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J Sanchez

Ryan Carnegie
Virginia Institute of Marine Science

P Warris

J Hill

J Davidson

See next page for additional authors

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**RISK CHARACTERIZATION FOR INTRODUCTION AND SPREAD OF MULTINUCLEATE SPHERE X (MSX) IN PRINCE EDWARD ISLAND, CANADA**

JAVIER SANCHEZ,1* RYAN B. CARNEGIE,2 PETER WARRIS,3 JONATHAN HILL,1 JEFF DAVIDSON1 AND SOPHIE ST-HILAIRE1

1Department of Health Management, Atlantic Veterinary College, University of Prince Edward Island, 550 University Avenue, Charlottetown, PE, Canada, C0A 1P3; 2Virginia Institute of Marine Science, College of William & Mary, P.O. Box 1346, Gloucester Point, VA 23502; 3PEI Aquaculture Alliance, 101 Longworth Avenue, Charlottetown, PE, Canada, C1A 5A9

**ABSTRACT** Multinucleate Sphere X (MSX) is an important pathogen of the eastern oyster (*Crassostrea virginica*) on the east coast of North America. This parasite is currently not present in Prince Edward Island (PEI), but there are concerns that it will spread from Cape Breton, Nova Scotia, where it was recently identified. Introduction of MSX to PEI would cause direct losses to the shellfish industry and it would have long-term implications for exports of oysters. The main goal of this study was to assess the risk of introduction and dissemination of MSX, as estimated by the number of movements of commercial oysters from three data sources. The in-degree (incoming) and out-degree (outgoing) of the contact network among bays, estuaries, and rivers were used to quantify the risks. For a single location on PEI, identification of up to 55 incoming movements to and from different locations within PEI were recorded. This suggests that if MSX was introduced it could be disseminated quickly. Movements of shellfish (oysters and mussels) from New Brunswick and Nova Scotia, which pose additional risks of pathogen introduction to PEI, were also identified. Two locations (New London Bay and the East-North-West River complex) were consistently ranked in the top quartile of incoming movements by the three data sources. In addition, two other locations (Bideford Area and Conway Narrows) were classified in the same quartile by only two of the data sources, suggesting that these four areas can be considered at high risk for pathogen introduction. Similarly, two locations were classified in the top quartile of outgoing movements (East-North-West River complex and Orwell Area) by the three data sources, whereas Bideford Area and Foxley River were only identified by two of the three data sources in the same quartile. These results indicate not only risk areas for pathogen introduction or dissemination, but also those areas having both high risk of introduction and high risk of dissemination (e.g., East-North-West River complex). Results of this study illustrate the potential consequences of MSX should *Haplosporidium nelsoni* reach PEI. Findings also highlight the need for the development of a system that captures all shellfish movements, which will be essential to mounting an effective response to pathogen introduction and mitigation of disease dissemination.

**KEY WORDS:** shellfish, oyster, movement, risk, dissemination, Multinucleate Sphere X, *Haplosporidium nelsoni*, pathway, network analysis

**INTRODUCTION**

Multinucleate Sphere X (MSX) disease, caused by *Haplosporidium nelsoni*, affects the eastern oyster (*Crassostrea virginica*) on the east coast of North America, from Florida to Nova Scotia (Stephenson & McGladdery 2003). This parasite is currently not present in Prince Edward Island (PEI), but there are concerns that it will spread from Cape Breton, Nova Scotia, where it was recently identified. Given that mortality associated with this disease in naïve populations can exceed 80% (Haskim & Ford 1979, Stephenson & McGladdery 2003), spread of this parasite to PEI will have long-term implications for the aquaculture and fisheries economies; the oyster industry in PEI generates approximately 6 million CAD per year [Department of Fisheries, Aquaculture and Rural Development (DFARD)—http://www.gov.pe.ca/].

The MSX is typically reported during the summer, and incubation periods can vary from 5 wk to as long as 10 mo, depending on the exposure dose, temperature, and salinity (Andrews 1968, Haskin & Andrews 1988). The optimal range of temperature for MSX is between 5° and 20°C. The temperature range in river systems and estuaries in PEI (Fig. 1) suggests that water temperatures between April and November are conducive to the clinical manifestations of MSX, and the mean water temperature in PEI seldom exceeds the threshold necessary for oysters to overcome infections (i.e., more than 20°C) (Ford 1985).

Salinity below 10 ppt is not conducive to survival of *Haplosporidium nelsoni* (Andrews 1968, Haskim & Ford 1982, Ford 1985, Hofmann et al. 2001), and water salinity in tidal river systems in PEI, where oyster populations are found, ranges between 26 ppt and 28 ppt (DFARD), which is optimal for survival of the pathogen.

The life cycle of *Haplosporidium nelsoni* is not completely understood; therefore, the host range for this pathogen has not yet been defined but, so far, this pathogen appears to only cause disease in the eastern oyster on the east coast of North America. It may also infect other oyster species, but generally does not cause serious disease, as has been shown in the Pacific oyster (*Crassostrea gigas*) (Friedman et al. 1991).

Transmission of *Haplosporidium nelsoni* under field conditions has been shown to occur via contaminated water (Chintala & Fisher 1991, Sunila et al. 2000, Ford et al. 2001). As attempts to transmit the pathogen under laboratory conditions (Canzonier 1968, 1974) have failed, it may require an intermediate host.

Reducing the risk of introduction and, if necessary, mitigating the impact of MSX in PEI requires that we identify pathways for introduction and spread. Although the parasite...
could conceivably be moved via water currents, this route of introduction would likely only transfer the pathogen short distances. This is supported by the finding that recolonization of previously MSX-free oyster reefs in the same estuary, during drought periods, can take up to 2 y (Haskin & Andrews 1988). In addition, *Haplosporidium nelsoni* has not spread from Bras d’Or Lakes in Cape Breton, Nova Scotia, to PEI despite its presence there for over 10 y, and there is no evidence that oysters in the Gulf of St. Lawrence are tolerant of MSX.

The species *Haplosporidium nelsoni* might be introduced to PEI via water transported within contaminated, nonsusceptible bivalves (i.e., mussels) or boats or both. There is currently an active cruise ship industry in the two largest ports in PEI, Charlottetown and Summerside, which is comprised of ships that arrive mostly from the eastern coast of the United States. There is also commercial shipping in other ports on PEI, including Georgetown and Souris in the eastern part of the island. Although these routes of introduction are possible, given current understanding of the biology of this parasite, its transfer via water moved with boats and contaminated nontarget bivalve hosts seems less plausible than other routes, such as the movement of large quantities of infected eastern oysters. Understanding the movements of oysters within and between bays in PEI and other provinces will allow prediction of the most likely risk of introduction and dissemination for this parasite. A social network analysis approach was used to quantify the risks of introduction and dissemination in PEI. Risk characterization was based on the number of movements among bays for the pathways identified from each data source. The in-degree and out-degree values, commonly used in social network analysis, were computed to characterize the risk of disease spread in animal populations (Dorjee et al. 2013, Thakur et al. 2014). The in-degree value represents the number of incoming movements from unique bays of origin and, therefore, was used to represent the risk of introduction (incoming movements). On the other hand, the out-degree value was calculated based on the number of unique bays to which a given bay sent products and was, therefore, representative of the risk of dissemination (outgoing movements). These two variables were created for each bay in each of the three datasets. These values were then used to identify, which bays had the highest risk of introduction and dissemination of pathogens via the movement of animals.

Descriptive statistics illustrate the movement of spat to different bays on PEI, the movement of oysters between leases during the grow-out period, and the movement of oysters for harvest. Descriptive statistics were calculated using Stata V13 (StataCorp 2013).

To identify the spatial location of places with high risk, two sets of maps were created to illustrate the introductions and disseminations for each of the three datasets. First, the approximate geo-coordinates of each bay or river system were captured using Google Maps by one of the coauthors (J. H.—industry expert). Then, these locations were entered into QGIS (Quantum Geographic Information System) analysis software to create the maps.
RESULTS

Forty bays, estuaries, and rivers were identified as sources or destinations of shellfish movements in this study. Although 24 of them received at least one incoming movement, 39 locations sent shellfish out to other locations. The mean number of total movements per location from the three data sources ranged from 0.33 to 35 (range: 1–55). The distribution of incoming movements indicated that two locations (New London Bay and the East-North-West River complex) ranked in the top quartile in the three data sources. On the other hand, Bideford Area, Hillsborough Bay, and Conway Narrows were ranked in the top quartile by two of the data sources. Similarly, the distribution of outgoing movements indicated that the Orwell Area and the East-North-West River complex were consistently classified in the top quartile by the three data sources, whereas Bideford Area and Foxley River were classified in the top quartile by only two of the data sources. The following locations had, on average, the highest number of total movements: New London Bay (mean = 35), Bideford Area (mean = 31), the East-North-West River complex (mean = 31), and Orwell Area (mean = 21). A detailed description of the results for each of the three data sources is presented below.

Survey of 2012 Processing

Eighteen of 31 processors responded to our survey (58% response rate). The proportions of respondents that processed the different types of shellfish products are summarized in Table 1. A total of 153 incoming and 158 outgoing movements were recorded in this survey. The majority of oysters were processed in or introduced to New London Bay (35.9%), Bideford Area (25.5%), Conway Narrows (16.3%), and the

| Type of product          | Number of processors (%) | Number of products | Number of processors (%) |
|--------------------------|--------------------------|--------------------|--------------------------|
| Wild oyster harvest,     | 11 (61.1)                | 1                  | 5 (27.8)                 |
| spring                   |                          |                    |                          |
| Wild oyster harvest,     | 9 (50.0)                 | 2                  | 3 (16.7)                 |
| fall                     |                          |                    |                          |
| Oyster aquaculture       | 14 (77.8)                | 3                  | 7 (38.9)                 |
| Softshell clams          | 3 (16.7)                 | 4                  | 2 (11.1)                 |
| Mussels                  | 8 (44.4)                 | 5                  | 1 (5.6)                  |

East-North-West River complex (12.4%) [Fig. 3 (risk of introduction) and Fig. 4A]. On the other hand, most of the oysters processed came from the East-North-West River complex (17.7%), Orwell Area (10.8%), Bedeque Bay (10.1%), Cascumpec Bay (7.6%), and Mill River (7.6%) [Fig. 3 (risk of dissemination) and Fig. 4B]. In this survey, the two areas highlighted as having introductions of oysters from the greatest number of different areas were Bideford Area and New London Bay (Fig. 3).

Industry Survey in 2014

Forty-eight (73.8%) of 65 oyster fishermen and growers, and 10 (71.4%) of 14 oyster processors responded to the survey. In general, oysters in the aquaculture industry were moved at least two times within the grow-out period (e.g., spat/seed in river A to grow-out in river B and then to processor site). There were cases where oysters were transferred several times between leases within an estuary, depending on individual circumstances. Wild oysters were moved at least once (to the processor), but could also have been moved to a relay site before going to a processor. Wild oysters may also have originated from several locations, including the Bideford River, as this is where the majority of the spat for enhancement of natural stocks originates.

A total of 198 incoming and 197 outgoing movements were identified in this survey. Similar to the previous survey, New London Bay and Bideford Area had the greatest number of sources of oyster introductions, 18.7% and 10.1% of total introductions, respectively. They were followed by Hillsborough Bay (9%) and the East-North-West River complex (8.6%). More than half of these introductions were harvest-sized oysters [Fig. 5 (risk of introduction) and Fig. 6A].

Foxley River and Orwell Area were identified as largest contributors to all outgoing movements, where oysters were shipped out to 26 (13.2%) and 24 (12.2%) different locations [Fig. 5 (risk of dissemination) and Fig. 6A]. The East-North-West River complex was also identified as a source for shipping out harvest-sized oysters (risk of dissemination) to numerous river systems on PEI (19 out 197). Bideford Area was identified as having incoming oyster movements from a number of sources, with many also being harvest-sized (17 out 197). As well, Bideford Area is the spat or seed

**Figure 2. Pathways for aquaculture oyster movements on PEI.** Seed and spat are purchased and transferred to a grow-out site. In some cases, oysters are moved among grow-out sites before being transferred to a processor for grading and shipping. In many cases processors have to hold oysters on leases for several weeks before they are marketed.

GIS Development Team 2014, QGIS Geographic Information System, Open Source Geospatial Foundation. Available at: http://qgis.osgeo.org, QGIS version number 2.4.0), and a series of density maps were created using the inverse weighted distance interpolation method to identify the areas that demonstrated higher risks of introduction and dissemination, where weights were proportional to the number of movements for each bay.

**TABLE 1.**

Proportion of responses by type of product and number of products, reported by 18 of 31 processors from PEI who participated in the 2013 survey.
source for many PEI rivers [Fig. 5 (risk of dissemination) and Fig. 6B].

Overall, locations that introduced and distributed the most market-sized animals included New London Bay, Bideford Area, and the East-North-West River complex (Fig. 5).

The I&T Permits between 2010 and 2013

The DFO I&T database contained 183 bivalve movements between 2010 and 2013 (Table 2). Almost 40% of the I&T permits were for oyster movements, and most of these were for juvenile animals. Oyster movements identified in the I&T permits between 2010 and 2013 are consistent with the data in the two surveys conducted in this study. The I&T database additionally identified several off-island introductions of bivalves (Table 3), half of which were of oysters originating in New Brunswick. Mussel movements occurred from all three Maritime Provinces and were predominantly for the purpose of harvesting (Table 3). Shellfish originating outside of PEI were sent to several bays in PEI, including some of the areas that distribute oysters within PEI, such as Bideford Area, Conway Narrows, the East-North-West River complex, and New London Bay (Table 3).

To estimate the risks of introduction and dissemination from the movements of oysters within PEI, all outgoing, off-island movements of products were excluded. A total of 63 incoming and 55 outgoing movements were recorded during this period. The variety of sources for oysters moved into and out of different rivers in PEI, based on I&T permits, were not the same as those identified in the other two surveys. For example, Bideford Area was not identified as having the most varied incoming sources of oysters on PEI in the I&T data (Fig. 7—risk of introduction). The I&T data did, however, identify New London Bay, the East-North-West River complex, Conway Narrows, and Hillsborough Bay as having many sources of incoming oysters [Fig. 7 (risk of introduction) and Fig. 8A]. Bideford Area (27.27%), along with Foxley River (21.8%), and Orwell Area (18.2%) were identified as disseminating high numbers of oysters to other bays and rivers on PEI, including a number of harvest-sized oysters [Fig. 7 (risk of dissemination) and Fig. 8B].

All of these areas were identified in the 2014 survey (Fig. 6B), and Bedeque Bay and Orwell Area were also identified in the 2012 processor survey (Fig. 4B).

DISCUSSION

To our knowledge, this is the first study that quantifies the movements of shellfish to provide an estimate of the risk of
Figure 4. Density map of the number of bays involved in the oyster movements captured with the 2012 survey. The size and lightness of the area is proportional to the number of bays: (A) receiving oysters (risk of introduction) and (B) sending oysters (risk of dissemination). Lighter areas on the map indicate greater levels of movement.
introduction and dissemination of *Haplosporidium nelsoni* in PEI. The number of animal movements identified by the PEI oyster industry, during the period when the water temperature and salinity was conducive for MSX infection acquisition and disease development (i.e., May to November), was very high and included several movements from outside of the province. The latter are of particular importance because the number of movements within PEI suggests that if an oyster pathogen is introduced to PEI its spread will be difficult to prevent. The three different sources of information used for this study highlighted similar areas of concern. The 2014 survey included more industry members than the 2012 survey, and provided information only partially collected from either the 2012 survey or DFO. For instance, in 2012 most of the Bideford Area was identified as having a high risk of introduction (Fig. 3). On the other hand, DFO data identified this area as at high risk for pathogen dissemination, based on oyster movements out of the area (Fig. 7). The 2014 survey also identified this area as at high risk for both introduction and dissemination (Fig. 6), which included seed/spat movement. Overall, New London Bay, Bideford Area, and the East-North-West River complex were classified within the top five risk areas (i.e., had both high risk of introduction and dissemination) by all three sources of information.

If MSX is introduced to PEI, the impact of this disease on the oyster industry will depend on how quickly and how widely the pathogen is disseminated. It is logical that the greater the number of introductions of oysters into a river system from unique areas, the more likely an introduction will occur. The risk of introduction of MSX to a river system, as determined in this study, increased as the number of oysters from different sources introduced to that system increased. If harvest-sized oysters are introduced to areas with oysters at other stages (i.e., spat), and these are subsequently transferred to a new geographic location for grow-out or harvest purposes, the risk of dissemination increases. The risk of dissemination is, therefore, based on the number of rivers or bays to which a system distributes oysters for grow-out or harvest purposes. If an area only receives oysters for harvest purposes then its risk of dissemination via animal movement is assumed to be lower than a site that receives and distributes oysters to other areas. Likewise, a river system from which oysters are moved out but not in is assumed to be less likely to become infected via animal movements and, therefore, less likely to spread pathogens. Both the number of sources of oysters moved into a river system or an area and the number of river systems or areas to which oysters were distributed were considered when identifying

### Figure 5. Distribution of the number of bays where oysters originated from (risk of introduction) and were shipped to (risk of dissemination). For example, Bideford Area received harvest aquaculture oysters from approximately 13 different bays and sent spat/seed to approximately 14 bays on PEI. Data were estimated from the 2014 survey, for all within-island movements according each pathway identified in Figure 2.
Figure 6. Density map of the number of bays involved in the oyster movements captured with the 2014 survey. The size and lightness of the area is proportional to the number of bays: (A) receiving oysters (risk of introduction) and (B) sending oysters (risk of dissemination). Lighter areas on the map indicate greater levels of movement.
river systems that may be more problematic for dissemination of MSX on PEI.

In general, areas with processing plants were identified as having a higher risk of becoming infected with MSX, as there are high volumes of animal movement into these systems. Since oysters are usually resoaked (in the spring wild oysters are relayed for 14 to 21 days before processing) in the water before processing, it is not possible to treat effluent from this process, which makes it difficult to reduce the risk associated with processors. If no oysters are moved out of these water bodies, then the risk of dissemination could be minimized, but in many cases oysters were moved from river systems where there were processors.

The surveys demonstrated that market-sized oysters were moved in and out of several river systems. The water areas that were highlighted in several of our surveys included New London Bay, Bideford Area, and the East-North-West River complex; however, it should be noted that we did not have full participation of all processors and producers, so some systems may not be represented in this study, and be at risk. These areas may pose a risk of spreading pathogens if the movement of animals occurs after the oysters have been in the area for some time. Further, with the exception of the East-North-West River complex, all other locations were identified as having received oysters from other provinces.

Of particular concern is the Bideford Area, which is one of the largest producers of spat in PEI, and provides seed for the enhancement program for wild PEI oysters. Oysters of different sizes are being moved back into the Bideford Area from other systems for grow-out purposes, as well as for harvest [Fig. 3 (risk of introduction) and Fig. 4A], which makes this system particularly vulnerable to the introduction and spread of MSX. Further, there were movements of oysters from New Brunswick, in 2011, to Bideford Area and Conway Narrows, an area close to Bideford Area. Should MSX be introduced into the Bideford Area, it would have the potential to be disseminated relatively quickly to many other water systems on PEI. Foxley River is also a system that provides spat/seed to many other areas on PEI; however, its overall risk of dissemination is determined to be less than the Bideford Area, based on the fact that it has significantly fewer introductions of oysters.

Any area that is used for spat recruitment has a high risk of dissemination because animals are moved to so many other areas. Yearling oysters are clearly susceptible to *Haplosporidium nelsoni* infection (Barber et al. 1991, Burreson 1995, Carnegie & Burreson 2011), and given an incubation period that may be 3–8 wk long (Ewart & Ford 1993), it is possible that spat could be transferred to other locations before it develops patent infections and disease.

It may also be that the intermediate host required for the life cycle of *Haplosporidium nelsoni* is not present on PEI; however, given the wide distribution of MSX on the east coast of the United States, and that the parasite exists as far north as Cape Breton, it is possible that the intermediate host is widely distributed, even in areas where the parasite is not yet present (Andrews 1983). Climate change has also been suggested as one of the main drivers of the northward migration of MSX (Hofmann et al. 2001).

Although the distribution of an intermediate host could be fundamental to the parasite’s successful transmission, dispersal of waterborne infectious parasite stages is unlikely to occur over long distances. Coarse-scale hydrodynamic data from DFO indicate that currents flow predominantly from PEI toward Cape Breton, which may also help explain why it has not moved from Cape Breton to PEI. As the intermediate host has not yet been identified it is not yet possible to predict the pathogen’s potential distribution within PEI and other maritime provinces.

In bays/estuaries that have dynamic movement patterns, as was demonstrated for PEI, timely detection of emerging pathogens is difficult. Given the volume of oyster movement in PEI between bays and river systems, MSX likely would survive in PEI, so efforts should be taken to reduce the risk of introduction and dissemination of this pathogen. Early diagnosis of *Haplosporidium nelsoni* is an essential step in control of this disease (Ford & Haskin 1988). Frequent sampling and sampling before introductions can reduce the risk of moving pathogens, but this strategy becomes logistically difficult to execute when there are as many movements of animals as were found in PEI, and there is always the risk of testing inaccuracies that result in false-negative results. Surveillance efforts should target higher-risk areas such as New London Bay, Bideford Area, and the East-North-West River complex. To reduce the dissemination potential of spat collection areas, sampling these areas before the dispersal of oysters might reduce the likelihood of *H. nelsoni* spread. Perhaps it is more important to reduce the...
risk of introduction into PEI, and so requiring testing for each product that is introduced from outside the province may help reduce the risk of introduction.

Another measure that could take some time to implement, but would help reduce the risk of disease transmission, would be to separate the areas used for processing from the areas used for spat collection. Over time, movements of oysters for extending the grow-out period could be reduced or coordinated to reduce connectivity between river systems.

A limitation of this study, which should be considered before making changes to the surveillance program for MSX, is the quantity of product at risk or the potential impact of introduction into specific areas. The quantity and frequency of movements were not considered in this study because this information could not be retrieved by all participants. Although many links between watersheds on PEI were identified, processors change suppliers and suppliers change buyers, so movement patterns may change over time. It is also likely that total oyster movements were underestimated, as not all growers, fishermen, and processors on PEI were interviewed for either year sampled. Further, 2011 DFO I&T permits were thought to represent only a small proportion of oyster movements within PEI during that year (all I&T permitted transfers were provided to us; however, it is generally believed that there is under-reporting of oyster transfers to this system), as many movements are known to occur without permits.

Despite these limitations, this study serves to illustrate PEI’s potential serious consequences should *Haplosporidium nelsoni* reach the island. Given the presence and epidemic occurrence of the pathogen in nearby Cape Breton, the ability of the parasite to complete its life cycle in PEI waters cannot be viewed as a biological impossibility, so long as the appropriate intermediate host is also present. Assuming this is, or at some point becomes, the case, the interconnectedness of producing areas described here suggests that *H. nelsoni* could quickly become established island-wide. For this reason, strong consideration should be given to minimizing the impact of the disease, which could include protecting industry through the proactive application of selective breeding for MSX resistance. Captive breeding programs to produce MSX-resistant strains of oysters began in the 1960s in Delaware Bay, NJ (Haskin & Ford 1979), and such MSX- (and *Perkinsus marinus*-) resistant lineages are in wide commercial use today (Ragone Calvo et al. 2003, Dégremont et al. 2012). Although the use of MSX-resistant oysters in PEI and elsewhere in Atlantic Canada may be an option, it would require several generations of oysters to

Figure 7. Distribution of the number of bays oysters were moved to (risk of introduction) and from (risk of dissemination). Data were obtained from the DFO I&T database (2010 to 2013) for all within-island oyster movements by stage of production (i.e., seed/spat or market-size).
Figure 8. Density map of the number of bays involved in oyster movements captured in the DFO I&T database (2010 to 2013). The size and lightness of the area is proportional to the number of bays: (A) receiving oysters (risk of introduction) and (B) sending oysters (risk of dissemination).
develop resistant strains as well as the careful use of quarantine to prevent the inadvertent spread of MSX and other pathogens with the use of MSX-selected oyster brood stock from the eastern United States or Nova Scotia. Planning ahead and development of the infrastructure and industry knowledge for this type of program will be a must if a lag in oyster production is to be avoided.

Finally, the exercise of collecting and mapping movements of commercial oysters from spat to harvest highlights the limitations of not having an automated system for capturing this type of information. The time required to collect the data necessary to conduct trace-backs would render this process inadequate for responding to an emergency pathogen introduction. An automated, computerized system that captures all movements will be essential to mounting an effective response to pathogen introduction and mitigation of disease dissemination.

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