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Characterization of *Renibacterium salmoninarum* and bacterial kidney disease 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

Bacterial kidney disease (BKD) is a persistent, debilitating condition that affects all species of salmonids in freshwater or marine phases, including Atlantic Salmon (*Salmo salar*). Experimental studies indicate that Pacific salmon such as Chinook (*Oncorhynchus tshawytscha*), Sockeye (*O. nerka*), and Chum Salmon (*O. keta*) are more susceptible to infection and disease than Atlantic Salmon or Rainbow Trout (*O. mykiss*). The etiologic agent, *Renibacterium salmoninarum*, can be transmitted horizontally and vertically, and infection or disease can be detected at all life history stages. Both symptomatic and asymptomatic fish shed bacteria, and ingestion of bacteria is likely the most common route of horizontal infection. Organisms, rather than the environment (e.g., sediments) are likely to serve as reservoirs for the bacterium, including non-salmonid marine fish such as prey (Pacific Herring (*Clupea pallasii*)), sympatric species (Threespined Stickleback (*Gasterosteus aculeatus*)), Shiner Perch (*Cymatogaster aggregata*)), and potential parasitic fish (Western River Lamprey (*Lampetra ayresii*)), Pacific Lamprey (*Entosphenus tridentatus*)). Infected but asymptomatic salmonids are another important reservoir. Although a minimum infectious dose in seawater has not been experimentally determined, Chinook Salmon can be infected with as few as 700 bacterial cells mL^{-1} when exposed for 24 hours. Although *R. salmoninarum* has relatively short persistence in unfiltered seawater (>50% loss within eight hours), association with organic particles such as feces can extend viability to many days. Determination of incubation period and minimum infectious dose depends upon exposure dosage, water temperature, and species of salmon. Typical incubation periods for susceptible species exposed by immersion can range from 21 to 50 days. Water temperature effects on incubation period and mortality exhibit a nonlinear pattern. In laboratory studies, pathogenicity increases as temperatures increase from 8°C, then declines at ~ 15°C and up through higher temperatures. These observations contrast with field observations of increased clinical signs at higher temperatures, but there are often confounding factors (e.g., smoltification, crowding, decreased dissolved oxygen) associated with field observations. Among the factors associated with dissemination of BKD and *R. salmoninarum*, anthropogenic actions are the leading cause. This has been supported both by genomic analyses and by epidemiology around management efforts to control the disease. While there are a range of hygiene and rearing practices available, sensitive and specific surveillance with sufficient temporal resolution is the initial tool for management, as it will provide information on the effectiveness of hygiene and biosecurity. When an outbreak occurs, management actions should be followed up by effectiveness monitoring. This process can provide credibility for the actions and help to minimize the risk of transmission from net pen stocks to wild salmon populations.

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. However, it is recognized that there are interactions between aquaculture operations and the environment (Grant and Jones, 2010; Foreman et al., 2015) and DFO will be conducting a series of environmental risk assessments under the Aquaculture Science Environmental Risk Assessment Initiative to address the identified environmental stressors resulting from aquaculture activities, starting with the release of pathogens from aquaculture sites.

In partial response to the outcome of Cohen (2012), DFO is conducting assessments of 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 to be followed by a synthesis.

PURPOSE OF DOCUMENT

This document intends to review, highlight, and interpret the existing literature on *Renibacterium salmoninarum* and bacterial kidney disease (BKD) of salmonids relevant to inform and support the assessment of the risk to Fraser River Sockeye Salmon due to *R. salmoninarum* on Atlantic Salmon farms in the Discovery Islands area, and subsequently in other regions, of British Columbia. Consequently, this document focuses on the data and information pertinent to the transmission, survival, pathogenicity, virulence and prevalence of *R. salmoninarum*.

OVERVIEW

Bacterial kidney disease (BKD) is a global disease of salmonids caused by the Gram-positive bacterium, *Renibacterium salmoninarum*. BKD has been known since 1930's, when it was initially identified as Dee Disease in wild Atlantic Salmon (*Salmo salar*) in Scotland; (Mackie et al., 1933) and then subsequently among Brook Trout (*Salvelinus fontinalis*) and Brown Trout (*Salmo trutta*) at a Massachusetts State (USA) hatchery (Belding and Merrill, 1935). The disease continues to impact both aquaculture production and conservation activities wherever it occurs, in spite of decades of research and management efforts (Flagg and Mahnken, 1995; Hoffnagle et al., 2003). BKD was a mandatory notifiable disease to the World Organisation for Animal Health (OIE) through 2006, but is no longer on the list, perhaps due to its widespread geographic distribution. It is still a voluntary notifiable condition in Canada, the United Kingdom (UK), and the United States (US) by producers. The dominant factors contributing to management difficulties are: (1) chronicity and subclinical infections; (2) vertical and horizontal transmission; (3) occurrence and infectivity in both freshwater and saltwater environments; and (4) a slowly growing life history that is relatively refractory to bacteriostatic antibiotics.

CHARACTERIZATION

DISEASE

Clinical disease

BKD can span a spectrum of manifestations from an acute, high mortality condition to a chronic, subclinical, and low morbidity form. The disease is often described as being similar to

mycobacterial diseases such as tuberculosis due to the intracellular infection, slow bacterial growth, granulomatous host reaction, and chronic inflammatory sequelae of BKD. However, acute disease can cause epizootic mortality, with production losses as high as 80% in Pacific salmon stocks and 40% among Atlantic Salmon stocks (Evenden et al., 1993). In addition to causing mortality directly, *R. salmoninarum* infection can increase susceptibility to other causes of mortality (i.e., gas bubble trauma in Chinook Salmon) (Weiland et al., 1999). Clinical manifestations of acute disease include abdominal distention with ascites, anemia, epidermal darkening, exophthalmia, muscular cystic cavities, and lethargy (Fryer and Sanders, 1981; Evenden et al., 1993). Surprisingly, fish often continue to attempt to take food into later stages of morbidity (Pirhonen et al. (2000); pers. obs.). Internally, the signs are focal to multifocal pale nodular lesions on the kidney, which are granulomas containing bacteria. Sometimes, granulomas are observed in the spleen, heart, and liver. Pseudomembranous layers may cover internal organs, and petechiae in the abdominal walls and viscera may occur (Fryer and Sanders, 1981; Evenden et al., 1993). There are serum and hematologic changes, most notably decreased hematocrit, hemoglobin, total plasma proteins, blood urea nitrogen and potassium (Suzumoto et al., 1977; Bruno, 1986; Bruno and Munro, 1986a) and increased cortisol (Mesa et al., 1999). If fish survive acute infection, a membranous glomerulonephritis can develop as a result from deposits of immunoglobulin and bacterial antigen in the kidney's filtering apparatus (Sami et al., 1992). In addition to mortality directly attributable to the pathogen, clinical disease can alter behaviour that increases vulnerability to piscivorous predation (Mesa et al., 1998). Although clinical disease does not appear to inhibit smoltification physiology (Mesa et al., 1999), it does reduce the behavioural preference for saltwater at smoltification (Seals Price and Schreck, 2003), possibly due to reduced osmoregulatory capacity (Moles, 1997). This reluctance to enter saltwater can force fish to remain near the surface in fresher water lenses or hold longer in brackish water, making them vulnerable to bird predators or compromising a successful migration to sea. Furthermore, reduced osmoregulatory capacity is consistent with observations of poor survival associated with seawater transitions (Banner et al., 1983; Sanders et al., 1992).

Subclinical disease

While the presence of the infectious agent, *R. salmoninarum*, or its antigens does not necessarily confirm the presence of disease, in the absence of clinical signs it is interpreted as a significant risk for subclinical disease, particularly for broodstock that can transfer the bacterium to the next generation. In free-ranging populations, asymptomatic but infected fish are commonly detected (e.g., Arkoosh et al., 2004; Chambers et al., 2008; Rhodes et al., 2011; Kristmundsson et al., 2016), and whether these individuals can be considered as being subclinically diseased is unresolved. Subclinical infections can occur during early stages of an acute infection before host responses (which are responsible for the signs) have developed, or can result from suboptimal conditions for acute disease development. For example, experimental infection of Atlantic Salmon at low temperatures can result in infection profiles similar to those observed in asymptomatic commercially reared fish (Lovely et al., 1994). The relatively slow growth of *R. salmoninarum* and its potential ability to become metabolically dormant has been speculated to underlie some subclinical infections (Hirvela-Koski et al., 2006), and may be related to host species that are relatively resistant in developing disease (Meyers et al., 1993). Subclinical infection can persist through the lifecycle of the fish, through spawning and transfer to progeny. Subclinically infected fish can shed bacteria and serve as a source for horizontal transmissions (Balfry et al., 1996). Furthermore, there can be subclinical costs that could reduce fitness and survival to reproduction. Subclinical fish can have slightly elevated stress hormones such as cortisol (Mesa et al., 1999) and poor responses to stressors such as hypoxia (Mesa et al., 2000). Given the strong antibody response to the dominant

surface protein of *R. salmoninarum* (Alcorn and Pascho, 2002) and that surface protein's immunosuppressive capabilities (e.g., Turaga et al., 1987a; Siegel and Congleton, 1997), it is also likely that there is some degree of immunosuppression in fish with subclinical disease.

Geographic extent

The geographic extent of BKD and *R. salmoninarum* is considered to be global among all species of salmonids examined from the Atlantic and Pacific Oceans, and even from the Black Sea (Savas et al., 2006). It is deemed endemic or enzootic in salmonids worldwide except in Ireland, Australia, New Zealand, and the former Soviet Union (Fryer and Sanders, 1981; Jonsdottir et al., 1998; Meyers et al., 2003; Chambers et al., 2008; Wiens, 2011). It has been detected in remote populations that have not had enhancement activities such as Canada's Northwest Territories (Souter et al., 1987), wild populations with limited hatchery influence (Mitchum et al., 1979), and many free-ranging marine populations of salmonids of the North American west coast (Meyers et al., 1993; Arkoosh et al., 2004; Rhodes et al., 2006; Nance et al., 2010; Rhodes et al., 2011; Sandell et al., 2015).

It was detected among wild fish in Scotland from the early 1930's through 1961, but there were no reports from 1962 to 2003. In 2003 a wild live Atlantic Herring (*Clupea harengus*) within an infected Atlantic Salmon net pen was reported positive (Murray et al., 2012), and in 2005-2007 free-ranging fish (Threespine Stickleback (*Gasterosteus aculeatus*)) and escaped farmed Rainbow Trout (*Oncorhynchus mykiss*) collected in the vicinity of trout farms were reported as BKD-positive (Wallace et al., 2011). In Norway, BKD among farms was high through the early 1990's (Dale et al., 2008), but prevalence dropped after a severe culling program. The most recent surveys of 31 Atlantic Salmon farms (3339 samples) and four Rainbow Trout farms (193 samples) in Norway did not detect any BKD (Gjevre and Lyngstad, 2017). During the early years of salmon farming in Chile (1990's), BKD was one of the main diseases affecting production, but the more recent use of vaccines may have limited its impact (Ibieta et al., 2011). In Canada, significant prevalence of infection was detected among wild Atlantic Salmon smolts and returning adults in the Margaree River watershed (Nova Scotia) in 1957 (15 to 40%), 1965-68 (27 to 45%), 1977 (Atlantic Salmon parr: 0 to 70%; freshwater Atlantic Salmon adults: 44% total; saltwater Atlantic Salmon adults: 33% total; Rainbow Trout parr: 10 to 100%) (Paterson et al., 1979).

ETIOLOGICAL AGENT

Description and behaviour

The etiological agent of BKD is the Gram-positive bacterium, *R. salmoninarum*. It is a small (0.3 to 1.5 µm long by 0.1 to 1.0 µm wide) diplococcobacillus (i.e., short rods that occur in pairs) that is not motile, not spore-forming, and not acid-fast (Young and Chapman, 1978; Sanders and Fryer, 1980). It is a facultative intracellular pathogen with an optimal temperature range in culture between 15-18°C. It was originally isolated by Ordal and Earp (Ordal and Earp, 1956), and assigned to the genus and species by Sanders and Fryer (Sanders and Fryer, 1980). It is a slowly growing bacterium (Benediktsdóttir et al., 1991), with an approximate 24 hour doubling time in broth culture (Rhodes et al., 2008) and fastidious in nutrient requirements (Ordal and Earp, 1956; Evelyn, 1977; Teska, 1994). Its current classification in the order Actinomycetales is based on high G+C content, cell wall constituents, and whole genome sequence (Sanders and Fryer, 1980; Gutenberger et al., 1991; Wiens et al., 2008).

Upon infection, the bacterium can be detected within phagocytes of the filtering organs (kidney, spleen) in less than an hour, then it appears in peripheral monocytes and macrophages within ten days (Young and Chapman, 1978; Bruno, 1986). Subsequently, bacterial cells can be

detected in phagocytes of the heart, central nervous system, and thymus (Bruno, 1986; Speare et al., 1993; Flaño et al., 1996). Uptake by phagocytes is probably facilitated by the bacterium's binding of complement component C3b (Rose and Levine, 1992) and its adherence to leukocytes (Wiens and Kaattari, 1991). There is evidence that intracellular *R. salmoninarum* can evade destruction within phagocytes (Bandín et al., 1993; Bandin et al., 1995), perhaps by inactivating the TNF-α proinflammatory pathway (Grayson et al., 2002), and then escape the phagosome into the cytoplasm (Gutenberger et al., 1997). By occupying an intracellular habitat, the bacterium can exploit nutrient availability while evading detection and destruction by the immune system.

Virulence factors are bacterial gene products or bacterial molecules that are involved in causing disease. Often virulence factors are involved in attaching or invading the host, evading or suppressing a host immune response, and tissue destruction. Toxins and extracellular enzymes are common virulence factors, and several proteases, haemolysins, and exotoxins have been found in or cloned from *R. salmoninarum* (Smith, 1964; Bruno and Munro, 1982, 1986c; Shieh, 1988; Evenden et al., 1990; Rockey et al., 1991; Grayson et al., 1995b), but none have been demonstrated to function as a virulence factor. Hosts often sequester circulating serum iron in an attempt to starve pathogens of this essential metabolic cofactor. In turn, pathogens often use counteracting molecules to scavenge and store it. Iron acquisition through production of siderophores and the utilization of complex iron molecules such as haemin has been documented in *R. salmoninarum*, fulfilling some criteria as virulence determinants (Bethke et al., 2016). Additional potential, but not yet demonstrated, virulence factors include iron reductase (Grayson et al., 1995a) and haemin sequestration molecules (Wiens et al., 2008).

The only definitive virulence factor is the abundant extracellular molecule, major soluble antigen (MSA), sometimes called p57 due to its molecular weight of 57 kDa. MSA is an immunodominant surface antigen that is also secreted into the surrounding environment (Getchell et al., 1985; Turaga et al., 1987b; Wiens and Kaattari, 1989). It stimulates a strong antibody response in fish and mammals (Bartholomew et al., 1991; Alcorn and Pascho, 2002). In fact, the commercially available anti-*Renibacterium* polyclonal antibody from SeraCare (KPL # 01-96-9) raised in goats is overwhelmingly directed against MSA. The genetics of MSA are unusual because it is encoded by multiple gene copies. There are at least two full copies (*msa1*, *msa2*) in all strains examined (O'Farrell and Strom, 1999; Rhodes et al., 2004a; Brynildsrud et al., 2016), and both copies are expressed (Rhodes et al., 2002) suggesting the MSA protein is important. There are three pieces of functional evidence supporting a virulence role for MSA: 1) a naturally attenuated strain of *R. salmoninarum* isolated from Rainbow Trout (Bruno, 1988) has dramatically reduced virulence and poor expression of MSA (Senson and Stevenson, 1999); 2) gene-specific disruption of either *msa1* or *msa2* results in dramatically increased survival (3.5- to 5-fold) over the original parental strain (Coady et al., 2006); and 3) strains with more than two copies of *msa* exhibit greater virulence over strains with only two copies (Rhodes et al., 2004a). The MSA protein may have multiple functions, based on both *in vitro* and *in vivo* observations. It has been associated with agglutination in a variety of assays (Daly and Stevenson, 1987, 1989, 1990; Wiens and Kaattari, 1991), and may be involved in suppression of various components of the host immune response (Rockey et al., 1991; Fredriksen et al., 1997; Siegel and Congleton, 1997; Densmore et al., 1998; Rhodes et al., 2009). The potential for an immunosuppressive role for MSA has influenced vaccine research interest away from including the protein in formulations (see below).

Genetic types/strains

Studies over the years have found little variation among isolates from divergent parts of the world or over time. *R. salmoninarum* isolates exhibit low antigenic heterogeneity (Bullock et al.,

1974; Fiedler and Draxl, 1986; Wiens and Kaattari, 1989), with only a few antigenic groups reported (Bandin et al., 1992; Wiens et al., 2002). Subsequent genome sequencing found a high degree of genetic homogeneity with copy number variation of certain genes such as MSA and the p22 gene (Brynildsrud et al., 2014; Brynildsrud et al., 2016), and isolates carrying copy number variants appear to originate from North America (Brynildsrud et al., 2016). Both genomic sequencing and genotyping (by multilocus variable number tandem repeats) identified two clusters among isolates from Norway, the UK, and North America. One smaller cluster derived from primarily wild Atlantic Salmon in the UK and Norway, including isolates associated with the original Dee Disease in Scotland. The majority of isolates was derived from Rainbow Trout, Atlantic Salmon, and Pacific salmon species, and were most likely distributed geographically as a result of aquaculture-associated movements (Matejusova et al., 2013; Brynildsrud et al., 2014). Although *R. salmoninarum* has a high degree of host switching (i.e., same isolates causing disease in multiple species of salmon), certain Chilean strains are associated with Coho Salmon (Bayliss et al., 2018). Genetic homogeneity suggests homogenous pathogenic potential, and experimental infections have shown that individual isolates can cause disease in multiple salmonid species (e.g., Evelyn et al., 1973).

DIAGNOSTIC METHODS

LETHAL SAMPLING

Diagnostic methods for BKD have evolved over the past 50 years from microscopic morphology through the use of antibody recognition of bacterial antigens to the application of nucleic acid detection, and some of the approaches effectively combine technologies (e.g., Teska et al., 1995). In spite of the array of techniques, there is no single method that can provide all the information for management and research needs (Elliott et al., 2015), and use of complementary techniques can be applied (Nance et al., 2010). While most of the methods are intended for lethally collected samples, there is growing interest in applying them to non-lethally collected samples (see below).

The most commonly used tissues for lethal sampling are kidney and spleen, while heart, liver, and gill are often included, especially for histological examination. Direct bacterial culture from internal organs or visible lesions onto a cysteine-containing medium including added antimicrobials to suppress faster growing contaminants such as fungi (Evelyn, 1977; Austin et al., 1983) is often considered the best standard diagnostic method. However, the extremely slow growth of *R. salmoninarum* can delay a diagnosis by at least ten days, and typically longer for subclinical cases. The use of antigen recognition by anti-*R. salmoninarum* antibodies was applied to tissue smears or imprints by direct or indirect fluorescent antibody technique (FAT), as well as for identification of cultured bacteria (Lee and Gordon, 1987). A subsequent refinement of the FAT method, the quantitative FAT (qFAT), allowed estimates of infection intensity (Cvitanich, 1994). Probably the most widely used antibody-based analysis is the enzyme-linked immunosorbent assay (ELISA), which can be performed in a 96-well format for multiple samples (Pascho and Mulcahy, 1987; Pascho et al., 1991). Assays using monoclonal or polyclonal antibodies have been published, and a commercially available polyclonal goat anti-*R. salmoninarum* antibody has allowed standardization of ELISA across laboratories, even though there is some minor cross-reactivity to other bacteria (Brown et al., 1995). The emergence of nucleic acid detection has ushered in end-point PCR for sensitive presence/absence detection (Chase and Pascho, 1998) and quantitative PCR methods for approximations of bacterial burdens (Powell et al., 2005; Chase et al., 2006; Rhodes et al., 2006; Gahlawat et al., 2009; Halaihel et al., 2009).

The following summary table was compiled from a representative list of BKD diagnostic methods papers. The intent of the table is to allow general comparison of the important features of a type of diagnostic test (detection limit, sensitivity, specificity) and the principal disadvantages, rather than provide a fine resolution description of every variant of each diagnostic method. In some cases, divergent diagnostic sensitivity and specificity values are from different published studies, that evaluated experimentally infected fish (Elliott et al., 2013) or naturally infected farmed fish with and without lesions (Jaramillo et al., 2017). The quantitative assignments (low, medium, high) are based on both published literature and clinical experiences.

Table 1. Summarized comparison of detection methods for Renibacterium salmoninarum derived from the more commonly cited publications (Bullock et al., 1980; Elliott and Barila, 1987; Meyers et al., 1993; Olea et al., 1993; Elliott and McKibben, 1997; Chase and Pascho, 1998; Cvitanich, 2004; Suzuki and Sakai, 2007; Elliott et al., 2013).

Method	Lower detection limit	Sensitivity[A]	Specificity[B]	Determine viability?	Disadvantages
Culture	10 – 501 cfu mL^{-1}	Low (0.38, 0.30 - 0.92)	High (1.00, 0.99 - 1.00)[2]	yes	Slow (weeks); susceptible to contamination
Membrane FAT	10 – 100 cells mL^{-1}	High	High	no	Ovarian fluid only
Tissue smear FAT	10^6 cells mL^{-1} homogenate	High (0.76)	High (0.85)	no	Poor correlation with other assays
Tissue smear qFAT	30 cells mg^{-1} tissue	Medium (0.29 - 0.85)	High (0.96)	no	Labor-intensive
ELISA	0.3 µg mL^{-1} bacterial protein	High (0.70 - 0.99, 0.21 - 0.97)	Medium to High (0.60 - 1.00, 0.97 - 1.00)	no	Not necessarily correlated to number of cells; false positives for vaccinated fish
Nested PCR	10 cells $reaction^{-1}$	Low (0.20)	High (0.90)	no	High stringency lab conditions required
Chase qPCR	5 cells $reaction^{-1}$	Low (0.25, 0.82 - 0.98)	High (1.00, 0.83 - 0.89)	no	Expensive reagents and equipment required
RT-qPCR	316 cells mg^{-1} tissue	Medium	High	yes	Expensive reagents and equipment required

[A] Relative to other methods in table; when available, calculated sensitivity or specificity value in parentheses.
[B] If colonies are tested with Gram stain and/or anti-*Renibacterium* antibody.

For the purposes of establishing disease-free status or health certification, methods from the World Organisation for Animal Health (OIE) or American Fisheries Society Fish Health Section could be applied. Prior to the removal of BKD as an OIE-listed disease in 2006, the 2003

Manual of Diagnostic Test for Aquatic Animals used ELISA and FAT for screening, and culture on KDM-2 and PCR for confirmation. Currently, diagnostic procedures from the latest (2016) "Blue Book" published on-line by the American Fisheries Society Fish Health Section can be applied. The latter manual is helpful because it includes methods for confirming clinical disease and detecting subclinical infections.

NON-LETHAL SAMPLING

Ovarian fluid has been widely screened for *R. salmoninarum* using an antibody detection method (membrane FAT; Elliott and Barila (1987)), and it can be collected by nonlethal methods for repeat spawners. A comparison of methods applied to blood, mucus, and urine-fecal exudate identified a nested PCR technique on the exudate as most similar to results from the kidney and spleen (Richards et al., 2017). The most comprehensive assessment is an extensive comparison of six detection methods (culture, immunohistochemistry, ELISA, direct fluorescent antibody technique, nested end-point PCR, Taqman quantitative PCR) applied to five nonlethally collected tissues (mucus, fin clips, gill filament, blood, kidney biopsy) (Elliott et al., 2015). Nested PCR and quantitative PCR of mucus exhibited high diagnostic sensitivity (98% and 92%, respectively) and specificity (89% and 98%, respectively), and correlated with kidney infection intensity. Furthermore, the methods could detect infection at least five months after challenge, indicating that mucus could be used for longitudinal surveillance (Elliott et al., 2015).

CASE DEFINITION

At the fish level, case definition is based on presentation of clinical signs (see "Clinical Disease" above) and laboratory diagnostics, such as those outlined for BKD in the American Fisheries Society Fish Health Section's "Blue Book". Boerlage et al. (2017) attempted to develop BKD-specific case definitions for farm management by a retrospective examination of New Brunswick Atlantic Salmon net pen industry records from 2006-2013 (production and mortality records, veterinary reports, diagnostic testing at site level, applied treatments at pen level), and using veterinary judgments as a reference standard. Assuming that incoming smolts to a seawater farm are initially disease-free, the authors were able to establish a threshold of 1 or 2% total mortality during a rolling four-week period as a useful case definition at either the pen- or site-level, to be used in combination with credible field observations, positive test results, and pen treatments (Boerlage et al., 2017). The choice to use a 1% or 2% mortality threshold depended on whether higher or lower sensitivity, respectively, was desired, because specificity was the same for both values.

In BC, 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, 2015b).

EPIDEMIOLOGY

RESERVOIRS

Environmental reservoirs for *R. salmoninarum* have not been characterized, although organic-rich sediments or fecal accumulations have good potential to serve as a reservoir due to *R. salmoninarum*'s ability to persist several weeks under eutrophic conditions (Balfry et al., 1996). Infected spawned out carcasses may also serve as a source of bacteria, either by pathogen release into the stream or by direct feeding of fry on carcasses. Although current practices for nutrient enhancement attempt to control disease transmission by restricting movements within a

watershed, guidelines based on pathogen testing thresholds could prevent spread from sources with suspected acute BKD problems.

While *R. salmoninarum* is known to cause disease in salmon and trout, the pathogen has been detected in some non-salmonid species without associated pathology or clinical signs. Although these detections can be considered as potential reservoirs, definitive transmission to salmonids has not been demonstrated. In freshwater, these species have included minnow, Threespine Stickleback (Wallace et al., 2011); Sea Lamprey (*Petromyzon marinus*) (Eissa et al., 2006); Burbot (*Lota lota*) (Polinski et al., 2010); and European Eel (*Anguilla anguilla*) (Chambers et al., 2008). Potential marine reservoirs include Pacific Hake (*Merluccius productus*), Pacific Herring (*Culpea pallasii*), Starry Flounder (*Platichthys stellatus*) (Kent et al., 1998); Western River Lamprey (*Lampetra ayresii*), Pacific Lamprey (*Entosphenus tridentatus)* (Rhodes et al., 2011); Fat Greenling (*Hexagrammos otakii*), Bartail Flathead (*Platycephalus indicus*), and Yesso Scallop (*Patinopecten yessoensis*) (Sakai and Kobayashi, 1992). Experimental infections of Pacific Herring, Shiner Perch (*Cymatogaster aggregata*) (Evelyn, 1993), and Sablefish (*Anoplopoma fibria*) (Bell et al., 1990) have also demonstrated the potential of non-salmonid species to carry the bacterium.

Asymptomatic but infected salmon and trout should also be considered as reservoirs, as these fish can shed viable bacteria (Balfry et al., 1996). Furthermore, infection status can shift to a clinical state due to environmental change such as a seasonal temperature shift (Sanders et al., 1978; Jones et al., 2007) or increased density (Mazur et al., 1993).

SUSCEPTIBLE SPECIES AND LIFE STAGES

Experimental testing for susceptibility and pathogenicity are typically conducted using intraperitoneal injection. Intraperitoneal injection circumvents barriers presented by skin and epithelial tissues, and directly challenges systemic cellular and humoral immunity. Injection is widely used because it permits strict dosage and exposure control with low inter-animal variability. In contrast, transmission testing by cohabitation of diseased fish with naive fish or immersion of naive fish in bacteria-laden water is a better analogue for natural horizontal transmission than injection exposures, but dosage control is more difficult, infection kinetics are much more variable, and disease progression is much longer (months vs weeks). Consequently, the number of reported experimental exposures by cohabitation and immersion are far fewer than those by injection.

Salmonids

The *Oncorhynchus*, *Salmo*, *Salvelinus*, and *Coregonus* genera are primary hosts for developing BKD from *R. salmoninarum* infections. In addition to well documented BKD in Chinook Salmon (*O. tshawytscha*), Sockeye Salmon, Atlantic Salmon, and Rainbow/Steelhead Trout, BKD has been reported in several other species listed in Table 2.

Table 2. Salmonid species in which bacterial kidney disease (BKD) has been reported.

Common name	Scientific name	Reference
Arctic Char	*Salvelinus alpinus*	Souter et al. (1987); Kristmundsson et al. (2016); Gudmundsdottir et al. (2017)
Atlantic Salmon	*Salmo salar*	Paterson et al. (1979); Paterson et al. (1981)
Black Sea Salmon	*Salmo trutta labrax*	Savas et al. (2006)
Brook Trout	*Salvelinus fontinalis*	Mitchum et al. (1979); Meyers et al. (1993)
Brown Trout	*Salmo trutta*	Mitchum et al. (1979); Chambers et al. (2008); Kristmundsson et al. (2016); Gudmundsdottir et al. (2017)
Bull Trout	*Salvelinus confluentus*	Jones and Moffitt (2004); Jones et al. (2007)
Chinook Salmon	*Oncorhynchus tshawytscha*	Earp et al. (1953); Hendricks and Leek (1975); Banner et al. (1986); Sanders et al. (1992); Holey et al. (1998)
Chum Salmon	*Oncorhynchus keta*	Banner et al. (1986); Sanders and Barros (1986); Sakai et al. (1992); Kent et al. (1998); Bethke et al. (2016)
Coho Salmon	*Oncorhynchus kisutch*	Earp et al. (1953); Banner et al. (1986); Sanders et al. (1992); Kent et al. (1998); Bethke et al. (2016)
Cutthroat Trout	*Onchorynchus clarki*	Banner et al. (1986); Meyers et al. (1993)
Dolly Varden	*Salvelinus malma*	Meyers et al. (1993)
Grayling	*Thymallus thymallus*	Meyers et al. (1993); Chambers et al. (2008)
Lake Trout	*Salvelinus namaycush*	Souter et al. (1987); Meyers et al. (1993)
Lake Whitefish	*Coregonus clupeaformis*	Faisal et al. (2010)
Masu Salmon	*Oncorhynchus masou*	Kawamura et al. (1977)
Pink Salmon	*Oncorhynchus gorbuscha*	Bell (1961); Banner et al. (1986); Kent et al. (1998)
Rainbow/Steelhead Trout	*Oncorhynchus mykiss*	Evelyn et al. (1973); Mitchum et al. (1979); Austin and Rayment (1985); Banner et al. (1986); Sanders et al. (1992)
Sockeye Salmon	*Oncorhynchus nerka*	Sanders et al. (1978); Banner et al. (1986)

Susceptibility to infection is not necessarily equivalent to susceptibility to disease. However, several comparative studies have observed differences in dosages required to cause morbidity and mortality, which could be inferred as difference in disease susceptibility. A caveat about these comparative studies is that exposures were by intraperitoneal injections, a route of exposure that circumvents barriers to natural infection, such as mucosal immunity. Nonetheless, the results from experimental susceptibility testing have been corroborated by hatchery and field observations. Table 3 below provides a summary of comparative susceptibility studies, with species arranged in relative order within each individual study.

Table 3. Summary of comparative susceptibility studies of morbidity and mortality to Renibacterium salmoninarum by intraperitoneal infection. All challenges were conducted in freshwater. Within each study, the tested species are arranged from most susceptible (left) to least susceptible (right). Summary drawn from Kawamura et al. (1977); Sanders et al. (1978); Sakai et al. (1991); Starliper et al. (1997); Jones et al. (2007). "ND" indicates not determined; M = months (age).

Reference	**Parameter**	**Study Results**							
Kawamura et al. (1977)	% visible kidney lesions	Chum	Chinook	steelhead	Masu				
		42.9%	13.3%	9.1%	0.0%				
Sanders et al. (1978)	% mortality at 15ºC	Sockeye	Coho	steelhead					
		100%	76.0%	49.0%					
Sakai et al. (1991)	% mortality for 2 doses	Chum	Masu	Coho	Rainbow Trout	Japanese Charr (*Salvelinus pluvius*)			
	2×10^8	100 %	91.0%	87.5%	94.4%	80.0%			
	2×10^7	98.1%	53.0%	72.0%	15.0%	16.0%			
Starliper et al. (1997)	Based on LD_{50} values for isolate 33209	Brook Trout (9M)	Atlantic Salmon (10M)	Coho (13M)	Chinook (9M)	Atlantic Salmon smolts (18M)	Brown Trout (17M)	Rainbow Trout (9M)	Lake Trout (10M)
		1.4×10^5	---	3.0×10^5	5.6×10^5	5.3×10^6	8.6×10^6	2.3×10^7	2.9×10^8
	Based on LD_{50} values for isolate A34	Chinook (9M)	Atlantic Salmon (10M)	Coho (13M)	Atlantic Salmon smolts (18M)	Rainbow Trout (9M)	Brook Trout (9M)	Lake Trout (10M)	Brown Trout (17M)
		1.9×10^1	5.2×10^2	3.2×10^3	5.7×10^3	1.6×10^4	5.4×10^5	8.6×10^5	6.5×10^6
Jones et al. (2007)	% survival for i.p. dose ~1.3×10^6 per fish	Chinook Salmon	Arctic Charr	Rainbow Trout	Lake Trout	Bull Trout (*Salvelinus confluentus)*			
	9.3 ºC	ND	63.0%	67.0%	99.0%	95.0%			
	14.8 ºC	ND	ND	70.0%	95.0%	98.0%			
	% survival for i.p. dose ~2.8×10^6 per fish	Chinook Salmon	Arctic Charr	Rainbow Trout	Lake Trout	Bull Trout (*Salvelinus confluentus)*			
	9.3 – 9.4 ºC	10.0%	76.0%	ND	68.0%	76-89%			
	14.8 ºC	ND	50.0%	ND	94.0%	96.0%			

Based on studies conducted in freshwater, Sockeye, Chinook and Chum salmon can be considered more susceptible, while Lake, Brown, Bull, Rainbow trout, and steelhead can be considered less susceptible. Furthermore, Sockeye Salmon exhibited 100% mortality across a wider temperature range (6.7 - 20.5°C) than Coho Salmon or steelhead, supporting its greater susceptibility under varying conditions (Sanders et al., 1978). Only one study included Atlantic Salmon, and both age groups (10-month smolts and 18-month juveniles) exhibited an intermediate susceptibility, similar to Coho Salmon (Starliper et al., 1997). There are also tissue-specific differences among species, where central nervous system involvement is more common in farmed Atlantic Salmon than in Chinook Salmon (Speare, 1997).

Certain life history stages of salmonids appear to be more vulnerable to infection and disease development than others, with peak vulnerabilities during emigration to sea water and returning to fresh water for spawning. There is considerable variation in results around the vulnerability of salmonids to developing disease during smoltification or upon entry into seawater. While increases in clinical BKD have been found associated with seawater emigration (Banner et al., 1983), gill Na^+K^+-ATPase activity does not differ between uninfected and infected Chinook Salmon during peak smoltification, suggesting monovalent ion regulation is not impaired (Mesa et al., 1999). However, decreases in total serum immunoglobulin and antibody production are associated with smoltification, suggesting this stage may be more susceptible to infection or disease development (Melingen et al., 1995a; Melingen et al., 1995b). Infected Coho Salmon have much poorer survival in saltwater relative to freshwater, and were unable to adapt to salt water if previously exercised (Moles, 1997). Approximately 20% of three species of salmon volitionally migrating down the Columbia River toward the Pacific Ocean were infected with *R. salmoninarum*, and BKD-associated mortality increased when these fish were transferred to salt water (Sanders et al., 1992).

Sexual maturation and progression to spawning is a second known period of increased susceptibility in the salmon life history. Because infection can be acquired at any point prior to spawning and disease progression can be slow, spawning individuals probably carry the highest risk of disease. The rise of sex hormones and glucocorticoids such as cortisol have widespread physiological effects due to multi-tissue distribution of receptors (e.g., Maule and Schreck, 1990). Cortisol and testosterone can directly suppress immune cell function (Tripp et al., 1987; Slater and Schreck, 1997), and cortisol can induce cell death among B cells (Weyts et al., 1998). Immunosuppression is present in sexually maturing Sockeye Salmon adults, which display declines in immune functions such as complement activity and number of circulating lymphocytes (Alcorn et al., 2002). Although returning and spawning adult salmon retain some selective immunocompetence (Schouten et al., 2013), this life history stage is clearly more vulnerable to infection and disease development.

Non-salmonids finfish

Naturally infected non-salmonid finfish have already been listed above as potential reservoirs; however, these reports typically do not include evidence of pathology or clinical signs. BKD clinical signs were reported in Ayu (*Plecoglossus altivelis*) that were cultured in proximity to infected Masu Salmon, demonstrating both potential horizontal transmission and non-salmonid disease (Nagai and Iida, 2002). Experimental intraperitoneal infection of Sablefish induced morbidity and mortality, showing the potential for BKD in a non-salmonid (Bell et al., 1990). *R. salmoninarum* can also induce morbidity and mortality in European Carp (Common Carp), *Cyprinus carpio* (Sakai et al., 1989) that were experimentally infected by injection.

Others

Detection of fish pathogens, including *R. salmoninarum*, have raised concerns that bivalves could serve as reservoirs (Starliper and Morrison, 2000). However, a study found that Blue Mussels (*Mytilus edulis*) collected from the intertidal zone of Departure Bay, Nanaimo, BC can remove a large proportion (>90%) of bacterial cells suspended in seawater within two hours under laboratory conditions (25‰, 15°C), and the molluscs appear to be inactivating >75% of the cells by passage through the digestive tract (Paclibare et al., 1994).

GENETIC SUSCEPTIBILITY

There are intraspecific variations in susceptibility, and clear differences in genetic susceptibility have been demonstrated in Chinook and Coho salmon. A derived Great Lakes (Wisconsin)

stock of Chinook Salmon exhibited reduced susceptibility to BKD compared to its parental Green River (Washington) stock, possibly due to protracted epizootics experienced by the Great Lakes stock from 1988 to 1992 (Purcell et al., 2008; Purcell et al., 2014). Three populations of BC Chinook Salmon displayed differential phenotypic susceptibility to BKD (Beacham and Evelyn, 1992). Among the three stocks, the Kitimat River stock exhibited the least susceptibility and the Quinsam River stock the highest susceptibility, with the Nitinat River stock intermediate. In a related observation, selection against *R. salmoninarum* was found to be unlikely to enhance growth or survival of Chinook Salmon stocks due to low heritability estimates (Johnson et al., 2003). A comparison of six stocks of juvenile Coho Salmon identified the Chehalis River and Eagle River Hatchery stocks as most susceptible, based on cumulative mortalities (McGeer et al., 1991). Coho Salmon with one homozygous transferrin genotype exhibited greater vulnerability to BKD than a second homozygous or the heterozygote genotype (Suzumoto et al., 1977). A comparison of two strains of juvenile Coho Salmon in BC found that one strain (Kitimat River) had higher resistance to BKD than the second strain (Robertson Creek) (Withler and Evelyn, 1990). Susceptibility for three problematic bacterial diseases in Atlantic Salmon found the highest estimated heritabilities for BKD ($h^2 = 0.34$), suggesting a significant genetic component to resistance to BKD (Gjedrem and Gjoen, 1995). However, that value may have been inflated because many of the mortalities due to BKD were also infected with *Aeromonas salmonicida*. Nonetheless, the dynamics of the mortality pattern was more typical of BKD than furunculosis, leading the authors to speculate that selective breeding against BKD was feasible (Gjedrem and Gjoen, 1995).

MECHANISM AND DYNAMICS OF TRANSMISSION

The etiological agent can be transmitted both horizontally and vertically, presenting different challenges for disease management at different life history stages. Horizontal transmission can pose an infection threat at any stage post-hatch because the most likely route is through the gastrointestinal tract, perhaps through ingestion of contaminated water, tissue or feces (Balfry et al., 1996). Observed and experimental infections of fish such as Pacific Herring by *R. salmoninarum* present another route of horizontal transmission (Evelyn, 1993; Kent et al., 1998). Epidermal or eye infections can occur through direct exterior contact (Hendricks and Leek, 1975; Hoffmann et al., 1984) or through skin breaches, such as those associated with tagging (Elliott and Pascho, 2001). Experimental testing suggests, however, that the gills are not a common entry site (McIntosh et al., 2000).

In freshwater, highly infected juvenile Chinook Salmon can shed a range of 6.5×10^3 to 3.8×10^5 colony forming units (cfu) per hour into the water (McKibben and Pascho, 1999), posing a mobile and dynamic source of infectious agent. Proximity of diseased fish and fish density are logical factors contributing to infection. A comparison of juvenile Chinook Salmon cohorts that were barged or were permitted volitional migration down the Columbia River observed that infection prevalence was always greater in the barged cohorts (Van Gaest et al., 2011). Challenge studies indicate that cohabitation of infected and naïve fish is more effective than simple immersion in suspended bacteria (Murray et al., 1992), indicating a possible role for behavioural interactions of hosts and/or greater infective potential of *in vivo*-passaged bacterial cells may contribute to horizontal transmission.

There is evidence for density-dependent horizontal transmission in free-ranging populations of Chinook Salmon in Puget Sound. Analysis of fish newly emigrated to seawater over a six-month period (May through October) found that the capture location was the strongest predictor of infection, regardless of fish origin based on coded wire tagging (Rhodes et al., 2006) or genetic stock (Rhodes et al., 2011). Furthermore, fish density correlated with infection prevalence and was a significant predictive factor (Rhodes et al., 2011). These observations coupled with the

schooling behaviour of emigrating juvenile salmon suggest that horizontal transmission could occur at this life history stage.

The potential for water currents to transport shed bacteria between farms depends on pathogen persistence and sufficient concentration for infection. Although *R. salmoninarum* is not spore-forming for long term persistence, experimental inoculations with cultured bacteria display viability up to four days in unfiltered river water and seven days in unfiltered seawater (Austin and Rayment, 1985; Balfry et al., 1996). If associated with organic-rich particles such as fecal material, it maintains viability up to 21 days in fresh water under laboratory conditions (Balfry et al., 1996). Bacteria shed from infected fish can be detected in water under laboratory conditions (e.g., McKibben and Pascho, 1999; Purcell et al., 2016), and can be found in net pen water as well (Balfry et al., 1996). Due to the aggregation properties of *R. salmoninarum*, it is likely that bacteria preferentially associate with particulates such as feces and become associated with the benthos or sediments, reducing the time in suspension (Austin and Rayment, 1985) and potentially limiting the range of waterborne distribution. Natural pressures, such as grazing by predators or competition with other microbial communities may also limit distribution of *R. salmoninarum* in the environment, as persistence in filter-sterilized river water or seawater is longer than in unfiltered water (Austin and Rayment, 1985; Balfry et al., 1996). Viability of *R. salmoninarum* in unfiltered seawater, was reduced to ~ 40% after eight hours, to ~ 1% after 24 hours, and remained ≤ 1% after seven days (Balfry et al., 1996). Although a decay rate for *R. salmoninarum* in unfiltered seawater has not been reported, the survival rate reported in Balfry et al. (1996) is equivalent to a decay constant of approximately 2.3 per day[A]. In sterile river water, *R. salmoninarum* can remain viable up to 20 weeks (Hirvelå-Koski, 2005). Nonetheless, potential for water current-based transmission is supported by the ineffectiveness of cage-based fallowing in a farm, whereas the fallowing the entire farm is effective (Murray et al., 2011b; Wallace et al., 2011; Murray et al., 2012).

Renibacterium salmoninarum can also be vertically transmitted across generations, a feature that complicates disease management. The bacterium can be detected on both the egg surface and in the ovarian fluid of infected broodstock (Evelyn et al., 1984), and intraovum infection could be experimentally induced by immersion of eggs in infected ovarian fluid prior to fertilization (Evelyn et al., 1986b) and by direct injection into eggs (Brown et al., 1990). Bacterial DNA can also be detected within eggs from naturally infected broodstock by PCR (Brown et al., 1994). Although experimental egg exposures suggested an infection threshold between 10^3 and 10^5 cells mL^{-1} in the immersion fluid, natural egg infections occur at concentrations as low as 10^2 cells mL^{-1} (Lee and Evelyn, 1989). Even though salmon eggs do contain the antibacterial enzyme lysozyme, this protection is less effective against *R. salmoninarum* than for other bacteria (Yousif et al., 1994). Intraovum infection prevalence can range from 8-10% for lower titer ovarian fluid (Lee and Evelyn, 1989) to 14-44% for high titer ovarian fluid (Evelyn et al., 1984; Lee and Evelyn, 1989), but can be lower depending on temperature and maternal bacterial burden (Evelyn et al., 1986b; Lee and Evelyn, 1989). There is also evidence that intraovum infection can occur well before the latter stages of development. Infected maturing oogonia have been observed in experimentally infected trout (Bruno and Munro, 1986b), and *R. salmoninarum* was detected by immunohistochemistry in as early as secondary oocytes of naturally infected Chinook Salmon more than a year prior to spawning (Rhodes, unpublished observations). Therefore, it is likely that nearly all stages of ovum development are vulnerable to infection *in vivo*.

[A] The decay rate was calculated as the slope of the least squares method linear regression applied to data points representing the percent survival of *R. salmoninarum* versus time in unfiltered seawater (see Figure 2 in Balfry et al. (1996)).

In addition to intraovum transmission of the infectious agent, another factor potentially influencing the likelihood of progeny infection is suppression of an immune response in the progeny by *R. salmoninarum*'s highly abundant extracellular protein called major soluble antigen (MSA) or p57 (see section above about the etiological agent). The presence of 100 ng of MSA in an egg is sufficient for higher susceptibility to infection in resulting fry possibly due to immunosuppression (Brown et al., 1996).

There is no conclusive evidence that the infection status of the male or the presence of bacteria in the milt contributes directly to intraovum infection (Evelyn et al., 1986b).

Probably the greatest contributor for long distance movement of BKD is anthropogenic transmission (Murray et al., 2011b; Murray et al., 2012; Brynildsrud et al., 2014). For example, analysis of Direct Action Orders (DAOs) imposed due to BKD in Scotland revealed that farm ownership had a strong association with infection in the absence of a geographic, environmental, or smolt origin pattern (Murray et al., 2011b; Murray et al., 2012). Genome analysis of isolates distributed over a wide geographic area and over four decades indicates that movement of *R. salmoninarum* strains across hemispheres is most likely due to human transport (Brynildsrud et al., 2014). In Chile, which has no indigenous salmonid populations, the distribution of *R. salmoninarum* was through the initiation of salmon farming, and the molecular evolution of the bacterium can be dated to at least four introductions and to shifts in rearing from primarily Coho Salmon to Atlantic Salmon (Bayliss et al., 2018).

Incubation period and shedding rates in Atlantic Salmon

In order to quantitatively address infection pressure attributable to Atlantic Salmon in the context of pathogen transfer risk assessment, it is useful to have estimates of incubation periods (i.e., time between initial infection and the development of disease signs) and shedding rates of *R. salmoninarum*.

Much of the direct experimental information on incubation periods and shedding rates have been collected from Chinook Salmon, rather than Atlantic Salmon, and some extrapolation across species may be necessary, being mindful of the differential species susceptibilities to BKD (e.g., Table 3 above).

The incubation period for BKD is expected to reflect the effects of two encounters with the fish immune system. The first is the mucosal immune system that is distributed throughout the surfaces in contact with the external environment, including the gastrointestinal tract, and is patrolled by phagocytic and lymphoid cells (Salinas, 2015). Microbes that can manage to penetrate the outer surface of epithelium or mucosa will be engaged and perhaps inhibited or destroyed by the mucosal immune system. The second encounter is with the systemic immune system, which is more familiarly known to the lay person as immunity mediated through internal organs and the peripheral circulation.

Incubation period

The majority of exposures to *R. salmoninarum* have been conducted through intraperitoneal injection (i.p.) to deliver a definitive quantity of infectious agent. However, i.p. injection circumvents the mucosal immunity involved in a natural exposure, so incubation times would be shorter and not necessarily be representative of natural exposure. Juvenile Sockeye Salmon (~86 g) injected with an isolate from a naturally infected sub-adult Rainbow Trout exhibited a mean time to death of 22 to 36 days (lower titer) or 17 to 31 days (higher titer), even though the cell concentrations differed by ten-fold (Evelyn et al., 1973). Small (20 g) Atlantic Salmon challenged i.p. with 4×10^4 cells per fish developed kidney and spleen lesions by six weeks, and ~ 50% mortality by 12 weeks (Griffiths et al., 1998). Intraperitoneal challenges of seawater-

adapted fall Chinook Salmon with 10^3 bacterial cells per fish can result in a median time to death ranging between 61 to >106 days, depending on the bacterial strain (Rhodes et al., 2004a). Juvenile Chum Salmon reared in either freshwater or seawater challenged i.p. with 1.5 x 10^6 cells exhibited similar mean time to death of 30.0 vs 25.6 days, respectively (Sakai et al., 1992). In addition to dosage effects, there are water temperature effects on incubation period, with shorter incubation to mortality (30-35 days) at temperatures > 11°C and longer periods (60-90 days) at temperatures between 7°C and 11°C (Sanders et al., 1978). These observations suggest that i.p. exposure has an incubation period ranging between 17 to over 106 days, depending on dosage, temperature, and which clinical sign is used as an endpoint.

Cohabitation and immersion challenge are more representative of natural exposure, but there are far fewer reported studies with this method due to the logistical difficulty and protracted duration required. An immersion challenge in freshwater with Chinook Salmon showed a clear dose-dependent effect. At doses 3 x 10^4 cells mL^{-1}, it took ~ 180 days to reach 5% cumulative mortality, while at doses > 3 x 10^4 cells mL^{-1}, 5% cumulative mortality was reached by ~ 80 days (Murray et al., 1992). Freshwater immersion challenge of Rainbow Trout using a dosage as low as 2 x 10^5 cells mL^{-1} for 24 hours can produce elevated *R. salmoninarum* antigens in kidney by 50 days, with the caveat that antigen is not necessarily indicative of viable infection (Pascho et al., 1997). Successful and robust freshwater immersion challenge conditions for Chinook and Coho salmon have used ~ 0.5 x 10^6 to 4 x 10^6 cells mL^{-1} for 22-24 hours (Mesa et al., 1999; Piganelli et al., 1999a; Elliott et al., 2015). Immersion exposure of Chinook Salmon could result in > 50% of the exposed fish with kidneys that were culture-positive by three weeks post-infection, although few fish (7%) displayed clinical signs by 21 weeks post-infection (Elliott et al., 2015). Again, depending upon the clinical sign used, incubation period for immersion challenge can range from 21 to 50 days.

In cases of vertical transmission, where the embryo is infected intraovum, disease signs often do not emerge until roughly six months after hatching, possibly due to a combination of changing temperatures and pathogen amplification time (Warren, 1983).

Shedding rates

Shedding bacteria by infected fish is the most common route for horizontal transmission, and Atlantic Salmon with BKD do shed *R. salmoninarum* (Griffiths et al., 1998). Although shedding rates are not known for Atlantic Salmon, measurements in Chinook Salmon might be applied as surrogate measurements (Table 4).

Feces is a significant source of infectious agent, with over 80% of infected Chinook Salmon in seawater shedding fecal *R. salmoninarum* (Balfry et al., 1996). Shedding in heavily infected Chinook Salmon could be detected more than nine months after transfer to seawater at a rate of 1 x 10^6 CFU g^{-1} feces (Balfry et al., 1996). Sub-clinically infected fish also shed *R. salmoninarum* in feces (Balfry et al., 1996) but levels were not reported. In a seawater net pen holding 14,000 heavily infected Chinook Salmon experiencing 80% of BKD-related mortalities, *R. salmoninarum* was detected at a concentration of 256.6 ± 179.1 *R. salmoninarum* cells mL^{-1} at 1 m depth (Balfry et al., 1996).

Experimentally infected juvenile Chinook Salmon (27 g) in fresh water began shedding bacteria as early as 12 days post-infection, although consistently elevated levels in the water were not achieved until 20 days post-infection (McKibben and Pascho, 1999). Calculated values[B] for

[B] Calculated values were made from data presented in Table 1 in McKibben and Pascho (1999). Estimates of shed bacteria were derived after 20 days post-infection using tanks where the majority of fish exhibited kidney ELISA OD(492) ≥ 1 (a widely accepted value for severely infected fish), assuming a 25% water turnover on the flow rate of 0.67 L min^{-1}.

heavily infected fish reported in McKibben and Pascho (1999) estimate shedding rates of 4.1 x 10^5 cells per fish per hour.

The highest level of mean individual shedding by individual juvenile Chinook Salmon (5 g) challenged with *R. salmoninarum* by injection was reported to be 2.1 x 10^5 cells per fish per hour at 91 days post-infection (Purcell et al., 2016). The authors also identified a positive correlation between bacterial loads in the kidney and the quantity of shed bacteria in Chinook Salmon, although that relationship did not hold at a higher temperature of 15°C. Such a correlation between kidney bacterial load and shedding levels suggests that sub-clinically infected or asymptomatic fish may shed at a slower rate, but that relationship has not been defined for any other salmonid species. While shedding by individual fish can range over five orders of magnitude (M. Purcell, USGS, Western Fisheries Research Center, 6505 NE 65th Street, Seattle, WA, pers. comm.), calculated values[C] for heavily infected fish based on this relationship estimate shedding rates of 6.5 x 10^6 and 3.1 x 10^6 cells per fish per hour at 8°C and 12°C, respectively. It is highly likely that variations in shedding rates are influenced by multiple host and environmental factors, resulting in a complex relationship.

Table 4. Reported and calculation shedding rates in juvenile Chinook Salmon exposed to Renibacterium salmoninarum. All experiments were conducted in freshwater and fish were challenged by intraperitoneal injections.

Species	Time post infection	Shedding rates (cells per fish per hour)	Reference
Chinook Salmon (27 g juveniles)	20 to 30 days post-infection (13°C)	4.1 x 10^5 (in heavily infected fish)	Calculated based on McKibben and Pascho (1999)
Chinook Salmon (5 g juveniles)	91 days post-infection (8°C)	2.1 x 10^5 (highest mean)	Purcell et al. (2016)
	14 to 112 days post-infection	6.5 x 10^6 (in heavily infected fish at 8°C)	Calculated based on Purcell et al. (2016)
		3.1 x 10^6 (in heavily infected fish at 12°C)	

Infectious and lethal doses in susceptible species

Minimum infectious or lethal doses of *R. salmoninarum* in Sockeye Salmon have not been determined. However, some experiments conducted in salmonids can be informative and be used as proxy data in risk assessments.

A classic approach in estimating infectious and lethal dosages is by intraperitoneal injection challenge, which can guarantee exposure level but is an unnatural exposure route. Therefore, extrapolation based on intraperitoneal injections is not a logical approach to estimating infectious or lethal doses for wild populations. For this reason, only immersion studies are considered.

[C] Calculated values were made from data presented in Figure 3 of Purcell et al. (2016). The derived log-log relationships between kidney bacterial load and shedding (goodness of fit $R^2 \geq 0.99$) were $\log_{10}$(shed bacterial cells $mL^{-1}h^{-1}$) = 0.39*$\log_{10}$(*R. salmoninarum* copies g^{-1} tissue) + 0.67 at 8°C and $\log_{10}$(shed bacterial cells $mL^{-1}h^{-1}$) = 0.50*$\log_{10}$(*R. salmoninarum* copies g^{-1} tissue) + 0.50 at 12°C. Fish with ≥ 10^8 gene copies per g kidney tissues (there are ≥ two gene copies per bacterial cell) were considered heavily infected.

Immersion challenge of Chinook Salmon for only 15 minutes using 3 x 10^4 cells mL^{-1} in fresh water caused 5% cumulative mortality up to six months later and 15% cumulative mortality up to a year later after fish were switched to seawater at six months (Murray et al., 1992). Chinook Salmon in freshwater can be infected with as few as 700 *R. salmoninarum* cells mL^{-1} when exposed for 24 hours (Elliott and Pascho, 1995). Chinook Salmon exposed to a higher dose (1 x 10^5 cells mL^{-1}) for 24 hours resulted in 11% mortality at 22 weeks after challenge (O'Farrell et al., 2000). Coho Salmon that were immersion challenged for 22 hours at a much higher dose (4.1 x 10^6 cells mL^{-1}) experienced 100% mortality by 22 days post-challenge (Piganelli et al., 1999b). Immersions that resulted in mortalities are summarized in Table 5.

Based on these observations, the lowest infectious dose of 700 cells mL^{-1} for 24 hours and the lowest lethal dose of 3 x 10^4 cells mL^{-1} for 15 minutes can serve as proxy for the minimum infectious and lethal doses respectively, with the caveat that these are freshwater exposure in Chinook Salmon. Due to the lack of seawater exposures by immersion, there are no credible minimum infectious or lethal dosages reported for marine salmonids.

Table 5. Lowest concentrations of Renibacterium salmoninarum that resulted in mortalities in exposed salmonids in immersion challenge studies. All challenges were conducted in freshwater.

Species	Challenge	*R. salmoninarum* concentration	Mortality	Time post-infection	Reference
Chinook Salmon	15-m immersion	3 x 10^4 cells mL^{-1}	~ 2.5%	80 days	Murray et al. (1992)
			5%	6 months	
			15%	12 months	
Chinook Salmon	24-h immersion	1 x 10^5 cells mL^{-1}	11%	22 weeks	O'Farrell et al. (2000)
Coho Salmon	22-h immersion	4.1 x 10^6 cells mL^{-1}	100%	22 days	Piganelli et al. (1999b)

Survival of etiological agent in aquatic environment

Renibacterium salmoninarum has significant genome reduction away from its nearest precursor, a soil bacterium-like ancestor (Wiens et al., 2008). Controlled studies measuring *R. salmoninarum* survival have found that the pathogen can persist up to several weeks under certain conditions.

Viable *R. salmoninarum* can be recovered up to 28 days in filter-sterilized river water (Austin and Rayment, 1985) and 8 to 14 days in filter-sterilized seawater (Paclibare et al., 1994; Balfry et al., 1996). Viability of the bacterial pathogen is much shorter in non-sterilized water, with survival of up to four days in unfiltered river water (Austin and Rayment, 1985) and seven days in unfiltered seawater (10ºC and 22‰) (Balfry et al., 1996).

As mentioned in a previous section, a decay rate was not reported for unfiltered seawater, but viability was reduced to ~ 40% by 8 hours, to ~ 1% by 24 hours, and remained ≤ 1% to seven days (Balfry et al., 1996). *R. salmoninarum* viability is high (i.e., 21 days) if associated with sediment or feces (Austin and Rayment, 1985), suggesting that a nutrient-rich or higher complexity environment might provide protection or buffering against environmental stress. Given its agglutinating nature (Daly and Stevenson, 1987; Bruno, 1988; Daly and Stevenson, 1989), *R. salmoninarum* most likely exists in the environment primarily in a particulate-associated form, and its hydrodynamics may be better modeled as larger particles (e.g., 5-50 µm) rather than as very tiny (i.e., < 1 µm) planktonic bodies.

VIRULENCE AND PATHOGENICITY

Morbidity and mortality under experimental conditions

The slowly progressive nature of BKD can influence interpretation of experimental mortality rates. Most exposure studies substitute dose for time (i.e., higher dose to get an earlier effect), and very few studies are conducted beyond 120 days post-challenge.

One study of juvenile Chinook Salmon immersion-challenged with 3 different doses (~ 3×10^4, 10^5, or 10^6 cells mL^{-1}) for ≤ 30 minutes achieved a maximum mortality of 15%, 50%, and 50%, respectively, by 350 days post-challenge (Murray et al., 1992). A prolonged (22 hours) immersion challenge of Coho Salmon to > 10^6 cfu mL^{-1} resulted in 100% mortality by 25 days post-challenge (Piganelli et al., 1999b). Therefore, any calculated mortality rates will depend upon challenge doses and duration of exposure. Calculation of case fatality rates requires a more dedicated type of study because the number of cases needs to be determined throughout the study (i.e., incidence), which is typically not done in BKD studies.

As mentioned in previous sections, water temperature modulates the epizootiology of BKD. The optimal temperature for *R. salmoninarum* growth in culture is 15-18°C. This is consistent with *in vivo* results showing higher mortality in Chinook Salmon at 14°C than at 9°C (Purcell et al., 2014), and the shortest time to death at temperatures > 12.2°C for Sockeye Salmon, Coho Salmon, and steelhead (Sanders et al., 1978). Similarly, LD_{50} estimates for Chinook Salmon, Bull Trout, Rainbow Trout, Lake Trout and Arctic Charr was an order of magnitude lower at 15°C than at 9°C (Jones et al., 2007). Even with the administration of the antibiotic erythromycin, onset of BKD mortality began earlier at 12°C than 8°C (Moffitt, 1992). However, the relationship between pathogenicity and temperature is not a simple linear one. For example, Purcell et al. (2016) discovered lower survival, greater tissue bacterial burdens, and higher shedding rates at 8°C compared to 12°C or 15°C.

Some of these temperature effects may also rely on differential immunological competence at different temperatures. A multiyear study of Sockeye Salmon (from smolting to sexual maturation) evaluated nonspecific and specific immune responses at two rearing temperatures, 8°C and 12°C (Alcorn et al., 2002). In general, nonspecific immune responses (complement activity, percent kidney phagocytes) were greater at the lower temperature, while specific immune responses (peripheral lymphocytes, antibody production) were greater at the higher temperature (Alcorn et al., 2002).

In addition to potential interactions of temperature effects on pathogen and host immunologic competence, a confounding factor in field settings (e.g., hatcheries) is co-infection. These may be triggered by changes in temperatures, as well as absolute temperatures, or seasonal shifts. Polymicrobial infections can be higher than expected in spawning Chinook broodstock at a time of year when water temperatures can be declining (Loch et al., 2012). Clearly, understanding temperature effects requires contextual information for interpretation.

Morbidity and mortality in wild fish populations

In offshore juvenile Chinook Salmon, infection by *R. salmoninarum*, either singly or in combination with other pathogens such as *Nanophyetus salmincola* is associated with reductions in growth metrics such as weight (Sandell et al., 2015).

Examination of 3,680 fish representing seven species of salmonids (Chinook Salmon, Chum Salmon, Coho Salmon, Pink Salmon, Sockeye Salmon, steelhead trout, Cutthroat Trout) collected offshore of Oregon and Washington states found < 4% infection in all species except Chinook Salmon, which had an infection prevalence of 11% (Banner et al., 1986). Interestingly,

only 2.8% (Chinook Salmon) and 0.3% (Coho Salmon) of the infected fish displayed clinical signs.

Similar to observations under experimental conditions, temperature appears to be a factor for infection in free-ranging populations. Regression analysis of data for juvenile Chinook Salmon throughout inland marine waters of Washington state identified increases in water temperature as a highly significant factor that increased infection likelihood (Rhodes et al., 2011).

There are no mortality data associated with *R. salmoninarum* infection in wild fish.

OCCURRENCE IN CANADA

As an annually notifiable disease under the National Aquatic Animal Health Program (NAAHP), Canadian laboratories are required to notify the CFIA regarding the suspicion or diagnosis of BKD once a year.

Table 6 summarizes the number of reports for *R. salmoninarum*/BKD per species and province between 2013 and 2016, inclusively. Although, these data support occurrence of *R. salmoninarum* and/or BKD in different species, it should be noted that data from certain province(s) are limited and likely incomplete because of lack of reporting from the provincial laboratory; data do not specify whether the sample originated from the wild or cultured fish; data do not specify if the detection is from a single fish or multiple fish; and data do not specify whether the sample originated from freshwater or seawater.

Table 6. Total Annually Notifiable Disease detections for Renibacterium salmoninarum/bacterial kidney disease submitted to the Canadian Food Inspection Agency (CFIA) between 2013 and 2017 per province. An additional 24 notifications for Salmo salar have also been reported for which the province is unknown. Source: CFIA, January 2018.

Common Name	Species	British Columbia	Manitoba	Ontario	Quebec	New Brunswick	Nova Scotia	Prince Edward Island	Newfoundland and Labrador	Yukon
Sablefish	*Anoplopoma fimbria*	1								
Lake Whitefish	*Coregonus clupeaformis*			1						
Deepwater Cisco	*Coregonus johannae*			1						
Pink Salmon	*Oncorhynchus gorbuscha*	2								
Chum Salmon	*Oncorhynchus keta*	3								
Coho Salmon	*Oncorhynchus kisutch*	174								
Rainbow Trout	*Oncorhynchus mykiss*	14	2	4	3		5	9	2	
Sockeye Salmon	*Oncorhynchus nerka*	49								
Pacific salmon	*Onchorhynchus spp.*	11								
Chinook Salmon	*Oncorhynchus tshawytscha*	149		4						
Atlantic Salmon	*Salmo salar*	269	2	4		34	19	3	56	
Brown Trout	*Salmo trutta*			4						
Arctic Char	*Salvelinus alpinus*				1					4
Brook Trout	*Salvelinus fontinalis*		1	3	7					
Lake Trout	*Salvelinus namaycush*			7						
Lake Trout x Brook Trout	*Salvelinus namaycush x Salvelinus fontinalis*			1						
Walleye	*Sander vitreus*			2						

WILD SALMONIDS IN BC

Since the first report of BKD in BC in the early 1970's (Evelyn et al., 1973), the disease or infection by *R. salmoninarum* has been found among wild and free-ranging populations in BC waters.

Kent et al. (1998) surveyed salmonid pathogens in ocean-caught fishes in BC. *R. salmoninarum* was detected by ELISA in 58% (45/77) of Chinook Salmon, 7% (25/339) of Chum Salmon, 42% (31/74) of Coho Salmon, 6% (25/402) of Sockeye Salmon, 26% (7/27) of Pink Salmon and 18% (17/96) of Atlantic Salmon captured >1 km from a salmon farm.

Juvenile Chinook Salmon collected by DFO's Canadian Program on High Seas Salmon (2002-2007) from the west coast of Vancouver Island to Southeast Alaska included ~5% of 334 fish with heavy infection with *R. salmoninarum* (Nance et al., 2010). On the other hand, marine-phase juvenile Fraser River Sockeye Salmon (1530 fish over 45 stocks) from 2010-2012

exhibited a 0% infection prevalence (Mahony et al., 2017). The authors, however, acknowledged that this finding contrasts with some DFO unpublished data (referenced in their discussion) suggesting a widespread occurrence of *R. salmoninarum* in spawning Fraser River Sockeye Salmon with *R. salmoninarum* prevalence ranging from 1 to 89% depending on the year and stock. The authors speculated that infections below the limit of detection of their end-point PCR method or loss of the infected juveniles from the population prior to marine migration may have been responsible for their inability to identify positive fish (Mahony et al., 2017).

SALMONID ENHANCEMENT PROGRAM

DFO launched the Salmonid Enhancement Program (SEP) in 1977 to increase the catch of salmon in BC and Yukon (DFO, 2015a). The SEP focuses on the production of Pacific salmon in hatcheries and spawning channels and also includes restoration and enhancement of habitat for fish production, as well as education and awareness programs to facilitate participation in cooperative fisheries and watershed stewardship activities (DFO, 2015a).

DFO SEP uses risk-based control measures to lower the risk of amplifying BKD through hatchery operations. These management activities are based on current science advice and are consistent with BKD management practices for Pacific salmon enhancement in neighbouring US states. As this pathogen is endemic in BC, screening efforts are aimed toward disease control as opposed to eradication. For known higher-risk stocks, *Renibacterium salmoninarum*-specific antibody-based screening enables hatcheries to select eggs with a lower chance of intra-ovum infection with the bacterium and break the vertical transmission pathway of infection. Additionally, husbandry measures designed to optimize fish health and welfare are employed. These general practices include egg disinfection, low rearing densities, predation deterrence, life stage appropriate nutrition, veterinarian directed disease prevention and treatment, etc. (C. MacWilliams, DFO, pers. comm., 2018).

Although not amenable to prevalence estimation, data provided by the Finfish Diagnostic Laboratory at the DFO Pacific Biological Station confirm the endemic nature of this pathogen, with *R. salmoninarum* infections found in returning Sockeye Salmon in all watersheds in BC, including the Fraser River watershed.

FARMED ATLANTIC SALMON

Renibacterium salmoninarum and BKD have been reported on Atlantic Salmon farms in BC through both diagnoses from the Fish Health Audit and Surveillance Program conducted by DFO (and by the BC provincial government prior to 2011) and by the industry through the reporting of Fish Health Events (FHEs).

British Columbia

Fish health information for farmed salmon in British Columbia is obtained through the Fish Health Audit and Surveillance Program conducted by DFO Aquaculture Management veterinarians and staff (as a continuation of the BC provincial government audit program prior to 2011) and submissions by industry (Wade, 2017).

Fish Health Audit Program

Each quarter, DFO audits a maximum of 30 farms through their Fish Health Audit and Surveillance Program (Wade, 2017). Between 2002 and 2016, a total of 1229 audits were conducted on Atlantic Salmon farms in all management regions of BC, representing an average of seven audits per month (range: 0 to 19 in a given month) (Jones, 2019).

DFO veterinarians provide farm-level diagnoses based on farm history, treatment history, environmental factors, mortality records, clinical presentation on farm, and results of diagnostic procedures performed on individual fish (DFO, 2018). These farm-level diagnoses are used to audit industry fish health reporting (DFO, 2018) and include both bacteriological and histological tools to confirm the presence of *R. salmoninarum*. The DFO audit program is not designed to capture incidence or prevalence, but diagnostics are indicative of the presence of the pathogen and/or disease in some individuals on farms.

Between 2002 and 2016, there were a total of 49 farm-level diagnoses of BKD in BC (Table 7), the majority (35%) from farms in Fish Health Surveillance Zone 3.3 (Broughton Archipelago).

Table 7. Summary of British Columbia Provincial (2002-2010) and Fisheries and Oceans Canada Aquaculture Management Fish Health Audit and Surveillance Program (2011-2016) farm-level diagnoses for bacterial kidney disease (BKD) in seawater-reared Atlantic Salmon in BC. Source: data provided by BC provincial government, 2010 and DFO Aquaculture Management Division, 2018. Numbers in parentheses represent the total number of individual farms with audit-based diagnoses for BKD.

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	1 (1)		1 (1)			1 (1)		1 (1)		4 (4)
2003							1 (1)	1 (1)		2 (2)
2004					1 (1)		3 (3)			4 (4)
2005						3 (2)	5 (5)			8 (7)
2006				2 (2)		3 (2)	1 (1)	2 (2)		8 (7)
2007				2 (1)		1 (1)		5 (4)		8 (6)
2008				2 (1)			2 (2)			4 (3)
2009						1 (1)	1 (1)			2 (2)
2010							1 (1)			1 (1)
2011							1 (1)			1 (1)
2012									1 (1)	1 (1)
2013				1 (1)			2 (1)			3 (2)
2014								1 (1)		1 (1)
2015			1 (1)					1 (1)		2 (2)
2016										0
$\Sigma_{subzone}$	**1 (1)**	**-**	**2 (2)**	**7 (4)**	**1 (1)**	**9 (4)**	**17 (10)**	**11 (7)**	**1 (1)**	**49**

Average fish weight on Atlantic Salmon farms with an audit-based farm-level BKD diagnoses between 2011 and 2015 ranged from 1.2 to 7.9 kg (n=8 audits in all BC) with an average of 4.1 kg. Average fish weights on farms during audits conducted before 2011 are not available.

Fish Health Events (FHEs)

A 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, 2015b).

Reporting FHEs began in the fall of 2002 but was not required in 2013 to the last quarter of 2015 (Wade, 2017). Between 2002 and 2016, a total of 57 FHEs attributable to BKD have been

reported on Atlantic Salmon farms in BC (Table 8), with the most reported (47%) in Zone 3.3 (Broughton Archipelago) and approximately 9% in Zone 3.2 (Discovery Islands) (Table 8).

Table 8. Summary of fish health events (FHEs) (2002-2017-Q1) associated with bacterial kidney disease in seawater-reared Atlantic Salmon in British Columbia. Dashes indicate no requirements to report FHEs. Source: data provided by DFO Aquaculture Management Division, 2018. Numbers in parentheses represent the total number of individual farms where there were 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			1 (1)		1 (1)				1 (1)	3 (3)
2004			1 (1)		2 (2)		4 (4)		3 (1)	10 (8)
2005						3 (3)	9 (8)			12 (11)
2006				1 (1)			2 (2)	3 (3)		6 (6)
2007				4 (4)			1 (1)			5 (5)
2008							4 (3)			4 (3)
2009						1 (1)				1 (1)
2010				4 (4)		1 (1)	1 (1)			6 (6)
2011				1 (1)			3 (2)			4 (3)
2012					2 (2)				1 (1)	3 (2)
2013	-	-	-	-	-	-	-	-	-	-
2014	-	-	-	-	-	-	-	-	-	-
2015	-	-	-	-	-	-	-	-	-	-
2016							1 (1)			1 (1)
2017							2 (1)			2 (1)
$\Sigma_{subzone}$	**0**	**0**	**2 (2)**	**10 (9)**	**5 (4)**	**5 (5)**	**27 (13)**	**3 (3)**	**5 (2)**	**57**

HEALTH MANAGEMENT

PREVENTION

Broodstock surveillance and culling

The surveillance and culling of broodstock, particularly females, is based on tissue or ovarian fluid levels of *R. salmoninarum* antigens and has been widely employed. The caveats to this approach are that *R. salmoninarum* antigens can persist at high levels in kidney and spleen of Rainbow Trout > 110 days in the absence of viable bacteria (Pascho et al., 1997), and that elevated levels of *R. salmoninarum* antigens in a river system can be absorbed through gills or drinking, and can be detected in nonclinical animals (Kristmundsson et al., 2016). If the risk of false positives is acceptably low, the approach has demonstrated efficacy in interrupting vertical transmission in Atlantic Salmon sea ranching (Gudmundsdottir et al., 2000) as well as in hatcheries for Pacific salmon (Meyers et al., 2003). Fortunately, the effects of culling on the heritability of resistance to BKD appear to be very low, and unlikely to impact the resistance of progeny (Hard et al., 2006).

The success of culling has been demonstrated in the Norwegian Atlantic Salmon industry, which experienced a peak of outbreaks from 1987 through 1993 when a severe broodstock culling

program based on kidney ELISA values was implemented. Annual outbreaks declined steadily until there were none reported in 2006 and 2007 (Dale et al., 2008).

Progeny segregation by broodstock screening

If the stock is valuable and cannot be culled at early egg stage, broodstock screening and segregation is an option. One approach screens female broodstock by kidney ELISA (Pascho and Mulcahy, 1987) and membrane filtration FAT of ovarian fluid (Elliott and Barila, 1987), sorts progeny into infection probability groups (e.g., high *vs* low) based on screenings, and rears the progeny groups separately. This approach applied to Chinook Salmon showed that by the smolt stage, the segregated progeny were on different infection trajectories (Pascho et al., 1991). However, a retrospective analysis speculated that intraovum immunosuppression could be an alternative explanation for the mortality trajectories (Hamel, 2005).

Disinfection of eggs

Disinfection of eggs can reduce surface bacteria, but cannot eliminate vertical transmission because it is not 100% effective and bacterial cells can be transferred inside the egg. The immersion in iodine at 100 to 500 mg L^{-1} for 15 to 20 minutes can inactivate most bacteria, although the agglutinating nature of *R. salmoninarum* can shield many cells and allow them to survive (Bullock et al., 1978; Evelyn et al., 1984). In addition, water hardening eggs in an erythromycin solution (50 mg mL^{-1}) to inactivate bacterial cells was also not entirely effective (Evelyn et al., 1986a).

Other prevention methods

Seawater maturation, instead of freshwater maturation, can lower prevalence of ELISA-positive broodstock, but whether this translated into a reduction in infection in progeny was not reported (Meyers et al., 1999).

IMMUNIZATION

Atlantic Salmon

Studies with Atlantic Salmon found that immunization with bacterins or killed bacterial cells did not stimulate protective antibodies. Although vaccination did reduce prevalence of clinical BKD, it did not reduce the prevalence of infected carriers (Paterson et al., 1981). Two avirulent mutants of *R. salmoninarum* lacking the requirement for L-cysteine and retaining expression of MSA showed promise as live vaccines in Atlantic Salmon (Daly et al., 2001), although there is always concern for reversion to a virulent phenotype. The most productive vaccine development for Atlantic Salmon has been the isolation of an *Arthrobacter* spp. from Chinook Salmon that produces an exopolysaccharide with antigenic similarity to *R. salmoninarum* (Griffiths et al., 1998). This vaccine may limit the horizontal transmission of *R. salmoninarum* by restricting the shedding of the pathogen in Atlantic Salmon (Griffiths et al., 1998). Eleven weeks following infection, *R. salmoninarum* was detected by PCR in tank water of control unvaccinated group tanks but not in the *Arthrobacter* spp (Rsx II) vaccinated fish (Griffiths et al., 1998). Fourteen weeks after infection, 85.8% the of unvaccinated control group were culture positive for *R. salmoninarum* compared to 15.7% in the Rsx II vaccinated fish (Griffiths et al., 1998).

This vaccine has shown to reduce BKD related mortalities in Atlantic Salmon during large scale trials (Salonius et al., 2005), and it is a commercially available product called Renogen (Griffiths and Kira, 2003). Salonius et al. (2005) report 5 to 14% cumulative mortality in Renogen-vaccinated Atlantic Salmon parr 2 months after injection-challenge with 1×10^4 cfu $fish^{-1}$ of *R.*

salmoninarum during a laboratory trial, while cumulative mortality in the unvaccinated control group reached 52%, suggesting a vaccine efficacy of 72 to 91% for this period. In a clinical field trial comparing vaccines in combination with Lipogen Forte®, a commercial vaccine against vibriosis, the Renogen + Lipogen Forte® was the only combination displaying significant efficacy against BKD (Burnley et al., 2010). Although the improved efficacy was slight (hazards ratio 0.68 for Lipogen Forte® alone compared to 0.71 for the combination vaccine), the impact was highly significant due to the scale (6,000 fish) and field trial nature of the experiment.

Pacific salmon

Although the commercial *Arthrobacter* vaccine Renogen has exhibited effectiveness in Atlantic Salmon (Salonius et al., 2005), it confers little or no protection for Pacific salmon (Rhodes et al., 2004b; Alcorn et al., 2005). The Chilean aquaculture industry used Renogen in the early 2000's for Coho Salmon, but the application was limited to only 5% of the fish and effectiveness could not be determined (Bravo and Midtlyng, 2007). However, application of Renogen alone or in combination with the killed MT239 (attenuated) strain of *R. salmoninarum* showed some promise as a therapeutic vaccine for Chinook Salmon that were already infected (Rhodes et al., 2004b).

Bacterin vaccination in Pacific salmon has not been encouraging in protecting against experimental infection in salmon or trout (Sakai et al., 1993). An extensive test of multiple vaccine formulations (killed strains of *R. salmoninarum*, recombinant MSA protein) against a cohabitation challenge in Chinook Salmon found no protection (Alcorn et al., 2005). These findings were especially disappointing because one of the candidate vaccines was a whole cell vaccine that was heat-treated to remove extracellular MSA (Piganelli et al., 1999a), a process that appeared to increase its immunogenicity (Wood and Kaattari, 1996). This heat-treated *R. salmoninarum* vaccine had exhibited encouraging results in earlier trials with Pacific salmon (Kaattari and Piganelli, 1997; Piganelli et al., 1999b). Adjuvants and immunostimulants have also been employed to initiate or boost immunogenicity of vaccines, with no or slight improvement in efficacy. These boosters have ranged from standard products such as Freunds complete or incomplete adjuvant to fermented biological products (Sakai et al., 1995) to DNA oligonucleotides (Rhodes et al., 2004b).

CHEMOTHERAPEUTICS AND EFFICACY

A wide variety of antibiotics/antimicrobials have been tested against *R. salmoninarum* over the decades, with variations in susceptibility dosages across the bacterial strains (Austin, 1985; Gutenberger et al., 1989; Bandin et al., 1991). Establishment of minimum inhibitory concentrations (MICs) for *R. salmoninarum* has been complicated by the lack of established standardized tests for pathogens with temperature optima < 37°C and that have slow replication rates.

Most of the tested antibiotics are bacteriostatic with a mode of action that slows bacterial growth through inhibition of protein synthesis. In the case of a slowly growing microorganism such as *R. salmoninarum*, these types of antibacterials may have limited long term effectiveness - the slower metabolism of the cell may persist beyond the effective dose half-life of the antibiotic. The pattern of recurrent morbidity and mortality is commonly reported in association with macrolide treatment (Rhodes et al., 2007). *R. salmoninarum* also has potential for a quiescent or very low metabolism state, and putative resuscitation genes have been identified in its genome (Wiens et al., 2008). An alternative *in vitro* growth morphology has also been characterized possibly representing a quiescent state (Hirvelå-Koski, 2005; Hirvela-Koski et al., 2006).

CONTROL AND TREATMENT

Surveillance

Clinical detection is easy because signs are evident and well defined, but late in disease progression. Subclinical detection requires an active surveillance program.

Between 2004 and 2010, Marine Scotland Science conducted surveillance specifically to control and eradicate BKD and to obtain guarantees for disease-free status in the European Community. Surveillance was conducted according to the following schedule outlined in Table 9 below (Munro, 2007).

Table 9. Veterinary surveillance for UK national control programme and additional guarantees for BKD allowed for under EU Directive 91/76/EEC. Source: Munro (2007).

type	# of fish / sample	frequency of sampling	screen method	confirmatory method
No broodstock	30	1x per year	ELISA	150 fish by culture
Broodstock	30	2x per year	ELISA	150 fish by culture

If a site was placed under Designated Area Orders (DAOs)/Confirmed Designation Notices, movement of fish was restricted and other remediation activities were required. The follow-up surveillance and criteria to lift the order required biennial site testing by ELISA, and confirmation of positive results placed sites under movement restrictions until no longer detectable, using one of two sampling models listed in Table 10 below (Munro, 2007).

Table 10. Surveillance models required for lifting DAO restrictions due to bacterial kidney disease in Scotland, 2004-2010. Source: Munro (2007).

model	free of clinical disease	Follow-up testing
A	2 years	2 x 150-fish samples for 2 years
B	4 years (30 fish sample per year + 2 inspections)	2 30-fish samples, 2x / year, for 2 years

These management actions require statistical estimates of infection prevalence which are often underestimates, but an analysis of impact based on a beta-binomial distribution concluded that the surveillance measures effected a reduction in clinical BKD for farmed Atlantic Salmon (Hall et al., 2015).

Fallowing

Attempts to determine the utility of fallowing individual freshwater net pens for Rainbow Trout failed to provide conclusive evidence of effectiveness, but did find that the likelihood of intra-farm transmission was high and swift, within a month of introducing naive fish (Wallace et al., 2011). However, other case studies of trout and salmon fallowing at only the farm level (rather than cage or pond) appear to be effective in eliminating infection at a farm (Murray et al., 2012). Fallowing between cycles of fish reduces disease outbreaks in subsequent generations, and fallowing with reduced generational overlap in broodstock operations helps to disrupt vertical transmissions (Bruno, 2004).

Isolation and fish movement restrictions

The effectiveness of isolation has been demonstrated in Scotland from the example of freshwater trout cages that have been under Designated Area Orders (DAOs)/Confirmed Designation Notices under Scottish Aquatic Animal Health Regulations continuously since 1981. These are sites with confirmed infection by *R. salmoninarum*, and are under fish movement restrictions. Although these freshwater trout cages are persistently infected, it has not spread to other farms, and the movement restrictions have shown the effectiveness of isolation (Murray et al., 2012).

In Canada, the movement of live aquatic animals is regulated by the Canadian Food Inspection Agency (CFIA) and DFO. Given that BKD is not a reportable disease for finfish in Canada (CFIA, 2018), only movement restrictions through the Introduction and Transfer licenses granted by DFO under Section 56 of the Fishery (General) Regulations apply. Additionally, in BC, companies are required to have Standard Operating Procedures (SOP) to address the movement of fish between facilities as a condition of license (DFO, 2015b; Wade, 2017).

INACTIVATION

Disinfection of all equipment, containers, and surfaces is a fundamental aspect of fish culture hygiene, although not all disinfectants are effective against *R. salmoninarum*. The iodophors Betadine and Wescodyne (25 mg L^{-1}) at neutral or slightly alkaline conditions can totally inactivate cells by five minutes (Ross and Smith, 1972). In Canada, the iodophor Ovadine is most widely used at 100 ppm for egg disinfection and 250 ppm for equipment and surface disinfection (P. Ackerman, DFO, pers. comm., 2018). Peroxy compounds, such as Virkon S, have been reportedly effective for non-porous surfaces and foot baths (Fraser et al., 2006). Tests with chlorine (free chlorine 0.8 to 1.0 mg L^{-1}) show that lower concentrations of cells (~ 2 x 10^6 cells mL^{-1}) require only one minute of contact time for 100% inactivation, while four-fold higher concentrations (~ 8 x 10^6 cell mL^{-1}) require ten minutes of contact time (Pascho and Ongerth, 2000). However, chlorine effectiveness can be attenuated by the presence of organic material, such as feces. Conditions for chlorine inactivation are also important, with pH 6 or 7 and a temperature 15°C being most effective (Pascho et al., 1995). Ensilage processing with a minimum of 24 hours at pH < 4 (e.g., formic acid) effectively eliminates *R. salmoninarum* from waste (Smail et al., 1993).

Although *R. salmoninarum* is sensitive to temperatures > 20°C, it can survive heating for 15 minutes at 65°C and for four hours at 50°C (Whipple and Rohovec, 1994). However, at lower pH (~ pH 4), it survives only one minute at 55°C (Smail et al., 1993).

CONCLUSIONS AND RECOMMENDATIONS TO MINIMIZE TRANSMISSION

The chronic nature of BKD and the occurrence of undetected subclinical infections can dictate different management decisions dependent upon circumstances. For example, there is a substantive Atlantic Salmon and Rainbow Trout aquaculture industry in Scotland, and both are affected by BKD. Marine Scotland Science has invested in both research and modeling to address questions about disease interactions between Atlantic Salmon and Rainbow Trout farms and efficient management practices (e.g., Murray et al., 2011b; Wallace et al., 2011). Modeling incorporating undetected subclinical infections indicated that effectiveness of control strategies would be different for Atlantic Salmon and Rainbow Trout farms (Murray et al., 2011a). Therefore, it is likely that management tactics should be customized to produce the desired outcome (i.e., reduction or elimination of infection) while minimizing negative impact on growers.

The leading tool for minimizing transmission is surveillance with high sensitivity and specificity and with sufficient temporal resolution to allow an early effective response. There are validated high sensitivity assays for detection and, with the possibility of nonlethal sampling, high frequency monitoring (e.g., monthly during higher water temperature months) is feasible and affordable.

Because outbreaks are usually linked to anthropogenic activities, biosecurity is the premier management tool for containment, and there are a host of guides (e.g., Fraser et al., 2006) for implementing controls on transmission of *R. salmoninarum* at different scales. The importance of hygiene between sites or farms is a cost-effective tool against undetected infections, and should be practiced even in the absence of outbreaks.

When a clinical outbreak occurs and a threshold for a management action is crossed, the action should be timely and thorough. Actions can include therapeutic treatment, fish or equipment movement restrictions, selective or broad depopulation, or a blend of action. Documentation and accountability for the action is critical for a good epidemiological outcome. A follow-up to the action, such as monitoring for infection at other pens at a farm, can provide credibility for the action, as well as inform future decisions.

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