefs of Europe.

GLOSSARY

- **Autonomous underwater vehicle (AUV):** an unmanned and untethered autonomous underwater vehicle that is used to carry out underwater research.
- **Baseline:** the condition of an area or native oyster population prior to an activity taking place.
- **Before-After-Control-Impact (BACI):** a survey design that monitors at a control site (unrestored) and impact site (location of oyster restoration) both before and after the construction of the oyster reef.
- **Bimodal:** having or involving two modes, such as having two maxima in statistical distribution.
- **Blue carbon:** refers to the carbon stored in marine ecosystems.
- **Broodstock:** the group of sexually mature native oysters used in aquaculture or in restoration projects for the purpose of reproduction and larval supply.
- **Cohorts:** a group of subjects with a common defining characteristic, for example age.
- **Cultch:** any substrate, such as rock or shell, that a juvenile native oyster is attached or may attach to.
- **Differential Global Positioning System (dGPS):** an enhanced version of the traditional Global Positioning System (GPS), which improves the accuracy of location (10s of cm accuracy).
- **Ecosystem service:** the benefits provided by nature.
- **Environmental DNA (eDNA):** genetic material obtained directly from environmental samples, such as sea water, which allows for the non-invasive identification of a suite of target species.
- **Epifauna:** a group of benthic organisms that live attached to hard surfaces such as rocks or shells. Epifauna include oysters, sponges, sea squirts and barnacles.
- **Infauna:** a group of benthic organisms that live burrowed into the bottom sediments of the ocean floor. Infauna include worms and clams.
- **Invertebrates:** any animal that lacks a backbone, such as mussels, oysters, shrimp and worms.
- **Larviparous:** an organism in which the fertilised eggs develop internally up to the larval stage, and are then released in their larval form.
- **LiDAR:** a remote sensing method that utilises laser light to densely sample the earth's surface and produce high accuracy measurements.
- **Poaching:** the taking of oysters illegally or without permission, as defined by regional or national legislation or a voluntary measure.
- **Pseudoreplicates:** samples or replicate measurements are not independent of one another and therefore result in invalid statistical tests if analysed.
- **Recruitment:** the settlement and survival of native oysters such that they contribute to the overall population.
- **ROV:** a remotely operated underwater vehicle that is used to carry out underwater research.
- **Sessile:** refers to an immobile organism that is fixed in one place.
- **Seston:** the particulate component moving in water, in the form of living organisms (plankton and nekton) and non-living material (plant debris and sediment).
- **Settlement:** the process whereby native oysters in the larval stages settle out from the water column onto suitable substrates and undergo metamorphosis, permanently cementing themselves to the surface.
- **Spat:** the term used to describe juvenile oysters that have attached to a hard substrate following the free-swimming larval phase.
- **Substrate:** the hard material, often shells, small stones or large rocks, that juvenile native oysters are able to settle upon. This can be naturally occurring or intentionally deployed to encourage recruitment settlement.



CHAPTER 1
MONITORING EUROPEAN NATIVE OYSTER RESTORATION PROJECTS: AN INTRODUCTION

CHAPTER AUTHORS
Joanne Preston, Alison Debney, Celine Gamble, William Sanderson, Philine zu Ermgassen.

OVERVIEW OF EUROPEAN NATIVE OYSTER RESTORATION

The habitat formed by the European Native Oyster (*Ostrea edulis*) was historically a dominant feature of European coastal and offshore waters. Over exploitation of native oysters through industrial fishing in the late 1800s and early 1900s was the primary driver of the degradation and decline of this habitat. Subsequently, poor water quality and introduced diseases have also taken their toll. Today, native oysters, which were once considered a cultural mainstay and staple food for people from all walks of life, are more or less forgotten inhabitants of Europe's coastal seas. It is only over the past decade, following on from successful restoration efforts in the United States of America and a growing understanding of the need to actively engage in recovering our degraded marine ecosystems, that restoration of wild native oyster habitats in Europe has been actively pursued. In just five years, between 2015 and 2021, the number of native oyster restoration projects in Europe has increased from five to 33 (see Figure 1.1). Restoration is now being attempted across much of the historical range of the species and in a range of habitat settings, including marinas, estuaries, near shore coastal waters and deeper offshore areas. While most restoration efforts are still in the pilot stage, oyster restoration practitioners range from nature conservation bodies to large companies (e.g. offshore wind farms), through to small community action groups.

Figure 1.1: *Ostrea edulis* restoration projects active in 2015, 2017 and 2021, illustrating an increased number of projects addressing native oyster restoration.

DEVELOPMENT AND RATIONALE FOR RESTORATION GUIDELINES

This handbook has been written to facilitate effective and consistent monitoring of native oyster restoration projects across Europe. It builds on the information provided in the European Native Oyster Habitat Restoration Handbook (Preston *et al*. 2020) and acts as a European extension to the American Oyster Habitat Restoration Monitoring and Assessment Handbook (Baggett *et al*. 2014). It has been developed by the Native Oyster Network – UK & Ireland and as part of the European Native Oyster Restoration Alliance (NORA) Monitoring Working Group in recognition of the need to adopt shared monitoring protocols across Europe.

The need to develop monitoring guidelines for the native oyster was identified because native oyster habitat most often occurs in the subtidal zone of soft-sediment coastal estuaries, bays and open coasts. The visibility in these locations is often low, while oyster habitat itself is often fragmented and of low relief. These factors make it far more challenging to monitor native oyster populations than densely aggregating, vertical-reef-building species such as the North American eastern oyster (*Crassostrea virginica*), for which the earlier monitoring handbook (Baggett *et al*. 2014) was primarily written.

Adoption of a shared monitoring approach will allow systematic comparison of key metrics for restoration across the biogeographic range of the native oyster, which spans many political and geographical jurisdictions. This handbook recommends a suite of monitoring metrics with the aim of enabling practitioners to assess the progress of their restoration project and therefore provide useful information to facilitate adaptive and evidence-driven management of their projects. It will facilitate a pan-European evidence base of restoration progress, extent, successes, failures and performance (see Figure 1.2). This handbook does not seek to provide a comprehensive review of all possible methods to address scientific research, nor is it intended that all projects undertake the full suite of monitoring outlined here (see Table 1.1).

Consistent and joined-up monitoring of native oyster restoration projects is necessary for a number of reasons. The widespread extirpation of the species has resulted in a lack of information regarding the nature and characteristics of undisturbed, well-established native oyster habitat. This loss of memory or evidence of a habitat is called a shifted baseline and it presents a major challenge for restoration goal setting (see Figure 1.3).

Not all environmental conditions will support high-level recruitment and reef formation; therefore, monitoring across the range of the native oyster is necessary to provide evidence on the potential of the habitat and the range of characteristics of undisturbed or restored native oyster habitat (see Box 1.2). This knowledge gap extends to the benefits to society and human well-being (ecosystem services) provided by native oyster habitat; these benefits are often the main drivers for restoration projects. A shared monitoring approach will also provide a greater evidence base regarding impacts on water quality, associated fish, biodiversity, and interactions with neighbouring coastal habitats.

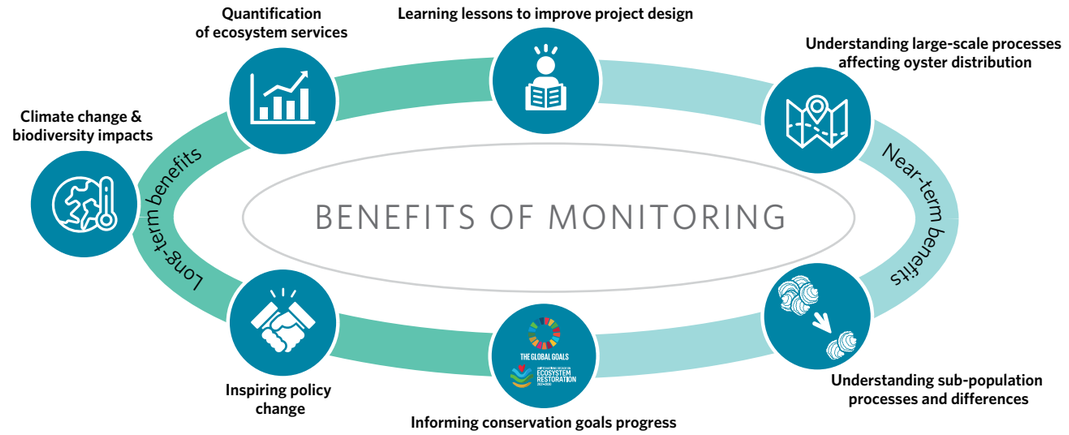


Figure 1.2: Monitoring allows not only for the basic performance of each reef to be assessed through time, but also assists with lessons learned. Consistently gathering monitoring information allows those data to be bundled to provide a critical evidence base in the long term for developing environmental policies.

BOX 1.1: WHAT IS RESTORATION?

For the purposes of this handbook, ecological restoration is defined as **"the process of establishing or re-establishing a habitat that in time can come to closely resemble a natural condition in terms of structure and function"** (Baggett *et al*. 2014). There are several terms used to describe activities that qualify as restoration, including habitat enhancement, habitat recovery, habitat regeneration and habitat creation. Across this spectrum, monitoring enables the measurement of changes in biodiversity, habitat structure, ecological integrity and ecosystem services. With time and continued monitoring of native oyster habitat restoration projects, our understanding of a healthy 'natural' or reference ecosystem will improve.

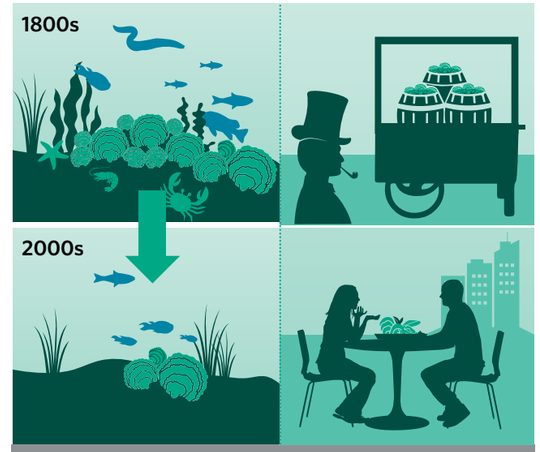


Figure 1.3: People's relationship and understanding of the European native oyster has changed over time since the widespread loss of the habitat and associated fishery. This shifting baseline applies to both the culinary value and the cultural value of the native oyster.

BOX 1.2: WHAT IS EUROPEAN NATIVE OYSTER HABITAT?

Given the degraded status of native oyster habitat throughout most of its range, and the lack of historical surveying prior to the habitat being impacted and largely extirpated, a comprehensive description of healthy native oyster habitat in the European context is lacking. Yet a definition of the habitat is critical to ensuring that the aims of habitat restoration are universally understood. Native oyster habitat can take a range of forms and include both oyster 'reefs' or 'beds' (see Table 1.1). Other terms historically used include 'oyster grounds', which relates to areas fished for oysters. **Native oyster habitat** is defined here as **'a substrate with a veneer of living oysters, providing high surface complexity, on a substrate which may be dominated by dead oyster shell.'**

The threshold density, height and spatial extent of native oyster that delineates an oyster reef is not clearly defined, both due to the lack of available baseline and because such thresholds are universally challenging to define for reefs (see Baggett *et al.* (2014) for further definitions in the US oyster context). OSPAR Commission (2009) has defined oyster beds as **"*Ostrea edulis* occurring at densities of 5 or more per m² on shallow mostly sheltered sediments (typically 0–10m depth, but occasionally down to 30m). There may be considerable quantities of dead oyster shell making up a substantial portion of the substratum."** It is clear from historical documents that native oyster habitat supports a distinct associated community of other species, which may in the future also prove useful in defining the habitat. For a description of the range of native oyster habitat currently observed in Europe, see Table 1.1 below.

Table 1.1: Oyster habitat definition and description (modified from Pouvreau *et al.* 2021b).

Criteria	Stage 0	Stage 1	Stage 2	Stage 3	Stage 4
Oyster habitat descriptor	**Mixed sediments**	**Mixed sediments featuring oysters**	**Functioning oyster habitat reefs and beds**		
Example					
Max density	0-1 ind/m²	1 to 5 ind/m²	5 to 10 ind/m²	10 to 20 ind/m²	> 20 ind/m²
Aggregation	None	Single/pair	Several individuals	Many individuals	Maximal
Size spectrum	1 cohort	1 or 2 cohorts	Several cohorts	Several cohorts	Many cohorts
Recruitment	< 0.1 ind/cm²	< 1 ind/cm²	< 10 ind/cm²	< 100 ind/cm²	> 100 ind/cm²
Oyster composition	Rolling - Buried	Fixed - Emerging	Small clusters	Big clusters	Biogenic reef structure
Habitat resilience	Minimal	Minimal	Low	Medium	High
Biodiversity	Low	Low	Medium	High	Very high
SER label	★	★★	★★★	★★★★	★★★★★

Monitoring is a critical element of managing restoration efforts, informing restoration practitioners whether a project is progressing as planned. Furthermore, without monitoring, restoration practitioners lack credibility in their assertions about the project and will not be able to build the evidence base for their funders and stakeholders. This is pivotal because partners and contributors are making significant investments and need evidence that their investments are well made. The goals behind restoration programmes can be varied, including intended gains in biodiversity, water quality improvement, management of carbon or provision of habitats that support commercially important species. Where possible, monitoring should be designed to address the aims of the restoration project (zu Ermgassen *et al.* 2016). In the longer term, this information is critical to understanding the 'return on investment' as well as project success or failure in general terms. Although the evidence for cost-benefit analysis of native oyster restoration is incomplete, it is an important consideration and could help leverage future funding. In some cases, such as Marine Protected Areas (MPAs), there may be supporting legislation that requires monitoring and reporting, with a legal imperative to provide monitoring evidence. In addition, as restoration ecology is a relatively new science, monitoring is also valuable because it can inform what works best and how restoration tools and designs can be improved. Finally, the standardisation of monitoring and the reporting of results can allow for powerful analyses across projects and enable learning opportunities that improve the implementation of future projects (see Figure 1.2).

This handbook has been written for all those involved in native oyster habitat monitoring. This includes restoration project managers, restoration practitioners, community projects, governmental bodies, 'citizen scientists' and charitable organisations.

HOW TO PLAN MONITORING AND USE THIS HANDBOOK

Deciding what to sample

A metric is a quantifiable measure that is used to track and assess the status of a specific process. In this handbook, we make recommendations for **universal, supplementary** and **restoration** goal-based metrics (see Box 1.3 for metric definitions), which can measure the progress of native oyster restoration in Europe against project goals. It is appropriate to include monitoring of the metrics which most directly reflect the aims of the project (see Table 1.2), that should be determined in partnership with restoration stakeholders (Preston *et al.* 2020).

For each of the metrics listed in this handbook, information is provided regarding:

- Required units for data collected.
- Primary and alternative methodologies for both subtidal and intertidal habitats.
- Recommended minimum frequency of sampling.
- Performance criteria.

Once the suite of metrics have been decided, practitioners can refer to the appropriate sections of the handbook; it is not necessary to read the entire handbook.

The stated frequency is the minimum amount of monitoring necessary for comparability and statistical rigour. Restoration practitioners are encouraged to monitor more frequently and for longer if doing so improves the resolution of their results and the ability to interpret them.

BOX 1.3: METRIC DEFINITIONS

Universal monitoring metrics outlined in this handbook have been identified as essential metrics that should be recorded by all native oyster restoration projects, regardless of the restoration goal(s) of that project. Sampling of the universal metrics allows for the basic performance of each reef to be assessed through time, while also allowing for comparisons of universal metrics with other projects across the native oyster range. Sampling of the universal environmental variables also provides important information that can aid in the interpretation of data collected during oyster habitat monitoring activities **(Chapter 2)**.

Supplementary monitoring metrics outlined in this handbook are desirable but not essential metrics that might be recorded by a native oyster restoration project. These provide supplemental and/or more detailed information concerning the performance of a given project; however, they are not essential to monitoring the progress of the project when working with budget or capacity limitations. The supplementary metrics relate to native oyster condition, survival, growth, reproduction and recruitment **(Chapter 3)**.

Restoration goal-based metrics are those that may not be necessary to assess if restoration is working, but can help determine if the underlying goals are being achieved, i.e. the provision of ecosystem services such as biodiversity gain, water quality enhancement or carbon storage. It is recommended that projects for whom these are primary goals should monitor directly for these outcomes. This can serve both to increase stakeholder understanding of the value of the restoration efforts and inform adaptive management to maximise the potential ecosystem service return of the project **(Chapter 4)**.

Table 1.2: Examples of monitoring metrics most relevant to shared native oyster habitat restoration goals (E indicates essential, S indicates supplementary).

Primary aim of the project	Universal metrics	Oyster growth	Reproduction/recruitment	Threats	Broodstock and oyster population enhancement	Fish and invertebrate biodiversity	Interactions with associated habitats	Water quality improvement	Socio-economic	Blue carbon
Recovery/re-establishment of historical habitat	E	S	S	S						
Increased biodiversity	E		S			E	S			
Supporting a sustainable oyster fishery	E	S	S	S	E					
Improved adjacent coastal habitats	E			S			E	S		
Improved water quality	E			S				E		
Increased fish and invertebrate abundance	E			S		E				
Increasing carbon storage	E	S	S	S						E
Restoring coastal jobs and culture	E			S					E	

DECIDING HOW TO SAMPLE

Once the relevant metrics have been identified, the next step is to determine which of the applicable methods is most suitable for the restoration project. The most appropriate method to apply can vary with the project depth and accessibility. Regulations may also apply in Marine Protected Areas (MPAs) that influence the most appropriate or permitted methods. Early partnership with the managing authority is recommended. Cost and available expertise will also play a role in selecting the optimal methods. Throughout these guidelines, a range of possible methods are therefore presented.

Where visibility at a site is low and diver-led surveys are not an option due to either unfavourable conditions or cost, it may be that the only remaining option is to sample using methods destructive to the seabed, e.g. by grab or dredge. The decision to adopt these methods, and methods requiring the sacrifice of living oysters, should not be taken lightly and should take into account the size and condition of the oyster population. Oyster habitat restoration relies on the cultch and living oysters remaining in situ and relatively undisturbed in order for the habitat to recover. In some settings, however, destructive sampling is effectively the 'best' option or an integral part of long-term and site-specific monitoring protocols. Some destructive sampling methods are therefore included in these guidelines.

Destructive methods have been identified with a warning triangle. It is important that all conservation and research activities adhere to high standards of animal welfare and animal and human ethics. A ethical review of the proposed monitoring activities should be considered.

Designing monitoring surveys

To measure the ecological changes and assess the performance of a project in meeting its restoration goals, monitoring should ideally be conducted to encompass pre-restoration, the short-term (e.g. 1-2 years), mid-term (e.g. 4-6 years) and long-term (10+ years) impact of activities, at both restored and control sites, ideally using a Before-After-Control-Impact (BACI) design (see Figure 1.4).

Where pre-restoration monitoring is not an option, the comparison between the restored and control sites is essential to be able to assess the impact of restoration activities.

A BACI design allows for differences between the control and the restoration site to be accounted for to some extent, and therefore for the impact of restoration to be identified with greater certainty, despite limited replication.

Control sites and the restoration site should be sampled concurrently. Control sites should have similar habitat characteristics to pre-restoration conditions (e.g. mixed sediments or sand substrate) and should also be:

- Close enough to the restored habitat to ensure comparable physical conditions (e.g. regarding currents, temperature, salinity, bathymetry).
- Distant enough to ensure that there is no direct interaction with the restored habitat (e.g. migration distance of fish and other transient biota, change in current patterns not influenced by the restoration site).
- Assessed concurrently with the restored habitat, using identical methods and effort.

Where possible, nearby natural reference sites (healthy, natural reefs characteristic of the restoration goal) should also be concurrently sampled, although this is unlikely to be an option for most restoration efforts in Europe, given the near extirpation of native oyster habitat.

HEALTH & SAFETY

Coasts are dynamic environments and present potential hazards, such as strong tidal flows, adverse weather, uneven substrates with trip hazards, deep mud and waterborne pathogens. Location-specific risk assessment must be conducted for each sampling site prior to surveys being commenced.

BIOSECURITY

Monitoring surveys should be designed to minimise the risk of spreading disease or non-native species. This should include implementing 'Check, Clean, Disinfect, Dry' protocols on all equipment (see Figure 1.5). Where multiple sites are being surveyed, careful planning regarding the order of sites visited based on their risk profiles should be undertaken. A comprehensive overview of biosecurity issues relating to native oyster restoration can be found in zu Ermgassen *et al.* (2020).

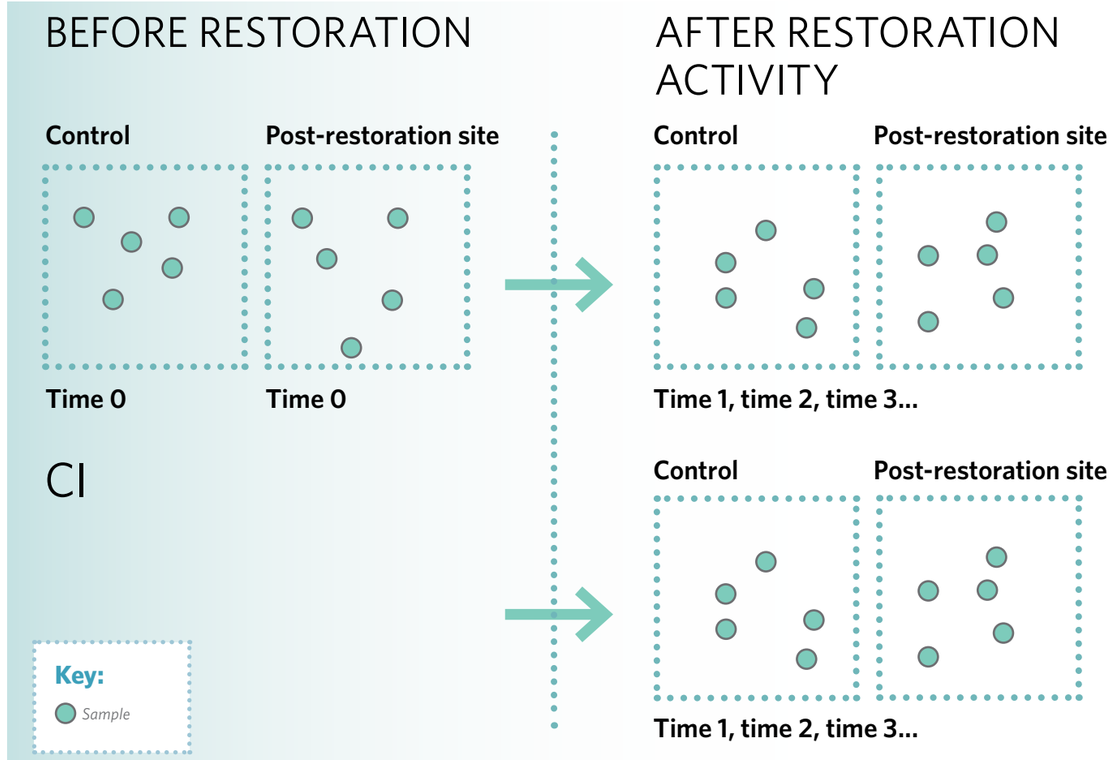


Figure 1.4: The Before-After-Control-Impact (BACI) survey design entails monitoring at a control site (unrestored) and impact site (location of oyster restoration) both before and after the restoration activity. Pre-restoration monitoring should be conducted at both the control and restoration site, both prior to and post-restoration.

The success and reputation of a restoration project can be negatively impacted by accidental introductions of invasive species and pathogens. Project equipment such as vans, boats and field kit can all be vectors for their transmission, which will ultimately damage the marine environment and wildlife.

CHECK **Check** your equipment, clothing and boats after carrying out fieldwork for fouling material. Ensure that you remove anything that you find and dispose of it in the appropriate manner.

CLEAN **Clean** all fieldwork items thoroughly with freshwater as soon as possible. Ensure that you pay attention to items such as fieldwork clothing, restoration equipment, trailer wheels and areas that are damp or hard to reach.

DIS INFECT **Disinfect** Where the risks are higher, include disinfection as part of cleaning procedures.

DRY **Dry** Ensure that you drain water from any water remaining on fieldwork items, and equipment such as a trailer and boat. Try to dry all equipment for as long as possible before next usage.

WATCH OUT FOR

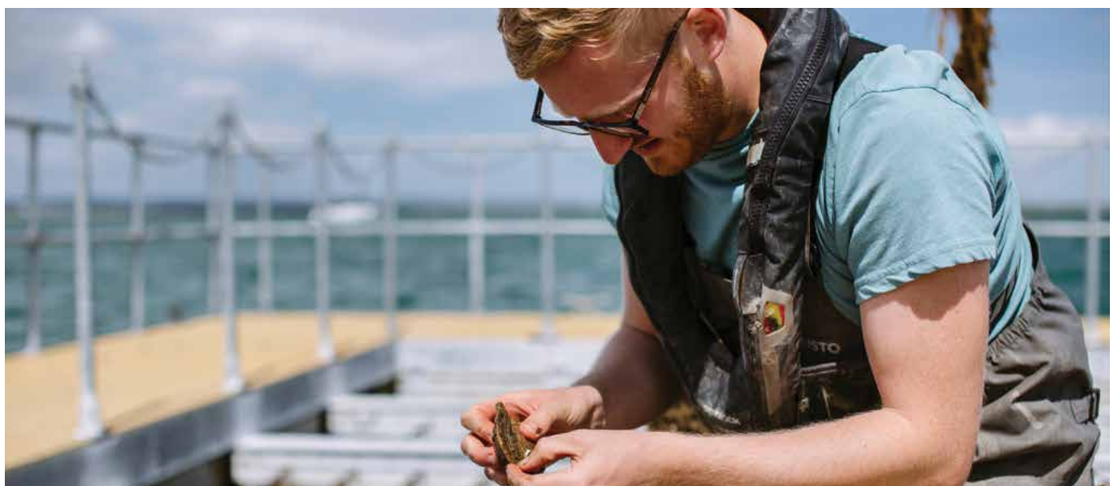

American slipper limpet *(Crepidula fornicata)*


Pacific oyster *(Crassostrea gigas)*


Carpet sea squirt *(Didemnum vexillum)*


American oyster drill *(Urosalpinx cinerea)*

Figure 1.5: Areas to be vigilant with when cleaning after carrying out fieldwork for native oyster restoration projects: 'Check, Clean, Disinfect, Dry' protocol.



The Solent Oyster Restoration Project carrying out monitoring on oyster cages in the Solent. (Photo: Luke Helmer).

BOX 1.4: SAMPLING TECHNIQUES

Random sampling

To eliminate bias when collecting samples, a true random sampling method must be employed. Samples should also be 'independent' so they cannot be considered pseudoreplicates. A common way to perform random sampling is to determine the area to be sampled, superimpose a numbered grid on to an aerial photo or a diagram of the area to be sampled (e.g. restoration area), then randomly select the locations to sample using a random number generator. Random number tables can be found online (e.g. https://www.random-generator.org.uk/numbers/) and in most statistics textbooks.

See example below of a numbered grid superimposed on a diagram of a native oyster habitat (in blue), with randomly selected sample locations noted in green.

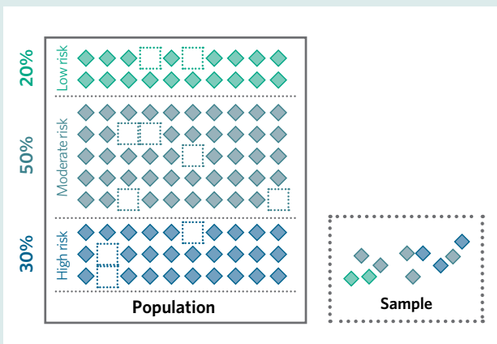
1	2	3	4	5	6	7	8	9	10
11	12	13	14	15	16	17	18	19	20
21	22	23	24	25	26	27	28	29	30
31	32	33	34	35	36	37	38	39	40
41	42	43	44	45	46	47	48	49	50

In some instances, it may be necessary to perform fixed sampling, in which the same marked locations are sampled at every monitoring event. The locations should initially be randomly determined.

Stratified random sampling

This is used when a habitat can be divided into zones (e.g. native oyster habitat, mudflat, seagrass). The area of each stratum should be defined and the locations within each stratum should be randomly determined as illustrated below.

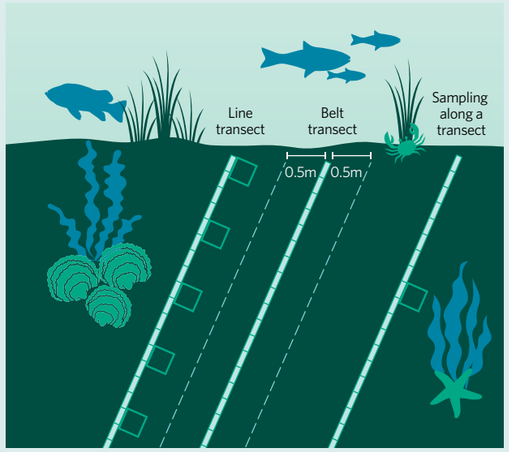
Those familiar with the statistical programme R could consider using the 'MBH design' package to develop spatially balanced/stratified random sampling designs. See: https://cran.r-project.org/web/packages/MBHdesign/index.html.



Systematic sampling, e.g. line and belt transects

This is used in locations with a known environmental gradient (e.g. salinity, distance to an existing native oyster population, tidal height) and to measure changes in species composition. A transect is placed along the gradient and quadrats are placed at regular intervals of horizontal or vertical distance. For low-density populations, belt transects can be used that record the occurrence of a species at a specified distance either side of the entire length of the transect.

For additional guidance we recommend that the restoration practitioner consult with a statistician or experienced ecologist, perhaps at a nearby academic institution, to assist with some of the more complex designs and analysis.



Determining sample size

Native oyster restoration projects vary in extent, restoration materials, morphology, form and tidal depth. As such, the number of samples (e.g. quadrat samples, core samples, lift net samples, etc.) to be taken per site for each metric will be different for each restoration project. Monitoring costs can be high, particularly when using advanced methods and/or when large areas need to be assessed. Attempts are therefore often made to keep sample replicates to a minimum, while still having enough data to make a robust assessment. In some instances, however, accurate estimates can require large numbers of samples (e.g. estimates of mean abundances in highly patchy populations; see **Chapter 3** for further explanation). Adequate replication is essential for meaningful conclusions to be drawn.

Reporting results

It is imperative that data are recorded using the required units and with an average (mean) and variance (standard error) so that data may be compared across projects. Further consideration of data management, record keeping and reporting are provided in **Chapter 5**.

CHAPTER 2
UNIVERSAL MONITORING METRICS

CHAPTER AUTHORS

Philine zu Ermgassen, Liz Ashton, Oscar Bos, Cass Bromley, Patricia Daly, Eric Harris-Scott, Tanja Hausen, Chris Hauton, Alice Lown, Sharon Lynch, Bernadette Pogoda, David Smyth, Oliver Tully, Ben Wray.

INTRODUCTION

Monitoring of the universal metrics should be undertaken for all European native oyster restoration projects. There are six **universal metrics:** four relating to project performance (project footprint, oyster habitat area, oyster density and oyster size frequency), and two **environmental metrics** (temperature and salinity). The recording and reporting of these metrics allows for project progress to be assessed, enables funders and statutory bodies to receive reports in a standard way, and facilitates comparison across projects.

Comparable monitoring data will ensure that lessons are learnt across projects, that progress towards national and international conservation goals can be determined, and that summary statistics are available across sites on national and European scales (see Figure 1.2, Chapter 1). As resource availability differs from project to project, a range of alternative methods are provided for each metric and each metric has an easily accessible and not too time-consuming method associated with it to ensure that all projects can collect data on the universal metrics.

METRIC 1: PROJECT FOOTPRINT AND OYSTER HABITAT AREA

An accurate measurement of the area of seabed impacted by the project activities is critical for reporting. Two separate measurements should be reported: **project footprint and oyster habitat area (see Figure 2.1).**

The project footprint is the maximum areal extent over which active restoration activity is planned or permitted. This measurement does not account for the inherent patchiness in oyster habitat coverage. In some cases, the project footprint may be very similar to the oyster habitat area (e.g. where cultch is relayed to a specified footprint; Figure 2.1, part b), whereas in other cases it may differ substantially (e.g. where broodstock are relayed along a boat track; Figure 2.1, part d). Practitioners should note that the actual footprint may differ from the planned footprint due to operational challenges and natural environmental conditions.

The restored habitat area provides the total (summed) areal extent over which the restoration activities have resulted in an increase in cultch cover and living oysters (see oyster habitat definition in Box 1.2). It should include any area over which oyster density and size distribution is assessed, such that multiplying the reported densities by the area provides the practitioners and funders with an estimate of the oyster population size of the restored habitat.

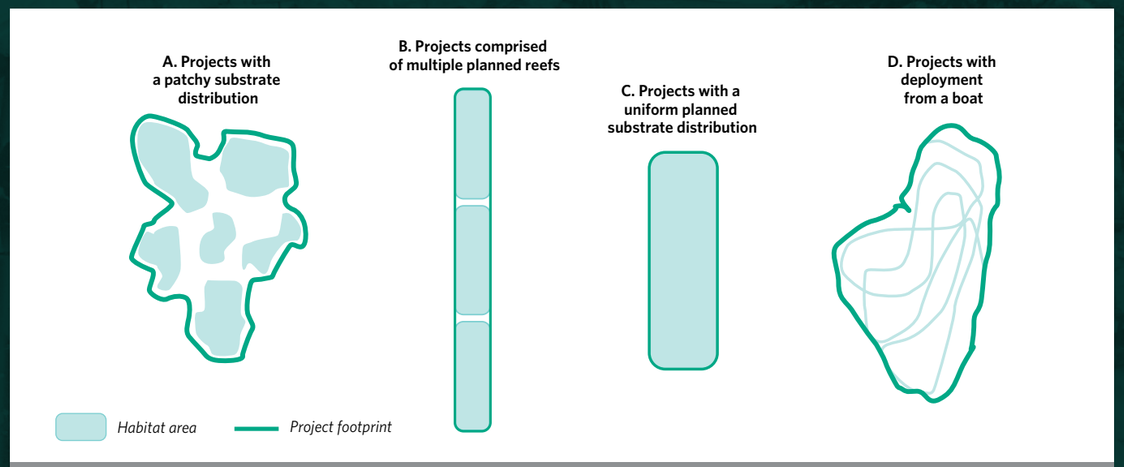


Figure 2.1: Examples of project footprints and their related area of restored habitat from projects with a range of designs. For projects with a high-relief placement of substrate, the project footprint may be the same as the habitat area. The figure shows: A) Projects with a patchy substrate distribution; B) Projects comprised of multiple planned reefs; C) Projects with a uniform planned substrate distribution; and D) Projects with deployment from a boat. (Figure adapted from Baggett *et al.* 2014.)

Required Units: m^2. Note the accuracy of the measuring device (e.g. ±0.5m).

Primary Subtidal Method: Area assessment by SCUBA diving.

SCUBA diving can be used to determine the project footprint and oyster habitat area (see Figure 2.1). Divers, followed by a boat, assess the edge of the project footprint visually and deploy small buoys at regular intervals along the edge of the reef. The boat crew plots the positions of the buoys using a handheld GPS. Alternatively, if hydrodynamic conditions (currents) allow, the divers can tow a surface buoy with a mounted portable GPS. GPS positions can then be entered into mapping software. Shell cover and oyster density should be considered when assessing the oyster habitat area (see Metrics 2 and 3, Chapter 2).

Alternative Method 1: Monitoring with underwater video.

Remotely Operated Vehicle (ROV) video transects or towed underwater video systems can be used to determine the project footprint and restored oyster habitat area (see Box 2.1 for methods). For mapping large areas, the project footprint and oyster habitat area will have to be interpolated between transects due to the defined field of view of this technique (lane width).

Alternative Method 2: Acoustic methods coupled with GPS.

The project footprint of shallow subtidal oyster habitat can be assessed from a boat using side-scan sonar coupled with GPS (see Box 2.1). The extent of the restored habitat can be determined by undertaking transects across the project footprint. Guidance as to how to determine the proper lane width for such surveys is provided in depth in Baggett *et al.* (2014). Given the low relief, low density nature of many native oyster habitats, the resulting data should be ground-truthed by video (see Box 2.2) and/or grab sampling (Metric 3, Chapter 2) to ensure that areas identified as native oyster habitat actually contain living oysters.

Alternative Method 3: Monitoring with AUV.

A more cost-intensive but highly efficient method of assessing the project footprint and associated oyster habitat area is the operation of an autonomous underwater vehicle (AUV), which can produce 3D maps of the reef and surrounding seafloor using a combination of underwater video and side-scan sonar data. AUVs are operated without cable and can dive independently over the habitat. Their application is limited only by inclement hydrodynamic conditions (e.g. current velocity).

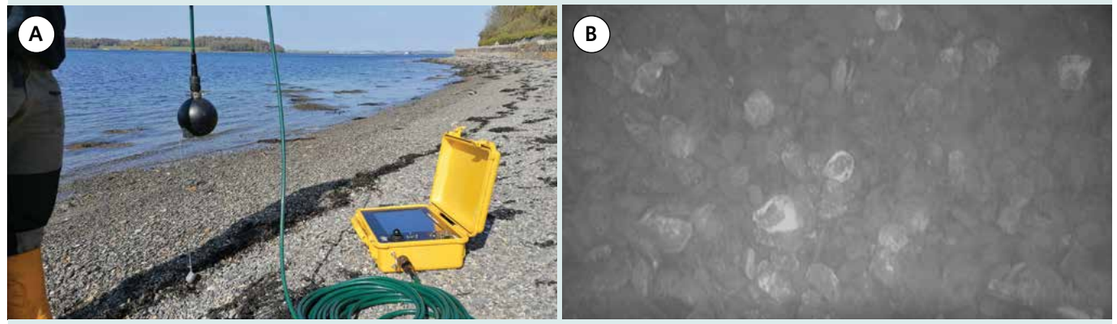
BOX 2.1: MONITORING WITH UNDERWATER VIDEO

Underwater video can be used to monitor a range of metrics, including Native oyster habitat area, shell cover and associated species community. Underwater video is operated either as drop down video (DDV) or as video tows with steel sledges. Current boat position (GPS coordinates) and water depth (per boat lead) should be recorded for georeferencing. Alternatively, video systems and ROVs can be equipped with acoustic systems and GPS to plot the position. If no advanced equipment is available, a handheld GPS or smartphone can be used to mark the position.

Laser pointers should be mounted and calibrated to determine the field of view. If capturing images of quadrats, the camera must be pre-positioned to include the entire quadrat within its field of view. Where water clarity allows, lighting should be used to increase the quality of the recording.

When operating a DDV, gently lower the system until it makes contact with the seabed. Upon contact, the rope will go slack. Wait for two to three minutes so that any sediment disturbed by the quadrat has settled before capturing the image. The DDV and quadrat should be recovered before moving to the next sampling location.

When towing ROVs or operating underwater video on a sled, the boat should drift (maximum speed of one knot) over the study site. The area covered (m^2) can be calculated from the distance travelled (course plot/average speed) and the field of view.

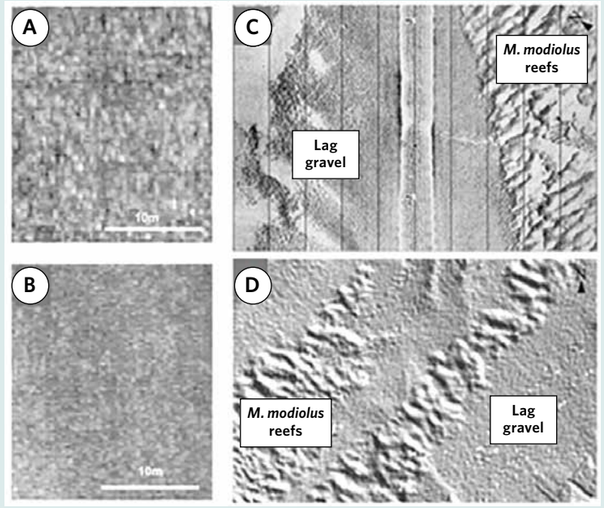


In Loch Ryan, video surveys were carried out using a Spyball DDV (image A) (Submertec, Model SB-MO), controlled using a battery-powered portable control unit (Submertec, Model SCBP) and the footage recorded on a laptop using a Dazzle USB video converter. The height of the camera from the local substratum was used to calculate the area of seabed in the field of view, and the height was controlled by using a plumb bob either 0.5 or 1m in length. Video still from the Spyball underwater camera in Loch Ryan (Lawrence Eagling 2014) (Image B).

BOX 2.2: MONITORING WITH ACOUSTIC METHODS

Acoustic methods include side-scan sonar, multibeam echosounders, SWATH hybrid types and singlebeam echosounder techniques such as RoxAnn. 'Hydroacoustic' monitoring methods can be used when the sound-reflection characteristics of the native oyster habitat differ from those of surrounding habitats. Differences might occur because the surface texture of the native oyster habitat is distinctive due to the clumping of shellfish or accumulation of shell. Additionally, the strength at which sound reflects off a seabed habitat can allow habitats that are rougher or softer to be picked out.

In studies of horse mussel (*Modiolus modiolus*) habitats in the north-east Atlantic, surface textures were reliably distinct in examples with high densities of bivalves where shell deposits had built up (panel D from side-scan imaging and panel E from multibeam imaging below; from Lindenbaum *et al.* 2008). Horse mussel beds also have a distinct texture at lower densities where clumps have formed (see panels A and B in the images below; from Sanderson *et al.* 2014). The overall effect is that these habitats can be confidently picked out on ground-truthed hydroacoustic surveys (see images C and D). Further details on acoustic mapping techiques can be found in Bundesamt für Seeschiffahrt und Hydrographie (BSH) (2016).



Primary Method for lower intertidal habitats: GPS mapping.

Walk the perimeter of the project footprint at low spring tide, marking the location frequently using the most accurate GPS available for the project. In the case of the oyster habitat area, mapping should be paired with assessments of oyster density (Metric 3, Chapter 2) and shell cover (Metric 2, Chapter 2), such that only areas visually deemed to be oyster habitat (see Box 1.2 and Figure 2.1) are mapped. A large quantity of data points or the use of track mode increases the accuracy of the measurement. Alternatively, there are a range of mobile phone apps that can track walking routes. Always check the reporting accuracy of the app and test its utility in advance of conducting monitoring activities.

The resulting coordinates should be entered into mapping software (e.g. ArcGIS, QGIS) and the location and shape of the perimeter should be plotted. If access to mapping software is limited, the maximum length of the area should be measured and marked out, and the width of the area should then be measured at regular intervals. A rough estimate of the total area can then be derived. Estimates derived in this way will not be as accurate as using a GPS around the perimeter. The location of the project and the oyster habitat should be marked on a map to allow for re-assessment.

Alternative Method 1: Aerial imaging by drones.

Images of the project area can be obtained from high-resolution aerial images, for example using a drone. The oyster habitat area may be identified in these images and imported to mapping software. It is important that appropriate geo referencing tools are used when obtaining aerial images, to allow for the image to be mapped and the scale of the image to be determined. The simplest form would be to use base stakes visible in the image and at known locations, or the presence of permanent features in the landscape. Local regulations regarding the use of drones should always be checked before it is determined whether this is an appropriate technology to use in the project.

Sampling Frequency: A pre-restoration baseline should be established followed by sampling within three months and annually thereafter. Additional measurements should be considered following storm events.

Performance Criteria: No performance criteria for project footprint. Oyster habitat areas should be stable or increasing habitat areal extent over time.

METRIC 2: SHELL COVER

Oyster spat require a suitable habitat for settlement. Generally, the native oyster itself is regarded as the best cultch for native oyster spat, but other molluscs such as mussels, scallops and clams have also been used. Living oysters should also be accounted for when monitoring shell cover as they stimulate settlement for oyster spat.

Required Units: % shell cover or shell volume (L/m^2).

Primary Method for subtidal habitats: Underwater visual survey (UVS).

Diver survey can be used to visually estimate the percentage of shell cover using photos of quadrats. See Box 4.1 in Chapter 4 for UVS methods. Cover can be estimated by plotting e.g. 50 random points on a photo, using photo analysis software, and determining what is under each point, similar to methods used in coral reef research.

Alternative Method 1: Drop down video (DDV).

An alternative method where conditions or cost prevent quadrat images being taken by divers is for images to be taken using DDV or a ROV (see Box 2.1).

Alternative Method 2: Box or grab cores.

The following method requires destructive sampling of the habitat. Such methods should be avoided if possible.

Where poor visibility prevents visual survey methods, the use box or grab cores to sample shell cover may be considered. Note that each grab sample should be accompanied by a GPS location and the sediment samples should be collected into labelled buckets. Detailed guidance on grab sampling is provided in Przeslawski *et al.* 2018. Black shell and material that is clearly not found on the seabed surface should be discarded. The volume of surface material can then be determined using the water displacement method.

Figure 2.2: Van-Veen grab from Station LR4, in Loch Ryan consisting of silty mud (Golding *et al.* 2015).

This method involves the use of either: (1) a bucket filled to the brim with water and placed within an empty container (to catch overflow) or, (2) a bucket filled to the level of a hole drilled three quarters of the way to the top and fitted with a tube to direct overflow from the hole to a container (see Figure 2.3). For each quadrat sample, the volume and number of live oysters should be enumerated separately from the cultch volume. Available cultch or oysters should be placed into the bucket and the volume of water displaced determined. The volume of water displaced is equal to the shell volume and should be reported as L/m^2 where the sample area is known, and as L/sample if the area sampled is unknown.

Primary Method for lower intertidal habitats: Quadrat survey.

Shell cover can be assessed relatively quickly by visually estimating the percentage cover of shell using quadrats or photos of quadrats. To allow documentation of trends in shell cover over time, it is recommended that a GPS reading and a photograph of the whole quadrat be taken at a fixed height and a fixed location in the middle of the quadrat. Standard height can be achieved by lining up with a 1m rule. If the same quadrats are also being assessed for oyster density or size frequency, photographs should be taken before any material is disturbed. To aid the determination of the coverage percentage, a quadrat with a delineated grid pattern can be used. Count the number of squares in the grid in which the shell is present, and from that, determine the percentage of the substrate within the grid covered by shell.

Sampling Frequency: Shell cover should be measured prior to the addition of cultch, immediately after laying and six months after laying. Thereafter annual sampling is recommended.

Performance Criteria: For projects identified as substrate-limited, an increase in shell cover for image-based approaches, or increase in shell volume per unit area for volume-based approaches. For recruitment-limited areas, shell cover and volume should not decrease.

Figure 2.3: Equipment used to determine cultch availability via the water displacement method. (Photo: West Wales Shellfishermans Association Ltd).

BOX 2.3: SHELL BUDGET

Shell produced by native oyster habitats can have many fates, including transport, breakdown dissolution or burial. For an area of native oyster habitat to be self-sustaining over time, it is necessary for the shell contributed by oysters to meet or exceed the shell lost. The balance between shell inputs and outputs can be expressed as a shell budget. Currently, there are few locations where the native oyster populations in Europe are accumulating habitat through growth, recruitment and natural mortality. Nevertheless, understanding the fate of cultch material and shell produced by living native oyster at restoration sites is important for understanding progress towards native oyster creating a self-sustaining habitat. The shell volume metric provides one of the building blocks for understanding shell accumulation or loss over time. Further details on how shell budget is determined in the eastern oyster can be found in Soniat *et al*. (2013). Methods are yet to be developed for the European native oyster.

METRIC 3: OYSTER DENSITY

Monitoring oyster density within the restored area is crucial to understanding the development of the habitat and has implications for disease transmission and reproductive success. Assessment of oyster density should take place over the oyster habitat area (see Box 1.2), such that multiplying the reported densities by the area provides the practitioners and funders with an estimate of the oyster population size of the restored habitat.

Oyster density is defined as the number of live oysters per unit area (individuals/m^2) and includes oysters > 35mm in shell height. Oysters smaller than 35mm are unlikely to be reliably and consistently sampled across sampling methods or sites and should therefore not be included in reported oyster densities, but may be included in size frequency assessments (Metric 4). As native oysters commonly have a patchy distribution, measurements must be distributed across the site to ensure reliable reporting of year on year trends.

Practitioners should consider the range of densities they are likely to encounter during monitoring. Where densities are extremely low, transect methods will yield better results than quadrat or grab methods, which sample small areas intensely.

Required Units: Mean density (individuals > 35mm/m^2) ± SE.

Primary Method for subtidal habitats: Quadrat sampling or transects by SCUBA diving.

See Box 4.1, Chapter 4 for diver methods.

Random transect positions can be planned in lower-density habitat areas (see Metric 1). Divers descend a shot-line and swim side-by-side while unreeling a transect tape (often 25m) with a distance pole at a tangent to count all the oysters in the area they pass over. In higher-density areas, quadrats can be deployed randomly along the transect line may be a more manageable survey method. Average density per unit area calculated in either case.

Alternative Method 1: Drop down video quadrats.

DDV sample locations should be randomly pre-assigned (see Box 1.4) and sampled as outlined in Box 2.1. Note that, given the cryptic nature of native oysters, oyster densities determined from images are likely to be lower than those determined by excavated quadrats. Where possible, sampling efficiency should be ground-truthed by divers (see Primary Method). The cryptic nature of the species means that towed video transects are not appropriate for determining densities.

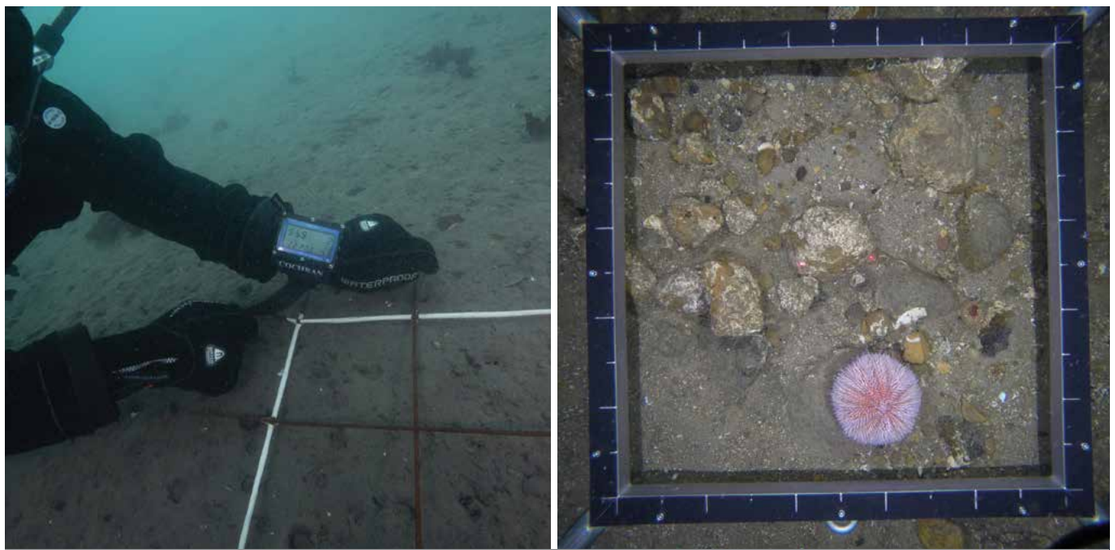


Figure 2.4: Image of a quadrat frame with camera and torch. (Photos: Bernadette Pogoda).

Alternative Method 2: Grab sampling.

The grab should be lowered at each randomly assigned GPS location, and the resulting sample should be processed either onboard or properly labelled and stored for processing later. If processing grab samples onboard, it is important that sampled oysters are returned to the seabed only once all samples have been taken. Each sample should be sieved using a 10mm sieve to remove sediment and the number of live oysters present should be recorded in order for a mean density per unit area (grab dimension) to be calculated. Using the mapped extent of oyster habitat or project footprint, the oyster population density can be estimated for the restored area.

Alternative Method 2: Microdredge.

If an area is too turbid and dynamic to survey visually and the population of oysters is sparse, surveying by dredge may be the only remaining option for sampling the population. Given the potential of dredges to impact larger areas of the restoration site and their variable and unpredictable sampling efficiency, careful consideration should be given to whether dredging is the most appropriate sampling method. Dredge data are challenging to compare between locations, both because of the different sediment types and because each dredge is unique. It is therefore key that the equipment, tow speed and tow duration are kept consistent within the site to allow for intra-site comparison. The tow distance should be recorded by GPS on the boat. Dredge efficiency varies from 5% to 30% for a standard ladder dredge, and often smaller individuals are caught with even lower efficiency. If an efficiency correction has been applied to the measured density, it must be clearly stated alongside the results.

Primary Method for lower intertidal habitats: Transect.

Intertidal areas are often zonal. This should be taken into account when developing a sampling design. Stratified random sampling with quadrats, or sampling along a transect is therefore the most appropriate option (Box 1.4). Quadrats should be placed across the area of restored habitat and excavated to the depth necessary to capture all living oysters. The number of oysters > 35mm in shell height should be recorded.

Where densities are low, the survey should be undertaken along a transect parallel to the low water mark and along the area of interest. A measuring tape should be laid out randomly parallel to the tide line and oysters > 35mm in shell height identified within one metre either side of the tape should be counted. The area sampled in each case should be reported.

Sampling Frequency: Density monitoring should be undertaken pre-restoration and at least once per year thereafter.

Performance Criteria: Increasing mean density of oysters > 35mm in shell height.

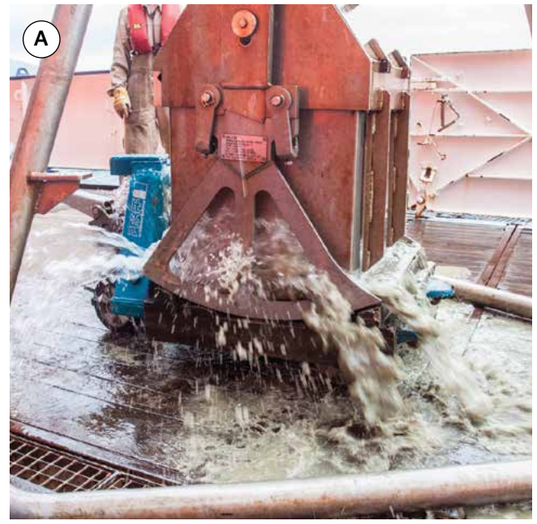


Figure 2.5: Grab sampler and box sieve. (Photos: Bernadette Pogoda).

METRIC 4: OYSTER SIZE FREQUENCY

The oyster size frequency distribution shows how the population is composed in terms of the age, number and size of oysters. This metric provides information on recruitment success, survival and growth rates (see also Metric 7 and Metric 13, Chapter 3) and may assist in the identification of key cohorts that may be sustaining a population. Oysters are frequently patchily distributed. A large number of samples may therefore be necessary to capture a sample that is representative of the population. Sampling methods may also result in biases in the sizes represented (e.g. dredges are known to capture smaller individuals less efficiently than large ones), and this should be considered both when selecting a sampling method and when interpreting the results.

The same methods that are used for assessing oyster density (Metric 3) can be used to collect oysters to assess of size frequency. All oysters in the sample, including the smallest oysters, should be counted and measured for this metric.

Required Units: Percentage of oysters or number of oysters measured in each size class.

Primary Method: Shell measurement using calipers.

Oyster size distributions should be obtained from living oysters, preferably using calipers, although, a ruler may also be used. Shell height should be measured as the distance from the umbo to the distal margin of the shell (see Figure 2.7). **Note that the terms shell height and length have been used inconsistently in the literature to date. It is important to report the measurements by the names illustrated in Figure 2.7 to avoid confusion.**

A minimum of 50 oysters should be measured in order to gain an insight into the size frequency. If large numbers of oysters are available, a random subsample of individuals

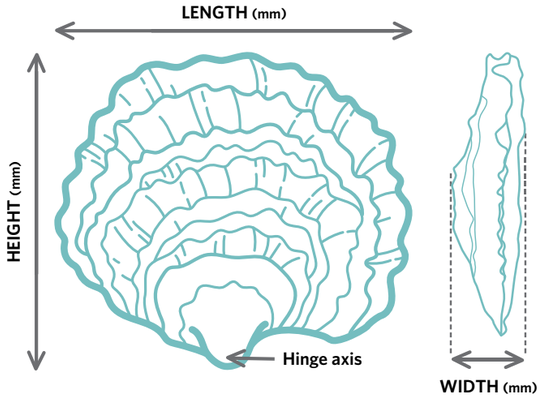


Figure 2.7: *Ostrea edulis* shell height, length, width and hinge axis.

should be measured (Baggett *et al.* 2014). Measurements should be made to the nearest mm. Data should be plotted into histograms with each measurement assigned into bins a maximum of 5mm in size. Data can be reported as absolute number (frequency) or % of individuals (e.g. 50 individuals in a size class ÷ 250 individuals measured = 20% of the population) (see Figure 2.6).

Sampling frequency: Biannual sampling in spring and autumn is recommended.

Performance criteria: The population is at least "bimodal" and shows signs of recent recruitment (see Figure 2.6).

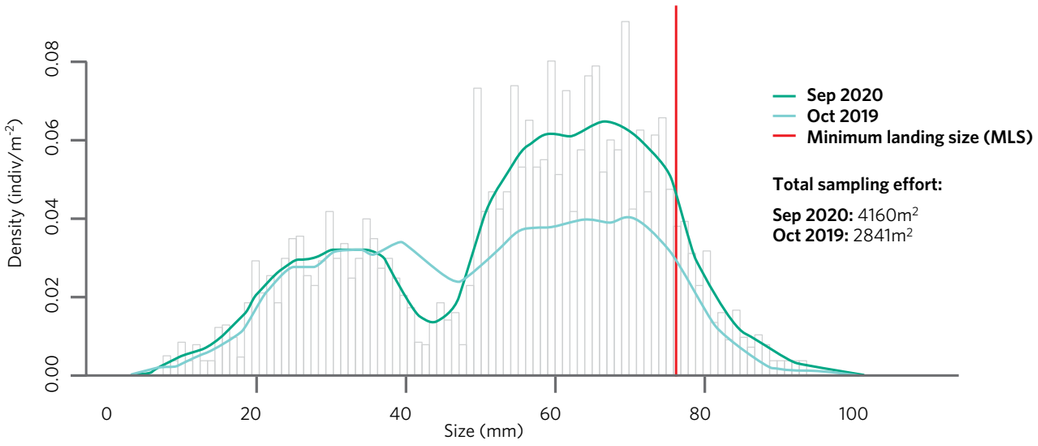


Figure 2.6: Survey data from Tralee Bay, Ireland reported as oysters/m² surveyed, illustrating a bimodal population structure. Note the absence of recent settlement (0-10mm). The increase in abundance from 2019 to 2020 is likely due to variation in dredge efficiency during the surveys rather than an increase in biomass. (Source: Marine Institute Ireland).

UNIVERSAL ENVIRONMENTAL METRICS

Measurements of universal environmental variables are important baseline information to explain the potential impact of their variability on restoration performance. The universal environmental variables are temperature and salinity (Baggett *et al.* 2014).

Continuous measurements recorded by data loggers provide the best insight into variation in the temperature and salinity over time. If continuous measurements are not possible, discrete measurements may be taken, but as the frequency of monitoring decreases, so does the potential for this data to inform restoration. To make the limitations of the data clear, practitioners should report the frequency and timing of sampling.

Some projects may find that they are sufficiently close and hydrodynamically similar to existing monitoring stations to use that data to inform restoration activities. In Europe, data collected by national and European monitoring authorities is made available on the EMODnet website.

METRIC 5: WATER TEMPERATURE

Temperature has an important influence on the physiology of the native oyster. Food intake, growth, spawning, larval development and survival are all related to temperature. In spring, native oysters begin to take in food and grow at temperatures above 7°C. Spawning and larval release are temperature-dependent (see Maathuis *et al.* 2020), and the survival of oyster parasites is also affected by temperature. As such, temperature has the potential to help explain the performance of restoration activities.

Required Units: °C, Accuracy ± 1°C.

Primary Method: Data logger or thermometer.

Temperature measurements using data loggers, thermometers, or other instruments should be conducted as close as possible to the restored oyster habitat. Permanently deployed *in situ* instruments with data loggers are low-cost and require minimum effort to install, but they do need to be regularly maintained and calibrated.

Sampling Frequency: Continuous water temperature measurements taken throughout the year at intervals of 15–60 minutes are recommended. If continuous data loggers are not available, water temperature measurements should be taken every time other sampling is performed or as often as possible. Additional measurements are recommended after storm events.

Performance Criteria: There are no performance criteria for this metric.

METRIC 6: SALINITY

Fluctuations in salinity have a significant influence on the stress level, food intake, growth, condition index and survival of native oysters. The native oyster prefers marine areas with a higher salinity (> 30psu). At water temperatures < 20°C it tolerates temporarily lower salinity levels of 16-19psu.

Required Units: ppt (parts per thousand) or psu. Accuracy ± 1ppt or 1psu. **Note:** Salinity measurements from instruments that use a conducivity ratio, such as CTDs, are unitless.

Primary Method: Data logger.

In areas with large salinity fluctuations or low values, regular salinity measurements using data loggers over a longer period of time are recommended. Loggers should be installed as close as possible to the restoration site. It is recommended that a thorough review of available salinity loggers be undertaken before a decision is made about which logger to use, as some are highly prone to drifting resulting in inaccurate measurements.

Alternative Method 1: Refractometer.

If it is not possible to obtain or deploy a data logger, salinity can be measured at low cost using a refractometer. Equipment-specific instructions should be used, but refractometers generally require a drop of water to be placed on the lens, after which the salinity can be read by holding the refractometer up to the eye.

Sampling Frequency: Continuous salinity measurements at intervals of 15–60 minutes throughout the growing season are preferred. If continuous data loggers are not available, salinity measurements should be taken every time other sampling is performed at the reef and after storm events.

Performance Criteria: There are no performance criteria for this metric.

BOX 2.4: PULSE EVENTS

Alongside the planned monitoring of the restoration site, practitioners should undertake regular surveillance for the unusual or unexpected. For example, abnormal levels of mortality, changes in native oyster growth or the absence of larval settlement can indicate that all is not as it should be. In instances where there are sudden high rates of mortality or recruitment failure, it is recommended that practitioners investigate the following:

Environmental conditions – e.g. temperature, salinity, turbidity and precipitation (see Metrics 5 and 6, Chapter 2). **Note:** salinity fluctuations may also be the result of water management activities.

Sedimentation – high rates of sedimentation can result in native oyster being smothered and/or make cultch unavailable for settlement by juveniles (see Metric 17, Chapter 3).

Pollution – storms (onshore and offshore), onshore activities or vessel activities may lead to sudden pulses of pollution entering systems.

Harmful algal blooms (HABs) – have the potential to become more prevalent with the changing climate and can cause sudden high mortality rates in shellfish, finfish and infauna. Areas where shellfish production occurs are regularly monitored and HAB alerts may be issued by national competent/monitoring authorities. HAB information may also be available from satellite monitoring and via the website of the relevant competent authority.

Disease – see Metric 15, Chapter 3. **Note:** If a disease outbreak is suspected, the relevant competent authority must be informed immediately, and the restoration project management team must ensure that biosecurity measures are in place to contain it.

Biotic factors – Predation and competition are part of the natural dynamics of any native oyster habitat. However, high densities of predators can severely affect the establishment of newly restored populations. The European tingle (*Ocenebra erinacea*), American tingle (*Urosalpinx cinerea)*, eider duck (*Somateria)* oyster-catcher (*Haematopus),* starfish (*Asterias rubens)*, green shore crab (*Carcinus maenas)* and several *Polydora* worms can all impact native oyster populations. The slipper limpet (*Crepidula fornicata)* is a major competitor for space and food, producing vast quantities of pseudofaeces, which inhibit native oyster larvae settlement, while the Pacific oyster (*Crassostrea gigas)* can also be considered a competitor.

Monitoring for predators can be incorporated into other biodiversity assessment protocols (see Section 4.2, Chapter 4). If predation is a concern, monitoring should be increased during spawning, when adult native oyster are particularly vulnerable, and continued at least once a month for the four months post-spawn.

Illegal Harvesting – if monitoring indicates that native oyster are missing but there is no evidence of mortality, (such as empty shells), then illegal harvesting should be investigated. To identify illegal harvesting in intertidal sites, sites should be routinely observed 30 minutes after low tide as this is the most likely time to witness poaching. Support from the fishing community, harbour patrols and the public is of paramount importance. Details of the poachers, any vessels or vehicles involved and the number of native oyster taken should all be recorded. It is not advisable to approach or challenge illegal harvesters. Where possible, it is important that restored broodstock oysters are clearly identifiable as belonging to a funded restoration programme. This allows restoration practitioners to have ownership and gives local authorities the power to police and prosecute illegal harvesters. Regular monitoring of native oyster within the commercial size range (50-80mm) may allow an evidence base to be built to assist with prosecution or action by the enforcement agencies.

CHAPTER 3
SUPPLEMENTARY MONITORING METRICS

CHAPTER AUTHORS
Stéphane Pouvreau, Jenna L. Alexander, Isabelle Arzul, Elisabeth Ashton, Oscar Bos, Cass Bromley, Tom Cameron, Mark Chatting, Patricia Daly, Chris Hauton, Maria Hayden-Hughes, Luke Helmer, Zoë Holbrook, Patrick Joyce, Louise Kregting, Alice Lown, Sharon Lynch, Verena Merk, Bernadette Pogoda, Joanne Preston, Chris Ranger, Ana Rodriguez-Perez, David Smyth, Oliver Tully, Ben Wray, Philine zu Ermgassen.

INTRODUCTION

While the universal metrics outlined in Chapter 2 provide the basics for assessing and communicating progress in restoration efforts, restoration practitioners may opt to gather more details on how and why progress (or otherwise) in restoration is being observed. The supplementary metrics outlined in this chapter provide insight into the condition, growth, survival, reproduction and recruitment of the European Native Oyster (*Ostrea edulis*), as well as delivering evidence of a wider range of potential drivers of restoration performance (see Figure 3.1).

Note: if any of the planned monitoring requires oysters to be sacrificed, it is worth considering whether additional measurements can be made on the individuals to gain the greatest amount of information from each. For example, it is possible to assess growth, condition, disease and gonad development from the same oyster.

METRIC 7: GROWTH RATE

Oyster growth rate is an important, non-lethal indicator of oyster productivity and an indicator that native oyster at the restoration site are experiencing suitable conditions for growth.

Required Units: Growth mm/year.

Primary Method: Growth rate from tethered, caged or marked individuals.

Oyster growth rate can be assessed by measuring the change (in mm) in shell height (see Figure 2.7, Chapter 2) in the same individual over time. Marked and individually identifiable native oyster should be placed in the same conditions as the restored population and protected from human interference. A minimum of 50 individuals is suggested, but larger samples are preferable. The method that best reflects the wider reef should be chosen, ensuring that the density and the condition are similar to those of the restored population. This method can be applied to native oyster of all size classes without restriction.

Oysters can either be tethered to strings laid on the seabed; placed in trays, suspended cages, oyster mesh bags; or fixed on benthic plates at a given density if SCUBA diving operations are possible (see Figure 3.2). For tethered individuals, line configuration should consist of a central rope running between anchors. The line should be marked at intervals that reflect the background oyster

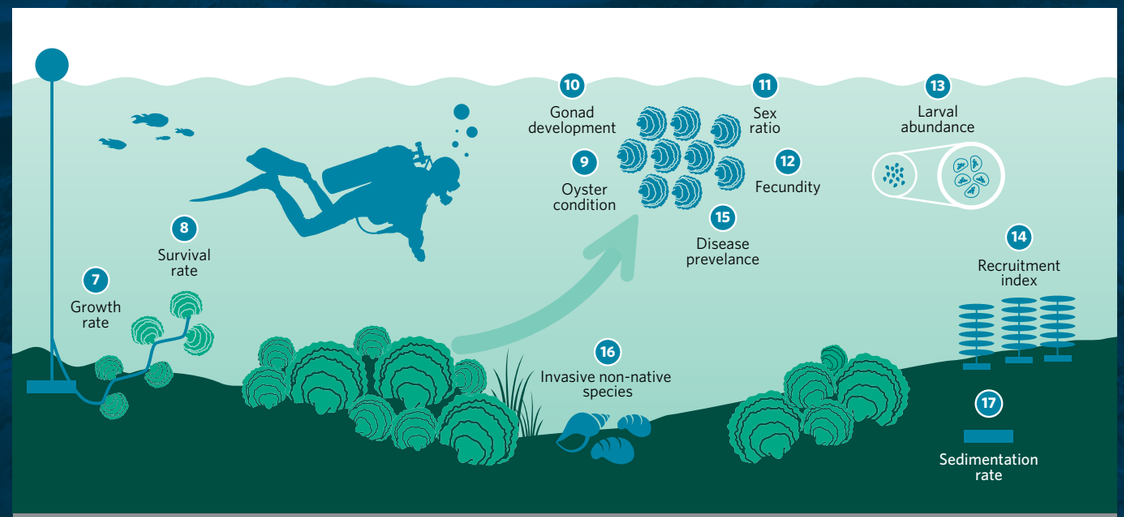


Figure 3.1: Supplementary monitoring metrics; numbers correlate to numbered metrics throughout Chapter 3.

density or desired test density and a 15cm loop knotted to the line for oyster attachment. At the end of each loop offshoot, an epoxy putty ball (e.g. Milliput©) is moulded onto each oyster, close to the hinge on the flat valve. Individual oysters should be measured before deployment and suitably marked for re-identification (e.g. a small label fixed into the epoxy ball). If using cages, trays or mesh oyster bags, these should be filled with marked oysters at the desired density and deployed at the study site at randomly selected locations. The height at which the gear is suspended should be recorded. Trays or mesh bags should have a large mesh size to reduce the impact of the gear on the growth rate. Gear should be regularly cleaned of biofouling.

If SCUBA diving operations are possible, oysters may alternatively be glued at the desired density to ceramic supports classically used for coral cuttings. The supports should be fixed onto a plate which is labelled on the X and Y axis and the plates anchored inside the restoration site (see Figure 3.2 and Figure 3.3). Images of each plate can then be taken with a standard GoPro system and the oyster sizes assessed with image software.

Alternative Method 1: Growth rate from size frequency measurements.

Native oyster larvae settle onto substrates at a particular time of year, usually in mid-summer, with long periods where no settlement occurs. This results in individual year classes (cohorts) of oysters being identifiable in size frequency distributions in random samples taken of the population, at least for the first- and second-year classes and before variability in growth leads to merging of year classes in size distributions of older cohorts. Growth and mortality (Metric 8, Chapter 3) of the first and second cohorts are key indicators of their eventual contribution to oyster biomass.

Recently settled native oyster can be detected and measured in the field in the autumn following the summer settlement (see Metric 14, Chapter 3). They will usually have settled on shell material on the seabed that can be sampled remotely using grabs or dredges, or in situ using SCUBA quadrat sampling (see Metric 3, Chapter 2).

Samples of shell or other material on which oyster spat have settled should be collected and numbers and size of spat on this material measured. A large sample of over 100 spats should be counted and measured with calipers. Larger samples will provide smoother data and more clearly identifiable cohorts. This should be repeated quarterly or even monthly to identify seasonality in growth and mortality (see Metric 8, Chapter 3). The resulting histogram of size data is a 'distribution mixture' and this can be decomposed to individual normal (or other) distributions, each representing a cohort of native oyster, at least for ages 0+ and 1+ (see Figure 3.4). This provides data on numbers, mean size and variance in size for each cohort, as well as the proportion of the population in each cohort. The methods for decomposing the size distribution mixture can be developed in Microsoft Excel or using R libraries (https://cran.r-project.org/web/packages/mixtools/index.html).

The annual growth rate is the difference between the mean sizes of successive cohorts. Shorter-term growth rates can be estimated by repeated sampling and modal progression analysis, e.g. by identifying increases in the average size of a given cohort between two sampling events (Growth ($mm.T^{-1}$) = $(U_{T2}-U_{T1})/T$) where U is mean size and T is time). Monthly sampling would enable seasonality in the growth rate to be identified and seasonalised growth parameters to be estimated.

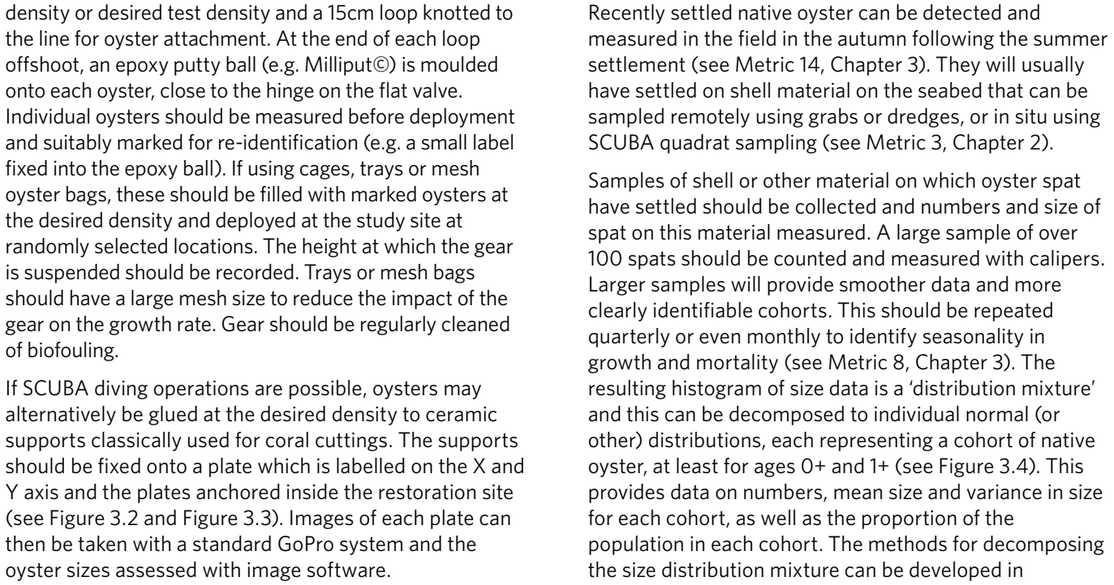


Figure 3.2: Experimental designs for monitoring growth rate (and also mortality rate) of marked *Ostrea edulis*. Different techniques can be applied: A and B) placed in suspended cages, trays and oyster mesh bags; C) tethered to strings laid on the sea bed; or D) and E) fixed on benthic plates directly on the bottom inside the restored site. (Photos: Luke Helmer, Stéphane Pouvreau, Matt Huber and Matt Uttley).

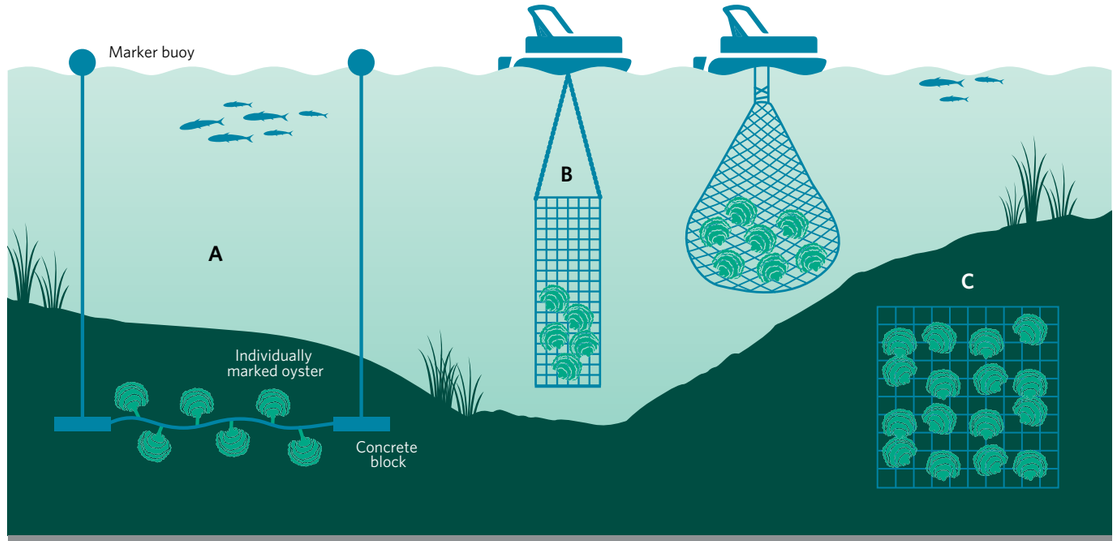


Figure 3.3: Experimental designs for monitoring growth rate (and also mortality rate): (A) tethered to strings laid on the seabed, (B) placed in suspended cages or mesh bags, and (C) fixed onto benthic plates.

Sampling Frequency: Ideally, native oyster should be measured every three months, in September, December, March and July, or at least once per year, preferably in July.

Performance Criteria: A growth rate in shell height of between 10-20mm per year is expected, especially within the first three years. As growth rate declines with size, expected values in older individuals will be lower (< 10mm per year).

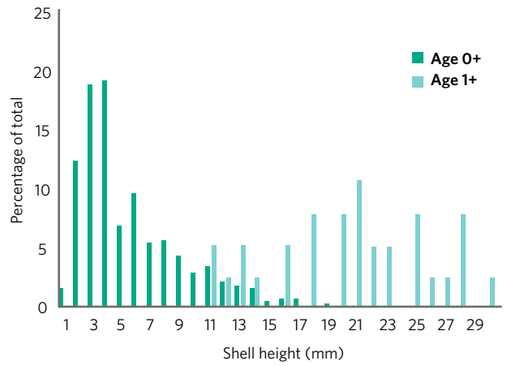


Figure 3.4: Example of size distribution of *Ostrea edulis* aged 0+ and 1+ settled on oyster shell cultch. (Source: Marine Institute IRL unpublished data).

METRIC 8: OYSTER SURVIVAL RATE

An increase in oyster biomass is a balance of growth and mortality. So, there are many situations where defining the oyster survival quantitatively can support adaptive management and assist in identifying arising issues. Note that natural mortality generally declines with size, at least in the absence of *Bonamia* and/or *Marteilia* (see Metric 15, Chapter 3).

Required Units: Survival (S) is the change in numbers of native oyster in a given population over time. Alternatively, mortality rate (M) is the rate of decline in numbers in time (generally in %). Generally, those rates are expressed as a percentage of the total amount.

Primary Method: Survival rate from tethered or caged individuals.

Survival can be determined by assessing the change in the number of living individuals over time from any of the methods outlined in Metric 7, Chapter 3.

Alternative Method: Survival rate from size frequency measurements.

Size frequency measurements provide data on numbers, mean size and variance in size for each cohort, as well as the proportion of population in each cohort. If the histogram data is standardised for sampling effort, then changes in numbers per cohort over time also provide information on mortality/survival between sampling events.

Survival (S) is obtained from the change in numbers of native oyster in a given cohort over time (N_{T2}/N_{T1} = survival) where T_1 and T_2 are sampling events 1 and 2. The instantaneous mortality rate (M), or the rate of mortality at any point in time between sampling events is given by the log (N_{T1}/N_{T2}).

Sampling Frequency: Ideally, native oyster should be assessed every 3 months, preferably September, December, March and July, or at least once per year, preferably in July.

Performance Criteria: There are no performance criteria associated with this metric.

METRIC 9: OYSTER CONDITION

Condition indices can be used to indicate the health of a population and provide an easy-to-assess status that is comparable across sites. This may be important for identifying poor environmental conditions and/or disease development.

Required Units: The condition index (CI) is unitless.

Primary Method: Biometric measurements.

The most common method for assessing the condition index is the ratio of tissue mass to shell mass. For greatest accuracy, the condition index can be calculated from dry weight data using the following equation: **CI = dry meat weight (g) *100/dry shell weight.** Dry tissue weight is determined by carefully excising the soft tissue from the shell and placing each onto a pre-weighed piece of foil. Samples should be labelled to ensure that shell and tissue pairs can easily be identified. Both tissue and shell samples should be dried in an oven at 100°C until they have reached a steady weight. This normally takes about 36 to 72 hours depending on the size of the oysters. Both parts should then be weighed to the nearest 0.1g.

Sampling Frequency: Condition index values follow a clear seasonal pattern. Variations are generated by the naturally changing food supply and physiological events (especially spawning, but also diseases). If sampling once per year, the condition index should be assessed for the same season, ideally in spring (pre-spawning) or late autumn.

Performance Criteria: A condition index of 2-5 (outside of the spawning period) indicates that the native oyster are in good health.

METRIC 10: GONAD DEVELOPMENT

Along with oyster density, growth rate and condition, gonad development status is a good indicator of potential larval production, swarming timing and (indirectly) reproductive efficiency within a given population. Gonads develop from many-branched ducts, from which numerous sacs contain the germinal cells. This development, which takes a few weeks, is generally divided into several stages for convenience. These stages depend on the method used, but correspond to resting, developing, mature, spawned and/or incubating, and reabsorbing (see Figure 3.5). Note that this metric also provides the sex ratio within the population (see Metric 11, Chapter 3)

Methodologies to monitor gonad development status will depend on budget, effort and access to required equipment. A representative sample of 30+ native oyster should be collected from the field. Sampling must be representative of the size distribution of the population (see Metric 3, Chapter 2). The percentage for each stage of maturity within the population is calculated by dividing the number of adults in each stage by the total number of oysters in the sample and then multiplying by 100.

Required Units: The percentage of each qualitative stage observed in the sample.

Figure 3.5: Gonad development stage of *Ostrea edulis*. The right valve has been removed and the pictures show the development of the gonad into the visceral mass all around the digestive gland. (Photos: Stéphane Pouvreau, Hélène Cochet, Luke Helmer).

Primary Method: Macroscopic observation of gonad development.

The oyster should be carefully opened and the gonad, which is found just under the right valve above the adductor muscle, examined. The gonad shape, colour and fullness will give the development status. Generally, the classification is composed of six potential stages (see Figure 3.5). Note that stages 1-4 are relevant for males and females, whereas stages 5-6 apply only to females.

Alternative Method: Microscopic observation of gonad development.

Microscopic observation of the gonad development stage requires access to expertise (histology facilities). Soft tissues are excised to produce a histological section as follows: a sagittal (parallel to the anterior-posterior axis), approximately 5mm thick section containing gill, gonad, digestive gland and mantle lobes is cut, fixed in Davidson's solution and embedded in paraffin. The embedded gonad is then sliced into 5µm thick sections and stained with Harris' haematoxylin and eosin. Gonad analysis is performed on the histological sections and each individual can be classified according to sex category and gonad development based on a scale according to the six stages: (0) inactive or resting gonad; (1) early gametogenesis; (2) advanced gametogenesis; (3) ripe gonad; (4) partially spawned gonad; and (5) reabsorbing gonad. Active stages (from 1-4) are shown in Figure 3.6.

The histological sections show the four different active gonad stages of both male (left) and female (right). **1. Early gametogenesis (A-B):** Gonad follicles are more spread into the connective tissue. Sexual cells become visible. **2. Advanced gametogenesis (C-D):** Gonad follicles are larger but connective tissue is already present and every cell type of the germ line is present. **3. Ripe gonad (E-F):** Juxtaposed large follicles occupy the entire area between the mantle and the digestive gland. Mature gametes are predominant. **4. Spawned gonad (G-H):** Gonad follicles are smaller than in the previous phase and separated by some connective tissue. Gametes had been released but a large amount of residual mature gametes remain in the follicle lumen.

Sampling Frequency: Monthly to biweekly sampling is required to follow gonad development during the active period.

Performance Criteria: Presence of 30-40% of mature females in stages 3 and 4 (see Figure 3.5) within the population when water temperature is above 16°C is expected and is indicative of a good reproductive efficiency. A low ratio of individuals in the advanced maturity stage would have negative consequences on the renewal of the population.

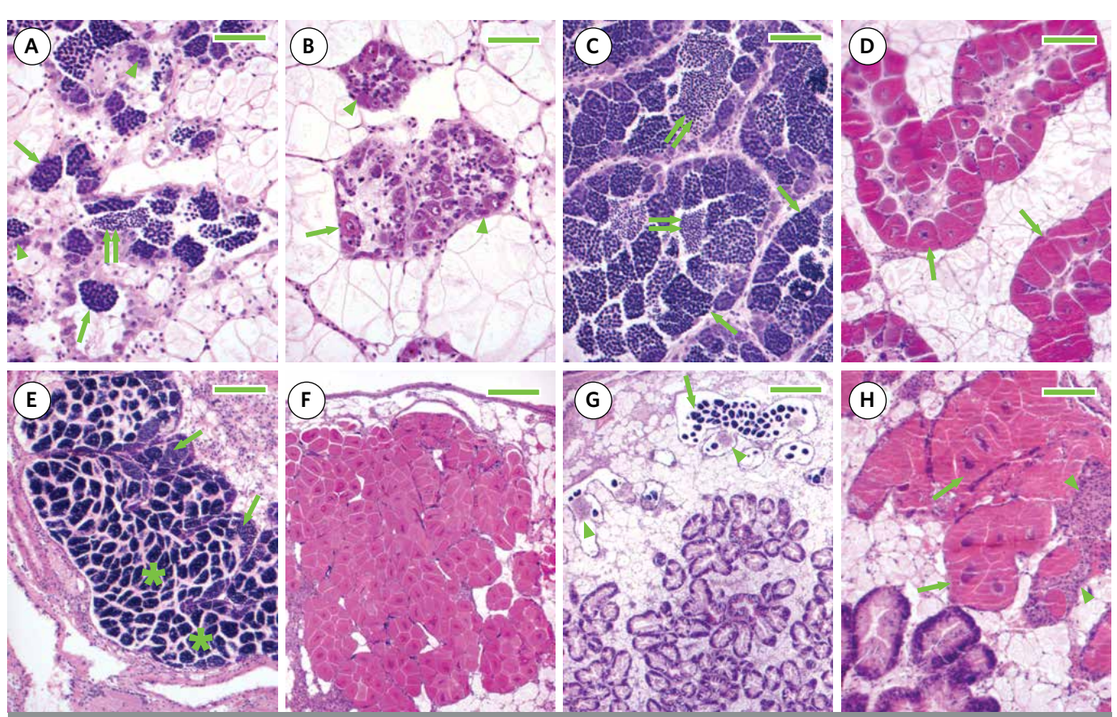


Figure 3.6: Microscopic observation of the gonad development stage of *Ostrea edulis*. The length correspondence of the scale bar in each micrograph is : A: 50µm; B: 50µm; C: 50µm; D: 100µm; E: 100µm; F: 200µm; G: 200µm; H: 100µm. (Source: da Silva *et al.* 2009).

METRIC 11: SEX RATIO

A skewed sex ratio has been recorded in some native oyster populations. Oysters are protandrous hermaphrodites and undergo multiple sex changes throughout their lives, however, an unbalanced sex-ratio, especially one with low female occurrence is cause for concern.

Methodologies to determine sex ratio will depend on budget, effort, and access to required equipment. Note that sex ratio can be assessed in oysters collected for other sampling to reduce impact into the population. Size of the sample should be high (30+) to ensure a fair estimation of the metric.

Sex ratio can also alternatively be determined from Metrics 10 and 12, Chapter 3.

Required Units: Sex ratio is unitless (ratio of females to males). It can also be expressed as a percentage of males (or females) into the population.

Primary Method: Sex ratio from direct gonad tissue sampling.

Sex ratio can be determined by direct observation of the gonadal tissue under a light microscope. Gonadal tissue is found just under the surface of the oyster body below the adductor muscle (see Figure 3.5). Gametes can be extracted by gently scratching the surface of this gonadal tissue and using a Pasteur pipette or other fine capillary tube to suck up a small sample. This sample can then be spread over a glass slide for observation under a simple light microscope or should be kept on ice and processed within 24 hours. Native oyster eggs are oval shaped (with a diameter > 50µm) when compacted into the gonadal tissue but expand and become round when added to saline or seawater. Native oyster sperm (regrouped in spermatid balls) become active and liberated when added to seawater.

BOX 3.1: HOW TO ANAESTHETISE AN OYSTER

Anaesthetic can be used to relax the adductor muscle using a relaxant such as 5% magnesium chloride ($MgCl_2$) in a seawater (30ppt) bath for a maximum of 3 hours until the valves open. It should be noted that a proportion of the native oyster will remain closed for durations exceeding this time (up to 6 hours), so it is potentially more time efficient to collect a greater number of individuals than the intended sample size. Anaesthetised oysters should be placed in seawater and protected until they have recovered and have fully closed.

Methods of tissue sampling that avoid sacrificing the individual are possible but require greater time and skill. Oysters can be anaesthetised following the methods in Box 3.1, or alternatively a fine 2mm drill should be used to make a hole in the right valve of the oyster shell without puncturing the oyster body tissue. Tissue sampling can be made with a Pasteur pipette or other fine capillary tube. Following tissue sampling, and after a period of recovery, both anaesthetised and drilled oysters can be returned to the seabed. Anaesthetised oysters are vulnerable until they have recovered and have fully closed, and although drilled oysters can repair their shell within days, the drill hole temporarily exposes the oyster to pathogens. These sampling methods could raise ethical concerns for the handling of oysters.

Sampling Frequency: Once per year just prior to the spawning season.

Performance Criteria: There are no performance criteria associated with this metric, but a balanced sex-ratio within the population is expected.

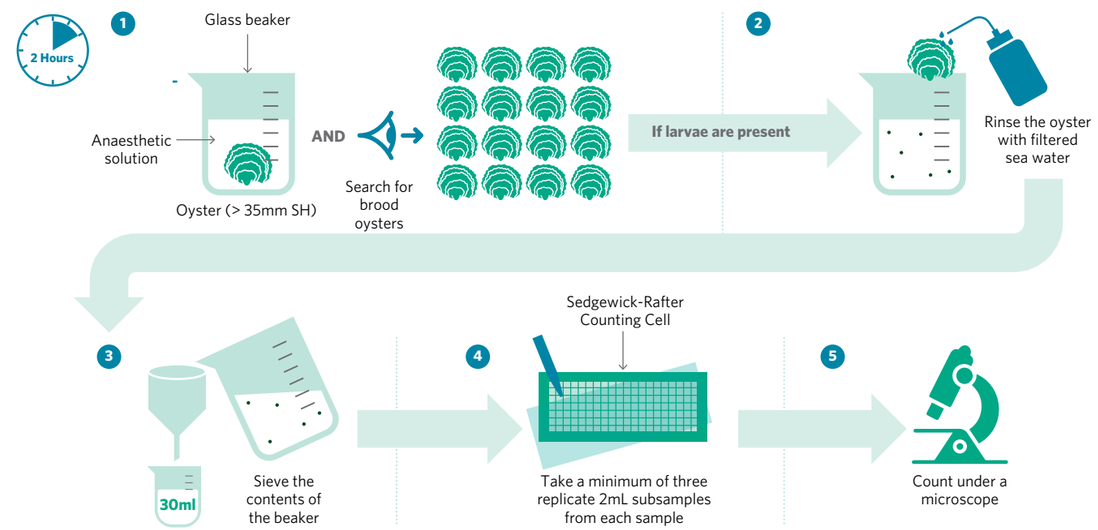


Figure 3.7: Methodology for fecundity metric; 1) submerge oyster (> 35mm shell height) in anaesthetic solution and examine broodstock oysters for brooding larvae, 2) rinse the oyster with filtered sea water and collect larvae in glass beaker, 3) sieve the contents of the beaker into 30mL universal container, 4) to count the larvae take a minimum of three replicate 2mL subsamples from each sample. It is recommended to use a Sedgewick-Rafter Counting Cell, 5) count the larvae present under a microscope.

METRIC 12: FECUNDITY

Fecundity is a measure of the number of larvae a female oyster produces. This metric provides information on whether native oyster are contributing towards a self-sustaining population. Fecundity can be impacted by environmental factors, both directly (e.g. pollutants), or indirectly by affecting oyster condition. If oysters are fecund, but recruitment is low, this is valuable information for adaptive management. Note that this metric also provides the sex-ratio within the population (see Metric 11, Chapter 3).

Required Units: Mean number larvae/brooding oyster (± SE) and the percentage of brooding adults to estimate the reproductive potential of the population.

Primary Method: Brooding larvae counting.

Collect a range of oysters from sexually mature size classes (> 35mm shell height). For information on calculating sample size see Box 1.4 in Chapter 1, however, a minimum sample of 30 oysters per location/time point is recommended. The brooding larvae can be sampled using either sacrificial or non-destructive sampling to minimise the impact on the broodstock population. For either technique, record the oyster height (mm) and whole wet weight (g), as fecundity increases with oyster age/size but is also influenced by oyster condition. Wet weight should be determined after biofouling organisms have been removed and the oyster has been patted dry.

Destructive sampling: Open the oyster carefully so as not to damage soft tissue or spill any brooding larvae from within the shell cavity. If the oyster contains a brood, use filtered seawater to rinse larvae from the mantle cavity into a container (e.g. glass beaker). Allow the larvae to settle at the base of the container, remove excess fluid, including the filtered seawater, using a pipette (see Figure 3.7). Transfer to a sample container (30mL volume should be sufficient), recording the total volume of larvae.

Non-destructive sampling: In the field, submerge each oyster in a small container (< 1 litre) of anesthetic solution (see Box 3.1). If larvae are present within the pallial cavity, rinse the larvae into the solution and seal the container for transport to the laboratory. The oyster can be revived by replacement in seawater before returning to the restoration site. In the laboratory, sieve the contents of the container over a 60µm mesh and transfer the concentrated larvae to a 30mL universal container. If not analysed immediately, preserve the larval sample with 70% ethanol (or 98% ethanol if molecular analysis is planned) and store at 4°C.

To count the larvae, take a minimum of three replicate 2mL subsamples from each sample. The recommended method for counting is to use a S50 plastic Sedgewick-Rafter Counting Cell (see Figure 3.7). Place a 1mL aliquot of the sample onto the slide and analyse under a compound microscope. A serial dilution may be required if the density of larvae is too high to count on the slide.

Count the larvae present within 10% of the randomly selected squares on the slide (n = 100). Replicate in triplicate for each sample, calculate the average larval density (larvae/mL) and multiply by the total sample volume for the number of larvae/oyster (taking into account any serial dilution factor). Note that automated larvae counting can be done using a coulter counter (e.g. MultisizerTM 3, Beckman Coulter Life Sciences, USA) through a qualified lab.

Sampling Frequency: Monthly sampling from May to August is recommended.

Performance Criteria: A healthy and growing population will include either a constant or increasing occurrence of % breeding oysters.

METRIC 13: LARVAL ABUNDANCE

Data on larval abundance in the water column can provide a range of useful information. Firstly, the presence of larvae is evidence of successful reproduction. Secondly, oyster settlement intensity is related to larval abundance in the water column. The presence of pediveliger larvae on and around restored beds is a particularly promising indicator. In the event of recruitment failure, knowing whether larvae were present in the water column, and at what stage, can be useful in determining whether pre or post settlement factors were responsible. Finally, knowing when larvae are present in the water column can also be useful for optimising the timing of substrate deployment for settlement.

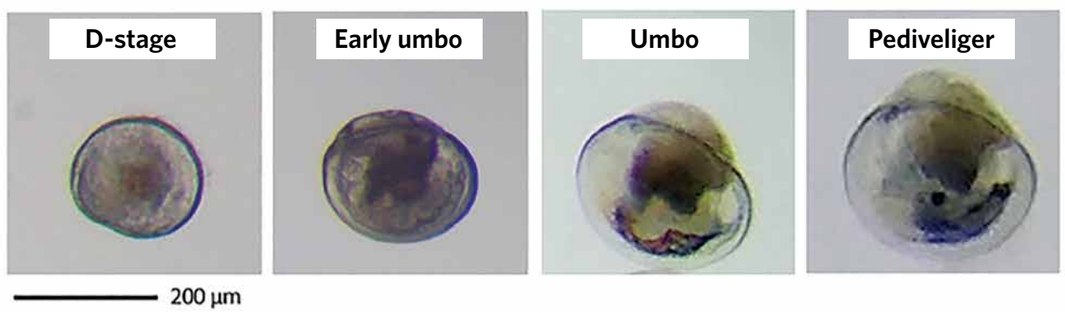


Figure 3.8: Life history of *Ostrea edulis* with larval morphology described in four stages: D-veliger (height = 160µm) Early umbo (height = 200µm) Umbo (height = 240µm); and Pediveliger (height = 260µm). (Photos: Ana Rodriguez-Perez).

Larvae are in the D-stage when newly released from the mother oyster and in the pediveliger stage when ready to settle (see Figure 3.8). The eyespot (the black dot in the middle of the shell) is a characteristic feature of larvae in the pediveliger stage. Note that shell height should be measured as the distance from the hinge to the distal margin of the shell, whereas shell length is the widest segment perpendicular to the height measurement.

Required Units: Larval abundance: the number of individuals per cubic meter (ind/m^3 ± SE).

Two stages of native oyster can be easily identified and counted separately in plankton samples:

1. Young veliger D-shape and early umbo larvae (shell height < 240µm).
2. Umbo and pediveliger larvae (shell height > 240µm).

Methods: For all methods listed below, sampling should be conducted at three different locations across the extent of the oyster habitat at regular intervals during the spawning season (from June to September in Europe). Each sampling event must be at approximately the same water column height and tidal stage (e.g. within 2 hours ± high tide).

Primary Method: Pump sampling.

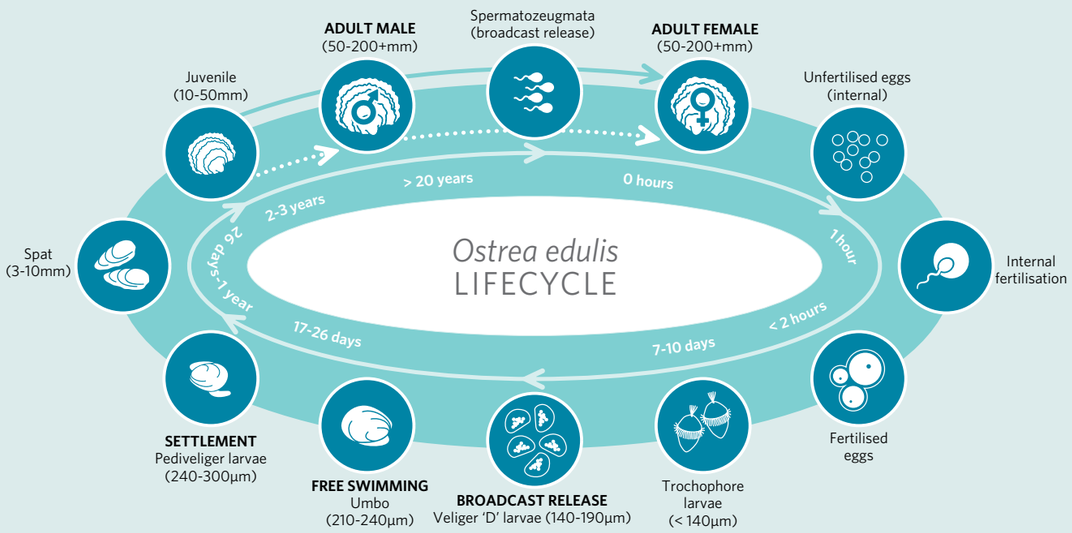
Sampling is conducted at one meter above the benthos at high tide (±2 hours), from plankton sample collected by filtering generally between 1000 to 2000 litres of seawater through 50-70µm plankton net by using a surface centrifugal pump (see Figure 3.9).

Alternative Method: Towed plankton sampling.

Plankton samples are collected by oblique vertical tows using a 500mm diameter, 50-70µm mesh plankton net (see Figure 3.9). Distance travelled by the net is calculated using a manual flow meter attached to the net mouth.

BOX 3.2: THE LARVAL LIFE OF *OSTREA EDULIS*

The larval phase among bivalves can be considered in three stages: (1) development from fertilisation to initiation of feeding; (2) free-swimming planktotrophic life; and (3) metamorphosis. The native oyster is a larviparous species that broods its young larvae for 7-12 days: Stage (1) from the trochophore to the first shelled stage (the Prodissochonch I veliger larva, namely PI) occurs within the mantle cavity of the mother. PI larvae are then released into the water (at a shell height > ~150µm). That precise and rapid moment constitutes the swarming phase. The free-swimming stage lasts approximately 10-12 days, during which larvae secrete the Prodissoconch II shell (PII), reaching a shell height of ~260µm (pediveliger larvae) before settling onto a substrate to start metamorphosis. Note that depending on water temperature, the development time can be shorter or longer. During this pelagic life stage, the shape of the larva becomes more spherical, and an umbo appears progressively at the hinge side. When PII larvae reach ~240µm, the umbo develops fully and can be easily observed: this stage is identified in plankton samples as 'evolved umbo larvae'. Then a functional foot, but also an eyespot appears; the larva reaches ~260µm and enters the pediveliger stage. That final stage indicates that larvae are fully competent to settle.



The lifecycle of Ostrea edulis from Preston et al. (2020) and Helmer et al. (2019).

The volume of seawater sampled is calculated using the formula $\prod r^2h$ (where r = radius of net mouth and h = distance towed). Distance towed (h) was obtained by multiplying the number of revolutions made by the manual flow meter (value at end of tow minus value at start of tow) multiplied by the manufacturer's constant (for this model 0.3, i.e. 10 revolutions = 3m towed) or using GPS navigation systems on the boat.

After collection, rinse the plankton net and transfer the retained material into a polyethylene bottle (2L) and fix by adding 50mL of alcohol (90%) or formaldehyde (4%) immediately upon collection.

At the laboratory, each sample is gently filtered, rinsed again with filtered seawater, and transferred into a graduated tube. After complete homogenization, three sub-samples of 1mL are then taken using a pipette and examined on a reticule slide (such as a Sedgewick Rafter counting cell, see Figure 3.7) under microscope at a magnification of 10X or above (see Box 3.3). Expertise is required to undertake the larval count as larvae of other bivalve species are also collected and confusion remains possible. An aliquot of 100 larvae is also used to estimate the ratio of evolved umbo larvae (shell height > 240µm) in the sample.

Bivalve larvae per sample is calculated by multiplying the mean count per three 1mL aliquots by the sample volume (mL) and dividing the result by the volume of water sampled.

Sampling Frequency: Larvae should be sampled once per week during the larval spawning season (approx. June-September but varies with location). The temperature sum (see Box 3.3) may be applied to inform when to start sampling.

Performance Criteria: There are no performance criteria for this metric.

BOX 3.3: TEMPERATURE SUM

Temperature sum is an important variable explaining larval abundance in modelled scenarios. It can be applied as a crude predictor for native oyster larval peak abundance in the water column following recently developed models. If spat collectors are deployed, it is advised to do so one to two weeks after maximum larvae numbers are detected.

Temperature sum is the sum total of degrees per day above 7°C in a calendar year. It is a transferable indicator of peak native oyster larval abundance because it is based on a 6.75-7°C gonad development threshold. Temperature sum has been shown to be a good predictor of the peak larval abundance at a site, although the exact value of the sum likely varies and should be independently verified by site (Maathuis *et al.* 2020). In Loch Ryan, Scotland, Scotland, the temperature sum was determined to be 617 degree-days (Chapman *et al.* 2021).

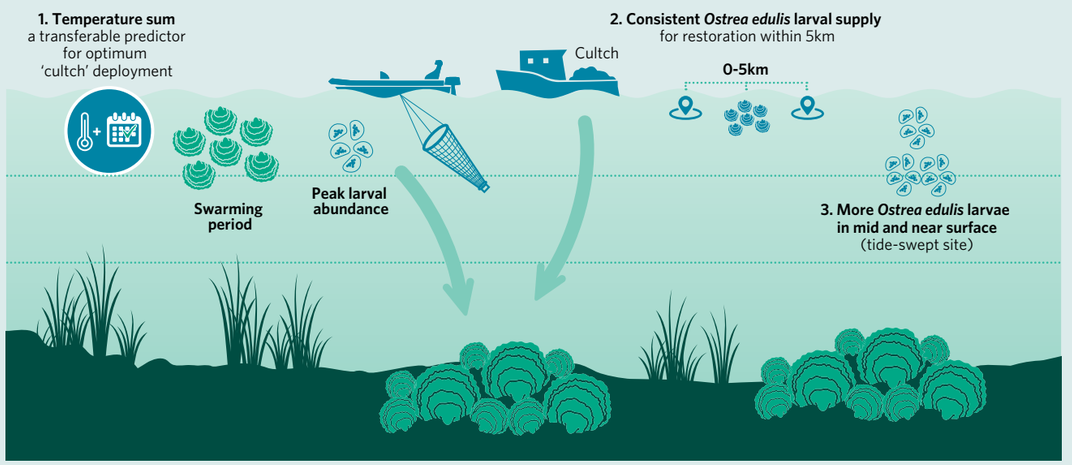


Illustration of use of temperature sum in practice, modified from Chapman *et al.* (2021).

BOX 3.4: RECRUITMENT VS SETTLEMENT

The native oyster has a two-stage life history: a planktonic stage where larvae are dispersed by currents and a sessile post-settlement stage, during which juveniles recruit into the adult population (see Figure in Box 3.2). Settlement refers to the moment when larvae metamorphose and attach to a hard substrate. This rapid event occurs some 10-15 days after larvae swarming. Recruitment is defined as the number of competent larvae that settle from the plankton, complete metamorphosis on substrata and survive over a prespecified time after settlement. Pre- and post-settlement factors impact the number of recruits. Pre-settlement factors include larval supply and mortality and availability of suitable substrate, which influences the amount of settlement. Post-settlement factors include predation and competition, which influence survival of recruits through post-settlement mortality.

Figure 3.9: Plankton sampling techniques used to collect *Ostrea edulis* larvae: Pump method and towed plankton net method. (Photos: Julien Coïc, Stéphane Pouvreau, Oscar Bos and Bernadette Pogoda).

METRIC 14: RECRUITMENT INDEX

Successful settlement, survival, and subsequent recruitment (see Box 3.4) are critical for the long-term persistence of restored populations. Evaluating recruitment during and after each reproductive season allows assessment of the health and potential of an oyster habitat. Two complementary methods can be conducted depending on objectives and available time: annual recruitment (onto the underlying reef or on cultch and a few months after the breeding season) and early recruitment (on benthic collectors deployed specifically during the reproductive period). The first method provides insight into oyster settlement and survival potential on the restored reef itself. The second method provides more information on the efficiency of reproduction, the best settlement time, and the number of settlement events in a season, the effects of environmental factors and also predation on the recruits.

Sampling dates should be kept as constant as possible across years and sites to allow for comparison or assessment of trends across space and time.

Required Units: Units are method specific and listed under each method.

Primary Method: Annual recruitment onto the reef or on cultch.

Required Units: Density of living < 1 year old recruits per m^2 (individuals/m^2 ± SE).

Method: Monitoring of annual recruitment may be made directly on the same excavated quadrat sampling outlined in the Size Frequency metric (Metric 4, Chapter 2) or onto cultch that has been deployed (see also Metric 7, Chapter 3). This assessment can be made directly in the autumn following settlement. By this time the spat should be ~10mm in shell height (see Figure 3.10).

To properly monitor for recruitment, excavated quadrat or clutch material covered 1m^2 must be carefully examined by eye and/or with the help of a binocular microscope depending on the size of the spat and/or the presence of other similar bivalves (*Anomia*, *Crassostrea*; see Figure 3.10 and Figure 3.11). Given the large shell surface area, and the likely patchy distribution of settlement, it is recommended that a random subsample, manageable in size given the resources available, be assessed from each quadrat. If possible, oysters collected for sampling purposes should be returned to the oyster habitat after the measurements are completed, in order to minimise the impact of sampling efforts.

Alternative Method: Seasonal recruitment on benthic collectors.

Required Units: Number of freshly settled post larvae per unit of substrate area over a fixed period of deployment time (e.g. ind/cm^2 ± SE).

Method: The alternative method (see Box 3.5) is a complementary more analytical method to estimate recruitment efficiency. It consists of deploying passive benthic collectors with standardized substrate just before

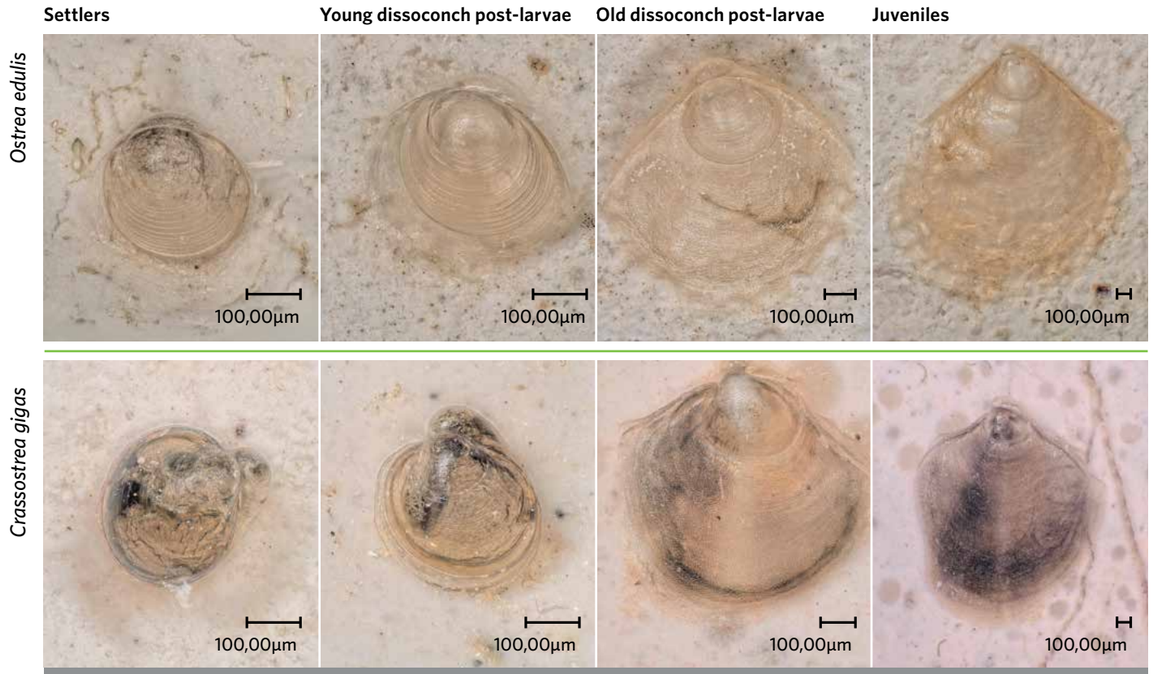


Figure 3.10: Development stages of the recruits for *Ostrea edulis* compared to those observed for *Crassostrea gigas*: Settlers (no dissoconch is visible, length < 300µm); Young dissoconch post-larvae (dissoconch is easily visible, but still narrow, 300µm < length < 500µm); Old dissoconch post-larvae (dissoconch becomes large, 500µm < length < 1,000µm) and Juveniles (very large dissoconch, length > 10,00µm). *Crassostrea gigas* post-larvae can be distinguished from *Ostrea edulis* larvae by a twisted asymmetry of the umbo. (Figure adapted from Pouvreau *et al.* 2021b).

settlement (see Figure 3.12) and during a short period of time (ideally two weeks). Counting is performed in the laboratory with a binocular microscope after collecting the benthic collectors from the sea. This analysis can yield detailed information on the development stages of the recruits (see Figure 3.10):

- **Settlers:** no dissoconch is visible, length < 300µm - 1 day post settlement.
- **Young dissoconch post-larvae:** dissoconch is easily visible, but still narrow, 300µm < length < 500µm - 1 week post settlement.
- **Old dissoconch post-larvae:** dissoconch becomes large, 500µm < length < 1000µm - between 1 and 2 weeks post settlement.
- **Juveniles:** very large dissoconch, length > 1000µm, > 2 weeks post settlement.

Sampling Frequency: Annual recruitment inside the bed should be evaluated at least once per year, ideally in spring. This allows recruits that have survived from the previous reproductive season to be more easily identified.

Seasonal recruitment on benthic collectors should be conducted several times across the reproductive season (see Box 3.5).

Performance Criteria: For annual recruitment onto cultch and/or within the habitat, increasing or reliably high levels of recruitment over multiple years.

For early recruitment, performance after a standardised period of 15 days exposure can be assessed according to the following scale (from Pouvreau *et al.* 2021b):

- **Recruitment < 0.1 ind/cm^2:** Recruitment is very low, the population is in danger of extinction if this value is observed several years consecutively and if mortality causes (predation, diseases, and pollution) are also high. Urgent conservation measure and restoration to be undertaken.
- **Recruitment between 0.1 and 1 ind/cm^2:** Low recruitment, urgent conservation measure and restoration to be undertaken. Very slow and uncertain recovery depending on juvenile mortality rates.
- **Recruitment between 1 and 10 ind/cm^2:** Intermediate recruitment, the application of conservation and restoration measures should allow a probable recovery of the population. A control of mortality rate is also necessary.
- **Recruitment > 10 ind/cm^2:** High recruitment, the application of light conservation and restoration measures should allow a very good recovery of the population, if subsequent mortality rate is not too high.
- **Recruitment > 100 ind/cm^2:** Very high recruitment, such values indicate an exceptional site deserving strong protection because it can play an important swarming role (spill-over effects). Of course, a control of mortality rate is also necessary.

BOX 3.5: TOWARD A UNIFYING EUROPEAN METHOD TO ESTIMATE AND COMPARE RECRUITMENT IN NATIVE OYSTER POPULATIONS

Deploying passive benthic collectors for a specified time during the reproductive season is more time-consuming than the simple counting of spat onto cultch or onto the restored habitat. However it provides detailed information on settlement events and timing across the breeding season, the effect of environmental factors on settlement and the recruitment index between sites and years. It can also be used as a rapid evaluation of a potential restoration site in terms of recruitment efficiency. This method can bring information equivalent to fecundity and larval abundance metrics (see Metrics 12 and 13, Chapter 3).

The benthic collectors used must meet several physical criteria to be attractive for oyster larvae, including (1) being physically and chemically similar to adult calcareous shells; (2) offering a coarse surface with fine relief; and (3) having a white colour as shells. It has also been shown that multi-plates 3D-structure with a narrow space between plates (< 2cm) and without inclination constitute a satisfying frame, allowing the availability of replicates. Lastly, the benthic collectors should not be deployed directly onto the sediment (to avoid problems of hypoxia and sedimentation), but some centimetres above it.

To meet all those criteria, the standardised benthic collector's method revised and proposed by Pouvreau *et al*. (2021a) for monitoring recruitment of native oyster consist uses a series (n=6) of rugged plates of a known surface area made of plastic (collectors cups used in shellfish farming) or cement (aragonites tiles used for aquarium). Furthermore, the plates have to be fully covered by a fine limed coating (2mm thick), which is obtained through a standard procedure (1kg of lime, 800mL of seawater and 15 days of drying time at temperature fixed between 20°C and 25°C in a high air renewal place). This step of coating is very important because it standardises the surface of the substrate (in terms of composition, texture, roughness and colour).

The benthic collectors should be deployed in replicate (n=2x6 plates) at the bottom of the seafloor near the native oyster habitat for a continuous period of 15 days, and no longer than 3 weeks. The operation can be repeated at regular time intervals from June to September to allow the seasonal dynamics in settlement to be observed.

The deployment of these benthic collectors can be made from a boat with a mooring, by SCUBA divers or on the shore in the low intertidal area at low tide. After 15 days, collectors are removed, gently rinsed and dried at the laboratory. Newly settled recruits are identified and counted by visually inspecting the top and bottom sides of each plate under standard binocular microscopes or more advanced 3D microscopes (e.g. KEYENCE VHX 6000). Care should be taken to avoid misidentification of other bivalve species. For each deployment date, a minimum of three plates from each of the two collectors (i.e. six cups in total) should be analysed to get a reliable mean and standard error. Recruitment should be reported as the number of individuals settled per cm^2 within 15 days of exposure (biweekly recruitment).

Figure 3.11: Series of images showing young *Ostrea edulis* spat on cultch (< 6 months old). **A**: Young spat on oyster shell, here *Ostrea edulis* can be confused with *Anomia epiphium*, i.e. the small white shells in the picture; note also that *Anomia ephippium* can be distinguished by looking carefully at the inferior valve of the prodissoconch shell, which is totally glued to the substrate. (Photo: Hélène Cochet). **B**: Young spat on a mussel shell. (Photo: Oscar Bos). **C**: Diagnostic features of *Ostrea edulis* spat include the presence of a white calcium streak lying vertically from the umbo towards the midpoint of the shell. (Photo: Luke Helmer). **D** and **E**: Young *Ostrea edulis* spat fixed onto the reef. (Photos: Matthias Huber).

METRIC 15: DISEASE PREVALENCE

Oyster disease is recognised as one of the main causes of oyster population decline in Europe. Two genera of parasites are prevalent, the haplosporidia (*Bonamia ostreae & B. exitiosa*) and the paramyxea (*Marteilia refringens*). *Bonamia spp.* are intracellular parasites that persist within the blood cells (haemocytes) of oysters, whilst *M. refringens* tends to be associated with the digestive tissues of oysters. Both parasites are defined as Notifiable Diseases within European (EC Council Directive 2006/88/EC, replaced by the new Animal health law in April 2021) and international legislation, the OIE aquatic code: https://www.oie.int/en/standard-setting/aquatic-code/access-online/. They are virtually impossible to treat or eradicate. Currently prevention of spread is the only viable option. Mortality is not immediate, but typically occurs after periods of environmental and/or physiological stress. The potential environmental effects on the disease development are an important feature to consider in any restoration project.

Other diseases of bivalves are also listed under EC Council Directive 2006/88/EC, including *Perkinsus marinus* and *Microcytos mackini*. Neither disease is yet to have been detected in Europe and there remains a legislative requirement by authority to monitor for the occurrence of these diseases. There has been a recent report that native oyster larvae and spat may be experimentally infected with oyster herpesvirus 1µvar (OsHV-1 µvar). Projects may therefore also consider monitoring for this virus, especially if co-located with Pacific oyster aquaculture.

Required Units: Parasite prevalence should be measured in % per total population (disease prevalence, %).

Primary Method: Histopathology and molecular biology.

Technical advice on, and minimum standards for, the detection of *Bonamia spp.* and *Marteilia refringens* of native oysters is based on the European Reference Laboratory for Mollusc Diseases. The detailed procedures can be found, for each potential disease, here: https://www.eurl-mollusc.eu/SOPs.

Sampling Frequency: Parasite prevalence should be assessed annually in spring and autumn. Any positive samples must be reported to the relevant national authority.

Performance Criteria: There are no performance criteria for this metric.

METRIC 16: INVASIVE NON-NATIVE SPECIES

Invasive non-native species (INNS) may impact restoration efforts and restoration practitioners should monitor for the following species: the slipper limpet *Crepidula fornicata*, the Pacific oyster *Crassostrea gigas*, the oyster drills *Ocinebrellus inornatus* & *Urosalpinx cinerea*, the Japanese wireweed *Sargassum muticum*. The carpet sea-squirt *Didemnum vexillum*, and the folded sea-squirt, *Styela clava* are also of concern, both because they smother surfaces and because they pose a serious biosecurity risk to areas where they are not yet recorded. Where the species listed above are present, surveys should be performed to assess their distribution and abundance (see Metric 19 in Chapter 4 for appropriate methods). The appropriate response to the data collected must then be determined in partnership with the relevant authorities (see zu Ermgassen *et al*. 2020).

Required Units: Number of individuals/m^2 for each species.

Primary Method: Grab sampling.

See Metric 18, Chapter 4.

Alternative Method: eDNA analysis.

See Metric 18, Chapter 4.

Sampling Frequency: Monitoring for invasive non-native species can be incorporated in assessment protocols required for biodiversity metrics (see Chapter 4).

Performance Criteria: There are no performance criteria for this metric.

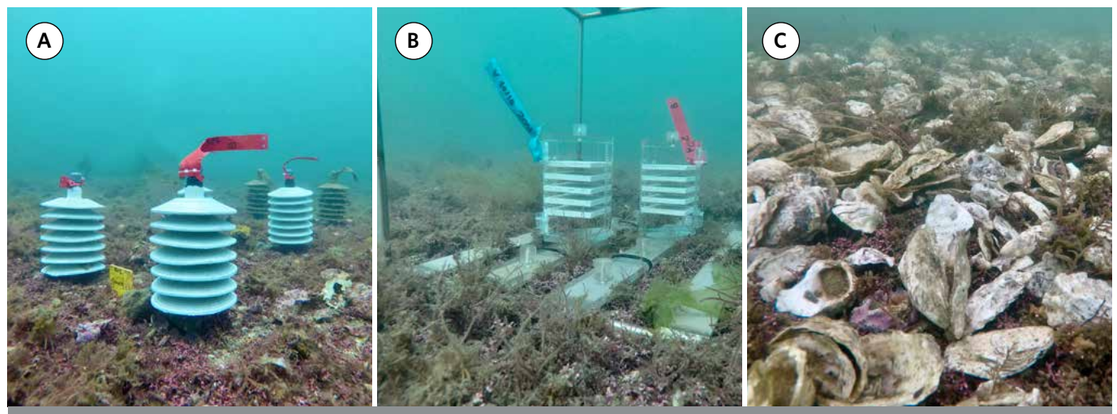


Figure 3.12: Deployment of benthic collectors and cultch in the vicinity of *Ostrea edulis* beds. A and B: benthic collectors screwed to a support fixed in the sediment. C: *Crassostrea gigas* cultch that has been deployed on the bottom a few days before the larval swarming. (Photos: Stéphane Pouvreau).

METRIC 17: SEDIMENTATION RATE

Sedimentation rate should have been considered carefully during site selection as high sedimentation can negatively affect survival, growth, and recruitment. Should a selected restoration site nevertheless have high or variable sedimentation, then sedimentation may need to be monitored. Sediment traps can be deployed on the shore and seabed to measure the degree of sedimentation at the restoration site.

Required Units: Dry weight/m^2 per day.

Primary Method: Sediment traps.

The sedimentation rate can be assessed by deploying sediment traps, which vary in design and cost. A relatively simple and inexpensive method is outlined here.

Traps are made by attaching a piece of AstroTurf© or a scouring pad to the inside of a plastic box or onto a thick perspex plate/tile. The AstroTurf© and scouring pad serve to restrict the resuspension of sediment, ensuring the collected bio-deposits remain on the collector pads within the container (see Figure 3.13).

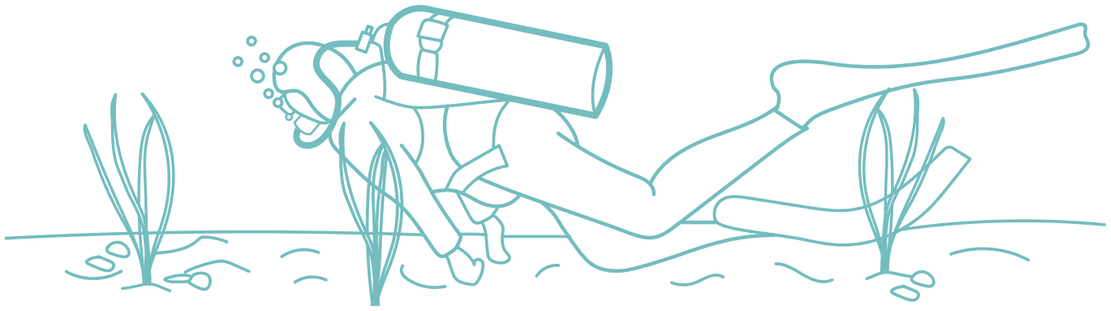
Prior to deployment the sediment trap should be covered to prevent contamination by placing a lid on each box or storing in a plastic zip-lock bag. Traps can be placed in the intertidal from the shore at low tide, or by divers in the subtidal. Once at the site, divers should remove the box lid and/or zip-lock bag in situ and anchor the trap to the sediment. Sediment traps should be deployed starting upstream and working downstream, such that traps do not capture sediment disturbed by the deployment of subsequent traps. Sediment traps should be recovered in less than 3 weeks as the efficiency of the sediment pads becomes variable if left in the environment for extended periods. When recovering the traps, begin at downstream sites first and work upstream to prevent disturbed sediments contaminating the collector pads. If using boxes, replace the lid firmly or alternatively place sediment traps carefully into zip lock bags while in-situ to prevent leaks and contamination.

In the laboratory, all sediment from the outside of the boxes should be rinsed off. The interior of the boxes and the collector pads should then be rinsed using filtered water until the water runs clear. All water used to rinse the trap should be collected in a single container which should then be vacuum filtered through a pre-weighed ashless GF/C Whatman filter paper (1.2µm pore size) and rinsed through with distilled water to wash away salt. The filter papers should be dried at approximately 80°C until weight has stabilised. Calculate the Total Particulate Matter (TPM) by: TPM = Final dry weight – filter paper weight. TPM can be standardised into g/m^2/day by dividing TPM by the size of the collection area and by the duration of time that the sediment traps were in the environment.

Sampling Frequency: Sedimentation rate should be considered four times a year, generally an assessment per season.

Performance Criteria: There are no performance criteria for this metric.

Figure 3.13: A sediment trap using a plastic box fixed onto the seabed (left) and a sediment trap being deployed by a diver, removing box lid (right). Photos sourced from Kent *et al.* (2017). (Photos: Robert Cook).



CHAPTER 4
RESTORATION GOAL-BASED MONITORING METRICS

CHAPTER AUTHORS
Joanne Preston, William Sanderson, Bernadette Pogoda, John M. Baxter, Thomas C. Cameron, Ian M. Davies, Monica Fabra, Jacob Kean Hammerson, Roland Krone, Tanja Hausen, Chris Hauton, Luke Helmer, Zoë Holbrook, George Kelly, Hannah Lee, Alice Lown, Charlie Mountain, Stéphane Pouvreau, Simon Reeves, David Smyth, Emma Ward, Philine zu Ermgassen.

INTRODUCTION

While European Native Oyster (*Ostrea edulis*) restoration in Europe has a strong impetus based on the historical declines and threatened status of the species, many restoration activities are also motivated by the potential to recover associated ecosystem functions and services delivered by healthy native oyster habitats. These include increased biodiversity, improved water clarity and quality, increased carbon draw-down, increased quality of adjacent vegetated habitats such as seagrass and saltmarsh, associated increases in fish production and overspill from restored populations resulting in increased oyster fisheries yield in adjacent areas.

Where ecosystem services or biodiversity gain are an explicit restoration goal, it is recommended that restoration practitioners monitor directly for these outcomes. This can serve both to increase stakeholder understanding of the value of the restoration efforts and inform adaptive management to maximise the potential ecosystem service return of the project.

BROODSTOCK AND OYSTER POPULATION ENHANCEMENT

Metrics that assess the status of the native oyster population are of relevance when aiming to restore or enhance native oyster over a larger area than the project footprint itself, either for habitat and biodiversity benefits, or to support a co-located sustainable oyster fishery. The recovery of nearby areas is achieved through higher densities of native oyster at the restored site providing larvae, which spread into the surrounding areas and seed nearby suitable habitats, a process referred to as overspill.

The following metrics provide insight into connectivity between the restoration site and nearby areas, and go some way to quantifying the effects of native oyster population enhancement.

METRIC 18: NEARBY-REEF OYSTER DENSITY AND ASSOCIATED SIZE-FREQUENCY DISTRIBUTIONS

Required Units: Mean oyster density: individuals/m^2. Size frequency: mean oyster shell height (mm), percentage (%) and/or number of measured oysters per size class; annual recruitment density of living < 1-year-old recruits per m^2 (individuals/m^2 ± SE).

Primary Method: See Metric 3 (Oyster Density) and Metric 4 (Oyster Size Frequency) in Chapter 2, and Metric 14 (Recruitment Index) in Chapter 3.

Monitoring should cover nearby locations both prior to and after restoration. While this information can provide useful inferences or correlation regarding the contribution to recruitment that a restored reef is providing to nearby habitat, it is not possible to absolutely confirm the restoration site as the source of this larvae and recruitment without further analysis (e.g. genetic relationships, larval distribution hydrodynamic models).

Sampling Frequency: Annually, between December and July, when newly settled oyster spat have grown to a size that can be seen with the naked eye (~5-10mm) and can be confidently identified as native oyster recruits (see Metric 14, Chapter 3).

Performance Criteria: A trend of increasing density of native oyster outside the restoration site and the appearance of cohorts in the size-class-frequency data, indicating repeated recruitment events over time.

FISH AND INVERTEBRATE BIODIVERSITY

Biodiversity is an important ecosystem function. In Europe, a number of restoration projects have defined increased biodiversity as a key goal of native oyster restoration. Aside from providing a direct measure of the biodiversity impacts resulting from restoration, monitoring biodiversity also provides insights into important ecological aspects of native oyster habitats, such as the presence of predators (to understand predator-prey relationships) and of invasive, potentially problematic species. Furthermore, oyster habitats in other geographies (USA, Australia) have been identified as essential fish habitat, supporting a suite of species both as nursery habitats and transient feeding grounds. Developing an evidence base in Europe of the associated community of the native oyster is critical for the development of any biodiversity and fishery related policy in the future.

Native oyster habitats can support a range of different species groups, including those living in the sediment (infaunal species), those living on their surface (epifaunal species), those interacting strongly with their three dimensional structure (small resident fish and invertebrates) and those temporarily visiting them as feeding grounds (transient species). No single sampling method can effectively sample all of these groups; this section therefore outlines a range of options that may effectively sample one or more of these groups. The most appropriate method for a project will depend on the aims of the restoration project and the resources and expertise available.

Identifying communities to fine taxonomic levels is highly time consuming and requires significant expertise. This is often not feasible in ad-hoc or larger scale surveys. Previous literature has shown that increasing the resolution of taxonomic identification in invertebrate communities often does not increase the understanding of biodiversity within an area. Where resources or expertise is lacking, practitioners may want to decide to focus on counting the number of species or groups present.

Licences and permission should be secured before conducting biodiversity surveys. All monitoring methods that may capture animals require ethical consideration and review to ensure the welfare of animals is considered and any potential harm reduced.

METRIC 19: INFAUNAL INVERTEBRATES

Infaunal species, such as clams, snails, polychaetes, flatworms and small crustaceans, live in the seabed and are especially common in soft sediments (see Figure 4.2). They contribute to biodiversity and their abundance is an indicator of ecosystem health.

Required Units: Number of species/sediment volume, with a list of the species/groups identified; biomass/species or groups/area sampled (fresh weight (FW) g/m^2).

Primary Method: Sediment cores.

Sediment cores (see Figure 4.1) can be collected in the intertidal or (by divers or operated from a boat) in the subtidal. The core tube should be driven into the sediment vertically and the enclosed sediment carefully cleaved, as close as possible to the penetrating edge of the tube. As soon as possible after collection, core samples should be sieved (e.g. mesh size: 1mm), and fixed (e.g. in buffered 4% formalin-seawater solution) for further processing. In the laboratory, all organisms should be identified to the lowest taxonomic level possible, and then enumerated and weighed.

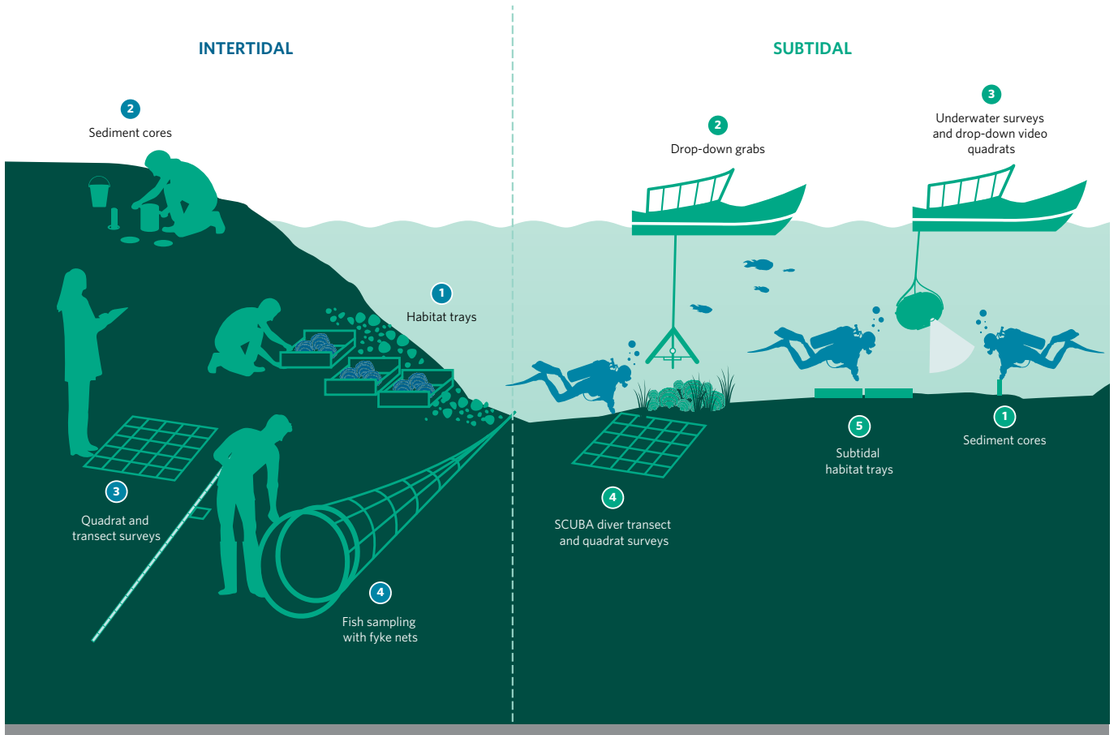


Figure 4.1: A schematic diagram depicting the different methods of sampling that can measure biodiversity in intertidal and subtidal habitats. **Subtidal:** (1) Sediment core and (2) Drop-down grap to for assessing infaunal invertebrate diversity (Metric 19). (3) Underwater surveys and drop-down video quadrats for larger mobile and transient fish and invertebrates (Metrics 21 and Metric 22). (4) Diver transect and quadrat surveys for underwater visual census for epifaunal sessile biodiversity (Metric 20), (5) Subtidal habitat trays for epifuanal diversity and small resident fish and invertebrates (Metrics 20 and 21). **Intertidal:** (1) Habitat trays, (2) Sediment cores, (3) Quadrat and transect surveys, (4) Fish sampling fyke nets (Metric 22).

Alternative Method 1: Grab sampling.

Grabs (e.g. Van Veen grab; area: 0.1m^2, weight: 90kg) or box corers are invasive methods and are usually applied in subtidal waters. These methods allow for a good volume of sediment to sort and analyse infauna next to the native oyster habitat or between oyster patches on soft sediments. However, they are difficult to use directly on native oyster habitat areas, as they might not grab/penetrate deep enough into the shelly sediment. Processing of samples should follow the methods described in the primary method.

Alternative Method 2: Environmental DNA (eDNA) analysis.

Fish and other organisms shed cells and excrete mucus, faeces or other material that can be detected in the environment through eDNA techniques. eDNA can be used to efficiently reveal the presence of target species at low population densities, and can be easily sampled at large scale. Cryptic species or juveniles that may otherwise be missed can be detected in this way. Sampling of sediment, water or epifauna (scrape samples, Metric 20) allows for eDNA analysis in the laboratory. It is important that samples are correctly fixed and stored immediately after sampling. DNA is processed using techniques such as metabarcoding, which allows for the identification (absence/presence) of a suite of target species. To identify taxa, amplified DNA fragments (barcodes) are compared to databases. Hence, species can only be identified if they have already been added to a database. While most European fish species are already present in public databases, many benthic species still need to be added. The technique is potentially powerful for biodiversity assessments of native oyster habitats, but does require further testing.

Sampling Frequency (for Metrics 19, 20, 21 and 22): At least once prior to restoration, and annually thereafter, when abundances of key species are highest.

Performance Criteria (for Metrics 19, 20, 21 and 22): A trend of higher biodiversity and abundance on restored sites, with the ultimate aim of having statistically higher biodiversity on restored sites relative to the preconstruction and control sites.

METRIC 20: EPIFAUNAL SESSILE INVERTEBRATES AND MACROPHYTES

The appropriate enumeration and recording of sessile epifauna and flora depends on the growth form and abundance of the species. Colonial species are assessed as percent cover, while solitary species and conspicuous fauna (including sessile invertebrates) are counted as number of individuals per area. Abundances of macrophytes are treated the same as colonial species and can sum up to more than 100% because of overlapping growth. Surveyors must be trained in the target species identification to ensure consistency of data over time.

Required Units: Number of species/m^2 with a list of the species identified, as well as the abundance as percent cover or count of individuals as appropriate.

Primary Method: Quadrat sampling or transects by SCUBA diving (see Metric 3, Chapter 2).

Alternative Method 1: Substrate trays.

This method is described in Metric 21, Chapter 4.

Alternative Method 2: Drop down video (DDV) quadrats.

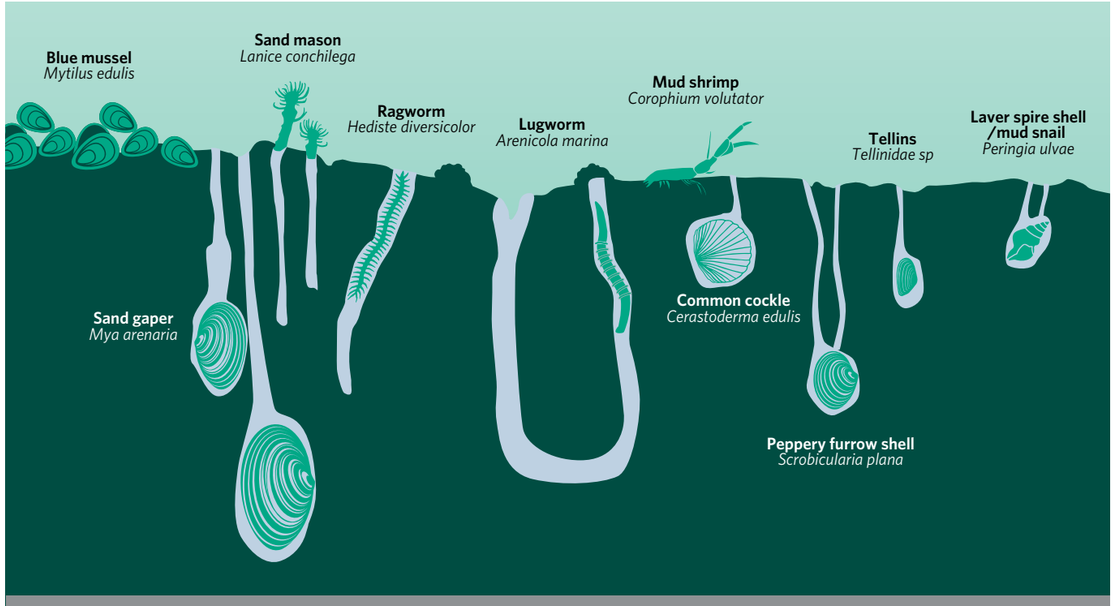


Figure 4.2: Examples of infaunal invertebrates, including blue mussel (*Mytilus edulis*), sand mason (*Lanice conchilega*), lugworm (*Arenicola marina*), peppery furrow shell (*Scrobicularia plana*) and mud snail (*Peringia ulvae*).

Sessile invertebrates and epifauna can be assessed through analysis of images of quadrats taken by DDV (see Box 2.1, Chapter 2) or by divers (see Box 4.1). Post-production analysis to assess/identify species and coverage should be carried out by a trained person, with the support of specific software tools, such as the web-based free image/video annotation software BIIGLE. Further guidance can be found in Metric 3, Chapter 2.

Alternative Method 3: eDNA analysis (see Metric 19, Chapter 4).

METRIC 21: SMALL RESIDENT FISH AND MOBILE INVERTEBRATES

Mobile fauna such as fish, crustaceans and snails live in the crevices of native oyster habitats, which can make them challenging to assess. Resident and mobile fauna contribute to biodiversity and their abundance is an indicator of ecosystem health. Many species will be potentially important prey for commercially important species, but may also include some species of commercial value (e.g. whelks).

Required Units: Individuals/m^2, and, if possible total length (mm) for length-frequency distribution/species. For fish sampling nets: catch per unit effort (CPUE (individuals/hour), with length (mm) by species.

Primary Method for Subtidal Habitat: Underwater video tools.

Underwater video systems are a powerful and efficient tool for monitoring macro zoobenthos if visibility allows. Depending on water depth, native oyster habitat area and vessel size, the use of remote underwater video (RUV) (see Metric 22, Chapter 4) or DDV (see Metric 1 and Box 2.1, Chapter 2) can be applied.

Alternative Method 1: Underwater visual census (see Box 4.1).

Alternative Method 2: eDNA analysis (see Metric 19, Chapter 4).

Alternative Method 3: Habitat units or trays. See primary method for lower intertidal habitat below. This method can be adapted to be subtidal if collected and deployed by divers. The trays can be analysed on board the research vessel, or transported back to a laboratory for further processing.

Primary Method for Lower Intertidal Habitat: Habitat units or trays (see Figure 4.3).

Tray sampling is a quantitative, non-destructive method for monitoring oyster growth (see Metric 7, Chapter 3) and associated biodiversity, including epifauna, mobile mesofauna and sessile organisms attached to the benthos. Trays filled with native oyster and optionally oyster shells as substrate are deployed at the study site at randomly selected locations. If the native oyster habitat has high relief, e.g. due to a rocky substrate, the trays should be deployed at different heights: along the crest, the base and in the directly adjacent sediment. Trays should be provided with holes for natural exchange with the surrounding sediment and should be placed in the sediment so that they are aligned with the environment. Their size and number should be scaled according to the size of the reef. When trays are removed, they should be capped and transported in fine mesh bags to avoid losing mobile organisms. All organisms should be identified to the lowest possible taxonomic level, counted and measured. Identification can be performed on site and organisms released after monitoring. Alternatively, the trays can be transported back to the laboratory and organisms preserved (e.g. in buffered 4% formalin-seawater solution) for future analysis.

METRIC 22: TRANSIENT FISH AND CRUSTACEANS

Larger fish and crustaceans may use native oyster habitat regularly (e.g. for feeding) or seasonally (e.g. for spawning). Such transient species are often fast swimmers; many display schooling behaviour, and as they only occasionally visit the habitat, they can be challenging to accurately assess. Many commercially important species are transient and are therefore important to monitor to build up an evidence base of the potential contribution of native oyster habitats in Europe.

Required Units: Individuals/m^2 and, if possible, total length (mm) for length-frequency distribution/species. For fish sampling nets: catch per unit effort (CPUE (individuals/hour), with length (mm) by species.

Primary Method for subtidal habitat: Remote underwater video (RUV) surveys.

Unbaited RUV surveys can be used depending on visibility. Underwater video camera sets (or GoPro, depending on battery capacity) are attached to a weighted frame (see Ebner and Morgan 2013), which is then deployed by a rope with an attached buoy. RUVs should be placed at random locations across the reef and on control sites, at least 50m apart. The GPS coordinates at each location should be recorded. After deployment, the video footage should be processed to measure fish abundance and diversity. The first arrival of each species and the maximum number of species in the first 10, 20, 30, 40, 50 and 60 minutes of each deployment should be recorded (see Willis, Millar and Babcock, 2000).

Alternative Method 1: Underwater visual census (see Metric 19 and Box 4.1 in Chapter 4).

Alternative Method 2: eDNA analysis (see Metric 19, Chapter 4).

Primary Method for Intertidal Habitat: Fish sampling nets.

Intertidal methods such as Fyke or Lift nets (see Figure 4.3) can be deployed by wading during low tide and left for 3-6 hours before collection after high tide. Double-ended Fyke nets have a length of net (length will be dependent on project size, but at least 10m in long) with a positively buoyant float line at the top and negatively buoyant weighted lead line at the bottom. At each end of the net, mesh tunnels are suspended by aluminum hoops of decreasing size, which capture the fish, and are secured by stakes. Fyke nets are able to capture fish swimming from either direction toward the nets. Lift nets are similarly deployed at low tides by stakes in a circular pattern, with the nets at the bottom. At high tide, the nets are lifted, encircling and trapping the fish. Both day and night sampling is recommended. The nets should be deployed at random locations on the native oyster habitat, and at locations on adjacent bare sediment as a control.

Net collection should be completed while the nets are still slightly submerged. It is important to ensure that fish are not left out of water, but removed carefully from the nets and placed in a container filled with seawater for measurement and identification before releasing. Ethical review will be required for all methods involving fish sampling.

Alternative Method 1: eDNA analysis (see Metric 19, Chapter 4).

Sampling Frequency:
At least once prior to restoration, and annually thereafter, when abundances of key species are highest.

Performance Criteria:
A trend of higher biodiversity and abundance on restored sites, with the ultimate aim of having statistically higher biodiversity on restored sites relative to the preconstruction and control sites.

BOX 4.1: UNDERWATER VISUAL CENSUS METHODS

The abundance of epifauna and mobile demersal megafauna (MDM) can be assessed by underwater visual censuses (UVCs) using SCUBA or snorkel techniques. UVCs generally work well at depths of up to 30m and at sites that do not have strong currents or poor visibility.

Sample length is controlled by a transect line. The sample length should be adapted to the appropriate diving time. Transect widths and heights are controlled by a spacer clipped to the line reel. The diver stops every marked metre along the transect to search and document the cubic metre, including the sea floor ahead for fishes and MDM (e.g. sea stars, crabs, fish) of 2cm or more in size.

1m markings across the transect line ensure that the surveyed area remains consistent. Critical habitat features such as shell cover, for example, can be recorded as part of the same operation by photographing 50 x 50cm quadrats randomly deployed along the same transect line, photographed in such a way that the numbers on the measuring tape are visible (quadrat sampling).

A variation to the UVC survey approach is to use the transect line to record a video of the seabed, keeping a consistent field of view (about 0.75-1m distance) and with the tape at the edge of the field of view as a reference. Analysis of the video is best achieved by using extremely slow movement along the transect line (e.g. a minimum of 20 minutes for 25m transect), sample collecting and identification in situ to support subsequent 'on-screen' identifications.

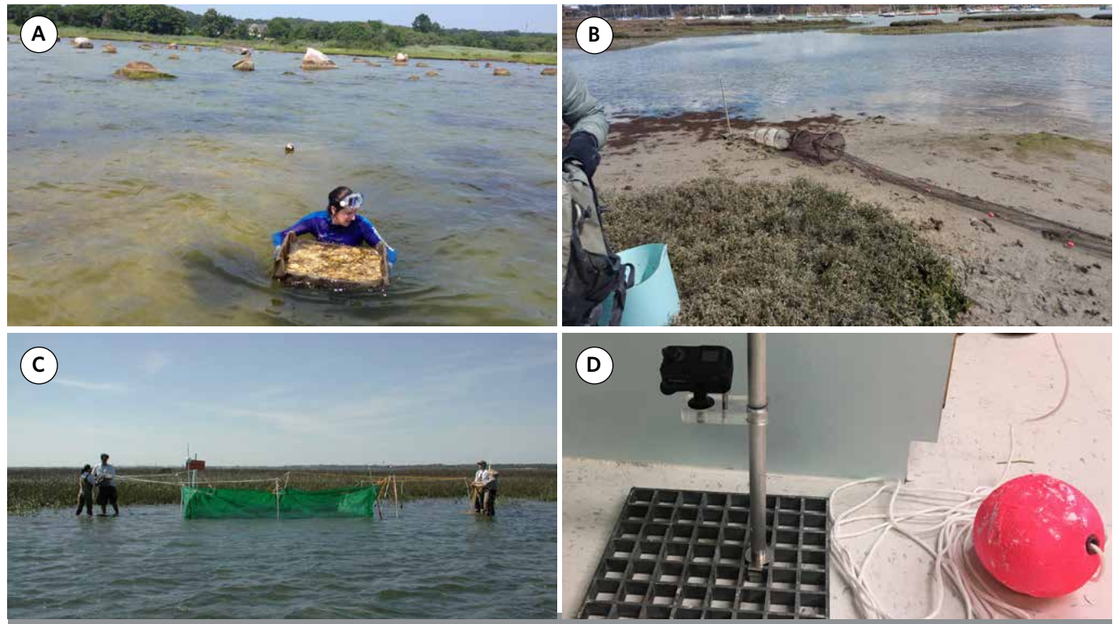


Figure 4.3: Survey methods used to monitor resident or transient fish and crustaceans: A) Habitat trays; B) Fyke nets; C) Lift nets and D) Remote underwater video (RUV). (Photos: Theresa Davenport, Charles Mountain, Jonathan Grabowski and Ronald Baker).

Table 4.1: Biodiversity assessment methods for different target groups, including advantages and limitations of their use.

Method	Positives	Limitations
Photographic methods (implemented by divers or after sampling)	Non-destructive method In situ time-saving method (more post-sampling processing effort with less time required underwater) Less training of divers/taxonomic knowledge required Reproducible results (data acquisition, data evaluation means post-sampling processing) Automation of the post processing possible (eg. for photo frame)	Underestimation of hidden, cryptic and well camouflaged taxa (mostly not detectable from images) Limited by visibility/turbidity Incapable of or limited in assessing hidden fractions of groups, particularly those within sediments or between crevices within more complex reefs (eg. epifauna, mobile in vertebrates, vertebrates) Expensive equipment in the case of photogrammatic methods Bias from divers/people processing photographs Artificial light could attract or deter certain taxa
Underwater video or remote underwater video	Non-destructive method In situ time-saving method (more post-sampling processing effort required) Less training of divers, less taxonomic knowledge required Unlimited sampling time Reproducible results (data acquisition, data evaluation means post-sampling processing)	Underestimation of hidden, cryptic and well camouflaged taxa (mostly not detectable form images) Limited by visibility/turbidity and strong currents Incapable of or limited in assessing hidden species and niches, particularly those within sediments or between crevices within more complex reefs (e.g. epifauna, mobile invertebrates, vertebrates) Depends on moderate drift velocity Artificial light could attract or deter certain taxa Expensive equipment in the case of photogrammetric methods
Visual quadrat surveys	Possible in areas of low visibility Applicable in intertidal (low tide) and subtidal (divers) Simple equipment	Extensive training required with limited availability of retrospectively identifying species Differences among divers must be considered Time consuming No biomass data
Underwater visual census (dive transects)		Extensive training required with limited availability of retrospectively identifying species Differences among divers must be considered Presence of the diver and use of artificial light could lead to attraction or deterence of certain taxa High time and manpower requirements, strong limitation by dive time
Trays and substrate baskets	Provides a snapshot of an entire trophic community (part of the reef) Coverage of all non-highly mobile faunal groups of the reef community (epifauna, infauna, slow mobile macrofauna)	Deployment can be complex depending on location Moderately invasive when being removed or recovered (also impacting the surrounding areas)
Sediment cores	Rapid sampling Methodologically simple Possible in areas of low visibility Applicable in intertidal (low tide) and subtidal (divers) Simple, low-cost equipment	Requires detailed and time-consuming post-sampling processing Small area sampled – may miss rare species Unsuitable on cultch and oyster reef cover or coarse grain size
Dredge surveys	Enables sampling over a large area Possible in areas of high turbidity Provides large samples, which increases detection of rarer species Possible to process samples accurately on deck with limited post-sampling processing effort required	Destructive method that may damage fragile species Not allowed in some areas Not suitable for assessing particularly rocky and/or shallow areas Restricted by dredge ring/ladder size with smaller species able to pass through the dredge
Grab surveys e.g. van Veen or day grab	Small footprint of impact - reduces impact on benthic environments Possible in areas of high turbidity Unlimited sampling time Provides insight into sediment changes alongside biodiversity information	Small area sampled – may miss rare species Unsuitable in rocky areas where grabs are unable to penetrate sediment, oysters or shell can prevent grab closure causing loss of sample Focus on infauna Destructive method which may damage fragile species
Remotely operated underwater vehicles and autonomous underwater vehicles	Non-destructive method Independent of divers Unlimited sampling time	Extensive training required High cost and complex technical demand No sampling of unidentified species possible
eDNA Sampling	Non-destructive method Possible in areas of high turbidity Independent of divers Unlimited sampling time Able to provide samples over a large area	Small footprint of impact – may miss rare species If ship-based, water sampling is not possible, divers are needed New technology Ground truthing highly recommended Currently cost intensive

INTERACTIONS WITH ADJACENT HABITATS

Native oyster habitat forms part of a wider continuum or mosaic of coastal and estuarine habitats that have experienced catastrophic losses of biodiversity and habitat over the last few hundred years – particularly saltmarsh and seagrass meadows. Saltmarsh and seagrass provide a range of ecosystem services, such as carbon sequestration and storage, essential fish habitat, sediment stabilisation and coastal protection (see Figure 4.4). These vegetated ecosystems, much like the native oyster, are threatened by a range of human activities and are much reduced from their historical extent. The presence of native oyster habitat can offer protection and mitigation from anthropogenic pressures impacting

neighbouring vegetated habitat. Native oyster have been shown to enhance saltmarsh growth and improve water quality, which in turn can improve the growth of submerged aquatic vegetation. By decreasing wave attenuation, oyster reefs in the USA reduced erosion of neighbouring saltmarsh habitats but this is yet to be quantified in a European context. See Figure 4.5 for examples of living shorelines and integrated marine habitat restoration practices.

METRIC 23: SHORELINE LOSS/GAIN (CHANGE IN SHORELINE POSITION)

Native oyster habitat with height (e.g. oyster reefs) can attenuate wave energy and enhance sedimentation, thereby potentially contributing to a reduction in shoreline loss or to shoreline gain.

Shoreline position can be mapped using a variety of techniques outlined below. Shoreline loss/gain is calculated by subtracting the current year's shoreline linear distance from the permanent base stake of the previous measurement's linear distance. Positive values indicate gain, while negative values indicate loss.

Required Units: Shoreline loss/gain (m/year).

Primary Method: Mapping the shoreline on foot.

Shoreline loss and gain can be assessed by repeated mapping of the same area over time. The maps can then be overlayed in mapping software, such as QGIS or ArcGIS, to determine the change in shoreline between surveys. Mapping of the shore can be achieved by one of two methods.

Using a handheld digital Global Positioning System (dGPS)

If a dGPS is available (accuracy 10's of cm), e.g. Garmin eTrex, the shoreline can be mapped by walking the shoreline edge adjacent to the proposed reef areas. Shoreline edge is generally characterised by significant change in habitat/sediment (see Figure 4.6). If possible, the entire shoreline edge within designated site boundaries should be walked, although distances of 100m either side of the proposed reef site are considered sufficient to measure change. Where the site is smaller or too fragmented to walk along, recordings of the available shoreline edge should be coupled with other measures, such as aerial imagery, or the low-tech option below reverted to.

Permanent perpendicular transects

If a dGPS is not available, measurements can be made relative to a pre-established transect marked out with permanent base stakes. Where it is not possible to install permanent base stakes, a constant GPS location can be used. Using a measuring tape, the distance from the base stakes placed inland to the shoreline edge should be walked, along the pre-established transects, and the total distance recorded. If the marsh is fragmented by areas of mudflat, a note should be made, but the transect to the furthest point of marsh in that orientation should be continued. When taking measurements, practitioners should keep the length of measuring tape taut. Data should be entered into mapping software, such as ArcGIS, and mapped over a georeferenced basemap. This process should be repeated for an equal distance of shoreline edge at the control or reference site.

Monitoring can be made efficient by measuring shoreline elevation (see Metric 24 , Chapter 4), plant density and percent cover (see Metric 25, Chapter 4) along the same transects.

Alternative Method 1: Using surveying instrumentation.

If a practitioner is familiar with basic surveying techniques/advanced surveying instrumentation (e.g. a Total Station or other instruments used to find horizontal and vertical angles and distances), they may perform a topographic survey along each of the transects using these instruments. A topographic survey allows the collection of shoreline loss/gain data and shoreline profile/elevation change data to be obtained simultaneously. Elevation/location measurements should be taken at regular intervals along each transect from the permanent base stakes to the shoreline edge (or continuing to the constructed reef if collecting information for the profile/elevation change metric). Data should be entered into mapping software, such as ArcGIS, and mapped over a georeferenced basemap.

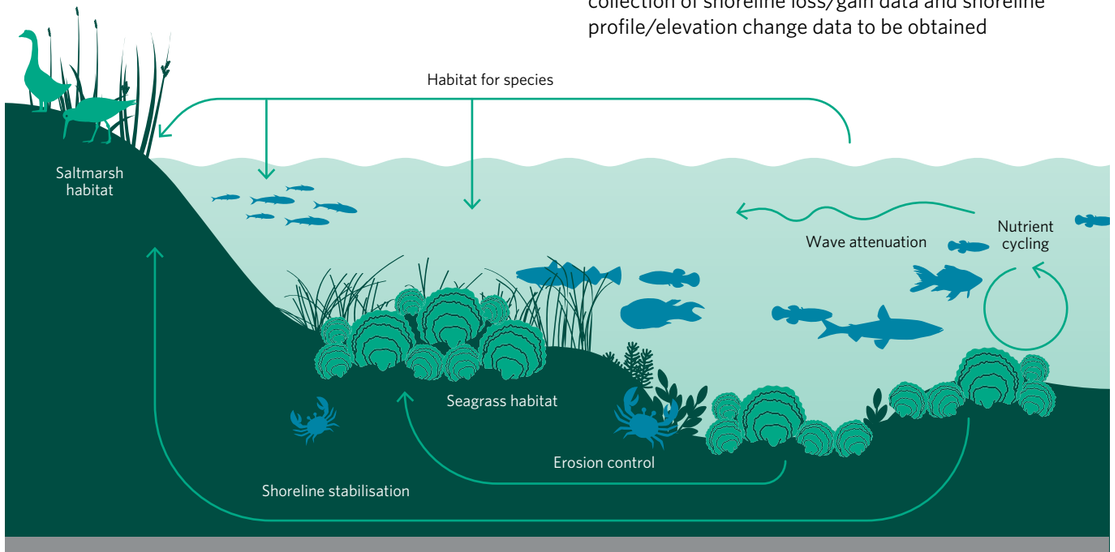


Figure 4.4: The seascape from saltmarsh, seagrass to oyster habitat, showing the possible interaction of ecosystem services.

Alternative Method 2: Using aerial photography.

Shoreline loss/gain can also be determined through aerial/satellite imagery. It is important to make sure that the images can be georeferenced, for example with permanent base stakes with markers of known dimensions that are visible from the air and the presence of a permanent feature. If possible, ortho-rectified aerial photographs (geometrically corrected to have a uniform scale) should be used.

Data should be entered into mapping software and mapped over a georeferenced basemap. Using the aerial images, the linear distance from the permanent base stakes to the shoreline edge along the transects should be measured. Aerial photography is also useful in communication to wider audiences, as it can be easier to interpret and connect with than raw data.

Sampling Frequency: Measurements should be taken once prior to construction, six months post-construction (to document the immediate post-construction project footprint and reef area), and annually thereafter. Additional measurements after events that could alter shoreline position (e.g. dredging events/large storms) are also recommended.

Performance Criteria: Trend of decreasing shoreline loss, or shoreline gain.

Figure 4.5: Examples of living shorelines and integrated oyster and saltmarsh restoration. A, B and C: Saltmarsh protection by 'oyster castles' with *C. virginica* recruits in Chesapeake Bay, Virginia, USA. (Photos: Luke Helmer and Joanne Preston). D and E: Biodegradable BESE systems placed in front of Saltmarsh in the Solent. F and G: Biodegradable BESE systems with *Ostrea edulis* oyster shell. (Photos: Solent Oyster Restoration Project/Charles Mountain). H: Natural Australian flat oyster (*Ostrea angasi*) reefs in Georges Bay, Tasmania. (Photo: Chris Gillies/The Nature Conservancy).

METRIC 24: SHORELINE PROFILE/ ELEVATION CHANGE

Required Units: Shoreline profile/elevation change (cm/year), shoreline slope (i.e. rise/run) (unitless).

For the methodologies listed below, it is suggested that a dGPS be used to mark the locations of the measurements taken.

Primary Method: Using surveying instrumentation.

Elevation change can be determined using traditional surveying equipment such as a surveyor's level, laser level and graduated rod, or ranging poles between two people (see Figure 4.6).

Where the practitioner has training in and access to advanced surveying instrumentation (e.g. a Total Station or other instruments used to find horizontal and vertical angles and distances), they may perform a topographic survey along each of the transects using these advanced surveying instruments. If using advanced surveying instrumentation, data for the shoreline profile/elevation metric and the shoreline loss/gain metric (previous section) can be obtained simultaneously.

Elevation measurements should be taken at standardised intervals (a minimum of every 5m along horizontal transects or 30cm change in vertical height) moving seaward from the permanent base stake to the native oyster habitat, recording location and elevation of the shoreline edge. It is also recommended that any notable change in elevation outside of the standardised distance between measurements be recorded. This process should be repeated for an equal linear distance of shoreline edge at the control site (see Figure 4.6).

The shoreline profile (elevation change data per measured point) for each transect should be plotted as a line graph, and an overall shoreline profile for the site can be obtained by calculating the mean elevation change of all transects for each measured point.

Sampling Frequency: Measurements should be taken once prior to construction, within three months post construction (to document the immediate post-construction project footprint and reef area) and annually thereafter. Additional measurements after events that could alter shoreline profile (e.g. dredging events/large storms) are also recommended.

Performance Criteria: Trend of decreasing slope and increasing mean elevation at the shoreline, or a decreased 'step/cliff' at the water's edge.

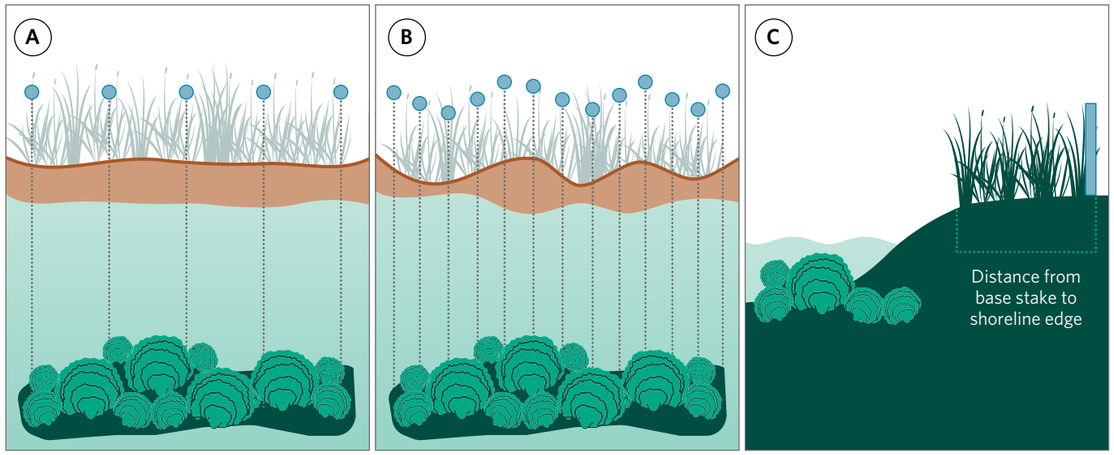


Figure 4.6: Overhead and cross-sectional views of example layouts of permanent transects (dashed lines) and base stakes (light blue dots). Shoreline edges (orange lines) with a low degree of sinuosity or irregularity (A) will require fewer permanent transects, whereas shoreline edges with a high degree of sinuosity and irregularity (B) will require more permanent transects. When measuring shoreline loss/gain (C), practitioners should measure the distance from the base stakes (light blue rectangle) to the shoreline edge. (Infographic modified from Baggett *et al.* 2014).

Figure 4.7: A characteristic shoreline edge in the Solent, showing the transition from saltmarsh to mudflat. The shoreline is considered to be the edge of the vegetation. Left: Aerial image of saltmarsh and mudflats with transects indicated by solid yellow lines and shoreline indicated by dashed line. Right: Typical edge of saltmarsh vegetation in the southern UK. (Photos: Charlie Mountain/Joanne Preston).

METRIC 25: DENSITY AND PERCENT COVER OF SALTMARSH/SEAGRASS PLANTS

Where a restoration project is specifically aiming to recover vegetated habitats associated with the native oyster habitat, monitoring of changes in the quality (density and percent cover) of these vegetated habitats should be conducted. The intertidal methods below can be used for saltmarsh, but this metric mostly applies to seagrass, referred to here as submerged aquatic vegetation (SAV).

Given the high degree of interannual variability in SAV cover and the numerous factors other than restored habitat that impact SAV habitat quality, a Before-After-Control-Impact (BACI) design (see Figure 1.4, Chapter 1) is essential for assessing the impact of oyster habitat on SAV. The sampling of SAV should be performed at the site adjacent to the restored native oyster habitat and at a non-restored control site with similar current and wave conditions.

Habitat quality data collected in this metric can be considered alongside results from Metrics 23 and 24 to characterise the impact of native oyster habitat restoration on adjacent habitats.

Required Units: Mean density (live shoots/m^2), mean percent cover (%) of each species present.

Primary Method for Intertidal Habitat: Quadrats.

A visual estimate of the percentage of ground covered by each species should be recorded at standardised intervals, using a 1 x 1m quadrat (or 0.5 x 0.5m quadrat if vegetation is dense). Percent cover can be estimated as described in Metric 2, Chapter 2. Ideally, quadrats should be placed along the same permanent transects as used for other shoreline measurements (e.g. shoreline edge and elevation, see Figure 4.6). In saltmarsh plots, the transect should range from ~4m inland, beyond the start of the saltmarsh, to the shoreline edge. For intertidal SAV, the transect should extend from mean high water springs (MHWS) to mean low water springs (MLWS).

In each quadrat, the number of SAV shoots present within a 0.25 x 0.25m subsection should also be counted to give an estimate of the SAV shoot density. Example photographs of various seagrass percent coverages may be found in Short *et al.* (2015). Where sampling on foot intertidally is logistically challenging, intertidal habitats can also be sampled subtidally at high tide.

For largely fragmented habitats, belt transects can be conducted for the entire permanent transect. All species present within 2m either side of the transect can be recorded at suitable intervals and their rough abundance noted by visual estimate using the semiquantitative seagrass scale outlined in Table 4.2 or a SACFOR scale (SACFOR codes are: S: superabundant; A: abundant; C: common; F: frequent; O: occasional; R: rare; see JNCC for more details). More focused transects can then be designated to all areas of interest, e.g. the marsh islands, using quadrats to determine live shoots per m^2 and percent cover of each species as detailed above.

Alternative Method 1: Aerial imagery and light detection and ranging (LiDAR).

Saltmarsh and SAV cover and distribution can be determined using an object-based image analysis approach (OBIA) with very high resolution (VHR) imagery, including aerial imagery, LiDAR, RGB and bathymetric data. This method is recommended when mapping large areas; however, as some species can appear spectrally similar and occur at the same depths, this method must be ground truthed using the quadrat methods mentioned above.

Monitoring should occur during a period of saltmarsh or SAV growth, which can vary spatially, e.g. in southern Britain from February/April to October. Data can be collected first hand through the use of drones or planes, but must be ground truthed. Therefore, visible markers of a known size and position are required. Data may also be obtained through various organisations, e.g. in the UK: the Channel Coast Observatory. Data can then be mapped using a Graphic Information System (GIS) software such as QGIS or ArcGIS.

Table 4.2: Semi-quantitative seagrass density scale. This scale can be applied to submerged aquatic vegetation density rating using visual estimates (either from transects or benthic video data). Modified from Lefebvre *et al.* (2009).

Rating	Description
0	No seagrass
1	Sparse coverage (only a few shoots within several video frames)
2	Patchy coverage (patches of seagrass and sand or intermediate density)
3	Dense seagrass but seabed visible (< 100% cover)
4	Continuous seagrass canopy (100% cover)

Primary Method for Subtidal Habitat: Underwater visual survey (UVS).

At least three transects should cross the area of SAV perpendicular to the native oyster habitat. The start and end of patches of SAV habitat should be noted along the transect, along with SAV percent cover and shoot density measurements collected from at least three quadrats along each transect. Quadrats should be assessed as outlined in the primary intertidal method above.

Alternative Method 1: Drop down video (DDV).

Percent cover data can be acquired at each sampling point, utilising a 0.5 x 0.5m video quadrat frame lowered to the seafloor from a vessel such as a boat or kayak (see Box 2.1, Chapter 2). A semi-quantitative density scale for SAV can be used to calculate a SAV density rating for the site (see Table 4.2). An average score across sets of images can then be calculated. For example, scores of (1+1+2)/3= 1.3 would indicate that the bed lies between sparse and patchy coverage.

Alternative Method 2: Acoustic remote sensing technology.

Where resource and practitioner expertise is proficient, acoustic remote sensing approaches can be utilised to determine density, coverage and presence of SAV (see Box 2.2, Chapter 2). Acoustic techniques utilising a Sediment Imager Sonar and Biosonics DT-X splitbeam echosounder can yield SAV density and percent cover data in sites of high turbidity where in situ observations and photographic/video measurements are not feasible or are of insufficient quality. Further to this, acoustic methods also have the potential to simultaneously collect supplementary data, including detailed bathymetry and SAV canopy height, without being affected by the presence of algae.

Sampling Frequency: Sampling of saltmarsh and SAV should be performed annually at the end of the peak growing season, which is between August and September in much of Europe.

Performance Criteria: A trend of increasing mean plant density and mean percent cover, with an ultimate goal of having statistically greater mean plant density and mean percent cover than pre-construction conditions and at the control site, or a mean density and mean percent cover that is roughly equal to that of a natural reference site.

WATER QUALITY IMPROVEMENT

Native oyster can positively impact water quality. By filtering both organic and inorganic particles from the water and either consuming or depositing them on the seafloor, native oyster draw down suspended material from the water column and increase the water clarity. This process enriches the sediments with carbon and nutrients. It also brings oxygenated and deoxygenated surfaces in close proximity, which promotes the denitrification of bacteria that convert biologically active forms of nitrogen (nitrates and nitrites) back into inert nitrogen gas (denitrification).

Water quality is a highly important and relevant ecosystem service associated with restoration activity. In response to EU legislation, national governments have committed to achieve Good Environmental Status (GES) of the marine environment (Marine Strategy Framework Directive MSFD, 2008/56/EC). A reduction in seston, chlorophyll-*a*, and nutrients in the water column is therefore a powerful statement in favour of native oyster restoration activity within eutrophic coastal systems.

Monitoring for denitrification is a complex and challenging scientific endeavour, and applicable methods are still in development. Methods for monitoring denitrification are therefore not addressed here, but interested practitioners are encouraged to contact academic institutions if they are interested in pursuing monitoring of this ecosystem service. A comprehensive review of the potential of this ecosystem service is provided in Kellogg *et al.* (2014).

The following methods focus on monitoring reduced turbidity and increased light penetration.

Suggested for reporting (in order of priority):

- Turbidity (see Metric 26: Light Penetration)
- Chlorophyll-*a*
- Total N (nitrate and nitrite)
- Total phosphate
- Breakdown ions of nitrate, nitrite, phosphate
- Total ammonium

METRIC 26: LIGHT PENETRATION

Light intensity decreases exponentially with water depth (according to the Beer-Bouguer-Lambert Law, see Box 4.2) and depends on the amount of light absorption by dissolved substances and suspended particles, such as phytoplankton and particulate organic matter (termed seston). Filter-feeding by oysters reduces seston concentration and can therefore be beneficial to submerged aquatic vegetation (SAV) where light penetration is a limiting factor to photosynthesis and growth. Changes in light penetration can be measured by measuring the depth of light penetration (e.g. via secchi disk or transparency tube) or by measuring light penetration changes with depth (e.g. with in situ or handheld light sensors) (see Figure 4.8). If a turbidity tube or handheld turbidity meter is available, the turbidity (or cloudiness of the water) can be measured as nephelometric turbidity units (NTU).

For all the methods detailed below, samples or readings should be taken at the centre of the native oyster habitat or control site, then both immediately up-current and down-current (e.g. 50m) – as depicted in Figure 4.9. A minimum of three light readings should be taken at each location. Readings must be taken at a consistent state of tide. Post slack high tide at the beginning of the ebb tide is recommended. The position, time, and tidal state should be recorded for all measurements.

Required Units: Units are method specific. **For In situ light sensors:** lux (lumen/m^2) is the unit of light intensity (or illuminance). **For secchi disk:** depth of disappearance (cm). **For transparency or turbidity tube:** depth of disappearance (cm) or NTU. **For hand held instruments:** lux (lumen/m^2) or NTU.

Primary Method: In situ light sensors.

Light sensors can be deployed in the intertidal or subtidal. Relatively inexpensive waterproof loggers can be used that measure temperature and light (e.g. UA-002-64 HOBO Waterproof Temperature/Light Pendant Data Logger). The light sensors must be cleaned and positioned horizontally and facing upwards. The sensor should be set to take continuous measurements every 5 seconds for an hour at the end of slack high tide (measuring from the start of the ebb tide).

BOX 4.2: VERTICAL EXTINCTION COEFFICIENT

The percentage of surface light absorbed or scattered within a vertical 1 metre column of water is called the Vertical Extinction Coefficient, a parameter symbolised by 'k' or Kd. To calculate K the ambient surface light intensity needs to be measured alongside two submerged sensors at known vertical heights apart. Coastal and estuarine water bodies are typically turbid with shallow light penetration and therefore have high K values.

Beer-Bouguer-Lambert Law states that:

$I(D) = I(0) * [e\text{-}Kd*D]$

where

I(D) = the intensity of light as a function of depth z

I(0) = the intensity of light at the surface (depth = 0 m)

k = the vertical extinction or attenuation coefficient = % surface light absorbed in 1m of vertical water column (units = 1/m)

D = depth (units = metres)

To calculate K, the difference in light intensity should be measured between the two submerged sensors over a known vertical distance to calculate the amount of light absorbed over 1m, then divided by the surface light value e.g. if the ambient surface light intensity is 250 Lux, and two submerged in situ sensors separated vertically by 20cm measure 62 and 114 Lux, K would =(114/62)*5/250 * 100 = 1.04.

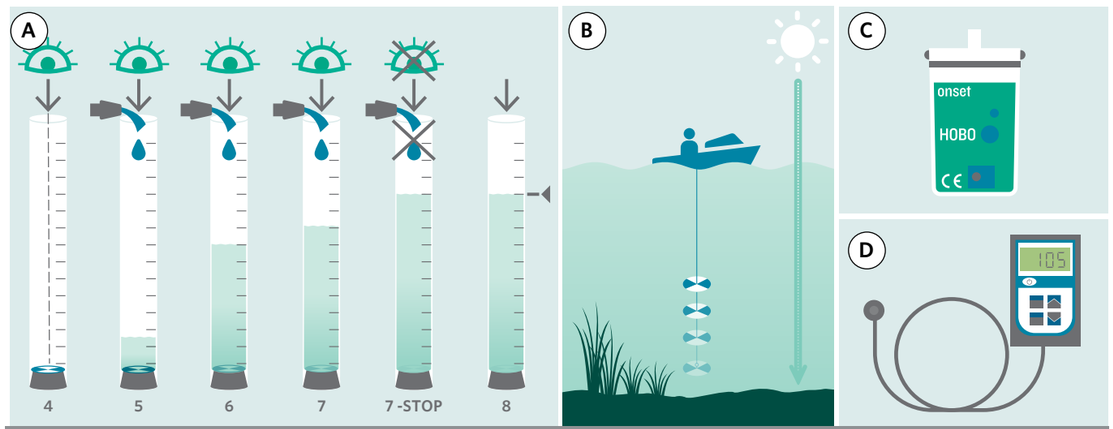


Figure 4.8: Measuring light penetration: A) Transparency tube; B) Secchi disk; C) In situ light sensor (HOBO logger); D) Handheld instrument.

At each location it is recommended to deploy:

- A calibration sensor to measure the ambient light above the surface. For intertidal sites this can be deployed at a height that will not be submerged at high tide. For subtidal sites, the ambient light sensor can be attached to the sampling vessel or a floating structure.
- A minimum of three 'habitat condition' sensors deployed 10cm above the native oyster or seabed habitat surface (or 10cm above the seabed in control or pre-deployment sites) positioned evenly across the middle of habitat.
- A 'K-coefficient' light sensor deployed at a known height above one of the seabed condition sensors (e.g. 20cm).

The sensors can be attached to poles of known height (intertidally) or deployed at a known depth attached to weighted and buoyed ropes if subtidal.

The calibration, habitat condition and K-coeffecient sensor lux readings can be used to calculate changes in light penetration using the methods outlined in Box 4.2.

Alternative Method 1: Secchi disk.

A 20 or 30cm diameter secchi disk (see Figure 4.8) attached to a weighted line with a graduated rope marked at 1cm intervals should be lowered until it is no longer visible from the surface. The length between the secchi disk and water surface (depth of disappearance) should be recorded. Readings should be taken in triplicate just above the native oyster habitat or control and immediately up- and down-current of the site, as described above. Slack tide high tide is recommended.

Alternative Method 2: Transparency or turbidity tube.

A transparency tube is a graduated cylindrical tube with secchi disk markings at its base (see Figure 4.8). Using a bucket, a sample of water should be collected above the surface of the native oyster habitat or seabed without disturbing the benthos. The sample should be homogenised by mixing it and pouring it into the transparency tube until the black and white base is no longer visible. The height on the tube at which the secchi disk is no longer visible should be recorded (depth of disappearance). Readings should be taken in triplicate using water from just above the native oyster habitat or seabed surface and immediately up- and down-current of the site, as described above. A turbidity tube is very similar but rather than cm graduations, it is marked in NTU.

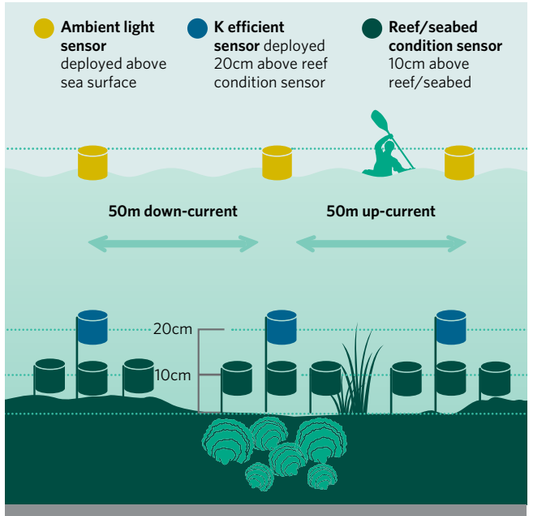


Figure 4.9: Locations and replicates of light penetration readings recommended on the reef and 50m up-current and down-current.

Alternative Method 3: Handheld instruments.

Reference readings of ambient air irradiance should be recorded, and this should be done at regular depth intervals if a light extinction curve is required. Otherwise, readings should be taken in triplicate just above the native oyster habitat surface and immediately up- and down-current of the site, as described above and depicted in Figure 4.9.

Sampling Frequency: Four times a year; once in each season.

Performance Criteria: A trend of increased light penetration on the restored oyster reef and immediately down-current.

METRIC 27: SESTON AND/OR CHLOROPHYLL-*A* CONCENTRATIONS

Concentrations of seston and chlorophyll-*a* are frequently used metrics to determine water quality, as well as selected nutrient concentrations (e.g. nitrate or phosphate).

Monitoring the concentration of seston and/or chlorophyll-*a* is achieved with water sampling at locations up-current and down-current of the native oyster habitat, or using in situ fluorometry methods.

Required Units: Total particulates (mg/L); organic content (%); chlorophyll-*a* (mg/L).

Primary Methods:

For water sampling and flow rates

For ex situ analysis, it is recommended that replicate (n = 3) water samples be taken at multiple (a minimum of three) locations within the restoration site (up-current, down-current and midpoint of the native oyster bed), pre- and post-construction at the restored bed, as well as at a control site or natural reference reef, at 5-10 minute intervals during both slack ebb and slack flood tide where possible. Water samples should be taken at mid-depth between the water surface and the native oyster bed surface. Each sample set should be accompanied by a measurement of the water depth (m) at the midpoint of the bed, the distance (m) between sample sites, and by water flow rates. Sampling bottles should be opened and closed at the given depth to avoid surface contamination. The use of Niskin bottles is recommended for subtidal oyster beds or in water with low visibility.

Flow rates may be determined by measuring the amount of time it takes for a drifter or float to cover a known distance and should be reported on a cm/s basis (see Grizzle *et al.* (2006) and Grizzle *et al.* (2008), for further information on measuring water flow in intertidal and shallow subtidal habitats). Flow rates may also be determined using an instrument such as an electromagnetic flow meter or an acoustic Doppler current velocimeter (Grizzle *et al.* 2018).

For seston concentration

At least ten sets of triplicate 1L water samples should be taken at each location. Each water sample should be filtered through 1µm GF/F or GF/C glass fibre filters, dried (at 40°C) to a constant weight (Nelson *et al.* 2004), and then cooled and weighed to determine total particulate matter (mg/L) (note that the dried filter weight should be subtracted from the total). To acquire organic content, material must be ashed at 450°C, cooled and weighed (Nelson *et al.* 2004). Dividing the total particulate value by the organic content value will give the percentage of organic content.

For chlorophyll-*a*

Fluorometry and spectroscopy are common methods for determining chlorophyll-*a* concentration in situ or in the laboratory. At least six sets of triplicate 0.05L water samples should be measured per location. Ideally, chlorophyll-*a* should be measured using the fluorometric method (see Parsons *et al.* 1984 and Welschmeyer 1994) and reported in µg/L.

Throughout the monitoring period, seston and/or chlorophyll-*a* concentrations ought to be statistically lower on the bed location, and immediately down-stream of the bed compared to concentrations immediately up-stream and at control locations.

For nutrients

A minimum of triplicate 1L water samples should be taken at each location across the restored native oyster habitat. It is recommended that nutrient (nitrate, nitrite, phosphate, ammonium) concentrations are analysed using techniques described by Parsons *et al.* (1984), or acquired by sending samples to a water quality laboratory (often affiliated with a government agency or an academic institution) for processing.

Sampling Frequency: Measuring water quality prior to restoration efforts and then post restoration efforts would determine whether restoration is positively impacting the area. Long-term monitoring with frequent sampling would be most beneficial, and would inform a project whether concentrations of nutrients or seston can explain an 'event', such as sudden mortality. Where budget and effort allow, weekly sampling is preferred. Quarterly sampling would discern seasonal differences.

Performance Criteria: A trend of decreasing total particulates, organic content and/or chlorophyll-*a* values is expected. The overall goal is to quantify statistically lower values at the restored habitat, compared to pre-construction conditions and to the control site, or ~equal to the natural reference site if available.

SOCIO-ECONOMIC BENEFITS OF NATIVE OYSTER RESTORATION

The ability to measure social and economic aspects of a project is essential to a holistic assessment of restoration project success. Marine restoration is a new and fast developing field. To ensure future support, it is important to provide evidence that restoration can deliver on the promised social, environmental and economic benefits.

Similarly, the social facets of restoration (e.g. engagement, stewardship and capacity building) are fundamental in building environmental optimism and shifting community focus away from ecosystem decline towards conservation, restoration and recovery. Finally, the economic aspects of restoration are central to assessing restoration feasibility and the long-term sustainability of restoration programmes.

METRIC 28: SOCIAL BENEFITS

The success and longevity of a project can often rely on local buy-in and support, be that from councils and regulators, local businesses, local residents or others. The amount or change in stewardship of native oyster habitats and the wider environment is therefore a key attribute to measure in restoration projects. Stewardship can be demonstrated through opportunities for local communities to be involved in restoration activities and to protect and care for shellfish reefs and the environment, and by illustrating that individuals and groups are motivated to cooperate and take part in the opportunities provided by restoration. Therefore, data on the number of community programmes, number of volunteers, attendance at events and metrics on media engagement should be collected by project managers through project tracking.

Required Units: Total number of project events; mean number of people/event; number of partner organisations; number of volunteers; total volunteer time, hours.

Primary Methods:

Number of project events: Total number of restoration project events; these can be categorised as public information, restoration activities (e.g. shell cleaning, shellfish deployment, construction), citizen science, fundraising, stewardship (e.g. friends of, land/coast-care) or technical advisory groups. This is a measure of the opportunities provided/developed by a project or programme.

Attendance at project events: The total number of people attending project events. This is a measure of active engagement by the local community in different aspects of the restoration project.

Community and partner organisations engaged: Demonstrates that the restoration project has built local capacity and engaged community and partner groups

Total number of volunteers: The number of volunteers contributing to different aspects of the project, i.e. citizen science or restoration activities. This can be extracted from the data collected for 'Attendance at project events'.

Volunteer hours: Tracks the number of hours committed by volunteers. A measure of volunteer effort on different project activities. Volunteer hours can be used to understand the value of volunteer 'labour', i.e. if the project paid appropriately skilled workers to undertake equivalent work. This is calculated by the median wage and an additional 15% for the 'employer contribution', payroll and administration costs converted to an hourly rate. The value of volunteer labour can then be used to establish the total value of volunteers leveraged by the project or the in-kind benefit.

Media engagement: This is a measure of how actively the wider community engages with a restoration project/ programme through traditional and social media. A reporting window should be decided on and engagement tracked, either manually by counting traditional media stories and manually tracking native analytics tools from social media platforms (e.g. Twitter analytics), by using free or paid apps (e.g. Khoros, Hootsuite, Google alerts, Mention, Newsmeter) to automate tracking of social and traditional media engagement.

Sampling Frequency: Project managers should monitor on an ongoing basis, using an event tracking spreadsheet and incorporating monitoring into standard monthly or reporting-period tracking workflows.

Performance Criteria: A trend of increasing engagement throughout the project timespan.

METRIC 29: ECONOMIC BENEFITS

With many projects reliant on a variety of funding streams with a range of agendas, the ability to express the impact that financing native oyster restoration can have on local economies and coastal populations, as well as ecologically, is extremely valuable. Many funding bodies view positively applications that have taken this kind of impact into consideration, with some even requesting that it be incorporated.

Required Units: Number of full time jobs; cost of restoration project (£ or €); total economic value (£ or €).

Primary Methods:

Total number of full-time jobs across the project: (local + national + International). Jobs across projects should be summarised per activity.

Estimated jobs per project can be standardised to a jobs-per-million £ or € invested. This metric is a standard reporting tool that is used when discussing the cost effectiveness of government spending. This jobs-per-million estimate enables comparison across projects with varying levels of expenditure (Edwards *et al.* 2013). In Australia, native oyster restoration has been shown to provide substantial numbers of jobs for the amount invested (see Figure 4.10).

Through progress reporting, project managers can capture the total number of people and hours that are employed directly or indirectly (in-kind) on the project across project activities. Also of interest, are the industry that the workers provide skills and knowledge from and also the occupation that the worker undertakes. This information enables the restoration project to demonstrate the industries that benefit from native oyster restoration and the occupations that support restoration activities.

Costs of restoration projects: Costs should be summarised across projects per activity and expense type.

Tracking of the financial costs or expenditure for restoration projects rarely happens and few projects and programmes systematically seek to record costs/expenses associated with restoration activities. Project expenditure is important to both private and public sector schemes. In the private sector, financial viability and profitability are important to the success of a project and motivate investors. For the public sector, assessments of financial cost are important when judging the level of resources and funding required to deliver a project. Restoration expenditures by industry are also important parameters for Input-Output (I/O) models that can be used to understand the economic impact of expenditure on investments such as habitat restoration.

I/O models are a standard approach to calculating employment impacts from government infrastructure investments (e.g. road and rail improvements and construction) and enable comparison to blue infrastructure initiatives, such as shellfish restoration. The benefit of I/O models over direct job estimates is that the models estimate direct, indirect and induced effects of spending rather than just direct effects.

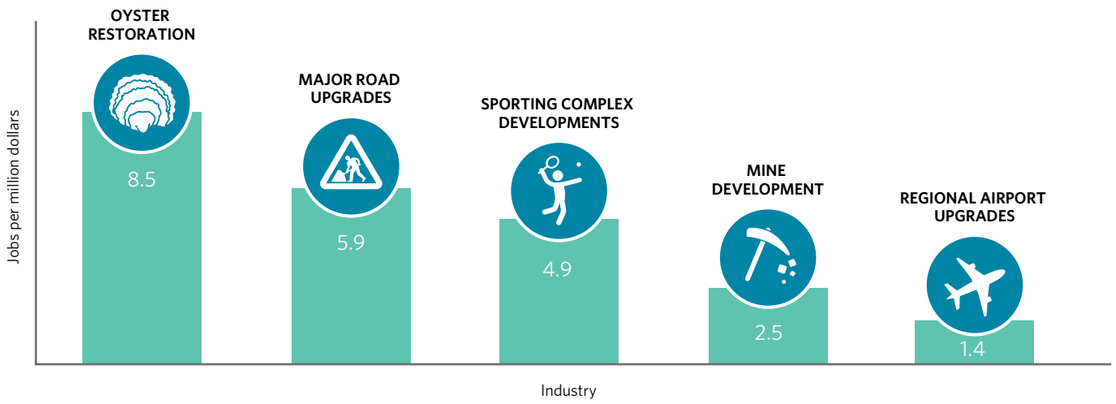


Figure 4.10: Economic modelling from South Australia showing the modelled full-time-equivalent job creation per million dollars invested in Australian native flat oyster restoration and other infrastructure projects, such as major road upgrades, sporting complex developments, mine development or regional airport upgrades. (Graph modified from Simon Reeves, The Nature Conservancy).

Costs are likely to vary greatly depending on the type of restoration technique used, site accessibility, length of monitoring and the development status of a country. Therefore, it is important to systematically track financial costs and expenses to enable better decisions to be made in terms of allocating resources to restoration and assessing the long-term sustainability of projects and programmes. Better systematic accounting for costs will also enable cost-benefit analyses to be carried out more frequently, and therefore provide opportunities to optimise decisions when allocating limited resources.

Costs/expenses should be tracked regularly throughout the project timeline. Many funders require financial audits as a final project deliverable, so regular accounting throughout the project timeline is essential. A project should decide on a reporting period and project managers should collate project expenditure from invoices, receipts and organisational expense management systems, i.e. Concur, for a reporting window. Costs/expenses should be captured across capital and operational activities.

Capital costs/expenses (CAPEX) are costs to implement a restoration initiative.

Operational costs/expenses (OPEX) are shorter-term costs to continue a restoration initiative.

Capital and operational cost categories are listed in Table 4.3.

Sampling Frequency: Project managers should monitor the jobs and costs on an ongoing basis, using an event tracking spreadsheet and incorporating it into the standard monthly or reporting-period project-tracking workflow , as collecting this data at the end of a project is challenging.

Table 4.3: Examples of capital costs/expenses (CAPEX) and operational costs/expenses (OPEX). (Modified from Spurgeon, 1999).

CAPEX	OPEX	Cost/expense sub-category
Feasibility	Management (to control and enhance the development of the site)	Administration (e.g. stationary, Internet)
Construction	Monitoring, evaluation and reporting	Equipment (e.g. boats, excavators, barges)
Site surveys	Maintenance (to maintain the site, e.g. reseeding, fixing damage)	Materials (e.g. rock, paper, shellfish)
Objective setting	Compliance (e.g. legal fees, permits i.e. MACA consent, development approval, etc.)	Labour (wages)
Design	Marketing and communications	Expenses (e.g. food, accommodation)
Tendering	Government relations	
Permitting	Finance/accounting	
Site preparation	Community/stakeholder engagement	
Stock (broodstock, seedstock)		
Transport (e.g. of materials and stock)		

Benefit valuation:

Native oyster habitats provide a vast array of benefits to humans in the form of goods and services (see Figure 4.11). Since few of the goods and services are traded in the market-place, they often do not have a monetary value. However, they can provide considerable socio-economic value, particularly when used sustainably.

The full range of benefits and value is often termed the 'total economic value'. Total economic value includes values based on direct and indirect use and non-use values as outlined in Figure 4.11. Many of the valuation methods, particularly for non-use and indirect use values, are complex and time consuming and are typically not applied for routine monitoring. For an overview of valuation techniques that go some way to capturing these non-use values, such as hedonic pricing, travel costs and contingent pricing, see Spurgeon (1999). While not directly assessing economic value, monitoring the metrics outlined in this handbook in a systematic manner will provide supporting data to enable economists to undertake valuation of the benefits derived from native oyster ecosystems.

Performance Criteria: Trend of the value arising from the restoration project being greater than the investment or cost of the project.

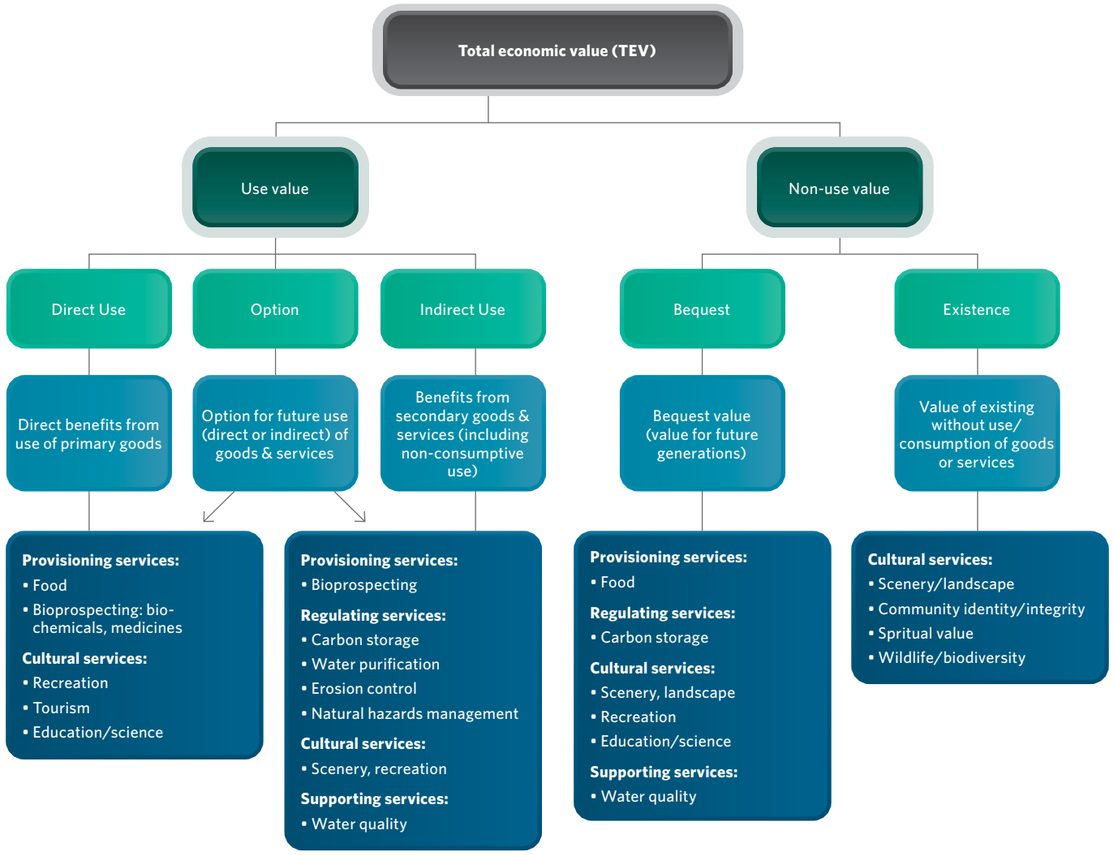


Figure 4.11. Application of a total economic value framework to ecosystem services (Figure modified from TEEB 2011).

Dutch Voordelta mixed oyster reef. (Photo: Floor Driessen, Bureau Waardenburg Ecology & Landscape).

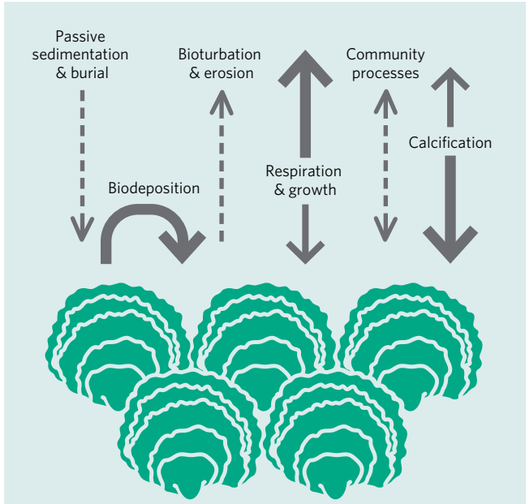


Figure 4.12: Conceptual carbon budget of *Ostrea edulis* (from Lee *et al.* 2020). Carbon deposition contributes to the carbon stock and is indicated by downward arrows. Once carbon release (indicated by upward arrows) is subtracted from the carbon stock accumulated over time, potential carbon sink value can be calculated. Arrow size gives an indication of the relative size of carbon flow. Biodeposition, respiration and growth, and calcification are the dominant processes considered here.

BLUE CARBON

While potential blue carbon captured by marine ecosystems is a widely acknowledged ecosystem service from saltmarshes, seagrasses and mangroves, the importance of other carbon stocks, such as fjordic seabed sediments, bivalve habitats and other biogenic reefs, is only just gaining recognition. As such, the monitoring of blue carbon within restoration projects can contribute to a local and global understanding of the potential importance of this service.

Native oyster contain organic carbon in soft tissues, inorganic carbon in shell material and cause deposition of carbon in sediment, either actively as a result of defaecation or passively as a result of turbulence (see Figure 4.12). Once processes such as the production of respiratory CO_2 are accounted for, it is possible to determine whether this native oyster related carbon is released or stored and if the habitat provides an overall carbon sink that contributes to climate change mitigation. While the role of bivalve habitats as carbon sinks is the subject of ongoing research, there is considerable interest in blue carbon restorative 'business models' for native oyster habitats and the core metric to monitor for either stored carbon or carbon sink is the carbon stock.

Carbon stock: Sedimentary carbon in the deposit (organic and inorganic carbon) + shell carbon in the deposit.

Carbon sink (i.e. possible contribution to climate change mitigation): (sediment carbon + shell carbon) - (carbon produced from respiration and shell production) per year.

METRIC 30: CARBON STOCK

As the carbon stock associated with a restored native oyster habitat is bound up in the oysters themselves and in the surrounding sediments, it is best assessed by surveying the native oyster population, associated shell and the carbon content of the surrounding sediments. Sediment accumulations of up to 2-3m have been observed in other types of subtidal shellfish beds over at least 150 years (see Figure 4.13). Change is therefore likely to be measurable at the cm scale per year.

The composition of the sediment can be compared before and after restoration and/or between oyster and non-oyster habitats using sampling designs such as BACI (see Chapter 1). To measure carbon stock, habitat extent (see Metric 1, Chapter 2) and habitat thickness (see Figure 4.13) also need to be estimated.

Required Units: Grams (g) of carbon per m^2 along with a measurement of sediment depth (m). Explanatory/contextual data on oyster density (ind/m^2), depth of habitat (m), total area of habitat (m^2), average oyster size (cm) or biomass (g per m^2) should also be reported (see Metric 2 and Metric 4, Chapter 2).

Primary Method: Sediment cores.

See Metric 19 and Figure 4.1 for core sampling. Buried shell material can obstruct the penetration of the cores; therefore, for oyster habitats, sturdy cores with diameters of > 10cm are recommended. When preparing and analysing samples for carbon, cores must be capped before being returned to the laboratory.

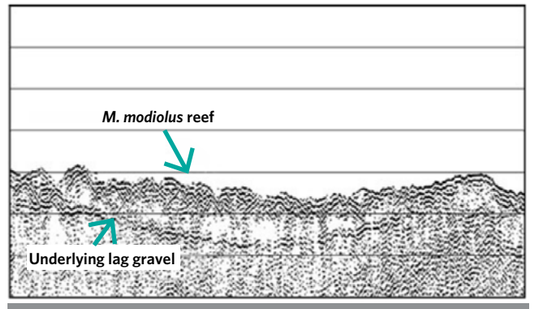


Figure 4.13: Carbon-rich sediments built-up over time beneath a subtidal shellfish reef (*Modiolus modiolus*). The image is a high resolution acoustic 'boomer' profile; like a slice-through of the seabed. The surface is visible, with underlying lag gravel and cobble. Horizontal scale lines are 1.5m apart, horizontal distance is ~220m (image from Lindenbaum *et al.* 2008).

In some biogenic habitats, carbon content has been found to vary throughout the thickness and across the extent of the habitat. It is therefore important that cores sample the total thickness of sediments that are affected by the overlying native oyster habitat, to ensure that variability is accounted for (Howard *et al.* 2014; Fodrie *et al.* 2017). Different sediment characteristics or variation in oyster density can indicate sub-habitat with spatial differences in carbon content that may require separate, 'stratified' sampling. Sediment samples should be analysed primarily for total carbon.

Calculating carbon stock

The volume of the core must be known and can be determined as follows: *Volume of sample (cm^3) = π x r2 x d (where r is the internal diameter of the core and d is the depth/length of the core sample).*

In the laboratory, the core sample total **wet weight** and subsample lengthways (half, quarter, etc.) should be recorded for processing. The sample should be dried in an oven at 60°C for 48 hours or longer until a constant weight is achieved and the total **dry weight** of the cooled sample recorded. The dry bulk density of each sample is the dry weight of total sediment divided by sediment volume:

Dry bulk density (g/cm^3) = mass of dry sediment (g) ÷ volume sampled (cm^3)

The next step is to sieve the sample to remove material > 2mm and separate shell fraction from small rocks/pebbles and **weigh total shell**. The < 2mm sediment fraction and > 2mm shell fraction should be homogenised with either a grinder or pestle and mortar. Shell material (> 2mm) first will need to be brushed or rinsed to remove sediment and epifauna.

Samples should be analysed for carbon content using a specialist elemental analyser, e.g. a 'CHN analyser' which simultaneously measures carbon, hydrogen and nitrogen. Procedures can be found in Howard *et al.* (2014). It is important that samples are analysed alongside a series of standards such as the organic compound acetanilide, benzoic acid as a nitrogen standard, and sediment standards, such as Sediment Standard B2178 (Elemental Microanalysis Ltd, Okehampton, UK) to check machine calibration and reproducibility.

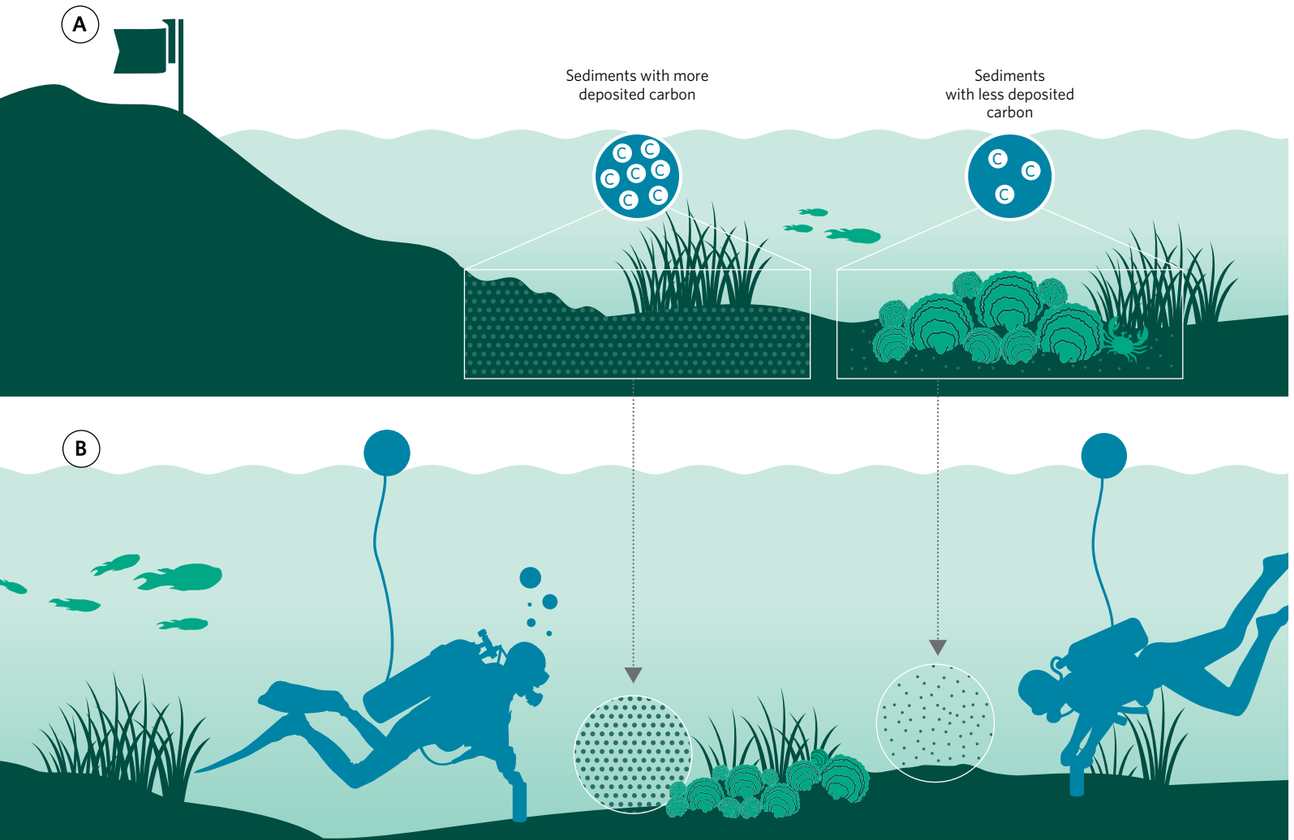


Figure 4.14: Monitoring the carbon store of an oyster restoration project. A) Conceptual sample stratification needs based on experience with other shellfish habitats. B) There are several established methods of core collection. However, sampling requiring stratification is likely to require in situ SCUBA sampling.

Other methods to calculate shell carbon content involve weight-loss by acidification with hydrochloric acid (see Howard *et al.* 2014). Shell carbon can also be estimated as 11.1% inorganic carbon and 0.5% organic carbon from the dry weight of shell (Fodrie *et al.* 2017).

At this point, the sum of the < 2mm sediment and > 2mm shell fractions should be used to calculate the percentage of total carbon in the sediment.

The sediment carbon density can then be determined for each core as follows:

Sediment carbon density (g/cm^3) = dry bulk density (g/cm^3) x (% C ÷ 100)

In order to derive carbon stock from the determined sediment carbon density, the volume of sediment associated with the area of restored native oyster habitat must be determined. The area and volume of restored native oyster habitat can be determined using sub-bottom profiling, side-scan sonar or 3D modelling from photogrammetry surveys (see Box 2.2, Chapter 2). For intertidal surveys, habitat perimeter may be determined using a handheld GPS and walking the habitat boundary at low tide, or through the use of drones to capture images for photogrammetry (see Metric 1, Chapter 2). These images should subsequently be processed using GIS software for aerial and volumetric measurements and photo modelling software such as Agisoft Premier Pro or Metashape.

The average core carbon content determined from the sampled sediment cores should be scaled to the area of native oyster habitat restored by multiplying the average carbon content of the sample by the volume of sediment in the restoration project. This number should then be converted to g of carbon per m^2 of the restoration site, to give the mean carbon stock of the site.

Sampling Frequency: Pre-restoration and then at 5 year intervals. In the meantime, it may be necessary to forecast the growth of carbon stocks by site-specific experimentation.

METRIC 31: CARBON SINK

Quantifying net carbon accumulation over time in native oyster habitats, and therefore its status as a sink, will be important for a project if climate change mitigation claims are to be supported. This has not yet been demonstrated for the native oyster. Estimation of carbon sink rates requires that the production of CO_2 in respiration and shell formation is accounted for, as well as the carbon stock.

Like any animal, native oyster produce respiratory CO_2 as they live and grow, and also as a result of the calcification process of their shells. The ratio of released CO_2 to deposited mineralised carbonate in shells is generally 0.6:1 in seawater. The overall metabolic production of CO_2 over a year is needed in calculations of carbon sink value.

Primary Method: Measuring carbon produced.

Changes in carbon dissolved in water can be monitored to measure carbon production rates. Generally, this is to determine the rate at which carbon is released during metabolic shell production processes.

Carbon production can be calculated using incubation studies using the methods from Tagliarolo *et al.* (2012), briefly described here.

Incubation units (~1L) are covered to prevent light penetration and photosynthesis during deployment at site. Seawater samples (250mL) are taken pre-incubation, making sure bottles are rinsed twice prior to sampling. Units containing oysters and no oysters (background respiration) are incubated for a known period of time at the restoration site or under similar environmental conditions. After 24 hours incubation, further water samples are taken and spiked with saturated $HgCl_2$ solution to a dilution of 0.04% for preservation (Dickson *et al.* 2007). Analysis of water samples for dissolved inorganic carbon can be achieved using a carbon dioxide coulometer or a dissolved inorganic carbon (DIC) coulometer: measurement of DIC can then be used to calculate respiration rate. To do this, the water sample is acidified with 2N H_2SO_4, off-gassing CO_2 which is carried to the coulometer cell. The gas reacts in the coulometer cell with an indicator solution that changes colour according to the amount of inorganic carbon present, reporting the value in micrograms. Samples are run alongside a set series of blanks and standards to check instrument calibration. Dissolved CO_2 reference material is provided by the Certified Reference Materials Laboratory, Scripps Institution of Oceanography, San Diego.

DIC can alternatively be calculated using pH, total alkalinity, temperature, salinity, phosphate and silicate concentrations. Pierrot *et al.* (2006) provide a useful Excel program that provides DIC output values.

Utilising DIC to calculate carbon production rate

DIC values are used to calculate carbon production rates that include respiration, but also a small amount from the chemical production of shell (see Tagliarolo *et al.* 2012):

carbon production = $(\Delta DIC \times v) \div (\Delta t \times 1{,}000) - G$

v is the net chamber volume (L), Δt is the incubation time (h), ΔDIC is the change in the total inorganic carbon concentration (mmol DIC L^{-1}), background respiration values from "blank" chambers without oysters should be subtracted prior to reporting this value and G is the net $CaCO_3$ flux measured. The potential carbon sink value can be estimated by subtracting the carbon production rate over the monitoring period from the carbon stock (Metric 30, Chapter 4) measured over the same monitoring period.

Measuring $CaCO_3$ flux – calcification rate changes in seawater total alkalinity can be used to determine net calcification rate (μmol $CaCO_3$ h^{-1}) using the alkalinity anomaly technique (Tagliarolo *et al.* 2012). Change in alkalinity is determined through Gran titration. $CaCO_3$ flux must be subtracted from measured change in DIC in order to calculate respiration rate.

$G = - ((\Delta TA \times v) \div (2 \times \Delta t))$

Performance Criteria: A trend of neutral or positive change in carbon stores compared to the control or, pre-restoration habitat.

CHAPTER 5
EVALUATION AND REPORTING

CHAPTER AUTHORS
Celine Gamble, Azra Glover, Joanne Preston, Philine zu Ermgassen.

INTRODUCTION

The evaluation and reporting phase of project monitoring is an opportunity to analyse and interpret the ecological impact and performance of restoration project activities. It is important to factor in time for analysis, evaluation and reporting of the data collected on the selected monitoring metrics. The evaluation of data from the metrics measured can inform decision-making, enable adaptive management and provide information on drivers of success and failure for other restoration projects. Evaluation and reporting are also essential for demonstrating to funders and others whether a project has reached the goals and targets set out at the beginning.

DATA COLLECTION AND MANAGEMENT

It is important that the monitoring metric data is collected and managed effectively to ensure that it is accurate and statistically valid. Once the relevant monitoring metrics have been selected, it is good practice to create clear and concise data collection sheets, tailored to fit each metric (see Table 5.1 for example data collection sheets). When collecting data in the field, consider using waterproof paper to record data to avoid losing important information. It is important to regularly upload, save and back up project data to reduce the potential of losing it. Online platforms such as OneDrive can also be used as a form of reliable storage.

The British Ecological Society's *Guide to Data Management in Ecology and Evolution* provides an outline of global best practices in data management. The principles outlined include standardised and consistent procedures to collect, process, check, validate and verify data.

DATA ANALYSIS AND EVALUATION

The restoration practitioner should establish a statistical design for data analysis as early as possible in the monitoring process. It should be noted that not all metrics lend themselves to statistical analysis, however, where applicable, the data should be compiled and summarised to find the mean, standard deviation, standard error, coefficient of variation and sample size (n). Metrics can be visualised by plotting graphs to observe how the metric has changed through time.

Data analysis can be carried out using Excel or statistical analysis programs such as R or Statistical Package for the Social Sciences (SPSS). Selecting and applying the most appropriate statistics can be challenging. It is recommended that practitioners without experience in analysing statistics seek academic partners to ensure the analyses are correctly applied. Such partnerships should start at the project planning stage to ensure that the data and sample sizes collected are suitable for the statistical analysis planned.

Evaluation is defined by the US National Research Council as a set of approaches and techniques used to make judgements about the effectiveness or quality of a programme or treatment; to improve its effectiveness; and to inform decisions about its design, development, and implementation (National Research Council 2010). To evaluate the results of the monitoring metrics is to evaluate the ecological impact and performance of the restoration activities in meeting their goals.

Ecological data is naturally noisy. It can therefore be challenging to determine whether the changes observed on the restoration site are significantly different from those in the control. Even if the mean size or density of oysters is greater on the restoration site than in the control, this may be due to chance as opposed to a genuine difference. Significance can be statistically determined. If two datasets are deemed to be statistically significantly different from one another, there is only a low probability that there is no difference between them. The use of statistics is therefore recommended.

REPORTING

The results of the statistical analysis and evaluation can be used to report on project progress with project team members and stakeholders. Project reporting products could take the form of annual progress summaries, scientific papers, technical reports or funder reports outlining the progress of the project with regard to the targets, goals and performance indicators. Other reporting mechanisms could include project outreach materials, such as infographics on key project progress metrics.

Furthermore, to contribute to the collective knowledge and progression of native oyster restoration across Europe, the Native Oyster Network - UK & Ireland and the Native Oyster Restoration Alliance welcome receiving information and monitoring data from oyster restoration projects.

CASE STUDY: BRINGING THE OYSTER REEFS BACK TO OYSTER HARBOUR PROJECT, ALBANY, WESTERN AUSTRALIA.

The detail and complexity of the evaluation and reporting can vary depending on the size, complexity and capacity of the oyster restoration project. One example of implementing a comprehensive monitoring, evaluation and reporting (MER) plan is the Bringing the Oyster Reefs Back to Oyster Harbour project in Albany, Western Australia. The project follows the MER principles of the Society for Ecological Restoration, the Open Standards for the Practice of Conservation and best practices for shellfish reef restoration. See Figure 5.1 for an overview of the project's monitoring, evaluation and reporting workflow.

The project collects monitoring data on key indicators, such as shellfish metrics (Metrics 1–6, 8, 14, 20–22, 28 and 29), baited remote underwater videos (BRUVs), and socio-economic indicators, that are aligned with the project's goals and objectives. The raw data is entered, processed, collated, and undergoes quality assurance and quality control (QAQC). For each performance indicator, the data is compiled and summarised, following which a score is calculated in reference to a benchmark and then standardised in the form of percentage. A trend for each indicator is also assessed by fitting the raw data for each reef as a linear model. If the metric is not significantly different to zero, the slope will then automatically be assigned to the 'Stable' category. If the slope is significant, the value of the slope coefficient is then placed into one of six categories.

The evaluation products are then used to produce maps, score and trend tables, score wheels and infographics, which aid communication with stakeholders in the form of progress summaries, technical reports, and annual project reports (see Figure 5.1).

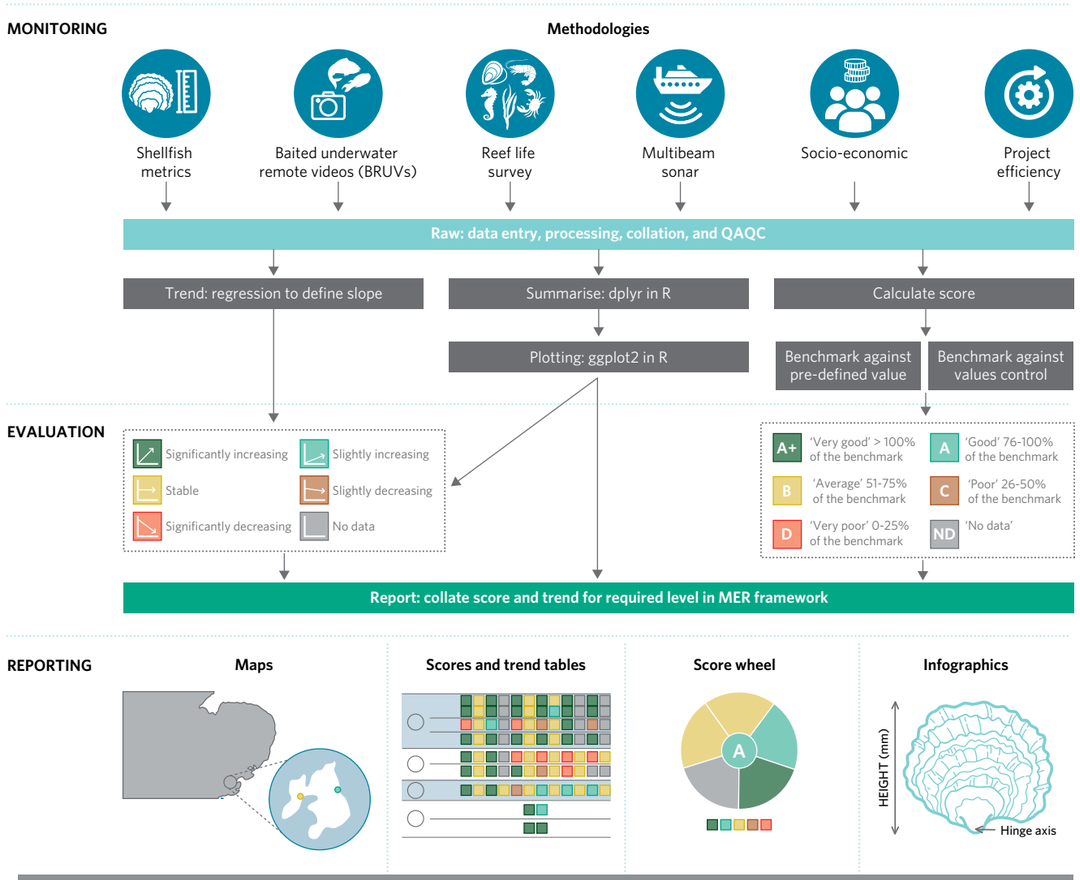


Figure 5.1: Restoration targets and MER workflow, Oyster Harbour Monitoring, Evaluation and Reporting plan 2018-2020 (Nedosyko and Gillies, 2019).

Table 5.1: Examples of data collection sheets for monitoring metrics

Oyster growth rate and size frequency						Record if measuring oyster condition					
Oyster	Location	Shell height (mm)	Shell length (mm)	Shell width (mm)	Weight (whole wet) (g)	Foil weight (g)	Foil + flesh (g)	Dry tissue weight (g)	Dry shell weight (g)	Condition Index	Gill/heart taken
1											
...											

Table 5.2: General project information collection sheet

Project name:		Site name:	
Contact name:		Contact email:	
Date:		Project partners:	
Project location:	Lat:	Long:	
Project status:	☐ Pre-planning phase ☐ Restoration in progress ☐ Complete ☐ Other:		
Project start date:		Project end date (or estimation):	Project duration:
Project budget:		No. of people engaged (or estimation):	
Project footprint (m^2):		Oyster reef area (m^2):	
Reef/bed height mean (cm):		Oyster density (individuals/m^2):	

REFERENCES

B

Baggett, L.P., Powers, S.P., Brumbaugh, R., Coen, L.D., DeAngelis, B., Greene, J. *et al.* (2014). *Oyster Habitat Restoration Monitoring and Assessment Handbook*. Arlington, VA: The Nature Conservancy. http://www.oyster-restoration.org/wp-content/uploads/2014/01/Oyster-Habitat-Restoration-Monitoring-and-Assessment-Handbook.pdf.

Bundesamt für Seeschiffahrt und Hydrographie (2016). *Guideline for Seafloor Mapping in German Marine Waters Using High-Resolution Sonars*. BSH No. 7201. Hamburg. https://www.bsh.de/download/Guideline-for-Seafloor-Mapping.pdf.

C

Chapman, E.C.N., Rodriguez-Perez, A., Hugh-Jones, T., Bromley, C., James, M.A., Diele, K. *et al.* (2021). Optimising recruitment in habitat creation for the native European oyster (*Ostrea edulis*): Implications of temporal and spatial variability in larval abundance. *Marine Pollution Bulletin* 170, 112579. https://doi.org/10.1016/j.marpolbul.2021.112579.

D

da Silva, P., Fuentes, J. and Villalba, A., (2009). Differences in gametogenic cycle among strains of the European flat oyster *Ostrea edulis* and relationship between gametogenesis and bonamiosis. *Aquaculture,* 287(3-4), pp.253-265.

Dickson, A.G., Sabine, C.L. and Christian, J.R. (eds.) (2007). *Guide to Best Practices for Ocean CO_2 Measurements.* PICES Special Publication 3. Sidney, BC: North Pacific Marine Science Organization. https://cdiac.ess-dive.lbl.gov/ftp/oceans/Handbook_2007/Guide_all_in_one.pdf.

E

Ebner, B.C. and Morgan, D.L. (2013). Using remote underwater video to estimate freshwater fish species richness. *Journal of Fish Biology* 82(5), 1592-1612. https://doi.org/10.1111/jfb.12096.

Edwards, P.E.T., Sutton-Grier, A.E. and Coyle, G.E. (2013). Investing in nature: Restoring coastal habitat blue infrastructure and green job creation. *Marine Policy* 38, 65-71. https://doi.org/10.1016/j.marpol.2012.05.020.

F

Fodrie, F.J., Rodriguez, A.B., Gittman, R.K., Grabowski, J.H., Lindquist, N.L., Peterson, C.H. *et al.* (2017). Oyster reefs as carbon sources and sinks. *Proceedings of the Royal Society B: Biological Sciences* 284(1859), 20170891. https://doi.org/10.1098/rspb.2017.0891.

G

Grizzle, R.E., Greene, J.K. and Coen, L.D. (2008). Seston removal by natural and constructed intertidal eastern oyster (*Crassostrea virginica*) reefs: A comparison with previous laboratory studies, and the value of in situ methods. *Estuaries and Coasts* 31(6), 1208-1220. https://doi.org/10.1007/s12237-008-9098-8.

Grizzle, R.E., Greene, J.K., Luckenbach, M.W. and Coen, L.D. (2006). A new *in situ* method for measuring seston uptake by suspension-feeding bivalve molluscs. *Journal of Shellfish Research* 25(2), 643-649. https://doi.org/10.2983/0730-8000(2006)25[643:ANISMF]2.0.CO;2.

Grizzle, R.E., Rasmussen, A., Martignette, A.J., Ward, K. and Coen, L.D. (2018). Mapping seston depletion over an intertidal eastern oyster (*Crassostrea virginica*) reef: Implications for restoration of multiple habitats. *Estuarine, Coastal and Shelf Science* 212, 265-272. https://doi.org/10.1016/j.ecss.2018.07.013.

H

Helmer, L., Farrell, P., Hendy, I., Harding, S., Robertson, M. and Preston, J. (2019). Active management is required to turn the tide for depleted *Ostrea edulis* stocks from the effects of overfishing, disease and invasive species. PeerJ 7, 6431. https://doi.org/10.7717/peerj.643.

Howard, J., Hoyt, S., Isensee, K., Pidgeon, E. and Telszewski, M. (eds.) (2014). *Coastal Blue Carbon: Methods for Assessing Carbon Stocks and Emissions Factors in Mangroves, Tidal Salt Marshes, and Seagrass Meadows.* Arlington, VA: Conservation International, Intergovernmental Oceanographic Commission of UNESCO, International Union for Conservation of Nature.

K

Kellogg, M.L., Smyth, A.R., Luckenbach, M.W., Carmichael, R.H., Brown, B.L., Cornwell, J.C. *et al.* (2014). Use of oysters to mitigate eutrophication in coastal waters. *Estuarine, Coastal and Shelf Science* 151, 156-168. https://doi.org/10.1016/j.ecss.2014.09.025.

Kent, F.E.A., Last, K.S., Harries, D.B. and Sanderson, W.G. (2017). In situ biodeposition measurements on a *Modiolus modiolus* (horse mussel) reef provide insights into ecosystem services. *Estuarine, Coastal and Shelf Science* 184, 151-157. https://doi.org/10.1016/j.ecss.2016.11.014.

L

Lee, H.Z.L., Davies, I.M., Baxter, J.M., Diele, K. and Sanderson, W.G. (2020). Missing the full story: First estimates of carbon deposition rates for the European flat oyster, *Ostrea edulis*. *Aquatic Conservation: Marine and Freshwater Ecosystems* 30(11), 2076-2086. https://doi.org/10.1002/aqc.3402.

Lefebvre, A., Thompson, C.E.L., Collins, K.J. and Amos, C. (2009). Use of a high-resolution profiling sonar and a towed video camera to map a *Zostera marina* bed, Solent, UK. *Estuarine, Coastal and Shelf Science* 82(2), 323-334. https://doi.org/10.1016/j.ecss.2009.01.027.

Lindenbaum, C., Bennell, J.D., Rees, E.I.S., McClean, D., Cook, W., Wheeler, A.J. *et al.* (2008). Small-scale variation within a *Modiolus modiolus* (Mollusca: Bivalvia) reef in the Irish Sea: I. Seabed mapping and reef morphology. *Journal of the Marine Biological Association of the United Kingdom* 88(1), 133-141. http://doi.org/10.1017/S0025315408000374.

M

Maathuis, M.A.M., Coolen, J.W.P., van der Have, T. and Kamermans, P. (2020). Factors determining the timing of swarming of European flat oyster (*Ostrea edulis L.*) larvae in the Dutch Delta area: Implications for flat oyster restoration. *Journal of Sea Research* 156, 101828. https://doi.org/10.1016/j.seares.2019.101828.

N

Nelson, K.A., Leonard, L.A., Posey, M.H., Alphin, T.D. and Mallin, M.A. (2004). Using transplanted oyster (*Crassostrea virginica*) beds to improve water quality in small tidal creeks: A pilot study. *Journal of Experimental Marine Biology and Ecology* 298(2), 347-368. https://doi.org/10.1016/S0022-0981(03)00367-8.

O

OSPAR Commission (2009). *Background Document for Ostrea edulis and Ostrea edulis Beds. Biodiversity* Series. London. https://www.ospar.org/documents?v=7183.

P

Parsons, T.R., Maita, Y. and Lalli, C.M. (1984). *A Manual of Chemical and Biological Methods for Seawater Analysis.* Oxford: Pergamon Press.

Pierrot, D., Lewis, E. and Wallace, D.W.R. (2006). MS Excel program developed for CO_2 system calculations. ORNL/CDIAC-105a. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, United States Department of Energy, Oak Ridge, TN.

Pouvreau, S., Cochet, H., Bargat, F., Petton, S., Le Roy, V., Guillet, T. *et al.* (2021a). Current Distribution of the Residual Flat Oysters Beds (*Ostrea Edulis*) Along the West Coast of France. SEANOE. Accessed 17 November 2021. https://doi.org/10.17882/79821.

Pouvreau, S., Cochet, H., Fabien, A., Arzul, I., Lapegue, S., Gachelin, S. *et al.* (2021b). *Inventaire, diagnostic écologique et restauration des principaux bancs d'huitres plates en Bretagne : le projet FOREVER. Rapport final. [Flat Oyster RecoVERy in France (FOREVER): The Final Report].* Ifremer. https://doi.org/10.13155/79506.

Preston J., Gamble, C., Debney, A., Helmer, L., Hancock, B. and zu Ermgassen, P.S.E. (eds.) (2020). *European Native Oyster Habitat Restoration Handbook*. London: The Zoological Society of London.

Przeslawski, R., Berents, P., Clark, M., Edgar, G., Frid, C., Hughes, L. *et al.* (2018).

Marine sampling field manual for grabs and box corers. In *Field Manuals for Marine Sampling to Monitor Australian Waters*. Przeslawski, R. and Foster, S. (eds.). Canberra: National Environmental Science Programme Marine Biodiversity Hub. Chapter 9. 172-195. https://www.nespmarine.edu.au/sites/default/files/_PUBLIC_/FieldManuals_NESPMarineHub_Chapter9_Grab_v1.pdf.

R

Reeves, S., Nedosyko, A. and Gillies, C. (2019). The Nature Conservancy Windara Reef Technical Report 2019. 10.13140/RG.2.2.31234.91841.

S

Sanderson, W.G., Hirst, N.E., Fariñas-Franco, J.M., Grieve, R.C., Mair, J.M., Porter, J. *et al.* (2014). *North Cava Island and Karlsruhe Horse Mussel Bed Assessment.* Scottish Natural Heritage.

Short, F.T., Coles, R.G. and Short, C.A. (2015). *SeagrassNet Manual for Scientific Monitoring of Seagrass Habitat.* Worldwide edition. Durham, NH: University of New Hampshire Publication.

Soniat, T.M., Colley, S.B., Cooper, N., Dahal, J. and Gallegos, J. (2013). *Sustainable Oyster Shellstock Training Handbook*. New Orleans, LA: University of New Orleans. https://data.oystersentinel.cs.uno.edu/resources/SoniatEtAl2013Handbook.pdf.

Spurgeon, J. (1999). The socio-economic costs and benefits of coastal habitat rehabilitation and creation. *Marine Pollution Bulletin* 37(8-12), 373-382. https://doi.org/10.1016/S0025-326X(99)00074-0.

T

Tagliarolo, M., Clavier, J., Chauvaud, L., Koken, M. and Grall, J. (2012). Metabolism in blue mussel: Intertidal and subtidal beds compared. *Aquatic biology* 17(2), 167-180. https://doi.org/10.3354/ab00464.

TEEB. (2011). The economics of ecosystems and biodiversity in national and international policy making. Earthscan, London and Washington, DC.

W

Welschmeyer, N.A. (1994). Fluorometric analysis of chlorophyll a in the presence of chlorophyll b and pheopigments. *Limnology and oceanography,* 39(8), 1985-1992.

Willis, T. J., Millar, R. B., & Babcock, R. C. (2000). Detection of spatial variability in relative density of fishes: comparison of visual census, angling, and baited underwater video. *Marine Ecology Progress Series,* 198, 249-260.

Z

zu Ermgassen, P.S.E., Gamble, C., Debney, A., Colsoul, B., Fabra, M., Sanderson, W. *et al.* (eds.) (2020). *European Guidelines on Biosecurity in Native Oyster Restoration*. London: The Zoological Society of London. https://nativeoysternetwork.org/wp-content/uploads/sites/27/2020/11/ZSL00161%20Biosecurity%20Handbook%20ONLINE.pdf.

zu Ermgassen, P.S.E., Hancock, B., DeAngelis, B., Greene, J., Schuster, E., Spalding, M. *et al.* (2016). *Setting Objectives for Oyster Habitat Restoration Using Ecosystem Services: A Manager's Guide*. Arlington, VA: The Nature Conservancy.

METRIC SUMMARY TABLE

Summary of the primary and alternative methods recommended for each metric. For more details, see Chapters 2, 3 and 4.

UNIVERSAL MONITORING METRICS				
METRIC	**METHODS**	**UNITS**	**FREQUENCY**	**PERFORMANCE CRITERIA**
METRIC 1: PROJECT FOOTPRINT AND OYSTER HABITAT AREA Project footprint is the maximum areal extent over which active restoration activity is planned or permitted. Restored habitat area describes the total areal extent in which the restoration activities have resulted in an increase in cultch cover and living oysters. Chapter 2, page 10.	**Primary subtidal method:** Area assessment by SCUBA diving, with either a boat crew following and plotting global positioning system (GPS) coordinates or with the diver towing a surface buoy with a mounted GPS navigator. GPS coordinates can then be entered into mapping software, such as QGIS or ArcGIS. **Alternative method:** Monitoring with underwater video and autonomous underwater vehicles (AUV) and acoustic methods, coupled with GPS. **Primary lower intertidal method:** GPS mapping by walking the perimeter of the project footprint at low spring tide and marking the location frequently using the most accurate GPS available to the project. **Alternative method:** Aerial imaging by drones.	m^2 (note the accuracy of the measuring device, e.g. ±).	Establish a pre-restoration baseline, followed by sampling within 3 months and annually thereafter. Consider additional measurements following storm events.	No performance criteria for project footprint. Oyster habitat areas should be stable or increasing habitat areal extent over time.
METRIC 2: SHELL COVER Oyster spat require suitable habitat for settlement. *Ostrea edulis* itself is generally regarded as the best cultch for *Ostrea edulis* spat, but other molluscs have also been used. Living oysters should also be accounted for when monitoring shell cover, as they stimulate settlement for oyster spat. Chapter 2, page 13.	**Primary subtidal method:** Underwater visual survey (UVS). SCUBA diver surveys can be used to visually estimate shell cover using photos and quadrats. **Alternative methods:** Drop down video (DDV) or box/grab cores. **Primary lower intertidal methods:** Quadrat survey. Visual estimates of shell cover using quadrats or photos of quadrats.	% shell cover or shell volume (L/m^2).	Should be measured before and after the addition of cultch, and 6 months after laying. This should be followed by annual sampling.	For substrate-limited areas, an increase in shell cover for image-based approaches, or increase in shell volume per unit area for volume-based approaches. For recruitment-limited areas, shell cover or volume should not

UNIVERSAL MONITORING METRICS				
METRIC	**METHODS**	**UNITS**	**FREQUENCY**	**PERFORMANCE CRITERIA**
METRIC 3: OYSTER DENSITY Oyster density is defined as the number of live oysters per unit area (individuals/m^2) and includes recruits (oysters > 35mm in shell height). Chapter 2, page 14.	**Primary subtidal method:** Quadrat sampling or transects by SCUBA diving. **Alternative methods:** DDV quadrats, grab sampling and use of a microdredge. **Primary intertidal method:** Transect. Quadrats should be placed across the area of restored habitat and excavated to the depth necessary to capture all living oysters. The count of all oysters > 35mm in shell height should be recorded.	Mean density (individuals > 35mm/m^2) ± standard error (SE).	Oyster density monitoring should be undertaken pre-restoration and at least once per year thereafter.	Increasing mean density of oysters > 35mm in shell height.
METRIC 4: OYSTER SIZE FREQUENCY Oyster size frequency distribution describes the composition of the population in terms of age, number and size of oysters. Chapter 2, page 16.	**Primary method:** Shell measurement with callipers or ruler. Shell height should be measured from a minimum of 50 oysters. If large numbers of oysters are available, a random subsample should be measured and data plotted in histograms.	% of oysters or number of oysters measured in each size class.	Biannual sampling in spring and autumn is recommended.	The population is at least bimodal and shows signs of recent recruitment.
METRIC 5: WATER TEMPERATURE Temperature has an important influence on the physiology of *Ostrea edulis*. The survival of oyster parasites is also affected by temperature, and temperature can therefore partly explain the performance of restoration activities. Chapter 2, page 17.	**Primary method:** Data loggers or thermometers (or other similar instrumentation) should be used to measure temperature as close as possible to the restored oyster habitat.	°C, accuracy ± 1°C.	Continuous measurements should be taken throughout the year at intervals of 15–60 minutes. If continuous loggers are not available, measurements should be taken as often as possible, for instance every time sampling for another metric is performed, and after storm events.	There are no performance criteria for this metric.

UNIVERSAL ENVIRONMENTAL METRICS				
METRIC	**METHODS**	**UNITS**	**FREQUENCY**	**PERFORMANCE CRITERIA**
METRIC 6: SALINITY Fluctuations in salinity influence the stress level, food intake, growth, condition index (CI) and survival of *Ostrea edulis*. Chapter 2, page 17.	**Primary method:** Data loggers should be used to taking regular salinity measurements. These should be installed as close as possible to the restoration site. **Alternative method:** Use a refractometer.	ppt (parts per thousand) or psu, accuracy ±1ppt or 1psu.	Continuous salinity measurements at intervals of 15-60 mins throughout the growing season are preferred. If continuous loggers are not available, then measurements should be taken every time other sampling is performed at the reef and after storm events.	There are no performance criteria for this metric.
METRIC 7: GROWTH RATE Oyster growth rate is an important, non-lethal indicator of oyster productivity and an indicator that oysters at the restoration site are experiencing suitable conditions for growth. Chapter 3, page 19.	**Primary method:** Growth rate from tethered, caged or marked individuals. Measure the change in shell height in the same individual over time. A minimum of 50 individuals is suggested, but larger samples are preferable. **Alternative method:** Use size frequency measurements to identify growth rate.	Growth mm/year.	Ideally measure oysters every 3 months (preferably September, December, March and July) or at least once per year (preferable in July).	A growth rate in shell height between 10-20mm per year is expected, especially within the first 3 years. As growth rate declines with size, expected values in older individuals will be lower (< 10mm per year).
METRIC 8: OYSTER SURVIVAL RATE Increase in biomass (i.e. the aim of restoration) is a balance of growth and mortality. There are many situations where quantitatively defining oyster survival can support adaptive management and assist in identifying any issues arising. Chapter 3, page 21.	**Primary method:** Survival rate from tethered, caged or marked individuals. Survival can be determined by assessing the change in the number of living individuals over time from any of the methods outlined in Metric 7. **Alternative method:** Use size frequency measurements to identify survival rate.	Survival (S) is the change in numbers of oysters in a given population over time. Alternatively, mortality rate (M) is the rate of decline in numbers over time (generally in %).	Ideally, oysters should be assessed every 3 months, (preferably September, December, March and July) or at least once per year, (preferably in July).	There are no performance criteria for this metric.

SUPPLEMENTARY MONITORING METRICS				
METRIC	**METHODS**	**UNITS**	**FREQUENCY**	**PERFORMANCE CRITERIA**
METRIC 9: OYSTER CONDITION Condition indices can be used to evaluate the health of a population and provide an easy-to-assess status which is comparable across sites. Chapter 3, page 22.	**Primary method:** Biometric measurements. The most common method for assessing condition index (CI) is the ratio of tissue mass to shell mass. The CI can be calculated from dry weight data using the following equation: CI = dry meat weight (g) *100/dry shell weight (g).	The CI is unitless.	CI values follow a clear seasonal pattern. Variations are generated by the naturally changing food supply and physiological events. If sampling once per year, the CI should be assessed for the same season, ideally in spring (pre-spawning period) or late autumn/winter.	A CI of 2–5 (outside the spawning period) indicates that the oysters are in good health.
METRIC 10: GONAD DEVELOPMENT Gonad development status is a good indicator of potential larval production, swarming timing and (indirectly) reproductive efficiency within a given population. Chapter 3, page 22.	**Primary method:** Macroscopic observation. The oyster should be carefully opened so the gonad, which is found just under the right valve above the adductor muscle, may be examined. The gonad shape, colour and fullness will reveal the development status. **Alternative method:** Microscopic observation of gonad development.	% of each qualitative stage observed in the sample.	Monthly to biweekly sampling is required to follow gonad development during the active period.	Presence of 30–40% mature females in stages 3 and 4 within the population when water temperature is above 16°C is expected and is indicative of a good reproductive efficiency. A low ratio of individuals in the advanced maturity stage would have negative consequences on the renewal of the population.
METRIC 11: SEX A skewed sex ratio has been recorded in some *Ostrea edulis* populations. Oysters are protandrous hermaphrodites and undergo multiple sex changes throughout their lives. However, an unbalanced sex ratio, especially one with low female occurrence, is cause for concern. Chapter 3, page 24.	**Primary method:** Sex ratio from direct gonad tissue sampling under a light microscope. Gametes can be extracted by gently scratching the surface of the gonadal tissue and using a Pasteur pipette or other fine capillary tube to suck up a small sample. This sample can then be spread over a glass slide for observation under a simple light microscope, or kept on ice and processed within 24 hours.	Unitless (ratio of females to males). It can also be expressed as a % of males (or females) in the population.	Once per year just prior to the spawning season, or with the same frequency as metric 10.	There are no performance criteria for this metric, but a balanced sex ratio within the population is expected.

SUPPLEMENTARY MONITORING METRICS				
METRIC	**METHODS**	**UNITS**	**FREQUENCY**	**PERFORMANCE CRITERIA**
METRIC 12: FECUNDITY Fecundity is a measure of the number of larvae produced by a female oyster. This metric provides information on whether the oysters are contributing towards a self-sustaining population. Chapter 3, page 25.	**Primary method:** Brooding larvae counting. Collect a range of oysters from sexually mature size classes (> 35mm shell height). A minimum sample of 30 oysters per location/time point is recommended. The brooding larvae can be sampled using either sacrificial or non-destructive methods.	Mean number larvae/ brooding oyster (± SE) and the % of brooding adults to estimate the reproductive potential of the population.	Monthly sampling from May to August is recommended.	A healthy and growing population will include either a constant or increasing occurrence of % breeding oysters.
METRIC 13: LARVAL ABUNDANCE The presence of larvae is evidence of successful reproduction. Oyster settlement intensity is related to larval abundance in the water column. Knowing when larvae are present can also be useful for optimising the timing of substrate deployment for settlement. Chapter 3, page 25.	**Primary method:** Pump sampling. Sampling is conducted at 1m above the benthos at high tide (±2 hours), from plankton samples collected by filtering generally between 1000 to 2000 litres of seawater through a 50–70µm plankton net by using a surface centrifugal pump. **Alternative method:** Towed plankton sampling.	The number of individuals per m^3 (individuals/m^3 ± SE).	Larvae should be sampled once per week during the larval spawning season (approximately June–September, but varies with location). The temperature sum may be applied to inform when to start sampling.	There are no performance criteria for this metric.
METRIC 14: RECRUITMENT INDEX Successful settlement, survival and subsequent recruitment are critical for the long-term persistence of restored populations. Evaluating recruitment during and after each reproductive season allows assessment of the health and potential of an oyster habitat. Chapter 3, page 28.	**Primary method:** Annual recruitment onto the reef or on cultch. Monitoring of annual recruitment may be carried out through the same excavated quadrat sampling outlined in Metric 4: Size Frequency (Chapter 2), or on cultch that has been deployed (see Metric 7, Chapter 3). To properly monitor for recruitment, excavated quadrat or clutch material covering 1m^2 must be carefully examined by eye and/or with the help of a binocular microscope, depending on the size of the spat and/or the presence of other similar bivalves. **Alternative method:** Seasonal recruitment on benthic collectors.	Density of living < 1-year-old recruits per m^2 (individuals/m^2 ± SE).	Annual recruitment inside the bed should be evaluated at least once a year, ideally in spring. This allows recruits that have survived from the previous reproductive season to be more easily identified.	For annual recruitment onto cultch and/or within the habitat, increasing or reliably high levels of recruitment over successive years.

SUPPLEMENTARY MONITORING METRICS				
METRIC	**METHODS**	**UNITS**	**FREQUENCY**	**PERFORMANCE CRITERIA**
METRIC 15: DISEASE PREVALENCE Oyster disease is recognised as one of the main causes of modern oyster population decline in Europe. Two genera of parasites are prevalent, the haplosporidia (*Bonamia ostreae* and *B. exitiosa*) and the paramyxea (*Marteilia refringens*). Chapter 3, page 31.	**Primary method**: Histopathology and molecular biology. Technical advice on, and minimum standards for, the detection of *Bonamia spp.* and *Marteilia refringens* of *Ostrea edulis* are based on the recommendations of the European Reference Laboratory for Mollusc Diseases. The detailed procedures for each potential disease can be found here: https://www.eurl-mollusc.eu/SOPs.	Parasite prevalence should be measured in % per total population (disease prevalence, %).	Parasite prevalence should be assessed annually in spring and autumn. Any positive samples must be reported to the relevant national authority.	There is no performance criteria for this metric.
METRIC 16: INVASIVE NON-NATIVE SPECIES Invasive non-native species may detrimentally impact restoration efforts and the surrounding environment. Restoration practitioners should monitor for the following species: *Crepidula fornicata, Crassostrea gigas, Ocinebrellus inornatus, Urosalpinx cinerea, Sargassum muticum, Didemnum vexillum* and *Styela clava.* Chapter 3, page 31.	**Primary method:** Grab sampling. See Metric 18, Chapter 4. **Alternative method:** eDNA analysis.	Number of individuals per m^2 for each species.	Monitoring for invasive non-native species can be incorporated in assessment protocols required for biodiversity metrics (see Chapter 4).	There are no performance criteria for this metric.
METRIC 17: SEDIMENTATION RATE Sedimentation rate should have been considered carefully during site selection, as high sedimentation can negatively affect survival, growth and recruitment. Chapter 3, page 32.	**Primary method:** Sediment traps. The sedimentation rate can be assessed by deploying sediment traps, which vary in design and cost. A relatively simple and inexpensive method is outlined on page 32, Chapter 3.	Dry weight (g)/m^2 per day.	Sedimentation rate should be considered 4 times a year, generally 1 assessment per season.	There are no performance criteria for this metric.

RESTORATION GOAL-BASED MONITORING METRICS				
Broodstock and oyster population enhancement				
METRIC	**METHODS**	**UNITS**	**FREQUENCY**	**PERFORMANCE CRITERIA**
METRIC 18: NEARBY-REEF OYSTER DENSITY AND ASSOCIATED SIZE-FREQUENCY DISTRIBUTIONS Chapter 4, page 33.	**Primary method:** See Metric 3 (Oyster Density) and Metric 4 (Oyster size frequency) in Chapter 2, and Metric 14 in Chapter 3 (Recruitment Index). Monitoring should take place in nearby locations both prior to and after restoration. It should be noted that confirming the restoration site as the source of the larvae and recruitment is not possible without further analysis.	Mean oyster density (individuals/m^2). Size frequency (mean oyster shell height (mm)). % and/or number of measured oysters per size class, and annual recruitment density of living < 1-year-old recruits per m^2 (individuals/m^2 ± SE).	Annually, between December and July when newly settled oyster spat have grown to a size that can be seen with the naked eye (~5–10mm) and can be confidently identified as *Ostrea edulis* recruits.	An increasing density of oysters outside the restoration site and the appearance of cohorts in the size class frequency data, indicating repeated recruitment events over time.
Fish and invertebrate biodiversity				
METRIC 19: INFAUNAL INVERTEBRATES Infaunal species such as clams, snails, polychaetes, flatworms and small crustaceans live in the substrate and are especially common in soft sediments. They contribute to biodiversity and their abundance is an indicator of ecosystem health. Chapter 4, page 34.	**Primary method:** Sediment cores. Sediment cores can be collected in the intertidal or (by SCUBA divers or operated from a boat) in the subtidal zones. The core tube should be driven into the sediment and the enclosed sediment should be carefully cleaved. Core samples should be sieved and stored in buffered 4% formalin-seawater solution for further processing. In the laboratory, all organisms should be extracted and identified to the lowest taxonomic level possible, then enumerated and weighed. **Alternative methods:** grab sampling and eDNA analysis.	Number of species per volume, as well as a list of the species/ groups identified; biomass per species/ group per volume (fresh weight (g)/m^2).	At least once prior to restoration, and a minimum of annually thereafter, when abundances of key species are highest.	A trend of higher biodiversity and abundance on restored sites, with the ultimate aim of statistically higher biodiversity on restored sites relative to the pre-construction and control sites.

RESTORATION GOAL-BASED MONITORING METRICS				
Fish and invertebrate biodiversity				
METRIC	**METHODS**	**UNITS**	**FREQUENCY**	**PERFORMANCE CRITERIA**
METRIC 20: EPIFAUNAL SESSILE INVERTEBRATES AND MACROPHYTES The appropriate enumeration and recording of sessile epifauna and flora depends on the growth form and abundance of the species. Chapter 4, page 35.	**Primary method:** Quadrat sampling or transects by SCUBA diving (see Metric 3, Chapter 2, page 14). **Alternative methods:** Use substrate trays, DDV quadrats or eDNA analysis.	Number of species per m^2 along with a list of the species identified, as well as the abundance as % cover or count of individuals as appropriate.	At least once prior to restoration, and a minimum of annually thereafter, when abundances of key species are highest.	A trend of higher biodiversity and abundance on restored sites, with the ultimate aim of statistically higher biodiversity on restored sites relative to the pre-construction and control sites.
METRIC 21: SMALL RESIDENT FISH AND MOBILE INVERTEBRATES Resident and mobile fauna contribute to biodiversity and their abundance is an indicator of ecosystem health. Many species will be potentially important prey for commercially important species, but may also include some species of commercial value (e.g. whelks). Chapter 4, page 36.	**Primary method for subtidal habitat:** Underwater video tools (if visibility allows). **Alternative methods:** Carry out an underwater visual census or eDNA analysis, or the use of habitat units or trays. **Primary method for lower intertidal habitat:** Habitat units or trays. All organisms should be identified to the lowest possible taxonomic level, counted and measured. Identification can be performed on site and organisms released after monitoring. **Alternative method:** The trays can be transported back to the laboratory and organisms preserved for future analysis.	Individuals per m^2, length (mm) for length-frequency distribution/ species.	At least once prior to restoration, and a minimum of annually thereafter, when abundances of key species are highest.	A trend of higher biodiversity and abundance on restored sites, with the ultimate aim of statistically higher biodiversity on restored sites relative to the pre-construction and control sites.

RESTORATION GOAL-BASED MONITORING METRICS				
Fish and invertebrate biodiversity				
METRIC	**METHODS**	**UNITS**	**FREQUENCY**	**PERFORMANCE CRITERIA**
METRIC 22: TRANSIENT FISH AND CRUSTACEANS Larger fish and crustaceans may use oyster habitat regularly or seasonally. It is important to monitor this use to build up an evidence base of the potential contribution of oyster habitats in Europe. Chapter 4, page 36.	**Primary method for subtidal habitat:** Remote underwater video surveys (RUVs). Unbaited RUVs can be used depending on visibility. An underwater video camera set to a fixed focal length of infinity should be attached to a weighted frame, which is then deployed by a rope with an attached buoy. RUVs should be placed at random locations across the reef and on control sites, at least 50m apart. Video footage should be processed to measure fish abundance and diversity. **Alternative method:** Carry out an underwater visual census or eDNA analysis. **Primary method for intertidal habitats:** Fish sampling nets. Intertidal systems such Fyke or Lift nets can be deployed by wading during low tide and left 3–6 hours before collection after high tide. Nets should be deployed at random locations on the oyster habitat, and at locations on adjacent bare sediment as a control. Net collection should be completed whilst the nets are still slightly submerged. Alternatively, eDNA analysis can be used.	Individuals per m^2, length (mm) by species. Catch per unit effort (individuals per hour), length (mm) by species.	At least once prior to restoration, and a minimum of annually thereafter, when abundances of key species are highest.	A trend of higher biodiversity and abundance on restored sites, with the ultimate aim of statistically higher biodiversity on restored sites relative to the pre-construction and control sites.

RESTORATION GOAL-BASED MONITORING METRICS				
Interactions with adjacent habitats				
METRIC	**METHODS**	**UNITS**	**FREQUENCY**	**PERFORMANCE CRITERIA**
METRIC 23: SHORELINE LOSS/ GAIN (CHANGE IN SHORELINE POSITION) Oyster habitat with height (e.g. oyster reefs) can attenuate wave energy and enhance sedimentation, thereby potentially contributing to a reduction in shoreline loss or to shoreline gain. Chapter 4, page 40.	**Primary method:** Mapping the shoreline on foot. Shoreline loss and gain can be assessed by repeated mapping of the same area over time, either using either a handheld digital Global Positioning System (dGPS) or with permanent perpendicular transects. The maps can then be overlayed in mapping software, in order to determine the change in shoreline between surveys. **Alternative methods:** Use surveying instrumentation or aerial photography.	Shoreline loss/gain (m per year).	Measurements should be taken once prior to construction, 6 months post-construction (to document the immediate post-construction project footprint and reef area) and annually thereafter. Additional measurements after events that could alter shoreline position (e.g. dredging events and large storms) are also recommended.	Trend of decreasing shoreline loss, or shoreline gain.
METRIC 24: SHORELINE PROFILE/ELEVATION CHANGE Chapter 4, page 42.	**Primary method:** Surveying instrumentation. Elevation change can be determined using traditional surveying equipment such as a surveyor's level, laser level and graduated rod, or ranging poles between two people. Where the practitioner has training in and access to advanced surveying instrumentation (e.g. a total station or other instruments used to find horizontal and vertical angles and distances), the practitioner may perform a topographic survey along each of the transects using these advanced surveying instruments.	Shoreline profile/ elevation change (cm per year), shoreline slope (i.e. rise/run) (unitless). It is suggested that a dGPS be used to mark the locations of the measurements taken.	Measurements should be taken once prior to construction, within 3 months post-construction (to document the immediate post-construction project footprint and reef area), and annually thereafter. Additional measurements after events that could alter shoreline profile (e.g. dredging events/ large storms) are also recommended.	Trend of decreasing slope and increasing mean elevation at the shoreline, or a decreased "step/cliff" at the water's edge.

RESTORATION GOAL-BASED MONITORING METRICS				
Interactions with adjacent habitats				
METRIC	**METHODS**	**UNITS**	**FREQUENCY**	**PERFORMANCE CRITERIA**
METRIC 25: DENSITY AND PERCENT COVER SALTMARSH/ SEAGRASS PLANTS Where a restoration project is specifically aiming to recover vegetated habitats associated with the oyster habitat, monitoring of changes to the quality (density and % cover) of these vegetated habitats should be conducted. Habitat quality data collected in this metric should be considered alongside results from Metric 23 and Metric 24 to characterise the impact of oyster habitat restoration on adjacent habitats. Chapter 4, page 43.	**Primary method for intertidal habitat:** Quadrats. A visual estimate of the % of ground covered by each species should be recorded at standardised intervals, using a 1m^2 quadrat (or 0.5m^2 quadrat if vegetation is dense). % cover can be estimated as described in Metric 2, Chapter 2. **Alternative methods:** Aerial imagery and light imaging, detection and ranging (LiDAR). **Primary method for subtidal habitat:** UVS. At least three transects should cross the area of submerged aquatic vegetation (SAV) perpendicular to the oyster s habitat. The start and end of patches of SAV habitat should be noted along the transect, along with SAV % cover and shoot density measurements collected from at least three quadrats along each transect. **Alternative methods:** DDV or acoustic remote sensing technology.	Mean density (live shoots per m^2), mean % coverage of each species present.	Sampling of saltmarsh and SAV should be performed annually at the end of the peak growing season, which is between August and September in much of Europe.	A trend of increasing mean plant density and mean percent coverage, with an ultimate goal of having statistically greater mean plant density and mean percent coverage than pre-construction conditions and at the control site, or a mean density and mean present coverage that is roughly equal to that of a natural reference site.

RESTORATION GOAL-BASED MONITORING METRICS				
Water quality improvement				
METRIC	**METHODS**	**UNITS**	**FREQUENCY**	**PERFORMANCE CRITERIA**
METRIC 26: LIGHT PENETRATION Light intensity decreases exponentially with water depth and depends on the amount of light absorption by dissolved substances and suspended particles such as phytoplankton and particulate organic matter (also termed seston). Filter-feeding by oysters reduces seston concentration and can therefore be beneficial to SAV where light penetration is a limiting factor to photosynthesis and growth. Chapter 4, page 45.	**Primary method:** In situ light sensors. Light sensors can be deployed in intertidal or subtidal habitats. Relatively inexpensive waterproof loggers that measure temperature and light can be used. The light sensors must be cleaned and positioned horizontally and facing upwards. Set the sensor to take continuous measurements every 5 seconds for an hour at the end of slack high tide (measuring from the start of the ebb tide). **Alternative methods:** Use a Secchi disk, a transparency/turbidity tube, or a handheld instrument.	Lux (lumen/m^2) is the unit of light intensity (or illuminance).	4 times a year, once in each season.	A trend of increased light penetration on the restored oyster reef and immediately down-current.
METRIC 27: SESTON AND/OR CHLOROPHYLL-*A* *CONCENTRATIONS* Concentrations of seston and chlorophyll-*a* are frequently used metrics to determine water quality, as well as selected nutrient concentrations (e.g. nitrate or phosphate). Monitoring the concentration of seston and/or chlorophyll-*a* is achieved with water sampling at locations up-current and down-current of the oyster habitat, or using in situ fluorometry methods. Chapter 4, page 46.	**Primary methods:** For water sampling and flow rates, see page 46. For seston concentration, chlorophyll-*a* and nutrients, see page 47.	Total particulates (mg/L); organic content (%); chlorophyll-*a* (mg/L).	Measuring water quality before and after restoration efforts would determine whether restoration is positively impacting the area. Long-term monitoring with frequent sampling would be most beneficial, and inform a project whether concentrations of nutrients or seston can explain an event such as sudden mortality. Where budget and resources are available, weekly sampling is preferred. Quarterly sampling would discern seasonal differences.	A trend of decreasing total particulates, organic content and/or chlorophyll-*a* values is expected. The overall goal is to quantify statistically lower values at the restored habitat, compared to pre-construction conditions and to the control site, or roughly equal to the natural reference site if available.

RESTORATION GOAL-BASED MONITORING METRICS				
Socio-economic benefits of native oyster restoration				
METRIC	**METHODS**	**UNITS**	**FREQUENCY**	**PERFORMANCE CRITERIA**
METRIC 28: SOCIAL BENEFITS The success and longevity of a project can often rely on local buy-in and support. A key attribute to measure for restoration projects is therefore the amount or change in stewardship of *Ostrea edulis* habitats and the wider environment. Stewardship can be demonstrated through opportunities for local communities to be involved in restoration activities and to protect and care for shellfish reefs and the environment, and the motivation of individuals and groups to cooperate and take part in the opportunities provided by restoration. Chapter 4, page 47.	**Primary methods:** The following information should be collected by project managers through project tracking: • Number of project events • Attendance at project events • Community and partner organisations engaged • Total number of volunteers • Volunteer hours • Media engagement	Total number of project events; mean number of people per event; number of partner organisations; number of volunteers; total volunteer time in hours.	Project managers should monitor on an ongoing basis, use an event tracking spreadsheet and make it part of standard monthly or reporting period tracking workflows.	A trend of increasing engagement throughout the project timespan.

RESTORATION GOAL-BASED MONITORING METRICS				
Socio-economic benefits of native oyster restoration				
METRIC	**METHODS**	**UNITS**	**FREQUENCY**	**PERFORMANCE CRITERIA**
METRIC 29: ECONOMIC BENEFITS With many projects reliant on a variety of funding streams with a range of agendas, the ability to express the impact that *Ostrea edulis* restoration can have on local economies and coastal populations, as well as ecologically, is extremely valuable. Many funding bodies view applications that have taken this kind of impact into consideration positively, with some even requesting that it be incorporated. Chapter 4, page 48.	**Primary methods:** Total number of full-time jobs across the project. Through progress reporting, project managers can capture the total number of people and hours employed directly or indirectly (in kind) on the project across project activities. **Cost of restoration projects:** Summarise costs across projects per activity and expense type. **Benefit valuation:** The full range of benefits and value is often termed the total economic value, and includes values based on direct and indirect use and non-use values.	Number of full-time jobs. Cost of restoration project, £ or €. Total economic value, £ or €.	Project managers should monitor on an ongoing basis, use a jobs-tracking spreadsheet and a contractor employment log. This should be part of standard monthly or reporting period workflows, as attempting to collect this data at the end of a project can be challenging.	Trend of the value arising from the restoration project being greater than the investment or cost of the project.
METRIC 30: CARBON STOCK The carbon stock associated with a restored oyster habitat is bound up in the oysters themselves and in the surrounding sediments. Therefore, carbon store is best assessed by surveying the oyster population, associated shell mass and the carbon content of the surrounding sediments. Chapter 4, page 51.	**Primary method:** Sediment cores. Buried shell material can obstruct the penetration of the cores so, for oyster habitats, sturdy cores with diameters ≥ 10cm are recommended. When preparing and analysing samples for carbon, cores must be capped before being returned to the lab. Cores should sample the total thickness of sediments that are affected by the overlying oyster habitat, to ensure that variability is accounted for. Different sediment characteristics or variation in oyster density can indicate subhabitat with spatial differences in carbon content that may require separate, "stratified" sampling. Sediment samples should be analysed primarily for total carbon.	Grams (g) of carbon per m^2, along with a measurement of sediment depth (m). Explanatory/contextual data on oyster density (individuals per m^2), depth of habitat (m), total area of habitat (m^2), average oyster size (cm) or biomass (g per m^2) should also be reported.	Pre-restoration and then at 5-year intervals. In the meantime, it may be necessary to forecast the growth of carbon stocks by site-specific experimentation.	There are no performance criteria for this metric.

RESTORATION GOAL-BASED MONITORING METRICS				
Socio-economic benefits of native oyster restoration				
METRIC	**METHODS**	**UNITS**	**FREQUENCY**	**PERFORMANCE CRITERIA**
METRIC 31: CARBON SINK Estimation of carbon sink rates requires that the production of CO_2 in respiration and shell formation is accounted for, as well as the carbon stock. Oysters produce respiratory CO_2 as they live and grow, and they also produce it during the calcification process of their shells. The ratio of released CO_2 to deposited mineralised carbonate in shells is generally 0.6:1 in seawater. The overall metabolic production of CO_2 over a year is needed in calculations of carbon sink value. Chapter 4, page 53.	**Primary method:** Measuring carbon produced. Changes in carbon dissolved in water can be monitored to measure carbon production rates. Generally, this is to determine the rate at which carbon is released during metabolic shell production processes. Carbon production can be calculated using incubation studies using the methods from Tagliarolo *et al.* (2012) detailed on page 53.	N/A	N/A	A trend of neutral or positive change in carbon stores compared to the pre-construction or control sites.