Fisheries and Oceans Canada | Pêches et Océans Canada
Ecosystems and Oceans Science | Sciences des écosystèmes et des océans

Canadian Science Advisory Secretariat (CSAS)

Research Document 2019/035

National Capital Region and Pacific Region

Characterization of piscine orthoreovirus (PRV) and associated diseases to inform pathogen transfer risk assessments in British Columbia

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June 2019

Canada

Foreword

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

Published by:

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

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

ISSN 1919-5044

Correct citation for this publication:

Polinski, M. and Garver, K. 2019. Characterization of piscine orthoreovirus (PRV) and associated diseases to inform pathogen transfer risk assessments in British Columbia. DFO Can. Sci. Advis. Sec. Res. Doc. 2019/035. v + 35 p.

Aussi disponible en français :

Polinski, M. et Garver, K. 2019. Caractérisation de l'orthoréovirus pisciaire (RVP) et des maladies associées pour guider les évaluations des risques de transfert d'agents pathogènes en Colombie-Britannique. Secr. can. de consult. sci. du MPO, Doc. de rech. 2019/035. v + 40 p.

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ABSTRACT

Piscine orthoreovirus (PRV) is a common and pervasively distributed virus of salmon. In Canada, nearly all sea-farmed salmon likely become infected with PRV prior to harvest and the virus has been detected in archived specimens dating back to at least the mid 1980's in British Columbia. Wild salmon (all species) also occasionally become infected with PRV. Detection is generally lower in wild populations than on farms, and not all salmon species are equally susceptible to PRV infection. Specifically, Sockeye Salmon appear mildly refractory compared to other species such as Atlantic Salmon. Among the wild Pacific salmon species in the Eastern Pacific, Coho and Chinook salmon have the highest prevalence of PRV (approximately 9% and 6%, respectively); this prevalence appears independent from whether fish were collected from locations in close proximity to salmon farming or from areas devoid of salmon farming. The cumulative prevalence of PRV detected in Sockeye Salmon of Western North America over the past decade is approximately 1.5% based on the sampling of nearly 7,000 specimens of which more than 6,000 were collected from British Columbia stocks. Nonetheless, laboratory studies demonstrate that PRV infected Atlantic Salmon (dependent upon stage of infection) can transmit virus to cohabitating Sockeye Salmon; although the minimum exposure time, dose, and whether such transmission requirements would be reached in natural environs remain unknown. In some farmed salmon, PRV has caused disease – namely, cardiopathy and/or anemia – particularly in Europe and Japan. In farmed salmon of British Columbia, on rare occurrences, PRV has been detected in diseased Atlantic and Chinook salmon where the virus may have contributed to or caused the disease. This includes at least one instance of severe cardiopathy in farmed Atlantic Salmon and one instance of anemia in farmed Chinook Salmon in the past decade. If or when disease may manifest as a result of PRV infection is not well understood, appearing to require complex etiological factors that include host, virus, and environmental components. Both regional as well as viral strain-specific variations in virulence have been documented, and disease has, as yet, only been identified in farmed salmon populations. Important to discussions of PRV in Canada is that PRV in the Eastern Pacific appears less virulent in comparison to PRV in the Eastern Atlantic, and experimental infection of Sockeye and Atlantic salmon with the PRV strain endemic to the Eastern Pacific has failed to manifest significant disease or impact respiratory function even though extreme systemic blood infections developed in both species. Furthermore, stressors such as smoltification, hypoxia, exhaustive chasing, or secondary viral (infectious hematopoietic necrosis virus) superinfection of salmon have not induced or enhanced this PRV virulence. Thus, neither the presence nor quantity of PRV generated during an infection is indicative of disease or physiological impairment in salmon of British Columbia.

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. Restoring funding to support federal ocean science programs to protect the health of fish stocks, to monitor contaminants and pollution in the oceans, and to support responsible and sustainable aquaculture industries in Canada has been identified as a top priority of the Minister of Fisheries, Oceans and the Canadian Coast Guard.

It is recognized that there are interactions between aquaculture operations and the environment (Grant and Jones, 2010). One interaction is the risk to wild salmon populations resulting from the potential spread of infectious diseases from Atlantic Salmon (*Salmo salar)* farms in British Columbia (BC) (Cohen, 2012). While several Atlantic Salmon farms are located within the migratory routes of Pacific salmon species, no risk assessment has been conducted to specifically determine the risk to wild fish populations associated with pathogens released from Atlantic Salmon farms.

DFO Aquaculture Management Division requested formal science advice on the risks of pathogen transfer from Atlantic Salmon farms to wild fish populations in BC. Given the complexity of interactions between pathogens, hosts and the environment, DFO will deliver the science advice through a series of pathogen-specific risk assessments followed by a synthesis.

PURPOSE OF THIS DOCUMENT

The information summarized in this document will assist in the environmental assessment of the risk to Fraser River Sockeye Salmon (*Oncorhynchus nerka*) due to the occurrence of piscine orthoreovirus (PRV) infection on Atlantic Salmon farms located in the Discovery Islands area of British Columbia. This document is designed to be a focused consideration on PRV as a potential causative or contributing agent of disease in salmon of British Columbia which might be presumed to occur and putatively impact Fraser River Sockeye Salmon. As a consequence, this document concentrates on data pertinent to the transmission, pathogenicity (potential for causing disease) and virulence (potential for disease severity) of PRV to Sockeye Salmon occurring in the Discovery Islands area.

GENERAL CONSIDERATIONS

Reovirus infections of farmed Atlantic and Pacific salmon are widespread and nearly all farmed stocks likely become infected at some time during a production cycle. The vast majority of these infections do not result in disease. Nevertheless, in some instances disease syndromes of salmon have been associated with aquatic reovirus infections; specifically, field and laboratory studies with piscine orthoreovirus (PRV) have identified an etiological link between all three known genetic PRV types and circulatory diseases: cardiopathy (heart disease) and/or anemia (insufficient number of red blood cell or hemoglobin) (Takano et al., 2016; Wessel et al., 2017; Vendramin et al., 2019).

Reovirus (both aquareovirus and orthorevirus) infections are also regionally ubiquitous in wild salmon, although prevalence in and across wild stocks are generally lower than among farms. To our knowledge, there is no direct evidence that reovirus infections (and specifically PRV infections) cause disease in populations of wild salmon. Nevertheless, indirect inference from the fact that reoviruses can sometimes cause disease in farmed salmon suggests that similar diseases may occur in wild salmon assuming all host, environmental, and pathogen specific factors can be fulfilled in a natural setting.

A chief consideration in assessing PRV related risks is that the potential for PRV to cause disease in farmed salmon appears to be a complex process with regional variability and high dependence on host, virus, and environmental factors (Garver et al., 2016a; Polinski et al., 2019). This complexity becomes further complicated by dynamic industry and natural field environments such as those found in the Discovery Islands Region of Canada. Recent scientific investigations have identified several putative factors involved in PRV-associated disease, but much is still unknown.

Importantly, PRV presents an atypical example of a microbial pathogen in that the quantity of virus generated during an infection is not an accurate predictor of whether a fish becomes diseased or how severe an associated disease becomes (Lund et al., 2017; Polinski et al., 2019; Zhang et al., 2019). This is counterpoint to most animal pathogens for which disease presence and severity is directly correlated with pathogen load. As a consequence, the risks associated with PRV on salmon health require careful and atypical considerations relative to other salmon pathogens currently of note in British Columbia.

In this document, we provide an overview of PRV and highlight its potential and variable ability to cause disease in salmon. We then review two disease states (cardiopathy and anemia) within farmed salmon of British Columbia for which there is indirect evidence that PRV might have the ability to be a contributing or causative factor. Finally, we discuss current knowledge about the potential interrelationship of PRV and these disease states in salmon of British Columbia and specifically how this relates to Sockeye Salmon. This review focuses considerably on one genogroup of PRV (PRV-1) because it is the only genogroup that has been detected in North America and is also the most well studied.

PISCINE ORTHOREOVIRUS

GEOGRAPHIC DISTRIBUTION AND GENETIC TYPES

PRV is a non-enveloped, double stranded RNA virus within the Reoviridae family (Palacios et al., 2010; Kibenge et al., 2013) that is distributed across multiple countries around the world (Figure 1). PRV has been generally accepted as a species within the orthoreovirus genus due to its 10 linear dsRNA segmented genome and phylogenetic ordination to other orthoreoviruses (Markussen et al., 2013). However, distinction between the orthoreovirus and aquareovirus

genus is currently not well defined and a need for taxonomic reassessment has been suggested given the common yet divergent ordination of PRV to both genera (Nibert and Duncan, 2013), the likely common ancestry of the two genera (Attoui et al., 2002), and the recent discovery of additional putative orthoreoviruses in multiple divergent fish lineages including cartilaginous fish (Shi et al., 2018). Of specific relevance to PRV is that this virus is phylogenetically distinct from other currently known species in both the aquareovirus and orthoreovirus genera with unique genotypic and phenotypic characteristics (Key et al., 2013; Roscow et al., 2018).

Within the current PRV species, more than 20 molecular isolations have yielded fully sequenced genomes. Phylogenetic analyses using amino acid and nucleotide sequences from multiple genomic segments suggest three distinct genogroups: PRV-1, PRV-2 and PRV-3 (Dhamotharan et al., 2018; Kuehn et al., 2018). Each genogroup appears to be loosely segregated by geographical and/or host species divisions, although exceptions exist, and to date isolates from multiple PRV genogroups have not been detected within a single individual host. Nevertheless, members of all three genogroups appear to specifically target salmon, have a proclivity for infecting red blood cells that lead to extensive systemic blood infections, and are suggested to be within a single genus based on current orthoreovirus taxonomic characterization (King et al., 2011; Markussen et al., 2013).

PRV-1

PRV-1 was first identified in Norway (Palacios et al., 2010) and has since been ubiquitously detected in that country (Lovoll et al., 2012; Wiik-Nielsen et al., 2016). PRV-1 is also commonly detected in farmed Atlantic Salmon from Canada, Chile, the United Kingdom, Ireland, Iceland, Germany, Denmark, France and the United States with an additional single detection from an Atlantic Salmon in Sweden (Table 1) (Biering and Garseth, 2012; Garseth et al., 2013; Kibenge et al., 2013; Marty et al., 2015; Siah et al., 2015; Garver et al., 2016b; Adamek et al., 2018; Labrut et al., 2018; Vendramin, 2019). Retrospective studies of archival specimens have identified a historical presence of PRV-1 in Atlantic Salmon in both Norway and Canada dating back to at least the mid 1980's, with presumed high prevalence in farmed populations during much of that time (Marty et al., 2015; Markussen et al., 2018). Phylogenetic comparisons of the PRV-1 S1 genomic segment – which codes the outer clamp protein σ3 of the viral capsid and displays high sequence heterogeneity between isolates – further suggests possible additional delineations within this genogroup. Specifically, PRV-1a and PRV-1b subgroups has been proposed (Kibenge et al., 2013). However, as more PRV-1 sequences become available, new preliminary evidence suggests that whole genome sequence comparisons may provide a clearer picture of PRV-1's divergent regional evolution than S1 alone (Siah et al., 2018), and may prove particularly significant as additional preliminary evidence suggests that segment reassortment may be occurring in Norway between subgroups; i.e., between PRV-1a and PRV-1b (Markussen et al., 2018). Of importance with regard to PRV in British Columbia is that there appears to be relatively high genome homology between PRV-1 isolates within the Eastern Pacific and that these isolates are notably distinct from isolates sequenced from PRV-1 in the Atlantic (Siah et al., 2015; Di Cicco et al., 2018; Siah et al., 2018; Polinski et al., 2019). It is currently unknown if or how much segment reassortment occurs between genomic PRV variants in British Columbia.

PRV-2

The second PRV genotypic variant (PRV-2) is currently only associated with Coho Salmon (*Oncorhynchus kisutch*) in Japan (Takano et al., 2016), and to date has not been detected in any other country or fish species. Although the historic prevalence of PRV-2 is unknown, the presence of a disease condition associated with PRV-2 in Japan known as erythrocytic inclusion

body syndrome or EIBS has been documented since at least the mid 1980's (Takahashi et al., 1992), suggesting PRV-2 has been present in Japan since that time. It is also possible, given the historic detection of EIBS in Chum (*Oncorhynchus keta*) and Masu (*Oncorhynchus masou*) salmon in Japan (Okamoto et al., 1992), that PRV-2 may also have had a historical and possibly current presence in these species but this has not been confirmed. PRV-2 is currently not known to occur in British Columbia or in the Eastern Pacific at large. Although an erythrocytic inclusion body syndrome associated with anemia (also abbreviated EIBS) has long been diagnosed in Coho and Chinook salmon of the North Eastern Pacific (Arakawa et al., 1989), PRV-2 RNA could not be detected in at least one population of Coho Salmon manifesting this North American version of EIBS in Washington State (M. Purcell, personal communication) indicating PRV-2 is unlikely to be the primary causative agent responsible for this syndrome in North America.

PRV-3

The third PRV genotypic variant (PRV-3) was identified in farmed Rainbow Trout (*Onchorhynchus mykiss*) in Norway (Olsen et al., 2015; Hauge et al., 2017) and has subsequently been reported in farmed Coho Salmon in Chile and in farmed Rainbow Trout in several European countries including Denmark, Scotland, Germany, France, and Italy (Dhamotharan et al., 2018; Labrut et al., 2018). PRV-3 has also been detected in Brown Trout (*Salmo trutta*) from Germany (Kuehn et al., 2018). The historic presence of PRV-3 in these countries is unknown. PRV-3 is also not known to occur in British Columbia at this time.

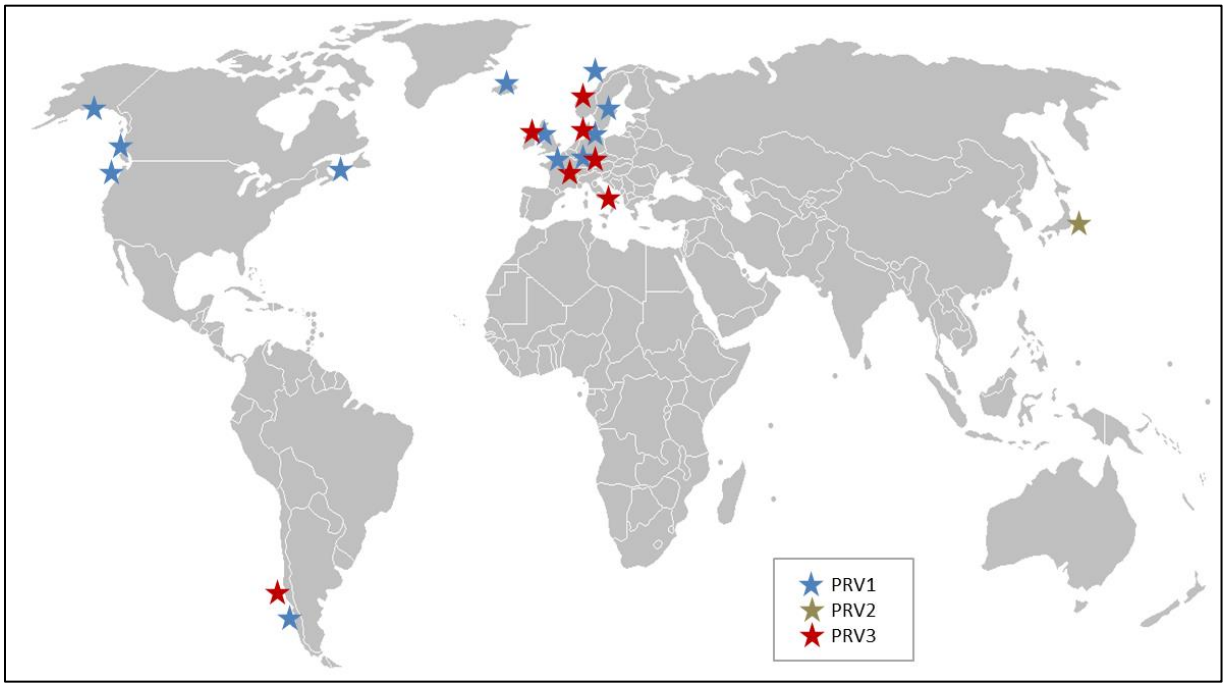

Figure 1. Detection of PRV in natural and farmed fish populations by country and/or geographic region.

HOST RANGE OF PRV-1

Natural infections and controlled laboratory exposure studies indicate PRV-1 predominately infects salmonid fish (Table 1). Occasional detection of PRV nucleic acid (RNA) has also been accomplished in some non-salmonid fish species of the North Atlantic and in Eulachon

(*Thaleichthys pacificus*) in the Pacific, although none have shown indication of being a primary ecological host and their capacity to replicate or transmit PRV-1 remains unknown.

Table 1. Fish species in which PRV-1 genetic material has been detected.

Species	Scientific name	Reference
Canada		
Atlantic Salmon	*Salmo salar*	Kibenge et al. (2013)
Sockeye Salmon	*Oncorhynchus nerka*	Miller et al. (2014)
Chinook Salmon	*Oncorhynchus tshawytscha*	Garver et al. (2016b)
Coho Salmon	*Oncorhynchus kisutch*	Marty et al. (2015)
Pink Salmon	*Oncorhynchus gorbuscha*	Marty et al. (2015)
Chum Salmon	*Oncorhynchus keta*	Kibenge et al. (2013)
Steelhead Trout	*Oncorhynchus mykiss*	Kibenge et al. (2013)
Cutthroat Trout	*Oncorhynchus clarkii*	Kibenge et al. (2013)
Dolly Varden Trout	*Salveinus malma*	Morton et al. (2017)
Eulachon	*Thaleichthys pacificus*	Hrushowy (2018)
United States		
Atlantic Salmon	*Salmo salar*	Warheit (2018)
Chinook Salmon	*Oncorhynchus tshawytscha*	Purcell et al. (2018)
Coho Salmon	*Oncorhynchus kisutch*	Marty et al. (2015)
Pink Salmon	*Oncorhynchus gorbuscha*	Marty et al. (2015)
Steelhead Trout	*Oncorhynchus mykiss*	Purcell et al. (2018)
Norway		
Atlantic Salmon	*Salmo salar*	Palacios et al. (2010)
Sea Trout	*Salmo trutta*	Garseth et al. (2013)
Great Silver Smelt	*Argentina silus*	Wiik-Nielsen et al. (2012)
Atlantic Horse Mackerel	*Trachurus trachurus*	Wiik-Nielsen et al. (2012)
Atlantic Herring	*Clupea harengus*	Wiik-Nielsen et al. (2012)
Capelin	*Mallotus villosus*	Wiik-Nielsen et al. (2012)
Chile		
Atlantic Salmon	*Salmo salar*	Kibenge et al. (2013)
Coho Salmon	*Oncorhynchus kisutch*	Godoy et al. (2016)
Iceland		
Atlantic Salmon	*Salmo salar*	Gunnarsdóttir et al. (2018)
Ireland		
Atlantic Salmon	*Salmo salar*	Rodger et al. (2014)
Faroe Islands		
Atlantic Salmon	*Salmo salar*	Markussen et al. (2018)
Germany		
Atlantic Salmon	*Salmo salar*	Adamek et al. (2018)

Species	Scientific name	Reference
France		
Atlantic Salmon	*Salmo salar*	Labrut et al. (2018)
Denmark		
Atlantic Salmon	*Salmo salar*	Vendramin (2019)
Sweden		
Atlantic Salmon	*Salmo salar*	Vendramin (2019)

CELLULAR TROPISM OF PRV-1

The primary cell type targeted by PRV in salmon is the erythrocyte (red blood cell). Unlike mammals, fish erythrocytes remain nucleated throughout their lifespan and thus possess the cellular components necessary for viral replication during all cellular life stages. PRV is detected with the highest prevalence in blood during most stages of infection relative to all other tissue types tested (Finstad et al., 2014; Garver et al., 2016a), and of the three types of blood cells (red blood cells, white blood cells and platelets), red blood cells appear to be the only cell type significantly infected (Wessel et al., 2015; Polinski et al., 2019). Amplification of both PRV-1 protein and genetic material occurs within erythrocytes (Finstad et al., 2014; Wessel et al., 2015) and erythrocytes have repeatedly been used to initiate experimental infections (Wessel et al., 2015; Polinski et al., 2019). This provides strong empirical evidence that infectious virus can be generated within this cell type. Secondary infections of cardiomyocytes (heart muscle cells), enterocytes (intestinal absorptive cells) and tissue-resident leukocyte-like cells (presumably macrophages) have also been reported (Di Cicco et al., 2017; Di Cicco et al., 2018). However, it is unclear as to whether or not PRV replication occurs within these cell types, and *in vitro* experimental infection of Atlantic Salmon heart endothelial (ASHe), epithelial (ASK) and fibroblast (BAASf) laboratory cells lines, as well as Rainbow Trout macrophage (RTS11) and approximately 20 other fish laboratory cells lines, have yet to effectively replicate PRV-1 under varied environmental conditions (Pham, Bols, Polinski and Garver, unpublished data). One laboratory cell line, GF-1, derived from the fin of orange-spotter grouper, *Epinephelus coioides*, showed cytopathic effects suggestive of viral replication after being inoculated with a homogenate containing PRV (Mikalsen et al., 2012). However, PRV was not visualized by electron microscopy (Mikalsen et al., 2012) and subsequent attempts to detect amplification of PRV in GF-1 cells using RT-qPCR proved negative (Garver et al., 2016b).

INFECTION DYNAMICS OF PRV-1

The kinetics of PRV-1 as observed in Atlantic Salmon indicates three distinct phases of infection: early entry and dissemination, peak systemic replication, and long-term persistence. In the first (early) phase of infection which typically lasts 2-3 weeks at 12°C, initial host entry, replication and dissemination of the virus into blood cells occurs. It is unknown where PRV first enters host cells, although it is likely through cells of the respiratory (gill) or enteric (gastrointestinal) epithelium as these sites are typical for reovirus entry. Mammalian orthoreoviruses first infect epithelial cells of the small intestine or lung prior to haematogenous dissemination (Boehme et al., 2013); and the recent detection of PRV in intestinal enterocytes (Di Cicco et al., 2018) indicates that a similar course of infection might be followed by PRV. Upon infection, the early replicative phase of mammalian reovirus likely dictates how much virus gets disseminated, ultimately setting the course and overall severity of infection (Lai et al., 2013). This first phase appears equally important with PRV infections and may account for discrepancies in total virus production occasionally observed following laboratory challenge of salmon with different PRV isolations with similar RNA loads, where a lag in replication of one

isolate appears to be the major difference between otherwise identical replication dynamics with blood cells (Polinski et al., 2019). A lack of PRV transmission via fish cohabitation at this early stage of infection also suggests that whatever cell type(s) PRV is initially infecting, it is not likely being shed into the environment to a high degree (Polinski et al., 2019).

In the second (peak) phase of infection that typically lasts 2-3 weeks at 12°C, substantial PRV replication within erythrocytes occurs along with the formation of cytoplasmic viral inclusions (Finstad et al., 2014; Wessel et al., 2015; Haatveit et al., 2017; Polinski et al., 2019) similar to those that develop during mammalian reovirus infection of well-established cell lines (Eichwald et al., 2018). The highest systemic blood loads of PRV occur during this period, and it is when innate virus recognition pathways of the host are most likely to become activated, although this activation can be variable and even nonexistent depending on PRV regional variants [summarized by (Polinski et al., 2019)]. Cohabitation challenges have shown substantial virus shedding at this time (Garver et al., 2016a; Wessel et al., 2017).

In the third (persistent) phase of infection, viral inclusions within erythrocytes disappear and a marked reduction in viral protein production occurs even though large quantities of genomic PRV material remain associated with the erythrocyte cell fraction (Haatveit et al., 2017; Lund et al., 2017; Polinski et al., 2019). The ability to recapitulate infectious replication of PRV from late stage infections has been readily accomplished by injecting lysed blood cell material into naïve fish (Polinski et al., 2019); however, poor viral transmission has also been demonstrated via cohabitation during this late infectious stage, suggesting natural shedding of virus might be minimal during persistent infections and may even cease entirely over time (Garver et al., 2016a). If heart inflammation occurs, it is typically observed early in the persistent infection phase, although in some instances heart inflammation has occurred just prior to this phase during the peak of infection (Lund et al., 2017; Wessel et al., 2017; Polinski et al., 2019). This inflammation can last for weeks to months depending on a number of factors, but ultimately appears to resolve in all cases even though PRV infections continue to persist (Di Cicco et al., 2017; Lund et al., 2017).

GENERAL PATHOGENICITY OF PRV

The perceived capacity of PRV to cause disease in many regards closely mirrors that of Avian orthoreovirus (ARV) in poultry. Namely, its impact varies widely from region to region and its ubiquitous nature is often associated with diseases for which a causative link cannot be established (Jones, 2000). It should be noted that in controlled experimental trials, PRV has (as yet) never caused clinical morbidity or mortality in salmon even during extreme blood infections (Garver et al., 2016a; Takano et al., 2016; Wessel et al., 2017; Polinski et al., 2019), nor has it contributed to clinical morbidity or mortality during experimental trials in accompaniment with stressors such as smoltification, viral co-infection, hypoxia, or exhaustive chasing (Garver et al., 2016a; Lund et al., 2016; Polinski et al., 2016; Lund et al., 2017; Zhang et al., 2019). However, all three genogroups of PRV can at the very least contribute to disease states in salmon of variable significance (Olsen et al., 2015; Takano et al., 2016; Wessel et al., 2017; Polinski et al., 2019; Vendramin et al., 2019). Thus, all three genogroups of PRV have pathogenic potential but low virulence due to the inability of extreme systemic infections to cause mortality or morbidity under controlled laboratory conditions.

PRV is typical for a reovirus, but unlike many other viruses, in that it does not directly lyse the cells it infects (Finstad et al., 2014; Wessel et al., 2015; Polinski et al., 2019). Rather, the pathogenic potential of PRV likely stems from the killing of infected cells via an adaptive (T-cell mediated) immune response by the host fish (Mikalsen et al., 2012; Yousaf et al., 2012; Zhang et al., 2019). In other words, PRV itself does not appear to inflict damage to host cells, but if host immune T-cells develop an ability to recognize PRV as a foreign invader, infected cells

become targeted by these sensitized T-cells for destruction. In some instances this appears to results in immune cells targeting infected cardiomyocytes and cardiac epithelial cells such as during heart and skeletal muscle inflammation (HSMI) (Mikalsen et al., 2012). In others instances, infected erythrocytes have been suggested to become targeted for destruction while passing through the liver or spleen such as possibly during jaundice/anemia of Chinook Salmon (*Oncorhynchus tschawytscha*) (Di Cicco et al., 2018). The mechanisms for initiating these adaptive host responses to PRV (if they can be confirmed) are unknown, and it is also unclear why some cell types are more selectively targeted for destruction than others in different instances; e.g., cardiomyocytes and not erythrocytes in Atlantic Salmon even though erythrocytes are the primary cell type infected (Zhang et al., 2019). Recent investigations have suggested that these mechanisms are highly variable with regard to the host species, host strain (possibly even the individual), and to the PRV isolate involved (Polinski et al., 2018; Wessel et al., 2018a). Current knowledge regarding the pathogenic potential of each PRV genogroup is outlined below.

PRV-1

At least one purification-based isolation of PRV-1 has been demonstrated to cause severe heart inflammation in farmed Atlantic Salmon of Norway (Wessel et al., 2017) and both PRV-1a and PRV-1b have been physically isolated from HSMI diseased fish in net-pen farm environments [for summary, see (Garver et al., 2016a)]. In Norwegian Atlantic Salmon aquaculture, HSMI is associated with morbidity, lethargy, and occasional mortality; it is considered one of the most significant transmissible diseases affecting industry production (Hjeltnes B et al., 2017).

The inflammation generated during HSMI is likely mediated by an adaptive cytotoxic T-cell response to PRV-1 antigen (Mikalsen et al., 2012). This hypothesis is supported by the increased presence of cytotoxic T-cells in the heart of HSMI diseased fish in accordance with increased transcription of their killing enzymes, e.g., granzyme-A (Mikalsen et al., 2012) and that cytotoxic T-cells are also responsible for reovirus-induced heart inflammation in mammals (London et al., 1990; Gujar et al., 2010). Nevertheless, the clinical severity of HSMI as seen on industry farms in Norway has not been recreated in controlled experimental conditions despite the generation of high-load PRV infections with or without hypoxic stress (Lund et al., 2017; Wessel et al., 2017) – indicating that factors specific to the commercial field environment in Norway contribute to HSMI. This is supported by previous finding that HSMI increases in prevalence with increasing cohort lifespan, infection pressure, cohort size, and local geography (Kristoffersen et al 2013). Heightened disease scenarios are also likely driven in part by host-specific factors as evidenced by the development of a strain of Atlantic Salmon in Norway that had less heart damage and higher survival but were not resistant to PRV infection (AquaGen, 2017; Emilsen et al., 2017); further supporting that host sensitivity to PRV may play a critical role in determining the severity of disease.

In Pacific Canada, PRV-1 has been suggested to be a contributing factor in a jaundice/anemia syndrome of farmed Chinook Salmon (Di Cicco et al., 2018) as well as severe cardiomyopathy and skeletal muscle inflammation in farmed Atlantic Salmon (Di Cicco et al., 2017; Di Cicco et al., 2018). Although it is probable that PRV can and occasionally does contribute to both conditions, the role for how or if PRV acts as the etiological mediator of these relatively rare diseases in BC is far from clear. Specifically, neither the jaundice/anemia syndrome nor severe cardiomyopathy has been successfully transmitted to naive Chinook or Atlantic Salmon in laboratory challenge trials in Pacific Canada despite the successful passage and development of high-load PRV blood infections within both species (Garver et al., 2016b; Polinski et al., 2019). This type of passage experiment is critical for establishing and identifying pathogenicity of a microbial agent (Fredericks and Relman, 1996), and the lack of virulence demonstrated by

high-load PRV infections on these occasions indicates that other critical etiological factors are necessary to establish these disease conditions. This is further supported by the low number of documented incidences of jaundice/anemia or HSMI-like cardiopathy compared to the high prevalence of PRV in farmed populations of Chinook and Atlantic Salmon in British Columbia, respectively. Additional investigations into the etiological factors for initiating these disease conditions is needed to determine if or how PRV may be involved.

PRV-2

In Japan, PRV-2 has been shown to cause an anemic condition of farmed Coho Salmon known as erythrocytic inclusion body syndrome or EIBS and has caused moderate anemia in Coho Salmon following experimental infection (Takano et al., 2016). Significant mortality has been historically attributed to EIBS in Japan during farming of Coho Salmon (Takahashi et al., 1992); although experimental challenges with PRV-2 have failed to cause mortality (Takano et al., 2016). The mechanisms behind PRV-2 pathogenicity are unknown, but as with PRV-1, factors specific to field environments appear to exacerbate the severity of disease and associated mortality (Takano et al., 2016). It may be hypothesized from work done with PRV-1 that a T-cell mediated sensitivity might be responsible for the anemia observed during EIBS in Japan via a mechanism of targeted destruction of infected erythrocytes as they pass through the liver or spleen. Of particular note in considering PRV-2 relative to other PRV genogroups is the staggering quantity of virus generated during peak infection (approximately one trillion genomic copies per mL blood) in both experimentally and naturally infected fish (Takano et al., 2016). These quantities appear to be 10 to 1,000 times greater than produced during PRV-1 infections of Atlantic Salmon (Garver et al., 2016a; Polinski et al., 2019; Zhang et al., 2019) and at least 1,000 to 10,000 times higher than the most robust PRV-1 infections reported in Pacific Sockeye Salmon (Polinski et al., 2016).

PRV-3

PRV-3 has been detected in association with an anemic/HSMI-like condition in farmed Rainbow Trout in Europe (Olsen et al., 2015) and a jaundice/anemia syndrome in farmed Coho Salmon in Chile (Godoy et al., 2016). PRV-3 was initially thought to also be the causative agent of a proliferative darkening syndrome (PDS) in Brown Trout in central Europe (Kuehn et al., 2018); however, subsequent investigation has demonstrated that PRV-3 is not the causative agent of PDS (Fux et al., 2019). Low to moderate mortality occurs in Rainbow Trout suffering from the anemic/HSMI condition (Olsen et al., 2015) although the role that PRV-3 plays in the development of these diseases remains unclear. As for PRV-3, it could be speculated to be driven by cytotoxic T-cell recognition. A laboratory study conducted to assess the pathogenicity of a Norwegian variant of PRV-3, demonstrated that PRV-3 infections of Rainbow Trout were capable of generating heart inflammation yet failed to recreate anemia (Vendramin et al., 2019). Consequently, the anemia observed in hatchery outbreaks may be due to a secondary factor triggering a more severe disease as is observed in the field (Hauge et al., 2017). Interestingly, exposure of Atlantic Salmon to PRV-3 isolated from Rainbow Trout revealed a capability for the virus to infect both salmonid species, but faster transmission, more notable antiviral response and more prominent heart pathology were observed in Rainbow Trout, suggesting host species-specific factors are important modulators of PRV-3 associated disease (Hauge et al., 2017).

REGIONAL VARIATIONS IN VIRULENCE (PRV-1)

In Norway, most farmed Atlantic Salmon become PRV positive, but only some develop disease. This does not appear to be dependent on systemic PRV load, and it is not clear why some farms experience high losses due to HSMI while others do not. Nevertheless, clinical outbreaks

of HSMI in farmed Atlantic Salmon of Norway are reasonably common (Kongtorp et al., 2004a; Kongtorp et al., 2004b; Kongtorp et al., 2006; Palacios et al., 2010), and laboratory challenge trials have demonstrated a clear ability for PRV to cause severe heart lesions (Wessel et al., 2017). Indeed, laboratory challenge trials in Norway routinely generate severe heart lesions in accompaniment with occasional skeletal muscle lesions similar to those observed on HSMI diseased salmon farms (Kongtorp et al., 2004b; Kongtorp and Taksdal, 2009; Mikalsen et al., 2012; Finstad et al., 2014; Lund et al., 2017).

In Pacific Canada, there is a strikingly divergent relationship regarding PRV and its association with disease. PRV appears to be highly prevalent in farmed Atlantic Salmon of Pacific Canada (Marty et al., 2015); yet, a clinical outbreak of HSMI as described in Norway (Kongtorp et al., 2004a; Kongtorp et al., 2004b) has never been reported. Two farm-level cases of HSMI-like disease have been suggested to date (Di Cicco et al., 2017; Polinski et al., 2019), but unlike in Norway, this disease could not be transmitted to naïve fish in a laboratory setting (Polinski et al., 2019). Indeed, PRV has failed to cause severe heart lesions or any severity of skeletal muscle inflammation following experimental challenge of Atlantic or Pacific salmon in Pacific Canada (Garver et al., 2016a; Polinski et al., 2019; Zhang et al., 2019). Ongoing laboratory investigations directly comparing PRV-1 isolated in both Norway and the Eastern Pacific have also preliminarily identified that the PRV-1 from the Eastern Pacific is of lower virulence to Norwegian Atlantic Salmon (Wessel et al., 2018a).

Host, virus, and environmental factors may all be responsible or contributing factors for this regional altered virulence of PRV. The relative contribution by each of these putative factors is currently unknown; however, there are at least three potentially significant phenotypic dissimilarities between Canadian and Norwegian PRV-1 that have been revealed through laboratory challenge trials (Figure 2). First, despite the similarity of Pacific Canada PRV and Norwegian PRV to produce high load viremia, Pacific Canada PRV remains absent from the plasma (Polinski et al., 2019) while Norwegian PRV can be detected at high loads in the plasma for up to six weeks following infection (Finstad et al., 2014; Wessel et al., 2017). Second, there is a considerable difference in scale regarding host recognition of PRV. Although direct comparisons between Canadian and Norwegian studies are limited, mean systemic and heart-specific antiviral responses increased no more than fivefold in Pacific Canada studies (Garver et al., 2016a; Polinski et al., 2019; Zhang et al., 2019) whereas in Norwegian challenges these genes increased 10-50 fold in the blood (Haatveit et al., 2017; Wessel et al., 2017) and more than 100 fold in the heart (Mikalsen et al., 2012). The comparative lack of antiviral response to Pacific Canada PRV compared to Norwegian PRV is further supported by the relative protection PRV has afforded fish challenged with a secondary virus (IHNV) in Norway (Vendramin et al., 2018) but not in Pacific Canada (Polinski et al., 2016). Lastly, in addition to the discrepancies concerning the severity of heart inflammation outlined above, the timing of PRV associated heart inflammation is also different between challenges conducted with PRV from these two countries. Specifically, by either injection or cohabitation exposure of PRV, heart inflammation (prevalence and severity) in Norwegian studies consistently begins around the time of peak systemic PRV load, reaches high severity 1-2 weeks later, and thereafter diminishes (Lund et al., 2017; Wessel et al., 2017). In contrast, increased prevalence of heart inflammation in Pacific Canada challenge trials did not occur until approximately 4 weeks after peak PRV systemic loads were reached and maintained high prevalence (although not severity) for prolonged periods of greater than 6-7 weeks (Polinski et al., 2019; Zhang et al., 2019). All challenges were conducted at approximately the same temperature (10-12°C).

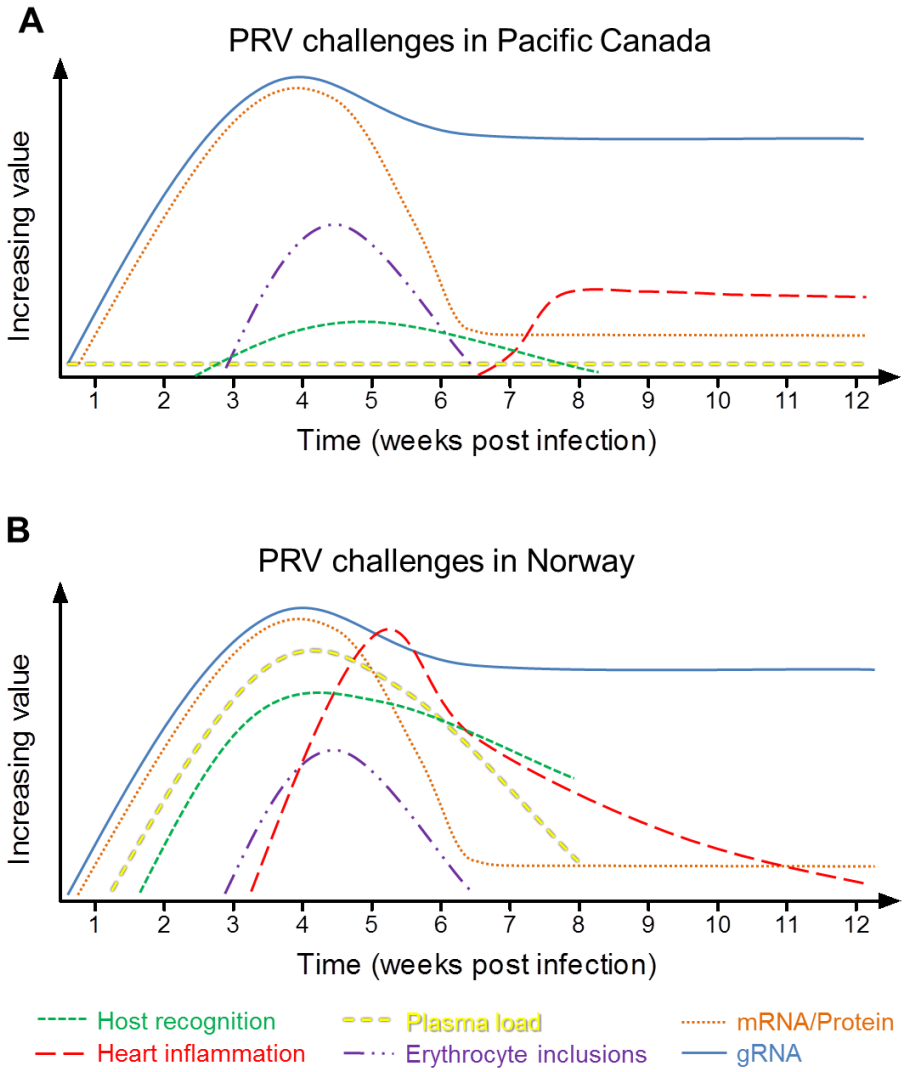

Figure 2. Contrast summary for general trends in PRV phenotypic infection dynamics between Norway and Canada laboratory challenge of Atlantic Salmon (taken from Polinski et al., 2019). In comparing challenge trials conducted in (A) Pacific Canada (Garver et al., 2016a; Polinski et al., 2019; Zhang et al., 2019) with results from similar challenge trials conducted in (B) Norway (Mikalsen et al., 2012; Finstad et al., 2014; Haatveit et al., 2017; Wessel et al., 2017). Note that some variance in experimental design exists between these experiments.

TRANSMISSION DYNAMICS (PRV-1)

Routes of Entry

PRV has been demonstrated to spread horizontally (from fish to fish) during laboratory cohabitation studies where PRV infections become evident in 100% of naïve fish (Garver et al., 2016a; Wessel et al., 2017). The route by which PRV enters naïve hosts remains unclear; however, fecal-oral transmission is a hallmark of many reoviruses and the presence of PRV-1 in feces of infected fish (Hauge et al., 2016) coupled with the demonstrated ability of PRV to infect naïve fish via anal intubation (Hauge et al., 2016) suggests fecal-oral transmission is at least one likely route for natural PRV entry. Experimental studies have also generated PRV infections following waterborne immersion (Kvamme et al., 2018). Given that direct horizontal transmission

of PRV can readily be accomplished, vector-mediated transmission (e.g., via a multicellular parasite) would present an unnecessary step in spreading PRV. Currently there is no evidence to suggest a vector is needed for PRV transmission.

Although the primary mode of PRV transmission is almost certainly horizontal, it is probable, given the systemic nature of PRV infections, that PRV contamination of sexual fluids permits the potential for egg-associated vertical (from parent to egg) transmission. Fish in freshwater hatcheries in both North America and Europe (Lovoll et al., 2012; Polinski et al., 2019) have become infected presumably via this method; however, a study following a population of Norwegian Atlantic Salmon brood fish and progeny from 2008 to 2011 found that PRV was not isolated in or on eggs collected from infected brood fish following a Buffodine® disinfection treatment (Wiik-Nielsen et al., 2012). Similarly, PRV could not be detected in juvenile Atlantic Salmon reared from iodine disinfected eggs at a commercial freshwater facility in British Columbia even though the Atlantic Salmon brood fish supplying the facility carried high systemic RNA loads of PRV (Polinski & Garver, unpublished data). As iodine disinfects egg surfaces but not the internal yolk or embryo, this data suggests that infectious PRV is not carried internally within fertilized eggs of salmon and that, if PRV is present on egg surfaces, iodine-based disinfection has the potential to be used to block infectious PRV transmission from parent to offspring.

Shedding

PRV infected salmon are considered a main transmission source of virus; yet it remains unknown as to how long and at what rates PRV is shed from an infected fish. Cohabitation studies where naïve salmon were introduced at different stages of PRV infection revealed that Atlantic Salmon recently infected with PRV were capable of transmitting virus but those in persistent stages of infection had reduced or ineffectual transmission to the naïve cohabitants (Garver et al., 2016a; Polinski et al., 2019). Therefore, it is inaccurate to presume that all PRV infected Atlantic Salmon are equally contagious and are likely to transmit virus and it remains unknown as to what factors may impact virus shedding. Laboratory studies in Pacific Canada have demonstrated that Atlantic Salmon were highly infectious after 4-6 weeks of becoming infected with PRV (Garver et al., 2016a) but horizontal transmission was reduced by 15 weeks (Polinski et al., 2019) and could not be accomplished after 44 weeks despite the ongoing persistence of PRV (Garver et al., 2016a). Based on these studies, it is hypothesized that natural horizontal transmission primarily occurs between 3-15 weeks following infection of a fish, after which the potential for natural shedding becomes severely reduced (Polinski et al., 2019). However, given the long term persistence of infectious particles within the blood of infected fish (Polinski et al., 2019), it is unknown if there are mechanisms which could increase shedding following periods of latency or if physical trauma resulting in blood loss could alternatively increase shedding of virus from fish with persistent PRV infections.

Environmental stability

It can be presumed that PRV maintains at least a minimum capacity to survive in water, as successful waterborne transmission has been demonstrated experimentally. Yet, the extent to which PRV can remain infectious in the natural marine environment remains unknown. Many environmental factors such as sunlight, organic load, and indigenous microbial populations can adversely affect virus stability to varying degrees dependent upon virus type (Pinon and Vialette, 2018). For instance, viruses surrounded with an envelope are generally more easily rendered inactive than viruses without an envelope (Fitzgibbon and Sagripanti, 2008). Being free of an envelope, PRV could be expected to have greater stability than, for example, the envelope containing aquatic virus infectious hematopoietic necrosis virus (IHNV) which showed

markedly reduced infectivity within hours of being held in natural seawater (Garver et al., 2013). In contrast, infectious pancreatic necrosis virus (IPNV), a nonenveloped aquatic fish virus, requires days to lose infectivity in estuarine water (salinity 11.5 ppt) (Toranzo et al., 1983). However, due to the fact that decay rates are highly conditional upon virus and environmental factors, survival studies specific to PRV are required to accurately define its duration of infectivity in seawater. To date, such studies have not been undertaken due to the lack of conventional culture methodologies to conveniently monitor and evaluate the infectivity of PRV. Furthermore, suitable proxy data are unavailable as viral stability measurements from culturable surrogates such as Chum Salmon aquareovirus, that shares both structural and genomic similarities with PRV, have not been performed.

Infectious potential

Preliminary evidence using PRV-1 from Pacific Canada suggests that $\leq 10^3$ PRV particles are sufficient to initiate infection by intra-peritoneal injection in Atlantic Salmon (M. Polinski, unpublished data). The minimum dose required to establish infection by immersion or ingestion is unknown, but the route of virus exposure, the host condition, stock, and species involved are all likely to play a role in the infectious potential of PRV. For example, Sockeye Salmon have exhibited detectable levels of PRV in blood as early as five days post intra-peritoneal injection (Polinski et al., 2016) while Sockeye continually cohabitated with PRV infected Atlantic Salmon did not acquire PRV blood infections until the 4th week of cohabitation (Garver et al., 2016a). Further, sentinel Sockeye Salmon showed substantially lower prevalence and intensity of PRV infections than in sentinel Atlantic Salmon of an equivalent exposure group after 4 weeks of cohabitation (Garver et al., 2016a), indicating that Sockeye Salmon are less susceptible to PRV than Atlantic Salmon and may require a more lengthy exposure period or dose to become infected. For newly smolted Pink Salmon (*Onchorhynchus gorbuscha*) (1 g), waterborne exposure to either 100 or 1,000 purified PRV particles per mL for one hour was insufficient to initiate infection (n=20) while an equivalent dose of 1,000 purified particles administered via intra-peritoneal injection established PRV infection in 90% of fish (n=10), suggesting a low susceptibility of Pink Salmon to waterborne infection (Richard, Polinski, and Garver, unpublished data). Refractivity to PRV-1 by immersion has also been preliminarily demonstrated in Sea Trout (*Salmo trutta*) relative to Atlantic Salmon in Norway (Kvamme et al., 2018). It is currently unknown if these reduced susceptibilities are dose and/or duration dependent.

Farmed-to-wild salmon transmission

Given the linkage with PRV to HSMI in Norwegian Atlantic Salmon farming, investigations have been conducted in Norway to evaluate the transmission of HSMI and PRV between neighboring farms and to wild fish populations. Sequence comparisons of PRV variants collected from farm and wild salmon in Norway revealed that PRV genotypes are similar regardless of host origin, suggesting that virus exchange is occurring between wild and farmed populations in Norway (Garseth et al., 2013; Madhun et al., 2018). However, neither the directionality nor the mechanism(s) responsible for exchanging PRV between farmed and wild populations are currently known. It has been postulated that interactions between wild and escaped farmed salmon, specifically when wild salmon migrate through aquaculture areas, may serve as potential mechanisms of virus perpetuation (Garseth et al., 2013). Nevertheless, comparisons of PRV prevalence in wild adult salmon from regions of northern Norway with differing farming intensity and disease frequency showed no association between salmon farming and the prevalence of PRV infection in wild salmon (Madhun et al., 2018).

In western North America, the high genome homology between PRV-1 isolates of farmed and wild salmon (Siah et al., 2015) suggests the presence of a common reservoir and/or exchange of virus between wild and farmed populations. Yet the contribution of salmon farms to potentially exchange PRV with wild fish is unclear. One study has hypothesized salmon farms may influence PRV prevalence in wild Pacific salmon after identifying a higher prevalence of PRV in wild salmon with a “high” exposure probability to salmon farms than in fish sampled from “low” farm-exposure regions (Morton et al., 2017); although it must be noted that the categorization for low and high farm exposure used in this study is highly speculative. In contrast, a study that compared detection of PRV in Coho Salmon from Alaska (an area devoid of net pen salmon aquaculture) to Coho Salmon from British Columbia (where salmon farms are present) identified no significant difference in PRV detection prevalence, suggesting salmon farming was contributing negligibly to PRV prevalence in these wild Coho stocks (Marty et al., 2015). Chinook Salmon also screened for PRV in Alaska (Purcell et al., 2018) similarly showed analogous detection prevalence and stock variability for PRV detection to Chinook Salmon of British Columbia (Marty et al., 2015) (Table 2); also suggesting that salmon farms are having minimal direct impacts to PRV prevalence in Chinook of the Eastern Pacific. Undoubtedly a multitude of factors are responsible for influencing PRV prevalence in wild salmon, which is clearly evident by the fact that PRV detection varies considerably across host species and even between cohorts of a particular species (Purcell et al., 2018). Consequently, longer time scale monitoring efforts in conjunction with molecular epidemiology studies are needed to fully appreciate the drivers of PRV infection in salmon population of western North America.

PREVALENCE IN WESTERN NORTH AMERICA

Molecular diagnostic screening has been applied in numerous surveillance programs that have identified the presence of PRV among farmed and wild salmon collected over the geographic range spanning Alaska to Washington. Analyses of archived salmon samples from 1974-2008 from British Columbia also revealed a long-term and common presence of PRV-1 in the Eastern Pacific with positive detections identified in samples dating back to 1987 and possibly as early as 1977 (Marty et al., 2015). Both farmed and wild fish stocks have been shown to become infected.

Farmed Atlantic Salmon

Once PRV becomes present on a salmon farm, it is expected to reach 100% prevalence within the population (Di Cicco et al., 2017; Polinski et al., 2019). In a temporal study of PRV at one Atlantic Salmon farm site in British Columbia, PRV was first detected 3 to 4 months following seawater entry and peaked at 100% several months later (Di Cicco et al., 2017). A second study also identified 100% PRV prevalence at a different British Columbia Atlantic Salmon farm site after fish had spent 3 months at sea (Polinski et al., 2019). More recently, a sampling survey of dead or dying fish collected in all aquaculture zones of BC demonstrated that time-at-sea was a significant predictor for the detection of PRV in Atlantic Salmon with prevalence increasing up to 18 month post seawater entry and declining thereafter (Laurin et al., 2019). Additionally, current ongoing research examining PRV prevalence on 13 Atlantic Salmon farms spread across British Columbia found that fish at all 13 sites became infected with PRV with a general onset within 100 and 200 day post seawater entry that was independent of location or time of stocking. Further, following initial infection, all 13 farms reached 100% infection prevalence within 100 days of first detection (Polinski and Garver, unpublished data).

Wild Pacific salmon

Either through experimental or natural infection, all five species of North American Pacific salmon have been presumed to be capable of supporting PRV infections as evidenced by the internal detection of PRV RNA in all five species (Table 1); however, surveys of wild Pacific salmon demonstrate that PRV detection can vary dramatically between species and stock. Across multiple independent surveys of Pacific salmon and trout, PRV was consistently detected in Chinook and Coho salmon as compared to Chum (*O. keta*), Pink, Sockeye, and steelhead trout (*O. mykiss*). Collectively across the studies, while understanding that there are no reported test performance characteristics for the various studies and that they differ in sampling protocols, life stage of fish sampled, analytical techniques and quality control stringencies, face value detection prevalence for Chinook and Coho salmon identified within these studies reached approximately 6% and 9%, respectively, while PRV detection prevalence in Pink Salmon remained below 4%, Sockeye around 1.4%, and Chum as well as steelhead less than 1% (Table 2).

Table 2. PRV-1 prevalence in North American Pacific salmon and trout species sampled in Alaska, British Columbia, and Washington. Numbers in parentheses represent the PRV positive fish per total number of fish sampled. Results are reported at face value without consideration of differences in sampling or assay design.

Species	**PRV-1 surveillance studies**						**Overall prevalence**
	(Marty et al., 2015)	(Purcell et al., 2018)	(Morton et al., 2017)	S. Johnson unpublished	Studies using Fluidigm BioMark™ assay[a]	Unpublished student theses[b]	
Sockeye Salmon	**0.3%** (1/371)	**0.0%** (0/394)	**9.3%** (21/225)	**0.0%** (0/717)	**1.6%** (67/4215)	**1.0%** (8/771)	**1.4%** (97/6693)
Chinook Salmon	**8.8%** (6/68)	**4.0%** (19/480)	**34.3%** (34/99)	**4.4%** (54/1232)	**2.8%** (9/325)	**2.4%** (1/41)	**5.5%** (123/2245)
Coho Salmon	**7.6%** (9/118)	**11.8%** (56/473)	**26.1%** (18/69)	**4.5%** (16/356)	**1.7%** (1/61)	--	**9.3%** (100/1077)
Pink Salmon	**0.0%** (0/313)	**0.4%** (1/243)	**25.0%** (27/108)	**0.0%** (0/70)	--	--	**3.8%** (28/734)
Chum Salmon	**0.0%** (0/101)	**0.0%** (0/287)	**7.5%** (5/67)	**0.0%** (0/135)	--	--	**0.8%** (5/590)
Steelhead Trout	--	**0.3%** (1/375)	**28.6%** (4/14)	--	--	**1.0%** (3/303) trout combined	**0.9%** (5/553)
Cutthroat Trout	--	--	**50.0%**(8/16)	--	--		--
Dolly Varden Trout	--	--	**10.0%** (1/10)	--	--		--

[a] (Jeffries et al., 2014; Miller et al., 2014; Bass et al., 2017; Teffer et al., 2017; Nekouei et al., 2018; Teffer et al., 2018; Thakur et al., 2019)

[b](Furey, 2016; Healy, 2017; Hrushowy, 2018; Stevenson, 2018)

Specific to Sockeye Salmon, 12 independent studies cumulatively indicate that the majority of samples positive for PRV nucleic acid were collected from returning adults (Table 3). A cumulative 0.3% (12/3911) of fry and juvenile fish were positive for PRV whereas 2.9% (85/2912) of returning adults were positive. This data also suggests that most PRV infections occurred at sea. PRV was detected on or in out-migrating smolts collected at the mouth of Queen Charlotte Strait and within the southern Queen Charlotte Sound (after presumed northward migration through the Discovery Islands/Johnson Strait) at a prevalence of 0.8% (7/833), whereas fry and parr had a nominally lower prevalence of 0.4% (4/1072) in freshwater, with most detections (3) occurring in a population of fry from Oweekeno Lake that is not associated with the Fraser River (Table 3). Similarly, the majority of PRV detections in adult Sockeye Salmon (63/85 total positives) occurred in one study which screened gill biopsies of returning adult fish migrating southwards through the Johnston Strait/Discovery Islands (Miller et al., 2014). Interestingly, liver samples taken at the same time of gill biopsies as well as subsequently in the Fraser River were negative for PRV; suggesting that the PRV on or in the gill tissues of these fish did not represent systemic infections nor did systemic infections likely develop before returning fish reached their spawning grounds.

Within the Fraser River, PRV has been detected in at least five stocks of Sockeye Salmon (Table 4), although sampling for many stocks has been limited and the single Nadina River sample positive for PRV nucleic acid was considered questionable by the authors (Marty et al., 2015). Further, it should again be noted that 63/68 positive PRV detections occurred as a result of gill biopsies taken from returning adults passing through the Johnstone Strait/Discovery Islands which did not appear to develop systemic infections (Miller et al., 2014).

Table 3. Detection of PRV in Sockeye Salmon of Alaska, British Columbia, and Washington by life stage. Numbers in parentheses represent the PRV positive fish per total number of fish sampled. Cumulative detection specific to Fraser River Sockeye Salmon (FRSS) stocks (where identified) is also presented; the 29 adult fish sampled in saltwater by Morton et al. are of unknown (possibly Fraser River) origin but not incorporated into the FRSS summary.

Data Source	Sockeye Salmon PRV prevalence				
	Fry	**Parr/smolt**		**Adults**	
	Freshwater	**Freshwater**	**Saltwater**	**Saltwater**	**Freshwater**
Marty et al. (2015)	--	0/30	--	--	1/341
Purcell et al. (2018)	--	--	--	--	0/394
Johnson (unpublished)	--	0/344	0/373	--	--
Morton et al. (2017)	--	1/1	3/90	0/29	17/105
Miller et al. (2014)	--	--	1/165	64[1]/531	1/498
Teffer et al. (2017)	--	--	--	--	0/112
Thakur et al. (2019)	--	--	--	--	0/652
Nekouei et al. (2018)	--	0/896	1/1110	--	--
Jeffries et al. (2014)	--	0/228	--	--	0/23
Stevenson (2018)	--	0/300	--	--	--
Furey (2016)	--	0/80	--	--	--
Hrushowy (2018)	3/89	--	3/205	--	2/97
Totals	**3.4% (3/89)**	**0.1% (1/1879)**	**0.4% (8/1943)**	**11.4% (64/560)**	**0.9% (21/2222)**
Totals (FRSS only)	**--**	**0.1% (1/1505)**	**0.2% (2/1258)**	**12.1% (64/531)**	**1.3% (19/1431)**

[1]63/155 detections of PRV from gill biopsies but 0/57 detections in liver tissues collected at same location

Table 4. Distribution of PRV detection across Fraser River Sockeye Salmon stocks (Jeffries et al., 2014; Miller et al., 2014; Marty et al., 2015; Furey, 2016; Morton et al., 2017; Teffer et al., 2017; Nekouei et al., 2018; Stevenson, 2018).

Stock screened for PRV	PRV screening results	
	Juveniles	Adults
Bowron	0/9	--
Cultus	1/62	--
Weaver	0/8	--
Portage	0/35	--
Early Stuart, Late Stuart & Misc.[1]	0/4	1/191
Quesnel	0/22	0/297
Horsefly	0/148	--
Mitchell	0/119	--
Blue Lead	0/1	--
Wasko-Roaring	0/16	--
Nahatlatch River	0/16	--
Fennell	0/1	--
Thompson	0/75	--
Raft	0/18	--
Upper Barrier	0/3	--
Birkenhead	0/77	0/11
Scotch	0/72	0/8
Seymour	0/134	--
Adams	1/370	0/2
Shuswap	0/398	49/304
Eagle	0/6	--
Little	0/5	--
Nadina	0/60	1[2]/60
Dolly Varden	0/86	--
Chilliwack Lake	0/34	--
Stellako	0/137	0/10
Gates	0/65	0/19
Big Silver	0/4	--
Pitt	0/79	--
Harrison	--	0/103
Chilko	0/1018	15/250

[1] This includes juvenile fish sampled from Sandpoint Creek, Five Mile Creek, Middle River, and Dust-Sinta Creek (n=1 per stock).

[2] Positive detection of PRV nucleic acid in only one of two technical replicates which was noted as inconclusive by the authors (Marty et al., 2015).

CARDIOPATHY OF SALMON

CAUSATIVE FACTORS

Cardiopathy refers to diseases of the heart that affects contractive functions and decreased capacity to circulate blood. These diseases have many causes and, in association with the global production of farmed salmon, a variety of cardiopathies have been described. Some occur as a result of non-transmissible conditions such as during cardiac remodeling and expansion due to chronic hypoxia stress (Simonot and Farrell, 2007) or as a result of congenital mutation (Becker et al., 2011). However, cardiopathy can also occur due to infectious and transmissible microbes. Specific to salmon, at least eight infectious agents are known to cause cardiopathic disease, although high-load systemic infections of virtually any moderately virulent pathogen has the potential to inflict damage to heart tissues:

- *Renibacterium salmoninarum* (Bruno, 1986)
- *Piscirickettsia salmonis* (Olsen et al., 1997)
- *Kudoa thyrsites* (Moran et al., 1999)
- *Ichthyophonus hoferi* (Kocan et al., 2006)
- *Yersinia ruckeri* (Rucker, 1966)
- Salmonid alpha virus (SAV) (Wiik-Nielsen et al., 2016)
- Piscine myocarditis virus (PCMV) (Haugland et al., 2011)
- Piscine othoreovirus (PRV) (Wessel et al., 2017)

Of these, six are endemic to British Columbia: *I. hoferi*, *K. thyrsites*, *P. salmonis*, *R. salmoninarum*, *Y. ruckeri* and PRV. In the event that the causative agent of a heart disease is not clearly identifiable, a diagnosis of idiopathic cardiopathy is assigned.

PREVALENCE AND IMPACT IN BRITISH COLUMBIA

General cardiopathy

Mild cardiopathy is prevalent in farmed salmon of British Columbia; however, severe cardiopathy impairing heart function is rare. Between 2006 and 2018, the Fish Health Auditing and Surveillance Program (FHASP) conducted by DFO Aquaculture Management Division has evaluated all major organs of nearly 6,000 Atlantic and 800 Pacific (majority Chinook; some Coho) salmon net-pen farming mortalities by histopathology including heart tissues. Mild to moderate cardiopathy occurred in 61% of Atlantic and 41% of Pacific salmon mortalities sampled during this period. However, this cardiopathy, mainly epi- and endocarditis, does not compromise heart or respiratory function (Lund et al., 2017; Zhang et al., 2019) and is not expected to adversely affect salmon health. Moderate to severe cardiopathy with a putative ability to negatively affect heart functioning was diagnosed in 7% and 3% of Atlantic and Pacific salmon mortalities, respectively. The severity was sufficient to be suggested as a putative cause or contributing factor to death in less than 3% of both Atlantic and Pacific salmon species (Figure 3). These percentages are representative of sites specific to the Discovery Islands region and are consistent with other independent studies which have corroborated the relatively widespread occurrence of generally mild cardiopathy in British Columbia salmon with little evidence for its contribution to morbidity or mortality over the past decade (Marty et al., 2015; Di Cicco et al., 2017). This is also consistent with previous diagnoses of prevalence for severe cardiopathy in farmed salmon from the early 1990's (Brackett et al., 1990; Brackett et al., 1991;

Brackett and Newbound, 1992; Brackett et al., 1992); suggesting that cardiopathy has likely caused or contributed to less than 0.4% cumulative mortality in farmed salmon in BC over the past 25 years. The proportion of this cardiopathy that is attributable to infectious diseases and specifically PRV is unknown, although multiple transmissible and non-transmissible factors are indicated to be involved in addition to PRV (Figure 3).

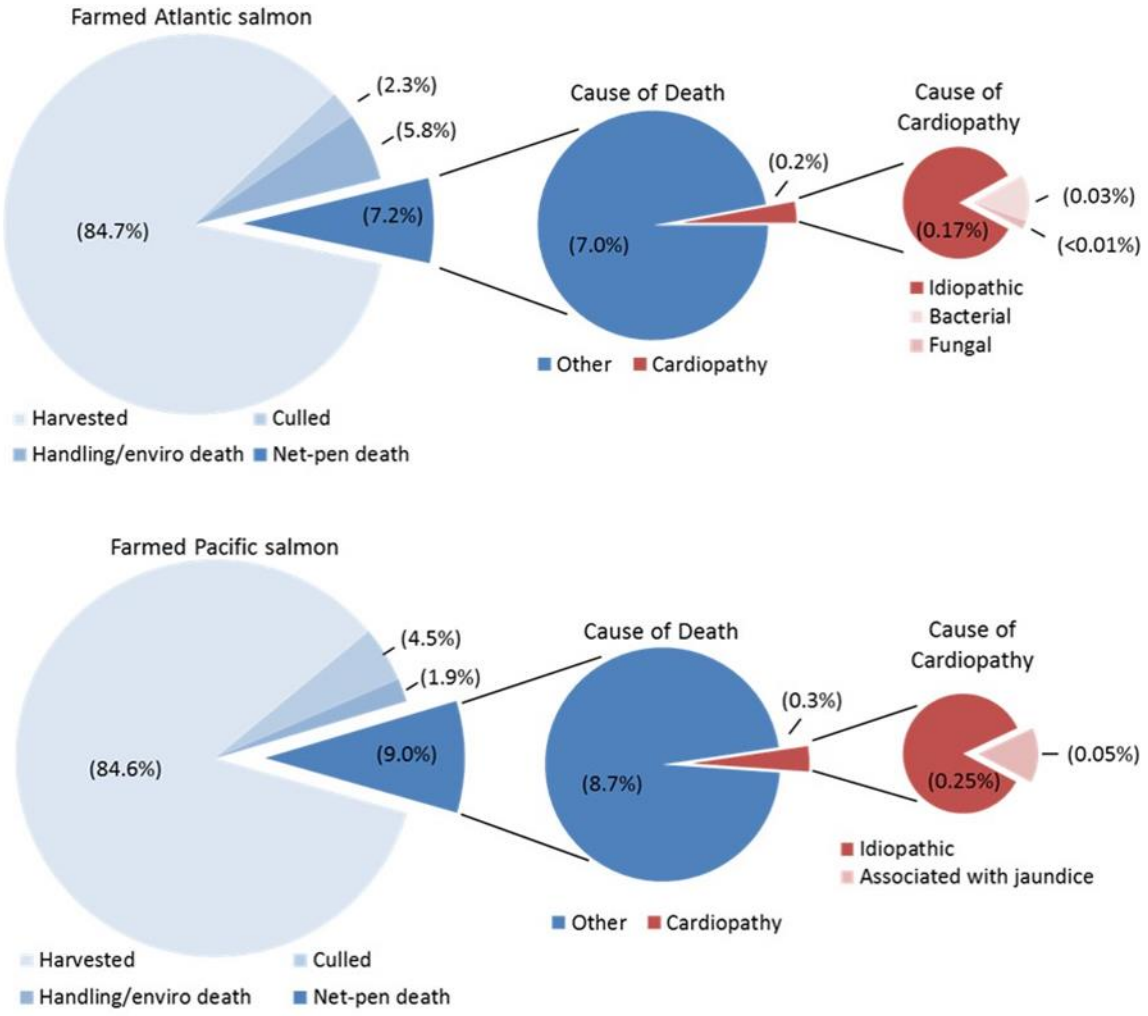

Figure 3. Cardiopathy as a marker of death in farmed salmon of British Columbia.Cumulative percent mortality of stocked fish per annum presented in left pie charts are extrapolated from the mean monthly mortalities reported across salmon farming industries between 2012-2018. Putative cause of death diagnoses from net-pen mortalities not associated with culling, handling, or environmental causes (e.g., low dissolved oxygen) is presented in the center pie charts based on FHASP data collected between 2006 and 2018. The putative causes of cardiopathy in these instances are presented in the right pie charts. All percentages are relative to total number of net-pen Atlantic or Pacific salmon stocked per annum during this time period.

The prevalence of cardiopathy in wild Pacific salmon is relatively unknown; however, a survey of 204 wild salmon of British Columbia in 2013 that included Pink, Chum, Chinook, Coho, and Sockeye salmon diagnosed mild cardiopathy in 12 fish (5.9%) but failed to identify significant (severe) cardiopathic disease (Marty et al., 2015). Similarly, severe cardiopathy that occurs in farmed Atlantic Salmon in Norway (e.g., HSMI, cardiomyopathy syndrome and pancreatic disease) has not been detected in wild Atlantic Salmon (Garseth et al., 2013), suggesting environmental components specific to intensive culture likely enhance the prevalence and severity of cardiopathy in salmon.

HSMI

The term HSMI, although foundationally descriptive, has evolved considerably in meaning over the past decade. Before a causative agent was known, the original diagnosis of HSMI was founded on a set of distinct clinical disease characteristics in Norwegian Atlantic Salmon farms during episodes of morbidity and/or mortality for which histopathology was used to confirm and differentiate this condition from other similar diseases; e.g., pancreatic disease or cardiomyopathy syndrome. By this original case definition, HSMI has never been reported in British Columbia:

"Affected fish are anorexic and display abnormal swimming behaviour. Autopsy findings typically include a pale heart, yellow liver, ascites, swollen spleen and petechiae in the perivisceral fat. Diagnosis of HSMI is presently based on histological examination. HSMI is characterised by extensive panmyocarditis and myositis, particularly involving red skeletal muscle. Morbidity may be very high, while mortalities are variable and may reach 20% in affected cages." (Kongtorp et al., 2004a).

Following the discovery of PRV in association with HSMI in Norway in 2010 (Palacios et al., 2010) and the subsequent demonstrated ability for PRV to cause severe heart inflammation (Wessel et al., 2017), the diagnosis for HSMI, although still exclusively based on histopathology, is generally accepted to be initiated by PRV. Many subclinical infections of HSMI have now been diagnosed in Norway, some even without the evidence of skeletal muscle inflammation, and although environmental and/or host contributing factors may explain the often exacerbated severity of HSMI in a field relative to laboratory setting, PRV appears to be the sole infectious agent associated with the unique set of histopathological criteria that defines HSMI in Norway (Palacios et al., 2010; Wiik-Nielsen et al., 2016; Wessel et al., 2017). To our knowledge, HSMI has never been used to classify a disease state in Norway where PRV has been confirmed to be absent.

Two recent studies from Pacific Canada have also used the term HSMI to classify subclinical heart disease of farmed Atlantic Salmon based on histopathology in accordance with their own definitions similar to those previously reported in Norway – namely, moderate to severe heart inflammation sometimes accompanied by skeletal muscle inflammation (Di Cicco et al., 2017; Di Cicco et al., 2018). Although the presumed commonality for the heart and skeletal muscle lesions in these Canadian studies relative to HSMI diagnosed in Norway is the causation by PRV, there is far less evidence in Canada to support that PRV is indeed the key component for initiating this relatively rare disease state; particularly given that these modified definitions have occasionally been observed in the absence of PRV (Marty and Bidulka, 2013; Di Cicco et al., 2018). Consequently, if HSMI diagnosis is based solely on histopathological heart lesions which can occur in the absence of PRV, then PRV cannot be assumed to be *the* causative agent of the disease, but rather one of multiple stand-alone or synergistic putative factors. Thus, if using the definition proposed by Di Cicco et al. (2017), HSMI in British Columbia can likely be used interchangeably with the terms 'moderate to severe cardiopathy' or 'idopathic cardiopathy' as described above. Throughout this review, we use the term HSMI only in cases which fit those

described by Wiik-Nielsen et al. (2016) where PRV appears to be the most likely primary causative factor.

ANEMIA OF SALMON

CAUSATIVE FACTORS

Anemia is a condition marked by a deficiency in red blood cells and/or hemoglobin that results in a reduced ability for blood to transport oxygen. Many factors can cause or contribute to anemia in fish including nutrient deficiencies, toxic agents and infectious pathogens (Witeska, 2015). Chronic disease, both infectious and non-infectious, can also result in anemia (Zarychanski and Houston, 2008). Specific to salmon, at least eight pathogenic organisms (including viruses, bacteria, and external parasites) are known to directly or indirectly contribute to anemia although this is almost certainly not a comprehensive list:

- Infectious salmon anemia virus (ISAV) (McBeath et al., 2015)
- Infectious hematopoietic necrosis virus (IHNV) (Amend and Smith, 1975)
- Piscine orthoreovirus (PRV) (Takano et al., 2016)
- *Aeromonas* sp. (Řehulka, 2002)
- *Flavobacterium columnare* (Řehulka and Minařík, 2007)
- *Vibrio anguillarum* (Harbell et al., 1979)
- *Ichthyophthirius multifiliis* (Abdel-Hafez et al., 2014)
- *Tetracapsuloides bryosalmonae* (Hoffmann and Lommel, 1984)

Of these, there are five relevant to salmon of British Columbia that reside in saltwater: IHNV, PRV, *Aeromonas* sp. *F. columnare* and *V. anguillarum.* However, although PRV is listed here, it must be noted that the only PRV isolate to induce anemia in salmon through experimental infection to date is that of PRV-2 from Japan (Takano et al., 2016).

It also must be acknowledged that the measures used to assess anemia (erythrocyte count, hemoglobin concentration and hematocrit) are at best indirect measures because they do not necessarily imply a lack of sufficient oxygen delivery. This is highly important when considering that, unlike for mammals, these measures can fluctuate substantially in healthy populations of fish. In salmon, the packed erythrocyte volume (hematocrit) can vary as much as 40% between individuals within a single cohort population (i.e., hematocrit ranging from 40-65% of total blood volume) without significant correlation to respiratory functioning (Zhang et al., 2019). For salmon, it has been suggested that functional anemia occurs when hematocrit drops below approximately 20-25% total blood volume (Simonot and Farrell, 2007; Clauss et al., 2008), although this estimate will likely vary depending on a variety of environmental and host-specific factors. Thus, clinical symptoms such as lethargy or other signs of morbidity and/or mortality are important characteristics for identifying fish which are truly anemic (i.e., have a loss in respiratory function) relative to those which may have a reduced hematocrit or hemoglobin relative to what is ‘typical’ for the species but remain physiologically uncompromised.

IMPACT AND PREVALENCE IN BRITISH COLUMBIA

Unexplained anemia (i.e., with the potential to be caused by an unknown pathogen such as PRV) is rarely documented in salmon of British Columbia. There were no veterinary diagnoses indicative of anemia based on excessive internal organ pallor or jaundice in farmed Atlantic

Salmon made as part of the FHASP within DFO between 2011 and 2017 that included 663 site visits and 4,344 sampled specimen carcasses. There were also no diagnoses of anemia in farmed Coho Salmon during this period (17 site audits involving 75 collected specimens), although it should be noted that subclinical anemia would likely be missed by visual inspection of moribund or recently deceased carcasses. In farmed Chinook Salmon, a condition referred to as Jaundice Syndrome was diagnosed by the FHASP veterinarian for 7 out of 479 carcasses (1.5%) in 5 of 95 site audits during this seven year period. Jaundice Syndrome is characterized by yellow discolouration of the skin resulting from excessive bilirubin in the blood due to red blood cell breakdown. Substantial red blood cell breakdown is needed to cause jaundice and can therefore be used as a proxy for identifying current or recent anemia. The prevalence of jaundice/anemia reported during the FHASP is similar to that reported previously in farmed Chinook Salmon of BC (< 1.5% jaundice-associated mortality per production cycle) that appears to have a seasonal (cold water temperature) component (Garver et al., 2016b). This seasonality is at least partially substantiated by a focused study involving 210 FHASP samples collected from Chinook Salmon in 2011-2013 by Di Cicco et al. (2018), where the authors noted what they classified as Jaundice Syndrome based on their own definitions using histopathology (a more liberal classification than previously used by government or industry veterinarians) in 14 fish (6.7%) that was restricted to two specific sampling events. The occurrence of unexplained anemia in wild Chinook Salmon or any other Pacific salmon species in British Columbia is unknown.

RELATIONSHIP OF PRV-1 AND DISEASE IN BRITISH COLUMBIA

Infectious disease investigations often draw on a multi-varied approach of field studies, epidemiological analyses, and laboratory experimentation to understand the biology and disease causing potential of an infectious agent. Ultimately the proof of disease causation rests on the concordance of scientific evidence, with one of the key components in establishing a causative link between agent and disease being the reproduction of disease when the purified agent is introduced into a naïve host. For disease investigations of PRV in Norway, Denmark and Japan, laboratory experimentation has demonstrated that specific disease conditions could be reproduced when either Atlantic or Pacific salmon species received one of the three genetic types of PRV (Takano et al., 2016; Wessel et al., 2017; Vendramin et al., 2019). These studies not only demonstrated specific PRV types to have disease causing potential but also importantly established that laboratory environments are a suitable surrogate for investigating PRV associated diseases. Consequently, to investigate the relationship of PRV-1 and disease in salmon of British Columbia, laboratory and field based studies were conducted similar to those carried out in countries where an etiological link of PRV with disease has been established. This section provides an overview of the studies investigating PRV-1 and its link to disease in British Columbia.

ATLANTIC SALMON

Experimental challenge trials using PRV-1 in Pacific Canada Atlantic Salmon have resulted in extreme PRV blood infections, but either failed to generate notable pathology (Garver et al., 2016a) or induced only minor to moderate heart inflammation – specifically, epi- and endocarditis (Polinski et al., 2019; Zhang et al., 2019). Further, the increased prevalence of minor heart inflammation induced by PRV (when it occurred) in these experiments was demonstrated to be inconsequential to normal heart and respiratory functioning (Zhang et al., 2019). However, an association of PRV to moderate to severe heart inflammation has been observed in a field environment based on a longitudinal study of one farm site with a high presence of moderate to severe cardiopathy which lasted for several months (Di Cicco et al.,

2017). The visualization of PRV in diseased hearts in this study in conjunction with activation of host cellular antiviral response pathways strongly suggested that PRV was contributing to the severity of cardiopathy observed. Thus, taken together, these studies suggest that PRV has the potential to exacerbate instances of severe cardiopathy in net-pen farmed Atlantic Salmon in British Columbia and may be a contributing etiological factor in establishing at least some instances of this relatively rare disease.

PACIFIC SALMON

Two PRV experimental challenge studies have been published exploring the disease causing potential of PRV-1 to Sockeye Salmon; both failed to identify an association with PRV and disease (Garver et al., 2016a; Polinski et al., 2016). A third laboratory study has also recently been completed with Sockeye Salmon that failed to generate anemia or notable pathology in heart, kidney, or liver tissues of infected fish; and, in the same manner as assessing physiological impacts of PRV on Atlantic Salmon (Zhang et al., 2019), demonstrated these infections to be inconsequential to Sockeye physiological respiratory functioning (Polinski et al., manuscript in preparation). For adult Chilko or Shuswap Sockeye Salmon returning to the Fraser River, it was identified that the presence of PRV on or in the gills of fish migrating through Discovery or Juan De Fuca sea channels had no significant effect on the likelihood that they would reach their spawning grounds (Miller et al., 2014). Coho Salmon challenged with PRV harvested from infected farmed Atlantic Salmon in British Columbia also failed to acquire notable pathology or anemia despite becoming infected with PRV (M. Purcell, personal communication). Lastly, experiments attempting to passage Jaundice Syndrome in Chinook Salmon in association with PRV failed to passage the disease despite successfully passaging PRV (Garver et al., 2016b). Nevertheless, similar to field observation of PRV contributing to severe cardiopathy in Atlantic Salmon, PRV has been visualized in diseased tissues of farmed Chinook Salmon experiencing Jaundice Syndrome (Di Cicco et al., 2018) which would suggest that PRV is capable of contributing to jaundice/anemia in captive fish and may be part of a more complex aetiology for establishing this disease state in Chinook Salmon. However, this assumption cannot be extrapolated to wild Chinook Salmon or other Pacific Salmon because captive and wild environments can cause different pathophysiology and susceptibilities.

DISEASE PREVENTION

In British Columbia, there have been no data to suggest PRV adversely affects aquaculture production of salmon. In Norway, however, HSMI is considered one of the most significant infectious diseases affecting Atlantic Salmon aquaculture (Hjeltnes B et al., 2017; Marine Harvest, 2017) and a number of strategies are being explored to mitigate this disease. Two experimental vaccination studies have been conducted; one using a formalin killed PRV vaccine (Wessel et al., 2018b) and one using a DNA vaccine expressing the non-structural proteins of PRV (Haatveit et al., 2018). Both demonstrated moderate protection against HSMI, although neither were protective against PRV infection. In addition to vaccination, work towards establishing a “HSMI-resistent” Atlantic Salmon strain has been undertaken (AquaGen, 2017), although similar to the vaccines mentioned above, these fish do not appear to be refractory to PRV but rather only HSMI (Emilsen et al., 2017). Furthermore, use of specific feed formulations, similar to the other treatments, has shown promise at reducing the effects of HSMI primarily through dietary immunomodulation but is not successful at eliminating PRV infections (Grammes et al., 2012; Martinez-Rubio et al., 2012).

SUMMARY

There has been a great deal of knowledge gained regarding the virology and ecology of PRV following its discovery nine years ago; and much of that knowledge has direct or indirect relevance for assisting in the assessment of risk to Fraser River Sockeye Salmon posed by the occurrence of PRV on Atlantic Salmon farms. The most critical research findings that can aid this risk assessment are summarized here:

- PRV is ubiquitous and highly prevalent in net-pen farmed salmon of British Columbia; it is also widely distributed in wild Pacific salmon but with less detection prevalence and more species/stock-specific variation.
- Farmed and wild salmon of British Columbia appear most likely to become infected with PRV as adults in saltwater although freshwater infections of fry/parr can and have occurred.
- Experimental infections with PRV in British Columbia generate high-load blood infections in both juvenile Atlantic and Pacific salmon but disease specific to PRV infection was not observed following experimental infection in any species challenged (Sockeye, Chinook, Coho, Pink and Atlantic).
- PRV is of lower virulence to Atlantic Salmon in British Columbia relative to PRV infections of Atlantic Salmon in Norway; both host and virus specific factors are likely involved in this altered virulence.
- Infections of PRV in Atlantic and Sockeye salmon of British Columbia have been demonstrated as inconsequential to respiratory function in the absence of moderate to severe pathology.
- Systemic PRV load is not indicative of whether a salmon will or will not develop a notable disease.
- PRV may contribute to or be a possible etiological component of severe heart inflammation in farmed Atlantic Salmon or jaundice/anemia syndrome in farmed Chinook Salmon in British Columbia; both conditions appear rare and likely have complex etiologies and have not been reported in wild Pacific salmon.
- There is currently no evidence to suggest that PRV causes disease in Sockeye Salmon, which appear less susceptible to infection relative to Atlantic Salmon in British Columbia.

Despite substantial gains in our understanding about PRV, there are also knowledge gaps concerning PRV virology and ecology that leave critical uncertainties. The environmental source(s) and transmission potential of PRV in ocean environments are unknown. Specifically, there are no current data on environmental shedding (quantity or duration) or the minimum exposure load (quantity or duration) to establish an infection in any salmon species. There is also a current lack of understanding for why PRV can show higher virulence in some instances compared to others. Lastly, in the instances where PRV has been linked to disease in farmed salmon, it is as yet unclear as to whether all host, environment, and viral specific factors of these diseases can manifest in the natural environment in British Columbia.

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