Information on MSX Extracted from the PEI Oyster Biosecurity Manual 2014

Prince Edward Island
AQUACULTURE ALLIANCE

MSX: The Facts

PEI SHELLFISH ARE PERFECT!

HELP KEEP THEM HEALTHY

MSX is a disease that affects both wild and cultured oysters but is **not a risk to human health**

Oyster mortality rates can reach **90% to 95%** but there are **no treatments**

Do not import live oysters from areas known or suspected to be positive for MSX

It is likely that people spread MSX by **moving infected oysters** and equipment

Report illegal transportation of shellfish into or within the province, call **Crime Stoppers:**

1-800-222-8477

Mass mortality reporting protocols

Purposes

This reporting protocol is a guide for PEI shellfish harvesters and processors of what to do in the case of discovering a mass mortality event or any other situation that may raise suspicion of a disease outbreak (e.g. animal health issues). These protocols, as part of the PEIAA Shellfish Biosecurity Manual, are for guidance only and do not supersede any relevant provincial or federal regulations.

Currently the CFIA has regulatory authority for Aquatic Animal Disease Investigations under NAAHP (National Aquatic Animal Health Program). Any detection of a reportable disease (such as MSX) must be reported to them by law. Not all mass mortality events or animal health issues are caused by disease outbreaks, there are a number of environmental conditions that may impact shellfish over a large area, including winter kill / high spring mortality, eutrophication / algae bloom leading to anoxic water conditions. Other diseases could also be present. However any mass mortality event or health issues should be reported so that its cause can be investigated.

Current industry practice is to contact the PEI DFARD regarding any possible shellfish health issues or mass mortalities. DFARD biologists investigate and communicate with other regulatory partners (Provincial Environment, DFO, CFIA etc.) as necessary. This practice is familiar to the industry and inter-governmental communication is effective, therefore this protocol is intended to formalise what is already occurring.

Reporting a Suspected Mortality

1. Upon discovery of a mass shellfish mortality or suspected aquatic animal health issue call:

XXX-XXX-XXXX?

2. Ask to speak to the Shellfish Biologist / Technician

3. Give them your name, phone number, location of the issue and the date and time discovered

4. If leaving a voicemail out of office hours clearly state your name, phone number, location of the issue and the date and time discovered.

Inter-governmental reporting

1. Upon receiving a mortality or suspected aquatic animal health issue notification the date and time of the call, caller identification and reported location should be recorded on the form below. Subsequent resulting correspondence and activities should also be logged on this form.

   If received via voice mail contact must be made with the reporter within two hours of receipt of the message. This should also be logged.

2. Determine the earliest opportunity that a designated representative will be able to assess the situation.

3. Using the contact list below inform the necessary organisations of the report and of the planned initial assessment.

4. If the report relates to a private aquaculture lease and was not reported by the lease holder the lease holder must be informed as soon as possible.

Note: This reporting protocol does not detail the procedures for sample collection, transport, testing etc. These protocols are in place and it is assumed here that they will be followed in an expedited manner.

Post assessment / testing communication

1. Once an initial assessment has been completed and samples have been dispatched for testing the lead biologist should:

    a. Contact the initial reporter and the leaseholder if on a private aquaculture lease;

    b. Using the contact list below inform the necessary organisations;

    to inform them that the initial assessment has taken place.

2. Upon receipt of laboratory testing results and assessment as to the cause of the mortality the lead biologist should:

    a. Contact the initial reporter or the leaseholder if on a private aquaculture lease;

    b. Using the contact list below inform the necessary organisations;

3. If a reportable disease is detected the lead biologist must:

    a. Contact the designated CFIA representative immediately;

    b. Contact the initial reporter or the leaseholder if on a private aquaculture lease.

Prince Edward Island
AQUACULTURE ALLIANCE

MSX Fact Sheet

What is MSX?

MSX (**M**ultinuclear **S**phere **X**) is a parasitic disease in cultured and wild American oysters caused by *Haplosporidium nelsoni.*

Is MSX a risk to human health?

No. MSX is not a risk to human health.

What are the signs of MSX?

- Valves slow to close when disturbed
- Decreased rate of growth, no new shell growth
- Extensive fouling along the inside left valve fringe
- Juvenile oysters may have pale digestive glands
- Oysters appear thin and watery, receding of the mantle
- Raised yellow-brown spots on internal valve surfaces

Oysters that are over 2 years old (over 38mm or 1.5" in length) are particularly affected.

Mortality rates can reach 90% to 95% in older oysters.

Is MSX found in Atlantic Canada?

Yes - MSX has been found in parts of Nova Scotia, within the Bras d'Or Lakes and along the Atlantic Coast of Cape Breton

How is MSX spread?

The complete life cycle of MSX is not known, therefore how infection is spread between molluscs is not fully understood.

It is likely that people can spread MSX by moving infected molluscs and contaminated equipment.

How is MSX treated?

There are **no treatment options** currently available for MSX

What measures should you take to prevent the introduction and spread of MSX?

- If you frequently handle or work with oysters, be aware of the signs of MSX and where it occurs in your area

- **Do not import live oysters from areas known or suspected to be positive for MSX**. It is illegal to transfer oysters from MSX positive areas to non-MSX positive areas, except to retail/market. This is controlled through Condition of Licence for harvesters/aqua-culturists in the MSX positive areas.

- Anyone transporting oysters should check with the appropriate federal or provincial departments to see if a license or permit is required

- An Introduction and Transfer (I&T) Licence from the receiving province is required for **any shellfish** entering the province that will be re-soaked or where processing/washing effluent will reach fish habitat.

- Excess oysters should never be returned to the water, either store in chilled storage or dispose of as per normal compost in your municipal garbage

- Shells that are removed should never be returned to the water, dispose of as per normal compost in your municipal garbage

- An I&T Licence is required for movements of native species of shellfish within PEI if the source area is infested with tunicates or any shellfish disease.

- An I&T Licence is required **for all** movements of non-native species of shellfish within PEI.

MSX is a **reportable disease** in Canada. This means that anyone who owns or works with aquatic animals, who knows of or suspects an MSX outbreak **is required by law** to notify the CFIA, 690 University Avenue, Charlottetown, PEI, C1E 1E3. Tel: 902-566-7290, Fax: 902-566-7334

To report illegal transportation of shellfish into or within the province please call Crime Stoppers at:

1-800-222-8477

MSX Risk assessment and mitigation

Introduction

MSX (Multinucleate Sphere X) is a major pathogen of the American oyster (*Crassostrea virginica*) on the east coast of North America. *Haplosporidium nelsoni,* the parasite responsible for the development of the disease, has not currently been detected in Prince Edward Island (PEI) waters, but there are concerns that it may spread from Cape Breton, Nova Scotia, where it has been identified.

The introduction of MSX to PEI would cause direct losses to the industry and it would have long term implications for the export of oysters. The main goal of this project was to investigate the movements of commercial oysters into and within PEI to assess the risk of introduction and dissemination of MSX.

Potential Pathways for introduction to PEI

1. **Movement of oysters from infected areas into PEI**
2. **Movement of other bivalves and associated organisms from infected areas into PEI**
3. Movement of boats between infected areas and PEI
   - Small crafts, recreational and fishing - Risk can be mitigated by increasing awareness of disease transfer potential
   - Large vessels - International laws should protect from contaminated ballast water
4. Movement of infected water
   - Very low probability that water from Cape Breton reaches PEI - Risk from this pathway could change if the distribution of MSX changes

Pathway #1 and 2 are considered the highest risk pathways of possible transmission.

Recommended mitigation for reducing the risk of introduction of MSX to PEI:

Do not import live oysters from areas known or suspected to be positive for MSX

Potential pathways for spreading MSX within PEI

A significant number of oyster movements between bays, estuaries and river systems within PEI were identified as part of this project; which suggests that if MSX was introduced it would be disseminated relatively quickly.

1. **Movement of oysters between bays**
   - Aquaculture
   - Fisheries
   - Recreational
2. Movement of other bivalves and associated organisms between bays
   - Aquaculture
3. Boat movement between bays
4. Water movement between bays

Pathway #1 is considered the highest risk pathway for spreading the disease

Several bays identified as having a high risk of introduction (Receive oysters and other bivalves from a number of other bays). Several bays were identified as having the potential for disseminating MSX (Send oysters out to many bays). Bays that have a high risk of introduction and the potential for dissemination are problematic. The precise location of these areas will vary on an annual / seasonal basis.

The current structure of the oyster fisheries and aquaculture industries render it impossible to avoid oyster movements and it is unlikely that the disease would be detected early enough to prevent its spread, especially as it has an incubation period of six to eight weeks.

Early detection of suspected mortality events are one key aspect to early detection of disease outbreaks

Please refer to the Mass Mortality Reporting Protocols in this manual or the wallet card provided for further details.

PEI Oyster Biosecurity Project
MSX Literature Review

Global distribution of the H. nelson

Haplosporidium nelsoni the parasite responsible for the development of MSX disease in the American oyster (*Crassostrea virginica*) has been detected along the eastern seaboard of North America, from Florida in the USA, to the Bras d'Or Lakes in Nova Scotia, Canada (Stephenson, *et al.,* 2003). *H. nelsoni* has also been detected using an un-validated PCR method in *C. virginica* samples collected in the Gulf of Mexico and the Caribbean Sea (Ulrich, *et al.,* 2007).

H. nelsoni was detected in cultured and wild Pacific oysters (*C. gigas*) in British Columbia, Canada during a surveillance project conducted by AAHD-CFIA. *H. nelsoni* has also been reported in the Pacific oyster, in California and Washington states in the USA, Japan and Korea (Burreson, *et al*., 2000), and in France (Renault, *et al*., 2000),

Life Stages

The known current life stages of MSX disease include: plasmodia, sporocysts and spores (Fig.1). The plasmodia are multinucleate and range in shape from spherical to oval with an overall size of 4 to 25 µm (Scro and Ford, 1990). The sporocysts typically measure roughly 28-54 µm and contain 8 to 50 spores (Couch *et al.* 1966). Spores range from 5.3 to 10.7 µm and the width from 4.8 to 7.5 µm. The spore is surrounded by a refractive capsule (1 µm thick) without projections or appendages. Spores are round with an operculum that extends laterally beyond the margin of the orifice to the margin of the capsule. The operculum is approximately 1 µm high. The spore nucleus is small (1.5 to 2.0 µm) and has a peripheral endosome of identical appearance to those in smaller plasmodia (Couch, *et al.,* 1966). During sporulation, sporocysts are found almost exclusively in the epithelium of the digestive diverticula of the oyster (Couch, *et al.,* 1966). The stages of the life cycle after spore release are uncertain, as transmission directly between oysters has not been successful in lab trials. (Bower *et al*. 1994). Attempted lab trials include:

1. Proximity to infected oysters or suspected oysters' alternate hosts.
2. Feeding with infected material both forced and as a suspension in the aquarium water
3. Inoculation into various sites such as muscle, heart, visceral mass and mantle cavity and
4. Implantation of infected gill and mantle tissue (Canzonier 1967, 1974).

The early life stages: gametes, fertilized eggs and larval stages of oysters do not appear to be susceptible to infection. All life stages after settlement are susceptible to infection, with young oysters (<1 yr) regularly producing spores and 75-85% of all infections reaching the advanced stage. Barber *et al*. 1991 examined a population of oysters and 30-35% of one year old spat were infected with the plasmodial stages of *H. nelson.* By month end all infections were advanced and by June 20, 83% of spat with advanced infections showed signs of sporulation. Sporulation is seasonal; spore production in June and July occurs in spat infected the previous late summer or fall. In spat that set and were infected earlier that summer, the period over which spores were found in the late summer and fall was prolonged. It appears that incubation is dependent on temperature and salinity (Andrews and Wood, 1967; Ford and Haskin, 1982).

The timing of maximum food supply for the oyster and stage in the parasites life cycle within the oyster is important in determining whether or not the parasite undergoes sporulation or density-independent growth of the plasmodia (Hofmann *et al*. 2001).

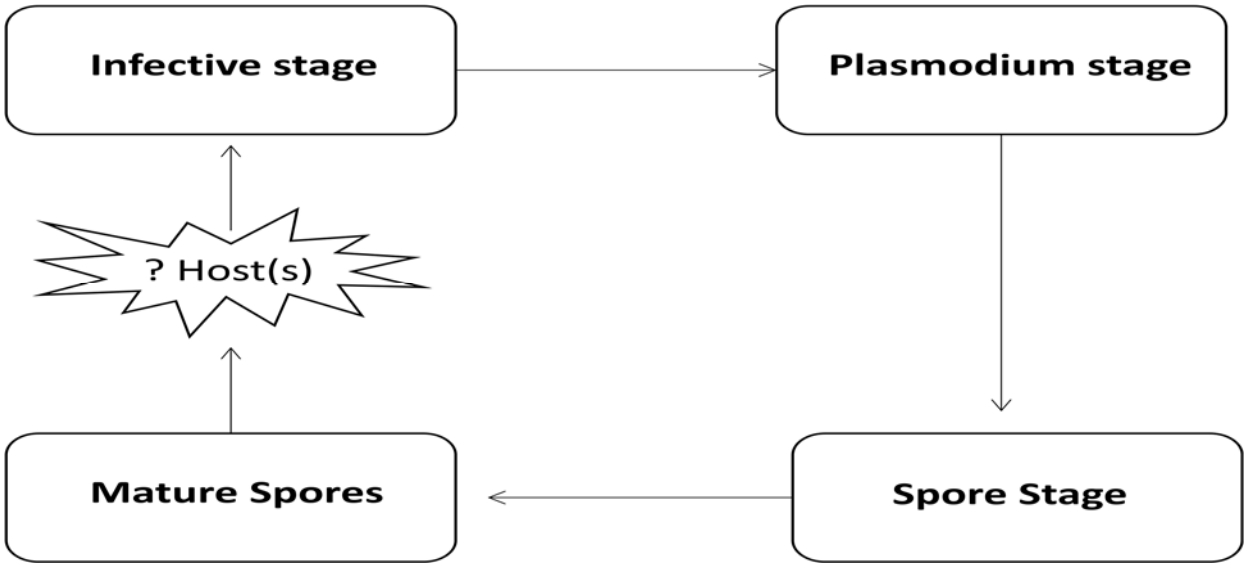

Figure 1. Life cycle stages of *Haplosporidium nelsoni* as seen in *Crassostrea virginica.* ? Host(s) indicates missing component of life cycle enabling the transfer of the MSX disease between infected and non-infected oysters.

Modes of transmission of H. nelsoni

It does not appear that vertical transmission between an infected oyster and gametes is unlikely to occur since gametes and life stages prior to settlement have not been found to be infected. The parasite is typically extracellular and has never been observed in eggs. (Ford 1992). Canzonier (1967, 1974) documented the variety of procedures attempted to transmit H. *Nelsoni* under controlled conditions in the laboratory. These include, proximity to infected oysters or suspected oysters alternate hosts, inoculation into various sites such as muscle, heart, visceral mass and mantle cavity, implantation of infected gill and mantle tissue, feeding with infected material both forced and as a suspension in the aquarium water. No evidence of direct or indirect transmission between oysters in a laboratory setting was found during the process of this literature search.

Sunila *et al.* (2000) reported that *H. nelsoni* can be transmitted to the American oyster *C. virginica* through water from an area infected with *H. nelsoni.* In their study, hatchery-raised, MSX-free juvenile oysters were placed in upweller tanks. Water to the tanks originated from the water column overlaying naturally infected oysters beds (prevalence of *H. nelsoni* infection of 17-57%). The water was filtered through a screen with 1mm^2 openings. After a period of 11 weeks MSX was diagnosed by histopathological analysis at a prevalence of 57% and with an increased mortality rate of 19%. This increased to 80% mortality after 16 weeks. This study demonstrated that *H. nelsoni* is transmissible via water-borne agents capable of passing through a 1mm filter. This is of particular importance as many of PEI's processing plants have screens in place to prevent the dispensing of invasive species into adjacent rivers and bays with their waste water. However the ability of the disease to be transmitted through a 1mm screen calls into question their effectiveness.

Infection

The portal of entry for *H. nelsoni* into the oyster is the epithelial lining of the gill and inner palp (Farley 1968). Once entry has been gained the plasmodia are localized within epithelia and restricted by base membranes. An intense cellular response characterized by infiltration of the affected tissue by hyaline hemocytes occurred when the parasites invaded the connective tissues and circulatory systems.

Infection typically occurs between late May and October along the mid Atlantic coast (Haskin *et al.* 1988). This period of infection was determined by importing disease-free seed-oysters from uninfected areas into those know to have high levels of *H. nelsoni* infections. The findings of these trials were that *H. nelsoni* was not found in winter-spring imports until late June and did not become common until early August each year. Oysters imported in June showed the same timing on infections as others imported the previous November. All showed the absence of *H. nelsoni* infections until about August and the same time of *H. nelsoni* losses in their first summer of exposure. It appears that the period of infectivity did not begin until late May or early June. These dates would likely translate to a slightly smaller window (July to October) in PEI given our colder winters.

Incubation period

There is considerable variation in the incubation period of MSX, depending on the time of year when the initial infection occurs. Introductions occurring in the early spring and progressing over the summer can take as little as 5-8 weeks, while infections occurring in the fall can take up to 10 months (Andrews 1966). Both salinity and temperature are key to the duration of the incubation period with warmer higher salinity conditions expediting the incubation period (Andrews and Wood, 1967; Ford and Haskin, 1982).

Clinical signs of MSX:

The clinical signs associated with MSX disease include dead or gaping oysters, general weakness in the abductor mussels ability to tightly close the shell and in response to a agitation stimulus, mantle recession and fouling of shell margins consisting of a 2-10mm band of fouling on the inner margin of the left shell. In addition to this fouling of the inner margins of the shell, oysters with a raised yellow-brown conchiolinous depositions are a strong indication of the presence of the *H. nelsoni*. Pale coloration of the digestive gland and a thin emaciated watery tissue are also indications of infection and disease (Farley 1968).

Progression of MSX disease

The stages of infection by *H. nelsoni* were defined into five categories by Farley (1975) as initial, intermediate, advanced terminal and remission. Initial infection occurred in epithelia of the gill, palp and water tubes prior to spreading into the connective tissue. Intermediate infection was characterized by infiltration of connective tissue in and adjacent to epithelia of gill and palp along with further progression into the oesophagus, stomach, gut, diverticula, and gonadal alveoli. Advanced infections are recognized by the infiltration of connective tissue and the circulatory system by hyaline hemocytes. Barber *et al.* (1991) suggest that in oyster less than 1 year old, spore formation occurs regularly and that spores are produced in at least 75-85% of all infections reaching this stage. Terminal infections showed histologically pykonosis and necrosis

of tissues before outward signs of death were apparent. Remission of the disease is observed via the reduction of infection intensity and infiltration. The localisation of *H. nelsoni* near the external epithelia; increased pigment cell formation and diapedesis and deposition of necrotic parasites and tissue against the shell, followed by external conchiolinous encapsulation

Mortality patterns and rates

Multiple studies have been carried out looking at the patterns of mortality within oysters infected with *H. nelsoni*. Andrews (1982) in a retrospective study of data collected in Virginia over 23 years reported that high prevalence of *H. nelsoni* was associated with high mortality and low prevalence of *H. nelsoni* was associated with low mortality. The seasonal timing of the introduction is more important then the length of exposure with regards to the first period of mortality (Couch and Rosenfield, 1968). Farley (1975) indicated that in the first year of infection mortality generally peaked between August and October. In the second year of exposure mortality occurs earlier in the summer with infections being observed from March- June.

Environmental Requirements:

Temperature:

The optimal temperature range for MSX is between 5^0 and 20^0 C with decreasing prevalence of the parasite above and below this range. This temperature range fits well with those observed in river estuary systems in PEI (Figure 2). Water temperatures in PEI estuaries are typically within the optimal range between April and November, indicating that the island oyster industry appears to be susceptible to MSX disease, as the average water temperature in PEI seldom reaches above the threshold necessary for oysters to rid themselves of the pathogen (i.e.> 20 C) (Ford 1985). However, during PEI winters water temperatures do drop below the 5 C mark for several months consecutively (Figure 2.), which has been shown to slow and reduce the level of infection within oysters considerably (Ford and Haskin 1982).

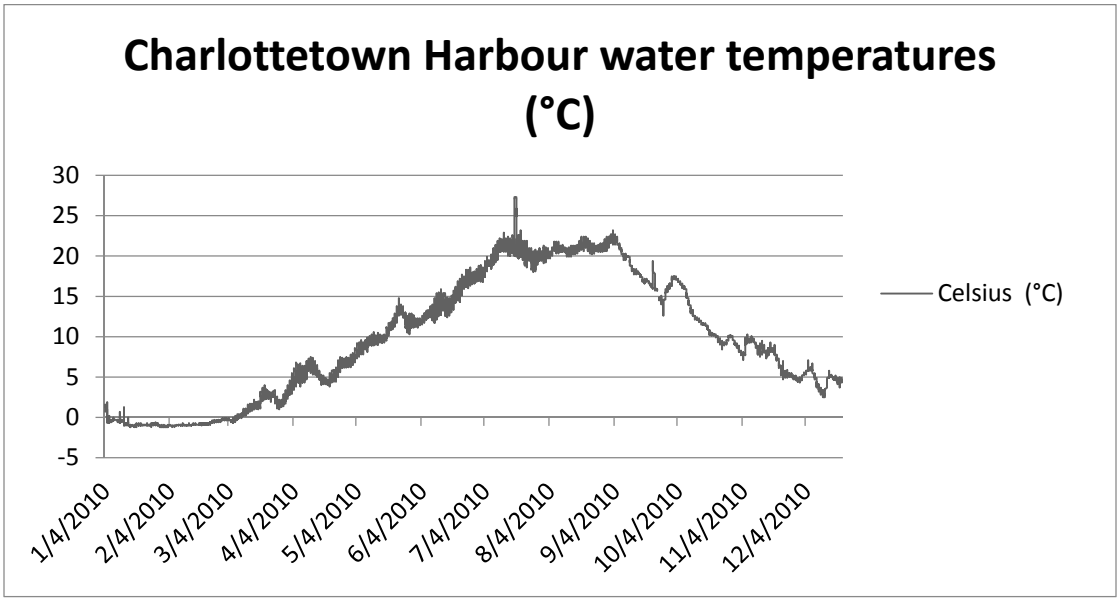

Figure 2. Water temperatures °C for Charlottetown Harbour for the year 2010.

Salinity:

Haskin and Ford (1982) illustrated a direct link between the salt gradient and the development of the parasite within an oyster in a retrospective study of data collected from 1958 to 1981. They found that the prevalence of infections falls in parallel with decreasing salinities. Suggesting a salinity threshold that has little effect on the distribution of infective stages of MSX or on their ability to infect, but that severely limits the parasite's capacity to develop once it has entered the oyster. They also observed that:

1. At a salinity level below 9 to 11 ppt MSX infections are rarely acquired. 10 ppt seems to be a minimum level needed for survival of the parasite;
2. Between 10 and 20 ppt, infections are acquired in a pattern that parallels the salinity gradient, but the development of these infections as well as the consequent mortality are greatly inhibited;
3. From 20 to 24 ppt appear to be ideal for the production, acquisition, and development of the MSX parasite in oysters;
4. Full parasite activity is manifest above 20 ppt but may decrease again above 30 ppt.

An in vitro study by Ford and Haskin (1988) determined that the destruction of the parasite begins when salinities reach 15ppt and increased exponentially as salinities fell to 9ppt at which point the maximum level of damage has occurred to the parasite. Most bays and estuaries on PEI appear to be optimal for MSX disease with regard to the salinity levels observed (fig 3.).

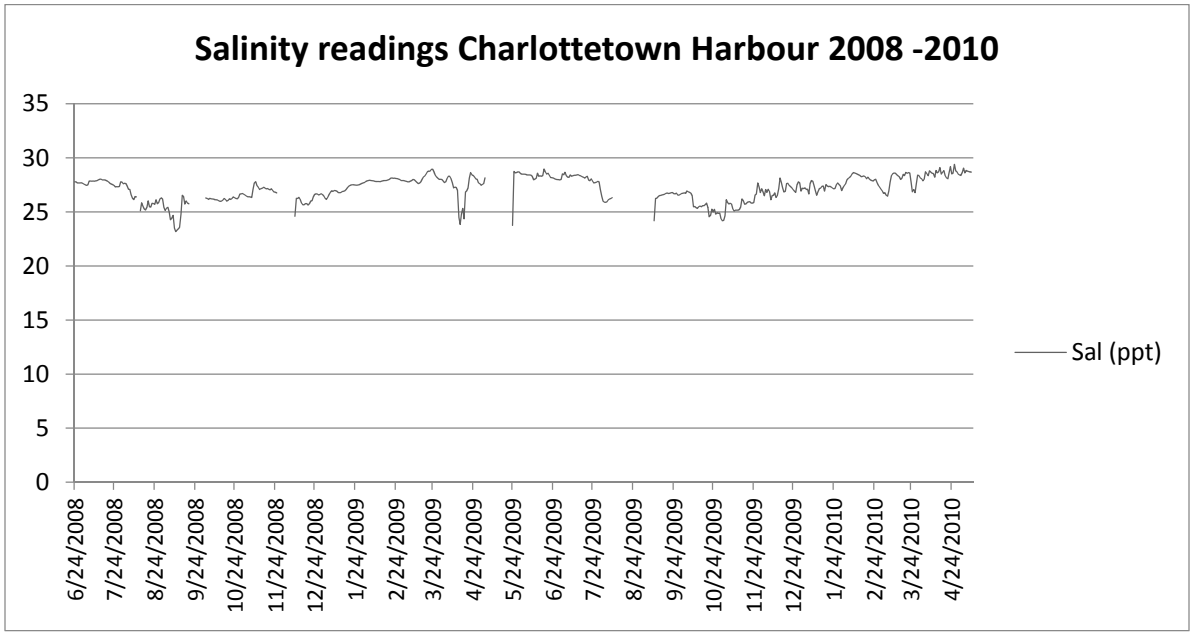

Figure 2. Water salinities (ppt) for Charlottetown Harbour for the years 2008 -2010.

Combined effect of salinity and temperature on infection and prevalence:

Ford (1985) further expanded on the previous understanding of low-salinity effects on MSX by defining more precisely the impact of various salinity levels on the parasite and the duration of time required for these changes to occur. To accomplish this, infected oysters from high (20-25%) were moved to 3 low salinities (5-15%) locations for 4 months, then returned to high salinity, MSX-free water. The results of the trial indicated that infections disappeared after 2 weeks of exposure at mean salinities of 10% or less and temperatures above 20°C. Infections did not reappear when oysters were returned to high salinity. The rate that parasites level decreased was faster with decreasing salinities from 15 to 5%. At 15% salinity patent infections disappeared within a month. At 10 and 5% salinity the loss occurred in 2 weeks.

A simulation model using field and environmental data collected from Chesapeake Bay over a 10 year period by Hofmann, *et al*. (2001), suggested that when a year with cool water temperatures (< 3 °C) was followed by a year of low salinity (< 15 ppt), *H. nelsoni* prevalence and intensity of infection were greatly reduced. Simulations showed that the disease returned with the return to average environmental conditions. The model was validated by comparing simulations from inputting data that was independent to that used to produce the model and comparing them to the known field events.

Soniat *et al.* (2009) conducted time–series analysis of temperature and salinity to determine patterns of disease in C. Virginica showed that disease prevalence and intensity in C. Virginica populations along the Gulf of Mexico were primarily regulated by salinity, whereas temperature determines the disease progression along the United States east coast. The authors suggested that one of the most important differences between Gulf of Mexico oyster populations and oyster population of the northeast coast of the United States is that epizootics in Gulf oyster populations are principally controlled by salinity and the effects of fresh water inputs, whereas epizootics in northeast populations are influenced more substantially by water temperatures. These results do not discount the importance of salinity in the northeast United States region, but rather emphasizes the critical importance of colder winter temperatures which are not observed in the Gulf of Mexico.

Other influencing environmental factors

Prevalence of H. nelsoni within C. virginica populations

The prevalence of *H. nelsoni* within a *C. virginica* population is highly variable with salinity levels playing a major role. At salinities above 15ppt a prevalence of 15-90% is common in the summer with winter prevalence averaging between 40-60%. The level of prevalence has been shown to follow a cyclical pattern which typically peaks every 6-8 years (Andrews and Wood. 1967, Ford & Haskin, 1982, Andrews, 1982). The level of prevalence does decrease slightly during the winter, but not to the same extent as the intensity of the infection (Ford and Haskin, 1982). Ford and Haskin (1982) conducted a retrospective study examining data collected over a 23 year period (1958-1981) from Delaware Bay and reported that in the fall *H. nelsoni* continue to proliferate in *C. virginica* with parasite levels peaking in December as water temperature approach 5°C. Activity and prevalence of the parasite slows from this point on as when water temperatures fall below 5°C.

Another key factor in determining the prevalence of *H. nelson* within populations of *C. virginica* is location of the oysters within the water column and the availability of food. Hofmann *et al.* (2001) indicated that the timing of the maximum food supply availability of the oyster and the particular stage of the life cycle the parasite is in are important factors in determining whether the parasite undergoes sporation or density-independent growth of the plasmodia.

Factors affecting infection between oyster populations

Since the secondary host(s) of the life cycle is not know at this time it is difficult to determine possible factors affecting the infection of one population of oysters from another. A study by Kratz *et al.* (1972) observed that an infected population of oysters in Wellfleet Harbor, Massachusetts did not spread to infect naive oyster populations in close proximity to a known infected populations. Indicating that local environmental conditions may play a large role in the spread of infection between populations of oysters.

There may also be some advantage for smaller sized oysters in withstanding infections of *H. nelson* due to their higher metabolic rates, which seems to enable them to be able to withstand prevalent dosages of the parasite (Krantz *et al.* 1972)

The depth within the water column that an oyster population is located could also influence its likely hood of becoming infected. Volety *et al* (2000) reported that prevalence and intensity of infection was significantly higher in oysters collected from greater than 90 cm of water depth than those collected at less than 45 cm of depth.

Development of MSX resistant C. virginica

Aquaculture

Along the North Eastern coast of North America several MSX resistant strains (AOFA, ARDN, ASOLD, AVA, BLA, CFX, NEHSL, DBHSLR) of *C. virginica* have been developed over the last thirty plus years. These strains were primarily developed through an intensive cross breeding program using disease survivors as brood stock. Some of the more successful strains were developed by Dr. Hal Haskin and Dr. Susan Ford at Rutgers University. These strains have recorded survival rates of up to 10 times those of naive oysters. The selection of resistant genes along with fast growing genes has allowed for the redevelopment of productive oyster grounds that had been abandoned previously as a result of the high mortality rates observed from MSX disease. Another advantage of these hatchery produced strains is that they are almost all triploid allowing for better conditioning and growth of the oyster since they don't have to put energy into spawning. The resistant capabilities of these oysters along with their fast growth rates allowed them to reach market size prior to the disease becoming lethal.

Commercial Fishery

Natural stocks of *C. virginica* have gradually developed some resistance to MSX along the Eastern seaboard, however without the help of selective cross breeding programs the process has been much slower taking over 30 years to reach only partial resistance and is only now starting to allow for a small scale commercial fishery. It is likely the reestablishment of any commercial fishery on PEI after a MSX out break would require the use of hatchery developed resistant brood stock as well as an extensive seeding program, similar to what was done for Malpeque disease.

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