Status and future of molluscan pathology in North America

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Abstract — Serious diseases have plauged the North American oyster industry for at least 75 years. These include Malpeque Bay disease, MSX disease, Perkinsiosis, nocardiosis, oyster velar virus disease and others. Some of these diseases have proven intractable since they are caused by infectious agents which have not yet been transmitted in the laboratory or which cannot yet be cultivated outside of the host animal. The increased importance of aquaculture of these species and the advent of modern molecular technologies in biology have provided both the impetus and the means to now advance the science of molluscan pathology.

The relative importance of each molluscan disease in North America and specific objectives to enhance the management of the diseases will be discussed. For example, the potential and actual application of antibody technologies and cytofluorometry to diagnose clinical stages of hemic neoplasia in Mytilus will be described. The use of molecular probes to detect the presence of infectious agents or the presence of abnormal host gene transcripts is another method which can potentially make health management more efficient.

The development of resistant strains of molluscs is an important aspect of health management which should receive more attention. Specific criteria of resistance must be identified and, if possible, correlated with in vitro measurements of functional aspects of immunity or with the presence of gene transcripts which are indicative of resistance. A priority list of recommended future research will be presented.

Serious diseases have plagued the North American oyster industry for at least 75 years. These include Malpeque Bay disease, MSX disease, Perkinsiosis, nocardiosis, oyster velar virus disease and others. Some of these diseases have proven intractable because they are caused by infectious agents which have not yet been transmitted in the laboratory or which cannot yet be cultivated outside of the host animal. The increased importance of aquaculture of molluscan species and the advent of modern molecular technologies in biology have provided both the impetus and the means to now advance the science of molluscan pathology. In the following treatment, the classic diseases affecting wild harvested populations of mollusces in North America are discussed as well as those diseases
which have been recently recognized as the intensive culture of molluscs is increasingly practised and advanced.

MALPEQUE BAY DISEASE

This is a widely known but poorly understood disease that caused severe mortalities in American oysters (*Crassostrea virginica*) in Malpeque Bay in the Canadian maritime province of Prince Edward Island starting in 1915 and continuing through the 1930s. The geographical expansion of the disease, first observed a year after substantial plantings of seed oysters imported from the United States, is considered evidence for an infectious cause of the disease. Over 90% of original stocks were reported to have succumbed to the disease. The oysters affected by the disease reportedly show visceral shrinkage, a translucent quality, reduced growth, and failure to spawn. The cause of Malpeque Bay disease has never been determined with certainty.

MSX DISEASE OF THE AMERICAN OYSTER
(*Crassostrea virginica*)

This disease is caused by *Haplosporidium nelsoni* (*Minchinia nelsoni*) referred to as multinucleate sphere unknown (MSX) (Haskin et al., 1966). The plasmodial form of the parasite invades all but the epithelial tissues of the oyster but apparently requires another host species (as yet unknown) in order to complete its life cycle. Sporulation is rarely observed in the epithelial tissues (Farley, 1965, 1967). MSX disease was first recognized in Delaware Bay in 1957. It destroyed the Delaware Bay industry with mortalities of oysters reaching 90% to 95% by 1960 (Andrews and Wood, 1967; Ford and Haskin, 1982). Resistance to the disease has occurred in some stocks of oysters subjected to continuing infection over the years (Haskin and Ford, 1982; Ford and Haskin, 1987). The disease has occurred in other locations in Atlantic North America and beginning in about 1980, a recurrence of the disease was observed in both Chesapeake and Delaware Bays, associated with a drought. Salinity and temperature are important in determining the severity of MSX disease (Ford, 1985). In general, the disease is rarely acquired below about 10 ppt (parts per thousand); salinities of about 15 ppt are required for the parasite to appear in substantial numbers in host tissues and serious mortalities occur only above about 20 ppt.

There is some indication that the disease may be limited above a salinity of 30 ppt. Diagnosis is based on histological examination or on the observation of parasites in preparations of oyster blood.

PERKINSIOSIS OF THE AMERICAN OYSTER
(*Crassostrea virginica*)

This disease is caused by *Perkinsus marinus* (*Apicomplexa*) (*Dermo-cystidium marinum, Labyrinthomyxa marina*) that infects almost all tissues
of the oyster (Mackin et al., 1950; Levine, 1978). Transmission occurs by
direct contact in water or by the gastropod vector, *Boonea impressa*, (White
et al., 1987) but is limited by the parasite’s inability to tolerate low salinities
and low temperatures (Andrews, 1966). The disease occurs during high
temperature months of the year and is more severe in highly concentrated
populations of oysters. Mortalities can reach 100 % and have been reported
to be 30-50 % in the first year and up to 95 % in the second year in oysters
introduced to an infected area. The disease does not cause serious
mortalities below salinities of 12 to 15 ppt but can persist in overwintering
oysters in salinities below 5 ppt. The disease has had a resurgence in the
last 5 years in the Chesapeake Bay. Diagnosis is facilitated by the use of
the fluid thioglycollate method which allows spores to enlarge vegetatively
in anaerobic conditions (Ray, 1966), and subsequently be manipulated to
undergo zoosporulation (Perkins and Menzel, 1966).

**SEASIDE HAPLOSPORIDIOSIS OF AMERICAN OYSTERS**
(*Crassostrea virginica*)

This disease is caused by *Haplosporidium costale* (*Minchinia costalis*).
In 1959 it was originally described as seaside organism (with the acro-
nym SSO) because of its occurrence in relatively high salinity waters on
the seaside coast of Virginia and Maryland in contrast to *Haplosporidium
nelsoni* (causing MSX disease) which is found in more inland waters such
as Chesapeake Bay (Andrews, 1982). The disease caused three years of
serious mortalities from 1959 to 1961 but has not been as severe and
recurrent a problem as MSX (Andrews et al., 1962; Andrews and Casta-
gna, 1978). Annual mortality rates can reach 50 % in seaside bays of
Virginia. The parasite infects all tissues of the oysters except the epithelium
and causes substantial synchronous mortalities when sporulation occurs.
Diagnosis is based on histological examination.

**BONAMIASIS OF THE EUROPEAN FLAT OYSTER**
(*Ostrea edulis*)

This disease, caused by *Bonamia ostreae*, infects blood cells of the
oyster. Serious mortalities occur in newly infected populations. The disease
is best known for its substantial impact on the European industry,
particularly in France, where it was first identified in 1979. It is transmitted
by water contact but close proximity to infected oysters is believed to be
necessary. The disease occurred in flat oysters in California in the 1960s,
but was then known as « microcell disease » (Katiansky et al., 1969;
Katiansky and Warner, 1974). The disease spread from a California
hatchery to Brittany, France, where it initiated the well known epizootic
(Pichot et al., 1980). Bonamiasis was transplanted to Washington state
from the California hatchery in the late 1970s and remains an important
disease in the Pacific Northwest (Elston et al., 1986). Some degree of
resistance to the disease has occurred in North American stocks of oysters
which have had long-term exposure (Elston et al., 1987a; Holsinger, 1988).
Studies in Washington state showed a 20%, 7% and 4% mortality rate, respectively, for 2-, 3- and 4-years-old infected oysters. The effects of the disease are mitigated in off-bottom culture. Bonamiosis can cause significant mortalities between 12° and 20°C but not at higher temperatures. A related Bonamia occurs in New Zealand. The possibility must be considered that Bonamia represents a cosmopolitan parasite of another host and that multiple infections have taken place in flat oysters around the world. Diagnosis is performed by an immunodiagnostic assay developed by the French or by histological analysis (Bucke and Feist, 1985).

VELAR VIRUS DISEASE OF PACIFIC OYSTERS
(Crassostrea gigas)

Oyster velar virus disease (OVVD) is known only as a hatchery disease, and is caused by an iridovirus (Elston and Wilkinson, 1985). It infects the epithelium of the velum, mouth, esophagus and, rarely, the mantle of the larvae. The disease has only been reported from Washington state. Since there has been extensive commerce of this oyster historically, it is likely that the disease is much more widespread than is presently known. Larvae of the Pacific oyster, Crassostrea gigas, are the only species and life stage known to be infected by the disease. Some similar viruses have been observed in adult Pacific and Portuguese oysters in France but their relationship to OVVD has not been determined. Oyster velar virus disease can cause nearly 100% mortality in affected hatchery tanks. The disease typically appears in the March to May time period, but it has also been reported throughout the summer. Observations of mortalities in the spring, always in larvae greater than 150 μm in shell length and at least 10 days post-spawning, when grown in the 25 to 30°C temperature range are suggestive of OVVD. Virus infected cells on the velum of sick larvae detach and form the characteristic « blisters ». Deciliation of the velum also occurs but it should be noted that loss of cilia can result from other causes. Presumptive diagnosis can be made histologically by observation of the intracytoplasmic inclusion bodies.

DENMAN ISLAND DISEASE OF THE PACIFIC OYSTER
(Crassostrea gigas)

Denman Island Disease, also referred to as « microcell » disease is a little studied but apparently important disease of the Pacific oyster (Quayle, 1961, 1982). The term « microcell » has also been used to refer to bonamiosis (caused by Bonamia ostreae) of the European flat oyster. Since the diseases and their causative microorganisms are unrelated, the term microcell should be abandoned to avoid further confusion between these two diseases. The causative agent of Denman Island disease infects the glycogen storage cells of the oyster and is now known as Mikrocytots mackini (Farley et al., in press). The disease was first reported from Pacific oysters, Crassostrea gigas, from Denman Island in British Columbia in 1960. Since then it has been noted at other sites in the Strait of Georgia
in British Columbia. Mortality rates of up to 53% in a single season have been reported but the severity fluctuates from year to year. Infection and loss to the disease increased at lower tide levels when oyster mortality was monitored at the 4.0, 2.5 and 1.0 foot levels (Bower, in press). The disease is characterized by the appearance of round, yellow to green lesions or pustules (1 to 3 mm in diameter) occurring on the body surface. Similar lesions occur in several other oyster diseases.

**PACIFIC OYSTER**  
(*Crassostrea gigas*) **NOCARDIOSIS**

Nocardiosis is a disease of the Pacific oyster, *Crassostrea gigas*. The causative agent was recently isolated and identified as a member of the genus *Nocardia* (Friedman et al., 1987). Previously the disease has been known as « fatal inflammatory bacteremia », « focal necrosis », and « multiple abscesses » (Glude, 1974; Elston et al., 1987b). The disease causes typical small, round yellow lesions (which are, in fact, abscesses) on the body surfaces of the oysters, often observed on gaping individuals. « Multiple abscesses » described from Matsushima Bay, Japan, appears to be the same as nocardiosis (Imai et al., 1965, 1968). On the west coast of North America, the disease has been reported from sites in California to British Columbia. The Pacific oyster, *Crassostrea gigas*, is the principal oyster affected by the disease, although a few specimens of the European flat oyster, *Ostrea edulis*, cultivated near areas of infected Pacific oysters have been reported to have a similar disease. The severity of the disease in individual oysters and the high prevalence in some populations suggest that it is an important mortality factor in some cases. In one study it was reported to occur in about 30% of oysters sampled from Washington sites during September and October. The disease is a summer and fall phenomenon, typically observed from August through November.

**HEMIC NEOPLASIA OF VARIOUS SPECIES OF BIVALVE MOLLUSCS**

Hemic or hemocytic neoplasia (HCN) is also referred to as hemic proliferative disease, leukocytic neoplasia, sarcomatous neoplasia, sarcomatoid proliferative disorder, disseminated sarcoma and atypical hemocyte condition. Research on *Mya arenaria* (the soft shell clam) has suggested that the disease is caused by a retrovirus, but this is not yet confirmed in other species (Oprandy et al., 1981). The disease is transplantable with whole cells and transmissible with cell free homogenate, in some species, at least (Elston et al. 1988a; Twomey and Mulcahy 1988). The disease affects many species throughout the world (Peters, 1988). The species of commercial importance which are affected in North America are *Crassostrea virginica*, *Mya arenaria*, *Mytilus edulis*, *Ostrea lurida* (Farey, 1969a, b; Frierman and Andrews, 1976; Elston et al., 1988b). Dense farmed populations appear to be 100% infected if individual animals are monitored over several months. Mortality rates due to the disease are
reported to approach 100% over an annual period in some species, but these high mortality rates have not been associated with the disease in natural populations. Typically, the disease is reported to have highest prevalence and intensity during fall and winter months. The prevalence drops in the spring and summer period, possibly because heavily infected individuals die in the winter and the disease does not start another cycle of increasing infection until autumn (Mix 1983). Diagnosis can be made by microscopic examination of blood for the enlarged, transformed cells or by histological examination of tissues. Mitotic figures are common among the affected cells in advanced cases of the disease. Recent research has demonstrated a repeatable pattern in the formation of aneuploid DNA content of the transformed cells. In some cases affected individuals fail to produce mature reproductive follicles.

VIBRIOSIS OF LARVAL AND JUVENILE MOLLUSCS

Vibriosis is an opportunistic disease of the larval stage of many, if not all, bivalve molluscs and is also known to affect juvenile stages of the red abalone, *Haliotis rufescens*, in North American abalone operations (Elston, 1984). In a properly managed hatchery there should be only minimal loss due to the disease. However, the disease has been associated with some significant mortalities (Elston et al., 1981). Three general sources of bacterial contamination and expansion exist in hatcheries: (1) from brood stock, (2) from algal cultures and (3) from incoming seawater or the seawater system. The disease probably results from a variety of species of *Vibrio*, the most notable of which is the recently described *Vibrio tubiashi* (Hada et al., 1984). It is likely that strains which have not received species designations are important in the disease as well.

HINGE LIGAMENT DISEASE OF JUVENILE BIVALVE MOLLUSCS

This disease causes erosion or destruction of the ligament that binds the two valves of bivalve molluscs together (Dungan, 1987; Dungan and Elston, 1988). Myxobacteria or « gliding bacteria », the causative agents of the disease, are known to have the ability to decompose many highly organized and complex biological structures made of protein such as the hinge ligament of bivalve molluscs (Dungan et al., in press). Infections with such bacteria are often found within the ligaments of juvenile clams, oysters or scallops which are dying in nursery areas. Once the ligament is destroyed, the mollusc is unable to open its valves for feeding and respiration. In addition, it is possible that the destruction of the ligament allows other bacteria to infect the tissues of the animals. Juvenile bivalves from the east and west coasts of North America have been examined and found to have the disease. It has been found in the following species: Pacific oyster (*Crassostrea gigas*), American oyster (*Crassostrea virginica*), European flat oyster (*Ostrea edulis*), hard clam (*Mercenaria mercenaria*), Japanese littleneck clam (*Tapes philippinarum*), Pacific razor clams (*Siliqua patula*) and bay scallops (*Argopecten irradians*). In many cases, aquaculturists have reported the complete loss of large groups of clams and oysters.
from this disease. Usually the bivalves affected by the disease are from settlement size to 1 cm in shell height. The smaller animals are probably more susceptible. No typical seasonal cycle of the disease has been determined. It can occur year-around possibly because juvenile molluscs are usually grown in a controlled environment, often with heated water. Research on the disease has shown that the hinge ligaments are degraded at an increasing rate as the water temperature increases over the range from 5°C to 20°C and that the normally hard ligament, when infected with the gliding bacteria, can become jelly-like at water temperatures as low as 10°C. The disease may be controlled by disinfection of the outer surface of the bivalves. The most effective disinfectant has been sodium hypochlorite. While treatments may have to be adjusted to meet individual circumstances, a suggested starting point is 25 parts per million sodium hypochlorite dip for three minutes daily.

OTHER DISEASES

There are many other reported diseases of bivalve molluscs in North America which are of lesser significance or are not extensively studied. As the field of molluscan pathology develops, some of these diseases will likely receive more attention from the research community.

APPLICATION OF NEW TECHNOLOGIES

There is clearly an opportunity to apply new technologies in order to advance the science of molluscan pathology and health management in mollusc husbandry. More basic research is needed to understand physiological and pathological processes in these animals. The need for invertebrate tissue culture is as great as ever for the study of obligate intracellular parasites and viruses. Molecular biological tools offer an opportunity to understand certain basic mechanisms in disease progression and to identify infectious agents. For example, use of gene probes in the study of bivalve hemic neoplasia may help reveal the relationship of this disease to other cancers and the potential presence of gene transcripts originating from an integrated viral genome. Use of monoclonal antibodies has potential for both fundamental and applied benefits. These probes can be used to identify epitopic relationships between cell and tissue types and thus indicate ontogenetic and pathogenic relationships of tissues. Monoclonal antibodies have recently been used to develop a diagnostic kit for the detection of *Bonamia ostreae* in commercial stocks of oysters (Mialhe et al., 1988a). Conceivably within the foreseeable future, it will be possible to transfer genes for specific traits into invertebrate animals. Research to achieve this objective should receive priority for the long term development of the invertebrate aquaculture industry. Already, on a technologically less complex plane, triploid oysters are being produced on a commercial scale and providing an improved product.
NEEDS IN MANAGEMENT OF HEALTH IN MOLLUSC HUSBANDRY

In addition to the need to utilize new technologies, there are several other important needs for the advancement of the applied side of this science. One important area is to develop accurate quantitative data on mortality rates and growth loss due to each disease. Surprisingly, little of this type of information is available. Without such information, the importance of some diseases is overstated while that of others may be understated. Perhaps even more importantly, such quantitative information should be available so that diseases can be prioritized by their economic impact and this information used to allocate research funds.

Another area is the formation of long term research programmes aimed toward the development of disease-resistant stocks of molluscs. Technologies for gene transfer, should they develop for invertebrates in the near future, may help shortcut this process. Nonetheless, we need to begin to identify desirable traits and combinations of traits in molluscs in order to understand how these affect the overall performance of the animals.

The science of molluscan pathology is on the threshold of remarkable new advances. The increasing numbers of investigators in the field and the use of new technologies are signs of the progress the field is beginning to experience. These advances will increase our understanding of fundamental biological processes in these animals, with potential application to pathological processes in higher animals, as well as increase our ability to manage the health of husbanded molluscs.

Acknowledgment. Research supported by the National Cancer Institute, National Institutes of Health, U.S. Public Health Service.


