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Characterization of *Moritella viscosa* and winter ulcer to inform pathogen transfer risk assessments in British Columbia

Joy Wade[1] and Lily Weber[2]

[1]Fundy Aqua Services Inc.
1859 Delanice Way
Nanoose Bay, BC V9P 9B3

[2]Fisheries and Oceans Canada
Aquaculture, Biotechnology and Aquatic Animal Health Science
200 Kent Street
Ottawa, ON K1A 0E6

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Foreword

This series documents the scientific basis for the evaluation of aquatic resources and ecosystems in Canada. As such, it addresses the issues of the day in the time frames required and the documents it contains are not intended as definitive statements on the subjects addressed but rather as progress reports on ongoing investigations.

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ABSTRACT

Winter ulcer is a disease caused by infection from the gram-negative bacterium, *Moritella viscosa*. It is commonly reported in farmed Atlantic Salmon (*Salmo salar*) in Norway, Iceland and to a lesser extent in Scotland. Disease has been reported in farmed Atlantic Salmon in British Columbia (BC) but no other fish species. It typically occurs during the winter, when water temperatures drop below 7-10ºC. Infected fish that survive the cold water period can recover once temperatures increase or if salinity decreases below 12-15 ppt. Although mortalities due to winter ulcer may be relatively low, it is considered a significant issue for animal welfare as well as loss of revenue due to downgrades caused by ulceration. Waterborne transmission of the bacteria is the most likely route of spread within a population, although only a few studies have been conducted using natural transmission routes. Environmental and husbandry related stressors have been identified as likely contributors to infection and disease. Differences in virulence between strains of *M. viscosa* as well as species specific strains have been identified. There is some indication that strains may also be regionally distinct. Little is known of the strain types or virulence of *M. viscosa* resulting in winter ulcer in farmed Atlantic Salmon in BC. Most of what is known about winter ulcer and the etiological agent is a result of studies conducted on farmed Atlantic Salmon and strains from Norway, Iceland and Scotland.

In BC, Fisheries and Oceans Canada (DFO) began screening both farmed Atlantic and Pacific salmons for *M. viscosa* as a part of the Fish Health Audit and Surveillance Program in 2012. From 2012 to 2018, there were 17 audit-based farm-level winter ulcer diagnoses made in the province. To date, no such diagnoses have been made in farmed Pacific salmon.

With the exception of three years (2013-2015), it has been a condition of licence to report Fish Health Events (FHE) on Atlantic Salmon farms to the Regulator since 2002. We cannot confirm when the Atlantic Salmon industry began testing for *M. viscosa*, nor if all the industry began screening at the same time. The first FHE attributable to *M. viscosa* was reported to DFO in 2011. From 2011 to 2012 and 2016 to 2018, there were 13 FHEs attributed to winter ulcer reported on Atlantic Salmon farms in BC.

INTRODUCTION

Fisheries and Oceans Canada (DFO) has a regulatory role to ensure the protection of the environment while creating the conditions for the development of an economically, socially and environmentally sustainable aquaculture sector. The development of an aquaculture science risk assessment framework was a commitment under the 2008 Sustainable Aquaculture Program (SAP) and builds upon the work initiated with the scientific peer-review validation of the Aquaculture Pathways of Effects (DFO, 2010) through the Canadian Science Advisory Secretariat (CSAS). This framework is a formalized approach to the provision of risk-based advice that is consistent with activities currently undertaken by Aquaculture Science and is a component of the overall Sustainable Aquaculture Program's Risk Management Framework.

It is recognized that there are interactions between aquaculture operations and the environment (Grant and Jones, 2010; Foreman et al., 2015). A series of environmental risk assessments is being conducted to address the following environmental stressors resulting from aquaculture activities: physical alteration of habitat structure; alteration in light; noise; release of chemicals and litter; release/removal of nutrients, non-cultured organisms, and other organic matter; release/removal of fish and; release of pathogens. Release of pathogens is the first of these stressors to be assessed.

In partial response to the outcome of Cohen (2012), DFO Aquaculture Management Division requested formal science advice on the risks of pathogen transfer from Atlantic Salmon farms to Fraser River Sockeye Salmon (*Oncorhynchus nerka*). Given the complexity of interactions between pathogens, hosts and the environment, DFO is delivering this science advice through a series of pathogen-specific risk assessments.

This paper characterizes *Moritella viscosa*, the causal agent of winter ulcer, and synthesizes the information relevant to conduct a risk assessment relevant to BC.

PURPOSE OF THIS DOCUMENT

The information summarized in this document will assist in the assessment of the risk to Fraser River Sockeye Salmon due to the transfer of *M. viscosa*, the causative agent of winter ulcer from Atlantic Salmon farms located in the Discovery Islands area of BC. The purpose of this document is not to be an exhaustive review of *M. viscosa* but rather focuses on the natural distribution of the pathogen and the characteristics that affect its transmissibility, pathogenicity and virulence to susceptible wild species occurring in the Discovery Islands area.

BACKGROUND

Winter ulcer has been recognized since the 1980s in Norway (Lunder, 1990; Lunder et al., 1995); it was first reported in Canada in New Brunswick (NB) in 1990 (Whitman et al., 1990). In BC, it was first reported by the industry on Atlantic Salmon farms in December 2011 and the Fish Health Audit and Surveillance Program started screening for the causative agent in 2012 (H. Manchester, DFO, 103 - 2435 Mansfield Drive, Courtenay, BC V9N 2M2, pers. comm., 2019).

The disease is considered endemic in farmed salmonids in North Atlantic countries (Benediktsdóttir and Heidarsdóttir, 2007) and as such, the majority of the literature is from this area. As it has only relatively recently been found in BC, there is little documentation or research specific to the disease in this geographic area.

METHODS

A literature search of peer-reviewed articles was undertaken using Google Scholar, Google, the USearch search engine through the University of Saskatchewan's library and, the Vancouver Island University search engine. The search engines have access to a variety of databases, including those commonly used in biology research including Web of Science, Ovid, and Scopus. The following search terms were used singularly: "winter", "ulcer", "winter ulcer" "*Moritella*", "viscosa", "*Moritella viscosa*", "*Vibrio viscos**", and most in combination with "Atlantic Salmon", "Sockeye Salmon", "Pacific salmon", "outbreak", "infection", "disease", "transmission", "biofilm", "mortality", "vaccine", "exposure", "British Columbia", "susceptible species".

A stronger emphasis was placed on literature published after 1980 due to accessibility reasons but primarily due to improved pathogen detection and elucidation methodologies. Relevant references cited in any of these papers were also retrieved for use. Non peer-reviewed literature, or "grey literature", was searched using Google with the same terms as listed above.

Commonly used fish disease reference books and manuals including: *Diseases of Seawater Netpen-Reared Salmonid Fishes* (Kent and Poppe, 1998)*; Diseases and Disorders of Finfish in Cage Culture* (Woo et al., 2002); *Fish Diseases and Disorders Vol. 3* (Woo and Bruno, 2011); *Bacterial Fish Pathogens Disease of Farmed and Wild Fish* (Austin and Austin, 2012) were also searched for relevant information.

Using many of the same search terms, specific searches of the following organization websites were conducted: Government of Canada, Food and Agriculture Organization of the United Nations (FAO).

Laboratory data and interpretation of results were requested from DFO, Aquaculture Management Division (AMD). Farm specific data including stocking times and fish biomass were also requested from AMD. As necessary, phone calls were conducted to supplement information provided through government records or industry reports.

CHARACTERIZATION

AGENT

Moritella viscosa (formally *Vibrio viscosus*) is the main aetiological agent of winter ulcer (Løvoll et al., 2009; Tunsjø et al., 2009; Björnsson et al., 2011; Karlsen et al., 2017a; Karlsen et al., 2017b). It is a gram-negative, psychrophilic, facultative anaerobic bacterium capable of both fermentative and respiratory metabolisms (Gudmundsdóttir and Björnsdóttir, 2007; Tunsjø et al., 2009; Björnsson et al., 2011). It is oxidase and catalase positive, requiring salt for growth; colonies are round, yellowish-translucent and viscous (Gudmundsdóttir and Björnsdóttir, 2007). There are seven species of *Moritella*, all are present in the marine environment (Urakawa, 2014). Although *Moritella* species have been isolated from seawater, sediments and wood block samples (Urakawa et al., 1998; Urakawa et al., 1999; Kim et al., 2008), to date, no reference could be found reporting the isolation of *Moritella viscosa* from environmental sources other than from fish. However, studies conducted under experimental conditions demonstrated that *Moritella viscosa* can survive and proliferate in an oligotrophic and cold environment suggesting that the bacterium would be capable of surviving in seawater (Benediktsdóttir and Heidarsdóttir, 2007; Tunsjø et al., 2007).

GEOGRAPHIC RANGE AND HOSTS

Salmonids

The main marine host, among economically important species is Atlantic Salmon (Toranzo et al., 2005). The disease has been reported in Atlantic Salmon in Norway (Salte et al., 1994; Lunder et al., 1995), Iceland (Benediktsdóttir et al., 1998), Scotland (Bruno et al., 1998); the Faroe Islands (I. Dalsgaard, pers. comm. in Grove et al. (2010)) and Ireland (ICES, 2005). Gudmundsdóttir and Björnsdóttir (2007) reported the disease in Atlantic Salmon in Denmark but this could not be confirmed with the references provided. It has been reported in farmed Atlantic Salmon in Atlantic (Whitman et al., 1990) and Pacific Canada (DFO, 2019a, c).

It has been reported in sea farmed and wild Rainbow Trout (*Oncorhynchus mykiss*) in Norway (Lunder, 1990 in Rørvik et al. (2000); Larsen and Pedersen, 1999 in Gudmundsdóttir et al. (2006); Grove et al. (2010)); and isolates from Icelandic Rainbow Trout have been used in Benediktsdóttir et al. (2000).

No reference could be found describing the bacterial isolation of *M. viscosa* or winter ulcer in Pacific salmon species. Winter ulcer has not been diagnosed in farmed Pacific salmon in BC (DFO, 2019b, a, c). *M. viscosa* has been detected in 2 of 2,006 juvenile Sockeye Salmon sampled along their out-migration route in the spring and summer of 2012 and 2013 using high-throughput microfluidics quantitative polymerase chain reaction (PCR) (Nekouei et al., 2018). Both detections were from the Discovery Islands area in 2013, no disease was reported (O. Nekouei, DFO, 200 Kent, Ottawa, ON K1A 0E6, pers. comm., 2019). Tissue samples included gills therefore external contamination cannot be excluded.

Non-Salmonids

Because of the importance of alternate species to Atlantic Salmon aquaculture in Norway and Iceland, experiments have been conducted to determine the susceptibility of Atlantic Halibut (*Hippoglossus hippoglossus*), Turbot (*Scophthalmus maximus*) and Atlantic Cod (*Gadus morhua*) to *M. viscosa*. All species have been found to be sensitive to *M. viscosa* infection, depending on the type of challenge.

Colquhoun et al. (2004) describes the first isolation of *M. viscosa* from farmed Atlantic Cod broodstock with skin lesions in Norway in 2002. Fish had been held in captivity from January to April when the isolation took place. Fish were post-spawn and in poor condition. Water temperature at the time of the investigation was 7°C but had recently been lower. Four of the 300 fish (average weight 5-6 kg) had skin lesions similar to that described as winter ulcer in Atlantic Salmon. *M. viscosa* was confirmed using phenotypical testing and PCR. No significant mortality or loss occurred (Colquhoun et al., 2004).

Challenge experiments were conducted on Icelandic Atlantic Cod (average weight 53 g) and Atlantic Halibut (average weight 44 g) using the Norwegian Atlantic Salmon *M. viscosa* isolate F288/95. Injection challenges induced systemic disease in Atlantic Cod and Atlantic Halibut; only Atlantic Cod were infected in bath challenges (Gudmundsdóttir et al., 2006).

Turbot (average weight 50 g) bath challenged with the same isolate as used by Gudmundsdóttir et al. (2006) resulted in 100% mortality at 10^7 cfu mL^{-1} and no mortality at 10^6 cfu mL^{-1} (Björnsdóttir et al., 2004). *M. viscosa* has also been isolated from farmed Norwegian Atlantic Cod broodstock displaying skin lesions (Colquhoun et al., 2004).

To date, *M. viscosa* has only been identified in one free ranging non-salmonid species, Lumpfish (*Cyclopterus lumpus*), which appeared clinically healthy (Benediktsdóttir et al., 2000).

It has also been reported to have been isolated from diseased Lumpfish, but no further information is provided (unpublished data in Einarsdottir et al. (2018)).

Moritella viscosa has been identified in European Plaice (*Pleuronectes platessa*) which were wild caught but held in captivity for five months before developing winter ulcer (Sorum et al., 2000).

GENETIC STRAINS

Heterogeneity between different strains of *M. viscosa* isolated from various locations in Norway and Iceland has been demonstrated through genotypical and serological studies (Benediktsdóttir et al., 2000; Heidarsdóttir et al., 2008).

Two phenotypic and genotypic clades, typical and variant, have been identified from an analysis of 40 different strains isolated from Atlantic Salmon (n=23), Rainbow Trout (n=11), Atlantic Cod (n=5) and Lumpfish (n=1) from different geographic locations (Grove et al., 2010). The typical (type) form, consistent with the type strain, NCIMB 13548, is isolated from Atlantic Salmon farmed in Norway, Scotland and the Faroe Islands. One isolate from farmed Norwegian Atlantic Cod clustered closely with the typical group (Grove et al., 2010). The variant form is isolated from Norwegian farmed Rainbow Trout, Icelandic farmed Rainbow Trout and Atlantic Salmon, Canadian farmed Atlantic Salmon, Icelandic Lumpfish and, some farmed Norwegian Atlantic Salmon (Grove et al., 2010). Two Canadian Atlantic Salmon isolates (Vvi-7 Sasa Canada and Vvi-11 Sasa Canada) were tested. It was not possible to determine their geographic origin.

In Norway, the typical strain does not appear to cause disease in Rainbow Trout while the variant strain rarely causes disease in Atlantic Salmon (Grove et al., 2010). The typical form has not been isolated from diseased Atlantic Salmon in Iceland or Canada, but there were few isolates tested (n=4 and n=2, respectively) (Grove et al., 2010). The variant strains can, however, cause disease in both Atlantic Salmon and Rainbow Trout in Canadian and Icelandic waters (Grove et al., 2010). The authors provide a hypothesized explanation of this result but as the sample sizes are so low they will not be discussed further here. This study could not determine if the typical and variant strains are ecologically limited by geography and host specificity but did provide further evidence of antigenic differences supported by Heidarsdóttir et al. (2008) and cross protection between Icelandic and Norwegian strains reported in Greger and Goodrich (1999).

Differences in virulence between typical and variant strains from Norwegian Atlantic Salmon were tested in Rainbow Trout (Karlsen et al., 2014a). In infection trials, Karlsen et al. (2014a) demonstrated the differences in virulence between typical and variant strains in unvaccinated Atlantic Salmon (average mass 110 g, n=200) and Rainbow Trout (average mass 46 g, n=200) in seawater in Norway. In bath challenges, fish were exposed for one hour to concentrations of either 1.2×10^6 cfu mL^{-1} of Atlantic Salmon isolate NVI 3632 (typical) or 5×10^5 cfu mL^{-1} of Rainbow Trout isolate NVI 5450 (variant). Mortality was recorded for 18 days post challenge.

Cumulative mortality reach 78% in Atlantic Salmon challenged with Atlantic Salmon isolate and 12% for Atlantic Salmon challenged with Rainbow Trout isolate (Karlsen et al., 2014a). Cumulative mortality reach 9% in Rainbow Trout challenged with Atlantic Salmon isolate and 12% in Rainbow Trout challenged with Rainbow Trout isolate (Karlsen et al., 2014a).

Incidence of ulceration between groups was significant. Eighty-eight percent of Atlantic Salmon and 73% of Rainbow Trout exposed to typical *M. viscosa* had ulceration; 71% of Atlantic Salmon and 58% of Rainbow Trout exposed to variant *M. viscosa* had ulceration (Karlsen et al., 2014a). No mortality or ulceration was reported in control fish.

Heidarsdóttir et al. (2008) suggest that based on their antigenic study new serotypes may emerge in new areas and that unnoticed serotypes may become dominant after vaccination against any another serotype.

Strain relationships have been further supported by a recent study by Karlsen et al. (2014b) which compared the genome sequences of 12 North Atlantic strains using pan genome and clustered regularly interspaced short palindromic repeats (CRISPR) analyses. Results indicated that in the strains tested, *M. viscosa* carries two distinct variants of the CRISPR-Cas subtype I-F systems and that CRISPR features are aligned with the four phylogenetic lineages identified in the genomic analysis (Karlsen et al., 2014b).

To date, no studies have been conducted which describe the virulence of BC Atlantic Salmon *M. viscosa* strains in BC Atlantic Salmon. A study has been funded in 2016 by DFO's Aquaculture Collaborative Research and Development Program (ACRDP) which compared isolates from both the Atlantic and Pacific coasts in challenge models; however, the results have not yet been published. Studies have been conducted on Norwegian Atlantic Salmon with Canadian isolates (Vvi-7 and Vvi-11) (Grove et al., 2010; Björnsdóttir et al., 2011); however, the origin is unknown. Björnsdóttir et al. (2011) determined that these two strains were nonvirulent under the conditions of their study.

INFECTION AND DISEASE

Winter ulcer is typically a disease of Atlantic Salmon reared in cold temperatures, usually during the winter (Sorum et al., 2000; Toranzo et al., 2005). It has been reported to occur when seawater temperatures drop below 7-10ºC (Gudmundsdóttir and Björnsdóttir, 2007; Grove et al., 2008; Heidarsdóttir et al., 2008; Tunsjø et al., 2009; Björnsdóttir et al., 2011; Olsen et al., 2011; Björnsdóttir et al., 2012).

The disease manifests as superficial skin lesions that can develop into skin ulcers on the scaled parts of the body surface (Benediktsdóttir et al., 1998; Tunsjø et al., 2009; Tunsjø et al., 2011). Fin rot, gill pallor and severe internal pathology may also be present (Björnsdóttir et al., 2004; Grove et al., 2008; Tunsjø et al., 2009). Diffuse or petechial (i.e., small spots) haemorrhages of internal tissue may occur (Jansson and Vennerström, 2014). Although mortalities may be low, the open ulcers facilitate the entry for other pathogens (Jansson and Vennerström, 2014).

Winter ulcer is a significant concern to salmonid aquaculture in Norway. It is the main bacterial infection and has not been eliminated by vaccination, antibiotics or management (Løvoll et al., 2009). The economic and ethical consequences of winter ulcer disease are serious in Norway and Iceland in particular (Jansson and Vennerström, 2014). In addition to the mortalities during grow-out, downgrades will occur at harvest resulting in significant economic losses (Grove et al., 2008; Jansson and Vennerström, 2014).

Compared to other systemic bacterial infections, winter ulcer results in relatively low mortality, less than 10% during an outbreak (Lunder et al., 1995; Olsen et al., 2011), although one study reported that mortalities may reach 40% (Hoffman et al., 2012). Even if infection does not result in mortality, winter ulcer can cause significant external damage making the fish unmarketable (Toranzo et al., 2005; Olsen et al., 2011). Fish that survive recover in the spring when temperatures increase (above 8ºC Lunder et al. (1995); above 10-12ºC Løvoll et al. (2009)) or when salinity falls below 12-15 ppt (Løvoll et al., 2009).

Disease infects both juvenile and adults (Lillehaug et al., 2003) but in Norway it most frequently affects fish in their first year at sea (Coyne et al., 2006). Bruno et al. (1998) reported winter ulcer in 2-3 kg Atlantic Salmon in Scotland. In a review of antibacterial records from Norwegian Atlantic Salmon fish farms (1991-2000), Lillehaug et al. (2003) report winter ulcer treatments in

137 cases. In 17 cases, treated fish weighed between 45 and 90 g, in 98 cases they weighed between 100 and 800 g. In the remaining 18 cases where fish were weighed, fish weighed between 1 and 4 kg (Lillehaug et al., 2003). On the east coast of Canada, farmed Atlantic Salmon typically get ulcers at sizes less than 1 kg (MacKinnon et al., 2019).

Reduced osmoregulatory abilities at low temperatures plays a role in disease development (Kent and Poppe, 1998).

Several studies have been conducted to try to determine the source(s) of bacterial entry resulting in ulcer formation. Karlsen et al. (2012) conducted lab experiments on both live and dead Norwegian Atlantic Salmon which suggested that skin ulcer formation resulted primarily from direct skin surface and wound colonization by exposure through water and not by the passage of bacteria from internal parts of the body. Similar results were reported in Lunder et al. (1995) in bath and cohabitation studies with Norwegian Atlantic Salmon. Björnsdóttir et al. (2004) and Gudmundsdóttir et al. (2006) showed that ulcers primarily formed at the inoculation site in intraperitoneal and intramuscular challenge studies.

Karlsen et al. (2012) also demonstrated that ulcer development can occur on the body posterior to the pectoral fins in fish exposed to *M. viscosa* in the head and gill region. It was suggested that under laboratory conditions the exposure may overwhelm some tissues such as the gills which under normal infection conditions could inhibit a systemic infection. This would explain what is seen under farm conditions where the bacterium would overwhelm the epidermal barrier, colonizing external surfaces or wounds, degrading tissues and localized ulcers develop (Karlsen et al., 2012). A systemic infection then may occur and would likely result in death (Karlsen et al., 2012). Those that did not develop a systemic infection were likely to recover when water temperatures increased (Karlsen et al., 2012) although ulcers could result in downgrades at harvest.

TRANSMISSION AND PERSISTENCE

Survival outside the host

Laboratory studies of Norwegian strains have demonstrated that *M. viscosa* can survive and proliferate in an oligotrophic environment similar to marine water (Tunsjø et al., 2007). In the lab, cell growth reaches and maintains higher densities for a longer period at 4ºC than 15ºC (Tunsjø et al., 2007). This poor stability at 15ºC has been suggested as a reason why infections are not seen at this temperature (Tunsjø et al., 2007). Cell yield was highest when cultured in salinities similar to seawater (3-4%); mortality was prolonged and greater when low temperature and low salinity (1-1.5%) occurred at the same time (Tunsjø et al., 2007).

Benediktsdóttir and Heidarsdóttir (2007) reached similar conclusions to Tunsjø et al. (2007). Growth and cell lysis of *M. viscosa* was studied at different temperatures (4,10 and 15ºC) and on different media (Benediktsdóttir and Heidarsdóttir, 2007). Growth rate was highest at 15ºC and lowest at 4ºC; cell density was highest, and cells were more stable at 4ºC than 15ºC (Benediktsdóttir and Heidarsdóttir, 2007). The authors suggest that the instability of cells at temperatures above 10ºC may be one of the factors responsible for their inability to infect fish at higher temperatures. Growth curves at 10ºC are presented in the paper. This study confirmed that cations prevented lysis because of interactions with cell envelope components and the ability to balance osmotic pressure of the cells (Benediktsdóttir and Heidarsdóttir, 2007). Although the addition of minerals to the marine broth media (Difco, 2216) did not influence growth rate and the prevention of lysis, the effect on long-term protection is unknown (Benediktsdóttir and Heidarsdóttir, 2007). The authors state that the minerals were added as it

was noted in other experiments that *M. viscosa* cells remain culturable on that media for up to a year. No further details or references to these studies were provided.

Biofilm

No papers were found which specifically described *M. viscosa* forming a biofilm; however, the adhesion mechanisms of *M. viscosa* (Norwegian strains) have been shown in the lab to be temperature regulated which may contribute to the temperature dependence of outbreaks (Tunsjø et al., 2009).

Transmission

True horizontal transmission has not been demonstrated for *M. viscosa*. In the study by Lunder et al. (1995), 169 fish were collected from farms with and without visible signs of winter ulcer, weights ranged from 70 to 3,000 g. Diseased fish with clinical signs were placed in tanks with apparently healthy fish from farms with no signs of disease. All apparently healthy fish had their adipose fin clipped. A cohabitation experiment was conducted in seawater at 10ºC. One hundred and two of the 169 fish had ulcers consistent with winter ulcer; however, ulcers were only found on the scale covered parts of the body most commonly in the area between the adipose fin, dorsal fin and urogenital pore (Lunder et al., 1995). Mechanical lesions were a predisposing factor to ulcer formation. Unfortunately there is the question as to whether or not the exposed fish were naïve as they came from a farm and *M. viscosa* is in the marine environment.

MacKinnon et al. (2020) conducted a series of transmission experiments in hatchery-reared Atlantic Salmon (133.8 g) using an Atlantic Salmon *M. viscosa* isolate derived from an outbreak in farmed Atlantic Salmon in New Brunswick when water temperature was 10ºC. A bath challenge was performed with 75 fish in three tanks, 25 fish per tank exposed for one hour at a concentration of 5.6 X 10^6 cfu mL^{-1} at 10.9°C. All fish developed ulcers after seven days. A delayed-challenge experiment was then conducted by diverting the water from the bath-challenge tank to the “delayed-challenge” tank with 25 naïve fish in each tank. No clinical signs of winter ulcer were observed and qPCR tests on skin and kidney samples were negative which suggests horizontal transmission was not demonstrated in this experiment (MacKinnon et al., 2020). One of the limitations of the study identified as such by the authors, was the inability to induce severe disease (systemic infection) or mortality in bath challenges. They provide likely reasons for this including the inability to transmit the pathogen via water. *M. viscosa* could not be detected in water sampled during the study. The study does report that the progression of disease and transmission after bath challenge is consistent with studies and field reports in Europe at lower temperatures.

Risk factors

Several pre-disposing factors or contributors to outbreaks have been suggested. Salte et al. (1994) proposed a role for dietary iron in the thrombotic process making the fish susceptible to opportunistic bacteria. Mechanically induced skin lesions have been identified as a predisposing factor for winter ulcer development (Wahli et al., 2003) and have been identified as a requirement in some studies for ulcer formation (Lunder et al., 1995). Mechanical handling or movement of fish has been associated with incidences of winter ulcer in farmed Atlantic Salmon in BC (T. Hewison and P. Whittaker, Grieg Seafood, 1180 Ironwood St, Campbell River, BC V9W 5P7, pers. comm., 2019). Water temperature, nutrition, general condition and husbandry (density, handling, opportunity for physical damage) appear to be related to the incidence of winter ulcer in farmed Atlantic Salmon in BC (B. Milligan, Cermaq Canada, 203-919 Island Highway, Campbell River, BC, Canada V9W 2C2, pers. comm., 2019).

Coyne et al. (2006) conducted a study on the effects of a florfenicol treatment on vaccinated Atlantic Salmon (average weight 300 g) six weeks post sea water transfer. A strong correlation was found between fish weights and health suggesting that within a cage population, infection, ulceration and death are restricted to the proportion of the population which had adapted poorly to the cage environment (Coyne et al., 2006). Results indicated that because the smaller fish were of poorer health, they consumed less or no feed, and were therefore not receiving the treatment. The results of the analysis are, however, complicated by a sea lice treatment which was undertaken on day six.

CO-INFECTION

Although *M. viscosa* is considered the causative agent of winter ulcer, in Norway contributing factors to winter ulcer outbreaks may be the presence of other bacteria in the environment (Jansson and Vennerström, 2014; Karlsen et al., 2014b). Despite several lab studies, *M. viscosa* remains the only bacteria shown to cause winter ulcer (Karlsen et al., 2012).

In Norway, co-infection with other bacteria is common, particularly *Tenacibaculum* spp. and *Allivibrio wodanis* (Smage in Powell and Podlasly (2015)). Various *Vibrio* spp. including *A. wodanis* and *T. maritimum* have been isolated from ulcers in cultured Atlantic Salmon from the north eastern Atlantic Ocean (Benediktsdóttir et al., 1998; Benediktsdóttir et al., 2000). In reviewing the DFO audit data, of the 17 winter ulcer farm-level diagnoses, two also had farm-level diagnoses of mouthrot. It has also been reported in farmed Atlantic Salmon on the east coast of Canada that after bacterial cultivation from winter ulcer *A. wodanis* dominated in the ulcer above the occurrence of *M. viscosa* (Whitman et al., 1990).

Toranzo et al. (2005) have hypothesized that *A. wodanis* may suppress the healing process of skin ulcers arising from the primary infection with *M. viscosa*; however, Hjerde et al. (2015) demonstrated that *A. wodanis* may inhibit growth of *M. viscosa*.

Karlsen et al. (2014b) undertook studies to determine both the lethal dose of a particular strain of *M. viscosa* as well as a bath challenge to determine the difference in survival between fish exposed to combinations of *A. wodanis* and *M. viscosa* both alone and in succession.

Lethal dose of 30-50% mortality was determined for 50 g Norwegian Atlantic Salmon smolt (Karlsen et al., 2014b). Three different doses ($3x10^5$, $1x10^6$ and $5x10^6$ cfu mL^{-1}) of *M. viscosa* (NVI 06/09/139) were tested. Fish were exposed for one hour in seawater and observed for three weeks post exposure; mortalities began at 8, 4 and 3 days post challenge, respectively; at three weeks post challenge cumulative mortality was 42%, 47% and 59%, respectively (Karlsen et al., 2014b).

For the main bath challenge, fish were exposed to one of five different challenge combinations of mono or co-cultured *M. viscosa* and/or *A. wodanis* namely: mono culture *M. viscosa* (no concentration indicated, 1-hour exposure; co-culture *M. viscosa* ($1x10^6$ cfu mL^{-1}, 1-hour exposure); co-culture *M. viscosa* ($1x10^6$ cfu mL^{-1}) plus *A. wodanis* ($1x10^6$ cfu mL^{-1}) for one hour, in duplicate; co-culture *A. wodanis* ($1x10^6$ cfu mL^{-1}) for 3-hour exposure then a 1-hour exposure to co-culture *M. viscosa* ($1x10^6$ cfu mL^{-1}); co-culture *A. wodanis* ($1x10^6$ cfu mL^{-1}) for a 3-hour exposure (Karlsen et al., 2014b).

Mortality post exposure was reported, and various internal organs were cultured for re-isolation of bacteria (Karlsen et al., 2014b). This study demonstrated that *A. wodanis* systemically infects Atlantic Salmon rapidly and mutually with *M. viscosa*. The authors hypothesize that *A. wodanis* colonization of scarified skin may influence the progression of *M. viscosa* infection; *A. wodanis* may modulate *M. viscosa* as pathogenesis is prolonged in the presence of *A. wodanis*. Although

both bacteria grow at low temperatures, the modulating ability of *A. wodanis* may be reduced at lower temperatures when *M. viscosa* cell density is greatest (Karlsen et al., 2014b).

Based on the results of Karlsen et al. (2014b) and Hjerde et al. (2015), it is possible that *A. wodanis* may have a specific role as a pathogen that is inhibiting the pathogenic activity of itself and *M. viscosa* through a bacteriocin. It appears that bacteriocin is only produced when *A. wodanis* cells are in close contact with *M. viscosa* cells in tissues or media (0.9% NaCL), not at higher salt concentrations (H. Sørum, Norwegian University of Life Sciences, Arboretveien 16, 1430 Ås, Norway, pers. comm., 2019). It is likely that *A. wodanis* reduces the virulence of *M. viscosa* extensively resulting in a chronic condition when these bacteria infect the fish together as opposed to the higher pathogenicity observed when *M. viscosa* infects Atlantic Salmon alone (H. Sørum, pers. comm., 2019). Throughout the history of winter ulcer occurrence in farmed Atlantic Salmon in Norway *A. wodanis* has been isolated from diseased fish. It has been documented that a bacteriocin is produced in Atlantic Salmon when *A. wodanis* is cultured together with *M. viscosa.*

REGIONAL DIFFERENCES

Winter ulcer has been recognized since the 1980s in Norway (Lunder, 1990; Lunder et al., 1995); it was first reported in Canada in New Brunswick (NB) in 1990 (Whitman et al., 1990) and; was not identified in BC until 2011 (see section below Occurrence in Farmed Salmon in BC).

Infection with *M. viscosa* resulting in winter ulcer in BC appears similar to that described in farmed Atlantic Salmon in Norway, Scotland and Iceland. The basic characteristics of disease and infection are similar between these two regions namely: onset at temperatures below 8-10^{o}C, recovery or no incidence of infection at temperatures greater than 10^{o}C, shallow wounds, isolation of *M. viscosa* (Lunder et al., 1995; Benediktsdóttir et al., 1998; Bruno et al., 1998; Sorum et al., 2000).

Infection and disease described on the east coast of Canada is more complex. Whitman et al. (1990) describe onset when water temperature was 8^{o}C, but mortality mostly attributed to “winter ulcer” continued until the end of September. As no temperatures were reported we referred to Brewer-Dalton et al. (2015) to obtain mean monthly temperatures for comparison. Brewer-Dalton et al. (2015) report mean monthly temperatures in September in the 4XS region of NB in the upper 12 m to be approximately 13^{o}C (range 9-18^{o}C).

Total mortalities reported by Whitman et al. (1990) during the event exceeded 31% even with antibiotic treatment. Cumulative mortality in Norwegian outbreaks is most commonly reported as approximately 10% (Lunder et al., 1995; Olsen et al., 2011) but has been reported as high as 40% (Hoffman et al., 2012). In addition, *Vibrio* spp. was isolated from this study in NB; biochemical and SDS-Page properties corresponded to those of *V. wodanis* (now *Allivibrio wodanis*) (Whitman et al., 1990).

There have been two studies published recently (MacKinnon et al., 2019; MacKinnon et al., 2020) which have helped address some of the knowledge gaps regarding skin ulcers in Atlantic Salmon in Atlantic Canada.

MacKinnon et al. (2019) conducted a review of the risk factors for the development of skin ulcers in farmed Atlantic Salmon in Atlantic Canada. They showed that the incidence steadily increases in the summer and fall at temperatures above 10^{o}C (MacKinnon et al., 2019). This study examined incidences of skin ulcer diagnoses which were based on gross clinical signs ranging in severity from raised scales to skin ulceration on the lateral side (MacKinnon et al., 2019). The pathogen was not identified in this study. One of the goals was to determine factors

associated with disease (MacKinnon et al., 2019). The descriptive analysis of findings from 29 farms (2014-2016) with a total of 312 cages were:

- The number of cages affected per farm ranged from 5 to 20. Only two farms had 100% of cages diagnosed with skin ulcers.
- Earliest onset of disease occurred four weeks post saltwater entry, the majority started ten weeks post saltwater entry.
- In 2014, outbreaks began in late July and ended mid-October; in 2015, outbreaks began in late July and subsided mid-January 2016.
- MacKinnon et al. (2019) found the pattern of disease is suggestive of point source exposures to the causative agent across farms. In some cases farms close together had outbreaks at similar times, in other cases they did not. In other instances farms spatially distant reported skin ulcers within one week of each other.
- Average mortality during outbreaks lasted eight weeks, some cages had high mortality for only one week, others up to 26 weeks.
- Most cages (n=73) were treated with antibiotics during the outbreaks and had higher average total percent mortality than untreated cages.
- 49 cages of fish were vaccinated against *M. viscosa*, 46 were not. The mean percent mortality was comparable between groups.
- During the outbreaks the minimum water temperature was 10.06^{o}C, maximum 13.36^{o}C.
- Average range of fish weight at sea water entry was 0.055-0.5 kg, weight at the start of the outbreaks ranged from 0.149-0.890 kg.
- All farms that were not treated with ivermectin for sea lice control did not experience skin ulcer outbreaks. Of the 17 farms that were treated with ivermectin, 12 reported ulcers.

Most predictors included in the study were not statistically associated with total percent mortality during outbreaks, possibly due to data limitations of the model (MacKinnon et al., 2019).

A second study, MacKinnon et al. (2020), reported the results of transmission experiments using *M. viscosa* isolated from a clinical Atlantic Salmon ulcer disease case in Atlantic Canada during an outbreak at 10^{o}C. The study was conducted on unvaccinated Atlantic Salmon smolt (133.8 g) from a New Brunswick hatchery. The results, in many ways, were similar to those reported in Europe. Importantly, however, the study was conducted at 10.9^{o}C and at temperatures above 10^{o}C skin ulcers could be induced. When the temperature was dropped to 8.5^{o}C, there was no effect on the proportion of fish affected or the severity of skin lesions. Low mortality during the study was related to the elevated water temperature.

These recent studies by MacKinnon et al. (2019) and MacKinnon et al. (2020) are important as they are beginning to highlight the similarities and differences between the ulcer disease identified in the 1990s in Atlantic Canada and winter ulcer in Europe. Importantly, they have shown that ulcers can be induced by a local *M. viscosa* strain in a local strain of Atlantic Salmon under experimental conditions. The conditions under which ulcers are induced or reduced, and the magnitude of mortality are, however, different.

VIRULENCE AND PATHOGENICITY

Little is known of the virulence factors and how the bacteria interacts with host cells during infection (Karlsen et al., 2014b). Pathogenicity studies have identified both cellular and

extracellular virulence factors (Bjornsdottir et al., 2009a; Bjornsdottir et al., 2009b; Tunsjø et al., 2009).

Moritella viscosa cells have rough-type lipooligosaccharides that are important antigens (Björnsson et al., 2011). A protective antigen is found in pathogenic strains (isolates from various geographic locations) in the outer membrane (Björnsson et al., 2011). An outer membrane protein (MvOmp1) of a pathogenic strain *M. viscosa* has been shown to be a major protective antigen of *M. viscosa*; this protein could be used to develop vaccines (Björnsson et al., 2011).

Infection and disease studies

There are a few laboratory studies which can help inform disease progression in Atlantic Salmon. None of these studies have been conducted on Atlantic Salmon from BC or using BC isolates of *M. viscosa*.

Bruno et al. (1998) conducted an experimental infection of Scottish Atlantic Salmon (approximately 150 g) with *M. viscosa* (MT 1887). Two groups of 30 fish were injected intraperitoneally to give an absorbance of 540 nm of 1.0 (Group A) and 0.71 (Group B). Five uninfected fish were marked intradermally and placed in each of Group A and B tanks. The experiment was conducted at 6-8°C. All moribund fish, uninfected fish and those remaining at the end of the study were sampled for histopathology and bacteriology. The study was terminated 28 days post infection. No mortality occurred in uninfected cohabiting fish (Bruno et al., 1998). The cumulative mortality in Group A was 46% and 20% in Group B (Bruno et al., 1998).

Lethal dose of 30-50% mortality was determined for 50 g Norwegian Atlantic Salmon smolt (Karlsen et al., 2014b). Three different doses ($3x10^5$, $1x10^6$ and $5x10^6$ cfu mL^{-1}) were tested and fish were exposed for one hour in seawater and observed for three weeks post exposure; mortalities began at 8, 4 and 3 days post challenge, respectively; at three weeks post challenge cumulative mortality was 42%, 47% and 59%, respectively (Karlsen et al., 2014b). The specific strain utilized in this lethal dose determination was not identified; however, the strains used in the remainder of the experiments were from Norwegian Atlantic Salmon.

Løvoll et al. (2009) describe the pathogen invasion and host response during a bath trial with unvaccinated Norwegian Atlantic Salmon (n=159, weight 80-110 g) and Atlantic Salmon isolate NVI 96/09/1016 ($7x10^5$ cfu mL^{-1} for one hour at 8.9°C). Mortality was first observed two days post challenge (8.9°C, 31-35 ppt). It is difficult to interpret the significance of the overall results for the purposes of the risk assessment as temperature was increased on day four post challenge and tanks were exposed to freshwater at eight days post challenge. This study will not be discussed further.

Outbreaks

There are few descriptions of winter ulcer outbreaks in Atlantic Salmon in the literature, no published descriptions of outbreaks could be found for BC. Most papers mention information regarding outbreaks but do not describe the event specifically. For example, Coyne et al. (2006) state that outbreaks are most common in post smolts in the first year at sea but provide no further description of the event. Benediktsdóttir et al. (1998) and Lillehaug et al. (2003) mention that outbreaks occur most often in Atlantic Salmon but have also been reported in Rainbow Trout.

Bruno et al. (1998) describe the first outbreak of winter ulcer in farmed Scottish Atlantic Salmon. Mortality occurred at “low temperature” in market sized (2-3 kg) Atlantic Salmon. Between

October and January mortality was 0.3, 0.7, 1.0 and 0.8% per month, total fish losses were 2.5% in four months.

MacKinnon et al. (2019) do provide, at length, a descriptive analysis of diagnoses of “ulcer” in Atlantic Salmon farms in NB. However, due to the regional differences described in the previous section they are not directly comparable to winter ulcer as described in farmed Atlantic Salmon from BC or the North East Atlantic Ocean.

DIAGNOSTIC METHODS

ISOLATION

Moritella viscosa often requires a long incubation time on test media, up to ten days (Toranzo et al., 2005). Properties of *M. viscosa* which are important in identification are: positive lysine decarboxylase and negative citrate, mannitol and sucrose reactions (Bruno et al., 1998; Benediktsdóttir et al., 2000; Sorum et al., 2000). It is typically isolated after prolonged incubation at 15^{o}C on tryptic soy agar with 2% NaCl and 10% horse blood or modified Anacker or Ordals medium (Bruno and Woo, 2002). MacKinnon et al. (2020) grew *M. viscosa* cultures at 4 ^{o}C for 14 days on blood agar media (2% NaCl) similar to Benediktsdóttir and Heidarsdóttir (2007) where strains were kept on agar plates made of Marine Broth from Difco and 1·2% (w/v) agar at 4°C. PCR can be used for confirmation (Coyne et al., 2006; Grove et al., 2008; Grove et al., 2010; Björnsdóttir et al., 2012).

CASE DEFINITION (BRITISH COLUMBIA)

The current case definition used by DFO Pacific Region, Aquaculture Management Division (2019) for the diagnosis of winter ulcer is:

“Winter ulcer is diagnosed in a farmed Atlantic Salmon population when the site is undergoing treatment for the disease or, if there is population level mortality attributable to the disease with fish displaying lesions (ulcers) occurring in the characteristic season (winter) and location on the fish (triangle formed by the dorsal, anal and pelvic fins) and any of:

- Positive culture of *M. viscosa* from margin of skin ulcers and/or kidney;
- Positive PCR for *M. viscosa* from characteristic skin ulcers or systemically;
- Intralesional rods visualized on histopathology from characteristic lesions”

INTERPRETING DIAGNOSTIC RECORDS

In BC, three sources of diagnostic data are available for farmed Atlantic Salmon: i) DFO’s Fish Health Audit and Surveillance Program (FHASP), ii) industry reported FHEs, and, iii) industry data. How these data are collected and for what purposes are described in Wade (2017). When making any diagnoses, lab results are interpreted in conjunction with gross pathology and syndromic information.

Winter ulcer is recorded as a condition of note in farmed Atlantic Salmon when there are observable characteristic lesions in a significant portion of the population (either alive or dead), but without population level mortality attributable to the disease. The confirmation required for the causative agent *M. viscosa* is the same as described in the case definition.

Skin ulcers under low temperature conditions with *M. viscosa* isolation have not been found in audits of Pacific salmon farms; however, the case definition would be the same as for Atlantic Salmon should it occur.

Skin ulcers are diagnosed in a farmed Atlantic Salmon population when the site is undergoing treatment for the disease or, if there is population level mortality attributable to the disease without a confirmed etiology.

Skin ulcers are recorded as a condition of note in farmed Atlantic Salmon when there are observable lesions in a significant portion of the population (either alive or dead), but without population level mortality attributable to the disease, and without a confirmed etiology.

Diagnosis is made based on the characteristic ulcers which should be differentiated from other physical wounds and ulcers caused by other bacteria (Kent and Poppe, 1998).

HEALTH MANAGEMENT

CONTROL AND PREVENTION

Prevention in Norway is to avoid management methods that may result in injuries pre-disposing to ulcers, use of vaccination and removal of infected fish (Hjeltnes, 2014; Bornø and Lie, 2015; Karlsen et al., 2017b).

Vaccine use and general efficacy

Beginning in the 1990s there was an oil-adjuvanted vaccine available against *M. viscosa* for Atlantic Salmon (Greger and Goodrich, 1999; Toranzo et al., 2005; Gudmundsdóttir and Björnsdóttir, 2007; Björnsson et al., 2011; Karlsen et al., 2017b). Initially the vaccine was not optimal, but efficacy has improved (Björnsson et al., 2011). Field (Gudmundsdóttir and Björnsdóttir, 2007) and lab vaccine trials (Greger and Goodrich, 1999) have been conducted with various isolates and species. No formal studies have been conducted with Canadian isolates or BC Atlantic Salmon.

Grove et al. (2008) estimates that 90 to 95% of all fish transferred to sea are now vaccinated against *M. viscosa*, and despite this, mortalities may still be high. It is presumed this is in reference to Norwegian aquaculture.

Vaccine efficacy is dependent on the characteristics of the infective strain, including antigenic differences between strains (Gudmundsdóttir and Björnsdóttir, 2007; Heidarsdóttir et al., 2008). In Norwegian winter ulcer vaccine formulations, components may differ depending on the growing region (Einarsdottir et al., 2018).

The isolate and species from which the isolate is derived also play a role in vaccine efficacy. For example, Einarsdottir et al. (2018) describe both bath and injection experiments with vaccinated Atlantic Salmon (50 g presmolt) and unvaccinated Lumpfish (5 g) in saltwater (35 ppt). In the bath trial, Lumpfish were exposed to a Lumpfish isolate (F6/15) or an Atlantic Salmon isolate (F112/17); Atlantic Salmon were exposed to the same isolates. Atlantic Salmon were found susceptible to the Lumpfish isolate, no *M. viscosa* could be isolated from Atlantic Salmon exposed to the salmon isolate. None of the Lumpfish exposed to the salmon isolate showed any signs of ulcers and no *M. viscosa* could be recovered. Lumpfish exposed to the Lumpfish isolate showed ulceration beginning 18 days post exposure; *M. viscosa* was isolated from all Lumpfish with ulcers and 47% of the kidneys from fish without any external signs 27 days post challenge. Lumpfish can therefore be asymptomatic carriers of *M. viscosa*. Lumpfish were resistant to the salmon isolate but the Atlantic Salmon could be infected with Lumpfish isolate (Einarsdottir et al., 2018).

In the injection trial, Atlantic Salmon injected with Atlantic Salmon isolate (F112/17) were protected against the salmon isolate but not Lumpfish isolate (F6/15) (Einarsdottir et al., 2018).

All infected Atlantic Salmon injected with the Lumpfish isolate died within seven days. Over a nine day period post injection, all Atlantic Salmon recovered from the injection of the salmon isolate and *M. viscosa* could not be isolated from these fish (Einarsdottir et al., 2018).

For the last five years (approximately), applications have been approved by Health Canada for special import and use of a multivalent vaccine (Pharmaq AJ5-3) including a Norwegian isolate of *M. viscosa* (B. Milligan, pers. comm., 2019). In the last two years (approximately) a monovalent vaccine has been available for trial from Elanco based on Canadian isolates tested in the ACRDP project described previously (B. Milligan, pers. comm., 2019). Most of the vaccine was/is being tested on the east coast, a trial is being conducted by one company on the west coast (B. Milligan, pers. comm., 2019). In BC, both the multivalent and monovalent vaccines appear to be approximately 50% effective (B. Milligan, pers. comm., 2019). Although the vaccine is a useful tool and is cost effective, winter ulcer remains an issue (B. Milligan, pers. comm., 2019).

Treatment

In Norwegian Atlantic Salmon farming, almost half of all antibiotic prescriptions are for the control of winter ulcer even though they do not effectively control mortalities related to disease (Coyne et al., 2004; Coyne et al., 2006; Løvoll et al., 2009). This may be in part due to the tendency of infected fish to stop feeding (Jansson and Vennerström, 2014) and are therefore not consuming the antibiotic. As disease may not result in a systemic infection it is important that the antibiotic also target the skin.

For BC farmed Atlantic Salmon, antibiotics have been traditionally prescribed for the treatment of gram-negative bacteria causing furunculosis, vibriosis, enteric redmouth (ERM) and stomatitis (Morrison and Saksida, 2013). While vaccination of fish against furunculosis, vibriosis and ERM has drastically reduced the need for antibiotics, the majority of antibiotics are prescribed in the treatment of mouthrot (Morrison and Saksida, 2013). Atlantic Salmon have been treated for winter ulcer (see Occurrence in farmed salmon in British Columbia).

The only antibiotics authorized in Canada in aquaculture are oxytracycline hydrochloride (Terramycin-Aqua), trimethoprim and sulphadiazine powder (Tribressen 40% powder), sulfadimethoxine and ormetoprim (Romet 30), and florfenicol (Aquaflor) (Health Canada, 2018).

With the goal of preventing winter ulcer, studies have tested the mitigation effects of urea added to feed to reduce osmotic stress (Kent and Poppe, 1998; Rørvik et al., 2000; Rørvik et al., 2001) and trimethylamine oxide to aid in fat digestibility (Rørvik et al., 2000). Positive effects associated with these mitigation measures were reported.

OCCURRENCE IN FARMED SALMON IN BRITISH COLUMBIA

In BC, winter ulcer has only been diagnosed in farmed Atlantic Salmon. All government held data are presented in reference to DFO Fish Health Surveillance Zones (Figure 1).

Three farms, Althorpe, Hardwicke and Shaw Point are included in fish health surveillance zone 3.2 on the Open Canada website, however, they are licensed in zone 3.3. They are included in fish health surveillance zone 3.2 in this document.

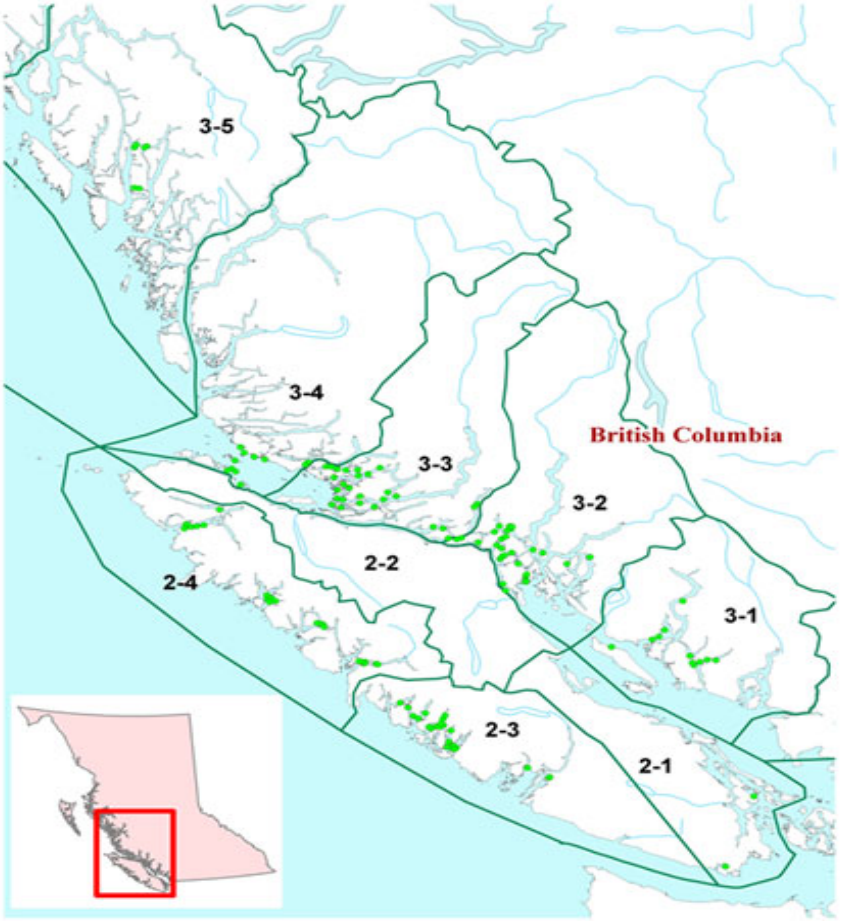

Figure 1. Map of Fisheries and Oceans Canada (DFO) Fish Health Surveillance Zones. Reproduced from Appendix 1-A (iii) Marine Finfish Aquaculture License, no date.

ATLANTIC SALMON

Moritella viscosa has been identified in BC farmed Atlantic Salmon through both diagnoses from the Fish Health Audit and Surveillance Program (FHASP) and by the industry through the reporting of FHEs. Criteria for diagnoses by DFO are provided in the Diagnostic Methods section. A summary of detections in all Fish Health Surveillance Zones are reported below.

Fish Health Audit and Surveillance Program

The FHASP is conducted by DFO's BC Aquaculture Regulatory Program (BCARP) as a continuation of the provincial program prior to DFO assuming regulatory authority. Each quarter DFO audits the routine monitoring and reporting of a maximum of 30 farms (Wade, 2017). During these audits samples are also taken for diagnostic testing as described in Wade (2017). The dataset has been truncated as DFO only began screening for *M. viscosa* in 2012.

Between 2012 and 2018, a total of 715 audits were conducted on active Atlantic Salmon farms in all Fish Health Surveillance Zones of BC. Overall, the fewest number of audits were conducted in December (n=31), and the highest in October (n=87) (Table 1).

Table 1. Total number and monthly average number of audits conducted on Atlantic Salmon farms in British Columbia from 2012-2018. Sources: Fisheries and Oceans Canada (DFO)-Aquaculture Management Division and Open Canada website as of May 29th, 2019. Updated from Jones (2019).

Month	Total number of audits conducted	Monthly average number (range) of audits conducted
January	58	8 (4-15)
February	78	11 (8-15)
March	40	6 (2-9)
April	64	9 (4-12)
May	73	10 (6-14)
June	47	7 (4-9)
July	75	11 (5-16)
August	61	9 (5-13)
September	44	6 (3-10)
October	87	12 (8-19)
November	57	8 (3-13)
December	31	4 (0-8)
Total	**715**	9 (0-19)

Through the audits, DFO veterinarians can diagnose “farm-level” winter ulcer based on farm history, environmental factors, mortality records, treatment history, clinical presentation and screening of individual fish or fish pools for infection by using histopathological examination and/or bacteriology.

The audits permit farm-level diagnoses of winter ulcer to be generated by DFO veterinarians as described in the Diagnostic Methods section. Seventeen farm-level winter ulcer diagnoses were made during fish health audits conducted between 2012 and 2018 in five Fish Health Surveillance Zones (Table 2). Winter ulcer has been diagnosed in 17/715 (2.4%) of all audits. Most farm-level diagnoses (9/17) were reported in zone 3.3, nine on eight farms.

Table 2. Summary of number of audit-based farm-level diagnoses of winter ulcer in seawater-reared Atlantic Salmon in British Columbia between 2012 and 2018. Values in parentheses are the numbers of unique farms on which farm-level audit-based diagnoses were made. Source: data provided by DFO Aquaculture Management Division and from the Open Canada website as of May 29th, 2019. Dashes: no audit.

Year	Fish Health Surveillance Zone and Sub-Zone									
	2.1	**2.2**	**2.3**	**2.4**	**3.1**	**3.2**	**3.3**	**3.4**	**3.5**	**Σ_{year}**
2012	-	-	3 (3)	0	0	0	2 (2)	0	0	5 (5)
2013	-	-	0	0	0	0	0	0	0	0
2014	-	-	0	0	0	1 (1)	2 (2)	1 (1)	0	4 (4)
2015	-	-	0	0	0	0	1 (1)	1 (1)	0	2 (2)
2016	-	-	0	0	0	0	1 (1)	0	0	1 (1)
2017	-	-	0	0	0	0	2 (2)	0	1 (1)	3 (3)
2018	-	-	0	0	0	0	1 (1)	0	1 (1)	2 (2)
$\Sigma_{subzone}$	**-**	**-**	**3 (3)**	**0**	**0**	**1 (1)**	**9 (8)**	**2 (2)**	**2 (2)**	**17**

Fish Health Events

A Fish Health Event (FHE) is defined as “a suspected or active disease occurrence within an aquaculture facility that requires the involvement of a veterinarian and any measure that is

intended to reduce or mitigate impact and risk associated with that occurrence or event" in the Marine Finfish Aquaculture Licence under the Fisheries Act (DFO, 2015).

FHE reporting, in general, began in the fall of 2002 (Wade, 2017). However, from 2013 until end of the third quarter of 2015 it was not a requirement to report events but became once again a condition of licence as of quarter four of 2015 (Wade, 2017). As a condition of licence, when a FHE occurs, the licence holder must take action to manage the event, evaluate the mitigation measures, submit a notification of FHE and therapeutic management measures to the Department (DFO, 2015).

We cannot confirm when the industry began testing for *M. viscosa*, nor if all the industry began screening at the same time. We know that DFO began screening through the FHASP in 2012. The first FHE attributable to *M. viscosa* was reported to DFO in 2011. The FHE data set has therefore been restricted to 2011 to 2018. These data should therefore be interpreted as the minimum number of FHEs.

Between 2011 and 2018 (excluding 2013 to 2015), a total of 13 FHEs attributed to winter ulcer were reported on Atlantic Salmon farms in BC (Table 3); eight occurred in quarter 1, four in quarter 2, and one in quarter four. In all events, fish in affected pens were treated.

Table 3. Summary of Fish Health Events (FHE) (2011-2018) attributed to winter ulcer in seawater-reared Atlantic Salmon in British Columbia reported by industry to Fisheries and Oceans Canada (DFO). Dashes indicate no requirement to report FHEs. Values in parentheses are the numbers of unique farms on which a FHE was reported. Sources: Aquaculture Management Division and from the Open Canada website as of June 6th, 2019.

Year	Fish Health Surveillance Sub-Zone									
	2.1	**2.2**	**2.3**	**2.4**	**3.1**	**3.2**	**3.3**	**3.4**	**3.5**	**Σ_{year}**
2011	0	0	0	0	0	0	1 (1)	0	0	1 (1)
2012	0	0	2 (1)	0	0	1 (1)	1 (1)	0	0	4 (3)
2013	-	-	-	-	-	-	-	-	-	-
2014	-	-	-	-	-	-	-	-	-	-
2015	-	-	-	-	-	-	-	-	-	-
2016	0	0	0	0	0	0	0	0	0	0
2017	0	0	0	1 (1)	2 (2)	0	2 (2)	0	0	5 (5)
2018	0	0	0	0	0	0	2 (2)	1 (1)	0	3 (3)
$\Sigma_{subzone}$	**0**	**0**	**2 (1)**	**1 (1)**	**2 (2)**	**1 (1)**	**6 (5)**	**1 (1)**	**0**	**13**

Mortality events

DFO (2015) defines a mortality event as "(a) fish mortalities equivalent to 4,000kg or more, or losses reaching 2% of the current stock inventory within a 24 hour period; or (b) fish mortalities equivalent to 10,000kg or more, or losses reaching 5% of the current stock inventory, within a five day period". As a condition of licence, any mortality event must be reported to DFO no later than 24 hours after discovery with details including facility name, fish cultured, number of dead fish, suspected proportion affected, suspected carcass biomass, probable cause, and action taken (DFO, 2015).

Between 2011 and 2018, there was one mortality event attributed to winter ulcer on an Atlantic Salmon farm in BC (DFO, 2019b). The mortality event occurred in zone 3.1 in 2018.

PACIFIC SALMON

Winter ulcer has not been diagnosed nor has *Moritella* sp. been reported in any of the 109 audits conducted through the FHASP on Pacific salmon farms (2012-2018). Audit testing for winter ulcer in farmed Pacific salmon began in 2012, when it did for Atlantic Salmon (H. Manchester, pers. comm., 2019). FHEs attributed to winter ulcer have not been reported in farmed Pacific salmon farms in BC. We do not know when the industry began testing.

KNOWLEDGE GAPS

Much of what is known about winter ulcer or *M. viscosa* is from studies in Atlantic Salmon from Norway, Iceland and to a lesser extent, Scotland; few of these studies have included isolates of *M. viscosa* from Canada. Knowledge gaps most important to the assessment of risk are:

- Transmission pathways;
- Natural reservoirs and vectors;
- Interactions between *M. viscosa* and other infectious agents in winter ulcer in Atlantic Salmon in BC;
- Identification of strains of *M. viscosa* from BC farmed Atlantic Salmon exhibiting clinical signs of disease;
- Determination of strain pathogenicity and geographical distribution;
- Susceptibility of Pacific salmon species and other Pacific marine species to *M. viscosa*;
- Environmental and biological factors contributing to infection and disease in Atlantic Salmon in BC; and
- Applicability of studies conducted on Atlantic Salmon from northeastern Atlantic farming regions using their regional strains to the BC context.

SUMMARY

Winter ulcer in BC is a relatively new phenomenon in Atlantic Salmon. It was first reported by the industry in December 2011 and FHASP started screening for the causative agent in 2012. From 2011 to 2018 (excluding 2013-2015), there have been 13 FHEs reported by industry. From 2012 to 2018, 17 farm level diagnoses resulted from audits. The occurrence of winter ulcer in farmed Atlantic Salmon in BC is not uncommon. With the available information, there is no evidence to date of winter ulcer or the causative agent in farmed Pacific salmon in BC.

Much of what is known about the disease and pathogen are from studies conducted on farmed Atlantic Salmon in Norway and Iceland. In the literature, most reports of winter ulcer or *M. viscosa* in salmonids are in Atlantic Salmon and sea farmed Rainbow Trout. No reference could be found describing the bacterial isolation of *M. viscosa* in Pacific salmon species but one reference reported the detection in two juvenile Sockeye Salmon using high-throughput microfluidics quantitative PCR.

Because of the paucity of winter ulcer and *M. viscosa* literature specific to Atlantic Salmon cultured in BC it is necessary to draw on studies conducted elsewhere. Infection with *M. viscosa* resulting in winter ulcer in BC appears most similar to that described in farmed Atlantic Salmon in Norway, Scotland and Iceland. The basic characteristics of disease and infection are similar between these two regions namely: onset at temperatures below 8-10^{o}C, recovery or no incidence of infection at temperatures greater than 10^{o}C, shallow wounds, isolation of *M.*

viscosa. It would therefore be reasonable to use results from studies conducted on these strains of Atlantic Salmon from these countries in the absence of BC specific data. Utilizing disease and infection data from the east coast of Canada appears less relevant to the BC context particularly as the onset of disease and conditions of progression are markedly different.

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