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European research project VivaldiProgressing crescendo on mollusc health

Early detection of pathogens affecting shellfish, strengthening the immune defences of oysters, identifying individuals that are more resistant to certain diseases and environmental factors with an impact on mortality episodes, etc. The European research project Vivaldi is coming to a close, with numerous scientific results and recommendations for better management of shellfish diseases.

Shellfish farming is a vital economic sector in Europe, employing more than 40,000 people. However, it must cope with recurring episodes of mortality. For instance, the OsHV-1 virus has been responsible for high rates of mortality in juvenile cupped oysters in various European Union member states, especially since 2008. Another pathogen, the bacteria *Vibrio aestuarianus*, has been linked to mortality episodes affecting adult cupped oysters in France and Ireland. Other farmed mollusc species have not been spared: for instance, the cockle populations in Galicia which have dramatically declined, linked to the presence of a parasite called *Marteilia cochillia*.



The results obtained enable to identify solutions to mitigate the impact of the diseases. © Ifremer

« Shellfish diseases know no frontiers and therefore, enhancing scientific collaboration interntionally is critical. A reference in ocean science and shellfish diseases in Europe, Ifremer coordinates Vivaldi - a Horizon 2020 project - to strengthen mutual learning across research and stakeholder communities, with 21 partners, from 11 countries », says François Houllier, CEO at Ifremer. Vivaldi has been initiated by Ifremer in 2016, following on from another European project, called Bivalife, also coordinated by the Institute. Vivaldi involves 21 partners from ten different countries. Nearly four years after its launch, the project is ending with a final conference held from 26 to 28 November 2019, bringing together all the project partners and stakeholders in Brest (France). "The results obtained will provide better knowledge about the pathogens affecting shellfish farms, enable us to better understand the factors influencing bivalve mortalities and identify solutions to prevent or mitigate the impact of these diseases", emphasized Isabelle Arzul, an Ifremer researcher (Laboratory of marine mollusc genetics and pathology, La Tremblade) and the coordinator of

the Vivaldi project. Special efforts for sampling and analyses were devoted to four key study sites: the Ebro delta and Ria de Vigo in Spain, Dungarvan bay in Ireland, and the Bay of Brest, in France.

Knowing more about the enemy

The first strand of the project improved knowledge about shellfish pathogens and their life cycles. For example, it was shown that there was not a single OsHV-1 virus within an infected oyster, but rather a "constellation of viruses": several variants with potentially different levels of virulence were detected in the same individual.

Another issue is that of "reservoirs", i.e. the "compartments" where pathogens can be present outside of the shellfish. They can be in water, sediments or other marine organisms. A study conducted during the project showed, for example, that the parasite *Bonamia ostreae*, which affects flat oysters, can survive for approximately two days in water.



The first strand of the project improved knowledge about shellfish pathogens. © Ifremer

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Finally, systems were developed which can detect the presence of pathogens in water, particularly before the onset of the infection. For instance, the analysis of submerged membranes made it possible to reveal the presence, in a given sector, of the OsHV-1 virus, but also of viruses which are human pathogens, like noroviruses.

Training immune defences

Contrary to what was long believed, molluscs have a sort of immune memory. This is seen in the form of patterns in the proteins, which are capable of recognising the pathogens the animal has been confronted with in the past and triggering defence mechanisms. Another strand of the project explored ways of stimulating this response. Oysters seem to defend themselves better against the OsHV-1 virus when they have been previously exposed to a molecule resembling the virus. This phenomenon could path the way to some sorts of immuno-stimulation. Better yet: this capability may be transmissible. Initial results seem to show that the offspring of oysters put in contact with the "stimulating" molecules survive a viral infection better, even when never previously exposed themselves.



Oysters seem to defend themselves better against the OsHV-1 virus when they have been previously exposed to a molecule resembling the virus. © Ifremer

Resistance lies in the genes



Oysters could be selected with respect to their resistance to some diseases without impacting their growth. © Ifremer

Within the same population, some individuals are particularly sensitive to pathogens whilst others are more resistant. Scientists studied the genes which could explain this difference in cupped oysters. Two methods were used. The first one consisted in exposing thousands of cupped oysters to a viral infection in the natural environment in order to determine their level of resistance. Their genotypes were then determined by sequencing to identify the regions of the genome involved in the resistance to diseases. To that end, several tens of thousands of genes were screened. The other strategy was to analyse numerous oysters in natural populations on several sites in Europe, both before and after a mortality episode. The identification of key genes could enable us to understand how oyster populations cope with chronic diseases and to select more resistant animals.

However, this selection must not be made to the detriment of genetic diversity or of other interesting characteristics (oyster size, taste qualities, etc.). Numerical simulations have made it possible to define good practices to be implemented in hatcheries, in order to limit the loss of genetic diversity. These simulations also confirmed that cupped oysters could be selected with respect to their resistance to some diseases without impacting their growth.

Cohabitation is good for oysters

The environment plays an important role in the emergence of marine mollusc diseases. The effects of temperature, salinity, acidity and nutrients, as well as cohabitation with other species, have been studied. It was thus shown that above 29°C, the OsHV-1 virus does not cause mortality in cupped oysters. The sea water's pH does not appear to have an impact on the ability of the virus to cause infection. Moreover, cohabitation with competing species, like mussels or ascidians, is beneficial to cupped oysters. Several mechanisms can explain this phenomenon. Firstly, competition for food. The oyster has fewer nutrients available, which reduces its development and can lessen the multiplication of the virus. Sharing of beneficial bacteria between species can also be another explanation for this observation. During an



The oysters' microbiota was modified in the presence of red algae. © Ifremer

experiment to cultivate cupped oysters in the presence of red algae, it was indeed shown that the animals' microbiota was modified and that their survival during infection by the OsHV-1 virus was improved.

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Good practices to fight disease

Numerical modelling tools were developed over the course of the project. Taking account of the hydrodynamics of each site studied and the modes of pathogen transmission, this type of model can simulate the dissemination of diseases and could be used to test the effectiveness of management measures.

Studies performed in Vivaldi have enabled us to identify practices which reduce the risk of introducing diseases and their associated mortalities. The results obtained have been shared and discussed and a manual containing these best practices is

being prepared, together with producers and competent authorities in the countries represented in the Vivaldi project. Some recommendations are general in scope, such as improving surveillance or not translocating shellfish in cases of mortality. Other measures must be adapted to each site, e.g. the dates and water temperature at which it is preferable to submerge spat vary from one region to another.

Finally, from the United States to China, via South Korea or New Zealand, the Vivaldi project has made it possible to create a network of international experts beyond our European borders, in order to share the results of research studies and information about the emergence of new diseases.



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About the Vivaldi project

Vivaldi is a European project launched in 2016, which will reach completion in early 2020. It is funded by the Horizon 2020 research programme and coordinated by Ifremer. There are 21 partners, mostly European, in the project: Ifremer (France), CNRS (France), Labogena DNA (France), SYSAAF (France), CSIC (Spain), IRTA (Spain), University College Cork (Ireland), National University of Ireland Galway (Ireland), Genova University (Italy), Trieste University (Italy), Padova University (Italy), Institute of Marine Research (Norway), NOFIMA AS (Norway), CEFAS (United Kingdom), University of Liverpool (United Kingdom), Queen's University Belfast (United Kingdom), Alfred Wegener Institute (Germany), Marine Institute (Ireland), Atlantium Technologies (Israel), Wageningen University (The Netherlands), National Veterinary Institute (Denmark).

The project is organised into six work strands with working groups coordinated by different institutes:

- Strand 1 (Pathogen diversity and cycles): Centre for Environment, Fisheries and Aquaculture (CEFAS, UK)
- Strand 2 (Bivalve functional response): Spanish National Research Council (CSIC, SP)
- Strand 3 (Genetic selection): Ifremer (FR)
- Strand 4 (Complex interactions between animal/environment/ pathogens): CNRS (FR)
- Strand 5 (Management measures): Research and Technology Institute for Food and Agriculture (IRTA, SP)
- Strand 6 (Sharing information): Ifremer (FR)