Appendix "B"

Water Sampling Progress Reports

Prepared by

Dr. Emiliano Di Cicco
Fish Health Expert for the Indigenous Monitoring and Inspection Plan Framework Agreements

eDNA study Report

Midsummer Island sampling on October 12, 2021

Dr. Emiliano Di Cicco

June 29, 2022

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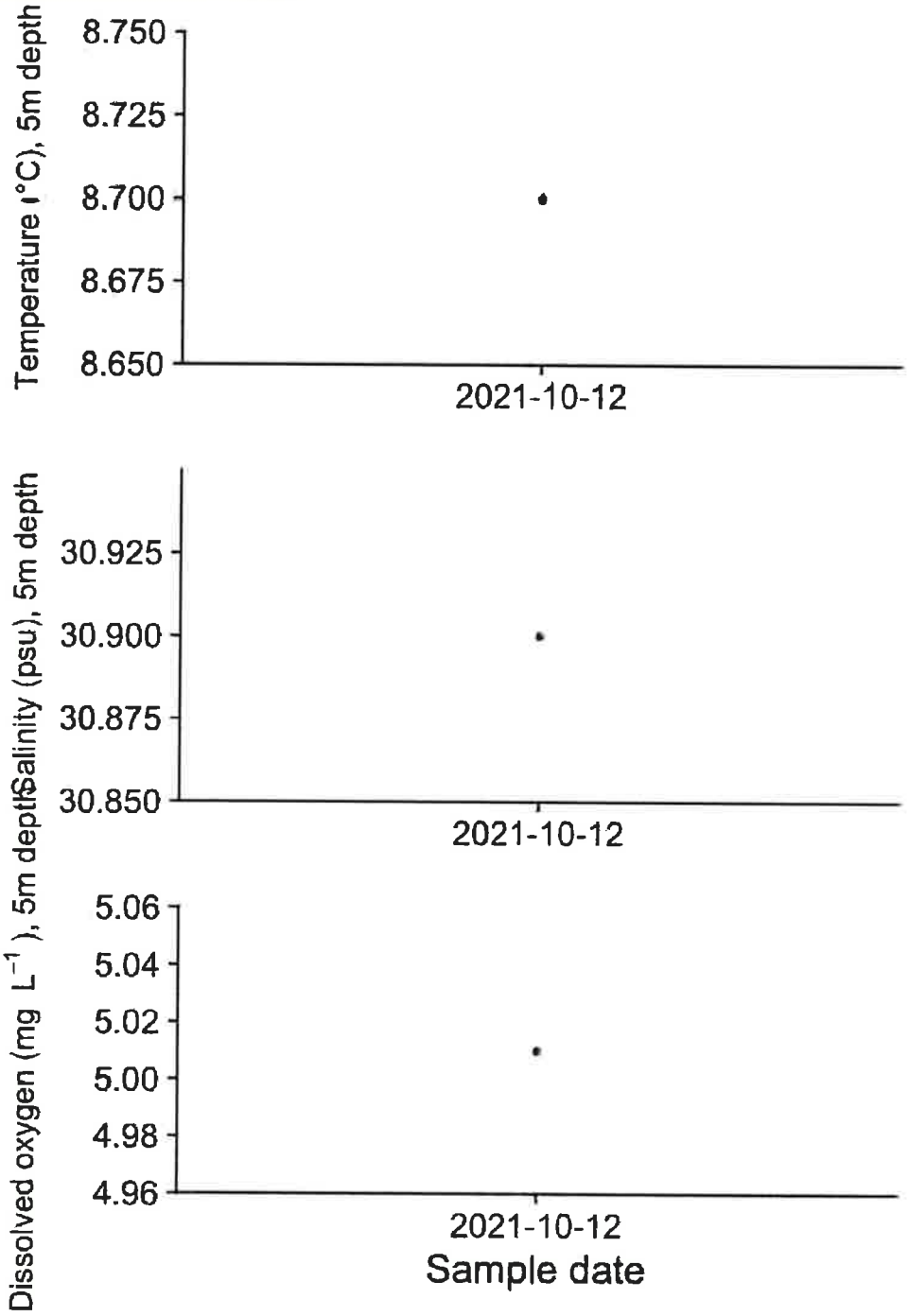
Executive summary

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.

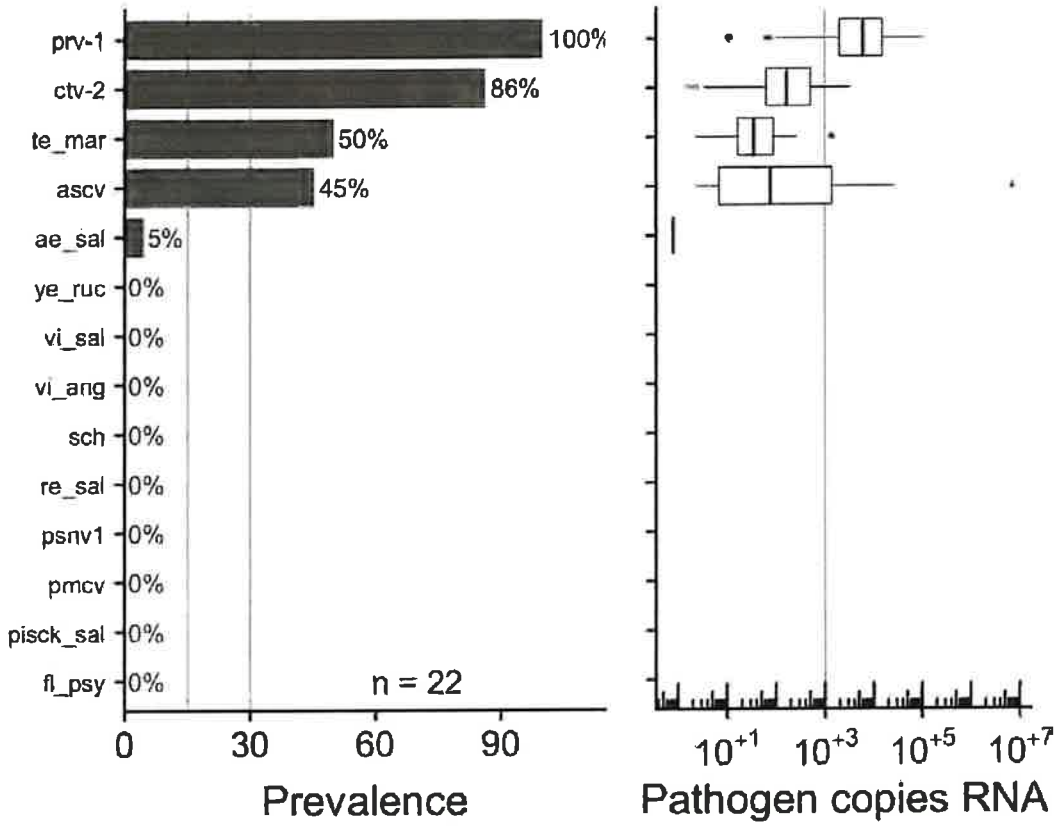
Histology samples have been sent to Wax-It Histo Ltd. to process and prepare slides, which have been read and scored by Dr. Di Cicco. A digital copy of each slide is available to the Company.

Environmental data

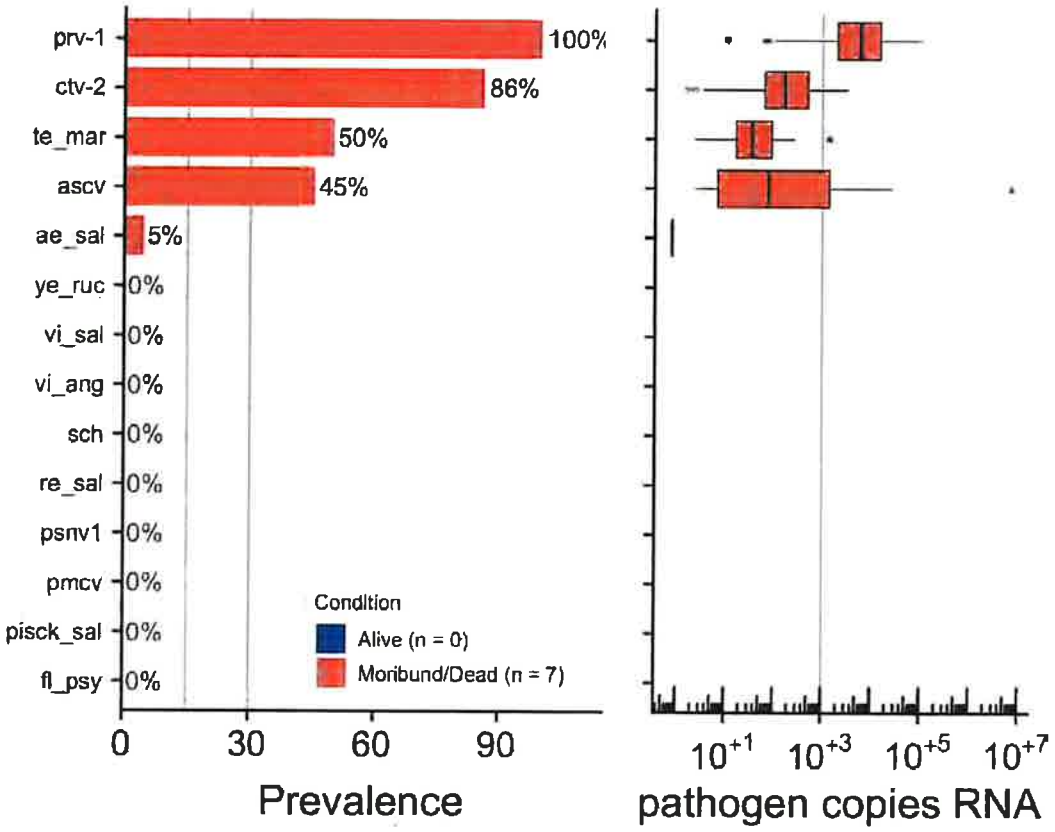


Water temperature (°C), salinity (ppm), and dissolved oxygen (mg/L) at a 5m depth. Certain sampling dates have no recorded environmental data, resulting in gaps in the plots.

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

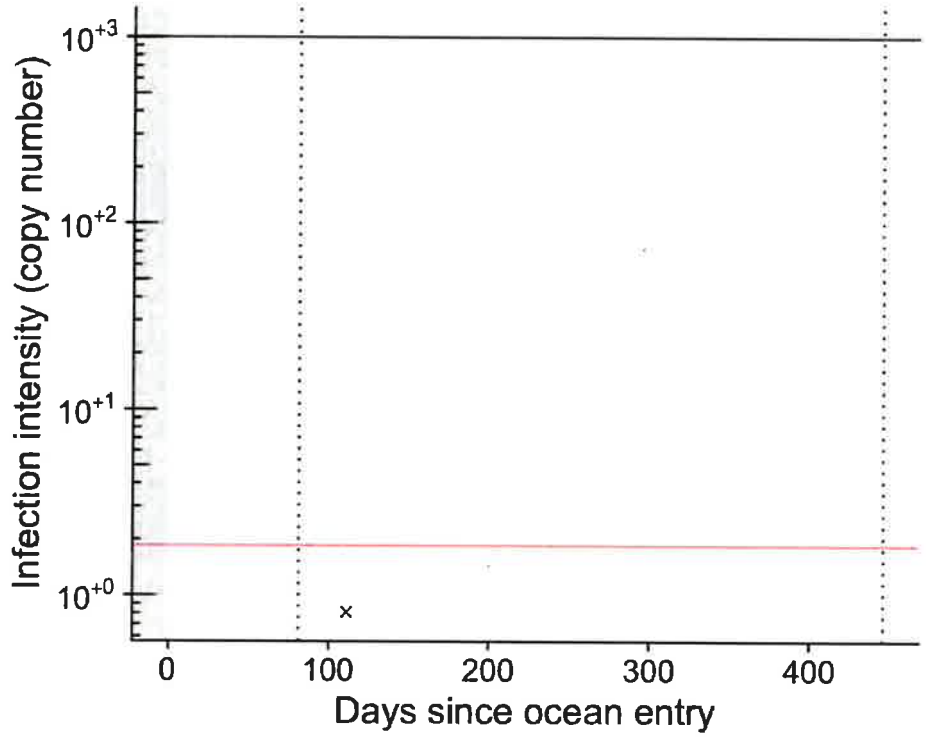
The following plots show individual infectious agent trends across all farm sites. In cases where sample size is sufficient, curves from a generalised additive model are included in the plot.

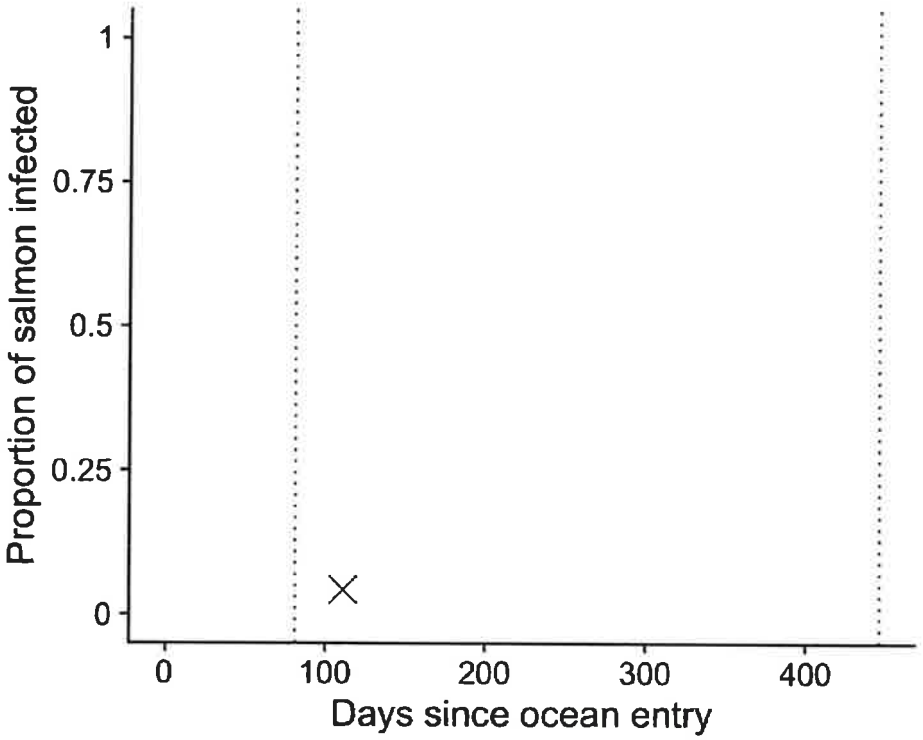
Grey circles represent live fish, and black X's represent dead/dying fish. Curves indicate mean predictions from a generalised additive model; blue and red correspond to live and dead/dying fish, respectively (shaded areas show 95% confidence regions). Left-hand grey region indicates freshwater hatchery 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.

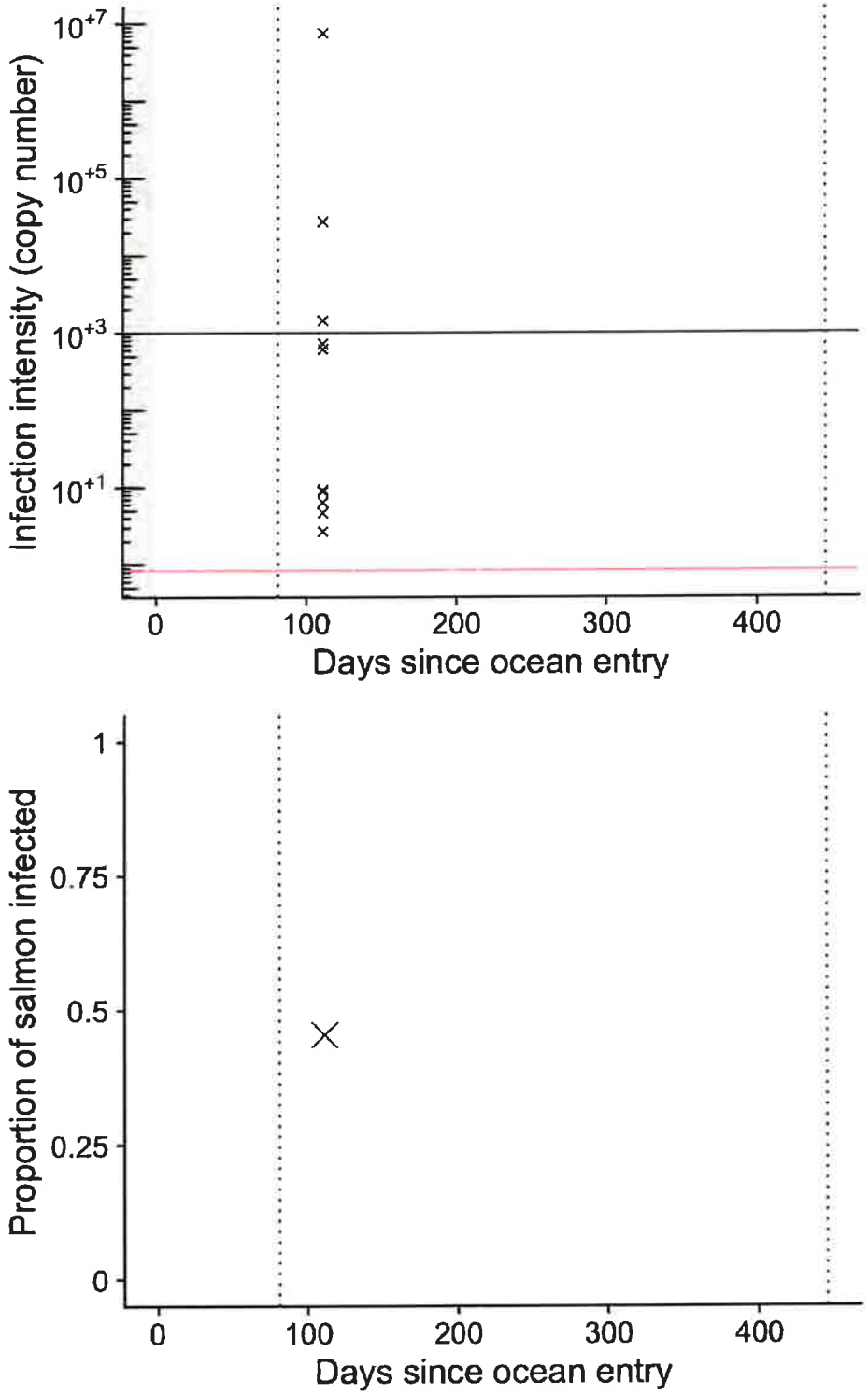
For proportion plots, grey circles show prevalence in live fish on each sampling date, and black X's show prevalence in dead/dying fish (symbol areas proportional to sample sizes).

Aeromonas salmonicida

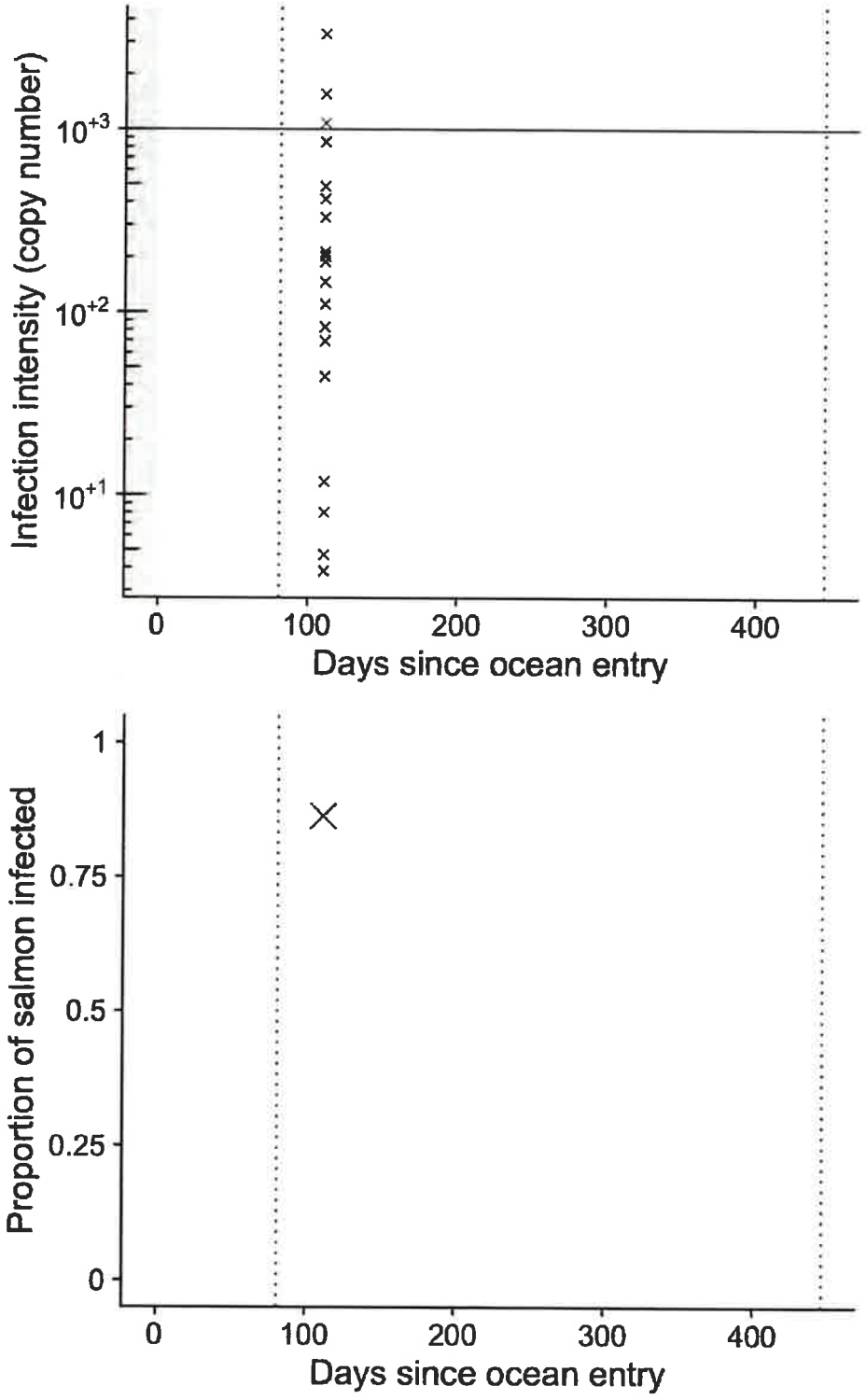




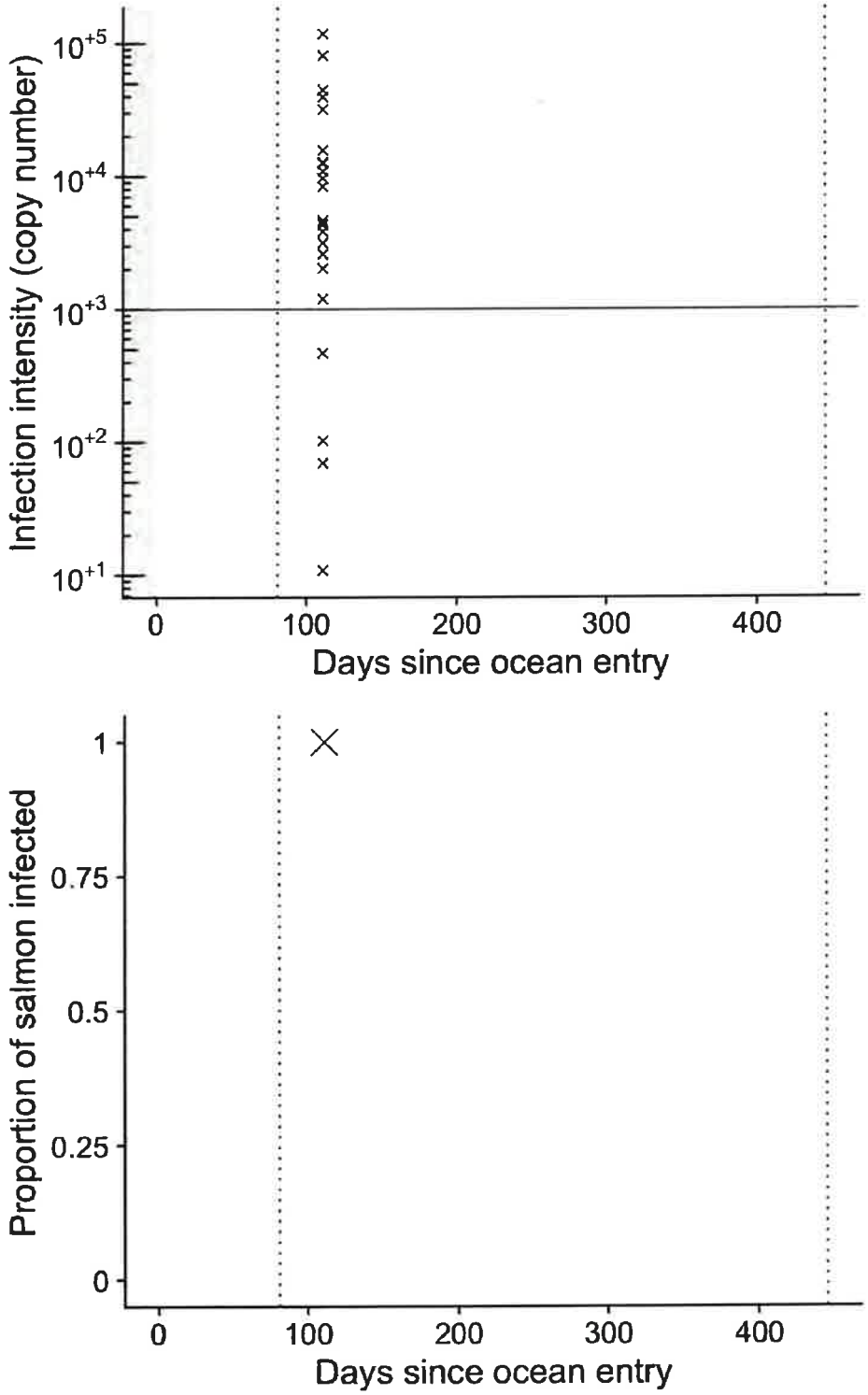
Atlantic salmon calicivirus



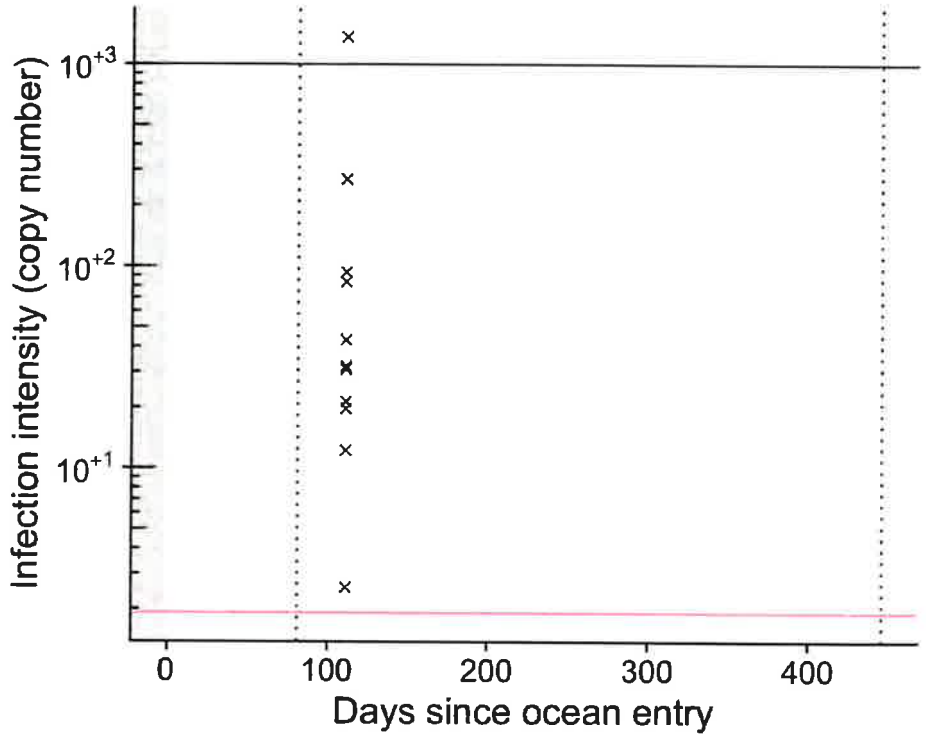
Cutthroat trout virus-2

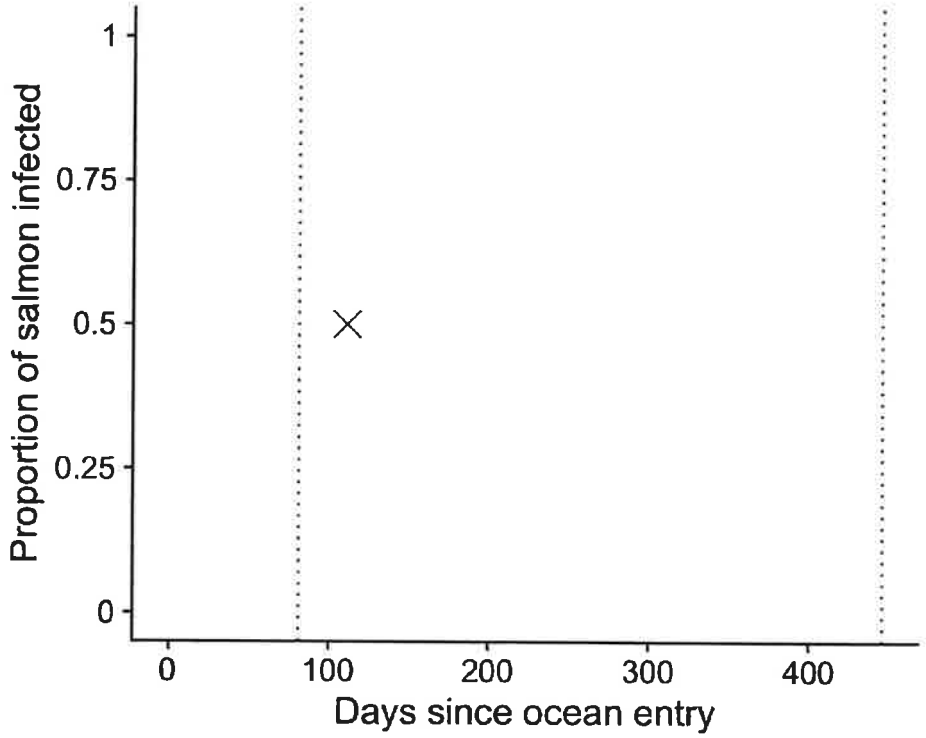


Piscine orthoreovirus



Tenacibaculum maritimum





Clinical signs

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

metric	N5016	N5017	N5018	N5019	N5020	N5021	N5022
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	N5016	N5017	N5018	N5019	N5020	N5021	N5022
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 Heam	2	3	2	2	3	2	1
W Pulpitis	1		2	1	1	1	2
Kidney							
Itis			1				
Osis					1		1
Cong Heam	2	1		1	1	1	
Interst Hyperplasia	2	1	2	1	1	1	1
Pancreatitis							
Pancreatitis		1					
Cnc							
Malacia						1	
Gliosis			1			1	
Cong Heam		2	1	1	1	1	2
Gills							
Itis	nv	nv	nv	nv	nv	nv	nv
Cong Heam	nv	nv	nv	nv	nv	nv	nv
Prolif	nv	nv	nv	nv	nv	nv	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	Myocardionecrosis (2), Localized Clots In Atrium (1), Eosinophilic Vacoules In Hepathocytes (1); Gills Very Old, Diffused Picnotic Nuclei In Hepatocytes And Enterocytes
N5021		Neuronal Vacuolization (2), Neuronal Chromatolisis (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 sligthly 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 Syngnamydia 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

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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.

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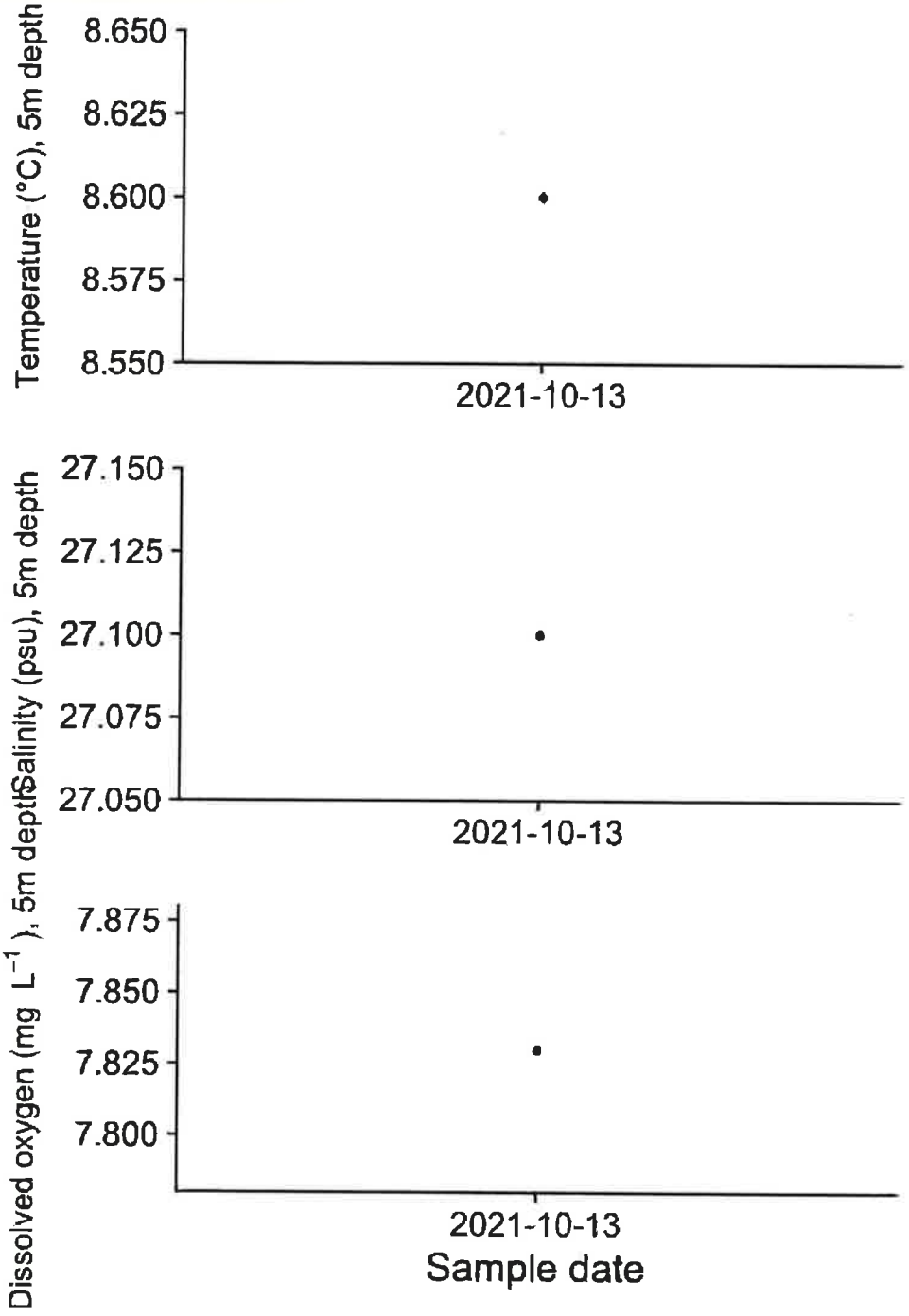

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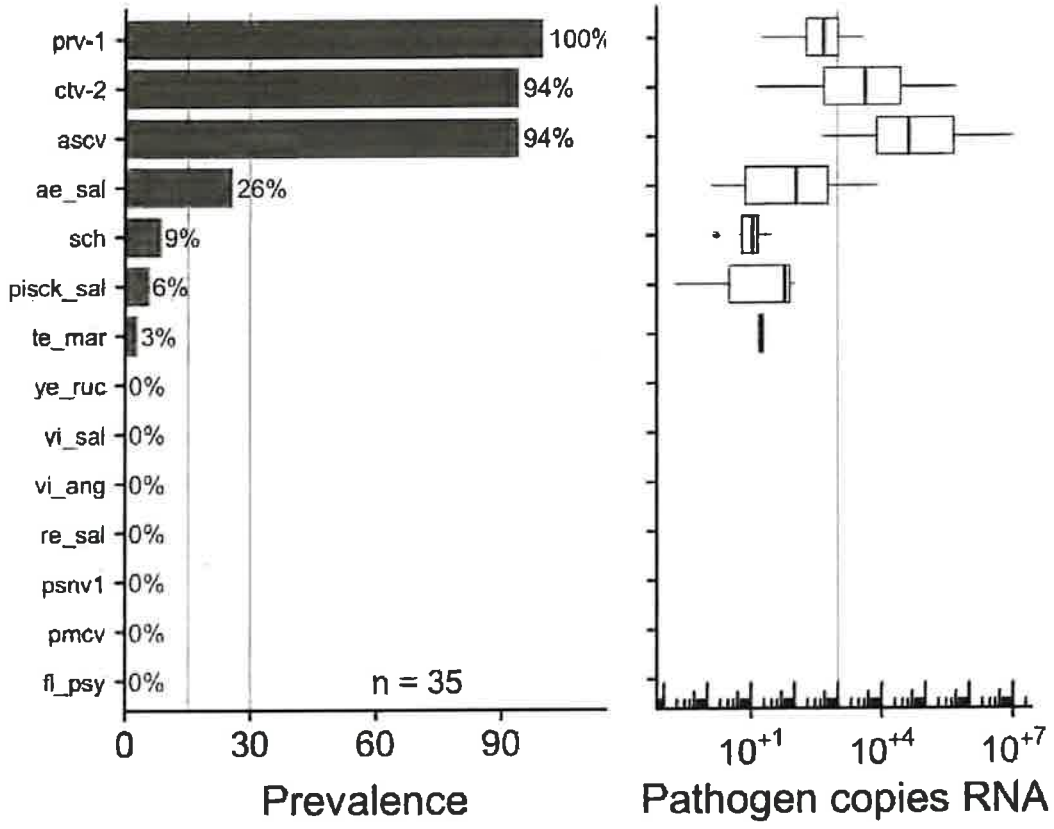
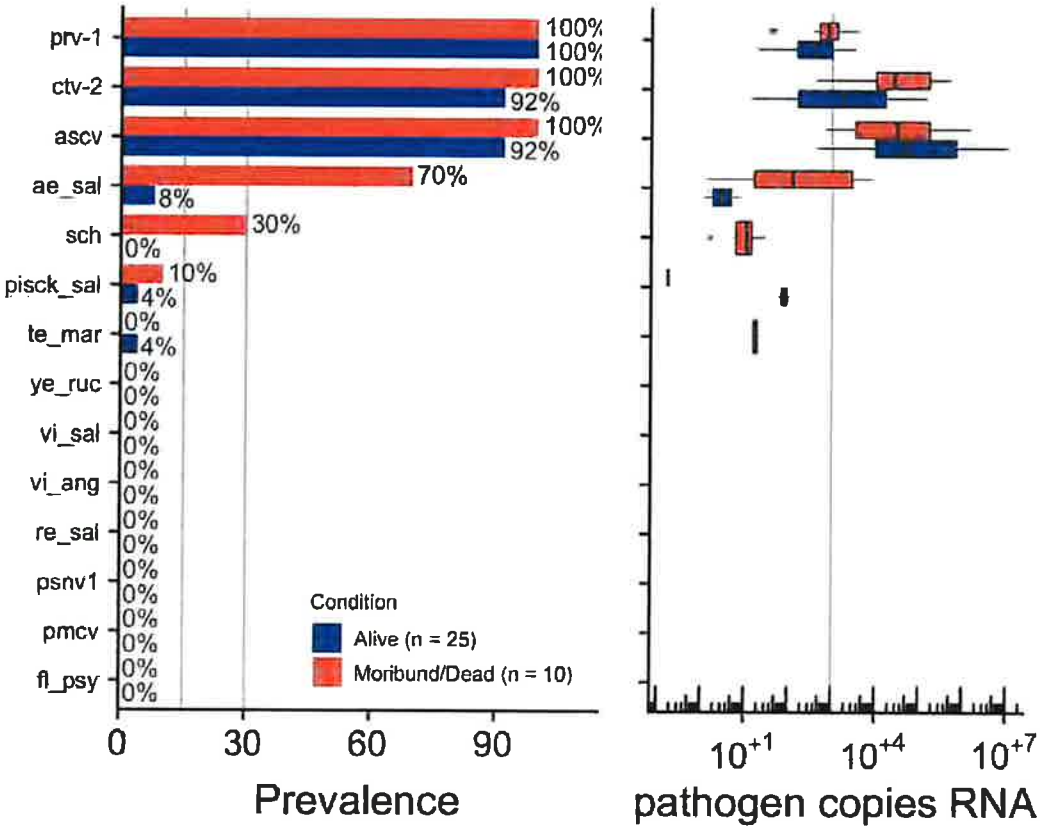


Infectious agent prevalence in samples collected on 2021-10-13.



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.

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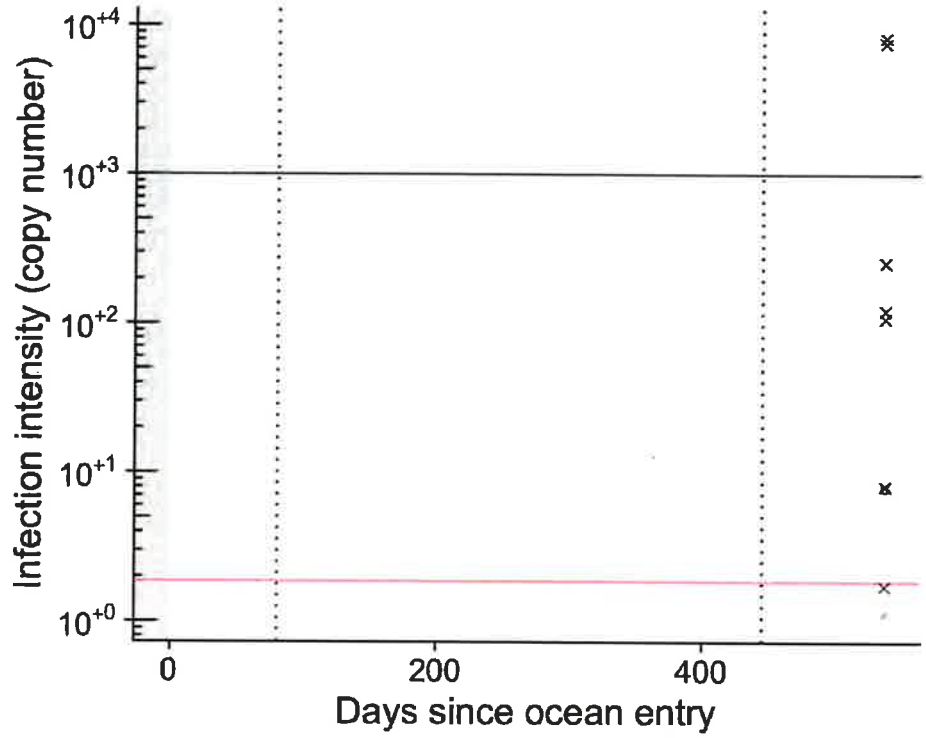
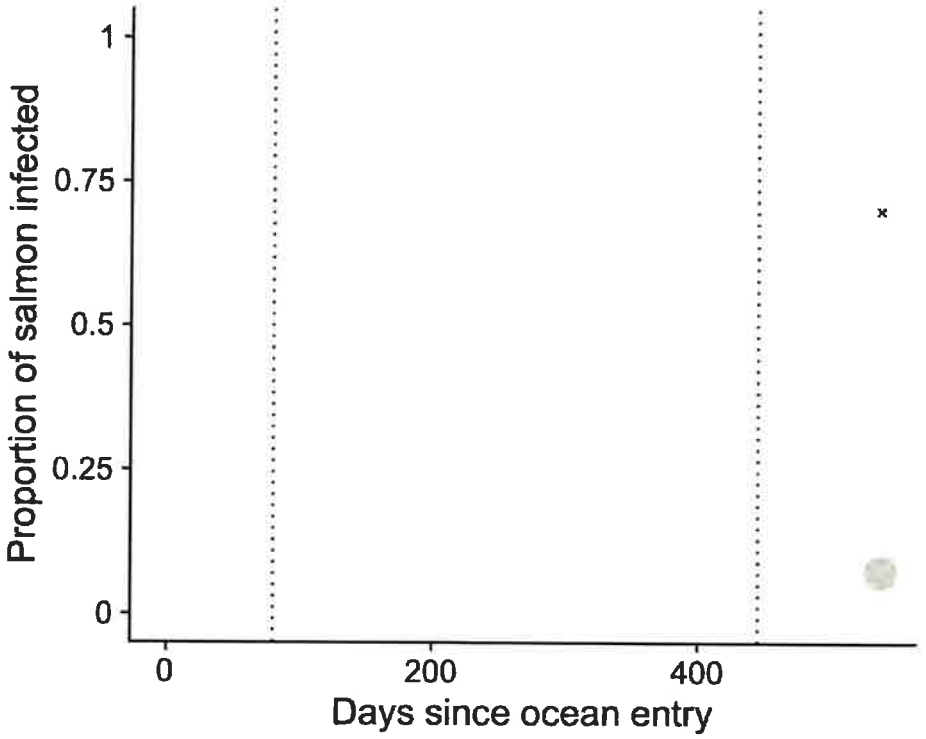
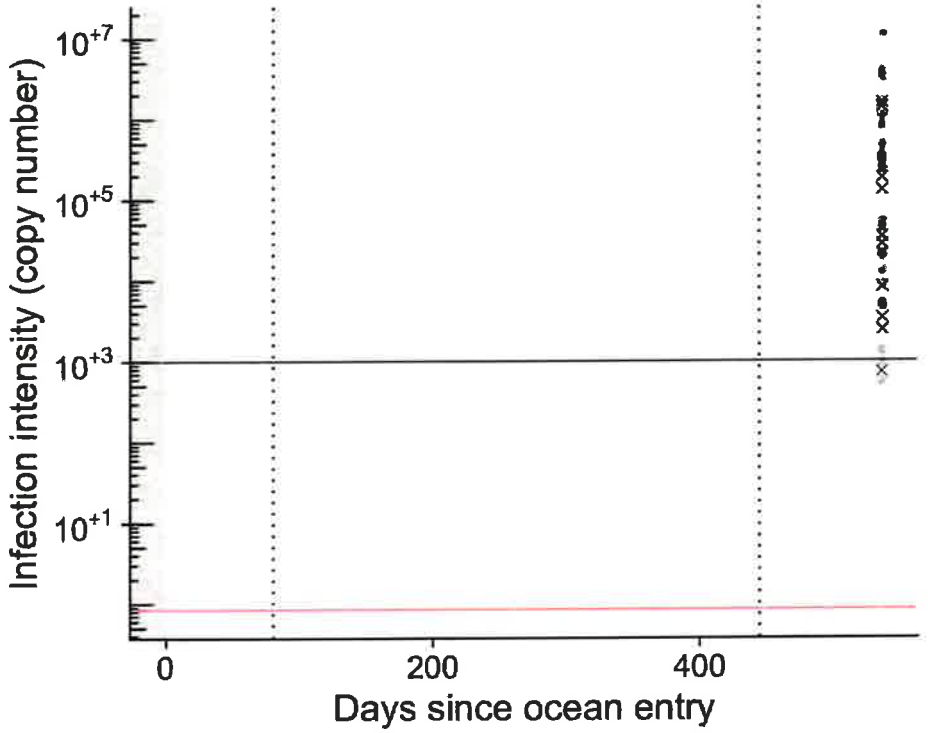
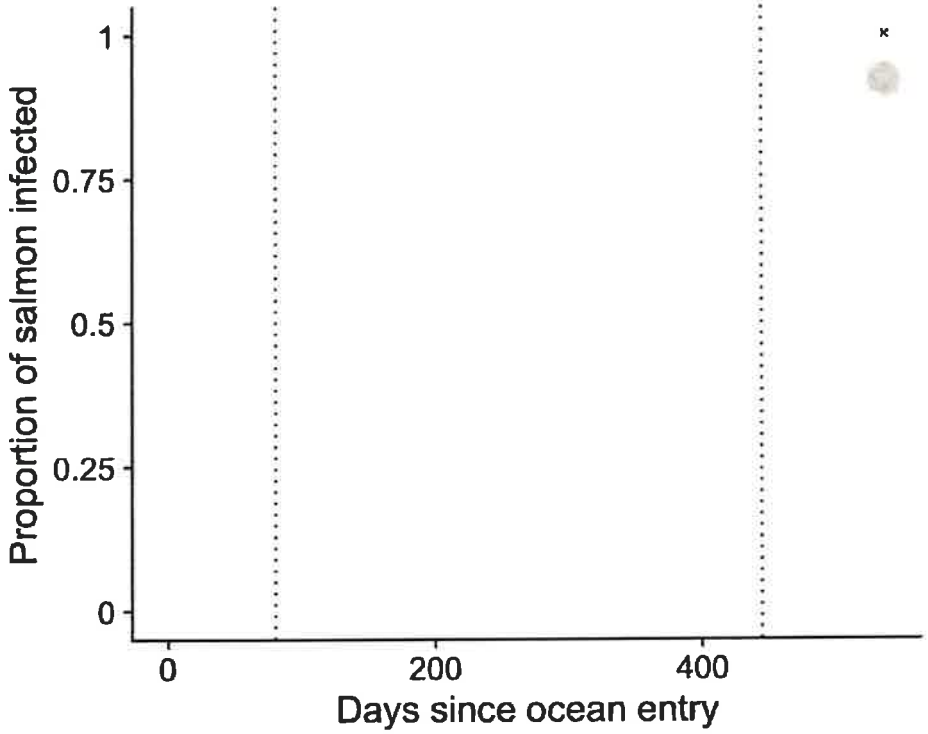


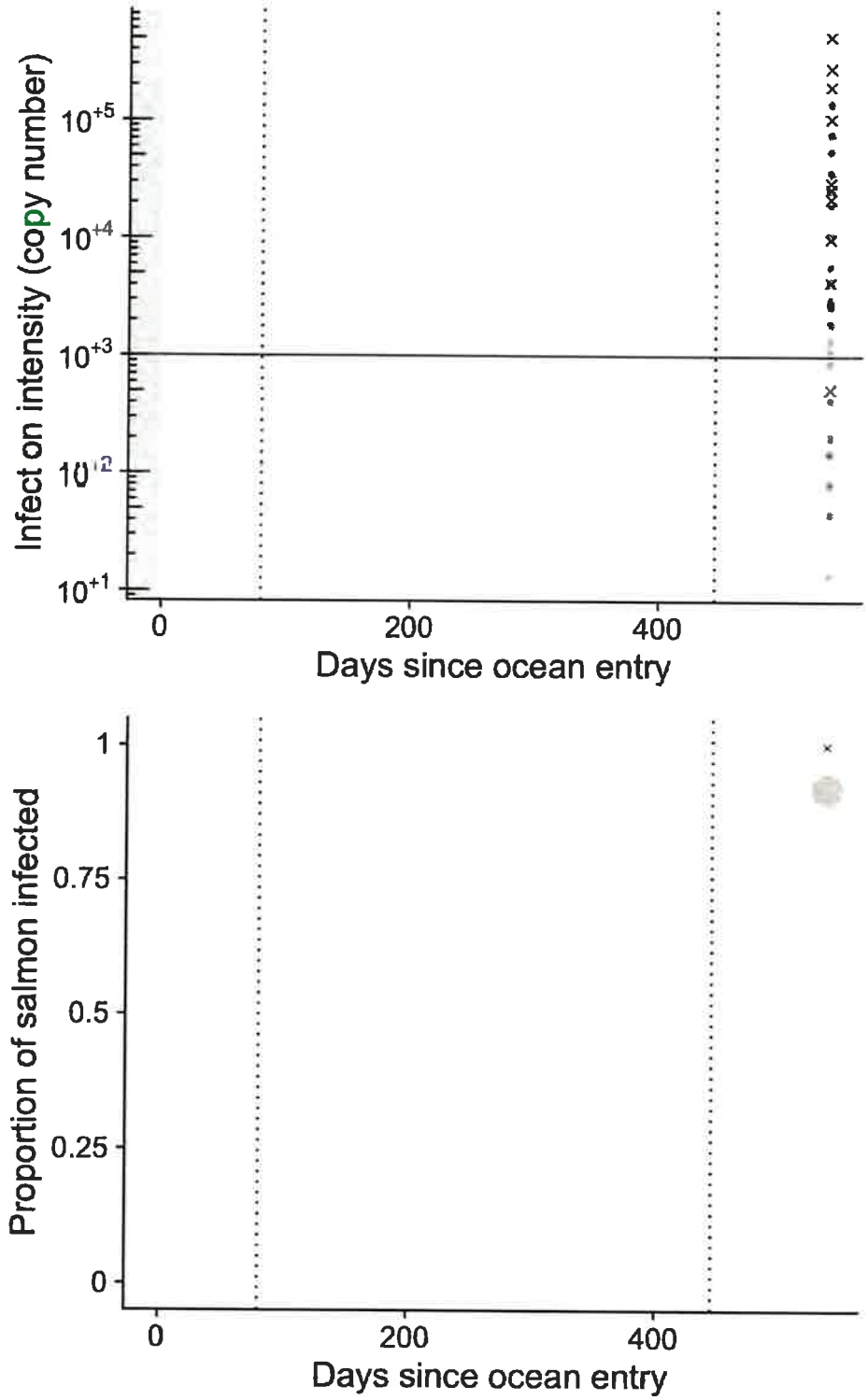


Atlantic salmon calicivirus

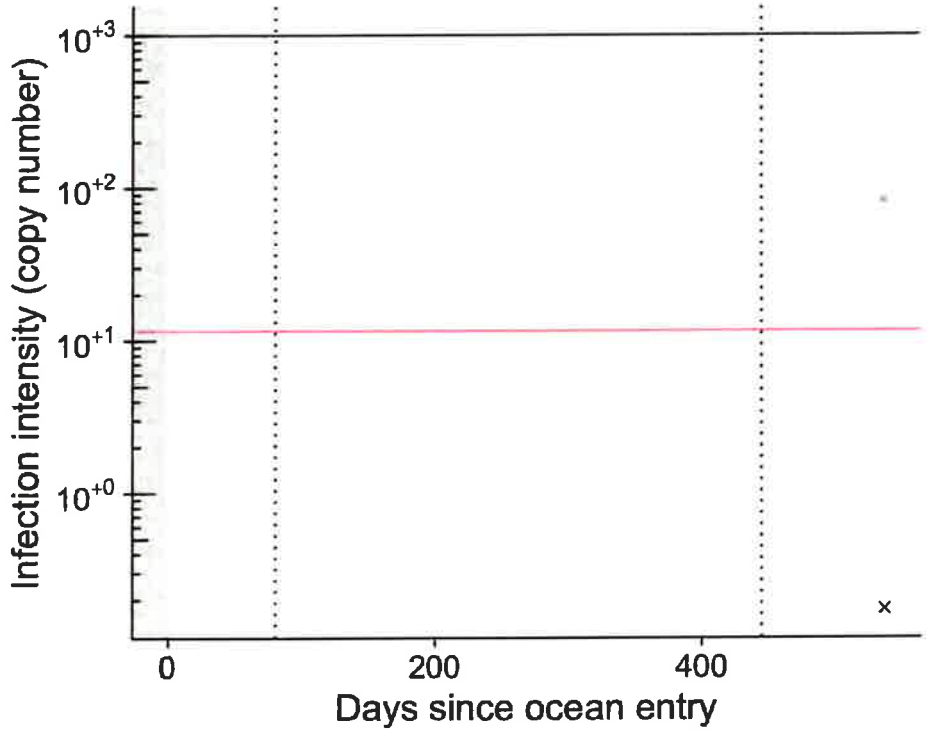


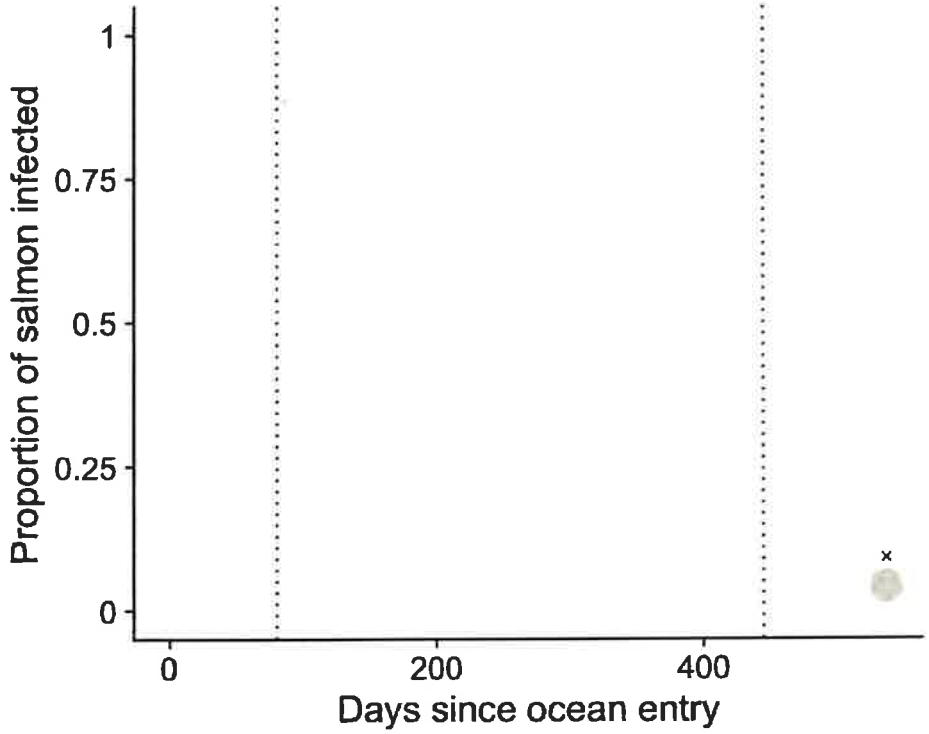


Cutthroat trout virus-2

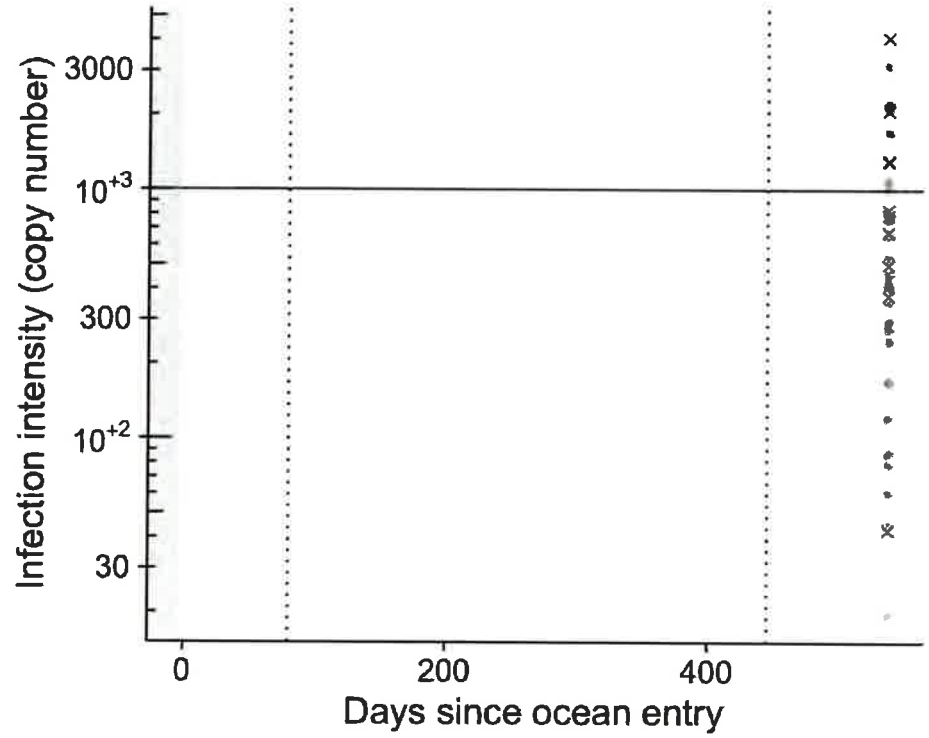


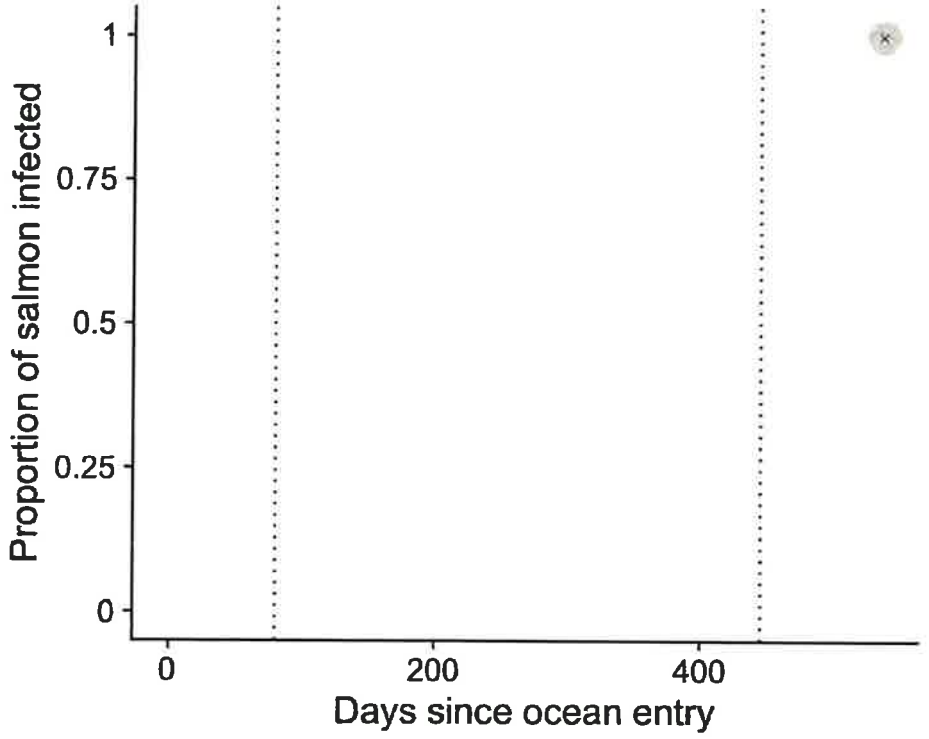
Piscirickettsia salmonis



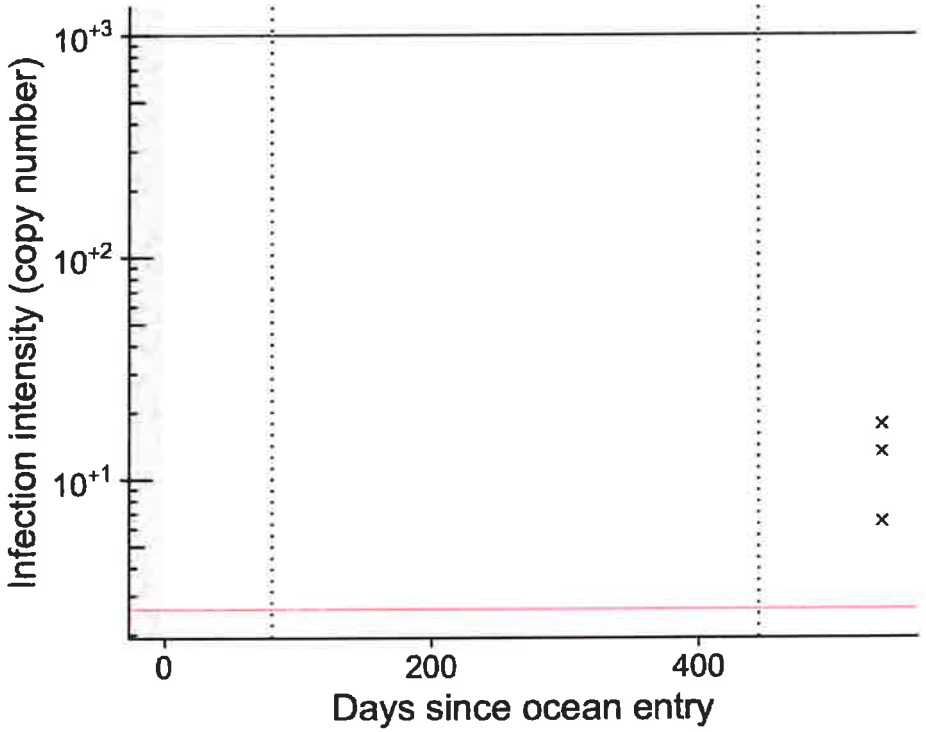


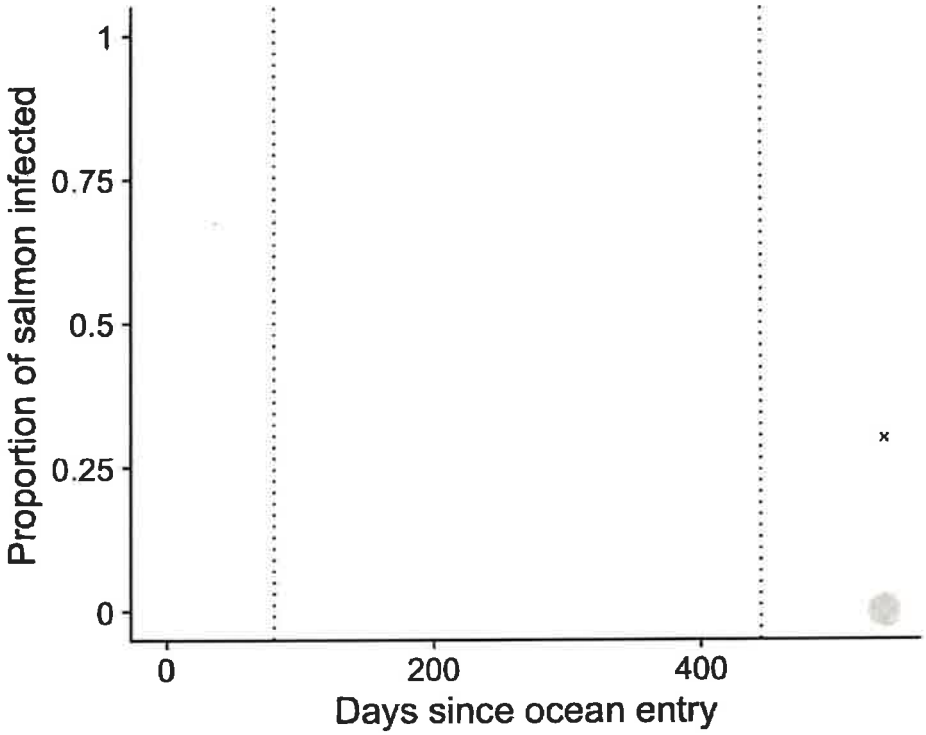
Piscine orthoreovirus



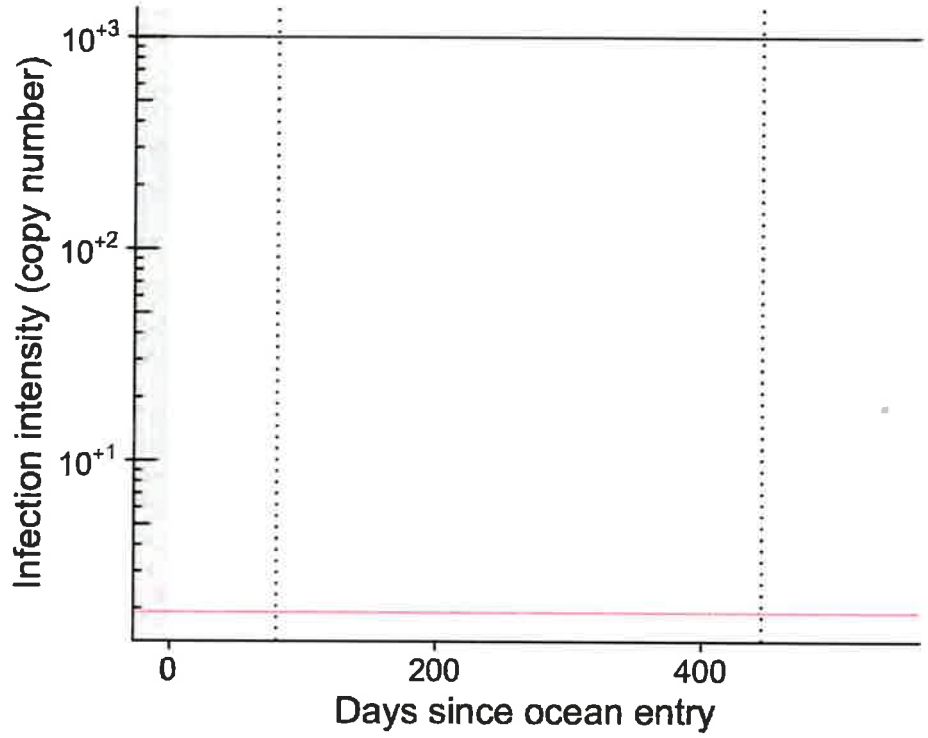


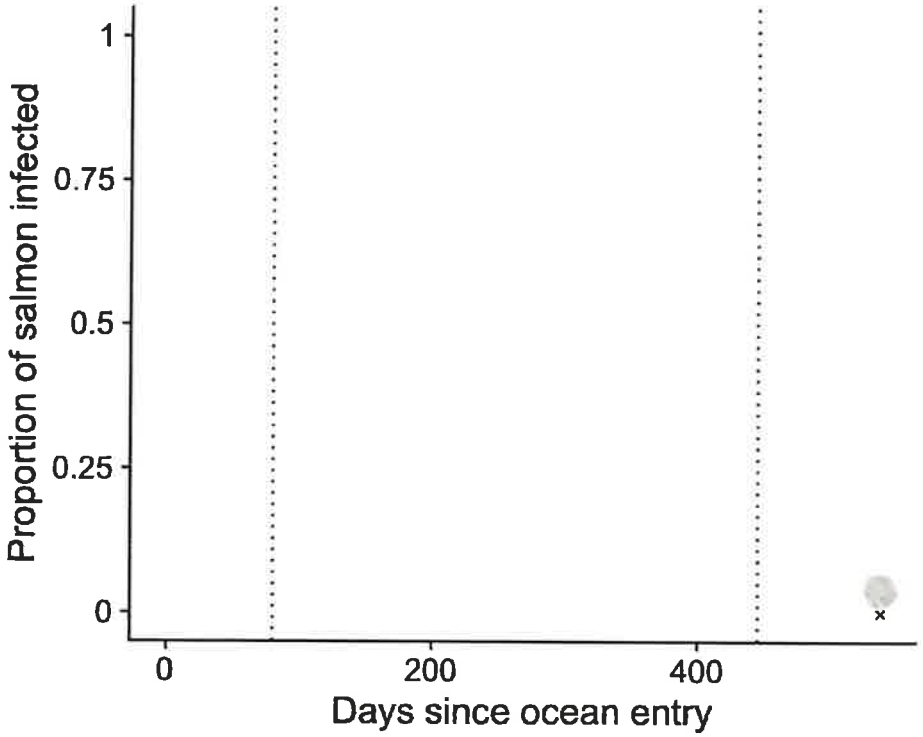
Candidatus Syngnamydia salmonis





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																				
Exopthalmia		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															
Intestine																				
Fluid Content					X															
Brain																				
Hemorrhages/Congestion	X			X	X															

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

metric	N5061	N5062	N5063	N5064	N5065	N5066	N5067	N5068	N5069	N5070	N5071	N5072	N5073	N5074	N5075
General															
Live						X	X	X	X	X	X	X	X	X	X
Poor Performer															
Moribund															
Mort	X	X	X	X	X										
Skeletal Deformity														X	
Exopthalmia															
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	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	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	Myocardionecrosis (2), Bacterial Colonies In Heart (2), Fibrotis Hyperplasia + Laciniae In Epicardium (2), Hepatocyte Apoptosis (1)
N5043	Furunculosis + Epitheliocystis	Epitheliocystis (2), Myocardionecrosis (1), Bacterial Colonies In Heart (1)
N5044		Epitheliocystis (3), Increase Fibrin In Spleen (2), Coag Necrosis Liver (2)
N5045	Furunculosis	Epitheliocystis (1), Neuronal Chromatolisis (1), Septic Thrombi In Atrium (2), Myocardionecrosis (2), Bacterial Colonies In Heart (1)
N5062		Myocardionecrosis (3)
N5063		Colangitis (1), Myocardionecrosis (2), Bacterial Colonies In Heart (3)
N5064	Furunculosis	Diffused Picnotic Nuclei In Hepatocytes And Enterocytes
N5065		Myocardionecrosis (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* Syngnamydia salmonis. Background level detection of *Piscirickettsia salmonis* and *Tenacibaculum maritumum* 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 Syngnamydia 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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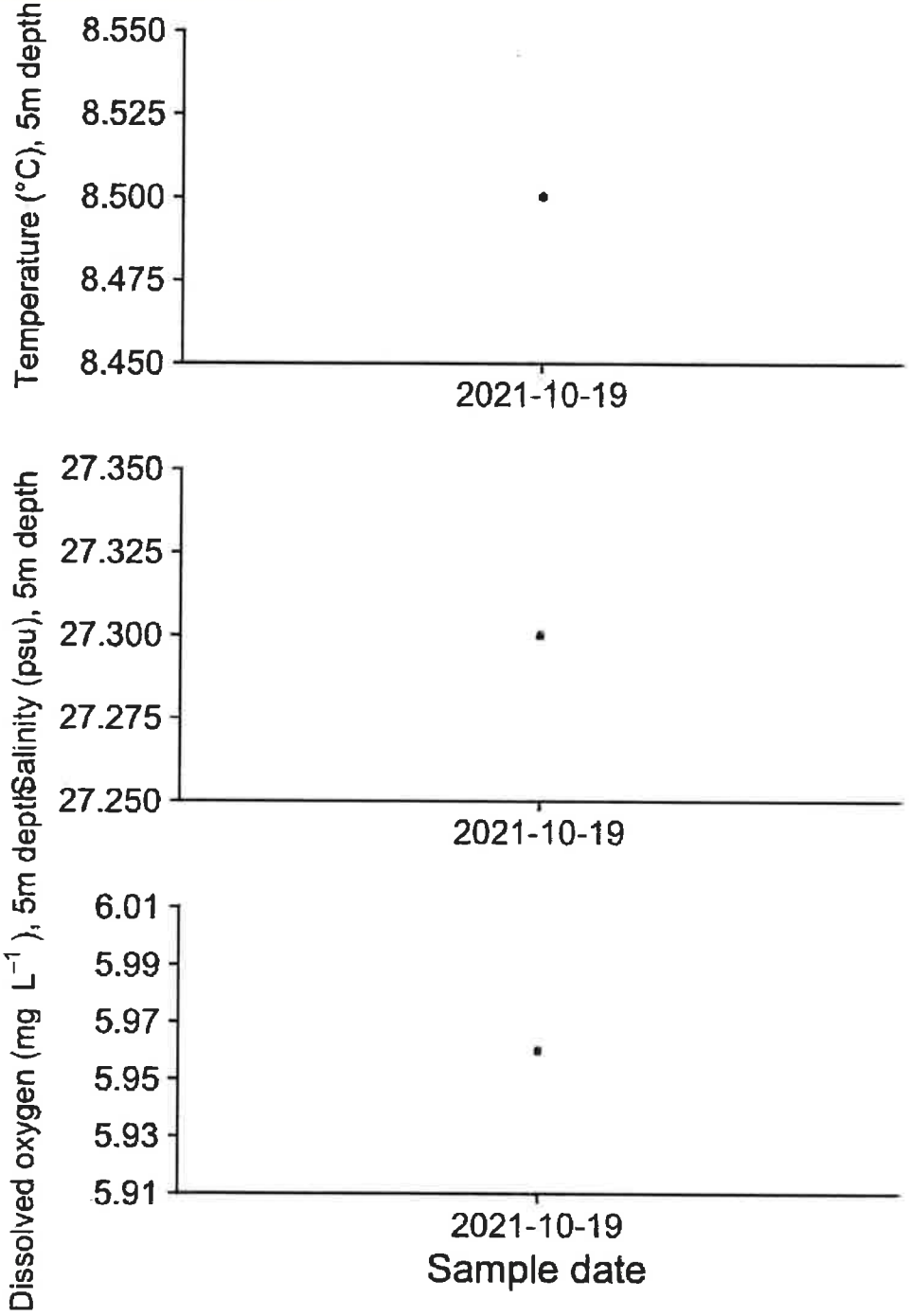
Executive summary

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.

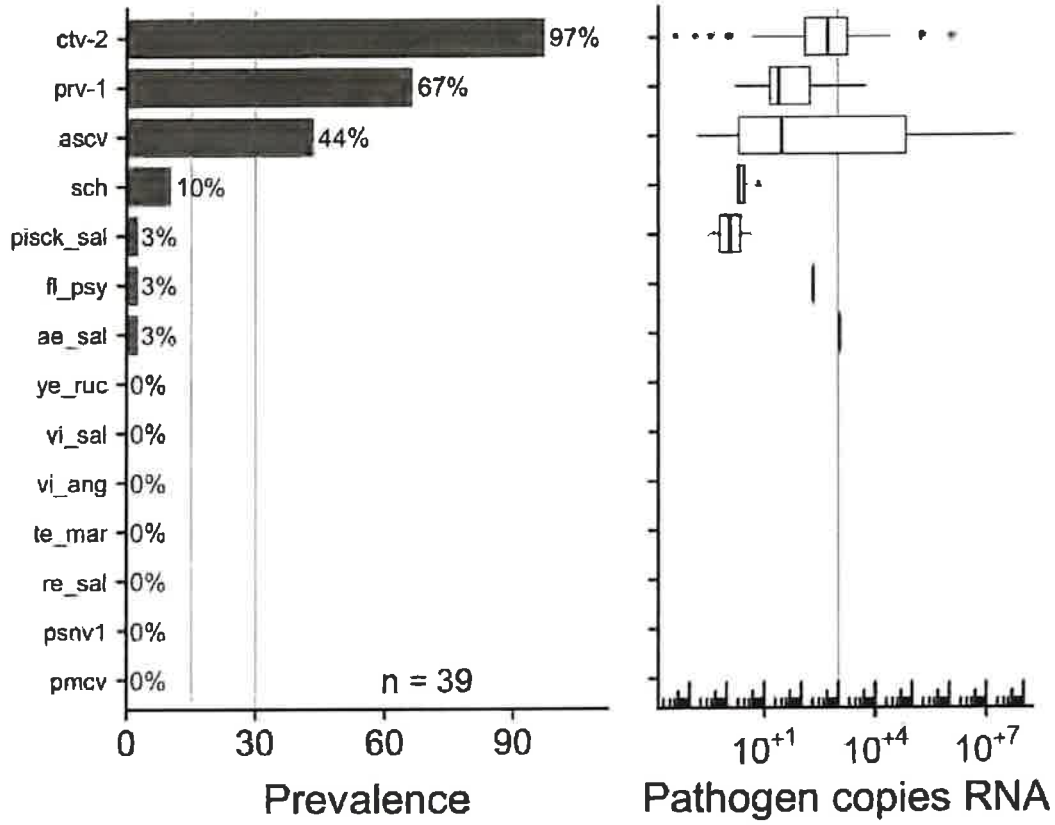
Histology samples have been sent to Wax-It Histo Ltd. to process and prepare slides, which have been read and scored by Dr. Di Cicco. A digital copy of each slide is available to the Company.

Environmental data

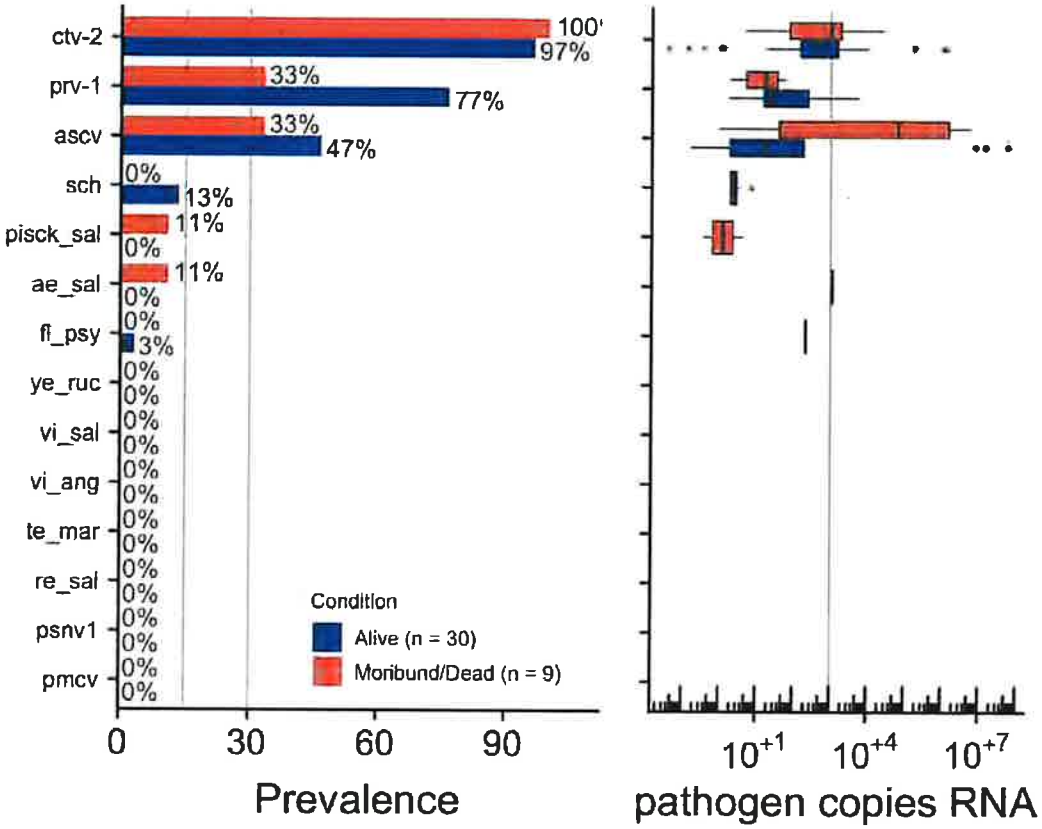


Water temperature (°C), salinity (ppm), and dissolved oxygen (mg/L) at a 5m depth. Certain sampling dates have no recorded environmental data, resulting in gaps in the plots.

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

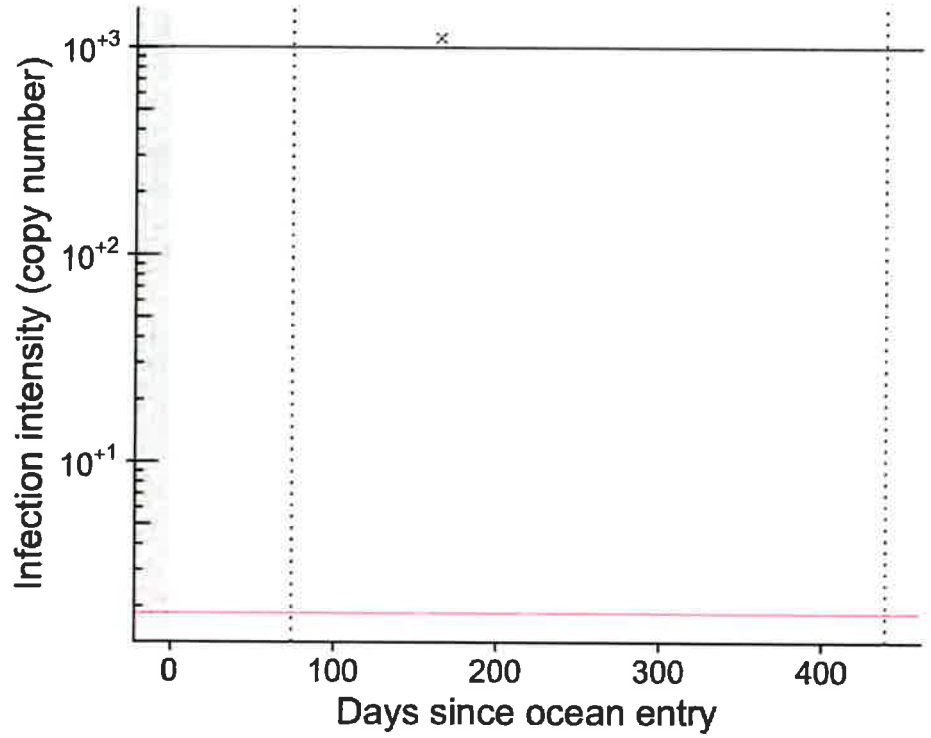
The following plots show individual infectious agent trends across all farm sites. In cases where sample size is sufficient, curves from a generalised additive model are included in the plot.

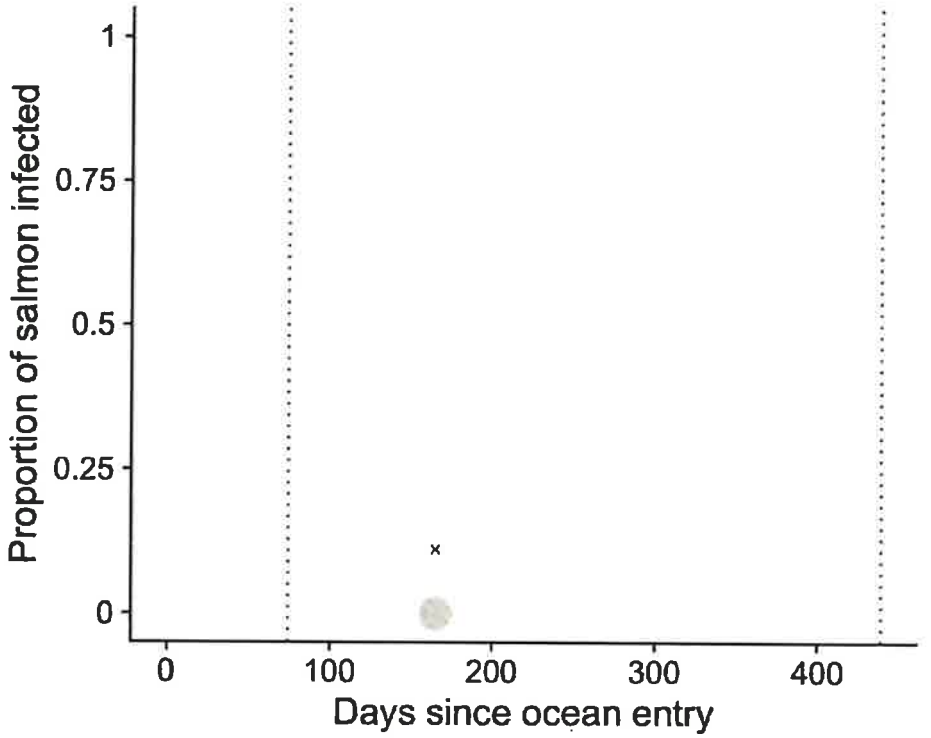
Grey circles represent live fish, and black X's represent dead/dying fish. Curves indicate mean predictions from a generalised additive model; blue and red correspond to live and dead/dying fish, respectively (shaded areas show 95% confidence regions). Left-hand grey region indicates freshwater hatchery 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.

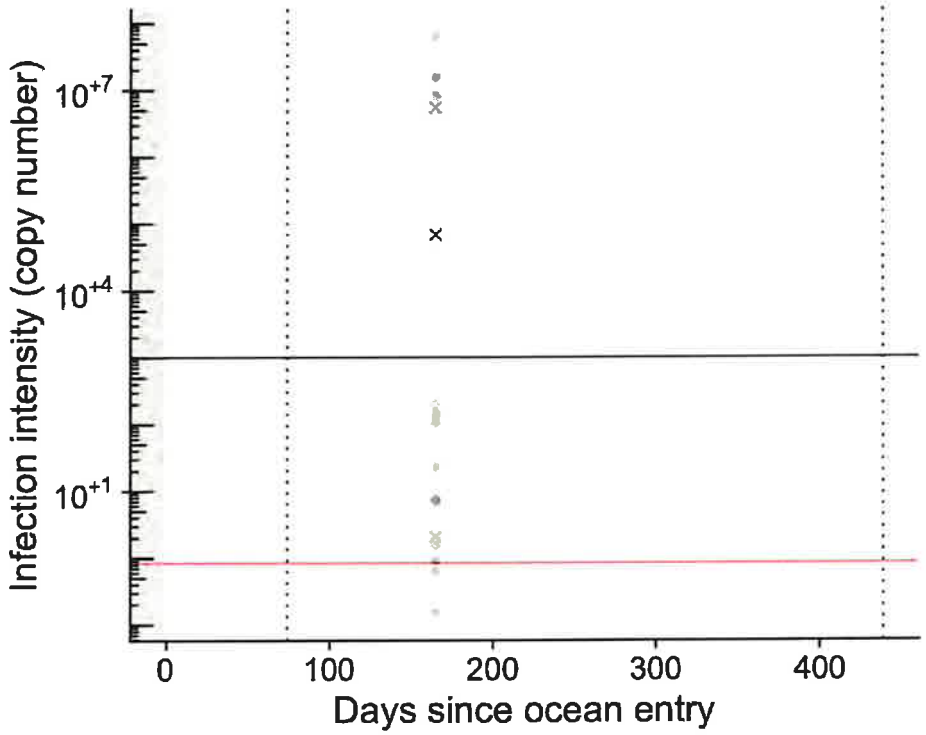
For proportion plots, grey circles show prevalence in live fish on each sampling date, and black X's show prevalence in dead/dying fish (symbol areas proportional to sample sizes).

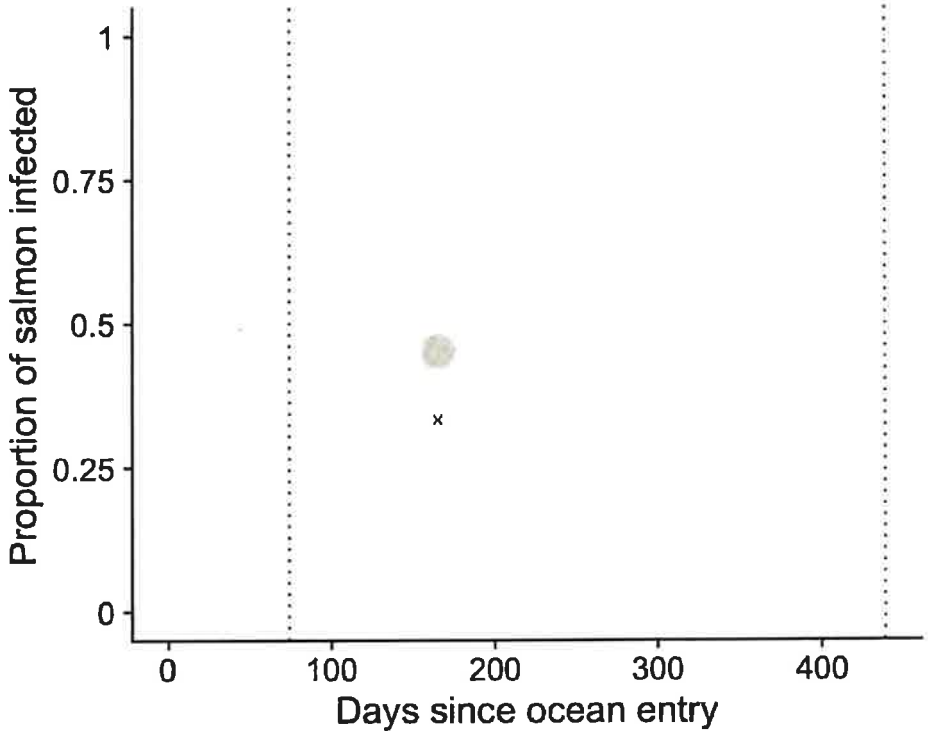
Aeromonas salmonicida



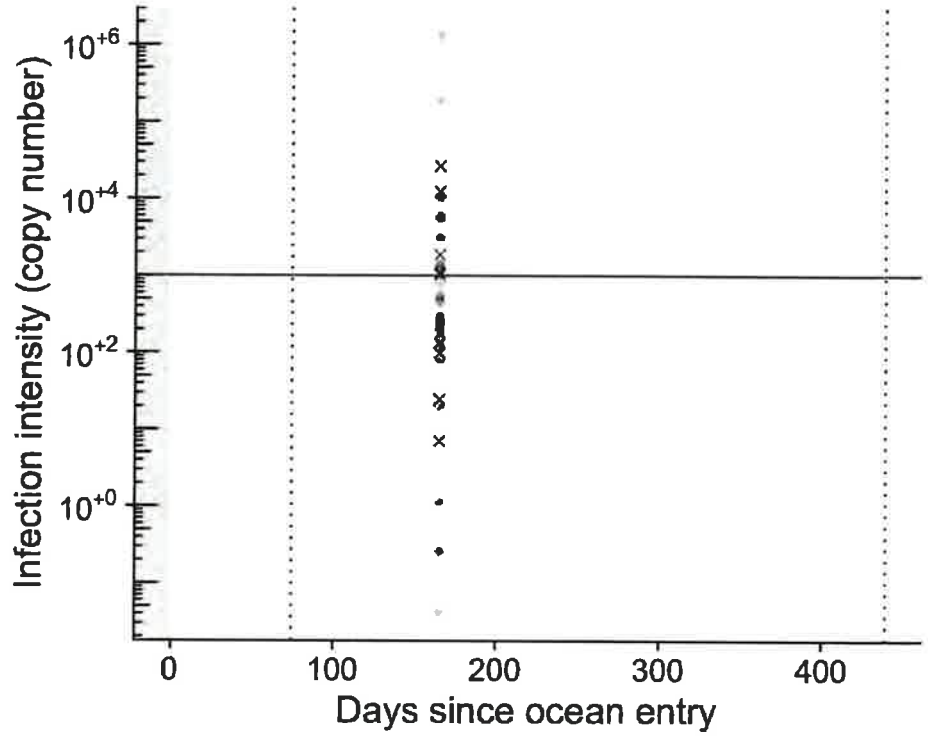


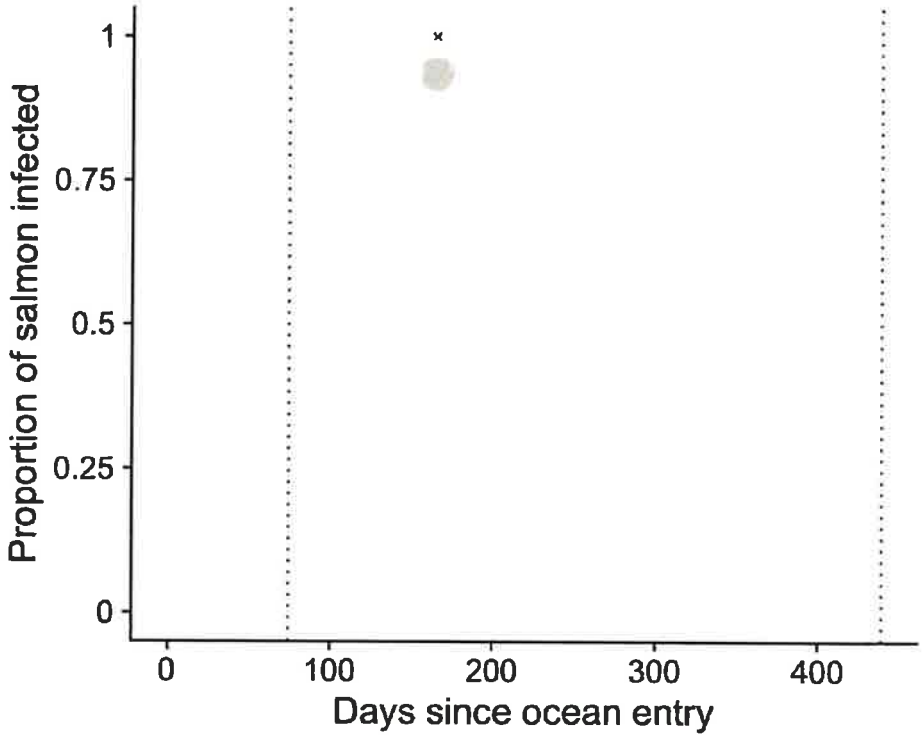
Atlantic salmon calicivirus



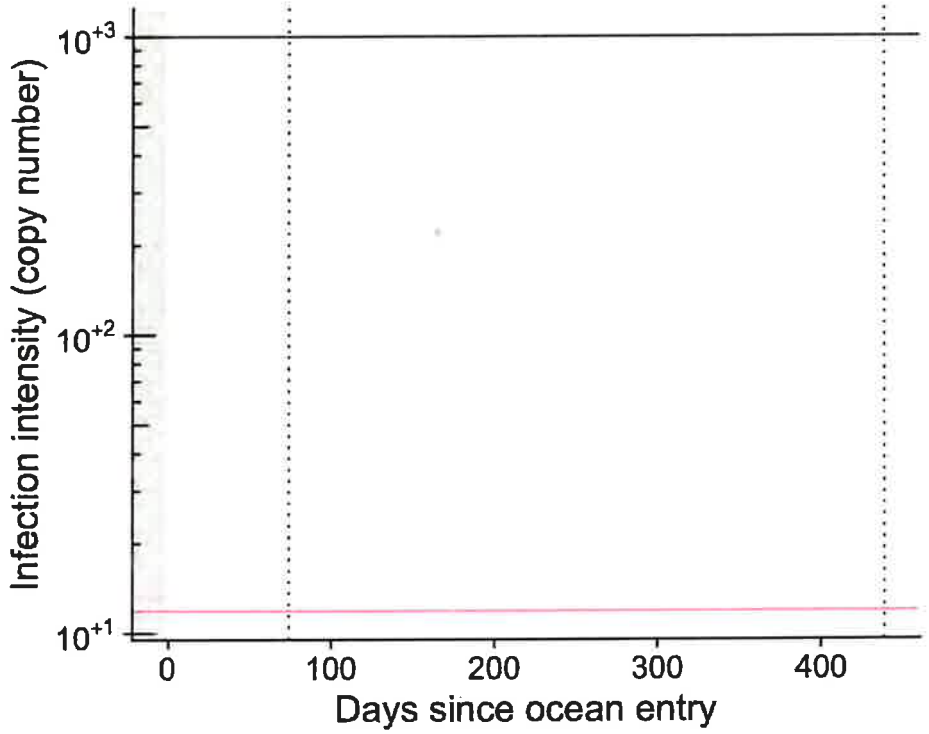


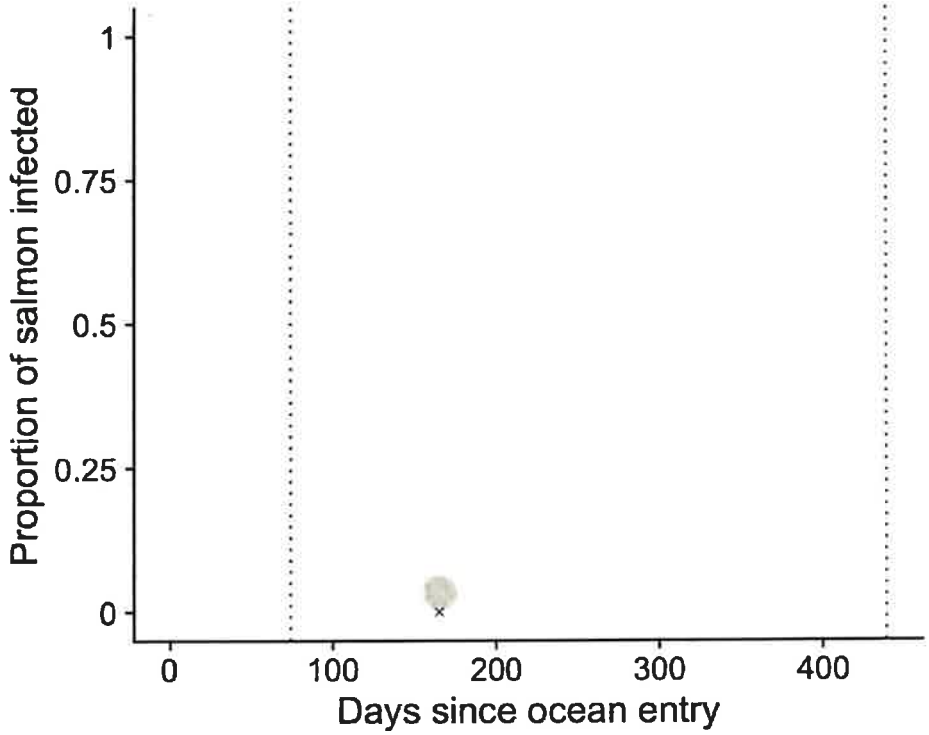
Cutthroat trout virus-2



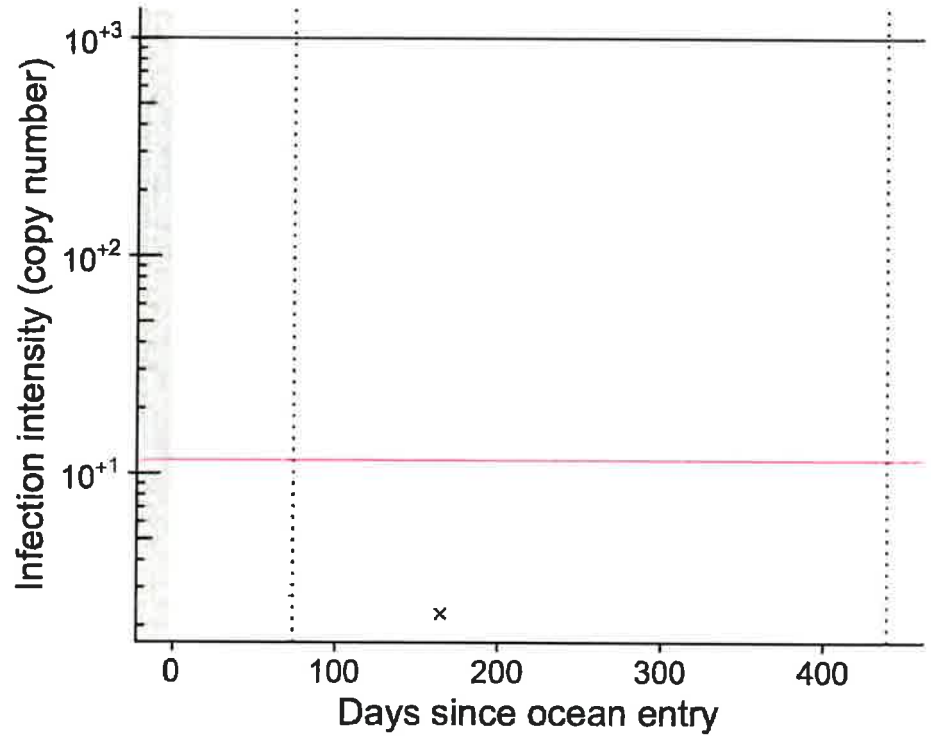


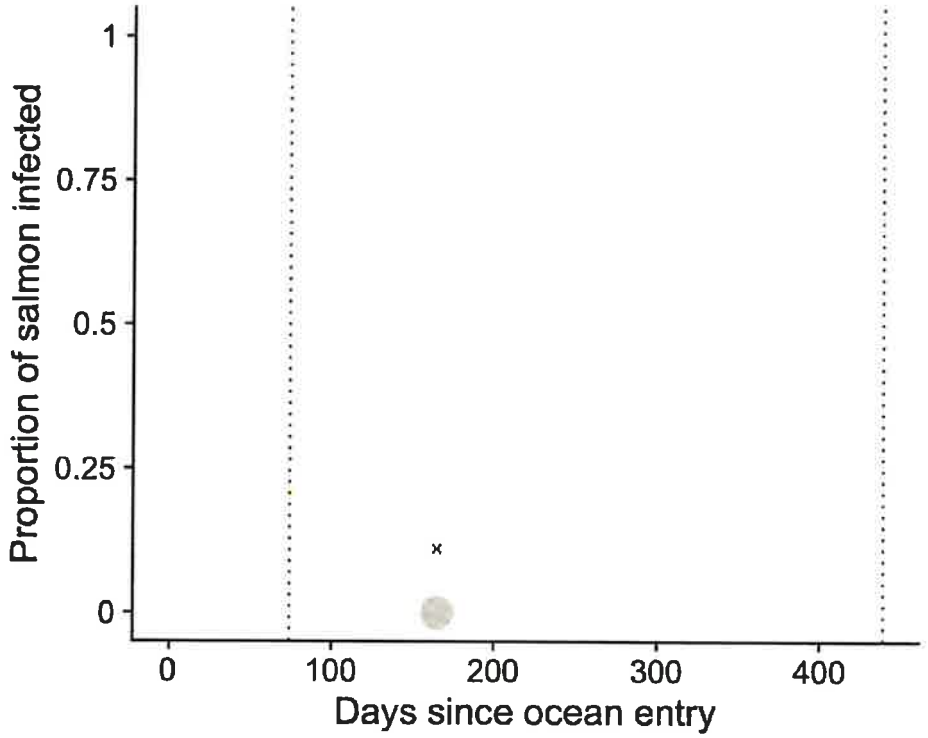
Flavobacterium psychrophilum



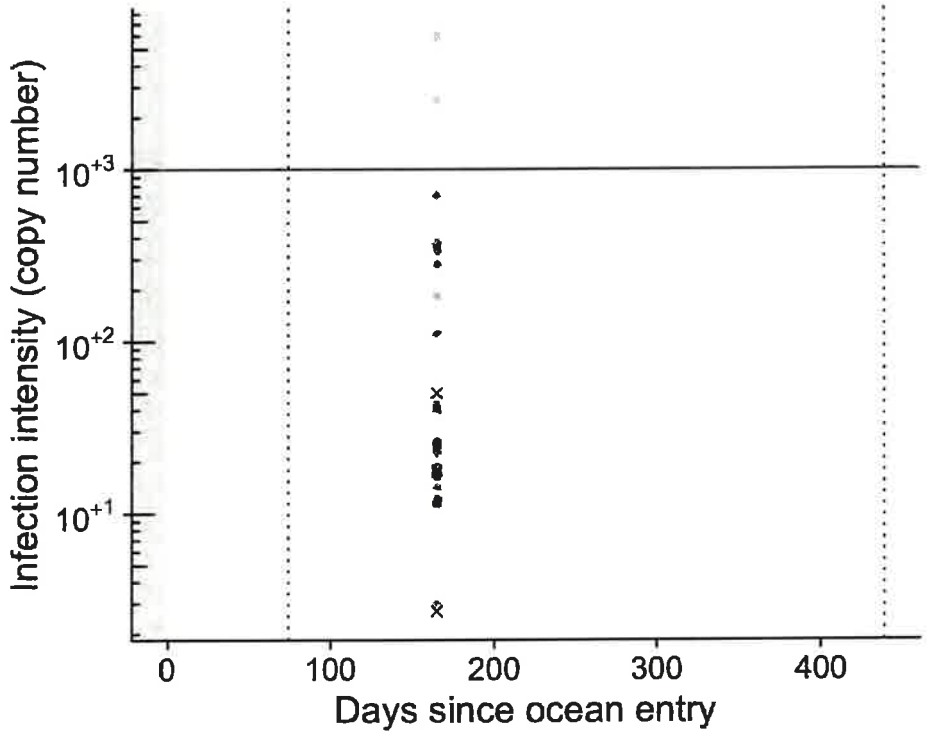


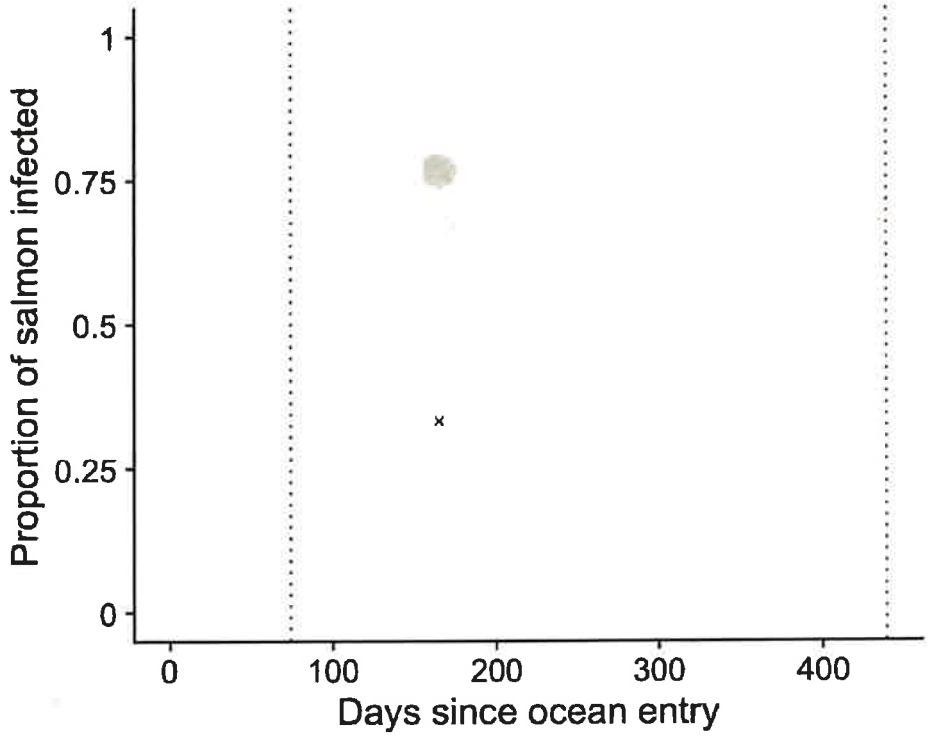
Piscirickettsia salmonis



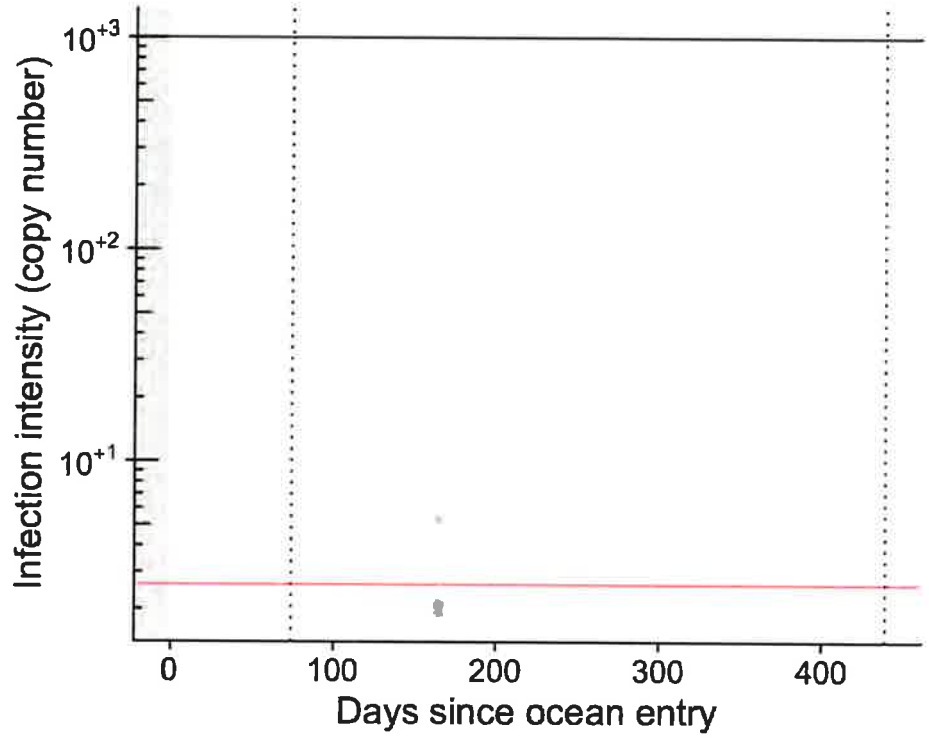


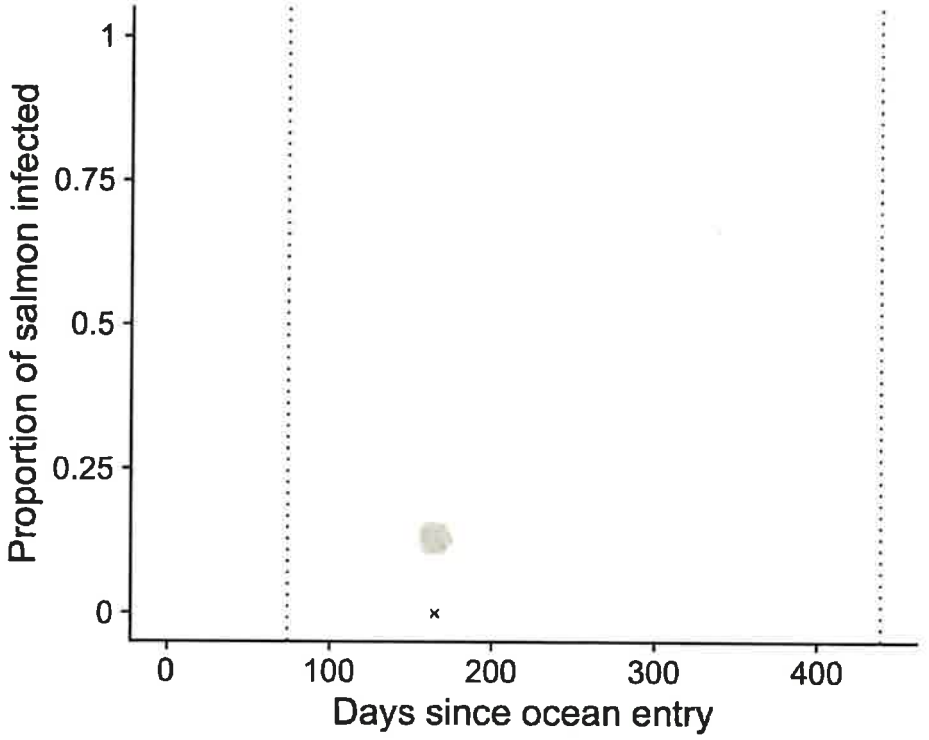
Piscine orthoreovirus





Candidatus Syngnamydia salmonis





Clinical signs

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

metric	N5081	N5082	N5083	N5084	N5085	N5086	N5087	N5088	N5089	N5090	N5091	N5092	N5093	N5094	N5095	N5096	N5097	N5098	N5099	N5100
General																				
Live					X	X	X	X					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	
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
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					
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	N5081	N5082	N5083	N5084	N5089	N5090	N5091	N5092	N5097
Heart									
Peri Epi	1		2	1	1	1	1	2	1
Myo		1	1				1	2	1
Liver									
Cong Haem	1	1			1	2			
Nec									1
Itis					1			2	
Spleen									
Cong Heam	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 Heam	1					1			
Interst Hyperplasia	1	1	2	2	1	1	2	2	2
Cnc									
Gliosis		1	1	1			2		1
Cong Heam	2	2	2	2	2	1			
Gills									
Itis	nv		nv	nv	nv	nv	nv	nv	
Cong Heam	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		Myocardionecrosis (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), Acumulation Eosiniphilic 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* Syngnamydia 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 conducible 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
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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
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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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Piscine orthoreovirus 12
Candidatus Syngnamydia salmonis 13
Tenacibaculum maritimum 14
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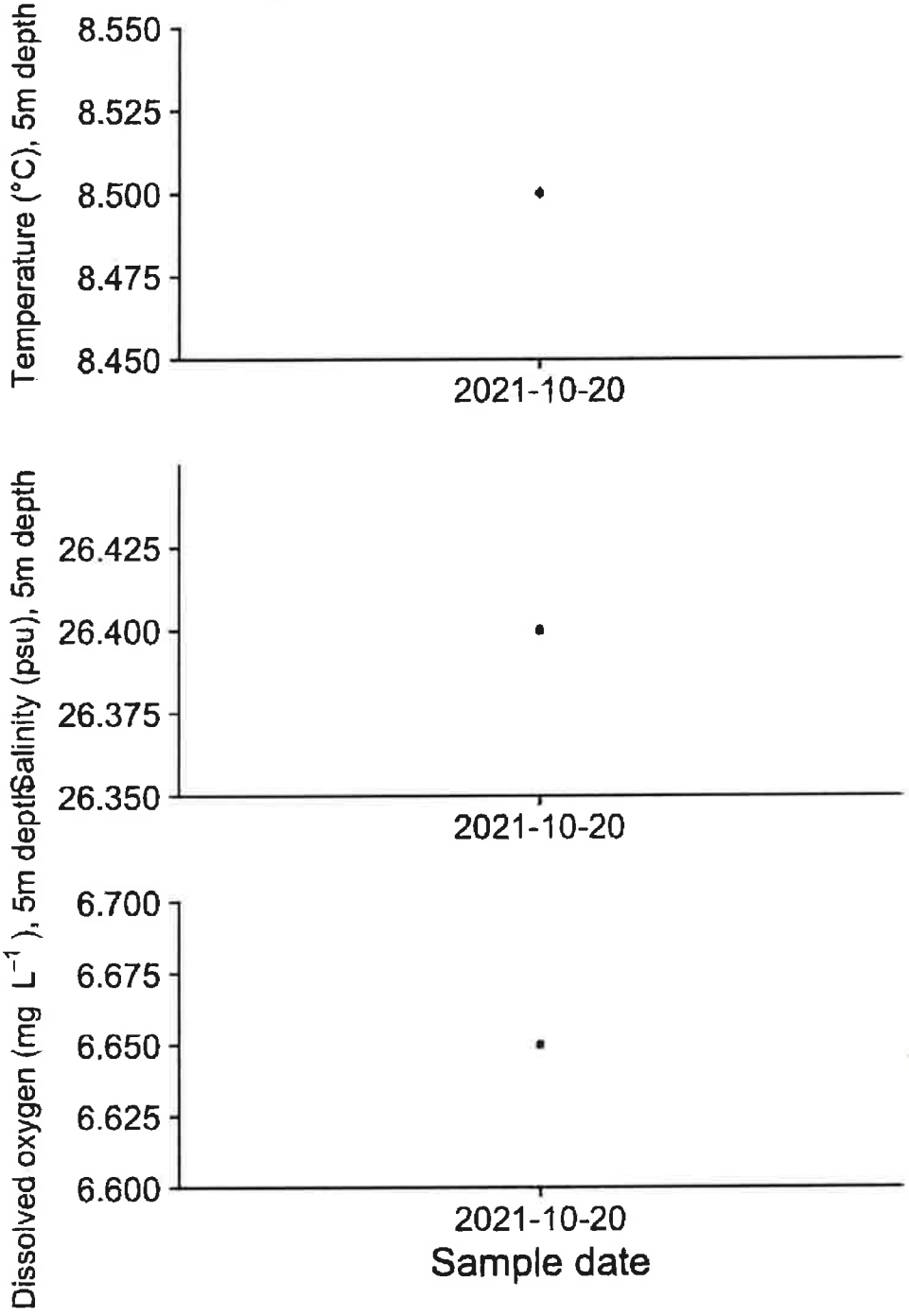
Executive summary

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.

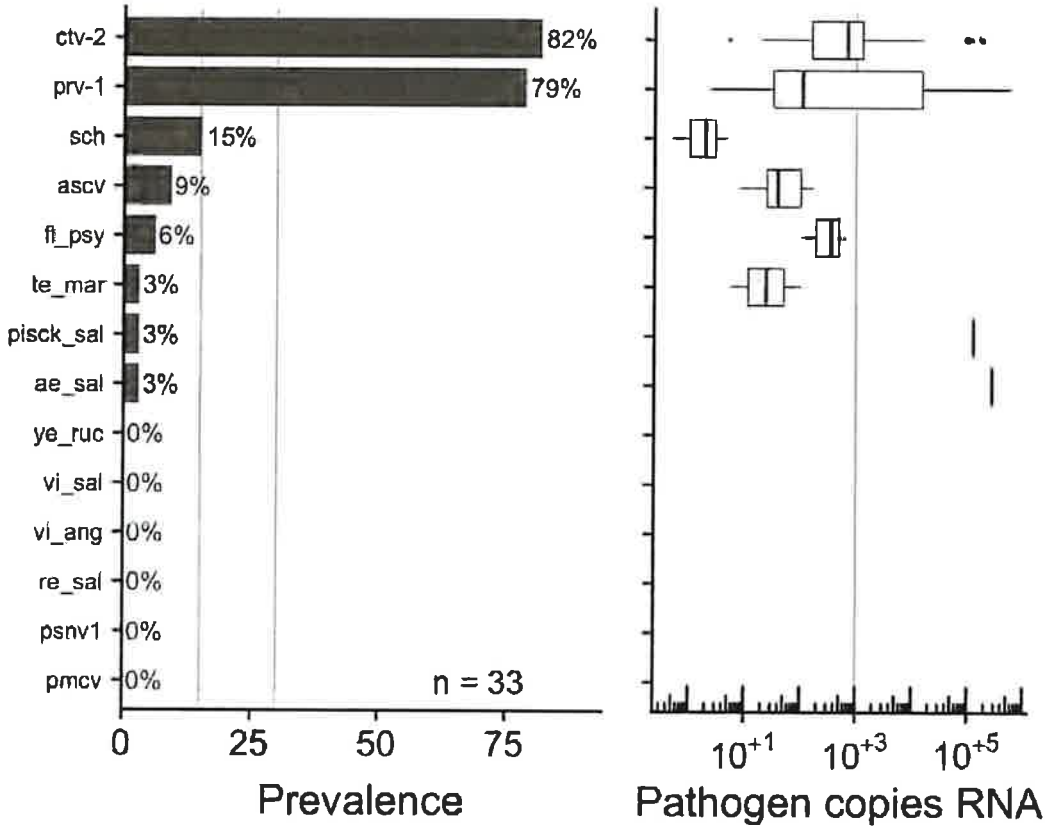
Histology samples have been sent to Wax-It Histo Ltd. to process and prepare slides, which have been read and scored by Dr. Di Cicco. A digital copy of each slide is available to the Company.

Environmental data

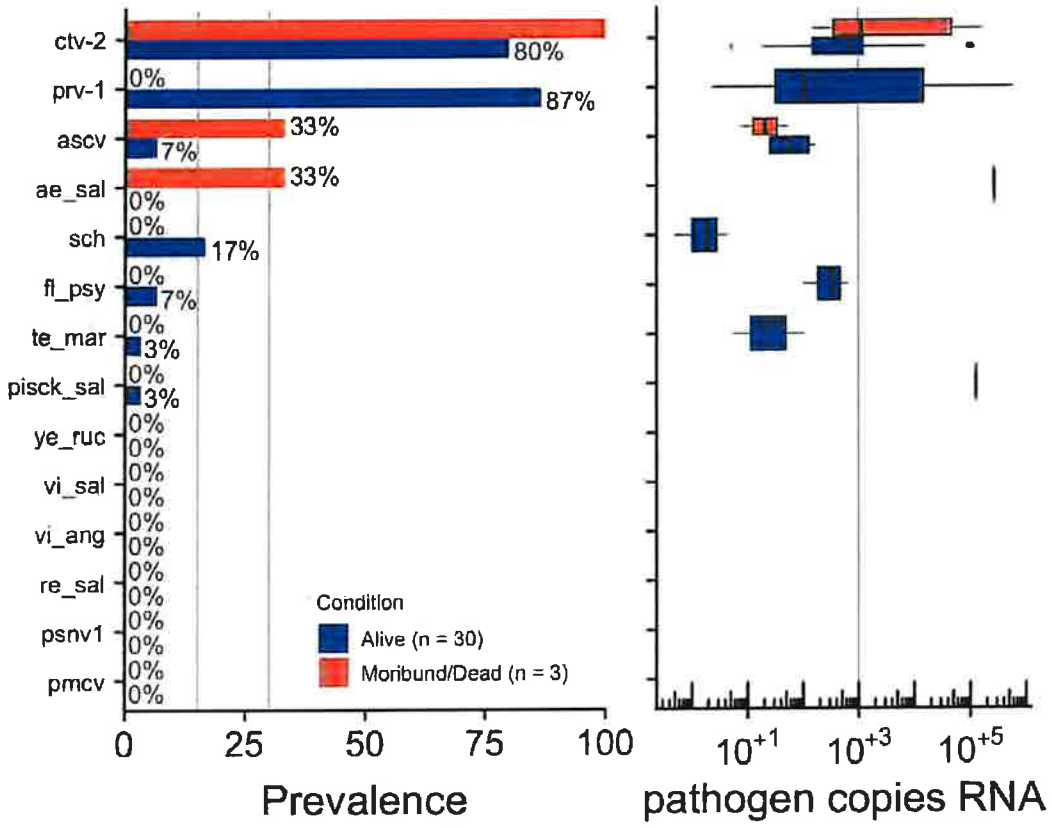


Water temperature (°C), salinity (ppm), and dissolved oxygen (mg/L) at a 5m depth. Certain sampling dates have no recorded environmental data, resulting in gaps in the plots.

Overall infectious agent prevalence



Infectious agent prevalence in samples collected on 2021-10-20.



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

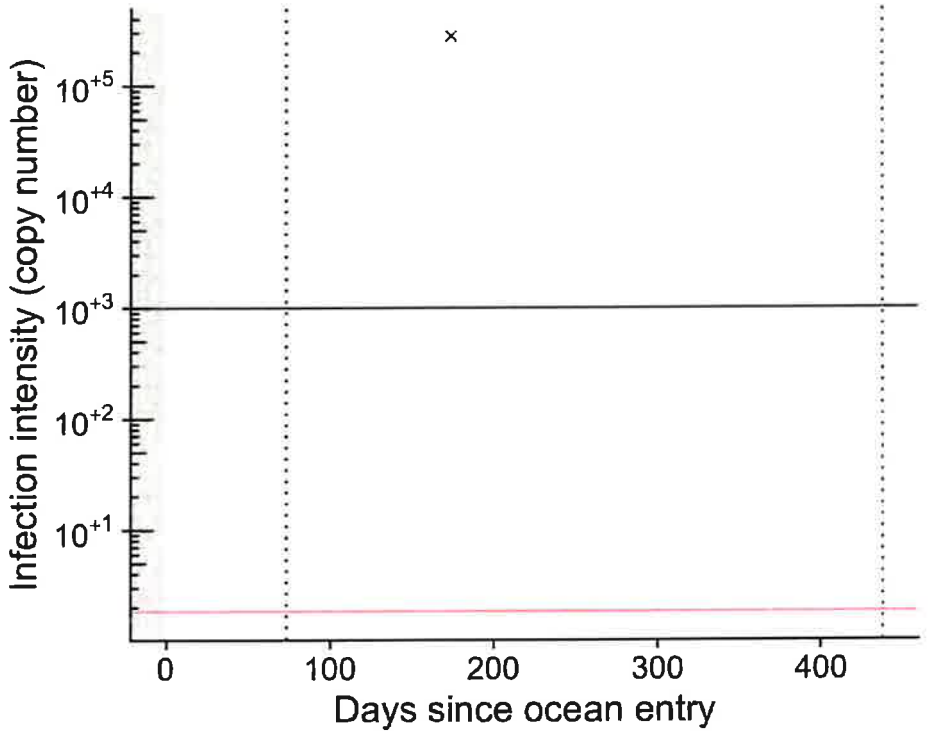
The following plots show individual infectious agent trends across all farm sites. In cases where sample size is sufficient, curves from a generalised additive model are included in the plot.

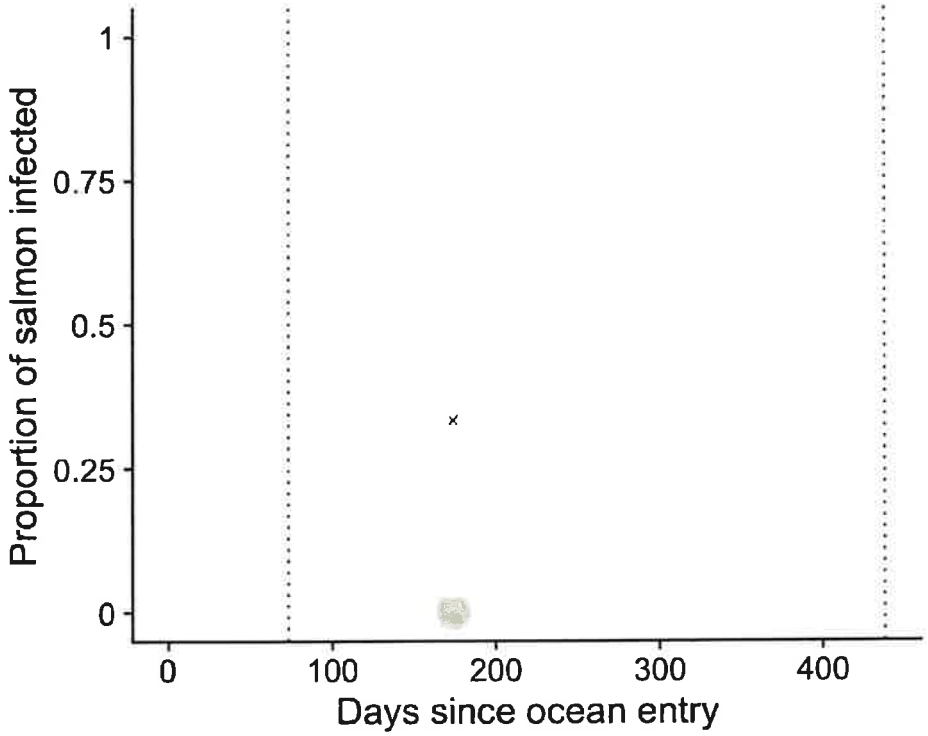
Grey circles represent live fish, and black X's represent dead/dying fish. Curves indicate mean predictions from a generalised additive model; blue and red correspond to live and dead/dying fish, respectively (shaded areas show 95% confidence regions). Left-hand grey region indicates freshwater hatchery 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.

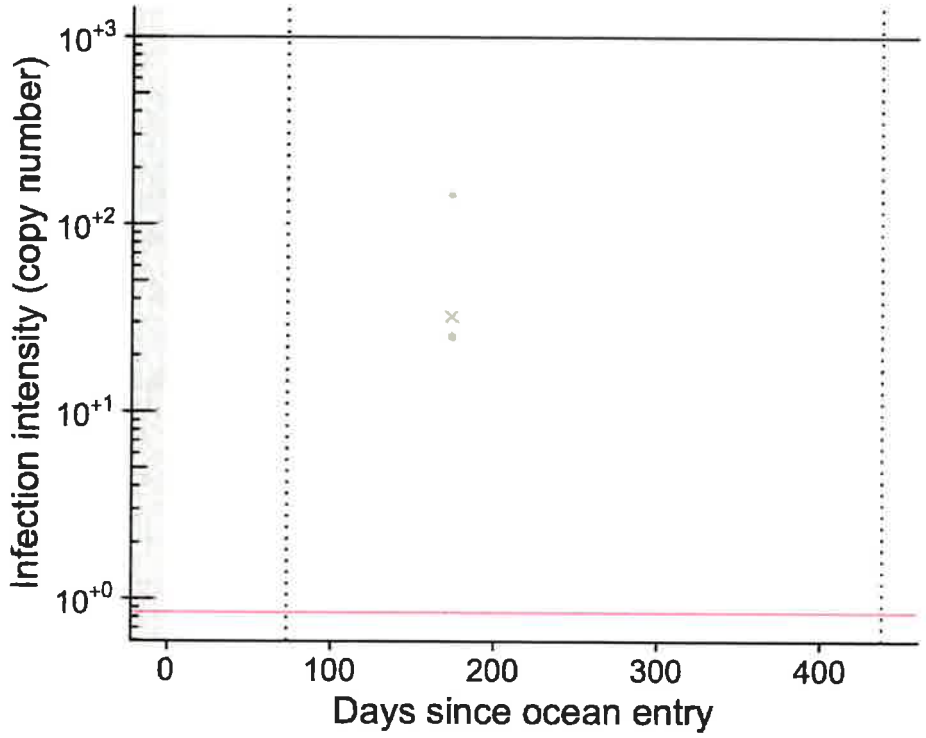
For proportion plots, grey circles show prevalence in live fish on each sampling date, and black X's show prevalence in dead/dying fish (symbol areas proportional to sample sizes).

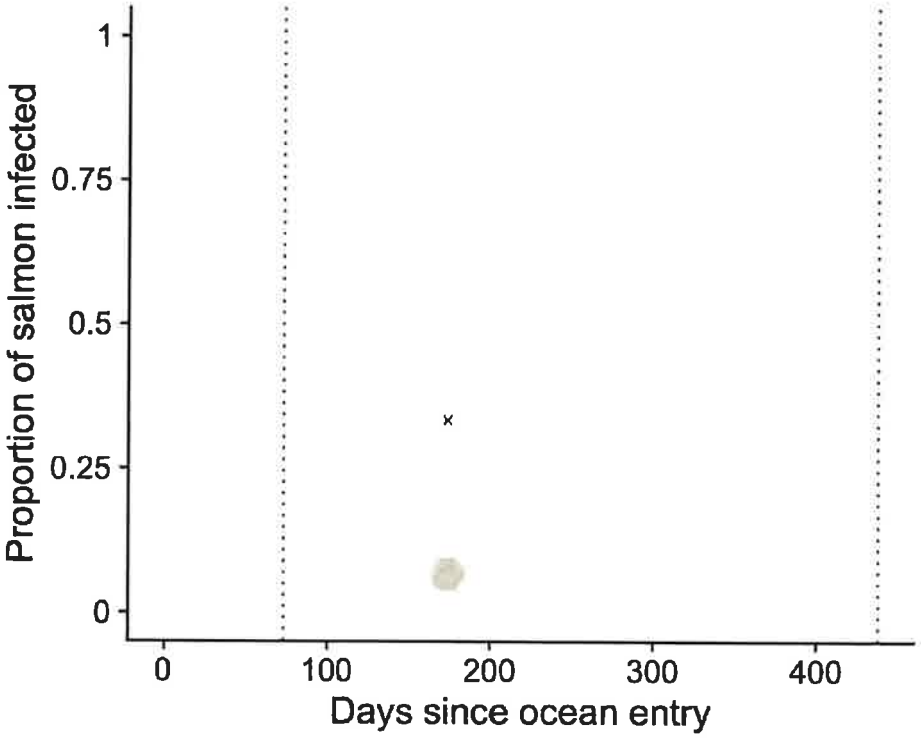
Aeromonas salmonicida



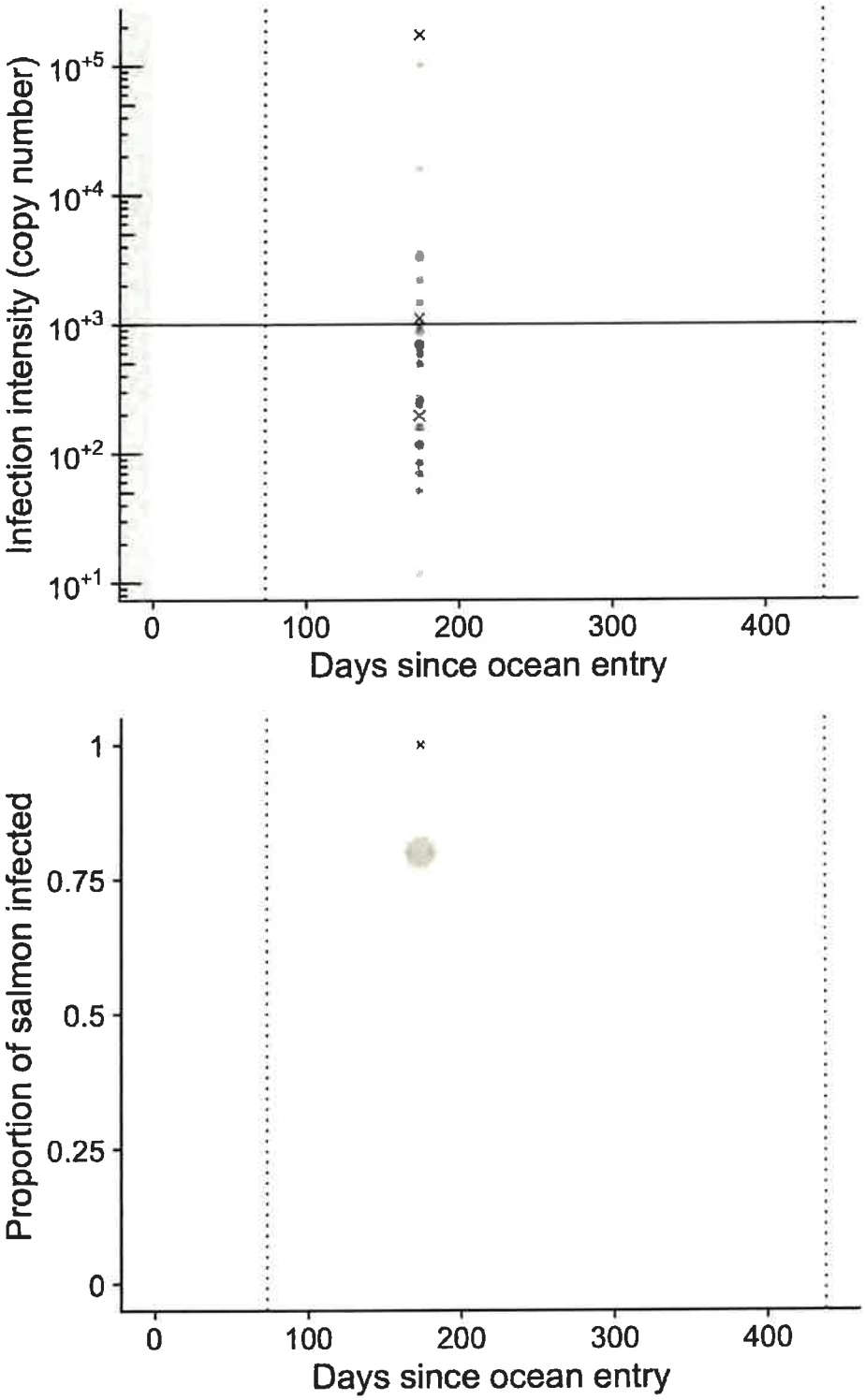


Atlantic salmon calicivirus

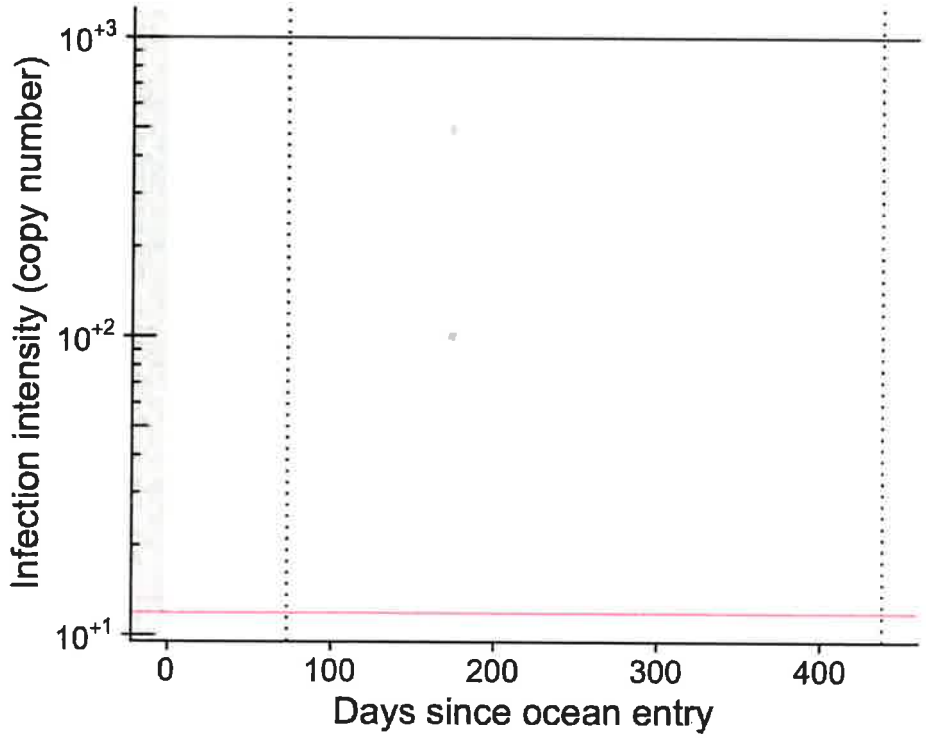


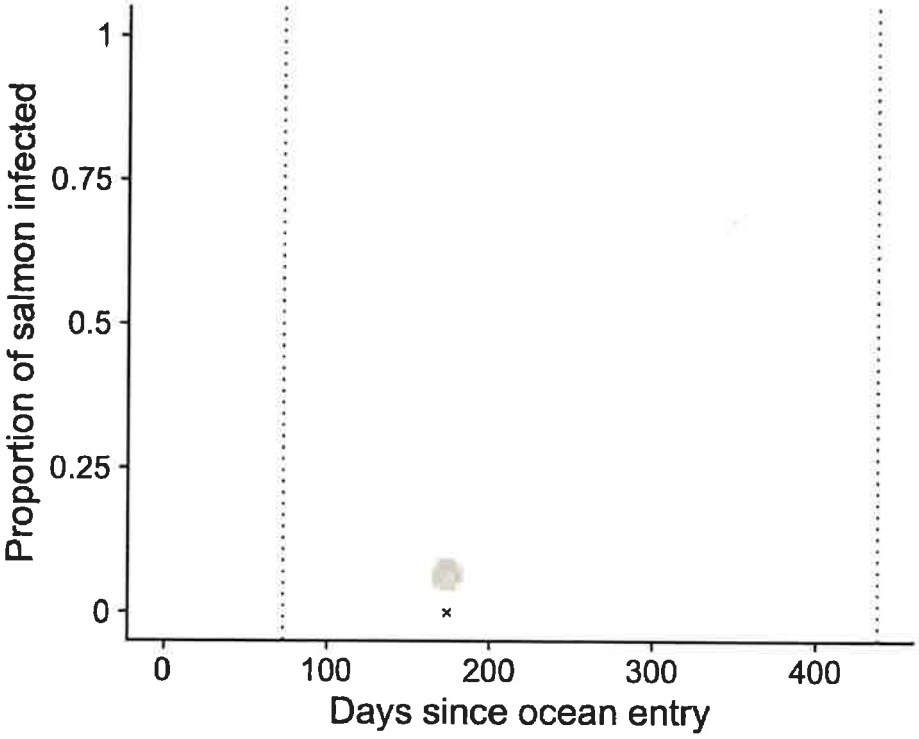


Cutthroat trout virus-2

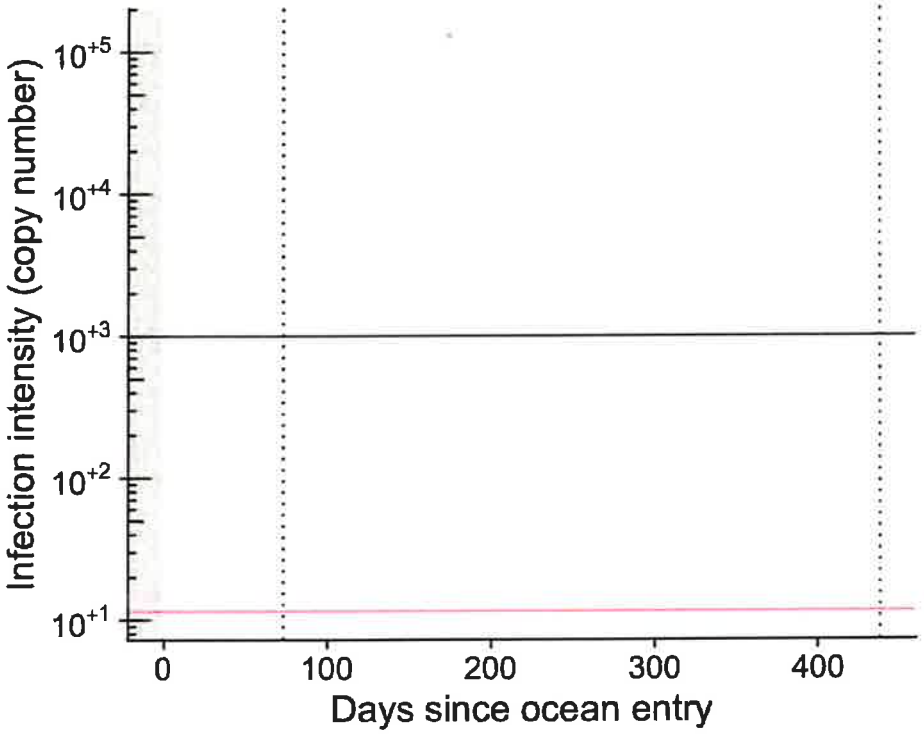


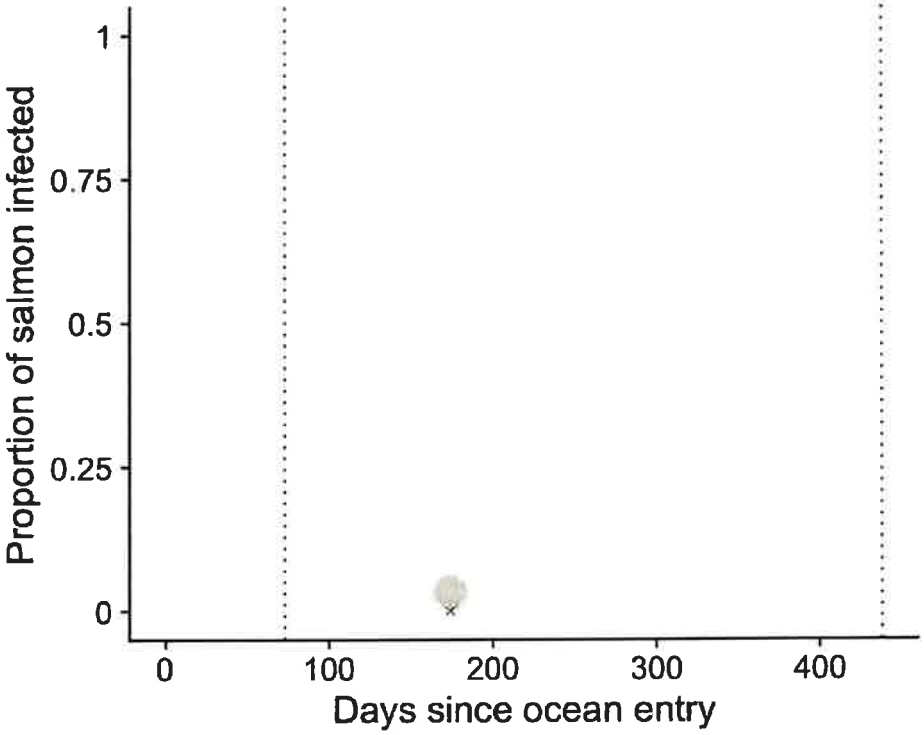
Flavobacterium psychrophilum



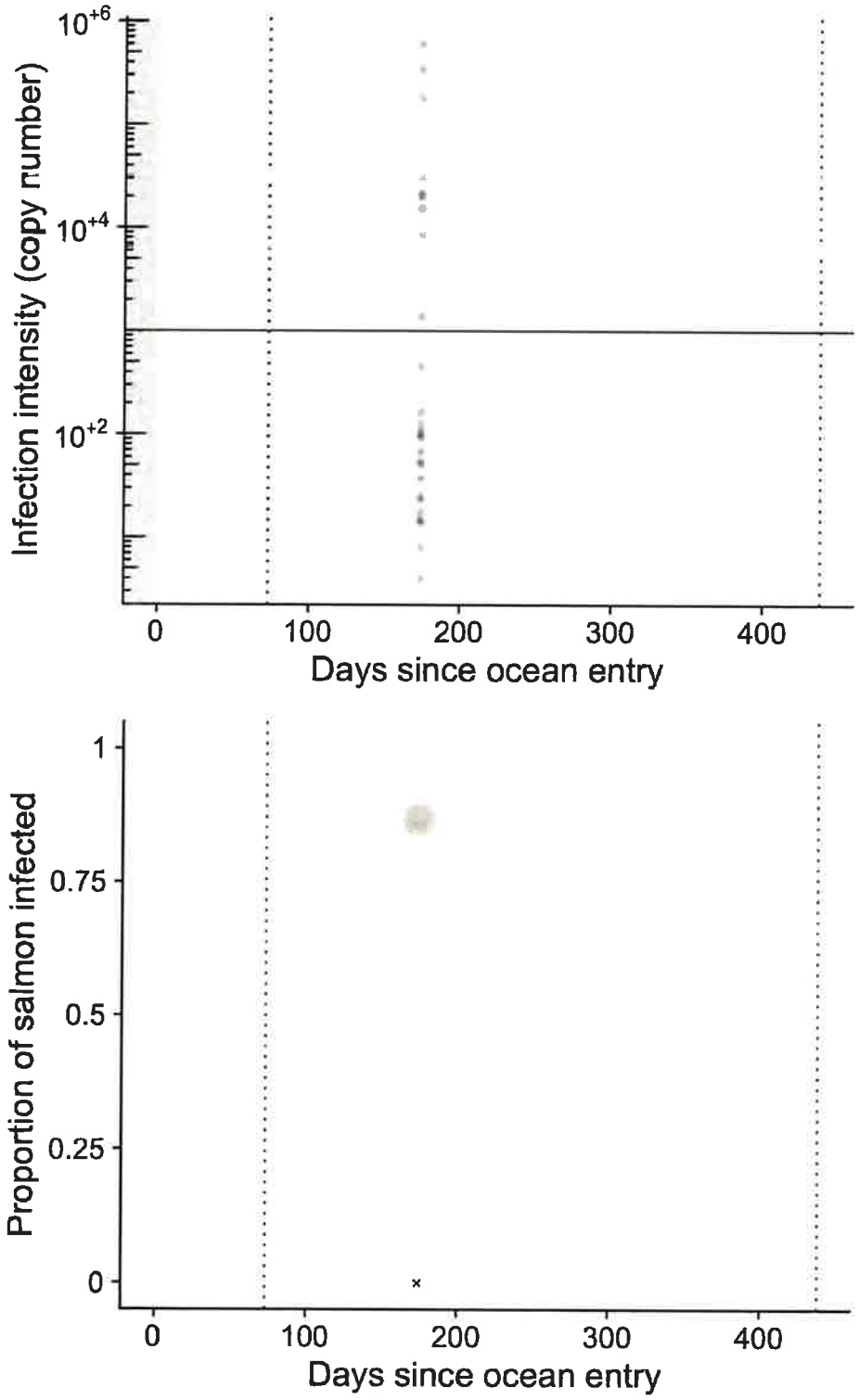


Piscirickettsia salmonis

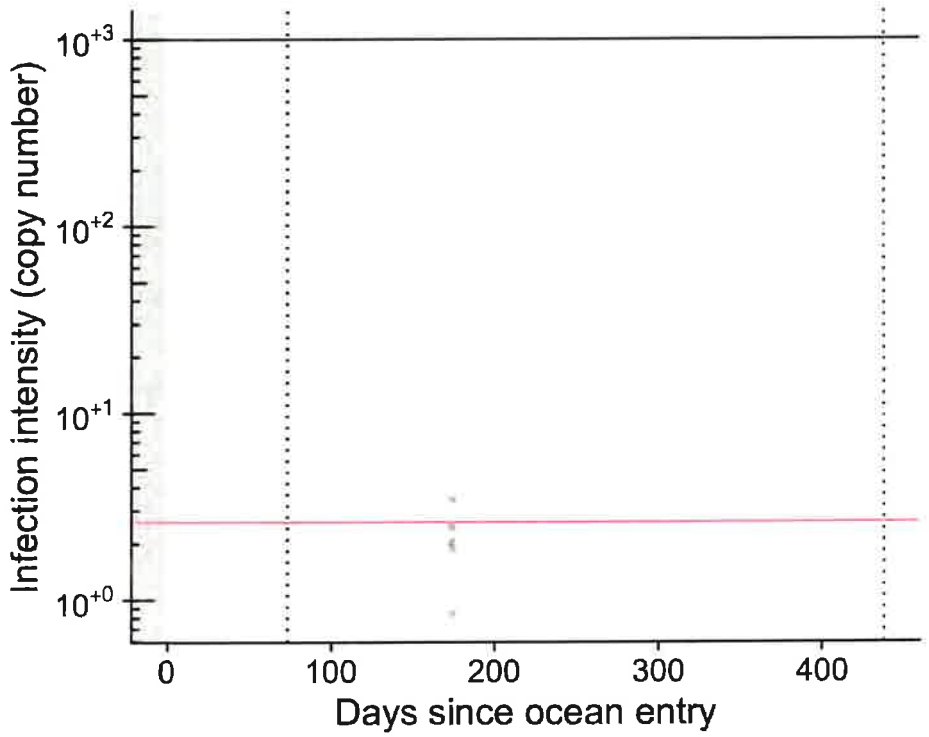


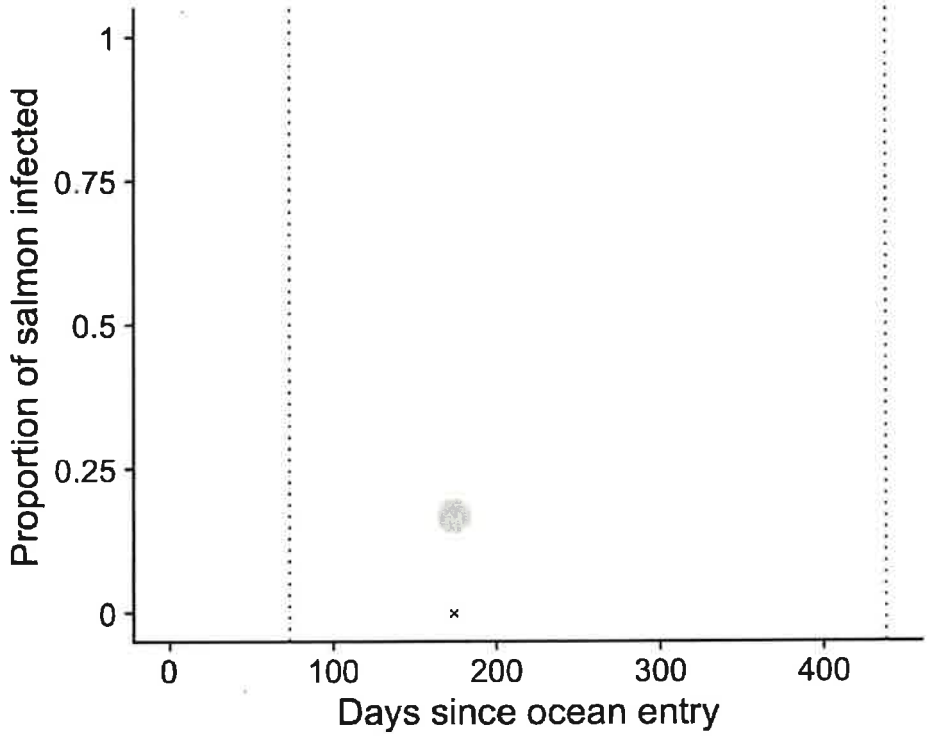


Piscine orthoreovirus

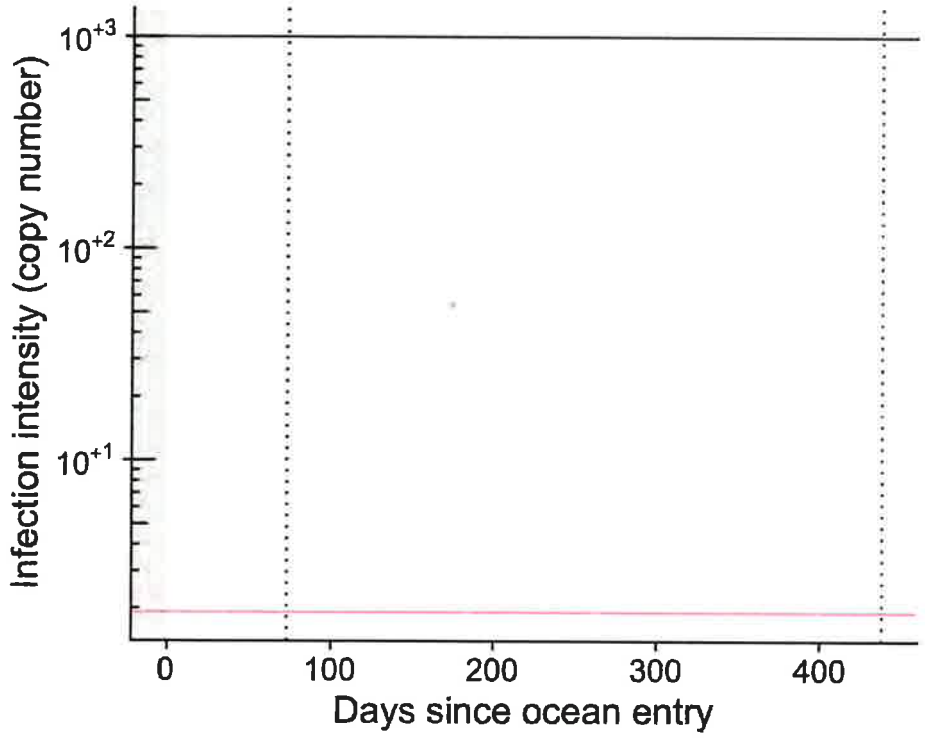


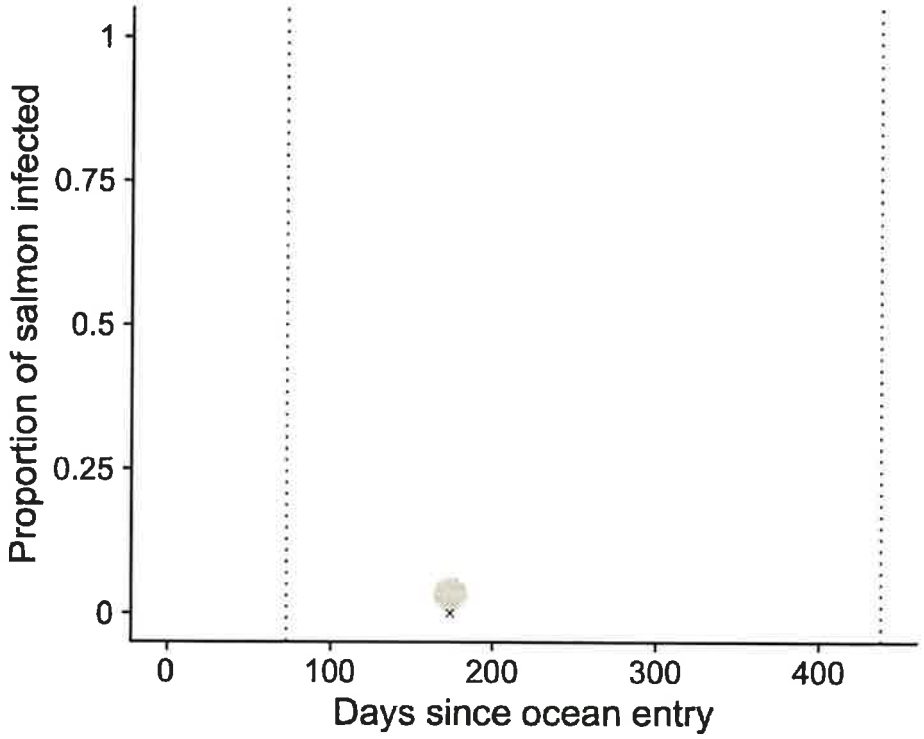
Candidatus Syngnamydia salmonis





Tenacibaculum maritimum





Clinical signs

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

metric	N5121	N5122	N5123	N5124	N5125	N5126	N5127	N5128	N5129	N5130	N5131	N5132	N5133	N5134	N5135	N5136	N5137	N5138	N5139	N5140
General																				
Live				X	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																				
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
Erosions		X	X								X	X	X
Nodules/White Spots												X	X
Abdominal Cavity													
Body Fat Content							X						
Spleen													
Enlarged	X	X				X		X			X		
Liver													
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	N5121	N5122	N5123
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* Syngnamydia 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 Syngnamydia 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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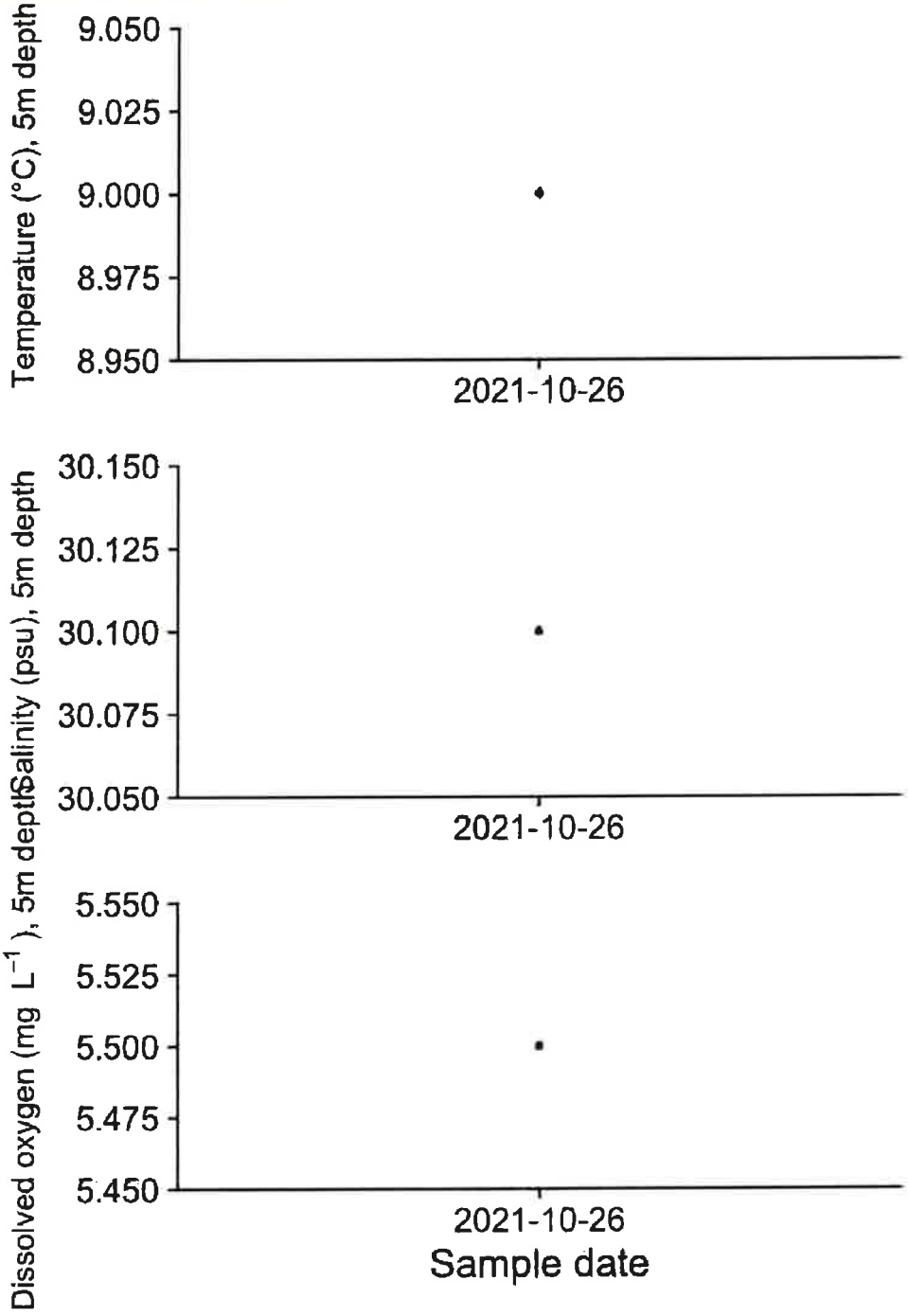
Executive summary

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.

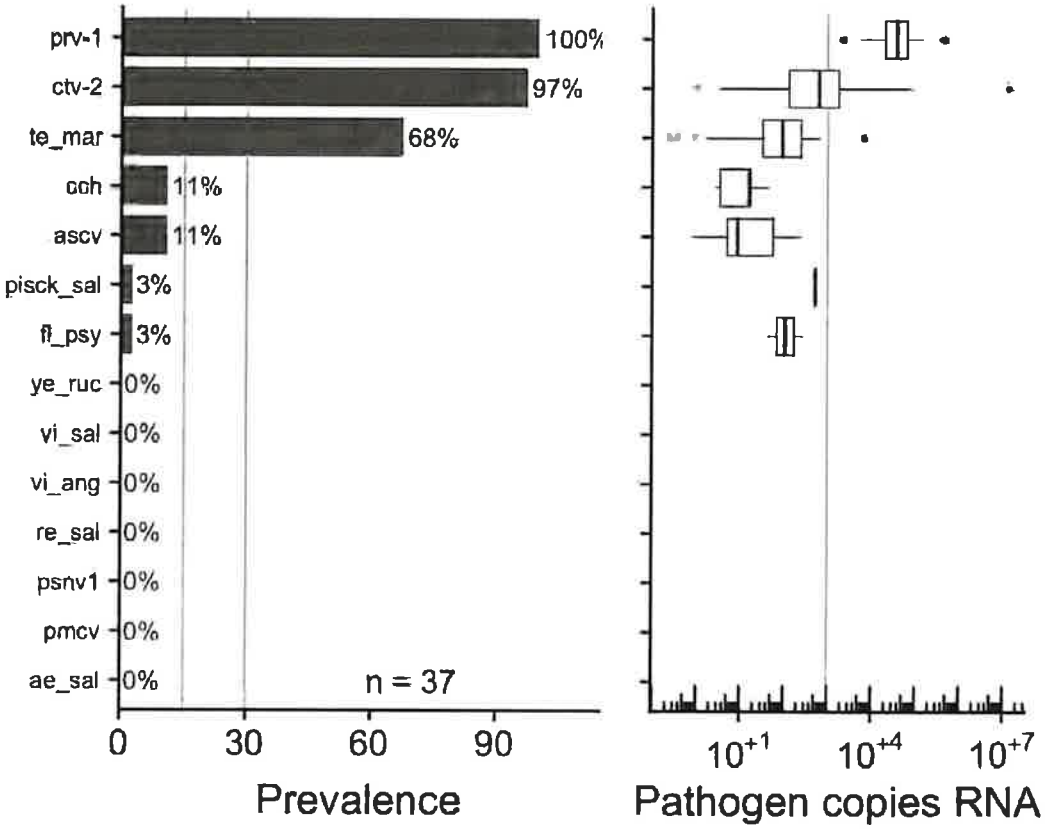
Histology samples have been sent to Wax-It Histo Ltd. to process and prepare slides, which have been read and scored by Dr. Di Cicco. A digital copy of each slide is available to the Company.

Environmental data

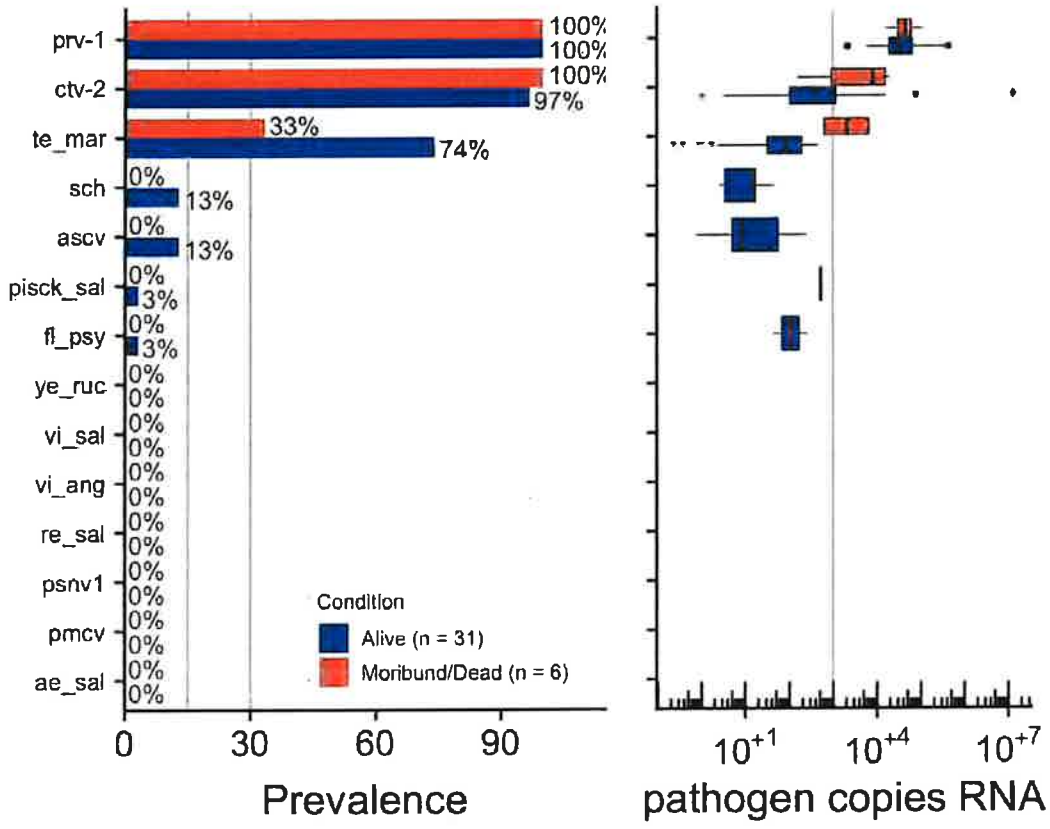


Water temperature (°C), salinity (ppm), and dissolved oxygen (mg/L) at a 5m depth. Certain sampling dates have no recorded environmental data, resulting in gaps in the plots.

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

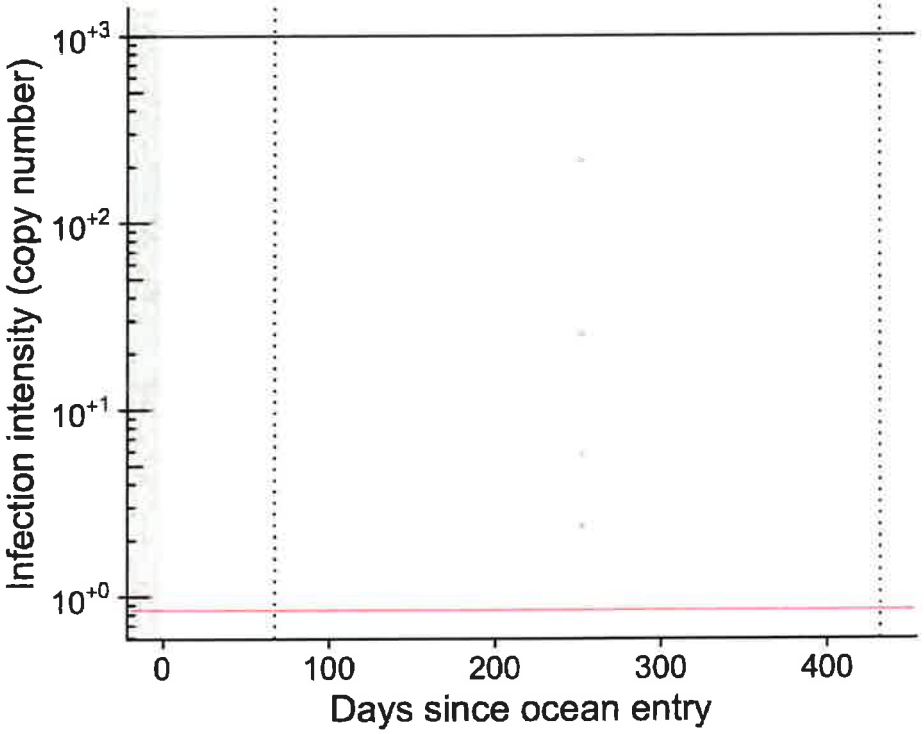
The following plots show individual infectious agent trends across all farm sites. In cases where sample size is sufficient, curves from a generalised additive model are included in the plot.

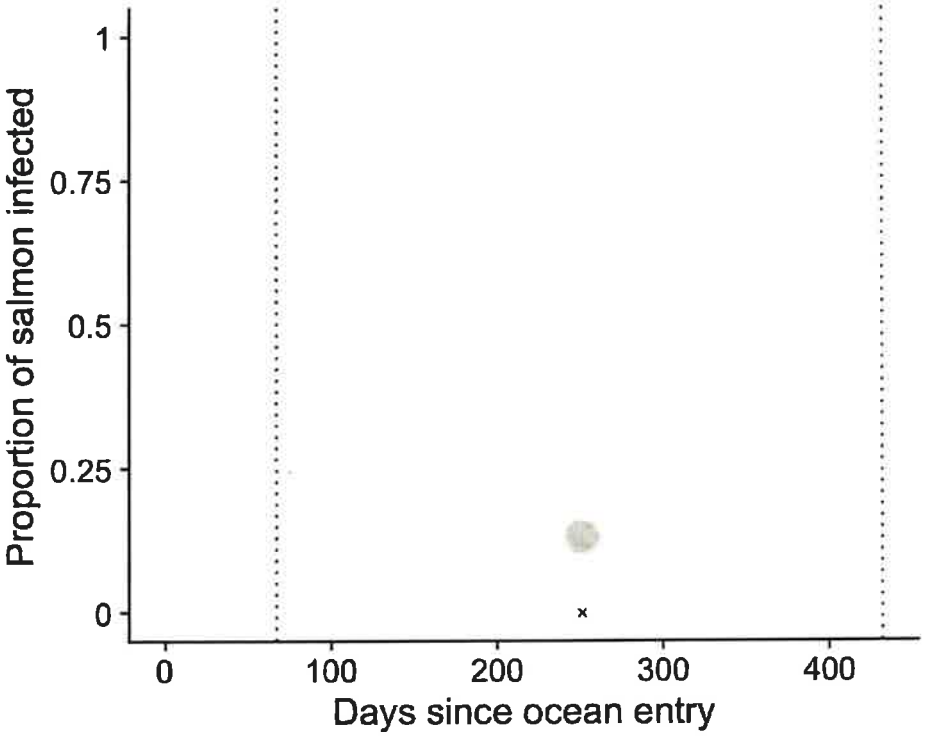
Grey circles represent live fish, and black X's represent dead/dying fish. Curves indicate mean predictions from a generalised additive model; blue and red correspond to live and dead/dying fish, respectively (shaded areas show 95% confidence regions). Left-hand grey region indicates freshwater hatchery 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.

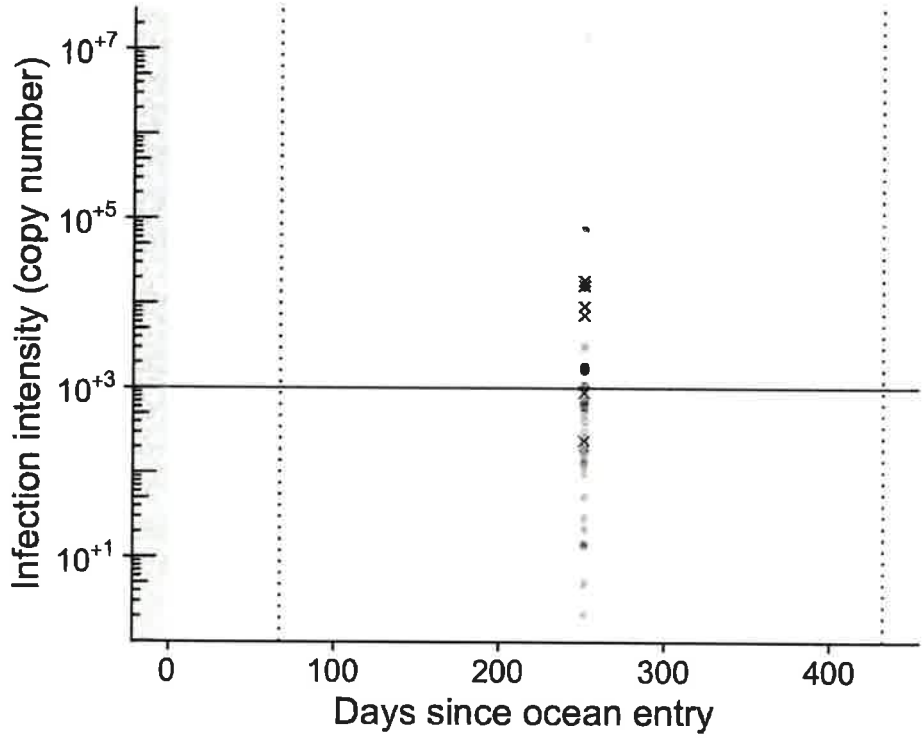
For proportion plots, grey circles show prevalence in live fish on each sampling date, and black X's show prevalence in dead/dying fish (symbol areas proportional to sample sizes).

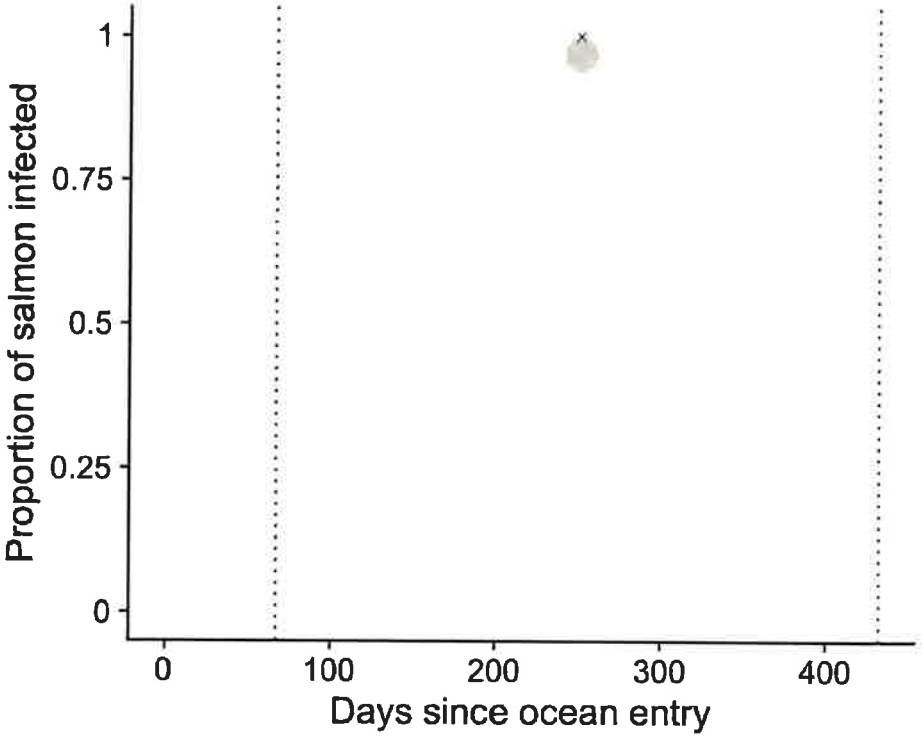
Atlantic salmon calicivirus



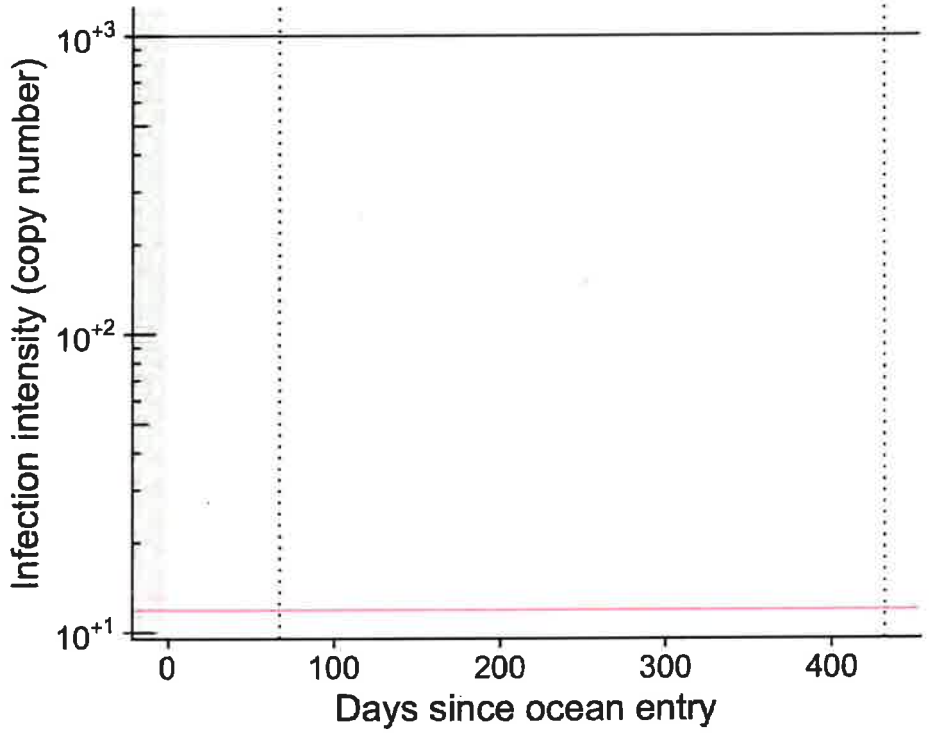


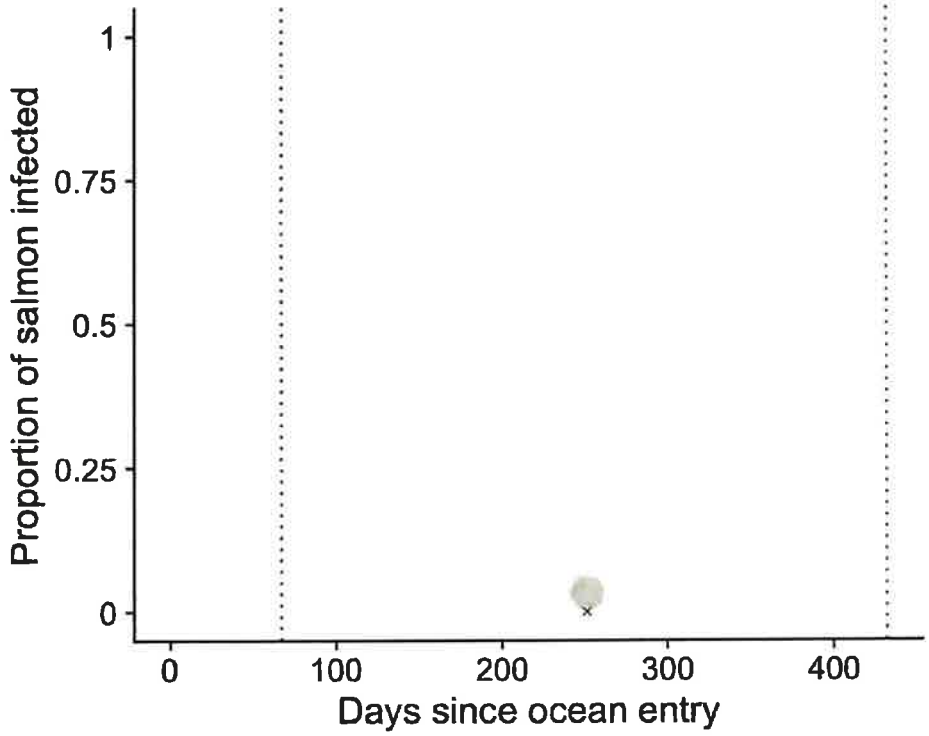
Cutthroat trout virus-2



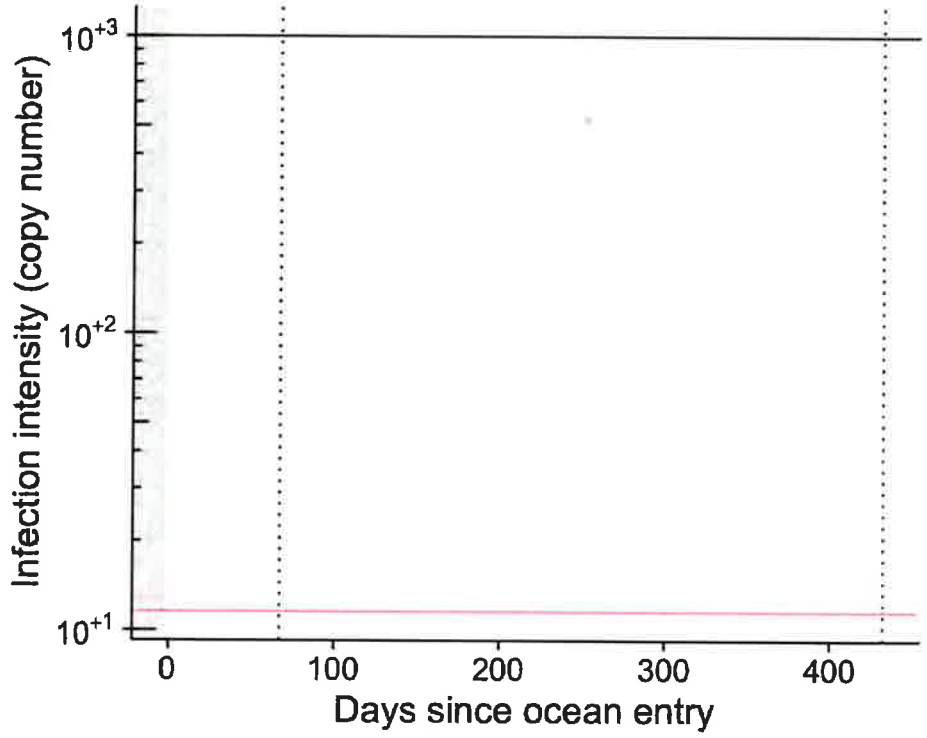


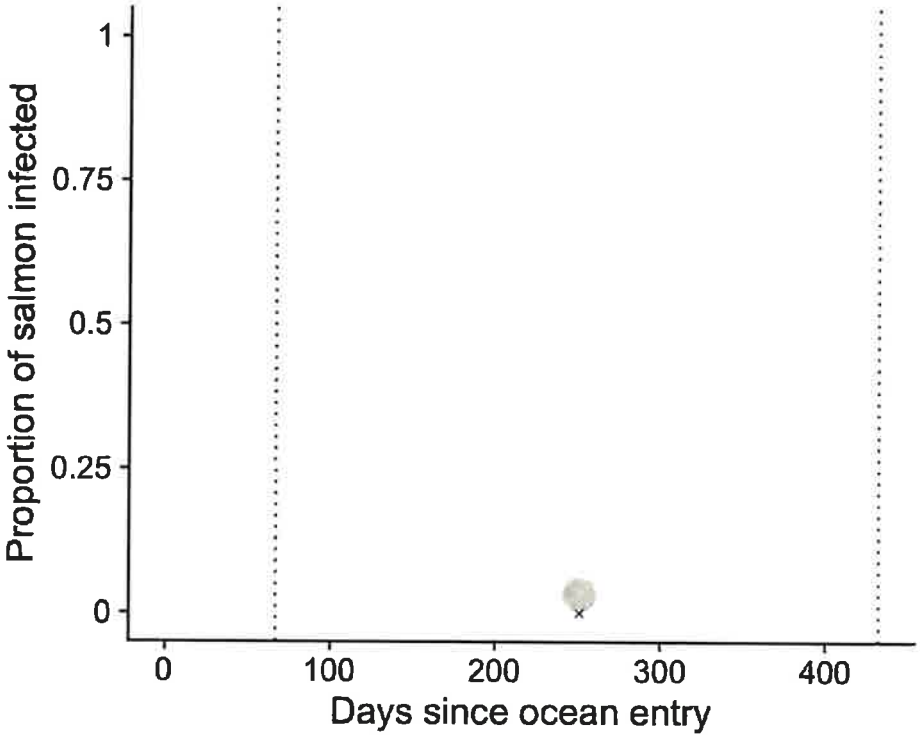
Flavobacterium psychrophilum



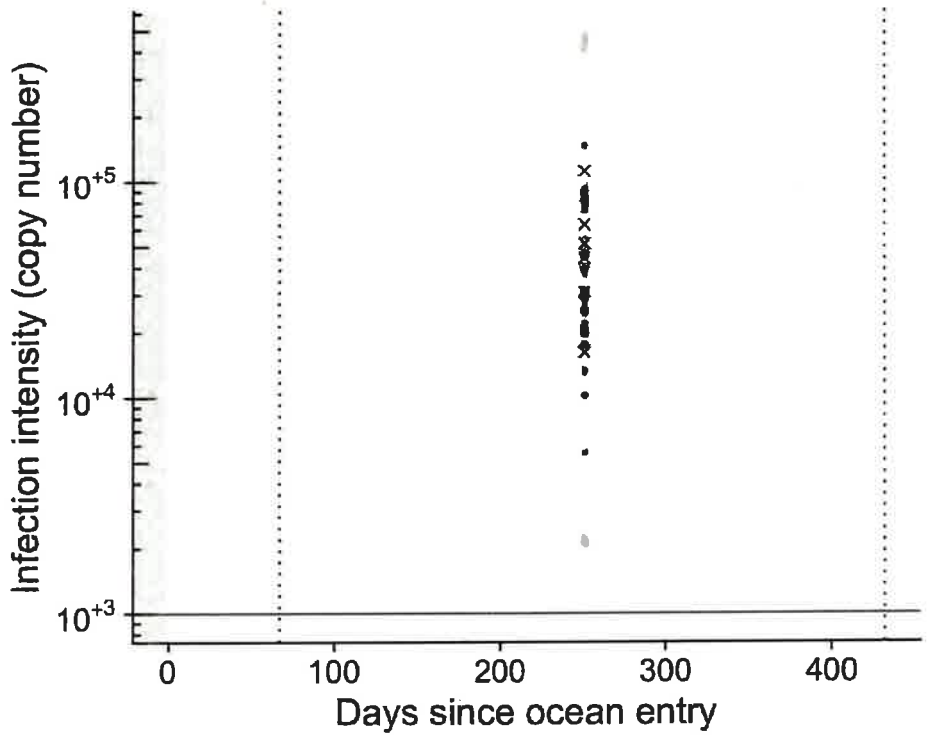


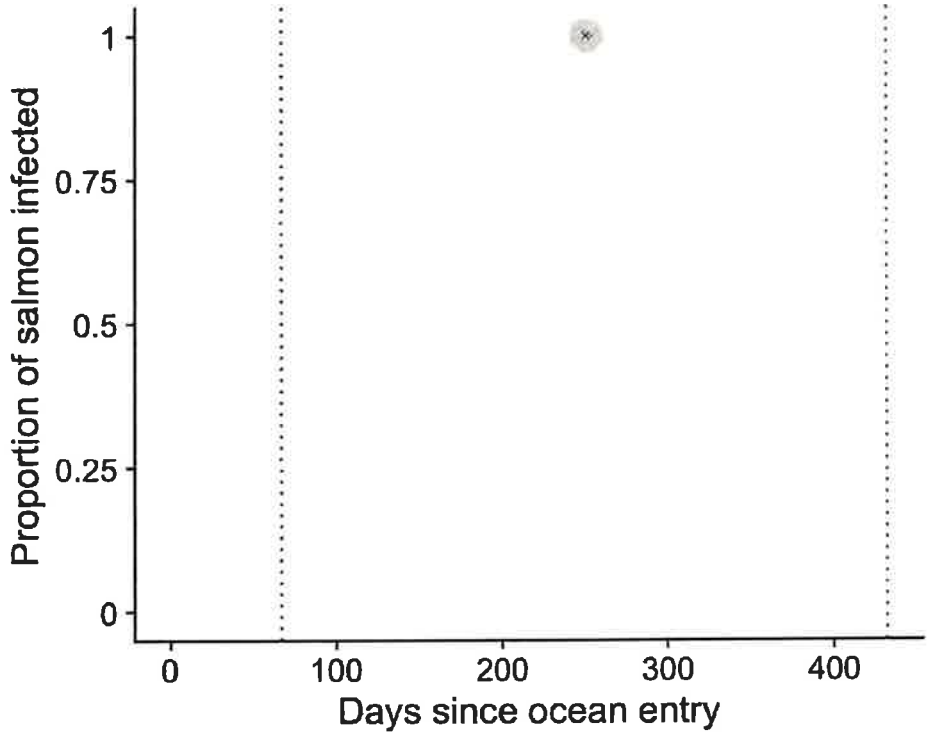
Piscirickettsia salmonis



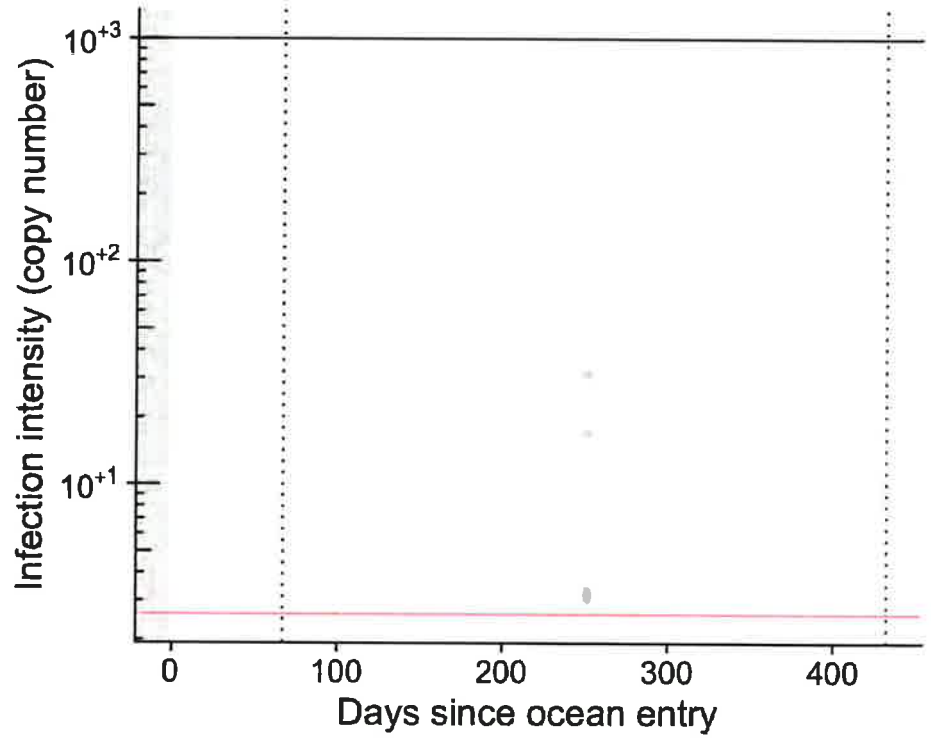


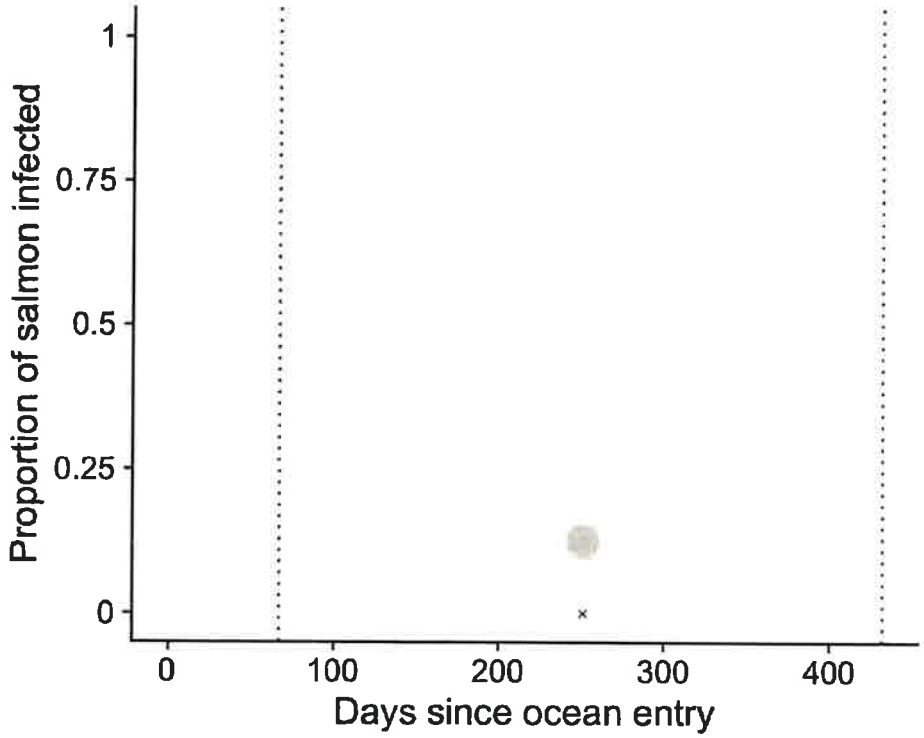
Piscine orthoreovirus



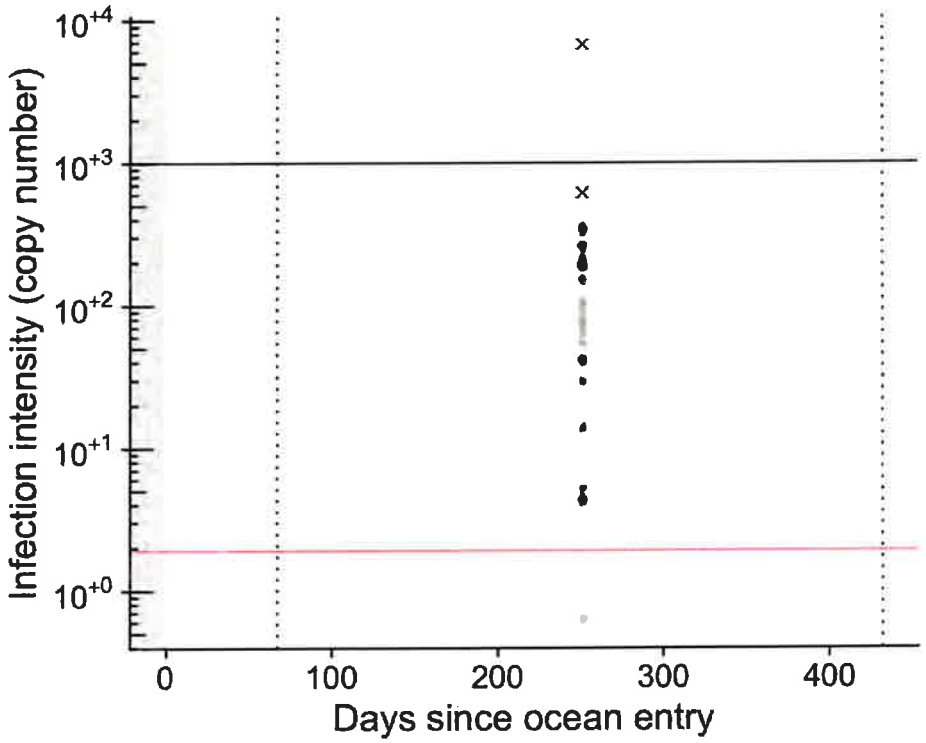


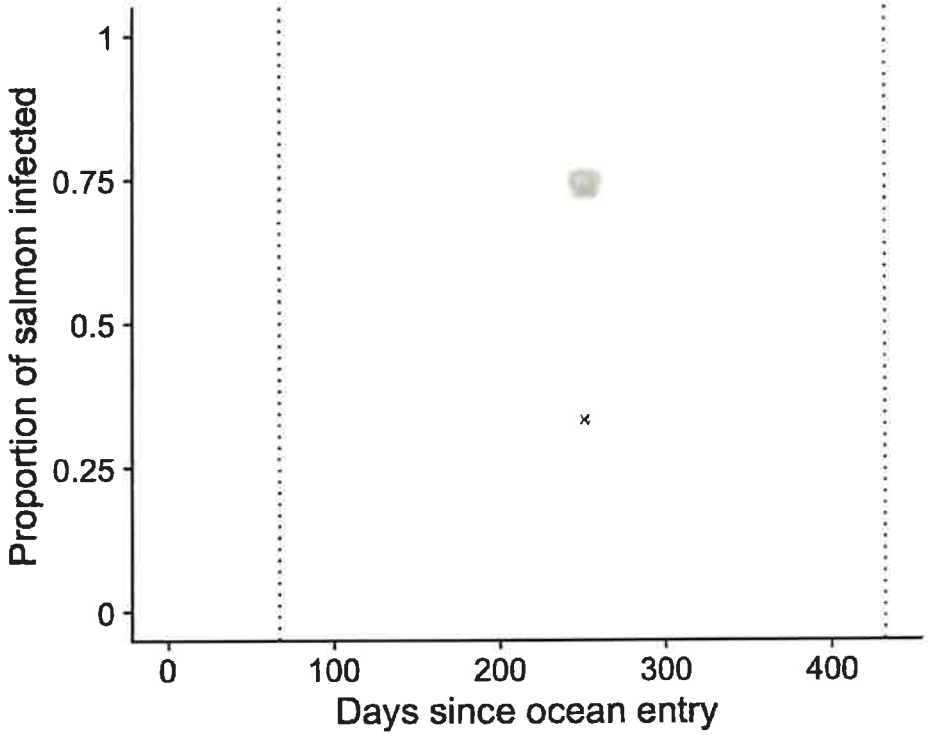
Candidatus Syngnamydia salmonis





Tenacibaculum maritimum





Clinical signs

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

metric	N5161	N5162	N5163	N5164	N5165	N5166	N5167	N5168	N5169	N5170	N5171	N5172	N5173	N5174	N5175	N5176	N5177	N5178	N5179	N5180
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																				
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	
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* Syngnamydia 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 myocardionecrosis, 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 Syngnamydia 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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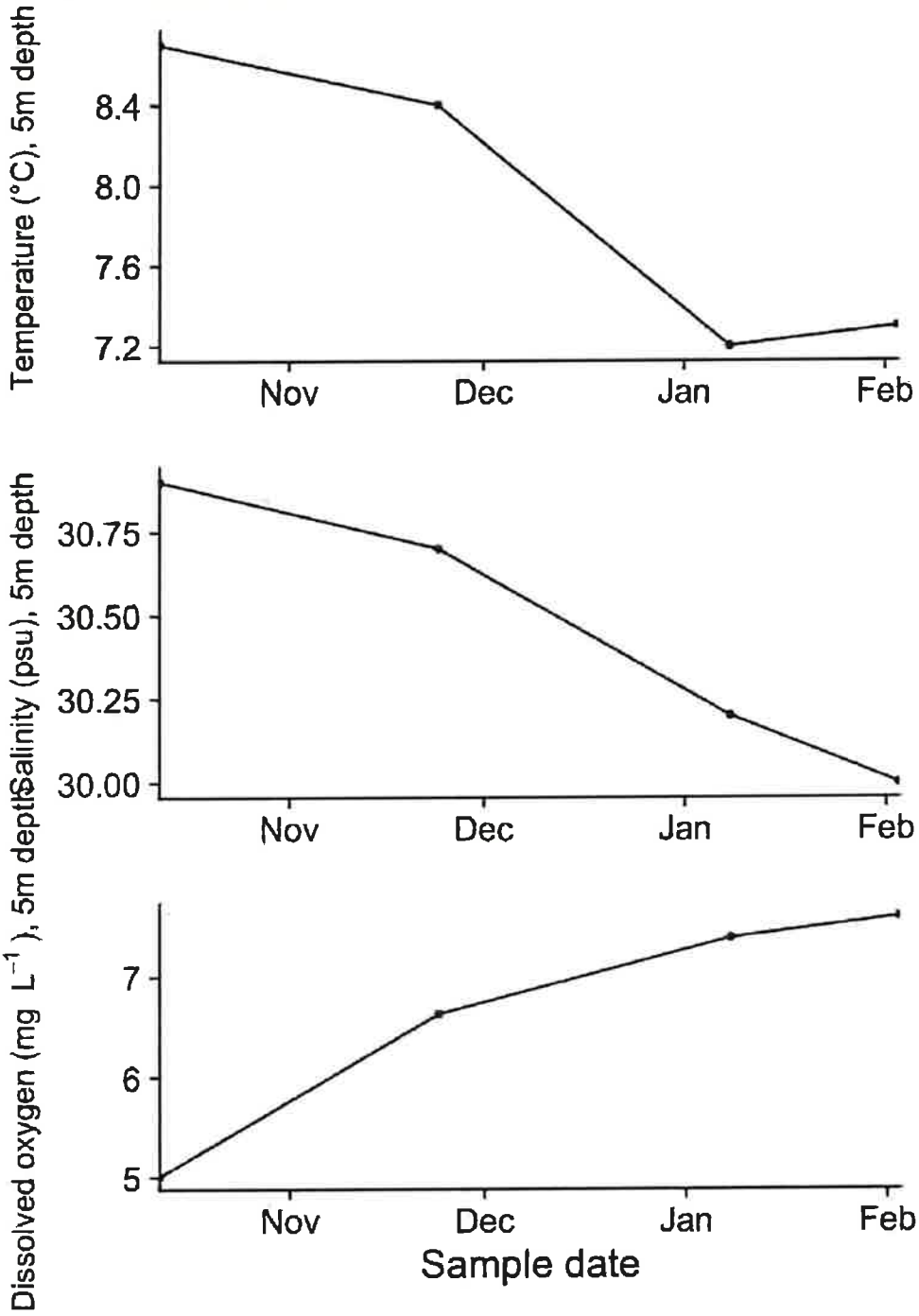
Executive summary

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.

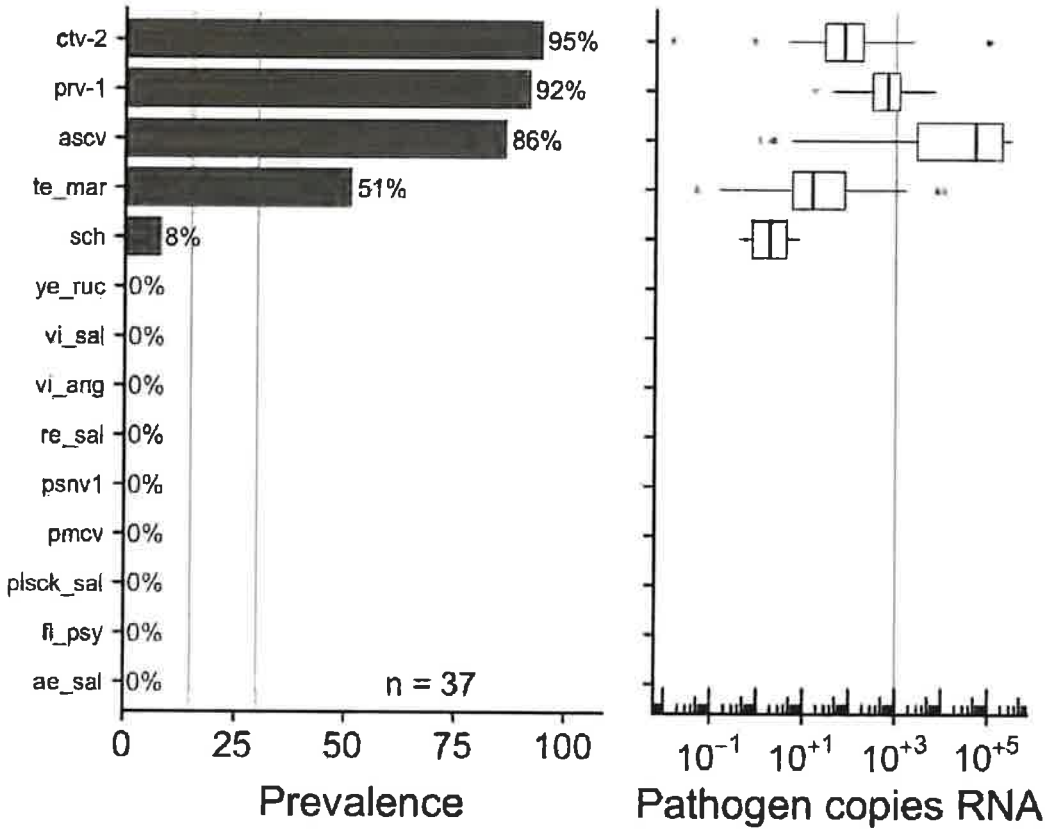
Histology samples have been sent to Wax-It Histo Ltd. to process and prepare slides, which have been read and scored by Dr. Di Cicco. A digital copy of each slide is available to the Company.

Environmental data

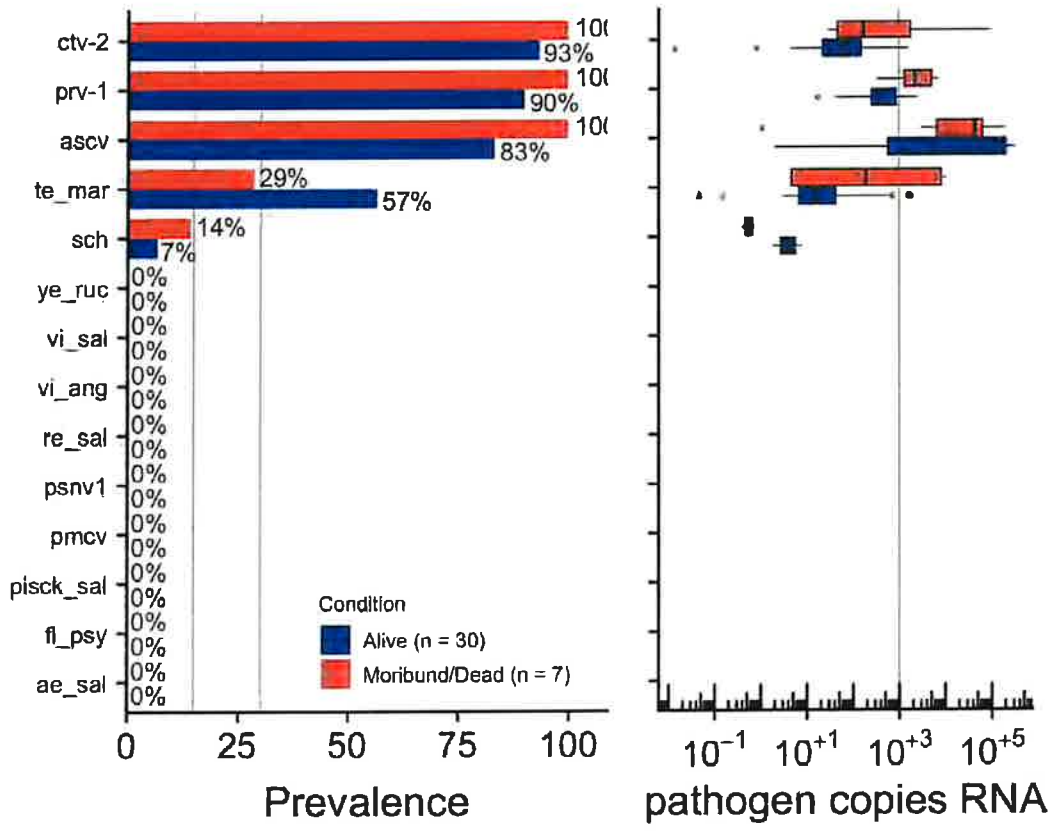


Water temperature (°C), salinity (ppm), and dissolved oxygen (mg/L) at a 5m depth. Certain sampling dates have no recorded environmental data, resulting in gaps in the plots.

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

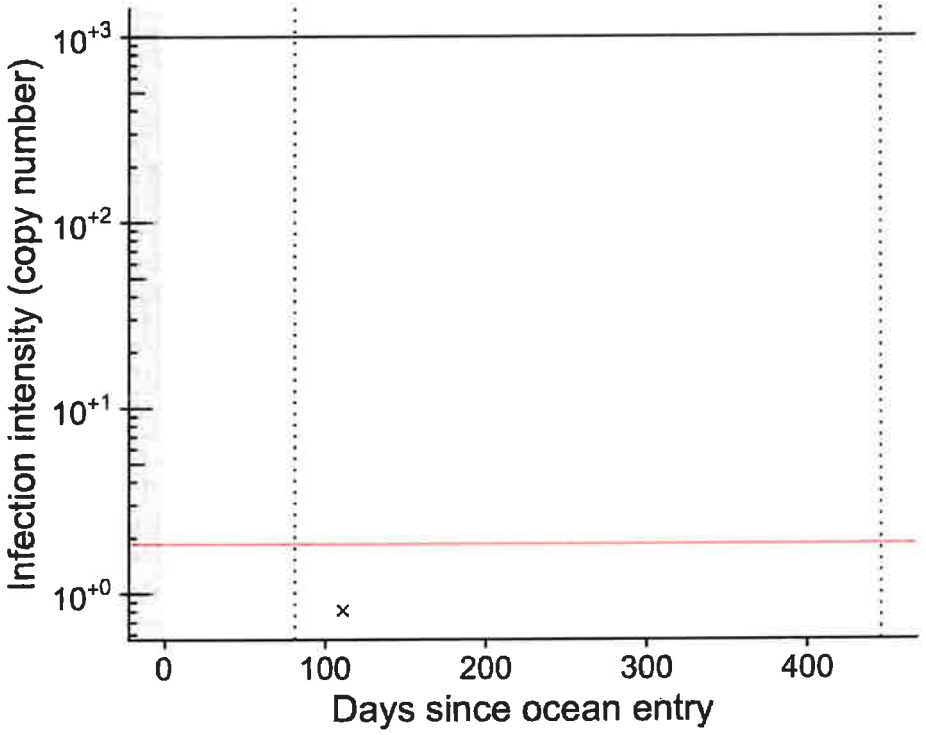
The following plots show individual infectious agent trends across all farm sites. In cases where sample size is sufficient, curves from a generalised additive model are included in the plot.

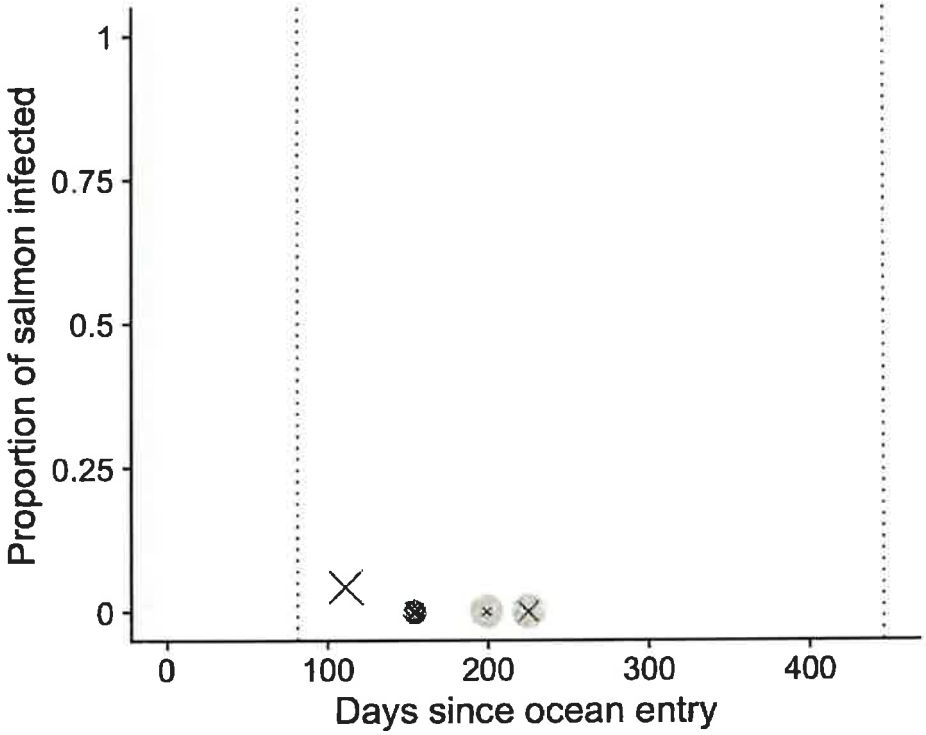
Grey circles represent live fish, and black X's represent dead/dying fish. Curves indicate mean predictions from a generalised additive model; blue and red correspond to live and dead/dying fish, respectively (shaded areas show 95% confidence regions). Left-hand grey region indicates freshwater hatchery 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.

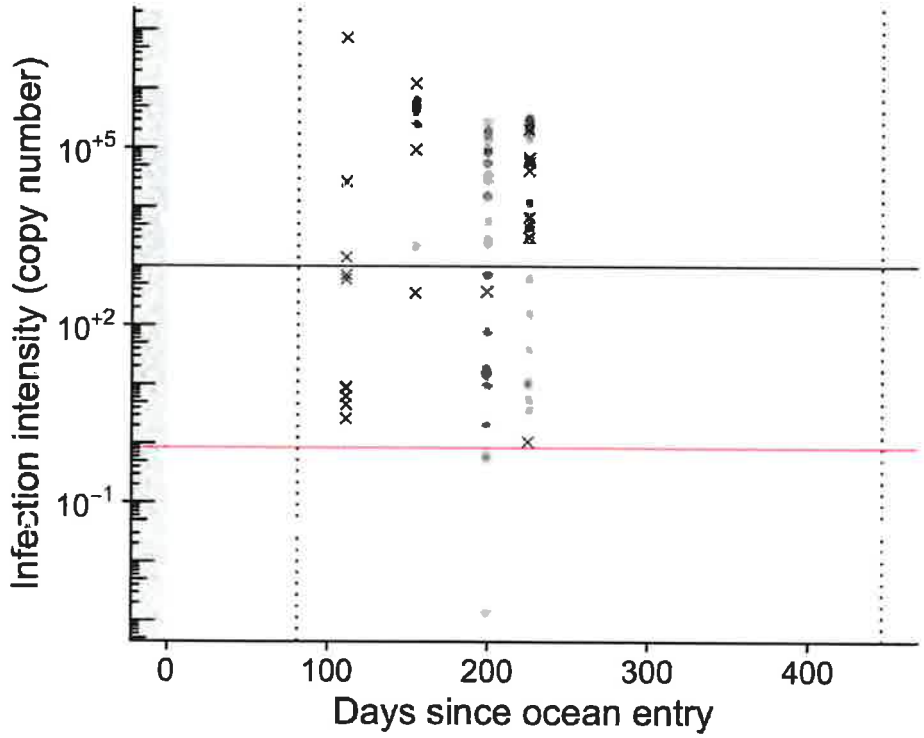
For proportion plots, grey circles show prevalence in live fish on each sampling date, and black X's show prevalence in dead/dying fish (symbol areas proportional to sample sizes).

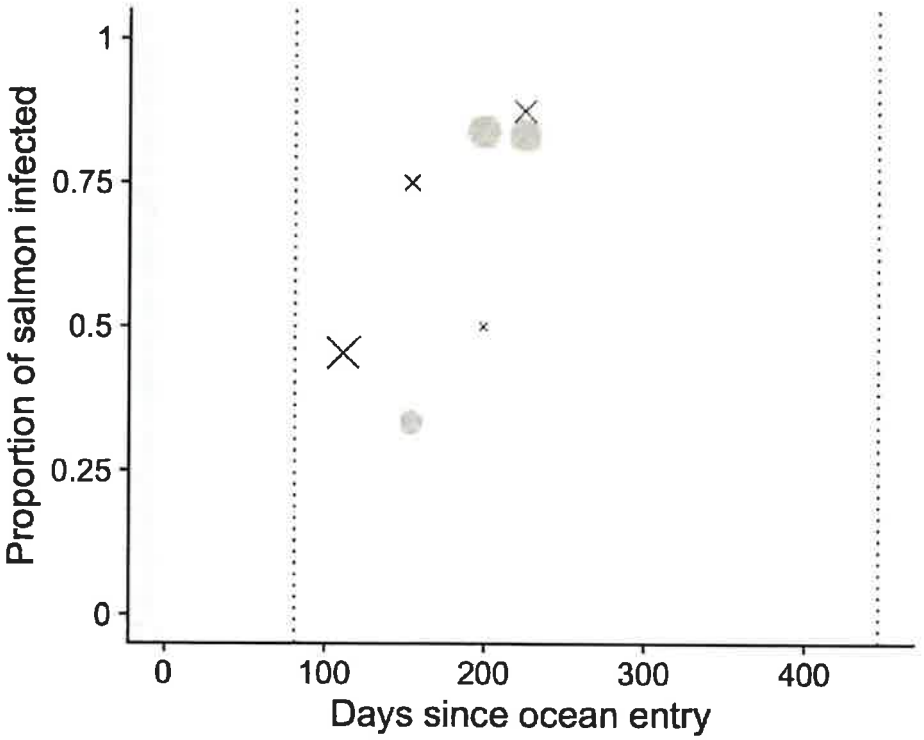
Aeromonas salmonicida



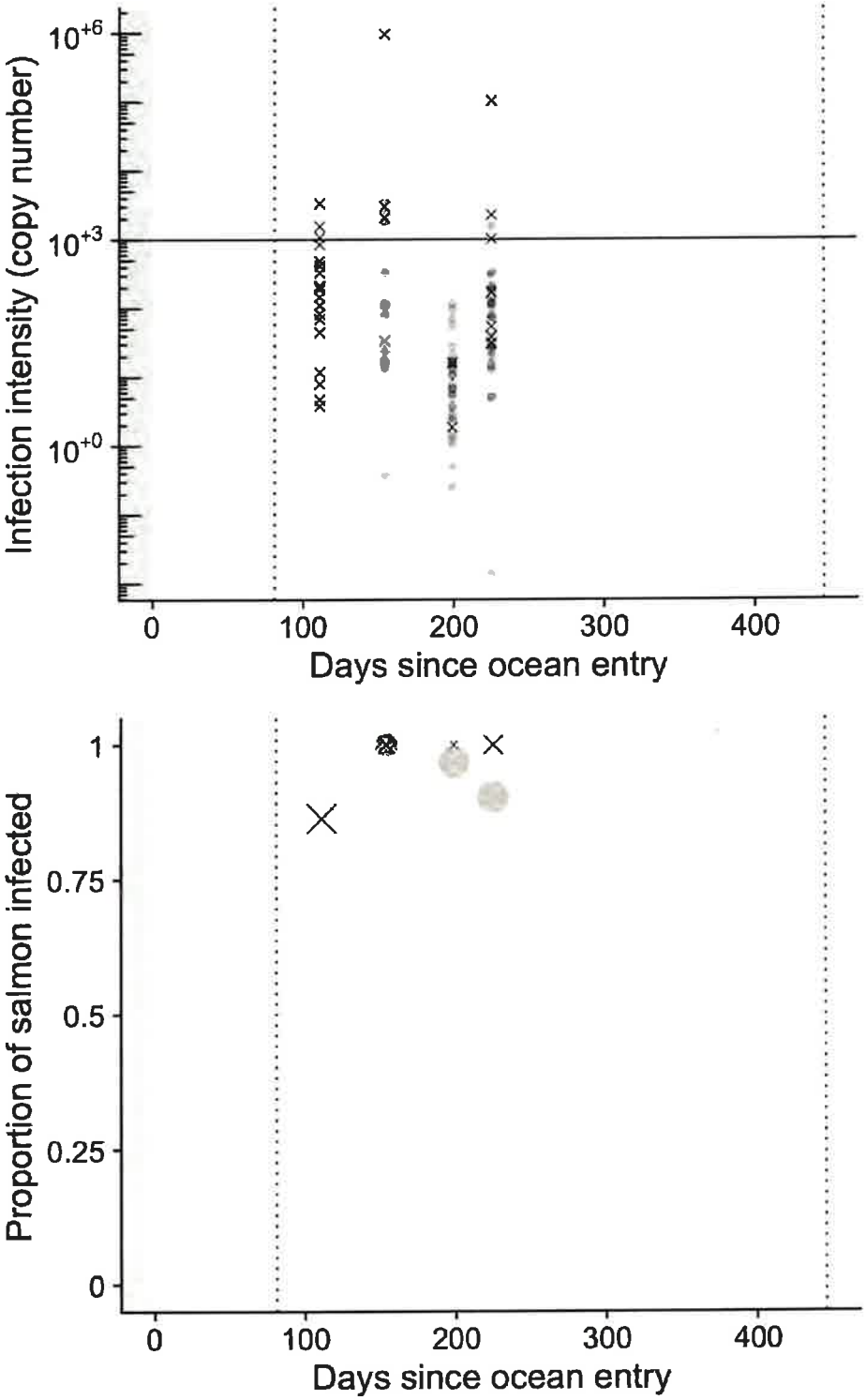


Atlantic salmon calicivirus

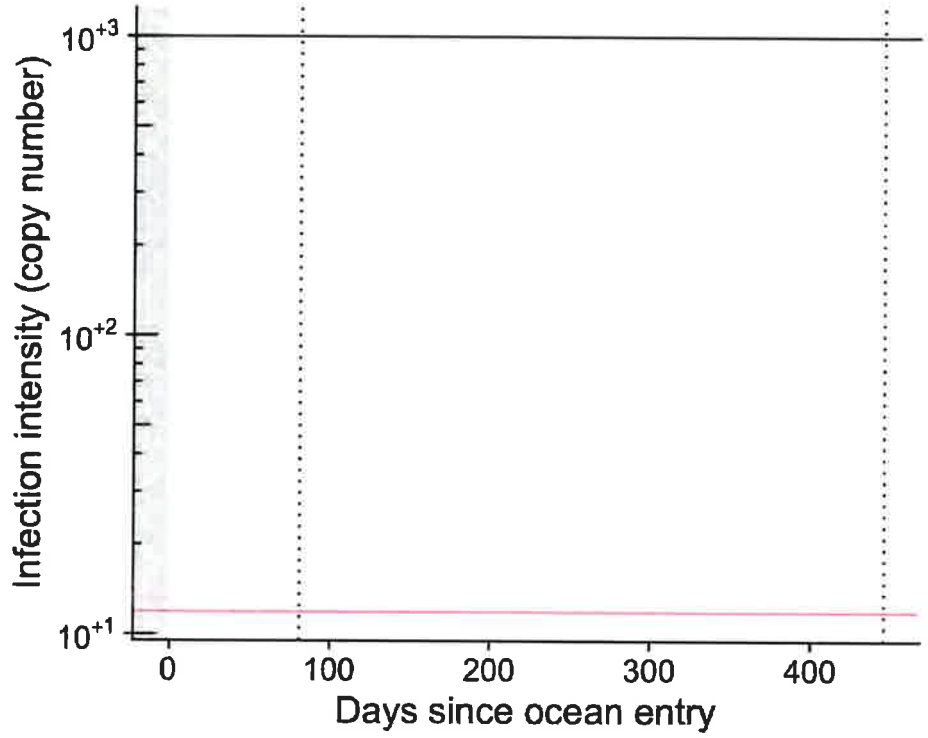


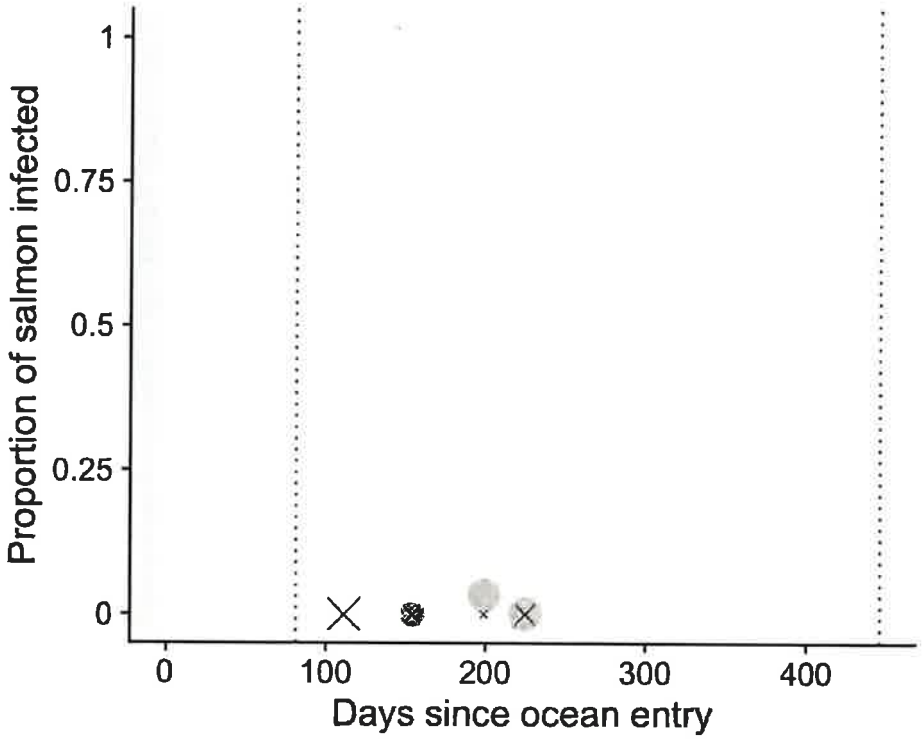


Cutthroat trout virus-2

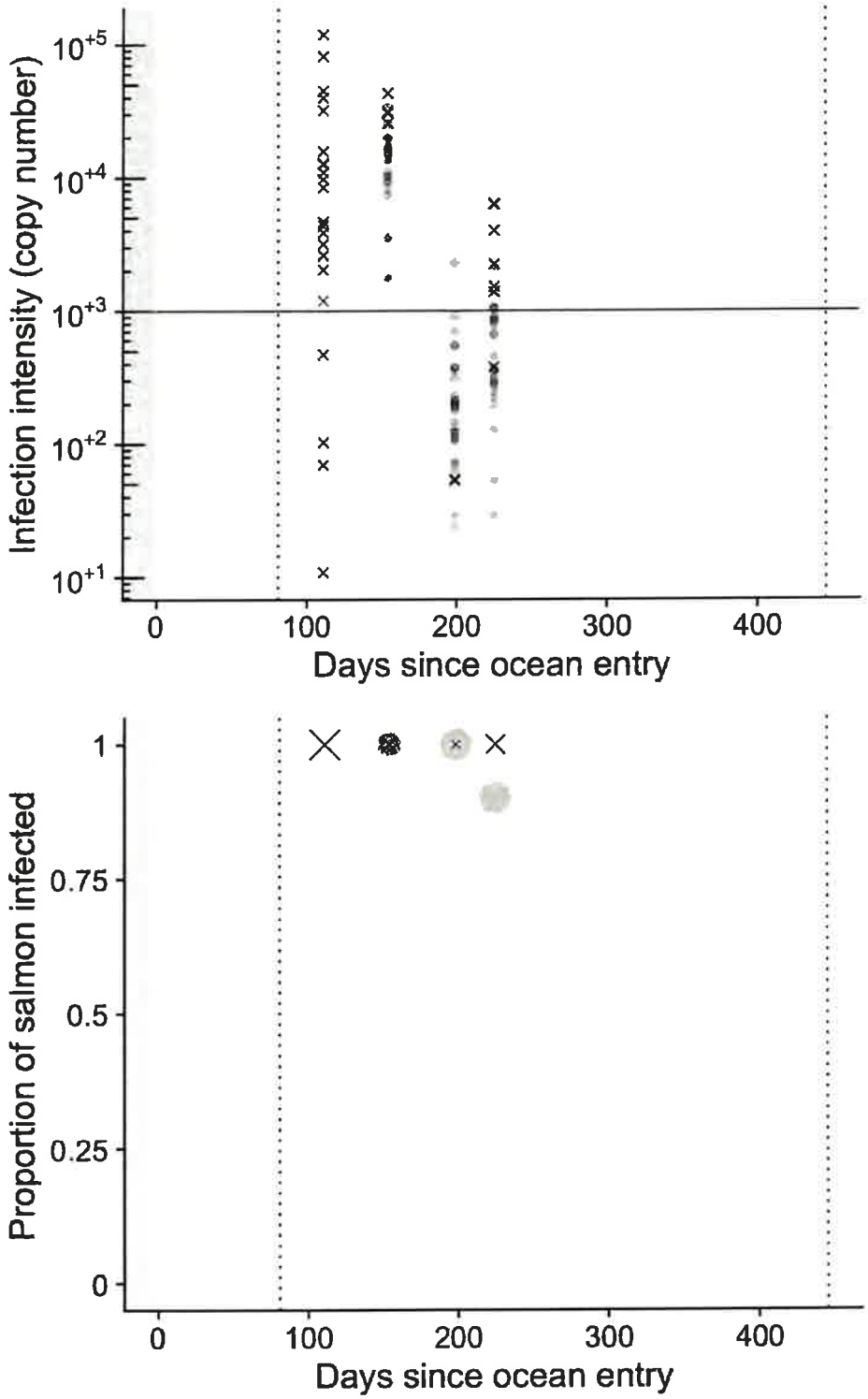


Flavobacterium psychrophilum

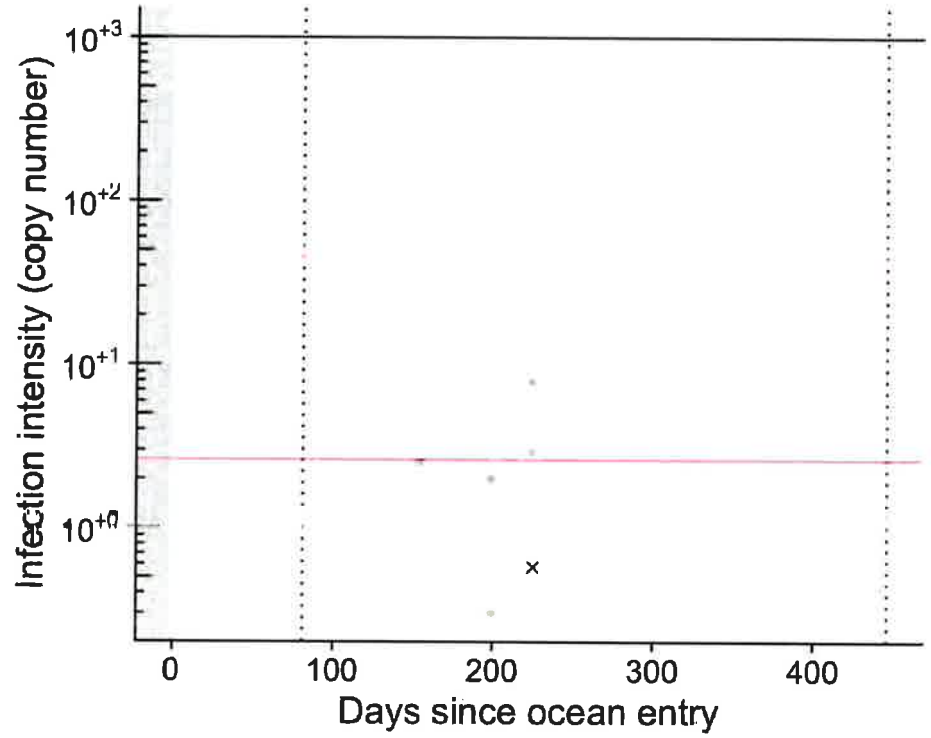


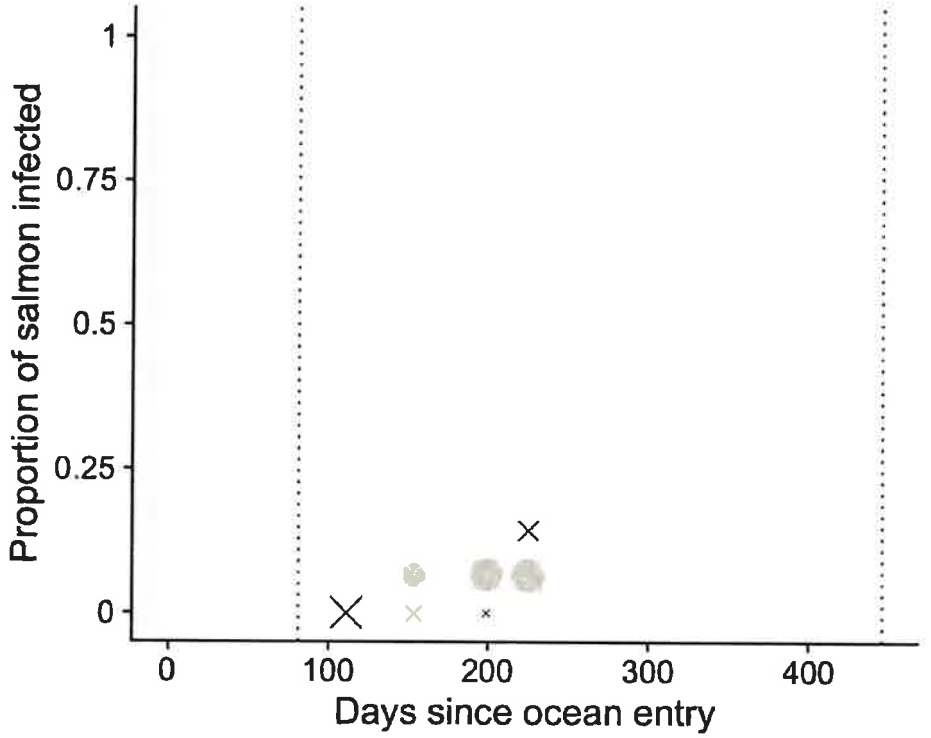


Piscine orthoreovirus

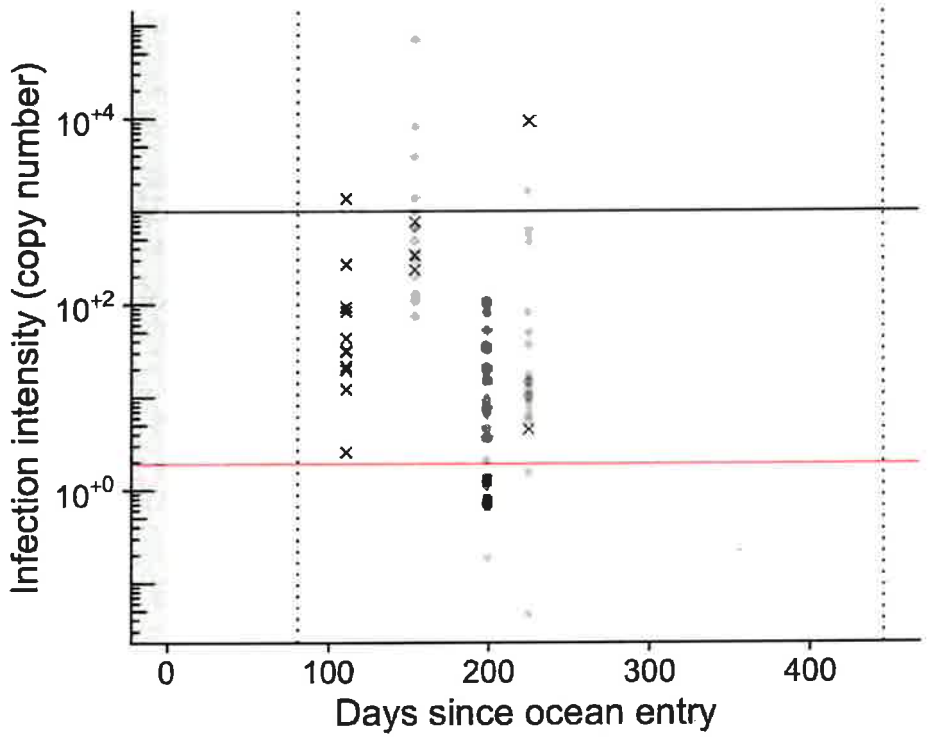


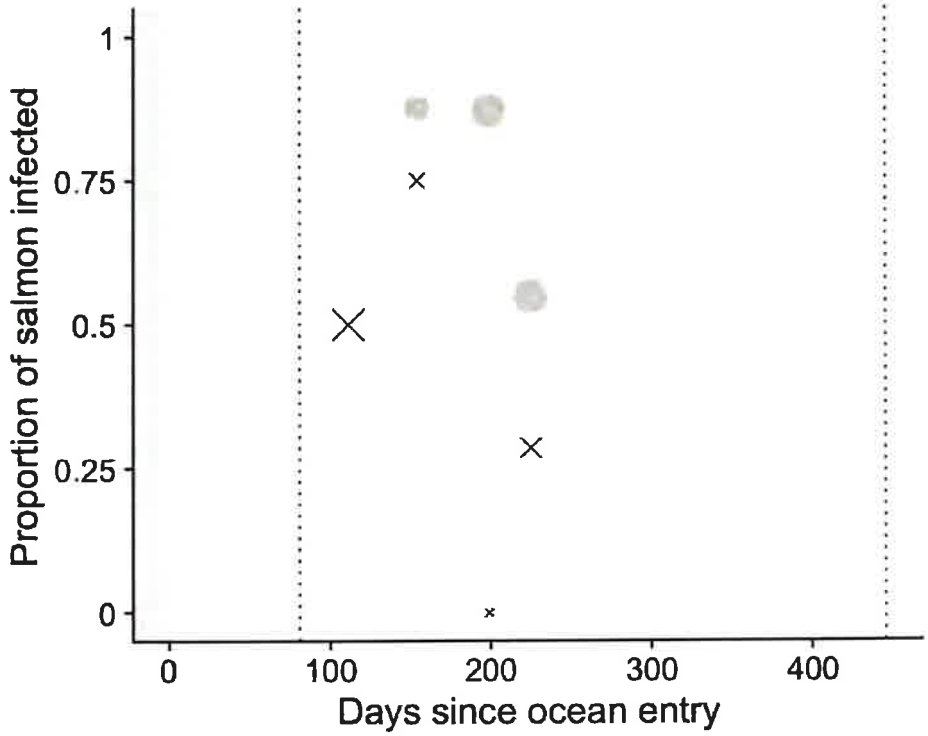
Candidatus Syngnamydia salmonis





Tenacibaculum maritimum





Clinical signs

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

metric	N5761	N5762	N5763	N5764	N5765	N5766	N5767	N5768	N5769	N5770	N5771	N5772	N5773	N5774	N5775	N5776	N5777	N5778	N5779	N5780
General																				
Live								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														
Exopthalmia							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														

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

metric	N5781	N5782	N5783	N5784	N5785	N5786	N5787	N5788	N5789	N5791	N5792	N5793	N5794	N5795	N5796	N5797	N5798
General																	
Live	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Poor Performer																	
Moribund																	
Mort																	
Exopthalmia	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	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	N5761	N5762	N5763	N5764	N5765	N5766	N5767	N5788
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 + Chromatolisis (1), Myocardionecrosis (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 Syngnamydia 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
asav	Atlantic salmon salivivirus	Virus	unknown	1
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 Syngnamydia 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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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.

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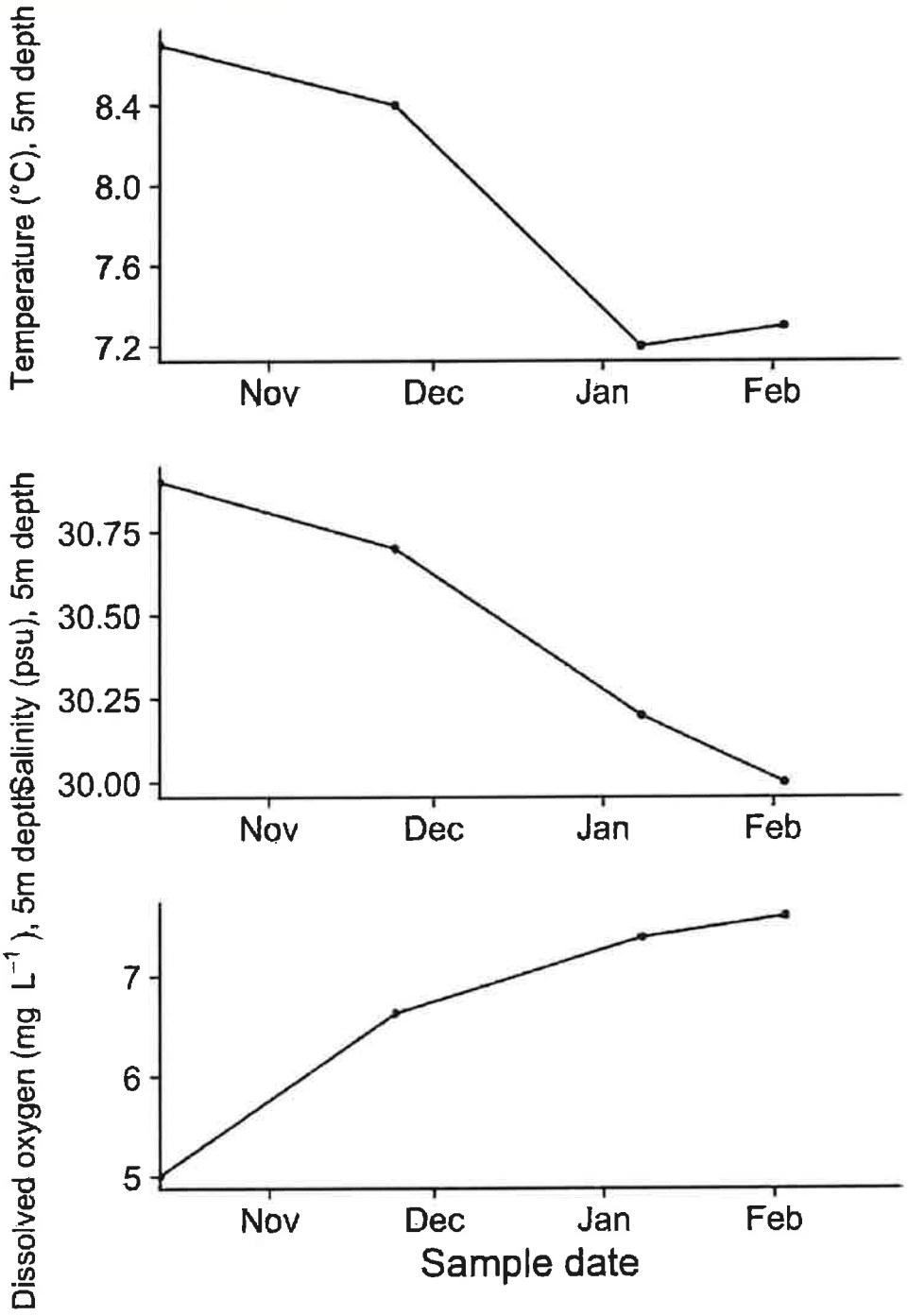
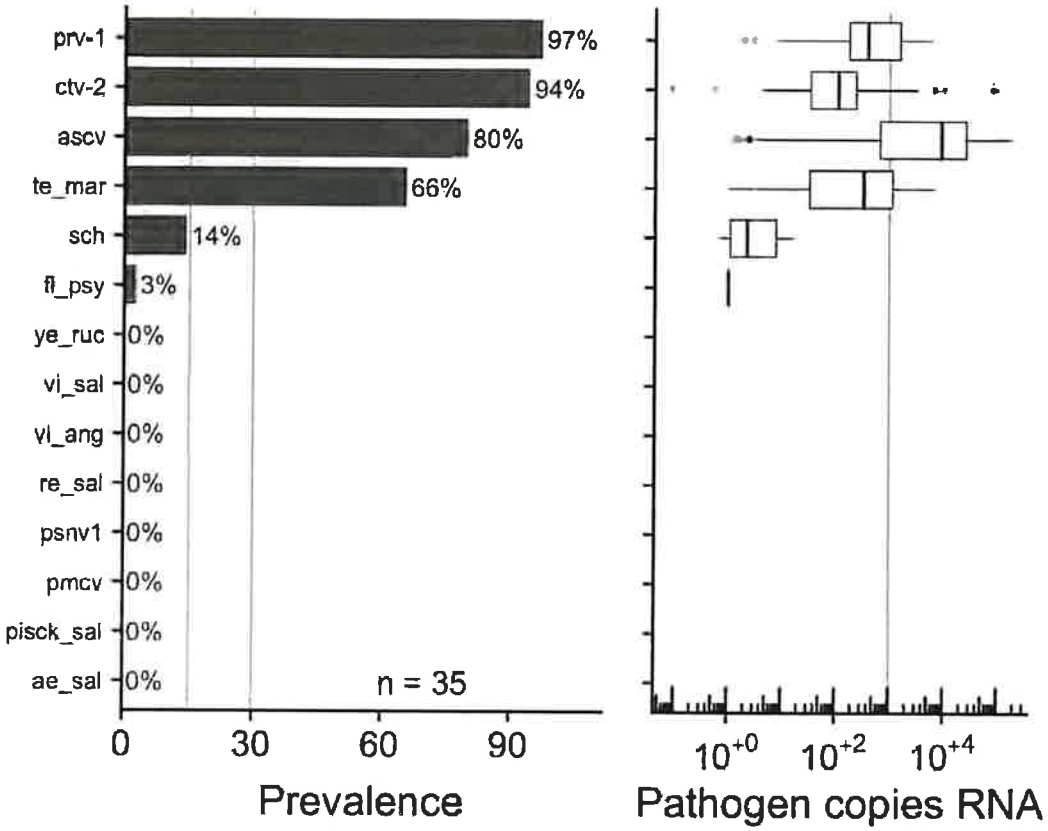
Environmental data

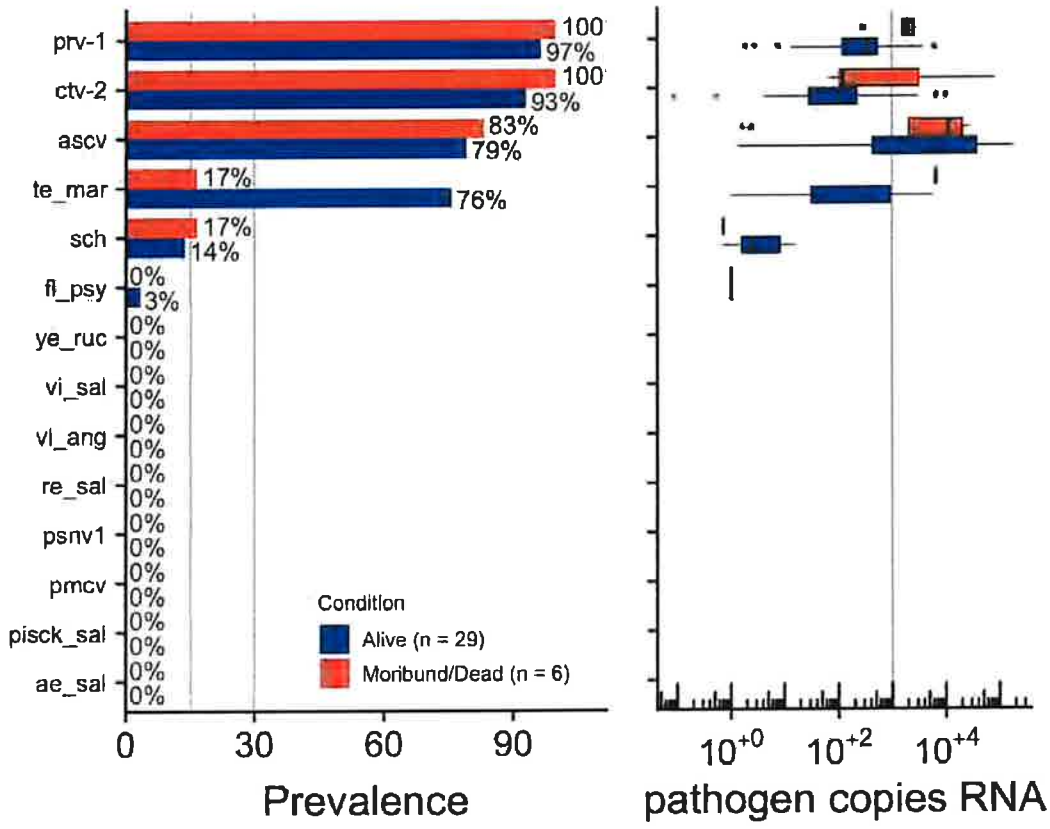


Water temperature (°C), salinity (ppm), and dissolved oxygen (mg/L) at a 5m depth. Certain sampling dates have no recorded environmental data, resulting in gaps in the plots.

Overall infectious agent prevalence



Infectious agent prevalence in samples collected on 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

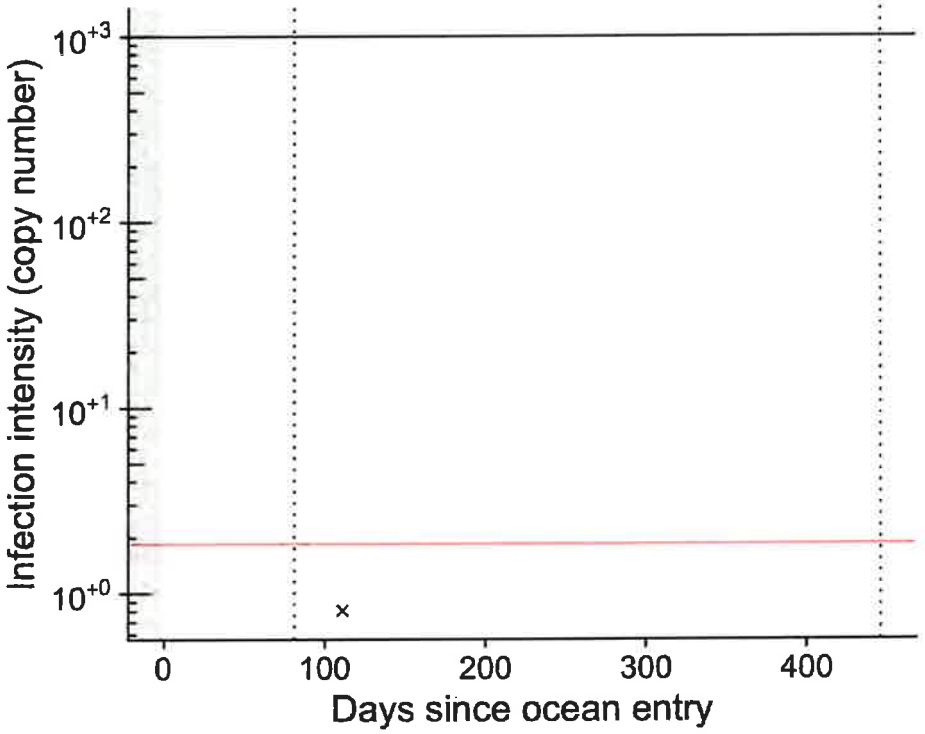
The following plots show individual infectious agent trends across all farm sites. In cases where sample size is sufficient, curves from a generalised additive model are included in the plot.

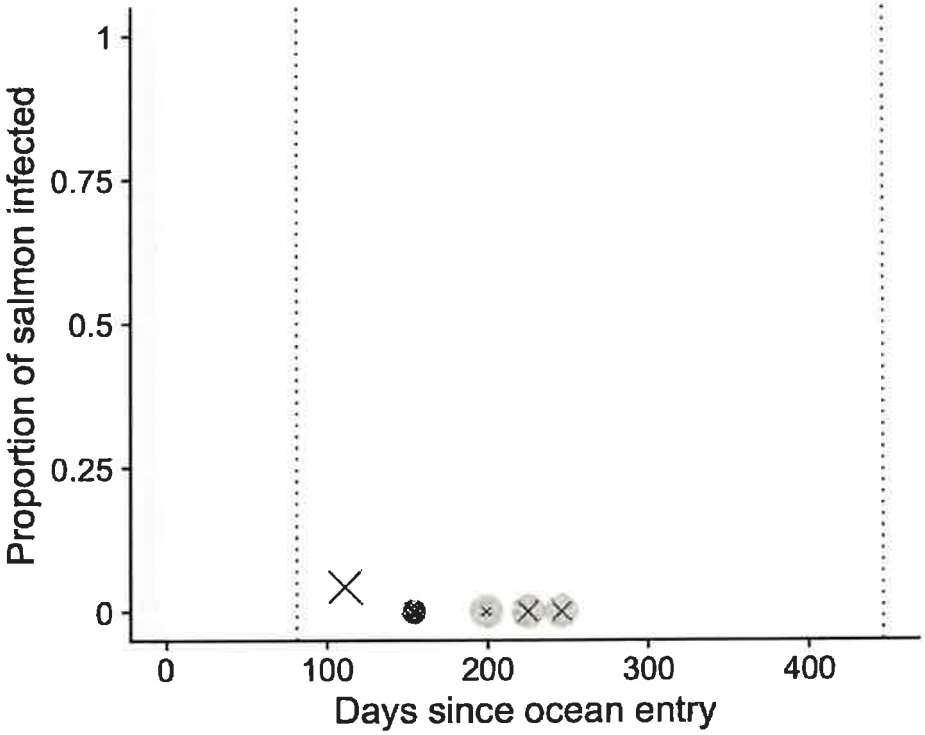
Grey circles represent live fish, and black X's represent dead/dying fish. Curves indicate mean predictions from a generalised additive model; blue and red correspond to live and dead/dying fish, respectively (shaded areas show 95% confidence regions). Left-hand grey region indicates freshwater hatchery 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.

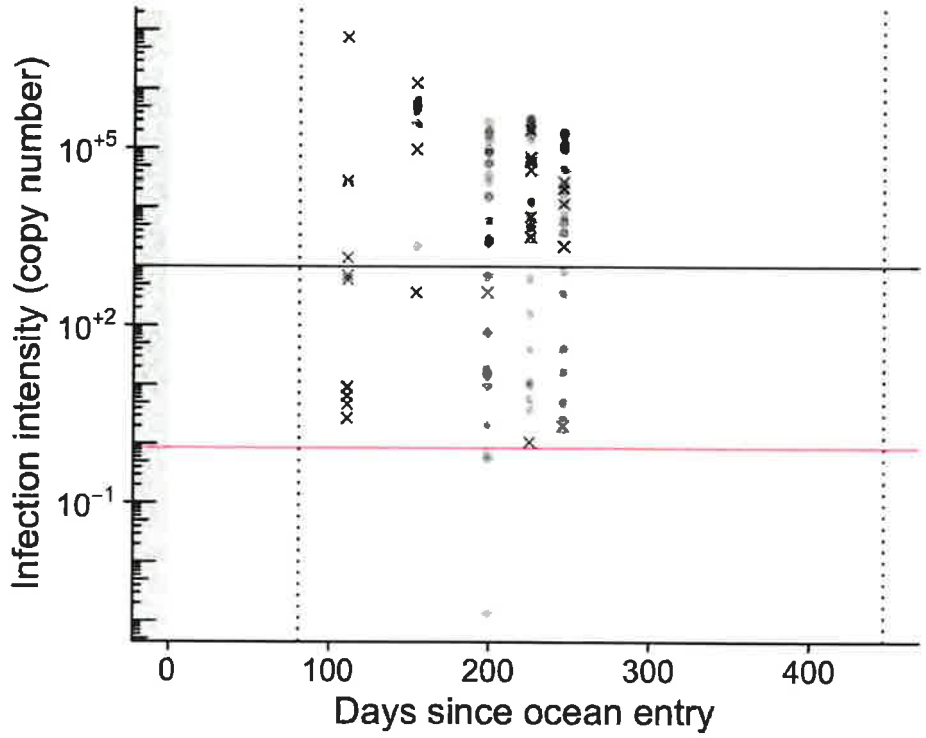
For proportion plots, grey circles show prevalence in live fish on each sampling date, and black X's show prevalence in dead/dying fish (symbol areas proportional to sample sizes).

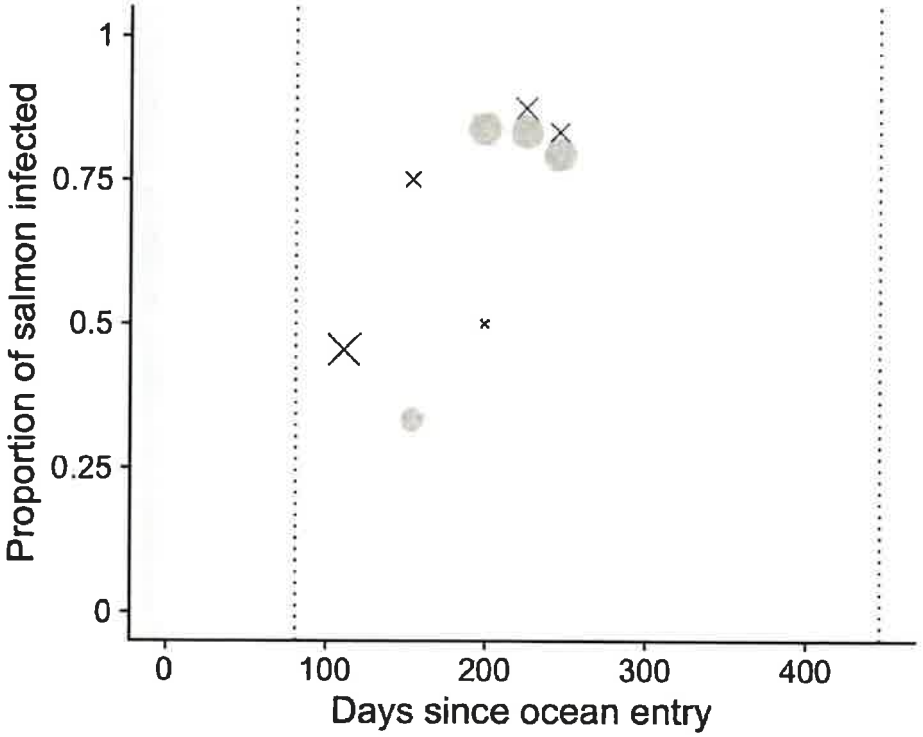
Aeromonas salmonicida



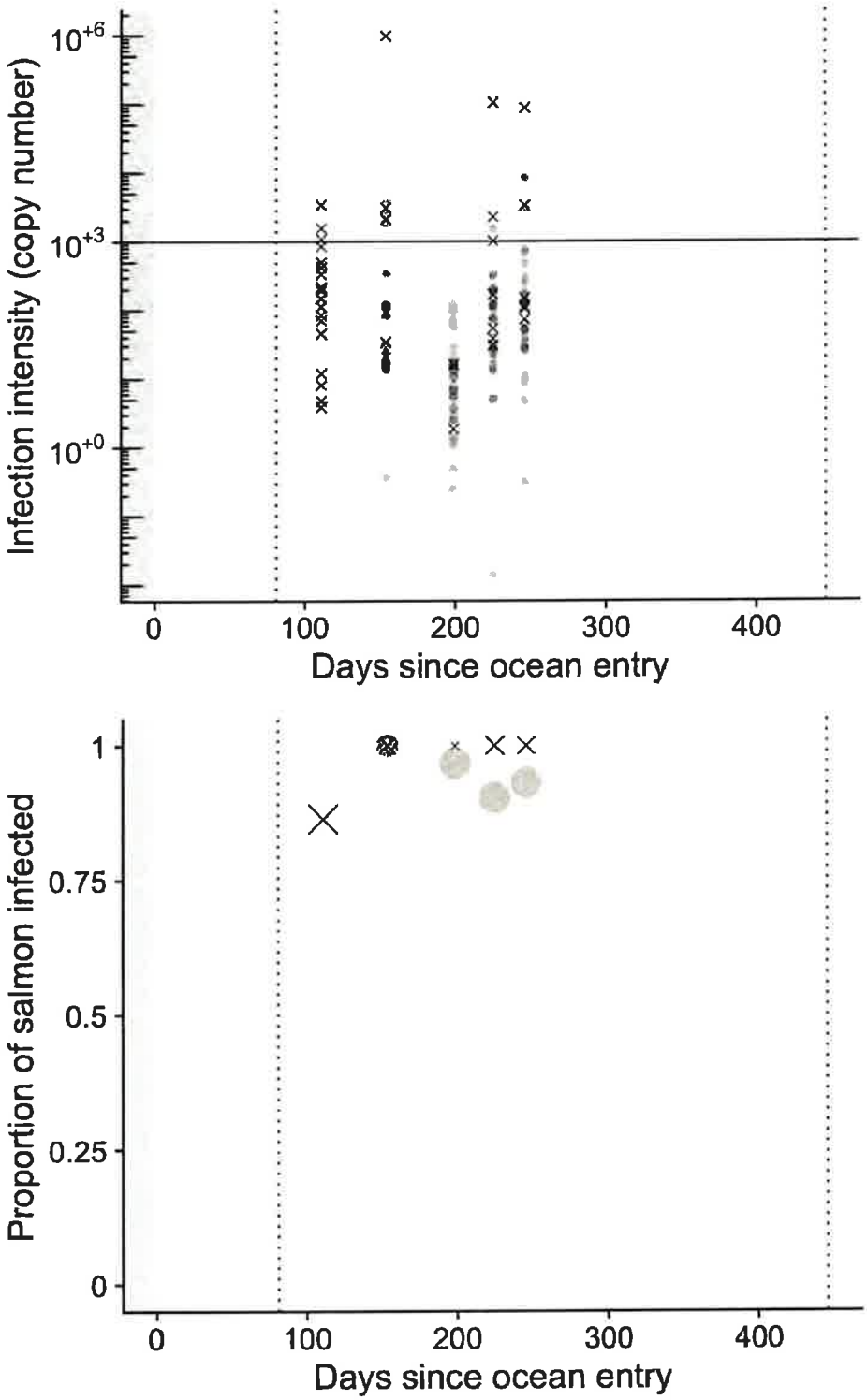


Atlantic salmon calicivirus

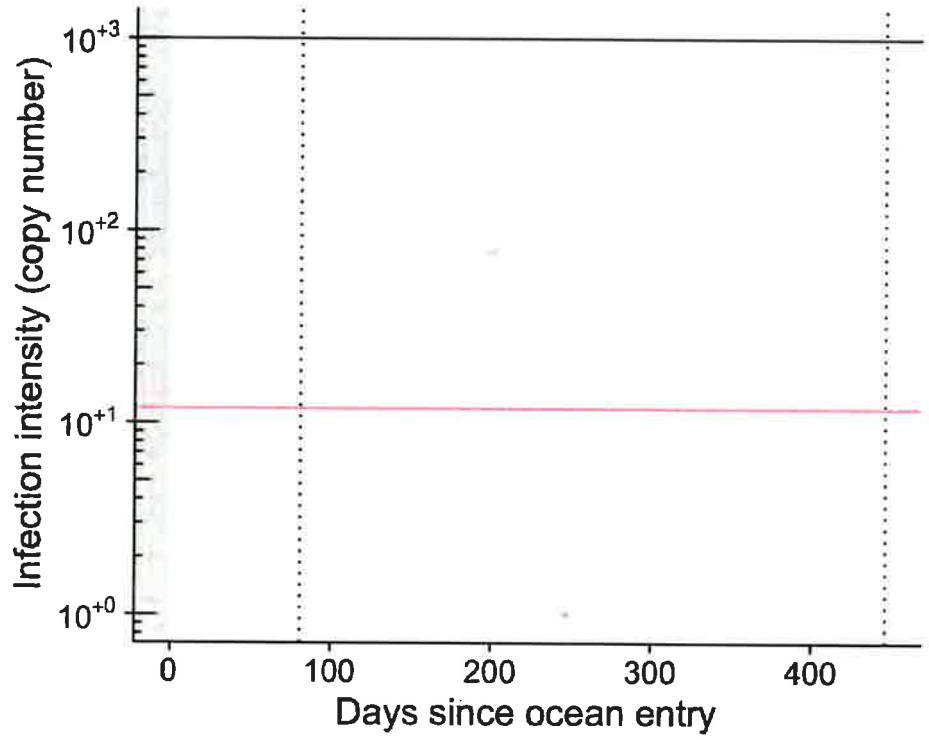


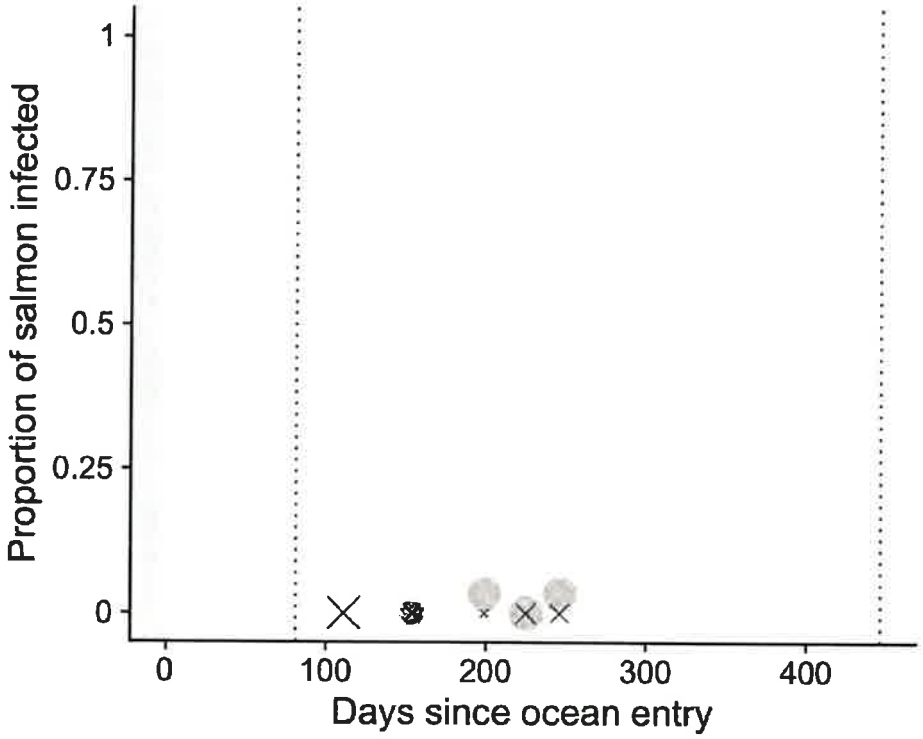


Cutthroat trout virus-2

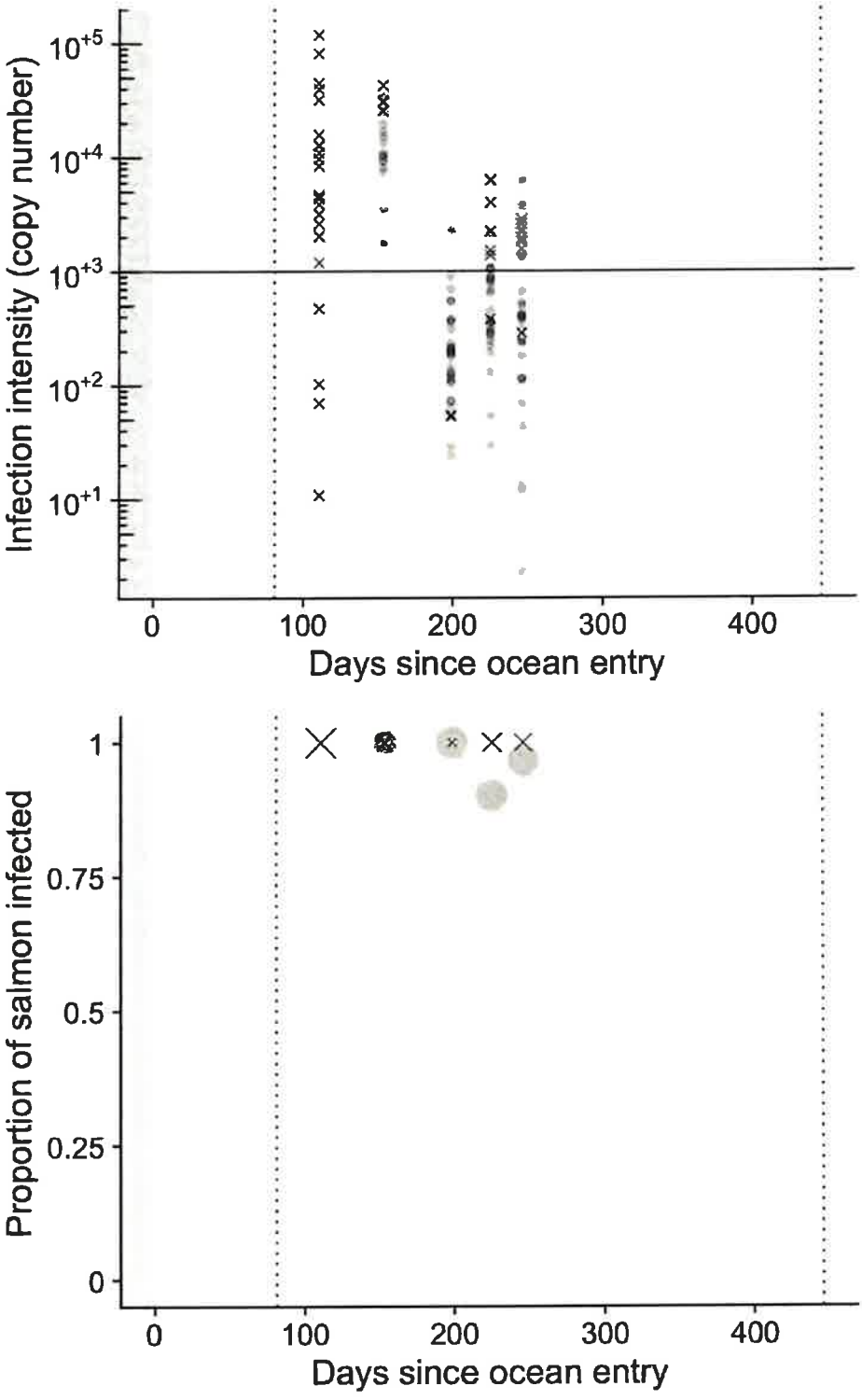


Flavobacterium psychrophilum

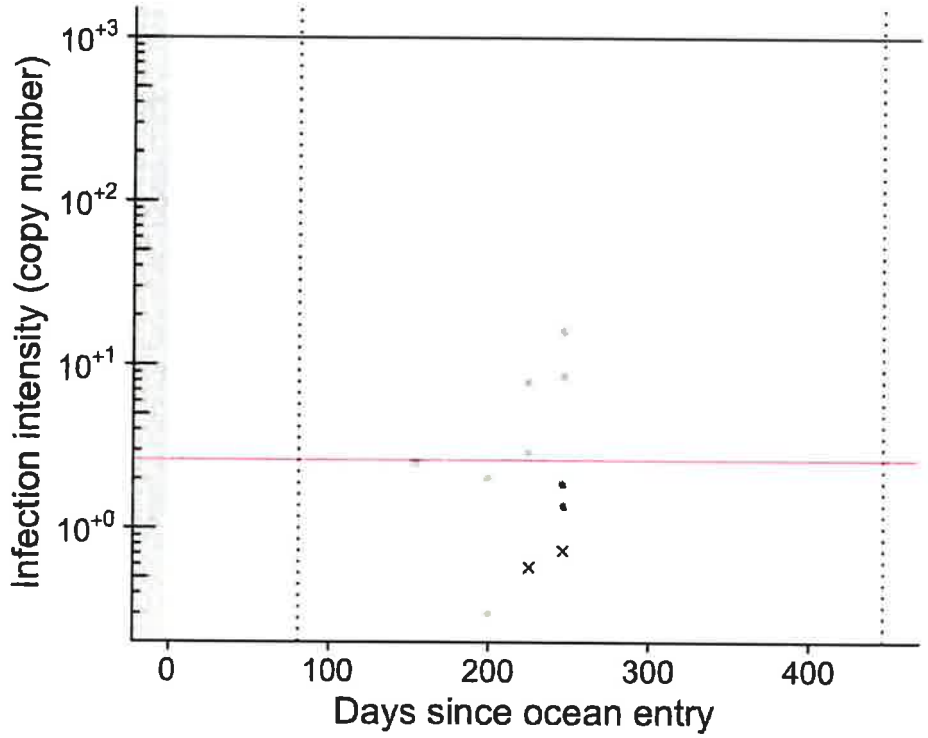


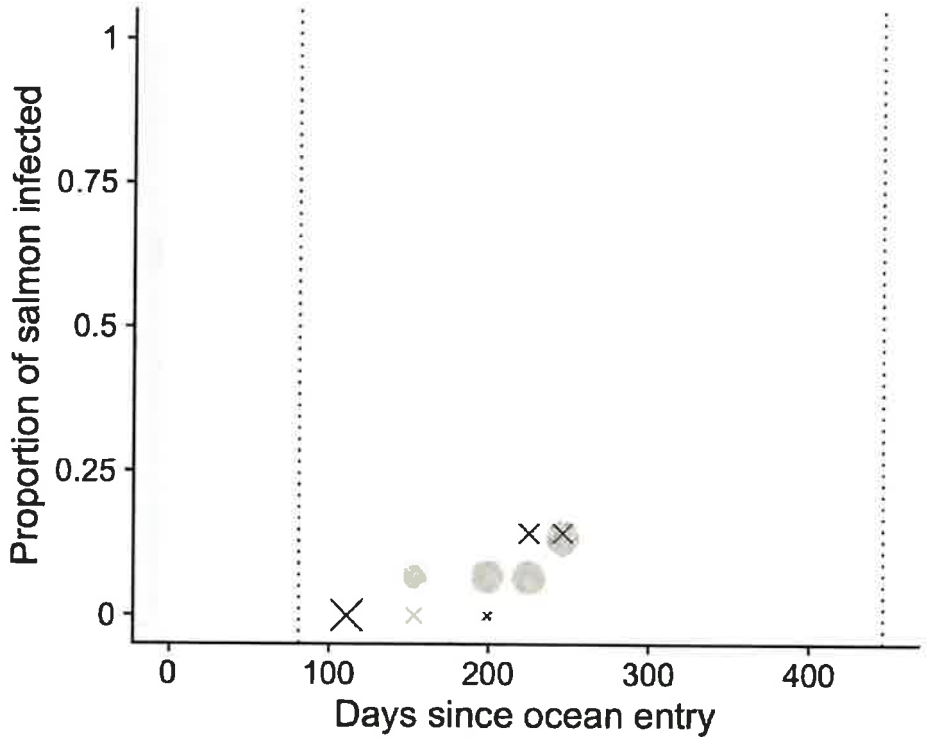


Piscine orthoreovirus

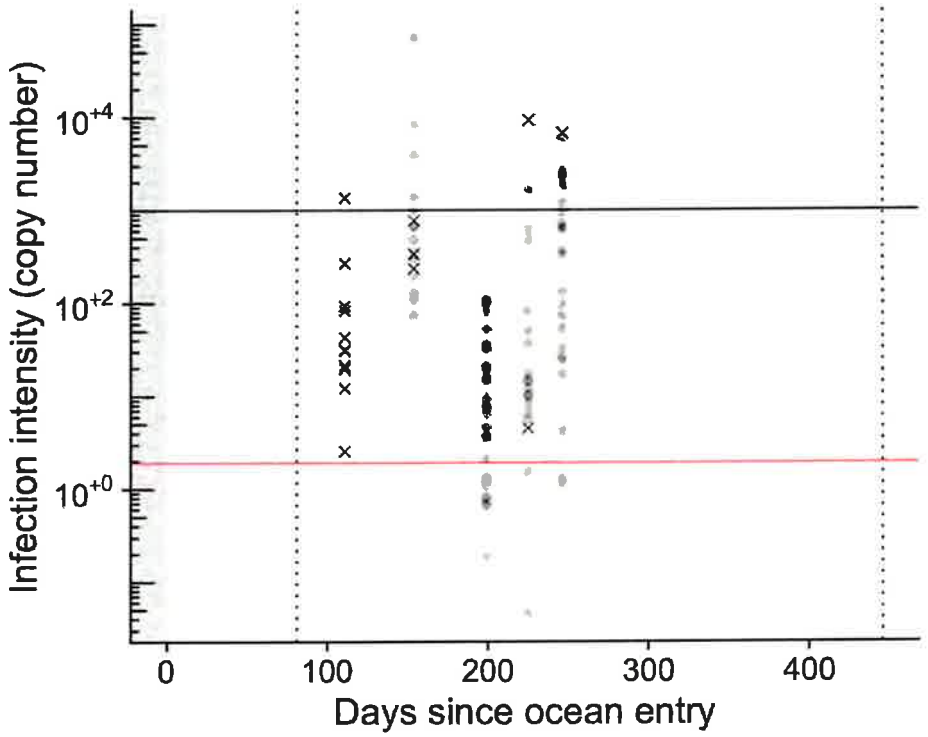


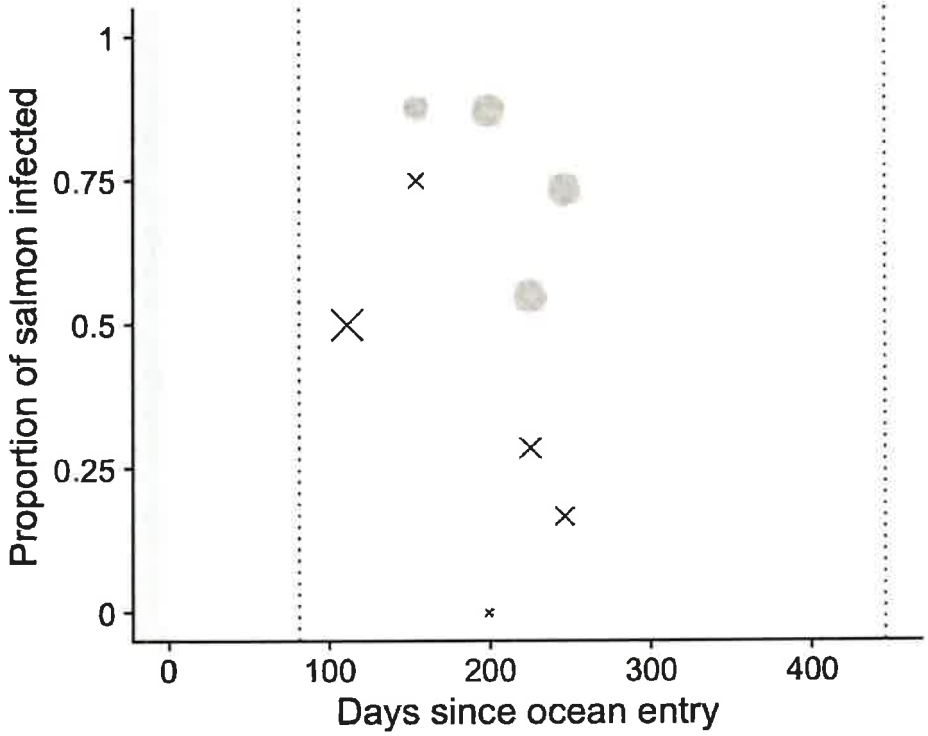
Candidatus Syngnamydia salmonis





Tenacibaculum maritimum





Clinical signs

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

metric	N5921	N5922	N5923	N5924	N5925	N5926	N5927	N5928	N5929	N5930	N5931	N5932	N5933	N5934	N5935	N5936	N5937	N5938	N5939	N5940
General																				
Live							X	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																
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	N5921	N5922	N5923	N5924	N5925	N5926	N5928	N5934	N5942	N5951
Heart										
Peri Epi	1		1	2	3	1		1	1	2
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 Chromatolisis (1)
N5924		Increased Fibrin In Spleen (2), Myocardionecrosis (2)
N5925	HSMI	Increased Fibrin In Spleen (2), Myocardionecrosis (2), Satellitosis (2), Neuronal Vacuolization (2), Neuronal Chromatolisis (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 Syngnamydia 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 Syngnamydia 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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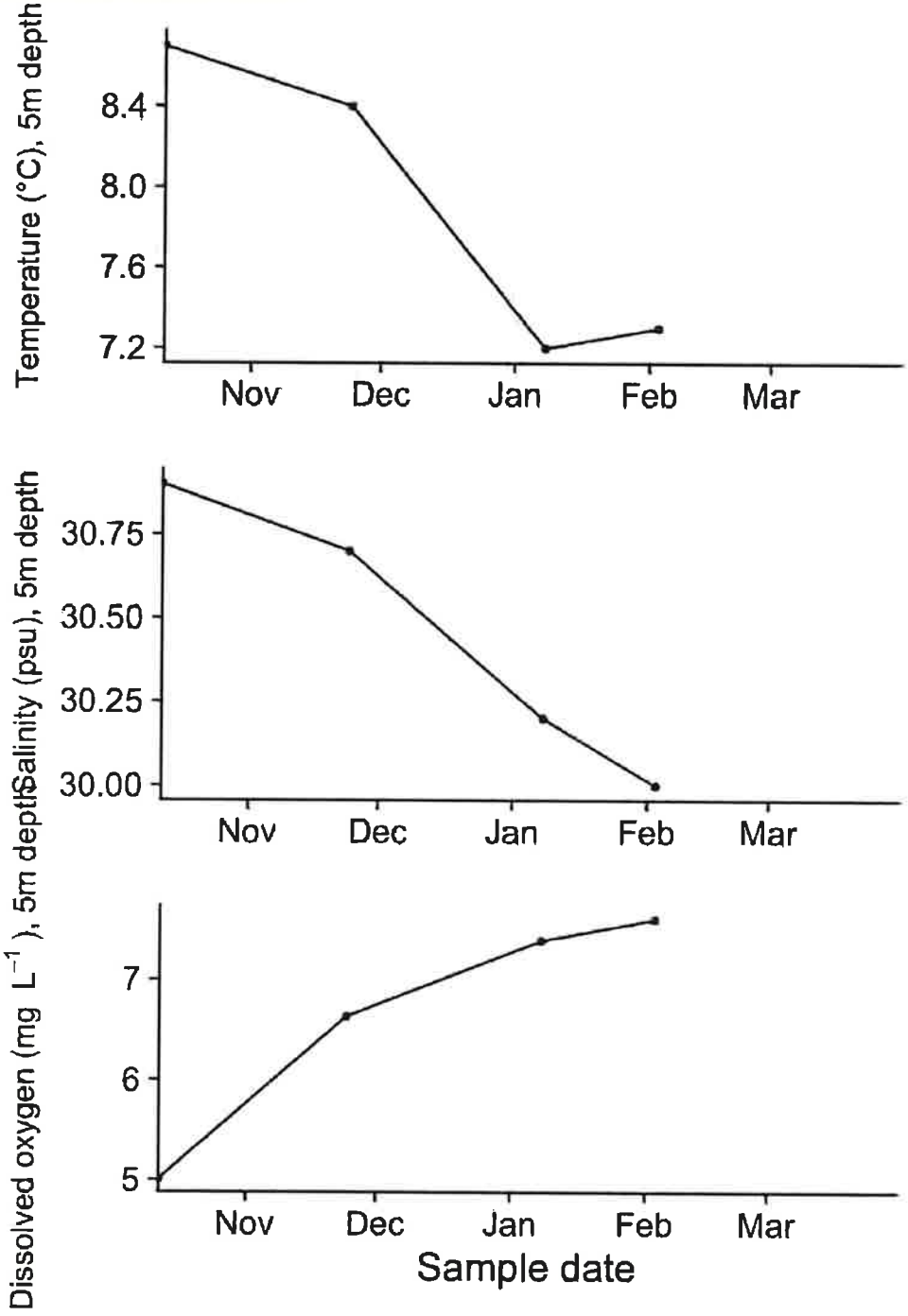

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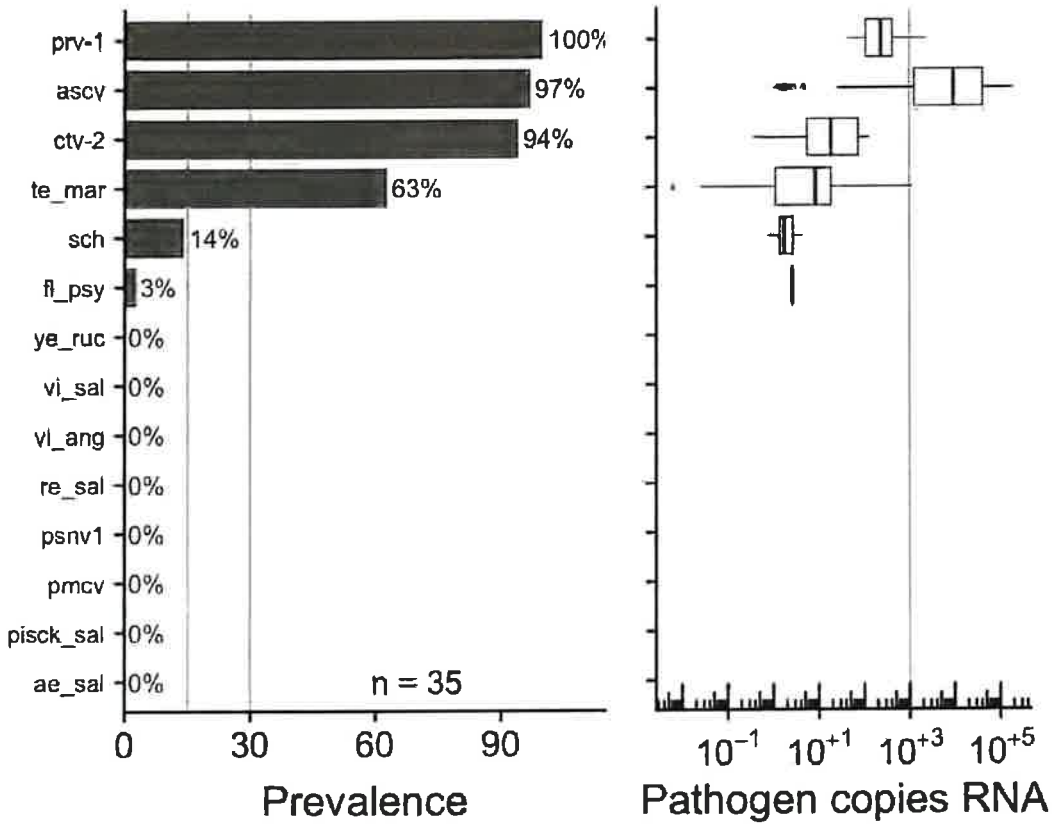
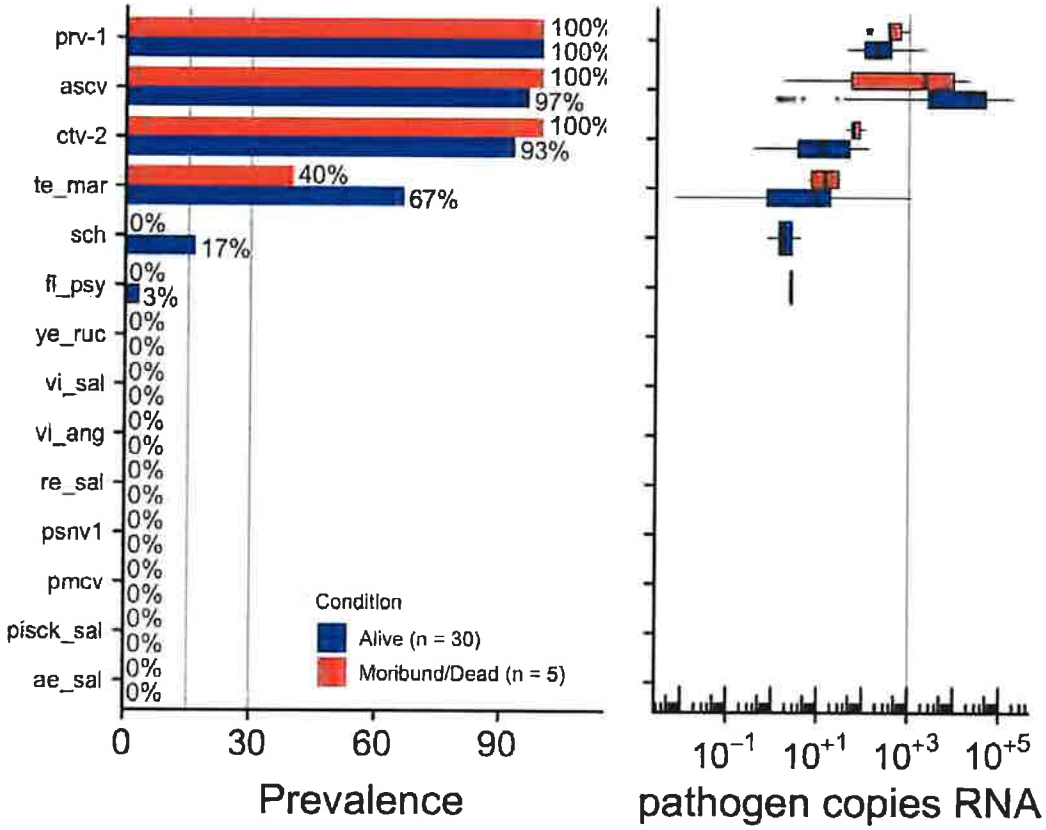


Infectious agent prevalence in samples collected on 2022-03-31.



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.

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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.

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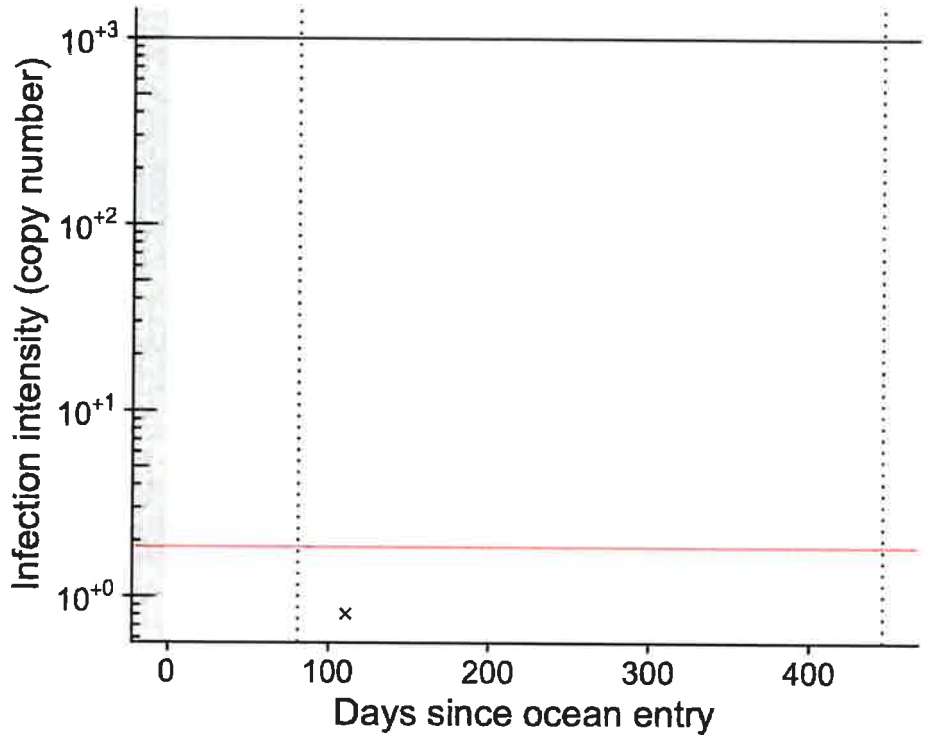
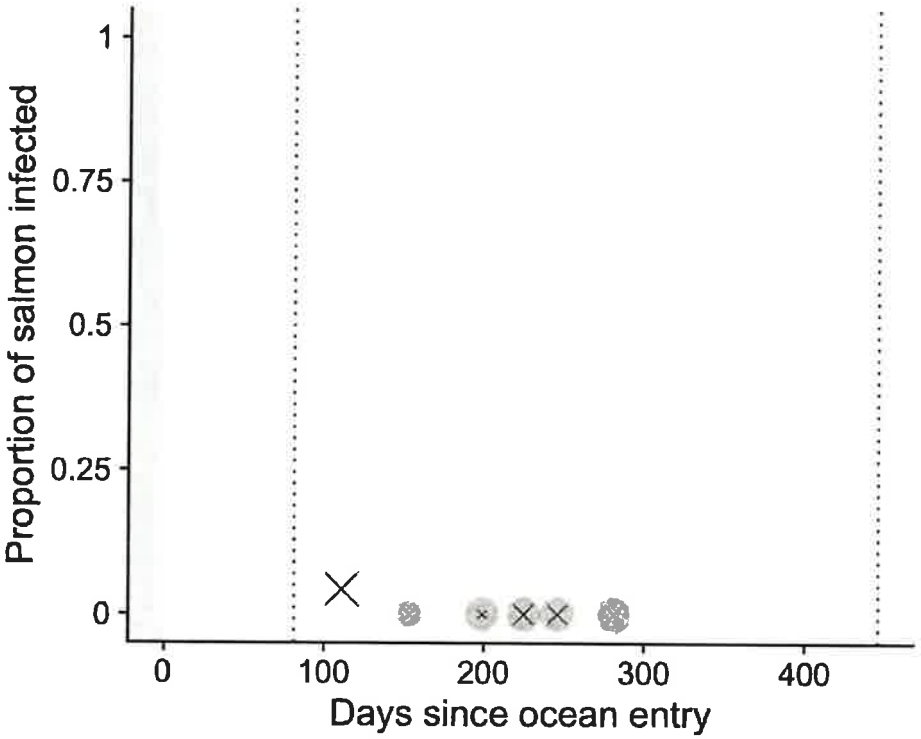
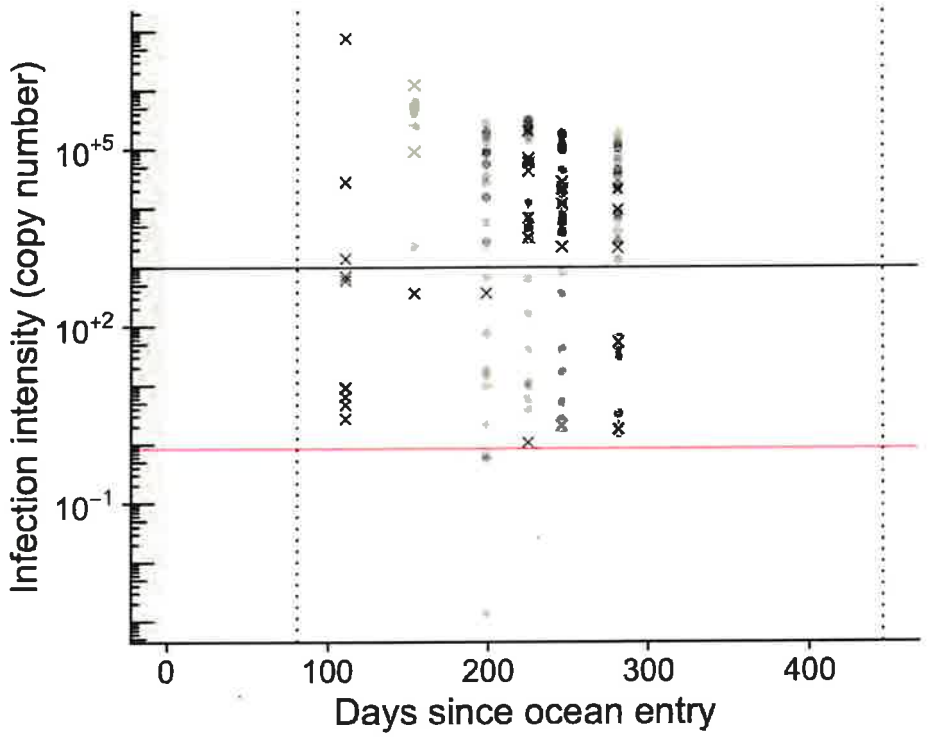
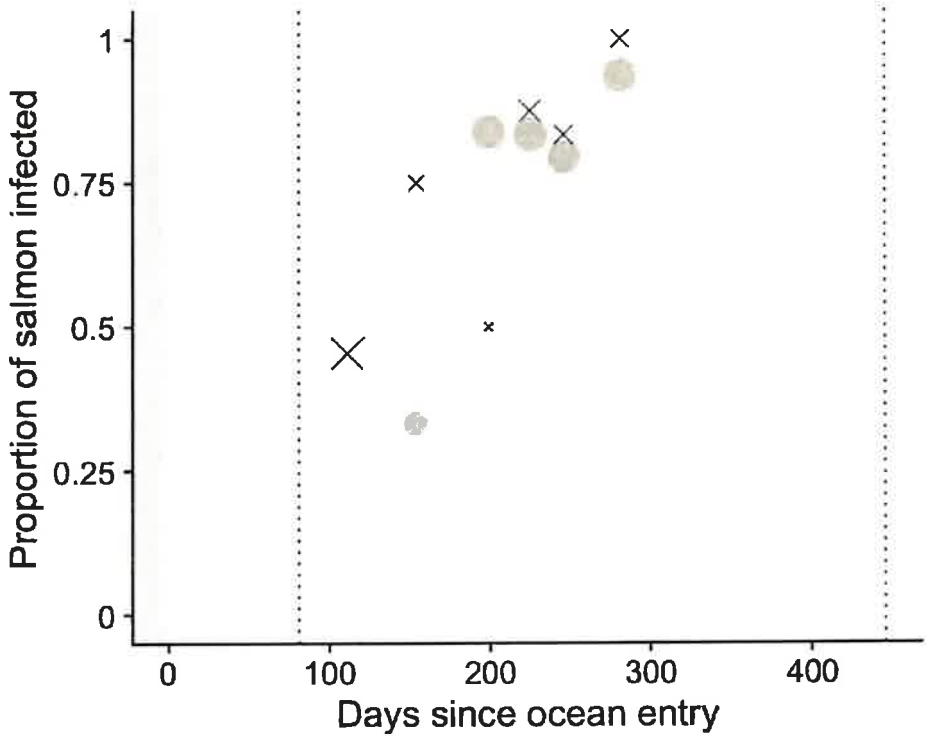




Atlantic salmon calicivirus





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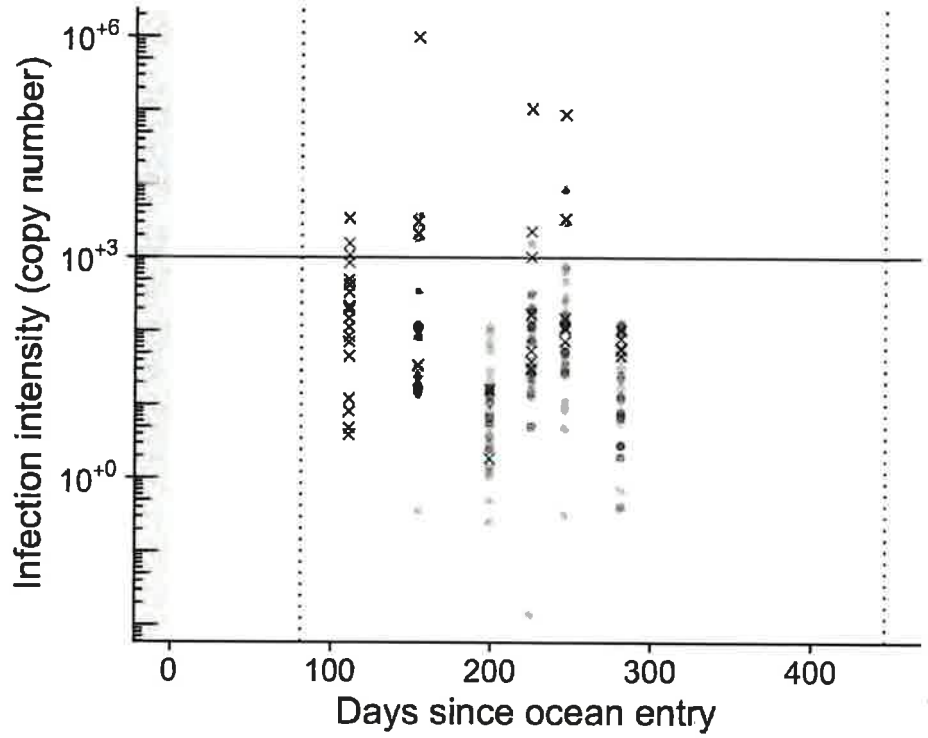

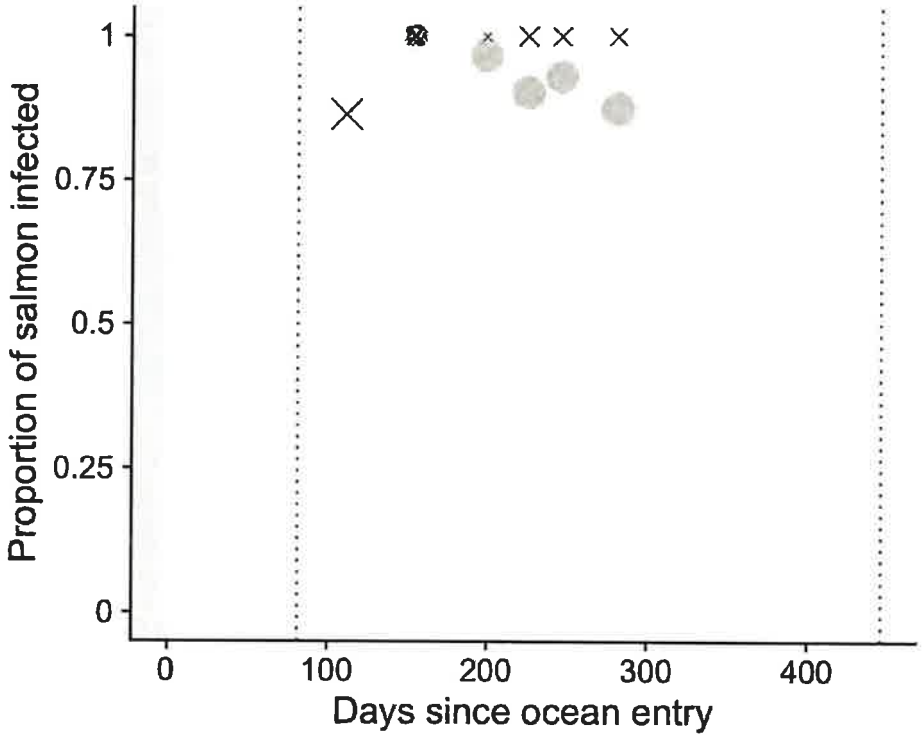

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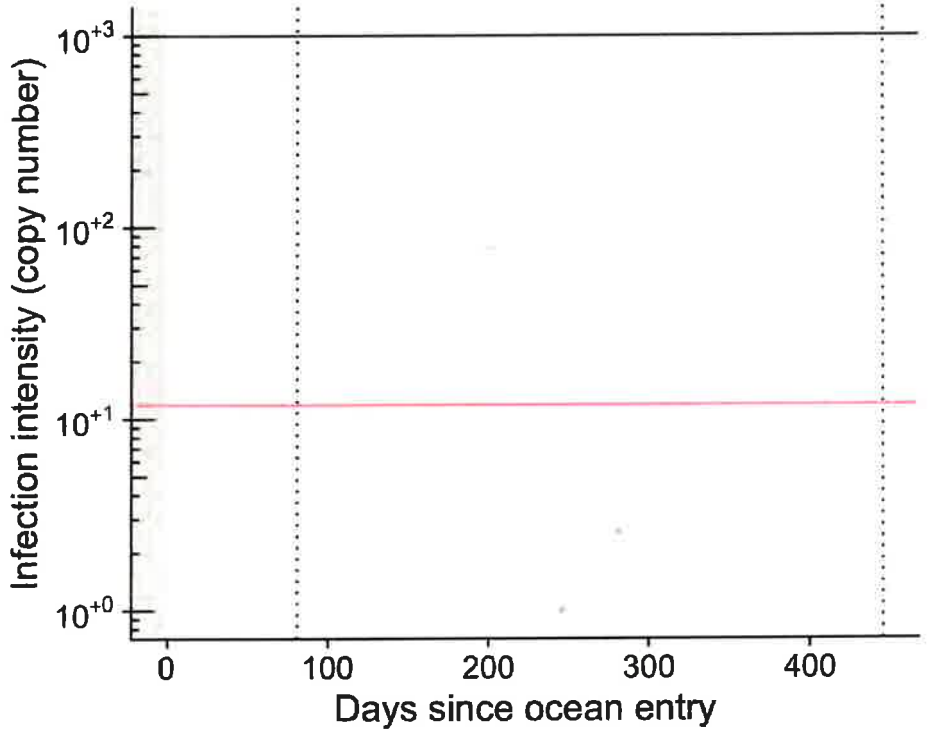

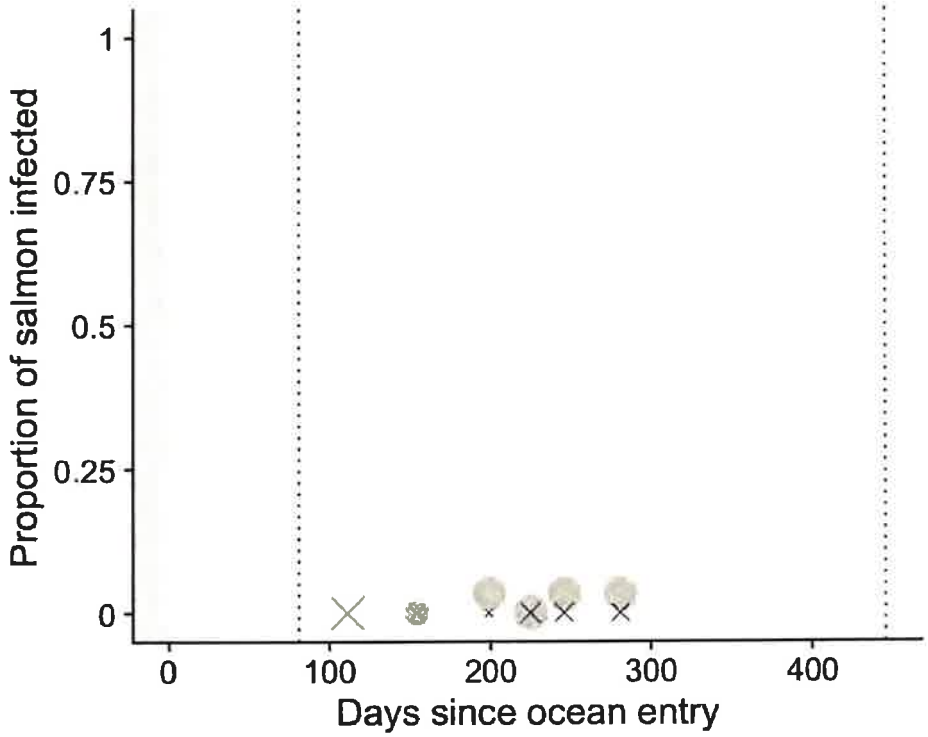

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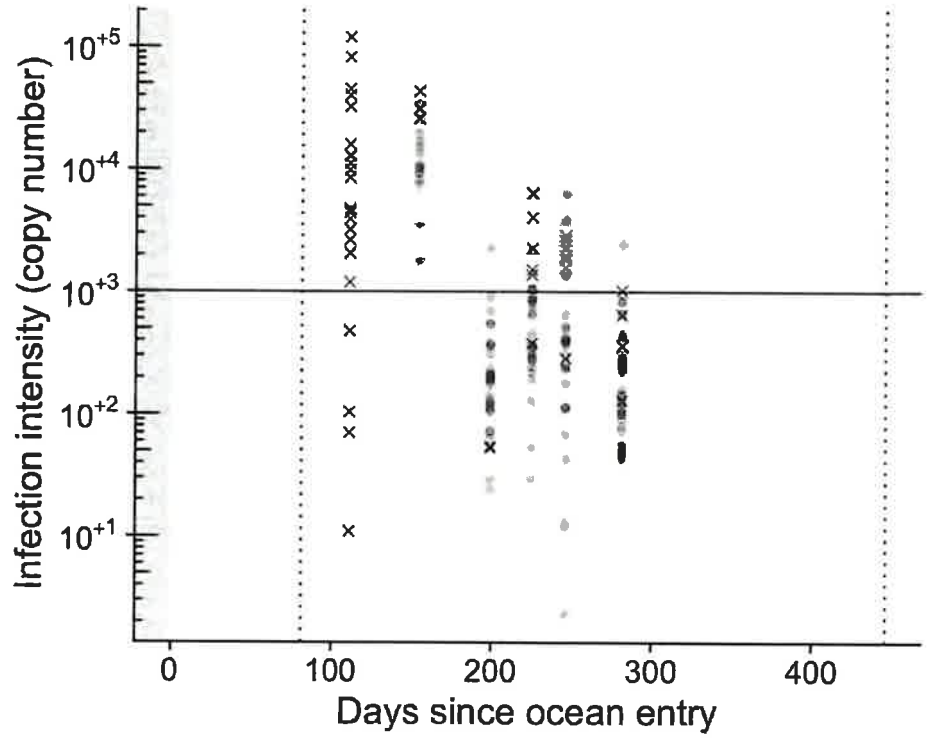
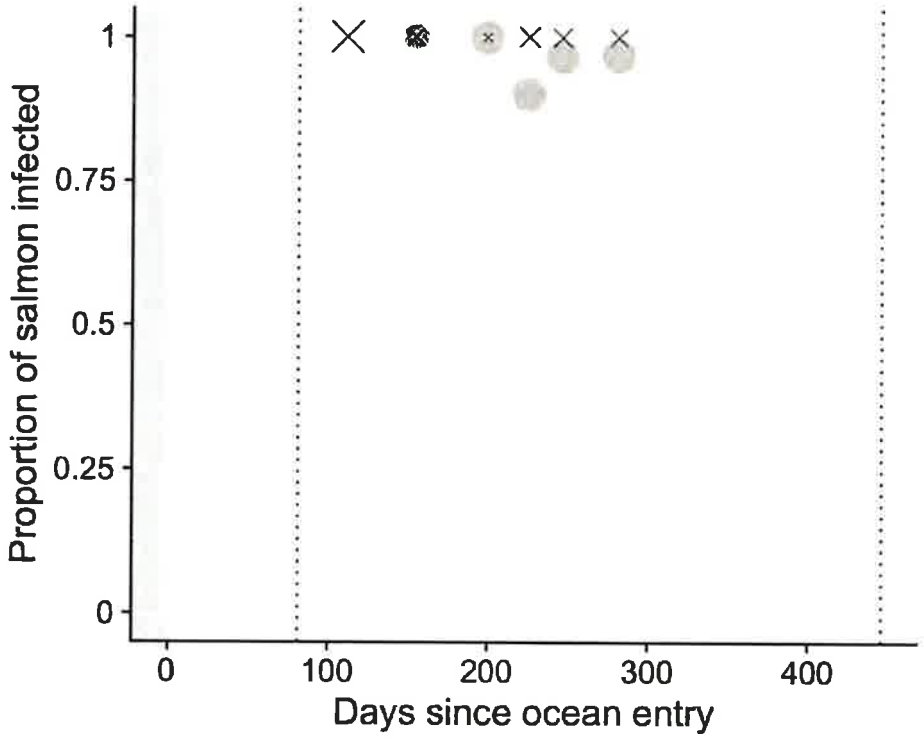
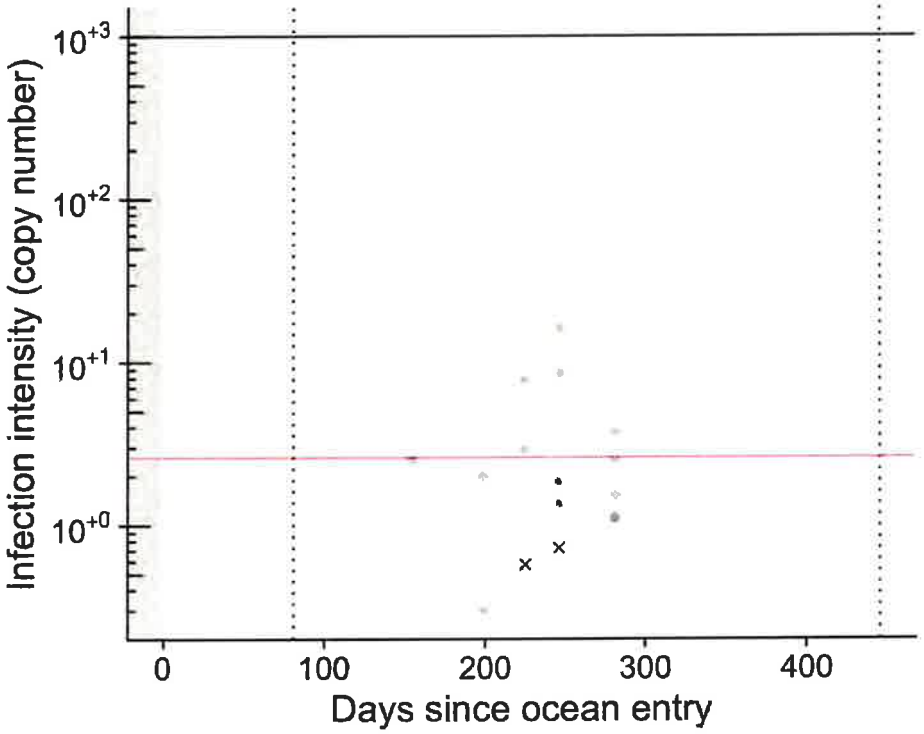
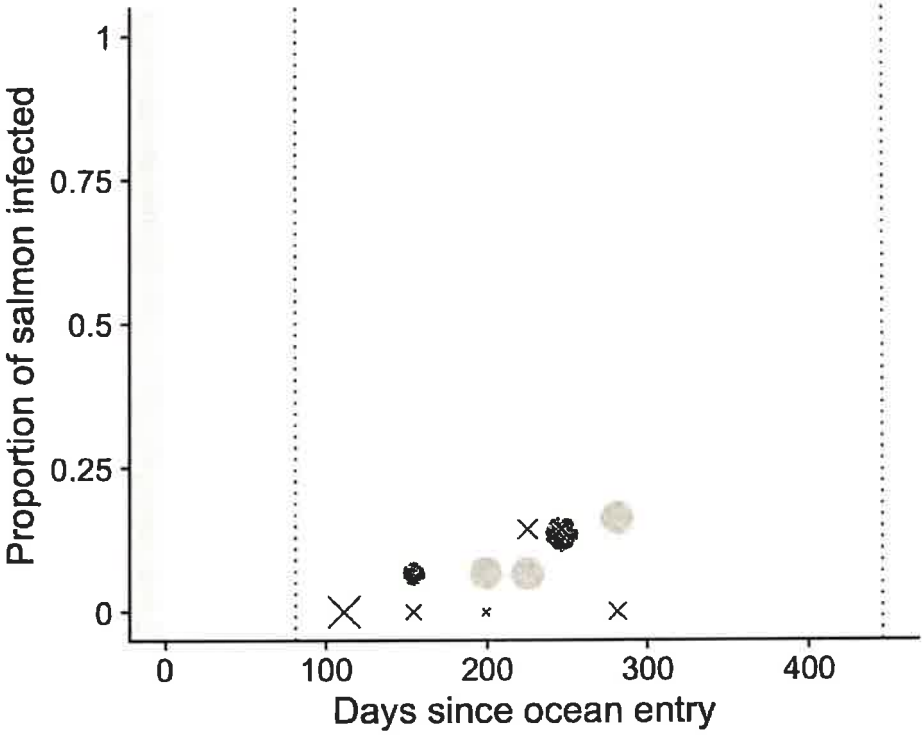


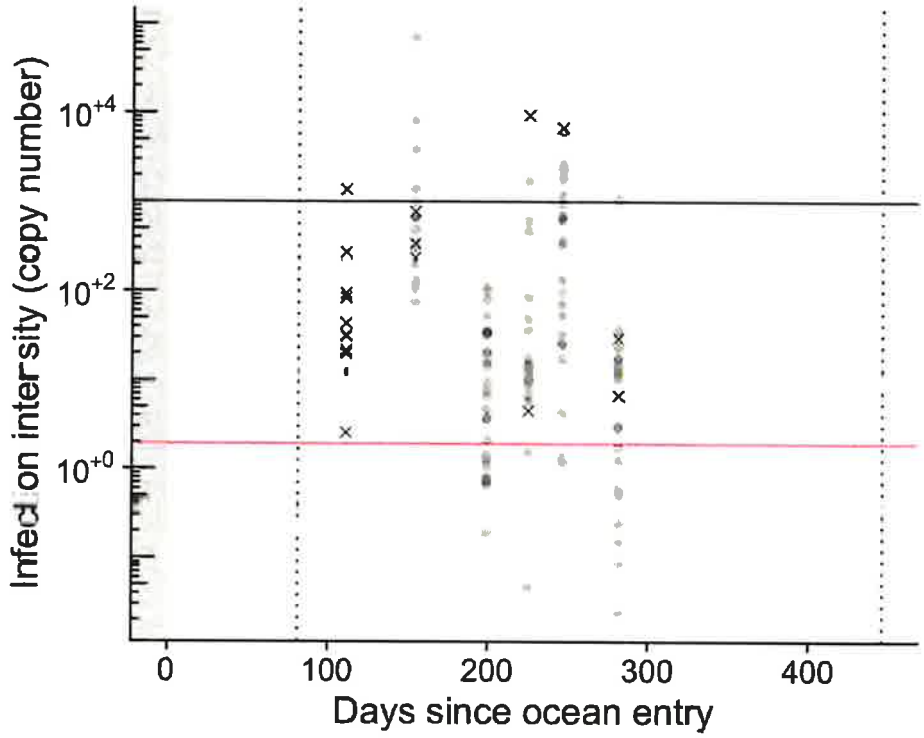


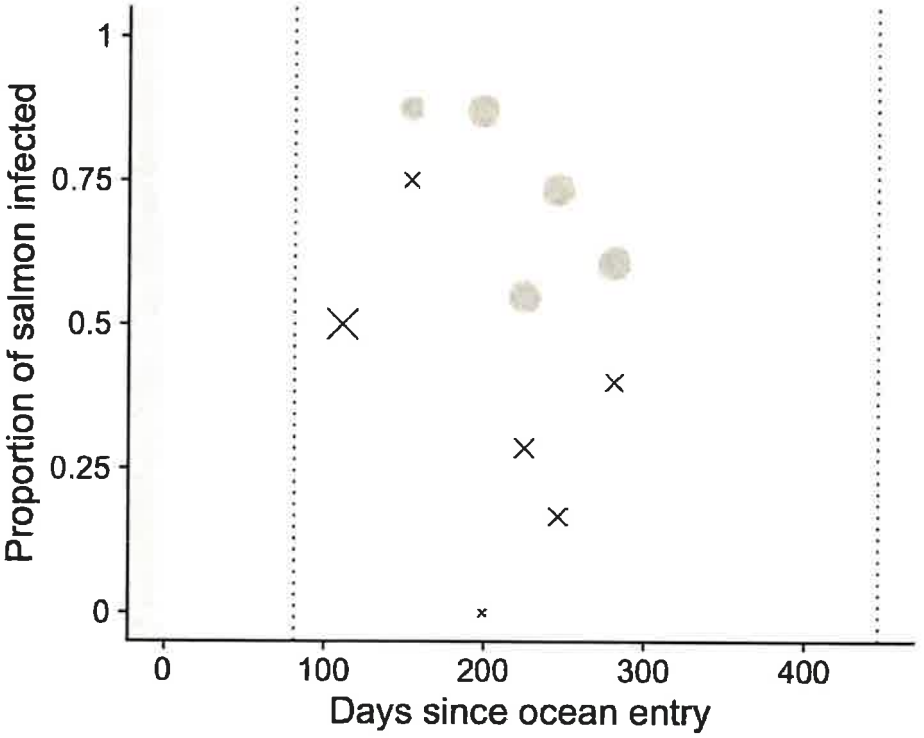
Candidatus Syngnamydia salmonis





Tenacibaculum maritimum





Clinical signs

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

metric	O6141	O6142	O6143	O6144	O6145	O6146	O6147	O6148	O6149	O6150	O6151	O6152	O6153	O6154	O6155	O6156	O6157	O6158	O6159	O6160
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															
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	O6161	O6162	O6163	O6164	O6165	O6166	O6167	O6168	O6169	O6170	O6171	O6172	O6173	O6174	O6175
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															
Enlarged		X	X		X					X		X			X
Green															
Heart															
Deformed	X														
Pale															X
Brain															
Hemorrhages/Congestion															

Histology

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

metric	O6141	O6142	O6143	O6144	O6145	O6148	O6155	O6170	O6175
Heart									
Peri Epi	1	1	2		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
Pig Inc									2
Kidney									
Osis			1			na			
Cong Heam		2				1			
Interst Hyperplasia	2	1	2	1	1	3	1	2	2
Interst Nec			1						
Glomitis						na			
Pancreatitis									
Pancreatitis	2								
Cns									
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.

he 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 s 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* Syngnamydia 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 Syngnamydia 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

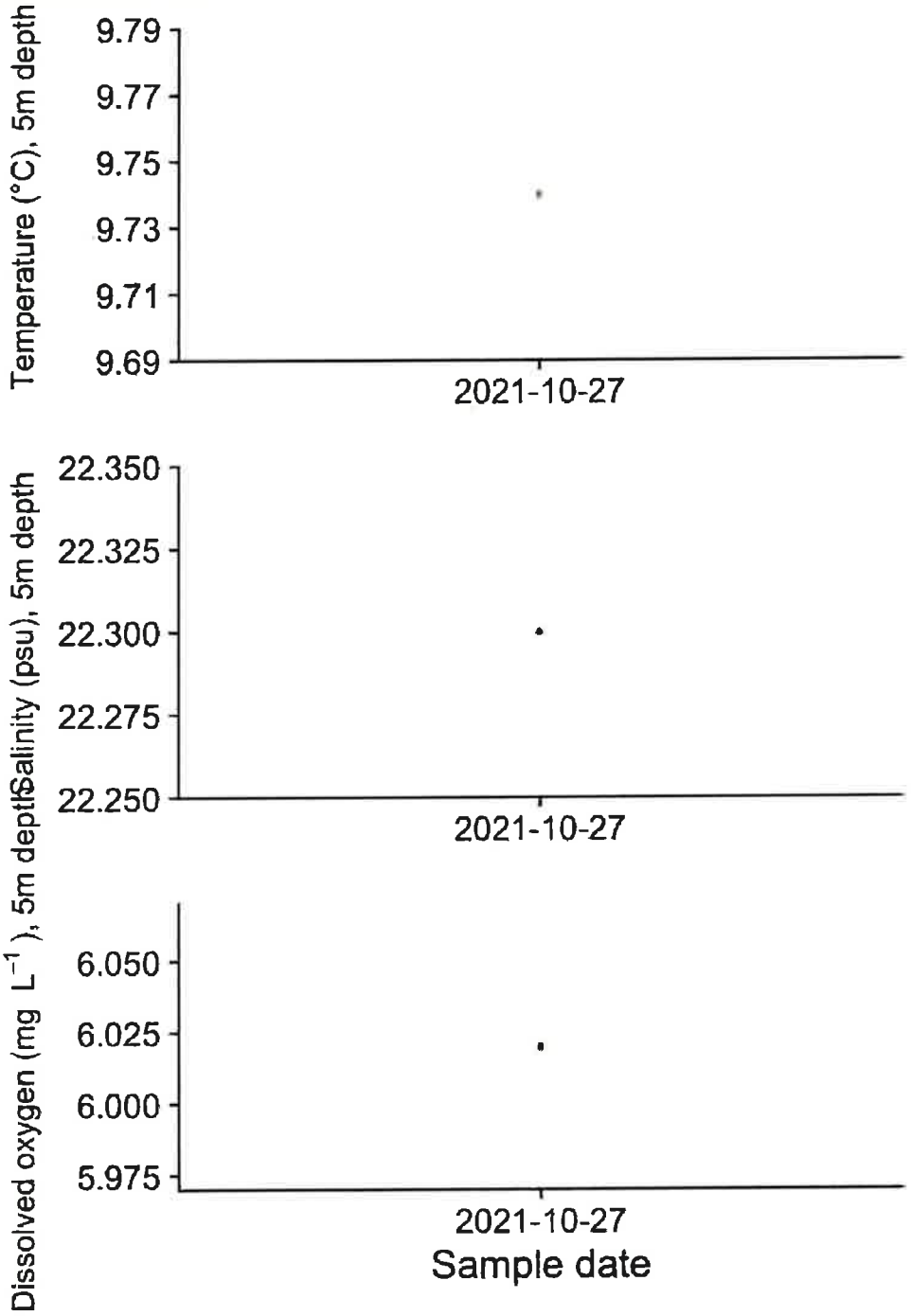
This report updates the Tenure Holder on the data collected and the testing and analysis conducted under the Water Sampling Research Program for the sampling event described below.

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.

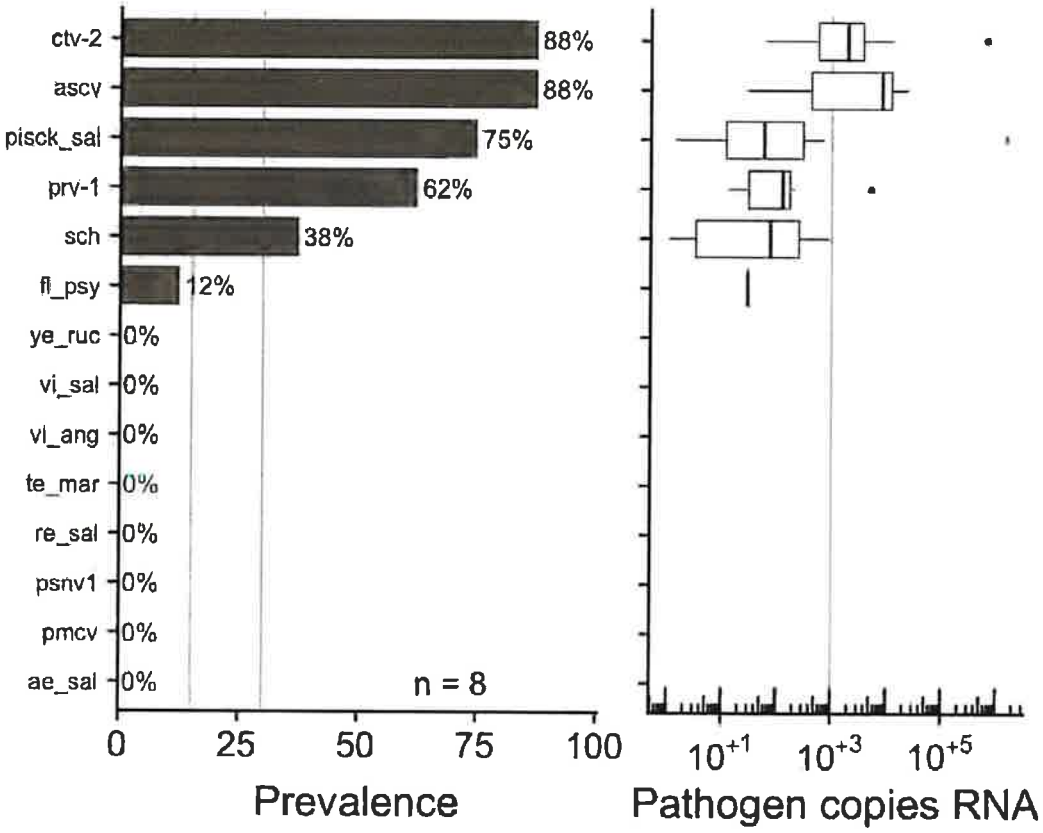
Histology samples have been sent to Wax-It Histo Ltd. to process and prepare slides, which have been read and scored by Dr. Di Cicco. A digital copy of each slide is available to the Company.

Environmental data

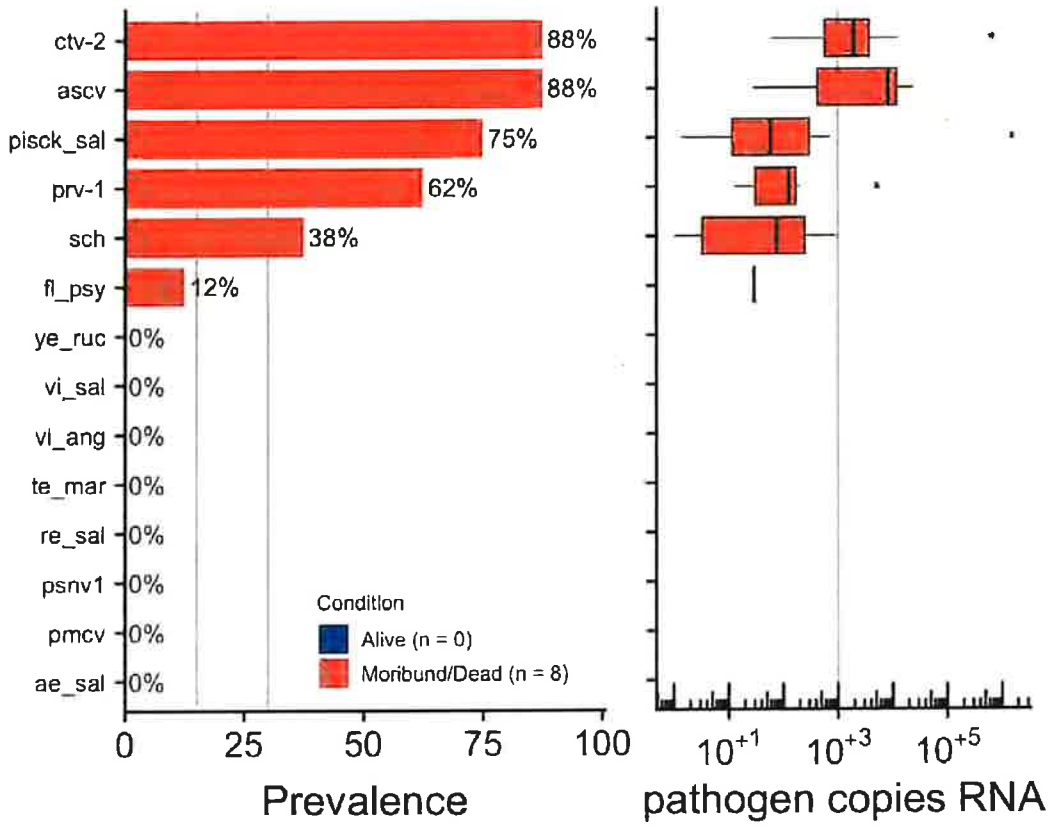


Water temperature (°C), salinity (ppm), and dissolved oxygen (mg/L) at a 5m depth. Certain sampling dates have no recorded environmental data, resulting in gaps in the plots.

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

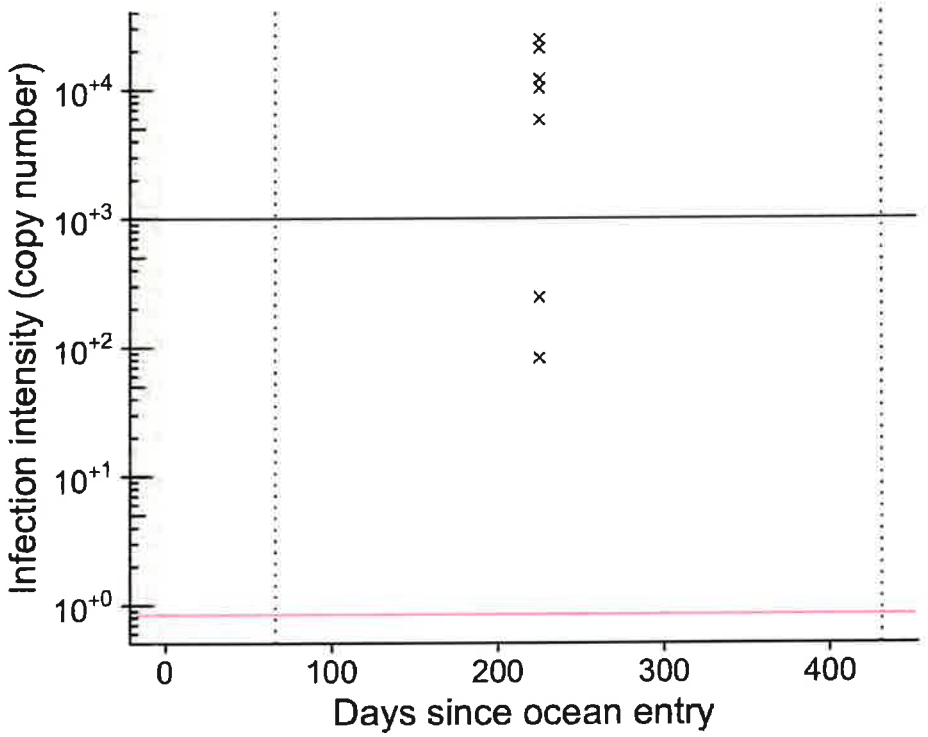
The following plots show individual infectious agent trends across all farm sites. In cases where sample size is sufficient, curves from a generalised additive model are included in the plot.

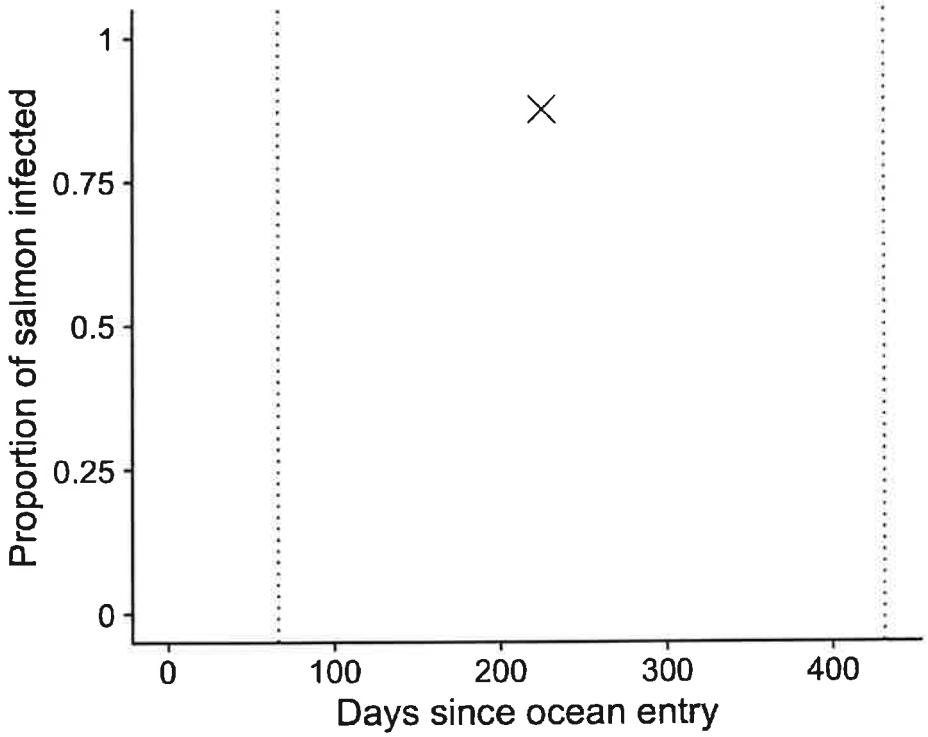
Grey circles represent live fish, and black X's represent dead/dying fish. Curves indicate mean predictions from a generalised additive model; blue and red correspond to live and dead/dying fish, respectively (shaded areas show 95% confidence regions). Left-hand grey region indicates freshwater hatchery 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.

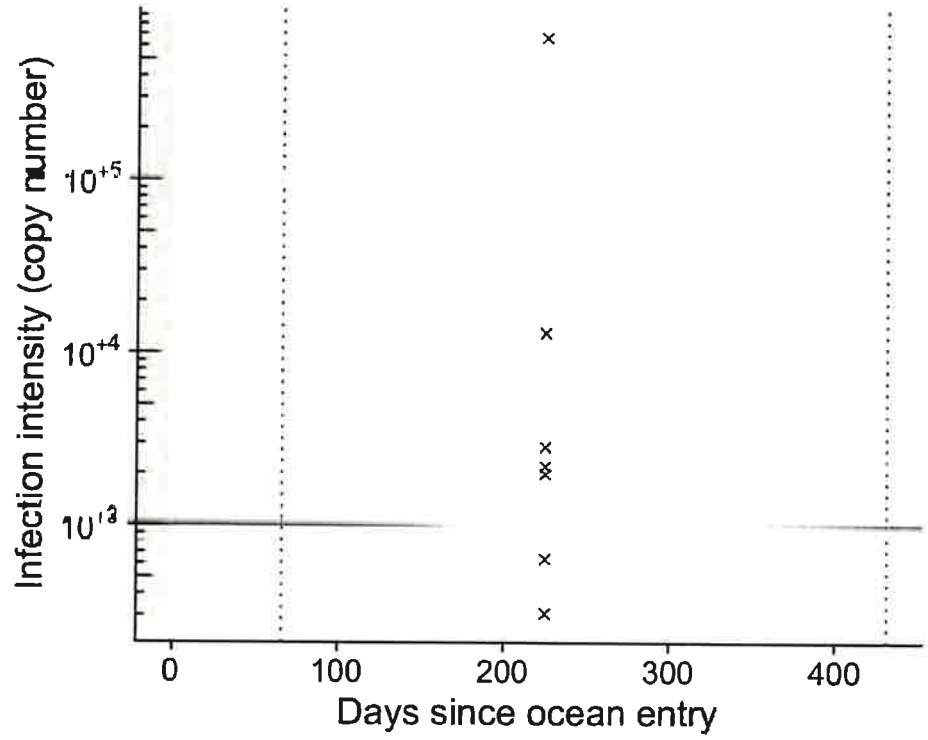
For proportion plots, grey circles show prevalence in live fish on each sampling date, and black X's show prevalence in dead/dying fish (symbol areas proportional to sample sizes).

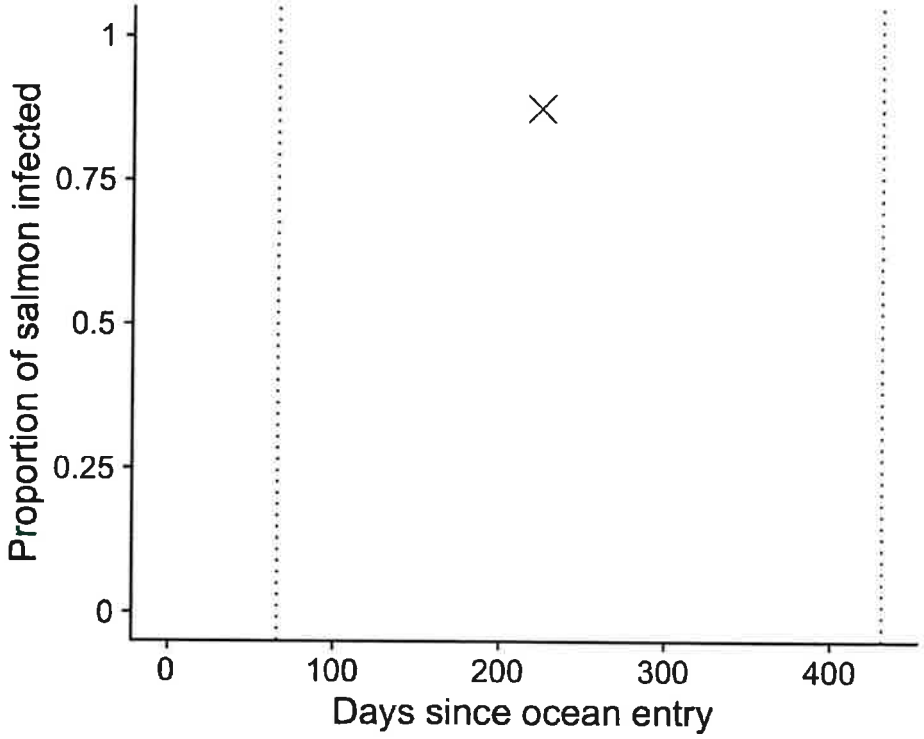
Atlantic salmon calicivirus



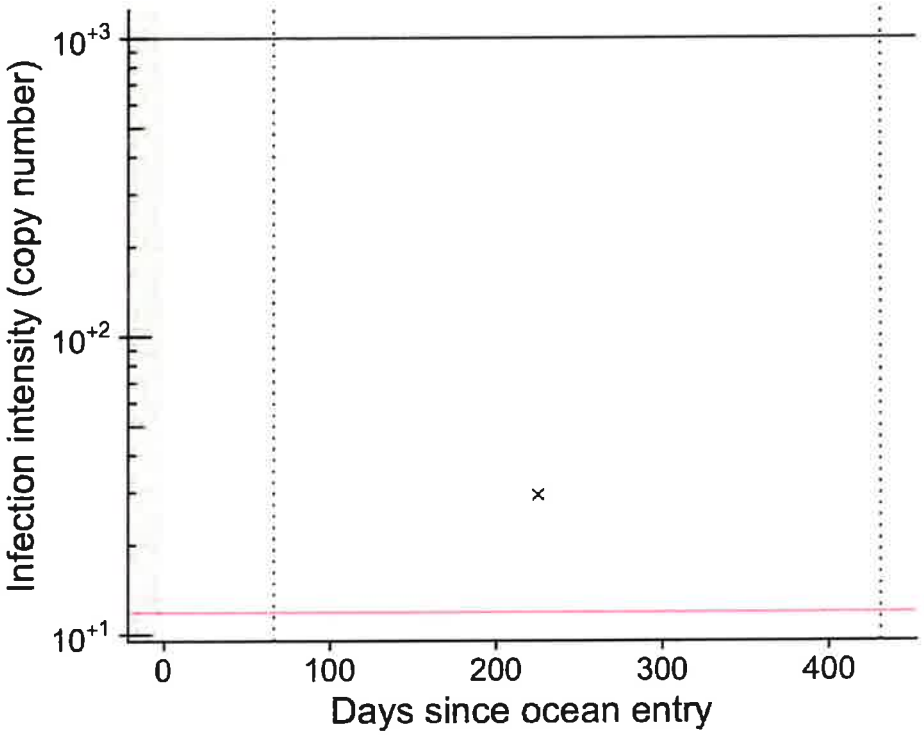


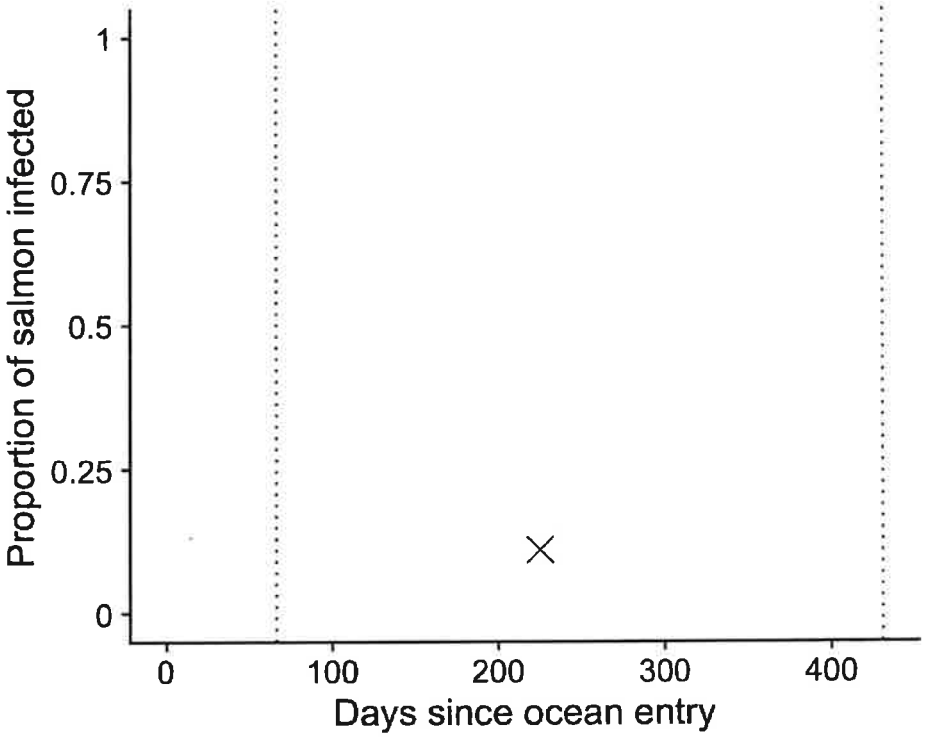
Cutthroat trout virus-2



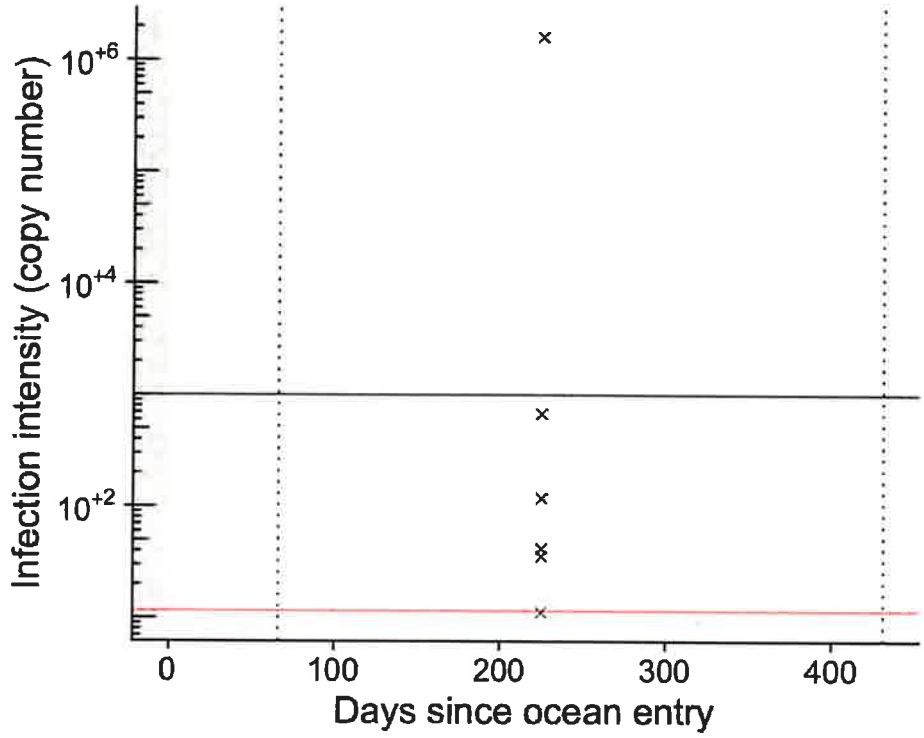


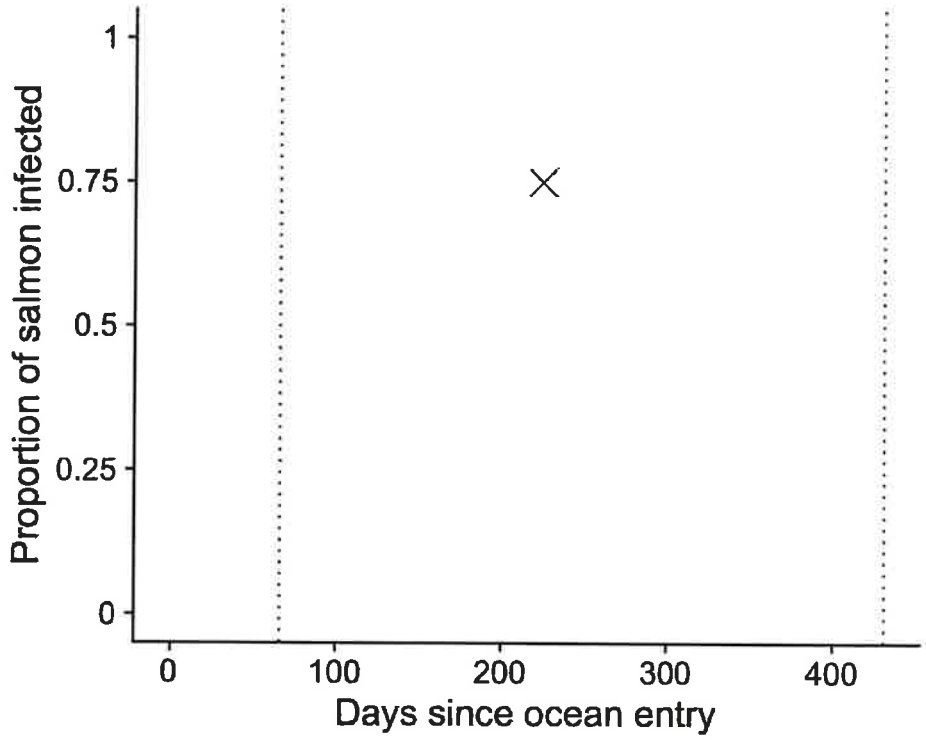
Flavobacterium psychrophilum



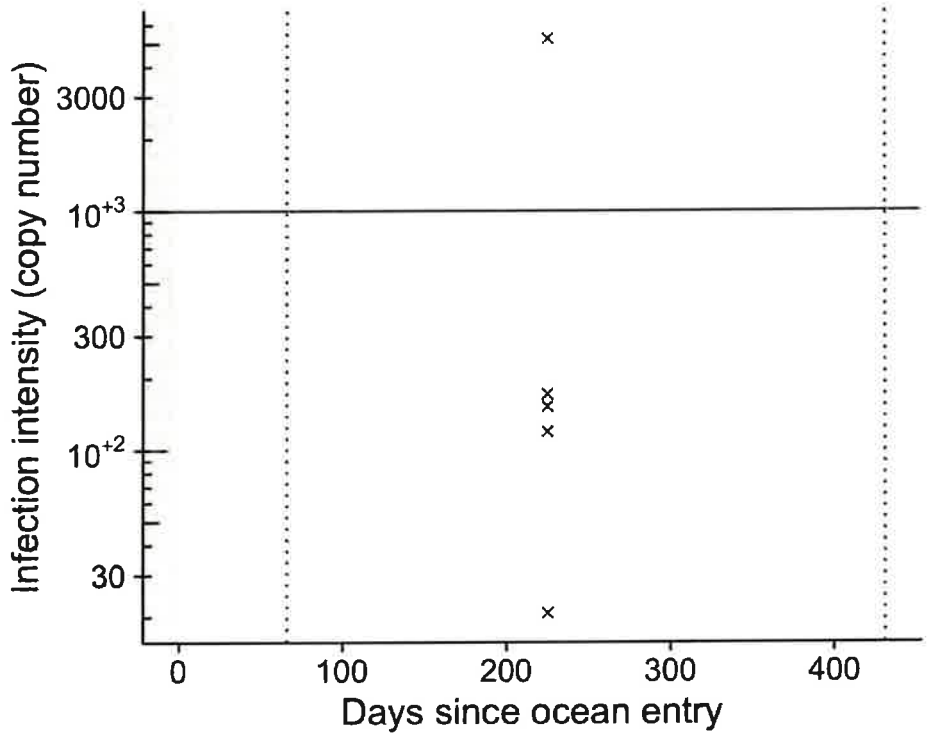


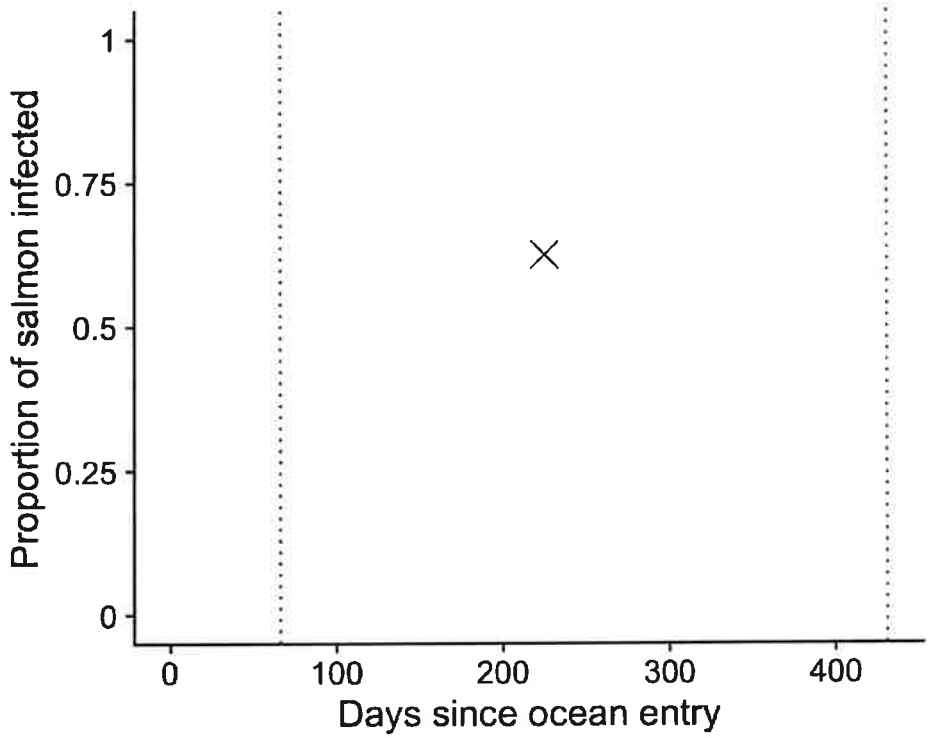
Piscirickettsia salmonis



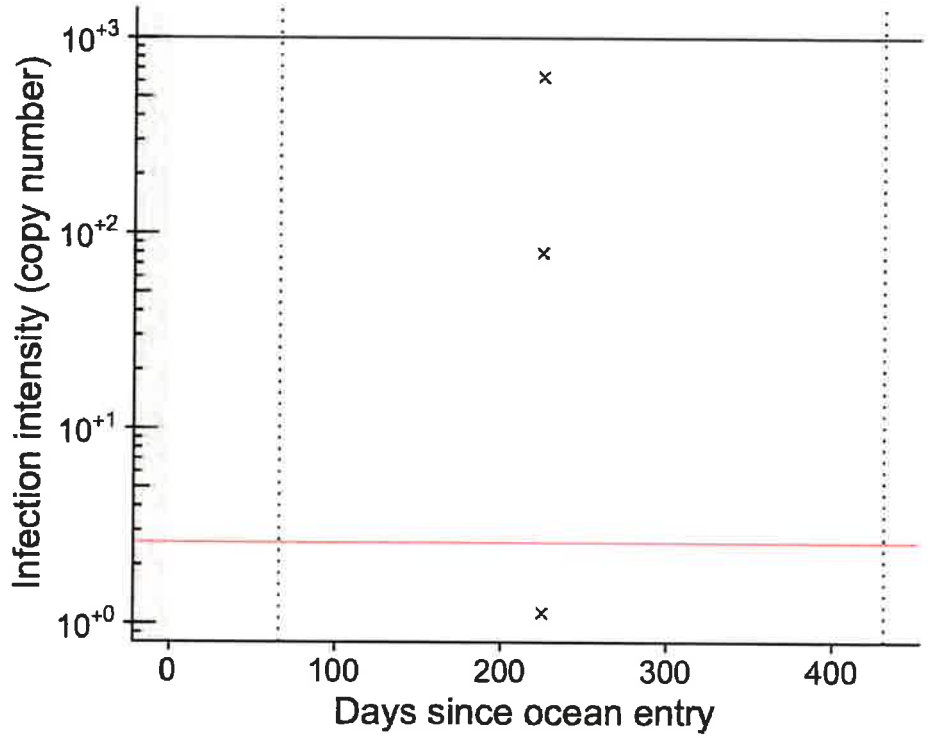


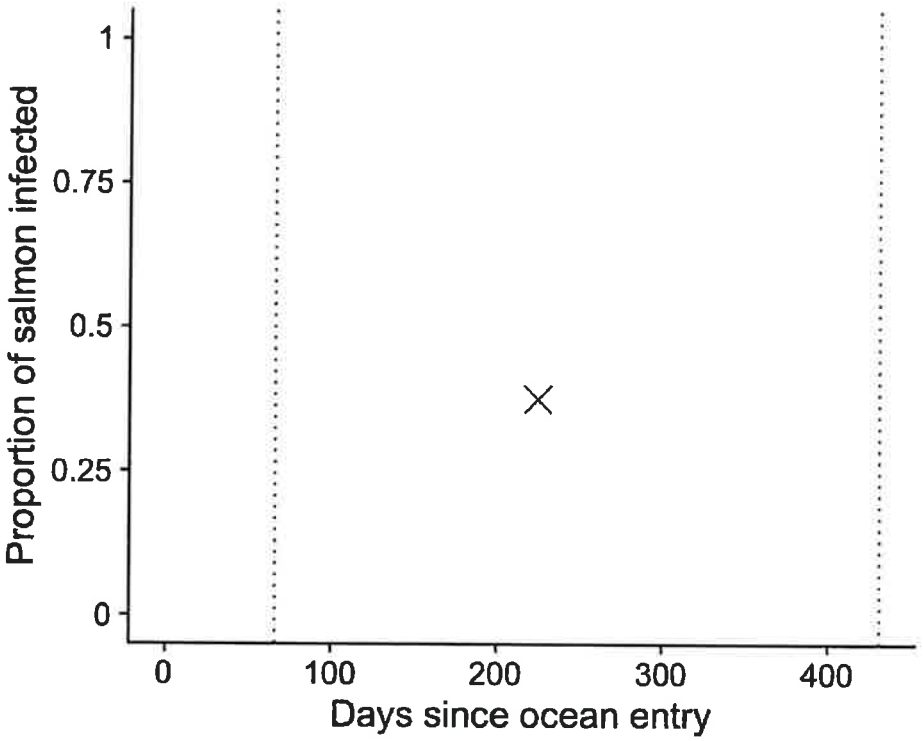
Piscine orthoreovirus





Candidatus Syngnamydia 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	N5201	N5202	N5203	N5204	N5205	N5206	N5209	N5210
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 Heam	2	2	2	3	3	3		3
Ellip Nec					1	1		2
W Pulpitis	2	2	1	1		2	2	1
Kidney								
Itis	1	2						
Osis		2						
Cong Heam	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 Heam	1	2	1	2		1		
Microsporidia								
Gills								
Itis	nv	nv	nv		nv		1	3
Cong Heam	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), Myocardionecrosis (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* Syngnamydia 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

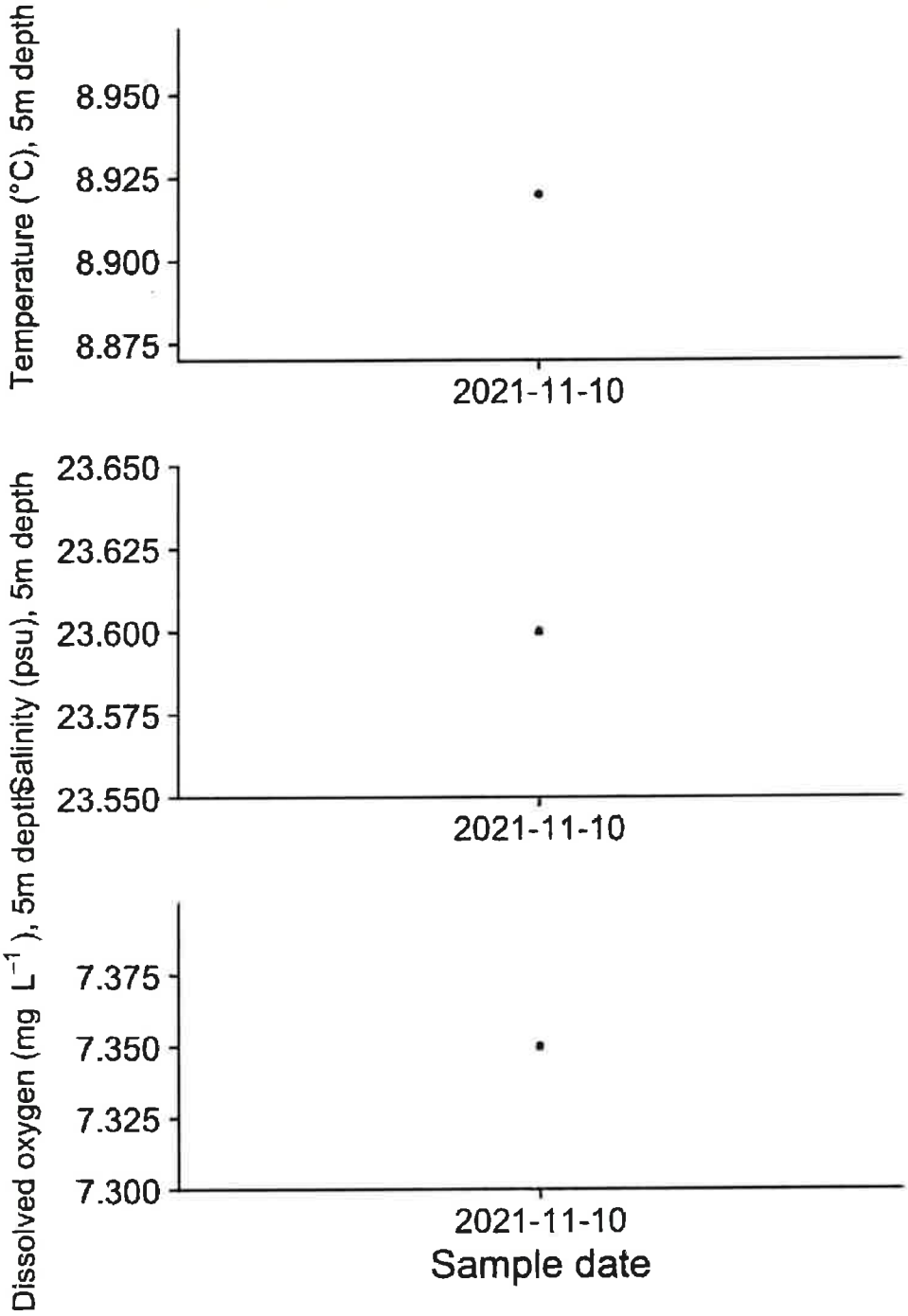
This report updates the Tenure Holder on the data collected and the testing and analysis conducted under the Water Sampling Research Program for the sampling event described below.

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.

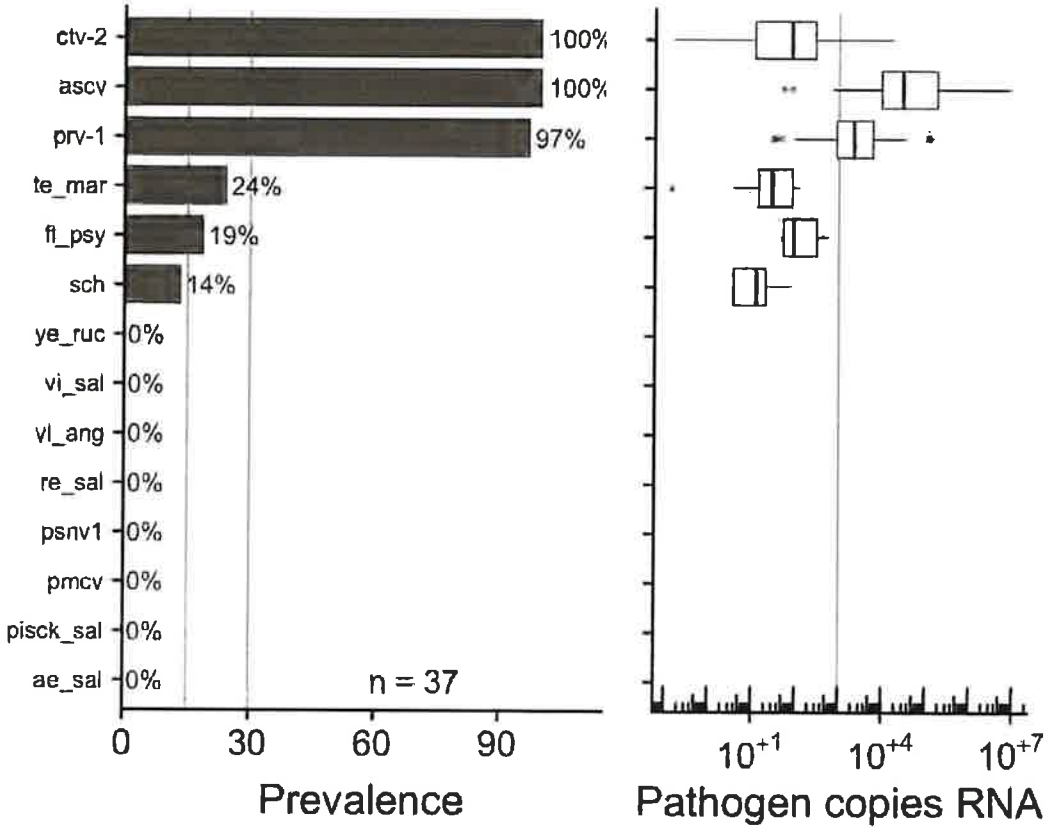
Histology samples have been sent to Wax-It Histo Ltd. to process and prepare slides, which have been read and scored by Dr. Di Cicco. A digital copy of each slide is available to the Company.

Environmental data

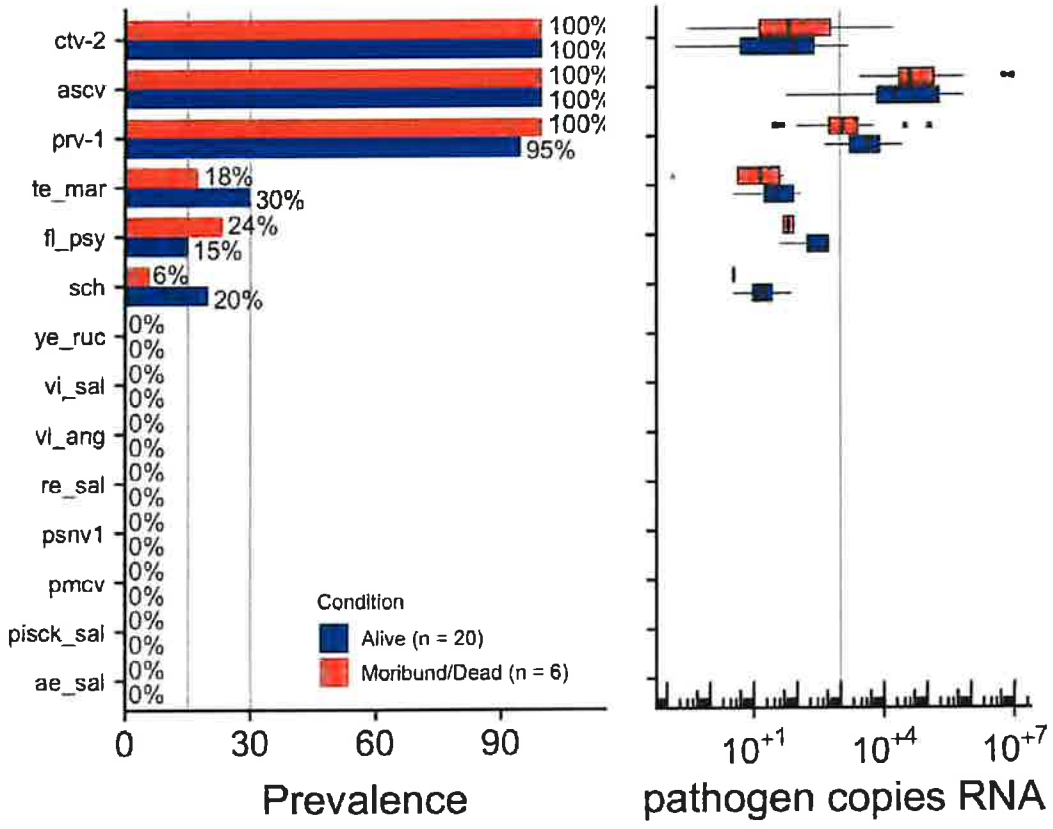


Water temperature (°C), salinity (ppm), and dissolved oxygen (mg/L) at a 5m depth. Certain sampling dates have no recorded environmental data, resulting in gaps in the plots.

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

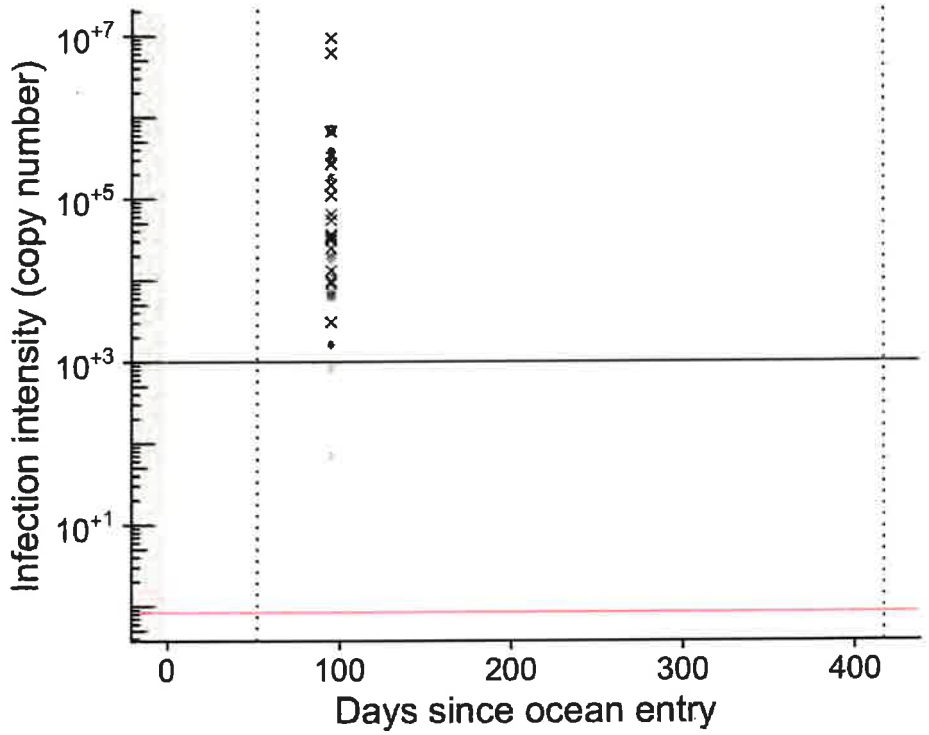
The following plots show individual infectious agent trends across all farm sites. In cases where sample size is sufficient, curves from a generalised additive model are included in the plot.

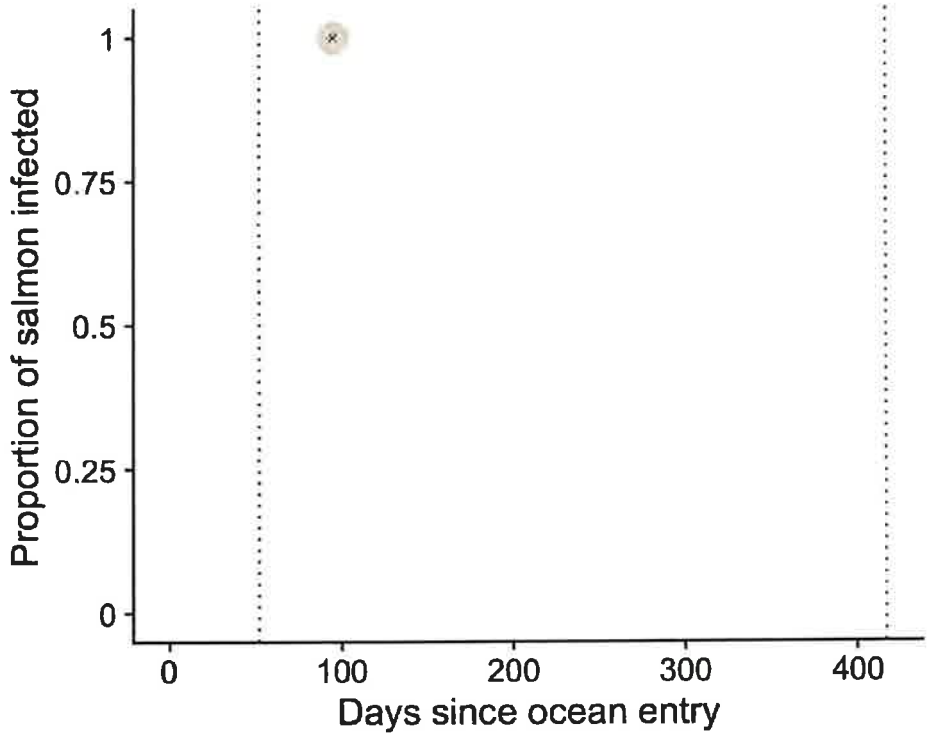
Grey circles represent live fish, and black X's represent dead/dying fish. Curves indicate mean predictions from a generalised additive model; blue and red correspond to live and dead/dying fish, respectively (shaded areas show 95% confidence regions). Left-hand grey region indicates freshwater hatchery 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.

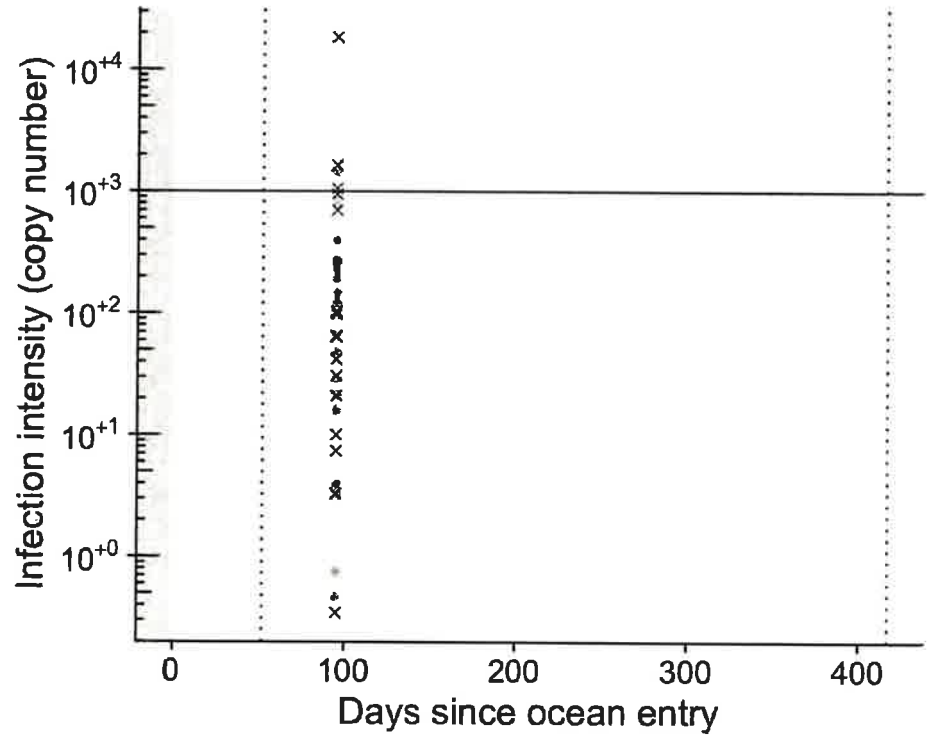
For proportion plots, grey circles show prevalence in live fish on each sampling date, and black X's show prevalence in dead/dying fish (symbol areas proportional to sample sizes).

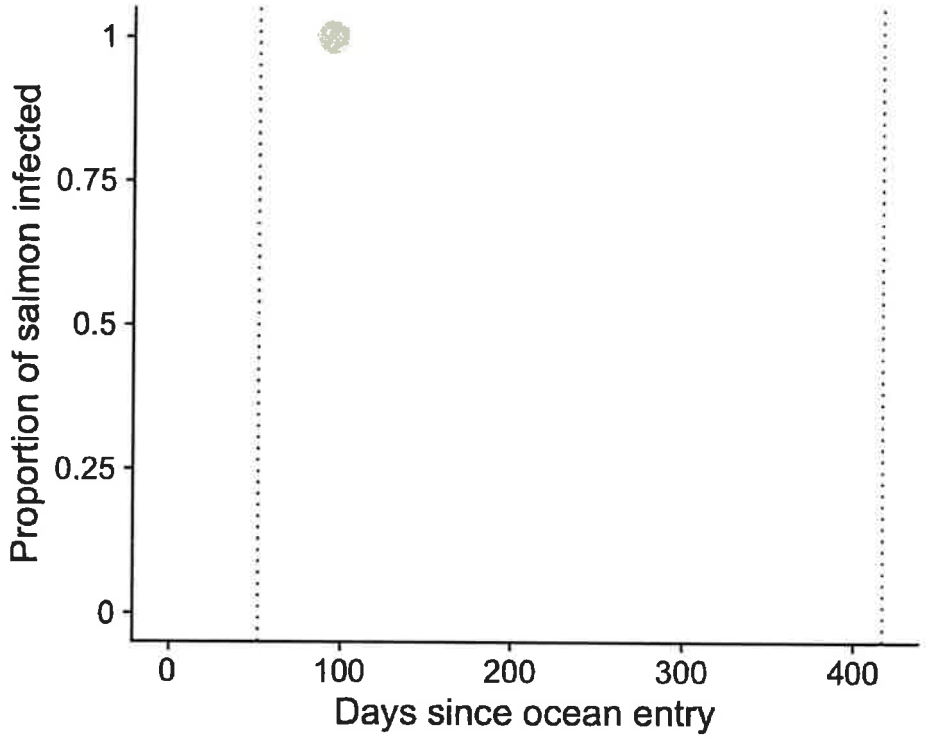
Atlantic salmon calicivirus



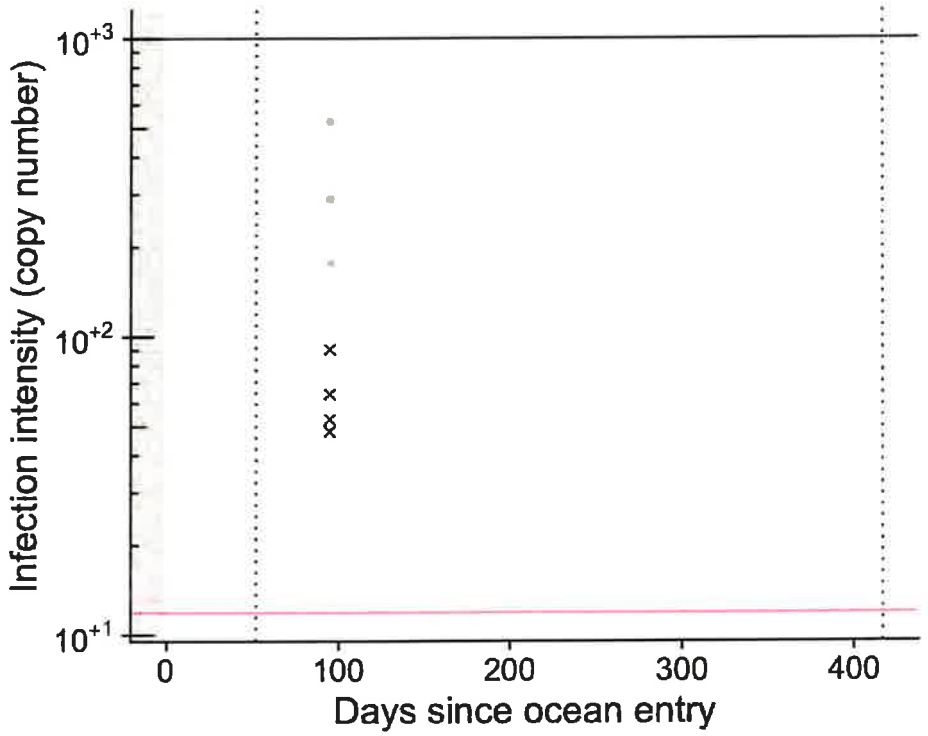


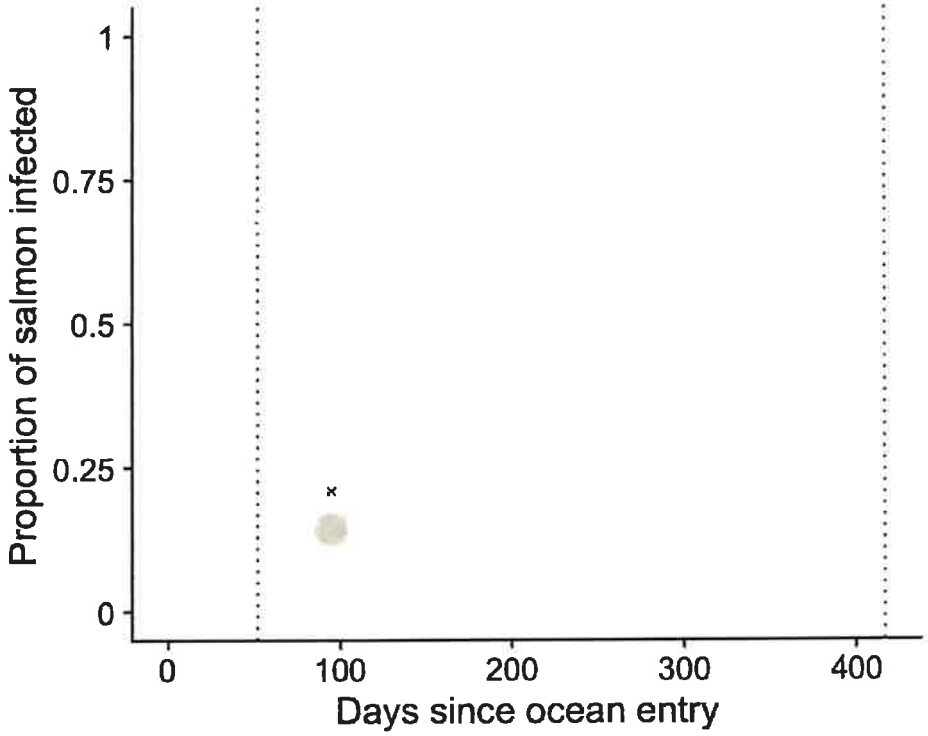
Cutthroat trout virus-2



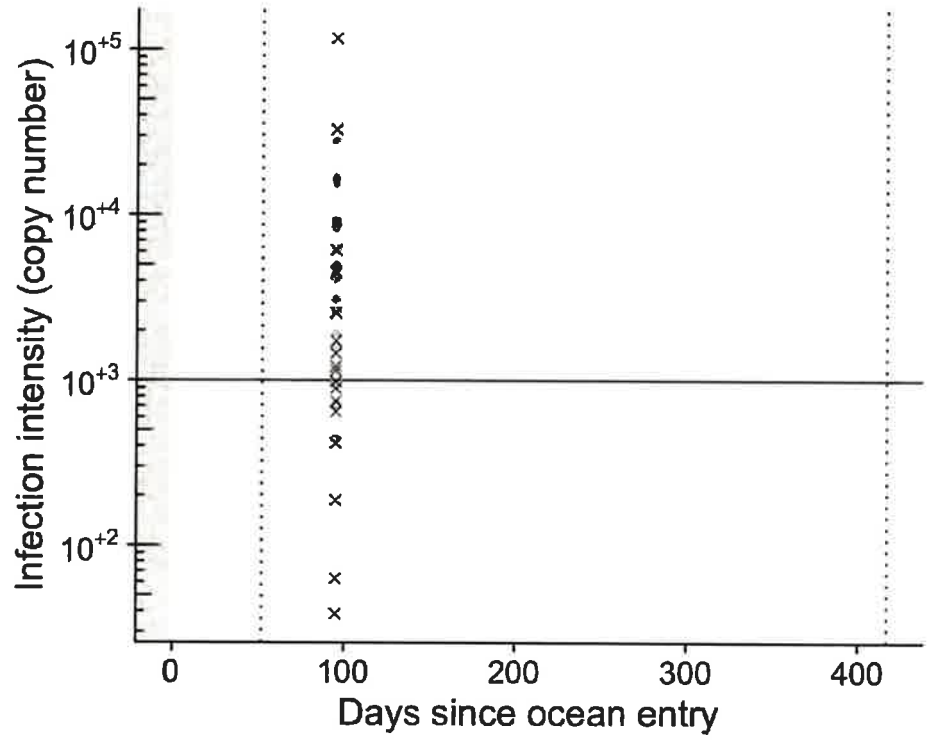


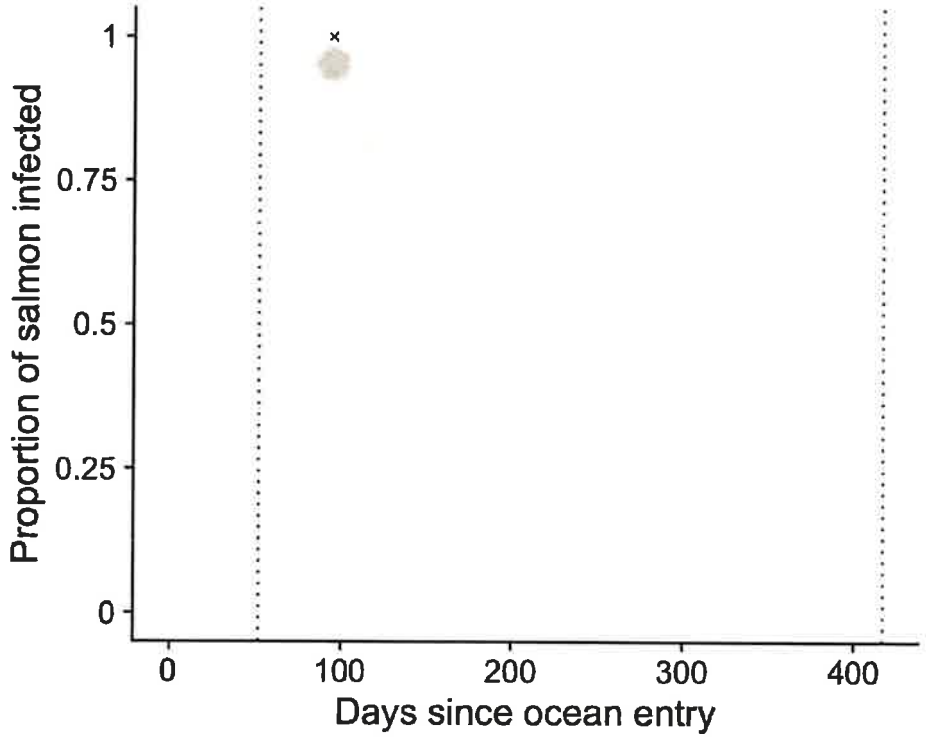
Flavobacterium psychrophilum



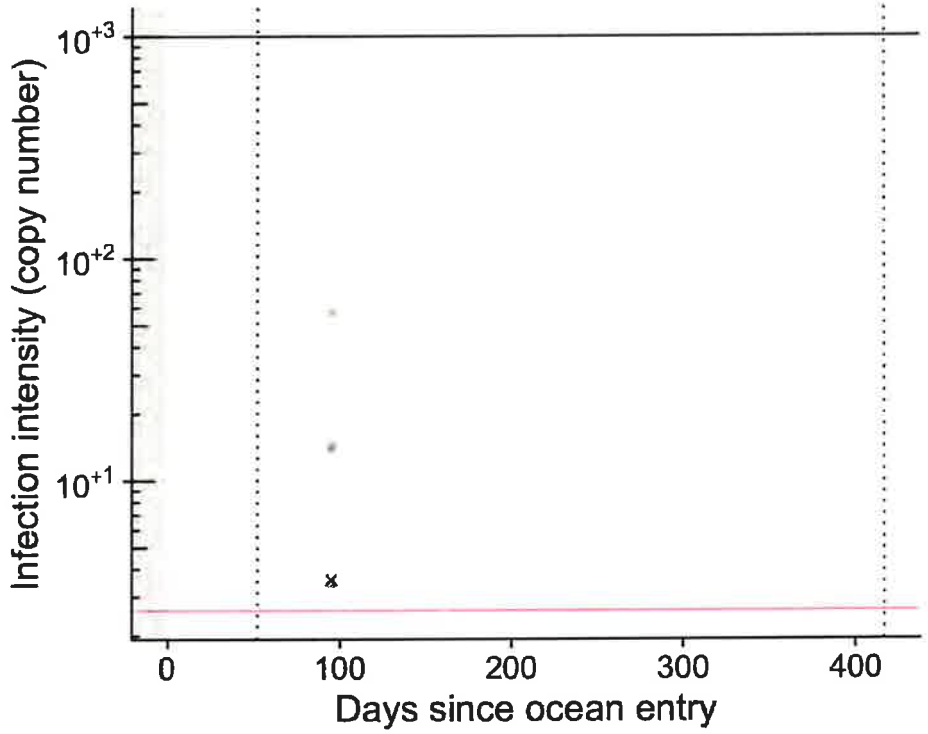


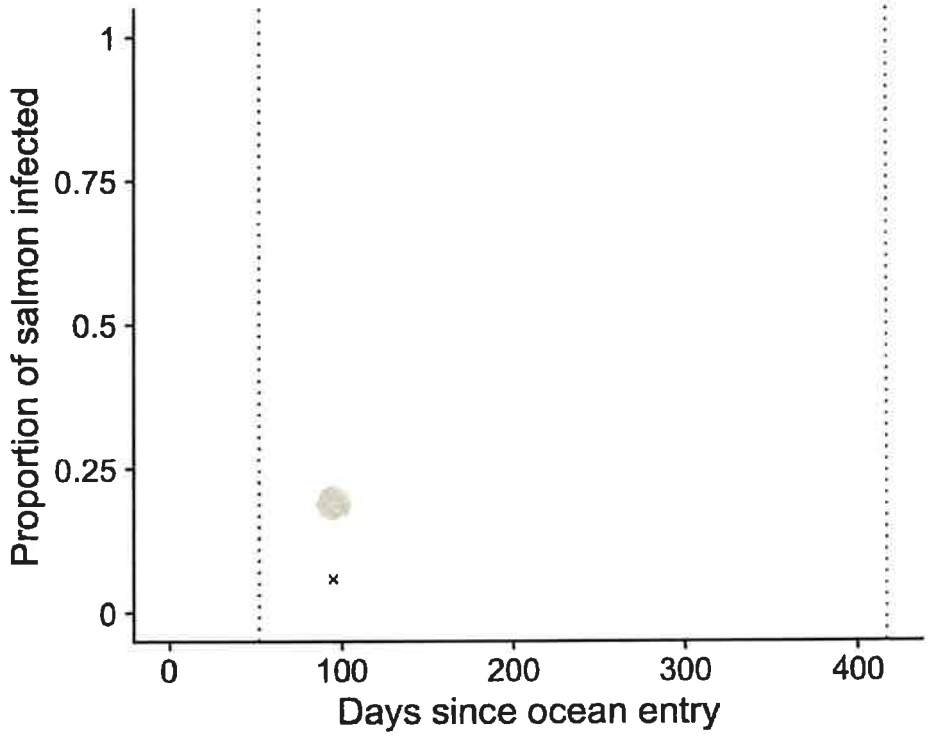
Piscine orthoreovirus



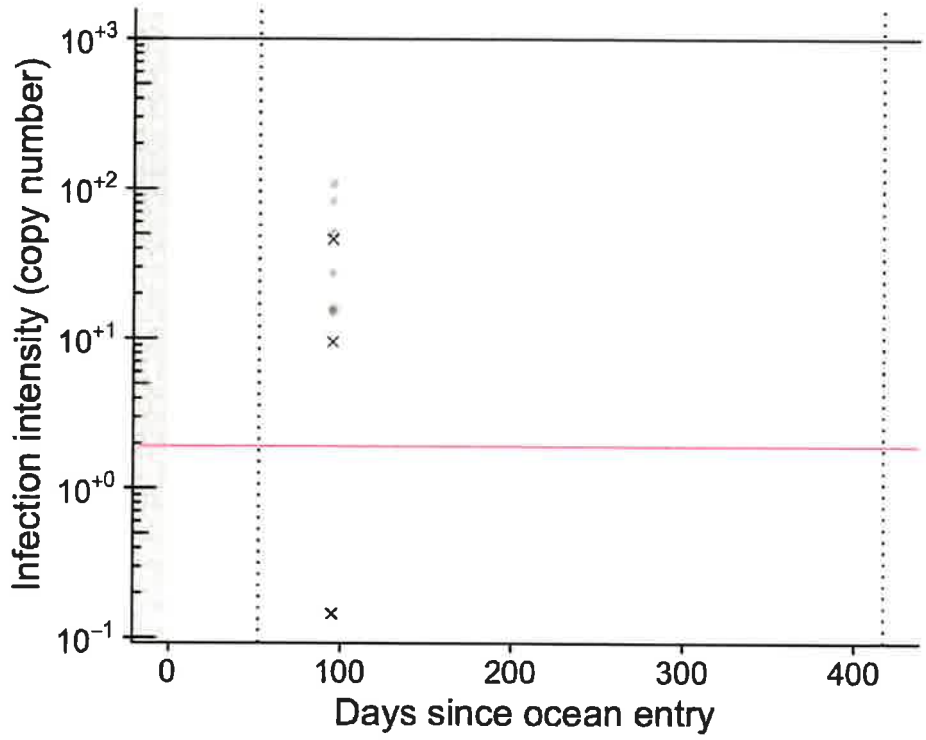


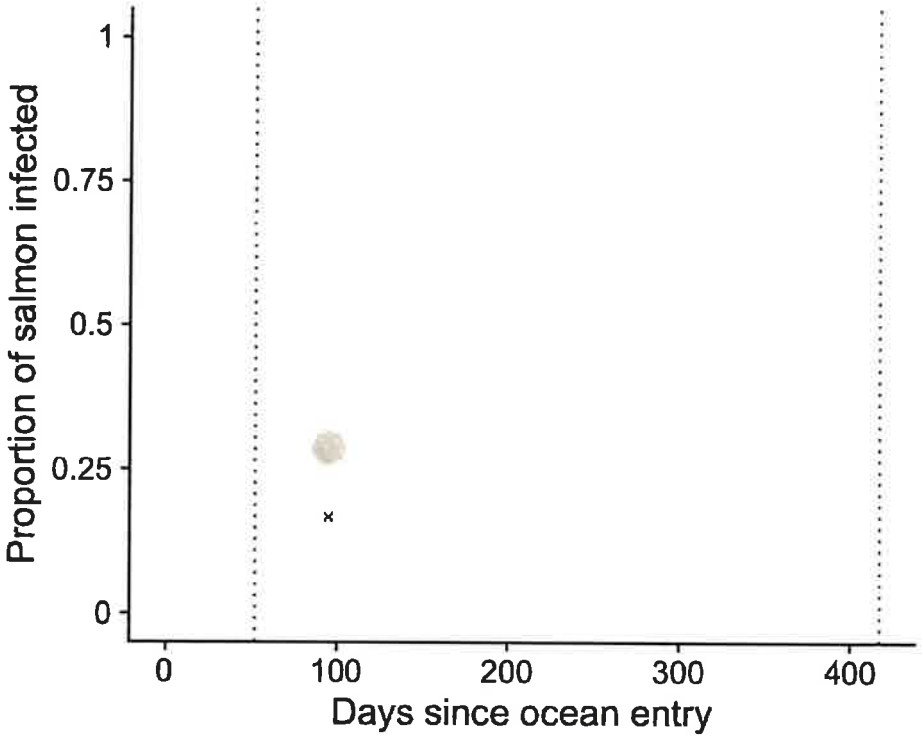
Candidatus Syngnamydia salmonis





Tenacibaculum maritimum





Clinical signs

Table 1: Clinical signs for specimens sampled on 2021-11-10

metric	N5241	N5242	N5243	N5244	N5245	N5246	N5247	N5249	N5250	N5251	N5252	N5263	N5264	N5265	N5266	N5267	N5268	N5269	N5270	N5271
General																				
Live								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																				
Enlarged				X			X			X										
Liver																				
Pale		X			X	X	X										X			
Yellow				X																
Gallbladder																				
Enlarged				X			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	N5272	N5273	N5274	N5275	N5276	N5277	N5278	N5279	N5280
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	N5241	N5242	N5243	N5244	N5245	N5246	N5247	N5248
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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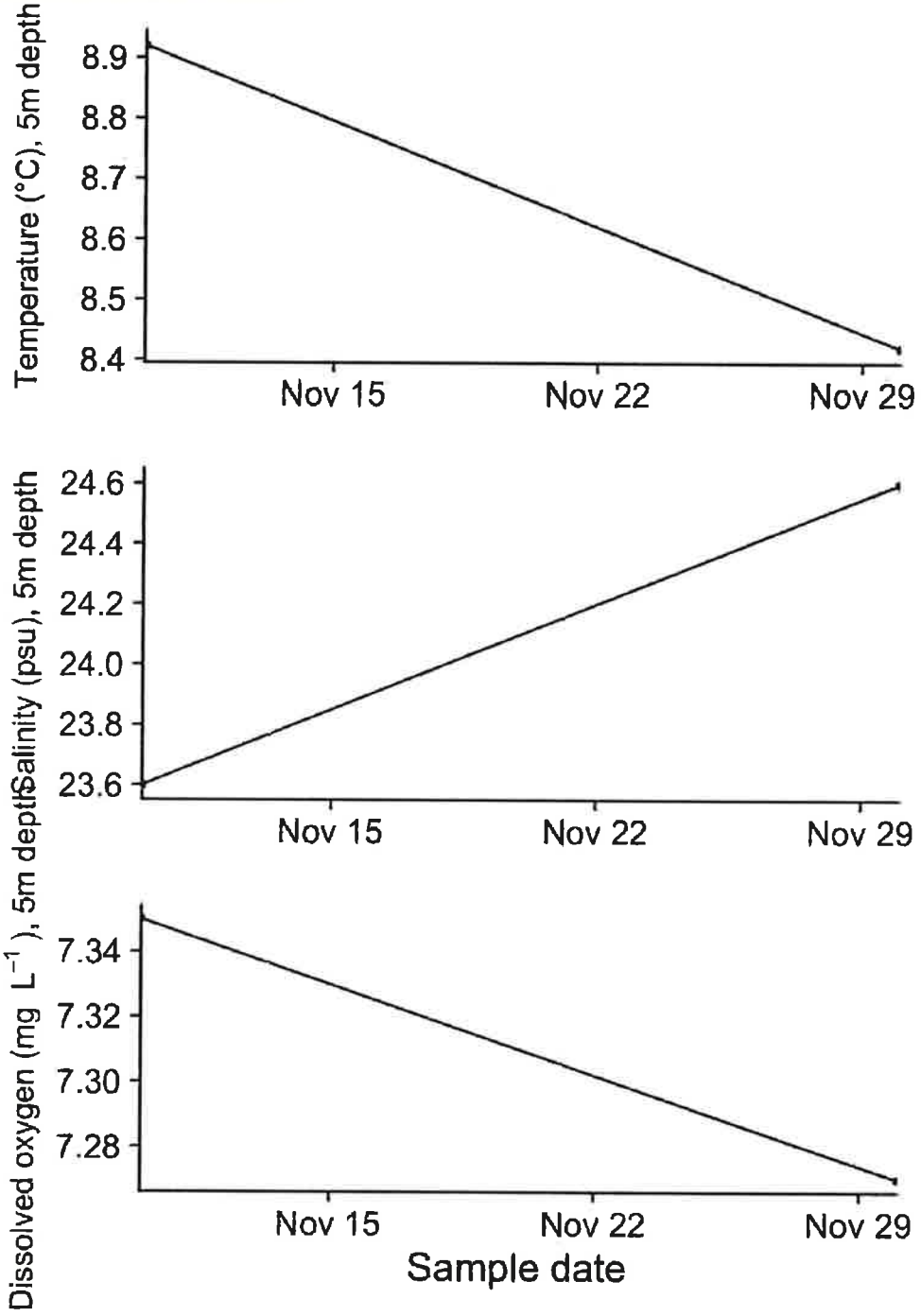
Executive summary

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.

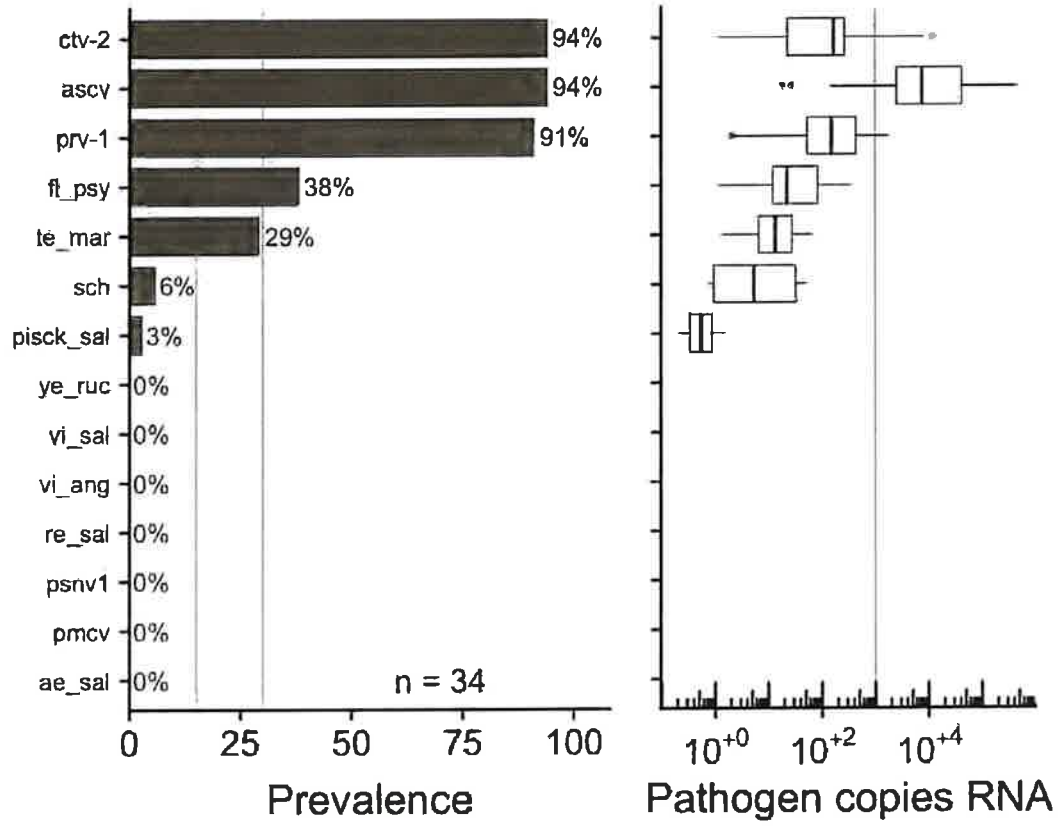
Histology samples have been sent to Wax-It Histo Ltd. to process and prepare slides, which have been read and scored by Dr. Di Cicco. A digital copy of each slide is available to the Company.

Environmental data



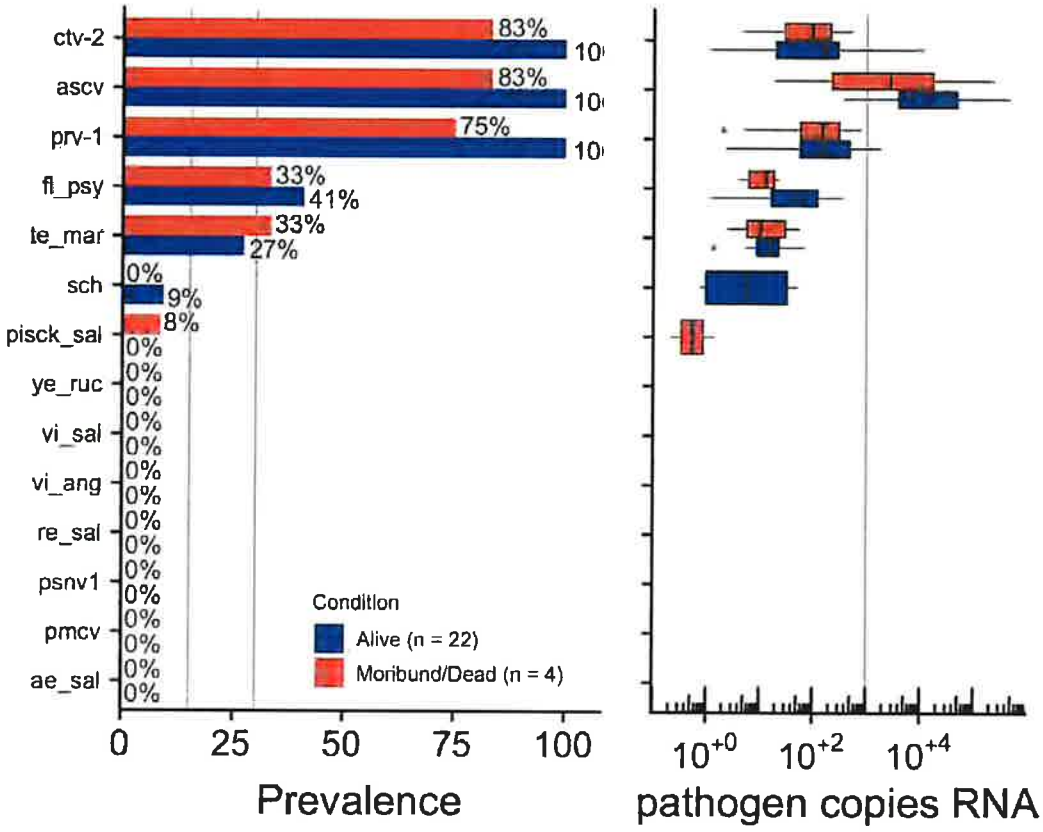
Water temperature (°C), salinity (ppm), and dissolved oxygen (mg/L) at a 5m depth. Certain sampling dates have no recorded environmental data, resulting in gaps in the plots.

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

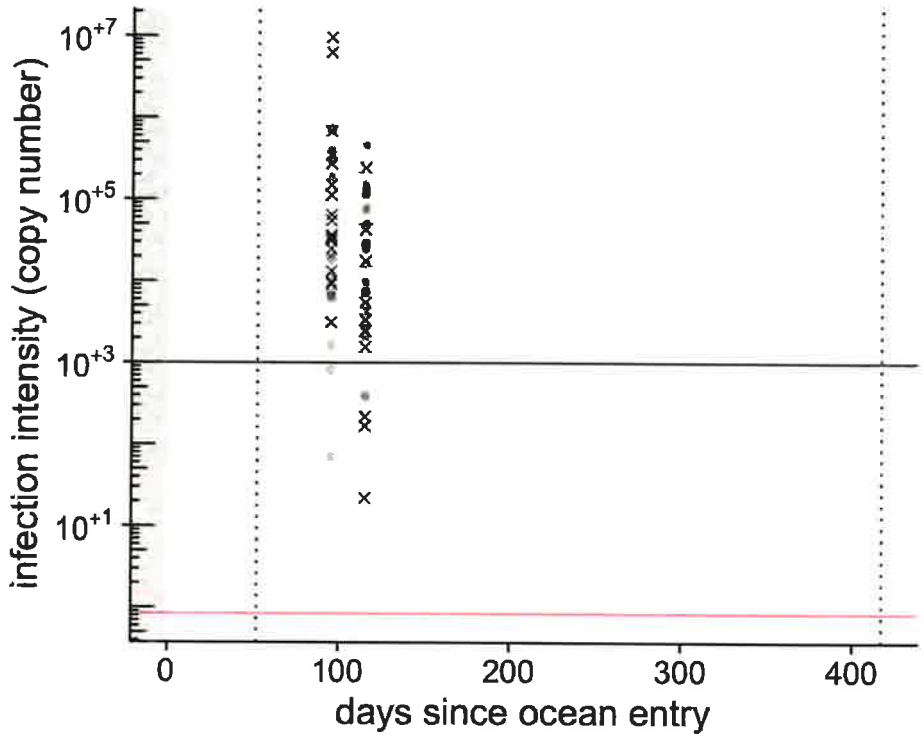
The following plots show individual infectious agent trends across all farm sites.

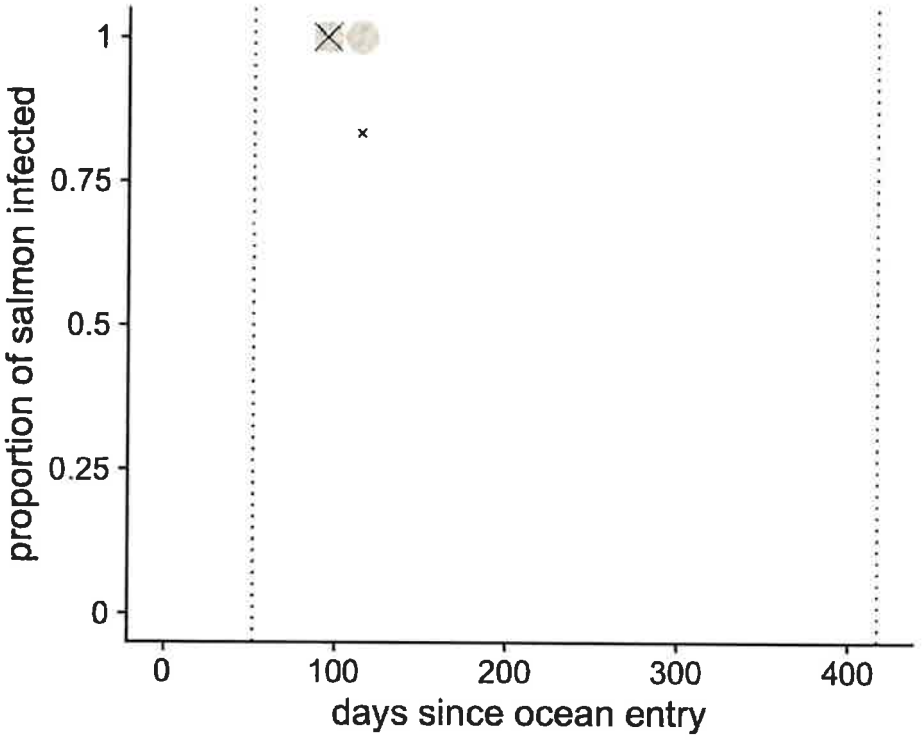
Grey circles represent live fish, and black X's represent dead/dying fish. Curves indicate mean predictions from a generalised additive model; blue and red correspond to live and dead/dying fish, respectively (shaded areas show 95% confidence regions). Left-hand grey region indicates freshwater hatchery 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.

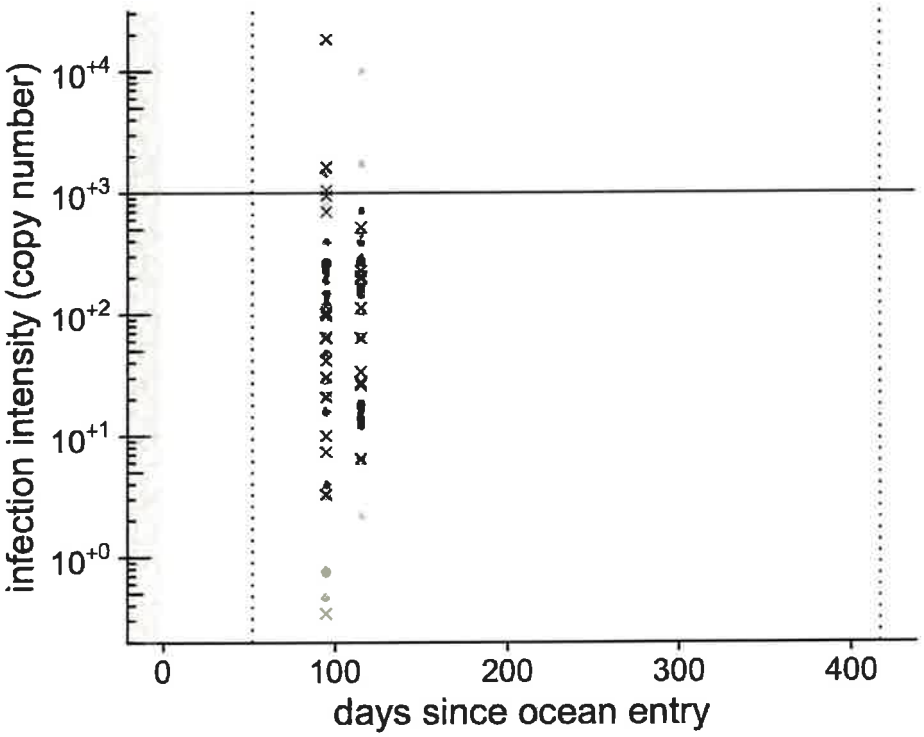
For proportion plots, grey circles show prevalence in live fish on each sampling date, and black X's show prevalence in dead/dying fish (symbol areas proportional to sample sizes).

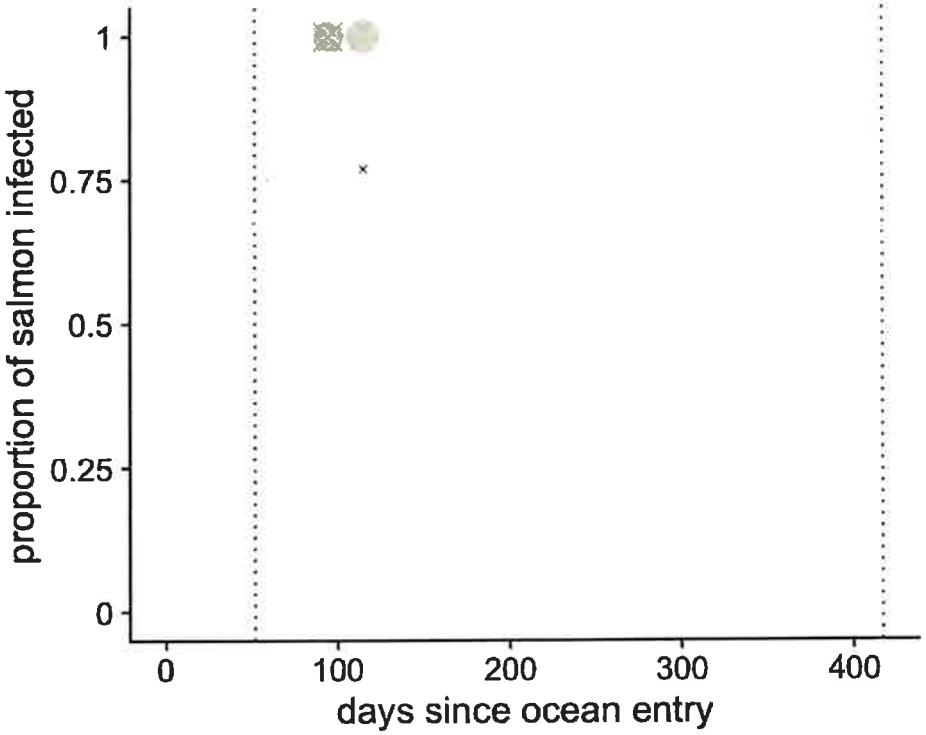
Atlantic salmon calicivirus



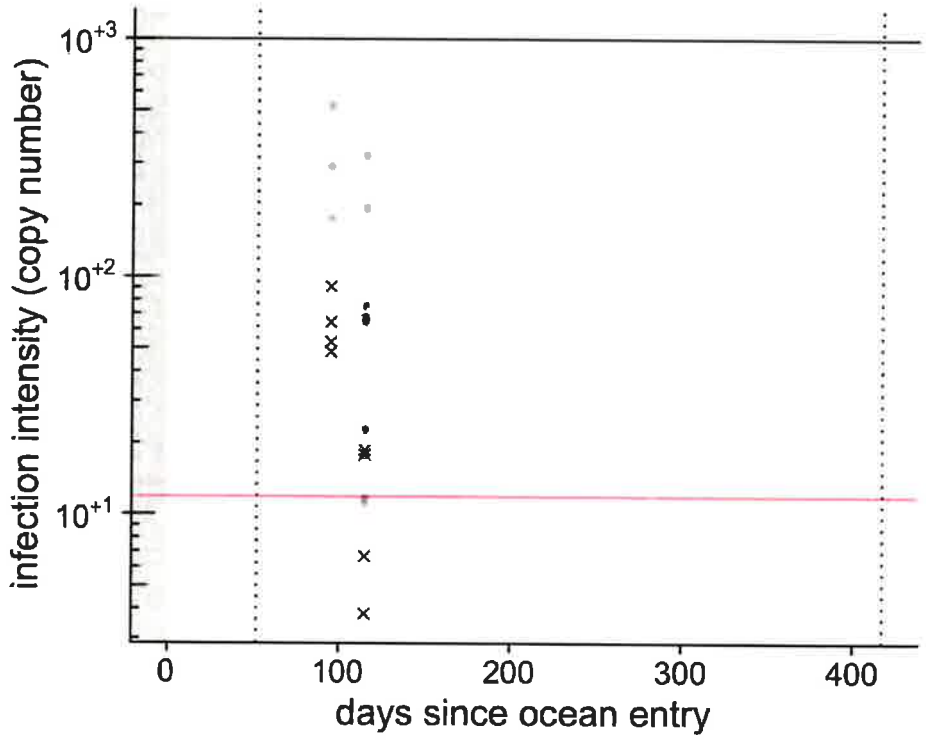


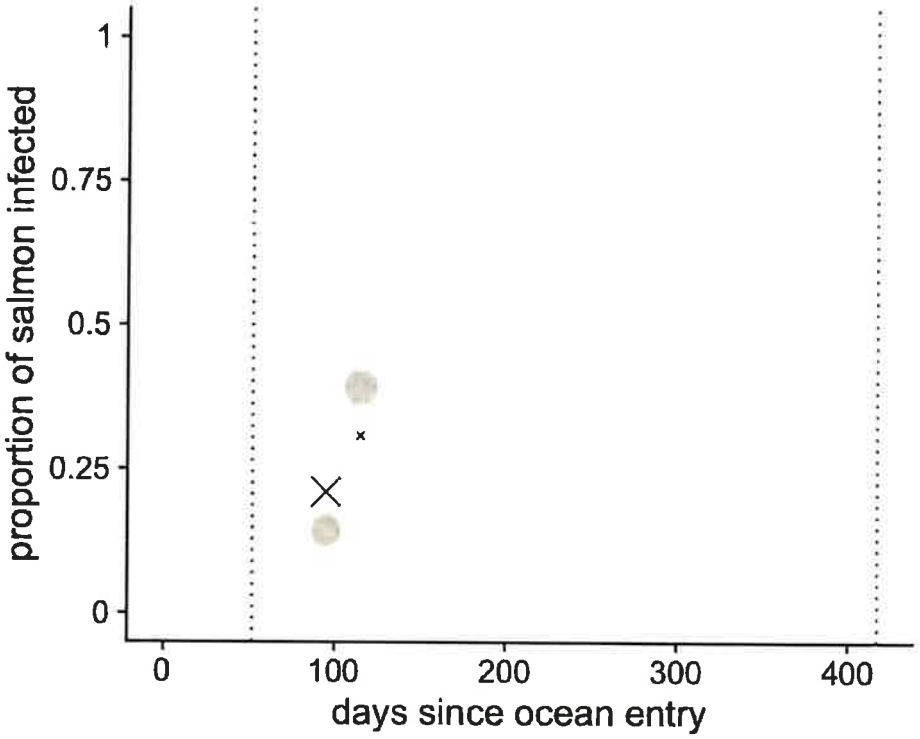
Cutthroat trout virus-2



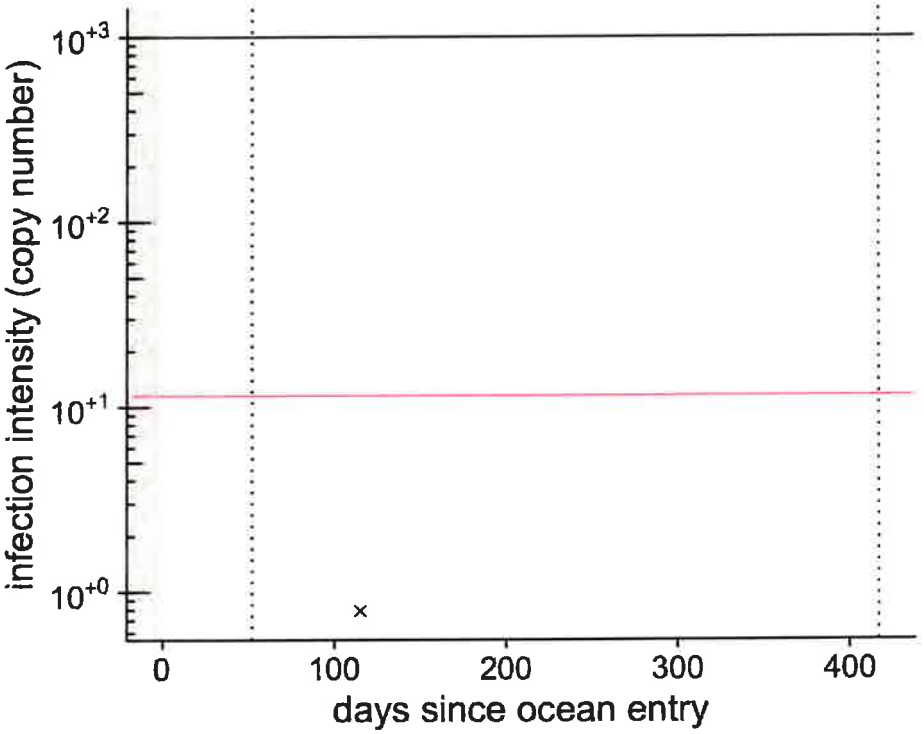


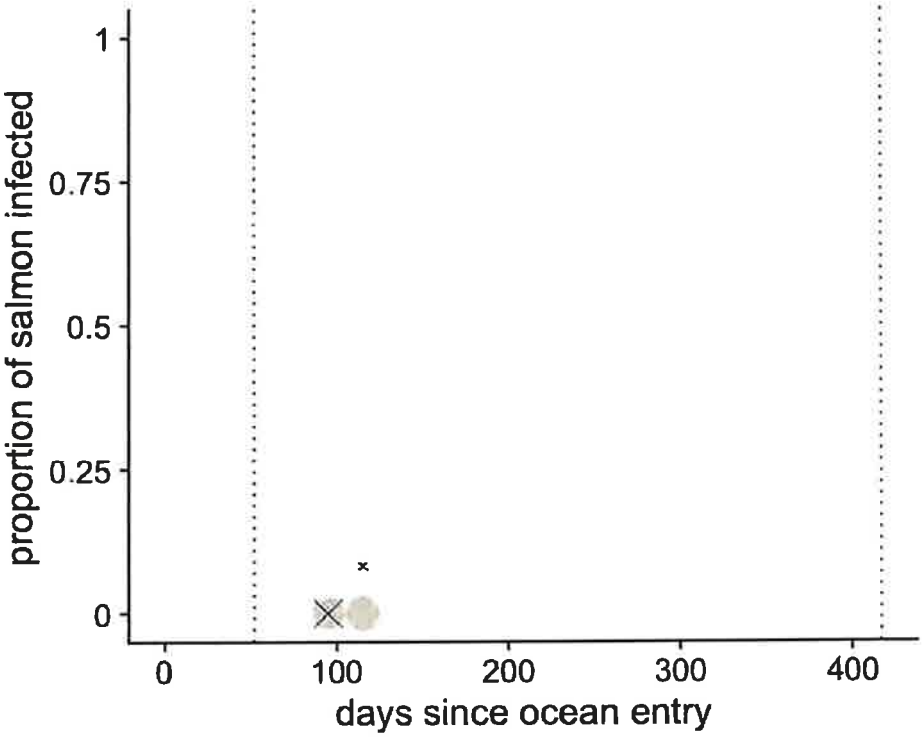
Flavobacterium psychrophilum



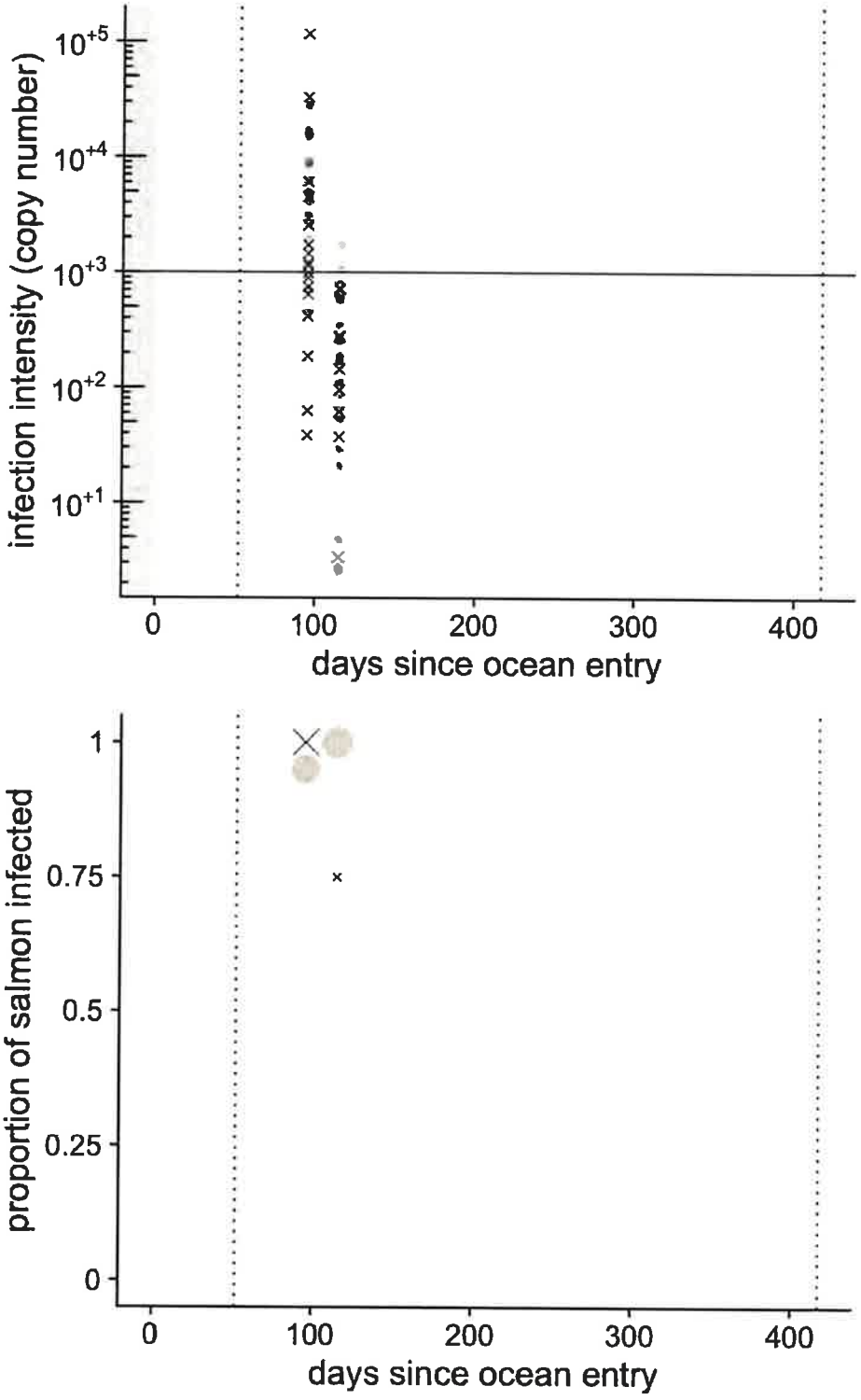


Piscirickettsia salmonis

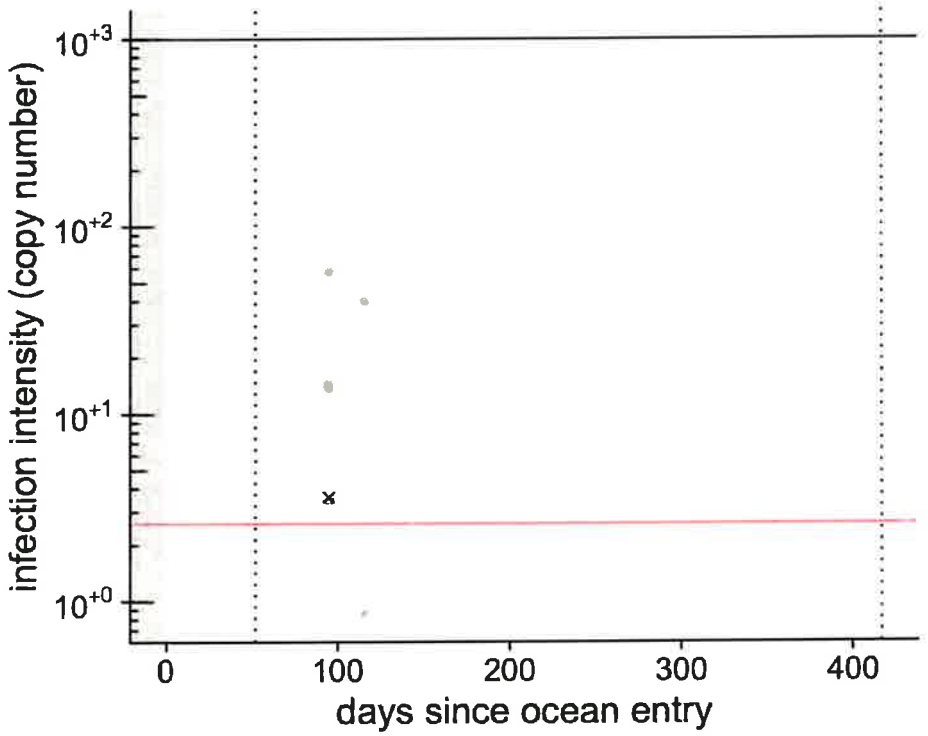


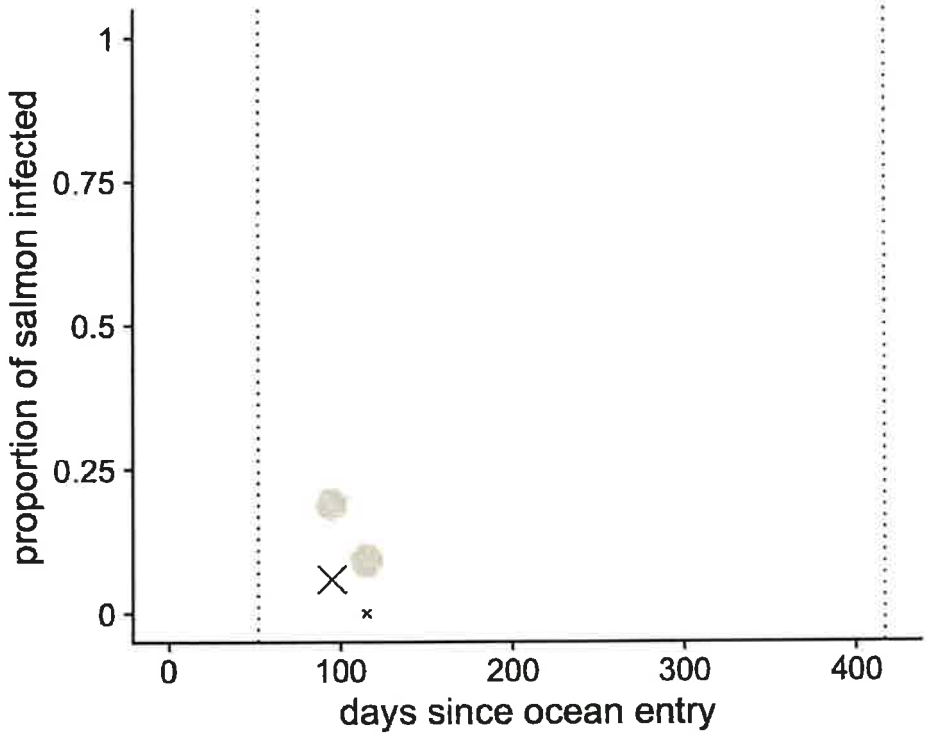


Piscine orthoreovirus

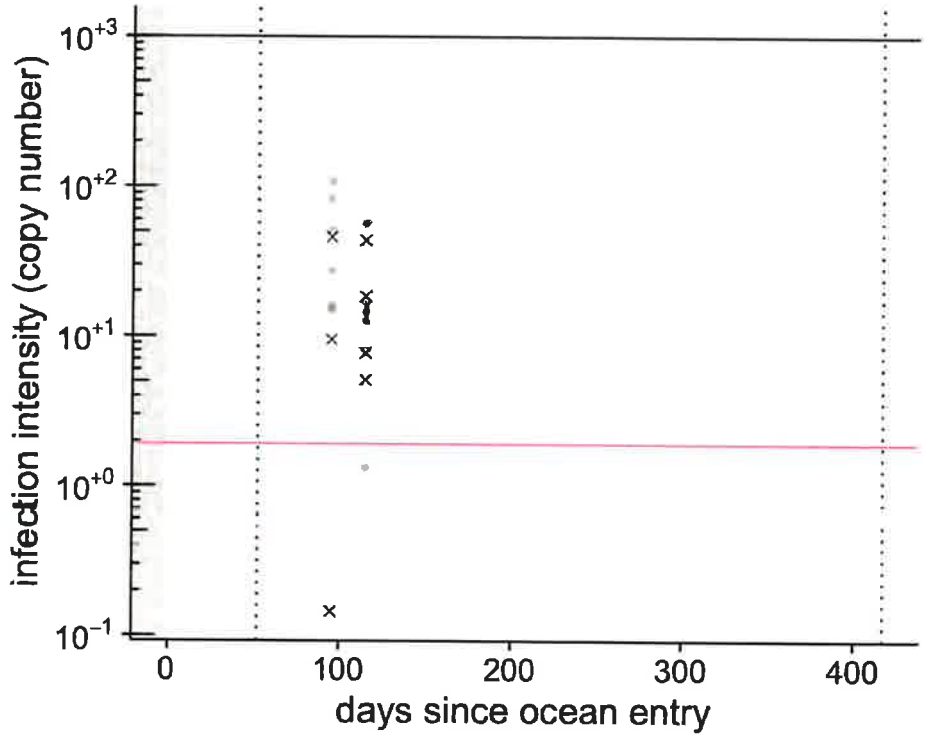


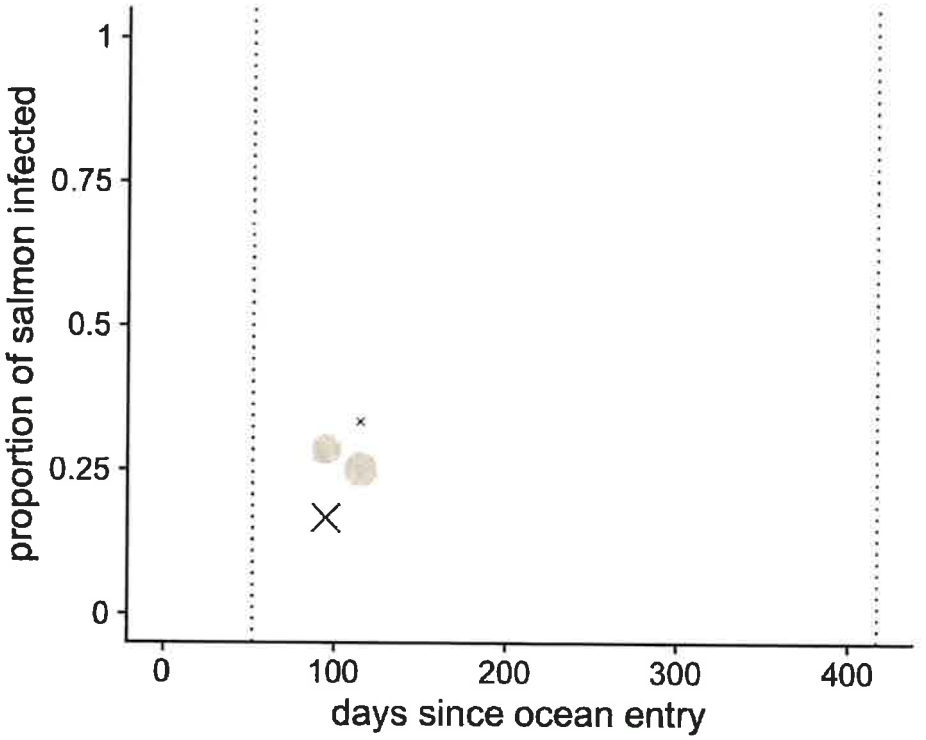
Candidatus Syngnamydia salmonis





Tenacibaculum maritimum





Clinical signs

Table 1: Clinical signs for specimens sampled on 2021-11-30

metric	N5361	N5362	N5363	N5364	N5366	N5367	N5368	N5369	N5370	N5371	N5372	N5374	N5375	N5376	N5377	N5378	N5379	N5380	N5381	N5382
General																				
Live										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	N5383	N5385	N5386	N5387	N5388	N5389	N5390	N5391	N5392	N5393	N5394
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	N5361	N5362	N5363	N5364	N5382
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	N5361	N5362	N5363	N5364	N5382
Necrosis Artefacts	2	3	3	1	

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* Syngnamydia 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 to the pattern of lesions detected, respective clinical signs and molecular data, are conducible 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 Syngnamydia 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 December 1, 2021

Dr. Emiliano Di Cicco

September 23, 2022

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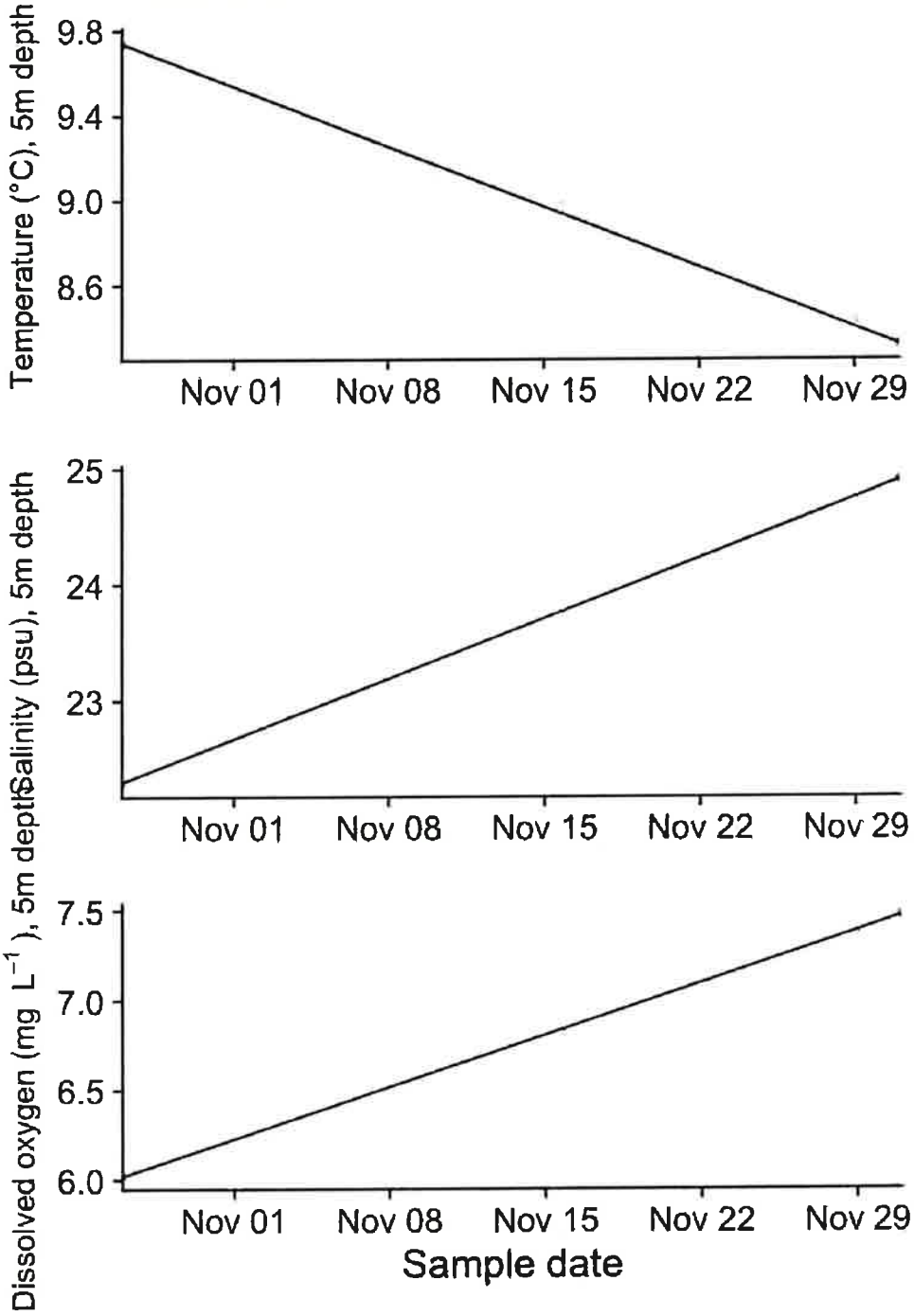
Executive summary

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.

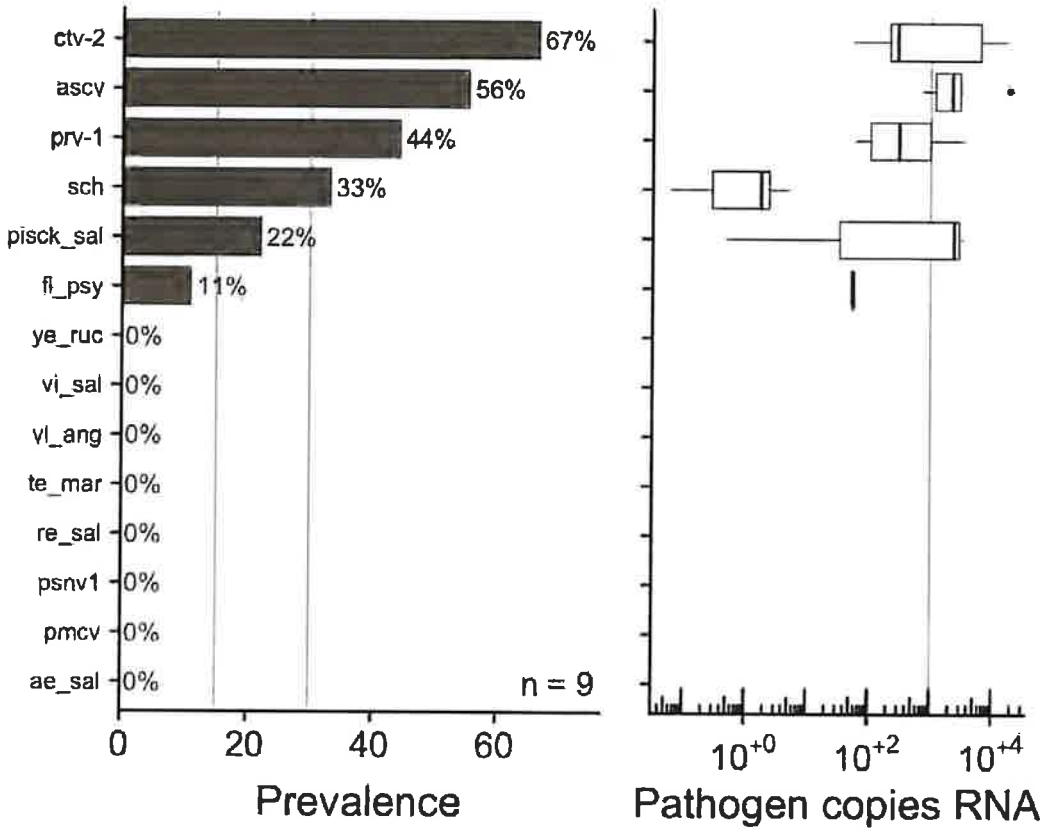
Histology samples have been sent to Wax-It Histo Ltd. to process and prepare slides, which have been read and scored by Dr. Di Cicco. A digital copy of each slide is available to the Company.

Environmental data

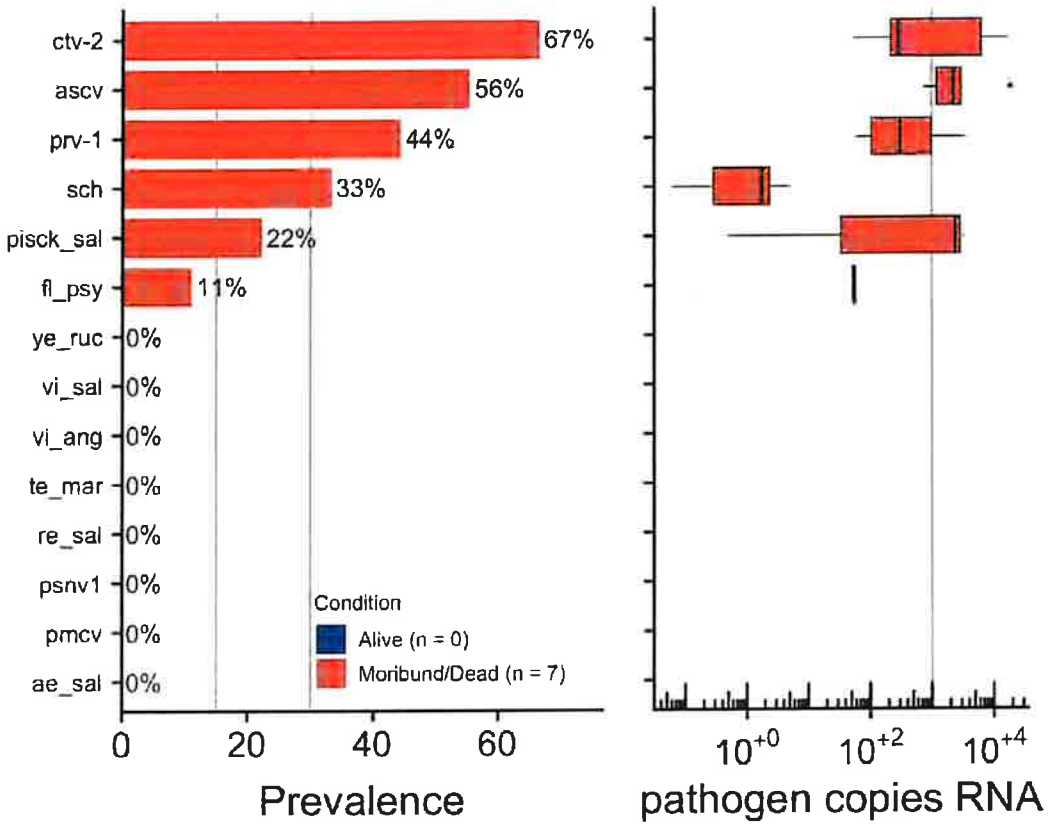


Water temperature (°C), salinity (ppm), and dissolved oxygen (mg/L) at a 5m depth. Certain sampling dates have no recorded environmental data, resulting in gaps in the plots.

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

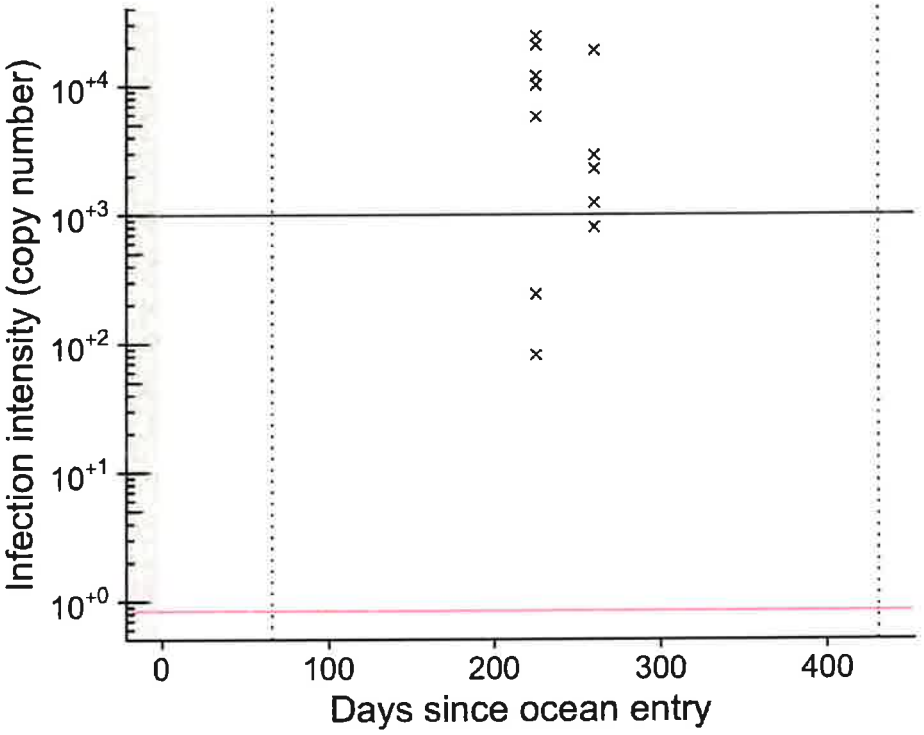
The following plots show individual infectious agent trends across all farm sites. In cases where sample size is sufficient, curves from a generalised additive model are included in the plot.

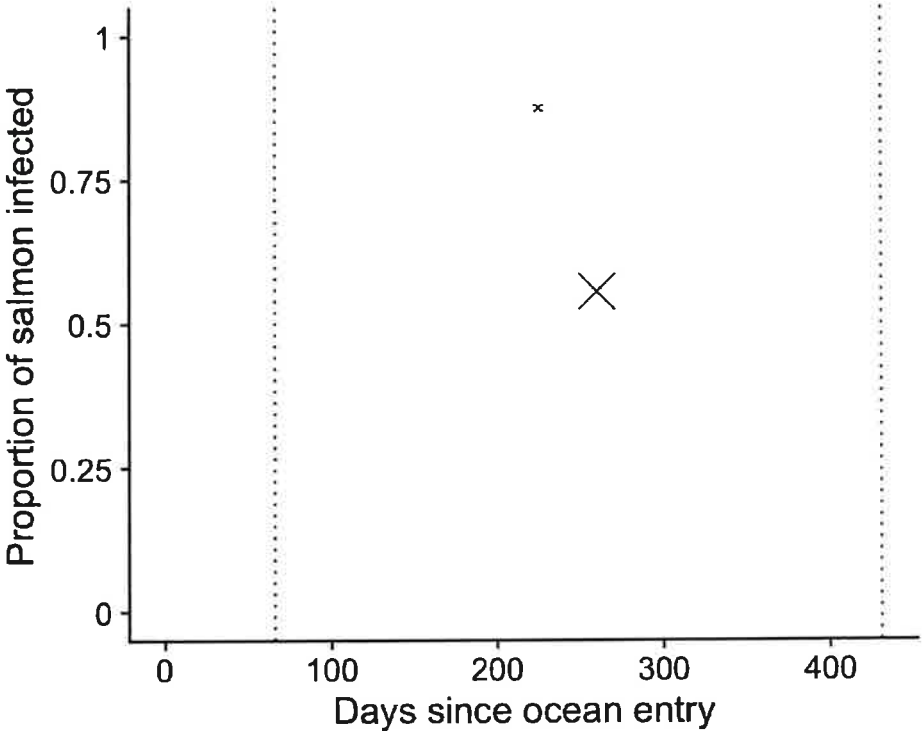
Grey circles represent live fish, and black X's represent dead/dying fish. Curves indicate mean predictions from a generalised additive model; blue and red correspond to live and dead/dying fish, respectively (shaded areas show 95% confidence regions). Left-hand grey region indicates freshwater hatchery 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.

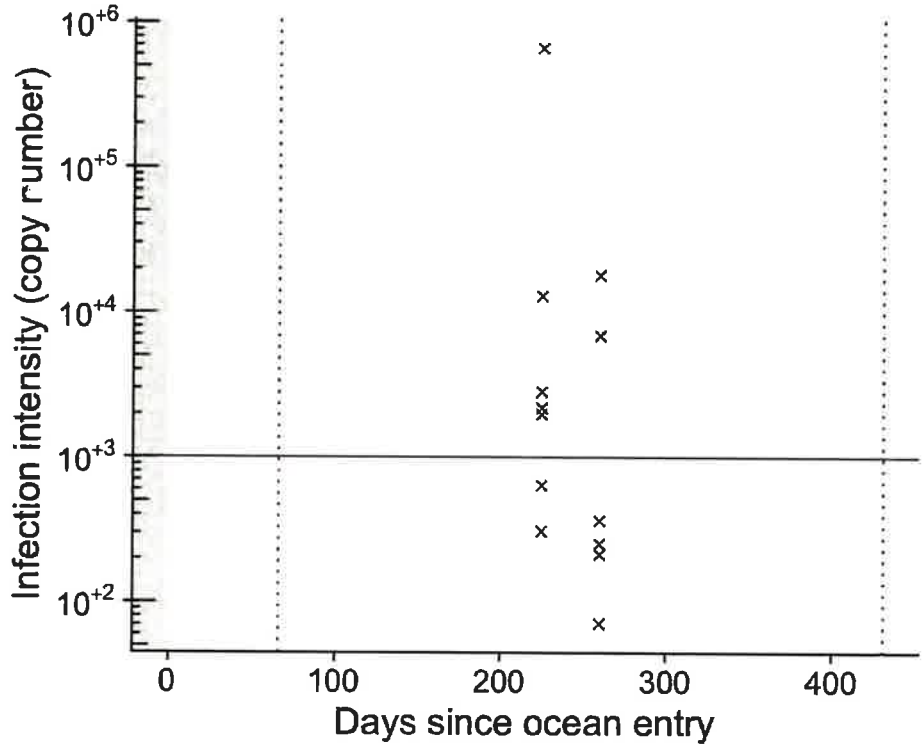
For proportion plots, grey circles show prevalence in live fish on each sampling date, and black X's show prevalence in dead/dying fish (symbol areas proportional to sample sizes).

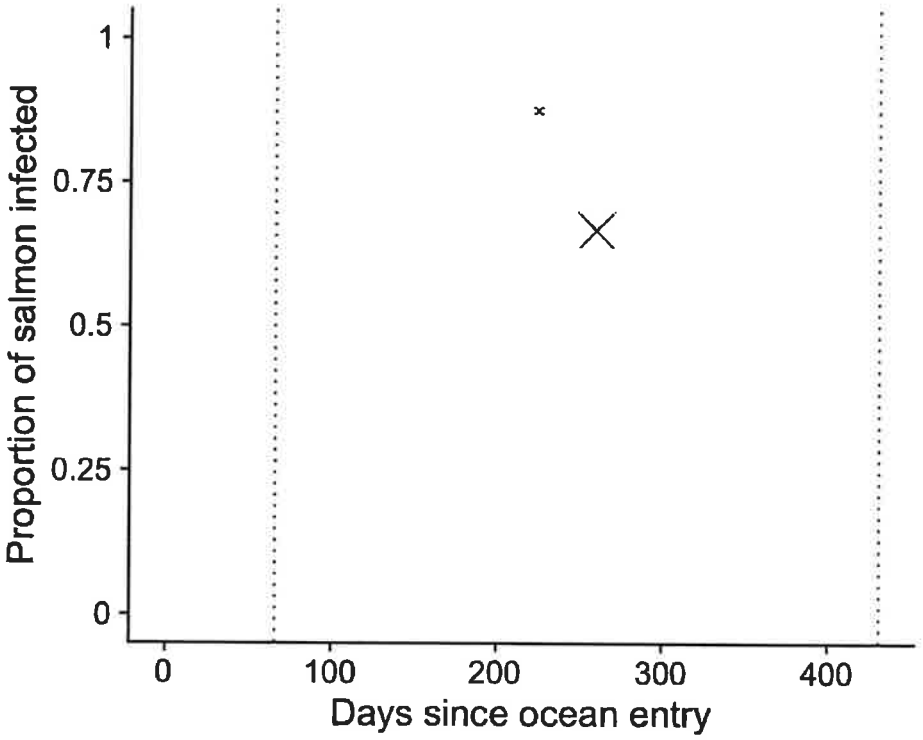
Atlantic salmon calicivirus



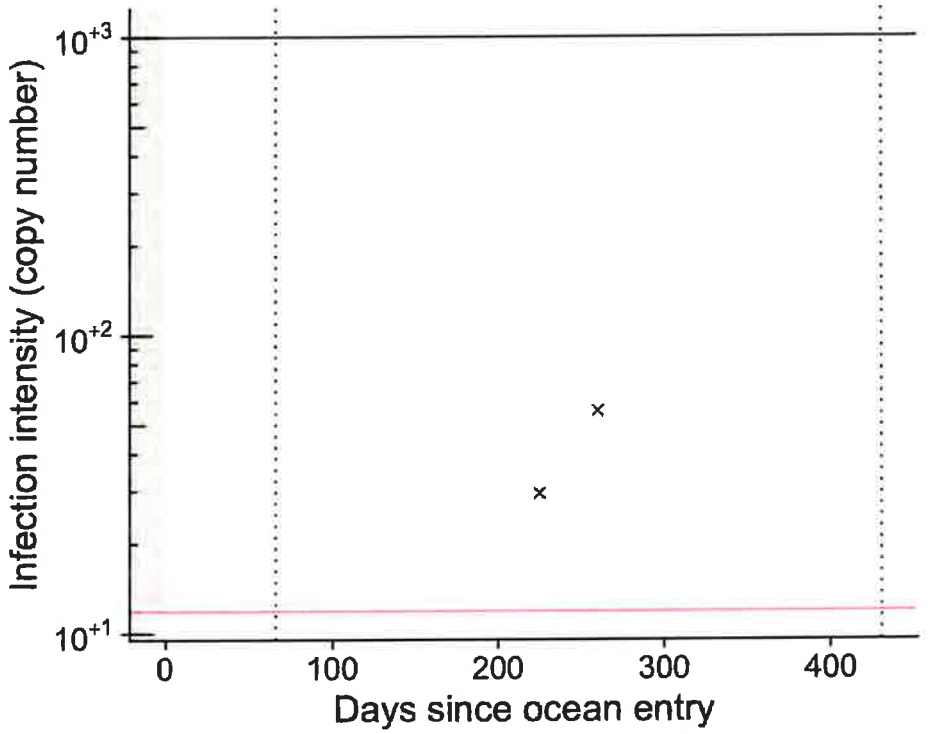


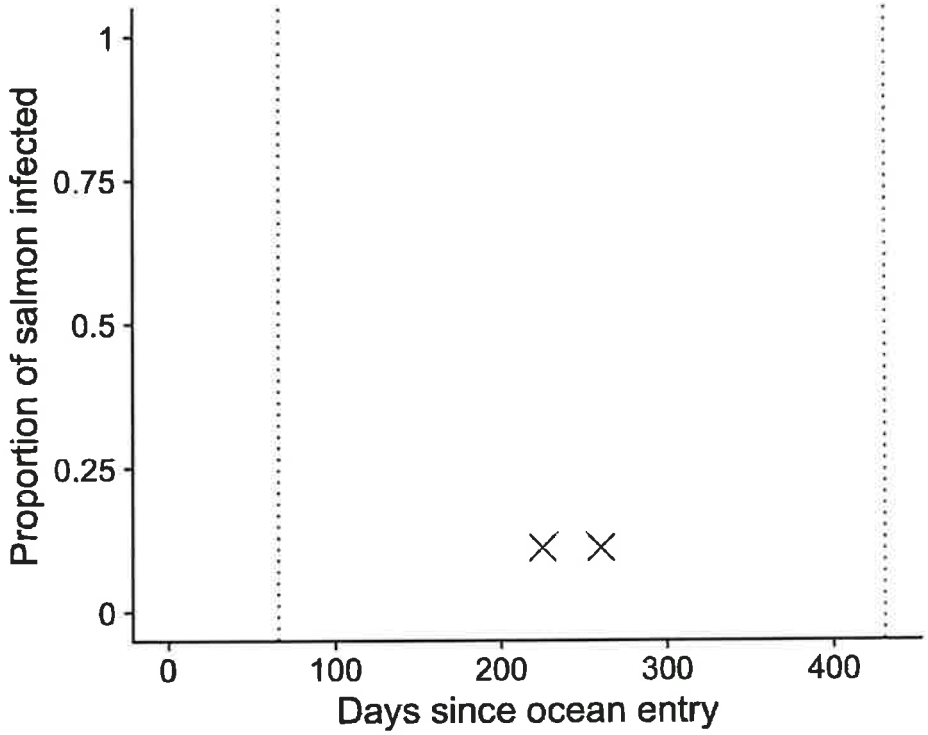
Cutthroat trout virus-2



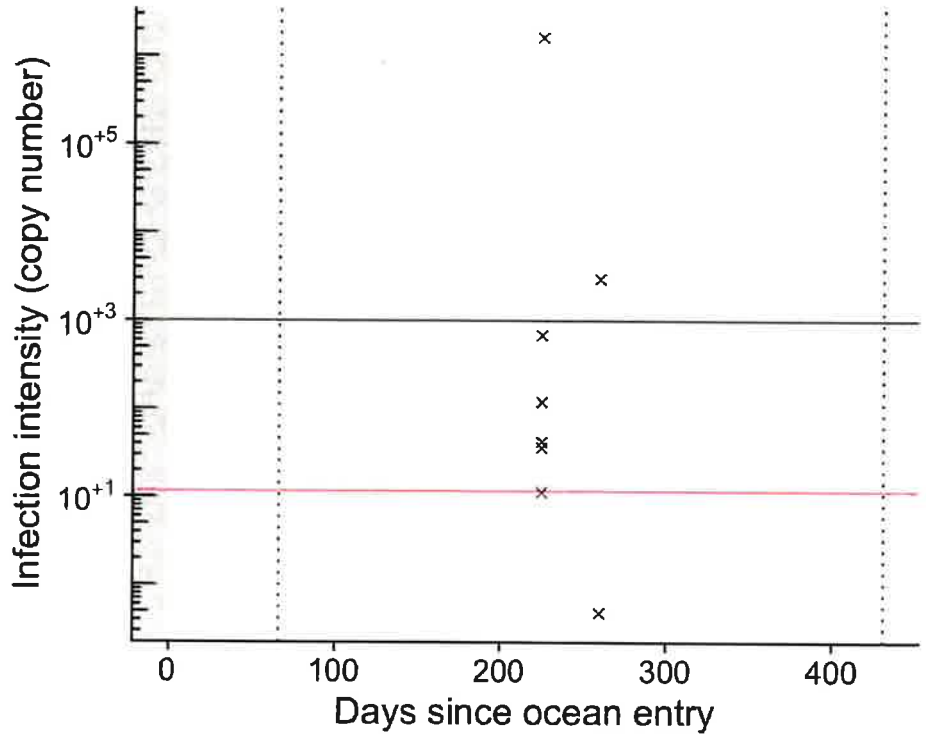


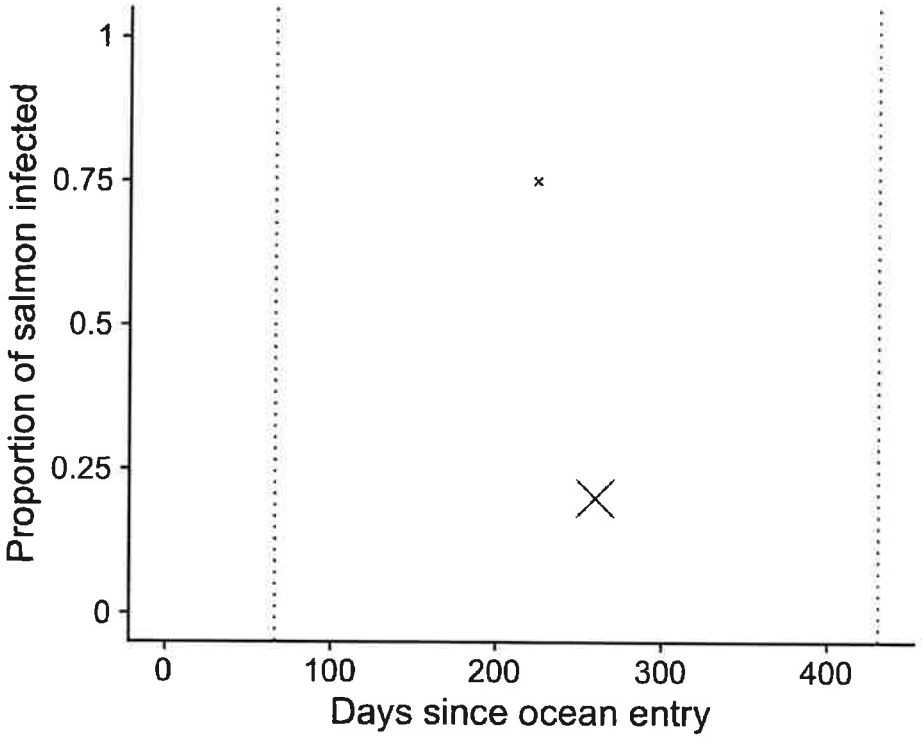
Flavobacterium psychrophilum



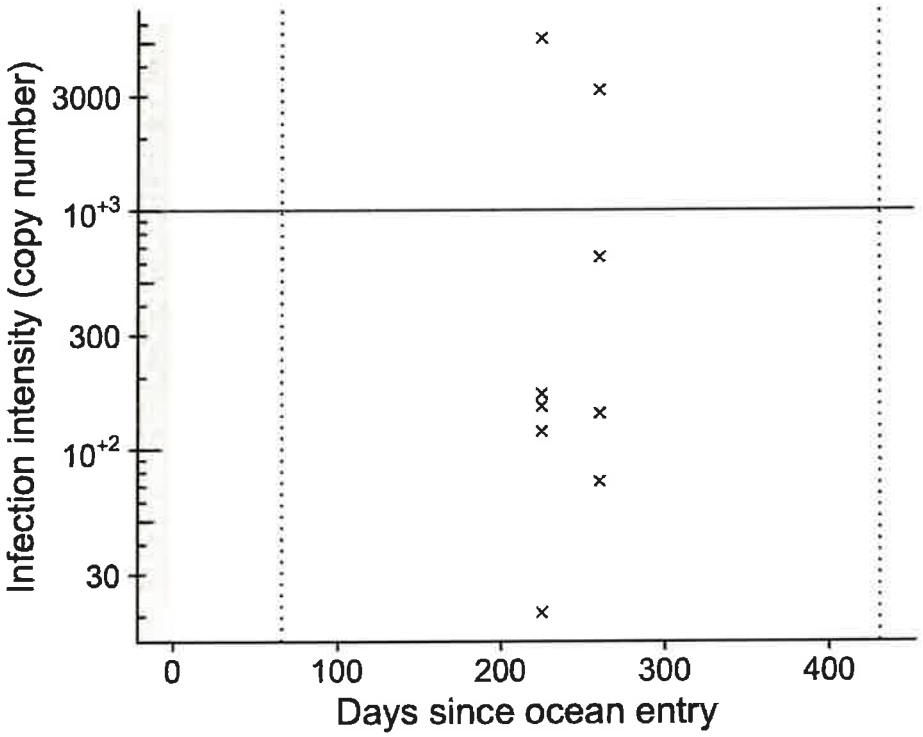


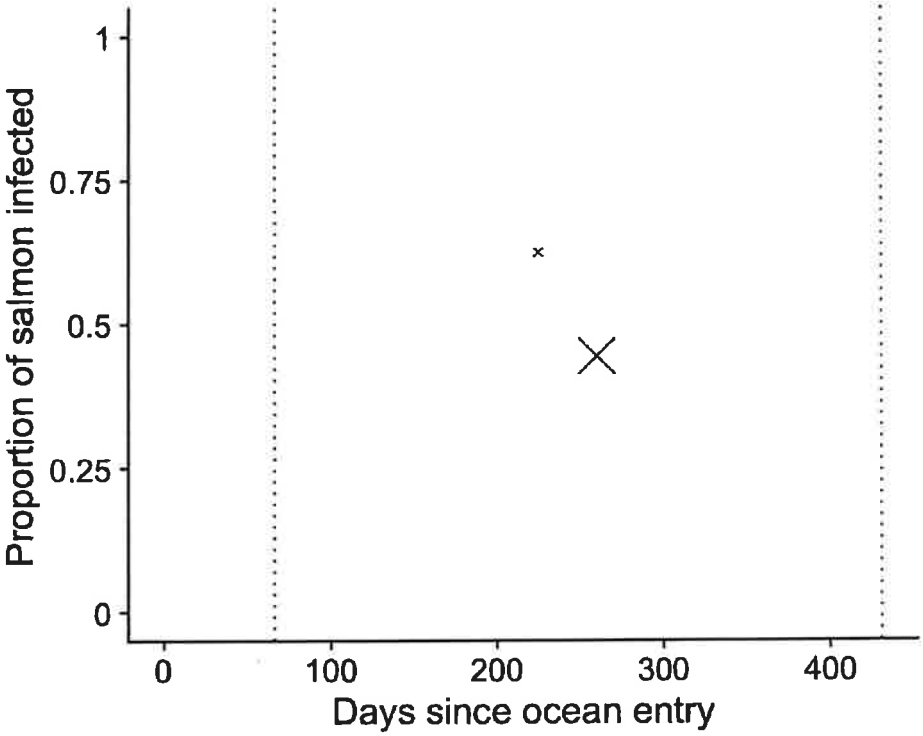
Piscirickettsia salmonis



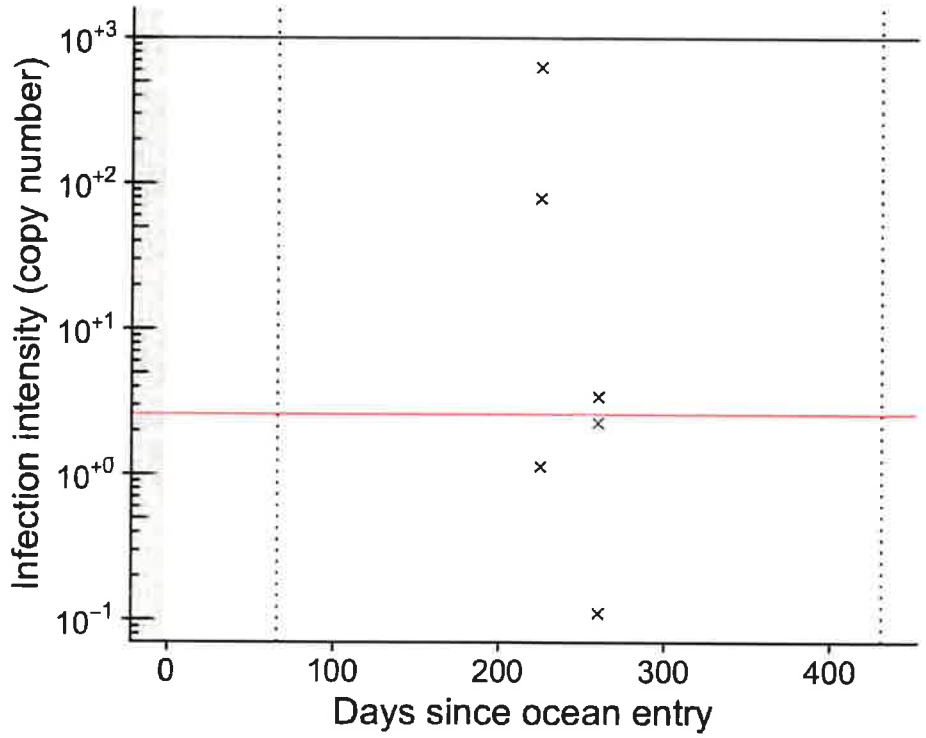


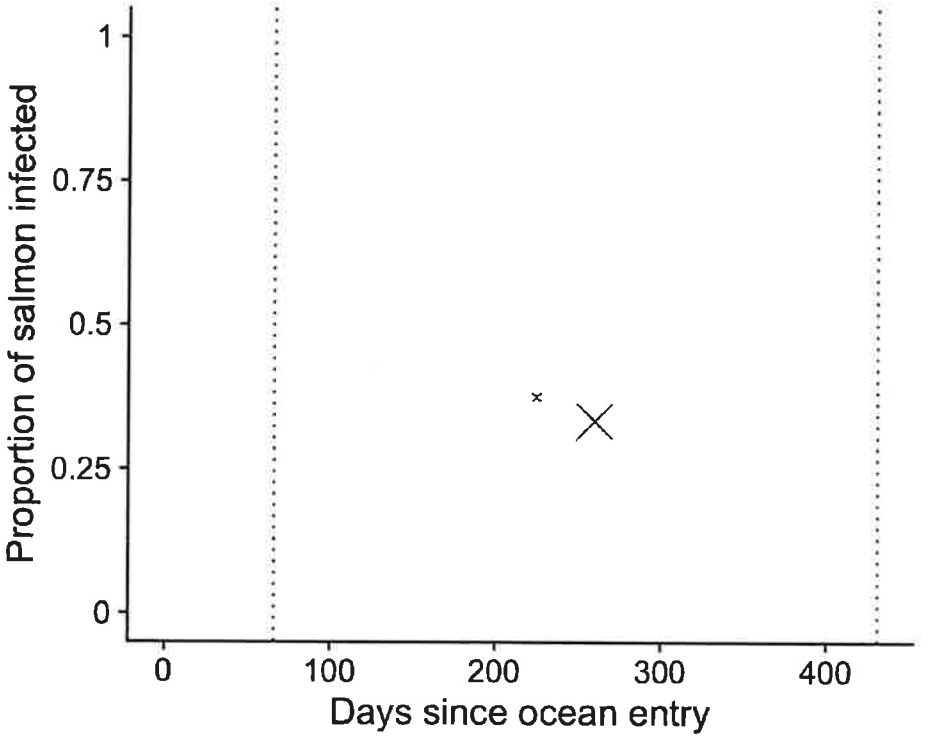
Piscine orthoreovirus





Candidatus Syngnamydia 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
Exopthalmia					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	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 exanimated 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 Syngnamydia 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 Syngnamydia 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.

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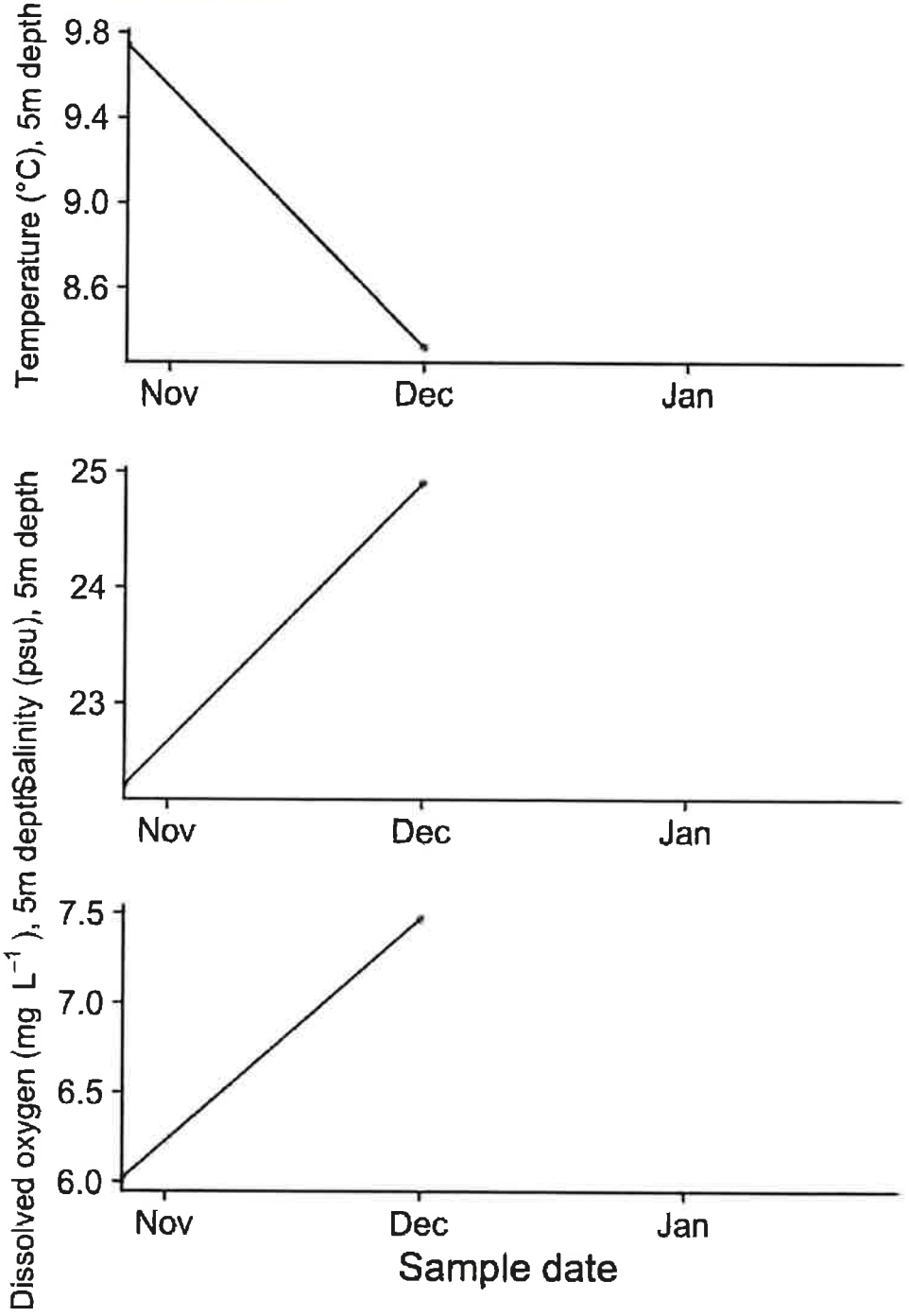
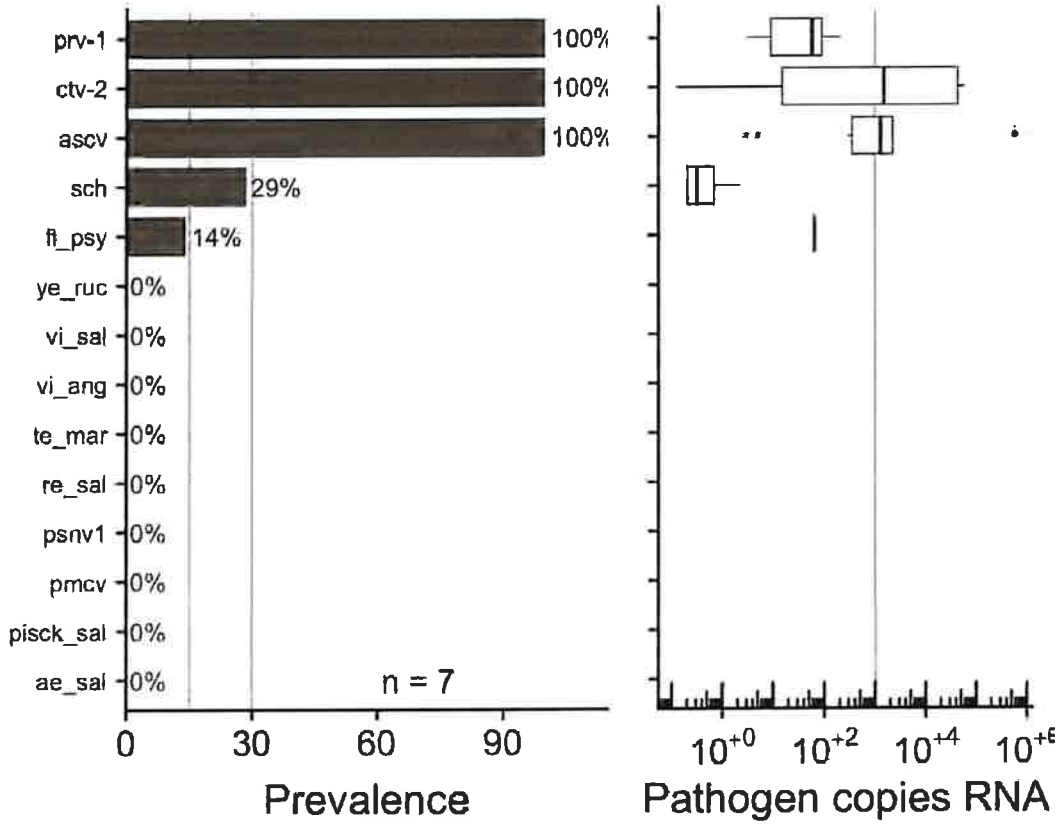
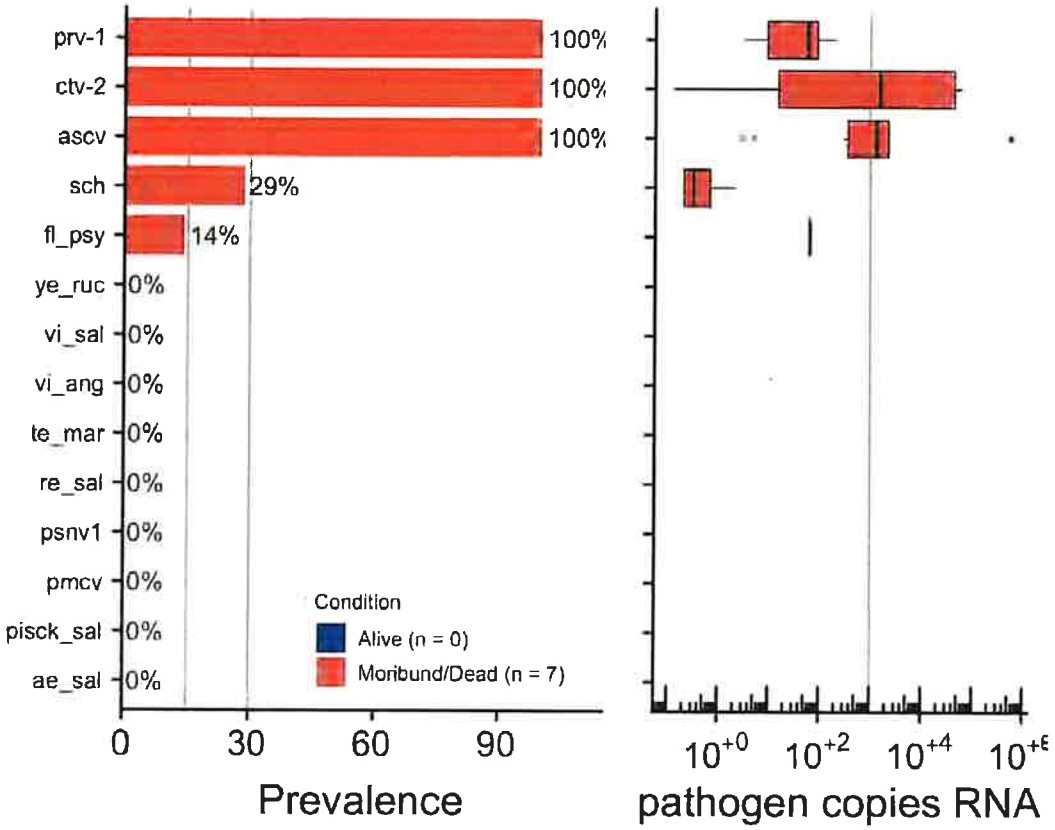


Water temperature (°C), salinity (ppm), and dissolved oxygen (mg/L) at a 5m depth. Certain sampling dates have no recorded environmental data, resulting in gaps in the plots.

Overall infectious agent prevalence



Infectious agent prevalence in samples collected on 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.

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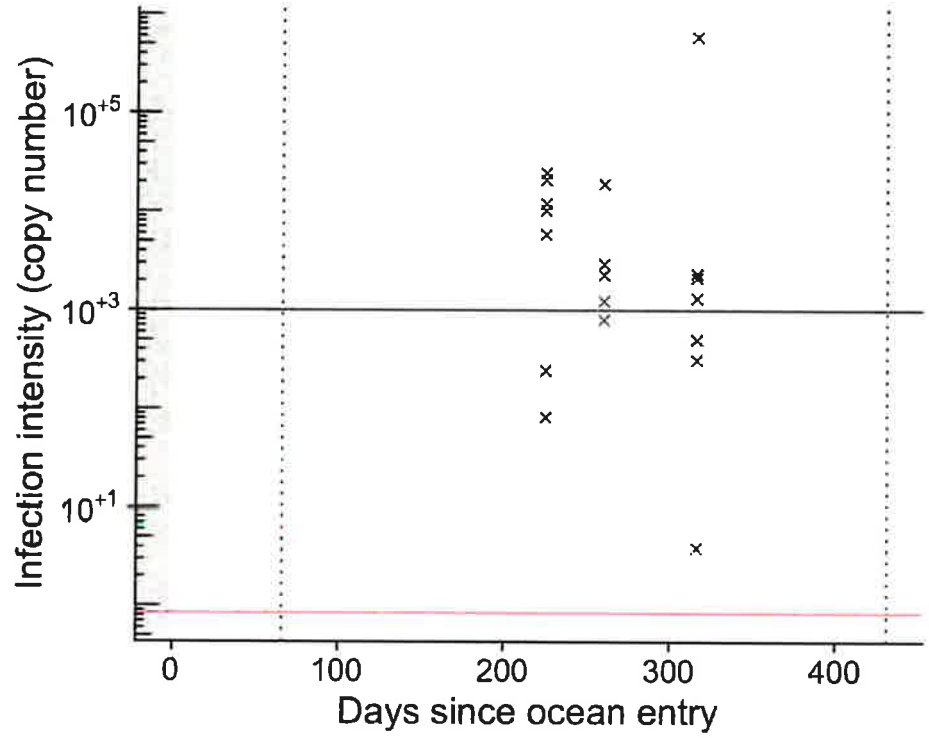
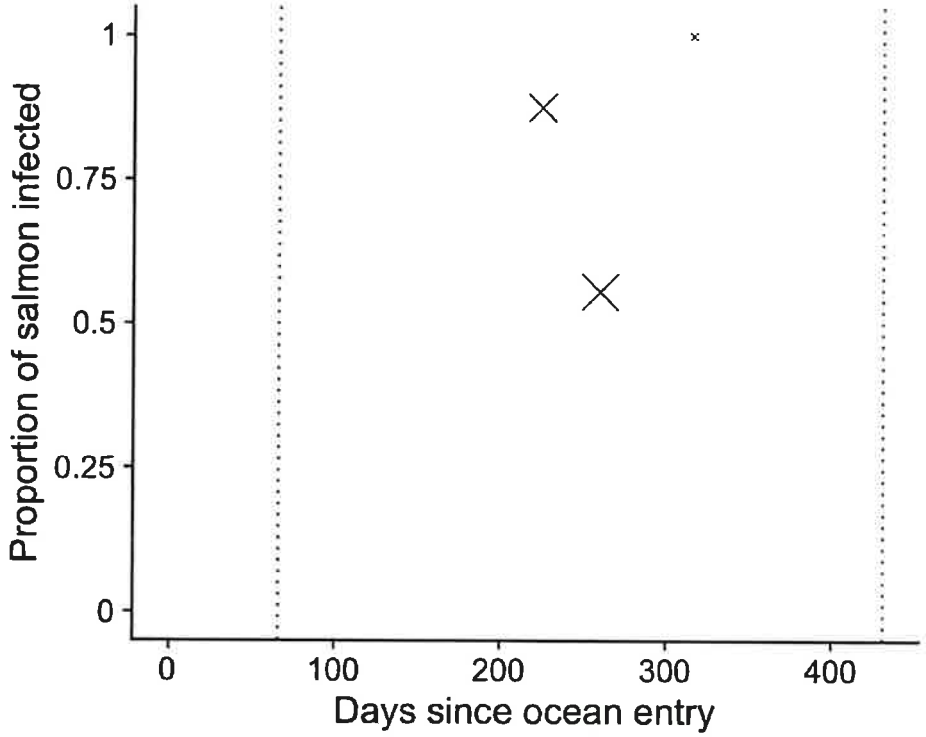
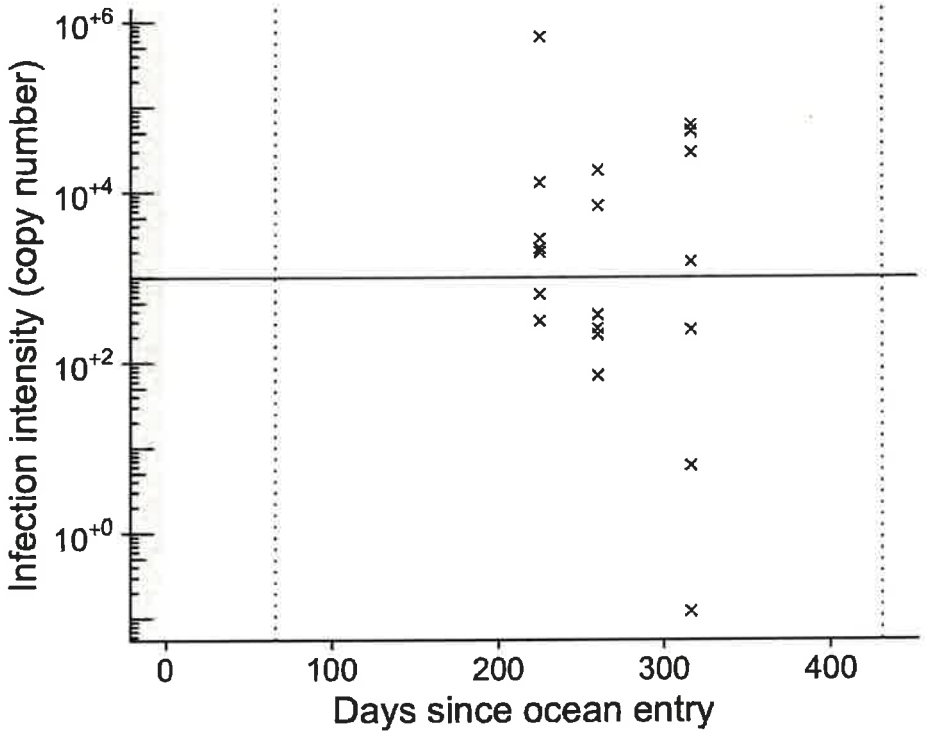
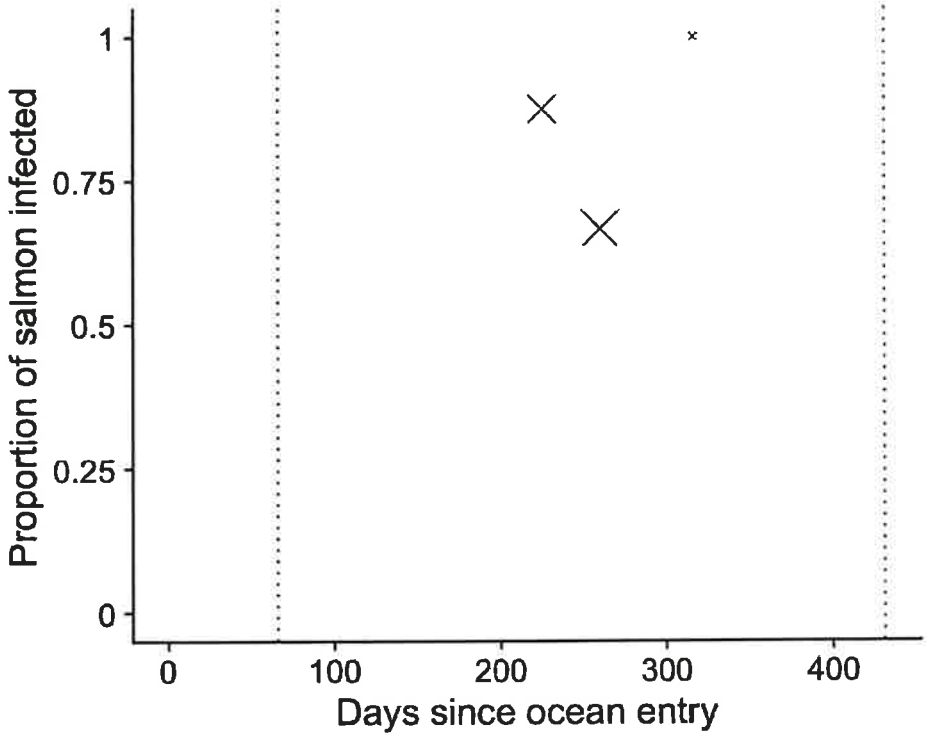
Atlantic salmon calicivirus



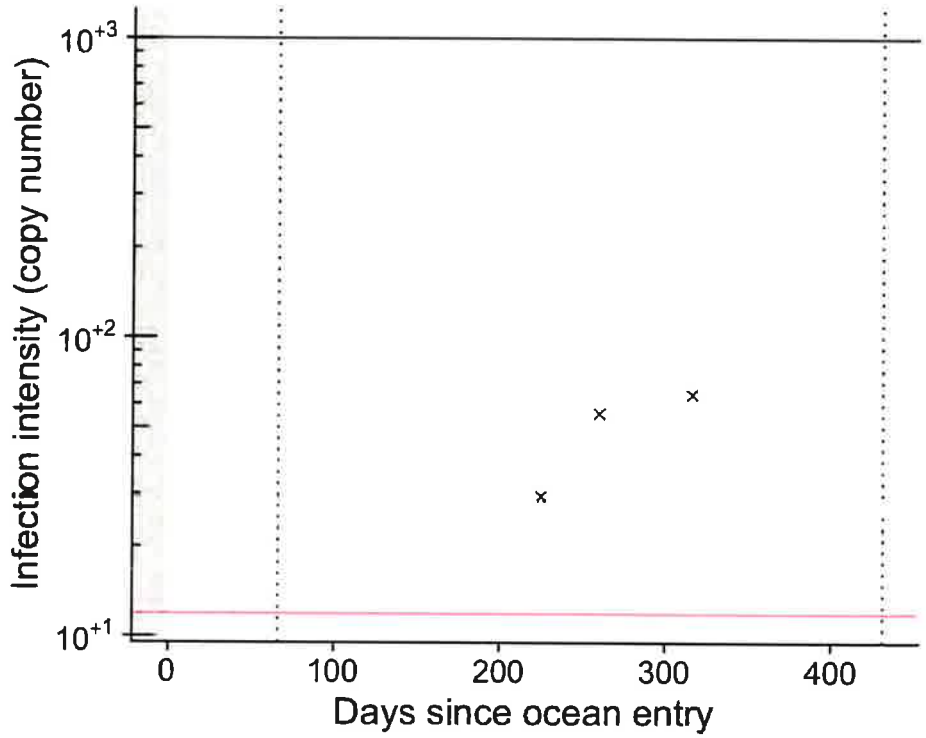


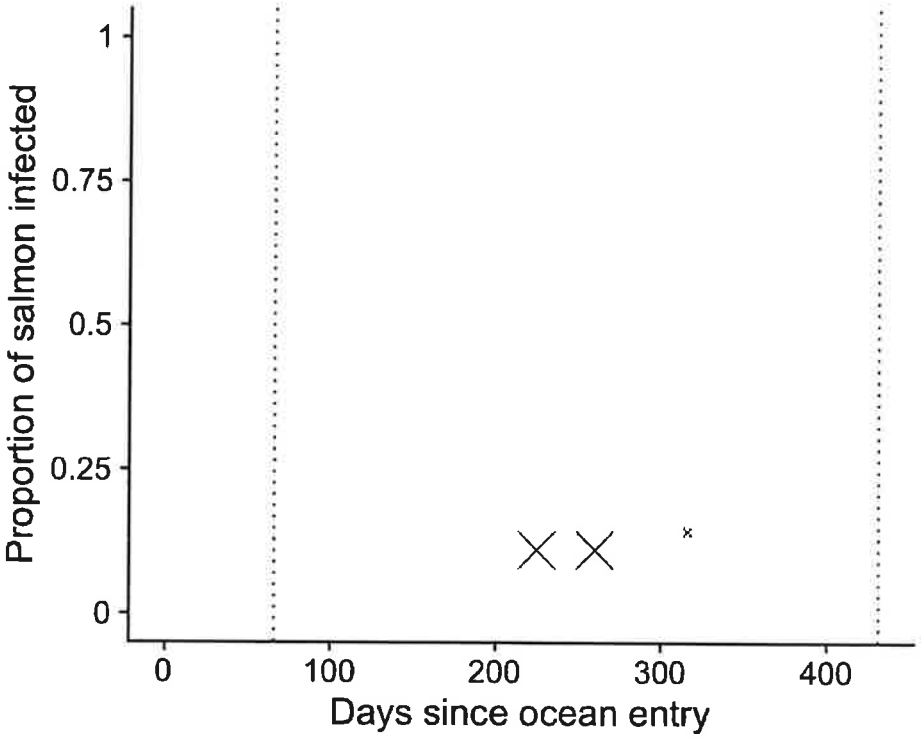
Cutthroat trout virus-2



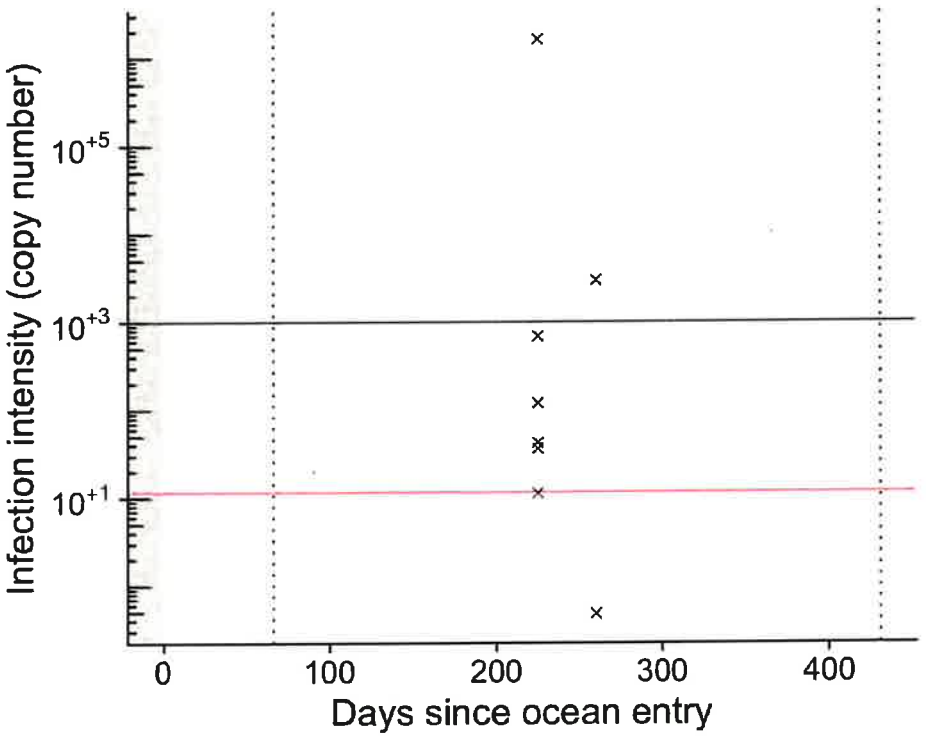


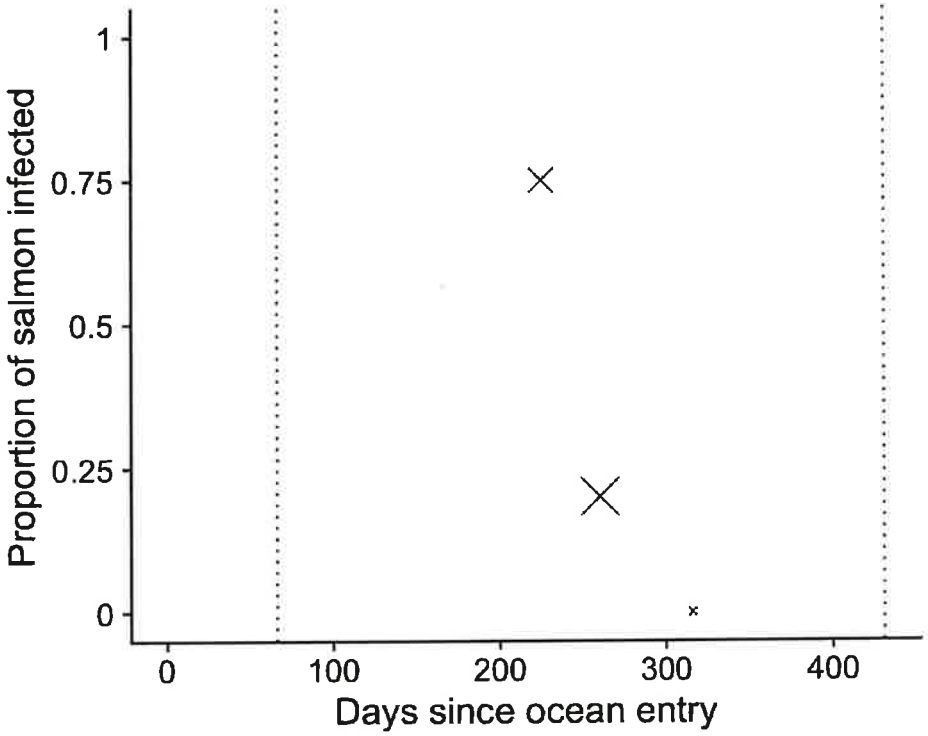
Flavobacterium psychrophilum



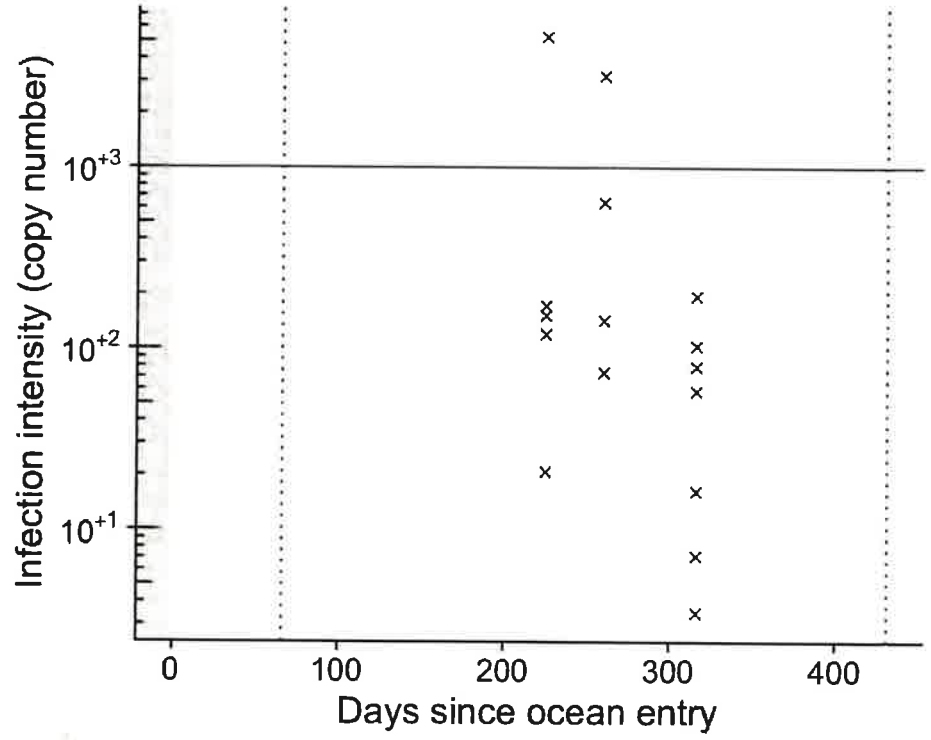


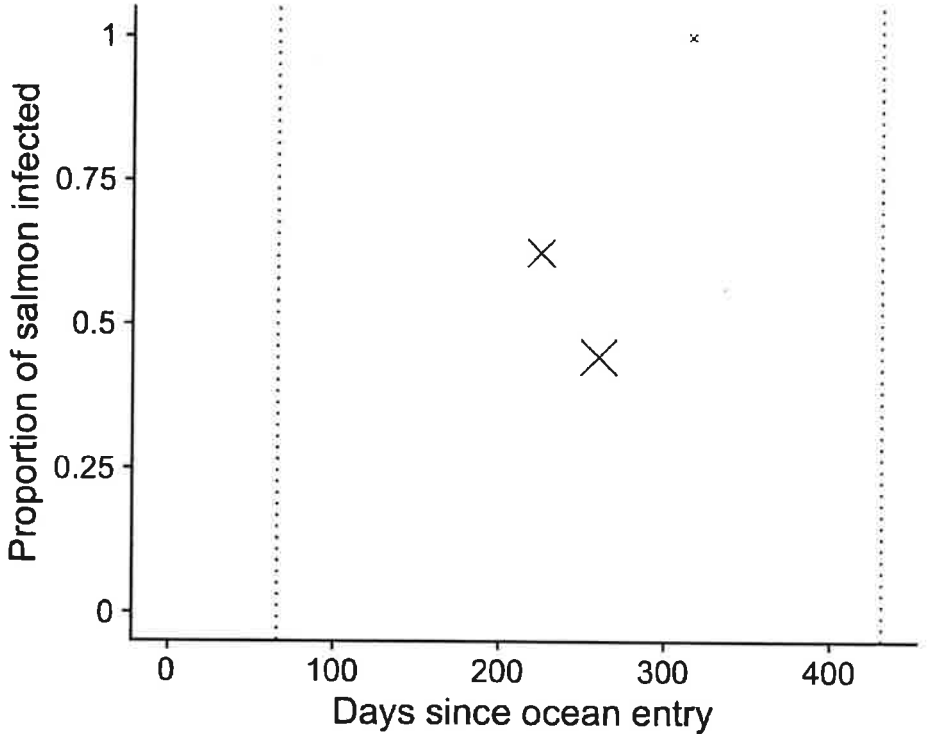
Piscirickettsia salmonis



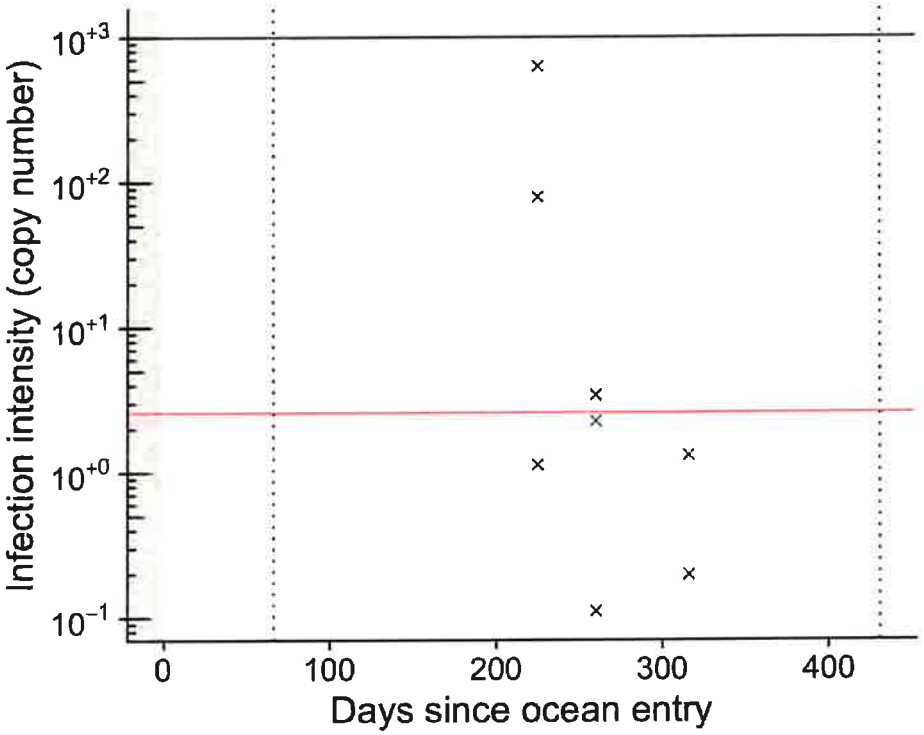


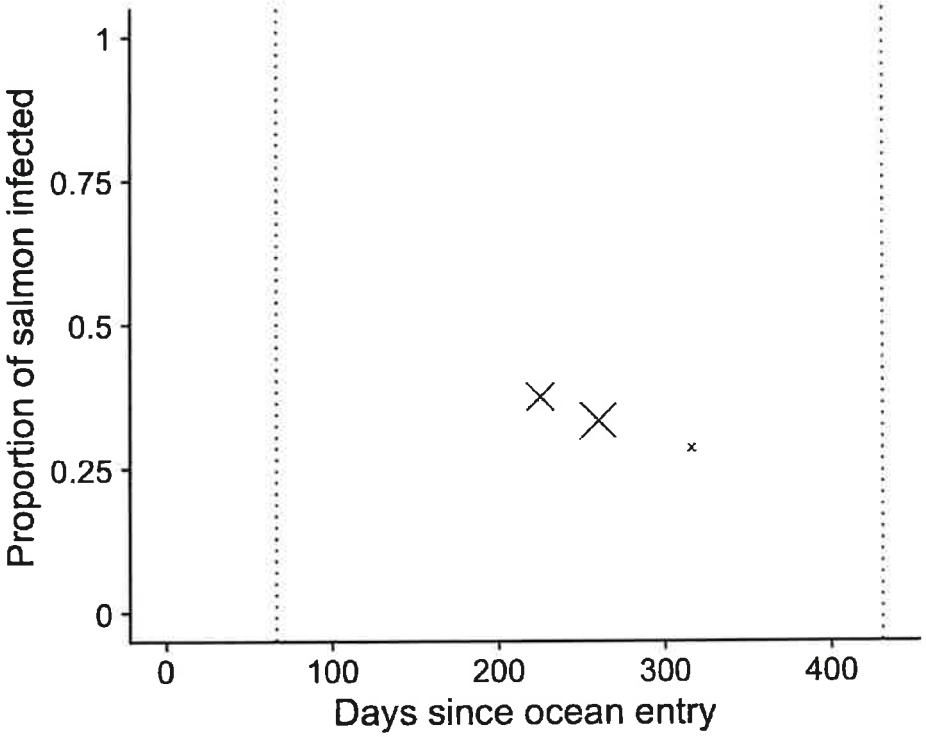
Piscine orthoreovirus





Candidatus Syngnamydia 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	N5721	N5722	N5723	N5724	N5725	N5726	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), Myocardionecrosis (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 exanimated 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* Syngnamydia 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 Syngnamydia 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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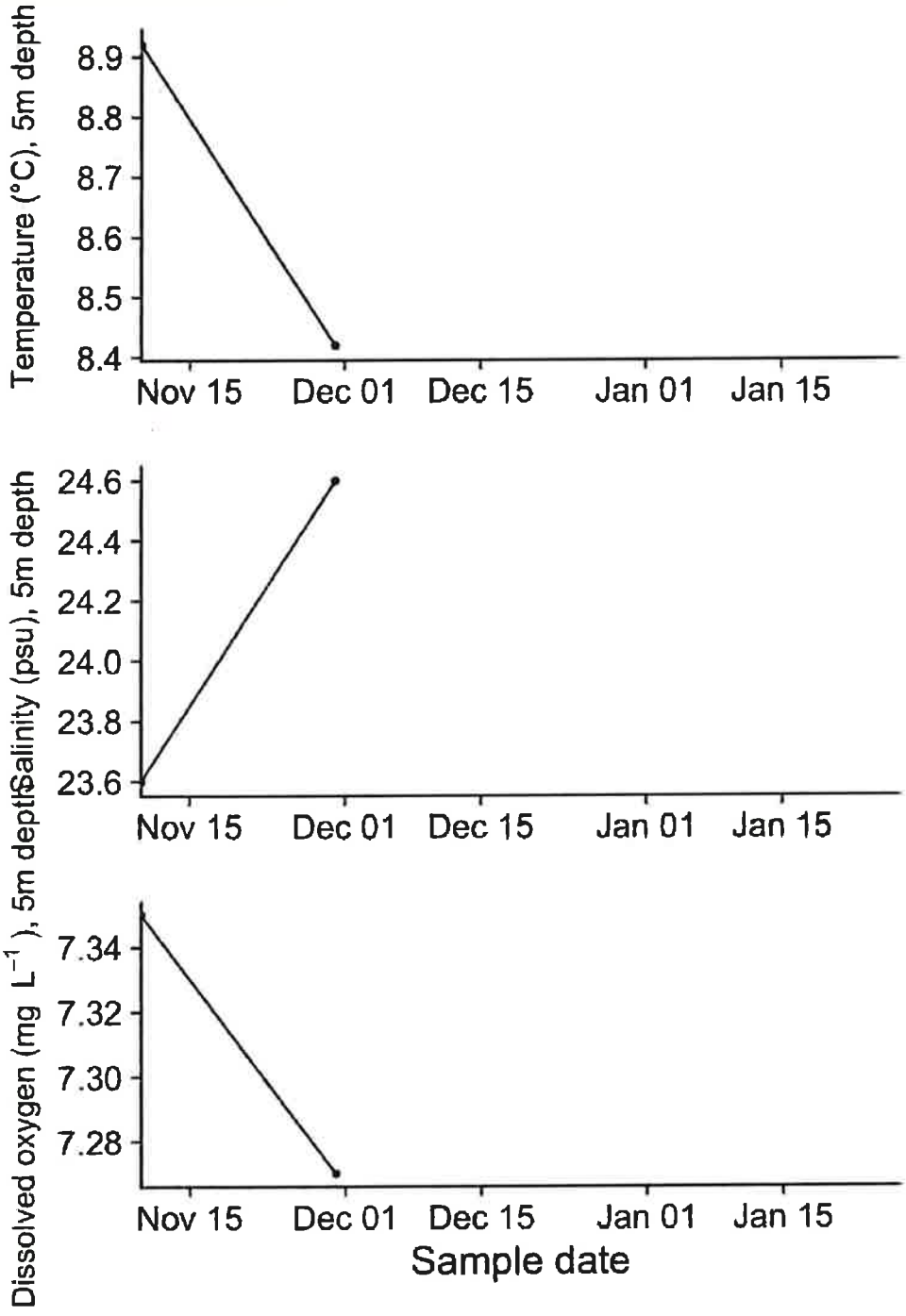
Executive summary

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.

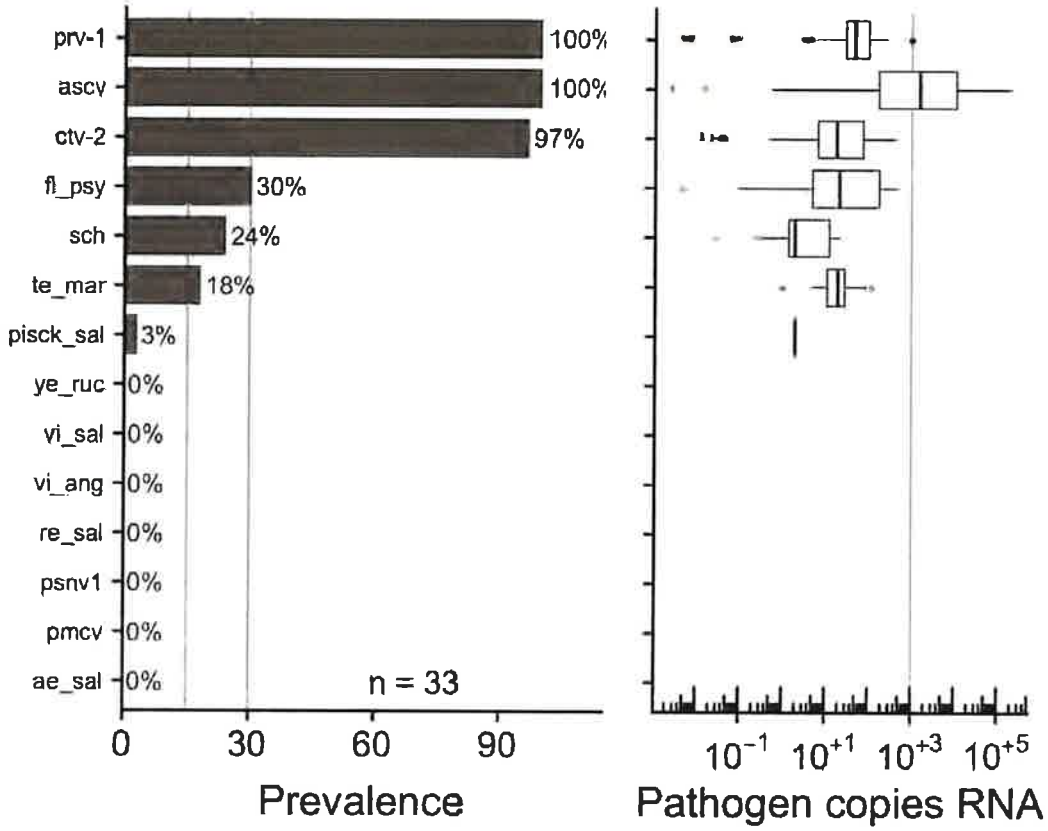
Histology samples have been sent to Wax-It Histo Ltd. to process and prepare slides, which have been read and scored by Dr. Di Cicco. A digital copy of each slide is available to the Company.

Environmental data



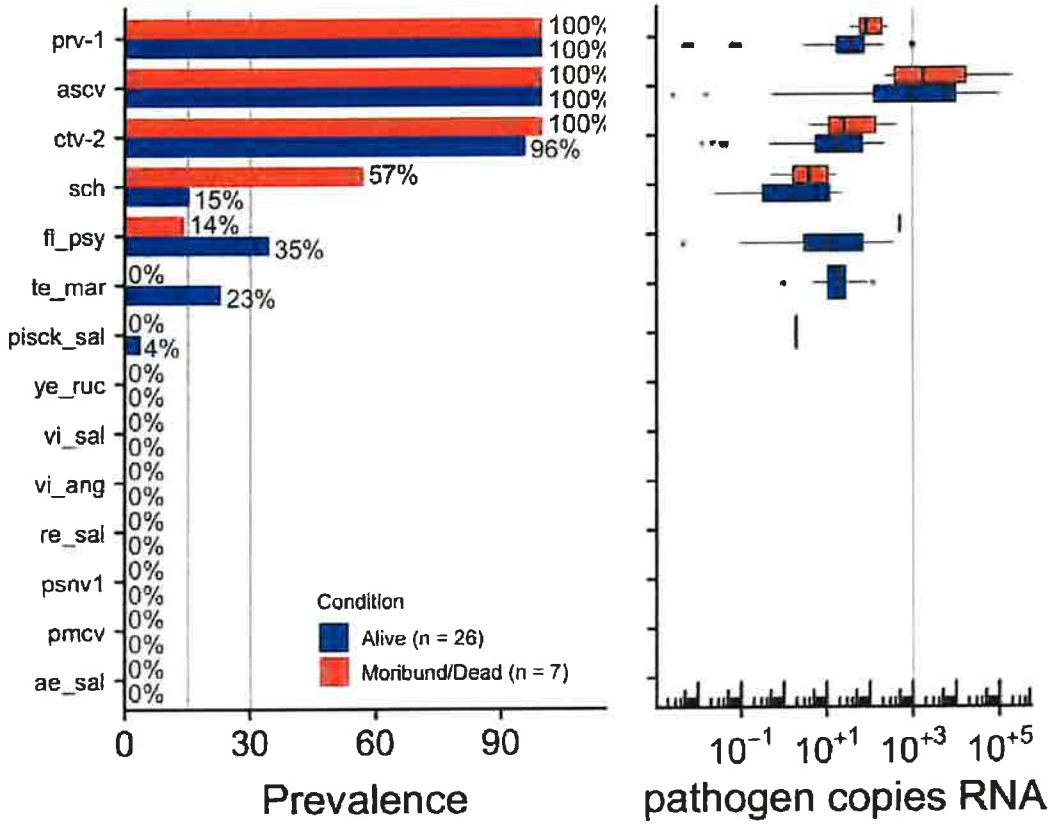
Water temperature (°C), salinity (ppm), and dissolved oxygen (mg/L) at a 5m depth. Certain sampling dates have no recorded environmental data, resulting in gaps in the plots.

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

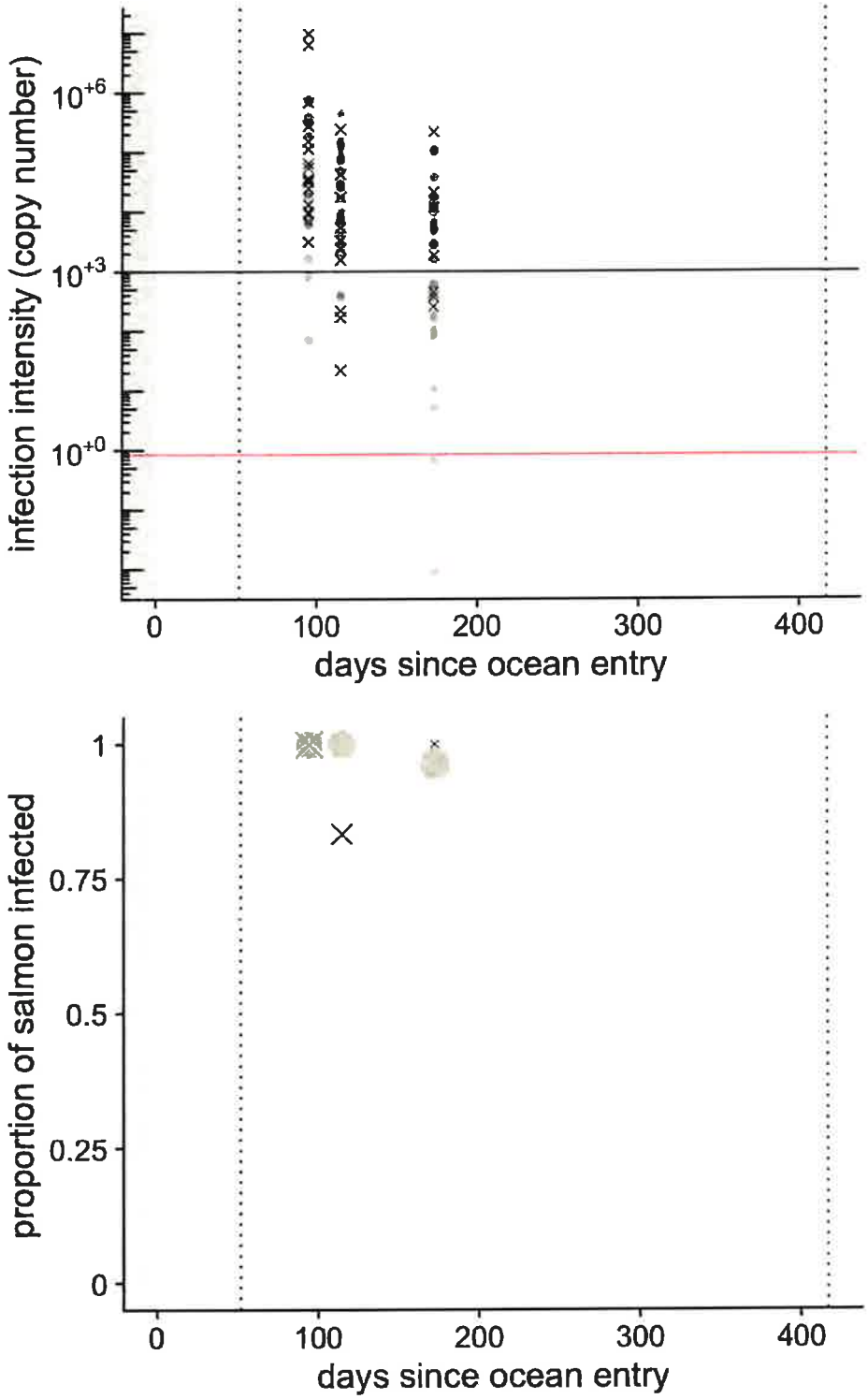
The following plots show individual infectious agent trends across all farm sites.

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.

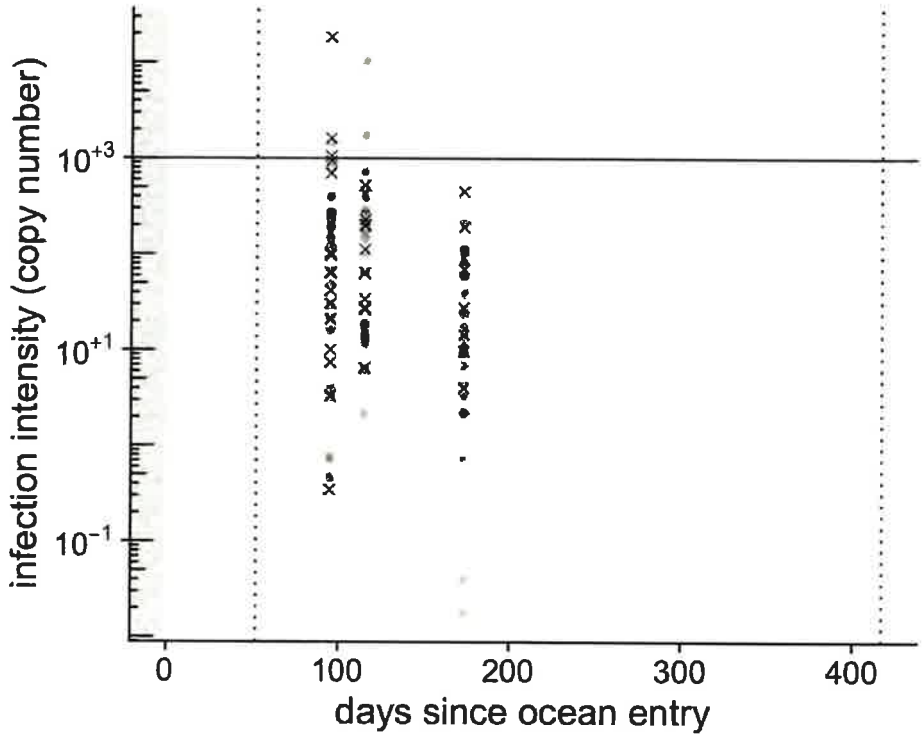
For infection intensity plots, horizontal red line indicates limit of detection (yielding ~90% true positive rate) for respective qPCR assay run in duplicate, while the horizontal black line indicates 1000 copies. Note log scale.

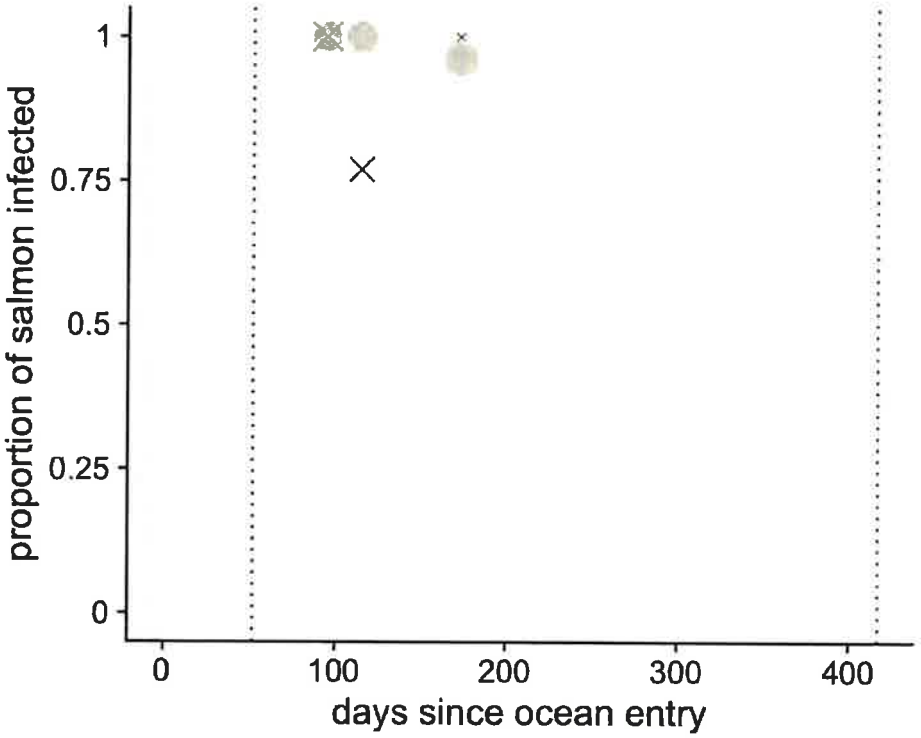
For proportion plots, grey circles show prevalence in live fish on each sampling date, and black X's show prevalence in dead/dying fish (symbol areas proportional to sample sizes).

Atlantic salmon calicivirus

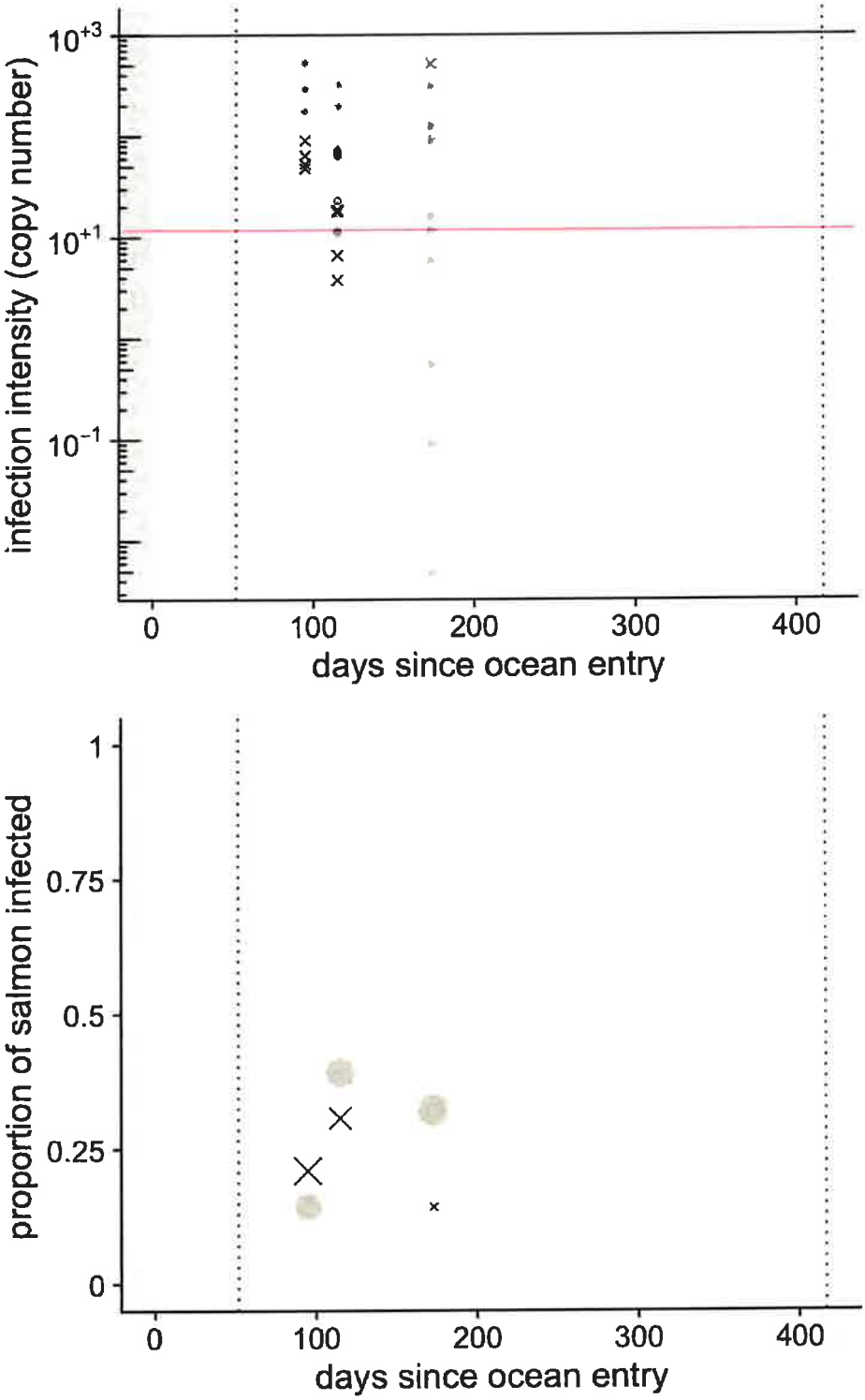


Cutthroat trout virus-2

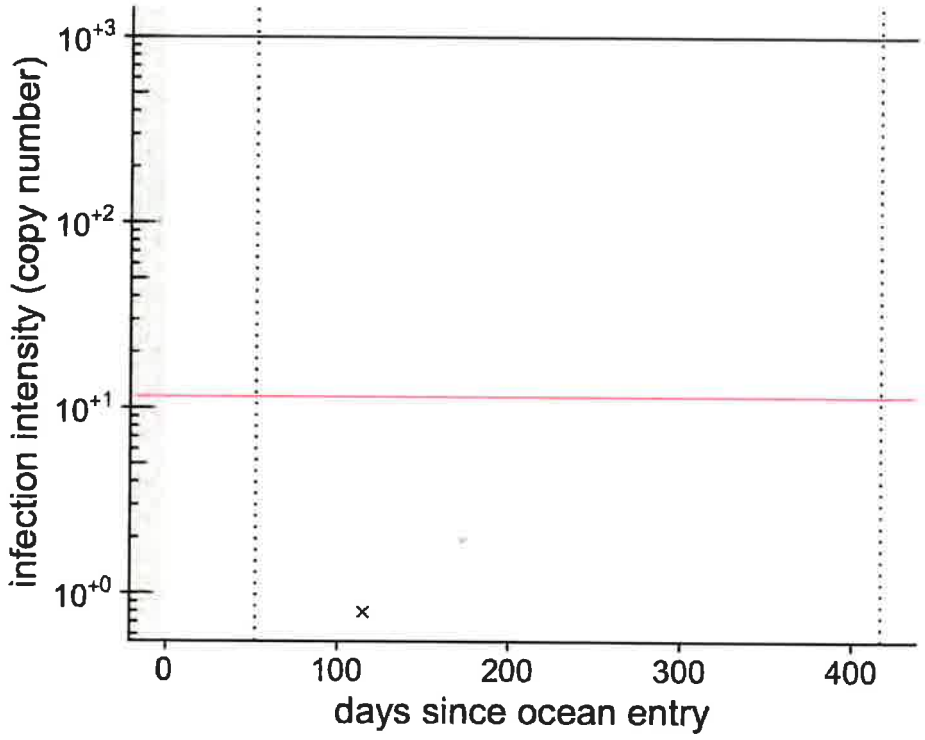


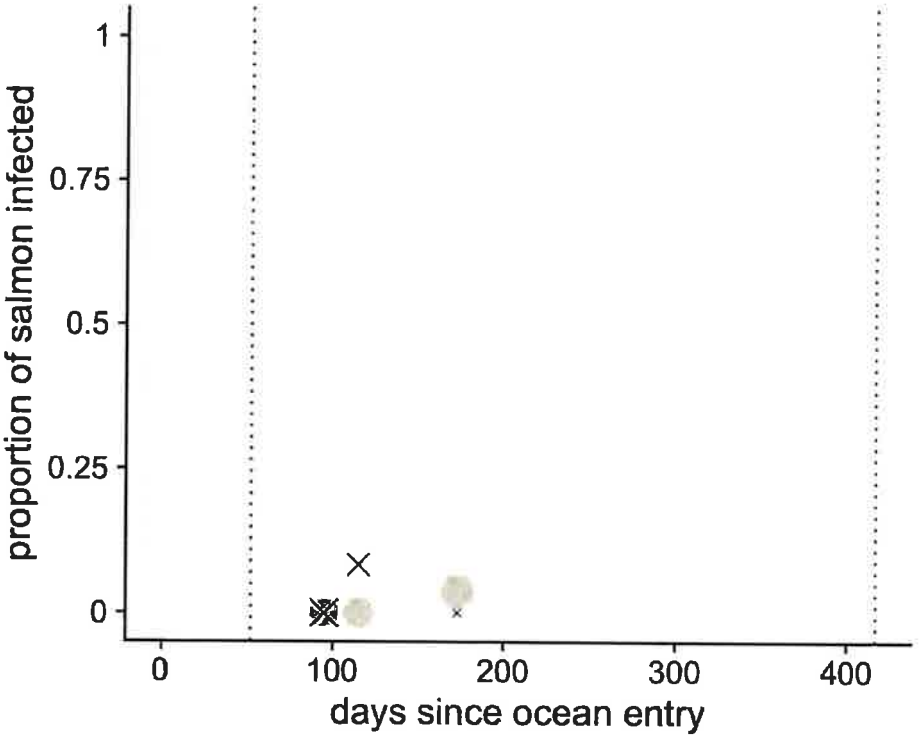


Flavobacterium psychrophilum

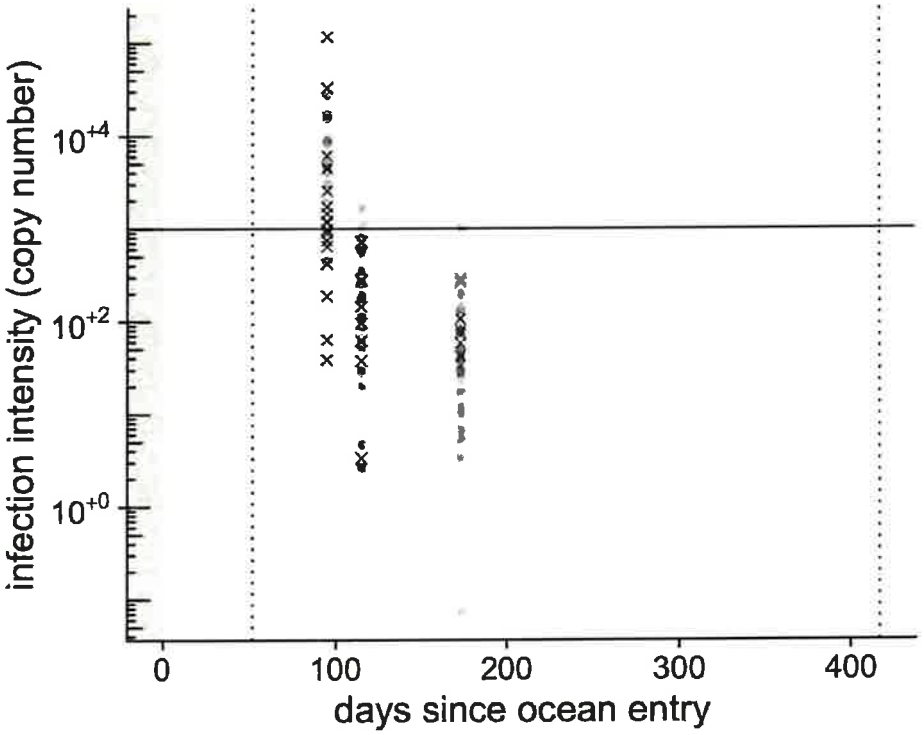


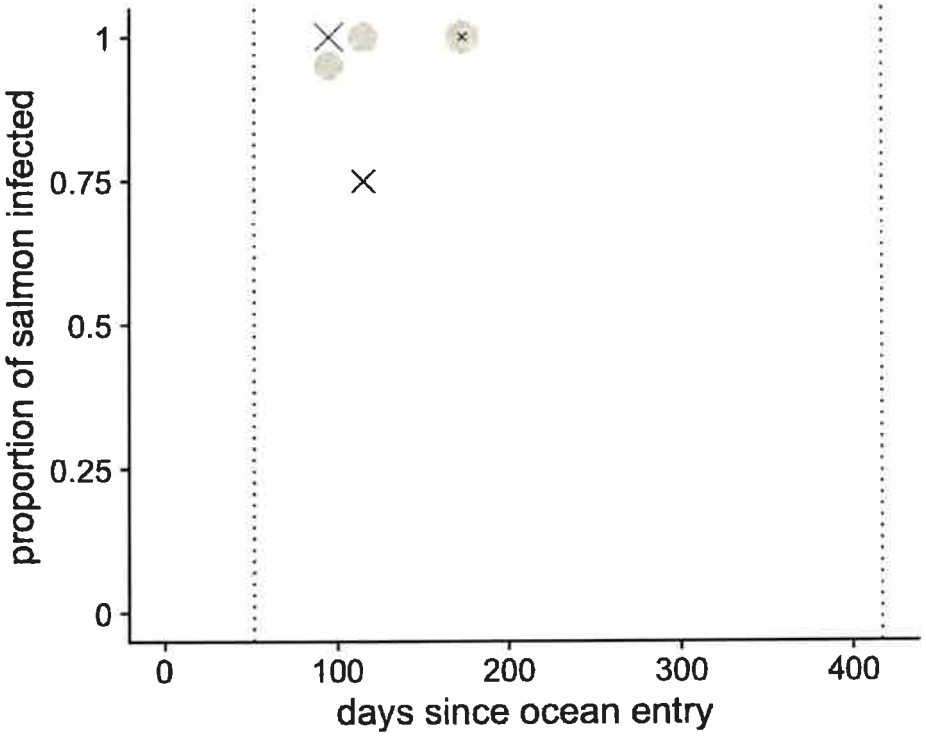
Piscirickettsia salmonis



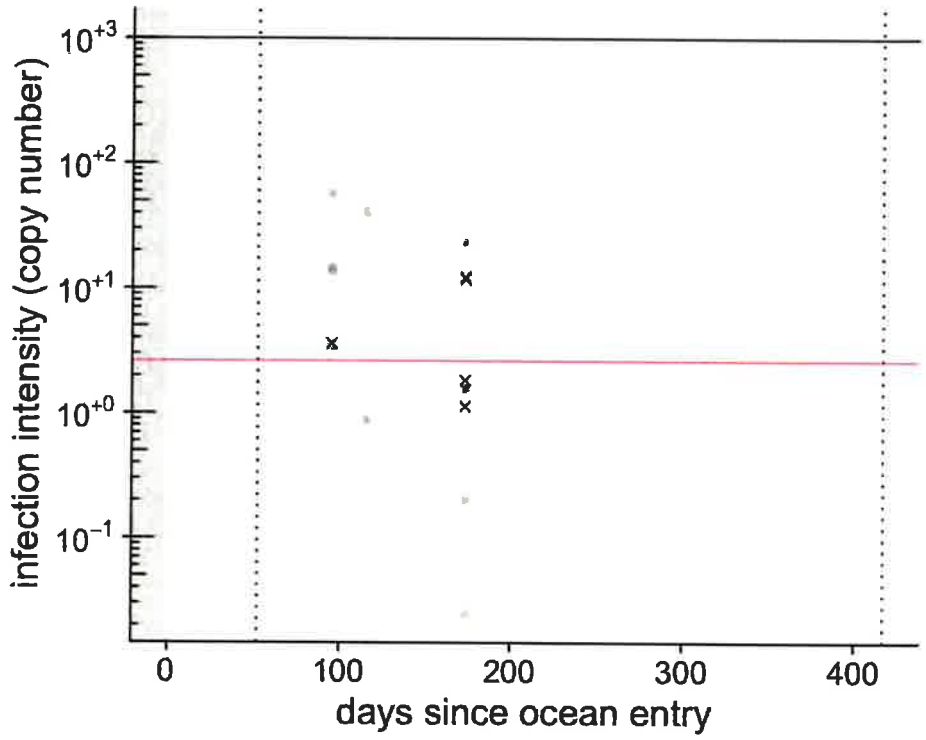


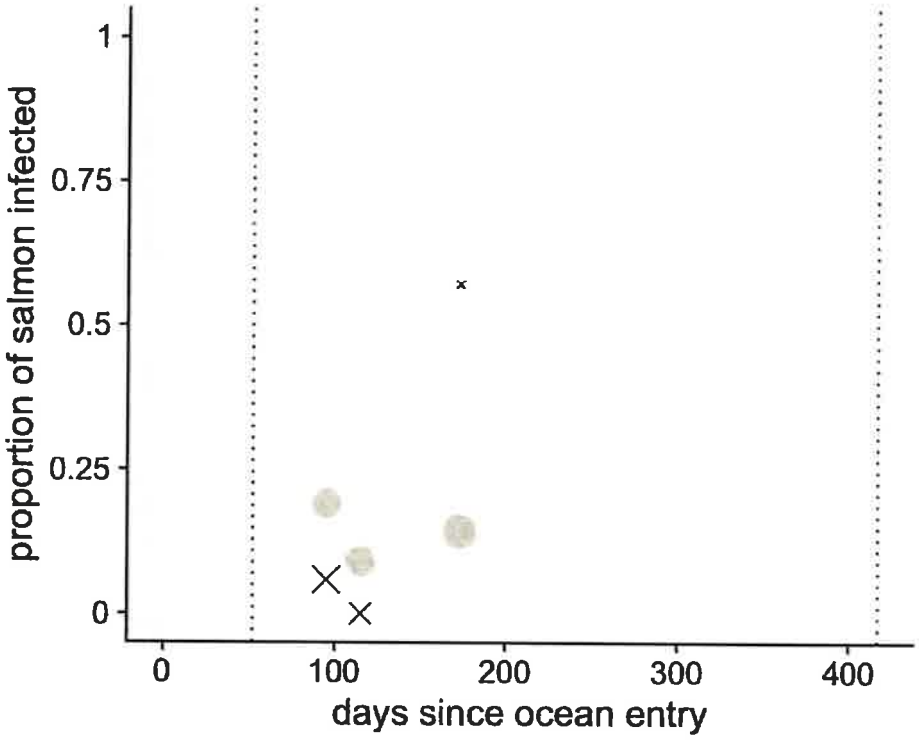
Piscine orthoreovirus



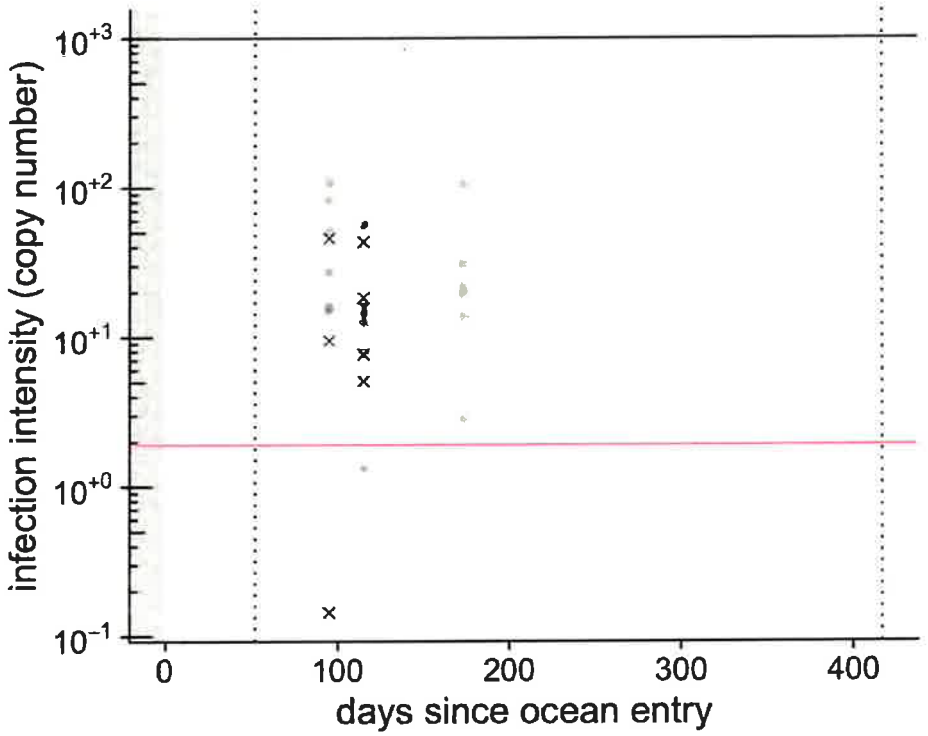


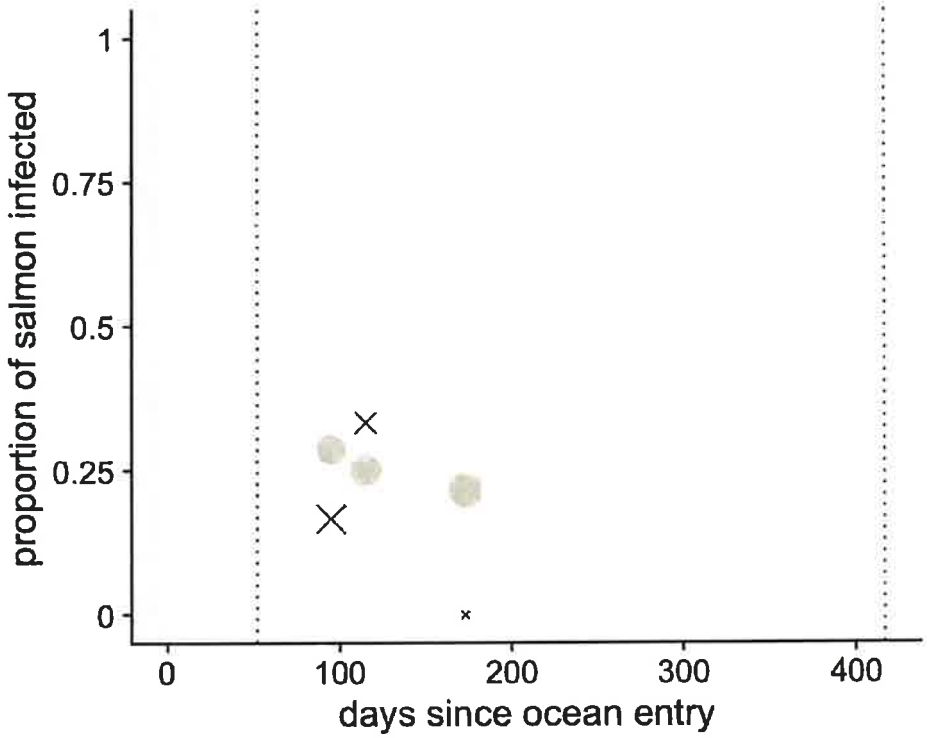
Candidatus Syngnamydia salmonis





Tenacibaculum maritimum





Clinical signs

Table 1: Clinical signs for specimens sampled on 2022-01-27

metric	N5705	N5704	N5703	N5681	N5682	N5683	N5684	N5685	N5686	N5687	N5688	N5689	N5690	N5691	N5692	N5693	N5694	N5695	N5696	N5697
General																				
Live	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											
Exopthalmia		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	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	N5698	N5699	N5700	N5701	N5702	N5706	N5709	N5711	N5712	N5713	N5714	N5715	N5710
General													
Live	X	X	X	X	X	X	X	X	X	X	X	X	X
Poor Performer													
Erratic Swimmer													
Moribund													
Mort													
Exopthalmia													
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		
Prolif		1	nv	nv	nv	nv		1
Skin_muscle								
Itis Nec						1		1
Tissue								
Necrosis Artefacts			2	2	2	1		

metric	N5681	N5682	N5683	N5684	N5685	N5686	N5687	N5689

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* Syngnamydia salmonis and *Tenacibaculum maritimum* prevalence was also significant (30%, 24% and 18%, respectively), with 57% of the morts testing positive to *Candidatus* Syngnamydia 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, conducible 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
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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
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vi_sal	Vibrio salmonicida	Bacteria	Cold water vibriosis	2
ye_ruc	Yersinia ruckeri (Enteric redmouth disease)	Bacteria	Yersiniosis (Enteric red mouth)	2