A Review of the 2019 Newfoundland and Labrador South Coast Cultured Atlantic Salmon Mortality Event

Submitted to:
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MARCH 16, 2020

With Corrections March 22, 2020

MARINE INSTITUTE

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Executive Summary

An extreme and unprecedented mass mortality event in Atlantic salmon occurred at 10 NHSF netpen sites in Fortune and Harbour Breton Bays, which are separated by a minimum of 60 km (33 nm), on the south coast of NL. Affected sites had either a cumulative mortality of 100% (n=6) or partial mortality (33 to 50%; n= 4) during August and September 2019. The Committee evaluated multiple data sources including reports from veterinary diagnostic laboratories, information provided by FLR in veterinary reports and NHSF data on water temperatures, adult lice counts at a single time point for each site, handling of fish for sea lice treatments, feeding protocols, and satellite images of the bays.

We concluded the most likely cause of mortality was a prolonged increase in water temperatures (measured as the number of August days with >18^{o}C at 0.5 m and >15^{o}C at 15 m). The high water temperatures and reduced dissolved oxygen levels during August led to asphyxiation (hypoxia) as the final cause of death. We speculate that the following series of events created a spiral of worsening conditions inside netpens. Salmon, by avoiding the warm surface temperatures, became crowded at the bottom of netpens and depleted oxygen from the water faster than it could be replenished, creating a hypoxic and stressful environment which caused salmon to die. Dead fish lying on the bottom of the netpen then tightened up the netpen, worsening the hypoxic environment. Also, fish handling during therapeutic treatments for sea lice stressed the salmon and increased their oxygen needs, worsening conditions further. An algal bloom in the area, as suggested by satellite imagery, would exacerbate the poor environmental conditions because algae remove oxygen from the water at night. A toxic algal bloom would make matters even worse. There was no evidence that infectious disease was a contributor to mortality except possibly for one site which had a confirmed case of infectious salmon anemia that preceded the mortality event.

The emergency response efforts of NHSF began immediately upon reporting the mortality event to FLR. However, the lack of a robust contingency plan for such an extreme event hindered NHSF's ability to begin mort removal activities quickly resulting in a two-week delay. Once the mort removal activities began there were several challenges associated with the removal of such large quantities of dead fish. Mort removal was impeded by several factors including, but not limited to: availability and mobilization of divers and seiners; Hurricane Dorian; the quantity and condition of the dead fish material which was difficult to handle; and the impact of the mass of dead fish on netpen shape and depth which made it challenging for divers to access some of the netpens. Based on our assessment of the response effort by NHSF and the Barry Group Inc. we have concluded that mort removal activities were completed as quickly as possible under extraordinary circumstances.

Ten concluding recommendations are made for improved reporting of mortality events by industry to government, a strengthened response plan and approaches to mitigate future occurrence of similar events.

Acronyms and Abbreviations

AAHD – Aquatic Animal Health Division

AVC – Atlantic Veterinary College

BCARD – British Columbia Aquaculture Regulatory Program

BKD – Bacterial Kidney Disease

CASD – Centre for Aquaculture and Seafood Development

CFIA – Canadian Food Inspection Agency

Committee – Review Committee Members: H. Burke, I. Gardner & A. Farrell

DO – Dissolved Oxygen in Water (as a percentage of 100% air saturation)

ECCC – Environment and Climate Change Canada

FLR – Department of Fisheries and Land Resources, NL

IFAT - Fluorescent antibody test

ISAv – Infectious Salmon Anemia Virus

MAMKA – Mi'kmaq Alsumk Mowimsikik Koqoey Association

ME – Mortality Event

MI – Marine Institute

Mort(s) – refers to mortality (mortalities) or dead fish

MUN – Memorial University of Newfoundland

NAIA – Newfoundland Aquaculture Industry Association

NHSF – Northern Harvest Sea Farms Newfoundland Ltd.

NSDFA – Nova Scotia Department of Fisheries and Aquaculture

NWD – New World Dairy

PCR – Polymerase Chain Reaction

PPM – Policy and Procedures Manual

SOP – Standard operating procedure

TOR – Terms of Reference

UBC – University of British Columbia

UPEI – University of Prince Edward Island

1. Terms of Reference (TOR)

At the request of the Department of Fisheries and Land Resources (FLR), the Marine Institute (MI) facilitated a "Review of the 2019 Newfoundland and Labrador South Coast Cultured Atlantic Salmon Mortality Event – As reported by NHSF (Northern Harvest Sea Farms Newfoundland Ltd - Mowi Canada East)". A review committee of subject-matter experts was engaged including Ms. Heather Burke, Director of MI's Centre for Aquaculture and Seafood Development, who coordinated the review on behalf of MI; Dr. Ian Gardner, Atlantic Veterinary College, UPEI; and Dr. Anthony Farrell, Canada Research Chair (Tier I) in fish physiology, culture and conservation at UBC. (Refer to section 2 for more detailed biographies of Committee members).

The mortality event (ME) occurred at 10 NHSF netpen sites in Fortune and Harbour Breton Bays on the South Coast of Newfoundland and Labrador. Six of those sites had 100% mortality and four sites had partial mortality. Mortality for the first affected site was reported to FLR on August 28, 2019, with another five sites reported shortly thereafter. By September 2, 2019 most fish at these six sites were considered "dead" or "moribund with behavioral changes" and 100% mortality occurred soon thereafter. The remaining four sites were added to the ME on October 11, 2019 and had exhibited partial mortality (i.e., 33-50% mortality) by this time. Live fish at three of the four partially-populated sites were subsequently harvested by November 22, 2019 using normal farm practices because they were nearly full-grown salmon. The remaining marine site is still populated with juvenile salmon. This was clearly an extreme and unprecedented episode of mass mortality with widespread consequences in NL.

The Committee's mandate included:

- Identifying the cause of the mortality event (ME);
- Making an assessment of the clean-up response effort to the mortality event;
- Making recommendations for improvements to:
  - The reporting requirements by industry to government; and
  - Response efforts by industry.
- Making recommendations to mitigate future occurrence of similar mortality events.

The scope of this report does not cover:

- An environmental assessment;
- A review/assessment of social license issues;
- Economic consequences and adverse effects on worker health

The complete Terms of Reference (TOR) are found in Appendix A (page 37).

2. The Review Committee

Heather Burke, Fisheries and Marine Institute of Memorial University of Newfoundland
Heather Burke is the director of the Centre for Aquaculture and Seafood Development (CASD), a position she has held since 2005. Ms. Burke leads a diverse team of research scientists, engineers, technologists, and graduate/post-doctoral students in providing multi-disciplinary scientific and technical expertise to industry, academia and government in areas of seafood processing, aquaculture and marine bioprocessing. Since joining the Marine Institute in 1998, Ms. Burke has been involved in more than 400 applied research projects for the seafood, aquaculture and marine bioprocessing sectors, and has facilitated numerous collaborations with provincial, national and international organizations. Her own research interests include the assessment and optimization of fisheries and aquaculture value chains, marine bioprocessing and biomass utilization, bio-production of crustacean extracts, and environmental impacts of aquaculture and their mitigation. Ms. Burke obtained her B.Sc. (1st Class Hons) in Biochemistry (Food Science) in 1994. She also holds master's degrees in Food Science (1996) and Business Administration (2003) from Memorial University where she is currently a PhD candidate in Environmental Science.

Dr. Ian Gardner, University of Prince Edward Island
Dr. Ian Gardner is a professor of epidemiology at the Atlantic Veterinary College, UPEI, and held a position as the Canada Excellence Research Chair in aquatic epidemiology at UPEI until September 2019. Dr. Gardner's research interests include diagnostic test evaluation, pathogen exchange between farmed and wild fish, risk factor studies and infectious disease modelling. He has authored more than 300 peer-reviewed scientific publications and reports, many of which are focused on the validation and application of diagnostic tests for terrestrial and aquatic animal diseases. Prior to moving to Canada in 2011, Dr. Gardner was a professor of epidemiology at the University of California, Davis, for 23 years. Dr. Gardner obtained his veterinary degree at the University of Sydney in 1975 and completed post-graduate training (Master of Preventive Veterinary Medicine and PhD) at the University of California, Davis, in 1988.

Dr. Anthony Farrell, University of British Columbia
Dr. Tony Farrell is a professor with the Department of Zoology and the Faculty of Land and Food Systems at UBC. He obtained his B.Sc. (1st Hons) at Bath University, England, and PhD at UBC. He held faculty positions at Mount Allison University and Simon Fraser University prior to his current position as Canada Research Chair (Tier I) in fish physiology, culture and conservation at UBC. His research aims to understand fish cardiorespiratory systems and apply this knowledge to salmon migratory passage, sustainable aquaculture and aquatic toxicology. He has trained nearly 80 M.Sc. students, PhD students and postdoctoral fellows. He has more than 450 research publications in peer-reviewed scientific journals that include pioneer works on the sub-lethal effects of sea lice and Piscine orthoreovirus on the physiology of juvenile wild salmon. Dr. Farrell has served on numerous committees related to aquaculture management and practice, including the federal government's Independent Expert Panel on Aquaculture Science, the B.C. government's Aquaculture Advisory Committee on Finfish Aquaculture; and the Department of Fisheries and Oceans' Canadian Science Advisory Secretariat.

Ms. Heather Burke facilitated the review, coordinated the acquisition of data and focused on the cleanup efforts following the salmon mortality. The independent external reviewers, Drs. Gardner and Farrell, focused primarily on health and disease aspects, and environmental and oceanographic factors, respectively. Both Drs. Farrell and Gardner have no conflicts of interest with NHSF and FLR and conducted this review independently of their respective academic research institutions. In the remainder of the document, the review committee is referred to as the Committee.

3. Hypotheses

To identify the cause(s) of the ME the Committee considered the following 3 hypotheses, alone or in combination.

1) **The disease hypothesis**: An infectious disease or diseases (including sea lice and infectious salmon anemia virus) triggered an epidemic, with up to 100% mortality at most sites that involved two year classes of salmon and occurred rapidly around the beginning of September 2019.

2) **The temperature hypothesis**: An unusual set of natural environmental conditions resulted in a water temperature that was too high for salmon to endure, with up to 100% mortality at most sites that involved two year classes of salmon and occurred rapidly around the beginning of September 2019.

3) **The hypoxic squeeze hypothesis**: An unusual set of natural environmental conditions triggered a series of events that lead to mass asphyxiation (suffocation) of salmon, with up to 100% mortality at most sites that involved two year classes of salmon and occurred rapidly around the beginning of September 2019.

4. Methodology

4.1 Our Review of Event Timelines

Timeline information was provided to the Committee by DFRL, NHSF and the Barry Group. Some of the timeline information was also collected during stakeholder consultations and from media publications/reports.

The Committee used this information to review the timelines of the ME to:
a) assess the reporting requirements and compliance by NHSF to FLR;
b) evaluate the response time of FLR in providing assistance to NHSF; and
c) assess the response time by NHSF, Barry Group and others involved in the mortality removal and site clean-up.

4.2 Our Review of Mort Removal Capacity

Mort removal capacity was estimated based on information provided by the Barry Group Inc. and NHSF. Specifically, number of netpens, estimated size and mass of dead fish per netpen, number of divers, average maximum daily dive time, maximum daily mort removal time, number of seiners available, seiner holding capacity, vessel loading and offloading rates, steam time to and from the rendering (fishmeal) plant and farm sites, and plant processing capacity were variables that were considered when estimating the minimum timeframe in which dead fish could reasonably be removed from all 10 aquaculture sites. Also included in this assessment were other unexpected delays such as abnormal weather events (a hurricane) and a diver stop-work order. A simplified calculation illustrating how we estimated the time requirement to complete mort removal from the 10 affected salmon sites is available in Appendix B (page 38).

4.3 Our Analysis of Diagnostic Reports

The purpose of the Committee's review of the diagnostic reports was to propose likely causes (risk factors) for the ME as described in the TOR (Appendix A – page 37). FLR provided the Committee with its interim report on December 23, 2019. Additional information and a final veterinary report were provided by FLR to the Committee on February 7, 2020. Diagnostic laboratory reports and site data were also provided by NHSF. During its review, the Committee also consulted with Amanda Borchardt (NHSF), Nicole O'Brien (FLR), and Dave Groman (AVC). These consultations were conducted in person, via telephone or via teleconference. Seven of the 10 affected sites (one site had a second sampling 2 weeks after the first) had extensive follow-up investigation by AAHD personnel including laboratory testing and site-specific results were evaluated qualitatively. The diagnostic reports were evaluated qualitatively because of the retrospective nature of the review, only 7 sites were subject to testing, and hypotheses were developed by the Committee after reviewing all the reports. The Committee also provided its opinion about the conclusions made in the final veterinary report.

4.4 Our Analysis of Environmental and Site Data

NHSF provided environmental and site data, including daily information for water temperatures and dissolved oxygen levels, number of fish per site, average size of fish per site, weekly mortalities, sea lice treatments (via feed and using bath immersion), reports based on camera observations, and feeding protocols. The purpose of the Committee's review of these proprietary data was to assess other possible causes (risk factors) of the ME in data that were not readily available from FLR. These data were made available for use by the Committee and were subject to a confidentiality agreement. The data covered the month of July 2019 for the first affected (index) site and for the month of August and the first two days of September for all 10 sites. With NHSF fully engaged in the clean-up operation during September and October, such data were not available from September 3 onwards. Therefore, our efforts to ascribe a cause to the ME primarily focused on those sites where there was 100% mortality. Data for one non-affected NHSF site were provided late in the evaluation for a qualitative comparison with the 10 affected sites.

Statistical analysis of the mortality, environmental and lice treatment data was limited because of their coarse nature, because most data were aggregated at a site level (not by netpen). In other cases, the weekly aggregate (not necessarily the specific day of collection) was provided. There was variation in the time periods over which water temperature data were reported, necessitating decisions about the most relevant times to be considered; ultimately we chose July 31 to August 31, if available. Also, while the day and the depth of the dissolved oxygen (DO) and temperature data were known, we had no knowledge of how representative this daily snapshot was on either spatial or temporal scales. We would expect that differences existed within a netpen due to variable influences of the tidal currents. The two main risk factors considered in the statistical analysis of mortality categories (100% or partial) were the number of days that August water temperatures exceeded critical thresholds (18^{o}C and 15^{o}C) and the handling of fish for sea lice treatment on days when the seawater temperatures were >18^{o}C.

Environmental data analysis included evaluation of:

- Timing of mortality during August (Figure 1), feeding, and handling of fish for sea lice treatments for early warning signs and associations.
- DO concentrations at sites at the surface (0.5 m), 5 m and 15 m.
- Temperature at sites at the surface (0.5 m), 5 m and 15 m. Statistical comparisons using the Mann-Whitney test of the number of days with elevated surface water temperatures (>18^{o}C at the surface and >15^{o}C at 15 m) were done between sites with 100% mortality and those with partial mortality (see statistical analysis section in the Glossary page 50).
- Timing of weekly fish mortality relative to the water temperature during August (Figure 2).
- Timing of spikes in weekly fish mortality at a site was compared with the timing of fish handling events required for sea lice bath treatments. Also, odds ratios were calculated (see statistical analysis section in the Glossary page 50) to measure how strongly associated handling of fish for treatments for sea lice was during the period of high surface water

temperatures (18^{o}C) in August with the subsequent occurrence of either 100% mortality or partial mortality at sites.

4.4.1 Our Calculation of Weekly Mortality Rates

We calculated weekly mortality rates (%) for August 2019 from the mortality numbers for each site that were provided by NHSF. These mortality counts were based on the sum of one to four dives per week per site for the period of July 29 –September 2. Therefore, the data used by the Committee were an aggregated weekly mortality count. The numerator for the calculation of weekly mortality rate was the number of fish that died during the week and the denominator was the estimated inventory of fish on August 20, 2019 as provided by NHSF. This value is a reasonable estimate of the site-specific average population at-risk of death during the subsequent two weeks. Use of this approximation may result in a slight underestimate of mortality risk in earlier weeks as it does not account for mortality effects of any elevated water temperatures from July 29 to August 20.

4.4.2 Our Analysis of Early Warning Signs of a Fish Mortality Event

Early warning signs of an ME (see Figure 1) were explored by assessing fish mortality rates during August using a finer level of discrimination than that used to trigger an ME. We used four lower thresholds: a background mortality rate (< 0.1% of fish per week), an increase above this background rate (0.1% to <0.5%), a spike in mortality (0.5% to <1%) and a warning level (>1%-2%). Background mortality was without consideration of variation attributable to life stage and time at sea, water temperature and salinity, management practices, and different pathogen profiles etc. A recently-published study from Norway[1] showed that spatio-temporal mortality patterns from 2014-2018 in farmed Atlantic salmon depended on multiple factors including months at sea, month of first stocking, and production zone.

Also, lacking more detail on exactly which day mortality occurred, we cannot discriminate between the weekly mortality count being a single or a cumulative event, a consideration that is central to the criteria used to define a fish ME (refer to Glossary – page 50) in the conditions of license.

4.4.3 Our Calculations of Fish Oxygen Needs in a Netpen

Calculations on the total oxygen needs of the salmon contained in a netpen at 10^{o}C and 20^{o}C were based on the water volume of the netpen (100 m circumference x 15 m deep at all sites)[2], estimated stocking density and fish mass for both year classes of salmon. By comparing this oxygen need to the DO content of the water inside the netpen, we could then calculate the water

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[1] Bang Jensen B, Qviller L, Toft N. Spatio-temporal variations in mortality during the seawater production phase of Atlantic salmon (Salmo salar) in Norway. J Fish Dis. 2020 Apr;43(4):445-457

[2] Netpen dimensions have been corrected to 100 m circumference x 15 m depth. Dimensions were previously incorrectly reported as 100 m (diameter) x 15 m depth.

turnover required to prevent a hypoxic condition developing inside the netpen as a direct result of the salmon removing oxygen from the water for their needs (refer to Appendix C - page 39).

4.5 Our Stakeholder Consultations

Consultations were conducted between December 2019 and March 2020 with about twenty stakeholders including industry, industry associations, academia, municipal, provincial and federal government departments (refer to Appendix D on page 43 for a list of people/organizations contacted). Interviews were conducted to fill data gaps, confirm timelines of events, ground truth data-sets, and identify similarities/differences in finfish aquaculture policies and procedures for different jurisdictions across Canada.

4.6 Our Site Visits

In January 2020, two of the Committee members conducted a site visit to five of the affected sites in Harbour Breton and Fortune Bays. The purpose of the site visit was to observe the current environmental conditions, confirm the extent of the clean-up, meet with NHSF management, better understand on-site practices, and assess logistical constraints that may have impacted clean-up response efforts.

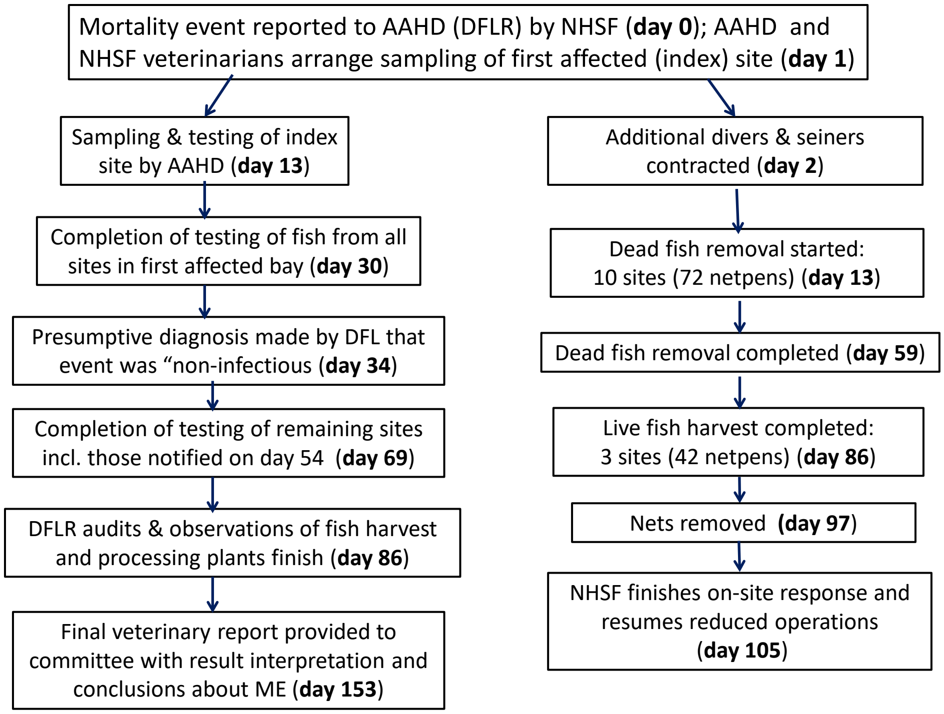
4.7 Our Review of Finfish Aquaculture Policies in Canada

The Committee reviewed the finfish aquaculture policies currently in use in NL and in other Canadian jurisdictions for a comparison of regulatory practices. Specifically, the Committee reviewed the policies and procedures in place in British Columbia and Newfoundland and Labrador and compared the roles and responsibilities of the federal government and the province in each jurisdiction.

5. Results and Discussion

5.1 Our Summary of the Mortality Event Timelines

The first ME was reported to FLR on August 28, 2019 (day 0) and AAHD and NHSF veterinarians made arrangements for sampling of the first affected (index) site the following day. The flowchart below shows the disease investigation timelines for FLR (left column) and the emergency response by NHSF (right column) based on the Committee's review of written reports and discussion with government veterinarians and with NHSF personnel. The reporting of events by NHSF to FLR (August 28 first site, September 3 another 5 sites, and October 11 an additional 4 sites which had partial survival) has been simplified in the chart. Sites reported on August 28 and September 3 had 100% mortality and the 4 remaining sites had partial mortality.



The Committee considers that this summary of times will be beneficial in future contingency planning and can be used to identify "bottlenecks" that can be more effectively managed in future "incident events" as defined in AP-17 Public Reporting, which was published by FLR on September 24, 2019. Incident events (see Glossary page 50 for definition) includes abnormal ME as described in this report.

One to two days after the notification of the ME, AAHD veterinarians were in the region with affected NHSF sites and were ready to respond. Hurricane Dorian arrived to the South Coast on September 8 and created unsafe water travel conditions to the netpen sites from September 7 - 9. AAHD vets were on site the first day of seiner activity on September 10 and they collected samples from two sites on that day. The remaining two sites were not sampled until September 25 and 27 (22 and 24 days after the second notification).

5.2 Possible Causes of the Mortality Event

5.2.1. Our Analysis of the Infectious Disease Hypothesis

Clinical signs and presumptive diagnosis by the NHSF veterinarian

The NHSF veterinarian (Dr. Borchardt) reported that the ME was of sudden onset and associated with increasing water temperatures (>15ºC) and decreasing dissolved oxygen (DO) levels. According to NHSF personnel on site, fish were reported as lethargic and behaving abnormally, and were congregating at the bottom of the netpens. Six of the 10 affected sites were reported as a "geographic cluster" over a seven-day time span (August 28 to September 3). Dr. Borchardt attested that no infectious diseases were detected prior to the ME at four of the first six affected sites in Fortune Bay and noted that there were no health concerns on the two other sites, which were recently stocked. Four sites, mostly in Harbour Breton Bay (60+ km away) were reported as being affected on October 11, 2019.

The presumptive diagnosis as described in press releases by NHSF was that the primary cause was a prolonged period of elevated water temperatures and decreased DO at affected sites that resulted in suffocation of fish.

After reviewing the data provided by NHSF, the Committee agrees that the pattern of disease occurrence reported to FLR was consistent with what is commonly termed a "common source" (e.g. a toxin associated with harmful algae, handling of fish during periods of elevated seawater temperature, deficits of a critical resource such as dissolved oxygen in the water in netpens). The pattern of mortality was <u>not consistent</u> with an infectious agent such as infectious salmon anaemia virus (ISAv) spreading site to site. Indeed, a cumulative mortality of 100% attributable to a pathogen never occurs because of innate immunity and/or recovery of some fish. In addition, only a single site had confirmed ISAv infection before the ME occurred. There was no clinical evidence of ISAv when any of the four affected sites in Fortune Bay reported as having a ME were tested for ISAv.

Investigation of causes of the ME by the Aquatic Animal Health Division (AAHD)
The primary questions to be answered as part of the AAHD investigation were: a) were clinical signs, gross pathology, histopathology, virology and bacteriology consistent with an infectious or non-infectious disease? and b) if infectious, what agents were likely involved?

Sampling and testing of moribund fish was done at seven of 10 affected sites, including all six sites that eventually experienced 100% mortality. These sites were sampled over 56 days, starting on September 10 and finishing on November 5 (refer to flow diagram on page 13). Priority was given to sampling sites in Fortune Bay. Live fish were difficult to obtain during the warm water temperatures due to the fish going deep in the cages. Divers and seiners were required to collect moribund fish in many cases. Three sites were not sampled (two sites were harvested and no live fish were available, and a third site was under quarantine for ISAv and was being harvested).

The number of samples for virology (including ISAv and culture for a range of important viruses), bacteriology and histopathology ranged from 6 to 15 (median = 10). This was consistent with AAHD protocols to sample at least 5 fish per site (or as directed by the coordinating veterinarian). Assuming that the prevalence of disease in the 5 sampled fish was conservatively 80% and not 100%, and the tests were at least 60% sensitive, this protocol would ensure 95% confidence of detecting the underlying disease condition. Protocols for site sampling procedures for a mass mortality/disease event/incident event (PM-P-323) and sample management (PM-P-303) were followed.

Fish were necropsied at the St. Albans laboratory mostly within 12 hours of collection and sent to the Atlantic Veterinary College (AVC), UPEI and/or RPC Fredericton, as appropriate. Grossly at the first four sites sampled, most fish were in good body condition, but gill damage was noted on several of the fish with haemorrhages, build-up of mucus and some pale areas on gill arches. Some scale loss and skin lesions were observed but the presence of sea lice alone was concluded to be insufficient to explain the acute onset of mortality observed.

A detailed description of laboratory findings is in the summary AAHD veterinary report which was provided to the Committee on February 7, 2020. That report included additional information that was not in the interim report which was received in December 2019. It is important to note, that the AAHD report included limited information on sea lice infestations, other than noting that average sea lice counts were below 6 adult females per salmon per site[3]. NHSF data showed that average counts were below 2 on all but two of the sites in late July/early August. Industry reporting of lice counts and treatments was not required at the time of the ME but will be implemented in January 2021. Dates and methods of lice treatment were not available to AAHD for evaluation.

The following three sections summarize the main findings at the first four sites sampled in Fortune Bay. Any differences in results from the remaining three sampled sites are noted.

Histopathological findings

Fixed tissues of gill, kidney, heart, liver, spleen and pyloric caeca were submitted from salmon to Dr. David Groman, a board-certified veterinary pathologist, at the AVC. The samples were provided without a history or description of the gross post-mortem findings but all fish were moribund at the time of collection to help maximize sample integrity for the pathologist. The principal finding across all sites was damage to the gills "consistent with hypoxia associated with exposure to a bath treatment or a toxic algal bloom". This comment was not included as a note on all accessions as no information was provided to link accessions to the ME on all sites. Some tissues that were examined, especially gills, showed mild to moderate autolysis indicating the fixation was delayed or incomplete but in general, were still of sufficient quality to provide useful information about the likely causes. There was no evidence of infectious agents in any of the internal body tissues or organs.

Toxicological testing

Testing for known toxins was <u>not</u> done as part of the ME because of a low index of suspicion. However, it is important to note that monitoring for plankton and harmful algae was not done at the NHSF sites and hence, cannot be "ruled out" as a possible factor in the ME. A satellite image of Harbour Breton Bay on September 21 showed a shadow which could have been a fish oil slick or an algal bloom (Figure 3). Additionally, satellite images from EOSDIS Worldview show a possible algal bloom in Fortune Bay starting August 31 and dissipating by September 3, 2019 (Figure 4).

Testing for viruses and bacteria

The AAHD's surveillance program for specific pathogens of salmon in NL includes testing for four viruses (ISAv, infectious pancreatic necrosis virus, infectious haematopoetic necrosis virus and

[3] Sea lice counts have been corrected and reported per salmon per site.

viral haemorrhagic septicaemia virus). Viral culture was done on 3 different cell lines known to support growth of pathogenic salmon viruses and all results were negative. Confirmation of ISAv was made at one site in the second affected bay before the onset of the ME and therefore may have contributed to mortality at this site. A second detection of ISAv was made at another site, 6 weeks after the ME and therefore would not be a contributing factor. Culture for a range of bacterial agents and specific testing for bacterial kidney disease (BKD) by PCR or indirect fluorescent antibody test (IFAT) were negative. Bacterial culture was also negative for three other endemic bacterial pathogens (atypical *Aeromonas salmonicida*, *Yersina ruckerii*, and *Vibrio anguillarum)*

In summary, the clinical presentation of the ME, gross signs at post-mortem of moribund fish and histopathology of tissues and organs, and negative laboratory results for known infectious agents were consistent with the hypothesis that the ME was non-infectious and likely attributable to hypoxia. Diagnostic testing for toxins was not done to "rule-out" their possible involvement. Data on lice bath treatments and environmental measurement were not available for Dr. O'Brien, AAHD's veterinary epidemiologist to review and were provided to the Committee by NHSF on request. Relevant findings from interpretation of those data are in section 5.2.2. Our Analysis of Environmental Factors.

<u>Recommendations</u>:

1. Veterinarians submitting samples in future mortality events should provide relevant history including clinical signs and gross necropsy findings so that the pathologist can review all relevant information to make a presumptive diagnosis. Sampling protocols should include documentation of chain of custody to minimize the risk of legal challenges to laboratory findings when samples from infection salmon anaemia virus (ISAv) cases are being handled in the laboratory at the same time as samples from a ME of similar magnitude to this event.
2. Strategies to maximize the quality of samples for histopathology (whether the disease is infectious or not) should be reviewed and strengthened.
3. Introduction of a random surveillance system of moribund fish by a government regulatory agency, as is done in British Columbia, should be considered as this would help with public confidence and trust of salmon companies and reduce the reliance on a ME being the only trigger for such surveillance.
4. Monitoring for harmful algal blooms during summer months when water temperatures are elevated would be useful for future assessments of any contributions to non-infectious ME similar to the present event. The timing of decisions to monitor blooms should be made by the company personnel.

5.2.2. Our Analysis of Environmental Factors

Based on our analysis of environmental and site data, specifically the on-site water temperatures (Figure 1) and DO data, the timing of the sea lice bath treatments and the weekly fish mortality counts reported by divers (Figure 2), we provide the following key observations.

1. Water temperatures at 0.5 m were $\geq$15°C m at all 10 farm sites for the entire month of August (Figure 2). Indeed, for the majority of days in August, the temperature at 0.5 m was $\geq$18°C at all sites except one. This exception had partial survival of the adult salmon that were subsequently harvested in November 2019.

2. Water temperatures at 5 m were $\geq$15°C at eight farm sites for the entire month of August (except 1-2 days at two sites) and for most sites the water temperatures at 5 m were $\geq$18°C for about half of the days in August (Figure 2). Moreover, the water temperatures at 15 m periodically reached $\geq$15°C, punctuated by saltwater intrusions at this depth, more so at some sites than at others. The exceptions in this regard were two sites where water temperatures at 15 m were <10°C for at least a third of the days in August (Figure 2) and these two sites had partial survival of both adult and juvenile salmon.

3. The only site that was not treated with a sea lice bath treatment during August had partial survival of adult salmon.

4. A site treated for sea lice in late July, just before the water temperature at 0.5 m increased to $\geq$15°C, had no increase in background fish mortality.

5. Two sites treated for sea lice in early August, just after the water temperatures at 0.5 m were >15°C, had spikes in fish mortality > 0.1% and > 0.5%.

6. Seven of eight sea lice bath treatments in mid-August were followed by spikes to around 1% in the weekly fish mortality. Fish mortality with the other lice treatment that did not require handling was > 0.1%.

7. Seven sea lice bath treatments in late August, when water temperatures at 0.5 m remained >15°C at all sites, were all associated with a subsequent weekly mortality >1%.

Our rough calculations of weekly fish mortalities ranged from 0.05% to 4.0% during August 2019, with one site exceeding 2% during the week of August 5 and three other sites exceeding 2% during the week of August 19 (Figure 1). According to the site license (see Glossary page 50) these weekly mortality rates would not have triggered a fish ME, as they would need to reach 5%.

Weekly mortality at all 10 sites approached 2% or reached 10% during the week of August 26 through to September 2. A fish mortality event at one site that was reported by NHSF to FLR on August 28 was in compliance with the site license. ME reports followed for other sites a few days later, again in compliance with the site license, after which a catastrophic 100% mortality occurred at six sites and partial mortality (33-50%) occurred at four other sites.

We had additional information about the index site in Fortune Bay which provides a longer temporal sequence of events at that location than at other sites. Briefly, the temperature at 0.5m rose rapidly from July 22 (12.9^{o}C) to July 28 (17.6^{o}C). During that week, oxygen saturation at 0.5m ranged from 84-102% and at 5m was 77-85%. The following week on July 31, fish in all netpens were handled for a lice treatment and the average count of gravid females 10 days earlier at this site was 1.8. The water temperature at 0.5 m was 17.3^{o}C on July 31 but 4 consecutive days of >18^{o}C at 0.5m followed and water temperatures at 5m were about 15^{o}C, with minimal change in oxygen saturations. Water temperature and oxygen saturation patterns were generally similar to those of other affected sites in Fortune Bay. The consecutive mort counts increased over the next 3 weeks (July 22-28; July 29-August 4; and August 5-11), but then decreased over the following 2 weeks. A major spike (3 to 4-fold increase over the week of August 5-11) occurred during the week of August 26-September 2. It was not possible to breakdown mortalities into pre-treatment days (July 29 and 30) vs post-treatment days (August 1 to 4) because daily mort data were not available for this site, as with other sites in the investigation.

In contrast, we received data from a single non-affected site operated by NHSF which was being farmed with deeper cages (25m). The site only had 2 August days where temperatures at 15m were >15^{o}C compared with sites in Fortune and Harbour Breton Bays whose median numbers of days of >15^{o}C temperatures were 10.5 and 5.5, respectively. The non-affected site had a mortality rate of 0.06% during the week of August 26 to September 2 which was lower than all 10 affected sites in the two bays during the same week. We present these summary data for transparency but make no conclusions because they may or may not be representative of all non-affected sites in NL.

Statistical comparisons of the number of days in August with surface water (0.5m) temperatures greater than 18^{o}C showed significantly (P=0.02) higher median values in farms with subsequent 100% mortality (24.5 days) compared to those with partial mortality (19.5 days). A similar result was found at 15m depths using a cutoff of >15^{o}C for the elevated temperatures as there were only 3 days when the temperatures reached 18^{o}C. In contrast, depth of water beneath cages was not a significant factor. This is not surprising because all the fish were in pens of the same depth and therefore did not have the opportunity to move to cooler water that would have been available in deeper cages (25m).

Handling of fish for lice treatment on days when surface water exceeded 18^{o}C was associated with 100% mortality (6/6 sites) compared with sites with partial mortality (1/4 sites). The odds ratio, which indicates how strongly the risk factor is associated with the 100% mortality, was 30.3. However, this result should be interpreted with caution as weekly spikes were not always evident immediately after treatment but handling of fish was likely to have been an additional stressor which further compromised health of already hypoxic fish.

Additional observations from our assessment of the environmental conditions during the ME are summarized in Appendix E (see page 44).

Satellite images

The images from EOSDIS Worldview clearly indicate a surface phenomenon in Fortune Bay that lasted for a few days from August 31 to September 2, 2019 (Figure 4). We were also provided a low resolution satellite image by C-Core Memorial University of Newfoundland which also shows a shadow in Harbour Breton Bay on September 21, 2019 (Figure 3). One possibility is that there was an algal bloom in Fortune Bay in late August-early September, as well as an algal bloom in Harbour Breton Bay in mid-September. While algae add oxygen to the water during daylight, they remove oxygen from the water at night, creating hypoxic conditions during a bloom. Thus, an algal bloom could have exacerbated the compromised environmental conditions in the netpens. A toxic algal bloom, on the other hand, could have directly killed salmon.

5.2.3. Our Conclusions about the Cause of the Mortality Event

Conclusion 1

Based on our evaluation of the available data, it became apparent that the pattern of mortality was inconsistent with an infectious agent that was spread site to site. Therefore, we rejected our disease hypothesis as the primary cause of the ME.

The unprecedently high water temperatures were certainly a major contributing factor to the ME, but Atlantic salmon can endure temperatures between 15-18^{o}C without other contributing factors. Therefore, we rejected our temperature hypothesis as the sole cause of the ME.

Instead, we found support for our hypoxic squeeze hypothesis: **An unusual set of natural environmental conditions triggered a series of events that lead to mass asphyxiation of salmon, with up to 100% mortality at some sites, that involved two year classes of salmon and occurred rapidly around the beginning of September 2019.**

Conclusion 2

Based on the Committee's review after receiving lice treatment data from NHSF, we have concluded that a significant contributing factor to elevated mortality at the index site in August was fish handling associated with bath treatments for sea lice during the period of high water temperature. At this site, there was clear evidence of a mortality spike (over the next 2 weeks) that followed a bath treatment on July 31. Water surface temperatures were close to 18^{o}C at the time of treatment, the bottom of the netpen, and even water temperature at 15 m, was nearly an unprecedented 15^{o}C during the first 8 days following treatment. Subsequently, temperatures at those depths, but not the surface temperatures, returned to below 10^{o}C and mortality decreased concurrently. The linking of handling of fish for lice treatments with water temperature data at all levels helped strengthen conclusions about possible mortality effects.

5.2.4. Our Proposed Sequence of Events Regarding the Cause of the Mortality Event

1. The main cause of the ME was likely a non-infectious, 'hypoxic squeeze' resulting from a worsening spiral of environmental insults within the netpens at all sites.
2. The main triggering event of the ME was high water temperatures, which at the surface were >15^{o}C on all days during August and > 18^{o}C on the majority of days in August in all but one site. Even at 5 m and 15 m, water temperatures exceeded 15^{o}C on some days during August. These less than optimal surface temperatures then triggered salmon to naturally seek cooler water near the bottom of the netpen.
3. Crowding of salmon at the bottom of the netpen then greatly increased the need for water exchange across the netpen walls to replenish the oxygen the salmon were removing from the water inside the netpen.
4. Crowding stress and agitation (because water at the bottom of the netpen (15 m) was >15^{o}C on some days during August) further increased the fish's oxygen needs. As water exchange across the netpen walls became inadequate, netpen water became progressively hypoxic.
5. Dissolved oxygen levels in netpen water reached their lowest concentration at all sites around August 20. The lowest levels recorded were >70%. Thus, 20-30% less oxygen was available to the salmon and the salmon were expending more energy to obtain their oxygen needs. Weekly fish mortality rates had now increased at all but two sites.
6. Dead fish (sometimes in the hundreds) that accumulated on the bottom of netpens between regular diver collections then collapsed the unsupported walls of the nylon netpens, greatly restricting the available water inside, and consequently restricting the much needed water exchange across the bottom of the netpen. Thus, less than timely fish removal would contribute to fish mortality.
7. Fish handling events during sub-optimal environmental conditions in netpens also contributed to increased fish mortalities. Indeed, fish handling during bath treatments for sea lice during the warm-water event throughout August were associated with spikes in weekly mortalities. Mortality due to stressful handling would likely be delayed mortality (see Glossary) and spread after several days post-event.
8. While the overall pattern of mortality was inconsistent with an infectious agent that was spread site-to-site, infectious salmon anemia virus infection likely contributed to a mortality spike at one site during July and August.
9. An algal bloom in the area, as suggested by satellite imagery, would have exacerbated the hypoxic conditions because algae remove oxygen from the water at night.

Our proposed sequence of events is consistent with the summary report from the AAHD veterinarian (Dr. O'Brien) who concluded that:

> "The abnormal mortality event was consistent with environmental factors including an increase in water temperatures and low dissolved oxygen. The environmental insults may have been exacerbated for sites that had possible contributing factors such as FLR-confirmed infectious salmon anaemia virus (ISAv) prior to the event, specifically site # 10. The environmental events resulted in abnormal fish behaviour with fish seeking cooler water and swimming to the bottom of the net in an avoidance behaviour. The sustained elevated water temperatures, concurrent low dissolved oxygen and fish behaviour combined to create a situation that resulted in hypoxia/anoxia leading to asphyxiation".

The AAHD veterinarian did not have access to all the environmental and sea lice bath treatment data that the Committee received and therefore would have been less likely to provide a plausible sequence of events that was informed by discussion with NHSF site personnel, divers and the company veterinarian. As with all field investigations, there were data limitations that should be taken into account when making inferences about causes and their effects. First, the site mortality data are confounded in time and space. All high mortality events were reported earlier than the partial mortality events. This may have been because NHSF resources were at maximum capacity and there was delayed reporting for logistical reasons associated with ensuring an expedient response at the first 6 affected sites. Finally, we only had access to data from a single unaffected site with a low mortality (0.06%) in the critical week of August 26 – September 2, when most other sites had substantially increased mortalities. Hence, we were unable to compare reasons why sites in these two bays had elevated mortality compared with sites with normal background mortality in other geographical locations.

Despite these limitations, we believe that our conclusions about causes of the ME are robust, biologically sound and the risk factors (high water temperatures and handling of fish for lice treatment) occurred before the mortality increased. The conclusions are well supported by coherent evidence from multiple sources, including histopathology of tissues, calculation of dissolved oxygen deficits, limited statistical evaluation, and a thorough review of clinical and diagnostic findings in the context of field experiences of the NHSF veterinarian.

5.3 Our Summary of the Timeline for Mort Removal, Disposal and Site Clean-up

Mort removal was primarily facilitated by divers and seiner activity under the direction of NHSF. Disposal methods included anaerobic digestion at NWD in Stephenville, NL and rendering by the Barry Group Inc. at its rendering plant in Burgeo, NL. These are currently the only options available in the province for mort disposal.

Site clean-up during and following mort removal and disposal was conducted by NHSF and included collection of surface fat using booms and pillow cases on all sites followed by removal of nets.

Mort Removal
Mort removal was a complicated and challenging process. The Committee used several variables (e.g. daily dive limitations for divers, # of seiners and seiner holding capacity (160-220 tonnes per vessel), number of netpens, estimated mass of dead fish per netpen, estimated vessel loading and offloading rates (10-50 t/hr and 10-15 t/hr), steam time to and from the rendering plant (24-26 hours), and plant processing capacity (10-15 t/hr)) to assess the amount of time it would likely take to remove about 5,000 t of mortalities from 10 netpen sites. The mortalities were spread across six sites with 100% mortality in 72 netpens and another 4 sites with 33-50% mortality in 56 netpens (estimated to be equivalent to 20.52 netpens with 100% mortality).

Based on the simplified calculation presented in Appendix B (refer to page 38), the Committee estimated that the time for mort removal from all 10 sites required about 46 days from the time that mort removal began (September 10, 2019). Therefore, under these adverse conditions, we believe the earliest all morts could have been removed from all 10 sites was sometime during the week of October 20, 2019. NHSF reported that all morts had been removed from all affected sites on October 25, 2019 which is within the estimated timeframe.

Seiner Activities
NHSF contacted the Barry Group Inc. (Barry's) on August 30, 2019 (Friday before a long weekend – Labour Day) to request three seiners for mort removal assistance. At the time of this request, Barry's had two seiners (Ocean Leader, Eastern Pride) undergoing retrofits for fishing. A third seiner (Nancy Jillian) was at sea in the Labrador Straits preparing to start fishing mackerel.

The Ocean Leader and Eastern Pride were the first seiners deployed from Corner Brook to assist NHSF with mort removal. However, the two seiners were not deployed until September 6, 2019 as they had to be retrofitted for mort removal activities. The retrofit took about one week and involved changing hoses, fittings, wiring and pumps from those used for fishing (i.e. live fish removal) to those that were appropriate for dead fish removal. For example, longer vacuum hoses (120-130 feet) were required to reach the bottom of the netpens. Due to Hurricane Dorian which created >30 m seas, mort removal did not begin until September 10, 2019.

The Nancy Jillian was called back from the Labrador Straits and arrived onsite to assist NHSF with mort removal on September 14, 2019. By September 26, 2019 NHSF requested a fourth seiner to help with mort removal. The Margaret Elizabeth was deployed September 27, 2019.

NHSF directed seiner activities and site clean-up activities. NHSF held daily conference calls with Barry's and the seiner captains to determine site priority each day. Priority was based on the condition of the fish at each site, weight or strain on the netpens at each site, with the goal of preventing netpen breakage. A site would not necessarily be completely cleaned out before seiners were directed to move to another site. The priority to this approach was to decrease the weight in the netpen to prevent collapse and avoid fish escapes.

Mobilizing seiners to remove fish

Based on the Barry Group's experience over the last 8-years responding to ISA events for Cooke Aquaculture and NHSF, the response time for deploying seiners for this type of situation is typically 1-week.

The main delays for the NHSF 2019 ME can be attributed to the following factors.

- NHSF did not have a contingency plan in place to have seiners on call during the warmer summer months, although NHSF standard practice is to have at least one seiner on-call in the winter to respond to "super-chill" events.
- The ME occurred at the start of a long holiday weekend, making it difficult to arrange seiners and divers to begin mort removal activities.
- At the time of the ME, the seiners were either actively fishing (1 seiner), or dry-docked being retrofitted (2 seiners) for mackerel fishing.
- The retrofit to equip the seiners for mort removal activities took about 1-week, therefore the earliest the first 2 seiners could be deployed was September 6.
    - The seiners arrived on site on September 7, however due to Hurricane Dorian, mort removal activities did not begin until September 10.
- "Mortalities" included a range of materials from whole dead fish, to partially decomposed fish to "sludge" making it difficult to remove this material from the netpens, and hence requiring more time to remove in comparison to removing live fish.
- During mort removal activities the use of vacuum pumps unavoidably resulted in additional water being recovered with the dead fish material. The fish material therefore required dewatering prior to loading aboard seiners and again before processing through the Burgeo rendering plant resulting in additional removal and processing time.

Diver Activities

Divers facilitated the removal of morts from netpens by using a vacuum system with extension hoses about 40-50 m long to reach the bottom of the netpen. These hoses were put in place by divers who would move the hose around in the netpen to the areas where the fish had accumulated. The biomass that was pumped out of the netpens contained live and dead whole fish, sludge and seawater. Divers used vacuum pumps to suck-up the dead fish which were deposited on a dewatering screen before going into the fish hold onboard the seiners.

During normal operations, NHSF engages 2 dive companies for mort removal which is conducted twice per week per site from May to November/December. Dives are conducted in teams of three divers under normal circumstances. During the ME a pool of independent divers were engaged through five dive companies. This created some confusion and delays in trying to arrange divers as multiple companies approached the same divers. Eventually, approximately twenty divers were brought in from NL, NS, NB (there were no divers available from BC). However, many of the divers were already working on other jobs at the time which caused an additional delay.

During the mort removal process diver safety was a major concern. Diving practices were inspected by outside agencies to ensure safety. Initially, Barry's was advised that the divers were not allowed to be in the netpen while they were pumping out the fish due to diver safety. However, there was no way to determine where the fish were in the netpen and pumping was going very slow. Eventually, a Diver Safety SOP was implemented by NHSF, which allowed the divers to be in the netpen to place the hoses in areas where fish could be pumped out.

On some sites, nets were weighed down by dead fish to depths well below 15 m that would require decompression dives to clean up. A component of the diver safety SOP that was implemented *ad hoc* during the clean-up included an assessment of each net pen to determine if the diver could do the dive safely based on the depth of the dead fish mass. Therefore, some netpens were not accessible by divers.

Each diver averaged about 6 hours per day in the water performing dead fish removal, with some variability depending on dive tables, dive depths and type of dive gear. There is no question that this is an arduous dive schedule day in and day out. No dive activities were permitted to take place after dark due to worker safety concerns, therefore the maximum mort removal time per day could not exceed 10-12 hours on average.

Mobilizing divers to remove fish

A number of issues have been identified as factors contributing to the delay in mobilizing divers.

- NHSF did not have a contingency plan to have additional divers on call beyond their normal needs for mort removal under "normal operations".
- Eventually 20 divers were brought in from five dive companies and 3 provinces.
    - Delay because the same diver was on call for several companies creating confusion
    - Travel time to get to sites from NS, NB and St. John's
    - Training time. Most divers had never dived in a fish pen and required training before doing a dive.
- Diver reports suggested that the mass of dead fish on the bottom of the netpens had pulled in the sides, even to an extent that divers could not easily get down to the dead fish, and reduced the available netpen volume.
- Decompression limits limited total dive time of any individual diver on a given day. Therefore, NHSF cycled through divers during the day, with maximum dive time averaging 6 hours per day.
- There were also some issues with communication between the divers, pump operators and the deck. Some divers had very good communication gear, while others had limited to no communication gear. Poor communication caused additional delays which resulted in slower pumping.

Rendering Activities

All morts collected by seiners were sent to Barry's rendering (fishmeal) plant in Burgeo, NL. The morts were processed according the Barry Group's 64-page CFIA approved standard operating procedure (SOP) for processing ISA-infected morts. Although the fish affected by this event were not considered to be diseased, Barry's followed their existing SOP as a precautionary measure.

The maximum processing capacity of the rendering plant was 15 tonnes/hour for fresh morts, for 20 hours processing capacity/day or 300 tonnes/day. However, older morts and sludge were more difficult to handle resulting in longer processing times in comparison to fresh morts. Additional delays were encountered because the waste water also had to be heated to 80^{o}C for 10 minutes before discharge as required by the rendering SOP for a pathogen/ISA event.

All fishmeal produced from the rendering process was tested for residual therapeutants as per the SOP and according to reports from both NHSF and Barry's, all fishmeal tested negative for these agents following the rendering process.

Due to the variable quality of the fish mortality material, the resulting fishmeal had lower yield and quality (i.e. lower protein content) than what would normally be produced. However, it did fit within the quality parameters for fishmeal, but likely at a lower price point than usual.

Anaerobic Digestion
For three of the sites which experienced only partial mortality, divers were used for mort removal at these sites, and seiners were not involved. Morts were removed by divers filling totes which were loaded onto trucks and shipped to New World Dairy (NWD) in Stephenville for anaerobic digestion. Fat was also skimmed from the surface at these sites using a boom and pillow cases. The collected fat was also sent to NWD for anaerobic digestion.

Site Clean-up
Site clean-up activities by NHSF included mort removal and disposal as described above, as well as the collection and removal of organic deposits from the affected sites. Recognizing that some of this organic matter could not be easily collected NHSF sought additional advice from experts within DFO, FLR and ECCC for guidance regarding environmental clean-up and monitoring. Based on the collective feedback, NHSF decided to engage MAMKA (Mi'kmaq Alsumk Mowimsikik Koqoey Association) to conduct environmental monitoring in the affected areas. MAMKA publicly released its interim report on November 29, 2019. MAMKA's key finding was that 2.9% (1,565 m) of the shoreline assessed (54,608 m) was impacted by the ME, and no impacted bird species were observed during their monitoring activities. MAMKA's final report is not yet available.

DFO also conducted at-sea and air surveillance throughout the fall of 2019 and the spring of 2020 to visually assess the areas affected by the ME. DFO reported that their surveillance activities to date have shown a few locations with organic deposits present, but no widespread impacts to the coastline. These findings are consistent with MAMKA's interim report.
Two of the Committee members visited 5 of the affected sites in January 2020. At that time there was no visible evidence of organic deposits on any of the sites visited or along the shoreline near these affected sites. However, this was not a formal environmental assessment which is beyond the scope of this report, and is mentioned here only as an observation.

Environmental monitoring by MAMKA and DFO are still ongoing. Based on the pending results additional clean-up activities may be undertaken by NHSF if necessary.

Recommendations:

1. NHSF should have a contingency plan to have seiners and divers on call beyond their normal operating requirements particularly during the warmer summer months.
2. Consideration should be given to using other options which do not rely on divers, such as the use of automated lift systems in netpens to allow more frequent, faster and safer methods of mort removal.
3. An established diver safety SOP should be included in the contingency plan. This should include minimum requirements and standards for communication equipment so that divers can effectively stay in contact with the pump operator and deck.
4. We recommend earlier reporting by site managers to the company veterinarian regarding the condition of fish on-site (e.g. when first signs of weak fish are noticed) which would allow NHSF to be more proactive, thus minimizing fish losses and the potential for adverse environmental impacts.
5. Proactive removal of compromised fish before environmental conditions that could lead to a worsening ME (i.e. live fish are easier to remove than dead fish).
6. FLR in collaboration with industry should evaluate the availability of additional mort disposal options in the province for large-scale non-infectious MEs. Capacity is currently limited to two options (i.e. NWD, Burgeo rendering plant).

5.4 Our Comments on the Reporting of the ME

On-going infectious salmon anaemia (ISA) investigations

As a preface to the Committee's comments, we acknowledge that AAHD was dealing with suspected cases of infectious salmon anaemia (ISA) reported on two sites. ISA is a reportable disease attributable to strains of ISA virus (ISAv) of differing pathogenicity and several ISAv outbreaks have been reported in the province since October 2017. As such, AAHD has criteria for confirmed and suspect cases of ISAv. Also, AAHD has a contingency plan for management of ISAv, including preventing spread of the virus, which site licensees must adhere to.

The AAHD administers the Finfish ISAv Surveillance Plan which requires at least monthly testing of sites by designated (company) veterinarians. An Enhanced Finfish ISAv Surveillance Plan with bi-weekly sampling (done jointly by designated veterinarians and FLR AAHD) follows detection or suspicion of ISAv. For the latter purpose, the AAHD veterinarian and staff were on one site on August 29-30 investigating ISAv. This site was added to the ME list on October 11, 2019 but no diagnostic testing was done because the site was to be depopulated. On September 9, a quarantine order was imposed on the site because of pathogenic ISAv and site was harvested using Standard Operating Procedures (SOPs) for High Risk Material. The AAHD approves SOPs associated with handling High Risk Material and will conduct biosecurity audits of these activities.

The AAHD personnel were unable to visit other sites for 72 hours after visiting possibly infected sites – this time period is required between sites/companies or between sites of different disease status to ensure biosecurity protocols are not breached. Hence, there was insufficient surge capacity (adequately trained technicians with field and/or laboratory experience) to respond to both the suspect ISAv detections and the unprecedented ME that occurred concurrently. Theoretically assuming that human resource capacity was not limiting, two well-trained technicians who each sampled two sites a week should have been able to complete the sample collection for the four sites in a week or if there were two technicians per visit, it would have required two weeks.

Finally, the quality of some of the sets of gill samples was variable as noted by Dr. Groman and sample collection and fixation of some fish soon after returning to land for histopathology might warrant consideration. In fairness, because of biosecurity concerns with ISAv, the focus was on the logistics of getting samples back and the same staff dissecting fish for distribution of specimens for testing at other laboratories.

Recommendations:
The Committee acknowledges that response to this case was a complicated one because it involved multiple sites in different geographic locations that were affected within a narrow time-window and with variable site-level mortalities. Had all the sites been affected by a reportable disease, which typically does not result in a 100% mortality and occurs within a tighter geographic boundary, a different and more timely investigational response might have occurred. We make the following recommendations in the context of helping ensure timely responses in future ME:

1. Although several steps have already been taken by FLR (see next paragraph), there is a need for a clearer differentiation of the roles and responsibilities of company veterinarians compared with government veterinarians in NL, including the individual and collective responsibilities of companies and government, and communication of information to the NL public who will likely be closely scrutinizing how the next ME is handled.

   Note: In the past week, the Committee became aware of an updated policy document for salmon disease surveillance that in future AAHD will conduct risk-based targeted, active surveillance in addition to surveillance performed by the designated (company) veterinarian who must make a surveillance visit to all sites at least once per month (Appendix F page 4747).

2. Specific questions that might be addressed, in relationship to the first point, for strengthening the implementation of the policy going forward include:
   a) who is responsible for the work-up of a case once ISAv or another reportable disease is ruled out and when does that transfer of responsibility officially happen?
   b) who should pay for any investigation by AAHD and associated laboratory testing costs in future ME events, when ISAv is not suspected or ruled it?
   c) should government veterinarians respond to any ME above an agreed-to threshold or should companies take the lead through the attending clinical veterinarian who is ultimately responsible for the care and control of animals. Also company vets

have details of history and presenting signs that would not be readily available to government veterinarians and this would be a valuable addition for laboratory diagnosticians.

3. If government veterinarians are to respond to any or all future ME of this magnitude, more resources are required as this will exceed the capacity of the current budget and human resources in place. These resources need to be clearly defined, including costs, as part of a contingency plan for future ME.

4. If government is to have a role in future ME, it would be prudent to consider employment of an experienced emergency response coordinator to optimize the deployment of human resources and equipment, liaise with other Departments and contractors, facilitate transport of fish to the laboratory and laboratory testing, and working with other professionals ensure that all personnel are adequately trained (and practiced) in response protocols. If done, this would allow AAHD veterinarians to dedicate their efforts to using epidemiological methods to assign putative causes (agent, host and environmental) to similar future events. Environmental and lice data will likely be available for January 2021 and this will require additional training in multivariable analysis of risk factors.

5. An investment in personnel training of an inspector/support team should be made proactively and a more detailed training plan developed before a major salmon industry expansion occurs. This must be done in times when such ME and disease events are not happening (commonly termed "peacetime"). Continuing education in the form of conferences, workshops and advance training is essential to maintain the skills of the AAHD to ensure they keep current with the latest knowledge pertinent to their responsibilities.

6. Although the ME was ultimately determined to be non-infectious, the reasons for the delayed response by AAHD should be examined to determine whether the actual response times could have been shortened given the available trained personnel. For example, would use of helicopters to transport sample and perhaps personnel improve response speed and sample quality?

7. The toll on AAHD personnel involved in investigation of ME such as this should not be overlooked. Human fatigue, compassion fatigue, social pressures, poor work-life balance and mental health issues likely affected both industry and government workers throughout an extreme and unprecedented ME episode and may continue to take a toll. We recommend that the government start an evaluation of adverse effects of the ME on worker health. This evaluation should quantify the magnitude of the issue but should offer solutions for those affected and be part of a contingency plan for similar future events.

8. The recent loss of a highly-skilled veterinarian from AAHD will have further negative impacts on those that have remained. Strategies for recruitment and retention of veterinarians and

fish health specialists should be critically evaluated to ensure that FLR is highly competitive in a national and international market where demand exceeds supply.

5.5 Review of Finfish Aquaculture Policies in Canada

Across Canada the federal and provincial governments have a shared responsibility for the governance of aquaculture activities. However, the roles and responsibilities of federal and provincial governments vary by province and are covered by bi-lateral agreements/MOU's.

Overall, Fisheries and Oceans Canada (DFO) has the lead federal responsibility for managing Canada's marine fisheries and protecting the oceans through the Aquaculture Activities Regulations of the *Fisheries Act.* Other federal departments which play a role in regulating Canada's aquaculture industry include Transport Canada, Environment Canada, Health Canada, the Canadian Food Inspection Agency, and Agriculture and Agri-Food Canada.

It should be noted that a detailed review of aquaculture policies and procedures was not possible due to time limitations.

5.5.1 British Columbia

In British Columbia, DFO has the main responsibility for regulating and managing aquaculture activities through the Pacific Aquaculture Regulations (2010) and the Aquaculture Activities Regulations (2015). Key activities under DFO's B.C. Aquaculture Regulatory Program (BCARP) include licensing, review of applications, consultations with First Nations and stakeholders, environmental monitoring, fish health management and public reporting.

The province is responsible for issuing Crown tenures (primarily leases or licences of occupation) that allow salmon farms to operate in provincially owned foreshore, nearshore and inland waters and conduct related activities on shore.

5.5.2 Nova Scotia

In 2014 the Final Report of the Independent Aquaculture Regulatory Review for Nova Scotia [The Doelle-Lahey Panel] was released. The main finding of this report was "that a fundamental overhaul of the regulation of aquaculture in Nova Scotia was called for". The Committee concluded that this overhaul should be guided by the idea that aquaculture that integrates economic prosperity, social well-being and environmental sustainability is one that is low impact *and* high value. By this, we mean aquaculture that combines two fundamental attributes: it has a low level of adverse environmental and social impact, which decreases over time; and from the use of coastal resources it produces a positive economic and social value, which is high and increases over time. As a result of this report, Nova Scotia revised its aquaculture regulations/policies in 2015.

The Committee contacted the Nova Scotia Department of Fisheries and Aquaculture (NSDFA) and requested a copy of the new policies and procedures manual. However, the Committee was advised that NSDFA does not have a publicly available policies and procedures manual associated with the changes made to the regulatory framework for aquaculture in Nova Scotia. Many of the policies are internal and in draft form and were not available for our review.

5.5.3 Newfoundland and Labrador

Fisheries and Oceans Canada (DFO) was notified of the mortality event (ME) on September 4 by DFO fishery officers who had become aware of the situation through fish harvesters in the area. After confirming with FLR and NAIA that the ME was not due to a fish health/disease issue, the department reviewed the Aquaculture Activities Regulations (AAR) of the *Fisheries Act* to determine where a non-infectious ME is within the current regulatory framework.

Under the AAR, licensed aquaculture facilities are required to report to DFO any fish morbidity or mortality, occurring outside of the facilities, and affecting wild fish populations, within 96 hours after the deposit of any drug or pest control product as described in the AAR (Section 13 of the AAR). However, the AAR does not have a provision for farmed fish morbidity or mortality events that occur within the lease boundaries of a facility (i.e. on the farm site).

The AAR has a provision in Section 3 which allows aquaculture facilities operating with an active license to deposit "deleterious substances" into the marine environment, under conditions set out in Sections 4-14. For the purpose of the AAR, a deleterious substance is defined as a drug, a pest control product, or biochemical oxygen demanding (BOD) matter.

At the time, DFO concluded that the NHSF 2019 ME fell within the AAR for normal aquaculture operations and that the release of organic waste (in this case, dead fish material), is a permitted activity for licensed aquaculture facilities.

As discussed in section 5.3 (page 21) NHSF sought additional advice from experts within DFO, FLR and ECCC for what they could do with environmental clean-up and monitoring. Based on the collective feedback, NHSF decided to engage MAMKA (Mi'kmaq Alsumk Mowimsikik Koqoey Association) to conduct environmental monitoring in the affected areas.

DFO conducted at-sea and air surveillance throughout the fall of 2019 and the spring of 2020 to visually assess the areas affected by the ME. DFO reported that their surveillance activities to date have shown a few locations with organic deposits present, but no widespread impacts to the coastline. These findings are consistent with MAMKA's interim report.

On October 11, 2019 FLR suspended NHSF's licenses for the 10 affected sites. Consequently, in the absence of an active license, the AAR does not apply. To further assess the impact of the ME on fish and fish habitat, DFO initiated environmental monitoring of the area to supplement the work MAMKA was doing. The DFO monitoring program is designed to determine how much organic material is remaining in the marine environment post-ME. This involves analyses of sediment samples, water samples and shellfish samples. The assessment is currently ongoing.

DFO continues to assess this incident with regard to potential impacts to fish and fish habitat, and will use the information gathered to inform on how improvements can be made going forward when addressing non-infectious aquaculture MEs within the regulatory framework of the AAR and the *Fisheries Act*.

FLR's New Aquaculture Policy and Procedures Manual

The 2007 version of the FLR Policy and Procedures Manual (PPM) which was in force at the start of the ME on August 28, makes no reference to reporting requirements for incident events, including mortality attributable to any type of harm, and the need for a contingency plan (e.g. fish removal, carcass disposal, clean-up including ensilage) to mitigate consequences of such an event. While we saw a response plan specifically for ISAv that had been jointly developed with CFIA, but we were unable to find a response plan for a non-infectious ME. As such, NHSF and FLR were inadequately prepared to respond rapidly to the incident evident which was not ISAv (or another OIE-listed disease).

After the ME had started, a new PPM was signed by the FLR Minister on September 24, 2019. The new PPM had been in development since 2017 when FLR committed to a comprehensive review and modernization of the provincial Aquaculture Licensing and Procedures Manual as part of The Way Forward on Aquaculture Sector Work Plan. The development of the new PPM was a collaborative effort between Newfoundland Aquaculture Industry Association (NAIA), industry and FLR. NAIA was regularly engaged on behalf of its members with DFRL during the development of the new PPM. In addition, a full-day stakeholder consultation session was held in Grand Falls-Windsor on August 6, 2019 to review policy changes with industry and other organizations. Six industry or related organizations participated in the finfish sector consultations. A public questionnaire was also placed online for stakeholders to provide feedback and was available during the period of August 6-19, 2019. A summary report of the consultation process, key feedback and a list of participations is available online at:
https://www.fishaq.gov.nl.ca/publications/pdf/Summary_Aquaculture_Governance_Consultation_2019.pdf

NAIA and its members were aware that the new PPM would be launched at the NAIA annual Cold Harvest conference on September 24, 2019. However, industry did not see the final version of the PPM until it was presented at the conference. Some of the policies had been revised following the August 6-19, 2019 consultation process but these revisions were not presented to industry in advance of the launch. As a result, some of the new procedures have already been updated to address industry concerns related to implementation of the new procedures.

The new PPM has 46 policies and procedures with a much greater emphasis on a broad range of fish health management procedures, site fallowing, and contingency plans. AP-17 Public reporting (effective date: September 24, 2009 and updated on November 4, 2020) is most relevant to the ME event. However, we do not feel that the new PPM would have helped in a major way with the recent ME. Language such as a "suspicious fish mortality" is inadequate and would benefit from inclusion of an objective criterion such as a threshold value for reporting.

Specifically, but briefly, the new PPM requires aquaculture licensees to openly and transparently report "incident events" (harms, abnormal mortality, or imminent threat) within 24 hours of detection, which is good. However, the description of an "incident event", including a ME is ambiguous and not based on any criteria or threshold that would provide clarity to end-users or any observer for that matter. Clearly, the date of the index case would be different if a specific threshold had been provided in advance – see the section on the case definition for the mortality threshold for more details. In addition, all licensees need to maintain an Incident Management System (which is not defined to our knowledge).

Other requirements in the new PPM include written notification to the Assistant Deputy Minister, FLR and reporting of the incident event within 24 hours through public communication. Since the ME and introduction of the new PPM, companies have been publicly reporting incidents on their own websites, as well as on the Newfoundland Aquaculture Industry Association's (NAIA) website. In addition, according to NAIA, their members are now communicating more openly with one another and trying to keep each other more informed about such "incident events". New policies (AP29) on Aquatic Animal Heath Surveillance (AP29) require a visit by a veterinarian to all active sites every 30-45 days and AP33 (Aquatic Animal Health Contingency Planning) provides the requirements for a contingency plan.

Recommendations:
The Committee recommends further refinement of existing salmon company contingency plans to prepare for a robust, thorough and timely response in the event that a similar mass mortality occurs this summer with goal of mitigating the social, financial, worker health, and ecosystem consequences. Ideally, the response plan should be "similar" across all salmon companies growing fish in NL, especially in regard to issues such a mort removal and disposal. Several of the issues raised in other sections pertain to contingency planning. The AAHD contingency plan should also be strengthened especially in the area of infrastructure and surge capacity and it may be prudent to stage a "practice event" to establish whether potential bottle-necks identified in this review have been rectified. A strengthened plan for more proactive and perhaps coordinated media communication strategy in such events is likely well justified.

6. Final Recommendations

1. There is a need for new netpens at NHSF sites based on two issues: (a) their structural integrity, and (b) depth.

(a) Verbal reports of the observations made with cameras lowered on a line during daily feeding that the nylon netpen structure lost its integrity with the walls pulling in due to the weight of dead fish on the bottom of the net. We recommend that all netpens should have stiffer wall structures to prevent such occurrences. Our understanding is that MOWI East has already begun replacing (and will eventual replace all of) its existing netpens (100 m circumference x 15 m depth)[4] with a netpen that has a strengthened wall design to resolve this issue.

(b) The netpen depth of 15 m was too shallow to provide an appreciable deep-water cold refuge for salmon should the surface water become unusually warm as it did in August 2019. Given that the temperature records for 2019 all show periodic cold seawater intrusions that varied from site to site, a larger and deeper netpen would solve the problem that existed during August 2019. Our understanding is that MOWI East has already begun replacing (and will eventual replace all of) its existing netpens with ones that are 140 m in circumference and 20 m deep, plus a 6 m cone on the bottom. Not only will these new netpens increase the netpen volume by almost 3-fold, from about 12,000 m^3 to about 35,500 m^3, they provide access to cold, deep water by being more than 5 m deeper and have an extra volume below 15 m (just over 11,000 m^3) that is almost equivalent to the entire volume of the old 15 m deep nylon netpens.[5] These calculations assume that stocking density remains unchanged, at around 3.6 kg/m^3. In our opinion, a deeper netpen would be a better option than a contingency plan that required either relocation of the netpens or a temporary aeration system, which in any event would require proof-of-principle testing before being adopted. NHSF sites have the water depth to accommodate the new netpens and they can be installed without any proof-of-principle testing.

2. Continuous online monitoring of environmental conditions is needed during the summer months, at a minimum, at NHSF sites.

The temperature and DO records made from a small boat during July and August were too limited (one value of each for a whole site rather than for each netpen and not always to the bottom of the netpen) and perhaps used different locations within a netpen. We recommend installation of more robust and more permanent recording devices onto each netpen, at least during the summer season, ones that measure temperature and DO at several depths, including at 20 m, and preferably on both sides of a netpen parallel to the

[4] Netpen dimensions have been corrected to 100 m circumference x 15 m depth. Dimensions were previously incorrectly reported as 100 m (diameter) x 15 m depth.
[5] Corrections were made to netpen volumes based on the corrected netpen dimensions.

prevailing tidal current. Water downstream of a school of fish always has a lower DO than upstream of the fish, but tidal currents will determine which side of a netpen is downstream and upstream. Any potential interactions among netpens also would become clear from such data.

3. Mortality at NHSF sites should be monitored more closely during summer months for earlier detection of warning signals associated with a changing marine environment.

Mortality spikes, as well as the appetite of fish, are dependent in part on observations made with a camera at the end of a line. The camera images we have seen from summer 2019 are poor. We recommend an upgrade of camera quality to greatly improve the images received at the surface. Permanently mounting cameras at strategic locations underwater may also improve image resolution.

4. While recognizing that August is a vacation month for Canadians, it is also the month for the hottest temperatures on salmon farms. Therefore, contingency plans developed by NHSF for the month of August should fully accommodate this challenge. For example, we recommend that sufficient divers should be on call for an emergency situation. In 2019, the frequency of dives did increase when there was a spike in mortality in early to mid-August but it was delayed response.

5. We recommend more accurate record keeping and analysis of fish mortalities than the current aggregated weekly average used by NHSF.

Our analysis for this report shows how much can be inferred from a closer tracking of mortality (e.g. internal use of lower thresholds for early warning signs), especially when combined with environmental data and fish handling events. More importantly, it would help resolve a concern about the minor practicality of the conditions of license for a "fish mortality event", which is triggered by either (a) mortalities greater than 0.05% per day for three consecutive days; or (b) fish mortalities equivalent to 4000 kg or more, or losses reaching 2% of the current facility inventory, within a 24 hour period; or (c) fish mortalities equivalent to 10,000 kg or more, or losses reaching 5%, within a five day period. These thresholds are acceptable as is NHSF's SOP to increase diving frequency following a spike in mortality on a farm, which was the case during August 2019. However, unless diving frequency is increased from twice a week (the norm for summer) to daily, conditions (a) and (b) cannot be met as soon as they occur. Likewise, if site mortality is reported as a weekly aggregated percentage, condition (c) cannot be met as soon as it occurs.

6. NHSF should review its salmon handling practices and policies in the context of fish welfare and health under extreme conditions which are likely to become the new norm, e.g. lice treatments in warm temperatures are very stressful on fish and may contribute to delayed mortality (refer to Glossary page 50 for definition).

7. Thresholds for mortality events, as described in recommendation 5, should be better defined by FLR based on broad stakeholder input and consideration of current thresholds used in British Columbia and internationally.

> Decisions about thresholds need to accommodate the consequences and prevalence of errors (false-positive and false-negative decisions). More stringent case definitions (lower mortality thresholds) will increase sensitivity but result in more false alarms. This issue should be addressed in the context of discussion about roles of government regulators and salmon companies in mortality investigation and whose responsibility it is to pay for these investigations – is it the public who pay taxes or is it the company's role as they accrue the primary financial benefits? This discussion is complicated by the on-going occurrence of ISA mortality events in NL and the need for FLR to comply with existing case definitions for national and ultimately international reporting of ISA cases.

8. The capacity of the Department of Fisheries and Land Resources (FLR) to carry out its regulatory mandate should be significantly enhanced with the salmon industry expansion while ensuring trust and confidence from the public. We recommend that FLR has

a) A veterinarian on site in St. Alban's.
b) A plan to bring in additional veterinarians with experience in salmon aquaculture from other jurisdictions, as needed.
c) More detailed description of information that should accompany lice counts or treatment data from the sites (e.g. whole sites vs. cage treatment (and cage number if the latter), date of and treatment used. Thresholds for what constitutes elevated lice counts need to be established at the netpen and site levels based on stakeholder consultation.
d) Potentially 4 technicians available in future ME to be able to sample 8 sites in 2 weeks allowing for the 72-hour rule. This only addresses the NHSF sites and would be more challenging if other company sites were affected.

9. A continuing education program is needed for AAHD technicians in St. Alban's to maintain and strengthen collection, processing and submission of tissue samples to meet acceptable standards in diagnostic testing, especially when human resources are limiting as occurred in this ME.

> This is most important in the case of specimens for histopathology where autolysis of tissues impedes diagnostic findings and for virus isolation where suboptimal handling, transportation and delays in delivery to the laboratory will increase the risk of false-negative results (failure to detect viruses such as ISAV or emerging viruses that should be routinely detectable in cell lines that are in current use by AAHD).[6]

10. While the NL salmonid aquaculture industry, through NAIA, does have a general fish management contingency plan in place to address mass mortality events, more robust company-specific contingency plans are needed. The NAIA contingency plan is a good foundation from which companies can customize their contingency plans based on their own unique situations. One important factor that the 2019 NHSF ME highlighted was the need for additional mort

[6] Text in parentheses has been reworded for clarity.

removal capacity and disposal options in the province. This event has been the largest ME in the province since the development of the salmonid aquaculture industry. As the industry continues to expand, and environmental conditions change, the industry will need expanded contingency plans to deal with future large scale non-infectious MEs. We recommend that the various government departments both federal and provincial work with industry and NAIA to address current and future requirements for timely mort removal and disposal.

Appendices

Appendix A – Terms of Reference

Background
At the request of the Department of Fisheries and Land Resources (FLR), the Marine Institute (MI) will facilitate a "Review of the 2019 Newfoundland and Labrador South Coast Cultured Atlantic Salmon Mortality Event – As reported by Northern Harvest Sea Farms (Mowi Canada East)".

Objective
To facilitate a review of the 2019 cultured Atlantic salmon mortality event at the Northern Harvest Sea Farms (Mowi Canada East) marine cage sites on the South Coast of Newfoundland and Labrador.

Scope of Work
The scope of work will include the following:

1.0 Establish a committee of independent external subject matter experts to conduct the review.
2.0 The committee will
- 2.1 Review the timelines of the event including:
  - 2.1.1 Reporting by Northern Harvest Sea Farms (Mowi Canada East) to FLR;
  - 2.1.2 Response time by Northern Harvest Sea Farms (Mowi Canada East), Barry Group and others involved in the clean-up.
- 2.2 Review the report provided by FLR's Chief Fish Health Veterinarian and other relevant information as provided by FLR such as available diagnostic, oceanographic, environmental data, etc. (pre-, during and post-event if possible). Relevant information available from other sources will also be reviewed;
  - 2.2.1 With the objective of identifying the cause of the mass mortality, and,
  - 2.2.2 To assess the clean-up response effort to the mortality event by Northern Harvest Sea Farms (Mowi Canada East), Barry group and others involved in the clean-up including;
    - 2.2.2.1 Course of action,
    - 2.2.2.2 Availability of resources such as infrastructure, logistical support, human, financial and other.
- 2.3 Make recommendations
  - 2.3.1 For improvements to the reporting requirements by industry to government,
  - 2.3.2 For improvements to response efforts by industry, and
  - 2.3.3 To prevent future occurrence.

Appendix B – Simplified Calculation of Time Needed to Complete Mort Removal

(1) The number of netpens (n) affected by the ME included:

- n_1 = 72 (100% mortality) = 72
- n_2 = 56 (33-50% mortality) = 20.52

(2) Total # of netpens (N) requiring mort removal was calculated using the following equation:

$$N = n_1 + n_2$$
$$= 72 + 20.52$$
$$= 92.52$$

(3) Average # of netpens (np) that could be cleaned per day was estimated based on a number of variables including:

a) Seiner holding capacity, loading and offloading capacity, turnaround time
b) Diver capacity
c) Plant processing capacity
d) Unexpected delays (e.g. diver stop work order)
e) Anecdotal information provided by NHSF site managers and Barry Group Inc.

$$np = 2$$

(4) Estimated # of days required to complete mort removal (M) from 92.52 netpens was calculated using the following equation:

$$M = N/np$$
$$= 92.52/2$$
$$= 46.26$$

Notes and assumptions:

1. The 56 netpens affected by partial mortality were estimated to be equivalent to 20.52 netpens with 100% mortality.
2. Netpens with smaller fish < 1 kg required about 2 hours for mort removal.
3. Netpens with larger fish > 2 kg required about 2 days for mort removal.
4. Maximum mort removal rate was approximately 2 cages per day.
5. Average mort removal time per day was 10-12 hours.

Appendix C – Oxygen Calculations

Oxygen calculations[7]

Dissolved oxygen (DO) measurements were limited to one measurement per site which is insufficient to properly understand what is happening at the level of each netpen. All the calculations that follow assume that the single measurement applies equally to all netpens in each site, which is unlikely to have been the case. The purpose of the calculations is to demonstrate the effects of changes in netpen shape, reduced DO levels associated with elevated water temperatures, and behavioural changes in the fish.

Nylon netpen: 100 m circumference X 15 m deep with flat bottom
Radius = 100/(2pi) = 15.91 m

Cage Volume = 15 m x pi x 15.91 m x 15.91 m = ~12,000 m^3 = **12,000,000 L**
(1 m^3 = 1000 L)

Two sites were used to calculate the representative total fish mass in a netpen. NHSF had provided an estimate of fish numbers for the beginning of August and the average mass of the fish at these sites, as well as the number of netpens per site. All pens were assumed to have the same fish mass to simplify the calculations.

Estimated fish mass per netpen (rounded to the nearest 100 kg):

2018 stock: largest = 3.8 kg average → 1,540,100 kg of fish in 14 netpens
= ~110,000 kg of adult fish per netpen

2019 stock: smallest = 0.5 kg average → 242,500 kg in 14 netpens
= ~ 17,300 kg of smolts per netpen

Oxygen needs for the fish mass:

At 10°C an Atlantic salmon routinely needs 100 mg O_2/h/kg → 100 x 110,000 mg O_2/h
~11 kg O_2/h per netpen for adults
→ 100 x 242,500 mg O_2/h
~1.8 kg O_2/h per netpen for smolts

Seawater contains 8.8 mg O_2/L at 10°C → 8.8 x 12,000,000 L per cage

[7] Netpen dimensions have been corrected to 100 m circumference x 15 m depth. Dimensions were previously incorrectly reported as 100 m (diameter) x 15 m depth. Corrections were made to netpen volume and oxygen calculations based on the corrected netpen dimensions. Also, assumptions used, and the purpose of the oxygen calculations are better defined.

Thus, 105 kg O_2 is available in an entire 15 m x 100 m netpen at 10°C

However, salmon die if oxygen content goes below 4 mg/L. Thus, to prevent salmon dying from hypoxia at 10°C, the oxygen available in a netpen should exceed 57.6 kg O_2 (4.8 x 12,000,000 mg O_2).

Therefore, if salmon fully utilize a netpen at 10°C, the water content of an entire netpen must completely turnover at least every 5 hours for adults and about every 24 hours for smolts to prevent salmon dying from hypoxia.

But to keep DO at 80% saturation at 10°C, only 21.1 kg O_2 (1.76 x 12,000,000) would be available in a netpen. Therefore, to keep good water quality at 10°C, the water content of an entire netpen must completely turnover at least every 2.5 hours for adults and about every 12 hours for smolts.

At 20°C, Atlantic salmon double their routine oxygen needs requiring:

→ ~22 kg O_2/h per cage of adults
→~3.6 kg O_2/h per cage of smolts

At 20°C, oxygen content of seawater decreases by 18% to 7.2 mg O_2/L. Thus, the available oxygen in a netpen is 47.2 kg O_2 to prevent salmon from dying and 17.3 kg O_2 to maintain good water quality.

Consequently, at 20°C the water content of an entire netpen must completely turnover either about every 2 hours for adults and about every half a day for smolts to prevent fish dying from hypoxia, or every 0.75 hours for adults and about every 5 h for smolts to maintain good water quality.

Now if salmon crowd into the bottom 5 m of a netpen to avoid warmer surface water, oxygen availability at 20°C diminishes either to 15.7 kg O_2 to prevent fish from dying or 5.8 kg O_2 to maintain water quality. Water turnover of an entire netpen to maintain good water quality is now about every 15 min for adults and 1.6 hours for smolts.

The above considerations are conservative because the salmon's oxygen requirement could easily increase, and perhaps double, as a result of either feeding and/or the stress of being crowded and becoming hypoxic. Furthermore, if fish start dying weighing down the bottom of the netpen causing it to become elliptical rather than circular, the available water is further reduced. The effect of a netpen collapsing to the shape of an ellipse was calculated in the following way. A 100 m circumference might collapse to an elliptical cross-section of about 24 m by 8 m, which yields a surface area of ~600 m^2 compared with a surface area of ~800 m^2 for a non-impacted netpen. For 15 m deep netpen, the total volume is reduced by about 25% to

~9,000 m^3 compared with a circular netpen.[8] Since divers said they could barely get through the collapsed netpen to retrieve fish, the ellipse could have been much more severe. Finally, when dead fish lie on the bottom of the netpen the effective area for water turnover through the net is reduced and decomposition will deplete oxygen in the water.

In view of the above rough calculations, the temperature measurements at the bottom of netpens that reached 19°C, and diver/camera observations of fish not occupying the upper portion of the netpens, being lethargic and being off feed, we propose the following series of cascading events during August 2019.

1. Surface temperatures that exceeded the optimal temperature stimulated a natural behavioural response of Atlantic salmon: to seek a cooler and more optimal water temperature at depth. This crowded the fish into a smaller net volume, increasing their stress level while decreasing the oxygen available to them.

2. As the water at the bottom of the netpen then increased in temperature, the fish's oxygen requirement increased to the point that water turnover through the wall of the netpen could not keep up with the rate at which fish were removing it from the water. The water at the bottom of the netpen then became progressively hypoxic, being worst at slack tide, adding more stress, causing more agitation and further spiraling the depletion of oxygen from the water. A single DO measurement at a variable location on the bottom of the netpen might not always pick up the severity and extent of the hypoxia. Some DO measurements at some sites did approach 70%. Lethargic fish at 18-19°C are likely to be hypoxic rather than too warm and normoxic.

3. Once the water at the bottom of the netpen had reach 18-19°C, the hypoxia-sensitive fish and the most agitated fish began to die. It is also possible that some fish died because they were temperature-sensitive.

4. Dead fish accumulated on the bottom of netpens at a rate that routine diving could not keep up with. These dead fish closed in the sides of the netpen (as observed), stressing the fish further, restricting water exchange to refresh the oxygen inside the netpen and even preventing salmon from swimming away from the bottom of the netpen.

5. A well-established phenomenon when salmon are severely stressed at a temperature >18°C is that they show delayed mortality sometimes several days after a single stress. (Indeed, in recognition of this unfortunate fact, DFO use 18°C as a trigger to stop in-river commercial fishing activity for sockeye salmon on the Fraser River). Thus, once triggered, mortality would ensue for several days even if water quality conditions improved.

6. Based on the above scenario, an expectation for sites where the temperature at the bottom of the netpen was less extreme is that fewer fish would have been hypoxia- and temperature-

[8] The ellipse calculation was corrected based on the corrected netpen dimensions resulting in a 25% volume reduction compared with a circular netpen.

stressed and died. This appears to be the case for the four sites where there was only partial mortality.

Appendix D – List of Stakeholders Consulted

STAKEHOLDER CONSULTATIONS

Elizabeth Barlow	**Chris Hendry**
Sustainability Director MOWI Canada East	Senior Fisheries and Aquaculture Advisor, NL Fisheries and Oceans Canada
Bill Barry	**Darren Ingersoll**
Founder, CEO Barry Group Inc.	Salt Water Production Manager MOWI Canada East
Joe Barry & David Barry	**Mark Lane**
Barry Group Inc.	Executive Director Newfoundland Aquaculture Industry Association
Dr. Amanda Borchardt	**Dr. Nicole O'Brien**
Fish Health and Welfare Director MOWI Canada East	Aquaculture Veterinarian Fisheries and Land Resources, NL
Carla Buchan	**Georgina Ollerhead**
Aquaculture Review Board Fisheries and Aquaculture, NS	Mayor Town of Harbour Breton
Murray Bungay	**Brian Rogers**
Site Manager Northern Harvest Sea Farms NL	Fisheries and Aquaculture Consultant Rogers Consulting Inc.
Susan Farquharson	**Kerra Shaw**
Executive Director Atlantic Fish Farmers Association	Senior Aquaculture Biologist, BC Fisheries and Oceans Canada
Mike Fudge	**Stephanie Synard-McInnis**
Site Manager Northern Harvest Sea Farms NL	Director Aquaculture Development Fisheries and Land Resources, NL
Dan Fudge	**Chris Williams**
Site Manager Northern Harvest Sea Farms NL	Site Manager Northern Harvest Sea Farms NL
Dr. David Groman	**Sherry Warren**
Fish Pathologist University of Prince Edward Island	C-CORE Memorial University of Newfoundland
	Dr. Daryl Whelan
	Chief Aquaculture Veterinarian Fisheries and Land Resources, NL

Appendix E - Additional Observations: Assessment of the Environmental Conditions

Key points from data analysis of mortality, feeding, site characteristics & lice counts

Early warning signs and associations

1. Spikes above the typical weekly percentage mortality were first detected during routine weekly dives at 5 sites (from ~0.3% to 2% in early August for sites 4, 6, 9, and 10 and in the last week of July for site 3). These four sites all contained salmon that averaged 3 kg or more and included two separate bay management areas.

2. During the week 12-18 August, percentage mortality increased further at sites 4, 9 and 10 while the mortality subsided somewhat at sites 3 and 4 (to just below 0.3%). During the same week, the percentage mortality at sites 1, 2 and 7 approached or exceeded ~0.3%. Site 7 had fish averaging >3 kg, while sites 1 and 2 had salmon averaging 0.5 kg. Thus, the spikes in mortality which started in large salmon, now included small salmon and extended to two other bay management areas. However, the percentage mortality remained at a background level at site 5 containing salmon < 1 kg and site 8 containing salmon >3 kg.

3. Feeding rate was reduced or stopped (*) between August 18 and August 20 at sites 1, 3*, 4*, 9 and 10.

4. During the week 19-25 August, percentage mortality increased further at all sites except at sites 5 and 8, where percentage mortality remained at a background level, and at site 4 where it decreased slightly. Feeding rate was decreased or stopped at all sites by August 26.

5. During the week 26 August – 3 September, percentage mortality increased at all sites, averaging ~5% and including sites 5 and 8, which had previously remained at a background level. Feeding had stopped at all sites by 3 September.

6. Mortality was 100% soon after September 3 at sites 1, 2, 3, 4, 6 and 7, sites that spanned three bay management areas.

7. At sites 5, 8, 9, and 10 at least 50% of the salmon survived. Three of these 4 sites were in a single bay management area and contained salmon >3 kg. These salmon were harvested between November 12 and November 22. The other site contained <1 kg salmon from which mortalities were removed and the remaining fish will be grown to harvest.

8. While spikes in mortality were first evident in larger fish and a greater total biomass per netpen, smaller fish held at a lower biomass per netpen eventually succumbed. But fish size and biomass per netpen did not predict which sites had fish that survived. Three sites in one bay management area with larger fish and biomass had fish surviving, whereas in another bay management area one site had all small salmon dying, while other sites had 100% mortality for large salmon.

9. Neither the first appearance of mortality spikes nor the extent of the mortality at a site was related to the minimum depth of water at that site.

10. Mortality at a site was unrelated to the number of gravid sea lice found on salmon between 21 July and 4 August, which was lowest for the small salmon at site 5 and highest for the large salmon at site 7. Site 8 had an average lice load and along with site 5 was the last site to show a spike in mortality, whereas nearby sites 9 and 10 developed a mortality spike earlier than site 8 with a slightly lower than average lice load. Sites with average lice loads could develop an early mortality spike and all die (e.g. site 2) or have some survivors (e.g. site 10).

Key points from data analysis of DO at sites

1. DO measured at the surface, at 5 m and at 15 m (started about 10 August) was always >70% saturation at all sites throughout July and August, with most recording being >90% saturation.
2. For some of the sites (3, 4, 6, 9 and 10) DO tended to be lowest at 15 m. DO tended to increase at 15 m if the water temperature at 15 m spiked below 10°C.
3. Around 20 August, all sites reached their lowest DO, which was generally at 15 m rather than at the surface or at 5 m, which would have made oxygen in the water 20-30% less available to the fish. This date was coincident with all but two of the sites (5 and 8) experiencing an increase in percentage fish mortality. Both of these sites generally had a DO of >80 % during this period, and surface temperatures hovered around 18-19°C.

Key points from data analysis of temperature at sites

Site 1: For the first 3 weeks of July, water temperature at site 1 was typically 10-14°C (averaging ~12°C) at the surface, 9-14°C (averaging ~10°C) at 5 m and 2-8°C (averaging ~5°C) at 15 m. Surface water temperature first exceeded 15°C on 25 July and first remained above 19°C on 13 August reaching 20°C on 22 August. The 5 m water temperature first exceeded 15°C on 28 July and first remained above 19°C on 11 August, and remained above 18°C on 14 August. At 15m, the water temperature reached nearly 18°C on 22 August, but there were periodic cold water intrusions (2-5°C).

Site 2: Surface water temperature was already at 17°C on 1 August and first exceeded 19°C, on 6 August reaching 20°C on 18 August. The 5 m water temperature was already at 15°C on 1 August and first reached 19°C on 19 August, and decreased below 17°C on 26 August. At 15m, the water temperature first exceeded 15°C on 13 August, reaching 17°C on 22 August and with periodic cold water intrusions (3-9°C).

Site 3: Surface water temperature was already at 17°C on 1 August and first exceeded 19°C, on 6 August reaching 20°C on 18 August. The 5 m water temperature was already at 15°C on 1 August and first reached 19°C on 19 August, and decreased below 17°C on 26 August. At 15m, the water temperature first exceeded 15°C on 13 August, reaching 17°C on 22 August and with periodic cold water intrusions (3-9^{0}C).

Site 4: Surface water temperature was already at >15^{o}C on 27 July and peaked at 19^{o}C on 19 August, remaining >17^{o}C. The 5 m water temperature reached 15^{o}C on 30 July and peaked at 19^{o}C on 19 August, remaining >17^{o}C. At 15m, the water temperature first exceeded 15^{o}C on 14 August, reaching 18^{o}C on 23 August and with few periodic cold water intrusions (7^{o}C).

Site 5: Surface water temperature was already at 15^{o}C on 27 July and first reached at 19^{o}C on 11 August, remaining >17^{o}C. The 5 m water temperature reached 15^{o}C on 30 July and peaked at 19^{o}C on 22 August, remaining >17^{o}C. At 15m, the water temperature first exceeded 15^{o}C on 8 August, peaking at 18^{o}C on 22 August and periodic cold water intrusions especially in early August (4-9^{o}C).

Site 6: Surface water temperature first exceeded 15^{o}C on 26 July and first reached at 20^{o}C on 3 August, remaining >17^{o}C and peaking at 20^{o}C on 20 August. The 5 m water temperature reached 15^{o}C on 31 July and peaked at 20^{o}C on 11 August and 19^{o}C on 17 August, remaining >17^{o}C. At 15m, the water temperature first exceeded 15^{o}C on 5 August, peaking at 18^{o}C on 23 August with few periodic cold water intrusions (3-9^{o}C).

Site 7: Surface water temperature remained above 15^{o}C on 27 July and first reached 19^{o}C on 2 August, remaining >18^{o}C and peaking at >19^{o}C on 12 and 20 August. The 5 m water temperature reached 15^{o}C on 31 July and peaked at 18^{o}C on 17 August, remaining >17^{o}C. At 15 m, the water temperature first exceeded 15^{o}C on 13 August, peaking at 18^{o}C on 23 August with a few periodic cold water intrusions (4-13^{o}C).

Site 8: Surface water temperature was already >15^{o}C on 1 August and exceeded 17^{o}C from 9 August, peaking at 19^{o}C on 18 August. The 5 m water temperature first reached 15^{o}C on 5 August and peaked at 18^{o}C on 20 August, remaining >15^{o}C. At 15m, the water temperature first exceeded 15^{o}C on 10 August, but cold water intrusions followed, keeping the temperature generally <13^{o}C, peaking at 15^{o}C or higher for a few days around 23 August with periodic cold water intrusions (3-12^{o}C).

Site 9: Surface water temperature was already >17^{o}C on 1 August and exceeded 19^{o}C on 18 August, remaining >15^{o}C. The 5 m water temperature was already >17^{o}C on 1 August and oscillated between 15-18^{o}C thereafter, except a cold water intrusion on 22 August. At 15 m, the water temperature first exceeded 15^{o}C on 10 August, but cold water intrusions followed, keeping the temperature generally <12^{o}C, but peaking at 15^{o}C on 16, 17, 23, 28 & 29 August.

Site 10: Surface water temperature was already >17^{o}C on 1 August and remained >18^{o}C from 5 to 24 August, peaking at 19^{o}C on 20 August. The 5 m water temperature had reached 15^{o}C on 1 August cold water intrusions kept it around 13^{o}C until 8 August when it remained above 16^{o}C until 23 August, peaking at 19^{o}C on 20 August. At 15 m, the water temperature only reached 15^{o}C on 10, 11, 12, 17 and 26 August, with cold water intrusions keeping the temperature generally <10^{o}C.

Appendix F – NL FLR Atlantic Salmon Surveillance Plan

Newfoundland and Labrador Department of Fisheries and Land Resources Atlantic Salmon Surveillance Plan

Each company must have a Designated Veterinarian to oversee aquatic animal health surveillance. This surveillance plan is a living document and will be reviewed and updated to reflect ongoing changes in the industry.

Disease Surveillance

Surveillance by facility/company Designated Veterinarians

- Every active Atlantic salmon aquaculture site within Newfoundland must have a licensed Designated Veterinarian oversee a surveillance visit a minimum of **once** per month (every 30 days).
- Samples are submitted to the provincial veterinary diagnostic laboratory in St. Alban’s. Please see Annex 1: Sampling Requirements.
- The Aquatic Animal Health Division recognizes that some aquaculture sites in the province are not accessible during certain months of the year due to weather and unsuitable water temperatures.

Surveillance by the Aquatic Animal Health Division of the Department of Fisheries and Land Resources

The Aquatic Animal Health Division will conduct a risk-based, targeted, active surveillance in addition to surveillance performed by the Designated Veterinarians. The Aquatic Animal Health Division may conduct multiple activities at the time of the surveillance visit. For example, a biosecurity audit and surveillance can occur at the same time.

Annex 1

Designated Veterinarian Monthly Surveillance

- Virology - pool of organs from a minimum of five moribund fish per site.
  - Organs to be included: kidney, heart, spleen, gill
  - Cell lines: Chinook Salmon Embryo, Epithelioma Papulosum Cyprini and Atlantic Salmon Kidney
- Pathogen specific testing
  - Kidney samples for molecular testing using polymerase chain reaction for Infectious Salmon Anaemia virus. Submitted in duplicate.
  - Kidney impression sides for test Infectious Salmon Anaemia virus using Immunofluorescent Antibody Testing. Submitted in duplicate
  - Kidney samples for archive at minus 80 degrees Celsius.

Aquatic Animal Health Division Surveillance

Quarterly site visits

- Virology - pool of organs from a minimum of five moribund fish per site.
  - Organs to be included: kidney, heart, spleen, gill
  - Cell lines: Chinook Salmon Embryo, Epithelioma Papulosum Cyprini and Atlantic Salmon Kidney
- Pathogen specific testing
  - Kidney samples for molecular testing using polymerase chain reaction for Infectious Salmon Anaemia virus.
  - Kidney impression sides to test for Infectious Salmon Anaemia virus using Immunofluorescent Antibody Testing.
  - Kidney samples for archive at minus 80 degrees Celsius.
- Bacterial screening
  - Bacteriology culture using blood agar, SKDM and TSA
  - Kidney impression slides to test for Bacterial Kidney Disease using Immunofluorescent Antibody Testing.
- Histology screening: spleen, kidney, heart, gill and liver.

Appendix G – List of Online Resources

FLR, Newfoundland and Labrador
https://www.fishaq.gov.nl.ca/aquaculture/index.html
https://www.assembly.nl.ca/Legislation/sr/statutes/a13.htm
https://assembly.nl.ca/Legislation/sr/regulations/rc961139.htm
https://www.fishaq.gov.nl.ca/publications/pdf/Sustainable_Aquaculture_Strategy_2014.pdf
https://www.fishaq.gov.nl.ca/publications/pdf/Summary_Aquaculture_Governance_Consultation_2019.pdf
https://www.fishaq.gov.nl.ca/aquaculture/pdf/Aquaculture_Risk_Assessment_Matrix.pdf
https://www.fishaq.gov.nl.ca/aquaculture/development.html
https://www.fishaq.gov.nl.ca/department/pdf/Canada_NL_Memorandum_Understanding_Aquaculture_Dev_1988.PDF
https://www.fishaq.gov.nl.ca/aquaculture/public_reporting/pdf/aquatichealth.pdf

DFA, Nova Scotia
https://novascotia.ca/fish/
https://nslegislature.ca/sites/default/files/legc/statutes/fisheries%20and%20coastal%20resources.pdf
https://novascotia.ca/fish/aquaculture/laws-regs/

DFA, British Columbia
https://www2.gov.bc.ca/gov/content/industry/agriculture-seafood/fisheries-and-aquaculture/aquaculture
https://www2.gov.bc.ca/assets/gov/farming-natural-resources-and-industry/agriculture-and-seafood/fisheries-and-aquaculture/aquaculture-reports/finfishaquaculturetechbriefing_june_20.pdf
https://www2.gov.bc.ca/gov/content/industry/agriculture-seafood/fisheries-and-aquaculture/aquaculture/fish-health

NAIA
https://naia.ca/application/files/8315/7616/4108/Northern_Harvest_Statement_for_Website.pdf
https://naia.ca/application/files/2815/7616/3865/Statement_by_the_Managing_Director_MOWI_-_revised.pdf

MAMKA
http://mamka.ca/wp-content/uploads/2020/01/MAMKA-2019_Seafarm_Temperature_Event.pdf

Satellite Images
https://worldview.earthdata.nasa.gov/?v=-65.17737089828985,40.78532088813561,-46.43030058578985,55.18801023600238&t=2019-09-01-T12%3A52%3A20Z&l=MODIS_Aqua_Chlorophyll_A,GHRSST_L4_MUR_Sea_Surface_Temperature,GHRSST_L4_G1SST_Sea_Surface_Temperature(hidden),GHRSST_L4_AVHRR-OI_Sea_Surface_Temperature(hidden),Reference_Labels(hidden),Reference_Features(hidden),Coastlines,VIIRS_SNPP_CorrectedReflectance_TrueColor(hidden),MODIS_Aqua_CorrectedReflectance_TrueColor(hidden),MODIS_Terra_CorrectedReflectance_TrueColor(hidden)

Glossary of Concepts and Terminology

1. Fish Mortality Events: Mortality thresholds for definition of a case

The conditions of license define a "fish mortality event" (ME) on NHSF salmon farms are the same as those used in British Columbia (BC). An ME is triggered by either:

(a) fish mortalities equivalent to 4000 kg or more, or losses reaching 2% of the current facility inventory, within a 24-hour period; or

(b) fish mortalities equivalent to 10,000 kg or more, or losses reaching 5%, within a five-day period.

We used these triggers for our analysis. Additionally, in BC, a ME must be reported within 24 hours of discovery and follow-up reporting must be done every 10 days until resolved.

We calculated weekly mortality rates for August using mortality numbers for each site that were provided by NHSF. Mortality counts were provided by NHSF based on 1-4 dives per week, but we were only given weekly mortality counts. Therefore, the numerator for the calculation was the number that died during the week and the denominator was the estimated live fish at the start of the month, which was an estimated inventory number from NHSF for August 2019. No adjustment was made for the cumulative mortality during August in the denominator, which results in a slight underestimate of the inventory mortality.

Importantly, we also examined for early warning signs of a fish ME (see Figure 1) by monitoring fish mortality rates during August using a finer level of discrimination using four lower thresholds: a background mortality rate (<0.1% of fish per week), an increase above this rate (0.1% to <0.5%), a spike in mortality (0.5% to <1%) and a warning level (>1% to 2%). Background mortality was without considering any variation attributable to life stage, water temperature and salinity, management practices, and different pathogen profiles etc. Also, without finer detail on when the mortality occurred, we cannot discriminate between the weekly mortality count being a single or a cumulative event, a consideration that is paramount to the two criteria used to define a fish ME in the conditions of license.

2. Incident Event as defined in AP-17 (licensing of sites): Any event that caused or under slightly different circumstances would have caused abnormal mortality, harm or an imminent threat to farmed finfish or shellfish, marine installation or structure or vessel on a licensed finfish or shellfish aquaculture site. It also includes any event that impairs the function of any equipment required to sustain aquatic fish health and prevention of escape, and any other event deemed to be reportable by the department

Requirements for reporting are as follows: "All finfish and shellfish operators shall report to the Aquatic Animal Health Division and the Aquaculture Development Division of the Department of Fisheries and Land Resources, any abnormal mortality event within 24 hours of the event occurring. The operator is required to submit to the Chief Aquaculture Veterinarian (CAV) and the Director of Aquaculture Development, Standard Operating Procedures for abnormal mortality removal for departmental review and approval and must demonstrate that other agencies with jurisdictional responsibilities have approved the Standard Operating Procedures. Written updates must occur to the CAV and the Director of Aquaculture Development every 10 days following the initial report. When the incident event and removal operations are complete, the final numbers of finfish or shellfish removed are to be reported in writing to the CAV and the Director of Aquaculture Development, and any other information deemed by the department to be reportable.

For all incident events, all licensed finfish and shellfish operators must provide a written notification to the Assistant Deputy Minister, Department of Fisheries and Land Resources, as soon as reasonably practicable but no later than 24 hours, after the operator becomes aware of any incident. Written notification must contain:

a) date and time of the incident or event;
b) licensed aquaculture operator name and licence number;
c) contact name and telephone number;
d) location of the site(s) impacted;
e) the finfish or shellfish species stocked on site;
f) the estimated number of finfish or shellfish species affected;
g) list of other agencies notified;
h) description of the incident event; description of site operations and relevant environmental conditions at the time of the incident event;
i) immediate response action(s) taken including implemented emergency measures;
j) planned response action to be taken; and
k) any other information deemed by the department to be reportable"

3. Oxygen availability in water

a) Dissolved oxygen (%) levels in water

Water with 100% dissolved oxygen (DO) is water that is fully saturated with air. DO is an index of the partial pressure of oxygen in the water, which is the driving force for the diffusion of oxygen across fish gills and for the binding of oxygen to hemoglobin in red blood cells. A lower DO (<100%; environmental hypoxia) reduces the amount of oxygen contained in water and reduces this driving force. To compensate, fish in hypoxic water pump more water over their gills to provide the oxygen that they need. Even so, lowering DO eventually will mean that arterial blood cannot become fully saturated with oxygen at the gills (hypoxemia). Consequently, fish suffer physiologically in hypoxic water.

b) Oxygen content of water

The amount of oxygen present in water is given by its oxygen content, information that DO% cannot provide unless water temperature and other factors, such as its salinity, are known. This is because, the oxygen content of water decreases as it is warmed and when it becomes more saline. For example, with a 10°C increase the oxygen content of seawater decreases by ~10%. Consequently, an Atlantic salmon at 20°C must double its gill ventilation rate to deliver the same amount of oxygen to its gills compared with one at 10°C, but also the routine oxygen needs will have doubled at 20°C (see 3a below). Therefore, the gill ventilation would need to quadruple to meet the routine oxygen needs of an Atlantic salmon at 20°C when compared with one at 10°C.

4. Oxygen needs of a fish

a) As temperature increases

Warming exponentially increases the routine oxygen needs of fish tissues (their routine metabolic rate, RMR) such that the tissue oxygen needs approximately double for every 10°C increase in temperature. This is a simple thermodynamic response of chemical and biochemical reaction rates. Thus, a fish at 20°C would double its oxygen uptake compared with a fish at 10°C primarily by (i) pumping more water across the gills per minute (increased total ventilation), (ii) pumping more blood around the body per minute (increased cardiac output), and (iii) removing more oxygen out of arterial blood as it passes through the blood capillaries (increased tissue oxygen extraction). However, each of these three variables is limited by how much it can increase.

b) The maximum oxygen uptake

Aerobic exercise triggers the greatest increase in oxygen uptake for athletic fishes like Atlantic salmon. An inactive, non-stressed and non-digesting Atlantic salmon at ~10°C has a routine oxygen uptake of ~ 100 mg O_2/h/kg, which can increase by about 4-fold during maximum prolonged swimming. The maximum oxygen uptake is termed maximum metabolic rate (MMR). The numerical difference between MMR and RMR is termed absolute aerobic scope (AAS = MMR - RMR), while the factorial difference between MMR and RMR is termed the factorial aerobic scope (FAS = MMR/RMR). A stressed fish will use up part of its FAS. A severely agitated fish can reach its MMR and use up all its AAS.

c) The effect of temperature on maximum oxygen uptake

Maximum oxygen uptake can increase only up to a certain level; further warming does not increase MMR. Because maximum oxygen uptake does not increase in proportion with the exponential increase in routine oxygen uptake during warming, an optimal temperature exists at which AAS is maximal. For Atlantic salmon the optimal temperature appears to be around 12-13°C. Also, FAS declines with temperature.

d) The effect of digestion on oxygen uptake

Fish increase their oxygen uptake to digest a meal. The total amount of oxygen used in digesting a meal is termed the specific dynamic action (SDA). A peak in oxygen uptake, about double the

RMR, occurs during digestion several hours after the meal is eaten, but the duration of SDA lasts many hours. Total SDA, peak SDA and the duration of SDA all increase with meal size.

e) The effect of temperature on specific dynamic action (SDA)

Increasing temperature decreases the duration of SDA, but increases the peak SDA. Thus, Atlantic salmon will need a FAS of at least 2 to attain the peak SDA. Yet, FAS decreases with temperature. Thus, salmon in warmer water than their optimum temperature may not have the required FAS to efficiently digest a meal. Consequently, Atlantic salmon 18°C often display poor appetite, food conversion efficiency and growth (e.g., Kullgren et al., 2013).

5. Temperature limits in fish[9, 10]

a) Thermal resistance

Thermal resistance of a fish to warming is commonly assessed by measuring the critical thermal maximum (CT_{max}), the maximum temperature that a fish can withstand for about a minute. To measure CT_{max}, a fish is warmed at a steady rate (0·3°C/min) until it can longer maintain a dorso-ventral orientation. The fish would die unless immediately revived in cooler water. CT_{max} is ~26°C for Atlantic salmon in 100% DO and acclimated to 10-12°C, a temperature similar to that found during early July on salmon farms in Newfoundland. Thermal resistance temperatures above CT_{max} involves death in seconds, not minutes, hours, days or weeks.

b) Thermal resistance and hypoxia

CT_{max} decreases when seawater is hypoxic. CT_{max} of Atlantic salmon decreases by about 2.5°C for a 25% decrease in water DO. Consequently, CT_{max} of Atlantic salmon becomes closer to 21°C when seawater has a 50% DO. The effect of hypoxia on CT_{max} is beyond a simple consideration of the 50% reduction in the water oxygen content because the 5°C difference between 21°C and 26°C only changes the water oxygen content by ~5%.

c) Thermal tolerance

Fish have long-term tolerance of temperatures lower than CT_{max}. Within their zone of thermal tolerance, fish have an optimal temperature range for growth, which may coincide with that for peak AAS; growth is slower above and below this optimal temperature range. For example, Atlantic salmon grow well at 12-13°C, but at temperatures above 15°C and certainly above 18°C, growth and long-term survival of Atlantic salmon are increasingly compromised. Similar to CT_{max}, this optimal temperature range would become compressed under hypoxic conditions.

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[9] Hvas et al. (2017). The effect of thermal acclimation on aerobic scope and critical swimming speed in Atlantic salmon, *Salmo salar*. J. Exp. Biol. 220, 2757-2764.
[10] Kullgren et al. (2013). The impact of temperature on the metabolome and endocrine metabolic signals in Atlantic salmon (*Salmo salar*). Comp. Biochem. Physiol. A 164, 44-53.

6. Delayed mortality in fish

That fish suffer delayed mortality after a stressful event has been recognized since the 1950's. Also, delayed mortality is particularly prevalent in salmonids, as recognized in a recent DFO Canadian Science Advisory Secretariat Report which addressed the longer term consequences to fishery escapements and by-catch. Simply, when a fish is chased, handled or captured by various fishing methods, mortality can follow up to many days later, but especially within the subsequent 24-48 h period even though the fish visually appear to have recovered from the stress. Indeed, it may take farmed salmon up to 15 h to recovery their oxygen uptake post-exhaustion, and even longer to recover their tissue metabolites. Prevalence of delayed mortality increases at supra-optimal temperatures in Pacific salmon, with 18°C being a particularly critical temperature in this regard.

Consequently, for our analysis of the water temperatures, we considered temperatures of 15°C, 18°C and 20°C as pivotal transition temperatures for Atlantic salmon: a green zone below 15°C, an amber zone between 15°C and 18°C, and a red zone between 18°C and 20°C (Figure 2). (While these temperature differences may appear rather small, remember that humans with a fever of 41°C are only 4°C above their normal temperature and should be heading to a hospital.) Atlantic salmon moving into an amber temperature zone are a few degrees Celsius away from catastrophic consequences, with no hospital at hand, and the possibility of hypoxic water exacerbating the temperature challenge. For completeness, our graphical analysis of the water temperatures also includes light blue (5-10°C) and dark blue (<5°C) zones because such temperatures are indicative of cold-water intrusions into the netpens.

7. Statistical analysis

The strength of the association between potential risk factors and whether the farm had total or partial mortality was measured with odds ratios and 95% confidence intervals. Odds ratios (OR) measure the relative odds in the group with the risk factor compared to the group without the risk factor. The formula for calculation of the OR is ad/bc which equals 15, based on cell count data (a=5, b=1, c=1, and d=3) below. Odds ratios of 1 indicate no association whereas the value of 15 indicates a strong positive association. The uncertainty about this value is large given that only 10 farms were affected in the mortality event and this is reflected in a wide 95% confidence interval (0.7 to 339)

Risk factor	Total mortality	Partial mortality
Present	5 (a)	1(b)
Absent	1(c)	3 (d)
	6	4

The P value is 0.08 which would lead to the conclusion that there is no statistical association between the risk factor and mortality because the P value is >0.05 but the power to find differences is small. Had the risk factor been present in one more farm (a=6, c=0) with total mortality, the statistical conclusion would have been stronger.

Risk factor	Total mortality	Partial mortality
Present	6 (a)	1(b)
Absent	0 (c)	3 (d)
	6	4

Where zeros cause problems with computation of odds ratios (here the denominator is zero), 0.5 is typically added to all cells (a, b, c, d) so in this case the odd ratio calculation is 6.5 x 3.5/(0.5 x 1.5) = 22.5/0.75 = 30.3 and the P value is 0.05.

The Mann-Whitney (MW) test is used to evaluate the significance of the difference between two independent samples where the data are continuous (e.g. depth of water beneath cages, number of days with surface water temperatures were above 18oC). It is the alternative for the *independent samples t-test* when the distribution of the samples is not Normal (and not bell-shaped). The MW test combines and ranks the data from sample 1 (e.g. 100% mortality) and sample 2 (e.g. partial mortality) and calculates a statistic on the difference between the sum of the ranks of samples 1 and 2. If the resulting P-value is small (P<0.05) then statistically significant difference between the two samples can be accepted.

Figures

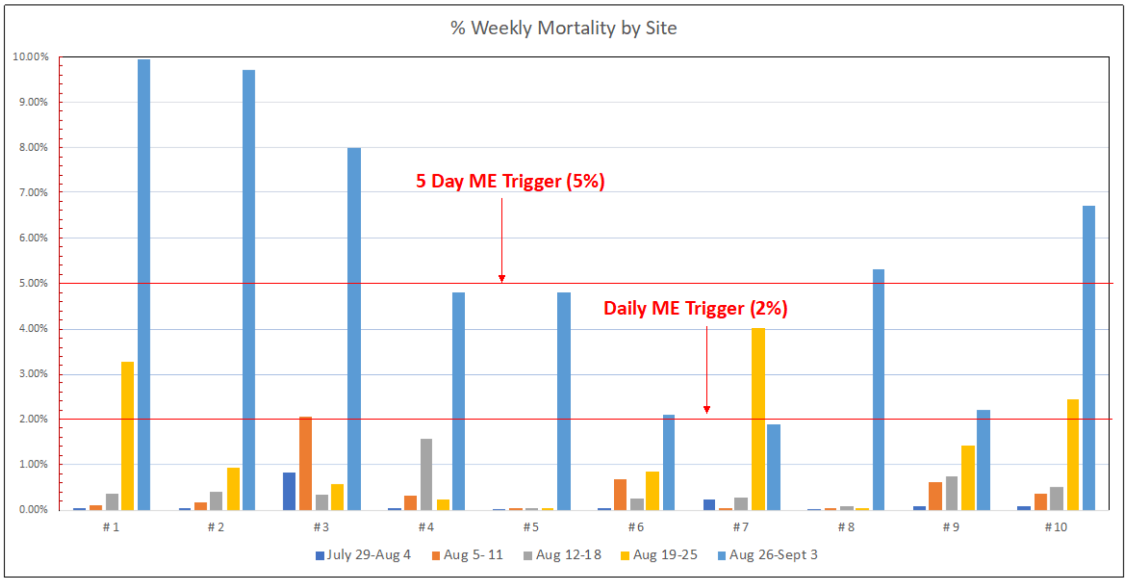

Figure 1. Percent weekly mortality by site July 29 – September 3, 2019.

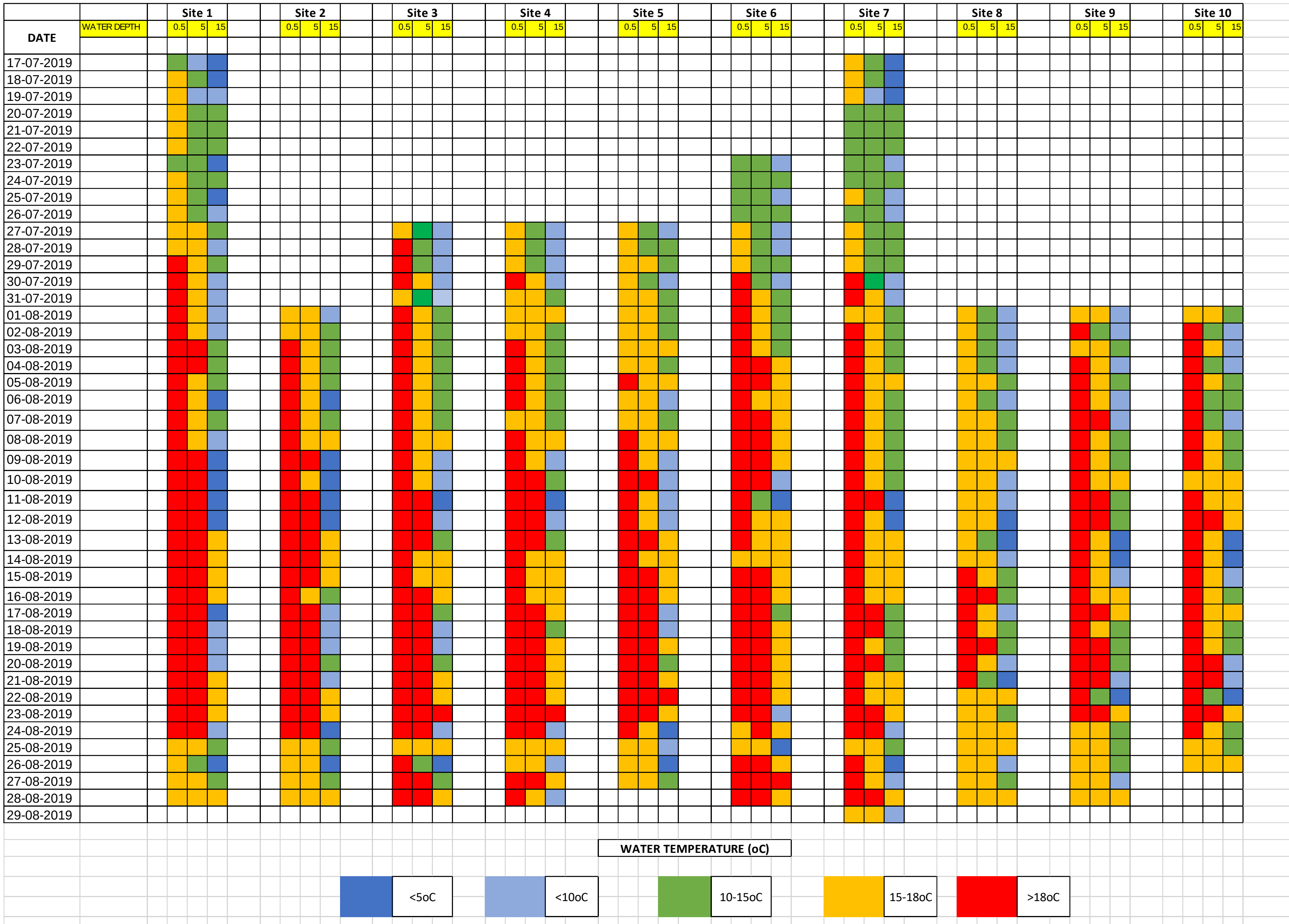

Figure 2. Water temperature during August 2019.

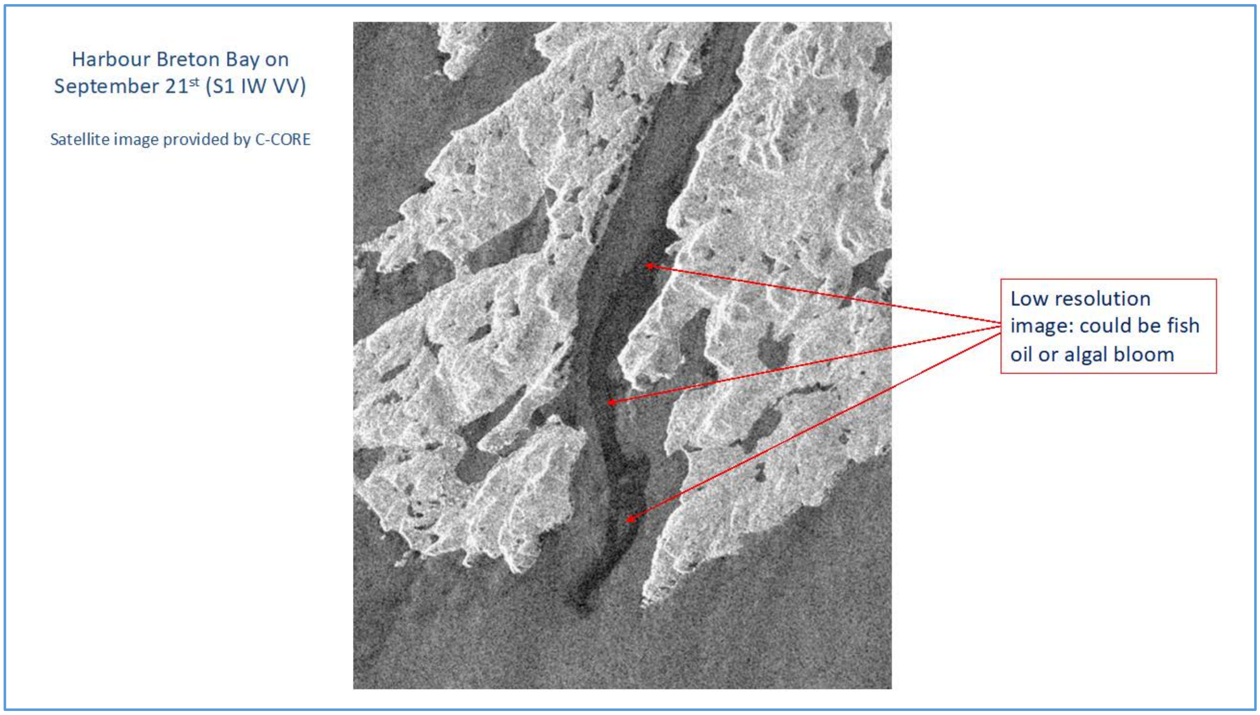

Figure 3. Satellite image of Harbour Breton Bay on September 21, 2019 showing possible fish oil slick or algal bloom

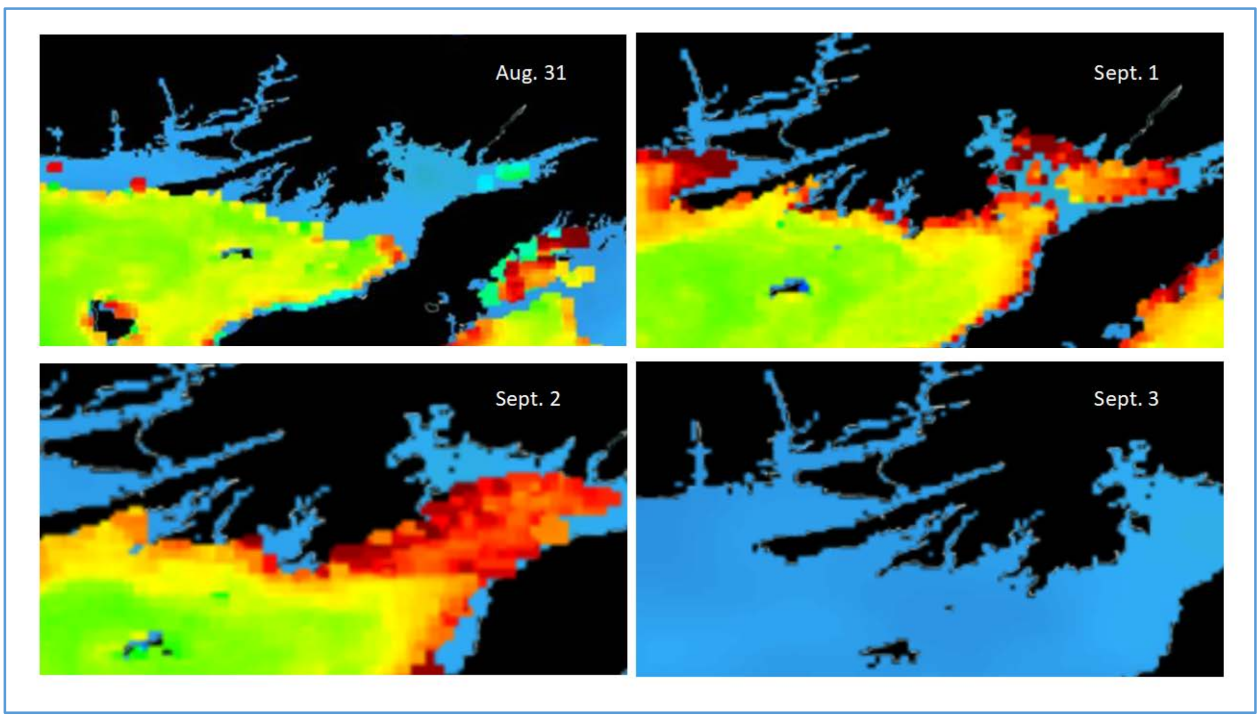

Figure 4. Satellite images of Fortune Bay showing possible algal bloom moving in on August 31, 2019 and dissipating by September 3, 2019.

(Images retrieved from worldview.earthdata.nasa.gov).

Acknowledgements

The Committee acknowledges the contributions of Northern Harvest Sea Farms Newfoundland Ltd - MOWI Canada East for providing site specific data, accommodating our visits to the affected farm sites, and answering our ongoing questions.

The Committee also thanks Bill Barry, CEO and Founder, the Barry Group Inc., as well as Joe Barry and David Barry for providing information regarding seiner activities and rendering activities that occurred during the mort removal process which helped us understand the various complexities of the mort removal and clean-up effort.

We also acknowledge the contributions of Nicole O'Brien, FLR Fish Health Veterinarian including the provision of FLRs fish health and diagnostic reports and her assistance with clarification of AAHD and FLR's role in investigating and assisting with fish mortality events.