From: Garver, Kyle
Sent: Thursday, July 28, 2022 2:54 PM
To: Ryall, Emily
Cc: Higgins, Mark
Subject: RE: ACRDP Final Report
Attachments: ACRDP final report signed cover page1.pdf; ACRDP Final Report _PRV in the sea_July28.doc

Hi Emily,
Please find attached the final report for ACRDP project 18-P-01. Note the signature page is included as a separate file.

Sorry for the delay.
Regards,
Kyle

From: Ryall, Emily <Emily.Ryall@dfo-mpo.gc.ca>
Sent: Wednesday, June 15, 2022 12:43 PM
To: Garver, Kyle <Kyle.Garver@dfo-mpo.gc.ca>
Cc: Higgins, Mark <Mark.Higgins@dfo-mpo.gc.ca>
Subject: ACRDP Final Report

Dear Kyle,

I hope this email finds you well.

This is a friendly reminder that the Final Report for your ACRDP project **18-P-01** *"Prevalence and transmission dynamics of Piscine Reovirus (PRV) in the marine environment"* is overdue and we ask that you submit it by **June 30, 2022**. The final reports are a requirement of the ACRDP funding and are needed for each ACRDP project to promote the transfer of knowledge, both to the industry partner(s) as well as the general public, through communication initiatives including online result summaries.

Please ensure you use one of the attached English or French templates for your report, and that it is signed by both yourself (as the Primary Investigator), as well as the Industry Proponent(s).

Should you have any questions, please don't hesitate to contact me.

Thanks very much.
Emily Ryall
Science Advisor, Aquaculture, Biotechnology and Aquatic Animal Health Science Branch
Fisheries and Oceans Canada / Government of Canada
Emily.Ryall@dfo-mpo.gc.ca / Tel: 343-548-2275

Conseillère scientifique, Direction des sciences de l'aquaculture, de la biotechnologie et santé des animaux aquatiques
Pêches et Océans Canada / Gouvernement du Canada
Emily.Ryall@dfo-mpo.gc.ca / Tel: 343-548-2275

Aquaculture Collaborative Research and Development Program (ACRDP)

FINAL REPORT FORM

PART 1 – GENERAL PROJECT ADMINISTRATION & REPORT APPROVALS

1. LEAD AUTHORITY INFORMATION

A. DFO LEAD AUTHORITY

Name and Title: Kyle Garver, Research Scientist; Address: Pacific Biological Station, 3190 Hammond Bay Road, Nanaimo, BC
Phone: 250 713 6422 ; E-mail: kyle.garver@dfo-mpo.gc.ca

B. INDUSTRY COLLABORATOR(S) (FULL LEGAL NAME) – if more than 3, please include on a separate sheet.

1. Company Name: Cermaq Canada ; Project Lead:
Address: 203-919 Island Highway
Phone: 250 286 0022 E-mail:

2. Company Name: ; Project Lead:
Address:
Phone: E-mail:

3. Company Name: ; Project Lead:
Address:
Phone: E-mail:

2. PROJECT INFORMATION

a. Project Number: 18-P-01

b. Project Title: Prevalence and transmission dynamics of piscine reovirus in the marine environment

c. Project Duration (in fiscal years): FY2018/19 – FY2019/2020

d. Final Report Check List

- ☐ A completed final report, containing project outcomes, and experimental results summary (pages 2 and 3).
- ☐ A list of (and where available, files for) presentations, publications, reports related to the project.
- ☐ Signature from the DFO authority (below)
- ☐ Approval and signature from industry lead project authorities (below)
- ☐ Final report submitted in hard copy and electronic copy (.doc preferred).

I hereby declare that all of the above and attached information contained within this final report is correct.

	Please Print Name CLEARLY	Title Cermaq	Date July 16/2022
Project proponent Signature 2	Please Print Name CLEARLY	Title	Date
Project proponent Signature 3	Please Print Name CLEARLY	Title	Date
DFO Project Lead Signature	Please Print Name CLEARLY	Email address	Date

Garver, Kyle
2022.07.28
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Aquaculture Collaborative Research and Development Program (ACRDP)

FINAL REPORT FORM

PART 1 – GENERAL PROJECT ADMINISTRATION & REPORT APPROVALS

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Name and Title: Kyle Garver, Research Scientist; Address: Pacific Biological Station, 3190 Hammond Bay Road, Nanaimo, BC
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Project proponent Signature 1	Please Print Name CLEARLY	Title	Date
Project proponent Signature 2	Please Print Name CLEARLY	Title	Date
Project proponent Signature 3	Please Print Name CLEARLY	Title	Date
DFO Project Lead Signature	Please Print Name CLEARLY	Email address	Date

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PART 2 – PROJECT OUTCOMES

1. Expenditures and variance from budget:

	Initial budget	Actual expenditure	Difference	Comments (if differences)
Industry $	**$69,000**	**$69,000**	**$0**	
Industry (in kind)	**$60,500**	**$60,500**	**$0**	
ACRDP ($)	**$222,176**	**$222,176**	**$0**	
Other DFO ($ and in kind)	**$67,000**	**$67,000**	**$0**	
Partners ($ and in-kind)				

2. Expertise developed during the project (e.g., within DFO, industry, graduate students etc.):

Multiple levels of highly qualified personnel within DFO, Academia, and Industry were trained during this project.

Within DFO, personnel trained include three technicians at the EG03, 04, and 05 levels as well as a postdoctoral fellow (SE-RES-01). Outside of DFO, two undergraduate students from Vancouver Island University received training and conducted honor theses on the project while one technician and one veterinarian received training from the industry partner.

3. Briefly describe the impact that your project will have on the Canadian aquaculture industry (*i.e.*, efficiencies, productivity, sustainability, diversity, economics, social, etc). If possible, please provide a financial estimate to quantify this impact.

Since the discovery of piscine orthoreovirus (PRV) in BC farmed salmon, the virus has engendered substantial public concern regarding its frequency of occurrence and whether it causes disease in the infected farms. The project herein determined the temporal and spatial occurrence of PRV across the BC salmon farming industry. Furthermore this study defined the load and persistence of PRV in infected Atlantic salmon and cumulatively identified that PRV-1 ubiquitously infects farmed Atlantic salmon in British Columbia during seawater production but rarely contributes to heart disease. These results are directly applicable in establishing best salmon farming

4. Identify any invention or innovation that may have resulted from this Project, including any new process or technique.

Laboratory methods to differentially quantify replicating PRV from persistent infections were developed during this project along with a method to universally detect all PRV genetic variants. These innovations were published in two separate peer reviewed journals.

- Polinski MP, Marty GD, Snyman HN, Garver KA. Piscine orthoreovirus demonstrates high infectivity but low virulence in Atlantic salmon of Pacific Canada. Sci Rep. 2019 Mar 13;9(1):3297. doi: 10.1038/s41598-019-40025-7.
- Zhao J, Vendramin N, Cuenca A, Polinski M, Hawley LM, Garver KA. Pan-Piscine Orthoreovirus (PRV) Detection Using Reverse Transcription Quantitative PCR. Pathogens. 2021 Nov 27;10(12):1548. doi: 10.3390/pathogens10121548. PMID: 34959503; PMCID: PMC8707331.

5. General Comments:

PART 3 – COMUNICATING RESULTS

- **The following sections should be completed in non-technical language suitable for the general public.**
- **This information will be used to develop a web project summary, a fact sheet on the project, and to provide information for the biennial Research and Development Review publication, and other articles.**
- **Please limit the information to approximately 5 pages**

1. PROJECT RESULTS SUMMARY: *Project summary and key conclusions.*

Please note: This text will be posted on the ACRDP project website, below the existing project description. Maximum 250 words.

Piscine orthoreovirus genotype-1 (PRV-1) is a virus commonly associated with Atlantic salmon aquaculture with regional variability in prevalence and association with disease. From August 2016 to November 2019, 2,070 tissue samples from 64 Atlantic salmon net-pen farm sites were collected during 302 sampling events from coastal British Columbia, Canada, and screened for PRV-1 using real-time qPCR. Nearly all populations became PRV-1 positive within one year of seawater entry irrespective of location, time of stocking, or producer. Cohorts typically became infected between 100-300 days at sea and remained infected until harvest (typically 500-700 days at sea). Heart inflammation, which is sometimes attributed to PRV-1, was also assessed in 783 production mortalities from 47 cohorts with known PRV status. Mild heart inflammation was common in mortalities from both PRV+ and PRV- populations (67% and 68% prevalence, respectively). Moderate and severe heart inflammation was rare (11% and 3% prevalence, respectively); however, mainly arose (88% of all occurrences) in populations with PRV-1. Detection of PRV-1 RNA was also accomplished in water and sediment for which methods are described. These data cumulatively identify that PRV-1 ubiquitously infects farmed Atlantic salmon in British Columbia during seawater production but rarely contributes to heart disease.

2. INTRODUCTION - *Project rationale (e.g., background information, why solving the problem was of interest to industry, project hypothesis and goals):*

Piscine orthoreovirus genotype-1 (PRV-1) has commonly been detected among net-pen farmed Atlantic salmon of British Columbia, Canada for more than a decade (Polinski et al., 2020). Most PRV-1 infections at commercial Atlantic salmon net-pen farms in western Canada have been hypothesized to be acquired at sea (Polinski et al., 2020b). In one temporal study of PRV-1 at a single farm site in 2013, the virus was first detected only after 3 to 4 months of seawater rearing (Di Cicco et al., 2017). A second study in 2017 also identified PRV-1 at a farm site after fish had spent 3 months at sea but not in the same cohort continually held in freshwater (Polinski et al., 2019). Lastly, a survey of dead or dying fish collected from net-pen farms in all aquaculture zones of British Cumbia from 2011-2013 demonstrated that time-at-sea was a significant predictor of PRV-1 occurrence (Laurin et al., 2019), suggesting a substantial infection pressure from seawater environs. It is currently unclear, however, as to what the seawater reservoir(s) for PRV-1 may be.

In Norway and Chile, PRV-1 has been identified as an etiological factor for the development of a disease known as heart and skeletal muscle inflammation (HSMI) which has

presented an industry production concern for nearly two decades (Kongtorp et al., 2004; Wessel et al., 2017). In British Columbia, PRV-1 appears far less associated with disease (Garver et al., 2016; Polinski et al., 2019; Zhang et al., 2019). Differences in viral genotype can at least partially explain this phenomenon (Wessel et al., 2020) which are also almost certainly further impacted by host and environmental factors (Polinski et al., 2020). Nevertheless, at least two instances of HSMI-like heart inflammation have been identified on Atlantic salmon farms in British Columbia (Di Cicco et al., 2017; Polinski et al., 2019a). In both instances, affected populations were PRV-1 positive; yet it is unclear as to whether PRV-1's presence was tangential or integral to these HSMI-like occurrences (Marty et al., 2020; Polinski et al., 2019). Disease causation has been confounded in British Columbia in part by a lack of disease transmissibility (Garver et al., 2016; Polinski et al., 2019; Zhang et al., 2019) and in part by the fact that occasionally Atlantic salmon with HSMI-like heart inflammation have been identified without detectable PRV-1 infections (Di Cicco et al., 2018).

In this study, we set out to define the current prevalence of PRV-1 across the BC Atlantic salmon farming industry, the putative reservoir (freshwater or marine) contributing to current net-pen infections, and if geographical location is suggested to have contributed to variations in prevalence, source of infection, or disease outcome. This included identifying the length of PRV-1 persistence and load dynamics in infected farmed populations as well as PRV-1's potential contribution to heart inflammation during Atlantic salmon seawater production. Lastly, we sought to identify if screening of PRV-1 genetic material from environmental sources – specifically seawater and sediment – could be used for identifying PRV-1's regional occurrence and if such methods could inform on PRV-1's potential transmission mechanisms.

3. METHODS - *Short summary of project methods (e.g., experimental and analytical procedures followed, deviations from the originally proposed methods):*

Sample collection

Between August 2016 and November 2019, 425 sampling events were conducted at 64 Atlantic salmon net-pen productions sites in British Columbia, Canada. Sites were grouped into 9 geographically distinct regions. (Fig. 1). For 302 out of the 425 total sampling events, fish tissues were collected and involved all three commercial Atlantic salmon producers operating in the study region. Additionally, seawater and benthic sediment was collected with all sample types screened for PRV-1 RNA. Heart histopathology was conducted and used to identify heart inflammation in populations with known or deducible PRV-1 status (i.e., individuals from populations testing or previously testing positive for PRV-1 were presumed positive, and individuals from populations at or prior to testing negative were presumed negative). PRV-1 was detected using real-time quantitative PCR (qPCR) based on previously published methods (Palacios et al., 2010; Polinski et al., 2019; Saksida et al., 2012). All histopathology was conducted blinded to PRV-1 infection status. Fourteen indices were scored as either being none/not present (0), mild (1), moderate (2) or severe (3). This included the two inflammatory indices of lymphohistiocytic epicarditis and endocarditis, which were combined to give a cumulative heart inflammatory score (0 = none; 1-2 = mild, 3-4 = moderate, 5-6 = severe). HSMI-like heart inflammation has previously been defined as having cumulative heart scores of 4-6 by this method (Di Cicco et al., 2018; Polinski et al., 2019).

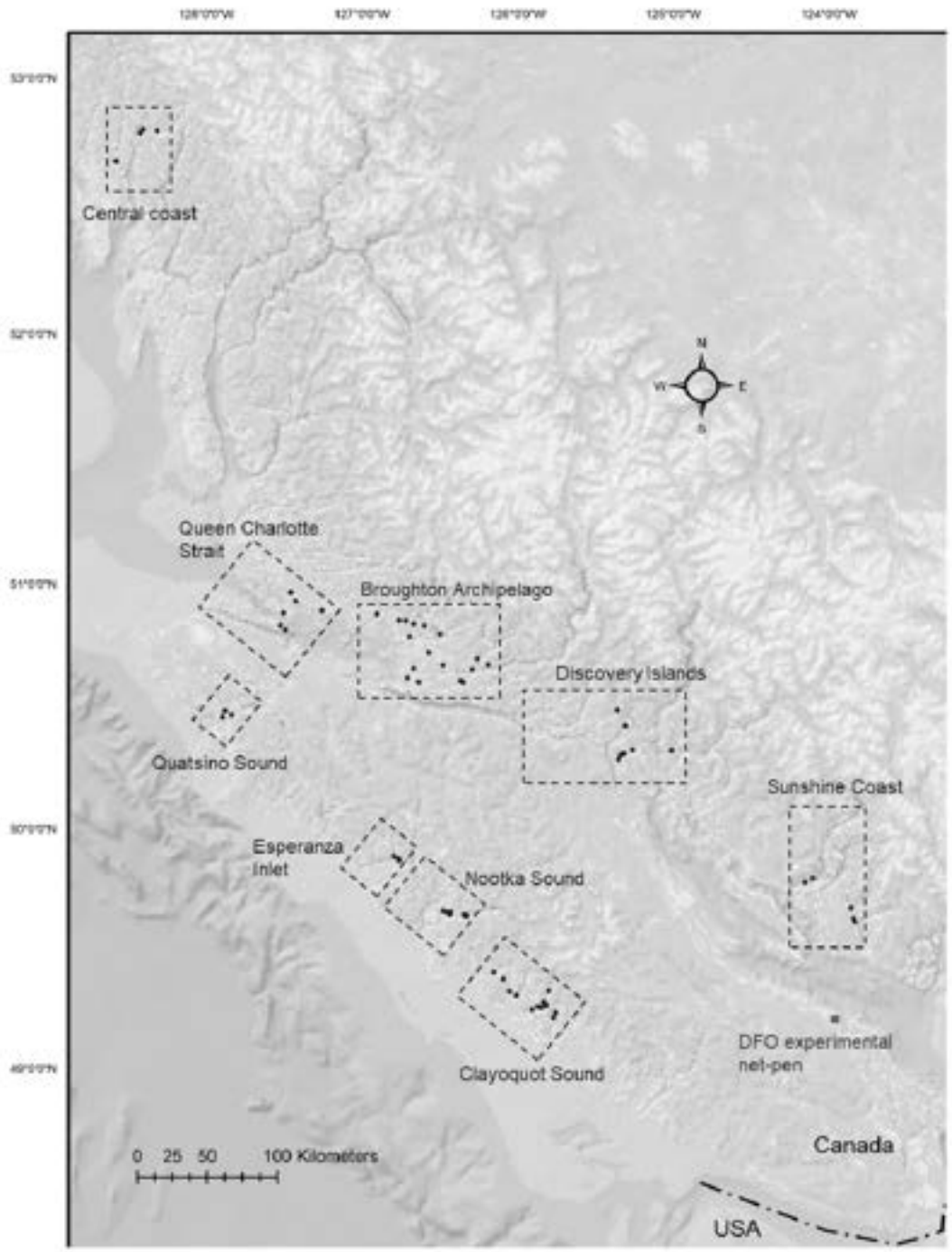

Fig. 1. Map of study region with indication of commercial (red circles) and DFO research (blue square) net-pen sites sampled in this study. Regional boundaries (dotted red lines) are also provided.

4. RESULTS - ***Key results (include graphs, data tables, photos (of publication quality – 300 dpi or more), etc. where applicable). PLEASE NOTE: If there are people in your pictures, please ensure that you have received approval to publish them:***

PRV prevalence on farms

PRV-1 RNA was identified at 51 of the 64 commercial net-pen farm sites sampled and in all 9 geographical regions in this study (Fig. 1; Fig. 2). PRV-1 prevalence was positively correlated with time at sea ($r = 0.28$; 95% CI 0.17-0.38; $p < 0.0001$) (Fig 2). In the first 90 days following fish stocking into the net-pens, PRV-1 was detected in less than 2% (1/59) of commercial sampling events. In the one event where PRV-1 was detected (2018), producer records indicated that PRV-1 had been detected in the freshwater facility late in 2017 where the fish were sourced. For fish populations which had been at sea in net-pens for more than 300 days, 96% (96/100) of sampling events identified PRV-1. Of the 4 instances where PRV was not detected after 300 days at sea, one population (in Nootka Sound) had been sampled 8 times (3-5 fish per time point) in 2018/2019 from 66 to 321 days at sea before the virus was finally detected in 3 of 6 individuals at 349 days at sea. In the second instance, 6/6 fish tested negative at 464 days at sea from a site in the Broughton Archipelago. Interestingly, this population had been transferred from another seawater site in the same region where at 214 days at sea 6 out of 10 individuals tested positive for PRV-1 one week before the transfer. No follow up testing was done at the origin site following transfer. The last 2 instances occurred at sites in the Central Coast region in October of 2017 and July of 2018 where fish had been at sea for 648 and 452 days, respectively. There were no other PRV-1 screenings conducted at these 2 sites.

Nearly all first detections of PRV-1 occurred in populations that had been at sea between 100-300 days irrespective of year, producer, region, or farm site (Fig. 2; Fig. 3). Sites became infected during almost all months of the year; however, two regions appeared to have relatively consistent seasonality for first detections, albeit at low temporal resolution. In the Broughton Archipelago, first detections occurred only in the latter half (July – December) of the calendar year in 2017, 2018, and 2019 (Fig. 3). In Nookta Sound, first detections occurred only in late August or early September in 2016 and 2019 when sampling was conducted. In contrast, no seasonality for first PRV-1 detection was observed in net-pens in the Clayoquot Sound where first detections occurred in almost all months of the year (Fig. 3).

There were 26 instances in this study where PRV-1 positive sites were resampled one or more time following first detection. PRV-1 was detected at resampling in all sites and during all resampling events. Prevalence was usually high (typically 100%) at resampling (Fig. 2). The longest period between first detection and last positive resampling in this study was 460 days.

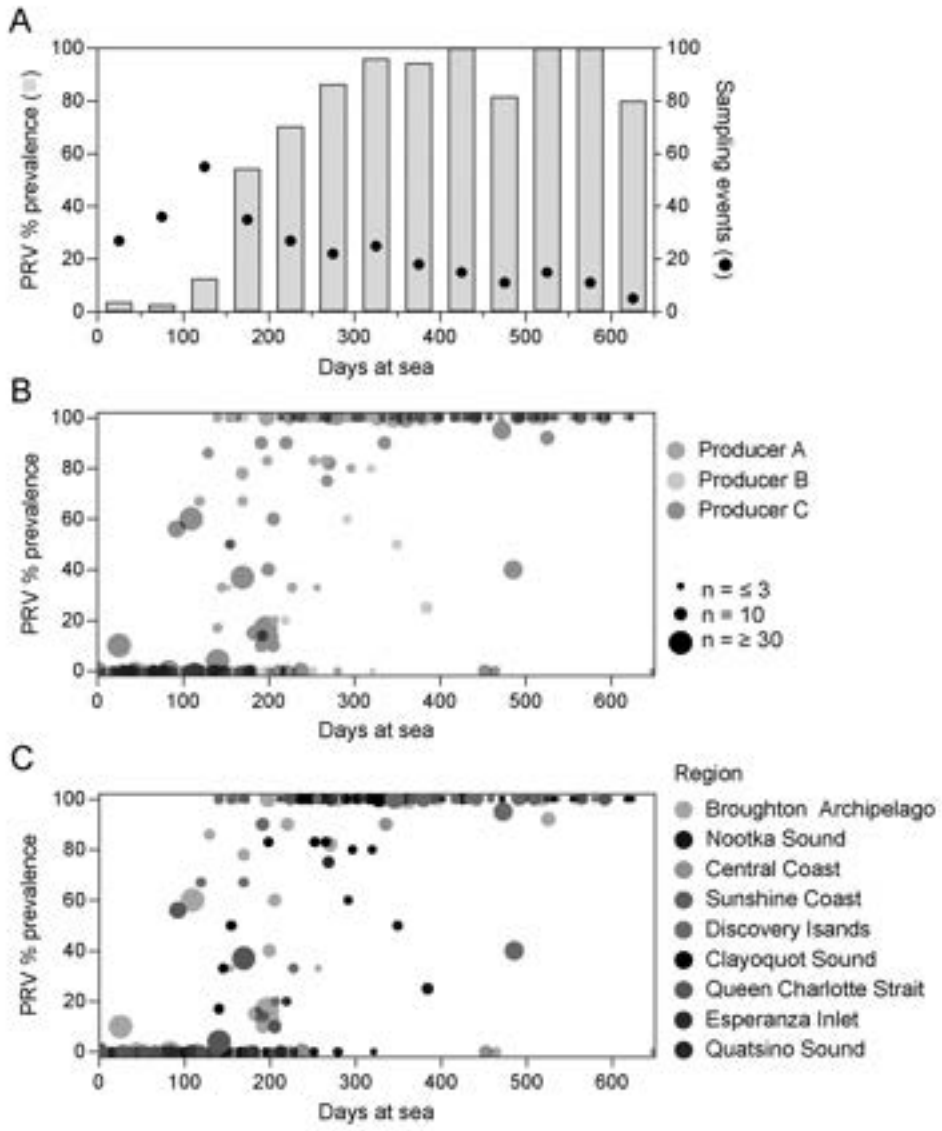

Fig. 2. (A) Prevalence of PRV-1 RNA in fish (bar) and number of sampling events (dots) identified for every 50 days at sea. PRV-1 RNA prevalence in relation to days at sea corresponding to (B) each of the 3 Atlantic salmon commercial producers in British Columbia as well as (C) the 9 geographical regions of production. Sample size (dot size) is indicated in both instances.

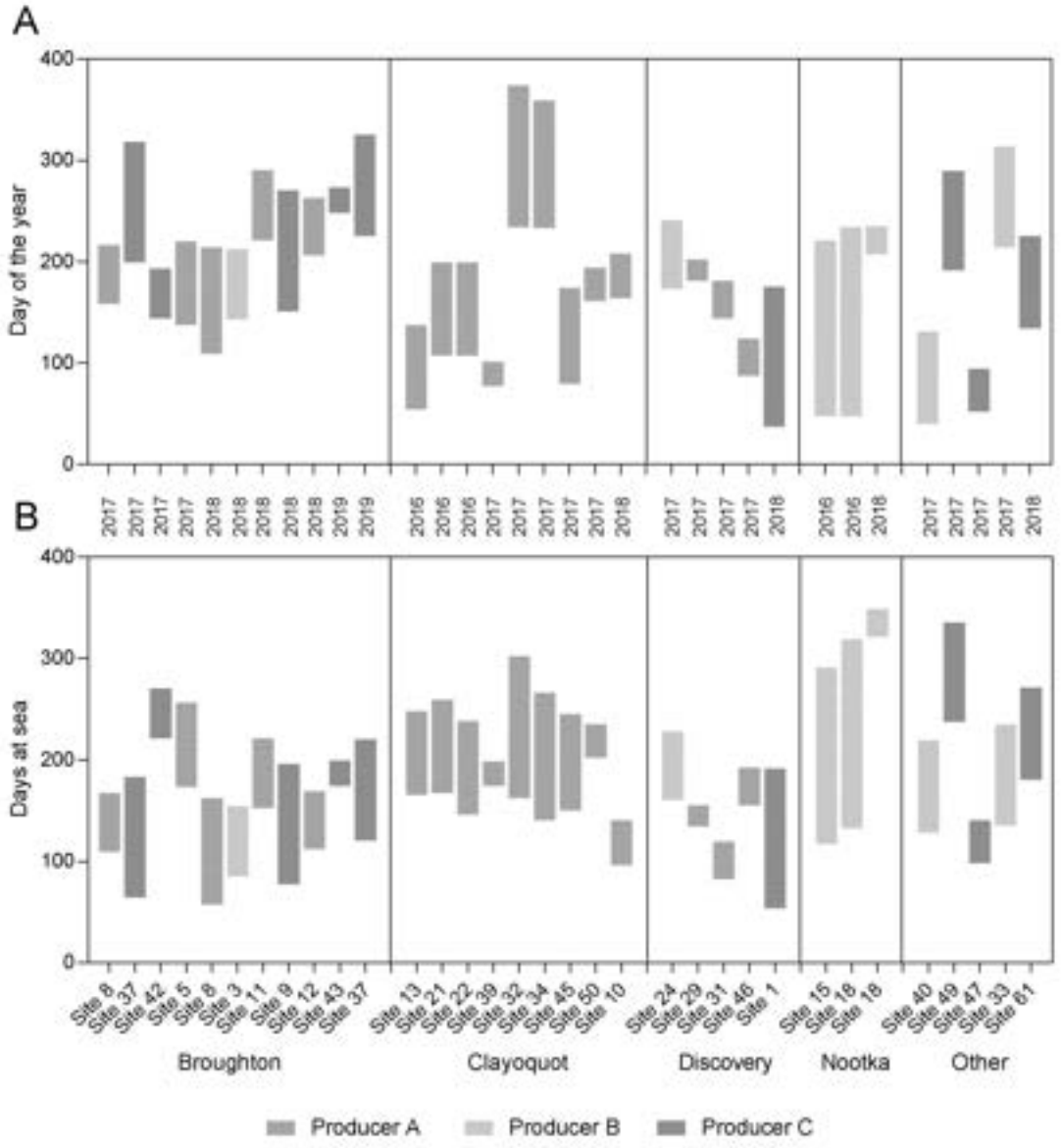

Fig. 3. The number of ordinal days (A) or days at sea (B) between the last negative and first positive PRV-1 sampling event (colored region) at 30 farm sites across the 9 geographical study regions between 2016 and 2019 where both negative and positive sampling events occurred. The producer (color) as well as the sampling year are indicated for each net-pen site.

PRV RNA loads in infected individuals

The mean relative PRV-1 blood load was similar between individuals from the normal (n = 126; mean Ct = 18.1 ± 5.2 SD), moribund (n = 33; mean Ct = 17.5 ± 5.0 SD) or mortality (n = 322; mean Ct = 18.6 ± 4.8 SD) populations of fish sampled from 19 farm sites operated by Producer A in this study ($p > 0.3$; Fig. 4A). Of the 16 individuals which had both blood and heart samples collected, Ct comparisons showed significantly lower Ct (higher PRV-1 RNA loads) in blood compared to heart samples in these individuals ($p < 0.0001$) with a mean Ct difference of approximately 5 (Fig. 4B). PRV-1 blood load was variable between individuals and populations in this study with the greatest variance occurring early

during the infection processes. However, variance decreased with increased time at sea with an overall mean blood Ct of 17 (Fig. 4C).

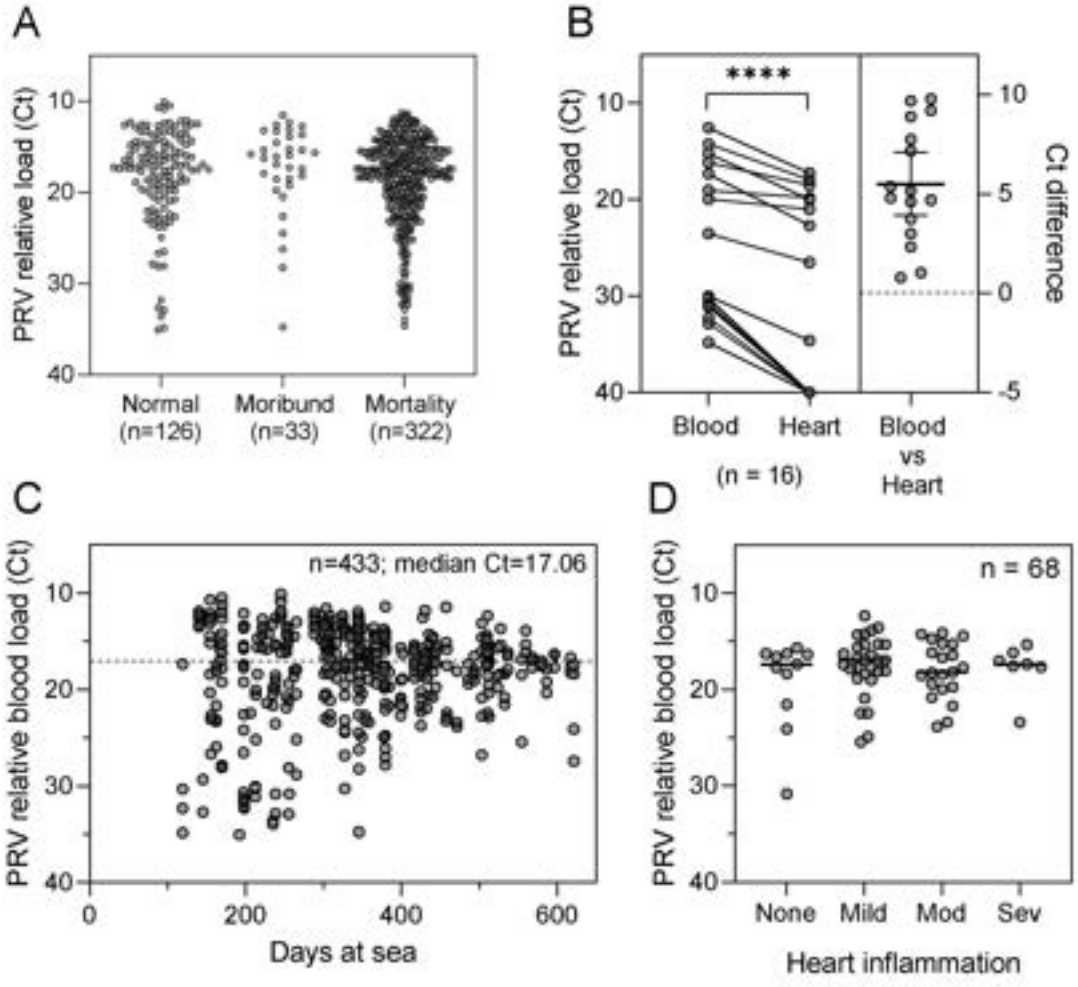

Fig. 4. (A) Individual (dot) PRV-1 RNA blood loads collected from either recent mortalities, moribund individuals, or members of the normal Atlantic salmon population across 19 commercial net-pen farms sites in British Columbia are contrasted by one-way ANOVA. (B) qPCR Ct values for blood and heart samples for 16 fish in which both blood and heart samples were simultaneously collected are compared by a paired T-test (**** = p-value < 0.0001. (C) the individual distribution (dots) and mean (dotted line) of qPCR Ct from all blood samples from a single commercial producer are presented relative to days at sea. (D) mean (line) and individual (dot) PRV-1 RNA blood loads of fish for which heart inflammation (combined epi- and endo-carditis) was assessed by histopathology and scored as either not present, mild, moderate, or severe by one-way ANOVA.

Heart inflammation in relation to PRV infection

There were 113 individual Atlantic salmon mortalities from Producer A collected from 21 net-pens where both PRV-1 blood screening results and matched heart histopathology were available from the same fish. Of these, 45 were PRV- and 68 were PRV+. There was no significant difference by rank ($p > 0.57$) or by cumulative distribution ($p > 0.98$) of heart pathology scores in PRV+ versus PRV- individuals. In the 68 PRV+ mortalities, relative viral blood load (Ct) was similar between fish with either mild, moderate, severe, or no heart inflammation ($p > 0.58$; Fig. 4D). Additionally, there were 148 sampling events conducted at producer B and C net-pens for which heart pathology was assessed: 57

events included fish presumed PRV- and 112 events included fish presumed PRV+ based on screening of alternate individuals within each cohort. Cumulatively, mild heart inflammation was common (60-77% prevalence) among net-pen reared salmon mortalities irrespective of PRV status or producer (Fig. 5). Moderate to severe heart inflammation was rare; however, in most instances occurred in PRV+ individuals. There were 24 out of a total of 783 production mortalities (3%) with heart inflammation considered to be HSMI-like (severity scores of 4-6). Of these, 23 were from PRV+ populations and 1 was from a PRV- population.

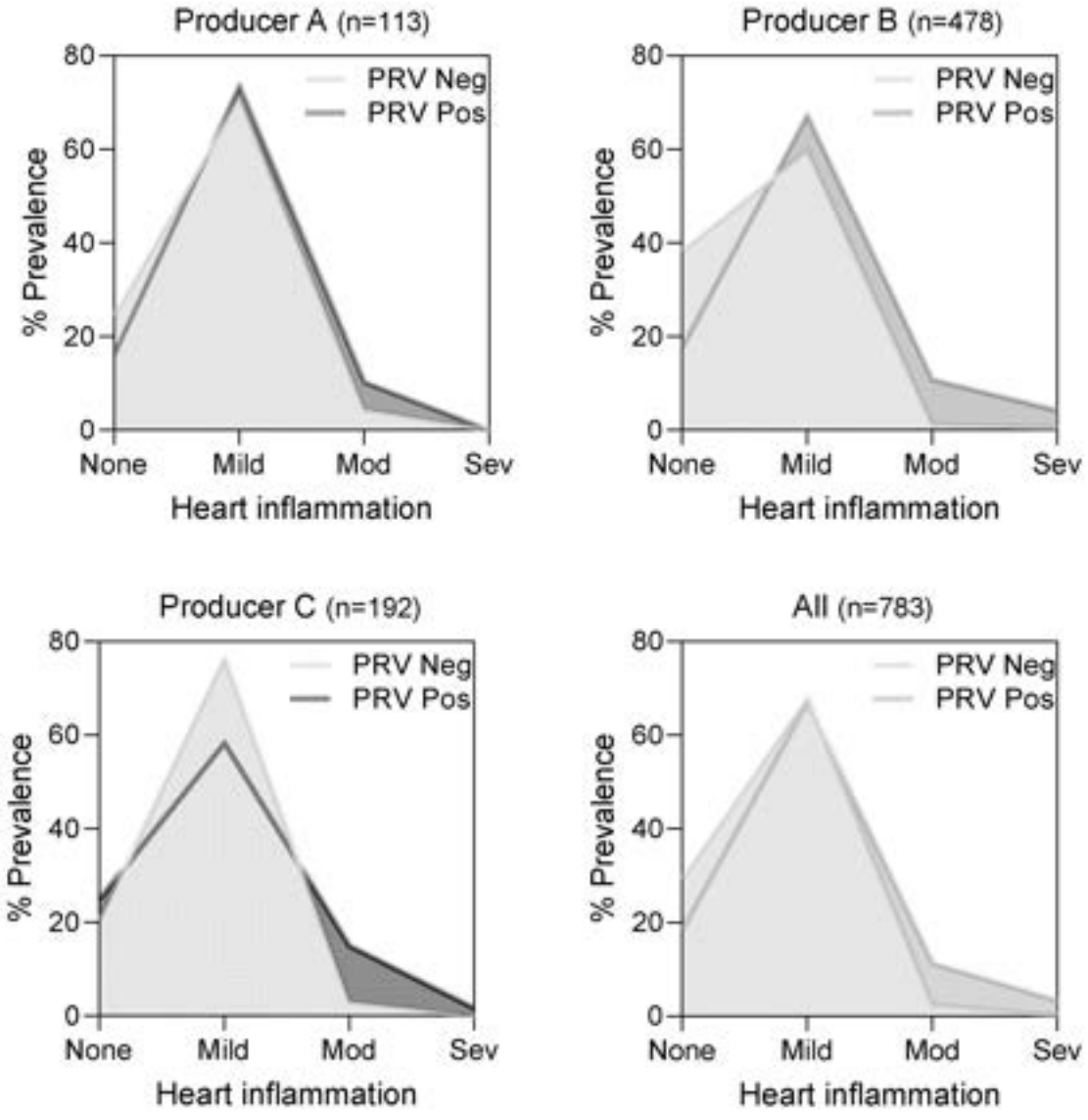

Fig. 5. Prevalence of heart inflammation in Atlantic salmon net-pen production mortalities across sites from all three regional producers is presented for both PRV-1 positive and PRV-1 negative populations. Heart inflammation (cumulative epi- and endo-carditis) as identified by microscopy as either not present (none), mild, moderate (mod) or severe (sev) (Supplemental file 1). For Producer A, heart inflammation scores and PRV-1 status were matched to individual fish. For Producers B and C,

PRV-1 status is indicated at the population level as fish screened for PRV-1 were not necessarily the same fish screened by histopathology for heart inflammation.

5. DISCUSSION – *Interpretation of results.*

This study confirmed PRV-1 infections of commercially produced Atlantic salmon in Western Canada are acquired at sea. The identification of seawater reservoirs of PRV-1 in all farming areas of Western Canada also verifies the widespread prevalence of the virus in the region (Polinski et al., 2020). Further, it is now clear that PRV-1 can and does perpetuate in coastal environments of British Columbia independent of commercial freshwater introductions. This implies that farmers currently have little control over if or when their fish become infected by PRV-1 once they are stocked to net-pens.

As salmon appear to be the primary (if not exclusive) long-term reservoir of PRV-1 in the Northeastern Pacific (Polinski et al., 2020), it is logical to assume that the seawater reservoir of PRV-1 is made up of commercial salmon stocks, wild stocks, or both. We hypothesize that both are likely. In arguing for commercial net-pen populations as a regional reservoir, we identified that nearly all net-pen sites have this potential given that nearly all showed evidence of becoming PRV infected and maintained these infections for sometimes well over a year. Although specific shedding and putative transmission from net-pens is unknown, previous experimental transmission studies (Garver et al., 2016; Polinski et al., 2019) coupled with the environmental seawater detection of viral nucleic acid in this study suggests that at least some shedding of viable virus from net-pens occurs. Additionally, data from this study indicate that in at least some regions such as the Clayoquot Sound – where PRV+ populations are present year-round, farms are within a few nautical miles of one another, and first detections occur in almost every month of the year – farm-to-farm transmission presents a likely contributor to PRV prevalence on farms in the region.

In other regions, an argument for a wild fish reservoir is better suited. For example, in Nootka Sound – where the region is fallowed for 2-3 months between production cycles, the nearest positive site is many nautical miles away, and first detections appear to occur seasonally and only after 10 or more months at sea – the source of infection seems likely to be from a wild migrating population of Chinook, Coho, or other Pacific salmon in the sound. This is supported by the fact that PRV-1 is common in both Chinook and Coho species of British Columbia (Polinski et al., 2020) and that transmission between (and therefore from) wild fish occurs independent of farms in the northeastern Pacific as evidenced by PRV-1 detection in populations from Alaska and southern Washington state where fish farming does not occur (Purcell et al., 2018). We therefore speculate that for most sites in this study area, both adjacent farms (where present) and wild salmon have the potential to act as an infection source of naive net-pen stocks and combine to form a regionally ubiquitous seawater reservoir for PRV-1 in coastal British Columbia.

Following experimental infection, PRV-1 has shown a tropism for red blood cells compared to heart tissues with no evidence of viral clearing in either sample type (Finstad et al., 2014; Garver et al., 2016). Our study confirms a blood tropism by PRV-1 during commercial net-pen production with little evidence that the virus is cleared from an infected population. Indeed, PRV-1 was detected in every sampling event at each of the 26 PRV+ sites where repeated sampling took place. The only putative evidence for viral clearing in this study was when PRV-1 was detected in 60% of samples at a Broughton site but not at a second Broughton

site 200+ days later after a fish transfer had occurred. Although this could represent the first putative evidence for clearing of PRV-1 from an infected population, it could also be possible that PRV+ fish were randomly excluded from the transfer or that testing produced a false-negative result. Currently, cumulative evidence supports the tentative conclusion that PRV-1 is not cleared from an individual or population of farmed Atlantic salmon once infected.

This study also confirms that for maximizing early detection, screening of blood relative to heart is recommended. During late-stage infection, either tissue would be acceptable due to the systemically high viral titers which are maintained in both sample types. These data in conjunction with data from previous laboratory challenge trials indicate that relative quantity can be utilized to give a rough dichotomous (early vs late) estimation of the infection stage and thereby the timing of infection. Specifically, low relative quantities (for example, blood Ct values >25 in this study) suggest that the fish was recently infected – likely within the past few weeks – whereas high relative quantities (i.e., blood Ct values <20 in this study) suggest that individuals have probably been infected for a considerable time – i.e., a month or more (Polinski et al., 2019; Zhang et al., 2019). In addition, this study confirms that relative PRV-1 load was not useful as a predictor of morbidity or mortality in a commercial net-pen setting which is similar to results of previous laboratory challenge trials (Polinski et al., 2019). This reiterates that PRV-1 load cannot be used as a disease proxy.

The Fish Health Auditing and Surveillance Program (FHASP) conducted by DFO Aquaculture Management Division previously evaluated heart tissues of nearly 6,000 Atlantic salmon net-pen farming mortalities from 2006-2018 and found 61% prevalence of mild to moderate heart inflammation (Polinski & Garver, 2019). We identified a similar percentage (68%) of mild heart inflammation in production mortalities in this study during an overlapping period (2016-2019). We further identified that this condition occurred equally in PRV+ and PRV- populations as well as PRV+ and PRV- individuals, indicating that PRV-1's contribution to the prevalence of mild heart inflammation has been minimal to non-existent during net-pen culture in Western Canada.

The 2006-2018 FHASP also identified heart inflammation with a severity sufficient to be a putative cause or contributing factor to death in approximately 3% of sampled mortalities, which is similar to what we identified here (3% with heart inflammation histopathology scores of 4-6) and is consistent with historical observations from the 1990's (Brackett et al., 1990, 1992). We were able to further identify that the supermajority (23 of 24) of these HSMI-like occurrences happened in populations that were infected with PRV-1, suggesting that PRV-1 was significantly contributing to this condition. Nevertheless, net-pen mortalities in recent years have accounted for approximately 7% of a total production cohort (Polinski & Garver, 2019). Thus, PRV-1 associated heart inflammation appears to have contributed to or caused approximately 0.2% mortality (i.e., 3% of 7%) in net-pen cultured Atlantic salmon of British Columbia during 20-month production cycles in the last few years, and likely has done so for the past 25 years. Noteworthy, however, is that 1/24 (4%) of observed HSMI-like heart inflammation in this study occurred in the absence of PRV-1, which has also been observed previously (Di Cicco et al., 2018). This indicates that at least in British Columbia, there are other factors independent of PRV-1 which can cause HSMI-like heart inflammation in farmed Atlantic salmon.

Environmental detection of PRV-1 nucleic acids has potential utility for expanding early detection when prevalence is low as well as increasing sensitivity to include material associated with non-resident hosts. Environmental RNA in marine systems has recently been shown to be unexpectedly stable, particularly when associated with biofilms (Wood et al., 2020) which may

be even further extended by protection from an intact viral capsid or subviral particle. In this study, PRV-1 was consistently identified from replicate sediment samples at an active farm both before and after the virus was detectable in fish as well as from a farm more than two months after infected fish had been removed. This suggests that either PRV-1 genetic material is environmentally stable in sediment for multiple months, or that a secondary local host reservoir is present at these locations. Further investigation will be needed to determine if either of these scenarios is valid, although we speculate prolonged environmental stability is more likely. It is also uncertain if this sediment-associated virus contains infectious particles, and if so, if they can act as an infection source for salmon held in net-pens meters above.

6. CONCLUSIONS & NEXT STEPS: *Resulting key improvements to sustainable aquaculture and scientific advancements, suggested next steps, future research/development/innovation needs:*

In conclusion, this study confirms the regionally widespread seawater occurrence of PRV-1 in net-pen reared Atlantic salmon of British Columbia where the majority of farms become infected within 100-300 days of stocking. Mild heart inflammation – which appears prevalent in farmed Atlantic salmon of British Columbia – was occasionally exacerbated by PRV-1, but with negligible impacts to overall morbidity/mortality during production. Environmental detection of virus was also demonstrated at farm sites but provided minimal insight into PRV prevalence and transmission potential within the region beyond what could be deduced from fish sampling.

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8. PROJECT COMMUNICATION: ***Copies (please attach) and references of publications, presentations (from conferences, etc.), reports, articles or other communications produced in reference to the project:***

Publications:

Polinski, M. P., Marty, G. D., Snyman, H. N., & Garver, K. A. (2019). Piscine othoreovirus demonstrates high infectivity but low virulence in Atlantic salmon of Pacific Canada. *Scientific Reports*, *9*(1). https://doi.org/10.1038/s41598-019-40025-7

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Reports:

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

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Presentations:

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Polinski MP, Johnson S, [redacted], Garver KA (2019) PRV – Investigations into Environmental Reserves. Aquaculture Canada: Collaborations on the Coast, 1-4 May, Victoria, CAN

9. **RELATING TO OTHER PROJECTS & PROGRAMS:** ***Please provide information on how this project might relate to other existing or new projects being supported by DFO, e.g. AIMAP or PARR projects.***

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