Situation of European mollusc production regarding diseases

I. Arzul

Ifremer, SG2M-LGPMM, EURL for Mollusc Diseases, La Tremblade, France

Abstract
The production of marine molluscs is an important part of the European aquaculture. Its growth has unfortunately been hindered over the recent years by mortality events, linked to pathogen organisms.

Surveillance of mollusc diseases in Europe includes active surveillance of regulated pathogens and non-regulated pathogens and passive surveillance based on mortality reports. However, mortality reporting relies on the good will of producers/fishermen and mostly concerns the Pacific cupped oyster *Crassostrea gigas*.

Since these last years, implemented surveillance programmes have allowed for better defining of the geographic distribution of some mollusc pathogens. This is the case of *Bonamia exitiosa,* a protozoan parasite previously considered exotic to Europe and which has been detected in different European countries since 2008. Moreover, these different active and passive surveillance programmes have contributed to characterising new pathogens including the protozoan parasite *Marteilia cochillia* in cockles in Spain and parasites of the genus *Mikrocytos* in France, Spain, The Netherlands and United Kingdom. Transfer of animals and depuration centers seem to contribute to the spread of mollusc pathogens. However, the source of these apparently new pathogens is often difficult to identify.

Introduction

The European shellfish industry enjoys a privileged position on the global scene. Its social dimension is essential, as it employs a high number of people in more than 8000 companies, mostly micro-companies.

Shellfish production in Europe mainly relies on the industrially produced mussels *Mytilus galloprovincialis* and *M. edulis* and in a lesser concern on oysters, *Crassostrea gigas*. Other species including clams *Ruditapes philippinarum*, cockles *Cerastoderma edule*, the flat oyster *Ostrea edulis* or scallops *Pecten maximus* are partly harvested from natural beds and represent a great potential for diversification. The frontier between wild and farmed animals is not easy to draw, some species being harvested from beds created by settlement of spat produced in hatcheries or spat collected from other geographic sites.

Behind this general picture, the European shellfish production displays diversity between European countries. Spain is the first producer of mussels *M. galloprovincialis*. France and Italy are the first producers of oysters *C. gigas* and manila clams *R. philippinarum*, respectively.

Although the production of flat oysters *O. edulis* is very low, less than 5000 t per year, it concerns

* Corresponding author's email: Isabelle.Arzul@ifremer.fr

a wide range of countries from Northern Europe to Spain and Croatia. Conversely, the production of cupped oyster *C. gigas* mostly concerns France, Ireland and The Netherlands.

Moreover, this picture is non-static and can evolve depending on several factors including economic, epidemiologic and ecologic factors. Diseases are recognised as a major threat for shellfish production. The history of the French oyster production perfectly illustrates the importance of diseases (Azema et al., 2015). The production of the European flat oyster *O. edulis* has been dramatically affected by two protozoan diseases due to *Marteilia refringens* initially described in 1968 in the Abers (Brittany) (Grizel et al., 1974) and *Bonamia ostreae* described for the first time in 1979 in Tudy Island (Brittany) (Pichot et al., 1979). The Portuguese cupped oyster *Crassostrea angulata* was the main produced oyster during the 20th Century but completely disappeared from the French coasts at the end of the 70ies. This economic disaster has been attributed to an infection with iridovirus. To face this critical situation, Pacific cupped oyster *C. gigas* was introduced from Canada and Japan. This species is now the main oyster species produced in France and more generally in Europe. Its production has reached up to 120,000 t until 2008 but has then started declining. This decline has been associated with high abnormal mortality of young oysters associated with the presence of the virus OsHV-1μvar. Since 2012, abnormal mortality events affecting adult oysters have also been reported in association with the bacteria *Vibrio aestuarianus* (Azema et al., 2015).

Other factors than diseases can contribute to modify the production of shellfish. For example the geographic distribution of the cupped oyster *C. gigas* has expanded since this last decade. This species was repeatedly introduced to Europe for aquaculture purposes in the second half of the 20th Century (Lallias et al., 2015), and has established wild populations in the Black Sea, the Mediterranean Sea and along the Atlantic European coasts, to Scandinavia (Nehring, 2011). Temperature conditions north of France were initially thought inappropriate for natural reproduction. Climate changes and broad ecophysiological tolerances of the species (Strand et al., 2011) are proposed to be the cause of the recently rapid northward expansion of the species from the Wadden Sea to Norway (Angles d'Auriac et al., 2017).

This natural expansion offers new possibilities for the production of *C. gigas* in Scandinavia and this example highlights the need to consider the production of shellfish not only at the national but at the European level.

Hatchery technology has allowed securing a reliable supply of 'spat' following inconsistent, short supply of 'wild-catch'. The development of industries farming shellfish species requires a consistent supply of spat. The commercial hatchery production of a diverse range of bivalve shellfish is thus essential and is being successfully undertaken around the world, predominately with oysters, clams, and to lesser degree mussels and scallops. Nevertheless, hatchery production strategies are greatly influenced by the environmental, and social economic environment.

In Europe, in 2012, there were 14 hatcheries producing cupped oysters, 14 producing clams, 8 producing abalone, 4 hatcheries also produced mussels and 3 *Pecten maximus* (Reproseed, 2012). These closed and semi closed establishments concentrate huge numbers of animals which are then spread in many sites for grow out. These establishments represent a high risk for pathogen spread.

Although molluscs are sessile organisms, farming activities contribute to move them several times during their life. Movements concern all age classes including spat from recruitment sites or hatcheries to sites suitable for growth; juveniles between different grow out sites and finally marketable adults. Movements of shellfish contribute to spread non expected associated species including pathogens and are recognised as the main risk factors contributing to disease spread (Thrush et al., 2017). Keeping shellfish in depuration centers before their consumption is commonly done for the issue of public health. However, effluents are generally not treated in order to avoid mollusc pathogen escape and some examples of pathogen spread from such establishments raise the need to implement efficient water treatment processes.

In a study carried out in Charente Maritime in France, oyster movements were characterised by type of farming activities (spat collection, adult growing, marketing…) and by season (Lupo et al., 2016). Results showed a seasonal variation of oyster transfers in all farming activities, with peaks of transfers in spring and autumn and different geographical patterns of oyster transfers between farming activities. Growing sites were shared by farms belonging to different farm categories which have different behavior related to oyster movements in time. This study has notably revealed that an oyster can be moved up to 9 times during its production cycle highlighting the importance of transfers in shellfish production, more particularly in oyster production (Lupo et al., 2016).

Disease control and regulation

Contrary to other animal production, the control of mollusc diseases cannot rely on the use of classical vaccines because these species do not produce antibodies. Most of the shellfish production takes place in the field, in open areas where disinfection and the use of treatment cannot be considered. By filtering the water column, bivalves concentrate particles including microorganisms and may act as carriers for many pathogens. Additionally, the lack of frontier between farms and between wild and farmed animals increases the difficulty to eradicate diseases. These observations highlight the importance of preventing the introduction and spread of pathogens in free zones. Nevertheless, once a pathogen is established, some measures can be implemented to mitigate the impact of the disease on farmed and wild populations. Such measures include the use of tolerant and resistant animals and stock management measures decreasing the risk of disease expression.

At the EU level, the animal health conditions governing the placing on the market of aquaculture animals and products are defined in Council Directive 2006/88/EC (European Commission, 2006). Because the animal health situation is not the same throughout the territory of the EU, the movement regulations are based on the concept of approved (disease free) zones and farms for non-exotic diseases. The Directive lays down the criteria and procedures for the granting, maintenance, suspension, restoration and withdrawal of approval of such zones and farms as well as certification requirements for

Table 1. List of diseases and susceptible species regulated at the European level (Directive 2006/088/EC).

	Diseases	Susceptible species
Exotic diseases	Infection with *Bonamia exitiosa*	*Ostrea angasi, O. chilensis*
	Infection with *Perkinsus marinus*	*Crassostrea gigas, C. virginica*
	Infection with *Mikrocytos mackini*	*Crassostrea gigas, C. virginica, Ostrea conchaphila and O. edulis*
Non exotic diseases	Infection with *Bonamia ostreae*	*Ostrea edulis, O. chilensis, O. conchaphila, O. denselamellosa, O. puelchana*
	Infection with *Marteilia refringens*	*Ostrea edulis, O. chilensis, O. angasi, O. puelchana, Mytilus edulis et M. galloprovincialis*
Diseases of importance at the national level	Infection with OsHV-1µvar	*Crassostrea gigas*

movement into disease free zones/farms. It also contains rules governing import from non-EU countries.

The Directive concerns any aquatic mollusc (all life stages included) reared in a farm or mollusc farming area including any aquatic animal from the wild intended for a farm or mollusc farming area. A mollusc farm can be a farm, a natural bed, a hatchery, nursery and depuration center. A mollusc farming area can be a, or part of, a bay, an estuary, a lagoon a zone in the intertidal zone or a group of ponds inland.

There are two categories of regulated diseases in the EU (Table 1):

- Exotic diseases: infection with *Bonamia exitiosa* (although it is present in EU : see section "mollusc diseases in Europe"), infection with *Perkinsus marinus* and infection with *Mikrocytos mackini*;
- Non exotic diseases: infection with *Bonamia ostreae* and infection with *Marteilia refringens*.

The Directive also gives the possibility to Member States to implement regulatory measures for diseases of importance at the national level. It is the case for infection with OsHV-1 µvar in Ireland and United Kingdom.

The European Parliament and the Council adopted the Regulation on transmissible animal diseases ("Animal Health Law") including aquatic animal diseases in March 2016. It will be applicable in 5 years. The animal health law aims at strengthening the enforcement of health and safety standards for the whole agri-food chain. Several delegated and implementing acts will be adopted by the Commission until April 2019 to make the new rules applicable.

Disease surveillance

In Europe, surveillance effort regarding mollusc diseases is different between Member States and partly depends on the amount and the diversity of the shellfish production.

Some countries implement active surveillance of pathogens regulated at the European level including *Bonamia ostreae*, *Marteilia refringens* and OsHV-1 μvar. In some Member States, this active surveillance aims at demonstrating and maintaining free status in zones or countries regarding one or several diseases. This is the case in the United Kingdom, Ireland, and Norway where there are free zones regarding bonamiosis and marteiliosis or Denmark free of marteiliosis (European Commission, 2009). The United Kingdom and Ireland have also established surveillance programmes regarding OsHV-1 μvar and have demonstrated freedom in part of their coasts (European Commission, 2014).

Other member states carry out monitoring of some diseases. For example, The Netherlands has monitored prevalence of bonamiosis in flat oysters *Ostrea edulis* in lake Grevelingen since 1988 (Engelsma et al., 2010). Such monitoring is very useful to better understand factors driving the dynamics of this disease and to identify potential management measures. Although the parasite *Perkinsus olseni* is not notifiable at the European level, it infects a wide range of molluscs including clams *Ruditapes philippinarum* and *R. decussatus* sometimes in association with mortality. Production of clams is an important part of Italian, Spanish and Portuguese aquaculture industries explaining why these countries carry out active surveillance regarding this parasite (Ruano et al., 2015).

In addition to active surveillance, many European countries carry out passive surveillance based on abnormal mortality reports. In case abnormal mortality is observed, it has to be reported to the Competent Authority which then might decide to organise sampling and send samples to recognised laboratories for presumptive diagnosis.

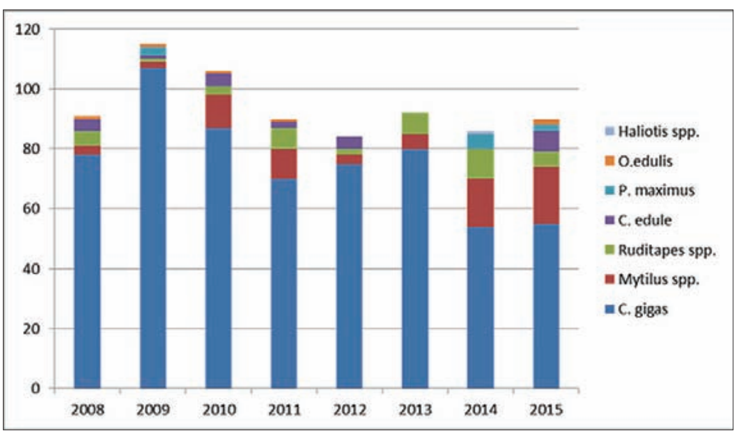

Figure 1. Number of mollusc mortality events investigated by the network of NRLs for mollusc diseases since 2008 by species.

However, mortality reporting relies on the good will of producers/fishermen (Lupo et al., 2012) and differs between mollusc species. Indeed, abnormal mortality reports in Europe mostly concern the Pacific cupped oyster *Crassostrea gigas,* and, in a lesser concern, mussels and clams (Figure 1). Very occasionally mortality is reported on other less accessible species including scallops *Pecten maximus* or flat oyster *Ostrea edulis*.

Mollusc diseases in Europe

Since these last ten years, the surveillance effort carried out in Europe has improved our knowledge of the situation of mollusc populations regarding diseases. It has notably contributed to better define the geographic distribution of some mollusc pathogens. For example, the protozoan parasite *Marteilia refringens* infects several bivalve species among which the flat oyster *Ostrea edulis* and mussels *Mytilus edulis* and *M. galloprovincialis*. For many years, the surveillance of marteiliosis was based on flat oysters only. Including mussels in surveillance programmes of *M. refringens* has contributed to detect it in new locations in the Adriatic Sea like in Montenegro and in Northern Europe (EURL mollusc diseases, 2017). The parasite was indeed detected for the first time in Sweden in 2009, South England in 2011, Norway and Northern Ireland in 2017.

Before 2007, the parasite *Bonamia exitiosa* was considered exotic to EU. However in 2007, Abollo et al. (2008) reported for the first time the presence of this protozoan species in Europe in flat oysters from Galicia. *B. exitiosa* is close to the endemic species *B. ostreae* and difficult to distinguish by histology or cytology. Since this description, it has been recommended to use not only histology or cytology (imprints) to detect *B. ostreae* but also molecular tools such as PCR and sequencing or species specific PCR assays. This approach has allowed detecting *B. exitiosa* (Figure 2) in Spain, France, Italy, United Kingdom and Croatia, sometimes in the context of mortality events but more often in the context of active surveillance programmes (Arzul et al., 2011).

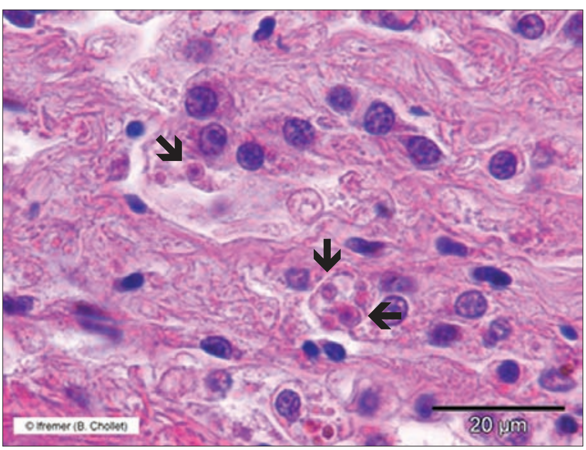

Figure 2. Presence of *Bonamia exitiosa* (arrows) in hemocytes of flat oyster *Ostrea edulis*. H&E stained section. Picture from B. Chollet.

In few Member States, surveillance programmes have been carried out to achieve and maintain free status regarding some pathogens in some zones or for the whole country. This surveillance effort has sometimes contributed to detect pathogens like OsHV-1µvar. This virus has been detected for the first time in the context of high mortality in France in 2008 and in Ireland the same year. In 2009, it was later detected in the United Kingdom, The Netherlands, Italy and Norway. Considering that the virus has been spread through movements of infected spat from France to other European countries, Ireland and United Kingdom have implemented surveillance programmes to demonstrate freedom in several bays/zones along their coasts and to prevent introduction of infected animals into these free zones. Despite regulatory measures in place, the virus has been detected in some free zones in both countries.

Some of these detections were associated with mortality events reported near depurations centers maintaining infected animals. Although such establishments present a high risk of introduction and spread of pathogens, there is presently no legislation regarding water treatment notably because there is no data on the efficacy of treatment on pathogen inactivation.

Similarly to OsHV-1µvar, *Bonamia ostreae* has been detected in previously free zones in Ireland and United Kingdom (Laing et al., 2014). Different hypotheses are suggested to explain these new detections for example through equipment or vessels used in different bays showing different status regarding the disease.

Because of the finding of *B. ostreae* in 2014 autumn sampling, Limfjorden in Denmark had lost its disease-free status in March 2015 (Madsen and Thomassen, 2015). In 1980, *B. ostreae* was already observed in relayed French oysters. However, the whole batch of French oysters was harvested and destroyed. In the 1990's, the native stock of flat oysters increased and a surveillance programme for *B. ostreae* and *Marteilia refringens* was initiated. Limfjorden gained its disease-free status regarding the two parasites in 2004. Regular samplings from three sites twice a year have then been done and neither of the parasites had been found, until the autumn sampling in 2014, where *Bonamia*-like cells were observed in sampled oysters confirmed as *B. ostreae* by PCR and sequencing. The origin of this emerging situation is unknown. The parasite could have remained in Limfjorden since 1980 at a very low and undetectable level of prevalence or could have been introduced through movements of other bivalve species including *Crassostrea gigas* or *Mytilus edulis.*

Finally, active and passive surveillance programmes carried out these last years have contributed to characterise new pathogens. For example, the protozoan parasite *Marteilia cochillia* was first detected in association with mortality of cockles on the Mediterranean coast in Spain (Carrasco et al., 2011). It has then been observed in the context of monitoring of wild populations of *Cerastoderma edule* (Villalba et al., 2014). The emergence of the parasite in Galician rias was concurrent to the decline of cockle beds and has contributed to the collapse of cockle fisheries.

Parasites of the genus *Mikrocytos* were also observed in the context of mortality events affecting different mollusc species including

Donax trunculus in France (Garcia et al. in press), *Ruditapes philippinarum* in Spain and The Netherlands or *Crassostrea gigas* in United Kingdom (Hartikainen et al., 2014). Characterisation of parasites in *D. trunculus* in France has revealed the presence of two distinct new species: *Mikrocytos veneroïdes* and *M. donaxi* (Garcia et al. in press). Although the parasite observed in *Crassostrea gigas* in United Kingdom induces macroscopic lesions and histological features similar to *M. mackini*, a parasite known to infect oysters in Canada, molecular works have revealed a new parasite species named *M. mimicus* (Hartikainen et al., 2014).

Conclusion and perspectives

The European production of molluscs is organised differently in Member States. Diseases are recognised as a major threat and disease management mostly relies on transfer regulations depending on the status of zones and countries regarding listed diseases, as described in the Directive 2006/088/EC. Surveillance effort is established by each Member State according to the importance of the production and is more or less balanced between active and passive surveillance. However, most of mortality reports concern the cupped oyster *Crassostrea gigas* suggesting that passive surveillance is less adapted to other mollusc species.

Over these last years, disease surveillance programmes carried out in Europe have allowed for improved knowledge regarding geographic range for some pathogens including *Bonamia exitiosa*. Detection of this parasite closely related to *B. ostreae* in new locations has required the development of new diagnostic tools discriminating between both species.

The apparent geographic spread of regulated pathogens such as OsHV-1 μvar or *Bonamia ostreae*, questions the routes of their dissemination. Transfer of animals, movements of other species acting as vectors or reservoirs and depuration centers seem to contribute to the spread of a number of mollusc pathogens. However, the source of their introduction is often difficult to identify.

New pathogen species have been characterised in the context of mortality investigations. The detection of *Marteilia cochillia* in cockles in Delta del Ebro, and then its emergence in Galicia and the characterisation of new *Mikrocytos* species in France and United Kingdom highlight the importance to combine histological and molecular approaches.

Mortality events are late signs and their report depends on the willingness of farmers. Complementary, there is a need for early warning tools. The interest of sensors and eDNA approaches is currently explored in the context of the European project VIVALDI which aims at preventing and mitigating mollusc diseases (Arzul et al., 2017).

Despite the implementation of the Directive 2006/88/EC on mollusc farms since 1 August 2008, the measures in place have not been successful in dealing with the risk posed by newly emerging, non-listed diseases across the EU. This should be taken into account in the context of the new Animal Health Regulation to ensure more rigorous disease control measures are implemented to protect this important sector.

Acknowledgements

The author is deeply grateful to the NRLs for mollusc diseases for their participation in annual meetings organised by the EURL: information shared during these events was very helpfull to prepare this synthesis. The author also wants to express her appreciation to the European Commission and more particularly to DG-Sante for its support of this work. Finally, the author warmely thanks her colleagues from the "Unite Technique" of the LGPMM for their daily collaboration

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