CHAPTER 3:
BIOSECURITY GUIDELINES FOR EUROPEAN NATIVE OYSTER HATCHERIES

CHAPTER AUTHORS
Bérenger Colsoul, **Monica Fabra**, Daniel Cowing, Chris Hauton, Bernadette Pogoda, Joanne Preston, William Sanderson, Åsa Strand, Kelsey Thompson, Raimund Weber and Joanne Preston.

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.

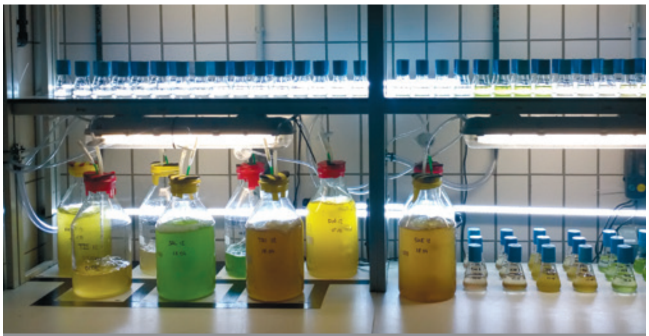

Figure 3.4: Microalgal culture in small volumes (intermediate phase in hatchery): 500ml up to 5l. Differences in colouration are due to the different species produced as well as their concentration. Photo: Bérenger Colsoul.

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).

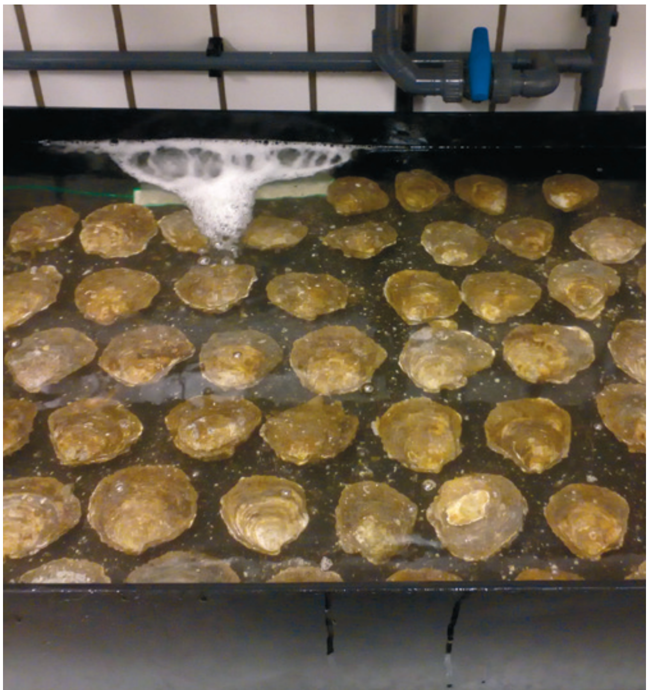

Figure 3.5: Broodstock conditioning: Oysters are cleaned and checked weekly. Photo: Bérenger Colsoul.

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.

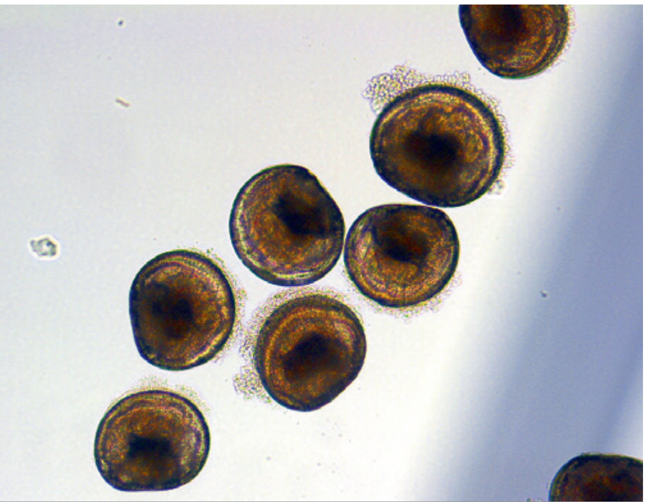

Figure 3.6: Larvae of *O. edulis*. Biological contamination must be controlled and minimised in order to optimise larval survival. Photo: Bérenger Colsoul.

- 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.

RESOURCES

Arthur RJ, Bondad-Reantaso MG, Subasinghe RP (2008) Procedures for the quarantine of live aquatic animals: a manual. (ed.) *FAO Fisheries Technical Paper 502*, pp. 1-89. Food and Agriculture Organization of the United Nations, Rome, Italy.

AquaNIS (2020) AquaNIS: Information System On Aquatic Non-Indigenous And Cryptogenic Species. Available at: http://www.corpi.ku.lt/databases/index.php/aquanis

Bonvarlet F (2011) Exigences réglementaires et recommandations concernant le traitement de l'eau vis-à-vis des agents pathogènes en entrée et en sortie d'écloseries conchylicoles [Regulatory requirements and recommendations for water treatment for pathogens entering and leaving shellfish hatcheries], MSc Thesis. National Veterinary School of Alfort, France.

Buss J, Wiltshire KH, Harris JO, Deveney MR (2020) Decontamination of *Bonamia exitiosa. Aquaculture*, 735210.

Caraguel CGB, Gardner IA, Hammell LK (2015) Selection and interpretation of diagnostic tests in aquaculture biosecurity. *Journal of Applied Aquaculture* 27, 279-298.

Carnegie RB, Arzul I, Bushek D (2016) Managing marine mollusc diseases in the context of regional and international commerce: policy issues and emerging concerns. *Philosophical Transactions of the Royal Society B* **371**, 1-11.

CEFAS (2015) Protocol for inspection and approval of purification (depuration) systems – England and Wales. (ed.) *CEFAS Report Version 9*, pp. 1-43. Centre for Environment Fisheries and Aquaculture Science, Weymouth, UK.

Cook, E.J., Macleod, A. Payne, R.D., and Brown, S. (2014) edited by Natural England and Natural Resources Wales (2015). Marine Biosecurity Planning – Guidance for producing site and operation-based plans for preventing the introduction and spread of non-native species in England and Wales.

Cook, E.J., Payne, R.D. & Macleod, A. (2014). Marine biosecurity planning – Identification of best practice: A review. Scottish Natural Heritage Commissioned Report No. 748. Council of the European Union (2006) Council Directive 2006/88/EC of 24 October 2006 on animal health requirements for aquaculture animals. Available at: https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX%3A32006L0088

Coz, R., Ragot, P. (2020). Interactions des activités de cultures marines avec le milieu marin – avec focus sur les habitats et espèces Natura 2000 [Interactions of marine cultivation activities with the marine environment – with focus on Natura 2000 habitats and species]. In *OFB Référentiel pour la prise en compte des activites de cultures marines dans la préservation de l'environnement marin* (Vol. 2, pp. 1-328). Paris, France: Office Français de la Biodiversité.

European Union Reference Laboratory for Mollusc Diseases (EURL) (2020) Standard Operating Procedures. Available at: https://www.eurl-mollusc.eu/SOPs

European Union Reference Laboratory for Mollusc Diseases (EURL) (2020) National Reference Laboratories of Europe, Main Activities. Available at: https://www.eurl-mollusc.eu/Main-activities/NRLs-network

Federal Agency for Nature Conservation (2020) Neobiota-Platform Germany. Available at: https://www.neobiota-plattform.de

Fitzsimons, J., Branigan, S., Brumbaugh, R.D., McDonald, T. and zu Ermgassen, P.S.E. (eds) (2019). Restoration Guidelines for Shellfish Reefs. The Nature Conservancy, Arlington VA, USA.

GB Non-Native Species Secretariat (NNSS) (2020) Available at: http://www.nonnativespecies.org

Geburzi J.C., McCarthy M.L. (2018) How Do They Do It? – Understanding the Success of Marine Invasive Species. In: Jungblut S., Liebich V., Bode M. (eds) YOUMARES 8 – Oceans Across Boundaries: Learning from each other. Springer, Cham. pp 109-124.

Hick P, Evans O, Looi R, English C, Whittington RJ (2016) Stability of Ostreid herpesvirus-1 (OsHV-1) and assessment of disinfection of seawater and oyster tissues using a bioassay. *Aquaculture* **450**, 412-421.

IUCN (2007) Guidelines for applying the precautionary principle to biodiversity conservation and natural resource management. Available at: https://www.iucn.org/sites/dev/files/import/downloads/ln250507_ppguidelines.pdf

Joint Nature Conservation Committee (JNCC) (2016) Marine Method Finder. Available at: https://hub.jncc.gov.uk/assets/d05e1bea-3453-4ac7-9c14-bac046cd05da.

Joint Nature Conservation Committee (JNCC) (2020) Marine Recorder. Available at: https://jncc.gov.uk/our-work/marine-recorder

Le Saux JC, Pommepuy M (2003) La purification des coquillages [The depuration of shellfish]. (ed.) *IFREMER Report: Risques sanitaires liés aux coquillages*, pp. 1-17. French Research Institute for Exploitation of the Sea, France.

Lee R, Lovatelli A, Ababouch L (2008) Bivalve depuration: fundamental and practical aspects. (ed.) *FAO Fisheries Technical Paper 511*, pp. 1-161. Food and Agriculture Organization of the United Nations, Rome, Italy.

Ministry of the Environment, Energy and the Sea (2020) French National Strategy on Invasive Alien Species. Available at: https://www.ecologie.gouv.fr/sites/default/files/17039_Strategie-nationale-especes-exotiques-invahissantes.pdf

Ministry of Environment and Sustainable Development (Ministerio de Ambiente y Desarrollo Sostenible) (2018) 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) Available at: https://www.minambiente.it/sites/default/files/archivio/normativa/dlgs_15_12_2017_230_specie_esotiche_invasive.pdf

National Biodiversity Network (GB) (2020) NBN Atlas. Available at https://nbnatlas.org

National Species Reintroduction Forum (2014). The Scottish Code for Conservation Translocations. Scottish Natural Heritage. 80 pp https://www.nature.scot/scottish-code-conservation-translocations).

Oidtmann BC, Thrush MA, Denham KL, Peeler EJ (2011) International and national biosecurity strategies in aquatic animal health. Aquaculture 1-2(19):22–33.

Official Journal of the European Union (2016) Transmissible animal diseases and amending and repealing certain acts in the area of animal health ('Animal Health Law'). Regulation (EU) 2016/429. Available at: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32016R0429&from=EN

Otero, M., Cebrian, E., Francour, P., Galil, B., & Savini, D. (2013). Monitoring marine invasive species in Mediterranean marine protected areas (MPAs): a strategy and practical guide for managers. *Malaga, Spain: IUCN, 136.* Available at: https://www.iucn.org/content/monitoring-marine-invasive-species-mediterranean-marine-protected-areas-mpas-a-strategy-and-practical-guide-managers

Palić D, Scarfe AD, Walster CI (2015) A standardized approach for meeting national and international aquaculture biosecurity requirements for preventing, controlling, and eradicating infectious diseases. *Journal of Applied aquaculture* **27**, 185-219.

Payne, R.D., Cook, E.J. and Macleod, A. (2014). Marine Biosecurity Planning – Guidance for producing site and operation-based plans for preventing the introduction of non-native species. Report by SRSL Ltd. in conjunction with Robin Payne to the Firth of Clyde Forum and Scottish Natural Heritage 39 pp. Available at: (includes templates and examples) https://www.nature.scot/sites/default/files/2019-02/Marine%20Biosecurity%20Planning.pdf

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

Rodgers C, Arzul I, Carrasco N, Furones Nozal D (2019) A literature review as an aid to identify strategies for mitigating ostreid herpesvirus 1 in *Crassostrea gigas* hatchery and nursery systems. *Reviews in Aquaculture* **11**, 565-585.

Scarfe AD, Cheng-Sheng L, O'Bryen PJ (2006) *Aquaculture Biosecurity: Prevention, Control, and Eradication of Aquatic Animal Disease*, Blackwell Publishing, Ames, USA.

Scottish Government (2017) 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. Available at: https://www.gov.scot/publications/loch-creran-community-biosecurity-action-plan

Spark E, Roberts S, Deveney M, Bradley T, Dang C, Wronski E, Walker M, Zippel B (2018) National biosecurity plan guidelines for Australian oyster hatcheries. (ed.) *PIRSA Fisheries and Aquaculture 2018*, pp. 1-66. Australian Government Department of Agriculture and Water Resources, Canberra, Australia.

Strand, M., Aronsson, M., & Svensson, M. (2018) Swedish Species Information Centre (SLU) Classification of alien species' effects on biological diversity in Sweden – ArtDatabank's risk list. Swedish Species Information Centre (SLU) Available at: https://www.artdatabanken.se/globalassets/ew/subw/artd/2.-var-verksamhet/publikationer/29.-artdatabankens-risklista/rapport_klassifisering_av_frammande_arter2.pdf

Swedish Agency for Marine and Water Management (SWAM) (2020) Invasive species information. Available at: https://www.havochvatten.se/arter-och-livsmiljoer/frammande-arter.html

Temple, S. (2014) North Western Inshore Fisheries and Conservation Authority Biosecurity Plan 2014 – 2019 Incorporating Marine Invasive Non-Native Species and Shellfish Disease. Available at: https://www.nw-ifca.gov.uk/app/uploads/NWIFCA-Biosecurity-Plan.pdf

Turrell, W.R., Brown, L., Graham, J., Gubbins, M.J., Hermann, G., Matejusova, I., and Robinson C. (2018) Selecting a bath treatment for the marine carpet sea squirt *Didemnum vexillum*, Kott 2002 in Scottish Shellfish Aquaculture. Scottish Marine and Freshwater Science Report Vol 9 No 12 https://data.marine.gov.scot/dataset/selecting-bath-treatment-marine-carpet-sea-squirt-didemnum-vexillum-kott-2002-scottish

World Organisation for Animal Health (OIE) (2020) Information on aquatic and terrestrial animal diseases, Aquatic Animal Health Code and OIE reference laboratories can be found at: www.oie.int. Available at: (https://www.oie.int/en/animal-health-in-the-world/information-on-aquatic-and-terrestrial-animal-diseases/