Final Conference

Brest (F)

26-28 November 2019

Proceedings

This project has received funding from the European Union's Horizon 2020 Research and innovation programme under grant agreement N° 678589

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Introduction to the VIVALDI project

Isabelle Arzul, Scientific coordinator of the VIVALDI project, Ifremer

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... 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*.

Shellfish diseases do not stop at borders and the stakes for shellfish health are international in scope. In this context, the Vivaldi European research project was 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. 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.

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.

Resistance lies in the genes

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, out of the genome's total three million 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 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.

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.

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)

Biography:

Isabelle Arzul is a marine parasitologist and pathologist whose research focuses primarily on the evolutionary ecology of marine diseases and the interactions between protozoan parasites and marine bivalves. She obtained her Veterinarian PhD from the Veterinarian school of Nantes in France before completing a PhD in virology at the University of Montpellier. During her PhD she investigated the diversity and virulence of a virus infecting oysters. Since 2002, she has been working at the Laboratory of Genetic and Pathology of Marine Molluscs at Ifremer La Tremblade on the mid-west coast of France. After coordinating the national network for the surveillance and the monitoring of bivalve diseases on the French coasts, she has been responsible for the EU Reference Laboratory for Mollusc Diseases in Europe, leading a network of 23 reference laboratories.

She has been involved in 5 European projects and since 2016 she has been coordinating the European H2020 project VIVALDI.

General poster presentations

1-VIVALDI: preventing and mitigating farmed bivalve diseases

Isabelle Arzul[1], David Bass[2], Antonio Figueras[3], Jean-Baptiste Lamy[1], Christine Paillard[4], Dolors Furones[5]

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Presenter : Isabelle Arzul

Abstract

Since 2016, VIVALDI project, supported by the European Union H2020 programme, has aimed to improve the sustainability and competitiveness of the European shellfish industry, which has been hit by a growing number of mortality cases over the recent years. To this end, tools and strategies to better prevent and mitigate the impact of bivalve diseases have been developed through 6 work packages:

- In the 1st work package, the diversity and lifecycles of some bivalve pathogens have been investigated. Environmental-based approaches such as passive sensors or the use of magnetic beads and electrochemical biosensors could be useful for pathogen surveillance and the development of early warning systems.
- In the 2nd work package, some key mechanisms involved in the response of bivalves during a pathogen infection have been identified. Several modulated genes were also found, which may help to identify potential markers related with resistance against diseases. VIVALDI has also demonstrated that stimulating bivalve immunity is possible.
- In the 3rd work package, oyster and clam families have been produced and challenged regarding their susceptibility to pathogens. Panels of well-selected SNP markers are now available for oysters and clams and have allowed us to identify markers associated with resistance to some pathogens and will optimise breeding programmes.
- In the 4th work package, the impact of environmental parameters on the development of bivalve diseases has been studied: for example biodiversity for infection with OsHV-1 and plankton for bacterial diseases. The characterisation of bivalve microbiota under different scenarios, including disease development or environmental perturbations, should contribute to the identification of bivalve health markers.
- In the 5th work package, a literature review and field studies have been carried out to identify the best husbandry practices to reduce mortality. The efficiency of UV treatment to remove pathogens, oyster gametes and larvae from the wastewater has been confirmed. Finally, our

risk ranking shellfish farm model could be used by competent authorities to implement risk-based surveillance of shellfish diseases.

- In the 6th work package, a stakeholders' analysis has allowed to map key stakeholders and better understand their relations. Their perception of the risks related to diseases was investigated. Recommendations to prevent mitigate and control bivalve diseases have been identified from the results of VIVALDI and will feed, together with existing guidelines, a manual for disease management and biosecurity, which is being co-constructed with a group of stakeholders.

Many studies have been carried out in VIVALDI and lay the foundations for future works notably on the impact of global change on bivalve diseases and also perspectives in the field of restoration of local bivalve species. Finally, VIVALDI has contributed to develop solutions, recommendations for a better implementation of the legislation. These outcomes will also bring helpful information for the evolution of this legislation.

2-A snapshot on VIVALDI through UNIPD activities

Paola Venier[1], Giuseppe Arcangeli[2], Luca Bargelloni[3], Isabelle Arzul[4], Estelle Delangle[4], Miriam Abbadi[2], Umberto Rosani[4,5], Morgan Smits[3,6]

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Presenters: Paola Venier, Giuseppe Arcangeli, Luca Bargelloni, Umberto Rosani, Morgan Smits

Abstract

The H2020 project VIVALDI (2016-2020) aims at an improved understanding of aquaculture-mollusk diseases to reinforce their prevention, control and management via research, innovation and fruitful networking with stakeholders. **Partner 1** is IFREMER-La Tremblade (Coordinator I. Arzul). **Partner 12** is University of Padova, a joint team including Istituto Zooprofilattico delle Venezie (IZSVE, G. Arcangeli) and the BCA and DIBIO departments. Activities performed within and beyond the VIVALDI consortium are summarized by work package. **WP1, WP4**. *Crassostrea gigas* spat deployment in lagoon waters and IZSVE monitoring of abnormal mollusk mortality allowed the in vivo propagation and direct shotgun genome sequencing of the Ostreid herpes virus microvariant OsHV-1-PT (overall observed oyster mortality did not exceed 50%, inocula equal or higher than $1x10^8$ OsHV-1 DNA copies were typically associated to oyster mortality; a genuine identification of viral reads among host reads was achieved without any virus purification step). **WP2**. Dual transcriptome sequencing, extended to publicly available datasets, was used to disentangle host-pathogen interactions and identify mollusk defense pathways and candidate markers, studying ADAR 1, MIF and antimicrobial peptides, among other gene functions. **WP3**. A real-time PCR assay (qPCR) was developed in collaboration with CSIC (Vigo, Spain) for the detection and quantification of *P. olseni* in clam gill tissue and hemolymph (*Ruditapes philippinarum*). Results obtained with the standard diagnosis methods Ray's fluid thioglycollate culture method (RFTM), polymerase chain reaction-restriction fragment length polymorphism assay (PCR-RFLP) and histopathology were compared. Although better results were obtained from gill than from hemolymph in the qPCR assays, especially at low infection levels of the parasite, significant lineal correlation between gill and hemolymph Ct values was observed. A field challenge experiment has been carried out in the lagoon of Venice where perkinsosis is endemic. Over 5,000 Manila clam spat were seeded in a controlled area and the prevalence of *Perkinsus olseni* was monitored every month by analyzing gill samples from 30 clams using the qPCR assay described above. After one year, when prevalence was approximately 50%, over 1,000 clams were collected from the field experiment and morphology, size, length, and sex were recorded. For each individual, total genomic DNA was extracted from gills and used to measure the amount of *P. olseni*. An aliquot of the same DNA was sent to Labogena (France) for genotyping. Genotyping was carried out using a SNP-chip including 250 SNPs selected from a large panel that had been previously produced by sequencing three pooled population samples of Manila clam. All recorded traits showed significant heritability and genetic correlations (Smits et al. in preparation). **WP5, WP6**. P12 supported the analysis of Italian stakeholders' opinions (two questionnaires) and is contributing to the preparation of a biosecurity manual. Overall, many scientific papers, one book chapter, one video interview on the oyster sector in Italy and one international symposium on mussels (AMMR 2019) have been produced.

Session 1: Studying pathogen diversity and improving tools for better surveillance

Keynote presentation: Pathogen detection and diagnostics in the light of microbial hyperdiversity

David Bass, VIVALDI Work package 1 leader (CEFAS-UK)

It is increasingly recognized that animals and plants have a wide diversity of small organisms, mostly microbes, associated with them as pathogens, commensals, mutualist symbionts, or somewhere along the 'symbiotic continuum' between these states. There are a range of techniques for investigating the diversity and function of these host-associated organisms (collectively, symbionts). Molecular methods for ascertaining the bacterial symbiome are well established, using 'universal' bacterial PCR primers. However, to investigate the symbiotic eukaryotes, an analogous use of eukaryote primers is not practical, as such primers usually amplify host DNA effectively to the exclusion of symbiont DNA. Two ways to avoid this are using 'anti-metazoan' primers, which avoid animal host gene amplification, and host-blocking primers, which exclude amplification of a specific host.

This presentation outlined a range of methods for pathogen detection, isolation, and diagnostics, and challenges for modern molecular diagnostics. It is now apparent that there is a very large diversity of pathogen lineages in the environment and associated with hosts. This means that standard diagnostics for many of these pathogens may not be able to distinguish between the target pathogen and its close relatives, as many diagnostics were designed in ignorance of this large diversity. Long-range PCR can be used to generate long/multi gene regions that provide more information and much higher resolution discrimination between lineages, better phylogenetic resolution to enable more accurate taxonomic definitions, and will be a valuable approach to designing better and more informative diagnostics in the future.

Metagenomic (shotgun) sequencing can be used to access genetic information from all symbiont types (bacteria, eukaryotes, viruses) in a sample, avoiding all amplification biases of PCR and accessing even highly divergent lineages. However, accurately interpreting metagenomic datasets pose significant challenges. Using RNA as a basis for pathogen detection, instead of DNA, can provide a more reliable indicator of activity, rather than simply presence, of pathogens, and therefore is a better proxy for inferring infection, at least in some cases. Laser dissection microscopy can be used to target pathogen cells, infected regions of tissue, and tissue showing host response to pathogens to increase the ratio of pathogen cells/infected tissue in a sample, allowing more focused investigations without obscuring the molecular pathogenetic signal with that from unaffected host tissue.

The talk also presented the concept of the pathobiome, which is the set of host-associated organisms (prokaryotes, eukaryotes, and viruses) associated with reduced health status, as a result of interactions between symbionts, host, and environment. This approach is supplementing the one-disease-one-pathogen paradigm, which is insufficient to explain many disease scenarios, particularly syndromic conditions.

Presenter's biography

Dr David Bass is an internationally leading researcher on protist biology and evolution, with expertise in molecular phylogenetics, eDNA, molecular taxonomy and diagnostics, protistan biodiversity and parasitology, and in molecular techniques for investigating microbial ecology. He employs a wide range of molecular (including 'omics) and pathology techniques to determine and characterise pathogens and parasites infecting finfish, shellfish and marine algae (including novel lineages and cryptic infections) and to assess disease risk by developing a new understanding of pathogen ecology. He is Past President of both the International Society for Protistology (ISOP) and Protistology-UK. He is a Principal Scientist at Cefas, where he is the 'pathogens and pathobiome' Topic Lead. He PI on the Defra-funded evidence and research and development contracts at Cefas, Cefas PI on a H2020 project investigating bivalve health and pathogens across several EU countries, Co-I on the GCRF-funded Global Seaweed Star project, aiming to secure the Future of the Seaweed Aquaculture Industry in developing countries, Co-I on a BBSRC-funded project investigating oilseed rape rhizosphere interactions as a possible cause of yield decline, and is Co-I on a BBSRC- Newton Fund Global Research Partnership in Aquaculture (£2.2million), that studied pond microbiomes to predict disease outbreaks (and AMR development) in aquaculture systems in India, Bangladesh and Malawi. He leads a project focusing on the metatranscriptomics of Highfield Experiment soils (with Rothamsted Research, BBSRC CCC scheme) and was a PI on the now complete BioMarKs EC Framework Programme. He has published over 100 ISI papers with over 7,200 citations (h-index=39). He was appointed Honoary Associate Professor at Exeter University in 2019.

How do OsHV-1 µvars stack up? : a comparison of multiple variants through experimental challenges

Colleen Burge (Institute of Marine and Environmental Technology, University of Maryland Baltimore County, USA)

A threat to global shellfish aquaculture industries is Pacific Oyster Mortality Syndrome (POMS) caused by microvariants of the Ostreid herpesvirus 1 (OsHV-1). POMS can kill all life stages of the Pacific oyster, Crassostrea gigas, and perhaps other bivalve species. Catastrophic impacts are associated with the global emergence and spread of POMS since 2008. Experimental challenges with naïve US oysters were conducted in a biosecure facility far from the ocean using multiple OsHV-1 variants including microvariants from France and Australia and OsHV-1 from California. An 'injection trial' (with ~12 month oysters) exposed to French and Australian µvars indicated that stocks of three important global oyster species, Crassostrea gigas (~20-95% mortality), Crassostrea sikamea (~22-55%), and Crassostrea virginica (~0-10%) are susceptible to both µvars; 1000 times higher viral loads were present in oysters that died. Interestingly, some species and stocks experienced differential mortality when exposed to the French and Australian µvars. One of the three C. gigas stocks experienced relatively low mortality when challenged with the Australian µvar (~20%) and higher mortality when challenged with the French µvar (~65%). Crassostrea sikamea experienced lower mortality when challenged with the French µvar (~22%) and higher mortality with the Australian µvar (~55%). A 'water trial' (with spat from the Molluscan Broodstock program) using French and Australian µvars and OsHV-1 from Tomales Bay, California corroborated that OsHV-1 µvars have increased transmissibility. Additionally, some lines used in this trial show low levels of mortalities again all variants but are tolerant to infection (conversely others experience high levels of mortality and high viral loads). A final trial showed the infectious nature and initial characterization of a new OsHV-1 variant detected recently in San Diego, California. To summarize, experimental challenges conducted with Crassostrea spp indicate that POMS may pose a threat to oyster industries globally and screening with multiple variants may be important in preparing shellfish industries for OsHV-1.

Presenter's biography:

Dr. Colleen Burge is a native to the coastal US, learning about oyster biology from a young age. Colleen is an Assistant Professor at the Institute of Marine and Environmental Technology with dual appointments at University of Maryland Baltimore County, Department of Marine Biotechnology and the University of Maryland Baltimore, Department of Immunology & Microbiology. Colleen received her BS (2002) and PhD (2010) in Aquatic & Fishery Sciences at the University of Washington. Colleen held two postdoctoral positions; her first was in the Department of Ecology & Evolutionary Biology at Cornell University and the second in the School of Aquatic & Fishery Sciences at the University of Washington. Colleen's research program, the "Aquatic Animal Health lab" focuses on marine host-pathogen-environment interactions including disease ecology, organismal physiology and immunology, and development of disease diagnostics

Genomic methodologies for bivalve pathobiome characterization and detection

Alberto Pallavicini (UNITS-IT)

Recent advances in DNA sequencing technology is enabling new quantitative insights into the microbial community diversity associated with human and animal tissues. It is now recognized that host associated microbial communities (also named the microbiota) are playing an important role in animal health by providing prominent services ranging from nutrient processing to protection from diseases. Marine bivalves host high microbial abundance and diversity and alteration of the microbiota due to stressful conditions and/or environmental changes was previously linked with a condition of a compromised health status and susceptibility to diseases.

We report the dynamics of microbial communities associated with a large number of *C. gigas* samples (525) collected within the collaborative EU project "VIVALDI" during recurrent mortality episodes at the three key European sites (Bay of Brest, Ebro delta and Dungarvan bay) were investigated by real-time PCR. 16S rRNA gene profiling of the microbial community was also conducted on a large number of contrasting (e.g. infected vs not infected) *C. gigas* samples (101) to support the "pathobiota" analysis and to extensively investigate the patterns and dynamics of oyster microbiota during mortality events. A new target enrichment next-generation sequencing protocol for the selective capturing, sequencing and classification of 884 phylogenetic and virulence markers of the marine microbial pathogenic community in oyster tissues was developed, providing, for the first time to our knowledge, comprehensive high taxonomic resolution analysis of the potential pathogenic microbial community (pathobiota) associated with *C. gigas*. Comparative analysis of contrasting *C. gigas* samples conducted using these methods revealed that oyster experiencing mortality outbreaks displayed signs of microbiota disruption associated with the presence of previously undetected potential pathogenic microbial species mostly belonging to genus *Vibrio* and *Arcobacter.* Hence, due to the extensiveness, geographic dimension and a new technological approach this study provides a solid background on the structure and dynamics of microbial communities associated to oyster disease outbreaks paving the way to future studies aimed to shed light on mechanisms underlying polymicrobial infections in *C. gigas.*

Presenter's biography:

Alberto Pallavicini is Associate Professor in Genetics at the University of Trieste. Professor Pallavicini has a B.S. degree in Biology from the University of Padua. He has more than 25 years of research and managerial experience in academic environment with emphasis on molecular biology, population genetics, transcriptomics and bioinformatics analysis, comparative and functional genomics with a record of more than 100 papers published in international scientific journals. He is member of the Italian Genetics Society, of the Italian Society of Developmental and Comparative Immunology and of the Molluscan Applied Research Italian Society.

Exploring OsHV-1 diversity at the gene and genome scale

Deborah Cheslett (Marine Institute-IE) and Benjamin Morga (Ifremer-F)

Since 2008, massive mortality outbreaks in *Crassostreae gigas* oyster spat have been reported in several Member States associated with the presence of a variant of Ostreid herpes Virus-1 (OsHV-1) termed Ostreid herpes Virus-1 µvariant. A better understanding of the diversity of the virus both across Europe and globally will allow for the development of better tools to aid rapid diagnosis and achieve better control of the spread of the disease. To this end two complementary studies were carried out: the first one was geographically and temporally focused investigated the diversity of OsHV-1 by sequencing two regions of the viral genome; the second one was based on a whole genome sequence approach and included samples from different parts of the world, collected at different points.

The first study on Irish and UK isolates and centred on analysis of the C2/C6 and ORF 49/50 regions. The UK and Ireland were the only countries to establish surveillance programmes for the virus and hence provided unique study sites. Mortality events occurred at four distinct locations in the UK between 2010 and 2015. Sequence analysis of two different regions of the genome (C2/C6 and ORF49/50) identified some minor differences between isolates from the same sites but significant differences, particularly in the ORF49/50 between the four locations which suggest that the initial outbreaks at the four locations are independent and likely to represent separate introductions. In Ireland, the impacted sites have continued to trade with France since the initial introductions of the virus and this is reflected in the diversity of isolates collected over the study period. Isolates collected in multiple locations share insertions and substitutions suggesting a common source whilst significantly divergent sequences are observed both at the site and within the same outbreak. A comparison of the UK and Irish isolates suggest connections which would also point to the source being the same in both countries as no trade exists between the impacted Irish and the UK sites.

Presenter's biographies:

Deborah Cheslett is the Shellfish Health Manager in the Fish Health Unit at the Marine Institute in Ireland. As such she oversees the diagnostic and research work programmes for the Irish National Reference Laboratories for Molluscan Diseases. The main focus of her work relates to investigation and management of diseases and mortality particularly relating to Pacific oysters. Since 2011, her research has focused principally on OsHV-1 and *Vibrio aestuarianus* which are the main diseases of concern for the Irish Pacific Oyster Industry.

Benjamin Morga validated a phD in the La Rochelle University in 2010. He then joined the agricultural Trypanosome Cell Biology Unit (TRYPA, Pasteur Institute) for a post doctorate during two years. He studied the role of the kinesin protein in the flagellum construction. In January 2014, he joined the Laboratory of Genetics and Pathology of Marine Molluscs at Ifremer, doing research on the OsHV-1 virus. In particular, he is working on the characterization of the diversity of the virus, of the pathogenesis and of the oyster's response to a viral infection.

Maldi-tof: a tool to help characterising bacteria in shellfish

Mirna Moussa-Pouly (Ifremer-F)

In mollusc aquaculture, a large number of *Vibrio* species are considered as major pathogens that can cause high losses in hatchery and field. Thus, development of effective techniques for rapid detection and accurate identification of *Vibrio* involved in mortality events appears important to build efficient mollusc diseases surveillance programs.

Phenotypic, biochemical and molecular techniques based on DNA amplification and sequencing are widely used for the identification of *Vibrio* species in the environment, but are time-consuming because of the use of different markers to differentiate closely related species. To provide the correct identification of unknown bacteria and species classification of environmental isolates, a tool is increasingly used, the matrix-assisted laser desorption/ionization time of flight spectrometry (MALDI-TOF MS), a proteomic method able to generate a specific proteomic bacteria profile in few seconds. Nevertheless, existing databases do not contain spectra for *Vibrio* associated with marine molluscs, consequently, we proposed to create a MALDI-TOF VibrioBase database containing spectra of *Vibrio* species potentially responsible for molluscs diseases.

121 bacterial strains were analyzed in this study, belonging to 25 species: *V. aestuarianus*, *V. cortegadensis*, *V. tapetis and* species of *Coralliilyticus*, *Harveyi*, *Mediterranei* and *Orientalis* clades. A total of 72 mass proteomic spectra per strain cultured in three different media were generated and analyzed to determine specific reference spectra for each strain in order to perform the MALDI-TOF MS database specific to marine molluscs. To increase the specificity of the reference spectra, complementary statistical methods (PCA clustering and CCI matrix) were used.

The first results showed that 96% of the reference spectra created composing the VibrioBase were well-identified based on existing databases. Furthermore, we observed a good discrimination at species and subspecies levels of bacteria of the genus *Vibrio*.

This VibrioBase is a first step that could enable the use of MALDI-TOF MS as a routine diagnostic tool for rapid bacterial identification in marine molluscs. This new database will be extended by including others strains of marine *Vibrio* as strains of *Splendidus* clade and will be made available for all interested laboratories.

Presenter's biography:

After a master's degree on health biology and genetic, Mirna Moussa-Pouly carried out a PhD in biology of organisms and microbiology at Institut Pasteur of Guadeloupe where she worked in particular on pathogenic free-living amoebae present in geothermal recreational waters by developing innovative detection methods of these organisms. Currently, at Ifremer, her work is focusing on the creation of a MALDI-TOF database to better identify and characterize *Vibrio* species potentially pathogenic in marine molluscs.

A-to-I RNA editing against Ostreid herpesvirus 1

Umberto Rosani (AWI-DE)

Adenosine deaminase enzymes of the ADAR family are conserved in metazoans. They convert adenine into inosine in dsRNAs and thus alter the structural properties and coding potential of their substrates. Acting on exogenous dsRNAs, ADAR1 exerts a pro- or anti-viral role in vertebrates and *Drosophila*. We traced 4 ADAR genes in 14 lophotrochozoan genomes and we classified them into ADAD, ADAR1 or ADAR2 based on phylogenetic and structural analyses of the enzymatic domain. Using RNA-seq and quantitative real time PCR data performed on *Crassostrea gigas* and *Haliotis diversicolor supertexta* samples infected by *Ostreid herpesvirus-1* or *Haliotid Herpesvirus-1*, respectively, we demonstrated both the over-expression of one ADAR1 gene during viral infection and the ADAR editing of viral dsRNAs. Single nucleotide variation profiles obtained by pairing RNA- and DNA-seq data from the same infected animals resulted to be almost entirely compatible with A-to-I editing (93-96 %). SNVs occurred at low frequency (1.8 % for OsHV-1 and 1.35-1.55 % for HaHV-1) in genomic hotspots denoted by the overlapping of protein coding genes on opposite DNA strands, and selectively concerned adenosines, according to their upstream neighbor nucleotide. Analysis of viral sequences indicated that, under the pressure of ADAR, the two *Malacoherpesviridae* genomes can have evolved to reduce deamination targets, further suggesting that ADAR1 exerts an antiviral role in mollusks. In conclusion, we reported for the first-time evidence of an extensive editing of *Malacoherpesviridae* dsRNAs performed by the host ADAR1 enzymes. The analysis of base neighbor preferences, structural features and expression profiles of molluscan ADAR1 support the conservation of its antiviral function among metazoans. This work was done in collaboration with Paola Venier (University of Padova, Italy), Chang-Ming Bai (Yellow Sea Fisheries Research Institute, China) and Maxwell Shapiro (Stony Brook University, USA).

Presenter's biography

Umberto Rosani is post-doc at the Alfred Wegener Institute (Sylt, Germany). He previously worked at the University of Padova, where he starts to explore genomic and transcriptomic approaches to disentangle host-pathogen interactions at molecular levels. His research covers the evolution of the components of the innate immune system, the study of the antiviral pathways in bivalves and the development of advanced approaches to study bivalve-associated and environmental viromes.

Bonamia ostreae in Limfjorden in Denmark: when and where?

Lone Madsen (Technical University of Denmark, National Institute of Aquatic Resources, Kgs. Lyngby)

Limfjorden in Denmark is recognized as a unique production area for the European flat oyster, *Ostrea edulis*. In later years, the Pacific oyster (*Crassostrea gigas*), an invasive species for the area, has been found to reproduce itself in Limfjorden too. Additionally, blue mussels (*Mytilus edulis*) are fished commercially in the area. A surveillance programme regarding *Bonamia ostreae* and *Marteilia refringens*, both being parasites known to be the causes of devasting diseases in flat oyster species, was set up in 2000, and up till 2014 more than 5600 flat oysters were screened for the parasites, all animals being negative. *Bonamia ostreae* was found in native flat oysters from Limfjorden for the first time in samples taken in 2014, with no observed elevated mortalities. The following years, the parasite has been found in flat oysters from several different areas of Limfjorden. Especially in one area of Limfjorden, the prevalence seen for *Bonamia* was high (50%), whereas the prevalences in other areas have been very low (3%). Screening of the two oyster species, as well as in some cases blue mussels, originating from the same area, both the area found to have high prevalence of *Bonamia* as well as an area found to have low prevalence of *Bonamia*, has been done. Methods used for the screening have primarily been molecular techniques, like PCR (Real-Time PCR as well as a conventional PCR), whereas histology and heart imprints have been used as confirmatory methods. Neither Pacific oysters nor blue mussels, originating from areas where *Bonamia* was found in flat oysters, have been found to harbour the parasite. Therefore, these results do not show any indication of any of the two mollusc species being passive vectors of the parasite, alhough it cannot be ruled out either, as this may require more samples than this investigation has covered.

Presenter's biography:

Dr. Lone Madsen is a senior researcher at DTU Aqua. She graduated in veterinary science in 1993 and obtained a PhD in fish diseases in 2001 from the Royal Veterinary and Agricultural University in Denmark. Her research focus is prevention of diseases, including phenotypic and genotypic characterization of bacterial fish pathogens and studies on pathogenesis and transmission as well as survival on and in the fish of the pathogens. Lone Madsen has been coordinator of the national reference laboratory for mollusc diseases in Denmark since 2002 and thereby is doing diagnostics as well as being involved in research projects within the field, covering diseases of parasitic, viral and bacterial origin.

Where can Bonamia ostreae and Marteilia refringens be found outside their bivalve host, Ostrea edulis

Nicolas Mérou (Ifremer-F)

Ostrea edulis is the native European flat oyster species. Since the late 1970's, French natural populations have been affected by two epizootic diseases: marteiliosis (1968) and bonamiosis (1979), caused by the protozoan parasites *Marteilia refringens* and *Bonamia ostreae* respectively. Nowadays, these diseases still cause significant mortality in natural oyster beds and in oyster farms. These last years, there has been a renewed interest for *O. edulis* because of its patrimonial, economic and ecological interests. In this context, a better understanding of parasite cycles is needed for the management of oyster populations. Although parasites development in flat oysters is well described, many questions remain about their behavior when shed in the environment. To contribute answering these questions, we have investigated the distribution of both parasites in the surrounding environment near the oysters in Rade of Brest (Brittany, France), known to be endemic regarding both diseases. For two years (2018-2020), field sampling has been carried out every season and included not only flat oysters but also sediment, plankton and other associated benthic species. DNA detection of both parasites in samples has been carried out by real-time PCR. Analyses are still in progress but first results reveal a different seasonal dynamics of parasite prevalence in flat oysters: *M. refringens* prevalence peaks in October whereas *B. ostreae* prevalence is the highest in April. Interestingly, *B. ostreae* has only been detected in flat oysters, contrary to *M. refringens* which could be detected in all the tested categories of samples. Winter seems to represent a key period in the cycle of *M. refringens* : the parasite is no longer detected in plankton and other bivalves and its detection is maximal in the sediment. Subsequent analyses based on RNA detection and histology will also be performed to characterize more deeply parasites in these environmental samples.

Presenter's biography:

Nicolas Mérou is a PhD student at Ifremer. He validated a two-year technical training course in Biological and Biochemical Analyzes at Montpellier University Institute of Technology, then joined the agricultural engineering school VetAgroSup (Clermont-Ferrand) in which he studied terrestrial agricultural productions (plants and animals) before specializing my course in aquaculture at AgroCampus Ouest (Rennes). Since October 2017, he is doing a PhD thesis at the Laboratory of Genetics and Pathology of Marine Molluscs, under the supervision of Dr. Isabelle Arzul and Dr. Stéphane Pouvreau. This PhD thesis involves an ecological approach to study *M. refringens* and *B. ostreae* cycle outside the flat oyster, *Ostrea edulis*.

Diversity of pathogens of molluscs in Mexico and surveillance tools

Jorge Caceres Martinez (Centro de Investigación Científica y de Educación Superior de Ensenada, México)

In Mexico we have a huge diversity of bivalve mollusk that has been locally consumed. However, the main species that support the production are the Eastern oyster *Crassostrea virginica*, Japanese oyster *Crassostrea gigas* and Cortez oyster *Crassostrea corteziensis*. Currently production is around of 50,000 metric tons per year. From which around of 80% corresponds to the Eastern oyster and 20% to the Cortez Oyster and the Japanese oyster in northwest Mexico. As it is natural there are several parasites and diseases that affect oysters such as Rickettsiales like bacteria, hypertrophy of gametes, trematodes, parasitic copepods, bacteria, ciliates etc. Fortunately, only two pathogens of major importance have been detected. The oyster herpesvirus (OsHV-1) with several varieties, that affect the pacific oyster and the protozoan *Perkinsus marinus* that affect some oyster species. There are several presumptive and confirmative diagnostic techniques for surveillance of OsHV-1, such as fresh analysis, histology, transmission electron microscopy, PCR, Real Time PCR, sequencing and in situ hybridization. For surveillance PCR and Real Time PCR are the usual techniques. The other pathogen is *P. marinus* which is present in the Gulf of Mexico infecting the Eastern oyster and the Cortez oyster in the pacific coast. As in the case of OsHV-1, there are several surveillance tools. However, PCR and RT PCR are the favorite techniques. In some cases, fluid of Thioglycolate culture media is also used for this propose. However, it is important to remembering that these techniques per se do not give us complete information. Presence of DNA of a particular parasite in one host do not means the presence of a living parasite or infection. Presence of one particular parasite alive in a host does not mean, necessarily infection. Presence of an infection related with a particular parasite does not mean necessarily mortality. Thus, one of the challenges for surveillance is the correct interpretation of results and the other is the expected spreading of diseases related with world overpopulation. The impact of this population to the environment is the greater in human history. Stressor conditions related to contaminants, inadequate physicochemical compounds, high levels of organic matter increase the risk for diseases. Additionally, the increasing temperature and ocean acidification is influencing in the equilibrium of host-parasite interrelationship, increasing the risk of diseases. Thus, the efforts of surveillance must be increased. Surveillance tools for detecting bivalve diseases must be in connection with environmental monitoring. Surveillance implicates not only the use of an ideal technique. It is necessary the integral analysis of information for a correct interpretation; and surveillance must be related with environmental monitoring.

Presenter's biography:

Jorge Caceres Martinez is a Doctor in Biological Sciences from the University of Santiago de Compostela, Spain, mention "Cum Laude", Senior Researcher "D" of the Center for Scientific Research and Higher Education of Ensenada and author of 85 articles, 10 book chapters and a book, including 235 participations in conferences. He has graduated 4 PhD students, 14 Masters and 3 Bachelor. He has directed 32 research projects. He belongs to the National System of Researchers level III. Member of the Mexican Academy of Sciences. President of the Western Society of Malacology from 2003-2004. Responsible for the Reference Laboratory of Mollusk Diseases of Mexico from 2002-2007. Founder and director of the Institute of Aquaculture Health, A.C. from 2002 to 2018.

Session 1 Poster presentations

3-Bonamia ostreae in Limfjorden in Denmark: when and where?

Lone Madsen[1]

[1]Technical University of Denmark, National Institute of Aquatic Resourcwes, Kgs. Lyngby, Denmark

Presenter: Lone Madsen

Abstract

Limfjorden in Denmark is recognized as a unique production area for the European flat oyster, *Ostrea edulis*. In later years, the Pacific oyster (*Crassostrea gigas*), an invasive species for the area, has been found to reproduce itself in Limfjorden, too. Additionally, blue mussels (*Mytilus edulis*) are fished commercially in the area. *Bonamia ostreae*, a parasite known to be the cause of devastating disease in flat oyster species, was found in native flat oysters from Limfjorden for the first time in samples taken in 2014, with no observed elevated mortalities. The following years, the parasite has been found in flat oysters from several different areas of Limfjorden. Especially in one area of Limfjorden, the prevalence seen for *Bonamia* was high (50%), whereas the prevalences in other areas have been very low (3%). Screening of the two oyster species, as well as in some cases blue mussels, originating from the same area, both the area found to have high prevalence of *Bonamia* as well as an area found to have low prevalence of *Bonamia*, has been done. Methods used for the screening have primarily been molecular techniques, like PCR (Real-Time PCR as well as a conventional PCR), whereas histology and heart imprints have been used as confirmatory methods. Neither Pacific oysters nor blue mussels, originating from areas where *Bonamia* was found in flat oysters, have been found to harbour the parasite.

4-Improving the characterisation of closely related parasites by long-range sequencing of ribosomal RNA

Chantelle Hooper[1], Joe Ironside[2], Ilze Skujina[2], Georgia Ward[3], Stephen Feist[1], David Bass[1]

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Presenter: Chantelle Hooper

Abstract

Marteilia spp. (Paramyxida) cause mortalities in bivalve species important to aquaculture around the globe. It is increasingly apparent that some very closely related paramyxid lineages are parasitologically distinct and should be considered distinct biological entities. However, standard approaches to molecular diagnostics are not sufficiently powerful to resolve these differences. Here we present a new approach to discriminating between such closely related lineages using long-range PCR and sequencing of multiple regions of the rRNA gene array. We focus on two examples: *Marteilia cochillia*, associated with mass mortalities in the cockle *Cerastoderma edule* in Spain and a similar, newly detected parasite of cockles in the UK, and *M. refringens* and *M. pararefringens*, parasites with different geographical ranges and infection dynamics in oysters and mussels. Methodology: The rRNA gene array for *Marteilia* spp. was amplified by long-range PCR using a newly designed hemi-nested PCR strategy, and then sequenced on Illumina and PacBio platforms. The resulting sequences were analysed phylogenetically, and the nature of the differences between them determined. Regions potentially suitable for new diagnostic assays were identified. Results: *Marteilia* rRNA amplicons of 4,300 bp were generated using 18S forward and 28S reverse primers using long-range PCR for both Welsh and Spanish cockles and UK mussels. Sequencing showed that the ITS2 region of *Marteilia* spp. was more variable than both the the 18S and ITS1 regions, and clearly distinguish between *M. cochillia* from Wales and Spain, and between *M. pararefringens* and *M. refringens*. Phylogenetic analyses based on longer gene regions provide a more robust evolutionary basis for lineage discrimination than the more commonly-used short diagnostic regions.

Conclusions: Short amplicons are increasingly being found to be insufficient for distinguishing closely related parasite lineages. Despite linages being closely related they are often biologically and parasitologically distinct, increasing the need for correct identification to prevent mis-characterisation. With costs decreasing and read-accuracy increasing, third-generation sequencing is becoming a more viable diagnostic tool that can access sequence data multiple genes in a single read, thereby increasing the confidence of parasite identification.

5-Drop in the Ocean: Molecular Approaches for Exploring the Diversity and Distribution of the Ascetosporea

Georgia Ward[2], Isabelle Arzul[4], David Bass[1], Deborah Cheslett[3], Stephen Feist[1], Matthew Green[1], Rose Kerr[1], Stein Mortensen[5], Stuart Ross[1], Grant Stentiford[1], Catherine Troman[2], Ander Urrutia[1]

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[3]Marine Institute, Galway, Ireland

[4]Ifremer, LGPMM, La Tremblade, France

Presenter: Georgia Ward

Abstract

The protistan class Ascetosporea (Rhizaria, Endomyxa) comprises five orders of parasites of aquatic invertebrates: Haplosporida, Paramyxida, Mikrocytida, Paradinida and Claustrosporida. The group includes a number of species known for their impact as pathogens of economically significant bivalves, the most notorious of which are the oyster pathogens *Bonamia ostreae* (Haplosporida), *Marteilia refringens* (Paramyxida) and *Mikrocytos mackini* (Mikrocytida). Traditionally species discovery and description has relied on microscopy studies of infected invertebrate tissues, and as such our understanding of the group is biased towards pathogens of commercially exploited or easily studied invertebrates, with comparatively little known of the diversity of Ascetosporea outside such hosts. Similarly the distribution of Ascetosporea in geographical regions with little or no aquaculture activity or dedicated research effort is unexplored. A paucity of fully characterised species, particularly within the orders Mikrocytida and Paradinida, and a lack of molecular data for many described species across the order means the extent of the genetic and morphological diversity of the group is largely unknown, as is the true host range of most species. This lack of molecular data has also hindered phylogenetic analyses, and so limited our insight into the relationships between morphology and phylogeny.

The use of order-specific PCR screens separately targeting haplosporids, paramyxids and mikrocytids has revealed novel sequence diversity in environmental sample types (including coastal and littoral water, sediments and planktonic samples), and suggested potential relationships between novel parasite lineages and invertebrate hosts including oysters, mussels, crabs and plankton. Pairing this molecular approach with traditional microscopy and *in situ* hybridisation techniques has led to the improved detection and characterisation of novel ascetosporean species, as well as proving invaluable in generating sequence data for those previously known only from morphological studies. Here we review recent advances in our understanding of the Ascetosporea, with particular focus on paramyxids, mikrocytids and haplosporids.

6-Passive samplers to capture viruses and bacteria in the marine environment

Françoise Vincent-Hubert[1], Candice Wacrenier[1], Benjamin Morga[2], Cyrielle Lecadet[2], Solen Lozach[1], Emmanuelle Quenot[1], Dominique Hervio-Heath[1], Soizick F. Le Guyader[1]

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Presenter: Françoise Vincent-Hubert

Abstract

Detection of bivalve and human water borne pathogens mostly relies on their detection in bivalve tissues and does not consider their presence in aquatic compartment. Indeed, restricted to a few microorganisms and an issue of water sampling, the detection of viruses and bacteria in the marine environment remains difficult. In order to facilitate their detection, we have developed a passive sampling system based on the adsorption capacities of different types of membranes. Our objective was to determine if passive samplers were sensitive enough to be used as an early warning system to prevent contamination of oyster production area. Different types of membranes, nylon, zetapor, low density polyethylene (LDPE), were deployed for one year (October 2017-october 2018) in an estuarine environment. The membranes were immersed for 48 hours and up to two weeks in seawater, and the DNA of several microorganisms were searched : noroviruses (NoV) genogroups (G) I and II, human viruses responsible for gastroenteritis, *Vibrio* spp and the species *Vibrio alginolyticus*, *Vibrio cholerae*, *Vibrio vulnificus*, *Vibrio parahaemolyticus*, potentially pathogenic to humans, and OsHV-1. Regardless of membrane type and immersion time, *Vibrio* spp. were detected and quantified throughout the year, while NoVs GII were detected and quantified in winter and spring, the period corresponding to the peak of gastroenteritis in the human population. After 48h of exposure, OsHV-1 DNA was detected every month from April to August mainly on zetapor membrane, in accordance to the period of the mortality caused by OsHV-1. Nylon membrane captured larger quantities of *Vibrio* spp while the three types of membranes detected equivalent amounts of NoV GII. Based on these data, passive samplers could have various applications from early warning system to the analysis of diversity of microorganisms by next-generation sequencing.

7-Magnetic beads as a strategy to capture and concentrate O*streid herpesvirus*-1 (OsHV-1) from *Crassostrea gigas* and seawater

Anna Toldrà[1], Karl B. Andree[1], Edgar Bertomeu[1], Ana Roque[1], Noèlia Carrasco[1], Ignasi Gairín[1], M. Dolors Furones[1], Mònica Campàs[1]

[1]IRTA, Sant Carles de la Ràpita , Spain

Presenter: Mònica Campàs

Abstract

Ostreid herpesvirus-1 (OsHV-1) has been involved in massive mortality outbreaks of Pacific *Crassostrea gigas* (Pacific oysters) throughout the world, causing important economic losses to aquaculture. Therefore, rapid isolation methods as well as more sensitive detection systems are highly desired to eventually minimise the impact of this disease.

In the present study, magnetic beads (MBs) coated with anionic polymer were used to capture OsHV-1. MBs were incubated with two types of matrices (oyster homogenate and seawater) prepared using naturally infected oysters collected from Fangar Bay (NW Mediterranean Sea). Adsorption of the virus on the MBs and characterization of the MB-virus conjugates was demonstrated by quantitative PCR (qPCR) (Fig. 1).

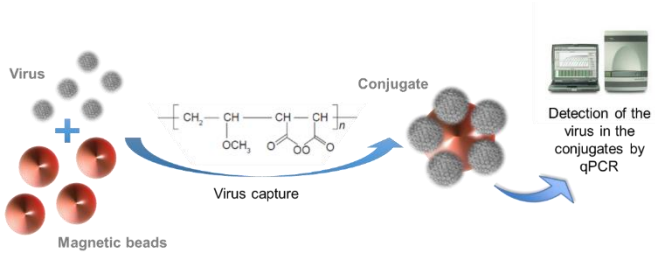

Fig.1 Capture of OsHV-1 by MBs and subsequent detection by qPCR.

To study the infectivity capacity of the captured virus, MB-virus conjugates (from both oyster homogenate and seawater) were injected into naïve spat oysters, using oyster homogenate and seawater with no MBs as positive controls, as well as bare MBs and sterile water as negative controls. Mortalities were induced after injection with MB-virus conjugates and in the positive controls, and no mortalities were recorded in the negative controls. Subsequent OsHV-1 DNA and RNA analysis of the oysters by qPCR and RT-qPCR, respectively, confirmed that the virus was the responsible for the mortality event and, consequently, the ability of the MBs to capture viable OsHV-1 particles. As a proof of concept, the utility of MBs as pre-concentrating agents was assessed using the oyster homogenate. Results indicated that MBs were able to pre-concentrate OsHV-1 particles at least 100 times. Afterwards, MBs were applied to the analysis of seawater during a depuration experiment. No OsHV-1 DNA was detected when using qPCR. Nevertheless, OsHV-1 DNA was detected when MBs were used prior qPCR, demonstrating that MBs were able to pre-concentrate OsHV-1 from seawater. The use of anionic polymer-coated MBs is a rapid, easy and cost-effective strategy to isolate OsHV-1 particles from complex matrices, and could be of great utility in research activities. Additionally, MBs are able to improve the limits of detection of qPCR by previously concentrating virus particles from seawater, thus getting closer to an early warning system.

8-An investigation into the existence of Oyster Herpesvirus-1 microRNAs

Barry Digby[1], Amanda Brechon[1], Ciar O'Toole[2], Aaron Golden[1], Deborah Cheslett[2], Terry Smith[1], Owen Donohoe[3,4]

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[2]Marine Institute, Galway, Ireland

[3]University of Liege, Liege, Belgium

[4]Athlone Institute of Technology, Athlone, Ireland

Presenter: Owen Donohoe

Abstract

Oyster Herpesvirus-1 (OsHV-1) is a pathogen of Pacific oysters (*Crassostrea gigas*) and a viral agent responsible for significant economic loss within the mollusc aquaculture industry across many regions. OsHV-1 is a member of the *Malacoherpesviridae* family, and like all herpesviruses, it is possible that it can exist as an asymptomatic latent infection, either in the absence of replication or with periodic low level replication. The primary goal of this project is to explore novel ways to overcome some of the problems we encounter when trying to detect OsHV-1 during latency. As with other viruses, OsHV-1 DNA levels are present at very low levels in latently infected hosts. This has important implications for molecular diagnostics methodologies, as it increases the chance of obtaining false negatives and underestimation of viral prevalence among latently infected populations. This is because there is no viral replication taking place at this stage. However, as observed with other herpesviruses, some OsHV-1 genes are expected to be transcribed during latency. Interestingly, latency associated transcripts (either non-coding or protein-coding) may be of considerable diagnostic value. In terms of non-coding latency associated transcripts, viral microRNAs (miRNAs) have been shown to be very prominent during latency. MiRNAs are small (~21 nt) single stranded non-coding RNAs that are involved in gene regulation and they are initially derived from longer precursor-miRNAs (pre-miRNAs) that exist as imperfect hairpin structures (prior to processing into small ~21nt mature miRNAs). Thus, in this study we explored the potential existence of OsHV-1 encoded miRNAs. As part of this process we sequenced over 440 million small RNA transcripts from OsHV-1 infected tissue from Pacific oysters and identified those that mapped to regions of the genome predicted to give rise to pre-miRNA secondary structures. However, despite the depth of sequencing, the amount of small RNAs mapping to potential pre-miRNA coding regions in the viral genome was very low and not sufficient to allow confident annotation of potential novel viral miRNAs. This was primarily due the fact that viral RNA only represented a very small fraction of total RNA reads from these *in vivo* samples. Given the low representation of viral RNA *in vivo* - as noted in studies involving the identification of miRNAs expressed by other herpesviruses, the use of an *in vitro* model would be more suited to this type of investigation. Such a model would facilitate a greater proportion of virally infected cells and thus greater representation of viral small RNAs in RNA-seq data in quantities sufficient for investigation into novel viral miRNA discovery and profiling.

9-Improved Enrichment Broth for Isolation of Shellfish-associated *Arcobacter*-like Species

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[1]IRTA-Aquaculture Center, San Carlos de la Rapita, Spain

[2]Universitat Rovira i Virgili (URV), Tarragona, Spain.

Presenter: Faiz Ur Rahman

Abstract

Arcobacter is a genus of gram negative, spiral shaped, non-spore forming, slender, motile, slightly curved rods, aero-tolerant bacteria belonging to ε-Proteobacteria subdivision, that was separated from the genus Campylobacter in 1991 by Vandamme et al. During isolation of bacteria on synthetic media, many bacterial cells can be present in a VBNC state (Viable but non-culturable) and thus are not detected due to insufficient growth promoting factors and nutrients, or due to other unknown reasons (Barbau-Piednoir et al., 2014). Until now, no official standard protocol exists for the isolation of *Arcobacter* from all or any specific kind of products or environment. The conventional pre-enrichment methods used commonly are time consuming that require at least 72-96 hours for the growth of culture (Levican et al., 2016). Despite the fact that 18 species out of a total of 29 *Arcobacter* species are isolated, from marine environment, there is still a lack of knowledge on diversity and presence of commensals as well as pathogenic *Arcobacter* found in marine invertebrates (Mizutani et al., 2019). The new taxonomic classification of the genus *Arcobacter* by Perez-Catalunya et al., (2018) into seven genera indicates that the knowledge of *Arcobacter* is still in its infancy and we need to focus more on their cultural strategies from different sources. New *Arcobacter* species can be isolated by developing new enrichment techniques or addition of specific nutrients required for their growth. The isolation of new *Arcobacter* species from shellfish is increasing (Salas-Masso et al., 2016) and these studies suggest the potential of a lot more novel *Arcobacter* species from the marine environment and shellfish. We tried to selectively cultivate *Arcobacter* associated with shellfish or their environment, by adding nutrients from shellfish and different levels of salinity to develop one or more efficient enrichment broths for isolation of shellfish-associated Arcobacters.

10-Potential genetic material exchanges between host and virus: study case *of C. gigas* and OsHV-1

Camille Pelletier[1], Serge Heurtebise[1], Nicole Faury[1], Lionel Degremont[1], Jean-Michel Escoubas[2], Jean Delmotte[2], Tristan Renault[3], Benjamin Morga[1], Jean-Baptiste Lamy[1]

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Presenter: Camille Pelletier

Abstract

Since the 90's, the cupped oyster Crassostrea gigas has suffered significant mortality events associated with the detection of the Ostreid Herpesvirus type 1. This virus is still present in the environment and is able to evolve rapidly by deletion of its genetic information. Moreover, the replication, capsid formation and DNA packaging steps of the Herpesvirus life cycle occur in the nucleus of oyster cells. In this context, we suspect some genetic material exchanges between C. gigas and OsHV-1. In our study, we have searched junctions between oyster and virus DNA to highlight chimeras (sequences composed by oyster and viral genome). Oysters infected by OsHV-1 in the natural environment or viral purified suspension were used to detect chimeras. Data obtained using Illumina Hiseq sequencing technologies were aligned to C. gigas and OsHV-1 (or OsHV-1 variants) references genomes. Aligned libraries of C. gigas and OsHV-1 were compared to find chimeras. Then, we used chimeras to describe the position of genetic material exchanges in the oyster and virus genomes. As a second step, an analysis of nucleotide content has been performed with chimeras previously identified in order to search for repeated nucleotide motifs. An annotation of chimeras or chimeric regions has been done to determine the biological function associated. According to the results, we identified genetic material exchanges between the oyster and OsHV-1 genome. 6000 chimeras were found, spread in particular positions of the references genomes used. Within OsHV-1 genome, 2000 chimeras are localized in repeated regions which contain telomeric repeats. In other herpesviruses (e.g. Human Herpesvirus-6), telomeric repeats are involved in the integration of the virus into the host. Inside the C. gigas genome, chimeras are localized in GC-rich regions (25% of chimeras have an aberrant GC%), which are possibly involved in DNA transcription promoters stability or are preferential recombination sites. These first results make us suspect a genetic material exchange between the virus and host genome. Finally, no chimera was detected in the analysis of sequences from purified viral suspension, indicating instead a possible integration of the OsHV-1 genome into the C. gigas genome. However, more analyses are needed to confirm this hypothesis. We propose to sequence infected samples using long reads approach.

Session 2: Understanding bivalve functional response for alternative methods of prevention and treatment

Keynote presentation: Bivalve disease management based on the knowledge of their immunity: the European advantage

Antonio Figueras, VIVALDI Work package 2 leader (CSIC-ES)

There are more than 15.000 molluscan species. They range from one millimeter to264 Kg. The commercial species cover a wide range of biological characteristics that cannot be overlooked when studying their immune response. These commercially important species are all filter feeders and can filter up to 7,5 liter per hour of water, getting in contact with millions of potential pathogens but they can live inside or on top of sandy or muddy sea beds or hanging from rocks or ropes in calm or quite exposed waters. Environmental pollution such as plastics (micro and nano) can influence their immune response. An established bivalve disease can only be "managed". In less than 10 years (2007-2016) the annual production of Pacific oyster in France went from 110.800 tons to 64.000 tons creating a tremendous socioeconomic and political crisis (FAO, 2018). Strikingly mussels (Mytilus galloprovincialis) were in the same ecosystem but did not die. The already available oyster, clam and the mussel genome (already submitted for publication) may boost new developments in the functional and comparative genomics of bivalves and their interaction with pathogens. We compared the results of in vivo and in vitro experiments using clams, oysters, cockles and mussels exposed to virus, bacteria and parasites. Several transcriptomes, proteomes and metabolomes were obtained and analyzed. Bivalve immune system is able to discern among virulent and non-virulent bacteria mounting a totally different response. Autophagy is a well conserved pathway in bivalves and almost all intervening genes have been detected in the oyster. Antimicrobial peptides are highly expressed in mussels and play an important role in their immune response. As all invertebrates, bivalves, lack an adaptive immune system, but they respond to pathogens, injuries or environmental stress in a very efficient manner. However, it is not well known if they are able to modify their immune response when they reencounter the same pathogen. Our results also show that after a first exposure to different pathogens or pathogen-associated molecular patterns (PAMPs) the second exposure led to changes at the transcriptional (i.e. control of the expression of pro-inflammatory transcripts), cellular (shift in the hemocyte population distribution), functional levels (inhibition of ROS production) and even phenotypic changes (higher survival rates when challenged with pathogens). These results suggest that a modified immune response after the second challenge allowed the mussels to tolerate rather than fight the infection, which minimized tissue damage.

Presenter's biography

Antonio Figueras: Research Professor of CSIC. BSc. Biology (1979), MSc. Biology (1979) and Ph.D. Biology (1984) Universidad de Santiago de Compostela. Director of the Spanish National Reference Laboratory Mollusc Diseases (UE Directive 95/70; Real Decreto 1043/1997) since 1996. Branch Official of European Association of Fish Pathologists in Spain since 1993. Member of the Research Advisory Board of Xunta de Galicia (2003-05). Vice-president of the Developmental and Comparative Immunology Society 2015-present. Professor of Postgraduate Studies (MSc) at Univ. Santiago, Vigo and La Coruña. Head of: Department Natural Resources, 1995-96; Department of Biotechnology and Aquaculture 2006-11. IIM, CSIC.

Director of the Institute of Marine Research, CSIC: 2001-05 and 2015-18. Vicepresident of Research and Technology Spanish National Research Council 2012-14.

Scientific activity: More than 250 scientific papers in SCI journals and 37 books and book chapters. H index. 51 (WOK). Advisor of 20 PhD. Principal Investigator (PI) of: 16 National Projects, 16 European Projects (in 2 also Coordinator), 7 International Projects and Integrated Actions. Member of the Editorial Board of Aquatic Living Resources. 2001- present. Coordinator of the CSIC Aquaculture network since 2004. Several Research Awards

Research interests: Diseases of aquatic organisms, mainly shellfish. Immune response of fish and shellfish and molecular basis of this response. Marine genomics. Marine bioactive compounds. Global climate change impact on aquaculture.

The dark matter of the genomes in marine bivalves: long non-coding RNAs as key players

Cristian Gallardo-Escárate (Interdisciplinary Center for Aquaculture Research-Chile)

Cancelled presentation

The complexity of the genomes in marine organisms and their functional information have been defined for only a small proportion of them. Here, close to ~1% of the genome is transcribed into protein-coding (mRNA) and non–protein-coding (ncRNA), where DNA elements controlling the gene expression involves ~0.5%. These facts suggest that the genome in marine species is dominated by a "dark matter," which is mostly nonfunctional. However, this portion of the DNA is pivotal for the evolutionary process and greatly exploited in bivalves such as mussel to face with the continuously changing marine environment. This study aimed to explore the role of ncRNAs in Bivalvia, reviewing the functional implications of long non-coding (lncRNAs) in the immune response of mussel exposed to pathogens and HABs, and how ncRNAs are modulated in individuals exposed to contrasting environments and also to marine pollution such as microplastics. Interestingly, the analysis of lncRNAs revealed that these transcripts are involved in relevant biological processes such as immune system and local adaptation. Herein, a comparative transcriptome analysis among different bivalve species was conducted. Our results provide the first identification of lncRNAs in Bivalvia and evidence that lncRNAs are key players in the biology marine organisms.

Immune priming and transgenerational immune priming in C. gigas

Caroline Montagnani (CNRS/Ifremer- F)

The major economic and environmental consequences of recurring mortalities affecting the Pacific oyster Crassostrea gigas have initiated many research projects aiming at understanding these phenomena. The solutions anticipated to deal with these mortalities are mainly based on mass selection breeding programs but preventive treatments are still lacking. However, over the last decade, studies have been accumulating revealing the adaptive capabilities of innate immunity, the only component of defense mechanisms in invertebrates. Numerous findings have shown that a wide range of invertebrates can develop innate immune memory (also called immune priming) leading to improved survival during a second encounter with a pathogen. Moreover, accumulating studies has brought new highlights on how the host immune system has been co-opted to establish and shape beneficial host-microbiota relationships contributing to the host health status and fitness notably through early interactions with the immune system. In this context, we undertook to study the possibilities of acting against mortalities by stimulating immune capacities of oysters.

Our results show that the exposure of oyster juveniles to an immunostimulant (a viral mimic called poly (I: C)) can lead to enhanced survival capacities (up to 100%) following OsHV-1 infection or during a mortality episode in the field. That protection is specific to viral protection as poly(I:C) fails to protect oyster against a pathogenic bacteria. This priming phenomenon is durable as it can last more than 4 months. Analysis of the molecular pathways underlying that protection using RNAseq, revealed that priming was based on the triggering of a strong and sustained antiviral response limiting replication of the virus, thus allowing the protection of oysters on the long term.

Moreover, we demonstrated that an early exposure to a non-infectious environmental microbiota could also lead to enhanced survival capacities towards the Pacific Oyster Mortality Syndrome. Originally, we could show that the impact of early microbial exposure on survival capacities extends to the next generation suggesting a multi-generational effect on offspring performances. Microbiota's impact as well as molecular and epigenetic determinants orchestrating these phenomena is currently investigated using integrative, high throughput approaches (microbiota analysis, transcriptomics, epigenetics).

Altogether these studies show evidence for within and trans-generational innate immune memory in the oyster leading to enhanced survival capacities following OsHV-1 infection or during a mortality episode in the field. They bring new insights into the oyster capacities to build an innate immune memory, its adaptive capacities and provide a platform to further explore novel strategies to help mitigate disease threats upon marine bivalves.

Presenter's biography

As a marine biologist, my general interest is to develop research project to formulate answers to current issues relating to the health of marine invertebrates and optimization of aquaculture production. My work focuses on the molecular interactions between the immune system of the Pacific oyster and its microflora either to defend its integrity or to promote the adaptation and creation of beneficial interactions within these complex ecosystems. More specifically, I currently investigate the molecular mechanisms of immune priming, or innate immune memory, in the oyster to develop novel strategies to protect oyster against recurrent diseases.

Macrophage migration inhibitory factor (MIF) in bivalve immunity

Paola Venier (UNIPD-IT)

Cytokines are small and heterogeneous proteins involved in cell-to-cell signalling. In vertebrates, they are secreted by a number of cell types, including immune cells, and they act via receptor-binding and intracellular signalling to modulate the expression of molecular effectors and the behaviour of target cells (i.e., growth, maturation, responses). Overall, cytokines can generate a complex interaction network eventually resulting in effective immune responses or even abnormal reactions, with organ and systemic effects. Macrophage migration Inhibitory Factor (MIF) is a pleiotropic cytokine, first described in relation to delayed hypersensitivity and macrophage functions and later recognized as an inflammatory cytokine and pituitary-derived hormone able to counteract glucocorticoids. MIF is currently studied also in relation to normal/abnormal cell growth, wound repair and inflammatory disorders.

Evidence for the existence of cytokines in invertebrate animals is relatively recent and few cytokine-like molecules have been identified also in bivalve mollusks, such as oysters and mussels. Here, we report compelling data (Fish and Shellfish Immunology, 93: 39–49, 2019) and new developments (Rosani et al. 2019 at http://www.isj.unimo.it/index.php/ISJ/article/view/557/447) related to bivalve MIF. Wide-range analyses of genome/transcriptome data available for 48 bivalve spp. allowed the identification of 137 MIF-like sequences. Most analyzed species displayed two MIF paralogue genes, namely MIF and D-Dopachrome tautomerase or D-DT, and the Mytilidae family was clearly characterized by the genomic expansion of D-DT genes. According to RNA-seq and RT-qPCR data obtained so far in M. galloprovincialis, the tissue-specific expression values of nine MgMIF-like genes did not provide enough support to MIF as a cytokine, instead suggesting an alternative role of MIF in highly specialized mussel tissues. A recombinant M. galloprovincialis MIF (MgMIF) protein produced in Pichia pastoris is now in use to ascertain the possible enzymatic activity (oxidoreductase and dopachrome methyl ester decarboxylase into 5,6-dihydroxyindole or DHI) and the transcriptomic effects of rMgMIF in mussel hemocytes and in human macrophages for comparison. U. Rosani, S. Domeneghetti, M. Gerdol, F. Vallese, E. Bortoletto, G. Zanotti, A. Pallavicini, R. Tavano participate in the study. Research supported by the H2020 project VIVALDI.

Presenter's biography

Paola Venier is associate professor in Microbiology at the Department of Biology, University of Padova (Italy) with knowledge and experience in Genetic toxicology and Genetics. She is author of 87 peer-reviewed papers (H index 32 at:

https://www.scopus.com/authid/detail.uri?authorId=7004589203

in addition to book chapters and communications to scientific meetings. She has been partner in Transborder cooperation and European FP6, FP7 and H2020 programmes. She was the main organizer of the international symposium Advances in Marine Mussel Research 2019 (AMMR 2019) and, as main research leading motif, she considers marine bivalves as holobionts in the frame of a changing environment.

Tissue lesions induced by OsHV-1 µVar and their evolution in time

Isabelle Arzul (Ifremer-F)

B. Chollet, B. Morga, L. Dégremont, M. Noyer, C. Dubreuil, D. Serpin, I. Arzul, C. Garcia

LGPMM-Ifremer La Tremblade

Previous works have reported the detection of OsHV-1 µVar by Real-Time PCR in different organs of oysters *Crassostrea gigas* during experimental infections and have identified gills and mantle as potential targets of the virus (Schikorski et al. 2011, Segarra et al. 2016). Although tissue necrosis and/or hemocyte infiltrations associated with nuclear abnormalities have been described in animals collected during mortality events (Hine et al. 1992, Renault et al. 1994 and 2001), few information is available regarding lesions associated with OsHV-1 µVar infection in histology. In order to better characterize lesions associated with the virus and its distribution in oyster tissues, an experimental infection mimicking the natural OsHV-1µvar infection pathway was carried out using 9 months old oysters produced in hatchery. First dead oyster was observed at Day 3 and 47% of oysters showed mortality after 8 days. A total of 30 oysters were collected twice a day on a 4 days period and tested by Real Time PCR for the detection and quantification of OsHV-1 (Martenot et al. 2010. Results showed an increase of the number of positive oysters and viral load with time and allowed classifying oysters depending on their level of viral infection. Oysters collected between 16h and 72h after infection were selected for histological and *in situ* hybridization analyses. Focal hemocyte infiltration was observed in lightly and more heavily infected oysters whereas necrosis and nucleus abnormalities appeared more prevalent with the development of the infection. In advanced infection, haemocytic infiltration was often associated with necrosis. Nuclear abnormalities consisted of marginated chromatin, pycnotic nucleus or nucleus fragmentation. These abnormal nuclear pictures were mainly observed in gills and mantle at the beginning of the infection and then increased and reached the connective tissues including in heart and nervous tissues. In highly infected oysters, severe necrosis was observed in many tissues /organs including muscular fibers contributing to a loss of tissular architecture. *In situ* hybridization appeared less sensitive than Real Time PCR allowing the detection of OsHV-1 DNA in oysters with a moderate and high viral load. Gills and mantle are the main organs where the OsHV-1 infection develops. However, in advanced infection, OsHV-1 DNA was detected in all the organs and in different cells including connective tissue cells, hemocyte-like cells but not in epithelial cells. This work highlights the interest of combining molecular and histology not only to better understand host and virus responses during an infection but also symptom/physiological dysfunction associated with the virus. Further work will be done to better characterize cell types infected with the virus and to better evaluate the impact of OsHV-1 on tissular architecture.

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Mytilus galloprovincialis immunity

Beatriz Novoa (CSIC-ES)

Mytilus galloprovincialis is the marine cultured species with the highest production in Europe. It has higher resistance against diseases than other bivalves such as oysters, clams or cockels. Almost half of the expressed genes in mussel hemocytes are antimicrobial peptides that could explain the high resistance of this species. Also, the complexity of the mussel genome that has been recently sequenced could be associated to this resilience. Myticin is one of the mussel antimicrobial peptides and myticin C has been reported to present high sequence variability. At functional level, myticin C has antibacterial and antiviral properties (inhibits OsHv replication in oyster hemocytes and also human herpes virus replication). Recently, we demonstrated that myticin C is also involved in tissue regeneration with a biotechnological potential since it increases the proliferation of human keratinocytes and regeneration of zebrafish wounds.

Presenter's biography

Beatriz Novoa is a Research Professor at the "Instituto de Investigaciones Marinas CSIC" and head of the "Immunology and Genomics" group. Graduated in Biology in 1989 and PhD in Biology from Universidade de Santiago de Compostela in 1993. She has been a postdoctoral researcher at the University of Aberdeen (United Kingdom) and has completed her training as a researcher at the University of Maryland, University of Maine, Harvard University and University of Pennsylvania (United States). She has published more than 190 scientific articles published in SCI journals, of which 85% correspond to journals included in the first quartile of its category, and 27 chapters of books / books. She has been Principal Investigator of National and European research projects and has received several research awards among which the "Academia Gallega de las Ciencias" prize (1993) and the "VI Premio Jacumar de Investigación en acuicultura**"** (2006). She is the director/co-director of 13 Doctoral Thesis and has also directed master's thesis and final year projects. In addition, she participates in doctoral programs in collaboration with different Universities. She has organized international congresses and seminars and has participated in several scientific committees of international congresses. In 2016, she has been named President of the International Society of Fish immunology Shellfish immunology. Her lines of research are focused on the study of the molecular basis of the immune response of fish and molluscs through gene expression analysis. In addition, she studies inflammatory processes associated with human diseases using the zebrafish (Danio rerio) as a model.

New advances in autophagy in *Crassostrea gigas*

Benjamin Morga (Ifremer-F)

Mortality outbreaks of young Pacific oysters, Crassostrea gigas, have seriously affected the aquaculture economy in several countries around the world. Although the causes for these mortalities outbreaks are complex, a viral agent was identified as the main factor, the ostreid herpesvirus 1 (OsHV-1). The mean to fight against the virus remains limited and Pacific oyster/virus interactions need to be further investigated. Recently, the results of several studies and the *C. gigas* genome sequencing have demonstrated the potential existence of several known mammals' antiviral pathways in the Pacific oyster. The autophagy pathway is involved in many cellular processes including immune defense. This pathway seems to be functional in the mantle of *C. gigas* and involve in the response of the Pacific oyster to several pathologies including viral diseases. As part of this work was to improve knowledge about the autophagy pathway mechanism in *C. gigas* and to decipher it modulation during the process of an infection by the virus OsHV-1. This work has highlighted a strong conservation of the pathway of autophagy at the molecular level. For the first time in *C. gigas*, autophagic structures were observed in haemocytes. This result has allowed to development new approaches to detect and monitor the regulation of autophagy in Pacific oyster. A monitoring of autophagy during an infection by the virus OsHV-1 showed that the viral replication is followed by a modulation of autophagy in the mantle and in haemolymph. Finally, a differential regulation of the autophagy pathway at the transcriptomic level in the mantle and haemolymph has been shown.

Presenter's biography

After having validated a phD in the La Rochelle University in 2010, I joined the agricultural Trypanosome Cell Biology Unit (TRYPA) for a post doctorat during two years. I studied the role of the kinesin protein in the flagellum construction. Since January 2014, I joined the Laboratory of Genetics and Pathology of Marine Molluscs. I am doing research on the OsHV-1 virus. In particular, I am working on the characterization of the diversity of the virus, of the pathogenesis and of the oyster's response to a viral infection.

Implication of the type IV secretion system in the pathogenicity of *Vibrio tapetis*, the etiological agent of Brown Ring Disease affecting the Manila clam *Ruditapes philippinarum*.

Alexandra Rahmani (CNRS LEMAR-F)

Vibrio tapetis is a Gram-negative bacterium that causes infections on mollusk bivalves and fish. The Brown Ring Disease (BRD) is an infection caused by V. tapetis that affects the Manila clam Ruditapes philippinarum. Recent studies have shown that the type IV secretion system (T4SS) gene cluster is exclusively found in strains of V. tapetis pathogenic to clams. However, whether the T4SS is effectively implicated during the infection process remains unknown. The aim of this study was to create and characterize a V. tapetis T4SS null mutant, in order to determine the role of T4SS in the development of BRD. This work demonstrates that the T4SS of V. tapetis plays a crucial role in the development of BRD in the Manila clam.

Presenter's biography

After a master's degree in microbiology at UPMC Sorbonne University, I continued my training with a PhD on pathogens and more particularly on the Vibrio tapetis bacteria responsible for brown ring disease in Manila clam in the context of the Vivalvi H2020 project at the "Laboratoire des Sciences de l'Environnement Marin", the LEMAR lab. I was able to conduct my research in order to better understand the mechanisms linked to this disease. Recently PhD graduated from the "Université de Bretagne Occidentale", I am currently pursuing my research at the CNRS, still on the subject of shellfish pathogens at the LEMAR lab in a more applied field.

Mucosal immunity in bivalves

Bassem Allam (School of Marine and Atmospheric Sciences-USA),

The growing economic importance of bivalves has been associated with an increased awareness of, and attention to, infectious diseases affecting these animals. Despite this information, there is a lack of understanding of factors affecting bivalve resistance to infections. Of great concern is the fact that most studies on bivalve (and molluscan in general) immunity focus on the circulating hemocytes and the humoral defense factors in the plasma while most relevant host-microbe interactions occur at mucosal interfaces. Mucus is produced from virtually all molluscan epithelia and plays an essential role in functions as diverse as lubrication, feeding, protection from environmental stress, and physical and biological barrier to infections. In bivalves, pallial organs (e.g. gill, mantle, palps) process a large volume of water and are exposed to the suite of waterborne microbes present within. Therefore, efficient microbial handling at these mucosal interfaces is a key factor for host homeostasis and health. This presentation highlights the role of mucosal immune factors in bivalve-microbe interactions. Available information underlines the diversity of immune effectors at bivalve mucosal interfaces and highlights the tailored immune response to pathogen stimuli. A fascinating discovery was that recognition molecules present in pallial mucosal secretions and known to play a major role in immunity (lectins) can be used by suspension-feeding bivalves to selectively capture and sort nutritious food particles and enhance energy gain. Finally, our research allowed the identification of mucosal hemocytes associated with pallial surfaces. These hemocytes, which can migrate to the circulatory system, occupy a niche similar to that of dendritic cells in vertebrates. Overall, current data underline the diversity and the dynamic nature of immune factors associated with bivalve mucosal interfaces. Unraveling host-microbe interactions at mucosal interfaces will likely lead to novel disease mitigation strategies.

Presenter's biography

Bassem Allam is the Marinetics Endowed Professor at Stony Brook University and Director of the Marine Animal Disease Laboratory (MADL). He earned his PhD from the European Institute for Marine Studies in Brest, France, and performed his postdoc at Rutgers University in New Jersey before joining Stony Brook in 2003. His research centers on the physiology and host-microbe interactions in shellfish, the resistance of these animals to infectious diseases, and how the environment affects these interactions. His work resulted in the publication of over 90 scientific publications and book chapters on various aspects of shellfish physiology and pathology.

Session 2 Poster presentations

11-Immune tolerance in *Mytilus galloprovincialis* haemocytes after repeated contact with *Vibrio splendidus*

Magalí Rey-Campos[1], Rebeca Moreira[1], Marco Gerdol[2], Alberto Pallavicini[2,3], Beatriz Novoa[1], Antonio Figueras[1]

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Presenter: Antonio Figueras

Abstract

Mediterranean mussels (*Mytilus galloprovincialis*) are sessile filter feeders that live in close contact with numerous marine microorganisms. As is the case in all invertebrates, mussels lack an adaptive immune system, but they respond to pathogens, injuries or environmental stress in a very efficient manner. However, it is not known if they are able to modify their immune response when they reencounter the same pathogen. In this work, we studied the transcriptomic response of mussel haemocytes before and after two consecutive sublethal challenges with *Vibrio splendidus*. The first exposure significantly regulated genes related to inflammation, migration and response to bacteria. However, after the second exposure, the differentially expressed genes were related to the control and inhibition of ROS production and the resolution of the inflammatory response. Our results also show that the second injection with *V. splendidus* led to changes at the transcriptional (control of the expression of pro-inflammatory transcripts), cellular (shift in the haemocyte population distribution) and functional levels (inhibition of ROS production). These results suggest that a modified immune response after the second challenge allowed the mussels to tolerate rather than fight the infection, which minimized tissue damage.

12-Transcriptomic response of *Mytilus galloprovincialis* gills to a bath infection with *Vibrio splendidus*

Amaro Saco[1], Magalí Rey-Campos[1], Antonio Figueras[1], Beatriz Novoa[1]

[1]Institute of Marine Research (IIM), National Research Council (CSIC), Vigo, Spain

Presenter: Beatriz Novoa

Abstract

Mussels (*Mytilus galloprovincialis*) are filter feeder animals that are constantly in contact with a wide range of microorganisms, some of which are potentially pathogenic to them. The fact that it is not clearly understood yet how mussels respond to pathogens made us focus on the mechanisms that these animals could use to deal with a bath infection with *Vibrio splendidus,* trying to replicate natural conditions. The study of the bacterial concentrations induced by the infection in different mussel tissues and their evolution showed that mussels can remove the pathogen from their body and from the tank water. Gills may have a central role in removing and cleaning all the bacteria that mussels filter therefore experiments were performed, analysing antibacterial and antiviral activity of mussel gills. In order to understand which processes are taking place on gills after an interaction with bacteria, a transcriptomic study was performed with control and infected mussel gills, 24 hours after the bath infection. A total number of 1156 differentially expressed genes was found. Genes contributing to immune response activation and its regulation with cytokines, cell adhesion and apoptosis were significantly up-regulated under the infection. Antimicrobial peptides genes such as mytimicin and defensin were among the down-regulated genes. These results showed that in gills processes related to the recognition of the infection and the activation and regulation of the innate immune response are taking place, revealing the important role that gills may play in the response against pathogens

13-Virulence differences between GFP-tagged pathogens and their parental strains for blue mussel (*Mytilus edulis*) larvae

Dongdong Wang[1], Gilbert Van Stappen[1], Nelia Mbewe[1], Nancy Nevejan[1]

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Presenter: Dongdong Wang

Abstract

Pathogens, especially vibrios, are largely responsible for larval diseases in shellfish aquaculture. Clarifying the mechanisms of Vibiro infections during the first hours is quite necessary for disease prevention. To make sure pathogens can be tracked *in vivo*, two strains of known pathogenic Vibrio of blue mussel (*Mytilus edulis*) larvae, *Vibrio hemicentroti* (ME09) and *V. anguillarum* (NB10) were labeled with green fluorescence protein (GFP), provided by *Escherichia coli* DH5α.

Following a previously developed challenge model (Eggermont, et al., 2017), healthy two-day-old D-larvae were challenged with the GFP-tagged pathogens and their parental strains in vivo, at concentration of 104, 105 and 106 CFU·ml-1, respectively. ME09-GFP and ME09 showed stronger toxicity to blue mussel larvae than NB10 and NB10-GFP. But all four strains showed less than 7%

mortality at day 1. Specifically, ME09-GFP and ME09 caused a high mortality from 48 h onwards and a mortality of 85% at day 4 was observed for all concentrations. There was, however, a very low mortality among NB10 and NB10-GFP treatments at day 2. A significant larval mortality was only observed and was concentration-dependent from day 3 onwards. Besides, compared to their parental strains, the GFP-tagged vibrios were less virulent because they obviously delayed blue mussel larvae mortality. As more energy is required for this extra protein, incorporation of GFP can be a metabolic burden for the bacterial cells (Allision & Sattenstall, 2007). In the future, the invasion pathway of GFP-labeled pathogens is set up to be determined and the homochromous histological damage can be followed.

14-*Ostreid herpesvirus* type 1 gene expression temporality revealed by RNAseq analyses of OsHV-1 infected cupped oyster (*Crassostrea gigas*) haemocytes

Margot Tragin[1], Nicole Faury[1], Jean-Baptiste Lamy[1], Sandy Picot[1], Tristan Renault[2], Benjamin Morga[1]

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[2]Ifremer, RBE, Nantes, France

Presenter: Margot Tragin

Abstract

Temporal expression patterns of herpesviruses at the entire genome scale were already demonstrated in mammals (herpes simplex virus 1 (Stingley et al. 2000), Rhesus Monkey Rhadinovirus (Dittmer et al. 2005)) and fish (anguillid herpesvirus 1, Beurden et al. 2013), but not yet in mollusks. Here, in vitro infections of *Crassostrea gigas* haemocytes by a viral suspension of OsHV-1 were performed and followed from 0 hours to 8 hours post contact by RNAseq. The data from 12 samples were analyzed by a home-coded bioinformatics pipeline and normalized to allow intra- as well as inter-sample comparisons. Along time series, an increasing number of reads, from 169 (right after contact) to 153521 (8 hours after contact, hpc), mapped the OsHV-1 µvar A reference genome (KY242785). Reads were distributed on the 142 gene predictions (132 Open Reading Frames, ORFs) of the virus reference genome and some reads especially mapped the ORF.IN regions, which are typical of µvar OsHV-1 variant. Temporal clustering normalized reads per gene predictions (gp) was performed on the data and revealed 4 main clusters in which 3 matched previous literature description. Four gp reached their maximum between 0 and 1 hpc corresponding to putative “immediate-early” genes, 7 gp (5 ORFs) showed a peak at 2 hpc corresponding to putative “early” genes, 29 gp in a cluster and 5 gp in another reached their maximum between 2 and 4 hpc corresponding to putative “late” and/or “early-late” genes. However, the last group of 5 gp was clustered with 5 other gp, which curves presented both a peak of normalized reads count at 20 minutes post contact and an increase at 8 hpc. These results clearly showed that OsHV-1 genes were temporally expressed, but they also suggested that the temporal clustering method seemed to gather genes according both the temporality and the intensity of normalized reads count. In the short term, RNAseq data from longer time series (from 0 to 24 hpc) will be analyzed. The new dataset will help confirming the very beginning of OsHV-1 gene expression temporality and it should allow to compare OsHV-1 temporal expression cycle infecting susceptible and resistant oyster haemocytes.

15-Comparison of gene expression and biological pathway response to *Oyster Herpesvirus*-1 (OsHV-1) infection among resistant and susceptible Pacific oyster populations

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Presenters: Owen Donohoe, Terry Smith, Benjamin Morga

Abstract

Oyster Herpesvirus-1 (OsHV-1) is a pathogen of Pacific oysters (*Crassostrea gigas*) that is responsible for significant economic loss within the mollusc aquaculture industry across many regions. The identification of Pacific oyster populations that show varying susceptibility to OsHV-1 present us with interesting subjects to use in the identification of specific biological characteristics that correlate with OsHV-1 resistance. In this study we looked at differences in gene expression and biological pathway perturbation between OsHV-1 resistant and non-resistant Pacific oysters in response to OsHV-1 infection. Gene expression in infected oysters was measured relative to corresponding non-infected controls at three time-points post infection (12, 24 and 144 hours post infection or hpi). In both populations, we observed statistically significant changes in gene expression in response to infection. The changes in expression were found to be dependent on population, (resistant vs. susceptible), and time post infection. This is also roughly correlated with the amount of viral material found in each sample, however for the most part, viral transcripts made up a small proportion of total transcript content. Furthermore, after gene expression analysis, genes were grouped into 127 different gene-sets corresponding to orthologous pathways. The expression data was used to identify gene-sets that were significantly differentially regulated in response to infection and observations were compared between resistant and susceptible populations. This revealed intrinsic differences in pathway responses in resistant vs. susceptible upon infection with OsHV-1. It was observed that the ribosome and oxidative phosphorylation pathways are ultimately up-regulated ($p<0.005$) in response to viral infection, but this response occurs earlier in resistant populations. Most notably, the phagosome pathway was, on average, upregulated in the resistant population at the 12hpi timepoint ($p<0.005$), but there is no such up-regulation in susceptible population. Phagocytosis is important for destroying pathogens and this key difference in the phagocytosis response may reflect differences in survival between the two populations. Furthermore, the autophagy pathway is down-regulated in both populations ($p<0.005$) at 12hpi, but only remains downregulated in the susceptible population at the 26hpi timepoint ($p<0.005$). Like, phagocytosis, autophagy plays an important role in the immune response, specifically in degrading pathogen proteins, thus contributing to antigen processing among other roles. It would be disadvantageous for cells not to up-regulate phagocytosis processes and or to down-regulate autophagy pathways in response to an infection. It is possible that such disadvantageous responses in the susceptible population may be caused by the virus itself. Viruses often inhibit key pathways in host immune responses as a way of evading detection and eradication. These pathways may be more sensitive to viral interference in susceptible population. Further work will be needed in order to explore these hypotheses further and or confirm these gene expression changes through additional experimental work.

16-Massive gene presence/absence variation in the mussel genome as an adaptive strategy: first evidence of a pan-genome in Metazoa

Marco Gerdol[1], Rebeca Moreira[2], Fernando Cruz[3], Jessica Gómez-Garrido[3], Anna Vlasova[4], Umberto Rosani[5], Paola Venier[5], Miguel A. Naranjo-Ortiz[4,6], Maria Murgarella[7,8], Pablo Balseiro[2,9], André Corvelo[3,10], Leonor Frias[6], Marta Gut[3,6], Toni Gabaldón[4,6,11], Alberto Pallavicini[1,12], Carlos Canchaya[7,8], Beatriz Novoa[2], Tyler S. Alioto[3,6], David Posada[7,8], Antonio Figueras[2]

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Presenters: Antonio Figueras, David Posada

Abstract

Mussels are ecologically and economically relevant edible marine bivalves, highly invasive and resilient to biotic and abiotic stressors causing recurrent massive mortalities in other species. Here we show that the Mediterranean mussel *Mytilus galloprovincialis* has a complex pan-genomic architecture, which includes a *core* set of 45,000 genes shared by all individuals plus a surprisingly high number of *dispensable* genes (~15,000). The latter are subject to presence/absence variation (PAV), i.e., they may be entirely missing in a given individual and, when present, they are frequently found as a single copy. The enrichment of *dispensable* genes in survival functions suggests an adaptive value for PAV, which might be the key to explain the extraordinary capabilities of adaptation and invasiveness of this species. Our study underpins a unique metazoan pan-genome architecture only previously described in prokaryotes and in a few non-metazoan eukaryotes, but that might also characterize other marine invertebrates.

Session 3: Genetic selection for disease resistance

Keynote presentation: Main achievements in VIVALDI on genetic selection for disease resistance or tolerance

Jean-Baptiste Lamy, VIVALDI Work package 3 leader (IFREMER-F)

Resistance to *OsHV1* infection in *Crassostrea gigas* has been proven to be a highly inheritable trait in both laboratory and field experiments, with a rather low genotype-environment component (Azéma, Lamy, et al. 2017; Azéma, Maurouard, et al. 2017; Dégremont et al. 2015). Such determinism is favorable to the implementation of genomic selection at the industrial scale to help to manage disease impacts. However, previous studies on the genomic determinism did not provide an unified view of the genes behind such trait. Early studies with low density genetic map showed that some QTL are family-specific and only two are shared between two families out of the three studied (Sauvage C. et al. 2010). More recently studies using SNP array (Gutierrez et al. 2017) showed small evidences with well identified QTLs across 21 families (Gutierrez et al. 2018) or 31 full-sib (Gutierrez et al. 2019; Camara et al. 2017) from two independents genetic backgrounds. Such findings are puzzling and need more investigations. In this studies, we set-up an F2 design using highly contrasted families regarding OsHV1 resistances (up to 7^{th} generation of selection) to derive 8 F2 families that were environmentally exposed to OsHV1 µVars infections in summer 2017 on Marennes-Oléron bassin. A careful monitoring of the mortality (most of mortality events occurred in less than 4 days) coupled with a sampling of early moribunds oysters and latest survivor allows us to have a highly contrasted phenotypical response to infection. In parallel, Grand-Parents (F0), parents (F1), 4 survivor and 1 moribund oyster per families (F2) were whole genome sequenced at low-coverage to build-up high-density linkage maps (>$1x10^6$ mapped SNPs) and characterize good SNPs quality. We also use Gutierrez array to genotype at 40625 loci 1536 F2 individuals with as much as possible a balanced representation between dead and alive individuals. The combination of linkage-maps, "in-house" oyster genome assembly (PacBio,ONT and Hi-C) allow to detect two mains QTLs plus a putative one. We make a proof-of-concept of the possibility to implement the genomic selection on such traits and the pitfalls using imputations approaches to improve the prediction. The join analysis of the GWAS experiment and temporal monitoring of allelic frequencies in natural populations before and after mortality events rise some common contigs, it strengths our findings.

Presenter's biography

Jean-Baptiste Lamy (M) PhD, is a junior researcher on quantitative genetics and genomics on shellfish species. His main academic interest is about the genotype-to-phenotype models that are crucial to understand adaptation or maladaptation phenomena. His main working area is to understand the molecular basis of the resistance/susceptibility to infectious disease (or more specific shellfish diseases as all and/ro auto-carcinogenesis). He uses genomic (bioinformatics) and simulation approaches on bivalves host and pathogen to understand how they interact during biotic interaction.

Biotic and abiotic factors shaping the genome of cockles (*Cerastoderma edule*) in the Atlantic area

Paulino Martinez (USC-Spain)

Production of edible cockle (C. edule) reached 24,626 tons in 2017 in Europe, being Spain, France and British Isles the main producers. In addition to food supplying, cockles provide many ecosystem services important for coastal areas. Suboptimal management and disease outbreaks, particularly Marteilia cochillia parasite, represent serious threatens for cockles in the Atlantic area. Here, using 2b-RADseq we tuned up a set of 9,309 SNPs to analyse the genetic structure of C. edule both at macro- and micro-geographic scales, as well as at a temporal scale (across consecutive cohorts). Twenty-eight samples including 746 individuals from 22 locations were studied. The recently assembled cockle genome was used as reference and more than 50% identified SNPs matched to a single genomic position. A practical molecular tool constituted by seven diagnostic SNPs was developed to distinguish edible and lagoon (C. glaucum) cockles, easily confounded at juvenile stages, which confirmed the pertaining of samples to C. edule. No significant genetic differences were detected between consecutive cohorts of 0+ individuals sampled at five locations (10 samples). Gene diversity per population ranged between 0.070 and 0.079 (average 0.074) and, despite a slight heterozygote deficit was systematically observed, none population deviated from panmixia. Pairwise genetic differentiation (FST) ranged between 0 and 0.067 (global FST = 0.034) and the Structure software identified two main population units above and below the 48º N parallel in the whole Atlantic Area. However, a refined analysis (DAPC, other likely K values, AMOVA) suggested additional substructuring in the northern (Irish, Wales and North Sea subgroups) and the southern (South Portugal, North Portugal/Spain subgroups) groups, thus totalling five population clusters. These five subgroups could represent a first approach for defining management units. Bayescan analysis identified 579 outlier loci at $P < 0.05$ against the neutral background (~ 5% of the total), mostly related to divergent selection. The Structure analysis performed only with those outliers detected again two main northern and southern groups, but other likely K values suggested a much more refined structuring. Three main consistent subgroups were identified in the northern group and a highly admixed constitution was shown in the south, excluding the southern Portuguese populations. This high admixture could be related to movement of stocks related to cockles production. No significant structuring was detected at microgeographic scale in Galicia (NW Spain), although slight evidences of isolation by distance were detected. Twenty-two outliers, all related to divergent selection, were identified in Galician samples, and one of them, separated the marteilia-free from marteilia-infected areas. Ongoing work involving abiotic (temperature, salinity, currents, oceanic fronts) and biotic (parasite, microbiota and non-indigenous species distributions) factors will allow to refine the structure looking for correlation of genetic diversity with environmental variables, and putatively identify candidate genes under selection by mining in the cockles genome.

Presenter's biography

Paulino Martinez is Professor of Genetics at the University of Santiago de Compostela. He is a a geneticist interested in the study of evolution and the architecture of genomes, and their applications in conservation genetics and genetic breeding programs. He works with different types of markers and statistical methodologies to estimate genetic structure and adaptive variation, as well as for phylogenetic reconstruction. His research is mostly focused on aquatic organisms, initially fish, but more recently mollusks and other production species. He is interested in technology transfer to companies and administration related to conservation genetics and genetic selection. He is actively working on the development of genomic tools for evolutionary and applied studies, such as genetic maps for the identification of QTL, studies of massive gene expression (oligo-microarrays, RNAseq) to identify candidate genes related to productive traits, and genotyping of Large-scale SNPs (RADseq) in population genomics or genomic selection projects.

First steps towards genomic selection in the Manila clam *Ruditapes philippinarum*

Morgan Smits, (UNIPD-IT / LEMAR-F)

While selective breeding has long since revolutionized the efficiency of production for terrestrial plant and animal species, this approach is still relatively new in aquaculture, especially for shellfish. Due to the inherent difficulties in treating infectious diseases in shellfish species, selective breeding for resistance to infection may be a sustainable solution to mitigate the negative impact of pathogens. In this work, VIVALDI partners investigated the feasibility of carrying out selective breeding for resistance to the parasite *Perkinsus olseni*, as well as for a number of other important production traits, in the Manila clam, *Ruditapes philippinarum*.

A hatchery-bred mixed-family cohort of *R. philippinarum* was produced by mass spawning of 109 broodstock, and progenies were raised as a single group. At one year of age, two batches of progenies were moved to two commercial clam production areas; one in Italy, with a high prevalence of *Perkinsus olseni*, and one in France, with no recorded disease. After one year on the field, about 1 000 progenies per site were individually measured for growth traits, sex, and parasite load, and DNA was genotyped for parentage assignment using a SNP array developed within the VIVALDI project.

Sire and dam representation was high (75-88%) in both sites, indicating a low risk of inbreeding and suggesting that genetic variability is maintained using a mass spawning design. Moderate heritability (0.20 – 0.46) was estimated for all traits, with sex and parasite load showing the highest heritability in the Italian site of Chioggia (0.42 and 0.52, respectively). Growth and parasite load showed no genetic correlation, indicating that breeding objectives can include both traits without negatively affecting either one. Genetic correlations for growth traits were not significant between sites, which suggests that family performance for any given trait differs by site. That said, simulations of potential genetic gain for total weight show that a selection carried out in the French site would still provide over 11% gain in total weight for clams grown at the Italian site.

Overall, these first estimations of genetic parameters for production traits in the Manila clam suggest a strong potential for selective breeding of both resistance to the parasite *P. olseni* and growth.

Presenter's biography

Morgan Smits is currently finishing her PhD, in international joint agreement between the University of Padova and the University of Brest, which focused on developing genomic tools to investigate disease resistance in the Manila clam. Her work was tightly linked with WP3 of the VIVALDI project, in which she followed the field studies put in place to evaluate selection potential for disease resistance and growth in clams.

Potential and optimization of breeding programs in Pacific oyster in presence of mortalities

Florian Enez (SYSAAF-F)

Pacific oyster production areas are regularly affected by some pathogens that cause mortalities and yield reductions. However growth traits are still important for farmers. Development of breeding programs is a solution to improve breeding performances and to limit the impact of pathogens. The estimation of genetic parameters is required to set up a breeding program. More than 650 families were produced with 65 sires and 70 dams by the French hatchery France Naissain in April 2016. Animals were transferred in summer 2016 to two rearing sites differently affected by pathogens, in Bourgneuf Bay and in Normandy. Respectively 1502 and 1388 oysters were phenotyped at commercial size during the winter 2019. They were weighted and a tissue sample was taken for DNA parentage assignment. Then shells and meet were weighted after opening. Genetic parameters were estimated by animal model with the reconstructed pedigree. We didn't observe different parents representation according to sites. Genetic correlations between sites were also high for most of traits (0.79 – 0.88). The impact of distinct pathogens affecting each site seems to be limited on family representation and traits. Heritability for growth was low (0.09 – 0.24), possibly due to environment or rearing practices. Growth traits were genetically correlated, but independent to the meet yield.

To conclude these results showed that a selection on growth traits and yield could be combined to improve these two traits. Moreover genetic by environment interaction was limited on family representation and performances at commercial size. Genetic progress generated in a site should be expressed in the other.

Presenter's biography

Florian Enez is a geneticist at SYSAAF (The French poultry and Aquaculture Breeding Technical Center), a non-profit professional association (French Law 1884) which groups together companies developing rational programs of genetic improvement and-or management applied to poultry, fish and oyster species and another species like fly. He assists French companies in their development of breeding programs.

Genomic signatures of selection across mass mortality events in European populations of Pacific oysters *C. gigas*

Mathias Wegner (AWI-DE)

Infection with ostreid herpes viruses (OsHv-1) have been causing mass mortalities and reeking havoc on production areas world wide. The resulting production losses have sparked several selective breeding programmes trying to improve resistance to infection. Recent studies showed however that there is genetic variation in OsHv-1 strains between different outbreak areas. Furthermore, it has been suggested that secondary infection by pathogenic bacteria contribute substantially to the observed mortalities. Together this means that the disease differs between regions suggesting that also the genetic determinants of resistance differ them. To test assess the role of environmental variation in disease we employ whole genome sequencing of pools of individuals (poolseq) pairing before and after mortality samples collected during mass mortalities across Europe (France, Germany, Ireland, Norway). We identified 67-109 outlier loci for all pairwise comparisons that showed either signatures of selective sweeps (fixation of major alleles) or allelic pull-ups (increase of minor alle frequency). While we could identify single nucleotide polymorphisms (SNPs) located in close genomic proximity for most pairwise comparisons, that also matched previous QTL-studies, the majority of outlier loci were unique to each location. This supports the polygenic architecture of OsHV-resistance, but also highlights the substantial selective differences between locations. Since the shared outlier loci probably only explained a relatively small variation in resistance, breeding programs should aim at location specific resistance rather than general resistance.

Presenter's biography

K. Mathias Wegner is an evolutionary ecologist based in the Waddensea Station of the Alfred Wegener Institute (AWI) on Sylt/Germany. He investigates the impact of parasitism and disease on host populations as well as the reciprocal selection on parasites and disease resulting from adaptation of the host, which ultimately fuels the coevolutionary arms race.

Selective breeding of Pacific oyster (*Crassostrea gigas*) for OsHV-1 resistance: impact of nutritional factors

Pauline Kamermans (Wageningen University-NL)

To investigate impact of family and larval diet on survival of *C. gigas* when exposed to OsHV-1 larvae of different families were reared with different algal diets. 40 Pacific oyster families were produced by single-pair mating 20 males and 40 females at the hatchery of Roem van Yerseke, the Netherlands. Larvae were reared in two groups with three replicate's: one group fed the standard algal diet and one group fed a diet in which 25% was replaced by *Nannochloropsis occulata*. It was hypothesised that a high selenium content in N. occulata would boost the immune system of the oyster. Spat was exposed to OsHV-1 through cohabitation with positive oysters in the laboratory and through rearing in an OsHV-1 infected area in the field. Oyster spat was successfully exposed to OsHV-1. Spat that was exposed to the virus in the lab showed mortality while there was high survival in the control group. No significant difference was found in survival between the two diets given in the larval phase both in the lab challenge and in the field challenge. Difference between the two diets may not have been large enough, as significant differences in selenium content were not observed. Oyster families showed differences in survival when exposed to the virus. Selection in lab conditions will improve survival in the field.

Presenter's biography

Dr. Pauline Kamermans has 32 years' experience in marine ecological and aquaculture research of which 25 years have been devoted to shellfish. Her present work focusses on shellfish aquaculture and restoration. The research is funded by the EU, the national and local government, shellfish farmers, nature organisations and wind farm developers. Kamermans has been working for Wageningen Marine Research since 2000, where she has carried out various projects on spat fall prediction, seed collection methods, development of hatchery and nursery techniques, preventing shellfish diseases, restoration of flat oyster beds and experiments on ecophysiological needs of shellfish at different life stages.

Imputation with shellfish genomes: pitfalls

Binyam Dagnachew (NOFIMA – NO)

Use of whole genome sequence data (WGS) is expected to improve identification of quantitative trait loci (QTL) and prediction of genomic breeding values. However, this requires sequencing of large number of individuals and is not cost efficient. However, strategically sequencing of few individuals and genotyping of others with low-density genotyping panels then impute to whole genome sequence has showed great potential in other species. The objective of this study was to determine accuracy of WGS imputation in Pacific oyster (*Crassostrea gigas*) and their application for genome-wide association studies (GWAS) and genomic selection (GS). WGS were available for 67 individuals (10 grandparents, 22 F1 and 35 F2) and 1,530 F2 were genotyped with SNP array. The WGS contains ~365 K markers and the genotype array has ~14K markers. The 14K genotypes were imputed to WGS using Beagle v5.0. Phenotypes for OsHV-1 infection were available for 1,530 F2 individuals. GWAS and GS for resistance for OsHV-1 were performed using imputed SNPs which had imputation accuracy (Beagle R^2) higher that 0.6. Imputation of 356K markers from 14K SNPs resulted an average Beagle R^2 of 0.52. After quality control, only 82K SNPs had a Beagle R^2 higher than 0.6. This poor imputation accuracies could be as a result of genome assembly for this species and not optimize selection of SNPs for the array. For example, comparing the genetic maps of sequence data and array data reveled that only the middle part of each chromosome is covered in the array data and that might contribute for the poor imputation accuracy. In addition, it was observed low pairwise linkage disequilibrium (LD) and faster decaying LD, which indicates that there are low number of shared haplotypes in this population and possible mapping errors. However, compared to the 14K, using imputed WGS amplified a putative QTL region and led to identification of more genomic regions against OsHV-1. On the other hand, use of imputed WGS did not improve genomic predication accuracies compared 14K. The results pointed out that even if the use of imputed WG sequence improved the power of QTL detection, improving the genomic resources of this species, such as better genome assembly, would help to improve imputation accuracies and hence use of genomic data in this species.

Presenter's biography

I was born in Ethiopia in 1982 and I completed my undergraduate degree in Ethiopia before I moved to Europe for further education. I earned my PhD in Animal Breeding and Genetics from Norwegian University of Life Sciences in 2012. Since then I have been working in different projects related to animal genetic improvements and currently, I hold a research Scientist position at NOFIMA, Norway.

Use and abuse of additive models in quantitative genetics

Arnaud Le Rouzic (CNRS-F)

Quantitative genetics gather various statistical frameworks to describe the genetic architecture of complex characters and predict their evolution, especially under artificial selection. The underlying models rely on classical linear predictions of gene effects and the partition of phenotypic variance into genetic and residual variances. It is known since more than one century that only a part of the genetic variance contributes to the heritability of the traits: the additive genetic variance, calculated as the sum of marginal effects of all loci involved in the trait. The part of the phenotypic variance due to within-locus interactions (dominance) and between-locus interactions (epistasis) does not affect the immediate response to selection, and has been largely ignored by quantitative geneticists. However, the strong connection between quantitative genetics and this additive model remains disturbing, because all other fields in biology point out that gene effects should not add up, and that gene interactions play a central role in formal genetics, systems biology, or evo-devo. Recently, new quantitative genetics models have emerged, trying to fill this gap and account for genetic interactions. This presentation illustrates two situations where interaction matters in quantitative genetics. The first case is the influence of gene-gene interactions (epistasis) on the long-term response to selection. When interactions are not random across all pairs or loci but rather tend to form a systematic pattern, epistasis can be directional, and strongly affect selection limits. Positive (or synergistic) epistasis tends to enhance the effect of alleles that increase the value of the trait; any selection in the direction of epistasis will see its efficiency enhanced during the response to selection. Inversely, negative (antagonistic) epistasis may make selection response more and more difficult, and selection limits would be reached very rapidly. Estimating the directionality of epistasis (a measurement that is still empirically difficult) would thus improve substantially the predictions of selection response. A second example where epistasis may matter is the case of the detection of complex interaction patterns in linkage or association QTL mapping studies. In particular, some loci displaying sign epistasis (inversion of the allelic effect depending of the genetic background) may be undetectable in additive QTL models. Nevertheless, running bidirectional QTL scans requires larger datasets, and raise considerable statistical power issues. In sum, new models and procedures making it possible to account for epistasis are emerging, pointing out the importance of accounting for non-additive effects in quantitative genetics. However, these models are also featured by substantial theoretical and empirical issues, illustrating how non-additive quantitative genetics remain an open research question.

Presenter's biography

Arnaud Le Rouzic is a CNRS researcher at the Evolution, Genomes, Behavior, and Ecology unit, in Gif-sur-Yvette, France. He is a theoretical evolutionary geneticist, interested in population and quantitative genetics and genomics. His research activity focuses on modeling the evolution of complex systems, either for theoretical purposes (e.g. in silico hypothesis testing) or for fitting statistical models on empirical data. His recent projects deal with the evolution of evolvability, including the evolution of genomes (transposable element dynamics) and the evolution of systems robustness (genetic canalization and phenotypic plasticity).

Session 3 Poster presentations

17-Efficiency of within-group mass selection on threshold trait and successive mass or index selection on continuous trait

Florian Enez[1], Pierrick Haffray[1]

[1]SYSAAF, Station INRA/LPGP, Rennes, France

Presenter: Florian Enez

Abstract

Within-group mass selection consists in combining the efficiency of independent mass selection within groups and the simplicity of inbreeding management by rotational crossing of those groups. The aims of this in silico study were to simulate a successive 1st mass selection on a threshold traits (T-trait) and a 2nd mass or index selection on a continuous trait (Q-trait) to evaluate the expected gains depending on heritabilities (h^2), genetic correlations (rg) and selection pressures.

Factorial mating was generated with 160 parents distributed in 4 groups reared in mixed family on 10 generations. Phenotypic values were based on the additive value of sire and dam, and micro-environment effect (only for T-trait). Selected individuals could be randomly sampled without Q-trait consideration, or selected by mass or BLUP selection on Q-trait, with or without inbreeding management based on parentage assignment. Threshold on the T-trait was fixed at 50% in the reference situation and cumulated selection pressure on the two traits at 4% from 4000 individuals. A rotational system was applied to cross groups and to produce the next generations. The effects of some h^2 (0.1; .25; 0.50), rg (-0.5; 0; 0.5) and selection pressure (0.50 and 0.25 on T-trait, 0.32 and 0.10 on Q-trait) on inbreeding and true breeding values (TBV) were estimated.

Selection on T-trait only had no significant impact on Q-trait. Adding selection on Q-trait after mass selection on T-trait had no impact on expected genetic gain for T-trait whatever rg and the selection method used for Q-trait improvement. Index selection with 32% selection pressure from estimated breeding values (EBV) on Q-trait increased gain on Q-trait in the same order than mass selection with higher inbreeding. When Q-trait h2 was limited (0.10), gain on Q-trait was lower (1-2% vs 19%) than when h2 was high (0.5). Inbreeding also increased faster. Introduction of inbreeding constrains limited up to 50% gain on Q-trait compared to mass or index selection. Inbreeding management seemed to be particularly useful when h^2 of Q-trait was more limited. Progress on T-trait was 50% higher with index selection on Q-trait and inbreeding management when initial pressure on T-trait was the highest (0.25 vs. 0.50), without effect on Q-Trait. Increase of pressure on Q-trait moderately impacted T-trait, but gain on Q-trait rose by 50%. When pressure was higher on a trait (0.25 vs. 0.50 on T-trait, 0.10 vs. 0.32 on Q-trait), the second trait wasn't impacted.

This work demonstrated that within-group multi-traits selection is an efficient strategy to improve simultaneously a threshold trait then a second quantitative trait with low-cost inbreeding management whatever rg is. Results shown possible simultaneous genetic gains when rg is -0.8. DNA parentage assignment in within-group multi-traits selection program may guarantee the genetic variability of the selected individuals. The effectiveness of such breeding program depends directly on the h^2 of the traits, their rg, and selection pressure and/or number of assigned individuals used to estimate EBV. Given its simplicity of implementation and effectiveness, both in terms of genetic improvement and inbreeding management, within-group multi-traits selection could be applied in breeding programs in aquaculture species.

Session 4: Understanding complex interactions between animal, environment, pathogens and health for risk assessment

Keynote presentation: Role of the environment in the interactions between bivalves, microbiota and pathogen: could the diversity of microbiota represent a relevant new indicator of the health status?

Christine Paillard, VIVALDI Work package 4 leader (CNRS-LEMAR-F)

In the context of climate change, an integrative approach, at the crossroads of disciplines, has been developed within WP4, involving not only the system, environment-host-pathogen, but also the microbiota. In molluscs, no major role of these microbial communities has really been identified, although many publications mention that they could be involved in the nutrition or immunity of their host. Combining experimental laboratory approaches and environmental monitoring in various key European sites (Dungarvan in Ireland, Thau lagoon and Brest Bay in France, Vigo Ria and Delta de l'Ebre in Spain and Gulf of La Spezia in Italy) we have undertaken to finely characterize the microbiota of four bivalves (oysters, mussels, clams and cockles) using new sequencing technologies, but also more targeted approaches (pathobiome, vibriome,.). The objective of these longer-term studies is to identify whether potential microbial signatures could be associated with good shellfish health, i.e. a profile of probiotic microorganisms that limit the multiplication of pathogens. A very complete characterization of the microbiota of each species has shown that the bivalve microbiota seems unique to each individual (barcode imprint) and specific to each compartment of the bivalve (digestive gland, hemolymph, extrapallial fluid...). Despite high inter-individual variability, the larger-scale comparison of the microbiota between the 4 bivalve species revealed a specific community for each species and also distinct from the surrounding habitat (water and sediment). However, environmental conditions (temperature, season, intertidal habitat) are major factors in the modulation of bivalve microbiota. During diseases or mortalities, we have also shown dysbiosis, often associated with a decrease in microbial diversity (alpha and/or beta diversity) could be detected in bivalves during mortality episodes, but we cannot conclude whether this imbalance is due to the disease or whether the bivalve has already shown a modification of microbial communities promoting the multiplication of the pathogen(s).

One of the most discriminating bio-indicators of health identified in this study concerns the measurement of both bacterial and protista microbial diversity. This result is perfectly in line with current research on human microbiomes.

In perspective, the identification of bio-indicators of bivalve health is being developed, by processing an even broader data set but also by validating the results obtained in the natural environment through laboratory experiments.

Presenter's biography

Christine Paillard is a CNRS research director (DR1 CNRS), she coordinates a research group "Environment-Host-Host-Pathogen-Microbiota Interactions EHPM in the Panorama team of LEMAR (Laboratory of Marine Environmental Sciences of the University of West Brest (UBO). Christine Paillard's research group is interested in how environmental factors modulate host-host-pathogen interactions, with clams and

abalones interacting with vibrios as the main model organisms; interests include ecophysiology, ecology, host-pathogen coevolutionary arm race, immunology and microbiology. Christine Paillard discovered *Vibrio tapeti*s as the bacterial agent responsible for brown ring disease in Manila clams while she was a PhD student at the University of Brest, France. Since then, she has been studying this pathogen, *Vibrio tapetis*, and how it interacts with its bivalve host using a wide range of cellular and molecular strategies. She is developing new approaches to compare two contrasted temperature-dependent diseases, the cold BRD in clams and the warmer abalone vibriosis due to *Vibrio harveyi*. She is studying adaptations of molluscs to temperature and acidification using experimental strategies combining integrative studies such as physiology, proteomics and next generation sequencing, with local and international collaborators. She is currently developing new approaches for the study of shellfish microbiotes.

Exploring the dilution of parasites and disease mitigation in oysters

Gorka Bidegain (University of the Basque Country-ES)

Eastern oyster (*Crassostrea virginica*) simulations were performed to investigate the effect of oyster density and its interaction with harvesting and vertical diffusion on the infectious particle (*Perkinsus marinus*) concentration in the water column and disease spread. Several disease models were developed and coupled into the Regional Ocean Modeling System (ROMS) model. The disease model results suggest that high oyster population densities and intensive culture with a harvest at a moderate rate remove disease-causing parasites from the environment, reducing oyster populations exposure to parasites, and eventually mitigating disease spread from aquaculture farms to wild oyster populations. The biophysical model of an estuarine channel with several reefs showed that for higher vertical diffusivity, parasites released from bottom infected reefs are mixed to the surface, drifted downstream with the surface outflow, mixed again to the bottom and filtered by outer reefs; inner reefs remain disease-free. For reduced vertical diffusivity, parasites are drifted upstream with the bottom inflow and accumulated in the inner reefs; outer reefs remain disease-free. Disease mitigation depend on the ability and density of oysters to filter arbitrarily large numbers of infective particles lowering per the capita exposure to safe levels (below the infective dose). These and further applications of these models could inform decision making in the management of filter-feeding shellfish populations impacted by marine diseases.

Presenter's biography

My research focuses on the development and use of mathematical models to study emerging marine diseases causing mass mortalities in marine invertebrates, with a special emphasis on the physical and biological mechanisms/processes that underlie the generation of outbreaks. I am particularly curious about the interaction between marine diseases and fisheries and the effect of climate change on disease transmission and spread. I am Assistant Professor at the Department of Applied Mathematics (University of the Basque Country, Spain). Previously, I worked as a postdoc at the University of Southern Mississippi/Old Dominion University (USA) and as a PhD student at the Environmental Hydraulics Institute (Spain).

The influence of surrounding species on the disease risk of *Crassostrea gigas* in oyster farming

Élyne Dugény (IFREMER-F)

Coastal marine ecosystems are highly productive and diverse areas where disease risk rely on host-pathogen-environment interactions. The effect of abiotic factors like temperature and salinity are already known as drivers of virulence and pathogenicity as reported in Pacific oyster *Crassostrea gigas*, and one of its pathogens, the ostreid herpesvirus OsHV-1. Although recent scientific literature suggests a link between species diversity and prevalence of infectious diseases, the effects of biotic factors such as abundance, richness and species diversity on disease risk in marine environments remain undescribed.

In this context, we aimed to investigate different kinds of relations between surrounding species occurring in oyster farms and individuals of Pacific oysters, in a disease context.

We tested hereby the effect of macroalgae (green, red and brown) on disease susceptibility of *C. gigas*. An experimental manipulation in controlled conditions was performed with young oysters placed in co-habitation with the selected algae and subsequently challenged with an infection phase by OsHV-1 µvar, in order to induce a mortality event. This experiment revealed that mortality of oysters was amplified with green algae and similar to control with brown and red algae. Therefore, the algal community influenced disease risk in oyster. Metabarcoding analyses revealed changes in bacterial communities for oysters held with green algae, that could explain the differences in survival. Indeed, infection lead by OsHV-1 consists in a polymicrobial disease inducing a repression of antibacterial defenses and consequently drives modifications in oyster microbiota and causes bacteraemia. Therefore, a destabilized microbiota could result in a higher mortality rate by making oysters more susceptible to pathogens. The identity and the role of bacterial strains characterized by sequencing remain to be determined. Our work will allow proposing mitigation measures for producers, based on reduction of green algae in their exploitations.

Presenter's biography

PhD student currently working in an Ifremer laboratory of Mollusc physiology, part of the LEMAR unity (Laboratoire de sciences de l'Environnement MARin). Ecologist by formation and graduate of a master's degree of sea science and marine environments from Sorbonne University, I combine experiments in controlled conditions and fieldwork to answer the questions I ask for my work. My research subject, implemented in the Workpackage 4 of the VIVALDI project, is about the influence of surrounding species on the Pacific oyster disease risk. I aim to understand the effect of co-habitation on oyster survival by testing associations with different organisms, to be able to propose better mitigation measures to face oyster's disease episodes.

Role of plankton in mediating Vibrio infections in bivalves

Luigi Vezzuli (UNIGE-IT)

Gram-negative bacteria of the *Vibrio splendidus* clade (e.g. *V. tasmaniensis* LGP32) and *Vibrio aestuarianus* have been associated to summer mortalities affecting the production of the Pacific cupped oyster (*Crassotrea gigas)* worldwide. We showed in previous studies that plankton organisms represent important environmental reservoirs for these bacteria. In addition, plankton species have been previously associated to transmission of pathogenic vibrios to humans and could potentially function as drivers of pathogen transmission also to oysters.

Build on this previous knowledge, this study aimed to determine whether pathogen interactions with plankton may favor pathogen transmission to the bivalve host. To this end, laboratory experiments were conducted to investigate whether different representative plankton substrates i.e. phytoplankton cells (*nannochloropsis gaditana*), marine snow particles and chitin fragments might represent suitable vehicle of infections for target pathogenic strains in the pacific oyster *C. gigas*.

Results from laboratory infection experiments showed that phytoplankton cells and marine snow particles promote *V. aestuarianus* 02/041 intake by *C. gigas* maintained under stressful conditions in the laboratory. Such intake is associated with a decrease in lysosomal membrane stability of oyster hemocytes indicating a compromised health status of infected oysters. In contrast, chitin particles did not appear to favor pathogen transmission to the bivalve host.

Transcriptomics analysis carried out on *Vibrio aestuarianus* 02/041 strains maintained in aquatic microcosms in the presence or absence of marine snow particles (MS) showed that interaction with MS promote upregulation of genes potentially involved in pathogenecity such as those encoding for colonization factors and bacterial proteases. In addition, fluorogenic enzymatic assays conducted under the same experimental conditions showed an increase in extracellular aminopeptidase activity in *V. astuarianus* 02/041 cells incubated with marine snow particles.

Results from these studies suggest that marine snow and plankton organisms (especially phytoplankton) might represent suitable vehicle of infections for *Vibrio* pathogens to their bivalve hosts.

Presenter's biography

Luigi Vezzulli obtained his PhD degree in Marine Sciences in 2003 from the University of Genoa. Since 2005 he was Assistant Professor of Microbiology at the University of Genoa, since 2018 is Full Professor. His research activity is carried out in the field of Marine Microbiology and is mainly focused on the study of human and nonhuman bacterial pathogens of the genus *Vibrio* in the marine environment. Since 2002, he has published 74 peer-reviewed scientific papers in international peer-reviewed journals, 27 of which is the first author and 6 is the last author (H-Index Scopus: 27).

All models are wrong but are some useful in managing shellfish health?

Edmund Peeler (CEFAS-UK)

Hydrodynamic models were constructed for four mollusc shellfish production sites which represented a range of bathymetric and climatic conditions: Dungarven Bay (Republic of Ireland), Bay of Brest (France), Ria de Vigo (Spain) and the Ebro Delta (Spain). The models were constructed using the Telemac software, and used existing bathymetry, climate and other data sources. The dominant processes influencing dispersal varied between sites. In Dungarven Bay and the Bay of Brest, tidal currents were most important whereas in Ria de Vigo stratification and upwelling were dominant, and in the Ebro Delta freshwater inputs were influential. To investigate the spread of diseases within the marine environment, passive tracers (without decay terms) were released within the domain from known aquaculture sites. The environmental conditions chosen for the simulation were typical of the summer months in each area, when the conditions are permissive for the pathogens of interest (e.g. oyster herpesvirus). The key conclusion from studies in Dungarven Bay is that release from within the bay (over a period of 48 hours) results in dispersal across the entire production area. Given the prevailing conditions, the bay is at greatest risk of pathogen introduction from disease outbreaks north of the bay. Production takes place in only one part of Alfacs Bay (Ebro Delta), along the north shore. Released particles remain in the embayment and only gradually disperse, thus release at any point will result in exposure to the entire production, but spread to other areas is unlikely. By contrast in the Bay of Brest there is considerable separation between 3 main production areas. Outbreaks in the western and northern clusters of the estuary may not result in spread to sites in the inner estuary (eastern cluster). In the Ria de Vigo, pathogen release in the outer part of the bay are rapidly dispersed under prevailing conditions and thus outbreaks in the outer bay are unlikely to result in spread to production in the inner bay. Similarly, it is unlikely that prevailing conditions will result in spread into the bay from neighbouring production areas. However, when pathogen was released from sites within the bay there was less dispersal, concentrations remained high during the simulations (48 hours). Therefore, contiguous spread between production beds is likely, however outbreaks within the bay are unlikely to result in spread out of the bay to neighbouring estuaries. There are clear limitations to the models developed. It was not possible to model plausible levels of particle release (which would require information on biomass, prevalence, excretion rates). Epidemiological modelling of disease processes within the bivalve populations was outside the scope of the project. Thus whilst robust estimates of likely pathogen dispersal have been obtained, we are limited in our interpretation of these data. It is not possible to model likely exposure levels and therefore likelihood that exposure would result in infection and thus a propagating epidemic. Nevertheless, the results provide useful insights into pathogen dispersal which can be used in both surveillance and spatial planning.

Presenter's biography

Dr Edmund Peeler is the principal epidemiologist at Cefas and currently is vice-president of the OIE Aquatic Animal Health Standards Commission. He has been employed at Cefas since 2001, where he has led development of aquatic animal epidemiology. He provides advice to the UK government on aquatic animal health policy. He has been involved in numerous UK government and EU funded research projects and has published over 60 peer-reviewed papers, focusing on the development of risk methods to support aquatic animal health policy making. He has been an invited speaker at science conferences in Europe, Asia, the Americas and Australia.

Impacts of environmental factors on pathogen development

Sarah Culloty (UCC-IE

The interactions between animal, environment, pathogen and health are complex. However, gaining a greater understanding of these associations will allow for better risk management. Predicted climate change scenarios and alterations in sea temperature and acidity may mean that bivalve molluscs will have thinner shells and therefore experience increased exposure to UV rays in the future. The effects of UV-B radiation on *Crassostrea gigas* and its associated pathogens, ostreid herpes virus (OsHV-1 and variants) and *Vibrio aestaurianus* were investigated at UCC. Results from laboratory and field trial studies showed that UV-B radiation negatively impacted survival of *C. gigas* spat, especially younger cohorts. UV-B exposure also had a negative impact on the pathogen *Vibrio aestuarianus,* but not ostreid herpesvirus microVar (OsHV-1 µVar). Overall, the findings from the UV radiation studies suggest that *C. gigas* spat held higher up the shore and with less emersion time in the intertidal are more susceptible to UV-B radiation compared to *C. gigas* spat lower on the shore. These results may indicate that a cumulative effect of other stressors experienced in a non-static environment that is the intertidal may be compounding the impact of UV-B on young oysters.

Presenter's biography

Sarah is the Head of the College of Science, Engineering and Food Science and Director of the Environmental Research Institute at UCC. She is also the principle investigator for the Aquatic Animal Health research group in the School of Biological, Earth and Environmental Sciences (BEES), UCC. Her main area of research is in ecological parasitology with a particular focus on molluscan diseases. The focus of her research centres on pathogen (macroparasites, protozoa, bacteria and viruses) life cycles, epidemiology, diagnostics and approaches to reducing impacts of disease. Recent research looks at the impact of potential climate change drivers on disease development in the marine environment. While at UCC, Sarah has won 20 research grants totalling over €5 million. She is currently working on the H2020 project Vivaldi, DAFM FIRM REPOSUS project and INTERREG projects BLUEFISH and COCKLES.

Eco-physiological indicators as clinical signs of *Crassostrea gigas* juveniles during a disease event

Marianne ALUNNO-BRUSCIA (IFREMER-F)

Recent studies on mortality outbreaks of juvenile oysters (<1-yr old) *Crassostrea gigas* have focused on the causative agents, *i.e.* the herpes virus OsHV-1 µVar and Vibrio spp., as well as on the factors that influence the disease transmission. But no clinical signs at the individual level of the host were available to describe its susceptibility to disease, beyond the very simple observation that animals were alive or dead.

In our study, we monitored the eco-physiological behaviour of individual oysters by using non-invasive methods. Filtration, respiration, and cardiac activity of seven oyster juveniles were monitored at the individual scale before (6 days), during (8 days) and after (13 days) a disease event. Repeated measures ANOVA were used to test whether these 3 indicators differed among the seven oysters over time and between individuals that survived or died during infection. Before infection, the cardiac activity was found to be significantly lower and more variable for individuals dying later during infection compared to their congeners that survived. This suggests that cardiac activity could be used as a predictive clinical sign. During infection, six over the seven individuals ceased abruptly their filtration activity for 40 hours at least, with a high variability in the cessation time of feeding; and four oysters died. After infection, the filtration and respiration rates of the three surviving oysters were similar to those measured before infection while their cardiac activity of was significantly lower than before infection. This would suggest that animals had not fully recovered.

Monitoring these non-invasive eco-physiological indicators on sentinel animals in the laboratory and in the field could provide precursory information on disease events and may be very helpful to better understand the interactions between the host and its pathogens. They could also be used as health indicators of *C. gigas* when exposed to other stressors (*e.g.* microplastics, toxic algae).

Presenter's biography

Marianne Alunno-Bruscia is a researcher working on ecophysiology and bio-energetics of bivalves by using Dynamic Energy Budgets (DEB). She develops non-invasive health indicators for bivalves in a context of disease or of other stressors (*e.g.* toxic algae, microplastics).

Oyster hemolymph is a complex and dynamic ecosystem hosting bacteria, protists and viruses.

Jean-Michel Escoubas (CNRS/IFREMER-F)

The impact of the microbiota on host fitness has so far mainly been demonstrated for the bacterial microbiome. We know much less about host-associated protist and viral communities, mostly due to technical issues. However, all microorganisms within the microbiome potentially interact with each other as well as with the host and the environment, which likely affects the host health. In this study we to characterize the entire microbial community (viruses, bacteria and protists) living in oyster hemolymph and we determine the impact of the oyster's genetic background and environmental parameters on the hemo-microbiota establishment and dynamics. To this end, we used five genetically differentiated oyster families, produced in hatchery and bearing contrasted phenotypes, especially in respect to summer mortality syndrome. These animals were then transplanted in natural environments (during infectious and non-infectious periods) in order to study the impact of the environment on the composition and dynamics of the hemo-microbiota. Our results demonstrate that hemolymph can be considered as an ecological niche hosting bacteria, protist and viral communities, each of them being controlled by different mechanisms. Overall, we found that hemolymph microbiota is more strongly shaped by environmental than by host factors. In addition, whereas we could not identify a common community structure in healthy animals, we found that disease outbreak was correlated with hemolymph invasion by OsHV-1 µVar virus, without significant modification of the others microbiota compartments. This work highlights the importance of considering all microbial compartments to better understanding the role of microbiota in animal health and disease.

Presenter's biography

JM Escoubas obtained his PhD in 1992 at the University Paul Sabatier (Toulouse, France) on work dedicated to bacterial mobile genetic elements. Then he moved to the USA (Brookhaven National Laboratory, New-York) for 2.5 years post-doctorate on light intensity regulation of nuclear and chloroplastic genes in a marine green alga. He entered the Centre National de la Recherche Scientifique in 1995 and work this then on invertebrate's immunity and hosts-pathogens interactions. He mainly worked on an insect pest crop and their associated pathogens (bacteria and parasitoid wasp) and pacific oyster immunity, microbiota and interactions with bacterial and viral pathogens.

Effects of *Perkinsus olseni* infection on growth, reproduction and mortality of juvenile Manila clams *Ruditapes philippinarum* on the south coast of Korea

Albert Choi Kwang-Sik (School of Marine Biomedical Science, Jeju National University-South Korea)

Fitness of Manila clam *Ruditapes philippinarum* is often affected by parasitic infection, especially by *Perkinsus olseni,* a protozoan endoparasite. In this study, we evaluated the impacts of *P. olseni* infection on *P. olseni*-free juvenile Manila clams by translocating the clams to an area of high level of *P. olseni*. *P. olseni*-free juveniles (11.6 mm in shell length, SL) were transplanted to the south coast and cultured in the intertidal bottom (IBC) and subtidal suspended cages (SSC) at 2 m depth. The shell and somatic growth, gonad maturation and mortality of IBC and SSC clams were monitored over 103 days from early summer to early fall. Shell and somatic growths of SSC clams were significantly faster than the IBC clams ($P<0.05$). At the end of the experiment, SSC clams reached to 29.0 mm in SL, while IBC clams become 18.0 mm in SL. After 44 days of rearing in subtidal in late July, first spawning of the juvenile observed and 80% of SSC clams were actively engaged in spawning in late August. In contrast, spawning in IBC clams was observed in late September (25%), although most of IBC clams were sexually immature. After 30 days of rearing in the intertidal, the *P. olseni*-free clams showed *P. olseni* infection, and the infection intensity and prevalence increased dramatically from July (1.6×10^4 cells/g tissue) to September (1.30×10^6 cells/g tissue). In contrast, *P. olseni* infection in SSC clams was observed at the end of the experiment in September (6.6×10^4 cells/g tissue, prevalence 60%). IBC clams showed a high level of mortality in late July (30%), and the mortality reached 65% at the end of the experiment. In contrast, clams in the subtidal suspended cage showed a comparatively low level of mortality ranging 3-15% over 103 days of rearing. In the intertidal, the dramatic increase in *P. olseni* infection coincided with a dramatic increase in mortality. It was believed that the observed differences in the growth and mortality between the IBC and SSC are closely linked to *P. olseni* infection, as a high level of *P. olseni* infection resulted in the observed mortality and slow growth and gonad maturation in the intertidal.

Presenter's biography

Kwang-Sik Choi (Albert) is a professor of the Department of Marine Life Science, Jeju National University (JNU) in South Korea. Albert has been working on various topics in molluscan biology, including *Perkinsus olseni* infections on reproduction and growth of Manila clams. Albert received his MS and Ph.D. from Dept. Oceanography, Texas A&M University (1987 and 1993)

Variations of perceptions across stakeholders in Europe, with regards to mollusc disease prevention

Coralie Lupo (Ifremer-F)

The European project VIVALDI (PreVenting and mItigating farmed biVALve DIseases) aims at increasing the sustainability and competitiveness of the shellfish industry in Europe, developing tools and approaches with a view to better preventing and controlling marine bivalve diseases. It will also help to identify the best communication strategies when it comes to disease management. In particular, better understanding of stakeholders' perceptions is needed to reach an overall commitment. This study aimed to explore the perceptions of stakeholders about mollusc disease risks and preventive practices.

Focus group discussions were engaged with stakeholders across different geographic locations in order to explore variation across the major shellfish producing countries in Europe. These discussions consisted in hierarchizing 13 preventive measures according to their perceived effectiveness, feasibility, cost and acceptability by different stakeholder groups, in different locations (Northern Ireland, France, Spain and Italy).

"Managing shellfish transfers" was revealed to be perceived as the most effective measure overall, and among farmers and scientists separately. It was also well regarded in terms of feasibility, cost and acceptability. Results highlighted that "managing shellfish transfers", "decreasing shellfish densities" and "increasing shellfish observation" and "testing shellfish for pathogens" had homogenous perceptions across locations and stakeholder groups, given the four criteria considered, and thus could be suitable measures to target for harmonization at the European level. Otherwise, despite evidence of regional beliefs, there was also considerable variation within single locations, most evident between the different stakeholder categories. Part of this variation may be explained by the differences in the priorities of different stakeholder groups, and the stakeholder networks within and between participating countries.

These results strongly suggest that these differences should be taken into account for prevention strategies to be successfully and sustainably implemented at the European level but also at the national level. For example, flexibility should be provided for the application of some measures perceived as not being feasible in some locations and leave the opportunity to replace this measure by another one. In other words, focus should be given to the obligation to achieve results (*i.e.* preventing disease introduction or spread) rather than the obligation to implement measures, whatever their effectiveness.

Presenter's biography

As a veterinary epidemiologist, Dr. Coralie Lupo is currently working at the French Research Institute for Exploitation of the Sea (IFREMER). Her first research activities concerned the modernisation of the French poultry meat inspection at slaughterhouse for the French Agency for food, environmental and occupational health & safety and then for the Ministry in charge of Agriculture. Since 2010, her research interests switched to aquatic animal epidemiology, with a focus on mollusc diseases. Her research focuses on surveillance strategies for early disease detection, modelling of disease control strategies as a decision support tool for stakeholders, and evaluation of stakeholders' perceptions and attitudes towards mollusc mortalities to support stakeholders' commitment to disease prevention.

Session 4 Poster presentations

18-Shifting of the digestive gland microbiota of oysters and clams according to their intertidal marine habitat

Clément Offret[1], Sauvann Paulino[1], Olivier Gauthier[1], Kevin Château[1], Adeline Bidault[1], Charlotte Corporeau[2], Philippe Miner[2], Bruno Petton[2], Fabrice Pernet[2], Caroline Fabioux[1], Christine Paillard[1], Gwenaelle Le Blay[1]

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Presenter: Gwenaelle Le Blay

Abstract

Marine intertidal bivalves face important habitat variations, depending on their position on the intertidal zone due to tidal cycles. Microbiota, including digestive microbiota, could provide a wide range of beneficial effects on host physiology and may play a key role in their ability to acclimatize. Currently, nothing is known about the effect of intertidal levels on microbiota, although it is well known that it affects bivalves' physiology. The aim of this study was to investigate how intertidal zone could affect digestive microbiota of two bivalve's species with different ecology, the Pacific Oyster *Crassostrea gigas* and the Manila Clam *Ruditapes philippinarum.* After 4 months of field implantation, the bivalves were sampled, and half of them were depurated for 14 days to remove transient microbiota. Surrounding seawater and sediment were also sampled from the intertidal zone. Based on 16S rRNA sequencing, different ecological niches harbored specific bacterial community and digestive microbiota was modified, according to the bivalve species and its intertidal position. Despite a strong modification of the digestive microbiota after depuration, intertidal effects were still observed, especially for clams, which conserve 15% of similar microbiota. Oysters digestive microbiota was more subjected to short-term environmental changes linked to seawater fluctuations. Nevertheless, a 4-month stay on the intertidal zone was enough to leave an environmental footprint on digestive microbiota of both bivalves.

19-Occurrence and distribution of two emerging pathogens, *Perkinsus olseni* and *Perkinsus chesapeaki*, from individual host to local scale (Arcachon bay, France)

Sarah Itoiz[1], Clara Mouronvalle[1], Morgan Perennou[1], Nelly Le Goïc[1], Adeline Bidault[1], Evelyne Derelle[1], Xavier de Montaudouin[2], Philippe Soudant[1], Aurélie Chambouvet[1]

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Presenter: Sarah Itoiz

Abstract

Parasitism is an important component of the natural ecosystem cycles. However, this equilibrium between hosts and parasites is today threatened by global changes illustrated by increases in prevalence and severity of infectious diseases. Although most of studies are restricted to single host-single parasite interaction, the reality is more complex with multiple parasitic species infecting a same individual host. However, the diversity and the ecology of these parasitic associations are still neglected leading to scientific gaps that urge to be overcome to understand ecological parasitic impacts on host populations. One of iconic marine parasite species, *Perkinsus olseni* (Perkinsozoa, Alveolata), classified on the OIE-list diseases since 2006, is considered as an emerging pathogen threatening ecosystems and aquaculture sustainability. In Europe, it occurred regularly along the Atlantic coastline causing mortalities in Portugal and Spain. Recently, another species, *P. chesapeaki*, never associated with mortality event, was recently detected and in some cases in association with *P. olseni*. In this context, it is critical to investigate the *in situ* diversity, distribution and abundance of Perkinsus spp. through different scales (from individual to local scale) to better understand their ecological traits and their impact on host populations. We sampled 250 clams from five contrasted sites from Arcachon Bay (SW France) which is an economically important area of shellfish production where perkinsosis is regularly detected. The genetic diversity of the Perkinsus species were determined directly from each individual organ tissues samples using Perkinsus-specific molecular amplification. We developed duplex qPCR TaqMan methodology to determine their distributions and abundances through the different scales. We highlighted for the first time both parasitic species co-occurring regularly in same individual host with different geographical distribution patterns. Taken together, this new knowledge will represent the first step to access in situ diversity and ecology of these parasitic protists in a context of the sustainable conservation policies for managing global diversity.

20-Effect of *Vibrio tapetis* infection on clam microbiota diversity

Cynthia Oliveira[1], Emilie Vanpeene[1], Adeline Bidault[1], Morgan Perennou[1], Philippe Miner[1], Clément Offret[1], Gwenaëlle Le Blay[1], Christine Paillard[1]

[1]Université de Brest, CNRS, IRD, Ifremer, LEMAR, Plouzané, France

Presenter : Cynthia Oliveira

Abstract

Vibrio tapetis is the pathogen causing Brown Ring Disease (BRD) to the Manila clam (*Ruditapes philippinarum)*. Symptoms of BRD are a brown deposit on the periostracal lamina and shell scars. Temperature affects BRD development and recovery and clam immune response. However, less is known about the relation between clam microbiota, *Vibrio tapetis* and the environment. The objectives of this study were: (i) to evaluate the effect of *V. tapetis* infection and environmental parameters on microbiota diversity and (ii) to assess the potential role of clam microbiota in BRD development, resistance or recovery. An experiment survey was carried out in trays with controlled parameters (temperature, antibiotics, *V. tapetis* or sterile seawater injections). Genomic DNA from clam tissues was extracted to study the microbial diversity through 16S rDNA sequencing. Clam microbiota composition highly differed according to the tissue at OTU and phylum levels. Digestive gland (GD) microbiota was very different from fluid microbiota, hemolymph (HLPH) and extrapallial fluids (FEP) as GD microbiota was dominated by *Tenericutes* (mostly *Mycoplasmatales*) while fluids microbiota was dominated by *Proteobacteria* and *Bacteroidetes*. Interindividual microbiota variability in GD and fluids was also important and could explain the different clam responses to *V. tapetis* injections. *V. tapetis* was detected in some asymptotic clams confirming that BRD symptoms are not enough for *V. tapetis* infection detection. The interaction between *V. tapetis* and clam microbiota is complex and this pathogen seems to affect clam microbiota differently according to environmental parameters such as temperature. Indeed, in FEP at 14°C, *V. tapetis* injections induced a decrease of α-diversity not observed at 21°C. Deeper analysis of microbial composition from asymptotic carrier clams and recovered clams could provide information of microbiota role into clam resistance to *V. tapetis* and to BRD recovery process.

21-Interactions between two protozoan parasites infecting the Manila clam *Ruditapes philippinarum*

Biyun Zhao[1], Kwang-Sik Choi[2], Kyungil Park[1]

[1]Kunsan National University, Republic of Korea

[2]Jeju National University, Republic of Korea

Presenter: Kyungil Park

Abstract

Protozoan parasites are ubiquitous in living organisms and are considered major agents of disease in bivalves. Current research on host–parasite interactions remains dominated by the study of "one host–one parasite" systems. However, numerous studies have revealed that two or more pathogens infect the same host, suggesting the occurrence of not only host–parasite interaction but also parasite–parasite interaction during infection. This paper mainly reports the *in vitro* growth of two parasites: *Perkinsus olseni* and Manila clam parasite unknown (MPX), which infect the Manila clam *Ruditapes philippinarum*. MPX showed a significantly lower growth performance than that of *P. olseni* when both parasites grew in the same medium. Low growth of MPX was also observed when they were grown in the extracellular products (ECPs) of *P. olseni*. In contrast, the growth of *P. olseni* in the ECPs of *P. olseni* was unaffected. These results suggest a crucial interaction between the two parasites, with the ECPs of *P. olseni* playing an important role in inhibiting the growth of MPX. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of the ECPs of *P. olseni* and MPX showed different protein patterns. *P. olseni* ECPs had four major bands, with molecular weights of 105, 55, 40, and 38 kDa, vis-à-vis those of MPX: 120, 70, 55, and 40 kDa. Furthermore, the ECPs of MPX and *P. olseni* differed considerably in the activities of large enzymes such as amylase and proteinase. We believe that certain enzymes are involved in parasite–parasite interaction. Thus, the enzyme characterization of these parasites is currently under investigation at our laboratory.

Session 5: Disease management measures and biosecurity

Application of genomic tools and perspectives

Romain Morvezen (SYSAAF-F)

The use of genomic tools in breeding programs can take many forms. The first one, famously used by the salmon industry, is marker-assisted selection (MAS). To do MAS, you first need to identify a strong link between phenotype (i.e. disease resistance) and genetic markers. The salmon industry MAS of Infectious Pancreatic Necrosis (IPN) resistance, a viral disease, is a textbook example of successfully applying genomic tools to improve genetic resistance of salmon and reduce the number of the disease outbreaks.

In the oyster industry, past project, confirmed by Vivaldi's results, showed that some disease resistance in mollusks (for example OsHV-1 resistance in cupped oyster) seems to be highly polygenic, with many genes along the genome contributing to resistance. In such a case, MAS is not possible, and genomic selection needs to be applied.

The concept of genomic selection (SG), initiated in 2001, has been gradually implemented in terrestrial animal species. Compared to conventional breeding, SG increases genetic progress by improving one or more of its parameters (accuracy, selection pressure, generation interval, preservation of variability) according to species and their selection patterns. For aquaculture, most salmon breeding companies have already taken the plunge, but also trout breeders such as Troutlodge or AquaChile. Projects are underway around the world on sea bass, sea bream, tilapia or shrimp or oyster.

The results of the project also highlight the challenge of phenotyping: well defined, reliable, repeatable phenotyping methods for disease resistance are key to establish link between genome and disease resistance. Without confidence in phenotyping, genetic gain will remain marginal. In any case, response to selection studies (testing selecting animals) will need to be conducted to validate genetic progress.

In conclusion, successful breeding for disease resistance in mollusk will be conditioned by having access to reliable phenotyping methods; by developing new genomic resources for species that are lacking; and by continued testing of selected animals to validate genetic gain.

Presenter's biography

Romain Morvezen is part of the SYSAAF (The French poultry and Aquaculture Breeding Technical Center), a non-profit professional association (French Law 1884) which groups together companies developing rational programs of genetic improvement and-or management applied to poultry, fish and oyster species and another species like fly. He is in charge of the development and implementation of genomic selection in the French aquaculture breeding programs.

Novel biotechnological strategies for the detection of ostreid herpesvirus

Mònica Campas (IRTA-ES)

Ostreid herpesvirus-1 (OsHV-1) has been involved in massive mortality outbreaks of *Crassostrea gigas* (Pacific oysters) throughout the world, causing important economic losses to aquaculture. Therefore, rapid isolation methods as well as more sensitive detection systems are highly desired to mitigate, prevent and control this disease. With this aim, IRTA has working on two approaches: the exploitation of magnetic beads (MBs) for the capture and pre-concentration of OsHV-1, and the development of an electrochemical biosensor for the detection of the virus.

In the first approach, MBs coated with an anionic polymer were incubated with two types of matrices (oyster homogenate and seawater) prepared using naturally-infected oysters collected from Fangar Bay (NW Mediterranean Sea). Adsorption of the virus on the MBs and characterization of the MB-virus conjugates was demonstrated by quantitative PCR (qPCR). These conjugates were able to infect naïve spat oysters, demonstrating the ability of MBs to capture viable OsHV-1 particles. Additionally, the same MBs were exploited as pre-concentrating agents. Results indicated that MBs were able to pre-concentrate OsHV-1 particles at least 100 times. Their applicability to the analysis of seawater was demonstrated in a depuration experiment. Whereas no OsHV-1 DNA was detected when using only qPCR, OsHV-1 DNA was detected when MBs were used prior qPCR, demonstrating that MBs were able to detect and pre-concentrate OsHV-1 from seawater.

In the second approach, an electrochemical biosensor for the detection of OsHV-1 was developed, based on the isothermal recombinase polymerase amplification (RPA) of the DNA and its subsequent detection via a sandwich hybridisation assay (SHA) on thin-film gold electrodes. For the RPA, specific primers were designed to render an amplicon with single-stranded DNA tails. For the detection step, a thiolated capture probe was immobilized on gold electrodes. Following hybridization of the RPA amplicon, electrochemical detection was achieved via addition of an HRP-conjugated reporter probe. A limit of detection (LOD) of ~200 target copies was achieved. The biosensor was applied to the detection of OsHV-1 in spat oysters from an infectivity experiment. A good agreement was found between the biosensor and qPCR quantifications demonstrating the reliability of the developed tool.

Summarising, the use of anionic polymer-coated MBs is a rapid, easy and cost-effective strategy to isolate OsHV-1 particles from complex matrices, and could be of great utility in research activities. Additionally, MBs are able to improve the limits of detection of qPCR by previously concentrating virus particles from seawater, thus getting closer to an early warning system. The biosensor is an alternative tool to detect OsHV-1 and it offers great potential to be integrated into microfluidic systems to develop compact devices that could be used to perform in-field analysis.

Presenter's biography

Dr. Mònica Campàs is researcher at IRTA, where she leads a research line on biosensors for the detection of toxins, toxic microalgae, viruses and bacteria. She obtained a BSc in Chemistry in 1996 and a PhD in Chemical Engineering in 2002 from the Universitat Rovira i Virgili. Her research interests lie in the development of bioanalytical tools and the study of their applicability. She has published 76 articles (63 SCI) and 11 book chapters, and has edited 1 book. She has been the principal investigator of 11 projects. She has directed 4 PhD thesis, 2 more being in progress.

Biosecurity measures and inactivation of pathogenic organisms in shellfish aquaculture facilities

Christophe Stavrakakis (Ifremer-F)

A part of the studies realized in the work package 5 about the "Performance evaluation of HOD (Hydro-Optic Disinfection) and laser in hatcheries, nurseries and SDP" were presented. Atlantium has manufactured a Collimated Beam Apparatus (CBA device) to inactivate by UV diverse pathogens of interest and provided a support in the calculation of the delivered dose. Atlantium purchased 2 HOD devices to Ifremer and IRTA in order to evaluate the performances close to the real conditions for different applications as explained below.

Mollusk experimental platform of Ifremer Bouin has participated in the setup of the inactivation of pathogens (OsHV-1, Vibrio splendidus and Vibrio aestuarianus), oyster gametes and larvae. With the objective to evaluate the performances of HOD to inactivate these targets, several experiments were performed at the laboratory scale and close to real conditions showing the high performances of the Atlantium technology. Ifremer compared the performances with other well-known processes such as chlorination and ozonation and innovative technologies for aquaculture with the use of membrane filtration. All experiments showed the high efficiency of the tested devices to protect Crassostrea gigas oyster breeding and to avoid the spread of non-endemic species in the environment, but ozonation and chlorination led to the production of potential toxic by-products.

IRTA worked on the inactivation of OsHV-1 in a pilot depuration plant with the aim of evaluating the role of these plants in the transmission of the virus in the ecosystem. This work evaluated the kinetics, survival and transmission capacity of OsHV-1 in depuration plants in oysters and mussel under different holding conditions, with and without Ultraviolet treatment. Additionally, anionic magnetic beads (MBs) were used to evaluate the presence of OsHV-1 in the seawater and were able to concentrate OsHV-1 up to 8 times. This work clearly demonstrated the correct operation of the HOD system in the inactivation of OsHV-1.

UCC worked on antimicrobial photodynamic therapies (aPDT treatments) such as Blue LED and Blue Laser technology to activate nontoxic photosensitisers for eradication of target microbial cells in microalgae, seawater and C. gigas seed. These therapies were shown to be successful in the reduction of pathogens (Vibrio spp.). The results of this study strongly suggest that there is a potential for using the aPDT treatments described in this study on hatchery microalgal feed and tank seawater even with oysters present. The aPDT therapies with nontoxic photosensitisers were shown to be non-destructive to the microalgal cells, the microbiome and the seed oysters. Furthermore, oysters administered aPDT treated microalgae continued feeding on the microalgae post-treatments, thus growth was not inhibited. aPDT has the potential to be used on a larger scale and could possibly work to reduce pathogen proliferation and disease outbreaks in aquaculture production systems.

Presenter's biography

Christophe Stavrakakis (39 years) obtained its Ph-D in engineering sciences (speciality in environmental processes) from Nantes University in 2007. He worked on the analysis and evolution of endocrine disrupters in wastewater treatment plants and tap water production. After that, during 6 years he worked in the French National Agency for Environment and Energy as project manager on industrial atmospheric pollution topics. Since 2013, he is working at Ifremer, a French marine institute, first as research engineer and then as laboratory manager, with the objective to develop original R&D projects in the sea water treatment field for shellfish production safety.

Is it possible to stimulate bivalves' immunity?

Rebeca Moreira (CSIC-ES)

To answer the question of whether it is possible to stimulate bivalve's immunity we have to take into account that invertebrates do not have an acquired immune system. It is, they do not produce antibodies, and therefore vaccination is not possible. But recent research has demonstrated that the antibodies are not the only immune memory available in nature. Innate immune system has also a certain degree of memory, and now, scientific research is discovering every year new species that possess it.

To know if mussels (*Mytilus galloprovincialis*) and oysters (*Crassostrea gigas*), two of the main cultured bivalve species in Europe, can be immunostimulated, different approaches have been undertaken. Bivalves were stimulated with glucans and poly IC, molecules mimicking bacterial, fungal or viral contact. Glucans showed certain degree of protection in mussels and oysters, and although limited, it is interesting to keep investigating future applications, especially in closed controlled environments such as hatcheries or nurseries. With regard to poly IC, oysters respond very well to this stimulus. Poly IC enhances the survival of oyster juveniles to almost 100% after a subsequent OsHV-1 infection, both in laboratory and in field conditions, and for periods up to 5 months. This is a very promising result that has been already published in scientific journals and has also gathered the attention of producers.

After these interesting outcomes new questions arise regarding the transfer of this protection to the next generations. This is a cutting edge scientific topic that is currently under research. And although recent results point to the fact that the innate immune memory can be indeed transferred to the offspring, these results are preliminary and require further investigation.

Therefore, the answer to the question "is it possible to stimulate bivalves' immunity?" is: yes, it is possible. And now the next steps are to translate these findings into applicable tools for the aquaculture sector (some of the VIVALDI results are currently being patented and could be available for the industry in a couple of years) and to continue the research to improve the knowledge about immune stimulation in aquacultured bivalve molluscs.

Presenter's biography

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I was born in 1981 in a small town in Galicia. In 2005 I graduated from the University of Vigo and completed my training with two Masters, in Biotechnology and in Life Sciences. In 2009 I joined the Immunology and Genomics group of the Marine Research Institute in Vigo to do my doctoral thesis, which obtained the Extraordinary Doctorate Award. Since then my work has been focused on genomics in bivalve's aquaculture and a very big and interesting project that we have recently finished is the mussel genome. I have published 19 scientific articles in SCI journals and 3 book chapters related to genomics, aquaculture and shellfish immunity.

Shellfish farming in an era of rapid changes

Fabrice Pernet (Ifremer-F)

The massive release of carbon dioxide (CO2) into the Earth's atmosphere by human activities over the past 150 years has warmed the atmosphere and the oceans. In addition to warming, the oceans absorb almost a quarter of anthropogenic CO2 emissions and become more acidic. Acidification of the oceans results in a decrease in the concentration of carbonate ions, a constituent of the calcium carbonate essential for shell growth. In addition, one of the main threats to oyster shellfish aquaculture is related to the risk of emerging pathogens that is increased by climate change and the weakening of farmed animals. Overall, climate change is expected to have a negative impact on shellfish culture. The annual impact of ocean acidification alone on European shellfish production was estimated at more than one billion USD in 2100. More generally, climate change will lead to variations in the goods and services provided by shellfish ecosystems and shellfish farming. It is therefore essential to develop adaptation and mitigation measures based on cultivation practices, genetic selection and environmental conservation and restoration.

Presenter's biography

Fabrice Pernet is a research scientist working at Ifremer (Brest, France) on ecology and physiology of marine invertebrates. Within the Vivaldi project, FP investigates the role of environmental factors on host-pathogen relationships. His work mainly focuses on the effect of ocean warming and acidification on mortality of oysters caused by the herpesvirus. He also addresses the impact of surrounding species on disease transmission.

A life in flux: effects of environmental parameters and extreme events on Pacific oyster performance and pathogen development

Sarah Culloty (UCC-IE)

There is now higher confidence in projected patterns of warming and extreme meteorological conditions. These climatic changes will create environmental stressors that effect pathogens and their hosts. As part of the Vivaldi work package deliverable; "Synthesis of the impact of environmental parameters and stressors on pathogen development or suppression" two studies were carried out at the University College Cork, Ireland. Firstly, the effects of turbidity, SPM and reduced salinity and secondly, the effects of fluctuating thermal stress on C. gigas spat performance (survival and stress response) and pathogen development were investigated. For these studies, naïve spat were placed in an OsHV-1 µVar endemic site for several months to encourage natural pathogen transmission prior to the trials. Comparing three levels of turbidity in conjunction with "increased precipitation conditions", it was found that, when SPM was high, and salinity was low, medium levels of turbidity (25 TU) caused the greatest C. gigas mortality rates and physical damage to gill tissue. However, it was observed that low salinity reduced OsHV-1 µVar infectivity. Investigating the effects of fluctuating thermal stress through short and sudden spells of extreme temperature (heat-wave and polar-snap simulations), results showed that C. gigas have a better tolerance to sudden drops in temperature, rather than sudden increases, and younger oysters, compared to older cohorts, had a higher tolerance to the freezing aerial temperatures. Results from these studies provide insights into the importance of shore height for oyster survival and the possible interactions of OsHV-1µVar with sediment flocs.

Presenter's biography

Sarah is the Head of the College of Science, Engineering and Food Science and Director of the Environmental Research Institute at UCC. She is also the principle investigator for the Aquatic Animal Health research group in the School of Biological, Earth and Environmental Sciences (BEES), UCC. Her main area of research is in ecological parasitology with a particular focus on molluscan diseases. The focus of her research centres on pathogen (macroparasites, protozoa, bacteria and viruses) life cycles, epidemiology, diagnostics and approaches to reducing impacts of disease. Recent research looks at the impact of potential climate change drivers on disease development in the marine environment. While at UCC, Sarah has won 20 research grants totalling over €5 million. She is currently working on the H2020 project Vivaldi, DAFM FIRM REPOSUS project and INTERREG projects BLUEFISH and COCKLES.

Public health and shellfish health implications

Bill Dore (Marine Institute-IE)

Bivalve molluscan shellfish can cause illness when consumed raw or likely cooked. Two well-recognised microbial risks associated with bivalve shellfish consumption include norovirus arising from sewage contamination and naturally occurring vibrio species such as *V. parahaemolyticus* or *V. vulnificus*. These pathogens have been associated with significant outbreaks of illness following oyster consumption. These risks are likely to increase in the future because of climate change and population growth. The world's population is set to reach 9.8 billion by 2050. Two obvious issues associated with population growth is an increasing pressure on wastewater treatment plants and increasing agriculture activity. These will both likely lead to increased contamination of shellfisheries if not carefully managed. In addition, increasing seawater temperatures will extend the range of marine *Vibrios*. Climate change and population growth are also likely to also impact on the health of shellfish themselves by increasing environmental stress. However, currently in the EU, public and animal health risks for shellfish are monitored separately under differing regulation. A one health approach employs a collaborative approach to attain optimal health for people and animals. Such an approach has been suggested for bivalve shellfish controls. In this presentation, I discuss the potential impact of climate change and population growth on public and shellfish health and, the potential to adopt a one health approach to monitoring these impacts.

Presenter's biography

Bill Dore is the manager of the Fish Health Unit in the Marine Institute, Ireland. He is responsible for Competent Authority activities for aquatic animal health in Ireland. Previously, He spent over 25 years working in the area seafood safety specialising in microbial contamination in Bivalve mollusc shellfish, particularly oysters. He worked in in the UK at CEFAS which was the European Reference Laboratory microbiological contamination in bivalve shellfish before moving to Ireland in 2004 to lead activities at the National Reference Laboratory.

Modelling spatial interactions for bivalve aquaculture

Cédric Bacher (Ifremer-F)

Mathematical models aim at representing a set of interactions between entities which drive the dynamics of a given system. I give examples which illustrate how models address spatial constraints and features with respect to the health of bivalves. The first example deals with the development of tools for Marine Spatial Planning for Aquaculture. A web based dynamic Geographic Information System combined to a model of oyster and mussel individual growth has been deployed for shellfish aquaculture in Normandy. It aims at helping end-users and decision-makers to optimize aquaculture performance and to select new potential sites by taking into account a number of environmental and regulatory constraints. The second example deals with oyster mortality outbreaks due to Vibrio aestuarianus infection in one aquaculture site. The model uses a population modeling approach considering bivalve populations at the level of aquaculture farms connected through the transport of pathogens. The model simulates the spatial and temporal dynamics of the infection. Simulations show that the susceptibility to mortality outbreak as well as distribution of pathogens is very much dependent on the spatial location of the initial infection as well as on the time of the year when the introduction takes place. The last example extends beyond aquaculture and aims at simulating the response of bivalve populations to temperature changes along the coast of Brittany using IPCC scenarios. It shows that oceanographic connectivity drives the population dynamics and local changes are likely to affect distant population structure. Simulations also show that seawater warming modifies the demographic structure of mussel populations and the phenology of the reproduction. In my conclusion, I emphasize that ecological concepts and agent based modeling framework open the door to the development of a new generation of models capable to predict the emergence of system properties at different scales. This framework would extend the set of interactions beyond target species and environment to social entities, e.g. shellfish farmers and decision makers.

Presenter's biography

My research covers several types and scales of ecological modelling: response of living organisms to environmental drivers using Dynamic Energy Budget theory, primary productivity and trophic networks, bioaccumulation of chemical contaminants, aquaculture production, integrated modeling and system approach, functional biodiversity. I have recently been involved in a project on the "Adaptation of oyster farming ecosystems to global change (GIGASSAT)", funded by the French National Research Agency, and in a project on the "Ecosystem Approach to making Space for Sustainable Aquaculture (Aquaspace)", funded by the H2020 European program.

Keynote presentation: Strategies for mitigating ostreid herpesvirus 1 in *Crassostrea gigas* hatchery and nursery systems

Dolors Furones, VIVALDI Work package 5 leader (IRTA-ES)

Disease prevention and management is a common worldwide necessity and, therefore, most countries and international organizations have to legislate and develop programs to prevent disease emergence and spread. Such legislative frameworks shape the approaches followed by the different countries in their pursuit of better disease management. However, since most of the relevant pathogens of bivalves in the European Union are not notifiable, there is a special need to provide technical strategies to manage such diseases. The VIVALDI project through its WP5 "Disease management measures and biosecurity", deals with implementation of tools developed by other areas in the project, such as early warning techniques (passive sensors and biosensors) and hydrodynamic models for pathogen spread. Moreover, knowledge on husbandry conditions to reduce mortalities, disinfection approaches in hatcheries and nurseries to avoid pathogens spread or shared gametes and farm risk ranking have been generated in order to assist stakeholders. VIVALDI ultimately intends to provide recommendations to avoid, mitigate and manage diseases better.

After a comprehensive retrospective overview of the work undertaken by the scientific community in the understanding of the risk factors associated with mortality in Pacific oysters, *Crassostrea gigas*, caused by ostreid herpesvirus 1 (OsHV-1), the project's results and their potential contributions to support recommendations for disease management strategies were analysed.

The main relevant interconnected factors that need to be considered to produce management control strategies are: the animal host (e.g. age, physiological state, selective breeding programmes), husbandry procedures (e.g. stocking density), the pathogen itself (e.g. pathogenicity, virulence, and diversity) and environmental effects (e.g. temperature). All these factors have been approached in VIVALDI, and they provide valuable background information for outlining the mitigation strategies needed by the industry, as well as in the context of surveillance and biosecurity programmes. These control mechanisms for hatchery or nursery areas are related to movement restrictions, water treatment, virus inactivation, the production calendar and practical farm management decisions. A revision of the main compartment factors and their potential contributions to provide recommendations is presented

Presenter's biography

I am a biologist, with a PhD in Fish Pathology (University of Plymouth, UK), which set up the basis of my professional career in aquatic animal health. My research focuses in Mediterranean aquaculture species, both finfish and molluscs. I am currently involved in five EU projects, such as VIVALDI and MedAID. I am also actively involved in contracts and research projects with companies working on aquaculture health. I have been working at IRTA since 1999, where I took management roles as Director of the IRTA_ Sant Carles Centre (1999-2018) and Head of its Aquaculture research programme (2008-2015). She is actively involved with stakeholders in Spain, being board member of Acuiplus cluster, the platform PTEPA and President of the Spanish Aquaculture Society, for a two years mandate.

The EU Animal Health Law (Regulation (EU) 2016/429) and its role in disease prevention and control in molluscs

Fiona Geoghegan (European Commission)

Regulation (EU) 2016/429 on transmissible animal diseases, the EU Animal Health Law (AHL) will apply in all Member States from 21 April 2021. The AHL sets out one single legal framework for EU animal health policy and replaces 39 Directives and Regulations, which are currently applicable in relation to diseases of terrestrial and aquatic animals.

The AHL will be supplemented by a number of delegated and implementing acts, which will, on aquatic animal health side, set out rules for:

- Disease surveillance, eradication, disease freedom, notification and reporting
- Disease awareness, preparedness and control
- Registration and approval of aquaculture establishments and transporters
- Movements of aquatic animals and certain products of animal origin within the EU
- Entry into the Union of aquatic animals and products of animal origin

The general approach, which has been taken in relation to the development of the new legislation, is to retain the concepts from the current legislation which have been successful and to improve those which have not served the purpose which was intended. In addition, the new legislation will also take scientific developments which have occurred since the application of Council Directive 2006/88/EC, into account.

The overall objective in relation to aquaculture establishments which grow molluscs is to simplify the legislation as much as possible, but to ensure the simplified legislation is fit-for-purpose and fully implemented in all Member States. There will also be a significant new emphasis on putting systems in place to recognise and control emerging diseases, such as those which have caused significant problems for the mollusc industry over the past decade. Operators and competent authorities must also play their part in the application of the legislation by being fully aware of their legal obligations and ensuring those obligations are met.

It is anticipated that training on the new legislation will commence for Member States in 2020 under the 'Better Training for Safer Food' platform.

Presenter's biography

Fiona has worked in the area of fish health for almost 30 years, most of which have been spent working with the Marine Institute in Ireland and more recently, with the European Commission. Her work with the Marine Institute included having responsibility for the National Reference Laboratories for diseases of fish, molluscs and crustaceans, as well as for the implementation of national and European fish health legislation. Currently, her main work in DG SANTE involves the drafting of relevant delegated and implementing regulations to supplement the rules set out in Regulation (EU) 2016/429 (the Animal Health Law).

Feedback on the exchange session with stakeholders

What are the main difficulties you meet regarding managing shellfish diseases?

Irish CA: In the context of oysters, Vibrio aestuarianus is the main problem at present. There is no legal support, no specific guidelines, to prohibit the movements from one bay to another for non-listed pathogens. Oyster seed comes mainly from France, when pathology starts in France it will easily transported to Ireland together with the seed. Ireland has guidance for non-explained mortalities over the normal level. For C.gigas related mortalities, trigger reporting levels have been introduced which are as follows: Seed – report at 30% or above, Half-grown – report at 10% or above, Adults – report at 10% or above.

Irish producer: Now, in Ireland Vibrio aestuarianus is the most important problem for oysters. It is impossible to limit the movements that can cause problems. Environmental factors have an impact but depend on ages and sizes. Simple solutions are necessary to be able to adapt to new or emergent diseases. This is a market- driven activity, which needs regulation.

French producer: Farmers know that mortality appear mainly some weeks after having stressed the oysters (3 weeks). The probability of mortality after having stressed the oysters is higher in summer than in winter. Movements are necessary.

French CA: Some of the limitations are the following, difficult access to the animals due to tides and meteorological conditions. Moreover it is difficult to identify diseases since there are not or very few visible symptoms, the briefness of the events. The implication of the shellfish aquaculture sector in the surveillance is necessary. In addition, the environment where shellfish is growing is a shared environment between different sectors; wild and farmed stocks are in the same environment, ballast water may be a thread for example. The density of the farmed animals is an important factor.

Spanish CA: Mussel production in Galicia is an example of success, 300,000 tones are produced every year by 3000 farms. There is genetic variability assured though collection of wild seed, this production shows its resilience and equilibrium. For oysters, we have not been able to copy the same model of production. There are not local hatcheries in Galicia; local hatcheries could allow obtaining oysters adapted to the environment capable to live together with the sickness.

Spanish producer: The aquaculture sector needs better training and improved access to information. Each aquaculture company should have a responsible of the management with a specific training that will implement good practices and better transport conditions. This specific training should be a requirement. Training should be adapted to the reality of the sector. Farmers have little access to scientific publications and their contents are difficult to understand. The type of information that could be especially relevant is for example: which are the environmental conditions that trigger mortality in front of a specific pathogen? Which are the symptoms? In addition, related to molluscs movements: Which is the situation related to aquatic health in the different shellfish growing areas of Europe? Before importing new seed, it will be useful to obtain a sample.

Italian CA: The situation in Italy is diverse, different depending on the geographical area. There was a narrow control related to *Marteilia* because this pathogen was an important economic problem. One of the difficulties for surveillance is that high mortalities are reached in very different environmental conditions depending on the pathogen. The official veterinarian conducts the sampling and sends the samples to the laboratory that will conduct the analysis. There is fear to lose markets by declaring affected production

Italian producer: Who has to pay the surveillance? It should not be the farmers. It is very difficult to understand all the legislation. A main point is capacity building. Another important issue is climate change, which is having a strong impact in some areas.

Irish farmer: hatcheries can do a lot to reduce mortality.

How is the surveillance of mollusc diseases organized? Explain the responsibilities of the different stakeholders categories and how do they interact with each other to implement this surveillance?

Irish CA: In Ireland, there are National measures in the National legislation for herpes virus. There are five disease free compartments or surveillance areas. There is a national organization for high mortalities. The reference laboratory conducts analysis of samples to investigate high mortality events. Regional inspectors conduct inspections every 2 or 4 years. For C.gigas related mortalities, trigger reporting levels have been introduced which are as follows: Seed – report at 30% or above, Half-grown – report at 10% or above, Adults – report at 10% or above.

French CA: the surveillance of events is conducted after receiving a declaration of farmers when they detect abnormal mortality. Then, samples are taken for analysis of known and emergent pathogens to know if the mortality is infective. Farmers inform the local authorities about these events, the "Prefect" decides if sampling has to be conducted, and Ifremer assures the coordination. Samples are sent to different laboratories for PCR analysis and histological analysis. It is difficult to characterize what is "High mortality". The system is not perfect. It is envisaged to put in place an early warning detection system. Since mortalities are frequent, there is a tendency to no declaration.

Spanish CA: veterinarians from the "Conselleria del Mar-Xunta de Galicia" conduct regular inspections in aquaculture farms to control the presence of pathologies, the register of movements, the accreditation of training and knowledge of farm workers, molluscs densities, immersion permits. The model is in equilibrium since 70 years ago.

Italian CA: The central authority is the Ministry of Health, which is coordinated with the regions where the experts in aquatic animal health of each farm contact the local official veterinarians when they detect symptoms for the mandatory declaration diseases and events of high mortalities. In the north of Italy the declaration system has improved a lot.

Irish CA: there are some areas with active surveillance, which correspond to those declared free.

Italian CA: Italy follows the European regulation for abnormal mortalities. The problem is the fear of the actions that will follow the declaration. We are all category III areas.

Spain: It is preferable not to change models that are performant. We should not be limited to one analysis, there are multiple factors. Forecasting models, selection of most adapted species, genetic variability, farming practices, surveillance…

France: regular surveillance was not able to provide an early warning of pathologies. It is necessary to find more performant systems for each pathogen and each species. It is necessary to determine what the areas at risk are and to implement the surveillance in those areas.

Some farmers try to improve the knowledge and others believe that nothing will improve. Once mortalities started, nothing can be done. The strategy is to leave it die and avoid cross contamination.

France Naissain: Hatcheries are conducting control on their water quality. Hatcheries are equipped with water treatment to avoid contamination from the environment. During the period at risk weekly analysis are conducted for herpes virus. Hatcheries have also been working to produce animals that are more resistant to herpes virus and the results now are good. Now, they are working on the resistance to Vibrio aestuarianus but heritability is much lower.

Spain (Rebeca): In Spain there is obligation to conduct analysis when there are mortalities. In Galicia, samples are collected every 2 months for mussels, clams and cockles. The prevalence of pathogens is recorded from those samples. In addition, Intecmar is in charge of the official control of the water quality in shellfish growing areas, different parameters are recorded. Farmers can also send samples to the national reference laboratory.

France (Ifremer): It is necessary to clarify that active surveillance is conducted in areas that are declared free of pathogens, in these areas active surveillance allows to protect the area.

Spain_ Asturias (Carmen Rodriguez): In small regions with low production it is possible to implement some measures in agreement with the farmers such as for example allow only the entrance of seed stock free of herpes virus from controlled hatcheries. But, what is the risk of introducing animals that are resistant to pathogens with the possibility of transmission of pathogens to the growing area?

What kind of biosecurity measures do you already implement in farms (including hatcheries, natural populations, depuration enters…)?

Irish producer: Since winter time is known to have lower risk, movements from one bay to another are mainly conducted during this time of the year. Farmers are conscious about reporting outbreaks to MI since they trust it is the best way to trace when a problem appears from where and when it appeared. Vibrio aestuarianus is now an important problem in some areas in Ireland. They try to find out which stock works well and which do not, and at what time of the year. Movement from one bay to another is an absolute necessity since at specific age animals grow well in an area and not in another, also because if all stock ages are in a single area and an outbreak happens all will be lost.

French producer: it is recommended not to mix different hatcheries in the same area. We have reduced stress in oysters and change husbandry practices to improve survival.

Spanish producer: there are not hatcheries in the Ebro delta. Mussel seed comes from Italy and Greece and oyster seed from France. Quarantine is not possible. Farmers follow a recommended calendar and try to mix mussel and oyster ropes decreasing densities and avoiding having mussels of commercial size during summer time when high temperature are a risk. IRTA provides training in different sessions during the year.

Italian producer: There are not special measures a part from densities, temperature. Climate change is having an important impact in Italy.

Conclusions by the moderators:

- There is a clear need for training.
- Environmental impact plays a key role in mortalities
- Surveillance must have a purpose.

Session 5 Poster presentations

22-Prediction of oyster herpes virus disease transmission using dispersion models in the Ebro delta

Andreu Fornós Bautista[1], Pablo Cerralbo Peñarroya[1], Manuel Espino Infantes[1], Noelia Carrasco[2], Karl B. Andree[2], Dolors Furones[2], Margarita Fernández Tejedor[2]

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Presenter: Margarita Fernández Tejedor

Abstract

Mortalities of *Crassostrea gigas* seed in Alfacs Bay during the years 2017 and 2018 have occurred in an irregular spatial pattern at the beginning of May. Rafts located at a short distance from each other and holding oyster spat from the same origin suffered very different mortality rates. The analysis of oyster tissue by qPCR showed the presence of OsHV in the affected rafts. A lagrangian dispersion model based on Ichthyop and Regional Modeling System (ROMS) was used to evaluate the possibility of infection of the different aquaculture rafts starting from a known hotspot, a raft holding infected spat. Different diffusion coefficients were tested to generate maps of probability of infection and its timing. The results obtained can explain the patchy pattern of mortalities in the Bay and are consistent when different diffusion coefficients are tested. These models can be improved if the density of viral particles in the water is known as well as using diffusion coefficients calculated though field experiments.

23-Electrochemical biosensor based on isothermal DNA amplification as a tool to detect Ostreid herpesvirus-1 (OsHV-1) in *Crassostrea gigas*

Anna Toldrà[1], Dolors Furones[1], Ciara K. O'Sullivan[2,3], Mònica Campàs[1]

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Presenter: Mònica Campàs

Abstract

Ostreid herpesvirus-1 (OsHV-1) has been involved in massive mortality outbreaks of Pacific oyster *Crassostrea gigas* throughout the world. Therefore, rapid, simple, low-cost and in-situ detection tools are highly needed to reduce economic loses to shellfish aquaculture. In this work, an electrochemical biosensor for the detection of OsHV-1 DNA based on isothermal recombinase polymerase amplification (RPA) and thin-film gold electrodes was developed.

The method involves two steps. For the RPA step, specific primers were designed to render an amplicon with single stranded DNA tails for its subsequent detection via a sandwich hybridization assay. For the detection step, a thiolated capture probe was immobilized on gold electrodes. Following hybridization of the RPA amplicon, electrochemical detection was achieved via addition of an HRP-conjugated reporter probe (Fig. 1). Prior to the electrochemical detection, a colorimetric assay was developed to test the feasibility of the approach and optimise the RPA conditions. Calibration curves were constructed using PCR-amplified OsHV-1 DNA. A limit of detection (LOD) of ~400 target copies was achieved by the colorimetric assay using the optimised RPA conditions. When the strategy was transferred to an electrochemical platform, the biosensor provided an LOD of ~200 target copies. The biosensor was applied to the detection of OsHV-1 in 16 spat oysters from an infectivity experiment. A good agreement was found between the biosensor and qPCR quantifications demonstrating the reliability of the developed method.

The biosensor is an alternative tool to detect OsHV-1 and it offers great potential to be integrated into microfluidic systems to develop compact devices that could be used to perform in-field analysis.

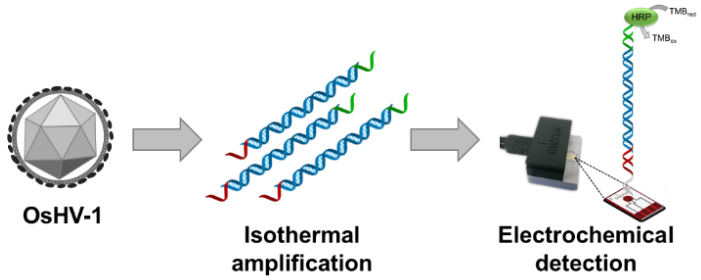

Fig. 1. Scheme of the biosensor strategy.