EUROPEAN GUIDELINES ON BIOSECURITY IN NATIVE OYSTER RESTORATION

NOVEMBER 2020

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NATIVE OYSTER NETWORK UK & IRELAND

NORA

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Disease and invasive non-native species have contributed to the decline of oyster habitats in Europe and reduced the ecological health of European waters. It is critical that restoration efforts mitigate the biosecurity risks arising from active restoration efforts.

Intertidal native oysters in Strangford Lough, Northern Ireland. Photo: José M. Fariñas Franco.

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In January 2020 the Native Oyster Network – UK & Ireland and Native Oyster Restoration Alliance co-hosted a "European Native Oyster Biosecurity Workshop" at Heriot-Watt University during which these guidelines were developed. The workshop was supported by Wetlands International European Association via the EU Life grant, John Ellerman Foundation, University of Portsmouth, and Environment Agency.

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EXECUTIVE SUMMARY

Across its indigenous range, there are growing efforts to reintroduce and restore the European native oyster, *Ostrea edulis*. Once widely distributed and plentiful, the native oyster is now threatened and declining through much of its range, and is extirpated as a habitat from most locations where reefs were recorded in the early 1800s.

At many locations, active intervention is necessary to recover native oyster reefs. Actions can include the introduction of oysters in order to increase the breeding population or the introduction of a substrate or medium for the oysters to settle on.

Translocation is the human-mediated movement of living organisms from one area, with release in another area. With translocation of both oysters and subtrate, there are a suite of risks associated including disease transfer and the introduction of invasive non-native species. It is not possible to undertake such introductions with zero risk. For this reason, the first step in undertaking restoration action is to consider whether alternatives to translocation exist in the form of local stock or materials. If alternatives are not available, the second step is to decide which biosecurity measures are appropriate and to ensure that all legislative responsibilities are met, including internationally and nationally agreed disease control measures.

Biosecurity is the implementation of management measures to reduce the probability of negative effects on ecosystems from biological organisms. Implementation of good biosecurity practices is fundamental in any ecosystem restoration and is the responsibility of the project. Biosecurity must be considered at every step of the restoration process.

This report presents biosecurity guidelines for conservation and restoration of native oyster habitat in Europe, based on current knowledge and experiences. The report sets out a series of steps developed to provide an objective, repeatable, transparent, and documented assessment of the risks and provides example approaches for their reduction. The guidelines have been prepared through a series of technical workshops led by the Native Oyster Restoration Alliance and the Native Oyster Network – UK & Ireland, bringing together experts and practitioners in the field. The guidelines should be used as part of a comprehensive risk assessment with a level of effort appropriate to the situation, national legislative framework and international guidance such as the IUCN Guidelines for Reintroduction and Other Conservation Translocations (2013), and the World Organisation for Animal Health (OIE) guidelines (Kock *et al.* 2010).

KEY SUMMARY POINTS:

- **The movement of people, equipment, materials, and oysters between locations carries with it the risk of moving potentially harmful organisms, such as diseases and invasive non-native species, to new sites.**
- **Translocation of material and oysters from one water body to another is never risk-free. Take time to plan and where possible, avoid or reduce the need to translocate.**
- **When translocating material or oysters, always plan for enough time and resources to undertake robust biosecurity measures to reduce the risk as far as possible.**
- **Never translocate material from a water body with an oyster disease or high impact invasive species present, to one where it is absent.**
- **Always undertake real-time assessment of the sites and oysters or cultch material, rather than assuming that existing test and survey results reflect actual status.**
- **Hatcheries producing certified oysters in disease-free areas can be used for restoration purposes while seed from hatcheries in disease positive areas should only be used locally.**
- **Hatchery raised stock should be treated as translocations if they have been in contact with the open water before being moved.**

Foreword
by Dr Bernadette Pogoda.

Scientist, Alfred Wegener Institute Helmholtz Centre für Polar and Marine Research: Shelf Sea Systems Ecology and Coastal Ecology, German coordinator for native oyster restoration; Member of the NORA advisory board.

Since the foundation of the Native Oyster Restoration Alliance (NORA) and the Native Oyster Network – UK & Ireland (NON) in 2017, we have seen a considerable increase in knowledge and in the number of publications related to native oyster restoration in Europe. We are approaching the UN decade on ecosystem restoration (2021-2030), and restoration efforts are rapidly gaining momentum. Against this background, the biosecurity guidelines aim to support restoration practitioners in scaling up efforts, and effective biosecurity is an essential part of achieving this responsibly and sustainably.

Biosecurity is examined here in two areas relevant for oyster restoration: the translocation of living animals and shell materials, and the production of seed oysters in hatcheries. These guidelines are intended as a reference for implementing the necessary biosecurity standards, which recognise the importance of the ecological health of our ecosystems and the responsibility we have when interacting with our environment via active restoration measures.

These guidelines represent valuable progress in understanding and managing the biosecurity risks inherent in native oyster restoration. Whereas previously biosecurity efforts may have been viewed as a barrier to action, it is hoped that the steps outlined here provide welcome guidance to achieve our goals in ecological restoration of native oysters or other shellfish species.

The guidelines should be seen as a living document which will benefit from further research and practical experience. I strongly encourage restoration practitioners to use and to disseminate the guidelines, and to take ownership of its further development in order to strengthen the positive impact of ecological restoration in our European seas.

I acknowledge all our partners, including practitioners, producers and academics, for the effort they have put into this collaborative document, as well as Germany and the United Kingdom for funding and supporting both networks.

I look forward to seeing more successful and progressive collaborations in the field of ecological restoration over the coming years.

CHAPTER 1
UNDERSTANDING BIOSECURITY IN NATIVE OYSTER RESTORATION

CHAPTER AUTHORS
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INTRODUCTION

Invasive Alien Species or Invasive Non-Native Species (hereafter INNS; see Box 1.1) and diseases are a significant threat to biodiversity throughout European waters. In the case of the native oyster (*Ostrea edulis*), the disease caused by *Bonamia ostreae* (see Figure 1.5), is a major driver of decline in remnant populations. Given the magnitude of the threat posed by both INNS and disease, biosecurity (see Box 1.1), is a critical aspect of work in the marine environment. In this chapter we introduce the threats posed by INNS and diseases, their possible pathways of introduction, and the legislative and policy background practitioners of native oyster restoration should familiarise themselves with. All activities involving site visits pose a possible biosecurity risk. Practitioners should therefore work with their partners to understand and mitigate these risks wherever possible. These guidelines provide an introduction, but are not a substitute for working with the relevant authorities to ensure that work on site exceeds the mandated requirements and we encourage practitioners to participate in dialogue with the relevant authorities from early on in the planning process.

The presence or introduction of INNS or diseases negatively impacts the conservation objectives for protected species and habitats, the biodiversity associated with a healthy biogenic habitat, and the livelihoods of other sea-users. INNS and diseases also threaten the success of native oyster restoration through inducing high mortality (e.g. from Bonamiosis), competition for food or space, by acting as predators/pests of native species, or even altering the local environment to a less suitable condition, as well as risking the reputation of restorative projects if they are in any way implicated. Since both INNS and translocated oysters can be vectors of disease that can have devastating impacts on the receiving areas, wild populations, and aquaculture stocks, it is important to understand and mitigate against risks of disease transmission.

BOX 1.1: KEY DEFINITIONS

Biosecurity is defined as preventive measures to reduce the risk of spread of infectious diseases, pathogens, pests, invasive non-native species, and modified organisms, and can include toxins and pollutants. The way the term is applied can depend on the context and over a range of scales, including local, national, and transnational, even global.

Cultch is any substrate, such as rock or shell, that a juvenile native oyster is attached or may attach to.

Disease is defined as a disorder of structure or function in an organism that produces specific signs that are not caused by physical injury alone.

Invasive Non-Native Species (INNS) are organisms that have been introduced deliberately or accidentally across a biogeographic boundary by humans, and which go on to have a negative ecological or economic impact in their new range. Not all non-native species become invasive. Many non-native species have little impact in their new range and may have positive economic benefits associated with them. INNS refers specifically to those species which do have a negative impact. INNS are also referred to as Invasive Alien Species (IAS) in Article 3 (2) and (3) of Regulation (EU) No 1143/2014 of the European Parliament where they are defined as: "an alien species whose introduction or spread has been found to threaten or adversely impact upon biodiversity and related ecosystem services".

Pathogen is defined as the biological agent (especially microorganisms such as bacteria, viruses) that cause disease in their host.

Translocation is the movement of populations of native oysters, adult, juvenile or larval, from one location to another that could be considered a different body of water.

Native oyster restoration as a pathway for INNS and disease

Shellfish movements have been a major vector of INNS and disease. Attempts to replenish oyster stocks in Europe through importing both native and non-native oysters (e.g. *Crassostrea virginica*, *Crassostrea angulata*) have been documented since the 1870s. Over the decades, many non-native species and diseases were inadvertently translocated with these introductions, some with severe negative effects for marine ecosystems. A number of the species introduced with live oysters have become highly invasive and a threat to the native oyster itself, including the American tingle *Urosalpinx cinerea*, the slipper limpet, *Crepidula fornicata* and the native oyster parasite *Bonamia ostreae* (see Figure 1.3 for examples). It is as a result of these lessons learned that legislation was developed, both nationally and internationally, to prevent future spread of INNS and disease. It is therefore critically important that practitioners familiarise themselves with the relevant legislation.

Native oyster restoration often involves the movement of people, equipment, cultch material and live shellfish between sites and as such represents numerous opportunities to accidentally move species between sites as well. Whilst such movements are by no means the only vectors of INNS or disease, efforts to restore native oyster populations must adopt rigorous biosecurity protocols in order to reduce the risk that an action with an intended positive ecological benefit, results in a negative impact.

Biosecurity as an integrated part of restoration practice

It is important to note that the responsibility for ensuring the highest possible level of biosecurity lies with the projects themselves. Inadequate biosecurity measures present a very real biological, ecological, and reputational threat. It must be recognised that it is not always possible to accurately identify threats to the environment prior to damage occurring, and that there is usually a time lag between arrival and recording of INNS and diseases. Therefore, vigilance is needed, even in areas where INNS or diseases are not yet recorded. As well as adhering to the legal requirements, projects should apply a "Precautionary Approach" to prevent harm being caused by accidental or poorly considered transfers. The IUCN has published "Guidelines for applying the precautionary principle to biodiversity conservation and natural resource management", which can helpfully be referred to.

It is the responsibility of the restoration practitioners to seek advice from the relevant authorities and ensure that they meet legal requirements. Failure to do so can result in legal consequences.

Figure 1.1: Solent Oyster Restoration Project staff carrying out fieldwork in marina sites in the Solent. Photo: Blue Marine Foundation.

Stop the spread **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.

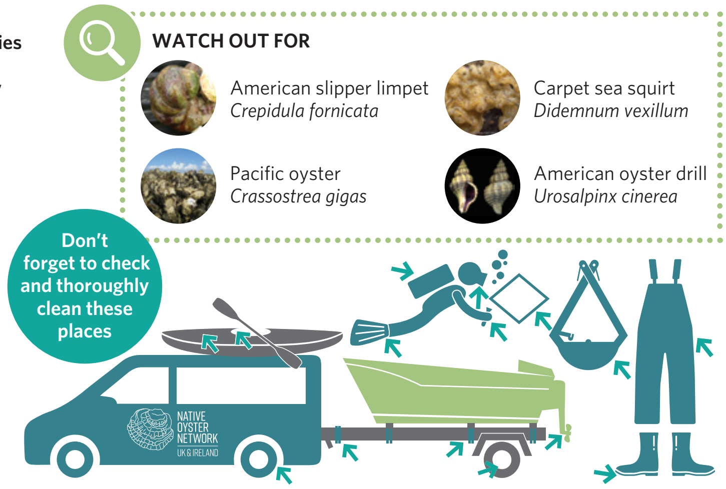


Figure 1.2: Biosecurity considerations to prevent transmission during restoration practice and fieldwork: Areas to be vigilant with when cleaning after carrying out fieldwork for oyster restoration projects: Check – Clean – Disinfect – Dry.

INNS and diseases can be moved between sites whenever people and equipment are moved, as well as when oysters or cultch material are placed in the water. As such it is important that all people participating in oyster restoration activities, including science and monitoring, comply with both standard 'Check, Clean, Disinfect, Dry' protocols, as well as with relevant European and national legislation relating to aquatic animal health when moving between sites (see Figure 1.2).

Check before leaving a site all equipment including wetsuits, vessels, boots, buckets etc. Remove all visible hitchhikers, sediment, and debris. If this occurs away from the site, ensure that all material is at least disposed of safely, and under no circumstances near a watercourse. Under circumstances of enhanced risk, disposal should be to a specified biological waste disposal route (possibly including incineration).

Clean all equipment including the vessel and bilge tank with freshwater. Do not let water drain back into the sea, as spores and eggs can persist for some time.

Disinfect under circumstances of increased risk, a biocide/disinfectant should also be used.

Dry all equipment thoroughly, ideally in sunlight, before moving to a new marine location.

All activities undertaken on the restoration site should be considered with regards to biosecurity. Further guidance on developing general marine biosecurity action plans, including useful worked examples were developed by Payne *et al.* 2014.

Native oyster restoration may include the translocation of cultch, spat attached to empty shells or pieces of shells (spat-on-shell), single spat (also called single seed oysters), juvenile or adult oysters. Each of these methods carries with it the risk that species and/or pathogens are also translocated. It is recommended that projects contact the relevant authority for advice on regulatory and licensing requirements early in the planning stage of any restoration project. This topic is covered in greater detail in Chapter 2.

Working with the public to understand risk

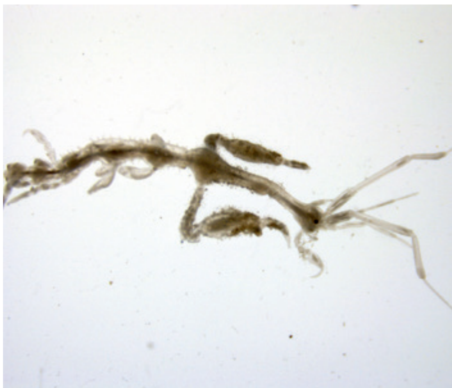
Native oyster restoration activities also present an opportunity for public engagement. Despite the many regulations in place to prevent the illegal release of oysters and the potential associated diseases and INNS into the wild, it is common practice in some coastal areas for individuals to store their live oysters in the water for a few days before consuming them, or to dump the fresh shells in the sea after a meal. Such activities may severely impact restoration and aquaculture activities and society at large, by contributing to the dispersal of pathogens and non-native species. These activities often take place both because the individuals are not aware of the risk and because they believe they may be doing something positive for the ecosystem; such an action is all the more likely if restoration efforts do not adequately communicate the risk of such activities. Restoration offers a unique opportunity to allow individuals to contribute positively to the recovery of a threatened species and a unique opportunity for the public to better understand coastal ecology and the practice of ecosystem restoration. Projects should not overlook these opportunities, in particular with regards to raising awareness of the risks associated with returning oysters or shells to the water without undertaking appropriate biosecurity measures.

Taxonomic group: Mollusca (*Bivalvia*)
Species: Pacific Oyster (*Crassostrea gigas*).
Impact: Competition, habitat change
Photo: Åsa Strand

Taxonomic group: Mollusca (*Gastropoda*)
Species: American slipper limpet (*Crepidula fornicata*)
Impact: Competition, habitat change
Photo: Zoë Holbrook

Taxonomic group: Ctenophora
Species: Warty comb jelly/Sea walnut (*Mnemiopsis leidyi*)
Impact: Predation (zooplankton), competition
Photo: Marco Faasse (CC BY-NC-SA 4.0 license)

Taxonomic group: Phaeista
Species: Japanese wireweed (*Sargassum muticum*)
Impact: Habitat change, competition
Photo: Prof. Bárbara Ignacio (CC BY-NC-SA 4.0 license)



Taxonomic group: Crustacea
Species: Japanese skeleton shrimp (*Caprella mutica*)
Impact: Competition
Photo: Joanne Preston

Taxonomic group: Tunicata
Species: Carpet sea squirt (*Didemnum vexillum*)
Impact: Competition, habitat change
Photo: Rosana Moreira da Rocha (CC BY-NC-SA 4.0 license)

Taxonomic group: Chlorophyta
Species: Green alga – "killer alga" (*Caulerpa taxifolia*)
Impact: Habitat change, competition
Photo: Boris Unger

Taxonomic group: Crustacea
Species: Acorn barnacle (*Hesperibalanus fallax*)
Impact: Competition
Photo: David Fenwick, APHOTOMARINE

Taxonomic group: Crustacea
Species: Brush-clawed/Asian shore crab (*Hemigrapsus penicillatus*)
Impact: Competition, predation
Photo: ffish.asia (CC BY 4.0 license)

Figure 1.3: A selection of high impact INNS listed as species (present and horizon) which have been selected for assessment of Good Environmental Status within GB waters, as required under the Marine Strategy Framework Directive. Please note that this list is for illustration only. Complete and current lists should be sought on national/local levels on a project by project basis. See Box 1.2 for some potential sources of information or contact the national competent authority for advice.

LEGISLATIVE OBLIGATIONS

The impacts of the introduction of shellfish diseases and INNS have long been acknowledged, and international institutions have developed legislation and relevant targets and reporting systems to address these threats. It is the responsibility of all restoration practitioners to ensure that they are aware of and adhere to relevant legislation on biosecurity to avoid falling foul of the law. Be aware that legislation and guidance functions on a variety of scales. Figure 1.4 illustrates the many levels of regulation relevant to oyster restoration. Seek the advice of the relevant authorities to ensure that the project adheres to all relevant guidance and the law.

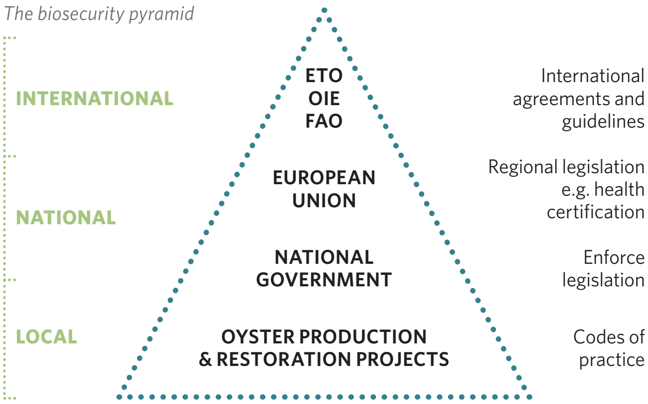


Figure 1.4: Legislation and policy regarding biosecurity function at a variety of scales, all of which projects should be aware of and seek advice on. Figure adapted from Oidtmann *et al.* (2011) Aquaculture 1-2: 22-33.

INNS and their biosecurity management

There have been few successful eradication attempts for marine non-native species or diseases in open waters. Therefore, the most effective method of control is to prevent their introduction.

A number of high-risk INNS are recognised in Europe (Figure 1.3, Box 1.2). The absence of a disease or species from the certifiable or high-risk list does not, however, mean that it is not a risk. While many non-native species have little or no impact on the receiving water bodies, it is difficult to predict which species will become problematic in an introduced range. Certain attributes related to both the life history of the species and the condition of the receiving site which can indicate the likelihood of species becoming problematic (see Geburzi and McCarthy 2018 for a good review), and invasion history from other locations can also be a useful indicator. Assessments of whether a species is likely to become invasive in a new location requires expertise. Fortunately, several databases exist for European, national, and regional records of non-native species, which projects can refer to for information. AquaNIS is an online information system for aquatic non-indigenous species introduced to marine, brackish, and coastal freshwater of Europe and neighboring regions. It includes regular updates and provides extensive species accounts, including biological traits and images to support the Marine Strategy Framework Directive (MFSD) and risk assessments for shipping and aquaculture. National platforms also provide important and up-to-date information (e.g. for Germany), or risk classification lists (e.g. the Swedish Species Information Centre (SLU)), whereas the IUCN have published a guide on INNS and their monitoring for the Mediterranean (Box 1.2). In the UK, the GB Non-Native Species Secretariat, a part of FERA/DEFRA, gives pragmatic advice and issues alerts for high impact INNS including specific marine advice and provides risk assessments for species. Further sources include: The UK Water Framework Directive Alien Species Alarm List and the EU Alien Species Regulation list of Species of Union Concern.

We recommend that projects check several platforms and ensure that the information they draw on in their risk assessment and planning is up to date. These lists and assessments can be used to identify which species are of particular concern when considering where to source oysters or cultch material from.

Every species introduced to a new area has the potential to become invasive. Therefore, while biosecurity protocols should prioritise the prevention of key identified problem species, projects should always strive to adhere to the precautionary principle and clean all materials and equipment moved, even if no INNS are believed to be present.

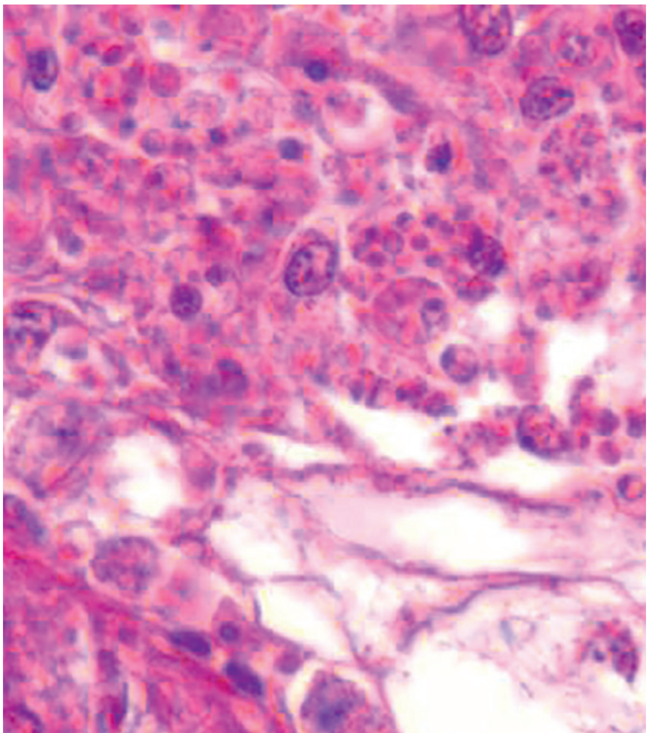


Figure 1.5: Histological slide depicting *Bonamia ostreae* cells infecting the tissue of a native oyster *Ostrea edulis*. Photo: B. Chollet/Ifremer.

BOX 1.2: EXAMPLES OF INTERNATIONAL, NATIONAL, AND SUBNATIONAL LEGISLATION AND GUIDELINES **RELATING TO INNS**

International:

Regulation (EU) 1143/2014 on invasive alien species (the IAS Regulation) entered into force on 1 January 2015, fulfilling Action 16 of Target 5 of the EU 2020 Biodiversity Strategy, as well as Aichi Target 9 of the Strategic Plan for Biodiversity 2011-2020 under the Convention of Biological Diversity.

Mediterranean – Monitoring marine invasive species in Mediterranean marine protected areas (MPAs): a strategy and practical guide for managers.

National:

France – French national strategy on invasive alien species. Reference documents for taking into account marine cultivation activities in the preservation of the marine environment (Coz & Ragot, 2020a, 2020b).

Italy – Decreto Legislativo 230/2017 – 'Disposizioni volte a prevenire e gestire l'introduzione e la diffusione delle specie esotiche invasive' (National strategies to prevent the introduction of invasive species).

Subnational:

Loch Creran Biosecurity Action Plan: A local community-level biosecurity plan set up in response to a high impact species in a shellfish growing waters.

Diseases and their biosecurity management

Native oysters are susceptible to numerous diseases which are still expanding their range in Europe and which are subject to monitoring efforts. It is critical that projects familiarise themselves with the notifiable diseases and the disease status of any locations where work is carried out. When working in shellfish growing waters consideration should also be given to the possible transmission between bivalve species. Some diseases, such as *Marteilia refringens* (including the recently proposed species *M. parafringens* sp. nov.) can be transmitted between native oysters and blue mussels (*Mytilus edulis*), and there are indications that OsHV-1 µvar can be transmitted between Pacific oysters (*Crassostrea gigas*) and native oysters.

There are several diseases which are of particular note in the context of native oyster restoration in Europe. These include the listed diseases (and the agents) of bivalves to the World Organisation for Animal Health (OIE) and/or to the European Commission (EC) (The Council Directive 2006/88/EC):

- Bonamiosis – *Bonamia ostreae* (OIE/EC – present in Europe),
- Bonamiosis – *Bonamia exitiosa* (OIE/EC – present in Europe),
- Marteiliasis – *Marteilia refringens* (OIE/EC – present in Europe),
- Denman Island Disease – *Mikrocytos mackini* (EC – not currently present in Europe),
- Herpes-like infection – Herpes virus OsHV-1-µvar (present in Europe) (notifiable in few zones in Ireland and UK only)

The law (Box 1.3) places an obligation on practitioners to report immediately any suspicion or confirmation of the presence of these listed diseases to the competent authorities. The competent authority should then investigate and decide what measures are to be taken. Measures may include an initial survey, the inclusion of a site-based risk assessment and biosecurity plan with contingency measures, as well as follow-up monitoring of the site as part of the licensing conditions of the plan or project and movement restrictions. In the case of the suspicion of the presence of a disease or non-native species, the practitioner must follow these steps:

- Report immediately to the competent authority.
- Adopt a precautionary approach – do not carry on operations that might contribute to further dispersal.
- Carry out risk assessments.
- Seek and follow advice from the relevant authorities. This may include not moving any material.
- It is important for restoration practitioners to be aware not only of the listed diseases and the requirements to follow the rules on translocations that apply internationally, but also to be mindful that there are a range of other parasites and pathogens to which the native oyster is susceptible, or may be a vector of. The following is a non-exhaustive list of known pathogens and parasites affecting the native oyster:
  - *Boccardia* (genus of),
  - *Cliona celata*,
  - *Cliona viridis*,
  - *Gyrodinium aureolum*,
  - *Haplosporidium armoricanum*,
  - *Herrmannella duggani*,
  - *Hexamita inflata*,
  - *Mytilicola intestinalis*,
  - *Nocardia crassostreae*,
  - *Ostracoblabe implexa*,
  - *Papovaviridae* (family of),
  - *Perkinsus mediterraneus*,
  - *Polydora* (genus of),
  - *Pseudoklossia* (genus of),
  - *Vibrio spp.* (e.g. *V. alginolyticus*, *V. anguillarum*, *V. coralliilyticus*, *V. neptunius*, *V. ostreicida*, *V. tubiashi*)

Haemic neoplasia may also affect oysters. In this case, no disease agent is observed, but the neoplastic cells may be infectious and cause significant mortalities.

It is the responsibility of the restoration practitioner to implement appropriate national disease prevention and management requirements and to report any unusual or unexplained mortalities, as well as any suspicion of occurrence of a listed or emerging pathogen, to the relevant authority for investigation.

Screening for diseases (see Figure 1.6) is usually carried out by national reference laboratories (European Union Reference Laboratory for Mollusc Diseases (EURL) (2020)) or other national institutions, depending upon the jurisdiction. OIE reference laboratories can be found on the World Organisation for Animal Health website (see resources section).

As with all introduced species, it is not possible to know before a disease is introduced, whether it will seriously impact in its introduced range. A disease may be subclinical in a population that has co-evolved with it, and therefore not apparent. Once transferred to a naive population it may cause high mortalities and disruption. For example, *Bonamia ostreae* was not known as a disease agent in its Californian source range but caused widespread mortalities in excess of 90% in its introduced range in Europe.

The guidance presented here is aimed at assisting native oyster restoration project managers and practitioners in ensuring that any restoration efforts in European waters are carried out responsibly. In order to ensure that all activities comply with the law, practitioners should work with the relevant authorities. Some biosecurity relevant resources from international, European, and national perspectives are given in Box 1.3.

Figure 1.6: Native oyster disease screening in University of Portsmouth lab. Photo: Luke Helmer.

Appropriate response in the event of unexpected mortality events

A critical aspect of biosecurity relating to disease management is monitoring of increased and unexplained mortality. During monitoring, restoration practitioners may notice unusual levels of mortality, changes in oyster growth, absence of larval settlement or increased or unexplained mortality. These may not have an immediate or obvious explanation and therefore require investigation. Disease is not the only cause of unexpected mortality. Pulse events, such as heavy rainfall can cause fluctuations in temperature, salinity, and turbidity, and may contribute to adult and spat mortalities, loss of planktonic larvae and cessation of reproductive activity. Storms can also increase pollution, horizontal advection, and abrasion, which can negatively impact oyster condition and possibly influence the prevalence of diseases such as infection with *Bonamia ostreae* where it is present. Because of the risk posed by disease, projects should always seek advice from the relevant authority regarding actions required in the event of an increased and unexpected mortality event.

As a guideline in instances where there are sudden, increased and unexplained high mortalities or recruitment failure, practitioners must report any abnormal mortality event to the authority and investigate the possible involvement of an infectious agent (as outlined in article 26 of the EU Directive 2006/88/EC, and also in Article 18 of Regulation 2016/429 which replaces 2006/88 in 2021). For that purpose, oysters including moribund ones (but not dead ones) should be sampled and processed according to recommendations of the European Union Reference Laboratory (EURL) for Mollusc Diseases in order to carry out histology, bacteriology and PCR for the detection of specific pathogens. These diagnostic analyses must be carried out by a recognised or agreed laboratory.

Going beyond legislative requirements and 'owning' the risk

Most existing national policies and legislative frameworks relevant to translocations for restoration are based on risk profiles of the aquaculture industry. It is important to understand that restoration carries potentially far higher risks because oysters go permanently back into the ecosystem. Routine monitoring by the government may be infrequent and reflective of a perceived low risk. In most cases, it will therefore be necessary for restoration projects to take responsibility for the biosecurity of their operations and apply a greater stringency than may be legally required. Maintaining a high level of biosecurity in restoration work is imperative both for ecological success, and to maintain a social license for such activities.

It is important to recognise that even with the most stringent testing and biosecurity procedures, it remains possible that a disease agent or INNS may become present at the restoration site where translocations have occurred (see Figure 1.7).

This may be because there is currently no 100% accurate method of disease-screening all translocated materials in a consignment.

1. Any biosecurity for the translocation of live oysters runs the risk that not all INNS individuals will be eliminated because, inevitably, the system must allow for the survival of the oysters.
2. Third-party activities in the area may have introduced a disease or INNS at or around the time of the project translocation.
3. The disease or INNS may have already been present and undetected in other biological reservoirs.

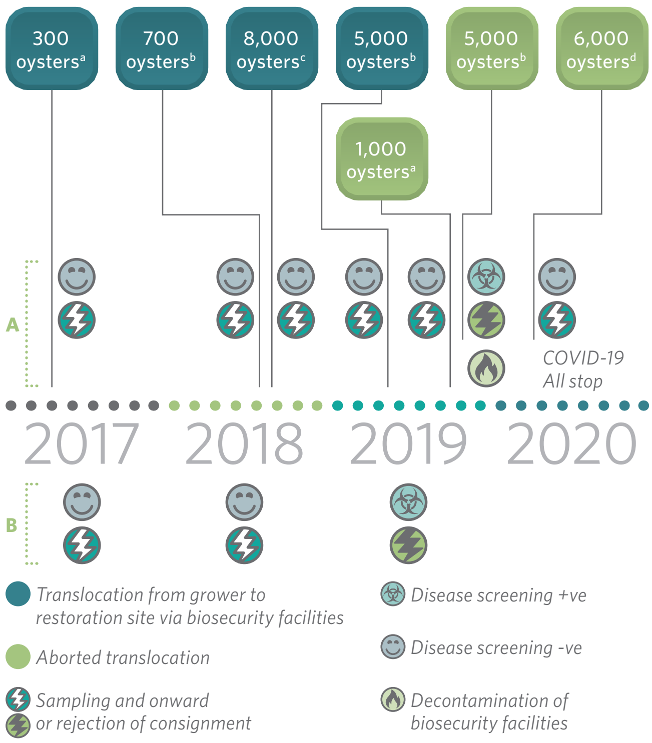


Figure 1.7: Schematic of biosecurity disease-screening activity of a restoration project based on a redacted but real case study. A = independent project-based testing of consignments translocated between oyster growers and the restoration site (via closed-circuit biosecurity holding facilities). B = project-based confirmatory testing of restoration site. All project testing (A&B) in addition to favourable (for disease) statutory government testing and accreditation of suppliers. Despite screening of all consignments, confirmatory annual screening of restoration site and rejection of consignment that tested positive for *Bonamia ostreae*, the restoration site tested positive in 2019 for said disease. Superscript letters indicate the four different suppliers.

BOX 1.3: EXAMPLES OF INTERNATIONAL, NATIONAL, AND SUBNATIONAL LEGISLATION AND GUIDELINES **RELATING TO DISEASE MANAGEMENT**

International:

Marine biosecurity has an international legislative framework: The European Union Member States, Council Directive 2006/88/EC (24/10/2006) sets out animal health requirements for aquaculture animals and products, and on the prevention and control of certain diseases in aquatic animals. The OIE Aquatic Animal Health Code (2019) provides standards for the improvement of aquatic animal health worldwide and the Regulation (EU) 2016/429 ('Animal Health Law') sets rules to control transmissible animal diseases and that have broad impacts on public or animal.

National:

UK – The Aquatic Animal Health (Scotland) Regulations 2009, AAH (England and Wales) Regulations 2009, and AAH (Norther) Regulations 2009 implement Council Directive 2006/88/EC (as amended) in the UK. NB: EU Directive 2006/88/EC will be replaced by Regulation 2016/429 from April 2021. Some useful advice on Marine Biosecurity Planning, INNS and marine diseases can be found at NatureScot and CEFAS.

Sweden – The translocation of organisms for aquaculture purposes (which also governs translocations of wild populations) is governed by the fisheries law (2 kap. 16 § Förordning (1994:1716) om fisket, vattenbruket och fiskerinäringen) complemented by SJVFS 2014:4 2 kap 7-10 § Statens jordbruksverks föreskrifter om djurhälsokrav för djur och produkter från vattenbruk and Fiskeriverkets föreskrifter (FIFS 2011:13) om utsättning av fisk samt flyttning av fisk i andra fall än mellan fiskodlingar. The general rules are that permission for culture and translocations cannot be granted for alien species, organisms with a contagious disease and some more specific cases.

Subnational:

On a regional level, Inshore Fisheries and Conservation Authorities or communities may produce Biosecurity Action Plans to manage shellfish (e.g. North western Inshore Fisheries and Conservation Authority Biosecurity Plan).

CHAPTER 2
BIOSECURITY GUIDELINES FOR NATIVE OYSTER AND CULTCH TRANSLOCATION

CHAPTER AUTHORS
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INTRODUCTION

Given that all translocations carry with them a risk of accidental introduction, it is important that **avoiding the risk by avoiding translocations be considered in project planning**. If projects decide to proceed with translocations despite the inherent risks, comprehensive protocols and actions to mitigate and reduce the risks presented should be developed on the project level. **It is critical that the relevant authorities are informed of all planned activities and projects seek advice from, and work in partnership with, the relevant authorities throughout the project**. Furthermore, projects should ensure that all required permits are obtained before the initiation of any restoration activities. Projects should seek to exceed the legally mandated standard, because the current framework was not developed with restoration (and hence the permanent placement) of native oysters in European waters in mind. And because the central aim of ecological restoration is to support ecosystem recovery and improve the environmental status of European waterbodies, which demands that a rigorous approach be applied.

Oyster habitat restoration in Europe is still in its infancy and the science to support best practice protocols has yet to be fully developed. This chapter seeks to outline the many considerations in planning a translocation activity and to provide some examples of solutions that have been used in oyster restoration to date. **While this chapter seeks to outline the considerations and provide some detail of existing practice, it is critical that project managers work with the relevant authorities to develop appropriate protocols in each case, and that validation of the efficacy of the actions undertaken are assessed on a case by case basis**. Project managers should be transparent about their protocols and share where possible, relevant validation data regarding the efficacy of the protocol. In this way the community of restoration practitioners can work together to refine approaches to ensure they are as effective and cost effective as possible.

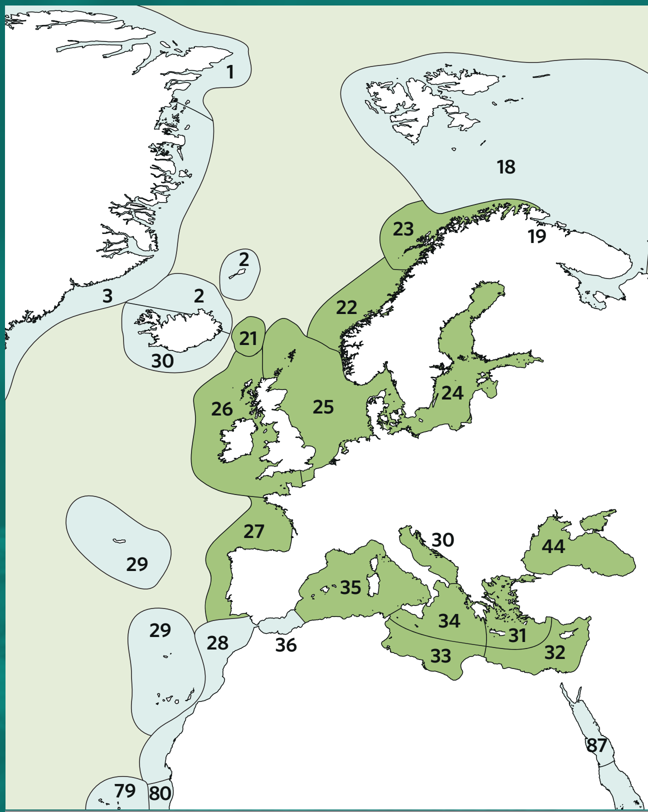

Figure 2.1: Marine ecoregions map adapted from Spalding *et al.* 2007. The known biogeographic range of *Ostrea edulis* is shaded in green. *Ostrea edulis* will only exist in areas within these ecoregions with suitable abotic and biotic habitat characteristics.

Considerations before translocation

It is critical when considering a translocation the following questions are addressed:

1. **Is translocation necessary?** Consider why translocation is the best option. Are there local stocks that could be used? Can the project timeline be adapted to allow for the use of 'on-site' or biosecure hatchery reared stock or local spat collection? If possible, do not translocate oysters.
2. **Are there local sources?** Identify potential local sources and if possible, use oysters from local sources and environments.
3. **If translocating, use the following hierarchy in selecting donor material to minimise risk:**
   i. **Do not consider donor sites outside of the historical native range of *Ostrea edulis* (see Figure 2.1).** While there are now populations of *O. edulis* outside of its native range (e.g. on the east coast of North America), reintroducing oysters from outside its native range should be avoided at all costs. This is to avoid the potential introduction of non-native species and diseases associated with the native oyster in its introduced range. As an illustration of the risk, the European presence of more than sixty species, native to the Pacific Northwest USA, can be attributed to movements of the Pacific oyster since the 1960's alone.

   ii. **Do not consider donor sites with high-risk invasive species or diseases that are not present at the receiving site.** Moving an oyster from an area where a listed disease is known to be present to a disease-free area is illegal where disease controls are applied. Similarly, releasing known INNS into the wild is a criminal offence in some countries. This aside, the ecological and societal risk of introducing either a disease or high-risk INNS into an area is unacceptable, given the possible impacts such action could result in.

   iii. **Minimise the physical distance between the donor and receiving site.** To reduce the risk of unknown diseases or INNS being introduced to an area, it is best to reduce the physical distance between the donor and receiving site. This may also have the additional benefit when moving live oysters, of maintaining any local or regional genetic structure in the oyster population (see Box 2.1).

   iv. **Avoid movements across latitudinal gradients.** The native oyster can be infected by a large number of pathogens (see list in Chapter 1). Within their co-evolved range and the local temperature regime, pathogens may have limited impact on their host. There is, however, a risk that pathogens may become more virulent when moved to a different environment (see Figure 2.2). As it is not possible to know which diseases may have an impact in the novel environment, and it is in any case challenging to screen for all known diseases, movement of oysters to a largely different environment is not recommended.

BOX 2.1: GENETIC IMPLICATIONS OF *OSTREA EDULIS* TRANSLOCATION

Human-mediated translocation of either wild or hatchery-produced *Ostrea edulis* has long occurred within the fisheries context but has, in the past decade, also been applied as a conservation management strategy for endangered species. The movement of live oysters from one location to another may pose an environmental risk due to not only the spread of diseases/pests, but also the genetic erosion of wild populations.

Translocation may induce an increase in genetic diversity in recipient populations, by mixing genetically divergent populations, and may reduce genetic divergence among geographically distant populations, hence possibly reducing the existence of adaptation to local conditions and consequently overall population fitness in recipient area populations. The impacts of translocation are therefore an important issue for the management of exploited or endangered species.

The selection of local sources should be prioritised, both to reduce the risks of accidentally introducing diseases/pests and to maintain genetic structure and adaptations present in the local population. Where the use of local stock is not an option, practitioners should consider genetics when selecting donor stock. The genetic structure for the native oyster at local and regional scales is currently the subject of ongoing investigation. It is therefore not included further in this report, but rather it is recommended that the current scientific literature be examined when seeking donor material.

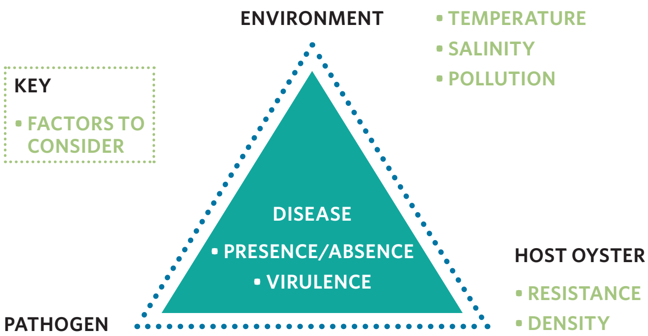

Figure 2.2: The disease triangle: The occurrence and severity of disease is a product of the host and pathogen co-occurring, and the environmental conditions being suitable for disease outbreak. Movement of oysters from one environmental regime to another therefore carries the risk that novel environmental conditions may favor the pathogen and that a previously subclinical disease in the oysters may become problematic.

Figure 2.3: Comparative survey of donor and recipient sites to establish INNS and disease transfer risks by W.G. Sanderson, DEEP Project. Photo: Richard Shucksmith.

Translocating live oysters

If translocation is necessary and potentially appropriate donor material has been identified, the next step is to undertake thorough biosecurity measures, under advice from the relevant authorities, to reduce the risk of accidental transfer of species. Initial risk assessments should be undertaken in order to understand the risk and map out the appropriate action. Assessment of risk should include consideration of ongoing activities in both the donor and receiving site.

It is critical that adequate time for completing comprehensive biosecurity measures is planned into the project. For example, undertaking field surveys to assess the suitability of donor sites should ideally take place in the summer, when species are most abundant and therefore likely to be encountered and identified (see Figure 2.3). Additionally, the time required to physically clean and screen oysters should be accounted for, as this can be a rate or scale limiting step. As an indicator, substantial epifaunal growth can mean that it takes one person one hour to clean 100 oysters. Projects should not seek to translocate a greater number of oysters than they have time to clean and check thoroughly. Translocating large numbers of oysters is an arduous and time-consuming process, and translocating more individuals increases the risk of unintended introductions. These realities should be considered when planning translocation activities.

Undertaking a risk assessment

The first steps in any risk assessment is risk identification or mapping and analysis. This may be facilitated by classifying risks according to different criteria, e.g. local/regional/global extent, continuous/instant, manageable/unmanageable, internal/external. The identified risks should then be analysed regarding likelihood and consequence. Moreover, to rank risks, they must first be comparable. This can be achieved through literature reviews and potentially expert consensus round table processes where the identified risks are prioritised and weighted. The different weights can then be combined in a structured way. High ranking risks, i.e. above the acceptance levels given by the risk evaluation, will proceed to treatment/management. As a rule of thumb, negative risks with high likelihood and low consequence should be treated with preventive measures, low likelihood and high consequence should be treated with mitigating measures and very high/catastrophic consequences should be avoided (see Cook *et al.* 2014 for further guidance). Bear in mind that risk assessment should be an ongoing, iterative, and adaptive process (see Figure 2.4).

Prior to translocating animals, it is important to consider the disease status of both the donor and recipient sites. Comprehensive existing OIE, EU and local regulation surrounding the testing, movement and monitoring of pathogens and disease should be adhered to as an absolute baseline with regards to decisions on movements. Details of legislation in EU areas is located on the European Commission website (see Box 1.2 and Box 1.3 in Chapter 1).

Recommended routes to compile relevant data for the donor and recipient site include:

- Search the confirmed designation notices for notifiable diseases and work with the competent authority to ensure that the site is a candidate donor site under animal health law and to verify compliance with the relevant aquatic animal health regulations.

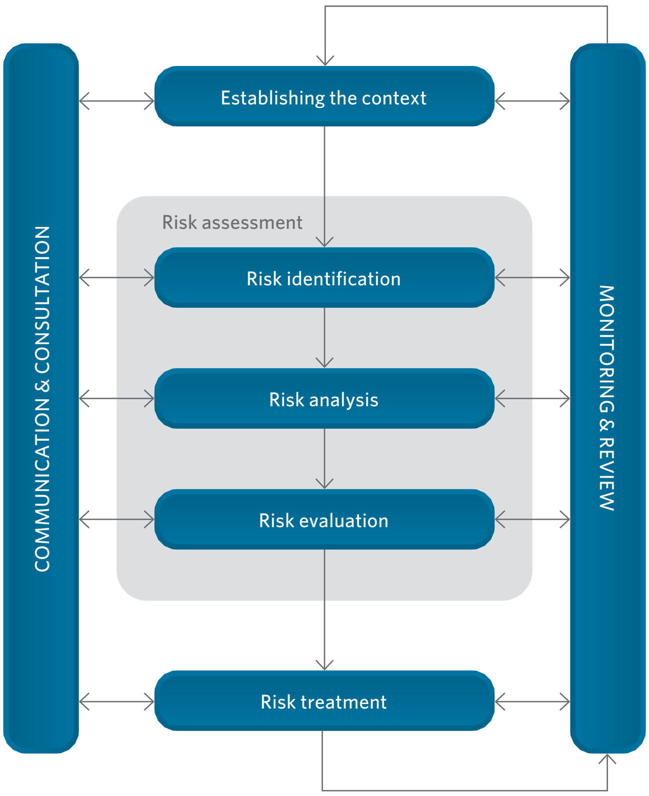

Figure 2.4: An overview of the steps required in undertaking a risk assessment. Risk assessment is an ongoing process and information derived from operations should be fed back to ensure that the assessment remains current.

BIOSECURITY MEASURES PLAN SCHEMATIC

1. Online assessment of INNS and notifiable shellfish diseases described in donor and recipient sites.

Websites to consult:
WoRMS, MarLIN, CABI, NBN atlas, NEMESIS

- INNS present at donor equal to or less than recipient.
- Disease status of donor site equal or less than recipient.

- High impact INNS/disease present at donor but not recipient.

No movement of oyster to take place.

2. Physical assessment of INNS and notifiable shellfish diseases at donor and recipient sites.

Particular attention paid to shellfish themselves.

- INNS present at donor equal to or less than recipient.
- Disease status of donor site equal or less than recipient.

- High impact INNS/disease present at donor but not recipient.

No movement of oyster to take place.

3. Plan to receive oysters from donor site.

Physical biosecurity measures put in place:

- Oysters to be monitored for INNS and signs of mass mortality.
- Oysters to be cleaned appropriately to remove epibionts.
- Water used to clean oysters either to enter the origin site or treated appropriately with chlorine or other.
- Plan for short-term contingency storage area if conditions prevent deployment.

- Epibionts removed or killed to satisfactory level so that risk of INNS transfer is removed.
- No signs of mass mortality.

- Individuals with compromised shells discarded at donor site.

- High impact INNS/disease present at donor but not recipient.

No movement of oyster to take place.

4. Receive oysters from donor site.

Transport oysters in most suitable conditions:

- Forecasted weather to be cool and unlikely to cause delays in marine vessel travel or travel by road.
- Transport to occur at a time of day as to avoid long traffic delays i.e. over night.
- Oysters to be transported in a damp and cool environment.

- No evidence of further epibiont growth.
- No signs of mass mortality.

- INNS observed.
- Large mortality event occurring during transport.

No deployment of oyster to take place.

5. Deploy oysters to seabed.

Figure 2.5: Decision tree outlining the factors to be considered when assessing suitable donor sites and whether to go ahead with relaying oysters into the recipient site. Figure modified from Blue Marine Foundation schematic.

Figure 2.6: Native oysters in hatchery in the Netherlands. Photo: Oscar Bos/Wageningen Marine Research.

- Contact the Statutory Nature Conservation Bodies or regulators directly for any non-native species abundance data from Water Framework Directive or Marine Protected Area surveys.
- Some useful data on non-native species can be found within the JNCC Marine Recorder snapshot from the National Biodiversity Network atlas, within the Federal Agency for Nature Conservation's information portal on alien and invasive species in Germany, SWAM (Swedish Agency for Marine and Water Management), or within the French National Inventory of Natural Heritage and in sources listed in Chapter 1.
- Consider which non-native species are present in areas with high connectivity to the donor areas (e.g. adjacent waterbodies, ports, or bays). Is there a high risk of these spreading into the donor site?

Survey the donor site

Once a potential donor site has been identified, the current disease status of the site should be confirmed through further testing. Legally mandated controls and disease testing, as well as biodiversity surveys, take place at insufficient frequency to ensure that the current disease/INNS status of a site is accurately reflected. Restoration practices should therefore endeavour to undertake their own field surveys and testing to ensure that the risk assessments are undertaken with the most current and relevant information.

Screening for disease

Pathogen screens should be done using recommended methods as specified in the OIE aquatic manual and as recommended by the EU legislation (Commission Implementing Decision (EU) 2015/1554 of 11 September 2015 laying down rules for the application of Directive 2006/88/EC as regards requirements for surveillance and diagnostic methods (notified under document C(2015) 6188). This should include all the notifiable diseases for the native oyster: Bonamiosis (*B. ostreae* and *B. exitiosa*) and Marteiliosis (*M. refringens*), as well as oyster herpes virus (see Chapter 1 for list of notifiable diseases). Sample sizes should follow or exceed those recommended OIE aquatic manual and EU legislation. In the aforementioned decision there are specific recommendations about the surveillance and diagnostic of *B. ostreae* and *M. refringens*. In addition to screening listed pathogens, general screening based on histology and bacteriology should be implemented (see EURL SOPs). Consideration should also be given to diseases which are not listed. Attention should therefore be paid to the general health of the oysters and the recent history of mortality at the donor site.

The OIE manual is a ready source of methods for screening, but it should be noted that this document can take considerable time to update and that there may be more appropriate, recent methods available in publication. When possible, the diagnostic analyses should be carried out by internationally recognised laboratories. In addition, it is prudent to use the appropriate national guidelines for disease monitoring to determine the number of individual samples required to 2nd para, sought from the relevant National Reference Laboratory.

Surveying for INNS

When undertaking the biodiversity survey to inform the project risk assessment, particular care should be paid to potential and high-risk INNS. As INNS include a full range of species with differing life histories, no one sampling protocol will be best suited to all potential species of interest. Project managers should therefore consider using a range of methods that cover: 1. species that are likely to have low densities and are dispersed and 2. species that are likely to have higher densities and/or be less patchily distributed. For example, grab sampling nested within drop down video may be suitable for some locations, whereas where the predominant habitats are dominated by epibiota, *in situ* surveys are more suitable, as some high risk species such as *D. vexillum* (see Figure 1.3 in Chapter 1) or *Styella clava*, are difficult to identify on video tows where there is already substantial epiphytic growth.

For detailed assessment of benthic fauna, a standard methodology (e.g. 0.1m^2 Day or Hamon grab, with samples sieved over 1mm sieve, and/or a drop-camera/towed video survey of the seabed) is commonly used by statutory bodies. Project managers can use the JNCC Marine Method Finder to identify suitable monitoring approaches for each habitat.

While biodiversity surveys, in particular in the intertidal, are a good way to engage local volunteers, it is critical that those undertaking the survey are trained to identify all the potential problem species and, equally, have enough knowledge to recognise species that 'don't-look-right' (i.e. a previously unrecorded INNS). This is because there are INNS we do not yet know about (cryptic and/or recent introductions). Furthermore, whilst positive identification for some species can be undertaken during the survey, project managers should ideally also collect samples for a full assessment of non-native species using lab-based specialist benthic taxonomists.

Once the site surveys have been undertaken, the initial risk assessment should be revisited with the updated information in mind. If an aggressive INNS such as *D. vexillum* or a notifiable shellfish disease is recorded at the donor site, then oysters should not be translocated from that site (see Figure 2.5). Should other non-native species be identified from previous data or surveys of the donor site, then a marine biosecurity plan should be written to identify measures that can reduce the risk of those non-native species being introduced. This may be required by regulators before consent is given for the translocation. Guidance on authoring such a plan can be found in Cook *et al.* 2014 (see resources).

Figure 2.7: Individual inspection of each oyster in closed biosecurity holding facilities. Oysters translocated to the restoration site after disease screening, cleaning, surface sterilisation and UV depuration. Photo: Phil Wilkinson/DEEP project.

PREPARING ADULT OYSTERS FOR TRANSLOCATION

Physical cleaning of oysters

If the origin and donor sites have been deemed suitable by the preceding steps, the oysters obtained for translocation should be first inspected and then physically cleaned to ensure no visible epibiota persists (see Figure 2.7). This process should be completed at the donor site pre-transport to ensure no epibiota is transferred elsewhere. It may be necessary to require suppliers to cost for this activity, which may be different to their normal aquaculture practices and restoration practitioners should therefore expect such oysters to be comparatively more expensive. Wastewater from the cleaning can be disposed of at the origin site rather than being transferred elsewhere. It is also recommended that treatment and transport of oysters takes place in the late autumn to late winter to minimise epibiotic growth.

As part of the visual inspection, a record of species present and in what number on the oyster shells should be recorded pre- and post-treatment. This is not only helpful as an audit trail to demonstrate statutory compliance but could contribute to the evidence base for best practice of future restoration work. Note that oysters with associated heavy infestations of boring sponges (e.g. *C. celata*, see Figure 2.8) will have holes which can be difficult to clean. Heavily undermined shells with many crevices should be discarded along with other oysters with physically compromised shells. These should be discarded responsibly at the donor site. If further fouling is found at a later stage, or if cleaning must occur remotely, material should be disposed of responsibly. Under circumstances of enhanced risk, disposal should be to a specified biological waste disposal route (possibly including incineration). During cleaning, care should be taken to ensure that there are no small bivalves hidden in the hinge-line of the oysters such as spat *Mytilus* (that could be the INNS *Mytilus trossulus* in some scenarios).

Physical cleaning can be done by hand (scrape/scrub off) and/or mechanical methods, such as cement mixers or shellfish cleaning machines. If using mechanical methods, large oysters can be tumbled in batches and so it is a more time-effective procedure for a large number. This treatment may not be suitable for smaller oysters.

Figure 2.8: The exterior (left) and interior (right) of a native oyster infested by a boring sponge (*Cliona celata*). Photos: Luke Helmer.

While cement mixers have been shown to be successful at removing epibiota in existing projects, they also found some or parts of organisms, such as holdfasts, may persist. Repeat treatment may be required. It is critical that, if mechanical treatment (as opposed to cleaning by hand) is undertaken, a large sample size of the treated oysters be examined by hand in order to determine that the epifauna have been effectively removed.

Following physical cleaning, oysters should be left to recover in running filtered seawater for a minimum of three days before undergoing chemical treatment. Subjecting them to immediate chemical treatment would put oysters with chipped shells at risk of unnecessary exposure and may result in increased oyster mortality. During this time, oysters also have the opportunity to "depurate" some of their internal microbiota. Disposal of water used in this phase should therefore be subject to biosecurity and chemical pollutant risk assessment and where necessary, treatment before disposal.

CHEMICAL TREATMENT OF OYSTERS

The purpose of chemical treatment is to kill any shell epibiota that may have survived the physical cleaning of the oysters and therefore reduce the risk of INNS transfer (see Figure 2.9). Remaining epibiota might include scraps of clonal organisms such as sponges, sea squirts or certain types of seaweed, as well as hardy spores and resting/reproductive stages of other organisms. As well as the oysters themselves, some organisms such as keel worms, barnacles and other bivalves can clamp-shut to avoid ingress of fluids: they are therefore theoretically able to survive the chemical treatment just as well as the oysters. Care should therefore be taken in the physical cleaning stage to make sure that the tubes of keel worms are removed or broken open, that barnacles are removed or broken open.

Various chemicals have been used for the surface sterilisation of oysters and they range in their expense and availability, including hypochlorite, formaldehyde, and commercial fish-farm treatments such as Virkon. There is not a clear evaluation of the relative effectiveness of different treatments, but the obvious abiding principle is that it should be toxic to the epibiota in the concentration and exposure time used. Exposure-times can vary, and bulk dunking methods have been used, akin to the use of a chip-fryer-basket. Dunking methods may be preferable and efficient with younger oysters (e.g. 10g) because the shells appear to seal-shut well. Sponging oysters with the chemical treatment (whilst using appropriate Personal Protective Equipment) might be deemed more appropriate in larger adult oysters where the gape of the shell may be worn or damaged and therefore less likely to seal well if fully submerged in a chemical bath.

Turrell *et al.* 2018 undertook a thorough review of the literature regarding chemical treatments of oysters in order to develop recommendations for moving *C. gigas* from an aquaculture site with a high risk INNS (*D. vexillum*). A complete review of the tested options and the resulting impacts on the target INNS and the shellfish are provided in their report. The method recommended for field tests as a result of the review was immersion in freshwater (salinity < 2ppt) for at least 24 hours.

Quarantine

Once oysters have undergone both physical and chemical external cleaning, it is important that the efficacy of the biosecurity protocol is quantitatively assessed and that internal contaminants are given an opportunity to be expelled. A quarantine period should therefore be imposed, during which the oysters are given time to recover from the treatment under controlled conditions, given time to be depurated of internal microorganisms, and can be monitored to assess the efficacy of treatment thus far. Given that it is not yet known whether the oysters have been successfully cleaned, water used in this period should be handled as potentially high-risk waste and should be disposed of accordingly. At this stage, oysters may be kept in closed circulation or flow through systems. Filtered water from the receiving site may be used for this stage. Ideally moderate numbers of oysters should be kept in each tank, and tanks should not share water circulation. In circumstances of high risk, it may also be desirable to use artificial seawater and u/v recirculation systems to 'flush' in-shell water and

Figure 2.9: Intermediate storage of scraped and rinsed oyster broodstock before their chlorination bath. Photo: Bérenger Colsoul.

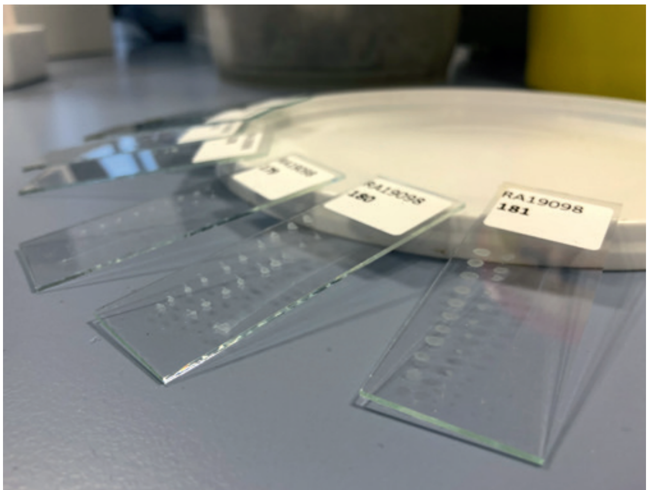

Figure 2.10: Smears of native oyster's heart for *Bonamia* screening. Photo: Chantelle Hooper.

decontaminate the oysters from waterborne organisms (**note:** "decontamination" will not "flush out" diseases). During this time individual batches of oysters can be screened and assessed independently (see Figure 2.10). The bottom of the tanks should be checked daily for evidence of recently dead organisms that may have crawled out of crevices or from within the shells. Anecdotally, it seems that slightly reduced salinities may encourage this process. Should any evidence of living associated biota be found, oysters should be subjected to further treatment. It should be noted that currently there is insufficient evidence regarding the effective duration of a quarantine period, but project-experience suggests that remnant live epibiota may take 3 days to emerge under these conditions, therefore a shorter period is not recommended.

Preparing spat for translocation

In the case of translocation of spat (settled larvae > =2mm or juvenile oysters < 10mm), it should be considered that the sensitivity of the young oysters may mean that many biosecurity treatments (both physical and chemical) are inappropriate and locally sourced spat from sites with the same (or better) disease and INNS status may be the only appropriate option. The relevant authorities should be informed throughout the process.

Spat on shell (or other substrate) is likely to have less well-established biofouling relative to adult oysters, having spent less time in the water. However, as young oysters are more sensitive to both physical and chemical cleaning methods, and the shell or substrate can be a vector of unwanted organisms, the potential risks associated with translocation of spat on shell are great. Therefore, as dictated by the precautionary principle, **spat which have been in contact with open water prior to translocation, should only be translocated locally**. Nevertheless, it is critical that the following ground rules be followed for all translocations: Without exception, animals must only be moved to recipient sites from donor sites with equal or higher health status. For example, moving native oysters from an OsHV-1 µvar positive to negative site, or moving animals from an area with close proximity to a Bonamiosis positive zone, should not take place.

Finally, it should be noted that cultch used in wild settings for translocation should be subject to the cultch biosecurity protocol below before being placed in the spatting pond or other structure.

DEPLOYING OYSTERS

Once all the steps have been taken to identify possible biosecurity risks and to address identified risks, it is critical that the effectiveness of the measures is assessed prior to oysters being relayed into the receiving site. Only when the restoration practitioner and that relevant authorities are confident that the associated risk is acceptable, should the translocation be completed (see Figure 2.11). Figure 2.5 provides an overview of the steps at which risks should be assessed.

Practice due diligence

There is currently no method that, when applied, renders living oysters completely biosecure for translocations. Although general protocols for cleaning aquatic organisms exist (see resources for some examples), protocols suitable for relaying of live oysters for ecological restoration have yet to be tested and confirmed effective. There are outstanding knowledge gaps regarding the efficacy of possible treatments, in particular if efforts involving translocations are scaled up. **It is therefore critical that each translocation attempt validates the efficacy of the biosecurity measures undertaken with a thorough screening of the treated oysters. The sample size should be large enough to ensure a high degree of confidence that the consignment of oysters has met the desired biosecurity standard.** Screening for epifauna should, as a minimum, involve visible examination of the shell and hinge. Screening for associated biota should as a minimum involve examination of the base of quarantine tanks for signs of recently emergent and dead individuals for several days.

Figure 2.11: Transport and relaying of oysters during restoration project. Photos: Åsa Strand.

While disease screening is one of the first steps undertaken in determining whether the stock is suitable for translocation from an approved donor site, a further and final screening for diseases may be undertaken before the stock are released into the wild. The rationale for this further final testing is that it is possible that oysters start to express the disease when under stress (e.g. having undergone treatment). Therefore, tests taken towards the end of the quarantine period may pick up disease presence overlooked in the initial stages.

Translocating cultch

When considering cultch translocation, it is critical that the following questions are posed.

1. **Is translocation necessary or are there local sources?** If possible, use cultch from local sources and environments to reduce the risk of introducing novel diseases or species.
2. **If translocating, consider the following factors when determining where to source cultch:**
   i. Do not accept any cultch from donor sites with high-risk invasive species or diseases are not present at the receiving site.
   ii. Use appropriately 'weathered' land-based or cooked/heat treated sources where possible (see Figure 2.12 and Figure 2.13).
   iii. Ensure that all cultch material is safe with regards to heavy metal content and other toxins.
   iv. Minimise the physical distance between the donor and receiving site.
3. **What is the waste designation and associated legislation for selected cultch?** If the cultch material is shell, cooked or otherwise, it will be necessary to weather the shell to ensure that all residual biology is rendered inert. There may be legislation pertaining to the storage of shell and the use of "animal waste". Check with the relevant authorities regarding waste management regulations.

Risk assessment

The first step in assessing the appropriateness of the identified source of cultch, is to undertake a risk assessment. If sourcing cultch from a known marine location, a survey of the cultch donor-site should be undertaken, following the same protocol as outlined for donor sites of live oysters (see above). For example, cultch material from a location with a known notifiable disease or high impact invasive species should only be considered for use in a site with a similar or lower status.

Clutch can also be purchased from aggregate suppliers. In such cases either the exact origin of the cultch may be unknown, or impractical to survey. In these cases, the supplier should be asked to provide information regarding whether the cultch material has been heat treated or weathered for any length of time or if any kinds of contaminants (heavy metals, organic chemicals, etc.) are present. Where this information is unknown, the material should be treated as though it were freshly extracted, and appropriate treatments applied.

The case study from the Essex Native Oyster Restoration Initiative (see Box 2.3) provides further details project managers may want to consider.

Treatment of cultch

Non-local and marine derived cultch material (shells or stones) must be treated in order to ensure that living marine organisms, spores or resting stages are not unwanted contaminants of the material. What is deemed suitable treatment should be agreed with the relevant authorities. One cost effective means is to weather (expose to the elements) the material for a minimum of 12 months, turning the shells every two months where material is deposited < 15m height, and twice monthly if deposited more deeply.

Assessment of effectiveness

As with all other stages of biosecurity practice, it is the responsibility of the project to ensure that the treatment has been effective in removing any unwanted organisms and spores. This may include visual examination of the material. As a general guideline, material should be weathered until there is no evidence of residual biology remains, dried or otherwise. An effective method of assessment and the appropriate sample size for assessing the status of the clutch material should be agreed with the relevant authority, whereas the methods outlined above have been shown to be effective under some circumstances, there is a limited scientific basis for establishing exact and reliable guidelines. There is therefore an emphasis throughout this document on practitioners assessing each step of the process. In order to optimise treatments moving forward, and promote cost effectiveness in restoration, further research is needed.

BOX 2.2: CONTRIBUTE TO IMPROVED BIOSECURITY GUIDELINES

Rendering living oysters' low risk for translocation from a biosecurity perspective is costly both in terms of time and money. The efficacy of actions and the investment required, in particular with regards to time and manpower, is not well documented. We therefore urge projects to submit their experiences to the NORA Secretariat or the Native Oyster Network – UK & Ireland.

Figure 2.12: Scallop shell weathered for a year and with no residual biology. Photo: Bill Sanderson, DEEP.

BOX 2.3: CASE STUDY: ESSEX NATIVE OYSTER RESTORATION INITIATIVE, U.K.

The Essex Native Oyster Restoration Initiative (ENORI) is a collaboration working towards the Essex estuaries having self-sustaining populations of native oysters. The restoration location is a designated Natura 2000 site and is a nationally important Marine Conservation Zone (MCZ). The MCZ is both substrate and recruitment limited for oysters. The project is unusual in benefitting from an adjacent oyster fishery that provided a locally-adapted broodstock, so translocation from further afield was not necessary. It was, however, necessary to translocate cultch from outside the waterbody to the restoration site. *B. ostreae* is present at this site.

Aggregate gravels were used to provide elevation off the seabed with shell cultch added as a top layer (see Figure 2.14). As it was not possible to confirm the source of marine gravels, a land-source aggregate was chosen from River Terrace Deposits quarried locally to the restoration site. For a local source of shell, a shell recycling initiative was set up to return the shell of the local fishery oysters. This was considered the lowest biosecurity risk for cultch translocation as the shell was originally removed from the same waterbody as the restoration site. However, there is a limited supply of local oyster shell and so other options were considered to achieve the volume of cultch required. Scallop, cockle, and blue mussel shell were sourced from national and European suppliers. Dialogue with suppliers was critical to confirm (where possible) the exact source of the shell and to understand risk. When shell is bought from an aggregate company, it should be noted that it is often not possible to know with certainty the geographical source as they are the 'middle-man' of the supply chain. Information such as how the shell had already been treated (if by heat for commercial shellfish processing, at what temperature and for how long) and how it was stored (location and duration) was also ascertained from suppliers.

Although this information was gathered where possible, it was agreed by ENORI that any shell (regardless of source and heat treatment) should be weathered outdoors, exposed to the elements, for a period of 12 months to ensure as far as possible that hitch-hikers and pathogens would not persist. Samples of the shell were visually inspected for living matter.

It is advised to secure storage sites as early as possible to avoid delays to deployment or multiple cultch handling and transport costs. The volume of cultch required can be substantial and to store it in a relatively thin layer and turn bi-weekly or monthly is a considerable undertaking. The resources (space, contractors, vehicles and potentially volunteers) that are required to deliver this should be considered as early in the planning process as possible and, importantly, built into project budgets.

Throughout the planning of the pilot restoration works, ENORI sought advice from shellfish health and INNS experts to ensure that the most appropriate risk management approach was adopted. On the advice of Cefas, a project record was set up that listed all the aggregate and shell used, the source, the treatment prior to delivery, the duration and location of storage, rotations and any risks associated with source and the restoration site. This was a valuable exercise for ensuring the appropriate steps were being taken by the project to minimise biosecurity risk and for any audits that may take place in the future.

Figure 2.13: Cultch for substrate enhancement, being weathered outside awaiting deployment. Photo: ENORI/ZSL.

Figure 2.14: Deployment of gravel cultch by Essex Native Oyster Restoration Initiative, UK. Photo: ENORI/ZSL.

CHAPTER 3:
BIOSECURITY GUIDELINES FOR EUROPEAN NATIVE OYSTER HATCHERIES

CHAPTER AUTHORS
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INTRODUCTION

Hatchery supplied oysters can be an alternative to translocation of oysters from oyster fisheries or spatting ponds. Hatchery supply introduces the advantage of oysters coming from controlled conditions, sterilised water and a pathogen free or known pathogen environment. Any oysters that leave the biosecure zone of the hatchery before being moved, i.e. if they have had contact with the external waterbody, should be considered translocations. The purpose of this chapter is twofold. First, it is intended to provide those seeking to purchase stock from a hatchery with the information required to understand the biosecurity issues relating to hatcheries. This is intended to help the project manager pose the relevant questions and understand the biosecurity status of the purchased stock. Second, this chapter is intended to assist those seeking to establish their own hatcheries in understanding the associated biosecurity requirements. Please note that not all steps outlined here are necessary at every location. While all steps should be considered, the decisions about which are applicable should take into account the local environmental conditions and activities, e.g. the local disease status and the disease status of the intended receiving site. These guidelines are intended for use in oyster restoration activities, and were not developed for commercial aquaculture activities, not seeking to supply the restoration market.

The Native Oyster Restoration Alliance (NORA) and Native Oyster Network – UK & Ireland (NON) and the European Aquaculture Society have stated that the limited availability of appropriate seed represents a limiting factor for the progress of many native oyster restoration projects across Europe. Where no reliable and large sources of wild seed are available and cannot be developed (e.g. through spatting ponds), reef restoration depends on seed brought in from different sources. This demand can be addressed by hatchery production. A hatchery is a farm where fish or shellfish are spawned, hatched, and kept until they are large enough to be transferred to grow out systems. Bivalve hatcheries have existed for over half a century and they are currently well-established in several countries.

Most of the global marine bivalve production (89%) comes from aquaculture while only 11% comes from wild fishery. Hatcheries can provide seed not only for aquaculture, but also for restoration purposes. Relaying of hatchery-produced seed, either set on shell or as singles, can supplement existing populations and contribute to shell reserves through growth, which in turn supports larval settlement and the recovery of natural populations.

If considering hatchery-produced seed, project managers should also consider that small seed are the cohort that suffer the highest mortalities, and that either large numbers of spat will be required, or that spat may require protection and support for a grow out phase in the receiving water body before being relayed to the reef. The choice will depend on the relative cost of newly settled spat compared to the cost of growing them to the larger size, and whether there are grow-out opportunities and appropriate infrastructure at the receiving site. Consideration should also be given to the genetic status of the hatchery reared stock (see Box 2.1). See European Native Oyster Habitat Restoration Handbook (Preston *et al.* 2020) for details on restoration techniques.

Considering the risks posed to native oysters, associated species and ecosystems through diseases or invasive non-native species (INNS) introductions, hatchery biosecurity must be prioritised and implemented. Hatchery production contains complex biological processes: broodstock (adult) conditioning and spawning, larval rearing (see Figure 3.1) and setting, and optional seed rearing to a larger size before delivery. Hatcheries usually also include extra facilities for the production of large quantities of microalgae to feed all stages of the production cycle. It is essential to be aware that diseases can affect any process and level of hatchery and farm operations.

Effective biosecurity is the basis for any successful production system as it reduces production risks, minimises problem-solving costs and improves production outcomes. Furthermore, disease prevention not only protects businesses, but also has wider benefits for the environment and for communities potentially devastated by a significant disease outbreak.

Biosecurity Measures Plan (BMP)

All aquaculture production businesses (APB's), including hatchery operations, must be authorised by the relevant authority, irrespective of scales of production. Licensing and permitting procedures depend on the respective hatchery characteristics such as site, region, species farmed, aim and scale of production.

An essential element for the authorisation process for new APB's or the renewal of existing licenses and already authorised APB's is the approved Biosecurity Measures Plan (BMP). The BMP describes defined measures to prevent or reduce the risk of introducing diseases/pests into the hatchery, spreading diseases/pests within the hatchery or the transferring diseases/pests from the hatchery to the aquatic environment. The BMP is reviewed and approved, including a site inspection, by the relevant authority. Regular inspections take place at predefined intervals to ensure that the hatchery is operating within its authorisation conditions and as defined within the BMP. It is critical that anyone establishing a hatchery is aware of the local requirements for the BMP. Understanding the structure of the BMP will also help the restoration practitioner understand the biosecurity information that is available and how to access it.

The BMP identifies and classifies diseases/pests and associated risks for site operations and oyster movements, providing the respective risk mitigation measures, via three steps:

1. Identification of major routes for potential disease/pest transmission in oyster hatcheries.
2. Risk assessment for each disease/pest transmission route.
3. Definition of measures to minimise the risk of disease/pest transmission.

Major routes of disease transmission

The identification and assessment of major routes (see Table 3.1), through which potential diseases/pests can be transmitted, considers three transmission levels:

- **Entry-level** – Transmission of disease/pest into the hatchery.
- **Internal level** – Transmission of disease/pest within the hatchery.
- **Exit-level** – Transmission of disease/pest from the hatchery to the environment.

Each level will consider the transmission potential of:

- Livestock i.e. broodstock, larvae, spat.
- Feed e.g. microalgae (cultures, concentrates).
- Water i.e. intake, discharge.
- Equipment and rearing infrastructure.
- People i.e. staff, visitors.
- Settlement substrates e.g. shells, sandstone reefs.

Table 3.1: Overview of potential disease/pest transmission routes in oyster hatcheries.

LEVEL OF TRANSMISSION	MEANS OF TRANSMISSION	ROUTES OF TRANSMISSION
Entry-level	Livestock	e.g. import of wild broodstock.
	Feed/algae	e.g. purchase of algal paste or starter cultures from external suppliers.
	Water	e.g. intake water.
	Equipment	e.g. admission of gear from outside the hatchery.
	People	e.g. entry to the hatchery by staff and visitors.
	Settlement substrates	e.g. transfer of shells.
Internal-level	Livestock	e.g. movement of broodstock, larvae or spat between production areas.
	Feed/algae	e.g. algal cultures.
	Equipment	e.g. sharing of gear between production areas.
	People	e.g. movement of staff between different production areas.
Exit-level	Livestock	e.g. discard of mortalities.
	Water	e.g. discard of water.
	Equipment	e.g. disposal of wastes.
	People	e.g. exit of the hatchery by visitors.

Figure 3.1: Larval rearing systems: Conical tanks in a marine bivalve hatchery in New Zealand (top). Cylindrical tubes at Ifremer's Argenton research center in France (bottom). Photos: Bérenger Colsoul.

Risks and risk assessment

The risk assessment analyses risks associated with each identified disease/pest transmission route. It includes the investigation and estimation of both likelihood and consequence of disease/pest transmission through each route (see Figure 3.2).

After this process, each risk is assigned to a specific category:

- Negligible (1-2) – No action required
- Low (3-5) – Ongoing monitoring required
- Medium (6-10) – Active management required
- High (12-15) – Intervention required
- Extreme (16-25) – Urgent intervention required

Medium, high, and extreme risks are considered as unacceptable and require implementation of management and intervention measures. Low risks need to be monitored over time. No action is required for negligible risks.

Risk management measures

In order to minimise identified disease/pest transmission risks, different types of risk management measures are defined: e.g. physical (infrastructure and equipment), procedural (production practices and training) or other supporting measures. These routine measures must be implemented in the daily hatchery operations.

Based on the risk assessment, each measure can be assigned to a specific risk category to prioritise the measures (see Table 3.2), in order to provide the highest degree of biosecurity:

- Category A – Failure to implement risk management measures may result in a **critical** risk of disease/pest transfer.
- Category B – Failure to implement risk management measures may result in a **high** risk of disease/pest transfer.
- Category C – Failure to implement risk management measures may result in a **moderate** risk of disease/pest transfer.
- Category D – Failure to implement risk management measures may result in a **low** risk of disease/pest transfer.

LIKELIHOOD RATING \ CONSEQUENCE RATING	*Insignificant*	*Minor*	*Moderate*	*Major*	*Catastrophic*
Remote	1	2	3	4	5
Unlikely	2	4	6	8	10
Possible	3	6	9	12	15
Likely	4	8	12	16	20
Certain	5	10	15	20	25

Figure 3.2: Risk assessment matrix, from Spark *et al.*, 2018.

Table 3.2: Example of BMP structure, summarising routes of disease/pest transmission, risk rating and biosecurity measures for the four different risk categories.

LEVEL OF TRANSMISSION	MEANS OF TRANSMISSION	ROUTE OF TRANSMISSION	RISK OF TRANSMISSION (FROM RISK ASSESSMENT)	RISK MANAGEMENT MEASURE	RISK CATEGORY
Entry-level	Livestock	e.g. import of wild broodstock.	Extreme	Keep broodstock in quarantine (in isolation in separate water and production area with appropriate biosecurity measures) before bringing into the main facility.	Category A (Critical)
Entry-level	People	e.g. entry to the hatchery by visitors.	High	All visitors must complete a biosecurity declaration on arrival to assess risk.	Category B (High)
Internal-level	Equipment	e.g. sharing of gear between production areas.	Medium	Do not move gear between its dedicated area to elsewhere in the hatchery.	Category C (Moderate)
Exit-level	People	e.g. entry to and exit from the hatchery.	Low	Ensure boots worn in the hatchery are not taken outside their designated production area. Visitors and staff to change into hatchery boots before entry.	Category D (Low)

FURTHER REQUIRED DOCUMENTATION

Record keeping

The authorisation conditions for an APB require a minimum level of record keeping. Good record keeping is necessary to demonstrate that biosecurity measures have been followed, in accordance with the hatchery biosecurity plan. In the event of a disease outbreak, these records can be used to trace the potential source of disease. They can also be used to review and improve hatchery practices and protocols. The records must be available for immediate inspection and in a format that can be copied for later analysis.

Three types of record must be taken:

- Movements record, i.e. date of movement, number of individuals, source, and destination:
  - Movement of broodstock to the hatchery.
  - Movement of broodstock, larvae and spat within the hatchery (between different biosecurity/production zones).
  - Movement of spat and adult oysters from the hatchery.
- Mortality record i.e. date, batch ID, number of mortalities, methods of disposal. Any unusual or mass mortality within the hatchery should be reported immediately to the relevant authority.

- Stock health and water quality record i.e. date, batch/treatment ID, parameters tested, methods of analysis:
  - Stock health and performance.
  - Tests and laboratory results associated with – clinical disease or for health certification purposes.
  - Water quality information.
- Revision record. This provides evidence to demonstrate the biosecurity plan is being maintained and is continually reviewed and updated (annually at minimum) based on:
  - Changed biosecurity threats.
  - Ongoing learnings and new available risk management tools.
  - Changes in hatchery practices.
  - Infrastructure upgrades.

Standard Operating Procedures (SOP)

Standard Operating Procedures (SOP) are supporting documents that provide detailed and clear instructions on how to complete either daily or emergency tasks, helping ensure every task is always carried out correctly, regardless of who is in charge. The SOP should contain:

- Title or reference code.
- Purpose and reason for having the procedure.
- List of the tasks.
- Definitions of any technical terms or acronyms used.

Emergency response plan

The emergency response plan is an essential document for every hatchery, providing clear guidelines and procedures to apply in case of a suspected and serious emergency. It must specify:

- Specific triggers for an emergency alert, e.g. massive mortality.
- Key emergency contacts.

Extraordinary biosecurity risk management measures that need to be implemented immediately when the emergency plan is activated (e.g. hatchery access, stock movement, disposal, and quarantine, etc.).

Biosecurity measures for native oyster hatcheries

All hatcheries have to produce a unique and personalised biosecurity plan, since they will have to deal with different biosecurity challenges. Nevertheless, each of the biosecurity measures listed in this section can be considered as a part of a generic standard approach and can be adapted to every native oyster hatchery. In cases where the broodstock are locally sourced and the oysters produced will be returned to the same water body, many of these steps may not apply. See Table 3.3 for example scenarios.

The following biosecurity measures should be considered as a basis on which existing native oyster hatcheries can help develop or confirm their protocols. Regarding developing and future hatcheries, it is important to note that this list is not exhaustive and therefore further research on potential risks needs to be conducted on a site-by-site basis.

ENTRY-LEVEL BIOSECURITY MEASURES

Livestock

- Be aware of diseases/pests affecting oysters at donor sites and keep up to date with current disease designations and conditions.
- Carry out an inspection of incoming broodstock (see Figure 3.3) and do not accept onto the hatchery batches of oysters showing clear signs of infection or unaccounted mortality. The entry of livestock into a native oyster hatchery is a critical phase where biosecurity aspects are combined with practical aspects of zootechnics and prophylaxis. The treatment of the fouling of native oyster broodstock is required in order to avoid undesirable colonisers, predators, parasites, and other associated species. Among these undesirables, colonisers, and associated species such as barnacles (e.g. *Semibalanus balanoides*), lugworms (*Arenicola marina*) or even Pacific oyster (*C. gigas*) can spawn at the same time as the native oyster. Nowadays, two methods are used in hatcheries for the screening and identification of internal parasites and pathogens: I. Sampling/destructive screening of a few individuals for histological analysis and PCR; II. Non-destructive screening by oyster anesthesia.
- Record all movements of broodstock on arrival (movements record previously described), in order to allow proper traceability.
- New stock should be kept in isolation in separate dedicated quarantine facilities, before introducing it into the hatchery, especially if the health status is unknown (wild stock).

Figure 3.3: Arrival of wild native oysters at a hatchery in Helgoland, Germany. Broodstock oysters are temporarily stored, before the one-to-one scraping, washing, chlorination bath, quarantine, biometrics and tagging. Photo: Bérenger Colsoul/AWI.

- The removal of fouling and epibiont for native oyster broodstock can be done both physically and chemically. These methods can vary between manual scraping or use of cement mixers, followed by a hyposaline (freshwater), hypersaline (brine), or chlorine bath. Water used in this process should be UV treated if possible and used water should be treated before disposal. See Chapter 2 for further guidance on cleaning.
- Hold broodstock in quarantine as long as necessary, keeping different batches/origins of oysters separate from each other. During the conditioning period, quarantine protocol should be followed with appropriate biosecurity measures. The quarantine measures generally include a purification phase. This can be very beneficial for the rest of the hatchery operations, as it can notably reduce the bacterial level present in the initial rearing water.
- Do not move any oysters that for any reason have not been approved for release from quarantine to the production zones of the hatchery. Remove and dispose of them in the case of health conditions not improving.

Water

Make sure the quality of water entering the hatchery is suitable for the production and that it is not contaminated/carrying pathogens.

- Water filtration down to 1µm, using bag or cartridge filters, also avoiding animal fouling potentially detrimental to the hatchery's facilities.
- Further sterilisation with UV lamps.
- Optional extra filtration by using ozone, pasteurisers, or other chemical treatments (e.g. chlorine, hydrogen peroxide, carbon filter, iodophors).
- Routine microbiological monitoring to give an indication on the effectiveness of such water filtration systems.

Feed

- Depending upon the specific hatchery's setup and layout, dedicate a separate production area to growing microalgae to feed oysters (see Figure 3.4). **Note:** for algal cultures, the following guidelines are suggested:
  - Having further filtration of the previously filtered incoming water to 0.2µm.
  - Use of certified master cultures, free from contamination (reputable collections).
  - Additional methods (if needed) to sterilise the water, including pasteurisation, chemical treatment, etc.).

  Microalgae can also be produced in ponds, which would require a review of current procedures.
- Certified manufactured feeds (e.g. algal paste) can be considered as an alternative source of food.

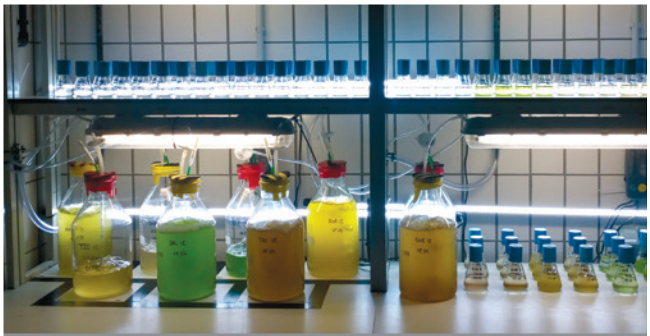

Figure 3.4: Microalgal culture in small volumes (intermediate phase in hatchery): 500ml up to 5l. Differences in colouration are due to the different species produced as well as their concentration. Photo: Bérenger Colsoul.

Equipment

Prior to entering the hatchery's production zones, clean, disinfect and assess for biosecurity risk any equipment and tanks brought onto the hatchery, including those coming from the quarantine area. As examples, disinfection can be carried out by using:

- Hypochlorite solution at 200ppm concentration, for 5 minutes.
- Approved iodophor solution containing iodine at 0.5 %, for 5 minutes.
- Any other disinfection procedure approved by the supervising Quarantine Officer (Arthur *et al.*, 2008).

People (staff, visitors, students)

- Make sure both staff and students understand they share the responsibility of maintaining biosecurity in the hatchery.
- Prior to working in the hatchery, train both staff and students on:
  - Hatchery biosecurity plan.
  - Emergency response plan.
  - Role-specific tasks (SOP).
- Clearly display to all visitors the hatchery biosecurity rules and entry conditions.
- Ensure all visitors complete a biosecurity declaration on arrival, reporting any potential for cross contamination from other shellfish or fishing related sites. Increase the level of prevention applied to high-risk visitors, previously visiting hatcheries located in different areas/ecoregions.
- Both visitors and staff should adhere to the hatchery BMP, and their access should be managed through access record and signage.
- To every person entering the hatchery, apply measures to prevent disease/pest transmission, providing appropriate PPE (Personal Protective Equipment) and disinfection stations (footbaths, hand sanitisers, etc.) on entry.
- Access to sensitive areas (e.g. quarantine room) should be restricted.

INTERNAL-LEVEL BIOSECURITY MEASURES

Livestock

- Examine stock health conditions by regular daily inspections and keep records (stock health record previously described) for inspections by relevant authorities (see Figure 3.5).
- In case of suspicious health status of livestock, isolate and hold the oysters in separate production zones or dedicated quarantine facilities. Run additional tests, inspections and inform the relevant authorities about the results.
- Remove mortalities from the production units as soon as they occur, in order to avoid the spread of potential infection. Store dead broodstock, larvae and spat, temporarily in a freezer, but try to avoid long-term storage of waste.
- Keep a daily record of mortalities (mortality record previously described) and inform the competent authorities in case of unusual mortality events.
- Keep a record of all movements of livestock between the different production areas of the hatchery, in order to allow proper traceability. To decrease the likelihood of infection, avoid moving or transferring oysters at periods likely to be stressful.
- Avoid having different simultaneous species in production in the same hatchery area.
- Keeping broodstock at low densities may reduce the risks of pathogen contamination and spreading of diseases. However, loss of genetic diversity, through inbreeding events, should be avoided, particularly when oysters are supplied for restoration purposes (see Box 3.1).

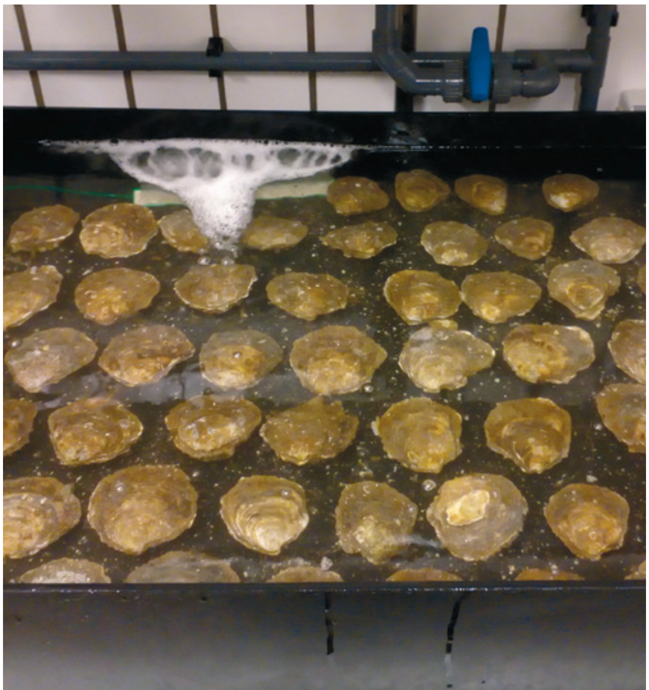

Figure 3.5: Broodstock conditioning: Oysters are cleaned and checked weekly. Photo: Bérenger Colsoul.

Water

- Manage the water flow in the hatchery in order to minimise the potential for diseases to spread within or between different production zones.
- Monitor and keep a daily record of water conditions within the hatchery (water quality record previously described).
- Carry out routine microbiological monitoring.

Feed

- Monitor and maintain the algal cultures, taking care of all the species present in the culture.

Equipment

- Keep the production lines (including pipework, tanks, tubing, valves, and pumps) separated between different production areas.
- Clean the production lines with chlorine regularly, with particular attention to the "dead-zones".
- Assign separate equipment to different production zones, or even to different treatments or health status if necessary.
- Organise a storage for the equipment in each production zone of the hatchery, in order to avoid cross-infection. Generally, these should be off the floor and away from "wet areas".
- If the equipment is used in multiple production zones, clean and disinfect it before and after moving it between zones. See previous section "Equipment" for disinfection methods.

People (staff, visitors, students)

- Manage the different production areas separately, assigning separate personnel to each zone. Staff should be assigned to production areas based on risk.
- In case of staff working in multiple production areas, or people visiting the hatchery, deal with less sensitive zones first, and high-risk zones or diseased animals last, with appropriate cleaning and disinfection protocols followed when moving between different zones. See previous section "People" for preventative measures.
- Access to sensitive areas (e.g. quarantine room) should be restricted to authorised personnel only.

Settlement substrates

Where hatcheries are producing non-single seed oysters, such as spat-on-shells, the following steps should be undertaken before using the substrates for larval settlement:

- Ensure the shells have been treated or aged appropriately for use as cultch.
- Sort the shells.
- Physically clean off dirt and remnants of fouling organisms.
- Sterilise the shells by chemicals (e.g. chlorine) or other sterilisation methods (e.g. autoclave).

For further guidance on appropriate cleaning of cultch, refer to the Chapter 2.

EXIT-LEVEL BIOSECURITY MEASURES

Livestock

- To ensure no infected oysters are transferred from the hatchery health certification is generally required (check with the competent authority for requirements). Protocols generally involve screening (sub-sampling or non-destructive screening method) broodstock and seeds before they leave the hatchery.
- Larvae are considered safe if prior biosecurity procedures are adhered to and monitoring results cause no concern (see Figure 3.6).
- In case of suspicious health status, oysters should be held in quarantine and additional tests/inspections should be undertaken.
- Record all movements of stock from the hatchery (movements record previously described) in order to allow proper traceability.
- Dispose of mortalities in a suitable and legal way as biological waste or incinerate them. Extra precautions must be taken if the death of a batch is suspected to be due to diseases. Certified sick oysters should be disposed separately from the rest of the waste.
- Record date and method of disposal in the mortality record.

Note: any product (larvae, spat, adult oysters) coming out of the hatchery, including transfers to nurseries, are considered included in the exit-level of disease/pest transmission potential route.

Water

- Make sure larvae are not spilled into the floor drain. Mesh screens/filters should be used and maintained.
- Filter hatchery's effluents in order to prevent the release of live or dead non-compliant products (gametes, larvae, spat, feed, faeces) in the environment, especially when flow-through systems are used. See previous sections "Water" for filtration and sterilisation methods.

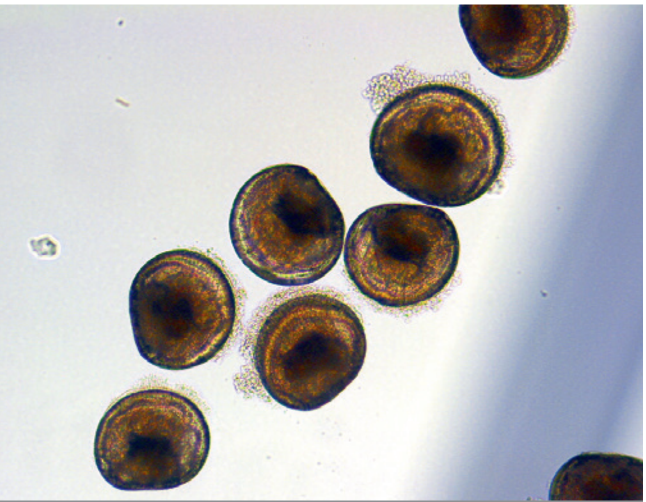

Figure 3.6: Larvae of *O. edulis*. Biological contamination must be controlled and minimised in order to optimise larval survival. Photo: Bérenger Colsoul.

- Treat water, which has been in contact with infected oysters (e.g. effluent from quarantine room) with chlorine and dispose of it separately.
- Keep a record of wastewater disposal (date, methods, treatment, effluent, etc.).
- Carry out periodical microbiological monitoring of the effluents.

Equipment

Clean and disinfect all the equipment coming out of the hatchery. See previous sections "Equipment" for disinfection methods.

People (staff, visitors, students)

- Measures to prevent spread of disease from the hatchery should be applied to every person exiting the hatchery, providing dedicated disinfection stations on exit. See previous section "People".
- After being inside the hatchery, both staff and visitors must avoid being in contact with any other hatchery, seafood processors or aquatic environment, located in a different ecoregion, on the same day or within the following 24 hours.

BOX 3.1: DUALISM BETWEEN BIOSECURITY AND GENETIC DIVERSITY

There is a link between disease susceptibility and physiological stresses caused by overcrowding. For this reason, the number of broodstock used in hatchery practices is frequently reduced to prevent disease outbreaks.

Unfortunately, this can increase the frequency of inbreeding, eventually resulting in a loss of genetic diversity in hatchery populations. The short-term success with reduced genetic diversity (boom) is manageable in food production aquaculture. In contrast, it is potentially highly problematic for restoration, where the aim is to form robust, self-sustaining, and therefore diverse populations. Loss of genetic diversity may lead to long-term failure (bust), with low survival of *Ostrea edulis* spat in the natural environment, after their translocation from the hatchery, due to their inability to adapt to local environmental conditions.

Hatchery biosecurity measures are being improved and prioritised across Europe, but the importance of genetic variability, essential for the success of *Ostrea edulis* restoration, is still underestimated.

Two conceptual scenarios and a case study

The level of biosecurity in native oyster hatcheries can range between very strict and moderate, depending both on the aim/purpose of the production, on the disease status of the donor stock and on the designation of the receiving site. The measures outlined are guidelines which can subsequently be adapted to each hatcheries' own needs, with some measures being applied in all circumstances, and others not. The local regulatory authority is responsible for mandating minimum standards that must be met.

In order to illustrate how the outlined measures may be applied under different conditions, two contrasting scenarios are provided in this section:

- **Scenario 1:** Production of **certified oysters** in hatcheries located within **disease-free areas**, for both aquaculture and restoration purposes.
- **Scenario 2:** Production of **uncertified oysters** in hatcheries located within **disease designated areas**, only for restoration purposes.

Table 3.3: Summary of the main differences in application of the general biosecurity measures, between the production of **certified oysters** in hatcheries located within **disease-free areas** (Scenario 1), and the production of **uncertified oysters** in hatcheries located within **disease designated areas**, only for restoration purposes (Scenario 2).

LEVEL OF TRANSMISSION	MEAN OF TRANSMISSION	SCENARIO 1: Example biosecurity measures in disease-free certified hatcheries	SCENARIO 2: Example biosecurity measures in uncertified hatcheries
Entry-level	Livestock	As a donor site, choose only areas free from diseases/pests.	Selecting a donor site as local as possible to the hatchery location will reduce the risk of bringing in new diseases or strains of disease, and may further benefit from existing disease-resistant broodstock.
		Accept only certified disease-free batches of oysters.	No need for certifications on health status of newcomer stock.
		At the end of the conditioning period in quarantine, screen the broodstock, by sampling or preferentially by non-destructive method, before moving it to the hatchery's production areas.	There is no need to run additional tests at the end of the conditioning period, especially in case of a local donor site.
	Water Feed Equipment	Ensure a high level of biosecurity inside the hatchery, complying with all the biosecurity guidelines, also applying additional measures if necessary.	No need for strict biosecurity measures.
	People	Strict compliance of hatchery's rules and conditions, making both staff and visitors observe all the biosecurity measures.	Strict biosecurity measures for visitors coming from different ecoregions as they could transfer new invasive non-native species onto the hatchery.
Internal-level	Livestock	Apart from routine biosecurity practices, consider additional preventive measures, such as the addition of probiotics rather than antibiotics.	Apply only prophylactic measures and regular monitoring of livestock health and fitness.

LEVEL OF TRANSMISSION	MEAN OF TRANSMISSION	SCENARIO 1: Example biosecurity measures in disease-free certified hatcheries	SCENARIO 2: Example biosecurity measures in uncertified hatcheries
Exit-level	Livestock	No restrictions on the choice of the receiving site.	Receiving sites have to be located in the same area as the hatchery and the donor site.
		In case of pathogen-free production, accurate screening of products for disease detection is necessary. Certification can be carried out via the National Reference Laboratories following the respective standard protocols (European Union Reference Laboratory for Mollusc Diseases (EURL) (2020) Standard Operating Procedures) within the different countries in Europe or by other laboratories approved by them.	No specific analysis is required in case of non-pathogen-free production. Carry out only regular screening, detecting, and removing only oysters clearly in a bad health status.
		Disease-free designated areas should be frequently tested if used as a source of seeds for certified hatcheries.	Movements of livestock (settlement substrates included) from restricted areas, require the permission of competent authorities.
	People	No specific restrictions for people who have been visiting disease-free hatcheries, unless they will visit other hatcheries located in different ecoregions. In this case, they should wait 24 hours before the next visit, in order to avoid transfer of INNS.	People who have been visiting hatcheries located in disease-designed areas should not be in contact with other hatcheries in the following 24 hours, change their clothes, and take all the necessary preventive measures.

The main differences between the two scenarios in Table 3.3 are related to the translocation process, concerning mainly livestock on entry and exit-level of disease transmission.

Translocation of native oysters can be reasonably undertaken in terms of biosecurity as long as they originate from areas which have an equal (or higher) health status as the receiving area. It is unnecessary and illegal to transfer oysters from a diseased area to a disease-free area.

Considering the above-mentioned translocation guidelines, all hatcheries included in Scenario 1 could receive oysters only from other disease-free areas in the same ecoregion, but hypothetically they could export oysters to areas of any disease designations.

Hatcheries included in Scenario 2 could not export oysters except to local areas. These hatcheries can indeed produce oysters only for restoration projects, which aim at replenishing local natural stocks, without involving any translocation process. They could, however, receive oysters from any area within the same ecoregion. It is advised to choose a donor site as local as the receiving site in order to avoid the risk of accidental introduction of diseases/pests. This 'local to local' scenario has the further potential benefit that any existing disease-resistance in the local population may also be maintained, maximising the chance of self-sustaining wild population of native oysters.

BOX 3.2: CLARIFICATIONS AND RESEARCH PRIORITIES

Whereas the methods outlined above draw on existing protocols and experience, hatchery rearing of the native oyster for ecological restoration purposes is still being developed. Therefore the guidelines should be used as a starting point and planned projects should consider scientifically documenting the steps taken within their own efforts, so as to contribute to future development of standard treatments, disease detection protocols and to increase the cost effectiveness of practices. Furthermore, since biosecurity practices and protocols are operated at different locations and latitudes, the practical information listed should be reinterpreted according to the environmental context (e.g. indoor, outdoor, temperatures), (re)validated (e.g. scarce or outdated data), or further developed in the case of new scenarios (e.g. reintroduction of the species in the German North Sea). The practical actions presented here were collated based on the specific needs of ecological restoration and are therefore to be distinguished from the actions and measures applied in commercial aquaculture.

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