

DISEASES AND PATHOLOGY OF AQUATIC ORGANISMS

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Summary

Disease is a natural feature of life. Relatively little research has been done on the

diseases of aquatic animals in the wild, and this article focuses mainly on cultured aquatic animals, with particular reference to the major groups cultured worldwide: the molluscs, crustaceans and fish. Whether a pathogen will cause disease or not, depends not only on the virulence of the pathogen, but also on the immune defences of the animal it attacks. Both sides of this relationship are influenced by environmental conditions and stressors, which can compromise the immune-competency and imbalance the host-pathogen interactions, causing disease. The presently known immune mechanisms of molluscs, crustaceans and fish are outlined. Approaches to prevention, diagnosis, and control of disease are presented, together with the technologies being developed and some of the needs for the future.

1. Introduction

This article focuses mainly on disease and pathology in cultured aquatic animals because while diseases occur in the wild, they are poorly documented, and are not easily controlled. Non-commercial species have received little attention. Commercial fishing tends merely to harvest and select healthy fish in good condition. Unless fish stocks undergo serious decline (more usually due to over-exploitation), there is little concern for diseases, which may occur.

One aspect of the pathology of wild aquatic species has received international attention over the past 25 years. It results from a considerable volume of circumstantial evidence, from many published studies, suggesting that certain diseases of fish and shellfish occur at higher prevalence where pollution occurs and the environment is degraded. Toxic chemicals may directly cause tissue damage and disease: e.g. carcinogens can cause tumours; or pollution may indirectly lead to disease by causing immune suppression. In studies worldwide, encouraged by the International Council for the Exploration of the Sea (ICES), national authorities have carried out baseline surveys of disease prevalence in coastal waters, and also surveyed areas of known pollution, such as some of the former marine dumping sites. Such studies have shown that elevated prevalence of ulcers and fin erosion may be observed in different species of fish, including cod, eels, and flatfish, where waters have a high organic content and are eutrophic. Skeletal abnormalities, such as spinal curvature and gill-raker deformities are associated with heavy metal, and with organochlorine contamination. Fish tumors of liver (hepatomas) and of skin (papillomas and carcinomas) have been associated with polluted waters containing pre-carcinogens or carcinogens. Laboratory studies to verify cause and effect are being pursued. In turn, the use of pathobiological monitoring of marine waters, using fish populations as biological monitors, is being refined.

More than 100 species of aquatic animal are now being cultivated and farmed, including amphibians, reptiles, sponges and echinoderms, but among the cultured species we focus on molluscs, crustaceans and fish, because these are the major cultured groups.

2. The Significance of Disease in Cultured Species

Parasites and pathology are part of the natural biology and functioning of ecosystems. Pathogens and parasites live at the expense of the host, divert host energy from growth and reproduction into disease resistance, and if the host resistance is overcome, may cause mortality. Whether a parasite or pathogen causes disease or not, depends on the

host genetics and its immune-competency. Thus parasites and pathogens, in the wild, can and may reduce host densities, but this reduction is part of the balance between reproduction and mortality; and while host populations fluctuate, they are maintained. In an undisturbed ecosystem it appears generally that host-pathogen interactions are balanced.

In contrast, fish and shellfish in aquaculture are removed from their natural niches, and while the total set of conditions under which they are cultured, replicates as far as is feasible the conditions in the wild, this is limited by practical and commercial constraints. Any change in living conditions may cause stress, and predispose the animal to disease. Disease requires not just a suitable host and a pathogen, but also a stressful environment to unbalance the host-pathogen relationship, and the host immune system to be overcome, at least temporarily, by a pathogenic factor. Environmental stress may be biological: for example due to overstocking; or chemical: such as due to excessive ammonia or other toxic pollutant; or physical: for example excessive temperature; or procedural: such as handling, or disease treatment. In aquaculture facilities therefore disease can be a significant factor dramatically decreasing animal production and commercial incomes.

2.1 The Diseases and Pathologies of Cultured Species

As aquaculture is being developed worldwide, the species being cultured, the geographic spread of the industry and the variety of environmental conditions in which culture is pursued, have all diversified at an increasing pace, particularly in the past 50 to 60 years. The species most widely cultivated include oysters, mussels, clams, marine shrimps, freshwater prawns, salmonid fish, cyprinids, tilapias, catfishes, snakeheads, milkfish, eels, cod, sea bass, sea bream, groupers, and redfish. The diseases of one group are usually different to those of other groups, and therefore need to be considered species by species. However, the causes include micro parasites (bacteria, fungi, viruses, and protozoan parasites), macro parasites such as ecto parasitic lice, or endo parasitic nematode worms, and non-infectious agents, such dietary or environmental chemical agents, which may cause pathology including neoplasia.

Transfers of cultured species from one environment to another, where they may be exposed to different environmental conditions, and particularly, where the transferred species are naïve to the parasites and pathogens occurring in the new areas, render them more prone to disease outbreaks. In addition, movement of cultured species into new areas can result in the introduction of their pathogens to the areas, where the resident hosts may be naïve and therefore very susceptible.

2.2 Diseases of Aquatic Molluscs

Shellfish farms have suffered from dramatic diseases due to pathogens. For instance, in 1860, a shortage of native flat oyster seeds, *Ostrea edulis*, prompted French farmers to import cupped oysters, *Crassostrea angulata*, from Portugal. In the 1910s, both species were equally produced until massive mortality struck *O. edulis*, favouring an increase in the culture of *C. angulata*. Impacted by a gill disease in the early seventies, *C. angulata* almost disappeared from the French coasts. The Pacific oyster, *Crassostrea gigas*, was

introduced at that time to restore the oyster industry. Subsequently two diseases, caused by the protozoans *Marteilia refringens* and *Bonamia ostreae*, spread in the late seventies and dramatically reduced the production of *O. edulis* all around the world. Now, *C. gigas* suffers from several diseases, limiting the natural recruitment. In fact, a large number of pathogens were identified in the different shellfish species, including bacteria, viruses, protozoa, and microsporidia.

Reports on pathogen-associated mortality of farmed invertebrates worldwide are numerous. For example, mortality of *C. gigas* was reported in Japan, California, France, Western Canada, New-Zealand and the USA. *O. edulis* suffered mortalities in France, caused by the protozoan *B. ostreae* and herpes-like virus. Another protozoan, *Bonamia* sp. devastated the populations of *Tiostrea chilensis* in New Zealand. *Crassostrea virginica* from Eastern USA suffer from several protozoa, including, *Perkinsus marinus* and a non-identified one responsible for juvenile-oyster-disease (JOD). The Sydney rock oyster, *Saccostrea commercialis*, was reported as infected by protozoan, *Marteilia sydneyi* in Australia; the giant clam, *Tridacna gigas*, by several bacteria belonging to the *Vibrio* genus also in Australia; the venerid clam, *Tapes decussatus*, by the protozoan *Perkinsus atlanticus* in Eastern Spain; the Manila clam *Ruditapes philippinarum*, by the bacterium *Vibrio tapetis* in France, Spain and Ireland; scallops by prokaryotic rickettsia in Northeastern USA and France, and by bacteria in North-western Spain; and mytilid mussels in Laguna Veneta, Italy, and by protozoan *M. refringens* in North-western Spain.

Another type of disease, which can occur in epizootics in bivalve molluscs, is sarcoma or hemic neoplasia. This has been recorded in oysters, cockles, mussels, and clams, of different species in many parts of the world, with prevalence up to 90%, and causing significant mortality. An unusual feature of the sarcomas is the polyploidy shown by the neoplastic cells. The aetiology is as yet unproven, but recent studies have shown that the sarcomas in the cockle are not only transplantable using cell transplants, but are also transmissible using cell-free ultra filtrate of neoplastic cell homogenates; this suggests that the aetiological agent may be a virus.

As techniques have developed, more and more viruses are implicated in diseases. For example, the Ostrei herpes virus 1 (OsHV-1) represents a new member of the third major class of herpes viruses associated with sporadic mortality in the Pacific oyster *C. gigas*. Its genome organization is similar to that of herpes simplex virus and human cytomegalovirus. Similarly, a herpes-like virus infects the larvae of Manila clam *Ruditapes philippinarum*, probably through induced apoptosis. In addition, viruses are probably involved in the so-called gill disease of the mussel *Mytilus trossulus* from Southern Baltic Sea.

2.3 Major Diseases of Crustaceans

Crayfish plague has occurred in many parts of the world. It is caused by the fungal micro parasite *Aphanomyces astaci*. It is believed to have originated in North America, where the crayfish (*Procambarus clarkii*, *Orconectes limosus*, *Pacifastacus leniusculus*) are resistant, but can act as carriers. In other countries the pathogen has been known to cause 100% mortality (in which species of crayfish?).

Viruses are the most common biological agents in the sea, numbering 10 billion per litre and viral infections are common in crustaceans. For instance, penaeid shrimp might be infected by more than 20 different viruses, some of them resulting in dramatic epizootics with considerable economical loses.

Infectious Hypodermal and Haematopoietic Necrosis Virus (IHHNV) is a single-stranded DNA parvovirus of 22 nm icosahedral in shape infecting shrimps on the Atlantic and Pacific coasts, in Asia and the Middle East. It has a wide host range, but is particularly virulent for the larvae and juveniles of *Penaeus monodon*, *L. vannamei*, and *L. stylirostris*. Its geographic distribution has been widening over recent years.

Yellow head disease (YHD) is a destructive viral infection in the giant tiger prawn *P. monodon*, causing mass mortality in cultured shrimp at grow-out stage. The first epizootic was recorded in Thailand in 1980. The virus is a cytoplasmic, rod shaped, enveloped particles of 175 nm in length and 50 nm in diameter, with a single-stranded RNA genome. It belongs to the new family roniviridae.

White spot syndrome is due to a large virus (WSSV), 250-300 nm in length and 150 nm in diameter, from the Nimaviridae family. WSSV was responsible for mass mortality, not only in the tiger prawn, but also other prawn species including *Marsupenaeus japonicus*, *P. penicillatus*, *Fenneropenaeus chinensis*, *F. merguiensis*, and *F. indicus*. It has the capacity to infect a wide range of aquatic crustaceans including salt, brackish and fresh water shrimp, crabs and crayfish. It has been isolated all around the world, from China, Japan, Southeast Asia, India, the Mediterranean Sea, the Middle East and both Americas. The virus is transmitted by predation on diseased individuals or via water, infecting the gills, lymphoid organ, cuticular epithelium, midgut and hepatopancreas. Mortalities occur generally within 5-10 days of infection.

White tail disease (WTD) causes a high mortality rate in the fresh water prawn *Macrobrachium rosenbergii*. Recently, the causal agent was isolated and appears to consist of a small single-stranded RNA virus of 25 nm in diameter, the *M. rosenbergii* nodavirus (*MrNV*) associated with a extra small single-stranded RNA virus-like particle of 15 nm in diameter (XSV). Strict association between the 2 particles to produce the disease has not yet been demonstrated.

Taura syndrome virus (TSV) was isolated from *Litopenaeus vannamei* during 1992 epizootics in the Taura river basin (Ecuador). It is a single-stranded RNA cytoplasmic particle of 32 nm in diameter closely-related to picornaviridae. Recently, it was accidentally introduced in Asia infecting locally produced shrimp, *Fenneropenaeus chinensis*.

2.4 Diseases of Fish

Diseases affecting fish have continued to emerge, as each new fish species is brought into culture. In salmon culture the viral diseases of Infectious Pancreatic Necrosis (IPN), Infectious Hemopoeitic Necrosis (IHN) and Viral Hemorrhagic Septicemia (VHS) are long-standing problems; likewise bacterial diseases such as Bacterial Kidney Disease (BKD), or parasitic disease such whirling disease, caused by the protozoan, *Myxobolus*

cerebralis. Spring Viremia (SVC) has been a feature of carp culture.

As culture methods have been developed for an ever-wider range of fish species, the range of diseases of concern has continued to grow. For example, the Channel Catfish Virus Disease (CCVD) clinically and economically affects catfish farming in the US. The causative herpes virus affects juveniles, causing high mortality, due to loss of osmotic balance. Survivors become symptomatic carriers. Another virulent problem causing high mortality, which emerged with the catfish industry, is Enteric Septicemia (Edwardsiellosis) caused by *Edwardsiella ictalurus*. Cultured sea bass, turbot, and groupers have been seriously affected by the nodavirus infection, Viral Nervous Necrosis (VNN), which can cause 100% mortality in juveniles.

As fish farming has extended geographically, fish species have been exposed to new pathogens; e.g. Piscirickettsiosis is a septicemia condition of salmonids, caused by a rickettsia, and resulting in mortalities up to 90%. It was first described in Coho salmon in Chile, but has since been recorded also in a range of other salmonids and in other countries. One of the most serious ecto parasitic problems in marine farming is that caused by sea lice. These copepods are to be found on all marine fish, but usually in very low numbers. In farms, the infestations can become devastating, and the host tissues are literally grazed away by the lice, causing damage, loss of osmoregulation and death. *Lepeophtheirus salmonis* is specific for salmon, but *Caligus* species have a wide host range. The sea lice problem is compounded by non-availability of effective, environmentally safe treatments.

Some diseases result from the culture methods or from diet; for example *Ichthyophonus* causes a fungal infection, which penetrates and destroys the flesh of farmed fish, which have been fed wet diet derived from fungal-infected marine trash fish. The disease renders the farmed fish un-saleable.

The number of viruses isolated from fish has grown in the last few years as fish diseases dramatically increased in aquaculture facilities. More than 50 different fish viruses have been isolated and tissue-culture adapted allowed molecular characterizations. They consisted mainly in five families: the Birnaviridae with the infectious pancreatic necrosis virus (IPNV), the Herpesviridae with the channel catfish virus (CCV), the Iridoviridae with the fish lymphocystis disease virus (FLDV), the Retroviridae with the walleye epidermal hyperplasia virus (WEHV1 and WEHV2), the salmon swim bladder sarcoma virus (SSSV) and the salmon leukemia virus (SLV), and the Rhabdoviridae with the infectious hematopoietic necrosis virus (IHNV) and viral hemorrhagic septicemia virus (VHSV). Viral diseases are untreatable and because effective vaccines for fish are not yet discovered, the only solution consisted in a great care to be exercised when moving fish or eggs from one country to another.

3. Host Pathogen Interactions

In all living creatures it is the role of the immune system to prevent pathogenic infections and maintain homeostasis. Little information is available concerning aquatic invertebrate defence capabilities.

For more than a century, phagocytosis has been recognized as an important phenomenon shared by all animals, involved not only in nutrition, but also in defence. The purpose of the activity is to neutralize and eliminate all foreign materials including inorganic particles, living organisms (pathogenic or non-pathogenic) as well as modified self-cells. In addition, vertebrates possess a very potent immune system, involving lymphocytes and immunoglobulins, capable of adapting to invading microorganisms. Invertebrates do not possess such acquired immunity. However, they possess an innate, non-adaptive immune system employing a large variety of circulating molecules: enzymes, cytolsins, anti-microbial peptides, lectins, etc. Circulating cells (hemocytes, macrophages, granulocytes, and hyalinocytes) can also mount phagocytic, cytotoxic or inflammatory responses.

When a parasite invades a host, multiple reactions occur, initiated both by the parasite in an attempt to survive and by the host to get rid of the parasite. Among the host reactions are their phagocyte capabilities, the synthesis of stress proteins and proteases, the presence of NO-synthesis activity, and the release of several anti-microbial peptides and of a range of hydrolytic enzymes including lysozyme and peroxidases.

3.1 Phagocytosis-associated Oxidative Killing

Mollusc hemocytes respond to appropriate stimuli with a burst of respiratory activity in a manner resembling the respiratory burst of mammalian phagocytes. The mechanisms involved in the intracellular killing of the phagocytised material encompass the generation of various reactive oxygen intermediates (ROIs) in the scallops *Patinopecten yessoensis* and *Pecten maximus*, the oysters *Crassostrea virginica*, *C. gigas*, and *Ostrea edulis*, the mussel *Mytilus edulis* and the clams *Mya arenaria* and *Mercenaria mercenaria*. Surprisingly, some clams, such the Manila clam *Ruditapes decussatus*, do not possess detectable ROIs coupled with phagocytosis.

According to some authors, the protozoan parasite, *Perkinsus marinus*, may either increase or suppress the production of ROIs in *C. virginica*. In addition, some pathogenic bacteria do not stimulate *C. virginica* hemocyte ROI production. Even if the molecular mechanism is not elucidated, it is supposed that at least some pathogens know the way to penetrate and develop in the host without triggering ROIs production.

3.2 Peroxynitrite Anions

In vertebrates, nitric oxide (NO) is an important molecule involved in normal physiological functions such as the regulation of vascular tone, cellular signalling in the brain, and elimination of pathogens in a non-specific immune response. *In vitro* bacterial clumping in *Mytilus edulis* hemolymph, in the presence or absence of inhibitory drugs or following stimulation by bacterial lipopolysaccharide extracts (LPS), revealed the release of NO by the hemocytes.

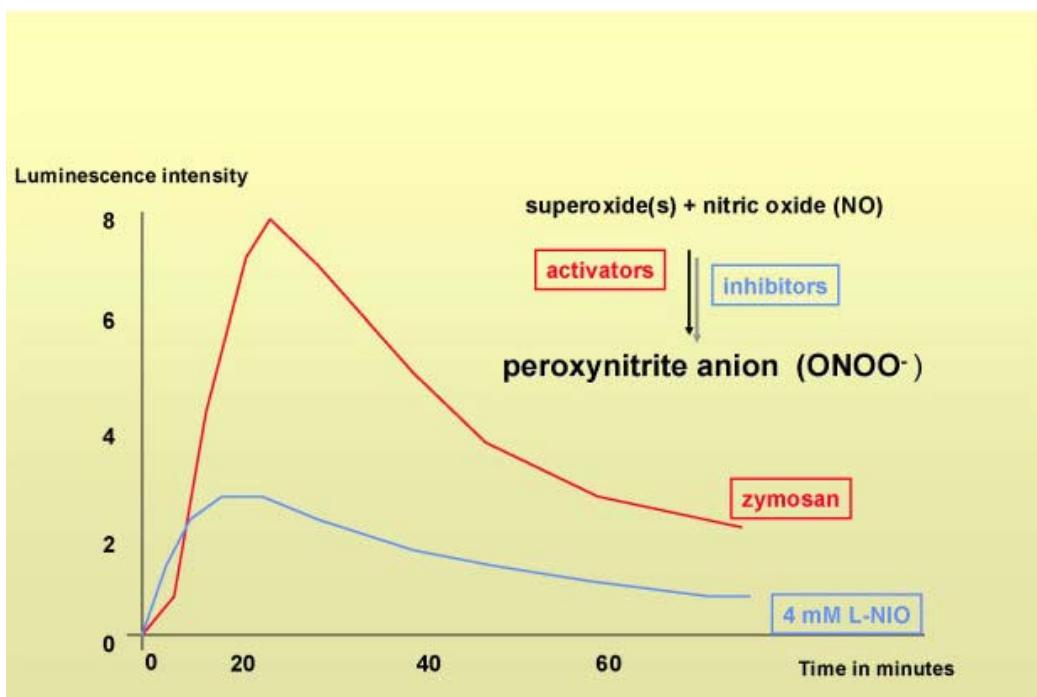


Figure 1. Phagocytosis-associated peroxynitrite in mussels. Stimulated by zymosan, the reaction is inhibited by 4mM of NIO.

NO is not toxic by itself, but in combination with super-oxide anions, synthesized during phagocytosis, it generates the peroxynitrite anion (ONOO^-), which is a highly toxic but extremely labile compound. Phagocytosis-associated peroxynitrite anion generation can be observed via chemiluminescence enhanced by bicarbonate anions. Addition of yeast membrane extracts (zymosan) increases the measured luminescence which peaks 25 minutes after contact and returns to baseline after one hour, as observed in the mussel, *Mytilus galloprovincialis*. (Figure 1) Partial inhibition by NO-synthesis inhibitors (NIO) demonstrated the involvement of other oxidative anions such as peroxynitrites, the only spontaneously luminescent NO derivatives.

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Biographical Sketches

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Philippe Roch was born 1949 in the French Basque country. Education at the Bordeaux University (France) with Thèse de Spécialité in Animal Biology (1973) and Thèse d'Etat és-Sciences in Biology (1980). Post-doctoral visitor at the UCLA Comparative Immunology lab of Professor Edwin L. Cooper (1981-1982). Permanent position at the Centre National de la Recherche Scientifique (CNRS) France. Found in 1992 the DRIM lab, a joint research unit between IFREMER, CNRS and the University of

Montpellier 2. Director of DRIM from 1993 to 2003. At present, leader of the Pathogens and Immunity lab at the Ecosystèmes Lagunaires joint research unit (CNRS-Univ Montpellier 2). Member of several scientific management councils. Teacher of advanced studies in several French and Italian Universities. Distinctions: CNRS bronze medal (1980), NATO fellowship (1981–1982) First Behring-Metchnikoff Immunology prize (1982). Lifelong member of the International Society for Developmental and Comparative Immunology, Société Française d'Immunologie, Société Zoologique de France, Fish Immunomodulators Network. Editorial Board member of Developmental and Comparative Immunology, Marine Biotechnology, Fish and Shellfish Immunology, Complementary and Alternative Medicine (*e*-CAM) and referee for numerous journals. Main scientific interests are in invertebrate immunology, principally the antimicrobial peptides.

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