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Characterization of *Yersinia ruckeri* and enteric redmouth disease (ERM) to inform pathogen transfer risk assessments in British Columbia

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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.

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ABSTRACT

Yersina ruckeri is a gram-negative enterobacterium that causes enteric redmouth disease (ERM), a septicemic bacterial disease of fishes. It is a common pathogen of salmonids and particularly Rainbow Trout (*Oncorhynchus mykiss*). All salmonid life history stages are susceptible, but the disease is most acute in Rainbow Trout fry and fingerlings and presents as chronic in older, larger fish.

Yersinia ruckeri and ERM are most commonly found in freshwater life history stages but have been reported from fish in the marine environment. *Y. ruckeri* is often found in freshwater salmonid hatcheries but can be prevented with proper egg disinfection and husbandry, including minimizing fish stress and vaccination. Should disease occur, it is readily and effectively treatable with antibiotics.

Yersinia ruckeri and ERM have been identified in both the freshwater and marine life history stages of Atlantic Salmon although, reports in the marine life history stage are not common. Outbreaks have occurred in Atlantic Salmon (*Salmo salar*) in both marine and freshwater. *Y. ruckeri* isolates from Sockeye Salmon (*O. nerka*) have been used experimentally but it was not possible, based on the literature, to determine if disease in this species has occurred. Although there are several genetic strains of *Y. ruckeri*, in cultured salmonids, ERM is mainly caused by the highly virulent, serotype O1a, biotype 1. Outbreaks have occurred in salmonids attributable to other serotypes but to date, there is no indication that new isolates or serotypes have been identified in Atlantic Salmon in North America.

INTRODUCTION

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

It is recognized that there are interactions between aquaculture operations and the environment (Grant and Jones, 2010; Foreman et al., 2015). A series of environmental risk assessments 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 partial response to the outcome of Cohen (2012), DFO Aquaculture Management Division requested formal science advice on the risks of pathogen transfer from Atlantic Salmon (*Salmo salar*) farms to Fraser River Sockeye Salmon (*Oncorhynchus nerka*). Given the complexity of interactions between pathogens, hosts and the environment, DFO is delivering this science advice through a series of pathogen-specific risk assessments followed by a synthesis. Pathogens which may be assessed were determined through the British Columbia Provincial and DFO Fish Health Audit and Surveillance Program (Audit Program) and Fish Health Events (FHEs) reported by the industry. 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 pathogen and, there must be evidence of temporal overlap of disease on Atlantic Salmon farms and presence of Fraser River Sockeye Salmon.

In 2014, the Department undertook the first of the series of pathogen risk assessments; to determine the risk to the diversity and abundance of Fraser River Sockeye Salmon due to infectious hematopoietic necrosis virus (IHNV) transfer from Atlantic Salmon farms in the Discovery Islands. The risk assessment was reviewed through the Canadian Science Advisory Secretariat peer review process and successfully completed in 2017 (Mimeault et al., 2017).

Four bacterial pathogens have been identified to undergo the next in the series of risk assessments, *Renibacterium salmoninarum*, *Aeromonas salmonicida*, *Yersinia ruckeri* and, *Piscirickettsia salmonis*. This paper synthesizes the information pertinent to *Y. ruckeri*, the causal agent of enteric redmouth disease (ERM).

PURPOSE OF THIS DOCUMENT

The information summarized in this document will assist in the assessment of the risk to Fraser River Sockeye Salmon due to the transfer of *Yersinia ruckeri*, the causative agent of enteric redmouth disease (ERM), from Atlantic Salmon farms located in the Discovery Islands area of British Columbia (BC). The purpose of this document is not to be an exhaustive review of ERM but rather focuses on the natural distribution of the pathogen and the characteristics that affect

its transmissibility, pathogenicity and virulence to susceptible wild species occurring in the Discovery Islands area.

PATHOGEN CHARACTERIZATION

GENERAL DESCRIPTION

Yersinia ruckeri is a gram-negative enterobacterium that causes enteric redmouth disease (ERM), a septicemic bacterial disease (Kumar et al., 2015). *Y. ruckeri* is approximately 0.75 µm in diameter and 1-3 µm in length and has a 3.7 Mb genome (Ewing et al., 1978; Navas et al., 2014; Kumar et al., 2015). *Y. ruckeri* enters the body of fish through the secondary gill lamellae where it spreads via the blood to internal organs (Kumar et al., 2015). It can but does not always cause subcutaneous hemorrhages at the corners of the mouth and in the gums and tongue (Barnes, 2011; Kumar et al., 2015). Behavioural changes include swimming at the water surface, lethargy and loss of appetite (Kumar et al., 2015). Clinical signs may include exophthalmia, darkening of the skin, splenomegaly and inflammation of the lower intestine and enlarged spleen (Kumar et al., 2015). Petechial hemorrhaging may occur on the surface of the liver, pancreas, pyloric caeca, swim bladder and in the musculature (Kumar et al., 2015). The spleen, kidney and liver may present with necrosis (Kumar et al., 2015). Significant heart pathology has been identified in Rainbow Trout during outbreaks which may partly explain the clinical symptoms such as slow swimming and lethargy (McArdle, 2014). Gill pathology can change with infection including hyperemia, oedema and desquamation of the epithelial cells of the secondary lamellae (Tobback et al., 2007).

ERM is an annually notifiable disease to the Canadian Food Inspection Agency (CFIA). That is, it is present in Canada and is a concern to some of Canada's trading partners. Only laboratories are required to contact CFIA upon suspicion or diagnosis of disease, and only once per year. Refer to Annually Notifiable Diseases webpage for more details.

GEOGRAPHIC RANGE

The causative agent was first isolated in Rainbow Trout from the Hagerman Valley in Idaho, USA (Ross et al., 1966). Barnes (2011) states that it is a safe assumption that *Y. ruckeri* will occur in fresh temperate waters wherever there are salmonid fish. Its range currently includes North and South America, Europe, Australia, New Zealand, South Africa, the Middle East, and China (Tobback et al., 2007; Austin and Austin, 2012; Shaowu et al., 2013). The global spread of the bacteria is likely the result of the movement of infected fish and fish products (Barnes, 2011); although Barnes et al. (2016) suggest that serotype 2 strains may have arisen from ancestral serotype 1 strains by a genetic change which has occurred several times and in different locations.

GENETIC STRAINS

Yersinia ruckeri has a 3.7 Mb genome with a 47% G + C ratio (Ewing et al., 1978; Navas et al., 2014) similar to other *Yersinia* species (Daligault et al., 2014; Navas et al., 2014). Strains of *Y. ruckeri* have been categorized into four serotypes with different sub-groups, two biotypes and outer-membrane protein types (Buller, 2014; Kumar et al., 2015). How these interrelate is outlined in Barnes (2011) and reproduced below in Table 1. Serotype O1 is divided into sub-groups O1a (serovar I, "Hagerman strain") and O1b (serovar III); serotype O2 (serovar II) is divided into three sub-groups namely, O2a, O2b and O2c; the other two serotypes are serotype O3 (serovar V) and serotype O4 (serovar VI) (Romalde and Toranzo, 1993). All serotypes occur in North America (Buller, 2014). *Y. ruckeri* is also categorized into one of two biotypes namely

biotype 1 (positive for motility and lipase secretion) and biotype 2 (negative for motility and lipase secretion) (Davies and Frerichs, 1989; Tobback et al., 2007; Evenhuis et al., 2009).

Most epizootics in salmonids are caused by serotype O1a (Romalde and Toranzo, 1993) including most naturally occurring outbreaks in Rainbow Trout (McCarthy and Johnson, 1982). Serotype O1a was considered the most virulent strain (McCarthy and Johnson, 1982) until the identification of new clonal groups with high virulence (Tinsley et al., 2011). These new clonal groups are described in the virulence section.

Table 1. Serotype scheme (columns 1-3) proposed by Romalde et al. (1993) in relation to O-antigen serogroup (column 4) from Stevenson et al. (1993) (compiled from Barnes (2011)).

Serotype	Subgroup	Former serovar	O-antigen serogroup
O1	a	I (Hagerman)	O1
	b	III (Australian)	O1
O2	a	II (Oregon)	O2
	b	II	O3
	c	II	O4
O3	n/a	V (Colorado)	O5
O4	n/a	VI (Ontario)	O6

HOSTS

Salmonids

Yersinia ruckeri hosts include both salmonid (Table 2) and non-salmonid (Table 3) species (Kumar et al., 2015); however, the most susceptible species is Rainbow Trout (Ross et al., 1966; Tobback et al., 2007; Meyers et al., 2008).

All salmonid life history stages are susceptible, but the disease is most acute in Rainbow Trout fry and fingerlings and presents as chronic in older larger fish (i.e., >12.5 cm) (Austin and Austin, 2012; Kumar et al., 2015).

ERM is considered one of the most significant diseases of freshwater trout aquaculture (Arias et al., 2007). Although most often reported in freshwater species or freshwater life history stages (i.e., parr), it can occur in salt water. ERM has been reported to occur in Atlantic Salmon smolt three to six weeks post saltwater transfer (Carson and Wilson, 2009). *Y. ruckeri* has been isolated and disease reported from 1-3 kg Atlantic Salmon from a marine farm in Norway (Sparboe et al., 1986) and *Y. ruckeri* has been isolated from one wild Atlantic Salmon found in freshwater after spending two years at sea in Scotland (Petrie et al., 1996). Clinical signs of disease typical of ERM were not found in this fish (Petrie et al., 1996). Arkoosh et al. (2004) report the isolation of *Y. ruckeri* from juvenile Coho Salmon (*O. kisutch*) and sub-yearling Chinook Salmon (*O. tshawytscha*) from estuaries in Washington and Oregon states, clinical signs of disease were not found. Farmed Chilean Coho Salmon have experienced outbreaks of *Y. ruckeri* (Bastardo et al., 2011a; Avendano-Herrera et al., 2017), it is assumed that these outbreaks occurred in freshwater as some are referred to as “freshwater farms”.

Table 2. Salmonid species from which Yersinia ruckeri has been isolated.

Common name	Scientific name	References
Arctic Charr	*Salvelinus alpinus*	Collins et al. (1996); Willumsen (1989)
Atlantic Salmon	*Salmo salar*	Petrie et al. (1996); Sparboe et al. (1986); Dear (1988); Rintamaki et al. (1986)
Brook Trout	*Salvelinus fontinalis*	Stevenson and Daly (1982)
Brown Trout	*Salmo trutta*	Arias et al. (2007); Fuhrmann et al. (1984)
Chinook Salmon	*Oncorhynchus tshawytscha*	McDaniel (1971); Arkoosh et al. (2004)
Coho Salmon	*Oncorhynchus kisutch*	Arkoosh et al. (2004); Avendano-Herrera et al. (2017)
Cutthroat Trout	*Oncorhynchus clarkii*	Reported in Daly et al. (1986)
Dolly Varden	*Salvelinus malma*	Reported in Daly et al. (1986)
Lake Trout	*Salvelinus namaycush*	Reported in Daly et al. (1986)
Northern Whitefish	*Coregonus peled*	Rintamaki et al. (1986)
Rainbow Trout	*Oncorhynchus mykiss*	Ewing et al. (1978); Fuhrmann et al. (1983); Stevenson and Daly (1982); Savvidis (1990); Timur and Timur (1991); Bastardo et al. (2011b)
Sockeye Salmon	*Oncorhynchus nerka*	Bullock et al. (1978)
Steelhead trout	*Oncorhynchus mykiss*	Reported in Daly et al. (1986)
Whitefish	*Coregonus muksun*	Rintamaki et al. (1986)

Sockeye Salmon

It is difficult to define the relative susceptibility of Sockeye Salmon to *Y. ruckeri* as compared to other salmonids based on the literature. It is equally difficult to determine if disease or outbreaks have occurred in Sockeye Salmon. It can be confirmed that isolates of *Y. ruckeri* from Sockeye Salmon have been used experimentally. The book *Fish Medicine* (Shotts and Nemetz, 1993) states that *Y. ruckeri* has been isolated and disease has been reported in many species including Sockeye Salmon, but there are no references to support this statement.

Austin and Austin (2012), *Bacterial Fish Pathogens Disease of Farmed and Wild Fish,* references “Pacific salmon” from Bullock et al. (1978) which in turn, does mention Sockeye Salmon. Bullock et al. (1978) compares isolates from different species and locations and refers to an isolate from Sockeye Salmon in Alaska. That is all the information they provide. It does not mention the age of the fish, freshwater or saltwater, if they exhibited clinical signs of disease or if there was an outbreak.

Austin and Austin (2012) also mention variation in immunity to *Y. ruckeri* among Coho, Sockeye and Pink salmon but provides no reference. There are three references cited in the paragraph, however: Raida and Buchmann (2008), Lamers and Muiswinkel (1984) and Johnson and Amend (1983b). There is no mention of Sockeye Salmon in Raida and Buchmann (2008), and Lamers and Muiswinkel (1984) is a book chapter about immune response in carp. Johnson and Amend (1983b) is an experimental study where Sockeye Salmon were infected with *Vibrio anguillarum* and Rainbow Trout were infected with *Y. ruckeri*. There was no mention of Pink or Coho salmon. This claim of variation in immunity among the three species could therefore not be substantiated.

In *Fish Diseases and Disorders Volume 3* (Horne and Barnes, 1999), Horne and Barnes list Sockeye Salmon in Table 12.1 “Species from which *Yersinia ruckeri* has been isolated”, referring to Dulin et al. (1976). Dulin et al. (1976) provides a list of susceptible species including Sockeye Salmon. A citation specific to Sockeye Salmon is not provided but rather two citations

to a list of species: Busch (1973) and Holt and Conrad (1970). Holt and Conrad (1970) does not mention Sockeye Salmon, only fall Chinook Salmon, winter steelhead trout (*O. mykiss*), Rainbow Trout and Cutthroat Trout (*O. clarkii*). Busch (1973) only mentions the use of Sockeye Salmon isolate as an inoculum component.

In the latest version of *Fish Diseases and Disorders Volume 3* (Barnes, 2011), the susceptibility table has been replaced by a table of the major fish species infected by geographic distribution. Sockeye Salmon are not included in the table. It could therefore be presumed that although *Y. ruckeri* has been isolated from Sockeye Salmon as it has been used in studies (i.e., Busch (1973) and Bullock et al. (1978)), the author does not consider it a major susceptible species. In the text in this chapter the author does state however that "confirmed clinical outbreaks have occurred in Rainbow/steelhead trout, Cutthroat Trout, Brown Trout, Brook Trout, Coho Salmon, Sockeye Salmon and Atlantic Salmon (Busch 1982)." There are several issues with this statement, first Busch (1982) is actually Busch (1983) and will be referred to as such. Second Busch (1983) states that "clinical isolation" has occurred in those species not "clinical outbreaks" and third; Busch (1983) is not the source of this citation but rather McDaniel (1975).

McDaniel (1975) is not listed in the Busch (1983) references. There are two other references for McDaniel though, McDaniel (1971) and McDaniel (1979). There is no reference to Sockeye Salmon in McDaniel (1971). McDaniel (1979) states that confirmed isolation has been made in Sockeye Salmon without any further information or a citation.

Therefore, with this weight of evidence, it was not possible to confirm outbreaks or disease in Sockeye Salmon but isolation of *Y. ruckeri* from Sockeye Salmon could be confirmed.

Non-salmonids

Although *Y. ruckeri* is primarily a salmonid pathogen, it has been isolated from many species of freshwater and marine non-salmonids (Table 3). It has also been isolated from human wounds (De Keukeleire et al., 2014), muskrat (*Ondatra zibetica*) (Stevenson and Daly, 1982), European otter (*Lutra lutra*) (Collins et al., 1996) and the greater black-backed gull (*Larus marinus*) (Willumsen, 1989). Snails, crayfish and sculpins have been suggested as transmitting agents for *Y. ruckeri* in a freshwater system in Idaho, but the authors acknowledged that there is no substantiating evidence to support these suggestions (Dulin et al., 1976).

Table 3. Non-salmonid fish species from which Yersinia ruckeri has been isolated.

Common name	Scientific name	Reference
Freshwater/catadromous		
Amur Sturgeon	*Acipenser schrencki*	Shaowu et al. (2013)
Burbot	*Lota lota*	Dwilow et al. (1987)
Carp	*Cyprinus carpio*	Fuhrmann et al. (1984)
Channel Catfish	*Ictalurus punctataus*	Danley et al. (1999)
Cisco	*Coregonus artedii*	Stevenson and Daly (1982)
Emerald Shiners	*Notropis atherinoides*	Mitchum (1981)
European Eel	*Anguilla anguilla*	Fuhrmann et al. (1983); Fuhrmann et al. (1984)
Fathead Minnows	*Pimephales promelas*	Michel et al. (1986)
Goldfish	*Carassius auratus*	McArdle and Dooley-Martyn (1985)
Nile Tilapia	*Oreochromis niloticus*	Eissa et al. (2008)
Perch	*Perca fluviatilis*	Valtonen et al. (1992)
Roach	*Rutilus rutilis*	Valtonen et al. (1992)
Rudd	*Scardinius erytnropthalmus hesperidicus*	Popovic et al. (2001)
Siberian Sturgeon	*Acipenser baeri*	Vuillaume et al. (1987)
Sturgeon	*Acipenser* spp.	CFIA (see Table 5)
Walleye	*Sander vitreus*	CFIA (see Table 5)
Marine		
Sablefish	*Anoplopoma fimbria*	CFIA (see Table 5)
Saithe	*Pollachius virens*	Willumsen (1989)
Seabass	*Dicentrarchus labrax*	Vigneulle (1984) in Bullock and Cipriano (1990)
Seabream	*Sparus auratus*	Vigneulle (1984) in Bullock and Cipriano (1990)
Turbot	*Scophthalmus maximus*	Vigneulle (1984) in Bullock and Cipriano (1990); Baudin-Laurencin and Tixerant (1985) in Michel et al. (1986)

It is likely that the report of *Acipenser* spp. in Table 3 and Table 5 is either Atlantic Sturgeon (*A. oxyrinchus*) or Shortnose Sturgeon (*A. brevirostrum*) based on the location of the report. As Sablefish is cultured in BC, it is unknown if the report in Table 3 and Table 5 is from the brackish water hatchery or from wild or farmed fish in the marine environment.

DIAGNOSTIC METHODS

Identification of infection is most often first made based on clinical signs (see General Description section) which are common to many other Gram-negative septicaemias (Barnes, 2011). There are no specific early signs of disease other than general septicaemia (Carson and Wilson, 2009). Small fish may die without exhibiting any clinical signs of disease (Barnes, 2011). Confirmation of infection is made by culture of spleen, heart and kidney on tryptone soya agar (TSA) sometimes supplemented with 5% blood (Barnes, 2011). Successful identification can also be made based on biochemical attributes and laboratory mini-kits (i.e., API 20E) (Santos et al., 1993) but these methods cannot identify to species (Barnes, 2011).

Antigen detection using specific antibodies in enzyme-linked immunosorbent assay (ELISA) is used in plate and rapid formats (Barnes, 2011). Latex-agglutination can be used to determine subclinical infections (Romalde et al., 1995). Additionally, several different polymerase chain reaction (PCR) specific tests for *Y. ruckeri* have been developed (Eissa et al., 2008). Some PCR assays (i.e., Gibello et al. (1999)) are particularly useful in detecting low levels of *Y. ruckeri* which may aid in the detection of asymptomatic carriers (Tobback et al., 2007).

Barnes (2011) recommends isolation in pure culture followed by identification by amplification and sequencing of the 16S rRNA gene using the universal bacterial primers 27F and 1492R (Lane (1991) in Barnes (2011)) as the gold standard. Identification using this method is most successful where pure cultures can be achieved (Barnes, 2011). Results should be considered along with case history, clinical signs and diagnostic PCR (Barnes, 2011).

At DFO's fish health laboratory at the Pacific Biological Station, isolation is made on TSA and confirmed with antibody agglutination (C. MacWilliams, DFO, 3190 Hammond Bay, Nanaimo, BC, Canada V9T 6N7, pers. comm., 2018). PCR is not used for confirmation and API 20 NE is not used as it does not readily distinguish between *Yersinia* species (C. MacWilliams, DFO, 3190 Hammond Bay, Nanaimo, BC, Canada V9T 6N7, pers. comm., 2018).

All three companies raising Atlantic Salmon in marine cages in the Discovery Islands area conduct bacteriological screening and testing as part of their fish health management procedures. Presumptive diagnosis of *Y. ruckeri* is based on bacteriological cultures of kidneys (with or without additional tissues) on TSA plates, followed by bacterial isolation and testing (Gram staining (-), cytochrome oxidase (-)). Definitive pathogen identification is done by a reference laboratory (see Wade (2017) for a description of laboratories used by the three companies).

TRANSMISSION

Mechanism

Yersinia ruckeri infection spreads horizontally between fish by direct contact with carriers or infected animals (Tobback et al., 2007; Eissa et al., 2008). Carrier states have been identified in Rainbow Trout (Rucker (1966) in Barnes (2011); Busch and Lingg (1975)) and steelhead trout (in freshwater) (Hunter et al., 1980).

Busch and Lingg (1975) recovered *Y. ruckeri* from asymptomatic carrier Rainbow Trout infected by immersion and found 25% of the fish could carry the bacterium in their lower intestines 45 days post infection at 14.5^{o}C; regular shedding and recurrent infection and mortality occurred within the population on a cyclic basis on 30-40 day intervals. This cyclical shedding precedes the reappearance of gross pathological changes and mortality by 3-5 days (Busch and Lingg, 1975). Recurrent mortality in naturally infected populations could therefore occur throughout the year where the periodicity and mortality levels may be altered by seasonal variations in water temperature, loading factors, handling, stress, natural resistance and population immunity (Busch and Lingg, 1975).

It has been demonstrated in freshwater that steelhead trout carriers can transmit *Y. ruckeri* to healthy fish when stressed (i.e., temperatures are increased to 25^{o}C) but unstressed fish carriers did not transmit *Y. ruckeri* (Hunter et al., 1980). Fish can be carriers for an extended period of time. For example, two months after an ERM outbreak, *Y. ruckeri* can be isolated from the feces of carrier Rainbow Trout (Rodgers, 1992).

Although it is likely that Atlantic Salmon can be carriers, only one example could be found in the literature which specified a possible carrier state. Petrie et al. (1996) describe the isolation of *Y.*

ruckeri (serotype 1) from one wild Atlantic Salmon in Scotland. They suggest, however, that as the gross clinical signs are not those generally associated with ERM, the fish was only lightly infected or possibly an asymptomatic carrier.

As chronically infected fish are carriers which periodically shed pathogen into the water and water is a mode of transmission, carriers are a reservoir of infection (Dulin et al., 1976). It has been suggested that although Atlantic Salmon are not the most susceptible species, and the freshwater phase is the most common in which to have mortality, that mortality can be an issue in a marine farm as Atlantic Salmon can be carriers and may develop disease when stressed after seawater transfer (Bullock et al., 1976). This phenomenon has been demonstrated in Australia/New Zealand where Atlantic Salmon smolt three to six weeks post saltwater transfer developed ERM (Carson and Wilson, 2009). In BC, the average weight of Atlantic Salmon diagnosed with ERM through the Audit Program (see Table 7) was 159 g (n=4 audits) (data provided by Aquaculture Management, DFO).

True vertical transmission of *Y. ruckeri* has yet to be confirmed. Sauter et al. (1985) as summarized by Tobback et al. (2007) have suggested that it could be vertically transmitted as *Y. ruckeri* was recovered from disinfected unfertilized Chinook Salmon eggs. The presence of *Y. ruckeri* DNA has been reported in unfertilized Chinook Salmon eggs and ovarian fluid; although it was not possible to confirm using cell culture (Glenn et al., 2014).

Mammals, birds, invertebrates, fish and humans can be considered possible vectors for *Y. ruckeri* (See Non-Salmonids section) (Bastardo et al., 2015). However, the main source of infection is considered to be the shedding of a large number (unspecified) of bacteria from carrier fish or infected fish in the feces (Rodgers, 1992; Barnes, 2011) which is transmitted via water to uninfected fish. It has been demonstrated that carrier fish, however, do not shed enough bacteria to cause infection unless they are stressed (Hunter et al., 1980), no clinical signs were observed. No studies could be found which estimated bacterial shedding rates from *Y. ruckeri* infected fish.

Incubation periods and contributing factors

Unless otherwise stated, throughout this section if no species is specified it is because none were identified in the references. It could be presumed that because ERM is principally a trout disease that un-speciated statements can be attributed to Rainbow Trout and in freshwater.

Incubation period varies depending on the virulence of the strain and environmental conditions (Busch, 1983). Incubation time for the development of disease varies inversely with water temperature and is affected by health and stress (Busch, 1983). At 15^{o}C, five to seven days are required for the incubation of disease in newly exposed populations (Busch, 1983). For populations which have already been exposed to *Y. ruckeri*, a stressful environment can result in mortality within three to five days (Busch, 1983). This is similar to incubation periods described by Bullock and Cipriano (1990) and Bullock (1984) which suggested that incubation time is five to ten days at 13-15^{o}C.

In a bath challenge, incubation period to first mortality in Atlantic Salmon (average weight 2.15 g) at 12.5^{o}C in spring water was determined to be ten days with a total mortality of 54% within 21 days (Bullock et al., 1976). In Rainbow Trout, water transmission of *Y. ruckeri* was reported to have a five day incubation period to first mortality and 52% loss in 19 days in spring water; the average size of the fish was not reported (Rucker (1966) in Bullock et al. (1976)).

Temperature and salinity can both influence the establishment and severity of infection (Altinok and Grizzle, 2001; Altinok, 2004). Severity of infection is highest when water temperatures are between 15 and 18^{o}C and decreases at 10^{o}C and below (Austin and Austin, 2012).

For example, disease or outbreaks have been reported in freshwater in Rainbow Trout in England at 8ºC (Roberts, 1983), in Denmark at 16ºC (Dalsgaard et al., 1984) and in Saskatchewan at 17ºC (Wobeser, 1973). In Turkey, in 16 ppt water, disease was detected in Rainbow Trout at 16ºC, and total mortality reached 3% (Karatas et al., 2004), duration of outbreak was not specified. Vuillaume et al. (1987) report disease in farmed freshwater Siberian Sturgeon at water temperatures of 17ºC; within eight days of noticing symptoms 10% of the fish had died. An outbreak of ERM in Atlantic Salmon in sea cages in Norway was reported to occur after handling and grading at 10ºC (Sparboe et al., 1986), no indication of severity of outbreak. Experimental evidence has shown that mortalities can be greatly reduced in Rainbow Trout by increasing salinity; mortalities were reduced from 96.5% in freshwater to 75% in 9 ppt salinity (Altinok and Grizzle, 2001).

Disease and infection in Atlantic Salmon

Of the reports of disease and infection in Atlantic Salmon, most occur in freshwater. Details of these reports have been summarized to aid in the risk assessment. Incubation and shedding rates in Atlantic Salmon could not be ascertained. *Y. ruckeri* is responsible for infections in Atlantic Salmon in many countries: Australia (Carson and Wilson, 2009; Costa et al., 2011), Chile (Bastardo et al., 2011a), Norway (Sparboe et al., 1986; Shah et al., 2012), Finland (Rintamaki et al., 1986), Scotland (Ormsby et al., 2016) and Canada. No studies could be found which estimated the minimum infectious dose of *Y. ruckeri* to cause ERM in fish.

Although it is most common to find disease in freshwater life history stages of Atlantic Salmon, for example, parr in Scotland (Dear, 1988), smolt in Norway (Shah et al., 2012) or broodstock in Finland (Rintamaki et al., 1986), outbreaks of ERM have occurred in Atlantic Salmon in Norway in sea water (approximate size 1-3 kg) (Sparboe et al., 1986). In BC, *Y. ruckeri* has been isolated by culture from samples collected through the Audit Program (see Occurrence in Canada).

The one record that could be found of *Y. ruckeri* in a wild Atlantic Salmon was from that of a returning female in Scotland (Petrie et al., 1996). Clinical signs of disease typical of ERM were not found in this fish (Petrie et al., 1996).

Survival in the environment

Yersinia ruckeri can survive and remain infective in the environment (Tobback et al., 2007). Because *Y. ruckeri* produces an overexpression of flagellar proteins, it easily adheres to hard surfaces and readily form biofilms (Coquet et al., 2002; Tobback et al., 2007). Biofilms have been suggested as a source of recurrent infection in Rainbow Trout farms (Tobback et al., 2007). *Y. ruckeri* has been isolated in pond algae and sediment of a fish pond (Coquet et al., 2002) as well as from water and sewage sludge (Dudley et al., 1980).

Survival in water may be dependent on salinity as *Y. ruckeri* can survive in fresh or brackish water for at least four months, but survival is greatly reduced in 35 ppt water (Barnes, 2011) as indicated by Thorsen et al. (1992). In laboratory studies it has been found that *Y. ruckeri* cultures can survive starvation in unsupplemented water for at least four months (Thorsen et al., 1992). At low salinities (0-20 ppt) there were no detectable changes in colony forming unit (CFU) during the first three days of starvation and only a small decrease over the next four months. In 35 ppt water, the survival was decreased below detection limits (3 CFU mL^{-1}) after 32 days. Similarly, optimal growth for *Y. ruckeri* strains was reported to occur at 5 and 15 ppt (Diler and Ekici (2003) in Karatas et al. (2004)).

A study examining the survival of *Y. ruckeri* in different environments found that it could survive for more than three months in a river, lake and estuary (Romalde et al., 1994; Austin and Austin,

2012). The persistence of culturable cells in sediment was greater than that in water and was also greater at 6°C than at 18°C (Romalde et al., 1994; Austin and Austin, 2012). Importantly, this study provided evidence that *Y. ruckeri* may be capable of surviving in the environment in a dormant or non-culturable state (Romalde et al., 1994; Austin and Austin, 2012). That is, the number of culturable cells increased for the first 15 days after *Y. ruckeri* was seeded into the experimental system and then decreased over a 100 day period; virulence, however, was maintained even when it could not be cultured (Romalde et al., 1994; Austin and Austin, 2012).

Two studies could be found which described the UV inactivation or degradation of *Y. ruckeri*. Liltved and Landfald (1996) was primarily a photoreactivation study but did provide dose survival data after irradiation; Liltved et al. (1995) conducted laboratory studies testing inactivation of bacterial fish pathogens using ozonation and UV irradiation.

Photoreactivation of irradiated *Y. ruckeri,* strain CCUG14190 was demonstrated in laboratory experiments (Liltved and Landfald, 1996). In order to achieve 99.9% inactivation, a dose of 5.3 $mWscm^{-2}$ was required when subjected to liquid holding recovery and 4.9 $mWscm^{-2}$ to withstand photoreactivation (Liltved and Landfald, 1996). Although these results are informative, caution is warranted when applied to a natural marine environment particularly as methods are not relatable to natural conditions (e.g., samples held in the dark and supplemented with caffeine) and experiments were carried out at 20°C in phosphate buffered saline (PBS). However, to conduct reactivation experiments *Y. ruckeri* was first irradiated. Bacterial suspensions in petri dishes were irradiated at 7°C using a 15 W low pressure lamp (3.5 W of 254 nmUV output). Dose survival curves calculated with changing irradiation times resulted in a 99.9% reduction in viable counts at a dose of 1.2 $mWscm^{-2}$ for *Y. ruckeri* (Liltved and Landfald, 1996).

By contrast, Liltved et al. (1995) report a 99.999% reduction in viable bacterial count (5 log reduction) at a UV dose of 2.7 mWs cm^{-2} in brackish water at room temperature. Experiments were conducted with stock cultures originating from infected Atlantic Salmon, no isolate numbers were provided. No measurements of salinity to define “brackish water” or specific temperatures other than “room temperature” were provided.

The differences in doses required for a 99.99% reduction in viable bacterial counts between these two studies are not unexpected due to differences in experimental conditions, particularly, salinity, temperature and bacterial strain.

VIRULENCE AND PATHOGENICITY

Morbidity and mortality under experimental conditions

Because Rainbow Trout is the most susceptible species to *Y. ruckeri*, most experimental challenges have been conducted on trout species. No challenge studies could be found with *Y. ruckeri* and Atlantic Salmon conducted in saltwater. Three challenge studies involving Atlantic Salmon in freshwater will be summarized: Haig et al. (2011), Cipriano et al. (1986) and Bullock et al. (1976). Studies involving intraperitoneal (IP) challenges have not been included as they are not considered a relevant transmission route under normal farm conditions.

Four different Atlantic Salmon isolates of *Y. ruckeri* of serotypes O1, O2 and O5 and one isolate of Rainbow Trout origin (serotype O1) were used in bath challenges for Atlantic Salmon and Rainbow Trout (Haig et al., 2011). Bath challenges were conducted at 16°C, in addition two groups of Atlantic Salmon fry challenged at 12°C with serotype O1 isolate 06059 and serotype O5 isolate 05094 (Haig et al., 2011). All challenges consisted of four hour exposures at the following final concentrations: Atlantic Salmon fry (0.4 g) 1.2 to 4.8 x 10^7 CFU mL^{-1}, Rainbow Trout fry (0.35 g) 1.2 to 4.8 x 10^7 CFU mL^{-1}, Rainbow Trout (150-250 g) 8.7 x 10^6 and 2.3 x 10^8 CFU mL^{-1} and Atlantic Salmon parr (5-10 g) 1.19 x10^7 and 1.3 x 10^8 CFU mL^{-1}. Experiments

were terminated 17 days post challenge for fry experiments and 33-35 days for parr and adult experiments. Results of challenges conducted at 16°C are summarized in Table 4.

Table 4. Summary of results of four hour freshwater bath challenge studies with Yersinia ruckeri isolates in Atlantic Salmon and Rainbow Trout at 16°C (Haig et al., 2011). Final concentrations of bacteria were 1.19 x10^{7} and 1.3 x 10^{8} colony forming units (CFU) mL^{-1} for parr; 1.2-4.8 x 10^{7} CFU mL^{-1} for fry and between 8.7 x 10^{6} and 2.3 x 10^{8} CFU mL^{-1} for adults.

Isolate	Serotype	Origin	Atlantic Salmon				Rainbow Trout			
			Fry (0.4 g)		Parr (5-10 g)		Fry (0.35 g)		Adult (150-200 g)	
			Mortality (%)	Infected survivors (%)	Mortality (%)	Infected survivors (%)	Mortality (%)	Infected survivors (%)	Mortality (%)	Infected survivors (%)
06041	O1	RT	60	100	59	20	34	12	78	60
06059	O1	AS	74	10	63	67	0	26	0	24
07039	O1	AS	40	90	17	60	2	40	0	9
06060	O2	AS	42	100	4	70	10	36	0	4
05094	O5	AS	33	70	13	33	2	45	0	0

This study demonstrates the differences in virulence of *Y. ruckeri* isolates between species and life stages in freshwater. For example, at 16°C, isolate 07039 may result in 40% mortality and 90% infected survivors in Atlantic Salmon fry but only 2% mortality and 40% infected survivors in Rainbow Trout fry (Table 4). No mortality was reported in the large (150-250 g) Rainbow Trout challenged with any Atlantic Salmon isolates. Importantly, when challenged with an isolate of Rainbow Trout origin, Atlantic Salmon fry and parr mortality was 60% and 59% respectively. By comparison, mortality of Rainbow Trout challenged with the same isolate was 34% (Table 4).

The study also demonstrates that water temperature affects disease severity and progression when Atlantic Salmon parr are challenged with certain isolates in freshwater. That is, mortality in Atlantic Salmon when exposed to isolate 06059 (O1 serotype Atlantic Salmon origin) and 05094 (O5 serotype Atlantic Salmon origin) was higher at 16˚C (74% and 33%, respectively) as compared to 12˚C (30% and 4%, respectively) (Haig et al., 2011). The first mortality when challenged at 16˚C was at day 3 (06059 isolate), compared to day 5 (05094 isolate) at 12˚C (Haig et al., 2011).

Because an epizootic of serovar 2 strain of *Y. ruckeri* occurred in Chinook Salmon, virulence testing of this strain was conducted on both Atlantic Salmon (36 g) and Brook Trout (52 g) in 30 second dip exposures to 1.4 x 10^{9} bacteria mL^{-1} of isolate 11.86 (sorbitol positive serovar 2) and 3.5 x 10^{9} bacteria mL^{-1} of isolate 11.40 (sorbitol negative serovar I) (Cipriano et al., 1986). Both isolates were virulent; mean mortality was 90% in Brook Trout and 70% in Atlantic Salmon challenged with isolate 11.40, and mean mortality was 75% in Brook Trout and 100% in Atlantic Salmon challenged with isolate 11.86 (Cipriano et al., 1986). What is particularly important about this study was that until this time, it was believed that sorbitol positive strains of *Y. ruckeri* were not pathogenic to fish (Cipriano et al., 1986).

Yersinia ruckeri isolates of Chinook Salmon and Rainbow Trout were used in two bath challenges with Atlantic Salmon (average weight 2.15 g) (Bullock et al., 1976). The serotypes were not indicated, challenges were conducted in spring water. Fish were placed in a 1500 mL beaker with 10^{7} bacteria for a 30 minute exposure; they were observed for 14 days post exposure (Bullock et al., 1976). In a second experiment, uninfected fish were held in aquaria receiving effluent from infected Rainbow Trout; fish were observed for 21 days post exposure; water temperature was 12.5˚C (Bullock et al., 1976). Total mortality in fish exposed to contaminated Rainbow Trout effluent was 54%. Mortality in fish exposed to Rainbow Trout

isolate was 50% in each of two trials. Mortality in fish exposed to Chinook Salmon isolate was 30% and 50% (Bullock et al., 1976). No control fish died from ERM (Bullock et al., 1976). Only about half of those salmon dying from ERM showed clinical signs such as hemorrhages in the mouth, operculum or bases of fins (Bullock et al., 1976). Changes in histopathology were consistent with ERM (Bullock et al., 1976). In this study, the incubation period in Atlantic Salmon was ten days and 54% of the fish died within 21 days (Bullock et al., 1976).

In addition to these challenge studies, *Y. ruckeri* has been isolated from ulcerative lesions of Atlantic Salmon broodstock held in freshwater at farms in Finland (Rintamaki et al., 1986); it was not isolated from the internal organs. Up to 80% of the fish examined were affected and mortality reached 4% (Rintamaki et al., 1986). Fish were also heavily infected with *Saprolegnia*. *Y. ruckeri* was also isolated from the ulcers and livers of 3/33 yearling Atlantic Salmon examined (Rintamaki et al., 1986). These fish were being held in freshwater. At a nearby estuary, *Y. ruckeri* was isolated from 5/5 Atlantic Salmon fingerlings (Rintamaki et al., 1986); whether they showed clinical signs of ERM was not indicated.

Busch (1973) as indicated in Busch (1983) states that in Rainbow Trout, the LD_{50} by immersion varies with size of fish (statement only, no data provided) and duration of exposure. Consistently, however, a one hour exposure to a 24 hour broth culture of serovar I isolate diluted to a density giving 50% transmittance at 640 nm results in 100% infection and 30-70% mortality in 28 days at 15^{o}C (Busch (1973) in Busch (1983)). In a similar study with Rainbow Trout, Bullock et al. (1981) showed that serovar I Hagerman (serotype O1a) was more virulent than serovar II or serovar III. In a waterborne challenge 10 g Rainbow Trout were exposed to 10^8 - 10^9 bacteria mL^{-1} for 90 seconds. Percent mortality in the serovar I challenge was 65 to 95%, 0-20% in serovar II and 0% in serotype III (Bullock et al. (1981) in Busch (1983)). The LD_{50} in this same challenge was 3.0 x10^5 bacteria mL^{-1} for serovar I and 1.0 x 10^7 bacteria mL^{-1} for serovar II.

Repeated vaccination of broodstock Atlantic Salmon females against *Y. ruckeri* serovar O2 (presume synonymous with serotype O2) does not reduce the mortalities in offspring under challenge conditions (Lillehaug et al., 1996). Passive immunity protection does not occur (Lillehaug et al., 1996). No laboratory studies with Sockeye Salmon could be found.

Outbreaks

Losses attributed to ERM of 10-15% over a growth cycle are common (Barnes, 2011). Outbreaks of ERM usually begin with low mortality and slowly escalate, often resulting in high mortality (Tobback et al., 2007; Barnes, 2011). Larger fish typically have chronic, slower infections, but this can reach epizootics if the fish are stressed or the infection is mismanaged (Barnes, 2011). It is assumed that all of these statements are in reference to Rainbow Trout and, likely under culture conditions, but could apply to any trout species in freshwater.

Outbreaks of ERM have been reported in freshwater under culture conditions in Coho Salmon, Cutthroat Trout (Dulin et al., 1976), Rainbow Trout (Ross et al., 1966; Wobeser, 1973), Chinook Salmon (Cipriano et al., 1986), Brown Trout (Arias et al., 2007), Atlantic Salmon (Dear, 1988; Shah et al., 2012), Coho Salmon (Avendano-Herrera et al., 2017) and Channel Catfish (Danley et al., 1999).

An outbreak of ERM was reported in Atlantic Salmon in Norway in sea water (approximate size 1-3 kg) (Sparboe et al., 1986). It is unknown how long the outbreak lasted, but fish were treated with antibiotics for ten days and total mortality did not exceed 5% (Sparboe et al., 1986). However, in Norway, outbreaks in Atlantic Salmon are most commonly associated with juveniles farmed in freshwater (Shah et al., 2012).

Outbreaks are generally cyclic in pattern and are more related to a farm rather than a season or geographic area (Dulin et al., 1976). Fish stress and strain virulence have been identified as the major determinants of severity of outbreaks (Tobback et al., 2007). It is generally accepted that ERM outbreaks are connected with stress caused by poor environmental conditions (low dissolved oxygen, high water temperature, poor water quality), transportation, handling, and stress due to other diseases (Rucker (1966) in Valtonen et al. (1992); McDaniel (1971); Roberts (1983); Dear (1988)). Outbreaks can occur quickly after a stress event, as early as three to five days (Dulin et al., 1976).

In cultured salmonids and natural outbreaks, ERM is mainly caused by serotype O1a, biotype 1 (Austin and Austin, 2012), the most virulent strain (McCarthy and Johnson, 1982). However, in recent years, new strains of *Y. ruckeri* have been reported as causal agents in outbreaks of vaccinated salmonids in different geographic areas (Bastardo et al., 2011a). Some were attributed to emergent non-motile, Tween 80 negative, biotype 2 isolates (Austin et al., 2003; Fouz et al., 2006; Arias et al., 2007) and others to serotype O2b (Romalde et al., 2003). Because these vaccines are failing in some Atlantic Salmon growing regions such as the United Kingdom (UK), vaccines are being modified to include new isolates (L.A. Laidler, Marine Harvest Scotland Ltd. in Haig et al. (2011)). Recently, a new O serotype, O8, in biotype I isolates from farmed Atlantic Salmon in Scotland was also identified (Ormsby et al., 2016). Recommendations by Ormsby et al. (2016) were to include serotype O1 and O8 in vaccines for Atlantic Salmon and Rainbow Trout in the regions.

To date, there is no indication that new isolates or serotypes have been identified in Atlantic Salmon in North America; serotype O1a, biotype 1 remains the type of concern. Biotype 2 has, however, been identified in the US in hatchery-reared Brown Trout in South Carolina (Arias et al., 2007).

OCCURRENCE IN CANADA

Although an exhaustive search for the occurrence in species in Canada was not conducted, *Y. ruckeri* has been identified in Burbot (Dwilow et al., 1987); Cisco and Muskrat (Stevenson and Daly, 1982); Rainbow Trout (Wobeser, 1973); and Whitefish, steelhead trout, Cutthroat Trout, Chinook Salmon, Lake Trout and Dolly Varden as referenced in Daly et al. (1986). This species list can be further expanded to include those reported to CFIA (2013-2017) (Table 5). As an annually notifiable pathogen, there is a requirement for laboratories to report the suspicion or diagnosis of the disease to the CFIA. The report identifies the species and province of origin of the sample. The data provided by CFIA does not specify the number of reports for each species (Table 5). Specific data about the samples such as fish size, source (wild or farmed) and environment (fresh or salt water) are not reported to CFIA. The majority of reports are from Atlantic Salmon from BC (58/123).

In addition, since 2005, DFO's fish health laboratory at the Pacific Biological Station has had five detections of ERM in Chinook Salmon and one in Coho Salmon from specimens taken in freshwater hatcheries (C. MacWilliams, DFO, 3190 Hammond Bay, Nanaimo, BC, Canada V9T 6N7, pers. comm., 2018).

Table 5. Total Annually Notifiable Disease detections for Yersinia ruckeri/ERM submitted to the Canadian Food Inspection Agency (CFIA) between 2013 and 2017 by province. Source: CFIA, January 2018.

Common name	Scientific name	British Columbia	Manitoba	Ontario	Quebec	New Brunswick	Nova Scotia	Newfoundland and Labrador
Arctic Charr	*Salvelinus alpinus*				1	1		
Atlantic Salmon	*Salmo salar*	58				23	3	12
Brook Trout	*Salvelinus fontinalis*			4				
Brown Trout	*Salmo trutta*			1	1			
Chinook Salmon	*Oncorhynchus tshawytscha*	1		1				
Sablefish	*Anoplopoma fimbria*	2						
Sturgeon	*Acipenser* spp.					1		
Rainbow Trout	*Oncorhynchus mykiss*	2		7	4			
Walleye	*Sander vitreus*		1					
Total		63	1	13	6	25	3	12

FARMED ATLANTIC SALMON

Yersinia ruckeri and/or ERM has been identified in Atlantic Salmon on farms in BC through both diagnoses from the Audit Program conducted by DFO (and by BC provincial government before December 2010) and by the industry through the reporting of Fish Health Events (FHEs). A summary of detections for BC and specifically in the Discovery Islands are reported below.

British Columbia

Fish Health Events

A Fish Health Event (FHE) is defined as “a suspected or active disease occurrence within an aquaculture facility that requires the involvement of a veterinarian and any measure that is intended to reduce or mitigate impact and risk that is associated with that occurrence or event” in the Marine Finfish Aquaculture Licence under the Fisheries Act (DFO, 2015).

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

Between 2002 and 2017 (excluding 2013-2015), there were a total of five FHEs on Atlantic Salmon farms attributed to ERM (Table 6) and all were reported during the months of February and March. These were only reported in Fish Health Surveillance Zones (Zones) 2.3, 3.3 and 3.4 in 2004, 2005, 2006 and 2017. No FHEs associated with ERM were reported in Zone 3.2.

Table 6. Summary of fish health events (2002-2017-Q1) associated with enteric redmouth disease in seawater-reared Atlantic Salmon in BC. Dashes indicate no requirements to report FHEs. Numbers in parentheses represent the total number of individual farms where there were reported FHEs.

Year	Fish Health 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)
2005			1 (1)							1 (1)
2006							1 (1)			1 (1)
2007										0
2008										0
2009										0
2010										0
2011										0
2012										0
2013	-	-	-	-	-	-	-	-	-	-
2014	-	-	-	-	-	-	-	-	-	-
2015	-	-	-	-	-	-	-	-	-	-
2016										0
2017							2 (2)			2 (2)
$\Sigma_{subzone}$	**0**	**0**	**1 (1)**	**0**	**0**	**0**	**3 (3)**	**1 (1)**	**0**	**5**

The Audit Program

The Audit Program is conducted by DFO's BC Aquaculture Regulatory Program (BCARP) as a continuation of the provincial program prior to DFO assuming regulatory authority. Each quarter DFO audits the routine monitoring and reporting of a maximum of 30 farms (Wade, 2017). During these audits, samples are also taken for diagnostic testing as described in Wade (2017).

Between 2002 and 2016, a total of 1229 audits (average 7 audits per month) were conducted on active Atlantic Salmon farms in all management regions of BC; the fewest number of audits were conducted in December (n=69), the highest number in February (n=129) (Jones, 2019).

The audits permit farm level diagnoses of ERM to be generated 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 and/or culture assay. Confidence in a diagnosis of infection increases with confirmatory testing, for example when histological findings are confirmed by positive culture assay. *Y. ruckeri* has been detected in 18 audits (1.4 %).

Between 2002 and 2016, there were a total of six farm-level diagnoses of ERM (Table 7). Approximately 67% of these occurred in Zone 3.3. In 2008 and 2011, audits also recognized the disease in Zones 2.3 and 3.2. No diagnoses of ERM were made in 2002 to 2005, 2009, 2010 and from 2013 to 2016.

Table 7. Summary of British Columbia Provincial and Fisheries and Oceans Canada Fish Health Audit and Surveillance Program diagnoses (2002-2016) for enteric redmouth disease in seawater-reared Atlantic Salmon in British Columbia. Numbers in parentheses represent the total number of individual farms from which there were audit-based diagnoses.

Year	Fish Health 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										0
2005										0
2006							1 (1)			1 (1)
2007							1 (1)			1 (1)
2008			1 (1)				1 (1)			2 (2)
2009										0
2010										0
2011						1 (1)				1 (1)
2012							1 (1)			1 (1)
2013										0
2014										0
2015										0
2016										0
$\Sigma_{subzone}$	**0**	**-**	**1 (1)**	**0**	**0**	**1 (1)**	**4 (3)**	**0**	**0**	**6**

HEALTH MANAGEMENT PREVENTION

As *Y. ruckeri* is primarily a freshwater fish health issue, it is difficult to determine from the literature what health management activities are valid in both freshwater and marine as the difference is rarely specified and therefore, due to the nature of the pathogen it is assumed to apply to freshwater. For example, Barnes (2011) states that outbreaks can be prevented by avoiding the use of infected stock and by disinfecting eggs properly. It is presumed that this statement is generally applied to freshwater culture.

Further, Barnes (2011) states that where it is endemic, it can be prevented by minimizing stress, which is achieved with good husbandry and high water quality, and by vaccinating fish. Factors that are known to contribute to clinical and sub-clinical infections are stress-related, namely handling, grading and excessive stocking densities (Barnes, 2011). Infection can occur without these stress factors, but they typically occur when suspended organic matter in the water is coupled with high temperatures and low dissolved oxygen (Bullock and Snieszko, 1975; Knittel, 1981). Again, these statements may be more appropriate for freshwater trout culture than marine Atlantic Salmon culture. However, it is likely that minimizing stress, in general, would be a prevention measure suitable for either culture environment and species.

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 SOPs are a requirement of license, compliance is recorded as a part of the Audit Program (Wade, 2017). On-farm water quality parameters including temperature, dissolved oxygen and salinity are monitored daily in order to implement mitigation measures as possible (e.g., air lifts).

CONTROL AND TREATMENT

Although ERM can often be prevented, it is primarily controlled with antibiotics and vaccines, and to a lesser extent, immunostimulants and probiotics (Tobback et al., 2007).

Antibiotics

Typical antibiotics used in the treatment of ERM include sulfamerazine, oxytetracycline, tribrissen and oxolinic acid (Inglis et al. (1995) in Barnes (2011)). The only antibiotics authorized in Canada in aquaculture are oxytracycline hydrochloride (Terramycin-Aqua), trimethoprim and sulphadiazine powder (Tribressen 40% powder), sulfadimethoxine and ormetoprim (Romet 30), and florfenicol (Aquaflor) (Health Canada, 2018). Antibiotic resistance can occur in *Y. ruckeri* with repeated short term treatments (Tebbit et al. (1981) in Rodgers (1991)).

Antibiotic treatments have been prescribed in four of the five ERM FHEs in BC.

Vaccines

ERM has been controlled for decades with a monovalent killed whole cell vaccine (Bastardo et al., 2011a; Bastardo et al., 2015). These types of vaccines are highly effective when administered to salmonids >1 g by immersion, automated immersion, shower-spray, orally or by injection (Amend et al., 1983; Johnson and Amend, 1983a, b). The remarkable stability of the ERM bacterin vaccine has recently been demonstrated when a 35-year-old vial of unopened vaccine was found and tested to be efficacious (Welch et al., 2017).

Duration of immunity of *Y. ruckeri* bacterins was tested in Rainbow Trout and Chinook Salmon fry exposed in bath treatments (Johnson et al., 1982). In Rainbow Trout, after 170 days, immunity dropped in fish vaccinated at 1.8 g, but similar decreases in immunity were not seen in bigger fish (3.2 g) until 280 days (Johnson et al., 1982). In Chinook Salmon, immunity started to decrease after 120 days for fish vaccinated at 2.8 g or less but those vaccinated at 5.2 g did not decrease until after 100 days (Johnson et al., 1982).

Most commercial vaccines are based only on serotype O1a (serovar I Hagerman strain) (Bastardo et al., 2015) which causes the majority of disease outbreaks (Barnes, 2011), but these vaccines afford some cross protection among other serotypes (Stevenson and Airdrie, 1984). In North America, serovar I vaccines appear to be cross protective preventing infection of either type; this has not been the case in Norway or South America where other serogroups are being added to the vaccine for better protection (Barnes, 2011). Cipriano et al. (1986) have identified serovar II as significant for hatchery Chinook Salmon in Illinois.

Ermogen® (manufactured by Elanco), is the only *Y. ruckeri* vaccine licenced for use in Canada. The efficacy of other vaccines such as AquaVac ERM or AquaVac Relera are not discussed here; see Deshmukh et al. (2012) for further details on protection using these vaccines. Ermogen® is a serotype O1 bacterin vaccine delivered via immersion to fish 2 g or larger. It was not possible to obtain efficacy data for Ermogen® as it is proprietary.

Vaccine breakdowns have been reported in farmed Rainbow Trout (Austin et al., 2003; Fouz et al., 2006; Calvez et al., 2014) and in farmed Brown Trout in the US (Arias et al., 2007). However, these outbreaks were mostly attributed to the presence of non-motile variant of serotype O1, biotype 2 (Austin et al., 2003; Fouz et al., 2006; Arias et al., 2007; Barnes, 2011; Calvez et al., 2014; Bastardo et al., 2015). A similar phenomenon has been reported in Spain in Rainbow Trout by serotype O2b (Romalde et al., 2003) and in Australia and Chile in farmed, vaccinated, Atlantic Salmon with serotype O1b/biotype 1 strains (Bastardo et al., 2011a; Bridle et al., 2012).

Barnes (2011) explains these vaccine breakdowns in that the protective immunity of the host is driving the evolution of novel pathogen variants that are not recognized by the immunized animals. Further, he states that because it has taken over 30 years of widespread use of the vaccine for this evolution to occur, it is a testament to the efficacy of the killed bacterin vaccine.

Under field conditions, vaccine efficacy varies depending on fish stress. Vaccines rarely prevent disease completely when fish are stressed (Pickering and Pottinger, 1987). If stress is minimized, the incidence of disease in vaccinated populations is low enough that no medication may be required (Horne and Robertson, 1987).

In BC, vaccination of Atlantic Salmon with Ermogen® is voluntary and practices vary among companies. Cermaq Canada and Marine Harvest Canada vaccinate all Atlantic Salmon raised in hatcheries supplied with surface water with Ermogen®. This represents approximately a third of each company's production (B. Milligan, Cermaq Canada, pers. comm., 2018; D. Morrison, Marine Harvest Canada, pers. comm., 2018). Grieg Seafood has always and continues to vaccinate 100% of their Atlantic Salmon with Ermogen® and considers the vaccine efficacious (P. Wittaker and T. Hewison, Grieg Seafood, pers. comm., 2018).

It is not possible to determine with the data available if any of the five ERM FHEs in BC have occurred in vaccinated fish.

KNOWLEDGE GAPS

Although risk assessments are conducted to inform decision making based on the best information available at the time, information specific to *Y. ruckeri* or ERM which is lacking, but could inform the pathogen transfer risk assessment include:

- Knowledge of the susceptibility of Sockeye Salmon to *Y. ruckeri*;
- Evidence of disease (or outbreaks) attributable to *Y. ruckeri* in Sockeye Salmon in saltwater (or freshwater);
- Evidence of the transmission of *Y. ruckeri* or ERM between any species of *Oncorhynchus* in the marine environment at any life history stage;
- Incubation rates, shedding rates, lethal doses and infectious doses in Atlantic Salmon and Sockeye Salmon in saltwater (or freshwater); and
- Efficacy data for Ermogen®.

SUMMARY

Yersinia ruckeri is an opportunistic pathogen that rarely causes disease in healthy, unstressed fish. ERM is primarily a freshwater disease of salmonids, although it has been found in some marine fish species. Outbreaks are common in Rainbow Trout. Outbreaks have occurred in freshwater in Atlantic Salmon. They have occurred in saltwater in farmed Atlantic Salmon in British Columbia. Outbreaks could not be confirmed in Sockeye Salmon but *Y. ruckeri* has been isolated from the species, it is not known if the isolates were from fish in the freshwater or marine life history phases.

Most mortalities and outbreaks are caused by serotype O1a *Y. ruckeri*. Recently, there have been outbreaks in fish vaccinated with this type of bacterin vaccine attributed to the non-motile variant of serotype O1/biotype 2 or serotype O2b or serotype O1b/biotype I. It does not appear that vaccine failures have occurred when the virulent strain was serotype O1a. Although all serotypes are present in North America, to date, only serotype O1a biotype 1 has been

identified in Atlantic Salmon in North America; biotype 2 has, however, has been identified in hatchery reared Brown Trout in South Carolina. In Canada, Ermogen® is the only vaccine currently available for the prevention of ERM; it is a serotype O1 bacterin vaccine.

Temperature and salinity greatly affect the establishment and severity of infection. The disease has been reported to be most contagious when water temperatures are between 15-20^{o}C; this is presumed to refer to freshwater species. An outbreak of ERM in Atlantic Salmon in sea cages in Norway was reported to occur after handling and grading at 10^{o}C.

Mortalities can be greatly reduced by increasing salinity (e.g., from 96.5% in freshwater to 75% in 9 ppt water). Incubation period varies depending on the virulence of the strain, environmental conditions and species. In a bath challenge in spring water, the incubation period in Atlantic Salmon (average weight 2.15 g) at 12.5^{o}C was determined to be ten days with a mortality of 54% within 21 days. Chronically infected fish can be carriers of *Y. ruckeri*, periodically shedding pathogen into the water and therefore serving as a reservoir for infection. However, it has been demonstrated in steelhead trout that unless stressed, known carriers did not transmit *Y. ruckeri*.

Yersinia ruckeri survives well in the environment outside of a host. It can survive for many months in fresh or brackish water after an outbreak; it can also survive for many months in sediment. It can be isolated from sewage and readily forms biofilms on hard surfaces. Survival in water is sensitive to temperature and salinity. In general, survival is greatest in salinities less than 15 ppt and at lower temperatures (i.e., 6^{o}C vs.18^{o}C).

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