

What's new in bivalve mollusc pathology? Overview of articles published in the last two years

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Abstract

This is an overview of articles on bivalve mollusc diseases published between the two last "International Conferences on Diseases of Fish and Shellfish" organised by the EAFP (September 2013 to September 2015). The need to limit the number of pages of this overview has involved selecting (and discarding) articles, prioritising by novelty and relevance, which entails an element of subjectivity.

Journal special issues

Highly relevant was the publication of special issues focused on this field by three journals (in chronological order): (1) "Microcell parasites of molluscs" (*Diseases of Aquatic Organisms* 110 (1-2), 2014), including recent research on protistan parasites of molluscs in the genera *Bonamia* and *Mikrocytos*; (2) "Molluscan Immunity" (*Fish & Shellfish Immunology* 46 (1), 2015), including reviews of many aspects of the immunology of molluscs as well as an announcement of the forthcoming effort to sequence the genome of the eastern oyster, *Crassostrea virginica*; and (3) "Pathogens and Disease Processes in Marine Molluscs" (*Journal of Invertebrate Pathology* 131, 2015), focusing on new knowledge about the pathogens of commercially important marine molluscs, expanding the scope to include associated topics important to understand disease processes. Articles included in these three special issues

will be key references in their respective fields for years.

Immune response

Bivalve immune response captured much attention in the reviewed period and numerous, excellent articles were published, mostly on host transcriptomic/proteomic changes associated with pathogens/diseases (viruses, bacteria, protistan, stress, etc), showing the complexity of the immune system of bivalve molluscs. Hopefully, advances in this field will be used to design strategies to fight diseases of economically relevant molluscs, such as identifying molecular markers of disease resistance. The list of articles is too long to address bivalve immunity in this overview; nevertheless, the increasing attention paid to apoptosis within bivalve immune context deserves to be mentioned (reviewed by Romero et al., 2015); the apoptotic effect of UV

radiation on haemocytes of the European flat oyster *Ostrea edulis* suggests that apoptosis is an important mechanism against stress factors (Gervais et al., 2015).

Viruses

The infection of the Pacific oyster *Crassostrea gigas* with the Ostreid herpesvirus 1 (OsHV1) is one of the bivalve mollusc pathology items standing out for the high number of articles published in the last two years, which reflects the economic relevance of this virus for the widespread production of *C. gigas*; this research effort, on a wide thematic spectrum, has resulted in useful knowledge to fight this disease. OsHV1 genetic variability within geographic and host ranges was reported from Mexico (Grijalva-Chon et al., 2013), South Korea (Hwang et al., 2013), China (Bai et al., 2015), Portugal and Spain (Batista et al., 2015) and The Netherlands (Gittenberger et al., 2015). Temporal variation of genetic profile was also detected in Spain (Andree et al., 2014) and Ireland (Morrisey et al., 2015). The widest perspective on genetic variability was provided by Mineur et al. (2015).

A genome analysis of OsHV1 focused on 40 ORFs allowed distinguishing virus variants (Martenot et al., 2013) while genotyping a microsatellite locus allowed differentiating clinical OsHV1 specimens (Renault et al., 2014a). Viral transcriptome was also addressed; analysis of the expression of 39 viral genes provided information on the virus cycle based on the kinetics of viral DNA replication and transcription (Segarra et al., 2014a, 2014b) and differences of gene expression were found between viruses infecting oyster spat and those infecting adults (Segarra et al. 2014c).

Regarding sampling procedures and diagnostic tools, new primers for PCR diagnosis were assayed (Lynch et al., 2013). Three procedures (qPCR, evaluation of mortality provoked by thermal challenge and qPCR during thermal challenge) were assessed to prevent mortality (Normand et al., 2014a). A centrifugation procedure was arranged to improve viral detection in water samples (Evans et al., 2014). A surveying protocol, using hydrodynamic dispersion models to determine epidemiological units, was designed (Pande et al., 2015). An *in situ* hybridisation assay for detecting OsHV1 mRNA was designed (Corbeil et al., 2015).

The pathogenesis of infection with OsHV1- μ var in oyster was also addressed. A proteomic approach showed cytoskeleton disorganisation, dysregulation of protein turnover, stress signals, dysregulation of steroid metabolism, and Warburg effect (Corporeau et al., 2014). Additionally, metabolic changes in the oyster were detected (Tamayo et al., 2014). A protocol for experimental infection (Paul-Pont et al., 2015) will allow further characterisation.

Research was also focused on the oyster immune response to OsHV1 and the search for oyster resistance associated genes. The analysis of oyster transcriptome across mortality episodes in the field, using microarray approach (Jouaux et al., 2013), and after experimental challenge with OsHV1- μ var, through Illumina RNA sequencing (He et al., 2015), showed oyster exhibit a strong and complex anti-viral response. Two MyD88 genes of the Pacific oyster involved in the anti-viral immune response were characterised (Du et al., 2013) and candidate genes to be used in marker-assisted selection for resistance were identified (Normand et al. 2014b). Induction of

oyster anti-viral response by challenging them with double strand RNA was demonstrated, involving TLR, MyD88, IRF, PKR genes and protecting oysters from OsHV1- μ var but not from *Vibrio splendidus* (Green and Montagnani, 2013); the anti-viral induction is influenced by temperature and host age (Green et al., 2014a) and is absent in a second challenge with double stranded RNA (Green et al., 2014b). Anti-herpesvirus activity of cavortin, the major Pacific oyster plasma protein, was demonstrated (Green et al., 2014c).

Host factors, especially size but also age, genotype (genetic selection) and ploidy (triploid/diploid) were shown to influence oyster susceptibility to OsHV1- μ var (Degremont, 2013); the percentage of somatic aneuploidy cells may be associated with oyster susceptibility (Batista et al., 2014). Regarding the influence of environmental factors on disease dynamics, seawater temperature above 16 °C favours disease progression and oyster mortality (Degremont, 2013; Petton et al., 2013, 2015; Renault et al., 2014b); seawater temperature also influences how long the virus keeps infective in the water (Martenot et al., 2015). Availability of good quality food (diatoms) decreases mortality risk (Pernet et al., 2014a), while the dinoflagellate *Alexandrium catenella* affects OsHV1-oyster interaction and reduces infection prevalence (Lassudrie et al., 2015). Field studies during OsHV1- μ var outbreaks associated with Pacific oyster mortality were performed in Italy (Domeneghetti et al., 2014), New Zealand (Keeling et al., 2014) and Australia (Paul-Pont et al., 2014), taking the chance to evaluate risk factors in Ireland (Clegg et al., 2014) and France (Pernet et al., 2014b).

Husbandry and management procedures were tested to minimise problems due to this disease. Bearing in mind all those host and environmental risk factors, mitigation strategies can be designed (Petton et al., 2015). Oyster mortality at nursery facilities can be prevented by treating pumped water, namely aging water for 48 h and filtration through 5 μ m, which supports the hypothesis that OsHV1 is carried on particles (Whittington et al., 2015a). Elevating growing height in intertidal oyster culture areas affected by this disease, in Woolloomare Bay, Australia, was effective for adult oysters but not for spat (Whittington et al., 2015b). Selective breeding for resistance appears as the most promising strategy (reviewed by Degremont et al., 2015a); oyster lines that had been selected for resistance against summer mortality (with a family-based design) were less susceptible to OsHV1- μ var than non-selected oysters (Degremont, 2013). Furthermore, successful results were attained through classic mass selection: 69% survival of the 4th generation selected oysters vs. 7% of non-selected ones (Degremont et al., 2015b). The heritability for oyster mortality ranged from 0.49 to 0.60 (Degremont et al., 2015c), which supports the potential of the selective-breeding strategy.

Bacteria

Most articles on bacteria and bivalve health were focused on particular species or groups although a few of them involved wide taxonomic spectrum. The search for probiotic bacteria that can be used for bivalve mollusc rearing has continued (Newaj-Fyzul et al., 2014; Escamilla-Montes et al., 2015). Bacteriological issues associated with bivalve hatcheries in Chile were reviewed by de la Fuente et al. (2015). The effects of temperature, temperature stress and challenge with a virulent *Vibrio* sp. strain on the microbiota of

the Pacific oyster *C. gigas* haemolymph were addressed analysing the microbioma (Lokmer and Wegner, 2015). Changes in bacterial microbiota were characterised through culture process of oysters *Crassostrea corteziensis*, *C. gigas* and *C. sikamea* (Trabal Fernández et al. 2014).

Regarding vibrios, articles describing new *Vibrio* species isolated from bivalve molluscs were published (González Castillo et al., 2014; Lasa et al. 2014; Prado et al., 2014b; Romalde et al., 2014; González Castillo et al., 2015). Hatchery larval mortality of scallops *Argopecten purpuratus* (Rojas et al., 2015) and of razor clams *Solen marginatus* (Prado et al., 2014a) caused by *Vibrio splendidus*-like strains, of European oysters *O. edulis* and Manila clams *Ruditapes philippinarum* associated with *Vibrio tubiashii europaeensis* (Prado et al., 2015) and of Pacific oysters *C. gigas* caused by *Vibrio coralliilyticus* and *V. tubiashii* (Travers et al., 2014; Richards et al., 2015) were reported. The involvement of *Vibrio* spp. in *C. gigas* summer mortality events, in naturalised populations (Thieltges et al., 2013; Wendling and Wegner, 2013) and culture areas (Barbosa Solomieu et al., 2015), was addressed. The invasive capacity of *Crassostrea gigas* in Europe was found to be linked with rapid evolution of resistance to local *Vibrio* spp. (Wendling and Wegner, 2015). The dynamics and pathogenicity of *Vibrio* communities in the haemolymph of *C. gigas* (Wendling et al., 2014) and those of *V. splendidus* and *V. aestuarianus* isolates in mussels (Romero et al., 2014) were studied. Regarding diagnosis, real-time PCR assays were designed for *V. coralliilyticus* (Wilson et al., 2013) and *V. aestuarianus* (McCleary and Henshilwood, 2015). Vibrios' extracellular products and virulence factors were also studied, namely in *V. tapetis* and *V. aestuarianus* (Madec et al., 2014)

and *V. tubiashii* (Mersni-Achour et al., 2014, 2015). Brown ring disease of Manila clams *R. philippinarum* was also subject of study, in particular the population structure, evolution and taxonomy of *Vibrio tapetis* (Balboa and Romalde, 2013; Balboa et al., 2014) and disease dynamics (Paillard et al., 2014).

Mollusc nocardiosis seemed to spread in Europe because *Nocardia crassostreae* was detected in oysters *O. edulis* and mussels *M. galloprovincialis* from Italy (Carella et al., 2014). A real-time PCR diagnostic assay was designed for *N. crassostreae* (Carrasco et al., 2013a).

Protista

Perkinsosis, bonamiosis, marteiliosis, and mikrocystosis are protistan diseases of bivalve molluscs that captured much attention in the reviewed period, but also QPX and other pathogenic protists were the subject of research. Especially relevant for fighting bivalve diseases, selective breeding programmes continue providing promising, even successful results, such as the cases of the infection of Sydney rock oyster *Saccostrea cucullata* with *Marteilia sydneyi* (Dove et al., 2013a, b), the infection of the European oyster *O. edulis* with *Bonamia ostreae* (Lynch et al., 2014), and the infections of the American oyster *C. virginica* with *Perkinsus marinus* and *Haplosporidium nelsoni* (Frank-Lawale et al., 2014). Furthermore, identification of molecular markers of resistance (Raftos et al., 2014; Nikapitiya et al., 2014) should contribute to improve those programmes.

Marteiliosis has reappeared in the bivalve disease scene due to mass mortalities of cockles *Cerastoderma edule* caused by the new species *Marteilia cochillia* (Carrasco et al., 2013b; Villal-

ba et al., 2014) and the detection of parasites of the genus *Marteilia* in Asia and America, namely the new species *M. granula* infecting the Manila clam *R. philippinarum* in Japan (Itoh et al., 2014) and PCR positive cases for *M. refringens* in oysters *C. gigas* and *C. corteziensis* in Mexico (Grijalva-Chon et al., 2015). New studies on the disease dynamics and parasite stages out of mollusc hosts, involving mussels in the case of France (Boyer et al., 2013; Arzul et al., 2014) and oysters in Australia (Adlard and Nolan, 2015), with the copepod *Paracartia grani* and the polychaete *Nephtys australiensis* as putative intermediate hosts, respectively, have contributed to a better knowledge of marteiliosis.

Regarding perkinsosis, a comprehensive review of host-pathogen interaction stands out (Soudant et al., 2013); within this context, putative virulence genes of *Perkinsus marinus* were shown to be modulated by oyster mucous (Pales-Espinosa et al., 2014). Experimental challenge of Manila clams *R. philippinarum* with *Perkinsus olseni* (Waki and Yoshinaga, 2013) and mass mortality of Manila clams in Italy caused by this parasite (Pretto et al., 2014) highlighted its pathogenicity. The population genetics of *P. marinus* was elucidated (Thompson et al., 2014a, b), and proteomic approaches were used to analyse both inter- and intra-species parasite variability (Fernández-Boo et al., 2014, 2015). Various surveys have shown *Perkinsus* spp. in new places and new hosts in Brazil (da Silva et al., 2014; Ferreira et al., 2015; Queiroga et al., 2015), western Mediterranean area (Carrasco et al., 2014; Valencia et al., 2014; Ramilo et al., 2015) and Australia (Dang et al., 2015).

As mentioned above, recent research on bonamiosis and mikrocytosis was thoroughly re-

viewed in a special issue of the journal *Diseases of Aquatic Organisms*. The scope of mikrocytids broadened as divergent lines were identified and diverse invertebrate hosts were found; in particular, the aetiological agents of two emerging diseases of oysters *C. gigas* and crabs *Cancer pagurus* from the United Kingdom were found to be two divergent new mikrocytid species, *Mikrocytos mimicus* and *Paramikrocytos canceri*, respectively (Hartikainen et al., 2014a). Additionally, *Mikrocytos mackini* was found in Pacific oysters *C. gigas* of Washington state, at lower prevalence and severity than previously reported from British Columbia, but it was not in native oysters *Ostrea lurida* (Elston et al., 2015). A real-time PCR diagnostic assay was designed for *M. mackini* (Polinski et al., 2015).

Regarding the infection of the hard clam *Mercentaria mercenaria* with QPX, the genome of this parasite was partially characterised and the influence of temperature in its gene expression was assessed (Garcia-Vedrenne et al., 2013).

A group of protists rarely mentioned in the literature of bivalve diseases, Amoebozoa, entered the stage with the detection of a parasitic amoeba in the female gonads of *Crassostrea gasar* from Brazil (Sühnel et al., 2014).

Pathogen DNA in environmental samples

Searching for pathogen DNA in environmental samples allowed detecting pathogen prints in remote disease-free locations as well as disclosing unsuspected pathogen diversity (new lineages, new branches in phylogenetic trees). That was the case of mikrocytids (Hartikainen et al., 2014a), haplosporidians (Hartikainen et al., 2014b) and perkinsids (Chambouvet et

al., 2014, 2015). This approach will be useful to get knowledge on many aspects of pathogens affecting bivalves, such as pathogen life cycle, phylogeny, geographic range, sanitary control and management and factors influencing disease dynamics (reviewed by Bass et al., 2015). New information on technical procedures was published (Bonilla et al., 2015; Schultz and Lance, 2015)

Cancer

The aetiology of disseminated neoplasia (leukaemia-like cancer) in bivalves has been a controversial issue for many years. Disease transmissibility (infectious character), occasional (questioned) experimental transmission with ultra-filtrated, homogenised tissues from affected molluscs, sporadic observation of viral particles in neoplastic cells, and high levels of reverse transcriptase activity in affected individuals of various bivalve species have been arguments to support retroviral aetiology (reviewed by Carballal et al., 2015). Two impacting contributions have changed the scope, a novel retrotransposon, called *Steamer*, was characterised and its DNA copy number per genome was found at enormously high levels in neoplastic cells of the softshell clam *Mya arenaria* (Arriagada et al., 2014), which explains the reverse transcriptase activity in affected individuals. Additionally, using three types of DNA markers (sequences of the *Steamer* integration sites, SNPs in mitochondrial genes and microsatellites in nuclear DNA), the genotype of neoplastic cells was found to be different from that of normal cells of the host clams *M. arenaria*, whereas the neoplastic cells of clams from dispersed locations in Canada and north eastern USA all have nearly identical genotypes; these results indicate that disseminated neopla-

sia is spreading between clams *M. arenaria* as a clonal transmissible cell derived from a single original clam (Metzger et al., 2015).

Other items

Unfortunately, articles devoted to many other items, such as environmental stress, climate change, health condition surveys, health management, bibliometry, and other group of pathogens (algae, metazoans, etc), have not been covered to avoid overpassing the allocated space.

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