THE PHYSIOLOGICAL ASSOCIATIONS BETWEEN INFECTIOUS AGENTS AND MIGRATING JUVENILE CHINOOK SALMON (*ONCORHYNCHUS TSHAWYTSCHA*)

by

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Abstract

The role of infectious diseases in the declining productivity of wild Chinook salmon (*Oncorhynchus tshawytscha*) in BC is poorly understood. In wild populations, it is difficult to study the effects of infectious diseases because they interact with environmentally induced stress and diseased fish are not often observed as many are likely predated upon or die out of view. The early marine life of Pacific salmon (*Oncorhynchus* spp.) is believed to be one of the key components of the declining populations. More focus on understanding the potential role of infectious agents during this life period is needed. My study assessed how infectious agents are associated with the physiology of migrating juvenile Chinook salmon upon their entry to marine waters by linking ancillary data, physiological responses and histopathological lesions with infectious agent detection. It is one of the first to study infectious agents carried by wild salmon through combining molecular, protein, and cellular levels of fish physiology information. Among 46 assayed infectious agent taxa, 26 were detected, including viruses, bacteria, and parasites. Fish from Columbia River system were found to have significantly higher infection burden than those derived from five other regional groups. I discovered and reported the associations between fish physiological conditions and five infectious agents, including *Ichthyophonus hoferi,* '*Candidatus* Branchiomonas cysticola', *Parvicapsula minibicornis, Ceratonova shasta,* and Piscine orthoreovirus (PRV). PRV, particularly, was recently reported in many salmon farms in BC as the suspected causal agent of two related diseases in both Atlantic and Chinook salmon, and has potential to be exchanged between farmed and wild populations. I further provided one of the first lines of evidence of potential impacts of PRV both on host genes and histopathology in the wild juvenile Chinook salmon. Understanding the relationships between infectious agents and salmon can help inform conservation and management practices.

Lay Summary

The early marine life of juvenile Pacific salmon (*Oncorhynchus* spp) is a critical period where salmon are thought to have very low survival, yet it is the least studied life period. Infectious agents are suspected to influence the health and survival of wild populations. I linked the infectious agents detected on juvenile Chinook salmon (*Oncorhynchus tshawytscha*) captured during their early marine life with three levels of fish physiology responses: gene expression, blood chemistry, and histopathology. Among 46 infectious agent taxa I screened for, 26 were detected. Fish from the Columbia River system were found to have significant higher infection burden than fish from any other regions. I identified five agents that had associations with fish physiological status, including one virus PRV which has been recently reported in several farms in BC and has potential to be exchanged with wild fish, although other routes of transmission are also possible.

Preface

This research was conducted as one component of the Strategic BC Salmon Health Initiative (SSHI) which is a multidisciplinary research program aiming at assessing the potential role of infectious disease in wild salmon declines through merging fields of studies including genomics, epidemiology, histopathology, virology, parasitology, fish health, veterinary diagnostics, and salmon ecology. I held primary responsibility for the study designs, part of the physiological analyses and full data analyses, as well as the preparation of manuscripts for submission. Throughout the process, I received supervision and guidance from my supervisor Dr. Scott Hinch and supervisory committee members Dr. Kristi Miller and Dr. Evgeny Pakhomov. I also received considerable support from my colleague Dr. Arthur Bass. All samples, including fish tissue and blood, were collected under a scientific fishing permit (MECTS # 2014-502-00249) issued to Pacific Region Department of Fisheries and Oceans (DFO) staff by the Government of Canada, DFO, Regional Director Fisheries Management. This work does not require an animal care protocol pursuant to an exemption contained in the Canadian Council on Animal Care (CCAC) guidelines applying to fish lethally sampled under government mandate for assessment purposes (4.1.2.2). Dr. Kristi Miller and her staff at the Molecular Genetics Lab (MGL) provided logistic assistance with sample selection and collection from fish captured and stored for DFO marine sampling program. The technicians Shaorong Li and Tobi Ming from MGL provided the full support of genomic laboratory work. David Patterson and his staff Jayme Hills and Miki Shimomura from the Fraser River Environmental Watch Program assisted with logistical support with the blood analysis work at the Fisheries and Oceans Canada West Vancouver Laboratory. Dr. Emiliano Di Cicco from Pacific Salmon Foundation and Dr. Hugh Ferguson provided professional veterinarian histopathology support. Individuals who were essential contributors to the conceptualization, development, or preparation of the manuscripts below are listed as coauthors on my data chapter manuscript.

Chapter 2: The Physiological Associations between Infectious Agents and Migrating Juvenile Chinook Salmon (*Oncorhynchus Tshawytscha*)

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Comments: This study was conducted and written by YW with assistance from ALB, SL, TJM, ED, and KMM, under the supervision and guidance of SGH who helped conceptualize the study and preparation of the manuscript.

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Chapter 1: Introduction

1.1 Infectious agents in marine ecosystem

Infectious disease is a natural component of both the terrestrial and the aquatic ecosystem. It constantly affects almost every life form. The emerging infectious diseases and their outbreaks such as Ebola and Middle East Respiratory Syndrome (MERS) have raised international attention in recent years and have suggested its potential as a current and major global health threat to humanity (Yang et al., 2017). International exchanges of human and goods have increased rapidly which leads to hidden dangers and potential spread of infectious diseases worldwide (Yang et al., 2017). This applies not only just in human society but also in almost every natural ecosystem. In conservation biology, infectious disease is now a bigger concern partly due to increasing human activities such as habitat alteration and translocation of infectious agents. For example, in terrestrial systems, host density is considered a determinant of infectious agent abundance, and crowding of animals in a limited place due to loss of habitat can place populations at risk for epidemics (Arneberg et al., 1998). This problem can be intensified when crowded individuals gradually exploit natural resources that could improve disease resistance (Lafferty & Gerber, 2002). In addition, habitat fragmentation can reduce gene exchange among populations and lower host immunity, which can lead to catastrophic outbreaks and ruin endangered species management when any exotic infectious agent is introduced (Lyles & Dobson, 1993).

Infectious disease is also a big concern in the marine ecosystem. Global fisheries have been under threat of human activities directly and indirectly for decades. The worldwide human population explosion and the growing demand for seafood in shifting

human diets have been the main driving forces behind habitat degradation and overexploitation of world's fish species (the Food and Agriculture Organization of the United Nations (FAO) 2016). Although the depressed status of wild fish stocks has provided an impetus for rapid growth in aquaculture (Naylor et al., 2000), both wild and farmed aquatic species face threats from infectious diseases (Krkošek, 2017). Farmed shellfish and finfish industries, including oysters, shrimp, abalone, and fishes, Atlantic salmon (*Salmo salar*) in particular, suffer from billions of losses due to infectious diseases (Lafferty et al., 2015). While the cost of infectious diseases in aquaculture is more observable, it is not easy to estimate the impacts of infectious diseases in wild populations, and such a topic has been controversial since there are very few available data to illustrate the role of infectious diseases in wild populations and the interrelationships between wild and farmed populations (Lafferty et al., 2015). Wild populations can provide the sources of infectious agents through water, food, or infected broodstocks, and regardless of the sources of infectious agents, the nature of high density and confinement in the farm may facilitate outbreaks of diseases (Bakke & Harris, 1998; Lafferty & Gerber, 2002; Lafferty et al., 2015). Wild populations can also receive diseases from farms through an intermediate carrier (such as birds), or escapees from the farm (McAllister & Owens, 1992; Naylor et al., 2000).

1.2 Pacific salmon (*Oncorhynchus* spp.) in the Pacific Northwest

Pacific salmon are ecologically and socially significant in the Pacific Northwest, but are facing multiple problems, including infectious diseases (Miller et al., 2014). They are heavily incorporated in human life: they are respected and utilized by indigenous people;

they are recreationally and commercially harvested; and they are an important connection between aquatic and territorial food webs (Haegen et al., 2004; Quinn, 2005; Schaepe, 2007). As anadromous fish, Pacific salmon start their life cycle by emerging in freshwater streams and lakes, migrating to the marine environment as they grow into smolts, spending a large portion of their life in ocean feeding grounds before they finally migrate back to their freshwater spawning areas, where they attempt to spawn and then die (Groot & Margolis, 1991). The complex life history involving both freshwater and saltwater system facilitates the high interannual variability in population abundance. However, despite typical fluctuations, some species and populations have experienced severe declines in recent decades (Beamish et al., 1999; Irvine & Fukuwaka, 2011). The declining trends pushed research efforts to focus on determining the factors impacting the survival and health conditions of wild Pacific salmon and the factors contributing to recent decreases in populations. Although the reasons behind the decline are still unclear, a number of potential factors have been discussed, which include predation and competition among species and stocks (Beamish et al., 2004; Irvine & Akenhead, 2013; Thomas et al., 2017), contact with aquaculture fish (Noakes et al., 2000), infectious diseases (Kent, 2011), human harvesting, habitat degradation and alteration (Beamish et al., 1995), and climate change (Irvine & Fukuwaka, 2011).

1.3 Declines in Chinook salmon (Oncorhynchus tshawytscha) southern populations

Chinook salmon is the largest species by size among all the Pacific salmon, having high economic and societal value in both commercial and recreational fisheries. Indigenous

people prize them spiritually and culturally. They are also important to non-human predators such as killer whales (*Orcinus orca*). Although killer whales predate on six salmonids species on the Pacific Northwest, around 65% of their prey was comprised of Chinook salmon (Ford et al., 1998). Killer whales are themselves very important ecologically and socially in the Pacific Northwest. In fact, southern resident killer whales are an endangered population (COSEWIC, 2008). Reduced prey availability of Chinook salmon is a potential factor in their decline (Hanson et al., 2010). It is for all these reasons that the status of Chinook salmon garners much attention among scientists and the public. The decline in Chinook salmon's productivity has been severe particularly in its southern distributions (Noakes et al., 2000; Riddell et al., 2013). In Canada, the total catch of Chinook salmon has been declining since the late 1990s (MacKinlay et al., 2004). Unlike the other four Pacific salmon species in Canada, Chinook salmon possesses two divergent life histories referred as "stream-type" and "ocean-type", reflecting variation in age at seaward migration, variation in time spent in freshwater, estuarine, and saltwater, and variation in age upon maturation (Groot & Margolis, 1991). This variability in life histories adds more complexity when studying this species. Over half of the populations of stream-type Chinook salmon are estimated to be extinct from California to British Columbia (BC) whereas a much smaller portion of ocean-type Chinook salmon populations is believed extinct (Gustafson et al., 2007). Understanding reasons behind the decline in populations across geographic and life history diversities are necessary to better inform Chinook salmon conservation.

1.4 The role of infectious agents in Pacific salmon

Among factors that are suspected to influence the survival of wild Pacific salmon, infectious diseases may be one of the least studied. The effect of infectious diseases is hard to observe in wild populations because the mortality of wild fish is rarely seen. Fish weakened by diseases can simply fall prey to predators such as birds, mammal and other fish, therefore, it is difficult to establish a relationship between an infectious disease and an infectious agent in wild (Miller et al., 2014). Much of our knowledge of infectious agents come from aquaculture, where Atlantic salmon is the main focus (Kent, 2011). Traditional diagnostic approaches of studying infectious agents require observation of abnormal signs, culturing of infectious agents and histopathology, often verified by molecular tests to the putative causative agent (Miller et al., 2014). Sometimes immunohistochemistry or in-situ hybridization are applied to locate the suspected infectious agents within the region of damage, or for novel diseases, a strict challenge study is used to establish Koch's postulates (1891) of causality (Miller et al., 2014).

However, infectious diseases can have significant impacts on Pacific salmon by interacting with other stressors such as extreme temperatures and pollutants which can weaken the immune system and impact fish survival (Barton, 2002). Wild fish often carry more than one infectious agent (K. M. Miller et al., 2014), which can potentially impact their physiological status in different ways. The migratory nature of Pacific salmon can be greatly influenced by infectious diseases, as there are several brief but critical times in the life history of salmon where large numbers of fish are all rapidly moving to new habitats, requiring acclimation to shifting environmental pressures. The high densities and strenuous nature of the migrations can be easily impacted by infectious diseases which can impair

physiological condition and swimming ability (Bakke & Harris, 1998). The anadromous nature of the juvenile smolt migration to the ocean or the adult return migration into freshwater is unique in that many infectious agents may lose pathogenicity when changing environmental salinity. However, some infectious agents may overcome the osmotic pressure when carried into a new environment, and a great number of them may become even more virulent in another environment (Miller et al., 2014). Infectious agents can have add-on effects during particular life history events, such as down-streaming migration and spawning. Chinook salmon smolt mortality during down-stream migration is estimated to be 11-17% annually in the Lower Columbia River and Estuary, and some smolt mortality by predation is associated with the bacterial infection of *Renibacterium salmoninarum* (Schreck et al., 2006). When adult salmon migrate back to the freshwater and converge into streams and lake to spawn, high density and immunosuppression induced by maturation hormones may facilitate disease transmission and progress (Miller et al., 2014; Pickering & Christie, 1980)

1.5 The impact of infectious agents in salmon early marine phase

The early marine life of Pacific salmon is believed to be the key to their general declining productivity (Beamish et al., 2010), yet it is one of the most difficult life history stages to study. Great efforts have been made on studying the down-stream and up-stream migration periods as the fish are more accessible in freshwater systems, but the large portion of salmon's life in the ocean is generally understudied (Drenner et al., 2012). Tracking studies confirm less direct measures such as catch per unit effort that substantial mortality is observed in the ocean, but also during the down-stream migration (Welch et al., 2011). The

local ocean conditions after smolts leave freshwater are suspected to be responsible for large levels of mortality (Sharma et al., 2013), however, it is not clear whether infectious agents are playing a role. There is evidence that epizootics can occur in marine smolts which can lead to drastic declines in some populations (Jones et al., 1997). In coastal BC, juvenile Chinook salmon carry an average of 3.7 infectious agents with a maximum of 10 (Tucker et al., 2018). They are hosts for a broad range of infectious agents, including 5 bacteria, 3 viruses, and 13 parasites - of these agents, 11 are suspected to have potential to impact the host at the population level (Tucker et al., 2018).

The southern BC coast contains numerous open net-pen farms for Atlantic salmon. These fish can carry infectious agents that could be passed onto wild Pacific salmon. For example, bacteria *R. salmoninarum* is the causative agent of one of the most commonly seen diseases in salmon farms in BC, bacterial kidney disease (BKD) (Laurin et al., 2019). This agent is highly prevalent among juvenile Chinook salmon in North Puget Sound (Rhodes et al., 2006) and various salmon species including sockeye and chum (*Oncorhynchus keta*) salmon are highly susceptible to this agent (Jones et al., 2007). This agent is known to have horizontal transmission during the juvenile neritic phase and can potentially be exchanged between hatchery and wild fish (Rhodes et al., 2011). However, the understanding of epizootiology of BKD remains limited due to difficulties in studying the agent *R. salmoninarum* in laboratory settings. Meanwhile, infectious agents could also be passed from wild to farmed fish. Infectious hematopoietic necrosis virus (IHNV) is a fish rhabdovirus that naturally occurred in freshwater wild populations in BC. It can cause infectious hematopoietic necrosis and is responsible for notable losses in Chinook salmon, sockeye salmon (*Oncorhynchus nerka*) and steelhead trout (*Oncorhynchus mykiss*) in the freshwater

captive breeding facilities in this region. It is also associated with mortality in down-stream migrations of smolts (Hershberger et al., 2013; Jeffries et al., 2014). The IHNV outbreaks have been associated with massive mortality in farms in BC and have caused $200 million economic loss during its first two outbreaks in BC (Garver et al., 2013). DNA vaccine was developed and applied in aquaculture to prevent massive loss (Anderson et al., 1996).

In order to better understand the role of infectious agents in wild salmon populations, coordinated surveillance activities to assess the prevalence and intensity of infections are generally followed with controlled laboratory studies to test hypotheses gained from the field (Hershberger et al., 2013; Miller et al., 2014), which can take considerable effort and time to develop. Another possible way to study the potential for impacts of infectious agents in wild salmon is to make use of the novel and emerging molecular technologies and combine these with different aspects of fish physiology that are traditionally used in empirical studies, such as blood chemistry and histopathology. Genetic population assessment uses a small number of genetic markers to identify population compositions of mixed population samples of Pacific salmon, which is applied routinely in salmon management (Beacham et al., 2008; Tucker et al., 2011, 2012). When this technology is applied at the individual level, it allows researchers to study the inter- and intra-population variances of infectious agents and host physiology and to determine the importance of genetic variation in associations between the infectious agent and fish physiology and mortality (Miller et al., 2014). In addition, infectious agents monitoring systems that incorporate the high-throughput quantitative polymerase chain reaction (HT-qPCR) technology enables rapid and highly sensitive screening of infectious agents across large sample sizes to determine the presence, prevalence, and intensity of infections (Miller et al., 2016). The same technology also

empowered host gene expression profiling to elucidate host response to multiple stressors through targeted gene 'biomarker' panels (Miller et al., 2014; Miller et al. 2017). Host gene expression can be simultaneously examined with infectious agents, by using multiple assays targeting aspects of host physiological conditions such as immune status, stress level, and osmoregulation in addition to assays to detect infectious agents (Jeffries et al., 2014; Teffer et al., 2017).

1.6 Thesis overview and research objectives

In an effort to better understand the potential impact of infectious agents on wild migratory juvenile Chinook salmon and ultimately their relationships to declining populations, my thesis investigated the associations of infectious agents with multiple aspects of physiological variance in juvenile Chinook salmon. My current work had three objectives. First, I described the presence and quantity of infectious agents detected on juvenile Chinook salmon among multiple natal region groups and sampling periods. Second, I paired infectious agent detection data with multi-layered physiological data, including blood chemistry, host gene expression profiles, and histopathology. Third, I examined if using host gene expression from multiple tissues can provide reliable supplementary information to histopathology to study infectious diseases in wild juvenile Chinook salmon. My hypotheses were: (1) Infectious agent profiles would vary among different natal region groups and sampling periods. (2) Some of the infectious agents would be associated with host physiological parameters including blood chemistry, gene expression, and histopathology. (3) Host gene expression results would show some agreement with histopathology results.

In Chapter 2, I examined prevalence and loads of infectious agents in juvenile Chinook salmon from six natal groups collected by marine sampling cruises in the southern coast of BC in the summer, fall and winter from fish sampled in 2012 to 2014. The objective of my sampling design was to get adequate sample sizes of fish with matching tissues for molecular, blood and histopathological analysis representing the first three seasons at sea with which to relate infectious agents with physiological changes. As such, given sample availability to meet this requirement, my sample design was not adequate to contrast agent profiles between years. Moreover, as migratory salmon from some natal regions are only represented in my samples for one or two seasons, it is impossible to balance the study design to ensure all natal regions are represented in all seasons. Hence, due to the biological reality of salmon migration, my design was best set up to examine variation between seasons for natal population groups migrating on the east and west coasts of Vancouver Island. However, in Chapter 2, because of the extreme variation in salmon productivity between populations in different regional groups of the BC coast, I chose to partition my findings by natal region group, while recognizing the inherent biological variance in the seasons in which fish from different natal region groups can be evaluated, My goal was to describe spatial and temporal patterns of infectious agents in juvenile salmon. I also related infectious agent loads to blood plasma parameters (lactate, glucose, chloride, sodium concentrations, and osmolality) and to host gene expression to examine broader physiological effects of infectious agents on juvenile salmon. Lastly, I compared the two layers of physiological results (plasma variables and gene expression) with histopathology results, which examine morphological damage and infectious agent localization, to help identify disease status in juvenile salmon. In Chapter 3, I highlighted how my work has overcome the traditional

difficulties in studying infectious agents in wild fish and expanded the understanding of the potential impacts of infectious agents in wild juvenile Chinook salmon. I assessed the novel method which synthesized the detection of infectious agents and the profiling of host gene expression through advance molecular technology and traditional laboratory methods including blood physiology and histopathology. I further outlined some of the limitations of my study and suggested improvements for future research. Lastly, I discussed potential implications of my results to future studies of wild fish, particularly to salmon management and conservation in the Pacific Northwest.

Chapter 2: The physiological associations between infectious agents and migrating juvenile Chinook salmon (*Oncorhynchus tshawytscha*)

2.1 Introduction

Pacific salmon are an iconic species on the west coast of North America and are important ecologically, nutritionally, economically, recreationally, and culturally (Willson & Hulupka 1995; Reimchen et al. 2003; Healey 2009; Pinkerton 1994; Lichatowich 1999). The productivity of Pacific salmon in the North Pacific Ocean is characterized by high interannual variability, however, and declines in some species and populations have been evident for decades (Beamish et al. 1999; Irvine & Fukuwaka 2011). Chinook salmon are in decline across a considerable portion of their southern distribution (Noakes et al., 2000; Riddell et al., 2013). Despite well-intentioned efforts to bolster their populations through hatchery supplementation, the total catch of Chinook salmon has been declining since the late 1990s, during which time over 20% of Canadian fisheries was contributed by hatchery program (MacKinlay et al., 2004). Although the reasons behind the decline in Chinook abundance are still in question, predation (Thomas et al., 2017), shifting marine conditions (Beamish et al., 1995), global climate change (Irvine & Fukuwaka, 2011), and disease (Miller et al. 2014) are suspected to contribute to the decline.

The early marine residence of Pacific salmon is a critical period where salmon are thought to have very low survival (Beamish et al., 2004, 2010, 2012; Duffy & Beauchamp, 2011), in some cases lower than that of down-river migration (Welch et al., 2011). Poor feeding conditions and high levels of predation could have large effects (Beamish et al.,

1995; Thomas et al., 2017), though the role of infectious agents could also be important (Bakke & Harris 1998) but have rarely been studied due to the difficulties in sampling dead or dying fish. Infectious agents (including viruses, bacteria, protozoa, and fungi) are considered to have sporadic impacts combined with other stressors in the environment due to their ability to rapidly reproduce and to influence the host as population size regulators and selective agents (Anderson & May 1979; Bakke & Harris 1998). Before entering the marine environment, juvenile salmon may perish as a consequence of infections contracted during their freshwater residence (Jeffries et al., 2014). Upon arriving in the marine environment, salmon encounter many new infectious agents (Tucker et al., 2018). Some agents carried by fish from freshwater to marine areas can have similar pathogenicity in the saltwater ecosystem, whereas others can be transmitted in a freshwater environment but become more virulent after encountering the ocean, such as Piscine orthoreovirus (PRV, Løvoll et al., 2012) and Piscine myocarditis virus (PMCV, Wiik-Nielsen et al., 2012). However, in wild populations, it is difficult to study the effect of infectious agents because diseased fish are rarely observed as most are predated upon or die unobserved (Bakke & Harris, 1998; Miller et al., 2014).

Mortality is highly observable in cultured settings, hence, much of our knowledge of infectious agents on salmonids comes from aquaculture where mortality is measurable (Kurath & Winton, 2011). An example of an infectious agent that has been mostly studied in aquaculture with the potential to impact salmonids in the early marine period is PRV, which in Atlantic salmon is the causative agent of heart and skeletal muscle inflammation (HSMI) (Di Cicco et al., 2017; Wessel et al., 2017), but for which various strains have also caused disease in Pacific salmon (Di Cicco et al. 2018). An outbreak typically occurs in ocean net

pens 5-9 months after sea transfer of Atlantic salmon (*Salmo salar*) (Kongtorp et al., 2004); a similar timed HSMI outbreak occurred in a salmon farm in BC (Di Cicco et al., 2017). More recent evidence shows the same strain of virus (PRV-1) that causes HSMI in Norway and in Canada is likely to cause jaundice/anemia in farmed Chinook salmon, which could potentially be a threat to wild populations (Di Cicco et al., 2018). In a study where marine-captured, first-year juvenile, Fraser River Chinook salmon were screened for 45 infectious agents, PRV was found in 7% of the population (Tucker et al., 2018). Moreover, 32 infectious agents were detected in this study, and several infectious agents displayed seasonal shifts of prevalence and load truncation, which was suspected to have the potential for impacting the host at the population level (Tucker et al., 2018). Although the prevalence of PRV was relatively low in Tucker et al., (2018), it increased in the spring and then decreased through the fall and winter, a pattern potentially reflecting mortality in juvenile Chinook. However, given these data, the actual impact of an infectious agent on wild juvenile salmon in their early marine residence is still unclear. Physiological information from infected wild fish is helpful to understand the likelihood that an infectious agent is linked to early marine mortality.

The advancement of molecular technology has opened more possibilities for studying infectious agents in wild populations than ever before. High-throughput quantitative polymerase chain reaction (HT-qPCR) enables rapid and highly sensitive screening of infectious agents across large samples (Miller et al., 2016). In addition, on platforms that amplify RNA sequences, the host gene expression can be simultaneously profiled through the inclusion of assays targeting aspects of host health, such as immune status, stress level, and osmoregulation (Jeffries et al., 2014; Teffer et al., 2017). Combining molecular

technology with traditional laboratory methods of studying fish physiology such as blood chemistry and histopathology can broaden our understanding of infectious agents carried by wild fish.

The primary objective of this thesis chapter is to assess how infectious agents impact the physiology of migrating Chinook salmon during their first year of marine residence. This research was incremental to the Tucker et al. (2018) study, focusing largely on the agents showing truncation in prevalence and/or load between seasons, and elucidating whether just prior to truncation, there was any evidence of potential physiological response or damage. As such, the sampling scheme in my research was set up to optimize the detection of these specific agents before and after truncation, rather than to balance spatial and temporal samples along the southern BC coast. Moreover, for inclusion in my study, availability of blood serum and formalin preserved tissues further narrowed the range of archived samples that could be utilized. In my study, HT-qPCR was applied to detect and quantify genetic material from known infectious agents. I compared infectious agent presence and load to fish physiological conditions including host transcriptional profiles, endocrine and osmoregulatory variation (e.g. glucose, lactate, ions and osmolality from blood serum), and evidence of cellular damage through histopathology. My thesis research examined juvenile Chinook salmon from a broad geographic area involving central mainland BC, Vancouver Island, and Washington State, therefore I was also able to examine infectious agent patterns both spatially and temporally, with some limitations. As sampling was not balanced between years, I did not attempt to make any assertions as to interannual variations of infectious agents or physiological data, although this is an obvious next step in our understanding of the role of infectious disease in salmon declines. This research is unique by the virtue of

combining three layers of physiological information: molecular (host gene expression), protein (blood plasma chemistry), and cellular (histopathology) of juvenile Chinook salmon in their early marine residence.

2.2 Methods

2.2.1 Fish, blood, and tissue collection

This study focused on juvenile Chinook salmon in their first year of marine residence. Samples were obtained from Fisheries and Oceans Canada (DFO) research sampling programs along the southern coast of BC (Figure 2.1). All samples were from winter, summer, fall of 2012, 2013 and 2014 (Appendix 1.1). The sampling methods are described in (Tucker et al., 2011, 2012, and 2018). Briefly, fish were captured by midwater rope trawl (DFO marine sampling vessels including primarily the CCGSW. E. Ricker) for 15–30 min at 5 knots and brought onboard. Juvenile Chinook salmon were haphazardly selected and length (mm) and mass (g) were measured. To ensure that only juvenile Chinook salmon were collected, seasonal size limits were applied as follows: May-August < 300mm, Oct-Nov <350mm, Feb-March <400mm.

Blood was collected from each individual with 1.0 ml syringe and 26 gauge needles. The needles and syringes were flushed with heparin solution prior to blood extraction from the caudal peduncle. The collected blood samples were centrifuged at 6,900 G for 5 minutes to isolate plasma for measurement of physiological parameters in the lab. Gill filament samples were collected from each individual using scissors and preserved in 95% ethanol for genetic natal population identification in the lab (Beacham et al., 2006). Samples of tissue were also taken from brain, gill, kidney, liver, and heart using scalpels between outside and inside tissues. In order to be consistent with tissue sample size, the whole brain and heart were taken, while tissues pieces between 20 mg to 30 mg were taken from other ograns. Each tissue was preserved in an individual vial of RNAlater (Qiagen, MD, USA), kept for 24

hours at 4°C and then frozen in -80°C; these samples were used for infectious agent detection and host gene expression profiling. Pieces of tissues from gill, muscle, heart, liver, spleen, kidney, pancreas, and central nervous system (CNS) were also collected into histology cassettes and preserved in 10% buffered formalin. We attempted to keep the size of tissue collected constant, hence in the case of small specimens, the whole organ may be included (e.g. spleen). In case of larger specimens, each tissue was then subsampled to a size not thicker than 4mm for at least one of the three dimensions to allow formalin to penetrate and fix the tissues quickly, avoiding subsequent artifact.

2.2.2 Laboratory methods

Molecular methods. Infectious agent detection and quantification and host gene transcriptome analyses were conducted at the Fisheries and Oceans Canada Pacific Biological Station (DFO-PBS, BC, Canada). High-throughput quantitative PCR (HT-qPCR) with TaqMan assays run on the Fluidigm BioMark HT microfluidics platform (Fluidigm, CA, USA) was applied to quantify the absolute and relative amount of nuclear acid of infectious agents and host stress- and immune-related host genes. This technology has been adopted for salmon research (Miller et al., 2016), and used in several studies of Pacific salmon featuring both juvenile and adult fish (Miller et al. 2014; Jeffries et al., 2014; Healy et al., 2018; Teffer et al., 2017, 2018; Bass et al., 2019). The platform performs independent PCR reactions for each of 96 samples against each of 96 assays for a total of 9,216 reactions. The specificity, sensitivity, and repeatability of the platform has been validated for use in salmon infectious agent detection and quantification (Miller et al., 2016)

In my study, BioMark dynamic arrays were run separately for infectious agents and host genes to maximize the number of agents and host genes surveyed. For infectious agent detection, 47 assays to 46 infectious agents (two assays to infectious salmon anemia virus) and one reference gene were selected to run in duplicate on each dynamic array. Each dynamic array contains pooled samples from brain, gill, kidney, liver, and heart, positive and negative processing controls, and six standard serial dilutions of artificial positive constructs (clones of DNA sequences corresponding to all infectious agent assays). For host gene expression, due to the variation of available sample size collected in the field, only a subset of gill samples (N=218) and a subset of liver samples (N=263) were used and they were run separately on different chips. At least one positive and one negative processing controls and six standard serial dilutions (made by pooling host cDNA using 1 µL from every sample) were also allocated on every chip. Eighty-nine host gene assays were selected to run as singletons based on their known contributions to immune response, general stress response, osmolality, thermal, and hypoxia stress (Table 2.1, Miller et al. 2016, 2017; Akbarzadeh et al., 2018), and a set of genes identified previously as a "mortality related signature" that has been predictive of wild adult salmon migration and spawning failure (Miller et al., 2011). A panel of host genes were also included that when co-expressed distinguish fish in a viral disease state (VDD) from a carrier or no virus state. This VDD panel of genes was developed and validated by Miller et al. (2017) using challenge studies of multiple viral agents.

Lab procedures for nucleic acid preparation and qPCR protocol are described in Miller et al. (2014, 2016) and the process has been applied in several recent studies (Jeffries et al., 2014; Bass et al., 2017; Teffer et al., 2017; Healy et al., 2018; Tucker et al., 2018). In short, every sample was first homogenized. For infectious agent detection, the aqueous phase

from multiple tissues was pooled. For host gene profiling, gill and liver tissue homogenates were used separately. The DNA and RNA were extracted from the aqueous phase, assessed for purity and normalized. The cDNA was then made from normalized RNA. For infectious agent monitoring, equal aliquots of cDNA and DNA were combined. Only cDNA was used for host transcriptome analyses. Because the BioMark platform uses a small volume (5 nL), samples were first pre-amplified with all target assay primers through a PCR cycling according to the Fluidigm protocol. Prior to qPCR cycling, excess or unincorporated nucleotides and primers were removed, and samples were diluted 5-fold. Cycle threshold(Ct) was determined in the BioMark Real-Time PCR software. Amplification curves of all reactions between each assay and each sample were visually evaluated for any abnormal curve shape. Assay efficiencies were calculated based on a fitted curve from serial dilutions. Assays with efficiencies less than 80% or greater than 120% or coefficients of determination (R^2) of the fitted curve less than 0.98 were removed from subsequent analyses. Host gene expression of gill and liver samples were normalized with the $2^{-\Delta\Delta Ct}$ method (Livak & Schmittgen, 2001) in which relative expression of each gene was calculated using the reference genes and the pooled sample made with all samples included. Gene expression data were normalized by subtracting the difference between the targeted gene and the mean of reference genes from the difference between the positive pooled sample and the mean of reference genes.

DNA natal population identification. At the DFO-PBS, fin clip samples preserved in ethanol were used to assess the population of origin following approaches in Beacham et al. (2006). Fish were grouped into six main natal region groups: West Coast of Vancouver Island, East Coast of Vancouver Island, Fraser River system (upper and lower Fraser River

and Thompson River), Mainland BC (including streams in Northern, Central and Southern mainland BC that were not included in the other five region groups), the Columbia River system (including the Columbia River and Snake River), and Washington (including tributaries to the Puget Sound and Strait of Juan de Fuca). Hereafter, they will be referred to as WCVI, ECVI, Fraser, Mainland, Columbia, and Washington respectively. In the analysis, I excluded any individuals from an unknown natal population or with a probability of assigned natal population less than 0.50.

Blood analyses. Blood samples were collected for a subset of juvenile Chinook salmon (N=213) to examine plasma concentrations of lactate, glucose, chloride, sodium concentrations, and osmolality. Immediately after thawing, the plasma layer was carefully transferred from the frozen 1500 μL centrifuge tube into a new 500 μL centrifuge tube by single-use pipet. Plasma glucose and lactate concentrations were measured using a YSI 2300 Stat Plus lactate/glucose analyzer (Yellow Springs Instruments, OH, USA). Chloride concentration and osmolality were measured as the average of the duplicates using a Model 4425000 Haake Buchler digital chloridometer and the Advanced Instruments 3320 freezing point osmometer, respectively. If the disagreement between the duplicates was greater than 3 mmol/L for chloride or 3 mOsm/kg for osmolality, measurements were repeated, and the average was taken from the two closest measurements. At a later date, transferred plasma samples were thawed again and were diluted at 1:100 dilution for sodium analysis by a BWB XP flame photometer. The photometer was calibrated against a four-point standard curve that was created using sodium standard solutions at every startup or after a change was observed during checks performed every 10 samples. If the difference between the two results was

greater than 6 mmol/L, the measurements were repeated, and the averages were calculated by the two closest results.

Histology. Based on the patterns of load truncation observed in Tucker et al. (2018) that could be consistent with mortality-related processes of juvenile salmon in the early marine environment, four infectious agents (*Ceratonova shasta, Parvicapsula minibicornis, Paranucleospora theridion*, and PRV) were chosen for histology analyses. Thirty-three histology samples that were positive for at least one of the four infectious agents from qPCR detection were used. Samples went through dehydration through a decreasing gradient of ethanol solutions. Samples were then embedded in paraffin wax and were cut to slides at 3.5 μm thickness. The slices were stained with standard hematoxylin and eosin (H&E). When the slices were examined, a subjective quantitative scoring system (1-mild, 2-moderate, 3-severe) was used to describe the damage levels of tissues from different organs. To localize the target agent in the host tissues, four samples showing relatively high loads of three infectious agents (*C. shasta, Parvicapsula minibicornis,* and PRV) and lesions in tissues were used for in-situ hybridization (ISH) staining (ACD, CA, USA) which used probes designed to hybridize to specific infectious agent RNA. The RNA-ISH was implemented using BASEscope (RED) (Advanced Cell Diagnostics, Newark, California, USA) according to the instructions from the manufacturer. In preparation for hybridization, the dewaxed samples used for the histopathological analysis were boiled and incubated each for 30 min in RNAscope target retrieval reagents (Advanced Cell Diagnostics, Newark, California, USA). The slides were then hybridized with a BASEscope probe against a portion of target agent genome segment (Advanced Cell Diagnostics, Newark, California, catalog #705151), to detect the target agent in the tissues. The finished slides went through the first round of

visual exam by the veterinarian Dr. Emiliano Di Cicco at PBS, and all slides were then shipped to the UK to be read and scored by Dr. Hugh Ferguson with three ranks: mild, medium and severe. All images of slides were taken by a camera system (Nikon Digital Sight DS-U3, Nikon, ON, Canada) attached to the Nikon Eclipse Ni microscope (Nikon, ON, Canada) and generated by a software Nikon NIS-Elements D4.30.01 64Mb.

2.2.3 Statistical analyses

All statistical analyses were performed in R statistical software, version 3.4.2 (R Core Team 2017).

Infectious agent load was defined as the amount of infectious agent nucleic acids in a given sample. The infectious agent Ct values were first averaged between replicates. In the case where an infectious agent was not positive for both replicates, no detection was assigned. The infectious agent Ct values were then converted to copy number using the standard curve method (Larionov et al., 2005). The RNA copy numbers were then log-transformed due to skewed distributions.

Limit of detection (LOD) was defined in Miller et al., (2016) as a cycle threshold (Ct) number above which true positive results was expected 95% of the time for a given assay. Due to the high sensitivity of the BioMark platform, this is a conservative estimate, so I retained the data exceeding the LOD but excluded the infectious agents that only had detections above LOD to reduce the chance of false detection.

For each individual, the richness of infectious agents was defined as the number of different infectious agent taxa detected on that individual. Because richness did not take the quantity of infectious agents per individual into account, I used Relative Infection Burden (RIB, Bass et al., 2019), a composite score that compares the load of each agent detected in an individual relative to the highest load in the population, and then sums the ranks. All loads used for calculating RIB in the current study were in log-transformed copy number. For each infectious agent, the prevalence was calculated as the percentage of positive detections within the entire study population.

To understand the temporal and spatial distributions of infectious agents, richness and RIB were calculated for each individual across the entire study population, and an ANOVA was applied to assess the effect of sampling periods and natal groups on RIB. Tukey's honest significant difference (HSD) *post hoc* multiple comparison tests were then applied to compare the difference of RIB among natal groups. In addition, prevalence and loads of each infectious agent across six main natal groups and six sampling periods were presented in figures.

Two-way ANOVAs were first used to assess if sampling periods and natal groups were associated with blood plasma parameters (lactate, glucose, chloride, sodium concentrations and osmolality). To look for correlations between infectious agents and fish physiology on the protein level, I employed a general linear model approach (R package *lme4,* Bates et al., 2015) using infectious agent loads as independent variables, blood plasma physiological parameters as response variables, and included sampling periods and natal groups as random factors. This analysis was only conducted for infectious agents that had more than ten detections in the entire study population. To reduce type 1 errors of false

detection, I adjusted all the *p* values using the false discovery rate (FDR) approach across all combinations of plasma parameters and infectious agents (Benjamini & Hochberg, 1995).

Because there were two types of tissues used for host gene expression profiling, the following statistical analyses were done in the same way for both gill and liver tissue separately. In order to explore any relationships between host gene expression and infectious agents, I employed a constrained ordination in the form of redundancy analyses (RDA). RDA assesses the amount of variation in one set of variables that can be explained by another set of variables, and visualizes the results in an ordination diagram (triplot) which summarize the interrelationships among samples, response variables, and explanatory variables. In the present case, the response variable was the host gene expression matrix, and the explanatory variable was the infectious agent matrix. Two separate RDA models were run for gill and liver gene expression using the R package *Vegan* (version 2.4-5, Oksanen et al., 2017). I also included dynamic array ID, sampling periods, and natal groups as partial terms which are often regarded as similar to random effects when put before other constraints of interests (in the present case infectious agent matrix, *vegan FAQ*, 2016) in the model (model: gill/liver gene expression matrix ~ dynamic array ID + sampling period + natal group + infectious agent matrix including all agents with more than five detections). Because the ordinary R^2 is often biased in RDA, an adjusted R^2 was used instead (Borcard et al., 2011). The significance of the entire model, significance of each canonical axis, and significance of each explanatory variable were tested by separate Monte Carlo permutation tests by terms which performed separate significance test for each term (Borcard et al., 2011; Legendre et al., 2011). The permutation test computed a *p*-value which is proportional to the

permuted values equal to or larger than the unpermuted true values of a one-tailed test, and it is widely implemented in community ecology studies (Borcard et al., 2011).

Because the RDA models I developed did not directly test for associations between each individual infectious agent and host gene expression, I implemented another analysis, which was a combination of principal component analysis (PCA) and general linear model (GLM). PCA is generally used for the reduction on dimensions of a multivariate data matrix. I ran PCA for gill and liver gene expression respectively using *prcomp()* function (the R base package). To assess the associations between infectious agent loads and host gene expression, the first five PCs (which represented over 50% variance cumulatively in both tissues) were used as response variables in general linear models (R package *lme4* Bates et al., 2015) with dynamic array ID, sampling periods, and natal groups included as random factors. Considering the limitation of the degree of freedom, this model was only applied to the infectious agents that had more than ten detections in the entire study population. The resulted *p* values were adjusted by the FDR approach across all combinations of the first five PCs and infectious agents separately by tissue to restrict the chance of type 1 error (Benjamini & Hochberg, 1995). An ANOVA was applied to test whether fish positive for lesions caused by PRV determined by histopathology were different from the rest of PRV positive fish regarding VDD signal related PCs.

2.3 Results

2.3.1 Infectious agent detection

Twenty-six of 46 assayed infectious agent taxa were detected in juvenile Chinook salmon (Table 2.2). Twenty-one infectious agents had an overall prevalence greater than 1%, including 2 viruses, 5 bacteria, and 14 parasites (Table 2.2). Among the ten infectious agents with greater than 10% prevalence, '*Candidatus* Branchiomonas cysticola' was the most prevalent and was found in 81% of the total population (Table 2.2).

Across the entire study population, the mean infectious agent richness was 4.02 (± SD 1.57) and the mean RIB was 1.54 (± SD 0.84). The infectious agent richness ranged from 0-10, with a median of 4. Fish from the Columbia River had both the highest mean richness of 5.39 (± SD 1.47) and the highest mean RIB of 2.37 (± SD 0.89) (Table 2.3). The Fraser River group had the second highest mean richness and mean RIB (Table 2.3). Fish caught in 2013 summer had both the highest mean richness of 5.38 (± SD 1.40) and the highest mean RIB of 2.21 (± SD 0.96) (Table 2.4). A two-way ANOVA indicated that natal group was the only significant factor associated with RIB, and there was no interaction between sampling periods and natal groups (ANOVAs: natal group, $F_{5,304}=6.120$, $p<0.001$; sampling period, $F_{5,304}=1.333$, $p=0.250$; natal group: sampling period, $F_{5,304}=1.258$, $p=0.243$). In Tukey's HSD *post hoc* multiple comparisons tests, significant differences were only found between Columbia and five other natal groups (Columbia-Fraser, Columbia-WCVI, Columbia-ECVI, Columbia-Mainland, $p<0.01$, Columbia-Washington, $p<0.05$, Figure 2.2).

Five infectious agents showed increasing prevalence from summer to winter, including one bacteria *Tenacibaculum maritimum,* 3 parasites *Ichthyophonus hoferi, Loma*

salmonae, Parvicapsula pseudobranchicola, and one virus Viral erythrocytic necrosis virus. One agent, Rickettsia-like organism (rlo), presented decreasing prevalence from summer to winter (Appendix 2.1). A few agents showed up only among fish from selected natal groups. For example, PRV had the prevalence of 17% and 13% among fish in the Washington group and WCVI group, however, it had less than 5% prevalence or no detection among fish from other natal groups (Figure 2.3a).

Two infectious agents, *Parvicapsula minibicornis* and *C. shasta* had very similar patterns of both prevalence and load. Both agents had high prevalence and wide ranges of loads among fish in the Fraser and Columbia groups, and low prevalence in other stocks (Figure 2.3b and Figure 2.3c). The loads of positive detections of *Parvicapsula minibicornis* and *C. shasta* were positively correlated in a simple linear regression ($b = 0.31$, $R^2 = 0.09$, $df = 61$, $p=0.01$).

2.3.2 Plasma Parameters

All five blood plasma parameters were influenced by natal groups and sampling periods except one (ANOVA: Glucose ~ sampling period, $F_{5,202}= 1.763$, $p=0.12$, details in Table 2.5). The five plasma parameter levels across six natal groups and six sampling periods are shown in Appendix 2.2. After the adjustments of *p* values, only one significant (significant level=0.05) correlation was found between infectious agent loads and blood plasma parameters: the load of *I. hoferi* was positively correlated with plasma sodium level (p-adjusted = 0.01) (Figure 2.4).

2.3.3 Host gene expression

The RDA models for gene expression in gill and liver (model: gill/liver gene expression matrix ~ dynamic array ID + sampling period + natal group + infectious agent matrix; including all agents with more than five detections) were both significant (permutation tests: gill $F_{32,262}$ =5.722, p=0.001; liver $F_{32,230}$ =5.722, p=0.001). The adjusted R^2 were 33.9% for gill samples and 31.4% for liver samples. All four explanatory terms were determined to be significantly associated with gene expression of both gill and liver samples (significant level $p<0.05$, details in Table 2.6). For gill samples, the first four canonical axes were significant (permutation tests, RDA1, $F_{1,262} = 58.764$, $p = 0.001$; RDA2, $F_{1,262} = 35.919$, $p = 0.001$; RDA3, $F_{1,262} = 27.673$, $p = 0.001$; RDA4, $F_{1,262} = 15.247$, $p = 0.001$), and accounted for 10.3%, 6.3%, 4.9%, and 2.7% variance separately and 24.2% cumulatively in gill gene expression. Similarly, the first four canonical axes in RDA with liver samples were also significant (permutation tests, RDA1, $F_{1,230} = 41.587$, $p = 0.001$; RDA2, $F_{1,230} = 35.762$, $p = 0.001$; RDA3, $F_{1,230} = 20.074$, $p = 0.001$; RDA4, $F_{1,230} = 14.198$, $p = 0.001$), and they accounted for 8.3%, 7.1%, 4.0%, and 2.8% variance separately and 22.2% cumulatively in the liver gene expression.

In general, the RDA overall ordinations for gill and liver samples presented similar patterns in terms of the relationships between host gene expression and infectious agents (Figure 2.5a, b). A RDA1 versus RDA2 ordination plot for gill tissue showed that PRV had a strong influence on multiple VDD genes, and it was very close to *Neoparamoeba perurans*. *Parvicapsula minibicornis* was in the opposite direction to most of the immunity and VDD genes but closer to two stress and osmoregulation related genes, HSP90ab1 and sepw1 (Figure 2.5a). A RDA1 and RDA2 ordination plot for liver tissue also revealed a close

relationship between PRV and VDD genes, as well as a separation between *Parvicapsula minibicornis* and most immunity and VDD genes. Similar to gill samples, PRV was adjacent to *N. perurans*. Genes including PCBL, SERPIN, sepw1, TF and HSP90a clustered in the same direction as *Parvicapsula minibicornis* (Figure 2.6a). As for the ordination plot made by RDA3 and RDA4, the gill model had *P. pseudobranchicola* and '*Ca.* B. cysticola' on the positive end close to genes such as FYB, HTA, HIF1A, IRF1, and *Parvicapsula minibicornis* on the negative end close to genes such as SERPIN, HSP90a, C3 (Figure 2.5b). Liver RDA3 and RDA4 showed PRV clustered with most of VDD and immune genes and were again very loosely related to *N. perurans*. *Parvicapsula minibicornis* and *C. shasta* seemed to have similar correlations with genes such as SERPIN, TCRb, IFNa, HSP90a, COMMD7, and JUN (Figure 2.6b).

For infectious agents that had similar positions on an ordination plot, I further examined relationships using simple linear regression for two pairs of agents with positive detections. There was no significant relationship between loads of positive detections of '*Ca.* B. cysticola' and *P. pseudobranchicola* ($b = 0.17$, $R^2 = 0.01$, $df = 146$, $p=0.25$), and no significant relationship between loads of positive detections of PRV and *N. perurans* ($b = 0.40$, $R^2 = 0.07$, $df = 4$, $p=0.62$).

RDA1 in both gill and liver sample models were related to a difference in natal group: WCVI, Mainland, Columbia, and Washington clustered on one side of RDA1 on and Fraser, ECVI were on the opposite side of RDA1 (Figure 2.5a and 2.6a). One gill sample array (plate c2494) and one liver sample array (plate c2486) were both positioned on the positive end of gill and liver sample RDA3 respectively.

For the results of the combined PCA and GLM, the first five PCs from gill PCA results accounted for 22.3%, 13.2%, 7.8%, 5.8%, and 4.5% of the variance in gill gene expression. The plots of gene loadings of the first five PCs are presented in Appendix 2.3 Only significant models (p-adjusted < 0.05) were reported as follows: Gill PC1 was positively associated with the load of '*Ca.* B. cysticola' (b=0.24, t=3.66, df=233.44, p-adjusted =0.01, Figure 2.7a). Gill PC4 was positively associated with PRV (b=0.42, t=3.99, df=13, p-adjusted=0.04, Figure 2.7b). Gill PC5 was positively associated with '*Ca.* B. cysticola' (b= -0.14, t= -5.20, df=230.52, p-adjusted<0.001, Figure 2.7c). The first five PCs from the liver PCA explained 19.4%, 12.0%, 7.3%, 5.5%, and 4.8% of the variance in liver gene expression. The plots of gene loadings of the first five PCs are presented in Appendix 2.4. The first five PCs were fitted into the same general linear model as gill gene expression data. Only one significant model (p-adjusted <0.05) was found which included liver PC5 and PRV (b= -0.69, t=-14.98, df=7.32, p<0.001, Figure 2.7d). Fish positive for lesions caused by PRV were not significantly different from the rest of PRV positive fish regarding VDD signal-related PCs: gill PC4 ($F_{1,13}$=1.76, p=0.21), and liver PC5 ($F_{1,12}$=1.87, p=0.20).

2.3.4 Histology

Evidence of lesions on host tissues potentially associated with all four agents (*C. shasta, Parvicapsula minibicornis, Paranucleospora theridion*, and PRV) was found, although no severe lesions significant enough to cause death were found in any fish examined. Most damage, if it occurred, was relatively mild, with only 24% of fish examined with lesions classified as medium (2). Nine fish examined were found to have no evidence of

lesions (Table 2.7). The majority of lesions observed were on spleen and kidney tissues, two tissues we did not examine for gene expression. The rates of lesions present in the spleen and kidney were 45% and 58% respectively. Noteworthy findings included: moderate lesions in the gastrointestinal system likely to be caused by *C. shasta* (n=1); mild lesions from developing stage of *C. shasta* in gill (n=1); mild heart lesions linked with PRV (n=1); mild spleen lesions associated with PRV (n=2); mild kidney lesions that were likely to be caused by PRV (n=6); moderate lesions in kidney associated with *Parvicapsula minibicornis* (n=1); and mild lesions in kidney associated with *Parvicapsula minibicornis* (n=1, details in Table 2.7). The associations between lesions and the suspected causal agent were supported by localization of the target agents near the lesions after applying ISH on the same set of slices that were used for H&E staining. *Parvicapsula minibicornis* was found in the host in both glomeruli and in the lumen of renal tubules (Figure 2.8). *C. shasta* was detected in the host lamina propria of the intestine (Figure 2.9) and in the host gill tissue (Figure 2.10). PRV was found in the host cardiomyocytes (Figure 2.11), spleen, posterior kidney, intestine and liver (Figure 2.13). PRV was widely distributed in the spleen, showing also blood congestion and hemosiderin deposits. The posterior kidney was also heavily infected with PRV with a few necrotic tubules. In the intestine and liver, PRV was also found in the enterocytes and hepatocytes respectively.

2.4 Discussion

2.4.1 Overview

My thesis study is the first to combine molecular, protein, and cellular data for fish physiology to study infectious agents carried by wild salmon. This thesis is also the first to report the associations between fish physiological condition and two infectious agents that were recently discovered to be relevant to salmon health, including '*Ca.* B. cysticola' and PRV. I provided some of the first evidence, through associations, of the potential impacts of PRV on both host genes and histopathology in the wild Chinook salmon that were highly consistent with observations in cultured fish of the same species. My results also broadened the knowledge of potential physiological impacts of several infectious agents that were previously suspected to be associated with salmon mortality, including *I. hoferi,* and *Parvicapsula minibicornis,* and demonstrated spatial geographic patterns in infection burden.

2.4.2 Infectious agent detection

Columbia River fish had the highest infectious agent richness and RIB of all natal groups. Numerous dams and reservoirs in the Columbia River and tributary system (Fish Passage Center, 2015), and its more southern latitude, makes this system generally warmer than the others in this study. Summer temperature has averaged above 20°C in the Columbia River with a maximum daily high of up to 24.8°C (USACE, 2004). Temperature is well known to be a critical factor in disease development in fish (Wedemeyer, 1996) for both impacting infectious agent growth and transmission and host physiological conditions (including their immune systems) (Marcogliese, 2001; Ray et al., 2012). In this study, 95%

of fish from the Columbia River system were positive for *C. shasta* and *Parvicapsula minibicornis*. These two agents were known to be associated with warmer temperature (Ray et al., 2012). The freshwater polychaete, *Manayunkia speciosa,* a host of *C. shasta* and *Parvicapsula minibicornis,* tends to aggregate near reservoir inflow areas, and sites below dams are likely to have elevated densities of parasite spores (Stocking & Bartholomew, 2007). In addition, compared to other natal groups, outmigrating juvenile fish from portions of the Columbia River system had to travel much longer distances to reach the area sampled, and relatively long exposure to novel saltwater agents might have contributed to higher infection burdens in the summer. As the majority of Columbia River origin fish continue migration to Alaska throughout the summer and fall, they are not observed off the west coast of Vancouver Island in all seasons, limiting our ability to analyze how infectious profiles change through time for this natal group. It is also possible that the large size of this system also accounted for higher infectious agent diversity comparing to smaller regions in my study.

In contrast to some of the large-scale sampling studies undertaken for infectious agent profiling in the Strategic Salmon Health Initiative, my results only used 315 fish from three years of sampling programs. A survey of the infectious agents detected in juvenile Chinook along with sockeye salmon originating from BC and Washington for five years from 2008 to 2012 was done by Miller et al. (2017). My study had similar results in terms of the number of infectious agent taxa detected in juvenile Chinook salmon despite the vastly greater sample sizes in their study (number of agent taxa with greater than 1% prevalence / Number of agent taxa screened for: Present study: 21/46, Sample size = 315; Miller et al., 2017: 21/46, Sample size = 1666). The mean and range of richness were similar between the present study

(mean=4.02, range=0~9) and the other study (Miller et al., 2017: mean between 3 and 4, range=0~10). Therefore, a smaller sample size similar to the current study may be sufficient to capture most of the infectious agent compositions. However, Miller et al. (2017) was able to detect the seasonal shifts in prevalence over time much more efficiently than the current study possibly due to the benefit of having a larger sample size. Among the few agents that I was able to see a consistent shift in overall prevalence over time, the increasing prevalence from summer to winter of three agents *I. hoferi, P. pseudobranchicola,* and *L. salmonae* was also noted in Miller et al., 2017.

2.4.3 Potential Physiological impacts of infectious agents

Piscine orthoreovirus (PRV)

Although PRV only had overall 5.08% prevalence, it was associated with the most obvious host gene responses both in my RDA and PCA+GLM analyses. In the RDA models of both gill and liver tissues, PRV was associated with VDD genes such as HERC6, RSAD, IFT5, 52Ro, CA054694, Mx, GAL 3. In the PCA+GLM analysis, the load of PRV was associated with gill PC4 and liver PC5 that both had a cluster of VDD genes loaded on the associated direction of the PC axis. My study is the first to highlight the important association between the presence of PRV and the upregulation of VDD genes in wild migrating juvenile Chinook salmon. Such a relationship in farmed Chinook salmon was recently confirmed by our group using fish from DFO farm audit program in BC (Di Cicco et al., 2018) and in farmed Atlantic salmon based both on the farm audit program and

longitudinal samples taken at a single farm undergoing an outbreak of HSMI (Di Cicco et al. 2018).

In my study, the load of PRV was relatively low compared with audit results in Chinook salmon farms (Di Cicco et al., 2018). The overall mean load of PRV in the current study was 141.03 copies per µg nucleic acids. Only one detection with load $>10^4$ copies per µg nucleic acids was present which was classified as high load in farm audits, and none of loads of PRV in the current study was as high as the minimum load of PRV detected in fish that were diagnosed as jaundice/anemia in Di Cicco et al., 2018. However, the VDD signal was still strongly associated with the load of PRV in liver PC5 despite the relatively lower loads. Moreover, the Di Cicco study also showed that milder lesions associated with earlier stages of the development of jaundice/anemia disease were present in fish not diagnosed with jaundice/anemia, but only in fish classified as VDD. In fact, their study hypothesized that the clinical observation of anemia relates to PRV-induced massive lysis of RBCs and jaundice to the toxic levels of hemoglobin causing necrosis of the kidney tubules, the latter of which represents a late stage of the disease. The wild fish in our study were not characterized for clinical signs during collection, so we cannot relate our data with clinical manifestations of a disease. However, the pathological data we have suggest that these wild fish were in an early stage of the development of jaundice/anemia. Whether fish with a late-stage disease would survive long enough to be sampled, and if they are physiologically compromised at early stages of disease development, are certainly questions worth pursuing in future.

PRV was recently proven to have a causal relationship with HSMI in Atlantic salmon (Wessel et al., 2017) which can cause up to 20% of cumulative mortality in an infected sea cage (Kongtorp et al., 2004). In BC, the only strain (PRV-1) found has no consistent

differences between Atlantic salmon with HSMI and Chinook salmon with jaundice/anemia, which suggests its ability to transmit diseases between two salmon species, and it implies a threat to migrating smolts due to its water-borne transmission and the farming intensity of both Atlantic salmon and Chinook salmon around Vancouver Island (Di Cicco et al., 2018). After finding higher prevalence of PRV in areas with greater densities of salmon farms, Morton et al., (2017) hypothesized that the pathogen was transferred from farmed Atlantic salmon to wild Pacific salmon. Among the fish positive for PRV in my study, 25% of them were caught at the locations identified as having a high exposure to farmed Atlantic salmon in Morton et al., (2017). The majority (56%) of PRV positive fish in my study were originated from Marble river which is part of the WCVI system. All of these Marble river fish were caught at Quatsino Sound, where six salmon farms are located and this site was not included for sampling in Morton et al., 2017. Although the actual impact of PRV on wild fish at the population level is unclear, the detection of PRV in combination with the VDD signal may be a good tool for monitoring because it is more sensitive in identifying a disease state compared to the traditional method of detecting diseases based on clinical signs (Di Cicco et al., 2018).

In my study, PRV shared ordination space with *N. perurans* in both gill and liver ordination spaces. There was no correlation between loads of PRV and *N. perurans*. However, all the *N. perurans* positive fish were also positive for PRV. *N. perurans* is a known agent of amoebic gill diseases in farmed Atlantic salmon and rainbow trout (*Oncorhynchus mykiss*) (Young et al., 2007; Young et al., 2008; Fringuelli et al., 2012). To my knowledge, this agent has not been studied in Pacific salmon in BC. Given that *N.*

perurans was only at 1.9% prevalence, the relationship between PRV and *N. perurans* needs further investigation in future studies.

On fish B2159, histology showed evidence that PRV was associated with mild lesions in heart and kidney tissues. In addition, I found heavy infections of PRV in the spleen, kidney, intestine, and liver in the same fish. Six fish in total were suspected to have lesions caused by PRV. Although fish positive for lesions caused by PRV were not significantly different from the rest of PRV positive fish regarding VDD signal related PCs, they appeared to group on the higher end of related PC axes. While the VDD signal was validated as an indicator of the presence of a disease state, this highly general viral disease response signature is not prognostic of the level of damage associated with an individual disease (Miller et al., 2017; Stevenson, 2018).

Candidatus Branchiomonas cysticola

'Ca. B. cysticola' was the most prevalent agents in the current study. It was correlated with gill PC1 and PC5. Based on the significance of the correlations, the strongest relationship was a negative association between this agent and gill PC5. On gill PC5, among the 13 genes with loadings lower than -0.1, seven of them were related to immunity (C1Qc, SAA, IGMS, IRF1, IL8, hep, TCRa) (Appendix 2.3). For example, C1Q chain B is considered to part of the acute phase response in rainbow trout (Gerwick et al., 2000). Serum amyloid protein a (SAA) is a major acute phase protein in mammals and its regulation is similar in Atlantic salmon (Bayne & Gerwick, 2001). Interleukin 8 (IL8) is a pro-inflammatory cytokine that responds to bacterial vaccines in pink salmon (*Oncorhynchus*

gorbuscha) and chum salmon (Fast et al., 2015). Hepcidin (hep) is an antibacterial peptide (Douglas et al., 2003), that responds to bacterial challenges in Atlantic salmon (Martin et al., 2006). *'Ca.* B. cysticola' is a type of bacteria that has been associated with gill disease in Norway, and is a common agent of gill epitheliocysts in farmed Atlantic salmon, which may be associated with mortality (Mitchell et al., 2013; Toenshoff et al., 2012). The load of this agent is associated with severity of proliferative gill inflammation (Mitchell et al., 2013). Therefore, the strong relationship with inflammatory genes such as C1Qc, SAA, IL8, and hep with increasing load of this agent is consistent with its purported role in gill inflammation. It is also suggested that this bacterium may be facilitated by other agents and appear as a secondary infection (Tengs & Rimstad, 2017).

Although in previous research conducted by our group, '*Ca.* B. cysticola' has been highly prevalent in adult Chinook salmon (Bass et al., 2017, 2019; Teffer et al., 2018) and out-migrating salmonid smolts (Healy et al., 2018; Stevenson, 2018), in most cases, there was no significant correlation between this agent and migration survival or any physiological indices. The exception was in Teffer et al., 2018, where higher loads of this agent were found in male Chinook salmon that died sooner in a cool water holding study. The present study was different from previous ones carried out in BC because it used the fish after they left the freshwater environment and before they matured. This life stage was more comparable to saltwater-farmed fish used in Toenshoff et al., 2012 and Mitchell et al., 2013. Therefore, the immune response observed in the current study may very well be associated with gill diseases. However, some researchers have suggested that as this bacterium is a member of the fish gill microbiota in healthy fish, it may not be pathogenic (Gunnarsson et al., 2017; Steinum et al., 2009). Hence, while the transcriptional data in my study is consistent with up-

regulation of inflammation in fish carrying high loads of this bacterium, histopathology will be required to demonstrate whether gill inflammation is occurring at the cellular level. If so, *in-situ* hybridization could be applied to resolve the spatial relationship between the bacterium and regions of inflammation. These are suggested next steps.

On the gill ordination plot '*Ca.* B. cysticola' had very similar positioning to agent *P. pseudobranchicola*. Similarly, on gill RDA4, '*Ca.* B. cysticola' and *P. pseudobranchicola* were close to each other and separate from other agents. *P. pseudobranchicola* is a myxozoan parasite that is associated with gill infection and potential impacts on swimming ability (Jørgensen et al., 2011; Karlsbakk et al., 2002). There was no significant correlation suggestive of concurrent infections between the positive detections of these two agents. Given that these two agents are both associated with gill diseases, they might have similar impacts on host genes in gill tissues. On the gill RDA3 by RDA4 ordination plot, these two agents were clustered with immune genes FYN-T-binding protein (FYB), HIV-1 Tat interactive protein (HTA), Interferon regulatory factor 1(IRF1), and T-cell receptor alpha (TCRa) and these genes are included in the MRS panel of genes that were predictive of migration and spawning fate of wild salmon (Miller et al., 2011).

Ichthyophonus hoferi

I. hoferi is a mesomycetozoan parasite of over 100 species of fish across marine, brackish and freshwater habitats (Rahimian & Thulin, 1996). It was prevalent among returning Chinook salmon in the Yukon River and was suspected to cause prespawn mortality (Kocan et al., 2004), and was recently reported in adult Fraser River Chinook

salmon (Bass et al., 2017). Its prevalence in spawning adult herring (*Clupea pallasii*) in Puget Sound (up to 58% of the population) may limit the maximum age of adult Pacific herring (Hershberger et al., 2002). After Chinook salmon ingest infected herring, this parasite can be found in several organs, including heart, liver, spleen, kidney, skeletal muscle or dermis. In response, the skeletal and cardiac muscle, dermis, liver and kidney become inflamed (Jones & Dawe, 2002). I hypothesize that the positive correlation of the load of *I. hoferi* and plasma sodium concentrations in juvenile Chinook was due to the potential loss of the ability to secrete sodium in a high saline environment, which might be related to impaired osmoregulation due to the pathogen's presence in the kidney. In sprat (*Sprattus sprattus*), high density of *I. hoferi* spores can be found in the kidney as well (Rahimian & Thulin, 1996). In contrast, a previous study by Rand & Cone (1990) found no effect on any blood chemistry parameter, including sodium, of experimentally infected rainbow trout. However, this study was conducted in a freshwater environment, and the salmon kidney functions differently in the freshwater as its purpose is to produce large volumes of dilute urine to maintain ions rather than to secrete ions (Clarke & Hirano, 1995).

Parvicapsula minibicornis

On both gill and liver RDA ordination plots, *Parvicapsula minibicornis* was similarly positioned to stress-related genes including HSP90a, (HSP90ab1, HSP90a, HSP90alike), SERPIN, sepw1, JUN, COX6B1, and Map3k14. HSP90a (Heat shock protein 90 alpha) and SERPIN (Serpin H1 precursor, also known as HSP47) are two types of heat shock proteins that are well known for protecting tissues from damage during exposures to stressors

including extreme temperature and extreme concentrations of ions (Martin & Gretchen, 1999; Palmisano et al., 2000; Akbarzadeh et al., 2018). JUN (Transcription factor) is related to cell apoptosis and its expression can be elevated by stress (Shaulian & Karin, 2002). Genes COX6B1 (Cytochrome c oxidase subunit 6B1) and sepw1 (Selenoprotein W) are linked to an antioxidant response and they can respond to extreme environmental challenges (Chen et al., 2013; Kim et al., 2015). Given that *Parvicapsula minibicornis* is a myxozoan parasite in the glomeruli of the kidney and is associated with mortality (Bradford et al., 2010), it might have impacts on the host osmo-equilibrium and may cause osmotic stress to the host. However, *Parvicapsula minibicornis* is not associated with any plasma parameters examined in my study, and had no significant impact on plasma ions in a study of infected adult sockeye salmon (Wagner et al., 2005).

Fish positive for *Parvicapsula minibicornis* might have experienced other forms of stress, such as thermal stress, since genes HSP90a , SERPIN, sepw1 and Map3k14 are known to be response genes after exposure to elevated temperature in salmonids (Akbarzadeh et al., 2018). Around 19% of fish positive for *Parvicapsula minibicornis* were from the Columbia River system which is known to have relatively elevated water temperatures (Fish Passage Center, 2015; Mantua et al., 2010). In adult sockeye salmon, high temperature leads to more severe *Parvicapsula minibicornis* infections and a higher chance of pre-spawn mortality (Wagner et al., 2005; Bradford et al., 2010). Temperature stress may facilitate the infection of *Parvicapsula minibicornis* in juvenile salmon while out-migrating as well. Higher temperature results in higher mortality and shorter days to death in infected *C. shasta* infected juvenile Chinook (Ray et al., 2012), and *Parvicapsula minibicornis* and *C. shasta* have very similar life history (Bartholomew et al., 2006). *Parvicapsula minibicornis*

was found to be isolated from most immune genes in both gill and liver RDA1 by RDA2 plots. Fish under stress could have impacted immune system function (Barton, 2002) and could be more susceptible to opportunistic diseases. Therefore, the suppression of immune response may be related to the infection of *Parvicapsula minibicornis* in my results.

Evidence of damage caused by *Parvicapsula minibicornis* was also confirmed by histology as well. Histology revealed lesions caused by this agent in the kidney with two degrees of glomerulonephritis observed including a moderate necorsis and a mild one that still showed a few morphological features of the host cells. The kidney is the known target tissue of *Parvicapsula minibicornis* (Bradford et al., 2010). The finding of lesions in the host kidney along with the presence of *Parvicapsula minibicornis* in the same tissue can indicate a disease status in the host, which agreed with the stress-related signals I discovered in the host genes.

Ceratonova shasta

In the current study, I did not find any direct associations between *C. shasta* and fish physiological condition. I only observed a positive relationship between the load of *C. shasta* and the load of *Parvicapsula minibicornis*. In addition, *Parvicapsula minibicornis* and *C. shasta* tended to have close positions on both gill and liver RDA ordination plots. *C. shasta* is a myxozoan parasite of fish intestine and it is commonly found in Chinook salmon in many freshwater systems in BC and Washington State (Fujiwara et al., 2011). The similar life history shared by *Parvicapsula minibicornis* and *C. shasta* (Bartholomew et al., 2006) and the fact that 82% of *C. shasta* positive fish were also positive for *Parvicapsula*

minibicornis probably explained the similar interrelationships between these two agents and the host gene expression.

Although I did not detect any physiological responses associated with *C. shasta* on the molecular (host gene) and protein (blood) levels, histology revealed that one individual had a moderate lesion in the gastrointestinal system that was likely to be caused by *C. shasta.* Histology also revealed that one fish had mild lesions including chlamydia-like aggregates and a suspected developing (pre-spore) stage of *C. shasta* at the tips of lamellae within the gills.

In addition to presenting the relationship between infectious agents and host gene expression, the RDA models also helped to understand the potential impacts of other environmental factors that might affect the host gene expression. In both gill and liver RDA models, sampling periods and natal groups had significant impacts on host gene expression, but the impacts were weaker than for infectious agents. RDA1 had a clear separation between Fraser, ECVI and WCVI, Mainland, Columbia and Washington. When plotting the capture locations of these fish on the map, the majority of Fraser, ECVI fish were caught on the inshore side of Vancouver Island from Queen Charlotte Strait to Strait of Juan de Fuca, and most of fish from WCVI, Mainland, Columbia and Washington were caught on the offshore side of Vancouver Island plus Queen Charlotte Strait. Neighbouring capture locations on the same side of the shore seemed to have a similar impact on the host gene expression regarding the RDA results. A large portion of variance accounted for in the statistical models was explained by the term “dynamic array ID” in both gill and liver RDA

analyses likely due to incomplete stratification in the sample layout on the dynamic arrays. This large inter-chip variance means that the biological results that I uncovered are conservative in their strength and likely would have accounted for even more of the physiological variation had this methodological issue not occurred.

To conclude, my research was novel because I studied the potential impacts of infectious agents on wild juvenile Chinook salmon through combining three layers of physiological data: molecular (host gene expression), protein (blood plasma chemistry), and cellular (histopathology). I specifically confirmed the potential impacts of *Parvicapsula minibicornis* and PRV on both molecular and cellular levels. Histopathology has been a traditional way of studying infectious agents and diseases in fish, but such methods require a stable environment to allow disease progress and measure mortality and while some lesions linked with specific agents were observed, associations with my other physiological metrics were weak. However, in wild environment, even weak effects of infectious agents on fish physiological condition and behavior can be crucial, if infections happen at a critical point in a salmon's life history that could impact survival (Bakke & Harris, 1998). My results supported the use of molecular methods to monitor the impact of infectious agents on wild populations, which can be applied to regular monitoring of infectious agents among Pacific salmon in the Pacific Northwest.

2.5 Chapter 2 tables

Table 2.1: Primer and probe sequences corresponding to assay for infectious agents and biomarkers used in HT-qPCR analyses on juvenile Chinook salmon *(Oncorhynchus tshawytscha)*. Amplification factor with * sign indicates this assay was excluded from statistical analysis for both gill and liver tissue due to a unacceptable amplification factor. Amplification factor with ** sign indicates this assay was excluded from statistical analysis only for liver tissue due to a large number of missing values in the samples.

Symbol	Infectious agent/ Host gene name	Assay Class	Type/ Function	Forward Primer Sequence (5'-3'), Reverse Primer Sequence (5'-3'), Probe Sequence (FAM-5'-3'-MGB)	Amplification Factor
52Ro	52 kDa Ro protein-2	Host gene	VDD	F: TGCACTATTGCCCAGTAACCAT R: TGCAAGAGGAGATGCCAACA P: AGTAGGATTCACAGAGAGTT	1.95
ACTB	Beta actin	Host gene	Growth	F: GAAATCGCCGCACTGGTT R: CGGCGAATCCGGCTTT P: TTGACAACGGATCCGGT	2.15
ALDOA	Aldolase A	Host gene	Glycolysis	F: CGTGATTCAGTGTTGTCATCTTGA R: TTCCTCCAGTGTTTTTTTCAGTCA P: AAGTACATGTGCCTTCTT	2.19
B2M	Beta-2-Microglobulin	Host gene	Immunity	F: TTTACAGCGCGGTGGAGTC R: TGCCAGGGTTACGGCTGTAC P: AAAGAATCTCCCCCCAAGGTGCAGG	2.88*
C1Qc	Complement C1q C Chain	Host gene	Immunity	F: CGCCGGTGAGTGGAATCTA R: CTTCTCCATCATGTGGTGTGCTA P: ACCTCCAAACATAGAAGAG	1.96
C3	Complement factor 3	Host gene	Immunity	F: ATTGGCCTGTCCAAAACACA R: AGCTTCAGATCAAGGAAGAAGTTC P: TGGAATCTGTGTGTCTGAACCCC	2.04
C7	Complement factor 7	Host gene	Immunity	F: ACCTCTGTCCAGCTCTGTGTC R: GATGCTGACCACATCAAACTGC P: AACTACCAGACAGTGCTG	2.43*
CA054694	Mitochondrial ribosomal protein (VAR1)	Host gene	VDD	F: CCACCTGAGGTACTGAAGATAAGACA R: TTAAGTCCTCCTTCCTCATCTGGTA P: TCTACCAGGCCTTAAAG	1.96
CA4	Carbonic anhydrase 4	Host gene	Growth	F: GGTCATTTTGGTTTTGTACACAGTCT R: CCTAGATATAGCTATCCACGTACTCACCTA P: TGATACGTGGTATAGAAAAG	1.98
CCL4	Chemokine (C-C motif) ligand 4	Host gene	Immunity	F: TCTCTTCATTGCAACAATCTGCTT R: ACAGCAGTCCACGGGTACCT P: CTACGCAGCAGCATT	1.98
CD4	cluster of differentiation 4	Host gene	Immunity/ MRS	F: CATTAGCCTGGGTGGTCAAT R: CCCTTTCTTTGACAGGGAGA P: CAGAAGAGAGAGCTGGATGTCTCCG	1.90

Symbol	Infectious agent/ Host gene name	Assay Class	Type/ Function	Forward Primer Sequence (5'-3'), Reverse Primer Sequence (5'-3'), Probe Sequence (FAM-5'-3'-MGB)	Amplification Factor
CD83	cluster of differentiation 83	Host gene	Immunity	F: GTGGCGGCATTGCTGATATT R: CTTGTGGATACTTCTTACTCCTTTGCA P: CACCATCAGCTATGTCATCC	2.04
CD8a	cluster of differentiation 8 subunit α	Host gene	Immunity	F: ACACCAATGACCACAACCATAGAG R: GGGTCCACCTTTCCCACTTT P: ACCAGCTCTACAACTGCCAAGTCGTGC	1.99
CD9	cluster of differentiation 9	Host gene	Immunity	F: CTTGATCTGTTTCATGAGGATGCT R: ACCTCCTCCTGTTGCTCCTAGA P: CAGCACACCAGGGC	2.00
CFTR-I	Cystic fibrosis transmembrane conductance regulator I	Host gene	Osmoregulation	F: GAGCTGTCAGAGAGGAAGTTCTCA R: GCAGCGACTCTTCAACCTGAT P: TGGTGCCCGAGGAC	1.95
CIRBP	Cold-inducible RNA-binding protein	Host gene	Stress/ Osmoregulation	F: GGGATGGTGGAGACCTTCTCT R: CAGAACCCACAGCGATCCTAA P: TTCTCTAGTCCACTGGGCT	N/A*
COMMD 7	COMM domain-containing protein 7	Host gene	Immunity/MRS	F: CAAAGCCAGTATGGACTGTTTCAG R: TTGTTTTCTGCTGCCCCTCTA P: ACCTGATCGCCAGTAGCATGAGCATGTAC	1.98
COX6B1	Cytochrome c oxidase subunit 6B1	Host gene	Stress/ Osmoregulation	F: GCCCCGTGTGACTGGTATAAG R: TCGTCCCATTTCTGGATCCA P: TCTACAAATCACTGTGCCC	2.00
DEXH	ATP-dependent RNA helicase	Host gene	VDD	F: CCATAAGGAGGGTGTCTACAATAAGAT R: CTCTCCCCCTTCAGCTTCTGT P: TGGCGCGCTACGTG	1.94
EF-2	Elongation factor 2	Host gene	Hypoxia	F: AGGTCACAGCCGCCCTTAG R: ACACAGTCTCTGTCTGCACACACA P: CGACTGCGTCTCAGGT	1.99
FK506	FK506-binding protein 10 precursor	Host gene	Thermal	F: ACTATGAGAATGCCCCCATCAC R: CTCGTCCAGACCCTCAATCAC P: CCTGGGAGCCAACAA	N/A*
FYB	FYN-T-binding protein	Host gene	Immunity/MRS	F: TGCAGATGAGCTTGTTGTCTACAG R: GCAGTAAAGATCTGCCGTTGAGA P: CTCAACGATGACATCCACAGTCTCCCC	1.92
GAL3	Galectin-3-binding protein precursor	Host gene	VDD	F: TTGTAGCGCCTGTTGTAATCATATC R: TACACTGCTGAGGCCATGGA P: CTTGGCGTGGTGGC	1.99
glut2	Glucose transporter 2	Host gene	Glycolysis	F: GGAACCTTACATCAACTGGCTACA R: GCAGTGGCCAGTAGTAGTCATTACC P: CTGGTATACTACTGAGTCAGG	2.01

Symbol	Infectious agent/ Host gene name	Assay Class	Type/ Function	Forward Primer Sequence (5'-3'), Reverse Primer Sequence (5'-3'), Probe Sequence (FAM-5'-3'-MGB)	Amplification Factor
HBA	Hemoglobin subunit α	Host gene	Hypoxia	F: GCCCTGGCTGACAAATACAGA R: GAGCAGGAACTGGAGTCCAATG P: ACCATCATGAAAGTCC	2.04
hep	Hepcidin	Host gene	Immunity	F: GAGGAGGTTGGAAGCATTGA R: TGACGCTTGAACCTGAAATG P: AGTCCAGTTGGGGAACATCAACAG	2.01
HERC6	Probable E3 ubiquitin-protein ligase HERC6	Host gene	VDD	F: AGGGACAACTTGGTAGACAGAAGAA R: TGACGCACACACAGCTACAGAGT P: CAGTGGTCTCTGTGGCT	1.98
HIF1A_3	Hypoxia-inducible factor 1-alpha	Host gene	Hypoxia	F: CACTACAACTTCTCCTCACTCACTCTGT R: AGCAGCCAAACTATAAGATCACTGATAC P: CTGCCCCTTTATTTGTCTC	2.06
HIF1A_6	hypoxia-inducible factor 1-alpha	Host gene	Hypoxia	F: AGAGGAGGCAGTGCTGTATTCAA R: GGGACAAGGCCCTCCAAT P: AGGGCCCTGACCATG	2.00
HIF1A_7	hypoxia inducible factor 1-alpha	Host gene	Hypoxia	F: TGGCAAATCTGCCTACGAATT R: GCAGGCTCTTGGTCACATGA P: ATCATGCCCTGGACTC	2.11
HSC70	Heat shock cognate 70 protein	Host gene	Stress/ Osmoregulation	F: GGGTCACACAGAAGCCAAAAG R: GCGCTCTATAGCGTTGATTGGT P: AGACCAAGCCTAAACTA	2.02
hsp90a	Heat shock protein 90 alpha	Host gene	Stress/ Osmoregulation	F: ATGACCCTCAGACACACTCCAA R: CCTCATCAATACCCAGTCCTAGCT P: CGCATCTACAGAATGA	1.95
HSP90ab1	Heat shock protein 90 alpha class b	Host gene	Stress/Themal	F: GACACGGTGTTGGGTTGGTT R: TTGCAGTCAACTCTCCATGCA P: TCATGTGCAACATAACAT	1.94
HSP90alike	Heat shock protein 90 alpha	Host gene	Stress/Themal	F: TTGGATGACCCTCAGACACACT R: CGTCAATACCCAGGCCTAGCT P: CCGAATCTACCGGATGAT	2.10
HTA	HIV-1 Tat interactive protein	Host gene	Immunity/MRS	F: CTTGTAACAGTTCGACATGGCTTATT R: TGGTGAAGCATTTCTGTATGTCAA P: TCTGTACTGAGCATCCCCGCACATTACA	1.96
IDH3B	Isocitrate Dehydrogenase 3 Beta	Host gene	Metabolism	F: AGAAATCTCTACCACAGCACTGTATCA R: GGCACGACTCAGGACTGTGA P: TGGATATCTGGCCTGTCAT	1.96
IFI44a	Interferon-induced protein 44 alpha	Host gene	VDD	F: CGGAGTCCAGAGCAGCCTACT R: TCCAGTGGTCTCCCCATCTC P: CGCTGGTCCTGTGTGA	1.99

Symbol	Infectious agent/ Host gene name	Assay Class	Type/ Function	Forward Primer Sequence (5'-3'), Reverse Primer Sequence (5'-3'), Probe Sequence (FAM-5'-3'-MGB)	Amplification Factor
IFIT5	Interferon-induced protein with tetratricopeptide repeats 5	Host gene	VDD	F: CCGTCAATGAGTCCCTACACATT R: CACAGGCCAATTTGGTGATG P: CTGTCTCCAAACTCCCA	2.02
IFNa	Interferon alpha	Host gene	Immunity	F: CGTCATCTGCAAAGATTGGA R: GGGCGTAGCTTCTGAAATGA P: TGCAGCACAGATGTACTGATCATCCA	2.03
IGFBP1	Insulin-like growth factor binding protein-1	Host gene	Growth	F: GGGTCCCTGCCACATTGAG R: TTCCTGCTGAGAGCTGGTTATCT P: CATGCAGCTCTGGAC	N/A*
IgMs	Immunoglobulin	Host gene	Immunity	F: CTTGGCTTGTTGACGATGAG R: GGCTAGTGGTGTTGAATTGG P: TGGAGAGAACGAGCAGTTCAGCA	2.05
IgT	Immunoglobulin tau	Host gene	Immunity	F: CAACACTGACTGGAACAACAAGGT R: CGTCAGCGGTTCTGTTTTGGA P: AGTACAGCTGTGTGGTGCA	2.02
IL-11	Interleukin 11	Host gene	Immunity	F: GCAATCTCTTGCCTCCACTC R: TTGTCACGTGCTCCAGTTTC P: TCGCGGAGTGTGAAAGGCAGA	1.91
IL-15	Interleukin 15	Host gene	Immunity	F: TTGGATTTTGCCCTAACTGC R: CTGCGCTCCAATAAACGAAT P: CGAACAACGCTGATGACAGGTTTTT	2.00
IL-17D	Interleukin 17D	Host gene	Immunity	F: CAACAGAAGTGCGAACGATG R: GATGCCACATCGCATAACAG P: TGGTCGAGTATCTTTCGTGTGTTTGC	2.17
IL-1B	Interleukin 1b	Host gene	Immunity	F: AGGACAAGGACCTGCTCAACT R: CCGACTCCAACTCCAACACTA P: TTGCTGGAGAGTGCTGTGGAAGAA	1.99
IL-8	Interleukin 8	Host gene	Immunity	F: GAGCGGTCAGGAGATTTGTC R: TTGGCCAGCATCTTCTCAAT P: ATGTCAGCGCTCCGTGGGT	1.89
IRF1	Interferon regulatory factor 1	Host gene	Immunity/MRS	F: CAAACCGCAAGAGTTCCTCATT R: AGTTTGGTTGTGTTTTTGCATGTAG P: CTGGCGCAGCAGATA	1.85
JUN	Transcription factor	Host gene	Stress/ Osmoregulation	F: TTGTTGCTGGTGAGAAAACTCAGT R: CCTGTTGCCCTATGAATTGTCTAGT P: AGACTTGGGCTATTTAC	2.15
KRT8	Cyclokeratin-8	Host gene	Immunity/MRS	F: CGATTGAGCGGCTGGATAA R: GCATTGTTTACCTTTGACTTGAATTG P: CCCCCTTCTCTACTCTCTTGCTCACCATTC	1.98

Symbol	Infectious agent/ Host gene name	Assay Class	Type/ Function	Forward Primer Sequence (5'-3'), Reverse Primer Sequence (5'-3'), Probe Sequence (FAM-5'-3'-MGB)	Amplification Factor
LdhaL	L-lactate dehydrogenase A chain-like	Host gene	Hypoxia	F: TTTGTTTAGTGTGTGCGAGAGTTG R: TCCGTGCACTTACGGTTAGTTTT P: CCAGAGCCATTCAGT	2.16
Ldhb	L-lactate dehydrogenase B-A chain-like	Host gene	Hypoxia	F: GTCACTGCTCCCATTTTACACTCTAG R: CCCAAACTCCCTCCCAGATAAC P: CTGTTCTTAGCTTCCC	N/A*
Map3k14	Mitogen-activated protein kinase 14-like	Host gene	Stress/ Osmoregulation	F: GCTCCCTGGGTTCATGGAT R: GCCTCCCTTCAGCAGAGACA P: CCAGCAATAGCTTATG	2.02
MHC1	Major histone compatibility complex 1	Host gene	Immunity	F: GCGACAGGTTTCTACCCCAGT R: TGTCAGGTGGGAGCTTTTCTG P: TGGTGTCCTGGCAGAAAGACGG	2.19
MHCII-B	Major histone compatibility complex clas II	Host gene	Immunity	F: TGCCATGCTGATGTGCAG R: GTCCCTCAGCCAGGTCACT P: CGCCTATGACTTCTACCCCAAACAAAT	1.99
MMP13	Matrix metalloproteinase 13	Host gene	Immune/ Growth	F: GCCAGCGGAGCAGGAA R: AGTCACCTGGAGGCCAAAGA P: TCAGCGAGATGCAAAG	1.99
MMP25	Matrix metalloproteinase 25	Host gene	Immunity/MRS	F: TGCAGTCTTTTCCCCTTGGAT R: TCCACATGTACCCACACCTACAC P: AGGATTGGCTGGAAGGT	2.03
MPDU1	Mannose-P-Dolichol Utilization Defect 1	Host gene	Metabolism	F: TGCTTGACCCCTTGATTATAGCTA R: GACCATAATCTAGAATGAAAACGCATT P: CTTCCTGGTTGTGTTCTG	1.95
Mx_onts	Antiviral protein	Host gene	VDD/MRS	F: CCACTTGCCAGAGCATGGT R: CGTAACTGCCCAGAGTGCAAT P: ATTCCCATGGTGATCCGCTACCTGG	2.01
NFX	Zinc finger NFX1-type	Host gene	VDD	F: CCACTTGCCAGAGCATGGT R: CGTAACTGCCCAGAGTGCAAT P: TGCTCCACCGATCG	1.96
NKA_a3	Na+/K+ ATPase subunit a3	Host gene	Stress/ Osmoregulation	F: GGAGACCAGCAGAGGAACAG R: CCCTACCAGCCCTCTGAGT P: AAGACCCAGCCTGAAATG	2.15
NKA_b1	Na+/K+ ATPase subunit b1	Host gene	Stress/ Osmoregulation	F: CGTCAAGCTGAACAGGATCGT R: CCTCAGGGATGCTTTCATTGGA P: CCTTGGCCTGAAGTTG	1.98
NKAa1-a	Na+/K+ ATPase subunit α-1a	Host gene	Stress/ Osmoregulation	F: TGGAATCAAGGTTATCATGGTCACT R: CCCACACCCTTGGCAATG P: ATCATCCCATCACTGCGA	2.06

Symbol	Infectious agent/ Host gene name	Assay Class	Type/ Function	Forward Primer Sequence (5'-3'), Reverse Primer Sequence (5'-3'), Probe Sequence (FAM-5'-3'-MGB)	Amplification Factor
NKAa1-b	Na+/K+ ATPase subunit α-1b	Host gene	Stress/ Osmoregulation	F: GCCTGGTGAAGAATCTTGAAGCT R: GAGTCAGGGTTCCGGTCTTG P: CCTCCACCATTTGCTCA	2.22*
NKAA1C	Na+/K+ ATPase subunit 1c	Host gene	Stress/ Osmoregulation	F: AGGGAGACGTACTACTAGAAAGCAT R: CAGAACTTAAAATTCCGAGCAGCAA P: ACAACCATGCAAGAACT	2.02
park7	Protein deglycase DJ-1	Host gene	Stress/ Osmoregulation	F: ACTGCAAGCAGCATGATCAACT R: TTGGCCTGTGTATCATAATGAACA P: CCCCACCTACTCAGC	2.19
PCBL	Precerebellin	Host gene	Immunity	F: TGGTGTTGCTTTGCTGTTGT R: GCCACTTTTGGTTTGCTCTC P: ATGGTTGAGACTCAGACGGAGAGTG	2.01
PDIA4	Protein disulfide-isomerase A4	Host gene	Thermal	F: TGAGGTGCAGGACTTTTTTAAGAA R: TCGTTGCTCTGTTTCCTGTGA P: ACATCCTGCCACTGGT	2.02
PgK3	Phosphoglycerate kinase 3	Host gene	Glycolysis	F: GGCAAAGTGCTCCCTAAGTTTC R: TAGAGAGCAGGGCTGGTGCTA P: CACCCTGCGCTTGT	2.09
PRAS	G-protein mRNA	Host gene	Immunity/MRS	F: GCAGGATGAGCAGAGGAAGAA R: GGCCTGGGCAATGTAACACT P: CCCCCTAAAGATGCAG	2.00
RPL31	60S Ribosomal protein L31	Host gene	Stress/Themal	F: GAGTACACGGTCAACATCCACAA R: CGAGGTGCCCTCCTCTTAAA P: CGCATACATGGCGTCT	2.06
RPL6	Ribosomal protein L6	Host gene	Immunity/MRS	F: CGCCACCACAACCAAGGT R: TCCTCAGCCTCTTCTTCTTGAAG P: AGATCCCCAAGACTCTGTCAGACGCCT	1.96
RSAD	Radical S-adenosyl methionine domain-containing protein 2	Host gene	VDD	F: GCCATTGCTGACAATACTGACACT R: GCCATTGCTGACAATACTGACACT P: GGGAAATTAGTCCAATACTGCAAAC	2.04
SAA	Serum amyloid protein a	Host gene	Immunity	F: GGGAGATGATTCAGGGTTCCA R: TTACGTCCCCAGTGGTTAGC P: TCGAGGACACGAGGACTCAGCA	2.01
SCG	secretogranin II [Ctenopharyngodon idella]	Host gene	Immunity/MRS	F: GGATGTGAAGAATCCAACACTGAT R: ACACCACTTCAAACTAGCCATACATT P: 6FAM-CGGCTGTATGTGCACTG-MGBNFQ	1.96
sepw1	Selenoprotein W	Host gene	Stress/ Osmoregulation	F: TGAGGATGAATTCCCAGGTGAT R: AAACCACCCAGAGGTTGAAGGT P: TTGAGATTACTGGTGAAAGC	2.03

Symbol	Infectious agent/ Host gene name	Assay Class	Type/ Function	Forward Primer Sequence (5'-3'), Reverse Primer Sequence (5'-3'), Probe Sequence (FAM-5'-3'-MGB)	Amplification Factor
SERPIN	Serpin H1 precursor	Host gene	Thermal	F: GAGGTCAGCGACCCAAAGAC R: GCCGTAGAGGCGGTTACTGAT P: CGGAACGTCACATGGA	2.02
SHOP21	Hyperosmotic protein 21	Host gene	Stress/ Osmoregulation	F: GCGGTAGTGGAGTCAGTTGGA R: GCTGCTGACGTCTCACATCAC P: CCTGTTGATGCTCAAGG	2.66*
SRK2	Tyrosine-protein kinase SKR2	Host gene	VDD	F: CCAACGAGAAGTTCACCATCAA R: TCATGATCTCATACAGCAAGATTCC P: TGTGACGTGTGGTCCT	2.12
STAT1	Signal transducer and activator of transcription 1-alpha/beta	Host gene	VDD/MRS	F: TGTCACCGTCTCAGACAGATCTG R: TGTTGGTCTCTGTAAGGCAACGT P: AGTTGCTGAAAACCGG	1.90
TCRa	T-cell receptor alpha	Host gene	Immunity	F: ACAGCTTGCCTGGCTACAGA R: TGTCCCCTTTCACTCTGGTG P: CAGCGCACACAAGGCTAATTCG	2.12
TCRb	T-cell receptor beta	Host gene	Immunity	F: TCACCAGCAGACTGAGAGTCC R: AAGCTGACAATGCAGGTGAATC P: CCAATGAATGGCACAAACCAGAGAA	2.11
TF	transferrin	Host gene	Immunity	F: TTCACTGCTGGAAAATGTGG R: GCTGCACTGAACTGCATCAT P: TGGTCCCTGTCATGGTGGAGCA	2.13
TNF	Tumor necrosis factor	Host gene	Immunity	F: CCCACCATACATTGAAGCAGATT R: GGATTGTATTCACCCTCTAAATGGA P: CCGGCAATGCAAAA	2.00
Tuba1a	Tubulin alpha-1A chain	Host gene	Growth	F: CTCTGCTGAGAAGGCCTACCAT R: AGCAGGCGTTGGTGATGTC P: AGCAGCTGTCTGTTGC	1.92
UBE2Q2	Ubiquitin-conjugating enzyme E2 Q2-like	Host gene	Growth	F: GGCAGGACCACTTGAACGTAA R: AGGCCTGCACTGAACCAGAT P: TGCTCATTCGGGTGCG	2.06
VHSV-P10	VHSV-induced protein-10 mRNA	Host gene	VDD	F: GCAAACTGAGAAAACCATCAAGAA R: CCGTCAGCTCCCTCTGCAT P: TGTGGAGAAGTTGCAGGC	1.97
VHSVIP4	VHSV-inducible protein-4	Host gene	VDD	F: TGGCTTCCCACATTGCAA R: CCTCCTCCCCCCTGCAT P: AGATGGAGACAGGAATG	1.88
ZAP7	Tyrosine-protein kinase ZAP-70	Host gene	Immunity/MRS	F: TCACCTCCGGACCTTTCATT R: CCATGTGGGAAGCCTTTTCTT P: 6FAM-TCTTGTATGGTTTTCCTCC-MGBNFQ	2.74*

Symbol	Infectious agent/ Host gene name	Assay Class	Type/ Function	Forward Primer Sequence (5'-3'), Reverse Primer Sequence (5'-3'), Probe Sequence (FAM-5'-3'-MGB)	Amplification Factor
78d16.1	S100 calcium binding protein	Reference Gene	Reference gene	F: GTCAAGACTGGAGGCTCAGAG R: GATCAAGCCCCAGAAGTGTTTG P: AAGGTGATTCCCTCGCCGTCCGA	1.95
COIL-p84	Coiled-coil domain-containing protein 84	Reference Gene	Reference gene	F: GCTCATTTGAGGAGAAGGAGGATG R: CTGGCGATGCTGTTCCTGAG P: TTATCAAGCAGCAAGCC	2.01
MrpL40	39S ribosomal protein L40	Reference Gene	Reference gene	F: CCCAGTATGAGGCACCTGAAGG R: GTTAATGCTGCCACCCTCTCAC P: ACAACAACATCACCA	1.98
ae_hyd	Aeromonas hydrophila	Infectious agent	Bacteria	F: ACCGCTGCTCATTACTCTGATG R: CCAACCCAGACGGGAAGAA P: TGATGGTGAGCTGGTTG	1.03
ae_sal	*Aeromonas salmonicida*	Infectious agent	Bacteria	F: TAAAGCACTGTCTGTTACC R: GCTACTTCACCCTGATTGG P: ACATCAGCAGGCTTCAGAGTCACTG	0.95
c_b_cys	*Candidatus* Branchiomonas cysticola	Infectious agent	Bacteria	F: AATACATCGGAACGTGTCTAGTG R: GCCATCAGCCGCTCATGTG P: CTCGGTCCCAGGCTTTCCTCTCCCA	0.95
fl_psy	*Flavobacterium psychrophilum*	Infectious agent	Bacteria	F: GATCCTTATTCTCACAGTACCGTCAA R: TGTAAACTGCTTTTGCACAGGAA P: AAACACTCGGTCGTGACC	0.86
sch	Gill chlamydia	Infectious agent	Bacteria	F: GGGTAGCCCGATATCTTCAAAGT R: CCCATGAGCCGCTCTCTCT P: TCCTTCGGGACCTTAC	1.09
mo_vis	*Moritella viscosa*	Infectious agent	Bacteria	F: CGTTGCGAATGCAGAGGT R: AGGCATTGCTTGCTGGTTA P: TGCAGGCAAGCCAACTTCGACA	0.96
pch_sal	*Piscichlamydia salmonis*	Infectious agent	Bacteria	F: TCACCCCCAGGCTGCTT R: GAATTCCATTTCCCCCTCTTG P: CAAAACTGCTAGACTAGAGT	0.97
pisck_sal	*Piscirickettsia salmonis*	Infectious agent	Bacteria	F: TCTGGGAAGTGTGGCGATAGA R: TCCCGACCTACTCTTGTTTCATC P: TGATAGCCCCGTACACGAAACGGCATA	0.93
re_sal	*Renibacterium salmoninarum*	Infectious agent	Bacteria	F: CAACAGGGTGGTTATTCTGCTTTC R: CTATAAGAGCCACCAGCTGCAA P: CTCCAGCGCCGCAGGAGGAC	0.96
rlo	Rickettsia-like organism	Infectious agent	Bacteria	F: GGCTCAACCCAAGAACTGCTT R: GTGCAACAGCGTCAGTGACT P: CCCAGATAACCGCCTTCGCCTCCG	0.95

Symbol	Infectious agent/ Host gene name	Assay Class	Type/ Function	Forward Primer Sequence (5'-3'), Reverse Primer Sequence (5'-3'), Probe Sequence (FAM-5'-3'-MGB)	Amplification Factor
te_mar	*Tenacibaculum maritimum*	Infectious agent	Bacteria	F: TGCCTTCTACAGAGGGATAGCC R: CTATCGTTGCCATGGTAAGCCG P: CACTTTGGAATGGCATCG	1.03
vi_ang	*Vibrio anguillarum*	Infectious agent	Bacteria	F: CCGTCATGCTATCTAGAGATGTATTTGA R: CCATACGCAGCCAAAAATCA P: TCATTTCGACGAGCGTCTTGTTCAGC	0.95
vi_sal	*Vibrio salmonicida*	Infectious agent	Bacteria	F: GTGTGATGACCGTTCCATATTT R: GCTATTGTCATCACTCTGTTTCTT P: TCGCTTCATGTTGTGTAATTAGGAGCGA	0.96
ye_ruc_glnA	*Yersinia ruckeri*	Infectious agent	Bacteria	F: TCCAGCACCAAATACGAAGG R: ACATGGCAGAACGCAGAT P: AAGGCGGTTACTTCCCGGTTCCC	1.04
de_sal	*Dermocystidium salmonis*	Infectious agent	Mesomycetozoean	F: CAGCCAATCCTTTCGCTTCT R: GACGGACGCACACCACAGT P: AAGCGGCGTGTGCC	1.01
ic_hof	*Ichthyophonus hoferi*	Infectious agent	Mesomycetozoean	F: GTCTGTACTGGTACGGCAGTTTC R: TCCCGAACTCAGTAGACACTCAA P: TAAGAGCACCCACTGCCTTCGAGAAGA	0.93
sp_des	*Sphaerothecum destructuens*	Infectious agent	Mesomycetozoean	F: GGGTATCCTTCCTCTCGAAATTG R: CCCAAACTCGACGCACACT P: CGTGTGCGCTTAAT	0.99
fa_mar	*Facilispora margolisi*	Infectious agent	Microsporidium	F: AGGAAGGAGCACGCAAGAAC R: CGCGTGCAGCCCAGTAC P: TCAGTGATGCCCTCAGA	0.99
lo_sal	*Loma salmonae*	Infectious agent	Microsporidium	F: GGAGTCGCAGCGAAGATAGC R: CTTTTCCTCCCTTTACTCATATGCTT P: TGCCTGAAATCACGAGAGTGAGACTACCC	1.04
nu_sal	*Nucleospora salmonis*	Infectious agent	Microsporidium	F: GCCGCAGATCATTACTAAAAACCT R: CGATCGCCGCATCTAAACA P: CCCCGCGCATCCAGAAATACGC	0.94
pa_ther	*Paranucleospora theridion*	Infectious agent	Microsporidium	F: CGGACAGGGAGCATGGTATAG R: GGTCCAGGTTGGGTCTTGAG P: TTGGCGAAGAATGAAA	0.93
ce_sha	*Ceratonova shasta*	Infectious agent	Myxozoan	F: CCAGCTTGAGATTAGCTCGGTAA R: CCCCGGAACCCGAAAG P: CGAGCCAAGTTGGTCTCTCCGTGAAAAC	0.93
ku_thy	*Kudoa thyrsites*	Infectious agent	Myxozoan	F: TGGCGGCCAAATCTAGGTT R: GACCGCACACAAGAAGTTAATCC P: TATCGCGAGAGCCGC	1.02

Symbol	Infectious agent/ Host gene name	Assay Class	Type/ Function	Forward Primer Sequence (5'-3'), Reverse Primer Sequence (5'-3'), Probe Sequence (FAM-5'-3'-MGB)	Amplification Factor
my_arc	*Myxobolus arcticus*	Infectious agent	Myxozoan	F: TGGTAGATACTGAATATCCGGGTTT R: AACTGCGCGGTCAAAGTTG P: CGTTGATTGTGAGGTTGG	0.96
my_ins	*Myxobolus insidiosus*	Infectious agent	Myxozoan	F: CCAATTTGGGAGCGTCAAA R: CGATCGGCAAAGTTATCTAGATTCA P: CTCTCAAGGCATTTAT	0.95
pa_kab	*Parvicapsula kabatai*	Infectious agent	Myxozoan	F: CGACCATCTGCACGGTACTG R: ACACCACAACTCTGCCTTCCA P: CTTCGGGTAGGTCCGG	1.02
pa_min	*Parvicapsula minibicornis*	Infectious agent	Myxozoan	F: AATAGTTGTTTGTCGTGCACTCTGT R: CCGATAGGCTATCCAGTACCTAGTAAG P: TGTCCACCTAGTAAGGC	0.95
pa_pse	*Parvicapsula pseudobranchicola*	Infectious agent	Myxozoan	F: CAGCTCCAGTAGTGTATTTCA R: TTGAGCACTCTGCTTTATTCAA P: CGTATTGCTGTCTTTGACATGCAGT	0.91
te_bry	*Tetracapsuloides bryosalmonae*	Infectious agent	Myxozoan	F: GCGAGATTTGTTGCATTTAAAAAG R: GCACATGCAGTGTCCAATCG P: CAAAATTGTGGAACCGTCCGACTACGA	0.98
gy_sal	*Gyrodactylus salaris*	Infectious agent	Platyhelminthes	F: CGATCGTCACTCGGAATCG R: GGTGGCGCACCTATTCTACA P: TCTTATTAACCAGTTCTGC	0.95
na_sal	*Nanophyetus salmincola*	Infectious agent	Platyhelminthes	F: CGATCTGCATTTGGTTCTGTAACA R: CCAACGCCACAATGATAGCTATAC P: TGAGGCGTGTTTTATG	0.95
cr_sal	*Cryptobia salmositica*	Infectious agent	Protozoan	F: TCAGTGCCTTTCAGGACATC R: GAGGCATCCACTCCAATAGAC P: AGGAGGACATGGCAGCCTTTGTAT	0.96
ic_mul	*Ichthyophthirius multifiliis*	Infectious agent	Protozoan	F: AAATGGGCATACGTTTGCAAA R: AACCTGCCTGAAACACTCTAATTTTT P: ACTCGGCCTTCACTGGTTCGACTTGG	1.00
ne_per	*Neoparamoeba perurans*	Infectious agent	Protozoan	F: GTTCTTTCGGGAGCTGGGAG R: GAACTATCGCCGGCACAAAAG P: CAATGCCATTCTTTTCGGA	1.01
sp_sal	*Spironucleus salmonicida*	Infectious agent	Protozoan	F: GCAGCCGCGGTAATTCC R: CGAACTTTTTAACTGCAGCAACA P: ACACGGAGAGTATTCT	0.98
ihnv	Infectious hematopoietic necrosis virus	Infectious agent	Virus	F: AGAGCCAAGGCACTGTGCG R: TTCTTTGCGGCTTGGTTGA P: TGAGACTGAGCGGGACA	0.98

Symbol	Infectious agent/ Host gene name	Assay Class	Type/ Function	Forward Primer Sequence (5'-3'), Reverse Primer Sequence (5'-3'), Probe Sequence (FAM-5'-3'-MGB)	Amplification Factor
ipnv	Infectious pancreatic necrosis virus	Infectious agent	Virus	F: GCAACTTACTTGAGATCCATTATGCT R: GAGACCTCTAAGTTGTATGACGAGGTCTCT P: CGAGAATGGGCCAGCAAGCA	0.86
isav7	Infectious salmon anemia virus	Infectious agent	Virus	F: CAGGGTTGTATCCATGGTTGAAATG R: GTCCAGCCCTAAGCTCAACTC P: CTCTCTCATTGTGATCCC	0.89
isav8	Infectious salmon anemia virus	Infectious agent	Virus	F: TGGGCAATGGTGTATGGTATGA R: GAAGTCGATGAACTGCAGCGA P: CAGGATGCAGATGTATGC	1.00
pspv	Pacific salmon parvovirus	Infectious agent	Virus	F: CCCTCAGGCTCCGATTTTTAT R: CGAAGACAACATGGAGGTGACA P: CAATTGGAGGCAACTGTA	0.93
pmcv	Piscine myocarditis virus	Infectious agent	Virus	F: AGGGAACAGGAGGAAGCAGAA R: CGTAATCCGACATCATTTTGTGA P: TGGTGGAGCGTTCAA	1.00
prv	Piscine orthoreovirus	Infectious agent	Virus	F: TGCTAACACTCCAGGAGTCATTG R: TGAATCCGCTGCAGATGAGTA P: CGCCGGTAGCTCT	1.01
sav	Salmon alphavirus 1, 2, and 3	Infectious agent	Virus	F: CCGGCCCTGAACCAGTT R: GTAGCCAAGTGGGAGAAAGCT P: TCGAAGTGGTGGCCAG	0.95
omv	Salmonid herpesvirus / Oncorhynchus masou herpes virus	Infectious agent	Virus	F: GCCTGGACCACAATCTCAATG R: CGAGACAGTGTGGCAAGACAAC P: CCAACAGGATGGTCATTA	0.98
ver	Viral encephalopathy and retinopathy virus	Infectious agent	Virus	F: TTCCAGCGATACGCTGTTGA R: CACCGCCCGTGTTTGC P: AAATTCAGCCAATGTGCCCC	0.94
ven	Viral erythrocytic necrosis virus	Infectious agent	Virus	F: CGTAGGGCCCCAATAGTTTCT R: GGAGGAAATGCAGACAAGATTTG P: TCTTGCCGTTATTTCCAGCACCCG	0.93
vhsv	Virus Viral hemorrhagic septicemia virus	Infectious agent	Virus	F: AAACTCGCAGGATGTGTGCGTCC R: TCTGCGATCTCAGTCAGGATGAA P: TAGAGGGCCTTGGTGATCTTCTG	0.95

Table 2.2: Forty-six infectious agents detection results among the entire study population of juvenile Chinook salmon (*Oncorhynchus tshawytscha*) (N = 315) (47 assays in total, two assays were used for infectious salmon anemia virus). The prevalence was the number of positive detections divided by total sample size (N = 315). Limit of detection (LOD, 95% detected <Ct) was defined in Miller *et al.*, (2016). The percentage beyond LOD was the number of positive detections beyond LOD divided by the number of total positive detections. Agents with 100% detection above LOD were excluded in any analysis.

Scientific Name	Type	Organism Abbreviation	Prevalence (%)	Load(Ct) (Mean±SD)	LOD	%beyond LOD
Aeromonas hydrophila	Bacteria	ae_hyd	-	-	-	-
Aeromonas salmonicida	Bacteria	ae_sal	-	-	-	-
Candidatus Branchiomonas cysticola	Bacteria	c_b_cys	80	19.12 ± 5.84	26.9	20.24
Flavobacterium psychrophilum	Bacteria	fl_psy	5.08	29.64 ± 1.42	28.3	75
Moritella viscosa	Bacteria	mo_vis	-	-	-	-
Piscichlamydia salmonis	Bacteria	pch_sal	0.95	8.53 ± 0.24	29.2	0
Piscirickettsia salmonis	Bacteria	pisck_sal	-	-	-	-
Renibacterium salmoninarum	Bacteria	re_sal	0.63	21.3 ± 11.63	26	50
Rickettsia-like organism	Bacteria	rlo	6.98	26.17 ± 3.99	26.5	59.09
Gill chlamydia	Bacteria	sch	19.68	29.22 ± 2.65	27.7	77.42
Tenacibaculum maritimum	Bacteria	te_mar	9.84	25.78 ± 3.59		0
Vibrio anguillarum	Bacteria	vi_ang	-	-	-	-
Vibrio salmonicida	Bacteria	vi_sal	-	-	-	-
Yersinia ruckeri	Bacteria	ye_ruc_glnA	-	-	-	-
Dermocystidium salmonis	Mesomycetozoean	de_sal	-	-	-	-
Ichthyophonus hoferi	Mesomycetozoean	ic_hof	12.7	22.21 ± 6.07	25.4	45
Sphaerothecum destructuens	Mesomycetozoean	sp_des	1.59	24.9 ± 4.22	27	40
Facilispora margolisi	Microsporidium	fa_mar	9.84	28.52 ± 6.02	29.1	70.97
Loma salmonae	Microsporidium	lo_sal	21.27	21.68 ± 7.24	26.1	47.76
Nucleospora salmonis	Microsporidium	nu_sal	0.95	28.76 ± 4.03	25.3	66.67
Paranucleospora theridion	Microsporidium	pa_ther	64.13	26.62 ± 3.59	28.6	41.09
Ceratonova shasta	Myxozoan	ce_sha	24.44	21.86 ± 5.09	28.2	15.58
Kudoa thyrsites	Myxozoan	ku_thy	3.17	24.11 ± 3.48	26.2	40
Myxobolus arcticus	Myxozoan	my_arc	13.02	19.13 ± 6.71	26.9	17.07
Myxobolus insidiosus	Myxozoan	my_ins	0.32	30.15 ± NA	26.6	100
Parvicapsula kabatai	Myxozoan	pa_kab	2.86	24.82 ± 2.92	27	44.44
Parvicapsula minibicornis	Myxozoan	pa_min	38.41	18.55 ± 5.49	28.7	4.13
Parvicapsula pseudobranchicola	Myxozoan	pa_pse	56.51	21.5 ± 3.74	25.7	28.09
Tetracapsuloides bryosalmonae	Myxozoan	te_bry	8.25	21.42 ± 6.54	25.4	38.46
Gyrodactylus salaris	Platyhelminthes	gy_sal	-	-	-	-
Nanophyetus salmincola	Platyhelminthes	na_sal	1.27	21.35 ± 2.21	25.8	0
Cryptobia salmositica	Protozoan	cr_sal	-	-	-	-
Ichthyophthirius multifiliis	Protozoan	ic_mul	3.49	29.84 ± 2.64	25.2	100

Scientific Name	Type	Organism Abbreviation	Prevalence (%)	Load(Ct) (Mean±SD)	LOD	%beyond LOD
Neoparamoeba perurans	Protozoan	ne_per	1.9	19.24 ± 5.6	26.9	16.67
Spironucleus salmonicida	Protozoan	sp_sal	-	-	-	-
Infectious hematopoietic necrosis virus	Virus	ihnv	-	-	-	-
Infectious pancreatic necrosis virus	Virus	ipnv	-	-	-	-
Infectious salmon anemia virus	Virus	isav7	-	-	-	-
Infectious salmon anemia virus	Virus	isav8	-	-	-	-
Salmonid herpesvirus / Oncorhynchus masou herpes virus	Virus	omv	-	-	-	-
Piscine myocarditis virus	Virus	pmcv	0.32	30.35 ± NA	26.3	100
Piscine orthoreovirus	Virus	prv	5.08	21.46 ± 7.69	25.4	43.75
Pacific salmon parvovirus	Virus	pspv	-	-	-	-
Salmon alphavirus 1, 2, and 3	Virus	sav	-	-	-	-
Viral erythrocytic necrosis virus	Virus	ven	13.97	25.27 ± 4.42	25.4	75
Viral encephalopathy and retinopathy virus	Virus	ver	0.95	21.23 ± 6.62	26.4	33.33
Virus Viral hemorrhagic septicemia virus	Virus	vhsv	0.32	23.13	31.8	0

Table 2.3: Summary table of infectious agent detection results of juvenile Chinook salmon (*Oncorhynchus tshawytscha*) captured by DFO marine sampling program from 2012 to 2014, grouped by natal groups.

Natal region group	Sample Size (N)	Number of Infectious agents detected (n)	Mean Richness	Richness SD	Mean RIB	RIB SD
Fraser	139	21	4.17	1.52	1.56	0.77
WCVI	72	20	3.96	1.46	1.37	0.83
ECVI	44	17	3.57	1.50	1.46	0.86
Mainland	27	17	3.33	1.62	1.37	0.82
Columbia	23	21	5.39	1.47	2.37	0.89
Washington	10	13	3.60	1.51	1.34	0.88

Table 2.4: Summary table of infectious agent detection results of juvenile Chinook salmon (*Oncorhynchus tshawytscha*) captured by DFO marine sampling program from 2012 to 2014, grouped by sampling periods.

Sampling period	Sample Size (N)	Number of Infectious agents detected (n)	Mean Richness	Richness SD	Mean RIB	RIB SD
2012-Summer	24	13	4.29	1.37	1.67	0.72
2013-Winter	67	20	3.75	1.57	1.40	0.86
2013-Summer	29	23	5.38	1.40	2.21	0.96
2013-Fall	65	20	3.75	1.58	1.46	0.89
2014-Summer	109	20	3.79	1.39	1.43	0.74
2014-Fall	21	17	5.00	1.58	1.70	0.76

Table 2.5: ANOVA results of natal group and sampling period effects on blood plasma parameters of juvenile Chinook salmon *(Oncorhynchus tshawytscha)* Significant results are in **bold**. (Significant level $p<0.05$)

		Natal group		Sampling Period	
	Mean±SD	$F_{5,202}$	p	$F_{5,202}$	p
Lactate (mmol L^{-1})	13.4±3.2	8.738	**<0.001**	15.786	**<0.001**
Glucose (mmol L^{-1})	3.3±1.2	7.104	**<0.001**	1.763	0.122
Sodium (mmol L^{-1})	171.1±14.7	9.818	**<0.001**	6.289	**<0.001**
Choride (mmol L^{-1})	155±17	6.281	**<0.001**	5.352	**<0.001**
Osmolality (mOsm kg^{-1})	373±31	9.386	**<0.001**	6.340	**<0.001**

Table 2.6: Summary for the Redundancy analysis (RDA) of gill gene expression (a) and liver gene expression (b) of juvenile Chinook salmon *(Oncorhynchus tshawytscha)* (model: gill/liver gene expression matrix ~ dynamic array ID + sampling period + natal group + infectious agent matrix including all agents with more than five detections). Significant p values are in **bold**.

(a) Gill gene expression data

Variable	DF	Variance	F	P
dynamic array ID	3	19.522	37.133	**<0.001**
sampling period	5	3.815	4.3536	**<0.001**
natal group	5	2.032	2.3196	**<0.001**
infectious agent	19	6.717	2.017	**<0.001**
Residual	262	45.914		

(b) Liver gene expression data

Variable	DF	Variance	F	P
dynamic array ID	3	16.444	27.547	**<0.001**
sampling period	5	4.959	4.985	**<0.001**
natal group	5	2.494	2.507	**<0.001**
infectious agent	19	6.339	1.677	**<0.001**
Residual	230	45.764		

Table 2.7: Summary of histopathological results of thirty-three histology samples that were positive for at least one of the four target infectious agents (*Ceratonova shasta, Parvicapsula minibicornis, Paranucleospora theridion*, and Piscine orthoreovirus (PRV)) among juvenile Chinook salmon *(Oncorhynchus tshawytscha)*. Sample number with * indicates they are the only individuals with images of hematoxylin and eosin (H&E) staining slices or In-Situ Hybridization (ISH) staining slices taken. Scores were assigned by the severity of the lesions in the host tissue (1-mild, 2-moderate, 3-severe). Scores in **bold red** were the lesions that were highly likely to be caused by the target agent. Abbreviations: GIT – gastrointestinal system, CNS – central nerve system.

Target Agent	Fish #	Heart	Liver	Spleen	Kidney	Pancreas	GIT	CNS	Gills	Skin/Muscle
C. shasta	B5041	-	-	1	1	-	-	-	-	-
C. shasta	B5061	-	1	1	2	-	-	-	-	-
C. shasta	B5066*	-	-	2	2	2	**2**	-	1	-
C. shasta	B5077	-	-	1	1	-	-	-	-	-
C. shasta	B5079	-	-	2	1	-	-	-	1	-
C. shasta	B5089*	-	-	-	2	-	-	-	**1**	-
PRV	B2157	-	-	-	**1**	-	-	-	-	-
PRV	B2159*	**1**	-	-	**1**	-	-	-	-	-
PRV	B2161	-	-	-	-	-	-	-	-	-
PRV	B2166	-	-	-	-	-	-	-	-	-
PRV	B2170	-	-	**1**	**1**	-	-	-	-	-
PRV	B2176	-	-	**1**	**1**	-	-	-	2	-
PRV	B2211	-	-	1	**1**	-	-	-	1	-
PRV	B5099	-	-	-	-	-	-	-	1	-
PRV	D5324	-	-	-	**1**	-	-	-	-	-
Paranucleospora theridion	B2208	-	-	2	1	-	-	-	1	-
Paranucleospora theridion	B5027	-	-	-	-	-	-	-	1	-
Paranucleospora theridion	B5040	-	-	1	-	-	-	-	1	-
Paranucleospora theridion	D5396	-	-	1	1	-	-	1	1	-
Parvicapsula minibicornis	B5083*	-	-	-	**2**	-	-	-	-	-
Parvicapsula minibicornis	B5101*	-	-	1	**1**	-	-	1	1	-
Parvicapsula minibicornis	B7057	-	1	1	-	-	-	-	-	-
Parvicapsula minibicornis	B7091	-	-	1	1	-	-	-	-	-
Parvicapsula minibicornis	D5322	-	-	-	-	-	-	-	-	-
Parvicapsula minibicornis	D5326	-	-	-	-	-	-	-	-	-
Parvicapsula minibicornis	D4229	-	-	-	-	-	-	-	-	-
Parvicapsula minibicornis	D4235	-	-	-	1	-	-	-	-	-
Parvicapsula minibicornis	D4236	-	1	-	-	-	-	-	-	-
Parvicapsula minibicornis	D4240	-	-	-	-	-	-	-	-	-
Parvicapsula minibicornis	D4274	-	-	-	-	-	-	-	-	-
Parvicapsula minibicornis	D4277	-	-	-	-	-	-	-	-	-
Parvicapsula minibicornis	D4278	-	-	-	-	-	-	-	-	-
Parvicapsula minibicornis	B2163	-	-	2	2	-	-	-	-	-

2.6 Chapter 2 figures

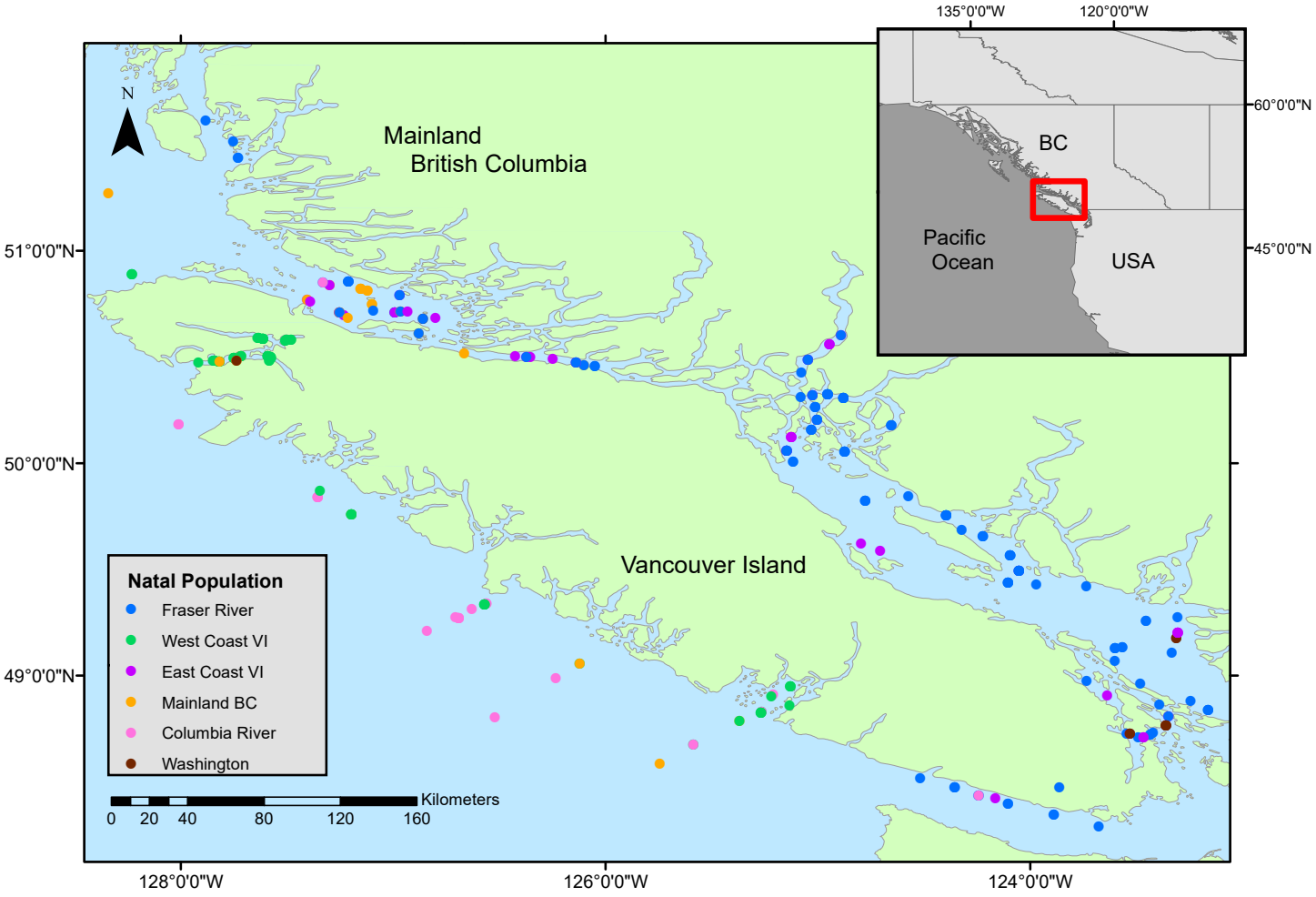


Figure 2.1: Capture locations of juvenile Chinook salmon *(Oncorhynchus tshawytscha)* captured by DFO marine sampling program from 2012 to 2014. Color represents fish natal groups.

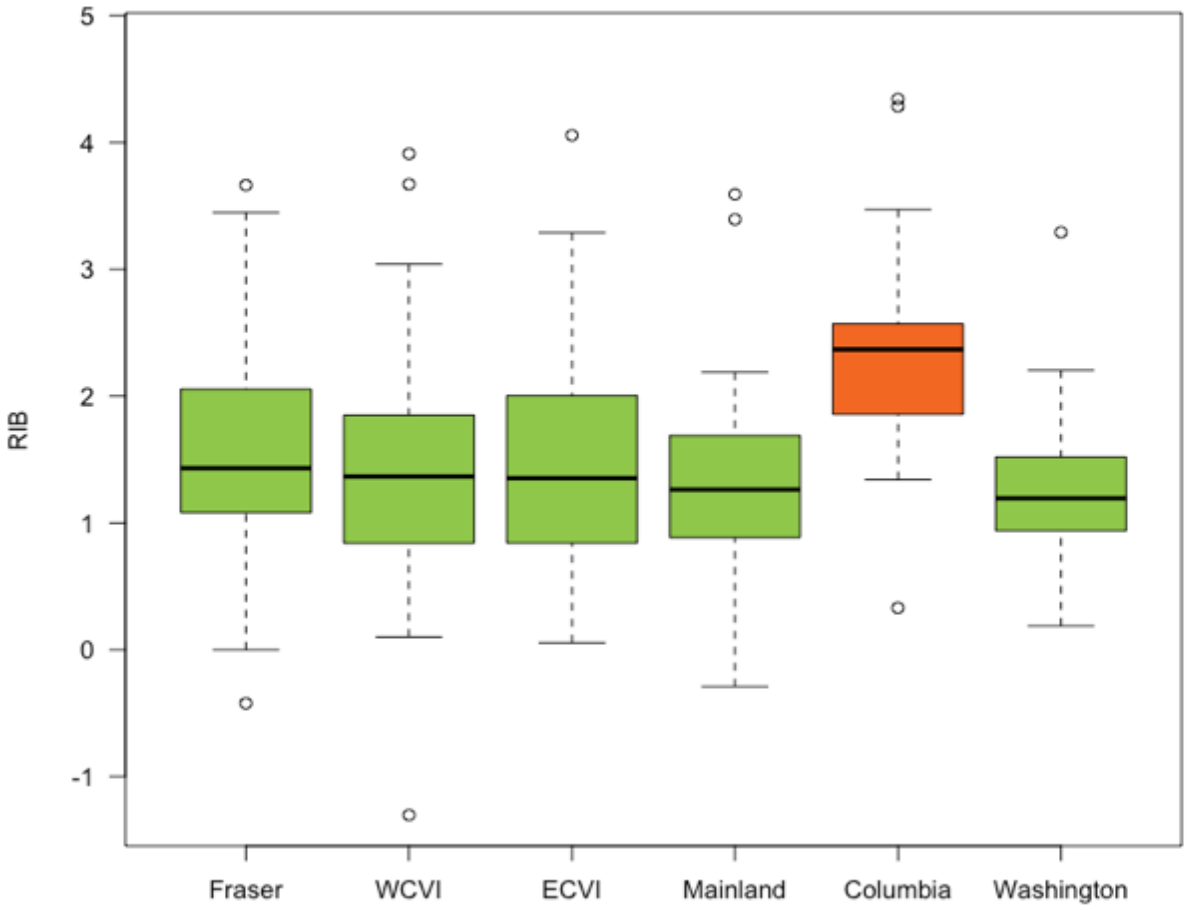
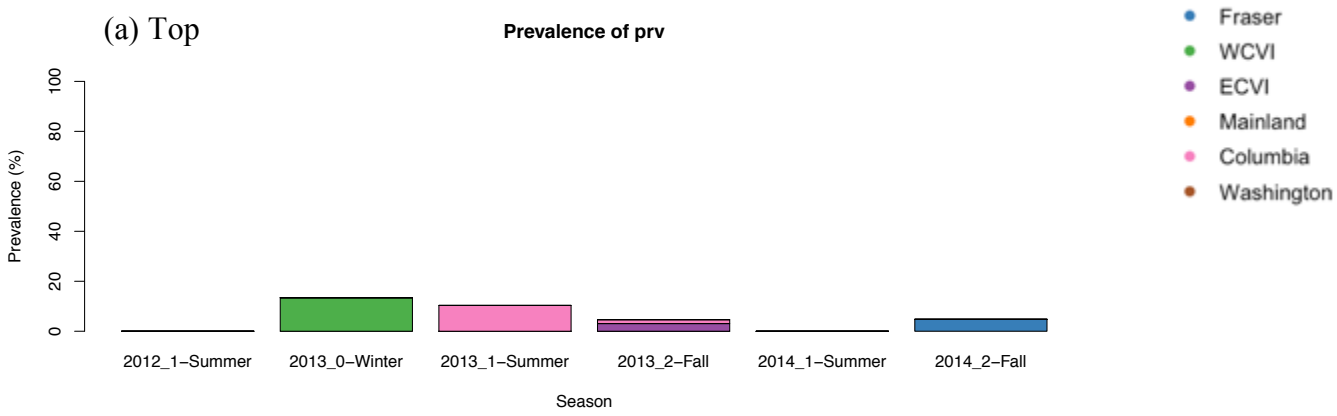
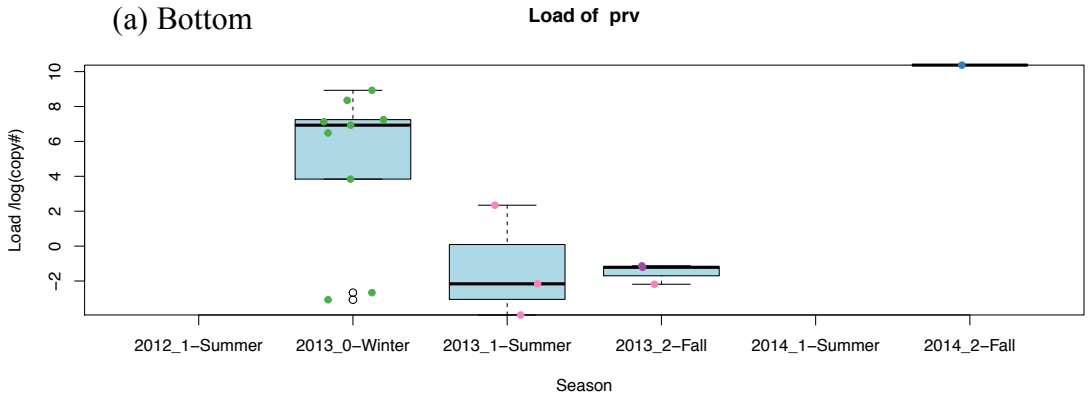


Figure 2.2: Infectious agent Relative Infection Burden (RIB) detected in juvenile Chinook salmon (*Oncorhynchus tshawytscha*) across six natal groups. Colors of boxes represent the result of a Tukey's HSD *post hoc* multiple comparisons test (confidence level 95%).
Natal groups abbreviations are: WCVI: West Coast of Vancouver Island; ECVI: East Coast of Vancouver Island; Fraser: Fraser River system (upper and lower Fraser River and Thompson River); Mainland: Mainland BC (including streams in Northern, Central and Southern mainland BC that were not included in the other five region groups); Columbia: Columbia River system (including Columbia River and Snake River); Washington (including tributaries to the Puget Sound and Strait of Juan de Fuca).

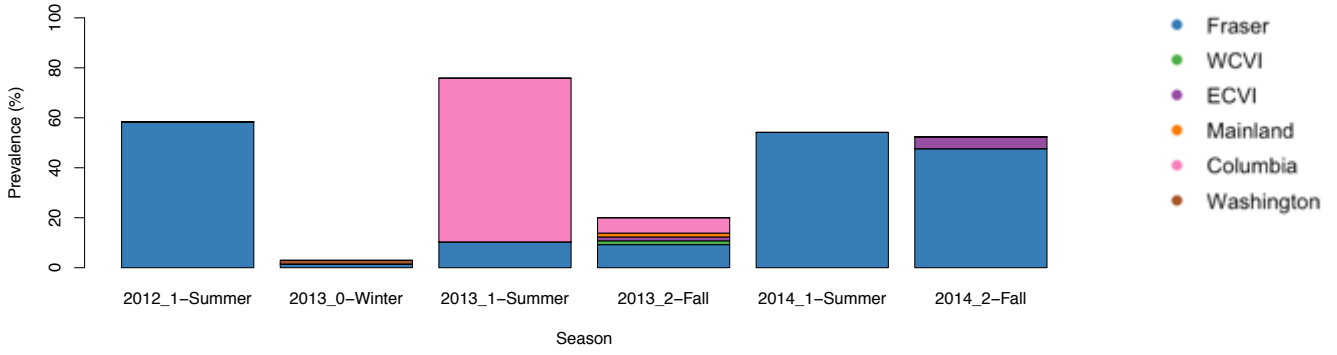
(a) Top



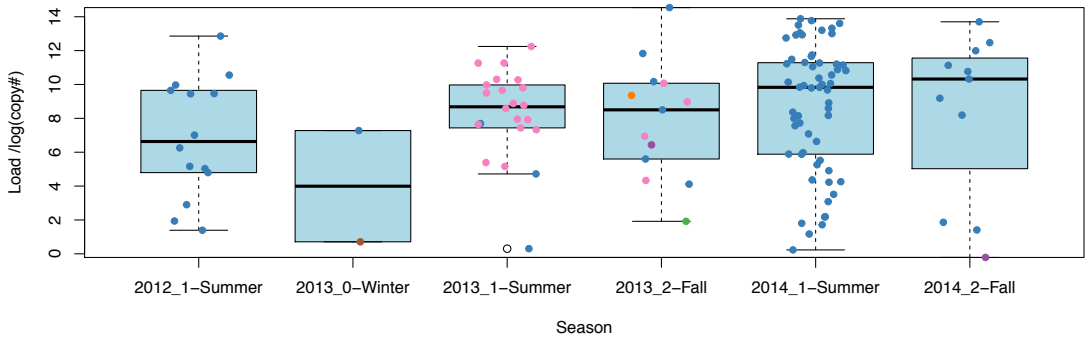
(a) Bottom



(b) Top



(b) Bottom



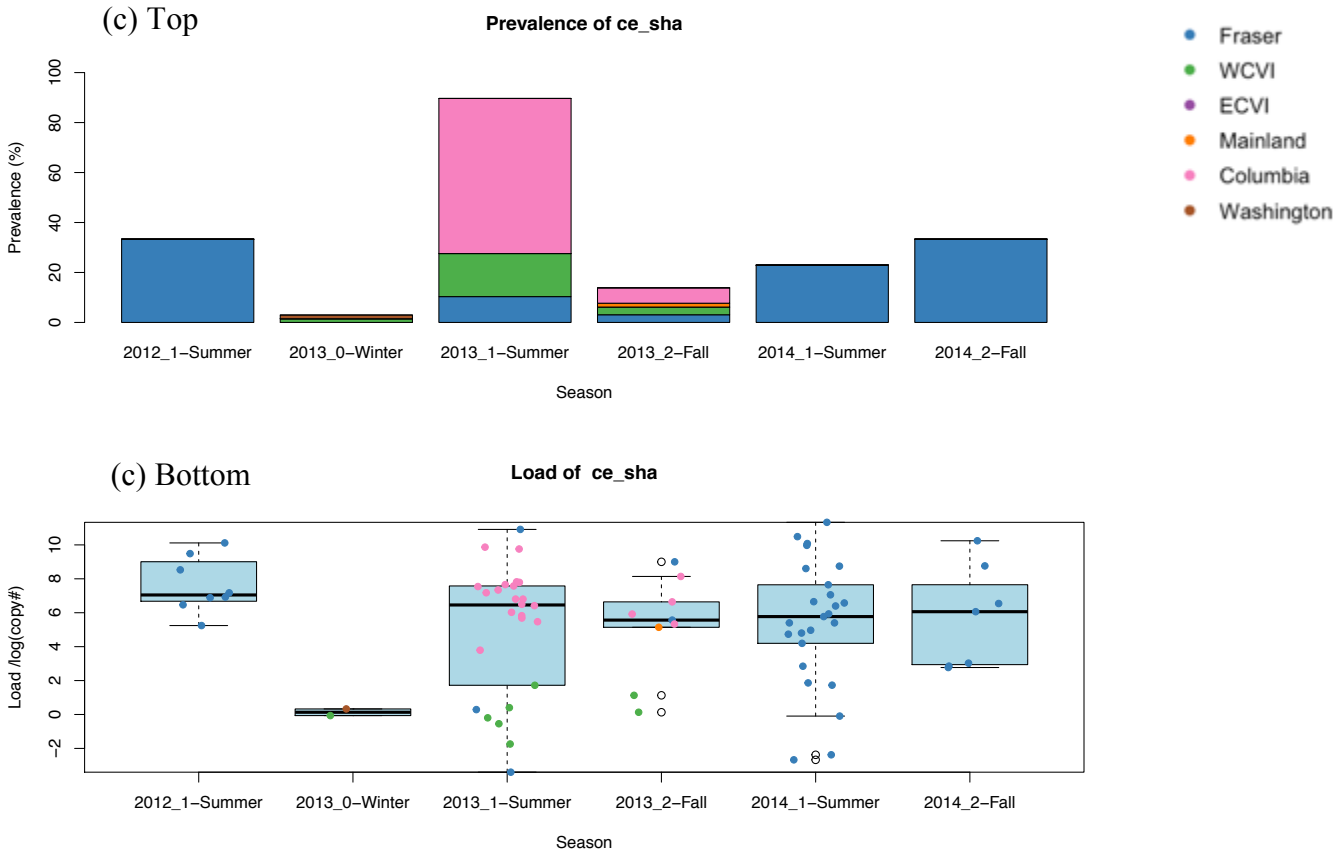


Figure 2.3: Prevalence (top) and load (bottom) of Piscine orthoreovirus (PRV, Figure 2.3a), *Parvicapsula minibicornis* (Figure 2.3b), and *Ceratonova shasta* (Figure 2.3c) among juvenile Chinook salmon (*Oncorhynchus tshawytscha*) captured by DFO marine sampling program from 2012 to 2014. In prevalence barplots (top), the total height of the stacked bars indicates the overall prevalence for the sampling period, and the colors indicate the proportion of the positives that are made up by each natal group. In load boxplots (bottom), the dots represent the load of each positive detection in log copy number, and the colors indicate the natal group. The whiskers are the range of load for the sampling period.
Natal group abbreviations are: WCVI: West Coast of Vancouver Island; ECVI: East Coast of Vancouver Island; Fraser: Fraser River system (upper and lower Fraser River and Thompson River); Mainland: Mainland BC (including streams in Northern, Central and Southern mainland BC that were not included in the other five region groups); Columbia: Columbia River system (including Columbia River and Snake River); Washington (including tributaries to the Puget Sound and Strait of Juan de Fuca).

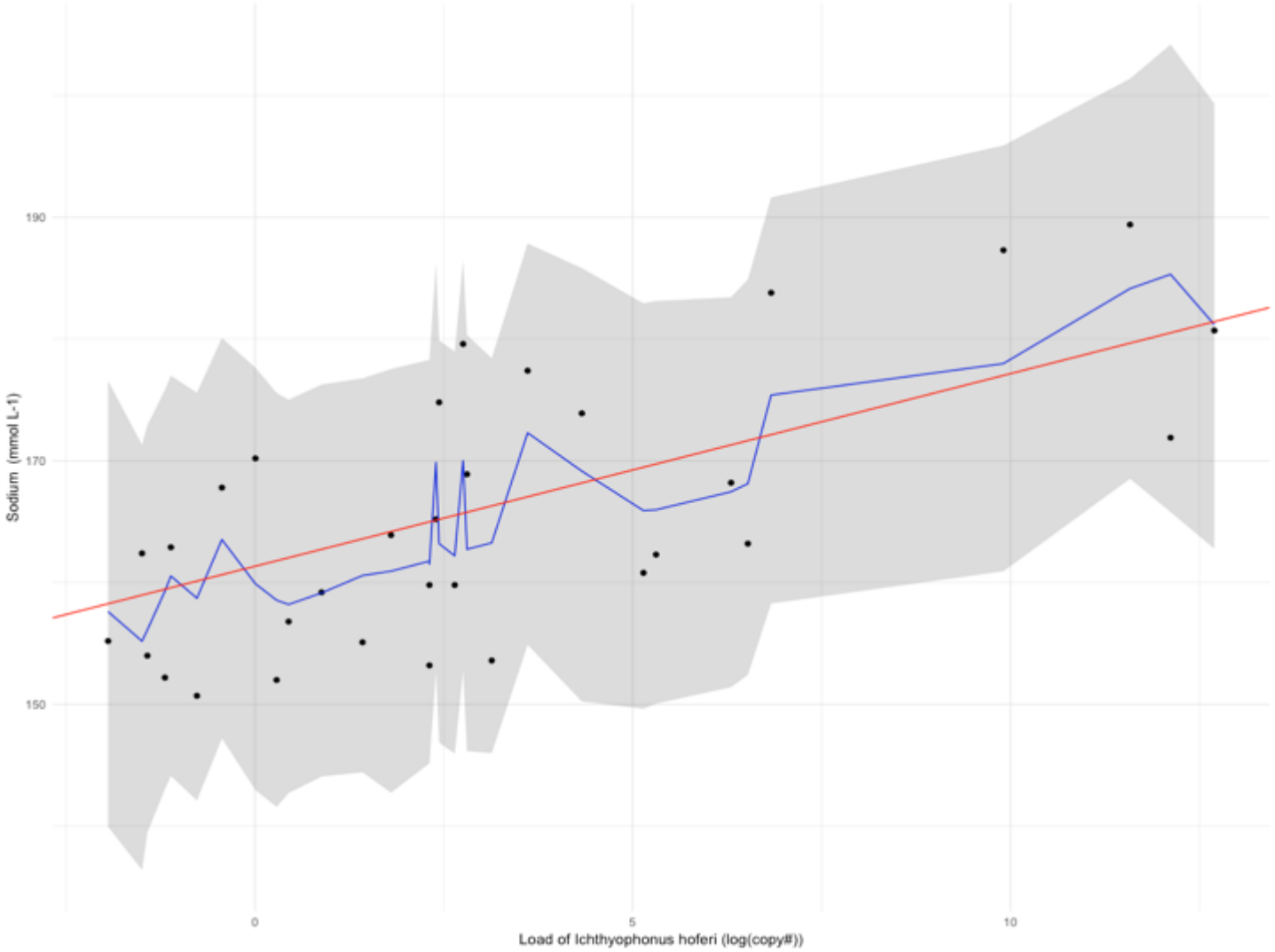
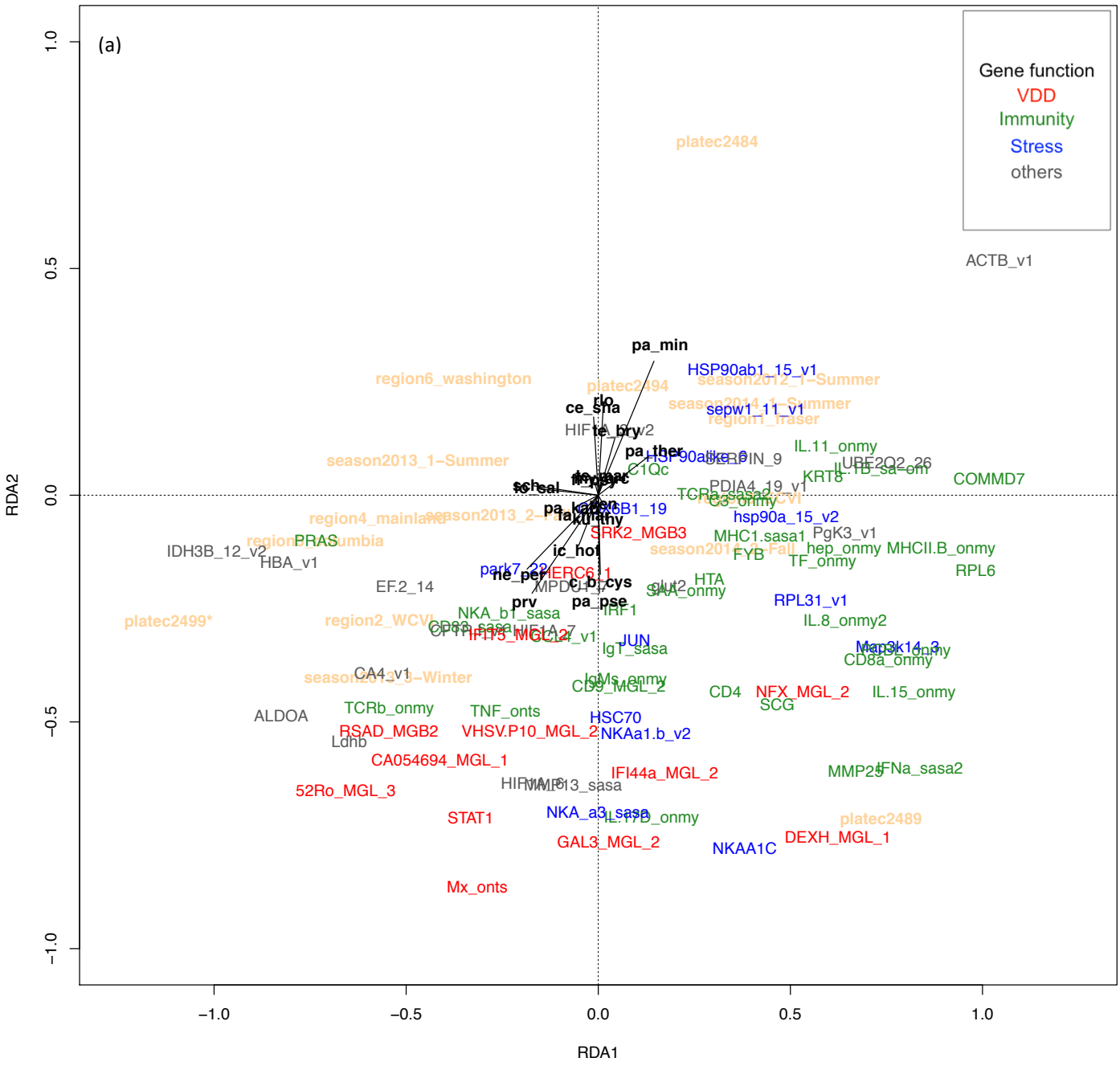


Figure 2.4: The load of *Ichthyophonus hoferi* was positively correlated with plasma sodium level in juvenile Chinook salmon (*Oncorhynchus tshawytscha*)). The red line represents the general linear mix effect model without any adjustments of random effects (Sodium ~ Load of *I. hoferi* + natal groups (random) + sampling period (random), b=1.583, p-adjusted < 0.01). The blue line represents the fitted sodium level by the same model and the grey area represents 95% confidence interval. Fitted values and confidence intervals were obtained by computing simulated distribution of all of the parameters including both fixed and random factors in the model for 999 times.


(a)
Gene function
VDD
Immunity
Stress
others
RDA1
RDA2
1.0
0.5
0.0
−0.5
−1.0
−1.0
−0.5
0.0
0.5
1.0
platec2484
ACTB_v1
pa_min
HSP90ab1_15_v1
region6_washington
platec2494
season2012_1−Summer
season2014_1−Summer
region1_fraser
sepw1_11_v1
ce_sna
HIF1a_bry_v2
pa_ther
IL.11_onmy
season2013_1−Summer
HSP90ab1_9
SLEP1N_9
UBE2Q2_26
C1Qc
IL.1B_sa_om
KRT8
COMMD7
PDIA4_19_v1
TCRa_sasa2
Cs_onmy
region4_mainland
season2013_2−Fall
hsp90a_15_v2
SRK2_MGB3
MHC1.sasa1
PgK3_v1
IDH3B_12_v2
PRAS
HBA_v1
ic_hot
season2014_2−Fall
FYB
hep_onmy
MHCII.B_onmy
TF_onmy
RPL6
park7_22
he_per
IERC6_1
EF.2_14
MPDU1_1
cb_cys
glut2
HTA
SAA_onmy
RPL31_v1
prv
pa_pse
IRF1
IL.8_onmy2
platec2499*
region2_WCVI
NKA_b1_sasa
CD83_sasa
IFI16_MGL_2
JUN
Ig1_sasa
CD8a_onmy
CA4_v1
season2013_3−Winter
IgMs_onmy
CD99_MGL_2
CD4
NFX_MGL_2
IL.15_onmy
SCG
ALDOA
TCRb_onmy
TNF_onts
HSC70
RSAD_MGB2
VHSV.P10_MGL_2
NKAa1.b_v2
Ldhb
CA054694_MGL_1
IFI44a_MGL_2
MMP25
IFNa_sasa2
52Ro_MGL_3
HIF1AP3_sasa
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NKAA1C
DEXH_MGL_1
Mx_onts

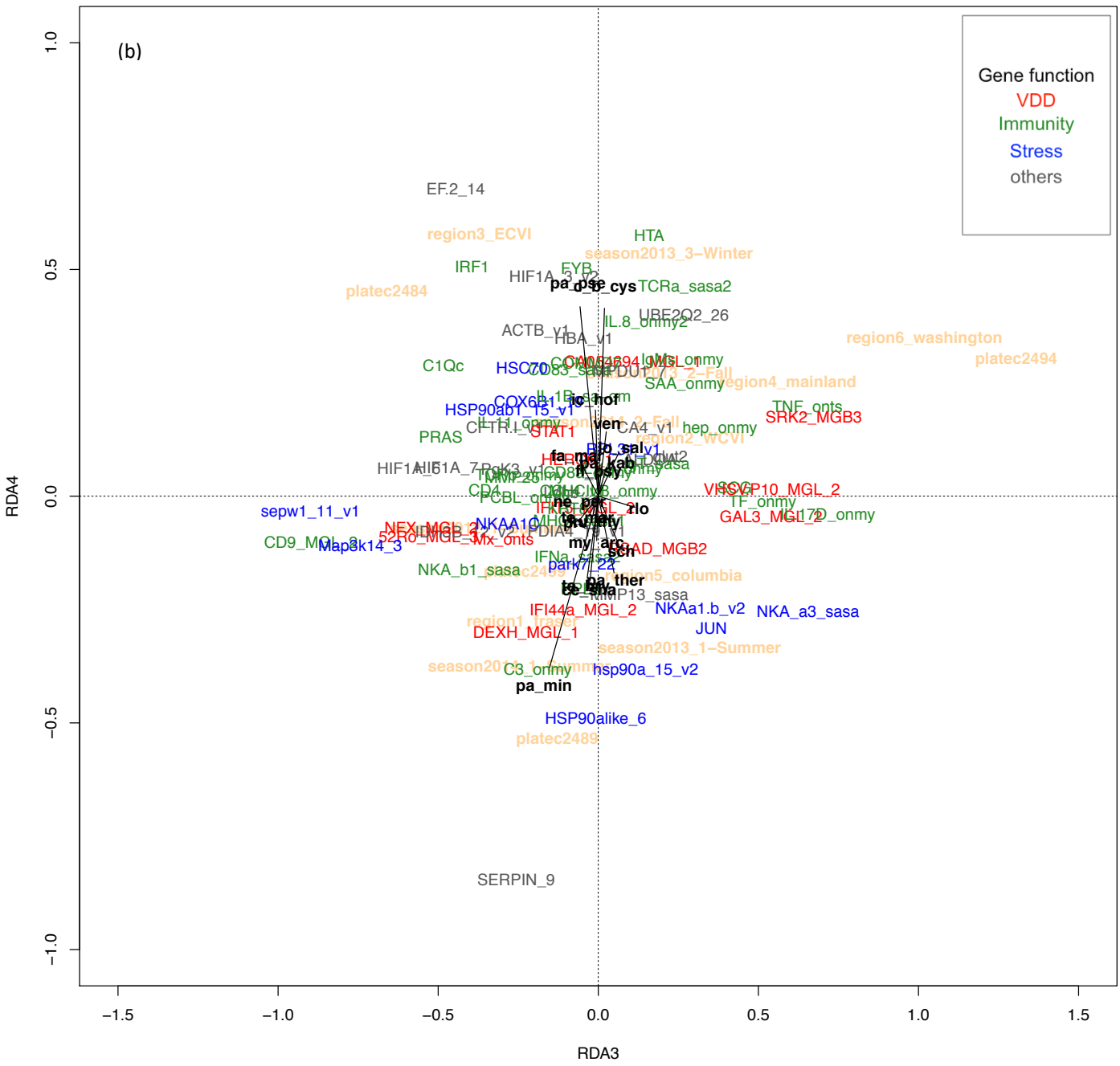
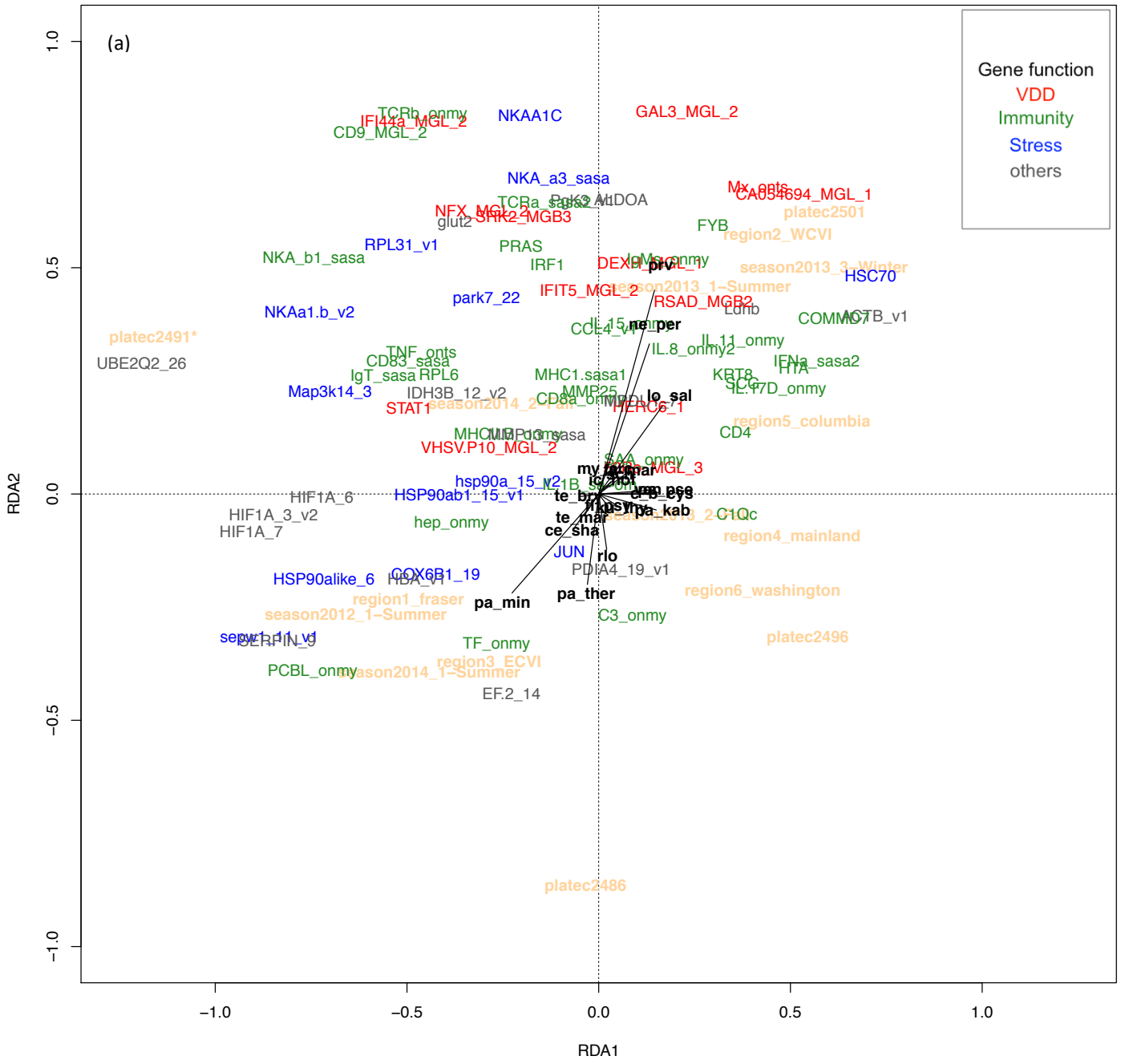


Figure 2.5: Redundancy analyses (RDA) ordination plot made by RDA1-RDA2(a) and RDA3-RDA4(b) of gill gene expression of juvenile Chinook salmon *(Oncorhynchus tshawytscha)* captured by DFO marine sampling program from 2012 to 2014. Model: gill gene expression matrix ~ dynamic array ID + sampling period + natal group + infectious agent matrix including all agents with more than five detections. RDA1, RDA2, RDA3, and RDA4 were all significant in the model. Gill host genes (response variable) are colored by their primary known functions, although many of them actually have multiple functions. Infectious agent (explanatory variable of interest) are shown by black lines. Other explanatory variables including dynamic array ID, sampling period and natal group are in bold light orange.


(a)
Gene function
VDD
Immunity
Stress
others
RDA1
RDA2
1.0
0.5
0.0
−0.5
−1.0
TCRb_onmy
IFI44a_MGL_2
CD9_MGL_2
NKAA1C
GAL3_MGL_2
NKA_a3_sasa
Mx_onts
CA034694_MGL_1
platec2501
FYB
region2_WCVI
NFX_MGL_2
SHK2_MGB3
glut2
RPL31_v1
NKA_b1_sasa
PRAS
IRF1
season2013_3_Winter
HSC70
park7_22
IFIT5_MGL_2
RSAD_MGB2
season2013_1−Summer
NKAa1.b_v2
platec2491*
UBE2Q2_26
TNF_onts
CD83_sasa
IgT_sasa
RPL6
MHC1.sasa1
IL.8_onmy2
IL_11_onmy
IFNa_sasa2
HTA
KRT8
SCG
Map3k14_3
IDH3B_12_v2
MMP25
STAT1
HERC6_1
region5_columbia
CD4
VHSV.P10_MGL_2
hsp90a_15_v2
HSP90ab1_15_v1
HIF1A_6
HIF1A_3_v2
HIF1A_7
hep_onmy
C1Qc
region4_mainland
JUN
PDIA4_19_v1
HSP90alike_6
COX6B1_19
region6_washington
region1_fraser
pa_min
pa_ther
C3_onmy
season2012_1−Summer
platec2496
TF_onmy
region3_ECVI
PCBL_onmy
season2014_1−Summer
EF.2_14
platec2486

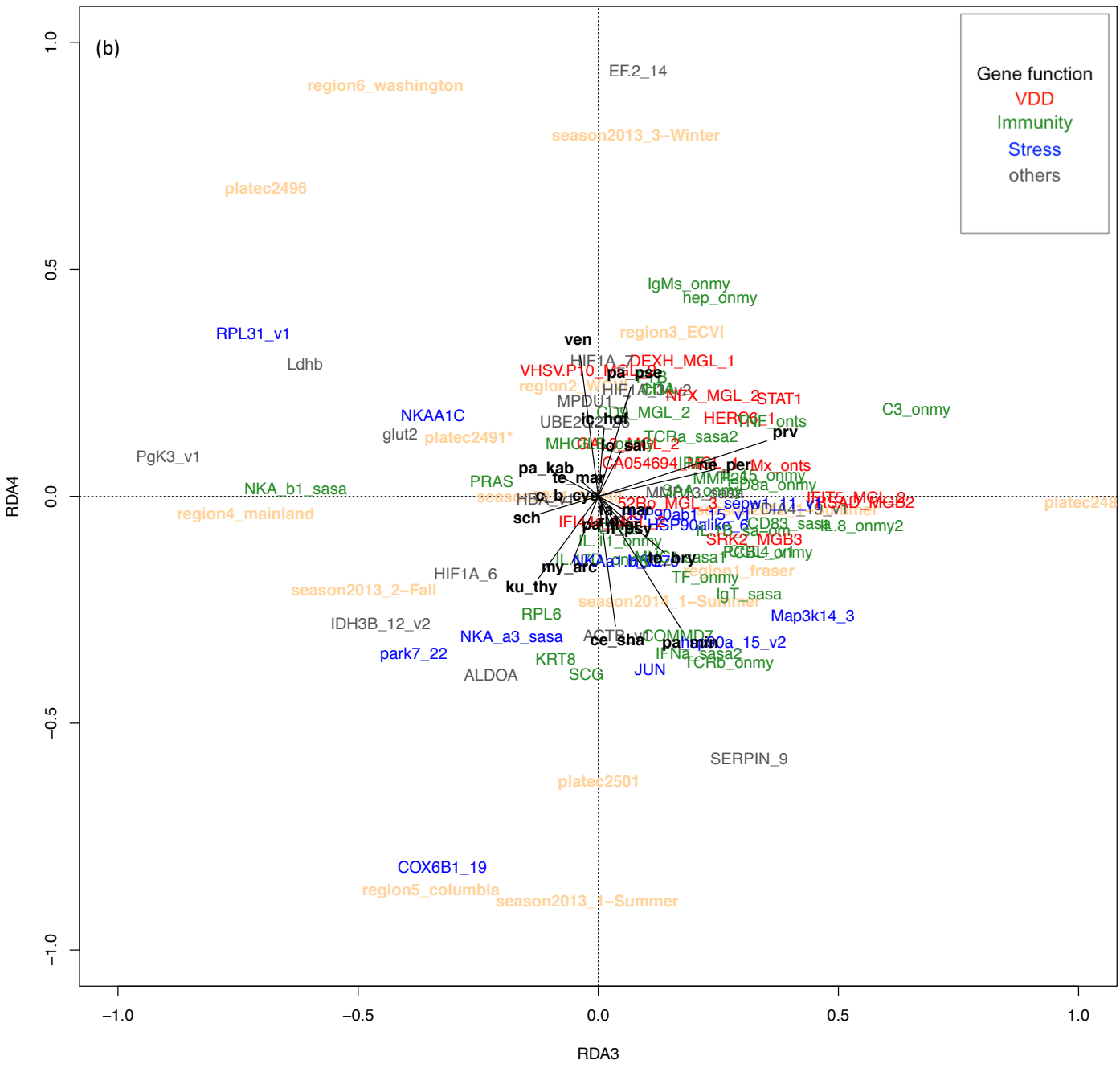


Figure 2.6: Redundancy analyses (RDA) ordination plot made by RDA1-RDA2(a) and RDA3-RDA4(b) of liver gene expression of juvenile Chinook salmon *(Oncorhynchus tshawytscha)* captured by DFO marine sampling program from 2012 to 2014. Model: liver gene expression matrix ~ dynamic array ID + sampling period + natal group + infectious agent matrix including all agents with more than five detections. RDA1, RDA2, RDA3, and RDA4 were all significant in the model. Liver host genes (response variable) are colored by their primary known functions, although many of them actually have multiple functions. Infectious agent (explanatory variable of interest) are shown by black lines. Other explanatory variables including dynamic array ID, sampling period and natal group are in bold light orange.

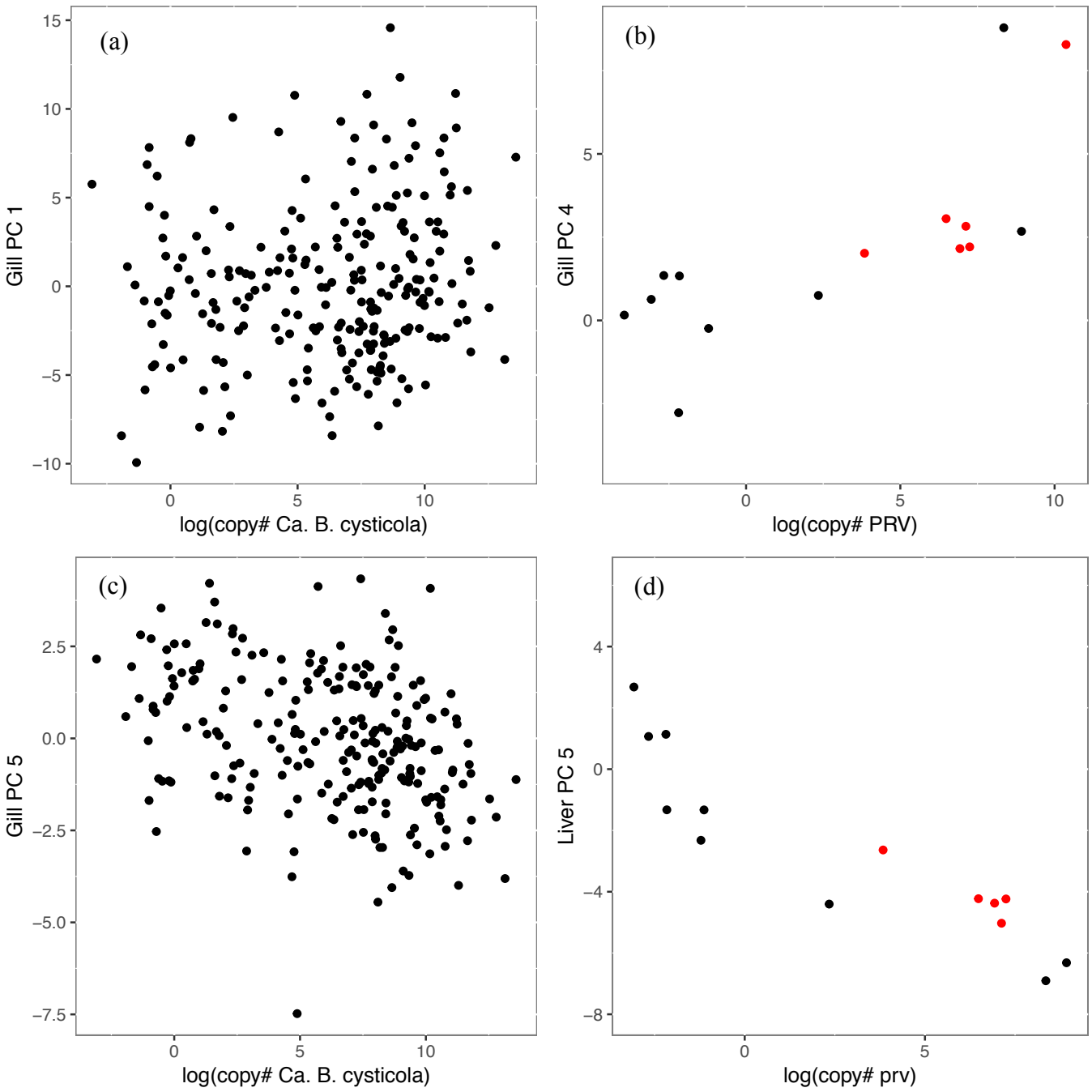


Figure 2.7: Relationships between infectious agent load and host gene expression PC of gill and liver sample of juvenile Chinook salmon *(Oncorhynchus tshawytscha)* captured by DFO marine sampling program from 2012 to 2014. General mixed effect models were applied as follows: PC ~ host gene expression experiment dynamic array ID (random) + sampling period (random) + natal group (random) + infectious agent load (fixed)). Only significant models (p-adjusted <0.05) are presented here. Plot (a) is between gill PC1 and the load of '*Candidatus* Branchiomonas cysticola'(p-adjusted = 0.01). Plot (b) is between gill PC4 and the load of Piscineorthoreovirus(PRV) (p-adjusted = 0.04). Plot (c) is between gill PC5 and the load of '*Ca.* B. cysticola'(p-adjusted < 0.001). Plot (d) is between liver PC5 and the load of PRV (p-adjusted <0.001). Red dots are the individuals used for histopathology analysis and found lesions caused by PRV.

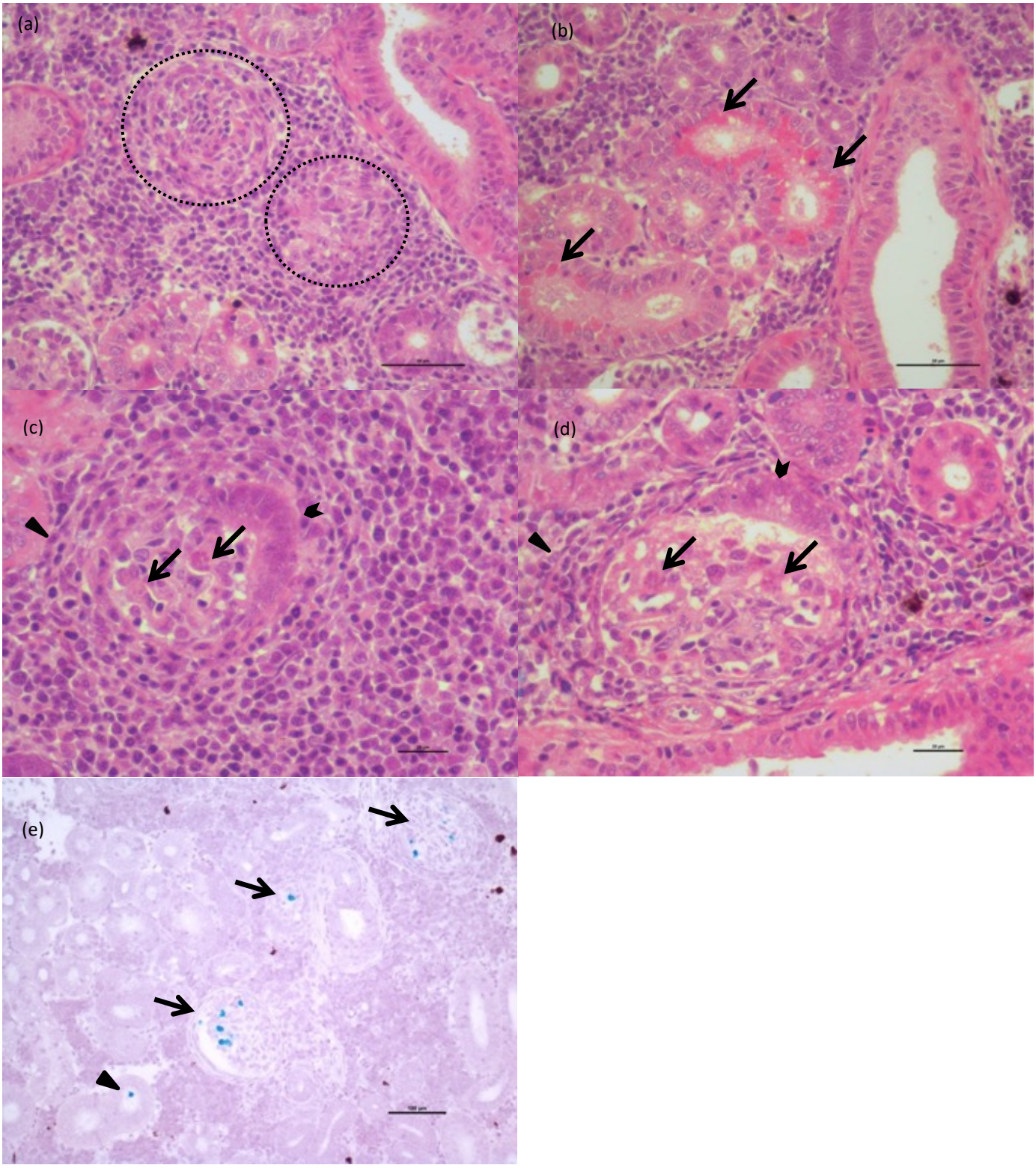


Figure 2.8: Moderate lesions (H&E) and *Parvicapsula minibicornis* detections (In-Situ Hybridization (ISH) staining) in kidney tissues in Fish B5083. (a) two different degrees of Glomerulonephritis: dashed line – the glomerulus on the right is in a more advanced stage of necrosis (moderate), while the one on the left still shows a few morphological features (mild) and generalized interstitial hyperplasia (in forty times magnification); (b) Eosinophilic Lipoproteic droplets (arrows) (in forty times magnification); (c) glomerulonephritis (triangle head), hypertrophy/hyperplasia of Bowman's capsule (arrowhead), *Parvicapsula minibicornis* pre-sporogonic forms (arrows) (in sixty times magnification); (e) *Parvicapsula minibicornis* (green) detection through ISH on both glomeruli (arrows) and in the lumen of renal tubules (arrowheads) (in twenty times magnification).

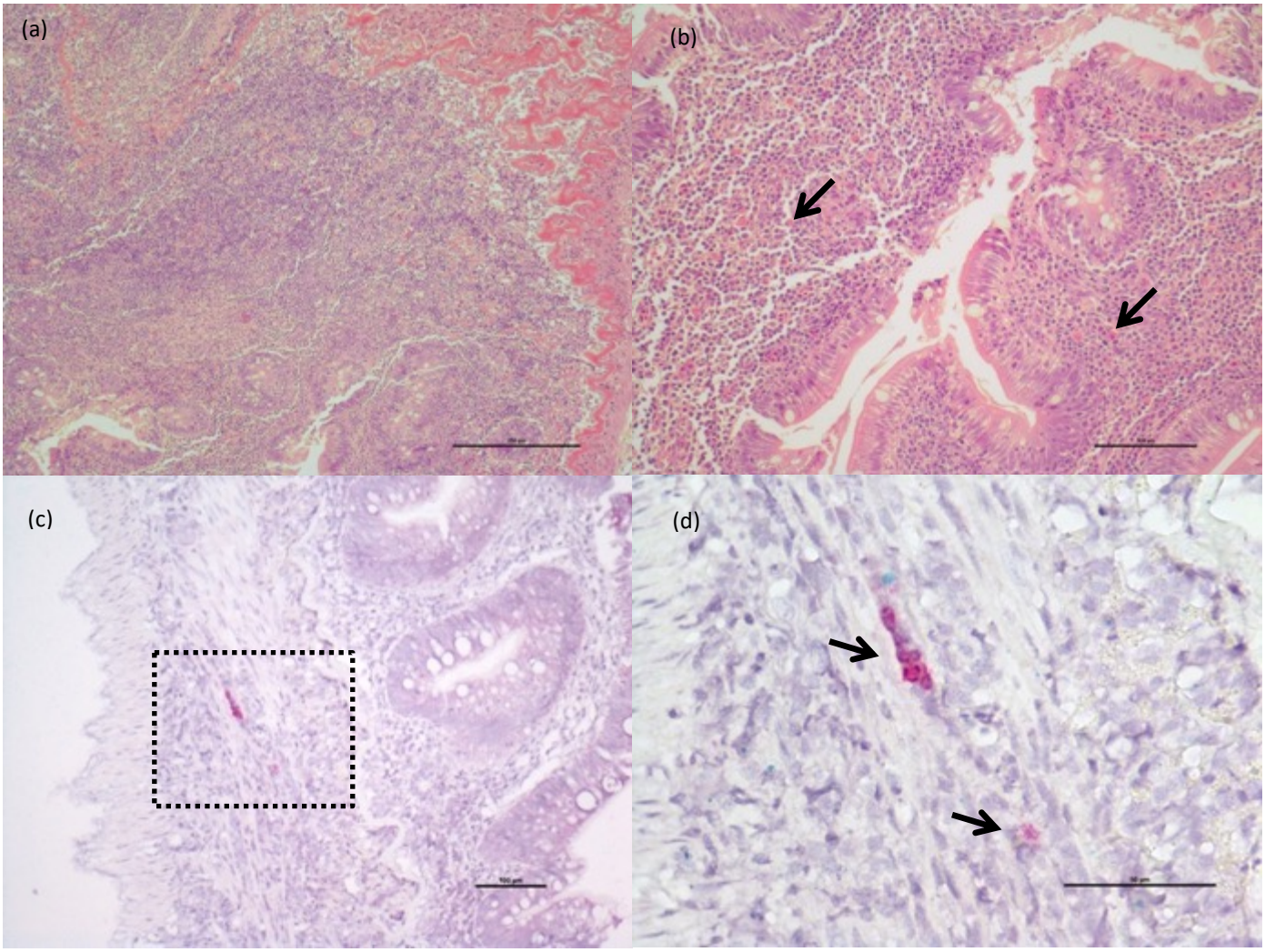


Figure 2.9: Moderate lesions (H&E) and *Ceratonova shasta* detections (In-Situ Hybridization (ISH) staining) in intestine tissues in Fish B5066. (a) Moderate Chronic Enteritis, affecting primarily the lamina propria in the intestine of Fish B5066 (in ten times magnification); (b) Moderate Chronic Enteritis, affecting primarily the lamina propria in the intestine of Fish B5066. Several heterophilic granulocytes are present (arrows) (in twenty times magnification); (c) *C. shasta* (red) detected through ISH in the lamina propria of the intestine, affected by chronic entetritis (in twenty times magnification); (d) details of dotted box in (c) (in forty times magnification).

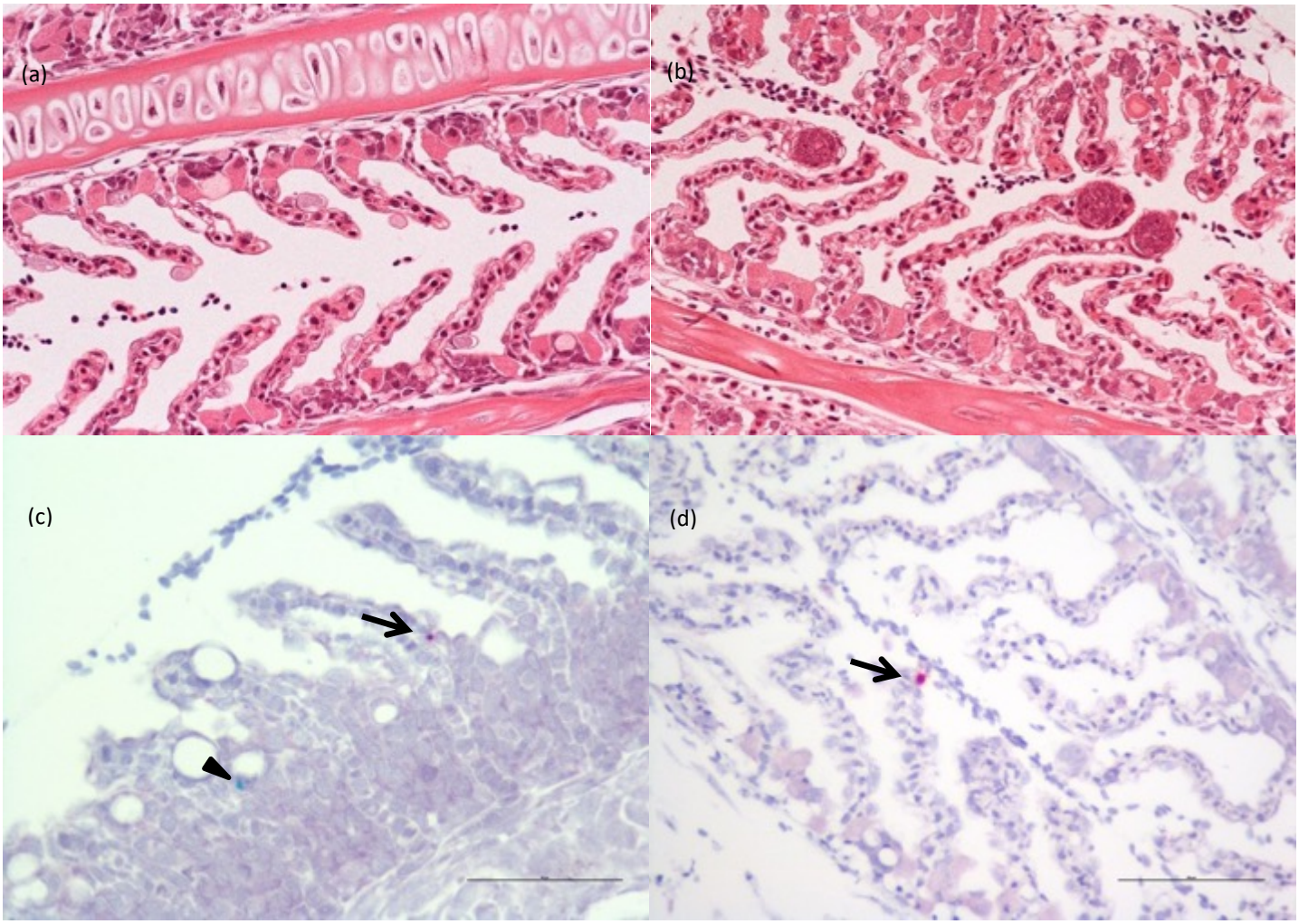


Figure 2.10: Mild lesions (H&E) and *Ceratonova shasta* detections (In-Situ Hybridization (ISH) staining) in gill tissues in Fish B5089. (a) *C. shasta* infection: chlamydia-like aggregates (epitheliocysts) in the lamellae in gills in Fish B5089; (b) *C. shasta* infection: suspected pre-spore aggregates at tips of lamellae in Fish B5089; (c) *C. shasta* (red) detected through ISH in gills in Fish B5089; (*Parvicapsula minibicornis* (green) is marked by arrowhead); (d) another *C. shasta* (red) detected through ISH in gills in Fish B5089;

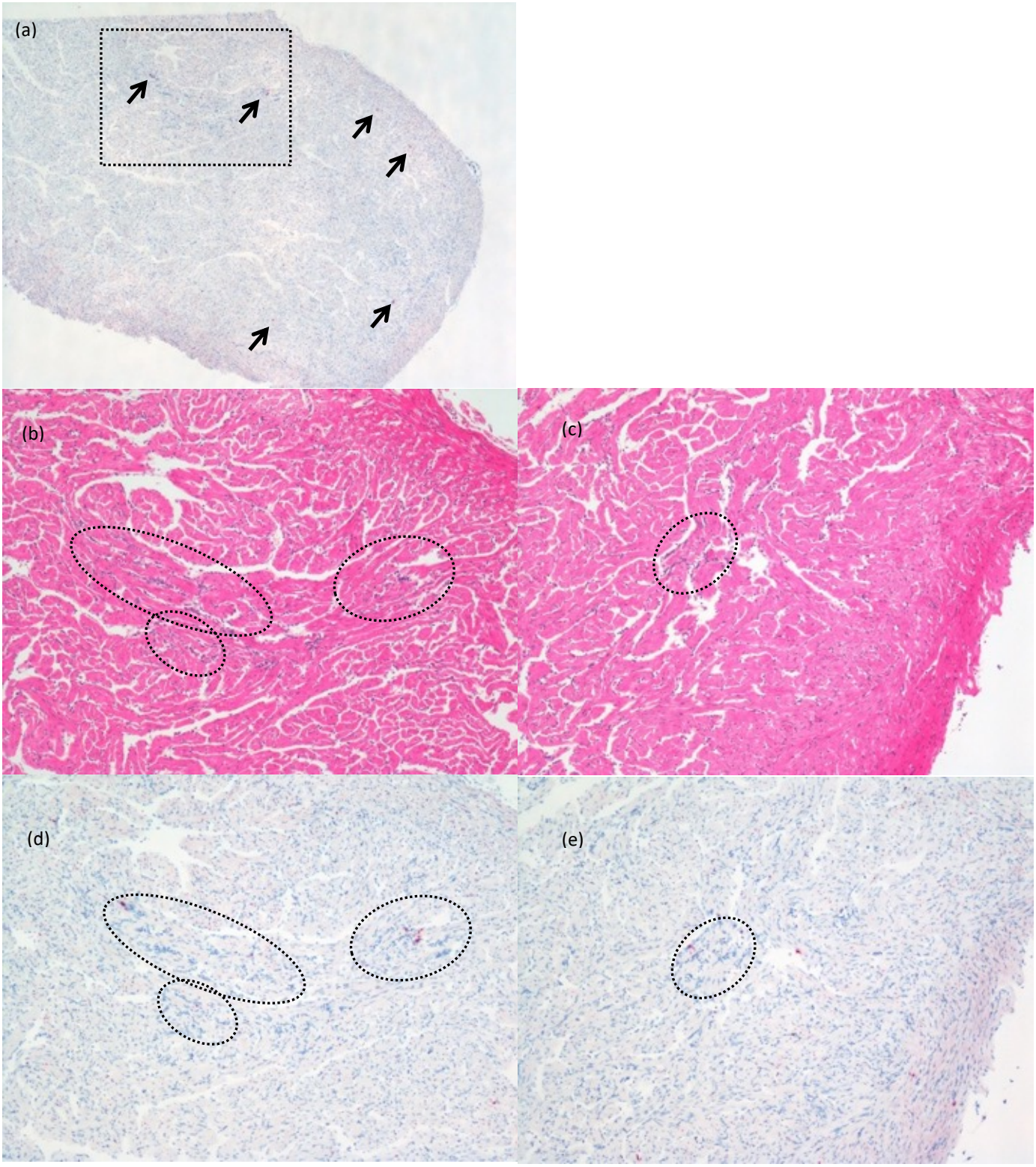


Figure 2.11: Mild lesions (H&E) and piscine orthoreovirus (PRV) detections (In-Situ Hybridization (ISH) staining) in heart tissue in Fish B2159. (a) PRV detections in heart tissue through ISH staining. PRV in cardiomyocytes (arrows) in both compact and spongy layers of the myocardium in the heart of Fish B2159. (in four times magnification) (b)&(c) Small, focal inflammatory infiltrates (dotted circles) in spongy myocardium (in ten times magnification).(d)&(e) Small, focal inflammatory infiltrates (dotted circles) including PRV in cardiomyocytes. (in ten times magnification)

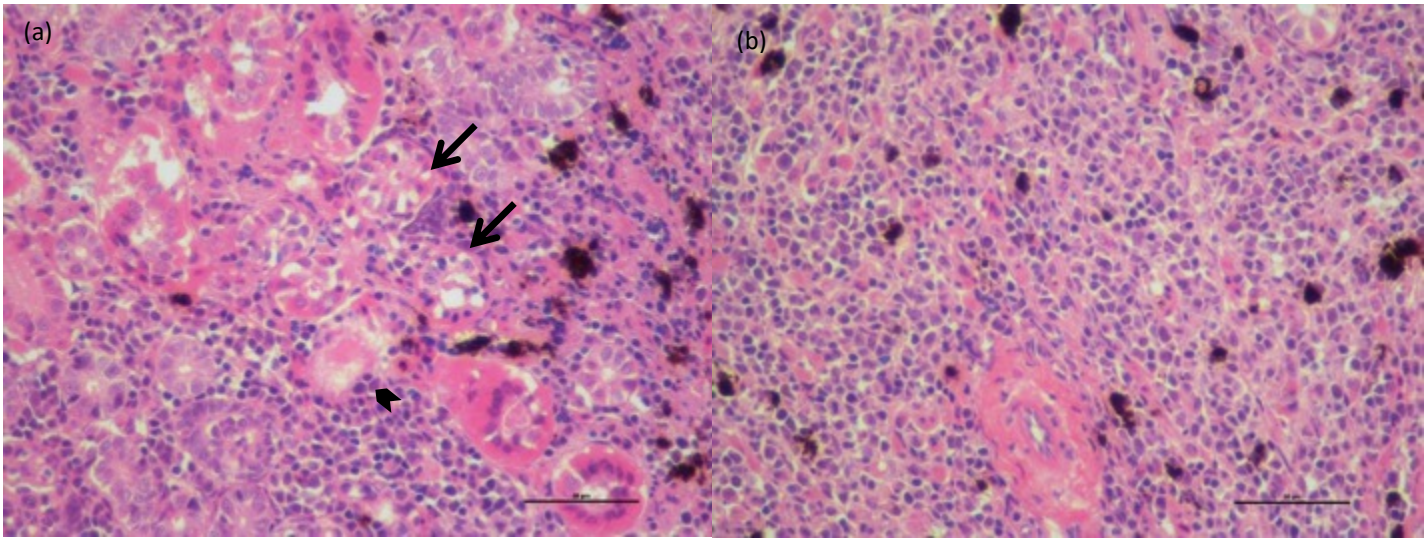


Figure 2.12: Mild leison (H&E) associated with piscine orthoreovirus (PRV) in kidney tissue in Fish B2159. (a) Renal tubular hydropic degeneration (arrows) leading to tubule necrosis (arrowhead) in the kidney of Fish B2159. (in forty times magnification) (b) Interstitial Hyperplasia associated with a "left shift" of erythropoietic population in the kidney of Fish B2159. (in forty times magnification)

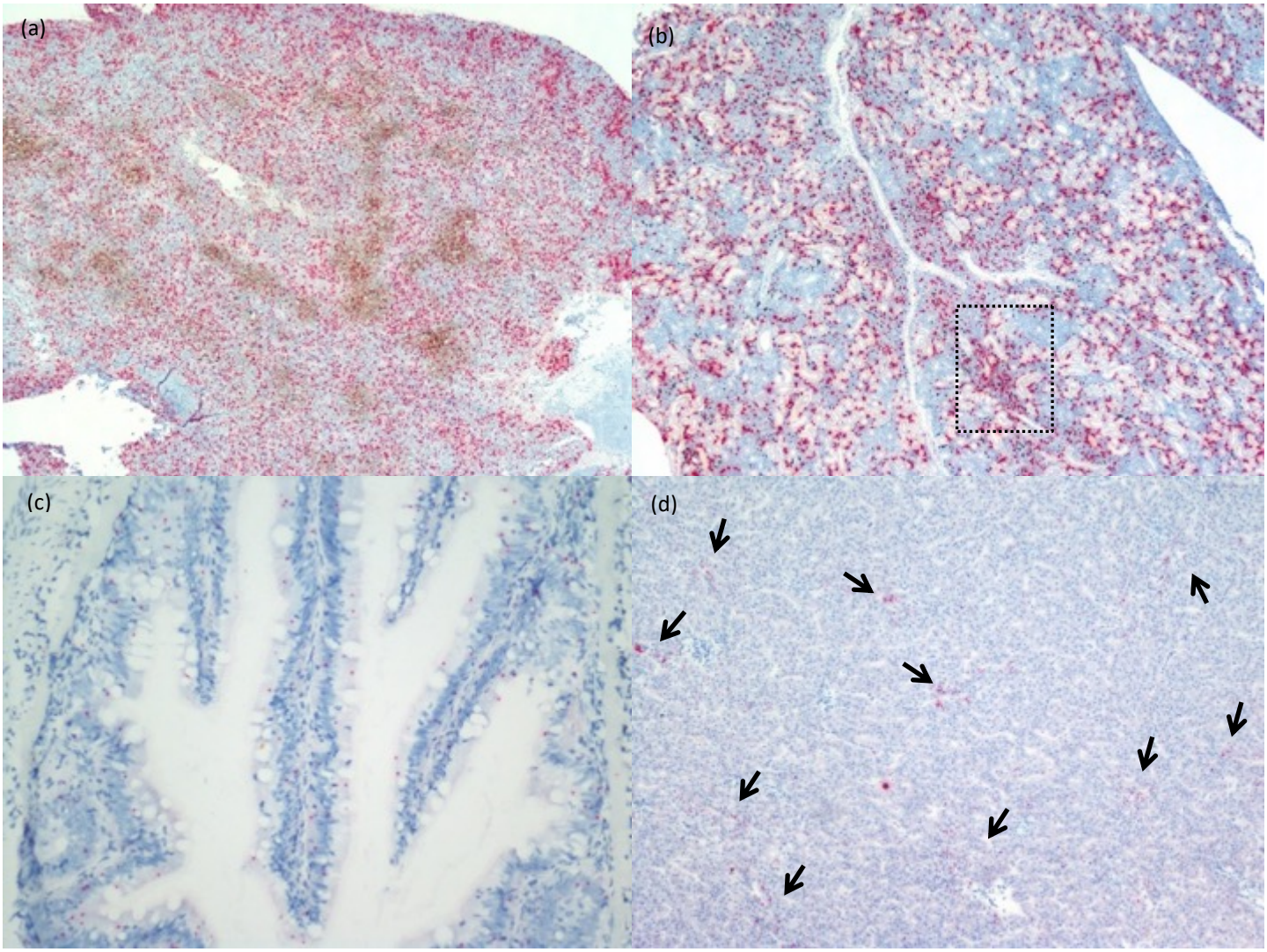


Figure 2.13: Infectious agent piscine orthoreovirus (PRV) detections by In-Situ Hybridization (ISH) staining in multiple tissues in Fish B2159. (a) the spleen was heavily infected by PRV (red), mostly in macrophages and RBCs, showing also blood congestion and hemosiderin deposits. (in four times magnification); (b) the posterior kidney (left) was highly infected with PRV (red), mostly in the macrophages and RBCs. It also showed a few necrotic tubules (dotted box); (c) the intestine shows several PRV bodies (red) in the enterocytes; (d) the liver showed small foci of PRV+ hepatocytes (arrows), often around small blood vessels.

Chapter 3: Conclusions, limitations and implications

3.1 Conclusions and limitations

3.1.1 Infectious agent detection

Infectious agents could be playing an important role in the decline of salmon populations, however, they are largely understudied (Hershberger et al., 2013; Miller et al., 2014). My study investigated the prevalence and load of 46 infectious agent taxa among six natal groups of wild juvenile marine Chinook salmon sampled along the southern coast of BC. I found higher infection burdens carried by fish from the Columbia River system and speculated this may be associated with higher temperature, and the presence of numerous dams and reservoirs in this system.

My sampling method was not able to adequately reflect on seasonal shifts of prevalence and associated loads due to discontinuous sampling periods and limited sample size of each sampling period. It was difficult to interpret the patterns of prevalence of individual agents because not necessarily the same group of fish was sampled. However, the foundation of my research was built upon a survey conducted by Tucker et al., (2018), where they define 11 agents carried by juvenile Chinook salmon originating from Fraser River system to have a potential population-level influence on the host, based on the hypothesis that concurrent decrease in prevalence and load truncation can indicate an infectious agent has potentials to impact the host at a population level. A future study design combining both

seasonal profiles of infectious agents and physiology of infected fish would be helpful in understanding the potential physiological impacts of infectious agents at a population-level.

3.1.2 Potential physiology impacts of infectious agents

I found evidence that at least five infectious agents were associated with physiological changes in juvenile Chinook salmon. Infectious agents can influence fish in various ways such as altering host gene functions (e.g. osmoregulation) and activating host immune response. Even weak effects can negatively affect survival if they happen at critical points in salmon's migratory life history (Miller et al., 2014). In wild populations, infectious agents can often interact with other environment-induced stressors. Some agents are known to be opportunistic and often benefit from the presence of other stressors that impact host immune system functions to facilitate their own replication (Barton, 2002).

In my study, I was not able to verify what caused the association between some agents and their host stress-response genes, which is a limitation of my study design. Additional host physiological information, for example, a better understanding of plasma cortisol levels, would have been helpful. However, collecting plasma cortisol information was not possible given the sampling approaches (trawling by research vessels). Excessive capture stress and handling is known to cause immediate elevation of plasma cortisol level (Pickering & Pottinger, 1989) as well as causing bias in other plasma characteristics. Therefore, future studies need to adopt alternative sampling approaches, those that capture juveniles in a more benign way such as with micro-seines (Godwin et al., 2015).

Another limitation of my study is the limited amount of sample involved in histopathology examination. Processing and reading histology slides are fairly costly, as it requires highly skilled expertise to read and interpret results. At the outset, the choice was made based on the findings of Tucker et al. (2018) to limit histopathological examination to the four agents showing the strongest truncations in prevalence and load distributions, which could be associated with mortality. For each, a limited number of fish with relatively high loads of target agents were selected for histology, due to logistic limitation. Future studies with a higher degree of involvement of histology and more randomized sampling design for histology examination would be desirable if there is a need to further investigating into the relationships between host gene expression and histopathology.

3.2 Potential implications

3.2.1 Conservation research implications

My results showed that host gene expression profile is sensitive and may be a great tool to study potential infectious agent impact on wild populations. The traditional method of studying infectious disease requires multiple steps, including the observation of abnormal behaviour, clinical signs, and mortality of infected fish, laboratory replication of infectious agents, and histopathological examination of cellular-level damage and identification of the suspected agent (Miller et al., 2014). It is difficult to complete all of these processes when studying diseases in wild populations because the current sampling method could not provide stable observation of infected fish (Miller et al., 2014). Therefore, even though alternative methods to study infectious agents in wild fish cannot replace the traditional methods, we

should still take the advantages of such methods to broaden our understanding of the role infectious agents may play in wild populations. My results presented two cases (*Parvicapsula minibicornis* and PRV) where physiological associations were found both through histopathology and host gene profiling. *Parvicapsula minibicornis* was associated with host stress response at the molecular level and histopathology confirmed damage in host kidney at the cellular level. PRV was related to viral immune response in both gill and liver, and lesions found in host heart, spleen, kidney were linked to PRV through histopathology. I hypothesized that host gene expression can be a supplemental tool to histopathology to study infectious agent impacts in wild fish. For a novel agent like PRV that has potential to exchange between wild and cultural fish and expand its distribution around the world (Di Cicco et al., 2018; Morton et al., 2017), different study methods may lead to a better chance to prevent potential loss it could bring to salmon economics and conservation.

In addition, I found considerable agreement in patterns and associations of host gene expression between gill and liver tissue through my RDA models. The gill and liver RDA results showed similarities between the presence of PRV and elevated VDD signals and the potential immune-suppression and stress responses associated with agent *Parvicapsula minibicornis*. Non-lethal gill biopsy has been widely used in research on wild fish and it is suggested to have only minimal impact on juvenile salmonid survival (Jeffries et al., 2014; Martinelli-Liedtke et al., 1999). My result provided evidence that taking the non-lethal gill samples in wild fish and incorporating with RDA models might be a good way to interpret the overall physiological condition of wild salmonids in terms of host gene expression.

3.2.2 Fisheries management implications

I profiled prevalence and loads of 26 infectious agent taxa including 4 viruses, 7 bacteria, and 15 parasites originating from both saltwater and freshwater. Considering the abundance of infectious agents that we detected and a great number of agents that we currently have not discovered, more frequent and systematic surveillance of infectious agents among wild fish is required to define the actual infectious agent abundance and potential threats in this region. The infection burdens varied among natal groups, which could help to inform population-specific management and conservation plans. Higher infection burden among fish from Columbia River system highlighted that infectious agents may be an important factor when considering conservation plan for populations from this region, and anthropological intervention such as continuing with hydropower development in this system may need to be considered with this factor.

I found five agents potentially interacted with multiple aspects of the physiology of juvenile Chinook salmon including osmoregulation, stress response, immune response, and specific viral immune response. These aspects of physiological conditions could influence juvenile early marine survival rate, although my study did not address survival. Estimating smolt survival estimates has been challenging for fisheries managers. Incorporating my results and results from other studies of infectious agents occurred in this region into calculating smolt survival estimates would help with reducing uncertainty. My thesis research was an exploration of not only the new methods but also the unknown impacts of infectious agents on wild salmon populations. The infectious agent profiles in different natal

groups and sampling periods, combined with associations between infectious agents and fish physiology presented in my results, can be used as a reference for the future studies with more focus on the impacts of specific agents to salmon on different levels. When the impact of infectious agents on wild mortality is clearer under future research, fisheries managers can adjust fishing plans based on the occurrences and abundance of infectious agents that are defined as high risk on specific populations, which can eventually benefit the fishery resources in a long run.

3.2.3 Aquaculture and hatchery management implications

My result suggested that management efforts should be more focused in regions with high fish farm density. My finding of the potential impacts of PRV on the physiology of wild fish, paired with other recent findings including this agent causing different diseases in Atlantic and Pacific salmon (Di Cicco et al., 2018) and evidence suggesting it potentially transferring from farmed Atlantic salmon to wild Pacific salmon (Morton et al., 2017), highlighted the threat PRV may have on the delicate southern Chinook populations, and can help to evaluate the impacts of fish farms around this water region. Most fish with high loads of PRV in my study were originated from Marble river which is part of the WCVI system. All of them were caught at Quatsino Sound, where salmon farm density is relatively high. Although the effect of fish farm was not particularly tested in my thesis research, the PRV infection rate was found related to exposure to salmon farms in Morton et al., 2017. While further research is needed in determining the actual impact of this agent on the wild

populations, monitoring the PRV occurence and abundance in the area with high fish farm density would be beneficial as historical records for references.

The impacts of hatchery should also be considered when evaluating the impact of infectious agents. Although it was not one of my primary objectives to investigate the difference of infectious agents among hatchery and wild fish, I did find a higher infection burden among hatchery fish compared to the wild fish. Hatchery fish were usually found larger during the same sampling event, and they had rapid movement through freshwater and shorter residency in the nearshore environment, while smaller wild fish usually spent over an extended period of time in the freshwater and nearshore environment (Thakur et al., 2018). Therefore, hatchery fish are speculated to encounter higher diversity of infectious agents soon after their rapid entry into the ocean, at the same time they are experiencing other physiological changes to adjust to the new environment and, thus, may be more vulnerable to additional stressors (Thakur et al., 2018). In my study, it is difficult to identify the contributing factors to the higher infection burden among hatchery fish. Future studies incorporating tracking methods would be helpful in determining the actual time hatchery and wild fish spend in both fresh and marine environment, and can provide a better understanding of the differences of infectious agent profile between hatchery and wild fish.

3.2.4 Climate change implications

The occurrence and progression of an infectious disease are influenced by factors present in the infectious agent, the host and the environment (Hershberger et al., 2013). There is a growing concern about the potential impacts of global warming and climate

change on infectious diseases, because the rising temperature can not only alter the development, survival rate, and transmission of the infectious agents, but also change the host susceptibility (Harvell et al., 1999, 2002; Lafferty et al., 2002; Miller et al., 2014). All river systems included in my study are experiencing warming at different levels, with the Fraser River summer temperature already warming by 1.5 °C since the 1950s (Patterson et al., 2007) and projected to be warmed up by 2°C in the future (Martins et al., 2011). Columbia River system might be the most affected considering it is already warmer than more northern systems in my study, and given the record that Columbia River has periods of summer days with river temperature above the critical 20 °C which rarely happens in other freshwater systems in my study (DeHart, 2018). Summer temperature in the Columbia River system is projected to keep rising and poses thermal stress to salmon (Mantua et al., 2010). Higher Columbia River water temperature results in slowed migration of adult Chinook salmon (Goniea et al., 2006), which may prolong their contact with freshwater infectious agents under a stressed condition.

My result highlighted the potential role infectious agent might be playing in the declining southern Chinook populations. There might be a synergistic effect of climate change and infectious agents (Hershberger et al., 2013; Miller et al., 2014). In the most recent COSEWIC Wildlife Species Assessments (COSEWIC, 2018), eight Chinook salmon populations have been listed as endangered, and they were all stream-type Chinook populations originated from the Fraser River system. In the US, one Chinook salmon population originated from the Columbia River system is listed as endangered under the Endangered Species Act. The Columbia River system and the Fraser River system had the highest infection agent richness and infection burden in my study. These two systems also

have more stream-type Chinook comparing to the rest of fish included in my study which were mostly from Vancouver Island. In Tucker et al. 2018, stream-type Fraser River Chinook was found to carrier more infectious agents than ocean-type when caught as juveniles in the ocean. Among the 11 agents that were determined to have potential associations with mortality, five were only found in stream-type fish, and another five were found to have higher prevalence in stream-type fish. Although there might be inherent differences in susceptibility, the stream-type Chinook juveniles do spend longer time including at least one summer when stream temperatures are extreme in these two freshwater systems (DeHart, 2018, Martins et al., 2011) at the same time they can encounter various fresh-water infectious agents. Once they move into the ocean, their bigger size could contribute to faster transition to a piscivorous diet faster than the ocean-type, which can be a source of salt-water infectious agents (Tucker et al., 2018).

It is highly probable that infectious disease is contributing to population declines, and research that can inform on which agents and diseases show the highest pathogenic potential may provide a means for mitigating, or at least predicting and managing around variance in early marine survival. Such information would be helpful to update management decisions and conservation plans through ways such as reducing uncertainty in models forecasting adult returns and intervention of diseases exchange among wild populations and wild versus farmed populations. Future studies continuing to explore the impacts of infectious agents under the changing environment will be beneficial to protecting this valuable species in the long run.

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Appendix

A.1 Appendix tables

A.1.1 Sampling information of juvenile Chinook salmon (*Oncorhynchus tshawytscha*) captured by DFO marine sampling program from 2012 to 2014.

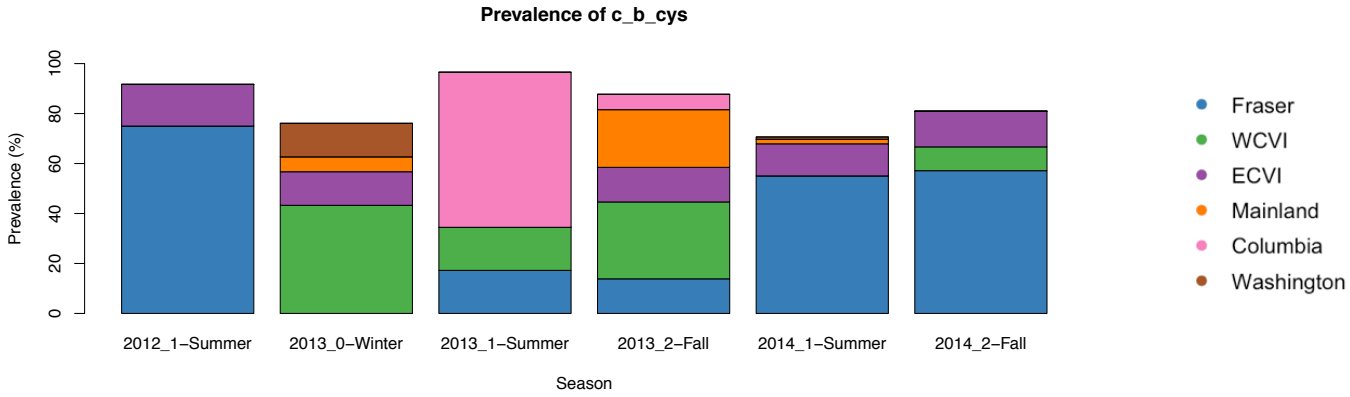
Sampling Month	Sample Size (N)	Fork Length Mean (mm)	Fork Length SD
2012 September	24	158	36
2013 March	67	231	34
2013 July	29	200	50
2013 October	34	208	39
2013 November	31	227	24
2014 June	2	154	1
2014 July	25	177	39
2014 September	82	176	36
2014 October	21	189	50

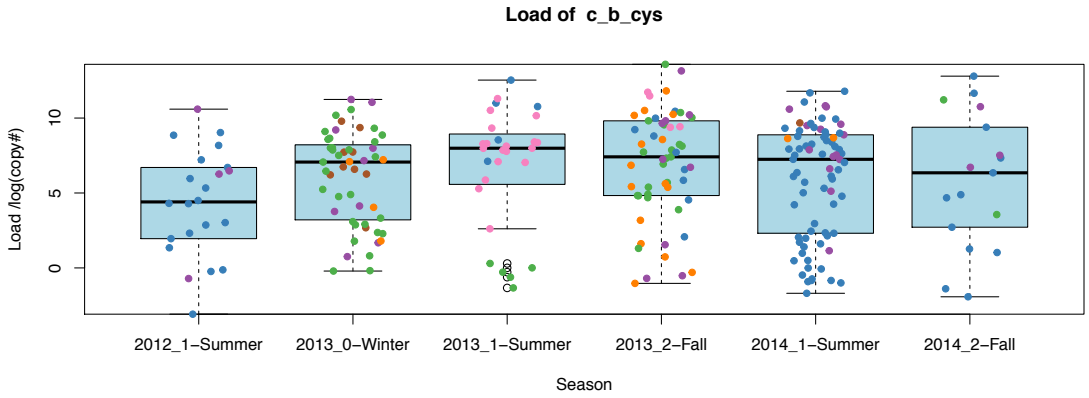
A.2 Appendix figures

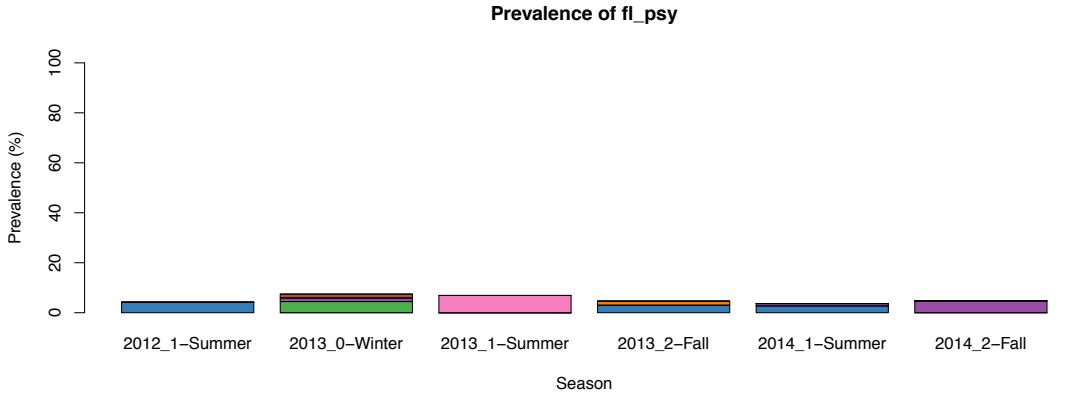
A.2.1 Prevalence (top) and load (bottom) of twenty-one infectious agent

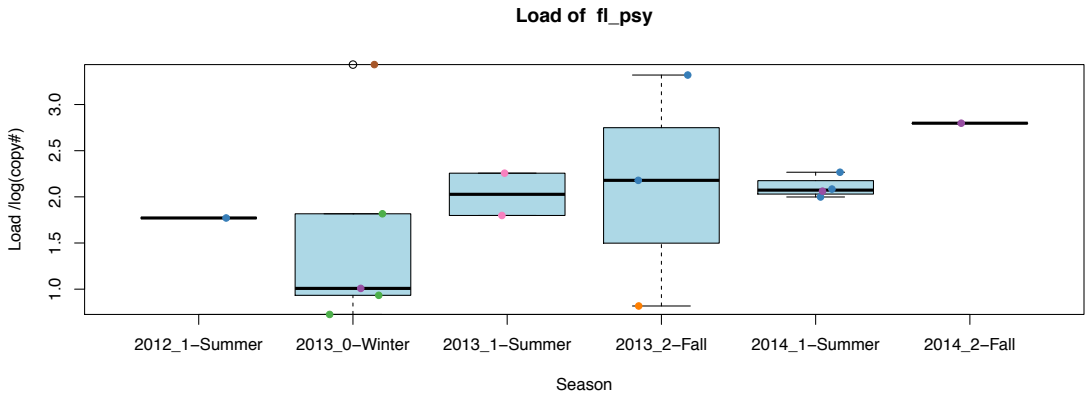
Figure A.2.1.1-21 are figures of prevalence (top) and load (bottom) of twenty-one infectious agent detected with prevalence higher than 1% among juvenile Chinook salmon (*Oncorhynchus tshawytscha*) captured by DFO marine sampling program from 2012 to 2014. In prevalence barplots (top), the total height of the stacked bars indicates the overall prevalence for the sampling period, and the colors indicate the proportion of the positives that are made up by each natal group. In load boxplots (bottom), the dots represent the load of each positive detection in log copy number, and the colors indicate the natal group. The whiskers are the range of load for the sampling period. Figures are in order the same order as infectious agent in Table 2.1, and infectious agent abbreviations are shown in Table 2.1. Infectious agents with detections only above LOD (limit of detection) are excluded.

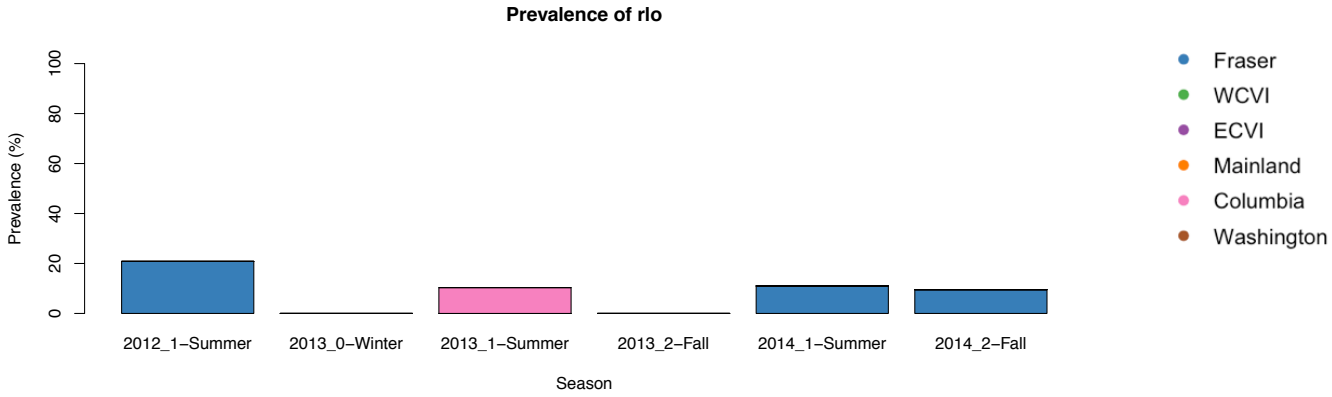
Natal group abbreviations are: WCVI: West Coast of Vancouver Island; ECVI: East Coast of Vancouver Island; Fraser: Fraser River system (upper and lower Fraser River and Thompson River); Mainland: Mainland BC (including streams in Northern, Central and Southern mainland BC that were not included in the other five region groups); Columbia: Columbia River system (including Columbia River and Snake River); Washington (including tributaries to the Puget Sound and Strait of Juan de Fuca).

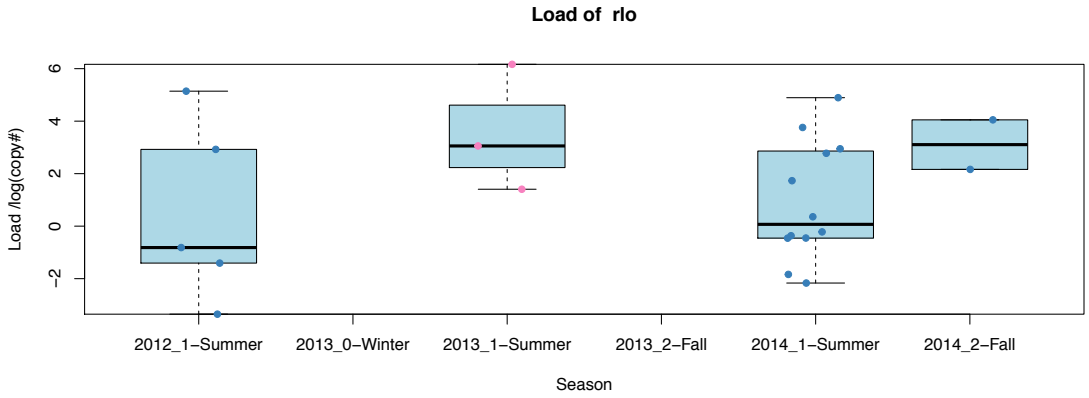

Prevalence of c_b_cys
Prevalence (%)
0
20
40
60
80
100
2012_1–Summer
2013_0–Winter
2013_1–Summer
2013_2–Fall
2014_1–Summer
2014_2–Fall
Season
Fraser
WCVI
ECVI
Mainland
Columbia
Washington

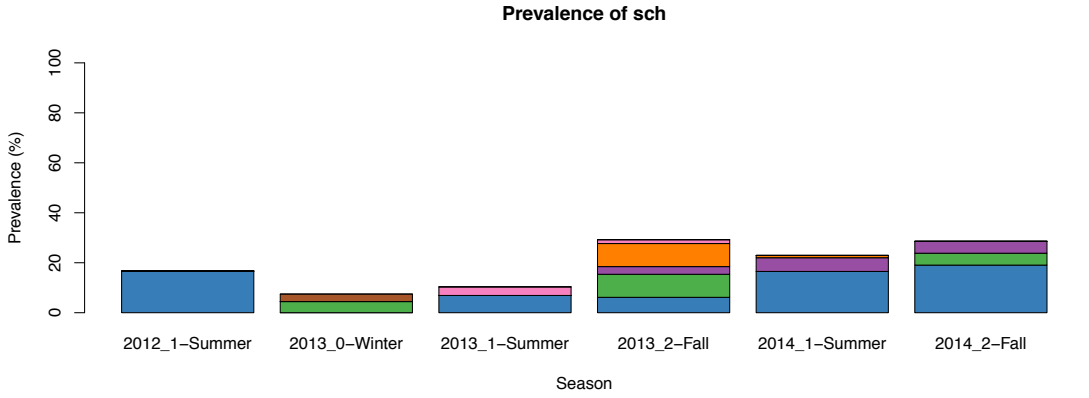

Load of c_b_cys
Load /log(copy#)
0
5
10
2012_1–Summer
2013_0–Winter
2013_1–Summer
2013_2–Fall
2014_1–Summer
2014_2–Fall
Season

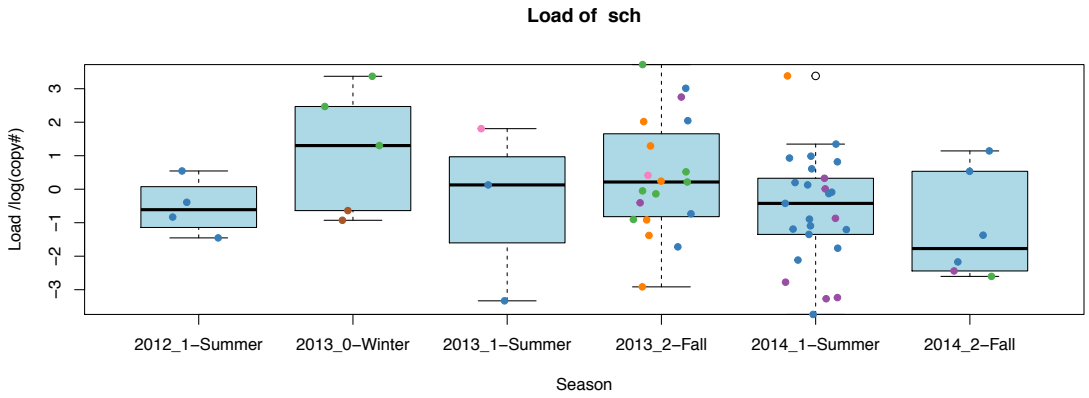

Prevalence of fl_psy
Prevalence (%)
0
20
40
60
80
100
2012_1–Summer
2013_0–Winter
2013_1–Summer
2013_2–Fall
2014_1–Summer
2014_2–Fall
Season

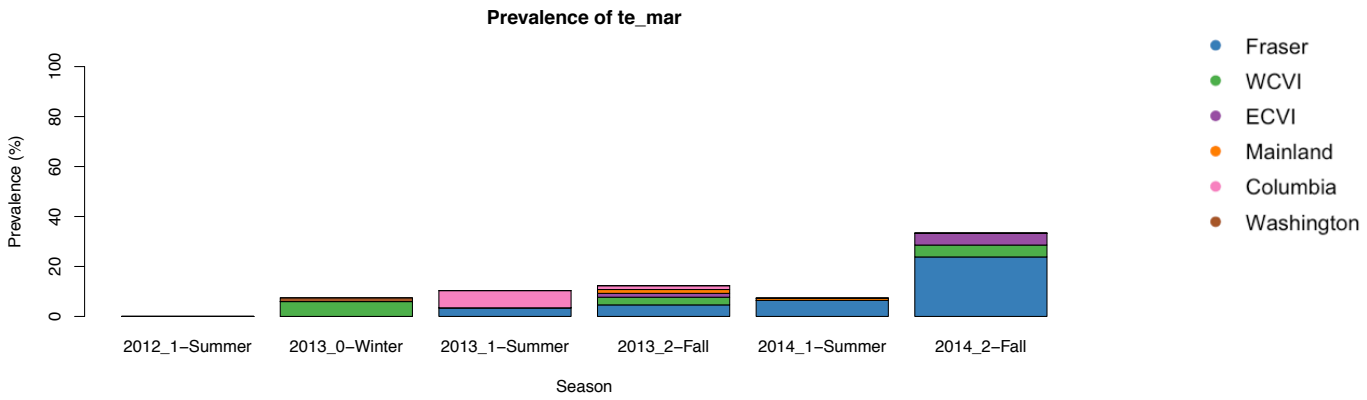

Load of fl_psy
Load /log(copy#)
1.0
1.5
2.0
2.5
3.0
2012_1–Summer
2013_0–Winter
2013_1–Summer
2013_2–Fall
2014_1–Summer
2014_2–Fall
Season

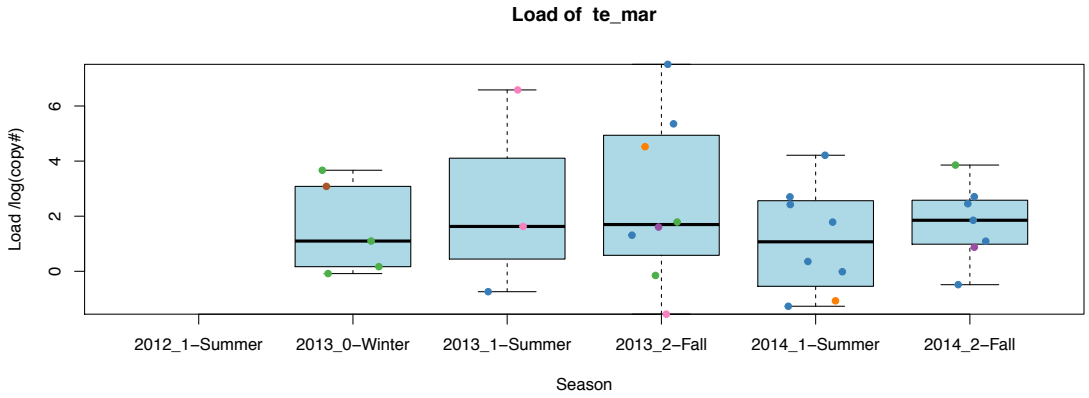

Prevalence of rlo
Prevalence (%)
0
20
40
60
80
100
2012_1–Summer
2013_0–Winter
2013_1–Summer
2013_2–Fall
2014_1–Summer
2014_2–Fall
Season
Fraser
WCVI
ECVI
Mainland
Columbia
Washington

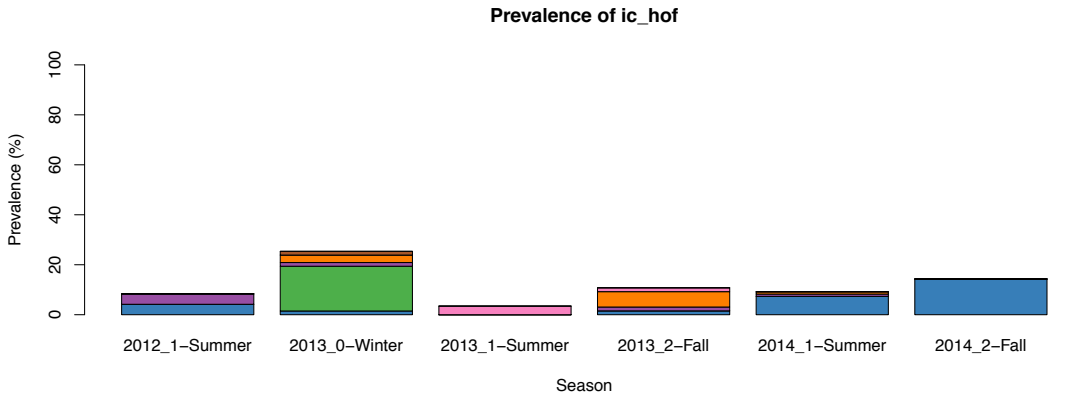

Load of rlo
Load /log(copy#)
−2
0
2
4
6
2012_1–Summer
2013_0–Winter
2013_1–Summer
2013_2–Fall
2014_1–Summer
2014_2–Fall
Season

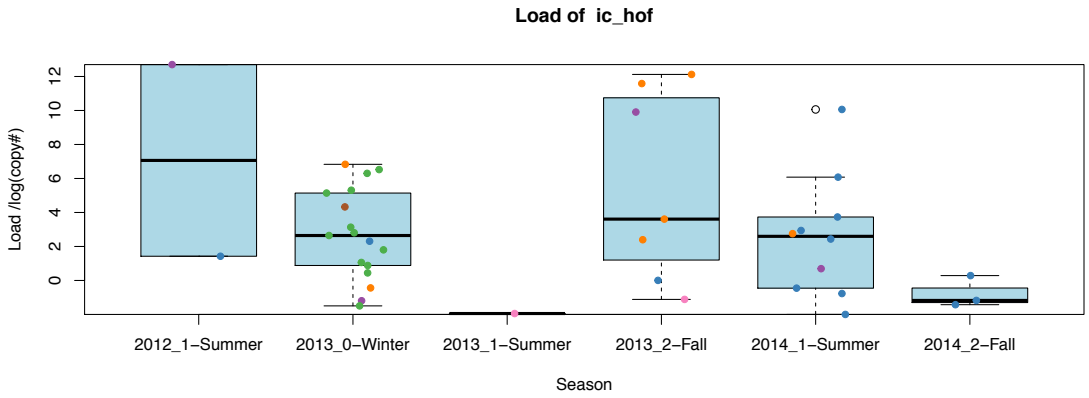

Prevalence of sch
Prevalence (%)
0
20
40
60
80
100
2012_1–Summer
2013_0–Winter
2013_1–Summer
2013_2–Fall
2014_1–Summer
2014_2–Fall
Season

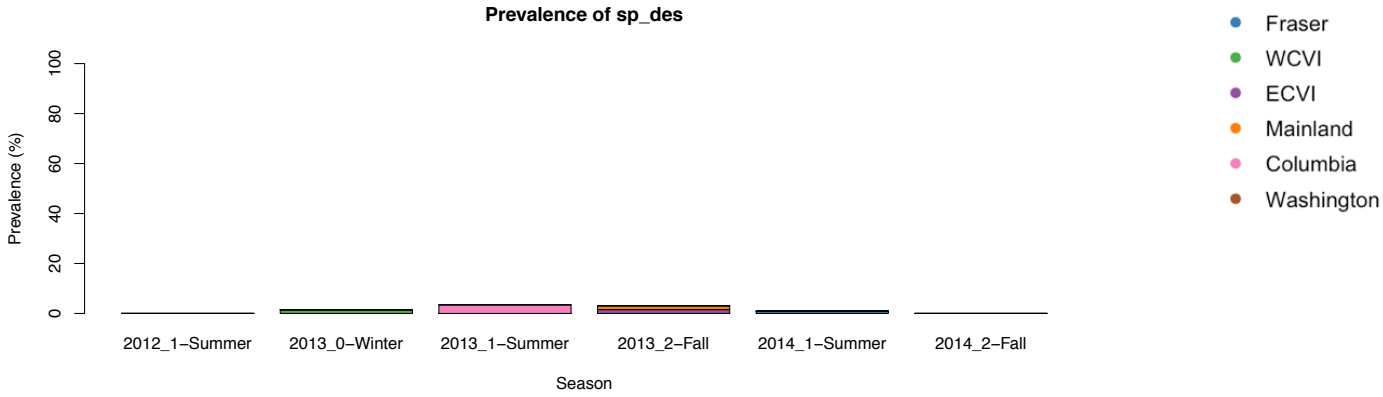

Load of sch
Load /log(copy#)
−3
−2
−1
0
1
2
3
2012_1–Summer
2013_0–Winter
2013_1–Summer
2013_2–Fall
2014_1–Summer
2014_2–Fall
Season

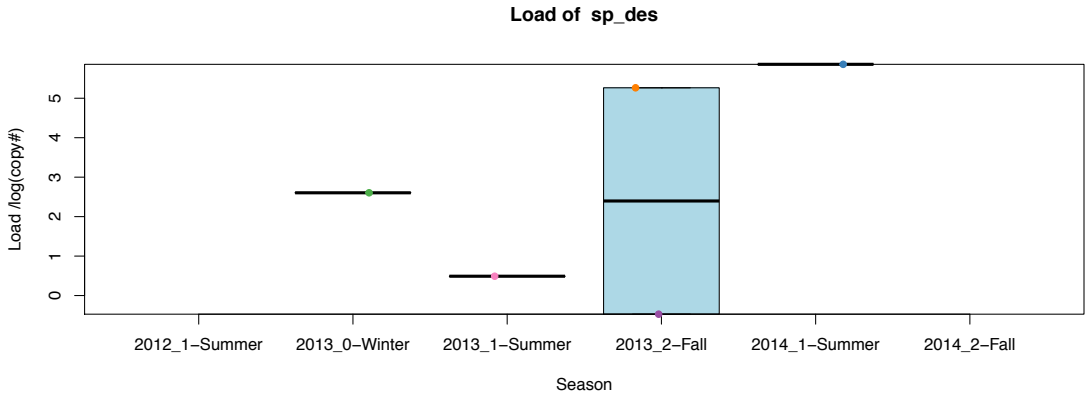

Prevalence of te_mar
Prevalence (%)
0
20
40
60
80
100
2012_1–Summer
2013_0–Winter
2013_1–Summer
2013_2–Fall
2014_1–Summer
2014_2–Fall
Season
Fraser
WCVI
ECVI
Mainland
Columbia
Washington

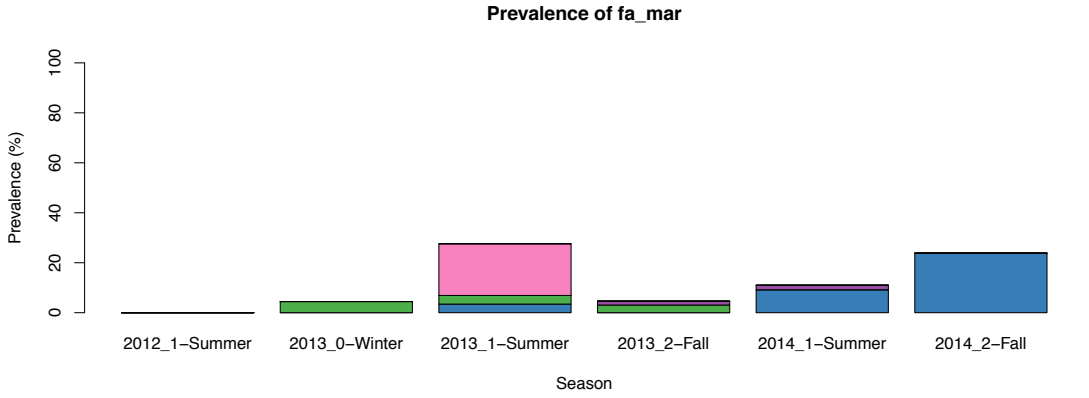

Load of te_mar
Load /log(copy#)
0
2
4
6
2012_1–Summer
2013_0–Winter
2013_1–Summer
2013_2–Fall
2014_1–Summer
2014_2–Fall
Season

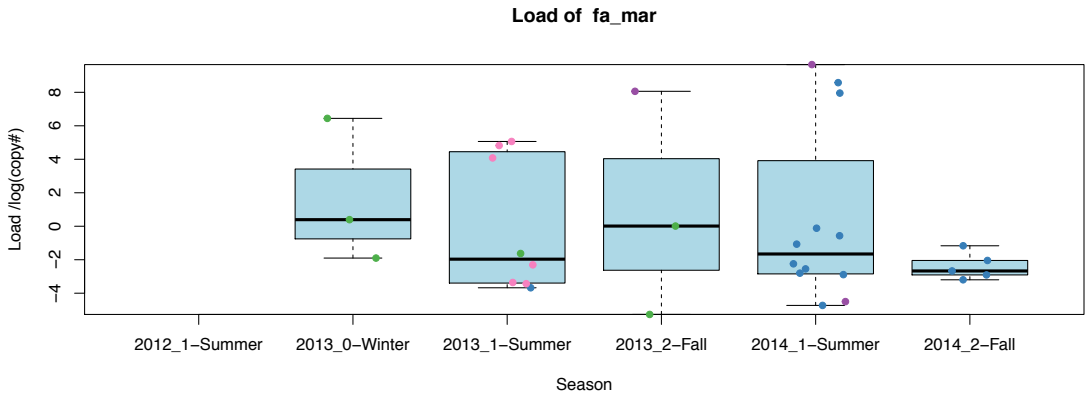

Prevalence of ic_hof
Prevalence (%)
0
20
40
60
80
100
2012_1–Summer
2013_0–Winter
2013_1–Summer
2013_2–Fall
2014_1–Summer
2014_2–Fall
Season

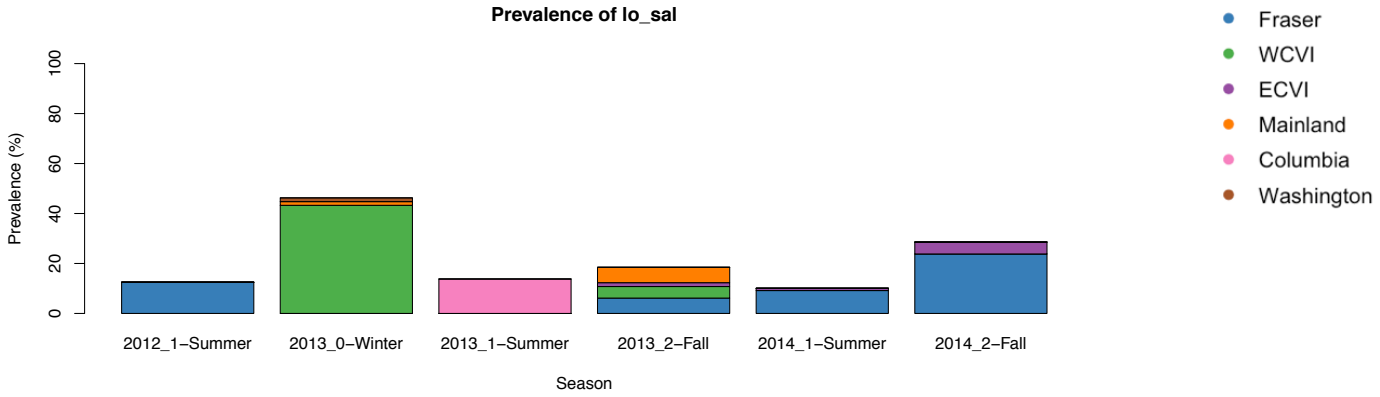

Load of ic_hof
Load /log(copy#)
0
2
4
6
8
10
12
2012_1–Summer
2013_0–Winter
2013_1–Summer
2013_2–Fall
2014_1–Summer
2014_2–Fall
Season

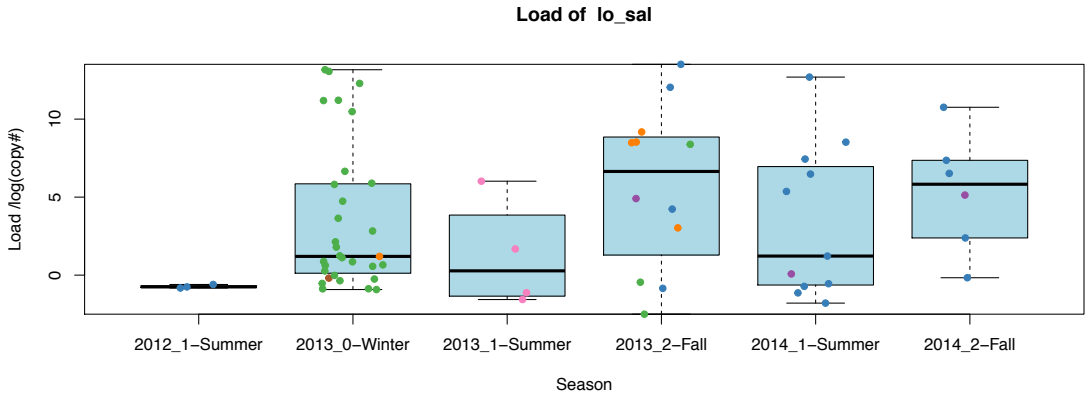

Prevalence of sp_des
Prevalence (%)
0
20
40
60
80
100
2012_1–Summer
2013_0–Winter
2013_1–Summer
2013_2–Fall
2014_1–Summer
2014_2–Fall
Season
Fraser
WCVI
ECVI
Mainland
Columbia
Washington

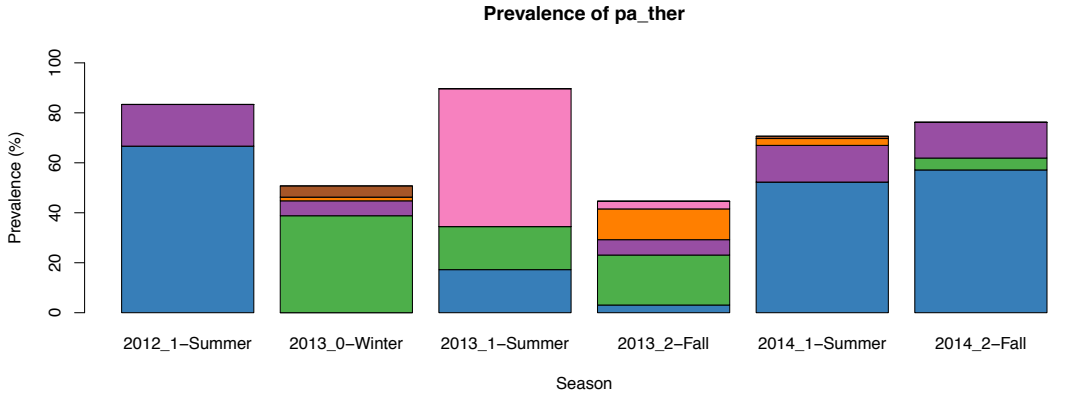

Load of sp_des
Load /log(copy#)
0
1
2
3
4
5
2012_1–Summer
2013_0–Winter
2013_1–Summer
2013_2–Fall
2014_1–Summer
2014_2–Fall
Season

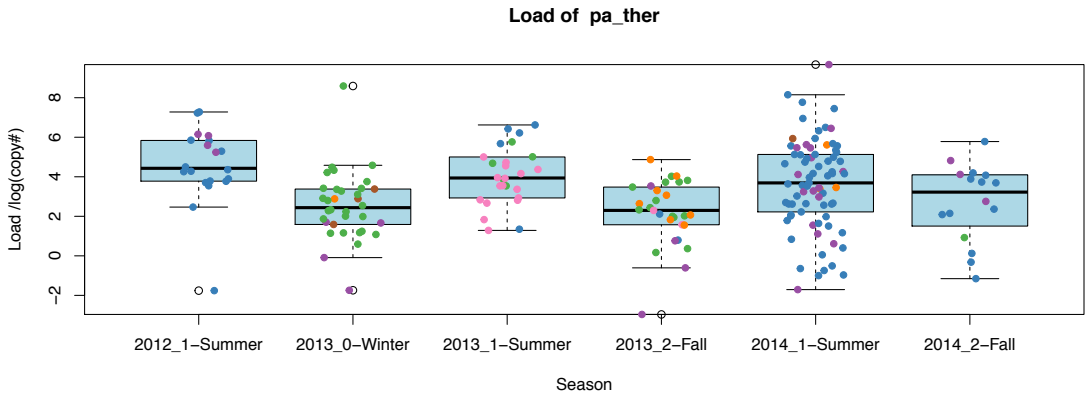

Prevalence of fa_mar
Prevalence (%)
0
20
40
60
80
100
2012_1–Summer
2013_0–Winter
2013_1–Summer
2013_2–Fall
2014_1–Summer
2014_2–Fall
Season

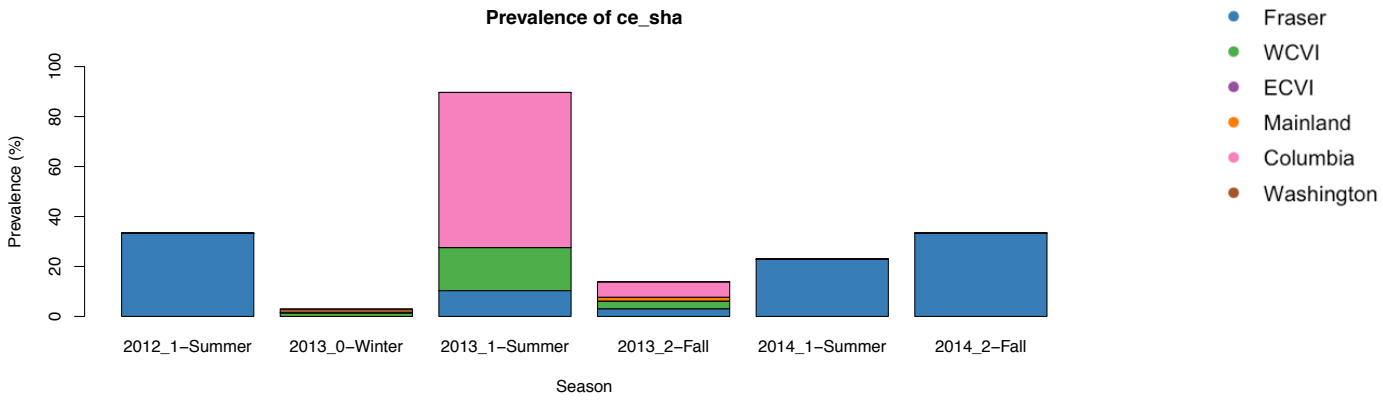

Load of fa_mar
Load /log(copy#)
−4
−2
0
2
4
6
8
2012_1–Summer
2013_0–Winter
2013_1–Summer
2013_2–Fall
2014_1–Summer
2014_2–Fall
Season

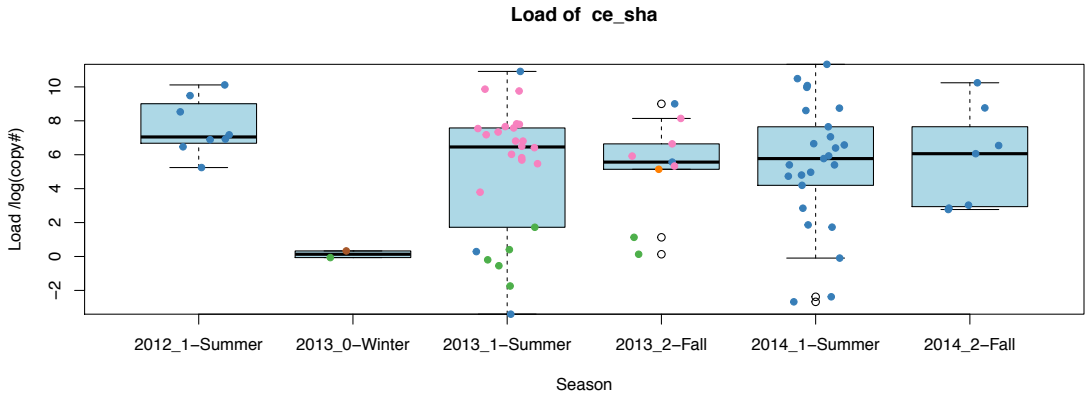

Prevalence of Io_sal
Prevalence (%)
0
20
40
60
80
100
2012_1–Summer
2013_0–Winter
2013_1–Summer
2013_2–Fall
2014_1–Summer
2014_2–Fall
Season
Fraser
WCVI
ECVI
Mainland
Columbia
Washington

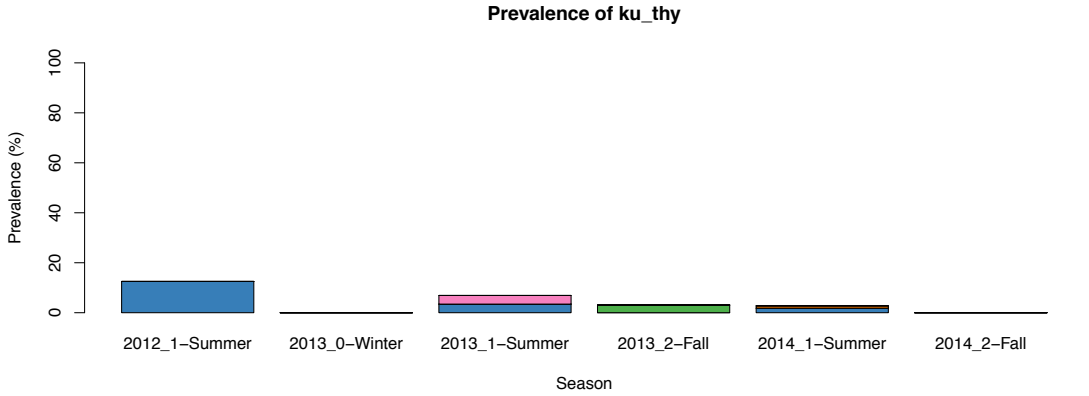

Load of Io_sal
Load /log(copy#)
0
5
10
2012_1–Summer
2013_0–Winter
2013_1–Summer
2013_2–Fall
2014_1–Summer
2014_2–Fall
Season

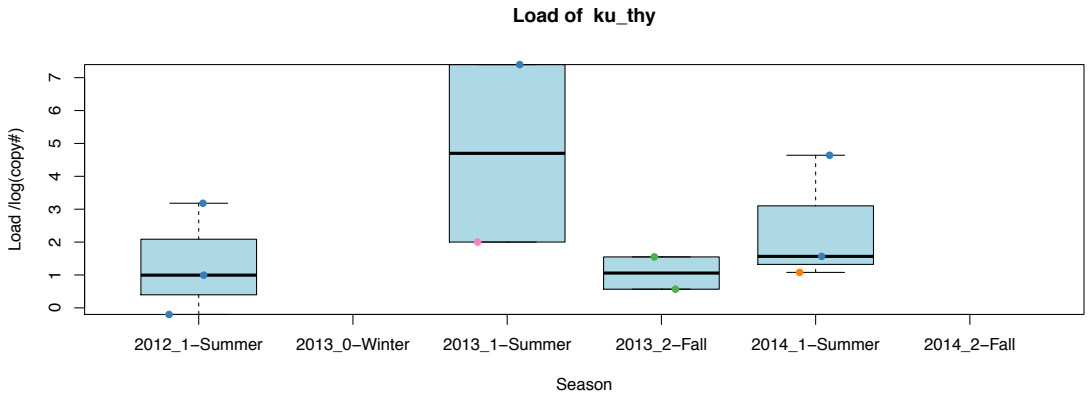

Prevalence of pa_ther
Prevalence (%)
0
20
40
60
80
100
2012_1–Summer
2013_0–Winter
2013_1–Summer
2013_2–Fall
2014_1–Summer
2014_2–Fall
Season

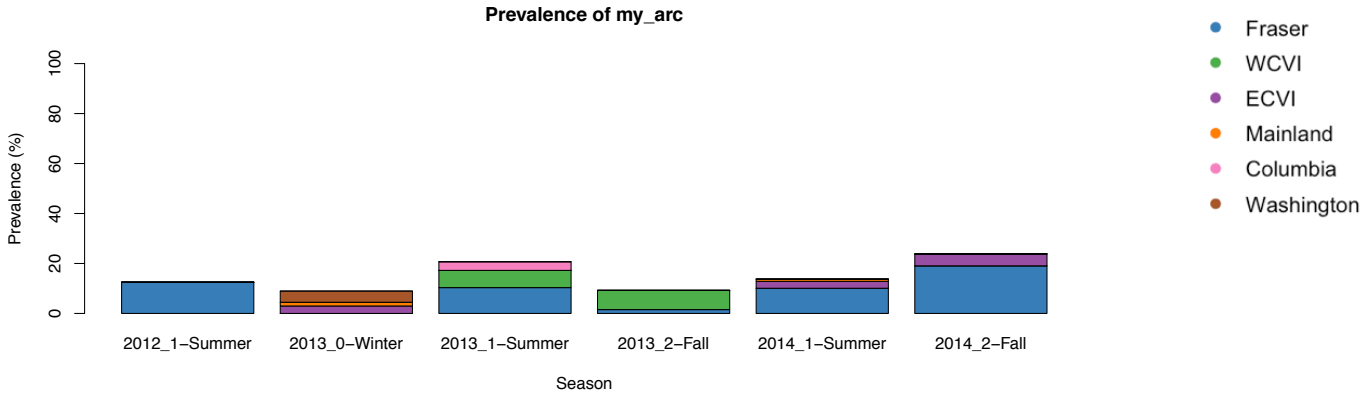

Load of pa_ther
Load /log(copy#)
−2
0
2
4
6
8
2012_1–Summer
2013_0–Winter
2013_1–Summer
2013_2–Fall
2014_1–Summer
2014_2–Fall
Season

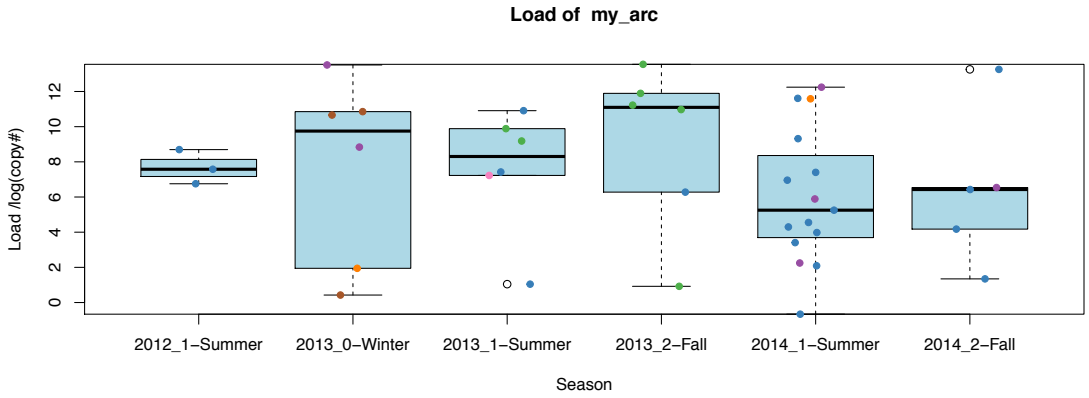

Prevalence of ce_sha
Prevalence (%)
0
20
40
60
80
100
2012_1–Summer
2013_0–Winter
2013_1–Summer
2013_2–Fall
2014_1–Summer
2014_2–Fall
Season
Fraser
WCVI
ECVI
Mainland
Columbia
Washington

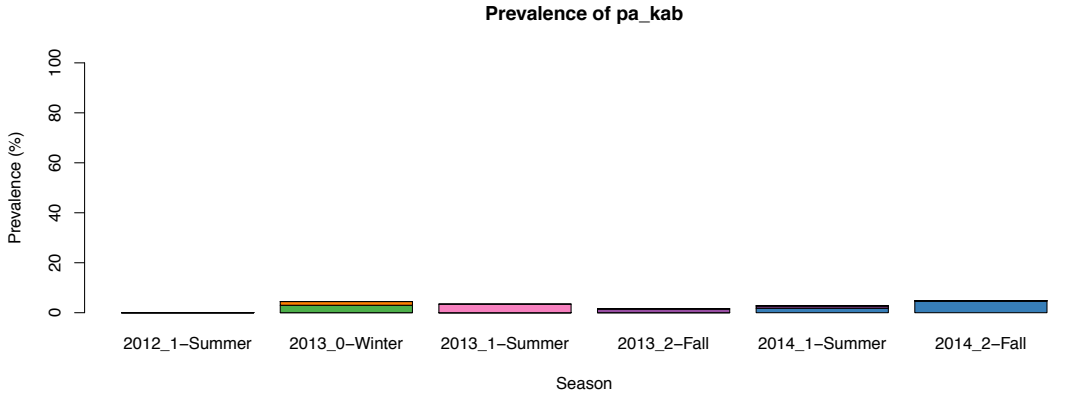

Load of ce_sha
Load /log(copy#)
−2
0
2
4
6
8
10
2012_1–Summer
2013_0–Winter
2013_1–Summer
2013_2–Fall
2014_1–Summer
2014_2–Fall
Season

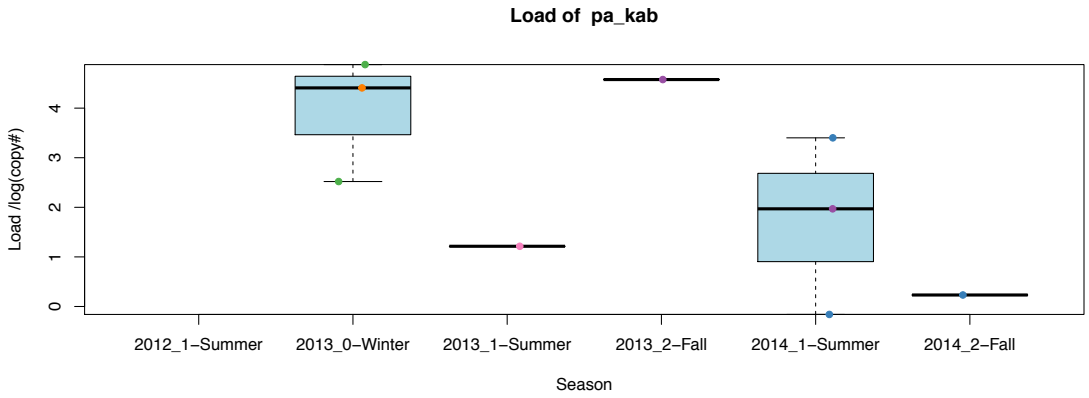

Prevalence of ku_thy
Prevalence (%)
0
20
40
60
80
100
2012_1–Summer
2013_0–Winter
2013_1–Summer
2013_2–Fall
2014_1–Summer
2014_2–Fall
Season

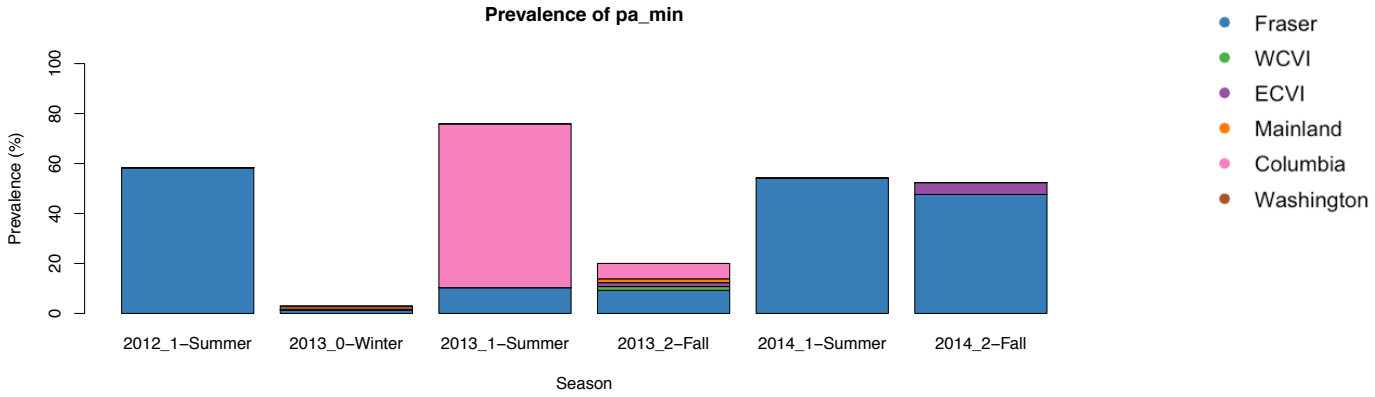

Load of ku_thy
Load /log(copy#)
0
1
2
3
4
5
6
7
2012_1–Summer
2013_0–Winter
2013_1–Summer
2013_2–Fall
2014_1–Summer
2014_2–Fall
Season

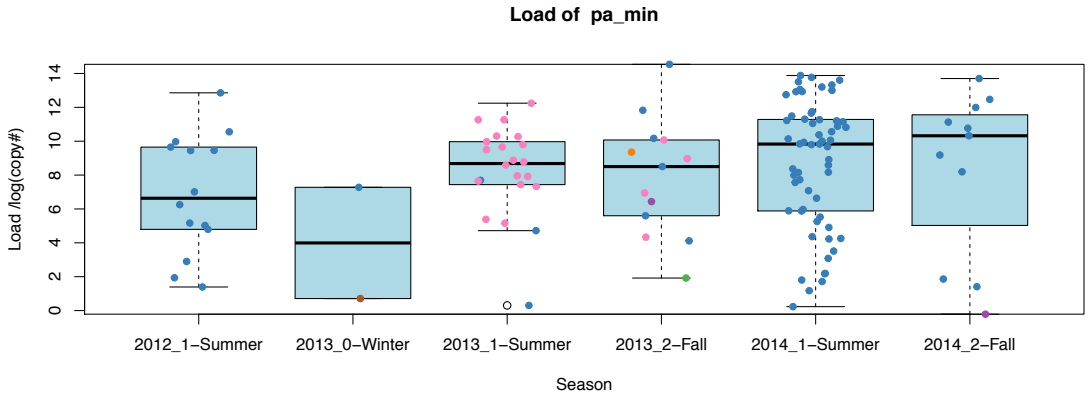

Prevalence of my_arc
Prevalence (%)
0
20
40
60
80
100
2012_1–Summer
2013_0–Winter
2013_1–Summer
2013_2–Fall
2014_1–Summer
2014_2–Fall
Season
Fraser
WCVI
ECVI
Mainland
Columbia
Washington

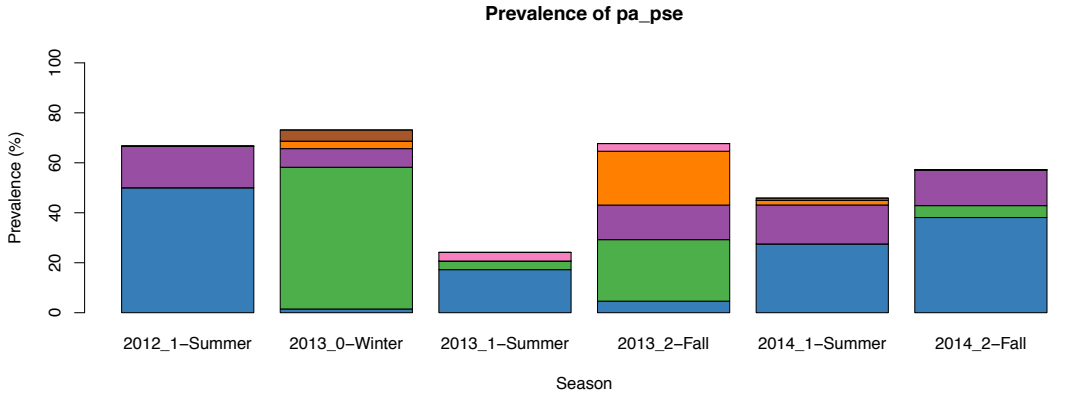

Load of my_arc
Load /log(copy#)
0
2
4
6
8
10
12
2012_1–Summer
2013_0–Winter
2013_1–Summer
2013_2–Fall
2014_1–Summer
2014_2–Fall
Season

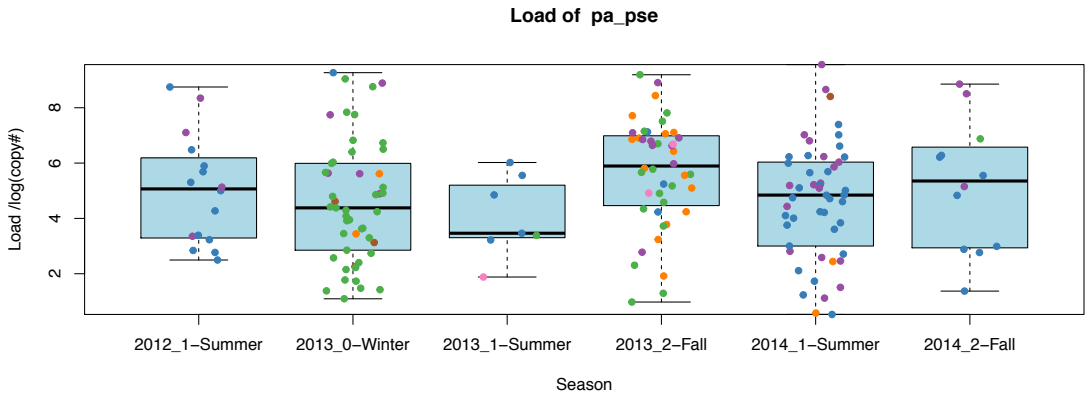

Prevalence of pa_kab
Prevalence (%)
0
20
40
60
80
100
2012_1–Summer
2013_0–Winter
2013_1–Summer
2013_2–Fall
2014_1–Summer
2014_2–Fall
Season

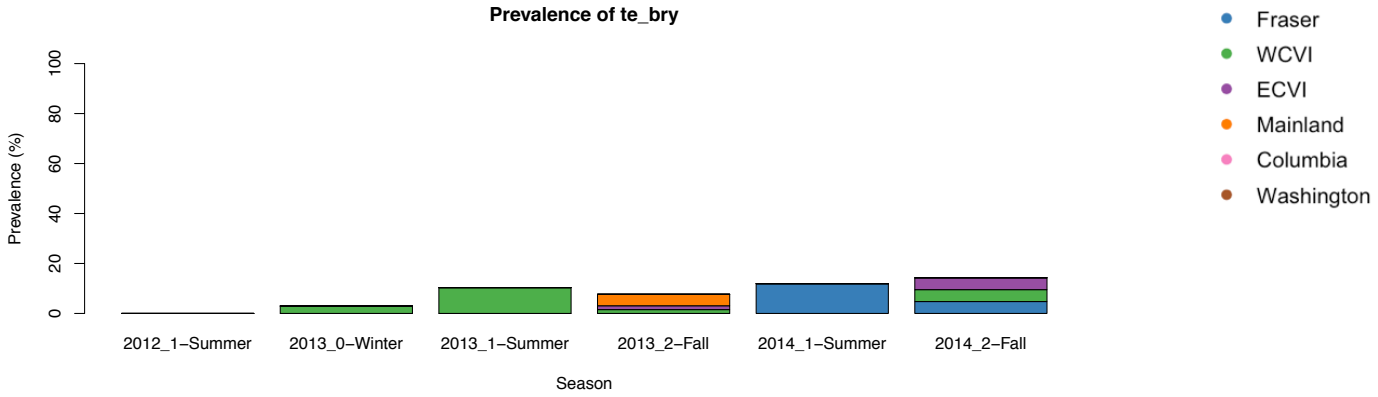

Load of pa_kab
Load /log(copy#)
0
1
2
3
4
2012_1–Summer
2013_0–Winter
2013_1–Summer
2013_2–Fall
2014_1–Summer
2014_2–Fall
Season

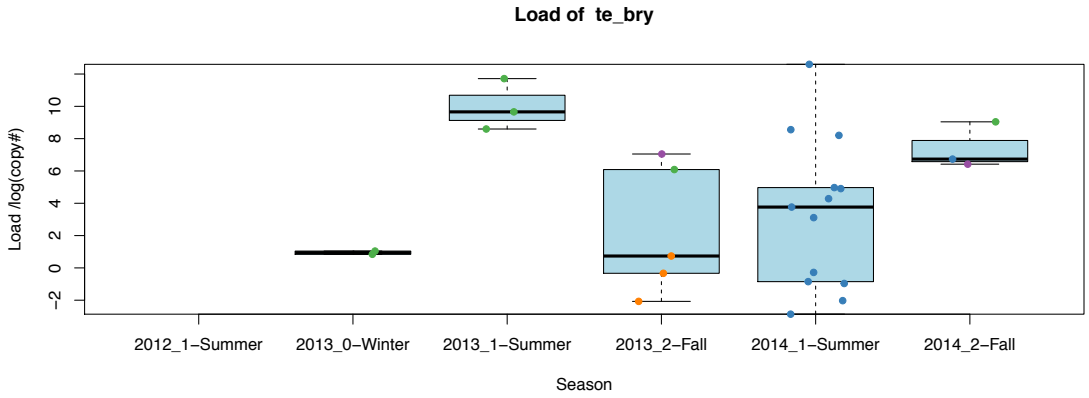

Prevalence of pa_min
Prevalence (%)
0
20
40
60
80
100
2012_1–Summer
2013_0–Winter
2013_1–Summer
2013_2–Fall
2014_1–Summer
2014_2–Fall
Season
Fraser
WCVI
ECVI
Mainland
Columbia
Washington

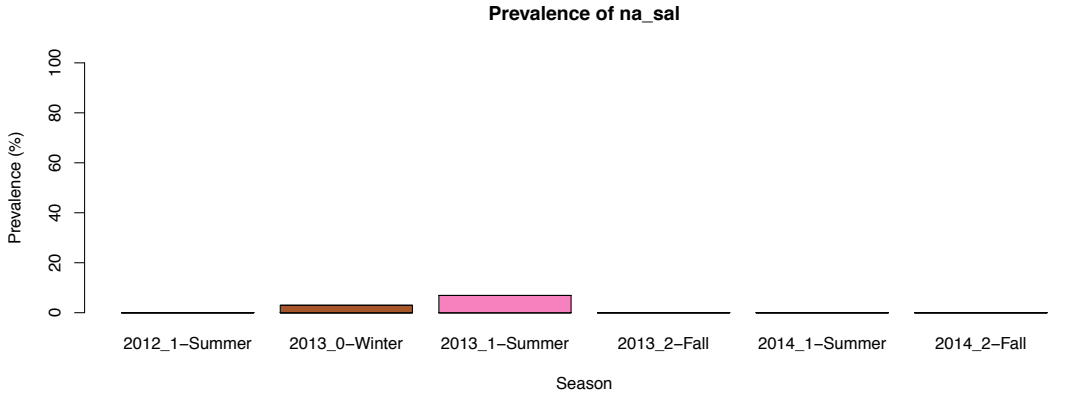

Load of pa_min
Load /log(copy#)
0
2
4
6
8
10
12
14
2012_1–Summer
2013_0–Winter
2013_1–Summer
2013_2–Fall
2014_1–Summer
2014_2–Fall
Season

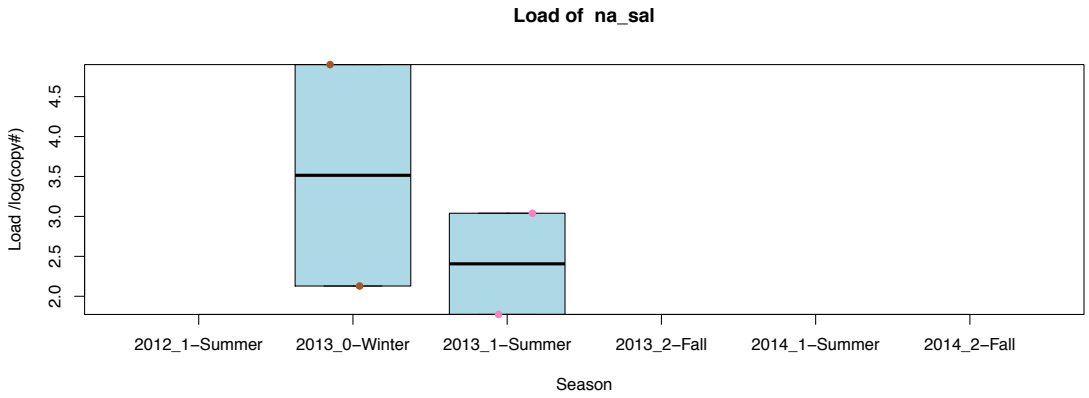

Prevalence of pa_pse
Prevalence (%)
0
20
40
60
80
100
2012_1–Summer
2013_0–Winter
2013_1–Summer
2013_2–Fall
2014_1–Summer
2014_2–Fall
Season

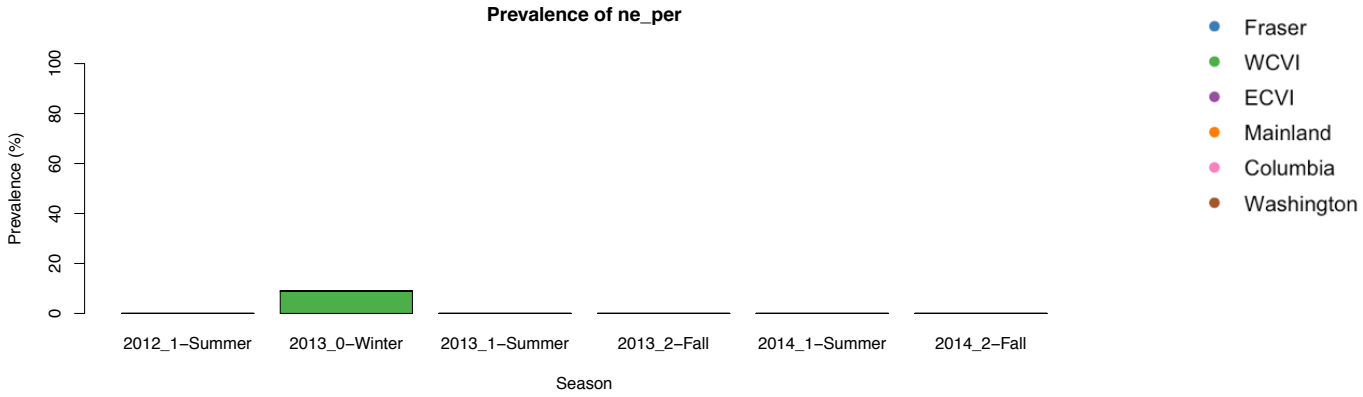

Load of pa_pse
Load /log(copy#)
2
4
6
8
2012_1–Summer
2013_0–Winter
2013_1–Summer
2013_2–Fall
2014_1–Summer
2014_2–Fall
Season

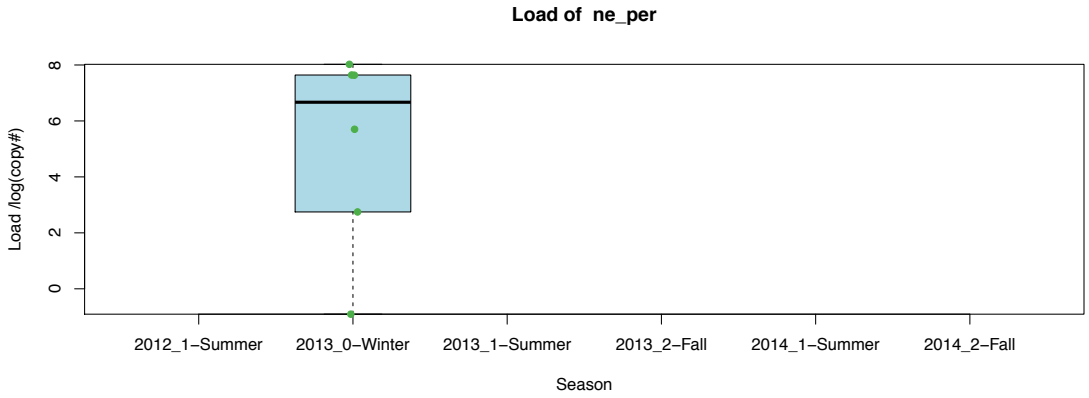

Prevalence of te_bry
Prevalence (%)
0
20
40
60
80
100
2012_1–Summer
2013_0–Winter
2013_1–Summer
2013_2–Fall
2014_1–Summer
2014_2–Fall
Season
Fraser
WCVI
ECVI
Mainland
Columbia
Washington

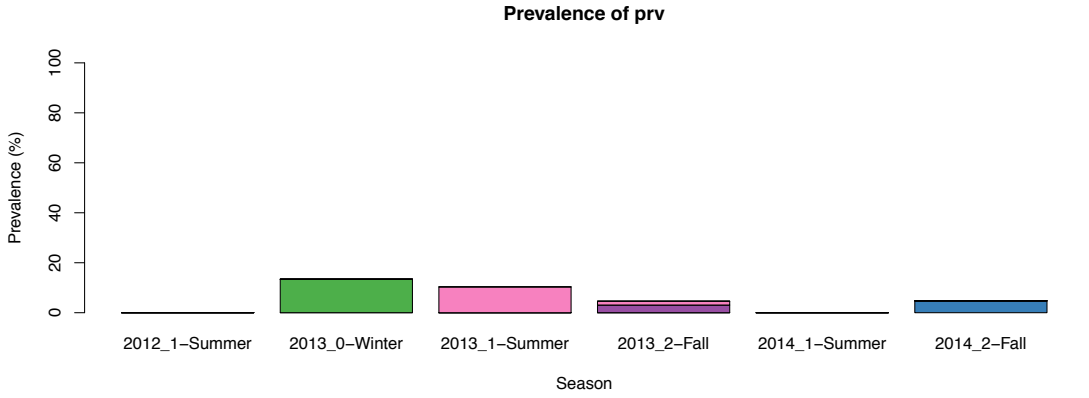

Load of te_bry
Load /log(copy#)
−2
0
2
4
6
8
10
2012_1–Summer
2013_0–Winter
2013_1–Summer
2013_2–Fall
2014_1–Summer
2014_2–Fall
Season

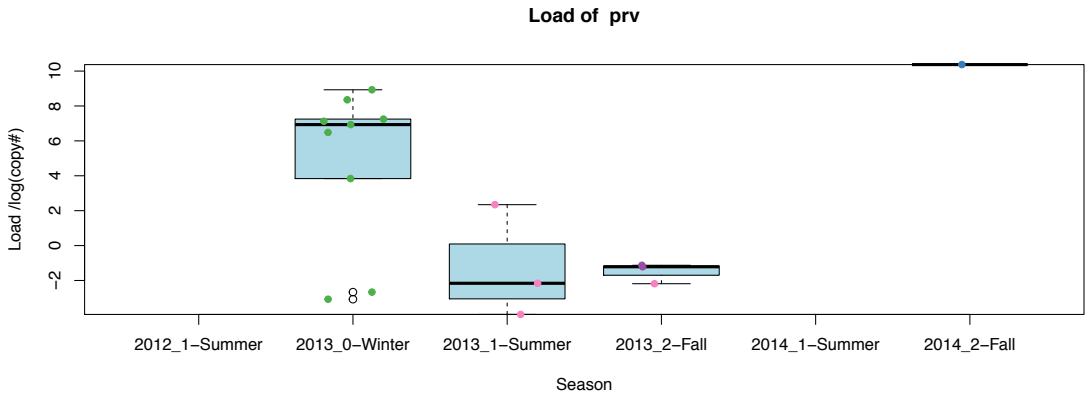

Prevalence of na_sal
Prevalence (%)
0
20
40
60
80
100
2012_1–Summer
2013_0–Winter
2013_1–Summer
2013_2–Fall
2014_1–Summer
2014_2–Fall
Season

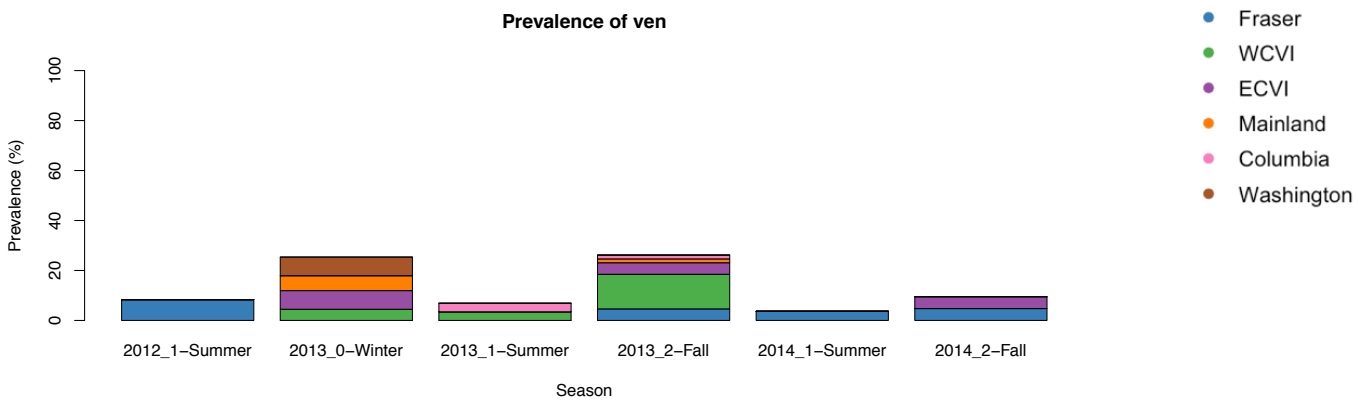

Load of na_sal
Load /log(copy#)
2.0
2.5
3.0
3.5
4.0
4.5
2012_1–Summer
2013_0–Winter
2013_1–Summer
2013_2–Fall
2014_1–Summer
2014_2–Fall
Season

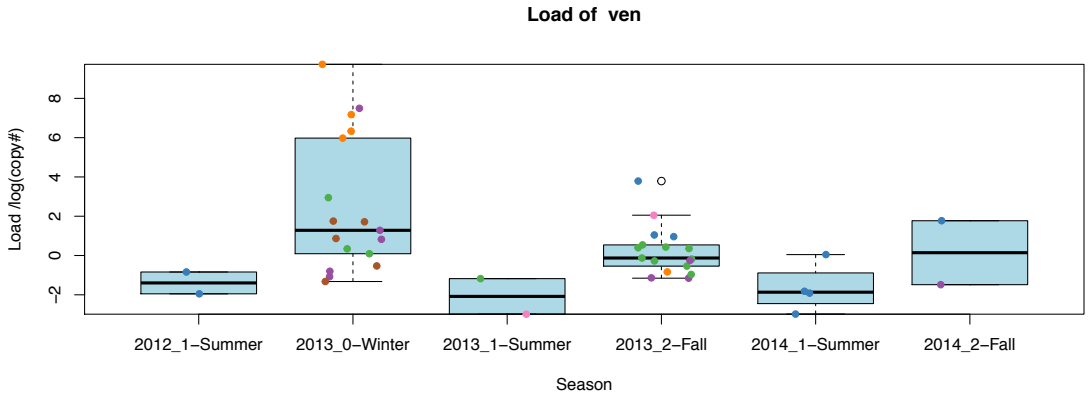

Prevalence of ne_per
Prevalence (%)
0
20
40
60
80
100
2012_1–Summer
2013_0–Winter
2013_1–Summer
2013_2–Fall
2014_1–Summer
2014_2–Fall
Season
Fraser
WCVI
ECVI
Mainland
Columbia
Washington


Load of ne_per
Load /log(copy#)
0
2
4
6
8
2012_1–Summer
2013_0–Winter
2013_1–Summer
2013_2–Fall
2014_1–Summer
2014_2–Fall
Season


Prevalence of prv
Prevalence (%)
0
20
40
60
80
100
2012_1–Summer
2013_0–Winter
2013_1–Summer
2013_2–Fall
2014_1–Summer
2014_2–Fall
Season

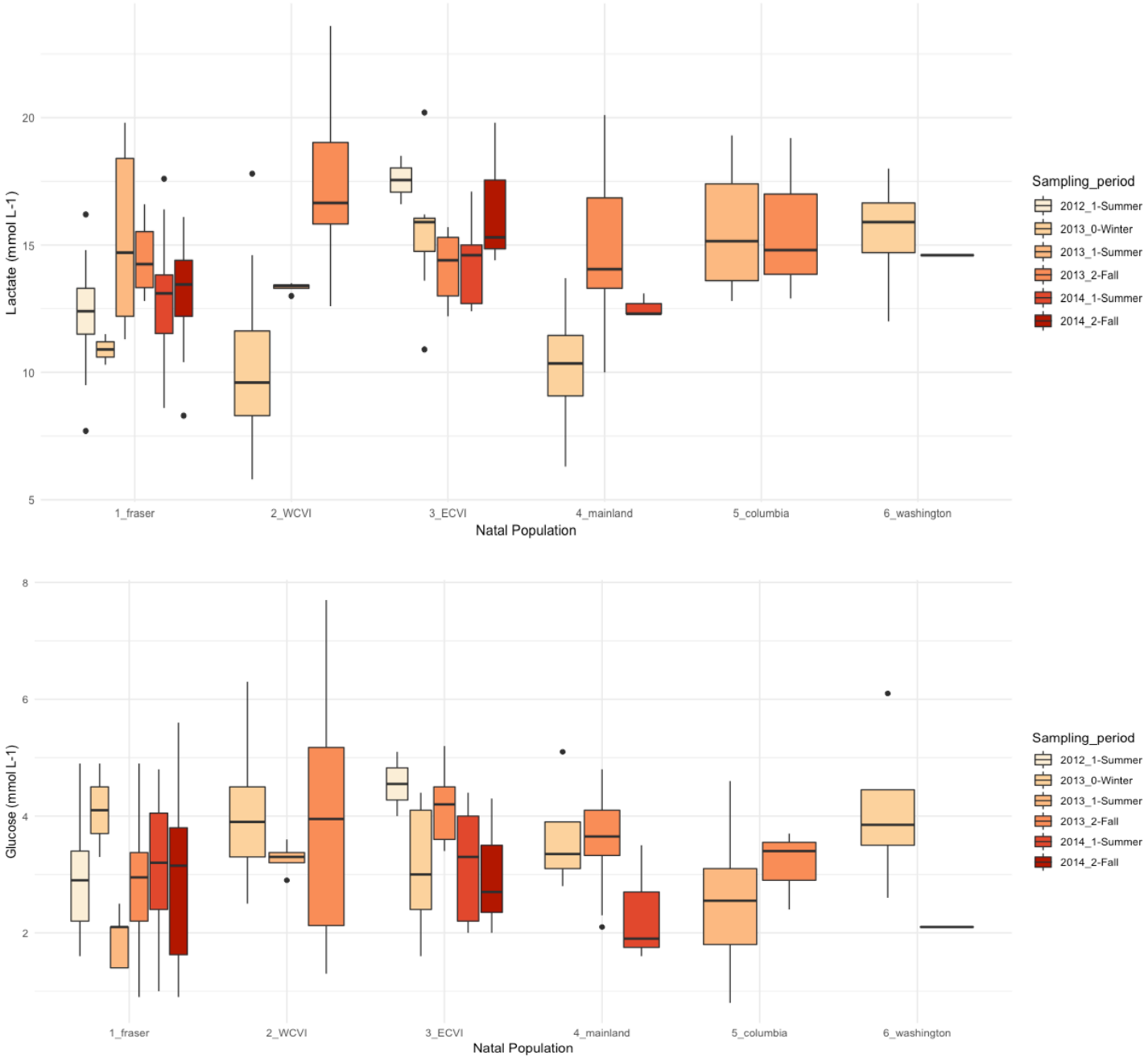

Load of prv
Load /log(copy#)
−2
0
2
4
6
8
10
2012_1–Summer
2013_0–Winter
2013_1–Summer
2013_2–Fall
2014_1–Summer
2014_2–Fall
Season

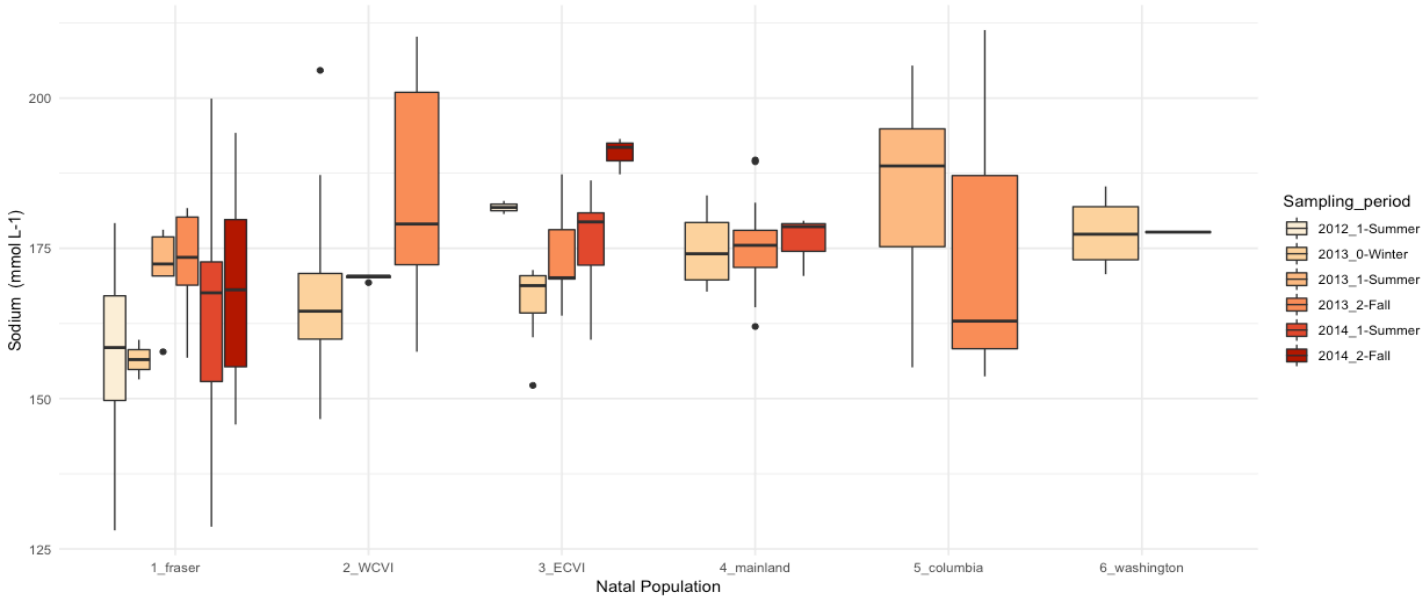

Prevalence of ven
Prevalence (%)
0
20
40
60
80
100
2012_1–Summer
2013_0–Winter
2013_1–Summer
2013_2–Fall
2014_1–Summer
2014_2–Fall
Season
Fraser
WCVI
ECVI
Mainland
Columbia
Washington

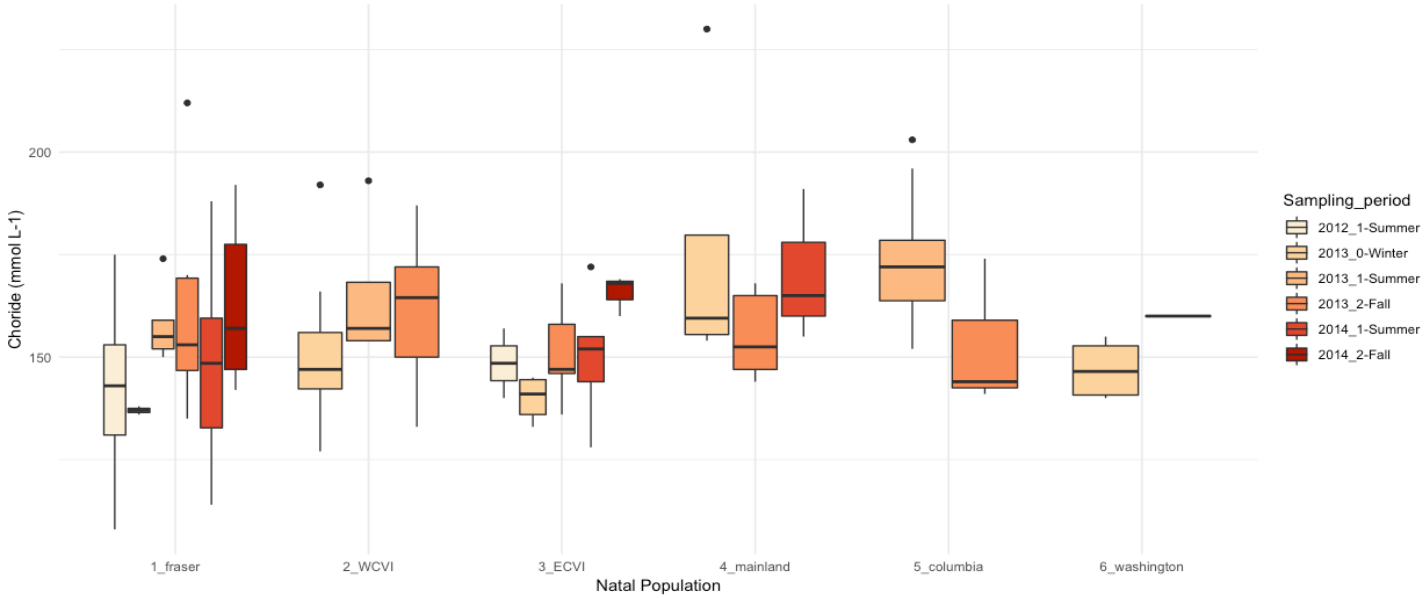

Load of ven
Load /log(copy#)
−2
0
2
4
6
8
2012_1–Summer
2013_0–Winter
2013_1–Summer
2013_2–Fall
2014_1–Summer
2014_2–Fall
Season

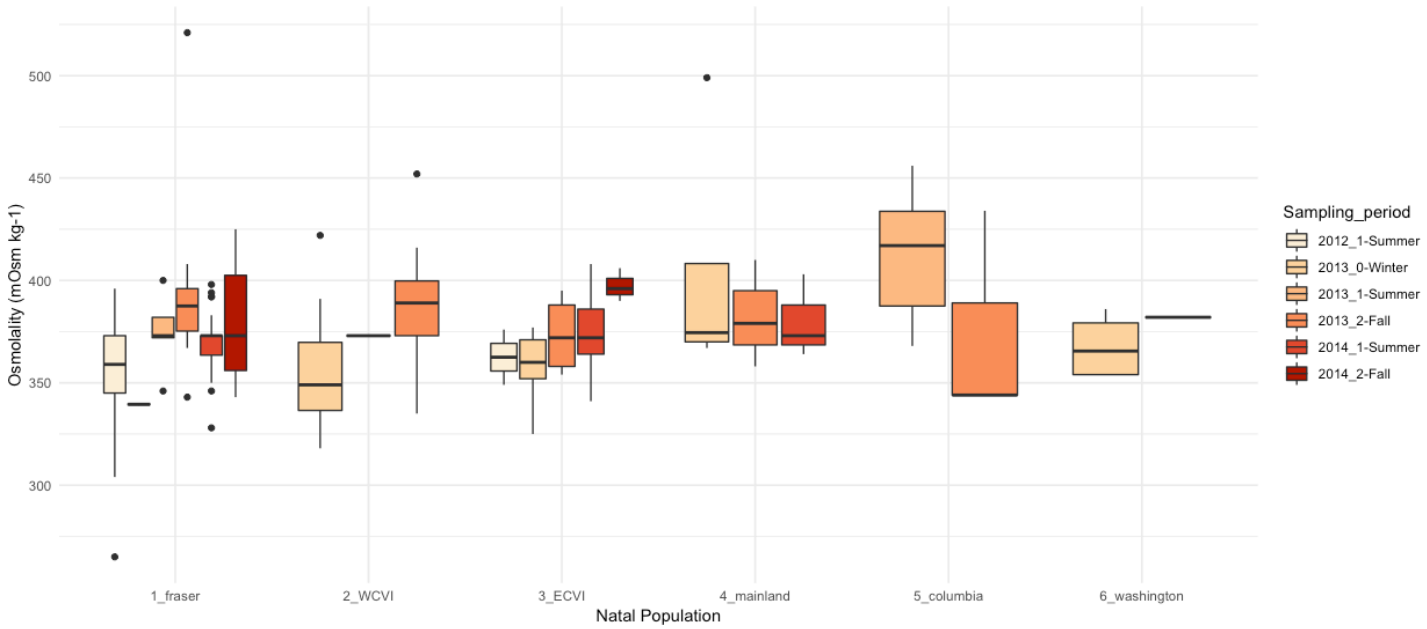
A.2.2 Five blood plasma parameters measured in juvenile Chinook salmon (*Oncorhynchus tshawytscha*) across six natal groups and six sampling periods. Error bars represent standard deviation.

Figure A.2.2.1-5 are boxplots of lactate, glucose, sodium, chloride, and osmolality level measured in blood plasma in juvenile Chinook salmon (*Oncorhynchus tshawytscha*) captured by DFO marine sampling program from 2012 to 2014. All five blood plasma parameters were influenced by natal group and sampling period except one parameter (ANOVA: Glucose ~ sampling period, F5,202= 1.763, p=0.12, details in Table 2.5).

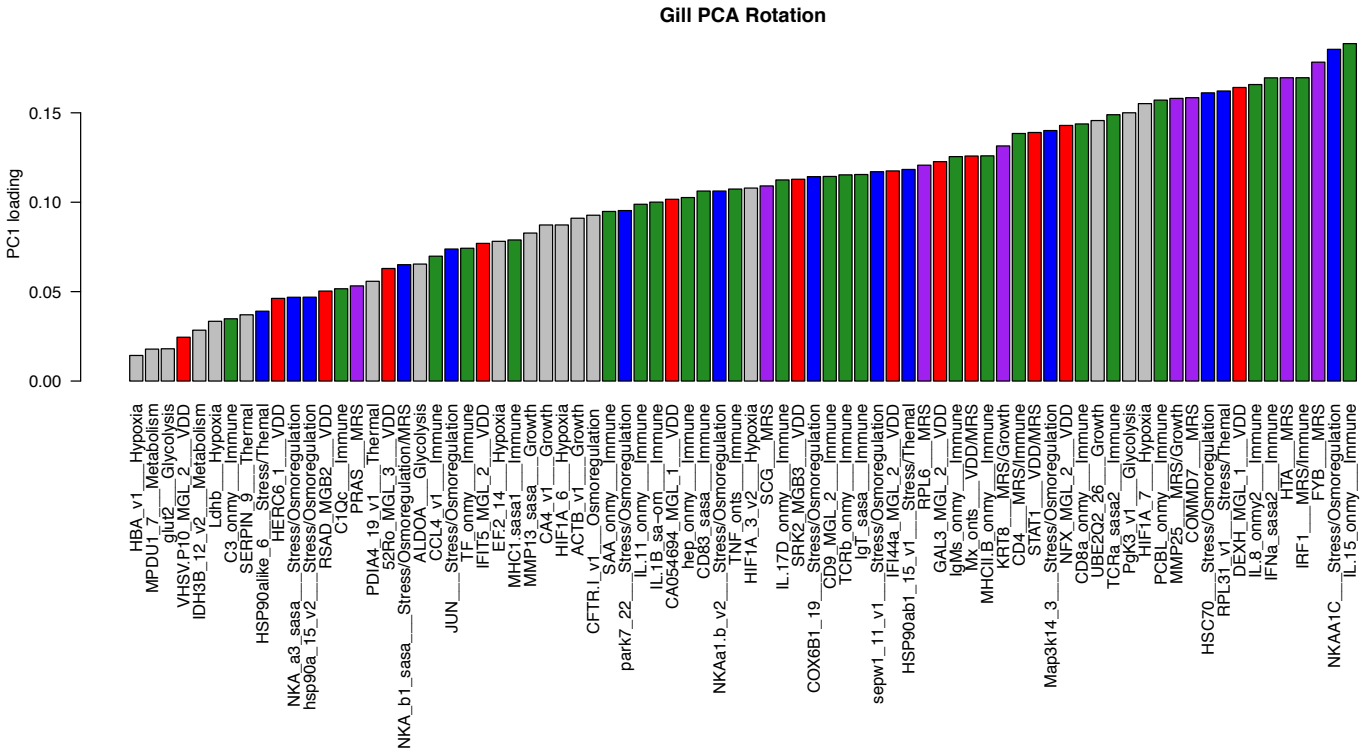


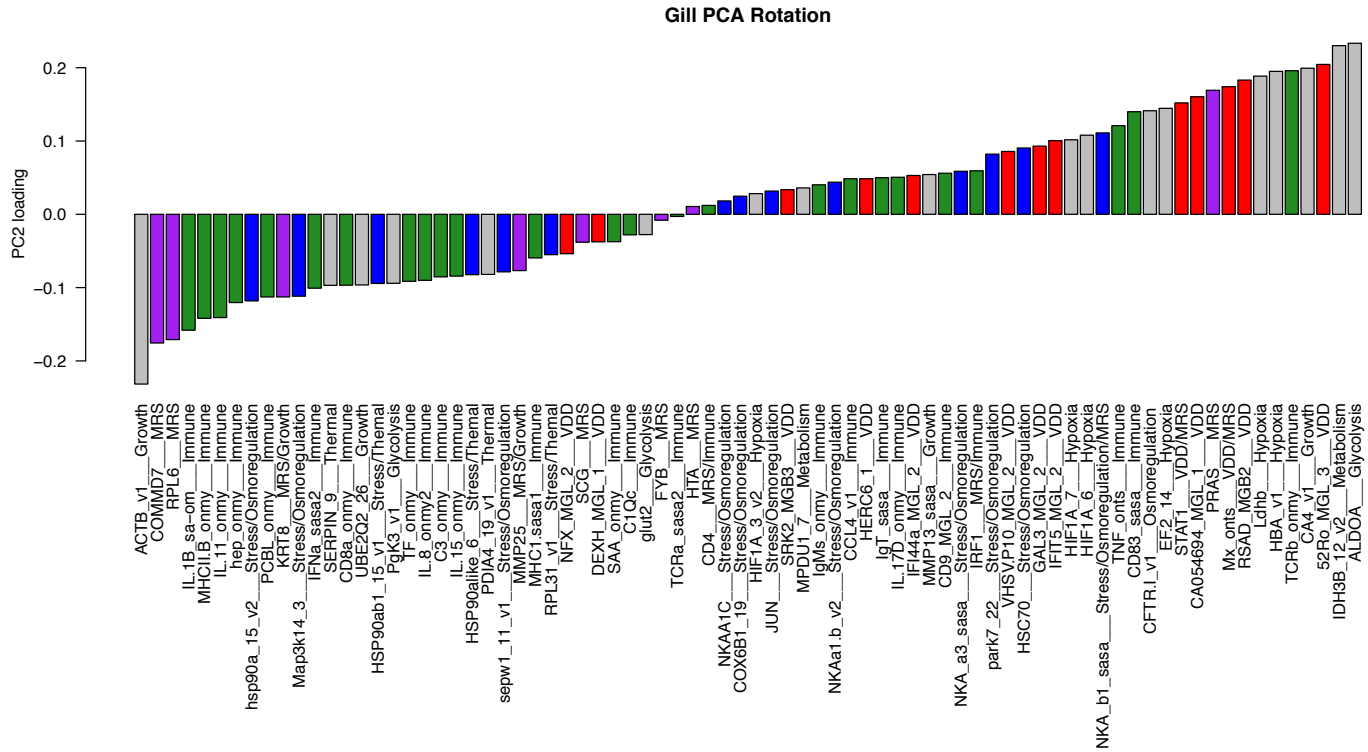

Sodium (mmol L-1)
200
175
150
125
1_fraser
2_WCVI
3_ECVI
4_mainland
5_columbia
6_washington
Natal Population
Sampling_period
2012_1-Summer
2013_0-Winter
2013_1-Summer
2013_2-Fall
2014_1-Summer
2014_2-Fall

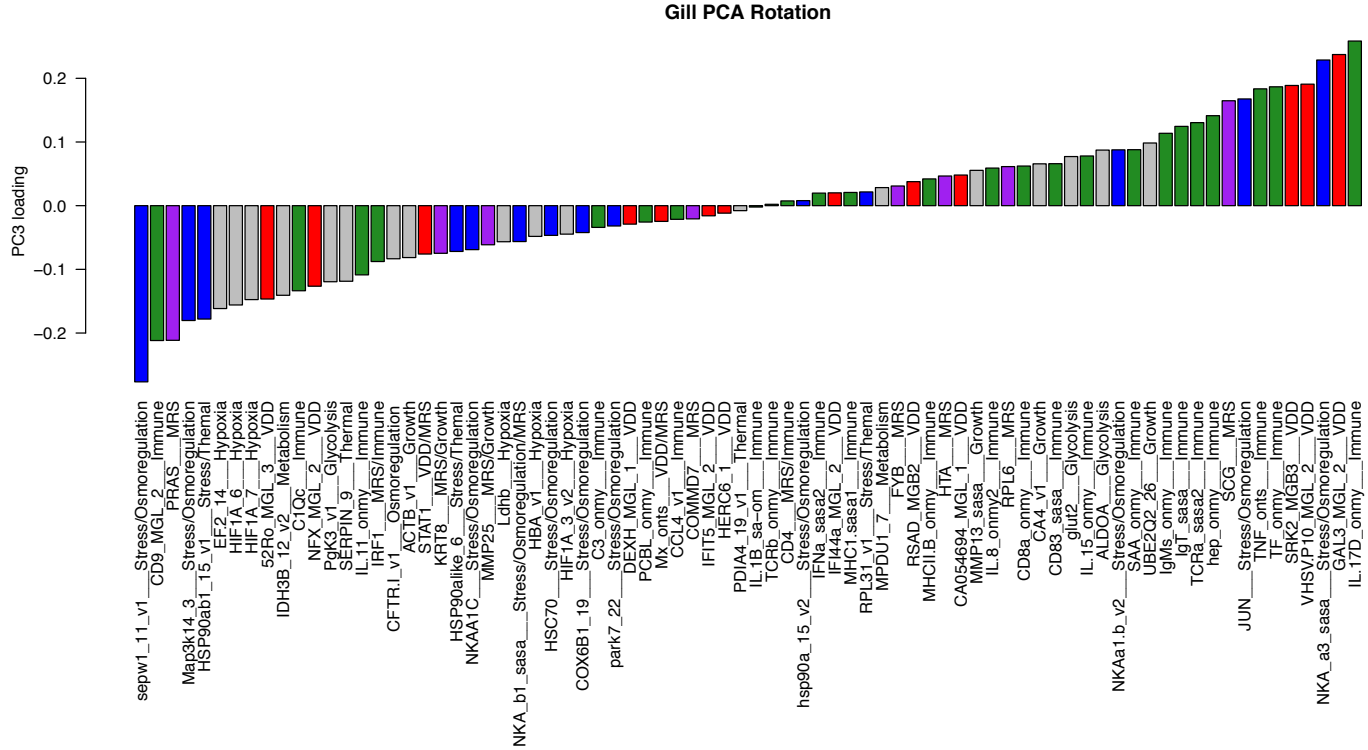

Choride (mmol L-1)
200
150
1_fraser
2_WCVI
3_ECVI
4_mainland
5_columbia
6_washington
Natal Population
Sampling_period
2012_1-Summer
2013_0-Winter
2013_1-Summer
2013_2-Fall
2014_1-Summer
2014_2-Fall

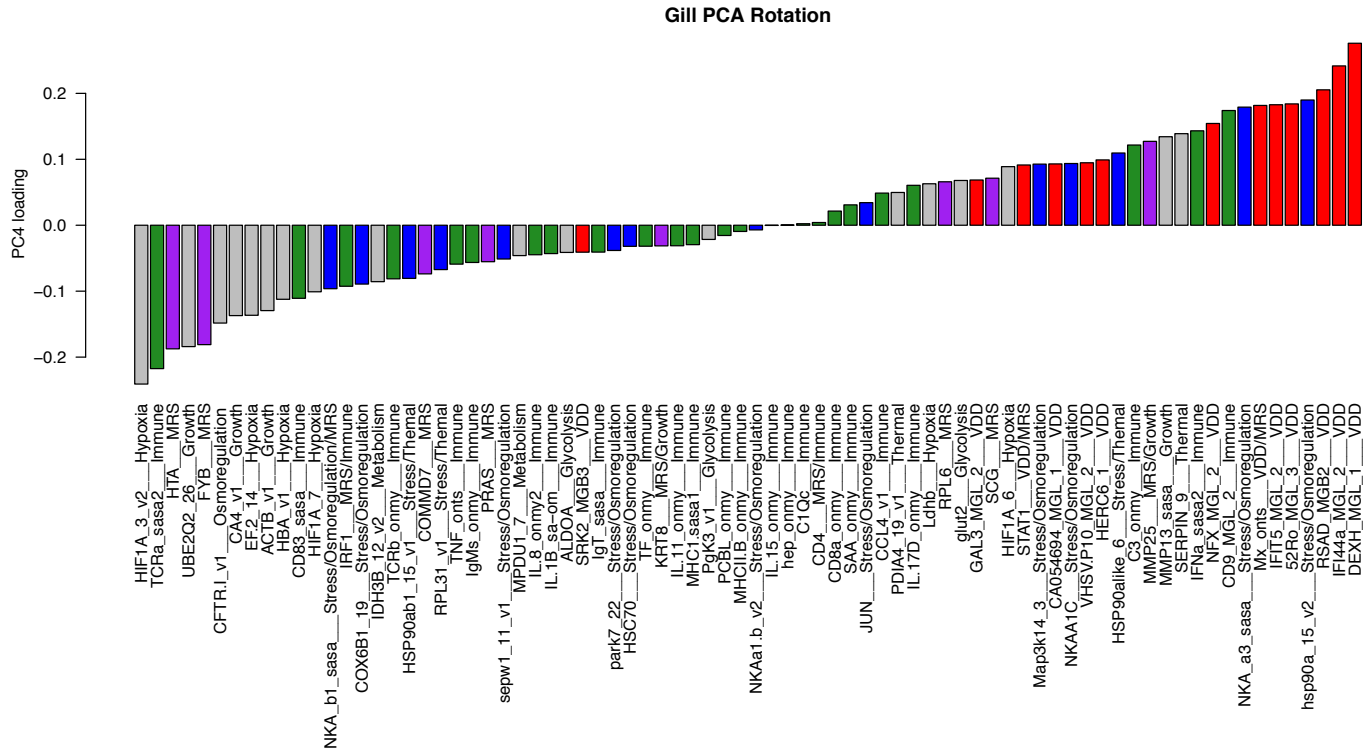

Osmolality (mOsm kg-1)
500
450
400
350
300
1_fraser
2_WCVI
3_ECVI
4_mainland
5_columbia
6_washington
Natal Population
Sampling_period
2012_1-Summer
2013_0-Winter
2013_1-Summer
2013_2-Fall
2014_1-Summer
2014_2-Fall

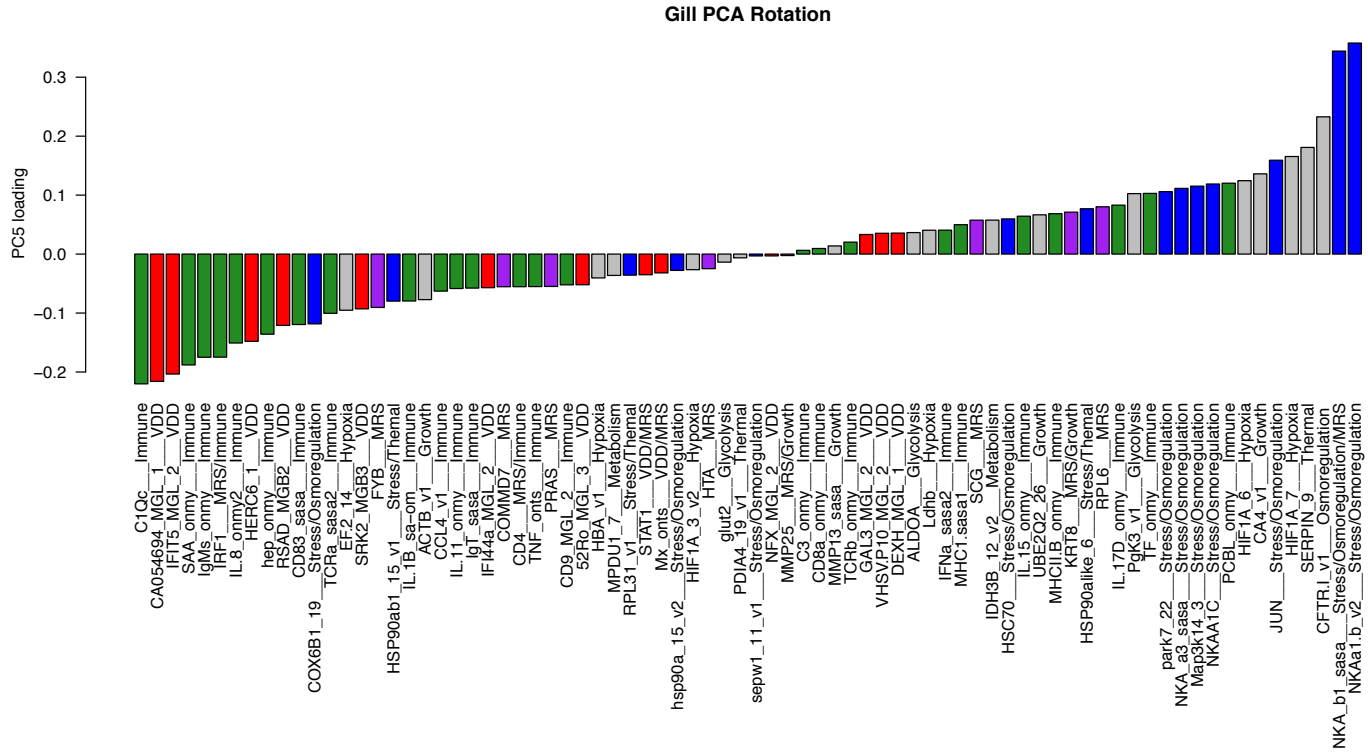
A.2.3 First five PC rotations of host gene expression PCAs in gill sample of juvenile Chinook salmon (*Oncorhynchus tshawytscha*). Host genes are colored by its primary known function: Red – Viral Disease Development (VDD), Green – Immunity, Blue – Stress, Purple –Mortality-Related Signature (MRS), Grey – other functions.



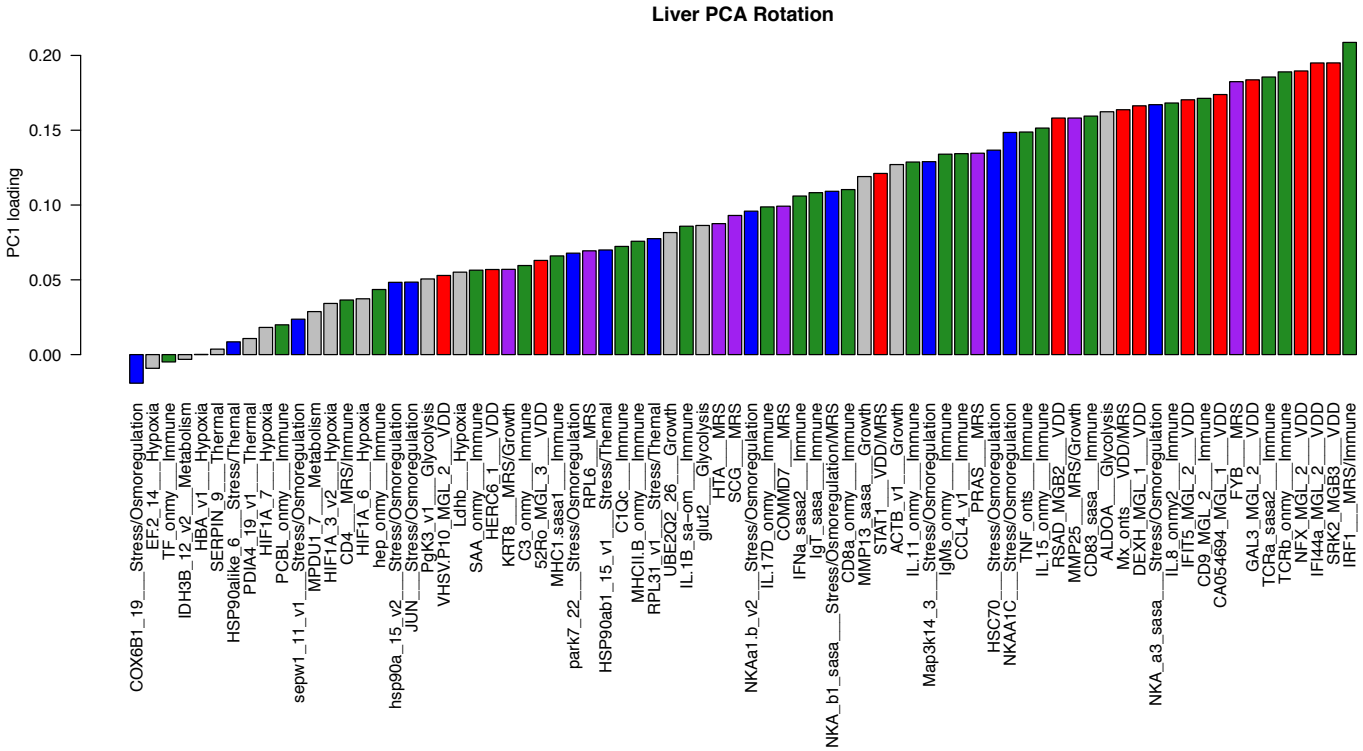

Gill PCA Rotation
PC2 loading
0.2
0.1
0.0
−0.1
−0.2
ACTB_v1___Growth
COMMD7___MRS
RPL6___MRS
IL_1B_sa−om___Immune
MHCII.B_onmy___Immune
IL_11_onmy___Immune
hep_onmy___Immune
hsp90a_15_v2___Stress/Osmoregulation
PCBL_onmy___Immune
KRT8___MRS/Growth
Map3k14_3___Stress/Osmoregulation
IFNa_sasa2___Immune
SERPIN_9___Thermal
CD8a_onmy___Immune
UBE2Q2_26___Growth
HSP90ab1_15_v1___Stress/Thermal
PgK3_v1___Glycolysis
TF_onmy___Immune
IL8_onmy2___Immune
C3_onmy___Immune
IL_15_onmy___Immune
HSP90alike_6___Stress/Thermal
PDIA4_19_v1___Thermal
sepw1_11_v1___Stress/Osmoregulation
MMP25___MRS/Growth
MHC1.sasa1___Immune
RPL31_v1___Stress/Thermal
NFX_MGL_2___VDD
SCG___MRS
DEXH_MGL_1___VDD
SAA_onmy___Immune
C1Qc___Immune
glut2___Glycolysis
FYB___MRS
TCRa_sasa2___Immune
HTA___MRS
CD4___MRS/Immune
NKAA1C___Stress/Osmoregulation
COX6B1_19___Stress/Osmoregulation
HIF1A_3_v2___Hypoxia
JUN___Stress/Osmoregulation
SRK2_MGB3___VDD
MPDU1_7___Metabolism
IgMs_onmy___Immune
NKAa1.b_v2___Stress/Osmoregulation
CCL4_v1___Immune
HERC6_1___VDD
IgT_sasa___Immune
IL_17D_onmy___Immune
IFI44a_MGL_2___VDD
MMP13_sasa___Growth
CD9_MGL_2___Immune
NKA_a3_sasa___Stress/Osmoregulation
IRF1___MRS/Immune
park7_22___Stress/Osmoregulation
VHSV.P10_MGL_2___VDD
HSC70___Stress/Osmoregulation
GAL3_MGL_2___VDD
IFIT5_MGL_2___VDD
HIF1A_7___Hypoxia
HIF1A_6___Hypoxia
NKA_b1_sasa___Stress/Osmoregulation/MRS
TNF_onts___Immune
CD83_sasa___Immune
CFTR.I_v1___Osmoregulation
EF2_14___Hypoxia
STAT1___VDD/MRS
CA054694_MGL_1___VDD
PRAS___MRS
Mx_onts___VDD/MRS
RSAD_MGB2___VDD
Ldhb___Hypoxia
HBA_v1___Hypoxia
TCRb_onmy___Immune
CA4_v1___Growth
52Ro_MGL_3___VDD
IDH3B_12_v2___Metabolism
ALDOA___Glycolysis

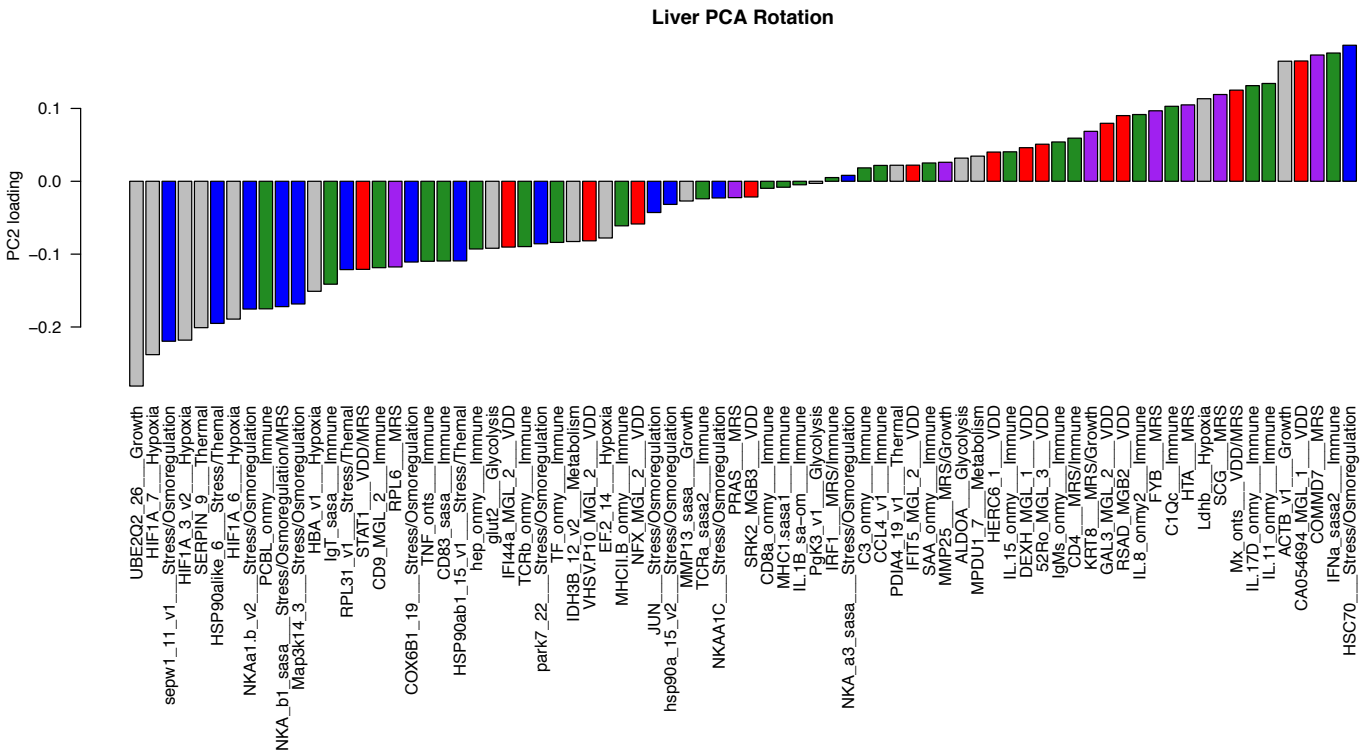

Gill PCA Rotation
PC3 loading
0.2
0.1
0.0
−0.1
−0.2
sepw1_11_v1___Stress/Osmoregulation
CD9_MGL_2___Immune
PRAS___MRS
Map3k14_3___Stress/Osmoregulation
HSP90ab1_15_v1___Stress/Thermal
EF2_14___Hypoxia
HIF1A_6___Hypoxia
HIF1A_7___Hypoxia
52Ro_MGL_3___VDD
IDH3B_12_v2___Metabolism
C1Qc___Immune
NFX_MGL_2___VDD
PgK3_v1___Glycolysis
SERPIN_9___Thermal
IL_11_onmy___Immune
IRF1___MRS/Immune
CFTR.I_v1___Osmoregulation
ACTB_v1___Growth
STAT1___VDD/MRS
KRT8___MRS/Growth
HSP90alike_6___Stress/Thermal
NKAA1C___Stress/Osmoregulation
MMP25___MRS/Growth
Ldhb___Hypoxia
NKA_b1_sasa___Stress/Osmoregulation/MRS
HBA_v1___Hypoxia
HSC70___Stress/Osmoregulation
HIF1A_3_v2___Hypoxia
COX6B1_19___Stress/Osmoregulation
C3_onmy___Immune
park7_22___Stress/Osmoregulation
DEXH_MGL_1___VDD
PCBL_onmy___Immune
Mx_onts___VDD/MRS
CCL4_v1___Immune
COMMD7___MRS
IFIT5_MGL_2___VDD
HERC6_1___VDD
PDIA4_19_v1___Thermal
IL_1B_sa−om___Immune
TCRb_onmy___Immune
CD4___MRS/Immune
hsp90a_15_v2___Stress/Osmoregulation
IFNa_sasa2___Immune
IFI44a_MGL_2___VDD
MHC1.sasa1___Immune
RPL31_v1___Stress/Thermal
MPDU1_7___Metabolism
FYB___MRS
RSAD_MGB2___VDD
MHCII.B_onmy___Immune
HTA___MRS
CA054694_MGL_1___VDD
MMP13_sasa___Growth
IL_8_onmy2___Immune
RPL6___MRS
CD8a_onmy___Immune
CA4_v1___Growth
CD83_sasa___Immune
glut2___Glycolysis
IL_15_onmy___Immune
ALDOA___Glycolysis
NKAa1.b_v2___Stress/Osmoregulation
SAA_onmy___Immune
UBE2Q2_26___Growth
IgMs_onmy___Immune
IgT_sasa___Immune
TCRa_sasa2___Immune
hep_onmy___Immune
SCG___MRS
JUN___Stress/Osmoregulation
TNF_onts___Immune
TF_onmy___Immune
SRK2_MGB3___VDD
VHSV.P10_MGL_2___VDD
NKA_a3_sasa___Stress/Osmoregulation
GAL3_MGL_2___VDD
IL_17D_onmy___Immune

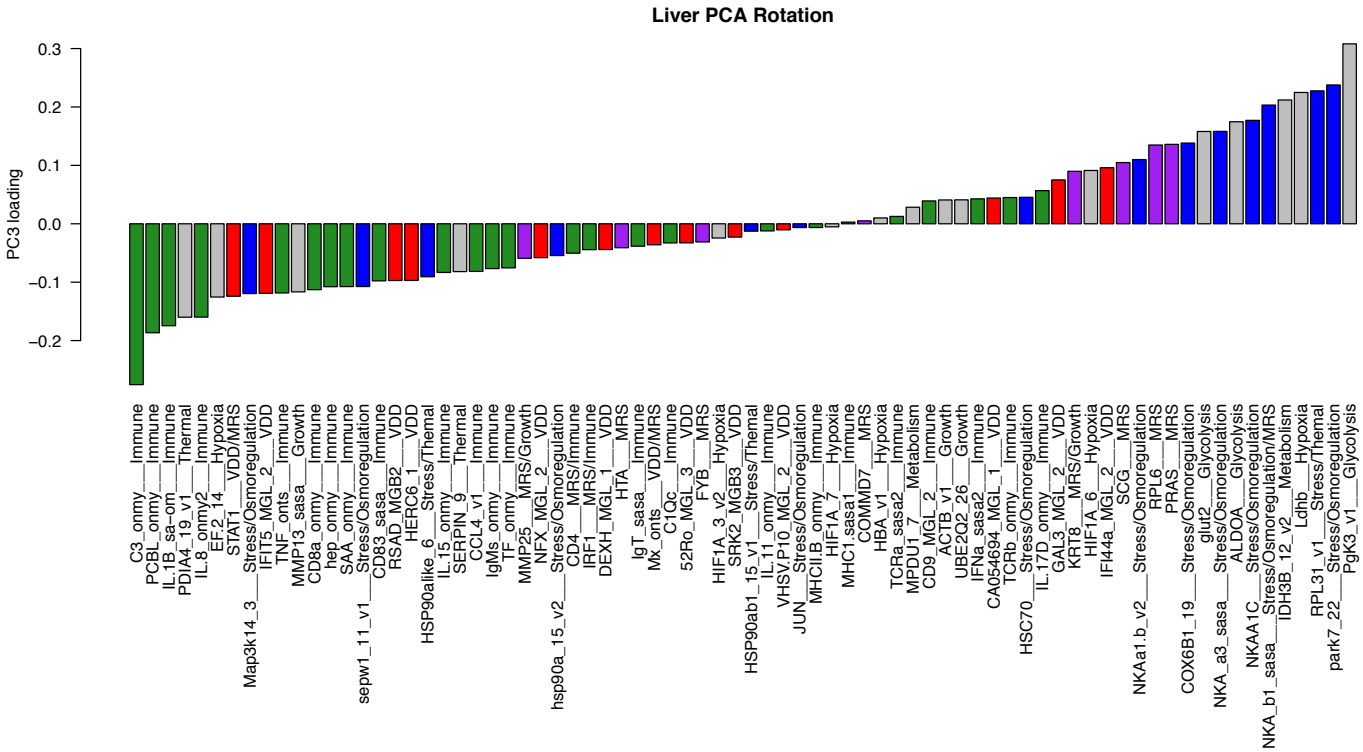

Gill PCA Rotation
PC4 loading
0.2
0.1
0.0
−0.1
−0.2
HIF1A_3_v2___Hypoxia
TCRa_sasa2___Immune
HTA___MRS
UBE2Q2_26___Growth
FYB___MRS
CFTR.I_v1___Osmoregulation
CA4_v1___Growth
EF2_14___Hypoxia
ACTB_v1___Growth
HBA_v1___Hypoxia
CD83_sasa___Immune
HIF1A_7___Hypoxia
NKA_b1_sasa___Stress/Osmoregulation/MRS
IRF1___MRS/Immune
COX6B1_19___Stress/Osmoregulation
IDH3B_12_v2___Metabolism
TCRb_onmy___Immune
HSP90ab1_15_v1___Stress/Thermal
COMMD7___MRS
RPL31_v1___Stress/Thermal
TNF_onts___Immune
IgMs_onmy___Immune
PRAS___MRS
sepw1_11_v1___Stress/Osmoregulation
MPDU1_7___Metabolism
IL.8_onmy2___Immune
IL.1B_sa-om___Immune
ALDOA___Glycolysis
SRK2_MGB3___VDD
IgT_sasa___Immune
park7_22___Stress/Osmoregulation
HSC70___Stress/Osmoregulation
TF_onmy___Immune
KRT8___MRS/Growth
IL.11_onmy___Immune
MHCI1.sasa1___Immune
PgK3_v1___Glycolysis
PCBL_onmy___Immune
MHCII.B_onmy___Immune
NKAa1.b_v2___Stress/Osmoregulation
IL.15_onmy___Immune
hep_onmy___Immune
C1Qc___Immune
CD4___MRS/Immune
CD8a_onmy___Immune
SAA_onmy___Immune
JUN___Stress/Osmoregulation
CCL4_v1___Immune
PDIA4.19_v1___Thermal
IL.17D_onmy___Immune
Ldhb___Hypoxia
RPL6___MRS
glut2___Glycolysis
GAL3_MGL_2___VDD
SCG___MRS
HIF1A_6___Hypoxia
STAT1___VDD/MRS
Map3k14_3___Stress/Osmoregulation
CA054694_MGL_1___VDD
NKAA1C___Stress/Osmoregulation
VHSV.P10_MGL_2___VDD
HERC6_1___VDD
HSP90alike_6___Stress/Thermal
C3_onmy___Immune
MMP25___MRS/Growth
MMP13_sasa___Growth
SERPIN_9___Thermal
IFNa_sasa2___Immune
NFX_MGL_2___VDD
CD9_MGL_2___Immune
NKA_a3_sasa___Stress/Osmoregulation
Mx_onts___VDD/MRS
IFIT5_MGL_2___VDD
52Ro_MGL_3___VDD
hsp90a_15_v2___Stress/Osmoregulation
RSAD_MGB2___VDD
IFI44a_MGL_2___VDD
DEXH_MGL_1___VDD

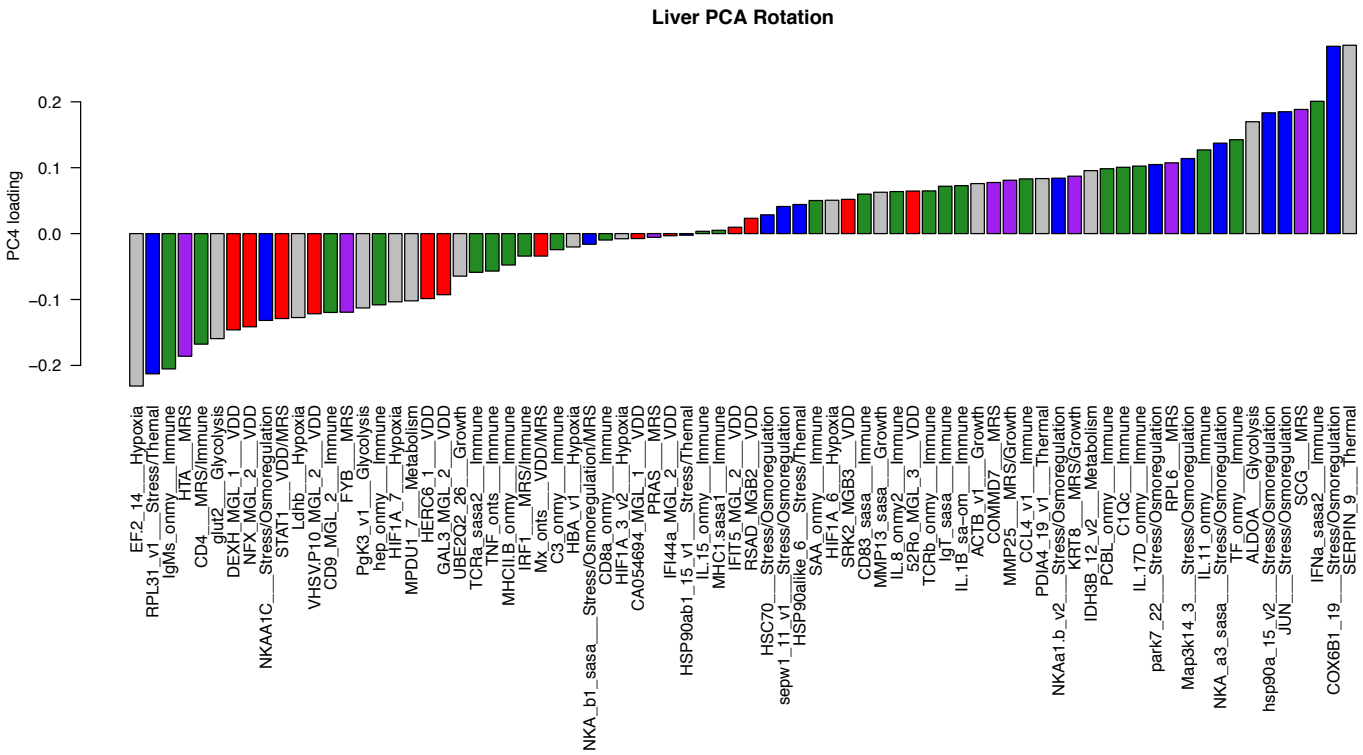

Gill PCA Rotation
PC5 loading
0.3
0.2
0.1
0.0
−0.1
−0.2
C1Qc___Immune
CA054694_MGL_1___VDD
IFIT5_MGL_2___VDD
SAA_onmy___Immune
IgMs_onmy___Immune
IRF1___MRS/Immune
IL.8_onmy2___Immune
HERC6_1___VDD
hep_onmy___Immune
RSAD_MGB2___VDD
CD83_sasa___Immune
COX6B1_19___Stress/Osmoregulation
TCRa_sasa2___Immune
EF2_14___Hypoxia
SRK2_MGB3___VDD
FYB___MRS
HSP90ab1_15_v1___Stress/Thermal
IL.1B_sa-om___Immune
ACTB_v1___Growth
CCL4_v1___Immune
IL.11_onmy___Immune
IgT_sasa___Immune
IFI44a_MGL_2___VDD
COMMD7___MRS
CD4___MRS/Immune
TNF_onts___Immune
PRAS___MRS
CD9_MGL_2___Immune
52Ro_MGL_3___VDD
HBA_v1___Hypoxia
MPDU1_7___Metabolism
RPL31_v1___Stress/Thermal
STAT1___VDD/MRS
Mx_onts___VDD/MRS
hsp90a_15_v2___Stress/Osmoregulation
HIF1A_3_v2___Hypoxia
HTA___MRS
glut2___Glycolysis
PDIA4.19_v1___Thermal
sepw1_11_v1___Stress/Osmoregulation
NFX_MGL_2___VDD
MMP25___MRS/Growth
C3_onmy___Immune
CD8a_onmy___Immune
MMP13_sasa___Growth
TCRb_onmy___Immune
GAL3_MGL_2___VDD
VHSV.P10_MGL_2___VDD
DEXH_MGL_1___VDD
ALDOA___Glycolysis
Ldhb___Hypoxia
IFNa_sasa2___Immune
MHCI1.sasa1___Immune
SCG___MRS
IDH3B_12_v2___Metabolism
HSC70___Stress/Osmoregulation
IL.15_onmy___Immune
UBE2Q2_26___Growth
MHCII.B_onmy___Immune
KRT8___MRS/Growth
HSP90alike_6___Stress/Thermal
RPL6___MRS
IL.17D_onmy___Immune
PgK3_v1___Glycolysis
TF_onmy___Immune
park7_22___Stress/Osmoregulation
NKA_a3_sasa___Stress/Osmoregulation
Map3k14_3___Stress/Osmoregulation
NKAA1C___Stress/Osmoregulation
PCBL_onmy___Immune
HIF1A_6___Hypoxia
CA4_v1___Growth
JUN___Stress/Osmoregulation
HIF1A_7___Hypoxia
SERPIN_9___Thermal
CFTR.I_v1___Osmoregulation
NKA_b1_sasa___Stress/Osmoregulation/MRS
NKAa1.b_v2___Stress/Osmoregulation

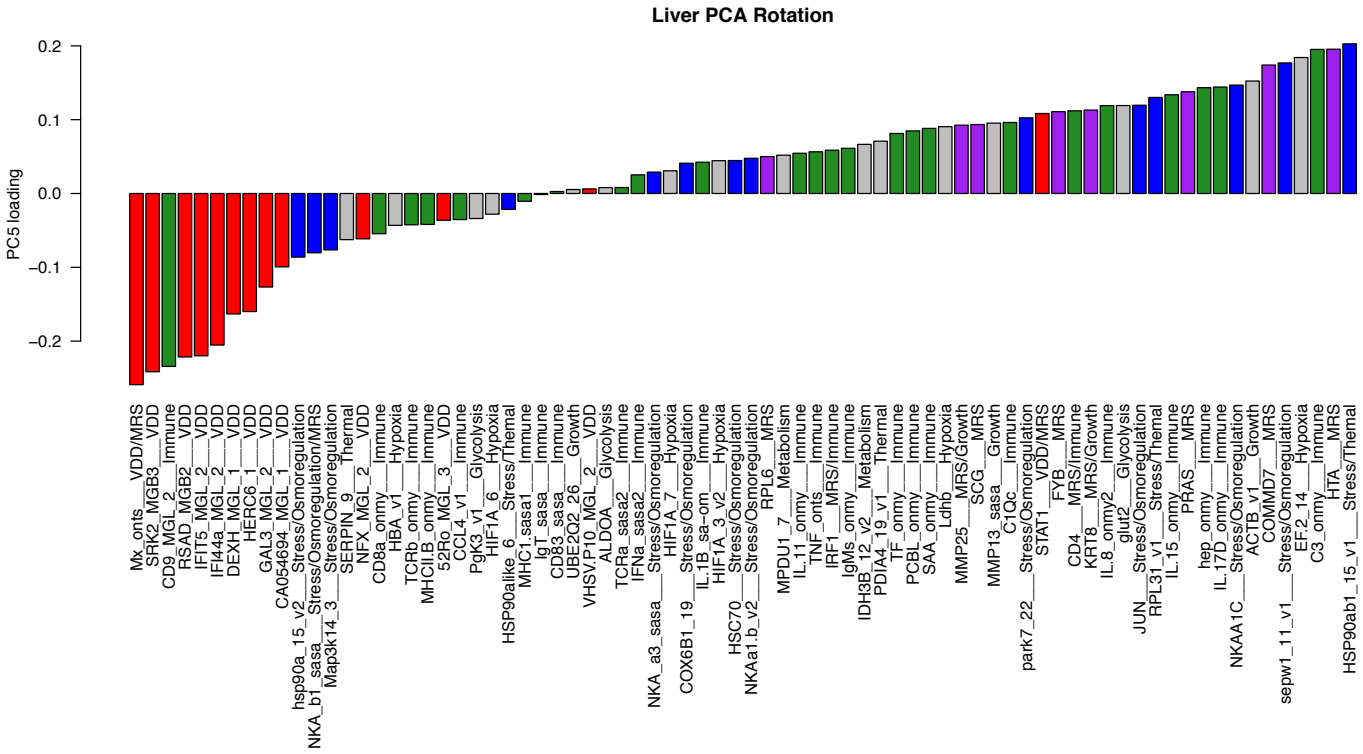
A.2.4 First five PC rotations of host gene expression PCAs in liver sample of juvenile Chinook salmon (*Oncorhynchus tshawytscha*). Host genes are colored by its primary known function: Red – Viral Disease Development (VDD), Green – Immunity, Blue – Stress, Purple –Mortality-Related Signature (MRS), Grey – other functions.




Liver PCA Rotation
PC2 loading
0.1
0.0
−0.1
−0.2
UBE2Q2_26___Growth
HIF1A_7___Hypoxia
sepw1_11_v1___Stress/Osmoregulation
HIF1A_3_v2___Hypoxia
SERPIN_9___Thermal
HSP90alike_6___Stress/Thermal
HIF1A_6___Hypoxia
NKAa1.b_v2___Stress/Osmoregulation
PCBL_onmy___Immune
NKA_b1_sasa___Stress/Osmoregulation/MRS
Map3k14_3___Stress/Osmoregulation
HBA_v1___Hypoxia
IgT_sasa___Immune
RPL31_v1___Stress/Thermal
STAT1___VDD/MRS
CD9_MGL_2___Immune
RPL6___MRS
COX6B1_19___Stress/Osmoregulation
TNF_onts___Immune
CD83_sasa___Immune
HSP90ab1_15_v1___Stress/Thermal
hep_onmy___Immune
glut2___Glycolysis
IFI44a_MGL_2___VDD
TCRb_onmy___Immune
park7_22___Stress/Osmoregulation
TF_onmy___Immune
IDH3B_12_v2___Metabolism
VHSV.P10_MGL_2___VDD
EF2.14___Hypoxia
MHCII.B_onmy___Immune
NFX_MGL_2___VDD
JUN___Stress/Osmoregulation
hsp90a_15_v2___Stress/Osmoregulation
MMP13_sasa___Growth
TCRa_sasa2___Immune
NKAA1C___Stress/Osmoregulation
PRAS___MRS
SRK2_MGB3___VDD
CD8a_onmy___Immune
MHC1.sasa1___Immune
IL_1B_sa−om___Immune
PgK3_v1___Glycolysis
IRF1___MRS/Immune
NKA_a3_sasa___Stress/Osmoregulation
C3_onmy___Immune
CCL4_v1___Immune
PDIA4_19_v1___Thermal
IFIT5_MGL_2___VDD
SAA_onmy___Immune
MMP25___MRS/Growth
ALDOA___Glycolysis
MPDU1_7___Metabolism
HERC6_1___VDD
IL_15_onmy___Immune
DEXH_MGL_1___VDD
52Ro_MGL_3___VDD
IgMs_onmy___Immune
CD4___MRS/Immune
KRT8___MRS/Growth
GAL3_MGL_2___VDD
RSAD_MGB2___VDD
IL_8_onmy2___Immune
FYB___MRS
C1Qc___Immune
HTA___MRS
Ldhb___Hypoxia
SCG___MRS
Mx_onts___VDD/MRS
IL_17D_onmy___Immune
IL_11_onmy___Immune
ACTB_v1___Growth
CA054694_MGL_1___VDD
COMMD7___MRS
IFNa_sasa2___Immune
HSC70___Stress/Osmoregulation


Liver PCA Rotation
PC3 loading
0.3
0.2
0.1
0.0
−0.1
−0.2
C3_onmy___Immune
PCBL_onmy___Immune
IL_1B_sa−om___Immune
PDIA4_19_v1___Thermal
IL_8_onmy2___Immune
EF2.14___Hypoxia
STAT1___VDD/MRS
Map3k14_3___Stress/Osmoregulation
IFIT5_MGL_2___VDD
TNF_onts___Immune
MMP13_sasa___Growth
CD8a_onmy___Immune
hep_onmy___Immune
SAA_onmy___Immune
sepw1_11_v1___Stress/Osmoregulation
CD83_sasa___Immune
RSAD_MGB2___VDD
HERC6_1___VDD
HSP90alike_6___Stress/Thermal
IL_15_onmy___Immune
SERPIN_9___Thermal
CCL4_v1___Immune
IgMs_onmy___Immune
TF_onmy___Immune
MMP25___MRS/Growth
NFX_MGL_2___VDD
hsp90a_15_v2___Stress/Osmoregulation
CD4___MRS/Immune
IRF1___MRS/Immune
DEXH_MGL_1___VDD
HTA___MRS
IgT_sasa___Immune
Mx_onts___VDD/MRS
C1Qc___Immune
52Ro_MGL_3___VDD
FYB___MRS
HIF1A_3_v2___Hypoxia
SRK2_MGB3___VDD
HSP90ab1_15_v1___Stress/Thermal
IL_11_onmy___Immune
VHSV.P10_MGL_2___VDD
JUN___Stress/Osmoregulation
MHCII.B_onmy___Immune
HIF1A_7___Hypoxia
MHC1.sasa1___Immune
COMMD7___MRS
HBA_v1___Hypoxia
TCRa_sasa2___Immune
MPDU1_7___Metabolism
CD9_MGL_2___Immune
ACTB_v1___Growth
UBE2Q2_26___Growth
IFNa_sasa2___Immune
CA054694_MGL_1___VDD
TCRb_onmy___Immune
HSC70___Stress/Osmoregulation
IL_17D_onmy___Immune
GAL3_MGL_2___VDD
KRT8___MRS/Growth
HIF1A_6___Hypoxia
IFI44a_MGL_2___VDD
SCG___MRS
NKAa1.b_v2___Stress/Osmoregulation
RPL6___MRS
PRAS___MRS
COX6B1_19___Stress/Osmoregulation
glut2___Glycolysis
NKA_a3_sasa___Stress/Osmoregulation
ALDOA___Glycolysis
NKAA1C___Stress/Osmoregulation
NKA_b1_sasa___Stress/Osmoregulation/MRS
IDH3B_12_v2___Metabolism
Ldhb___Hypoxia
RPL31_v1___Stress/Thermal
park7_22___Stress/Osmoregulation
PgK3_v1___Glycolysis


Liver PCA Rotation
PC4 loading
0.2
0.1
0.0
−0.1
−0.2
EF2_14___Hypoxia
RPL31_v1___Stress/Themal
IgMs_onmy___Immune
HTA___MRS
CD4___MRS/Immune
glut2___Glycolysis
DEXH_MGL_1___VDD
NFX_MGL_2___VDD
NKAA1C___Stress/Osmoregulation
STAT1___VDD/MRS
Ldhb___Hypoxia
VHSV.P10_MGL_2___VDD
CD9_MGL_2___Immune
FYB___MRS
PgK3_v1___Glycolysis
hep_onmy___Immune
HIF1A_7___Hypoxia
MPDU1_7___Metabolism
HERC6_1___VDD
GAL3_MGL_2___VDD
UBE2Q2_26___Growth
TCRa_sasa2___Immune
TNF_onts___Immune
MHCII.B_onmy___Immune
IRF1___MRS/Immune
Mx_onts___VDD/MRS
C3_onmy___Immune
HBA_v1___Hypoxia
NKA_b1_sasa___Stress/Osmoregulation/MRS
CD8a_onmy___Immune
HIF1A_3_v2___Hypoxia
CA054694_MGL_1___VDD
PRAS___MRS
IFI44a_MGL_2___VDD
HSP90ab1_15_v1___Stress/Themal
IL.15_onmy___Immune
MHC1.sasa1___Immune
IFIT5_MGL_2___VDD
RSAD_MGB2___VDD
HSC70___Stress/Osmoregulation
sepw1_11_v1___Stress/Osmoregulation
HSP90alike_6___Stress/Themal
SAA_onmy___Immune
HIF1A_6___Hypoxia
SRK2_MGB3___VDD
CD83_sasa___Immune
MMP13_sasa___Growth
IL.8_onmy2___Immune
52Ro_MGL_3___VDD
TCRb_onmy___Immune
IgT_sasa___Immune
IL.1B_sa−om___Immune
ACTB_v1___Growth
COMMD7___MRS
MMP25___MRS/Growth
CCL4_v1___Immune
PDIA4_19_v1___Thermal
NKAa1.b_v2___Stress/Osmoregulation
KRT8___MRS/Growth
IDH3B_12_v2___Metabolism
PCBL_onmy___Immune
C1Qc___Immune
IL.17D_onmy___Immune
park7_22___Stress/Osmoregulation
RPL6___MRS
Map3k14_3___Stress/Osmoregulation
IL.11_onmy___Immune
NKA_a3_sasa___Stress/Osmoregulation
TF_onmy___Immune
ALDOA___Glycolysis
hsp90a_15_v2___Stress/Osmoregulation
JUN___Stress/Osmoregulation
SCG___MRS
IFNa_sasa2___Immune
COX6B1_19___Stress/Osmoregulation
SERPIN_9___Thermal


Liver PCA Rotation
PC5 loading
0.2
0.1
0.0
−0.1
−0.2
Mx_onts___VDD/MRS
SRK2_MGB3___VDD
CD9_MGL_2___Immune
RSAD_MGB2___VDD
IFIT5_MGL_2___VDD
IFI44a_MGL_2___VDD
DEXH_MGL_1___VDD
HERC6_1___VDD
GAL3_MGL_2___VDD
CA054694_MGL_1___VDD
hsp90a_15_v2___Stress/Osmoregulation
NKA_b1_sasa___Stress/Osmoregulation/MRS
Map3k14_3___Stress/Osmoregulation
SERPIN_9___Thermal
NFX_MGL_2___VDD
CD8a_onmy___Immune
HBA_v1___Hypoxia
TCRb_onmy___Immune
MHCII.B_onmy___Immune
52Ro_MGL_3___VDD
CCL4_v1___Immune
PgK3_v1___Glycolysis
HIF1A_6___Hypoxia
HSP90alike_6___Stress/Themal
MHC1.sasa1___Immune
IgT_sasa___Immune
CD83_sasa___Immune
UBE2Q2_26___Growth
VHSV.P10_MGL_2___VDD
ALDOA___Glycolysis
TCRa_sasa2___Immune
IFNa_sasa2___Immune
NKA_a3_sasa___Stress/Osmoregulation
HIF1A_7___Hypoxia
COX6B1_19___Stress/Osmoregulation
IL.1B_sa−om___Immune
HIF1A_3_v2___Hypoxia
HSC70___Stress/Osmoregulation
NKAa1.b_v2___Stress/Osmoregulation
RPL6___MRS
MPDU1_7___Metabolism
IL.11_onmy___Immune
TNF_onts___Immune
IRF1___MRS/Immune
IgMs_onmy___Immune
IDH3B_12_v2___Metabolism
PDIA4_19_v1___Thermal
TF_onmy___Immune
PCBL_onmy___Immune
SAA_onmy___Immune
Ldhb___Hypoxia
MMP25___MRS/Growth
SCG___MRS
MMP13_sasa___Growth
C1Qc___Immune
park7_22___Stress/Osmoregulation
STAT1___VDD/MRS
FYB___MRS
CD4___MRS/Immune
KRT8___MRS/Growth
IL.8_onmy2___Immune
glut2___Glycolysis
JUN___Stress/Osmoregulation
RPL31_v1___Stress/Themal
IL.15_onmy___Immune
PRAS___MRS
hep_onmy___Immune
IL.17D_onmy___Immune
NKAA1C___Stress/Osmoregulation
ACTB_v1___Growth
COMMD7___MRS
sepw1_11_v1___Stress/Osmoregulation
EF2_14___Hypoxia
C3_onmy___Immune
HTA___MRS
HSP90ab1_15_v1___Stress/Themal