

FEDERAL COURT

BETWEEN:

'NAMGIS FIRST NATION

APPLICANT

AND:

MINISTER OF FISHERIES, OCEANS AND THE CANADIAN COAST GUARD,
MOWI CANADA WEST INC. AND CERMAQ CANADA LTD.

RESPONDENTS

AFFIDAVIT OF GIDEON MORDECAI

I, Dr. Gideon Mordecai, Research Associate, of the City of North Vancouver in the Province of British Columbia, SWEAR (OR AFFIRM) THAT:

A. ACADEMIC BACKGROUND

1. I hold a PhD in Biological Sciences from the University of Reading (2016), a Master of Research (Distinction) in Marine Biology from the University of Plymouth (2012) and a Bachelor of Science (First class honours) from the University of Southampton (2010).
2. I have worked in the field of viral ecology for 13 years. I began my career working on phytoplankton viruses based at the Marine Biological Association (Plymouth, UK) from 2011 to 2012, and continued at the same laboratory (in partnership with the University of Reading, UK) to work on insect viruses for my PhD from 2013 to 2016.
3. Since completing my PhD, I held the position of a Mitacs Post-doctoral Fellow (co-funded by the Pacific Salmon Foundation) within the Department of Earth, Ocean and Atmospheric Sciences at the University of British Columbia ("UBC") from 2016 to 2019, where I applied next generation sequencing techniques for virus discovery in salmon. I then went on to receive a Liber Ero Fellowship and worked within the Department of Medicine at UBC where my work specialised in using viral genome

sequencing to investigate the transmission dynamics of salmon viruses, including the Piscine orthoreovirus ('**PRV**').

4. I currently hold a Research Associate position within the Institute for Fisheries and Oceans at UBC, where I am partnered with the Pacific Salmon Foundation. I am part of a collaboration which is applying innovative molecular techniques to determine the cumulative factors that influence the survival of Pacific salmon. My research applies viral genome sequencing to assess the transmission risk of pathogens, such as PRV, posed to wild Pacific salmon by Atlantic salmon aquaculture in British Columbia.

5. I was recently selected and named as one of Action Canada's Fellows for my commitment to Canada and a demonstrated engagement with public policy in June 2024.

6. I have co-authored and published approximately 30 peer-reviewed papers with a total of more than 1500 citations. These include papers concerning fish viruses and PRV.

7. I am an expert in viral ecology with a focus on assessing viral diversity using genomic sequencing technology. Through my expertise in this area, I am qualified to, among other things, assess the diversity of viruses and use this to inform the risks posed to biodiversity, fisheries and aquaculture operations in British Columbia.

8. My current *curriculum vitae* further sets out my qualifications. Attached as Exhibit "A" is a copy of my *curriculum vitae*.

B. INITIAL REPORT

9. I was previously retained and prepared an expert report dated November 19, 2020 (my "**Initial Report**"), in the above noted proceedings for the Applicant, 'Namgis First Nation ("**Namgis**"). My Initial Report provided answers to questions about the following:

- (a) the use of the terms "endemic" and "epidemic";

- (b) the taxonomy of viruses;
- (c) the taxonomy of PRV;
- (d) the origins of PRV;
- (e) determining the pathogenicity and virulence of viruses, including PRV;
- (f) the peer-review process used in scientific publishing; and
- (g) the accuracy of certain scientific literature contained in Certified Tribunal Record for the Minister of Fisheries, Oceans and the Canadian Coast Guard's (the "**Minister**") October 3, 2019, decision not to test for PRV before issuing licences under s. 56 of the *Fishery (General) Regulations*, SOR/93-53 (the "**CTR**").

10. I have been retained by MacKenzie Fujisawa LLP, legal counsel for 'Namgis', in the above noted proceedings, to prepare a supplementary expert report to my Initial Report that provides answers to questions about the following:

- (a) summarize developments in scientific literature on PRV since 2019;
- (b) identify and highlight key developments, findings and advancement in scientific research on PRV since 2019;
- (c) the unpublished manuscript of Dr. Kristi Miller-Saunders disclosed by the Department of Fisheries and Oceans ("**DFO**") regarding the first detection of PRV in British Columbia; and
- (d) water sampling progress reports diagnosing heart skeletal muscle inflammation in Pacific salmon in the Broughton area.

11. Attached to as Exhibit "**B**" is a copy of my supplementary expert report, which I adopt as my evidence (my "**Supplementary Report**"). In preparing my Supplementary Report, I have consulted the scientific literature on this topic attached as Appendix 5 of my Supplementary Report. The letter of instructions, supplementary letter of instructions, second supplementary letter of instructions I received from MacKenzie

Fujisawa LLP can be found at Appendices 1, 2 and 3 of my Supplementary Report, respectively.

C. PRIOR INTERACTIONS WITH DFO

12. Prior to my Initial Report, I participated as an expert advisor on matters of science in the engagement between 'Namgis and the Minister regarding the Minister's current policy not to prohibit the issuance of licenses under s. 56 of the *Fishery (General) Regulations*, (the "PRV Policy") As part of that engagement, I attended two meetings in the summer of 2019 between 'Namgis and DFO. I also attended, as an observer, a DFO meeting regarding its Fish Health Technical Working Group in or around September 6, 2020.

13. Beginning in October 2016, I also collaborated with Dr. Kristi Miller-Saunders, Head of Salmon Genetics at DFO, and her research group within DFO. This work included, among other things, the following:

- (a) fieldwork respecting sampling of wild salmon;
- (b) generating and analysing data relating to (but not limited to) novel virus discovery, infectious agent prevalence, and virus phylogenetics;
- (c) designing experiments such as designing assays for viruses, using genome sequences to build phylogenies;
- (d) writing papers;
- (e) supervising students (for example, a student I co-supervised published a paper on the distribution and phylogeny of Erythrocytic Necrosis Virus); and
- (f) attending meetings.

14. The research I have carried out with Dr. Miller-Saunders and her group primarily focuses on the study of infectious agents in salmon, and together we have co-authored 14 peer-reviewed papers. These include two papers on virus discovery, and we are continuing this work by identifying more viruses and attempting to determine their role in

wild salmon health. We have also co-authored several papers concerning PRV, including an assessment of PRV in Atlantic salmon on the East Coast (Teffer *et al*, 2020), and an investigation on the role of PRV in jaundice/anemia in Chinook (Di Cicco *et al*, 2018). In these collaborations, my role has largely been to carry out analysis of viral genomes generated by Dr Miller-Saunders' laboratory. Most notably, together, we published a paper on the subject of transmission of PRV between farmed and wild salmon (Mordecai *et al*, 2021).

15. On or about April 6, 2022, I presented on emerging viruses in West Coast Vancouver Island Chinook Salmon at a DFO workshop as part of its Marine Risk Assessment for Natural-Origin West Vancouver Island Chinook Salmon.

16. On or about May 5, 2022, I testified before the Standing Committee on Fisheries and Oceans for its investigation into the use of science in decision-making at DFO.

17. In 2023, I acted as a scientific advisor to First Nation Wild Salmon Alliance and Kwiakah First Nation as part of their consultation with DFO on the transition from open-net pen salmon aquaculture in coastal B.C. waters.

D. GENERAL INFORMATION

18. Attached as Exhibit "C" is a copy of my signed Certificate Concerning the Code of Conduct for Expert Witnesses in Form 52.2.

19. I provide this affidavit for use as evidence, for the benefit of the Federal Court of Canada, and for no improper purpose.

**SWORN (OR AFFIRMED) BEFORE)
ME at the City of Vancouver in the)
Province of British Columbia on)
September 25, 2024.)**

A Commissioner for Taking Affidavits
for British Columbia)

) DR. GIDEON MORDECAI

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This is **Exhibit "A"** referred to in the affidavit of Dr. Gideon Mordecai sworn before me at the City of Vancouver this 25th day of September, 2024.



A Commissioner for taking Affidavits for the Province of
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Employment

2022- present	Research Associate – Institute for the Oceans and Fisheries, University of British Columbia
2021- 2022	Interim Manager, Salmon Ecological Health Program, Pacific Salmon Foundation
2019 -2021	Liber Ero Postdoctoral Fellow – University of British Columbia
2016 –2019	Post-Doctoral Fellow (MITACS/ Pacific Salmon Foundation) – University of British Columbia

Education

2024-present	Public Policy Forum's Action Canada Fellowship
2023-present	Simon Fraser University Non-Profit Management Certificate
2013-2016	University of Reading / Marine Biological Association of the UK PhD Thesis title: Diversity in emerging honey bee viruses.
2011-2012	University of Plymouth / Marine Biological Association of the UK MRes Marine Biology [Distinction] Dissertation title: Investigating the presence of the <i>Emiliania huxleyi</i> Virus transcriptome in haploid <i>Emiliania huxleyi</i> cells.
2007-2010	University of Southampton BSc Marine Biology & Oceanography [First class honours]

Publications & Patents

29. **Mordecai G** et al. Comment on a perspective: Molecular detections of new agents in finfish—Interpreting biological significance for fish health management. (2024). *Journal of Aquatic Animal Health*
28. **Mordecai G** et al. Is scientific inquiry still incompatible with government information control? A quarter century later. (2023). *Canadian Journal of Fisheries and Aquatic Sciences. Selected as Editor's choice and was the #1 most read article in the Canadian Journal of Fisheries and Aquatic Sciences of 2023*
27. Di Cicco E,...**Mordecai G** et al. Tenacibaculosis in wild-caught, captive Chinook salmon (*Oncorhynchus tshawytscha*) in British Columbia, Canada (2023). *BioRxiv*.
26. **Mordecai G** et al. Assessing the role of Piscine orthoreovirus in disease and the associated risk for wild Pacific salmon (2023). *BMC Biology*.
25. **Mordecai G et al.** (2022) Detection and phylogenetic assessment of PRV-1 via sampling of biological materials released from salmon farms in British Columbia. *Canadian Journal of Fisheries and Aquatic Sciences*.
24. Bass AL, ... **Mordecai G** et al. (2022) Identification of infectious agents in early marine Chinook and Coho salmon associated with cohort survival. *FACETS*.
23. Deeg C,... **Mordecai G** et al. (2022) Way out there: pathogens, health, and condition of overwintering salmon in the Gulf of Alaska. *FACETS*.
22. McLaughlin A, ... **Mordecai G** et al. (2022) Genomic epidemiology of the first two waves of SARS-CoV-2 in Canada. *eLife*.

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21. Kuhn JH, ... **Mordecai G** et al. (2021) 2021 Taxonomic update of phylum *Negarnaviricota* (*Riboviria*: *Orthornavirae*), including the large orders *Bunyavirales* and *Mononegavirales*. *Archives of Virology*
20. Montaya V, ... **Mordecai G** et al. (2021) Variable routes to genomic and host adaptation among coronaviruses. *Journal of Evolutionary Biology*.
19. Bateman A.D, ... **Mordecai G** et al. (2021) Descriptive multi-agent epidemiology via molecular screening on Atlantic salmon farms in the northeast Pacific Ocean. *Scientific Reports*.
18. **Mordecai G** et al. (2021) Aquaculture mediates global transmission of a viral pathogen to wild salmon. *Science Advances*.
17. Shea D, ... **Mordecai G** et al. (2020) Environmental DNA (eDNA) from multiple pathogens is elevated near active Atlantic salmon farms. *Proceedings of the Royal Society B*
16. **Mordecai G** & Hewson I (2020) Coronavirus in the Sea. *Frontiers in Microbiology*.
15. Highfield A, ... **Mordecai G** et al. (2020) Detection and Replication of Moku Virus in Honey Bees and Social Wasps. *Viruses*.
14. Teffer AK, ... **Mordecai GJ** et al. (2020) A molecular assessment of infectious agents carried by Atlantic salmon at sea and in three eastern Canadian rivers, including aquaculture escapees and North American and European origin wild stocks. *FACETS*.
13. **Mordecai G** et al. (2020). Discovery and surveillance of viruses from salmon in British Columbia using viral immune-response biomarkers, metatranscriptomics and high-throughput RT-PCR. *Virus Evolution*.
12. **Mordecai G** et al. (2019). Endangered wild salmon infected by newly discovered viruses. *eLife*.
11. Pagowski VA, **Mordecai GJ** et al. (2019). Distribution and Phylogeny of Erythrocytic Necrosis Virus (ENV) in Salmon Suggests Marine Origin. *Viruses*.
10. Di Cicco E, ... **Mordecai G** et al. (2018). The same strain of *Piscine orthoreovirus* (PRV-1) is involved in the development of different, but related, diseases in Atlantic and Pacific Salmon in British Columbia. *FACETS*.
9. Brettell LE, **Mordecai G**, et al. (2017). Novel RNA Virus Genome Discovered in Ghost Ants (*Tapinoma melanocephalum*) from Hawaii. *Genome Announcements*.
8. Jones S, ... **Mordecai G** et al. (2017). The Genome of the Beluga Whale (*Delphinapterus leucas*). *Genes*.
7. Kevill J ... **Mordecai G** et al. (2017). ABC Assay: Method Development and Application to Quantify the Role of Three DWV Master Variants in Overwinter Colony Losses of European Honey Bees. *Viruses*.
6. **Mordecai G** et al. (2017). Schrödinger's Cheshire Cat: Are Haploid *Emiliania huxleyi* Cells Resistant to Viral Infection or Not? *Viruses*.
5. Brettell L, **Mordecai G** et al. (2017). A Comparison of Deformed Wing Virus in Deformed and Asymptomatic Honey Bees. *Insects*.

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4. **Mordecai G** et al. (2016) Moku virus; a new *Iflavirus* found in wasps, honey bees and Varroa. *Scientific Reports*.

3. **Mordecai G** et al. (2016) Superinfection exclusion and the long-term survival of honey bees in Varroa-infested colonies. *The ISME Journal*. (ISME Journal 'top ten' paper, ranking=first)

2. **Mordecai G** et al. (2016) Diversity in a honey bee pathogen: first report of a third master variant of the Deformed Wing Virus quasispecies. *The ISME Journal*. (ISME Journal 'top ten' paper, ranking=fifth)

1. **Mordecai G** et al. (2011) Litter in submarine canyons off the west coast of Portugal. *Deep Sea Research Part 2: Topical Studies in Oceanography*.

Patent: Schroder D, Mordecai G (2016) A method of Preventing Infection of hymenopterous insects of the superfamily *Apoidea*.

Grants & Awards

- 2024 Evidence for Democracy's Evidence advocate of the month
2023 British Columbia Salmon Restoration and Innovation Fund, (grant writer and project team member to raise \$1M awarded to the Pacific Salmon Foundation and Ha'oom Fisheries Society - *Identifying factors that influence early marine survival of WCVI Chinook salmon*)
2022 Sitka Foundation (Lead grant writer to raise \$127,600 awarded to the Pacific Salmon Foundation to support the Salmon Health Program)
2021 Pacific Salmon Commission Southern Fund (\$79,500 - *Role of Pacific salmon Nidovirus undermining post-release survival of hatchery Chinook: application of salmon Fit-Chips*)
2019 Liber Ero Fellowship
2016-18 Mitacs accelerate postdoctoral fellowship
2017 Royal Entomological Society 'Alfred Russel Wallace Award' Runner up
2014 CB Dennis Trust Travel Grant
2010 Southampton Uni. School of Ocean and Earth Science Progression Scholarship (£1000)

Research and Teaching Activity

- 2024 **Webinar** for the Broughton Aquaculture Transition Initiative – "[The Truth About Open-Net-Pen Salmon Farms](#)"
2023 **Invited seminars** at the University of Calgary – "Towards a One Health perspective of zoonotic disease; a coronavirus case study" and "Emerging viruses – on the edge".
2022 **Presentation** to the WCVI Rebuilding: Marine Risk Assessment for Chinook Salmon
2021 **Instructor** eDNA workshop, Tofino
2021 **Guest Teaching Lecture** for the UBC Marine Microbiology Undergraduate course
2021 UBC Institute of Fisheries – Oceans and Fisheries **Seminar**, "The underwater epidemic; emerging viruses in wild Pacific salmon". [Video available](#)
2020 Guest **teaching seminar** for "AQUA 505 Ecological Sustainability of Aquaculture" (for UBC aquaculture graduate certificate)
2020 **Presentation** for the Puget Sound Marine disease working group 'Coronaviruses in the sea'
2020 **Presentation** at the American Society for Virology "Reoviruses and their hosts virtual workshop"
2019 UBC Institute of Fisheries – Oceans and Fisheries **Seminar**, 'A genomic view of viruses in farmed salmon in BC'
2019 UBC Biodiversity Research Centre - Biodiversity Research **Seminar**, 'Endangered wild salmon infected by newly discovered viruses'
2019 Guest **Teaching seminar** for the Ecology of Infectious Marine Diseases, Friday Harbor Laboratories, WA.
2019 Beatty Biodiversity Bliss **seminar** 'Emerging viruses of Salmon in British Columbia'
2019 **Chair** for the 2019 Gordon Research Seminar in Marine Molecular Ecology

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- 2018 **Presentation** at the 8th International Symposium on Aquatic Animal Health, “Novel Arenaviruses associated with disease are widely distributed in Chinook and Sockeye salmon.”
- 2018 **Guest Teaching Lecture** for the UBC Marine Microbiology Undergraduate course
- 2017 **Presentation** at Marine Molecular Ecology Gordon Research Conference, “Evidence for previously unknown viruses in farmed and wild Salmon in British Columbia”
- 2017 **Poster** at the Genome BC 15th Annual Genomics forum, “Evidence for previously unknown viruses in salmon from British Columbia”.
- 2016 **Workshop**; Porecamp, week long training course on Oxford Nanopore MinION sequencing technology, Falmouth, UK.
- 2016 **Poster** presented at the 8th Aquatic Virus Workshop, Plymouth, UK.
- 2016 **Presentation** at the Microbiology Society Annual Conference, Virus workshop: Positive strand RNA viruses, Liverpool, UK.
- 2016 **Presentation** at the Plymouth Marine Science & Education Foundation (PlyMSEF) annual student conference, Plymouth Marine Lab, Plymouth UK.
- 2015 **Article** in the British Bee Journal entitled ‘Implications of RNA virus quasispecies; determining the cellular and tissue tropism of Deformed Wing Virus’.
- 2015 **Presentation** at Reading University Graduate Symposium, Reading, UK.
- 2015 **Poster** presented at the British Beekeepers Association Spring Convention 2015, Harper Adams University, Shropshire, UK.
- 2014 **Poster** presented at the 19th International Bioinformatics Workshop on Virus Evolution and Molecular Epidemiology’, Rome, Italy.

Scientific Outreach & Training

- 2023 **Blog post** “The Case for an Independent Science Advisory Body at Fisheries and Oceans Canada (DFO)” written for the Evidence for Democracy “Perspective on Scientific Integrity” blog series
- 2023 **Scientific advisor** to First Nation Wild Salmon Alliance and Kwiakah First Nation for consultation with the Federal government on the transition from open-net pen salmon aquaculture in coastal B.C. waters
- 2022 Union of British Columbia Indian Chiefs **invited presentation** - Special Session on Protecting Wild Salmon
- 2022 **Testimony** to the Standing Committee on Fisheries and Oceans
- 2022 Ongoing provision of **scientific advice** to the First Nation Wild Salmon Alliance and shíshálh Nation on the disease risk posed by open-net salmon farming.
- 2022 **Workshop**, Liber Ero Policy Training
- 2021 **Presentation** to the First Nations Summit Meeting “Genomics research to improve the health of wild salmon”
- 2021 **Article** in The Marine Biologist Magazine “Fish farming fuels global movement of pathogens”
- 2021 **Presentation** to the Union of British Columbia Indian Chiefs “Genomics research to improve the health of wild salmon”
- 2021 **Media Article** in Canadian Geographic “Tracking salmon viruses”
- 2021 **Media Article** in the Conversation Canada “Fish farms transmit viruses to endangered wild Pacific salmon, new evidence shows”
- 2021 **Expert witness** for Homalco First Nation and Tla’amin Nation for judicial review by Mowi Canada West Inc. et al. to reverse the Minister of Fisheries, Oceans and the Canadian Coast Guard’s decision to phase out open-net salmon farming in the Discovery Islands area by June 20, 2022
- 2021 **Workshop**, Liber Ero Environmental Law workshop
- 2020 **Expert witness** for ‘Namgis First Nation to assist the Federal court for Judicial Review of the Minister of Fisheries, Oceans and the Canadian Coast Guard’s PRV Policy.

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- 2019 **Expert advisor** to the 'Namgis First Nation during engagement with the Minister of Fisheries, Oceans and the Canadian Coast Guard
- 2019 **Scientific advisor** to "The Last Salmon Run", a National Geographic funded multimedia documentary photography project
- 2019 **Workshop**, Liber Ero Conflict Resolution and Facilitation training
- 2018 **Workshop**, Ocean Leaders program 'Engaging with Policymakers' and 'Risk Analysis and Decision-making'
- 2018 **Workshop**; COMPASS scientific communication workshop
- 2016 **Presentation** at the Devon Apicultural Research Group (DARG) Annual meeting, Yelverton, UK
- 2016 **Co-founder** of the Plymouth PubhD group, where researchers have 10 minutes to explain their research to the public in a pub

This is **Exhibit "B"** referred to in the affidavit of Dr. Gideon Mordecai sworn before me at the City of Vancouver this 25th day of September, 2024.



A Commissioner for taking Affidavits for the Province of
British Columbia

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Expert Report for 'Namgis First Nation

'Namgis First Nation v. Minister Of Fisheries, Oceans and the Canadian Coast Guard et al., Federal Court No. T-1798-19.

By Dr. Gideon Mordecai
September 24 2024

Expert Report for 'Namgis First Nation

By Dr. Gideon Mordecai
September 24 2024

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Introduction

This is an independent expert report prepared in response to a request by 'Namgis First Nation ("Namgis") for *'Namgis First Nation v. Minister Of Fisheries, Oceans and the Canadian Coast Guard et al.*, Federal Court No. T-1798-19.

The report is to provide the court with background and context relevant to Piscine orthoreovirus (PRV). This report responds to the "Issues" provided to me by 'Namgis's legal counsel in a letter dated July 30, 2024 and in supplementary letters dated 21 August 2024 and 16 September, 2024. These letters of engagement are attached as appendices 1, 2 and 3.

Qualifications

I am a Research Associate at the University of British Columbia's Institute for the Oceans and Fisheries. My research focuses on the ecology of viruses—their interactions with each other, their hosts, and the environment. Although my initial undergraduate and Masters training was as a marine biologist, my PhD work applied next generation sequencing (a modern genomic sequencing approach) to explore the diversity of viruses associated with population declines in honey bees and other pollinators.

In 2016, I moved to Canada for a postdoctoral position at the University of British Columbia, where I discovered 15 new viruses in salmon. I was later awarded a Liber Ero Fellowship, which supports emerging scientists to conduct and communicate research that informs applied conservation and management issues relevant to Canada. By examining the genetic diversity of pathogens, my research aims to understand the emergence and spread of infectious diseases. Some of my most recent research involves applying viral genome sequencing to assess the disease transmission risk posed to wild Pacific salmon by Atlantic salmon aquaculture in BC. I have authored or co-authored approximately 30 peer-reviewed publications with a total of more than 1500 citations.

In my current role as a Research Associate within the Institute for the Oceans and Fisheries, I am partnered with the Pacific Salmon Foundation, and am part of a collaboration that is using innovative molecular technologies to determine the cumulative factors that influence the survival of Pacific salmon. Please see Appendix 4 for a copy of my CV.

Issues

These are the issues I was asked to investigate, copied from the Letter of Instructions dated July 30, 2024:

*Please provide a chronological and factual summary of **all** peer-reviewed literature published on Piscine orthoreovirus ("PRV") since September 2019 (the "**Research Summary**") which is relevant to the regulation of the open net-pen feedlots of Atlantic salmon in British Columbia:*

- a. In the Research Summary, please identify and highlight publications you deem key developments, findings and advancements in the scientific research on PRV as are relevant to the regulation of open net-pen feedlots of Atlantic salmon in British Columbia.
- b. For each peer-reviewed paper in your Research Summary, please provide a brief factual summary of the paper, highlighting the key contributions that paper makes to the scientific research on PRV.
- c. As part of the Research Summary, please provide a conclusion summarizing key developments in scientific research of PRV since 2019 and the current state of scientific research on PRV relevant to the regulation of open net-pen feedlots in British Columbia.

In addition, I was also asked to investigate the following issues laid out in the supplemental letter:

As part of the Research Summary portion of your report, please consider and provide a brief factual summary of the following documents, highlighting the key contributions the documents each make to the scientific research on Piscine orthoreovirus (“PRV”):

- 1. the unpublished manuscript of Dr. Kristi Miller-Saunders disclosed by the Department of Fisheries and Oceans regarding the first detection of PRV in British Columbia (the “**Manuscript**”); and
- 2. the Broughton Area Transition Initiative water sampling progress reports diagnosing heart skeletal muscle inflammation in Pacific salmon in the Broughton area (the “**Sampling Reports**”).

Summary of key findings since 2019

In general, there have been substantial advances in our understanding regarding PRV-1 in BC and the risk posed to wild Pacific salmon since 2019.

- Since 2019 the scientific literature has confirmed that PRV-1 originates from the Atlantic region, and was likely introduced into the region by Atlantic salmon aquaculture.
- PRV-1 is now very common on Atlantic salmon farms.
- In some cases PRV-1 causes disease on Atlantic salmon farms.
- There is very compelling evidence that the virus is transmitted from farmed Atlantic salmon to wild Pacific salmon
- A body of evidence links PRV-1 to disease in Chinook salmon.
- There is emerging evidence that this disease may be having a population level impact on Chinook salmon.

Overview of Research on PRV Published Since 2019

Concepts

The research summary considers a considerable number (47) of peer reviewed publications (including one manuscript in press and one in review) on PRV since September 2019 which have added to the overall understanding of the virus with knowledge that may be relevant to its management. Awareness of the concepts below is required in order to comprehend these advances.

Pathogenicity & Virulence

Pathogenicity refers to the ability of an organism, such as a virus, bacterium, or parasite, to cause disease in a host. It can vary between species, meaning that an agent might be pathogenic to one species but not to another.

Pathogenicity and virulence are related concepts with different meanings, and they are often incorrectly used interchangeably. While pathogenicity is the potential ability of an infectious agent to produce disease, virulence is the degree of severity of the disease.

Aside from the pathogen itself, additional factors such as host characteristics (e.g., genetic makeup, immune status, age, and overall health) and other biological or environmental factors (e.g., temperature, presence of co-infections) can significantly influence severity of disease. Understanding the interplay between these variables is crucial in accurately assessing and managing the risks associated with infectious diseases.

Disease challenge and epidemiological studies

Pathogenicity is often determined through challenge studies, where the host is deliberately exposed to the agent under controlled conditions to assess the likelihood and severity of disease development. Challenge studies can be used to study the infection, disease progression and immune response of the host to the pathogen.

The field of epidemiology is the study of infections and disease in populations in the real-world, rather than individuals in a lab.

Each approach has its own strengths and weaknesses, and both approaches complement each other. While lab-based challenge trials enable formal satisfaction of disease causation, the complex reality of different disease outcomes that occur in ecological settings are difficult to replicate in the lab. Epidemiological studies provide a view of how diseases spread and impact populations in real-world settings.

A useful analogy could be the study of the COVID-19 pandemic caused by the SARS-CoV-2 virus. Lab based human challenges, where young and healthy adults are exposed to the virus thankfully have not led to any severe disease or mortality. However, epidemiological studies in populations show that COVID-19 can lead to severe disease and mortality, especially in individuals with comorbidities (other medical conditions). Relying only on the lab-based studies would lead to a false sense of security over the risk the virus poses to public health. At the same time, the lab-based studies have greatly advanced our understanding in very relevant infection dynamics and immunology.

In 1965, Bradford Hill published 9 criteria to help determine if epidemiological associations are causal (1). These criteria included (among others) the strength and consistency of the association in order to evaluate whether an observed epidemiological association could indicate a causal relationship.

Origin and transmission dynamics

Phylogenetics is the study of the evolutionary relationships among organisms, often via analyzing the diversity of their genetic sequences. When applied to infectious diseases, phylogenetics relies on studying the genome sequence of the pathogen, which can help trace the origin of a pathogen, revealing its evolutionary history and how it has spread over time.

Phylogenetic analysis can identify the ancestral source of a pathogen, such as a specific animal reservoir or a geographic region where the pathogen first emerged. By comparing genetic sequences of different strains, the timeline of when the pathogen first appeared can be estimated.

Phylogenetics also helps in understanding the transmission dynamics of a disease, showing how a pathogen spreads between individuals, populations, jumps between species, or is introduced into a new region. By analyzing genetic similarities and differences among strains found in different hosts, researchers can map out the transmission pathways and track the movement of the pathogen across regions.

Viral spillover

Viral spillover refers to the event where a virus that typically circulates in one species crosses species barriers and infects a new host species. This process can lead to the emergence of new diseases in the newly exposed host.

Virus inactivation

Inactivation of viruses involves strategies to eliminate or neutralize the virus, preventing it from causing infection or spreading. These can include heat, UV light, or chemical treatments. Regardless of the method, they all disrupt the structure or function of the virus to disrupt infection.

Background on PRV

Introduction

In 2010, Piscine orthoreovirus (PRV) was discovered in Norway in farmed Atlantic salmon suffering with the disease heart and skeletal muscle inflammation (HSMI) (2). HSMI, the disease PRV causes in Atlantic salmon (3), is a concern in aquaculture because it impacts profitability and fish welfare. The mortality associated with HSMI ranges from negligible up to 20% of a farm population over the production cycle (4). PRV is now known to be exceedingly common on salmon farms globally, with an estimated 400 million infected individuals per year in Norway alone (5). PRV is also extremely common on Atlantic salmon farms in BC (6, 7), serving as a source of transmission to wild Pacific salmon (7–9).

PRV was first detected in British Columbia (BC) in 2012 in farmed Chinook exhibiting jaundice/anemia (10). In Pacific salmon, PRV-related diseases include anemia (complications from a low number of red blood cells) and in extreme cases, jaundice (a yellow discoloration) due to damage to the liver and kidney.

In the same year (2017) that a study in Norway determined PRV was the cause of HSMI (3), HSMI was diagnosed in BC Atlantic farms, and that diagnosis of HSMI was associated with PRV (11) (in epidemiology, an association refers to a relationship between an exposure and a disease outcome). Upon diagnosing HSMI on farms in BC, Di Cicco et al. (11) made the association between HSMI and PRV through a study investigating disease lesions on farms in BC through a statistical correlation between severity of lesions and the presence of PRV. The “longitudinal” nature of study (i.e. over a whole production cycle) allowed for the diseases progression over time to be tracked, and a temporal association with PRV to be made. In addition, the study used a staining technique to co-localize the virus with the disease lesions.

PRV diversity, epidemiology and pathology

Nearly 15 years after the discovery of PRV, there is now an accumulated body of research and better understanding of PRV genetic diversity, epidemiology and pathology. PRV currently comprises three known strains: PRV-1, PRV-2, and PRV-3. PRV-1 originates in the Atlantic Ocean (7, 12). It is the only causal agent of Heart and Skeletal Muscle Inflammation (HSMI) (3, 13). HSMI is commonly diagnosed in European Atlantic salmon farms (2, 4, 14). PRV-1 is the only strain that has been detected in BC, with genetic evidence suggesting multiple introductions into the region (in this context, an introduction refers to an instance where species or genetic lineages are brought into a new area from different sources or populations) (7, 15).

Phylogenetic analysis (the investigation of how organisms are related through evolution, typically by examining the variation in their genetic sequences) can be used to group PRV-1 into two major groups or sub-strains, commonly referred to as PRV-1a and PRV-1b (7, 16, 17) (within each strain of PRV, variations in genomic sequences can categorize the sequences into distinct sub-strains, also known as subtypes/sub-genotypes, sub-groups). Only PRV-1a has been detected in BC.

All three strains of PRV have been identified and proven to be causative agents of disease (3, 18–20). PRV-2 was first detected in Japan, in farmed coho salmon with a disease called erythrocytic inclusion body syndrome (EIBS) (19, 20). A related virus was recently discovered in diseased coho salmon in Alaska (21), although uncertainty remains regarding the origin of this new lineage, and its pathogenicity in different species of Pacific salmon. PRV-3 causes a disease similar to heart and skeletal muscle inflammation (HSMI) in farmed rainbow trout in Europe (18).

PRV-1 is the cause of Heart and Skeletal Muscle Inflammation (HSMI) in Atlantic salmon, initially demonstrated through a challenge trial with the PRV-1b sub-strain (3). Consequently, different isolates (a viral isolate is a specific sample of a virus separated from a mixed population) of PRV-1 were shown to vary in virulence (the degree of severity of the disease) in Atlantic salmon (13). Even the PRV isolates which were observed to be less virulent on a group level, were still observed to cause lesions (abnormal changes or damage to tissues) severe enough to qualify as HSMI in some, but not all of the individual fish challenged (13, 22). These observations established a cause and effect relationship between a BC isolate of PRV-1a and mild to moderate inflammatory heart lesions in Atlantic salmon, and corroborating epidemiological (i.e. real world) evidence of HSMI on farms in BC (11). All together these findings present both laboratory (disease challenge) and epidemiological (real-world) evidence that the lineage of PRV-1 in BC causes disease in Atlantic salmon.

Risk to Pacific salmon

A significant question is the potential of PRV to negatively affect BC's native Pacific salmon populations. Some argue that PRV-1 poses low concern based on disease challenge studies (mostly in species other than Chinook) (23–27). Epidemiological evidence suggests a different picture, with PRV-1a linked to jaundice/anemia in farmed and wild Chinook salmon in BC (28, 29). The disease jaundice/anemia is a consequence of rupture of red blood cells infected by the virus, and shares gross (i.e. changes observable with the naked eye) and microscopic pathological similarities with diseases that other strains of PRV have been shown to cause or are associated with in other Pacific salmonid species. That is, all three strains of PRV have shown the potential to cause rupture of infected blood cells, which are the main targets of the infection. In some cases, this can lead to a jaundiced (i.e. yellow) appearance and organ damage due to the release of toxic levels of hemoglobin (the protein contained in red blood cells) (18–20, 28, 30, 31).

Key developments in understanding of PRV since 2019

Below is an overview of the current state of knowledge. For a complete list of research published since 2019, please see Appendix 5 - Chronological Research Summary. This summary includes 47 peer-reviewed (including one *in press* and one under review manuscript) which I determined may be relevant to the management of PRV in BC since the PRV policy was established in 2019. These are based on the assumption that any findings relevant to the origin, transmission, epidemiology, virulence and pathogenicity of PRV-1 are relevant to the management of the virus in BC. The summary also incorporated unpublished reports provided

in the letter of instructions which include the Miller study on jaundice/ anemia (Appendix 6), and the BATI fish health reports (Appendix 7).

I have been informed by legal counsel from 'Namgis that DFO has not reconsidered its PRV policy since 2019. If the PRV policy has not been updated since 2019, then it could not have considered or incorporated any of this updated knowledge.

Origin

Key development since 2019 – It is now confirmed that PRV-1 originates from the Atlantic region and is a foreign virus introduced to BC

Upon its discovery in BC there was debate about the origin of PRV and whether the virus was native to the region or a foreign virus that was introduced to the region. There are now several papers published since 2019 which concluded that the virus originates from the Atlantic region (7, 12), corroborating a very early study (pre-2019) which made the same conclusion based on the first PRV genomes sequenced in BC (17).

Despite this agreement of an Atlantic origin, there is disagreement in the scientific literature over the timing of the arrival of PRV-1 to BC (12, 32). Additionally, Phylogenetic evidence supports the introduction of PRV-1 to the region on at least two separate occasions (see Figure 1). The initial introduction was approximately 35 years ago, and the second is associated with a farm in Washington which imported Icelandic eggs, and was detected in escaped Atlantic salmon in 2016 (7, 15).

The discovery of a lineage of PRV-2 in Alaska suggests there may be other, and more historic lineages of PRV in the region, although this is an area of very recent research, and lots of uncertainty remains around the origin and evolutionary history of this lineage of PRV. However, despite this finding of a new lineage in Alaska, the lineage present on farmed salmon in BC is the PRV-1 lineage introduced from the Atlantic region.

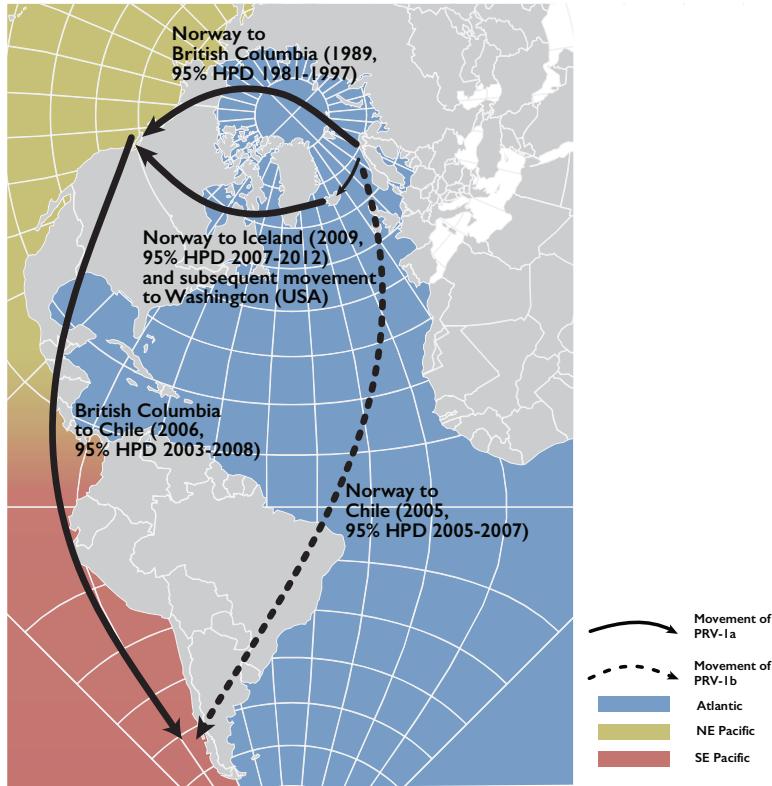


Figure 1. Adapted from Mordecai et al. 2021. Schematic representation of the global emergence of PRV-1. Two separate introductions of PRV into the North East Pacific are shown. Arrows depict estimated translocations of PRV-1 lineages based on phylogenetic reconstruction are labelled with estimated dates of each transmission event. The 95% highest posterior density (HPD) shows the range of dates where there's a 95% chance the real date falls (i.e. these values are analogous to a confidence interval).

Much of the discussion on the timing of the arrival focuses on a detection of PRV from an archived steelhead trout sample from 1977, which if real, could suggest an earlier introduction of PRV (32). However, this detection is contentious, and its reliability has been questioned since the detections were very weak and not readily repeated. To help to confirm if PRV-1 was present in BC in 1977, a peer-reviewed study would need to detect and sequence archival PRV from the 1977 sample, ensuring there are enough checks and controls in place to rule-out contamination and a false positive result. Contamination in this context is when genetic material from other sources accidentally mixes with the sample – a common problem due to the high sensitivity of PCR (a molecular test that amplifies small amounts of DNA to detectable levels). A difficulty with working with archival samples is that valid detections can often be weak due to sample degradation over time. Ruling out contamination as the source of these weak detections requires a carefully designed study with adequate controls.

Siah et al. (12) mention that partial PRV sequences from the 1977 samples were added to GenBank (an online sequence database). There is no peer-review standard for submitting

samples to Genbank. These sequences could potentially represent confirmation that the 1977 samples did indeed contain PRV. However, these sequences were not assessed in Siah et al., and the methods, protocols and study design used to obtain them are not described. These genetic sequences obtained from the 1977 sample are very similar to modern day PRV sequences, suggesting that more recent PRV material may have unintentionally contaminated the older sample. Gradual genetic change due to the natural evolution of the virus over time is expected to occur, so the very high similarity is somewhat of a red flag.

Regardless, even if the archival samples do represent that PRV was present in BC in 1977 (which remains uncertain), the potential presence of PRV at that time does not preclude subsequent introductions from the Atlantic into BC. Sequencing studies show that there have likely been several such introduction events, and that one of these introduced lineages is the likely ancestral source of the virus found on salmon farms in BC today.

Relevance for the Regulation of PRV in BC

The peer reviewed papers published since 2019 confirm that PRV is a foreign virus that was likely introduced into the Pacific region by Atlantic salmon farming. The introduction of a foreign virus raises management concerns for wild Pacific salmon, especially considering that salmon farms continue to sustain high levels of PRV infections. There is concern that since PRV is a virus that was only relatively introduced to the region, Pacific salmon did not evolve alongside this virus, and therefore it poses an additional disease risk. The disease impact of the virus has not been fully determined in all Pacific species (although what is known is reviewed below). Also, the virus appears to have a negative population level impact although there is considerable uncertainty on the size of the impact. A growing body of evidence (also described below) indicates that PRV-1 poses a risk to Chinook and coho salmon and there is a possible risk to other species.

Transmission dynamics

Key development since 2019 – PRV is transmitted from salmon farms to wild Chinook salmon

Epidemiological studies of Atlantic salmon in marine net-pens in BC carried out post-2019 confirm that PRV-1a becomes near ubiquitous on farms (6, 7, 33), and is detected in biological materials (i.e. waste and tissues sourced from Atlantic salmon farms), the sediment and in the water adjacent to Atlantic net-pens (6, 34). While an early study rarely detected PRV-1 in water samples (35), this appears to be a result of the method applied, since in more recent studies PRV-1 is very commonly detected (36). Additionally, PRV-1 has been detected in the effluent at salmon processing plants (34), and the effluent has been demonstrated to remain infectious despite the treatment applied (Appendix 8). Infection clusters (i.e. areas where there are high density of infections) in wild salmon occur regionally around Vancouver Island, including in regions where salmon farming takes place (9, 37). Alongside other aquaculture related infections, PRV-1 infection in wild fish was found to be more likely to occur, and at higher infection densities, in fish collected closer to active aquaculture facilities (8).

There are numerous lines of evidence (both genetic and epidemiological) that indicate that PRV-1 is transmitted between farmed and wild salmon; A) Infection in wild Pacific salmon is correlated with proximity to farms (7, 8), and PRV is often (but not exclusively) detected in wild fish in regions where farming occurred (9, 37) and B) farmed and wild salmon often share the same viral lineage (7). All together, these lines of evidence are significant since they strongly suggest infections in wild salmon are transmitted from farmed Atlantic salmon to wild Pacific salmon, as a direct result of high rates of infection on farms.

Relevance for the Regulation of PRV in BC

These results suggest that PRV-1 infection in wild fish is directly linked to the very common outbreaks of PRV-1 on Atlantic salmon farms in BC.

These findings are relevant to the management of PRV in BC since they show that PRV-1 infections are exceedingly common on farms. They also suggest that these high rates on farms result in pathogen spillover to wild fish populations which share the same waters. An additional route of transmission includes the effluent of processing plants, despite best attempts to treat the effluent to inactivate the virus. The patterns of infection in wild fish as well as similar identity of the virus in farmed and wild populations strongly suggest pathogen spillover towards wild populations.

Pathogenicity & Virulence

Key development since 2019 – PRV isolate from BC shown to cause disease lesions in Atlantic salmon, a finding corroborated by the multiple diagnoses of HSMI (the disease caused by PRV-1) on farms in BC.

There are now several lines of evidence finding that the lineage of PRV-1 in BC can cause disease in Atlantic salmon. Wessel et al. 2020 provided perhaps the most direct and compelling evidence that all the different isolates of PRV tested (including one from BC) were pathogenic, i.e. they caused heart lesions (See Figure 2) (13).

Wessel et al. 2020 established that while all the different isolates tested were the cause of heart lesions, they varied in virulence (i.e. disease severity). The PRV-1a isolate found in BC caused lesions diagnostic of HSMI, but in a lower proportion of infected individuals than the most virulent PRV-1b isolate dominant in Norway (13).

What remains undetermined is what factors these differences in virulence can be attributed to. Although variations in virulence among isolates in this challenge study were in some cases linked to specific viral genetic mutations, the phylogenetic placement of PRV-1 lineages was not the primary factor influencing virulence (13). Instead, it appears factors in addition to viral lineage likely influence virulence, such as environmental conditions or host factors.

Adding to the evidence that the lineage of PRV-1 in BC causes disease in Atlantic salmon is a study from before 2019 in which Atlantic salmon in open-net pens in BC were diagnosed with

HSMI (11). Post-2019, there are now multiple reports of HSMI diagnoses or lesions diagnostic of HSMI on farms, including by the BATI on-farm fish health monitoring (Appendix 7) (11, 38).

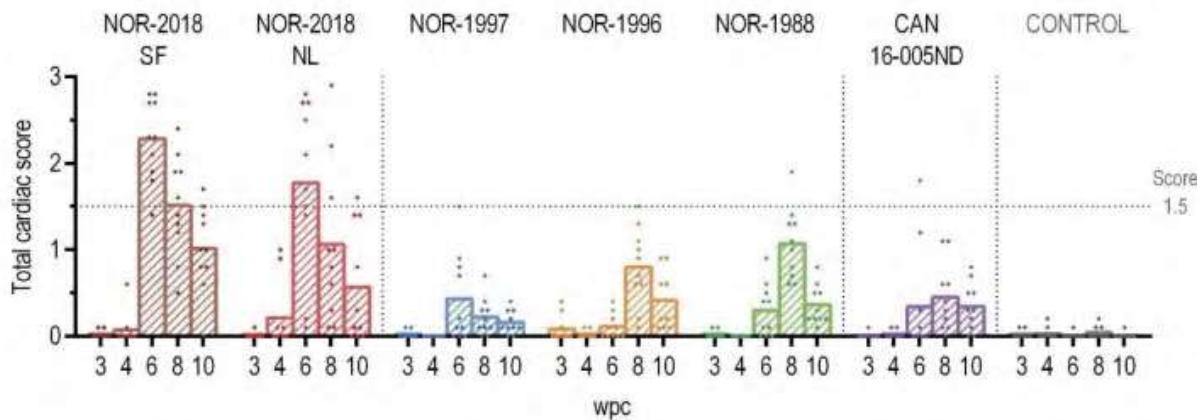


Figure 2. Adapted from Wessel et al. 2020. The chart demonstrates that all the different isolates of PRV-1 are pathogenic, and how virulence varies between isolates. The vertical axis is the heart lesion score, showing the severity of the heart lesions from 0-3. The bottom horizontal axis illustrates the weeks post challenge (i.e. time since exposure). Each coloured group (labelled at the top) is a different PRV isolate, and the grey group on the far right are the negative controls. The Canadian isolate is labelled CAN 16-005ND (shown in purple, second from right). Note that all of the controls have lesion scores close to 0, while all tested isolates led to heart lesions of varying severity. The BC isolate showed lesions of severity consistent with the Norwegian strains. The important takeaway from this is that challenge with a BC isolate caused disease lesions. The severity was lower or as low as the other Norwegian isolates. We don't know if this is due to implicit differences due to the isolates themselves. It is expected that these differences between isolates will differ in different hosts (i.e. in Pacific species, or different strains of Atlantic salmon).

Note that the presence of PRV does not always lead to HSMI outbreaks on farms. It is well understood that infection with PRV-1 will not necessarily lead to disease in all instances. The concept of asymptomatic spreaders (i.e. infected individuals that can pass on the virus, but show no disease symptoms) is now common knowledge as a result of the COVID-19 pandemic (39), but there are many examples of this phenomenon, beginning with the example of Typhoid Mary, a cook who unknowingly spread typhoid and subsequently became the text-book example of how asymptomatic carriers can play a significant role in the spread of infectious diseases (40).

Relevance for the Regulation of PRV in BC

The peer reviewed evidence published since 2019 shows that the lineage of PRV in British Columbia causes disease lesions diagnostic of HSMI. These findings are relevant to the regulation of PRV in British Columbia as together they provide evidence to inform whether or not PRV

should be regulated as a disease agent. The presence of asymptomatic infected individuals does not reliably inform the risk to populations or determine the pathogenicity of a virus.

Key development since 2019 – PRV-1 continues to be tightly associated with disease in farmed Chinook salmon.

In an epidemiological study, PRV-1a has been closely associated with jaundice/ anemia in farmed Chinook (28). This work built upon the understanding garnered from an unpublished study conducted in 2012 which marked the initial discovery of PRV in the province and also linked the virus to jaundice anemia in farmed Chinook. The draft of this manuscript was publicly released when the Office of the Information Commissioner compelled DFO to release it. What we now know about the tight association between PRV and jaundice/ anemia is consistent with the observations described in the draft paper.

Challenge studies in BC and Washington (with PRV-1a in Pacific salmon species) have not observed mortality or clinical jaundice/anemia (24, 25, 27) – although these studies did show some early signs of disease progression towards jaundice/anemia. Similarly, a challenge study in sockeye concluded that the effect of PRV on the host is of little consequence (41). However, the reliability of these conclusions have been questioned considering the study found PRV infection appears to have a modest impact on the blood cells as well as a specific measure of metabolism of sockeye. The relatively small sample sizes in the study and low statistical power also raised concerns (low power results in a reduced ability to detect a true effect or difference when it exists) (42–44).

Taken all together, my interpretation of these studies is that while PRV-1a likely can cause disease in Pacific salmon (most notably Chinook salmon); the clinical signs of jaundice anemia (i.e. the most severe signs of the disease) observed in farmed Chinook salmon, tightly linked with PRV infection (28), are not easily reproduced in challenge experiments. It is unclear if this is because of differences in environmental conditions or host factors, or because only a small number of infected individuals develop the most severe disease. Post-2019 observations of early signs of jaundice/ anemia in juvenile wild Chinook salmon corroborate the pre-2019 evidence from on farms, and suggest that wild fish experience at least early stages of the disease (29).

Other developments since 2019 include an emerging understanding in similarities of PRV-related disease in Pacific salmon. Instead of the inflammatory heart disease PRV causes in Atlantic salmon, PRV appears to cause a different type of disease in Pacific species. The disease in Pacific salmon is characterised by the virus resulting in the rupture of red blood cells, and the associated issues this causes for the host. These diseases share similarities across PRV strains in different Pacific salmon species – all include anemia as a result of the blood cells being infected by PRV and eventually rupturing (20, 21, 28, 31, 45). Altogether, this worldwide perspective, with some contributions since 2019, add to the body of evidence to support the hypothesis that PRV-1 causes jaundice/ anemia in Chinook salmon.

This global perspective of PRV related diseases across different species supports the analogy criterion of the Bradford Hill Criteria. Hill suggested that when there is strong evidence linking a specific agent to a specific disease, less evidence is needed to demonstrate a causal relationship for a similar agent in a related host (46). In this case, the analogy is even stronger because the diseases not only involve related agents and hosts, but also share similar mechanisms and types of disease.

While evidence is mounting that PRV-1 causes disease in Chinook salmon, more research is needed to demonstrate a cause-and-effect relationship. Further, more research is needed to determine potential links to disease in other Pacific salmon species.

Relevance for the Regulation of PRV in BC

The mounting evidence linking PRV to disease in Pacific species of salmon are highly relevant to the regulation of PRV in British Columbia. This emerging evidence from around the world (some of which is since 2019) linking PRV to disease in Pacific species support the previous findings that PRV-1 is closely linked to the disease jaundice/ anemia in Chinook salmon. Together this evidence strongly suggests that PRV-1 is likely to cause disease in wild fish populations. This evidence therefore informs whether or not PRV is a disease agent and if PRV poses a risk to wild fish populations.

The challenge trials conducted in BC did not reproduce this disease – however, a negative result from a laboratory challenge study should not be grounds for dismissing strong epidemiological evidence. This very concept is captured by Bradford Hill's criteria for causation, in which Hill cautioned against placing undue weight on isolated experiments, emphasizing that lack of laboratory evidence of disease should not invalidate any epidemiological associations.

Epidemiological evidence suggesting PRV poses a risk to wild Pacific salmon populations

Key development since 2019 – PRV has been linked to early signs of disease in wild Chinook salmon, and is linked to poorer survival and body condition in Chinook and coho populations

Determining the population level impact of PRV-1 infection on wild salmon is challenging due to the complex and variable nature of salmon populations and difficulties attributing PRV-1's specific effects from other environmental and biological factors (and their associated cumulative impacts). Nevertheless, correlational analyses have linked PRV to poorer survival and body condition in Chinook and coho populations (9, 47), and early signs of jaundice/ anemia have been observed in juvenile wild Chinook (29), also suggesting a potential effect on wild Chinook populations.

Relevance for the Regulation of PRV in BC

Similarly to the evidence linking PRV-1 to disease in farmed Chinook, evidence of the same disease pathway in wild Chinook, as well as associations with poorer survival and body condition are the best available evidence to suggest that PRV-1 likely causes disease in wild fish populations.

The associations described above are some the strongest possible available evidence to demonstrate such an effect. These are associations and not a firm cause-and-effect relationship, since it would be unreasonable to expect anyone to firmly establish a cause-and-effect relationship of population level harm given the complexity of natural ecosystems, and the inherent lack of a control population. Additionally, wild fish are exposed to multiple stressors—such as environmental changes, predation, and other pathogens. The long timescales and broad geographical ranges involved in population-level studies make it exceedingly challenging to isolate the specific contribution of a virus to observed declines or changes in wild fish populations.

Conclusions

Since 2019 the scientific literature has confirmed that PRV-1 originates from the Atlantic region, and was likely introduced by aquaculture. PRV-1 infections are now very common on farms, where in some cases it causes disease. Importantly, there is very compelling evidence that PRV-1 is transmitted from farmed Atlantic salmon to wild Pacific salmon, and there is a body of evidence linking the virus to disease in Chinook salmon. Finally, there is emerging evidence that this disease may be having a population level impact on Pacific salmon.

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41. M. P. Polinski, *et al.*, Innate antiviral defense demonstrates high energetic efficiency in a bony fish. *BMC Biology* **21**, 112 (2021).
42. G. Mordecai, *et al.*, Assessing the role of Piscine orthoreovirus in disease and the associated risk for wild Pacific salmon. *BMC Biol.* **21**, 114 (2023).
43. M. P. Polinski, *et al.*, Response to “Assessing the role of Piscine orthoreovirus in disease and the associated risk for wild Pacific salmon.” *BMC Biol.* **21**, 115 (2023).
44. S. Nakagawa, M. Lagisz, Next steps after airing disagreement on a scientific issue with policy implications: a meta-analysis, multi-lab replication and adversarial collaboration. *BMC Biol.* **21**, 116 (2023).
45. H. Hauge, *et al.*, Infection experiments with novel Piscine orthoreovirus from rainbow trout (*Oncorhynchus mykiss*) in salmonids. *PLoS One* **12**, e0180293 (2017).
46. K. M. Fedak, A. Bernal, Z. A. Capshaw, S. Gross, Applying the Bradford Hill criteria in the 21st century: how data integration has changed causal inference in molecular epidemiology. *Emerg. Themes Epidemiol.* **12**, 14 (2015).
47. A. L. Bass, *et al.*, Identification of infectious agents in early marine Chinook and Coho salmon associated with cohort survival. *Facets* **7**, 742–773 (2022).

Appendix 1

SEAN P. JONES*
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*Law Corporation

OUR FILE NO. N1243-011

MACKENZIE FUJISAWA LLP

BARRISTERS & SOLICITORS

July 30, 2024

VIA EMAIL: gidmord@gmail.com

1590 Gravely Street
North Vancouver, BC V7P 2A9

Attention: Dr. Gideon Mordecai

Dear Dr. Mordecai:

Re: Letter of Instructions — Expert Opinion for '*Namgis First Nation v. Minister Of Fisheries, Oceans and the Canadian Coast Guard et al.*, Federal Court No. T-1798-19

We write to confirm your engagement by MacKenzie Fujisawa LLP to prepare a supplementary independent expert report to your initial independent expert report dated November 19, 2020 (the "Initial Report"), previously prepared for and filed in the above noted matter. As we have discussed, our client, 'Namgis First Nation (the "Client"), has applied for judicial review of the Minister of Fisheries, Oceans and the Canadian Coast Guard's (the "Minister") decision not to test for Piscine orthoreovirus ("PRV") before granting licences to introduce or transfer fish into the marine environment (the "PRV Policy"). We have previously provided you with a copy of the certified tribunal record (the "CTR") for the Minister's October 3, 2019, decision to re-affirm the PRV Policy.

CODE OF CONDUCT FOR EXPERT WITNESSES

Before preparing your report, please carefully read the enclosed Code of Conduct for Expert Witnesses (the "Code"). This Code explains your duty to the court, along with your obligation to be impartial and objective, and sets out the mandatory content to be included in your report. In accordance with the *Federal Courts Rules*, you will be required to execute a certificate noting that you have read the Code and agree to be bound by its terms.

Please feel free to contact us if you have any questions about these instructions, including any questions about section 3 or other parts of the Code.

CONFIDENTIALITY AND PRIVILEGE

Your report and our communications throughout this assignment are "solicitor-client privileged", "litigation privileged", and confidential. This means that any draft reports, memos to file, e-mails, notes and so on will have the same privileges attached to them. Please mark all e-mails, letters, reports, and any other communications to us as "**Privileged and Confidential**". Please do not discuss the report with anyone except for us or 'Namgis representatives.

Privilege over your file may, however, be deemed to be waived once your report is filed with the Court. This means that opposing parties may seek, and may be granted access, to your file for the purposes of cross-examining you. In any event, you should always treat your file as privileged and confidential unless we advise you otherwise.

{N1243/0011/00733730.3}}

SCOPE OF REPORT

Your supplementary report is to provide the Federal Court with up-to-date background and context relevant to the Minister's October 3, 2019, decision to re-affirm the PRV Policy which became available following since you prepared the Initial Report. We have the following instructions and comment with respect to the format of your report, and the assumed facts upon which it is to be based.

1. Terms of Engagement

You should append this letter to your expert report, outlining your terms of engagement in the report. If additional instructions are required, then supplementary letters should also be attached to your report.

2. Qualifications

You are required to state your professional and scientific qualifications pertaining to your scientific and technical education, training and experience. This is an integral part of your report and should contain a detailed history as it pertains to your area of expertise and the subject matter of your opinions. Your detailed *curriculum vitae* should be attached as an appendix.

3. Assumed Facts and Documents Reviewed

The facts upon which your opinions are based must be explicitly set out in the body of your independent expert report.

4. Issues

Based on the assumed facts and documents reviewed, we request your insight and independent objective opinion concerning the following:

1. Please provide a chronological and factual summary of all peer-reviewed literature published on PRV since September 2019 (the "**Research Summary**") which is relevant to the regulation of the open net-pen feedlots of Atlantic salmon in British Columbia:
 - a. In the Research Summary, please identify and highlight publications you deem key developments, findings and advancements in the scientific research on PRV as are relevant to the regulation of open net-pen feedlots of Atlantic salmon in British Columbia. You may use your Initial Report as a guide for determining which issues are relevant.
 - b. For each peer-reviewed paper in your Research Summary, please provide a brief factual summary of the paper, highlighting the key contributions that paper makes to the scientific research on PRV.
 - c. As part of the Research Summary, please provide a conclusion summarizing key developments in scientific research of PRV since 2019 and the current state of scientific research on PRV relevant to the regulation of open net-pen feedlots in British Columbia.
2. Please provide a report on the enclosed Disease Agent Assessment forms disclosed by DFO for DFO's assessment of PRV (the "**DAA Report**") to determine, in your professional opinion, if

DFO's conclusion that the strain (or strains) of PRV found in British Columbia are not disease agent(s) for the purpose of regulating open net-pen feedlots of Atlantic salmon.

5. Discussion

You should set out your independent objective opinions in the same order as the issues are presented above. References should be made to the relevant assumed facts in all cases where necessary and technical documents, standards, guidelines, publications, and so on should also be referred to where considered or relied upon in forming your opinions.

6. Appendices

You should include the following documents as appendices to your independent expert report:

3. this instruction letter;
4. your qualifications (*curriculum vitae*); and
5. if you refer to any significant documents in your independent expert report which may include scientific reports, academic articles or any other documents you deem relevant in the exercise of your independent judgment, then those significant documents.

I trust all the above is satisfactory and if so, would ask that you sign a copy of this letter confirming that and return it to me at your earliest convenience. We look forward to working with you on this important matter.

Yours truly,

MACKENZIE FUJISAWA LLP



Per:

SEAN P. JONES

SPJ:azm

Encl. Disease Agent Assessment Forms released by DFO under the *Access to Information Act*
Code of Conduct for Expert Witnesses

{N1243/0011/00733730.3}}

I agree to the terms set out above in this letter



Dr. Gideon Mordecai

Date: 31 July 2024

Appendix 2

SEAN P. JONES*
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*Law Corporation

OUR FILE NO. N1243-011

MACKENZIE FUJISAWA LLP

BARRISTERS & SOLICITORS

August 21, 2024

VIA EMAIL: gidmord@gmail.com

1590 Gravely Street
North Vancouver, BC V7P 2A9

Attention: Dr. Gideon Mordecai

Dear Dr. Mordecai:

Re: Letter of Instructions — Expert Opinion for ‘*Namgis First Nation v. Minister Of Fisheries, Oceans and the Canadian Coast Guard et al.*, Federal Court No. T-1798-19’

This letter provides supplemental instruction to our initial letter of instructions dated July 30, 2024 (the “**Initial Instructions**”). We have the following additional instructions and comments with respect to the assumed facts upon which your report is to be based.

As part of the Research Summary portion of your report, please consider and provide a brief factual summary of the following documents, highlighting the key contributions the documents each make to the scientific research on Piscine orthoreovirus (“**PRV**”):

1. the unpublished manuscript of Dr. Kristie Miller-Saunders disclosed by the Department of Fisheries and Oceans regarding the first detection of PRV in British Columbia (the “**Manuscript**”); and
2. the Broughton Area Transition Initiative water sampling progress reports diagnosing heart skeletal muscle inflammation in Pacific salmon in the Broughton area (the “**Sampling Reports**”).

In addition to the documents identified in the Initial Instructions, you should include the following documents as appendices to your independent expert report:

1. this supplementary instruction letter;
2. the Manuscript; and
3. the Sampling Reports.

Additionally, given the large quantity of relevant papers published since 2019 on PRV, please retain the introduction and conclusion of the Research Summary portion of your report, but combine them into one section and rename that new section “Overview of Research on PRV Published Since 2019”. Please retain the chronological summary of the individual papers published since 2019, but relocate this

{N1243/0011/00739704.2}}}

summary to an appendix. We believe that this will result in a more concise and reader-friendly report for the Court.

I trust all the above is satisfactory and if so, would ask that you sign a copy of this letter confirming that and return it to me at your earliest convenience. We look forward to working with you on this important matter.

Yours truly,

MACKENZIE FUJISAWA LLP



Per:

Sean P. Jones

SPJ:azm

Encl. Excerpts from ATIP A-2017-01222 released by DFO under the *Access to Information Act*
Water Sampling Progress Reports

I agree to the terms set out above in this letter



Dr. Gideon Mordecai

Date: 23 Aug 2024

Appendix 3

SEAN P. JONES*
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*Law Corporation

OUR FILE NO. N1243-011

MACKENZIE FUJISAWA LLP
BARRISTERS & SOLICITORS

September 16, 2024

VIA EMAIL: gidmord@gmail.com

1590 Gravely Street
North Vancouver, BC V7P 2A9

Attention: Dr. Gideon Mordecai

Dear Dr. Mordecai:

Re: Letter of Instructions — Expert Opinion for ‘*Namgis First Nation v. Minister of Fisheries, Oceans and the Canadian Coast Guard et al.*, Federal Court No. T-1798-19’

This letter provides supplemental instruction to our initial letter of instructions dated July 30, 2024 (the “Initial Instructions”) and supplemental instructions dated August 21, 2024 (the “Supplemental Instructions”). We have the additional instructions and comments regarding the assumed facts and documents upon which your report is to be based.

Please omit your opinion on issue 2 identified in the Initial Instructions from your independent expert report. This refers to your opinion regarding the Disease Agent Assessment forms disclosed by DFO for its assessment of PRV in British Columbia and regulating open net-pen feedlots of Atlantic salmon.

In addition to the documents identified in the Initial Instructions and the Supplemental Instructions, you should include this supplementary instruction letter as an appendix to your independent expert report.

I trust all the above is satisfactory and if so, would ask that you sign a copy of this letter confirming that and return it to me at your earliest convenience. We look forward to working with you on this important matter.

Yours truly,

MACKENZIE FUJISAWA LLP


Per:

Sean P. Jones

SPJ:azm

{N1243/0011/00743711}

I agree to the terms set out above in this letter



Dr. Gideon Mordecai

Date: 23 September 2024

Appendix 4

Gideon Mordecai, PhD
(+1) (778) 680 8545, gidsmord@gmail.com

Employment

2022- present	Research Associate – Institute for the Oceans and Fisheries, University of British Columbia
2021- 2022	Interim Manager, Salmon Ecological Health Program, Pacific Salmon Foundation
2019 -2021	Liber Ero Postdoctoral Fellow – University of British Columbia
2016 –2019	Post-Doctoral Fellow (MITACS/ Pacific Salmon Foundation) – University of British Columbia

Education

2024-present	Public Policy Forum's Action Canada Fellowship
2023-present	Simon Fraser University Non-Profit Management Certificate
2013-2016	University of Reading / Marine Biological Association of the UK PhD Thesis title: Diversity in emerging honey bee viruses.
2011-2012	University of Plymouth / Marine Biological Association of the UK MRes Marine Biology [Distinction] Dissertation title: Investigating the presence of the <i>Emiliania huxleyi</i> Virus transcriptome in haploid <i>Emiliania huxleyi</i> cells.
2007-2010	University of Southampton BSc Marine Biology & Oceanography [First class honours]

Publications & Patents

29. **Mordecai G** et al. Comment on a perspective: Molecular detections of new agents in finfish—Interpreting biological significance for fish health management. (2024). *Journal of Aquatic Animal Health*
28. **Mordecai G** et al. Is scientific inquiry still incompatible with government information control? A quarter century later. (2023). *Canadian Journal of Fisheries and Aquatic Sciences. Selected as Editor's choice and was the #1 most read article in the Canadian Journal of Fisheries and Aquatic Sciences of 2023*
27. Di Cicco E,...**Mordecai G** et al. Tenacibaculosis in wild-caught, captive Chinook salmon (*Oncorhynchus tshawytscha*) in British Columbia, Canada (2023). *BioRxiv*.
26. **Mordecai G** et al. Assessing the role of Piscine orthoreovirus in disease and the associated risk for wild Pacific salmon (2023). *BMC Biology*.
25. **Mordecai G et al.** (2022) Detection and phylogenetic assessment of PRV-1 via sampling of biological materials released from salmon farms in British Columbia. *Canadian Journal of Fisheries and Aquatic Sciences*.
24. Bass AL, ... **Mordecai G** et al. (2022) Identification of infectious agents in early marine Chinook and Coho salmon associated with cohort survival. *FACETS*.
23. Deeg C,... **Mordecai G** et al. (2022) Way out there: pathogens, health, and condition of overwintering salmon in the Gulf of Alaska. *FACETS*.
22. McLaughlin A, ... **Mordecai G** et al. (2022) Genomic epidemiology of the first two waves of SARS-CoV-2 in Canada. *eLife*.

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21. Kuhn JH, ... **Mordecai G** et al. (2021) 2021 Taxonomic update of phylum Negarnaviricota (*Riboviria: Orthornavirae*), including the large orders *Bunyavirales* and *Mononegavirales*. *Archives of Virology*
20. Montaya V, ... **Mordecai G** et al. (2021) Variable routes to genomic and host adaptation among coronaviruses. *Journal of Evolutionary Biology*.
19. Bateman A.D, ... **Mordecai G** et al. (2021) Descriptive multi-agent epidemiology via molecular screening on Atlantic salmon farms in the northeast Pacific Ocean. *Scientific Reports*.
18. **Mordecai G** et al. (2021) Aquaculture mediates global transmission of a viral pathogen to wild salmon. *Science Advances*.
17. Shea D, ... **Mordecai G** et al. (2020) Environmental DNA (eDNA) from multiple pathogens is elevated near active Atlantic salmon farms. *Proceedings of the Royal Society B*
16. **Mordecai G** & Hewson I (2020) Coronavirus in the Sea. *Frontiers in Microbiology*.
15. Highfield A, ... **Mordecai G** et al. (2020) Detection and Replication of Moku Virus in Honey Bees and Social Wasps. *Viruses*.
14. Teffer AK, ... **Mordecai GJ** et al. (2020) A molecular assessment of infectious agents carried by Atlantic salmon at sea and in three eastern Canadian rivers, including aquaculture escapees and North American and European origin wild stocks. *FACETS*.
13. **Mordecai G** et al. (2020). Discovery and surveillance of viruses from salmon in British Columbia using viral immune-response biomarkers, metatranscriptomics and high-throughput RT-PCR. *Virus Evolution*.
12. **Mordecai G** et al. (2019). Endangered wild salmon infected by newly discovered viruses. *eLife*.
11. Pagowski VA, **Mordecai GJ** et al. (2019). Distribution and Phylogeny of Erythrocytic Necrosis Virus (ENV) in Salmon Suggests Marine Origin. *Viruses*.
10. Di Cicco E, ... **Mordecai G** et al. (2018). The same strain of *Piscine orthoreovirus* (PRV-1) is involved in the development of different, but related, diseases in Atlantic and Pacific Salmon in British Columbia. *FACETS*.
9. Brettell LE, **Mordecai G**, et al. (2017). Novel RNA Virus Genome Discovered in Ghost Ants (*Tapinoma melanocephalum*) from Hawaii. *Genome Announcements*.
8. Jones S, ... **Mordecai G** et al. (2017). The Genome of the Beluga Whale (*Delphinapterus leucas*). *Genes*.
7. Kevill J ... **Mordecai G** et al. (2017). ABC Assay: Method Development and Application to Quantify the Role of Three DWV Master Variants in Overwinter Colony Losses of European Honey Bees. *Viruses*.
6. **Mordecai G** et al. (2017). Schrödinger's Cheshire Cat: Are Haploid *Emiliania huxleyi* Cells Resistant to Viral Infection or Not? *Viruses*.
5. Brettell L, **Mordecai G** et al. (2017). A Comparison of Deformed Wing Virus in Deformed and Asymptomatic Honey Bees. *Insects*.

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4. **Mordecai G et al.** (2016) Moku virus; a new *Iflavirus* found in wasps, honey bees and Varroa. *Scientific Reports*.

3. **Mordecai G et al.** (2016) Superinfection exclusion and the long-term survival of honey bees in Varroa-infested colonies. *The ISME Journal*. (ISME Journal 'top ten' paper, ranking=first)

2. **Mordecai G et al.** (2016) Diversity in a honey bee pathogen: first report of a third master variant of the Deformed Wing Virus quasispecies. *The ISME Journal*. (ISME Journal 'top ten' paper, ranking=fifth)

1. **Mordecai G et al.** (2011) Litter in submarine canyons off the west coast of Portugal. *Deep Sea Research Part 2: Topical Studies in Oceanography*.

Patent: Schroeder D, Mordecai G (2016) A method of Preventing Infection of hymenopterous insects of the superfamily *Apoidea*.

Grants & Awards

- 2024 Evidence for Democracy's Evidence advocate of the month
2023 British Columbia Salmon Restoration and Innovation Fund, (grant writer and project team member to raise \$1M awarded to the Pacific Salmon Foundation and Ha'oom Fisheries Society - *Identifying factors that influence early marine survival of WCVI Chinook salmon*)
2022 Sitka Foundation (Lead grant writer to raise \$127,600 awarded to the Pacific Salmon Foundation to support the Salmon Health Program)
2021 Pacific Salmon Commission Southern Fund (\$79,500 - *Role of Pacific salmon Nidovirus undermining post-release survival of hatchery Chinook: application of salmon Fit-Chips*)
2019 Liber Ero Fellowship
2016-18 Mitacs accelerate postdoctoral fellowship
2017 Royal Entomological Society 'Alfred Russel Wallace Award' Runner up
2014 CB Dennis Trust Travel Grant
2010 Southampton Uni. School of Ocean and Earth Science Progression Scholarship (£1000)

Research and Teaching Activity

- 2024 **Webinar** for the Broughton Aquaculture Transition Initiative – "[The Truth About Open Net-Pen Salmon Farms](#)"
2023 **Invited seminars** at the University of Calgary – "Towards a One Health perspective of zoonotic disease; a coronavirus case study" and "Emerging viruses – on the edge".
2022 **Presentation** to the WCVI Rebuilding: Marine Risk Assessment for Chinook Salmon
2021 **Instructor** eDNA workshop, Tofino
2021 **Guest Teaching Lecture** for the UBC Marine Microbiology Undergraduate course
2021 UBC Institute of Fisheries – Oceans and Fisheries **Seminar**, "The underwater epidemic; emerging viruses in wild Pacific salmon". [Video available](#)
2020 Guest **teaching seminar** for "AQUA 505 Ecological Sustainability of Aquaculture" (for UBC aquaculture graduate certificate)
2020 **Presentation** for the Puget Sound Marine disease working group 'Coronaviruses in the sea'
2020 **Presentation** at the American Society for Virology "Reoviruses and their hosts virtual workshop"
2019 UBC Institute of Fisheries – Oceans and Fisheries **Seminar**, 'A genomic view of viruses in farmed salmon in BC'
2019 UBC Biodiversity Research Centre - Biodiversity Research **Seminar**, 'Endangered wild salmon infected by newly discovered viruses'
2019 Guest **Teaching seminar** for the Ecology of Infectious Marine Diseases, Friday Harbor Laboratories, WA.
2019 Beatty Biodiversity Bliss **seminar** 'Emerging viruses of Salmon in British Columbia'
2019 **Chair** for the 2019 Gordon Research Seminar in Marine Molecular Ecology

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- 2018 **Presentation** at the 8th International Symposium on Aquatic Animal Health, “Novel Arenaviruses associated with disease are widely distributed in Chinook and Sockeye salmon.”
- 2018 **Guest Teaching Lecture** for the UBC Marine Microbiology Undergraduate course
- 2017 **Presentation** at Marine Molecular Ecology Gordon Research Conference, “Evidence for previously unknown viruses in farmed and wild Salmon in British Columbia”
- 2017 **Poster** at the Genome BC 15th Annual Genomics forum, “Evidence for previously unknown viruses in salmon from British Columbia”.
- 2016 **Workshop**; Porecamp, week long training course on Oxford Nanopore MinION sequencing technology, Falmouth, UK.
- 2016 **Poster** presented at the 8th Aquatic Virus Workshop, Plymouth, UK.
- 2016 **Presentation** at the Microbiology Society Annual Conference, Virus workshop: Positive strand RNA viruses, Liverpool, UK.
- 2016 **Presentation** at the Plymouth Marine Science & Education Foundation (PlyMSEF) annual student conference, Plymouth Marine Lab, Plymouth UK.
- 2015 **Article** in the British Bee Journal entitled ‘Implications of RNA virus quasispecies; determining the cellular and tissue tropism of Deformed Wing Virus’.
- 2015 **Presentation** at Reading University Graduate Symposium, Reading, UK.
- 2015 **Poster** presented at the British Beekeepers Association Spring Convention 2015, Harper Adams University, Shropshire, UK.
- 2014 **Poster** presented at the 19th International Bioinformatics Workshop on Virus Evolution and Molecular Epidemiology’, Rome, Italy.

Scientific Outreach & Training

- 2023 **Blog post** “The Case for an Independent Science Advisory Body at Fisheries and Oceans Canada (DFO)” written for the Evidence for Democracy “Perspective on Scientific Integrity” blog series
- 2023 **Scientific advisor** to First Nation Wild Salmon Alliance and Kwiakah First Nation for consultation with the Federal government on the transition from open-net pen salmon aquaculture in coastal B.C. waters
- 2022 Union of British Columbia Indian Chiefs **invited presentation** - Special Session on Protecting Wild Salmon
- 2022 **Testimony** to the Standing Committee on Fisheries and Oceans
- 2022 Ongoing provision of **scientific advice** to the First Nation Wild Salmon Alliance and shíshálh Nation on the disease risk posed by open-net salmon farming.
- 2022 **Workshop**, Liber Ero Policy Training
- 2021 **Presentation** to the First Nations Summit Meeting “Genomics research to improve the health of wild salmon”
- 2021 **Article** in The Marine Biologist Magazine “Fish farming fuels global movement of pathogens”
- 2021 **Presentation** to the Union of British Columbia Indian Chiefs “Genomics research to improve the health of wild salmon”
- 2021 **Media Article** in Canadian Geographic “Tracking salmon viruses”
- 2021 **Media Article** in the Conversation Canada “Fish farms transmit viruses to endangered wild Pacific salmon, new evidence shows”
- 2021 **Expert witness** for Homalco First Nation and Tla’amin Nation for judicial review by Mowi Canada West Inc. et al. to reverse the Minister of Fisheries, Oceans and the Canadian Coast Guard’s decision to phase out open-net salmon farming in the Discovery Islands area by June 20, 2022
- 2021 **Workshop**, Liber Ero Environmental Law workshop
- 2020 **Expert witness** for ‘Namgis First Nation to assist the Federal court for Judicial Review of the Minister of Fisheries, Oceans and the Canadian Coast Guard’s PRV Policy.

Gideon Mordecai, PhD

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- 2019 **Expert advisor** to the 'Namgis First Nation during engagement with the Minister of Fisheries, Oceans and the Canadian Coast Guard
- 2019 **Scientific advisor** to "The Last Salmon Run", a National Geographic funded multimedia documentary photography project
- 2019 **Workshop**, Liber Ero Conflict Resolution and Facilitation training
- 2018 **Workshop**, Ocean Leaders program 'Engaging with Policymakers' and 'Risk Analysis and Decision-making'
- 2018 **Workshop**; COMPASS scientific communication workshop
- 2016 **Presentation** at the Devon Apicultural Research Group (DARG) Annual meeting, Yelverton, UK
- 2016 **Co-founder** of the Plymouth PubhD group, where researchers have 10 minutes to explain their research to the public in a pub

Appendix 5

Appendix 5. Chronological Research Summary

Literature Selection Method

The chronological literature review below contains peer-reviewed papers published since October 2019 which are relevant to the regulation of open net-pen aquaculture of Atlantic salmon in British Columbia, or the risk posed by open net-pen aquaculture to wild salmon. As described above, PRV-1a is the strain of the virus present in BC, so the majority of the manuscripts cited are studies on that specific strain. In certain instances, studies on other strains of PRV are included since similarities (e.g. in pathology) may be informative.

Review papers, comments and perspectives are included, but are described as such to indicate that they describe a summary or discussion of previously published research.

There are a small number of manuscripts which are currently under review or *in press* and in these instances the status of the manuscript at the time of writing is described.

In certain cases immunology studies and surveillance studies of other PRV strains in other regions were omitted.

I have made some assumptions in determining if these peer-reviewed publications are informative to the regulation of PRV in BC. These are:

- I assume that any publications that inform the key concepts I identified in my report are relevant to the regulation of PRV in BC. These key concepts include origin, transmission dynamics, pathogenicity & virulence, as well as the concept of epidemiological studies.
- I assume that analogous evidence from different regions is useful. E.g. I assume that knowledge gained from the study of aquaculture operations in Norway is useful to inform similar operations in BC. Similarly, I assume that there is at least some cross-species/ cross strain relevance. I.e. research abroad can be extrapolated to Pacific salmon species (*Oncorhynchus* spp.), despite differences in ecology, viral strain, and immune responses.
- I assume that all knowledge comes with a certain level of uncertainty. Where there are disagreements in the literature, I have tried to make this clear. I also have tried to describe any instances where there is considerable uncertainty that remains.

2019 (Oct-Dec)

[Malik et al. 2019](#) (1)- A laboratory challenge trial finds that PRV-1 establishes a productive, persistent infection in Atlantic salmon, particularly in blood cells. This study suggests that under certain infection conditions, PRV-1 particles may be continuously produced and released, a result that may be important in terms of management of transmission of PRV between populations.

2020

[Cartagena et al. 2020](#) (2)- PRV-1b and PRV-3a are detected in farmed Coho salmon with Jaundice syndrome in Chile. This adds to the evidence linking PRV to this disease mechanism in Pacific species of salmon.

[Dhamotharan et al. 2020](#) (3) - Disease challenge study investigating the infection dynamics and which tissues are infected by PRV-1 in experimentally infected Atlantic salmon. This study investigated the localization of the virus to different tissues and variation of PRV-1 genomic and protein levels at different times post-exposure. Similar to Malik et al. 2019, the study confirmed viral persistence in blood cells after the virus is clear from heart tissue. This study is relevant to the regulation of PRV in BC since it describes a persistent infection in Atlantic salmon, i.e. an infection which continues over time. This may inform the risk posed by infected Atlantic salmon on farms in BC.

[Jia et al. 2020](#) (4) - Review of infectious agent monitoring in wild salmon in British Columbia. PRV-1 was detected in all species and life stages (juvenile and adult) of Pacific salmon species (Chinook, chum, coho, pink and sockeye salmon, as well as Steelhead) present in BC waters. This broad distribution raises questions about the role of PRV-1 in the health and survival of these species.

[Marty et al. 2020](#) (5) - Epidemiological study investigating PRV infection dynamics and disease in Atlantic salmon hatcheries and farms in BC over a period of approximately 1.5 years. The study concludes that PRV-1 persistently infects the marine stage of Atlantic salmon production. The study includes a farm stocked from a PRV positive hatchery.

The amount of PRV in fish was only compared with the median (the middle) PRV level for each sample cohort, rather than between all sampling events, meaning it is hard to discern if the amount of PRV in fish with disease lesions differed from those without across all samples. The study concluded that there was no association between the level of PRV and heart lesions.

Despite this, the authors do acknowledge some of the heart lesions observed might be a result of infection with PRV-1, based on their similarity to lesions observed under controlled laboratory conditions.

The authors do not use the term HSMI, their reasoning being that the clinical signs and mortality observed in BC are less severe than in Norway and that HSMI did not occur in challenges with BC isolates of PRV. Instead, they use “morphologic diagnoses or the summary diagnosis of idiopathic cardiopathy rather than HSMI”. I.e. these authors diagnose the specific lesions associated with HSMI, but do not diagnose HSMI in name based on their view that clinical signs and a cause and effect relationship are required to diagnose the disease.

[Pham et al. 2020](#) (6) - In an attempt to develop an in-vitro PRV culture system (where fish cells infected with PRV are grown in vials). This study tested 31 fish cell lines (fish cells that grow and multiply in vials) from various tissues and cell types, yet showed no consistent changes or were able to sustain increases in the amount of PRV-1, indicating all cells tested

were nonpermissive for PRV (i.e. the cells do not support the continued replication of PRV) . Having a cell culture system for a virus is desirable since this provides a controlled environment to observe and manipulate the virus. This is especially useful for preparing pure isolates for challenge studies, as well as for developing vaccines and developing diagnostics. This study is relevant to the regulation of PRV in BC since it shows the difficulties in culturing PRV to achieve a pure isolate for further study. Note, growing a pathogen in pure culture is one of Koch's postulates for determining causality. Since PRV is (at the time of writing) a non-culturable organism, it cannot be strictly defined as the causative agent of disease using Koch's postulates. The inability of Koch's postulates to determine causality for non-culturable organisms are one of the major limitations of Koch's postulates.

Polinski et al. 2020 (7) - PRV review paper summarizing a decade of research and giving an overview of what was known at the time including similarities between all three strains of PRV. It includes a comprehensive review of the biology of PRV including physical characteristics, phylogeny, cell tropism (the types of cell PRV infects), infection dynamics, pathogenicity, transmission, shedding (the release of virus by a host as a result of infection), disease prevention, environmental stability and farmed to wild transmission. It also considers the geographic distribution of the different viral strains, as well as host range and situation in different geographic regions and detections in wild fish.

Although the paper describes challenge studies which failed to reproduce disease, and questions if PRV is the cause of HSMI and jaundice/ anemia in Pacific Canada, it does concede that it "is probable that PRV-1a can and occasionally does contribute to both" HSMI and jaundice/ anemia. I.e. this review paper appears to recognise that PRV-1a can cause disease. This is relevant to the management of PRV-1 because DFO's disease agent assessment concludes that PRV-1 is not a disease agent, despite DFO's own scientists concluding that it can contribute to disease.

This review is similar to CSAS Research Document 2019/035, although the peer-reviewed version was altered and with the input of two additional authors.

Purcell et al. 2020 (8) - PRV-1 laboratory challenge in Chinook, coho and rainbow trout. PRV-1 replicated in all species, but with negligible mortality. In the PRV-1 infected groups, inclusion bodies (viral inclusion bodies are aggregates of protein in the cell, often viral 'production factories') were observed in blood cells. And, at some of the time points, hematocrit (the proportion of red blood cells in the blood) was significantly decreased. Anemia was not observed. Mild heart lesions were reported in both the challenged and control fish. This challenge study is relevant to the regulation of PRV – although it did not replicate the severity of disease that PRV-1 is linked to in epidemiological studies, it did confirm that PRV-1 results in the rupture of blood cells in Pacific species, an early sign in the same disease pathway that in more severe cases leads to jaundice/ anemia.

Shea et al. 2020 (9) - Assessment of pathogen environmental DNA (also known as eDNA, genetic material collected from environmental samples e.g. from water) in relation to salmon farms in coastal British Columbia. PRV-1 was only detected once, which the authors attribute to possible technical or biological explanations related to the nucleic acid extraction

method. Detection of pathogens in the water adjacent to farms is significant because it indicates that farms are a source of pathogens to the environment.

Siah et al. 2020 (10) - Genome sequencing (i.e. determining the complete genetic sequence of an organism's genome) study of PRV-1 to examine the temporal and geographic range of the virus. The study found that PRV-1 originates from the Atlantic region. The estimated timing of the introduction from the Atlantic was inconclusive, depending if estimates were based on a larger dataset of partial genome sequences, or a smaller dataset of complete genomes. The origin of PRV-1 as being from a different region may be considered important since it shows that PRV-1 is not a natural component of the Pacific ecosystem. This study is relevant to the regulation of PRV, since it confirms the virus is Atlantic in origin. As an introduced virus, it was not a part of the natural diseases that Pacific salmon would have faced in their evolutionary history of Pacific salmon, and therefore it may pose an increased disease risk to these populations.

Siah et al. 2020 (11) - The development of a PCR assay to differentiate different PRV-1 lineages (i.e. different sub-strains of PRV-1). (PCR is a laboratory technique used to amplify and detect specific DNA or RNA sequences, enabling the identification and quantification of genetic material with high sensitivity and specificity). This study is relevant to the regulation of PRV since it described the development of a method to conduct surveillance of different lineages of the virus.

Teffer et al. 2020 (12) - Surveillance study of infectious agents in Atlantic salmon in Atlantic Canada, (aquaculture escapees and North American and European origin wild stocks). European and North American origin fish at sea shared similar PRV lineages, suggesting transmission between these populations. This study describes the transmission dynamics of PRV between different populations in the Atlantic region, which may have some relevance to the management of the PRV in BC.

Wessel et al. 2020 (13) - Laboratory challenge trial comparing six PRV-1 isolates (a viral isolate is a specific sample of a virus separated from a mixed population), including one isolate from BC. Virulence varied between isolates, with some causing more severe disease than others, and virulence was not attributed directly to a particular genetic lineage or evolutionary branch of the virus, suggesting factors other than genetic lineage are influencing the severity of the disease.

There was variability in lesion severity between individuals, but all isolates caused at least mild to moderate disease lesions. This is relevant to the regulation of PRV in BC, since it described a causal relationship between an isolate of PRV from BC and disease lesions in Atlantic salmon.

Wessel et al. 2020 (14) - Study investigating methods for the inactivation of PRV (i.e. strategies to eliminate or neutralize the virus). Iodine treatment, extreme pH levels, Virocid disinfectant, and UV (at a certain minimum dose) are effective for virus inactivation. The virus was highly resistant to heat inactivation. Knowledge of how to inactivate PRV may be important for informing biosecurity measures.

2021

[Bateman et al. 2021](#) - (15) - Multi-year and multi-agent screening of farmed salmon in British Columbia. PRV prevalence (the percentage of a population positive for PRV) increased to near ubiquity over time. In the majority of cases, PRV-1 prevalence and intensity (how much of the virus is in the fish tissues) were not elevated in dead/dying fish compared to live fish, with the exception of one of the cohorts. PRV-1 was detected in freshwater hatcheries. This study is relevant to the regulation of PRV since it found that at the time of the study, PRV infection occurred at the freshwater stage of production, and subsequently transferred to the marine environment.

[Godoy et al. 2021](#) (16)- Phylogenetic assessment of PRV (examining the genetic relationships) based on publicly available sequences of the S1 and M2 genomic segments (two different regions of the PRV genome). Although it does not represent a big advance in our understanding, I considered this study relevant to the regulation of PRV in BC since it confirms the analytical method used to classify different PRV lineages is valid.

[Malik et al. 2021](#) (17)- Infection with PRV-3 in Atlantic salmon induced cross-reacting antibodies (immune system proteins that can recognize and bind to related viruses) which block any subsequent PRV-1 infection. This protection provided by previous PRV-3 infection was greater than the protection provided by an inactivated PRV-1 vaccine (a type of vaccine made from virus that has been killed or otherwise rendered non-infectious). Infection with PRV-2 does not have such a pronounced protection effect. This research may be considered relevant to the regulation of PRV in BC since it shows that the vaccination does not fully protect against HSMI or block PRV infection.

[Polinski et al. 2021](#) (18) - (This paper is described below, see Mordecai et al. 2023, Polinski et al. 2023 and Nagamata et al. 2023)

[Mordecai et al. 2021](#) (19)- Phylogenetic assessment of PRV-1, which concluded that Atlantic salmon aquaculture facilitated the spread of PRV-1 from Europe to BC, approximately 30 years ago. The paper found that salmon farms are a source of infection for wild fish; wild Chinook salmon were more likely to be infected with PRV when they were closer to salmon farms, and genomic analysis found that farmed and wild salmon share the same viral variants, suggesting continual transmission.

[Vatne et al. 2021](#) (20) - Norwegian phylogenetic study examining the geographical distribution of PRV lineages in Norwegian Aquaculture operations. The study grouped the different lineages according to putative links to virulence; low, high and unknown. The “high-virulence” group was more common in mid and northern regions. This study is relevant to the regulation of PRV in BC since it revealed that the genetic underpinnings determining PRV virulence may be linked to several segments of the PRV genome. A better understanding PRV in the Norwegian aquaculture industry, including how it differs between different regions may help to inform its management in BC, since many of the issues are very similar. This study may also explain why, based on the lineage circulating in certain

regions, HSMI outbreaks appear to be more common and in some cases severe in certain regions of Norway compared to in BC, where only the lower virulent lineage is present.

[Zhao et al 2021](#) (21) - Development of a PCR assay (a laboratory technique used to amplify and detect specific DNA or RNA sequences) that can detect all three PRV strains (1, 2 and 3) known at the time of the study. This study is relevant to the regulation of PRV in BC since it described a new assay that could be used to aid surveillance PRV, including strains which have not yet been detected in BC, but are known to be present in the Pacific region (e.g. PRV-2).

2022

[Bass et al. 2022](#) (22) - Using a multi-year dataset of detections of 59 different infectious agents in Chinook and Coho salmon in British Columbia, PRV-1 and *Tenacibaculum* were the two pathogens most strongly associated with poorer survival and poorer body condition (body mass relative to size, a good indicator of fish health) in Chinook & coho salmon. This is relevant to the regulation of PRV in BC, since it indicates a population level impact of PRV to wild fish.

[Polinski et al. 2022](#) (23) - Nearly all Atlantic salmon on farms became infected with PRV, a finding consistent with previous studies. Although moderate and severe heart lesions mainly arose in populations with PRV, the study did not link infection with heart inflammation, but note, a restrictive study design prevented the paper from being able to link disease and infection in individual fish for all the samples, and to therefore corroborate previous work that identified PRV from BC as the likely cause of heart disease in Atlantic salmon. Additionally, the study describes detecting PRV-1 in the sediment and waters adjacent to Atlantic salmon net pens. These results confirm that PRV is very common on farms and is found in the waters adjacent to farms. This study is relevant to the regulation of PRV in BC since it implicates salmon farms as a source of PRV to the environment.

[Meyers et al. 2022](#) (24) - See Mordecai et al. 2024 and Meyers et al. 2024 below.

[Mordecai et al. 2022](#) (25) - Most BC salmon farms are releasing PRV to the environment via biological tissues. It is suggested that the release of tissue could possibly attract wild fish and facilitate transmission. PRV is also present in the effluent from salmon processing plants. Consistency in the PRV lineage for each company suggests freshwater hatcheries may be the source of infection. This study is relevant to the regulation of PRV in BC since it describes potential pathways of transmission of PRV between farmed to wild salmon.

2023

[Bass et al. 2023](#) (26) - Study examining the spatial distribution of 56 infectious agents in more than 10,000 juvenile salmon. Clusters of PRV infected wild individuals (described as 'hotspots') were identified in Columbia River salmon in the Spring and Summer. In the fall and winter, PRV 'hotspots' were detected in wild salmon in the inlets of West Coast of Vancouver Island, which was suggested to be a result of transmission from aquaculture

operations in the region, although the potential role of salmon enhancement hatcheries as a source of transmission was also discussed.

[Kannimuthu et al. 2023](#) (27) - PRV-1 challenge study comparing infection and disease in Atlantic salmon and brown trout. This study is relevant to the regulation of PRV in BC since it adds to the evidence that PRV-1 is a generalist (i.e. it can infect lots of different fish species, including in this case brown trout), but that the life stage and challenge method can influence the severity of these infections.

[Kannimutuhu et al. 2023](#) (28) - Challenge study in Norway to examine the infectious cycle of PRV-1 in Atlantic salmon during their development from fry to parr stage. No mortalities were observed during the 65 week challenge period, despite high loads of PRV which persisted throughout the trial. Heart lesions peaked at 6 and 8 weeks post infection and were resolved after 12 weeks. Cohabitation experiments at 10 and 31 weeks showed limited PRV transmission. The relatively quick recovery period may cast doubt on the reliability of challenge studies carried out in Canada which have been criticized for sampling regimes that likely missed the peak of infection, (29, 30). This study is relevant to the regulation of PRV in BC since it adds to the evidence that PRV infections in Atlantic salmon are persistent (i.e. occur over a long time period).

[Lolarte-Murillo et al. 2023](#) (31) - Genomic characterization of PRV-3 associated with jaundice syndrome in farmed Coho salmon in Chile. This study is relevant to the regulation of PRV in BC since it adds to the evidence linking PRV to jaundice anemia across PRV strains and Pacific salmon species. I.e. it contributed to the analogy criteria described above.

[Polinski et al. 2023](#) (32) - In a peer-reviewed response to Mordecai et al. 2023 (see below), Polinski et al. argue that PRV induced metabolic changes (shifts in how an individual uses energy) are not biologically relevant despite the statistical differences, and the changes as a result of PRV infection are “at most a small and temporary”. This study is relevant to the regulation of PRV in BC since it helps to lay out the differing opinions and perspectives that exist on the risk PRV poses to Pacific salmon populations. Regardless of which perspective is correct, it leaves no doubt that there is some level of uncertainty regarding the risk posed by PRV-1 to sockeye salmon.

[Madhun et al. 2023](#) (33) - Surveillance of migratory smolts for PRV-1 in Norway. PRV-1 prevalence was 4.6%, but varied across regions. PRV-1 prevalence was lowest in the region with highest aquaculture intensity. The authors conclude that the results suggest no apparent association between fish farming operations and PRV infections, but also acknowledge that more long-term studies of all salmon life-stages are needed to evaluate this risk. This study is relevant to the regulation of PRV in BC since a better understanding of the transmission dynamics of PRV in Norway, e.g. between farmed and wild populations, may inform the situation in BC.

[Mordecai et al. 2023](#) peer-reviewed response to [Polinski et al. 2021](#)(18, 34)
Polinski et al. (18) aimed to determine metabolic costs of viral infection in sockeye salmon, concluding that PRV is of little consequence to sockeye. The peer-reviewed response by Mordecai et al. (34) found that their study contained statistical flaws, failed to integrate

knowledge about diseases caused by PRV in other salmonids, and did not consider ecological realities that likely affect disease outcomes. The response concludes that overall, the data from Polinski et al. (18) are not adequate to support the conclusions drawn, and in some instances the findings actually suggest that PRV may cause ecologically relevant physiological impairment (i.e. disease) in sockeye.

This study is relevant to the regulation of PRV in BC since, similar to Polinski et al. 2023, it describes the differing opinions and perspectives that exist on the risk PRV poses to Pacific salmon populations. In this case, it suggests that PRV may pose a risk to sockeye salmon, although further research is needed.

[Nakagawa et al. 2023](#) (35) - A peer-reviewed commentary focusing on the scientific debate between Polinski et al. and Mordecai et al. (see above). Nakagawa et al. generally agree that the low statistical power and the other issues raised by Mordecai et al. are a serious issue, and suggest a 'registered multi-lab replication with adversaries' is needed to settle the debate. Similar to above, this study provides another perspective from scientists not directly involved in the research. They call for further research and they side with a more precautionary approach finding that the conclusions in Polinski et al. 2021 are not entirely supported by their data and analyses.

[Polinski et al. 2023](#) (36)- Surveillance of PRV-1 in freshwater hatcheries in BC including Atlantic, Chinook and coho between 2019 and 2021. Detections were minimal, and the study concluded that commercial and enhancement hatcheries in BC contribute minimally to PRV-1 in salmon in BC. This result is relevant to the regulation of PRV in BC since in the past, PRV has been detected in freshwater hatcheries. An understanding of the transmission dynamics of the PRV between different populations is necessary for its regulation.

[Rozas-Serri et al. 2023](#) (37) - Surveillance of archived samples for PRV-1 in Chile. The earliest detection was in 1994, 17 years before HSMI was first described in the region in 2011. This study helps to date the arrival of PRV to Chile, which appears to be prior to the phylogenetic estimates. This study is relevant to the regulation of PRV in BC since it describes methods which could be applied to the study of archival samples in BC, and also sheds light on the global transmission of PRV between regions.

[Turcotte et al. 2023](#) (38) - Screening of PRV-1 in out migrating salmon in BC. Chinook salmon had the highest PRV-1 prevalence, although there was lots of seasonal and spatial variability in detections. The study corroborated Bass et al. 2022 in finding that PRV was negatively correlated with body condition, but their interpretation was that this may be a consequence of life-history/behavioral differences rather than the difference being a result of infection. This result is relevant to the regulation of PRV in BC since it adds to the evidence that PRV may have a population level impact on wild Pacific salmon.

[Wang et al. 2023](#) (39) - Examination of physiological changes at the molecular, metabolic and cellular level associated with infectious agent monitoring in first year at sea Chinook. There was a strong molecular response to viral disease and pathological change consistent with jaundice/anemia associated with Piscine orthoreovirus was observed – the first

evidence that wild juvenile Chinook may experience a similar PRV related disease to that observed in farmed Chinook. This result is relevant to the regulation of PRV in BC as it suggests that infection in wild salmon populations may be linked to, at minimum, early signs of disease, which could explain why PRV is correlated to poorer survival.

2024

[Bass et al. 2024](#) (40) - Surveillance study to identify factors associated with infection. Infectious agents linked to aquaculture (including PRV-1) were more likely to be detected in wild fish detected closer to active aquaculture. These results are relevant to the regulation of PRV in BC since they add to the evidence that PRV is transmitted from farmed to wild salmon.

Bass et al. *under review* (41) Indigenous Monitoring and Inspection Plan (IMIP) study applying environmental DNA (eDNA) to characterize the release of infectious agents, including PRV-1 from Atlantic salmon aquaculture and the consequential exposure of Pacific salmon. Infectious agents were more likely to be detected in waters around salmon active farms, as were Chinook salmon eDNA. Alongside a few other pathogens, PRV-1 was identified as risk to Pacific salmon exposed to marine net-pen aquaculture

[Eckstrand et al. 2024](#) (42) - Discovery of a new PRV lineage in Alaska associated with a disease outbreak in coho salmon. The new virus has almost 90% genome identity to PRV-2, and approximately 70% identity to PRV-1 and PRV-3, suggesting it is a previously unknown Pacific lineage. The new virus was closely linked to a disease similar to that caused by PRV-2 in Japanese coho (erythrocytic inclusion body syndrome), and also reminiscent of the disease lesions, tissue distribution and anemia associated with PRV-1 infection in Chinook salmon in BC. This study is relevant to the regulation of PRV in BC since it adds to the ‘analogy’ evidence linking PRV to disease in Pacific species, but also suggests that it is possible that PRV-2 (or a lineage closely related to PRV-2) is present in the region.

Krkosek et al. *in press, accepted for publication*, expected publication date October 16 2024 (43) - A review paper examining the risk posed by pathogens prevalent in Atlantic salmon farms in BC, with a focus on PRV, *Tenacibaculum*, and sea lice. This review paper is relevant to the regulation of PRV in BC since it summarises major increases in the understanding of the risk posed by aquaculture associated pathogens (many of which are post-2019).

[Madhun et al. 2024](#) (33) - Surveillance of Norwegian farm salmon escapees for five viral infections. Over 90% of escaped fish were infected with at least one virus. PRV-1 was detected at a prevalence of 75%. This paper is relevant to the regulation of PRV-1 since the technologies used in Norwegian aquaculture are similar to in BC, and therefore the risk of escapees (which coincidentally are commonly infected with viruses) is similar.

[Meyers and Hickey, 2024](#) (44) - In a peer-reviewed response to Mordecai et al. 2024 (see below), Meyers and Hickey maintain that PRV-1a poses low risk to Pacific salmon. The

exchange of commentaries between authors are useful to inform the regulation of PRV in BC since they lay out the diversity of perspectives and the conflicting evidence in assigning the etiology of PRV-1.

[Mordecai et al. 2024](#) (45) peer reviewed comment on a perspective from [Meyers & Hickey, 2022](#). (Meyers and Hickey 2022; Mordecai et al. 2024) - Meyers & Hickey discusses the challenges in interpreting the biological significance of molecular detections of infectious agents. They use PRV as an example of a virus that “caused needless public concerns”, and conclude that the lineage of PRV in British Columbia is not a significant disease-causing agent in Pacific salmonids. In a peer-reviewed response, Mordecai et al. 2024 documented various concerns, and included a synopsis describing why Meyers & Hickey’s conclusion regarding PRV may be unfounded. In brief, Mordecai et al. explain how the focus on strict cause-and-effect relationships can overlook ecological complexity and ignore modern approaches to causal evidence. Mordecai et al. recommend a weight-of-evidence approach based on various lines of inquiry (including molecular methods and epidemiological evidence) rather than strictly following a single set of criteria in a laboratory setting to determine causality. Mordecai et al. proposed some ‘guiding principles’ to be considered in the precautionary management of infectious agents found in wildlife via molecular screening. They argue that a weight-of-evidence approach (incorporating epidemiological evidence) will provide a more robust assessment of whether a causal effect is likely to exist in real-world conditions compared to attempting to establish a causal relationship under laboratory conditions. Similar to above, these commentaries are relevant to the regulation of PRV in BC since they help to lay out the various lines of evidence for the differing perspectives on the risk posed by PRV-1 to wild fish.

[Solarte Murillo et al. 2024](#) (46) - Identifies reassortment (the exchange of genetic material between viruses with segmented genomes within a single host) as a key driver of PRV evolution and changes in virulence. This is relevant to the regulation of PRV in BC since it identifies a mechanism through which PRV can evolve and increase its genetic diversity which could potentially increase virulence.

[Takano et al. 2024](#) (47) –An investigation of coho salmon diagnosed with erythrocytic inclusion body syndrome likely caused by PRV-2. This study is relevant to the regulation of PRV in BC since it contributes to the ‘analogy’ criteria described above, but also is relevant to the potential risk to coho salmon from the new PRV-2 lineage discovered in Alaska.

[Vatne et al. 2024](#) (48) - Sequencing based epidemiological study on farms in Norway. The study found multiple introductions of PRV-1 to the study region, but that also there was a high level of genetic similarity among the viruses detected. Two of the three sites differed in PRV lineage to the previous production cycle, and the authors suggested that coordinated breaks in production (fallowing) could be an effective strategy for reducing the transmission of PRV-1 between different production cohorts. HSMI associated mortality was observed at all sites, regardless of the genotype detected, highlighting the complexity in associating viral lineages with mortality. I.e. the concept that there is a genotype of PRV that does not cause HSMI and associated mortality is not supported. This is relevant to the regulation of PRV in BC since it suggests that even the so called ‘lower virulence’ isolates of PRV can

cause HSMI outbreaks on farms. It also informs how biological control measures could be used to try and control PRV outbreaks between production cycles.

Unpublished reports

The reports described below were provided in the letter of instruction and are included as appendices 6 and 7.

Appendix 6: Miller et al. Histopathology and genomic characterization of idiopathic jaundice and anemia syndrome in cultured Chinook salmon (*Oncorhynchus tshawytscha*)

This draft manuscript (publicly released in March 2022 when the office of the information commissioner compelled DFO to release it) investigated the potential cause of jaundice anemia which was observed at the same time of chronic mortalities on Chinook salmon farms on the West Coast of Vancouver Island in 2010/2011. The clinical sign of the disease observed included yellowing of the skin, pale gills (anemia) and pale livers, and microscopic lesions were also observed. The disease was statistically association with detection of PRV. Gene expression signatures (the use of a gene's instructions to produce molecules for specific roles) suggested a viral role in the disease

These results are significant because they document the first detection of PRV in BC, and provide strong epidemiological evidence that PRV is likely the cause of jaundice anemia in Chinook salmon. Although this study was never published, the knowledge surely informed a later study which tightly associated PRV with the disease (including through co-localisation of the virus and disease lesions).

The unpublished study was pivotal in identifying and associating PRV with a specific disease in Chinook salmon, marking the first detection of the virus in BC. This early identification was crucial for understanding the potential threat PRV poses to Pacific salmon populations. Although it was not published, the results informed many subsequent studies, and opened many avenues of research into PRV in BC, including the first published detection of PRV in BC (49).

Appendix 7: Reports from IMIP

These reports document the result from on farm 'fish health' sampling in the Broughton Archipelago between October 2021. These include environmental data (temperature, salinity, dissolved oxygen), infectious agent monitoring, observations of clinical signs, histology scoring and any comments or diagnoses. Of note, is the very high prevalence of PRV (as high as 100% in some cases, and often at high viral loads (the amount of virus present), as well as diagnoses of HSMI made by the pathologist (Dr Emiliano Di Cicco). These reports are relevant to the regulation of PRV in BC since they reveal that outbreaks of HSMI are more common on farms than was previously described.

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Appendix 6

Information Commissioner's final report

Institution: Fisheries and Oceans Canada

OIC file number: 3218-01365

Institution file number: A-2017-01222 /DSP

Date: December 21, 2021

Complaint

The complainant disputes the Department of Fisheries and Oceans' (DFO) decision to withhold, under the *Access to Information Act*, information in response to a request for copies of all communications, over a specified timeframe, between certain named DFO employees, the Pacific Salmon Foundation, and/or "to or from" a named DFO employee, related to piscine reovirus, heart and skeletal muscle inflammation, the Creative Salmon Company Ltd., or jaundice syndrome. DFO refused to disclose portions of the requested records based on: section 14 (Federal-provincial affairs), subsection 16(2) (Facilitating the commission of an offence), subsection 19(1) (Personal information), paragraph 20(1)(b) (Confidential third-party financial, commercial, scientific or technical information), paragraph 20(1)(c) (Financial impact on third party), paragraph 21(1)(a) (Advice or recommendations), paragraph 21(1)(b) (Accounts of consultations or deliberations), section 23 (Legal advice or litigation privilege) and section 68 (Publicly available material).

During the investigation, the complainant informed the Office of the Information Commissioner (OIC) that its investigation should be limited to DFO's refusal to disclose information within a draft report and draft manuscript at pages 63-66, 69-71, 73-130, 264-266, 268-292 of the responsive records ("the information at issue") based on paragraphs 20(1)(b) and (c) of the Act. However, following DFO's additional claim that this information also warrants redaction under paragraph 18(c) (Government scientific or technical information obtained from research), the OIC's investigation further considered DFO's refusal of access based on this additional exemption. The investigation did not review DFO's refusal to disclose personal information based on subsection 19(1).

Investigation

Paragraph 18(c): Government scientific or technical information obtained from research

Paragraph 18(c) allows institutions to refuse to release scientific or technical information stemming from government research that, if disclosed, could jeopardize government researchers' chance to publish their findings first.

To claim this exemption, institutions must show the following:

- The information is scientific or technical.
- This information was obtained through research by a government employee or officer.
- Disclosing the information could threaten the exclusive rights of government researchers to publish the results of their research first.
- There is a reasonable expectation that this harm could occur—that is, the expectation is well beyond a mere possibility.

When these requirements are met, institutions must then reasonably exercise their discretion to decide whether to release the information.

Does the information meet the requirements of the exemption?

DFO relied on paragraph 18(c), concurrently with paragraphs 20(1)(b) and (c), to withhold:

- portions of a draft final report (“draft Final Project Report”); and
- nearly the entirety of a draft manuscript, and an additional copy of certain supplemental tables and figures to that draft manuscript (“draft Manuscript”).

These records were prepared pursuant to an Agreement, dating back to July of 2011, between the Crown and a third party, Creative Salmon Ltd., for a collaborative research project on the “Genomic characterization of jaundice-associated mortality events in cultured Chinook salmon” (“the Agreement”).

In reviewing the information at issue and considering the representations received, I am satisfied that at least the bulk of the information is both scientific in nature and was obtained through research of a government employee, so as to satisfy the first two criteria of paragraph 18(c). I am, however, not satisfied that the remaining criteria of paragraph 18(c) are met.

Although the Agreement and other records gathered during the OIC's investigation establish that final versions of the Final Project Report and Manuscript were initially intended to be published, those records raise serious doubt as to whether there was any continued intention to publish these records by the time the access request was made to DFO. This would seem to be supported by the fact that, when initially responding to the request, DFO did not raise paragraph 18(c) as a basis for withholding the information at issue.

The Agreement, which was said to expire on or before July 31, 2012, identifies the Final Project Report as a deliverable to the Agreement and further provides that "time" is "of the essence with respect to all deliverables ...". A Manuscript was then to be prepared based on the Final Project Report at the project's end.

Based on information provided by DFO, initial drafts of the Final Project Report and Manuscript were first circulated, respectively, on April 17, 2012, and April 4, 2014. Nonetheless, as of the date of DFO's receipt of the access request, on March 22, 2018, no final version of either record had been approved.

In reviewing the Agreement, including its terms regarding intellectual property, confidentiality and publication, there is no apparent restriction on the disclosure and/or publication of either the Final Project Report, Manuscript, or drafts thereof, in the absence of the Creative Salmon Ltd's agreement or consent. Nonetheless, more than 9 years since the circulation of a first draft of the Final Project Report, and more than 7 years since the circulation of the first draft of the Manuscript, there is still no indication of any significant progress in the approval and/or publication of either record.

No evidence was provided to the OIC of any continued intention to publish either a final version of the Final Project Report or Manuscript, much less any evidence supporting the contention that the disclosure of the information at issue could reasonably be expected to threaten the exclusive rights of government researchers to publish the results of their research first.

The Treasury Board Secretariat's *Access to Information Manual* informs institutions that in order for information to qualify for exemption under paragraph 18(c), it must establish that "...the officer or employee must be actively engaged in the research with a reasonable expectation of publication". In the present instance, I am not convinced that this is the case.

In the absence of any representations, much less representations supported by evidence, of any continued active engagement in the research for which DFO claims disclosure could deprive the officer or employee of priority of publication, DFO has not established that the third requirement of paragraph 18(c) is met. It has equally failed to establish a clear and direct connection between disclosure of the information at issue and a risk of harm that is

well beyond the merely possible. (*Merck Frosst Canada Ltd. v. Canada (Health)*, 2012 SCC 3, paras. 197, 206).

Had the information met the requirements of the exemption, did the institution reasonably exercise its discretion?

As DFO has not established that the information at issue falls within the scope of paragraph 18(c), it was not necessary for me to further consider whether DFO reasonably exercised its discretion when refusing disclosure. I do however note that DFO made no representations regarding the discretion to disclose information falling within the scope of paragraph 18(c), much less how discretion was reasonably exercised based on a consideration of all relevant factors.

Therefore, even if DFO had established that the information at issue falls within the scope of paragraph 18(c) - which it did not do - DFO would still not have established that it was or continues to be justified in refusing access under this exemption.

Paragraph 20(1)(b)

Paragraph 20(1)(b) requires institutions to refuse to release confidential financial, commercial, scientific or technical information, provided to a government institution by a third party (that is, a private company or individual, but not the person who made the access request).

To claim this exemption, institutions must show the following:

- The information is financial, commercial, scientific or technical;
- The information is confidential;
- The third party supplied the information to a government institution; and
- The third party has consistently treated the information as confidential.

When these requirements are met, and the third party to whom the information relates consents to disclosure, the institution must then reasonably exercise their discretion to decide whether to release the information.

Institutions must also reasonably exercise their discretion to decide whether to release the information for public health or public safety reasons, or to protect the environment, when both the following circumstances exist:

- Disclosure of the information would be in the public interest; and

- The public interest in disclosure clearly outweighs any financial impact on the third party, any prejudice to the security of the third party's structures, networks or systems, or competitive position, or any interference with its contractual or other negotiations.

Does the information meet the requirements of the exemption?

As previously noted, DFO relied on paragraph 20(1)(b) to withhold the same information claimed to be exempted under paragraph 18(c).

Based on the representations received and information gathered during the OIC's investigation, I am not satisfied that *each* of the requirements of paragraph 20(1)(b) are met.

While I accept that the redacted information largely consists of "scientific information", so as to satisfy the first criteria of paragraph 20(1)(b), neither DFO nor Creative Salmon Ltd. established other criteria needed to demonstrate the applicability of this exemption.

The second criteria – that the information is "confidential" – requires that the information:

- a) not be available from sources otherwise accessible by the public;
- b) originate and be communicated in a reasonable expectation of confidence that it will not be disclosed; and
- c) be communicated, whether required by law or supplied gratuitously, in a relationship between government and the party supplying it that is either a fiduciary relationship or one that is not contrary to the public interest, and which relationship will be fostered for public benefit by a confidential communication. (*Canada (Information Commissioner) v. Canada (Canadian Transportation Accident Investigation and Safety Board)*, 2006 FCA 157, para. 72).

Although there is no evidence that the information redacted under paragraph 20(1)(b) is in the public domain, I am not satisfied that the information at issue originated and/or was communicated in a reasonable expectation of confidence that it would not be disclosed.

In this regard, while both DFO and Creative Salmon Ltd asserted that pursuant to the Agreement, both the Final Project Report and Manuscript are to remain confidential until finalized and published, I was directed to no specific term(s) within the agreement to support this claim.

For instance, while the Agreement contains a confidentiality clause which states that “technology data or other information related to the Project shall be deemed confidential...”, this clause is subject to a *proviso* which states:

...this confidentiality obligation shall not apply to the Party who owns the Intellectual Property in such Information, and in the case of DFO, this confidentiality obligation shall be subject to the access to information and privacy protection legislation, including the *Access to Information* and the *Privacy Act*.

With respect to ownership of Intellectual Property, the Agreement provides, *inter alia*, that:

...biological material and organisms arising, acquired, and produced under the Agreement belong to the Minister;

and

...Research IP [defined as research and other activity under the Agreement and any parts of that IP] that is created, developed or produced by DFO employees in the course of their employment, or with any intellectual contribution or direction from DFO employees shall belong to Canada, under the control and administration of the Minister. ...;

In the absence of any representations or cogent explanations to the contrary, these terms suggest that the Crown’s Research Intellectual Property (IP) under the Agreement is extraordinarily broad.

Meanwhile, nothing in the Agreement’s terms with respect to publication, appear to restrict either the disclosure and/or publication of a party’s own Research Intellectual Property. As for “other information produced under the Agreement”, the terms of the Agreement only provide that a party seeking to disclose and/or publish such information must first give the other party a chance to review and request that this information not be disclosed; however, any request that the information be withheld cannot exceed a period of one year.

Therefore, the terms of the Agreement and evidence provided to my office only suggests that:

- the draft Final Project Report and Manuscript are the intellectual property (IP) of the Canadian Government;
- the confidentiality clause does not apply to this intellectual property and does not oust the applicability of the Act; and
- nothing prohibits or restricts DFO’s disclosure.

Based on the above, it has not been established that the information at issue originated and was communicated in a reasonable expectation of confidence that it would not be disclosed.

As previously noted, the final requirement needed to establish confidentiality by an objective standard requires that the information be communicated in a relationship between government and the party supplying it that is either a fiduciary relationship or one that is not contrary to the public interest, and which relationship will be fostered for public benefit by a confidential communication. DFO merely asserted that this requirement was met. Meanwhile, the Creative Salmon Ltd. stated:

.... third parties will be dissuaded from participating in research projects such as this if their IP can be mined and released prior to publication. This would have a dulling effect on future research projects, a result that would stifle innovations and development, contrary to one of the purposes of the Act ...

This representation, however, was not supported by any cogent explanation of what if any of the information at issue is Creative Salmon Ltd.'s own IP and/or could facilitate the "mining" of its IP, so as to dissuade others from participating in projects of this kind.

I am not satisfied that either DFO or Creative Salmon Ltd. has established that the information at issue was communicated within a relationship fostered for public benefit by maintaining confidentiality.

Turning to the third criteria of paragraph 20(1)(b) -- that the third party supplied the information to a government institution – the case law under the Act has repeatedly distinguished between information supplied by a third party and independent observations made based on information that has been supplied (see, for example: *Merck Frosst v. Canada (Minister of Health)*, 2012 SCC 3, at paras. 152-158; *Hibernia Management and Development Company Ltd. v. Canada – Newfoundland and Labrador Offshore Petroleum Board and the Information Commissioner of Canada*, 2012 FC 417).

In keeping with this case law, while the draft Final Project Report and Manuscript may have been prepared with the benefit of the Creative Salmon Ltd.'s fish samples or data, I am not satisfied that the substance of the draft Final Project Report and Manuscript is likewise information that Creative Salmon Ltd. supplied. In the absence of convincing representations, supported by cogent evidence, that clearly identifies information within the draft Final Project Report and Manuscript that was directly supplied by Creative Salmon Ltd. to DFO with no input from DFO officials, I conclude that the third criteria needed to demonstrate the application of paragraph 20(1)(b) is also not met.

As for the final criteria of paragraph 20(1)(b), Creative Salmon Ltd. in its representations asserted that the information has consistently been treated as confidential. Although I have no basis for questioning this assertion, the information can only be withheld under paragraph 20(1)(b), if *all* other criteria previously discussed are also met.

It has not been established that any of the information at issue satisfies each of the requirements of paragraph 20(1)(b). Therefore, I cannot accept that any of the information warrants being withheld under this exemption.

Paragraph 20(1)(c) - Financial impact on third party

Paragraph 20(1)(c) requires institutions to refuse to release information that, if disclosed, could reasonably be expected to have a substantial financial impact on a third party (that is, a private company or individual, but not the person who made the access request) or harm its competitive position.

To claim this exemption with regard to financial impact on a third party, institutions must show the following:

- Disclosing the information could result in substantial financial loss or gain to the third party.
- There is a reasonable expectation that this harm could occur—that is, the expectation is well beyond a mere possibility.

To claim this exemption with regard to competitive position, institutions must show the following:

- Disclosing the information could injure the competitive position of the third party.
- There is a reasonable expectation that this prejudice could occur—that is, the expectation is well beyond a mere possibility.

Does the information meet the requirements of the exemption?

As previously noted, paragraph 20(1)(c) was also relied on to withhold all of the information claimed to be exempted under paragraph 18(c) and paragraph 20(1)(b).

Neither DFO, nor Creative Salmon Ltd. established that there is a reasonable expectation, well beyond a mere possibility, that the disclosure of the information at issue could result in the harms alleged.

DFO's representations in support of the application of paragraph 20(1)(c) to withhold the information at issue were limited to alleged harms arising from a loss of the opportunity to publish. According to DFO: publication would benefit the creators of the study (i.e. *vis à vis* access to future funding from various sources based on prior publication, increased professional stature and respect engendered by scientific discovery and publication); therefore, disclosure, prior to publication, could reasonably be expected to: "...direct subsequent funding [available from various sources] away from those who create it and reward those who obtain this information and utilize the results in their work which may or

may not credit those who created it."

These representations fall well short of establishing a risk of harm described in paragraph 20(1)(c). As discussed in relation to paragraph 18(c), DFO failed to establish any continued intention to publish and/or ongoing risk of compromising a right of first publication of scientific results. In turn, it cannot establish any direct and clear link between disclosure of the information at issue and a substantial financial loss or gain to Creative Salmon Ltd. arising from the loss of benefits accruing from publication, much less establish that such an injury is reasonably expected and therefore well beyond the merely possible.

Notably, Creative Salmon Ltd. did not allege harms resulting from a loss of the right to publish; it did however maintain that disclosure would result in a harm described in paragraph 20(1)(c).

Like DFO's representations, these representations fall well short of establishing the requirements of paragraph 20(1)(c). Creative Salmon Ltd. failed to offer any explanation, much less any cogent explanation supported by evidence, of how the harm alleged could reasonably be expected to occur to the point of being well beyond the merely possible.

In light of the above, neither DFO nor Creative Salmon Ltd. established that disclosure of the information at issue could reasonably be expected to result in substantial financial loss or gain to Creative Salmon Ltd., or be injurious to its competitive position, so as to fall within the scope of paragraph 20(1)(c). No clear and direct connection between disclosure of the information at issue and a risk of harm that is well beyond the merely possible, was shown. (*Merck Frosst Canada Ltd. v. Canada (Health)*, 2012 SCC 3, paras. 197, 206)

If any of the information could fall within the scope of paragraphs 20(1)(b) or (c), did the institution reasonably exercise its discretion under subsection 20(6)?

Although it was not established that any of the information at issue falls within the scope of paragraphs 20(1)(b) or (c), were this not the case, in my view, DFO would have been required to consider disclosure under subsection 20(6).

During the investigation, DFO dismissed the applicability of this provision, stating:

...none of the information withheld under section 20 could reasonably pertain to public health, safety or the protection of the environment. The information is scientific study of and measurements of jaundice in cultured (farmed) aquaculture salmon in a laboratory. These fish were grown and raised by [the third party], they are not wild salmon, never sold to the public and pose no threat to the environment.

DFO's claim that because the information at issue studies/measures jaundice in farmed salmon, it does not engage matters of public health, public safety or the protection of the

environment, is undermined by previously published scientific studies, including two studies co-authored by the project lead and principal author of the draft Final Project Report and Manuscript. These studies, conclude, *inter alia*, that: migratory Chinook salmon may be at more than a minimal risk of disease from exposure to the high levels of PRV occurring on salmon farms. (see: <https://www.psf.ca/news-media/prv-virus-may-cause-disease-chinook-salmon>); and “PRV-1 is now an important infectious agent in critically endangered wild Pacific salmon populations, fueled by aquacultural transmission.” (see: <https://advances.sciencemag.org/content/7/22/eabe2592>)

Similarly, recent testimony of several witnesses before the Standing Committee on Fisheries and Oceans raised issues regarding the risks of disease and pest transfers from farmed salmon to wild fish. Notably, that Committee recommended, within its June 22, 2021 report entitled, Pacific Salmon: Ensuring the Long-term Health of Wild Populations and Associated Fisheries, that:

...Fisheries and Oceans Canada improve its data transparency practices, including making information available to the public without needing approval from industry and corporate stakeholders.
(<https://www.ourcommons.ca/Content/Committee/432/FOPO/Reports/RP11345845/foporp05/foporp05-e.pdf> at page 19)

Based on the above, had any of the information at issue fell within the scope of paragraph 20(1)(b) or (c), it would have been incumbent on DFO to consider disclosure under subsection 20(6), based on a consideration of all relevant factors.

Result

The complaint is well founded.

Recommendations

I recommend that the Minister of Fisheries and Oceans:

1. Disclose the information at issue in full, with the exception of personal information withheld at the time of DFO’s receipt of the request under subsection 19(1); and
2. Email a copy of the response letter to the Office of the Information Commissioner’s Registrar (Greffre-Registry@oic-ci.gc.ca).

On November 10, 2021, I issued my initial report to the Minister of Fisheries and Oceans setting out my recommendations.

On December 10, 2021, the Director of the Access to Information and Privacy Division of DFO gave me notice that DFO would be implementing my recommendations.

I have provided Creative Salmon Ltd. with this report. Institutions must abide by the terms of subsection 37(4) when disclosing any records in response to my recommendations.

Section 41 of the Act provides a right to any person, excepting institutions, who receives this report to apply to the Federal Court for a review. Complainants must apply for this review within 35 business days after the date of this report. When they do not, third parties may apply for a review within the next 10 business days. The person who applies for a review must serve a copy of the application for review to the relevant parties, as per [section 43](#).



Caroline Maynard
Information Commissioner of Canada



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PROTECTED A

Your file - Votre référence

Our file - Notre référence

A-2017-01222 / DSP

March 18, 2022

Mr. Tony Allard
c/o Ms. Katherine Bellett
MLT Aikens LLP
Suite 1800, 335 Burrard Street
Vancouver BC V6C 2G8

(By epost: KBellett@mltaikins.com)

Dear Mr. Allard:

As a result of your complaint to the Office of the Information Commissioner of Canada, we have completed a subsequent review of the documents originally processed in response to the request you submitted under the Access to Information Act for:

- 1. For the period of November 1, 2017 to January 1, 2018, all information and records under the control of Fisheries & Oceans Canada consisting of any communication between any of the following parties: (a) Dr. Kristi Miller (or Dr. Kristi Miller-Saunders), Research Scientist, Fisheries & Oceans Canada; (b) Carmel Lowe, Regional Director, Science, Fisheries & Oceans Canada; (c) Wayne Moore, Director General, Fisheries & Oceans Canada; (d) Nathan Taylor, Division Manager, Fisheries & Oceans Canada; and (e) Brian Riddell, Pacific Salmon Foundation, related to any of the following topics: (i) piscine reovirus (or PRV); (ii) heart and skeletal muscle inflammation (or HSMI); (iii) the Creative Salmon Company, Ltd.; or (iv) jaundice or jaundice syndrome.**

- 2. For the period of November 1, 2017 to January 1, 2018, all information and records under the control of Fisheries & Oceans Canada consisting of any communication to or from Jay Parsons, Director, Aquaculture Science, Ecosystems Aquaculture Management, Fisheries & Oceans Canada related to the topics of (a) Creative Salmon Company, Ltd.; or (b) jaundice or jaundice syndrome.”**

Fisheries and Oceans Canada has subsequently removed its reliance on paragraphs 18(c) and 20(1)(b) & (c) of the Act and is releasing the information originally withheld under these sections to you. Please find enclosed a complete copy of the final release package.

Please be advised that the original exemption provisions subsection 14(a), paragraph 16(2)(c), subsection 19(1), paragraphs 21(1)(a), 21(1)(b) and section 23 of the Act invoked in our original response are still applicable. Material excluded pursuant to subsection 68(a) continues to be withheld as well. A copy of the relevant sections is attached.

.../2

Canada

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Please be advised that you are entitled to submit a complaint to the Information Commissioner of Canada concerning the processing of your request within sixty (60) days of receipt of this notice. In the event you decide to avail yourself of this right, your notice of complaint can be submitted online at - <https://www.oic-ci.gc.ca/en/submitting-complaint>, or by mail to:

Office of the Information Commissioner of Canada
30 Victoria Street
Gatineau, QC K1A 1H3

Should you have any questions, please contact Dave St-Pierre at 613-804-6075 or dave.st-pierre@dfo-mpo.gc.ca.

Yours sincerely,

César Kagame
Director
Access to Information and Privacy Secretariat

Enclosures:

1. Access to Information Act: Applicable exemption/exclusion provisions
2. Final release package: pages 1 to 1970

c.c. Office of the Information Commissioner of Canada

Access to Information Act

14(a) FEDERAL-PROVINCIAL CONSULTATIONS OR DELIBERATIONS

14. The head of a government institution may refuse to disclose any record requested under this Act that contains information the disclosure of which could reasonably be expected to be injurious to the conduct by the Government of Canada of federal-provincial affairs, including, without restricting the generality of the foregoing, any such information on federal-provincial consultations or deliberations.

16(2)(c) METHODS EMPLOYED TO PROTECT BUILDINGS, STRUCTURES OR SYSTEMS

16. (2) The head of a government institution may refuse to disclose any record requested under this Act that contains information that could reasonably be expected to facilitate the commission of an offence, including, without restricting the generality of the foregoing, any such information on the vulnerability of particular buildings or other structures or systems, including computer or communication systems, or methods employed to protect such buildings or other structures or systems.

19(1) PERSONAL INFORMATION

19. (1) Subject to subsection (2), the head of a government institution shall refuse to disclose any record requested under this Act that contains personal information as defined in section 3 of the Privacy Act.

21(1)(a) ADVICE OR RECOMMENDATIONS

21. (1) The head of a government institution may refuse to disclose any record requested under this Act that contains advice or recommendations developed by or for a government institution or a Minister of the Crown.

21(1)(b) CONSULTATIONS OR DELIBERATIONS

21. (1) The head of a government institution may refuse to disclose any record requested under this Act that contains an account of consultations or deliberations involving officers or employees of a government institution, a minister of the Crown or the staff of a minister of the Crown.

23 SOLICITOR-CLIENT PRIVILEGE INFORMATION

23. The head of a government institution may refuse to disclose any record requested under this Act that contains information that is subject to solicitor-client privilege.

68(a) PUBLISHED MATERIAL

68. This Act does not apply to published material or material available for purchase by the public.

Supplemental Table 1. TaqMan assay references for infectious agents assessed on the Fluidigm BioMark™ HD platform. See Miller et al. 2015 for full references and TaqMan assay details.

Infectious Agent	Type	Assay Abbreviation	Assay Reference
<i>Aeromonas hydrophila</i>	Bacterium	ae_hyd	Lee <i>et al.</i> 2006
<i>Aeromonas salmonicida</i>	Bacterium	ae_sal	modified from Keeling <i>et al.</i> 2013
<i>Candidatus Branchiomonas cysticola</i>	Bacterium	c_b_cys	Mitchell <i>et al.</i> 2013
<i>Flavobacterium psychrophilum</i>	Bacterium	fl_psy	Duesund <i>et al.</i> 2010
<i>Gill chlamydia</i>	Bacterium	sch	Duesund <i>et al.</i> 2010
<i>Piscichlamydia salmonis</i>	Bacterium	pch_sal	Nylund <i>et al.</i> 2008
<i>Piscirickettsia salmonis</i>	Bacterium	pisck_sal	Corbeil <i>et al.</i> 2003
<i>Renibacterium salmoninarum</i>	Bacterium	re_sal	Powell <i>et al.</i> 2005
<i>Rickettsia-like organism</i>	Bacterium	rlo	Lloyd <i>et al.</i> 2011
<i>Vibrio anguillarum</i>	Bacterium	vi_ang	Miller <i>et al.</i> 2015
<i>Vibrio salmonicida</i>	Bacterium	vi_sal	Miller <i>et al.</i> 2015
<i>Nanophyetus salmincola</i>	Fluke	na_sal	Miller <i>et al.</i> 2015
<i>Ceratomyxa shasta</i>	Parasite	ce_shasta	Hallett and Bartholomew 2006
<i>Cryptobia salmositica</i>	Parasite	cr_sal	Miller <i>et al.</i> 2015
<i>Dermocystidium salmonis</i>	Parasite	de_sal	Miller <i>et al.</i> 2015
<i>Facilispora margolisi</i>	Parasite	fa_mar	Miller <i>et al.</i> 2015
<i>Gyrodactylus salaris</i>	Parasite	gy_sal	Collins <i>et al.</i> 2010
<i>Ichthyophonus hoferi</i>	Parasite	ic_hof	White <i>et al.</i> 2013
<i>Ichthyophthirius multifiliis</i>	Parasite	ic_mul	Miller <i>et al.</i> 2015
<i>Kudoa thysanites</i>	Parasite	ku_thy	Funk <i>et al.</i> 2007
<i>Loma sp.</i>	Parasite	lo_sal	Miller <i>et al.</i> 2015
<i>Myxobolus arcticus</i>	Parasite	my_arc	Miller <i>et al.</i> 2015
<i>Myxobolus cerebralis</i>	Parasite	my_cer	Kelley <i>et al.</i> 2004
<i>Myxobolus insidiosus</i>	Parasite	my_ins	Miller <i>et al.</i> 2015
<i>Neoparamoeba perurans</i>	Parasite	ne_per	Fringuelli <i>et al.</i> 2012
<i>Nucleospora salmonis</i>	Parasite	nu_sal	Foltz <i>et al.</i> 2009
<i>Paranucleospora theridion</i>	Parasite	pa_ther	Nylund <i>et al.</i> 2010
<i>Parvicipula kabatai</i>	Parasite	pa_kab	Miller <i>et al.</i> 2015
<i>Parvicipula minibicornis</i>	Parasite	pa_min	Hallett and Bartholomew 2009
<i>Parvicipula pseudobranchicola</i>	Parasite	pa_pse	Jørgensen <i>et al.</i> 2011
<i>Sphaerothecum destructuens</i>	Parasite	sp_des	Miller <i>et al.</i> 2015
<i>Spironucleus salmonicida</i>	Parasite	sp_sal	Miller <i>et al.</i> 2015
<i>Tetracapsuloides bryosalmonae</i>	Parasite	te_bry	Bettge <i>et al.</i> 2009
Atlantic salmon paramyxovirus	Virus	aspv	Nylund <i>et al.</i> 2008
Infectious hematopoietic necrosis virus	Virus	ihnv	Purcell <i>et al.</i> 2013
Infectious pancreatic necrosis virus	Virus	ipnv	Clouthier <i>et al.</i> 2014
Infectious salmon anemia virus	Virus	Snow7	Snow <i>et al.</i> 2006
Infectious salmon anemia virus	Virus	isav8	LeBlanc <i>et al.</i> 2010
Pacific salmon parvovirus	Virus	pspv	Miller <i>et al.</i> 2015
Piscine myocarditis virus (CMS)	Virus	pmcv1	Wiik-Nielsen <i>et al.</i> 2013
Piscine reovirus (HSMI)	Virus	prv	Wiik-Nielsen <i>et al.</i> 2012
Salmon alphavirus 1, 2, and 3	Virus	sav	Andersen <i>et al.</i> 2007
Salmonid herpesvirus / <i>Oncorhynchus</i>	Virus	omv	Miller <i>et al.</i> 2015
Viral encephalopathy and retinopathy	Virus	ver	Korsnes <i>et al.</i> 2005
Erythrocytic necrosis virus	Virus	env	Purcell <i>et al.</i> 2016
Viral hemorrhagic septicemia virus	Virus	vhs1	Jonstrup <i>et al.</i> 2013
Si:dkey-78d16.1 protein [<i>Danio rerio</i>]	Housekeeping	hkg	Miller <i>et al.</i> 2015

Supplemental Table 2. Stocking information for farms A and B.

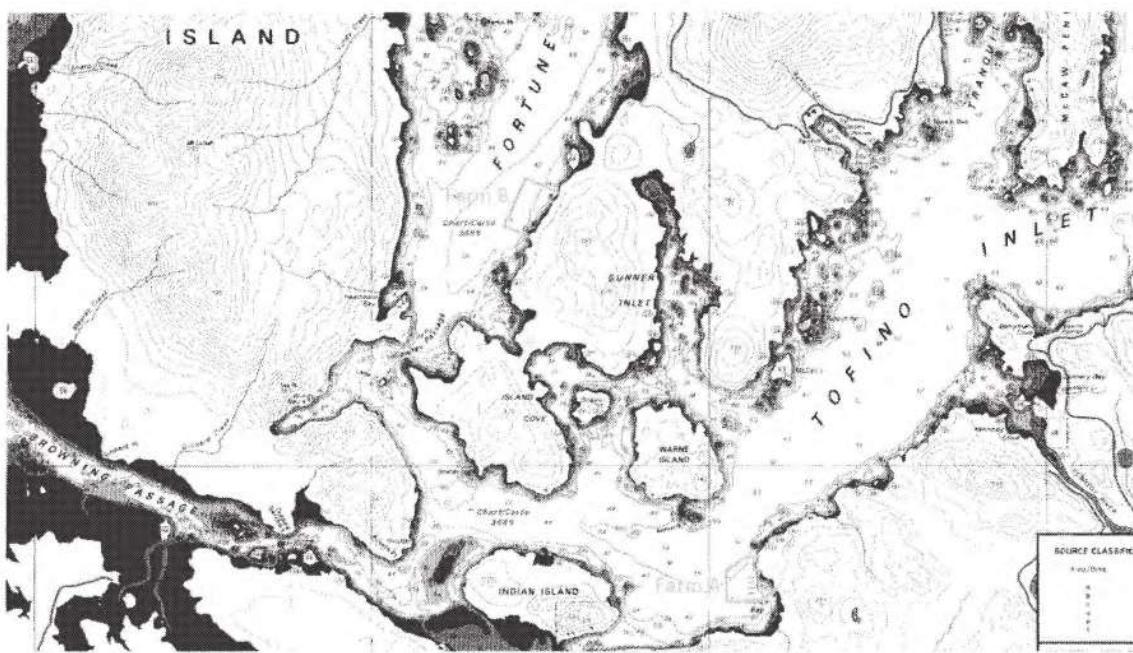
Farm A	Farm B
May, 2004- February 2006	October 2005 - May 2007
May 2008 - January 2010	September 2007 - August 2009
May 2010 - January 2012	September 2009 - October 2011

Supplemental Table 3. Classification of individual fish based on hierarchical cluster analyses driven by t-tests of liver and kidney transcriptional (microarray) data between "healthy" fish (classified as those with no BKD and no anemia or jaundice) versus fish with external signs of jaundice, anemia, or jaundice/anemia combined. Note that fish not scored for anemia were removed from this analysis. While the t-test was restricted to this group, all samples run were clustered based on the significant features resolved. Cluster "A" is the cluster highly loaded with "compromised" fish and cluster "B" with "healthy" controls. Cluster "a" was an intermediate sub-cluster that contained a combination of control, BKD infected, and jaundice/anemia fish; fish in this cluster did not show a strong pattern of segregation between the top up- and down-regulated genes (indicated by the darker coloring in Supplemental Figure 4). Under the "positive" and "negative" columns, the presence of each of the "indicators" scored in the study are shown as follows: J=jaundice, A=anemia, H=histological lesions associated with jaundice syndrome, PRV=piscine reovirus with $C_t < 26$, BKD=bacterial kidney disease. Fish 1069 was questionably BKD positive.

Fish #	Liver Jaundice Only Analysis	Liver Anemia Only Analysis	Liver Jaundice or Anemia	Kidney Jaundice Only Analysis	Kidney Anemia Only Analysis	Kidney Jaundice/Anemia	FINAL SCORE	Positive	Negative	Site
# genes at q<0.05	1736	523	4301	1997	2394	3760				
1002	A	A	a	A	A	a	J-A-H-PRV		A	
1003	A	A	A	A	A	A	J-A-H-PRV		A	
1010	A	A	A	A	A	A	J-A-PRV		A	
1011	A	A	A	ND	ND	ND	J-A-PRV		A	
1050	A	A	A	A	A	A	J-H-PRV		A	
1051	A	A	A	A	A	a	J-H-PRV		A	
1052	A	A	A	A	A	A	J-H-PRV		A	
1053	A	A	A	A	A	A	J-H-PRV		A	
1054	A	A	A	A	A	A	J-H-PRV		A	
1055	A	A	A	A	A	A	J-H-PRV		A	
1056	A	A	A	A	A	a	J-H-PRV		A	
1000	A	A	A	A	A	A	A-H-PRV	J	A	
1005	A	A	A	A	A	A	A-H	J-PRV	A	
1001	B	A	a	A	A	a	Intermediate	A-H	J-PRV	A
1013	A	A	a	A	A	a	Intermediate	BKD	J-A-PRV	A
1012	A	A	a	A	A	a	Intermediate	BKD	J-A-PRV	A
1006	A	A	a	B	A	a	Intermediate	J-A	PRV	B
1004	A	A	a	B	A	a	Intermediate	H-PRV	J-A	A
1068	B	B	B	B	A	B	Intermediate	JA-H-PRV	B	
1060	B	B	B	B	B	B	B	PRV (Kidney)	J-A-H	A
1009	B	B	B	B	B	B	B		J-A-PRV	B
1064	B	B	B	B	B	B	B		JA-H-PRV	A
1007	B	B	B	a	ND	ND	B	J-A-BKD	PRV	B
1008	B	B	B	B	ND	ND	B		J-A-PRV	B
1057	B	B	B	B	B	B	B		JA-H-PRV	A
1058	B	B	B	B	B	B	B		JA-H-PRV	A
1059	B	B	B	B	B	B	B		JA-H-PRV	A
1061	B	B	B	B	B	B	B		JA-H-PRV	A
1062	B	B	B	B	B	B	B		JA-H-PRV	A
1063	B	B	B	B	B	B	B		JA-H-PRV	A
1065	B	B	B	B	B	B	B		JA-H-PRV	A
1066	B	B	B	B	B	B	B		JA-H-PRV	A
1067	B	B	B	B	B	B	B		JA-H-PRV	B
1069	B	B	B	B	B	B	B	BKD	J-A-H-PRV	B
1070	B	B	B	B	B	B	B		J-A-H-PRV	B
1071	B	B	a	B	B	B	B		J-A-H-PRV	B

Supplemental Table 4. P-values of correlations of IJAS-related indicators with Principal Components 1-5 derived from microarray studies in liver and kidney. R indicates rank order of significance of each component (PC1 and PC2) in each tissue.

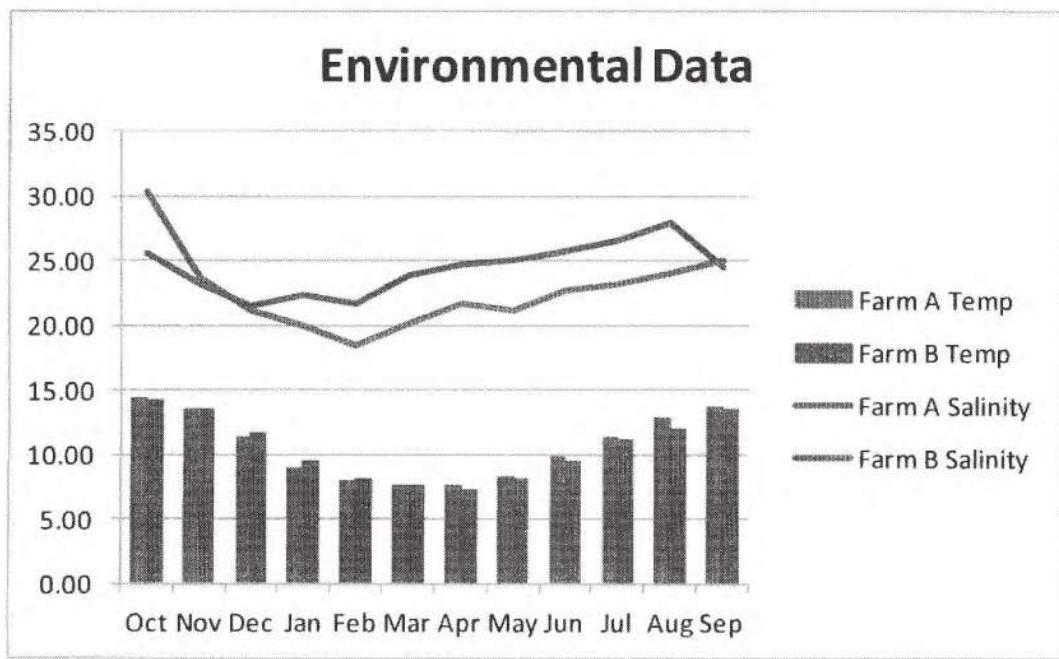
	PC1	R	PC2	R	PC3	PC4	PC5
LIVER							
Percentage Variance	25.2		14.6		7.1	5.2	4.3
Combined Indicator	8.24E-06	1	1.36E-03	4			
Jaundice/Anemia	1.08E-05	2	8.10E-03	5			
Jaundice			2.03E-02				
PRV(<26)	9.46E-04	4	7.14E-04	2			
Summed Histo	1.30E-02	5	3.01E+04	1			
Farm	1.56E-02	6					
SSF			1.20E+03	3			
SCN	7.67E-05	3	2.25E+02	7			
SNM							
HDD			9.17E-03				
MEG			2.22E+02	6		8.11E-05	
KIDNEY							
Percentage Variance	20.2		11.7		7.6	6.1	5.0
Combined Indicator	6.09E-03	1	4.39E-06	1			
Jaundice/Anemia			1.13E-03	5			
Jaundice			4.75E-05	3			
PRV(<26)			2.37E-06	2			
Summed Histo	4.36E-02	2	4.21E-04	4			
Farm			3.40E-02				
ICN							
ISH			1.61E-02				
IFB							
RTN	1.40E-02	3	1.47E-02				
MGN							



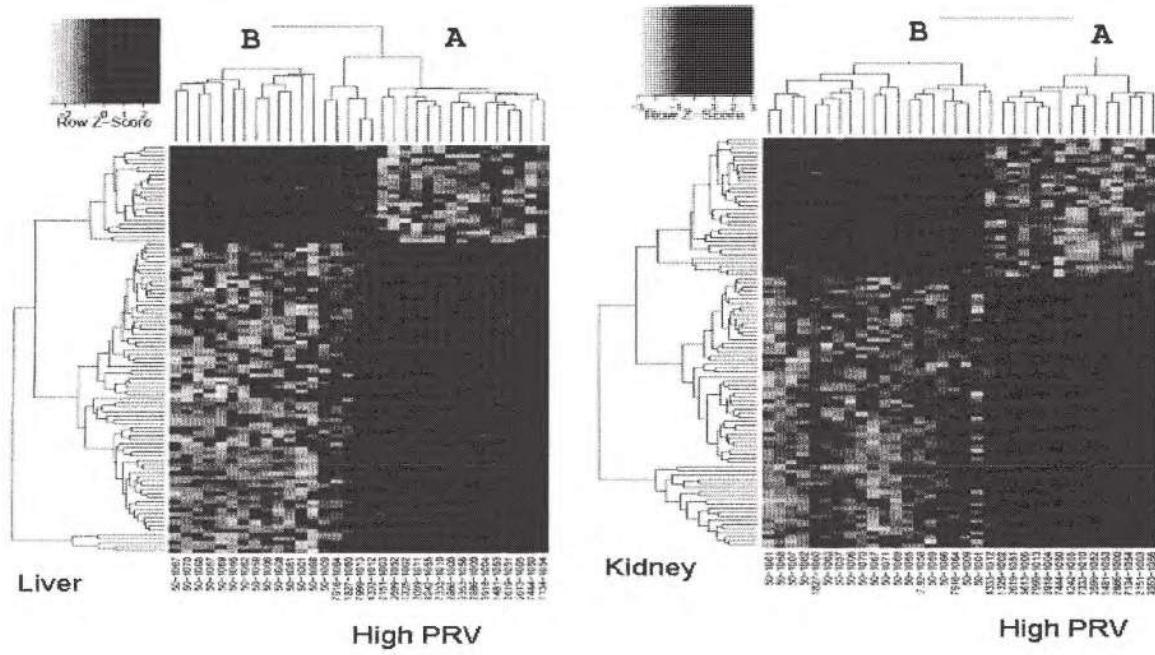
Supplemental Figure 1. Location of farm sites. Farm A (Indian) showed was located at the mouth of Tofino Inlet and showed the highest incidence of Jaundice whereas Farm B (Dawley) showed only a low incidence of Jaundice.

Pen #2 January 2011	Pen #4 November 2010	Pen #6 November 2010	Pen #8 January 2011
6	2	2	6
Pen #1 October 2010	Pen #3 January 2011	Pen #5 December 2010	Pen #7 December 2010
1	6	4	4

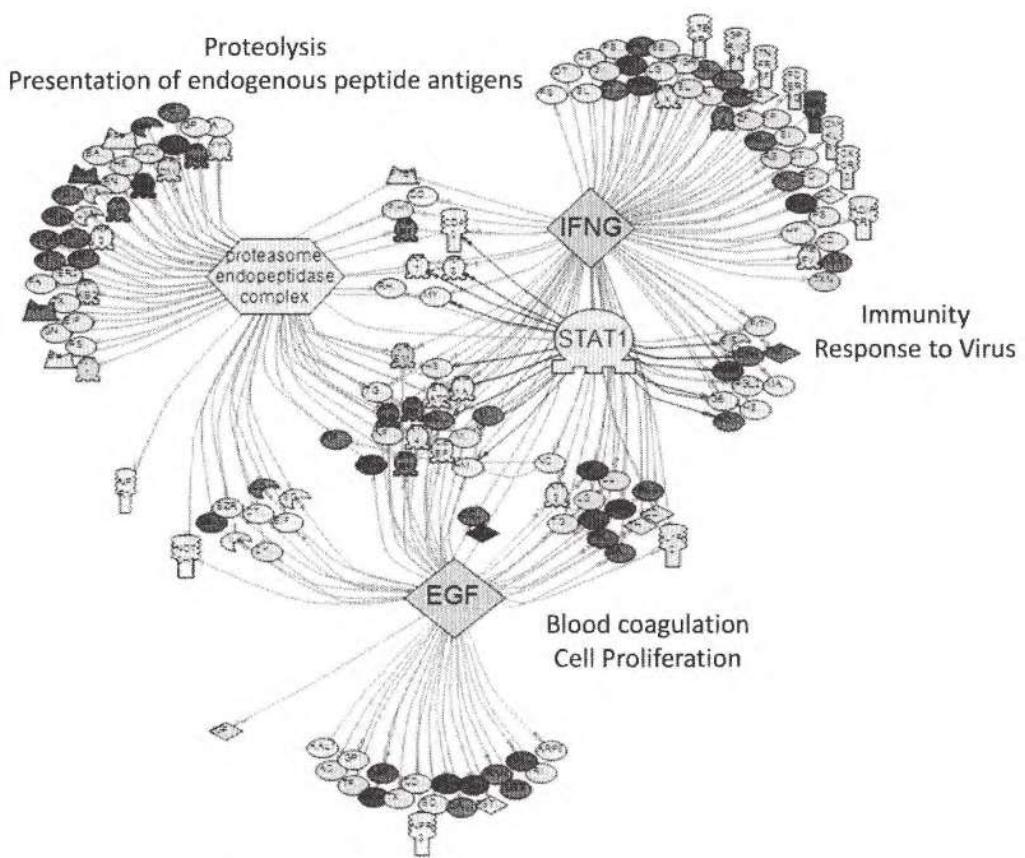
Supplemental Figure 2. The farm cage system at Farm A. Pens 1 and 2 were closest to land. The dates and large numerals list the month, year, and order when IJAS was first observed in the pens.



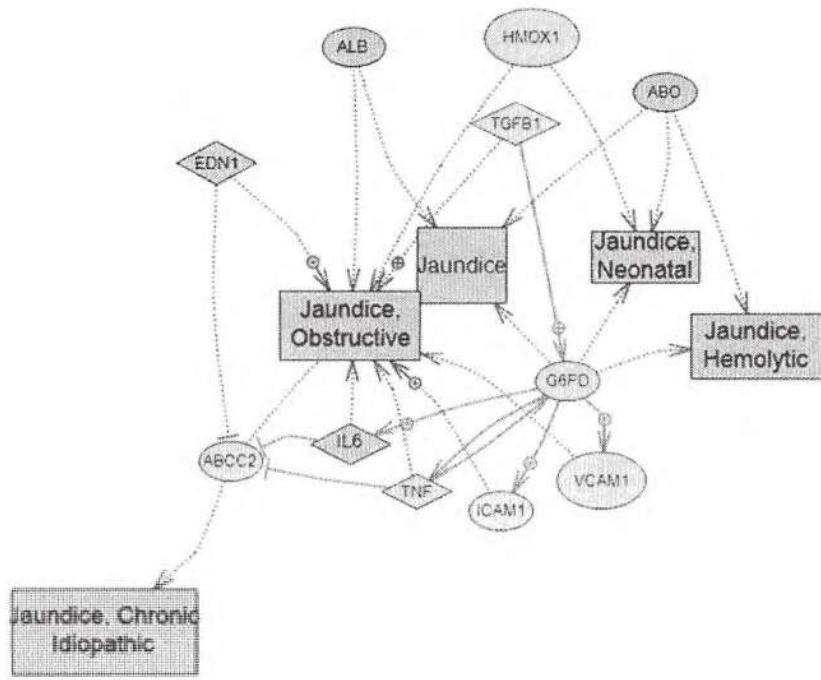
Supplemental Figure 3. Mean water Temperature and Salinity for Farm A and B
(May 2010 - Sept 2011) at 6 m.



Supplemental Figure 4. Hierarchical clustering of liver (left) and kidney (right) samples based upon gene-lists significantly correlated with PRV loads (5% FDR). The top 100 ranked gene features were used in the cluster plots. Fish with Ct<26 (relatively high PRV loads) generally clustered in "A" while those with no or relatively low PRV loads generally clustered in "B"; fish 1005 was a notable exception and clustered within "A" for both tissues but did not carry a high PRV load. Fish 1013, a BKD suspect fish (from gross examination), clustered in "A" for kidney and between "A" and "B" for liver. Genes are shown on the vertical axis, with yellow denoting genes down-regulated, blue up-regulated. Individual fish are clustered on the horizontal axis. This figure shows the results for all samples run on microarrays (not just histology samples).



Supplemental Figure 5. Schematic showing top transcriptional regulators (highlighted in yellow) of genes in liver tissue significantly ($q < 0.001$) associated with jaundice syndrome; analysis is based on "enriched subnetworks" of jaundice/anemia dataset in PathwayStudio. Red indicates up-regulated genes, blue down-regulated genes; stronger coloration represents higher fold-change difference. Top regulators as identified in Pathway Studio in grey were not on the array.



Supplemental Figure 6. Schematic showing activation of genes associated with jaundice in mammals. Genes up-regulated in jaundice/anemia-positive liver tissue (based on combined indicator dataset) are in red. Genes in grey were not on the array. Analysis from PathwayStudio and MedScan.

1
2 Histopathology and genomic characterization of idiopathic
3 jaundice and anemia syndrome in cultured Chinook salmon
4 (*Oncorhynchus tshawytscha*)

5
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Comment [D1]: to revise
with current address

32
33 **Keywords:** jaundice syndrome, IJAS, anemia, salmon, aquaculture, piscine orthoreovirus,
34 microarray, gene expression profiling, anti-viral

35 **Abstract**

36
37 A study linking functional genomics, qRT-PCR detection of microbes, histopathology,
38 veterinary diagnostics and epidemiology was undertaken to explore possible origins of an
39 idiopathic jaundice and anemia syndrome (IJAS) observed concomitantly with steady
40 low-level over-winter mortality of Chinook salmon (*Oncorhynchus tshawytscha*) at two
41 ocean farms in British Columbia, Canada. IJAS was predominantly diagnosed through
42 clinical presentation--yellowing of the skin, pale gills (anemia), and pale livers, with onset
43 of cases in the fall when both water temperature and salinity were declining. Over the
44 past decade, the farm site with the lower salinity profile and lower flushing rate had a
45 higher prevalence of IJAS than the higher salinity farm site, implicating environmental
46 factors. Significant microscopic lesions include renal tubular epithelial cell hydropic
47 degeneration and necrosis, hepatocellular hydropic degeneration and single cell necrosis,
48 and splenic parenchymal fibrin. Quantitative RT-PCR assays for 46 infectious agents
49 revealed a statistically significant association of the piscine orthoreovirus (PRV) with IJAS,
50 and no additional viruses were revealed by next generation sequencing (NGS). Salmon
51 microarrays (cGRASP 44K Agilent arrays) contrasting gene expression in liver and kidney
52 of fish with and without evidence of jaundice revealed a genomic signature comprised of
53 thousands of genes in each tissue. Microarray analyses driven by the clinical presence of
54 IJAS, combined microscopic lesions associated with IJAS, PRV load, and a metric
55 combining all three 'indicators' revealed highly congruent signatures that together
56 explained up to 40% of the overall variation in gene expression in unsupervised analyses.
57 Functionally, the strongest effects were on immune response—including induction of
58 interferon-related pathways and “response to virus”, proteolysis, metabolism, apoptosis
59 and cell cycle. Combined, we hypothesize that IJAS is virally-mediated, possibly with the
60 involvement (direct or indirect) of PRV.

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66 **Introduction**

67
68 Chinook salmon farmed in the Tofino Inlet region of Vancouver Island on the
69 western coast of Canada have suffered low levels of mortality during the fall through
70 winter associated with a unique clinical presentation, hereafter called idiopathic jaundice
71 and anemia syndrome (IJAS). External signs include mild to severe yellow discolouration
72 of the skin, most evident on the abdomen and around the eyes, and very pale gills
73 indicating anemia. Affected fish are usually in good body condition. Internal signs include
74 pale livers and empty stomachs indicating reduced feeding. Other organs have no gross
75 lesions. The clinical presentation is very different from marine anemia syndrome,
76 another Chinook salmon disease, which typically presents with splenomegaly,
77 renomegaly, and anemia without jaundice (Kent and Poppe 1998).

78 The cause of IJAS is not known. Hypotheses include a pathogen, exposure to a
79 negative environmental influence, possibly an undefined toxin, or some combination of
80 variables. Traditional diagnostic tests have not identified a causative pathogen. Tests
81 including classical bacterial culture, viral cell culture, PCR, blood assessment and
82 histopathology have yielded negative results for pathogens known to occur in BC salmon,
83 including *Renibacterium salmoninarum* (the cause of bacterial kidney disease [BKD]),
84 *Vibrio* sp (the cause of vibriosis), *Nucleospora salmonis* (the cause of marine anemia),
85 viral hemorrhagic septicemia virus (VHSV), infectious haematopoietic necrosis virus
86 (IHNV), infectious salmon anemia virus (ISAV), erythrocytic necrosis virus (ENV), and
87 *Loma salmonae*.

88 Little is known of the epidemiology of the condition. It affects fish that have been
89 in sea water for more than 6 months and therefore is not considered to be related to
90 smolt quality. There appears to be a seasonal pattern to this condition with clinical signs
91 and mortalities primarily in late fall/early winter (December), spiking in the winter and
92 decreasing by early summer. Fish affected by this condition are primarily, but not
93 exclusively, localized to farm sites where freshwater influences are strong, suggestive of
94 an environmental influence. Among farms operated by Creative Salmon in British
95 Columbia (BC), Canada, IJAS occurs in most of the generations stocked at the freshwater-

96 influenced farm even though the company operates single year class sites with a fallow
97 period before re-stocking. Typically one or two pens of fish are more severely affected,
98 but the condition usually occurs in several of the pens. The mortality levels in the most
99 heavily affected pens typically are several fold greater than in other pens on the farm.
100 For example in January 2011, the single affected pen of an eight-pen site
101 disproportionately made up 35% of all the mortalities. Of the dead fish examined from
102 this single pen, over 77% of the fish had IJAS. Total mortality attributed to this syndrome
103 has not been fully assessed although at certain times of the year it can be a significant
104 cause of mortality.

105 Currently no tools are available to manage the problem. Herein, in addition to use
106 of traditional veterinary diagnostics, epidemiology, and histopathology, we undertook a
107 transcriptome study to explore the molecular underpinnings of the syndrome and
108 applied a high throughput, quantitative microfluidics PCR platform to assess the
109 association between the syndrome and dozens of infectious agents associated salmon
110 diseases worldwide. The ultimate goal was to move closer towards identifying the cause
111 of jaundice that will enable the farms to track, predict, and/or mitigate this syndrome.

112
113

114 **Methods**

115

116 **Collections**

117 Collections were made from two farm sites (A and B) located on the west coast of
118 Vancouver Island. Farm A had the greatest mortality associated with IJAS (Supplemental
119 Fig. 1). Farms were stocked with Chinook salmon (Big Qualicum and Robertson Hatchery
120 stocks) and were sampled over a period of four months when clinical signs of jaundice
121 syndrome, later identified as IJAS, were prevalent on farm A. At the time of collection, all
122 fish were subject to gross necropsy to identify clinical signs of jaundice, as well as other
123 potential diseases. Moribund or recently dead fish on farm A were collected by divers,
124 and "healthy" swimming reference fish were collected from net pens using hook and line
125 or during harvest in farms A and B. Live fish were killed by a sharp blow to the head.

126 Tissue samples from all fish were preserved in 10% neutral buffered formalin for
127 histopathology and in RNAlater for molecular analysis. RNAlater preserved samples were
128 kept at 4 °C for 24 hours, then transferred to -80 °C until use.

129 Samples from 36 fish were collected over the course of the study (Table 1). At
130 farm A, where jaundice accounted for approximately 1% cumulative mortality, fish were
131 collected across three dates: Feb 7, April 27, and May 24, 2011; collections included 11
132 dying/freshly dead fish with external signs of jaundice and 3 with signs of anemia but not
133 jaundice, and 13 reference fish with no signs of jaundice or anemia (although two had
134 BKD). At farm B, 2 live fish with signs of jaundice and anemia were sampled (diagnosed
135 by a different veterinarian than on farm A) and 7 reference fish were collected on April
136 27 and May 24.

137 Mortality and environmental data were examined from both sites to describe the
138 epidemiological patterns of IJAS.

139

140 Histopathology

141 Formalin-preserved tissue samples were embedded in paraffin, sectioned at 3
142 µm, and stained with haematoxylin and eosin (H&E). Microscopic features were
143 categorized and scored as none (0), mild/small amounts (1), moderate (2), or
144 severe/abundant (3) as previously described (Marty, Heintz & Hinton 1997; Marty &
145 Heintz 2010). Optimization of photomicrograph illumination and colour balance used
146 published methods (Marty 2007). Samples were processed blinded by a single
147 pathologist (GDM).

148 Histopathology was performed on samples collected Feb 7 and May 24.

149 Inadequate preservation of samples collected on April 27, 2011 (6 healthy and 2 jaundice
150 syndrome fish) precluded their analysis by histopathology. Hence, histopathology was
151 limited to 15 healthy and 13 freshly dead (less than 12 hours)/sick fish.

152

153 Molecular Analyses

154 *RNA extraction, cDNA hybridization, and microarray experimental design*

155 Total RNA was extracted from RNAlater-preserved samples of liver, kidney, heart,
156 spleen and gill tissues. The functional genomics study was performed on liver and kidney,
157 previously identified by histological analyses as having the highest level of cell damage in
158 IJAS fish. The cGRASP (<http://web.uvic.ca/grasp/>) salmonid 44K gene oligonucleotide
159 array (Agilent, Santa Clara, CA) was applied to identify genes that were differentially
160 expressed in correlation with IJAS. The array is comprised of approximately 22,000 60-
161 mer oligonucleotides that are 95% conserved between rainbow trout (*Oncorhynchus*
162 *mykiss*) and Atlantic salmon (*Salmo salar L.*), plus an additional 14,866 Atlantic salmon
163 and 5,661 rainbow trout contiguous sequence assemblies, resulting in a microarray with
164 large transcript representation and very low redundancy.

165 For RNA extraction, tissues were homogenized with stainless steel beads in 600 µl
166 of TRI-reagent (Ambion, Austin, TX) on a MM301 mixer mill (Retsch Inc., Newtown, PA) in
167 a 96 tube format. 300 µl of each homogenates was placed in a well on a 96-well plate,
168 diluted in half with additional TRI-reagent, 75 µl of BCP was added and mixed via
169 vigorous shaking, incubated for 15 minutes and centrifuged. 100 µl aliquots of the top
170 aqueous layer were pipetted into Greiner (supplier) U-bottom 96-well plates and
171 extracted using Magmax™-96 for Microarrays Kits (Ambion, Austin, TX) with a Biomek
172 NXP (Beckman-Coulter, Mississauga, ON) automated liquid-handling instrument using
173 the manufacturer's "No-Spin Procedure". RNA yield and purity were determined by
174 measuring the A₂₆₀ and A₂₆₀/A₂₈₀ ratio of the eluate on the Beckman-Coulter DTX 880
175 spectrophotometer. RNA concentration was normalized to 300 ng/µl and stored at -80
176 °C.

177 Amplification and labeling steps were performed simultaneously in a single 96-
178 well plate. Aliquots of 1 µg total RNA were amplified using the Amino Allyl
179 MessageAmp™II-96 kit (Ambion, Austin, TX) according to manufacturer's instructions.
180 Dye coupling reaction was performed using 5 µg of amino-allyl aaRNA (cRNA) and Alexa
181 dyes (Invitrogen, Carlsbad, CA) where experimental samples were fluorescently tagged
182 with Alexa 555 and the pooled reference labeled with Alexa 647, as per manufacturer's
183 instructions (Ambion, Austin, TX.). Fluorescently labeled experimental and reference

184 samples were assessed for quantity and efficiency of dye incorporation via
185 spectrophotometry on a Nano-Drop ND-1000 spectrophotometer (Agilent, Santa Clara,
186 CA). Prior to array hybridization, 825 ng of Alexa555-labelled experimental sample and a
187 825 ng aliquot of the Alexa647-labelled pooled reference sample were fragmented using
188 25X fragmentation buffer in the presence of 10X blocking agent (Agilent, Santa Clara, CA)
189 for 30 minutes at 60 °C. Immediately prior to hybridization, fragmented cRNA
190 sample:reference mixture was diluted in half with 2X hybridization buffer HI-RPM
191 (Agilent, Santa Clara, CA).

192 Microarrays were run on liver and kidney samples from all fish sampled in the
193 project, although a few samples did not pass microarray quality assessments and were
194 removed for one or both tissues. Thirty-five liver and thirty-six kidney samples were
195 competitively hybridized on microarrays with a reference control made by combining
196 aliquots of cRNA from all experimental liver and kidney tissues samples. The reference
197 control is required to normalize variance in concentration of probes on the array, as well
198 as array to array variability and is thus not meant to represent an experimental sample
199 (i.e. this is different from the “reference” fish that do not show signs of IJAS which were
200 also run individually on arrays and contrasted with IJAS positive fish). Fragmented cRNA
201 in 2X HI-RPM buffer was loaded onto each 4x44k Agilent array using quad hybridization
202 chambers in the Tecan-HS4800 Pro Hybridization Station (Tecan Trading AG,
203 Switzerland). Slides were processed as follows: initial wash step (aCGH prehybridization
204 buffer) 1 min at 65 °C; sample injection at 63 °C with agitation; hybridization for 17 h at
205 63 °C at high viscosity agitation mode; two washes (GE wash 1 with 0.005% Triton-X102)
206 of 1 min at 23 °C with a 1 min soak time between washes; two high stringency washes of
207 1 min at 37 °C with a 1 min soak time (GE wash 2 with 0.005% Triton-X102 and 0.01%
208 surfactant); followed by slide drying at 30 °C for 2 min.

209 *Signal detection, normalization and statistical testing*

210 Slides were scanned at 647 and 555 nm using the Tecan LS Reloaded scanner
211 (TecanTrading AG, Switzerland) using the Automated Gain Control for laser intensity

212 adjustments and the Array-Pro Analyzer software according to manufacturer's
213 instructions. Images were quantified using Imagene software (BioDiscovery, El Segundo,
214 CA, www.biodescovery.com) and spots with poor quality or no signal (<2 standard
215 deviations from background) at both wavelengths were flagged.

216 Expression data were managed using a local installation of BASE [19822003]
217 which was customized slightly to support Imagene two-file formatting. Each slide was
218 normalized in BASE using the print-tip LOESS method.

219 The number of missing values, mean signal-to-noise (SNR) log-ratio and quality
220 metrics from arrayQualityMetrics [19106121] and arrayQuality [19544454] in
221 Bioconductor were used to assess slide quality (www.bioconductor.org; Gentleman
222 2004; Kauffmann *et al.* 2009). Slides were removed from further experimental analysis if
223 two or more plots were flagged on the arrayQualityMetrics report after data
224 normalization, if more than 40% missing spots were identified by the SNR and missing
225 spots report, if there were more than 30% missing spots and an experimentally low SNR
226 value as identified by the SNR and missing spots report, or if the slide had a lack of spatial
227 uniformity as identified by the plots from the arrayQuality package and substantiated by
228 the spatial plots score of the raw microarray data in the arrayQualityMetrics report.

229 Data for each retained slide were further processed to remove poor-quality spots.
230 Flagged spots were treated as missing, as were spots with a SNR < 2. In each dataset
231 (liver and kidney), in a set of slides considered a single data set, probes with more than
232 50% missing values were removed. For procedures such as principal components analysis
233 (PCA) and hierarchical clustering that require matrices without missing values, missing
234 data were imputed using an average of the existing probe intensities. Missing values
235 were unmodified for ANOVAs and T-tests.

236 All data were \log_2 transformed and an intensity ratio was computed by taking the
237 differences in log transformed intensities between the experimental sample and
238 reference sample. Log-transformed intensity ratios were used in all statistical analyses
239 performed using R: A language and environment for statistical computing (Development
240 Core Team 2011). The data were analyzed using supervised (t-tests and ANOVAs) and

241 unsupervised PCA approaches. Multiple comparisons were controlled for using the
242 Benjamini and Hochberg False Discovery Rate (FDR) procedure (Benjamini and Hochberg
243 1995) implemented in the p.adjust function within the stats library in R. Features were
244 identified as being differentially expressed if their p-values were significant at the 5% FDR
245 level.

246 Veterinary diagnostics based on external fish appearance noted two common
247 observations among the dying fish at farm A, jaundice and anemia. Microarray data were
248 explored to test the hypothesis that jaundice and anemia were not part of the same
249 physiological phenomenon – i.e. anemia was independent of jaundice. If this were the
250 case, we would expect that unique genomic signatures would be resolved when jaundice
251 and anemia were independently assessed relative to “healthy” control fish, and that
252 when assessed together as “diseased” fish relative to “healthy” controls, the genomic
253 signature would be weakened.

254 Fish with external signs of 1) jaundice, 2) anemia, and 3) jaundice and/or anemia
255 were contrasted with “healthy controls” via T-tests performed separately for each of
256 liver and kidney tissues. Healthy controls were fish with no signs of BKD and that did not
257 carry anemia alone (for the jaundice analysis) (Table 1). No fish were characterized as
258 only showing jaundice, but a number of fish were not assessed for anemia and those fish
259 were excluded from the anemia analysis (Table 1).

260 Clinical signs of IJAS, histological lesions associated with IJAS, and high loads of
261 piscine orthoreovirus (PRV) were used to drive supervised analyses of the data while PCA
262 followed by correlation analyses was conducted to identify the percentage of the
263 variation in the data accounted for by indices of IJAS and viral infection. Functional
264 analyses were performed using Pathway Studio version 9.0 (Ariadne Genomics; Nikitin et
265 al. 2003).

266 *Survey of Infectious Agents*

267 The Molecular Genetics Laboratory at the Pacific Biological Station has been
268 developing a novel high throughput approach to microbe surveillance based on TaqMan

269 assays applied on a microfluidics platform (Fluidigm BioMark™ Real-Time PCR System;
270 Fluidigm corp., San Francisco, CA). This platform is capable of simultaneously assessing
271 96 assays across 96 samples (96.96 dynamic array) or 48 assays across 48 samples (48.48
272 dynamic array). A complete analytical validation has been performed on 45 salmon
273 microbe assays applied on the BioMark platform (Miller et al. 2015). All assays performed
274 to high standards, showing high sensitivity (generally <10 copies per well are detectable),
275 and specificity and repeatability similar to other quantitative PCR platforms.

276 We employed the BioMark platform to assess the presence and load of 46
277 infectious agents (viruses, bacteria, fungal and protozoan parasites) expected or known
278 to associate with diseases in salmon worldwide (Supplemental Table 1), performed using
279 previously described methods (Miller et al. 2014 and 2016). The quantification cycle (Cq,
280 also known as the threshold cycle or C_t) was determined using Fluidigm Real-Time PCR
281 Analysis software 3.0.2 (Fluidigm). Controls included no-template negatives, a reference
282 pool containing a mixture of all samples run across multiple dynamic arrays, serial
283 dilutions of artificial construct positive controls, and a housekeeping gene (78d16.1) that
284 has been highly stable across multiple microarray studies (KM unpublished data). The
285 results were exported as csv files to GenEx (www.multid.se) for data preparation and
286 statistical analysis. Data from multiple dynamic arrays were combined within GenEx and
287 the average of the duplicated samples calculated. Samples amplifying products from only
288 one or two triplicate microbe assays were treated as negative.

289 T-tests were performed to determine associations of genes and infectious agents
290 with IJAS-positive fish. Results from the single infectious agent assay that was strongly
291 correlated with IJAS were subsequently verified in duplicate for the full set of tissues
292 (kidney, gill, spleen and heart) on the ABI 7900 platform using standard qPCR methods
293 (Evans et al. 2012) and through next-generation sequencing on an Illumina HiSeq™
294 platform, which yielded a full genome sequence of the infectious agent across multiple
295 samples.

296 *Sequence Validation of Piscine OrthoReovirus*

297 Three samples with high loads of PRV were selected for high throughput
298 sequencing (HTS) of RNA to resolve the full genome sequence of PRV. Ribosomal RNA
299 was removed from total RNA using the RiboMinus Invitrogen Eukaryote kit for RNA (Life
300 Technologies, Carlsbad, CA). The RNA-Seq library was prepared using the NEBNext Ultra
301 RNA Library prep kit (New England BioLabs, Ipswich, MA) with an average fragment size
302 of 250 bp, and was paired-end sequenced with 100 bp reads on the Illumina HiSeq
303 analyzer (Illumina, San Diego, CA).

304 Sequence analysis was performed using the Partek Flow software (Partek Inc. St.
305 Louis, MO, USA). Adaptors and bases with Phred quality scores <30 were trimmed from
306 both ends and reads less than 25bp were removed. The remaining reads were aligned to
307 the PRV genome segments of the Norwegian isolate Salmo/GP-2010/NOR (Palacios et al.
308 2010) using the BWA-mem aligner with default parameters. Finally, consensus sequences
309 were generated utilizing variant callers (FreeBayes and SamTools), chromosome
310 visualizations through reference alignments and Sequencher 5.1 software (Gene Codes
311 Corporation, Ann Arbor, MI). The consensus sequences were blasted against all
312 available PRV sequences in Genbank (*Nucleic Acids Research*, 2013 Jan;41(D1):D36-42) to
313 identify their closest matches and mismatches across each segment.

314

315 **Results**

316 Epidemiological Analysis of IJAS

318 Annually during February from 2004 through 2012, IJAS occurred at both farms,
319 but it was more common at farm A than farm B (Supplemental Fig. 1). Over eight years
320 both farms raised three generations of Chinook salmon (Supplemental Table 2). Farm A
321 was stocked with smolts in the spring, whereas farm B was stocked with smolts in the
322 fall.

323 IJAS is fairly easily diagnosed by characteristic external signs (Fig. 1). Chinook
324 salmon with clinical signs of IJAS are commonly referred to as yellow fish due to external
325 yellow coloration of the abdominal and periorbital region (Fig. 1a). Most often, the gills

326 are very pale, indicating severe anemia (Fig. 1b), and the fish have no food in the
327 stomach. The viscera have no signs of hemorrhaging or other abnormalities, although in
328 some cases, the liver is yellow (Fig. 1b-c). Typically, neither slow swimmers nor increased
329 morbidity are observed with this syndrome.

330 Mortalities attributed to this syndrome appear to have a seasonal pattern. In the
331 2010-2012 generation at farm A, monthly mortalities with clinical signs of IJAS first
332 occurred in the fall (October), eight months after seawater entry (Fig. 2). Signs were first
333 seen in single pen (Pen 1, the index pen) and within three months all the pens had
334 mortalities with signs of IJAS (Supplemental Fig. 2). Mortality associated with IJAS peaked
335 4-5 months after the first detection of IJAS on the farm. Mortality associated with IJAS
336 tended to decrease in the summer, with very few to no fish having clinical signs in
337 August/September. Fish from 3 pens (2, 7 and 8) presented with clinical signs again in
338 November 2011, however prevalence was low (<0.1%). The staggered pattern in the
339 peak monthly prevalence suggests possible pen-to-pen transmission of an infectious
340 agent.

341 Progression through the system was not linear: pens 2 and 3 were the last to
342 develop clinical disease even though they were adjacent to the index pen (pen 1).
343 Therefore, risk factors other than proximity to an affected population might be
344 important. For farm A (2010-12 generation), IJAS-associated monthly mortality was
345 highly variable among the pens, with fish in Pen 6 having almost 20 times higher
346 cumulative mortality than fish in Pen 1 (3.4% vs. 0.02%) (Fig. 2). Over the time that farm
347 A had fish, the index pen had the lowest cumulative mortality associated with IJAS. Pen 6
348 at farm A differed from the other pens at farms A and B in that it was a mixed stock (Big
349 Qualicum River and Robertson Creek cross) as well as mixed-sex with up to 25% males.
350 All other pens were stocked with all female Big Qualicum strain.

351 For the three generations we studied, the proportion of fish that died with signs
352 of IJAS was about 1% at farm A and about 0.1% at farm B. Prevalence of IJAS at farm A
353 cannot be determined before February 2005 because dead fish with signs of jaundice
354 syndrome were included in an 'other' category. As a consequence, total cumulative

355 mortalities attributed to this syndrome based on the records for the 2004-2005
356 generation was only 0.26%; however farm biologists (M. Tchipeff, personal
357 communication) suggest that the levels were likely closer to 0.70%. In 2007-2009
358 cumulative mortality due to IJAS on farm A was 1.0%, and from 2010-2011 it was 1.2%. .
359 Though total cumulative mortality due to IJAS was quite low, during some months this
360 syndrome was the most common cause of death noted in a pen. In the low prevalence
361 site (farm B), cumulative prevalence for the 3 generations was 0.03%, 0.02% and 0.10%
362 respectively.

363 Water flows and the degree of freshwater influence differed between the two
364 farms: Farm B has considerably greater water flows than farm A, and although water
365 temperatures do not differ between farm A and B, farm A has a 2 ppt. average lower
366 salinity than farm B (Supplemental Fig. 3).

367

368 Histopathology

369 Among the 28 fish examined by histopathology, 13 had evidence of IJAS: nine had
370 jaundice and anemia, three had anemia without jaundice, and one was moribund with
371 neither jaundice nor gross evidence of anemia. The other 15 fish were relatively healthy
372 reference fish. Lesions that clearly separated the 13 sick fish from the reference fish
373 occurred in the kidney (Fig. 3A), liver (Fig. 3B), and spleen, but no single lesion occurred
374 in all of the sick fish. Among the differentiating lesions, only splenic parenchymal fibrin
375 (PFB) occurred exclusively among jaundiced fish (5 of 9 affected) but not in sick fish
376 without jaundice (0 of 4 affected). Parenchymal fibrin deposits also occurred in the
377 kidney (IFB) of sick fish (5 of 13 affected), but one of these fish did not have jaundice or
378 gross anemia.

379 The most severe lesions associated with IJAS were all acute and probably of less
380 than 48 hours duration. They included (Table 2): 1) renal tubular epithelial hydropic
381 degeneration (xxx; 4 of 13); 2) moderate to severe renal tubular coagulative necrosis
382 (RTN; 7 of 13); 3) hepatocellular hydropic degeneration (HHD; 6 of 13); and 4)
383 hepatocellular single cell necrosis (SCN; 6 of 13). Five of the thirteen fish had mild

Comment [D2]: Gary, I do not see this listed in table 2. Your names should be the same between the text description and the table. I actually don't see any kidney lesion in Table 2 that is present in precisely 4 fish, and this lesion was not listed on the revised report you provided. Did this one simply get missed?

384 hyperplasia of haematopoietic cells in the kidney (ISH); this included four of nine fish
385 with jaundice and one of five fish with anemia but not jaundice. The increase in
386 haematopoietic cells might have taken more than 2 days to develop, but it probably did
387 not take more than a week to develop.

388

389 Functional genomics assessment of fish with IJAS

390 In both liver and kidney tissue, analysis combining anemia and jaundice produced
391 the strongest signature (largest number of significant genes) (Supplemental Table 3). This
392 finding supports the hypothesis that anemia and jaundice were related. For analyses
393 driven by anemia alone and by jaundice alone, three approaches were applied to assess
394 the degree of correlation between the resultant genomic signatures. First, a bootstrap
395 re-sampling correlation analysis was performed on the respective gene lists, and
396 obtained a correlation of >99.9% (i.e. less than 0.1% chance that the correlation in gene
397 loadings in these analyses would be due to chance). Second, the degree of overlap in the
398 top 100, 500 and (for kidney) 1000 genes was assessed for each of liver and kidney. For
399 liver, taking the top 100 significant genes for anemia alone, 96% were also significant for
400 jaundice, and the top 500 yielded 86% overlap with jaundice significant genes. For
401 kidney, the top 100 anemia alone genes yielded 92% overlap with jaundice, while the top
402 500 was 74%, and top 1000 was 65%. Third, hierarchical cluster analysis was performed
403 based on all samples (including those removed from statistical analyses because they
404 were not analyzed for anemia) to determine whether clustering of fish was divergent in
405 each of the analyses (as would be expected if they were uncorrelated physiological
406 phenomena). This latter analysis clusters both genes and individuals according to their
407 degree of correlation. Fish clearly clustered into two groups, with one (A) highly loaded
408 with compromised fish, and the other (B) containing the "healthy" controls
409 (Supplemental Table 3 and Fig. 4). Two of the three fish with anemia only (1000 and
410 1005) clustered consistently in the "A" grouping no matter which drivers were used in
411 the analysis (anemia, jaundice, or anemia/jaundice), while the third (1001) was more
412 variable, clustering with "B" in the liver jaundice alone analysis, and with an intermediate
413 sub-cluster "a" for both combined analyses.

414 Taken together, these analyses suggest that clinical signs of anemia and jaundice
415 are part of the same syndrome (IJAS). We note, however, that anemia is not
416 pathognomonic; hence, the presence of anemia alone in a group of fish showing no signs
417 of jaundice is not likely to be diagnostic for IJAS.

418 Hierarchical cluster analysis revealed a small number of "intermediate" fish that
419 did not cluster consistently between analyses. These included two fish from farm A that
420 were positive for BKD (1012, 1013) which often clustered with "A", but in both combined
421 analyses were in the intermediate "a" cluster that did not show a strong pattern of
422 differential regulation for the top 100 significant genes (i.e. no strong demarcation of
423 "blue" and "yellow" genes denoting up- and down-regulated genes differentiating the
424 two main clusters) (Fig. 4). One fish that was positive for jaundice and anemia (1006) on
425 farm B was also intermediate in the jaundice/anemia clustering and was in the "B"
426 cluster for the kidney jaundice analysis and the "A" cluster for other analyses. This fish,
427 as well as fish 1007 which clustered consistently with "B", was collected during harvest
428 and diagnosed by a Fish health expert; these fish did not die of jaundice and were also
429 not assessed by histopathology. These data suggest that jaundice in farm B is
430 physiologically different from that in farm A, at a considerably weaker stage of
431 development, or may have been misdiagnosed. One moribund "control" fish (1004) from
432 farm A with no external signs of jaundice or anemia also occupied an intermediate
433 position in the jaundice/anemia analysis and clustered with "B" for the kidney jaundice
434 analysis and "A" for remaining analyses. This fish had severe renal tubular necrosis, the
435 lesion most consistently associated with IJAS (see below), and it was strongly positive for
436 the PRV (described below).

437 A T-test between the two farm sites, performed to determine the relative role of
438 transcriptional variance stimulated by environmental differences between sites, did not
439 yield a highly significant list of genes that were differentially expressed between the
440 farms, with only 39 genes in liver and 3 in kidney significant at 5% FDR. These data
441 suggest that environmental differences alone were not likely to be causative of IJAS.

442 Survey of Infectious agents

444 One infectious agent, PRV, occurred at high viral loads in liver (Fluidigm
445 [threshold cycle] $C_t < 20$) that were correlated with IJAS ($R^2 = 0.649$, $p = 2.35 \times 10^{-7}$). No other
446 infectious agents showed any correlations with IJAS, nor were most observed at
447 appreciable copy numbers (data not shown). Additional agent detections, generally low
448 load, were observed across IJAS and control fish of the DNA virus ENV (41-55%
449 prevalence, higher prevalence and load in site B; $p < 0.01$), parasites *Paranucleospora*
450 *theridion* (aka *Desmoozan leptophtherii*; 6-36%, with higher prevalence in site B; $p < 0.05$
451 kidney), *Loma* sp. (8-5%), *Nucleospora salmonis* (3-6%), and bacterium *Renibacterium*
452 *salmoninarum* (6-28%) in liver and kidney tissues, respectively.

453 Quantitative RT-PCR with the PRV assay was additionally performed on the ABI
454 7900 platform using template RNA from gill, heart, kidney, liver and spleen tissue, with
455 each tissue run in duplicate (Tables 1 and 2). Efficiency of this assay on both platforms
456 was high, and highly similar (Fluidigm: $E = 0.99$, slope = -3.35, $R^2 = 0.998$; ABI: $E = 0.98$,
457 slope = -3.36, $R^2 = 0.997$). There was a strong correlation between PRV C_t determined on
458 Fluidigm BioMark versus the ABI 7900 platform ($R^2 = 0.956$, $p = 3.15 \times 10^{-13}$), although C_t
459 values were 6.5 cycles lower on the Fluidigm (Fig. 5). The limit of detection (LOD) on the
460 Fluidigm was between 28-29 C_t , above which results were not 100% repeatable (Miller et
461 al. 2015). The LOD on the 7900 was around 35 C_t .

462 There was a notable 5 C_t breakpoint in the PRV data between fish with clinical
463 signs of IJAS and those that were asymptomatic (Fig. 5). Fish with $C_t < 26$ on the ABI 7900
464 (indicating higher viral loads) carried multi-tissue infections at high load (Tables 1 and 2),
465 and all but one of these fish died of IJAS. Most fish with ABI 7900 $C_t \geq 30$ had no external
466 signs of jaundice or significant jaundice-associated microscopic lesions, but two fish
467 (1001 and 1005) with anemia but not jaundice had $C_t \geq 30$. These two fish also showed
468 variation in other indicators (1001 was not positive for any histological lesions in kidney,
469 1005 had only a weak kidney lesion and was not scored as jaundice positive).

470 Two non-moribund fish from farm B diagnosed by a Creative Salmon fish health
471 technician as having IJAS (1006 and 1007) also had PRV $C_t \geq 30$ (Table 1). These fish did
472 not cluster transcriptionally with fish carrying IJAS in farm A, suggesting that they were

473 physiologically distinct (Fig. 4). Unfortunately, the histology samples were not available
474 to confirm or refute whether they showed signs of IJAS at the cellular level.

475

476 **Sequence Validation of Piscine OrthoReovirus**

477 NGS was utilized to validate the detection of PRV in three samples from farm A;
478 two Chinook salmon spleen tissues, 1053 (spleen; PRV ABI Ct of 19) and S1003 (spleen;
479 PRV ABI Ct of 18.9) and 1 heart tissue 1056 (heart; PRV ABI Ct of 18.6). These samples
480 were processed on one Illumina HiSeq lane and generated 62,940,929, 72,372,861 and
481 62,769,388 post trim reads with average quality scores of 34.12, 34.24 and 34.19,
482 respectively. One of these samples (1003) was collected on February 7, 2011, while the
483 other two (1053 and 1056) were collected on May 24, 2011. Both 1056 and 1053, which
484 represented IJAS mortality and moribund fish, respectively, were diagnosed with lesions
485 and cause of death related to renal tubular necrosis (RTN), while 1003 represented an
486 IJAS fish that was diagnosed with cause of death due to single cell necrosis (SCN) of the
487 liver.

488 1056 generated 680,041 total alignments (0.55% of total reads) to the Norwegian
489 PRV Salmo/GP-2010/NOR 10 segment reference genome (Palacios et al. 2010) which
490 resulted in 100% coverage of the PRV genome and an average coverage of 2,724.05
491 reads. 1003 generated 2,388,384 total alignments (1.63% of total reads) which resulted
492 in 100% coverage of the PRV genome and an average coverage of 9,543.44 reads while
493 1053 generated 4,291,467 total alignments (3.44% of total reads) which resulted in 100%
494 coverage of the PRV genome and an average coverage of 17,146.50 reads. The only
495 other virus observed in these samples was ENV, a result that was expected based on
496 qPCR data.

497 Overall, the PRV genomes of 1056, 1003, and 1053 were more homologous to
498 one another than any other published sequences. Segments L1 (core shell protein), L3
499 (core RdRp protein), M2 (outer shell protein) and S1 (outer clamp protein) displayed no
500 variation (0%/0 SNPs) between the three samples, while segment L2 (core turret protein)
501 displayed the most (<1.0 %/ 3 SNPs). In contrast, segments M2 (outer shell protein) and

502 S1 (outer clamp protein) displayed the most divergence to the Norwegian reference
503 genome (3.2% in both cases), while S4 (outer fiber protein) displayed the least (0.7%).

504 Phylogenetic analysis of segment S1 revealed that the three isolates from this
505 study grouped into the sub-genotype Ia, which contains all of the Canadian PRV strains
506 reported to date. Blast searches revealed that our three Chinook PRV S1 segments were
507 identical to BCinoc3 (KR872635), isolated from a farmed Chinook salmon diagnosed with
508 jaundice syndrome from the same region (West Coast of Vancouver island-DFO Area 124)
509 in 2012, as well as WFSKFH12_14 (KR478638), a previously published PRV genome
510 isolated in March 2014 in a wild Columbia River Coho.

511 The PRV genome isolated from the jaundiced farmed Chinook samples herein
512 displayed <1% divergence (0-10SNPs/segment) over all 10 segments when compared to
513 those characterized from farmed Atlantic salmon on the east coast of Vancouver Island,
514 B.C., B5690 (PRV+/HSMI+) and B7274 (PRV+/HSMI-) from Miller et al. (2017). Only PRV
515 segment S3 (non-structural RNA protein) from Chinook 1056 and 1003 was identical to
516 our previous published isolate B5690 (KX851967). Over the three Chinook PRV isolates a
517 majority of the segments were most homologous to those isolated from wild Coho
518 salmon on the West Coast. Six segments (L1, L2, L3, M1, S1 and S3) were most
519 homologous to WSKSH12_14, isolated from Columbia River in March 2014, 3 segments
520 (M2, M3 and S2) were most homologous to BCJ19943_13, isolated from DFO area 127 in
521 August 2013, while segment S4 was most homologous to VT06062012-358 (KC715687),
522 isolated from a Canadian farmed Atlantic salmon in June 2012.

523 PRV segment consensus sequences for 1056, 1003 and 1053 were deposited
524 into Genbank under the accession number series x to x.

525 **526 Additional Microarray analyses**

527 Further analyses of the microarray data were conducted to determine which
528 measured variables (indicators) correlated with IJAS presentation in our study provided
529 the greatest physiological resolution (measured by gene expression profiling) among
530 salmon sampled on the farms. Among the indicators tested were the gross scores of

531 jaundice/anemia, the individual molecular lesions correlated with IJAS in each tissue, a
532 summed histology score whereby histology scores (0-3) were summed for syndrome-
533 correlated lesions within each tissue, and PRV C_t (contrasting ABI 7900 C_t<26 versus all
534 other samples) (see Table 2 for indicators). These analyses were only performed on fish
535 with histology data.

536 For analyses in both liver and kidney, the combined indicator score—which took
537 into account whether fish were jaundice/anemia positive, were histology positive (for at
538 least one lesion listed in Table 2; note these are not pathognomonic), and carried high
539 loads of PRV—generally provided the greatest transcriptional resolution between fish
540 sampled on the farms, with 3,449 features differentially regulated in liver and 3,864 in
541 kidney (FDR <0.05; Table 3). These results indicate that all three of these indicators may
542 be contributing to the transcriptional (i.e. physiological) shifts associated with disease on
543 the farms. Hierarchical clustering revealed that in both liver and kidney, all fish that were
544 positive for all three indicators clustered tightly together ("A" positive cluster) (not
545 shown). Fish that were positive for two indicators also grouped in the "A" cluster,
546 regardless of which indicator was not present. Three kidney samples were positive for
547 only a single indicator, and two of the three clustered in the "B" (negative) grouping of
548 samples. One of these (1060) was positive only for PRV but did not carry high loads in all
549 tissues. The other (1065) had only one of the analysed lesions—mild renal tubular
550 necrosis—which was not considered diagnostic for IJAS. The three samples with anemia
551 but not jaundice (1000, 1001, and 1005) grouped within the "A" (positive) cluster, again
552 suggesting that anemia, while not pathognomonic, is a physiological change that can be
553 associated with IJAS.

554 While jaundice alone did not generally provide as high a resolution of the
555 microarray data, as in our previous analysis based on all available samples (including
556 those without histopathology), the combined scoring of jaundice and anemia provided a
557 high degree of resolution in kidney (3,994 features) and liver (2,926 features) (Table 3).
558 These data suggest that fish may experience the physiological shifts associated with IJAS

559 before external or internal yellowing is detectable, and that some of these may be
560 resolved if signs of anemia are present at the farm level.

561 PRV load was also a powerful indicator resolving transcriptional variation among
562 fish for liver (3,352 features) and kidney (2,212 features) tissues (Table 3; Supplemental
563 Fig. 4). The summed histology scores provided a weaker, albeit still strong, signal for both
564 liver and kidney tissues (1,612 and 1,280 features, respectively). In general, the individual
565 histological lesions were the least powerful, likely because fewer fish were affected by
566 single lesions (reducing power). Renal tubular necrosis in kidney and single cell necrosis
567 in liver had the strongest single lesion-derived transcriptional signals; however these also
568 affected the most fish.

569

570 Functional Analysis of Microarray Data

571 Within tissues, there was a high degree of overlap between the significant gene
572 lists generated from each of the indicators analyzed, not unexpected given the extensive
573 overlap in positive samples. Using PathWay StudioTM, functional analyses were
574 conducted on each of the gene sets shown in Table 3 using three analytical approaches:
575 (1) gene-set enrichment analysis, which ranks features by their relative fold change
576 between positive and negative samples and contrasts their gene ontologies (which map
577 the biological and molecular processes for which each gene is active) with those of the
578 entire gene list to identify processes that are over-represented in highly differentially
579 expressed gene features; (2) pathway enrichment analysis, which identifies the key
580 biological processes represented in only the most highly significant gene features; and (3)
581 sub-network enrichment analysis, which identifies the key transcriptional regulators
582 significantly associated with the highly significant genes.

583 Functional analyses of each of the datasets also showed a high degree of
584 congruence (Tables 4 and 5), especially between analyses using the three top indicators
585 (jaundice/anemia, summed histology, PRV load). The following description of the
586 functional data largely reflects the signal from the combined indicator score; some of the
587 deviations in functional resolution among analyses are also presented. The presentation
588 reflects differential regulation between IJAS positive versus negative fish; hence

589 processes that are up-regulated or stimulated are more highly expressed in IJAS positive
590 fish.

591
592 *Liver*

593 None of the functional pathways from Pathway Studio™ analysis were significant
594 at p<0.001 when jaundice alone was used as a driver; all other drivers elicited some
595 physiological signal, and the signal based on jaundice and anemia together was quite
596 strong. Again, this indicates that transcriptionally, the external classification of jaundice
597 alone does not yield a strong physiological signal in the data—i.e. IJAS may be present
598 before external yellowing is apparent. Given that four fish classified as belonging in
599 transcriptional cluster “A” (Supplemental Table 3; Fig. 4) were jaundice negative (three of
600 which were anemia positive), perhaps this is not unexpected.

601 Functional analysis of gene features significantly correlated with each of the
602 remaining IJAS indicators revealed a strong down-regulation of most metabolic processes
603 and weak down-regulation of response to nutrient (Table 4). Alternately, proteolysis,
604 especially ubiquitin-requiring processes in the proteasome, was powerfully up-regulated
605 in IJAS fish. “Response to virus” was highly significant in all but the SSF gene-set
606 enrichment analyses (Table 4 and Supplemental Fig. 5). Specific viral-infection-related
607 pathways revealed in pathway- and subnetwork-enrichment analysis (not shown in Table
608 4) included viral replication, type-I interferon response, STAT signaling, viral infectious
609 cycle, positive regulation of viral transcription, and response to exogenous dsRNA (note
610 reoviruses are dsRNA viruses). Antigen presentation was also up-regulated in most
611 analyses, while acute phase response, important in early immune responses to infectious
612 agents, was down-regulated. DNA damage response was highly stimulated in all gene-set
613 enrichment analyses. The molecular signature also showed that many genes and
614 pathways associated with jaundice/hepatitis in mammals were up-regulated in livers
615 with IJAS (Supplemental Fig. 6).

616
617 *Kidney*

618 Gene-set enrichment analysis in kidney tissue was similar between the three key
619 indicators. In general, the functional signature in kidney tissue showed a more powerful
620 up-regulation of immune and virally-related processes, with 8 of the top 10 key
621 regulators involved in immune stimulation and response to virus (Table 5; Fig. 6). This is
622 consistent with the primary role of kidney tissue in immune-related processes. As in liver,
623 response to virus (kidney $p < 10^{-5}$; Table 5), type-I interferon-mediated signaling, response
624 to interferon gamma, viral reproduction, and response to double stranded RNA (pathway
625 enrichment analysis not shown) were strongly up-regulated in kidney tissue. Additional
626 immune pathways stimulated in kidney included negative regulation of defense response
627 to virus by host, regulation of viral genome replication, and positive regulation of viral
628 transcription (pathway enrichment analysis not shown). As observed in liver, DNA
629 damage response was also up-regulated in affected kidneys.

630 Proteolysis was also highly up-regulated in kidney in some analyses, and similar
631 shifts in proliferative and apoptotic cell cycle processes as observed in liver were
632 observed in kidney. Alternately, the metabolic signal was weaker, more variable among
633 analyses drivers, and showed considerable variation in patterns of stimulation/down-
634 regulation differing between the jaundice/anemia and RTN driven analyses and others. In
635 general, jaundice/anemia, summed histology, and RTN signatures showed strong
636 stimulation in processes associated with proteolysis while other analyses showed down-
637 regulation of a variety of metabolic processes.

638 Heme biosynthesis and degradation to bilirubin generally takes place in the liver.
639 Interestingly, while we did not observe disruption of heme metabolism in liver tissue,
640 although bile acid metabolism and heme oxidation were both affected, we did observe
641 down-regulation of heme biosynthesis in kidney, along with a down-regulation of
642 porphyrin biosynthesis and metabolism, an important constituent of heme. Perhaps
643 these processes are down-regulated due to the excess in the heme byproduct bilirubin
644 that is excreted in the kidney (i.e. as a negative feedback loop).

645
646
647 Unsupervised Analyses of microarray data

648 Principle Components Analysis (PCA) is an unsupervised approach to data analysis
649 that can be used to identify the major expression trajectories in the data. When
650 combined with correlation analysis of measured variables against the rank order of the
651 individuals along each principal component (PC), one can determine the relative
652 contribution of measured variables to the overall physiological variation among
653 individuals, essentially identifying the major associations between physiological and
654 molecular change. We conducted a correlation analysis between each of the indicators
655 and with farm site against the top five PCs (Supplemental Table 4). Indicators associated
656 with IJAS were highly correlated with PC1 and PC2 for both liver and kidney, which
657 combined explained roughly 40% of the overall variation within the data for liver and
658 32% of the variation for kidney. This suggests that IJAS was the most powerful driver of
659 molecular physiological change in the fish included in the study. The relative contribution
660 of indicators varied between PC1 and PC2 in both tissues. In liver, the combined indicator
661 and jaundice/anemia were more associated with PC1 while PRV and histological lesions
662 were more associated with PC2. In kidney, the combined indicator and renal tubular
663 necrosis were exclusively associated with PC1 while many indicators contributed
664 significantly to PC2. As with the supervised analysis, single cell necrosis in liver and renal
665 tubular necrosis in kidney were associated with the most powerful transcriptional
666 response among the individual lesions.

667 The patterns of variation associated with the indices driving variation in PC1 and
668 PC2 clearly differentiate all samples, including outliers (Fig. 7). Sample 1001, which had
669 anemia (evident by pale gills) and significant liver lesions without jaundice and no
670 appreciable PRV or lesions in the kidney, was distributed on the extreme positive ends of
671 PC1 and PC2 (PC1 +, PC2 +) (Fig. 6). This placement aligns with the general pattern that
672 jaundice/anemia is most closely associated with PC1 positive samples, and PRV and
673 histology positives are more associated with PC2 negatives. Fish 1060 was high load PRV
674 positive and jaundice/anemia and histology negative; it clustered as PC2 negative (with
675 other PRV positive samples) but at the extreme negative end of PC1 with
676 jaundice/anemia negative fish. These data may suggest that if the virus were to be

677 causative of jaundice (which we cannot determine in this association-based study) the
678 pathogenic effects leading to IJAS may not be present in this fish. Another fish (#1065)
679 with mild renal (intra)tubular necrosis clustered well within the negatives for all
680 indicators, potentially suggesting that the lesion in this fish had a different pathogenesis
681 than renal tubular necrosis in the other fish.

682

683 Osmoregulatory assessment of gill tissue

684 There was no association observed in gill transcription of any of the three Na-K
685 ATPase isoforms analyzed and IJAS ($p>0.05$) or farm site ($p>0.05$).

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688 Discussion

689

690 Pacific salmon showing clinical signs of jaundice have been observed associated
691 with low level mortality in Chinook salmon marine netpens in Canada for over a decade,
692 and references to yellow salmon from Washington to Alaska can be found on the internet
693 across a range of Pacific salmon species (Pink, Chum, Sockeye, Chinook). Herein, we
694 describe the clinical, epidemiological, microscopic, and molecular pathology of an
695 idiopathic jaundice and anemia syndrome, dubbed IJAS, in Chinook salmon farmed on
696 the West Coast of Canada.

697 Our study showed that the pattern of infection on Chinook salmon farms
698 between years and pens was more consistent with the activity of an infectious agent
699 than an environmental cause, although the low mortality rates suggest that if an
700 infectious agent were involved, it may not be highly virulent. Molecular data on the
701 expression of osmoregulatory genes did not support the hypothesis that the syndrome
702 was related to salinity on the farm, although the sporadic occurrence of infection
703 between pens did suggest unknown risk factors are likely involved.

704 The histopathology showed strong involvement of the liver and kidney, with more
705 minor lesions in the spleen. In the kidney and liver, the most notable lesions were
706 necrotic (RTN in kidney and SCN in liver). Necrosis is the additive effect of multiple

707 independent biochemical events activated by severe depletion of cell energy stores
708 (Rana *et al.* 2001). The transcriptional signature associated with IJAS in general and
709 specifically with necrosis in these two tissues is consistent with this mechanism, as both
710 tissues showed strong disruption of metabolic pathways. Protease activation, also a
711 major factor in both tissues, and oxidative stress, particularly associated with RTN, can
712 also lead to changes typical of necrosis (*ibid*). RTN is generally caused by injury to the
713 renal tubular epithelial cells by schema or cytotoxic agents (*ibid*). Acute tubular necrosis
714 can also a result from viral infection, including viral hepatitis (Wilkinson *et al.* 1978), but
715 also avian influenza A (Chan 2002) and simian virus 40 (Sheffield *et al.* 1980). In salmon,
716 acute tubular necrosis has been associated with infectious salmon anemia virus
717 (Bouchard *et al.* 1999) and hemorrhagic kidney disease (Byrne *et al.* 1998). Massive liver
718 necrosis, particularly in relation to viral hepatitis, is often associated with fibrin
719 deposition in the hepatic sinusoids causing a disturbance in the blood coagulation
720 equilibrium (Mochida and Fujiwara 1999). IJAS fish showed a high prevalence of and
721 damage from SCN, with more moderate observations of hepatic fibrin deposition.
722 Transcriptionally, cellular necrosis (RTN and SCN) appeared to drive the largest molecular
723 shift consistent with observed disruptions in cell cycle and apoptosis; 'response to virus'
724 was also significantly associated with these lesions.

725 Both the histopathology and the molecular signature were consistent with
726 cellular and molecular shifts associated with viral hepatitis in other species. Viral
727 hepatitis in mammals is associated with inflammation, fibrin deposition, and necrosis
728 (Levy *et al.* 2000), all processes that were apparent in the histological lesions associated
729 with IJAS. Moreover, functional analyses showed that many genes and pathways
730 associated with jaundice/hepatitis in mammals were up-regulated in livers of Chinook
731 salmon positive for IJAS. The up-regulation of DNA damage, ubiquitin-protein ligase
732 associated with mitotic cell cycle, and apoptosis is consistent with cellular necrosis
733 observed in jaundice livers. Excess fibrin deposition, notable from the histopathology and
734 the powerful up-regulation of FOS-like antigen 2 which has been implicated as a
735 regulator of fibrosis (Roy *et al.* 2010), can stimulate inflammatory pathways; livers with

736 evidence of excess hepatic sinusoidal fibrin deposition also showed significant
737 stimulation of genes that in mammals are associated with platelet and mast cell
738 activation. Assuming that these processes are also representative of the stimulation of
739 thrombocytes (fish equivalent of platelets) and eosinophilic granular cells (fish equivalent
740 of mast cells), these data suggest that the stimulation of inflammatory processes may be
741 most prominently associated with livers undergoing excess fibrin deposition. Finally,
742 during jaundice, the function of the liver is disturbed when there is a deposit of bile
743 pigments in excess of what can be processed, and bilirubin concentrations – derived
744 from the breakdown of hemoglobin – increase in the blood, causing the skin to turn
745 yellow. We speculate that the strong down-regulation of bile acid metabolism may be
746 associated with the build-up of excess bilirubin in the blood that causes IJAS in the
747 salmon. We propose that future studies should examine blood samples to determine if
748 excess bilirubin is observed in association with IJAS in salmon, as has previously been
749 demonstrated in association with erythrocytic inclusion body syndrome (EIBS) in
750 Japanese coho (Sakai et al. 1994) and acute haemolytic anemia in Chilean Coho (Smith et
751 al. 2006), both of which are also associated with jaundice. Interestingly, in the EIBS study,
752 Sakai and colleagues showed dysfunctional bile pigment excretion in conjunction with
753 hemolysis was responsible for hyperbiliruninemia in Coho Salmon.

754 Functional analysis of the IJAS transcriptome showed that response to nutrient
755 was weakly down-regulated in affected fish, potentially suggesting that the down-
756 regulation of metabolism could result from anorexia or reduced feeding of fish suffering
757 from this syndrome. This observation is consistent with the fact that fish that died of IJAS
758 did not have food in their stomachs and had probably been off feed for at least a few
759 days, possibly longer. However, fatty acid oxidation and gluconeogenesis are generally
760 stimulated under prolonged starvation, and both of these processes were down-
761 regulated, potentially suggesting that fish were not at an advanced stage of anorexia.

762 The strong up-regulation of proteolysis can also be enhanced under anorexia, as
763 the break-down of proteins is used for energy generation. However, the dominant
764 proteolytic pathways affected were those associated with mitotic cell cycle, also strongly

765 up-regulated, and the presentation of endogenous peptide antigen, important in the
766 immune response to intra-cellular pathogens. The up-regulation of proteolysis is also
767 consistent with tissue necrosis and cellular damage observed as the most significant
768 lesions associated with IJAS. Viruses can also co-opt the ubiquitin proteolytic pathway to
769 facilitate their own reproduction process (Kloetzel 2001). In mouse reovirus infections,
770 the ubiquitin-proteasome pathway can contribute to cytopathology and disease (Mbisa
771 2002).

772 The liver is the primary tissue for detoxification. The microscopic lesions
773 associated with IJAS were hypothesized to result from acute to sub-acute toxin exposure
774 at the cellular level. The lesions and clinical history are most consistent with those toxins
775 coming from a viral infection and the associated inflammatory mediators rather than an
776 exogenous environmental toxin. This is consistent with the genomic functional analysis of
777 IJAS, where there is no evidence of an enhanced response to exogenous toxins in the
778 liver. Indeed, both xenobiotic metabolism and response to drugs were highly down-
779 regulated in all gene-set enrichment analyses.

780 Patterns connected with immunity in the functional signatures associated with
781 IJAS were highly consistent with a response to viral infection. The strong activation of
782 viral-responsive genes was notable in the analyses driven by virtually all indicators.
783 Analyses of top transcriptional regulators of the most significantly differentially regulated
784 genes were strongly biased towards regulators involved in immunity and viral-response,
785 especially in kidney tissue. In both liver and kidney, the virus-specific innate pathway
786 involving type-I interferon response and STAT signaling was strongly up-regulated and
787 indicative of a Th1 cellular immune response (Dostert *et al.* 2005). Other responses
788 specific to viral activity that were up-regulated in both tissues included 'response to
789 virus' (e.g. MX1, IFIT1, IFI44, EIF2AK2, DDX58, IRF3, MYD88, IRF7, RSAD2, STAT1, STAT2,
790 IFIH1), exogenous dsRNA response (e.g. DDX58 and TLR3), viral
791 transcription/replication/viral infectious cycle (e.g. EIF2aK2, RPSA, RPL7 up-regulated;
792 RPL1, RPL12, RPLB6, RPL30, RPL25, RAN down-regulated), and viral reproduction (e.g.
793 NUP98, PEG3, PEMD12, PSMA1, PSMB6, PSMD3, GTF2B). In kidney, key regulators of the

794 capped cellular mRNA translation system (E1F4G1, E1F4G2, EIF4G3, EIF4E, EIF4A2) and
795 the 25-A Rnase L system (EIF2S1, DDX58, IFIH1) often targeted by viruses to limit
796 transcriptional response in the host were also strongly up-regulated. Moreover, the
797 down-regulation of acute phase response and up-regulation of antigen presentation are
798 consistent with an advanced stage immune response. DNA damage response was highly
799 stimulated in all gene-set enrichment analyses in both kidney and liver. While DNA
800 damage can derive from a nonspecific response to single cell necrosis (consistent with
801 SCN and interstitial cell necrosis [ICN]), it can also be co-opted by viruses to facilitate viral
802 replication (Chaurushiya and Weitzman 2009).

803 Krasnov and colleagues (2011) conducted a study that contrasted gene
804 expression profiles among Atlantic salmon experimentally challenged with four different
805 virally-mediated diseases (heart and skeletal muscle inflammatory syndrome [HSMI],
806 infectious salmon anemia [ISA], infectious pancreas necrosis [IPN], and cardiomyopathy
807 syndrome [CMS]), as well as synthetic double stranded RNA poly(I:C), and identified a
808 suite of genes that were commonly up-regulated in response to viruses in salmon—
809 termed “Viral Responsive Genes” (VRG). They identified 117 gene features that were \geq 1-
810 fold up-regulated in at least three of the five challenge experiments, and 25 features that
811 were up-regulated in all treatments. Many of the VRGs identified in the Krasnov study
812 were also significantly associated with IJAS; in fact, approximately 20% of the top 100
813 genes up-regulated (fold-changes between 4-32) in kidney and liver were annotated to
814 VRGs (combined indicator driven analysis). Fifteen of the 25 most consistent VRG's
815 contained matching annotations on the cGRASP 44K array that passed quality-control
816 analyses in our study, with all but one (PRDM9) significantly up-regulated in the
817 combined indicator analyses in liver and/or kidney. Eleven of the consistent VRG's were
818 up-regulated in both tissues, including genes defined by Krasnov as having specialized
819 antiviral functions (DHX58, RSAD2, HERC3, and HERC6), immune function (CD9), viral-
820 responsive genes with unknown viral function (IFI44, IFIT5, and SACS), and genes with an
821 “unknown viral role” (PRIC285, ZNFX1, and RTP3). Over the broader VRG list, 60 unique
822 genes were annotated and passed quality assessments on the cGRASP 44K array, with 35

823 of these significant in at least one tissue, 23 significant in both tissues. This is similar to
824 number of genes overlapping in our array that were enhanced in heart tissue nine weeks
825 after challenge with HSMI in their study. HERC6, IRF7, NMI, DXH58, RSAD2, STAT1, GVIN1
826 “anti-viral” genes, MHC class I antigen, CD9 “immune function” genes, BANF2, DCK,
827 EIF4G1, EIF4G, CH25HA “viral responsive/non-immune” genes, IFI44, IFIT5, RNF213, SACS
828 “viral responsive/unknown viral role” genes, and RTP3 and ZNFX1 “unknown viral role”
829 genes were up-regulated in our study and were among the VRG up-regulated in week 9
830 of the Krasnov HSMI challenge study.

831 In a recent study by our lab (Miller et al. 2017), we identified a suite of co-
832 expressed genes (biomarker panel) upregulated consistently in salmon in an active
833 disease state due to infection by a suite of RNA viruses (infectious hematopoietic necrosis
834 virus [IHNV], IPNV, ISAv, PRv, piscine myocarditis virus [PMcv]). This biomarker panel,
835 surveyed via high throughput qPCR, could distinguish fish with latent/inactive viral
836 infections from those associated with viral disease development (VDD), and fish
837 diagnosed with viral versus bacterial diseases, even when there was a background of
838 other infectious agents, and was effective across multiple tissues, even those that are not
839 the primary infective target of the virus. Interestingly, while fish with IJAS were not used
840 in the discovery of this panel, most of the genes showing the strongest co-regulation
841 among liver and kidney tissues, described above, were among the top 11 viral disease
842 development (VDD) biomarkers. Moreover, we showed that fish diagnosed with jaundice
843 (IJAS herein), including samples from the study herein and those from a DFO regulatory
844 audit program, were readily classified as being in a “VDD” state. These data offer strong
845 evidence that IJAS is a virally-mediated disease.

846 Given the proposed infectious etiology of IJAS, we conducted a broad association
847 study that included 46 salmon infectious agents to discern if any agents known or
848 suspected to associate with disease in salmon worldwide were associated with IJAS. The
849 high throughput microfluidics platform identified only one agent, PRv, that was
850 associated with IJAS, although there were detections of other agents within both farm
851 samples, some more prevalent in farm B (ENV and *P. theridion*). Further analyses across

852 multiple tissues revealed a statistically significant association of PRV with IJAS, and NGS
853 confirmed that the PRV sequence was highly similar to that identified previously on the
854 West Coast of Canada by Kibenge and colleagues (2013). Moreover, bioinformatics for
855 viral discovery on the NGS data revealed no other uncharacterized viruses across three
856 IJAS samples (analyzed by Drs. Graham Raby and Joe Derisi, University of California San
857 Francisco). However, from this study alone, based on only a single outbreak of IJAS, we
858 can only conclude an association existed between PRV and IJAS in these fish; we cannot
859 make any assertions on causation.

860 Reoviruses are non-enveloped viruses that contain a segmented double-stranded
861 RNA genome. PRV is phylogenetically most similar to viruses in the Orthoreovirus genus,
862 with a genome consisting of 10 segments (Palacios *et al.* 2010). The only other reoviruses
863 known to infect salmon are in the Aquareovirus genus, which contain 11 segments.
864 Although aquareoviruses infect salmon, they are not known to associate with any lesions
865 or disease, but may mediate resistance to other viruses (LaPatra *et al.* 1995).

866 The discovery of PRV, first reported in 2010 (Palacios *et al.* 2010), was based on
867 NGS of Norwegian salmon in sea net pens afflicted with HSMI. While low loads of PRV
868 are ubiquitously observed on Norwegian salmon farms, at high viral loads there is a
869 strong association with severity of HSMI in naturally and experimentally infected fish
870 (Palacios *et al.* 2010; Løvoll *et al.* 2012; Finstad *et al.* 2012, 2014), although occasionally
871 fish with high loads of PRV and no HSMI have been observed (Garseth *et al.* 2012).
872 Finstad and colleagues (2014) further found that in early phases of infection, loads of
873 PRV were higher in the blood than any other organ, and were particularly concentrated
874 in erythrocytes whereby under electron microscopy, inclusion bodies consistent with
875 erythrocytic inclusion body syndrome (EIBS) were observed; challenges based on
876 erythrocytes infected with PRV resulted in an earlier, stronger development of
877 microscopic lesions associated with HSMI (Finstad *et al.* 2014). A cause and effect
878 relationship between PRV-I and HSMI was demonstrated in 2017 by Wessell *et al.*
879 Moreover, HSMI, in association with the west PRV-I, was recently diagnosed in farmed
880 Atlantic Salmon in BC (Di Cicco *et al.* 2017).

881 In Chinook salmon with IJAS, we did not observe any of the classical microscopic
882 changes in the heart associated with HSMI in European Atlantic salmon, which include
883 epi-, endo- and myocarditis and myocardial necrosis (Kongtorp *et al.* 2004), a finding
884 confirmed in analysis by Dr. Trygve Poppe from the Norwegian School of Veterinary
885 Science and Dr. Hugh Ferguson from St. Georges University. However, there are some
886 parallels in the timing, location, and clinical signs of the two diseases. Both HSMI and IJAS
887 generally impact cultured salmon in saltwater, with peak appearance between 5-9
888 months post sea-transfer, with cessation of feeding before the low level mortalities occur
889 (Biering and Garseth 2012). There is also some evidence that liver disease may be a
890 diagnostic feature of HSMI in some cases and with reovirus infections in other species
891 (Kongtorp *et al.* 2006; Haller *et al.* 1995), although the types of lesions observed in these
892 cases may be more consistent with the severe effects on the heart. Kongtorp and
893 colleagues (2006) also observed cellular infiltrates surrounding the bile ducts in fish with
894 HSMI, although this can be observed commonly in association with many different
895 diseases. Finally, Wiik-Nielsen and colleagues (2011) also observed that anorexic fish with
896 HSMI often had pale gills and discoloured livers, consistent with the clinical presentations
897 of IJAS.

898 While classical HSMI has never been diagnosed in a Pacific Salmon species, a
899 jaundice disease that also contains mild HSMI-like lesions and anemia has been described
900 in farmed Rainbow Trout in Norway (Olsen *et al.* 2015) and farmed Coho Salmon in Chile
901 (Godoy *et al.* 2016). Both of these diseases have been associated with a novel strain of
902 PRV (PRV-II, aka PRV-Om in Hauge *et al.* 2017), 85% similar to that causing HSMI (PRV-I)
903 in Atlantic Salmon, although a cause and effect relationship has not yet been established.
904 Alternately, a variant of PRV-II has been established as the causative agent of EIBS
905 disease in Coho Salmon in Japan (Takano *et al.* 2016). Jaundice and anemia has also been
906 clinically observed in Japanese Coho with EIBS, but there are no published descriptions of
907 the histopathology.

908 PRV-I is the only strain of PRV that has been identified in BC salmon, both in
909 Atlantic and Pacific salmon species (Marty *et al.* 2014, Siah *et al.* 2015; Miller,

910 unpublished data). Thus far attempts to transmit jaundice syndrome (IJAS) or HSMI to BC
911 salmon by injecting filtered tissue homogenates from IJAS affected Chinook salmon (with
912 PRV) or from Atlantic Salmon tissues containing high PRV loads, into of healthy Atlantic,
913 Sockeye and Chinook salmon have not resulted in the transmission of the lesions
914 consistent with peak stages of either disease (Garver et al. 2015, 2016). Given that a
915 cause and effect relationship has been established with the same viral strain of PRV and
916 HSMI in Norway (Wessell et al. 2017), and that HSMI has recently been diagnosed on a
917 BC salmon farm (Di Cicco et al 2017), the difficulty demonstrating the development of
918 HSMI in these challenges suggests that the BC variant of PRV-I may be less virulent than
919 that in Norway, despite the fact that they share >97% homology. Until a challenge model
920 in BC is developed that verifies the already established relationship with HSMI, one
921 cannot be sure whether the negative results for IJAS are real or due to an insufficient
922 challenge model.

923 In conclusion, using a multidisciplinary approach to fish health research on IJAS
924 impacting cultured Chinook salmon on the west Coast of British Columbia, we set out to
925 determine whether an environmental toxicant or a virus was more likely to be causative.
926 All levels of analysis supported a viral causation. Although PRV was correlated by load
927 with IJAS, and there is some precedence for a similar relationship with other PRV strains
928 and diseases similar to IJAS, we cannot discern with these data whether PRV is causative
929 of the syndrome, whether PRV replication is concomitant but not causal in fish with IJAS,
930 or whether PRV might contribute to the development of IJAS without being the sole
931 cause.

932 In addition to further challenge research, there are numerous additional avenues
933 for follow-up studies required to fully understand the etiology of IJAS. Association studies
934 based on additional years' data would provide greater support for the consistency of any
935 PRV-jaundice association. Additional diagnostic testing of blood is required to better
936 understand the cause of anemia associated with the syndrome. Immunohistochemistry
937 and/or in situ studies could determine if PRV is localized in around the necrotic lesions in
938 kidney and liver tissue. Finally, given the differences in prevalence of this syndrome over

939 almost a decade of observations among farms, it will also be important to better define
940 risk factors contributing to the syndrome.

941
942

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949

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1097 **Figure Captions**

1098 Figure 1. Clinical presentation of fish with idiopathic jaundice and anemia syndrome
1099 (IJAS).

1100
1101 Figure 2. Monthly mortality for idiopathic jaundice and anemia syndrome (IJAS) and the
1102 total cumulative mortality at Farm A. The water temperature and salinity at 6 m is also
1103 shown - shaded area is the period when jaundice was observed.

1104
1105 Figure 3. Histopathology of idiopathic jaundice and anemia syndrome (IJAS). In A)
1106 Kidney and B) Liver, each row represents sections from each of three different Chinook
1107 salmon from farm A; black box in the left image outlines the area shown at greater
1108 magnification in the right image. H&E stain unless stated otherwise. A) Boxes a and b
1109 show renal tubules in a reference fish with basal nuclei and an apical brush border.
1110 Boxes c and d show a recently dead fish (microarray # 1004) with no gross signs of
1111 anemia or jaundice. A few renal tubular epithelial cells have hydropic degeneration
1112 (arrowheads) with apical anisomorphic cytoplasmic vacuoles. Other renal tubules are
1113 necrotic and filled with cellular debris that is hypereosinophilic and fairly uniform to
1114 globular (*). Boxes e and f show a moribund fish with jaundice and anemia. Several
1115 renal tubules are necrotic and filled with cellular debris that is hypereosinophilic and
1116 fairly uniform to globular (*). B) Boxes a and b show that most of the hepatocytes in a
1117 reference fish have moderate numbers of cytoplasmic glycogen vacuoles (open
1118 arrowheads) that stain positive for PAS (insets, magenta staining). Boxes c and d show
1119 that most of the hepatocytes in a fish with anemia but no jaundice have hydropic
1120 degeneration (*) with moderate numbers of fairly uniform foamy cytoplasmic vacuoles
1121 that distend the vascular pole. Boxes e and f show that most of the hepatocytes in a fish
1122 with anemia but no jaundice have hydropic degeneration (*) with moderate numbers of
1123 anisomorphic foamy cytoplasmic vacuoles, and a few scattered hepatocytes are
1124 undergoing single cell necrosis (arrowheads) with characteristic pyknosis and contracted
1125 hypereosinophilic cytoplasm.

1126
1127 Figure 4. Hierarchical clustering of liver (left) and kidney (right) samples based upon gene
1128 lists significantly correlated with jaundice/anemia (top 100 genes). Individuals are
1129 clustered on the x-axis, with 1-denoting jaundice/anemia (Note in some fish, anemia was
1130 not assessed; see Table 2), X-denoting anemia alone, and 0-denoting no jaundice or
1131 anemia. Genes are clustered on the y-axis, with yellow denoting up-regulated genes, blue
1132 down-regulated. Cluster "B" was highly loaded with fish with no outward signs of
1133 jaundice and/or anemia, while "B" was highly loaded with jaundice/anemia positive fish.
1134 Sub-cluster "a" were fish that were more intermediate in nature, with a more limited
1135 pattern of variation based on the top 100 genes (generally not showing the strongly
1136 down-regulated genes in "A". These fish were a mixture largely of controls, BKD positive
1137 fish, and jaundice/anemia fish from Farm-site B. One jaundice/anemia fish and one
1138 anemia only fish from Farm-site A were also in cluster "a".
1139
1140 Figure 5. Plot showing correlation between liver PRV RT-PCR assays run on two
1141 platforms: Fluidigm (y-axis) and the ABI 7900 (x-axis). Data were highly correlated
1142 ($R^2=96.5$, $p<10^{-12}$). Only fish with C_t values on both platforms ($n = 19$) are shown; not
1143 shown are 10 samples with no C_t on the Fluidigm platform and seven samples with no C_t
1144 on either platform. Fish classified externally as jaundiced or anemia positive are shown in
1145 blue; fish without signs of jaundice or anemia in red. The cluster of jaundice-presenting
1146 fish is circled. Fish 1004, which was not classified as jaundice or anemia positive but
1147 clustered with the high PRV C_t group was also classified at RTN positive through
1148 histology, and was transcriptionally classified in the A cluster containing IJAS.
1149
1150 Figure 6. Top transcriptional regulators (highlighted in yellow) in combined indicator
1151 (jaundice/anemia, histology, PRV<26) driven analysis of kidney tissue; analysis based on
1152 "enriched subnetworks" of genes significant at $q<0.001$ in PathwayStudio. Up-regulated
1153 genes are in red, down-regulated in blue; stronger color indicates higher fold-change
1154 difference. Regulators in grey are not on the array. Eight of the top 10 regulators are

1155 shown (not shown are L-peptidase and bZip transcription factor). All regulators were
1156 significant at P<0.0005.

1157

1158 Figure 7. Plot of PC1 versus PC1 in kidney showing the distribution of samples labeled by
1159 the Combined Indicator, with samples with 0 positive indicators represented by closed
1160 circles; 1 positive, open circles; 2 positive, open squares; and three positive, open
1161 diamonds. Outliers 1001 (positive jaundice only), 1065 (positive RTN histology only), and
1162 1060 (positive PRV only) are labeled.

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1168 Table 1. Data table showing fish sampled at two farm sites (A, B). Fish were scored for
1169 external signs of jaundice, anemia, other (e.g. BKD, hemorrhage), and reference samples
1170 (each scored "1" if positive, "0" if negative, "ND" for no data), histological lesions
1171 diagnosed as the probable cause of death or morbidity ("ND" indicates no histopathology
1172 was performed), and Threshold Cycle (C_t) values (average of duplicate samples) of RT-
1173 PCR analysis for piscine orthoreovirus (PRV) run on the Fluidigm (liver) and ABI 7900
1174 (liver and kidney) quantitative PCR instruments. C_t values from the ABI-7900 that were
1175 below 26 (indicating higher viral loads) are highlighted in red, C_t values between 26-30
1176 are highlighted in gold. $C_t > 30$ were not necessarily repeatable on the ABI 7900 Fluidigm,
1177 and may, in fact, be negative.

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Fish #	Site	Date sampled	Jaundice	Anemia	Other	Reference	Cause of Death	Fluoride Liver CT	Abi Liver CT	Abi Kidney CT	Abi Gill CT	Abi Heart CT	Abi Spleen CT
1050	A	24/05/2011	1	ND	0	0	HHD	17.1	25.2	23.0	23.7	22.7	24.4
1051	A	24/05/2011	1	ND	0	0	RTN	17.9	23.9	22.2	23.2	20.7	19.5
1052	A	24/05/2011	1	ND	0	0	HHD	17.2	24.0	21.4	24.7	20.5	29.2
1053	A	24/05/2011	1	ND	0	0	RTN	20.0	25.5	21.6	21.5	19.1	19.0
1054	A	24/05/2011	1	ND	0	0	PFB	17.4	24.5	22.2	24.7	22.0	20.8
1055	A	24/05/2011	1	ND	0	0	RTN/ICN	16.7	23.5	21.5	21.0	20.6	nil
1056	A	24/05/2011	1	ND	0	0	RTN	18.4	24.8	19.2	21.8	18.6	19.3
1002	A	07/02/2011	1	1	0	0	RTH/HHD	15.1	22.3	21.9	24.7	20.7	20.4
1003	A	07/02/2011	1	1	0	0	RTN	18.3	24.6	21.4	22.8	20.7	18.9
1010	A	27/04/2011	1	1	0	0	ND	17.5	24.4	22.2	22.8	21.9	20.4
1011	A	27/04/2011	1	1	0	0	ND	18.1	24.0	21.3	21.7	20.8	20.7
1006	B	27/04/2011	1	1	0	0	Percussion	nil	35.9	32.9	34.4	nil	34.8
1007	B	27/04/2011	1	1	0	0	Percussion	nil	nil	35.9	34.0	nil	33.3
1000	A	07/02/2011	0	1	0	0	RTN	18.4	24.6	22.3	23.4	20.3	22.6
1001	A	07/02/2011	0	1	0	0	HHD	nil	33.7	31.3	28.8	33.9	31.9
1005	A	07/02/2011	0	1	0	0	SCN/HHD	27.7	34.6	32.2	30.4	35.7	34.7
1004	A	07/02/2011	0	0	Hem	0	RTN	16.7	23.7	22.7	22.6	nil	22.6
1060	A	24/05/2011	0	0	0	1	none	24.5	30.0	23.5	26.4	27.5	25.7
1062	A	24/05/2011	0	0	0	1	Percussion	nil	nil	30.0	27.5	29.3	29.4
1064	A	24/05/2011	0	0	0	1	none	27.2	32.4	29.6	29.5	28.2	27.5
1061	A	24/05/2011	0	0	0	1	none	nil	nil	31.7	30.5	32.3	32.3
1063	A	24/05/2011	0	0	0	1	none	nil	35.8	34.0	27.8	32.6	33.3
1012	A	27/04/2011	0	0	BKD	1	ND	26.1	33.8	32.8	30.9	30.8	32.3
1013	A	27/04/2011	0	0	BKD	1	ND	27.7	32.6	29.6	30.8	31.7	30.3
1057	A	24/05/2011	0	0	0	1	Percussion	nil	37.1	35.4	30.9	34.3	31.8
1058	A	24/05/2011	0	0	0	1	none	28.0	35.3	33.6	32.2	nil	33.0
1059	A	24/05/2011	0	0	0	1	Percussion	nil	34.9	31.3	30.6	31.5	28.0
1065	A	24/05/2011	0	0	0	1	Percussion	nil	35.6	nil	nil	31.8	34.7
1066	A	24/05/2011	0	0	0	1	Percussion	nil	nil	35.9	35.9	nil	35.7
1008	B	27/04/2011	0	0	0	1	Percussion	nil	nil	34.2	34.3	32.0	35.8
1009	B	27/04/2011	0	0	0	1	Percussion	nil	33.8	34.4	33.9	29.4	34.5
1067	B	24/05/2011	0	0	0	1	Percussion	nil	35.8	33.4	34.3	35.8	35.2
1068	B	24/05/2011	0	0	0	1	Percussion	nil	nil	32.9	nil	34.5	33.8
1069	B	24/05/2011	0	0	BKD	1	Percussion	nil	34.4	33.8	34.5	32.7	34.4
1070	B	24/05/2011	0	0	0	1	Percussion	nil	nil	35.9	28.7	nil	33.5
1071	B	24/05/2011	0	0	0	1	Percussion	nil	37.9	34.7	35.2	34.7	33.9

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Histology Scoring Key:

1183 RTN – renal tubular coagulative necrosis (kidney)

1184 HHD – hepatocellular hydropic degeneration (liver)

1185 PFB – parenchymal fibrin (spleen)

1186 ICN – interstitial cell necrosis (kidney)

1187 Percussion – fish killed by a blow to the head causing brain hemorrhage

1188 RTH = renal tubular epithelial hydropic degeneration

1189 SCN = hepatocellular single cell necrosis/apoptosis

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1193 Table 2. Detailed data on histological analyses, showing microscopic findings (scored on
 1194 an increasing intensity scale of 0-3) with high correlation scores with jaundice/anemia
 1195 (combined) and piscine orthoreovirus (PRV) C_t values from ABI 7900, and cluster group
 1196 in microarray analyses, performed using jaundice/anemia (first two columns) or PRV C_t
 1197 value as measured variables (C_t <26 versus all others). C_t values reflect the average of
 1198 duplicate assays, with values <26 indicating high viral loads (10⁴-10⁶ copies per µl) and
 1199 highlighted in red, and values between 26 and 30 indicating low loads and highlighted in
 1200 gold. ND indicates no data.

Fish Number	Collection Date	Site	Jaundice	Anemia	Liver CT	Summed Histology Score Combined Index Score	Kidney CT	KAtly	RTN	ISH	IFB	ICN	MGN	Summed Histology Score Combined Index Score	Gill CT	GAtly	ECH	HAtly	Spleen CT	PFB	LNR	Kidney Microarray Group	PRV Liver Microarray Group	PRV Liver Pathology Group	PRV Kidney Microarray Group			
1055	May 24, 2011	A	X	ND	23.5	1	0	0	2	C	3	3	21.5	1	2	0	1	2	1	6	3	21.0	2	20.6	0	0		
1051	May 24, 2011	A	X	ND	23.9	1	0	0	0	1	3	22.2	1	3	1	0	0	1	5	3	23.2	2	25.7	1	0			
1052	May 24, 2011	A	X	ND	24.0	1	2	1	0	0	4	3	21.4	2	0	1	1	0	1	3	3	24.7	2	26.0	1	0		
1054	May 24, 2011	A	X	ND	24.5	1	0	1	0	0	1	3	22.2	1	0	0	1	0	0	3	3	24.7	2	22.0	0	0		
1056	May 24, 2011	A	X	ND	24.8	1	0	0	0	1	3	19.2	0	3	0	0	0	0	3	3	21.8	1	18.6	0	0			
1050	May 24, 2011	A	X	ND	25.2	1	2	1	0	0	1	3	23.0	2	0	1	0	0	1	3	3	23.7	1	22.7	0	1		
1053	May 24, 2011	A	X	ND	25.5	1	0	0	0	0	2	21.6	1	3	0	0	0	0	3	3	21.5	1	19.6	0	0			
1000	February 7, 2011	A	0	X	24.5	1	0	0	0	0	1	3	22.3	ND	0	0	0	0	0	0	3	2	23.4	ND	20.3	0	ND	
1001	February 7, 2011	A	0	X	33.7	1	3	0	0	0	4	2	31.3	ND	0	0	0	0	0	0	0	0	0	28.5	ND	33.0	0	ND
1002	February 7, 2011	A	X	X	22.3	1	3	0	0	0	4	3	21.9	ND	1	0	0	0	0	0	2	2	24.7	ND	25.7	0	ND	
1003	February 7, 2011	A	X	X	24.5	2	1	0	0	0	3	3	21.4	ND	3	0	0	0	0	0	2	2	22.5	ND	20.4	1	0	
1004	February 7, 2011	A	0	0	23.7	2	0	0	0	0	2	2	22.7	ND	3	0	0	0	0	0	2	2	22.5	ND	0	0	ND	
1006	February 7, 2011	A	0	X	34.5	2	2	0	0	0	4	2	32.2	ND	0	1	0	0	0	0	4	2	36.4	ND	26.7	0	ND	
1050	May 24, 2011	A	0	0	30.0	0	0	0	0	0	0	0	23.8	1	0	0	0	0	0	1	3	26.4	0	0	0	0		
1064	May 24, 2011	A	0	0	32.4	0	0	0	0	0	0	0	25.6	1	0	0	0	0	0	1	3	26.7	0	0	0	0		
1059	May 24, 2011	B	0	0	24.4	0	0	0	0	0	0	0	33.6	0	0	0	0	0	0	0	0	0	28.5	0	0	0		
1059	May 24, 2011	A	0	0	34.0	0	0	0	0	0	0	0	31.3	0	0	0	0	0	0	0	0	0	34.5	0	0	0		
1058	May 24, 2011	A	0	0	35.3	0	0	0	0	0	0	0	33.8	0	0	0	0	0	0	0	0	0	30.0	1	0	0		
1095	May 24, 2011	A	0	0	35.6	0	0	0	0	0	0	0	34.0	0	0	0	0	0	0	0	0	0	32.2	1	0	0		
1063	May 24, 2011	A	0	0	35.8	0	0	0	0	0	0	0	34.0	0	0	0	0	0	0	0	0	0	31.6	0	0	0		
1087	May 24, 2011	B	0	0	35.8	0	0	0	0	0	0	0	33.4	0	0	0	0	0	0	0	0	0	34.7	0	0	0		
1057	May 24, 2011	A	0	0	37.1	0	0	0	0	0	0	0	35.4	0	0	0	0	0	0	0	0	0	36.9	1	0	0		
1071	May 24, 2011	B	0	0	37.9	0	0	0	0	0	0	0	34.7	0	0	0	0	0	0	0	0	0	35.2	1	0	0		
1062	May 24, 2011	A	0	0	nil	0	0	0	0	0	0	0	30.0	0	0	0	0	0	0	0	0	0	27.5	0	0	0		
1070	May 24, 2011	B	0	0	nil	0	0	0	0	0	0	0	35.9	0	0	0	0	0	0	0	0	0	34.7	1	0	0		
1069	May 24, 2011	A	0	0	31.7	1	0	0	0	0	0	0	31.7	0	0	0	0	0	0	0	0	0	30.5	1	0	0		
1066	May 24, 2011	A	0	0	35.9	1	0	0	0	0	0	0	35.9	1	0	0	0	0	0	0	0	0	35.7	0	0	0		
1068	May 24, 2011	B	0	0	nil	0	0	0	0	0	0	0	32.9	0	0	0	0	0	0	0	0	0	34.5	0	0	0		

Histology Scoring Key:

- 1203 SCN – hepatocellular single cell necrosis
- 1204 HHD – hepatocellular hydropic degeneration
- 1205 SSF – hepatic sinusoidal fibrin
- 1206 SCM – hepatic sinusoidal congestion
- 1207 KAtly – kidney autolysis
- 1208 RTN – renal tubular necrosis
- 1209 ISH – interstitial cell hyperplasia, kidney
- 1210 IFB – interstitial fibrin, kidney
- 1211 ICN – interstitial cell necrosis, kidney
- 1212 MGN – membranous glomerulonephritis
- 1213 GAtly – gill autolysis
- 1214 HAtly – heart autolysis
- 1215 ECH – endocardial cell hypertrophy
- 1216 PFB – parenchymal fibrin, spleen
- 1217 LKR – leukocytic karyorrhexis, spleen

1218 Table 3. Statistical analysis of microarray data, including only samples that contained
1219 histology. Three key indicators associated with IJAS have been defined in this study: PRV
1220 C_t<26, presence of jaundice and/or anemia, and presence of one of the histological
1221 lesions associated with jaundice (see Table 3 for lesion abbreviations). Microarray
1222 analysis was driven by each of these indicators, as well as a combined indicator score
1223 whereby each indicator was given a score of 1 if positive, and indicators were summed
1224 (maximum of 3 if all were positive, 0 if none were positive; the combined score ignored
1225 jaundice only as an indicator, as jaundice/anemia was a more powerful transcriptional
1226 signature), and a summed histology score, which took the individual lesion scores (minus
1227 KAtly, which is a measure of tissue integrity assumed to correlate with postmortem cell
1228 lysis) defined in Table 3 (by tissue) and summed them. In this way, intensity of damage
1229 was taken into account, as individuals with more lesions or higher scores for individual
1230 lesions were ranked higher than those with single less damaging lesions. # Positive
1231 samples indicates those samples positive for the analysis driver in question. # PS entities
1232 is the number of genes with gene identifiers that could be mapped in for functional
1233 analysis in Pathway Studio. ND indicates no data.

1234

Analysis Driver	# Positive samples	# Significant Probes
Liver		
Combined Indicator Score	13	3449
PRV <26	11	3352
Jaundice	9	1017
Jaundice/Anemia	12	2926
Summed Histo Score	12	1612
SCN	12	1913
SSF	3	218
HHD	6	33
Kidney		
Combined Indicator Score	15	3864
PRV <26	12	2212
Jaundice	9	632
Jaundice/Anemia	12	3994
Summed Histo Score	12	1280
KAtly	9	135
RTN	9	541
ISH	5	70
IFB	3	4
MGN	3	0

1235
1236

1237 Table 4. Gene-set enrichment analysis showing biological processes differentially
1238 regulated in the liver in analyses driven by the combined indicator analysis
1239 (jaundice/anemia, PRV, histology), summed histology (of "IJAS-associated" liver lesions),
1240 jaundice/anemia, PRV load, and individual lesions SCN or SSF. Processes for each analysis
1241 significant at $P<10^{-05}$ contain three asterisks (***) $, 10^{-05} < P < 10^{-04}$ (**), and $10^{-04} < P < 10^{-03}$
1242 (*).
1243

			Liver Combined Indicator	Liver Summed Halo	Liver PRV CT 26	Liver Jaundice/Aemia	Liver SCN	Liver SSF
Viral/Immune/Inflammatory								
response to virus	Viral/immune	Up-regulated	++	++	++	++	+	++
Presentation Of Endogenous Peptide Antigen	Viral/immune	Up-regulated	+	++	++	+	+	++
mast cell activation	Viral/immune	Up-regulated	+	++	++	+	+	++
platelet activation	Viral/immune	Up-regulated	+	++	++	+	+	++
platelet degranulation	Viral/immune	Up-regulated	+	++	++	+	+	++
interspecies interaction between organisms	Viral/immune	Up-regulated	+	++	++	+	+	++
peroxisome	Viral/immune	Down-regulated	++	++	++	++	++	++
complement activation	Viral/immune	Down-regulated	+	++	++	+	+	++
complement activation, classical pathway	Viral/immune	Down-regulated	+	++	++	+	+	++
acute-phase response	Viral/immune	Down-regulated	++	++	++	+	+	++
Blood-Heme								
Bile acids metabolism [alternative pathway]	Blood	Down-regulated	++	++	++	+	++	++
heme oxygenase	Blood	Down-regulated	++	++	++	+	++	++
Heme oxidation	Blood	Down-regulated	++	++	++	+	++	++
Cellular								
DNA damage response, signal transduction by p53								
class mediator resulting in cell cycle arrest	Cellular	Up-regulated	++	++	++	++	++	++
regulation of apoptosis	Cellular	Up-regulated	++	++	++	++	++	++
positive regulation of ubiquitin-protein ligase activity involved in mitotic cell cycle	Cellular	Up-regulated	++	++	++	++	++	++
regulation of ubiquitin-protein ligase activity involved in mitotic cell cycle	Cellular	Up-regulated	++	++	++	++	++	++
anaphase-promoting complex-dependent proteasomal ubiquitin-dependent protein catabolic process	Cellular	Up-regulated	++	++	++	++	++	++
negative regulation of ubiquitin-protein ligase activity involved in mitotic cell cycle	Cellular	Up-regulated	++	++	++	++	++	++
M-G1 transition of mitotic cell cycle	Cellular	Up-regulated	++	++	++	++	++	++
G1-S transition of mitotic cell cycle	Cellular	Up-regulated	++	++	++	++	++	++
Adipocytokine Signaling	Metabolic	Up-regulated	++	++	++	++	++	++
ther development	Cellular	Down-regulated	++	++	++	++	++	++
Carcinogenesis	Cellular	Variable	+	+	+	+	+	+
Sclerosis	Cellular	Variable	+	+	+	+	+	+
Response								
response to nutrient	Metabolic	Down-regulated	+	+	+	+	+	+
response to amino acid stimulus	Response	Up-regulated	+	++	++	+	+	++
response to stress	Response	Variable	+	+	+	+	+	+
response to hypoxia	Response	Variable	+	+	+	+	+	+
response to peptide hormone stimulus	Response	Variable	+	++	++	+	+	++
Response to starvation	Response	Variable	++	++	++	+	+	++
Toxicant								
xenobiotic metabolic process								
response to drug								
drug metabolic process								
Metabolic								
proteasome complex	Metabolic	Up-regulated	++	++	++	++	++	++
proteolytic system involved in cellular protein catabolic process	Metabolic	Up-regulated	+	+	+	+	+	+
endopeptidase activity	Metabolic	Up-regulated	+	+	+	+	+	+
regulation of cellular amino acid metabolic process	Metabolic	Up-regulated	++	++	++	++	++	++
RNA metabolic process	Metabolic	Up-regulated	++	++	++	++	++	++
Biosynthesis of cholesterol	Metabolic	Up-regulated	+	+	+	+	+	+
cellular nitrogen compound metabolic process	Metabolic	Up-regulated	++	++	++	++	++	++
mRNA metabolic process	Metabolic	Up-regulated	+	+	+	+	+	+
Pentose-phosphate shunt	Metabolic	Up-regulated	++	++	++	++	++	++
Branched amino acid metabolism	Metabolic	Down-regulated	+	+	+	+	+	+
carbohydrate metabolic process	Metabolic	Down-regulated	++	++	++	++	++	++
catalytic activity	Metabolic	Down-regulated	++	++	++	++	++	++
cellular lipid metabolic process	Metabolic	Down-regulated	++	++	++	++	++	++
cholesterol homeostasis	Metabolic	Down-regulated	+	+	+	+	+	+
fatty acid metabolic process	Metabolic	Down-regulated	+	++	++	+	+	+
Fatty acid oxidation	Metabolic	Down-regulated	+	++	++	+	+	+
insulin Action	Metabolic	Down-regulated	+	++	++	+	+	++
gluconeogenesis	Metabolic	Down-regulated	++	++	++	++	++	++
glucose metabolic process	Metabolic	Down-regulated	++	++	++	++	++	++
Glucose metabolism	Metabolic	Down-regulated	++	++	++	++	++	++
Glutathione metabolism	Metabolic	Down-regulated	++	++	++	++	++	++
Lipid metabolic process	Metabolic	Down-regulated	++	++	++	++	++	++
Metabolism of estrogens and androgens	Metabolic	Down-regulated	++	++	++	++	++	++
omega-3-fatty acid metabolism	Metabolic	Down-regulated	++	++	++	++	++	++
omega-6-fatty acid metabolism	Metabolic	Down-regulated	++	++	++	++	++	++
oxidation-reduction process	Metabolic	Down-regulated	++	++	++	++	++	++
Serine and Glycine metabolism	Metabolic	Down-regulated	++	++	++	++	++	++
Inositol biosynthetic process	Metabolic	Down-regulated	++	++	++	++	++	++

1245 Table 5. Gene-set enrichment analysis showing biological processes differentially
1246 regulated in the kidney in analyses driven by number of positive indicators (jaundice,
1247 PRV, histology), histology alone (combined "associated" kidney lesions),
1248 jaundice/anemia, PRV infection, and individual lesions. Processes for each analysis
1249 significant at $P<10^{-05}$ contain three asterisks (***) $, 10^{-05} < P < 10^{-04}$ (**), and $10^{-04} < P < 10^{-03}$
1250 (*). Only pathways significant at $10^{-05} < P < 10^{-04}$ in at least one analysis are shown.

1251
1252
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1254

				Kidney	Combined Indicator					
				Sustained-Histo	PRV CT 26	JDR	Jardine	RTN	ISH	KATY
Viral/immune										
response to virus	Viral/Immune	Up-regulated	++	++	++	++	++	++	++	+
viral	Viral/Immune	Up-regulated	++	++	++	++	++	++	++	+
interspecies interaction between organisms	Viral/Immune	Up-regulated	++	++	++	++	++	++	++	+
Presentation of Endogenous Peptide Antigen	Viral/Immune	Up-regulated	++	++	++	++	++	++	++	+
cytokine activity	Viral/Immune	Up-regulated	++	++	++	++	++	++	++	+
immune response	Viral/Immune	Up-regulated	++	++	++	++	++	++	++	+
Blood	Viral/Immune	Variable	++	++	++	++	++	++	++	+
positive regulation of angiogenesis	Blood	Up-regulated	++	++	++	++	++	++	++	+
Bile acids metabolism (alternative pathway)	Blood	Down-regulated	++	++	++	++	++	++	++	+
heme biosynthetic process	Blood	Down-regulated	++	++	++	++	++	++	++	+
heme oxidation	Blood	Down-regulated	++	++	++	++	++	++	++	+
porphyrin metabolic process	Blood	Down-regulated	++	++	++	++	++	++	++	+
porphyrin biosynthetic process	Blood	Down-regulated	++	++	++	++	++	++	++	+
heme binding	Blood	Variable	++	++	++	++	++	++	++	+
Cellular										
regulation of apoptosis	Cellular	Up-regulated	++	++	++	++	++	++	++	+
apoptosis	Cellular	Up-regulated	++	++	++	++	++	++	++	+
cell proliferation	Cellular	Up-regulated	++	++	++	++	++	++	++	+
positive regulation of ubiquitin-protein ligase activity	Cellular	Up-regulated	++	++	++	++	++	++	++	+
involved in mitotic cell cycle	Cellular	Up-regulated	++	++	++	++	++	++	++	+
DNA damage response, signal transduction by p53 class	Cellular	Up-regulated	++	++	++	++	++	++	++	+
mediator resulting in cell cycle arrest	Cellular	Down-regulated	++	++	++	++	++	++	++	+
brush border membrane	Cellular	Variable	++	++	++	++	++	++	++	+
Sclerosis	Cellular	Variable	++	++	++	++	++	++	++	+
Cirrhosis	Cellular	Variable	++	++	++	++	++	++	++	+
aging	Cellular	Variable	++	++	++	++	++	++	++	+
Adipocytokine Signaling	Cellular	Variable	++	++	++	++	++	++	++	+
Toxicant										
xenobiotic metabolic process	Toxicant	Down-regulated	++	++	++	++	++	++	++	+
response to inorganic substance	Toxicant	Down-regulated	++	++	++	++	++	++	++	+
drug metabolic process	Toxicant	Down-regulated	++	++	++	++	++	++	++	+
response to ethanol	Toxicant	Variable	++	++	++	++	++	++	++	+
response to drug	Toxicant	Variable	++	++	++	++	++	++	++	+
response to organic cyclic compound	Toxicant	Variable	++	++	++	++	++	++	++	+
Response										
response to peptide hormone stimulus	Response	Up-regulated	++	++	++	++	++	++	++	+
response to mechanical stimulus	Response	Down-regulated	++	++	++	++	++	++	++	+
response to hypoxia	Response	Down-regulated	++	++	++	++	++	++	++	+
response to stress	Response	Variable	++	++	++	++	++	++	++	+
response to oxidative stress	Response	Variable	++	++	++	++	++	++	++	+
response to glucocorticoid stimulus	Response	Variable	++	++	++	++	++	++	++	+
response to cold	Response	Variable	++	++	++	++	++	++	++	+
Metabolism										
proteolysis involved in cellular protein catabolic process	Metabolism	Up-regulated	++	++	++	++	++	++	++	+
regulation of cellular amino acid metabolic process	Metabolism	Up-regulated	++	++	++	++	++	++	++	+
RNA metabolic process	Metabolism	Up-regulated	++	++	++	++	++	++	++	+
glucose metabolic process	Metabolism	Up-regulated	++	++	++	++	++	++	++	+
glutathione metabolic process	Metabolism	Down-regulated	++	++	++	++	++	++	++	+
tetrapyrrole biosynthetic process	Metabolism	Down-regulated	++	++	++	++	++	++	++	+
Glutathione metabolism	Metabolism	Down-regulated	++	++	++	++	++	++	++	+
Tetrapyrroles biosynthesis	Metabolism	Down-regulated	++	++	++	++	++	++	++	+
oxidation-reduction process	Metabolism	Down-regulated	++	++	++	++	++	++	++	+
imidazoles metabolism	Metabolism	Down-regulated	++	++	++	++	++	++	++	+
cellular amino acid metabolic process	Metabolism	Down-regulated	++	++	++	++	++	++	++	+
purine base metabolic process	Metabolism	Down-regulated	++	++	++	++	++	++	++	+
Aspartate metabolism	Metabolism	Down-regulated	++	++	++	++	++	++	++	+
Arachidonic acid metabolism	Metabolism	Down-regulated	++	++	++	++	++	++	++	+
Mannose metabolism	Metabolism	Down-regulated	++	++	++	++	++	++	++	+
cellular nitrogen compound metabolic process	Metabolism	Variable	++	++	++	++	++	++	++	+
Ser/Gly/Thr/Cys metabolism	Metabolism	Variable	++	++	++	++	++	++	++	+
catalytic activity	Metabolism	Variable	++	++	++	++	++	++	++	+
Glucose metabolism	Metabolism	Variable	++	++	++	++	++	++	++	+
glycolysis	Metabolism	Variable	++	++	++	++	++	++	++	+
nucleobase, nucleoside and nucleotide metabolic process	Metabolism	Variable	++	++	++	++	++	++	++	+
Tryptophan metabolism	Metabolism	Variable	++	++	++	++	++	++	++	+
carbohydrate metabolic process	Metabolism	Variable	++	++	++	++	++	++	++	+
Transport										
Glucose import	Transport	Down-regulated	++	++	++	++	++	++	++	+
substrate-specific transmembrane transporter activity	Transport	Down-regulated	++	++	++	++	++	++	++	+
transmembrane transporter activity	Transport	Down-regulated	++	++	++	++	++	++	++	+
sodium ion transport	Transport	Down-regulated	++	++	++	++	++	++	++	+
ion transmembrane transporter activity	Transport	Down-regulated	++	++	++	++	++	++	++	+
transporter activity	Transport	Down-regulated	++	++	++	++	++	++	++	+
electron carrier activity	Transport	Down-regulated	++	++	++	++	++	++	++	+
transmembrane transport	Transport	Variable	++	++	++	++	++	++	++	+
Other	Transport	Variable	++	++	++	++	++	++	++	+
platelet alpha granule membrane	Other	Up-regulated	++	++	++	++	++	++	++	+
translation initiation factor activity	Other	Up-regulated	++	++	++	++	++	++	++	+
protein homodimerization activity	Other	Variable	++	++	++	++	++	++	++	+
transferrin	Other	Variable	++	++	++	++	++	++	++	+
transaminase activity	Other	Down-regulated	++	++	++	++	++	++	++	+
glutathione transferase activity	Other	Down-regulated	++	++	++	++	++	++	++	+
sympporter activity	Other	Down-regulated	++	++	++	++	++	++	++	+
sugar binding	Other	Down-regulated	++	++	++	++	++	++	++	+
pyridoxal phosphate binding	Other	Down-regulated	++	++	++	++	++	++	++	+

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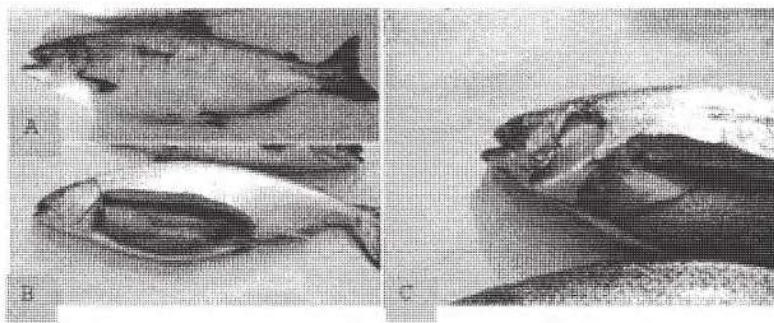
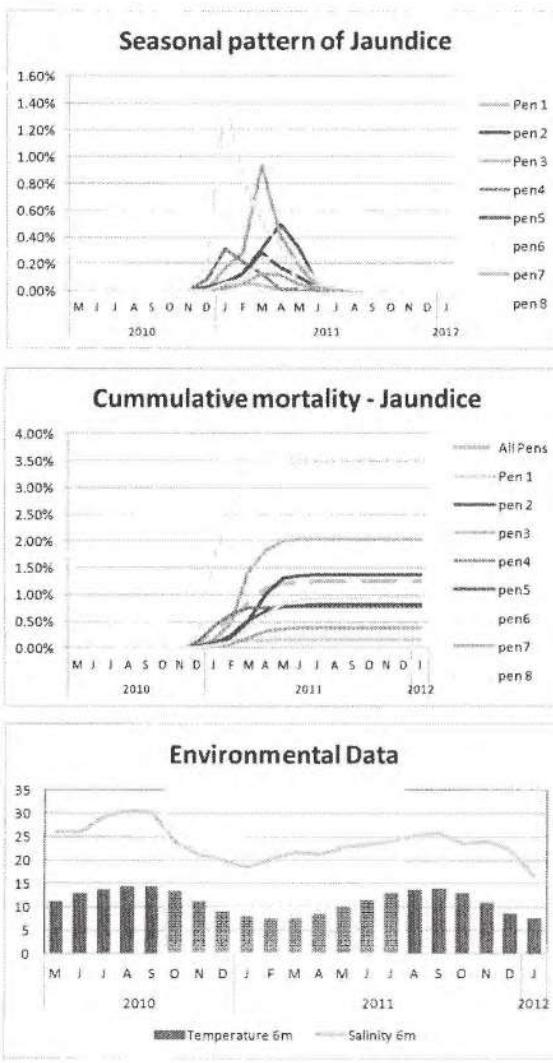


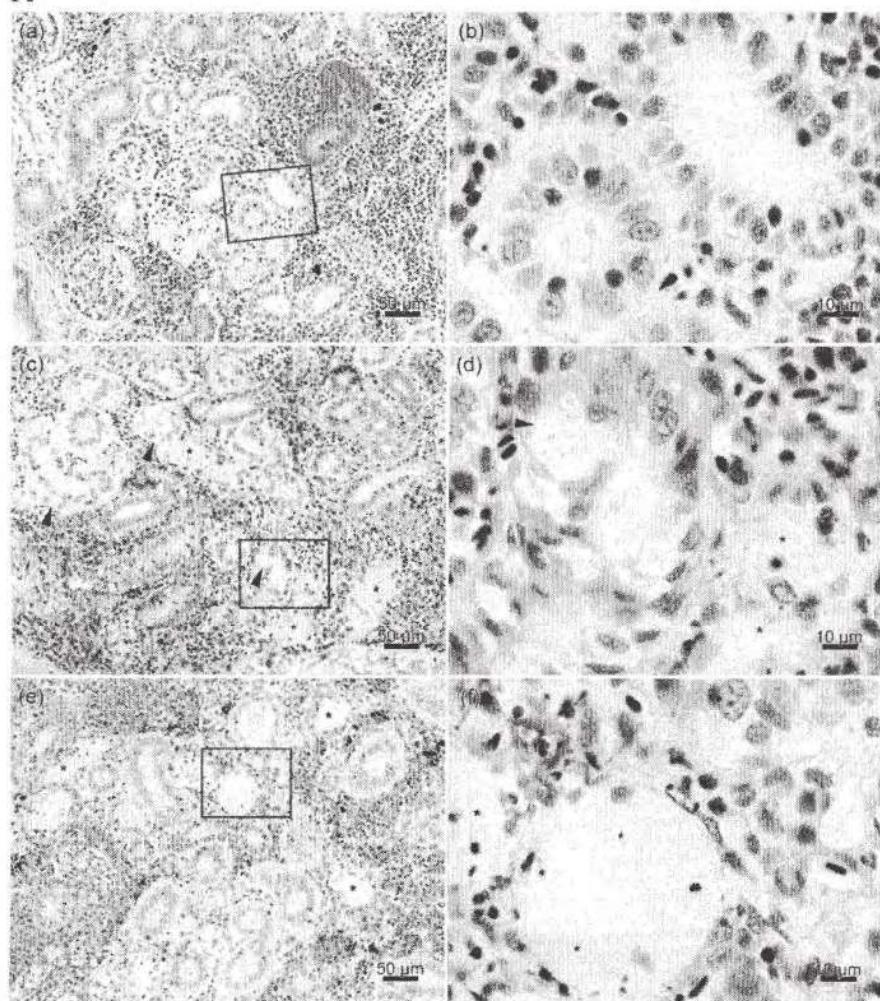
Figure 1. Clinical presentation of fish with Idiopathic jaundice and anemia syndrome.



1276
 1277
 1278 Figure 2. Monthly mortality for idiopathic jaundice and anemia syndrome (IJAS) and the
 1279 total cumulative mortality at Farm A. The water temperature and salinity at 6 m is also
 1280 shown - shaded area is the period when Jaundice was observed.
 1281

1282

A



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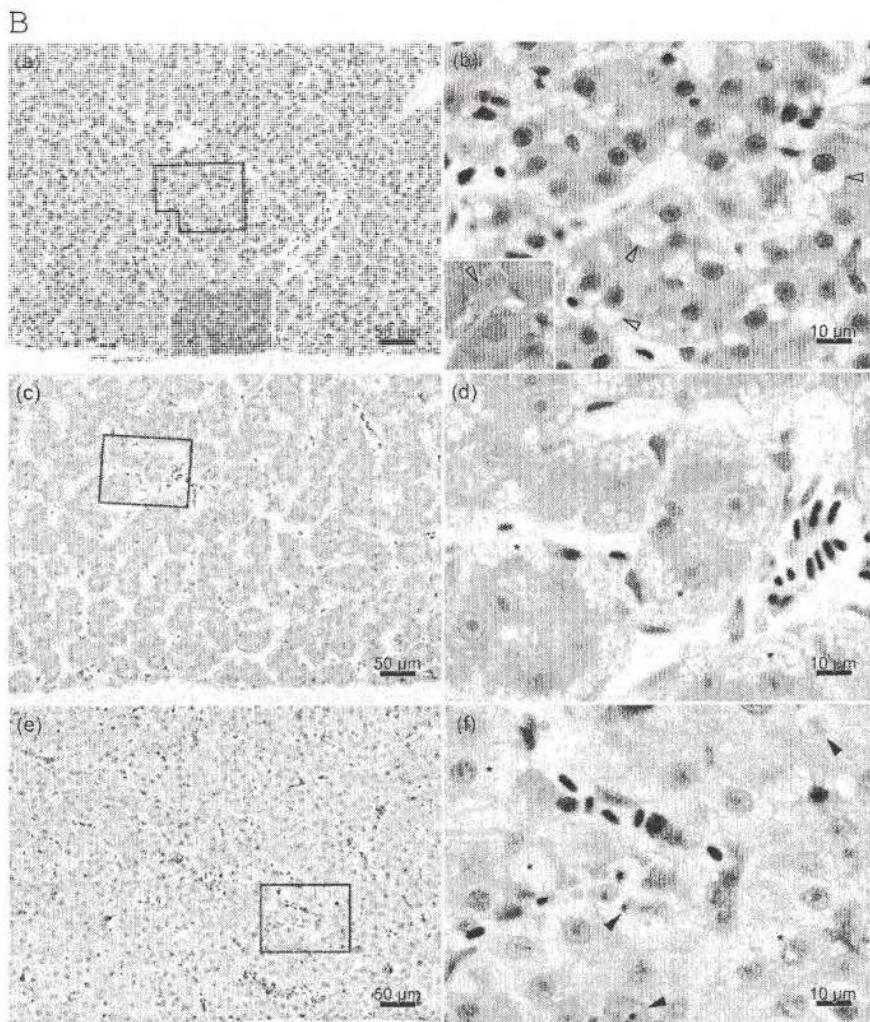
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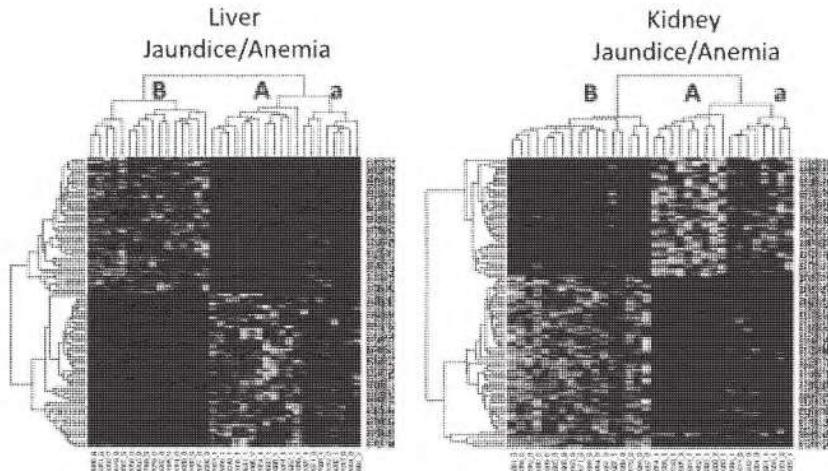


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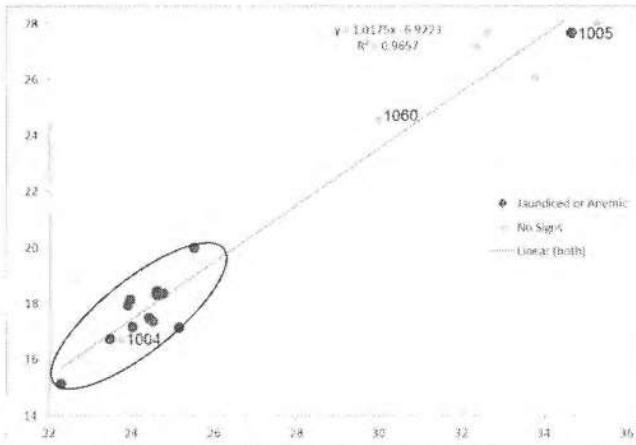
Figure 3. Histopathology of IJAS. In A) Kidney and B) Liver, each row represents sections from each of three different Chinook salmon from farm A; black box in the left image outlines the area shown at greater magnification in the right image. H&E stain unless stated otherwise. A) Boxes a and b show renal tubules in a reference fish with basal nuclei and an apical brush border. Boxes c and d show a recently dead fish (microarray # 1004) with no gross signs of anemia or jaundice. A few renal tubular epithelial cells have

1294 hydropic degeneration (arrowheads) with apical anisomorphic cytoplasmic vacuoles.
1295 Other renal tubules are necrotic and filled with cellular debris that is hypereosinophilic
1296 and fairly uniform to globular (*). Boxes e and f show a moribund fish with jaundice and
1297 anemia. Several renal tubules are necrotic and filled with cellular debris that is
1298 hypereosinophilic and fairly uniform to globular (*). B) Boxes a and b show that ost of
1299 the hepatocytes in a reference fish have moderate numbers of cytoplasmic glycogen
1300 vacuoles (open arrowheads) that stain positive for PAS (insets, magenta staining). Boxes
1301 c and d show that most of the hepatocytes in a fish with anemia but no jaundice have
1302 hydropic degeneration (*) with moderate numbers of fairly uniform foamy cytoplasmic
1303 vacuoles that distend the vascular pole. Boxes e and f show that most of the hepatocytes
1304 in a fish with anemia but no jaundice have hydropic degeneration (*) with moderate
1305 numbers of anisomorphic foamy cytoplasmic vacuoles, and a few scattered hepatocytes
1306 are undergoing single cell necrosis (arrowheads) with characteristic pyknosis and
1307 contracted hypereosinophilic cytoplasm.

1308
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1310
 1311 Figure 4. Hierarchical clustering of liver (left) and kidney (right) samples based upon gene
 1312 lists significantly correlated with jaundice/anemia (top 100 genes). Individuals are
 1313 clustered on the x-axis, with 1-denoting jaundice/anemia (Note in some fish, anemia was
 1314 not assessed; see Table 2), X-denoting anemia alone, and 0-denoting no jaundice or
 1315 anemia. Genes are clustered on the y-axis, with yellow denoting down-regulated genes,
 1316 blue up-regulated. Cluster "B" was highly loaded with fish with no outward signs of
 1317 jaundice and/or anemia, while "B" was highly loaded with jaundice/anemia positive fish.
 1318 Sub-cluster "a" were fish that were more intermediate in nature, with a more limited
 1319 pattern of variation based on the top 100 genes (generally not showing the strongly
 1320 down-regulated genes in "A". These fish were a mixture largely of controls, BKD positive
 1321 fish, and jaundice/anemia fish from Farm-site B. One jaundice/anemia fish and one
 1322 anemia only fish from Farm-site A were also in cluster "a".
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1328 Figure 5. Plot showing correlation between liver C_t 's from PRV RT-PCR assays run on two
 1329 platforms: Fluidigm (y-axis) and the ABI 7900 (x-axis). Data were highly correlated
 1330 ($R^2=96.5$, $p<10^{-12}$). Only fish with C_t values on both platforms ($n=19$) are shown; not
 1331 shown are 10 samples with no C_t on the Fluidigm platform and seven samples with no C_t
 1332 on either platform. Fish classified externally as jaundice- or anemia-positive are shown in
 1333 blue; fish without signs of jaundice or anemia in red. The cluster of jaundice-presenting
 1334 fish is circled. Fish 1004, which was not classified as jaundice or anemia positive but
 1335 clustered with the high PRV C_t group was also classified at RTN positive through
 1336 histology, and was transcriptionally classified in the A cluster containing IJAS fish. Fish
 1337 1005 was classified as anemia positive, not jaundice positive.

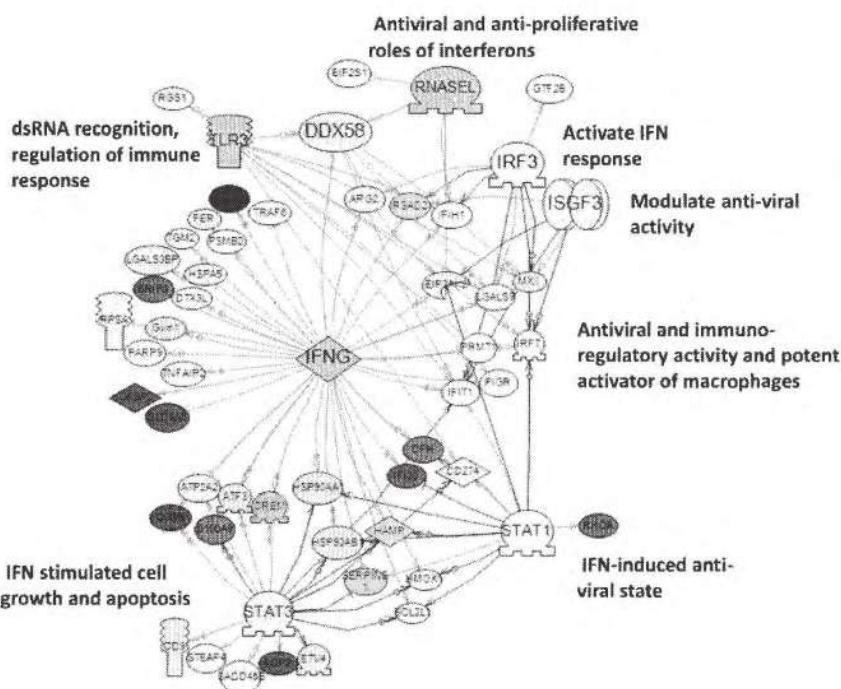
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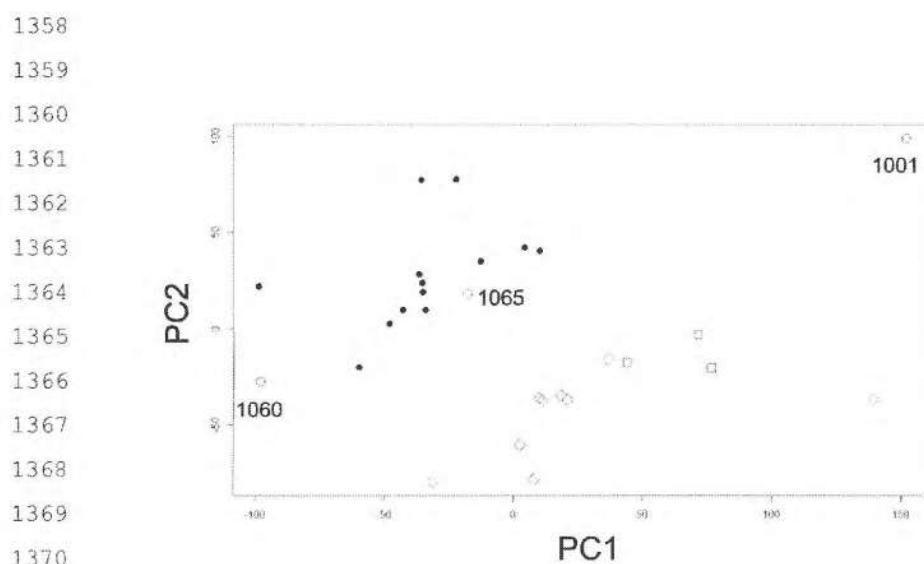
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1350 Figure 6. Top transcriptional regulators (highlighted in yellow) in combined indicator
1351 (jaundice/anemia, histology, PRV<26) driven analysis of kidney tissue; analysis based on
1352 "enriched subnetworks" of genes significant at $q < 0.001$ in PathwayStudio. Up-regulated
1353 genes are in red, down-regulated in blue; stronger color indicates higher fold-change
1354 difference. Regulators in grey are not on the array. Eight of the top 10 regulators are
1355 shown (not shown are L-peptidase and bZip transcription factor). All regulators were
1356 significant at $P < 0.0005$.
1357



1373 Figure 7. Plot of PC1 versus PC2 in kidney showing the distribution of samples labeled by
 1374 the Combined Indicator, with samples with 0 positive indicators represented by closed
 1375 circles; 1 positive, open circles; 2 positive, open squares; and three positive, open
 1376 diamonds. Outliers 1001 (positive jaundice only), 1065 (positive RTN histology only), and
 1377 1060 (positive PRV only) are labeled.
 1378

1379 Highlights

- 1380 • Veterinary diagnostics, epidemiology, histopathology, functional genomics and high
1381 throughput microbe monitoring was employed to determine whether an idiopathic
1382 jaundice and anemia syndrome (IJAS) affecting Chinook salmon in net pens on the west
1383 coast of British Columbia was more likely mediated by viral or toxicant-driven factors
- 1384 • The syndrome was characterized by consistent, low level overwinter mortality associated
1385 with a yellowing of the abdomen and periorbital region and pale gills
- 1386 • Epidemiology was consistent with an infectious mechanism, with potential contribution
1387 of salinity and other unidentified environmental factors
- 1388 • Renal tubular and interstitial cell necrosis, hepatocellular hydropic degeneration, and
1389 splenic parenchymal fibrin were the top cellular lesions associated with the syndrome
- 1390 • Functional genomic analysis using a 44K feature salmon oligonucleotide array revealed a
1391 genomic signature that was notably viral, with strong stimulation of proteolysis, anti-viral
1392 response, and apoptosis in both the liver and kidney
- 1393 • Quantitative PCR of 16 infectious agents revealed a single virus, the piscine
1394 orthoreovirus, was associated with the syndrome, but no determination as to its role in
1395 the syndrome was established

ACRDP Final Project Report

PART I

1. Project #:

2. Project Title:

Genomic characterization of jaundice-associated mortality events in cultured Chinook salmon

3. Project Duration:

1 April, 2011 – 31 March, 2012

4. Project Leader, contact information:

Project Manager

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5. Industry partner(s):

Creative Salmon Company Ltd.
PO Box 265
Tofino, British Columbia
250-725-2884

s.19(1)

6. Expenditures and variance from budget:

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	Contribution	Initial budget	Actual expenditure	Difference
Industry \$	6,000	6,000	6,000	0
Industry (in kind)	16,200	16,200	16,200	0
ACRDP (\$)	72,758	72,758	72,758	0
Other DFO (\$ and in kind)	4,000	4,000	4,000	0
Partners (\$ and in-kind)	1,750	1,750	1,750	0

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

- Identification of thousands of genes associated with the jaundice syndrome in liver and kidney tissue.
- Identification of piscine reovirus genomic signature that is highly consistent with the signature associated with the jaundice syndrome. Functional analysis of this signature reveals strong effects on immune response, proteolysis, metabolism, and cell cycle.
- Characterization of the epidemiology of jaundice syndrome, showing higher prevalence over multiple years at farm A than B, and low, but consistent levels of accumulative mortality occurring during Autumn/winter.
- With these data alone, we cannot determine whether the association of PRV with jaundice is through a cause and effect relationship; this will require a challenge study using cell free lysates or cultured virus. However, the lack of any indication of toxin activity within the genomic signature associated with jaundice reduces the likelihood that an environmental toxin is the main cause.

8. General Comments:

This project was undertaken to determine whether a jaundice syndrome associated with low-level mortality at one of Creative Salmon's farms was more likely caused by a viral infection or an environmental toxin. As a consequence of the low prevalence of the syndrome, only a small number of samples were able to be collected and evaluated in this study. The approach was to use salmon microarrays to determine the gene expression signature associated with the jaundice syndrome, contrasting fish from two farm sites with and without evidence of jaundice. Jaundice syndrome was predominantly diagnosed through clinical presentation (yellowing of the skin, pale gills [anemia], and pale livers).

We added to this project by including quantitative RT-PCR screening of 14 infectious agents, including 7 viruses. This approach yielded a highly significant association between jaundice syndrome and piscine reovirus (PRV). Genomic signatures resolved by statistical analyses driven by the presence of Jaundice syndrome, combined histological lesions associated with jaundice syndrome, PRV

load, and a metric combining all three indicators were highly congruent, with the three indicator metric providing the greatest transcriptional resolution among samples. These data suggest that diagnosis of the condition, as revealed by transcriptional variation, is most reliable if at least two of these indicators are present.

Reoviruses are non-enveloped viruses that contain a segmented double-stranded RNA genome. PRV is phylogenetically most similar to viruses in the orthoreovirus genus, with a genome consisting of 10 segments. Reoviruses in the Aquareovirus genus contain 11 segments; they infect salmon but are not associated with any lesions or disease.

There appears to be some discussion as to the effects of piscine reovirus infections. Piscine reovirus has been purported to be the causative agent of heart and skeletal muscle inflammatory (HSMI) syndrome in Atlantic salmon in Europe (Palacios et al. 2010; Finstad et al. 2012), whereby high viral loads in salmon in the ocean appears to cause HSMI in naturally and experimentally infected fish (Løvoll et al. 2012; Finstad et al. 2012). However, one report has found brood fish sampled in freshwater with abundant PRV in head kidney (not heart), that contained no evidence of the heart lesions traditionally associated with HSMI (Garseth et al. 2012).

We did not observe any of the classical heart lesions associated with HSMI in European Atlantic salmon, which include epi-, endo- and myocarditis and myocardial necrosis (Kongtorp et al. 2004) in Chinook salmon suffering from the Jaundice syndrome in BC. This finding was confirmed by a second opinion by Dr. Trygve Poppe from the Norwegian School of Veterinary Science.

The functional genomic signature associated with PRV load showed a strong induction of interferon and apoptosis pathways in kidney and liver tissue, which have also been associated with reovirus infections in mammals (Forrest and Dermody 2003). Reoviruses have also been associated with an immunosuppressive response in their host (Sharma et al. 1994).

Although a similar virus (PRV) may be associated with both jaundice syndrome in Chinook salmon and HSMI in Atlantic salmon in Europe, the clinical and histological presentations for these two conditions differ considerably between the species. While at the surface, these results may seem highly incongruent, they are not inconsistent with observations of other reovirus infections. In fact, in mice and humans reoviruses have been studied extensively due to the high degree of tissue tropism resultant from small mutations of the virus (reviewed in Forrest and Dermody 2003). Hence, speculation that different strains of PRV could be causative of pathological changes in the heart (HSMI) in European Atlantic salmon versus the liver and kidney (Jaundice syndrome) in Western North American Chinook salmon is not inconsistent with differential reovirus impacts on other species. Alternatively, PRV might replicate in fish with HSMI or jaundice

syndrome, but not be the cause. Or, PRV might contribute to the development of HSMI or jaundice syndrome without being the sole cause.

PART II

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

A Creative salmon farm-site on the west coast (farm A, Fig 1) has experienced consistent low level mortality with a unique clinical presentation of mild to severe yellow discolouration of the skin (jaundice) and pale gills. The cause has not been identified using standard diagnostic methods, but is hypothesized to be of either viral or environmental toxin origin. The project used a functional genomics approach to elucidate the genes differentially expressed in association with jaundice syndrome. The goal was to increase our understanding of the syndrome. The project also aimed to conduct a thorough epidemiological study to better understand why some farms are more affected and to determine the overall level of mortality attributable to the condition. The ultimate goal was to move closer towards identifying the cause of jaundice that will enable the farms to track, predict, and/or mitigate this syndrome.

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

Collections were made from two farm sites located on the west coast of Vancouver Island, A and B ,with farm A showing the highest incidence of mortality associated with the jaundice syndrome (see epidemiology) (Fig 1). Moribund or recently dead fish on farm A were collected by divers, and “healthy” swimming reference fish were collected from net pens using hook and line or during harvest in Farm B. From all fish, RNA was extracted from tissue samples of liver, kidney, heart, spleen and gill. The functional genomics study employed a 44K gene oligonucleotide salmonid microarray to identify genes correlated with the jaundice syndrome. Histopathology was done on 15 healthy and 13 freshly dead (less than 12 hours)/sick fish; incorrect preservation of samples collected on April 27, 2011 (6 healthy and 2 sick fish) precluded their analysis by histopathology. RNA was extracted from tissue samples of liver, kidney, heart, spleen and gill. The functional genomics study employed a 44K probe oligonucleotide salmonid microarray to identify genes correlated with the jaundice syndrome.

Thirty-five liver and thirty-six kidney samples were run on the arrays against a reference control containing RNA from all experimental samples and both tissues. The reference control is required to normalize variance in concentration of probes on the array, as well as array to array variability and is thus not meant to represent an experimental sample (i.e. this is different from the “reference” fish that do not show signs of jaundice which were also run individually on arrays and

contrasted with sick fish). After normalization, arrays were analysed statistically using T-tests to identify genes associated with jaundice syndrome, histological lesions associated with the jaundice syndrome, and with high loads of piscine reovirus (see below), and principal components analysis (PCA) was conducted to identify the major physiological trajectories of the data. Functional analyses were performed using Pathway Studio version 9.0.

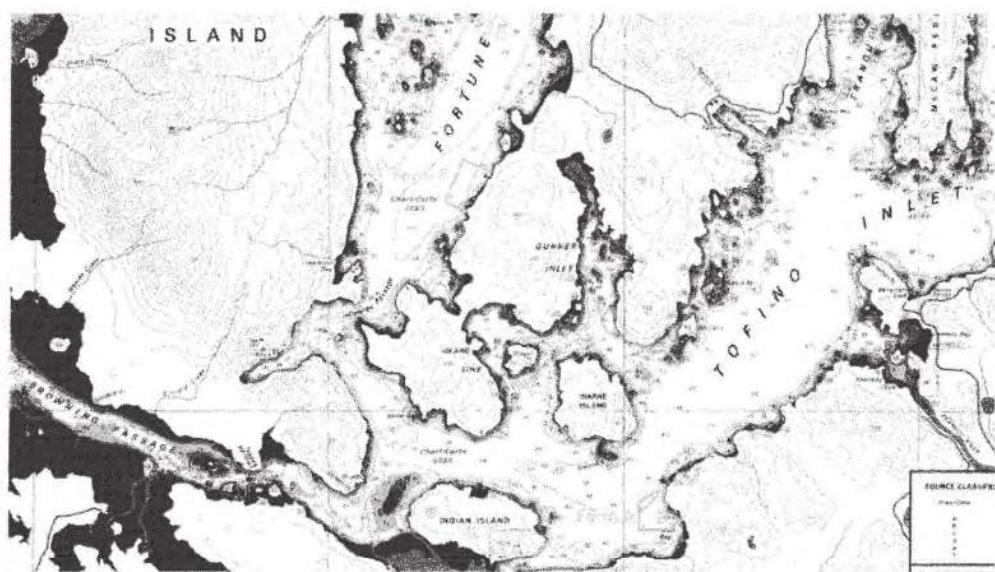


Figure 1. Location of Creative Salmon farm sites.

Quantitative RT-PCR was performed on a subset of host genes in gill tissue aimed at elucidating potential environmental effects (most notably salinity) on gene expression associated with jaundice. Gill tissue was not run on the arrays.

The only deviation from the original plan was the addition of a Fluidigm BioMark scan of infectious agents in liver tissue using published RT-PCR TaqMan assays for 13 infectious agents identified in association with mortality events in salmon and 1 newly identified microbe for which the association with disease is unknown. Correlation analyses were performed with each microbe surveyed to determine if any were associated with the jaundice syndrome. Piscine reovirus (PRV) is the only tested infectious agent that was correlated with the jaundice syndrome. Therefore, additional study was done to validate the PRV results. The other infectious agents were not considered further for this study. Additional study included ABI 7900 RT-PCR validation of PRV in liver, kidney, gill, spleen and heart tissues. Microscopic lesions that occurred with PRV CT's < 26 (indicating higher viral loads) were also identified.

11. Key results (include graphs, data tables, photos, etc. where applicable):

- A. Detailed deliverables of project

I. Epidemiological Analysis of the Jaundice Syndrome

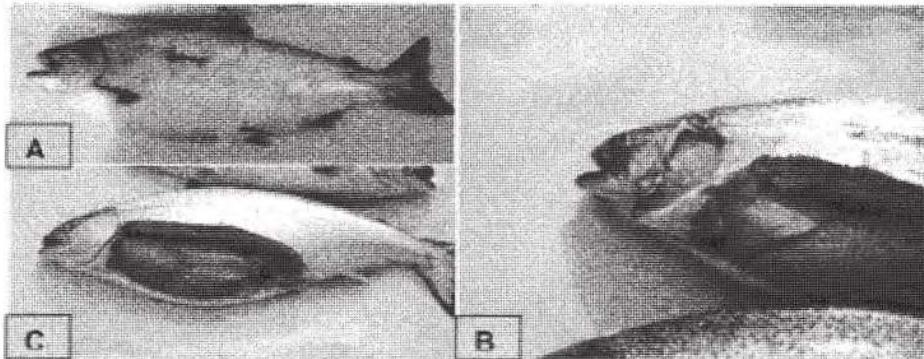


Figure 2. Clinical presentation of fish with Jaundice syndrome.

Farm level data collected from 2004 - 2012 (February) from two farms operated by Creative Salmon were examined by _____ from the BC Centre for Aquatic Health Sciences, Campbell River BC. These farms are located in Clayoquot Sound (Fig 1). Jaundice syndrome occurs at both sites, but it is more common at Farm A than Farm B. We examined both mortality data and environmental data from both sites to describe the epidemiological patterns of jaundice. Over the eight years both farms raised three separate generations of Chinook salmon (Table I). Stocking into Farm A consisted of spring entry smolts, while at Farm B, stocking occurred in the fall.

Farm A	Farm B
May, 2004- February 2006	October 2005 - May 2007
May 2008 - January 2010	September 2007 - August 2009
May 2010 - January 2012	September 2009 - October 2011

Table I. Stocking information for Farm A and B.

Jaundice syndrome is fairly easily diagnosed based on characteristic external signs (Fig 2). Chinook salmon with clinical signs of jaundice syndrome are commonly referred to as yellow fish. The fish exhibit external yellow coloration of the abdominal and periorbital region (Fig 2a). Most often, the gills are very pale, indicating severe anemia (Fig 2b). The fish appear anorexic with no food in the stomach. There are no signs of hemorrhaging or other abnormal findings in the viscera, although in some cases, the liver may have a jaundiced appearance (Fig 2b-c). Typically, neither slow swimmers nor increased morbidity are observed with this syndrome. Mortalities attributed to this syndrome appear to have a seasonal pattern. Monthly mortalities in the 2010-12 generation at Farm A with clinical signs of jaundice first occurred in the fall (October), eight months after seawater entry (Fig 3). Signs were first seen in Pen 1, and within three months all the pens had mortalities with signs of jaundice (Fig 4). Peak mortality occurred 4-5 months

after onset. Prevalence tends to decrease in the summer, with very few to no fish having clinical signs in August/September. Fish from 3 pens (2, 7 and 8) began to present with clinical signs again in November 2011, however prevalence was low (<0.1%). The staggered pattern in the peak monthly prevalence suggests possible pen to pen transmission of an infectious agent.

The fact that the progression through the system was not linear—pens 2 and 3 were the last to show clinical disease even though they were adjacent to pen 1—suggests that there may be other risk factors in play besides proximity to a diseased population. Monthly pen-level mortality for Farm A (2010-12 generation) showed that jaundice syndrome-associated mortality can vary considerably between pens with fish in Pen 6 having almost 20 times higher cumulative mortality than fish in Pen 1 (3.4% vs. 0.02%) (Fig 3). Incidentally, the index pen (Pen 1; the first pen to show signs of the syndrome) had the lowest cumulative mortality associated with this condition. Pen 6 differed from the other pens at Farm A and B in that it was a mixed stock (Big Qualicum and Robertson Creek cross) as well as mixed-sex with up to 25% of the population being males. All other pens were stocked with all females, Big Qualicum strain.

Prior to February 2005, no separate mortality classification for jaundiced fish was used; instead, fish with jaundice were included in an 'other' category. As a consequence, total cumulative mortalities attributed to this syndrome based on the records for the 2004-05 generation is estimated to be only 0.26%, however biologists (M. Tchipeff, personal communication) suggests that the levels were likely closer to 0.70%. In 2007-09 cumulative mortality due to jaundice syndrome on farm A was 1.0% and in 2010-11 it was 1.2%. Even though total cumulative mortality due to jaundice syndrome was quite low, during some months this syndrome can be the most significant cause of death noted in a pen. In the low prevalence site (Farm B), cumulative prevalence for the 3 generations was 0.03%, 0.02% and 0.10% respectively.

Several factors (including management and environmental) differ between the farm with the higher jaundice mortality (Farm A) and the farm with lower prevalence (Farm B). The first is stocking time. Farm B historically stocks in the autumn while Farm A stocks in the spring-time. The role of saltwater entry may need to be more closely examined to determine if there is any association between exposure and susceptibility of the fish. Environmental factors also differ between Farm A and Farm B. Farm B has considerably higher water flows than Farm A. Although the water temperatures do not differ between Farm A and B, Farm A has lower salinity than Farm B (Fig 5) by an average of 2 ppt.

In summary, some of the epidemiological trends appear to be:

- Overall prevalence is low, even on the more affected farm. However, the syndrome can account for 50% of the losses in a month in a single pen.

- There does not seem to be a significant change in prevalence between generations
- Significant pen to pen variation in prevalence.
- Seasonal pattern
 - Onset occurs in the fall, when both salinity and temperatures are declining
 - This seasonal pattern occurs regardless of whether fish are stocked the previous spring or fall.
- The farm with the lower salinity profile and lower flow (flushing) has a higher prevalence than the farm with higher salinity and flow (flushing).
- The pattern of occurrence in different pens is sporadic (i.e. not pen to adjacent pen) suggesting unknown risk factors. The low prevalence indicates that if the cause is infectious, the agent does not spread rapidly.
- Causative aetiology had not been identified using standard diagnostic methods, but new research elucidating a genomic signature consistent with viral activity and not an environmental toxicant response, and the identification of a candidate virus provides direction for future research.

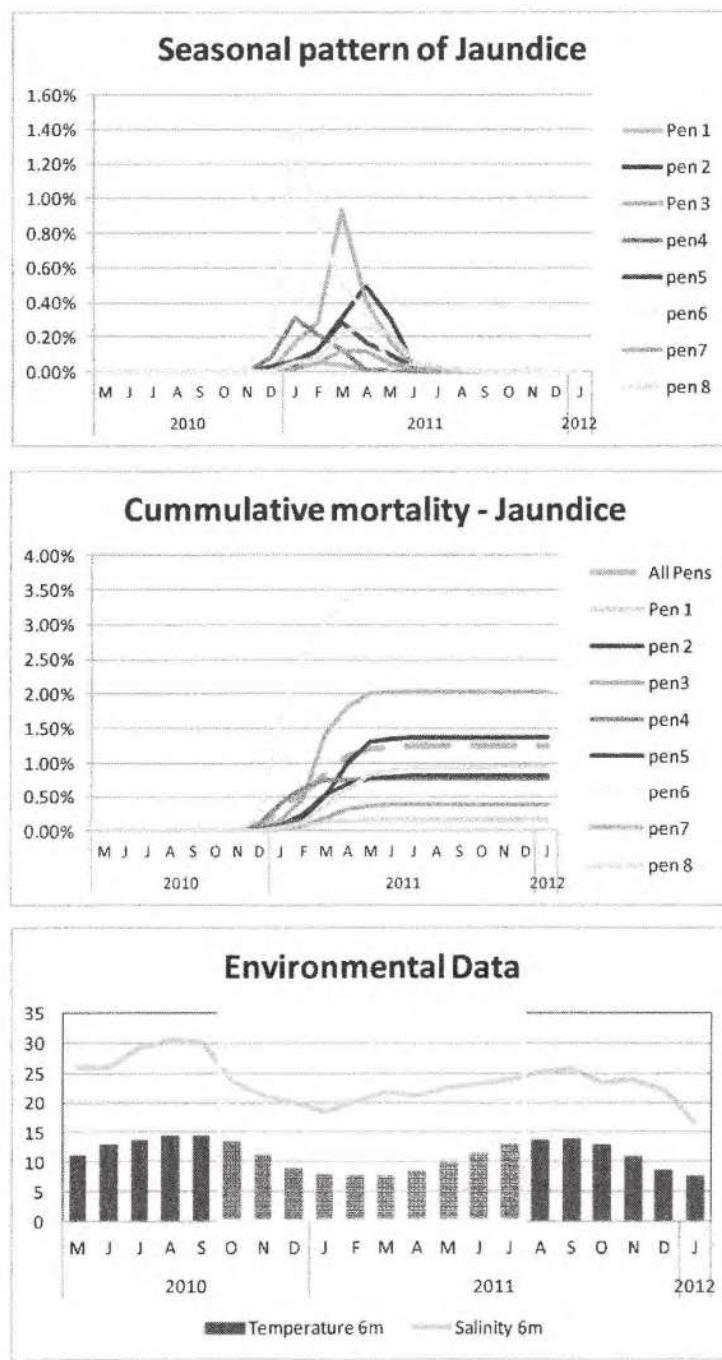


Figure 3. Monthly mortality for Jaundice syndrome and the total cumulative mortality at Farm A. The water temperature and salinity at 6m is also shown - shaded area is the period when Jaundice was observed.

Pen #2 January 2011	Pen #4 November 2010	Pen #6 November 2010	Pen #8 January 2011
Pen #1 October 2010	Pen #3 January 2011	Pen #5 December 2010	Pen #7 December 2010

Figure 4. The farm cage system at Farm A. Pens 1 and 2 were closest to land. The dates indicate the month when Jaundice syndrome was first observed in the pen.

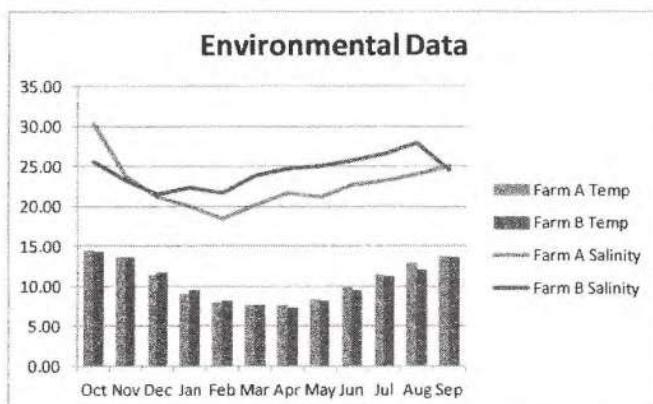


Figure 5. Mean Temperature and Salinity for Farm A and B (May 2010 - Sept 2011) at 6m.

II. Collections of tissues from jaundiced and healthy fish

Samples from 36 fish were collected over the course of the study (Table II). At farm A, where jaundice accounted for approximately 1% cumulative mortality, 11 fish with external signs of "jaundice", 3 with signs of anemia but not jaundice, and 13 reference fish that had no signs of jaundice or anemia (some had bacterial kidney disease, BKD) were collected across three dates: Feb 7, April 27, and May 24, 2011. At farm B, 2 fish with signs of jaundice and anemia and 7 reference fish were collected on April 27 and May 24.

Dr. Gary Marty, BC Animal Health Lab, performed histopathology on samples collected Feb 7 and May 24. The most severe lesions associated with jaundice syndrome involved necrosis and fibrin deposition, both of which result from acute tissue damage. Top lesions included renal tubular necrosis and interstitial cell necrosis in kidney, hepatocellular hydropic degeneration, a measure of sub-lethal cell damage in the liver, and parenchymal fibrin in spleen (Table III), with each of the salmon dying of jaundice containing at least one of these lesions with a severity of moderate to severe (score of 2-3). Moderate to severe renal tubular necrosis was considered perhaps the most classical histological sign of jaundice

syndrome, but two fish, 1065 and 1004, had this lesion with no outward signs of jaundice. However, microarray and viral data (see below) for fish 1004 was similar to jaundice syndrome fish. Other lesions more common in fish with the syndrome than in healthy fish include in liver, hepatocellular single cell necrosis, hepatic sinusoidal fibrin, hepatic sinusoidal congestion, and hepatocellular megalocystosis/karyomegaly; in kidney, interstitial cell hyperplasia, interstitial fibrin, and membranous glomerulonephritis; in heart, endocardial cell hypertrophy; and in spleen, leukocytic karyorrhexis indicative of white blood cell turnover (Table III). Many of these lesions could also be broadly classified as necrotic or involving fibrin deposition in other tissues. Most of the lesions are indicative of an acute process—probably no more than a few days from onset to death. This pattern is consistent with the clinical experience that sick fish are rarely detected before they die. Samples from fish with jaundice also had greater autolysis in the kidney, gill, and heart.

III. Functional genomics assessment of jaundiced fish

Microarrays were run on liver and kidney samples from all fish sampled in the project. Veterinarian diagnostics based on external fish appearance noted two common observations among the dying fish at Farm A, jaundice and anemia. There was some question as to whether these observations were related to the same syndrome, or constituted distinct disease states. We explored the microarray data to test the hypothesis that jaundice and anemia were not part of the same physiological phenomenon—i.e. anemia was not associated with the jaundice syndrome. If this were the case, we would expect that unique genomic signatures would be resolved when each of jaundice and anemia were assessed relative to “healthy” control fish, and that when they were assessed together as “diseased” fish relative to “healthy” controls, the genomic signature would be weakened.

We contrasted separately in each of liver and kidney fish with external signs of 1) jaundice, 2) anemia, and 3) jaundice or anemia with “healthy controls”. Healthy controls were fish with no signs of BKD and that did not carry anemia alone (for the jaundice analysis). There were no fish characterized as jaundice alone, but a number of fish were not assessed for anemia—these fish were only removed for the anemia analysis (Table II).

In both liver and kidney tissue, the strongest signature (most significant genes) was observed when anemia and jaundice were combined (Table IV), which did not support the hypothesis that these observations were unrelated. For analyses driven by anemia and jaundice alone, we applied three approaches to assess the degree of correlation between the resultant genomic signatures: 1) we ran a bootstrap re-sampling correlation analysis on the respective gene lists, and obtained a correlation of >99.9% (i.e. less than 0.1% chance that the correlation in gene loadings in these analyses would be due to chance). 2) We assessed the

degree of overlap in the top 100, 500 and (for kidney) 1000 genes for each of liver and kidney. For liver, taking the top 100 significant genes for anemia, 96% were also significant for jaundice, top 500 yielded 86% overlap with jaundice significant genes. For kidney, the top 100 anemia genes yielded 92% overlap with jaundice, while the top 500 was 74%, and top 1000 was 65%, 3) we ran hierarchical cluster analysis putting all samples not used in the statistical analysis back in for sample clustering, and determined whether clustering of fish was divergent in each of the analyses (as would be expected if they were uncorrelated physiological phenomena). This analysis clusters both genes and individuals according to their degree of correlation. Table IV shows the classification of fish based on these analyses, with "A" clusters referring to clusters highly loaded with compromised fish, and "B" clusters containing the "healthy" controls, and Figure 6 shows the hierarchical clusters associated with the combined jaundice/anemia analyses in liver and kidney. Two of the three anemia only fish (1000 and 1005) clustered consistently in the "A" cluster no matter which drivers were used in the analysis (anemia, jaundice, or anemia/jaundice), while the third (1001) was more variable, clustering with "B" in the liver jaundice alone analysis, and with an intermediate sub-cluster "a" for both combined analyses. These data do not support the hypothesis that jaundice and anemia are derived from separate physiological mechanisms.

Taken together, these analyses suggest that anemia and jaundice are associated, and together they may drive the physiological changes culminating in the disease state of the jaundice syndrome. Hence, for the remaining analyses, we combine jaundice/anemia as a single metric for our analyses. We note, however, that anemia is not pathognomonic; hence, the presence of anemia alone in a group of fish showing no signs of jaundice is not likely to be diagnostic for jaundice syndrome.

Hierarchical cluster analysis revealed a small number of "intermediate" fish that did not cluster consistently between analyses. These included two fish from farm A that were positive for BKD (1012, 1013) which often clustered with "A", but in both combined analyses were in the intermediate "a" cluster that did not show a strong pattern of differential regulation for the top 100 significant genes (i.e. no strong demarcation of "blue" and "yellow" genes denoting down- and up-regulated genes differentiating the two main clusters). One fish that was positive for jaundice and anemia (1006) on farm B was also intermediate in the jaundice/anemia clustering and was in the "B" cluster for the kidney jaundice analysis and the "A" cluster for other analyses. This fish, as well as fish 1007 which clustered consistently with "B", was collected during harvest and diagnosed by a different veterinarian (from those at farm A); these fish did not die of jaundice and were also not assessed for histopathology. These data potentially suggest that jaundice in Farm B is physiologically different from that in Farm A, at a considerably weaker stage of development, or may have been misdiagnosed. One "control" fish (1004) from farm A with no external signs of jaundice or anemia also occupied an intermediate position in the jaundice/anemia analysis and

clustered with "B" for the kidney jaundice analysis and "A" for remaining analyses. Histological analysis showed severe renal tubular necrosis, the lesion most consistently associated with the jaundice syndrome (see below). This fish was also positive for the piscine reovirus (described below).

A t-test between the two farm sites, performed to determine the relative role of transcriptional variance stimulated by environmental differences between sites, did not yield a highly significant list of genes that were different between the farms, with only 39 genes in Liver and 3 in Kidney significant at 5% FDR. These data suggest that environmental differences alone were not likely to be causative of the jaundice syndrome.

Fish #	Site	Date sampled	Jaundice	Anemia	Other	Reference	Cause of Death	Fluidigm Liver CT	ABI Liver CT	ABI Kidney CT	ABI Gill CT	ABI Heart CT	ABI Spleen CT
1050	A	24/05/2011	1	ND	0	0	HHD	17.1	25.2	23.0	23.7	22.7	24.4
1051	A	24/05/2011	1	ND	0	0	RTN	17.9	23.9	22.2	23.2	20.7	19.5
1052	A	24/05/2011	1	ND	0	0	HHD	17.2	24.0	21.4	24.7	20.5	29.2
1053	A	24/05/2011	1	ND	0	0	RTN*	20.0	25.5	21.6	21.5	19.1	19.0
1054	A	24/05/2011	1	ND	0	0	PFB	17.4	24.5	22.2	24.7	22.0	20.6
1055	A	24/05/2011	1	ND	0	0	RTN,ICN	16.7	23.5	21.5	21.0	20.6	nil
1056	A	24/05/2011	1	ND	0	0	RTN	18.4	24.8	19.2	21.8	18.6	19.3
1002	A	07/02/2011	1	1	0	0	RTH,HHD	15.1	22.3	21.9	24.7	20.7	20.4
1003	A	07/02/2011	1	1	0	0	RTN	18.3	24.6	21.4	22.8	20.7	18.9
1010	A	27/04/2011	1	1	0	0	ND	17.5	24.4	22.2	22.8	21.9	20.4
1011	A	27/04/2011	1	1	0	0	ND	18.1	24.0	21.3	21.7	20.8	20.7
1006	B	27/04/2011	1	1	0	0	Percussion	nil	35.9	32.9	34.4	nil	34.8
1007	B	27/04/2011	1	1	BKD	0	Percussion	nil	nil	35.9	34.0	nil	33.3
1000	A	07/02/2011	0	1	0	0	RTN	18.4	24.6	22.3	23.4	20.3	22.6
1001	A	07/02/2011	0	1	0	0	HHD	nil	33.7	31.3	28.8	33.9	31.9
1005	A	07/02/2011	0	1	0	0	SCN,HHD	27.7	34.6	32.2	30.4	35.7	34.7
1004	A	07/02/2011	0	0	Hern	0	RTN	16.7	23.7	22.7	22.6	nil	22.6
1060	A	24/05/2011	0	0	0	1	none	24.5	30.0	23.5	26.4	27.5	25.7
1062	A	24/05/2011	0	0	0	1	Percussion	nil	nil	30.0	27.5	29.3	29.4
1064	A	24/05/2011	0	0	0	1	none	27.2	32.4	29.6	29.5	28.2	27.5
1061	A	24/05/2011	0	0	0	1	none	nil	nil	31.7	30.5	32.3	32.3
1063	A	24/05/2011	0	0	0	1	none	nil	35.8	34.0	27.8	32.6	33.3
1012	A	27/04/2011	0	0	BKD	1	ND	26.1	33.8	32.8	30.9	30.8	32.3
1013	A	27/04/2011	0	0	BKD	1	ND	27.7	32.6	29.6	30.8	31.7	30.3
1057	A	24/05/2011	0	0	0	1	Percussion	nil	37.1	35.4	30.9	34.3	31.8
1058	A	24/05/2011	0	0	0	1	none	28.0	35.3	33.6	32.2	nil	33.0
1059	A	24/05/2011	0	0	0	1	Percussion	nil	34.9	31.3	30.6	31.5	28.0
1065	A	24/05/2011	0	0	0	1	Percussion	nil	35.6	nil	nil	31.8	34.7
1066	A	24/05/2011	0	0	0	1	Percussion	nil	nil	35.9	35.9	nil	35.7
1008	B	27/04/2011	0	0	0	1	Percussion	nil	nil	34.2	34.3	32.0	35.8
1009	B	27/04/2011	0	0	0	1	Percussion	nil	33.8	34.4	33.9	29.4	34.5
1067	B	24/05/2011	0	0	0	1	Percussion	nil	35.8	33.4	34.3	35.8	35.2
1068	B	24/05/2011	0	0	0	1	Percussion	nil	nil	32.9	nil	34.5	33.8
1069	B	24/05/2011	0	0	BKD	1	Percussion	nil	34.4	33.8	34.5	32.7	34.4
1070	B	24/05/2011	0	0	0	1	Percussion	nil	nil	35.9	28.7	nil	33.5
1071	B	24/05/2011	0	0	0	1	Percussion	nil	37.9	34.7	35.2	34.7	33.9

Histology Scoring Key:

RTN – renal tubular necrosis (kidney)

HHD – hepatocellular hydropic degeneration (liver)

PFB – parenchymal fibrin (spleen)

ICN – interstitial cell necrosis (kidney)

Percussion – fish killed by a blow to the head causing brain hemorrhage

RTH = renal tubular hydropic degeneration

SCN = hepatocellular single cell necrosis/apoptosis

Table II. Data table showing fish sampled at two farm sites (A, B). Fish were scored for external signs of jaundice, anemia, other (e.g. BKD, hemorrhaging), and reference samples (each scored "1" if positive, "0" if negative, "ND" for no data), histological lesions diagnosed as the probable cause of death or morbidity ("ND" indicates no histopathology was performed), and Threshold Cycle (CT) values (average of duplicate samples) of RT-PCR analysis for Piscine Reovirus (PRV) run on the Fluidigm (liver) and ABI 7900 (all tissues) quantitative PCR instruments. CT's from the ABI-7900 that were below 26 (indicating higher viral loads) are highlighted in red, CT's between 26-30 are highlighted in gold. CT's >30 were not necessarily repeatable on the ABI 7900 Fluidigm, and may, in fact, be negative, but are shown for completeness as these data came too late to incorporate into the analysis, we show the original scoring in the table. Note that while the cause of death for fish 153 is noted as RTN, this fish was sampled as a moribund, rather than a fresh mortality, with percussion the actual "cause" of death.

Fish Number	Collection Date	Site	Jaundice	Anemia	Lower CT	SCN	HHD	SSF	SCM	MEG	MGN	Summed Histology Score	Combined Index Score	Kidney CT	Katty	Gill CT	Gatty	Heart CT	Spleen CT	PFB	LKR	Liver Microarray Group	Kidney Microarray Group	PRV Liver Microarray Group	PRV Kidney Microarray Group					
1055	May 24, 2011	A	X	ND	23.5	1	0	0	0	0	0	3	3	21.5	1	2	0	1	3	21.0	2	20.6	0	0	A	A	A			
1051	May 24, 2011	A	X	ND	23.8	1	0	0	0	0	0	1	3	22.2	1	3	1	0	0	23.2	2	20.7	1	0	19.5	0	A	A		
1052	May 24, 2011	A	X	ND	24.0	1	2	1	0	0	0	4	3	21.4	2	0	1	0	1	3	24.7	2	20.5	1	0	29.2	1	A	A	
1054	May 24, 2011	A	X	ND	24.5	1	0	1	0	0	2	3	22.2	1	0	0	1	2	0	3	24.7	2	22.0	0	0	20.6	2	A	A	
1056	May 24, 2011	A	X	ND	24.8	1	0	0	0	0	0	1	3	19.2	0	3	0	0	0	3	21.8	1	18.6	0	0	19.3	0	A	ND	
1050	May 24, 2011	A	X	ND	25.2	1	2	1	0	1	5	3	23.0	2	0	1	0	0	0	1	3	23.7	2	22.7	0	1	24.4	0	A	A
1053	May 24, 2011	A	X	ND	25.5	0	0	0	0	0	0	2	3	21.6	1	3	0	0	0	3	21.5	1	19.1	0	0	19.0	0	A	A	
1000	February 7, 2011	A	0	X	24.6	1	0	0	0	0	0	1	3	22.3	ND	3	0	0	0	3	3	23.4	ND	20.3	0	ND	22.6	0	A	A
1001	February 7, 2011	A	0	X	33.7	1	3	0	0	0	4	2	31.3	ND	0	0	0	0	0	1	28.8	ND	33.9	0	ND	31.9	0	A	A	
1002	February 7, 2011	A	X	X	22.3	1	3	0	0	0	4	3	21.9	ND	1	1	0	0	0	2	3	24.7	ND	20.7	0	ND	23.4	1	A	A
1003	February 7, 2011	A	X	X	24.6	2	1	0	0	0	3	3	21.4	ND	3	0	0	0	0	2	22.8	ND	26.7	0	ND	18.9	0	A	A	
1009	February 7, 2011	A	0	0	23.7	2	0	0	0	0	2	2	22.7	ND	3	0	0	0	3	2	22.6	ND	ND	0	0	22.6	0	A	A	
1005	February 7, 2011	A	0	X	34.6	2	2	0	0	0	4	2	32.2	ND	0	1	0	0	0	4	2	30.4	ND	35.7	0	ND	34.7	0	A	A
1060	May 24, 2011	A	0	0	30.0	0	0	0	0	0	0	0	23.5	1	0	0	0	0	0	1	26.4	1	27.5	0	0	25.7	0	B	I	
1064	May 24, 2011	A	0	0	32.4	0	0	0	0	0	0	0	29.6	1	0	0	0	0	0	0	29.5	1	28.2	0	0	27.5	0	B	B	
1069	May 24, 2011	B	0	0	34.4	0	0	0	0	0	0	0	33.8	0	0	0	0	0	0	0	34.5	1	32.7	0	0	34.4	0	B	B	
1059	May 24, 2011	A	0	0	34.9	0	0	0	0	0	0	0	31.3	0	0	0	0	0	0	0	30.6	1	31.5	0	0	28.0	0	B	B	
1058	May 24, 2011	A	0	0	35.3	0	0	0	0	0	0	0	33.6	0	0	0	0	0	0	0	32.2	1	nil	0	0	33.0	0	ND	B	
1065	May 24, 2011	A	0	0	35.6	0	0	0	0	0	0	0	nil	0	1	0	0	0	0	1	1	nil	1	31.8	0	0	34.7	0	B	B
1063	May 24, 2011	A	0	0	35.8	0	0	0	0	0	0	0	34.0	0	0	0	0	0	0	0	0	27.8	1	32.0	0	0	33.3	0	ND	B
1067	May 24, 2011	B	0	0	35.8	0	0	0	0	0	0	0	33.4	0	0	0	0	0	0	0	34.3	1	35.8	0	0	35.2	0	B	B	
1057	May 24, 2011	A	0	0	37.1	0	0	0	0	0	0	0	35.4	0	0	0	0	0	0	0	30.9	1	34.3	0	0	31.8	0	B	B	
1071	May 24, 2011	B	0	0	37.9	0	0	0	0	0	0	0	34.7	0	0	0	0	0	0	0	35.2	1	34.7	0	0	33.9	0	0	ND	B
1062	May 24, 2011	A	0	0	nil	0	0	0	0	0	0	0	30.0	0	0	0	0	0	0	0	27.5	0	29.3	0	0	29.4	0	0	B	B
1070	May 24, 2011	B	0	0	nil	0	0	0	0	0	0	0	35.9	0	0	0	0	0	0	0	28.7	1	nil	0	0	33.5	0	0	B	B
1061	May 24, 2011	A	0	0	nil	0	0	0	0	0	0	0	31.7	0	0	0	0	0	0	0	30.5	1	32.3	0	0	32.3	0	0	B	B
1066	May 24, 2011	A	0	0	nil	0	0	0	0	0	0	0	35.9	1	0	0	0	0	0	0	35.9	1	nil	0	0	35.7	0	0	B	B
1068	May 24, 2011	B	0	0	nil	0	0	0	0	0	0	0	32.9	0	0	0	0	0	0	0	34.6	0	0	33.8	0	0	B	B		

Histology Scoring Key:

SCN – hepatocellular single cell necrosis
 HHD – hepatocellular hydropic degeneration
 SSF – hepatic sinusoidal fibrin
 SCM – hepatic sinusoidal congestion
 Katty – kidney autolysis
 RTN – renal tubular necrosis
 ISH – interstitial cell hyperplasia
 IFB – interstitial fibrin
 ICN – interstitial cell necrosis
 MGN – membranous glomerulonephritis
 Gatty – gill autolysis
 Hatty – heart autolysis
 ECH – endocardial cell hypertrophy
 PFB – parenchymal fibrin
 LKR – leukocytic karyorrhexis

Table III. Detailed data on histological analyses, showing microscopic findings (scored on an increasing intensity scale of 0-3) with high correlation scores with jaundice/anemia (combined) and Piscine Reovirus (CT values from ABI 7900), and cluster group in microarray analyses, performed using jaundice/anemia (first two columns) or Piscine Reovirus (PRV) CT value as measured variables (CT<26 versus all others). CT values reflect the average of duplicate assays, with values <26 indicating high viral loads (10^5 - 10^6 copies per μ l) and highlighted in red, and values between 26 and 30 indicating low loads and highlighted in gold. CT's > 26 do not correlate with the jaundice syndrome, and may indicate a carrier state of the virus. When comparing fish with HSMI and fish with no disease, the suggested PRV CT cutoff value for HSMI diagnosis in Atlantic salmon is 28 (Løvoll et al. 2012). ND indicates no data.

Fish #	Liver Jaundice Only Analysis	Liver Anemia Only Analysis	Liver Jaundice or Anemia	Kidney Jaundice Only Analysis	Kidney Anemia Only Analysis	Kidney Jaundice/Anemia	FINAL SCORE	Positive	Negative	Site
# genes at q<0.05	1736	523	4301	1997	2394	3760				
1002	A	A	a	A	A	a	J-A-H-PRV			A
1003	A	A	A	A	A	A	J-A-H-PRV			A
1010	A	A	A	A	A	A	JA-PRV			A
1011	A	A	A	ND	ND	ND	J-A-PRV			A
1050	A	A	A	A	A	A	J-H-PRV			A
1051	A	A	A	A	A	a	J-H-PRV			A
1052	A	A	A	A	A	A	J-H-PRV			A
1053	A	A	A	A	A	A	J-H-PRV			A
1054	A	A	A	A	A	A	J-H-PRV			A
1055	A	A	A	A	A	A	J-H-PRV			A
1056	A	A	A	A	A	a	J-H-PRV			A
1000	A	A	A	A	A	A	A+H-PRV	J		A
1005	A	A	A	A	A	A	A-H	J-PRV		A
1001	B	A	a	A	A	a	Intermediate	A-H	J-PRV	A
1013	A	A	a	A	A	a	Intermediate	BKD	J-A-PRV	A
1012	A	A	a	A	A	a	Intermediate	BKD	J-A-PRV	A
1006	A	A	a	B	A	a	Intermediate	J-A	PRV	B
1004	A	A	a	B	A	a	Intermediate	H-PRV	J-A	A
1068	B	B	B	B	A	B	Intermediate	J-A-H-PRV		B
1060	B	B	B	B	B	B	PRV (Kidney)	J-A-H		A
1009	B	B	B	B	B	B		J-A-PRV		B
1064	B	B	B	B	B	B		J-A-H-PRV		A
1007	B	B	a	B	B	B		J-A-BKD	PRV	B
1008	B	B	B	ND	ND	ND		J-A-PRV		B
1057	B	B	B	B	B	B		J-A-H-PRV		A
1058	B	B	B	B	B	B		J-A-H-PRV		A
1059	B	B	B	B	B	B		J-A-H-PRV		A
1061	B	B	B	B	B	B		J-A-H-PRV		A
1062	B	B	B	B	B	B		J-A-H-PRV		A
1063	B	B	B	B	B	B		J-A-H-PRV		A
1065	B	B	B	B	B	B		J-A-H-PRV		A
1066	B	B	B	B	B	B		J-A-H-PRV		A
1067	B	B	B	B	B	B		J-A-H-PRV		B
1069	B	B	B	B	B	B	BKD	J-A-H-PRV		B
1070	B	B	B	B	B	B		J-A-H-PRV		B
1071	B	B	a	B	B	B		J-A-H-PRV		B

Table IV. Classification of individual fish based on hierarchical cluster analyses driven by t-tests of liver and kidney transcriptional (microarray) data between "healthy" fish (no BKD, no anemia only for jaundice alone analysis, no fish not scored for anemia for anemia analysis [see Table II]) versus fish with external signs of jaundice, anemia, or jaundice/anemia combined. Cluster "A" is

the cluster highly loaded with "compromised" fish and cluster "B" with "healthy" controls. Cluster "a" was an intermediate sub-cluster that contained a combination of control, BKD infected, and jaundice/anemia fish; fish in this cluster did not show a strong pattern of segregation between the top up- and down-regulated genes (indicated by the darker coloring in the figure). Under the "positive" and "negative" columns, the presence of each of the "indicators" scored in the study are shown as follows: J=jaundice, A=anemia, H=histological lesions associated with jaundice syndrome, PRV=piscine reovirus with CT<26, BKD=bacterial kidney disease. Fish 1069 was questionably BKD positive.

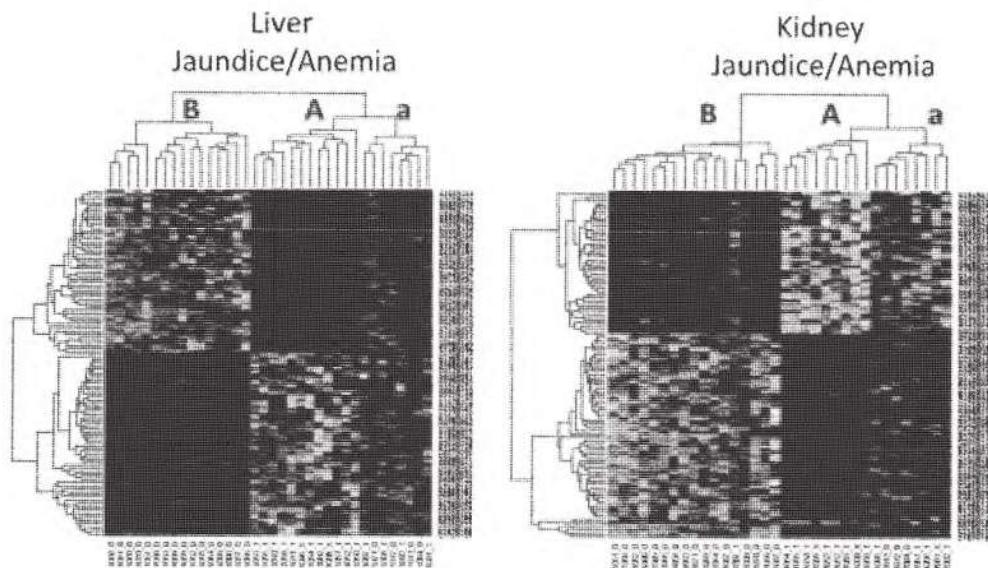


Figure 6. Hierarchical clustering of liver (left) and kidney (right) samples based upon gene lists significantly correlated with jaundice/anemia (top 100 genes). Individuals are clustered on the x-axis, with 1-denoting jaundice/anemia (Note in some fish, anemia was not assessed; see Table II), X-denoting anemia alone, and 0-denoting no jaundice or anemia. Genes are clustered on the y-axis, with yellow denoting up-regulated genes, blue down-regulated. Cluster "B" was highly loaded with fish with no outward signs of jaundice and/or anemia, while "B" was highly loaded with jaundice/anemia positive fish. Sub-cluster "a" were fish that were more intermediate in nature, with a more limited pattern of variation based on the top 100 genes (generally not showing the strongly down-regulated genes in "A"). These fish were a mixture largely of controls, BKD positive fish, and jaundice/anemia fish from Farm-site B. One jaundice/anemia fish and one anemia only fish from Farm-site A were also in cluster "a".

IV. Survey of Infectious agents (added to project)

The Molecular Genetics Laboratory has been developing a novel high throughput approach to microbe surveillance based on TaqMan assays applied on a microfluidics platform (Fluidigm BioMark; Fluidigm corp., California). This

application is at the developmental stage of a project that will be undertaken to conduct an extensive inventory of microbes carried by salmon in BC, and is intended to include microbes associated with, or suspected to associate with disease in salmon throughout the world. We had been developing this platform at the same time we were undertaking this Chinook salmon jaundice study, and decided to apply the assays largely to understudied microbes that we had up and running on the platform to determine if any of these microbes were correlated with jaundice. TaqMan assays for 4 bacterial pathogens, 7 viruses, and 3 intracellular parasites known or assumed to associate with disease and mortality in salmon were obtained from the primary literature (with the exception of a parvovirus sequence recently identified in our lab). These assays were performed simultaneously in triplicate on all liver samples using the Fluidigm BioMark system. One infectious agent, piscine reovirus (PRV), occurred at high viral loads in liver (Fluidigm [critical threshold] CT's <20) that were correlated with jaundice syndrome (scored as a combination of clinical presentation of the syndrome (jaundice +/- anemia and incorporating histology results) ($R^2=0.649$, $p=2.35\times 10^{-7}$). No other infectious agents showed any correlations with jaundice, nor were most observed at appreciable copy numbers.

Quantitative PCR with Piscine Reovirus (PRV) was additionally performed on the ABI 7900 platform using template RNA from gill, heart, kidney, liver and spleen tissue, with each tissue run in duplicate (Tables II and III). Efficiency of this assay on both platforms was high, and highly similar (Fluidigm: $E=0.99$, slope=-3.35, $R^2=0.998$; ABI: $E=0.98$, slope=-3.36, $R^2=0.997$). There was a high correlation between PRV CT's determined on Fluidigm BioMark versus the ABI 7900 platform ($R^2=0.956$, $p=3.15\times 10^{-13}$), although CT's were 6.5 cycles lower on the Fluidigm (Fig 7). The limit of detection (LOD) on the Fluidigm was between 28-29 CT's, above which results were not 100% repeatable. The LOD on the 7900 was around 35 CT's.

There was a notable 5 CT breakpoint in the data between fish showing clinical signs of the jaundice syndrome and those that were asymptomatic (Fig 7). Fish with CT's < 26 on the ABI 7900 (indicating higher viral loads) carried multi-tissue infections at higher loads (Tables II and III), and all but one of these fish died of jaundice syndrome. Most fish with CT's >30 had no external signs of jaundice or significant jaundice-associated histological lesions, but two fish (1001 and 1005) showing some evidence of jaundice syndrome had CTs >30. These two fish also showed variation in other indicators (1001 was not positive for any histological lesions in kidney, 1005 had only a weak kidney lesion and was not scored as jaundice positive). Two non-moribund fish from Farm B diagnosed by a Creative Salmon fish health technician as having jaundice syndrome also had PRV CT's ≥ 30 . These fish (from Farm B) did not cluster with fish carrying the jaundice syndrome in Farm A, suggesting that they were physiologically distinct. Unfortunately, the histology samples were not preserved properly, so no histopathology data were available to confirm or refute whether they showed signs of the jaundice syndrome at the cellular level.

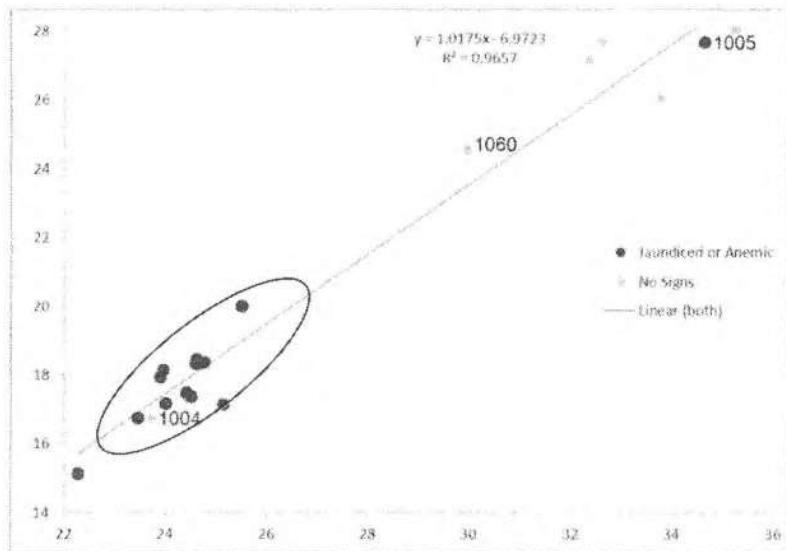


Figure 7 Plot showing correlation between liver PRV RT-PCR assays run on two platforms: Fluidigm and the ABI 7900 (liver tissue only). Data were highly correlated ($R^2=96.5$, $p<10^{-12}$). Only fish with CT values on both platforms ($n=19$) are shown; not shown are 10 samples with no CT on the Fluidigm platform and seven samples with no CT on either platform. Fish classified externally as jaundiced or anemia positive are shown in blue; fish without signs of jaundice or anemia in red. The cluster of jaundice-presenting fish is circled. Fish 1004, which was not classified as jaundice or anemia positive but clustered with the high PRV CT group was also classified at RTN positive through histology, and was transcriptionally classified in the A cluster containing jaundice syndrome fish. Fish 1005 was classified as anemia positive, not jaundice positive.

V. Additional Microarray analyses

We conducted further analyses of the microarray data to determine which measured variables (indicators) correlated with jaundice presentation in our study provided the greatest physiological resolution (measure by gene expression profiling) among salmon sampled on the farms. Among the indicators tested were the veterinary scores of jaundice/anemia, the individual histological lesions correlated with jaundice in each tissue, a summed histology score whereby histology scores (0-3) were summed for syndrome-correlated lesions within each tissue, and PRV CT (contrasting CT<26 versus all other samples) (see Table III for indicators). These analyses were only performed on fish that contained histology data.

For analyses in both liver and kidney, the combined indicator score—which took into account whether fish were jaundice/anemia positive, histology positive (for at least one lesion listed in Table III; note these are not pathognomonic), and carried high loads of PRV—generally provided the greatest transcriptional resolution between fish sampled on the farms, with 3,449 differentially regulated genes in liver and 3,864 in kidney (FDR <0.05; Table V). These data indicate that all three of these indicators may be contributing to the transcriptional (i.e. physiological) shifts associated with disease on the farms. Hierarchical clustering (not shown) revealed that in both liver and kidney, all fish that were positive for all three indicators clustered tightly together ("A" positive cluster). Fish that were positive for two indicators also grouped in the A cluster, regardless of which indicator was not present. Three kidney samples were positive for only a single indicator, and two of the three clustered in the B (negative) grouping of samples. One of these (1060) was positive only for PRV but did not carry high loads in all tissues. The other (1065) was scored positive for RTN, but this lesion was weak as it was only observed in a single tubule. All three samples with anemia but not jaundice (1000, 1001, and 1005) grouped within the A (positive) cluster, again suggesting that anemia, while not pathognomonic, is a physiological change that can be associated with the jaundice syndrome.

While jaundice alone did not generally provide as high a resolution of the microarray data, the combined scoring of jaundice and anemia provided a high degree of resolution in kidney (3,994 genes) and liver (2,926 genes). These data suggest that fish may experience the physiological shifts associated with the jaundice syndrome before external or internal yellowing is detectable, and that some of these may be resolved if signs of anemia are present.

PRV load was also a powerful indicator resolving transcriptional variation among fish for liver (3,352 genes) and kidney (2,212 genes) tissues (Table V; Fig 8). The summed histology scores provided a weaker, albeit still strong, signal for both liver and kidney tissues (1,612 and 1,280 genes, respectively). In general, the individual histological lesions were the least powerful, likely because fewer fish were affected by single lesions (reducing power). RTN in Kidney and SCN in liver

had the strongest single lesion transcriptional signals; however these also affected the most fish.

Analysis Driver	# Positive samples	# Significant Probes
Liver		
Combined Indicator Score	13	3449
PRV <26	11	3352
Jaundice	9	1017
Jaundice/Anemia	12	2926
Summed Histo Score	12	1612
SCN	12	1913
SSF	3	218
HHD	6	33
Kidney		
Combined Indicator Score	15	3884
PRV <26	12	2212
Jaundice	9	632
Jaundice/Anemia	12	3994
Summed Histo Score	12	1280
KAty	9	135
RTN	9	541
ISH	5	70
IFB	3	4
MGN	3	0

Table V. Statistical analysis of microarray data, including only samples that contained histology. Three key indicators associated with the jaundice syndrome have been defined in this study: PRV CT<26, presence of jaundice and/or anemia, and presence of one of the histological lesions associated with jaundice (see Table III for lesion abbreviations). Microarray analysis was driven by each of these indicators, as well as a combined indicator score whereby each indicator was given a score of 1 if positive, and indicators were summed (maximum of 3 if all were positive, 0 if none were positive; the combined score ignored jaundice only as an indicator, as jaundice/anemia was a more powerful transcriptional signature), and a summed histology score, which took the individual lesion scores (minus KAty, which is a measure of tissue integrity assumed to correlate with postmortem cell lysis) defined in Table III (by tissue) and summed them. In this way, intensity of damage was taken into account, as individuals with more lesions or higher scores for individual lesions were ranked higher than those with single less damaging lesions. # Positive samples indicates those samples positive for the analysis driver in question. # PS entities is the number of genes with gene identifiers that could be mapped in for functional analysis in Pathway Studio. ND indicates no data.

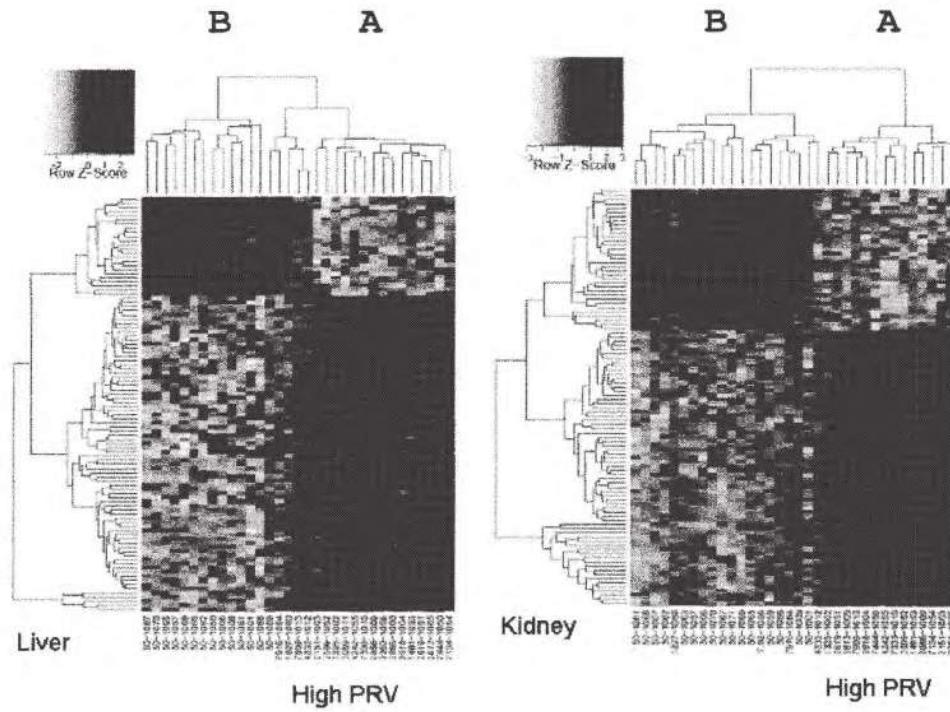


Figure 8. Hierarchical clustering of liver (left) and kidney (right) samples based upon gene-lists significantly correlated with PRV loads (5% FDR). The top 100 ranked genes were used in the cluster plots. Fish with high PRV loads ($CT < 26$) generally clustered in "A" while those with no or low loads generally clustered in "B"; fish 1005 was a notable exception and clustered within "A" for both tissues but did not carry a high PRV load. Fish 1013, a BKD positive fish, clustered in "A" for kidney only. Genes are shown on the vertical axis, with yellow denoting genes up-regulated, blue down-regulated. Individual fish are clustered on the horizontal axis. This figure shows the results for all samples run on microarrays (not just histology samples).

V.i. Functional Analysis of Microarray Data

Within tissues, there was a high degree of overlap between the significant gene lists generated from each of the indicators analyzed, not surprising given the extensive overlap in positive samples. Using PathWay Studio, we conducted functional analyses on each of the gene sets shown in Table V using three analytical approaches—(1) gene-set enrichment analysis, which ranks genes by their relative fold change between positive and negative samples and contrasts their gene ontologies (which map the biological and molecular processes for which each gene is active) with those of the entire gene list to identify processes that are over-represented in highly differentially expressed genes; (2) Pathway enrichment analysis, which identifies the key biological processes represented in only the most highly significant genes; and (3) Sub-network enrichment analysis, which identifies the key transcriptional regulators significantly associated with the highly significant genes.

Functional analyses of each of the datasets also showed a high degree of congruence (Tables VI and VII), especially between analyses using the three top indicators (jaundice/anemia, summed histology, PRV load). The following description of the functional data will largely reflect the signal from the combined indicator score, but will point out some of the deviations in functional resolution among analyses. The presentation reflects differential regulation between positive versus negative fish; hence processes that are up-regulated or stimulated are more highly expressed in positive fish.

Liver

None of the functional pathways from Pathway StudioTM analysis were significant at $p<0.001$ when jaundice alone was used as a driver; all other drivers elicited some physiological signal, and the signal based on jaundice and anemia together was quite strong. Again, this indicates that transcriptionally, the external classification of jaundice alone does not yield a strong physiological signal in the data. Given that four fish classified as belonging in transcriptional cluster A (Table III; Figure 6) were jaundice negative (three of which were anemia positive), perhaps this is not surprising.

Functional analysis of genes significantly correlated with each of the remaining jaundice syndrome indicators revealed a strong down-regulation of most metabolic processes (Table VI). Response to nutrient was also weakly down-regulated, potentially suggesting that the down-regulation of metabolism could result from anorexia or reduced feeding of fish suffering from this syndrome. This observation is consistent with the fact that fish that died of jaundice syndrome did not have food in their stomachs and had probably been off feed for at least a week. However, fatty acid oxidation and gluconeogenesis are generally stimulated under prolonged starvation, and both of these processes were down-regulated, potentially suggesting that fish were not at an advanced stage of anorexia.

Proteolysis, especially ubiquitin-requiring processes in the proteasome, was powerfully up-regulated in positive fish (Table VI). Proteolysis can also be enhanced under anorexia, as the break-down of proteins is used for energy generation. However, the dominant proteolytic pathways affected were those associated with mitotic cell cycle, also strongly up-regulated, and the presentation of endogenous peptide antigen, important in the immune response to intra-cellular pathogens. The up-regulation of proteolysis is also consistent with tissue necrosis and cellular damage observed as the most significant lesions associated with the jaundice syndrome. Viruses can also co-opt the ubiquitin proteolytic pathway to facilitate their own reproduction process (Kloetzel 2001). In mouse reovirus infections, the ubiquitin-proteasome pathway can contribute to cytopathology and disease (Mbisa 2002).

"Response to virus" was highly significant in all but the SSF gene-set enrichment analyses (Table VI). Specific viral-infection-related pathways revealed in pathway

and subnetwork enrichment analysis (not shown in Table VI) involved in viral replication, type-I interferon response, STAT signaling, viral infectious cycle, positive regulation of viral transcription, and response to exogenous dsRNA (note reoviruses are dsRNA viruses). Antigen presentation was also up-regulated in most analyses, while acute phase response, important in early immune responses to infectious agents, was down-regulated. DNA damage response was highly stimulated in all gene-set enrichment analyses, and consistent with findings of cell damage (HHD) and possibly necrosis (SCN). While DNA damage could derive from a nonspecific response to single cell necrosis, we note that it is often co-opted by viruses to facilitate viral replication (Chaurushiya and Weitzman 2009).

The molecular signature showed that many genes and pathways associated with jaundice/hepatitis in mammals were up-regulated in livers with the jaundice syndrome. Viral hepatitis in mammals is associated with inflammation, fibrin deposition, and necrosis (Levy et al. 2000), all processes that were apparent in the histological lesions associated with salmon jaundice syndrome. The up-regulation of DNA damage, ubiquitin-protein ligase associated with mitotic cell cycle, and apoptosis is consistent with cellular necrosis observed in jaundice livers. Excess fibrin deposition, notable from the histopathology, can stimulate inflammatory pathways; livers with evidence of excess hepatic sinusoidal fibrin deposition also showed significant stimulation of genes that in mammals are associated with platelet and mast cell activation. Assuming that these processes are also representative of the stimulation of thrombocytes (fish equivalent of platelets) and eosinophilic granular cells (fish equivalent of mast cells), these data suggest that the stimulation of inflammatory processes may be most prominently associated with livers undergoing excess fibrin deposition. Finally, during jaundice, the function of the liver is disturbed when there is a deposit of bile pigments in excess of what can be processed, and bilirubin concentrations – derived from the breakdown of hemoglobin – increase in the blood, causing the skin to turn yellow. We speculate that the strong down-regulation of bile acid metabolism may be associated with the build-up of excess bilirubin in the blood that causes the jaundice syndrome in the salmon. We propose that future studies should examine blood samples to determine if excess bilirubin is observed in association with the jaundice syndrome in salmon.

The liver is the primary tissue for detoxification. The microscopic lesions associated with the jaundice syndrome were hypothesized to result from acute to sub-acute toxin exposure at the cellular level. The lesions and clinical history are most consistent with those toxins coming from a viral infection and the associated inflammatory mediators rather than an exogenous environmental toxin. This is consistent with the genomic functional analysis of the jaundice syndrome, where there is no evidence of an enhanced response to exogenous toxins in the liver. Indeed, both xenobiotic metabolism and response to drugs were highly down-regulated in all gene-set enrichment analyses (Table VI).

			Liver Combined Indicator	Liver Summed Histo	Liver PRV GT 26	Liver Jaundice/Anemia	Liver Jaundice	Liver SCN	Liver SSF
Viral/Immune/Inflammatory									
response to virus	Viral/immune	Up-regulated	**	**	***	***		***	
Presentation of Endogenous Peptide Antigen	Viral/immune	Up-regulated	*	**	**	*		*	
mast cell activation	Viral/immune	Up-regulated							
platelet activation	Viral/immune	Up-regulated							
platelet degranulation	Viral/immune	Up-regulated	*	*	*	*	*	**	
interspecies interaction between organisms	Viral/immune	Up-regulated							
peroxisome	Viral/immune	Down-regulated	**	**	***	***	**	***	
complement activation	Viral/immune	Down-regulated							
complement activation, classical pathway	Viral/immune	Down-regulated							
acute-phase response	Viral/immune	Down-regulated							
Blood-Heme									
Bile acids metabolism (alternative pathway)	Blood	Down-regulated	**	**	***	*		**	***
irinotecan metabolism	Blood	Down-regulated	**	**	**		**	***	
Heme oxidation	Blood	Down-regulated	**	**	**		**	***	
Cellular									
DNA damage response, signal transduction by p53									
class mediator resulting in cell cycle arrest	Cellular	Up-regulated	***	***	***	***		***	
regulation of apoptosis	Cellular	Up-regulated	***	***	**	***		***	
positive regulation of ubiquitin-protein ligase activity									
involved in mitotic cell cycle	Cellular	Up-regulated	**	**	***	**		**	
regulation of ubiquitin-protein ligase activity involved in mitotic cell cycle	Cellular	Up-regulated	*	**	**	**		**	
anaphase-promoting complex-dependent proteasomal ubiquitin-dependent protein catabolic process	Cellular	Up-regulated	**	**	**	**		**	
negative regulation of ubiquitin-protein ligase activity involved in mitotic cell cycle	Cellular	Up-regulated	**	**	**	**		**	
M-G1 transition of mitotic cell cycle	Cellular	Up-regulated	**	**	**	**		**	
G1-S transition of mitotic cell cycle	Cellular	Up-regulated	*	**	*	*		*	
Adipocytokine Signaling	Metabolic	Up-regulated	**	**	***	**	**	**	
liver development	Cellular	Down-regulated	**	**	*	**	**	**	
Cirrhosis	Cellular	Variable							
Sclerosis	Cellular	Variable	**	*	*	*	**	**	
Response									
response to nutrient	Metabolic	Down-regulated	*	*	*	*		*	
response to amino acid stimulus	Response	Up-regulated	*	**	*		**		
response to stress	Response	Variable	*					**	
response to hypoxia	Response	Variable	*	*	*		**	*	
response to peptide hormone stimulus	Response	Variable		**	*		*		
Response to starvation	Response	Variable	**	*	**		*		
Toxicant									
xenobiotic metabolic process									
response to drug									
drug metabolic process									
Metabolic									
proteasome complex	Metabolic	Up-regulated	***	***	***	***	***	*	
proteolysis involved in cellular protein catabolic process	Metabolic	Up-regulated	*	*	**	*	*	**	
endopeptidase activity	Metabolic	Up-regulated	*	*	**	*		**	
regulation of cellular amino acid metabolic process	Metabolic	Up-regulated	***	***	***	***	***		
RNA metabolic process	Metabolic	Up-regulated	**	**	**	**			
Biosynthesis of cholesterol	Metabolic	Up-regulated	**	**					
cellular nitrogen compound metabolic process	Metabolic	Up-regulated	***	***	***	***	***		
mRNA metabolic process	Metabolic	Up-regulated	**	**	*				
Pentose-phosphate shunt	Metabolic	Up-regulated	**	**	**	**			
Branched amino acid metabolism	Metabolic	Down-regulated	*	**	*				
carbohydrate metabolic process	Metabolic	Down-regulated	**	*	*				
catalytic activity	Metabolic	Down-regulated	**	**	**	**	**	**	
cellular lipid metabolic process	Metabolic	Down-regulated	*	*	***	*			
cholesterol homeostasis	Metabolic	Down-regulated	*	*	**				
fatty acid metabolic process	Metabolic	Down-regulated							
Fatty acid oxidation	Metabolic	Down-regulated	*						
Insulin Action	Metabolic	Down-regulated	*	**	*				
gluconeogenesis	Metabolic	Down-regulated	**	**	**				
glucose metabolic process	Metabolic	Down-regulated	***	***	***	***	***		
Glucose metabolism	Metabolic	Down-regulated	***	***	***	***	***		
Glutathione metabolism	Metabolic	Down-regulated	**	*	**				
lipid metabolic process	Metabolic	Down-regulated	**	*	**				
Metabolism of estrogens and androgens	Metabolic	Down-regulated	***	***	*				
omega-3-fatty acid metabolism	Metabolic	Down-regulated	**	*	**				
omega-6-fatty acid metabolism	Metabolic	Down-regulated	**	*	**				
oxidation-reduction process	Metabolic	Down-regulated	***	***	***	***	***		
Serine and Glycine metabolism	Metabolic	Down-regulated	**	**	**	**	**		
triglyceride biosynthetic process	Metabolic	Down-regulated							

Table VI. Gene-set enrichment analysis showing biological processes differentially regulated in the liver in analyses driven by the combined indicator analysis (jaundice/anemia, PRV, histology), summed histology (of "jaundice syndrome-associated" liver lesions), jaundice/anemia, PRV load, and individual lesions SCN or SSF. Processes for each analysis significant at $P<10^{-05}$ contain three asterisks (***)�, $10^{-05} > P < 10^{-04}$ (**), and $10^{-04} > P < 10^{-03}$ (*).

Kidney

Gene-set enrichment analysis in kidney tissue was similar between the three key indicators. In general, the functional signature in kidney tissue showed a more powerful up-regulation of immune and virally-related processes, consistent with its role as a primary immune tissue (Table VII). In fact, most of the transcriptional regulators of genes differentially affected in kidney tissue were involved in the regulation of immunity (e.g. IRF3, IFNG, JAK, TLR3, interferon, DDX58, STAT1, IRF1, and others). As in liver, response to virus (Table VII), type-I interferon-mediated signaling, response to interferon gamma, viral reproduction, and response to double stranded RNA (pathway enrichment analysis not shown) were strongly up-regulated in kidney tissue. Additional immune pathways stimulated in kidney included negative regulation of defense response to virus by host, regulation of viral genome replication, and positive regulation of viral transcription (pathway enrichment analysis not shown). As observed in liver, DNA damage response, often co-opted by viruses and consistent with microscopic findings of cell necrosis (RTN, ICN), was also up-regulated in affected kidneys.

Proteolysis was also highly up-regulated in kidney in some analyses, again consistent with roles in mitotic cell cycle and presentation of endogenous peptide antigen. The metabolic signal was weaker, more variable among analyses drivers, and showed considerable variation in patterns of stimulation/down-regulation differing between the jaundice/anemia and RTN driven analyses and others. In general, jaundice/anemia, summed histo, and RTN signatures showed strong stimulation in processes associated with proteolysis while other analyses showed down-regulation of a variety of metabolic processes.

Heme biosynthesis generally takes place in the liver, where heme is also broken down to bilirubin. Interestingly, while we did not observe disruption of heme metabolism in liver tissue, although bile acid metabolism and heme oxidation were both affected, we did observe down-regulation of heme biosynthesis in kidney, along with a down-regulation of porphyrin biosynthesis and metabolism, an important constituent of heme. Perhaps these processes are down-regulated due to the excess in the heme byproduct bilirubin that is excreted in the kidney (i.e. as a negative feedback loop).

			Kidney	Combined Indicator
			Kidney Summed Histo.	Kidney PRV CT 26
			Kidney JDR	Kidney Jaundice
			Kidney RTN	Kidney ISH
			Kidney KATy	
Viral/immune				
response to virus	Viral/immune	Up-regulated	*** ++ **	*** ***
viral	Viral/immune	Up-regulated	**	**
interspecies interaction between organisms	Viral/immune	Up-regulated	**	**
Presentation of Endogenous Peptide Antigen	Viral/immune	Up-regulated	** ++ *	**
cytokine activity	Viral/immune	Up-regulated	**	**
immune response	Viral/immune	Variable	*** *** **	*** **
Blood				
positive regulation of angiogenesis	Blood	Up-regulated	* + * *	**
Bile acid metabolism (alternative pathway)	Blood	Down-regulated	*** ***	**
heme biosynthetic process	Blood	Down-regulated	**	*
heme oxidation	Blood	Down-regulated	*** ***	
porphyrin metabolic process	Blood	Down-regulated	*** *** **	*
porphyrin biosynthetic process	Blood	Down-regulated	*** *** **	*
heme binding	Blood	Variable	** ++ *	*
Cellular				
regulation of apoptosis	Cellular	Up-regulated	* + * **	*
apoptosis	Cellular	Up-regulated	* + * **	**
cell proliferation	Cellular	Up-regulated	** ++ *	***
positive regulation of ubiquitin-protein ligase activity involved in mitotic cell cycle	Cellular	Up-regulated		**
DNA damage response, signal transduction by p53 class mediator resulting in cell cycle arrest	Cellular	Up-regulated	* + * **	*
brush border membrane	Cellular	Down-regulated		*
Sclerosis	Cellular	Variable		**
Cirrhosis	Cellular	Variable		*
aging	Cellular	Variable	*** *** **	***
Adipocytokine Signaling	Cellular	Variable	*** *** **	**
Toxicant				
xenobiotic metabolic process	Toxicant	Down-regulated	*** *** **	**
response to inorganic substance	Toxicant	Down-regulated	** + *	
drug metabolic process	Toxicant	Down-regulated	*** **	
response to ethanol	Toxicant	Variable	* + * *	*
response to drug	Toxicant	Variable	**	*
response to organic cyclic compound	Toxicant	Variable	*** *** **	**
Response				
response to peptide hormone stimulus	Response	Up-regulated	* + * ** * *	*
response to mechanical stimulus	Response	Down-regulated	*** *** * **	**
response to hypoxia	Response	Down-regulated	*** *** * **	**
response to stress	Response	Variable	*** *** * *	**
response to oxidative stress	Response	Variable	* + *	***
response to glucocorticoid stimulus	Response	Variable	*** *** *	**
response to cold	Response	Variable	* + *	**
Metabolism				
proteolysis involved in cellular protein catabolic process	Metabolism	Up-regulated	** ++ *	**
regulation of cellular amino acid metabolic process	Metabolism	Up-regulated	* + * + **	*
RNA metabolic process	Metabolism	Up-regulated	* + * + **	*
glucose metabolic process	Metabolism	Up-regulated		**
glutathione metabolic process	Metabolism	Down-regulated	** ++ **	** + **
tetrahydroleurobiotic process	Metabolism	Down-regulated	** ++	
Glutathione metabolism	Metabolism	Down-regulated	** ++ **	** + **
Tetrahydroleurobiotic	Metabolism	Down-regulated	** + *	
oxidation-reduction process	Metabolism	Down-regulated	*** ++ **	*** + **
imidocetan metabolism	Metabolism	Down-regulated	*** ***	
cellular amino acid metabolic process	Metabolism	Down-regulated		**
purine base metabolic process	Metabolism	Down-regulated		**
Aspartate metabolism	Metabolism	Down-regulated		*
Arachidonic acid metabolism	Metabolism	Down-regulated		**
Mannose metabolism	Metabolism	Down-regulated		**
cellular nitrogen compound metabolic process	Metabolism	Variable	*** *** * **	**
Ser/Gly/Thr/Cys metabolism	Metabolism	Variable	*** ++ * + **	**
catalytic activity	Metabolism	Variable	* + *	**
Glucose metabolism	Metabolism	Variable		**
glycolysis	Metabolism	Variable		**
nucleobase, nucleoside and nucleotide metabolic process	Metabolism	Variable		**
Tryptophan metabolism	Metabolism	Variable		**
carbohydrate metabolic process	Metabolism	Variable		**
Transport				
Glucose import	Transport	Down-regulated	* + *	**
substrate-specific transmembrane transporter activity	Transport	Down-regulated	* + **	* *
transmembrane transporter activity	Transport	Down-regulated	* + *	**
sodium ion transport	Transport	Down-regulated	*** ++ *	**
ion transmembrane transporter activity	Transport	Down-regulated	* + *	**
transporter activity	Transport	Down-regulated	*** ++ *	***
electron carrier activity	Transport	Variable	* + *	**
transmembrane transport	Transport	Variable	***	**
Other				
platelet alpha granule membrane	Other	Up-regulated	**	*
translation initiation factor activity	Other	Up-regulated	*** +	
protein homodimerization activity	Other	Variable	*** ++ ***	
starvation	Other	Variable	* + **	
transamidase activity	Other	Down-regulated	*	**
glutathione transferase activity	Other	Down-regulated	* + *	**
symporter activity	Other	Down-regulated	* + *	***
sugar binding	Other	Down-regulated	*** ++ *	*
pyridoxal phosphate binding	Other	Down-regulated	*** ++ *	*

Table VII. Gene-set enrichment analysis showing biological processes differentially regulated in the kidney in analyses driven by number of positive indicators (jaundice, PRV, histology), histology alone (combined "associated" kidney lesions), jaundice/anemia, PRV infection, and individual lesions. Processes for each analysis significant at $P < 10^{-05}$ contain three asterisks (***) $, 10^{-05} > P < 10^{-04}$ (**), and $10^{-04} > P < 10^{-03}$ (*). Only pathways significant at $10^{-05} > P < 10^{-04}$ in at least one analysis are shown.

Finally, the occurrence of similar shifts in proliferative and apoptotic cell cycle processes as observed in liver tissue is consistent with the cellular signs of necrosis and occasionally observed interstitial cell hyperplasia (ISH), although the ISH samples alone did not have the statistical power to reveal these processes as significant.

V.ii Unsupervised Analyses of microarray data

Principle Components Analysis (PCA) is an unsupervised approach to data analysis that can be used to identify the major expression trajectories in the data. When combined with correlation analysis of measured variables against the rank order of the individuals along each principle component (PC), one can determine the relative contribution of measured variables to the overall physiological variation among individuals, essentially identifying the major drivers of physiological change. We conducted a correlation analysis between each of the indicators and with farm site against the top five PCs (Table VIII). Indicators associated with the jaundice syndrome were highly correlated with PC1 and PC2 for both liver and kidney, which combined explained roughly 40% of the overall variation within the data for liver and 32% of the variation for kidney. This suggests that the jaundice syndrome was the most powerful driver of physiological change in the fish included in the study. The relative contribution of indicators varied between PC1 and PC2 in both tissues. In liver, the combined indicator and jaundice/anemia were more powerfully associated with PC1 while PRV and histological lesions were more associated with PC2. In kidney, the combined indicator and RTN were exclusively associated with PC1 while many indicators contributed significantly to PC2. As observed in the supervised analysis, SCN in liver and RTN in kidney produced the most powerful transcriptional response among the individual lesions.

By plotting PC1 against PC2 in kidney tissue, one can easily visualize the differential distribution of outliers and speculate about what drives their variance (Figure 9). Sample 1001, which had anemia (evident by pale gills) without jaundice and no appreciable PRV or lesions in the kidney, was distributed on the extreme positive ends of PC1 and PC2 (PC1 +, PC2 +). This placement actually aligns with the observed patterns, as jaundice/anemia is most closely associated with PC1 positive samples, and PRV and histology positives were strongly associated with PC2 negatives. Fish 1060 was PRV positive and jaundice/anemia and histology negative; it clustered as PC2 negative (with other PRV positive samples) but at the extreme negative end of PC1 with jaundice/anemia negative fish, suggesting that the pathogenic effects leading to

jaundice syndrome (if the virus is causative of jaundice) may not be present. Fish number 1065 was positive for RTN in only one tubule, and it clustered well within the negatives for all indicators, potentially suggesting that the lesion in this fish had a different pathogenesis than renal tubular necrosis in the other fish. A re-examination of this slide showed that while a few tubules in fish 1065 had individual necrotic cells, in other fish with RTN, the entire tubule was necrotic.

	PC1	R	PC2	R	PC3	PC4	PC5
LIVER							
Percentage Variance	25.2		14.6		7.1	5.2	4.3
Combined Indicator	8.24E-06	1	1.36E-03	4			
Jaundice/Anemia	1.08E-05	2	8.10E-03	5			
Jaundice			2.03E-02				
PRV(<26)	9.46E-04	4	7.14E-04	2			
Summed Histo	1.30E-02	5	3.01E+04	1			
Farm	1.56E-02	6					
SSF			1.20E+03	3			
SCN	7.67E-05	3	2.25E+02	7			
SNM							
HDD			9.17E-03				
MEG			2.22E+02	6		8.11E-05	
KIDNEY							
Percentage Variance	20.2		11.7		7.6	6.1	5.0
Combined Indicator	6.09E-03	1	4.39E-06	1			
Jaundice/Anemia			1.13E-03	5			
Jaundice			4.75E-05	3			
PRV(<26)			2.37E-06	2			
Summed Histo	4.36E-02	2	4.21E-04	4			
Farm			3.40E-02				
ICN							
ISH			1.61E-02				
IFB							
RTN	1.40E-02	3	1.47E-02				
MGN							

Table VIII. Correlations of Jaundice syndrome-related indicators with Principal Components 1-5. R indicates rank order of significance.

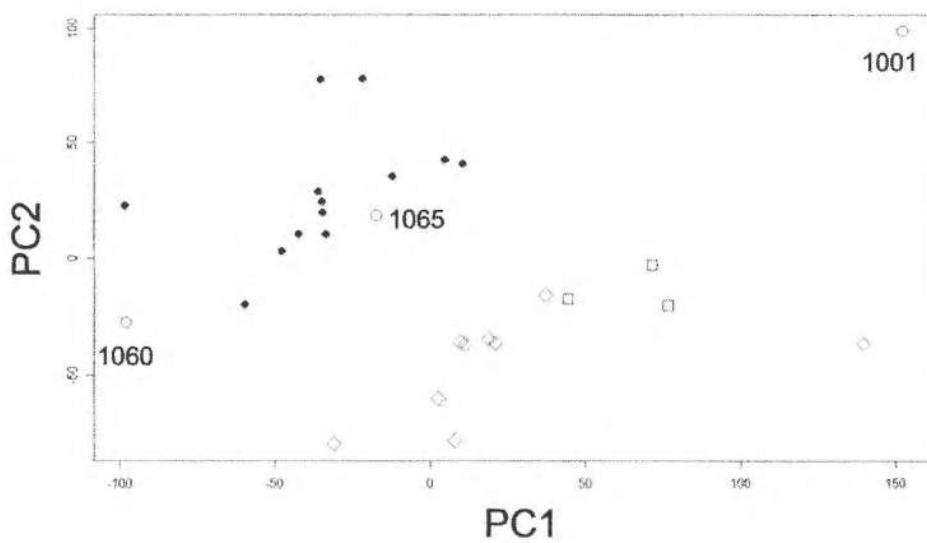


Figure 9. Plot of PC1 versus PC1 in kidney showing the distribution of samples labeled by the Combined Indicator, with samples with 0 positive indicators represented by closed circles; 1 positive, open circles; 2 positive, open squares; and three positive, open diamonds. Outliers 1001 (positive jaundice only), 1065 (positive RTN histology only), and 1060 (positive PRV only) are labeled.

VI. Osmoregulatory assessment of gill tissue

RNA from gill tissue was used as a template for quantitative PCR (qPCR). TaqMan assays for three Na-K ATPase isoforms were assayed on Fluidigm BioMark. Individually, these genes were not correlated with jaundice syndrome in gill tissue. These genes were also not correlated with the farms, despite the relatively low saline environment of farm A.

VII. References

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VIII. Recommendations to industry on next steps

- Laboratory challenge study will cell free size-filtered lysates to establish whether the piscine reovirus can cause signs of jaundice in BC Chinook salmon.
- Include other diagnostic testing such as hematocrit, blood assessment to better understand the cause of the anemia associated with the syndrome
- Better define risk factors contributing to this syndrome

12. Resulting key improvements to sustainable aquaculture and scientific advancements:

- Assessment of a potentially powerful novel diagnostic tool - genomic characterization as a diagnostic tool for fish health
- Illustrating a multidisciplinary approach (genomics, standard veterinary diagnostic techniques, histopathology, epidemiology) in attempt to solve fish health issues

13. Suggested next steps, future research/development/innovation needs:

- Conduct laboratory studies to assess the role of PRV load and the potential to elicit jaundice presentation or disease.

- If PRV is shown in follow-up studies to be causative of the Jaundice syndrome,
 - Routine monitoring of PRV and biomarkers for disease could enable more precise tracking of the virus and disease progression.
Biomarkers alone could be useful if PRV is not causative.
- Whole genome sequencing of nucleic acids (DNA and RNA) of affected fish to gain the full sequence of PRV in BC
- Phylogenetic analysis of the full sequence of the piscine reovirus here in BC to determine its relationship with European strains.

14. Copies of publications, reports or articles produced in reference to the project:

N/A

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

- High throughput microbe screening on the Fluidigm BioMark system was developed and applied during the course of this study, although it was not principally motivated or financed by this study.

PART III

Declaration:

I _____ have completed the report and declare that to the best of my knowledge the report is accurate.

Signature

Date

Approved by:

DFO Project Authority

Date

Industry Project Authority

Date

Appendix 7

eDNA study Report

Midsummer Island sampling on October 12, 2021

Dr. Emiliano Di Cicco

June 29, 2022

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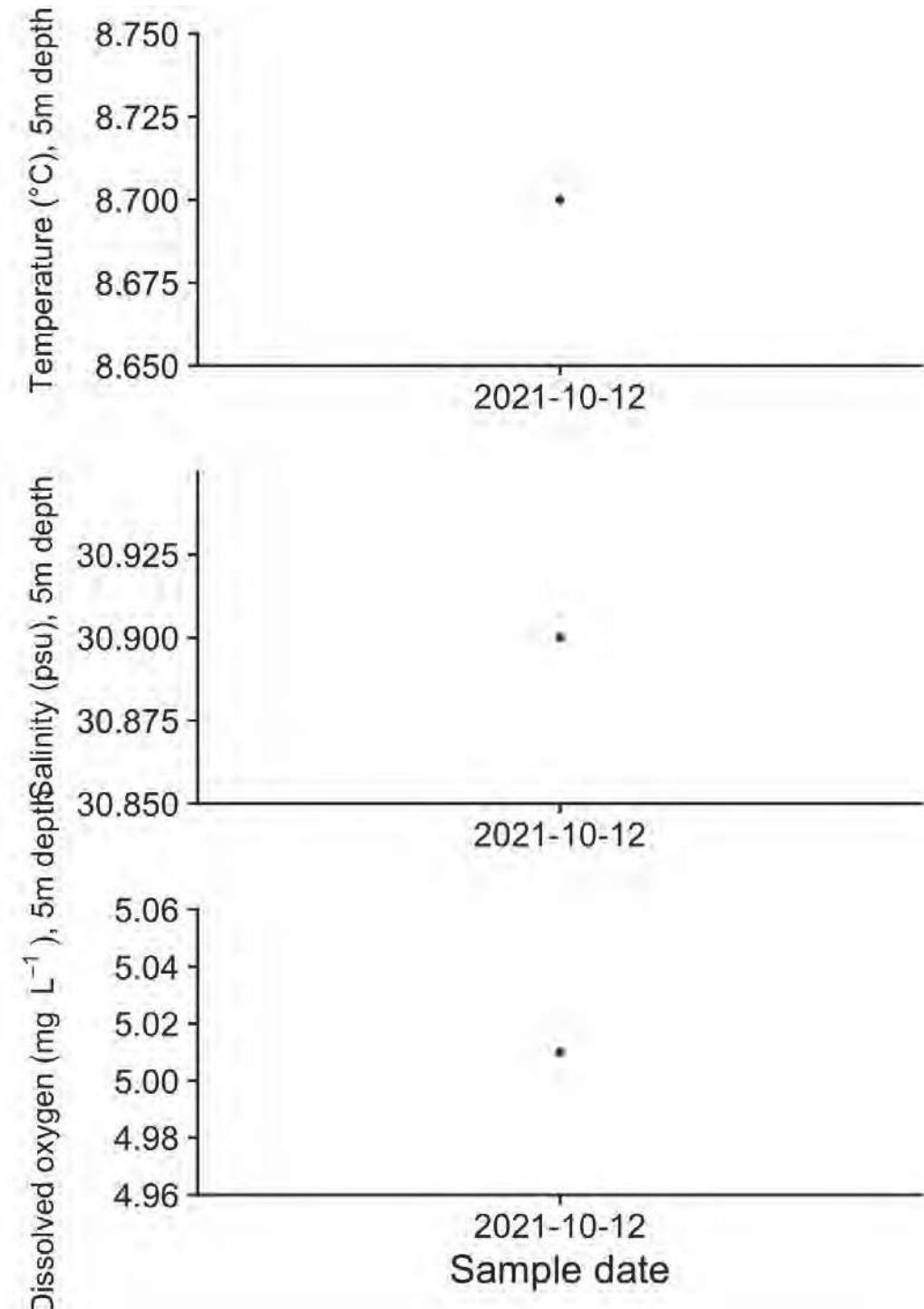
Executive summary

Premise

On October 12, 2021, 22 samples were collected by BATI and Mowi crew during a sampling event at Midsummer Island (Mowi Ltd.). 22 Atlantic salmon subadults were collected from the Midsummer Island farm site, including 15 live and 7 moribund/dead fish. All live fish were euthanized with TMS overdose prior to dissection with the exception of the moribund fish, which were administered a blow to the head. Portions of gill, liver and anterior kidney were collected in triplicate for molecular testing (preserved in RNA later) from all the fish, while all the moribund/dead fish also underwent collection of tissues (gills, spleen, liver, heart, anterior and posterior kidney, pyloric caeca, skeletal muscle + skin, brain) for histological analysis. Clinical notes and gross lesions were noted and reported for every fish. One aliquot has been provided to the Company MOWI Fish Health, another aliquot is stored at the BATI Field Office, and a third aliquot is stored at DFO - PBS. This latter aliquot has been tested for the presence and load of the agents indicated in the IMIP agreement as well as the agents indicated in the eDNA study agreement. Each sample has been extracted and tested individually. Negative and positive controls were run. A housekeeping gene was also included to assess the quality of the RNA extracted.

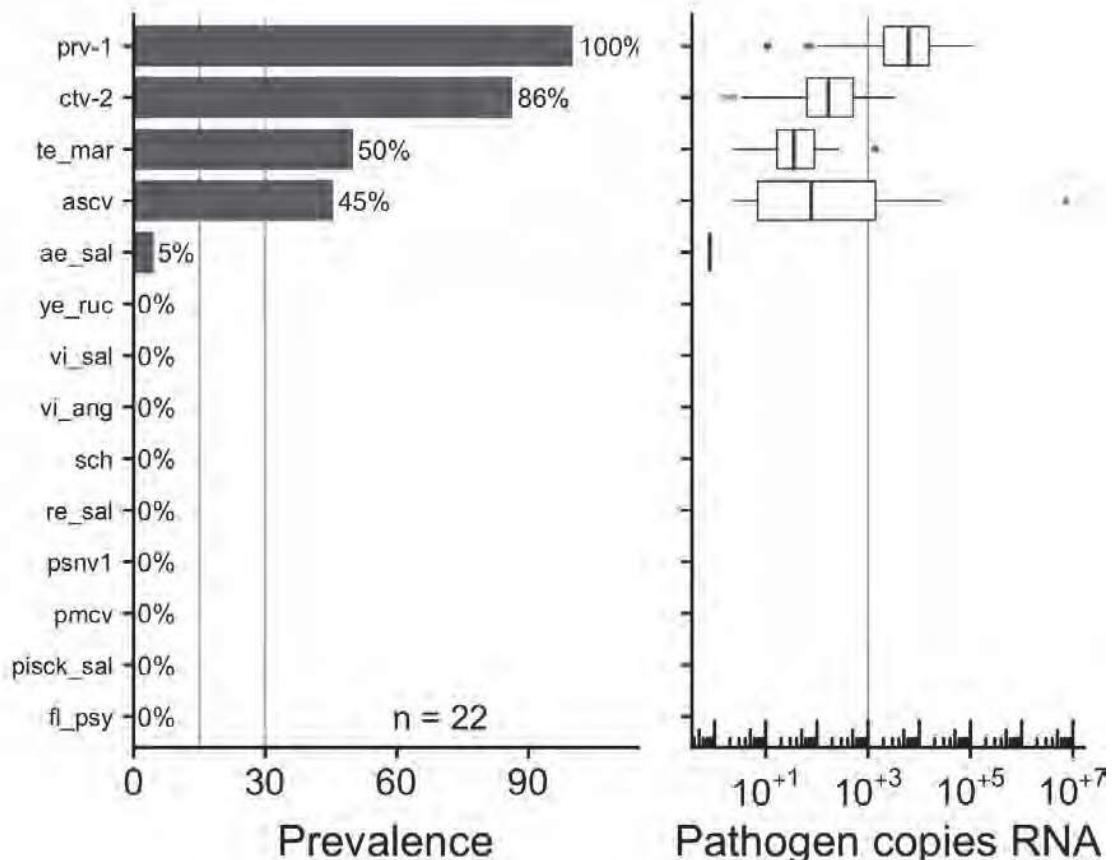
Histology samples have been sent to Wax-It Histo Ltd. to process and prepare slides, which have been read and scored by Dr. Di Cicco. A digital copy of each slide is available to the Company.

Environmental data

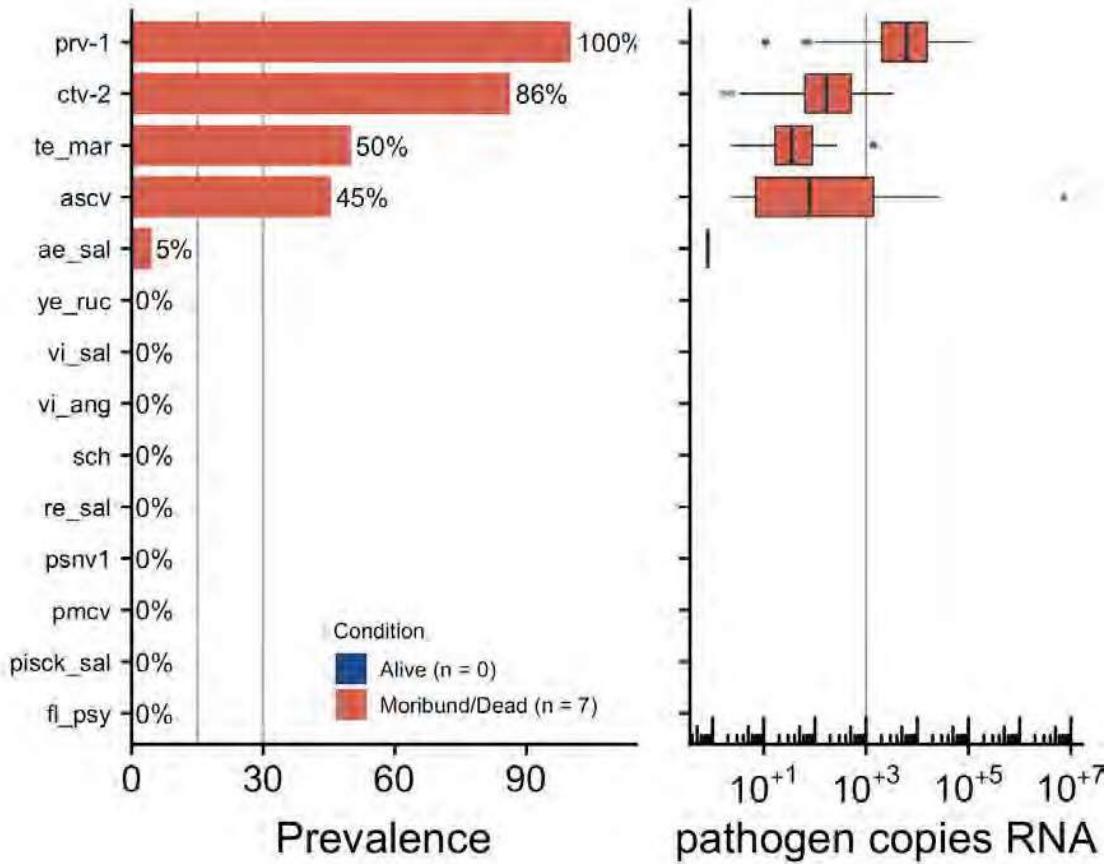


Water temperature ($^{\circ}\text{C}$), salinity (ppm), and dissolved oxygen (mg/L) at a 5m depth. Certain sampling dates have no recorded environmental data, resulting in gaps in the plots.

Overall infectious agent prevalence



Infectious agent prevalence in samples collected on 2021-10-12.



Infectious agent prevalence in samples collected on 2021-10-12, split by mortality status at time of sampling. Any specimens that were not confirmed to be either moribund or live at the time of generating this report are excluded from this figure.

Individual infectious agent trends

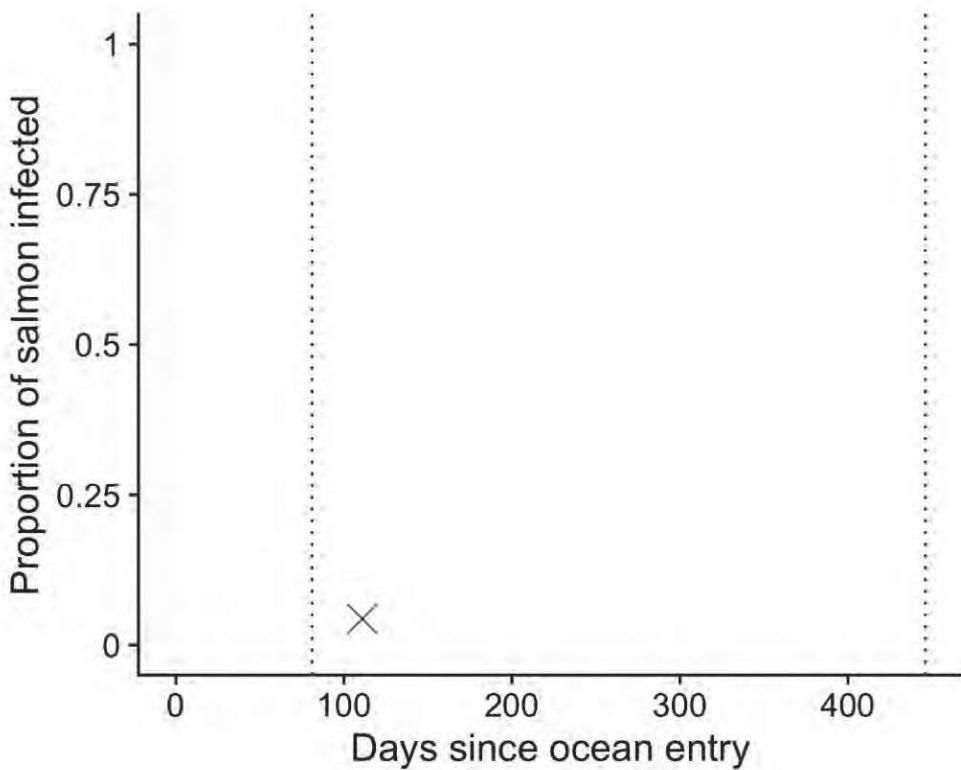
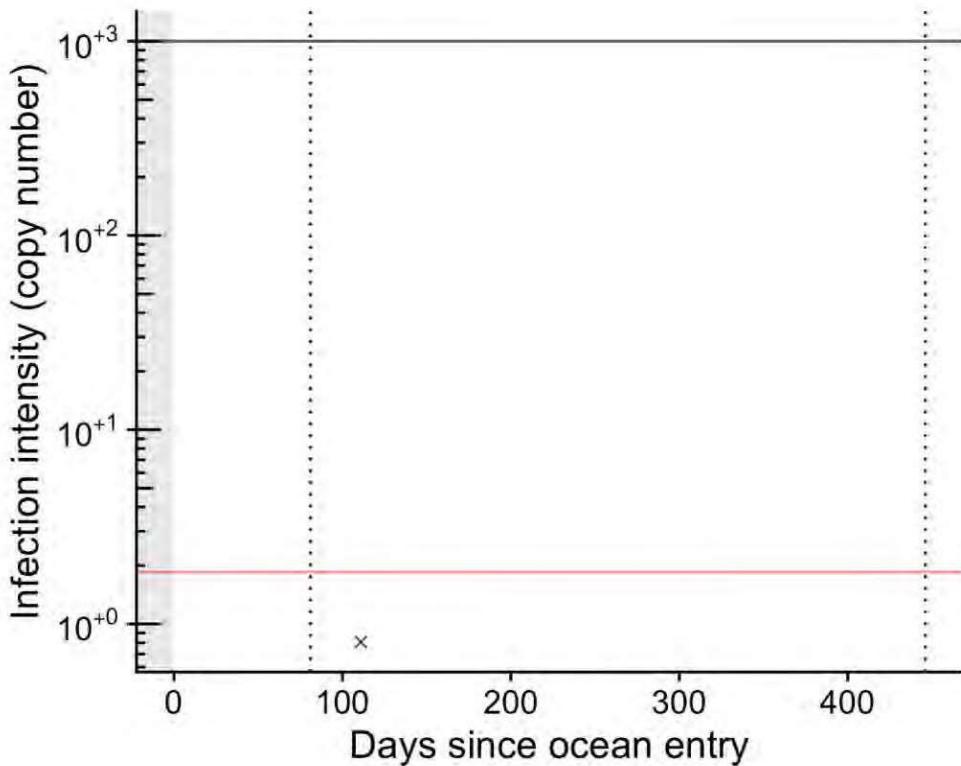
The following plots show individual infectious agent trends across all farm sites. In cases where sample size is sufficient, curves from a generalised additive model are included in the plot.

Grey circles represent live fish, and black X's represent dead/dying fish. Curves indicate mean predictions from a generalised additive model; blue and red correspond to live and dead/dying fish, respectively (shaded areas show 95% confidence regions). Left-hand grey region indicates freshwater hatchery residence, grey regions on x-axis indicate period of transfer to another site, and vertical dotted lines correspond to January 1st.

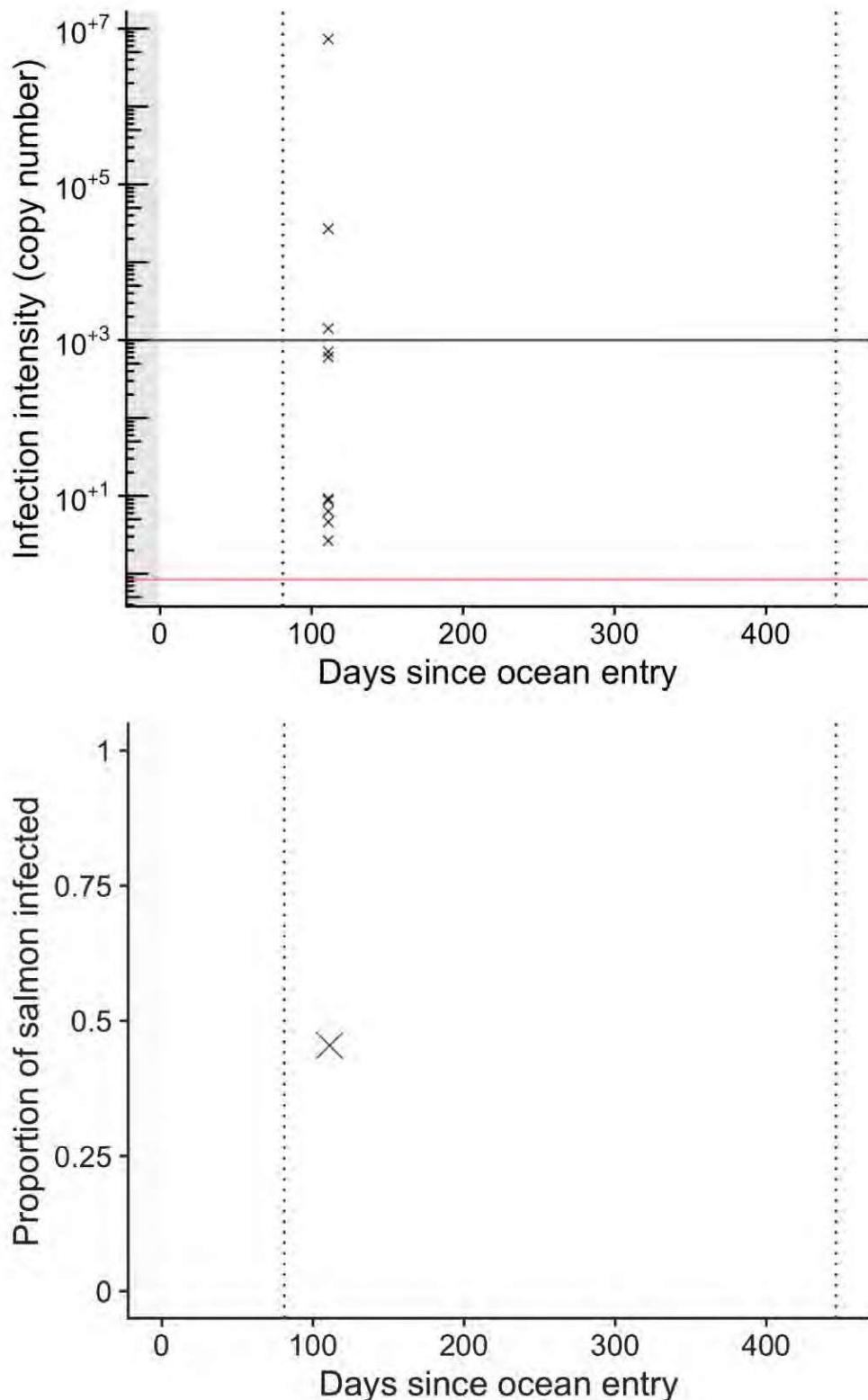
For infection intensity plots, horizontal red line indicates limit of detection (yielding ~90% true positive rate) for respective qPCR assay run in duplicate, while the horizontal black line indicates 1000 copies. Note log scale.

For proportion plots, grey circles show prevalence in live fish on each sampling date, and black X's show prevalence in dead/dying fish (symbol areas proportional to sample sizes).

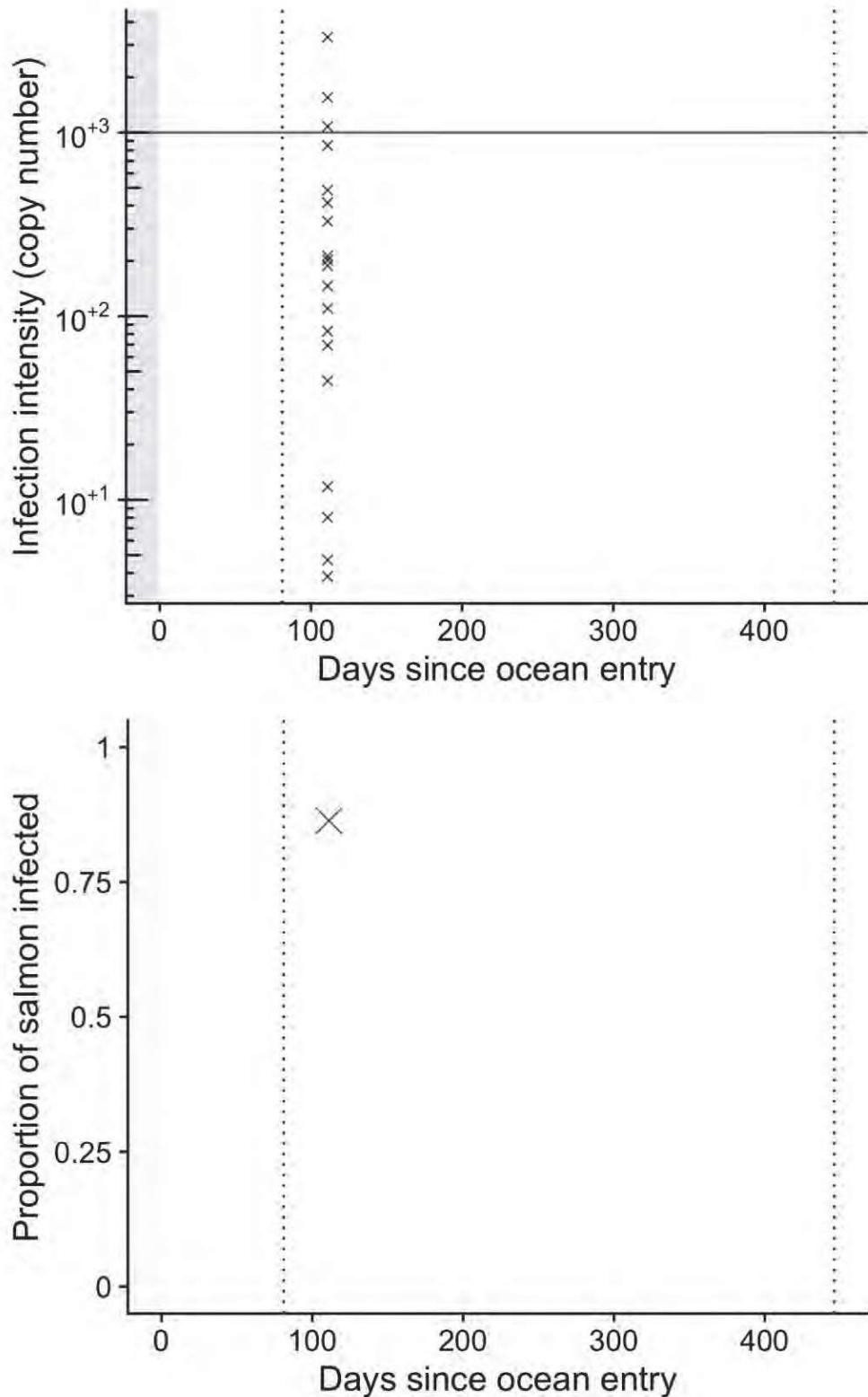
Aeromonas salmonicida



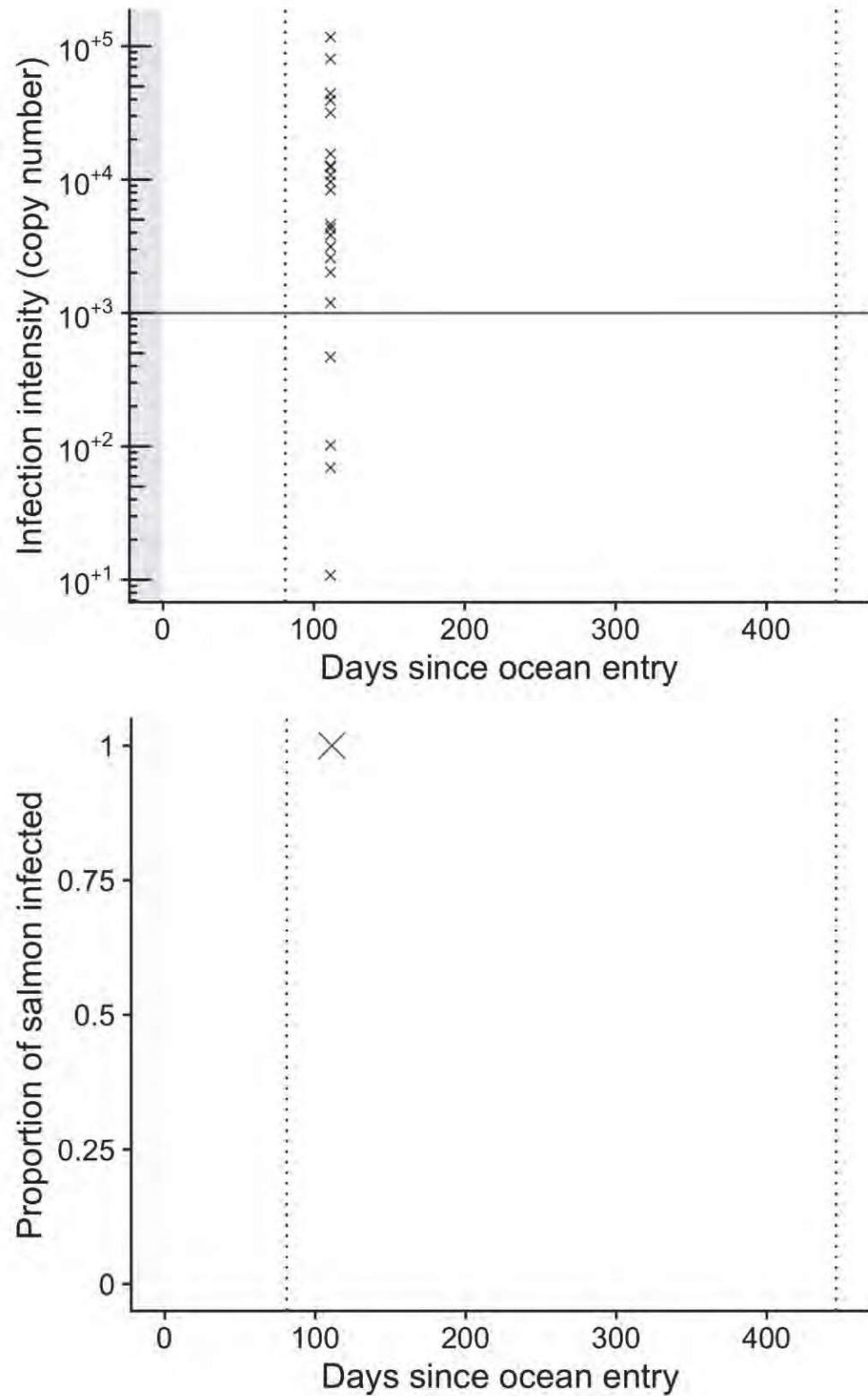
Atlantic salmon calicivirus



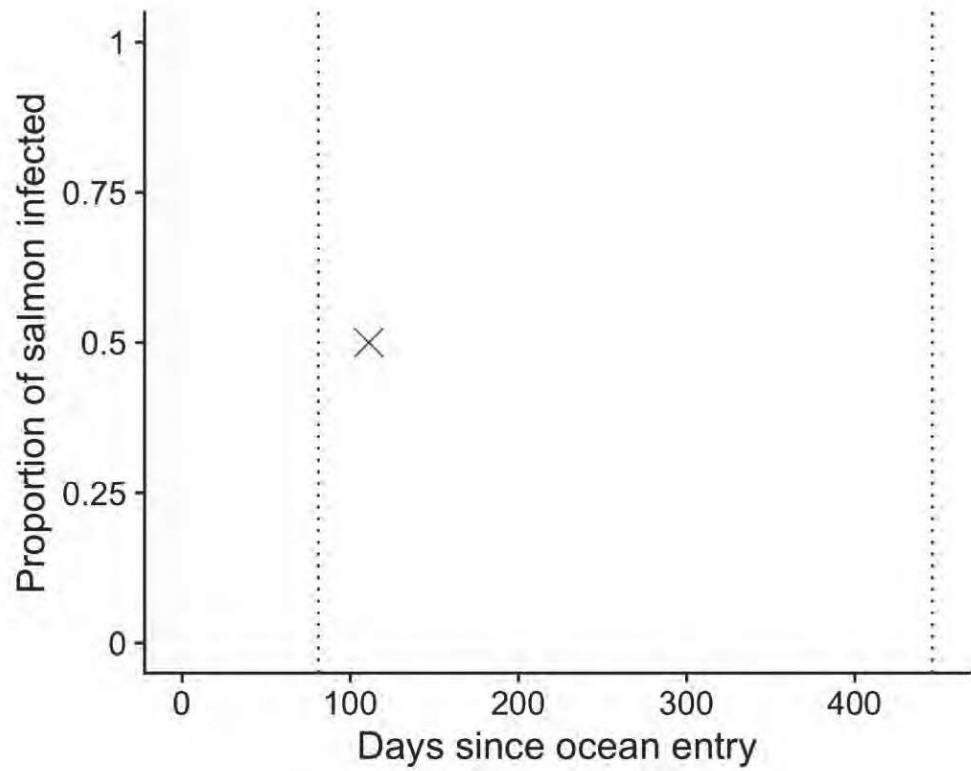
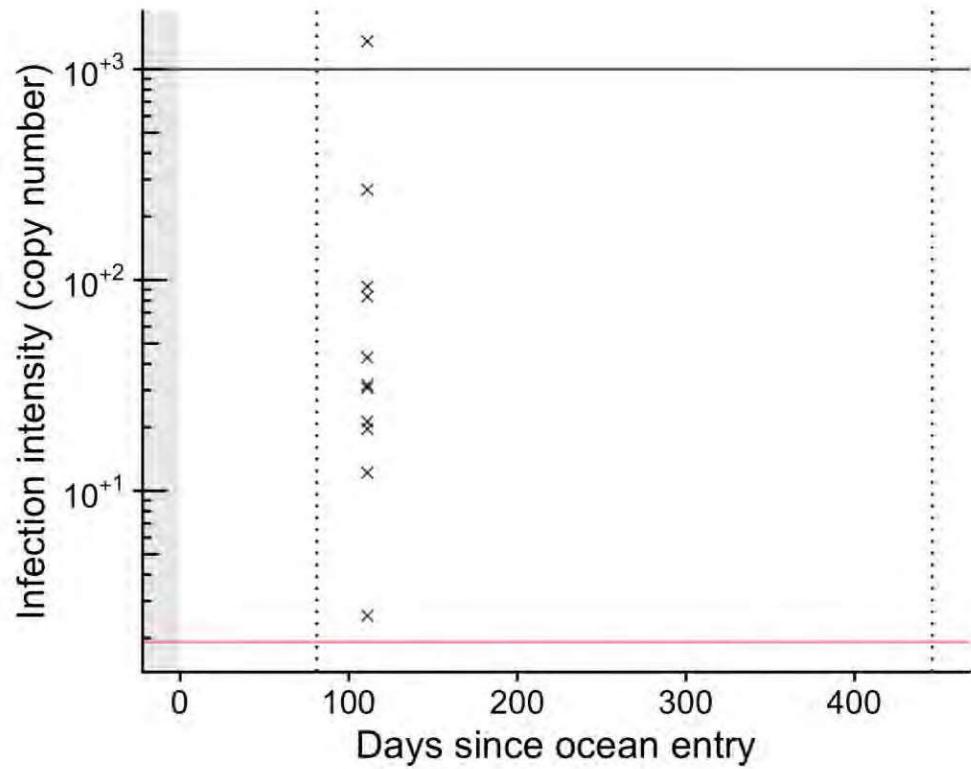
Cutthroat trout virus-2



Piscine orthoreovirus



Tenacibaculum maritimum



Clinical signs

Table 1: Clinical signs for specimens sampled on 2021-10-12

metric	N5022	N5021	N5020	N5019	N5018	N5017	N5016
General							
Mort	X	X	X	X	X	X	X
Skin & Fins							
Erosion					X		X
Muscle							
Nodules/White Spots							X
Abdominal Cavity							
Ascites							X
Hemorrhages							X
Liver							
Nodules/White Spots					X		X
Heart							
Hemorrhages							X
Brain							
Hemorrhages/Congestion					X		X

Histology

Table 2: Histology scores for specimens sampled on 2021-10-12

metric	N5022	N5021	N5020	N5019	N5018	N5017	N5016
Heart							
Peri Epi	1		1		2	1	1
Myo	1		1		2		
Liver							
Cong Haem	2	1		1	1	1	2
Nec					1		
Itis	1						
Spleen							
Cong Hearn	2	3	2	2	3	2	1
W Pulpitis	1		2	1	1	1	2
Kidney							
Itis			1				
Osis					1		1
Cong Hearn	2	1		1	1	1	
Interst Hyperplasia	2	1	2	1	1	1	1
Pancreatitis							
Pancreatitis		1					
Cnc							
Malacia						1	
Gliosis			1			1	
Cong Hearn		2	1	1	1	1	2
Gills							
Itis	nv						
Cong Hearn	nv						
Prolif	nv						
Skin_muscle							
Itis Nec					1		
Tissue							
Necrosis Artefacts	2	2	2	2	2	2	2

Diagnoses and Comments

Table 3: Diagnoses and comments for specimens sampled on 2021-10-12

DFO ID	Diagnosis	Comments
N5016		Peribiliary Immune Activation (2), Inflammatory Foci In Liver (1), Vaccine Induced Granulomatous Splenitis (1); Gills Very Old
N5017		Vaccine Peritonitis (1); Gills Very Old
N5018		Peribiliary Immune Activation (1); Gills Very Old
N5019		Peribiliary Immune Activation (1); Gills Very Old, Diffused Picnotic Nuclei In Hepatocytes
N5020 HSMI		Myocardioneclerosis (2), Localized Clots In Atrium (1), Eosinophilic Vacoules In Hepatocytes (1); Gills Very Old, Diffused Picnotic Nuclei In Hepatocytes And Enterocytes
N5021		Neuronal Vacuolization (2), Neuronal Chromatolysis (1); Gills Very Old, Diffused Picnotic Nuclei In Hepatocytes And Enterocytes
N5022		Gills Very Old, Diffused Picnotic Nuclei In Hepatocytes And Enterocytes

Conclusions

The sampling collection was incomplete (i.e. only one pen of live fish was sampled, plus most clinical data have been lost) due to technical and organizational issues linked to the beginning of the project. Nevertheless, here below is a summary and evaluation of the findings from the sampled fish.

The farm was inspected in its entirety: most fish were behaving normally, although several individuals appeared lethargic. The mortality per pen reported by the company resulted slightly higher than the normal. Clinically, numerous individuals among the sampled fish showed fin erosion as well as skin erosion/ulcers. This finding is compatible with the delousing treatments and manual/mechanic handling operations carried out over the last period. Several fish (either live or moribund/morts) also showed enlarged spleen during the dissection procedures, as well as pale liver/heart in some instances. Brain congestion and hemorrhages was pretty common too.

Molecular testing results show that the totality of the fish tested resulted positive to PRV, and at high load in some instances. *Tenacibaculum maritimum* was also present in 50% of the fish, while *Aeromonas salmonacida* was observed at background level.

Histopathologically, the moribund/morts samples collected showed an overall pattern of systemic congestive modifications with immunological/inflammatory response, affecting primarily spleen, kidney and liver. One individual also showed a pattern of lesions' severity and distribution (as well as clinical signs and gross lesions) consistent with the diagnosis of Heart and Skeletal Muscle Inflammation (HSMI), according to ICES diagnostic standards (ICES 2012) (1). However, according to current DFO standard, this would count as "provisional diagnosis", as a laboratory challenge trial hasn't been performed.

Given the overall situation, the molecular results and clinical/pathological findings, a close monitoring of the operations during the next visit at this site is highly recommended.

1. Heart and skeletal muscle inflammation (HSMI) of farmed Atlantic salmon (*Salmo salar L.*) and the associated Piscine reovirus (PRV) (ices.dk)

Appendix

Glossary of infectious agents

Agent abbr.	Full agent name	Agent type	Disease	Ranking
ae_sal	Aeromonas salmonicida	Bacteria	Furunculosis	2
ascv	Atlantic salmon calicivirus	Virus	unknown	4
ctv-2	Cutthroat trout virus-2	Virus	unknown	4
fl_psy	Flavobacterium psychrophilum	Bacteria	Bacterial cold water disease	3
pisck_sal	Piscirickettsia salmonis	Bacteria	Piscirickettsiosis (SRS)	2
pmcv	Piscine myocarditis virus	Virus	Cardiomyopathy syndrome	1
prv-1	Piscine orthoreovirus	Virus	HSMI-EIBS-Jaundice/anemia	
psnv1	Pacific salmon nidovirus-1 (CoV)	Virus	unknown	4
re_sal	Renibacterium salmoninarum	Bacteria	Bacterial kidney disease	2
sch	Candidatus Syngnathia salmonis	Bacteria	Gill chlamydia	3
te_mar	Tenacibaculum maritimum	Bacteria	Marine flexibacteriosis (mouth/fin rot)	2
vi_ang	Vibrio anguillarum	Bacteria	Vibriosis	2
vi_sal	Vibrio salmonicida	Bacteria	Cold water vibriosis	2
ye_ruc	Yersinia ruckeri (Enteric redmouth disease)	Bacteria	Yersiniosis (Enteric red mouth)	2

eDNA study Report

Doctor Islets sampling on October 13, 2021

Dr. Emiliano Di Cicco

June 29, 2022

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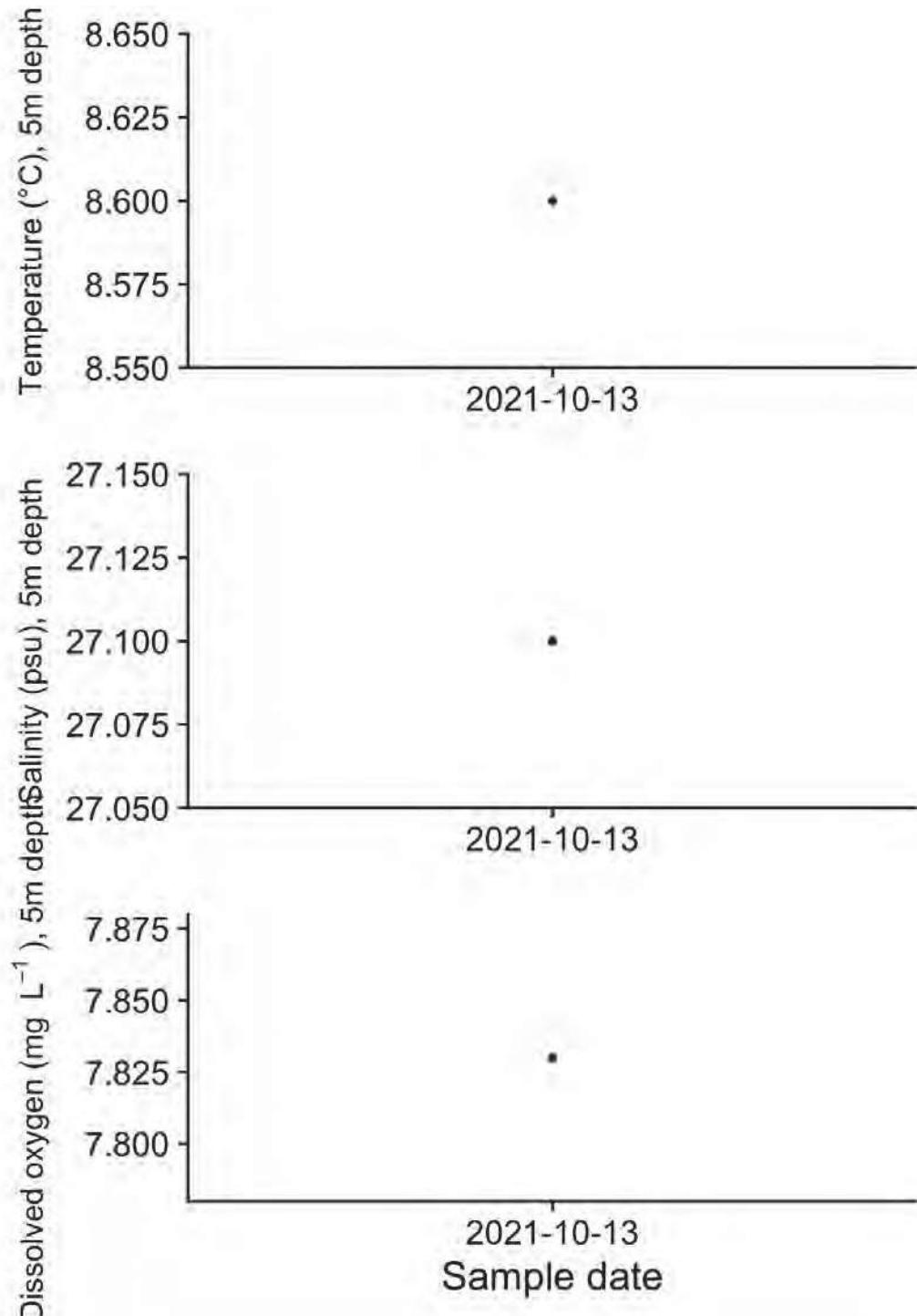
Executive summary

Premise

On October 13, 2021, 35 samples were collected by BATI and Mowi crew during a sampling event at Doctor Islets (Mowi Ltd.). 35 Atlantic salmon subadults were collected from the Doctor Islets farm site, including 25 live and 10 moribund/dead fish. All live fish were euthanized with TMS overdose prior to dissection with the exception of the moribund fish, which were administered a blow to the head. Portions of gill, liver and anterior kidney were collected in triplicate for molecular testing (preserved in RNA later) from all the fish, while all the moribund/dead fish also underwent collection of tissues (gills, spleen, liver, heart, anterior and posterior kidney, pyloric caeca, skeletal muscle + skin, brain) for histological analysis. Clinical notes and gross lesions were noted and reported for every fish. One aliquot has been provided to the Company MOWI Fish Health, another aliquot is stored at the BATI Field Office, and a third aliquot is stored at DFO - PBS. This latter aliquot has been tested for the presence and load of the agents indicated in the IMIP agreement as well as the agents indicated in the eDNA study agreement. Each sample has been extracted and tested individually. Negative and positive controls were run. A housekeeping gene was also included to assess the quality of the RNA extracted.

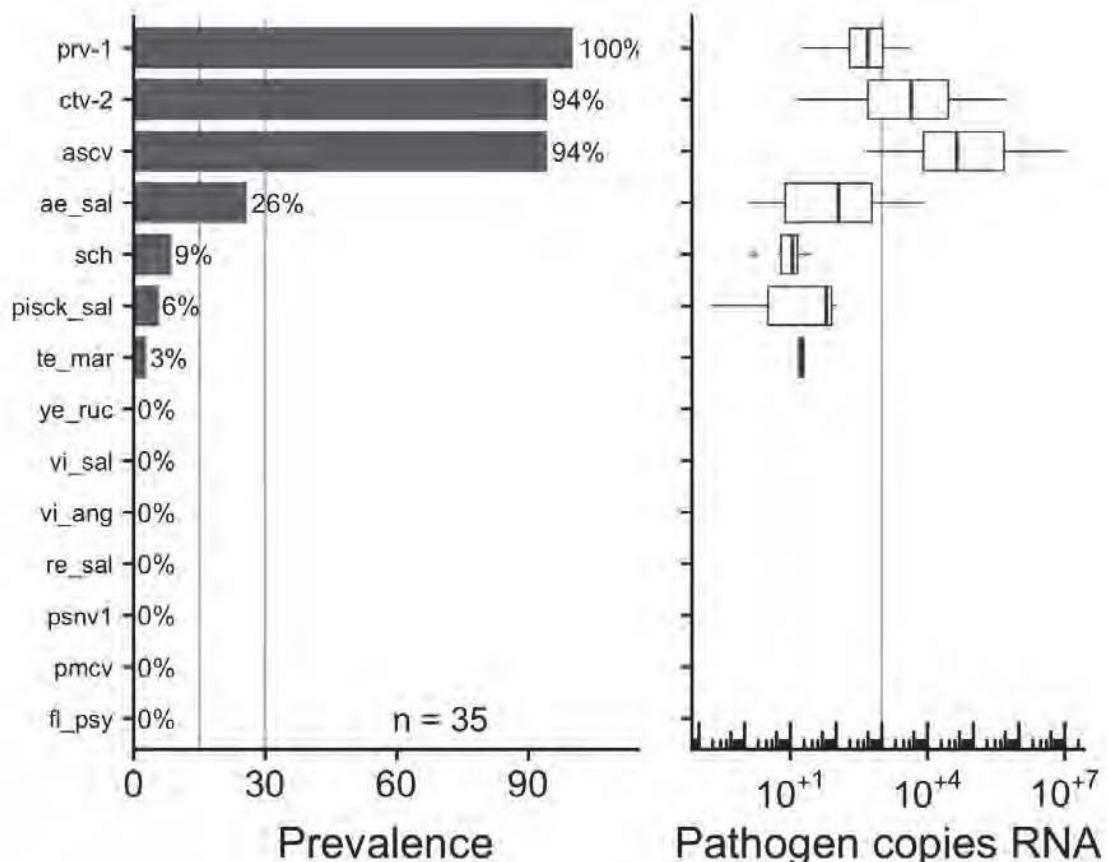
Histology samples have been sent to Wax-It Histo Ltd. to process and prepare slides, which have been read and scored by Dr. Di Cicco. A digital copy of each slide is available to the Company.

Environmental data

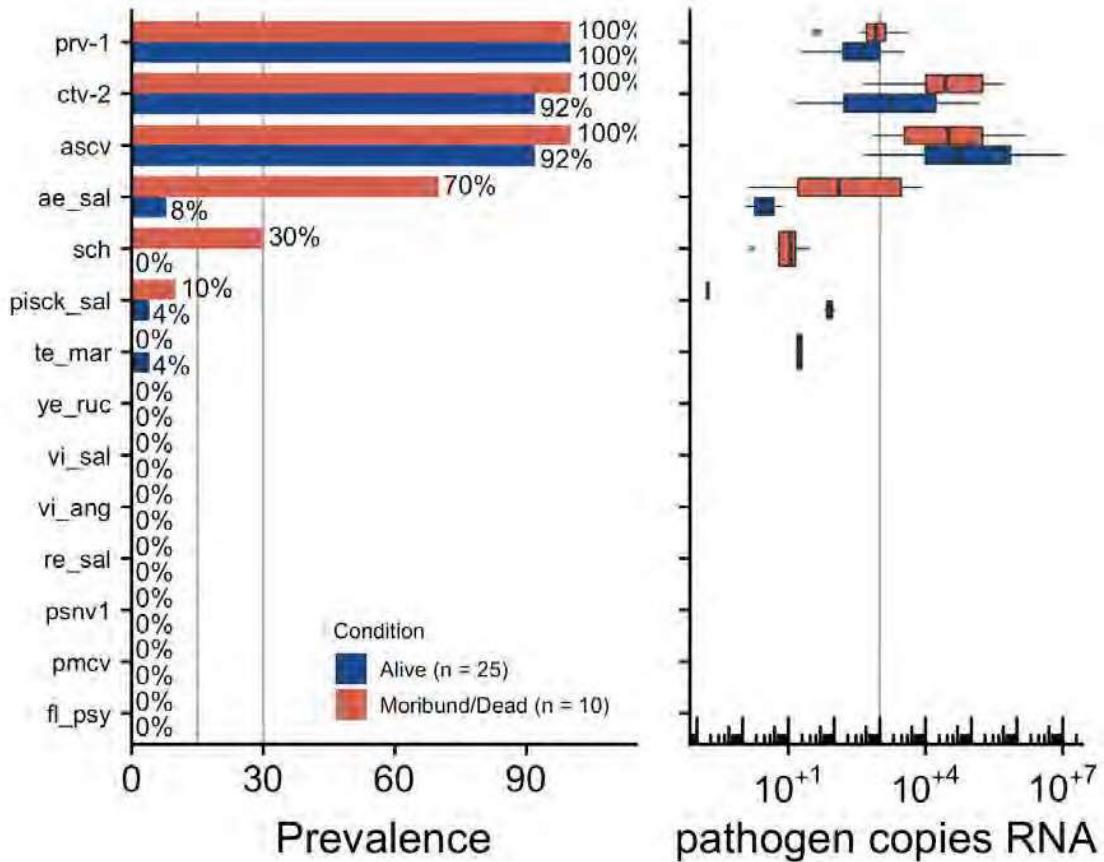


Water temperature ($^{\circ}\text{C}$), salinity (ppm), and dissolved oxygen (mg/L) at a 5m depth. Certain sampling dates have no recorded environmental data, resulting in gaps in the plots.

Overall infectious agent prevalence



Infectious agent prevalence in samples collected on 2021-10-13.



Infectious agent prevalence in samples collected on 2021-10-13, split by mortality status at time of sampling. Any specimens that were not confirmed to be either moribund or live at the time of generating this report are excluded from this figure.

Individual infectious agent trends

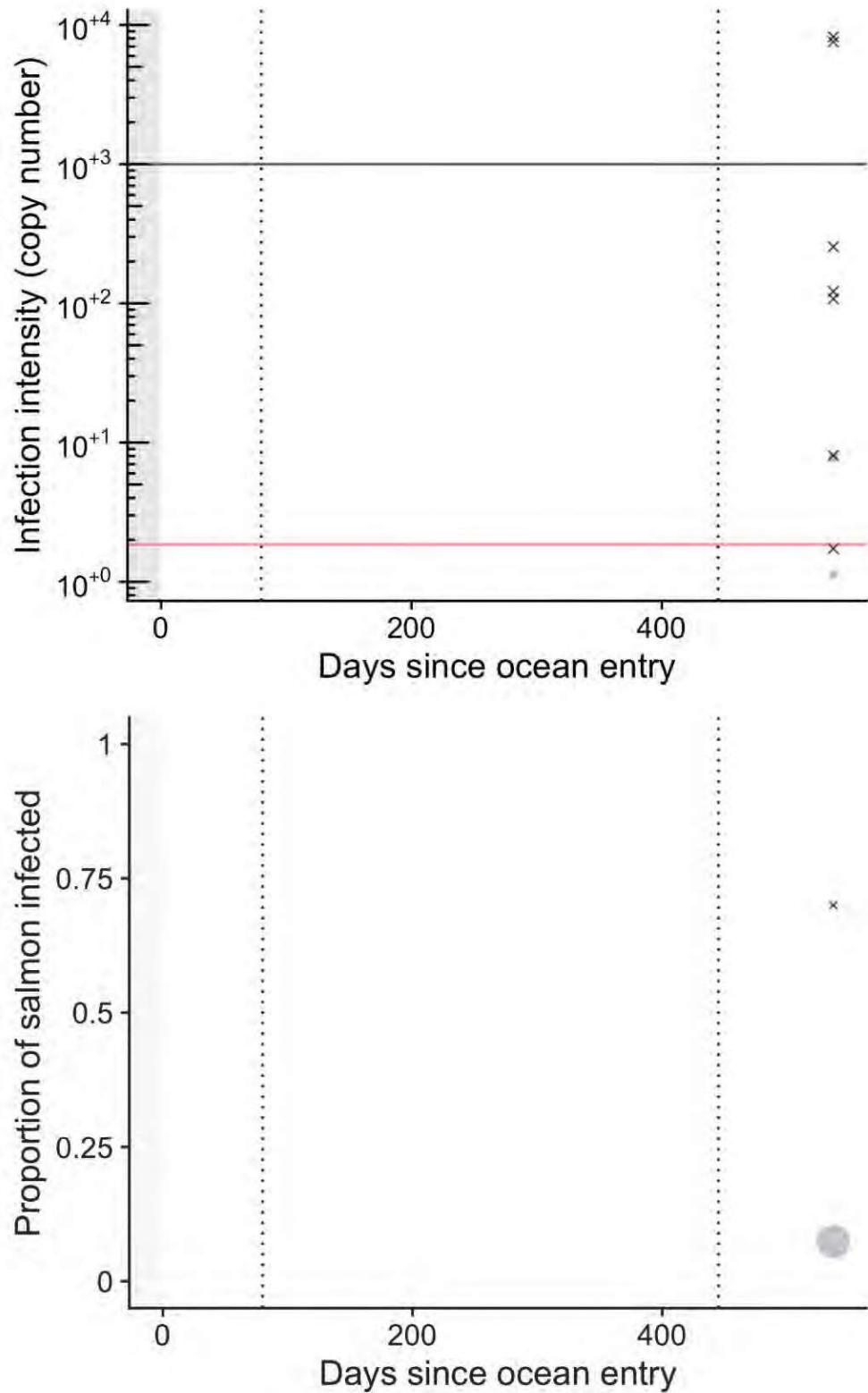
The following plots show individual infectious agent trends across all farm sites. In cases where sample size is sufficient, curves from a generalised additive model are included in the plot.

Grey circles represent live fish, and black X's represent dead/dying fish. Curves indicate mean predictions from a generalised additive model; blue and red correspond to live and dead/dying fish, respectively (shaded areas show 95% confidence regions). Left-hand grey region indicates freshwater hatchery residence, grey regions on x-axis indicate period of transfer to another site, and vertical dotted lines correspond to January 1st.

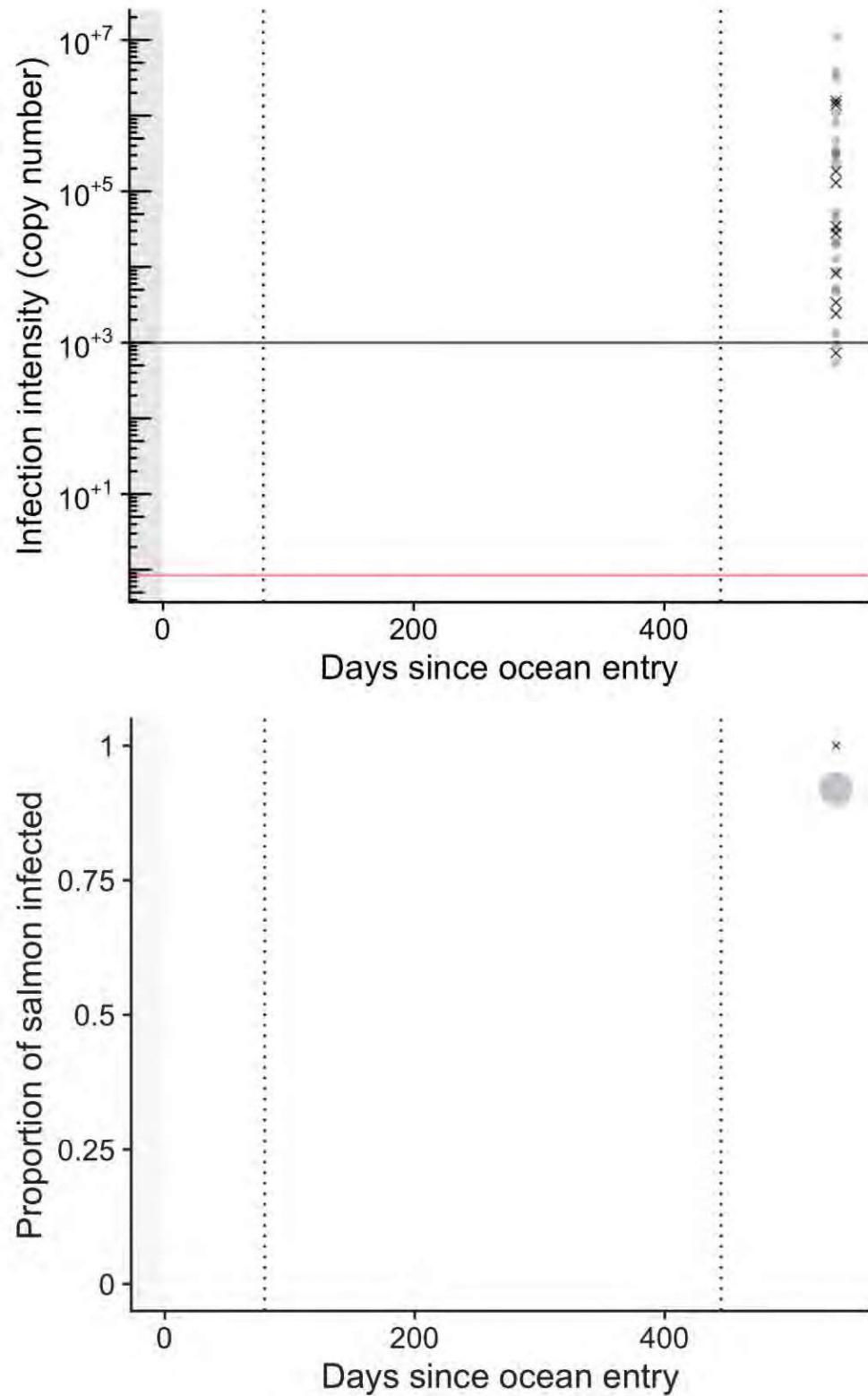
For infection intensity plots, horizontal red line indicates limit of detection (yielding ~90% true positive rate) for respective qPCR assay run in duplicate, while the horizontal black line indicates 1000 copies. Note log scale.

For proportion plots, grey circles show prevalence in live fish on each sampling date, and black X's show prevalence in dead/dying fish (symbol areas proportional to sample sizes).

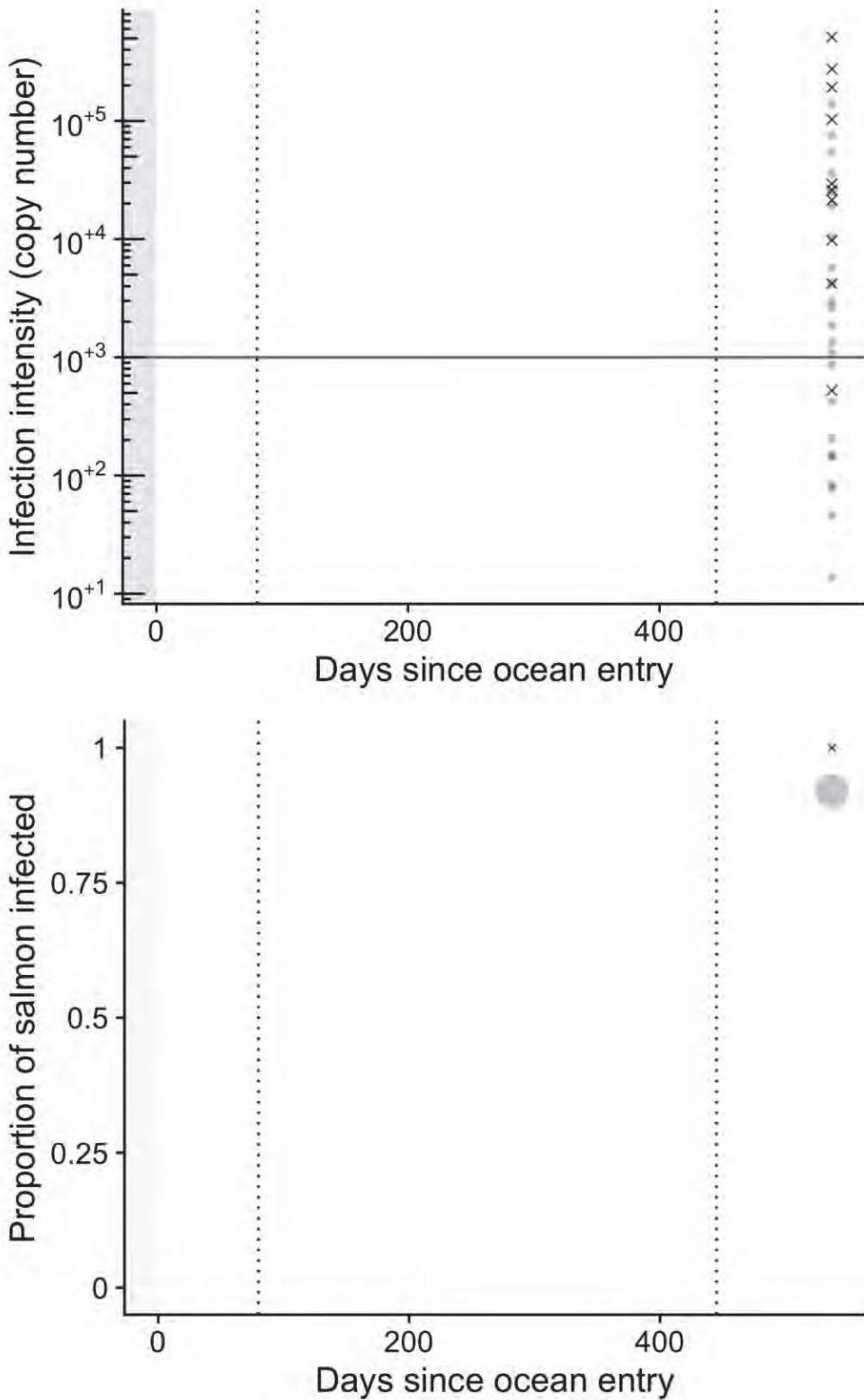
Aeromonas salmonicida



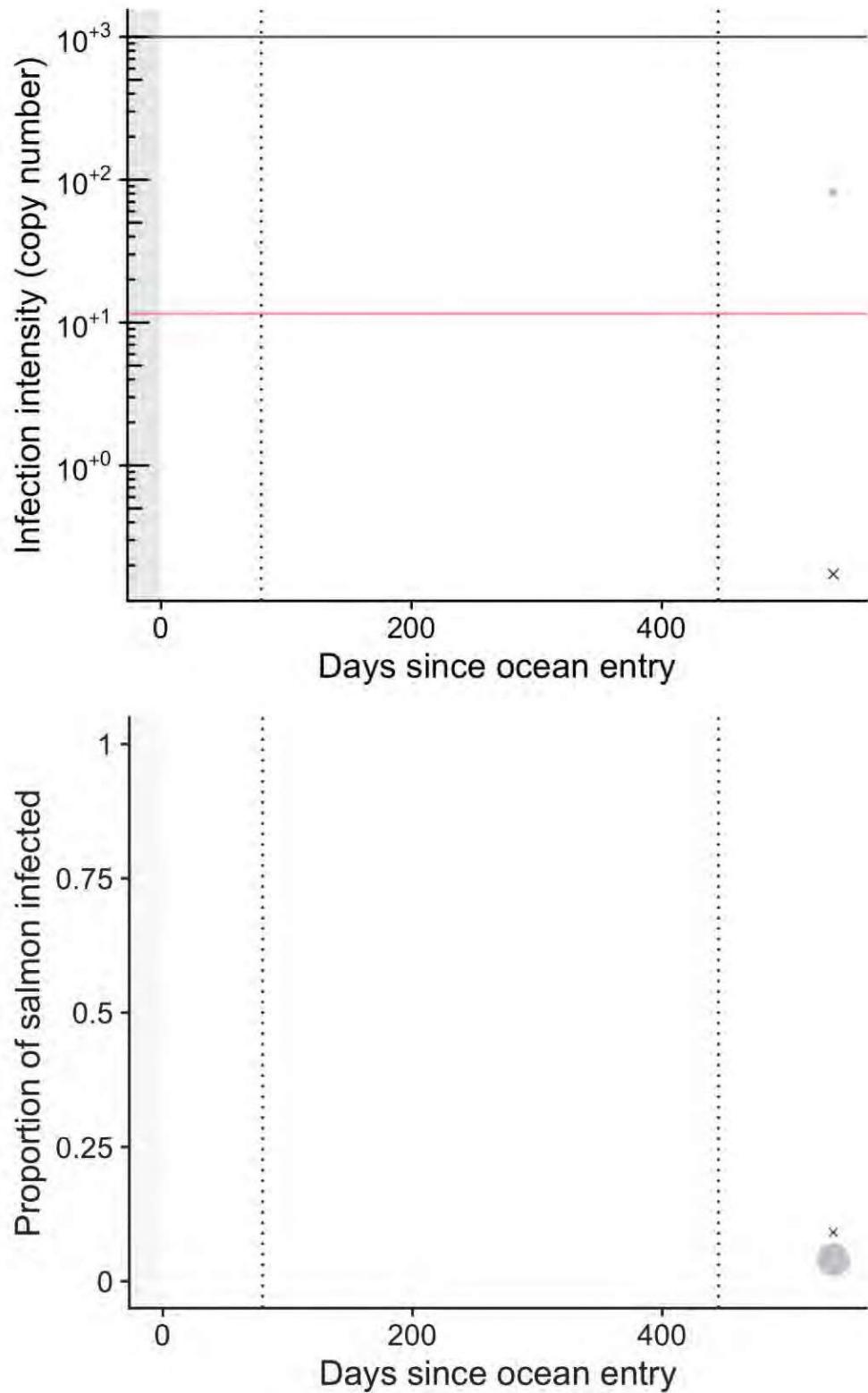
Atlantic salmon calicivirus



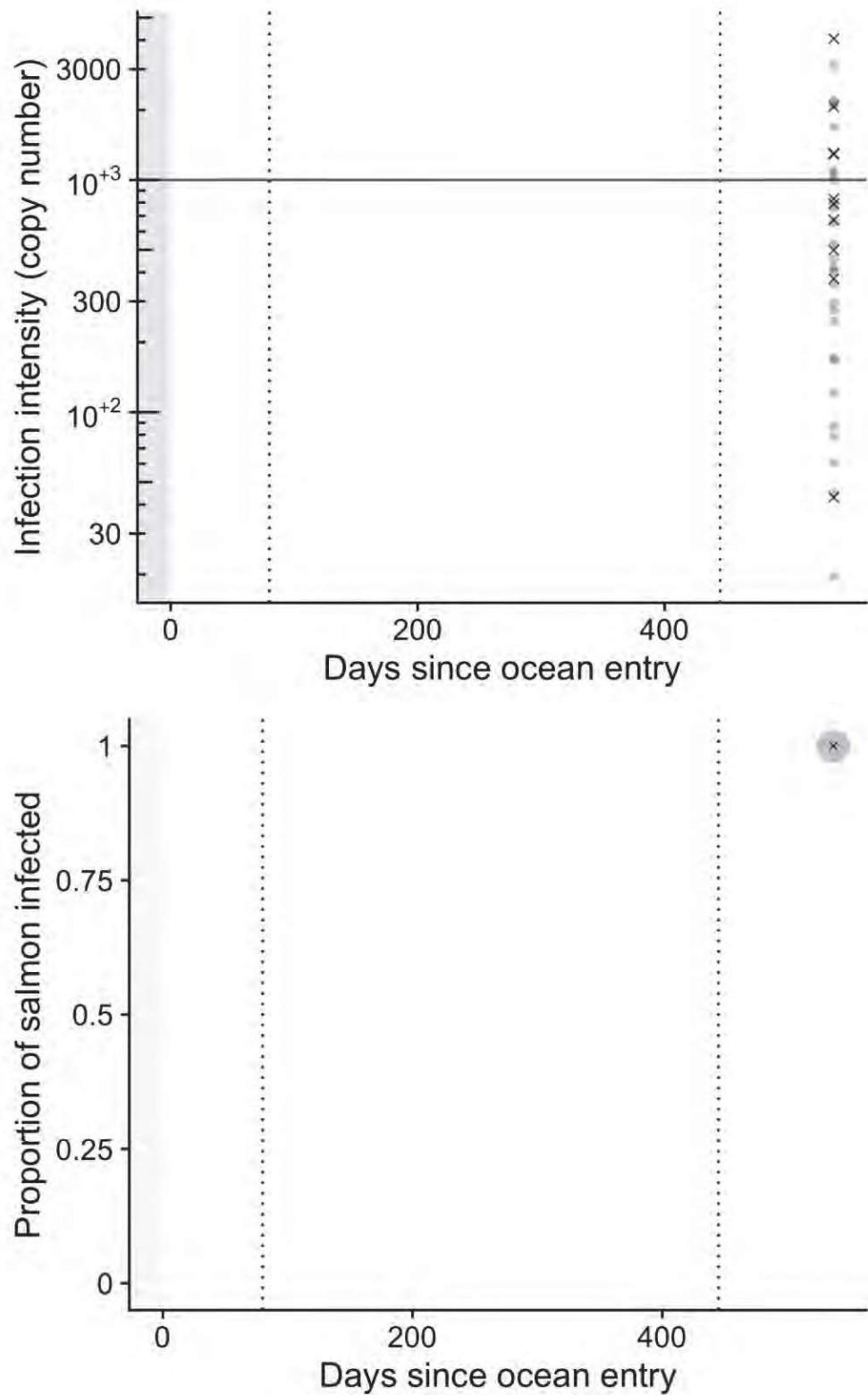
Cutthroat trout virus-2



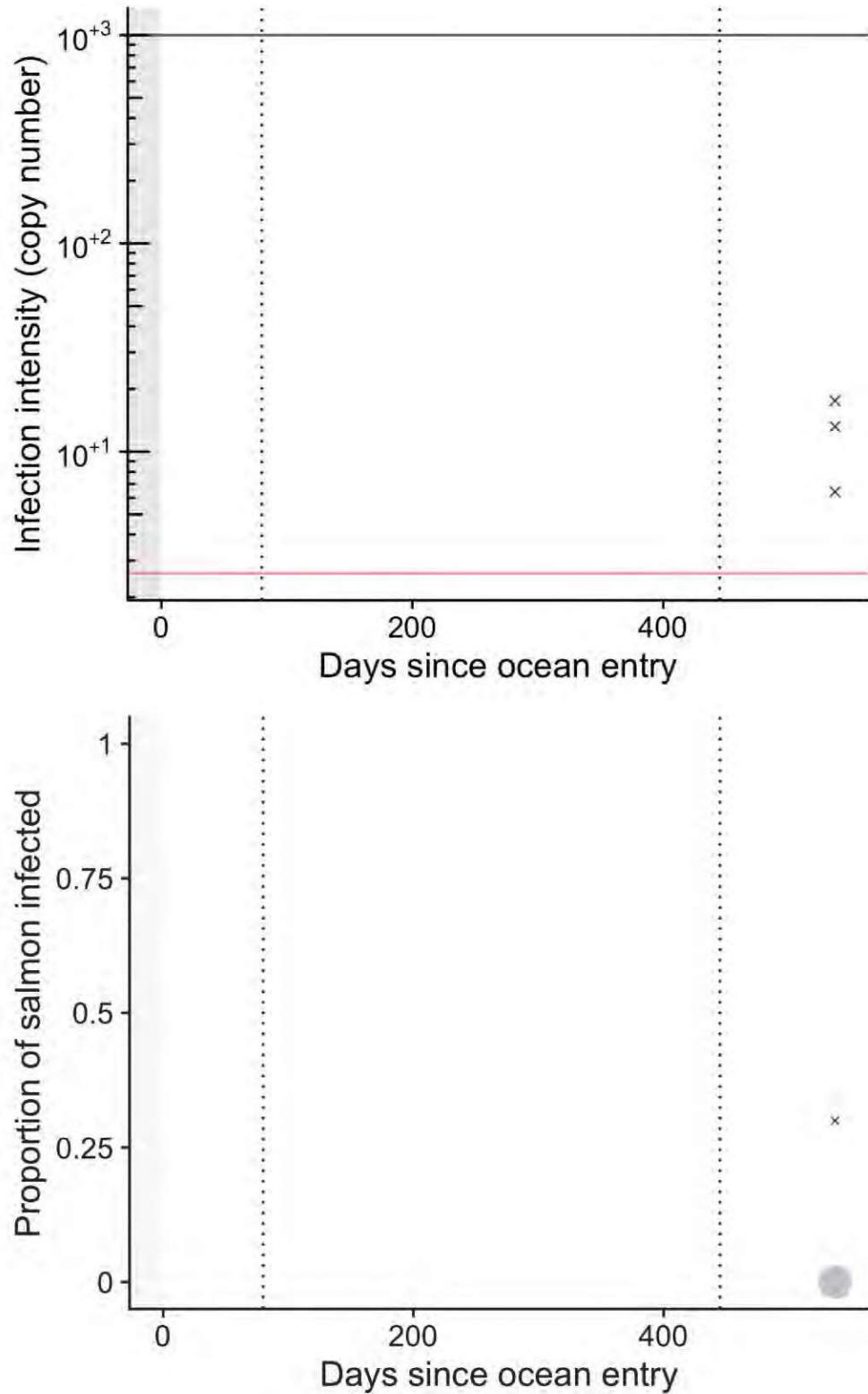
Piscirickettsia salmonis



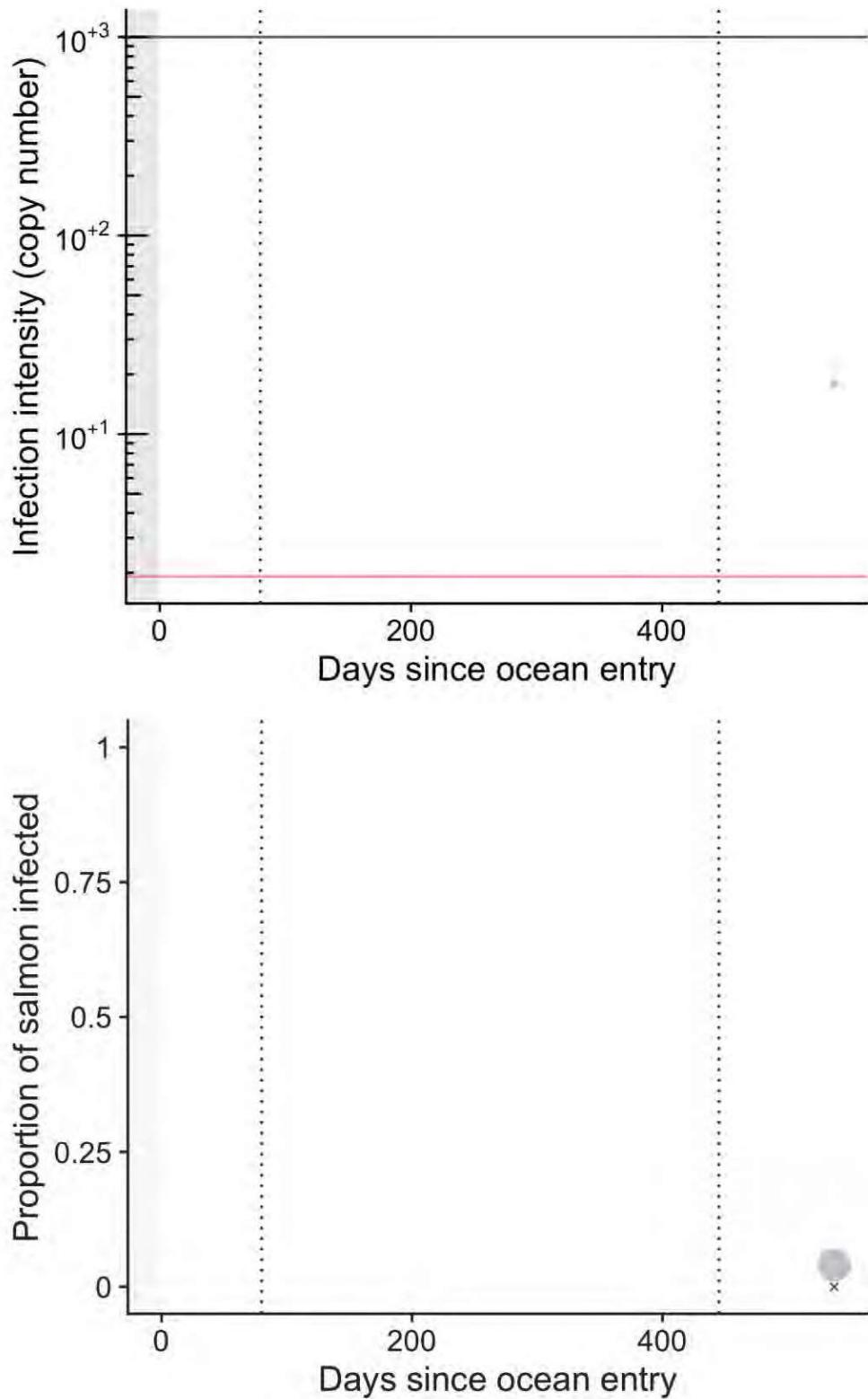
Piscine orthoreovirus



Candidatus Syngnathia salmonis



Tenacibaculum maritimum



Clinical signs

Table 1: Clinical signs for specimens sampled on 2021-10-13

metric	N5041	N5042	N5043	N5044	N5045	N5046	N5047	N5048	N5049	N5050	N5051	N5052	N5053	N5054	N5055	N5056	N5057	N5058	N5059	N5060
General																				
Live						X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Poor Performer					X															
Moribund		X																		
Mort	X		X	X	X															
Skeletal Deformity																				
Exophthalmia		X																X		
Cataract/Corneal Opacity																				
Skin & Fins																				
Hemorrhages		X																		
Erosion	X					X												X		
Excess Mucous								X							X			X		
Parasites																				
Gills																				
Short Operculum							X													
Pale														X	X	X		X	X	
Erosions	X	X					X							X	X			X		
Parasites		X																		
Muscle																				
Boils																				
Nodules/White Spots						X				X									X	
Parasites						X														
Abdominal Cavity																				
Adhesions					X															
Hemorrhages	X																X		X	
Parasites				X																
Spleen																				
Dark			X																	
Nodules/White Spots																				
Adhesion Hyp Capsule		X												X						
Liver																				
Yellow			X			X														
Nodules/White Spots	X			X			X													
Parasites	X			X	X	X	X													
Intestine																				
Fluid Content							X													
Brain																				
Hemorrhages/Congestion	X					X	X													

Table 2: Clinical signs for specimens sampled on 2021-10-13

metric	N5075	N5074	N5073	N5072	N5071	N5070	N5069	N5068	N5067	N5066	N5065	N5064	N5063	N5062	N5061
General															
Live							X	X	X	X	X	X	X	X	X
Poor Performer															
Moribund															
Mort	X	X	X	X	X										
Skeletal Deformity															X
Exophthalmia															
Cataract/Corneal Opacity						X									
Skin & Fins															
Hemorrhages						X									
Erosion			X												X
Excess Mucous															
Parasites							X								
Gills															
Short Operculum															
Pale	X	X				X	X	X	X		X	X		X	X
Erosions			X	X		X	X	X	X		X	X	X	X	X
Parasites															
Muscle															
Boils						X			X	X				X	X
Nodules/White Spots					X		X	X	X					X	X
Parasites															
Abdominal Cavity															
Adhesions															
Hemorrhages					X		X	X	X						
Parasites															
Spleen															
Dark															
Nodules/White Spots														X	
Adhesion Hyp Capsule															
Liver															
Yellow															
Nodules/White Spots						X								X	
Parasites						X									
Intestine															
Fluid Content															
Brain															
Hemorrhages/Congestion	X	X				X									

Histology

Table 3: Histology scores for specimens sampled on 2021-10-13

metric	N5041	N5042	N5043	N5044	N5045	N5061	N5062	N5063	N5064	N5065
Heart										
Peri Epi		2	1		1			2	1	
Myo	1	1			1		1	2	3	1
Liver										
Cong Haem	1	1		1		2	3	1		
Nec		1	1	2	1				1	1
Spleen										
Cong Heam	2				2	1				2
Ellip Nec		2	2							1
W Pulpitis	2	2	2	3	1		1	2	3	
Pig Inc						2			1	
Cap Prolif				2						
Kidney										
Itis					3			2	1	
Osis										1
Cong Heam	2		1	1		1				
Interst Hyperplasia	3	1	2	2	1	2	1	2	1	2
Pancreatitis										
Pancreatitis	na									
Enteritis										
Enteritis	na					na				
Cnc										
Gliosis					1		1			
Cong Heam	1	1		2		2	1			1
Gills										
Itis			1		1					
Prolif	2	1	1	1	2	1	1	1	1	
Skin_muscle										
Itis Nec							1	1	1	
Tissue										
Necrosis Artefacts	1			1	1	2	2	2	2	2

Diagnoses and Comments

Table 4: Diagnoses and comments for specimens sampled on 2021-10-13

DFO ID	Diagnosis	Comments
N5041		Squamous Hyperplasia In Gills (2), Epitheliocystis (3), 1 Small Thrombus In The Heart
N5042	Furunculosis	Myocardioneclerosis (2), Bacterial Colonies In Heart (2), Fibrosis Hyperplasia + Laciniae In Epicardium (2), Hepatocyte Apoptosis (1)
N5043	Furunculosis + Epitheliocystis	Epitheliocystis (2), Myocardioneclerosis (1), Bacterial Colonies In Heart (1)
N5044		Epitheliocystis (3), Increase Fibrin In Spleen (2), Coag Necrosis Liver (2)
N5045	Furunculosis	Epitheliocystis (1), Neuronal Chromatolysis (1), Septic Thrombi In Atrium (2), Myocardioneclerosis (2), Bacterial Colonies In Heart (1)
N5062		Myocardioneclerosis (3)
N5063		Colangitis (1), Myocardioneclerosis (2), Bacterial Colonies In Heart (3)
N5064	Furunculosis	Diffused Picnotic Nuclei In Hepatocytes And Enterocytes
N5065		Myocardioneclerosis (2); Diffused Picnotic Nuclei In Hepatocytes And Enterocytes

Conclusions

In order to support the eDNA study, below is provided further evaluation of the results of testing from the Fish Health Report.

The sampling collection was slightly incomplete (i.e. only 10 live fish was sampled from the second pen, instead of 15) due to technical and organizational issues linked to the beginning of the project. Nevertheless, here below is a summary and evaluation of the findings from the sampled fish.

The farm was inspected in its entirety: most fish were behaving normally. The mortality per pen reported by the company resulted significantly higher than the normal. Mortality was attributed by the company primarily to gills issues and furunculosis. Clinically, some individuals among the sampled fish showed fin/skin erosion and hemorrhages, and gills erosion was very common. Several fish (either live or moribund/morts) also showed enlarged spleen during the dissection procedures. Some morts presented muscle lesions (i.e. boils and ulcers) typical of furunculosis. Brain congestion and hemorrhages was common too.

Molecular testing results show that the totality of the population resulted positive to PRV. Over two third of the morts was infected with *Aeromonas salmonicida* (while only 8% of the live fish, and overall prevalence at 26%), and a significant portion of morts was also infected with *Candidatus Syngnathia salmonis*. Background level detection of *Piscirickettsia salmonis* and *Tenacibaculum maritimum* was also reported.

Histopathologically, the moribund/morts samples collected showed an overall pattern of systemic congestive modifications with immunological/inflammatory response, affecting primarily spleen and kidney. Bacterial colonies (presumptively attributed to *A. salmonicida*) systemically distributed among the organs were also a recurrent finding. A diagnosis of furunculosis could be attributed to most of the morts collected. The gills were often impaired with inflammatory foci and epithelial cells proliferation.

Appendix

Glossary of infectious agents

Agent abbr.	Full agent name	Agent type	Disease	Ranking
ae_sal	Aeromonas salmonicida	Bacteria	Furunculosis	2
ascv	Atlantic salmon calicivirus	Virus	unknown	4
ctv-2	Cutthroat trout virus-2	Virus	unknown	4
fl_psy	Flavobacterium psychrophilum	Bacteria	Bacterial cold water disease	3
pisck_sal	Piscirickettsia salmonis	Bacteria	Piscirickettsiosis (SRS)	2
pmcv	Piscine myocarditis virus	Virus	Cardiomyopathy syndrome	1
prv-1	Piscine orthoreovirus	Virus	HSMI-EIBS-Jaundice/anemia	
psnv1	Pacific salmon nidovirus-1 (CoV)	Virus	unknown	4
re_sal	Renibacterium salmoninarum	Bacteria	Bacterial kidney disease	2
sch	Candidatus Syngnathia salmonis	Bacteria	Gill chlamydia	3
te_mar	Tenacibaculum maritimum	Bacteria	Marine flexibacteriosis (mouth/fin rot)	2
vi_ang	Vibrio anguillarum	Bacteria	Vibriosis	2
vi_sal	Vibrio salmonicida	Bacteria	Cold water vibriosis	2
ye_ruc	Yersinia ruckeri (Enteric redmouth disease)	Bacteria	Yersiniosis (Enteric red mouth)	2

eDNA study Report

Humphrey Rock sampling on October 19, 2021

Dr. Emiliano Di Cicco

June 29, 2022

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Flavobacterium psychrophilum	10
Piscirickettsia salmonis	11
Piscine orthoreovirus	12
Candidatus Syngnathus salmonis.....	13
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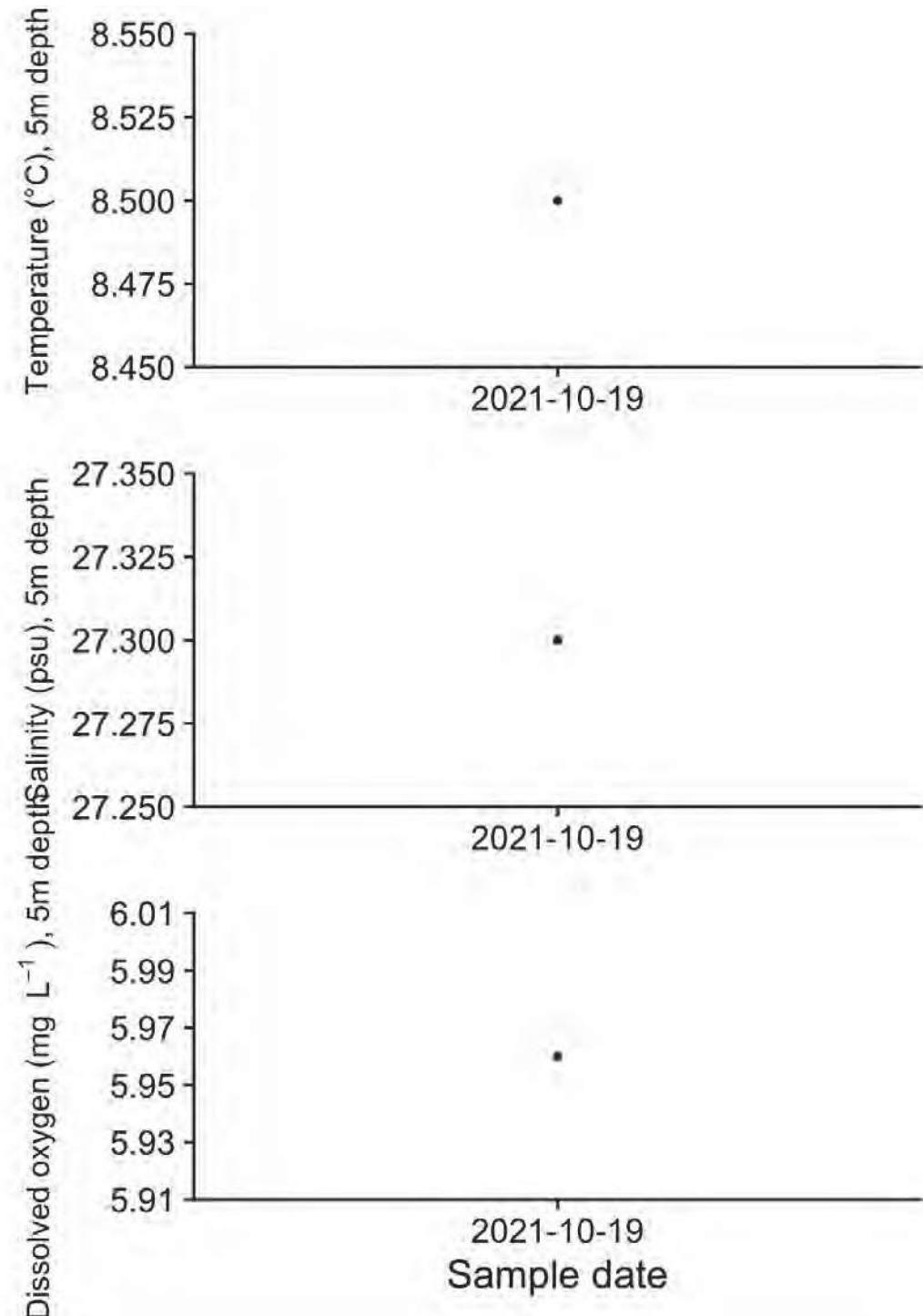
Executive summary

Premise

On October 19, 2021, 39 samples were collected by BATI and Mowi crew during a sampling event at Humphrey Rock (Mowi Ltd.). 39 Atlantic salmon subadults were collected from the Humphrey Rock farm site, including 30 live and 9 moribund/dead fish. All live fish were euthanized with TMS overdose prior to dissection with the exception of the moribund fish, which were administered a blow to the head. Portions of gill, liver and anterior kidney were collected in triplicate for molecular testing (preserved in RNA later) from all the fish, while all the moribund/dead fish also underwent collection of tissues (gills, spleen, liver, heart, anterior and posterior kidney, pyloric caeca, skeletal muscle + skin, brain) for histological analysis. Clinical notes and gross lesions were noted and reported for every fish. One aliquot has been provided to the Company MOWI Fish Health, another aliquot is stored at the BATI Field Office, and a third aliquot is stored at DFO - PBS. This latter aliquot has been tested for the presence and load of the agents indicated in the IMIP agreement as well as the agents indicated in the eDNA study agreement. Each sample has been extracted and tested individually. Negative and positive controls were run. A housekeeping gene was also included to assess the quality of the RNA extracted.

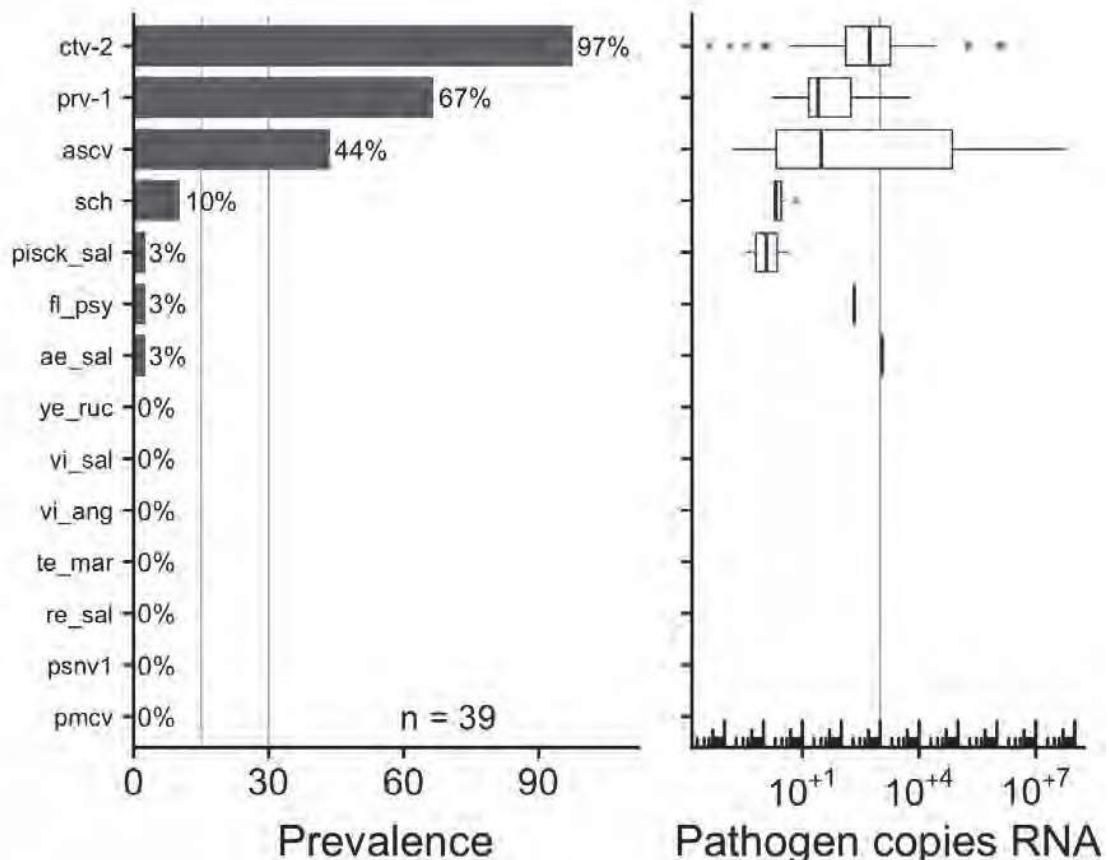
Histology samples have been sent to Wax-It Histo Ltd. to process and prepare slides, which have been read and scored by Dr. Di Cicco. A digital copy of each slide is available to the Company.

Environmental data

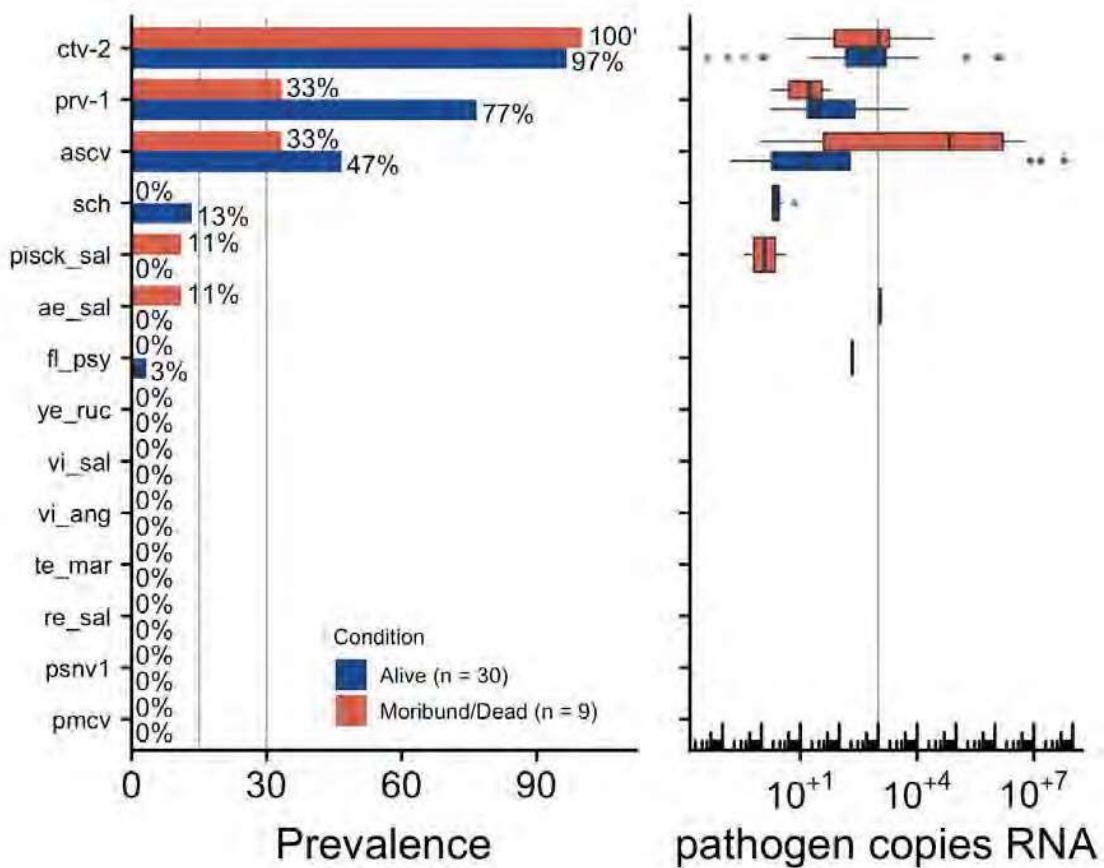


Water temperature ($^{\circ}\text{C}$), salinity (ppm), and dissolved oxygen (mg/L) at a 5m depth. Certain sampling dates have no recorded environmental data, resulting in gaps in the plots.

Overall infectious agent prevalence



Infectious agent prevalence in samples collected on 2021-10-19.



Infectious agent prevalence in samples collected on 2021-10-19, split by mortality status at time of sampling. Any specimens that were not confirmed to be either moribund or live at the time of generating this report are excluded from this figure.

Individual infectious agent trends

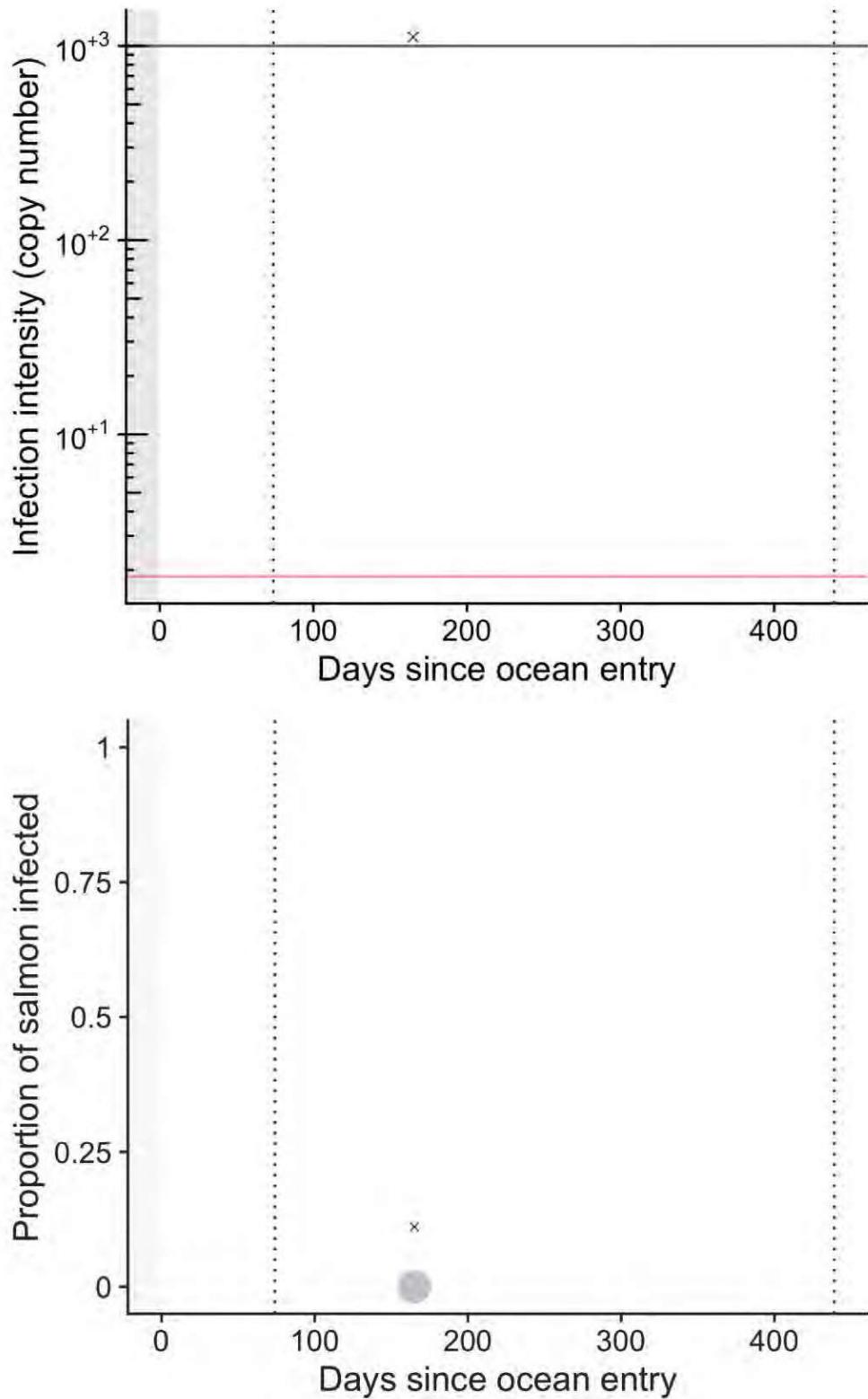
The following plots show individual infectious agent trends across all farm sites. In cases where sample size is sufficient, curves from a generalised additive model are included in the plot.

Grey circles represent live fish, and black X's represent dead/dying fish. Curves indicate mean predictions from a generalised additive model; blue and red correspond to live and dead/dying fish, respectively (shaded areas show 95% confidence regions). Left-hand grey region indicates freshwater hatchery residence, grey regions on x-axis indicate period of transfer to another site, and vertical dotted lines correspond to January 1st.

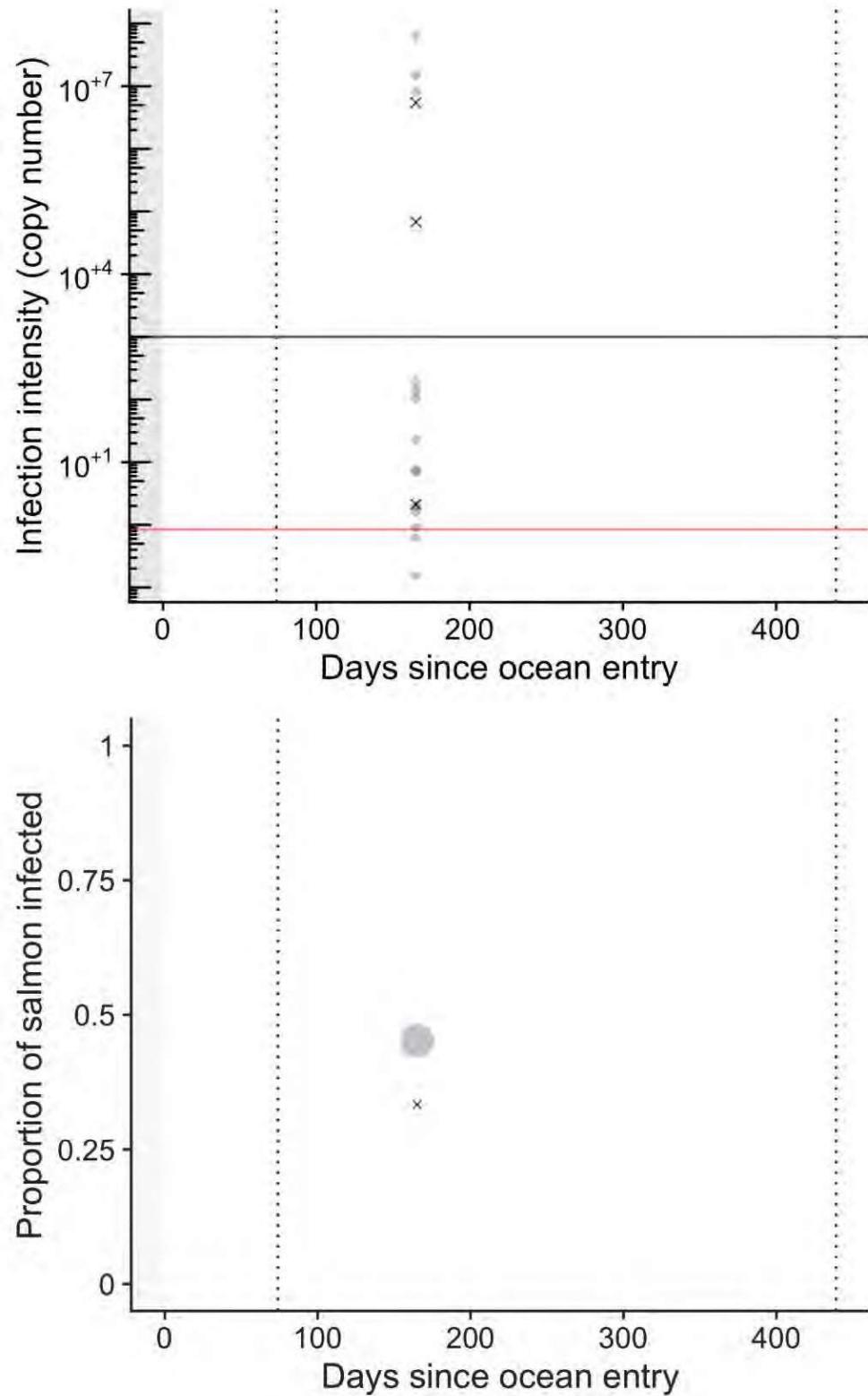
For infection intensity plots, horizontal red line indicates limit of detection (yielding ~90% true positive rate) for respective qPCR assay run in duplicate, while the horizontal black line indicates 1000 copies. Note log scale.

For proportion plots, grey circles show prevalence in live fish on each sampling date, and black X's show prevalence in dead/dying fish (symbol areas proportional to sample sizes).

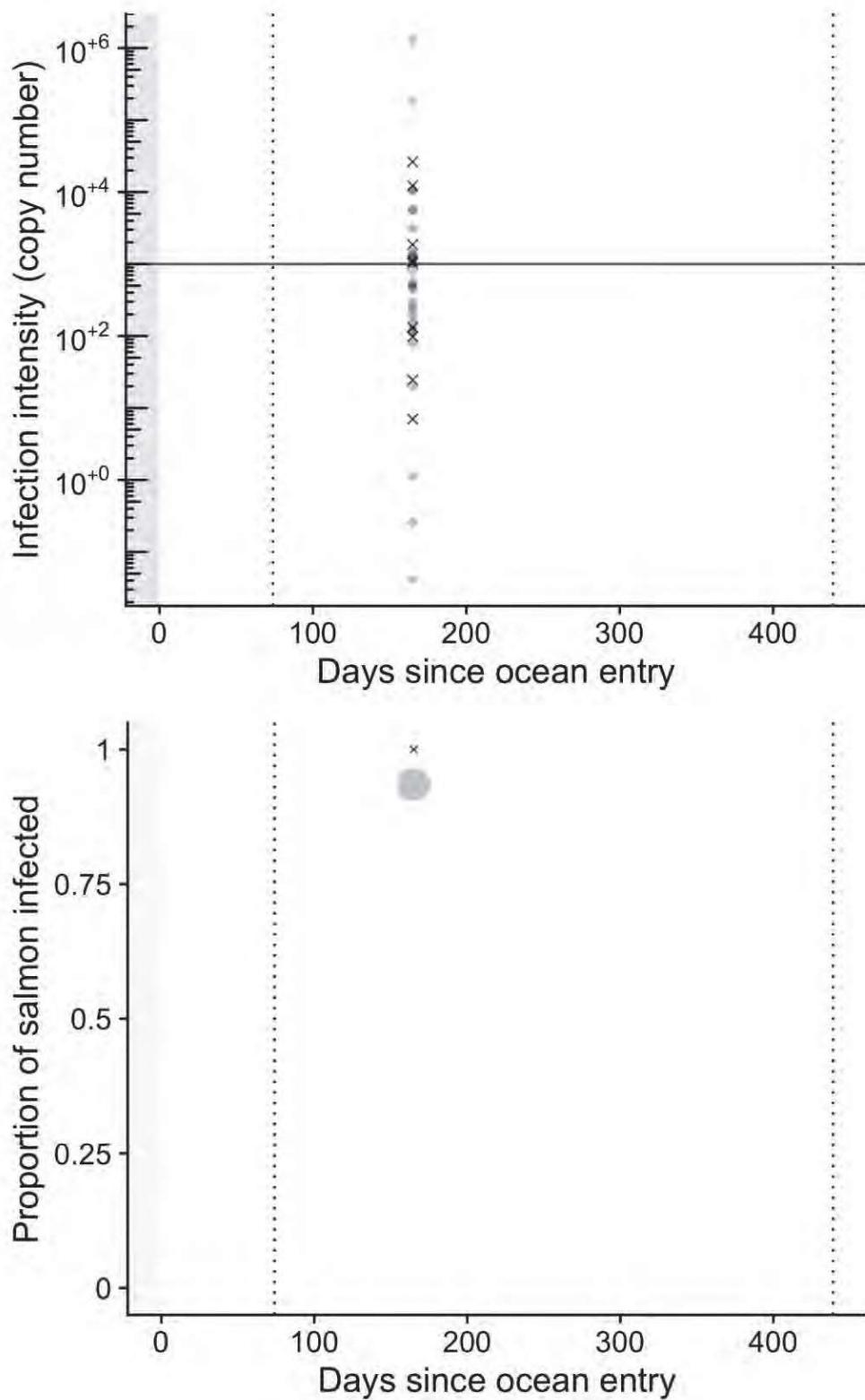
Aeromonas salmonicida



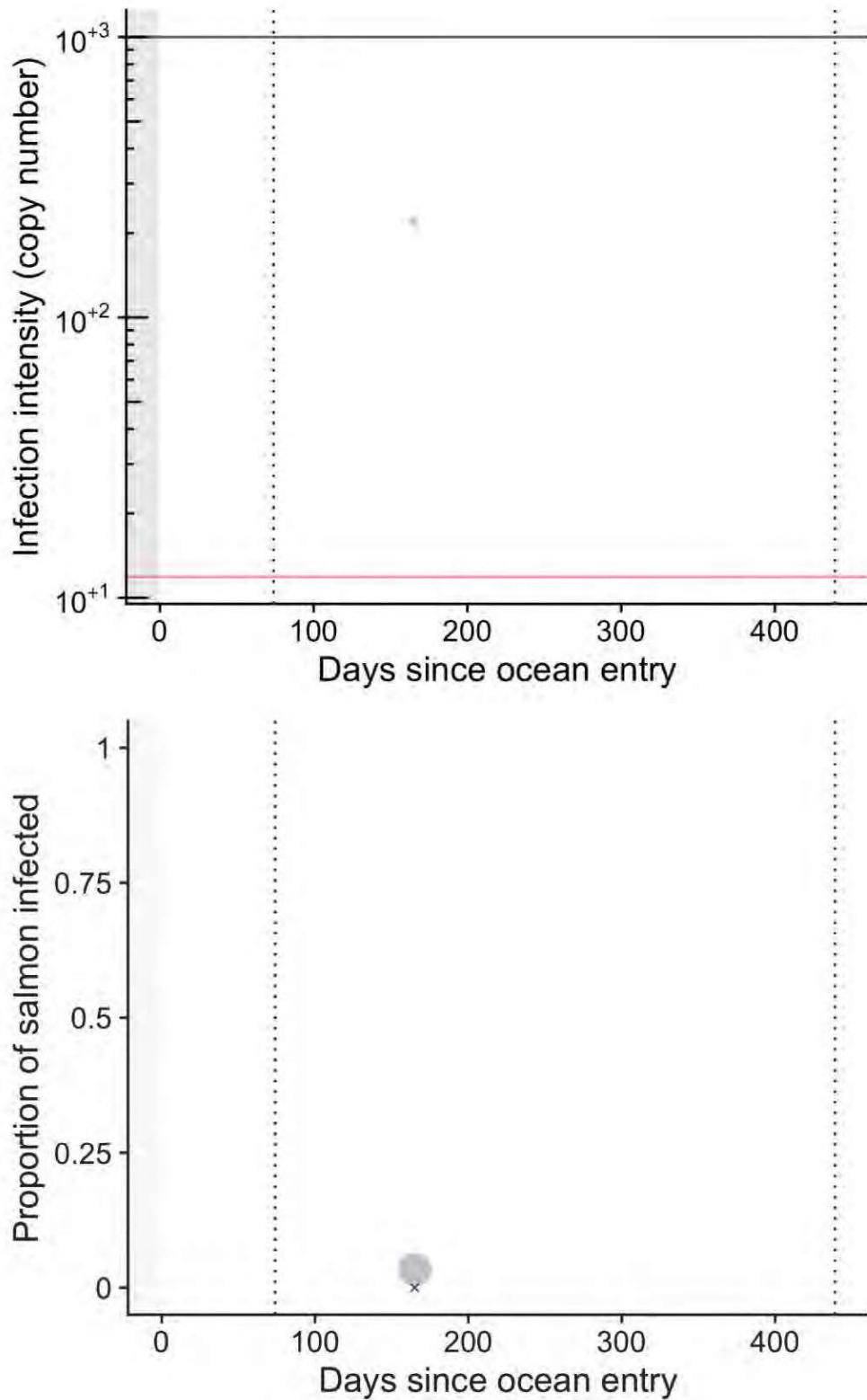
Atlantic salmon calicivirus



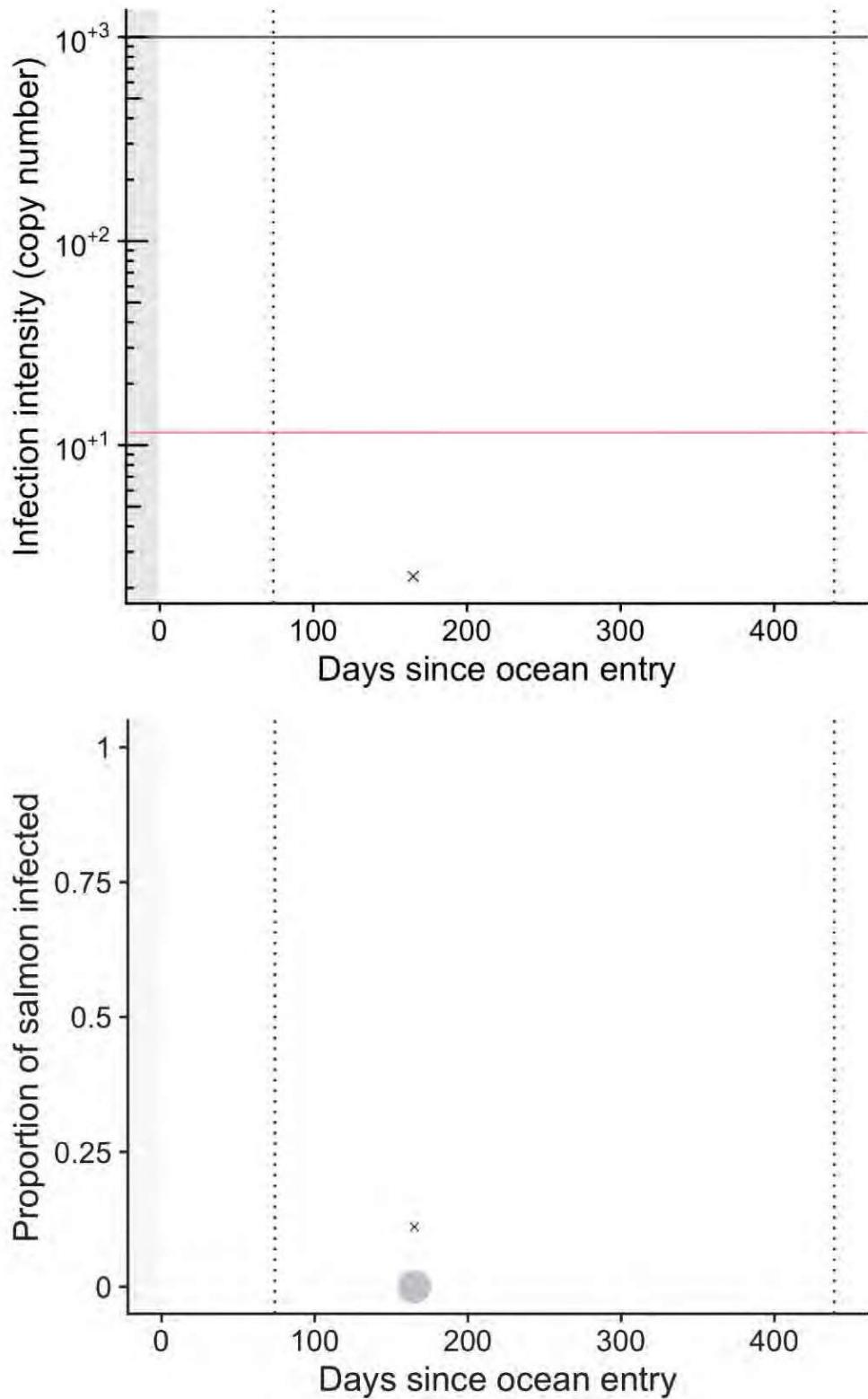
Cutthroat trout virus-2



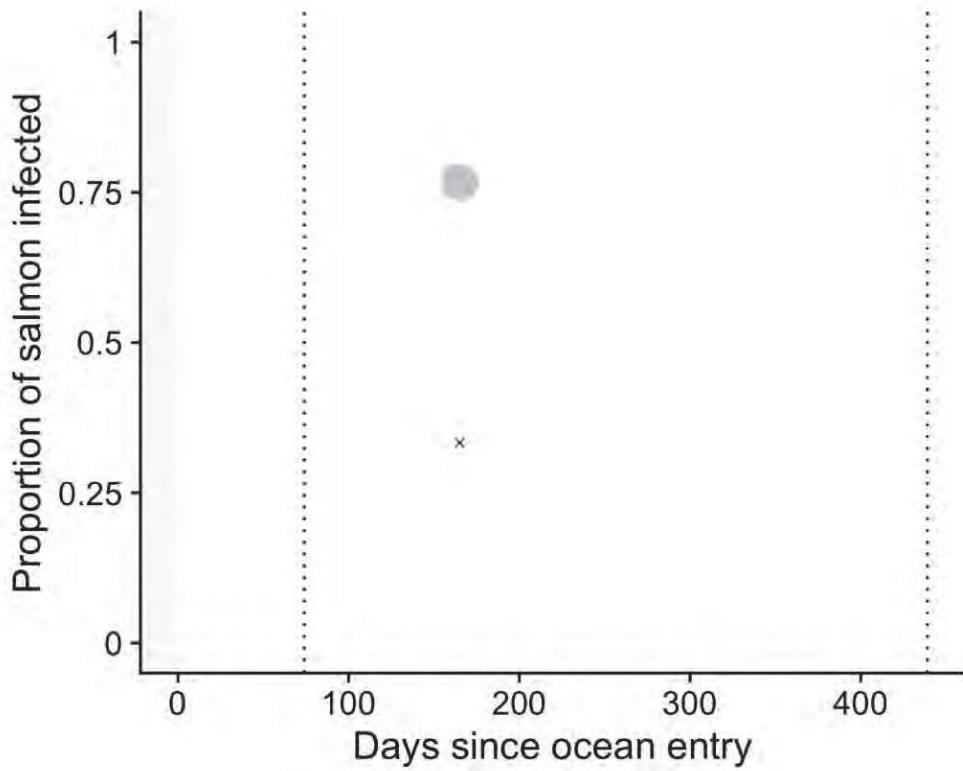
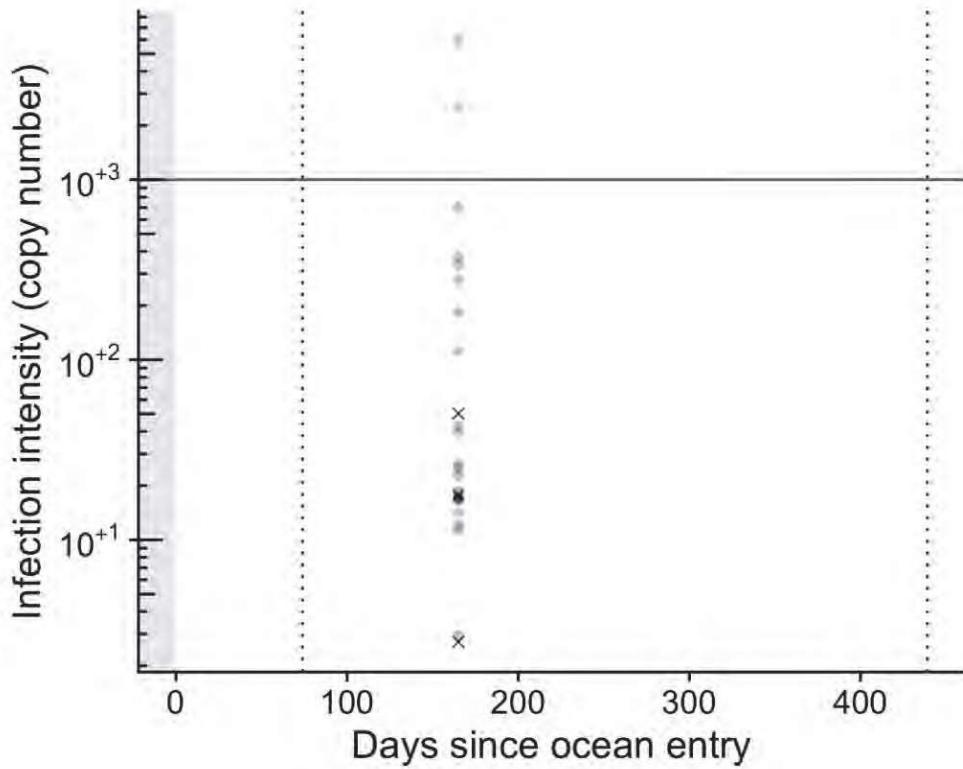
Flavobacterium psychrophilum



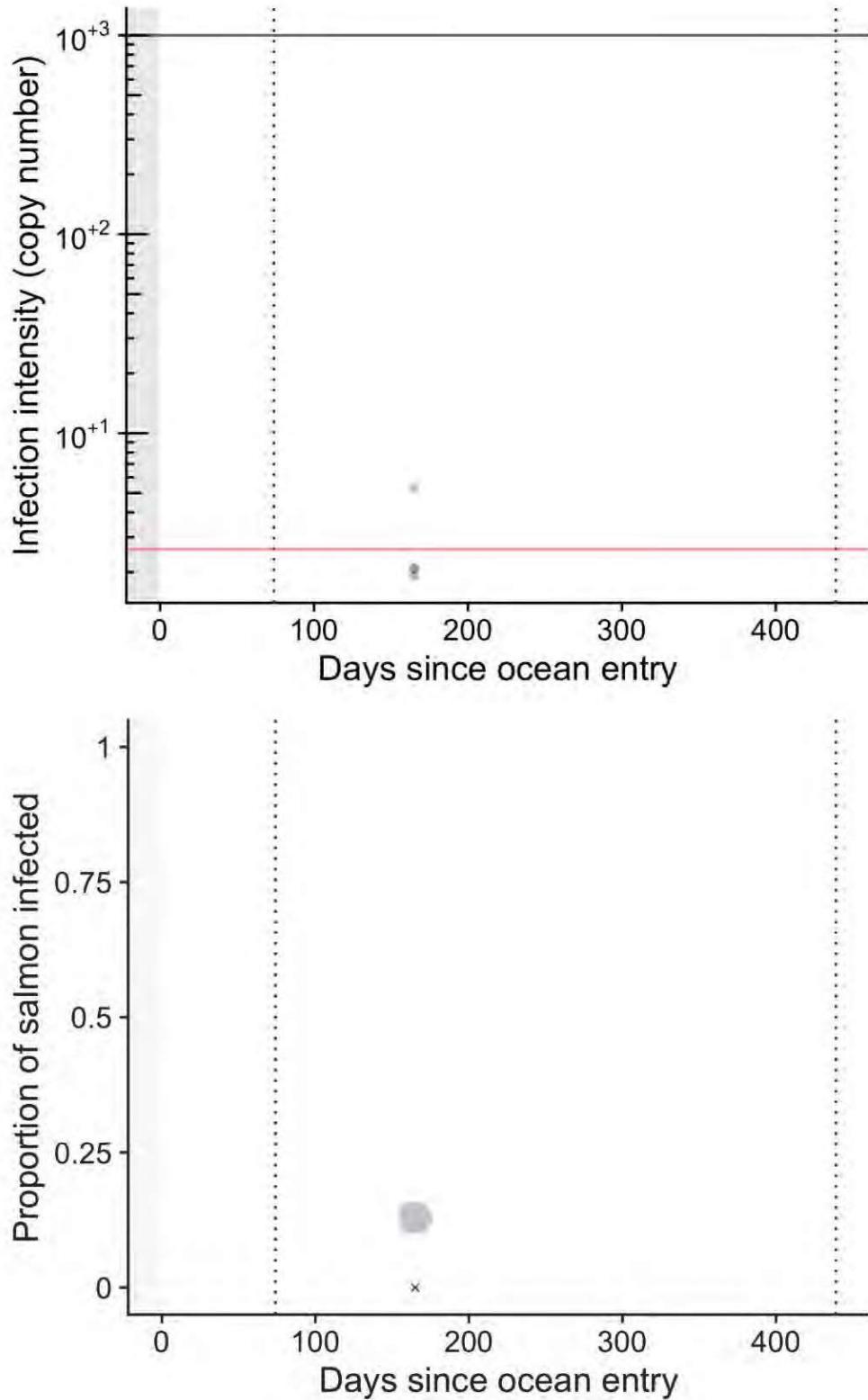
Piscirickettsia salmonis



Piscine orthoreovirus



Candidatus Syngnathia salmonis



Clinical signs

Table 1: Clinical signs for specimens sampled on 2021-10-19

metric	N5100	N5099	N5098	N5097	N5096	N5095	N5094	N5093	N5092	N5091	N5090	N5089	N5088	N5087	N5086	N5085	N5084	N5083	N5082	N5081		
General																						
Live						X	X	X	X											X	X	X
Poor Performer			X	X									X									
Moribund										X												
Mort	X	X	X	X							X	X	X								X	
Skin & Fins																						
Erosion																					X	
Fungus						X																
Lost Scales					X		X															
Parasites																			X			
Gills																						
Short Operculum			X				X		X			X	X	X								
Erosions	X						X	X	X										X		X	
Nodules/White Spots																						
Abdominal Cavity																						
Body Fat Content								X	X	X				X				X	X	X	X	X
Adhesions		X																X				
Spleen																						
Enlarged	X	X	X	X							X	X		X						X		
Liver																						
Pale																		X				X
Gallbladder																						
Enlarged				X							X		X				X		X		X	X
Heart																						
Pale																		X				X
Blood Clots/Hemopericardium					X							X	X								X	
Brain																						
Hemorrhages/Congestion	X	X	X	X							X	X	X	X								

Table 2: Clinical signs for specimens sampled on 2021-10-19

metric	N5101	N5102	N5103	N5104	N5105	N5106	N5107	N5108	N5109	N5110	N5111	N5112	N5113	N5114	N5115	N5116	N5117	N5118	N5119
General																			
Live	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Poor Performer																			
Moribund																			
Mort																			
Skin & Fins																			
Erosion	X		X																
Fungus																			
Lost Scales																			
Parasites																			
Gills																			
Short Operculum																			
Erosions							X				X		X		X		X		X
Nodules/White Spots		X											X		X		X		X
Abdominal Cavity																			
Body Fat Content	X		X	X	X		X		X		X	X			X	X			
Adhesions																			
Spleen																			
Enlarged				X						X			X			X			
Liver																			
Pale																			
Gallbladder																			
Enlarged		X			X		X												
Heart																			
Pale																			
Blood Clots/Hemopericardium																			
Brain																			
Hemorrhages/Congestion																			

Histology

Table 3: Histology scores for specimens sampled on 2021-10-19

metric	N5097	N5092	N5091	N5090	N5089	N5084	N5083	N5082	N5081
Heart									
Peri Epi	1		2	1	1	1	1	2	1
Myo		1	1					1	2
Liver									
Cong Haem	1	1			1	2			
Nec									1
Itis					1			2	
Spleen									
Cong Haem	1								2
Ellip Nec							1		
W Pulpitis		1	1	1	1	2	1	2	2
Pig Inc					1		1		2
Kidney									
Itis		1						2	
Osis		1							
Cong Haem	1						1		
Interst Hyperplasia	1	1	2	2	1	1	2	2	2
Cnc									
Gliosis		1	1	1			2		1
Cong Haem	2	2	2	2	2	2	1		
Gills									
Itis	nv		nv	nv	nv	nv	nv	nv	
Cong Haem	nv		nv	nv	nv	nv	nv	nv	
Prolif	nv	1	nv	nv	nv	nv	nv	nv	1
Tissue									
Necrosis Artefacts	2	2	2	2	2	2	2	2	

Diagnoses and Comments

Table 4: Diagnoses and comments for specimens sampled on 2021-10-19

DFO ID	Diagnosis	Comments
N5081		Neuronal Vacuolization (1), Peribiliary Immune Activation (1), Hepatocyte Apoptosis (1); Gills Very Old
N5082		Myocardioneerosis (1), Vac Deg Hepatocytes (2), Increase Fibrin In Spleen (2)
N5083		Neuronal Vacuolization (1); Gills Very Old
N5084		Peribiliary Immune Activation (1), Perivascular Inflammatory Cuffs In Brain (1), Inflammatory Foci In Atrium (2); Gills Very Old
N5089		Peribiliary Immune Activation (2); Gills Very Old
N5091		Peribiliary Immune Activation (1), Increase Fibrin In Spleen (2); Gills Very Old
N5092	Furunculosis	Bacterial Colonies In Gills And Heart (2), Perivascular Inflammatory Foci In Liver (2), Myocardial Necrosis (1)
N5097		Neuronal Deg + Gliosis (1), Renal Erythrophagocytosis (2), Accumulation Eosinophilic Material In Hepatocytes (1), Vac Deg Hepatocytes (2), Peribiliary Immune Activation (1)

Conclusions

In order to support the eDNA study, below is provided further evaluation of the results of testing from the Fish Health Report.

The sampling collection was completed. Available moribund/mort fish from control pen and secondary pen were collected. Here below is a summary and evaluation of the findings from the sampled fish.

The farm was not inspected in its entirety, due to the configuration of the site (i.e. circular pens). A full inspection would be timely demanding, and it doesn't appear to be practical as it's very difficult to observe the fish underwater when brightness is not adequate. However, most fish in the inspected pens were behaving normally. The mortality per pen reported by the company resulted in line with the normal standard expected for such a site. Clinically, some individuals among the sampled fish showed gills erosion and nodules. Several fish (either live or moribund/morts) also showed enlarged spleen, pale and heart and liver. Enlarged gall bladder was common amongst the morts as well. Brain congestion and hemorrhages were pretty common too.

Molecular testing results show that 67% of the samples collected resulted positive to PRV, particularly live fish. Background level detection of *Aeromonas salmonicida* and *Piscirickettsia salmonis* (amongst mortalities) as well as *Candidatus Syngnathus salmonis* and *Flavobacterium psychrophilum* (amongst live fish) was also reported.

Histopathologically, the moribund/morts samples collected showed an overall pattern of systemic congestive modifications with immunological/inflammatory response, affecting primarily spleen and kidney. Mild alterations consistent with early stages development of HSMI were common, primarily amongst the morts (enlarged spleen, pale liver and heart, associated with mild epicarditis and liver degeneration). Bacterial colonies in the heart was found in one of the morts, likely conducive to *A. salmonicida* infection. Brain hemorrhages was a common finding in morts, as well as neuronal alterations (vacuolization and gliosis).

Appendix

Glossary of infectious agents

Agent abbr.	Full agent name	Agent type	Disease	Ranking
ae_sal	Aeromonas salmonicida	Bacteria	Furunculosis	2
ascv	Atlantic salmon calicivirus	Virus	unknown	4
ctv-2	Cutthroat trout virus-2	Virus	unknown	4
fl_psy	Flavobacterium psychrophilum	Bacteria	Bacterial cold water disease	3
pisck_sal	Piscirickettsia salmonis	Bacteria	Piscirickettsiosis (SRS)	2
pmcv	Piscine myocarditis virus	Virus	Cardiomyopathy syndrome	1
prv-1	Piscine orthoreovirus	Virus	HSMI-EIBS-Jaundice/anemia	
psnv1	Pacific salmon nidovirus-1 (CoV)	Virus	unknown	4
re_sal	Renibacterium salmoninarum	Bacteria	Bacterial kidney disease	2
sch	Candidatus Syngnathia salmonis	Bacteria	Gill chlamydia	3
te_mar	Tenacibaculum maritimum	Bacteria	Marine flexibacteriosis (mouth/fin rot)	2
vi_ang	Vibrio anguillarum	Bacteria	Vibriosis	2
vi_sal	Vibrio salmonicida	Bacteria	Cold water vibriosis	2
ye_ruc	Yersinia ruckeri (Enteric redmouth disease)	Bacteria	Yersiniosis (Enteric red mouth)	2

eDNA Study Report

Sargeaunt Passage sampling on October 20, 2021

Dr. Emiliano Di Cicco

June 29, 2022

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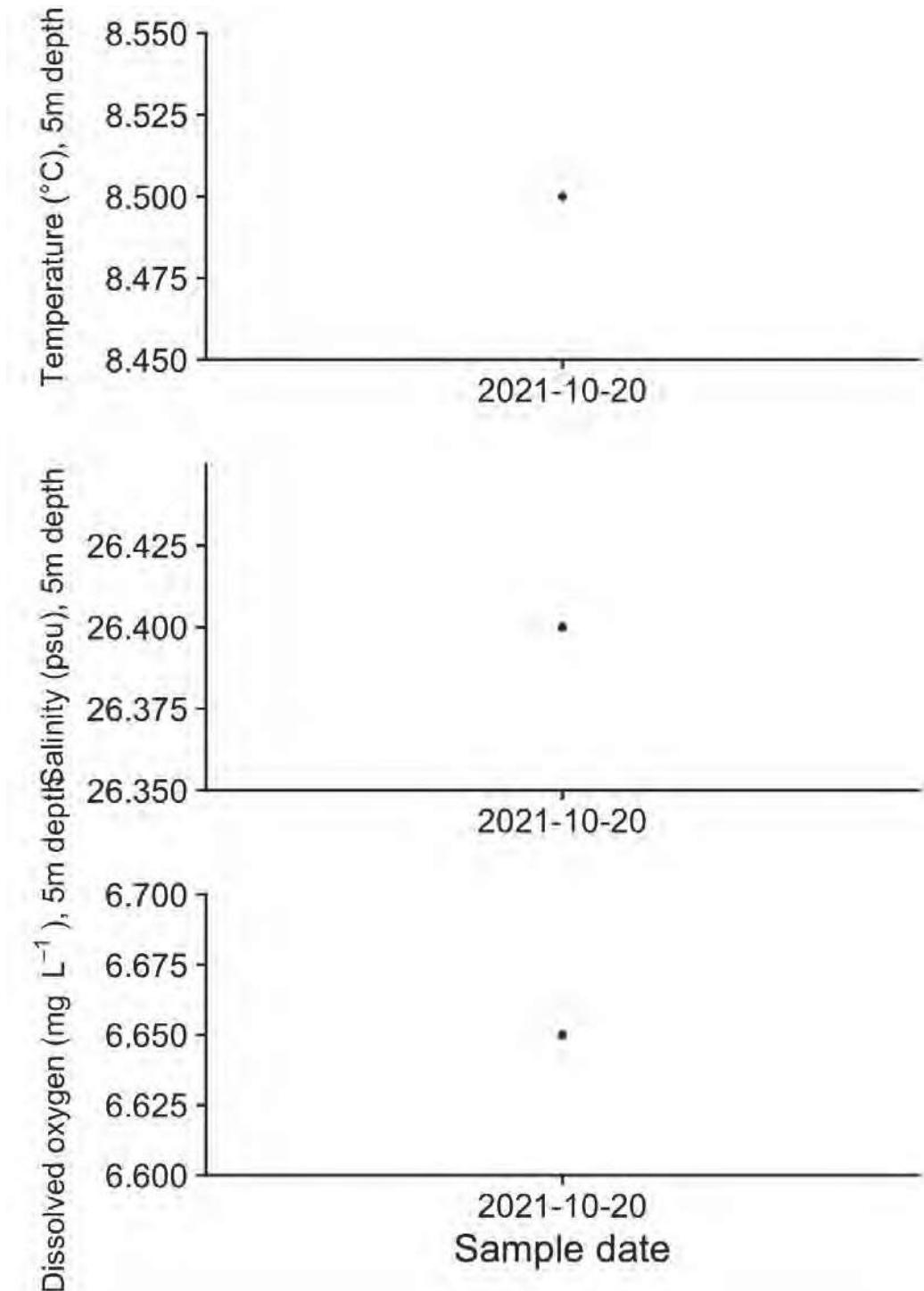
Executive summary

Premise

On October 20, 2021, 33 samples were collected by BATI and Mowi crew during a sampling event at Sargeaunt Passage (Mowi Ltd.). 33 Atlantic salmon subadults were collected from the Sargeaunt Passage farm site, including 30 live and 3 moribund/dead fish. All live fish were euthanized with TMS overdose prior to dissection with the exception of the moribund fish, which were administered a blow to the head. Portions of gill, liver and anterior kidney were collected in triplicate for molecular testing (preserved in RNA later) from all the fish, while all the moribund/dead fish also underwent collection of tissues (gills, spleen, liver, heart, anterior and posterior kidney, pyloric caeca, skeletal muscle + skin, brain) for histological analysis. Clinical notes and gross lesions were noted and reported for every fish. One aliquot has been provided to the Company MOWI Fish Health, another aliquot is stored at the BATI Field Office, and a third aliquot is stored at DFO - PBS. This latter aliquot has been tested for the presence and load of the agents indicated in the IMIP agreement as well as the agents indicated in the eDNA study agreement. Each sample has been extracted and tested individually. Negative and positive controls were run. A housekeeping gene was also included to assess the quality of the RNA extracted.

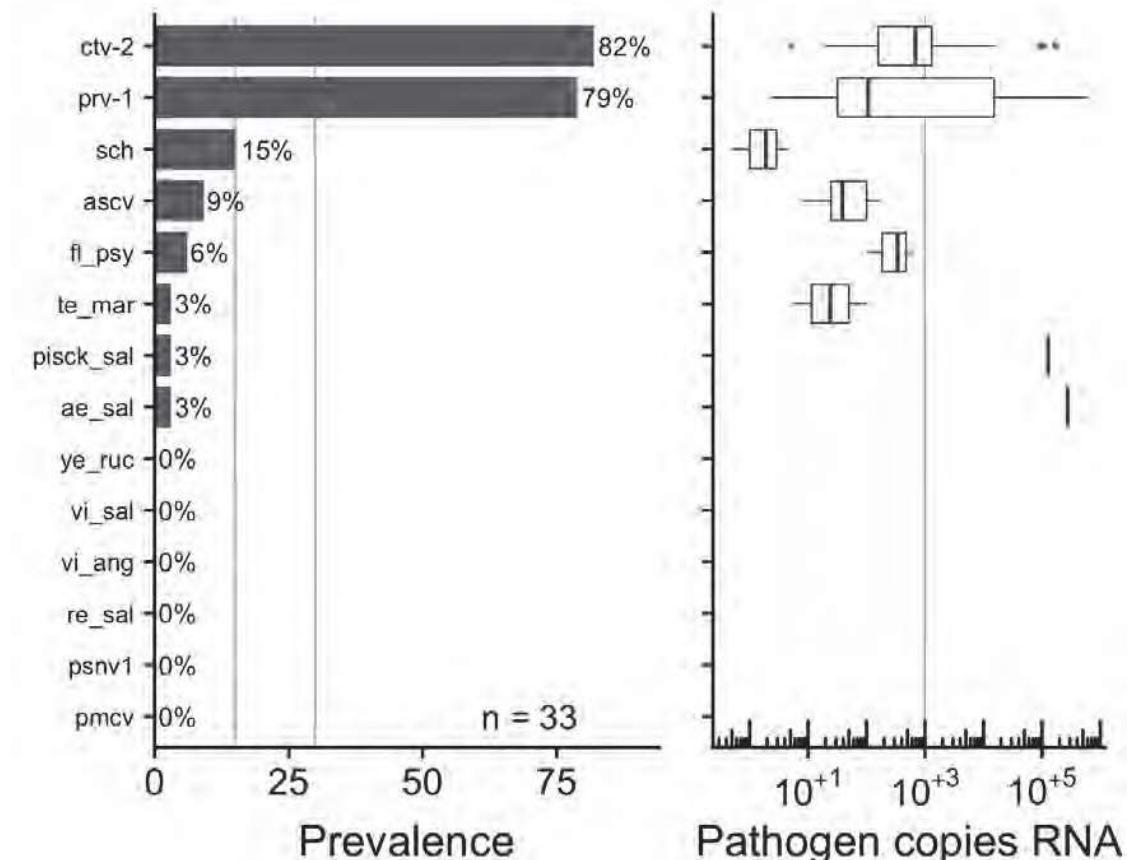
Histology samples have been sent to Wax-It Histo Ltd. to process and prepare slides, which have been read and scored by Dr. Di Cicco. A digital copy of each slide is available to the Company.

Environmental data

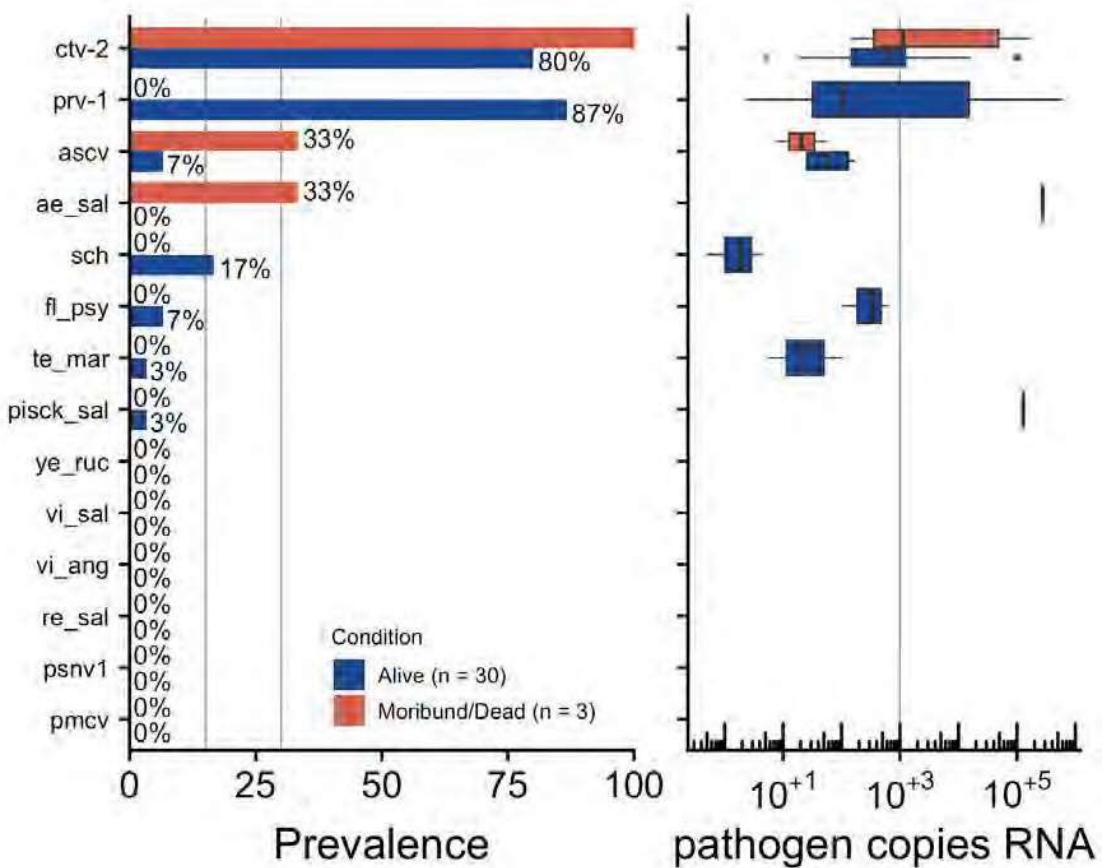


Water temperature ($^{\circ}\text{C}$), salinity (ppm), and dissolved oxygen (mg/L) at a 5m depth. Certain sampling dates have no recorded environmental data, resulting in gaps in the plots.

Overall infectious agent prevalence



Infectious agent prevalence in samples collected on 2021-10-2



Infectious agent prevalence in samples collected on 2021-10-20, split by mortality status at time of sampling. Any specimens that were not confirmed to be either moribund or live at the time of generating this report are excluded from this figure.

Individual infectious agent trends

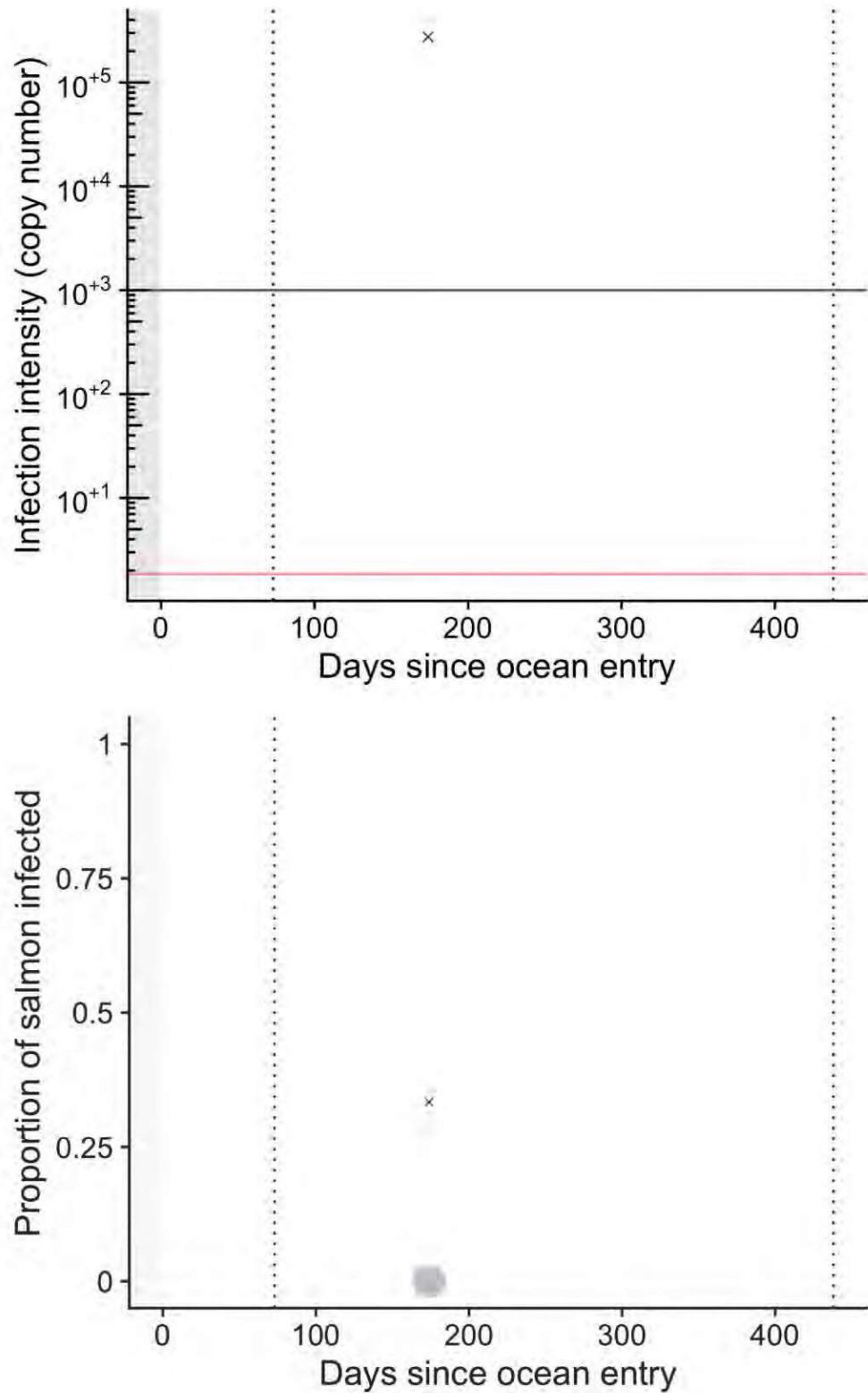
The following plots show individual infectious agent trends across all farm sites. In cases where sample size is sufficient, curves from a generalised additive model are included in the plot.

Grey circles represent live fish, and black X's represent dead/dying fish. Curves indicate mean predictions from a generalised additive model; blue and red correspond to live and dead/dying fish, respectively (shaded areas show 95% confidence regions). Left-hand grey region indicates freshwater hatchery residence, grey regions on x-axis indicate period of transfer to another site, and vertical dotted lines correspond to January 1st.

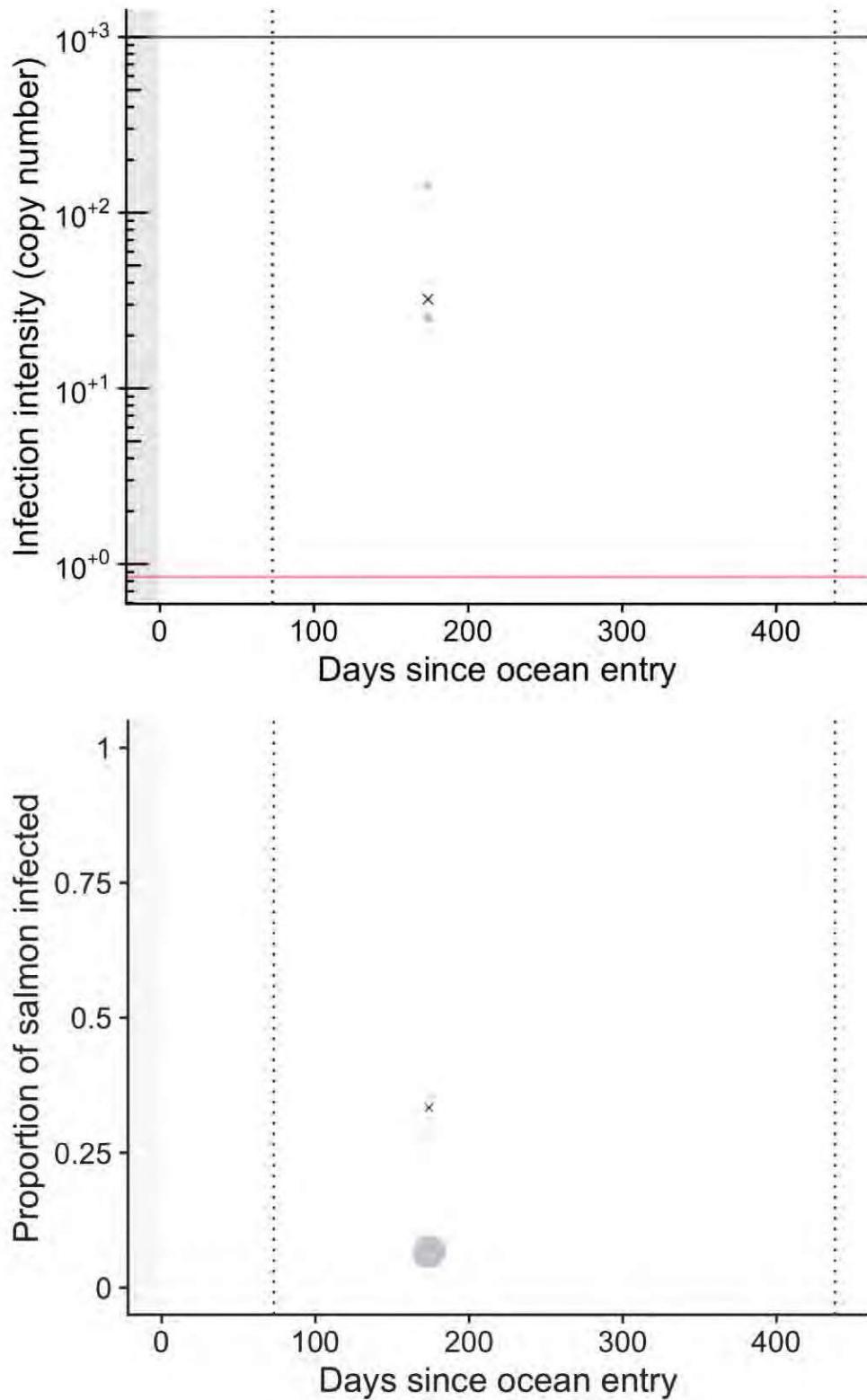
For infection intensity plots, horizontal red line indicates limit of detection (yielding ~90% true positive rate) for respective qPCR assay run in duplicate, while the horizontal black line indicates 1000 copies. Note log scale.

For proportion plots, grey circles show prevalence in live fish on each sampling date, and black X's show prevalence in dead/dying fish (symbol areas proportional to sample sizes).

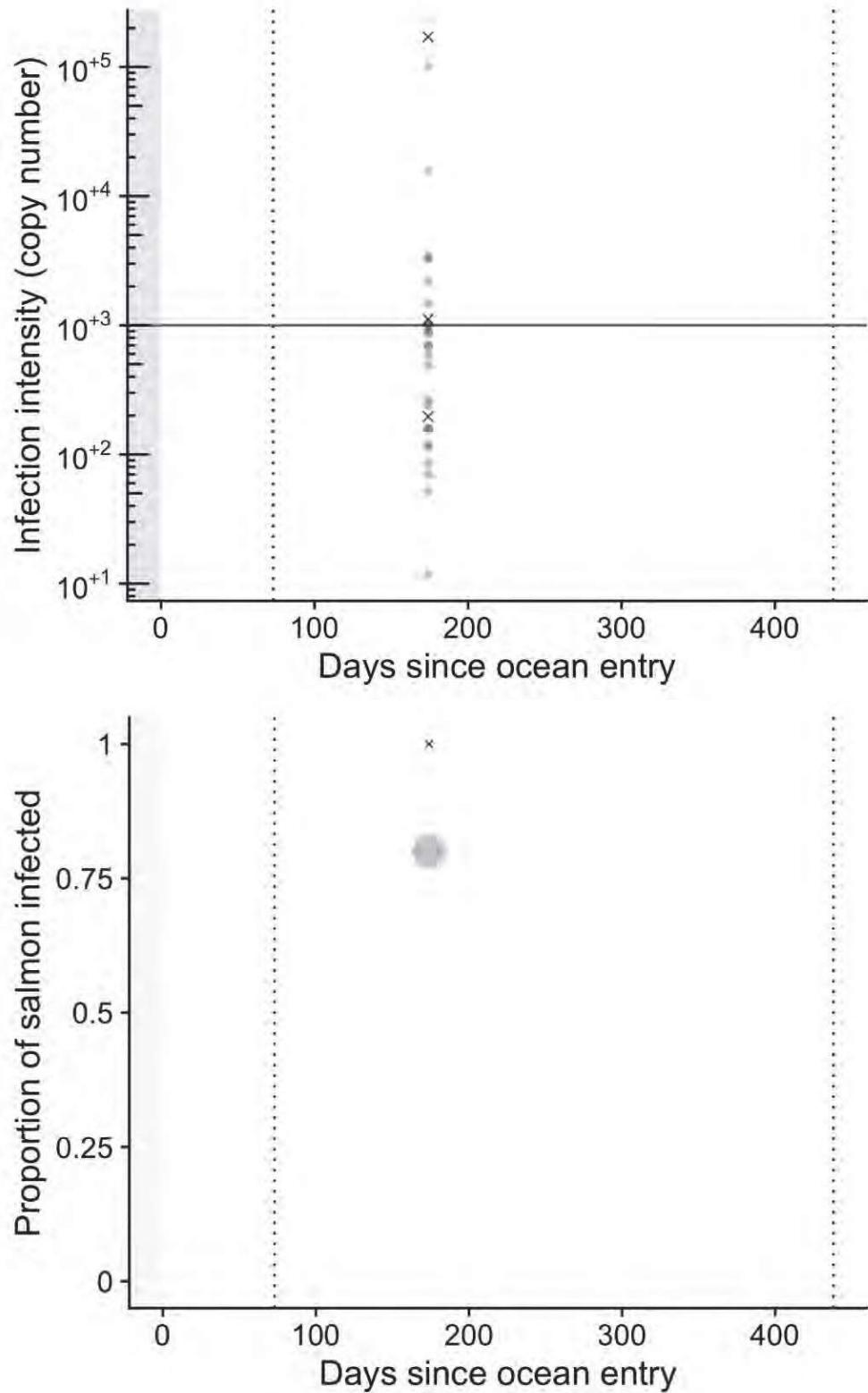
Aeromonas salmonicida



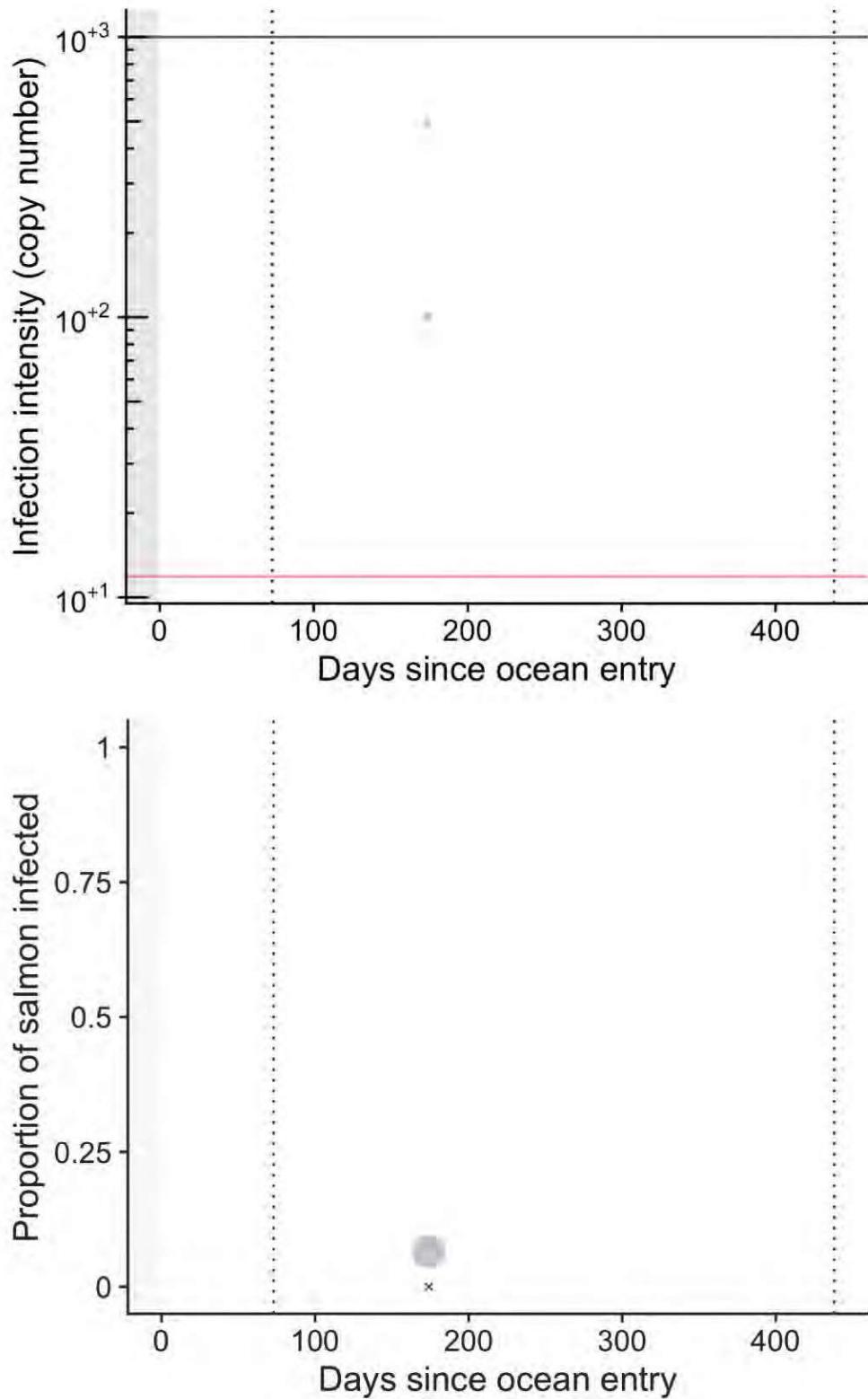
Atlantic salmon calicivirus



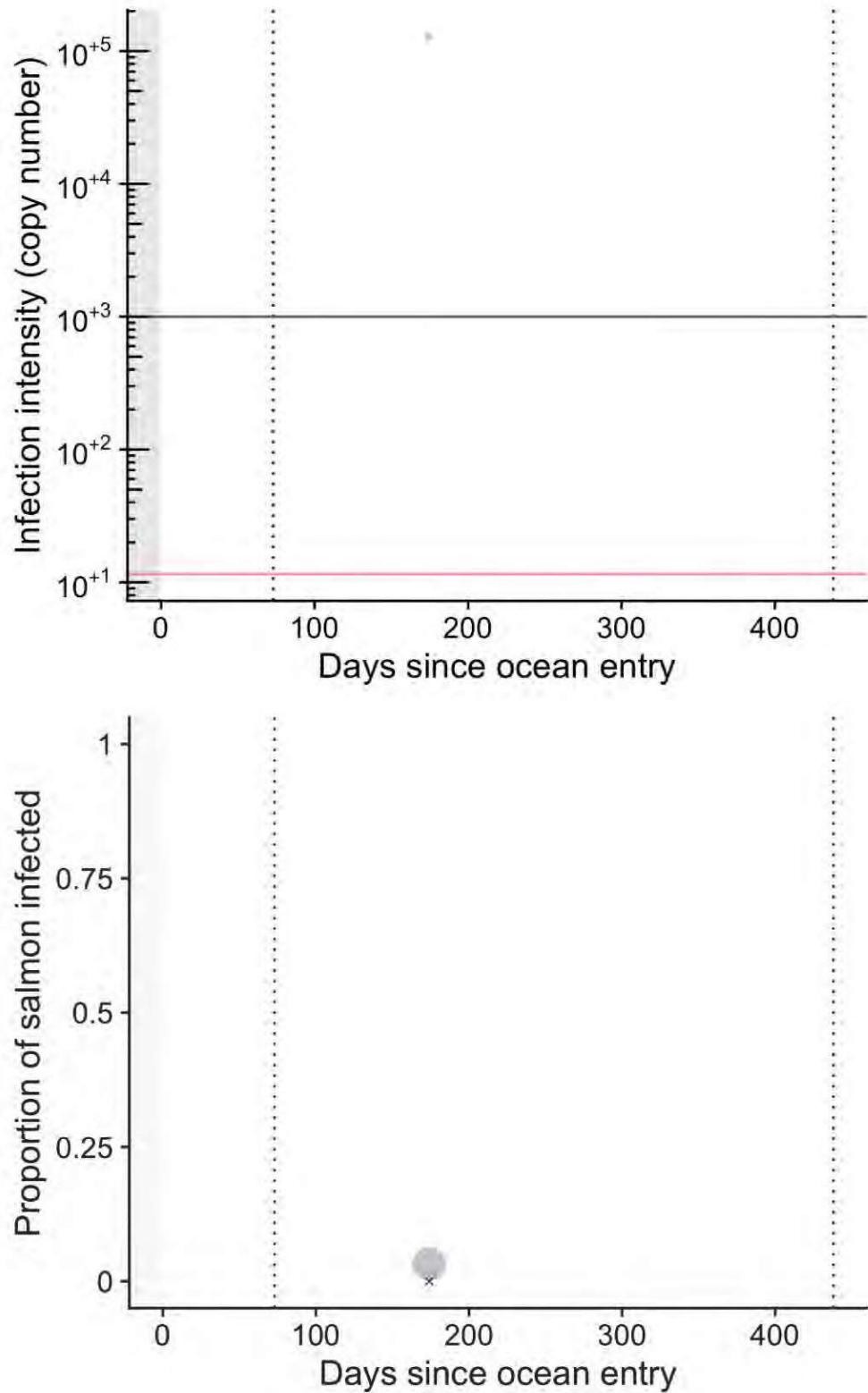
Cutthroat trout virus-2



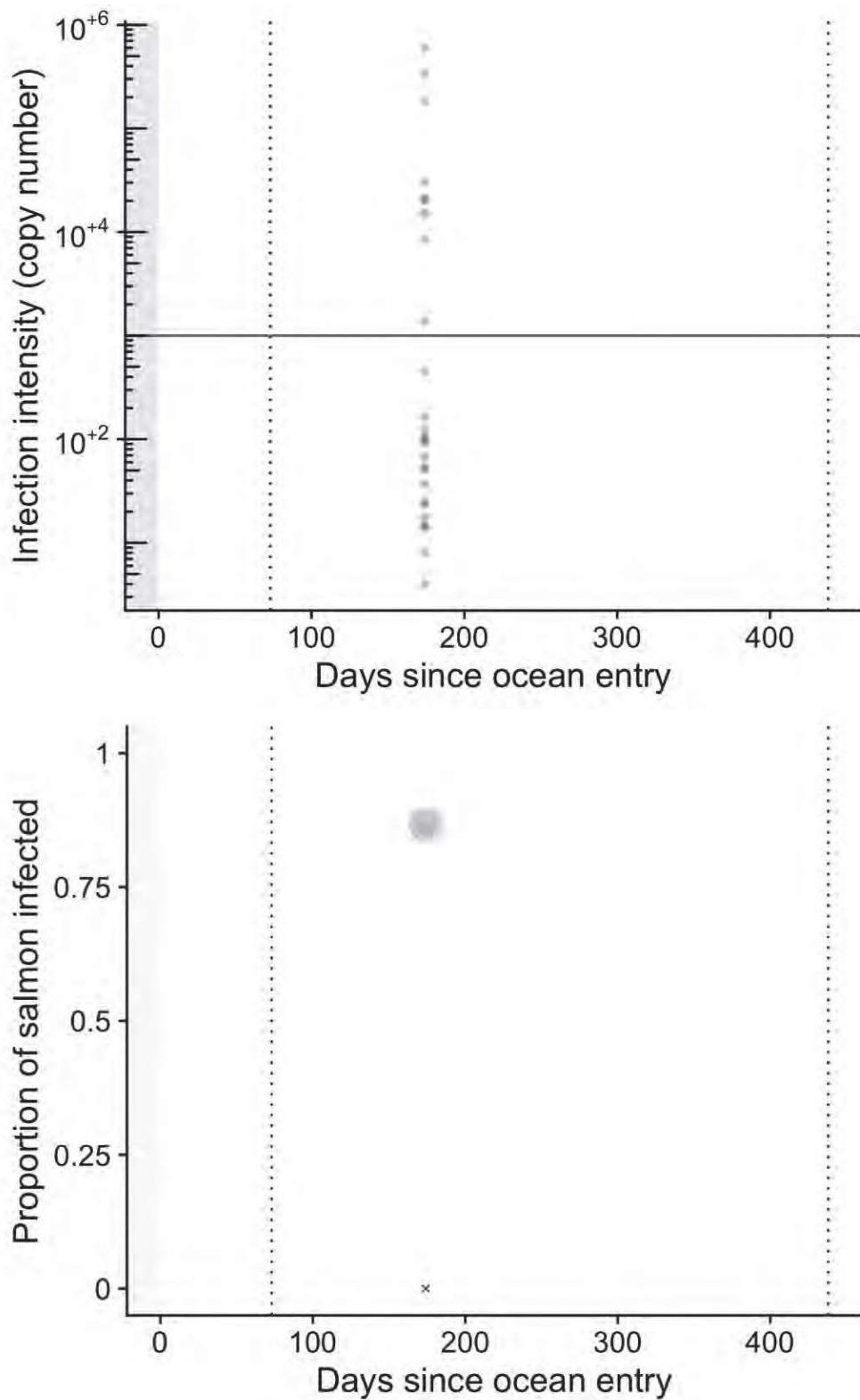
Flavobacterium psychrophilum



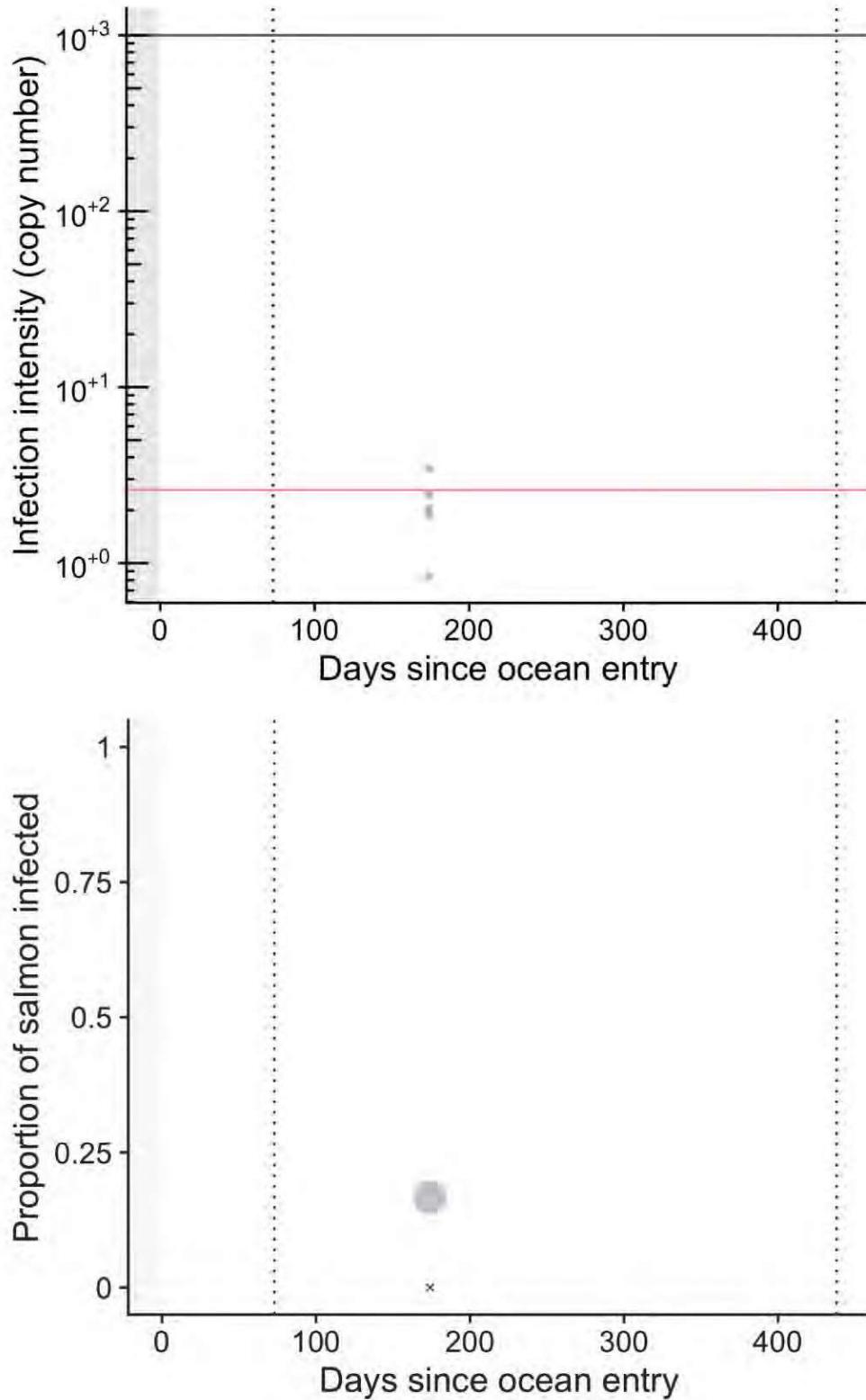
Piscirickettsia salmonis



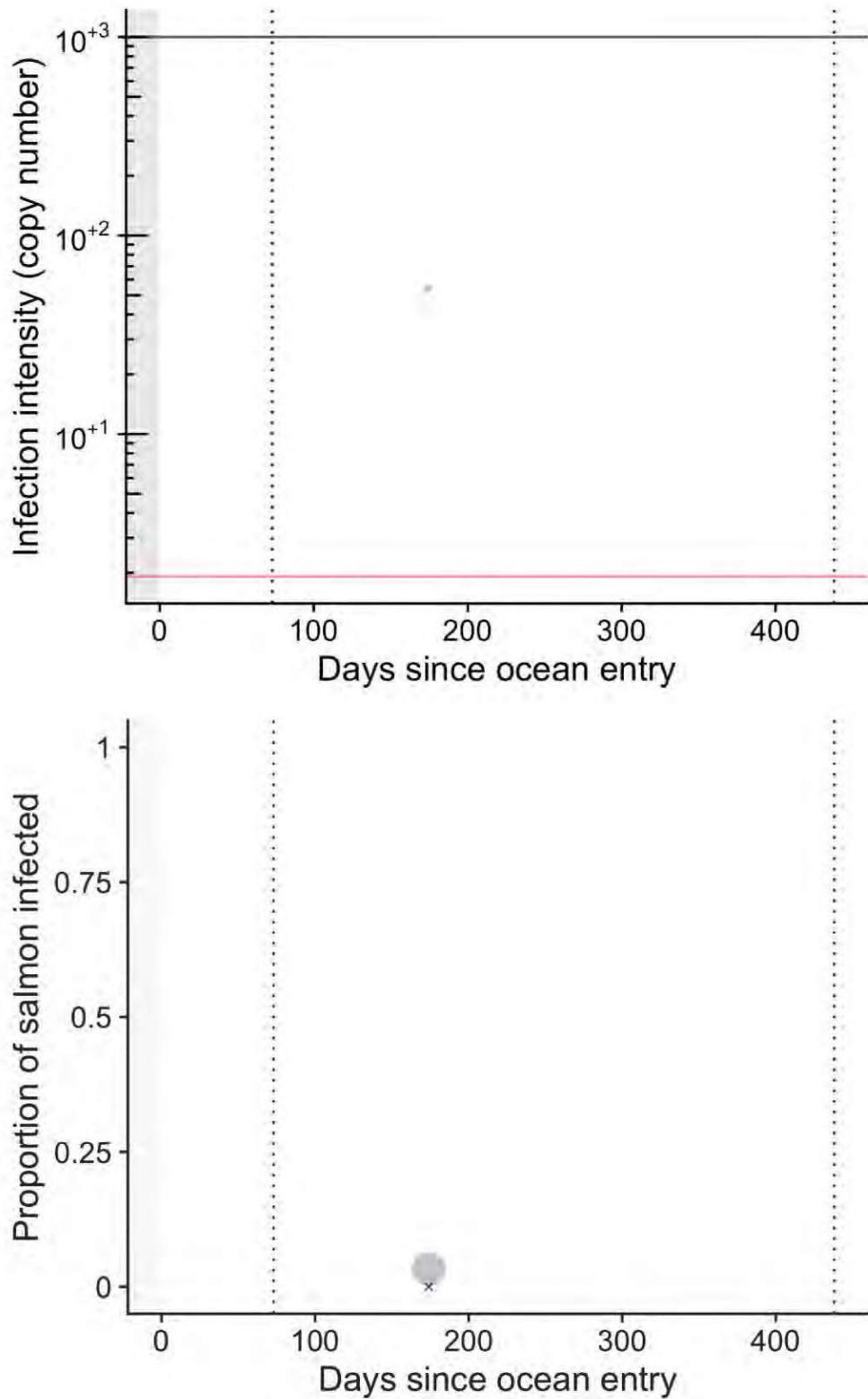
Piscine orthoreovirus



Candidatus Syngnathia salmonis



Tenacibaculum maritimum



Clinical signs

Table 1: Clinical signs for specimens sampled on 2021-10-20

metric	N5140	N5139	N5138	N5137	N5136	N5135	N5134	N5133	N5132	N5131	N5130	N5129	N5128	N5127	N5126	N5125	N5124	N5123	N5122	N5121
General																				
Live					X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Poor Performer		X																		
Mort	X	X	X																	
Skin & Fins																				
Erosion		X																		
Gills																				
Short Operculum			X					X		X		X	X					X		
Erosions			X				X					X		X				X		
Nodules/White Spots												X	X					X		
Abdominal Cavity																				
Body Fat Content			X				X	X	X									X		
Spleen										X	X								X	
Enlarged	X	X	X							X	X								X	
Liver																				
Pale				X																
Gallbladder																				
Enlarged																				
Heart																				
Blood Clots/Hemopericardium	X	X	X																	
Intestine																				
Hemorrhages/Congestion	X																			
Brain																				
Hemorrhages/Congestion	X	X																		

Table 2: Clinical signs for specimens sampled on 2021-10-20

metric	N5141	N5142	N5143	N5144	N5145	N5146	N5147	N5148	N5149	N5150	N5151	N5153	N5154
General													
Live	X	X	X	X	X	X	X	X	X	X	X	X	X
Poor Performer													
Mort													
Skin & Fins													
Erosion													
Gills													
Short Operculum		X	X				X				X	X	X
Erosions		X	X							X	X	X	
Nodules/White Spots											X	X	X
Abdominal Cavity													
Body Fat Content							X						
Spleen													
Enlarged	X	X				X		X		X			
Liver							X						
Pale								X					
Gallbladder													
Enlarged				X									
Heart													
Blood Clots/Hemopericardium													
Intestine													
Hemorrhages/Congestion													
Brain													
Hemorrhages/Congestion													

Histology

Table 3: Histology scores for specimens sampled on 2021-10-20

metric	N5123	N5122	N5121
Heart			
Peri Epi	1		3
Myo	1		
Liver			
Cong Haem	1		
Nec			1
Itis	1		1
Spleen			
W Pulpitis	2	2	2
Cap Prolif			2
Kidney			
Itis		1	
Osis		nv	
Interst Hyperplasia	1	1	1
Interst Nec		nv	
Glomitis		nv	
Cns			
Itis			na
Cnc			
Malacia			na
Gliosis			na
Cong Heam			na
Microsporidia			na
Gills			
Itis	nv	nv	
Cong Heam	nv	nv	
Prolif	nv	nv	
Tissue			
Necrosis Artefacts	2	3	1

Diagnoses and Comments

Table 4: Diagnoses and comments for specimens sampled on 2021-10-20

DFO ID	Diagnosis	Comments
N5121	Vac Deg Liver (2); Gills Very Old	
N5122	Bacterial Colonies In Several Organs (1); Very Old Fish	
N5123	Eosinophilic Granules In Kidney Tubules (1), Deg Vac Liver (2)	

Conclusions

In order to support the eDNA study, below is provided further evaluation of the results of testing from the Fish Health Report.

The sampling collection was completed. No morts were available from the control pen, while available moribund/mort fish from secondary pen and an additional pen were collected. Here below is a summary and evaluation of the findings from the sampled fish.

The farm was not inspected in its entirety, due to the configuration of the site (i.e. circular pens). A full inspection would be timely demanding, and it doesn't appear to be practical as it's very difficult to observe the fish underwater when brightness is not adequate. However, most fish in the exanimated pens were behaving normally and in good physical condition. The mortality per pen reported by the company resulted within normal range for this site. Clinically, the majority of the fish appeared in good physical condition, although a significant number of live fish presented short operculum, with gills abnormalities (erosion and/or nodules). Enlarged spleen was a frequent finding on live fish. On the other hand, most morts showed enlarged and dark spleen, enlarged gall bladder, pale liver, frequently associated with hemopericardium and congested brain

Molecular testing results show that PRV was prevalent in the majority of the fish tested (79%) even at high load, while bacterial infection (*Aeromonas salmonicida*, *Piscirickettsia salmonis*, *Candidatus Syngnathus salmonis*, *Tenacibaculum maritimum* and *Flavobacterium psychrophilum*) were observed at a significantly lower rate, just above what would be considered a background level of detection. However, *Aeromonas salmonicida* was present in higher prevalence in morts than life fish.

Histopathologically, one of the morts presented bacterial colonies in several organs in absence of immune reaction in the tissue and in other immune-reactive organs, a pattern compatible with furunculosis. Of the remaining morts, nonspecific lesions were observed.

Appendix

Glossary of infectious agents

Agent abbr.	Full agent name	Agent type	Disease	Ranking
ae_sal	Aeromonas salmonicida	Bacteria	Furunculosis	2
ascv	Atlantic salmon calicivirus	Virus	unknown	4
ctv-2	Cutthroat trout virus-2	Virus	unknown	4
fl_psy	Flavobacterium psychrophilum	Bacteria	Bacterial cold water disease	3
pisck_sal	Piscirickettsia salmonis	Bacteria	Piscirickettsiosis (SRS)	2
pmcv	Piscine myocarditis virus	Virus	Cardiomyopathy syndrome	1
prv-1	Piscine orthoreovirus	Virus	HSMI-EIBS-Jaundice/anemia	
psnv1	Pacific salmon nidovirus-1 (CoV)	Virus	unknown	4
re_sal	Renibacterium salmoninarum	Bacteria	Bacterial kidney disease	2
sch	Candidatus Syngnathia salmonis	Bacteria	Gill chlamydia	3
te_mar	Tenacibaculum maritimum	Bacteria	Marine flexibacteriosis (mouth/fin rot)	2
vi_ang	Vibrio anguillarum	Bacteria	Vibriosis	2
vi_sal	Vibrio salmonicida	Bacteria	Cold water vibriosis	2
ye_ruc	Yersinia ruckeri (Enteric redmouth disease)	Bacteria	Yersiniosis (Enteric red mouth)	2

eDNA Study Report

Swanson Island sampling on October 26, 2021

Dr. Emiliano Di Cicco

June 29, 2022

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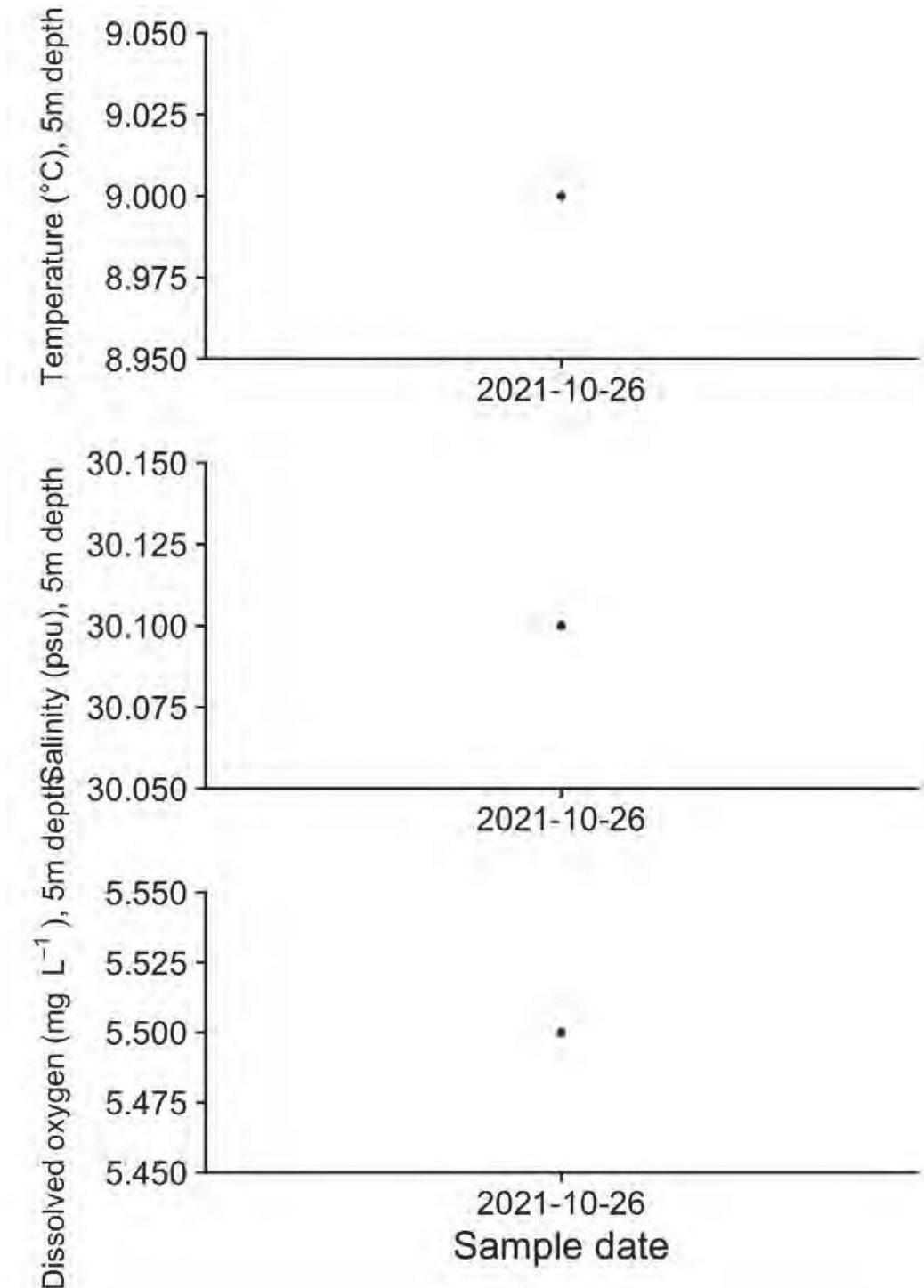
Executive summary

Premise

On October 26, 2021, 37 samples were collected by BATI and Mowi crew during a sampling event at Swanson Island (Mowi Ltd.). 37 Atlantic salmon subadults were collected from the Swanson Island farm site, including 31 live and 6 moribund/dead fish. All live fish were euthanized with TMS overdose prior to dissection with the exception of the moribund fish, which were administered a blow to the head. Portions of gill, liver and anterior kidney were collected in triplicate for molecular testing (preserved in RNA later) from all the fish, while all the moribund/dead fish also underwent collection of tissues (gills, spleen, liver, heart, anterior and posterior kidney, pyloric caeca, skeletal muscle + skin, brain) for histological analysis. Clinical notes and gross lesions were noted and reported for every fish. One aliquot has been provided to the Company MOWI Fish Health, another aliquot is stored at the BATI Field Office, and a third aliquot is stored at DFO - PBS. This latter aliquot has been tested for the presence and load of the agents indicated in the IMIP agreement as well as the agents indicated in the eDNA study agreement. Each sample has been extracted and tested individually. Negative and positive controls were run. A housekeeping gene was also included to assess the quality of the RNA extracted.

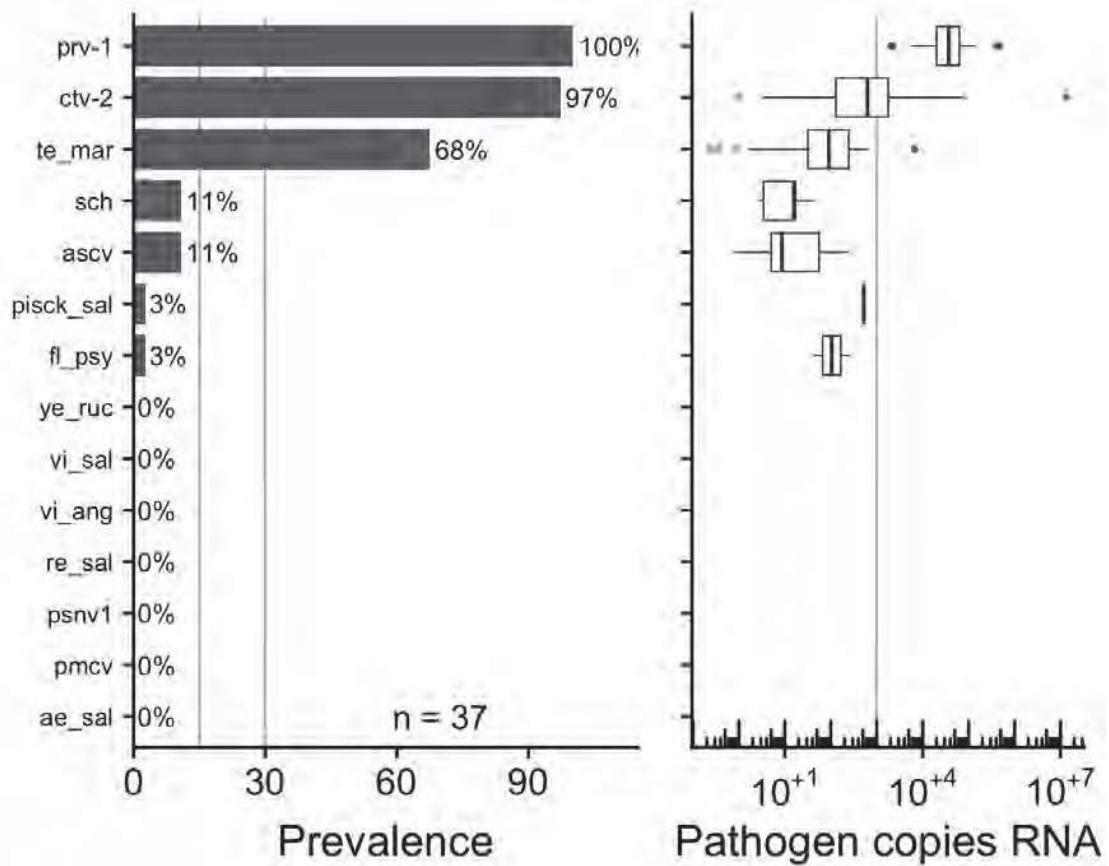
Histology samples have been sent to Wax-It Histo Ltd. to process and prepare slides, which have been read and scored by Dr. Di Cicco. A digital copy of each slide is available to the Company.

Environmental data

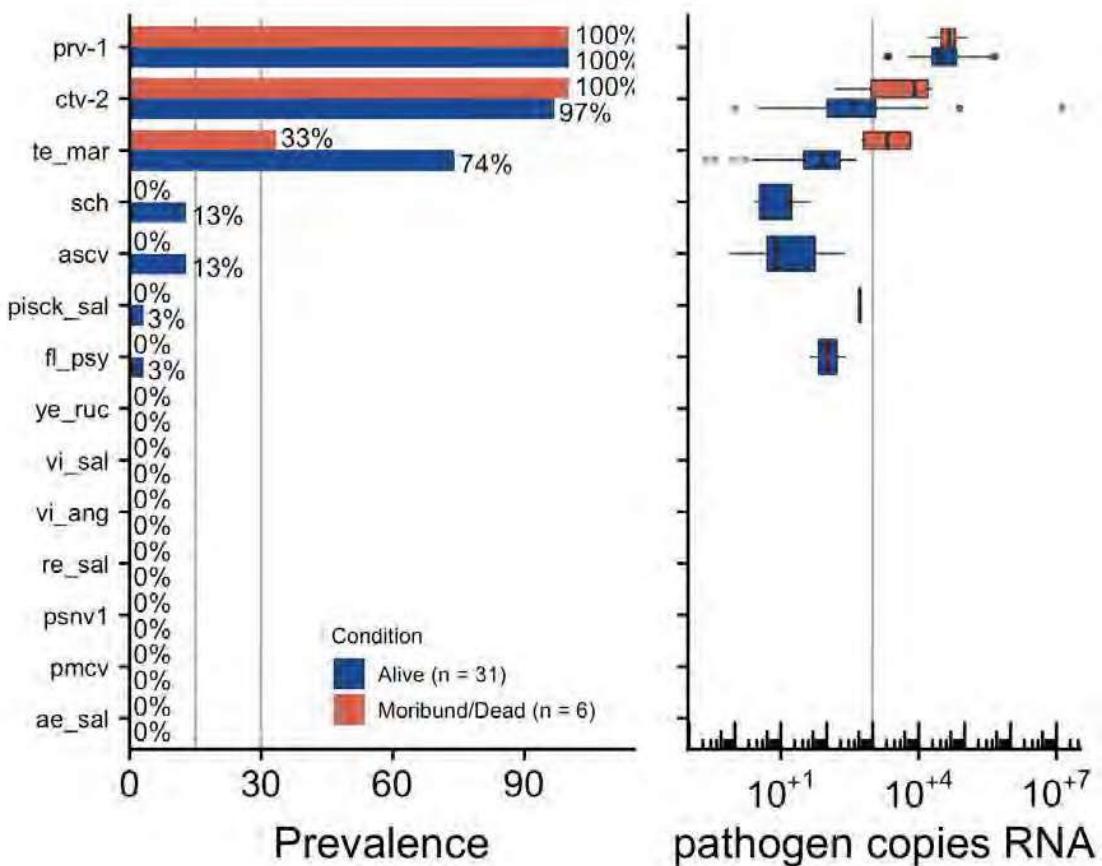


Water temperature (°C), salinity (ppm), and dissolved oxygen (mg/L) at a 5m depth. Certain sampling dates have no recorded environmental data, resulting in gaps in the plots.

Overall infectious agent prevalence



Infectious agent prevalence in samples collected on 2021-10-26.



Infectious agent prevalence in samples collected on 2021-10-26, split by mortality status at time of sampling. Any specimens that were not confirmed to be either moribund or live at the time of generating this report are excluded from this figure.

Individual infectious agent trends

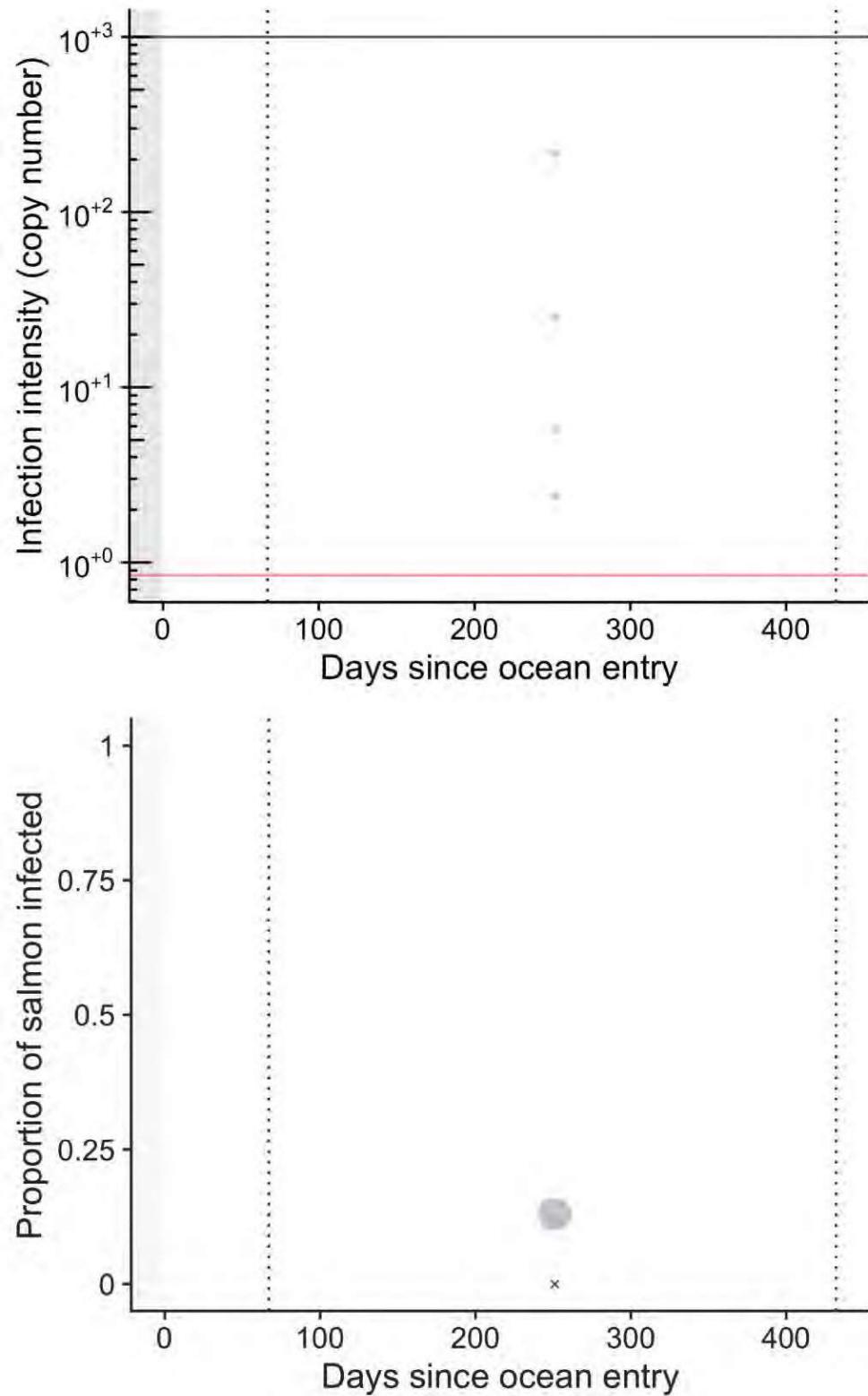
The following plots show individual infectious agent trends across all farm sites. In cases where sample size is sufficient, curves from a generalised additive model are included in the plot.

Grey circles represent live fish, and black X's represent dead/dying fish. Curves indicate mean predictions from a generalised additive model; blue and red correspond to live and dead/dying fish, respectively (shaded areas show 95% confidence regions). Left-hand grey region indicates freshwater hatchery residence, grey regions on x-axis indicate period of transfer to another site, and vertical dotted lines correspond to January 1st.

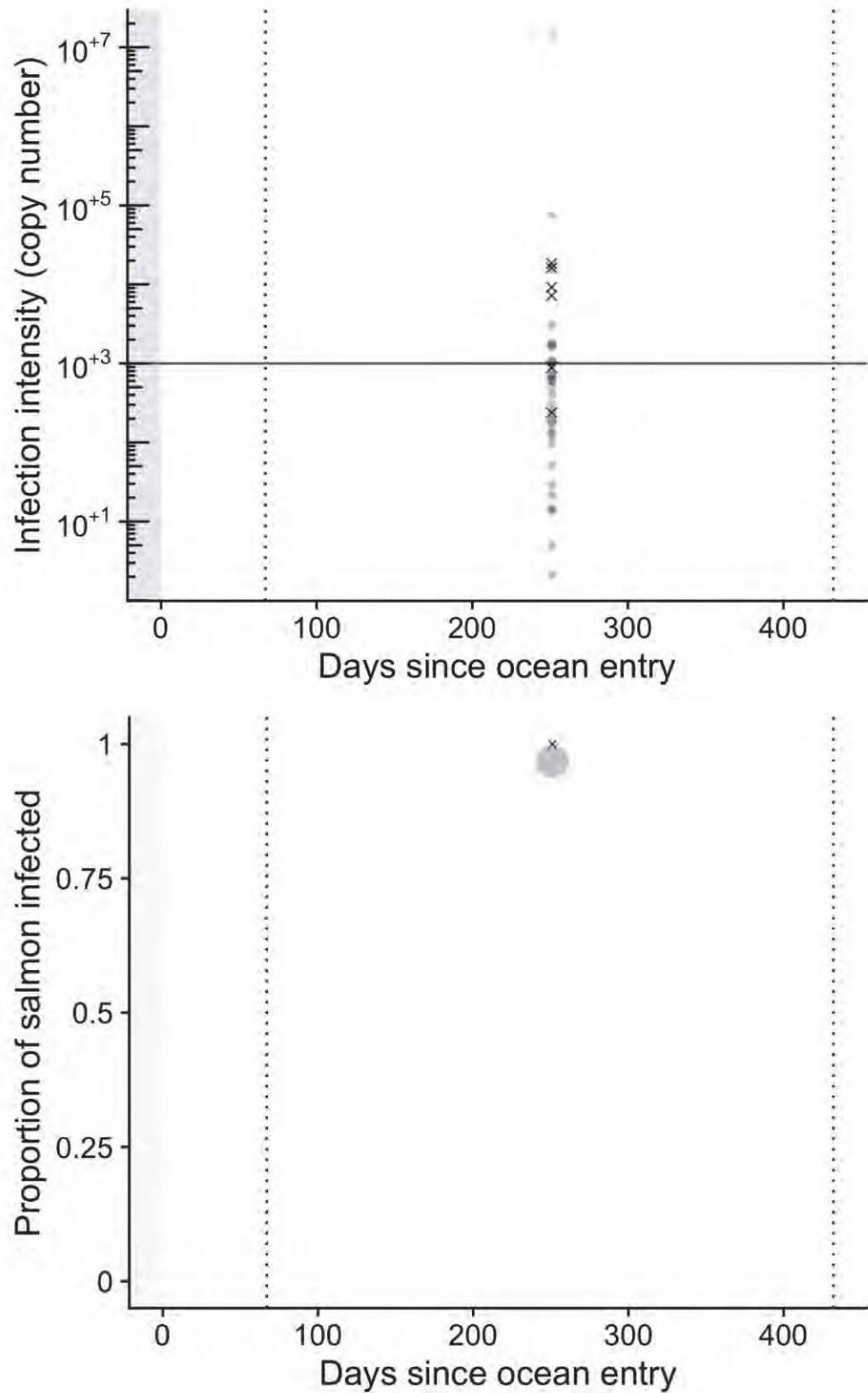
For infection intensity plots, horizontal red line indicates limit of detection (yielding ~90% true positive rate) for respective qPCR assay run in duplicate, while the horizontal black line indicates 1000 copies. Note log scale.

For proportion plots, grey circles show prevalence in live fish on each sampling date, and black X's show prevalence in dead/dying fish (symbol areas proportional to sample sizes).

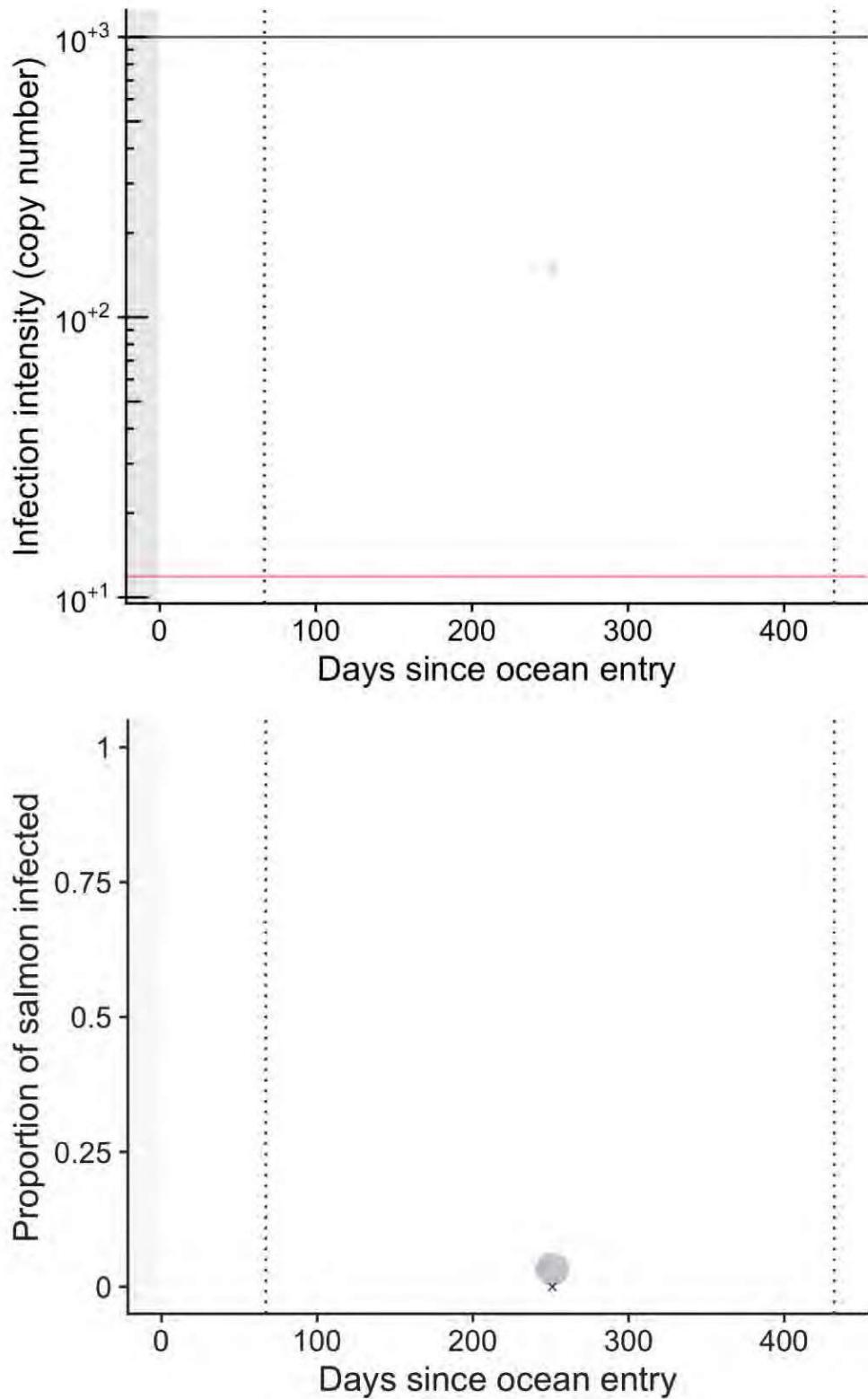
Atlantic salmon calicivirus



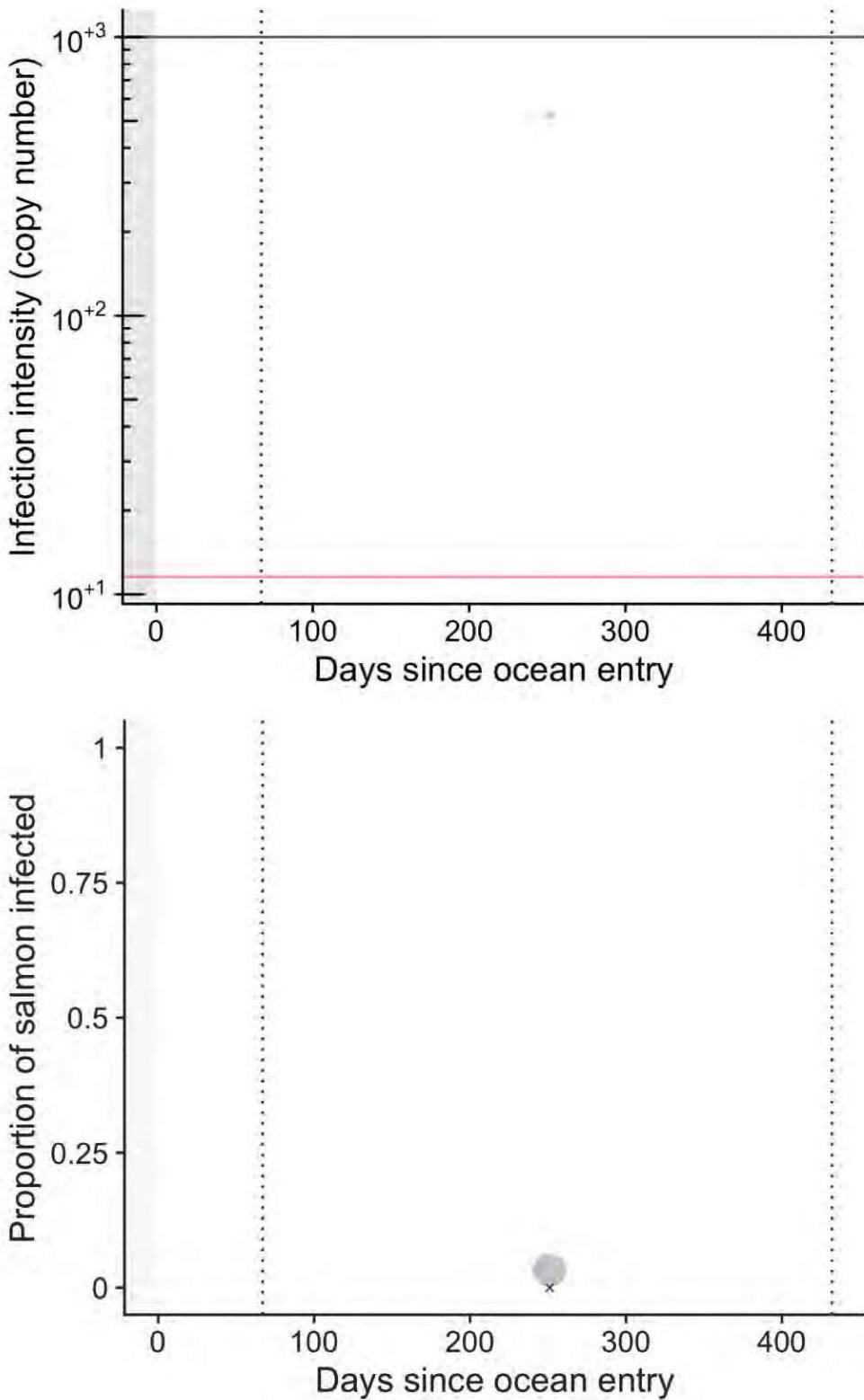
Cutthroat trout virus-2



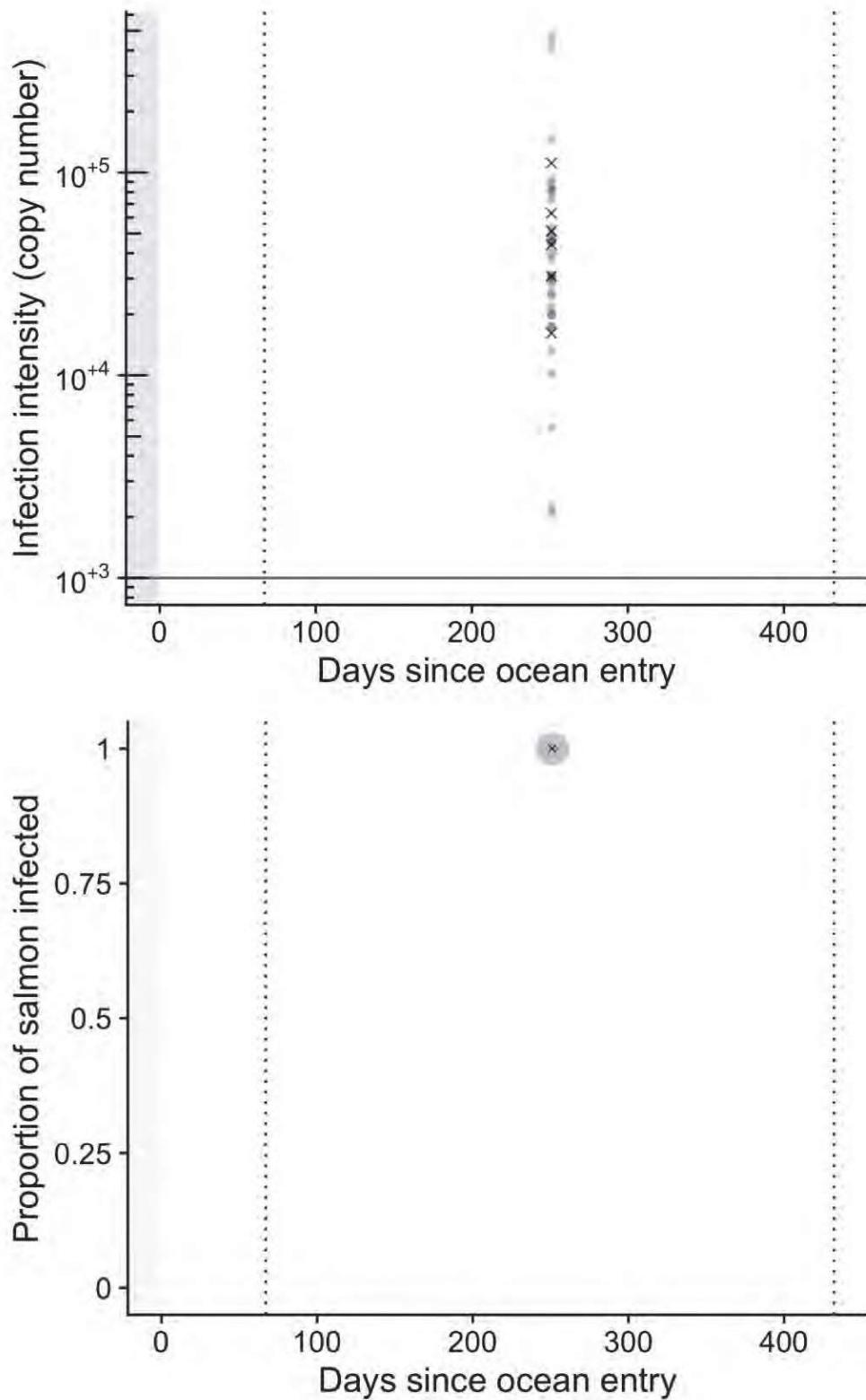
Flavobacterium psychrophilum



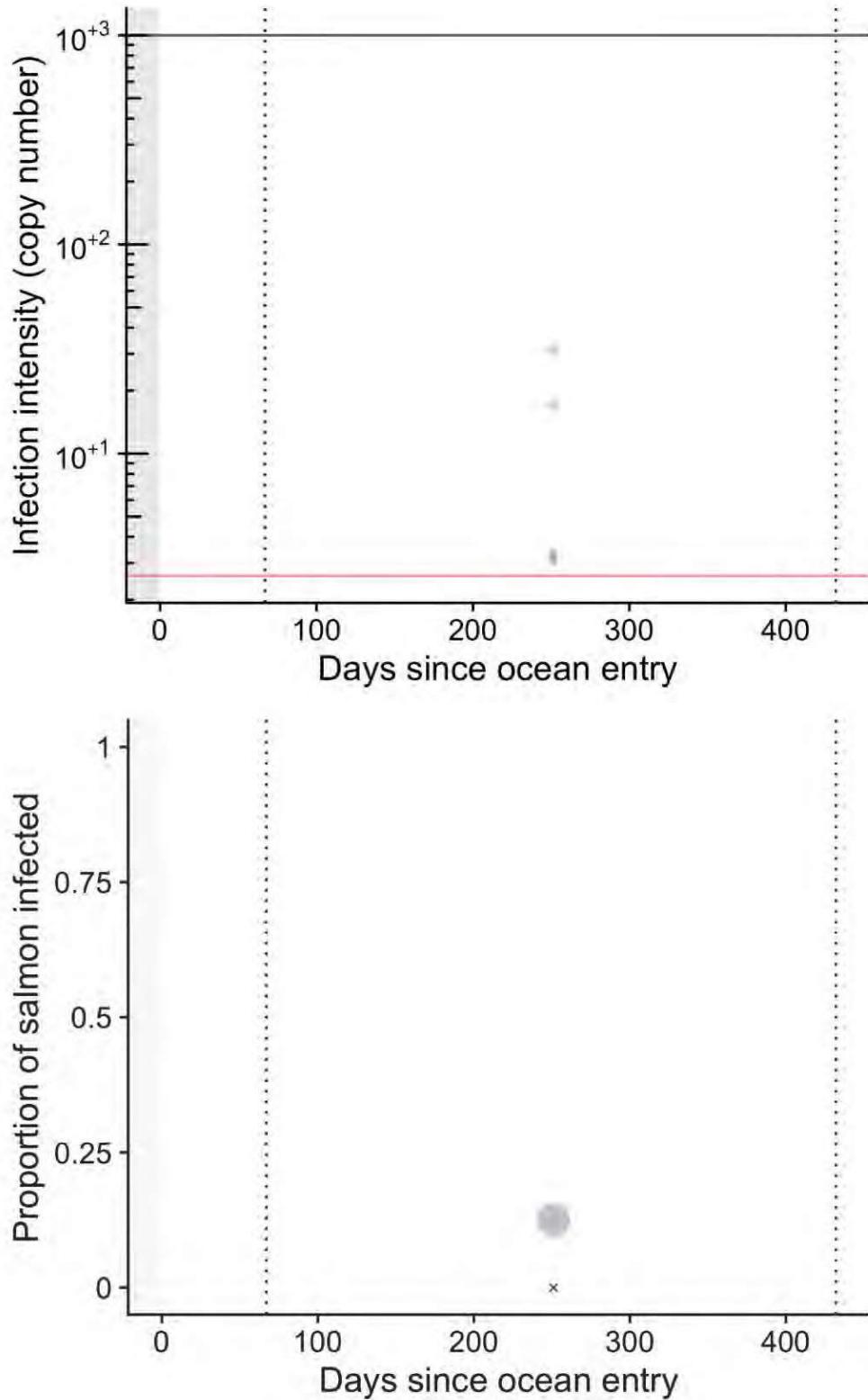
Piscirickettsia salmonis



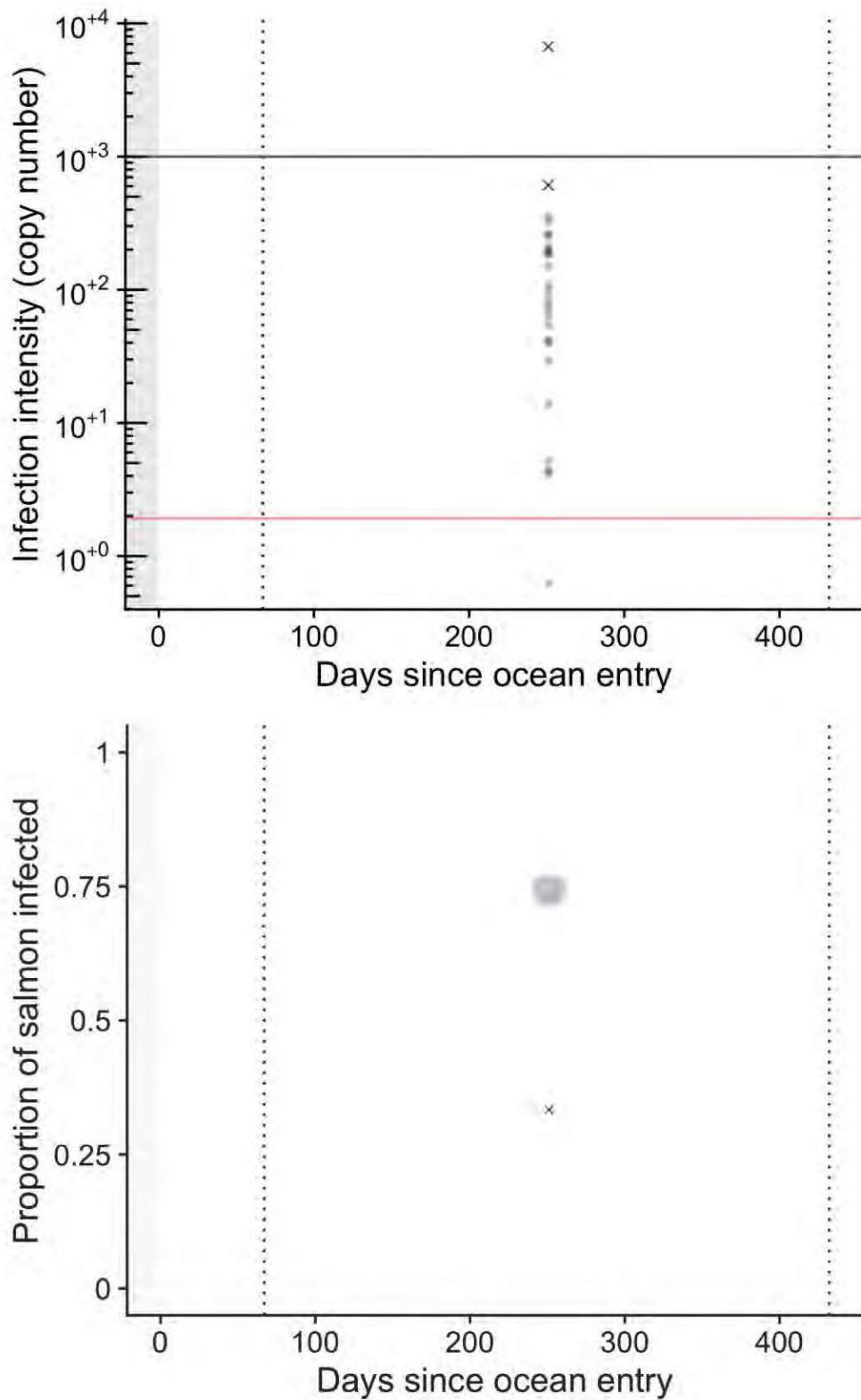
Piscine orthoreovirus



Candidatus Syngnathia salmonis



Tenacibaculum maritimum



Clinical signs

Table 1: Clinical signs for specimens sampled on 2021-10-26

metric	N5180	N5179	N5178	N5177	N5176	N5175	N5174	N5173	N5172	N5171	N5170	N5169	N5168	N5167	N5166	N5165	N5164	N5163	N5162	N5161
General																				
Live					X	X	X	X			X	X	X	X	X	X	X	X	X	X
Poor Performer		X																		
Mort	X	X	X	X					X	X										
Skin & Fins					X															
Erosion						X														
Lost Scales							X													
Gills																				
Short Operculum		X						X												
Pale										X	X									
Erosions																				
Nodules/White Spots																				
Abdominal Cavity																				
Adhesions								X				X		X		X		X		X
Ascites		X	X																	
Spleen																				
Enlarged		X	X	X	X			X		X	X					X		X	X	X
Liver																				
Pale		X	X	X						X										X
Nodules/White Spots																				
Gallbladder																				
Enlarged																				
Green																				
Heart																				
Enlarged					X															
Pale		X	X	X																
Blood Clots/Hemopericardium				X																
Kidney																				
Nodules/White Spots																				
Intestine																				
Hemorrhages/Congestion									X											
Brain																				
Hemorrhages/Congestion		X	X		X					X										

Table 2: Clinical signs for specimens sampled on 2021-10-26

metric	N5181	N5182	N5183	N5184	N5185	N5186	N5187	N5188	N5189	N5190	N5191	N5192	N5193	N5194	N5195	N5196	N5197
General																	
Live	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Poor Performer	X					X											
Mort																	
Skin & Fins																	
Erosion																	
Lost Scales																	
Gills																	
Short Operculum	X	X	X			X				X	X			X	X		
Pale			X		X					X				X			
Erosions			X											X			
Nodules/White Spots				X		X											
Abdominal Cavity																	
Adhesions		X	X	X		X				X	X			X			X
Ascites																	
Spleen																	
Enlarged			X	X			X			X							X
Liver																	
Pale							X										
Nodules/White Spots							X										
Gallbladder																	
Enlarged							X										
Green							X										
Heart																	
Enlarged																	X
Pale							X										
Blood Clots/Hemopericardium																	
Kidney																	
Nodules/White Spots							X										
Intestine																	
Hemorrhages/Congestion																	
Brain																	
Hemorrhages/Congestion																	

Histology

Table 3: Histology scores for specimens sampled on 2021-10-26

metric	N5161	N5162	N5163	N5164	N5169	N5170	N5186
Heart							
Peri Epi	3	3	3		3		2
Myo	3	3	3		3		2
Liver							
Nec			1				1
Itis					1		3
Spleen							
Cong Heam	2	2	2	2	2		1
Ellip Nec	1	2	2		3		1
W Pulpitis			1		2	1	3
Kidney							
Itis					1	na	3
Osis	1	1		1	na	1	2
Cong Heam				1	na		
Interst Hyperplasia	2	2	3	2	na	2	2
Interst Nec					na		
Glomitis					na		
Pancreatitis							
Pancreatitis						1	
Cns							
Itis					na		
Cnc							
Malacia					na		
Gliosis		1		1	na		
Cong Heam	1	1		2	na		
Microsporidia					na		
Gills							
Itis	nv	nv		nv	nv	nv	
Cong Heam	nv	nv		nv	nv	nv	
Prolif	nv	nv		nv	nv	nv	
Skin_muscle							
Itis Nec	1	2	1		2		
Tissue							
Necrosis Artefacts	1	2	1	3	1	2	

Diagnoses and Comments

Table 4: Diagnoses and comments for specimens sampled on 2021-10-26

DFO ID	Diagnosis	Comments
N5161	HSMI	Old Fish
N5162	HSMI	Myonecrosis (3); Old Fish
N5163	HSMI	Single Cells Necrosis In Liver (1) + Orange Pigm (1), Renal Erythrophagocytosis (2)
N5164		Very Old Fish
N5169	HSMI	Kudoa In Muscle (1)
N5170		Increase Fibrin In Spleen (2); Old Fish
N5186	Visceral Mycosis	Vaccine Peritonitis (1)

Conclusions

In order to support the eDNA study, below is provided further evaluation of the results of testing from the Fish Health Report.

The Fish Health sampling collection was completed. Available moribund/mort fish from the control pen and secondary pen were collected.

The farm was inspected in its entirety: the fish have been subject to several consecutive treatments to control sea louse density, and that was evident in their behavior: while part of the population was behaving normally, a significant portion of it appeared lethargic, laying on one side of the net, facing upstream to the tidal current. A noteworthy number of poor performers was also observed. Reporting from the company indicated mortality that was slightly elevated above what would normally be expected for such a site. Clinically, short operculum (with rare gill alterations) and enlarged spleen were the common findings in the live fish sampled. Morts and moribund fish showed several alterations, including enlarged spleen, pale liver and pale heart, as well as ascites in some instances. Brain congestion and hemorrhages were also reasonably common in morts.

Molecular testing results indicate PRV present in 100% of the fish tested, even at high load in few fish. *Tenacibaculum maritimum* was also quite prevalent (68% of fish tested; 74% of live fish and 33% of morts), and one individual passed 1000 gene copies per µg RNA. Background level of *Candidatus Syngnathia salmonis*, *Piscirickettsia salmonis* and *Flavobacterium psychrophilum* was observed in the live fish.

Histopathologically, four of the seven moribund/mort fish collected showed severe epi/myocarditis with myocardioneclerosis, infiltrating myositis localized to the red fibers, spleen congestion and spleen/kidney immune activation, a pattern of lesion severity and distribution that, associated with clinical signs and gross lesions observed and reported above (as well as the detection of PRV), is consistent with the diagnosis of Heart and Skeletal Muscle Inflammation (HSMI), according to ICES diagnostic standards (ICES 2012) (1). However, according to current DFO standard, this would count as “provisional diagnosis”, as a laboratory challenge trial hasn’t been performed.

One of the moribund (poor performer) fish also showed lesions consistent with the diagnosis of visceral mycosis, with granulomas containing fungal hyphae systemically distributed in the different organs collected.

Given the overall situation, the molecular results and clinical/pathological findings suggest that the farm population has been experiencing a case of subclinical HSMI, caused by PRV and likely triggered by the frequent delousing treatments. Following up the evolution of such case is recommended. Follow-up investigation of the potential spread of visceral mycosis in the population (and particularly in numerous poor performers present in the farm) would also be highly recommended: visceral mycosis is an infection disease (caused by opportunistic fungal pathogens of the genera *Exophiala* spp. and *Ochroconis* spp.) documented in BC since at least 2017, and capable to induce significant mortality (2).

1. Heart and skeletal muscle inflammation (HSMI) of farmed Atlantic salmon (*Salmo* *salar* L.) and the associated Piscine reovirus (PRV) (ices.dk)
2. Visceral mycoses in Atlantic salmon (*Salmo* *salar*): The role of opportunistic fungal pathogens in fish health and mortality in salmon aquaculture systems (dfo-mpo.gc.ca)

Appendix

Glossary of infectious agents

Agent abbr.	Full agent name	Agent type	Disease	Ranking
ae_sal	Aeromonas salmonicida	Bacteria	Furunculosis	2
ascv	Atlantic salmon calicivirus	Virus	unknown	4
ctv-2	Cutthroat trout virus-2	Virus	unknown	4
fl_psy	Flavobacterium psychrophilum	Bacteria	Bacterial cold water disease	3
pisck_sal	Piscirickettsia salmonis	Bacteria	Piscirickettsiosis (SRS)	2
pmcv	Piscine myocarditis virus	Virus	Cardiomyopathy syndrome	1
prv-1	Piscine orthoreovirus	Virus	HSMI-EIBS-Jaundice/anemia	
psnv1	Pacific salmon nidovirus-1 (CoV)	Virus	unknown	4
re_sal	Renibacterium salmoninarum	Bacteria	Bacterial kidney disease	2
sch	Candidatus Syngnathia salmonis	Bacteria	Gill chlamydia	3
te_mar	Tenacibaculum maritimum	Bacteria	Marine flexibacteriosis (mouth/fin rot)	2
vi_ang	Vibrio anguillarum	Bacteria	Vibriosis	2
vi_sal	Vibrio salmonicida	Bacteria	Cold water vibriosis	2
ye_ruc	Yersinia ruckeri (Enteric redmouth disease)	Bacteria	Yersiniosis (Enteric red mouth)	2

eDNA Study Report

Midsummer Island sampling on February 3, 2022

Dr. Emiliano Di Cicco

Feb 21, 2023

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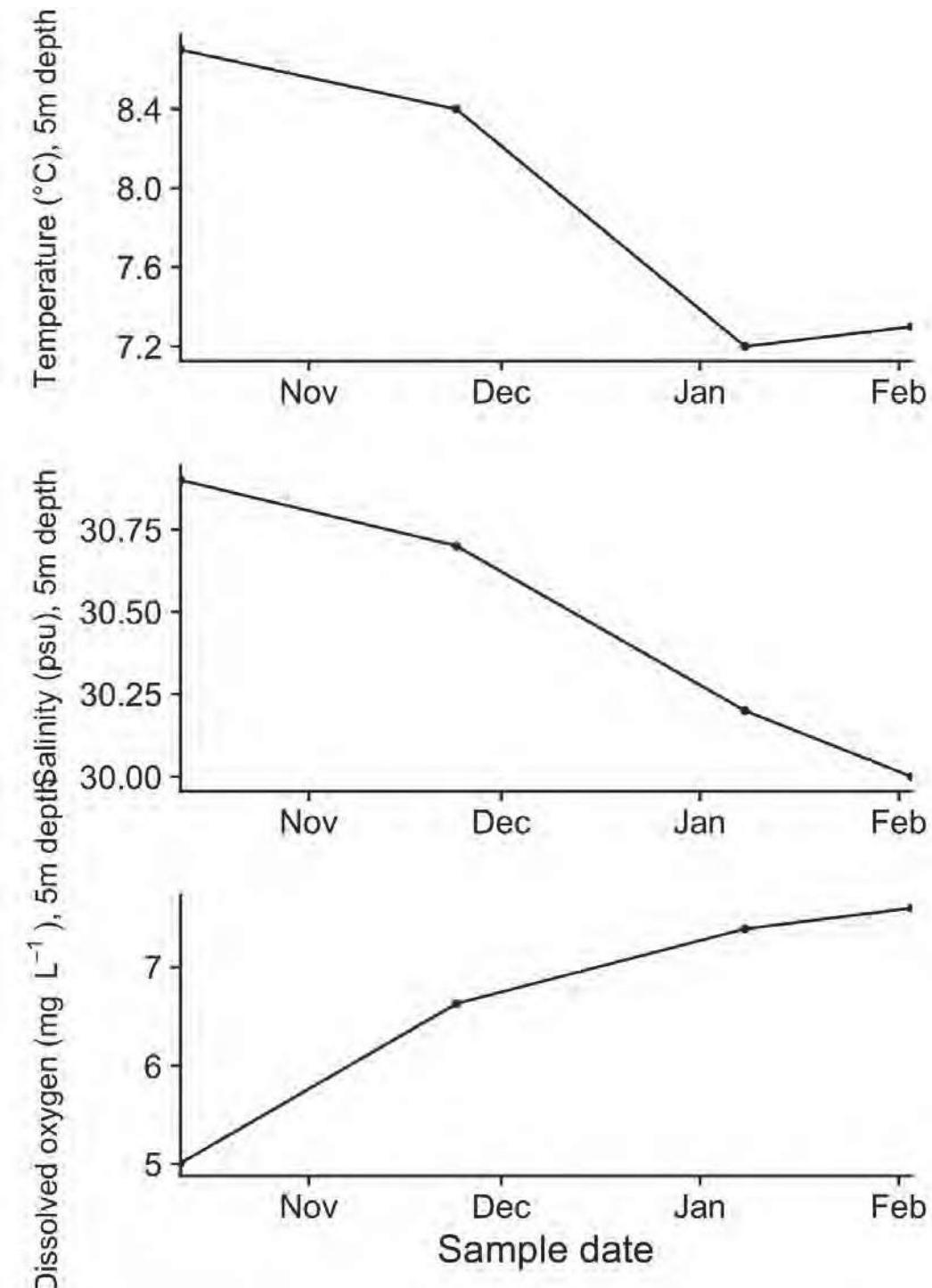
Executive summary

Premise

On February 03, 2022, 37 samples were collected by BATI and Mowi crew during a sampling event at Midsummer Island (Mowi Ltd.). 37 Atlantic salmon subadults were collected from the Midsummer Island farm site, including 30 live and 7 moribund/dead fish. All live fish were euthanized with TMS overdose prior to dissection with the exception of the moribund fish, which were administered a blow to the head. Portions of gill, liver and anterior kidney were collected in triplicate for molecular testing (preserved in RNA later) from all the fish, while all the moribund/dead fish also underwent collection of tissues (gills, spleen, liver, heart, anterior and posterior kidney, pyloric caeca, skeletal muscle + skin, brain) for histological analysis. Clinical notes and gross lesions were noted and reported for every fish. One aliquot has been provided to the Company MOWI Fish Health, another aliquot is stored at the BATI Field Office, and a third aliquot is stored at DFO - PBS. This latter aliquot has been tested for the presence and load of the agents indicated in the IMIP agreement as well as the agents indicated in the eDNA study agreement. Each sample has been extracted and tested individually. Negative and positive controls were run. A housekeeping gene was also included to assess the quality of the RNA extracted.

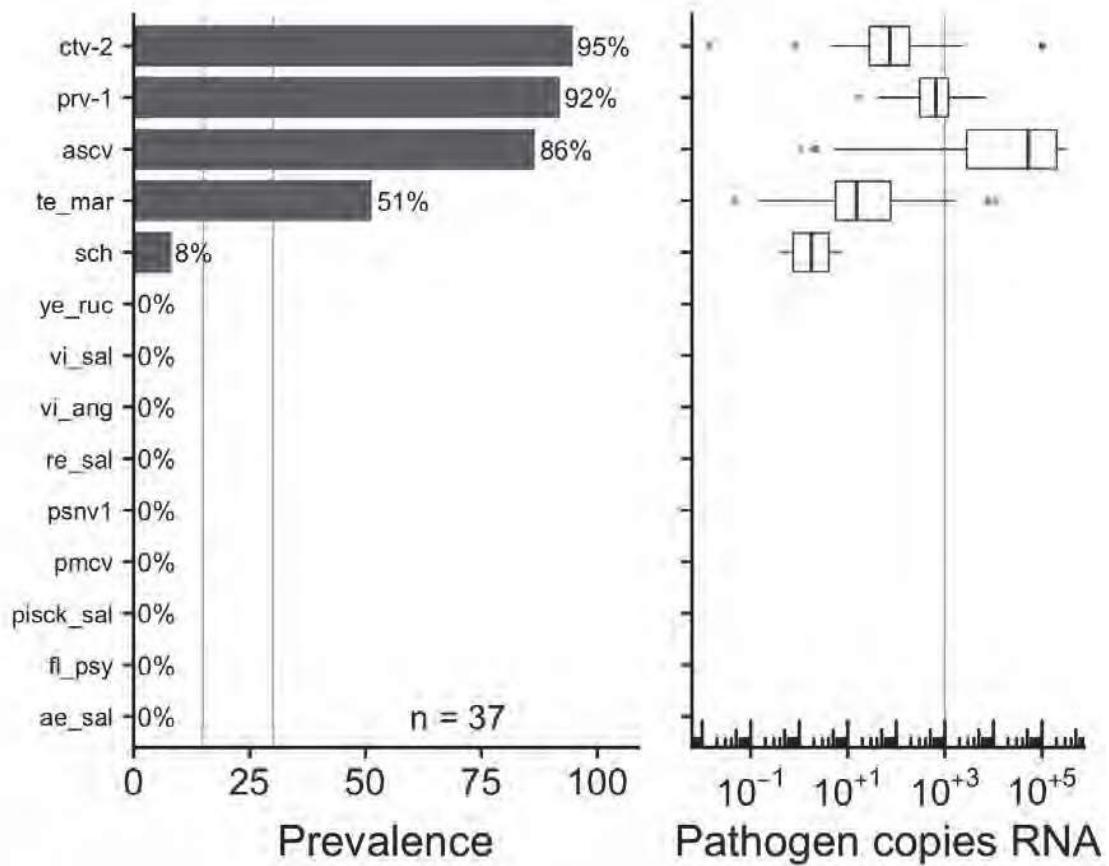
Histology samples have been sent to Wax-It Histo Ltd. to process and prepare slides, which have been read and scored by Dr. Di Cicco. A digital copy of each slide is available to the Company.

Environmental data

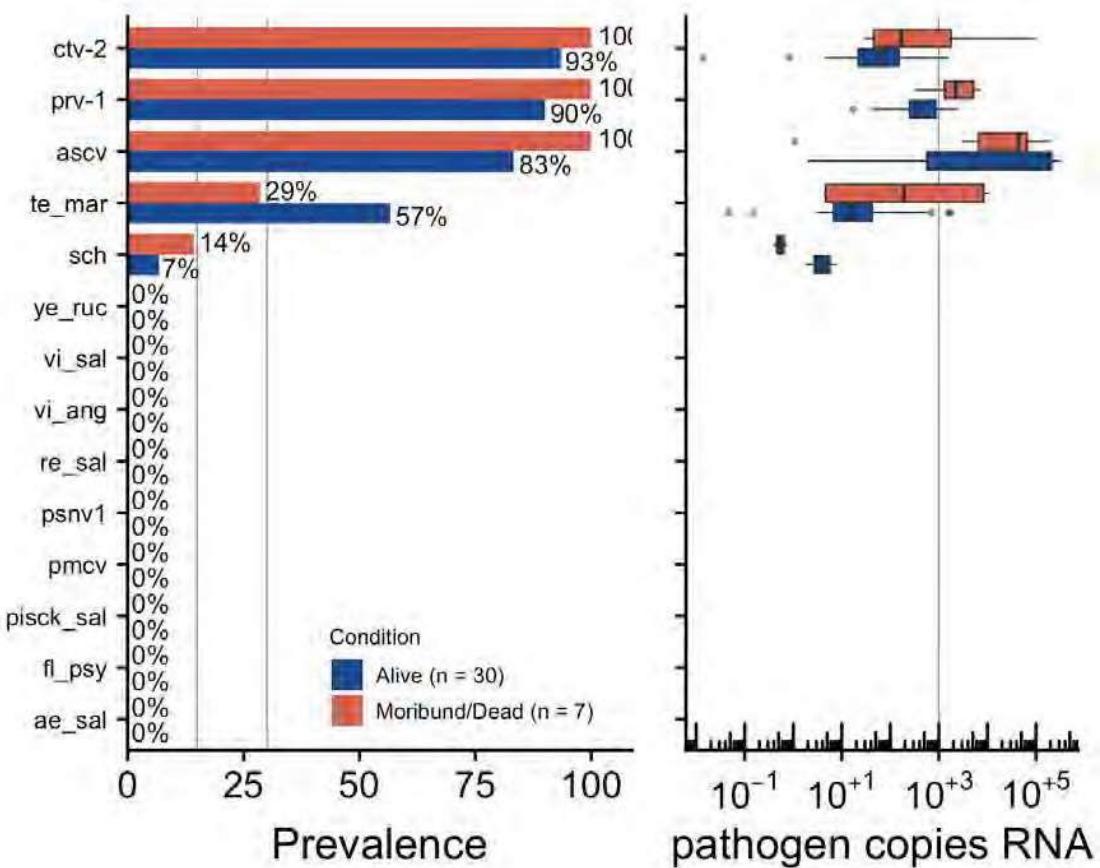


Water temperature ($^{\circ}\text{C}$), salinity (ppm), and dissolved oxygen (mg/L) at a 5m depth. Certain sampling dates have no recorded environmental data, resulting in gaps in the plots.

Overall infectious agent prevalence



Infectious agent prevalence in samples collected on 2022-02-03.



Infectious agent prevalence in samples collected on 2022-02-03, split by mortality status at time of sampling. Any specimens that were not confirmed to be either moribund or live at the time of generating this report are excluded from this figure.

Individual infectious agent trends

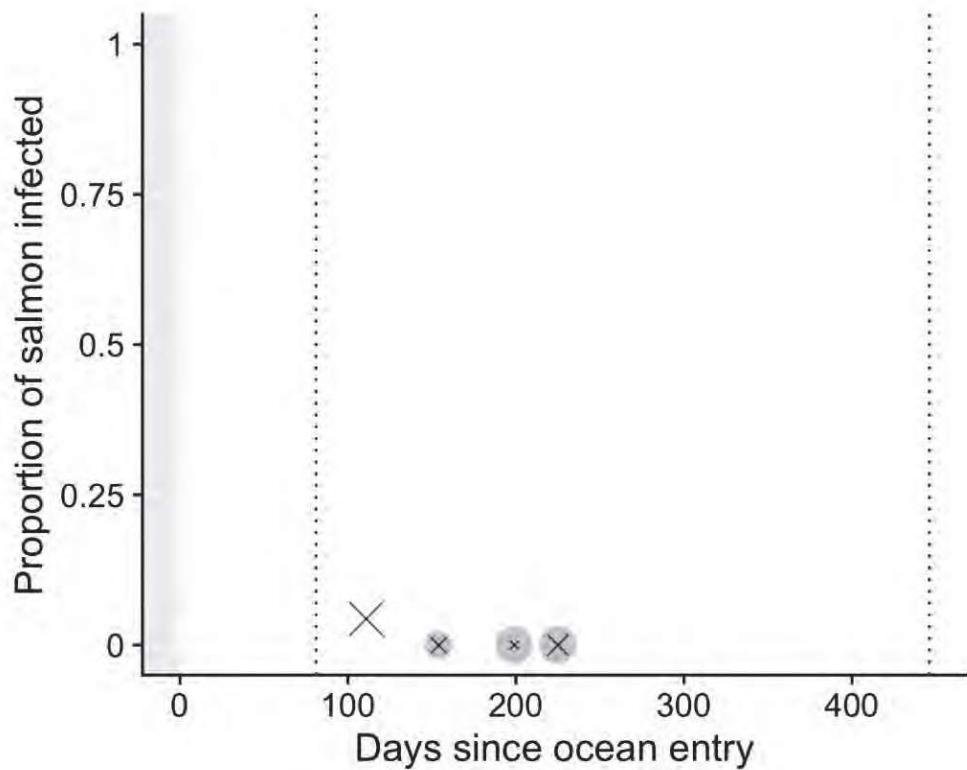
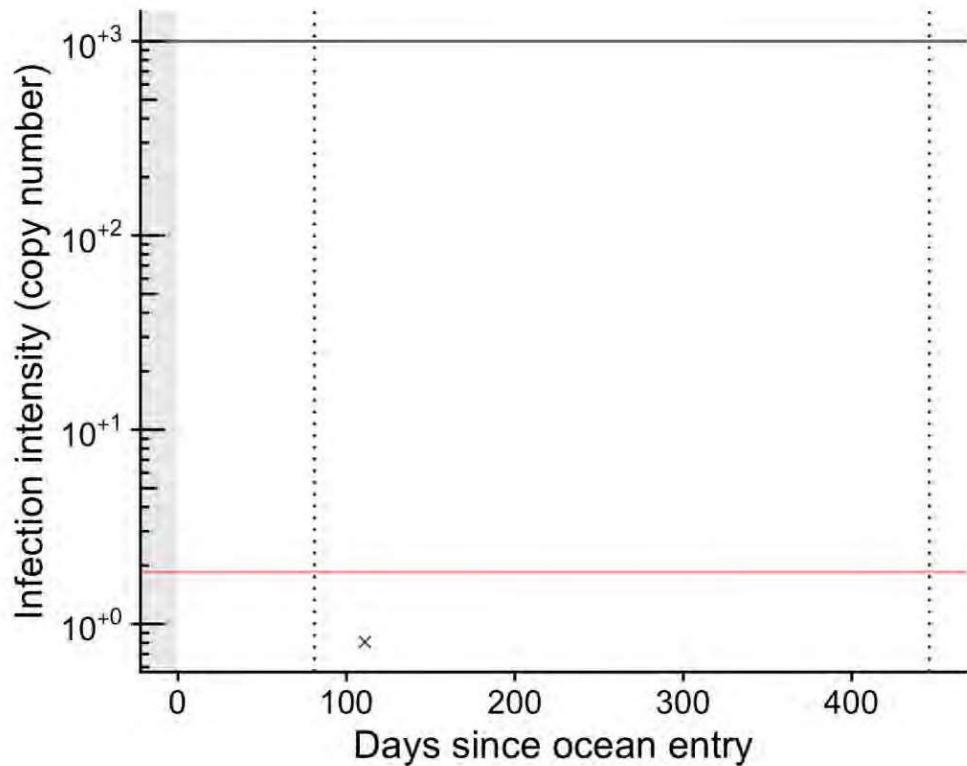
The following plots show individual infectious agent trends across all farm sites. In cases where sample size is sufficient, curves from a generalised additive model are included in the plot.

Grey circles represent live fish, and black X's represent dead/dying fish. Curves indicate mean predictions from a generalised additive model; blue and red correspond to live and dead/dying fish, respectively (shaded areas show 95% confidence regions). Left-hand grey region indicates freshwater hatchery residence, grey regions on x-axis indicate period of transfer to another site, and vertical dotted lines correspond to January 1st.

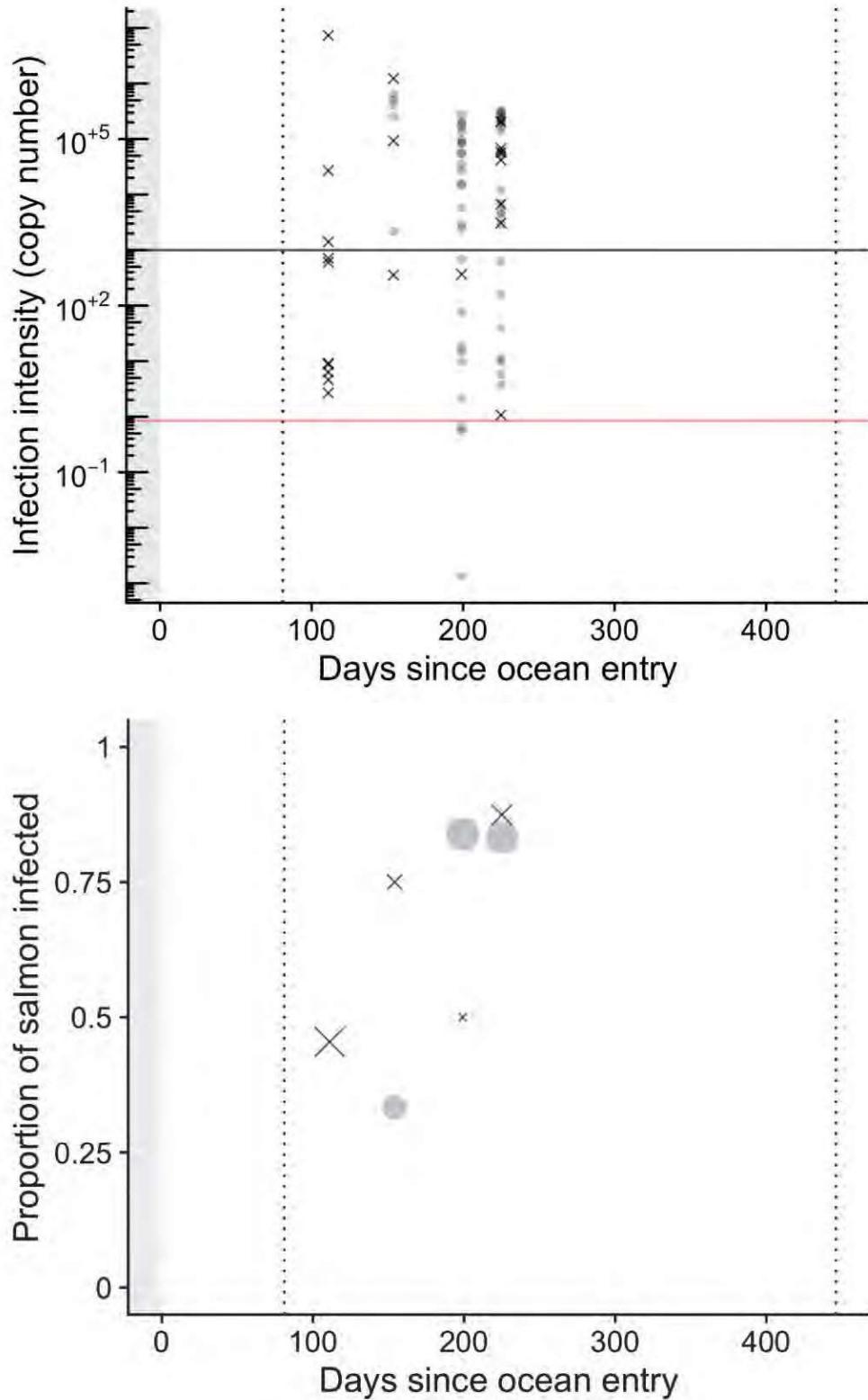
For infection intensity plots, horizontal red line indicates limit of detection (yielding ~90% true positive rate) for respective qPCR assay run in duplicate, while the horizontal black line indicates 1000 copies. Note log scale.

For proportion plots, grey circles show prevalence in live fish on each sampling date, and black X's show prevalence in dead/dying fish (symbol areas proportional to sample sizes).

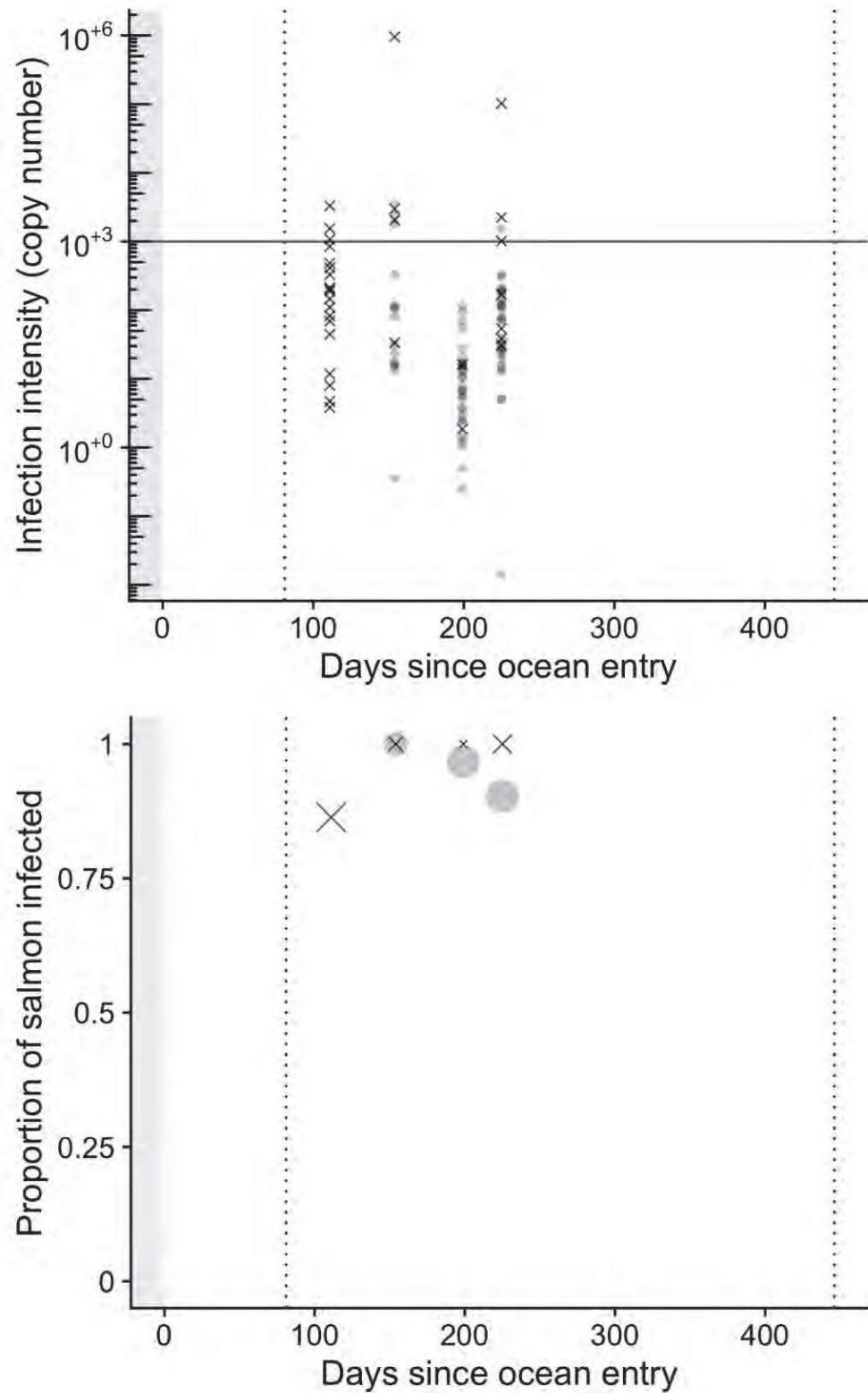
Aeromonas salmonicida



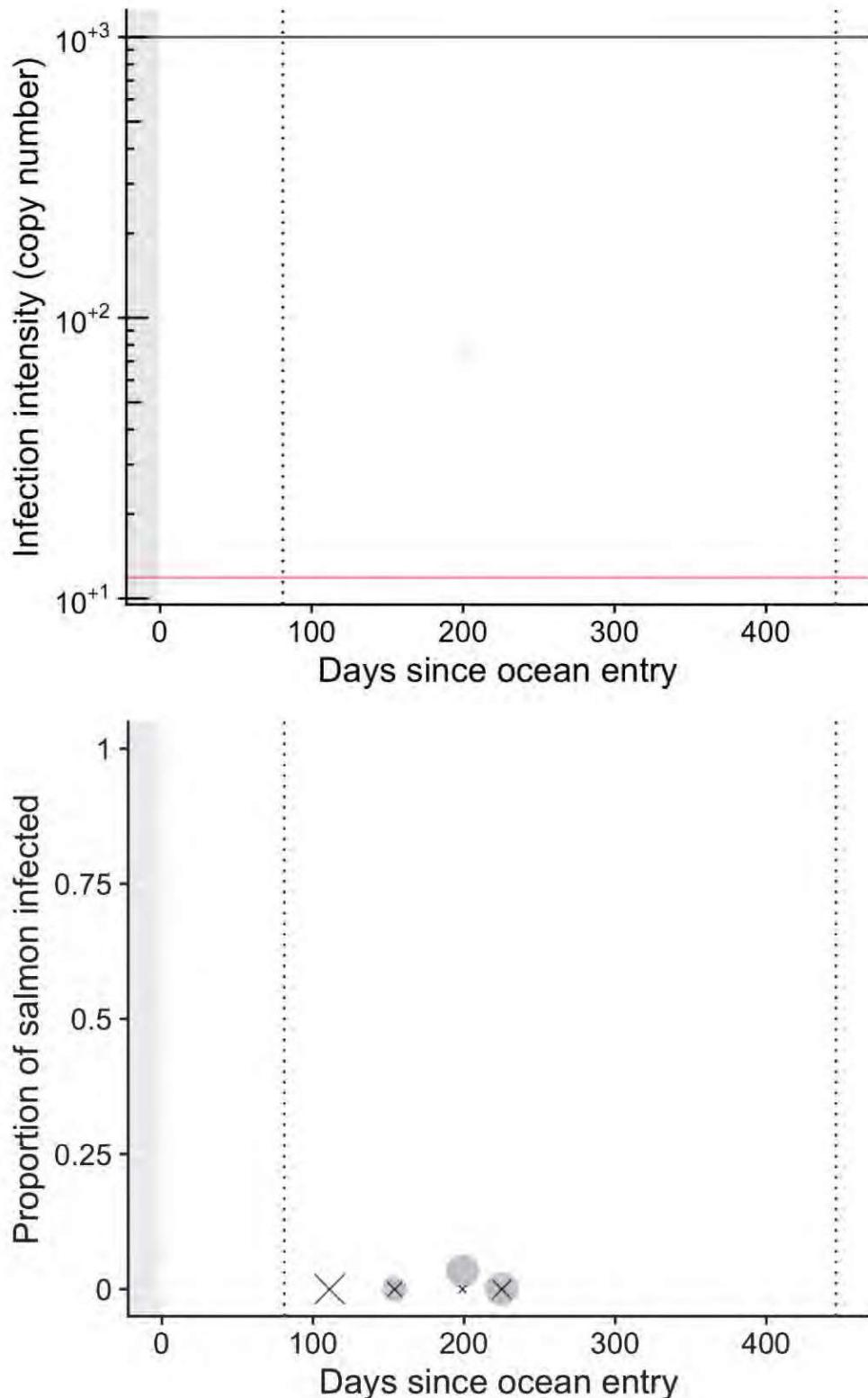
Atlantic salmon calicivirus



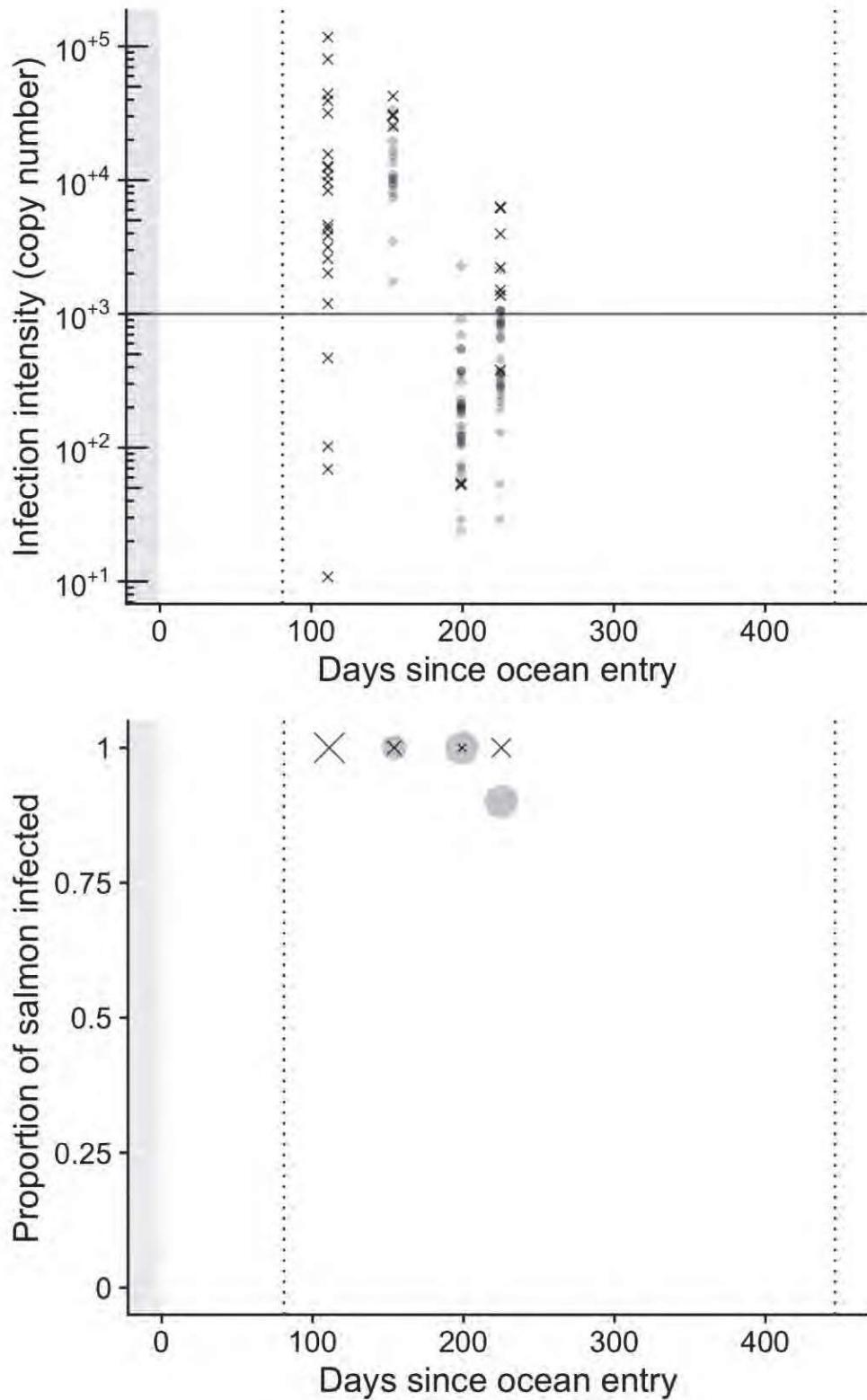
Cutthroat trout virus-2



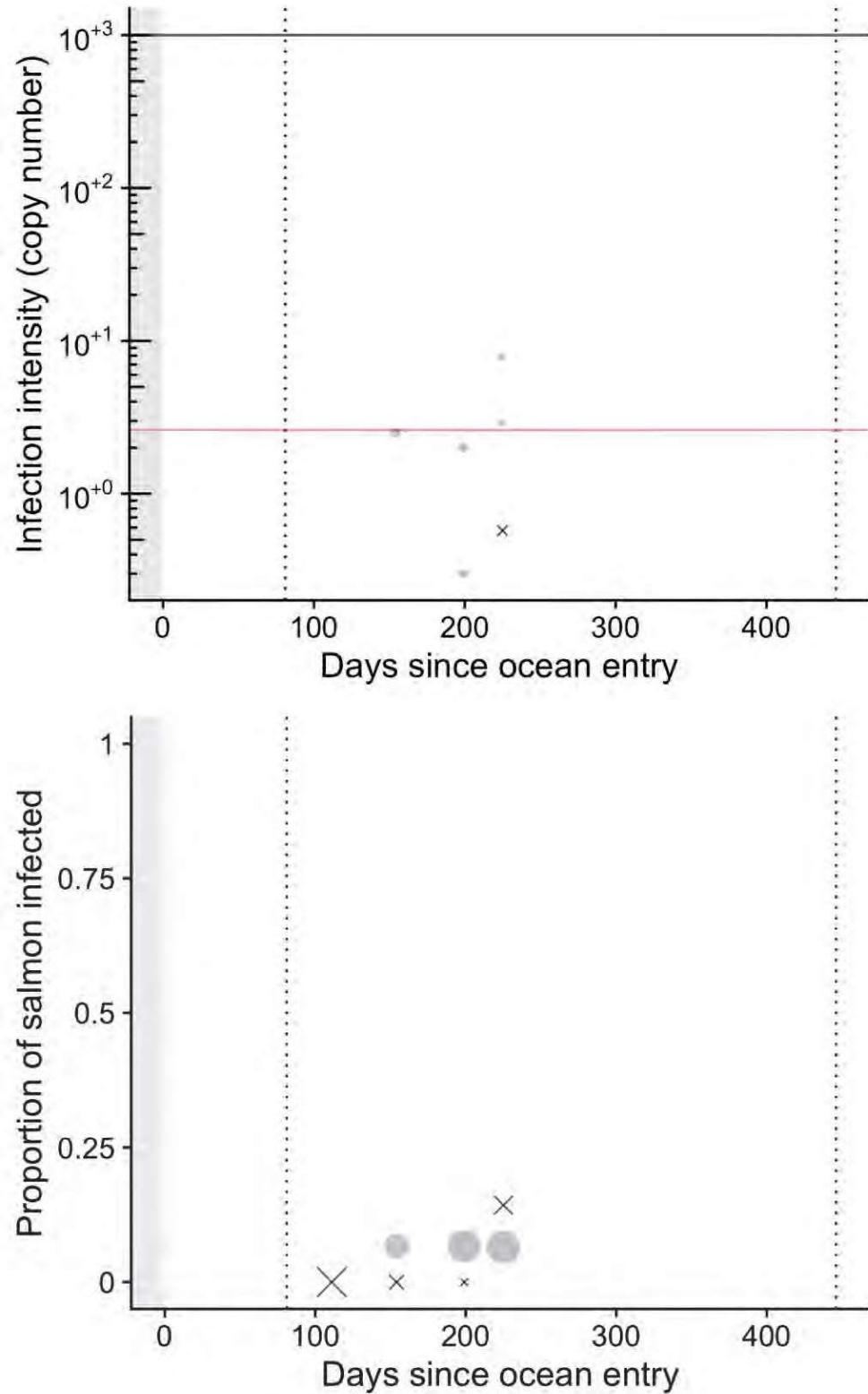
Flavobacterium psychrophilum



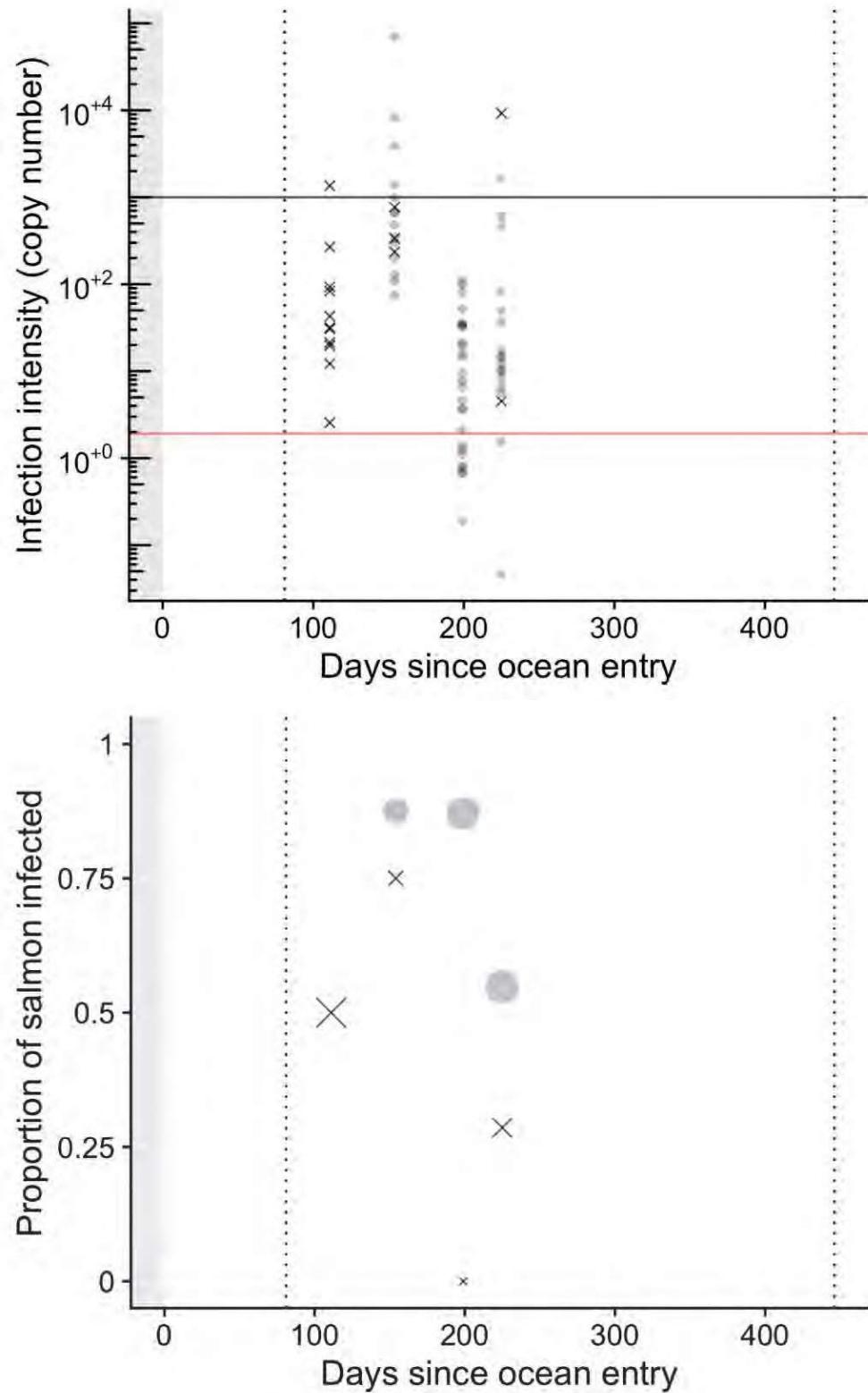
Piscine orthoreovirus



Candidatus Syngnathia salmonis



Tenacibaculum maritimum



Clinical signs

Table 1: Clinical signs for specimens sampled on 2022-02-03

metric	N5780	N5779	N5778	N5777	N5776	N5775	N5774	N5773	N5772	N5771	N5770	N5769	N5768	N5767	N5766	N5765	N5764	N5763	N5762	N5761	N5760
General																					
Live							X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Poor Performer						X															
Moribund	X	X	X				X														
Mort				X	X	X															
Exophthalmia							X											X			
Cataract/Corneal Opacity																					X
Skin & Fins																					
Erosion	X	X			X																X
Ulcers	X	X																			
Parasites							X														
Gills																					
Short Operculum																					
Pale																					
Erosions																					
Nodules/White Spots																					
Muscle																					
Hemorrhages																					
Abdominal Cavity																					
Body Fat Content			X															X		X	
Adhesions	X							X		X							X	X	X	X	X
Ascites								X													
Hemorrhages								X													
Spleen																					
Enlarged	X	X				X	X	X		X										X	X
Nodules/White Spots								X													
Liver																					
Enlarged									X												
Pale	X	X				X	X	X	X								X	X			X
Dark																			X		
Hemorrhages/Congestion																					
Nodules/White Spots								X													
Gallbladder																					
Enlarged								X											X		X
Heart																					
Deformed		X						X													X
Enlarged								X													
Pale	X								X												
Kidney																					
Nodules/White Spots									X												
Brain																					
Hemorrhages/Congestion						X	X	X													

295
000673

Table 2: Clinical signs for specimens sampled on 2022-02-03

metric	N5798	N5797	N5796	N5795	N5794	N5793	N5792	N5791	N5790	N5789	N5788	N5787	N5786	N5785	N5784	N5783	N5782	N5781
General																		
Live	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Poor Performer																		
Moribund																		
Mort																		
Exophthalmia	X																	
Cataract/Corneal Opacity										X								
Skin & Fins																		
Erosion	X																	
Ulcers																		
Parasites																		
Gills																		
Short Operculum		X																
Pale											X							
Erosions			X								X							
Nodules/White Spots																X		
Muscle																		
Hemorrhages				X														
Abdominal Cavity																		
Body Fat Content			X			X												
Adhesions	X					X						X					X	
Ascites																		
Hemorrhages			X								X							
Spleen																		
Enlarged			X	X	X	X					X	X						X
Nodules/White Spots																		
Liver																		
Enlarged																		
Pale							X			X	X							
Dark																		
Hemorrhages/Congestion			X															
Nodules/White Spots																		
Gallbladder																		
Enlarged		X	X			X						X				X		X
Heart																		
Deformed				X	X	X						X						
Enlarged												X						
Pale																		
Kidney																		
Nodules/White Spots																		
Brain																		
Hemorrhages/Congestion																		

Histology

Table 3: Histology scores for specimens sampled on 2022-02-03

metric	N5788	N5767	N5766	N5765	N5764	N5763	N5762	N5761
Heart								
Peri Epi	1	1	3	2	2		1	1
Myo			1	1	2		2	2
Liver								
Cong Haem	1	1		1				2
Nec		2		1	2	1		
Itis		2	1				3	1
Spleen								
Cong Heam		2		3	3			1
Ellip Nec					3	1		
W Pulpitis	1	2	1	1		2	2	1
Pig Inc		2		2				
Kidney								
Itis							3	
Cong Heam		2		2		2		
Interst Hyperplasia	1		1	1	2	2	2	1
Cnc								
Gliosis			1		1			
Cong Heam			2	2	2	3		
Gills								
Itis				nv	nv	nv		
Cong Heam				nv	nv	nv		
Prolif				nv	nv	nv		1
Skin_muscle								
Itis Nec				1			1	1
Tissue								
Necrosis Artefacts				3	3	2		

Diagnoses and Comments

Table 4: Diagnoses and comments for specimens sampled on 2022-02-03

DFO ID	Diagnosis	Comments
N5761		Peribiliary Immune Activation (1)
N5762		Peribiliary Immune Activation (1), Vac Deg Liver + Single Cell Apoptosis (3)
N5763	Early Hsmi	Erythrophagocytosis (1). Peribiliary Immune Activation (2)
N5764	Early Hsmi	Old Fish
N5765	HSMI	Satellitosis (1). Neuronal Vacuolization + Chromatolysis (1), Myocardioneerosis (3), Myocardium Hemorhages (1), Vac Deg Liver (2)
N5766		Hemosiderin (2), Increased Fibrin In Spleen (2)
N5767	Visceral Mycosis + Moderate Hsmi Myocarditis	
N5788	Early Hsmi	

Conclusions

In order to support the eDNA study, below is provided further evaluation of the results of testing from the Fish Health Report.

The sampling collection was complete, with fish collected from the control pen as well as the secondary pen. The farm was inspected in its entirety: most fish were behaving normally, although several individuals appeared lethargic. The mortality per pen reported by the company resulted slightly higher than the normal. Clinically, a significant number of moribund/morts showed external lesions (skin erosions and ulcers). Several fish (either live or moribund/morts) also showed enlarged spleen during the dissection procedures, as well as pale liver/heart in some instances. One individual presented with white nodules in liver and kidney, associated with ascites and internal hemorrhages. Brain congestion and hemorrhages was pretty common too. Molecular testing results show that almost the totality of the fish tested (92%) resulted positive to PRV (100% of the morts/moribund fish), and at high load in some instances. *Tenacibaculum maritimum* was also present in 51% of the fish (57% of the live fish, but in high load in some individuals), while *Candidatus Syngnathus salmonis* was observed at background level. Histopathologically, the moribund/morts samples collected showed an overall pattern of systemic congestive modifications with immunological/inflammatory response, affecting primarily heart, spleen, kidney and liver. In one individual, the pattern of lesions' severity and distribution (as well as clinical signs and gross lesions) consistent with the diagnosis of Heart and Skeletal Muscle Inflammation (HSMI), according to ICES diagnostic standards (ICES 2012). However, according to current DFO standard, this would count as "provisional diagnosis", as a laboratory challenge trial hasn't been performed. Four more fish (include a live one) presented similar lesions, but at an slightly earlier stage of development, either on the epicardium or in the myocardium. These fish would be classified as early stage of HSMI. Furthermore, in one fish the early HSMI lesions overlapped with the presence of numerous granulomas in the liver and kidney, which were suggestive of a visceral mycosis. Given the overall situation (subclinical HSMI + a case of visceral mycosis + high incidence of *T. maritimum*), the molecular results and clinical/pathological findings, a close monitoring of the operations during the next visit at this site is highly recommended.

Appendix

Glossary of infectious agents

Agent abbr.	Full agent name	Agent type	Disease	Ranking
ae_sal	Aeromonas salmonicida	Bacteria	Furunculosis	2
ascv	Atlantic salmon calicivirus	Virus	unknown	4
ctv-2	Cutthroat trout virus-2	Virus	unknown	4
fl_psy	Flavobacterium psychrophilum	Bacteria	Bacterial cold water disease	3
pisck_sal	Piscirickettsia salmonis	Bacteria	Piscirickettsiosis (SRS)	2
pmcv	Piscine myocarditis virus	Virus	Cardiomyopathy syndrome	1
prv-1	Piscine orthoreovirus	Virus	HSMI-EIBS-Jaundice/anemia	
psnv1	Pacific salmon nidovirus-1 (CoV)	Virus	unknown	4
re_sal	Renibacterium salmoninarum	Bacteria	Bacterial kidney disease	2
sch	Candidatus Syngnathia salmonis	Bacteria	Gill chlamydia	3
te_mar	Tenacibaculum maritimum	Bacteria	Marine flexibacteriosis (mouth/fin rot)	2
vi_ang	Vibrio anguillarum	Bacteria	Vibriosis	2
vi_sal	Vibrio salmonicida	Bacteria	Cold water vibriosis	2
ye_ruc	Yersinia ruckeri (Enteric redmouth disease)	Bacteria	Yersiniosis (Enteric red mouth)	2

eDNA Study Report

Midsummer Island sampling on February 24, 2022

Dr. Emiliano Di Cicco

February 21, 2023

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Piscine orthoreovirus	11
Candidatus Syngnathus salmonis.....	12
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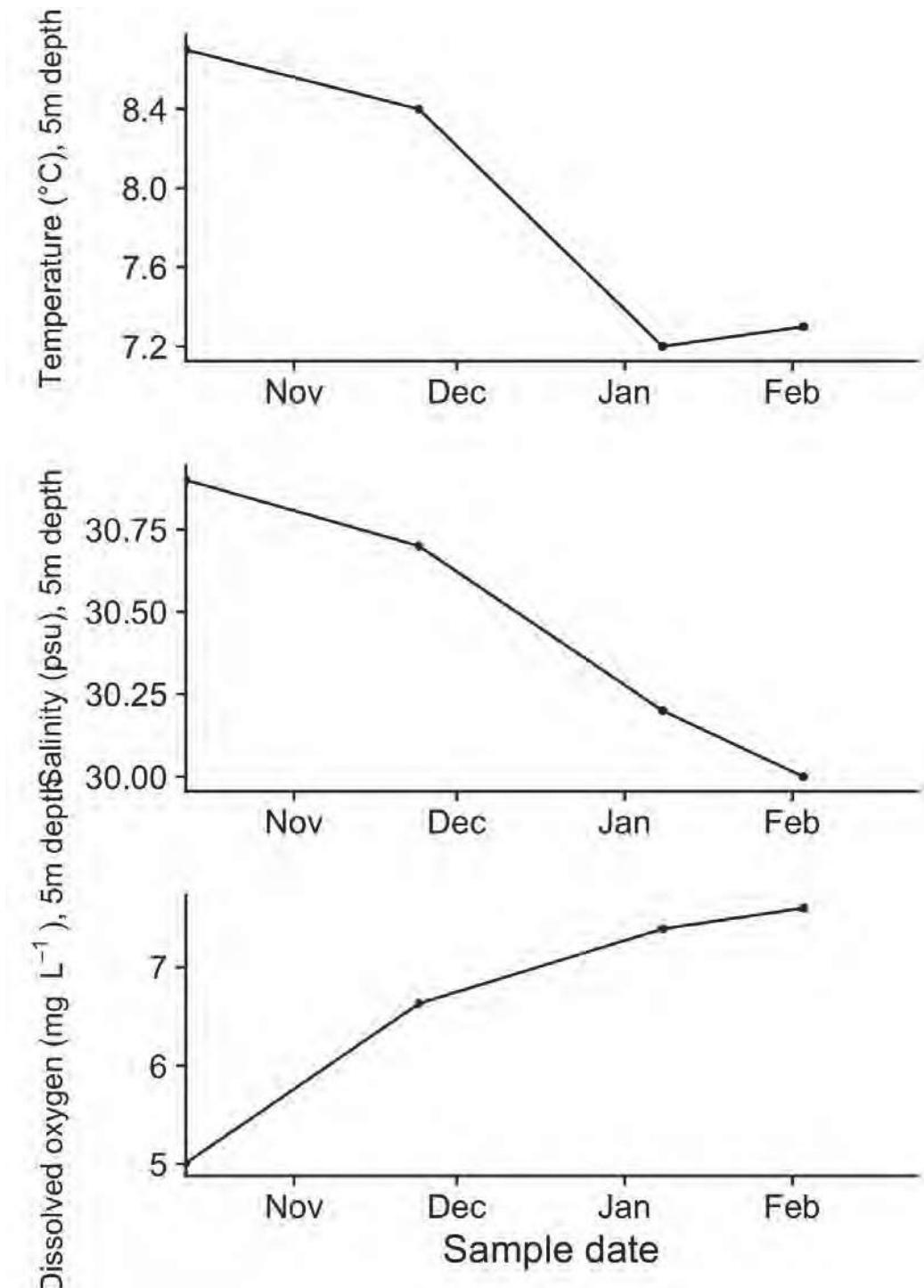
Executive summary

Premise

On February 24, 2022, 36 samples were collected by BATI and Mowi crew during a sampling event at Midsummer Island (Mowi Ltd.). 36 Atlantic salmon subadults were collected from the Midsummer Island farm site, including 30 live and 6 moribund/dead fish. All live fish were euthanized with TMS overdose prior to dissection with the exception of the moribund fish, which were administered a blow to the head. Portions of gill, liver and anterior kidney were collected in triplicate for molecular testing (preserved in RNA later) from all the fish, while all the moribund/dead fish also underwent collection of tissues (gills, spleen, liver, heart, anterior and posterior kidney, pyloric caeca, skeletal muscle + skin, brain) for histological analysis. Clinical notes and gross lesions were noted and reported for every fish. One aliquot has been provided to the Company MOWI Fish Health, another aliquot is stored at the BATI Field Office, and a third aliquot is stored at DFO - PBS. This latter aliquot has been tested for the presence and load of the agents indicated in the IMIP agreement as well as the agents indicated in the eDNA study agreement. Each sample has been extracted and tested individually. Negative and positive controls were run. A housekeeping gene was also included to assess the quality of the RNA extracted.

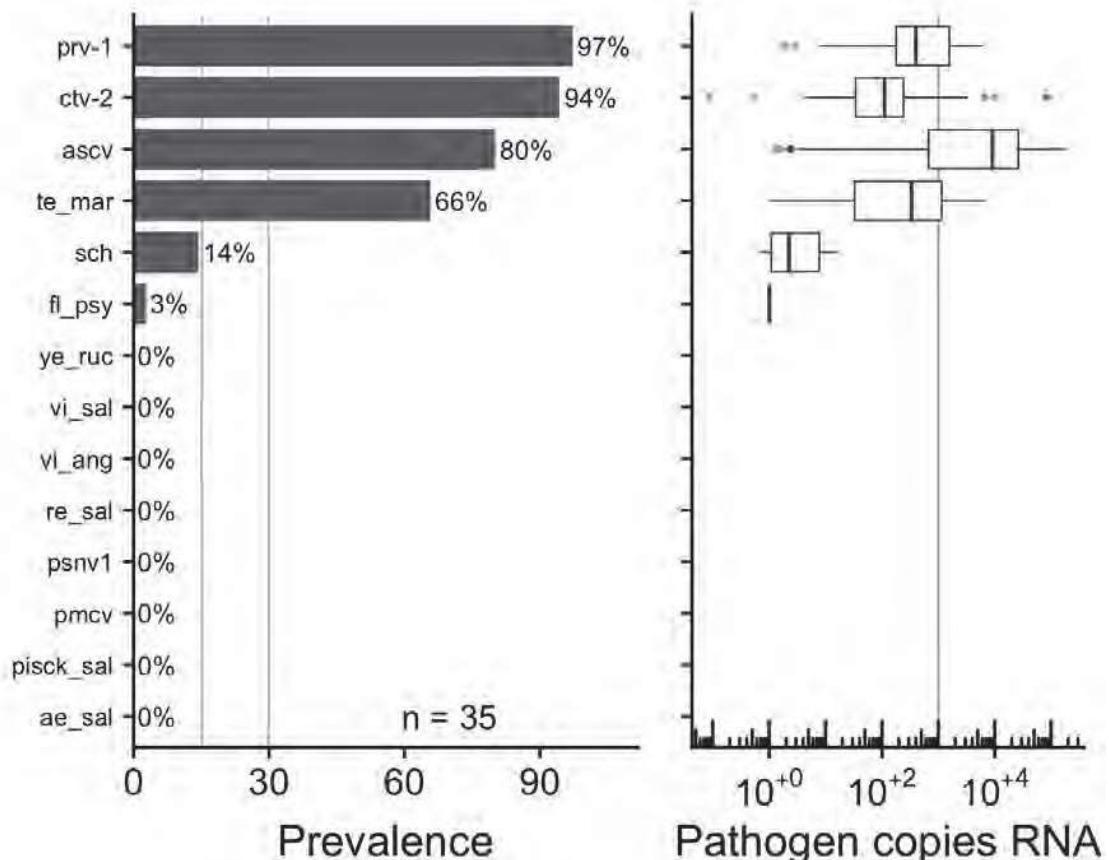
Histology samples have been sent to Wax-It Histo Ltd. to process and prepare slides, which have been read and scored by Dr. Di Cicco. A digital copy of each slide is available to the Company.

Environmental data

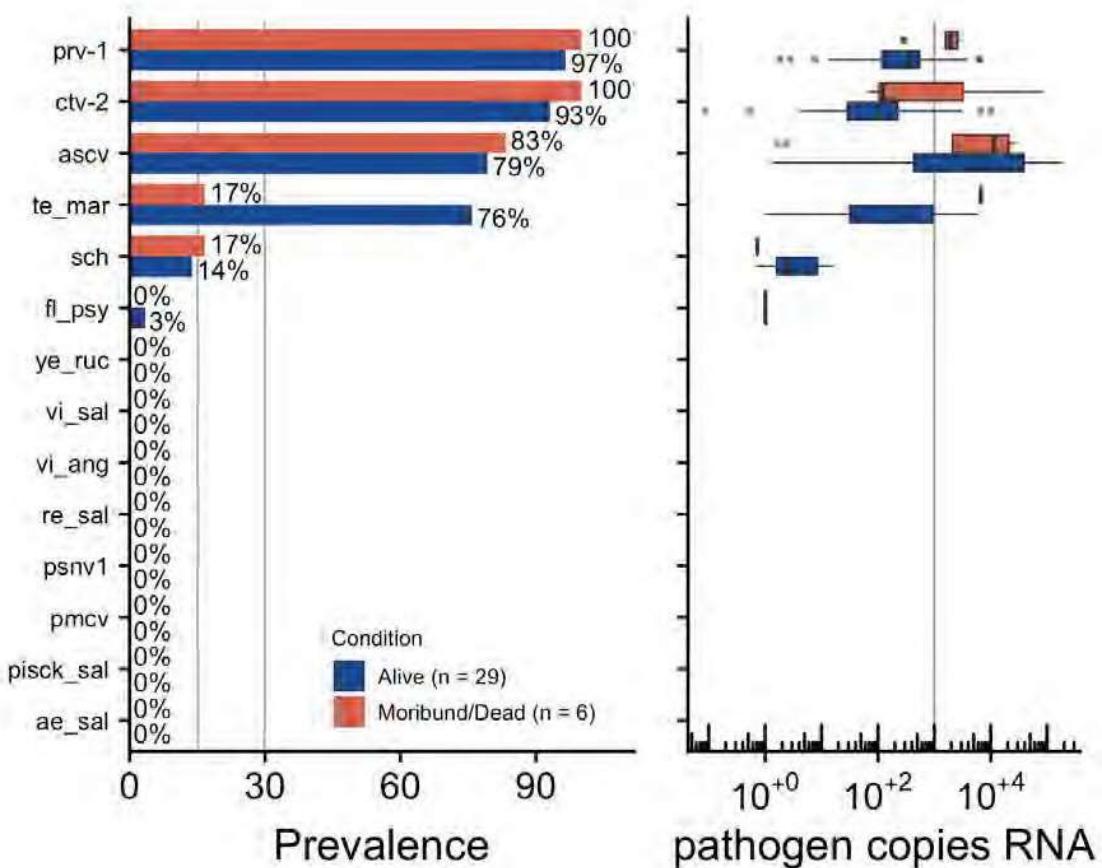


Water temperature ($^{\circ}\text{C}$), salinity (ppm), and dissolved oxygen (mg/L) at a 5m depth. Certain sampling dates have no recorded environmental data, resulting in gaps in the plots.

Overall infectious agent prevalence



Infectious agent prevalence in samples collected on 2022-02-24



Infectious agent prevalence in samples collected on 2022-02-24, split by mortality status at time of sampling. Any specimens that were not confirmed to be either moribund or live at the time of generating this report are excluded from this figure.

Individual infectious agent trends

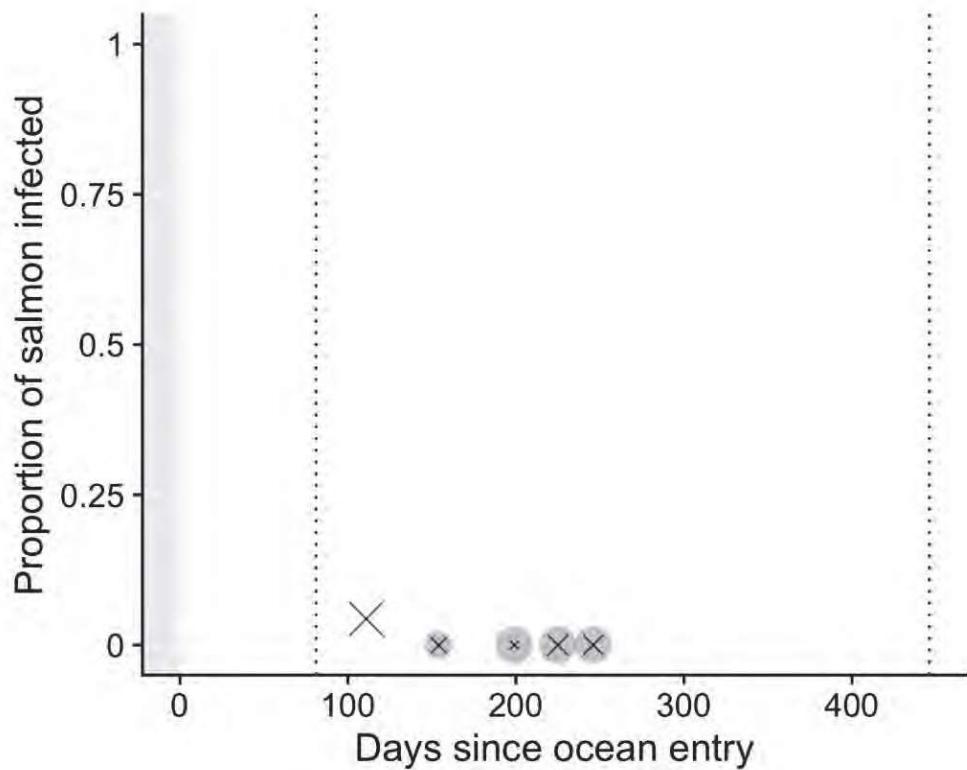
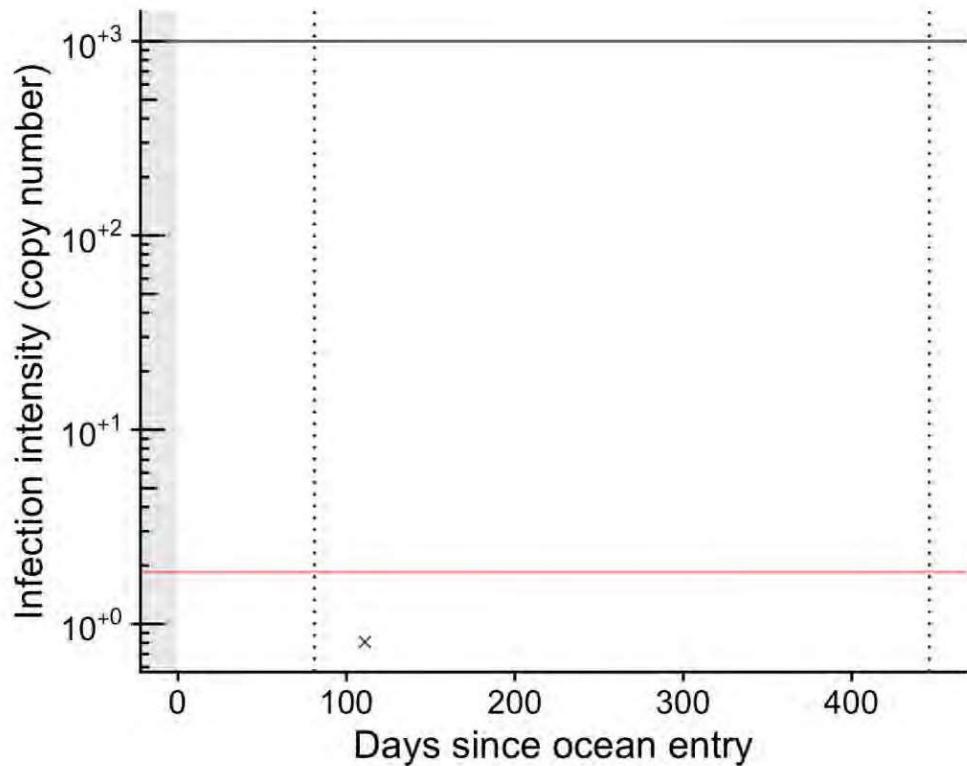
The following plots show individual infectious agent trends across all farm sites. In cases where sample size is sufficient, curves from a generalised additive model are included in the plot.

Grey circles represent live fish, and black X's represent dead/dying fish. Curves indicate mean predictions from a generalised additive model; blue and red correspond to live and dead/dying fish, respectively (shaded areas show 95% confidence regions). Left-hand grey region indicates freshwater hatchery residence, grey regions on x-axis indicate period of transfer to another site, and vertical dotted lines correspond to January 1st.

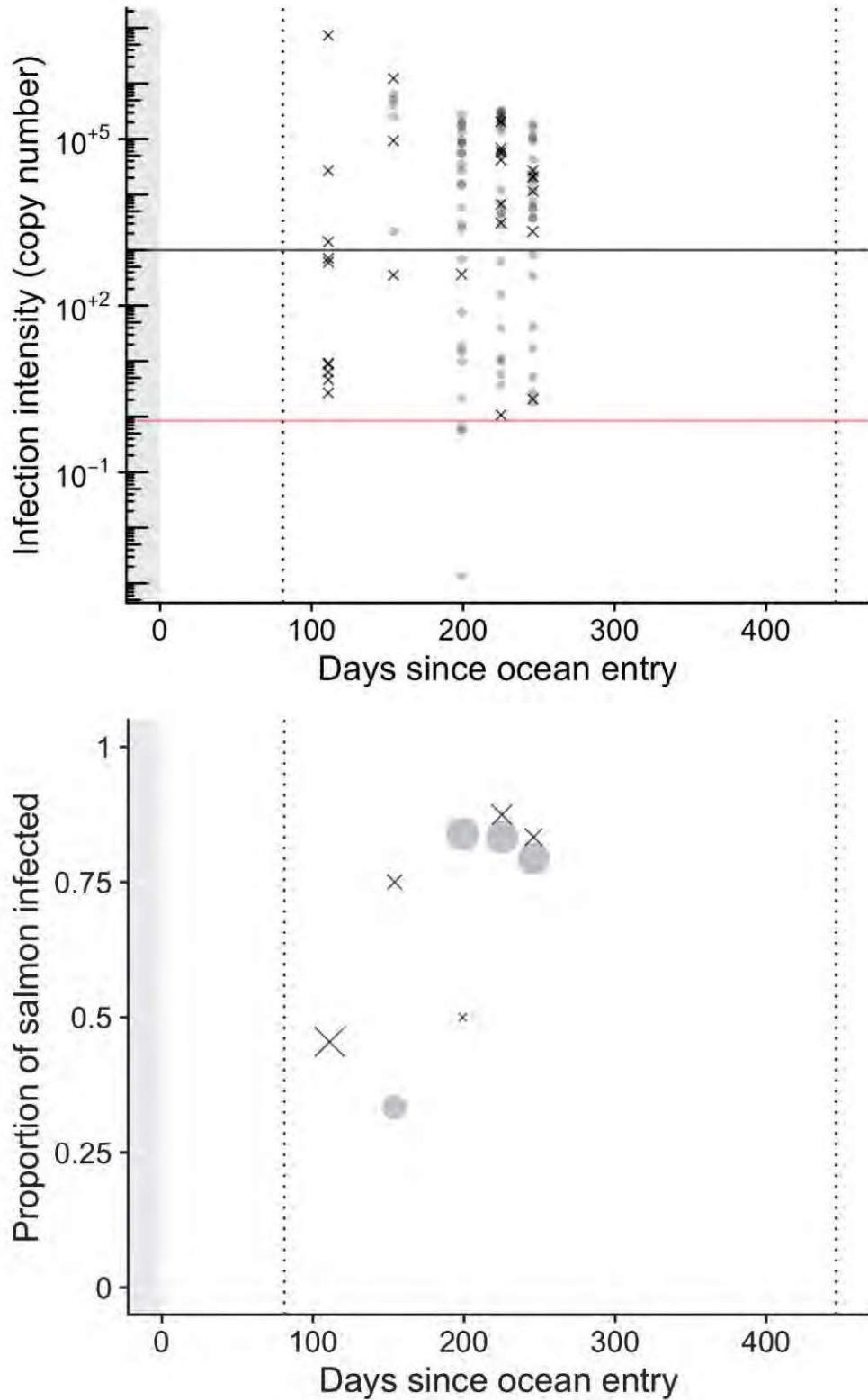
For infection intensity plots, horizontal red line indicates limit of detection (yielding ~90% true positive rate) for respective qPCR assay run in duplicate, while the horizontal black line indicates 1000 copies. Note log scale.

For proportion plots, grey circles show prevalence in live fish on each sampling date, and black X's show prevalence in dead/dying fish (symbol areas proportional to sample sizes).

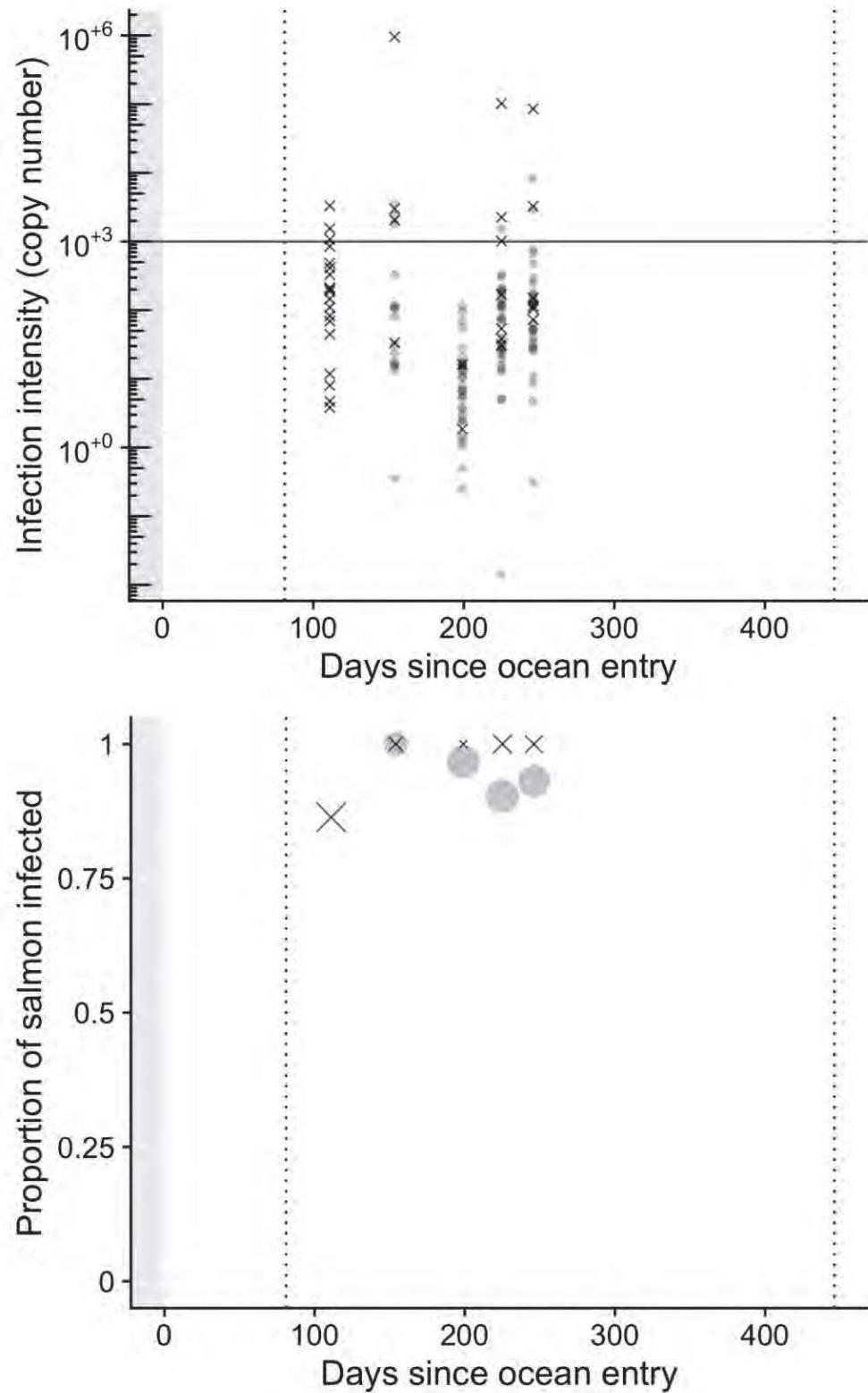
Aeromonas salmonicida



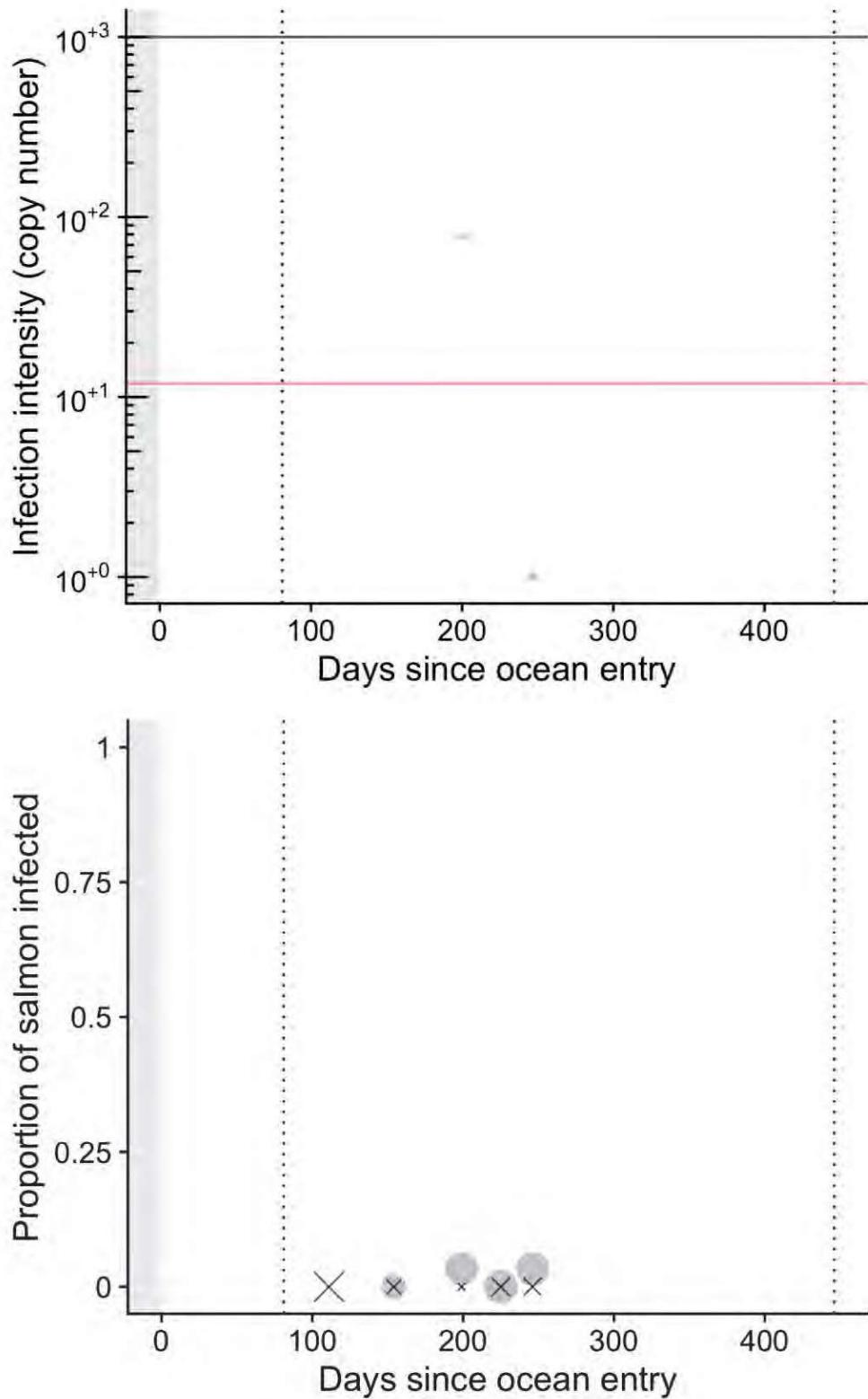
Atlantic salmon calicivirus



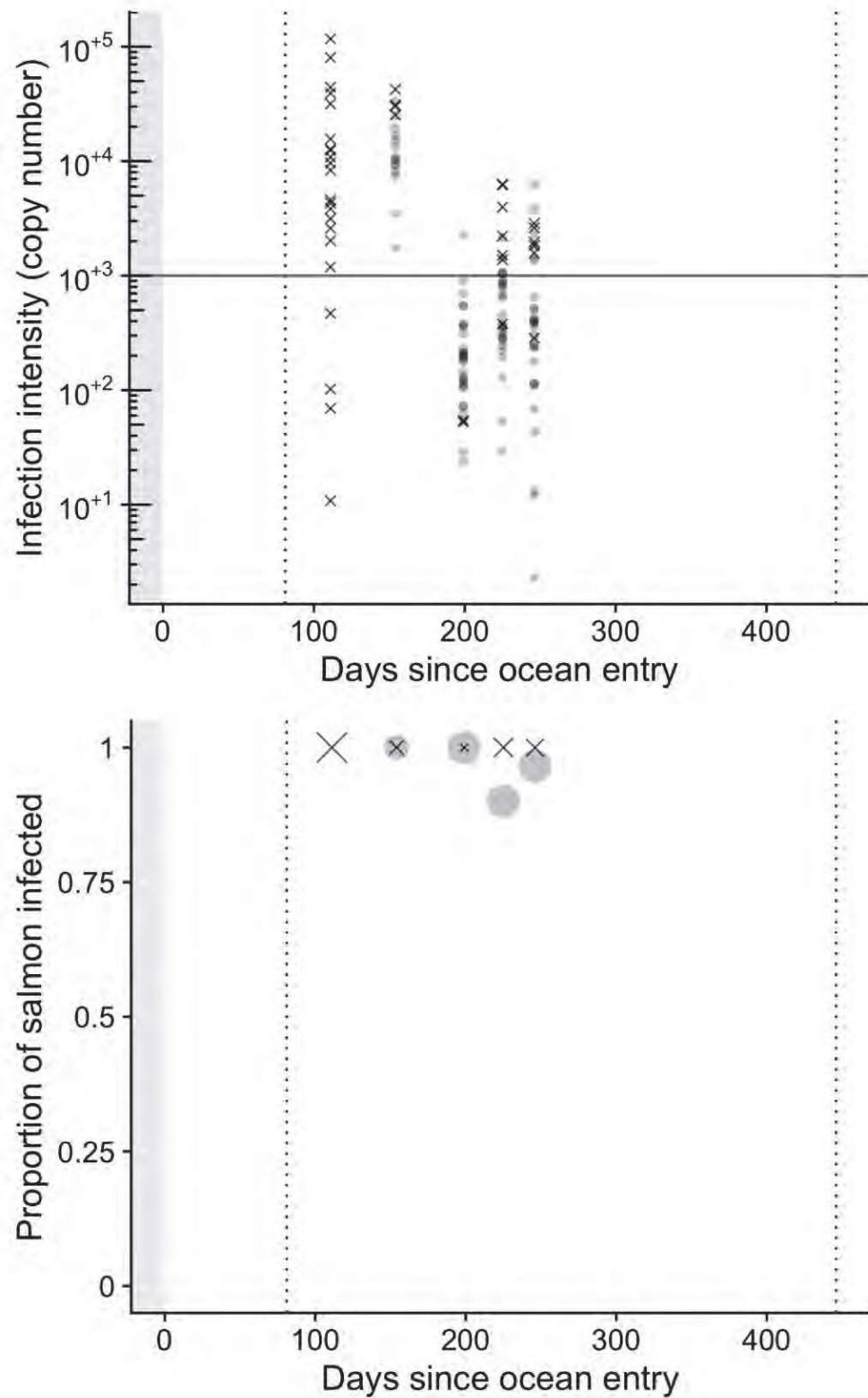
Cutthroat trout virus-2



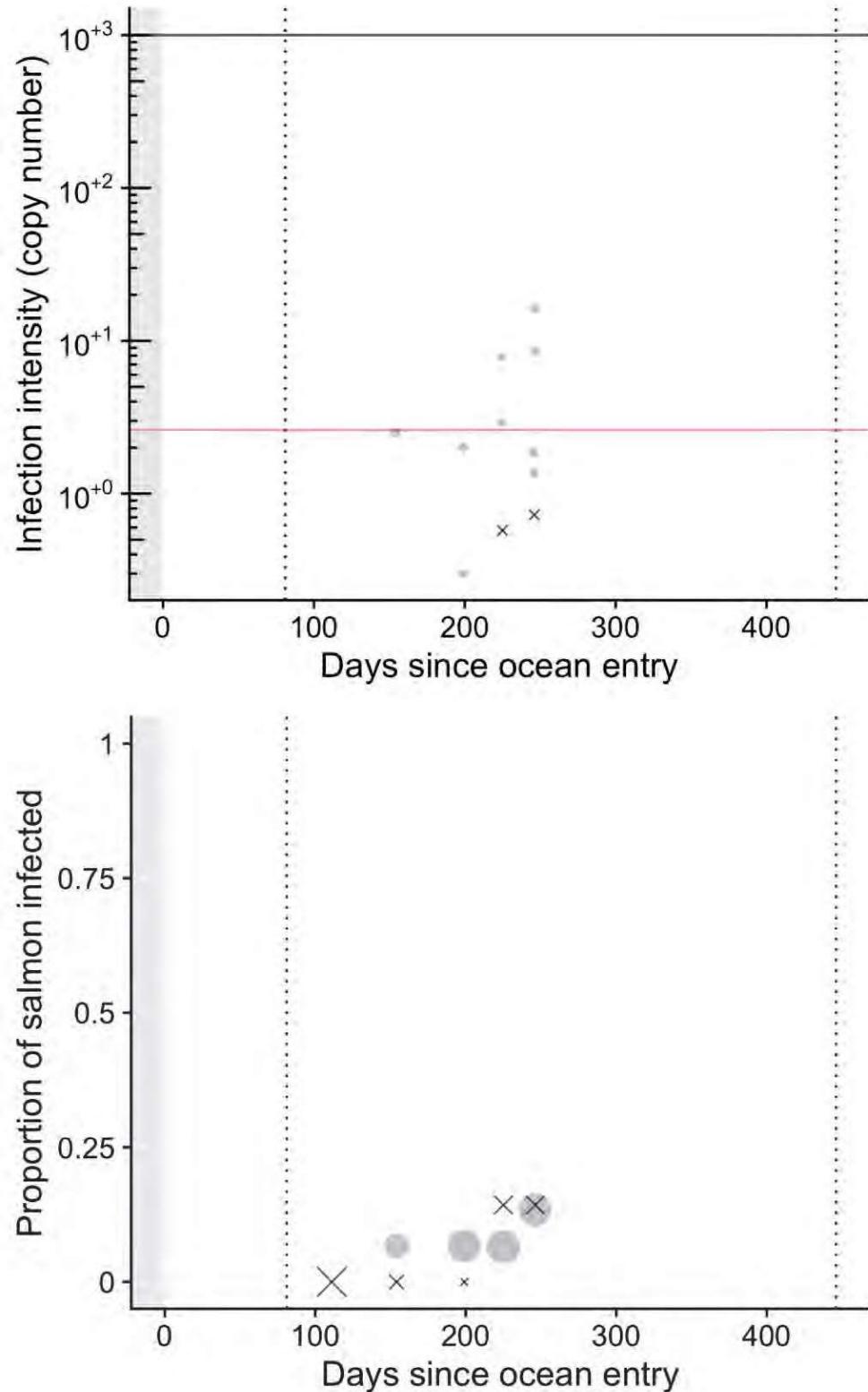
Flavobacterium psychrophilum



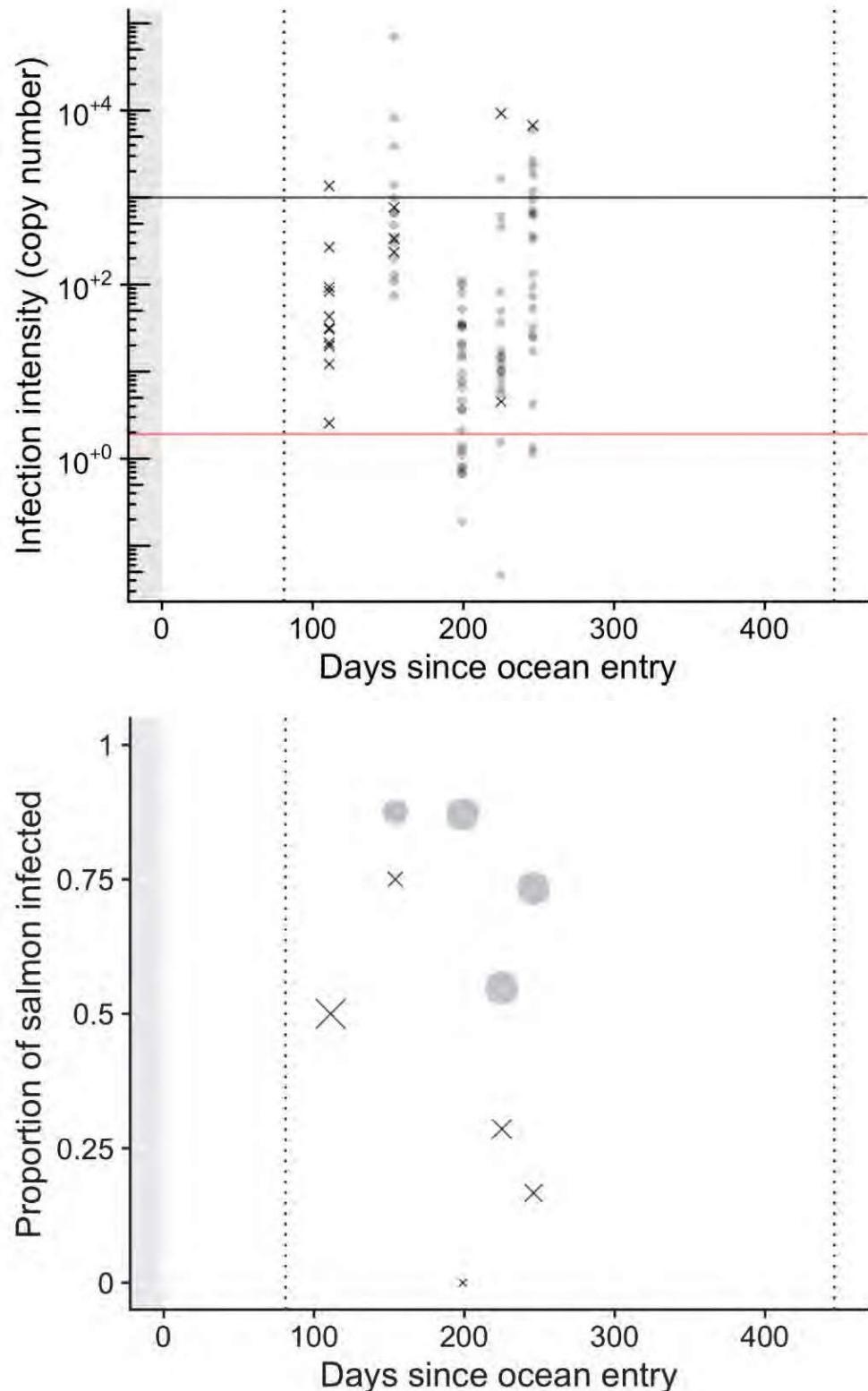
Piscine orthoreovirus



Candidatus Syngnathia salmonis



Tenacibaculum maritimum



Clinical signs

Table 1: Clinical signs for specimens sampled on 2022-02-24

metric	N5940	N5939	N5938	N5937	N5936	N5935	N5934	N5933	N5932	N5931	N5930	N5929	N5928	N5927	N5926	N5925	N5924	N5923	N5922	N5921
General																				
Live								X	X	X	X	X	X	X	X	X	X	X	X	X
Poor Performer		X				X														
Slow Swimmer	X																			
Emaciated					X															
Moribund	X																			
Mort		X	X	X	X	X														
Skin & Fins																				
Lost Scales				X																
Gills																				
Short Operculum																				
Pale																				
Erosions												X								
Nodules/White Spots			X																	
Abdominal Cavity																				
Adhesions											X	X	X	X	X	X			X	
Ascites					X															
Spleen																				
Enlarged	X		X	X	X	X														
Liver																				
Pale	X					X	X													
Gallbladder																				
Enlarged	X	X	X	X	X	X				X	X		X	X	X	X		X	X	X
Green		X	X	X	X	X							X		X			X	X	
Heart																				
Deformed							X		X									X		
Enlarged									X									X	X	
Pale		X		X	X															
Blood Clots/Hemopericardium				X																
Brain																				
Hemorrhages/Congestion			X	X	X	X	X													

Table 2: Clinical signs for specimens sampled on 2022-02-24

metric	N5941	N5942	N5943	N5944	N5945	N5946	N5947	N5948	N5949	N5950	N5951	N5952	N5953	N5954	N5955	N5956
General																
Live	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Poor Performer																
Slow Swimmer																
Emaciated		X														
Moribund																
Mort																
Skin & Fins																
Lost Scales																
Gills																
Short Operculum											X	X				
Pale		X														
Erosions		X								X						
Nodules/White Spots		X														
Abdominal Cavity																
Adhesions									X							
Ascites																
Spleen																
Enlarged			X		X						X					
Liver								X								
Pale									X							
Gallbladder																
Enlarged	X			X						X			X	X		
Green		X														
Heart																
Deformed		X							X		X					
Enlarged		X				X										
Pale																
Blood Clots/Hemopericardium																
Brain																
Hemorrhages/Congestion																

Histology

Table 3: Histology scores for specimens sampled on 2022-02-24

metric	N5951	N5942	N5934	N5928	N5926	N5925	N5924	N5923	N5922	N5921
Heart										
Peri Epi	1		1	2	3	1			1	1
Myo	1	1	1	1	2	1		2		1
Liver										
Cong Haem			1	1						
Nec		1	1	1	2	1		1		
Itis	1				1		1	2	1	1
Spleen										
Cong Heam	1		1			1	2	2		1
Ellip Nec		2	2				1			
W Pulpitis	1	1	1	1	1	2	2	2	2	2
Kidney										
Osis					1					
Cong Heam				2					1	1
Interst Hyperplasia	1	2	2	1	1	2	1	1	1	1
Pancreatitis										
Pancreatitis							na	na	na	na
Enteritis										
Enteritis							na	na	na	na
Cns										
Itis							na	na	na	na
Cnc										
Malacia							na	na	na	na
Gliosis		2		1	1		na	na	na	na
Cong Heam	2	2		2	2		na	na	na	na
Microsporidia							na	na	na	na
Gills										
Itis		nv	1		nv					
Cong Heam		nv			nv					
Prolif	nv	3			nv	2		1	1	
Skin_muscle										
Itis Nec				1	1					
Tissue										
Necrosis Artefacts		2	1	1	2					

Diagnoses and Comments

Table 4: Diagnoses and comments for specimens sampled on 2022-02-24

DFO ID	Diagnosis	Comments
N5921		Peribiliary Immune Activation (1)
N5923		Satellitosis (2), Neuronal Vacuolization (2), Neuronal Chromatolysis (1)
N5924		Increased Fibrin In Spleen (2), Myocardioneclerosis (2)
N5925	HSMI	Increased Fibrin In Spleen (2), Myocardioneclerosis (2), Satellitosis (2), Neuronal Vacuolization (2), Neuronal Chromatolysis (1)
N5926		Erythrophagocytosis In Liver(1) And Kidney (2), Hemosiderin In Spleen (1)
N5934	Recoverng Hsmi	Deg Vac Liver (2)

Conclusions

In order to support the eDNA study, below is provided further evaluation of the results of testing from the Fish Health Report.

The sampling collection was incomplete, with fish collected from the control pen as well as the secondary pen. However, no morts were retrieved from the control pen due to the temporary removal of the mort pump in this pen. Pens 7, 8, 9 and 10 were treated with FW bath (Tromoy) the previous day, so they were excluded from the mort collection to decrease bias for mechanical injuries.

The farm was inspected in its entirety: the fish in the untreated pens were behaving normally, and low mortality was reported. However, there were several poor performers and still a few lethargic fish facing the wall of pens (particularly in pen 2 and 3). The mortality was significant higher in the treated pens, and several fish from these pens presented with external lesions.

Clinically, the gall bladder was enlarged in several fish, likely due to pre-treatment fasting. Heart deformities were also unusually common. Among the morts, pale liver and heart, enlarged spleen and brain congestions were the most common and significant findings, while skin lesions and gill alteration were pretty rare.

Molecular testing results show that almost the totality of the fish tested (97%) resulted positive to PRV (100% of the morts/moribund fish), and at high load in some instances. *Tenacibaculum maritimum* was also present in 66% of the fish (76% of the live fish, but in high load in some individuals). *Candidatus Syngnathus salmonis* was observed in 14% of the fish samples, while *Flavobacterium psychrophilum* was detected at background level.

Histopathologically, the moribund/morts samples collected showed an overall pattern of mild to moderate systemic congestive modifications with immunological/inflammatory response, affecting primarily heart, spleen, kidney, brain and liver. In one individual, the pattern of lesions' severity and distribution (as well as clinical signs and gross lesions) consistent with the diagnosis of Heart and Skeletal Muscle Inflammation (HSMI), according to ICES diagnostic standards (ICES 2012). However, according to current DFO standard, this would count as "provisional diagnosis", as a laboratory challenge trial hasn't been performed. Two more (live) fish presented similar lesions, but at an slightly earlier stage of development, either on the epicardium or in the myocardium. These fish would be classified as either early stage of HSMI, or recovering phase. Brain lesions were also common, as gliosis/satellitosis + neuronal vacuolization and chromatolysis and/or moderate congestion.

Given the overall situation (subclinical HSMI + high incidence of *T. maritimum*), the molecular results and clinical/pathological findings, a close monitoring of the operations during the next visit at this site is highly recommended.

Appendix

Glossary of infectious agents

Agent abbr.	Full agent name	Agent type	Disease	Ranking
ae_sal	Aeromonas salmonicida	Bacteria	Furunculosis	2
ascv	Atlantic salmon calicivirus	Virus	unknown	4
ctv-2	Cutthroat trout virus-2	Virus	unknown	4
fl_psy	Flavobacterium psychrophilum	Bacteria	Bacterial cold water disease	3
pisck_sal	Piscirickettsia salmonis	Bacteria	Piscirickettsiosis (SRS)	2
pmcv	Piscine myocarditis virus	Virus	Cardiomyopathy syndrome	1
prv-1	Piscine orthoreovirus	Virus	HSMI-EIBS-Jaundice/anemia	
psnv1	Pacific salmon nidovirus-1 (CoV)	Virus	unknown	4
re_sal	Renibacterium salmoninarum	Bacteria	Bacterial kidney disease	2
sch	Candidatus Syngnathia salmonis	Bacteria	Gill chlamydia	3
te_mar	Tenacibaculum maritimum	Bacteria	Marine flexibacteriosis (mouth/fin rot)	2
vi_ang	Vibrio anguillarum	Bacteria	Vibriosis	2
vi_sal	Vibrio salmonicida	Bacteria	Cold water vibriosis	2
ye_ruc	Yersinia ruckeri (Enteric redmouth disease)	Bacteria	Yersiniosis (Enteric red mouth)	2

eDNA Study Report

Midsummer Island sampling on March 31, 2022

Dr. Emiliano Di Cicco

February 21, 2023

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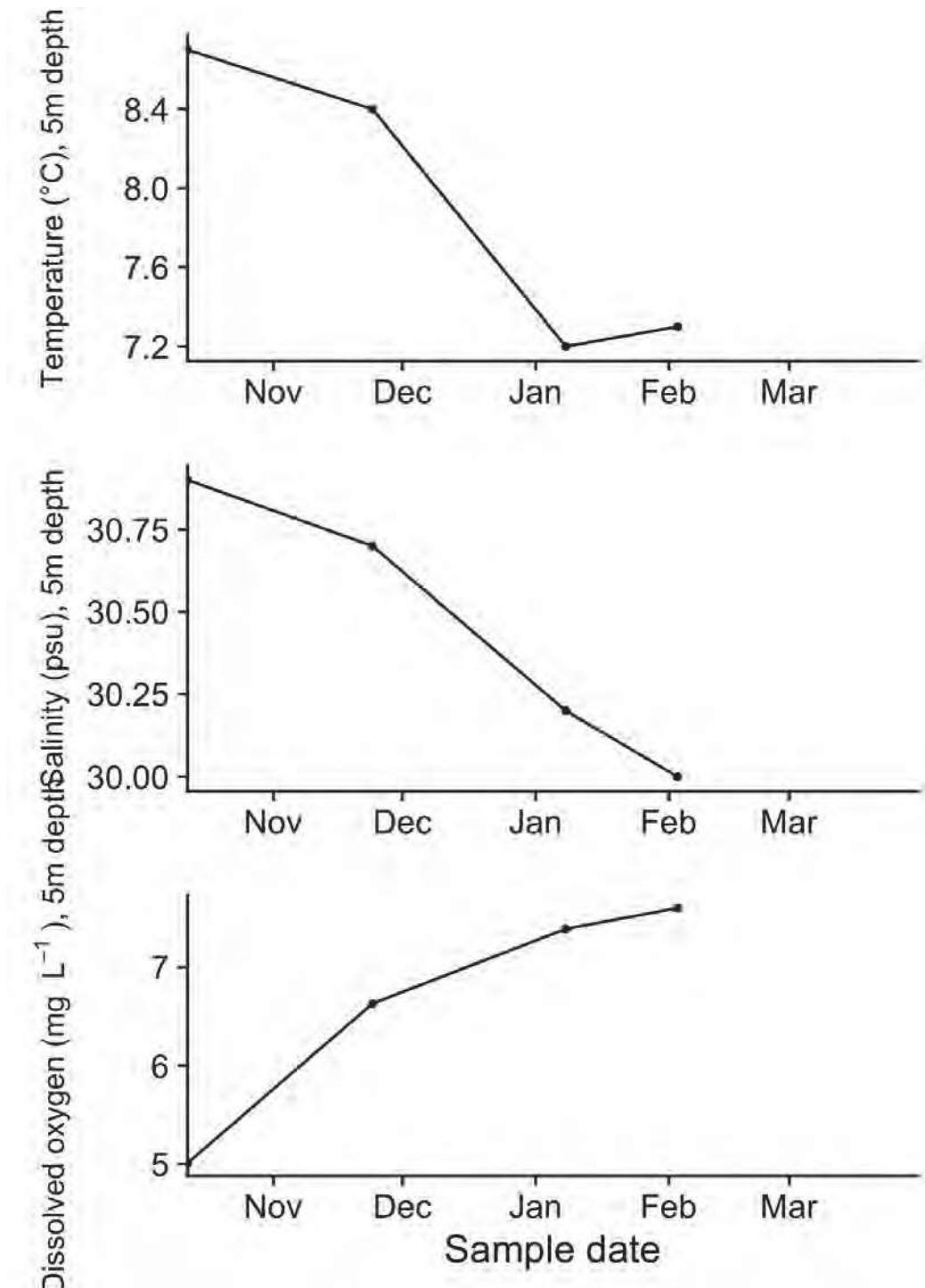
Executive summary

Premise

On March 31, 2022, 35 samples were collected by BATI and Mowi crew during a sampling event at Midsummer Island (Mowi Ltd.). 35 Atlantic salmon subadults were collected from the Midsummer Island farm site, including 30 live and 5 moribund/dead fish. All live fish were euthanized with TMS overdose prior to dissection with the exception of the moribund fish, which were administered a blow to the head. Portions of gill, liver and anterior kidney were collected in triplicate for molecular testing (preserved in RNA later) from all the fish, while all the moribund/dead fish also underwent collection of tissues (gills, spleen, liver, heart, anterior and posterior kidney, pyloric caeca, skeletal muscle + skin, brain) for histological analysis. Clinical notes and gross lesions were noted and reported for every fish. One aliquot has been provided to the Company MOWI Fish Health, another aliquot is stored at the BATI Field Office, and a third aliquot is stored at DFO - PBS. This latter aliquot has been tested for the presence and load of the agents indicated in the IMIP agreement as well as the agents indicated in the eDNA study agreement. Each sample has been extracted and tested individually. Negative and positive controls were run. A housekeeping gene was also included to assess the quality of the RNA extracted.

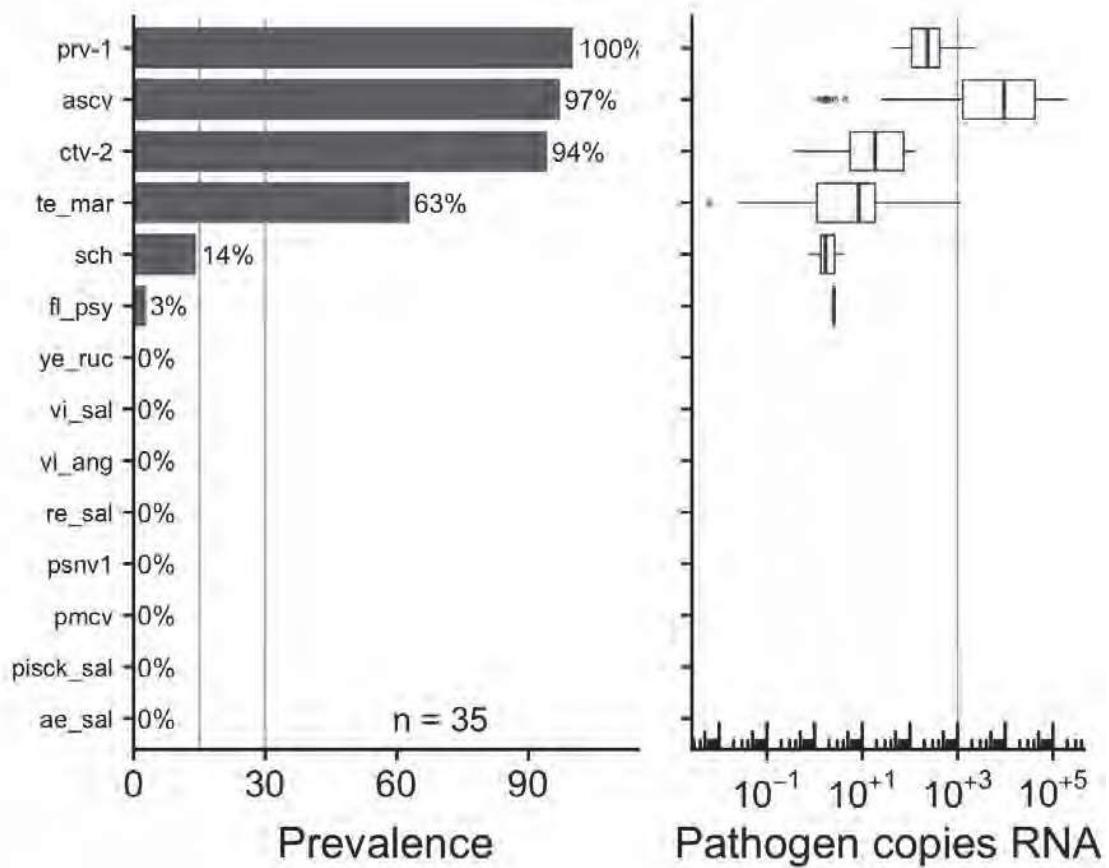
Histology samples have been sent to Wax-It Histo Ltd. to process and prepare slides, which have been read and scored by Dr. Di Cicco. A digital copy of each slide is available to the Company.

Environmental data

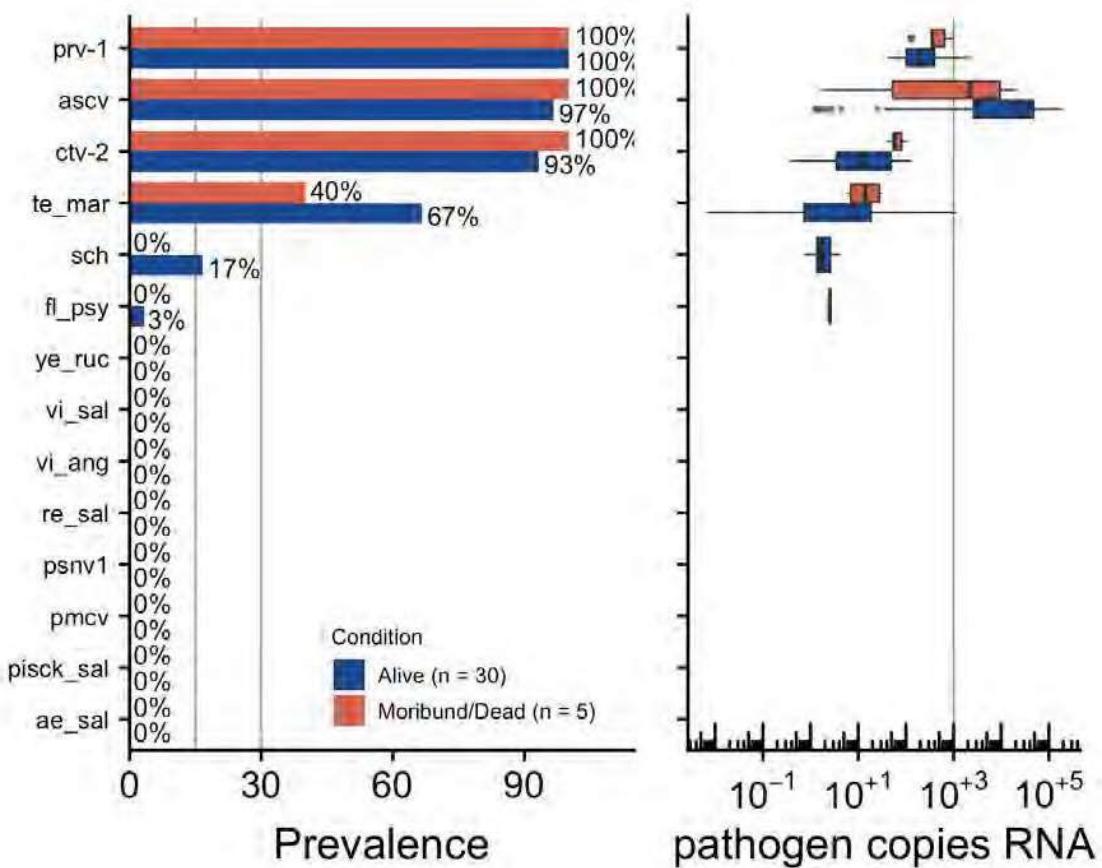


Water temperature ($^{\circ}\text{C}$), salinity (ppm), and dissolved oxygen (mg/L) at a 5m depth. Certain sampling dates have no recorded environmental data, resulting in gaps in the plots.

Overall infectious agent prevalence



Infectious agent prevalence in samples collected on 2022-03-31.



Infectious agent prevalence in samples collected on 2022-03-31, split by mortality status at time of sampling. Any specimens that were not confirmed to be either moribund or live at the time of generating this report are excluded from this figure.

Individual infectious agent trends

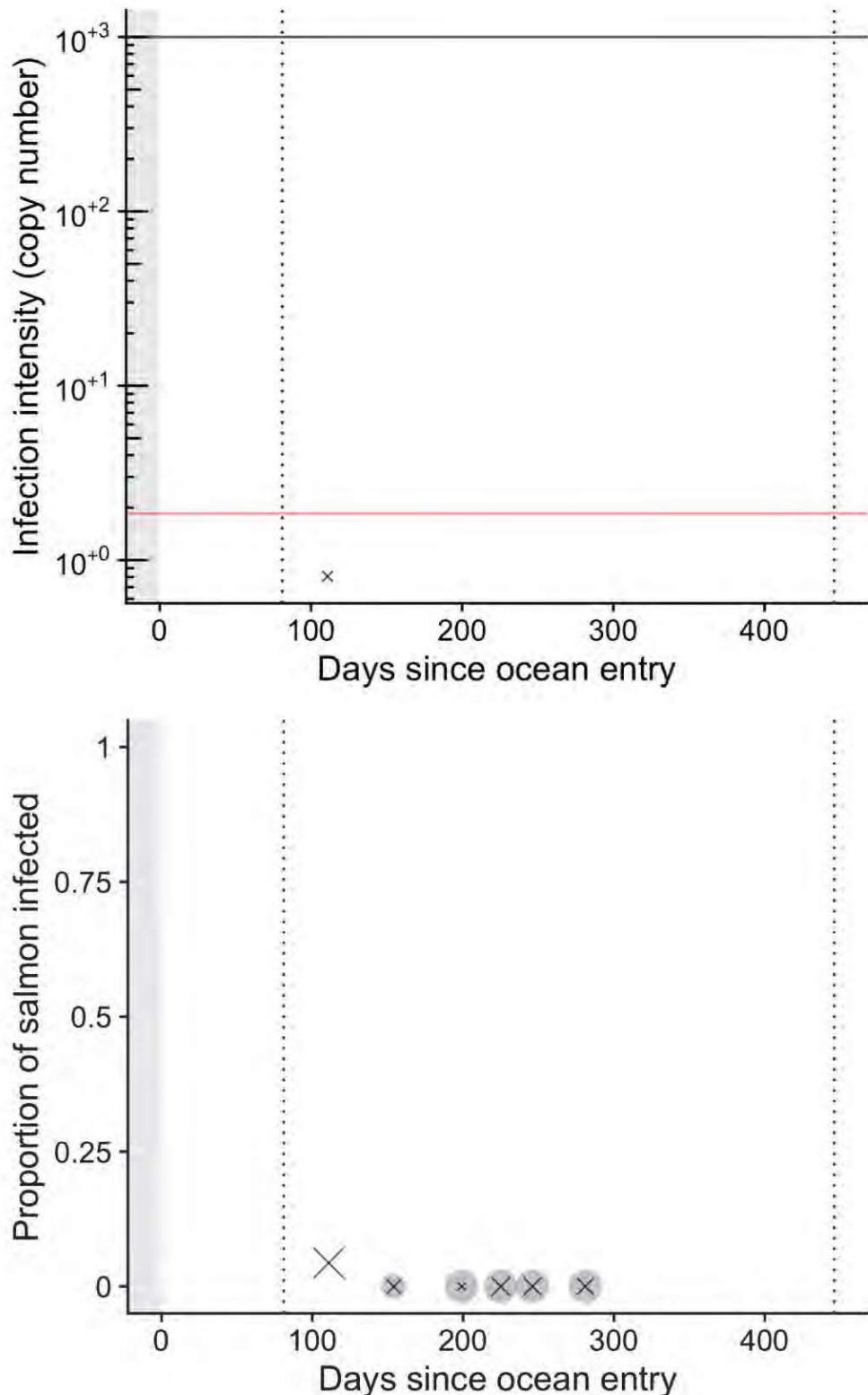
The following plots show individual infectious agent trends across all farm sites. In cases where sample size is sufficient, curves from a generalised additive model are included in the plot.

Grey circles represent live fish, and black X's represent dead/dying fish. Curves indicate mean predictions from a generalised additive model; blue and red correspond to live and dead/dying fish, respectively (shaded areas show 95% confidence regions). Left-hand grey region indicates freshwater hatchery residence, grey regions on x-axis indicate period of transfer to another site, and vertical dotted lines correspond to January 1st.

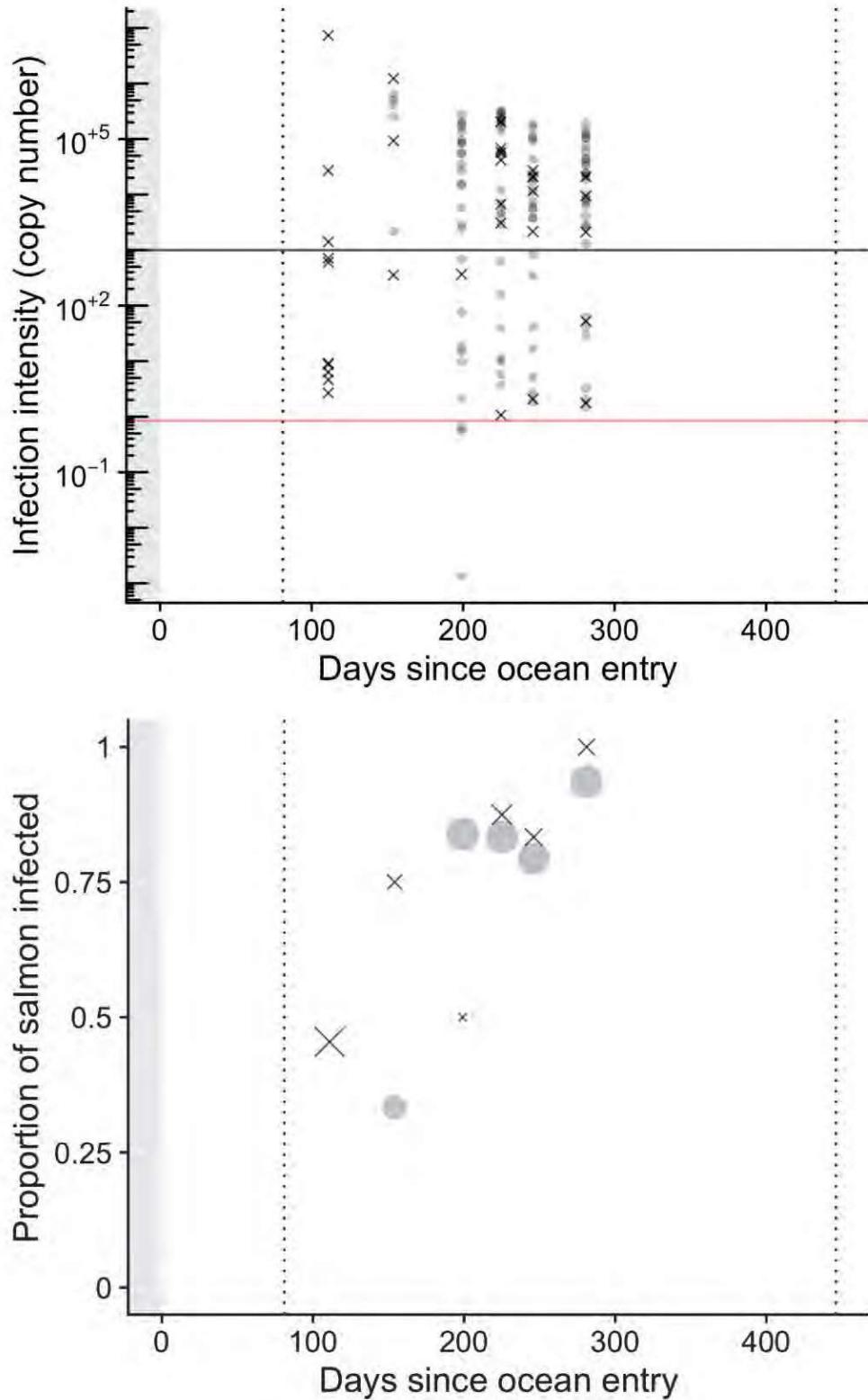
For infection intensity plots, horizontal red line indicates limit of detection (yielding ~90% true positive rate) for respective qPCR assay run in duplicate, while the horizontal black line indicates 1000 copies. Note log scale.

For proportion plots, grey circles show prevalence in live fish on each sampling date, and black X's show prevalence in dead/dying fish (symbol areas proportional to sample sizes).

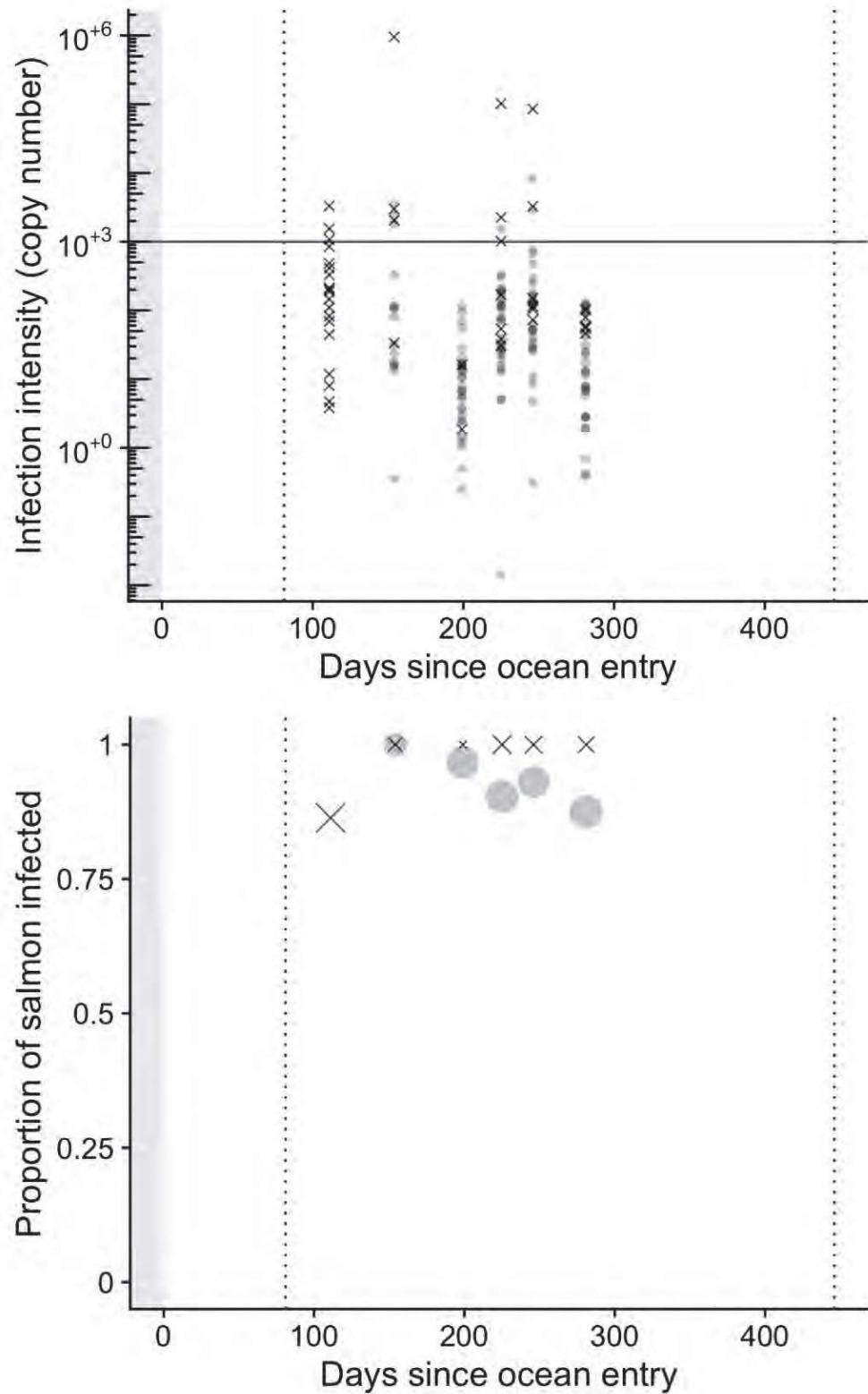
Aeromonas salmonicida



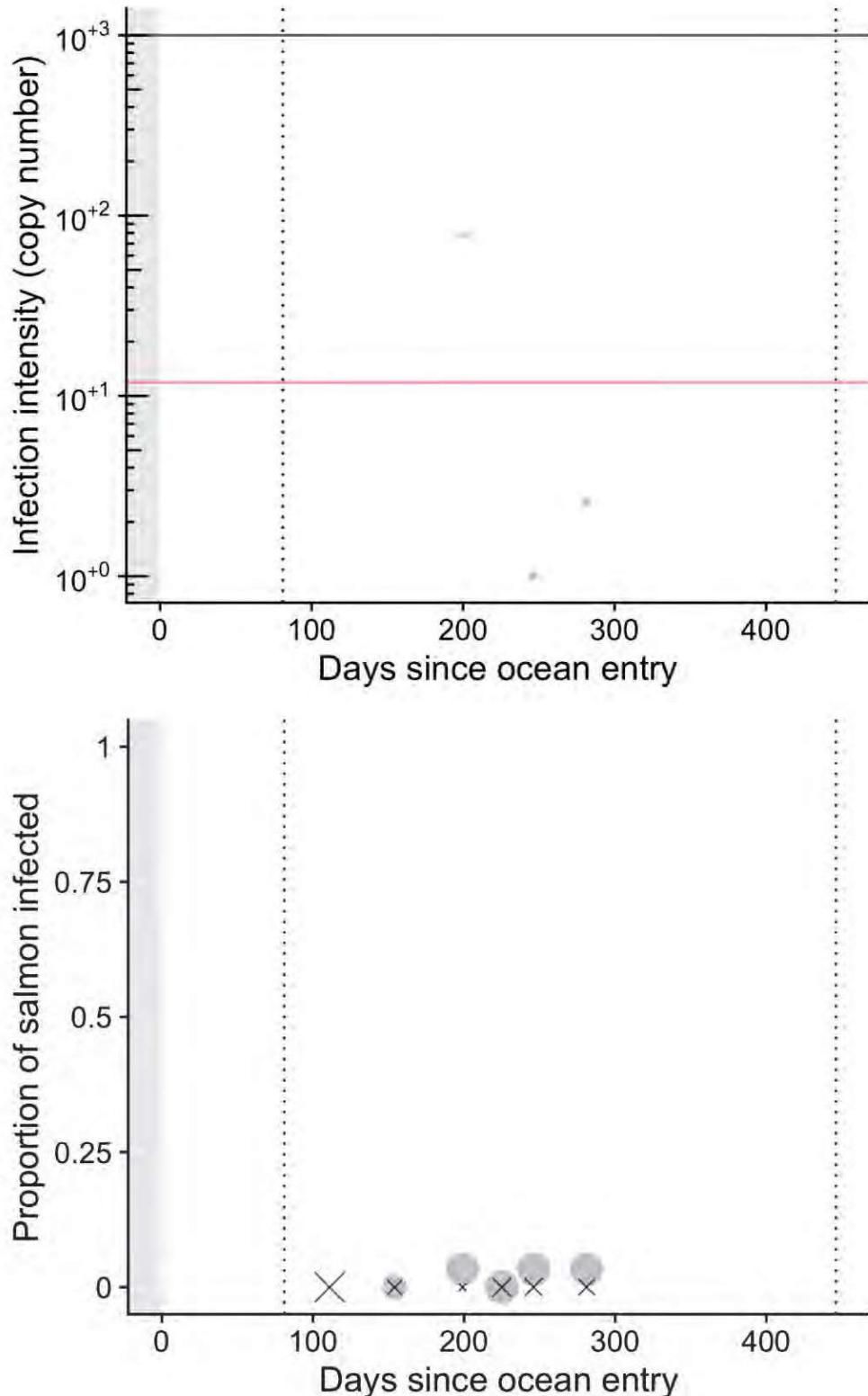
Atlantic salmon calicivirus



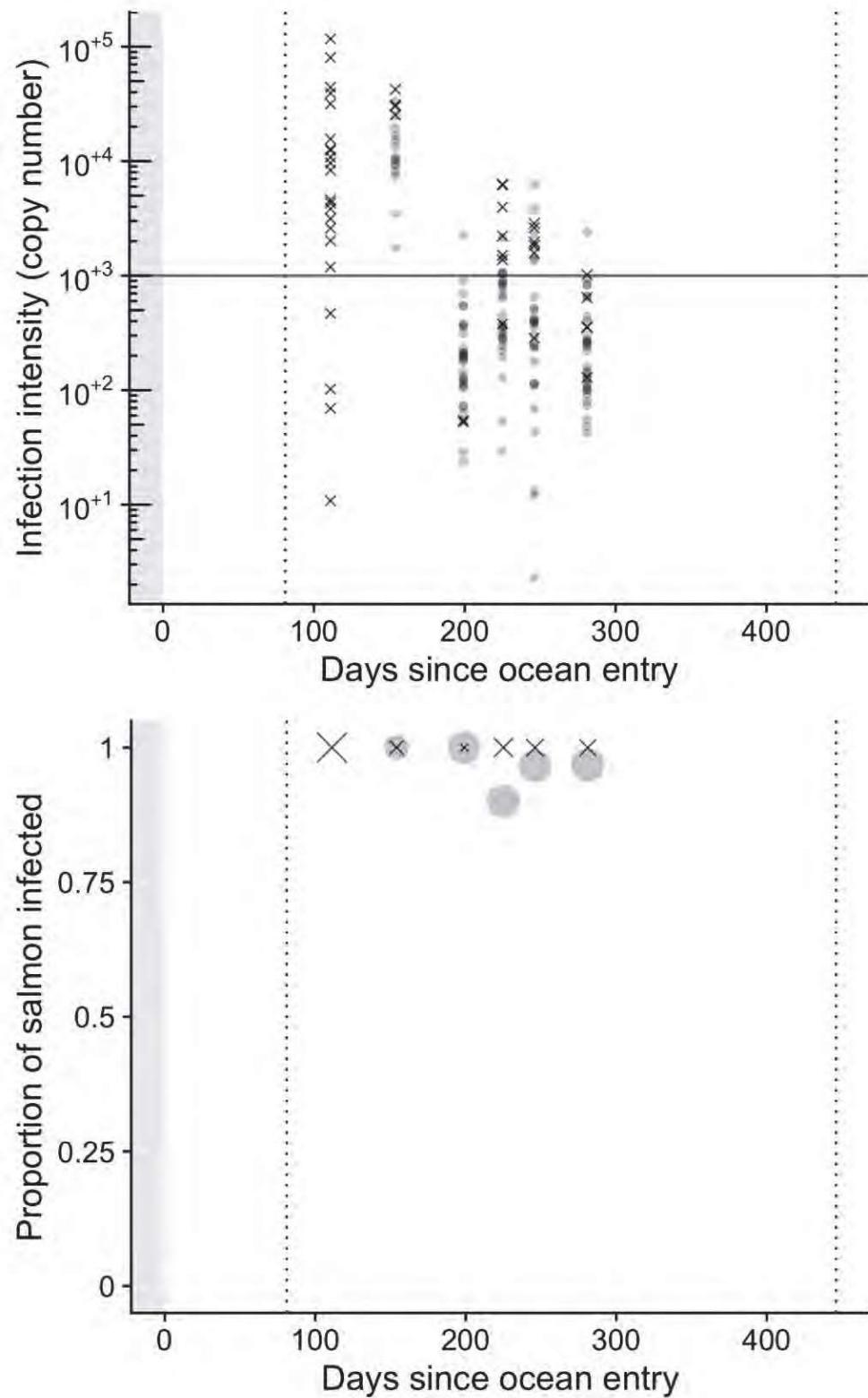
Cutthroat trout virus-2



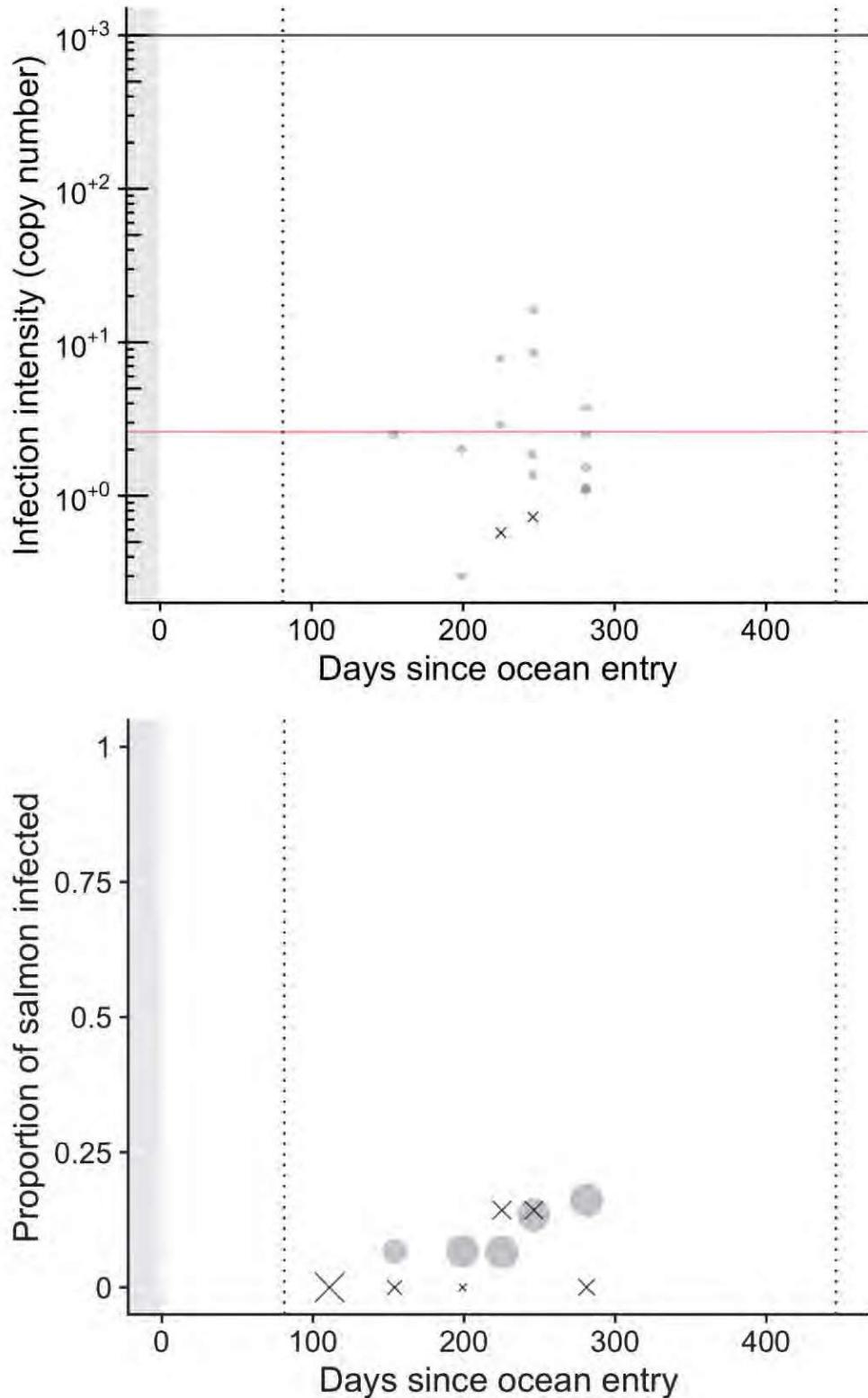
Flavobacterium psychrophilum



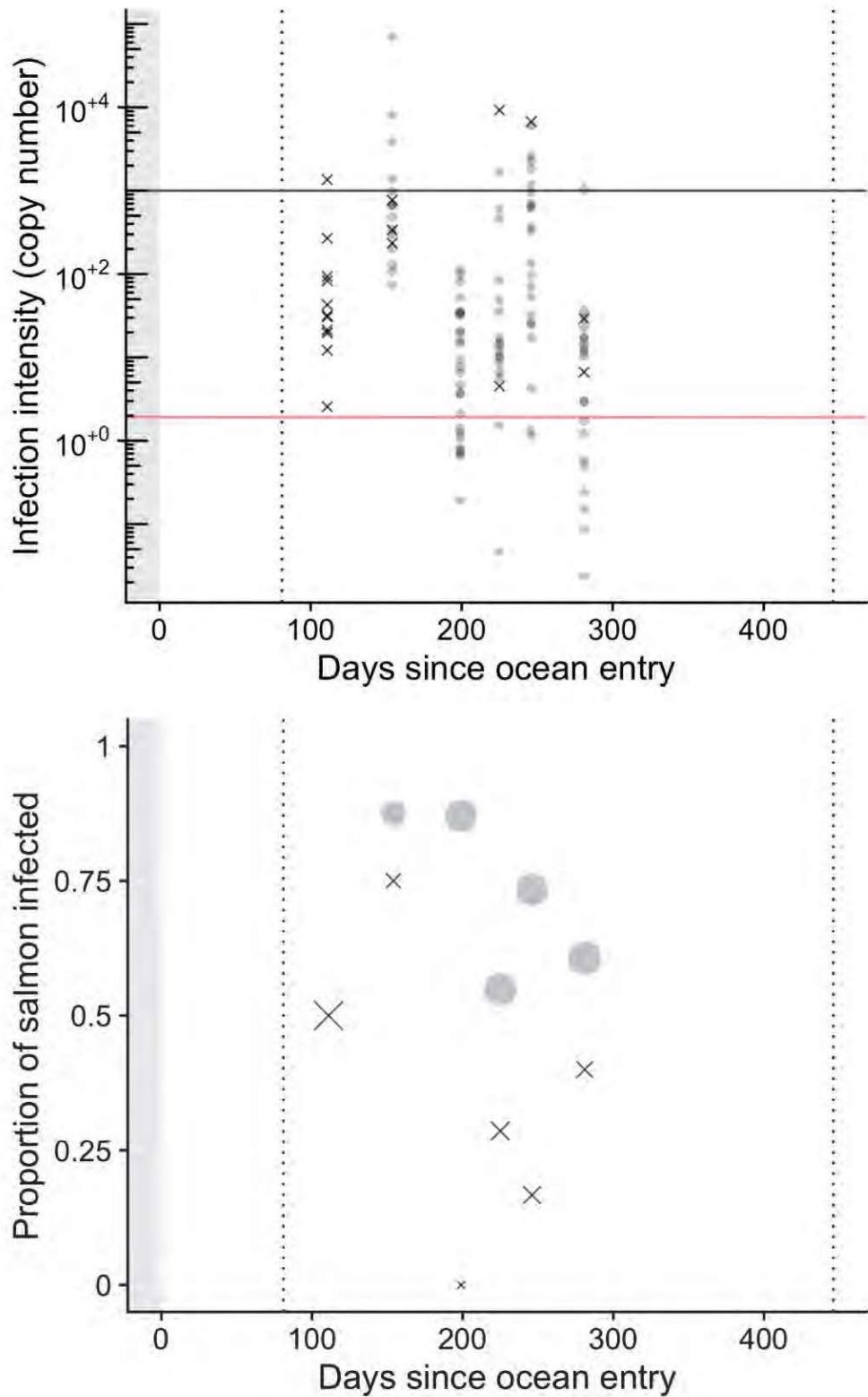
Piscine orthoreovirus



Candidatus Syngnathia salmonis



Tenacibaculum maritimum



Clinical signs

Table 1: Clinical signs for specimens sampled on 2022-03-31

metric	06141	06142	06143	06144	06145	06146	06147	06148	06149	06150	06151	06152	06153	06154	06155	06156	06157	06158	06159	06160
General																				
Live								X	X	X	X	X	X	X	X	X	X	X	X	X
Poor Performer							X													
Moribund	X																			
Mort		X	X	X	X															
Skin & Fins																				
Erosion				X	X															
Ulcers					X															
Parasites																				
Gills																				
Erosions		X				X														X
Nodules/White Spots							X	X	X				X			X		X	X	
Abdominal Cavity																				
Body Fat Content																				X
Adhesions										X							X	X		
Spleen																				
Enlarged		X		X	X				X		X			X		X	X	X	X	
Liver																				
Yellow			X																	
Gallbladder																				
Enlarged			X	X	X	X				X	X	X					X	X		
Green		X	X	X																
Heart																				
Deformed																				
Pale																				
Brain																				
Hemorrhages/Congestion			X		X	X														

Table 2: Clinical signs for specimens sampled on 2022-03-31

metric	06175	06174	06173	06172	06171	06170	06169	06168	06167	06166	06165	06164	06163	06162	06161
General															
Live	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Poor Performer															
Moribund															
Mort															
Skin & Fins															
Erosion															
Ulcers															
Parasites							X	X							
Gills															
Erosions					X			X	X						
Nodules/White Spots		X							X	X		X			X
Abdominal Cavity															
Body Fat Content					X										
Adhesions										X		X			
Spleen															
Enlarged	X					X		X	X	X	X	X			X
Liver															
Yellow															
Gallbladder					X	X	X				X	X			X
Enlarged															
Green															
Heart															
Deformed	X														
Pale															X
Brain															
Hemorrhages/Congestion															

Histology

Table 3: Histology scores for specimens sampled on 2022-03-31

metric	06141	06142	06143	06144	06145	06148	06150	06155	06170	06175
Heart										
Peri Epi	1	1	2		1	1	1	1	1	1
Myo	1		1			2	1	2	1	
Liver										
Cong Haem		1								
Nec				1	2					1
Itis	1					2	1	1	2	
Spleen										
Cong Heam	1			1		2		2	2	
W Pulpitis	2	1	3		1	2	1	2	2	2
Pig Inc										2
Kidney										
Osis			1			na				
Cong Heam		2				1				
Interst Hyperplasia	2	1	2	1	1	3	1	2	2	2
Interst Nec			1							
Glomitis						na				
Pancreatitis										
Pancreatitis	2									
Cns						na	na	na	na	
Itis						na	na	na	na	
Cnc										
Malacia						na	na	na	na	
Gliosis		1				na	na	na	na	
Cong Heam	3	2			2	na	na	na	na	
Microsporidia						na	na	na	na	
Gills										
Itis			nv	nv	nv					
Cong Heam		nv	nv	nv						
Prolif		nv	nv	nv	3	1		1		
Skin_muscle										
Itis Nec							1		1	
Tissue										
Necrosis Artefacts		2	2	2	2					

Diagnoses and Comments

Table 4: Diagnoses and comments for specimens sampled on 2022-03-31

DFO ID	Diagnosis	Comments
O6142		Increased Fibrin In Spleen (3)
O6143		Hemorrhages In Visceral Fat (2), Increased Fibrin In Spleen (1)
O6145		Increased Fibrin In Spleen (2)
O6148		Vac Deg Liver (1), Peribiliary Immune Activation (2)
O6170		Peribiliary Immune Activation (1)
O6175		Erythrophagocytosis (1), Peribiliary Immune Activation (2)

Conclusions

In order to support the eDNA study, below is provided further evaluation of the results of testing from the Fish Health Report.

The sampling collection was complete, with fish collected from the control pen as well as the secondary pen.

The farm was inspected in its entirety: overall the fish were behaving normally. However, high mortality (due to predation – sea lions) was reported. Some of the pens were also going to be treated with FW bath (Tromoy) in the following weeks.

Clinically, gill erosions and white nodules, as well as enlarged spleen and gall bladder were observed in a significant portion of the fish collected. Skin and fin erosions and ulcers were reported in the morts.

Molecular testing results show that the totality of the fish tested (100%) resulted positive to PRV, and at high load in some instances. *Tenacibaculum maritimum* was also present in 63% of the fish (67% of the live fish, but in high load in one individual). *Candidatus Syngnathus salmonis* was observed in 14% of the fish samples (only in some live fish), while *Flavobacterium psychrophilum* was detected at background level (only in some live fish).

Histopathologically, the moribund/morts samples collected showed an overall pattern of mild to moderate immunological/inflammatory response, affecting primarily heart, spleen, kidney and liver. Brain congestion was also common. The alterations reported in the heart are suggestive of an improvement of the condition with respect of the subclinical HSMI status previously reported, although mild to moderate inflammation in the heart was still observed in virtually all the samples analyzed by histology.

Given the overall situation (receding subclinical HSMI + high incidence of *T. maritimum*), the molecular results and clinical/pathological findings, a close monitoring of the operations during the next visit at this site is highly recommended.

Appendix

Glossary of infectious agents

Agent abbr.	Full agent name	Agent type	Disease	Ranking
ae_sal	Aeromonas salmonicida	Bacteria	Furunculosis	2
ascv	Atlantic salmon calicivirus	Virus	unknown	4
ctv-2	Cutthroat trout virus-2	Virus	unknown	4
fl_psy	Flavobacterium psychrophilum	Bacteria	Bacterial cold water disease	3
pisck_sal	Piscirickettsia salmonis	Bacteria	Piscirickettsiosis (SRS)	2
pmcv	Piscine myocarditis virus	Virus	Cardiomyopathy syndrome	1
prv-1	Piscine orthoreovirus	Virus	HSMI-EIBS-Jaundice/anemia	
psnv1	Pacific salmon nidovirus-1 (CoV)	Virus	unknown	4
re_sal	Renibacterium salmoninarum	Bacteria	Bacterial kidney disease	2
sch	Candidatus Syngnathia salmonis	Bacteria	Gill chlamydia	3
te_mar	Tenacibaculum maritimum	Bacteria	Marine flexibacteriosis (mouth/fin rot)	2
vi_ang	Vibrio anguillarum	Bacteria	Vibriosis	2
vi_sal	Vibrio salmonicida	Bacteria	Cold water vibriosis	2
ye_ruc	Yersinia ruckeri (Enteric redmouth disease)	Bacteria	Yersiniosis (Enteric red mouth)	2

Preliminary Report on Water Sampling Research

Cypress Harbour sampling on October 27, 2021

Dr. Emiliano Di Cicco

June 29, 2022

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Executive summary

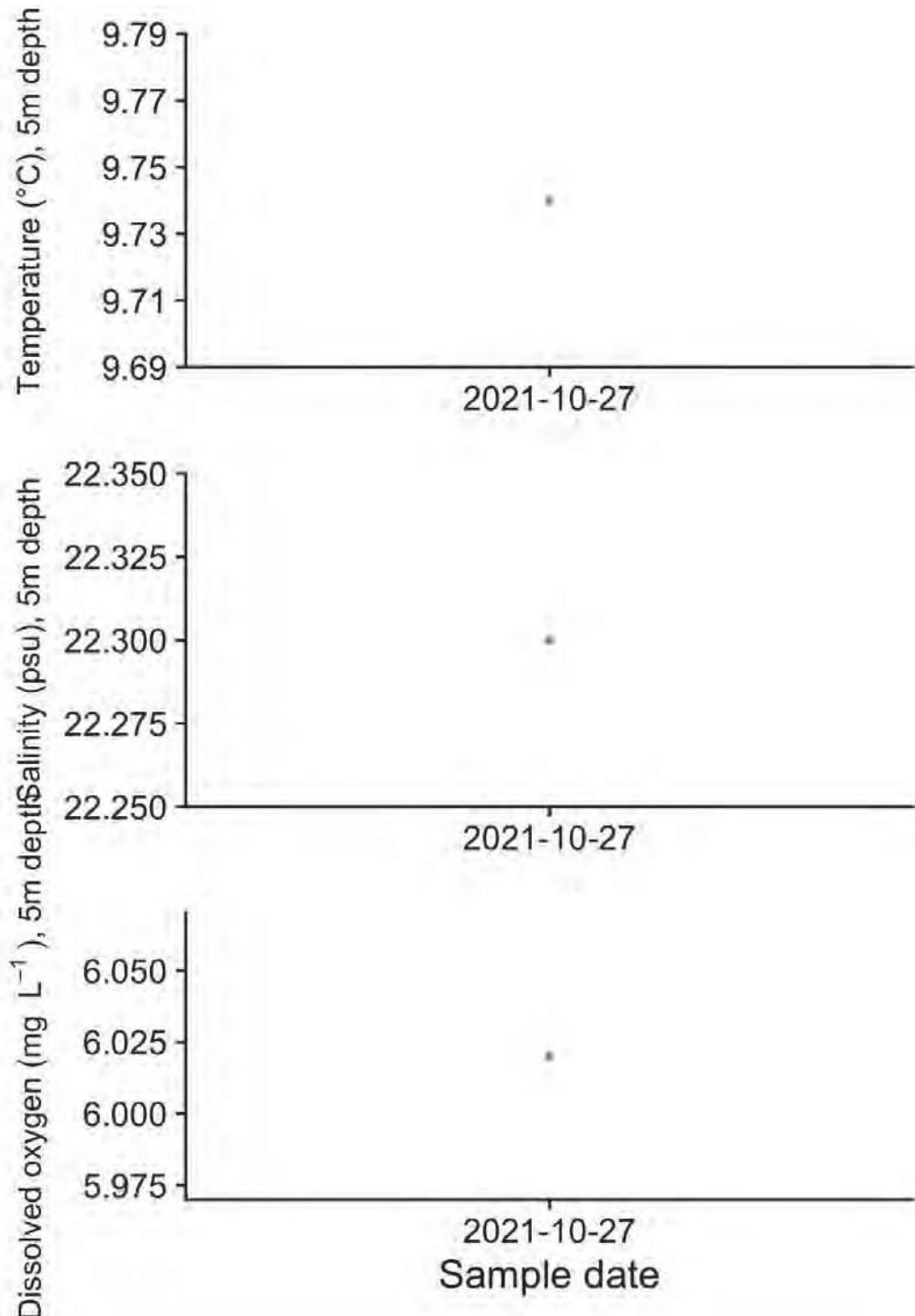
This report updates the Tenure Holder on the data collected and the testing and analysis conducted under the Water Sampling Research Program for the sampling event described below.

Premise

On October 27, 2021, 8 samples were collected by BATI and Cermaq crew during a sampling event at Cypress Harbour (Cermaq Ltd.). 8 Atlantic salmon subadults and matures were collected from the Cypress Harbour farm site, including 0 live and 8 moribund/dead fish. All live fish were euthanized with TMS overdose prior to dissection with the exception of the moribund fish, which were administered a blow to the head. Portions of gill, liver and anterior kidney were collected in triplicate for molecular testing (preserved in RNA later) from all the fish, while all the moribund/dead fish also underwent collection of tissues (gills, spleen, liver, heart, anterior and posterior kidney, pyloric caeca, skeletal muscle + skin, brain) for histological analysis. Clinical notes and gross lesions were noted and reported for every fish. One aliquot has been provided to the Company Cermaq Fish Health, another aliquot is stored at the BATI Field Office, and a third aliquot is stored at DFO - PBS. This latter aliquot has been tested for the presence and load of the agents indicated in the IMIP agreement as well as the agents indicated in the eDNA study agreement. Each sample has been extracted and tested individually. Negative and positive controls were run. A housekeeping gene was also included to assess the quality of the RNA extracted.

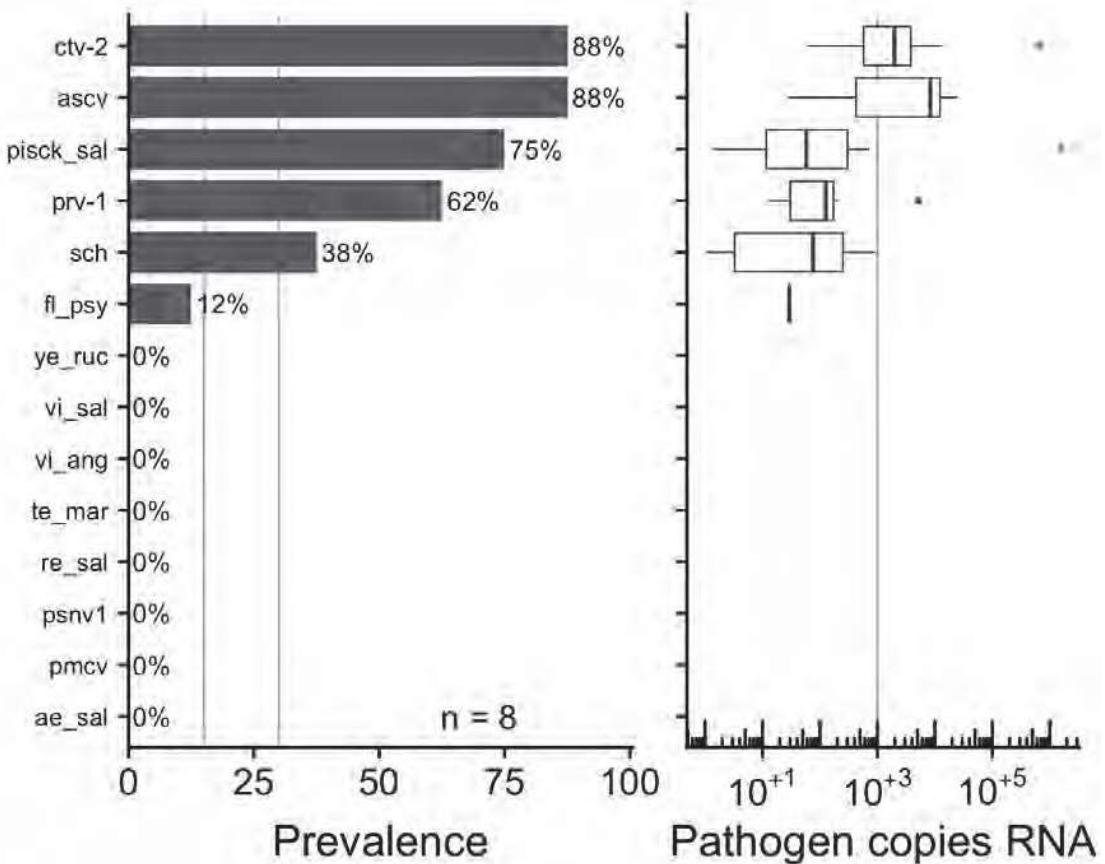
Histology samples have been sent to Wax-It Histo Ltd. to process and prepare slides, which have been read and scored by Dr. Di Cicco. A digital copy of each slide is available to the Company.

Environmental data

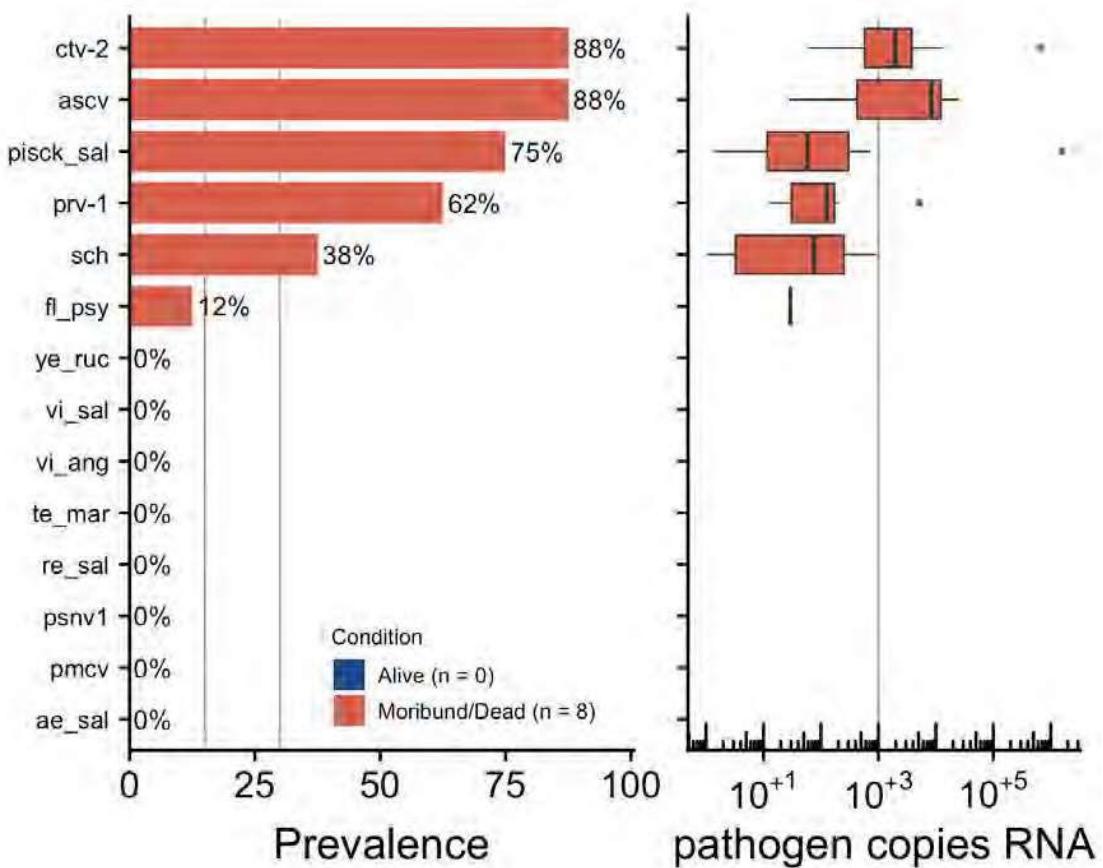


Water temperature ($^{\circ}\text{C}$), salinity (ppm), and dissolved oxygen (mg/L) at a 5m depth. Certain sampling dates have no recorded environmental data, resulting in gaps in the plots.

Overall infectious agent prevalence



Infectious agent prevalence in samples collected on 2021-10-27



Infectious agent prevalence in samples collected on 2021-10-27, split by mortality status at time of sampling. Any specimens that were not confirmed to be either moribund or live at the time of generating this report are excluded from this figure.

Individual infectious agent trends

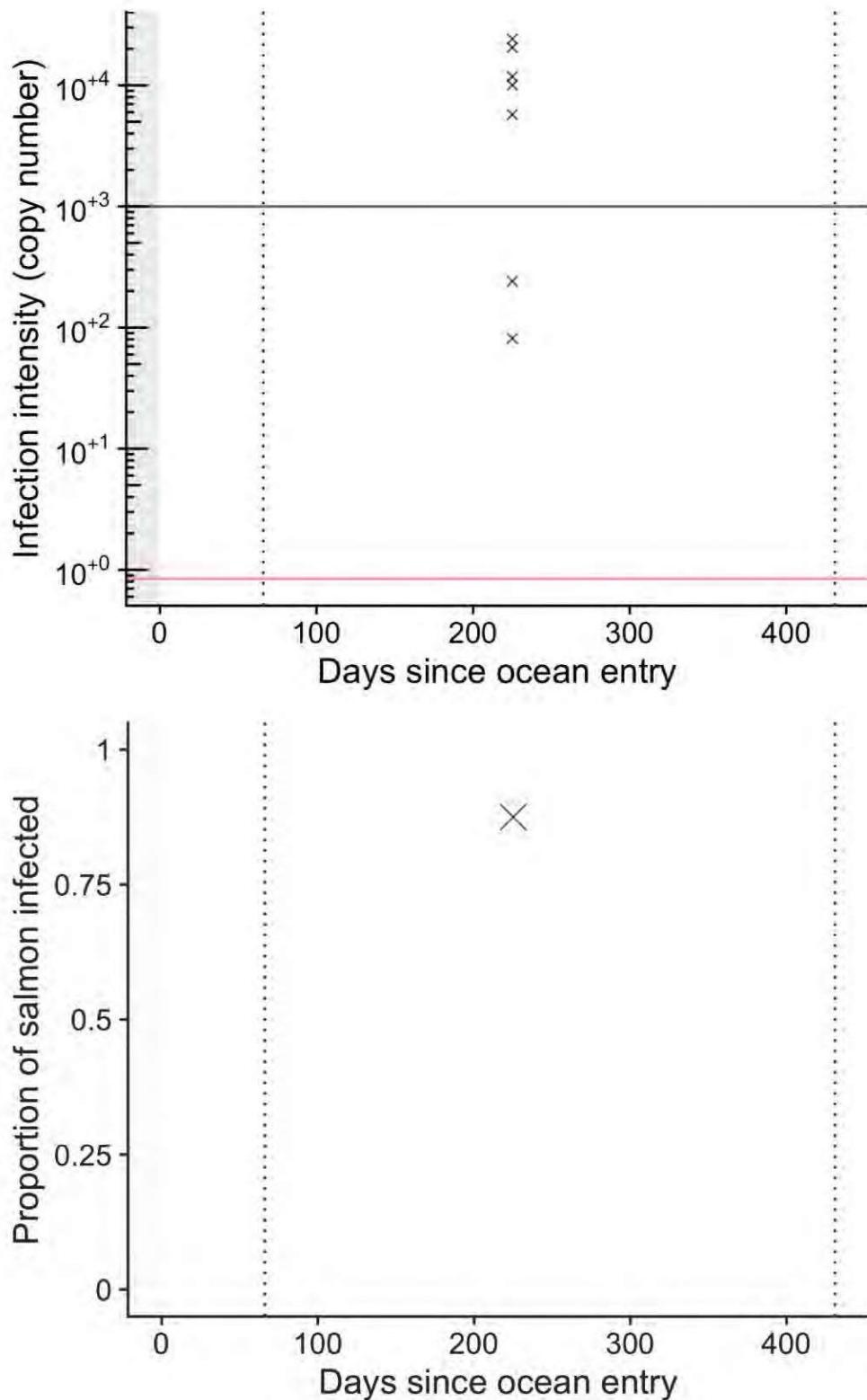
The following plots show individual infectious agent trends across all farm sites. In cases where sample size is sufficient, curves from a generalised additive model are included in the plot.

Grey circles represent live fish, and black X's represent dead/dying fish. Curves indicate mean predictions from a generalised additive model; blue and red correspond to live and dead/dying fish, respectively (shaded areas show 95% confidence regions). Left-hand grey region indicates freshwater hatchery residence, grey regions on x-axis indicate period of transfer to another site, and vertical dotted lines correspond to January 1st.

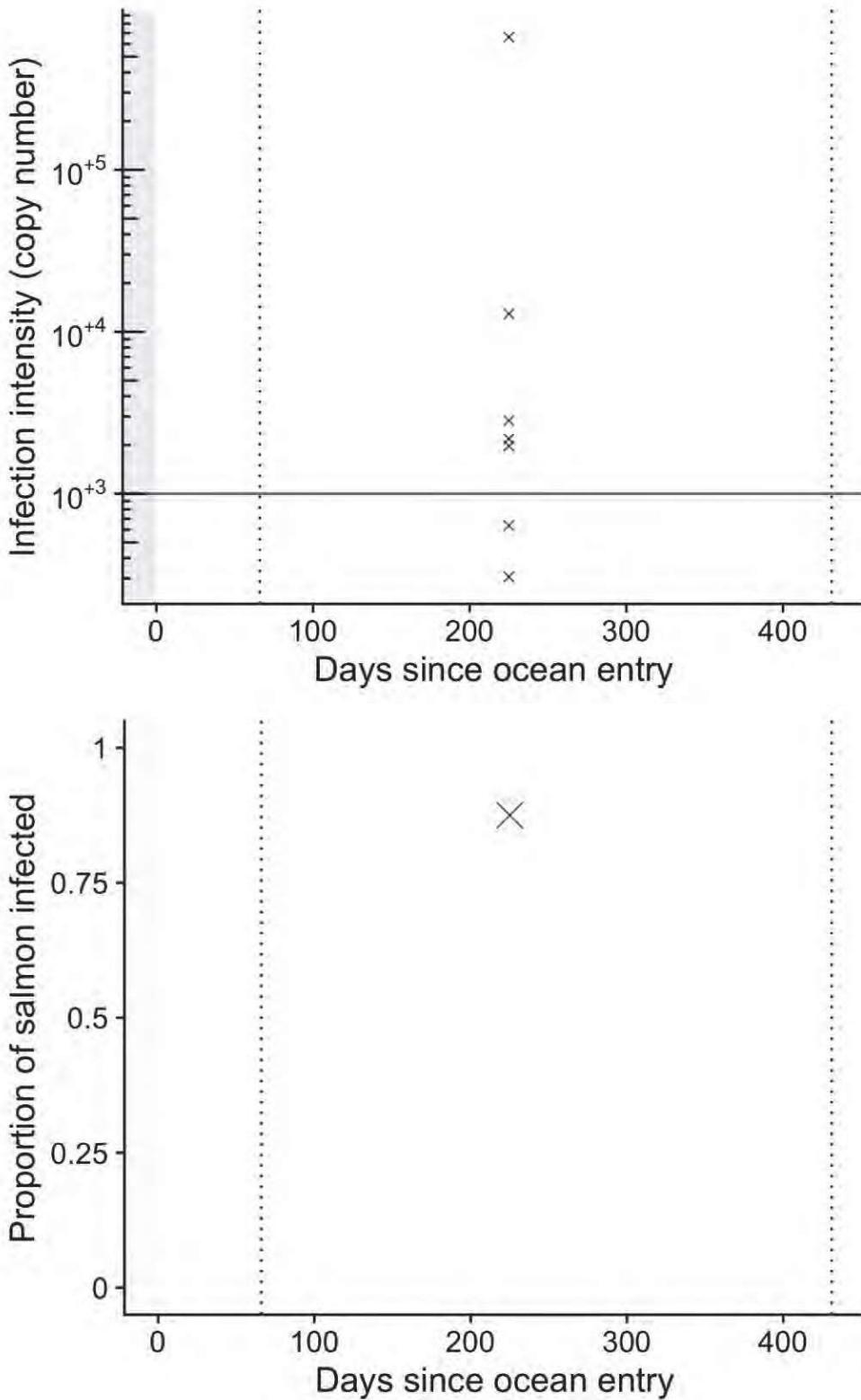
For infection intensity plots, horizontal red line indicates limit of detection (yielding ~90% true positive rate) for respective qPCR assay run in duplicate, while the horizontal black line indicates 1000 copies. Note log scale.

For proportion plots, grey circles show prevalence in live fish on each sampling date, and black X's show prevalence in dead/dying fish (symbol areas proportional to sample sizes).

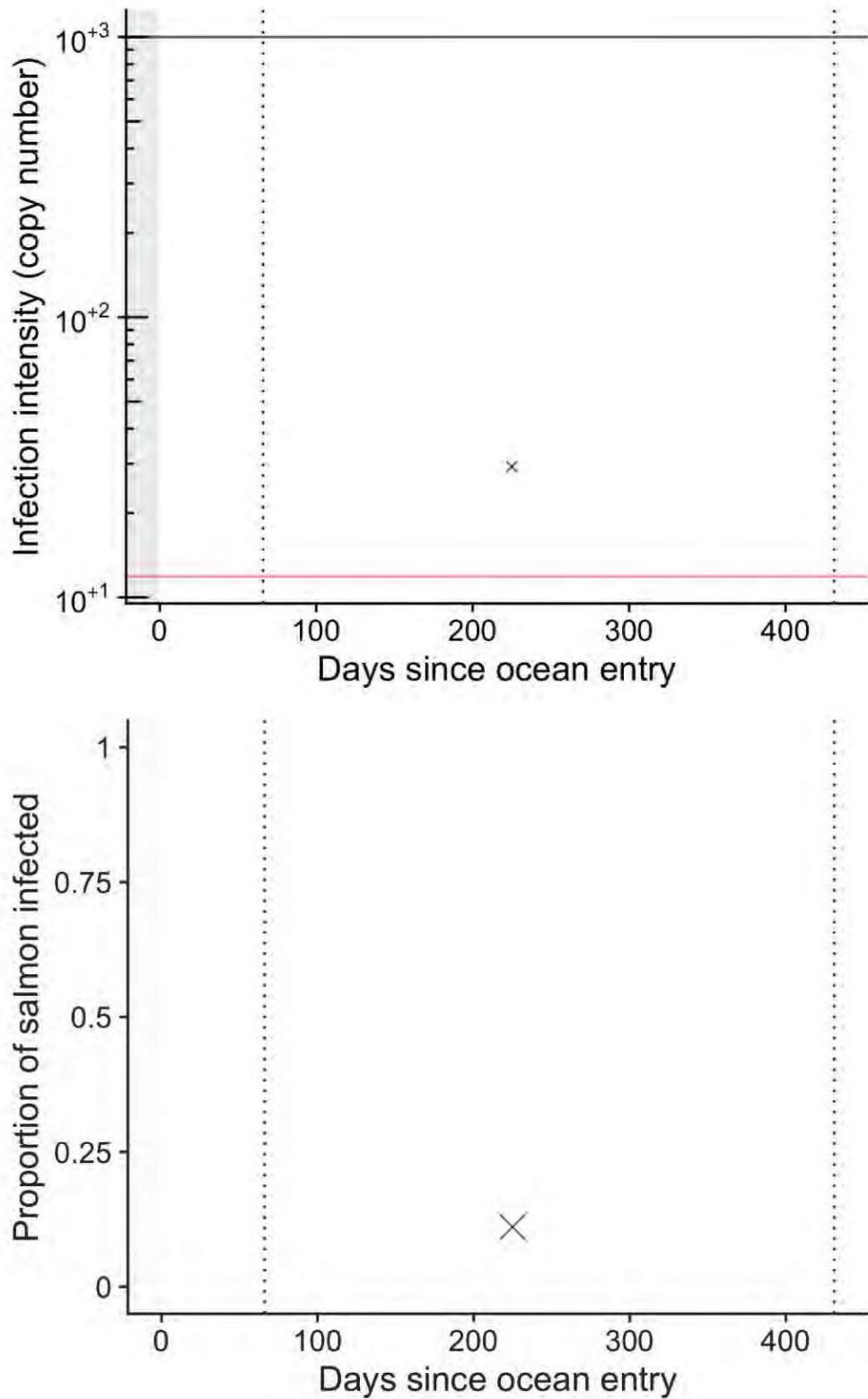
Atlantic salmon calicivirus



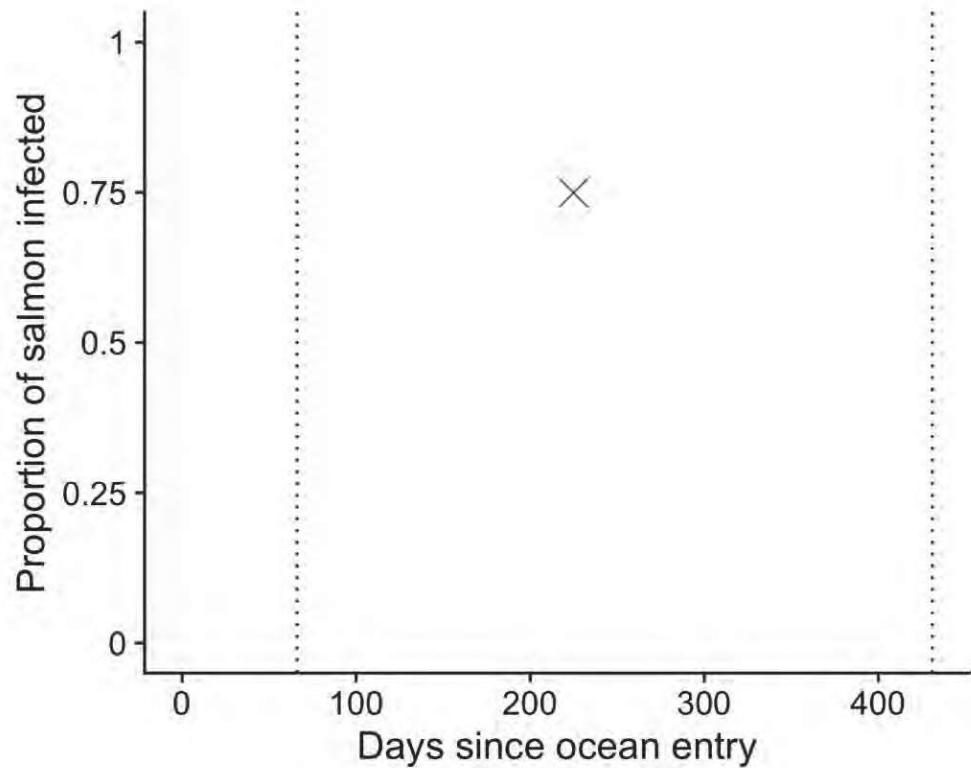
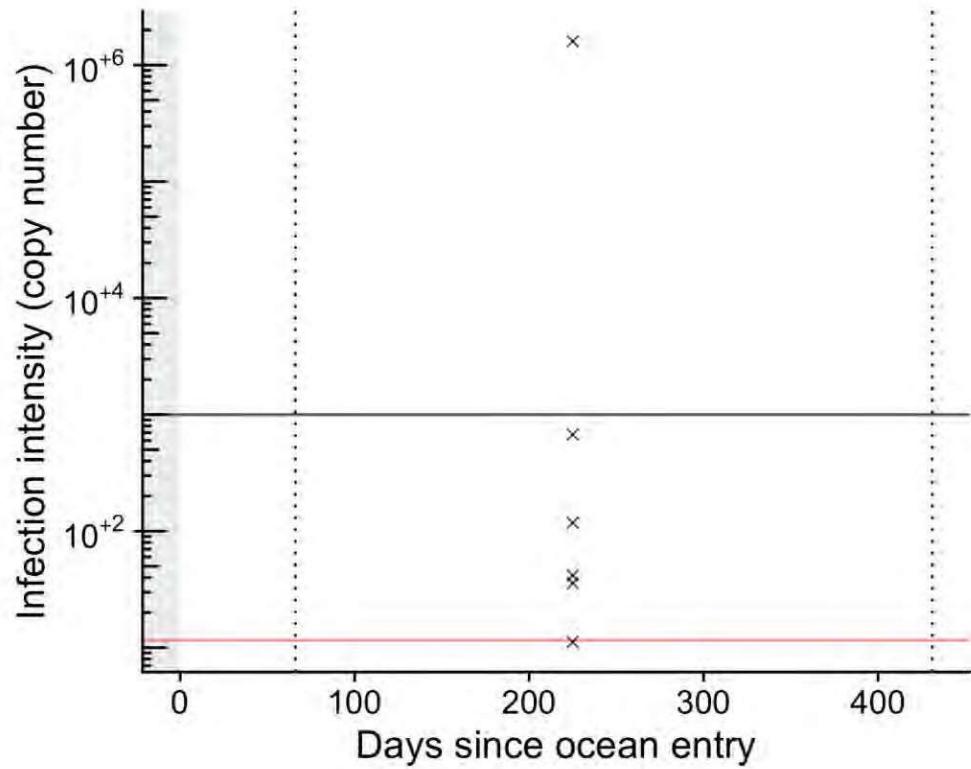
Cutthroat trout virus-2



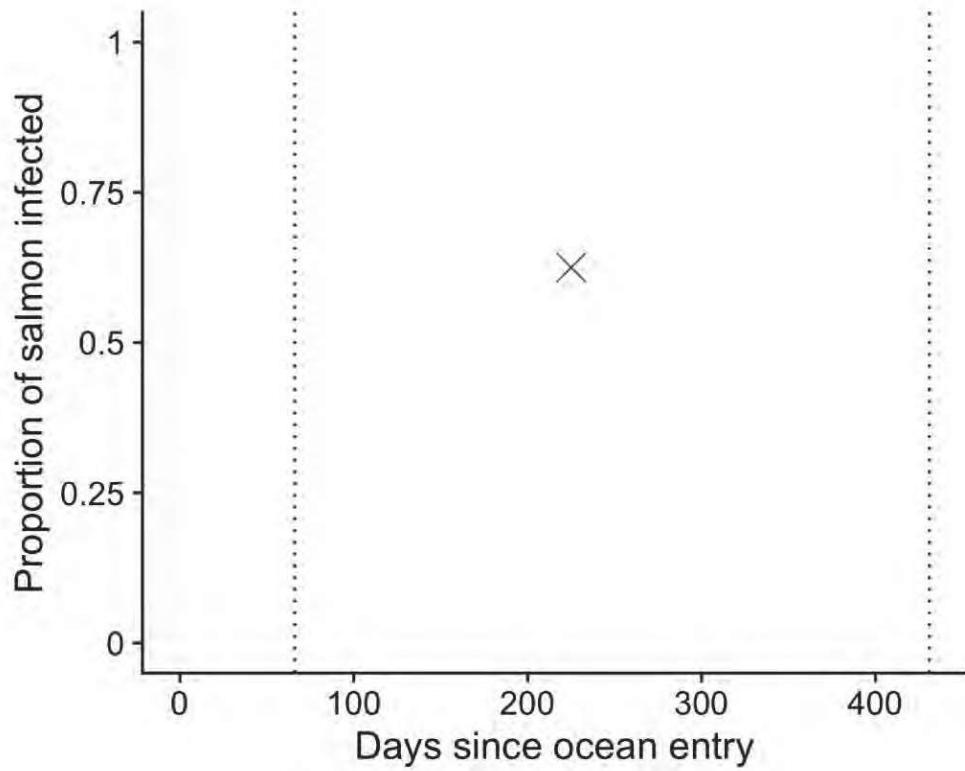
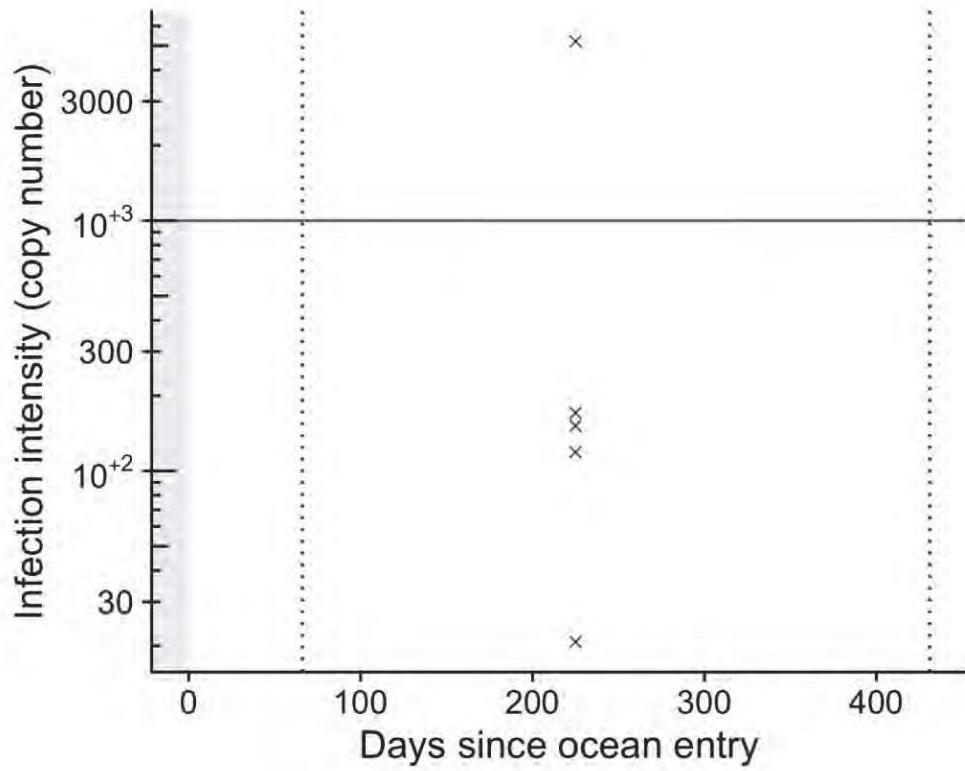
Flavobacterium psychrophilum



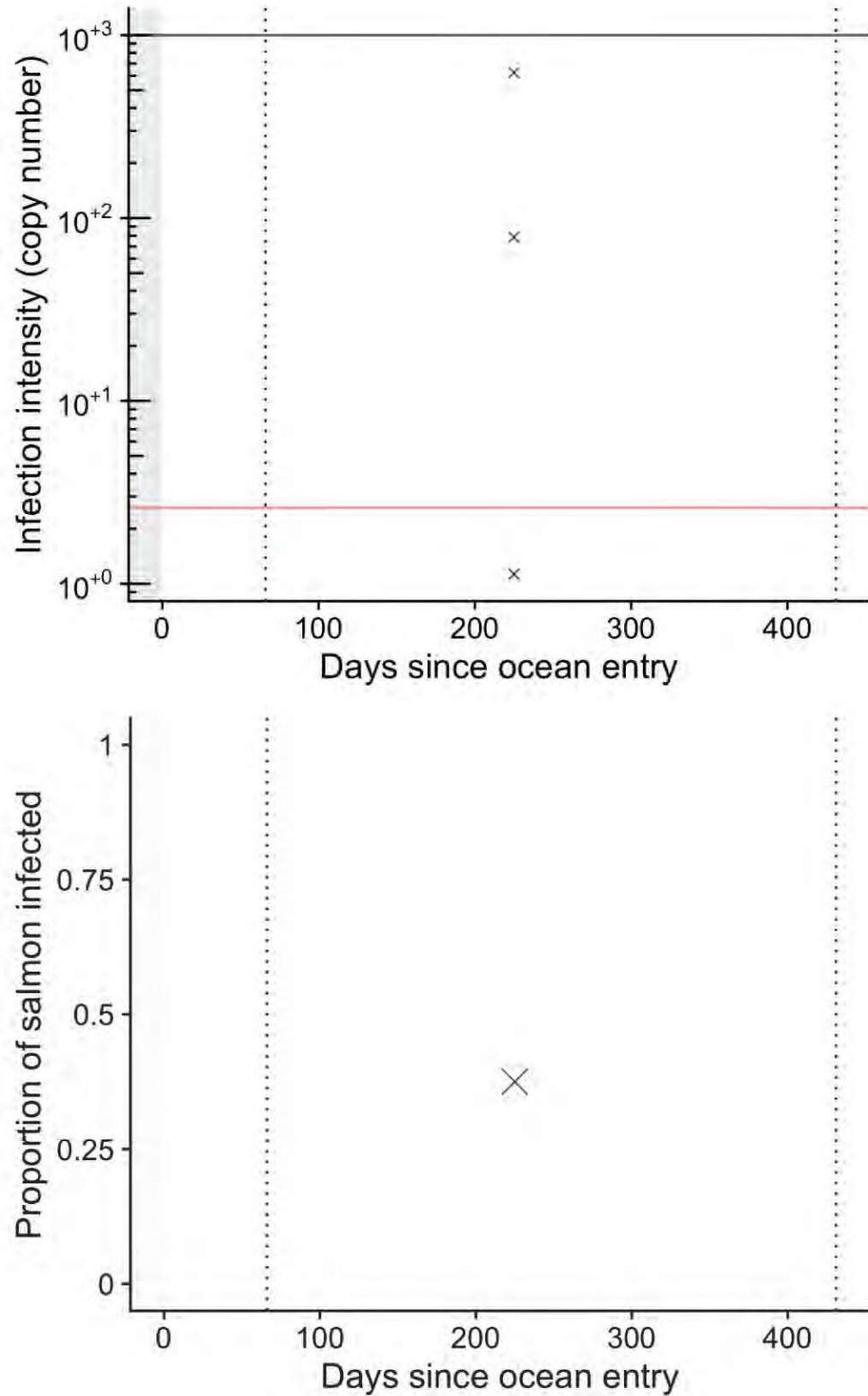
Piscirickettsia salmonis



Piscine orthoreovirus



Candidatus Syngnathia salmonis



Clinical signs

Table 1: Clinical signs for specimens sampled on 2021-10-27

metric	N5201	N5202	N5203	N5204	N5205	N5206	N5209	N5210
General								
Mort	X	X	X	X	X	X	X	X
Skin & Fins								
Parasites							X	
Gills								
Excess Mucous	X							X
Erosions		X						
Nodules/White Spots	X	X						
Abdominal Cavity								
Adhesions					X	X	X	
Ascites						X		
Spleen								
Enlarged	X	X		X	X	X	X	
Liver								
Dark	X			X				
Nodules/White Spots							X	
Heart								
Deformed			X					
Kidney								
Nodules/White Spots							X	
Brain								
Hemorrhages/Congestion	X	X	X	X				X

Histology

Table 2: Histology scores for specimens sampled on 2021-10-27

metric	N5210	N5209	N5206	N5205	N5204	N5203	N5202	N5201
Heart								
Peri Epi		2						
Myo		2		2				1
Liver								
Cong Haem				2	1			
Nec	2	2	1	1	1	1	1	1
Itis		2						
Spleen								
Cong Haem	2	2	2	3	3	3		3
Ellip Nec					1	1		2
W Pulpitis	2	2	1	1			2	2
Kidney								
Itis	1	2						
Osis		2						
Cong Haem	1	2	1	1				
Interst Hyperplasia	2	2	1	1	1	1		1
Interst Nec		1						
Pancreatitis								
Pancreatitis		1						
Cns								
Itis		3		3				
Cnc								
Malacia		2		1				
Cong Haem	1	2	1	2		1		
Microsporidia								
Gills								
Itis	nv	nv	nv		nv		1	3
Cong Haem	nv	nv	nv		nv			
Prolif	nv	nv	nv	1	nv	1	1	3
Skin_muscle								
Itis Nec		1		1				
Tissue								
Necrosis Artefacts	2	2	2	1	2	1		

Diagnoses and Comments

Table 3: Diagnoses and comments for specimens sampled on 2021-10-27

DFO ID	Diagnosis	Comments
N5201		Single Cells Necrosis In Liver (2) + Orange Pigm (1), Peribiliary Immune Activation (1)
N5202	Piscirickettsiosis	Perihepatitis (2) + Orange Pigm (2), Increase Fibrin In Spleen 2)
N5203		Increase Fibrin In Spleen (1)
N5204	Parasitic Encephalomyelitis	Increase Fibrin In Spleen (2). Hemorrhages In Pancreas/Intestine (2), Myocardioneclerosis (2)
N5205		Myonecrosis (2), Vac Deg Liver (2)
N5206		Thrombi In Gills (1)
N5209		Single Cells Necrosis In Liver (1) + Orange Pigm (1); *N5029 On The Slides
N5210		Myonecrosis (2), Orange Pigm (2)

Conclusions

The sampling collection was completed. This is a particular farm, due to the presence of different generations of brood stock reared in the same site. The disposition of the cages in the farm is also atypical, and the fish undergo frequent grading and subdivisions. No live fish were collected, as per agreement with the company, but available moribund/mort fish from all the pens were collected. Here below is a summary and evaluation of the findings from the sampled fish.

The farm was inspected in its entirety. Most fish in the examined pens were behaving normally. The morts are collected once a week by divers, therefore an estimation of the mortality rate is less accurate and indicative of the overall conditions of the fish. However, the mortality per pen reported by the company resulted in line with the normal standard expected for such a site. Clinically, most examined fish showed excess of mucous, gills erosion and/or nodules as well as enlarged spleen and congested brain. Dark liver was observed in two fish, while white nodules in liver and kidney were observed in another individual.

Molecular testing results show that about 75% of the individual tested were positive to *Piscirickettsia salmonis* and 62% to PRV. *Candidatus Syngnathidae salmonis* was identified in 38% of the fish, while background level detection was observed for *Flavobacterium psychrophilum*.

Histopathologically, significant blood engulfment of the spleen, mild liver necrosis and immune activation of spleen and kidney were the predominant alterations. One individual also developed inflammatory lesions in heart and brain suggestive of piscirickettsiosis, while an encephalomyelitis induced by a parasitic infection (likely unidentified microsporidian) was also observed in another fish.

Preliminary Report on Water Sampling Research

Sir Edmund Bay sampling on November 10, 2021

Dr. Emiliano Di Cicco

June 29, 2022

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Executive summary

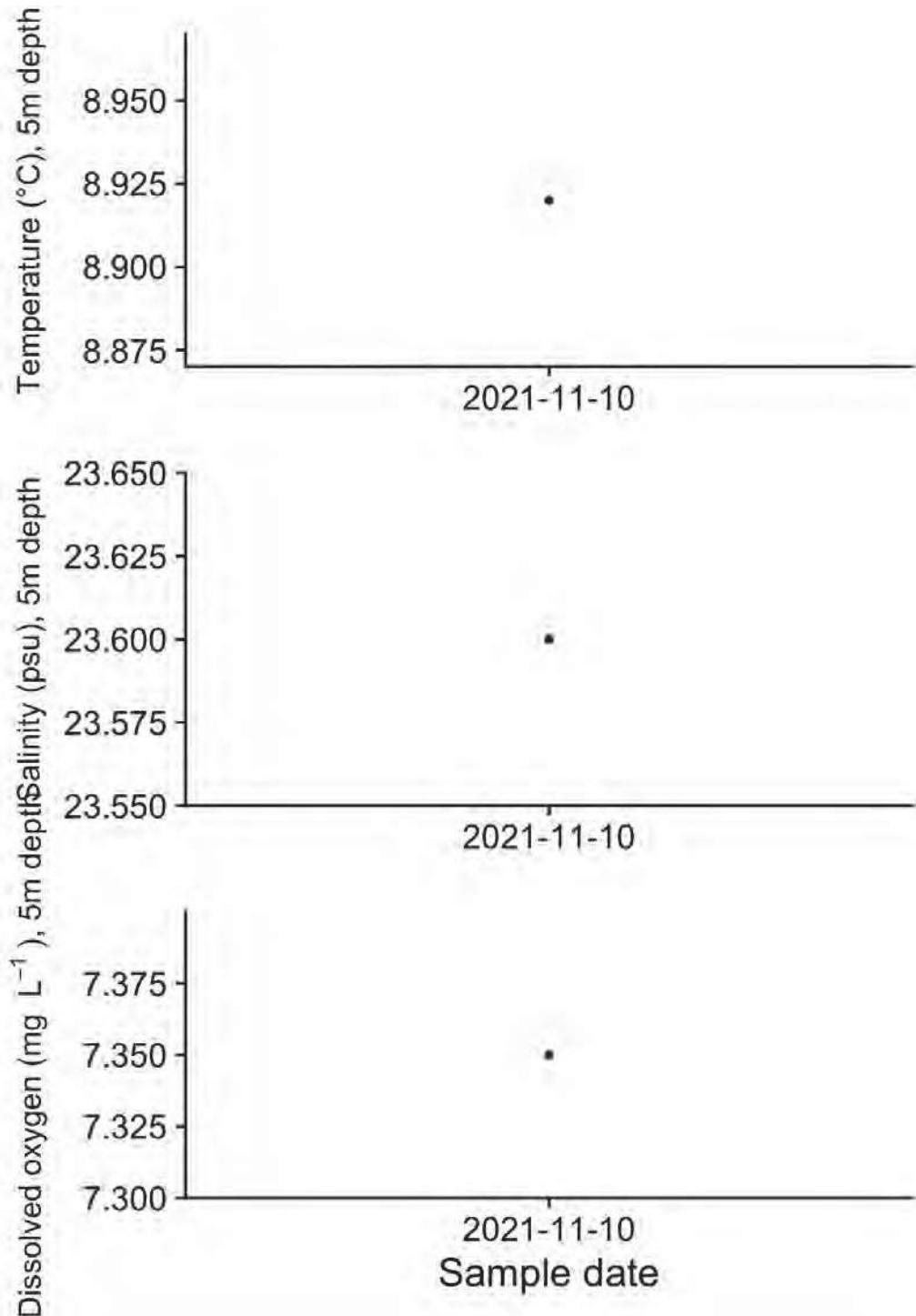
This report updates the Tenure Holder on the data collected and the testing and analysis conducted under the Water Sampling Research Program for the sampling event described below.

Premise

On November 10, 2021, 40 samples were collected by BATI and Cermaq crew during a sampling event at Sir Edmund Bay (Cermaq Ltd.). 40 Atlantic salmon subadults were collected from the Sir Edmund Bay farm site, including 22 live and 6 moribund/dead fish. At the time of generating this report, 12 samples have not yet been confirmed as live or moribund/dead due to data loss. All live fish were euthanized with TMS overdose prior to dissection with the exception of the moribund fish, which were administered a blow to the head. Portions of gill, liver and anterior kidney were collected in triplicate for molecular testing (preserved in RNA later) from all the fish, while all the moribund/dead fish also underwent collection of tissues (gills, spleen, liver, heart, anterior and posterior kidney, pyloric caeca, skeletal muscle + skin, brain) for histological analysis. Clinical notes and gross lesions were noted and reported for every fish. One aliquot has been provided to the Company Cermaq Fish Health, another aliquot is stored at the BATI Field Office, and a third aliquot is stored at DFO - PBS. This latter aliquot has been tested for the presence and load of the agents indicated in the IMIP agreement as well as the agents indicated in the eDNA study agreement. Each sample has been extracted and tested individually. Negative and positive controls were run. A housekeeping gene was also included to assess the quality of the RNA extracted.

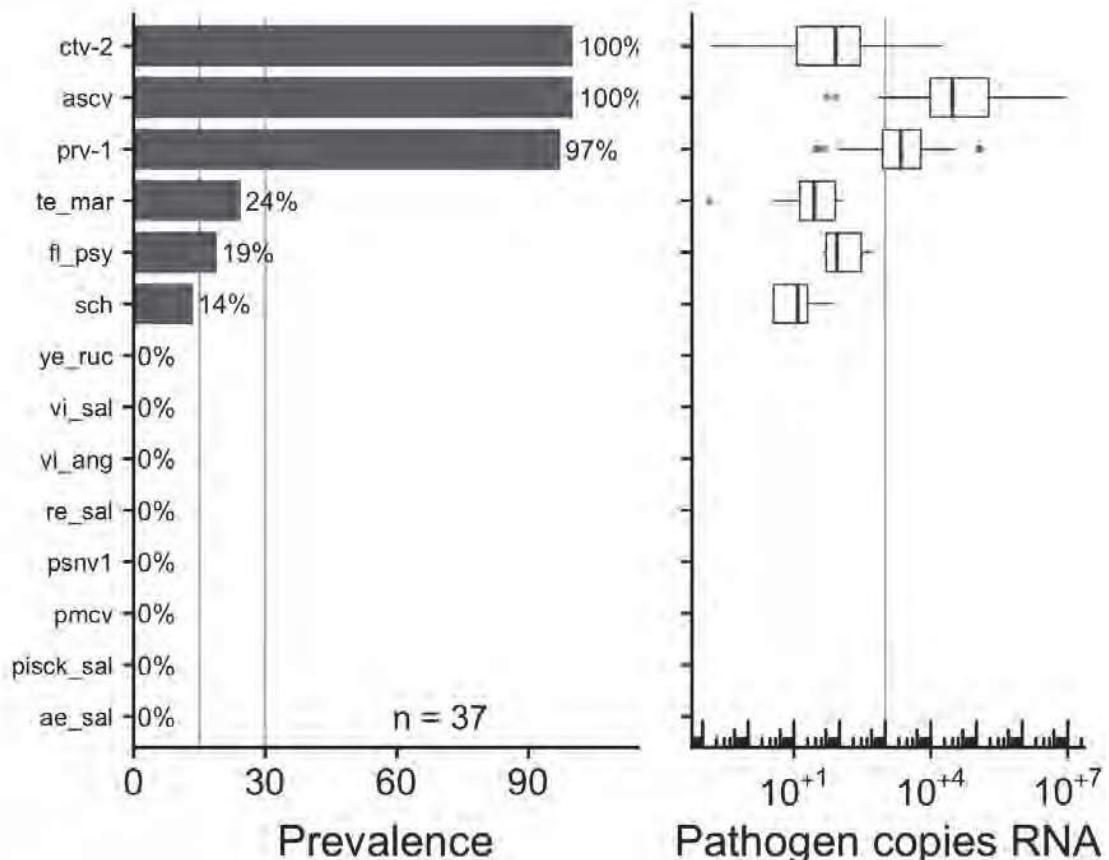
Histology samples have been sent to Wax-It Histo Ltd. to process and prepare slides, which have been read and scored by Dr. Di Cicco. A digital copy of each slide is available to the Company.

Environmental data

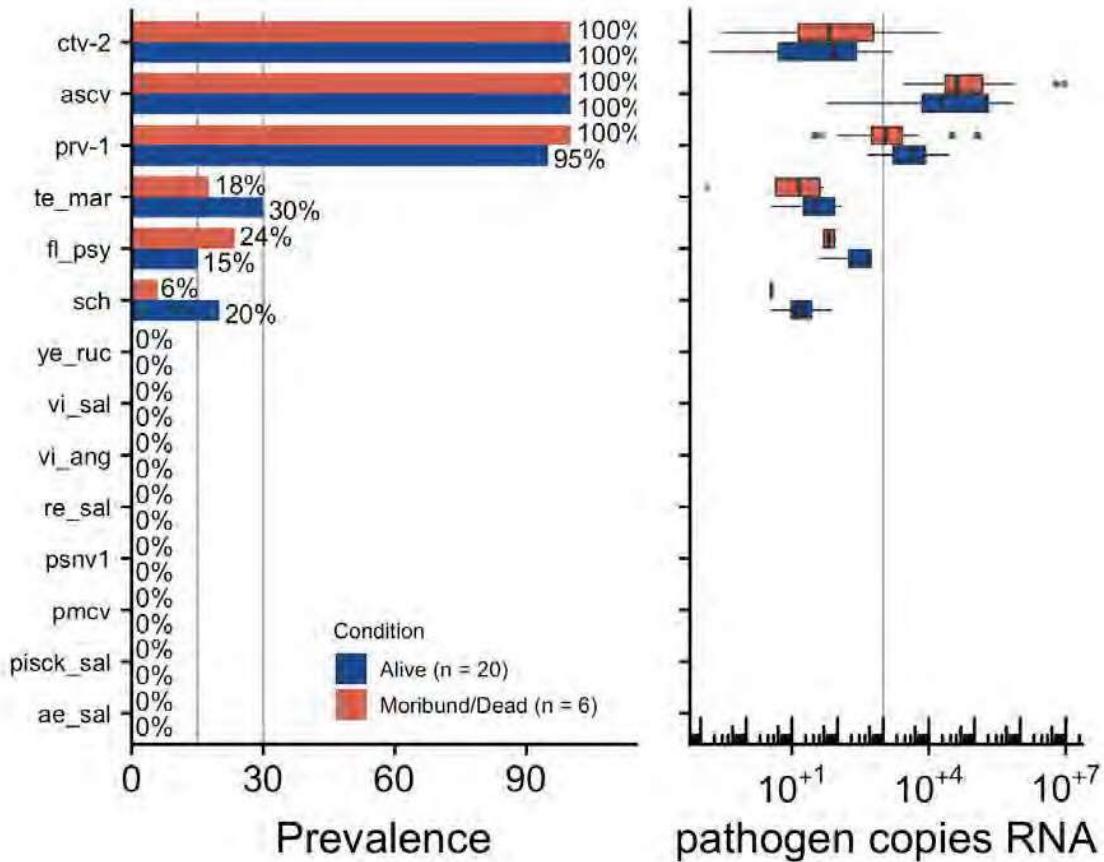


Water temperature ($^{\circ}\text{C}$), salinity (ppm), and dissolved oxygen (mg/L) at a 5m depth. Certain sampling dates have no recorded environmental data, resulting in gaps in the plots.

Overall infectious agent prevalence



Infectious agent prevalence in samples collected on 2021-11-10.



Infectious agent prevalence in samples collected on 2021-11-10, split by mortality status at time of sampling. Any specimens that were not confirmed to be either moribund or live at the time of generating this report are excluded from this figure.

Individual infectious agent trends

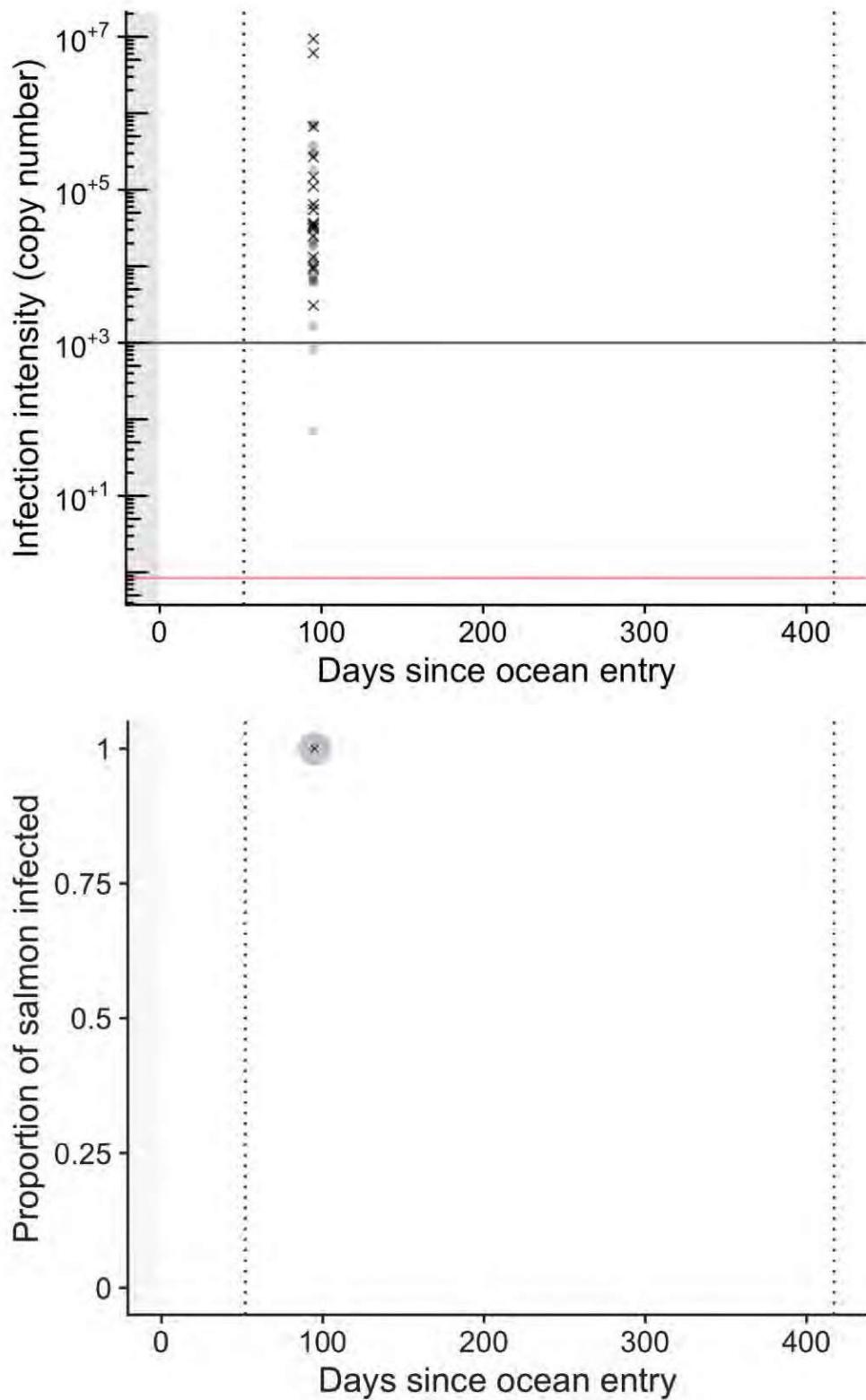
The following plots show individual infectious agent trends across all farm sites. In cases where sample size is sufficient, curves from a generalised additive model are included in the plot.

Grey circles represent live fish, and black X's represent dead/dying fish. Curves indicate mean predictions from a generalised additive model; blue and red correspond to live and dead/dying fish, respectively (shaded areas show 95% confidence regions). Left-hand grey region indicates freshwater hatchery residence, grey regions on x-axis indicate period of transfer to another site, and vertical dotted lines correspond to January 1st.

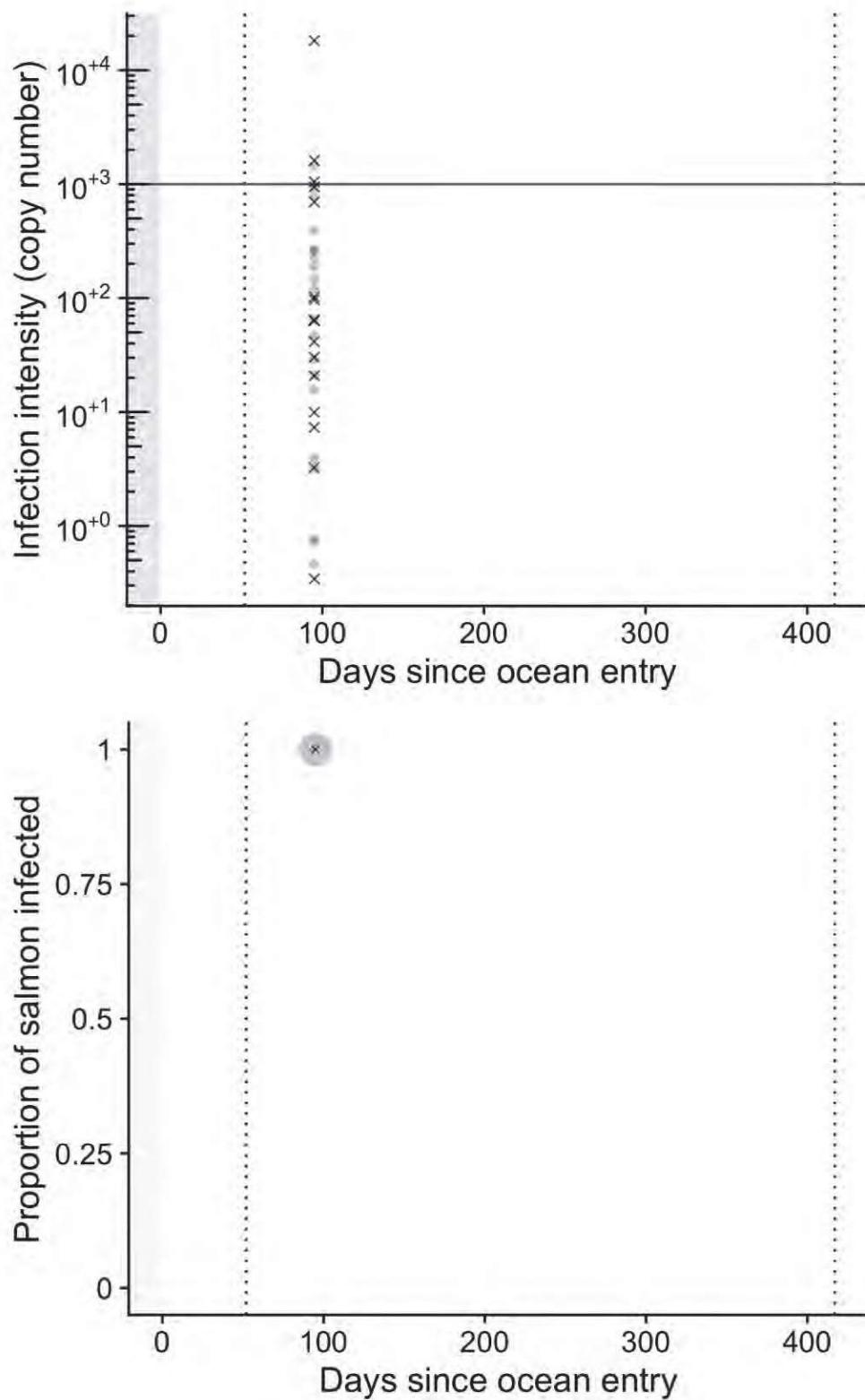
For infection intensity plots, horizontal red line indicates limit of detection (yielding ~90% true positive rate) for respective qPCR assay run in duplicate, while the horizontal black line indicates 1000 copies. Note log scale.

For proportion plots, grey circles show prevalence in live fish on each sampling date, and black X's show prevalence in dead/dying fish (symbol areas proportional to sample sizes).

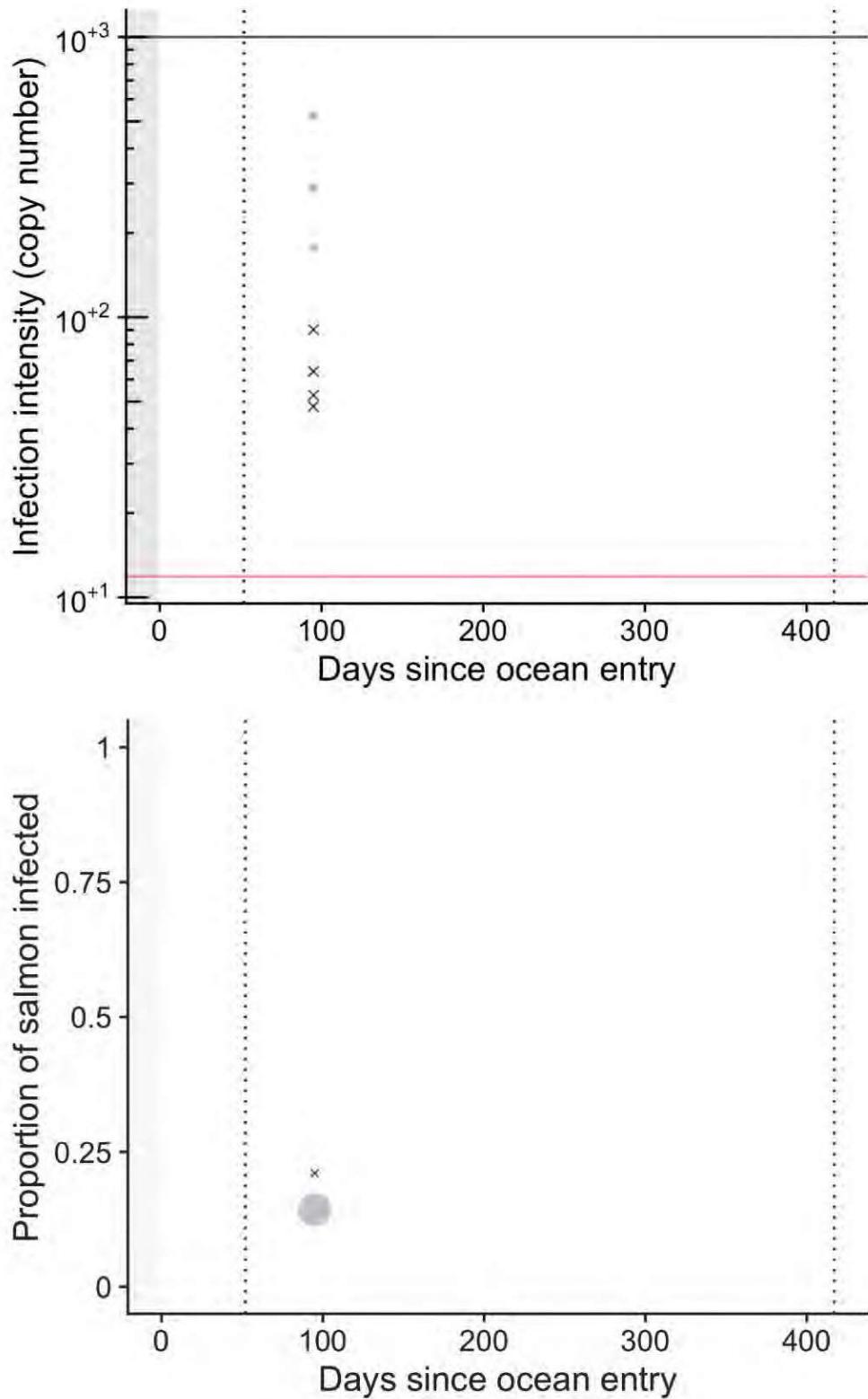
Atlantic salmon calicivirus



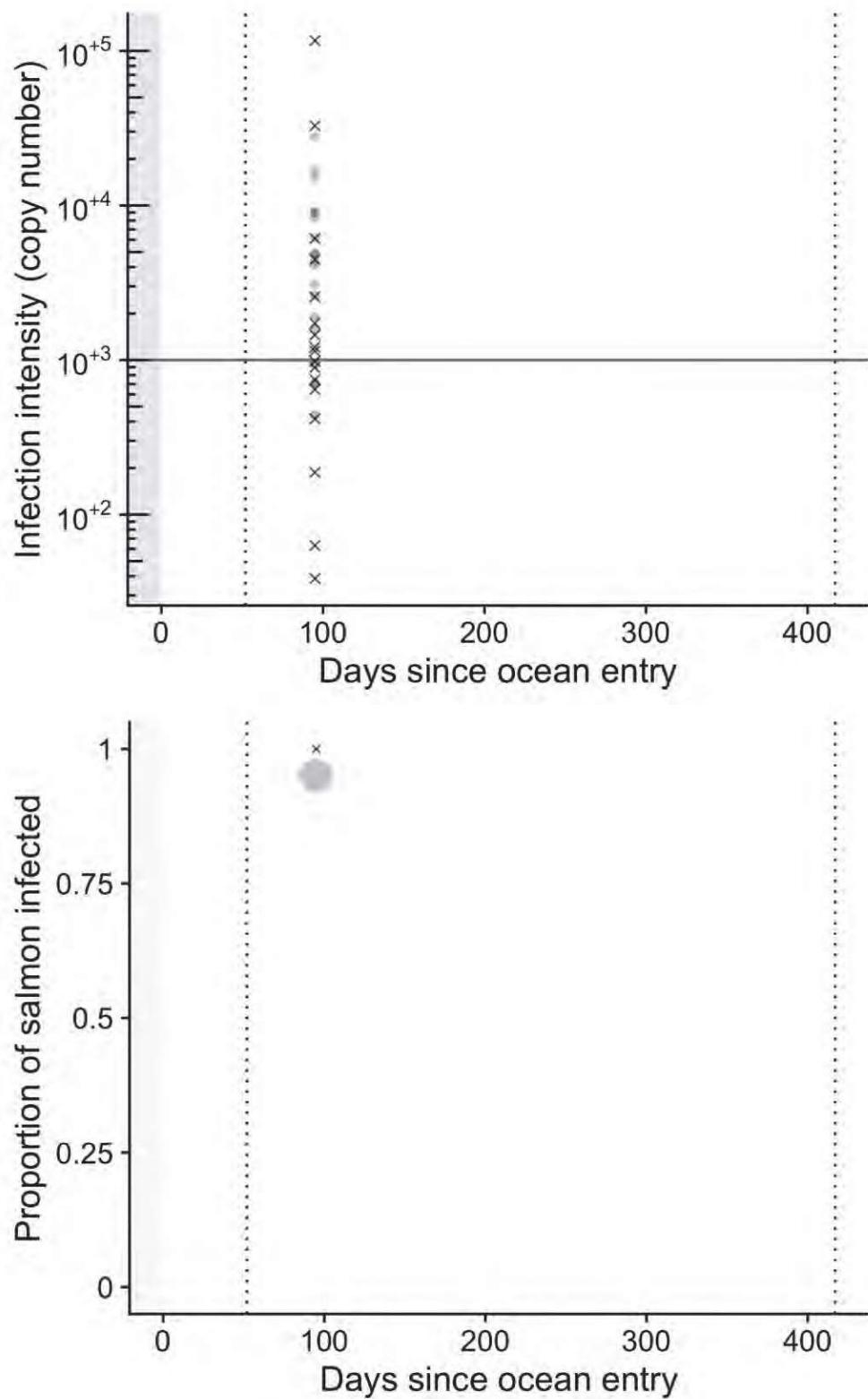
Cutthroat trout virus-2



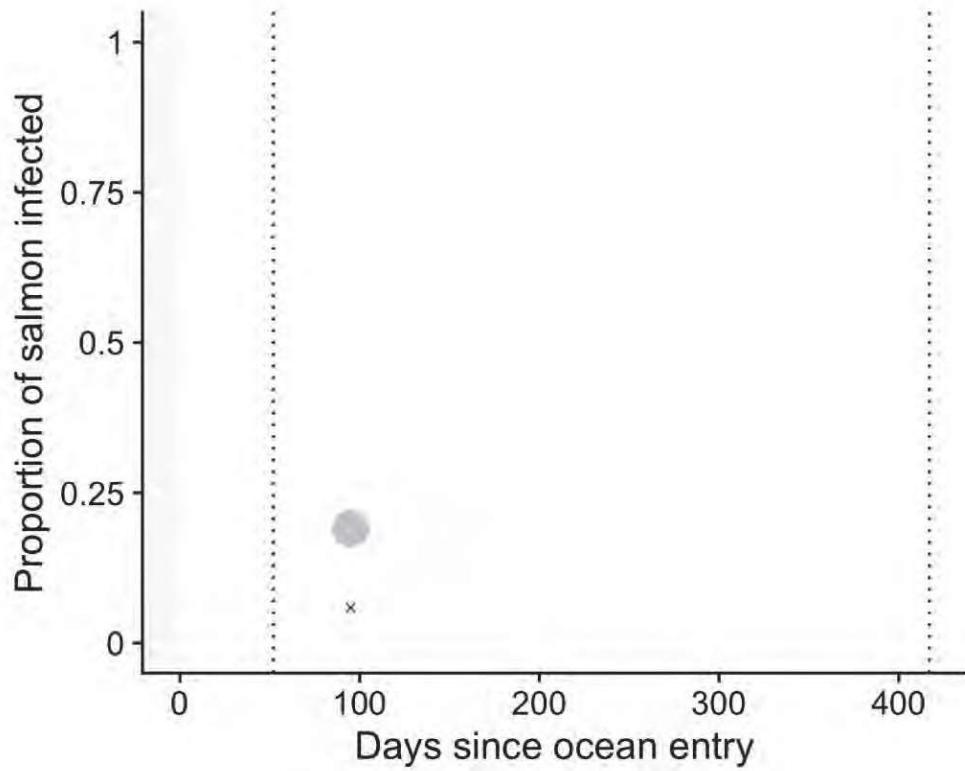
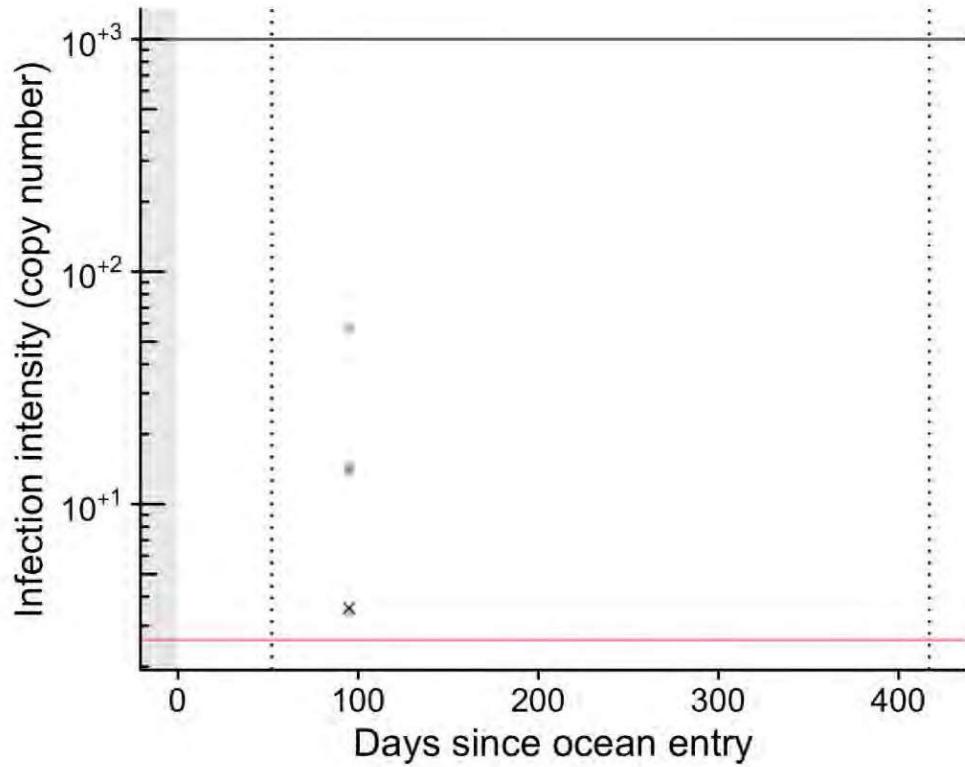
Flavobacterium psychrophilum



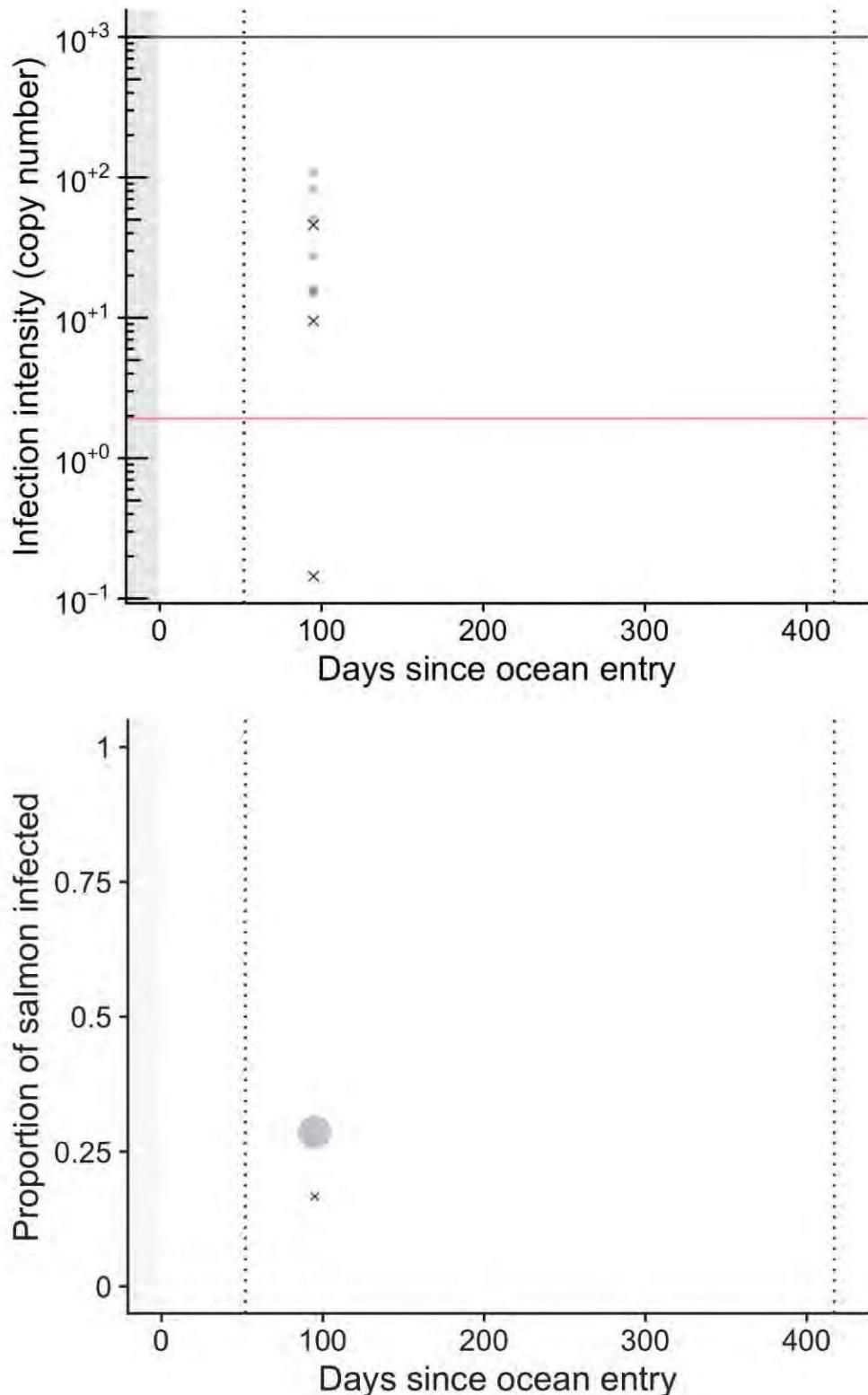
Piscine orthoreovirus



Candidatus Syngnathia salmonis



Tenacibaculum maritimum



Clinical signs

Table 1: Clinical signs for specimens sampled on 2021-11-10

metric	N5271	N5270	N5269	N5268	N5267	N5266	N5265	N5264	N5263	N5252	N5251	N5250	N5249	N5248	N5247	N5246	N5245	N5244	N5243	N5242	N5241
General																					
Live							X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Poor Performer			X	X																	
Moribund	X	X																			
Mort			X	X	X	X															
Gills																					
Short Operculum																					X
Excess Mucous			X																		
Erosions																					X
Nodules/White Spots																					X
Abdominal Cavity																					
Adhesions							X			X			X								X
Spleen								X		X		X									
Enlarged								X													
Liver									X												
Pale			X					X	X	X											X
Yellow				X																	
Gallbladder										X											X
Enlarged								X			X										
Green								X			X										
Heart																					
Enlarged									X												
Pale									X												
Intestine																					
Hemorrhages/Congestion									X												
Brain																					
Hemorrhages/Congestion	X			X	X			X													

Table 2: Clinical signs for specimens sampled on 2021-11-10

metric	N5280	N5279	N5278	N5277	N5276	N5275	N5274	N5273	N5272
General									
Live	X	X	X	X	X	X	X	X	X
Poor Performer									
Moribund									
Mort									
Gills									
Short Operculum						X	X		
Excess Mucous									
Erosions									
Nodules/White Spots							X		
Abdominal Cavity									
Adhesions			X						
Spleen									
Enlarged			X						
Liver									
Pale									
Yellow									
Gallbladder									
Enlarged				X					
Green									
Heart									
Enlarged									
Pale									
Intestine									
Hemorrhages/Congestion									
Brain									
Hemorrhages/Congestion									

Histology

Table 3: Histology scores for specimens sampled on 2021-11-10

metric	N5248	N5247	N5246	N5245	N5244	N5243	N5242	N5241
Heart								
Peri Epi	1	1	2					
Myo	1		1			1		
Liver								
Cong Haem	1							
Nec	1							
Itis	1							
Spleen								
Cong Heam	1	1						1
Ellip Nec		1		2			1	
W Pulpitis	1	1	2	1	1	1	1	1
Kidney								
Osis				1				
Cong Heam					2			
Interst Hyperplasia	1	1	1	1	1	2		1
Enteritis								
Enteritis	na				nv			nv
Cnc								
Gliosis	1		2			1		1
Cong Heam	2			2		1		1
Gills								
Itis	nv			nv	nv	nv	nv	nv
Cong Heam	nv			nv	nv	nv	nv	nv
Prolif	nv			nv	nv	nv	nv	nv
Tissue								
Necrosis Artefacts	2			3	3	3	2	3

Diagnoses and Comments

No diagnoses to report.

Conclusions

The Fish Health sampling collection was completed. Available moribund/mort fish from the control pen and secondary pen were collected. However, a technical issue caused the data loss in a portion of the fish sampled.

The farm was inspected in its entirety: the fish appeared in good conditions, with normal behavior. Reporting from the company indicated mortality that was within the normal range expected for this site. Clinically, just a few instances of short operculum (with rare gill alterations) and enlarged spleen were reported in live fish. Morts and moribund fish showed a wider array of lesions, including enlarged spleen, pale liver, and enlarged gall bladder. Brain congestion and hemorrhages were also reasonably common in morts.

Molecular testing results indicate PRV present in 95% of the fish tested, even at high load in few fish. *Tenacibaculum maritimum* was also observed (24% of fish tested; 30% of live fish and 18% of morts), along with *Flavobacterium psychrophilum* (19%) and *Candidatus Syngnamydia salmonis* (145), at lower degree.

Histopathologically, there was no specific pattern of lesions that would indicate a specific diagnostic differential in the mort fish analyzed. The lesions were in general mild or moderate, including inflammatory or congestive modifications, particularly occurring in spleen and kidney. A mild reactive epicarditis was relatively common, as well as gliosis and brain congestion and hemorrhages.

eDNA Study Report

Sir Edmund Bay sampling on November 30, 2021

Dr. Emiliano Di Cicco

September 22, 2022

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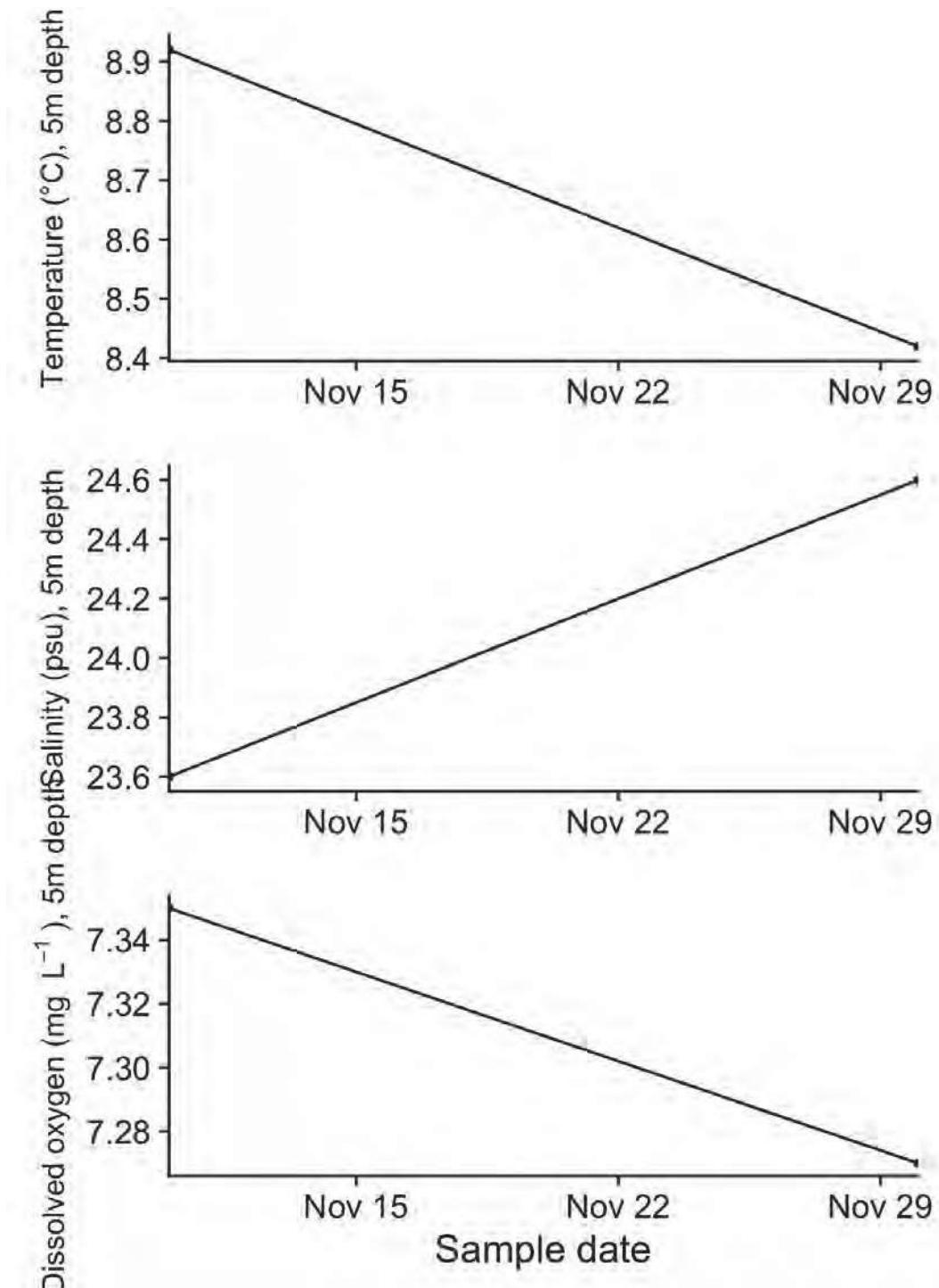
Executive summary

Premise

On November 30, 2021, 34 samples were collected by BATI and Cermaq crews during a sampling event at Sir Edmund Bay (Cermaq Ltd.). 34 Atlantic salmon subadults were collected from the Sir Edmund Bay farm site, including 30 live and 4 moribund/dead fish. All live fish were euthanized with TMS overdose prior to dissection with the exception of the moribund fish, which were administered a blow to the head. Portions of gill, liver and anterior kidney were collected in triplicate for molecular testing (preserved in RNA later) from all the fish, while all the moribund/dead fish also underwent collection of tissues (gills, spleen, liver, heart, anterior and posterior kidney, pyloric caeca, skeletal muscle + skin, brain) for histological analysis. Clinical notes and gross lesions were noted and reported for every fish. One aliquot has been provided to the Company Cermaq Fish Health, another aliquot is stored at the BATI Field Office, and a third aliquot is stored at DFO - PBS. This latter aliquot has been tested for the presence and load of the agents indicated in the IMIP agreement. Each sample has been extracted individually and tested for the presence of 15 pathogenic agents. Negative and positive controls were run. A housekeeping gene was also included to assess the quality of the RNA extracted.

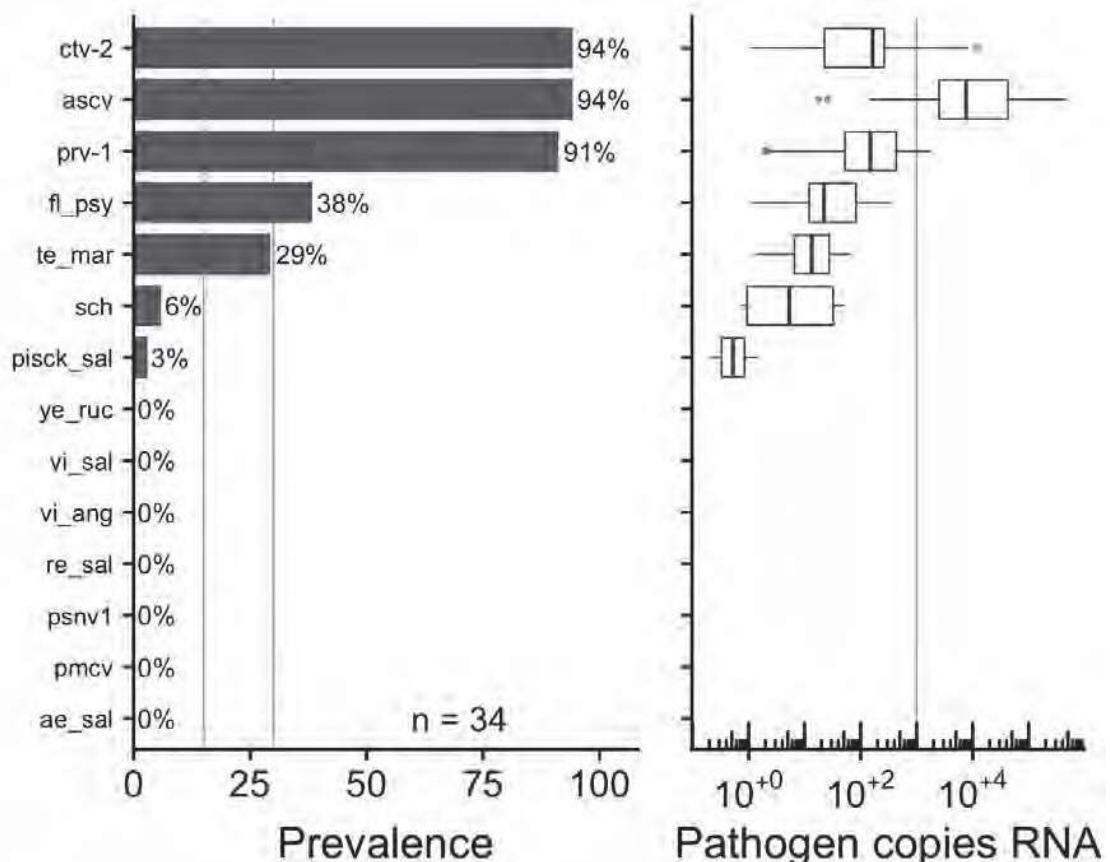
Histology samples have been sent to Wax-It Histo Ltd. to process and prepare slides, which have been read and scored by Dr. Di Cicco. A digital copy of each slide is available to the Company.

Environmental data



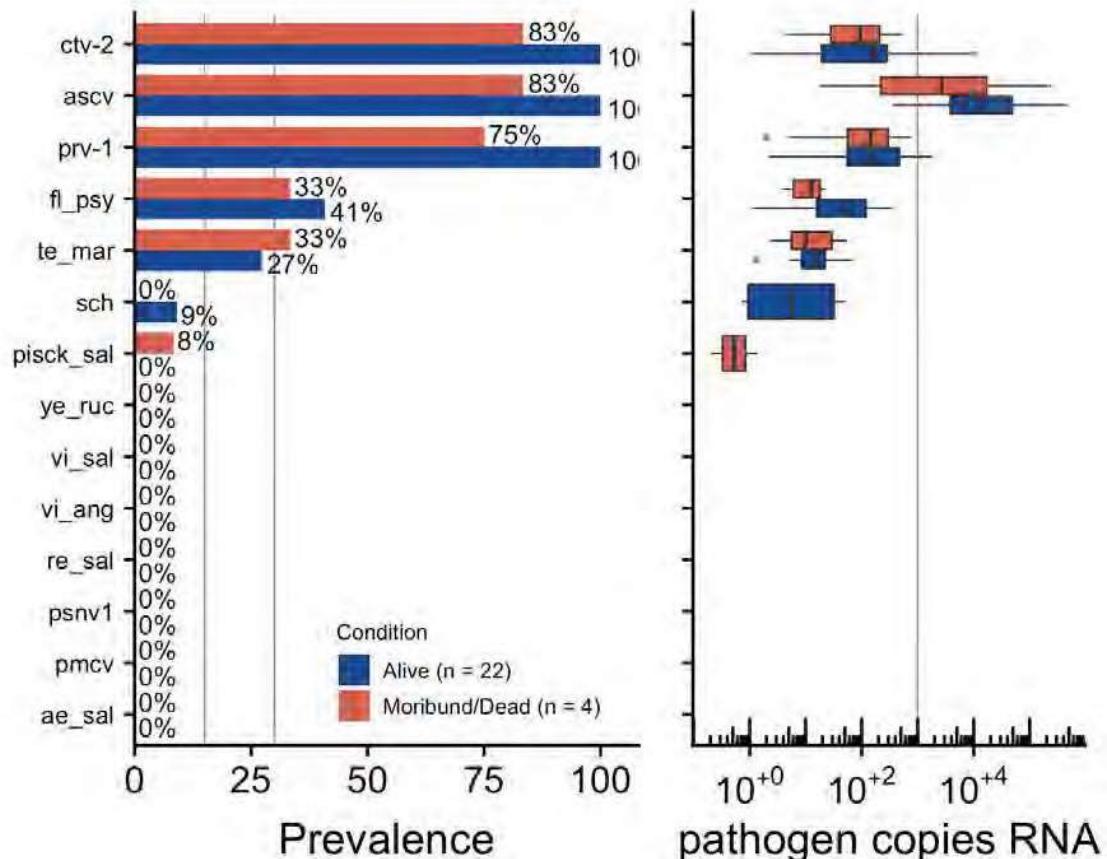
Water temperature ($^{\circ}\text{C}$), salinity (ppm), and dissolved oxygen (mg/L) at a 5m depth. Certain sampling dates have no recorded environmental data, resulting in gaps in the plots.

Overall infectious agent prevalence



Infectious agent prevalence in samples collected on 2021-11-30.

Prevalence in healthy vs. moribund/dead fish



Infectious agent prevalence in samples collected on 2021-11-30, split by mortality status at time of sampling. Any specimens that were not confirmed to be either moribund or live at the time of generating this report are excluded from this figure.

Individual infectious agent trends

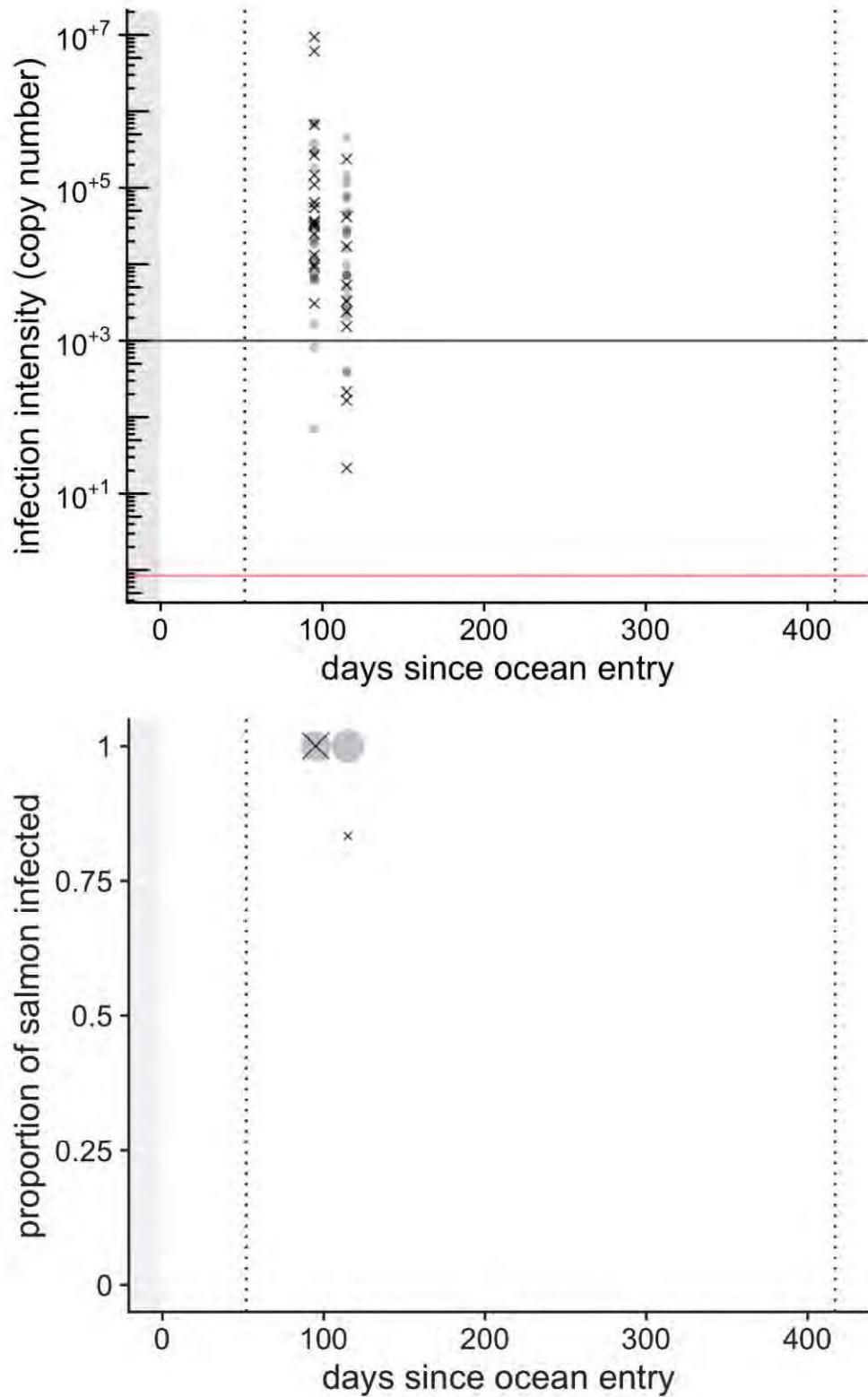
The following plots show individual infectious agent trends across all farm sites.

Grey circles represent live fish, and black X's represent dead/dying fish. Curves indicate mean predictions from a generalised additive model; blue and red correspond to live and dead/dying fish, respectively (shaded areas show 95% confidence regions). Left-hand grey region indicates freshwater hatchery residence, grey regions on x-axis indicate period of transfer to another site, and vertical dotted lines correspond to January 1st.

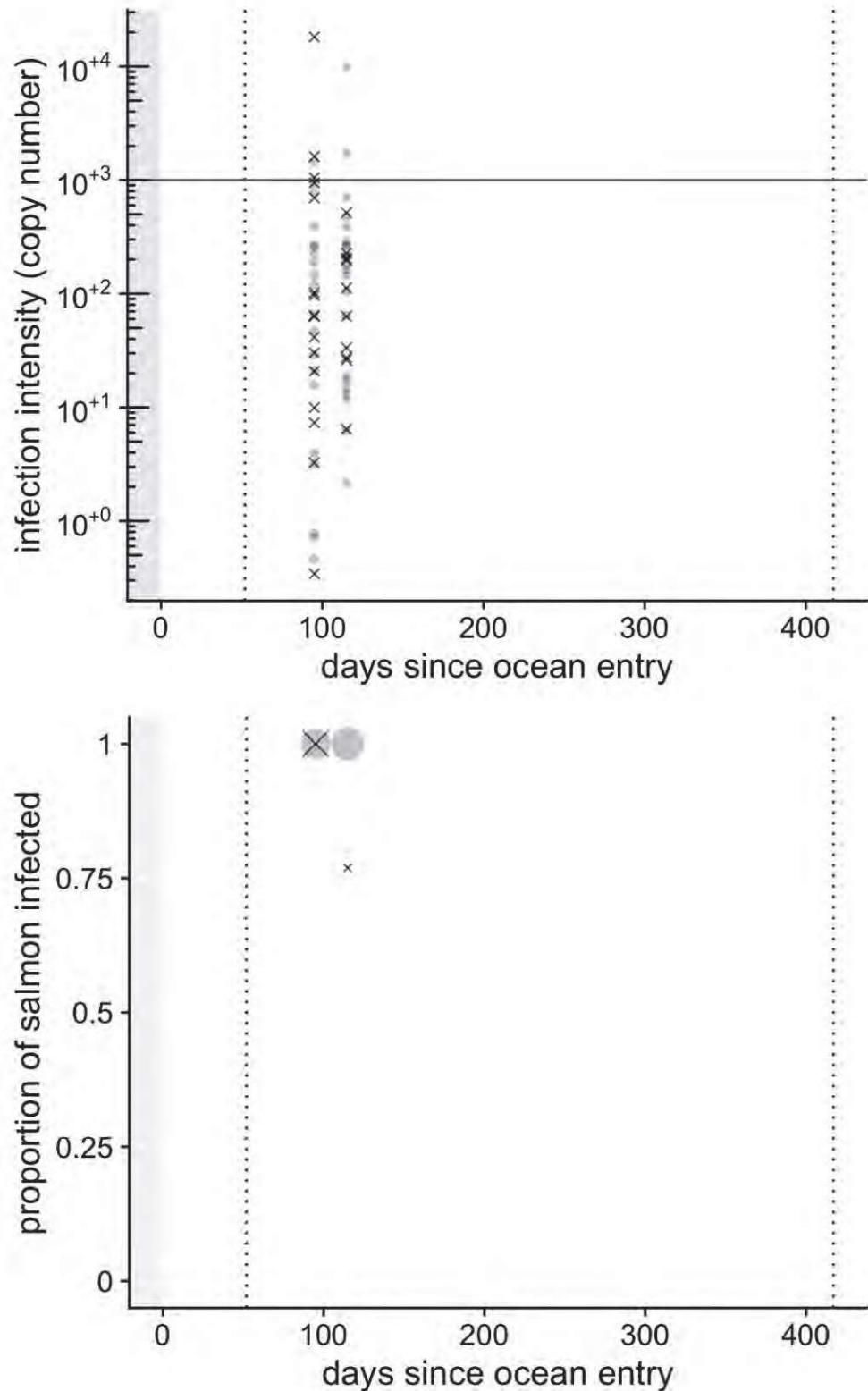
For infection intensity plots, horizontal red line indicates limit of detection (yielding ~90% true positive rate) for respective qPCR assay run in duplicate, while the horizontal black line indicates 1000 copies. Note log scale.

For proportion plots, grey circles show prevalence in live fish on each sampling date, and black X's show prevalence in dead/dying fish (symbol areas proportional to sample sizes).

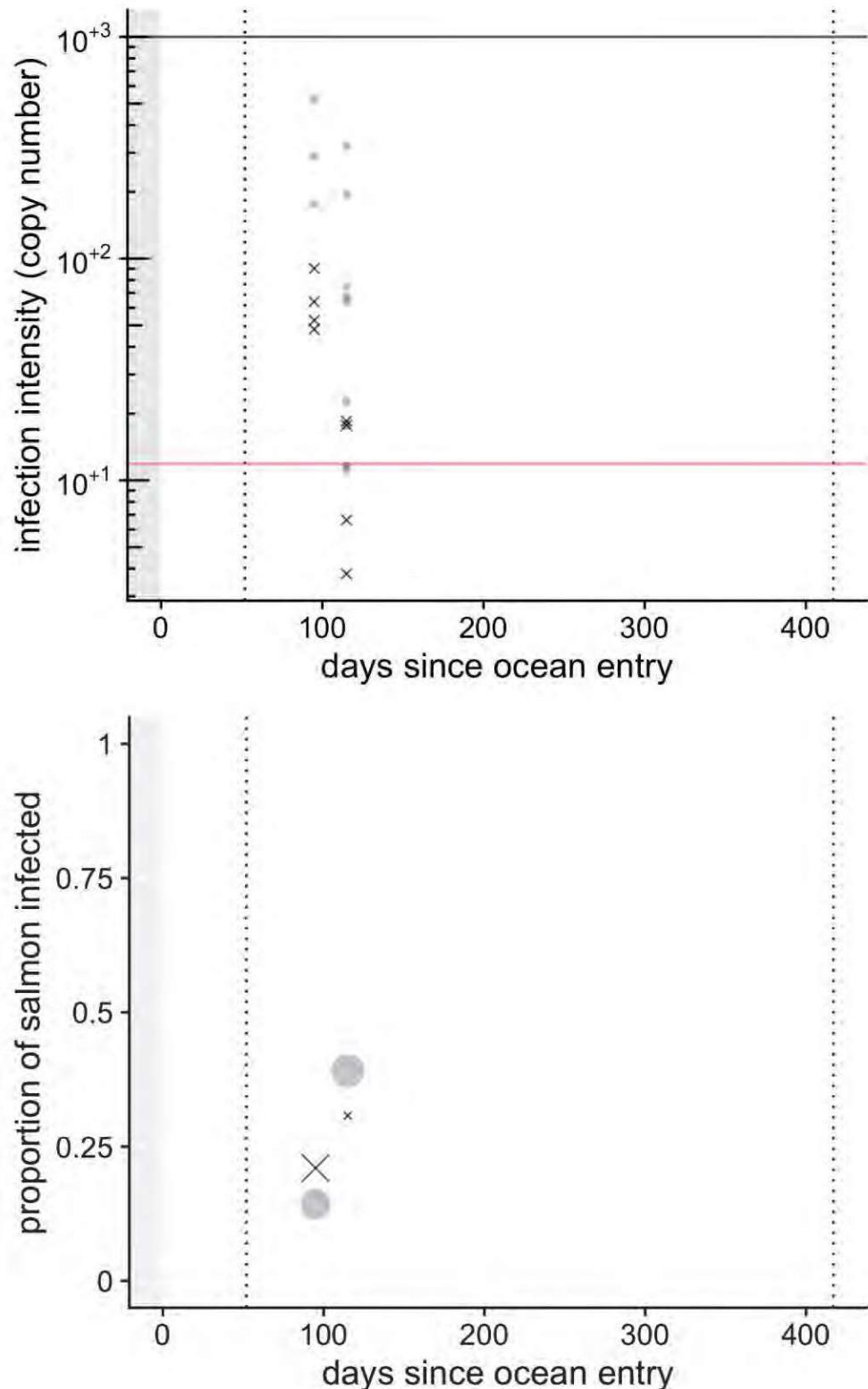
Atlantic salmon calicivirus



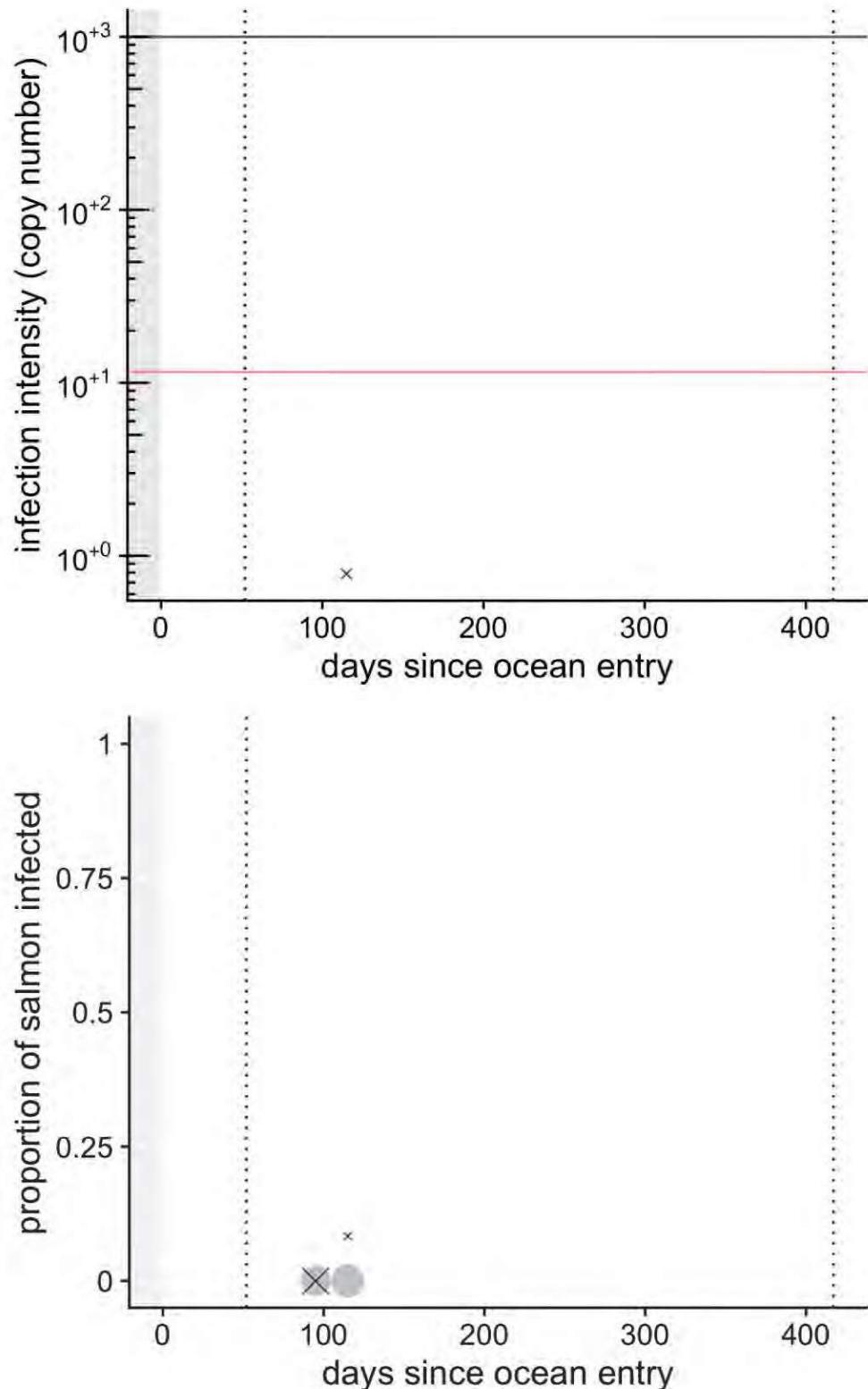
Cutthroat trout virus-2



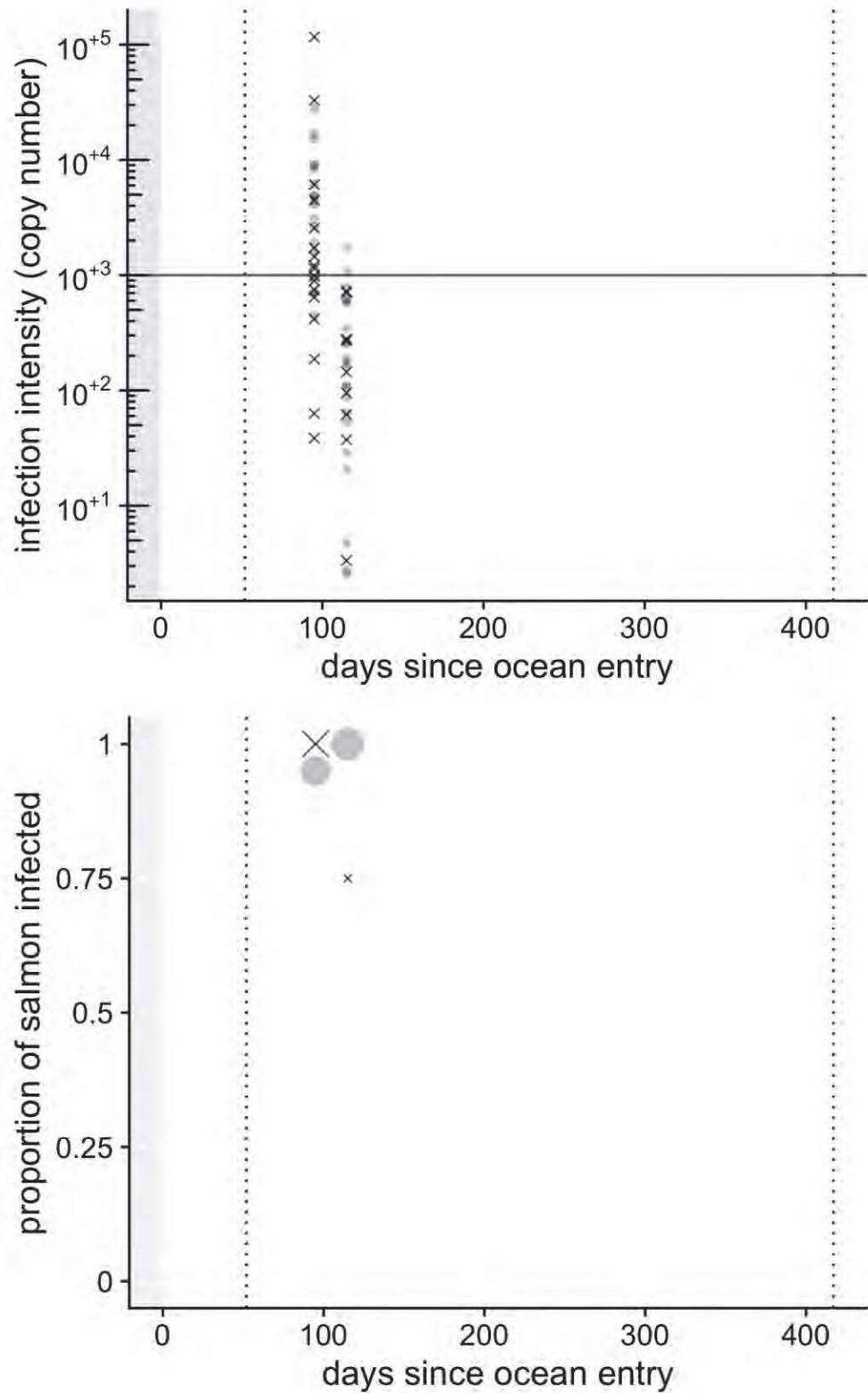
Flavobacterium psychrophilum



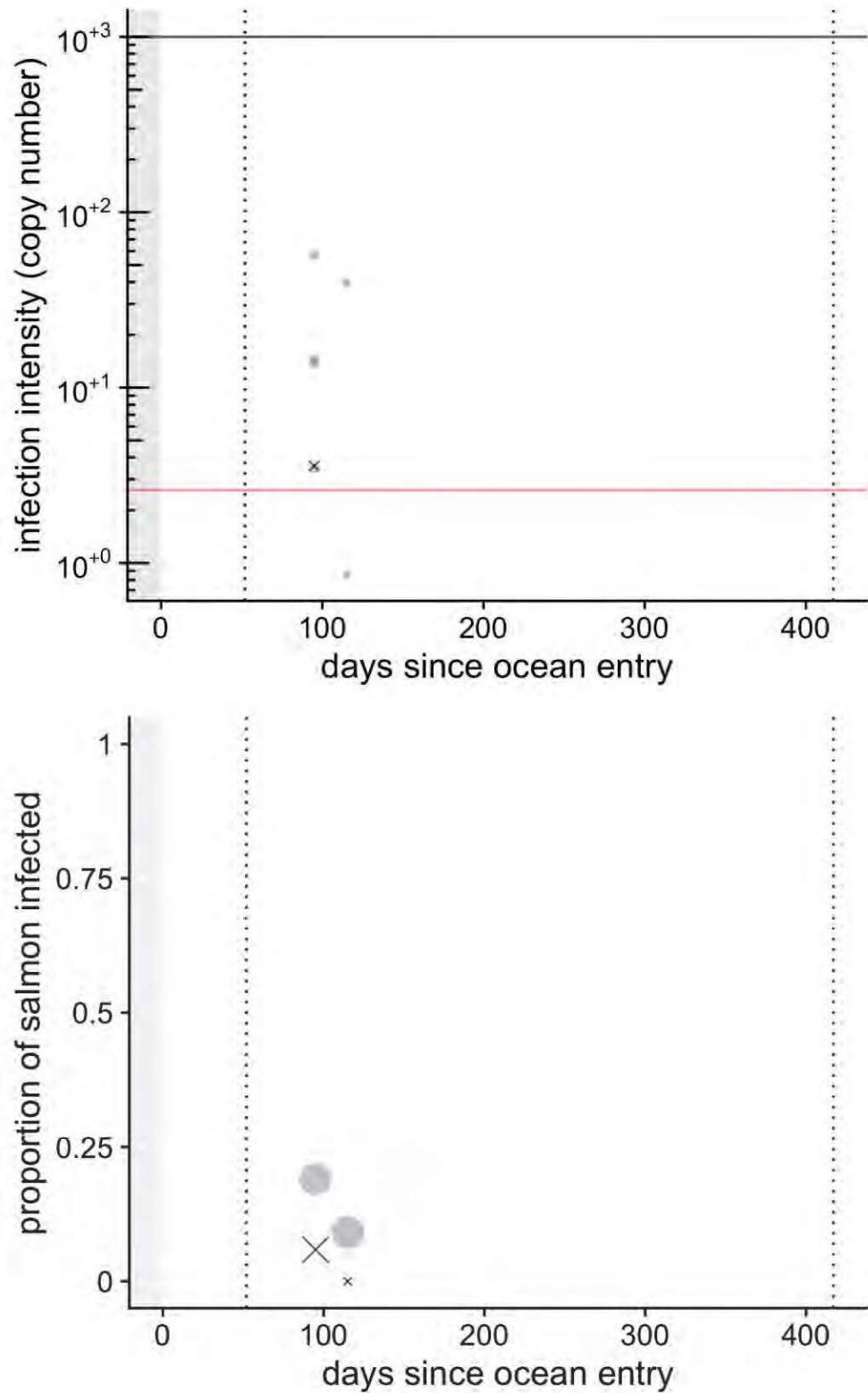
Piscirickettsia salmonis



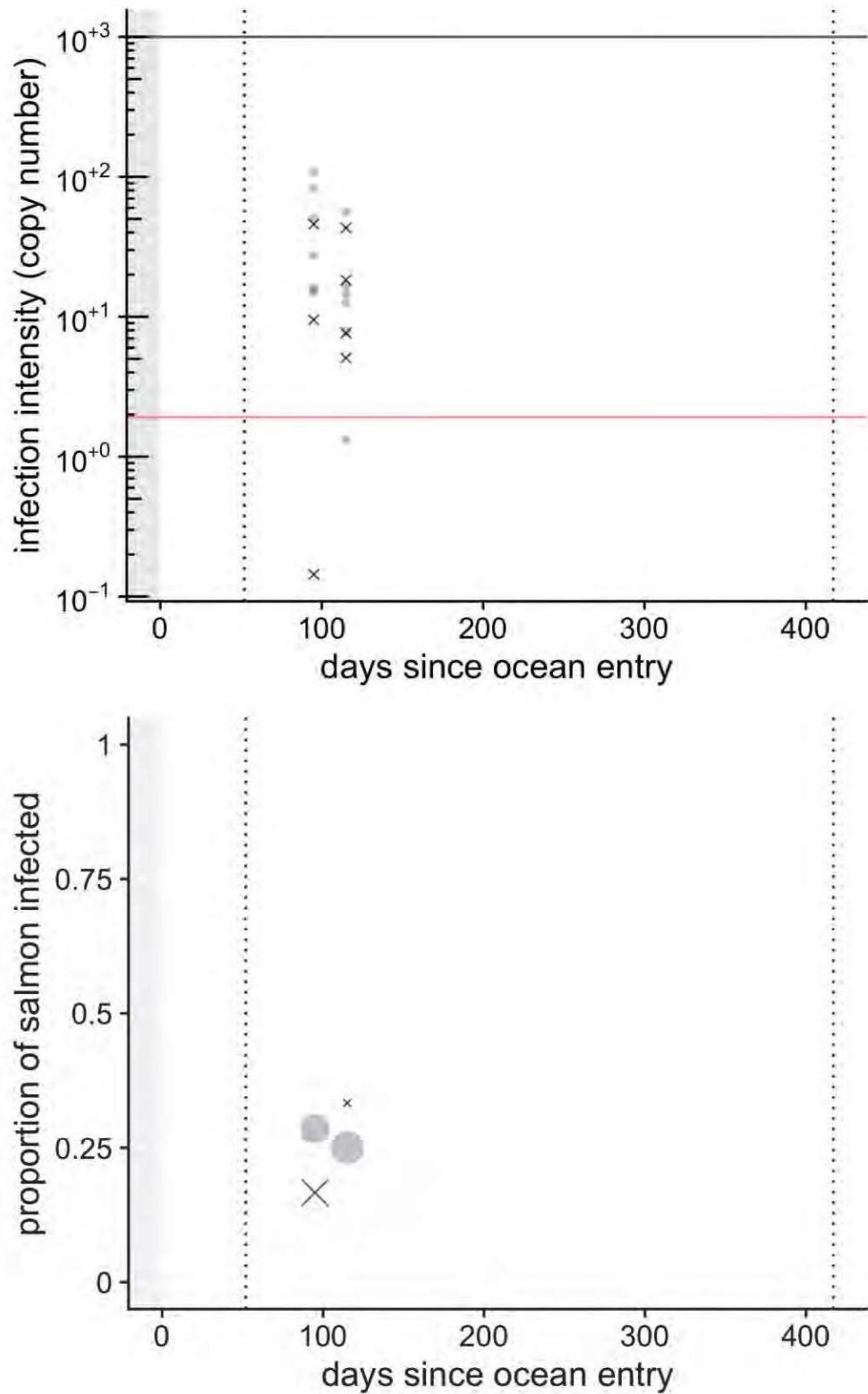
Piscine orthoreovirus



Candidatus Syngnathia salmonis



Tenacibaculum maritimum



Clinical signs

Table 1: Clinical signs for specimens sampled on 2021-11-30

metric	N5382	N5381	N5380	N5379	N5378	N5377	N5376	N5375	N5374	N5372	N5371	N5370	N5369	N5368	N5366	N5367	N5364	N5363	N5362	N5361
General																				
Live								X	X	X	X	X	X	X	X	X	X	X	X	X
Poor Performer	X																			X
Mort	X	X	X	X																
Skin & Fins																				
Ulcers				X																
Gills																				
Short Operculum																			X	X
Pale														X						
Erosions									X									X	X	X
Nodules/White Spots																				X
Abdominal Cavity																				
Adhesions					X												X			X
Ascites			X																	
Spleen																				
Enlarged				X			X	X										X		X
Liver																				
Pale	X																			
Yellow					X															
Hemorrhages/Congestion			X																	
Gallbladder																				
Enlarged					X			X	X	X	X							X		
Green																				
Heart																				
Deformed			X																	X
Pale	X	X	X	X																
Brain																				
Hemorrhages/Congestion				X																

Table 2: Clinical signs for specimens sampled on 2021-11-30

metric	N5394	N5393	N5392	N5391	N5390	N5389	N5388	N5387	N5386	N5385	N5383
General											
Live	X	X	X	X	X	X	X	X	X	X	X
Poor Performer											
Mort											
Skin & Fins											
Ulcers											
Gills											
Short Operculum			X								
Pale											
Erosions								X			
Nodules/White Spots						X	X				
Abdominal Cavity											
Adhesions											
Ascites											
Spleen											
Enlarged						X	X				X
Liver											
Pale											
Yellow											
Hemorrhages/Congestion											
Gallbladder											
Enlarged							X				
Green											X
Heart											
Deformed											
Pale											
Brain											
Hemorrhages/Congestion											

Histology

Table 3: Histology scores for specimens sampled on 2021-11-30

metric	N5382	N5364	N5363	N5362	N5361
Heart					
Peri Epi	3		3	1	2
Myo	1		1	1	2
Liver					
Cong Haem		1	3		na
Nec		1	2		na
Itis		1			na
Bdh					na
Spleen					
Cong Heam			3		1
Ellip Nec			1		
W Pulpitis	2			2	1
Pig Inc					
Cap Prolif					
Kidney					
Itis					na
Osis	2	2	1		na
Cong Heam					na
Interst Hyperplasia	2	2	2	1	na
Interst Nec	1	1	1	1	na
Glomitis					na
Pancreatitis					
Pancreatitis					na
Enteritis					
Enteritis					na
Cns					
Itis					na
Cnc					
Malacia					na
Gliosis					na
Cong Heam		1			na
Microsporidia					na
Gills					
Itis		nv	nv	nv	na
Cong Heam		nv	nv	nv	na
Prolif	2	nv	nv	nv	na
Skin_muscle					
Itis Nec					na
Tissue					

metric	N5382	
N5361	2	3
N5362		3
N5361	2	3

DFO ID	Diagnosis	Comments
N5361	Bacterial Myocarditis	Bacterial Colonies In Heart (2)
N5362		Peribiliary Immune Activation (1)
N5363	Early HSMI	Erythrophagocytosis In Liver(1)
N5382	Viral Pancarditis (Hsmi?)	Live Fish With Deformed Heart

Conclusions

In order to support the eDNA study, below is provided further evaluation of the results of testing from the Fish Health Report. The Fish Health sampling collection was completed. Available moribund/mort fish from the control pen and secondary pen were collected.

The farm was inspected in its entirety: the fish appeared in good conditions, with normal behavior. Reporting from the company indicated mortality that was within the normal range expected for this site.

Clinically, gills anomalies were more common in live fish (a few instances of short operculum, with rare gill erosions and nodules), along with enlarged spleen and gall bladder. Morts showed a wider array of lesions, particularly internally, including enlarged spleen, pale liver or hemorrhages, pale heart, ascites and enlarged gall bladder.

Molecular testing results indicate PRV present in 91% of the fish tested, even at high load in few fish. A rise in *Flavobacterium psychrophilum* prevalence was also observed (38% of fish tested; 41% of live fish and 33% of morts), along with *Tenacibaculum maritimum* (29%). *Candidatus Syngnathus salmonis* and *Piscirickettsia salmonis* were present at background levels.

Histopathologically, the lesions were in general mild or moderate, including inflammatory or congestive modifications, particularly occurring in spleen and kidney. Kidney interstitial hyperplasia and necrosis, spleen pulpitis and mild myo/endocarditis were the most common findings. However, one individual showed bacterial myocarditis as likely cause of death (no *Aeromonas salmonicida* or *Piscirickettsia salmonis* detected), while two fish (including a live individual sampled), showed sign of a viral myocarditis. This finding, along with the pattern of lesions detected, respective clinical signs and molecular data, are conducive to early development stages of HSMI.

Appendix

Glossary of infectious agents

Agent abbr.	Full agent name	Agent type	Disease	Ranking
ae_sal	Aeromonas salmonicida	Bacteria	Furunculosis	2
ascv	Atlantic salmon calicivirus	Virus	unknown	4
ctv-2	Cutthroat trout virus-2	Virus	unknown	4
fl_psy	Flavobacterium psychrophilum	Bacteria	Bacterial cold water disease	3
pisck_sal	Piscirickettsia salmonis	Bacteria	Piscirickettsiosis (SRS)	2
pmcv	Piscine myocarditis virus	Virus	Cardiomyopathy syndrome	1
prv-1	Piscine orthoreovirus	Virus	HSMI-EIBS-Jaundice/anemia	NA
psnv1	Pacific salmon nidovirus-1 (CoV)	Virus	unknown	4
re_sal	Renibacterium salmoninarum	Bacteria	Bacterial kidney disease	2
sch	Candidatus Syngnathida salmonis	Bacteria	Gill chlamydia	3
te_mar	Tenacibaculum maritimum	Bacteria	Marine flexibacterios (mouth/fin rot)	2
vi_ang	Vibrio anguillarum	Bacteria	Vibriosis	2
vi_sal	Vibrio salmonicida	Bacteria	Cold water vibriosis	2
ye_ruc	Yersinia ruckeri (Enteric redmouth disease)	Bacteria	Yersiniosis (Enteric red mouth)	2

eDNA Study Report

Cypress Harbour sampling on December 1, 2021

Dr. Emiliano Di Cicco

September 23, 2022

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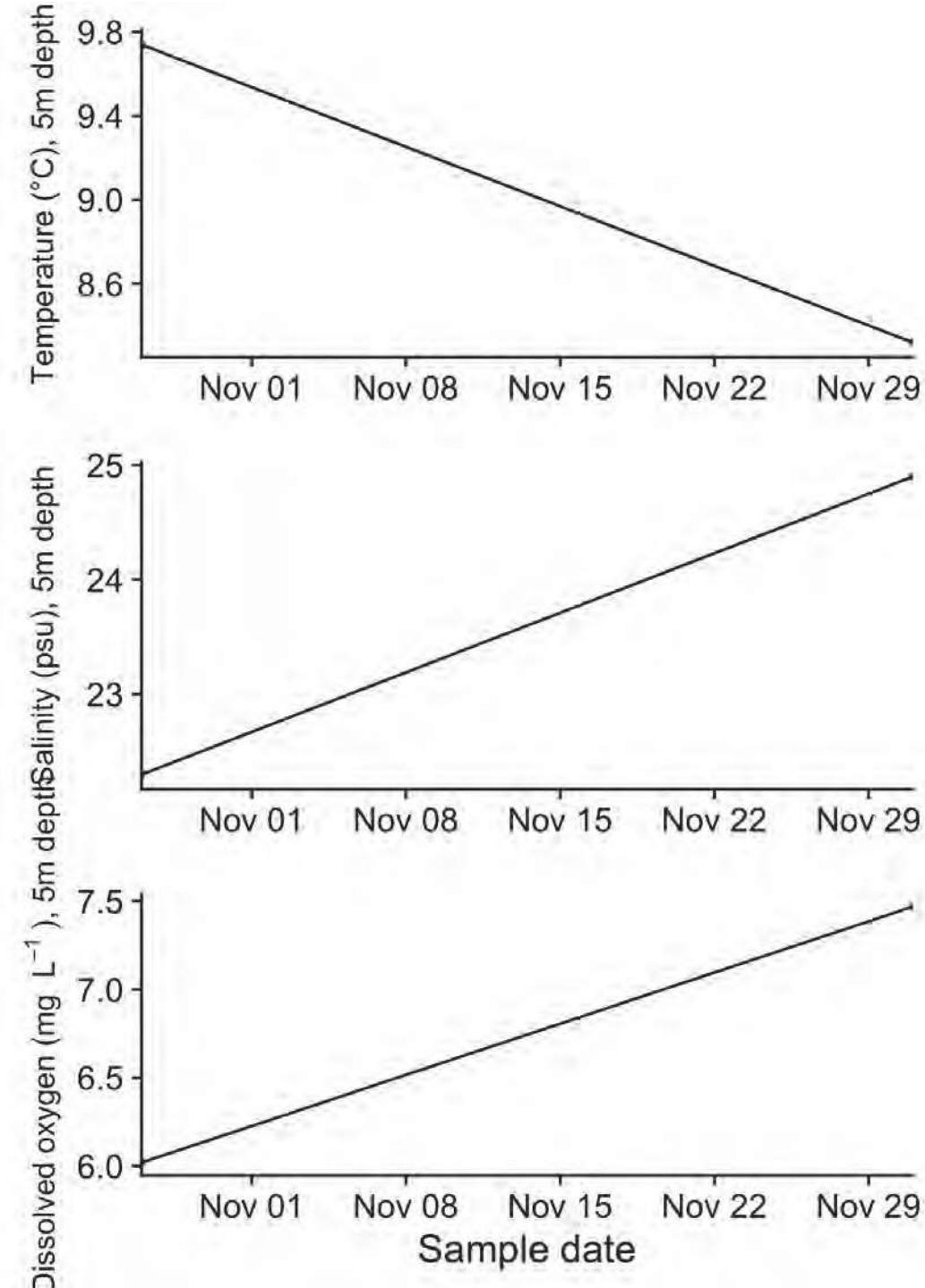
Executive summary

Premise

On December 01, 2021, 9 samples were collected by BATI and Cermaq crew during a sampling event at Cypress Harbour (Cermaq Ltd.). 9 Atlantic salmon subadults and matures were collected from the Cypress Harbour farm site, including 0 live and 9 moribund/dead fish. At the time of generating this report, 2 samples have not yet been confirmed as live or moribund/dead. All live fish were euthanized with TMS overdose prior to dissection with the exception of the moribund fish, which were administered a blow to the head. Portions of gill, liver and anterior kidney were collected in triplicate for molecular testing (preserved in RNA later) from all the fish, while all the moribund/dead fish also underwent collection of tissues (gills, spleen, liver, heart, anterior and posterior kidney, pyloric caeca, skeletal muscle + skin, brain) for histological analysis. Clinical notes and gross lesions were noted and reported for every fish. One aliquot has been provided to the Company Cermaq Fish Health, another aliquot is stored at the BATI Field Office, and a third aliquot is stored at DFO - PBS. This latter aliquot has been tested for the presence and load of the agents indicated in the IMIP agreement as well as the agents indicated in the eDNA study agreement. Each sample has been extracted and tested individually. Negative and positive controls were run. A housekeeping gene was also included to assess the quality of the RNA extracted.

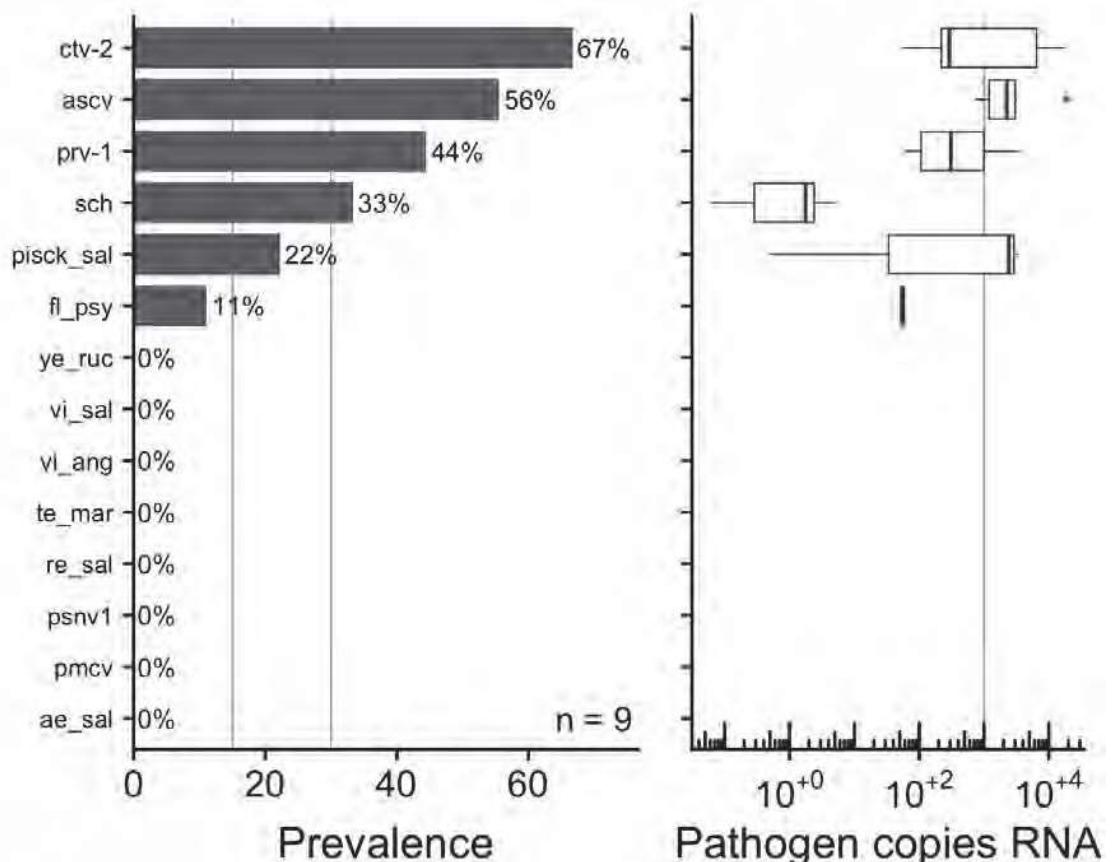
Histology samples have been sent to Wax-It Histo Ltd. to process and prepare slides, which have been read and scored by Dr. Di Cicco. A digital copy of each slide is available to the Company.

Environmental data

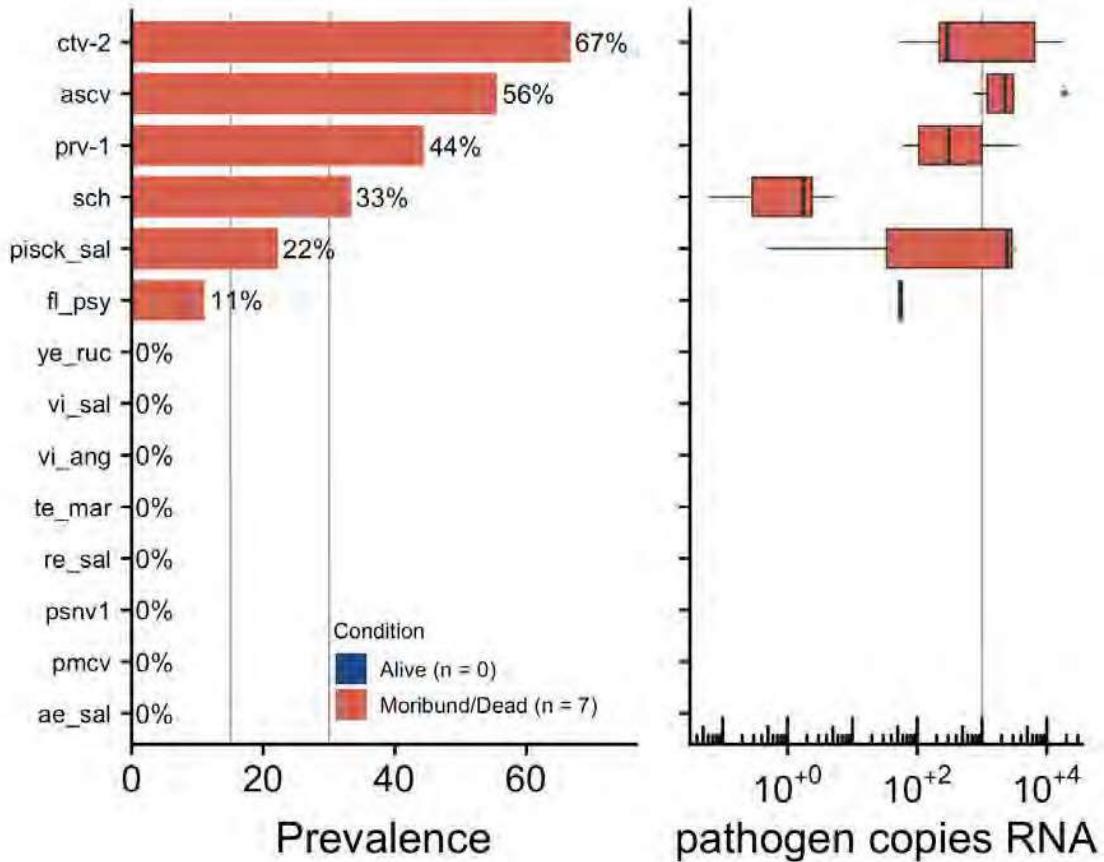


Water temperature ($^{\circ}\text{C}$), salinity (ppm), and dissolved oxygen (mg/L) at a 5m depth. Certain sampling dates have no recorded environmental data, resulting in gaps in the plots.

Overall infectious agent prevalence



Infectious agent prevalence in samples collected on 2021-12-01.



Infectious agent prevalence in samples collected on 2021-12-01, split by mortality status at time of sampling. Any specimens that were not confirmed to be either moribund or live at the time of generating this report are excluded from this figure.

Individual infectious agent trends

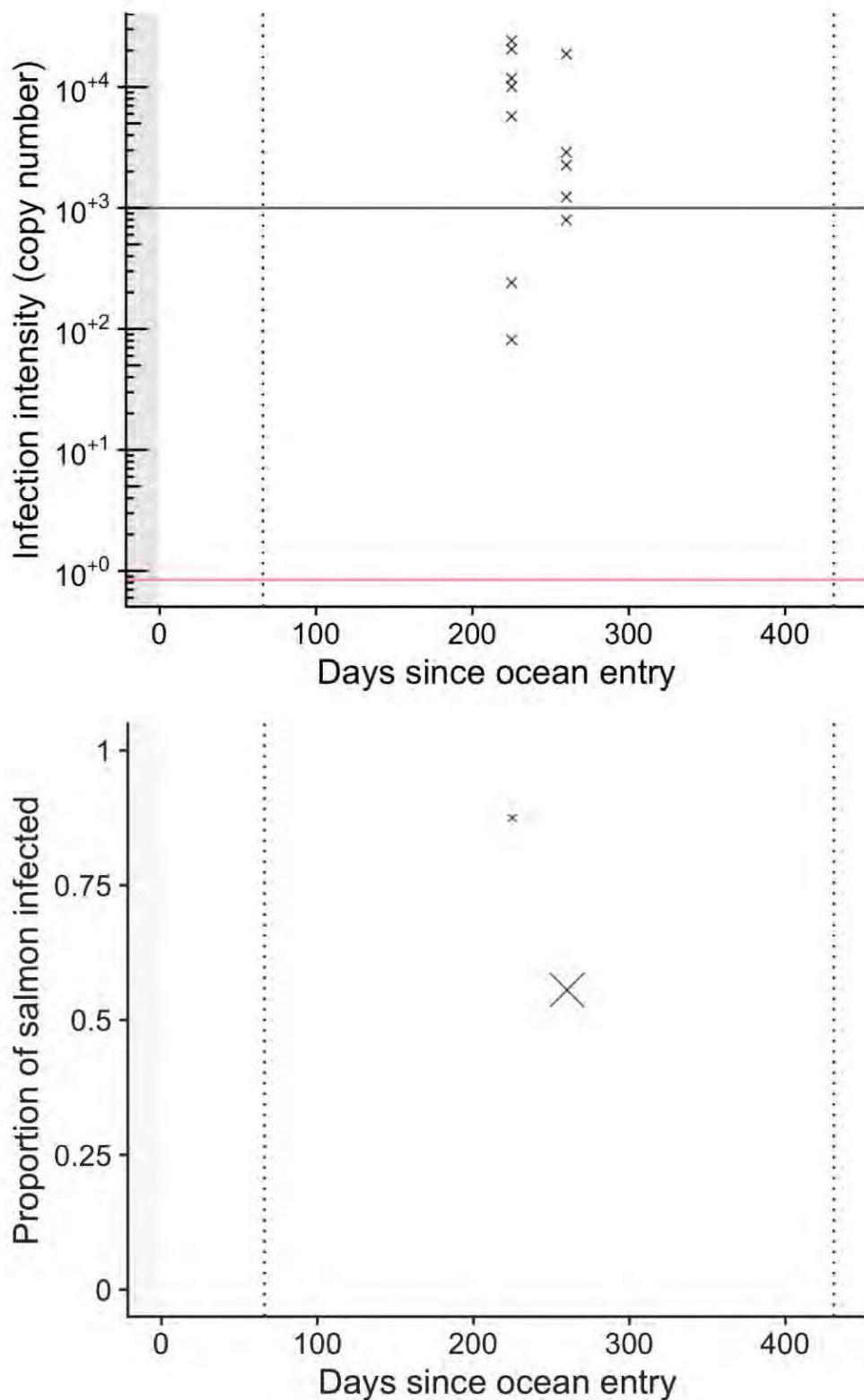
The following plots show individual infectious agent trends across all farm sites. In cases where sample size is sufficient, curves from a generalised additive model are included in the plot.

Grey circles represent live fish, and black X's represent dead/dying fish. Curves indicate mean predictions from a generalised additive model; blue and red correspond to live and dead/dying fish, respectively (shaded areas show 95% confidence regions). Left-hand grey region indicates freshwater hatchery residence, grey regions on x-axis indicate period of transfer to another site, and vertical dotted lines correspond to January 1st.

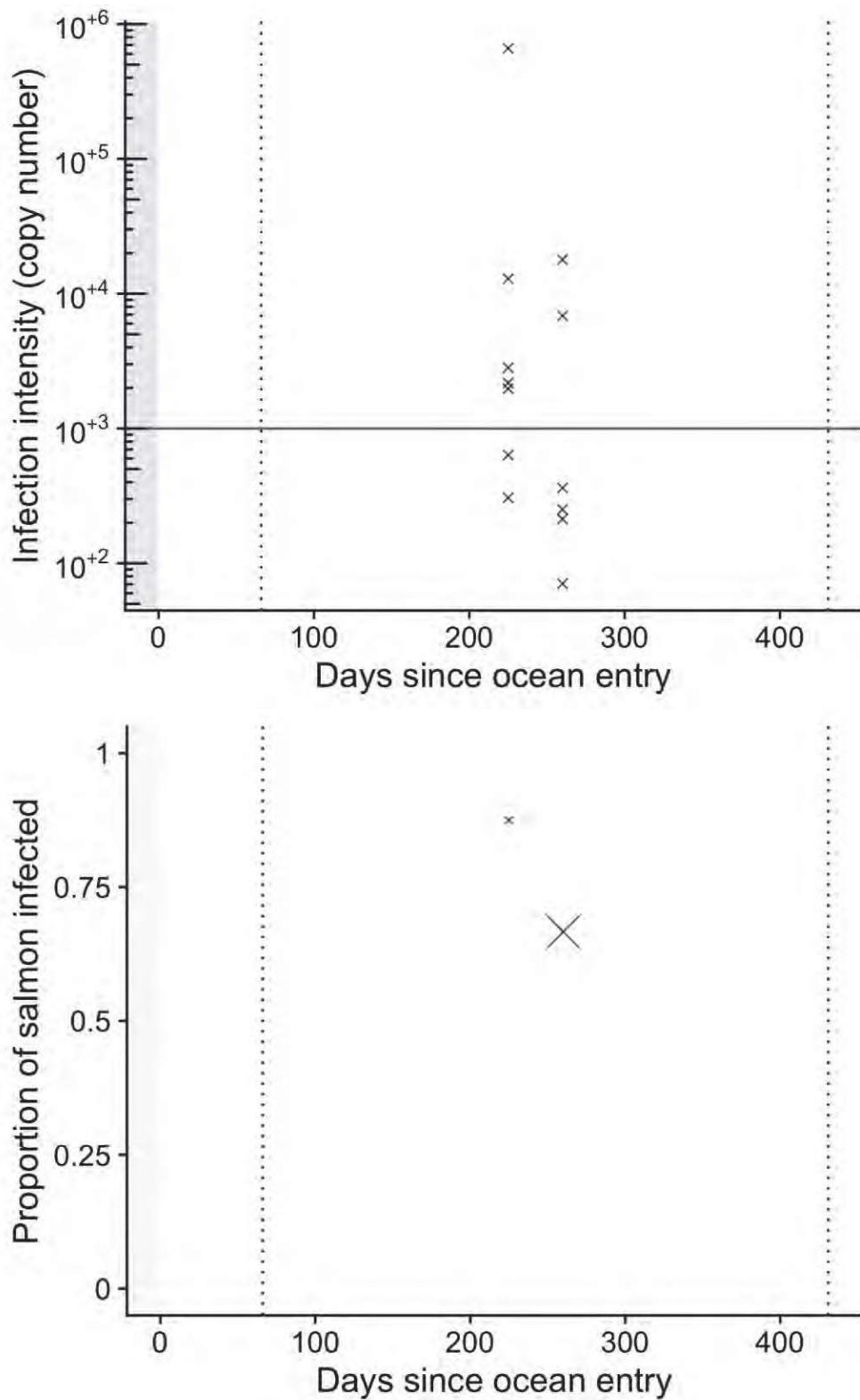
For infection intensity plots, horizontal red line indicates limit of detection (yielding ~90% true positive rate) for respective qPCR assay run in duplicate, while the horizontal black line indicates 1000 copies. Note log scale.

For proportion plots, grey circles show prevalence in live fish on each sampling date, and black X's show prevalence in dead/dying fish (symbol areas proportional to sample sizes).

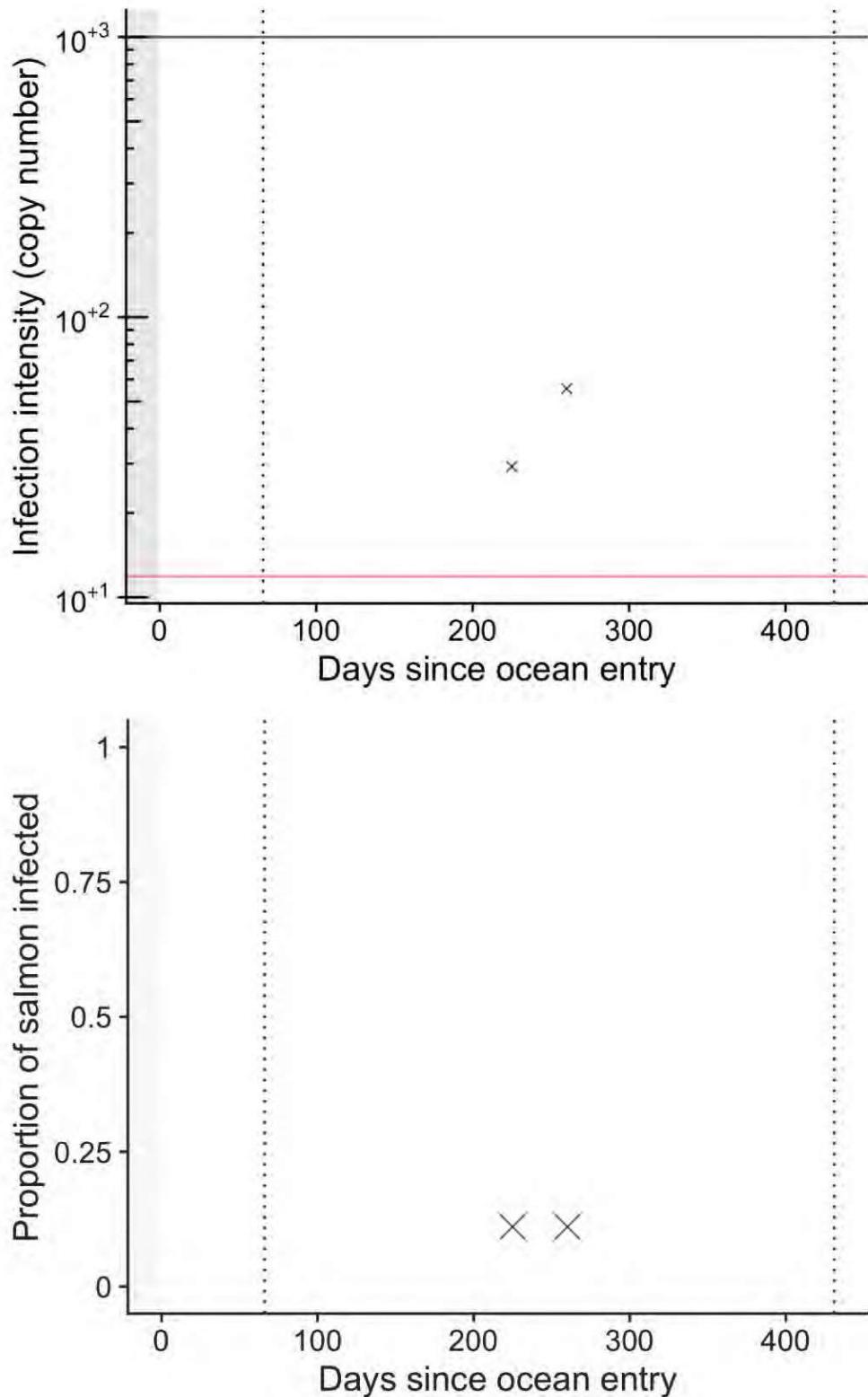
Atlantic salmon calicivirus



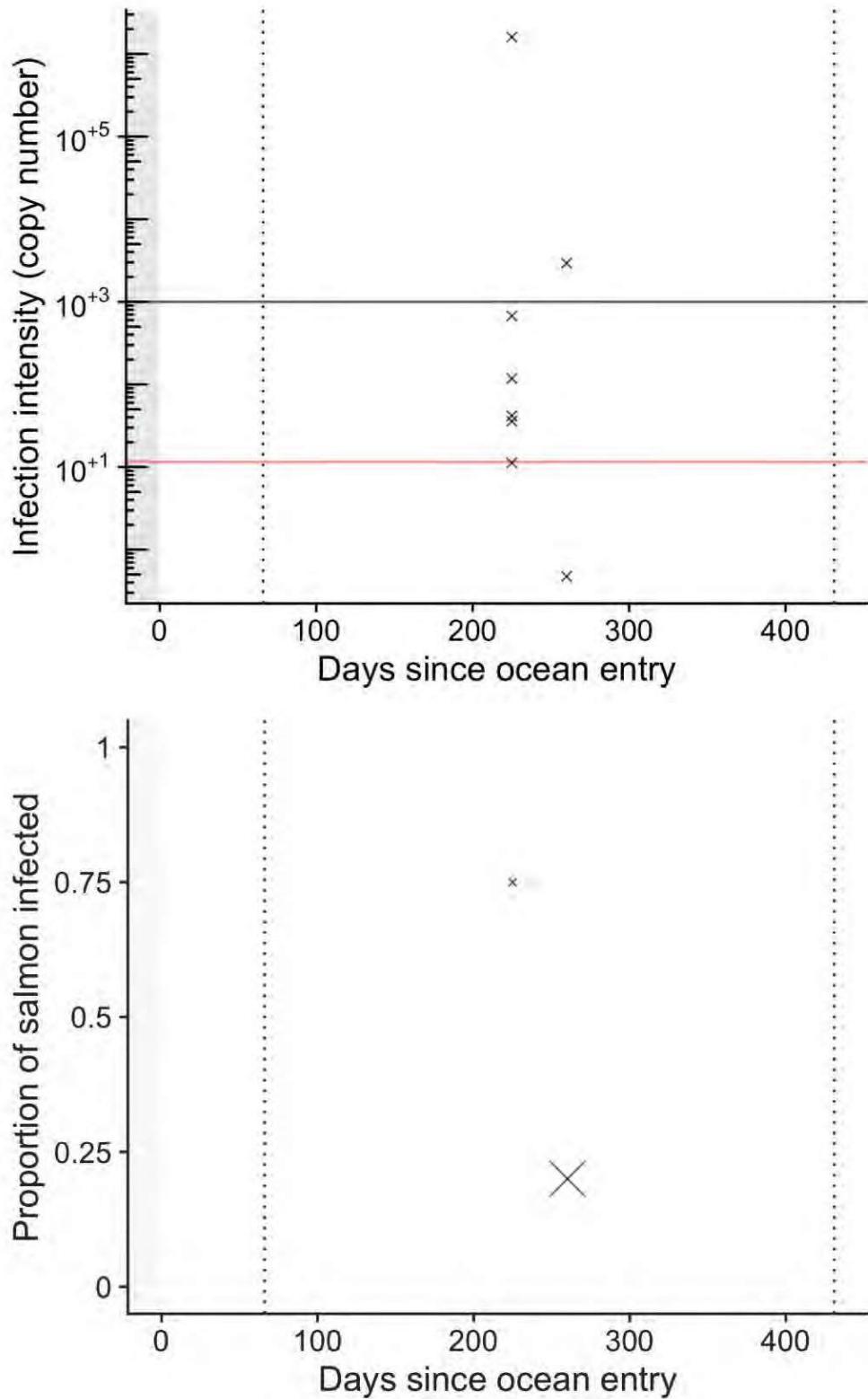
Cutthroat trout virus-2



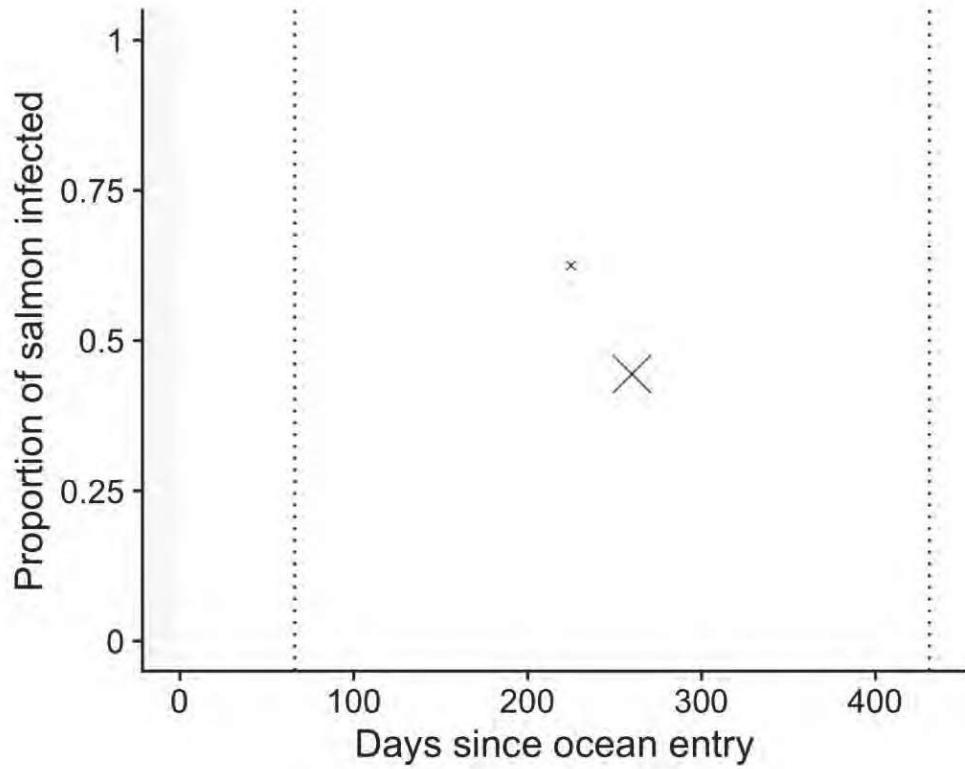
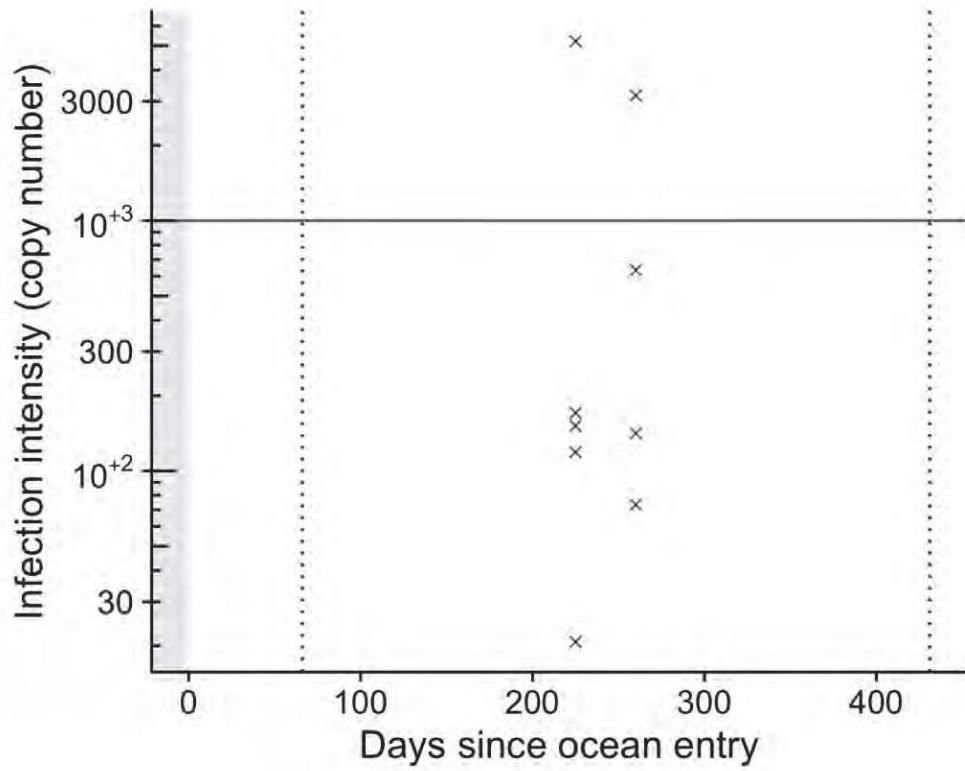
Flavobacterium psychrophilum



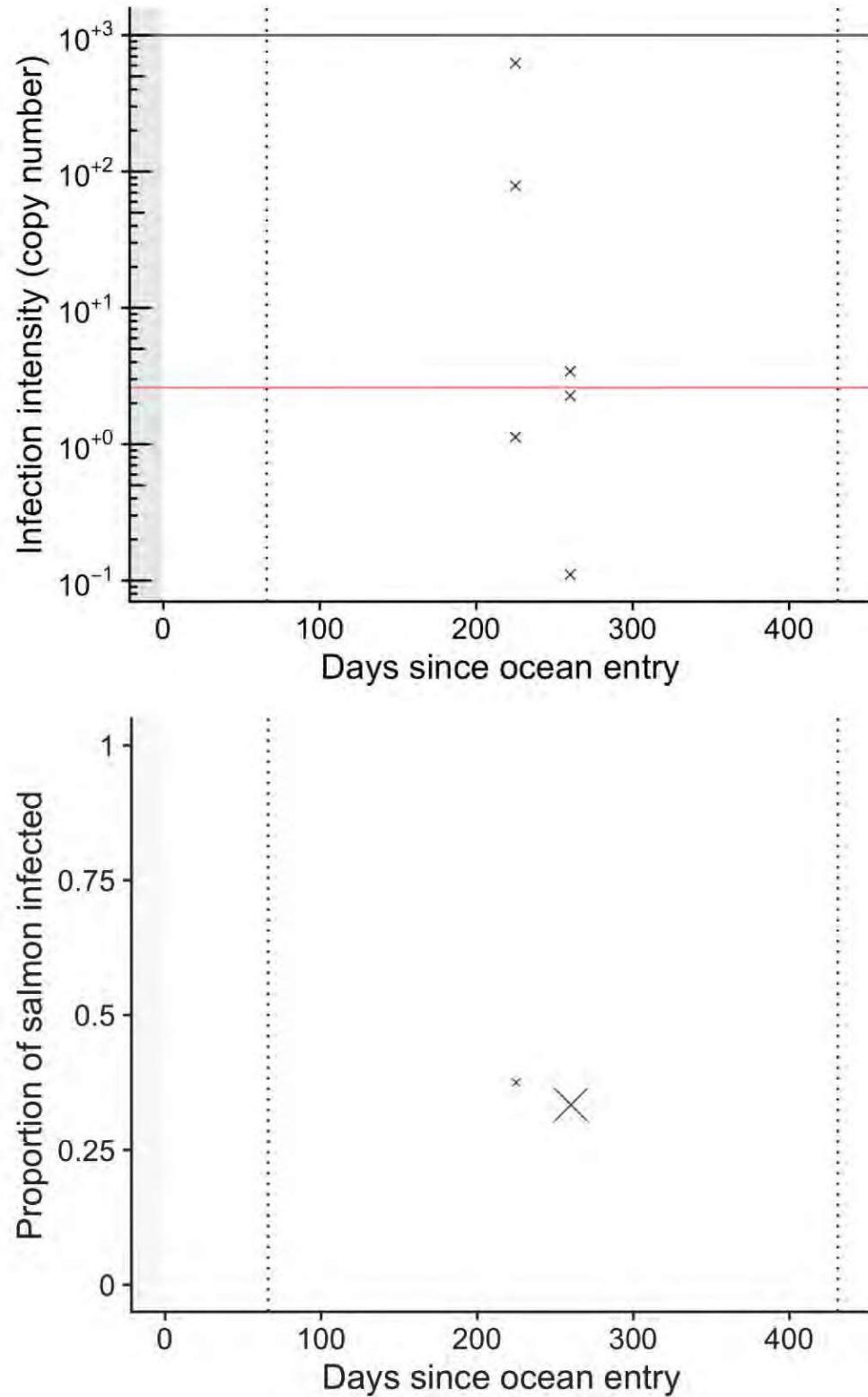
Piscirickettsia salmonis



Piscine orthoreovirus



Candidatus Syngnathia salmonis



Clinical signs

Table 1: Clinical signs for specimens sampled on 2021-12-01

metric	N5401	N5402	N5403	N5404	N5405	N5406	N5407	N5409	N5410
General									
Moribund	X								
Mort		X	X	X			X	X	X
Exophthalmia					X				
Skin & Fins									
Erosion		X							
Ulcers		X							
Gills									
Short Operculum					X				
Erosions		X		X					
Nodules/White Spots	X	X					X	X	
Abdominal Cavity									
Adhesions					X	X			
Spleen									
Enlarged					X	X	X	X	X
Liver									
Pale	X								
Dark			X				X	X	X
Hemorrhages/Congestion					X				
Nodules/White Spots							X		
Gallbladder									
Enlarged		X							
Green						X		X	X
Heart									
Pale					X	X			
Blood Clots/Hemopericardium						X			
Intestine									
Hemorrhages/Congestion				X				X	
Brain									
Hemorrhages/Congestion			X			X		X	X

Histology

Table 2: Histology scores for specimens sampled on 2021-12-01

metric	N5401	N5402	N5403	N5404	N5405	N5406	N5407	N5409	N5410
Heart									
Peri Epi					1				
Myo					1				1
Liver									
Cong Haem				1		2	2	1	1
Nec		1	1			1	1		
Itis	1	1			1				
Spleen									
Cong Heam						3	2		
Ellip Nec				1	1				
W Pulpitis	2	2	1	2		2	1	2	1
Pig Inc									1
Kidney									
Osis		1		1	1	1	1		1
Cong Heam				1	2	1		1	1
Interst Hyperplasia	1	1	2	2	2	1	1	1	1
Interst Nec		1					1		
Cnc									
Gliosis	1								
Cong Heam				1	1		1	1	1
Gills									
Itis			nv		nv	nv		2	
Cong Heam		nv			nv	nv			
Prolif		nv	1	nv	nv			1	1
Tissue									
Necrosis Artefacts			3		3	2	1	2	1

Diagnoses and Comments

Table 3: Diagnoses and comments for specimens sampled on 2021-12-01

DFO ID	Diagnosis	Comments
N5401		Erythrophagocytosis (2)
N5402		Granuloma In Liver (1)
N5403	Piscirickettsiosis	Peribiliary Immune Activation (1)
N5404		Congestion + Hemorrhages In Pancreas (2), Myonecrosis (1), Peribiliary Immune Activation (1)
N5405		Increase Fibrin In Spleen (2), Peribiliary Immune Activation (1)
N5410		Congestion + Hemorrhages In Pancreas (2), Eosinophilic Granules In Kidney Tubiules (1)

Conclusions

In order to support the eDNA study, below is provided further evaluation of the results of testing from the Fish Health Report. The sampling collection was completed. This is a particular farm, due to the presence of different generations of brood stock reared in the same site. The disposition of the cages in the farm is also atypical, and the fish undergo frequent grading and subdivisions. No live fish were collected, as per agreement with the company, but available moribund/mort fish from all the pens were collected. Here below is a summary and evaluation of the findings from the sampled fish.

The farm was inspected in its entirety. Most fish in the examined pens were behaving normally. The morts are collected once a week by divers, therefore an estimation of the mortality rate is less accurate and indicative of the overall conditions of the fish. However, the mortality per pen reported by the company resulted in line with the normal standard expected for such a site, with the exception of two pens that apparently showed significant mortality to be attributed to predators (i.e. sea lions). Typical lesions from predation episodes were observed in most morts, but it was not clear whether such lesions were the cause or the consequence of the mortality induced by the predators. Clinically, most fish showed gills erosion and/or nodules as well as enlarged spleen and dark/congested liver and green gall bladder. Congestion in the intestine was observed in two individuals, and ascites in one individual. Brain congestion and hemorrhages were pretty common too.

Molecular testing results show that about 44% of the individual tested were positive to PRV, while 22% were positive to *Piscirickettsia salmonis*. *Candidatus Syngnathida salmonis* was identified in 32% of the fish, while background level detection was observed for *Flavobacterium psychrophilum*.

Histopathologically, a congestive and inflammatory pattern of alterations affecting the liver, spleen, kidney, brain and abdominal fat was prominent in most samples, although pathognomonic lesions were not observed. One individual presented a large granuloma in the liver. Given the elevated incidence of *Piscirickettsia salmonis*, it's quite likely that most lesions were induced by such agent

Appendix

Glossary of infectious agents

Agent abbr.	Full agent name	Agent type	Disease	Ranking
ae_sal	Aeromonas salmonicida	Bacteria	Furunculosis	2
ascv	Atlantic salmon calicivirus	Virus	unknown	4
ctv-2	Cutthroat trout virus-2	Virus	unknown	4
fl_psy	Flavobacterium psychrophilum	Bacteria	Bacterial cold water disease	3
pisck_sal	Piscirickettsia salmonis	Bacteria	Piscirickettsiosis (SRS)	2
pmcv	Piscine myocarditis virus	Virus	Cardiomyopathy syndrome	1
prv-1	Piscine orthoreovirus	Virus	HSMI-EIBS-Jaundice/anemia	
psnv1	Pacific salmon nidovirus-1 (CoV)	Virus	unknown	4
re_sal	Renibacterium salmoninarum	Bacteria	Bacterial kidney disease	2
sch	Candidatus Syngnathia salmonis	Bacteria	Gill chlamydia	3
te_mar	Tenacibaculum maritimum	Bacteria	Marine flexibacteriosis (mouth/fin rot)	2
vi_ang	Vibrio anguillarum	Bacteria	Vibriosis	2
vi_sal	Vibrio salmonicida	Bacteria	Cold water vibriosis	2
ye_ruc	Yersinia ruckeri (Enteric redmouth disease)	Bacteria	Yersiniosis (Enteric red mouth)	2

eDNA Study Report

Cypress Harbour sampling on January 26, 2022

Dr. Emiliano Di Cicco

September 23, 2022

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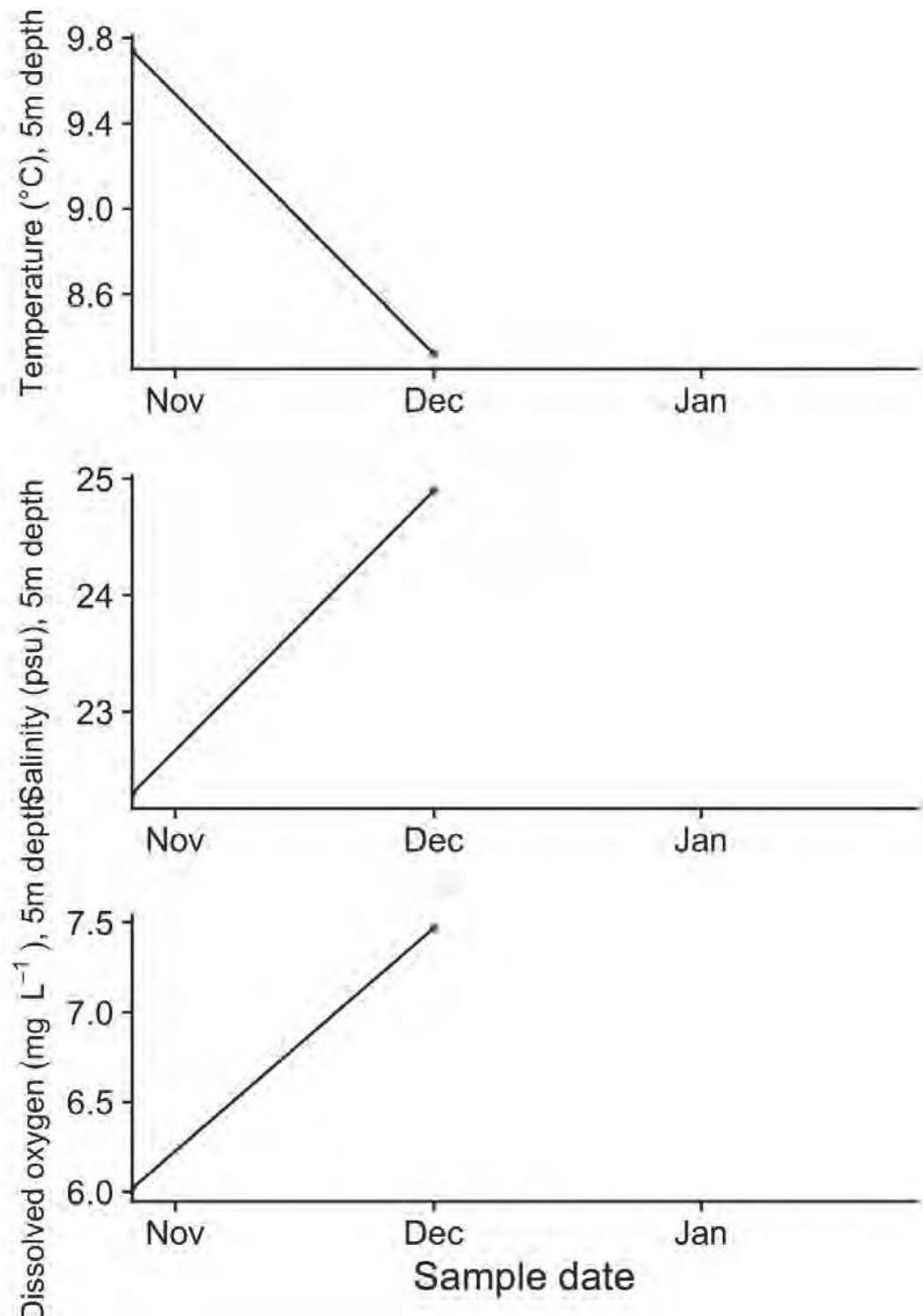
Executive summary

Premise

On January 26, 2022, 7 samples were collected by BATI and Cermaq crew during a sampling event at Cypress Harbour (Cermaq Ltd.). 7 Atlantic salmon subadults and matures were collected from the Cypress Harbour farm site, including 0 live and 7 moribund/dead fish. All live fish were euthanized with TMS overdose prior to dissection with the exception of the moribund fish, which were administered a blow to the head. Portions of gill, liver and anterior kidney were collected in triplicate for molecular testing (preserved in RNA later) from all the fish, while all the moribund/dead fish also underwent collection of tissues (gills, spleen, liver, heart, anterior and posterior kidney, pyloric caeca, skeletal muscle + skin, brain) for histological analysis. Clinical notes and gross lesions were noted and reported for every fish. One aliquot has been provided to the Company Cermaq Fish Health, another aliquot is stored at the BATI Field Office, and a third aliquot is stored at DFO - PBS. This latter aliquot has been tested for the presence and load of the agents indicated in the IMIP agreement as well as the agents indicated in the eDNA study agreement. Each sample has been extracted and tested individually. Negative and positive controls were run. A housekeeping gene was also included to assess the quality of the RNA extracted.

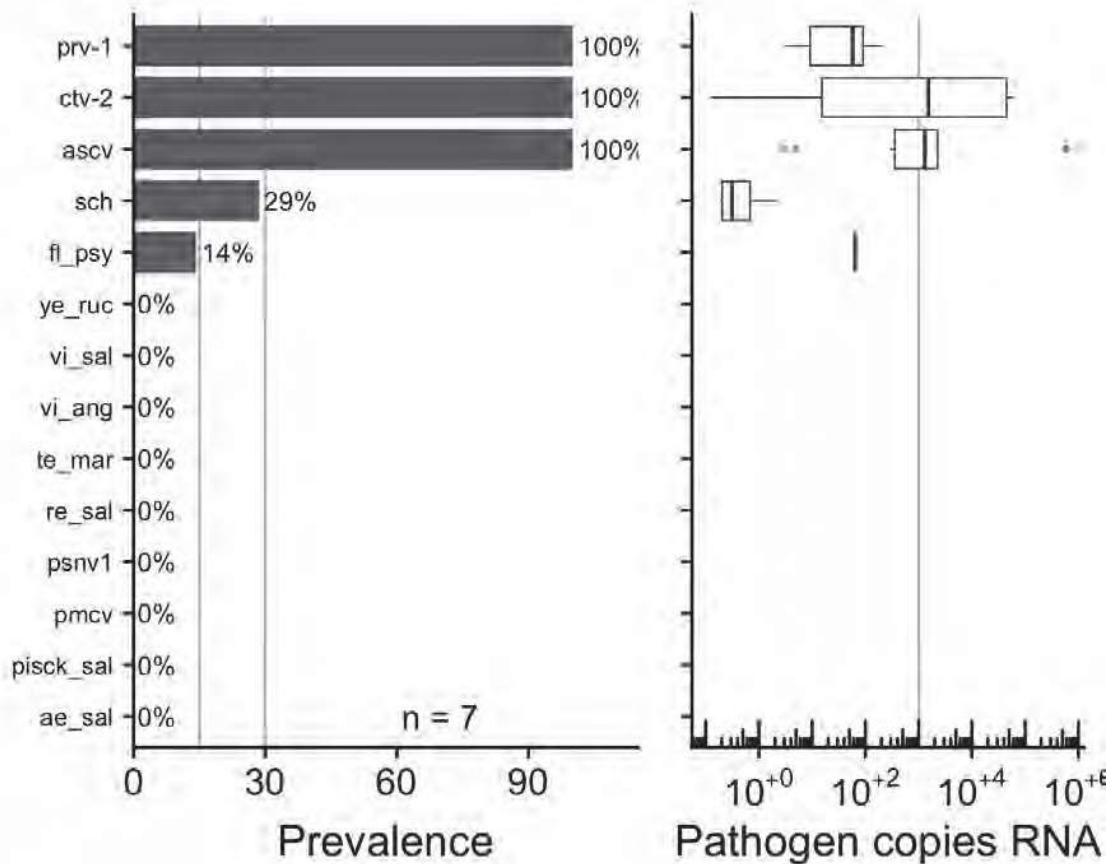
Histology samples have been sent to Wax-It Histo Ltd. to process and prepare slides, which have been read and scored by Dr. Di Cicco. A digital copy of each slide is available to the Company.

Environmental data

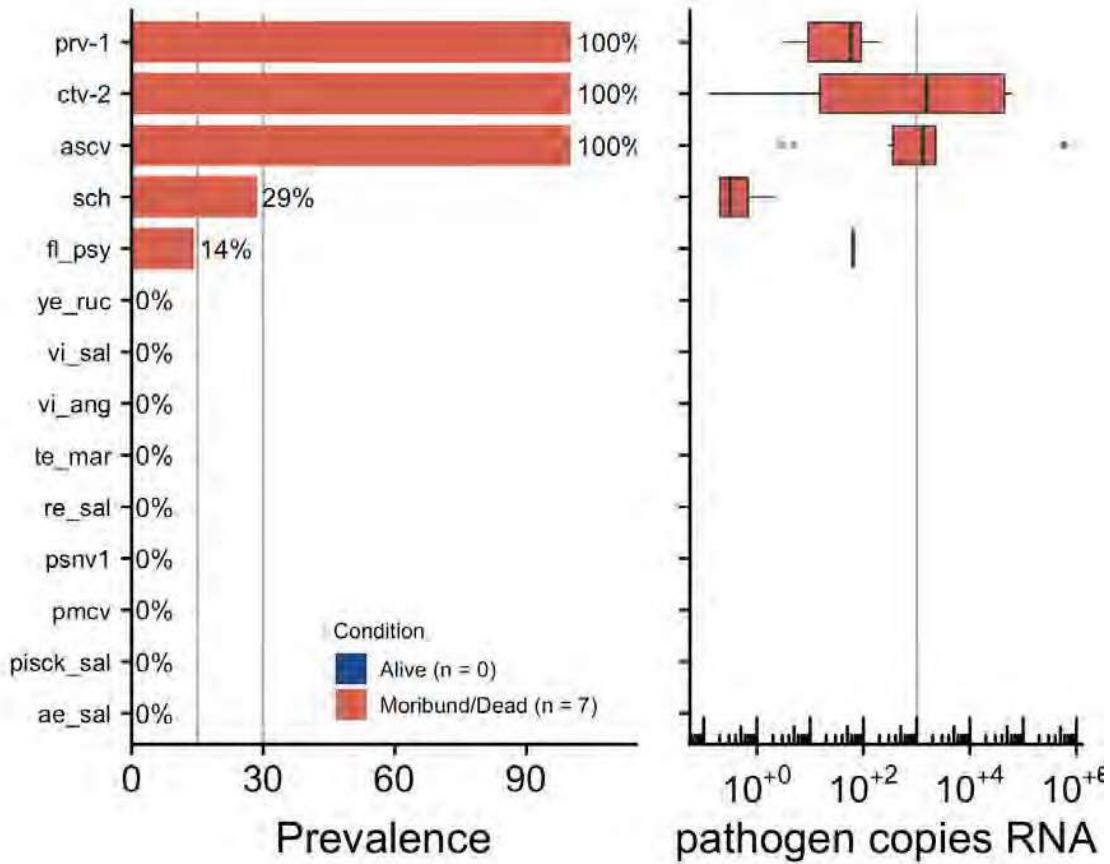


Water temperature ($^{\circ}\text{C}$), salinity (ppm), and dissolved oxygen (mg/L) at a 5m depth. Certain sampling dates have no recorded environmental data, resulting in gaps in the plots.

Overall infectious agent prevalence



Infectious agent prevalence in samples collected on 2022-01-26.



Infectious agent prevalence in samples collected on 2022-01-26, split by mortality status at time of sampling. Any specimens that were not confirmed to be either moribund or live at the time of generating this report are excluded from this figure.

Individual infectious agent trends

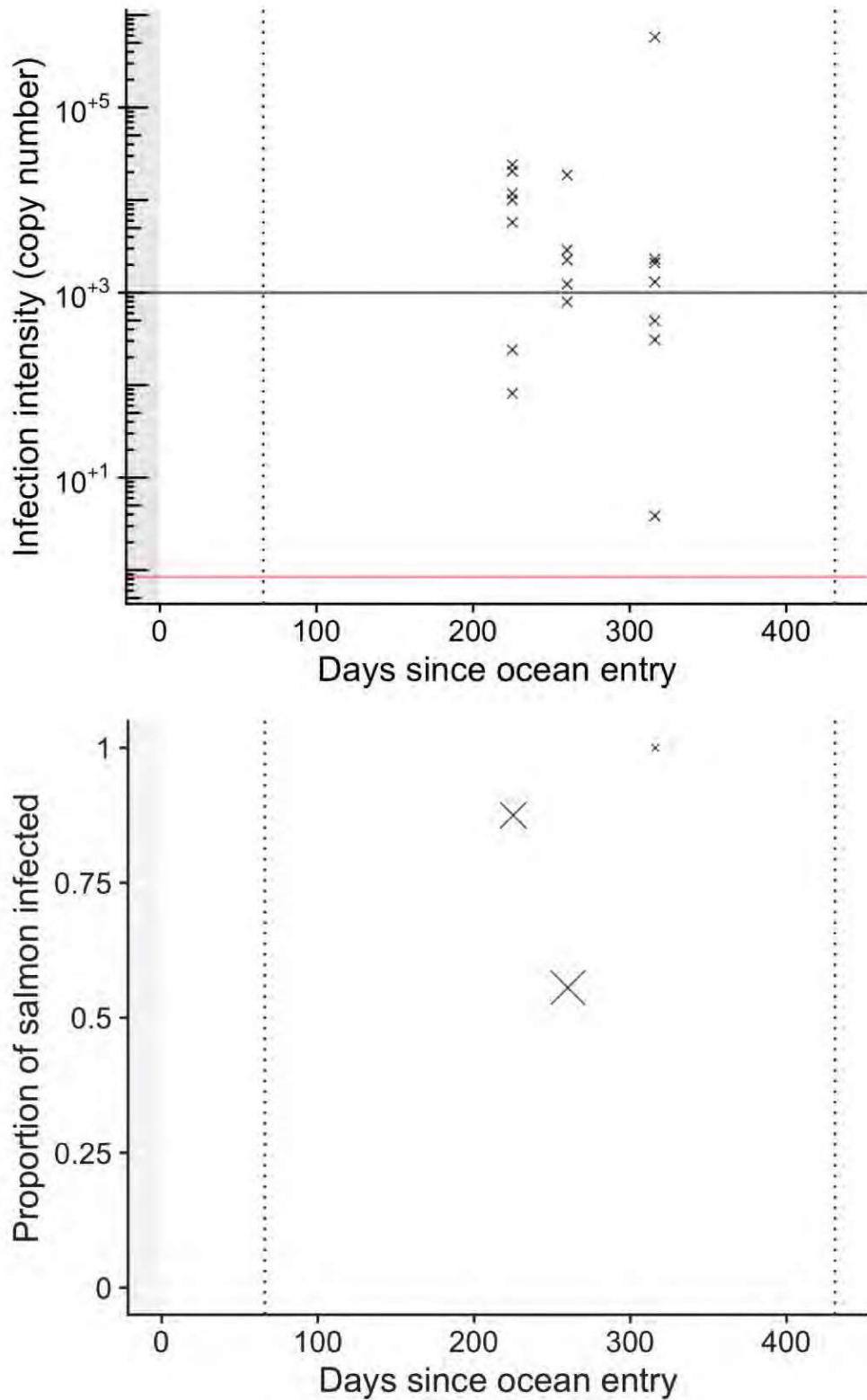
The following plots show individual infectious agent trends across all farm sites. In cases where sample size is sufficient, curves from a generalised additive model are included in the plot.

Grey circles represent live fish, and black X's represent dead/dying fish. Curves indicate mean predictions from a generalised additive model; blue and red correspond to live and dead/dying fish, respectively (shaded areas show 95% confidence regions). Left-hand grey region indicates freshwater hatchery residence, grey regions on x-axis indicate period of transfer to another site, and vertical dotted lines correspond to January 1st.

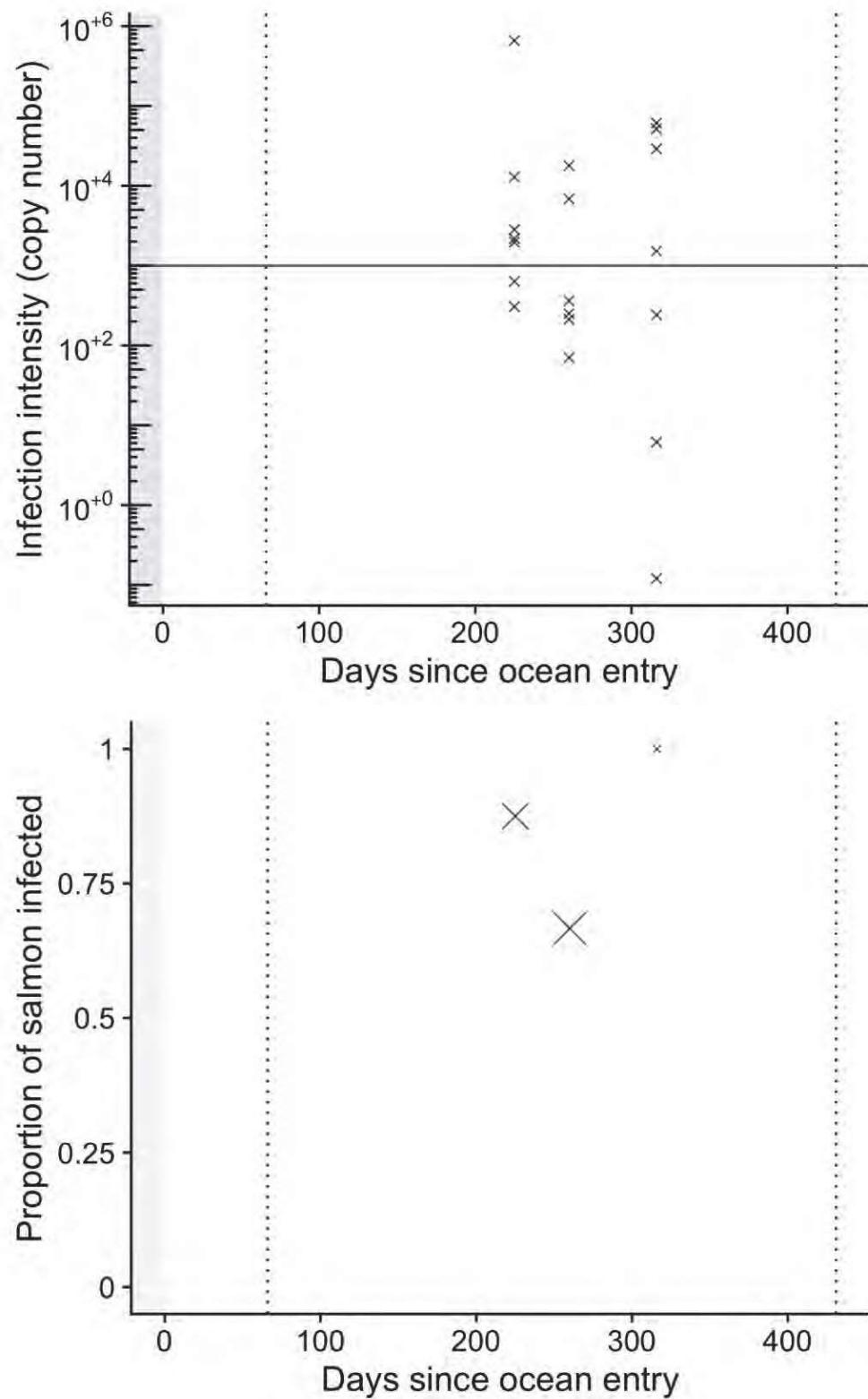
For infection intensity plots, horizontal red line indicates limit of detection (yielding ~90% true positive rate) for respective qPCR assay run in duplicate, while the horizontal black line indicates 1000 copies. Note log scale.

For proportion plots, grey circles show prevalence in live fish on each sampling date, and black X's show prevalence in dead/dying fish (symbol areas proportional to sample sizes).

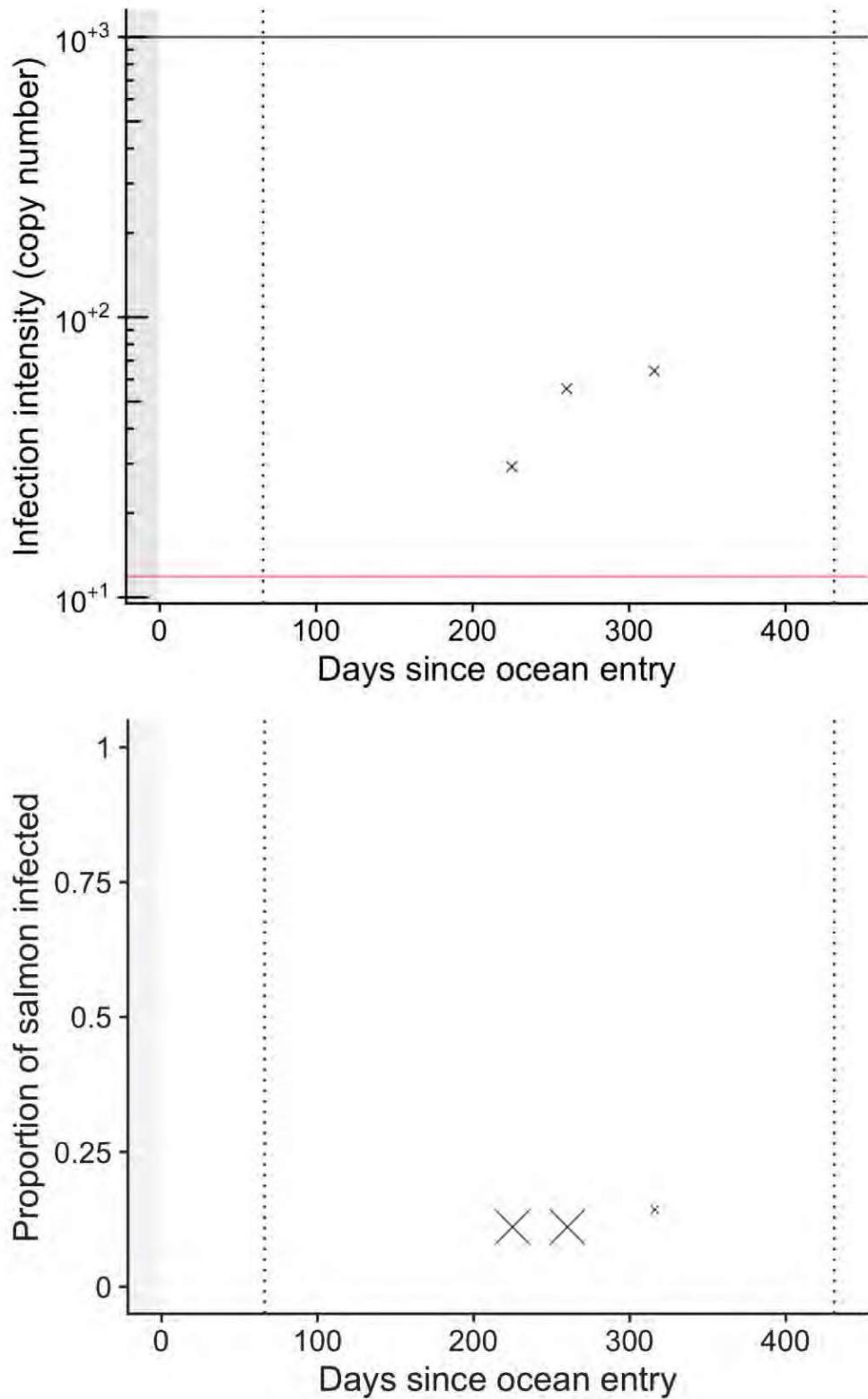
Atlantic salmon calicivirus



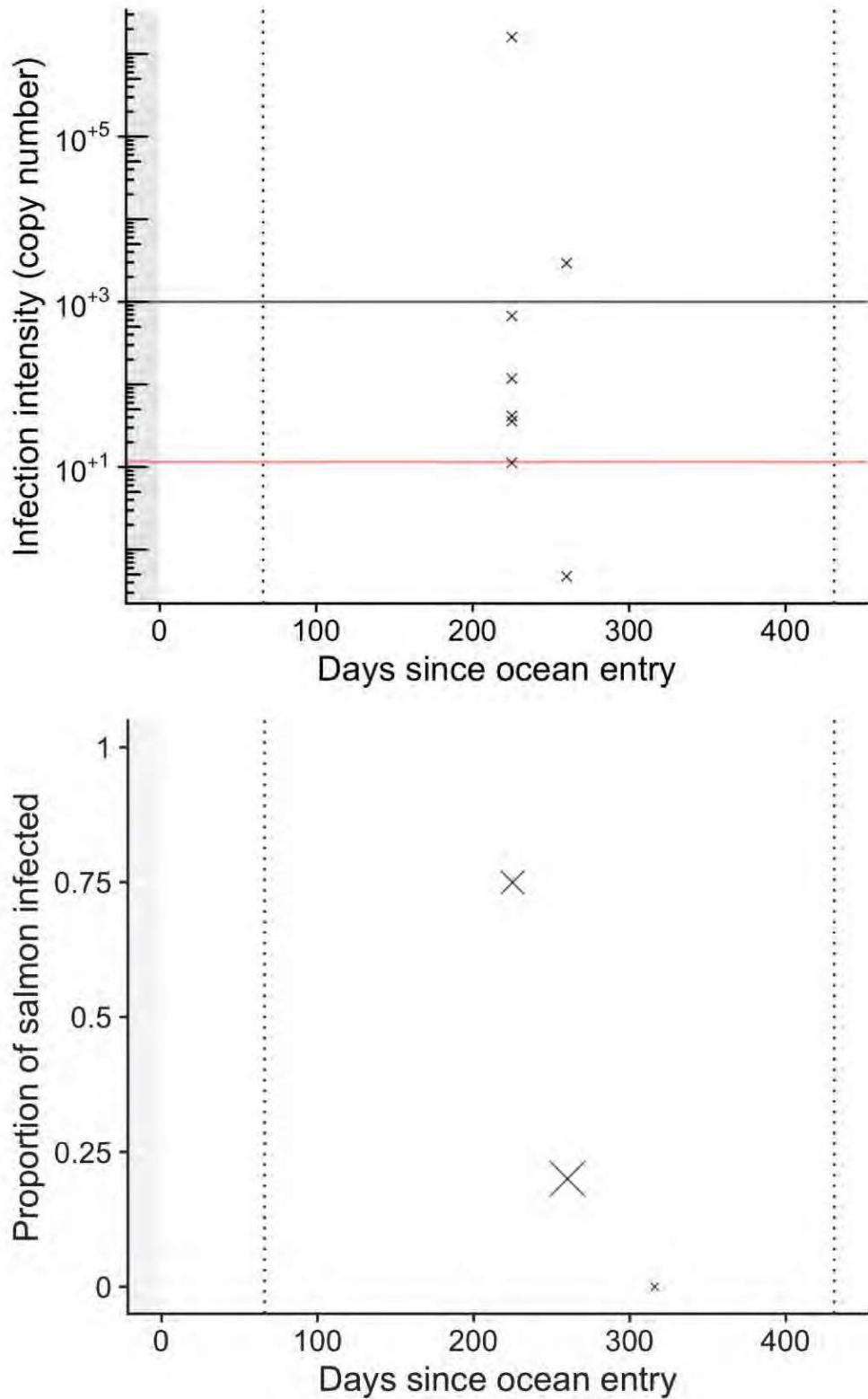
Cutthroat trout virus-2



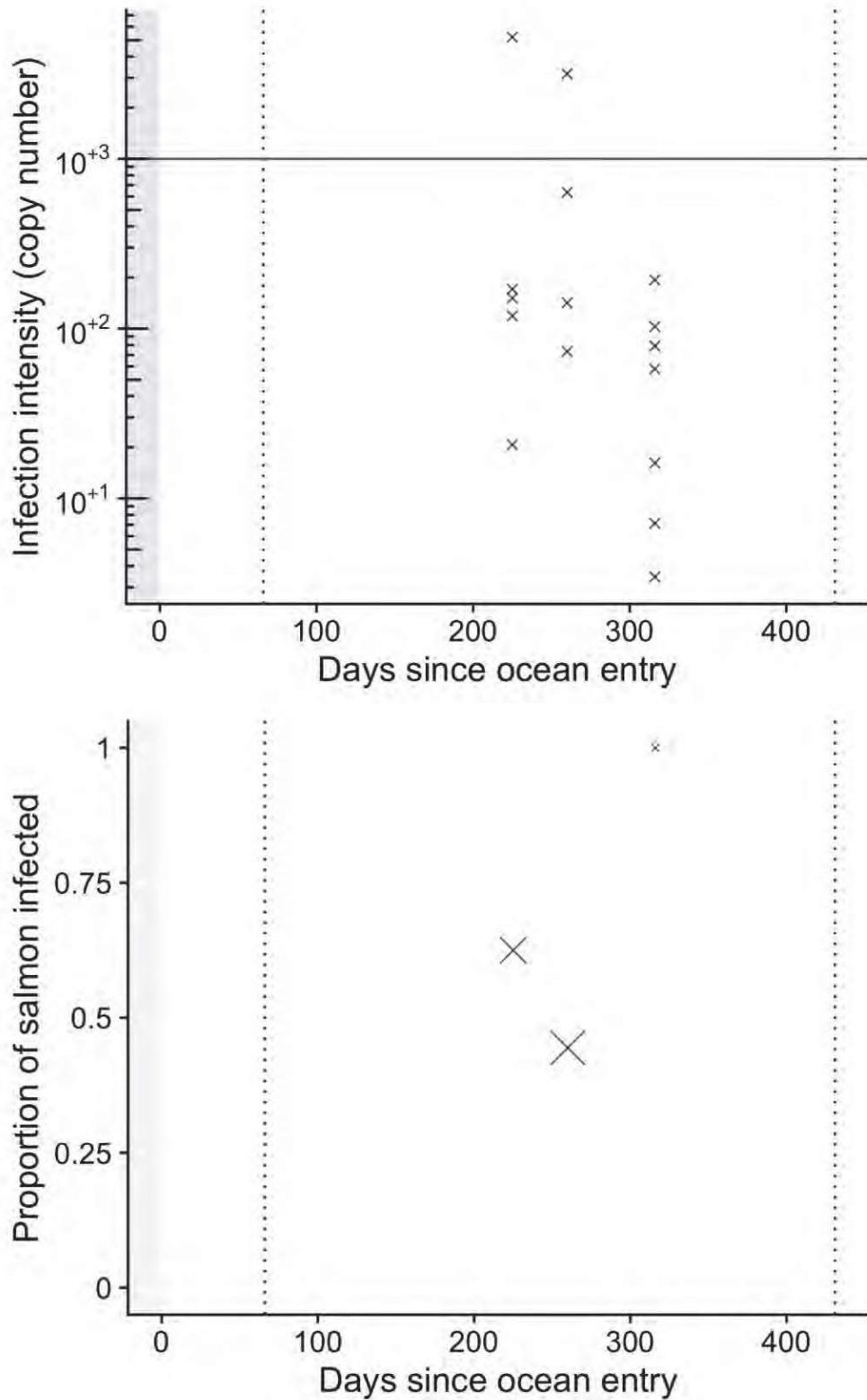
Flavobacterium psychrophilum



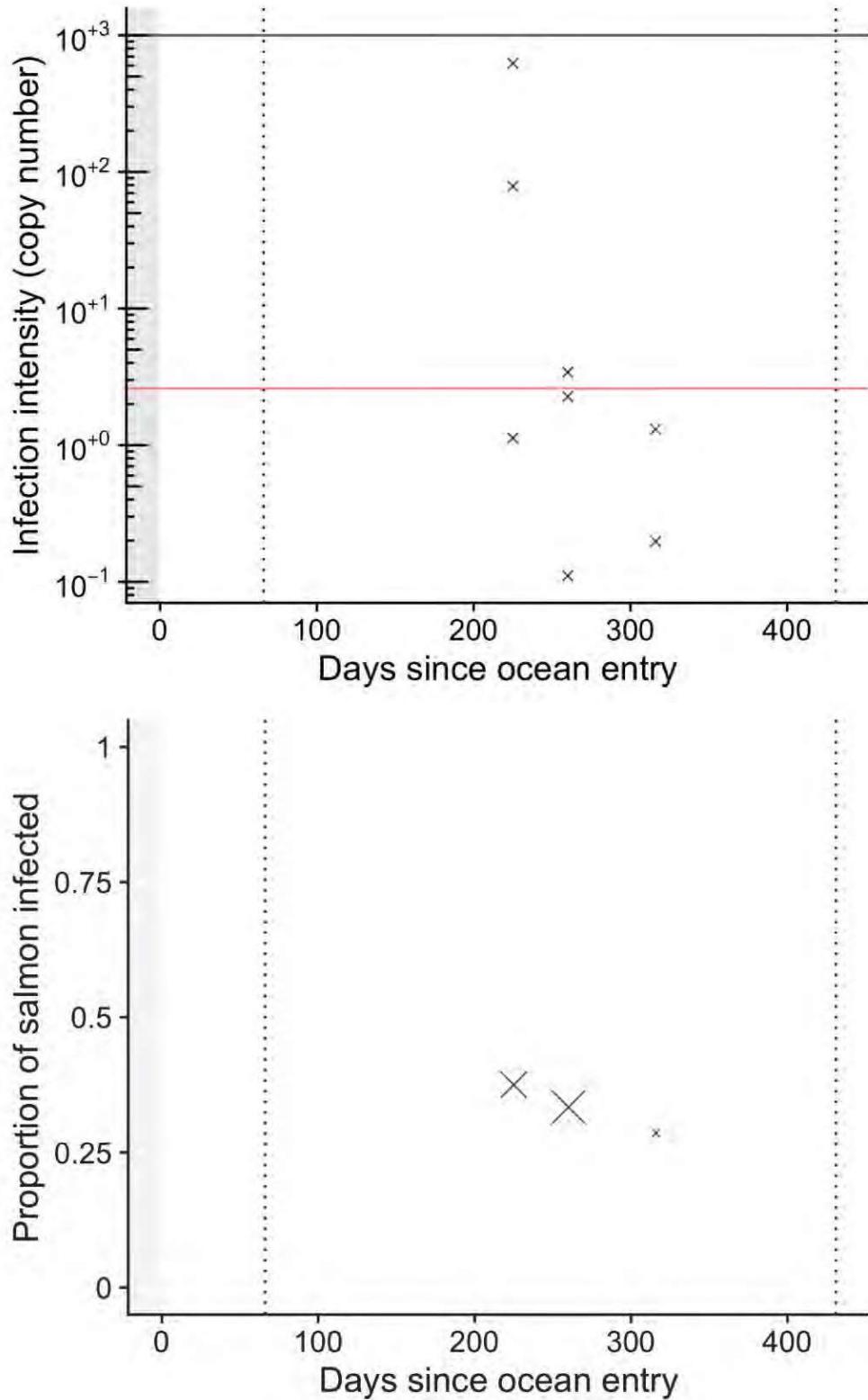
Piscirickettsia salmonis



Piscine orthoreovirus



Candidatus Syngnathia salmonis



Clinical signs

Table 1: Clinical signs for specimens sampled on 2022-01-26

metric	N5721	N5722	N5723	N5724	N5725	N5726	N5727
General							
Moribund	X						
Mort		X	X	X	X	X	X
Skeletal Deformity	X						
Skin & Fins							
Erosion	X	X			X	X	X
Ulcers	X	X		X	X	X	X
Lost Scales	X				X		
Gills							
Pale	X				X	X	X
Excess Mucous		X					
Muscle							
Hemorrhages		X	X		X		X
Boils	X						
Abdominal Cavity							
Adhesions	X	X					X
Ascites	X			X	X		
Hemorrhages		X	X	X			
Spleen							
Enlarged	X	X	X	X		X	X
Liver							
Pale	X		X	X			X
Hemorrhages/Congestion						X	
Gallbladder							
Enlarged	X	X		X		X	
Green		X				X	
Heart							
Pale					X	X	X
Kidney							
Pale	X						
Intestine							
Hemorrhages/Congestion							X

Histology

Table 2: Histology scores for specimens sampled on 2022-01-26

metric						N5727	
Heart							
Peri Epi	1			2	2		1
Myo	1	2					
Liver							
Cong Haem		2			2	1	1
Nec			1	1		1	1
Spleen							
Cong Heam	2	2	2	2	2	2	2
Ellip Nec			1		2		2
W Pulpitis	2	1	1	2	1	2	1
Pig Inc		1					
Kidney							
Itis		1					
Cong Heam		2					
Interst Hyperplasia	1	2	2	2	2	2	1
Cnc							
Cong Heam		1			2		2
Gills							
Itis		nv	nv		nv	nv	nv
Cong Heam		nv	nv		nv	nv	nv
Prolif		nv	nv		nv	nv	nv
Skin_muscle							
Itis Nec		2					2
Tissue							
Necrosis Artefacts		2	2		2	2	2

Diagnoses and Comments

Table 3: Diagnoses and comments for specimens sampled on 2022-01-26

DFO ID	Diagnosis	Comments
N5722	Bacterial Colonies In The Skin (2), Myocardioneerosis (2)	
N5723	Fibrotic Fringes In Epicardium (2)	
N5724	Kudoa In Muscle (1), Erythrophagocytosis (1)	
N5727	Hemorrhages In Intestine And Pancreas (2), Peribiliary Immune Activation (1)	

Conclusions

In order to support the eDNA study, below is provided further evaluation of the results of testing from the Fish Health Report.

The sampling collection was completed. This is a particular farm, due to the presence of different generations of brood stock reared in the same site. The disposition of the cages in the farm is also atypical, and the fish undergo frequent grading and subdivisions. No live fish were collected, as per agreement with the company, but available moribund/mort fish from all the pens were collected. Here below is a summary and evaluation of the findings from the sampled fish.

The farm was inspected in its entirety. Most fish in the examined pens were behaving normally. The morts are collected once a week by divers, therefore an estimation of the mortality rate is less accurate and indicative of the overall conditions of the fish. However, the mortality per pen reported by the company resulted significantly elevated in the pens containing the younger fish (i.e. transferred the previous year). No predation mortality was reported anymore.

Clinically, there was a high incidence of skin ulcers and erosions, muscle hemorrhages and enlarged spleen. Other findings commonly observed in the fish collected include ascites and hemorrhages in the abdominal cavity, pale liver (+/- hemorrhages) and pale heart.

Molecular testing results show that about 100% of the individual tested were positive to PRV. *Candidatus Syngnathia salmonis* and *Flavobacterium psychrophilum* were also detected at 29% and 14% prevalence, respectively.

Histopathologically, a congestive/hemorrhagic and inflammatory pattern of alterations, distributed in most internal organs and associated with skin lesions, was observed, and was suggestive of a septicemic condition. However, no differential bacterial species among the ones tested in our IMIP panel was detected, with the exception of a single individual showing a low amount of *F. psychrophilum* in the tissues. On the other hand, *Vibrio ordalii* (causative agent of atypical vibriosis), or other bacterial pathogens, should not be completely ruled out.

Appendix

Glossary of infectious agents

Agent abbr.	Full agent name	Agent type	Disease	Ranking
ae_sal	Aeromonas salmonicida	Bacteria	Furunculosis	2
ascv	Atlantic salmon calicivirus	Virus	unknown	4
ctv-2	Cutthroat trout virus-2	Virus	unknown	4
fl_psy	Flavobacterium psychrophilum	Bacteria	Bacterial cold water disease	3
pisck_sal	Piscirickettsia salmonis	Bacteria	Piscirickettsiosis (SRS)	2
pmcv	Piscine myocarditis virus	Virus	Cardiomyopathy syndrome	1
prv-1	Piscine orthoreovirus	Virus	HSMI-EIBS-Jaundice/anemia	
psnv1	Pacific salmon nidovirus-1 (CoV)	Virus	unknown	4
re_sal	Renibacterium salmoninarum	Bacteria	Bacterial kidney disease	2
sch	Candidatus Syngnathia salmonis	Bacteria	Gill chlamydia	3
te_mar	Tenacibaculum maritimum	Bacteria	Marine flexibacteriosis (mouth/fin rot)	2
vi_ang	Vibrio anguillarum	Bacteria	Vibriosis	2
vi_sal	Vibrio salmonicida	Bacteria	Cold water vibriosis	2
ye_ruc	Yersinia ruckeri (Enteric redmouth disease)	Bacteria	Yersiniosis (Enteric red mouth)	2

eDNA Study Report

Sir Edmund Bay sampling on January 27, 2022

Dr. Emiliano Di Cicco

September 23, 2022

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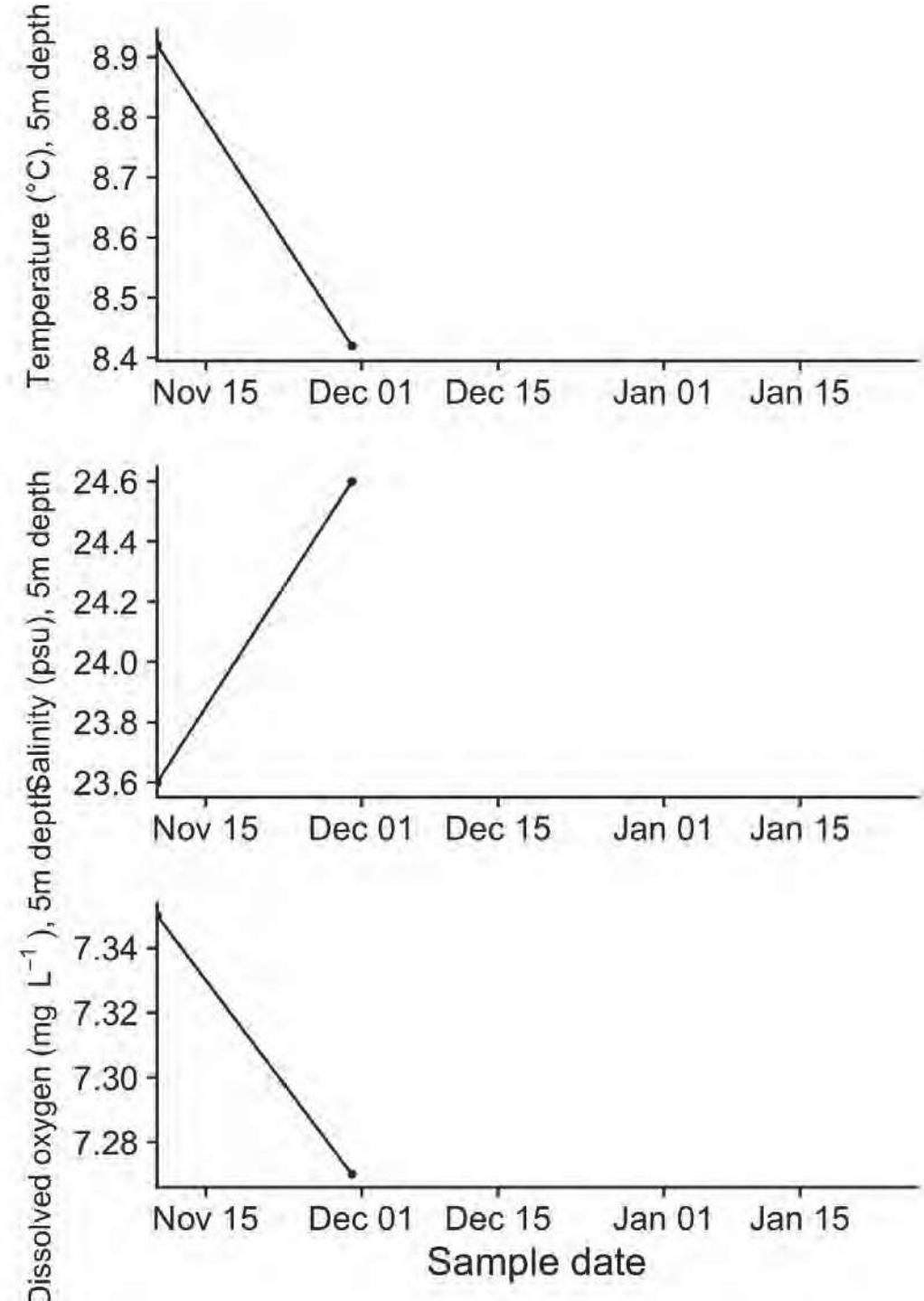
Executive summary

Premise

On January 27, 2022, 33 samples were collected by BATI and Cermaq crews during a sampling event at Sir Edmund Bay (Cermaq Ltd.). 33 Atlantic salmon subadults were collected from the Sir Edmund Bay farm site, including 26 live and 7 moribund/dead fish. All live fish were euthanized with TMS overdose prior to dissection with the exception of the moribund fish, which were administered a blow to the head. Portions of gill, liver and anterior kidney were collected in triplicate for molecular testing (preserved in RNA later) from all the fish, while all the moribund/dead fish also underwent collection of tissues (gills, spleen, liver, heart, anterior and posterior kidney, pyloric caeca, skeletal muscle + skin, brain) for histological analysis. Clinical notes and gross lesions were noted and reported for every fish. One aliquot has been provided to the Company Cermaq Fish Health, another aliquot is stored at the BATI Field Office, and a third aliquot is stored at DFO - PBS. This latter aliquot has been tested for the presence and load of the agents indicated in the IMIP agreement as well as the agents indicated in the eDNA study agreement. Each sample has been extracted and tested individually. Negative and positive controls were run. A housekeeping gene was also included to assess the quality of the RNA extracted.

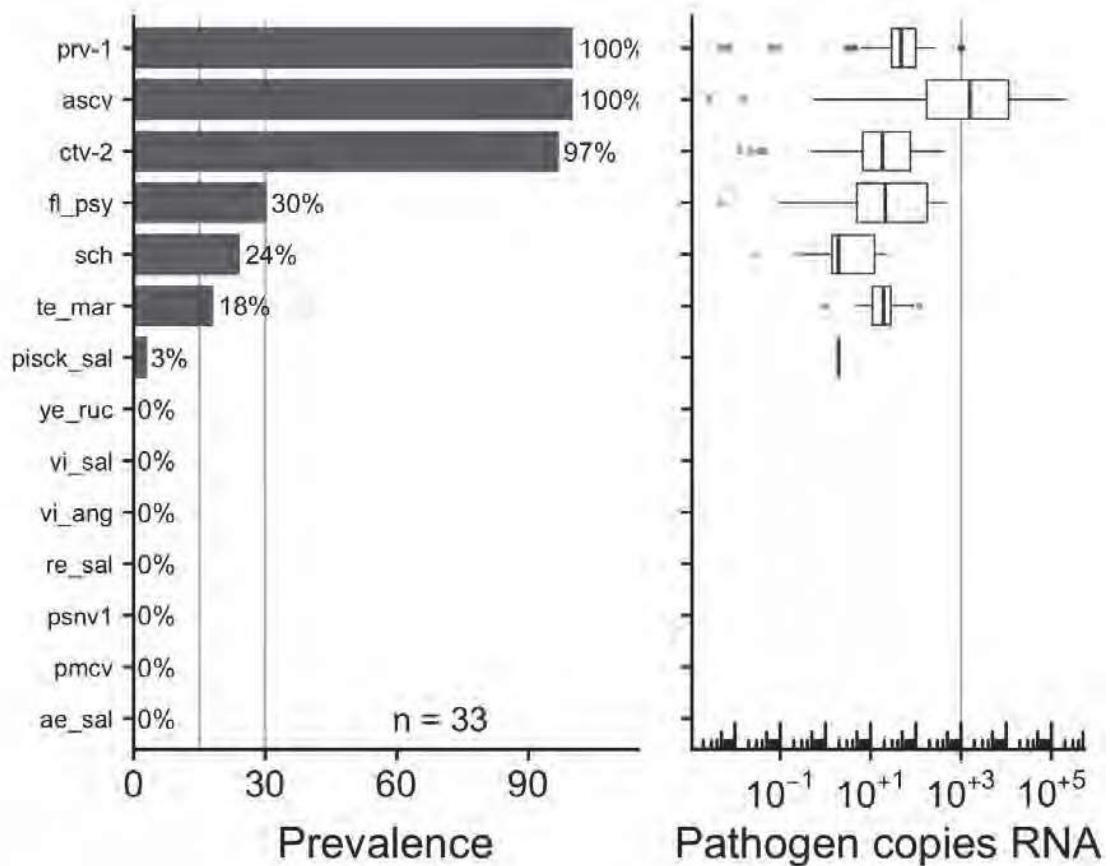
Histology samples have been sent to Wax-It Histo Ltd. to process and prepare slides, which have been read and scored by Dr. Di Cicco. A digital copy of each slide is available to the Company.

Environmental data



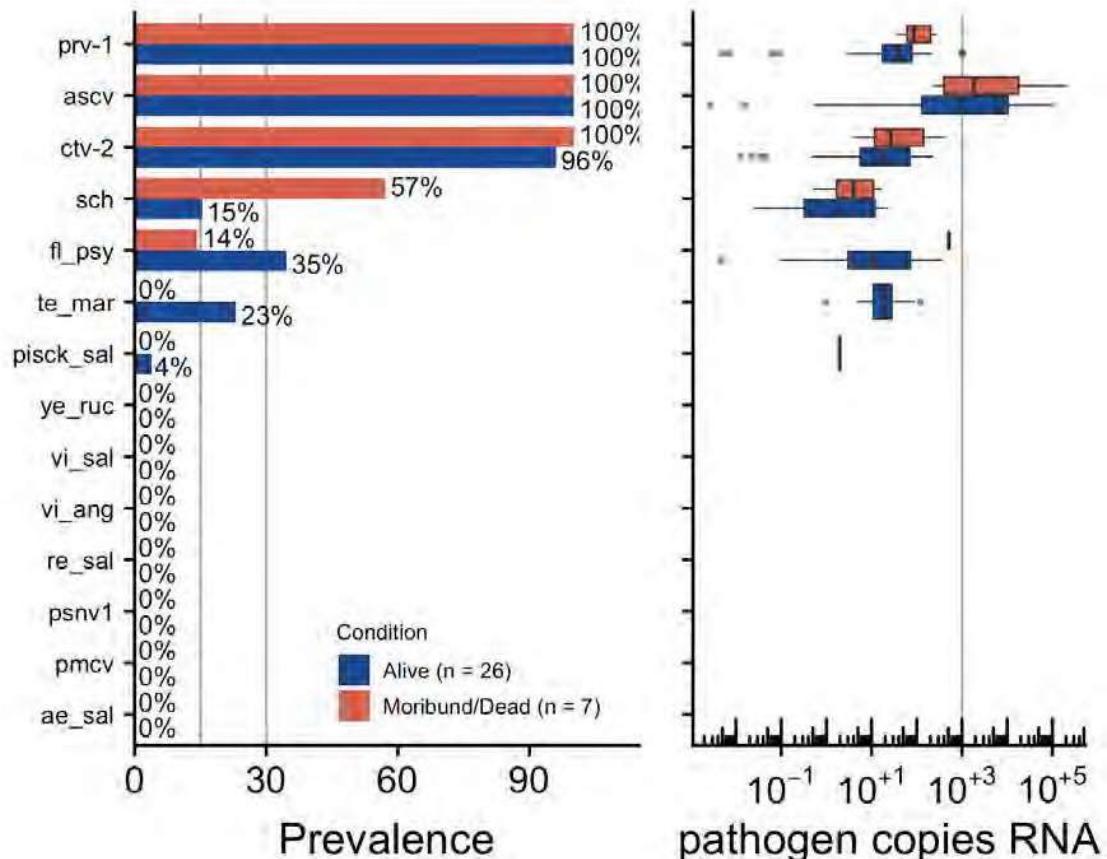
Water temperature ($^{\circ}\text{C}$), salinity (ppm), and dissolved oxygen (mg/L) at a 5m depth. Certain sampling dates have no recorded environmental data, resulting in gaps in the plots.

Overall infectious agent prevalence



Infectious agent prevalence in samples collected on 2022-01-27

Prevalence in healthy vs. moribund/dead fish



Infectious agent prevalence in samples collected on 2022-01-27, split by mortality status at time of sampling. Any specimens that were not confirmed to be either moribund or live at the time of generating this report are excluded from this figure.

Individual infectious agent trends

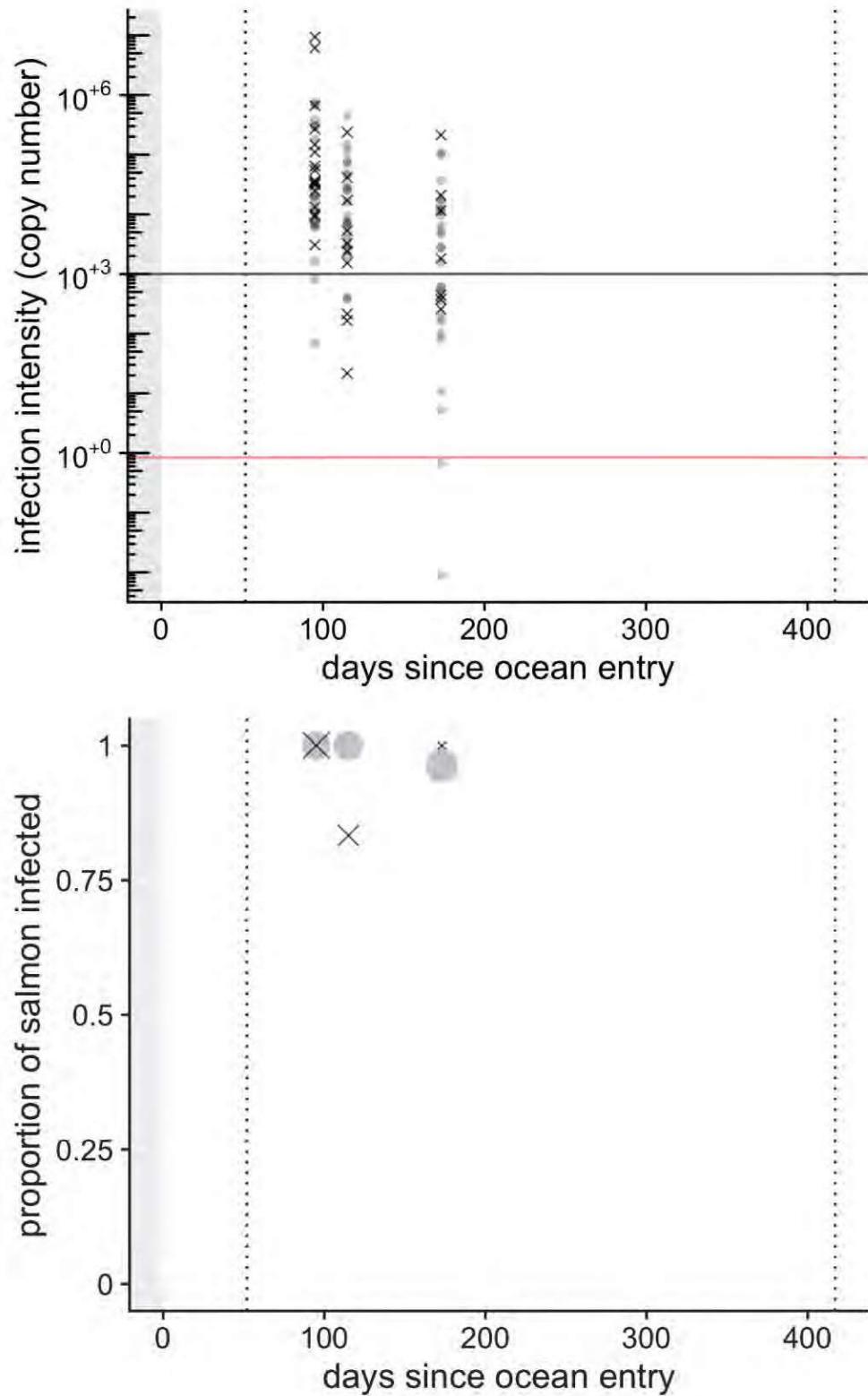
The following plots show individual infectious agent trends across all farm sites.

Grey circles represent live fish, and black X's represent dead/dying fish. Curves indicate mean predictions from a generalised additive model; blue and red correspond to live and dead/dying fish, respectively (shaded areas show 95% confidence regions). Left-hand grey region indicates freshwater hatchery residence, grey regions on x-axis indicate period of transfer to another site, and vertical dotted lines correspond to January 1st.

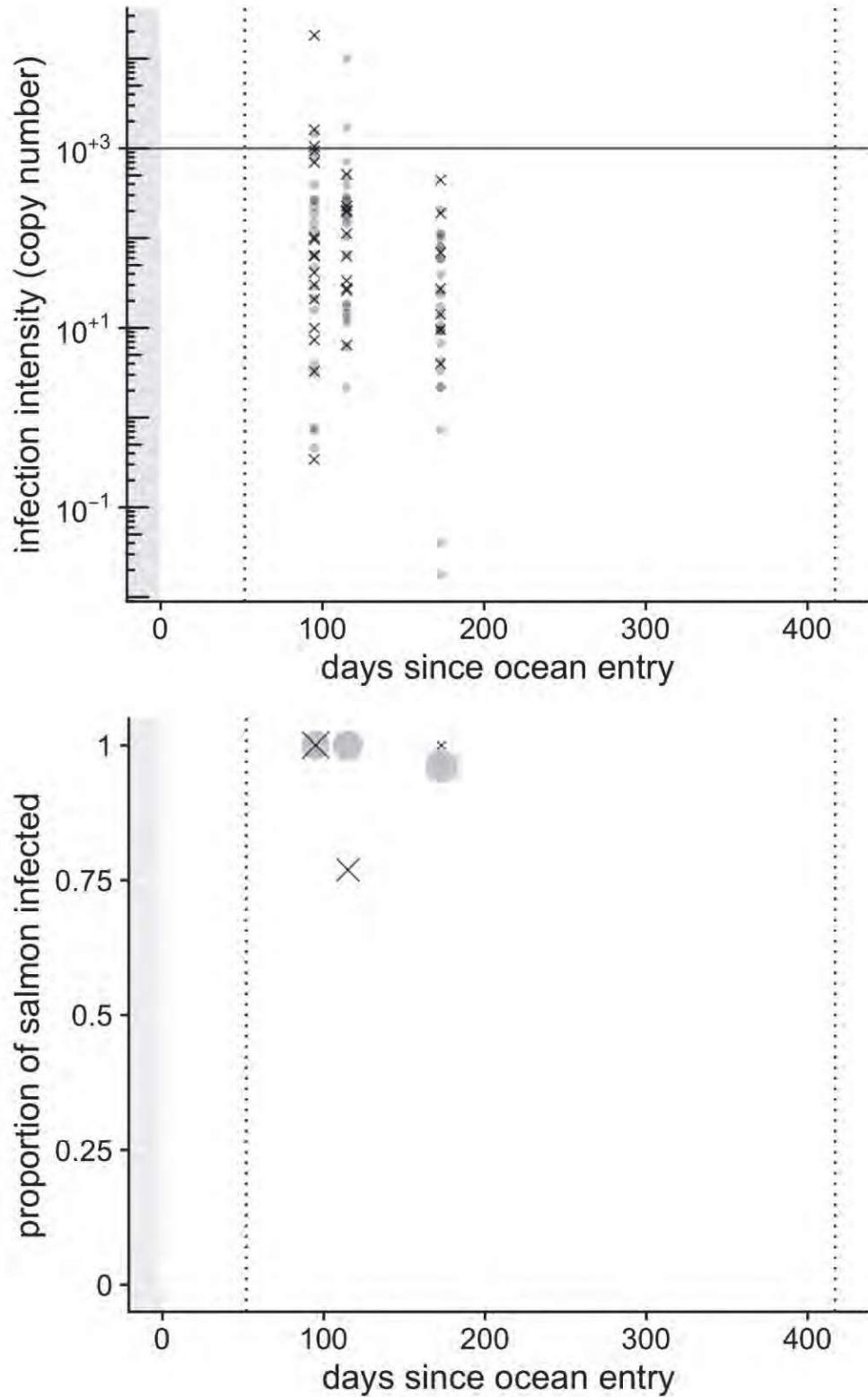
For infection intensity plots, horizontal red line indicates limit of detection (yielding ~90% true positive rate) for respective qPCR assay run in duplicate, while the horizontal black line indicates 1000 copies. Note log scale.

For proportion plots, grey circles show prevalence in live fish on each sampling date, and black X's show prevalence in dead/dying fish (symbol areas proportional to sample sizes).

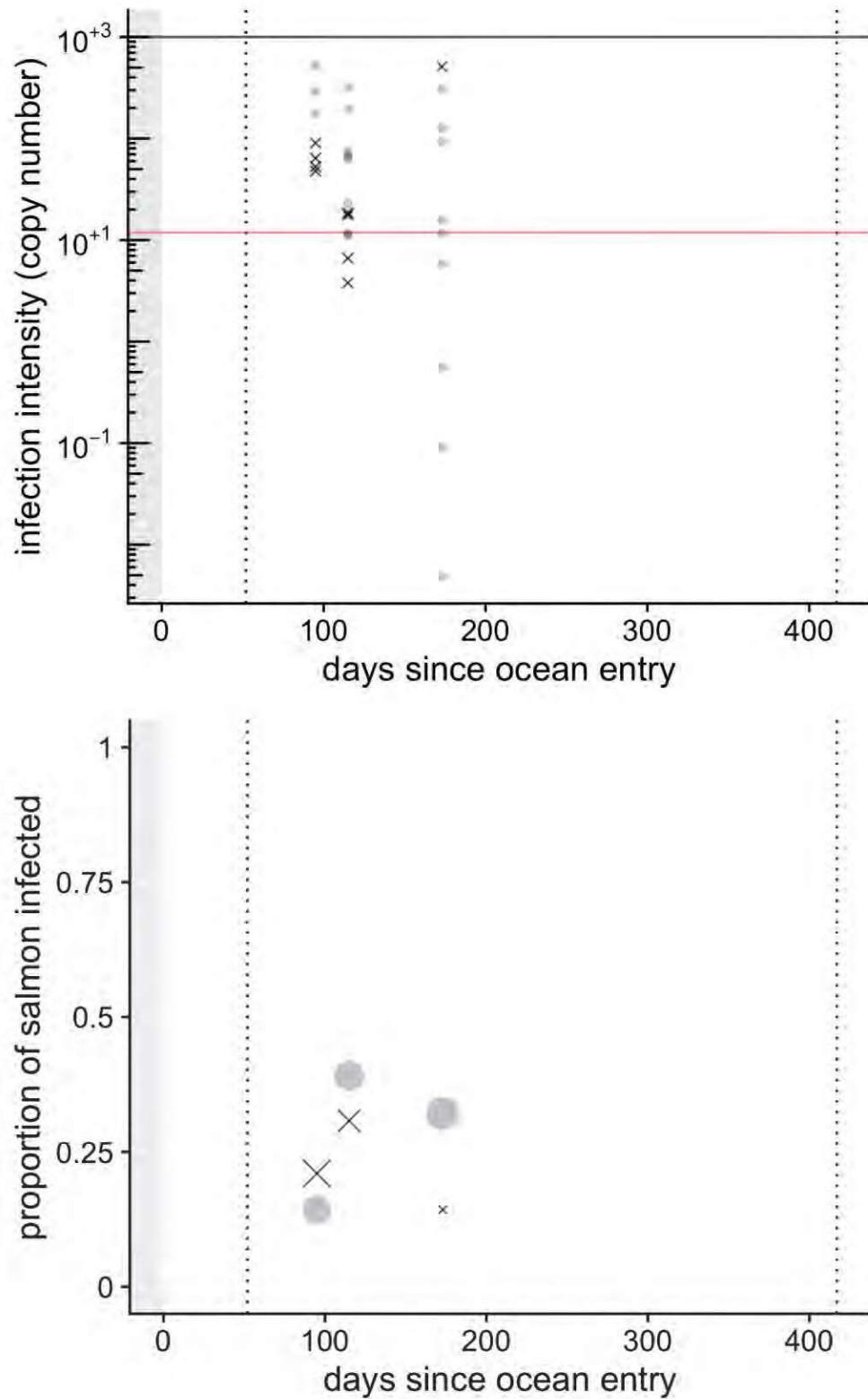
Atlantic salmon calicivirus



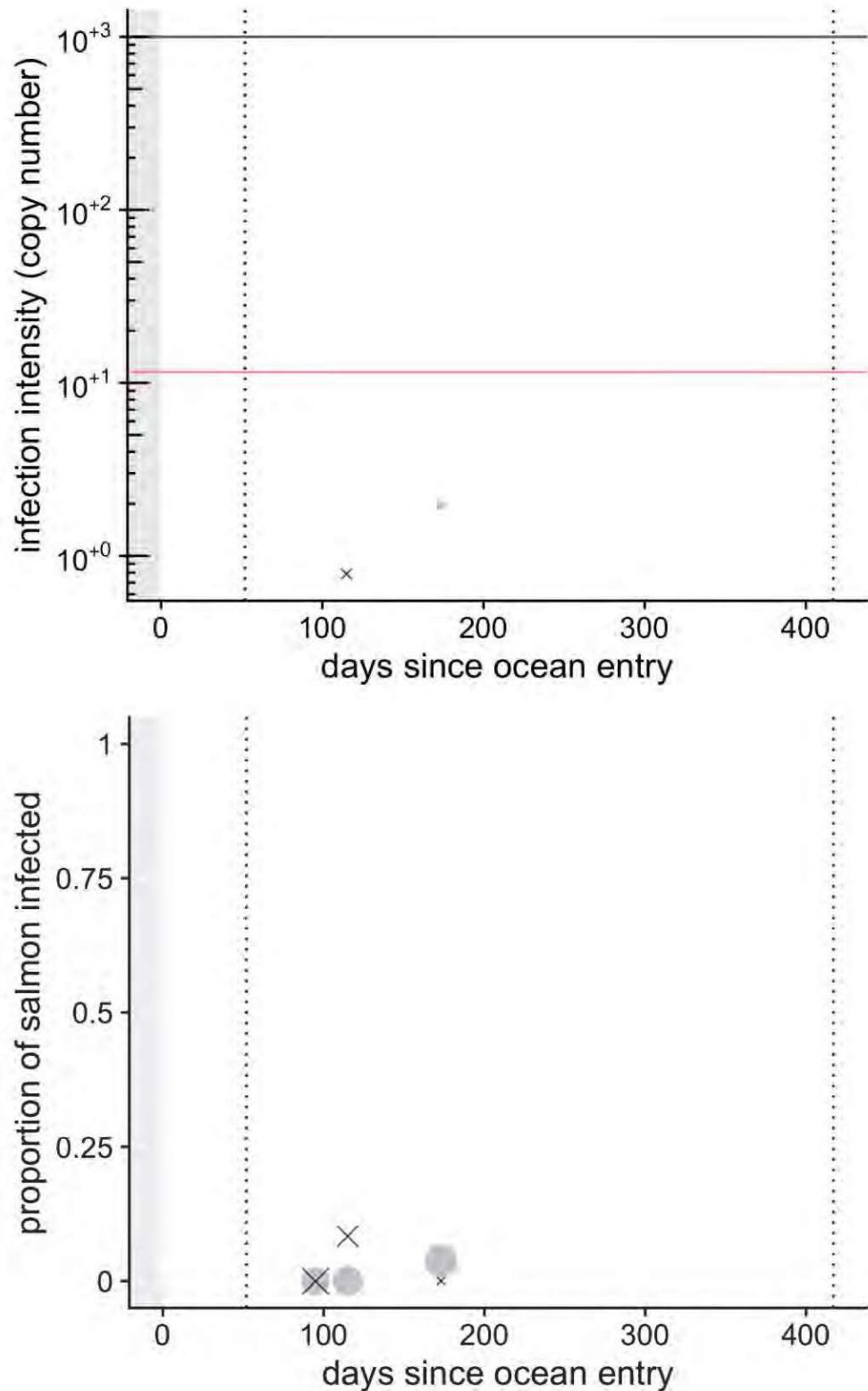
Cutthroat trout virus-2



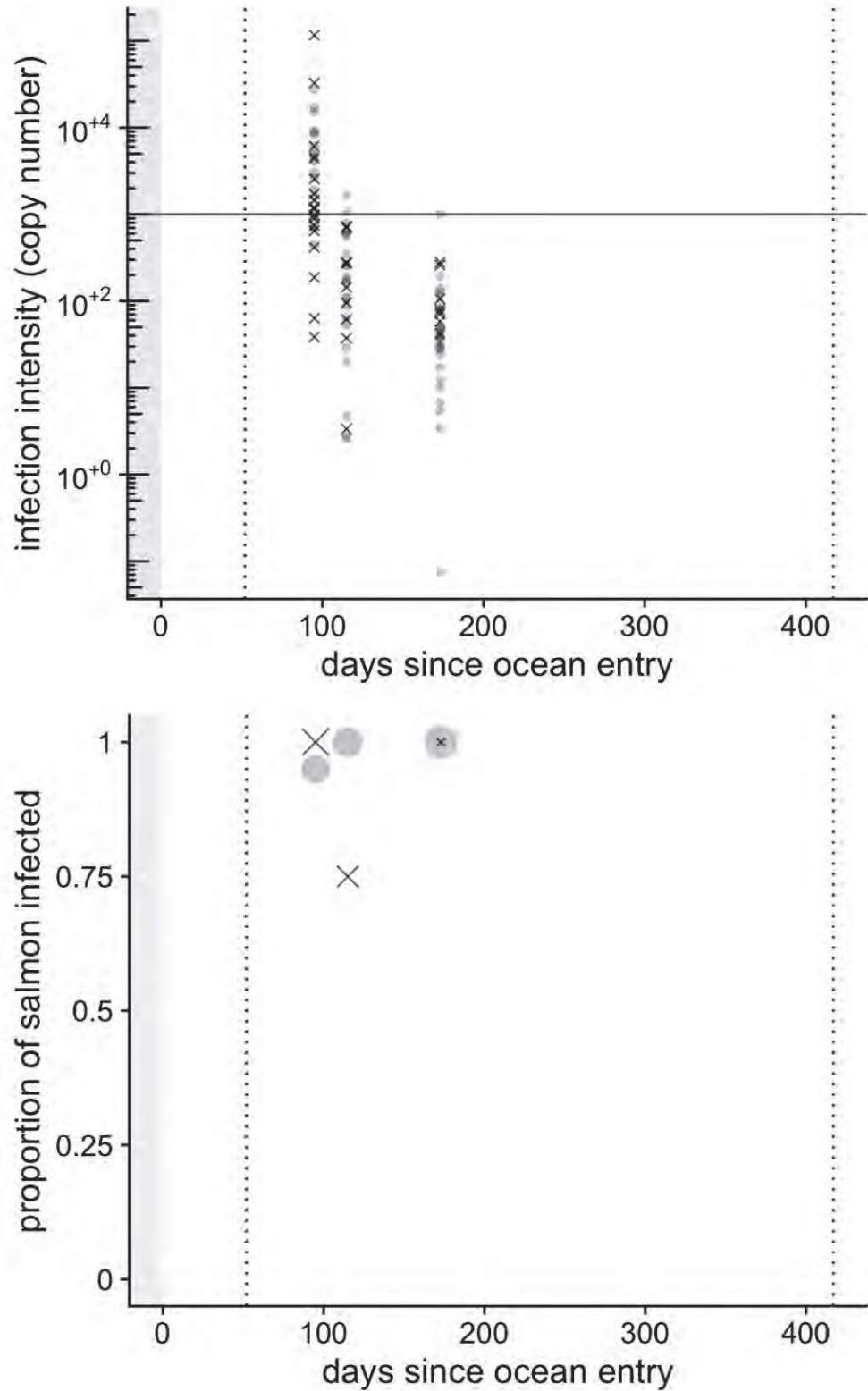
Flavobacterium psychrophilum



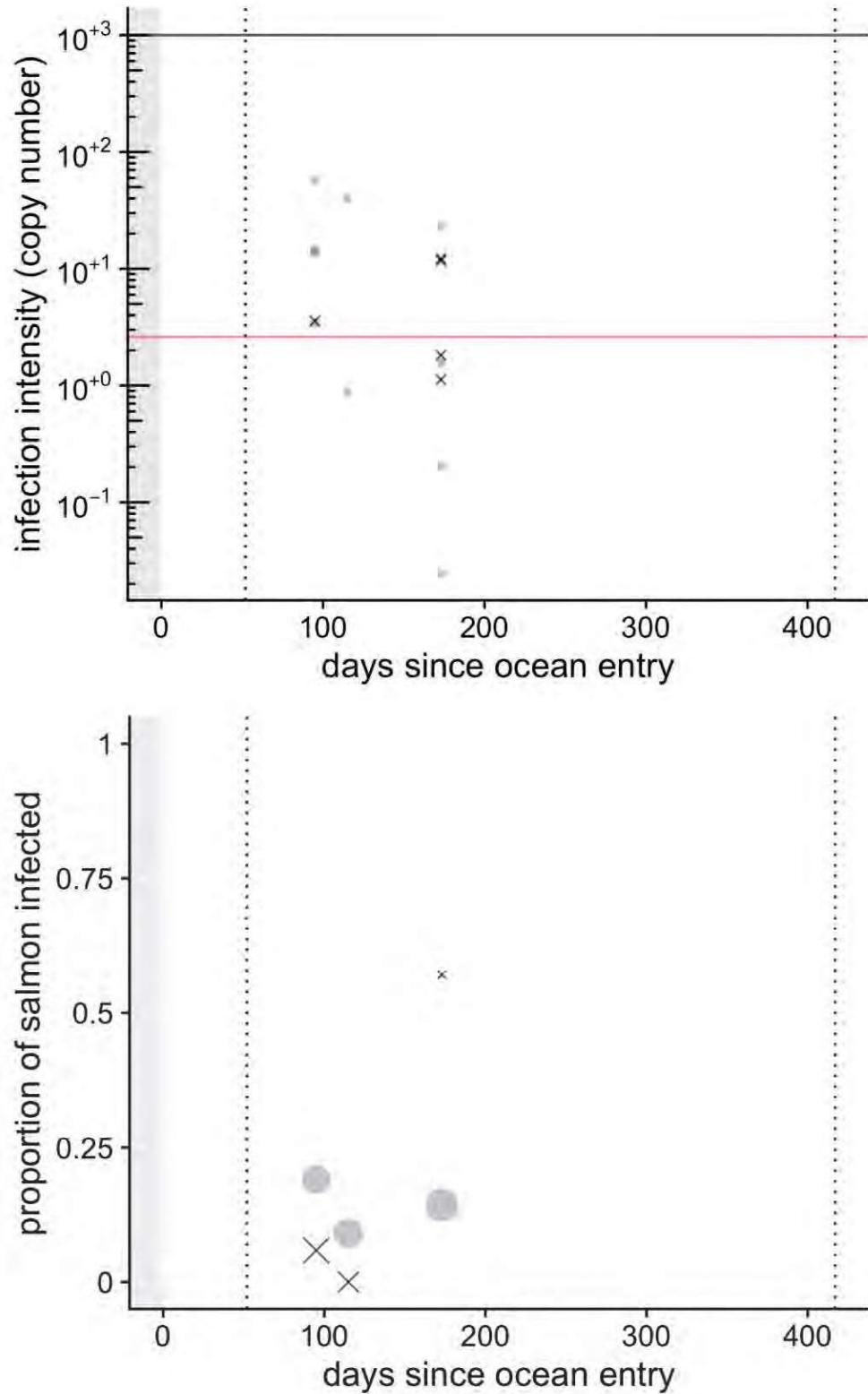
Piscirickettsia salmonis



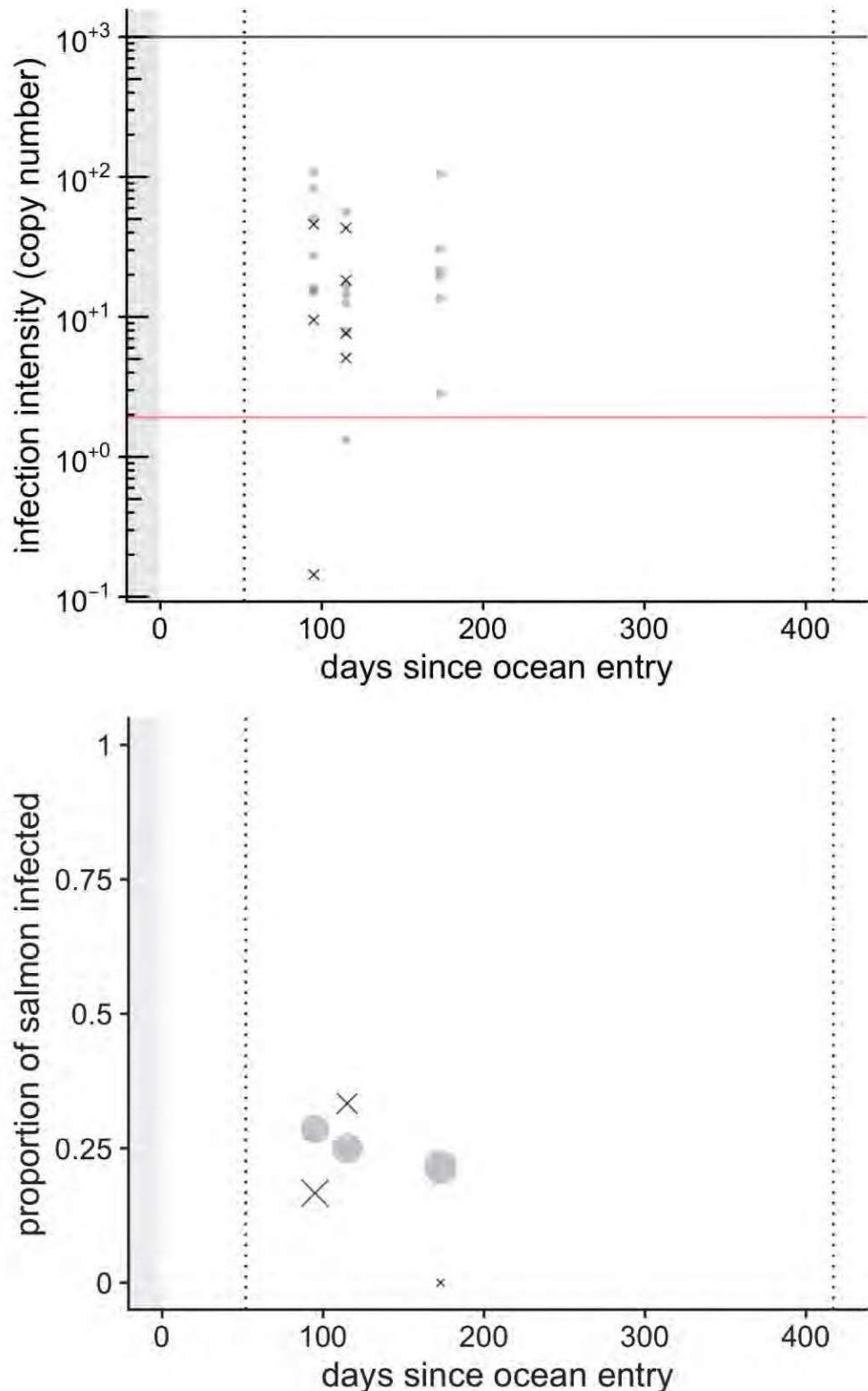
Piscine orthoreovirus



Candidatus Syngnathia salmonis



Tenacibaculum maritimum



Clinical signs

Table 1: Clinical signs for specimens sampled on 2022-01-27

metric	N5697	N5696	N5695	N5694	N5693	N5692	N5691	N5690	N5689	N5688	N5687	N5686	N5685	N5684	N5683	N5682	N5681	N5703	N5704	N5705
General																				
Live	X	X	X						X	X	X	X	X	X	X	X	X	X	X	X
Poor Performer								X												
Erratic Swimmer				X																
Moribund			X							X										
Mort					X	X	X	X	X											
Exophthalmia		X																		
Skin & Fins																				
Hemorrhages	X																			
Erosion	X	X	X																	
Ulcers											X									
Lost Scales								X											X	
Parasites	X			X																
Gills																				
Short Operculum	X		X																X	
Erosions	X																	X		
Nodules/White Spots				X																
Muscle																				
Hemorrhages							X													
Abdominal Cavity																				
Body Fat Content	X	X																		
Adhesions	X	X	X		X				X	X								X	X	X
Ascites						X														
Spleen																				
Enlarged	X	X		X			X	X	X		X		X	X						X
Dark					X															
Liver																				
Pale						X	X		X				X					X		X
Dark					X				X											
Gallbladder																				
Enlarged					X			X	X	X	X	X					X	X	X	
Green						X	X		X											
Heart																				
Deformed				X	X															
Pale								X	X						X					
Kidney																				
Enlarged Swollen							X													
Brain																				
Hemorrhages/Congestion						X	X		X											

Table 2: Clinical signs for specimens sampled on 2022-01-27

metric	N5710	N5715	N5714	N5713	N5712	N5711	N5710	N5709	N5708	N5707	N5706	N5705	N5704	N5703	N5702	N5701	N5700	N5699	N5698
General																			
Live	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Poor Performer																			
Erratic Swimmer																			
Moribund																			
Mort																			
Exophthalmia																			
Skin & Fins																			
Hemorrhages																			
Erosion																			
Ulcers																			
Lost Scales																			
Parasites												X	X					X	X
Gills																			
Short Operculum																			X
Erosions												X		X				X	
Nodules/White Spots													X						
Muscle																			
Hemorrhages																			
Abdominal Cavity																			
Body Fat Content								X											
Adhesions												X	X	X	X				
Ascites																			
Spleen																			
Enlarged												X	X						
Dark																			
Liver																			
Pale																			
Dark																			
Gallbladder																			
Enlarged	X							X				X	X					X	
Green																			
Heart																			
Deformed																			
Pale														X					
Kidney																			
Enlarged Swollen																			
Brain																			
Hemorrhages/Congestion																			

Histology

Table 3: Histology scores for specimens sampled on 2022-01-27

metric	N5681	N5682	N5683	N5684	N5685	N5686	N5687	N5689
Heart								
Peri Epi	1	2	1	1		2	1	2
Myo		1	1		1	1	1	1
Liver								
Cong Haem		1			2			
Nec					1	1	1	1
Itis	1	1				1	1	1
Spleen								
Cong Heam		2	2	3	1	1	1	2
W Pulpitis	2	1	1	1	1	2		2
Pig Inc								2
Cap Prolif		2						
Kidney								
Cong Heam		1		2	2			
Interst Hyperplasia	1	1	1		1	2		2
Pancreatitis								
Pancreatitis								na
Enteritis								
Enteritis								na
Cns								
Itis								na
Cnc								
Malacia								na
Gliosis						1		na
Cong Heam	1	2	2	1	3			na
Microsporidia								na
Gills								
Itis			nv	nv	nv	nv		1
Cong Heam		2	nv	nv	nv	nv		
Prolif	1	nv	nv	nv	nv	nv		1
Skin_muscle								
Itis Nec					1			1
Tissue								
Necrosis Artefacts			2	2	2	1		

metric								N5689
								N5687
								N5686
								N5685
								N5684
								N5683
								N5682
								N5681

Diagnoses

DFO	ID	Diagnosis	Comments
	N5681		Increase fibrin in spleen (1), peribiliary immune activation (1)
	N5682	Visceral Mycosis	Hemorrhages on peritoneum (2), granuloma in liver and spleen (1)
	N5683		Peribiliary immune activation (1)
	N5685		Increase fibrin in spleen (3)
	N5686	Early HSMI	
	N5687		Steatosis (1)
	N5689	Early HSMI	Peribiliary immune activation (1), erythrophagocytosis (1)

Conclusions

In order to support the eDNA study, below is provided further evaluation of the results of testing from the Fish Health Report. The Fish Health sampling collection was completed. Available moribund/mort fish from the control pen and secondary pen were collected.

The farm was inspected in its entirety: the fish showed normal behavior. Reporting from the company indicated mortality that was within the normal range expected for this site.

Clinically, gills anomalies were more common in live fish (a few instances of short operculum, gill erosions and nodules). Enlarged spleen and gall bladder were prevalent findings in both live and morts, while morts showed also a wider array of lesions, particularly internally, pale or dark liver, pale and /or enlarged heart, ascites, swollen kidney and brain hemorrhages/congestion.

Molecular testing results indicate PRV present in the totality (100%) of the fish tested, even at high load in one fish. The prevalence of *Flavobacterium psychrophilum*, *Candidatus Syngnathus salmonis* and *Tenacibaculum maritimum* prevalence was also significant (30%, 24% and 18%, respectively), with 57% of the morts testing positive to *Candidatus Syngnathus salmonis*, and 35% and 23% of the live fish positive to *Flavobacterium psychrophilum* and *Tenacibaculum maritimum*, respectively). *Piscirickettsia salmonis* was present at background levels.

Histopathologically, the lesions were in general mild or moderate, including inflammatory or congestive modifications, particularly occurring in spleen and kidney. Brain hemorrhages/congestion and mild/moderate epicarditis associated with mild myo/endocarditis were also very common findings. These latter findings, associated with the pattern of lesions detected, the clinical signs observed and the molecular detections are suggestive of an early development stages of HSMI in at least two individual fish sampled (including a live fish). On the other hand, another fish presented granulomas in liver and spleen, conducive to visceral mycosis.

Appendix

Glossary of infectious agents

Agent abbr.	Full agent name	Agent type	Disease	Ranking
ae_sal	Aeromonas salmonicida	Bacteria	Furunculosis	2
ascv	Atlantic salmon calicivirus	Virus	unknown	4
ctv-2	Cutthroat trout virus-2	Virus	unknown	4
fl_psy	Flavobacterium psychrophilum	Bacteria	Bacterial cold water disease	3
pisck_sal	Piscirickettsia salmonis	Bacteria	Piscirickettsiosis (SRS)	2
pmcv	Piscine myocarditis virus	Virus	Cardiomyopathy syndrome	1
prv-1	Piscine orthoreovirus	Virus	HSMI-EIBS-Jaundice/anemia	NA
psnv1	Pacific salmon nidovirus-1 (CoV)	Virus	unknown	4
re_sal	Renibacterium salmoninarum	Bacteria	Bacterial kidney disease	2
sch	Candidatus Syngnathida salmonis	Bacteria	Gill chlamydia	3
te_mar	Tenacibaculum maritimum	Bacteria	Marine flexibacteriosis (mouth/fin rot)	2
vi_ang	Vibrio anguillarum	Bacteria	Vibriosis	2
vi_sal	Vibrio salmonicida	Bacteria	Cold water vibriosis	2
ye_ruc	Yersinia ruckeri (Enteric redmouth disease)	Bacteria	Yersiniosis (Enteric red mouth)	2

Appendix 8



Fisheries
and Oceans

Pêches
et Océans

Title: Viral survival and infectivity in effluent from a fish processing plant

Report by:

Kyle Garver, Ph.D.
Aquatic Animal Health Section
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Fisheries & Oceans Canada
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Nanaimo, BC V9T 6N7

Email: Kyle.Garver@dfo-mpo.gc.ca

Overview: Fish processing plant effluent has the potential to be a point source of organics and infectious agents. While organic material loads are relatively easily measured, assessing the infectivity of aquatic pathogens in discharged effluent can be challenging. Consequently, the risk of processing effluent as a point source in the spread of endemic aquatic pathogens is not easily quantified. In particular, viral agents known to be common to cultured salmon in British Columbia such as piscine orthoreovirus (PRV), has raised concern regarding the potential release of live virus through fish processing effluent. In this study, we examine the efficacy of a dissolved air floatation (DAF) treatment system installed at Brown's Bay Processing Plant in reducing the load of PRV in effluent generated from the processing of farmed Atlantic salmon infected with PRV.

Experimental Design:

Immersion exposure

Approximately eighty to one hundred liters of DAF treated effluent was collected every hour from 9 am to 2 pm on April 11, 2022 from Browns Bay Processing Plant while farmed Atlantic salmon were being processed at the plant. The collected effluent was transported in insulated totes to the Pacific Biological Station where it was acclimated to 10-12°C overnight. The following morning, 200 liters of the DAF treated effluent was placed into a circular tank. In another tank, 100 liters of DAF treated effluent was mixed with 100 liters of saltwater (32 ppt, 10-12 °C). In each tank, 10 Atlantic salmon were immersed in the static effluent baths for 2 hours after which saltwater (32 ppt, 10-12 °C) was added to each tank at a rate of 7 liters per minute. Fish were monitored daily for 6 weeks at which time, all fish were euthanized and blood was collected and analyzed for the presence of PRV.

Injection exposure

Twenty milliliters of DAF treated effluent and twenty milliliters of untreated blood water, collected during the April 11, 2022 processing of Atlantic salmon, were each centrifuged at 400 G for 5 minutes. Post centrifugation, the aqueous phase was removed and passed through a 0.45 micron filter whereby the resulting filtrate were used as inoculums in the injection exposure groups. In one tank, ten Atlantic salmon received an intraperitoneal injection of 500 microliters of the DAF treated effluent while in a second tank ten Atlantic salmon received an intraperitoneal injection of 500 microliters of the blood water. The injected fish were placed in saltwater (32 ppt, 10-12 °C) and monitored daily for four weeks. At the end of four weeks, fish were euthanized and blood was collected and analyzed for the presence of PRV.

Screening for PRV

Blood was obtained from a caudle puncture using a 1 milliliter syringe and 22 gauge needle and transferred to a 1.5 milliliter microtube on ice. Total RNA was extracted from 100 microliters blood, untreated blood water and DAF treated

effluent in Trizol reagent (Life Technologies) as per manufacturer's instructions that implemented a 5 millimeter steel bead and TissueLyser II (Qiagen) operating for 2 minutes at 25 hertz. A portion of eluted RNA (1.0 µg) was denatured for 5 minutes at 95°C, immediately cooled to 4°C, and reverse-transcribed using a High-Capacity cDNA Reverse Transcription kit (Life Technologies) following the manufacturer's instructions. Resulting cDNA was used directly as template for qPCR analysis in a StepOne-Plus real-time detection system (Applied Biosystems) using previously described primers and TaqMan probe (Zhao et al. 2021). Samples were assayed in duplicate and were considered positive if both technical replicates reported a Ct value < 40 cycles, inconclusive if only one technical replicate reported a Ct value < 40 cycles, or negative if both technical replicates failed to fluoresce beyond the preset threshold (ΔRn 0.01) in 40 cycles.

Results:

No mortality or external signs of disease were noted in any of the treatment groups over the entire duration of the experiment. Blood sampled from ten Atlantic salmon that remained unexposed to effluent proved negative for PRV demonstrating the stock of fish used in the effluent exposure experiment were free of PRV prior to exposure. Four to six weeks post effluent exposure, Atlantic salmon tested positive for PRV in the blood. A summary of PRV RT-qPCR screening results across all treatment groups is shown in Table 1.

Table 1. Number of PRV positive, inconclusive and negative RT-qPCR results in blood samples collected from Atlantic salmon either immersed or intraperitoneally injected with DAF treated processing plant effluent or blood water.

Route of Effluent Exposure	Treatment group	PRV RT-qPCR result (n=10/treatment)
Immersion	DAF treated effluent	5 positive 3 inconclusive 2 negative
	1:1 Diluted DAF treated effluent (1 Seawater: 1 DAF treated effluent)	4 positive 6 negative
Injection	Blood water	10 positive
	DAF treated effluent	4 positive 6 negative

Conclusion:

DAF treated processing plant effluent showed a 100-fold reduction in the load of PRV in comparison to untreated blood water. Nevertheless, the treated effluent did contain infectious PRV, as Atlantic salmon exposed to effluent either through a 2 hour static immersion or via an intraperitoneal injection, acquired PRV blood infections. Consequently, the DAF treatment process installed at Browns Bay Processing plant will require additional disinfection processes to eliminate infectious PRV in blood water generated during the processing of PRV infected Atlantic salmon.

References:

1. 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.

Appendix 9

450

000828

Glossary of terms

Association: Refers to a relationship or link between a factor (like a behavior, exposure, or pathogen) and a health outcome (like a disease or condition). It indicates that these two variables occur together more frequently than would be expected by chance, but it does not necessarily imply a direct cause-and-effect relationship.

Cause and effect: In a disease context, cause and effect refers to the relationship where a specific factor, such as a pathogen or environmental exposure, directly triggers the onset of a disease. For instance, the virus (cause) leads to an infection and related symptoms (effect).

Epidemiology: The study of how diseases and health conditions spread, their causes, and their impact on populations. It examines diseases in real-world settings, rather than individuals in a lab.

Genetic Diversity: The range of genetic variations within a particular group or population

Isolate: A viral isolate is a specific strain of a virus obtained from a particular sample, separated from others within a mixed population.

Lineage: Refers to a group of organisms that share a common evolutionary origin descent, connected by ancestor/descendant relationships.

Pathogenicity: The ability of an organism to cause disease in a host.

Pathology: The study of disease, including its causes, effects. Relatedly, histopathology is the practice of examining tissues under a microscope to identify abnormalities and diagnose diseases. It focuses on the microscopic changes in tissue structure and function caused by disease.

PCR: Polymerase chain reaction (PCR) is a laboratory technique used to amplify and detect specific DNA or RNA sequences, enabling the identification and quantification of genetic material with high sensitivity and specificity)

Phylogenetics: The scientific field examining the evolutionary relationships between different individuals or species. This is typically done based on their genetic information. It involves constructing "phylogenetic trees" which are diagrams that illustrate how various species are related through common ancestors. This helps scientists understand the evolutionary history and genetic connections among species or individuals.

Reassortment: The exchange of genetic material between viruses with segmented genomes within a single host, resulting in the production of new viral lineages with a combination of genetic segments from both parent viruses.

Strain: A strain of a virus is a genetic variant within a virus species that may differ in characteristics like virulence or transmissibility.

Sub-strain: A further genetic variant within a virus strain.

Virulence: The degree to which a pathogen can cause disease or damage in a host.

This is **Exhibit "C"** referred to in the affidavit of Dr. Gideon Mordecai sworn before me at the City of Vancouver this 25th day of September, 2024.



A Commissioner for taking Affidavits for the Province of
British Columbia

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IN THE FEDERAL COURT OF CANADA

BETWEEN:

‘NAMGIS FIRST NATION

APPLICANT

AND:

MINISTER OF FISHERIES, OCEANS AND THE CANADIAN COAST GUARD,
MOWI CANADA WEST INC. and CERMAQ CANADA LTD.

RESPONDENTS

CERTIFICATE CONCERNING CODE OF CONDUCT FOR EXPERT WITNESSES

I, Dr. Gideon Mordecai, having been named as an expert witness by the Applicant, certify that I have read the Code of Conduct for Expert Witness set out in the schedule to the *Federal Court Rules* and agree to be bound by it.

July 30, 2024



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