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# *Piscine orthoreovirus* Genotype-1 (PRV-1) in Wild Pacific Salmon of British Columbia, Canada: 2011–2020

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**Abstract:** *Piscine orthoreovirus* genotype 1 (PRV-1) is an endemic virus to the Northeastern Pacific that infects both wild and farmed salmon. To better understand PRV-1 prevalence and transmission pathways in the region, we targeted out-migrating juvenile Pacific Salmon in the Strait of Georgia and Johnstone Strait in British Columbia, Canada, for PRV-1 molecular screening with an emphasis on Chinook (*Oncorhynchus tshawytscha*) and Coho (*Oncorhynchus kisutch*) salmon. A total of 4031 individuals were screened for PRV-1 and we identified an overall virus prevalence of 3.5% from 2011 to 2020. PRV-1 was absent in enhancement facilities and freshwater catchments and predominately found in the marine environment. The detection of PRV-1 varied greatly between species, year and stock of origin, but cumulatively identified that life history and migratory behaviors likely influenced viral prevalence. Specifically, Chinook salmon, which demonstrate long residence time in the Strait of Georgia relative to other species, had the highest PRV-1 prevalence in this study (7.4%). Varying stock composition and regional detection between year classes within the Strait of Georgia further indicated that the sources for Chinook infection were highly variable between years. These findings enhance our understanding for PRV-1 transmission in the region and more generally between/within salmon species.

**Keywords:** *Piscine orthoreovirus* (PRV); *salmon*; North Pacific; British Columbia

**Key Contribution:** This study expands current understanding for PRV-1 distribution in wild Pacific salmon and highlights potential limitations and trends for PRV-1 transmission in coastal environments.

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**Citation:** Turcotte, L.D.M.; Bradshaw, J.C.; Polinski, M.P.; Johnson, S.C. *Piscine orthoreovirus* Genotype-1 (PRV-1) in Wild Pacific Salmon of British Columbia, Canada: 2011–2020. *Fishes* **2023**, *8*, 252. https://doi.org/10.3390/fishes8050252

Academic Editor: Fei Ling

Received: 14 April 2023
Revised: 28 April 2023
Accepted: 4 May 2023
Published: 10 May 2023



## 1. Introduction

*Piscine orthoreovirus* (PRV) is a common and widely distributed virus of salmonids that has been noted with particularly high prevalence in aquaculture environments. Although of general low virulence, PRV has contributed to significant disease manifestation in cultured fish in some instances—the most significant being heart and skeletal muscle inflammation (HSMI) in farmed Atlantic salmon of Norway and Chile which has been associated with significant economic losses to the industry over the past two decades [1].

PRV is currently classified into three recognized subtypes based on phylogenetic analysis: PRV-1, PRV-2 and PRV-3, and was first described in [1–3]. Of these, PRV-1 is the only subtype endemic to wild and farmed salmonids of the Northeast Pacific (Alaska, British Columbia and Washington state), as reviewed in [4]. Field surveys and laboratory challenge trials further indicate that all species of anadromous salmon found in the Northeast Pacific can serve as hosts for PRV-1, as reviewed in [4,5].

In the Northeastern Pacific, PRV-1 ubiquitously infects farmed Atlantic (*Salmo salar*) and Chinook (*Oncorhynchus tshawytscha*) salmon, with the majority of farms becoming infected within 100 to 300 days of stocking [6–9]. PRV-1 infections are also commonly reported

in juvenile and adult wild Pacific salmon, especially Chinook and Coho (*Oncorhynchus kisutch*), albeit at a generally lower and highly variable prevalence [4,5,10].

Natural infections and controlled laboratory exposure studies have identified that the putative host range of PRV-1 in the Northeast Pacific Ocean includes all species of Pacific salmon, Steelhead trout (*Oncorhynchus mykiss*), Cutthroat trout (*Oncorhynchus clarkii*), Dolly Varden trout (*Salvelinus malma*) and Atlantic salmon, as reviewed in [4]. In seawater, both wild and farmed salmon have been suggested to serve as reservoirs of PRV-1 in the Northeast Pacific [8]. In freshwater, historical reservoirs of PRV-1 have been reported in commercial Atlantic salmon hatcheries [11]. However, changes in health management practices in recent years, such as the screening of broodstock for PRV-1, has resulted in a decline in PRV-1 prevalence in these facilities despite increased screening [12] and no positive detections have occurred since 2018 [13]. PRV-1 has also been singularly reported in juvenile wild salmon in the Northeast Pacific and in naturally spawned Sockeye salmon (*Oncorhynchus nerka*) fry collected in freshwater [10,14]. The potential for these stocks to have acted as regional reservoirs is currently unknown. Outside of a single putative infection of Eulachon (*Thaleichthys pacificus*) [14], non-salmonid hosts of PRV-1 have not been identified in the Northeast Pacific and only salmonids are considered epidemiologically important hosts of PRV-1 in the region [4].

The aims of this study were to (i) expand the spatial, temporal and host prevalence data available for PRV-1 infections in wild Pacific salmon in British Columbia (BC) waters, (ii) to obtain samples for future genome sequencing (iii) identify relative PRV-1 loads in infected individuals and tissues, (iv) evaluate PRV-1 in relation to geographically identifiable origin stocks where available and (v) compare PRV-1 prevalence in relation to fish size/condition and coastal residence time for populations of Chinook salmon where cumulative detection allowed for robust comparisons (i.e., involving more than 100 cumulative PRV-1 detections). This allowed for us to specifically examine how spatial and temporal factors, stock composition and body size are related to PRV-1 prevalence.

## 2. Material and Methods

### *2.1. Sample Collection*

We examined fish samples collected over the period of 2011 to 2020, which included: (1) submissions for routine diagnostic testing from the department of Fisheries and Oceans (DFO)'s salmon-enhancement hatcheries; (2) archived wild juvenile salmon from DFO's salmon marine interactions program; (3) archived diagnostic samples collected by non-DFO groups; and (4) samples of out-migrating Chinook, Coho and Sockeye salmon, of which were collected during juvenile salmon surveys (Table 1). These collections represented high geographical and temporal regional diversity and were obtained using a variety of sampling techniques in order to maximize our likelihood for identifying putative regional non-uniformity in the PRV-1 distribution. In this study, we use the term juvenile salmon to describe smolts collected in freshwater including pre-releases from hatcheries, as well as fish in their first year in the marine environment (spring ocean-entry through to the end of the following winter, i.e., April through to March). Adult salmon in this study were defined by having a fork length greater than 40 cm caught in marine waters or pre-spawning individuals at enhancement facilities.

**Table 1.** The prevalence of Piscine orthoreovirus (PRV-1) in different life-history stages of wild Pacific salmon collected from 2011 to 2020 in British Columbia. The sample region/description acronyms for locations are Strait of Georgia (SOG), West Coast Vancouver Island (WCVI), Lower Fraser River (LFR) and Discovery Islands (DISC). The lower and upper 95% confidence intervals for prevalence were calculated using the Wilson method [15].

| Species | Life Stage | Survey # | Sample Month | Sample Region/Description | Sample Size (N) | No. of Individuals Testing Positive | Prevalence (%) | Lower 95% CI | Upper 95% CI |
|---|---|---|---|---|---|---|---|---|---|
| Chinook | Freshwater pre-release | 2015-02 | June | Quinsam | 25 | 0 | 0.0 | 0.00 | 13.32 |
| | | 2016-02 | June | Quinsam | 15 | 0 | 0.0 | 0.00 | 20.39 |
| | | 2018-B | March | Eby St-Terrace | 25 | 0 | 0.0 | 0.00 | 13.32 |
| | | 2018-D | March | Spius creek, Coldwater | 25 | 0 | 0.0 | 0.00 | 13.32 |
| | | 2018-E | March | Spius creek | 24 | 0 | 0.0 | 0.00 | 13.80 |
| | | 2018-F | April | Inch creek, Chilko | 25 | 0 | 0.0 | 0.00 | 13.32 |
| | | 2018-G | April | Chehalis, Chilko | 25 | 0 | 0.0 | 0.00 | 13.32 |
| | | 2018-H | April | Chehalis-Summer red | 25 | 0 | 0.0 | 0.00 | 13.32 |
| | | 2018-J | April | Tenderfoot, Namquam | 25 | 0 | 0.0 | 0.00 | 13.32 |
| | | 2018-K | April | Tenderfoot, Ashley | 25 | 0 | 0.0 | 0.00 | 13.32 |
| | | 2018-O | April | Nitnat | 24 | 0 | 0.0 | 0.00 | 13.80 |
| | Total: | | | | 263 | 0 | 0.0 | - | - |
| | Juvenile | 2012-01 | May | SOG | 50 | 0 | 0.0 | 0.00 | 7.13 |
| | | 2012-02 | June | SOG | 42 | 0 | 0.0 | 0.00 | 8.38 |
| | | 2015-05 | June | SOG | 11 | 0 | 0.0 | 0.00 | 25.88 |
| | | 2015-16 | May–June | SOG | 218 | 4 | 1.8 | 0.72 | 4.62 |
| | | 2015-19 | October | WCVI | 87 | 11 | 12.6 | 7.21 | 21.24 |
| | | 2016-07 | May | SOG | 78 | 1 | 1.3 | 0.23 | 6.91 |
| | | 2016-08 | June | SOG | 45 | 2 | 4.4 | 1.23 | 14.83 |
| | | 2016-12 | July | SOG | 25 | 0 | 0.0 | 0.00 | 13.32 |
| | | 2018-04 | September–October | SOG | 318 | 26 | 8.2 | 5.64 | 11.71 |
| | | 2019-02 | June | SOG | 179 | 1 | 0.6 | 0.10 | 3.10 |
| | | 2019-03 | September–October | SOG | 281 | 51 | 18.1 | 14.08 | 23.08 |
| | | 2020-02 | September–October | SOG | 260 | 22 | 8.5 | 5.65 | 1.48 |
| | Total: | | | | 1594 | 118 | 7.4 | - | - |
| | Adult Returns | 2016-161 | November | Puntledge | 25 | 1 | 4.0 | 0.71 | 19.54 |
| | | 2016-170 | November | Omega, Robertson | 40 | 3 | 7.5 | 2.58 | 19.86 |
| | | 2017-110 | August | Tenderfoot, Chekamus | 26 | 0 | 0.0 | 0.00 | 12.87 |
| | | 2017-121 | August | Tenderfoot | 8 | 0 | 0.0 | 0.00 | 32.44 |
| | | 2017-143 | September | Chilko | 25 | 0 | 0.0 | 0.00 | 13.32 |
| | | 2017-163 | October | Puntledge | 24 | 0 | 0.0 | 0.00 | 13.80 |
| | | 2017-167 | October | Robertson | 36 | 2 | 5.6 | 1.54 | 18.14 |
| | | 2017-181 | November | Puntledge | 58 | 1 | 1.7 | 0.31 | 9.14 |
| | Total: | | | | 242 | 7 | 2.9 | - | - |
| | Total Chinook: | | | | 2099 | 125 | 6.0 | 5.02 | 7.05 |

**Table 1.** *Cont.*

| Species | Life Stage | Survey # | Sample Month | Sample Region/Description | Sample Size (N) | No. of Individuals Testing Positive | Prevalence (%) | Lower 95% CI | Upper 95% CI |
|---|---|---|---|---|---|---|---|---|---|
| Chum | Juvenile | 2011-07 | March–May | WCVI | 60 | 0 | 0.0 | 0.00 | 6.02 |
| | | 2012-01 | May | SOG | 25 | 0 | 0.0 | 0.00 | 13.32 |
| | | 2012-02 | June | SOG | 50 | 0 | 0.0 | 0.00 | 7.13 |
| | Total Chum: | | | | 135 | 0 | 0.0 | 0.00 | 2.77 |
| Coho | Freshwater | 2018-A | March | Kitimat | 25 | 0 | 0.0 | 0.00 | 13.32 |
| | | 2018-C | March | Oilfield, Prince Rupert | 25 | 0 | 0.0 | 0.00 | 13.32 |
| | | 2018-I | April | Inch creek, Stave | 25 | 0 | 0.0 | 0.00 | 13.32 |
| | | 2018-L | April | Quinsam | 25 | 0 | 0.0 | 0.00 | 13.32 |
| | | 2018-M | April | Fanny Bay | 25 | 0 | 0.0 | 0.00 | 13.32 |
| | | 2018-N | April | Nitnat | 25 | 0 | 0.0 | 0.00 | 13.32 |
| | | 2018-Q | April | Chilliwack, Coldwater | 25 | 0 | 0.0 | 0.00 | 13.32 |
| | | 2018-R | April | Quatse | 25 | 0 | 0.0 | 0.00 | 13.32 |
| | Total: | | | | 200 | 0 | 0.0 | - | - |
| | Juvenile | 2015-16 | May | SOG | 1 | 0 | 0.0 | 0.00 | 79.35 |
| | | 2016-07 | July | SOG | 1 | 1 | 100 | 20.65 | 100.00 |
| | | 2016-08 | June | SOG | 2 | 0 | 0.0 | 0.00 | 65.76 |
| | | 2018-04 | September | SOG | 152 | 1 | 0.7 | 0.12 | 3.63 |
| | | 2019-02 | June | SOG | 142 | 4 | 2.8 | 1.10 | 7.02 |
| | | 2019-03 | September | SOG | 128 | 3 | 2.3 | 0.80 | 6.66 |
| | | 2020-02 | September | SOG | 169 | 1 | 0.6 | 0.10 | 3.28 |
| | Total: | | | | 595 | 10 | 1.7 | - | - |
| | Adult Returns | 2017-195 | August | Oilfield | 15 | 0 | 0.0 | 0.00 | 20.39 |
| | | 2017-191 | September | Kitimat | 50 | 2 | 4.0 | 1.10 | 13.46 |
| | | 2017-192 | September | Nitnat | 25 | 0 | 0.0 | 0.00 | 13.32 |
| | | 2017-212 | September | Tenderfoot | 50 | 1 | 2.0 | 0.35 | 10.50 |
| | | 2018-203 | September | Kitimat | 50 | 4 | 8.0 | 3.15 | 18.84 |
| | | 2018-209 | September | Nitnat | 25 | 0 | 0.0 | 0.00 | 13.32 |
| | Total: | | | | 215 | 7 | 3.3 | - | - |
| | Total Coho: | | | | 1010 | 17 | 1.7 | 1.05 | 2.68 |
| Pink | Juvenile | 2012-01 | May | SOG | 20 | 0 | 0.0 | 0.00 | 16.11 |
| | | 2012-02 | June | SOG | 50 | 0 | 0.0 | 0.00 | 7.13 |
| | Total Pink: | | | | 70 | 0 | 0.0 | 0.00 | 5.20 |
| Sockeye | Juvenile | 2014-RST | April–May | LFR | 112 | 0 | 0.0 | 0.00 | 3.32 |
| | | 2016-RST | April–May | LFR | 232 | 0 | 0.0 | 0.00 | 1.63 |
| | | 2012-01 | May | DISC | 135 | 0 | 0.0 | 0.00 | 2.77 |
| | | 2014-04 | May–June | DISC | 127 | 0 | 0.0 | 0.00 | 2.94 |
| | | 2015-05 | May–June | DISC | 43 | 0 | 0.0 | 0.00 | 8.20 |
| | | 2016-07 | May–June | DISC | 68 | 0 | 0.0 | 0.00 | 5.35 |
| | Total Sockeye: | | | | 717 | 0 | 0.0 | 0.00 | 0.53 |
| | GRAND TOTAL: | | | | 4031 | 142 | 3.5 | 3.00 | 4.14 |

#### 2.1.1. Freshwater: Hatchery and Lower Fraser River

A total of 457 tissue samples of pre-spawning adult Chinook and Coho salmon, which were collected at BC-salmon-enhancement hatcheries in 2016–2018, were obtained from DFO's fish health diagnostic laboratory in Nanaimo B.C. Samples of head kidney from both Chinook and Coho salmon were aseptically removed at the hatchery and stored at −20 °C. These samples were collected as part of routine bacterial kidney disease screening within the Fisheries and Oceans Canada salmon-enhancement program and were made available to use in this study.

We were also able to access 463 samples of pre-release juvenile Chinook and Coho salmon smolts collected at BC-salmon-enhancement hatcheries in 2015, 2016 and 2018 (Table 1). These samples, which consisted of head kidney, were aseptically removed after euthanasia, preserved in 1.0 mL of RNA later (Thermo Fisher, Waltham, MA, USA) and stored at −20 °C.

We also screened 344 out-migrating juvenile Fraser River Sockeye salmon that were obtained from DFO's salmon marine interactions program, caught using a rotary screw trap (RST) at Mission in the Lower Fraser River in 2014 and 2016 (Table 1). The collection methods are described in Neville et al. [16]. Whole fish were individually bagged, immediately frozen on dry ice and stored at −80 °C. In the laboratory, specimens were partially thawed, and samples of kidney and brain were collected and stored back at −80. A combination of either the kidney or brain (Supplemental Table S1) was processed for PRV-1 screening, as described below. These tissues were originally collected for a viral surveillance project and used as a case study to illustrate the importance of a sample size in Infectious Hematopoietic Necrosis virus prevalence reporting [17]. All freshly frozen tissue samples were processed for RNA extraction within approximately 3 months of sampling.

#### 2.1.2. Marine Waters: Strait of Georgia/Lower Johnstone Strait

Fisheries and Oceans Canada routinely conducts juvenile salmon surveys in the Strait of Georgia (SOG) and Lower Johnstone Strait (JS). We relied upon this program for access to 2401 samples of juvenile salmon from the SOG/lower JS. During the 2011–2020 period of this study, purse seine and trawl surveys were conducted in May through to early July—when juvenile Pacific salmon of all species are present in the SOG. Trawl surveys, which focus on juvenile Chinook and Coho salmon, were conducted later in the year (September to October), as these species remain in the SOG/JS for longer periods than Chum (*Oncorhynchus keta*), Pink (*Oncorhynchus gorbuscha*) or Sockeye salmon [16,18–23]. Purse seine and trawl samples were collected using a standardized track line monitored by the salmon marine interactions program within Fisheries and Oceans Canada [16]. In the Discovery Islands, an additional 219 samples of juvenile Chinook and Coho salmon were collected by beach seining in 2015 (Table 1).

Once caught, juvenile salmon were euthanized with a lethal dose of Tricaine methane sulfonate (MS-222), individually frozen on dry ice and stored at −80 °C or −20 °C until dissection. In some cases where small numbers of fish were collected (<10 individuals) in trawl surveys, samples of either spleen or kidney were aseptically removed in the field and preserved in 1.0 mL of RNA Later (Thermo Fisher).

#### 2.1.3. Marine Waters: West Coast Vancouver Island

We screened 147 samples from the West Coast of Vancouver Island for this study. These included samples of 60 Chum salmon caught in 2011 in Muchalat and Esperanza Inlets, which were originally submitted to the BC Center for Aquatic Health Sciences for diagnostic testing. These samples were a mix of internal organs that had been archived as homogenates and stored at −80 °C. We were also able to access 87 juvenile Chinook salmon, of which were collected during a DFO trawl survey in 2015 in Clayoquot Sound. These fish were euthanized with a lethal dose of MS222, individually frozen on dry ice in the field and archived at −80 °C.

#### 2.1.4. Laboratory Dissections

When necessary, frozen whole fish were allowed to partially defrost, and tissues were aseptically removed in the laboratory. RNA was extracted immediately, or samples were re-frozen and stored in −80 °C until RNA was extracted.

### *2.2. RNA Extraction and Reverse Transcription*

Total RNA was extracted from each sample (approximately 100 mg) using Trizol® Reagent (Life Technologies, Carlsbad, CA, USA) following the manufacturer's instructions. Specifically, samples were thawed at room temperature and immediately homogenized using one 5 mm stainless steel bead (Qiagen, Hilden, Germany) and a TissueLyzer II (Qiagen) for 2 min at 25 Hz. Phase separation was completed using 100 µL 1-Bromo-3-chloropropane (Sigma Aldrich, St. Louis, MO, USA) per 1 mL of Trizol®. Total RNA was quantified, and its purity was assessed using a NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific). Total RNA was normalized to 1000 ng/µL (quantities varied) in DNAse- and RNAse-free water (Life Technologies) and denatured for five minutes at 95 °C prior to reverse transcription. Complimentary DNA (cDNA) was synthesized using a High-Capacity cDNA reverse transcription kit (Applied Biosystems, Waltham, MA, USA) in 20 µL reactions following the manufacturer's instructions.

### *2.3. PRV-1 qPCR Screening*

As all PRV subtypes do not grow in cell culture, molecular-based diagnostic methods are routinely used. The molecular assays which have been developed and validated are subtype-specific and not cross-reactive to each other [24]. In this study, the cDNA generated in Section 2.2 was used as a template in a PRV-1 real-time qPCR assay, which targets the L1 segment and is suitable for the detection of all known genetic variants of PRV-1 [1]. Assays were performed on a Step One Plus real-time detection system (Applied Biosystems) using previously described primers and probes [1] (Supplemental File, Table S2). Briefly, each 15 µL reaction contained 1.0 µL of cDNA template, 0.4 µM of each forward and reverse primer (IDT, Coralville, IA, USA), 0.3 µM PisReol Probe (Applied Biosystems) and 1× Taq Man® Universal PCR Master Mix (Applied Biosystems). Cycling conditions were 95 °C for 10 min, followed by 40 cycles of 95 °C for 10 s and 60 °C for 30 s [25]. Samples were assayed in duplicate and were considered positive if both technical replicates reported a cycle threshold (Ct) value < 40 cycles or negative if both technical replicates failed to fluoresce beyond the preset threshold (Δ Rn 0.01) in 40 cycles. Duplicate reactions containing $10^7$ copies of the 482 bp double-stranded DNA gBLOCK fragment (Integrated DNA Technologies) consisting of a sequence targeted by the qPCR primer and probe [26] (Supplemental File, Table S2) were incorporated into each run to ensure stable amplification efficiency and to act as a positive template control.

### *2.4. Genetic Stock Identification*

Juvenile Chinook salmon were identified to source and stock by the molecular genetics laboratory at the Pacific Biological Station in Nanaimo, B.C., following the methods outlined in Beacham et al. [27]. Briefly, deoxyribonucleic acid (DNA) samples obtained from the fin tissues were surveyed for 15 microsatellites and the resulting genotypes were scored using GeneMapper (Applied Biosystems, Foster City, CA, USA). Genetic stock analyses for Chinook were assigned from a baseline composed of 295 stocks [27] and a minimum probability of 70% stock assignment. When an individual's results returned with less than 70% probability, but when the first 2 probabilities resulted in over 70% and were identified to the same region, they were assigned to a regional stock such as East coast Vancouver Island (ECVI), NSOG (Bute, Homanthko, Phillips) or Fraser River. Over 75% of Chinook salmon sent for genomic analyses were assigned a stock or regional stock, and the remainder were reported as unknown.

## 3. Results

In this study, 4031 Pacific Salmon collected from fresh and marine waters of BC were screened for the presence of PRV-1 over a 10-year period (Table 1). Findings specific to each individual species, life stage and stock (where applicable) are presented separately in the following sections.

### *3.1. Prevalence of PRV-1 in Juvenile Chum, Pink and Sockeye Salmon*

We obtained samples of juvenile Chum (2012 $n$ = 60), Pink (2012 $n$ = 70) and Fraser River Sockeye salmon (2012 $n$ = 135; 2014 $n$ = 127; 2015 $n$ = 43; 2016 $n$ = 68) from the northern areas of the SOG including the Discovery Islands and Chum salmon (2011 $n$ = 75) from Muchalat and Esperanza Inlets on the West Coast of Vancouver Island (WCVI). All these areas support the active commercial net-pen production of Atlantic Salmon. PRV-1 was not detected in any of these samples, nor was PRV-1 detected in juvenile Sockeye salmon (2014 $n$ = 112; 2016 $n$ = 232) collected in the lower Fraser River (Table 1, Supplemental File, Table S1).

### *3.2. Prevalence of PRV-1 in Coho Salmon*

For returning adult Coho salmon sampled during spawning at regional enhancement hatcheries, we found a low prevalence of PRV-1 in 2017 (Oilfield = 0%, $n$ = 15; Nitinat = 0%, $n$ = 50; Kitimat = 4%, $n$ = 50 and Tenderfoot = 2%, $n$ = 65) and 2018 (Nitinat = 0%, $n$ = 50 and Kitimat 4%, $n$ = 50) (Table 1). PRV-1 loads in infected individuals also were observed to be relatively low ($2.5 \times 10^2$ to $6.7 \times 10^3$ copies/µg total RNA; Ct range 26–31) (Supplemental File, Table S1). PRV-1 was not detected in the pre-release freshwater juvenile Coho salmon from eight enhancement facilities ($n$ = 25 each; 200 total) collected in 2018. These hatcheries are distributed from Chilliwack northwards to Kitimat, BC and include hatcheries on both coasts of Vancouver Island (Table 1). PRV-1-infected brood fish were identified in 2017 at the Kitimat hatchery; however, PRV-1 was not detected in juveniles produced from this brood year ($n$ = 25) sampled in 2018.

For juvenile Coho salmon caught in marine waters, a total of 595 individuals were screened for PRV-1 with most of these fish collected in the SOG and lower JS in 2018 (September 0.7% prevalence, $n$ = 152), 2019 (June: 2.8%, $n$ = 142; September 2.3%, $n$ = 128) and 2020 (September 0.6%, $n$ = 169). The overall PRV-1 prevalence was 1.7% across all years with loads ranging between approximately $1.8 \times 10^1$ and $1.8 \times 10^3$ copies/µg total RNA (Ct values 28 to 35) (Table 1, Supplemental File, Table S1).

### *3.3. Prevalence of PRV-1 in Chinook Salmon*

Adult Chinook salmon were collected at spawning from five enhancement hatcheries in the fall of 2016 and 2017. PRV-1 was detected in four out of eight locations, albeit at a low prevalence, ranging from 0% to 7.5% (overall prevalence of 2.9%; $n$ = 242) and with relatively low loads (median Ct 31.1 or $2.3 \times 10^2$ copies/µg total RNA; range 19.6–36.6 Ct or 6 to $4.5 \times 10^5$ copies/µg total RNA) (Table 1, Supplementary Table S1). The hatcheries which had positive detections were located on both the west (Omega, Robertson) and east coasts (Puntledge) of Vancouver Island. PRV-1 was not detected in pre-release Chinook salmon collected at eight enhancement hatcheries located across BC in 2015, 2016 and 2018 ($n$ = 262) (Table 1).

Most PRV-1 detections in Chinook salmon from this study were identified in juveniles collected in seawater with an overall prevalence of 7.4% across all dates and areas (SOG, JS and WCVI) (Table 1, Supplemental File, Table S1). This indicated that Chinook salmon appeared to have the highest PRV-1 infection rates for any of the Pacific salmon species tested in this study. Nevertheless, the prevalence had high temporal and regional variability. Specifically, there was a low prevalence (% of host individuals infected) of PRV-1 in juvenile Chinook salmon collected in the SOG in May–July over multiple years (2012—0%, $n$ = 92; 2015—1.7%, $n$ = 229; 2016—2.0%, $n$ = 148 and 2019—0.6%, $n$ = 179) (Table 1 and Figure 1). In contrast, samples obtained from the SOG in September during overlapping-sample periods

revealed a much higher detection rate (2018—8.2%, $n$ = 318; 2019—18.1%, $n$ = 281 and 2020–8.5%, $n$ = 260). Juvenile Chinook salmon collected in October 2015 from the WCVI (Clayoquot Sound) also had a high PRV-1 prevalence of 12.6% ($n$ = 87).

The relatively high prevalence of PRV-1 in juvenile Chinook salmon sampled in this study provided an opportunity to further examine PRV-1 distribution and aspects of transmission within this species. Specifically, we considered how the PRV-1 load, host stock of origin and host condition (weight and condition factor) may contribute to the epidemiology of PRV-1 in the region.

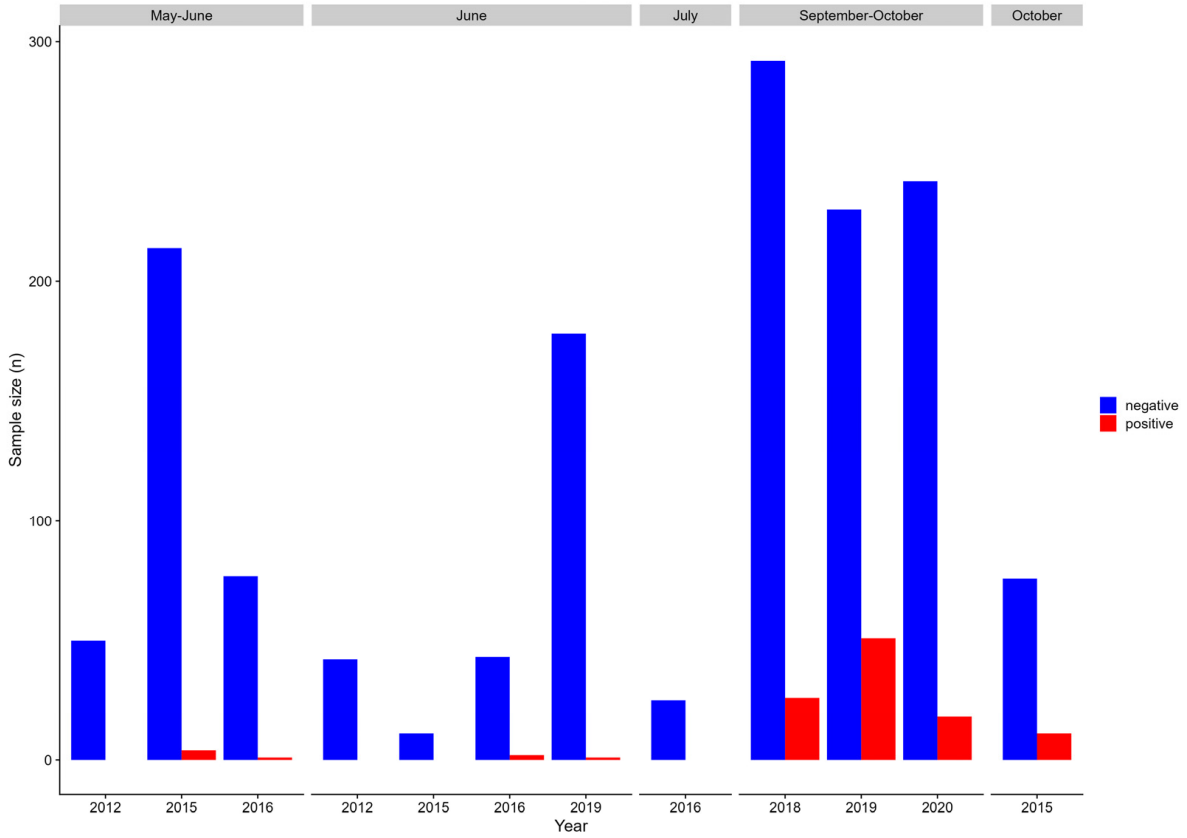


**Figure 1.** The numbers of juvenile Chinook salmon (*Oncorhynchus tshawytscha*) screened and tested negative and positive for *Piscine orthoreovirus*-1 (PRV-1) in relation to the month and year of sampling. Samples were obtained using various fishing techniques (May–June (Purse seine), June (mid-water trawl), July (Purse seine), September–October (mid-water trawl) and October (mid-water trawl)) in the Strait of Georgia (SOG) and Lower Johnstone Straight (JS) between 2012 and 2020, and Clayoquot Sound, West Coast of Vancouver Island (WCVI), only in October 2015.

#### 3.3.1. Relative PRV-1 Loads in Juvenile Chinook Salmon

Regardless of the year, the Ct values—which are an inverse relative quantity of PRV load on an exponential scale—of infected individuals collected in June and July in the SOG were all relatively high (Cts > 33; loads < approximately 60 copies/µg total RNA). Individuals collected in the SOG in September and October had a broader range of PRV-1 loads, with some fish having over one-order-of-magnitude-higher load detected in the spring (~40% with Cts < 30; > approximately 500 copies/µg total RNA), indicative of a cumulative increased PRV-1 load for infected individuals over the summer months (Figure 2).

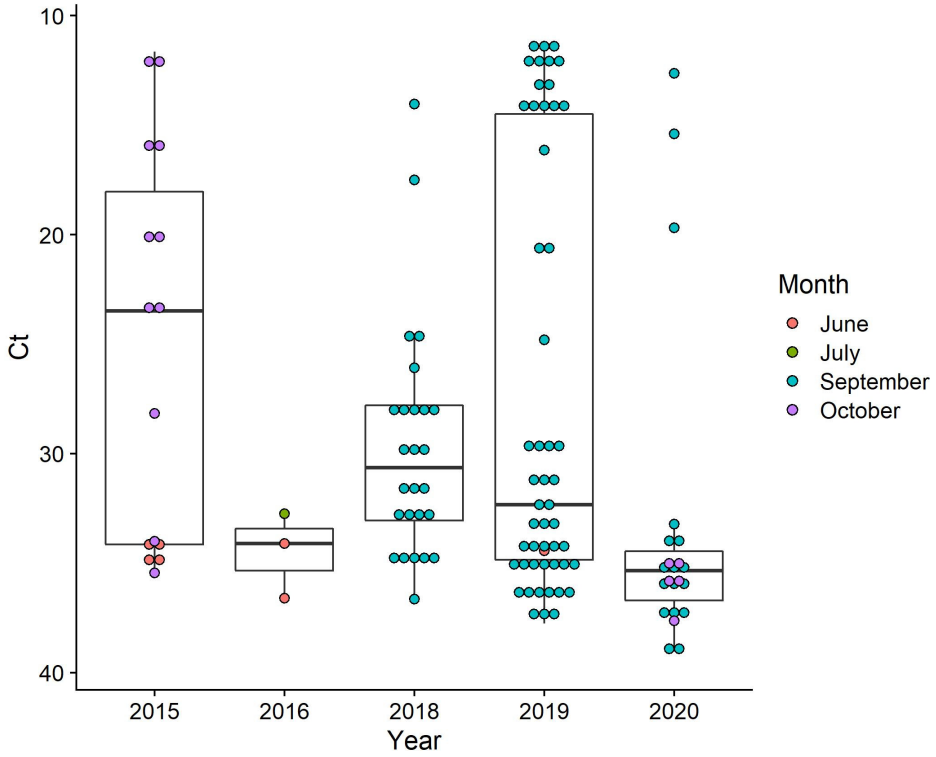


**Figure 2.** Ct value boxplots for juvenile Chinook salmon (*Oncorhynchus tshawytscha*) screened for *Piscine orthoreovirus*-1 (PRV-1) in the Strait of Georgia (SOG) and Lower Johnstone Strait (JS) in 2015, 2016, 2018–2020 and in Clayoquot Sound (WCVI) in October 2015, with individual Ct values colored by catch month. Box spread is the 25th to 75th percentile of Ct values with the median bar; the whiskers show the spread of the Ct values, and outliers are included as dots only.

#### 3.3.2. Stock Composition of PRV-1-Infected Juvenile Chinook Salmon

In 2018 and 2019, PRV-1-infected juvenile Chinook salmon were only caught in the northern SOG (Discovery Islands). Of these, 96% and 76%, respectively, belonged to stocks which originate from watersheds that enter directly into the northern SOG and JS (Figure 3). This was contrasted in 2020, where the majority (88%) of PRV-1-infected juvenile Chinook were caught in the southern-to-central SOG and the Juan de Fuca Strait (JDF). PRV-1-positive individuals in 2020 mostly belonged to stocks originating from the Fraser River (Fraser River—22%, Chilliwack/Harrison—11% and South Thompson—11%) or Washington state (33%). Specifically, positive individuals caught in the central and southern SOG all originated from Fraser River, while those caught in the JDF were from Washington state.

#### 3.3.3. Relationship between Body Weight and Condition Factor and PRV-1 Infection Status in Juvenile Chinook Salmon

Using combined data from our September/October 2018, 2019 and 2020 mid-water trawl surveys, PRV-1 was detected across the spectrum of both weight and condition factors of Chinook salmon in this study. However, the size of Chinook salmon at seawater entry can be highly variable between stocks and years [28], thus potentially confounding cross-yearly comparisons. To normalize for potential size differences between stock cohorts and years, individual fish weights and condition factors were assessed as a relative percentage of each respective cohort and stock year. By this method, we found that 71.4% and 87.1% of PRV-1-infected individuals fell within the 25th and 50th percentile for weight and condition factor, respectively (Figure 4), indicating that most PRV-1 infections occurred in the smaller, lower-conditioned Chinook within each cohort.

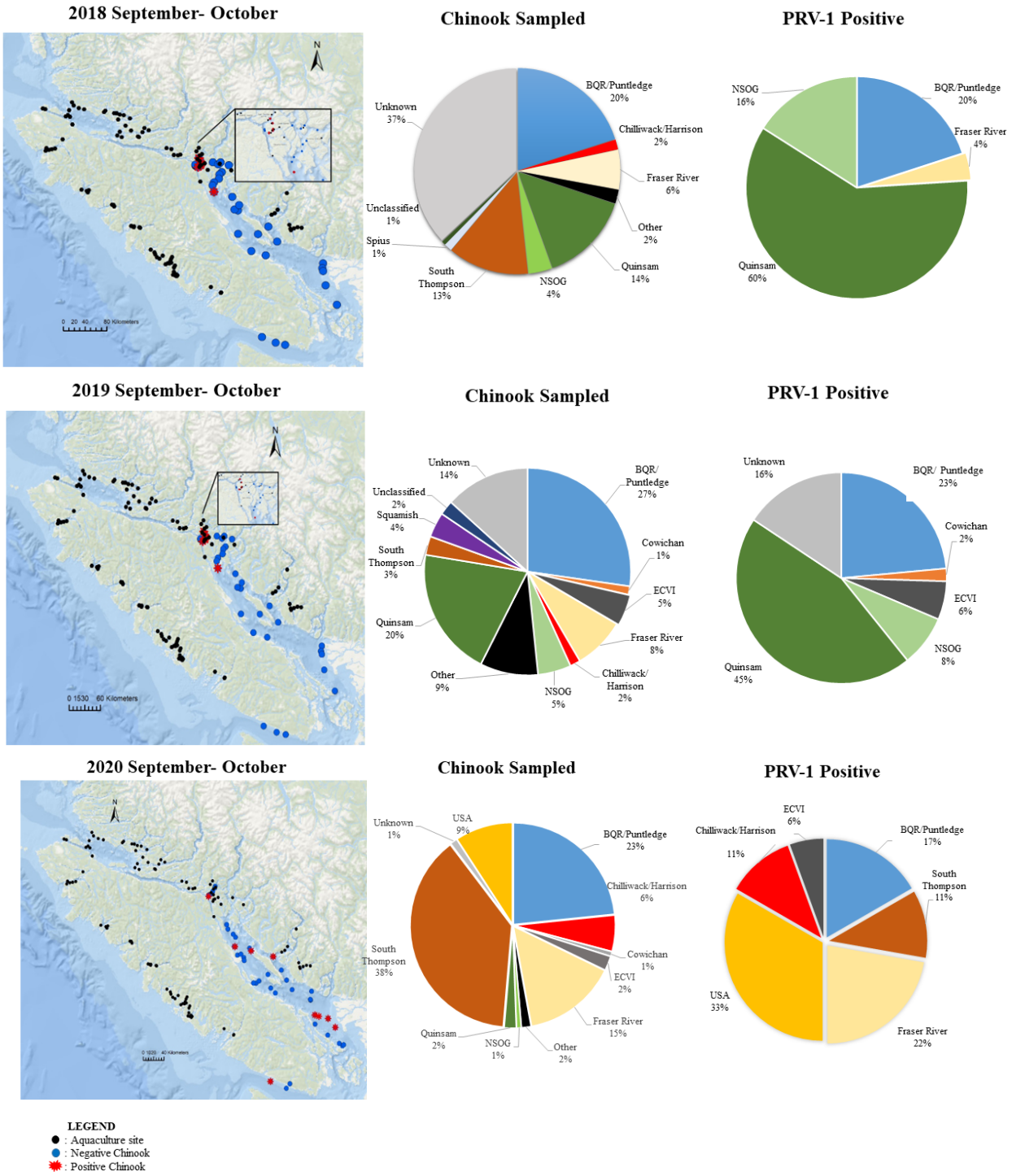


**Figure 3.** Catch locations and stock composition of *Piscine orthoreovirus-1* (PRV-1)-positive (red symbol) and -negative (blue circle) juvenile Chinook salmon from the September–October in 2018 (top row), 2019 (middle row), and 2020 (lower row) mid-water trawl surveys in relation to finfish Atlantic aquaculture sites (black dots). Stock composition notes: unknown—individuals for which stock composition was not determined; East Coast Vancouver Island (ECVI)—defined as ECVI when genetic results returned > 70% probability, but when the first 2 probabilities resulted in over 70% probability with both assigned to ECVI watersheds; Fraser River—all watersheds excluding those which are reported separately; Northern Strait of Georgia (NSOG)—includes stocks from Bute, Homanthko and Phillips watersheds; the USA—stocks originating from Washington state.

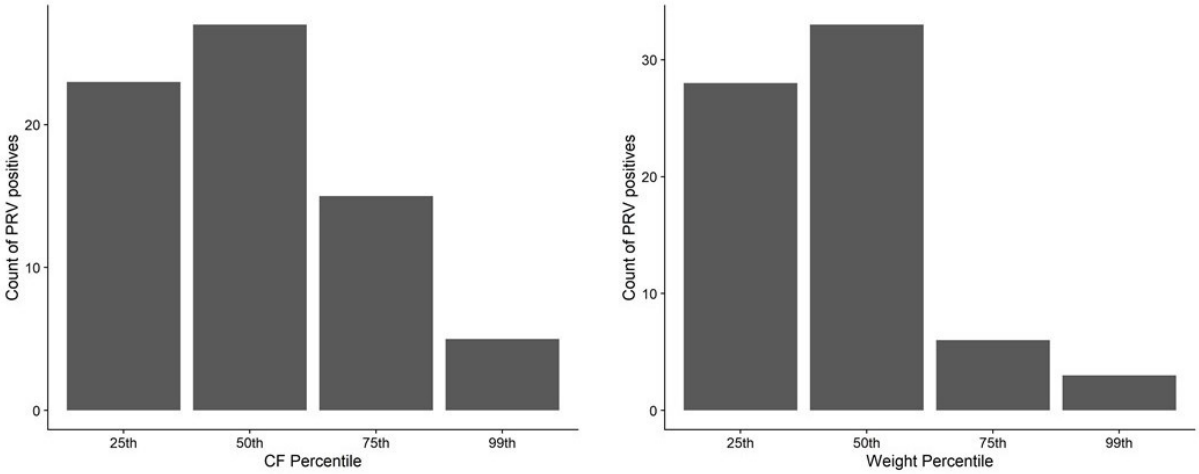


**Figure 4.** Condition factor (CF) and weight (W) distribution of juvenile Chinook (*Oncorhynchus tshawytscha*) salmon testing positive for *Piscine orthoreovirus-1* (PRV-1). Fish were caught in the Strait of Georgia (SOG), lower Johnstone Strait (JS) and Juan de Fuca Strait (JDF) during mid-water trawl surveys in September–October of 2018 ($n$ = 26), 2019 ($n$ = 51), and 2020 ($n$ = 22). Fish were initially assigned into percentiles according to weight and condition factor separately for each stock and year of collection.

## 4. Discussion

The overall prevalence of PRV-1 across all species and life histories reported in our study (3.5% from 2011–2020) is nearly identical to the overall PRV-1 prevalence reported by Purcell et al. [29] from pre-spawning Pacific salmon and trout collected in Washington and Alaska, USA, in 2012–2014 (3.4% of 2252 fish). It is also in general agreement with cumulative data for PRV-1 detection in Pacific salmon of the Northeastern Pacific over the past two decades, as documented by more than 15 independent studies (cumulative 3.1% of 12,992 fish, as reviewed by [4]). These data cumulatively support that an endemic, widespread and continuous low-level prevalence of PRV-1 has been maintained throughout the Northeast Pacific in anadromous Pacific salmonids throughout recent decades. Our findings, however, are unaligned with many of the reported prevalences in juvenile and adult Pacific salmon from Morton et al. [30]. In general, these authors report a much higher prevalence and a broader host distribution in juvenile salmon than reported in this and other studies in the Northeast Pacific. These inconsistencies could be attributed to differences in sample timing, sampling locations or diagnostic test methods.

PRV-1 was not detected in juvenile Chum, Pink or Fraser River Sockeye salmon collected in marine waters of the SOG and lower JS, juvenile Chum salmon from Muchalat and Esperanza Inlets WCVI, or in juvenile Fraser River Sockeye salmon caught in freshwater in the lower river in this study. Previous studies also failed to detect PRV-1 in juvenile/sub-adult and adult Chum salmon collected within the same general time frame in adjacent areas [29,31,32], and did not detect PRV-1 in juvenile Pink salmon caught in April and May of 2008 in the Broughton Archipelago or in May of 2013 in the JS [32,33]. Similar results have been obtained in juvenile Sockeye salmon in adjacent areas [32,34,35], except for a single detection in 2013 [36].

Thus, these data indicate a low (<1%) prevalence of PRV-1 in juvenile Sockeye salmon in BC and, particularly in consideration of the rapid rate at which juvenile Sockeye migrate through coastal waters [37], are likely a species that has contributed minimally to PRV-1 prevalence in the region in recent years. Although cumulatively a relatively small sample size ($n$ = 1668), these data suggest that these specific species and life stages have had a minimal contribution to regional PRV-1 prevalence over the past two decades and therefore appear to have a low likelihood of further contributing to PRV-1 persistence in the region.

A previous investigation into the freshwater occurrence of PRV-1 within Pacific salmon stocks of the federal-salmon-enhancement program indicated a minimal occurrence of PRV-

1 during early freshwater salmon life stages in recent years [13]. Our study supports this previous finding as we did not detect PRV-1 in any juvenile freshwater fish samples; this is supported by the findings of Thakur et al. [38] and Tucker et al. [39]. This finding further supports that most infections with PRV-1 occur following entry to the sea and that the primary reservoirs of PRV-1 are in seawater environments. Nevertheless, we also identify that some freshwater infection sources may be present, at least for some species such as Chinook. Specifically, we identified a low prevalence of PRV-1 in juvenile Chinook salmon collected in the SOG in May and early June (Table 1). Chinook salmon primarily enter the SOG starting in late April through July and were therefore resident in seawater for approximately 1 to 1.5 months (30 to 45 days) at their time of capture in May. In the Northeast Pacific, farmed Chinook salmon, which were stocked in May, were first identified as positive for PRV-1 in late August (3 months) [6], and farmed Atlantic salmon populations generally became PRV-1-positive only after a period of 100–300 days at sea, i.e., after more than 3 months at sea [8]. Based on these time frames, it is possible that at least some of the PRV-1-positive juvenile Chinook salmon caught in May through to early June represent infections acquired in freshwater, assuming infections at the population level occurred 3 months prior.

Seawater reservoirs of PRV-1 within the Northeastern Pacific appear to be regionally ubiquitous [8,29]. Nevertheless, infectivity at the species level appears far more specific. A cross-study trend for PRV prevalence within wild Pacific salmonids indicates that the highest PRV-1 prevalence occurs in Chinook and Coho salmon relative to the other species, as reviewed in [4]. Although our study ultimately supports this finding, it additionally identifies potential regional, life-history and/or temporal shifts in relative occurrence of PRV-1 within these two species when comparing fish caught in British Columbia vs. adjacent American (Alaska and Washington, USA) coastal waters. Specifically, Purcell et al. [29] identified the highest PRV-1 prevalence when returning Coho salmon (11.8%) relative to returning Chinook salmon (4.0%) in Alaska and Washington during 2012–2014. This is contrasted with data from our study where we identified comparatively low PRV-1 prevalence in both returning adult Coho (3.3%) and Chinook (2.9%) salmon in British Columbia during the overlapping period of 2012–2020. This highlights that the 2–3 times higher PRV-1 prevalence in returning adults to Washington/Alaska relative to British Columbia may be due to divergent infection pressures for these adjacent stocks. Given that most infections of PRV appear to be acquired at sea, our data would suggest possible variations in behavior/spatial distributions of these stocks while at sea, thereby resulting in segregated PRV-1 reservoirs. A further phylogenetic analysis of PRV-1 samples collected from these different stocks would likely provide greater insight for how PRV-1 is being regionally circulated among Pacific salmon populations.

Anadromous Chinook salmon had the highest PRV-1 prevalence of all Pacific salmon screened in our study, where the majority of the detections which occurred in seawater adapted juvenile populations in coastal waters. PRV-1 detections in this study occurred as early as May in near-shore areas and were maintained or even enhanced in resident Chinook salmon juveniles through the summer and into the fall season. Laboratory co-habitation studies have demonstrated that PRV-1 shedding primarily occurs early after infection, corresponding to the periods of highest PRV-1 loads, after which there is a reduction in shedding during the persistent phase of infection when PRV-1 loads are low [25]. In this study, a total prevalence of 7.2% in juvenile Chinook with Ct values ranging from 11.3 to 38.9 suggests that transmission may be occurring early during out-migration in the marine environment. Increased viral loads in some Chinook, particularly later in the season (September and October), suggest that these fish may also be shedding an infectious virus and may act as a reservoir for infection. It is possible that infected Coho salmon may also serve as a seawater reservoir, although this is likely to be at a lesser extent than Chinook based on life-history movements and an overall PRV-1 prevalence of 1.7% across all years and months.

In this study, juvenile Chinook salmon collected in the SOG in late September/early October had a higher PRV-1 prevalence when compared to fish caught in May and June. The increase in PRV-1 prevalence with time at sea is similar to what is commonly observed in farmed salmon in BC [8,40]. Most juvenile Chinook salmon entered the SOG in late April through to June and were therefore resident in seawater for approximately 3 to 4 months (90 to 120 days) at their time of capture in late September. As described above, other studies on juvenile Chinook salmon have not identified an increase in PRV-1 prevalence with time at sea, which may be due, at least in part, to their reporting of data combined across years and over broad geographical areas.

Migration behavior differs amongst Pacific Salmon species, stocks and life-histories [18,19,22,23]. Specifically, the out-migration of juvenile Sockeye indicates a relatively fast migration through the SOG, ranging between 14 and 54 days [16,20,37]. Like Sockeye, juvenile Pink and Chum salmon also use epipelagic waters as their primary feeding areas and are thought to rapidly transit through the SOG [21]. In contrast, Chinook and Coho salmon have relatively long residency times in coastal water before heading into open waters [19,22]. Coho salmon have been shown to stay within 200–400 km of their natal stream between June and December [19], and coded wire tag (CWT) data revealed juvenile Chinook remain even closer within a 100–200 km range irrespective of their life history [22]. Our results in September of 2018 and 2019 are consistent with this behavioral migratory pattern, as Big Qualicum River (BQR)/Puntledge and Quinsam River Chinook stocks are more prevalent around the Discovery Island aquaculture farms in September to October, which are within the described range of their natal streams. The higher prevalence of PRV-1 in these populations may be related to the proximity of natural seawater reservoirs of PRV-1. Specifically, salmon farms, fish-processing plants or contact with additional resident salmonids may provide an infectious source for Chinook and Coho. We further hypothesize that once infections are established in at least some individuals, prolonged residencies in small areas may further propagate PRV-1 transmission within cohorts and possibly back to farmed stocks. Further testing as to the validity of this hypothesis is warranted.

It is recognized that commercial and enhancement salmon hatcheries, sea cage sites and fish-processing plants have a potential to serve as the sources of PRV-1 infections to wild and farmed salmon in the Northeast Pacific and therefore contribute to the maintenance of PRV-1 in coastal waters, as reviewed in [4,8,9,40]. However, it has been demonstrated here and in other studies that the populations of wild Pacific salmon carry PRV-1 in areas which are devoid of commercial salmon farming and processing activities [9,29,32]. Although the integration of farmed or processed fish into the regional transmission and persistence of PRV-1 is highly likely, the consideration of the stock composition of PRV-1-positive Chinook salmon in 2020 identifies that multiple persistence and transmission mechanisms likely exist for PRV-1 in the region. Specifically, PRV-1-positive Chinook in the SOG in 2020 came mostly from areas devoid of salmon farms. It should therefore be recognized that there are a number of factors that may be responsible for PRV-1 transmission. We suspect the most salient to be: (i) differences in susceptibility to infection between species (infectious dose), (ii) differences in life histories, migratory and/or other behaviors (especially those which effect proximity to and/or residence time in the vicinity of PRV-1 reservoirs), and (iii) differences in enhancement activities between species.

Based on farm and laboratory challenge data for Atlantic salmon, Polinski et al. [8] hypothesized that relatively low PRV loads (Ct values > 25) may indicate that fish were recently infected (likely within the past few weeks), whereas high loads (blood Ct values < 25) indicate individuals which have probably been infected for a considerable time (a month or more). In this study, the Ct values of infected juvenile Chinook salmon collected in June and July in the SOG were all relatively high (Cts > 33), indicating low PRV-1 loads suggestive of relatively recent infections. As most salmon do not appear to clear PRV-1 infections for at least two years [8], it is therefore congruent that the PRV-1 prevalence and load increased in individuals collected in the SOG between September and October, where many fish had Ct values at least one order-of-magnitude lower than loads detected in the

spring. Nevertheless, some infections in the fall still had relatively high Cts, indicating that new infections were still likely occurring in the fall. This would indicate that seawater infection pressure for Chinook appears soon after seawater entry and continues throughout the summer and fall of the first year, although as noted previously, some infections seen shortly after seawater entry in May could represent infections acquired in freshwater.

In this study, we reported that although PRV-1 was detected across the spectrum of both weight and condition ranges, higher Chinook salmon proportions (71.4% and 87.1%) and PRV-1-infected juvenile Chinook salmon fell within the 25 and 50th percentiles for weight and condition factor, respectively. A similar observation was reported by Bass et al. [41] for Chinook and Coho salmon in that they identified a variety of pathogens which had moderate-to-strong negative correlations with body condition or survival for one host species in one season, whereas PRV-1 had the most consistently negative associations with body condition in both host species across seasons. There is a robust body of laboratory challenge studies that have assessed the physiological consequences and disease-causing potential of PRV-1 from the Northeast Pacific to Atlantic, Chinook, Coho and Sockeye salmon [25,26,42–45]. These studies have identified that all these species support the replication of PRV-1, but reported no significant mortality, pathology or physiological consequences of infection. Thus, it is unclear why PRV-1 infections occur more frequently in smaller and poorly conditioned Chinook salmon, given that the correlation is unlikely to be directly attributable to PRV-1 virulence. It is possible that this might be a consequence of life-history/behavioral differences in these small or poor conditioned fish relative to their larger or older counterparts, which puts them at a greater risk for PRV-1 exposure and infection. Alternatively, Chinook salmon in the smaller size classes may more commonly be co-infected with other infectious agents, which in themselves may be responsible for lower condition factors [41]. To our knowledge, no studies have purposely examined the effect of co-infections on the susceptibility of salmon to infection with PRV-1 in salmon. Therefore, it is unknown whether co-infections with other agents might influence susceptibility to PRV-1 infection; this is an area which warrants further investigation.

## 5. Conclusions

Our study contributes to the current body of evidence for a small proportion (~6%) of Chinook and Coho salmon in BC coastal waters have been infected with PRV-1 over the past decade. However, our study also provides support that PRV-1 is rarely, if ever, propagated to subsequent generations through BC enhancement hatcheries or during natural spawning events in the region. Although it appears that freshwater may not be an important reservoir for PRV-1 in BC, there remains uncertainty with respect to the importance of PRV-1 transmission at spawning in the continued persistence of PRV-1 in the Northeast Pacific at large. Studies to determine the risk that infected adults will transmit PRV-1 to their offspring, the conditions under which transmission occurs and the health consequences of transmission are warranted.

The majority of Chum, Pink and Sockeye samples that we examined were from juveniles caught in the SOG and adjacent waters in May and June. Assuming PRV-1 infection pressures are associated with seawater environs, the generally low occurrence of PRV-1 in Chum, Pink and Sockeye juveniles may be in part due to the relatively short period of time (≈60 days maximum) in seawater at the time of sampling. However, the well-supported literature for the low PRV-1 prevalence in returning adults of these species (particularly Sockeye) suggests that later infections are also rare. Thus, these species do not appear to be a major reservoir for PRV-1 in the region, particularly as it seems unlikely that fish becoming infected at sea would either clear infections once established or have a higher probability for premature mortality, as reviewed by [4].

In comparison, juvenile Chinook and Coho salmon remain resident in the SOG until much later in the year; annual surveys for these species also include sampling in mid-September to early October. In both species, the prevalence of PRV-1 was highest in the September/October samples, thus suggesting something about these species-specific

behaviors or that the near-shore environment contributes to the regional maintenance of PRV-1.

With respect to juvenile Chinook salmon in 2018 and 2019, most of the PRV-1-infected juvenile Chinook salmon were caught in the vicinity of salmon farms in the northern SOG/lower JS with most of these fish belonging to stocks/stock complexes which originate from that area—suggesting that PRV-1 transmission may be occurring between farmed and wild fish. However, in September 2020, the majority of PRV-1-infected fish were caught across a much broader geographical and stock/stock complex range. These results provide support for the presence of multiple PRV-1 reservoirs in the Northeast Pacific, some independent of farms.

The Pacific strain of PRV-1 has been shown to have low pathogenicity, with infections causing no significant mortality, pathology or physiological consequences [25,42–45]. Interestingly, in our 2018, 2019 and 2020 September/October samples of juvenile Chinook salmon, the overall prevalence of PRV-1 was 11.5% and the majority of these infected fish fell in the 25 and 50th percentiles for weight and condition factor, a result which is similar to that obtained by Bass et al. [41]. This would suggest that behaviors associated with early development or fish with poor body condition factors are associated with PRV-1 transmission, or alternatively, that currently unconsidered environmental factors associated with near-shore environments may potentially increase the virulence of PRV-1 enough to impact the fish condition or other causative factors associated with the size and poor condition in Chinook, thus making them more susceptible to PRV-1 infection. Future monitoring programs of wild salmon for infection with PRV-1 should take into consideration the biology/ecology of the host and the biology/epizootiology of PRV-1 when planning sampling programs.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fishes8050252/s1.

**Author Contributions:** L.D.M.T. collected samples, performed RNA extractions, PRV-1 molecular screening, assembled maps and figures and drafted the manuscript. J.C.B. performed RNA extractions on Sockeye salmon, statistical analysis, aided in figure development and manuscript edits. M.P.P. designed diagnostic methodologies and contributed to data interpretation. S.C.J. conceived the study, acquired funding, and participated in data analysis and interpretation. All authors have read and agreed to the published version of the manuscript.

**Funding:** Funding for this work was provided by Fisheries and Oceans Canada Aquaculture Ecosystems Interactions Program and the Program for Aquaculture Regulatory Research.

**Institutional Review Board Statement:** The tissues used in this study were collected as part of ongoing juvenile salmon surveys or routine fish health diagnostic testing. The collection of samples for these purposes 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 a government mandate for stock assessment population estimates and standardized disease monitoring.

**Data Availability Statement:** The data that support the findings of this study are available are provided in the manuscript and Supplemental Files.

**Acknowledgments:** The authors would like to thank Chrys-Ellen Neville (DFO), Christy Thompson (DFO), Canadian Coast Guard (CCG) staff on the W.E. Ricker and John Franklin, fishing vessels Sea Crest Staff and the Nordic Queen. We would also like to thank all of the RST staff, SEP and community hatcheries for providing samples.

**Conflicts of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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