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Survey of Infectious Agents Detected in Juvenile Chinook and Sockeye Salmon from British Columbia and Washington

by

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Abstract: The contribution of infectious diseases to annual variations in salmon survival in the ocean is poorly understood, largely owing to the inability to observe mortality. We developed a novel microfluidics quantitative PCR system to survey 46 infectious agents (viruses, bacteria, fungal and protozoan parasites), known or suspected to cause disease in salmon worldwide, in 96 samples at once. The platform was applied to juvenile Sockeye (*Oncorhynchus nerka*) and Chinook (*O. tshawytscha*) Salmon sampled in southern British Columbia from 2008-2013. Twenty-one infectious agents were detected at a prevalence greater than 1% in ocean-migrating Chinook Salmon, and 17 in Sockeye Salmon. Among species, the most commonly observed agents were the bacterium *Candidatis Branchiomonas cysticola*, parasites *Myxobolus arcticus*, *Paranucleospora theridion*, and *Parvicapsula minibicornis*, and erythrocytic necrosis virus.

Introduction

Most of what we understand about the role of infectious disease in salmon survival comes from studies of cultured fish, in hatcheries and ocean net pens, where mortality is observable. In these high density rearing systems, agents causing acute diseases are generally most impactful, directly causing high levels of mortality in a relatively short period of time. We expect, however, that in wild migratory salmon, where densities are lower, such acute agents would have more limited transmission potential, and therefore a reduced impact at the population level. Instead, agents associated with chronic diseases that can be maintained and spread within and among populations over longer periods of time carry a higher potential for impact, especially if they compromise the physiological performance of fish (Bakke and Harris 1998). In fact, direct mortality induced by infectious disease may be overshadowed by the indirect effects on swim performance, feeding ability, visual acuity, and behaviour that increase vulnerability to predation (Miller et al. 2014). Under this scenario, it may be quite difficult to sample migratory fish in a late stage of disease development if predators remove them rapidly once they become compromised.

Most surveys of infectious agents in migratory salmon have been limited to parasites either grossly evident or observable through microscopy (e.g. Margolis and Arthur 1979; Ferguson et al. 2012); few studies have been undertaken to document microparasite species (viruses, bacteria, fungal and protozoan parasites), especially in the early marine environment (but see Arkoosh et al. 2004). We had developed a molecular means to rapidly quantitate dozens of micro-parasitic infectious agents at once using the microfluidics quantitative (q)PCR Fluidigm BioMark^{TM} HT platform. We populated the platform with assays to 46 infectious agents known or suspected to cause diseases in salmon worldwide, including those associated with emerging diseases of farmed salmon in Norway which had never been surveyed in salmon in North America. We applied this platform to 1,876 juvenile Chinook and 630 juvenile Sockeye Salmon migrating from freshwater (FW) to saltwater (SW) and then along the southern British Columbia (BC) coast; sampling spring through summer in the ocean for Sockeye, and spring through the first winter at sea for Chinook Salmon. This characterization was conducted to provide a

baseline of information on the range of agents detected in BC salmon rather than focussing specifically on disease, as we cannot know from the detection of an agent, alone, whether there is any manifestation of disease.

Methods and Materials

Sample collection and analysis

Juvenile Sockeye and Chinook Salmon were collected on a migratory trajectory from freshwater natal rearing areas, the lower Fraser River (Sockeye Salmon only), and the marine environment within the Salish sea, the west coast of Vancouver Island, and the central BC coast, and the north coast of BC/Southeast Alaska (Figure 1). In FW, sampling occurred at the time of smolt release at salmon hatcheries, and through beach seining within natal lakes or dip netting at smolt fences. In the lower Fraser River, collections were conducted via a rotary screw trap placed at Mission. In the marine environment, fish were sampled off an ocean trawler contracted by DFO in the spring and during marine surveys performed on the CCGS WE Ricker or F/V Viking Storm during the summer (June/July), fall (Sept/Nov), and winter (Feb/Mar). For Chinook Salmon, our survey included fish captured from 2008-2012 whereas for Sockeye Salmon, only fish captured in 2013 were assessed. Table 1 shows the seasonal and annual distribution of samples used in our survey.

In most instances, salmon were dissected in the field, on ice in hot weather, within 30 minutes of capture, and liver, gill, heart, kidney, and brain were removed and preserved in RNALater. Instruments were disinfected in bleach and ethanol between fish. Tissues in RNALater were kept at -4^{o}C overnight and then frozen in liquid nitrogen dewars, followed by -80^{o}C freezers until extraction. Tissues were homogenized separately in Trizol buffer and tissue homogenates were combined for individual fish for RNA and DNA extraction, as outlined in Miller et al. (2014, 2016). As our agents of interest included RNA viruses, we needed to combine RNA and DNA for our quantitative assessments across the diverse range of agents under study, as described previously (Miller et al. 2014).

To facilitate highly sensitive detection of agents within the microfluidics system, samples underwent an amplification-based enrichment step before being purified and loaded onto the dynamic arrays for the Fluidigm BioMark^{TM} platform. Microfluidic infectious agent monitoring included 12 bacteria, 22 parasites, and 12 viral species (Table 2). Technical details on the processing of samples on this platform are provided in Miller et al. (2016).

The cycle threshold (Ct) for each assay in each sample was determined from the amplification curves. A salmon housekeeping gene (hkg) was included as a control to ensure sample quality, and samples with hkg Ct>20 were removed from further analysis. A Ct cut-off of 27, representing approximately 2-5 copies per µl starting material, was used for samples to be classified as positive (see assay-specific limits of detection in Miller et al. 2016). Only samples with detections in both duplicate assays were considered positive.

Genetic stock identification was applied for individual identification to stock for all samples collected in mixed stock environments (Beacham et al. 2005, 2006). Samples were chosen to cover a broad geographic distribution of stocks, from Columbia River in

Washington (Chinook) north to Babine River/Atnarko in British Columbia (Sockeye). A total of 630 Sockeye Salmon sampled from FW (286) and SW (344) environments in the spring through fall of 2013 were included in the analysis (Table 1). A total of 1876 Chinook Salmon sampled from FW (220) and SW (1656) environments (Table 1)

Data analysis

Prevalence of infectious agents was calculated over the entire survey for each of Chinook and Sockeye Salmon. We restrict our presentation of infectious agents only to those detected in at least one species at >1%. Infectious agent diversity was calculated as the number of agents detected within a sample.

Results

Infectious agent diversity within individuals peaked between 3-4 agents, with a range for both species between 0 and 10 (Figure 2). Agent diversity in freshwater was 1.5 for Chinook and 2.5 for Sockeye Salmon, and rose to 4.0 in the marine environment for both species.

Twenty-two of the 46 agents were detected in Chinook and/or Sockeye Salmon at a prevalence >1% across FW/SW environments (Figure 3). Twenty-one of these were found Chinook Salmon in SW, 13 of which were first detected >1% in FW. Among the 21 agents detected, 5 were bacteria, and 14 fungal and protozoan parasites, and 2 viruses. Alternately, in Sockeye Salmon, twelve of the 17 agents detected in >1% of fish in SW were detected first in FW. Among the 17 detected agents, 4 were bacteria, 11 fungal and protozoan parasites, and 2 viruses.

Sixteen agents were detected at a prevalence of at least 1% in both species, including 4 bacteria, 11 parasites, and 1 virus (Figure 3). The fluke *Nanophyetus salmincola*, bacterium *Renibacterium salmoninarum*, and virus Viral hemorrhagic septicemia virus (VHSV) were exclusively detected in Chinook Salmon. Microsporidian parasites *Facilispora margolisi* and *Kudoa thyrsites* and the Piscine OrthoReovirus (PRV) were detected in both species, but only at a prevalence >1% in Chinook Salmon. Alternately, the Pacific Salmon Parvovirus (PSPV) was only detected at a prevalence >1% in Sockeye Salmon.

The bacterium *Candiditis Branchiomonas cysticola* was the most prevalent agent in both species, detected in >75% of fish sampled. *Myxobolus arcticus* was also detected in over 75% of Sockeye, but only 35% of Chinook Salmon. Other agents common to both species included parasites *Parvicapsula minibicornis* and *Paranucleospora theridion*, both at prevalence $\geq$25% across species. *Ichthyophonus hoferi*, *Loma salmonae*, and *Parvicapsula pseudobranchicola* were more prevalent in Chinook Salmon, while *Parvicapsula kabatai* and *Ichthyophthirius multifiliis* were more common in Sockeye Salmon.

In Chinook Salmon, shifts in agent prevalence could be calculated over multiple seasons in the ocean (Figure 4), although care should be taken in the interpretation of these shifts as we were not necessarily sampling the same group of fish (i.e. stock and geographic effects may have confounded these variances). Increasing prevalence over time/season was noted for 4 parasites (*I. hoferi*, *P. pseudobranchicola*, *L. salmonae*, and

Tetracapsuloides bryosalmonae). Decreasing prevalence over seasons was noted for 4 parasites (*C. shasta*, *P. kabatai*, *P. minibicornis*, and *P. theridion*) and 2 bacteria (gill chlamydia [sch] and rickettsia-like organism [rlo]). An arced distribution, where prevalence rose and then fell, was observed for 1 parasite (*M. arcticus*) and 2 viruses (erythrocytic necrosis virus [ENV] and VHSV).

Substantial numbers of Chinook Salmon sampled in the summer were either of Fraser River origin or Columbia River origin, allowing us to contrast agents in migratory salmon between these two major drainages. We found that agent diversity and load was moderately higher for stocks from the Columbia drainage than the Fraser drainage, largely owing to higher prevalence and load of *C. shasta* and *P. minibicornis* in Columbia River Chinook (100% prevalence for both).

Discussion

Chinook and Sockeye Salmon smolts entering the marine environment clearly encounter a range of infectious agents over the course of their first few months at sea. This was demonstrated both by the finding that all agents observed in FW were also detected in the marine environment, where agent diversity by fish rose for both species. In Chinook Salmon, agent diversity remained relatively similar over the seasons in the ocean.

Below, we provide an overview of the most common agents identified in our sampling, and some details about their known or expected associations with disease in salmon.

Bacterium *C. B. cysticola* was the most prevalent infectious agent in both species, and was the only bacterium detected at a prevalence >10%. In Chinook Salmon, while 40% of salmon leaving FW carried this agent, prevalence reached 80-90% in SW. *C.B. cysticola* is associated with proliferative gill inflammation in sea pens in Norway, but its exact role in the development of the disease has not been established (Toenshoff et al. 2012). The bacterium is highly prevalent in Norway and Ireland (Mitchell et al. 2013), and was first identified in BC salmon by Miller et al. (2014), but has since been observed across most salmon species, in smolts and adults (Miller, unpublished data).

Infectious agents infecting out-migrating smolts were dominated by fungal and protozoan parasites, many of which were myxozoans, a class of cnidarian parasites transmitted through an alternate invertebrate host (typically an annelid worm or bryozoan). Perhaps the most important of these is *Ceratomyxa (*aka *Ceratonova) shasta*, a highly virulent myxozoan parasite endemic in salmon-bearing rivers throughout the Pacific Northwest (Fujiwara et al. 2011). The alternate oligochaete host resides in FW and estuarine environments. *C. shasta* is known to cause disease in salmon within the FW/estuarine environment, with enhanced pathogenicity under elevated water temperatures (Ray et al. 2012); there is also some evidence that the parasite can still induce ceratomyxosis disease in SW (Ching and Mundy 1984). This parasite was more prevalent in Chinook (17%) than Sockeye (2%) Salmon. In Chinook Salmon, *C. shasta* was most prevalent in spring (39%), after smolts entered the marine environment, decreasing gradually over the seasons, to 6% over winter. The parasite was particularly prevalent in Columbia River stocks, with 100% of sampled Columbia River Chinook

carrying this highly pathogenic parasite. In contrast, only 25% of Fraser River stocks carried the parasite in early marine environment.

Parvicapsula minibicornis is a myxozoan parasite that uses the same alternate oligochaete host as *C. shasta*, with the distribution of this agent in Chinook Salmon highly similar to that described for *C. shasta*. However, unlike *C. shasta*, *P. minibicornis* was also observed at an appreciable prevalence in Sockeye Salmon (29 vs 17% overall), primarily in Fraser River origin fish, suggesting either a differential distribution among watersheds or a higher susceptibility in Sockeye Salmon. As with C. *shasta*, all Columbia River origin Chinook Salmon tested positive for this parasite, and virtually all at considerable parasite abundance (>10^4 copies per µl); alternately, the parasite was detected in 45% of Fraser River stocks, but generally at low abundance in individual fish (10^1 copies per µl). *P. minibicornis* causes lesions in kidney tissue, has been associated with pre-mature mortality in return-migrating adult Sockeye Salmon in the Fraser River (Bradford et al. 2010), has been observed in out-migrating Chinook Salmon smolts (True et al. 2009), and has been associated with increased risk of predation by Rhinoceros Auklets in the marine environment (Miller et al. 2014).

Parvicapsula pseudobranchicola, a marine-transmitted myxozoan parasite, was the most prevalent parasite in Chinook Salmon, with sharply escalating levels of infection from ocean entry to the winter period, reaching >90% of infected salmon over winter. Alternately, detection in Sockeye Salmon was minimal. *P. pseudobranchicola* infects the pseudobranchs of gill tissue and has been associated with gill disease in farmed salmon (Karlsbakk et al. 2002), as well as impacts on swim performance and visual acuity (Jørgensen et al. 2011). *P. pseudobranchicola* was first detected in North America by our team and shown to be associated with increased risk of predation of juvenile Sockeye Salmon (Miller et al. 2014).

Parvicapsula kabatai was first described in kidney tissue from adult Pink Salmon (*O. gorbuscha*) in BC, and is a close relative to *P. pseudobranchicola* (Jones et al. 2006). In our survey, the parasite was more prevalent in Sockeye than Chinook Salmon smolts. Like others in the genus *Parvicapsula*, *P. kabatai* was associated with enhanced risk of predation of Sockeye Salmon smolts by Rhinoceros Auklets (Miller et al. 2014).

Myxozoan parasite *Myxobolus arcticus* is known to infect juvenile salmon in FW, and was the most prevalent parasite in Sockeye Salmon (68%), largely owing to its strong presence in Fraser River origin stocks. It was also prevalent in Chinook Salmon (35%), in which the highest prevalence was observed in spring and summer in the ocean. This parasite infects the brain, and stays with the infected host throughout their life-cycle (Urawa et al. 1998). *M. arcticus* has been associated with abnormal swimming behavior (Moles and Heifetz 1998), but direct impacts of disease on mortality are limited.

Tetracapsuloides bryosalmonae was the least prevalent of the myxozoan parasites, generally maintaining a prevalence under 10%. This highly virulent parasite, which is the causative agent of proliferative kidney disease, has been shown to exert population-level impacts in fry and smolts in FW (Sterud et al. 2007), being especially impactful under high water temperatures (Ferguson 1981). Less is known about its impact in SW.

Paranucleospora theridion (aka *Desmozoon lepeoptherii*), a microsporidian parasite transmitted by sea lice in SW (Nylund et al. 2010), was equally prevalent in Chinook and Sockeye Salmon, infecting ~30% of juveniles in SW. In Chinook, prevalence decreased sharply over winter, during a period when this parasite is known to

associate with enhanced mortality in Norwegian farmed salmon (Nylund et al. 2011). The parasite, transmitted through sea lice, is among the multiple agents associated with proliferative gill disease in Norway (ibid), but its impact in BC salmon has not been assessed.

Loma salmonae is a microsporidian parasite detected in >30% of Chinook Salmon in SW, but only minimally observed in Sockeye Salmon. The parasite, first detected in our samples in FW, can cause microsporidial gill disease in farmed BC salmon, typically occurring in late summer/early fall (Speare and Lovy 2012). In Chinook Salmon from our study, *L. salmonae* peaked in load over the winter period, possibly suggesting a high degree of activity/replication occurring over the winter period in migratory juvenile salmon.

Ichthyophonus hoferi is a mesomycetezoan parasite that causes ichthyophonosis, a systemic mycosis disease impacting a wide array of fish species (Sindermann and Chenoweth 1993), and is transmitted to salmon through their prey (Jones and Dawe 2002). The parasite was more common in Chinook than Sockeye Salmon, gradually increasing in prevalence in the marine environment. *I. hoferi* has been associated with substantial mortality in net-pen reared Chinook Salmon (Harrell et al. 1986), and possibly post-smolt Atlantic Salmon in Russia (Zubchenko and Karaseba 2002). The parasite can also cause abnormal spiral swimming behavior when infecting the central nervous system (Dorier and Degrange 2001), potentially increasing susceptibility of affected fish to predation.

Four viruses were detected in migratory juvenile salmon. A novel unpublished DNA virus identified by our research team via high throughput sequencing, Pacific Salmon Parvovirus (PSPV), was detected at high prevalence (>75%) in Sockeye Salmon, but virtually absent in Chinook Salmon. PSPV was observed in high prevalence and load in out-migrating Sockeye Salmon smolts, and continued to be highly prevalent in the early marine environment. While we know this virus is infectious (Miller, unpublished data), we do not yet know if it causes disease.

Erythrocytic necrosis virus, a DNA iridovirus, was the second most prevalent virus in migratory juvenile salmon. In Chinook Salmon, ENV peaked in summer/fall at a prevalence of ~30%, and decreased in prevalence over winter. ENV causes severe anemia associated with inclusion body syndrome (EIBS) in numerous marine fish, most notably causing severe disease and mortality in herring (Purcell et al, 2016), but can also cause disease in salmon (Haney et al. 1992), with potential consequences on tolerance to low oxygen environments and disruption in osmoregulation (MacMillan et al, 1980).

Piscine Orthoreovirus (PRV) was only detected in 4% of Chinook Salmon, and less than 1% of Sockeye Salmon. This double-stranded RNA virus has been associated with an emerging heart inflammatory disease (heart and skeletal muscle inflammation) in farmed Atlantic salmon in Norway (Palacios et al. 2010), recently also diagnosed on a BC salmon farm (Di Cicco et al. 2017). Various strains of the virus have also been associated with jaundice-related diseases, some also with heart inflammation, in Pacific salmon species (Coho in Chile [Godoy et al. 201] and Japan [Takano et al. 2016], Rainbow Trout (O. mykiss) in Norway [Olsen et al. 2015]). A similar jaundice disease occurs on farmed Chinook Salmon in BC, and is associated statistically with PRV (Miller, unpublished data), but its role in the development of the disease is yet to be established.

It is important to note that many highly virulent viral agents that have devastated the aquaculture industry in salmon farming regions throughout the world, namely infectious salmon anemia virus (ISAV), infectious pancreatic necrosis virus (IPNV), salmon alphavirus (SAV), and *Oncorynchus masou* herpesvirus (OMV), were not detected among the >2,500 fish assessed in our survey. The assays used to detect these regulatory agents were all approved by the World Organization for Animal Health (http://www.oie.int/) and capable of detecting all known variants.

While these data should not be interpreted as evidence of disease, they provide the most comprehensive baseline to date of the range of viruses, bacteria, and microparasites detected on wild BC and Washington salmon during the critical early marine phase of their migration, a time that is associated with high mortality and predictive of year-class strength (Beamish and Mahnken 2001). Future studies will examine the distribution of these agents over a decade of sampling, contrasting years of good and poor productivity, stocks of high and low conservation concern, ocean- and stream-type life-histories, and hatchery- versus wild-origin salmon to discern whether patterns of specific agents are correlated with indices of poor early marine survival.

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Chinook Salmon

Season	Sample	N
Freshwater		210
Spring		
2009		
	Vancouver Island Hatcheries	57
2010		
	Vancouver Island Hatcheries	19
2012		
	Vancouver Island Hatcheries	39
	Fraser River Hatcheries	95
Saltwater		1666
Spring		212
2008		
	West Coast Vancouver Island	23
2009		
	Central Coast BC	7
	West Coast Vancouver Island	3
	Salish Sea	8
2010		
	Salish Sea	1
2011		
	North Coast BC + SE Alaska	3
	Central Coast BC	4
	West Coast Vancouver Island	15
	Salish Sea	64
2012		
	Salish Sea	84
Summer		436
2009		
	West Coast Vancouver Island	1
	Salish Sea	40
2010		
	Salish Sea	83
2011		
	Central Coast BC	10
	Salish Sea	116
2012		
	Salish Sea	186
Fall		711
2008		
	North Coast BC + SE Alaska	12
	Central Coast BC	85
	West Coast Vancouver Island	21
	Salish Sea	52
2009		
	North Coast BC + SE Alaska	75
	Central Coast BC	42
	West Coast Vancouver Island	130
	Salish Sea	38
2010		
	North Coast BC + SE Alaska	48
	Salish Sea	64
2011		
	Central Coast BC	9
	West Coast Vancouver Island	27
	Salish Sea	108
Winter		307
2008		
	West Coast Vancouver Island	4
	Salish Sea	12
2009		
	North Coast BC + SE Alaska	31
	Central Coast BC	3
	Salish Sea	29
2010		
	Central Coast BC	4
	West Coast Vancouver Island	80
	Salish Sea	89
2011		
	Central Coast BC	5
	West Coast Vancouver Island	30
	Salish Sea	20
	Grand Total	1876

Sockeye Salmon

Season	Sample	N
Freshwater		286
2013		
	Babine River	10
	Chilko Smolt Fence	56
	Snootlie Hatchery	20
	Lower Fraser River	200
	Saltwater	344
2013		
	Central Coast BC	133
	Salish Sea	211
	Grand Total	630

Table 1. Distribution of Chinook and Sockeye Salmon samples in Southern BC and Alaska assessed for 46 infectious agents.

Agent full name	Type	Organism abbreviation	Primer F	Primer R	Probe
Aeromonas hydrophila	Bacterium	ae_hyd	ACCGCTGCTCATTACTCTGATG	CCAACCCAGACGGGAAGAA	TGATGGTGAGCTGGTTG
Aeromonas salmonicida	Bacterium	ae_sal	TAAAGCACTGTCTGTTACC	GCTACTTCACCCTGATTGG	ACATCAGCAGGCTTCAGAGTCACTG
Candidatus Branchiomonas cysticola	Bacterium	c_b_cys	AATACATCGGAACGTGTCTAGTG	GCCATCAGCCGCTCATGTG	CTCGGTCCCAGGCTTTCCTCTCCCA
Flavobacterium psychrophilum	Bacterium	fl_psy	GATCCTTATTCTCACAGTACCGTCAA	TGTAAACTGCTTTTGCACAGGAA	AAACACTCGGTCGTGACC
Gill chlamydia	Bacterium	sch	GGGTAGCCCGATATCTTCAAAGT	CCCATGAGCCGCTCTCTCT	TCCTTCGGGACCTTAC
Piscichlamydia salmonis	Bacterium	pch_sal	TCACCCCCAGGCTGCTT	GAATTCCATTTCCCCCTCTTG	CAAAACTGCTAGACTAGAGT
Piscirickettsia salmonis	Bacterium	pisck_sal	TCTGGGAAGTGTGGCGATAGA	TCCCGACCTACTCTTGTTTCATC	TGATAGCCCCGTACACGAAACGGCATA
Renibacterium salmoninarum	Bacterium	re_sal	CAACAGGGTGGTTATTCTGCTTTC	CTATAAGAGCCACCAGCTGCAA	CTCCAGCGCCGCAGGAGGAC
Strawberry disease	Bacterium	rlo	GGCTCAACCCAAGAACTGCTT	GTGCAACAGCGTCAGTGACT	CCCAGATAACCGCCTTCGCCTCCG
Vibrio anguillarum	Bacterium	vi_ang	CCGTCATGCTATCTAGAGATGTATTTGA	CCATACGCAGCCAAAAATCA	TCATTTCGACGAGCGTCTTGTTCAGC
Vibrio salmonicida	Bacterium	vi_sal	GTGTGATGACCGTTCCATATTT	GCTATTGTCATCACTCTGTTTCTT	TCGCTTCATGTTGTGTAATTAGGAGCGA
Yersinia ruckeri	Bacterium	ye_ruc	TGCCGCGTGTGTGAAGAA	ACGGAGTTAGCCGGTGCTT	AATAGCACTGAACATTGAC
Nanophyetus salmincola	Fluke	na_sal	CGATCTGCATTTGGTTCTGTAACA	CCAACGCCACAATGATAGCTATAC	TGAGGCGTGTTTTATG
Ceratomyxa shasta	Parasite	ce_sha	CCAGCTTGAGATTAGCTCGGTAA	CCCCGGAACCCGAAAG	CGAGCCAAGTTGGTCTCTCCGTGAAAAC
Cryptobia salmositica	Parasite	cr_sal	TCAGTGCCTTTCAGGACATC	GAGGCATCCACTCCAATAGAC	AGGAGGACATGGCAGCCTTTGTAT
Dermocystidium salmonis	Parasite	de_sal	CAGCCAATCCTTTCGCTTCT	GACGGACGCACACCACAGT	AAGCGGCGTGTGCC
Facilispora margolisi	Parasite	fa_mar	AGGAAGGAGCACGCAAGAAC	CGCGTGCAGCCCAGTAC	TCAGTGATGCCCTCAGA
Gyrodactylus salaris	Parasite	gy_sal	CGATCGTCACTCGGAATCG	GGTGGCGCACCTATTCTACA	TCTTATTAACCAGTTCTGC
Ichthyophonus hoferi	Parasite	ic_hof	GTCTGTACTGGTACGGCAGTTTC	TCCCGAACTCAGTAGACACTCAA	TAAGAGCACCCACTGCCTTCGAGAAGA
Ichthyophthirius multifiliis	Parasite	ic_mul	AAATGGGCATACGTTTGCAAA	AACCTGCCTGAAACACTCTAATTTTT	ACTCGGCCTTCACTGGTTCGACTTGG
Kudoa thyrsites	Parasite	ku_thy	TGGCGGCCAAATCTAGGTT	GACCGCACACAAGAAGTTAATCC	TATCGCGAGAGCCGC
Loma salmonae	Parasite	lo_sal	GGAGTCGCAGCGAAGATAGC	CTTTTCCTCCCTTTACTCATATGCTT	TGCCTGAAATCACGAGAGTGAGACTACCC
Myxobolus arcticus	Parasite	my_arc	TGGTAGATACTGAATATCCGGGTTT	AACTGCGCGGTCAAAGTTG	CGTTGATTGTGAGGTTGG
Myxobolus cerebralis	Parasite	my_cer	GCCATTGAATTTGACTTTGGATTA	ACCATTCATGTAAGCCCGAACT	TCGAAGCCTTGACCATCTTTTGGCC
Myxobolus insidiosus	Parasite	my_ins	CCAATTTGGGAGCGTCAAA	CGATCGGCAAAGTTATCTAGATTCA	CTCTCAAGGCATTTAT
Neoparamoeba perurans	Parasite	ne_per	GTTCTTTCGGGAGCTGGGAG	GAACTATCGCCGGCACAAAAG	CAATGCCATTCTTTTCGGA
Nucleospora salmonis	Parasite	nu_sal	GCCGCAGATCATTACTAAAAACCT	CGATCGCCGCATCTAAACA	CCCCGCGCATCCAGAAATACGC
Paranucleospora theridion	Parasite	pa_ther	CGGACAGGGAGCATGGTATAG	GGTCCAGGTTGGGTCTTGAG	TTGGCGAAGAATGAAA
Parvicapsula kabatai	Parasite	pa_kab	CGACCATCTGCACGGTACTG	ACACCACAACTCTGCCTTCCA	CTTCGGGTAGGTCCGG
Parvicapsula minibicornis	Parasite	pa_min	AATAGTTGTTTGTCGTGCACTCTGT	CCGATAGGCTATCCAGTACCTAGTAAG	TGTCCACCTAGTAAGGC
Parvicapsula pseudobranchicola	Parasite	pa_pse	CAGCTCCAGTAGTGTATTTCA	TTGAGCACTCTGCTTTATTCAA	CGTATTGCTGTCTTTGACATGCAGT
Sphaerothecum destructuens	Parasite	sp_des	GGGTATCCTTCCTCTCGAAATTG	CCCAAACTCGACGCACACT	CGTGTGCGCTTAAT
Spironucleus salmonicida	Parasite	sp_sal	GCAGCCGCGGTAATTCC	CGAACTTTTTAACTGCAGCAACA	ACACGGAGAGTATTCT
Tetracapsuloides bryosalmonae	Parasite	te_bry	GCGAGATTTGTTGCATTTAAAAAG	GCACATGCAGTGTCCAATCG	CAAAATTGTGGAACCGTCCGACTACGA
Atlantic salmon paramyxovirus	Virus	aspv	CCCATATTAGCAAATGAGCTCTATCTT	CGTTAAGGAACTCATCATTGAGCTT	AGCCCTTTTGTTCTGC
Infectious hematopoietic necrosis virus	Virus	ihnv	AGAGCCAAGGCACTGTGCG	TTCTTTGCGGCTTGGTTGA	TGAGACTGAGCGGGACA
Infectious pancreatic necrosis virus	Virus	ipnv	GCAACTTACTTGAGATCCATTATGCT	GAGACCTCTAAGTTGTATGACGAGGTCTCT	CGAGAATGGGCCAGCAAGCA
Infectious salmon anemia virus	Virus	isav7	TGGGATCATGTGTTTCCTGCTA	GAAAATCCATGTTCTCAGATGCAA	CACATGACCCCTCGTC
Infectious salmon anemia virus	Virus	isav8	TGGGCAATGGTGTATGGTATGA	GAAGTCGATGAACTGCAGCGA	CAGGATGCAGATGTATGC
Pacific salmon parvovirus	Virus	pspv	CCCTCAGGCTCCGATTTTTAT	CGAAGACAACATGGAGGTGACA	CAATTGGAGGCAACTGTA
Piscine myocarditis virus	Virus	pmcv	TTCCAAACAATTCGAGAAGCG	ACCTGCCATTTTCCCCTCTT	CCGGGTAAAGTATTTGCGTC
Piscine reovirus (HSMI)	Virus	prv	TGCTAACACTCCAGGAGTCATTG	TGAATCCGCTGCAGATGAGTA	CGCCGGTAGCTCT
Salmon alphavirus	Virus	sav	CCGGCCCTGAACCAGTT	GTAGCCAAGTGGGAGAAAGCT	TCGAAGTGGTGGCCAG
Oncorhynchus masou herpes virus	Virus	omv	GCCTGGACCACAATCTCAATG	CGAGACAGTGTGGCAAGACAAC	CCAACAGGATGGTCATTA
Viral encephalopathy and retinopathy virus	Virus	ver	TTCCAGCGATACGCTGTTGA	CACCGCCCGTGTTTGC	AAATTCAGCCAATGTGCCCC
Erythrocytic necrosis virus	Virus	env	CGTAGGGCCCCAATAGTTTCT	GGAGGAAATGCAGACAAGATTTG	TCTTGCCGTTATTTCCAGCACCCG
Viral hemorrhagic septicemia virus	Virus	vhsv	ATGAGGCAGGTGTCGGAGG	TGTAGTAGGACTCTCCCAGCATCC	TACGCCATCATGATGAGT

Table 2. TaqMan assays to infectious agents monitored. See Miller et al. (2016) for original assay references.

Figure 1. Map showing the geographic extent of collection areas in British Columbia. Image attribution: @ 2016 Google Image Landsat/Copernicus, Data SIO, NOAA, US Navy, NGA, GEBCO.

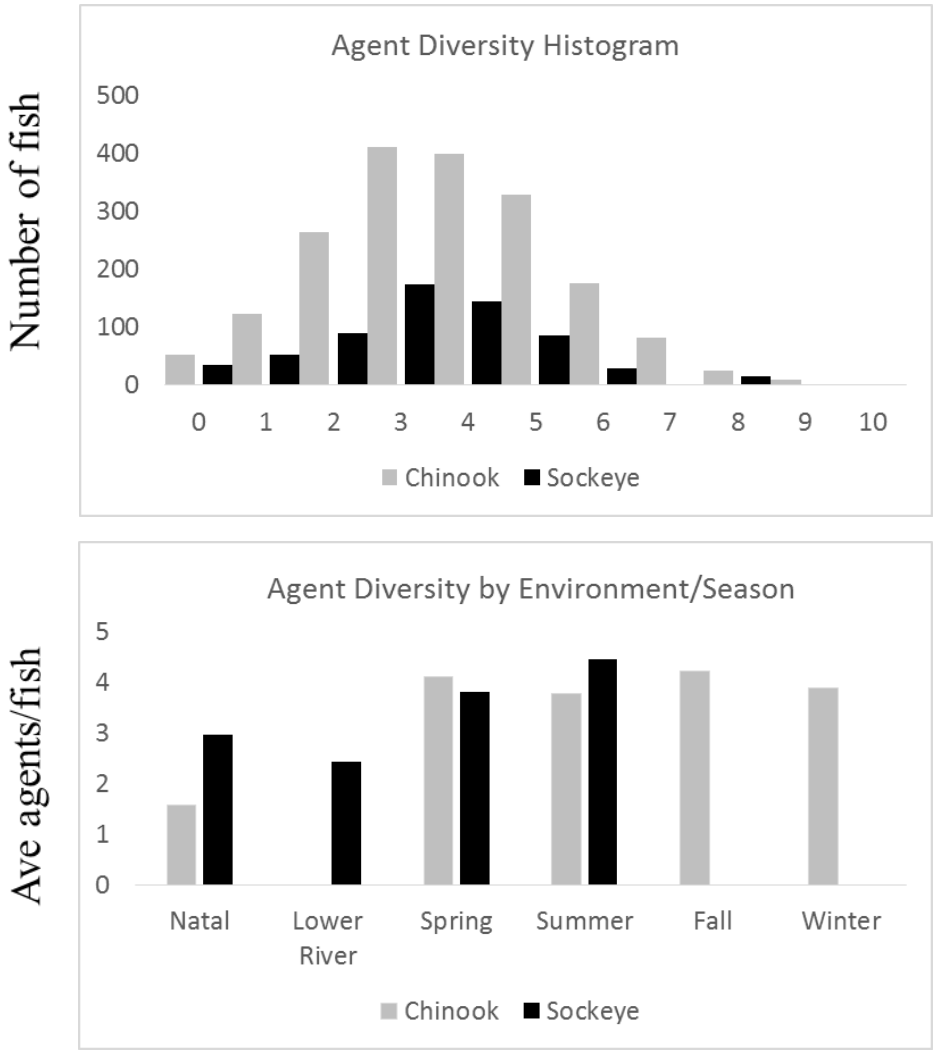

Figure 2. Infectious agent diversity shown as a histogram over all samples (top), and by environment and season (bottom). All freshwater samples were collected at natal environments, either in hatcheries (all Chinook, one Sockeye) or natural streams/lakes, or in the lower Fraser River. Seasonal samples all took place in the ocean. A maximum of 10 agents were observed within a single fish for both species.

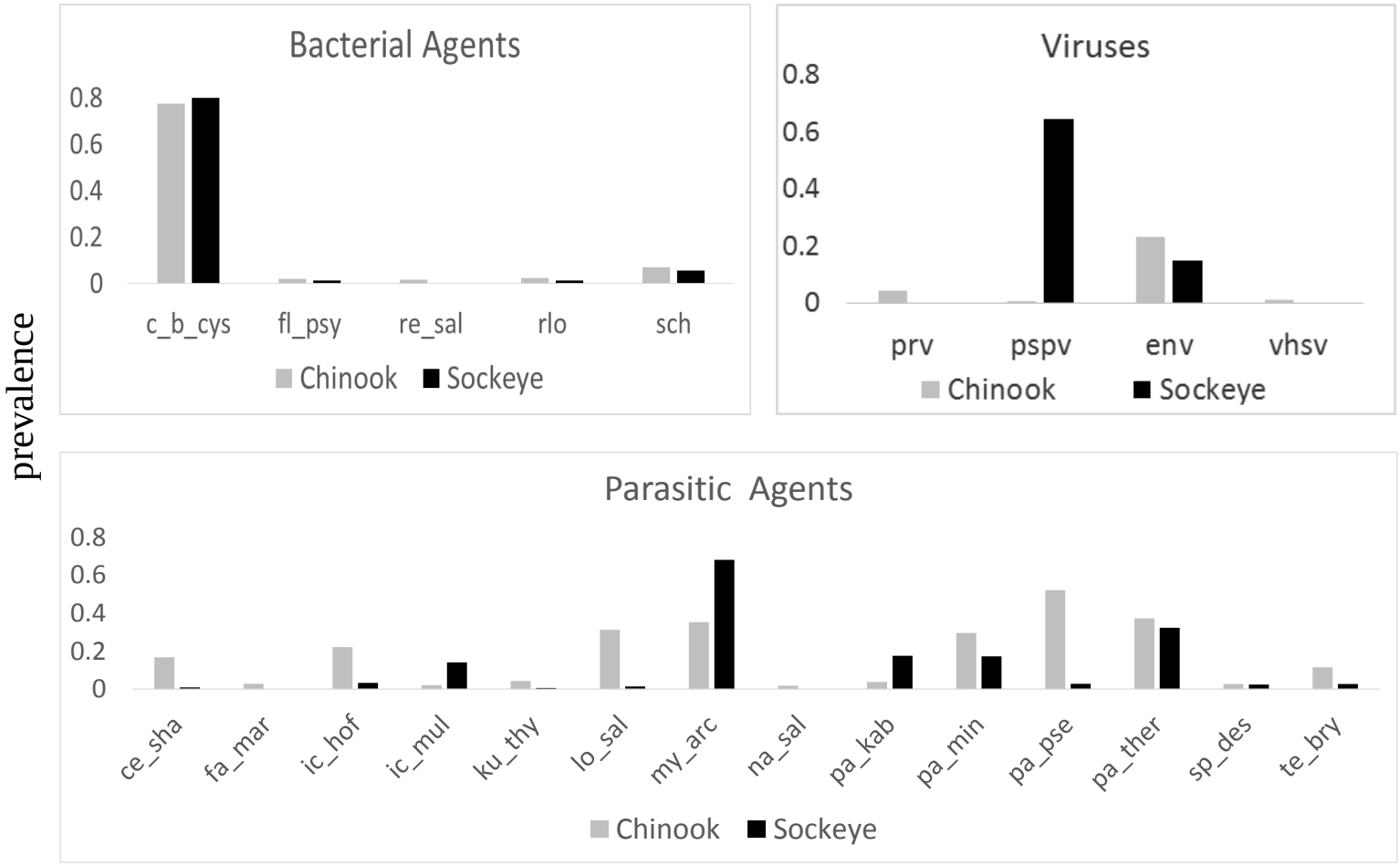

Figure 3. Prevalence of infectious agents detected in at least 1% of Chinook and/or Sockeye Salmon juveniles collected in southern British Columbia and originating from Washington and British Columbia stocks. Agent abbreviations are shown in Table 2.

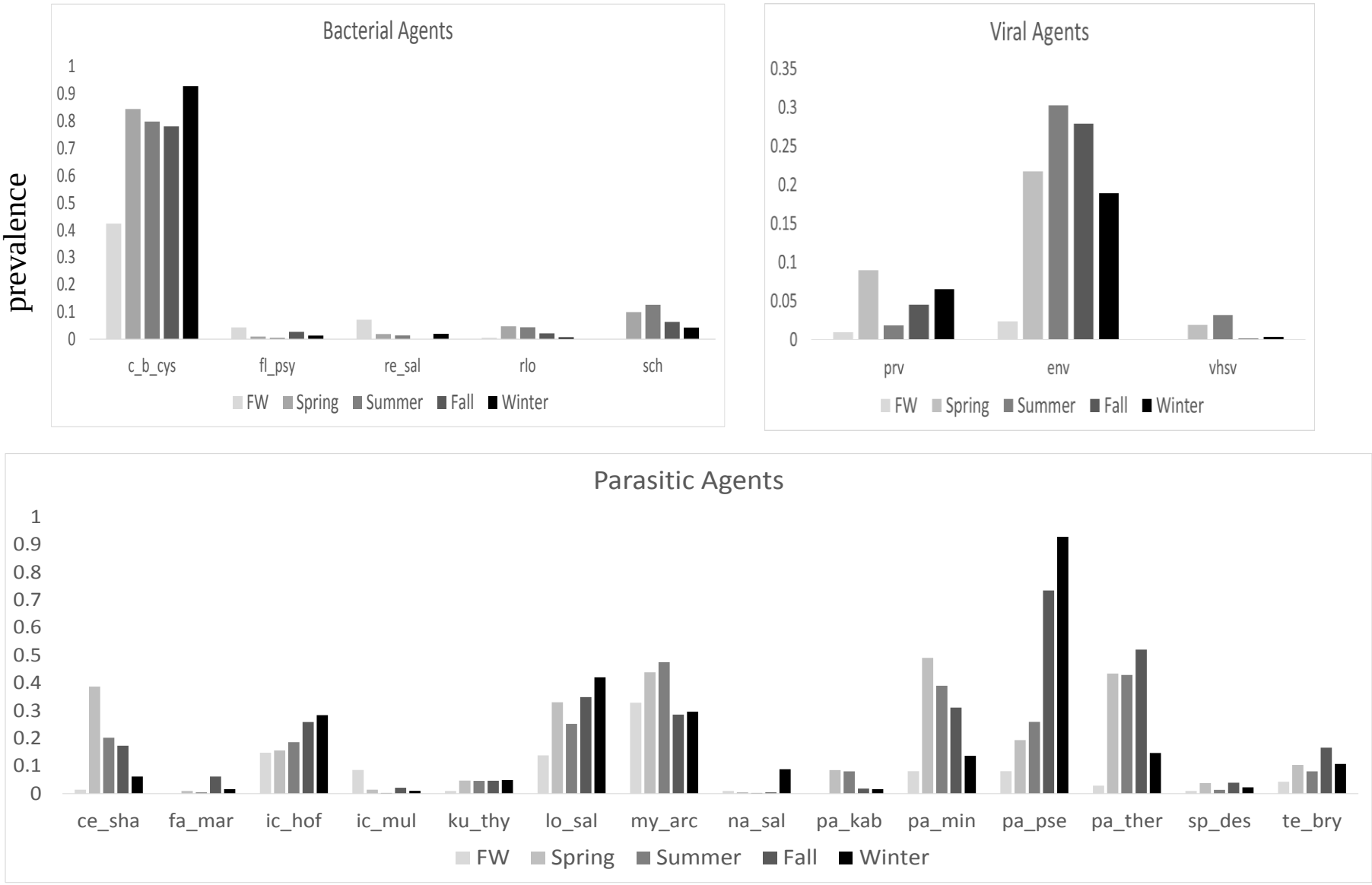

Figure 4. Distribution of infectious agents detected in Chinook Salmon across freshwater (FW) and saltwater environments and over seasons within the marine environment. Agent abbreviations are shown in Table 2.