Diseases and Parasites of Bivalves

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Molluscs are an important source of food and form a major fishery around the world, contributing significantly to the coastal economy. Molluscan aquaculture has been developing rapidly in recent decades, reaching 14 million t in 2006, accounting for approximately 27% of the total world aquaculture production (FAO 2009). One of the greatest advantages of cultured molluscs, is that these species are filter feeders and do not require any external feed inputs, thereby minimizing the major input costs as well as the environmental impacts. Moreover, by removing the suspended organic matter from the waters, molluscan farming through continuous filtration may also help to reduce eutrophication of estuaries, bays and near-shore waters.

Mussels are bivalve molluscs found attached to hard substratum in marine/brackish waters and are farmed worldwide. The two commercially important species of mussels in India are the Green mussel *Perna viridis* and the Brown mussel *Perna indica*, of which *P. viridis* enjoys a wider distribution. In India, initially the mussel resources supported only traditional sustenance fishing from the existing natural beds. But later, technology for farming the green mussel, *P. viridis*, was developed by the Central Marine Fisheries Research Institute. The present estimate of farmed mussel production from India indicates that it has risen to one among the top 10 mussel farming countries in Asia with latest figures of about 20,000 tonnes (CMFRI Annual Report, 2009-10) of which the major contribution is from the west coast of India, mainly from the state of Kerala. During the past decade, commercial mussel culture activity in the open ocean and estuaries along the south west coast of India, especially in Kerala and Karnataka has picked up in a big way. The growth of mussel farming in Kerala in recent years has been phenomenal, with the number of estuarine mussel farms increasing exponentially in Kasargode, Kozhikode, and Malappuram districts. Since feed inputs are not required, the profitability of the technology has attracted many entrepreneurs as well as women self-help groups towards mussel culture and the trend will continue during the years to come.

Diseases are the most important limiting factors for any aquaculture venture. Usually, the risks involved, increase with the intensification of the culture activities. As in the case of other aquacultured species, diseases have destroyed commercial shellfish culture many times world over. Parasitic

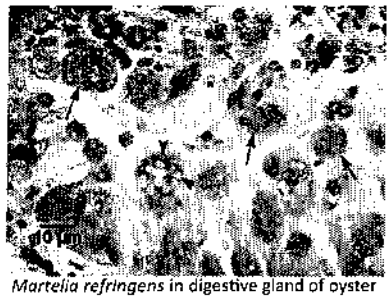
protozoa (e.g. *Bonamia ostrea and Martelia refringens)* have caused major problems for the oyster industry in Europe, wiping out the entire populations of European oysters from many geographical regions (Villalba et al., 2004). Over the past few decades, world mollusc production has been adversely affected by a number of diseases and International Disease Commission (OIE) has listed the diseases caused by *Bonamia ostreae, B. exitiosa, Haplosporidium nelsoni, Marteilia refringens, Mikrocytos mackini, Perkinsus marinus, P. olseni, Xenohaliotis californiensis* and Abalone viral mortality as the most important diseases in mollusks and interestingly, except *X. californiensis,* all are caused by protozoan parasites.

Perkinsosis or Dermo disease caused by the protozoan parasite, *Perkinsus marinus* in *Crassostrea virginica* and various other species of bivalves in tropical and subtropical waters worldwide and can cause mortalities up to 95%. The infection is usually systemic, proliferation of parasites causes disruption of connective and epithelial tissues leading to abscesses formation and finally mortality. *P. marinus* has also been reported from the mussel, *Mytilus edulis. P. olseni*, another OIE listed pathogen is known to have a wide host range including mussels and enjoys a worldwide distribution. It was believed that the Indian bivalves were totally free from all the OIE listed pathogens (NACA, 2009), but the report of *P. olseni* infection in the pearl oyster populations along the southeast coast of India (Sanil et al, 2010) proved otherwise indicating the possibility of a disease outbreak due to protozoan parasites.

Hypnospores of *Perkinsus olseni* in *Pinctada fucata*

Trophozoites in tissues

Marteiliosis (Digestive gland disease), though not reported from Asia-pacific region so far, is a serious disease infecting bivalves in Europe. The parasite, Marteilia *refringens*, destroys the digestive gland, the metabolic center of the animal, leading to severe mortalities. *M. refringens* is known to infect mussels also. *Marteilia sydneyi* infections in *Crassostrea commercialis* are capable of causing heavy mortalities in Australia.



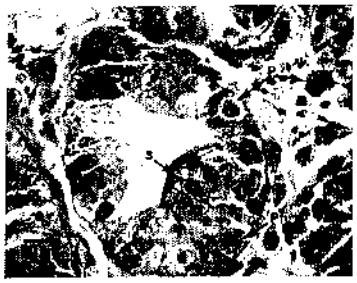
Martelia refringens in digestive gland of oyster

Marteilioides chungmuensis in *Crassostrea gigas*

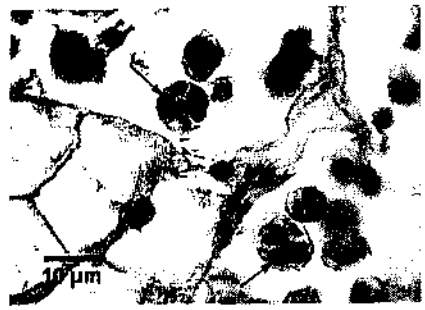
Marteilioides infection of gills caused by Marteilioides *branchialis* affects *Saccostrea commercialis* in Australia. Severe gill lesions can cause significant econonmic losses. *Marteilioides chungmuensis* infects oocytes thereby reducing the reproductive output in *C. gigas* in Japan and Korea

Mikrocytosis or microcell disease caused by parasites of the genus Microcytos. *Microcytos mackini* infects the vesicular connective tissue cells leading to abscess formation and tissue necrosis in the mantle of Pacific Oysters. *M. roughleyi* causes systemic infection of haemocytes leading to gills, connective tissues, gonads and digestive tract in Australian Oysters.

Mikrocytos mackini in *Crassostrea gigas*



Haplosporidian nelsoni in the digestive gland of *C. virginica*



Bonamia ostreae in haemocytes

Bonamiosis or **Haemocell disease** is one of the important protozoan infections affecting different species of *Crassostrea* and *Ostrea* and is caused by *Bonamia ostreae* and *Bonamia* sp. The parasites cause extensive destruction of haemocytes leading to lesions/perforated ulcers on gills and mantle resulting in large scale mortalities in Europe and Australia.

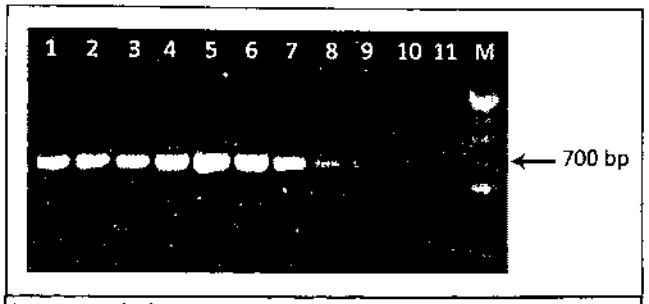
Haplosporidiosis is an important disease infecting *C. virginica* from the Atlantic coast. *Haplosporidium nelsoni* causes MSX disease and *H. coastale* causes SSO disease. *H. nelsoni* was also reported from *C. gigas* from Europe and South East Asian countries. Parasitic forms similar to *H. coastale* were also reported from pearl Oysters in Australia. *H. nelsoni* occurs extracellularly in the connective tissues and digestive gland epithelia and is associated with a brown discolouration of gill and mantle tissues, leading to the gill and digestive gland dysfunction causing mortalities. *H. coastale* causes severe seasonal mortalities in oysters. Haplosporidium infections are reported from mussels also.

Xenohaliotis californiensis is an intracellular prokaryote under the family Rickettsiaceae, occurring in the epithelium of the intestinal tract of Abalones. It causes the disease known as "withering syndrome" of abalones, causing mortality. Infected abalones are discoloured and weakened and can be detached easily from the substrate. They do not attempt to right themselves when turned upside down.

Diagnosis: Since most of the pathogens of mollusca are protozoans, diagnosis is difficult. The clinical signs and symptoms need not be apparent always. Depending up on the nature of the host-parasite interactions, the expression of signs & symptoms vary in different hosts and hence the

absence of symptoms does not indicate the absence of pathogens. The sensitivities of the various tests also vary greatly especially in the case of latent infections and carrier/reservoir hosts.

Agarose gel electrophoresis of the amplified products of the PCR using *Perkinsus* genus specific ITS 85 & ITS 750 primers



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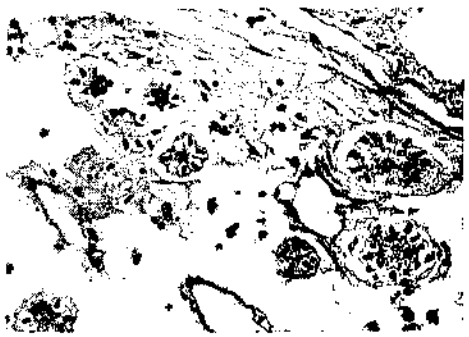
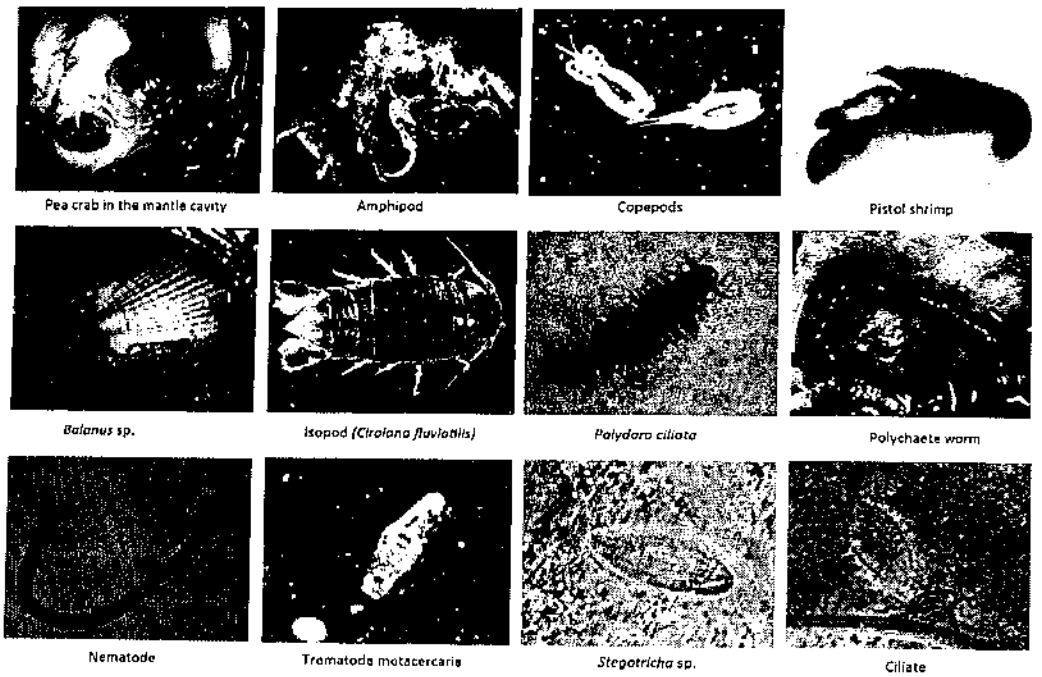
In the case of *Perkinsus* infection, RFTM assay, the routine test to diagnose Perkinsus infections cannot be considered as a confirmatory test. Low infections will go undetected as sometimes the presence of *Pseudo perkinsus*, a dinoflagellate, gives false positive results. Similarly histology preparations also will not give a clear indication of the disease in case of low infections. But the use of DNA based diagnostic techniques has revolutionized the arena of diagnosis. Use of PCR & nested PCR techniques can detect very low level of infections with pathogens. These techniques have very high levels of specificity (up to the level of sub species & strains depending up on the primers used) and sensitivity and are now widely used in the diagnosis of almost all other diseases with great success.



Nematopsis cysts in the gill tisues

In the Indian context, molluscan aquaculture has gained importance only recently and hence information on the various diseases affecting them are totally lacking. The limited information available on the pathogens/parasites of bivalves in India is limited to the reports of Copepod parasites belonging to different genera, infestations by *Myotomus ostrearum* in *Crassostrea gryphoides*, *Bucephalopsis haimeanus* in *C. madrasensis* and *Tylocephalum* sp. in *Pinctada fucata* and disease conditions associated with bacterial infection in *P. fucata* and *C. madrasensis*. Preliminary studies conducted at CMFRI have indicated the prevalence of various parasitic infections. Infections with *Anicistrocoma* like ciliates and *Steigotricha* sp. infecting the digestive tubules, *Ricketsia* like organisms, ectocommensal ciliates, *Trichodina* sp. turbellarians, copepodes, nematodes, trematode metacercaria, *Balanus* sp., polychaete worms, amphipods, isopods, *Modiolus sp.*, pea crabs, pistol shrimp, *Polydora* sp. and *Pinnotherus* sp. were also recorded. Infection with the developmental stages of *Nematopsis* sp. was also been observed in *P.viridis*.

The information on OIE listed pathogens of molluscs in India is limited to the only report on the occurrence of *Perkinsus olseni* in the pearl oyster, *Pinctada fucata* from the southeast coast of



India. Subsequently *Perkinsus olseni* was observed in many other bivalve species from Indian waters. But information on various other OIE listed protozoan diseases, which are widely studied and reported abroad and of great concern worldwide, is completely lacking from this part of the continent. Many mollusks are known to carry pathogens like *E. coli* and noroviruses which are hazardous to human health and hence there is hesitation among the importing countries to accept products free of these pathogens.

Aquaculture and its intensification always promote the spread of diseases from one geographic area to another. Movement of aquacultured stocks/larvae is the main factor responsible for the geographical dispersion or spread of pathogens/diseases to uncontrollable levels. The lessons learnt from the rise and fall of the shrimp culture in India due to the White Spot Syndrome Virus (WSSV) pandemic should be an eye opener for us. It is a typical example where the sudden appearance of diseases in epizootic proportions wiped out the sustainability and profitability of the entire industry within a short span of 3 years during 1994 to 1997. As there was no health management plan in place, preventive strategies could not be employed to save the industry. The significant increase in world trade, transport and tourism has contributed to the spread of many pathogens worldwide. Intentional (aquaculture) or unintentional introductions of species into new geographical areas have also greatly increased the threat of associated pathogen spread.

As the culture activities spread to more and more areas, the spat collected from the wild will not be sufficient and will have to depend on spat from the hatcheries. In the production of healthy and disease free larvae, the role of hatcheries is very important for the large scale production/ propagation

of the candidate species for aquaculture and mussel culture is not an exception. If the brood stock used for hatchery propagation are carriers/reservoirs of diseases, the result will be disastrous with the large scale dispersion of the pathogens throughout the farming area and a wide geographical region may be affected as happened in the case of the shrimp farming industry. This should not be allowed to be repeated in the case of mussel culture.

Control strategies: Prevention is always better that cure. Once an epizootic occurs in an aquatic habitat, the chances of eradication and control are limited. In fact, there are no examples, to date, of any molluscan disease agent being actively eradicated from an open-water system. Since most of these diseases do not have any treatment, taking preventive measures is the only option. This is worth considering while conducting risk-benefit analyses for new species, stocks, growing techniques or habitats. Another factor which is worth considering is the changes in the climate and variations in the global temperature which will definitely reflect in the altered occurrence/pattern/virulance of various diseases/epizootics in years to come. Though trade, transport and tourism have increased the pace of spread in aquatic pathogens, still many isolated populations/ beds may be free from infections. Such populations which can serve as disease free stocks need to be profiled for the bivalve propagation and farming. Approaches proposed include early detection of the disease, possible identification of disease free stocks, restricted transfers of stocks, selective breeding for disease-resistant stocks, and treatment. Unfortunately, none of these approaches have fully succeeded, in part because of the lack of information on the fundamental aspects of the biology of both parasites and hosts. It should be assured that spat for culture should be sourced from disease free regions to avoid lateral spread of the existing diseases. Sufficient care should be taken to produce spat from disease free brood stock or else the result will a disastrous spread of the diseases into the entire culture systems under wide geographic regions. This is possible only by thoroughly screening the animals for the various diseases.

As the mussel farming in the country is in the initial phase of expansion, we have an opportunity to put a scientific health management strategy in place, without waiting for a disaster in the form of a disease outbreak to happen. In this context, it is highly essential to specifically identify and document the various OIE listed pathogens/parasites present in the mussel populations, both wild and already farmed in various geographical areas so that strategies can be drawn to prevent/ control their spread at later stages. A proactive strategy for prevention of diseases is the only viable alternative in aquaculture disease management for which the basic pathogen profile of the species is very important.