Bivalife

Improving European mollusc aquaculture: disease detection and management

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State of the art

Aquaculture is a dynamic activity in Europe where the growth rate of the sector has been around 4% during the last decade. Europe was the third largest contributor with around 4.2% of world aquaculture production. European aquaculture is characterised by a focus on a limited number of species including oysters and mussels being raised at an industrial level. The marine bivalve industry has grown to be very important for many regions of the European Union contributing substantially to social and economic activity in the coastal zones. The yearly European oyster production is the 126 000 tonnes, France being the leading Member State (80 000 tonnes/year). The mussels *Mytilus edulis* and *M. galloprovincialis* are the bivalve species with the highest production output in Europe (491 000 tonnes/year). Spain is the second world producer and the top European producer with an output of 300 000 tonnes/year (*M. galloprovincialis*).

For some years, the European shellfish industry recognised a rather slow growth, compared to other aquaculture activities. European shellfish farming, relying directly upon natural environments and feeding resources, is facing various risks and limiting factors which include primarily, as well as in every farmed species, infectious diseases.

Historically, infectious diseases have seriously affected the marine bivalve industry in Europe in various occasions. In the early 1970's, the French oyster industry in particular faced a severe blow when irido-like virus infections led to the almost total extermination of the Portuguese oyster, *Crassostrea angulata*, in French and European Atlantic waters. In the late 1960's and 1970's, devasting effects on the European flat oyster, *Ostrea edulis*, production were reported related to two protozoan diseases, bonamiosis and marteilisois. Since 2008 massive mortality outbreaks affecting *C. gigas* oysters are reported in different Member States. These are attributed to a combination of adverse environmental factors together with the presence of ostreid herpesvirus type 1 (OsHV-1) and the presence of bacteria belonging to the genus *Vibrio*.

The control of farmed shellfish health is one of the key elements to maintain the competitiveness and to increase the sustainability of the industry as a whole. The Council Directive 2006/88/EC "on animal health requirements for aquaculture animals and products thereof and on the prevention and control of certain diseases in aquatic animals", underlines the necessity for the development of the aquaculture sector in the European Union to increase the awareness and preparedness of the competent authorities and aquaculture production business operators with respect to the prevention, control and eradication of aquatic animal diseases.

General and specific objectives of the Bivalife project

The two core objectives of the Bivalife project were (i) to provide innovative knowledge on pathogens infecting oysters and mussels and (ii) to develop practical approaches for the control of infectious diseases and resulting mortality outbreaks these pathogens induce.

The project addressed the major issue identified by the European Commission on the risk of pathogen transfer and infectious disease outbreak occurrence among marine bivalve molluscs, through the specific objectives:

- *(i) improve, validate and transfer existing methods for detection and identification of Pacific oyster and mussel pathogens;*
- *(ii) search and characterise Pacific oyster and mussel pathogens in relation to the presence or absence of mortality;*
- *(iii) assess the relationship between the presence of Pacific oyster and mussel pathogens and environmental risk factors in the development of mortality or disease;*
- *(iv) investigate mechanisms allowing Pacific oyster and mussel pathogens to survive outside the host;*
- *(v) identify pathogen intrinsic virulence factors and effects on host defence mechanisms;*
- *(vi) develop methods and recommendations for pathogen control and eradication in Europe.*

The project focused on three mollusc species, namely the Pacific oyster *Crassostrea gigas* and two mussel species *Mytilus edulis* and *M. galloprovincialis*, the most important species in terms of European production. The pathogens the project selected are mainly pathogens associated with mortality outbreaks in Pacific oysters, *C. gigas*. The targeted pathogens were the virus OsHV-1, *Vibrio* species including *V. splendidus* and *V. aestuarianus*, as well as the parasite *Marteilia refringens* and the bacterium *Nocardia crassostreae.* Epizootic diseases, like those that have devastated the oyster culture industry in Europe have not been encountered by the mussel culture industry. Mussels effectively appear less sensitive to a large range of well known infectious diseases. Consequently, mortality outbreaks have more rarely been reported in Europe in the mussel species, *Mytilus galloprovincialis* and *M. edulis*.

Bivalife validated and updated efficient methods for identification and detection of relevant pathogens that affect oysters and/or mussels. OsHV-1 and *Vibrio* species have been detected in association with mortality outbreaks affecting the Pacific oyster in Europe. *Marteilia refringens* is reported being associated with mortality of mussels in some cases while it does not seem to be able to develop in *C. gigas*. *Nocardia crassostreae* infects the Pacific oyster and was recently reported in The Netherlands in association with mortality of Pacific oysters.

The project studied the relationship between presence of a given pathogen and its actual implication in observed mortality through the development of a database related to site and pathogen characterization and the identification of any potential determinants related to disease manifestation. Pathogens are often necessary but not sufficient cause of disease, which is an outcome of interaction between host, pathogen and environment. The final objective is the use of a common methodological approach shared by Member States to identify and quantify potential risk related to the presence of relevant pathogenic agents in Europe.

Considering that information about Pacific oyster and mussel pathogens outside their hosts is lacking, the project explored the mechanisms that allow pathogens to resist outside the host based on

(i) defining criteria and methods to measure pathogen survival,
(ii) searching for infectious (viable) pathogens in different compartments in the field,
(iii) and monitoring survival of targeted pathogens in laboratory assays.

Bivalife also delivered new information on the pathogenicity of relevant pathogens through comparing contrasted biological material (infected versus non-infected individuals, resistant versus susceptible groups, susceptible versus non-susceptible species) originating from experimental trials.

Bivalife also developed efficient methods and techniques aimed at eradicating pathogens in controlled culture conditions. Practical methods and general recommendations were drawn from knowledge acquired during the course of the project.

Key drivers to improve disease diagnosis: harmonisation of diagnosis techniques for oyster and mussel pathogens through method transfers and validation (inter-laboratory assays)

Effective health management measures are based on diagnosis procedures such as: (i) a systematic approach to disease monitoring including the early detection of early infected animals, (ii) the use of sensitive and rapid diagnosis techniques, (iii) the collection of diagnosis data, and (iv) the establishement of diagnositic laboratories using standardised procedures with samples collected according to defined rules. Mollusc health surveillance/control programmes require both adequate legislation and standardised procedures for pathogen diagnosis. Valid laboratory results are essential for diagnosis, surveillance and trade. Molecular techniques for diagnosing bivalve infectious agents have been developed during the last decade. Real-time Polymerase Chain Reaction (PCR) techniques are already used for routine detection of pathogens infecting oysters and mussels including OsHV-1, *V. splendidus*, *V. aestuarianus*, *N. crassostreae* and *M. refringens*. Although they are now moving from a developmental phase in specialized laboratories for research purposes to routine application, molecular tools need formal validation. These diagnostic tools are not standardized and differences exist in the quality of reagents quality and preparation, in controls, as well as in the interpretation of results.

Obviously, the use of a “standardized” diagnostic tool for routine analysis should allow the implementation of a calibrated and controlled process in laboratories. It is recognised that for such purpose, studies conducted in parallel with the same isolates in several laboratories would be necessary.

For this purpose, the transfer and the validation of already existing molecular tools for notifiable and non-notifiable pathogens (*M. refringens*, OsHV-1, *V. splendidus*, *V. aestuarianus* and *N. crassostreae*) were performed during the course of the project based on interlaboratory comparison assays involving several laboratories.

Control of animal movements and transfers

Specific objectives of Bivalife were (i) determination of the geographical distribution of certain known oyster and mussel pathogens in Europe based on the use of validated techniques, (ii) identification of putative emerging pathogens in Europe related to mortality outbreaks, (iii) production of a data base related to site and pathogen detection, (iv) identification of relevant potential determinants related to disease manifestation and (v) development of practical methods and general recommendations for infectious disease control drawn from knowledge acquired during the course of the project

Considering that the trade of live molluscs is a major cause of epizootics, restriction of animal movements is a basic way to control infectious diseases. Transfer regulations at international and European levels have been developed in order to avoid the introduction of animals from an enzootic area to a pathogen-free area.

Minimum measures for the control of certain diseases affecting bivalve molluscs were established by the Council Directive 2006/88/EC. This Directive lays down harmonised animal health provisions for the placing of aquaculture animals and products thereof on the market, including specific provisions applicable to species susceptible to certain exotic and non-exotic diseases (notifiable). In Pacific oysters infectious diseases are frequently reported in larvae and spat and are involved in mortality outbreaks. These diseases are not currently notifiable diseases subjected to specific control measures under EU or WOAH (World Organisation for Animal Health) legislation. Although, pathogens associated with these mortalities, mainly virus and bacteria, generate important economic losses, their impact may be misunderstood or seriously underestimated.

During the course of Bivalife, relevant - mainly non-notifiable - pathogens (OsHV-1, *V. splendidus*, *V. aestuarianus*, *N. crassostreae* and *M. refringens*) were searched in samples collected from France, Ireland, Italy, Spain and The Netherlands. Information was obtained about the presence/absence of these pathogens in the main oyster and mussel producing Members States using previously validated techniques.

Understanding complex interactions between animal, pathogen and environment: development of tools to measure pathogen survival and detection of viable/infective relevant pathogens in different compartments (animals, water, sediment)

Reducing the impact of pathogens is likely to rely on knowledge of their biology, their life cycle and the mechanisms that allow them to survive outside the host. Marine bivalves including oysters and mussels are typically reared in estuarine environments frequently subjected to fluctuations of environmental factors such as temperature, salinity and pollution. Outside the host, the environment of pathogens is thus composed of many different types of factors. The pressure of different stressors, including variations of salinity and temperature, may lead to potentially irreversible alterations of pathogens and host metabolism.

Viable/infective relevant pathogens were searched in different types of samples (water, sediment, zooplankton, phytoplankton and macrofauna) collected from France, Ireland, Italy, Spain and the Netherlands. In order to complete information about the detection and the viable/non viable status of pathogens in different compartments in the field, the effects of physico-chemical parameter (e.g.temperature, salinity, UV) variations on pathogen survival were monitored based on laboratory trials.

Understanding immunity of aquacultured mollusc bivalve species : identification of relevant host defense mechanisms in contrasted biological material and identification of virulence factors in oyster and mussel pathogens

The development of an infectious disease results from an imbalance between the host and the pathogen due to different factors including internal factors of both protagonists (virulence of the pathogen, susceptibility of the host). During the last decade, various molecular techniques including mRNA differential display, Suppressive Subtractive Hybridization (SSH) and Expressed Sequence Tag (EST) libraries have been applied in bivalves in order to identify immune genes and to assess the level of gene expression. About 50,000 ESTs from bivalves are now available in various databases. Such data provides the basis for understanding the role of the innate immune system against various pathogens and may be useful in defining selection markers for improved resistance to infections.

In order to better understand pathogenesis and pathogen resistance, the innate immune response was studied in oysters in oysters and mussels from experimental trials. Contrasted biological material (infected versus non-infected individuals, resistant versus susceptible individuals (selected oyster and mussel families), susceptible versus non-susceptible species) was compared targeting genes of interest.

Developing and adopting new pathogen inactivation methods

Although hatchery technology is constantly being improved, significant production problems including infectious diseases must be solved before hatcheries become a major supplier of juveniles for the industry. Mollusc hatcheries are unique in having closed facilities that enhance disease control capabilities.

Hatcheries may produce checked progeny, namely specific pathogen free, and reduce circulation of infected stocks. Moreover, they may have a pivotal role in the development and implementation of health management strategies based on improved resistance to infectious diseases.

The involvement of hatcheries in the control of infectious diseases would offer producers the possibility to contribute proactively in the early detection of possible adverse conditions for bivalve growth and survival before a major problem occurs, contributing to the minimisation of the effects of disease outbreak. Adequate control of larvae and spat from hatcheries in controlled facilities may avoid costly epizooties. Moreover, the availability of efficient tests for relevant pathogen detection may facilitate screening of brood stock, spat and larvae before commercial transactions and therefore constitute a guarantee of product quality for oyster producers.

Creating a controlled growth environment during the inland growth cycle of the shellfish by reducing the presence of pathogens through the use of a disinfection barrier is one of the main objectives for sustainable aquaculture.

The disinfection barrier is a state of the art disinfection technology based on Ultra-Violet (UV) light. UV light has the ability to inactivate all microorganisms without creating collateral damage in the environment. The inactivation level is defined by the amount of germicidal UV light that each microbe absorbs during the exposure time in the UV system. The necessary dose level is "microbe dependent" - meaning that each microbe has its characteristic inactivation dose. Relying on advanced fiber optic principles, the Hydro Optic Disinfection™ (HOD) technology "traps" light photons to provide uniform UV dose distribution within the reactor and eliminate low dose escape routes. The HOD technology has proven field capabilities in the aquaculture farming industry such as in the salmon egg production, hatcheries and smolts, as well as in cod farms, sea bass and sea bream industries and in shrimp industries.

Bivalife developed and promoted (i) diagnosis efficacy (ii) integrated bio-security measures and (iii) efficient methods and techniques aimed at eradicating pathogens that meet the time constraints experienced under hatching conditions, and are of the utmost importance for efficient control of diseases in bivalve hatcheries. The project implemented the HOD technology to block each potential contamination source at the pre-defined inactivation level (UV dose). This activity focused on defining the UV germicidal dose to inactivate the predefined pathogens, selectively inactivate pathogens in the live feed without affecting the live feed itself.

Bivalife promoted a re-inforced collaboration among those stakeholders involved in mollusc health issues:

(i) Research institutes that developed relevant knowledge on oyster and mussel pathogens and interactions with their hosts;

(ii) European national laboratories involved in mollusc disease diagnosis and control, which aim is to support farmers in the implementation of control measures and bio-safety procedures;

(iii) a high tech SME that bring its extensive knowledge, methodology and technology in the field of new disinfection capabilities;

(iv) and the EU shellfish producers, who are in the front-line and face the negative impact of pathogens and diseases on their livestocks, will be beneficiary of the results through the participation of their representatives to Bivalife's exploration and development meetings.