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Characterization of *Piscirickettsia salmonis* and salmonid rickettsial septicaemia to inform pathogen transfer risk assessments in British Columbia

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Foreword

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

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ABSTRACT

Infection with the facultative, intracellular Gram-negative bacterium *Piscirickettsia salmonis* causes piscirickettsiosis (SRS), a potentially severe septicaemia of farmed marine fish in many parts of the world. The bacterium has a broad host range and several species of non-salmonid and salmonid fishes are susceptible to the infection and disease. There are no data on susceptibility in Sockeye Salmon (*Oncorhynchus nerka*), but Chinook (*O. tshawytscha*), Coho (*O. kisutch*) and Pink (*O. gorbuscha*) Salmon and Rainbow Trout (*O. mykiss*) are susceptible, as are Atlantic Salmon (*Salmo salar*). Waterborne movement of the bacterium is the most likely route of transmission, although there are no data on shedding rate or minimum infectious dose in any species. Environmental and husbandry-associated stressors contribute to the severity of the disease. The development of control strategies has been hampered by a poor understanding of the transmission, epidemiology and virulence factors of *P. salmonis*, and by an absence of efficacious vaccines and reliable antibiotic therapies. In British Columbia (BC), SRS has been reported in farmed Atlantic and Chinook salmon since the 1980s. There are very few data on the occurrence of *P. salmonis* in wild salmonids in BC. Nucleotide sequences from BC isolates of *P. salmonis* are remarkably similar to those from Chile and Europe. In conclusion, the wide host range of *P. salmonis* indicates a high likelihood that Sockeye Salmon will be susceptible. SRS outbreaks appear to be triggered by environmental stressors, whether of environmental or farm origin. Studies on *P. salmonis* are required to confirm susceptibility and pathogenesis in Sockeye Salmon, to determine salmon species-specific bacterial shedding rates and to characterize the attenuation of bacterial viability under natural conditions.

INTRODUCTION

Fisheries and Oceans Canada, consistent with the Government of Canada as a whole, is moving toward the implementation of science informed risk-based models for decision making. The development of an aquaculture science risk assessment framework was a commitment under the 2008 Sustainable Aquaculture Program (SAP) and builds upon the work initiated with the scientific peer-review validation of the Aquaculture Pathways of Effects (APOE) (2009) through the Canadian Science Advisory Secretariat (CSAS). This framework is a formalized approach to the provision of risk-based advice that is consistent with activities currently undertaken by Aquaculture Science, and is a component of the overall Sustainable Aquaculture Program's Risk Management Framework.

The National Capital Region (NCR) Aquaculture Science group implemented the Aquaculture Science Environmental Risk Assessment Initiative (Initiative) to determine the risks to the environment and wild fish due to stressors resulting from the aquaculture activities as validated through a peer-review process of the pathways of effects (DFO, 2010).

A series of environmental risk assessments will be conducted to address the following environmental stressors resulting from aquaculture activities: physical alteration of habitat structure; alteration in light; noise; release of chemicals and litter; release/removal of nutrients, non-cultured organisms, and other organic matter; release/removal of fish and; release of pathogens. Release of pathogens is the first of these stressors to be assessed.

In 2014, the Department began a series of risk assessments to assess the risk to Fraser River Sockeye Salmon (*Oncorhynchus nerka*) due to pathogen transfer from Atlantic Salmon (*Salmo salar*) farms located in the Discovery Islands area of British Columbia (BC). Pathogens which may be assessed were determined from the audit data collected by the Department and fish health events reported by the industry. In order for a pathogen to be considered for a risk assessment, there must be evidence that the pathogen caused disease on Atlantic Salmon farms in the Discovery Islands, there must be evidence of Sockeye Salmon susceptibility to the disease caused by the pathogen and there must be evidence of temporal overlap of disease outbreaks on Atlantic Salmon farms and Fraser River Sockeye Salmon migration.

Following the successful Canadian Science Advisory Secretariat peer review of the assessment of the risk to Fraser River Sockeye Salmon due to Infectious Hematopoietic Necrosis Virus transfer from Atlantic Salmon farms in the Discovery Islands, the same framework will be applied to assessing the risks associated with other pathogens known to have caused disease on Atlantic Salmon farms in that area. Four bacterial pathogens have been identified to undergo formal risk assessments, and in preparation for initiating the next risk assessment, the scientific information on the characteristics of each pathogen is being collated and analyzed. These bacteria are: *Renibacterium salmoninarum*, the causal agent of bacterial kidney disease (BKD); *Aeromonas salmonicida*, the causal agent of furunculosis; *Yersinia ruckeri*, the causal agent of enteric redmouth (ERM) and *Piscirickettsia salmonis*, the causal agent of piscirickettsiosis.

PURPOSE OF DOCUMENT

This document summarizes the relevant information pertaining to *P. salmonis* and salmonid piscirickettsiosis (SRS) within the context of an environmental risk assessment to determine the risk to Fraser River Sockeye Salmon due to pathogen transfer from Atlantic Salmon farms located in the Discovery Islands area of BC.

CHARACTERIZATION

DISEASE (DESCRIPTION AND GEOGRAPHICAL RANGE)

Piscirickettsiosis is a disease of farmed salmonids reported first in Chile as Coho Salmon (*O. kisutch*) syndrome (Bravo and Campos, 1989). Clinical presentations include acute, sub-acute or chronic depending on the isolate and the host (Otterlei et al., 2016), as well as on season, water temperature and husbandry activities, as discussed below. In Chile, clinical disease is most likely to be first reported, depending on species, between four and seven months following transfer to sea (Rees et al., 2014). Similarly, while epizootics occur year-round, the incidence of disease is elevated during warmer months (Rees et al., 2014). Using data from Chile, the latter study estimated a lower threshold for clinical disease between 9 and 11°C. Cumulative mortality in Coho Salmon ranged from 20 to 30% and up to 90% in some cases (Bravo and Campos, 1989). In Chile, the disease also occurs in Atlantic Salmon, Chinook Salmon (*O. tshawytscha*) and Rainbow Trout (*O. mykiss*) (Cvitanich et al., 1991), and has been observed to follow fluctuating environmental conditions including extreme water temperatures and algal blooms (Branson and Diaz-Munoz, 1991). Information from 2012 indicates that in Chile, piscirickettsiosis was the most frequently diagnosed pathogen in seawater-reared salmonids (54.4% of cases) and the highest cause of mortality associated with infectious disease during grow-out in seawater net pens (69.4% of infectious mortalities in Atlantic Salmon, 60.3% in Coho Salmon and 94.6% in Rainbow Trout) (Rozas and Enriquez, 2014). The impact of the disease to the Chilean economy was estimated at 250 M US$, in addition to the loss of market access of Chilean farmed salmon due to concerns over antibiotic residues (Henriquez et al., 2016).

Similar rickettsial septicaemias of farmed Atlantic Salmon have been reported from Eastern and Western Canada, Norway, Scotland and Ireland (Brocklebank et al., 1993; Rodger and Drinan, 1993; Grant et al., 1996; Olsen et al., 1997; Cusack et al., 2002), all with considerably lower rates of morbidity and mortality than reported in Chile. In Norway, outbreaks occurred between August and December and tended to follow an algal bloom on a farm (Olsen et al., 1997). In Eastern Canada, Cusack et al. (2002) reported daily mortality rates of up to 0.22% in two affected cages during an outbreak of rickettsial septicaemia that occurred in farmed Atlantic Salmon between September and March. The Canadian outbreak followed a period of very heavy rainfall and unusually strong winds (Cusack et al., 2002).

Clinical signs of SRS include darkening, lethargy, inappetence or erratic behaviour including uncoordinated swimming and swimming near the surface or pen netting. Affected fish may display a generalized pallor and focal skin lesions including raised scales, nodules, or ulcers. Clinical signs may be absent in affected fish (Branson and Diaz-Munoz, 1991; Cvitanich et al., 1991; Almendras and Fuentealba, 1997).

Gross and microscopic pathological lesions are consistent with a septicaemia and the earliest descriptions are those of Branson and Diaz-Munoz (1991) and Cvitanich et al. (1991), who used the name “salmonid rickettsial septicaemia”. Gross pathology involves several organs and tissues. In particular, swollen, discoloured kidney and splenomegaly are occasionally accompanied by ascites with exophthalmia and by hemorrhages of the skin, visceral organs, skeletal muscle and brain (Schafer et al., 1990; Cvitanich et al., 1991). In severe cases, the liver is pale occasionally with circular yellowish, subcapsular nodules up to 2 cm in diameter (Almendras and Fuentealba, 1997; Fryer and Mauel, 1997).

The most prominent microscopic lesions are found in liver, kidney, spleen and intestine and are broadly classified as inflammatory and necrotic. Microscopic pathological changes are also evident in brain, skeletal muscle, skin, heart, gills and ovary (Almendras and Fuentealba, 1997).

ETIOLOGICAL AGENT (DESCRIPTION AND GENETIC TYPES/STRAINS)

SRS is caused by infection with *P. salmonis*, a Gram-negative, non-motile, coccoid-like organism (0.5 to 1.5 – 2.0 µm) that occurs individually or in groups, intracellularly in susceptible fish hosts. Prior to the formal description of *P. salmonis* and an understanding of its true taxonomic affinity, the causative agent of SRS was referred to as a "rickettsia-like organism (RLO). There are numerous references to infections with RLOs in fish: some are now known to be *P. salmonis*, some are *Francisella* spp. and some are not yet identified (Lannan and Fryer, 1994; Fryer and Mauel, 1997; Mauel et al., 2007). *Piscirickettsia salmonis* belongs to the Class Gammaproteobacteria (Fryer et al., 1992), which also includes the genera *Francisella*, *Coxiella* and *Legionella,* while bacteria considered true rickettsia (genera *Rickettsia*, *Neorickettsia*, *Cowdria*, *Anaplasma* and *Ehrlichia*) belong to the Alphaproteobacteria. The original description of the organism was based on isolates from SRS-affected Coho Salmon in Chile. *Piscirickettsia salmonis* has been isolated from or identified in cases of SRS in Scotland, Ireland, Norway and in Eastern and Western Canada.

There is evidence of genetic and phenotypic heterogeneity among isolates of *P. salmonis*. Five isolates (type strain LF-89, Chile; SLGO-94, Chile; C1-95, Chile; ATL-4-91, Western Canada; NOR-92, Norway) were shown to be similar based on 16S ribosomal DNA, internal transcribed spacer and 23S ribosomal DNA sequences, whereas a sixth strain (EM-90, Chile) was found to differ (Mauel et al., 1999). Similar heterogeneity was revealed among Irish, Scottish and Canadian isolates (Reid et al., 2004). A more recent analysis of ribosomal DNA and housekeeping gene sequences showed that Chilean isolates clustered into two genotypes (LF-89-like and EM-90-like), both associated with SRS in the Chilean aquaculture industry (Otterlei et al., 2016). This study also revealed a dissimilarity of two isolates from Western Canada from either Chilean genotype. Saavedra et al. (2017) confirmed the presence of two genotypes in Chilean aquaculture and suggested the genotypes differed with respect to host preference, growth kinetics *in vitro*, and sensitivity to antibiotics and salmon serum. A complete genome sequence for one *P. salmonis* isolate (Pulgar et al., 2015) will accelerate the characterization of this organism and its interactions with the host. A more recent study concluded that an EM-90-like isolate was more virulent in Atlantic Salmon post-smolts than an LF-89-like isolate (Rozas-Serri et al., 2017). In another study involving the intraperitoneal injection of Coho Salmon, three *P. salmonis* isolates (LF-89, NOR-92 and ATL-4-91) were shown to differ in virulence (House et al., 1999). The Chilean isolate (LF-89) caused the most rapid onset of mortality and reduced mean days-to-death compared with the Canadian or Norwegian isolates.

A comparative assessment of the relative virulence of geographic isolates by using more natural routes of transmission is required. Despite the evidence for genetic heterogeneity among numerous *P. salmonis* isolates from several countries, available sequencing data are insufficient to predict the observed phenotypic heterogeneity. The relative importance of industry-level, farm-level and environmental parameters (see Transmission section, below) in influencing the apparent virulence heterogeneity among *P. salmonis* isolates in various regions requires further research.

DIAGNOSTIC METHODS

Diagnosis of SRS is based on the recognition of characteristic clinical signs and gross and microscopic lesions in a susceptible species as described above, combined with the detection of *P. salmonis* by one of several methods including isolation in culture, microscopy, serology or nucleic acid analysis.

CULTURE

P. salmonis was first isolated onto an established Chinook Salmon embryo cell line (CHSE-214) maintained in antibiotic-free cell culture medium (Fryer et al., 1990; Cvitanich et al., 1991). The isolate initially cultured in CHSE-214 cells was shown to replicate in established cell lines from four other salmonid species and two cyprinid species, but not in those from a centrarchid or an ictalurid species (Fryer et al., 1990). A later study showed that the Brown Bullhead (*Ameiurus nebulosus*) cell line (BB) was susceptible to *P. salmonis*, but the development of cytopathic effect occurred only after 42 days, compared with six days for the CHSE-214 line (Almendras et al., 1997). Although 12 cell lines, including one from an amphibian and one from an insect, are recognized as being permissive to *P. salmonis* replication (Table 1), it is not clear whether all are equally suitable for the primary isolation of the organism.

The discovery that *P. salmonis* replicates on enriched solid-phase media (Mauel et al., 2008; Mikalsen et al., 2008) supported a hypothesis that this is a facultative intracellular organism (Gomez et al., 2009). Subsequent demonstrations of bacterial replication in numerous defined solid- and liquid-phase media confirmed the facultative nature of the bacterium (Vera et al., 2012; Yanez et al., 2012; Henriquez et al., 2013; Yanez et al., 2013; Makrinos and Bowden, 2017). Most of the latter observations confirmed bacterial growth following inoculation from infected cell cultures, and there are few cases (e.g., Mikalsen et al., 2008) in which the utility of defined solid- or liquid-phase media has been demonstrated for the primary isolation of *P. salmonis* from infected fish.

MICROSCOPY AND SEROLOGY

The presence of *P. salmonis* may also be determined by microscopic detection in tissue imprints or histological sections stained with Gram, Giemsa, methylene blue or other suitable stain (Bruno et al., 2013; Rozas and Enriquez, 2014). Non-specific binding of the fluorochrome acridine orange provided a rapid method for the presumptive identification of *P. salmonis*, either in tissue imprints or following isolation into in cell culture (Lannan and Fryer, 1991, 1993). The development of a polyclonal antibody raised against the type strain of *P. salmonis* (Lannan et al., 1991) formed the basis of a specific fluorescent antibody test (FAT) that has been used in laboratories around the world. By bleaching interfering melanin granules, the application of microwave irradiation reduced over eight-fold the time required for development of the FAT (Larenas et al., 1996). This antibody is also effective as an immunohistochemical reagent when used to detect the bacterium in histological sections from salmonids and non-salmonids (Alday-Sanz et al., 1994; Steiropoulos et al., 2002). Since then, monoclonal antibodies (MAbs) were shown to be useful in detecting several isolates of *P. salmonis* by fluorescence microscopy in cultured cells or fish tissue imprints (Jamett et al., 2001) and by Enzyme-Linked Immuno-Sorbent Assay (ELISA) (Aguayo et al., 2002). Panels of MAbs may be useful in describing the serological heterogeneity of *P. salmonis* throughout its range as a guide to vaccine development.

Table 1. Host susceptibility to Piscirickettsia salmonis by infection of cultured cells or whole animal.

Species		Susceptibility		Geographic region[1]
		Cell line (tissue/organ)	Animal	
Oncorhynchus tshawytscha	Chinook Salmon	CHSE-214 (embryo)	+	SAP,NAP
Oncorhynchus kisutch	Coho Salmon	CSE-119 (embryo)	+	SAP,NAP
Oncorhynchus mykiss	Rainbow Trout	RTG-2 (gill), RTS11 (spleen)	+	SAP
Oncorhynchus keta	Chum Salmon	CHH-1 (heart)	?	
Oncorhynchus gorbuscha	Pink Salmon	---	+	NAP
Salmo salar	Atlantic Salmon	SHK-1 (head kidney), ASK (kidney)	+	SAP,NAP,NAA,EU
Pimephales promelas	Fathead Minnow	FHM, EPC (skin)	?	
Ameiurus nebulosus	Brown Bullhead	BB (trunk tissue)	?	
Xenopus laevis	African Clawed Frog	XTC-2 (epithelial)	?	
Spodoptera frugiperda	Fall Armyworm	Sf21(ovary)	?	
Atractoscion nobilis	White Seabass	---	+	NAP
Cyclopterus lumpus	Lumpfish	---	+	EU
Dicentrarchus labrax	European Seabass	---	+	EU
Epinephelus melanostigma	Blackspot Grouper	---	+	SEA
Eleginops maclovinus	Patagonian Blenny	---	+	SAP
Odontesthes regia	Chilean Silverside	---	+	SAP
Helicolenus lengerichi	Rockfish	---	+	SAP
Pinguipes chilensis	Chilean Sand Perch	---	+	SAP
Sebastes capensis	Cape Redfish	---	+	SAP
Prolatilus jugularis	Pacific Sand Perch	---	+	SAP
Salilota australis	Tadpole Codling	---	+	SAP
Stromateus brasiliensis	SW Atlantic Butterfish	---	+	SAP
Paralichthys microps	Flounder	---	+	SAP
Genypterus maculatus	Black Cusk-Eel	---	+	SAP

[1]SAP: South America Pacific; NAP: North America Pacific; NAA: North America Atlantic; EU: Europe; SEA: Southeast Asia

POLYMERASE CHAIN REACTION (PCR)

PCR technology is used to rapidly confirm the presence of relatively small concentrations of pathogen genomic DNA in animal tissue, which is generally considered to be evidence of infection. Conventional polymerase chain reaction (PCR) assays were used to amplify and subsequently sequence the entire 16S ribosomal RNA (rRNA) gene of *P. salmonis* (Fryer et al., 1992). A nested PCR assay also targeting the 16S rRNA gene, detected fewer than one tissue culture infective dose ($TCID_{50}$) of *P. salmonis* and correctly identified the bacterium in infected kidney or spleen (Mauel et al., 1996). Subsequently, a PCR that amplified a partial 16S rRNA gene, along with the more variable internal transcribed spacer (ITS) and 23S rRNA gene, was used to diagnose *P. salmonis* from serum samples of fish with or without clinical signs of the infection (Marshall et al., 1998). Similarities among *P. salmonis* isolates can be inferred from the nucleotide sequences of PCR amplification products (Mauel et al., 1999; Heath et al., 2000). A modification to primer sequence and reaction conditions improved the sensitivity of the conventional PCR assay (Sakai et al., 2010). The conventional PCR has also been modified to function as a multiplex diagnostic tool for the simultaneous detection of DNA from *P. salmonis* and three other bacterial fish pathogens (Tapia-Cammas et al., 2011). A real-time (TaqMan) PCR assay (Corbeil et al., 2003) was able to detect *P. salmonis* DNA in culture supernatants diluted to approximately 0.5 $TCID_{50}$/mL, indicating its sensitivity approximately equaled that of the conventional nested assay. Formalin-fixed pathogen nucleic acids may be amplified by real-time PCR from archival paraffin-embedded histological samples (Karatas et al., 2008). A solid-phase PCR variant for the detection of *P. salmonis* DNA has been reported (Del Rio et al., 2016). DNA oligonucleotides, similar to those used in PCR assays, once labelled with a chromogenic or fluorescent marker, are useful for the microscopic detection of *P. salmonis* DNA by in-situ hybridization assays in histological sections (Venegas et al., 2004).

EPIDEMIOLOGY

RESERVOIR

P. salmonis is a pathogen of marine fish with a remarkably broad host-range that includes salmonid and non-salmonid species (Table 1). No species have been identified as natural reservoirs of the infections in farmed salmon.

SUSCEPTIBLE SPECIES AND LIFE STAGES

Salmonids

The salmonid species known to be susceptible to infection with *P. salmonis* and in which clinical signs of SRS have been reported include Pink (*O. gorbuscha*), Coho, Chinook and Atlantic Salmon and Rainbow Trout. There are no reports from Sockeye or Chum (*O. keta*) salmon although the bacterium replicates in a cell line originated from Chum Salmon heart. Initial reports from Chile indicated that SRS was a disease of Coho Salmon (Branson and Diaz-Munoz, 1991; Cvitanich et al., 1991), it is now acknowledged that all salmon species farmed in Chile (Atlantic Salmon, Coho Salmon, Chinook Salmon and Rainbow Trout) are susceptible to SRS. Laboratory data suggests that Coho Salmon are more susceptible than Rainbow Trout when assessed by differential mortality (Smith et al., 1996) and more susceptible than Atlantic Salmon when assessed by clinical signs (Garces et al., 1991). There is one report of a natural SRS outbreak in 60 to 90 day old, 8 to 10 cm Coho Salmon in freshwater farms (Gaggero et al., 1995); however, no studies have explicitly tested susceptibility to infection or disease as a function of fish age.

Non-salmonid finfish

Diagnostic PCR assays and/or serological methods have been used to confirm or provide presumptive evidence of *P. salmonis* in clinically diseased White Seabass (*Atractoscion nobilis*), European Seabass (*Dicentrarchus labrax*), Blackspot Grouper (*Epinephelus melanostigma*) and Lumpfish (*Cyclopterus lumpus*) (see Table 1; Chen et al. (2000); Steiropoulos et al. (2002); Arkush et al. (2005); McCarthy et al. (2005); Timur et al. (2005); Marcos-Lopez et al. (2017)). The bacterium has also been detected by PCR in apparently healthy marine fish collected in Southern Chile (Table 1; Contreras-Lynch et al. (2015)).

Others

Rickettsia-like organisms (RLO) are frequently observed in aquatic invertebrates including molluscs and crustaceans (Gollas-Galvan et al., 2014). PCR was used to detect *P. salmonis* DNA in marine bacterioplankton samples (Mauel and Fryer, 2001) and in epifaunal Blue Mussels (*Mytilus edulis*) collected from net pens during an outbreak of SRS in Atlantic Salmon in Western Canada (S. Jones, Fisheries and Oceans Canada, unpub. obs.).

TRANSMISSION

Mechanism and dynamics of transmission

Laboratory data and epidemiological data from farms in Chile and Norway support horizontal transmission of *P. salmonis* among fish and among farms (Cvitanich et al., 1991; Garces et al., 1991; Almendras et al., 1997; Rees et al., 2014; Price et al., 2017). Evidence from Rainbow Trout suggests that a Chilean strain of *P. salmonis* enters the host via intact skin and gills and that damaged skin may exacerbate disease severity, presumably by increasing the rate of bacterial uptake (Smith et al., 1999). Uptake of *P. salmonis* via intact skin, gill and intestinal epithelium was later demonstrated in Coho Salmon and the resulting mortality was dose dependent and consistently less that that elicited by intraperitoneal injection (Smith et al., 2004). In contrast, a Scottish strain of the bacterium failed to cause mortality for 42 days following a skin inoculation procedure in Atlantic Salmon (Birkbeck et al., 2004). The latter study, however, did not confirm whether the bacterium was present in the skin following exposure. The esophagus has also been implicated as a site for uptake of the bacterium in Rainbow Trout (Smith et al., 2015). Rozas and Enriquez (2014) conclude that skin is the most effective route of bacterial entry into the host, whereas Rozas-Serri et al. (2017) suggest uptake via gill is important. The bacterium may be shed via bile, feces and urine (Rozas and Enriquez, 2014). While there is experimental evidence for vertical transmission in Rainbow Trout (Larenas et al., 1997; Larenas et al., 2003), the significance of these findings within commercial salmon production is not clear and the infection is rarely observed in freshwater-reared salmonids (Fryer and Hedrick, 2003). In Chile, epidemiological modelling has provided insight into factors associated with pathogen transmission. The probability of SRS on a farm was directly related to temperature, time that stock have spent in seawater and the number and distance of SRS-infected neighbours (Rees et al., 2014). In that study, the distance effect between farms ranged from 7.5 to 10 km.

Incubation period and shedding rates in Atlantic Salmon

As with any salmon pathogen, the incubation period will depend on the age and species of salmon and on its general condition, on the dose or infection pressure and strain of the pathogen and its route of inoculation and on environmental conditions, especially temperature, at the time of exposure. Rozas and Enriquez (2014) estimated an incubation period of 10 to 14 days for *P. salmonis* based on a combination of laboratory studies and clinical evidence from

multiple species. Rozas-Serri et al. (2017), based on a cohabitation study in Atlantic Salmon, suggest the incubation period ranges from 15 to 20 days, depending on bacterial strain.

Although the horizontal transmission data cited above are consistent with shedding of *P. salmonis* from infected fish, neither the timing of shedding during infection nor the rate of shedding has been described. While it is not unreasonable to expect low level shedding of *P. salmonis* from apparently healthy infected fish, there is little evidence to support this possibility. Larenas et al. (2005) detected *P. salmonis* in 22 of 100 five-gram, healthy Coho Salmon by using the fluorescent antibody test. The report stated that these fish had acquired the infection by vertical transmission from infected brood fish. In ten of these fish the bacterium was detected only in feces and in 11 fish it was detected only in kidney. The bacterium was detected in kidney and feces in 1 of 100 fish. It is not known whether *P. salmonis* shed in feces were viable nor is it clear why the bacterium was not detected in the feces from a higher proportion of kidney positive fish.

Minimum infectious/lethal doses in susceptible species

Mortality data following intraperitoneal (i.p.) injection of *P. salmonis* demonstrates the virulence of the organism, with some variation among strains (Garces et al., 1991; House et al., 1999). Groups of Coho Salmon were injected with inocula ranging from $10^{3.3}$ to $10^{5.3}$ $TCID_{50}$ per fish (Garces et al., 1991). More than 80% of fish died in all groups, precluding the calculation of lethal dose 50% (LD_{50}) values. Similarly, House et al. (1999) used i.p. injection of 12 g Coho Salmon maintained in freshwater to compare the virulence of *P. salmonis* isolates from Chile, Canada and Norway. The study concluded that virulence varied among strains from the Chilean LF-89 (most) to the Norwegian NOR-92 (least). However, intraperitoneal injection is a severe and un-natural route of challenge and data generated using these methods are of limited value in understanding natural mechanisms of transmission and pathogenesis. No studies have estimated the minimum (infectious or lethal) doses of *P. salmonis* necessary to cause SRS or mortality in fish through exposure routes that mimic natural transmission pathways.

Survival of etiological agent in marine environment

Piscirickettsia salmonis is a pathogen of marine fish which survives poorly in freshwater (Lannan and Fryer, 1994). In a laboratory study, survival of the bacterium was influenced by temperature and salinity. Viability decreased between 5° and 20°C; at the warmest temperature viable bacteria were not detected after one week while after three weeks, viable bacteria were detected in the 5° and 10°C groups. Survival of *P. salmonis* in seawater over a period of 10 to 15 days was equal to or greater than that in tissue culture medium at 5°, 10° or 15°C. No viable bacteria were detected following incubations in freshwater of any duration or at any temperature (Lannan and Fryer, 1994). Despite this observation, Almendras et al. (1997) demonstrated horizontal transmission of *P. salmonis* in freshwater to cohabitant Atlantic Salmon in the absence of contact, suggesting that the bacterium is protected from osmotic degradation by mucus, feces or other material sloughed from infected fish. The apparent tendency of *P. salmonis* to form biofilms may further enhance its survival in marine environments (Marshall et al., 2012).

VIRULENCE AND PATHOGENICITY

Morbidity/mortality under experimental conditions

Whether by intraperitoneal injection, cohabitation or direct immersion, the clinical signs of SRS have been reproduced in experimentally infected fish and Koch's Postulates have been satisfied in several salmon species (Rozas and Enriquez, 2014; Rozas-Serri et al., 2017).

In contrast to injection, exposure of naïve salmon by immersion in cultured bacteria is a more natural route of transmission which permits the quantification of transmission parameters. Birkbeck et al. (2004) exposed Atlantic Salmon to 1 x 10^5 $TCID_{50}$ mL^{-1} of a Scottish isolate of *P. salmonis* for 60 min. After 18 d, a total of 1 of 10 fish died. In another study, Rainbow Trout were immersed for 15 min in 10^5 $TCID_{50}$ mL^{-1} of a cultured Chilean strain of *P. salmonis* (Smith et al., 2015). In that study, a total of 95% of Rainbow Trout died between 9 and 24 days following exposure and no other doses were tested.

Morbidity/mortality in wild fish populations

There are no morbidity or mortality data associated with *P. salmonis* infection in wild fish.

INCIDENCE AND PREVALENCE IN BRITISH COLUMBIA

WILD FINFISH POPULATIONS

Piscirickettsiosis was reported in seawater-reared Pink Salmon in a research aquarium in Nanaimo in 1970 and 1978 (Evelyn et al., 1998). Evidently these were wild-caught salmon (Brocklebank et al., 1992); however, neither the origin of the infection nor its prevalence was reported. Injection of infected tissue from the Pink Salmon caused mortality in Pink, Chinook and Coho salmon and the bacterium was transmitted to naïve cohabitants from infected Pink Salmon (Evelyn et al., 1998). Molecular evidence of *P. salmonis* genomic DNA was reported in fewer than 1% of adult Fraser River Sockeye Salmon returning to spawn in freshwater (Chilko, n = 57; Lake Shuswap, n = 125) (Miller et al., 2014). No evidence of DNA from this pathogen was reported in 561 marine-caught Chinook Salmon of Fraser River origin (Tucker et al., 2018) or in 1666 marine-caught Chinook Salmon or in 344 marine-caught Sockeye Salmon from several origins (Miller et al., 2017). The latter two studies only reported positive results if they occurred at prevalence greater than 1%. These reports do not support Chinook or Sockeye Salmon serving as reservoirs of *P. salmonis* infection.

FARMED SALMON

There are anecdotal reports of SRS in farmed Coho and Chinook salmon from the 1980s (Brocklebank et al., 1992; Evelyn et al., 1998). The first well-described outbreak occurred in farmed Atlantic and Chinook salmon maintained in separate cage systems at a single site near Vancouver Island in 1991 (Brocklebank et al., 1992; Brocklebank et al., 1993). The outbreak occurred in the autumn, approximately six weeks after a *Heterosigma* bloom. The Atlantic Salmon were summer-entry smolts averaging 400 to 500 g and daily mortality in two pens (n = 8500 / pen) during October increased from 0.01% to 0.06% with a cumulative mortality of 8%. Moribund Atlantic Salmon collected in November displayed clinical signs consistent with a septicaemia. In January, fish were treated with in-feed oxytetracycline. Following treatment, daily mortality in Atlantic Salmon dropped from 0.66% to 0.02% (Brocklebank et al., 1993). In Chinook Salmon, internal signs were evident by December but mortality was negligible. An antibiotic-free tissue homogenate prepared from affected salmon induced cytopathology in

cultured cells and the infected cells gave a positive IFAT reaction with antibodies raised against *P. salmonis*, and presence of the agent was confirmed by PCR (Mauel et al., 1996, 1999).

British Columbia

Fish health information for farmed salmon in BC is obtained in two ways: fish health audits conducted by DFO Aquaculture Management veterinarians and staff (prior to 2011, audits were performed by the BC Provincial Government), and quarterly and annual submissions to DFO by industry, as a condition of licence (see Wade, 2017). Between 2002 and 2016, a total of 1229 audits were conducted on Atlantic Salmon farms in all management regions of BC (Table 2) representing an average of seven audits per month (range: 0 to 19). During this time, the fewest audits were conducted in December (69) and the highest number in February (129). The audits permit farm level diagnoses of SRS by DFO veterinarians 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 or PCR assay. Confidence in a diagnosis of infection increases with confirmatory testing, for example when histological findings are confirmed by PCR.

Between 2002 and 2016, there were a total of 36 farm-level diagnoses of SRS (Table 3). Approximately 56% of these occurred in Fish Health Zone (Zone) 2.3 (Figure 1; Southwestern Vancouver Island). Between 2004 and 2009, audits also recognized the disease in Zones 2.4, 3.1 and 3.3. No diagnoses were made in 2010, 2011 and 2012. In 2016, SRS was diagnosed at the farm level for the first time in Zone 3.2 (Discovery Islands) (Table 3).

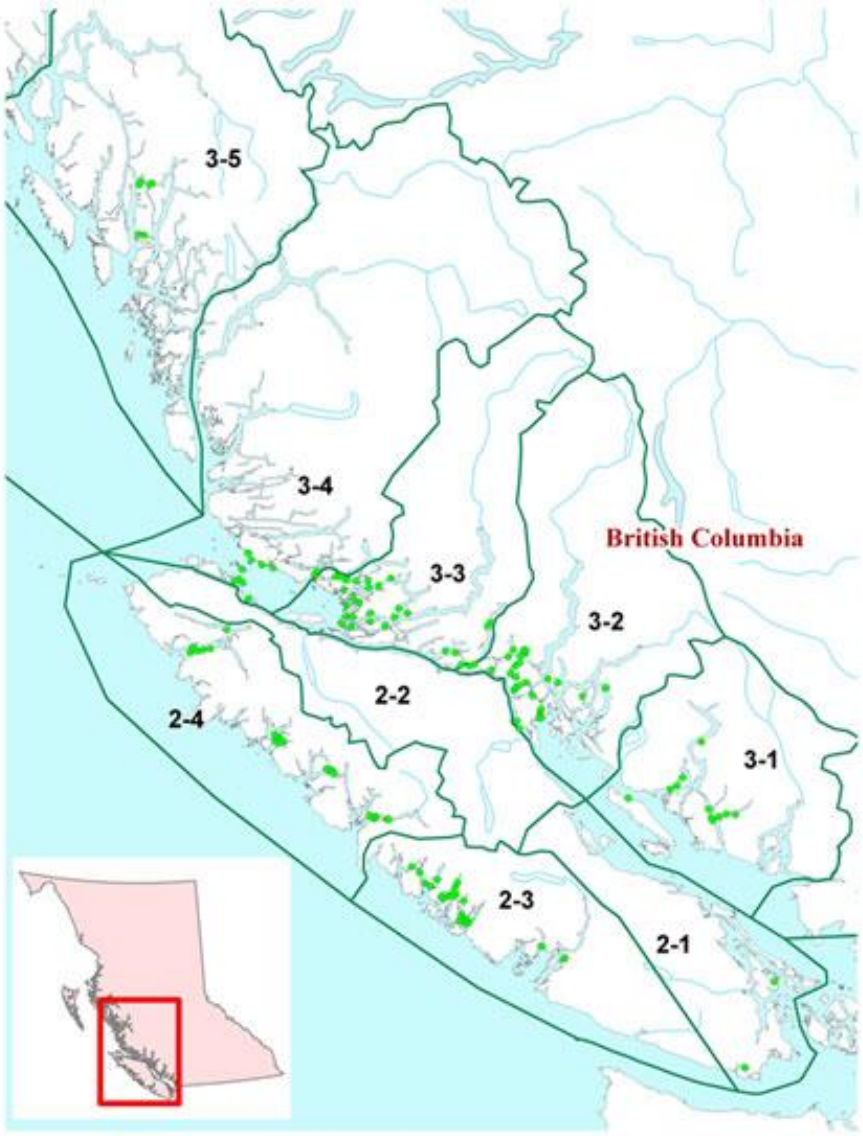

Figure 1. Map of DFO Fish Health Zones (reproduced from Appendix I-A (iii) Marine Finfish Aquaculture Licence 2015-2016).

Table 2. Total number and monthly average number of audits (BC provincial government (2002-2010) and DFO- Aquaculture Management Directorate (AMD) (2011-2016)) conducted on Atlantic Salmon farms in BC.

Month	Total number of audits conducted in British Columbia	Mean number (range) of audits conducted between 2002 and 2016
January	84	6 (0-11)
February	129	9 (0-13)
March	90	6 (0-14)
April	124	8 (0-19)
May	97	6 (0-14)
June	85	6 (0-12)
July	93	6 (0-12)
August	112	7 (0-14)
September	97	6 (0-13)
October	122	8 (0-13)
November	127	8 (0-13)
December	69	5 (0-8)
Total	**1229**	**NA**

Table 3. Summary of BC provincial government (2002-2010) and DFO-AMD (2011-2016) audit-based farm-level diagnoses of piscirickettsiosis (SRS) in seawater-reared Atlantic Salmon in British Columbia. Values in parentheses are the number of farms on which farm-level audit-based SRS diagnoses were reported.

Year	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										0
2004			1 (1)		1 (1)					2 (2)
2005			2 (2)		2 (1)		3 (2)			7 (5)
2006			1 (1)	1 (1)						2 (2)
2007			1 (1)							1 (1)
2008			1 (1)							1 (1)
2009			1 (1)							1 (1)
2010										0
2011										0
2012										0
2013			4 (3)							4 (3)
2014										0
2015			6 (6)	2 (2)	3 (3)					11 (11)
2016			3 (3)			3 (2)	1 (1)			7 (6)
$\Sigma_{subzone}$	**0**	**-**	**20 (8)**	**3 (3)**	**6 (3)**	**3 (2)**	**4 (3)**	**0**	**0**	**36**

Table 4. Summary of Fish Health Events (FHEs) (2002-2017) associated with piscirickettsiosis (SRS) reported by industry in seawater-reared Atlantic Salmon in BC. Dashes indicate no requirements to report FHEs. Values in parentheses are the numbers of farms on which a FHE attributable to SRS was reported.

Year	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			8 (5)							8 (5)
2003			1 (1)							1 (1)
2004			5 (3)							5 (3)
2005			1 (1)		1 (1)		2 (2)			4 (4)
2006			2 (2)							2 (2)
2007			3 (1)							3 (1)
2008			3 (3)		1 (1)					4 (4)
2009										0
2010										0
2011										0
2012										0
2013	-	-	-	-	-	-	-	-	-	-
2014	-	-	-	-	-	-	-	-	-	-
2015	-	-	-	-	-	-	-	-	-	-
2016			2 (2)		3 (3)		1 (1)			6 (6)
2017			2 (2)				1 (1)			3 (3)
$\Sigma_{subzone}$	**0**	**0**	**27 (10)**	**0**	**5 (4)**	**0**	**4 (3)**	**0**	**0**	**36**

Between 2002 and 2017, there were a total of 36 Fish Health Events (FHEs) in Atlantic Salmon attributed to SRS (Table 4). Approximately 75% of these occurred in Zone 2.3, 14% in Zone 3.1 and 11% in Zone 3.3. No FHEs associated with SRS occurred in Zone 3.2. Some events were associated with low mortality (data not provided) and did not require treatment with antibiotics. When required, treatment (oxytetracycline or more rarely florfenicol) was effective in reducing mortality.

HEALTH MANAGEMENT

PREVENTION

Strategies to prevent SRS in farmed salmon include stress reduction combined with husbandry practices that attempt to optimize biosecurity (e.g., limiting movement of staff and equipment among sites, reducing stocking density) and may include the use of effective disinfectants (Muniesa et al., 2018). In Chile, fallowing between fish cohorts at a site for a minimum of three months may be beneficial in reducing the risk of SRS re-emergence (Price et al., 2017). Although it has been reported that *P. salmonis* DNA persists in seawater in the vicinity of farms for up to 40 days following harvest of SRS-affected salmon (Olivares and Marshall, 2010), the source of this DNA was not confirmed. There is some evidence from salmon breeding programs that a wide range of susceptibility to infection is observed among Atlantic Salmon families (Dettleff et al., 2015). Further, the identification of quantitative trait loci through methods such as genomic predictions may accelerate progress in the selection of SRS resistance in farmed Atlantic Salmon in Chile (Bangera et al., 2017). These data indicate that selective breeding programs may provide increased resistance to SRS in Chilean stocks of farmed salmon.

IMMUNIZATION AND EFFICACY

The persistence of *P. salmonis* in the infected host is partly related to its ability to survive within host phagocytes (Rojas et al., 2009; Rojas et al., 2010). Infection within macrophages may also modulate the capacity of the infected host to mount an immune response (Tacchi et al., 2011), enhancing survival of the bacterium (Rise et al., 2004). A consequence of immune modulation may be to increase the susceptibility of fish with SRS to secondary infections (Lhorente et al., 2014). Vaccination provides an opportunity to increase host resistance to *P. salmonis* infection in healthy smolts prior to natural exposure to the bacterium. Experimental vaccines based on inactivated whole cell antigens of *P. salmonis*, with or without adjuvants, gave contradictory results in laboratory challenges (Smith et al., 1997; Birkbeck et al., 2004). Using production data from Chile, Jakob et al. (2014) reported significantly reduced mortality and delayed onset of SRS among vaccinated Atlantic Salmon that received an oral boost. The latter study also showed that the onset of SRS was delayed in vaccinated Rainbow Trout compared with controls. Evensen (2016) estimated that in excess of 25 commercial SRS vaccines are available to the market in Chile, most of which being inactivated, whole-cell formulations with adjuvant. Apparently these formulations, which often include antigens against other pathogens, provide short-term protection under laboratory conditions but there is little evidence that they protect against SRS through to harvest under production conditions (Evensen, 2016). Sub-unit and DNA-based vaccines require knowledge of protective antigens for which research is presently underway. An important challenge to optimizing the efficacy of SRS vaccines is improving our understanding of appropriate protective immune responses to intracellular bacterial pathogens in salmon. At present, vaccination does not offer significant protection against SRS in Chilean farmed salmon.

Although data are not available, some companies in BC have tested Renogen®, the live vaccine licenced for use against bacterial kidney disease, for efficacy against SRS. No other commercial vaccine with proven efficacy against SRS is available in Canada.

CONTROL AND TREATMENT

The intracellular habitat of *P. salmonis* partially shelters the bacterium from the antimicrobial activity of chemotherapy resulting in a need for more frequent treatments at higher antibiotic doses, despite the sensitivity of *P. salmonis* to a wide range of antibiotics *in vitro* (Fryer et al., 1990). Between 82% and 90% of all antibiotic use in Chilean aquaculture was prescribed for the treatment of SRS (Rozas and Enriquez, 2014; Price et al., 2018). In Chile there appears to be a relationship between salmon production biomass and the rate of antibiotic use (g drug / tonne production). Thus, the rate of antibiotic use was minimized in 2009 and 2010 when salmon biomass declined due to outbreaks of infectious salmon anaemia (Rozas and Enriquez, 2014). In production facilities, a range of sensitivities to antimicrobial treatments among salmon species and regions within Chile may indicate the risk of increased resistance to antibiotics in *P. salmonis* (Rozas and Enriquez, 2014; Yanez et al., 2014). More recent research indicates a need to initiate antibiotic treatment when SRS-associated mortality is low (Price et al., 2016). Furthermore, fish weight, species, water temperature and time since treatment predict the likelihood of therapeutic antibiotic concentrations occurring in tissues (Price et al., 2018; Price et al., 2019). In BC, in-feed florfenicol or oxytetracycline is administered under veterinary prescription following observations of clinical signs of SRS.

CONCLUSIONS AND RECOMMENDATIONS TO MINIMIZE TRANSMISSION

A total of 36 FHEs in Atlantic Salmon were attributed to SRS between 2002 and 2017. Approximately 75% of these occurred in Zone 2.3, 14% in Zone 3.1 and 11% in Zone 3.3.

Although *P. salmonis* has been diagnosed in farmed salmon in the Discovery Islands region of BC (Table 3), clinical SRS has not been reported (Table 4), and there were no FHEs associated with SRS. The vast majority of information on *P. salmonis* and SRS is derived from research related to salmon aquaculture in Chile. While this is informative with respect to understanding the biology of the bacterium and its interactions with the host, important differences between Chile and BC with respect to the magnitude and operation of the industry will limit the extent to which risk may be extrapolated to the BC context. The wide host range of *P. salmonis* indicates a high likelihood that Sockeye Salmon will be susceptible, despite the absence of direct evidence of infection in this species. It is useful to recognize that SRS outbreaks are often triggered by elevated water temperature and other environmental stressors, whether of environmental or farm origin.

Important information that will inform a risk assessment is still lacking. These include:

- Confirmation of susceptibility and pathogenesis in Sockeye Salmon;
- Salmon species-specific bacterial shedding rates;
- Characteristics of the attenuation of bacterial viability under natural conditions;
- The entire Fish Health Event dataset should be examined more carefully to test the hypothesis that SRS outbreaks tend to follow stressful events such as periods of elevated water temperature, algal blooms, hypoxic conditions or sea lice treatments.

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