Recommendation on the biosecurity of shellfish hatcheries and nurseries

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1. Background

Shellfish farming, the main products of which are mussels, oysters, clams and cockles, is a vital economic sector in Europe, employing over 40,000 people. In 2020, this sector produced 584.3 thousand tonnes of shellfish for a value of 1,167.3 million euro thanks to 6,183 companies[1]. These are small-scale operations (90% of shellfish businesses employed fewer than 10 people in 2020) with a high employment rate and play an important role in the socio-economic fabric of coastal areas (STECF, 2023).

Unfortunately, the European shellfish industry is faced with recurring episodes of mortalities that are undermining its economy, development and its very survival. These episodes impact all stages of growth, from spat to commercial-size shellfish. In the 1970s, the industry was severely destabilised by the virtual disappearance of the flat oyster *Ostrea edulis* due to the parasites *Marteilia refringens* and *Bonamia ostreae* (Grizel, 1985). Production of the Portuguese oyster *Crassostrea angulata* also fell drastically in the 1960s-1970s due to iridovirus-like viruses. From 1992 onwards, mortality episodes were regularly reported in larval and juvenile Pacific hollow oysters, *Crassostrea gigas*, with the identification of Ostreid herpesvirus type 1 (OsHV-1) throughout Europe (Garcia, 2011; Morrisey, 2015; Renault, 2018). Then, in 2008, the appearance of a particular genotype of this virus triggered a massive increase in the mortality of young hollow oysters in the various member states of the European Union (Soletchnik, 2009). Other species of molluscs have not been spared. For example, cockle populations have been in marked decline in Galicia since 2008, due to the presence of the *Marteilia* parasite. These crises illustrate the vulnerability of shellfish farming to epizootics, but also the need for professionals in this sector to adapt in order to ensure the survival of shellfish farming.

Faced with these episodes of mortality, the European shellfish industry is organising itself and identifying several avenues of adaptation, ranging from the rearing of new species, such as the introduction of the Pacific oyster *Crassostrea gigas* in the 1970s, to changes in farming practices and the use of hatchery and nursery spat. In fact, to compensate for the difficulties in collecting natural spat due to mortalities and the depletion and/or overexploitation of natural beds (Dubert, 2017), more and more shellfish farmers are sourcing shellfish from hatcheries and shellfish nurseries capable of supplying shellfish, particularly hollow oysters, at the spat stage to supplement or even replace their stock. The term “spat” refers to the early juvenile stage of bivalve development, from the larva to its attachment to a surface with metamorphosis (Dubert, 2017). For marine-cultivated species, spat is either wild or hatchery-bred. The hatchery houses the shellfish reproduction phase under controlled conditions, with rooms dedicated to the storage and maturation of broodstock and the rearing of larvae and juveniles (VIVALDI, 2021) up to 1 mm in size. The nursery accommodates the early phase of the shellfishes growth, from larval attachment onwards. Marketable oyster spats are 6 mm in size when they leave the nurseries.

The main species produced in hatcheries and nurseries in the European Union are hollow oysters, flat oysters and clams. Around 40 commercial hatcheries[2], mostly located in France (AGRESTE, 2022) and Spain, are involved in this production. There are also a dozen hatcheries in various European countries whose aim is to restore natural flat oyster beds. Hatcheries usually also include a nursery phase. It is difficult to estimate the supply of hatchery spat within the European shellfish industry. Nevertheless, for oysters in France, hatchery spat will account for at least 42% of spat production in 2022 (AGRESTE, 2022).

[1] EU Member States DCF and FAO data submission, 2022
[2] AAC members data, 2024

To meet the demands of professionals and offer quality products that take into account the various animal health issues, shellfish hatcheries and nurseries have developed genetic selection programmes and implemented animal health management measures to safeguard their facilities and production.

This recommendation focuses on the biosecurity of shellfish hatcheries and nurseries, and the means implemented to guarantee pathogen-free spat.

2. Justification

Shellfish hatcheries and nurseries produce shellfish in high densities in a controlled environment. Hatchery activities include conditioning broodstock and maturing them through to reproduction, and producing large quantities of microalgae to feed all stages of the production cycle (Prado, 2010).

These early stages of production are sensitive to pathogens and require optimal water quality with precise physicochemical conditions for enhanced productivity. In this regard, the application of biosecurity measures is essential to limit the introduction of pathogens and their dissemination inside and outside the breeding unit, as well as to prevent the risk of the biocontamination of humans and of the environment. Particular attention is also paid to seawater, the common link between the various production compartments (Dubert, 2017).

These measures, which are easier to implement in hatcheries and nurseries than in open and shared shellfish beds, include:

- A search for pathogens in animals, adapted to individual growth stages and production stages,
- Adapting water treatment to the volumes used and the animal health risks identified,
- And in hatcheries, complete physical separation between breeding stock and spat.

A. Animal health risk management plan

Through their animal health risk management plan, validated by the competent authorities via the issuance of an animal health licence, hatcheries and nurseries describe the measures defined to prevent or reduce the risks of introducing and spreading diseases in their facility, as well as the risks of transferring diseases from their facility to the environment. This plan identifies and classifies the diseases and risks associated with the various site operations and shellfish movements in 3 stages:

1. Identification of the main routes of potential disease/parasite transmission within the facility,
2. Risk assessment for each disease/parasite transmission route,
3. Definition of measures to minimise the risk of disease transmission.

Various types of measures are implemented: physical measures linked to infrastructure and equipment, procedural measures (production practices and training) or other support measures. For example, shellfish hatcheries and nurseries keep up-to-date records of the movements, mortality and health of their stock, as well as a record of water quality.

B. Animal health: prevention, control and correction

Firstly, preventive measures adapted to the production stage are implemented, such as cleaning and quarantining broodstock, bio-compartmentalisation, and regular disinfection of equipment. At the same time, hatcheries and nurseries are applying control measures. Professionals plan sampling strategies and histological, bacteriological and virological analyses according to the animal health risks targeted, monitor the state of health and behaviour of the animals (abnormal mortality rates and signs of disease) and immediately inform the competent authorities as soon as they suspect the presence of a listed disease. As a last resort, hatcheries and nurseries can use corrective actions such as destroying batches contaminated by pathogens or affected by mortalities. All of these steps are recorded in mortality and health registers maintained by companies (SENC, 2019).

Routinely, when there are signs of disease or abnormal mortality, the pathogens listed by the Animal Health Law[3] and by the World Organisation for Animal Health in its health code for aquatic animals[4] are tested according to the species produced: *Mikrocytos mackini*, *Perkinsus marinus*, *Bonamia ostreae*, *Bonamia exitiosa*, *Marteilia refringens*, *Perkinsus olseni*, *Xenohaliotis californiensis* and abalone ganglioneuritis. In addition to reportable pathogens, the appearance of other pathogens is regularly monitored at the various stages of production, such as OsHV-1 virus and bacteria of the *Vibrio* genus, which are recognised as major factors in hatchery mortality (Richards, 2015).

For example, in the case of *Vibrio*, hatcheries focus on preventing infections through preventive measures such as the curative treatment of incoming broodstock and its separation from spat using a specific water system, and identification and control of potential sources of *Vibrio* (e.g. microalgal feeds and seawater) (Colsoul, 2020), since this bacterium is permanently present in the marine environment. Hatcheries can resort to using antibiotics in the event of widespread infection, but they are rarely used due to their potentially negative impact on the environment and the risk of developing long-term resistance (Dubert, 2017). Furthermore, antibiotics reduce bacterial diversity and competition between species, which can favour the development of opportunistic or resistant bacteria (Dubert, 2016). Some alternatives to antibiotics are currently being investigated, such as the use of phages (Kim, 2020) in particular to prevent contamination of algal crops (Le, 2020) and the use of probiotics to increase resistance to Vibrio (Karim, 2013; Sohn, 2016).

As far as OsHV-1 is concerned, zootechnical practices in hatcheries and nurseries play a major role in limiting POMS disease. They range from adapting densities according to growth stage, reducing the duration of the larval phase, optimising handling and controlling the temperature, which is kept well below 16°C, the temperature at which mortality episodes can be triggered.

In terms of analysis, reportable pathogens, which are essentially parasites, are tested by histology. Although this technique takes a long time to obtain results (2 weeks minimum), it can detect both reportable and emerging parasitic agents. Nevertheless, further analysis is required to identify the species. To this end, PCR-based diagnostic techniques have been developed to obtain rapid, targeted results, but with the disadvantage of targeting only the pathogen of interest, with no information on the infectious status. Also, pathogen detection in shellfish can be limited by the sensitivity of analytical methods. To prevent the risk of the transmission and spread of diseases in hatcheries and nurseries, it is therefore essential to diagnose any pathogens affecting shellfish quickly and accurately. This requires the development of appropriate analysis methods and increased knowledge

[3] Règlement délégué (UE) 2018/1629 de la Commission du 25 juillet 2018 modifiant la liste de maladies figurant à l'annexe II du règlement (UE) 2016/429 du Parlement européen et du Conseil relatif aux maladies animales transmissibles et modifiant et abrogeant certains actes dans le domaine de la santé animale (« législation sur la santé animale »)

[4] Code sanitaire pour les animaux aquatiques (2017) - organisation mondiale de la santé animale

of the risks of pathogen emergence (as defined by the Animal Health Law), as well as of the transmission, virulence and pathogenicity of already known populations.

Analytical strategies differ between hatcheries and nurseries. In hatcheries, given the physical separation of the different growth stages and the preventive measures applied to broodstock and microalgae, pathogen analyses of spat are carried out at the beginning and end of spawning. Depending on the results, corrective action is taken to limit the spread of identified pathogens. In nurseries, given the densities and quantities of water used, analyses targeting OsHV-1, *Vibrio aestuarianus* and the *Splendidus* group are generally carried out on a weekly basis. They must guarantee the absence of OsHV-1 virus detection and a low *Vibrio* bacterial load. In all cases, analyses are systematically carried out as soon as signs of disease and abnormal mortality appear.

In summary, the biosecurity of livestock is essentially guaranteed by preventive measures and control, with the adaptation of zootechnical practices. However, there's another key factor in preventing the onset and spread of disease: water treatment.

C. Controlling water quality

Hatcheries and nurseries manage the various stages of the shellfish production cycle. As we saw earlier, they differ not only in terms of biosecurity measures for shellfish, but also in the quantities produced and the volumes of water pumped to supply the tanks. In fact, larvae and small spat are produced in covered structures, with a water supply requiring low flows, whereas nursery spat may be produced in uncovered structures with high water flows.

Hatchery water quality is monitored in all of the compartments, from the broodstock room to the micro nursery, as well as at the pumping station. Each workshop (broodstock stabling, microalgae production, larval rearing, etc.) has its own water circuit supplied by the same controlled water source. Potential risks of biological and chemical contamination are investigated, and physicochemical criteria are monitored and modified as required. Hatcheries are supplied with pumped or reconstituted seawater. In France, at the Polder de Bouin dyke, hatcheries pump seawater from saltwater aquifers, guaranteeing good water quality, but lacking the necessary nutrients.

Pumped seawater can vary widely in quality (salinity, turbidity, etc.) depending on the location of the hatchery and its water intake. Therefore, hatcheries generally have a settling tank, which is regularly cleaned of deposits and algae blooms. Pumped from this basin, the water is filtered, heated and then sterilised by an ultraviolet (UV) system. Water treatment with UV radiation ensures a non-selective reduction in the micro-organism load of treated water (SENC, 2019), as well as a reduction in the bacterial and viral load. The UV lamps most commonly used in aquaculture are low-pressure lamps. The UV dose delivered depends on: the type/number of lamps, its age, the water flow rate through the reactor, the quality of the water to be disinfected, and particularly its transmittance. This technology is fairly easy to implement, but its effectiveness varies according to the type of micro-organism to be eliminated. Ifremer research tested the impact of low-pressure UV systems on the OsHV-1 virus and the *V. aestuarianus* bacterium in non-turbid water: a target dose of 40 mJ/cm^2 ensures 5 to 6-log inactivation of the viral load, i.e. 99.999% of the initial load, and no bacterial growth after UV irradiation, also with 6-log abatement, i.e. 99.999%[5].

Companies are constantly working to improve their water treatment processes according to their needs, either in-house or through collaborative projects between scientists and professionals. One

[5] Communication Ifremer lors du groupe focal CCA « biosécurité sanitaires des écloseries et nurseries conchylicoles » du 25/03/2024

example is the SOAP project (2020-2023, EMFF), one of the objectives of which was to study the impact and performance of two coupled processes (ultrafiltration and adsorption on activated carbon) for the disinfection and chemical decontamination of seawater upstream of shellfish farms. Other recent initiatives aim to study the biosecurity of farms and the protection of animals from the larval to adult stage against pathogens (parasites, viruses and bacteria) and algal blooms, such as the BIOPAR project (France, EMFAF), which will demonstrate the effectiveness of water treatment processes in eliminating shellfish parasites.

In addition to water treatment on entry, it is important to manage water renewal within the different compartments of the hatchery because, due to their nature as filter feeders, bivalves act as a bacterial and viral reservoir and can release bacteria and viruses into seawater (Prado et al., 2014a) even without showing any sign of disease. Vibriosis in hatcheries can thus be controlled by reducing the bacterial load in the water, generally by increasing the water renewal rate in the tanks to prevent epidemics. For example, the water in the larvae and spat tanks is renewed every 2 days after filtration and UV sterilisation.

In nurseries, the water flow rates to be treated are very high. As a result, hatchery-style water treatment is neither technically nor economically feasible. However, the nurseries operate in a closed circuit, with the pumped water passing through a settling tank and a sand filter with partial water renewal. In order to guarantee pathogen-free spat, in addition to applying measures such as bacteriological and virological controls, and the destruction of contaminated or dead animals, nurseries work on the purification of spat before they are sold. These purification systems, fed by smaller volumes of water than the nursery tanks, are equipped with the same water treatment processes as the hatcheries. Companies are currently carrying out in-house experiments to optimise system sizing and adapt purification times to the initial bacterial or viral load that can be detected by analysis in shellfish prior to immersion.

D. Disease-free status of shellfish hatcheries and nurseries

Thanks to all the elements set out below, a hatchery or nursery can, according to Article 37 of Regulation (EU) 2016/429[6], apply for recognition of "disease-free" status for reportable diseases as a compartment. To achieve this, the facility must provide proof of the following points, among others:

- Demonstrate the impossibility of introducing into the facility the listed disease(s) subject to the application,
- Have a single, common biosecurity management system to guarantee the absence of the listed disease(s),
- Obtain an animal health licence from the authority in charge of animal movements.

Once this status has been approved, the facility must maintain it by complying with the points below and implementing a monitoring programme in line with the disease profile and the risk factors involved.

This status guarantees the absence of pathogens within the facility, thus facilitating the movement and transfer of animals from the facility to another facility, zone or country. The European Commission lists on its website the territories, zones or compartments awarded disease-free status.

[6] Regulation (EU) 2016/429 of the European Parliament and of the Council of 9 March 2016 on transmissible animal diseases and amending and repealing certain acts in the area of animal health (« Animal Health Law »)

However, the acquisition of free status is not adapted to the new practices developed, such as obtaining tolerant/resistant and pathogen-free spat from parents from an infected area. For example, a recent study (Kamermans, 2023) showed that hatchery production of *Bonamia*-free and potentially *Bonamia*-tolerant/resistant flat oyster larvae and spat is possible from parents collected in a *Bonamia*-infected area. Using a non-destructive selection method, only *Bonamia*-free broodstock were selected for reproduction. However, regulations exclude a hatchery from *Bonamia*-free status if the broodstock do not come from a *Bonamia*-free zone.

3. Recommendations

In conclusion, hatcheries and nurseries are designed to meet high biosafety standards in order to supply the European shellfish industry with quality shellfish spat while maintaining the genetic diversity of the species. In order to recognise and continuously improve the biosecurity performance of hatcheries and nurseries, the Aquaculture Advisory Council recommends the following actions:

To the European Commission:

- In consultation with the competent authorities in member countries and representatives of the European shellfish industry, revise the criteria for defining the disease-free status for hatcheries and nurseries, taking into account innovations such as obtaining disease-tolerant/resistant and disease-free larvae and spat through the non-destructive selection of pathogen-free broodstock from a zone that is not pathogen-free,
- In consultation with scientists and representatives of the European shellfish industry, develop a certification/label that recognises the production of spat free of one or more pathogens by the hatchery and/or nursery concerned. In particular, this requires:
    - Support applied research projects, carried out by scientists and shellfish companies, on the biosecurity of shellfish production in controlled environments through water treatment in recognition of the proven effectiveness on the pathogens studied,
    - Develop rapid, high-performance analysis methods with enhanced sensitivity to detect the pathogens of interest at the earliest stages of production.

Support work on stimulating immunity in bivalve molluscs, where initial results are proving interesting for hatcheries and nurseries, and work linked to genetic selection.

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