Strategic Salmon Health Initiative: Defining the role of infectious disease in survival of migratory salmon and interactions with cultured salmon

Kristi Miller
Head, Molecular Genetics
Fisheries and Oceans Canada

PACIFIC SALMON FOUNDATION

Genome BritishColumbia

Fisheries and Oceans Canada

NSERC CRSNG

Challenges with our understanding of disease impacts on wild populations

- We rarely observe wild fish die, they simply disappear
- Acute versus chronic infections versus carrier states
- Many infectious agents never assessed in wild populations or in BC salmon, especially in the marine environment
- Potential for interactions between cultured and wild fish
- Impacts may vary by environment, species, and stocks
- Cumulative impact of stress and disease

Miller et al. 2014 Evolutionary Applications

Sub-lethal effects of infection may be more detrimental in wild than cultured fish

Swim performance and predation

Feeding

Ion homeostasis

Behavioral shifts in migration timing and speed

Strategic Salmon Health Initiative

Discover the pathogens and potential diseases that may undermine the productivity and performance of BC salmon, their evolutionary history, and the potential role of exchanges between wild and cultured salmon

- Quantitative assessment of 47 potential pathogens in in >26,000 salmon
- Wild, enhancement hatchery, and farmed salmon
- Combines traditional and novel approaches to study disease
- Novel genomic technologies and systems biology approaches
- Considers both lethal and sub-lethal impacts of infection

Strategic Salmon Health Initiative

Reverse traditional approach starting with disease—

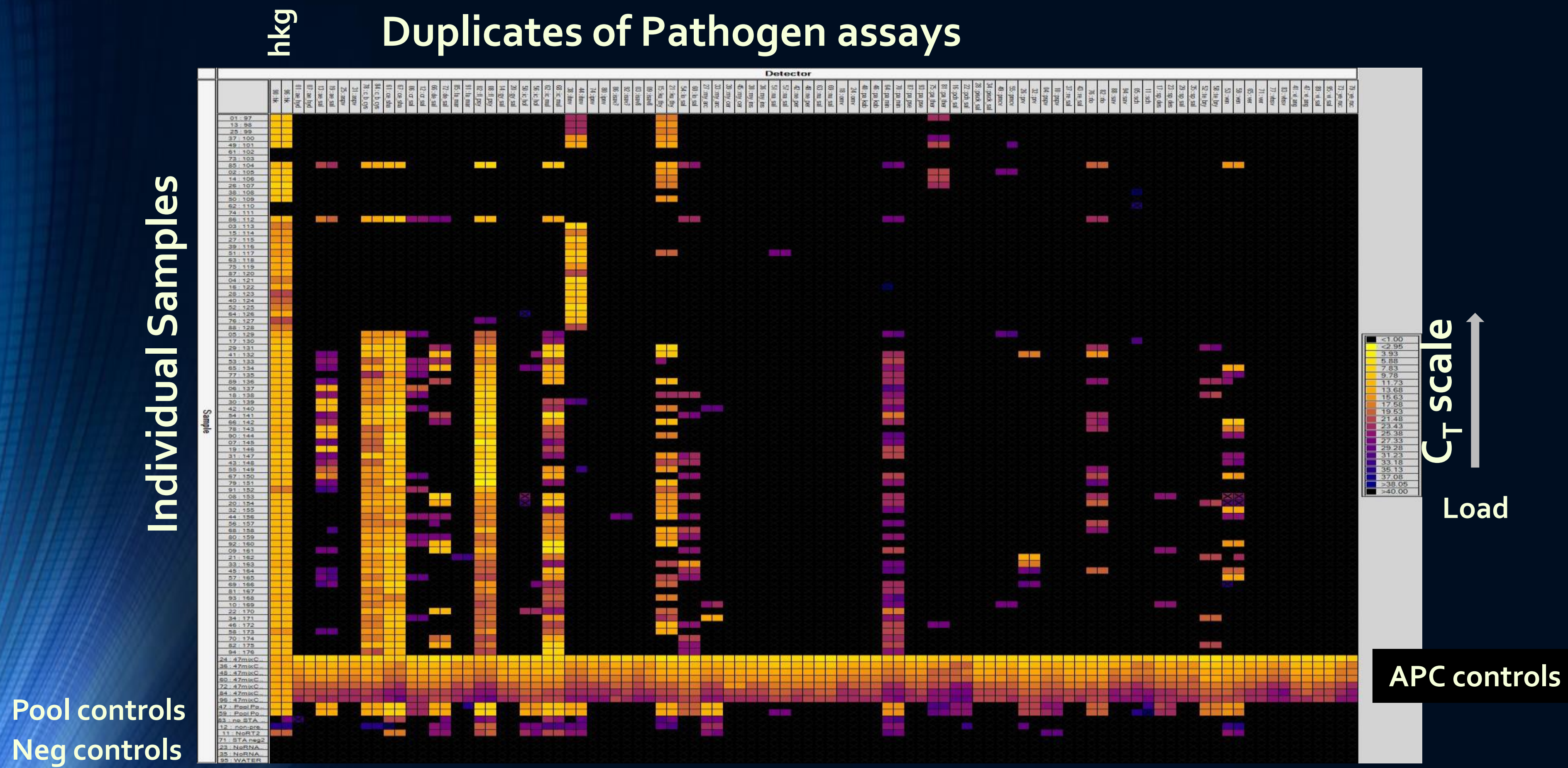
1) Broad-based quantitative infectious agent monitoring
2) Pathogenic potential
    a) physiological impacts at molecular, protein and cellular levels
    b) organismal impact via tracking, predation and holding/challenge studies
3) Novel pathogen discovery
    a) Construction of disease networks to identify potential diseases with unknown etiological agents

High Throughput infectious agent monitoring

Identify the infectious agents detected in BC salmon, their temporal and spatial distributions, and the locations in which they are transmitted

Fluidigm BioMark^{TM}:

Infectious agent monitoring heatmap



Salmon Pathogen Monitoring Platform

- TaqMan assays to 47 agents suspected or known to cause disease in salmon world-wide
- 10 OIE-listed salmon pathogens

12 viruses

Virus	Abbreviation
Atlantic salmon paramyxovirus	aspv
Infectious hematopoietic necrosis virus	ihnv
Infectious pancreatic necrosis virus	ipnv
Infectious salmon anemia virus	isav7 / isav8
Oncorhynchus masou herpes virus	omv
Pacific salmon parvovirus	pspv
Piscine myocarditis virus	pmcv
Piscine reovirus	prv
Salmon alphavirus	sav
Viral erythrocytic necrosis virus	ven
Viral encephalopathy and retinopathy virus	ver
Viral hemorrhagic septicemia virus	vhsv

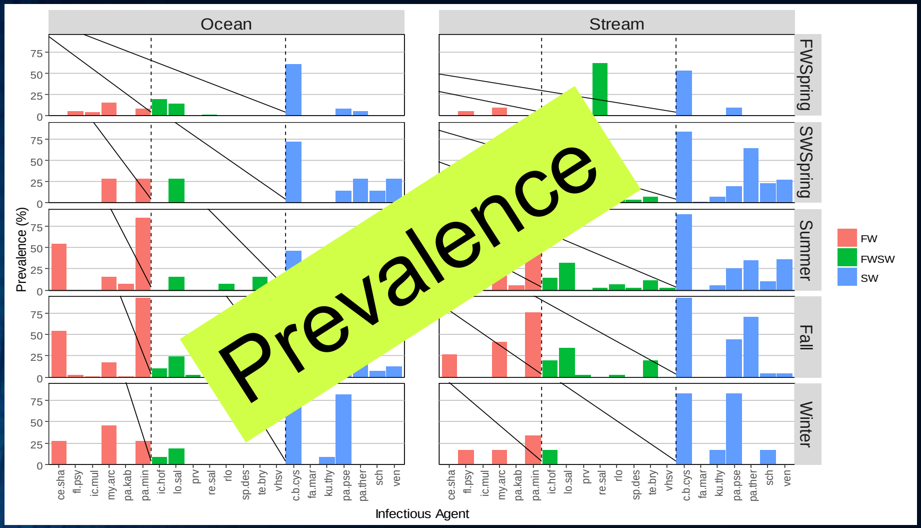
13 bacteria

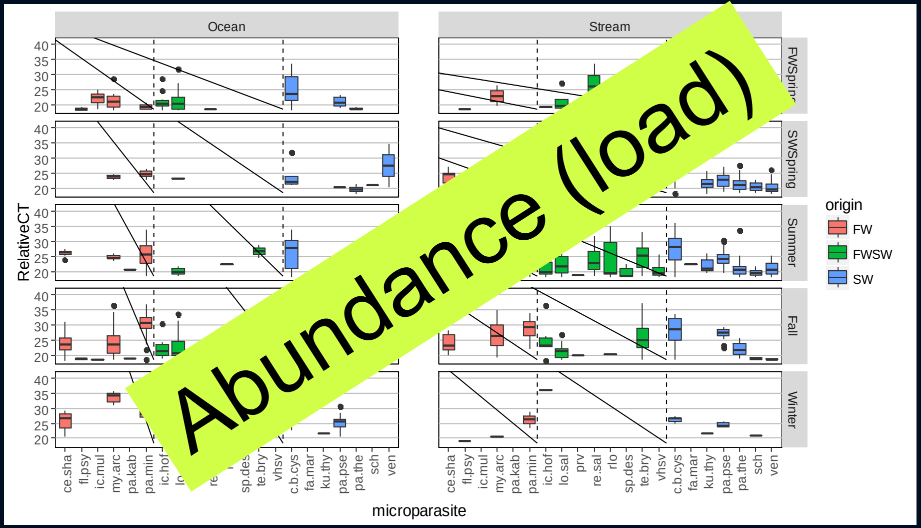
Bacterium	Abbreviation
Aeromonas hydrophila	ae_hyd
Aeromonas salmonicida	ae_sal
Candidatus Branchiomonas cysticola	c_b_cys
Flavobacterium psychrophilum	fl_psy
Gill *chlamydia*	sch
Piscichlamydia salmonis	pch_sal
Piscirickettsia salmonis	pisk_sal
Renibacterium salmoninarum	re_sal
Rickettsia-like organism	rlo
Vibrio anguillarum	vi_ang
Vibrio salmonicida	vi_sal
Yersinia ruckeri	ye_ruc
Tenacibaculum meritimum	Te_mer

22 parasites

Parasite	Abbreviation
Ceratomyxa shasta	ce_sha
Cryptobia salmositica	cr_sal
Dermocystidium salmonis	de_sal
Facilispora margolisi	fa_mar
Gyrodactylus salaris	gy_sal
Ichthyophonus hoferi	ic_hof
Ichthyophthirius multifiliis	ic_mul
Kudoa thyrsites	ku_thy
Loma salmonae	lo_sal
Myxobolus arcticus	my_arc
Myxobolus cerebralis	my_cer
Myxobolus insidiosus	my_ins
Nanophyetus salmincola	na_sal
Neoparamoeba perurans	ne_per
Nucleospora salmonis	nu_sal
Paranucleospora theridion	pa_ther
Parvicapsula kabatai	pa_kab
Parvicapsula minibicornis	pa_min
Parvicapsula pseudobranchicola	pa_pse
Sphaerothecum destructuens	sp_des
Spironucleus salmonicida	sp_sal
Tetracapsuloides bryosalmonae	te_bry

Infectious agent monitoring identifies the shifting prevalence, abundance (load), and diversity of infectious agents through smolt and adult migration





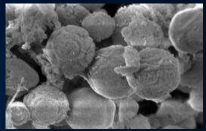
Strahan Tucker

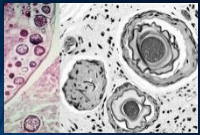
Chinook Smolt Infectious Agent Detections

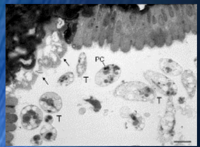
FW

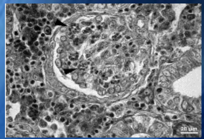

Parvicapsula


Icthyopthirius


Sphaerothecum


Ichthyophonus


Ceratomyxa


Parvicapsula

24 of 45 agents with prevalence >1%

8 agents prevalence of <1%

16 of 32 agents originate in freshwater (FW)

5 viruses
8 bacteria
19 parasites

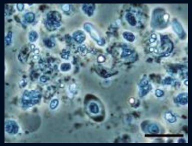
52 fish "agent-free" (most FW)

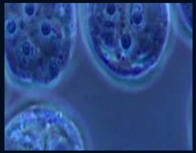
Infectious agent richness increases from FW (average 1.5 agents/fish) to SW (average 4 agents/fish)

Maximum agent richness was 10/fish

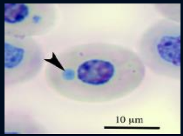
SW


Paranucleospora

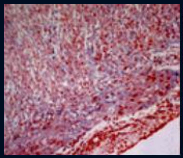

Parvicapsula


Tetracapsuloides


Rickettsia



VEN


PRV

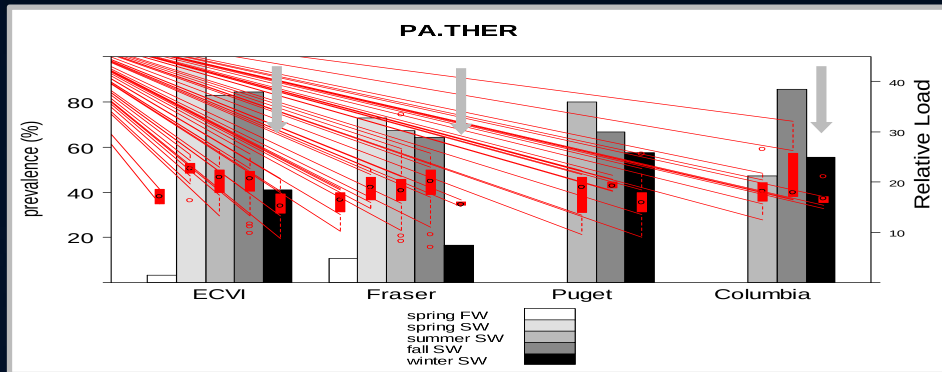
Strahan Tucker

Agents Commonly observed in Salish Sea Chinook Salmon

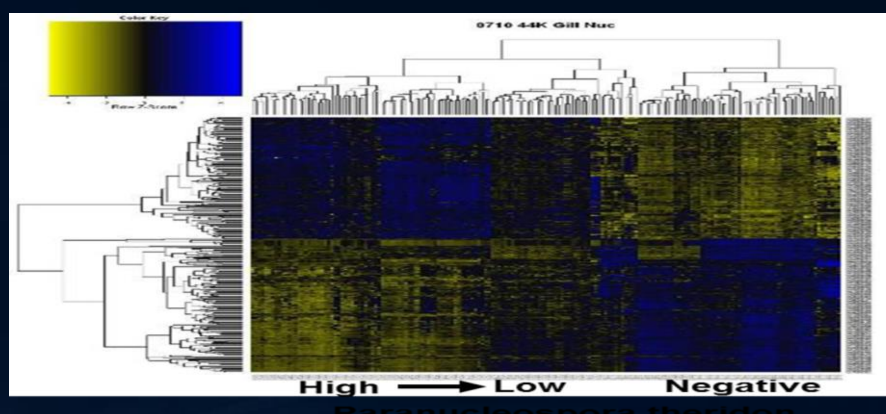
Paranucleospora theridion

SW microsporidian transmitted through sea lice

Proliferative gill disease (PGD) in European salmon



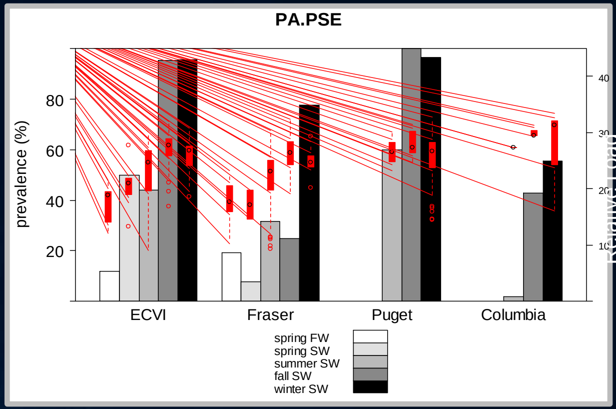
Sharp reduction in prevalence/load in winter consistent with timing of disease Oct-Feb in Europe



Powerful stimulation of immune response in Pacific salmon smolts

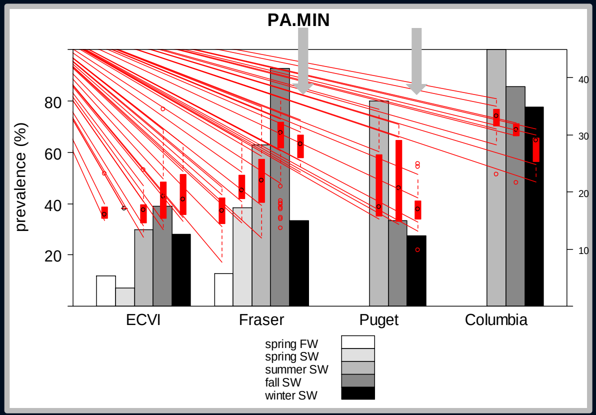
Microbes Commonly observed in Salish Sea Chinook Salmon

Parvicapsula pseudobranchicola
SW-transmitted myxozoan
PGD, vision impairment and anorexia in European salmon

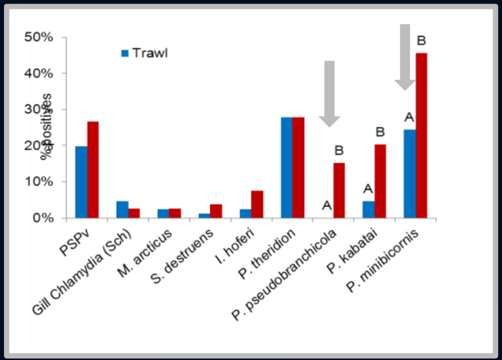


High prevalence and load fall/winter

Parvicapsula minibicornis
FW- transmitted myxozoan
Kidney disease



High prevalence/load Fraser- Columbia
Reduced prevalence and load truncation winter

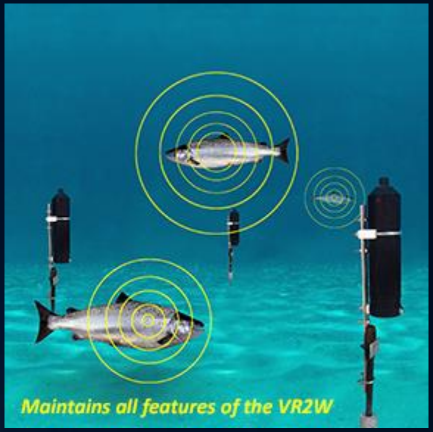


Increased Auklet predation risk in QC Strait sockeye

Linking infectious agents with disease

Which infectious agents carried by migratory salmon are actually impacting them?

Establishing Linkages with Survival



Tracking Studies

Predation Studies

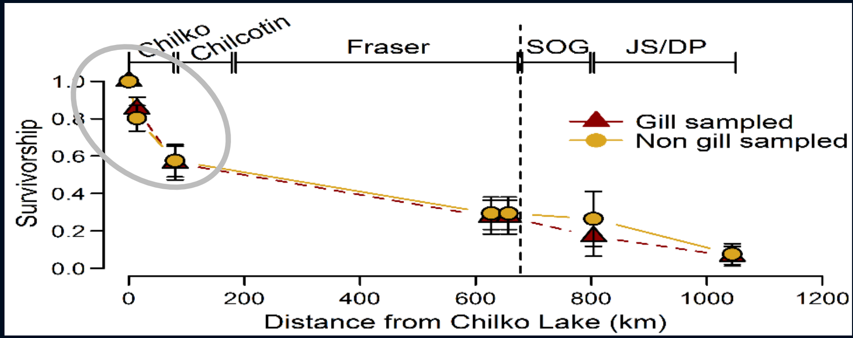
But… linking pathogens with disease in migratory fish is more difficult

Acoustic Tracking Studies

Downstream Migration Survival in Chilko Sockeye Smolts

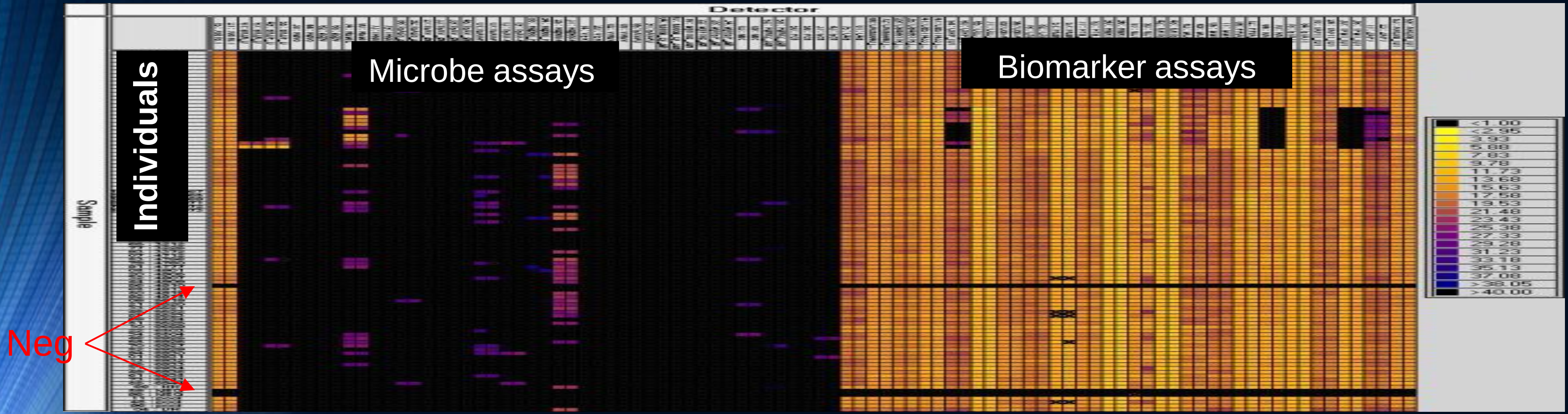
Ken Jeffries

Chilko Smolt



40% "Immediate Mortality" in the Chilcotin

Merging microbe monitoring with host gene expression profiling



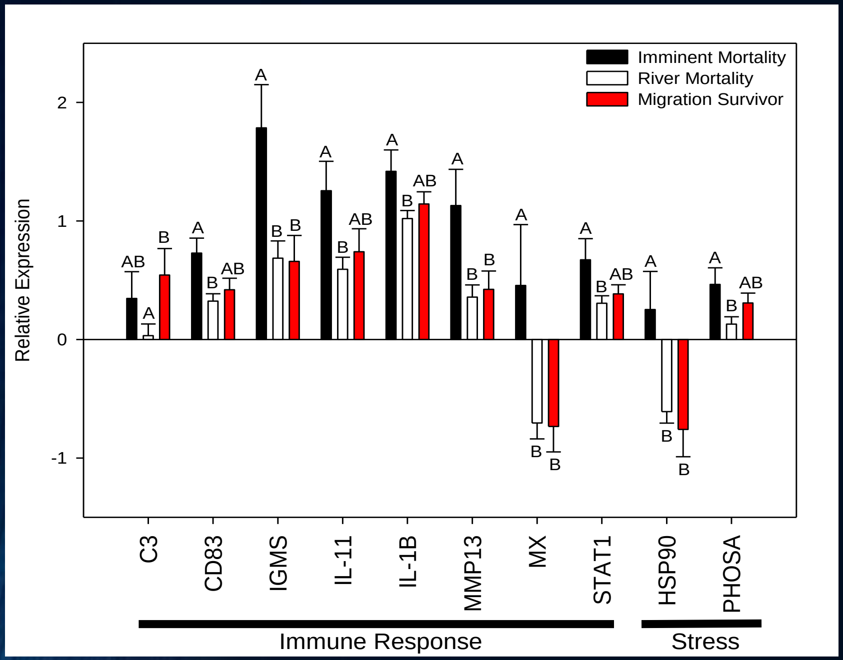
Powerful means to quickly identify microbes associated with immune stimulation, indicative of the potential for host damage/pathogenicity

Jeffries et al. 2014
Molecular Ecology

Acoustic Tracking Studies

Downstream Migration Survival in Chilko Sockeye Smolts

Ken Jeffries

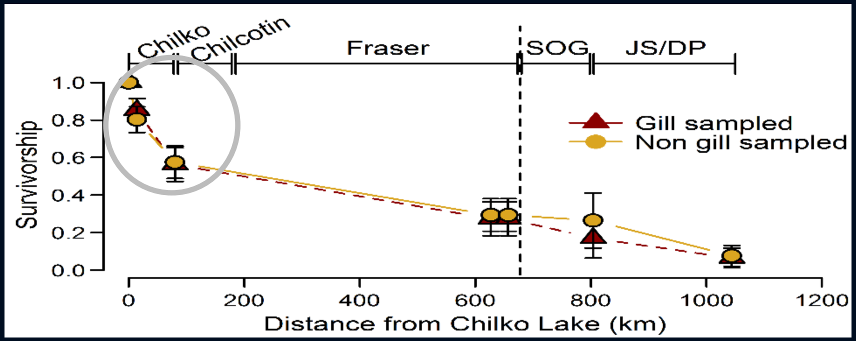


10 (of 50) immune and stress genes associated with imminent mortality in the Chilcotin River

Anti-viral
T-cell activity
Antibody
Inflammation
Stress

Infectious Hematopoetic Necrosis Virus

Associated with stimulated immune-related signature and high, early losses of smolts



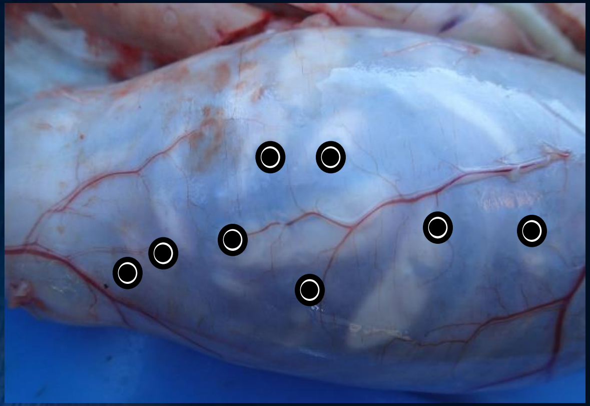
Fish with high IHNV loads disappear immediately and most with low-moderate loads don't make it to the ocean

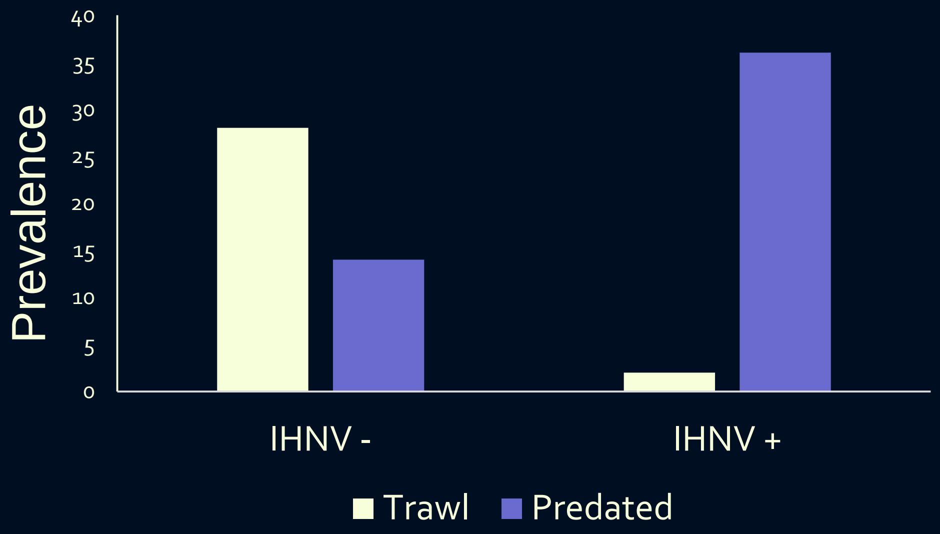
But how?...

Jeffries et al. 2014
Molecular Ecology

Predation Studies

Bull trout predation during downstream migration in Chilko Sockeye smolts





Chilko smolts with IHNV had 34 times greater odds of being consumed by bull trout than negative fish

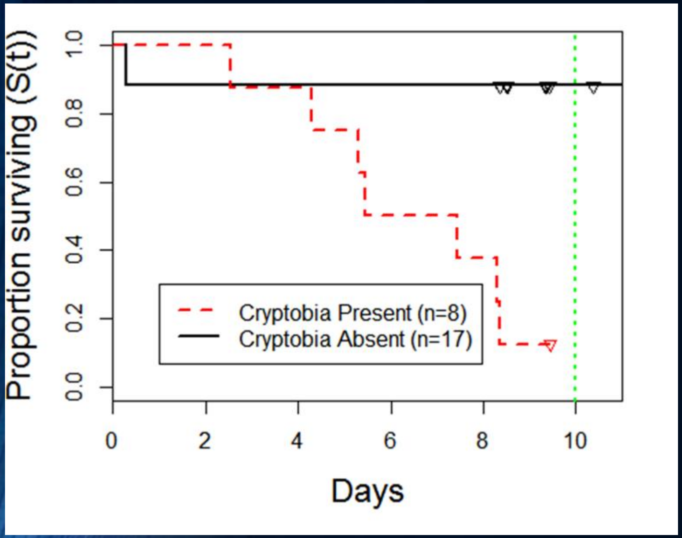
Predation decreased the infective burden in the populations—thereby increasing population health

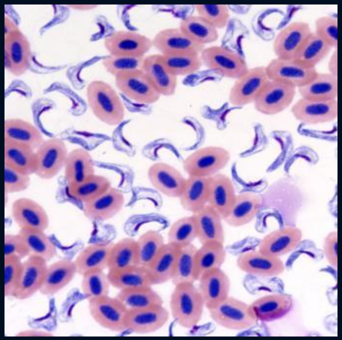
Nathan Furey
In prep

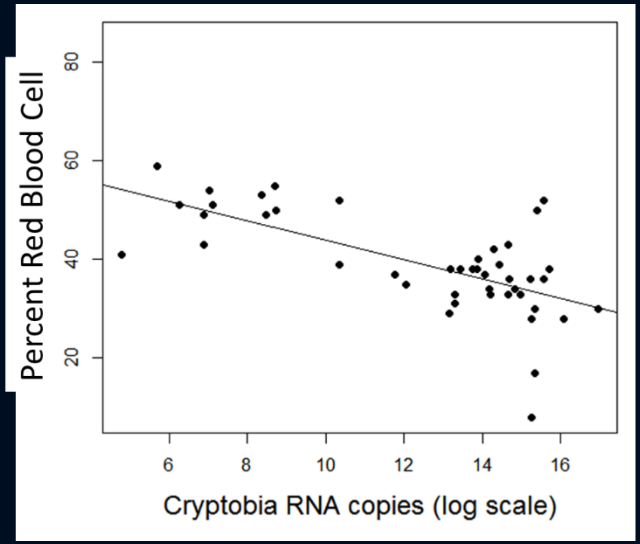
Holding Study
Adult salmon

Amy Teffer

2013
Chilliwack River
Chinook Salmon







C. salmositica infection reduced suvival

C. salmositica infection impacts blood physiology

Cryptobia salmonistica

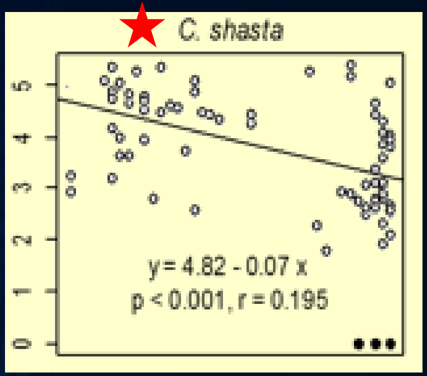
FW Transmitted
Blood Flagellate Causing Anemia

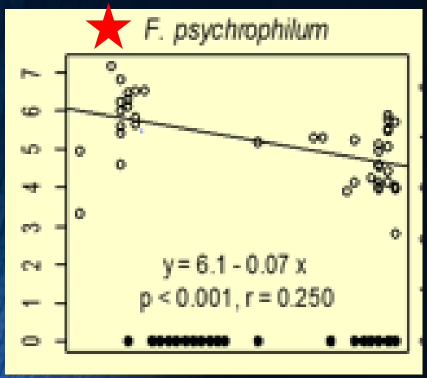
Amy Teffer
In prep

Microbes impacting fate associated with negative physiological impacts at molecular level

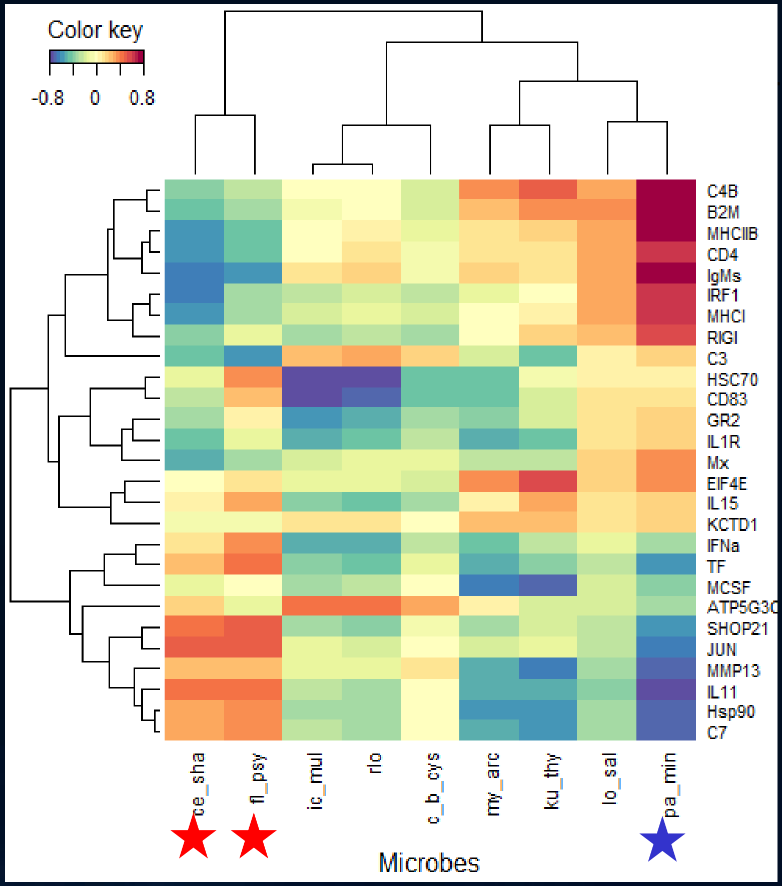
Amy Teffer

Early Stuart Adult Sockeye Salmon Holding Study



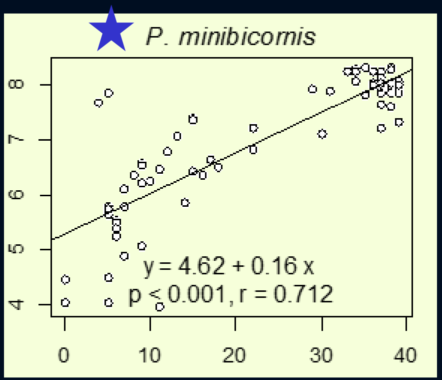


Days survived



oma salmonae

Delayed Mortality:
Adaptive Immunity
Antibody production



Early Mortality:
Stress, inflammation, tissue damage, immunosuppression

Transcriptional Signatures

Amy Teffer
In prep

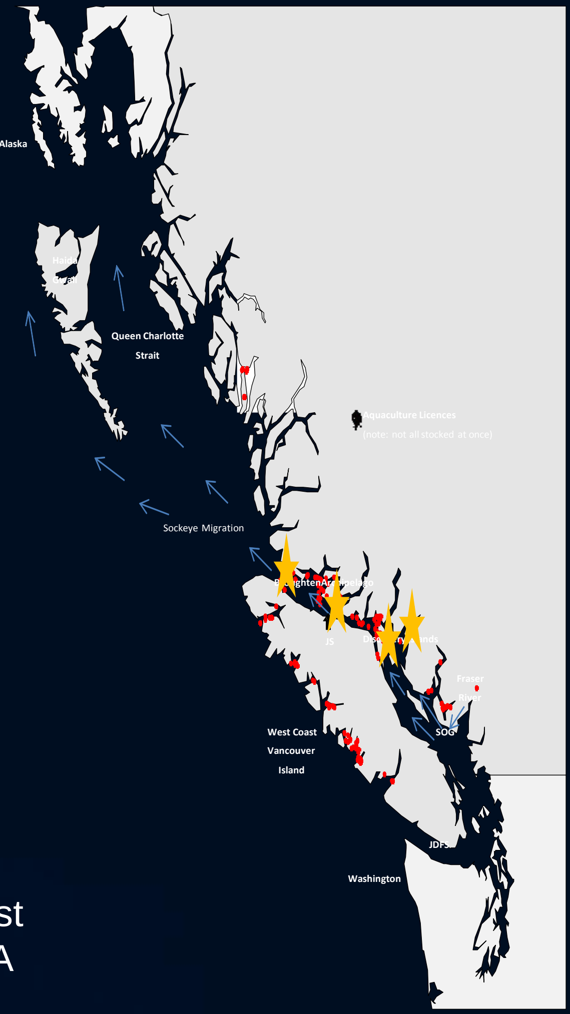
Aquaculture Collections

Longitudinal Farm Sampling:

Healthy and moribund aquaculture fish from 4 ocean farms sampled regularly throughout ocean production cycle (~2500 fish)—healthy comparators with wild fish

Audit program

930 samples from 2011-2013 provide larger spatial/temporal scale to assess microbes associated with dying salmon



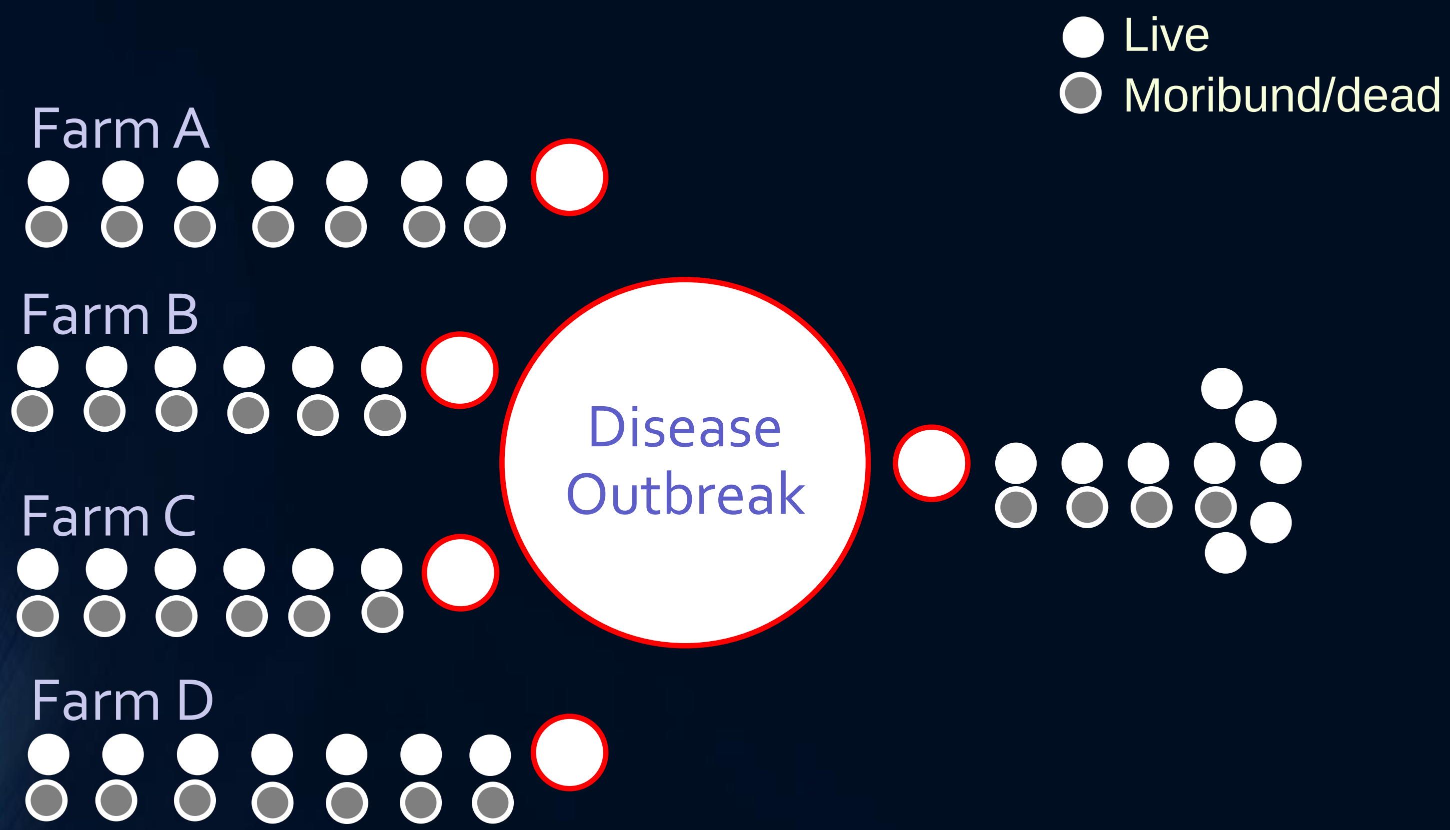
Working with Cermaq and Marine Harvest
Material Transfer Agreement with BCFSA

Longitudinal Farm Study Identifies Emerging Salmon Disease

Clinical pathology
Cellular pathology
Molecular profiling
Pathogen monitoring

Di Cicco et al. 2017
PLOS ONE

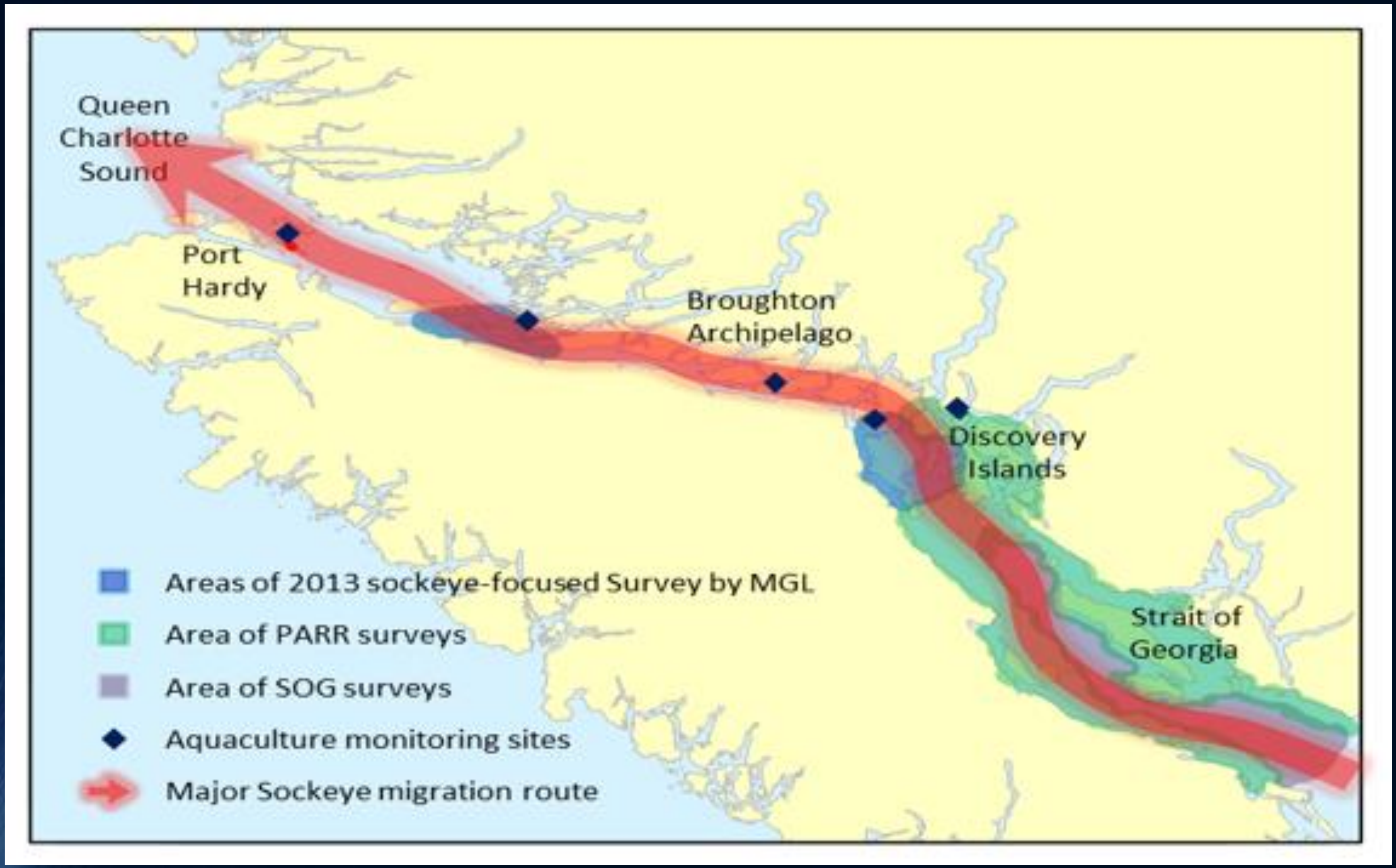
SSHI Longitudinal Farm Sampling



Fine-scale temporal sampling to uncover cellular and molecular processes associated with to disease development and recovery

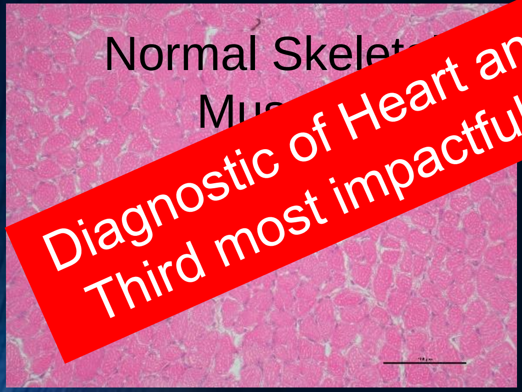
Collection of live fish provides best comparator to samples of migratory salmon

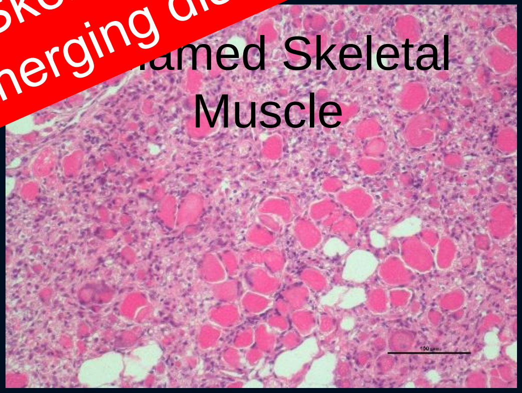
Aquaculture-Wild Interactions



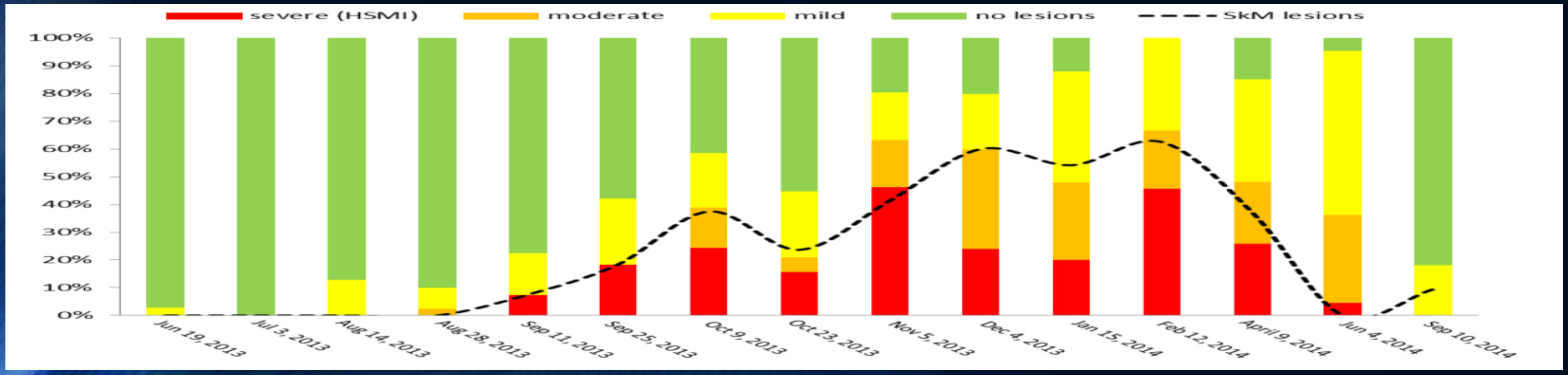
Sampled four geographically dispersed farms over the entire ocean production cycle along the migration pathway of wild salmon emanating from the Fraser River

Histopathological Investigations – Farm A

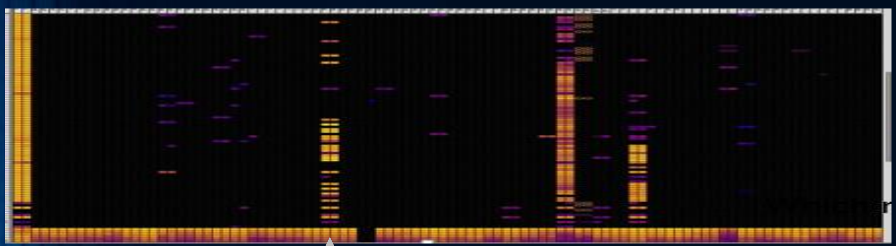




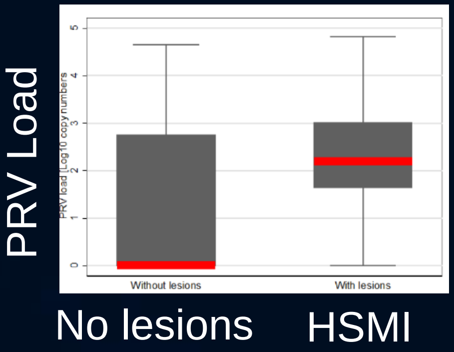
Diagnostic of Heart and Skeletal Muscle Inflammation (HSMI) disease
Third most impactful emerging disease in farmed Norwegian salmon



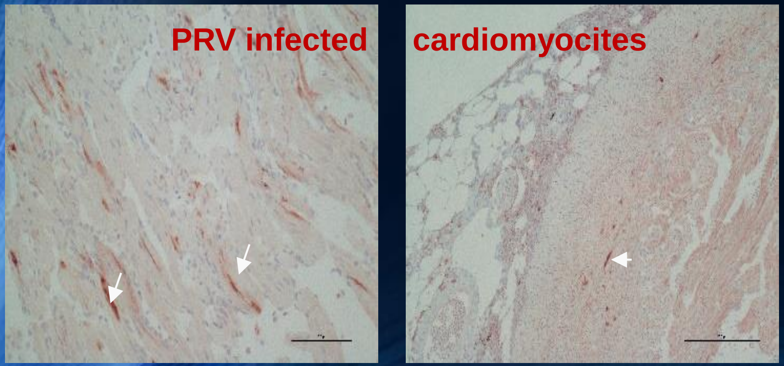
Longitudinal study resolves full developmental pathway of HSMI



High throughput pathogen monitoring:
Identify the shifting pathogen distributions in the heart

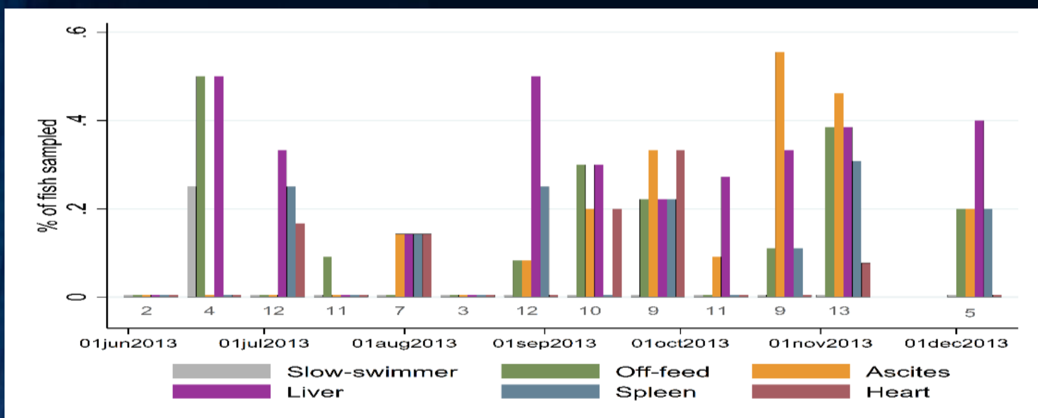


Epidemiological Analyses:
Identify **PRV Pathogen loads** correlated with **lesion scores**

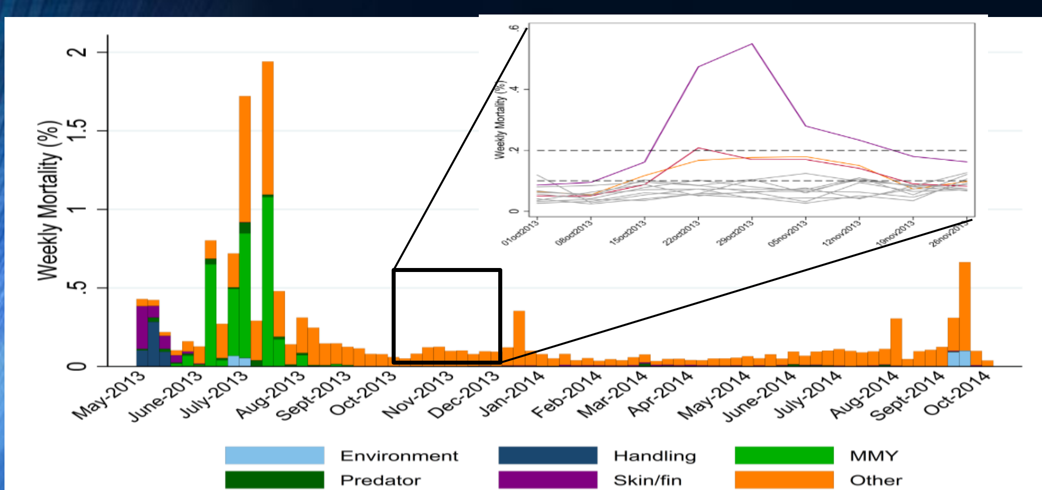


Immunohistochemistry:
Localization of PRV pathogen within the area of tissue damage

Longitudinal study resolves full developmental pathway of HSMI

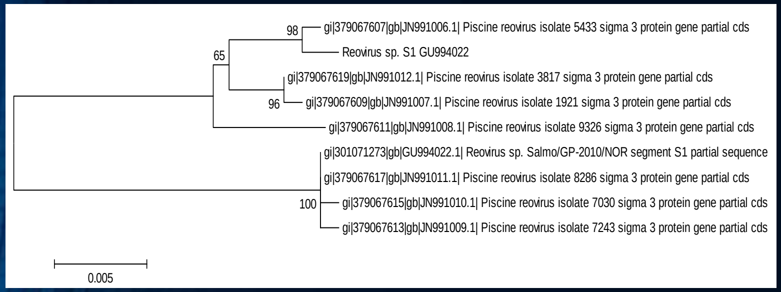


Clinical data:
Consistent with HSMI outbreaks in Norway



Mortality data:
Minimal impact on survival of fish on the farm

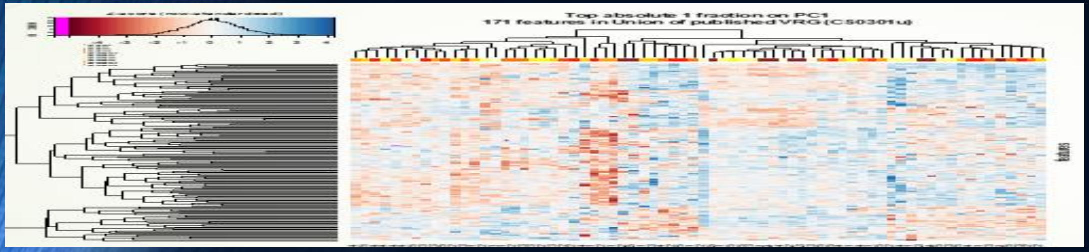
Longitudinal study resolves full developmental pathway of HSMI



High throughput sequencing:

Full viral genome sequencing identifies PRV strain 99.9% similar to sequences previously observed in wild-migrating BC salmon

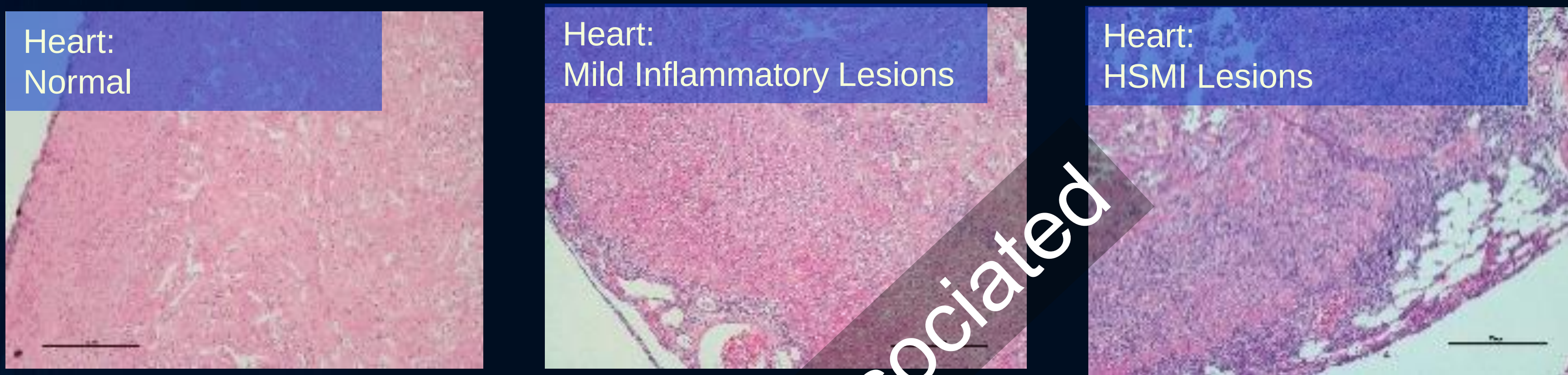
Identifying viral transcriptome shifts over disease cycle



Transcriptomics (RNA-seq) (Underway):

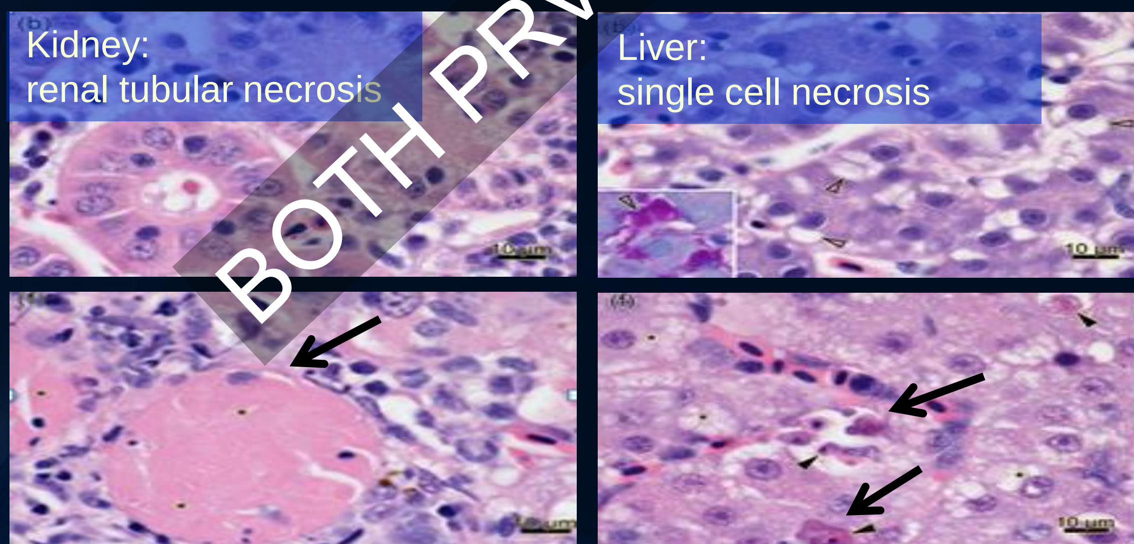
Does the transcriptional profile match HSMI in Norway?

Viral Diseases in BC Farmed Salmon



Heart and Skeletal Muscle Inflammation in Atlantic Salmon

BOTH PRV-associated



Jaundice Syndrome in Chinook Salmon

PRV highly prevalent in farmed fish (~70% of farm audit samples)

Virus increases in prevalence over the first 6 months in the ocean, while the two diseases associated with it occur ~8 months post ocean-entry

PRV detected, but NOT common, in migratory smolts

Virus increases from summer through fall/winter in Chinook and Sockeye salmon; 7% overall in Chinook and 3% in Sockeye

Our program has NOT detected viruses

ISAv – Infectious Salmon Anemia virus
IPNv – Infectious Pancreatic Necrosis virus
Omv -- Oncorynchus Masu Herpesvirus
Sav – Salmon Alphavirus
PMCv – Piscine Myocarditis Virus
ASPv – Atlantic Salmon Paramyxovirus

Infective agents can be present in the absence of disease

- Farmed salmon

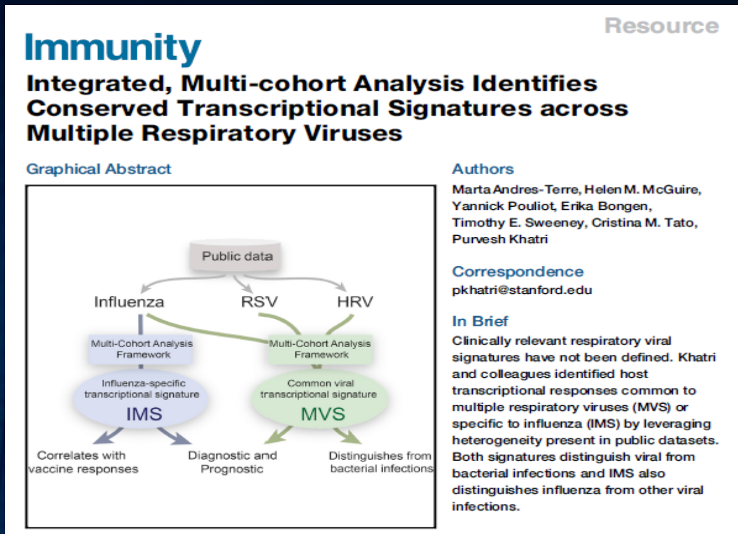
- 39% of Atlantic salmon and 52% of Chinook salmon with high loads of *Renibacterium salmoninarum* diagnosed with BKD
- 58% of Atlantic salmon and 31% of Chinook salmon with high loads of *Piscirickettsia salmonis* diagnosed with Rickettsiosis
- 35% of Atlantic salmon with high loads of *Tenacibaculum maritimus* diagnosed with Mouth Rot
- 30% of Chinook salmon audits with high PRV loads diagnosed with Jaundice/anemia
- 13% of Atlantic salmon audits with high PRV loads diagnosed with HSMI

How do we link PRV and other potential pathogens with disease if migratory fish disappear soon after they are physiologically compromised?

THE ANSWER:

Develop molecular methods to recognize early developing disease states

Molecular Disease Diagnostics – human medicine



Authors

Marta Andres-Terre, Helen M. McGuire, Yannick Pouliot, Erika Bongen, Timothy E. Sweeney, Cristina M. Tato, Purvesh Khatri

Correspondence

pkhatri@stanford.edu

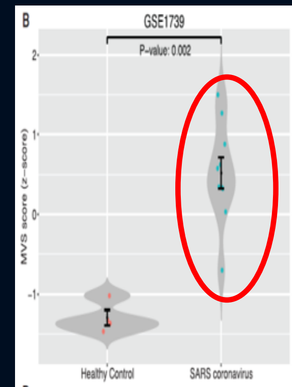
In Brief

Clinically relevant respiratory viral signatures have not been defined. Khatri and colleagues identified host transcriptional responses common to multiple respiratory viruses (MVS) or specific to influenza (IMS) by leveraging heterogeneity present in public datasets. Both signatures distinguish viral from bacterial infections and IMS also distinguishes influenza from other viral infections.

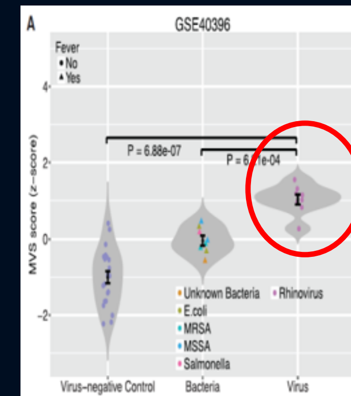
Andres-Terre et al., 2015, Immunity 43, 1199–1211
December 15, 2015 ©2015 Elsevier Inc.
http://dx.doi.org/10.1016/j.immuni.2015.11.003

Discovery

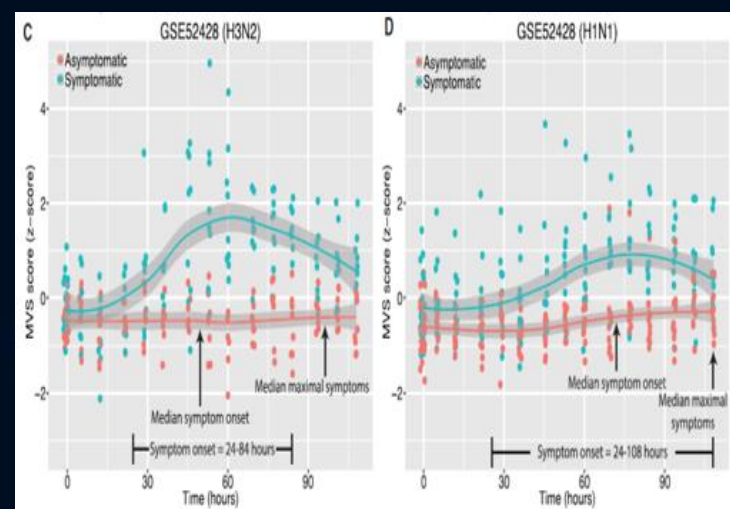
Validation



viral vs. healthy



viral vs. bacterial



Pre-symptomatic viral disease development

- Mined public transcriptome studies
- Identified diagnostic biomarker signatures for
  - respiratory viral disease
  - specific to influenza virus disease

Viral Disease Development [VDD]
Salmon RNA viruses

VDD – predictive for all viruses

Multiple viruses

Krasnov 2011	Skjseol 2011	LeBlanc 2010
ISAV PMCV PRV IPNV	IPNV	ISAV
Atlantic	Atlantic	Atlantic

Published signatures

Union (532 features)

GÜNTHER ANALYTICS
Data Analysis, Modeling and Simulation

Exploration Analysis

Gene Shaving, Sparse Independent PCA

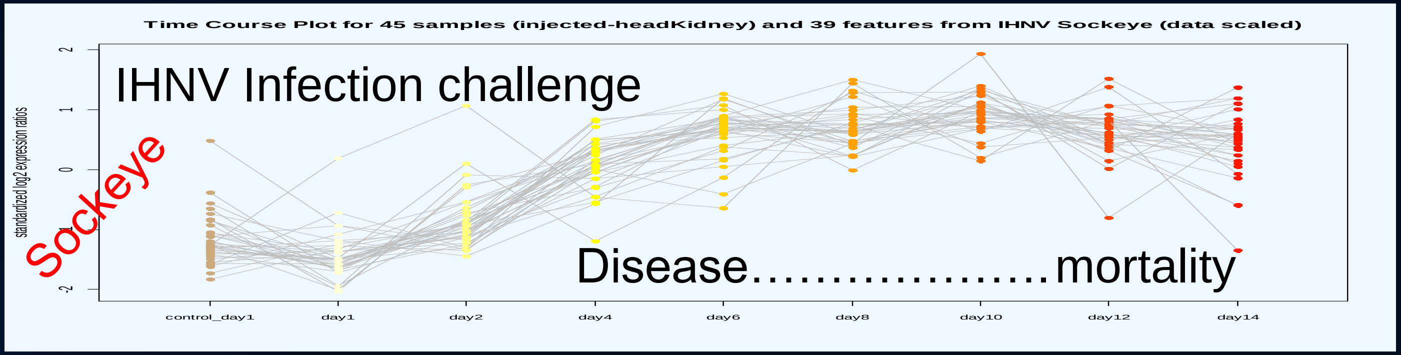
Multiple salmon species

Purcell 2011	Miller 2007
IHNV	IHNV
Rainbow Trout	Sockeye Chum Atlantic Chinook

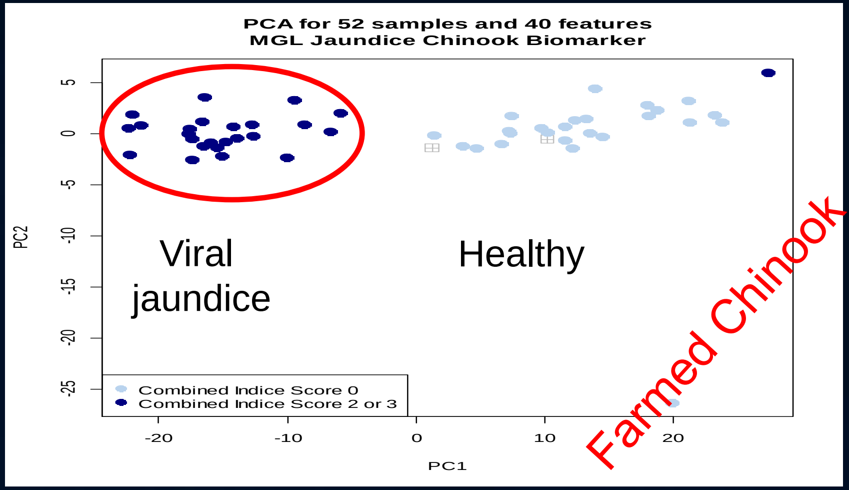
VDD 44 biomarker panel

Molecular biomarkers – early disease detection

Molecular biomarkers identify development of viral disease state



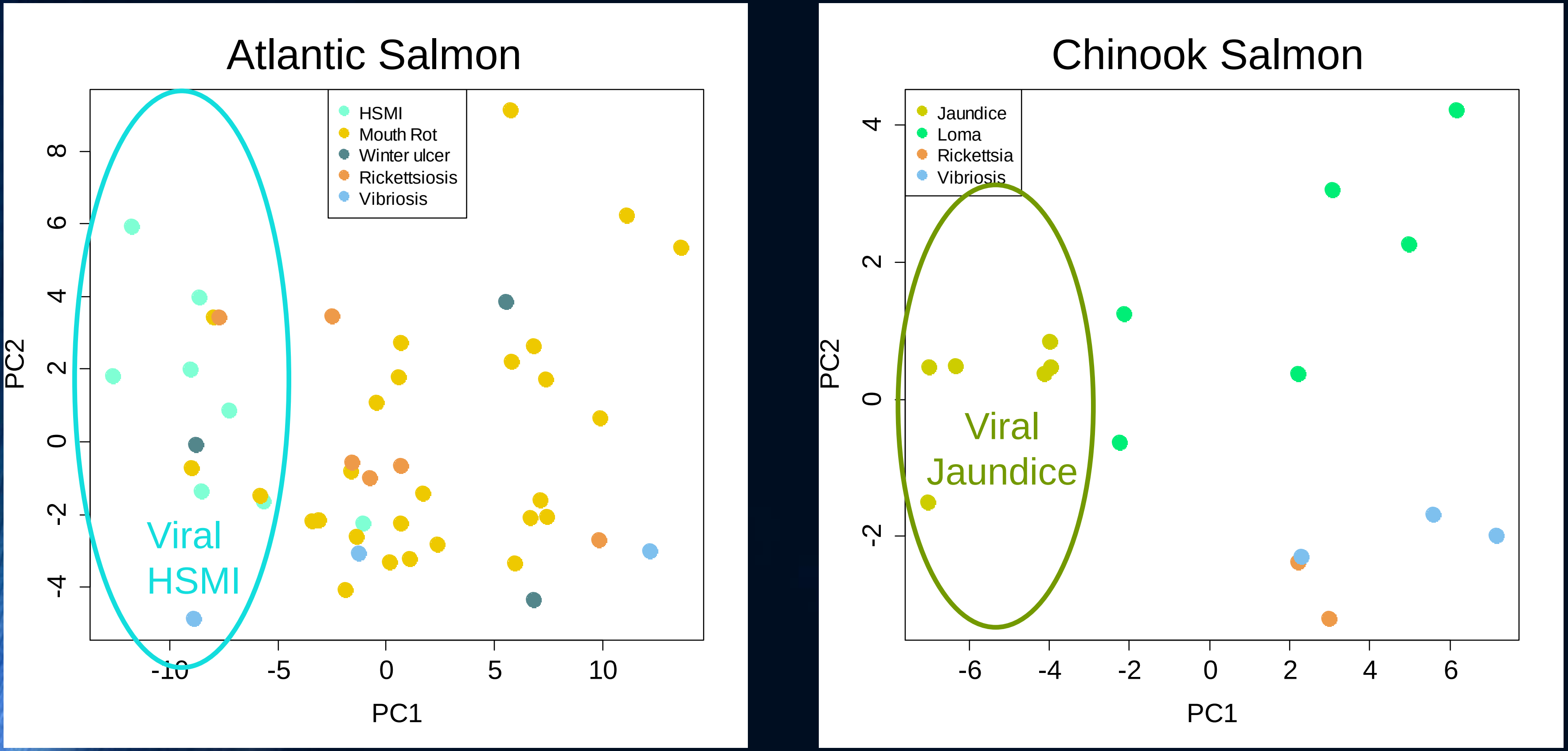
TIME



Miller et al. In Review
Conservation Physiology

Molecular biomarkers – early disease detection

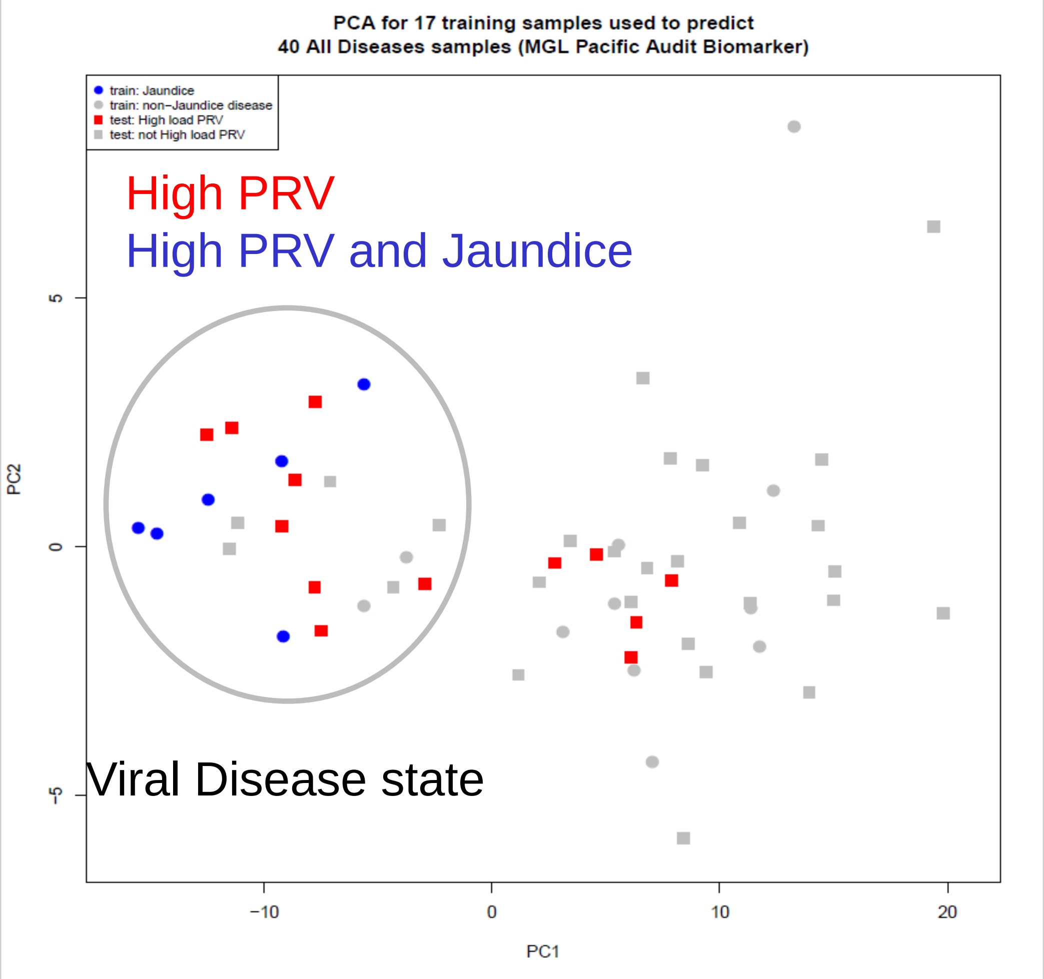
Molecular biomarkers identify differentiate fish with viral versus bacterial diseases



Mixed Tissues, Dead sampled fish,
Diagnosed through Veterinary Pathology

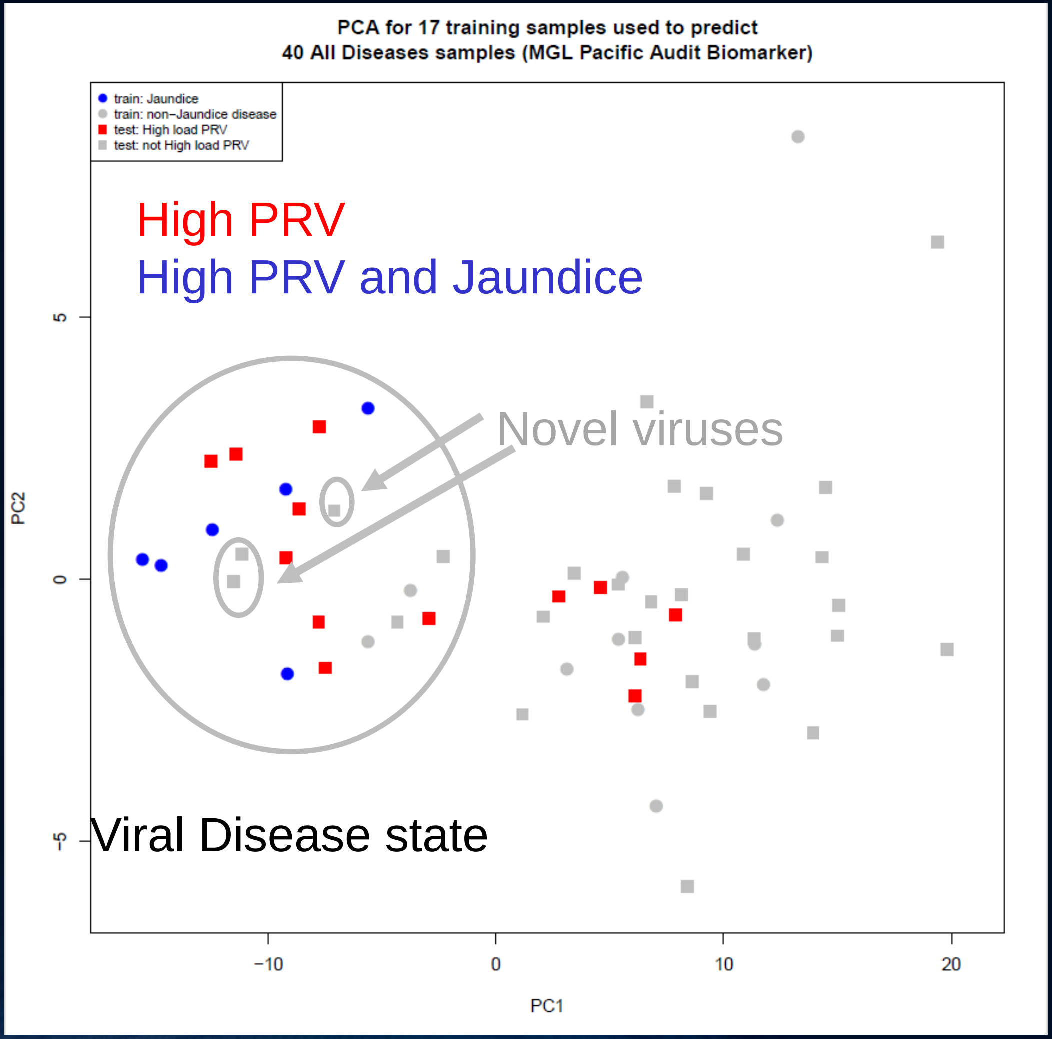
Molecular biomarkers – early disease detection

80% of Chinook salmon containing high loads of PRV are in a “viral disease state”
50% of which are diagnosed with jaundice/anemia
Wild Chinook salmon show the same 80% pattern

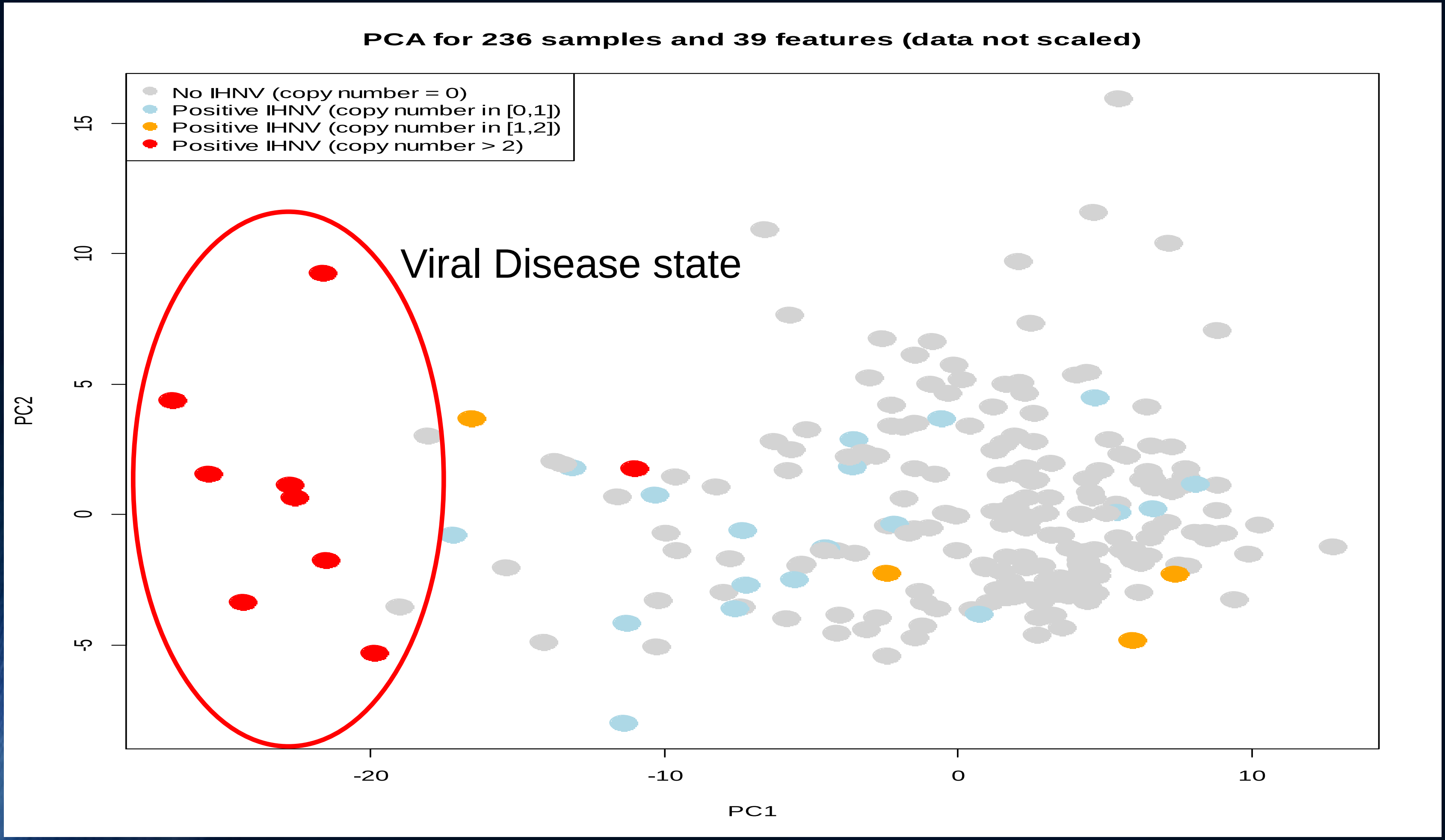


Molecular biomarkers – early disease detection

VDD Panel can be used to identify fish with uncharacterized viruses associated with a developing disease state



Wild Chilko Sockeye Smolts with high loads of IHNv in a viral disease state



- Move from “bug hunting” to “molecular disease diagnostics”
- Non-destructive gill biopsies — ideal for application with tracking studies

Key Findings to date

- Migratory salmon – natural exposure to wide array of potential pathogens
  - Half come from freshwater
  - An array of pathogens have already been associated with poor migratory success
  - Infection status enhances risk of predation
  - Fungal and protozoan parasites most commonly associate with poor survivorship outcomes

- Farmed salmon – The PRV-associated diseases HSMI and jaundice are present on BC salmon farms, but many other viral diseases are absent
  - We do not know:
    - Impacts on wild Pacific Salmon
    - How wide-spread
    - Industry-wide impacts on survival of farmed salmon
    - If factors in addition to PRV are required to initiate disease

SSHI: Linkages between Science and Regulation

- Develop innovative high throughput diagnostic tools for monitoring by regulators and industry
- Identify pathogens and diseases that pose biosecurity risks to Pacific salmon
- Contribute to DFO risk assessments associated with pathogen transmission from salmon farms and salmon enhancement hatcheries
- Inform disease management practices on farms and in salmon enhancement hatcheries
- Inform policies on minimal testing required to release smolts to the ocean
- Inform policies on the placement of farms within the migratory pathways of wild salmon

Strategic Salmon Health Initiative:
Project Team Acknowledgements

Fisheries and Oceans Canada

Genomics: Karia Kaukinen, Angela Schulze, Shaorong Li, Tobi Ming, Amy Tabata, Norma Ginther

Histopathology: Emiliano Di Cicco (PSF), Hugh Ferguson (Scotland)

Epidemiology/Sequencing: Raph Vanderstichel, Ian Gardner (UPEI), Curtis Suttle (UBC)

Salmon Ecology / Physiology: Scott Hinch, Tony Farrell (UBC), Stephen Cooke (Carlton), David Patterson, Strahan Tucker

Students/PDFs: Art Bass, Amy Teffer, Ken Jeffries, Nathan Furey, Aimee Lee Houde, Katrina Cook, Jacqueline Chapman, Christine Stevenson, Steve Healey, Yovela Wang, Gideon Mordecai, Emilie Lauren, Krishna Thacur, Dianna Jarmillo

Project Leads: **Brian Riddell (PSF)** and Kristi Miller (DFO)

Genome British Columbia

NSERC CRSNG

Questions?

PACIFIC SALMON FOUNDATION

Genome BritishColumbia

Fisheries and Oceans Canada | Pêches et Océans Canada

ATLANTIC VETERINARY COLLEGE · UNIVERSITY OF PRINCE EDWARD ISLAND

CVER

UPEI Centre for Veterinary Epidemiological Research

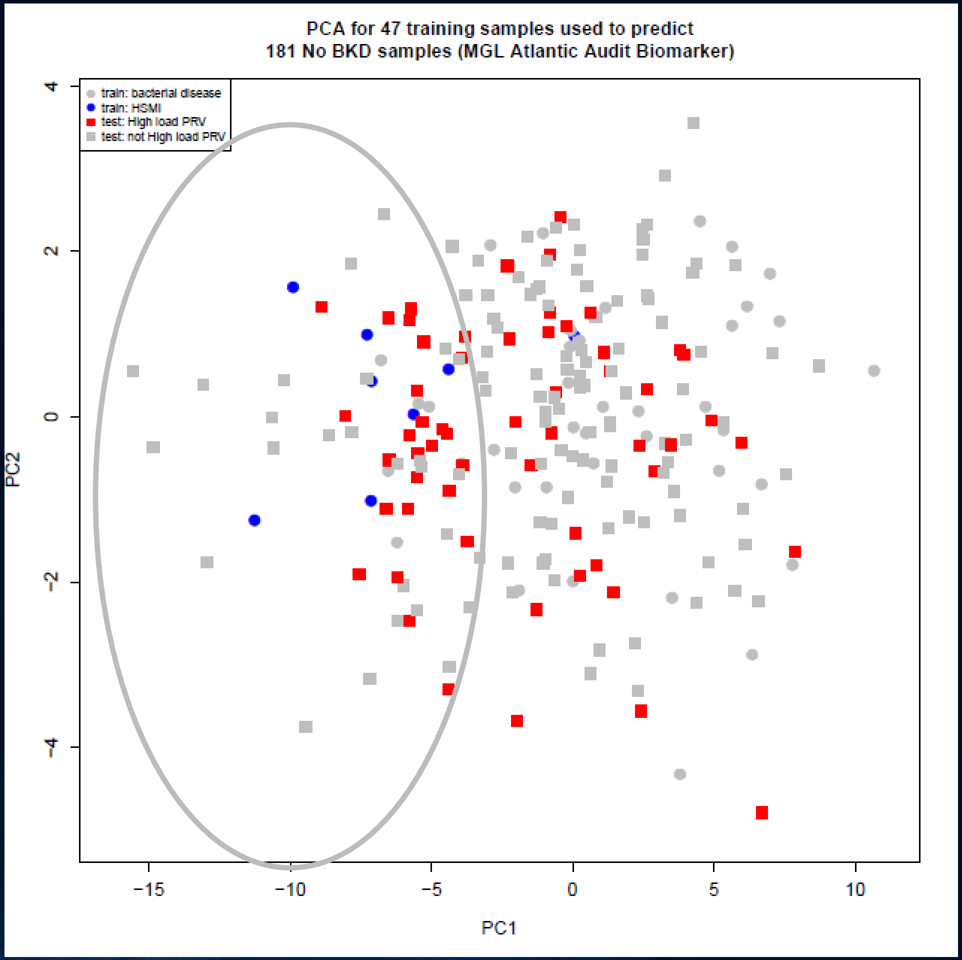
FLUIDIGM

Molecular biomarkers – early disease detection

50% of Atlantic salmon containing high loads of PRV are in a "viral disease state"

25% of which are diagnosed with jaundice/anemia

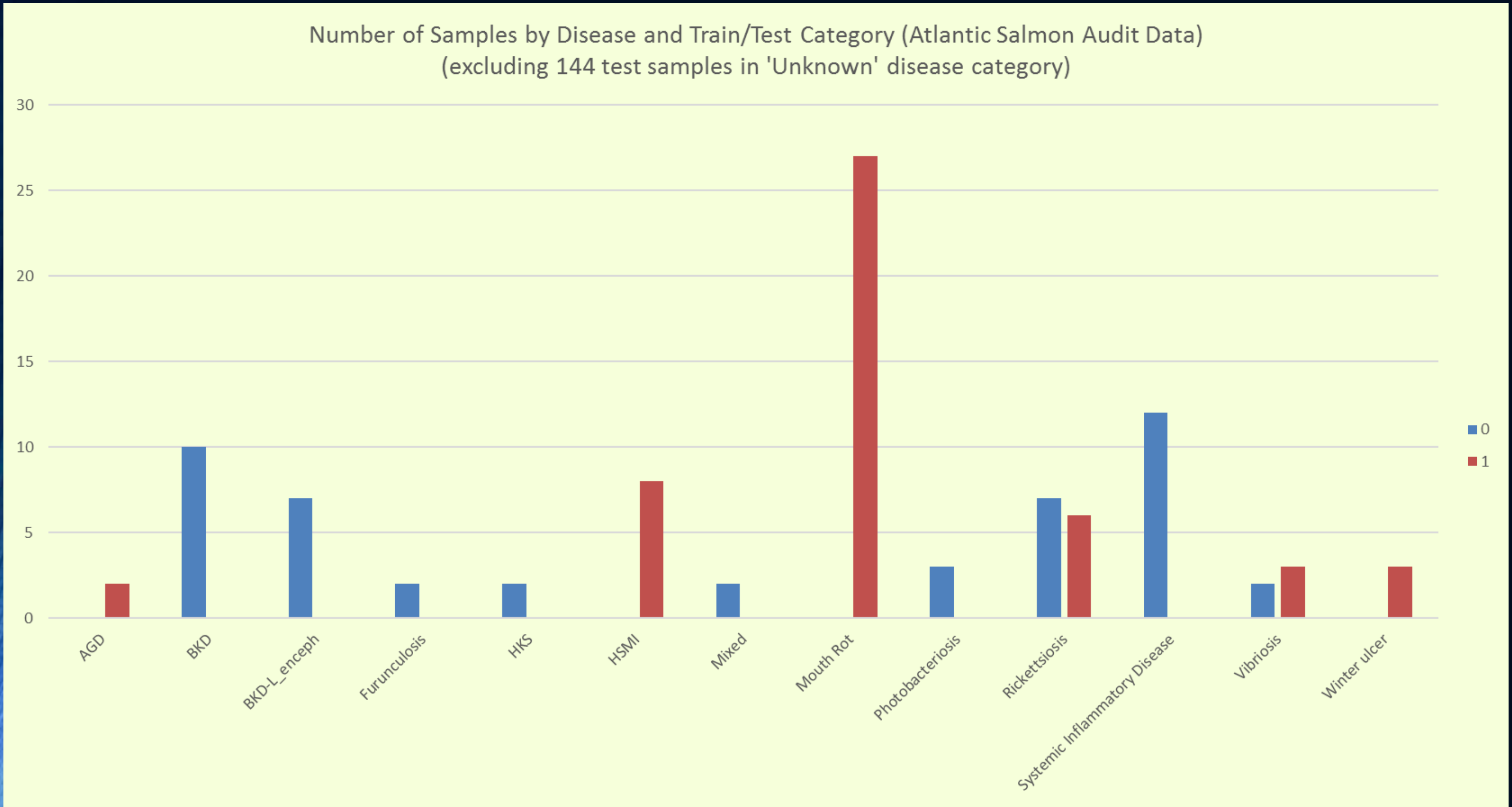
34% of dying chinook are in a VDD state—half with unknown viral associations



What do we know about PRV impacts on Pacific Salmon?

- Jaundice and anemia has been reported in farmed BC Chinook salmon, *Oncorhynchus tshawytscha*, coincident with high load PRV infections
- Jaundice and HSMI lesions associated with PRV infection has also been observed in farmed Rainbow Trout (Norway; Olsen et al. 2015) and Coho salmon (Chile – Godoy et al. 2016; and Japan – Takano et al. 2016).
- A BC challenge study failed to reproduce mortality, clinical signs, or the lesions found on fish dying of jaundice on the farms (Garver et al. 2015).
- However, the study identified mild lymphohistiocytic endocarditis lesions (early stage inflammation of the heart) exclusive to challenged fish and in all the three species tested (Chinook [60%], Sockeye [70%] and Atlantic [20%] salmon).
- Japanese study found cause and effect relationship between a novel strain of PRV and EIBS associated jaundice in Coho salmon (Takano et al. 2016)
- At this point, it would be remiss to discount these global findings in our assessments of “risk” of PRV to Pacific salmon

Diagnostic Distrubution of Atlantic salmon audits



Diagnostic Distrubution of Chinook salmon audits