Fisheries and Oceans Canada | Pêches et Océans Canada
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Canadian Science Advisory Secretariat (CSAS)

Research Document 2019/016

National Capital Region

Characterization of *Aeromonas salmonicida* and furunculosis to inform pathogen transfer risk assessments in British Columbia

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October 2019

Canada

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.

Published by:

Fisheries and Oceans Canada
Canadian Science Advisory Secretariat
200 Kent Street
Ottawa ON K1A 0E6

http://www.dfo-mpo.gc.ca/csas-sccs/
csas-sccs@dfo-mpo.gc.ca

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ISSN 1919-5044

Correct citation for this publication:

Boily, F., Malcolm, G. and Johnson, S.C. 2019. Characterization of *Aeromonas salmonicida* and furunculosis to inform pathogen transfer risk assessments in British Columbia. DFO Can. Sci. Advis. Sec. Res. Doc. 2019/016. vi + 39 p.

Aussi disponible en français :

Boily, F., Malcolm, G. et Johnson, S.C. 2019. Caractérisation de la bactérie Aeromonas salmonicida et de la furonculose pour informer les évaluations des risques de transfert d'agents pathogènes en Colombie-Britannique. Secr. can. de consult. sci. du MPO. Doc. de rech. 2019/016. vi + 41 p.

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ABSTRACT

Aeromonas salmonicida is a gram negative bacterial pathogen known to cause disease in wild and cultured fish from freshwater, brackish and seawater. Due to its almost worldwide distribution and its economic importance to salmonid farming and conservation, *A. salmonicida* has been the focus of over 100 years of research. The subspecies *A. salmonicida salmonicida*, referred to as "typical *A. salmonicida"*, is associated with acute to chronic septicemic disease called "furunculosis" in salmonids. Other *A. salmonicida* subspecies are referred to as "atypical *A. salmonicida*" and may cause ulcerative and systemic diseases in a wide range of fish taxa, including salmonids. Typical and atypical strains of *A. salmonicida* are endemic in British Columbia (BC). Pathogen transmission is horizontal and all salmonid life stages are susceptible to infection. In endemic areas, furunculosis often develops following stress but covert and subclinical infections may occur in fish which become reservoirs (carriers) of the pathogen. Experimental studies demonstrated that infected, diseased and dead Atlantic Salmon (*Salmo salar*) shed large numbers of *A. salmonicida* cells. Although shedding by carriers is known to occur, patterns of shedding and the number of cells shed have not been determined. Survival of *A. salmonicida* in the marine environment could range between 2 and 26 days (median = 6 days) for temperature and/or salinity conditions occurring in the Discovery Islands, based on published studies using culture methods. Changes in virulence and infectivity of *A. salmonicida* cells in the environment are unknown, but based on published starvation studies, cells entering a "dormant" or non culturable but viable state (NCBV) have failed to induce infection and disease when injected into Atlantic Salmon. The relative susceptibility of Atlantic and Pacific salmon species is unknown but interspecific and intraspecific variations in resistance to furunculosis have been documented. No minimum infectious and lethal doses were found in the literature; these parameters would likely vary with factors related to the host, the pathogen and the environment. As *A. salmonicida* is endemic to BC, all three companies operating Atlantic Salmon farms in the Discovery Islands area vaccinate all their fish against furunculosis prior to seawater transfer, and implement disease surveillance and biosecurity measures at all stages of the production cycle to reduce the risks of disease.

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. Canada has the potential to double its aquaculture production over the next ten years (Noakes, 2018). The development of an aquaculture science risk assessment framework was a commitment under the 2008 Sustainable Aquaculture Program (SAP). This framework is a formalized approach to the provision of risk-based advice that is consistent with activities currently undertaken by Aquaculture Science. The risk framework builds upon the work initiated with the scientific peer-review validation of the Pathways of Effects for Finfish and Shellfish Aquaculture in 2009 through the Canadian Science Advisory Secretariat (CSAS). The Aquaculture Pathways of Effects describes the potential effects of aquaculture activities on the environment and identifies stressors. These stressors include: alteration in physical structure of habitat, alteration of light, increased noise, release of chemicals, release or removal of nutrients, non-cultured organisms and other organic material, release or removal of fish, and release of pathogens (DFO, 2010).

In partial response to the outcome of the Cohen Commission (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 followed by a synthesis. Pathogens to be assessed were determined from farm audit data collected by DFO and Fish Health Events (FHEs) reported by the industry. For an environmental risk assessment to be considered for a particular pathogen, there must be evidence that the pathogen has caused disease on Atlantic Salmon farms in the Discovery Islands area and Sockeye Salmon are likely susceptible to the pathogen, as well as there must be potential for spatial and temporal overlap between the pathogen released from Atlantic Salmon farms and migrating Fraser River Sockeye Salmon (FRSS).

The first risk assessment was completed for the Infectious Hematopoietic Necrosis Virus (IHNV) and was scientifically peer-reviewed in December 2017. Four bacterial pathogens known to cause systemic diseases on Atlantic Salmon farms in the Discovery Islands, namely *Aeromonas salmonicida*, *Piscirickettsia salmonis*, *Renibacterium salmoninarum* and *Yersinia ruckeri*, are the next pathogens to undergo risk assessments. This paper synthesizes the relevant information on *A. salmonicida*, the causal agent of furunculosis, for the purpose of the risk assessment.

PURPOSE OF THE DOCUMENT

This document summarizes the relevant information on the bacterial fish pathogen *Aeromonas salmonicida*, the causative agent of furunculosis, including its epidemiology and the current health management practices in British Columbia (BC). This information informs and supports the assessment of the risk of *A. salmonicida* transfer from farmed Atlantic Salmon to wild Fraser River Sockeye Salmon in the Discovery Islands, BC (Mimeault et al., 2019).

PATHOGEN CHARACTERIZATION

DESCRIPTION OF *AEROMONAS SALMONICIDA* AND GENETIC STRAINS

Aeromonas salmonicida is a gram-negative, non-spore-forming, non-motile, fermentative, cytochrome oxidase positive, rod bacterium (Bernoth, 1997; Cipriano and Austin, 2011; Austin and Austin, 2016). *Aeromonas salmonicida* is presently assigned to five subspecies: *salmonicida*, *smithia*, *achromogenes*, *masoucida* and *pectinolytica* (Dallaire-Dufresne et al.,

2014; Austin and Austin, 2016; Menanteau-Ledouble et al., 2016). Morphological and biochemical characteristics have been used to distinguish between evolutionarily close subspecies (Austin and Austin, 2016). The subspecies *salmonicida* is commonly referred to as "typical *A. salmonicida"*, whereas other subspecies and isolates deviating phenotypically from the typical form are commonly referred to as "atypical *A. salmonicida"* (Hiney and Olivier, 1999; Reith et al., 2008; Austin and Austin, 2016). Although the production of a brown water-soluble pigment when grown on tryptone soy agar (TSA) has been considered a major diagnostic feature associated with typical *A. salmonicida,* some variations occur in pigment production, motility, and other phenotypic characteristics, many of which being related to growth conditions (Hiney and Olivier, 1999; Austin and Austin, 2016).

Unlike typical *A. salmonicida*, which is generally considered to be a phenotypically homogeneous group, larger variations exist amongst the atypical subspecies with respect to their biochemistry, growth and virulence (Bernoth et al., 1997; Wiklund and Dalsgaard, 1998; Cipriano and Austin, 2011; Austin and Austin, 2016). Atypical *A. salmonicida* strains deviate from the classical description of the taxon in a number of biochemical, physiological and genetic properties. Reasons commonly used to describe an isolate as "atypical" include: lack or altered pigment production, catalase and oxidase-negativity, nutritional fastidiousness, slow growth, and isolation from non-salmonid hosts (Austin and Austin, 2016).

A standardized system of classification of *Aeromonas* species and subspecies needs to be developed to ensure the proper assignment of isolates of *Aeromonas* to the various species and subspecies (Colston et al., 2014). The genetic diversity within the *Aeromonas* genus and relationships between species and sub-species are being reassessed using whole genome sequencing (Colston et al., 2014). This approach is being used to improve our understanding of mechanisms driving phenotypic variability such as differences in virulence and antibiotic resistance (see Rasch et al., 2007; Schwenteit et al., 2011) and to discover and facilitate the assignment of new isolates to species and subspecies (see Rouleau et al., 2018).

Genetic Strains

The most striking feature of typical *A. salmonicida* strains infecting salmonids is their homology (Dalsgaard et al., 1994; Hiney and Olivier, 1999). There are large numbers of *A. salmonicida* subsp. *salmonicida* strains (also called "substrains") identified worldwide, but traditional typing methods based on biochemical and antigenic characteristics of typical isolates do not show enough variation to be used as epidemiological markers (Dalsgaard et al., 1994). The various techniques that have been used (more or less successfully) to differentiate between *A. salmonicida salmonicida* substrains are described in Hiney and Olivier (1999). More recently, Bartkova et al. (2017) demonstrated that whole genome sequencing could successfully be used to study the epidemiology, evolution and genetic variation responsible for differences in virulence phenotypes among the homogeneous *A. salmonicida salmonicida* population in Denmark, and Attéré et al. (2015) found with genotyping analyses that 15 of 27 (56%) of the European isolates harboured differences in their small plasmid repertoire, compared to six of the 126 Canadian isolates (5%). Plasmids found in *A. salmonicida* are responsible for differences in antibiotic resistance and virulence phenotypes and these may be transferred from other *Aeromonas* spp. such as *A. bestiarum* (Vincent et al., 2014; Tanaka et al., 2016; Trudel et al., 2016). In British Columbia, the genetic diversity of *A. salmonicida* isolates is unknown but is under study as part of project undertaken by the Marine Environmental Research Program (MERP) to support diagnostic test evaluation and epidemiological studies (Siah, 2018).

DISEASE

Furunculosis is a septicemic bacterial disease found principally in salmonids and is caused by infection with *A. salmonicida*. Although the "typical" form of furunculosis is attributed to *A.*

salmonicida salmonicida, many older scientific studies did not characterize isolates below the species level and may include some “atypical” strains (Hiney and Olivier, 1999; Austin and Austin, 2016). We included these historical studies on *A. salmonicida* conducted on salmonids to cover the range of pathogen strains and characteristics. Furunculosis is named after the characteristic “furuncle” or boil-like lesions that develop in chronically infected individuals.

Clinical Signs

In salmonids, infection with *A. salmonicida* can result in peracute (very severe disease of short duration often without apparent clinical signs), acute (rapid onset and/or a short course) or chronic (persistent or long-lasting) disease (Hiney and Olivier, 1999; Cipriano and Austin, 2011; Menanteau-Ledouble et al., 2016). In addition, covert infections can occur, in which asymptomatic carriers can serve as sources of infection. Furuncle-like lesions are limited to the chronic disease state. See Menanteau-Ledouble et al. (2016) for a review of the clinical and histopathological signs associated with various strains *A. salmonicida* in salmonids and non-salmonids.

Peracute Disease

The peracute form of furunculosis has been most commonly reported in early life history stages (fry and fingerlings in freshwater) (Brocklebank, 1998). In this form of the disease, affected fish show no clinical signs other that rapid death and occasional slight exophthalmia and darker colouration (McCarthy and Roberts, 1980; Hiney and Olivier, 1999; Cipriano and Austin, 2011; Oidtmann et al., 2013; Austin and Austin, 2016).

Acute Disease

Acute furunculosis is usually reported in parr, smolts and salmonids during their first year in sea cages. Acute disease appears as generalized bacterial septicemia characterized by external hemorrhagic lesions at the base of the fins and oral cavity, dark colouration, lack of appetite, erratic swimming, lethargy and high mortalities, with death occurring within two to three days (Brocklebank, 1998; Hiney and Olivier, 1999; Cipriano and Austin, 2011; Oidtmann et al., 2013; Austin and Austin, 2016). The rapid onset and short course of the disease makes development of furuncles in acute disease uncommon (McCarthy and Roberts, 1980; Hiney and Olivier, 1999).

Chronic Disease

Chronic disease is typically reported in older fish (subadults and adults) that have become more refractive to the disease or in more resistant species, such as Rainbow Trout (*O. mykiss*) (Brocklebank, 1998; Hiney and Olivier, 1999; Cipriano and Austin, 2011). Chronically diseased fish are lethargic, anorexic and show slight darkening of the skin, slight exophthalmia, congested blood vessels at the base of fins, bloody discharge from the nares and in many instances the development of characteristic furuncles which are dark raised tumefactions containing serosanguinous fluid and necrotic tissues in the musculature (Hiney and Olivier, 1999; Cipriano and Austin, 2011; Oidtmann et al., 2013; Austin and Austin, 2016). Upon rupture, furuncles leave open deep ulcers and release large numbers of bacteria to the environment, thereby contributing to the spread of the infection (Hiney and Olivier, 1999; Cipriano and Austin, 2011; Oidtmann et al., 2013). Mortality rates in chronically infected fish can be low and diseased fish may recover and become carriers (Oidtmann et al., 2013).

Covert Infections/Carrier States

Covert infections with typical *A. salmonicida* are clinically unapparent and can persist in fish populations until stress induces clinical furunculosis or can remain silent in carrier fish (Hiney and Olivier, 1999). Carrier states may be established in fish that survived infection (McCarthy

and Roberts, 1980; Austin and Austin, 2016). They can also result when fish already infected with *A. salmonicida* are vaccinated against this bacterium (Hiney, 1995).

Covertly infected fish (carriers) shed the bacteria and are able to transmit furunculosis to susceptible fish in cohabitation experiments (Hiney and Olivier, 1999; Cipriano and Austin, 2011; Austin and Austin, 2016). Conditions under which shedding occurs and the frequency and duration of shedding events are not known. Covertly infected fish can carry *A. salmonicida* externally (i.e., mucus, gills), within the gut or within internal organs (McCarthy, 1977; Hiney et al., 1994; Cipriano et al., 1996; Hiney and Olivier, 1999; Austin and Austin, 2016). Stress related to poor environmental conditions such as high or changing water temperatures, low dissolved oxygen, poor handling and crowding can trigger the development of furunculosis in carriers (Mackie et al., 1935; McCarthy, 1977; Hiney and Olivier, 1999; Bruno et al., 2013).

Macroscopic Lesions

At necropsy, acutely infected fish may show punctate hemorrhage on the heart, and in visceral and parietal walls of the body cavity and reproductive organs (Hiney and Olivier, 1999; Cipriano and Austin, 2011). The spleen and kidney may be enlarged and friable, and the liver may be pale and show sub-capsular hemorrhage and necrosis, and severe congestion of the intestine can also be present (Scott, 1968; Hiney and Olivier, 1999; Cipriano and Austin, 2011; Austin and Austin, 2016).

Chronically infected fish may show abdominal effusion, splenomegaly, kidney necrosis, hemorrhage in the liver, intestines, pyloric caeca and gills, general visceral congestion and peritonitis, and liquefactive hemorrhagic lesions (furuncles) under the skin and in the skeletal musculature. Rupture of furuncles results in large and deep ulcerative lesions (McCarthy and Roberts, 1980; Brocklebank, 1998; Hiney and Olivier, 1999; Cipriano and Austin, 2011; Oidtmann et al., 2013; Austin and Austin, 2016).

Microscopic Lesions

Histopathological lesions vary with disease course. In peracute and early stages of furunculosis, microcolonies of *A. salmonicida* can be found in several organs such as kidney, spleen, heart, muscle and gills, with limited or no tissue reaction, although necrosis may become extensive in late and chronic stages of the disease (McCarthy and Roberts, 1980; Oidtmann et al., 2013).

Sublethal Effects of *A. salmonicida* Infection

Documented sublethal effects due to furunculosis are scarce. No publications were found specifically on the effects of chronic furunculosis. However, some insight can be drawn in the course of subclinical and clinical phases of acute experimental infections. For instance, Yi et al. (2016) reported that infection with *A. salmonicida* can significantly reduce the swimming performance in half kilogram Atlantic Salmon, and can affect some blood chemistry parameters. These authors observed that at day 4 post infection (intramuscular = i.m. injection), the mean critical swimming speed was 22% lower in experimentally infected fish, compared to the control group (i.m. injected with saline). This difference increased until death of the infected fish at 6 dpi. A similar pattern was observed for exhaustion time (Yi et al., 2016).

Clinical pathology can inform about the physiological state of infected fish. Yi et al. (2016) found significant differences in the blood chemistry between infected and uninfected fish. These observations are consistent with altered homeostasis, including decreased oxygen carrying capacity and swimming performance in infected and diseased fish. In Atlantic Salmon experimentally infected with *A. salmonicida*, Ellis et al. (2007) observed a surge in cortisol release into the water several days prior to the initiation of mortalities. Such cortisol release

likely results from the elevated blood cortisol levels associated with stress in acutely diseased fish (Ellis et al., 2007).

GEOGRAPHIC RANGE

Aeromonas salmonicida is found worldwide. Typical *A. salmonicida* has been reported on all continents, with the exception of Australia, New Zealand and South America where only atypical forms have been reported (Bernoth et al., 1997; Stone et al., 1997; Hiney and Olivier, 1999; Bravo, 2000; Godoy et al., 2010; Georgiades et al., 2016).

Aeromonas salmonicida is endemic in Canada and furunculosis is an annually notifiable disease to the Canadian Food Inspection Agency (CFIA). Laboratories contact the CFIA upon suspicion or diagnosis of furunculosis or *A. salmonicida*. Refer to the Annually Notifiable Diseases on CFIA's webpage for more details.

HOSTS

Aeromonas salmonicida has been reported to infect salmonid and non-salmonid fish species living in freshwater, brackish water and seawater (Evelyn, 1971; Wiklund and Dalsgaard, 1998; Hiney and Olivier, 1999; Diamanka et al., 2013; Coscelli et al., 2014; Long et al., 2016; Menanteau-Ledouble et al., 2016).

Host species in Canada with suspected or diagnosed cases of furunculosis are listed in Table 1. These are from reports to the CFIA made between 2013 and 2017, and are summarized by province. Sample details such as fish size, source (wild or farmed), environment (fresh or salt water), and *A. salmonicida* strains or subspecies involved, are not available. Of the 21 identified fish taxa, seven were non-salmonid species (Table 1). Of those, most diagnosed or suspected furunculosis cases are from BC salmonids (154 of 295).

*Table 1. Number of diagnoses or suspected cases of furunculosis (*Aeromonas salmonicida*) received by the Canadian Food Inspection Agency (CFIA) between 2013 and 2017 by province and host species. An additional notification for Salmo salar has also been reported for which the province is unknown. Source: CFIA, January 2018.*

Species	Scientific name	British Columbia	Manitoba	Ontario	Quebec	New Brunswick	Nova Scotia	Prince Edward Island	Yukon
Wolf-eel	*Anarrhichthys ocellatus*	1							
American Eel	*Anguilla rostrata*					1	1		
Sablefish	*Anoplopoma fimbria*	13							
White Sucker	*Catostomus commersoni*			1					
Atlantic Cod	*Gadus morhua*					1			
Atlantic Halibut	*Hippoglossus hippoglossus*							1	
Smallmouth Bass	*Microptera dolomieu*					1			
Cutthroat Trout	*Oncorhynchus clarkii*	1							
Pink Salmon	*Oncorhynchus gorbuscha*	1							
Chum Salmon	*Oncorhynchus keta*	1							
Coho Salmon	*Oncorhynchus kisutch*	13		4					
Rainbow Trout	*Oncorhynchus mykiss*	14		13	7				
Sockeye Salmon	*Oncorhynchus nerka*	6							
Pacific salmon	*Oncorhynchus spp.*	1							
Chinook Salmon	*Oncorhynchus tshawytscha*	3		17					
Atlantic Salmon	*Salmo salar*	112			16	2			
Brown Trout	*Salmo trutta*				1				
Arctic Char	*Salvelinus alpinus*	2	1		1	1			3
Brook Trout	*Salvelinus fontinalis*			5	43	1			
Lake Trout	*Salvelinus namaycush*			2					
Lake Trout x Brook Trout	*Salvelinus namaycush x Salvelinus fontinalis*			4					
Unspecified					3				

Salmonids

All salmonid species are considered susceptible to *A. salmonicida* infection and furunculosis (Kent, 2011). For the purposes of this document, we define susceptibility as the risk of becoming infected, regardless of whether infection causes disease under a given situation.

There are interspecific differences in susceptibility of salmonids to furunculosis (Cipriano and Heartwell, 1986; Pérez et al., 1996; Hiney and Olivier, 1999). Rainbow Trout (*Oncorhynchus mykiss*) are generally considered to be most resistant, whereas other species of trout and

Atlantic Salmon are considered more susceptible based on mortality rates following challenge (McCarthy, 1977; Cipriano and Heartwell, 1986; Bernoth et al., 1997).

All species of Pacific salmon in freshwater can become infected with *A. salmonicida* (Nikl et al., 1991; Beacham and Evelyn, 1992a; Beacham and Evelyn, 1992b; Nikl et al., 1993). However, there are no studies comparing the relative susceptibility of Pacific salmon species. McCarthy (1983) reported high (82-100%) mortality rates in all five Pacific salmon species following bath challenge with a virulent strain of typical *A. salmonicida* whereas Rainbow Trout exposed to a higher concentration of the same strain experienced lower (60%) mortality rates.

Infection with *A. salmonicida* and the development of disease can occur in all life stages of fish. Age differences in susceptibility to furunculosis have been reported, but results are inconsistent (Mackie et al., 1935; McCarthy and Roberts, 1980; Bakke and Harris, 1998; Hiney and Olivier, 1999; Roberts, 2012). There are insufficient data to assign different risks to different life history stages of Pacific salmon.

Atypical furunculosis has been reported in several fish species including salmon species in freshwater (Coho Salmon *Oncorhynchus kisutch*, Pink Salmon *Oncorhynchus gorbuscha*), in seawater (Chum Salmon *Oncorhynchus keta*, Sockeye Salmon), or in both freshwater and seawater (Atlantic Salmon) (Evelyn, 1971; Olivier, 1992; Bernoth, 1997; Hiney and Olivier, 1999).

Non-salmonids

Globally, *A. salmonicida* has been isolated from a growing number of freshwater and marine non-salmonid hosts (Wiklund and Dalsgaard, 1998; Diamanka et al., 2013; Coscelli et al., 2014; Long et al., 2016; Menanteau-Ledouble et al., 2016). In the Northeast Pacific, furunculosis caused by typical or atypical *A. salmonicida* has been diagnosed or suspected in Rocky Mountain Whitefish (*Prosopium williamsoni*) (Duff, 1932), Dolly Varden (*Salvelinus malma*) (Duff, 1932), Sablefish (*Anoplopoma fimbria*) (Evelyn, 1971), Pacific Herring (*Clupea pallasi*) (Evelyn, 1971; Traxler and Bell, 1988), Lingcod (*Ophiodon elongatus*) (Kent et al., 1998), Eulachon (*Thaleichthys pacificus*) found in the net pens with infected salmon (Novotny, 1975), and Wolf Eel (*Anarrhichthys ocellatus*) (Table 1). Given the taxonomically broad non-salmonid host range reported in other areas, it is likely that there are other non-salmonid hosts present in BC that are susceptible to *A. salmonicida* which have not been identified.

OCCURENCE OF *A. SALMONICIDA* AND FURUNCULOSIS IN BC

WILD SALMONIDS

Freshwater

Furunculosis is an enzootic disease in BC; natural outbreaks were first reported in wild Rocky Mountain Whitefish (*Prosopium williamsoni*), Dolly Varden (*Salvelinus malma*) and Cutthroat Trout (*Onchorhynchus clarki*) in freshwater in the early 1930's (Duff, 1932). Since then, infection with *A. salmonicida* and furunculosis has been commonly reported in Pacific salmon from hatcheries and adults prior to spawning (Hoskins and Hulstein, 1977; Stephen et al., 2011). While *A. salmonicida* is enzootic, the prevalence of infection is variable, as recent studies have not detected *A. salmonicida* in hatchery reared smolts from Chilliwack, Middle Shuswap, Spius Creek (Tucker et al., 2018) or from Cowichan hatchery (Thakur et al., 2018).

Coastal waters and upstream migration

Prevalence of infection with *A. salmonicida* in wild Pacific salmon has most often been determined in returning adults in freshwater, by which time they have experienced a number of stressors including the adaptation to freshwater, physiological changes associated with sexual maturation and crowding (Stoddard, 1993; MacDiarmid, 1994). Infections observed in adults in spawning condition may have been acquired in freshwater as juveniles and carried to sea, or in seawater, or on re-entering freshwater to spawn (Evelyn, 1971).

Prevalence of *A. salmonicida* from Pacific salmon collected at or near spawning grounds using non-random sampling designs from 1972 to 1993 suggest that the prevalence of *A. salmonicida* in adult Pacific salmon in spawning condition is relatively low (6% overall, 1.1 - 1.5% for Sockeye Salmon) (see Stoddard, 1993; MacDiarmid, 1994).

The prevalence of *A. salmonicida*/furunculosis in Pacific salmon in estuarine and marine environments from various studies, using a range of sampling and detection methods, is summarized in Table 2. Data from pathogen surveys should be interpreted with caution given that *A. salmonicida* is difficult to isolate from healthy carriers and that culture based methods likely underestimate the prevalence of infection in wild fish. In addition, non-culture based methods may detect the pathogen or its components but do not provide information about the viability and pathogenicity of the infectious agent.

Table 2. Summary of available information about the prevalence of Aeromonas salmonicida *in wild salmon caught in coastal waters and early upstream migration. ND = not determined; O = Offshore; N = near marine net pens; FW = freshwater; †variable number of fish per sample – the number of fish screened for* Aeromonas salmonicida *would range between 1740 to 2380 for Chinook Salmon, and from 300 to 620 for Coho Salmon; * the positive sample (i.e., 1 of 7 fish) was from a juvenile Chinook Salmon near net pens; ** pathogen screening included* Aeromonas salmonicida *but only prevalence greater than 1% were reported in the study.*

Reference	Description	Species	Life stage	N	Prevalence (%)
Arkoosh et al. (2004)	Chinook and Coho salmon sampled from estuaries in Washington and Oregon States, USA between 1996 and 2001. *A. salmonicida* was detected by culture methods and confirmatory immunological assays.	Chinook Salmon	Sub-yearling	Variable†	0 to 5
		Coho Salmon	Juveniles	Variable†	0 to 2
Kent et al. (1998)	Survey of salmonid pathogens in wild fish (includes 48 taxa) captured near (<0.5 km) marine net pens or offshore in the coastal waters of BC away (>1 km) from net pens. *A. salmonicida* was detected by culture. Note: sampling dates unknown. It is not known whether these data are different from those in MacDiarmid (1994).	Chinook Salmon	ND*	7 (N)	14.3*
		Chum Salmon	ND	300 (O)	0
		Sockeye Salmon	ND	333 (O)	0
		Pink Salmon	ND	10 (O)	0
Miller et al. (2014)	Sockeye Salmon post-smolts collected from a trawl survey within Queen Charlotte Sound, BC. *A. salmonicida* screened by qRT-PCR.	Sockeye Salmon	Post-smolts	86	0

Reference	Description	Species	Life stage	N	Prevalence (%)
Miller et al. (2017)	Juvenile Sockeye Salmon sampled from spring through summer in 2013 from Central Coast BC (n=133) and Salish Sea (n=211) and Chinook Salmon (n=1666) sampled from 2008 to 2012 from Southern BC. *A. salmonicida* screened on the (q)PCR Fluidigm BioMark™ HT platform.	Sockeye Salmon	Juveniles	344	0 to 1**
		Chinook Salmon	Juveniles	1666	0 to 1**
MacDiarmid (1994)	Wild Sockeye Salmon and Chum Salmon sampled in the coastal environment (DFO, unpublished data). *A. salmonicida* by culture. Sampling dates unknown.	Sockeye Salmon	Before FW entry	300	0
		Chum Salmon	Before FW entry	300	0
Stone et al. (1997)	Surveys of wild Sockeye Salmon spawners at an early stage of sexual maturation in the Fraser River, in 1993 and 1995. (T. Evelyn, pers. comm. 1996; DFO, unpublished data)	Sockeye Salmon	Adults	502 (in 1993) 345 (in 1995)	2.2 (in 1993) 0.3 (in 1995)
Thakur et al. (2018)	Samples of juvenile Cowichan River Chinook Salmon near Cowichan Bay. *A. salmonicida* screened by qPCR assay.	Chinook Salmon	Juvenile	431	0
Tucker et al. (2018)	Juvenile Chinook Salmon sampled spring to winter from 2008-2012 in the Strait of Georgia. *A. salmonicida* screened by qRT-PCR.	Chinook Salmon	Juvenile	561	0 to 1**

ATLANTIC SALMON FARMS IN BC

Based on reporting of Fish Health Events (FHEs), results from the Department of Fisheries and Oceans (DFO) Fish Health Audit and Surveillance Program and the Province of British Columbia fish health audits (for the period prior to January 2011) *Aeromonas salmonicida* and/or furunculosis has been detected on Atlantic Salmon farms in BC.

In BC, outbreaks of furunculosis are now uncommon in farmed Atlantic Salmon, due to improved fish health management practices in hatcheries and marine production sites, improved diagnostic methods and the availability of effective vaccines.

Fish Health Events

A Fish Health Events (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).

Reporting FHEs began in the fall of 2002, but was not required in 2013 until the last quarter of 2015 (Wade, 2017). Between 2002 and 2017, a total of 61 FHEs attributed to furunculosis were reported on Atlantic Salmon farms in BC (Table 3). Most (32.8%) of these were reported in Fish Health Surveillance Zone 2.3 (Southwest Vancouver Island). Nine (14.8%) were reported in Zone 3.2 (Discovery Islands) the most recent in 2017; none were reported on Hardwicke, Althorpe or Shaw Point farms (in Zone 3.3).

*Table 3. Summary of fish health events (FHEs) (2002-2017-Q1) attributed to furunculosis in seawater-reared Atlantic Salmon in British Columbia. Dashes indicate no requirements to report FHEs. Numbers in parentheses represent the total number of individual farms where there were reported FHEs. Source: data provided by DFO Aquaculture Management Division, 2018. *indicates FHEs with reported non-pigmenting or atypical* Aeromonas salmonicida*.*

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						2 (1)	1 (1)		2 (1)	5 (3)
2003			1 (1)	4 (3)	2 (1)	3 (2)	1 (1)	1 (1)		12 (9)
2004			2 (2)	4 (1)					2 (2)	8 (5)
2005					2 (1)		1*(1)			3 (2)
2006							1 (1)			1 (1)
2007			1 (1)	1 (1)			1 (1)			3 (3)
2008			4 (4)				1*(1)			5 (5)
2009			6 (5)			1 (1)				7 (6)
2010			5 (3)	4 (2)		2 (2)				11 (7)
2011								1 (1)		1 (1)
2012							1 (1)			1 (1)
2013	-	-	-	-	-	-	-	-	-	-
2014	-	-	-	-	-	-	-	-	-	-
2015	-	-	-	-	-	-	-	-	-	-
2016			1 (1)							1 (1)
2017					1 (1)	1*(1)	1 (1)			3 (3)
$\Sigma_{subzone}$	**0**	**0**	**20 (13)**	**13 (6)**	**5 (1)**	**9 (6)**	**8 (6)**	**2 (1)**	**4 (2)**	**61**

DFO's Fish Health Audit Program

The Fish Health Audit Program is conducted by DFO's BC Aquaculture Regulatory Program (BCARP) and is a continuation of the BC provincial audit program that DFO assumed regulatory authority of in January 2011. In each quarter, DFO audits the routine monitoring and reporting of up to 30 farms (Wade, 2017). During these audits samples of recently dead fish (fresh silvers) are taken for diagnostic testing as described in Wade (2017). Between 2002 and 2016 a total of 1229 audits were conducted on Atlantic Salmon farms across all Fish Health Zones in BC, representing an average of 7 audits per month (range: 0 to19) (Jones, 2019). Over this period the month with the highest total number of audits was February (129), and the month with the least number of audits was December (69) (Jones, 2019).

Through the audits, DFO veterinarians can diagnose farm-level infections of furunculosis 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.

Farm-level diagnoses of furunculosis on BC Atlantic Salmon farms were made in five of 15 years (2002 and 2016). There were a total of 11 farm-level diagnoses, none of which occurred in the Discovery Islands (described in more details below) (Table 4). Approximately 36% of these occurred in Zone 2.3 (Southwest Vancouver Island).

Table 4. Summary of BC Provincial (2002-2010) and DFO-Aquaculture Management Directorate (AMD) audit-based (2011-2016) farm-level diagnoses of furunculosis in seawater-reared Atlantic Salmon in British Columbia. Source: data provided by the BC provincial government, 2010, and DFO Aquaculture Management Division, 2018. NA: 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}**
2002										0
2003			2 (1)	2 (2)			1 (1)	2 (2)		7 (6)
2004										0
2005							1 (1)			1 (1)
2006										0
2007										0
2008										0
2009			1 (1)							1 (1)
2010			1 (1)							1 (1)
2011										0
2012										0
2013				1 (1)						1 (1)
2014										0
2015										0
2016										0
$\Sigma_{subzone}$	**0**	**NA**	**4 (3)**	**3 (3)**	**0**	**0**	**2 (1)**	**2 (2)**	**0**	**11**

DIAGNOSTIC METHODS AND CASE DEFINITION

DIAGNOSTIC METHODS

Presumptive diagnosis of infection with *A. salmonicida* can be made on the basis of the clinical signs and disease course, gross lesions, the fish species and life stage involved and the clinical history of the aquaculture facility (Cipriano and Austin, 2011).

Definitive diagnosis of furunculosis is based on isolation and identification of *A. salmonicida* using culture-based methods and nonculture methods, including serodiagnostics (i.e., Enzyme-Linked Immunosorbent Assay (ELISA), agglutination tests, direct or indirect fluorescent antibody techniques (FAT, iFAT)), molecular techniques (DNA and PCR assays), and Denaturing Gradient Gel Electrophoresis (DGGE) which can be used for the non-lethal detection of the bacterium from mucus in fish, and histopathological lesions (Austin and Austin, 2016). *Aeromonas salmonicida* is easily isolated and cultured from skin lesions, mucus, blood and kidney of diseased fish (Cipriano et al., 1992; Cipriano and Austin, 2011; Roberts, 2012). However, bacterial culture is not considered effective for the isolation of *A. salmonicida* from unstressed asymptomatic carriers (Hiney et al., 1994).

Although the traditional description of (typical) *A. salmonicida* is a non-motile, gram-negative rod, producing a brown water-soluble pigment on tryptone containing agar, does not grow at 37^{o}C, and produces catalase and oxidase (Austin and Austin, 2016); exceptions do occur. For example, the production of the brown diffusible water-soluble pigment by *A. salmonicida salmonicida* may be delayed up to ten days under suboptimal culture conditions (Roberts, 2012; Austin and Austin, 2016), and may be inhibited by the presence of other bacteria (Hiney and Olivier, 1999). In addition, non-pigmented variants may arise when the pathogen is maintained in culture for extended periods (Evelyn, 1971; Austin and Austin, 2016), and oxidase negative

isolates have been reported (Hiney et al., 1994). Atypical subspecies such as *A. salmonicida achromogenes* may also produce pigment which is regulated by quorum sensing (Schwenteit et al., 2011). Given the above, identification of typical *A. salmonicida* should not rely too heavily on pigment production (Austin and Austin, 2016; Gudmundsdottir and Bjornsdottir, 2017). However, growth of atypical strains tends to be more fastidious, requiring agar plates which are supplemented with blood, and colonies are non or slow-pigmenting (Austin and Austin, 2016).

Diagnostic Methods used by DFO's Fish Health Audit Program

DFO's audit program utilizes the Animal Health Centre (AHC) in Abbotsford, BC, for diagnostic testing support. The AHC is accredited by the American Association of Veterinary Laboratory Diagnosticians (AAVLD). Diagnostic procedures on each sampled fish include gross examination and histopathology by light microscopy of gills, heart, liver, spleen, head kidney, trunk kidney, pyloric caeca, exocrine pancreas, mesenteric adipose tissue and brain. With respect to bacterial pathogens, DFO's audit program conducts routine bacterial culture on blood agar, tryptone soy agar (TSA) and TSA plates with salt before sending the isolates to the AHC, when deemed necessary for identification (Erin Zabek, Animal Health Centre, BC Ministry of Agriculture, 1767 Angus Campbell Road, Abbotsford, BC, V3G 2M3, pers. comm., 2018). The target tissue for bacteriology is the kidney, but lesions may also be plated. Plates with bacterial growth are subcultured and single colonies submitted for identification using biochemical and molecular tests, if required (Erin Zabek, Animal Health Centre, pers. comm., 2018).

No diagnostic test is 100% specific and sensitive; in some cases in BC, identification of *A. salmonicida* isolates based on culture/biochemical methods differed from molecular tests results. This has led the BC aquatic animal health community to conclude that the diagnostic tests currently used in BC are not 100% specific for *A. salmonicida*. Depending on the diagnostic test method, other subspecies of *A. salmonicida* (atypical forms), as well as other species of *Aeromonas* such as *A. bestiarum*, have been identified in farmed Atlantic Salmon in BC. There is an ongoing program to accurately identify and genetically characterize pathogenic strains of *Aeromonas* in fish present in BC waters (Siah, 2018). The goal of this program is to develop validated diagnostic tools that will identify and distinguish between the different species and/or the genetic types/subspecies of *A. salmonicida* present in BC fish.

DFO's Case Definition of farm-level furunculosis

During an audit, DFO's veterinarians will diagnose furunculosis in an Atlantic Salmon population when the site is under treatment for the disease or when sampled fish show septicaemia with characteristic histologic lesions, with isolation or identification of the causative bacterium from tissues, and population level losses to the disease (I. Keith, DFO, 103-2435 Mansfield Drive, Courtenay, BC V9N 2M2, pers. comm., 2018).

EPIDEMIOLOGY

TRANSMISSION

Routes of Transmission and Infection

Horizontal transmission is the main mode of infection (Roberts, 2012). Susceptible fish can become infected through contact with: infected fish, contaminated water and/or contaminated equipment (McCarthy and Roberts, 1980; Roberts, 2012). Horizontal transmission of *A. salmonicida* is known to occur in brackish and seawater (Scott, 1968; Evelyn, 1971; Novotny, 1978; Smith et al., 1982). On marine salmon farms, increased contact through stock movement and inter-year class transfers, and high stocking densities have all been factors that contributed

to the spread of *A. salmonicida* in the past (Bruno, 1986; Ogut et al., 2004). Smith et al. (1982) also reported successful hydrodynamic transmission of *A. salmonicida* between marine cages on Atlantic Salmon farms in Ireland. However, fish health management practices and regulations, as described below, have evolved to limit the risk of transfer of *A. salmonicida* from hatcheries to sea cages and within and between farms.

The precise routes by which *A. salmonicida* enters and disseminates within hosts are not fully understood (Bernoth et al., 1997; Austin and Austin, 2016). It is possible that routes could differ within species between resistant and non-resistant hosts, as well as between different species of hosts (McCarthy, 1977; Bartkova, 2016). Entry can occur through the gills and epidermal mucus layer (Bruno, 1986; Svendsen and Bogwald, 1997; Ferguson et al., 1998; Bartkova, 2016), fins (Bartkova, 2016), gastro-intestinal tract (McCarthy, 1977; Ringo et al., 2004; Jutfelt et al., 2006) and wounds/abrasions (McCarthy, 1977; McCarthy, 1983; Svendsen and Bogwald, 1997; Roberts, 2012).

Although *A. salmonicida* is known to occur within the ovaries and testes of infected fish, there is no evidence for true vertical transmission (intra-ovum) of this bacterium (Austin and Austin, 2016). Egg surfaces from infected individuals may be contaminated, but there is no evidence that such eggs survive or that *A. salmonicida* persists throughout incubation (McCarthy, 1977; Austin and Austin, 2016), although contamination can occur at any stage from the water. Biosecurity practices, including egg disinfection and the use of incoming water from a fish free water source, control pathogens in hatcheries (DFO, 2016).

Bacterial Shedding

Aeromonas salmonicida is shed from fish at most stages of infection. Fish with clinical furunculosis (with or without furuncles) shed cells via their urine, faeces and from ruptured furuncles. Furuncles in the musculature of Atlantic Salmon can contain from 10^8 to 10^{10} colony-forming units (cfu) or cells of *A. salmonicida* mL^{-1} of necrotic tissue, and, upon rupture, release these cells into the environment (McCarthy, 1977; Rose et al., 1989). Bacterial shedding also occurs before the onset of clinical signs from asymptomatic carriers (Hiney and Olivier, 1999), from clinically infected fish (Rose et al., 1989; Ögüt, 2001) and from dead hosts (McCarthy, 1977; Rose et al., 1989). The few laboratory studies that have examined shedding of *A. salmonicida* are described below and summarized in Table 5.

In Rainbow Trout infected experimentally with *A. salmonicida* through i.m. injections, McCarthy (1977) found that 10^3 viable *A. salmonicida* cells mL^{-1} were shed into the tank water on the fourth day post injection (dpi), when the majority of infected fish died. To determine the viability of *A. salmonicida* in dead fish, McCarthy (1977) removed all surviving fish from the tank and monitored shedding from dead fish. *Aeromonas salmonicida* was isolated from tissues of dead fish up to 32 dpi and viable cells were still present in the water for a further eight days despite an open flow, perhaps due to contamination of the tank sides from which viable cells were gradually released (McCarthy, 1977).

Rose et al. (1989) examined the infectivity of *A. salmonicida* in Atlantic Salmon by bath challenges (10^5 cfu mL^{-1}) and subsequently determined shedding rates from infected and from recently dead fish. Median shedding rates for recently dead Atlantic Salmon were 4.1×10^4 cfu $fish^{-1}$ h^{-1} in freshwater, and 1.7×10^6 cfu $fish^{-1}$ h^{-1} in seawater (Table 5). Shedding could be demonstrated two days prior to onset of mortalities (sea water bath exposure) at which time 200 to 3,800 cfu mL^{-1} were found in the water. None of bath challenged fish had developed furuncles. To assess shedding rates from fish with furuncles, fish were challenged by i.m. injection. Median shedding rates for small live fish were 1.3×10^7 cfu $fish^{-1}$ h^{-1}, and 5.4×10^7 cfu $fish^{-1}$ h^{-1} for large fish at day of death (Table 5).

Exposure to infected cohabitants is considered the best method to mimic natural infections. In a cohabitation study, seven donor Atlantic Salmon smolts intraperitoneally (= i.p.) infected with 1.3 x 10^3 cells $fish^{-1}$ were housed with cohabitant naïve Atlantic Salmon smolts in 400-litre freshwater tanks with a water exchange rate of 1 to 1.5 volumes per hour (Enger et al., 1992). *Aeromonas salmonicida* was detected in the water before the donor fish began to die. The highest concentrations (10^5 cells mL^{-1}) were reported at the onset of donor fish mortalities (4 dpi) at the surface microlayer. While the number of *A. salmonicida* in this layer decreased with time, it remained above the surface microlayer detection limit of 10^3 cells mL^{-1} through to the end of the 15-day trial. *Aeromonas salmonicida* was first detected in samples from 10 cm in the water column (detection limit 12 cells mL^{-1}) at two days post infection (<10^2 cells mL^{-1}) increasing to approximately 10^3 cells mL^{-1} at day 5 and rapidly declining thereafter. The bacterium was not detected after the last donor fish had died (eight days). These authors reported that the cohabitants did not seem to shed bacteria to the same extent as the donor fish (i.p. infected) since *A. salmonicida* was not detected in the water column throughout the period when cohabitants were dying (9 to 15 days). This suggests that the infection route may have an effect on patterns of shedding.

Pérez et al. (1996) challenged Rainbow Trout with bath concentrations of *A. salmonicida* ranging from 10^4 to 10^8 cfu mL^{-1} for a 12-hour period in freshwater. None of the fish challenged with 10^7 cfu mL^{-1} or less died. However, 4/8 and 8/8 of the fish were found positive, following carrier testing for *A. salmonicida* at 29 dpi in the 10^6 and 10^7 cfu mL^{-1} exposures, respectively. Infected survivors were estimated to shed an average of 3.5 X 10^4 to 10^5 cfu $fish^{-1}$ h^{-1} after a stress test with an i.m. injection of dexamethasone. Dead fish from the 10^8 cfu mL^{-1} dose were estimated to shed an average of 10^5 cfu $fish^{-1}$ h^{-1} (Table 5). The duration over which bacterial shedding occurred was not determined.

Table 5. Aeromonas salmonicida *shedding rates from experimentally infected Atlantic Salmon and Rainbow Trout. dpc: days post challenge, dpi: days post infection, nd: not determined*

Species mean size (g)	n	Salinity	Exposure Route (concentration)	Shedding rates (cfu $fish^{-1}$ h^{-1})		Notes (References)
				Median/ Average	Range/ SD	
Atlantic Salmon 25.8 g	6	SW	Bath 12 hr/day (10^5 cfu/mL)	$1.7x10^6$ (median)	$1.7x10^5$ - $1.1x10^7$	From freshly dead fish (Rose et al., 1989)
Atlantic Salmon 23.3 g	4	SW	Intramuscular injection (10^3 cfu)	$1.3x10^7$ (median)	$5.7x10^5$ - $2.1x10^7$	From live fish, at 5 dpi (one day before death) (Rose et al., 1989)
Atlantic Salmon 1200 g	2	SW	Intramuscular injection (10^5 cfu)	$5.4x10^7$ (median)	$9.0x10^6$ - $6.4x10^8$	From freshly dead fish (5 & 13 dpi) (Rose et al., 1989)
Atlantic Salmon 6.9 g	3	FW	Bath 12hr/day (10^5 cfu/mL)	$4.1x10^4$ (median)	$1.7x10^4$ - $7.0x10^4$	From freshly dead fish (Rose et al., 1989)
Rainbow Trout nd	nd	FW	Intramuscular injection (3.4×10^4 cfu)	nd	nd	Dead fish (McCarthy, 1977)
Rainbow Trout 25 g	8	FW	12 hr Bath (10^6 cfu/mL)	$3.5x10^4$ (average)	SD = $1.8x10^4$	Survivors of infection (29 dpc) following injection of dexamethazone (Pérez et al., 1996)
Rainbow Trout 25 g	4	FW	12 hr Bath (10^7 cfu/mL)	10^5 (average)	SD = $8x10^4$	Survivors of infection (29 dpc) following injection of dexamethazone (Pérez et al., 1996)
Rainbow Trout 25 g	8	FW	12 hr Bath (10^8 cfu/mL)	10^5 (average)	SD = $2.3x10^4$	Dead Fish (29 dpc) (Pérez et al., 1996)

VIRULENCE AND PATHOGENICITY

Experimental infections

Strains of *A. salmonicida* can differ markedly in their virulence. For example, McCarthy (1983) bath challenged Coho Salmon with very high doses (1/100 dilution of broth culture) of four strains of *A. salmonicida.* Mortalities were 100, 68, 36 and 0% for strains AS-1, AS-4, AS-3, and AS-5, respectively. Sakai (1985) determined the lethal dose 50% (LD_{50}) of four wild strains of *A. salmonicida*, originally isolated from fish with furunculosis, to Sockeye Salmon and Rainbow Trout by injection. The LD_{50}'s reported as the i.p. dose (number of viable cells per fish) ranged from 2.4×10^4 to $>10^8$. It is also well known that within strains, differences in virulence seen in laboratory challenge trials can arise due to the techniques used to maintain and grow *A. salmonicida*.

In BC, the strains of *A. salmonicida* have not yet been fully characterized, but are the subject of ongoing research (Siah, 2018).

For the purposes of this section we define “infectious dose” as the amount of pathogen required to cause infection under environmental conditions favourable to the host and “minimum lethal dose” as the smallest number of cells required to establish an infection that results in disease and death of any individual within a population.

No published records were found on the infectious dose or minimum lethal dose of *A. salmonicida* specific to Sockeye Salmon. However, data abound from challenge studies conducted to develop standardize challenge conditions for the testing of therapeutants and vaccines. Fish have been exposed under laboratory conditions to various infectious doses (rarely the minimum) using a variety of exposure routes: injections (intraperitoneal, intramuscular), intra-gastric administration and bath challenges, and cohabitation with infected donor fish. These studies have used a variety of strains of *A. salmonicida*, most frequently, strains known to be highly virulent.

Culture conditions such as stocking density, water exchange rates, temperature, dissolved oxygen, etc. will affect the infective dose of *A. salmonicida* fish are exposed to and ultimately whether fish become infected and the severity of disease. The effects of stocking density on the dynamics of furunculosis in Chinook Salmon fry has been examined using laboratory cohabitation challenges and modelling (Ogut et al., 2004; Ogut et al., 2005; Ogut and Reno, 2005; Ogut and Bishop, 2007). These authors demonstrated: that the transmission coefficient and the disease-specific mortality of furunculosis is dependent on host density with the highest furunculosis-specific mortality rates at the highest densities, that different host densities result in different patterns of disease with acute disease developing at high densities and chronic disease at low densities. Ogut et al. (2004) reported a relationship between host density and survival following cohabitation challenge. At the lowest stocking density (0.05 g L^{-1} or kg m^{-3}) there was no detectible transmission of *A. salmonicida* over a 33 day period. At slightly higher stocking densities, cumulative mortalities were still low, ranging from 1 to 3 % at 0.15 kg m^{-3} to 1 to 6 % at 0.32 kg m^{-3} over a 23 day period Ogut et al. (2004).

The results from laboratory challenge trials conducted in fresh and saltwater which used bath challenge or intra-gastric administration are summarized in Table 6. Data from one cohabitation challenge in which bacterial levels in the water were determined during the challenge is also included (Ogut and Reno, 2005).

Table 6. Summary of bath, intra-gastric and cohabitation Aeromonas salmonicida *laboratory challenges. Different virulent strains of* Aeromonas salmonicida *and host species were used in these challenges.* [A] *Carriers were identified by carrier testing,* [B] *indicates values representing percent morbidity,* [C] *mortalities had ceased for 7 days prior to end of challenge,* [D] LD_{50} *for this isolate in Chinook Salmon,* [E] *dose of* Aeromonas salmonicida *at the start of the cohabitation* [F] *infection of exposed cohabitants could occur from direct and indirect contacts nd: not determined, NS: not specified unnamed strain.*

Bath Challenges

Species (weight (g), sample size)	**Salinity (duration of exposure)**	***A. salmonicida* concentration (strain)**	**Time post-infection to first mortality (challenge duration)**	**Cumulative Mortality % (% of survivors carrying *A. salmonicida*)[A]**	**Reference**
Atlantic Salmon (75-115 g, n=10)	SW (daily 12 h exposure for 7 days)	10^2 cfu mL^{-1} (423)	--- (17 days)	0% (0/10: 0%)	(Rose et al., 1989)
Atlantic Salmon (75-115 g, n=10)	SW (daily 12 h exposure for 21 days)	10^2 cfu mL^{-1} (423)	24 (31 days)	20% (0/8: 0%)	(Rose et al., 1989)
Atlantic Salmon (20-32 g, n=10)	SW (1 day)	$3x10^4$ cfu mL^{-1} (423)	9 (19 days)	90% (0/1: 0%)	(Rose et al., 1989)
Atlantic Salmon (20-32 g, n=10)	SW (2 days)	$3x10^4$ cfu mL^{-1} (423)	--- (19 days)	0% (0/10: 0%)	(Rose et al., 1989)
Atlantic Salmon (20-32 g, n=10)	SW (3 days)	$3x10^4$ cfu mL^{-1} (423)	8 (19 days)	70% (0/3: 0%)	(Rose et al., 1989)
Atlantic Salmon (20-32 g, n=10)	SW (1 day)	$3x10^5$ cfu mL^{-1} (423)	12 (19 days)	80% (0/2: 0%)	(Rose et al., 1989)
Atlantic Salmon (20 -32 g, n=10)	SW (2 days)	$3x10^5$ cfu mL^{-1} (423)	9 (19 days)	70% (0/3: 0%)	(Rose et al., 1989)
Atlantic Salmon (20-32 g, n=10)	SW (3 days)	$3x10^5$ cfu mL^{-1} (423)	8 (19 days)	50% (0/5: 0%)	(Rose et al., 1989)
Atlantic Salmon (20-32 g, n=7)	FW (1 day)	$3x10^5$ cfu mL^{-1} (423)	7 (19 days)	60% (not tested)	(Rose et al., 1989)
Atlantic Salmon (ND, n=80)	FW (30 min)	10^6 cfu mL^{-1} (A449)	nd (66 days)	60%[B] (nd: some survivors infected)	(Dacanay et al., 2006)
Atlantic Salmon (120 g, n=80)	FW (40 min)	10^6 cfu mL^{-1} (A449)	nd (66 days)	44%[B] (nd: some survivors infected)	(Dacanay et al., 2010)
Rainbow Trout (25 g, n=8)	FW (12 h)	10^4 and 10^5 cfu mL^{-1} (AI_{130B})	--- (19 days)	0% (0/8: 0%)	(Pérez et al., 1996)

Species (weight (g), sample size)	Salinity (duration of exposure)	*A. salmonicida* concentration (strain)	Time post-infection to first mortality (challenge duration)	Cumulative Mortality % (% of survivors carrying *A. salmonicida*)[A]	Reference
Rainbow Trout (25 g, n=8)	FW (12 h)	10^6 cfu mL^{-1} (AI_{130B})	--- (19 days)	0% (4/8: 50%)	(Pérez et al., 1996)
Rainbow Trout (25 g, n=8)	FW (12 h)	10^7 cfu mL^{-1} (AI_{130B})	--- (19 days)	0% (8/8: 100%)	(Pérez et al., 1996)
Rainbow Trout (25 g, n=8)	FW (12 h)	10^8 cfu mL^{-1} (AI_{130B})	nd (19 days)	100%	(Pérez et al., 1996)
Rainbow Trout (33 g, n=8)	FW (1 h)	$6x10^6$ cfu mL^{-1} (NS)	4 (28 days)	70%	(Villumsen and Raida, 2013)
Rainbow Trout (2.7g, n=30)	FW (60 min)	$2x10^7$ cfu mL^{-1} (AS-1)	nd (14 days)	60%	(McCarthy, 1983)
Sockeye Salmon (5.7 g, n=21)	FW (20 min)	$8x10^4$ cfu mL^{-1} (AS-1)	nd (14 days)	95%	(McCarthy, 1983)
Sockeye Salmon (5.7 g, n=21)	FW (20 min)	$1x10^4$ cfu mL^{-1} (AS-1)	nd (14 days)	76%	(McCarthy, 1983)
Sockeye Salmon (5.7 g, n=22)	FW (20 min)	$5x10^5$ cfu mL^{-1} (AS-1)	nd (14 days)	50%	(McCarthy, 1983)
Sockeye Salmon (0.6 g, n=30)	FW (60 min)	$7x10^4$ cfu/mL (AS-1)	nd (14 days)	85%	(McCarthy, 1983)
Pink Salmon (1.2 g, n=30)	FW (60 min)	$7x10^4$ cfu mL^{-1} (AS-1)	nd (14 days)	82%	(McCarthy, 1983)
Coho Salmon (9 g, n=30)	FW (60 min)	$1x10^4$ cfu mL^{-1} (AS-1)	nd (14 days)	86%	(McCarthy, 1983)
Chum Salmon (1 g, n=30)	FW (60 min)	$7x10^4$ cfu mL^{-1} (AS-1)	nd (14 days)	100%	(McCarthy, 1983)
Chinook Salmon (2.7 g, n=30)	FW (60 min)	$7x10^4$ cfu mL^{-1} (AS-1)	nd (14 days)	90%	(McCarthy, 1983)
Chinook Salmon (5.9 g, n= 150)	FW (15 min)	$1.6x10^4$ cells mL^{-1} (76-30)	3 and 5 (11 and 13 days)	11.3% and 47.3% (two replicates)	(Beacham and Evelyn, 1992b)
Chinook Salmon (6.3 g, n=150)	FW (15 min)	$1.6x10^4$ cells mL^{-1} (76-30)	3 and 5 (11 and 13 days)	49.2% and 57.4% (two replicates)	(Beacham and Evelyn, 1992b)
Chinook Salmon (6.9 g, n=150)	FW (15 min)	$1.6x10^4$ cells mL^{-1} (76-30)	3 and 5 (11 and 13 days)	76.7% and 80.5% (two replicates)	(Beacham and Evelyn, 1992b)
Chinook Salmon (<20 g, n=900)	FW (15 min)	$4.8x10^3$ cells mL^{-1} (76-30)	approx. 1-2 days (78 days)	30%	(Beacham and Evelyn, 1992a)
Coho Salmon (<20 g, n=840)	FW (15 min)	$3.4x10^5$ cells mL^{-1} (76-30)	approx. 3 days	50%	(Beacham and Evelyn, 1992a)

Species (weight (g), sample size)	Salinity (duration of exposure)	*A. salmonicida* concentration (strain)	Time post-infection to first mortality (challenge duration)	Cumulative Mortality % (% of survivors carrying *A. salmonicida*)[A]	Reference
			(18 days)		
Chum Salmon (<20 g, n=900)	FW (15 min)	$3.4x10^4$ cells mL^{-1} (76-30)	6 days (19 days)	80%	(Beacham and Evelyn, 1992a)
Coho Salmon (10.8 g, n=50)	FW (15 min)	$4.6x10^5$ cfu mL^{-1} (76-30)	nd (20 days)	96%	(Nikl et al., 1991)
Chinook Salmon (4 g, n=50)	FW (15 min)	$6.8x10^4$ cfu mL^{-1} (76-30)	nd (21 days)	17%[C]	(Nikl et al., 1993)
Chinook Salmon (6.5 g, n=50)	FW (15 min)	$2.5x10^5$ cfu mL^{-1} (76-30)	nd (18 days)	39%[C] and 70%[C] (two replicates)	(Nikl et al., 1993)
Chinook Salmon (30-35 g, n=30)	FW (15 min)	$3.6x10^6$ cfu mL^{-1} [D] (NS)	4 days (30 days)	46.7 ± 13.3% (ND: survivors not infected)	(Roon et al., 2015)

Intra-gastric Challenges

Species (weight (g), sample size)	Salinity (duration of exposure)	*A. salmonicida* concentration (strain)	Time post-infection to first mortality (challenge duration)	Cumulative Mortality % (% of survivors carrying *A. salmonicida*)[A]	Reference
Atlantic Salmon (70-115 g, n=8 per dose)	SW	10^1 to 10^4 cfu mL^{-1} (423)	--- (14 days)	0% (0/8 per dose: 0%)	(Rose et al., 1989)
Atlantic Salmon (70-115 g, n=8)	SW	10^5 cfu mL^{-1} (423)	--- (14 days)	0% (4/8: 50%)	(Rose et al., 1989)
Atlantic Salmon (70-115 g, n=8)	SW	10^6 cfu mL^{-1} (423)	--- (14 days)	0% (0/8: 0%)	(Rose et al., 1989)
Atlantic Salmon (70-115 g, n=8)	SW	10^7 cfu mL^{-1} (423)	nd (14 days)	12.5% (3/7: 43%)	(Rose et al., 1989)
Rainbow Trout (150 g, n=8 per dose)	FW	10^4 , 10^5, orto 10^7 cfu mL^{-1} (AI_{130B})	--- (19 days)	0 % (0/8 per dose: 0%)	(Pérez et al., 1996)

Cohabitation Challenge

Species (weight (g), sample size)	Salinity (duration of exposure)	*A. salmonicida* concentration (strain)	Time post-infection to first mortality (challenge duration)	Cumulative Mortality % (% of survivors carrying *A. salmonicida*)[A]	Reference
Chinook Salmon (2 g, n= approx. 2924)	FW	Single donor 3 dpe ca. 10^2 -10^4 cfu mL^{-1} [E, F] (NS)	5 (10 days)	100%	(Ogut and Reno, 2005)

There is a high level of variability in mortality rates within and between comparable bath challenges with *A. salmonicida*. Further, it is difficult to extrapolate laboratory challenge results to potential outcomes in the field due to the inherent variability of natural systems, ranging from the strain of fish and pathogen, prior exposure, the effects of handling, fish rearing and environmental conditions at the farm, and differences between wild and cultured fish.

Based on freshwater challenges of Sockeye Salmon the minimum lethal dose based on a short bath exposure (≤ 20 min) is $<10^4$ cfu mL^{-1} under laboratory conditions (McCarthy, 1983). Twenty minute bath exposure to higher doses resulted in > 50% mortality.

Twelve hour exposures (conducted daily over a seven days) of 75 to 115 g Atlantic Salmon to 10^2 cfu mL^{-1} *A. salmonicida* in seawater failed to cause mortalities(Rose et al. 1989). In contrast, exposure of two gram Chinook Salmon to a dose of 10^2 cfu mL^{-1} by cohabitation resulted in 100% mortality over a ten-day period (Ogut and Reno, 2005).

Oral-fecal transfer may play a role in the transmission of *A. salmonicida*. However, intra-gastric administration of ≤10^6 cfu mL^{-1} *A. salmonicida* to Atlantic Salmon failed to cause mortalities, but resulted in relatively high proportions of the exposed fish becoming carriers (Rose et al., 1989).

Outbreaks in the Marine Environment

In marine farms

Outbreaks of furunculosis have been described for both Atlantic Salmon and Pacific salmon marine farms, in particular before the introduction of efficient injectable vaccines in the 1990's. In marine Atlantic Salmon farms, Smith et al. (1982) documented furunculosis outbreaks in smolts with latent infection (carriers) originating from a hatchery with a history of furunculosis outbreaks and in smolts originating from a hatchery without furunculosis. Within two days after sea entry (spring), smolts known to be carriers of *A. salmonicida* from their hatchery developed overt furunculosis, with approximately 40-50% mortality in ten days (i.e., between 2-12 days post sea water transfer) and a cumulative mortality of approximately 70% by the end of the summer. Mortalities in adjacent marine cages stocked with smolts from the furunculosis-free hatchery began 20 days after exposure to the diseased stock. Initial mortalities were relatively low at first (0.1 to 0.2% per day) but reached 3-4% per day by 23 days after the first mortalities occurred, with cumulative mortalities of about 70% by the end of the summer (Smith et al., 1982). Smith et al. (1982) did not mention any treatments or other measures undertaken at the farm to manage furunculosis. The duration of the outbreaks cannot be determined, but a duration of several months is likely in presumably unvaccinated and untreated Atlantic Salmon smolts. A second marine farm supplied with smolts from the same hatcheries reported covert infections and ~40% mortalities within 20 days post sea water transfer. Fish on this farm were eradicated. The farm was re-stocked with furunculosis-free smolts and an outbreak of

furunculosis occurred the following month but with relatively low mortality (~5%) (time period not specified). Since this outbreak occurred six months after the removal of the infected smolts cohort, Smith et al. (1982) postulated the source of this outbreak may have been previously uninfected smolts that became carriers in the vicinity of the furunculosis-affected smolts before their removal from the farm, feral fish, or contaminated sediments.

In a Pacific salmon marine farm located in Puget Sound (Washington State, USA), Novotny (1978) documented epizootics of vibriosis and furunculosis in Chinook Salmon. Chinook Salmon (average 5.4 g) were directly transferred from freshwater to seawater in June. Epizootics of vibriosis and furunculosis appeared in July with mortality rates generally ≥0.1% per day. Despite periodic antimicrobial treatments and decreasing water temperatures in the fall, furunculosis cases were recurrent, and by November, the cumulative mortality had reached 80% most of which was attributable to furunculosis (Novotny, 1978). It is not mentioned whether furunculosis persisted after November. Novotny (1978) indicated that, by February, the total cumulative mortality of experimental Chinook Salmon had reached 93.5%, of which 3.2 to 15.7% could be attributable to vibriosis alone based on other Chinook Salmon stocks where only vibriosis outbreaks occurred. Therefore, this would indicate a cumulative mortality of 77.8 to 90.3% for combined “baseline and furunculosis” mortality. Novotny (1978) attributed the failure to control diseases in those young Chinook Salmon to environmental stress of low dissolved oxygen, presence of furunculosis carriers, high loading densities, and osmotic stress in juvenile Chinook Salmon that were too small for seawater transfer.

In wild Pacific salmon

No epizootics of furunculosis have been reported in wild Pacific salmon from the marine environment. No studies were found on chronic and sublethal effects of *A. salmonicida* infection and disease in wild Pacific salmon.

SURVIVAL IN THE ENVIRONMENT

Studies have shown that *Aeromonas salmonicida* can survive for some time outside of its host in fresh, brackish and marine waters and sediments; however, there is little evidence to support growth outside of hosts (reviewed in Hiney et al., 2002). The ability of *A. salmonicida* to persist in the aquatic environment is important with respect to understanding patterns and the risk of transmission between hosts. Survival of this bacterium under a variety of conditions has been estimated using a number of techniques such as culture/colony formation, direct microscopic methods, flow cytometry, and molecular methods such as PCR. These studies have generated a wide range of estimates ranging from short survival times (Rose et al., 1990b) to prolonged survival of the pathogen cells into putative adaptive mechanisms such as the “dormant” or “non-culturable but viable” (NCBV) state (Effendi and Austin, 1995; Austin and Austin, 2016). However, it is important to remember: 1) that these techniques measure different aspects of survival, 2) that these methods are not always in agreement when applied to the same samples, and 3) that detection by some of the methods is not a good predictor of cell viability/infectivity (Smith et al., 2003). Therefore, to estimate survival of *A. salmonicida* in natural brackish and seawater, we relied on culture-based studies because other methods (indirect and direct counts) do not distinguish between live and dead *A. salmonicida* cells. In addition, the potential of *A. salmonicida* cells in a dormant or NCBV states to revert to an infective form has not been demonstrated (Rose et al., 1990a; Effendi and Austin, 1995), even though such cells were shown to be alive. Bacterial survival in water is dependent upon many factors and their interactions including temperature, salinity, visible and ultraviolet radiation, presence of dissolved and particulate organic matter, and biotic factors (predation, competition and loss to bacteriophages). Factors identified as affecting *A. salmonicida* in brackish and marine environment are summarized below.

Temperature and Salinity

The survival of *A. salmonicida* in seawater at different salinities and temperatures has been studied by many authors. For the purpose of this document and subsequent risk assessment (Mimeault et al., 2019), only survival studies with experimental conditions most relevant to environmental conditions found in the Discovery Islands area were summarized in Table 7. Therefore, results from experimental studies conducted in sterile seawater (Rose, 1990; Rose et al., 1990b; Effendi and Austin, 1991; Fernandez et al., 1992; Effendi and Austin, 1994; Husevag, 1995; Perez et al., 1995; Ferguson et al., 1998), or linked to fomites (Rose, 1990; Effendi and Austin, 1994) were not included in Table 7.

Mean water temperatures in the Discovery Islands area vary from 6°C to 14°C and the mean salinity varies from 23 to 30 parts per thousand (ppt) (Chandler et al., 2017). Experimental results indicate that, when held in non-sterile seawater at temperatures of 11 to 15^{o}C and/or salinities of 23 to 35 ppt, survival of *A. salmonicida* generally ranged between 2 and 26 days (median = 6 days). However, inoculum concentrations varied between studies (10^4 to 10^8 cfu mL^{-1}), which likely contributed to the wide range of reported survival times and decay rate constants. If we exclude the extremely high decay rate constant (10.28 day^{-1}) calculated by Rose (1990) using a subset of Lund's (1967) data, for which experimental methods could not be verified, the average and lowest decay rate constants for *A. salmonicida* from non-sterile brackish and marine environments are estimated at 2.09 day^{-1}, and 0.66 day^{-1}, respectively.

Longer survival times have been reported in sterile seawater (Rose, 1990; Rose et al., 1990b; Effendi and Austin, 1991); however, this may be caused by the absence of biotic factors such as competing species of bacteria (described below).

There are few data on how infectivity and virulence of *A. salmonicida* changes over time following release into the environment. Rose et al. (1990b) collected aliquots of *A. salmonicida* (strain MT 432) from experiments which used non-sterile seawater to examine survival. These aliquots, which were obtained six days after viable counts had reach zero, were injected i.m. into naïve Atlantic Salmon. Clinical disease did not occur and they were unable to isolate *A. salmonicida* from injected fish.

pH

No studies were found on the effect of pH on survival of *A. salmonicida* cells in seawater or freshwater.

Visible and Ultraviolet Radiation

Ultraviolet (UV) and visible components of sunlight likely play a role in the inactivation or degradation of *A. salmonicida* in surface waters (Liltved and Landfald, 2000); however, no studies were found which focused on solar inactivation of *A. salmonicida*.

Table 7. Survival of Aeromonas salmonicida *in brackish and marine waters. The presence of viable bacterial cells was assessed by culture using a variety of different media. [A] Decay rate constants were estimated using data from Figures listed with the reference, and were calculated by fitting a least squares linear regression to a plot of the natural logarithm of bacterial concentration (cfu mL^{-1}) versus time, where the negative slope of the line is the decay rate constant per unit time. A high k value represents a rapid decay or short survival time. ND = not determined*

Temp. (ºC)	Salinity (ppt)	Strain	Initial inoculum (cfu mL^{-1}) – Survival (day no longer detected by culture)	Incubation Conditions	Decay rate constant per unit time (k day^{-1})	References
ND	24	ND	10^8 – 2 days	Non-sterile	10.280	Lund 1967 cited in (Rose, 1990) Table 5.3
ND	33	ND	10^8 – 5 days	Non-sterile	4.089	Lund 1967 cited in (Rose, 1990) Table 5.3
11-13	23	Strepto-mycin resistant	10^7 – 26 days	Non-sterile (dialysis bags)	0.658[A] 0.693	(McCarthy, 1977) Fig. 1 McCarthy (1977) cited in (Rose, 1990) Table 5.3
11-13	35	Strepto-mycin resistant	10^7 – 10 days	Non-sterile (dialysis bags)	1.987	McCarthy 1977 cited in (Rose, 1990) Table 5.3
14	33	MT 432	10^5 – 7 days	Non-sterile (flask)	1.564	(Rose, 1990) Fig. 3.1 Table 5.2
14	33	MT 432	10^5 – 7 days	Non-sterile (dialysis bags)	1.660	(Rose, 1990) Fig. 3.1 Table 5.2
14	33	MT 432	10^4 – 6 days	Non-sterile (broth culture)	1.357[A]	(Rose et al., 1990b) Fig. 2
14	33	MT 432	10^4 – 7 days	Non-sterile (shed bacteria)	1.522	(Rose, 1990) Fig. 3.2 Table 5.2
15	33	MT 432	10^4 – 4 days	Non-sterile	2.803	(Rose, 1990) Fig. 3.5 Table 5.2
15	33	MT 432	10^5 – 7 days	Non-sterile	1.959[A]	(Rose, 1990) Fig. 3.6
15	34	MT 393	10^4 – 4 days	Non-sterile Sea cage Water	2.308	(Rose, 1990) Fig. 3.5 Table 5.2
15	34	MT 432	10^4 – 3 days	Non-sterile Sea cage Water	3.380	(Rose, 1990) Fig. 3.5 Table 5.2
15	25	256/91	10^7 – approx. 5 days	Non-sterile (Dark)	3.197[A]	(Effendi and Austin, 1994) Fig. 4

Environmental Microorganisms

In laboratory and field studies, natural microbial biota has been shown to reduce the recovery (a proxy of survival) of *A. salmonicida* from environmental samples*,* a pattern consistent in both freshwater and seawater*.* Effendi and Austin (1991) reported that survival, as determined by colony counts, was influenced by potential inhibitory microorganisms in sea water which can outcompete or antagonize growth of *A. salmonicida*. In non-sterile seawater, the number of culturable *A. salmonicida* cells declined within three hours from an initial concentration of 10^4-10^6 cell mL^{-1} and could not be cultured on basal marine agar (BMA) plates after six days due to the presence of inhibiting or outcompeting native bacteria such as *Acinetobacter* sp. and *Aeromonas hydrophila*. Whereas in autoclaved sea water, numbers of viable *A. salmonicida* cells generally increased and were still viable after 24 days (Effendi and Austin, 1991). In addition, they observed that survival of *A. salmonicida* in seawater in the presence of microorganisms (*Acinetobacter* sp., *Aeromonas hydrophila*, *Chromobacterium* sp., *Escherichia coli*, *Flavobacterium* sp. *Pseudomonas* sp.) is further adversely influenced by shaking, which favoured the growth of inhibitory microorganisms.

Sediments

In fresh and saltwater environments, sediments are an important environmental reservoir of *A. salmonicida* with this bacterium being transported to the sediments by faeces and carcasses of infected/diseased fish. With respect to the saltwater environment, (Enger and Thorsen, 1992) examined bacterial abundance in the water column (surface, 1 and 3 meter depths) and in the sediments within and around net pens during an furunculosis epizootic of unspecified severity. The highest cell counts of *A. salmonicida* were reported in the surface microlayer (average abundance of 4.3 x $l0^3$ cells mL^{-1} and in the sediments sampled beneath the farm (average abundance of 2.2 x 10^6 cells mL^{-1}) (Enger and Thorsen, 1992). Although there are several studies of survival of *A. salmonicida* in freshwater sediments we are aware of only one study that has examined survival in marine sediments (see Effendi and Austin (1994)). Similar to that reported for non-sterile seawater it appears that survival is reduced in non-sterile sediments (sand, mud) (11 days) when compared to sterile sediments (>22 days) at 15°C and 25 ppt (Effendi and Austin, 1994). These periods are considerably shorter than reported in freshwater sediments which range from approximately one to nine months (McCarthy, 1977; Michel and Dubois-Darnaudpeys, 1980; Sakai, 1986).

We are unaware of any studies that have examined changes in *A. salmonicida* pathogenicity over time in marine sediments.

Fomites and vectors

Aeromonas salmonicida can adhere to a variety of hard substrates used in aquaculture and adherence to these materials may play a role in survival (Carballo et al., 2000). Effendi and Austin (1994) examined survival of this bacterium on wood held in water of 15°C and 25 ppt under sterile and non-sterile conditions in the dark. Similar to what was reported for survival in seawater, survival times were lower under non-sterile conditions (decrease of 10^7 to 10^2 cells per mL^{-1} at 14 days) when compared to sterile conditions (decrease of 10^6 to 10^4 cells per mL^{-1} at 21 days). In the same non sterile marine microcosm, survival of *A. salmonicida* was lower (<10 days) on seaweed (*Ascophyllum nodosum* and *Fucus vesiculosus*) than on wood (Effendi and Austin, 1994). Under experimental conditions, McCarthy (1977) found that *A. salmonicida* was able to survive on both wet and dry contaminated nets for at least six days when those were kept at 10°C, as well as on contaminated dry net that were previously dipped several seconds into 1% hypochlorite solution. More recently, Virsek et al. (2017) isolated *A. salmonicida* from microplastics in the marine environment using molecular methods.

Marine invertebrates are potential mechanical vectors as they may carry *A. salmonicida* externally or internally, or both. However, Effendi and Austin (1994) have shown experimentally that *A. salmonicida* (strains 256/91 and AS20) is relatively short-lived in and on marine benthic invertebrates (Common Marine Hermit Crabs, *Pagurus bernhardus*; European Lobsters, *Homarus vulgaris*; Laver Spire Shell, *Hydrobia ulvae*; and Spiny Starfish, *Marthasterias glacialis*) as the bacterium could not be recovered after two days post exposure (dpe). However, *A. salmonicida* was isolated from the body surface of experimentally exposed common starfish (*Asterias rubens*) at 7 dpe (Effendi and Austin, 1994).

Aeromonas salmonicida has also been isolated from marine zooplankton and from sea lice (*Lepeophtheirus salmonis*) sampled at a fish farm experiencing a furunculosis outbreak (Nese and Enger, 1993). In addition, Novak et al. (2016) demonstrated that sea lice (*Lepeophtheirus salmonis*) exposed to seawater containing *A. salmonicida* or collected from infected Atlantic Salmon, can become infected and could transmit the pathogen to naïve young Atlantic Salmon under specific laboratory conditions. It is not known whether *A. salmonicida* can multiply in sea lice, therefore, its role as a mechanical or biological vector for the transmission of *A. salmonicida* had not been defined (Novak et al., 2016).

Fish undoubtedly play a major role in the transmission of furunculosis, where diseased and dead fish, but also healthy carriers, are sources of *A. salmonicida* (McCarthy, 1977; Hiney and Olivier, 1999; Austin and Austin, 2016). Wild fish entering salmon marine net pens or in their vicinity can be infected with *A. salmonicida.* In the North Atlantic, species of Wrasse (Labridae) used as cleaner fish in salmon farms can be infected with typical and atypical strains of *A. salmonicida*, most likely by cohabitation (Treasurer and Laidler, 1994) (Laidler et al., 1999). On the U.S. Pacific coast, Novotny (1975) isolated *A. salmonicida* from a diseased Eulachon (*Thaleichthys pacificus*) found in a net pen with infected salmon, and in coastal British Columbia, Evelyn (1971) isolated an aberrant strain of *A. salmonicida* from a Sablefish (*Anoplopoma fimbria*) and from cultured Pacific salmon. Novotny (1975) and Evelyn (1971) speculated that Eulachon and Sablefish may have acquired their respective *A. salmonicida* infection from captive Pacific salmon. Yet, an atypical *A. salmonicida* was also isolated from a Lingcod (*Ophiodon elongatus*) caught well away from salmon farms (Kent et al., 1998).

HEALTH MANAGEMENT

BIOSECURITY

Effective farm management practices are crucial to prevent introduction of pathogens on a farm, and to control disease in the event of an outbreak. The Aquatic Animal Health Code of the World Organization for Animal Health (the OIE) defines biosecurity as a set of management and physical measures designed to reduce the risk of introduction, establishment and spread of pathogenic agents to, from and within an aquatic animal population.

Husbandry protocols such as cleaning and disinfecting of equipment and hard surfaces, use and maintenance of footbaths are in place on BC farms as standard operating procedures (Wade, 2017). As these standard operating procedures (SOPs) are a requirement of license, compliance is recorded as a part of the audit program (Wade, 2017). SOPs are produced and reviewed in accordance to DFO aquaculture licensing requirements. Details about SOPs and other health management practices specific to Atlantic Salmon farms in BC are provided by Wade (2017).

PREVENTION, CONTROL AND TREATMENT

Immunization and Vaccine Efficacy

The introduction of efficient commercial vaccines in the 1990's and improved husbandry practices resulted in a dramatic reduction in furunculosis outbreaks and a corresponding decline in chemotherapeutic treatment against the disease in BC (Noakes et al., 2000; Morrison and Saksida, 2013).

Immersion vaccines are generally unsuccessful for the prevention furunculosis (Midtlyng, 1997). Injectable (intraperitoneal or intramuscular) formulations prepared from whole *A. salmonicida* bacterin with an oil-based adjuvant are very efficacious but cannot be delivered to very small fish (Midtlyng, 1997; USDA, 2014). As a consequence, young salmon exposed to *A. salmonicida* at the hatchery can acquire the infection before vaccination and are at risk of developing furunculosis, or they may become covertly infected and carry the pathogen when transferred to their marine grow-out sites (Munro and Waddell, 1984; Hiney, 1995).

Vaccine efficacy depends on the *A. salmonicida* strains used in the vaccine and the wild strains. In experimental challenges, Atlantic Salmon vaccinated against atypical *A. salmonicida* were not protected against the typical strain, but vaccination against the typical strain conferred relative protection against atypical *A. salmonicida* (Gudmundsdóttir and Gudmundsdóttir, 1997; Gudmundsdóttir and Björnsdóttir, 2007). Other considerations include proper host condition (immunocompetency) prior to vaccination and adequate vaccination protocols (USDA, 2014).

In Canada, three vaccines are currently authorized for use against furunculosis in salmonids grown in Canada (CFIA, 2018), namely: 1) Forte Micro® (*Aeromonas salmonicida-Vibrio anguillarum-ordalii-salmonicida* bacterin); 2) Forte VII® (Infectious Salmon Anaemia killed virus vaccine, *A. salmonicida*, *Vibrio anguillarum-ordalii-salmonicida* bacterin); and 3) Alpha Ject Micro 4® (*A. salmonicida-Listonella* (*Vibrio*) *anguillarum-Vibrio salmonicida* bacterin). Data about the efficacy of commercial vaccines used in Canada under field conditions are not available. In British Columbia, vaccination is not a requirement of license and is done on a voluntary basis (Wade, 2017).

Inactivation

Inactivation of *A. salmonicida* can be achieved by an array of chemical and physical processes. Chemical disinfectants are used to inactivate *A. salmonicida* on objects, hard surfaces and equipment, and in egg disinfection (McCarthy and Roberts, 1980; Bowker et al., 2016; Wade, 2017).

Virkon® is the effective disinfectant used by all companies on material and equipment, including vessels, in BC (Wade, 2017). It is generally applied, in diluted form, to all equipment and in footbaths to meet company SOPs (Wade, 2017). Prior cleaning of surfaces is usually required since many disinfectants can be inactivated by organic matter (Bowker et al., 2016). After disinfection, equipment is stored in a dry and proper location (Wade, 2017). Transmission of *A. salmonicida* can be prevented when equipment such as fish nets are properly disinfected (McCarthy, 1977).

Disinfectants can also be used to inactivate microorganisms living on the surface of fish eggs. Eggs are required to be disinfected in BC as part of the Salmonid Health Management Plan (SHMP) (DFO, 2015; Wade, 2017). Egg disinfection can be conducted either at the broodstock facility or once the gametes enter the hatchery (DFO, 2015).

Ultraviolet (UV) treatment is often used to disinfect freshwater for use in hatcheries. Bullock and Stuckey (1977) studied the effects of UV on known concentrations of *A. salmonicida* in spring water and water containing suspended organic matter. UV irradiation at dosages of 4500 µW s

cm^{-2} killed 99.83-100% of test strains in spring water while dosages of 11,600-29,700 µW s cm^{-2} and 3300-5300 µW s cm^{-2} killed 99.93-100% and 99-99.94% of test strains respectively in water containing organic matter (Bullock and Stuckey, 1977). Kimura et al. (1976) report a 99.99% reduction in viable cells of *A. salmonicida* in a continuous laboratory pond water flow system at a UV dosage of 23,100 µW s cm^{-2}. Sako and Sorimachi (1985) indicated that a UV dosage of 3.4 x 103 µW s cm^{-2} was effective for killing 99.9% of viable *A. salmonicida* cells in freshwater.

The external presence of *A. salmonicida* on fish, primarily on gill surfaces, can be controlled with the use of topical disinfectants such as Chloramine T (Cipriano et al., 1996).

Although not an active inactivation process, fallowing of aquaculture site is known to reduce infection pressure between production cycles. During a furunculosis outbreak in a fish farm, *A. salmonicida* cells shed in fish faeces and body remains sink into sediments where they can survive and reach high abundances (Enger and Thorsen, 1992). Length of survival of *A. salmonicida* varies greatly between studies, from two days in non-sterile seawater (Lund (1967) cited in Rose (1990)) to >10 days in mud from non-sterile marine microcosm (Effendi and Austin, 1994) to prolonged survival up to nine months in sterilized river mud (Michel and Dubois-Darnaudpeys, 1980).

Antimicrobial Agents

The introduction of efficacious vaccines and improved husbandry practices resulted in a dramatic reduction in the use of antibiotics to treat furunculosis (Burka et al., 1997; Alderman and Hastings, 1998; Noakes et al., 2000). However, vaccination does not guarantee full protection against targeted pathogens and vaccination failures do occur. When vaccination or other preventive measures fail, outbreaks of furunculosis are often managed with antibiotics (McIntosh et al., 2008). The use of antibiotics is efficacious at reducing furunculosis-related mortalities (Nordmo et al., 1994); however, carrier fish may still be present in the population after treatment. Antibiotic treatments have been prescribed in all furunculosis related Fish Health Events in the Discovery Islands area.

Successful treatment of furunculosis relies on the early detection and early administration of suitable antibiotics through feed (Hoskins and Hulstein, 1977). As sick fish become anorexic and therefore not medicated, the treatment goal is to prevent disease spread to healthy stock. There are currently only three antibiotics approved in Canada to control furunculosis in aquatic animals destined for use as food: florfenicol (Aquaflor®); sulfadimethoxine/ormetoprim (Romet® 30); and oxytetracycline hydrochloride (Terramycin-Aqua©) (Health Canada, 2018). Antibiotics are prescribed by a veterinarian, delivered through feed and their use must be reported to DFO (Morrison and Saksida, 2013).

Control Measures in Development

The use of various supplements such as pro- and prebiotics to control infections has received consideration in recent years (Menanteau-Ledouble et al., 2016). Robertson et al. (2000) found the probiotic *Carnobacterium* sp. to be inhibitory to *A. salmonicida* and Rainbow Trout and Atlantic Salmon that received the probiotic showed improved survival after 14 days compared to a control group (Robertson et al., 2000). Other studies have demonstrated the ability for isolates of *Lactobacilllus plantarum, L. fermentum* (Balcazar et al., 2008) and *Lectobacillus pentosus* H16 (Garces et al., 2015) to compete against *A. salmonicida*. Despite the promising results from these isolated studies; it is still unknown how well probiotics would benefit in a large-scale intensive production (Menanteau-Ledouble et al., 2016). More research is needed before the reliability and cost-effectiveness of probiotics can be demonstrated (Menanteau-Ledouble et al., 2016).

Selective breeding for fish strains that are resistant to *A. salmonicida* has been considered as more permanent solution to reduce mortalities caused by furunculosis (Gjedrem and Gjoen, 1995; Menanteau-Ledouble et al., 2016). When breeding for resistant strains there are concerns with the possibility to unwillingly select for detrimental aspects (Menanteau-Ledouble et al., 2016) such as increased susceptibility to infectious salmon anemia (ISA) (Kjoglum et al., 2008).

Bacteriophage therapy is another promising avenue for the biological control of bacterial diseases in farmed fish. Typing of bacteriophages was successfully used as a technique to identify substrains of *A. salmonicida salmonicida* (Hiney and Olivier, 1999; Austin and Austin, 2016; Menanteau-Ledouble et al., 2016), and *in vitro* characteristics of bacteriophages *of A. salmonicida* have been documented, but research on the use of bacteriophage to fight bacterial infections has been neglected (Morrison and Rainnie, 2004). Bacteriophage therapy research is likely to increase with development of antibiotic resistance and environmental issues in aquaculture.

KNOWLEDGE GAPS

Aeromonas salmonicida is the most studied of fish pathogen, yet many knowledge gaps were identified from the literature review; which if understood, would help refine the risk assessment and improve advice for decision makers. These include knowledge of the:

- subspecies and strains of *A. salmonicida* circulating in populations of farmed Atlantic and Fraser River Sockeye Salmon;
- epidemiological relationships between *A. salmonicida*/furunculosis, farmed Atlantic Salmon and Sockeye Salmon, and environmental conditions; epidemiology of *A. salmonicida* (pathogen transmission) in wild Sockeye Salmon throughout the life cycle;
- consequences of sublethal infection/disease at the fish and the population level in Sockeye Salmon and other Pacific salmon species;
- relative importance of carrier fish, farmed and wild, as reservoirs *of A. salmonicida*; characterization of shedding rates and patterns of shedding in carriers;
- population prevalence of *A. salmonicida* in farmed Atlantic Salmon and wild Sockeye Salmon at every life stage;
- direct and indirect mortality attributable to furunculosis in wild fish;
- incubation times, shedding rates, minimum infectious concentrations and lethal doses of *A. salmonicida* strains circulating in BC in Atlantic Salmon and Sockeye Salmon, under different environmental conditions; and
- efficacy of commercial vaccines used by companies farming Atlantic Salmon, including efficacy under laboratory and, more importantly, under field conditions.

SUMMARY

Furunculosis is a septicemic bacterial disease found principally in salmonids in freshwater, brackish and marine environments, caused by infection with *Aeromonas salmonicida*. The pathogen is transmitted horizontally. Information about interspecific and intraspecific variability in susceptibility within and across salmonid species is limited, however, all salmonid species are considered susceptible to *A. salmonicida* infection and disease.

Furunculosis has been reported on marine Atlantic Salmon farms in BC, however there were no audit-based farm-level diagnoses attributable to furunculosis in the Discovery Islands area between 2002 and 2016. Effective health management practices by marine aquaculture

companies such as vaccination of all fish prior to seawater transfers, disease surveillance and biosecurity measures such as disinfection of eggs and farm equipment, likely limit the occurrences of *A. salmonicida* and Fish Health Events attributed to furunculosis. Despite these management practices, vaccinated fish may still develop furunculosis after a stressful event if they were infected with *A. salmonicida* prior to vaccination.

Data about the importance of carrier fish as reservoirs of *A. salmonicida* and shedding rates from covert infections are lacking, but infected diseased and dead fish are known to release large amounts of the bacterium in the environment. The survival of *Aeromonas salmonicida* in the marine environment is influenced by factors such as temperature, salinity, radiations, and the presence of particulate organic matter and environmental microorganisms. Survival of *A. salmonicida* has also been documented in marine sediments.

The minimum infectious and lethal doses of *A. salmonicida* to Atlantic and Pacific salmon are unknown; however, bath challenge studies reporting the lowest infectious and lethal doses could be used as a proxy for the purposes of the risk assessment.

Despite the lack of data, transmission of *A. salmonicida* infection from farmed Atlantic Salmon to wild Fraser River Sockeye Salmon can be mitigated with pathogen and disease control measures in the farmed salmon population. This can be accomplished at every stage of the production cycle.

ACKNOWLEDGMENTS

We would like to thank David Bruno, Ahmed Siah, Caroline Mimeault, Ingrid Burgetz and Jay Parsons for reviewing the manuscript.

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