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Characterization of *Tenacibaculum maritimum* and mouthrot to inform pathogen transfer risk assessments in British Columbia

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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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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Mouthrot is a disease unique to salmonids of British Columbia (BC) and the United States (US) Pacific Northwest. Recently, the causative agent has been confirmed as *Tenacibaculum maritimum* in laboratory challenges with Norwegian Atlantic Salmon (*Salmo salar*) and isolates from farmed BC Atlantic Salmon exhibiting clinical signs of disease. *Tenacibaculum maritimum* is a gram-negative, filamentous bacterium which, in regions outside BC and the US Pacific Northwest, causes tenacibaculosis, an infection in many marine fish species globally. The clinical signs and gross pathology associated with strains of *T. maritimum* causing mouthrot and tenacibaculosis are different. Because of these differences and that mouthrot is found only in this region where the risk assessment is focused, it is important to separate out information pertaining to *T. maritimum* causing mouthrot and *Tenacibaculum* spp. causing tenacibaculosis.

Between 2002 and 2018 there were 106 farm-level mouthrot diagnoses made as a result of 1446 fish health audits conducted on Atlantic Salmon farms in BC. Mouthrot has been diagnosed every year since 2003 and from all Fish Health Surveillance Zones where audits were conducted. Although there was no requirement to report Fish Health Events (FHEs) between 2013 and 2015, from 2002 to 2013 and 2015 to 2018 there were 537 FHEs attributed to mouthrot 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 (*Salmo salar*) 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. Pathogens which may be assessed were determined through the British Columbia Provincial and DFO Fish Health Audit and Surveillance Program (Audit Program) and Fish Health Events (FHEs) reported by the industry.

In 2014, the Department undertook the first of the series of pathogen risk assessments; to determine the risk to the diversity and abundance of Fraser River Sockeye Salmon due to infectious hematopoietic necrosis virus (IHNV) transfer from Atlantic Salmon farms in the Discovery Islands. The risk assessment was reviewed through the Canadian Science Advisory Secretariat peer-review process and successfully completed in 2017 (Mimeault et al., 2017). In 2018, similar risk assessments were conducted on four bacterial pathogens, namely *Renibacterium salmoninarum*, *Aeromonas salmonicida*, *Yersinia ruckeri* and, *Piscirickettsia salmonis*. In early 2019, the Department undertook a risk assessment of Piscine Orthoreovirus (PRV) and in this next set of assessments is determining the risk to wild Fraser River Sockeye Salmon in the Discovery Islands associated with two more bacterial infections found on Atlantic Salmon farms in the area, winter ulcer (*Moritella viscosa*) and mouthrot (*Tenacibaculum maritimum*). This paper synthesizes the information pertinent to *T. maritimum*, the causal agent of mouthrot.

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 *T. maritimum*, the causative agent of mouthrot from Atlantic Salmon farms located in the Discovery Islands area of British Columbia (BC). The purpose of this document is not to be an exhaustive review of *T. maritimum*, 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

Mouthrot is a significant issue for farmed Atlantic Salmon in BC; treatment of mouthrot is the greatest use of anti-microbials by the industry (Morrison and Saksida, 2013; T. Hewison in Powell and Podlasly, 2015). Chronic mortality due to mouthrot can be as high as 15% (Anonymous, 1996; Ostland et al., 1999). One company reports the annual cost of outbreaks at over $1.6 million (T. Hewison in Powell and Podlasly, 2015).

Mouthrot has been noted in farmed salmon smolts (no species specified) in Puget Sound since the late 1980s (Frelier et al., 1994); outbreaks attributed to mouthrot have been reported in farmed Atlantic Salmon smolt reared in saltwater cages as early as 1994 and identified as *Flexibacter maritimus* (Ostland et al., 1999). *T. maritimum*, the causative agent, was only confirmed in 2018 (Frisch et al., 2018a; Frisch et al., 2018b), but it had been suspected as at least a contributor to the disease for many years. Although the recent confirmation of the causative agent is a significant gain, mouthrot is considered a complex disease that likely involves many contributing factors including environmental conditions, geographic location, fish condition and potentially other bacteria.

Tenacibaculosis is a term for an infection caused by bacteria in the genus *Tenacibaculum*. Because it can refer to infections caused by many different species of *Tenacibaculum* with varying clinical signs, it is important to be consistent with terminology. For example, tenacibaculosis in Atlantic Salmon in Norway is most often the result of an infection with *T. finnmarkense*. It is considered an ulcerative disease characterized by frayed fins, skin lesions and mouth erosion and, commonly identified in mixed culture with *Moritella viscosa* (Olsen et al., 2011; Bornø and Lie, 2015; Småge et al., 2016; Småge et al., 2018). In Atlantic Salmon cultured in Atlantic Canada, similar clinical signs have been reported to those described in Norway, although not necessarily in conjunction with skin ulcers (S. Leadbeater, DFO, 125 Marine Science Dr., St. Andrews, NB E5B 0E4, pers. comm., 2019). A recent laboratory study has confirmed that the causative agent for tenacibaculosis to be *T. finnmarkense* (S. Leadbeater, pers. comm., 2019).

The clinical signs and gross pathology associated with strains of *T. maritimum* causing mouthrot and infections with *Tenacibaculum* resulting in tenacibaculosis are different and described in the pathogen characterization section. In this paper we will endeavor to separate out the literature pertaining to mouthrot and tenacibaculosis with a focus on the mouthrot literature.

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: "mouthrot", "mouth rot", "tenacibaculosis" "myxobacteria", "stomatitis", "*Tenacibaculum",* "*Flexibacter maritimus**", "yellowmouth" and most in combination with "Atlantic Salmon", "Sockeye Salmon", "Pacific salmon", "outbreak", "infection", "disease", "transmission", "biofilm", "mortality", "vaccine", "exposure", "British Columbia', and "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) and fish health manuals by Hicks (1989) and Kent (1992) 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 was requested from DFO, Aquaculture Management Division (AMD). As necessary, phone calls were conducted to supplement information provided through government records or industry reports.

CHARACTERIZATION

DISEASE AND AGENT

Mouthrot

In BC, mouthrot is commonly reported as bacterial stomatitis, myxobacterial stomatitis or ulcerative stomatitis, which are all non-specific terms referring to a bacterial infection of the mouth. It was first officially described in the early 1990s as a unique bacterial infection of the mouth of Atlantic Salmon in the US Pacific northwest and BC (Ostland et al., 1999).

Until recently, the causative agent or agents of mouthrot were unknown but there was evidence to support that *T. maritimum* was a contributing bacterium namely Ostland et al. (1999) and Frelier et al. (1994). It is through recent work by Frisch et al. (2017; 2018a; 2018b) with isolates of mouthrot from BC farmed Atlantic Salmon, that it can be confirmed that *T. maritimum* is the causative agent. For the remainder of this document, the use of the term mouthrot is synonymous with strains of *T. maritimum* causing mouthrot, but not all *T. maritimum* strains.

Tenacibaculum maritimum (formally *Flexibacter maritimus)* is an aerobic, gram-negative, gliding, filamentous bacterium (Wakabayashi et al., 1986; Suzuki et al., 2001; Avendaño-Herrera et al., 2006d); Family Flavobacteriaceae, genus *Tenacibaculum*, species *maritimum* (Loch and Faisal, 2015). The family *Flavobacteriacea*e is comprised of over 100 genera within the phylum *Bacterioidetes* (Bernardet and Nakagawa, 2006; Bernardet, 2010; Ludwig et al., 2010; Zhang et al., 2012). Members of the *Bacteroidetes* division can be found in most habitats on earth (Kirchman, 2002). Within the *Bacteroidetes* division, the *Cytophaga-Flavobacteria* cluster are present in both fresh and marine waters (Kirchman, 2002). In most marine habitats this cluster is the most abundant of all bacterial groups (Kirchman, 2002).

Tenacibaculum maritimum requires at least 30% seawater in the medium to grow (Wakabayashi et al., 1986). Mouthrot isolates have been shown to require a minimum of 50% seawater in the medium to grow (Ostland et al., 1999). It is a naturally occurring member of the marine bacterial community (Habib et al., 2014).

Clinical signs and gross pathology of Atlantic Salmon infected with mouthrot include: lethargy, emaciation and anorexia, some fish may exhibit head shaking or flashing (Kent, 1992). Yellow bacterial mats around the palate, teeth and vomer are present early in an infection (Kent, 1992). As disease progresses, fish develop multiple ulcers in the mouth with large yellow bacterial

mats (Kent, 1992; Frelier et al., 1994). Lesions are characteristically associated with regions of dentition including premaxilla, dentary, vomer and palatine bones and may result in tooth loss (Frelier et al., 1994). Lesions may extend to the branchial arches and esophagus; in severe cases the lower and upper jaw may be completely eroded (Kent, 1992). Severely infected fish cease feeding (Kent, 1992). This clinical manifestation, the presence of plaques associated with the teeth, has not been reported in any other Atlantic Salmon farming regions even where *T. maritimum* is present (Frisch et al., 2018b).

Tenacibaculosis caused by *Tenacibaculum maritimum*

Tenacibaculosis is a term used for infections in marine fishes caused by several *Tenacibaculum* spp*.,* and not only *T. maritimum*. It is associated with the diseases: salt water columnaris disease, gliding bacterial disease of sea fish, bacterial stomatitis, eroded mouth syndrome and black patch necrosis (Avendaño-Herrera et al., 2006d). The pathology of tenacibaculosis differs from that of mouthrot.

Pathology of tenacibaculosis associated with infection with *T. maritimum* includes: lesions on the body surface such as ulcers, necrosis, eroded mouth, frayed fins and tail rot, and sometimes necrosis on the gills and eyes (McVicar and White, 1979; Campbell and Buswell, 1982; Baxa et al., 1986; Devesa et al., 1989; Alsina and Blanch, 1993; Chen et al., 1995; Handlinger et al., 1997; Ostland et al., 1999; Cepeda and Santos, 2002; Jansson and Vennerström, 2014).

GEOGRAPHIC RANGE AND HOSTS

Mouthrot

Mouthrot has been identified in farmed Atlantic Salmon in BC (Ostland et al., 1999) and Washington State (Frelier et al., 1994), in farmed Rainbow Trout in Washington State (Frelier et al., 1994) and in farmed Chinook Salmon (*O. tschawytscha*) in BC (see Fish Health Audit and Surveillance Program).

Mouthrot has not been diagnosed in any farmed Coho Salmon (*O. kisutch*) in BC (DFO, 2019a, c). Mouthrot was not found described for any wild fish species in BC or Washington State. Kent and Poppe (1998) mention that cage-reared Arctic Char (*Salvelinus alpinus*) have been afflicted with the infection, but provide no geographic origin or citation to be able to confirm.

No reference could be found describing the bacterial isolation of *T. maritimum* or mouthrot in Sockeye Salmon. However, Nekouei et al. (2018) report the detection of *T. maritimum* in 5 of 2,006 juvenile Fraser River Sockeye Salmon screened using high-throughput microfluidics quantitative polymerase chain reaction (PCR). Tissue samples included gills, therefore external contamination cannot be excluded.

Tenacibaculum maritimum has been isolated from body lesions and gills (not mouths) of Chinook Salmon and White Seabass (*Atractoscion nobilis)* (Chen et al., 1995) and body lesions (not mouths) of Northern Anchovy (*Engraulis mordax*) and Pacific Sardine (*Sardinops sagax)* from California (Table 1) (Chen et al., 1995). None of these fish had plaques typical of mouthrot.

There are two other reports (Sawyer, 1976; Hilger et al., 1991) which are sometimes attributed to *T. maritimum* and/or mouthrot in the literature, but the information to support a diagnosis of mouthrot is weak. The first report is that of a “yellow pest” found in young-of-the-year Atlantic Cod (*Gadus morhua*) in the nearshore area of the German Wadden Sea (Hilger et al., 1991). It does not appear that this is mouthrot as the pathology is different and the bacterium was able to grow on a freshwater medium. The second report is an outbreak of “myxobacterial disease” in Coho Salmon smolt in Maine, US (Sawyer, 1976). As with Hilger et al. (1991), the pathology

described in Sawyer (1976) is not akin to that described for mouthrot and the bacteria grew on media with both 50% seawater as well 1.5% NaCl alone. *Tenacibaculum maritimum* requires seawater to grow (Wakabayashi et al., 1986; Ostland et al., 1999; Toranzo et al., 2005; Avendaño-Herrera et al., 2006d), which is consistent with the findings in Sawyer (1976), but it is known that no growth occurs in the presence of NaCl alone (Santos et al., 1999; Avendaño-Herrera et al., 2006d).

Tenacibaculum maritimum

Tenacibaculum maritimum is found worldwide in many marine finfish species (Table 1). It has been identified in salmonids including Rainbow Trout (*O. mykiss*) in Australia and in the USA (Frelier et al., 1994; Soltani et al., 1996; Handlinger et al., 1997), farmed Chinook Salmon in California and New Zealand (Frelier et al., 1994; Chen et al., 1995), and Atlantic Salmon in Spain (Pazos et al., 1993; Ferguson et al., 2010), Australia (Soltani and Burke, 1994; Handlinger et al., 1997; Powell et al., 2004), Chile (Apablaza et al., 2017), Scotland (Ferguson et al., 2010), Washington State (Frelier et al., 1994) and BC (Hicks, 1989; Frisch et al., 2017).

Table 1. Examples of teleost host species or suspected host species for Tenacibaculum maritimum *and their geographic origin.*

Common name	Species	Country	Reference
Salmonids			
Atlantic Salmon	*Salmo salar*	Spain	Pazos et al. (1993) in Ferguson et al. (2010)
		Australia	Soltani and Burke (1994); Handlinger et al. (1997); Powell et al. (2004)
		Canada (British Columbia)	Hicks (1989); Kent (1992); Ostland et al. (1999); Frisch et al. (2017)
		Chile	Apablaza et al. (2017)
		Scotland	Ferguson et al. (2010)
		USA (Washington)	Frelier et al. (1994)
		Norway	PHARMAQ-Analytiq, 2017
		Ireland	Downes et al. (2018)
Chinook Salmon	*Oncorhynchus tshawytscha*	USA (California)	Chen et al. (1995)
		New Zealand	Brosnahan et al. (2018)
Coho Salmon	*Oncorhynchus kisutch*	Canada	Nekouei et al. (2019)
Rainbow Trout	*Oncorhynchus mykiss*	Australia	Soltani et al. (1996); Handlinger et al. (1997)
		USA (Washington)	Frelier et al. (1994)
Sockeye Salmon	*Oncorhynchus nerka*	Canada	Nekouei et al. (2018)
Non-salmonids			
Barramundi	*Lates calcarifer*	Singapore	Labrie (2008)
		Australia	Soltani et al. (1996)
Black Bream	*Acanthopagrus butcheri*	Australia	Handlinger et al. (1997)

Common name	Species	Country	Reference
Black Damselfish	*Neoglyphieodon meles*	Ornamental	ICES (2019)
		Egypt	Abd El-Galil and Hashiem (2011)
Black Seabream	*Acanthopagrus schlegeli*	Japan	Masumura and Wakabayashi (1977); Wakabayashi et al. (1984); Wakabayashi et al. (1986)
Blackspot Seabream	*Pagellus bogaraveo*	Spain	Castro et al. (2007)
Broomtail wrasse	*Cheilinus lunulatus*	Egypt	Abd El-Galil and Hashiem (2012)
Dover Sole	*Solea solea*	UK	McVicar and White (1979, 1982); Bernardet et al. (1990)
		Netherlands	Habib et al. (2014)
		Spain	Avendaño-Herrera et al. (2004b)
European Seabass	*Dicentrarchus labrax*	France	Pepin and Emery (1993); Bernardet et al. (1994)
		Malta	Bernardet (1998)
		Italy	Salati et al. (2005)
		Greece	Kolygas et al. (2012)
Gilthead Seabream	*Sparus aurata*	Spain, Italy	Avendaño-Herrera et al. (2004a); Avendaño-Herrera et al. (2004c); Salati et al. (2005)
		Greece	Kolygas et al. (2012)
Greenback Flounder	*Rhombosolea tapirine*	Australia	Soltani et al. (1996) ; Handlinger et al. (1997)
Japanese Flounder	*Paralichthys olivaceous*	Japan	Baxa et al. (1986)
Japanese Pufferfish	*Takifugu rubripes*	Japan	Rahman et al. (2014)
Lumpfish	*Cyclopterus lumpus*	Norway	Småge et al. (2016)
Northern Anchovy	*Engraulis mordax*	USA (California)	Chen et al. (1995)
Olive Flounder	*Paralichthys olivaceus*	Korea	Jang et al. (2009)
Orbicular batfish	*Platax orbicularis*	French Polynesia	Bardon-Albaret et al. (2016)
Pacific Sardine	*Sardinops sagax*	USA (California)	Chen et al. (1995)
Picasso Triggerfish	*Rhinecanthus assasi*	Ornamental	ICES (2019)
		Egypt	Abd El-Galil and Hashiem (2011)
Red Seabream	*Pagrus major*	Japan	Masumura and Wakabayashi (1977)
Rock Bream	*Oplegnathus fasciatus*	Japan	Wakabayashi et al. (1986)
Sand Tiger Shark	*Carcharias taurus*	Italy	Salati et al. (2005); Florio et al. (2016)
Sharpsnout Bream	*Diplodus puntazzo*	Italy	Salati et al. (2005)

Common name	Species	Country	Reference
Six-toothed bream	*Dentex dentex*	Italy	Salati et al. (2005)
Senegalese Sole	*Solea senegalensis*	Portugal	Cepeda and Santos (2002); Avendaño-Herrera et al. (2004c); Avendaño-Herrera (2005)
		Spain	Cepeda and Santos (2002); Avendaño-Herrera et al. (2004c); Avendaño-Herrera (2005)
Striped Trumpeter	*Latris lineata*	Australia	Carson et al. (1992); Handlinger et al. (1997)
Tub Gurnard	*Chelidonichthys lucerna*	Italy	G. Magi (Unpubl. data in Avendaño-Herrera et al. (2006d))
Turbot	*Scophthalmus maximus*	Spain	Devesa et al. (1989); Alsina and Blanch (1993); Avendaño-Herrera et al. (2004a); Avendaño-Herrera et al. (2004c)
		Chile	Bernardet (1998); Lopez et al. (2009)
		France	Habib et al. (2014)
		Norway	Olsen et al. (2017)
		Italy	Magi et al. (2007)
Wedge Sole	*Dicologoglossa cuneate*	Spain	Chen et al. (1995); Lopez et al. (2009)
White Seabass	*Atractoscion nobilis*	USA (California)	Chen et al. (1995); Salati et al. (2005)
White Seabream	*Diplodus sargus*	Italy	Salati et al. (2005)
Yellow-eye Mullet	*Aldrichetta forsteri*	Australia	Handlinger et al. (1997)
Yellowtail	*Seriola quinqueradiata*	Japan	Baxa et al. (1988b, 1988a); Avendaño-Herrera et al. (2006d)

Tenacibaculum maritimum has been detected in several jellyfish species. It has been detected molecularly from the medusa *Phialella quadrata* near a Scottish Atlantic Salmon farm (Ferguson et al., 2010). Using scanning electron microscopy (SEM), DNA extraction and PCR, *T. maritimum* was confirmed in the mouth of Mauve Stingers (*Pelagia noctiluca*) from the Irish Sea (Delannoy et al., 2011). *Tenacibaculum maritimum* DNA was detected in a low number (4/26) of jellyfish at low levels using real-time PCR from preserved jellyfish species (*Muggiaea atlantica* and *Phialella quadrata*) collected in the marine waters surrounding Ireland (Fringuelli et al., 2012).

Although both Delannoy et al. (2011) and Ferguson et al. (2010) detected *T. maritimum* by PCR and sequencing, it is difficult to determine whether the bacterium was present in the mouth of the jellyfish, or if it was merely attached to the surface of the jellyfish or present in water surrounding the jellyfish. The authors state that the threadlike structures in the mouth are *T. maritimum*, however, the morphology presented in the SEM micrograph does not resemble *T. maritimum*. In a study by Småge et al. (2017), it was shown by using both transmission electron microscopy (TEM) and SEM that a similar Cnidarian jellyfish has similar threadlike structures in the mouth region, but that these structures are cilia, and not bacteria (*Tenacibaculum*).

Tenacibaculum maritimum has been isolated both internally and externally from sea lice (*Lepeophtheirus salmonis*) removed from farmed Atlantic Salmon in BC (Barker et al., 2009).

The experiment did not determine if sea lice released *T. maritimum*. Strains were not identified but it was stated that it grew well on Tyes Agar with 3% sea salt.

GENETIC STRAINS

Tenacibaculum maritimum is found globally; however, mouthrot is not. It has only been described in cultured salmonids in BC and Washington State and until recently there has been little knowledge of the genetics of *T. maritimum* associated with mouthrot.

Frisch et al. (2017) genotyped *T. maritimum* isolates from farmed Atlantic Salmon in BC and subsequently compared them to other sequence types of the same species and of other species within the genus *Tenacibaculum*. Although much genetic research has been completed on *T. maritimum* strains from Europe, Asia and Australia (Habib et al., 2014), it provides the first information on the genetic profile of *T. maritimum* associated with Atlantic Salmon exhibiting clinical signs of mouthrot. Details of the bacterial isolation, PCR and sequencing and genetic analysis are described in Frisch et al. (2017).

The results of Frisch et al. (2017) showed that *T. maritimum* isolates from Atlantic Salmon with clinical mouthrot belonged to two distinct sequence types (STCan1, STCan2) different from those previously published by Habib et al. (2014) and that the strains were more closely related to a Norwegian Lumpfish strain (NLF-15, reared at 12ºC) and a Chilean Atlantic Salmon strain (Ch-2402, reared at 14ºC). The Canadian isolates were from Atlantic Salmon reared at temperatures ranging from 8.7-14.7ºC. Ostland et al. (1999) report that all mouthrot isolates grew at temperatures ranging from 12-30ºC whereas only the reference strains (ATCC 43397, ATCC 43398T) could grow at 34ºC, no isolates grew at 5ºC or 37ºC.

Frisch et al. (2017) in conjunction with results of geographic analysis of *T. maritimum* by Habib et al. (2014) have suggested that the genetic relationships between isolates of *T. maritimum* globally, may be distributed by temperature which is reflected in their geographic location. Results from Habib et al. (2014) suggest endemic distribution of strains is not linked to international movement of fishes.

This importance of temperature for strain survival may be reflected in the culture temperatures cited in the literature. Apablaza et al. (2017) report that *T. maritimum* (Ch-2402) isolated from Chilean Atlantic Salmon was able to grow at 8ºC, well below the lower range of growth (15ºC) for type species NCIMB 2514^{T} reported by Suzuki et al. (2001), which is typically cultured at 25ºC (Bernardet et al., 1994).

Most studies outside BC and Washington State report culture temperatures for *T. maritimum* in the low to mid 20ºC, much higher than those in Frisch et al. (2017) or Apablaza et al. (2017). For example: 25ºC for Seabass isolates in Spain (Bernardet et al., 1994), Japanese Flounder in Japan (Baxa et al., 1986), Senagalese Sole in Spain (Cepeda and Santos, 2002) and reference strains from various species in Japan (Watanabe and Nishimura, 2010); 22-25ºC for various species in California (Chen et al., 1995), 22ºC for reference strain NCIMB2163 and jellyfish isolates from the Irish Sea (Delannoy et al., 2011); 20.5ºC for Wedge Sole in Spain (Lopez et al., 2009); 20-25ºC for Atlantic Salmon strain 89/4762 from Australia (van Gelderen et al., 2009; van Gelderen et al., 2011). The temperature range for *T. maritimum* is reported as between 15-35ºC and the optimal temperature of 30ºC (Avendaño-Herrera et al., 2006d).

DIAGNOSTIC METHODS

MOUTHROT

In the 1990s, *Flexibacter maritimus* (now *T. maritimum*) was found to be associated with bacterial stomatitis in Atlantic Salmon in BC (Ostland et al., 1999) and until recently (Frisch et al., 2017; Frisch et al., 2018a; Frisch et al., 2018b), the causative agent of mouthrot has been elusive. The main reason why the causative agent had not been confirmed was because it is very difficult to culture and isolate as other faster growing bacteria would routinely outcompete *T. maritimum*. The addition of kanamycin to the media used for bacterial isolation significantly improved success through the reduction in growth of other bacteria allowing for studies (Frisch et al., 2017; Frisch et al., 2018a; Frisch et al., 2018b) to demonstrate the role of *T. maritimum* as the causative agent of mouthrot. A *Tenacibaculum*-specific agar named KABAMA has been developed that aids in the differentiation between *T. maritimum* and other *Tenacibaculum* spp. by their colony characteristics (Småge et al., unpublished, presented at CAHS Tenaci 2 workshop in Campbell River, BC, October 29, 2019).

Case definition

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

"Mouthrot 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 gross pathology and histopathology consistent with the disease.

- Characteristic gross pathology is yellow plaques in the mouth, gill rakers and/or palate.
- Characteristic histopathology is the visualization of filamentous bacteria (consistent with *Tenacibaculum* sp.)."

Interpreting diagnostic records

In BC, three sources of diagnostic data are available for farmed Atlantic Salmon: DFO audit data, DFO Fish Health Event (FHE) data, and 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.

Mouthrot diagnoses from audit testing refer to "mouthrot (filamentous myxobacteriosis)" or "mouth myxobacteriosis". Specific pathogens are not identified.

FHEs attributed to mouthrot may refer to any one of the following: "bacterial stomatitis", "bacterial stomatitis/ulcers", "infectious stomatitis", "mouthrot", "*T. maritimum*", "*Tenacibaculum*", "myxobacterial infection" or left blank. "Myxobacterial infection" may include "Cytophaga-Flexibacter-Flavobacter-Myxobacteria", "*Flexibacter* sp.", "*Mycobacterium marinus*", "not provided", "unknown" or left blank.

Because the diagnoses do not provide insight into a causative agent, as it has only recently been confirmed, it is important to understand how a diagnosis is made.

During an audit, mouthrot is identified through gross pathology, specifically, yellow mouth plaques (H. Manchester, DFO, Fisheries and Oceans Canada, 103-2435 Mansfield Drive, Courtenay, BC V9N 2M2, pers. comm., 2019). Standard audit tissues including kidney, heart, liver, spleen, gill, pyloric cecae (Wade, 2017), are then sent to the provincial Animal Health Centre in Abbotsford (lab) for analysis. The Centre is an American Association of Veterinary Laboratory Diagnosticians (AAVLD) accredited full service diagnostic lab.

Until recently, there had been no evidence that mouthrot could spread systemically, and therefore any bacteria isolated from organ tissues was not directly attributed to a mouthrot infection. The recent study by Frisch et al. (2018a) has demonstrated that mouthrot caused by *T. maritimum* can become systemic.

Starting in 2008, during an audit, jaws from fish with plaques in the mouth (or suspect plaques) were sent to the lab for histology. A maximum of three jaws (from three different fish) are submitted per audit. From these samples, the lab determines if there is inflammation and presence of filamentous bacteria, which is typical of fish with clinical signs of mouthrot. The type(s) of filamentous bacteria is not identified by the lab. Typically, filamentous bacteria do not spread systemically. Because they are found in the jaws of fish with typical mouthrot plaques and inflammation is found, it is believed they are contributing to the disease.

Therefore, depending on when a diagnosis was made (i.e., pre- or post-2008), a diagnosis of mouthrot may be based on presence of plaques as observed on the farm or presence of plaques as observed on the farm with filamentous bacteria and inflammation in the jaw as determined in the lab. In addition, mouthrot is accompanied with elevated mortality on the farm with a large proportion of the mortality having visible mouth plaques; once treatment is initiated it will be reported as a FHE to DFO (H. Manchester, DFO, pers. comm., 2019). If the diagnosis was myxobacterial infection, bacterial stomatitis or any other bacterial infection without plaques, it is not possible to say definitively that the fish had mouthrot. If a diagnosis of mouthrot is made, there is no doubt it was mouthrot as plaques were present.

TENACIBACULOSIS CAUSED BY *TENACIBACULUM MARITIMUM*

The presumptive diagnosis for tenacibaculosis is based on clinical signs and in this case, gross external lesions (see General Description) and presence of long, thin, rod-shaped bacteria in wet mounts or Gram preparations from gill or skin lesions (Avendaño-Herrera et al., 2006d). Definitive diagnosis is supported by the isolation of *T. maritimum* colonies on specific media followed by determination of at least a limited number of morphological and biochemical characteristic or molecular DNA-based methods (Avendaño-Herrera et al., 2006d).

As it is difficult to distinguish *T. maritimum* from other phylogenetically and phenotypically similar species, the use of PCR is important both to aid in diagnoses and in the development of vaccines (Toranzo et al., 2005; Fernandez-Alvarez et al., 2018).

INFECTION AND DISEASE

MOUTHROT

Mouthrot is considered primarily a disease of concern for Atlantic Salmon smolts. Mouthrot can be detected in smolt as early as six weeks after sea water entry (Hicks, 1989) and mainly affects smolt in their first year at sea (Anonymous, 1996 in Ostland et al. (1999)), likely physiologically related not size dependent. Frisch (2018) stated that clinical signs of mouthrot can be observed on farms as early as two days post seawater transfer. It is likely that environmental factors contribute to the incidence and development of mouthrot. An association with high-salinity water was reported in Kent and Poppe (1998) and Frelier et al. (1994). Mouthrot has been reported at temperatures ranging from 8.7-14.7^{o}C in BC (Frisch et al., 2017).

Some farms in BC are more prone to outbreaks than others, but it is not known why (Powell and Podlasly, 2015). Understanding environmental factors which may (or may not) contribute to disease and outbreaks is seen as a priority to understanding the disease (Powell and Podlasly, 2015). Correlations with temperature and salinity are discussed in subsequent sections.

How Atlantic Salmon smolt contract mouthrot is unknown. *Tenacibaculum maritimum* is an opportunistic bacteria and handling is a major contributing factor to disease (Salati et al., 2005; Avendaño-Herrera et al., 2006d; Kolygas et al., 2012). Some pre-disposing factors which have been suggested include: feeding on hard pellets, fish biting net surfaces and stress-induced lesions in the mouth (Kent and Poppe, 1998). Abrading of periodontal tissue by feeding on spiny crustaceans including crab larvae and amphipods has also been hypothesized as an entry point for bacteria (Kent and Poppe, 1998). Recently, it has been possible to detect *T. maritimum* internally through reverse transcription PCR (RT-PCR) and bacteriology, and it is suggested that one possible entry point would be the teeth (Frisch et al., 2018a). There is still little understanding of the mechanism of infection.

Mortality due to mouthrot can sometimes reach up to 15% (Anonymous, 1996; in Ostland et al., 1999). The mechanism by which Atlantic Salmon smolt are killed by *T. maritimum* in this region remains unknown (Frisch et al., 2018a).

TENACIBACULUM MARITIMUM

Both adults and juveniles are affected by *T. maritimum*; however, younger fish suffer a more severe form of the disease (Toranzo et al., 2005). Infections have been documented in association with handling events such as counting, size-classification and transport (Alsina and Blanch, 1993; Cepeda and Santos, 2002) and recent transfer to sea cages (Wakabayashi et al., 1984; Pepin and Emery, 1993).

Other factors attributed to outbreaks include: high water temperature (Wakabayashi et al., 1984; Handlinger et al., 1997) or rapid increases in water temperature (Devesa et al., 1989); existing gill pathology related to feed quality and management (Handlinger et al., 1997); and physical damage to the skin (Chen et al., 1995). These factors are similarly noted by Avendaño-Herrera et al. (2006d).

Prevalence and severity of disease is often reported at temperatures higher than 15ºC and salinities 30-35 ppt as well as in conditions of low water quality (Avendaño-Herrera et al., 2006d). It has been suggested that because saltwater is needed to culture the bacteria, that low salinities (<15 g/L) may aid in the prevention of outbreaks in some strains isolated from Atlantic Salmon in Australia (Soltani and Burke, 1994). In Australia it has been associated with high temperatures (21ºC), sunny cloudless days and poor feeding management (Handlinger et al., 1997).

In the laboratory, abrasion of the gill epithelium has been shown to increase the severity and rate of disease progression in Atlantic Salmon smolt, depending on the strain of *T. maritimum* used (Powell et al., 2004). Infections with *T. maritimum* are reported in association with other infections including amoebic gill disease (Powell et al., 2005).

Tenacibaculum maritimum infection has been reported in association with gill damage caused by jellyfish stings in Atlantic Salmon in Australia (Handlinger et al., 1997).

Tenacibaculum maritimum (CH-2402) has been isolated from gill tissue from Chilean Atlantic Salmon during a harmful algal bloom in 2016 which resulted in a mass mortality event (Apablaza et al., 2017). The authors do acknowledge that although the bacteria could have contributed to the pathology, the bacteria may also be attributed to secondary infections from the environment. This isolate, CH-2402, was reported as one of the two most closely related isolates to those derived from Atlantic Salmon in BC exhibiting clinical mouthrot in a recent study by Frisch et al. (2017).

TRANSMISSION

Because so few studies have been published on mouthrot, information pertaining to transmission is focused on *T. maritimum* in general, not *T. maritimum* strains causing mouthrot, unless specified.

Reservoirs

Although no natural reservoirs have been confirmed, *T. maritimum* can be isolated from cultures of sediments and water exposed to infected fish (Santos et al., 1999). There have also been studies which suggest jellyfish may be a host or vector for *T. maritimum* (Handlinger et al., 1997; Ferguson et al., 2010; Delannoy et al., 2011). This is contrary, however, to the report by Downes et al. (2018) who found that *T. maritimum* was present in farmed Atlantic Salmon prior to a jellyfish bloom.

Members of the family Flavobacteriaceae (to which *T. maritimum* belongs) constitute one of a few dominating bacterial linages in phytoplankton-associated bacterial communities that are important for carbon recycling (Teeling et al., 2012; Teeling et al., 2016; Bohórquez et al., 2017). Although it is not specifically stated for *T. maritimum* in the literature, it is important to mention that these bacteria are likely present in the natural environment and may not require a specific vector to spread between hosts.

In lab studies conducted to determine what factors contribute to the survival and infection in the aquatic environment, Avendaño-Herrera et al. (2006a) suggest that *T. maritimum* may also be present in a viable but non-culturable state (VBNC) like other marine fish pathogens. In sterile seawater, *T. maritimum* (PC424.1) was able to survive in a culturable state for more than five months, whereas in natural seawater, it survived only five days. The authors attribute this decline in survivability to the inhibitory effect of the natural microbiota. This antagonistic interaction has been documented in other studies and has been suggested as the reason why isolation of the bacteria from wounds is difficult.

Environmental parameters

There is little known about the survival of *T. maritimum* causing mouthrot in the environment. What is known about survival is largely based on environmental factors correlated with diagnoses of mouthrot, for which there is little published documentation. Warm water temperatures and high salinity have been suggested as two factors associated with mouthrot. Hicks (1989) suggest water temperatures of 16-18^{o}C may be contributing factors to the development of mouthrot. Frelier et al. (1994) report disease outbreaks from April to July 1990 and 1991 in farmed salmon in Puget Sound at 8 and 12^{o}C and 29 and 32 ppt. Mortality and morbidity were observed in smolts 3-8 weeks post seawater entry. It is difficult to determine from the paper if a disease outbreak occurred on the Chinook Salmon farm or only on the Atlantic Salmon and Cutthroat Trout farms. They report necropsy survey results for each year for Atlantic Salmon (13.4 and 9% affected), Rainbow Trout (11.5 and 9.5% affected) and Chinook Salmon (0% affected).

Ostland et al. (1999) describe the bacteria isolated from fish with clinical signs of mouthrot, but do not provide any details of the outbreak such as temperature, salinity or mortality. The outbreak occurred in caged Atlantic Salmon near Campbell River and fish were sampled from March 1994 to July 1995.

In a multi-factorial study of various bacterial pathogens and amoebic gill disease in farmed Atlantic Salmon in Ireland, Downes et al. (2018) found that detections of *T. maritimum* from gills were significantly correlated with temperature demonstrating seasonality. Over the duration of

the study, water temperature ranged from 8.4-17.9°C (Downes et al., 2018). An increase in prevalence was observed in the summer and fall of the first year of production followed by a decline in the winter (Downes et al., 2018). Prevalence increased as water temperatures increased the following year (Downes et al., 2018). Additionally, Downes et al. (2018) found no effect on the presence of the bacterium after freshwater treatments.

The isolates used by Frisch et al. (2017) were derived from fish with clinical signs of mouthrot being reared at temperatures ranging from 8.7-14.7°C. As stated previously, the results of genotyping of these isolates showed that these Canadian isolates were most closely related to colder water strains from Lumpfish in Norway (12°C) and Atlantic Salmon in Chile (14°C). It has been suggested that the genetic relationships between isolates of *T. maritimum*, globally, may be distributed by temperature which is reflected in their geographic location (Habib et al., 2014; Frisch et al., 2017). It is therefore reasonable to assume that temperature affects survival in the wild; however, temperature ranges or sensitivities are unknown.

In the lab, it has been demonstrated that *T. maritimum* requires seawater to grow (Suzuki et al., 2001). Challenge studies with Atlantic Salmon and Rainbow Trout exposed at low salinity (15 ppt) resulted in low mortality (Soltani et al., 1996; Handlinger et al., 1997).

Biofilms

Tenacibaculum maritimum is adhesive and can therefore create biofilms on hard surfaces (Declercq et al., 2013; Frisch et al., 2017; Frisch et al., 2018a; Frisch et al., 2018b). The bacterium produces substantial amounts of “slime” allowing it to adhere to hydrophobic surfaces, which may explain why it can adhere to external fish tissues (Avendaño-Herrera et al., 2006d). van Gelderen et al. (2010) suggested that the adhesive nature of *T. maritimum* is associated with pathogenicity and virulence, as more adhesive isolates appears more pathogenic based on their studies. This is also true for the Canadian strains with TmarCan 16-1 being the most adhesive (S. Småge, Cermaq Group, Dronning Eufemiasgate 16, Oslo, Norway, 0191, pers. comm., 2019).

There is thought that the formation of biofilms by the bacteria may aid in adhesion to the fish and/or fish’s tissues (Vinogradov et al., 2003; Avendaño-Herrera et al., 2006c). *Tenacibaculum maritimum* has been shown to adhere to the mucous of European Seabass, Gilthead Seabream and Turbot, with no antibacterial activity detected in the mucous (Magarinos et al., 1995).

A recent study has demonstrated the importance of understanding the role of biofilms in the transmission of disease. The kinetics of various *T. maritimum* strains tested (none from BC) suggest that the inert surfaces of aquaculture settings can harbour biofilms and serve as transient reservoirs for the bacteria (Levipan et al., 2019).

Biofilms on tooth enamel have been described in farmed Atlantic Salmon in Puget Sound with what was then termed ulcerative stomatitis (Frelier et al., 1994), which we now recognize as mouthrot.

Horizontal transmission

Horizontal transmission has been demonstrated in the laboratory. Frisch et al. (2018b) conducted cohabitation and horizontal transmission experiments with 40 g Norwegian Atlantic Salmon smolt and isolates derived from BC Atlantic Salmon with clinical signs of mouthrot. The details of this study are described in the next section.

VIRULENCE AND PATHOGENICITY

Experimental models

Many infection models and studies have been conducted to reproduce tenacibaculosis in many commercially cultured marine species (Baxa et al., 1986; Powell et al., 2004; Avendaño-Herrera et al., 2006c; Nishioka et al., 2009; van Gelderen et al., 2010, 2011; Failde et al., 2013; Mabrok et al., 2016). These studies cannot be applied to mouthrot for several reasons: mouthrot and tenacibaculosis are clinically different; there are significant antigenic differences among BC isolates and between BC isolates and *T. maritimum* reference strains (Ostland et al., 1999); and recent studies have demonstrated the differences in the genetics, antibody response and pathology of BC strains of *T. maritimum* compared to other strains which result in tenacibaculosis (Frisch et al., 2017; Frisch et al., 2018a; Frisch et al., 2018b).

There is one published study (Frisch et al., 2018b) which attempted to induce mouthrot with *T. maritimum* isolates derived from Atlantic Salmon with clinical signs of mouthrot from BC in order to develop a bath challenge model, in addition co-habitation and transmission experiments were conducted. Laboratory holding conditions and animals were the same for all experiments, most notably: Norwegian Atlantic Salmon, 12^{o}C experimental water, 12-h photoperiod (except when smoltifying) and when smolt were transferred from freshwater to saltwater prior to the bath challenge salinity was increased to 34 ppt over the first 24-hr period. Three challenge model experiments were conducted and are summarized below, further details can be found in (Frisch et al., 2018b); an accompanying paper (Frisch et al., 2018a) describes the pathology of the bath infected fish. Key findings of both papers are described herein.

Atlantic Salmon smolt from Norway were used in the experiments, all challenges were concluded three weeks post exposure. The first challenge experiment showed that disease could be replicated in the lab and that the *T. maritimum* type species strain (NCIMB 2154^{T}) was not as pathogenic as TmarCan15-1, one of the Canadian strains. Disease was mainly seen in fish exposed to the highest concentration for the longest duration (Frisch et al., 2018b).

To determine virulence among isolates, four Canadian isolates (TmarCan16-5, TmarCan16-1, TmarCan16-6 and TmarCan15-1) were tested, each at two different concentrations and exposures. Two isolates (TmarCan16-1 and TmarCAn16-6) had 100% mortality at both concentrations and exposures (Frisch et al., 2018b). Onset of mortality began as early as day 3. In these four challenges which resulted in 100% mortality, all fish were dead a maximum of 13 days post exposure (Frisch et al., 2018b). No mortality was reported for the challenge with TmarCan16-5 (7.3 x 10^{6} cells/mL) at 5 or 7.5 hr exposures. Depending on the concentration and exposure, challenges with TmarCan15-1 resulted in a minimum of 40% mortality and maximum of 70% mortality (Frisch et al., 2018b). No mortality was reported in either control group. The third experiment confirmed similar results to that reported for the second experiment. Overall, isolate TmarCan15-1 produced the most reproducible challenge model (Frisch et al., 2018b). These three studies demonstrate the differences in pathogenicity among isolates and the importance of understanding which strains are present on the farm. For example, TmarCan16-1 caused 100% mortality in a bath concentration as low as 6.36 x10^{5} cells/mL whereas challenges with TmarCan16-2 at concentrations of 1.28 x10^{7} cells/mL could not induce disease at all (Frisch et al., 2018b).

The cohabitation and horizontal transmission experiment was conducted with 40 g Norwegian Atlantic Salmon. Three strains were tested in duplicate, TmarCAn15-1 at 1.68 x10^{7} cells/mL, TmarCan16-5 at 1.78 x 10^{7} cells/mL and TmarCan16-1 at 8.75 x 10^{5} cells/mL at a five-hour exposure. Controls were a marine broth and no exposure. Twenty shedding fish and 40

cohabitating fish were used in each group. The experiment was concluded three weeks after the final exposure. Shedding rates were not reported.

Cumulative percent mortality of shedding fish (range 84-100%) and cohabitation fish (range 27-100%) varied among strains (Frisch et al., 2018b). Depending on the strain, mortality began as early as two days post exposure for shedding fish and six days post exposure for cohabitation fish (Frisch et al., 2018b). Control fish had no clinical signs of disease or mortality. This study demonstrates the horizontal transmission of these strains between Norwegian Atlantic Salmon smolt.

Frisch et al. (2018a) compared the microscopic pathology of Atlantic Salmon smolts bath infected with *T. maritimum* isolates (TmarCan15-1, TmarCan16-1 and TmarCan16-5) as reported in Frisch et al. (2018a) to findings from natural outbreaks. Samples were taken of mouth, gill and skin lesions of diseased fish infected via bath exposure to Canadian isolates (TmarCan15-1, TmarCan16-1, TmarCan16-5). Primary mouth lesions typical of mouthrot were reported in exposed fish (Frisch et al., 2018a). Skin lesions were reported more commonly in the experiment than in natural outbreaks, it was suggested this was an effect of dose and/or handling during the experiment. Another important finding was that *T. maritimum* exposure under these conditions resulted in a systemic infection confirmed by internal detection using real-time RT-PCR and isolation of the bacteria in the kidney.

Through the sequencing of the complete *T. maritimum* genome, Perez-Pascual et al. (2017) have provided some indications of potential virulence mechanisms for the bacteria. In particular, they identified genes likely involved in immune escape, invasion, colonization, destruction of host tissues and nutrient scavenging (Perez-Pascual et al., 2017). Of particular relevance may be that most of the predicted toxins which were identified from the *T. maritimum* isolates used are not found in the genomes of other common pathogenic *Tenacibaculum* species. It should be noted that the study did not include isolates of *T. maritimum* from British Columbia.

Outbreaks

Although incidences of high mortalities or outbreaks of mouthrot are referred to in many original documents (e.g., Hicks, 1989; Ostland et al., 1999); only one published paper, Frelier et al. (1994) could be found which describes an outbreak. Initial increases in mortality attributed to mouthrot have been reported to spike as early as seven to 14 days post seawater entry and again when fish reached approximately 400 g (Ness (2015) in Powell and Podlasly (2015)).

Frelier et al. (1994) report on an outbreak of mouthrot in Puget Sound in 1990 and 1991. The study was conducted in response to reports of high mortality in Atlantic Salmon smolt up to six months post sea water entry on four farms in Puget Sound, Washington. Fish were stocked when they were seven to 12 month old smolts at 50-80 g.

Smolt from three farms: one Atlantic Salmon farm, one Chinook Salmon farm and one Rainbow Trout farm, in Puget Sound were examined for necrotizing stomatitis (mouthrot) in 1990 and 1991. Disease outbreaks occurred from April to July at 8-12^{o}C and 29-32 ppt salinity. Cumulative cage mortality of smolt with characteristic oral lesions during the first six weeks post introduction was between 5-10%, but as high as 30%. Fish were examined from April to August in each of 1990 and 1991.

Disease was not identified in any of the Chinook Salmon (n=241) in either year. In Atlantic Salmon smolt, ulcerative stomatitis was reported in 13.4% of the 627 fish examined in 1990 and 9% of the 670 fish examined in 1991; in Rainbow Trout, it was reported in 11.5% of the 260 fish examined in 1990 and 9.5% of the 26 fish examined in 1991. During 1990 and 1991, the average proportion of mortality attributable to ulcerative stomatitis was 10% when considering

all locations. Frelier et al. (1994) state that this may be an underestimate based on observations of farm workers. The authors recognize that species other than Atlantic Salmon were affected, but they affirm that the data on these species are insufficient to estimate a relative susceptibility by species. The authors could also not attribute any factors responsible for initial bacterial colonization or promotion of bacterial proliferation. *Caprella* sp. was sometimes noted in the oral cavity of smolts, but it was not consistently found. Particular farm practices such as net size/shape or pellet size could also not be attributed to outbreaks.

HEALTH MANAGEMENT

CONTROL AND PREVENTION

As the factors contributing to mouthrot remain largely a mystery, preventing, controlling and treating becomes increasingly difficult, particularly as the causative agent was only recently identified. It is not known what effects different stocking regimes may have on incidence of disease or what environments are best suited for infection and disease proliferation. Many factors have been suggested as contributors to infection and disease such as biofilms and feed type, but we do not know what role such factors may play. Without understanding these and other interactions, prevention is not possible. At most, it is likely that stress plays a role in disease as it does in many other infections and minimizing stress is possible. It has been suggested that there may be benefits to in-feed immune boosting agents (Powell and Podlasly, 2015) although to date, none have been tested.

Vaccines

There are no commercially available vaccines for *T. maritimum* in Atlantic Salmon (Frisch et al., 2018b). Experimental vaccines for several strains of *T. maritimum* have been tested for tenacibaculosis in Atlantic Salmon in Australia (Carson et al., 1993; Carson et al., 1994; van Gelderen et al., 2009) none of which proved highly effective.

Frisch et al. (2018b) undertook studies to induce mouthrot with *T. maritimum* isolates derived from Atlantic Salmon with clinical signs of mouthrot from BC in order to develop a bath challenge model with the goal of testing whole-cell adjuvant vaccines. An experimental vaccine was tested, but under the study conditions did not protect the fish (Frisch et al., 2018b). A vaccine is being tested in Norway and is being tested in BC in farmed Atlantic Salmon; however, efficacy is low (B. Milligan, Cermaq Canada, 203-919 Island Highway, Campbell River, BC, Canada V9W 2C2, pers. comm., 2019).

Studies have also been undertaken for the development of vaccines for other commercially important marine species elsewhere (Salati et al., 2005; Khalil et al., 2018). FM 95, a flexibacteriosis vaccine, was patented in Spain and is currently the only protective vaccine for *T. maritimum* used commercially (Santos et al., 1999; Frisch et al., 2018b). It is a Turbot vaccine and because of the serological diversity of *T. maritimum*, it would not be effective in other species (Toranzo et al., 2005).

Treatment

Different drugs and chemicals for the treatment of *T. maritimum* infections have been tested on various fish species including hydrogen peroxide (H_2O_2), bronopol and stabilized chlorine dioxide, all with varying success.

H_2O_2 has been tested as a potential treatment for Turbot with infections with *T. maritimum* (strain PC424.1) as well as its efficacy as a chemical disinfectant in killing *T. maritimum*

(Avendaño-Herrera et al., 2006b). This is a strain which grows at high temperatures (20°C) (Avendaño-Herrera et al., 2006b). Experimentally infected Turbot were placed in tanks with H_2O_2 at a concentration of either 30 ppm, 240 ppm or untreated for 30 min. There was a 99% reduction in culturable *T. maritimum* in the highest concentration and 83.9% in the lowest (Avendaño-Herrera et al., 2006b). However, both groups of fish had a cumulative mortality of 100% within seven days post challenge (Avendaño-Herrera et al., 2006b). Although a 30 min exposure at 240 ppm may be effective at removing a high proportion of bacteria it appears the stress accelerates mortality (Avendaño-Herrera et al., 2006b).

In the same study, four different concentrations of H_2O_2 (30, 60, 120 and 240 ppm) and three exposures (15 min, 30 min and 24 hr) were tested for bactericidal effect. *Tenacibaculum maritimum* was completely killed at the two highest concentrations in the 15 min exposure (Avendaño-Herrera et al., 2006b). At lower concentrations and the same exposure, the treatment was incompletely effective. No *T. maritimum* cells could be found at any concentration for both the 30 min and 24-hr exposures (Avendaño-Herrera et al., 2006b). After 24 hr, the 240 and 30 ppm concentrations were tested to see if reactivation could occur. Recovery did not occur at 240 ppm; however, at 30 ppm the pathogen recovered to 10^3 CFU/mL (Avendaño-Herrera et al., 2006b).

Bronopol and stabilized chlorine dioxide have been shown to be effective at inhibiting *T. maritimum* and non-lethal to five marine species cultured in Japan (Watanabe and Nishimura, 2010). Their suitability as a mouthrot treatment is not likely as the exposures tested were for several hours and the strains of *T. maritimum* used were derived from Japanese fish.

Interestingly, in vitro studies with various chemotherapeutic agents and bacterial strains from different hosts and geographical regions exhibit a similar pattern of susceptibility; however, field results are not always similar even when using the same drug (Avendaño-Herrera et al., 2006d).

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). Mortalities decrease within two to three days once treatment begins (B. Boyce, MOWI Canada West, 124-1334 Island Highway, Campbell River, BC V9W 8C9, pers. comm., 2019; T. Hewison and P. Whittaker, Grieg Seafood, 1180 Ironwood St., Campbell River, BC V9W 5P7, pers. comm., 2019).

The only antibiotics authorized for use in Canadian aquaculture are oxytracycline hydrochloride (Terramycin-Aqua), trimethoprim and sulphadiazine powder (Tribressen 40% powder), sulfadimethoxine and ormetoprim (Romet 30), and florfenicol (Aquaflor) (Health Canada, 2018). Typically, florfenicol and the potentiated sulfonamides are used to treat mouthrot.

Antibiotic resistance has been demonstrated in various strains of *T. maritimum* tested from Turbot and Senegalese Sole under culture conditions in Spain (Avendaño-Herrera et al., 2005; Avendaño-Herrera et al., 2006d; Avendaño-Herrera et al., 2007).

OCCURRENCE IN BRITISH COLUMBIA

FARMED SALMON

Mouthrot has been reported in farmed Atlantic Salmon in BC through both the Audit Program conducted by DFO (and by BC provincial government before December 2010) and by the industry as Fish Health Events (FHEs) and mortality events. Criteria for diagnoses are provided

in the Diagnostic Methods section. A summary of mouthrot diagnoses in all Fish Health Surveillance Zones is reported below.

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.

Mouthrot has only been diagnosed in farmed Chinook and Atlantic Salmon in British Columbia. All government held data are presented in reference to DFO Fish Health Zones (Figure 1).

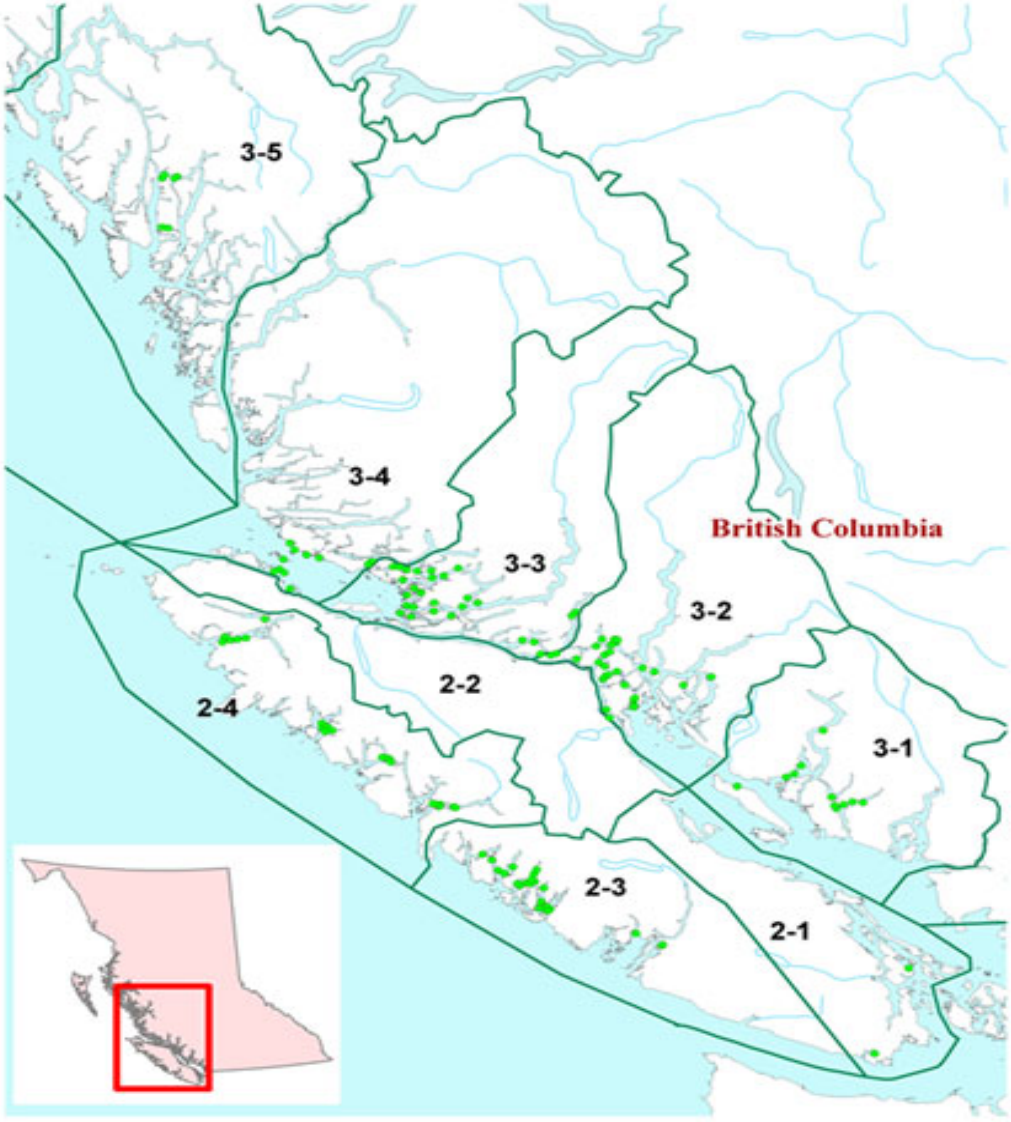

Figure 1. Map of Fisheries and Oceans Canada (DFO) Fish Health Surveillance Zones. Reproduced from Appendix 1-A (iii) Marine Finfish Aquaculture License.

Fish Health Audit and Surveillance Program

The Fish Health Audit and Surveillance Program (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).

Between 2002 and 2018, a total of 1446 audits (average seven audits per month) were conducted on active Atlantic Salmon farms in all Fish Health Surveillance Zones of BC; the fewest number of audits were conducted in December (n=74), the highest number in October (n=155) (Table 2).

Table 2. Total number and monthly average number of audits conducted on Atlantic Salmon farms in British Columbia from 2002-2018. 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	Mean number (range) of audits conducted
January	109	7 (0-15)
February	152	9 (0-15)
March	96	6 (0-14)
April	146	9 (0-19)
May	118	7 (0-14)
June	97	6 (0-12)
July	123	7 (0-16)
August	130	8 (0-14)
September	103	6 (0-13)
October	155	9 (0-19)
November	143	9 (0-13)
December	74	4 (0-8)
Total	**1446**	**7 (0-19)**

Atlantic Salmon

In BC, 106 farm-level mouthrot diagnoses were made during fish health audits conducted between 2002 and 2018. Mouthrot has been diagnosed every year since 2003 and from all Fish Health Surveillance Zones where audits were conducted (Table 3). Mouthrot has been diagnosed in 106/1446 audits (7.3%).

Table 3. Summary of British Columbia provincial government (2002-2010) and Fisheries and Oceans Canada (DFO) - Aquaculture Management Division (2011-2018) audit-based farm-level diagnoses of mouthrot in farmed Atlantic Salmon. 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 indicate no audits.

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}**
2002	0	-	0	0	0	0	0	0	-	0
2003	0	-	1 (1)	1 (1)	0	0	1 (1)	0	-	3 (3)
2004	-	-	2 (2)	0	0	0	1 (1)	1 (1)	0	4 (4)
2005	-	-	0	1 (1)	0	2 (2)	1 (1)	0	0	4 (4)
2006	-	-	0	3 (2)	0	0	2 (2)	1 (1)	0	6 (5)
2007	-	-	1 (1)	1 (1)	0	8 (7)	0	0	1 (1)	11 (10)
2008	-	-	3 (3)	1 (1)	0	3 (3)	2 (2)	3 (2)	0	12 (11)
2009	-	-	3 (3)	0	1 (1)	4 (3)	1 (1)	2 (1)	0	11 (9)
2010	-	-	0	0	0	0	1 (1)	0	-	1 (1)
2011	-	-	1 (1)	3 (3)	0	0	1 (1)	2 (2)	1 (1)	8 (8)
2012	-	-	2 (2)	0	0	0	1 (1)	1 (1)	0	4 (4)
2013	-	-	1 (1)	1 (1)	1 (1)	0	1 (1)	2 (1)	0	6 (5)
2014	-	-	0	1 (1)	1 (1)	1 (1)	1 (1)	2 (2)	0	6 (6)
2015	-	-	1 (1)	2 (2)	0	1 (1)	3 (3)	0	0	7 (7)
2016	-	-	3 (3)	3 (3)	0	0	0	4 (2)	0	10 (8)
2017	-	-	1 (1)	0	0	0	1 (1)	1 (1)	0	3 (3)
2018	-	-	2 (2)	4 (4)	0	1 (1)	1 (1)	1 (1)	1 (1)	10 (10)
$\Sigma_{subzone}$	**0**	**-**	**21 (12)**	**21 (14)**	**3 (2)**	**20 (13)**	**18 (9)**	**20 (7)**	**3 (3)**	**106**

Pacific salmon

Mouthrot has not been diagnosed at the farm-level in Pacific salmon in BC through the FHASP between 2002 and 2018.

However, in 2009, jaw plaques consistent with mouthrot were identified in three small (15-20 g) Chinook Salmon from a farm in Discovery Passage (Fish Health Zone 3.2, Figure 1) (H. Manchester, DFO, pers. comm. 2019). The audit took place in July 2009 and given the small size of the fish, it is likely they were transferred to sea in June of the same year. The lab report describes the lesions as “stomatitis, ulcerative with abundant filamentous bacteria”; mouth plaques were noted for each fish. No internal organ lesions were noted. The farm-level diagnosis for this audit was bacterial kidney disease (BKD), however, mouthrot was present. Mouthrot in Chinook Salmon is not typically seen in audits (H. Manchester, DFO, pers. comm. 2019).

It is important to note that these same microscopic findings contributing to a diagnosis of mouthrot in Chinook Salmon (i.e., stomatitis, ulcerative with abundant filamentous bacteria) would be described for Atlantic Salmon smolts.

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 that is associated with that occurrence or event” in the Marine Finfish Aquaculture License under the Fisheries Act (DFO, 2015).

FHE reporting 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 license 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).

Atlantic Salmon

Between 2002 and 2018, there were a total of 537 FHEs attributed to mouthrot reported on Atlantic Salmon farms in BC (Table 4). All Fish Health Surveillance zones and subzones with FHE disease data (zones 2.3, 2.4, 3.1 to 3.5) have reported FHEs attributed to mouthrot, with most being reported in zone 2.4 (130 of 537).

Table 4. Summary of Fish Health Events (FHEs) (2002-2018) attributed to mouthrot in farmed Atlantic Salmon in British Columbia reported by industry. Dashes indicate no requirement to report FHEs. Values in parentheses are the numbers of unique farms on which a FHE was reported. Sources: Fisheries and Oceans Canada (DFO) - Aquaculture Management Division and from the Open Canada website as of June 6th, 2019.

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}**
2002	0	0	1 (1)	0	0	1 (1)	1 (1)	0	0	3 (3)
2003	0	0	14 (6)	7 (3)	0	1 (1)	3 (2)	1 (1)	0	26 (13)
2004	0	0	12 (5)	3 (2)	0	5 (4)	1 (1)	13 (6)	3 (2)	37 (20)
2005	0	0	14 (8)	8 (3)	0	26 (7)	1(1)	3 (2)	6 (1)	58 (22)
2006	0	0	13 (5)	5 (3)	0	11 (5)	3 (3)	20 (6)	0	52 (22)
2007	0	0	2 (1)	18 (3)	2 (1)	29 (9)	2 (1)	0	1 (1)	54 (16)
2008	0	0	12 (7)	24 (3)	1 (1)	3 (3)	2 (1)	3 (2)	0	45 (17)
2009	0	0	4 (3)	9 (6)	4 (2)	15 (6)	1 (1)	1 (1)	0	34 (19)
2010	0	0	6 (6)	8 (4)	3 (2)	17 (5)	10 (6)	8 (2)	1 (1)	53 (26)
2011	0	0	7 (6)	20 (4)	3 (1)	7 (4)	5 (3)	23 (6)	1 (1)	66 (25)
2012	0	0	5(5)	12 (2)	3 (1)	0	9 (3)	9 (3)	0	38(14)
2013	-	-	-	-	-	-	-	-	-	-
2014	-	-	-	-	-	-	-	-	-	-
2015	-	-	-	-	-	-	-	-	-	-
2016	0	0	5 (5)	8 (8)	0	0	9 (9)	2 (2)	2 (2)	26 (26)
2017	0	0	5 (5)	2 (2)	3 (2)	3 (3)	3 (3)	3 (3)	1 (1)	20 (19)
2018	0	0	4 (4)	6 (6)	0	1 (1)	9 (9)	3 (3)	2 (2)	25 (25)
$\Sigma_{subzone}$	**0**	**0**	**104 (17)**	**130 (14)**	**19 (4)**	**119 (20)**	**59 (18)**	**89 (8)**	**17 (4)**	**537**

Pacific salmon

Between 2002-2018, excluding the years 2013-2015, two FHEs (2002 and 2009) attributed to mouthrot were reported in farmed Chinook Salmon in BC, both in Fish Health Zone 3.1 (BCSFA, 2010; DFO, 2019a).

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 facility inventory, within a 24 hour period; or (b) fish mortalities equivalent to 10,000kg or more, or losses reaching 5%, 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, a total of four mortality events have been attributed to mouthrot on Atlantic Salmon farms in BC (DFO, 2019b). Two of them occurred on one farm in Zone 2.3, once in 2014 and once in 2016, and the other two occurred on a farm in Zone 3.3, both in 2018. Although attributed to infectious diseases, factors other than diseases, such as water quality, could also have contributed to these mortality events.

KNOWLEDGE GAPS

Because of its commercial importance, much effort has been spent to study the pathology, genetics, transmission pathways, etc. of strains of *T. maritimum* causing tenacibaculosis. Even with such efforts there remains many basic knowledge gaps. Although we can learn from these studies, it clear that *T. maritimum* infections causing mouthrot do not exhibit the same pathology as tenacibaculosis in other species and regions. More work is required in order to understand the differences in *T. maritimum* strains in this region which cause mouthrot. Knowledge gaps most important to the assessment of risk are:

- Transmission pathways
- Natural reservoirs and vectors
- Interactions between strains of *T. maritimum* causing mouthrot and other infectious agents
- Virulence mechanisms
- Susceptibility of Atlantic Salmon to different strains of *T. maritimum* causing mouthrot
- Susceptibility of Pacific salmon species and other Pacific marine species to strains of *T. maritimum* causing mouthrot
- Strain pathogenicity and geographic distribution
- Environmental and biological factors contributing to infection and outbreaks in Atlantic Salmon

SUMMARY

The mouthrot literature is not abundant, much of the basic epidemiology of the disease is unknown. It was not until 2018 that the causal agent was confirmed and Koch's postulates fulfilled. As comprehensive as these studies are, much work remains to be done to understand strain pathogenicity, geographic distribution, reservoirs, transmission pathways and contributing factors. It is a complex disease for which we know little.

As there is abundant literature on some of these aspects of *T. maritimum* globally, it is possible to utilize this information to assess the risk of transfer from farmed Atlantic Salmon to Fraser River Sockeye Salmon. However, there are important differences between strains of *T. maritimum* causing mouthrot and those which cause tenacibaculosis which should be considered including: different clinical signs; lower culture temperatures for mouthrot strains of *T. maritimum*; there are significant antigenic differences among BC isolates and between BC isolates and *T. maritimum* reference strains; and differences in the genetics, antibody response and pathology of BC strains of *T. maritimum* compared to other strains which result in tenacibaculosis.

It is likely that environmental factors such as temperature and salinity affect the incidence of mouthrot, specific ranges are unknown; however, outbreaks have occurred in Puget Sound when water temperatures were between 8 and 12°C and 29-32 ppt salinity. The temperature range for *T. maritimum* is reported as between 15-35°C and the optimal temperature of 30°C.

Recently it has been shown that *T. maritimum* isolates from fish with clinical mouthrot are more closely related to a Norwegian Lumpfish strain (NLF-15, reared at 12°C) and a Chilean Atlantic Salmon strain (Ch-2402, reared at 14°C). The Canadian isolates were from fish reared at temperatures ranging from 8.7-14.7°C.

Tenacibaculum maritimum causing mouthrot has been demonstrated to transfer horizontally between fish in a cohabitation experiment. Bath challenges with several BC isolates have been able to reproduce disease in Atlantic Salmon smolt resembling that of mouthrot.

Between 2002 and 2018, 106 farm-level mouthrot diagnoses were made as a result of fish health audits conducted on Atlantic Salmon farms in BC. From 2002 to 2013 and 2015-2018, 537 Fish Health Events attributed to mouthrot were reported on Atlantic Salmon farms in BC.

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