Living and Working with MSX, an Oyster Industry Perspective

August 21 and 22, 2024

Credit Union Place, Summerside, PEI

Prepared for the Prince Edward Island Aquaculture Alliance

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Contents

Introduction:

Living and Working with MSX, an Oyster Industry Perspective was a workshop hosted by the Prince Edward Island Aquaculture Alliance, Prince Edward Island Shellfish Association, Prince Edward Island Seafood Processors Association and Prince Edward Island Oyster Processors Association in response to the recent finding of multinucleate sphere X (MSX) in waterways around Prince Edward Island (PEI).

The workshop provided an update on the current MSX situation in PEI and brought together local fishers, aquaculturists, processors, and researchers with those from other regions along the eastern seaboard who operate in MSX-infected waters or experienced an MSX outbreak in the past (Virginia, Maine and Nova Scotia) along with provincial and federal government agencies and funders. Day 1 of the workshop focused on industry's perspectives on MSX, while Day 2 focused on broodstock programs, hatcheries, and research.

Attendees were encouraged to listen, learn and engage in discussions. Participation was encouraged by opening up the floor to questions after presentations or enabling panel discussions, all facilitated by Peter Warris, Executive Director of the Prince Edward Island Aquaculture Alliance. At the end of each day, attendees were asked to list priorities for industry to adapt in the presence of MSX.

These proceedings provide a summary of the presentations, question and answer periods, panel discussions as well as the priorities identified by participants. They are not intended as a word-for-word transcript, but an assembly of the most pertinent information. For additional context and information, the agenda and the presentations have been appended to this document (Appendix A), as have listings of priorities identified by participants on each day of the workshop (Appendices B, C).

We thank our sponsors and funders: Innovation PEI, Genome Atlantic, Farm Credit Canada, and Prince Edward Island Department of Fisheries, Tourism, Sport, and Culture. We also thank the speakers who lent their expertise to this workshop, Atlantic Aqua Farms for funding some of their staff travel expenses, Credit Union Place for the use of their facility and catering as well as Pater Audio for their technical support.

Day 1: Industry Perspectives on MSX, Presentation Summaries and Q&A

MSX Sampling and Surveillance

Kim Gill, Director of Aquaculture, PEI DFTSC

PDF – https://www.aquaculturepei.com/wp-content/uploads/2024/09/Gill-Task-Force.pdf

Video - https://youtu.be/-kCVkYCKO90

The core role of the Aquaculture Division of the Department of Fisheries, Tourism, Sport, and Culture (DFTSC) is to provide advice, assistance and information to support the aquaculture and shell fishing industries. It includes a number of technical programs which monitor algae, biofouling organisms, spatfall and shellfish growth at sentinel locations. It has financial programs to support industry growth and innovation. The small technical group at DFTSC also provides other biological services, including water sampling, in partnership with Fisheries and Oceans Canada (DFO), Canadian Food Inspection Agency (CFIA) and Environment and Climate Change Canada (ECCC), for the Canadian Shellfish Sanitation Program (CSSP); and MSX surveillance and shellfish mortality investigations to complement other surveillance work.

A pilot project was started in 2019 to survey for MSX, dovetailing off of DFO surveillance work which was ending at this time. For this surveillance, which continued up to the finding of MSX in Island waters (June 2024), oysters were collected in August or September at a number of sites. Originally, only six (6) areas were sampled, but the number of sites grew to seventeen as a reflection of industry growth. 30 to 60 oysters were sampled per site; and they were screened at the Atlantic Veterinary College (AVC) using histology. As of 2023, there was no MSX detected under this surveillance program.

DFTSC also investigates mortality events reported by fishers or aquaculturists. In the past, DFTSC has typically responded to about 3-5 mortality reports per year. This type of investigation includes a questionnaire or interview that collects information on the timing, product, husbandry practices, environment, and any other input that may be relevant. Water quality information is collected, and the percentage mortality of the event is assessed. Samples are also collected for histology screening. Any abnormalities detected through histological analysis are sent for polymerase chain reaction (PCR) testing. Histology is where tissues are sectioned, stained, and examined under the microscope for any tissue changes, conducted at the AVC, and PCR is a molecular technique that determines whether or not MSX DNA is present. This is conducted at the Research and Productivity Council (RPC) in Fredericton.

To give a bit of background on where things are today, a mortality was reported in mid-June in Bedeque Bay by fishers. Histology results indicated suspicion of MSX. CFIA was notified and the case definition for MSX was met on July 11 with confirmatory histology, PCR and sequencing. Additional surveys have identified six areas that have tested positive for MSX and have been declared Primary Control Zones (PCZ) by CFIA: Bedeque Bay, Lennox/Bideford/Conway, New

London/Stanley Bride, Boughton River, Darnley basin, and Percival Bay/River. The leases that tested positive have been quarantined.

Currently, staff at DFTSC continues to sample oysters from sites to test for MSX. This testing does not duplicate but complements the existing CFIA testing. CFIA testing follows a strict procedure that looks at epilinks while the DFTSC testing looks at areas most important to industry, such as fall fishing beds. DFTSC worked with the PEI Shellfish Association to identify significant sites for the fall fishery for sampling and has also sampled spat collection areas in Bideford and Orwell-Vernon. A distribution map shows sites that have been sampled by DFTSC (not CFIA). All have tested negative for MSX so far.

Next steps are to increase testing within PCZs, to determine the distribution of MSX to try to help limit its spread. DFTSC will conduct follow-up testing in Bedeque Bay to look at enhancement oysters spread in mid-August and do some follow up sampling of the original mortality event. DFTSC will also look at non-trace out areas and work with the associations to determine areas that should be sampled.

Question: Is there any sense of the level or mortality in Bedeque Bay and other areas?

Answer: No, we have not been back to Bedeque Bay yet and do not know the level of mortality. There are different ways to assess mortality rates, and we need to build the methodology. For other areas, we have not heard of large-scale mortality from industry. If people see mortality, we want to hear about it, so we can stay on top of it.

Question: In Bedeque Bay, how many tested positive?

Answered by Aaron Ramsay (Aquaculture Biologist, DFTSC): We collected eighty oysters from each of the five sites sampled. We sent ten from each to RPC and AVC. All five were positive for PCR, four out of five were suspect using histology.

Answered by Danielle Williams (CFIA): For CFIA testing for the five sites (62 oysters/site), the June testing showed a 42% prevalence, while a month later, the prevalence was >90%.

Question: Of the positives identified, were they cage culture or bottom?

Answer: The original identification was from wild beds. CFIA cannot give details of locations of other positives. There have been sites with cage culture identified as positive that were traced back from bottom oysters.

Question: What is the plan moving forward for sampling in Bedeque Bay?

Answer: The province is working with Dr. Roland Cusack, who did the sampling in Cape Breton during their outbreak, and he has suggested using diving for sample collection; however, this may be challenging for Bedeque's conditions (i.e., higher turbidity). There are two pieces to the work at Bedeque Bay: 1) we will go back to the original five sites to assess mortality; and 2) we will follow the enhancement stocks that were negative when they were placed on the sites.

Question: Do growers have to pay for testing in their leases?

Answer: Some industry members are testing on their own. There were costing estimates that were distributed that came from RPC. If anyone wants to send oysters to them, they can. For the provincial surveillance work, this is not something we expect growers to pay for.

CFIA Surveillance Plan for MSX

Dr. Danielle Williams, Aquatics Regional Veterinary Officer, CFIA

PDF – https://www.aquaculturepei.com/wp-content/uploads/2024/09/Williams-CFIA.pdf

Video - https://youtu.be/cSKeIva6AIg

CFIA has a different approach to sampling relative to DFTSC, but the two approaches are complementary, and the agencies work together.

To date, CFIA has sampled thirty-nine sites, for which MSX has not been detected at 16 sites, MSX was detected at 17 sites and six results are pending. Testing starts with PCR that indicates the presence of *Haplosporidium*, histology confirms the presence of *Haplosporidium,* and sequencing confirms that it is *H. nelsoni*. The locations for the sampling have been determined by: trace out of oyster movements (where oysters from an MSX positive area have been moved to); trace in of oyster movements (where oysters from an MSX positive area may have originated from); locations where sick oysters have been reported; proximity relative to positive sites; and determination of PCZ boundaries. With respect to the trace in/trace out work, information from industry is critical. CFIA has been following a protocol to contact growers to determine potential oyster movements up to a year ago. Open communication is very important to understand the spread.

In order to determine whether MSX is present is a numbers game and is dependent on how many oysters in a population have MSX. The percentage of oysters that have MSX determines the number of samples that have to be taken in order to detect it. The more samples taken, the more likely to find it, if it is there. As an example, if 5% of oysters have MSX, sixty-two samples would be required to detect it; if 2% of oysters have MSX, 170 to 175 samples would be required to detect it. CFIA is using sixty-two samples to determine if MSX is present in at least 5% of the population. So, stating that MSX was not detected means that MSX is not in more than 5% of the population. Not detected therefore does not mean that MSX is not present at any level. In interpreting the results, a number of things are considered, including how many oysters are in close proximity, the age of the oysters, and the condition of the oysters. MSX is a challenging disease to manage because it has an unknown intermediate host and the time from infection to time to positive detection varies greatly. Finally, the environment can impact time to positive detection (lower salinity and colder water slows down spread).

All of the trace-outs from Bedeque Bay have been completed. Testing oysters from trace-ins is currently in progress.

Future sampling will focus on water bodies not previously tested and there will be repeat testing in areas which previously did not have MSX detection, because of the potential lag time between introduction and detection (up to 10 months). The pattern of spread is being assessed by a technical specialist team. This is what happens during the time that an area is a PCZ. The goal is to determine the boundaries of spread in order to establish what area is to be declared as an Infected Area.

Once an area has been declared an Infected Area (the step after declaration of a PCZ, once boundaries have been established), it is a permanent label[1] and surveillance will not continue since MSX cannot be removed from the environment. It survives in areas without oysters. In a declared Infected Area, controls will be in place for movements, with movement allowed between infected areas (with CFIA permits) and within an infected area (without CFIA permits). Oysters will also be able to leave an Infected Area for human consumption without a CFIA permit, provided the processing is done in an Infected Area. Note that Introductions and Transfer (I&T) permit requirements from DFO will still apply for all oyster movements.

Outside of this work, CFIA has an Eastern Shellfish Surveillance Program which has been ongoing since 2015. As part of this program, shellfish samples are collected twice a year to check for presence of *Haplosporidium nelsoni* and *Mikrocytos mackini*. In addition to this, CFIA relies on industry to report potential disease states of stocks.

MSX Primary Control Zone Permit Process

Joe Boutilier, Shellfish Specialist for the CSSP

PDF of Infographic

An infographic was produced to detail the requirements and process for obtaining a permit to move restricted animals and things into, out of or within a PCZ. There are four options for permits, as described in the infographic, with the option required determined by the item being moved and the direction of movement into, out of, or within the PCZ. It is notable that there is no allowance for movement of oysters or spat out of a PCZ into a non-PCZ for culture, relay, depuration or release (i.e., enhancement) into waterways. The intention is to have permit applications move to an online process as soon as possible.

CFIA encourages industry to get on their email list to get regular updates on the MSX situation and permitting process. People who are interested are encouraged to contact CFIA (email: cfia.ATLAHPermitting-DelivranceDePermisSAATL.acia@inspection.gc.ca or phone: 902-370-1368).

[1] It was clarified that the boundaries of a Primary Control Zone can be reduced or increased, based on new information; however, the boundaries of an Infected Area are considered permanent.

The U.S. history with MSX disease in eastern oysters

Dr. Ryan Carnegie, Professor of Marine Science, Virginia Institute of Marine Science (VIMS)

PDF – https://www.aquaculturepei.com/wp-content/uploads/2024/09/Carnegie-MSX-USperspective-FINAL.pdf

Video - https://youtu.be/xyAZ7m-Bfdk

MSX was an exotic pathogen, likely introduced from Asia, appearing in Chesapeake Bay in 1859, having previously appeared in Delaware Bay in 1957. It has moved north since this time, appearing in Cape Breton, NS, in 2002 and in the Damariscotta River in Maine in 2010.

The oyster fishing industry was a very lucrative industry which peaked in the 1850's when overfishing began to impact outcomes. Even up to the fifties and sixties, there were robust harvests in Virginia, from natural reefs and from transplants from James River. It was estimated that 1.5 billion oysters were harvested in 1959, prior to the arrival of MSX. When MSX emerged, more than 95% mortality was experienced on the reefs and planting grounds. The outbreaks in the 1950's and sixties were devastating to the economy, but also to the ecology. Oysters that had previously filtered the water and provided a solid substrate and shell reefs disappeared, to be replaced with mud.

Oysters are thriving now, but it took a long time for recovery in Chesapeake Bay. In part, because industry focused on looking at the potential for other species, with the hard clam being the best candidate. It was thought that the wild oysters would not recover since oysters in the disease areas were not healthy enough to produce spat. As a result, spat from oysters in low salinity areas, where there was no selective pressure for disease resistance, would spawn and reseed the disease areas, only to be killed by the parasite. However, more recent reductions in the salinity dynamics (less fluctuations) enabled resistance to develop in wild populations. But a rebound in population numbers was complicated by the appearance of *Perkinsosis* (i.e., Dermo).

In Delaware Bay, a breeding program was initiated in the 1960 and this strategy was eventually applied in Chesapeake Bay as well. Today, wild oysters are doing better, but primarily due to a natural evolution of disease resistance and more harvest controls and better management – such as the rotation of harvest areas and maintenance of sanctuary areas that allow the growth of natural broodstock to help with repopulation. This recovery took more than 50 years.

Recovery from the later MSX outbreaks in New York and New England took much less time. This is likely because there was aquaculture occurring in these areas, with hatchery reared, selected stocks available. Adaptation was quicker with the cultured stocks. There are also fewer low salinity populations to dilute the stocks with non-resistant spat.

Currently, oysters are doing well in Virginia, with disease resistant, genetically selected stocks. However, there was a recent outbreak of MSX in Chesapeake Bay in the past few months, in both the bottom beds and aquaculture stocks. This is likely because the prevalence of MSX had

decreased in the area and reduced the selective pressure for resistant stocks. We also continue to have low salinity, non-resistant oysters seed the beds. Moving forward, we will have to determine how to maintain a high disease resistance, even when the prevalence of MSX in the wild is low.

Question: How long does it take between the appearance of MSX and high mortality?

Answer: For high mortality, you need to have the right combination of host, environment, and pathogen. Sometimes it is very quick, but some cases present no mortality, or it takes a long time to reach a mortality event. In some areas, MSX was detected decades prior to a major mortality event. This variability may have to do with the intermediate host and its presence.

Question: Do you have a sense of how much local adaptation is important? There may be adaptation to other things which may be important for resilience. Is resistance the same everywhere?

Answer: Do not know; however, it is important to ensure local adaptations are considered/safeguarded to ensure continued local resilience (i.e., cold-water adaptations of PEI oysters in comparison to Virginia oysters).

Question: In a breeding program, can you test for resistance?

Answer: Have not yet identified the markers for resistance

Question: With regards to the funding challenge, how important is the restoration of the oyster beds recognized to access funding, as a factor of cultural/biological/ecological significance and to provide support in the conversation around the use of hatcheries to repopulate?

Answer: There can be an application for the use of hatcheries to repopulate. Shell planting with seeding is better than shell only. There is funding for restoration programs, with their recognized positive biological/ecological impacts and ability to reconnect people to the oyster. Triploids can be used to get around any genetic concerns.

Question: Can you give a basic overview on who is doing the breeding program and how this is connected to the hatcheries and how seed is getting out?

Answer: Leave this to Jessica Small of the Aquaculture Genetics and Breeding Technology Center (ABC) to explain later today.

MSX in the Bras D'Or, Industry Perspective

Robin Stuart

PDF – https://www.aquaculturepei.com/wp-content/uploads/2024/09/Stuart-MSX-Cape-Breton.pdf

Video - https://youtu.be/SjyIE9Y4y7M

Historically, Cape Breton has had an oyster industry, but it has been small scale relative to New Brunswick or Prince Edward Island. It has been a combination of bottom leases, commercial fishery and food fishery. Most oysters exist near the shore in less than 4 feet of water since it is too muddy in deeper water.

The oyster fishery has been a "political football", with people turning to oyster fishing during economic downturns. Prior to World War I, the stocks were greatly depleted, and importation of stock occurred from New England, introducing Malpeque disease. It took only one generation to develop resistance to Malpeque disease. The closure of the mines and steel plants in the 2000's resulted in a lot of unemployment and a turn back to oyster fishing. This greatly depleted the beds, so they were not in great shape prior to the arrival of MSX in 2002. However, aquaculture was just starting to boom at this time with plans to transfer high numbers of seed to promote expansion. Then MSX was found, and the transfer of seed was stopped.

MSX was first found in Dena's Pond. Here high mortality which lessened closer to shore was noted and was thought to be Dermo. Samples were taken and sent away for assessment and VIMS confirmed MSX. It was collectively decided to stop fishing in the Bras D'Or, and movement of stocks was stopped.

Stocks were closely surveyed for three years. Losses were concentrated in the St. Patrick's Channel area initially, but it spread to other areas over three years and leases belonging to larger growers were completely wiped out.

There were some sites outside the Bras D'Or that tested positive for MSX, but never experienced mortality. These areas had low over-winter water temperatures in common.

Looking forward for PEI, it is important to recognize that prompt communication to the growers and fishers from government is crucial; and research should have high industry input and be collaborative and shared. Those who have experienced MSX in the past should be consulted. It may make sense to use the Bras D'Or for research if movement around PEI is too restrictive.

Temperature and salinity data for areas are crucial to determine the best opportunities for surviving MSX. And hatcheries will be crucial to develop tolerant stocks since sick oysters do not spawn. And it is important to have a quick method to screen for MSX. It would be nice to know the intermediate host, but the industry may die waiting for its discovery. PEI should be looking at the potential to farm oysters in areas with suitable conditions, even if MSX is present. Triploids oysters may help with this by allowing the oysters to reach a critical size faster.

Question: How long was the lag between discovery of MSX and mortality?

Answer: It depended on the area. In Nyanza Bay, oysters introduced early in the season were infected by the end of the season, with high mortality seen the next year. But there have been spores found in oysters elsewhere in Cape Breton that did not experience mortality (St. Ann's Bay, Mira). Mortality could be in a year, could be in six years.

Question: Did you see success with off-bottom culture?

Answer: Joe Googoo has been growing oysters in floating bags in a barachois pond. These bags get frozen-in in the winter. In other places, we can see mortality on bottom, but not in floating gear. Floating gear may be an opportunity.

Question: You mentioned that the site you dove had less mortality in the shallower areas?

Answer: Shallower water typically has more changeable temperature and salinity. This may impact mortality. But we need to understand winter data too through the use of in-situ/real-time data collection probes/equipment. That may be what is controlling the parasite.

Question: There is a lot of aquaculture in shallow water, will this help?

Answer: Research has only been funded in recent years. Can potentially do trials in the Bras D'Or on this since we can move around stocks.

The Unpredictability of MSX: Opportunities for Industry and Research

Dr. Rod Beresford, Associate Professor Biology, Cape Breton University

PDF – https://www.aquaculturepei.com/wp-content/uploads/2024/09/Beresford-Opportunities.pdf

Video - https://youtu.be/Y6sWzUnQuAI

Oyster aquaculture was just starting to take hold in the early 2000's, when MSX hit. Its infection in the Bras D'Or wiped out 80% of oyster production in Nova Scotia. Work has been done since this time.

We know that temperature can impact the progression of *H. nelsoni*. In the US, the trend is for less parasite activity after a cold winter. In the lab, we can see an impact of higher temperature, with 4- weeks of holding at a higher temperature resulting in a reduction in mortality and prevalence of MSX. It is uncertain if this can have a practical application, but it is notable. Food availability, by contrast, does not seem to impact the progression of the parasite.

Although lab experiments may be useful and have a better ability to control various factors (i.e., temperature, salinity, etc.), field experiments are a must as it appears a number of environmental factors may impact results.

Work to evaluate resistance to MSX was done in the Bras D'Or. A few areas in the lake had MSX, according to screening, but had variable levels of die off, if any at all. So, the question was asked as to whether the surviving oysters are resistant or are the conditions not right to promote a mass mortality (i.e., virus inactivity/dormant). Oysters from these areas were moved to Nyanza Bay, where MSX essentially wiped out everything. By December, almost all oysters moved to Nyanza had significant infection, and most died after a year in Nyanza. This occurred despite populations in the origin sites still surviving.

Work was also done to look at spatial distribution of MSX in MacDonald's Pond, a small pond in the Bras D'Or that had a high prevalence of MSX, but low mortality. Several locations in this pond were chosen and differences in prevalence of MSX infection were seen. These may be attributable to wave and wind action differences at the sites.

Work in the field has also suggested that we may find places where oysters will survive, or maybe even where the infection may recede. A good example is a site in Aspy Bay where MSX has not been detectable since a sandbar closed the area off.

Moving forward, there may be opportunities to use a warm water treatment to reduce infection, or the variability of the environment for oyster in floating cages may keep the parasite at bay. Colleagues are important, as are conversations and having a number of eyes review research proposals, in order to pin down the best testing regime to try. Hatchery production of seed is likely the way of the future and, with the help of AFF, we have recently purchased an on-land hatchery to try.

Question: After the trials in Nyanza, was anything done with the survivors?

Answer: Anything left had to be destroyed.

Question: Did you move any oysters from Aspy Bay to MacDonalds Pond?

Answer: No, we were not allowed.

Question: Can you tell us about testing capacity at CBU?

Answer: The Verschuren Centre has a service capacity for testing. It is not accredited, but it has the capability. $28/sample. Ideally, 2-3 fully accredited labs should be available in the region, for capacity and contingency purposes.

Question: Does MacDonalds Pond freeze?

Answer: Yes, it does. And winter conditions may impact outcomes.

MSX Surveillance and Mortality – Understanding Disease and Impacts

Dr. Roland Cusack, C&H Aquatic and Laboratory Veterinary Services Ltd

PDF – https://www.aquaculturepei.com/wp-content/uploads/2024/09/Cusack-MSX-PEI-190824.pdf

Video - https://youtu.be/9Wn02KkNcJM

The management strategies for MSX will have to consider many things. The disease outcomes will be different for different sites, depending on the management strategies and environmental conditions. We do not understand many aspects of the disease triad for MSX. There are holes in our knowledge about this pathogen, the immunity of the host, as well as how the environmental conditions affect disease. You need to survey for this disease, now and into the future, including for morbidity and mortality.

Management of health for wild and domestic animals is not new and a lot is known about controlling animal disease, but it may be a new concept for many in the shellfish farming and fishing sectors. When new diseases emerge and become established in the environment (endemic) they can be managed. For example, in terrestrial animals a disease like Avian influenza, affecting wild and farmed birds, can be managed well and the industry continues to prosper. There have also been a number of aquatic endemic diseases that have emerged and are managed very successfully in the finfish farming industry.

Unlike bacteria and virus which tend to replicate rapidly, causing rapid infection, the MSX pathogen is a parasite with an indirect life cycle. Parasites typically have a slower reproductive rate than other pathogens, with a longer lag phase between the detection of infection and having the disease. Just because you are seeing infection, does not mean you will have disease or high associated mortality. And if we can cut off the intermediate host, we can affect the disease outcome.

Understanding the distribution via surveillance will be important. In NS, a lot of surveillance was done between 2002 and the mid 2010's, across the province. Currently, DFO and CFIA do surveillance focused on the processing sector and the province of NS has begun a new surveillance program for the shellfish sector.

We know that MSX spread rapidly in Cape Breton. Although the initial infection was found at Dena's Pond, it was quickly found at other areas. The infected areas are known to be the Bras D'Or Lake and outside the Bras d'Or Lake in MacDonald's Pond, St. Ann's and North and South Harbour Aspy Bay, all in Cape Breton.

There are three case studies from Nova Scotia that can be looked at to gain insight on MSX.

Case 1: To look at mortality rate due to MSX in NS, MSX positive and negative sites were selected in the Bras D'Or Lake. Nyanza Bay was the positive site. Chapel Island was the negative control site. Four 100 X 100 grids, separated by 10m strips were surveyed at each site. Every tenth live

oyster was collected by diver, to determine the prevalence of infection, and the site was revisited after 77 days to determine the mortality rate. Sixty-four percent mortality was seen at Nyanza after 77 days. Histology confirmed the mortality was caused by MSX. The control site (Chapel Island) had about 13% mortality rate with histology indicating that these oysters lacked feed and had other parasites that likely contributed to their mortality. Reminding us that even in areas where MSX may occur it is important to determine the cause of mortality and not assume it is from MSX.

Case 2: There were several sites outside of the Bras D'Or Lake that also tested positive for MSX. Oysters had been transferred out of the Bras D'Or Lake to these sites prior to knowing MSX was in the Lake, and it is likely that MSX existed prior to its detection in 2002. This emphasizes the importance of doing surveillance and turning results around rapidly so informed decisions on oyster movements can be made. In St. Ann's, an MSX positive site outside of the Bras D'Or, we removed as many oysters from the bottom as we could with divers. MSX has not been detected again in the area. Since the removal of these oysters MSX has not been redetected and oysters' population in wild beds are quite abundant there at this time. It is notable that the environmental conditions in St. Ann's are very different from the Bras D'Or, so it may not have been the oyster removal that reduced the progression of MSX, but this could be considered as a means to manage it.

Case 3: In the Aspy Bay area, there was detection of MSX in 2003. Oysters were harvested out. This may have lowered the infective pressure. In 2006, MSX prevalence was seen at 3.3% by histopathology with no mortality noted. There was increased prevalence observed in 2010 (16-22%), but no mortality. In 2012, significant mortality began. This exemplifies the fact that the time frame from detection to mortality can vary and in our cases from as low as 17 weeks to 10 years.

There are management strategies that can be implemented. Restrictions on movement from infected areas in Cape Breton curtailed spread. Environment is less easy to control, except via site selection. Results for disease outcomes can be severe, moderate, or mild. An area that is infected may not show clinical impacts or it may show them many years down the road. But this lag time may be shortened, and more disease seen if more infected oysters are brought into an area.

MSX is an endemic disease, but it can be managed with various control strategies and whole industries working together. Surveilling for disease, including the morbidity and mortality rate is critical. Environmental conditions matter. Quick growth, early harvest, and lower salinities will improve outcomes. Off bottom or enhanced growing methods technologies for growing oysters may provide good environmental conditions, enhance immunity, and allow better outcomes. Applied genetic selection to improve MSX tolerance will be an important long-term solution.

Question: For an infected area, could bringing seed in from an infected area speed up the disease?

Answer: It depends. If there is only one infected site in an area, there may not be a lot of infective pressure. And you may want to manage accordingly. Do you want to bring in more? Or manage what you have? It is a different scenario if everything is positive.

Question: Will a dead MSX oyster spread to other oysters?

Answer: Yes. There may be spores in the dead oyster that are released.

MSX in Maine, Industry Perspective

Jeff Auger, VP of Operations, Atlantic Aqua Farms & Nellie Brylewski, General Manager, Muscongus Bay Aquaculture

PDF – https://www.aquaculturepei.com/wp-content/uploads/2024/09/Auger-MSX-in-Maine.pdf

Video - https://youtu.be/-oSqxgXUzX0

The Damariscotta River has been a site for growing oysters for thousands of years. It is currently the highest concentration of aquaculture in the state with about 100 FT employees. The area generates about 75-85% of the oysters in Maine using a variety of growing techniques, bottom and surface.

About ten years ago, at the end of July, a processor noted a few dead oysters in their product. Samples were sent away for analysis, and they tested positive for MSX. Other farms in the river tested their oysters and also came back positive for MSX. Within the next month, the industry started to see 60% mortality and above, with an eventual overall state estimate of 80-90% mortality. Farms harvested as many oysters as they could in August and September. What was not harvested died.

Farmers acted collaboratively, held meetings, and shared information and practices. Communication was key. Tools initially implemented included biosecurity audits and pathogen specific actions plans. But these were not as critical as the communication that occurred between farms. This allowed the accurate identification of the situation and led to increased communication moving forward. It was the growers themselves that requested quarantining of the river from other areas in the state. This facilitated good dialogue between industry and regulators.

At the time, we had two hatcheries on the river which provided seed to the farms. They were able to get disease resistant stock from VIMS and Rutgers, as well as survivors from the outbreak for use as broodstock. The broodstock facility was a quarantine facility to keep in line with biosecurity protocols. At this time, the farms looked into the use of triploids, but the offspring hybrids had faster growth anyway, so triploids were not necessary. Hatcheries currently use broodstock collected from the river crossed with broodstock from lines maintained at universities and using oysters with MSX resistance is the standard used moving forward. A new disease may be on the horizon and the tool to deal with that will be hatcheries.

Currently in Maine, there are three MSX-positive areas with movement restricted to within the zones. Maine has been able to show that they have disease free oysters, which allows export to some other states, depending on the receiving state's restrictions. Bu they are still not allowed to move oysters outside of the restricted zones within Maine. There is increased pressure to do so, in a safe way, for oysters with low prevalences of MSX since MSX is assumed to be everywhere in the state now. Hatcheries on the river, producing disease resistant seed, which are demonstrated to be disease free and have biosecurity processes in place can move spat out of the zone.

To note, the pathology criteria are regulated at the state level, not at the federal level, therefore each State has their own regulatory framework to follow and adhere to.

A nearby state, Rhode Island, allows the import of seed with clean pathology, or growers there can bring in seed to MSX positive areas, as long as the prevalence is not higher than the receiving area. But to support such a program, the testing is expensive.

There is also a Regional Shellfish Seed Biosecurity Program (RSSBP). Proper management practices, that are part of this program, can ensure with a high degree of confidence that seed coming out of a hatchery does not have disease.

Key messages regarding the experience in Maine are the importance of communication. and that hatcheries are key.

Question: I am curious about the biosecurity program to enable transport of seed from a controlled zone to an uncontrolled zone.

Answer: This had more to do with the hatchery itself. Since we were importing broodstock from out of state, we needed to protect local water bodies. We had to redo the broodstock room to allow water treatment in and out (i.e., UV & filters) and we had to invoke a high level of pathology testing through the hatchery and nursery process to ensure we were maintaining healthy stock.

Question: Can resistant oysters pass the disease to clean oysters?

Answer: Yes. Disease-resistant/tolerant oysters can harbour the parasite.

MSX in Virginia, Industry Perspective

Chris Smith, Mobjack Bay Lease Holdings

PDF – https://www.aquaculturepei.com/wp-content/uploads/2024/09/Smith-MSX-in-Virginia-an-Industry-Perspective.pdf

Video - https://youtu.be/2t04d7gzlZE

Mobjack Bay is located about 30 miles from the mouth of Chesapeake Bay. Its salinity runs from about 18-20 ppt. The area has a lot of food availability for oysters. Historically, it has large numbers of wild oyster beds and an active wild oyster fishery with a high level of aquaculture activity. Because of the location and salinity, the area is always subject to disease pressure from MSX (i.e., sporadic pressures) and Dermo (i.e., constant pressures). However, over the past ten years, these diseases have not hampered growth.

A graph of oyster landings reflects the impact of Dermo and MSX on landings due to past disease events. However, there has also been a significant rebound in production, including within: the fishery off public beds that relies on natural recruitment; oysters that are dredged off privately leased bottom, sometimes planted with shell, sometimes planted with seed on shell (remote setting); and finally, intensive cultivation that uses hatchery seed using a variety of gear. Disease resistant broodstock are distributed by VIMS to commercial hatcheries that spawn the animals to produce larvae and seed. Virginia leads the east coast of the US in oyster production, with more than 500,000 bushels a year. This is occurring in the presence of MSX. Wild populations now largely have a degree of disease resistance and cultured populations have been selected for disease resistance.

Processes used on the farm to deal with the disease include using oysters that have been selected for disease resistance. Also, farms keep detailed planting records to be able to track cages to year class and genetics, which are important to know if there is an issue. Third, there is an active disease testing program. Much of it focuses on seed to enable transfers, but there is also a lot of testing on the farm, to know if there is a mortality event that is associated with disease. We know that Seed oysters rarely have a Dermo infection; but it is sometimes seen in larger seed; while MSX has never been seen in seed oysters. In larger oysters, Dermo is in about 15 to 20% of the population; but MSX infections are relatively rare.

The biosecurity program gets state regulators, shellfish pathologists and hatchery operators on the same page to facilitate seed transfers, and it encourages the use of best management practices. This program is now recognized by some state regulators. Small seed reared in a biosecure hatchery that follows BMPs should be considered as a biosecure product. Clean pathology reports for the past three years are also necessary for the Regional Shellfish Seed Biosecurity Program (RSSBP). Finally, detailed record keeping on broodstock source, spawn data, maintenance logs, and others allow trace-back. An audit of the RSSBP occurs annually.

The intention of the RSSBP is to facilitate seed transfers. In the US, there is no nation-wide standard. Management is at the state level. All states require some kind of transfer permit. Requirements for these transfers require careful management of disease testing since there can be a lag between testing and the report and different states have different standards for how long a report is valid (30-60 days). Relationships between growers and regulators are necessary. Generally, bio-secure seed is small seed (i.e., retained on a 1 mm screen). However, this means a nursery stage (up-weller) is necessary for recipients of seed. This can be a bottleneck.

There was a recent mortality event (2023) in a 2022-year class found at a grower who uses on-bottom culture. It was originally thought to be a mortality event associated with high summer temperatures. But in the fall, the grower noticed high mortality (70%) in cages, with no growth in the oysters. Samples were sent for assessment and came back positive for MSX. Records were traced and showed that the stock was a low salinity lineage that does not have much resistance to MSX. Fortunately, the next year's seed was a different stock, with MSX resistance. This stock is doing well and is being harvested now. At the time of this event, public and private fishing grounds were also experiencing mortality at 10-15% above normal, but public harvest numbers were still good in 2023.

Another event occurred at a spat-on-shell producer with mortality across several leases grown in low salinity water. One lease in particular had high mortality noticed in September, but the event likely occurred in April or May. It was a dry year, and it is likely that higher salinity water allowed the pathogen to move farther up the bay. The plan is to use higher salinity, resistant lines moving forward.

Three takeaways from the experience in Virginia include the following: 1) Virginia has a thriving oyster aquaculture industry and wild oyster fishery in the presence of pathogens; 2) documentation, record keeping and monitoring for disease can catch problems early; and 3) resistance via breeding or via natural selection are critical for a thriving oyster industry.

Question: Do you focus on oyster broodstock that is close to where you want to grow it, so you do not have surprises in your outcomes?

Answer: Regional adaptations to the environment are important. And resistance in one area may not be the same in another.

Question: Is all of the breeding program being done through VIMS? Rutgers is available too. Are there others?

Answer: We do some in-house. Most of the larger hatcheries do some in-house breeding. There is a Northeast Breeding Consortium that has been put together recently.

Question: Do you do triploids or diploids, or both? What is your opinion?

Answer: In Virginia, triploid production is the vast majority (i.e., 95%) of the stock grown in gear. We find there is a growth advantage, and there are marketing purposes. But triploids need lots

of feed and are not suitable for all sites (i.e., difficult to rear in variable conditions, need near "perfect" conditions for good growth). So, the use of triploids should be a site-to- site decision. In other states, fewer triploids are used.

Answer from Jessica Small: All of the diploid and triploid resistant material is available to hatcheries. We typically distribute broodstock in the fall to hatcheries.

Breeding at ABC: Past, present and future directions

Jessica Small, Director, Aquaculture Genetics & Breeding Technology Center, VIMS (virtual)

PDF – https://www.aquaculturepei.com/wp-content/uploads/2024/09/Small-ABC-Breeding-Presentation-08202024.pdf

Video - https://youtu.be/wNMF1WJ9u8Q

The appearance of MSX and Dermo really devastated the industry, and the state of Virginia recognized this. Through a legislative initiative in 1998, the Aquaculture Genetics and Breeding Center (ABC) was created at VIMS.

Early years focused on bringing in different stocks that were disease resistant. VIMS got some of the MSX resistant strains from Rutgers. Dermo exposed/resistant stocks were imported from the Gulf of Mexico. Over a few years, these lines were propagated and in-water tested (i.e., planted 50,000 individuals and hand-picking the survivors after elapsed time). They were also crossed to each other. This resulted in many different lines. But managing the data and crossing and raising the different lines was untenable, so the lines coalesced based on genetic background. This resulted in three lines: DBY (Delaware Bay), XB (Cross Breed), LA (Louisiana). Between 2008-2014, these lines underwent mass selection (growing out large numbers from each line in an area that was intentionally stressful, due to disease pressure, low salinity, and other stress conditions). The best of the offspring was hand-selected and bred. Their offspring were distributed to industry for use as broodstock. This was very successful. The selected stocks showed better growth in all environments and improved survival under disease pressure (both MSX and Dermo), relative to wild stocks. Today, these mass selected lines are still available. Some mass selection is still occurring, but at a lower intensity than before.

Family breeding also occurs and has occurred since 2004. This incorporated the mass selected lines (superlines) as well as wild founders from a number of sites. Wild material was crossed with the superlines to create families, where each family is a group of individual oysters which share the same parents (one mom, one dad). This allows determining what benefits are conferred by a single parent. Theory associated with quantitative genetics is applied to calculate estimated breeding value (EBV) based on phenotype in the field and family information. A high level of record keeping is required to enable tracing families/pedigree and how they perform. This all informs parental selection for the future.

There is a “library” of genetic information kept in the field. Up to five different year classes are available. Parents selected for crosses are based on estimated breeding values (EBV) and a multi-trait selection index. Inbreeding must also be considered when trying to manage gains. This is done via a theory called optimal contributions. Typically, about one hundred diploid families are spawned. The testing locations include low salinity (down to 5-6 ppt) and multiple moderate salinity sites with disease pressure. These are grown out for 18-30 months then brought back in for assessment. The traits of interest include survival, growth, meat yield, and shape. The value of each of these traits are weighted, with survival and growth weighted highest. There is a strong genotype-environment interaction seen so that the traits have to be tracked according to their environment (low versus high salinity). Genetic gains can be seen relative to founder populations.

The intention of the family program is to make lines that are improved, even relative to the mass selected material, since in family breeding there is a more precise ability to manipulate traits. The top five families from the moderate salinity areas are bred together to make a best line for a moderate salinity environment: HNRY (“Henry”). Similarly, this is done for low salinity areas, creating a LILY line. These lines are incrementally improved every year.

Triploids are widely used in the Viriginia and Maryland areas. Tetraploids are needed for the creation of triploid stocks and both tetraploid and triploid families have been created to enable determination of genetic correlation between triploid and tetraploid traits. This helps to select which tetraploid parents will most likely confer desired traits like growth to triploid offspring.

ABC is currently also working on finding genomic tools, like genetic markers, to assist with selection. A SNP (single nucleotide polymorphism) tool has been developed to screen wild populations and selected lines and develop a 66K breeder’s array. This 66K array has been used for the past two years and been used to genotype about five thousand oysters that have been phenotyped (known growth, meat yield, shape). We have also developed protocols to tag and non-lethal biopsy broodstock candidates.

The use of genotyping enables getting individual information on traits of interest. This can more precisely select potential broodstock, since it allows selection on an individual level, instead of on a family level and avoids the variability of performance seen at a family level. Ultimately it makes selection of broodstock more precise.

At ABC, disease monitoring is not regularly conducted. ABC uses survival in a disease-rich environment to assume disease resistance. In the future, ABC will select families and challenge them in the field where Dermo and MSX are endemic. Oysters will be closely screened for survival in the field, pathogen prevalence, and host response. The hope is to learn how to breed for pathogen specific resistance/ tolerance for both Dermo and MSX.

Question: Is there any possibility of importing biological material to here from VIMS or Rutgers? E.g., to cross and MSX resistant VIMS oysters with a wild oyster from PEI to accelerate the production of an MSX resistant broodstock? It may be a bad idea, as you would want to ensure you keep as many local PEI adaptations (i.e., cold water) as possible.

Answer: This is not a bad idea. You have material in the field that has gone through a disease event. But it should be done in a controlled manner (i.e., biosecure facility) to properly compare with wild stocks.

Question: How long would it take to generate oyster varieties that are resistant to diseases? Would there be trade-offs?

Answer: We saw significant improvement regarding survival in three generations. Consideration of trade-offs is important. You have to be careful. Something with high resistance may have other undesirable traits – e.g., may not be marketable, may be slow growing. So, you have to balance trade-offs.

Question: When you do field trials, is everything that survives considered resistant, or do you assess prevalence to get a measure of exposure?

Answer: We have ancillary information regarding Dermo and MSX pressure at a filed site but have not been looking at an individual level. A new project will look at things at an individual oyster level to determine if the survivors actually got the disease.

Question: How would MSX resistant individuals be selected: from the field or by laboratory settings?

Answer: Laboratory trials of MSX are not typically very successful, because of the intermediate host issue. Survivors from a field exposure will also be exposed to more than just the pathogen. There is more confidence that the oyster will do ok in a real-world situation if selected from the field.

Day 2: Hatcheries and Research, Presentation Summaries and Panel Discussions

PEI Task Force on MSX

Kim Gill, Director of Aquaculture, DFTSC

PDF – https://www.aquaculturepei.com/wp-content/uploads/2024/09/Gill-Task-Force.pdf

Video - https://youtu.be/gzikkXBktBA

When MSX was found earlier this year (July), DFTSC looked to the potato wart experience. This included having the province coordinate communication with industry until eventually CFIA became engaged. This has evolved to once-a-week stakeholder sessions with CFIA. The province's role has shifted and resulted in the formation of the MSX Task Force.

This task force is co-chaired by DFTSC and the DFO Area Office and has as main members the industry groups: PEI Shellfish Association, PEI Aquaculture Alliance, PEI Seafood Processors Association and Oyster Processors Association.

The purpose is to keep open communications with industry, including communicating up to CFIA, and to support industry around adapting to MSX. This communications role will continue. Any drop in the marketplace will be managed if it arises. The research and development piece will be key. There have been a lot of suggestions for R&D that should get done. The task force will try to coordinate this to make sure there is no duplication and to make sure research is done for all of industry. This group will also tackle emerging issues, such as leasing, particularly for processors and other issues that arise.

Meetings are twice a week with variable agendas. One of the positive outcomes has been the identification of CFIA contacts for industry to allow consistency. This has been very helpful. Media or communications requests are also handled. Marketing and other industry concerns will continue to be discussed.

Moving forward, there will be more communication, more research and development coordination, and handling any industry needs that pop up.

Question: When should industry expect to hear about results regarding what they can do in the future regarding seed (i.e., transfers)?

Answer: Do not know. This has not yet been looked at in this group. It is a bigger question. Some questions may need to get passed to someone else. But it is a recognized industry need.

Question: What we need now will be different than what we will need years from now. If we start building today (i.e., broodstock program, hatcheries, etc.), we are 9-10 years out before we have

resistant seed.

Answer: Yes, it will take time, but hopefully shorter than 9-10 years.

Answer from Peter Warris (ED of PEIAA): Yesterday and today is the start of that response which needs to be collaborative with the sectors.

Supplying Safe Oyster Seed: A commercial perspective from Virginia

Michael Congrove, Oyster Seed Holdings, Inc.

PDF – https://www.aquaculturepei.com/wp-content/uploads/2024/09/Congrove-PEI-MSX-Workshop.pdf

Video - https://youtu.be/qkmv2_t5e40

Oyster Seed Holdings is in the mid-Atlantic and deals with MSX and Dermo. It is strictly a hatchery located in a small, protected bay with close connections to the main stem of Chesapeake Bay and the mouth of a river. We sell over 100 million single seed annually and more than 750 million eyed larvae annually. In addition to producing product for sale, we are dedicated to the hatchery craft and pushing forward operations in hatchery technology in general and solving problems as they come up. Oyster Seed Holdings also does advocacy for the industry, including having a food truck that serves farm raised oysters to promote customers and provide a hatchery-to-farm-to-table experience to advocate for aquaculture as a valuable use of a shared space.

Production here uses stocks predominantly from ABC with a high proportion of triploids for highly productive areas. The breeding work has been very important for the mid-Atlantic region. The resistance now found in the wild populations is a lot more recent than in cultured stocks, because cultured stocks had selected strains to work with. The volunteer biosecurity program (RSSBP), including third party audits, has been important. But we still have long term pathology screening program in place because of the interstate transfer process. Primarily, transfers of seed are “like” to “like” for disease status but there are other possible hatchery supply paradigms, including: no pathogens at either the hatchery or the farm; no pathogens at the hatchery, but maybe at the farm (still low risk transfer); pathogens at both the hatchery and the farm – requires more oversight to consider disease prevalence considerations; and finally, potentially, transfers from hatchery in an area with pathogens to an area without. The biosecurity program can accommodate all transfer paradigms, except the last one which requires more caution. But we are trying to explore this potential, to expand our market. The Gulf of Mexico is a potential market that is seed limited which we could supply, but MSX is not endemic there. So, we are verifying a safe protocol for safe transfer of seed by working with a bio-contained facility in that area. Gulf broodstock and Virgina broodstock have been conditioned at the hatchery, spawned and larvae reared up to a bottle nursery stage, which is a very small size – so at a low risk for contracting disease. These larvae are sent 1-day post-set and 7 days post-set to the biosecure facility, with disease sampling at both ends of the transfer. We are also tracking survival. All disease testing

has tested negative. But there are questions remaining to create this safe/effective protocol to be confident in the disease screening techniques utilized.

Another potential we are exploring to overcome the restriction of moving seed to an area with no pathogens is to use a portable hatchery facility to rear larvae at the receiving site. We have a prototype and have operated two of these. There are situations where this could be useful, but there are recognized losses in economy of scale.

Mallet Oyster Hatchery: Present and future solutions for the PEI Industry

Martin Mallet, Co-owner & Hatchery Manager, Mallet Oysters

PDF – https://www.aquaculturepei.com/wp-content/uploads/2024/09/Mallet-Oyster-Hatchery.pdf

Video - https://youtu.be/V24ChLEngFw

The first seed from Mallet Oysters was sold to PEI in 2015, and there has been a continuous presence ever since. Seven years ago, at a similar workshop in PEI, Mallet Oysters shared that hatchery seed was already a commercial reality in the Maritimes, but with the precaution that nurseries were a limiting factor with regards to hatchery seed use, and that hatcheries need reliable customers. They cannot only be used in times of low wild spat supply. Communication with the hatchery regarding needs is necessary because of planning that is involved in hatchery production. This same message is repeated today.

Based on conversations, PEI should expect to need about eighty million for off bottom culture and forty million for the fishery. The current supply of seed is probably about thirty million, already supplying a significant fraction of the off-bottom industry. So, there is already a commercial seed supply occurring. And, as stated seven years ago, nursery capacity is still the limiting factor. At Mallet Oysters, capacity for seed production is eighty million of 1 mm graded seed, but the nursery can only hold thirty million. Current PEI nurseries can probably hold about thirty-five million, leaving a twenty-five million gap in nursery capacity based on current hatchery production at Mallet Oysters. Another necessary consideration is that there is local expertise being developed regarding handling hatchery seed. This is important since hatchery seed is very different from wild seed and expertise is required.

In the short term, with MSX, we can speculate that wild set and hatchery seed will continue to supply the industry. But if hatcheries need to produce more, they need to plan for this and the best time to order seed for next year is generally the fall previous.

For the medium/long term, development of MSX resistant stocks will be important. Breeding for resistance can occur via letting natural resistance to develop, but this is difficult to predict. Hatchery assisted (selected) breeding can accomplish this faster. Mallet Oysters has been running a breeding program for a decade, with a fourth generation now available. Results of cohorts in

the field, show high growth with good shape, and estimates have reduced growth period by a year. But we do not have good, controlled growth trials in specific areas with a baseline control strain for comparison. For Mallet Oysters, we are looking to build on the increased growth seen in the existing strain since faster growth is already a mitigation strategy for MSX. We also want to incorporate MSX resistance into the program. Our breeding program structure will allow for doing this, even without moving animals out of the PCZs.

This selection will be enhanced using genomic tools. Mallet Oysters is completing a project that developed an SNP chip, genomic selection models specific to our breeding program and a wild population study. Results so far indicate low inbreeding with high SNP heritability for measured traits, indicating that our program will respond well to selective breeding. The wild population study looked at oysters throughout the gulf region and shows genetic similarity between areas, including between areas of PEI and areas in NB.

Things to consider include the fact that breeding takes time, so containment of MSX will be important to give time to develop a resistant oyster. Collaboration will be important, and information should be shared, where possible. Most of the questions will require fieldwork. There will be a need to coordinate this and share protocols. Also, success is not a single strategy with a single winner. There are lots of ways for competitors to work together with broodstock licensing as an example of how this could play out.

The genetics of disease resistance

Tiago Hori, Director of Innovation, Atlantic Aqua Farms

PDF – https://www.aquaculturepei.com/wp-content/uploads/2024/09/Haori-Genetics-of-disease-resistance.pdf

Video - - https://youtu.be/tI4HTBrOWrU

All organisms have an energy budget that has to be divided between essential things and other things. When there is a demand that is not predicted, the animal has to adapt. Disease is one of those unpredictable stressors that will require adaptation and an unexpected demand for resources. The oyster may increase its food consumption to compensate, but this ability may be limited. So, instead the oyster will limit other things as trade-offs. This is why the environment is so important. Environment is the main drag on energy and is very inconsistent between regions and within regions.

Disease resistance is not a trait that will be consistent between environments, and it is very complex. We have to think of things in terms of the environment and the cost that it takes to be resistant. The cost is often growth. And this can be highly variable. So, it may be best to have multiple lines and multiple breeding programs to ensure that the industry can prosper in the presence of MSX.

Optimization can be achieved in many ways and be the result of many smaller optimizations steps. This is likely the case for MSX resistance which will not only be resistance to the parasite, but also the resistance to the environment. Disease resistance is one of the most complex traits. Different environments will require different breeding programs. Selected animals will not perform the same way in different environments. Therefore, origin of broodstock is very important.

Breeding programs are expensive and are a long-term commitment (i.e., 20+ years) and ownership in genetics is challenging. This question should be addressed honestly and up front to figure out the rights to use the genetics and licensing fees. And protecting interests does not preclude cooperation.

There are a couple of models as to how breeding programs could work for shellfish. In the US, there are breeding programs that supply hatcheries with broodstock with agreements and fees in place for the use of the seed. In NZ, there was a government funded breeding program for mussels that then got passed to industry and is now in the hands of a single company.

The separation of the breeding program and the hatcheries may be a good thing since both need to be established, and they are two very different things. There is no point in having selected lines without hatchery capacity. Also, hatcheries fail so it is essential to have redundancy in hatchery capacity. And do not forget that nursery capacity is the bottle neck. In PEI, we should also consider remote setting. We can produce a lot more eyed larvae than seed. Some of the limitations highlighted include production capacity, performance assessment capacity, maintenance of multiple family lines, and nursery capacity.

This will need to be a cross province and cross industry effort. We have to be frank and honest about questions regarding IP and who pays for the development so it is fair, and things can move forward quickly with minimal conflict.

Bideford Shellfish Hatchery, Lennox Island First Nation

Adrian Desbarats, Ulnooweg Development Group

PDF – https://www.aquaculturepei.com/wp-content/uploads/2024/09/Desbarats-Bideford-Shellfish-Hatchery.pdf

Video - https://youtu.be/I3SR_f_y2js

Lennox Island First Nation is the owner of the Bideford Shellfish Hatchery. This facility is located in Bideford, PEI. It has a long history, was originally owned by DFO, and later the province. It was instrumental in helping industry recover from Malpeque disease. After this, the hatchery was not operated for some time, then handed over to Lennox Island which restarted the hatchery to stabilize and strengthen the oyster aquaculture industry.

The hatchery is currently about five thousand square feet but is in a building that has room to expand. It was originally designed as a static system that could produce ten million seed/year in

multiple batches. It was converted to flow through to increase larval stocking densities and an improved algae production system was installed to increase production capacity to thirty million per year in two batches. There is space to increase capacity to sixty million per year provided there is investment in additional down-wellers and algae production capacity.

Incoming water goes through a series of filters down to one micron and is heated and UV treated. All critical components are redundant to reduce the possibility of mechanical failure. Additional temperature control can be applied. Lines in the hatchery are pressurized to allow water availability on demand, and there is an additional UV treatment prior to the water going to the algae and larvae. There is a wet lab, a carboy room, an algae/larval room, a nursery room for everything post-set with down-wellers and then up-wellers. The seed leaves the hatchery at 1-2mm in size.

Lennox Island also has a grow-out operation and has reached a critical mass and technical staff.

The breeding program required for MSX resistant seed will provide other opportunities including producing triploids to increase growth rates and avoid drops in market quality due to spawning, providing resilience in the face of other diseases (e.g. Dermo or others), and providing more consistent supply in the face of climate change.

Hatchery Panel Discussion

Tiago Hori, Martin Mallet, Adrian Desbarats, Michael Congrove (virtual), Chris Smith (virtual), Jessica Small (virtual)

Video - https://youtu.be/9KGymE9jULE

Question: Any idea what the receptor in the oyster is which permits infection by the parasite?

Tiago: This is an example of something that will require people to work together. We know very little about this bug.

Martin: The precise mechanisms of infection and resistance are not known. But this is more of a "nice to have" piece of information. Focusing on the outcomes (survival) have been successful for other breeding programs so this should be our focus. Knowing the mechanism of infection will not impact how we go about breeding for resistance.

Tiago: A lack of understanding provides barriers, but we can still produce resistant lines.

Question: What is the capacity of the trailer hatchery? (to Michael Congrove)

Michael: The design capacity is for ten million seed per season (750,000 750 um -1mm seed per run, one long season). But none have met this goal. So much is dependent on the site, and it takes some time (a couple of years) to figure out how to operate a hatchery on a site to get to capacity.

Tiago: I would expect it to take up to three to five years for a hatchery to reach capacity consistently, if starting from scratch.

Adrian: That assumes experienced staff.

Chris: That is also my experience. Getting a hatchery off the ground and working well does not happen overnight.

Question: There is no lab/disease model, but it is not impossible to develop one. How much will it support shortening the time frame to develop a resistant line?

Tiago: I do not see that a disease model will accelerate things, but it will be important for helping to understand environmental effects, pathogenicity and other things.

Jessica: One of the challenges is the intermediate host issue. Models for infection with other parasites are easier. The intermediate vector with MSX makes things challenging and this needs to be understood.

Martin: What do lab trials add to the operationalization of a breeding program?

Jessica: Lab trials at ABC have been in collaboration with others and my program has not used them for selection. The families that have been trialed in the lab for Dermo do not show a strong genetic correlation for survival in the field. There is value in lab trials, but it will never replace a field challenge. I am not sure yet how to incorporate lab trials.

Question: What can farms do to make the pathogen less resistant to surviving?

Chris: Moving farm to a lower salinity location has been discussed. But the scale is probably impractical for most. Some producers have done this – moved their farm to a low salinity location. Another option is to grow faster oysters to get to markets faster before the disease impacts the oysters.

Question: There is no low salinity here (PEI) – we are mid 20's. What about air drying? Will that have an effect on the pathogen?

Chris: If the farm practices affect the intermediate host, it may interrupt the infection cycle. But we do not know.

Question: Tiago, you mentioned it would cost about a million a year for the hatchery? Has anyone been in contact for these projects?

Peter Warris: That is why we are here.

Martin: Tiago was referring to costs for a breeding program and there is funding for that via a variety of agencies.

Adrian: To get money to do a one off would be relatively easy. But a breeding program is long term, and it is unclear how this gets funded. Interested to hear from Jessica how this gets funded? Is it private/ government?

Jessica: For ABC, the state gives about 53% of the funding. The rest comes from revenue from licensing broodstock and grant funding. We need royalty revenue to operate the breeding program. Rutgers has a licensing structure as well. Amount from licensing varies according to production but is usually about 20% to 25% for ABC. Not an ideal funding structure.

Question: If MSX hit hard, seeing 80-90% mortality elsewhere, has anything been put together in case that happens?

Peter Warris: Not at the moment. We have spoken to both levels of government about that, but it is difficult to ask for support for an impact that has not happened.

Question: This could happen anywhere from weeks to years.

Peter: There is a clear demonstration of support from the provincial and federal governments that they will be there to support the industry. If/when that starts to happen, the awareness and need should be there, hopefully to enable to pull the money together quickly.

Martin: We have talked a lot about hatchery need but have not talked a lot about the hatchery demand side, which is important too. Who is buying the seed, what price are they willing to pay, etc. There is risk on both sides, and we need to focus on the demand side as well. We are where we are because we have steady, good clients that have supported us. How does that look in an MSX world where the grower could lose their income? Will they be buying seed? How does that translate to how hatcheries operate?

Question: How many generations of natural selection will it take to get resistant stock?

Tiago: If we require three generations and have a two-year generation cycle, need six years to get 80% resistance. In the wild, this is hard to predict since it depends on the selection pressure.

Question: What can be done to reduce this time?

Tiago: Oysters that are not challenged can dilute the population with non-resistant seed. This may not be a problem here (PEI) where do not see salinity variations. But we would not, on purpose, play too much with the pathogen. We are really at the mercy of the pathogen which is unreliable.

Chris: In terms of wild stock restoration, often want the genetics to be from the area that is being restored and many groups do not want to introduce genes from selected stocks.

Tiago: Waiting for natural resistance is not necessarily a viable strategy. Natural resistance can vary across the population, like seen in the Bras D'Or Lake example.

Question: What is the potential for using hypochlorous acid for water treatment or treatment of MSX contaminated oysters?

Tiago: From a hatchery perspective, I would never use bleach on intake water.

Adrian: Lennox has one micron filtration, followed by UV treatment and we would do this every time. This process is supported by literature. I would never put anything in the supply line like ozone, hypochlorous acid, any of those things, because you have to get rid of the residual which could otherwise kill the larvae or algae. It is not worth the risk.

Question: Is there, within the Atlantic Region, currently a hatchery or hatchery technician training course?

Adrian: There are some programs and some capacity like at Dal in Truro. But we need something more translatable to industry.

Tiago: VIMS has training courses which may be a good fit for right now. Looking forward, training locally would be good.

Jessica: This VIMS training goes through my program on an annual basis and there is a model to bring people in from outside of the US for short training courses. The typical program is 5 months and includes training and internship, often with industry.

Chris: There are a lot of people in the industry that have taken that program. It is excellent.

Genome Atlantic

Britta Fiander, Director Innovation Programs, Genome Atlantic

PDF – https://www.aquaculturepei.com/wp-content/uploads/2024/09/Fiander-Genome-Atlantic.pdf

Video - https://youtu.be/zESFxnbrlgs

How do you determine the research solution and put it in the hands of industry? I'll describe how Genome Atlantic works to explore those initiatives.

Genome Atlantic is a not-for-profit, funded by provincial and federal governments. So, we can work with industry at no cost. Our main mandate is to connect industry with genomics technologies. There has been about $150 million dollars in regional R&D investment to date involving company to company and company to academic collaborations.

Genome Atlantic has worked with oysters and shellfish, in general, including having worked with L'Etang Ruisseau Bar and Atlantic Aqua Farms for selective breeding, Verschuren Center for rapid detection of disease in oysters, and the use of environmental DNA for pathogen or invasive species surveillance.

Genome Atlantic does not do research but connects researchers or organizations with industry or the people who need the tools. We can help get a project up and going and put it into place. This may mean conducting research to develop new tools, or using already established tools. Genome Atlantic can help with concept development and partnership development. We specialize in funder identification to determine how to fund a program. We support peer review of a proposal and can do a friendly review, and can help manage the process and timelines, providing project management support to keep projects on track. This includes making sure that the benefits intended for industry land in the hands of the industry. Genome Atlantic can also provide assistance with collaborative research agreements, IP agreements and licensing.

We connect you with both funders and the people who can do the work. We work with all sizes of companies in a number of different ways, whether to participate in a project or enable you to simply observe what is going on in a project.

Current Research and Future Ideas in the US

Ryan Carnegie, Professor of Marine Science, VIMS

PDF – https://www.aquaculturepei.com/wp-content/uploads/2024/09/Carnegie-ResearchPriorities-FINAL.pdf

Video - https://youtu.be/1-4_LLGVXww

The first research question is what is, this intermediate host. Our inability to identify this is limiting our ability to manage this disease. It may look like it there have been 67 years of no success, but we have not been looking for the host for this long. Most of the early period was in the pre-molecular era when we did not have the tools to look at the environmental essays to search for the host. Also, the success in developing resistant lines did not incentivize searching for the intermediate host. There is a limited amount of funds going towards mollusc health and it has not been focused on the intermediate host. In Canada, the heterogenous distribution of the pathogen in space (especially the evidence for microenvironments that seem to be more conducive to the disease) and the advanced tools now available represent a new opportunity to research the pathogen and find the intermediate host.

It is also important to have a . d understanding of our tools for detecting MSX and what outcomes of testing really mean. PCR tools are generally more sensitive at detecting MSX. But, if we want to look at the dynamics of the disease in the oysters, we are not sure what the PCR results are telling us. We can generate a lot of PCR-positives without necessarily seeing histological positives in the oysters. PCR-positives can be generated in molluscs that we know do not get infected by the parasite. We need to be clear on how we are applying these tools. Are PCR positives that are not showing histological impacts actually infections that are meaningful with regards to understanding how the disease is working and with regards to how we are managing the disease?

What is the role of environment driving epizootics? Temperature and salinity clearly impact MSX dynamics, but what else could be happening? Trends in MSX prevalence in naïve oysters, relative to resistant oysters in an MSX hot zone suggest that the intermediate host is doing better and better in the marine environment today, maybe due to climate warming. Wherever MSX hangs out is doing better over time in these disturbed, eutrophic hypoxic systems. What is the role of this in driving outbreaks? Is it simply stressing the oysters more, or is it creating a more favorable environment for the intermediate host? Are there aquaculture practices that are more or less favorable to outbreaks?

What is the basis for resistance evolution in natural populations: Wild oyster samples in James River show that MSX prevalence boomed early on, then decreased, then increased again. And resistance obviously developed. What is the genetic basis for this resistance in wild oysters? What is the molecular basis for this evolution?

What is the role of the oyster in transmission? Are oysters themselves capable of transmitting the parasite? We do not know. We rarely see sporulation in oysters. We cannot directly transmit spores from oyster to oyster. We do not know that the products of the infection in the oyster go anywhere in maintaining the parasite life cycle. There are indications that the parasite can cycle in the absence of oysters. It is important to understand this to effectively manage the disease without putting unnecessary obstacles in the way for reasonable commerce.

Can eDNA monitoring be useful? All of the molecular arrays for pathogen detection have been developed for and validated in their oyster hosts and not in complex marine systems. Environmental DNA (eDNA) may enable us to economically, efficiently and rapidly understand the dynamics of the parasite in the marine environment.

Using a screwdriver for a chisel: using tools for other intended purposes

Rod Beresford, Associate Professor Biology, CBU

PDF – https://www.aquaculturepei.com/wp-content/uploads/2024/09/Beresford-Future-Research.pdf

Video - https://youtu.be/LhmIcXxjf7E

There are a number of research questions to be examined for the MSX puzzle. When doing so, it is important to keep an open mind and listen to others, both in terms of what they know and in terms of how they can contribute in collaboration to improve work. Collaboration will be important. With that in mind, here are a number of research questions being worked on in our lab.

Sampling for MSX involves not just sampling a portion of the population but also a portion of the animal. With MSX, there are sometimes localized infections, meaning it can be in one spot on the oyster and not through the animal. With such localized infections, could a representative

tissue sample miss it? In order to examine this, we looked at homogenizing the whole animal, then sampling the homogenate. Preliminary results suggest that this may result in a higher number of positives than if just a representative tissue sample is used. This will be repeated with a larger sample size.

In looking for eDNA to look for MSX, there are many questions to consider. How should you collect it? How long should the collectors go out for? Where should you look for it? Could the DNA degrade? Could the presence of a spike in another organism predict a spike in MSX? Early trials using whiffle balls filled with cheese cloth as collectors have successfully detected oyster DNA. This needs to be explored further but could be a very useful tool.

Can we detect MSX using a rapid PCR test similar to what was used to detect COVID in wastewater? It may be used as an early warning for the presence in the environment, prior to an outbreak. But extraction of DNA is proving to be the tricky part since these tests and the processes used typically are for bacteria or viruses, not protozoans. Procedures for this are currently under development. Ideally this could be used as a field test with rapid return of results (3 hours).

When holding oysters, can a thermal treatment practically be used to reduce mortality? We have shown that a thermal treatment in the lab can reduce prevalence of MSX. Could this be combined with other parameters to increase its effectiveness? And does this knowledge have any use on a large scale?

In a 3-year trial conducted at 12 sites across the Bras D'Or Lake, suspended culture reduced mortality in the oysters in most sites. There was some variation, but this may have been due to variation among the sites, or due to husbandry differences. There was a trend which needs to be explored further.

On the hatchery side of things, we have purchased and are starting up an Ocean-On-Land, self-contained hatchery. This phase of production will bring new questions. One that we are working on is whether there are more optimal local algae species for the hatchery phase.

As some final thoughts on research, it is important to recognize that everyone has something to contribute – industry, regulators, researchers. In particular, there is invaluable information from people who are on the water every day. Let's work together and pull on the same side. Collaboration is very important and keeping eyes and ears open is important. There will be short term and long-term successes and failures, but this is the long game, and it will take some time to know what is really happening.

Research Panel Discussion

Rod Beresford, Tirosh Shapira (Scientist at ONDA), Ryan Carnegie. Jessica Small

Video - https://youtu.be/7g5qBfByYRM

Question: Has anyone looked at changes to water inputs from the human side? Could on-land behaviour changes have suppressed something that previously competed with the parasite?

Ryan: We do not really have a good sense of how these other influences may be playing a role in disease trends. It may now be possible to get a better handle on this through research and long-term data sets and modelling. But we have not done this.

Rod: If you look in the Bras D'Or where oyster leases often are, there is very little human activity around them. They are pretty isolated.

Question: There are areas close by (e.g., across the water in NB) that are not testing positive. If you had to guess, would you say the intermediate host is not present, or are they not infected yet? And can that be used in the search for the host?

Rod: It is a combination of many things: is the parasite present; is the parasite present in something; is the parasite present at high enough numbers to cross a threshold; are the conditions right? It could be a combination of any numbers of things. I do not know.

Question: Has there been an effort to incorporate quantitative methods, looking at epigenetics or RNA-level things?

Ryan: Regarding epigenetics: it has been a blind spot in the US for MSX. There is only one lab working in the mollusc realm with epigenetics.

Jessica: I am not personally looking at this, but, tangentially, there are people looking at it at VIMS in the sense of the difference between triploids and diploids and diet stress. It is going on but is not being incorporated into the breeding program.

Ryan: Are we getting any closer to understanding the loci that might behind resistance?

Jessica: I am not aware of any studies yet. We would like to start this. This has been done with Dermo challenges. But it is unlikely we will find a strong single marker. Disease resistance will be polymorphic loci of small effect. But the studies are in progress.

Question: There have been many papers regarding low salinity. Would land based holdings using low salinity water be a possible mitigation?

Rod: We did not do low salinity work since we focused on things the growers could incorporate at their farms. But in Nyanza Bay, where there is the lowest salinity at times due to influences of a couple of rivers, oysters on the bottom all died. Oysters at the surface also had some mortality.

Question: As a disaster management plan, could we put oysters in a low salinity land-based facility?

Rod: I do not know. It may be worth trying. And there may be an opportunity to combine temperature and salinity.

Adrian: Regarding the land-based approach, we have to be pragmatic. Would this be feasible?

Question: How far along are we to identify the secondary host?

Rod: We do not know.

Question: what about doing the genome of the MSX?

Tirosh: The question is how helpful would it be? Are there better places to invest money, for example for better detection systems? Would this be more useful? It can be done but depends on how much money and how many people we put on it.

Question: Was the MSX removal technology validated?

Rod: It was repeated several times. Sometimes MSX remained detectable, sometimes it did not; but the prevalence dropped, and the oysters survived.

Question: Is there anything that can be done to reduce the amount of MSX in the water, or kill it?

Rod: We know the parasite was at all the locations we tested in the Bras D'Or Lake. Eradication is not an option, so you need to ask how do you produce a saleable product? How do you keep the farm operational?

Question: Can air drying longer help? Or air drying right away?

Rod: Cages were flipped every two weeks in the Bras D'Or experiments. This may have had an effect. Oysters in the fridge for four months also showed reduced prevalence.

Question: Oysters go dormant for four months but are exposed to the bottom in fall and spring.

Rod: There were positives on the top and bottom. The difference was the mortality.

Question: You can have MSX for years and have no mortality?

Rod: Yes. If you look at one of the areas in the Bras D'Or, there has been MSX in the area for years with no mortality, but the prevalence has remained low. The Bras D'Or in general has a salinity of 18-22 ppt.

Question: For surface culture, would putting in fast growing, clean seed give a chance of success? Some hatchery seed will produce 60% of three inch or larger oyster in less than 2 years. Would this give a better chance of success?

Rod: On bottom, in the Bras D'Or, it takes 6-7 years. Almost all oysters grown at the surface reached market size in 3 years. If the oysters grow faster and do not get so infected they might be harvestable before they die. This might be an option.

Question: One of the things that will hold people back from hatchery seed is the cost.

Rod: Consider that for the past three years, in the Bras D'Or, people tried to collect seed, and there has been none.

Question: Have any other models been looked at in other parts of the world? There could be two or three intermediate hosts. Is there a similar model elsewhere that could be used to help solve the MSX life cycle model?

Ryan: There are a lot of possibilities. For haplosporidians, there is no life cycle that has been solved. There is limited information.

Question: Can Rod speak to the value of broader collaboration and buy-in by individuals versus small individual projects to try and address a problem that has broader industry application.

Rod: I cannot emphasize enough about the importance of knocking on people's doors. Someone has to extend the question. You get ahead by sharing plans, making partners. There needs to be an Atlantic regional strategy.

Tirosh: The collaborations when executing the experiments are great. But collaborations should also come when forming the research questions. Otherwise, the research will go nowhere. We need to establish the priorities to justify the investment.

Industry Priority Identification

At the end of each day of the workshop, participants were asked to identify key priorities and record them on a form. Suggested categories for these priorities included research, economic support, programs, adaptations to new equipment, and adaptations to existing processes. The intent was to identify questions that need to be answered in order to help businesses operate, whether in the fishery, in aquaculture, or as a processor. Participants were asked to categorize each priority according to the industry it is most applicable to fishery, aquaculture, processor. The recorded priorities were presented to the room at the of Day 1 and the sheets were collected. On Day 2, this request was repeated, but directed at industry only, with the intent to identify the top three priorities for moving forward. A synopsis of these activities follows.

Primary priorities

Raw listings of identified priorities are attached as Appendices B and C. These, as well as discussions that occurred through the workshop, were used to identify primary priorities for the fishing, aquaculture and processing industries.

A repeated theme throughout the workshop was the importance of collaboration and input from all industries and parties, within PEI and beyond its borders. The sharing of information and collective agreement of parties in the US was repeatedly said as being necessary for recovery of the oyster fishery and aquaculture industries. MSX is a cross-industry, cross-regional problem and its management and recovery will advance more quickly with cooperative efforts.

The most popular priority for both Days 1 and 2 of the workshop was the development of MSX resistant seedstocks that are locally adapted. Both fishers and aquaculturists emphasized the importance of this need. It was widely recognized that MSX resistant stocks have been critical for revitalizing the fishing and aquaculture industries in the eastern US and will be needed to ensure a future for the oyster industries in PEI and potentially elsewhere in Atlantic Canada. Although some participants asked that this be done “for next season,” it was evident from discussions by the invited experts that this process will likely take at least six years since it will require several generations of breeding. This is a complex goal, requiring a breeding program and hatchery and nursery capacity. The ownership structure of the genetics of the resistant strains, the best options for distribution of the genetic line to industry to ensure widespread accessibility, and the potential for increased cost of hatchery produced stock all need to be determined. The expense of such an effort should not be underestimated. It will require long-term investment in research, infrastructure, and technical capacity.

The second most prominent priority identified by all industries is more surveillance and testing that will help to determine the distribution of MSX, its prevalence in affected areas and resultant mortality. There is, understandably, a lot of concern about the uncertainty that currently exists and industry views surveillance as being a way to increase understanding of what is currently happening and what is to come. The industries recognize that MSX can be present in the absence of significant mortality events, and therefore would like to have all MSX positive areas identified

as quickly as possible across the Island. The aquaculture industry would, additionally, like to gain a better understanding of distribution of MSX in affected areas, including in terms of its prevalence relative to environmental parameters (water temperature profile, salinity, and depth), stock location (surface grown or bottom culture or wild beds) and husbandry methods. This information, gained early could help to develop harvest management plans and best practices for growing oysters in the presence of MSX. One of the recognized constraints for surveillance is the unavailability of rapid, local testing. Increased testing capacity, was therefore identified as a need. Hand-in-hand with mortality and MSX prevalence surveillance is the need to better understand the environmental parameters associated with the sample sites to enable correlating patterns of prevalence, mortality, and water quality.

Research on husbandry techniques that could lower MSX prevalence and/or reduce mortality associated with the MSX parasite was a common priority identified by aquaculturists. Outcomes from trials conducted in the Bras D'Or Lake indicate that growing oysters at the surface may reduce mortality in areas that are MSX positive. This sparked a lot of interest and discussion regarding what aspect of surface growing techniques (husbandry of cages, winter temperature, salinity fluctuations, time on bottom, short term temperature exposure, others) caused the reduced mortality. Other lab trials at Cape Breton University suggested that the prevalence of MSX may be reduced by changing the environment or temperature.

Research on the potential to diversify the aquaculture industry was an additional priority repeated for both days. This avenue was pursued in Virginia as a means to reduce the impact of oyster diseases and may have potential in PEI.

Regulatory changes that could support operations in the presence of MSX was a common theme proposed as a priority by all industries, particularly on Day 1 of the workshop. Suggestions included: simplify paperwork, expand seed collection areas, increase access to surface culture leases, develop a buy-back program for licenses and re-examine relay practices.

Determining the intermediate host was a prominently requested research item after Day 1 of the workshop. However, this was listed only once on Day 2 and described as a long-term goal. The two-day workshop featured several discussions on the value of intermediate host information. Understanding this aspect of the MSX parasite was generally recognized to be helpful with applying controls for movement of the parasite; however, this knowledge will likely not change the short term and long-term needs of the industries, nor accelerate the development of resistant stocks. As a result, this was described as a "nice to know" item, rather than a "need to know" item. This conclusion is supported by results in the eastern US where the oyster fishing and aquaculture industries have rebounded in the absence of identifying the intermediate host.

The need for funding to attain the priorities identified by industry and to support the industries affected by MSX was repeated numerous times. Support at all levels of government will be required to secure operations now, and in days to come. An economic impact assessment of oyster sector stakeholders may be helpful to give context to the significance of MSX to the Island.

Next steps

In order for the industries to effectively manage through the current MSX crisis, it is important to distinguish between what can be done immediately to reduce impacts and what needs to be done to ensure future sustainability of the industries. Proposed actions that will be required to achieve industry priorities have been categorized in this manner. It is important to recognize that this listing reflects the impressions given at the two-day workshop. Additional collaborative discussions on the best path forward to more clearly delineate an actionable plan are required in concert with all industries, government agencies, potential funders and researchers.

Short term actions

- Continued communication with industry by provincial and federal departments to facilitate transition of the industry to the new era of working with MSX.
- Continued collaboration and discussions between and within the fishery, aquaculture, and processing sectors on PEI to determine research questions and refine short term and long terms goals.
- Continued collaboration and discussions with other regions that have experienced an MSX outbreak in the past.
- Collaboration and partnerships with local academia and regulators to develop plans for investigating research questions.
- Development and execution of an enhanced surveillance program for MSX that will clarify province wide distribution in public fishery beds and aquaculture leases.
- Development and execution of an enhanced surveillance program that will identify local distribution patterns for MSX in MSX-positive areas to support the development of practices that can assist the industry to operate in the presence of the parasite.
- Determination of the ownership, development, and funding model for a locally selected strain(s) of MSX resistant broodstock.
- Determination of the ownership, development, and funding model for the hatchery production of MSX resistant seedstock.
- Research on husbandry procedures and environmental parameters that reduce the prevalence of MSX and/or mortality of oysters in MSX positive areas.
- An examination of current regulatory constraints for oyster fishers and growers to determine if there are any restrictions that impede harvesting and growing oysters that may be safely temporarily eased in order to enable operations to survive.
- Continued, ongoing and enhanced technical resource support for fishers, growers, and processors.
- Assessment of the potential for a licence buy-back program for fishers.

Long term actions:

- Development and execution of a breeding program(s) to produce a locally adapted, MSX resistant strain(s) of oyster, for both aquaculturists and the public fishery.

- Assessment of the potential and process for public oyster bed restoration.
- Assessment of the potential for alternative species.
- Increase in the local capacity of hatchery production of oyster seed (infrastructure, training, customer base development).
- Assessment of the need for subsidizing seed purchase.
- Increase in the local capacity for nursery grow out of oyster seed (infrastructure, training).
- Training in hatchery techniques, nursery techniques, other changes required to support needed changes in equipment and processes.
- Identification of the intermediate host/vector and mechanism of infection.

Appendix A

Workshop Agenda (with Video links)

Agenda - Day 1: Industry Perspectives on MSX

8:30-8:45	Welcome Remarks – Hon. Cory Deagle, Minister. PEI Department of Fisheries, Tourism, Sport, and Culture (DFTSC) - https://youtu.be/W7A8IvtbrBs
8:45-9:00	Day 1 Objectives – Peter Warris, Executive Director, PEI Aquaculture Alliance (PEIAA)
9:00-9:30	MSX Sampling and Surveillance – Kim Gill, Director of Aquaculture, PEI DFTSC - https://youtu.be/-kCVkYCKO90
9:30-10:00	CFIA Surveillance – Dr. Danielle Williams, Aquatics Regional Veterinary Officer, Canadian Food Inspection Agency (CFIA) - https://youtu.be/cSKeIva6AIg
10:00-10:30	History of MSX in the US, Ryan Carnegie, Professor of Marine Science, Virginia Institute of Marine Science (VIMS) - https://youtu.be/xyAZ7m-Bfdk
10:30-11:00	Coffee Break
11:00-11:30	MSX in Bras d'Or, Industry Perspective – Robin Stewart - https://youtu.be/SjyIE9Y4y7M
11:30-12:00	The Unpredictability of MSX: Opportunities for Industry and Research – Dr. Rod Beresford, Associate Professor Biology, Cape Breton University (CBU) - https://youtu.be/Y6sWzUnQuAI
12:00-12:30	Surveillance and Mortality Monitoring in NS – Dr. Roland Cusack - https://youtu.be/9Wn02KkNcJM
12:30-13:30	Lunch
13:30-14:00	MSX in Maine, Industry Perspective - Jeff Auger, VP of US Operations Atlantic Aqua farms & Nellie Brylewski, General Manager, Muscongus Bay Aquaculture - https://youtu.be/-oSqxgXUzX0
14:00-14:30	MSX in Virginia, Industry Perspective, Chris Smith, Mobjack Bay Lease Holdings - https://youtu.be/2t04d7gzlZE
14:30-15:00	Breeding at ABC: past, present and future directions – Jessica Small, Director, Aquaculture Genetics & Breeding Technology Center, VIMS (Virtual Presentation) - https://youtu.be/wNMF1WJ9u8Q
15:00-16:00	Breakout Groups - Oyster Industry Sector Priorities
16:00-16:30	Breakout Group Reports and Discussion

Agenda - Day 2: Hatcheries and Research

8:30-8:45	Day 2 Objectives – Peter Warris, Executive Director, PEIAA
8:45-9:00	PEI Task Force on MSX – Communication and Coordination of the Industry Led Response – Kim Gill, Director of Aquaculture, DFTSC - https://youtu.be/gzikkXBktBA
9:00-9:20	Supplying Safe Seed in the Presence of MSX - Michael Congrove, Oyster Seed Holdings (Virtual Presentation) - https://youtu.be/qkmv2_t5e40
9:20-9:40	Mallet Hatchery: Present and future solutions for the PEI Industry - Martin Mallet, Co-owner & Hatchery Manager, Mallet Oysters - https://youtu.be/V24ChLEngFw
9:40-10:00	The genetics of disease resistance - Tiago Hori, Director of Innovation, Atlantic Aqua Farms - https://youtu.be/tI4HTBrOWrU
10:00-10:20	Bideford Shellfish Hatchery, it's capacity and the role that hatcheries could play in recovery from MSX - Adrian Desbarats, Ulnooweg Development Group - https://youtu.be/I3SR_f_y2js
10:20-10:40	Q&A with Hatchery Panel – https://youtu.be/9KGymE9jULE
10:40-11:00	Coffee Break
11:00-11:10	Genome Atlantic - Britta Fiander, Director, Innovation Programs, Genome Atlantic - https://youtu.be/zESFxnbrlgs
11:10-11:30	Current Research and Future Ideas in the US – Ryan Carnegie, Professor of Marine Science, VIMS - https://youtu.be/1-4_LLGVXww
11:30-11:50	Using a Screwdriver for a Chisel: Using Tools for Other Intended Purposes - Dr. Rod Beresford, Associate Professor Biology, CBU - https://youtu.be/LhmIcXxjf7E
11:50-12:10	Q&A with Research Panel - https://youtu.be/7g5qBfByYRM
12:10-13:00	Breakout Groups - Industry Priorities Review and Prioritisation
Workshop Closes	

Appendix B

Participant Priorities Listing, Day 1 (August 21, 2024)

At the end of Day 1 of the *Living and Working with MSX, an Oyster Industry Perspective* workshop, all participants were asked to identify key priorities for research, economic support, programs, adaptations to new equipment, and adaptations to existing processes, categorized according to the industry it is most applicable to: fishery (F), aquaculture (A), processor (P). A raw listing of the stated priorities and considerations for the priority, organized by theme, follows. The identified associated industry is indicated, according to the afore-mentioned abbreviations, where possible.

Monitoring

1) MSX Surveillance and Testing
    - Widespread public bed testing and monitoring - compete survey of entire province to know where we stand (F)
    - Coordinated testing to establish boundaries for current positive zones and to track movement spread; tests to determine how much area adjacent to PCZ is affected.
    - Sample and observe Bedeque extensively (August to November) to determine what level of mortality.
    - Gather baseline data to prove mortalities were due to MSX and not husbandry related (A)
    - Data compilation of occurrence and prevalence of MSX in surface versus off bottom versus bottom leases (A)
    - Long term surveillance program (including mortality rates, prevalence, re-testing of negative sites)
    - More testing capacity for PEI/NB/NS (F); local accredited MSX testing (F/A)

2) Environmental monitoring
    - Base-line environmental data amongst the surveillance sites.
    - More environmental/ water quality monitoring all over PEI
    - Real Time data water quality monitoring across PEI, NS (F)
    - Collect salinity data on top foot of leases.

Research & development

1) Hatchery/breeding program/MSX resistant seed development (most common priority across all sectors) (A/F/P)
    - Seed for both aquaculture and fishing industry (A/F)
    - Must be reliable and cost effective (F/A)
    - Breed a local, tolerant oyster.
    - Consider resistance, growth, survival, temp/salinity tolerance.

- "Atlantic Canadian Oyster Breeding Program" (A)
- Provincial/federal funded or co-owned by government, industry, or academia (rather than private) (A)
- Broodstock could be distributed to hatcheries.
- Can do test trials in hatcheries in PCZ to create resistant strain under supervision of researchers.
- Develop algal strains for oyster hatchery that are similar to what oysters are feeding on here.
- Need to understand equipment/water filtration pertaining to hatchery, water treatment plans + systems (A)
- Establishment of sanctuaries or zones for the establishment of broodstock
- Development of multispecies hatchery (A)
- Training required for hatchery techniques - spawning/algae production/facility operation (A), for up-wellers (A)

2) Increased understanding of the MSX-parasite and its infection
   - Identification of intermediate host/vector and mechanism of infection (F/A)
     - Macroinvertebrate study done for genetic analysis to cross match between genes of MSX in Oysters and different species of macroinvertebrates. Sampling should be done in all three regions of the water body (epipelagic, Mesopelagic, Bathypelagic, benthic, to tally with the three types of leases issued to the farmers.
   - Profile the genetic and phenotypic characteristics that imbue resistance (A)
   - Genomic research - comparison of resistant and non-resistant strains
   - Genomic research of MSX isolates (sequencing)
   - Can MSX spread by other bivalves? (F/A/P)

3) Husbandry and Best Management Practices
   - Determine factors (environment/husbandry/production practices) that cause MSX susceptibility (A) and/or MSX related mortalities (F/A); research MSX tolerance of surface-grown oyster – most research seems to be related to bottom culture (A); research on moving product – ice, salinity & water depth? (F/A/P); determine if there are less risky times of year to transfer oysters (F/A/P); research on moving product – salinity & water depth? Will a hot water dip at high temperature or high temp air dry work? (A)
   - Do a practical mitigation strategies assessment; develop processes and guidelines with Island knowledge and experience (common-sense based) (F/A/P)
   - Best management practices for PCZ to maximize growth, reduce spread and sell product; remove diseases oysters from water to remove/reduce spread? (F/A/P) What

about if spread seed in PCZ in public beds so initially higher number of oysters out of which a certain percentage will survive?
- Solutions for red zone farms to move product to clean water processors.
- Assistance setting up water treatment plans and systems.
- What is the shelf life of oysters infected by MSX?
- Develop basic biosecurity program - “Atlantic Region Shellfish Biosecurity Program.”
- Determine MSX eradication techniques (A)
- Explore the use of Hypochlorous Acid (HOCI) for treating incoming and effluent waters in processing plants, as well as testing its effects on MSX-contaminated oysters (P)

4) Industry diversification
    - Quahogs; razor clams; others
    - Hatcheries important to support diversification.

Strategy and collaboration

- Taskforce entity responsible to develop the path forward, and ensure it gets executed – populated with industry stakeholders, government, First Nations. Task Force should have a dedicated project lead.
- Develop a Centre for Shellfish Health with scientists and graduate students to lead an industry-informed research program to collaborate with others (CBU, VIMS)
- Involvement of AVC/UPEI and NRC
- Maritime Collaborative Shellfish Group (A)
- Support for field research and experimental sites

Communication and education

- Education is needed to prevent spread (F/A)
- Ensuring disease is not spread when seed is moved – potential for using seed that is low cost but not clean since mortality not seen in early stages (A)
- Increase communication and information flow from government to industry and industry to industry re. sample results, mortality observations (F/A/P)

Funding supports

- Economic risk support to help processors purchase product over the winter (P)
- Economic support for industry as we work to rebuild.
- Financial assistance for wild fishers (F)
- Financial support for growers that experience MSX mortalities (A)
- Financial support for MSX testing (A)
- Financial support to implement biosecurity measures (F/A/P)
- Investment in nursery capacity (upwelling systems)

- Funding for hatcheries
- Financial support for breeding strategy, breeding plan & MSX resistant broodstock

Regulatory supports

- Simplify paperwork.
- MSX seems more prevalent on the bottom rather than on the surface – so makes sense to revisit the moratorium on switching bottom leases to off-bottom (A)
- Regulatory support to access strains or research samples
- Retirement (buy back) of “active” licences from wild fishery (F)
- Allowance for wild industry to access surface culture leases (F)
- If people have to abandon Bideford River, will DFO allow new seed collection areas in cleaner waters?
- Fishers might get seed shortage this year, but next year can they go to other areas for seed collection?
- Offshore aquaculture applications
- Holding leases in PCZ areas for processors who are in clean areas.
- Do people have the ability to hold seeds for others?

Appendix C

Industry Priorities Listing, Day 2 (August 22, 2024)

At the end of Day 2 of the *Living and Working with MSX, an Oyster Industry Perspective* workshop, industry participants were asked to identify key priorities for research, categorized according to the industry it is most applicable to: fishery (F), aquaculture (A), processor (P). A raw listing of the stated priorities and considerations for the priority, organized by theme, follows. The identified associated industry is indicated, according to the afore-mentioned abbreviations, where possible.

Monitoring

1) MSX Surveillance and Testing (F X 2; A X 3)
    - Increase testing done (F)
    - Test all waters of PEI. Not just trace in, trace out (F)
    - Increased sampling and surveillance that can be shared with industry (prevalence and mortality (A)
        - Learn about spread and transmission.
        - Utilize families/genetics as part of this to look at potential for resistance.
    - Monitoring and data management systems (A)
    - Test kits that can be deployed in the field (A)

Research & development

1) Hatchery/breeding program/MSX resistant seed development (most common priority across all sectors) (F X 4; A X 5; U)
    - Hatchery support and development for public beds
        - Plan to restore high mortality areas.
    - Hatcheries and support for development of aquaculture facilities with disease resistant stock
        - Hatchery capacity, upwelling capacity, breeding program, remote setting; subsidy for seed prices
        - Non-profit government hatchery to supply the whole industry.
        - Develop MSX-resistant seed in a way that is affordable (industry owned hatchery co-op, government funding, etc.
        - Remote setting start-ups
        - Breeding and genetic analysis
    - Not sterile triploids

2) Husbandry and Best Management Practices (A X 4)
    - Field research to trial/experiment various techniques in areas that are hit first to develop Best Management Practices on farms that can benefit others as it is spread.

- Practical steps, short term, to keep MSX positive oysters alive (temperature, air drying, salinity, etc.) and good communication of these.
- Determine what surface culture practices will reduce the spread of MSX (UV, reduced salinity, higher temperatures, cage flipping schedule, temporary relocation to low salinity area, timing of sinking and raising of cages, timing of oyster transfers, land-based storage potential over winter)
- Testing seed – rapid testing of seed in in bags

3) Industry diversification (A)

4) Increased understanding of the MSX-parasite and its infection. (Identified as a long-term priority.) (A)
   - Determine the intermediate host and how its presence can be mitigated or how to prevent contraction of MSX by oysters.

Communication, education, and strategy

1) Additional communication and education in order to inform priorities (A)

2) Establish a board to help choose priorities (A)

3) Aquaculture technical advisors to help/support farmers with changes in equipment, processes, disease, etc. (A)

4) Complete economic impact assessment of oyster sector stake holders with the onset of MSX situation in PEI

Regulatory supports

1) Buy-back program for fishers as catches decrease (F)

2) Reduce the distance requirement for relaying oysters to increase relay capacity, recognizing that validation of food safety through testing will still be required (A)

3) Regulatory approvals to enable surface culture leases to sink oysters prior to harvest (A) (and equipment assistance if this is approved)