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

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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
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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 >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
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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™:
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

### 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

### 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

### 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

12 viruses
22 parasites
13 bacteria

[Image 0x0 to 720x540]
Infectious agent monitoring identifies the shifting prevalence, abundance (load), and diversity of infectious agents through smolt and adult migration.
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
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

**Parvicapsula minibicornis**
- FW-transmitted myxozoan
- Kidney disease

High prevalence and load fall/winter

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

Increased Auklet predation risk in QC Strait sockeye
Linking infectious agents with disease
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

Infectious Hematopoetic Necrosis Virus
Associated with stimulated immune-related signature and high, early losses of smolts

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

Anti-viral
T-cell activity
Antibody
Inflammation
Stress

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.
Holding Study
Adult salmon

2013
Chilliwack River
Chinook Salmon

Cryptobia salmonististica
FW Transmitted
Blood Flagellate Causing Anemia

C. salmositica infection impacts
blood physiology

C. salmositica infection reduced survival

Amy Teffer
In prep
Microbes impacting fate associated with negative physiological impacts at molecular level

Early Stuart Adult Sockeye Salmon Holding Study

Early Mortality:
Stress, inflammation, tissue damage, immunosuppression

Transcriptional Signatures

Delayed Mortality:
Adaptive Immunity
Antibody production

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
SSH1 Longitudinal Farm Sampling

Disease Outbreak

Farm A

Farm B

Farm C

Farm D

Live
Moribund/dead

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

Normal Heart

Mild Lesions

Severe Lesions

Normal Skeletal Muscle

Inflamed Skeletal Muscle

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?
Kidney: renal tubular necrosis
Liver: single cell necrosis
Heart: Normal
Heart: Mild Inflammatory Lesions
Heart: HSMI Lesions
Kidney: renal tubular necrosis
Liver: single cell necrosis

Jaundice Syndrome in Chinook Salmon

Heart and Skeletal Muscle Inflammation in Atlantic Salmon

Viral Diseases in BC Farmed 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
Infected salmon 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

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

Pre-symptomatic viral disease development
Viral Disease Development [VDD]
Salmon RNA viruses

VDD – predictive for all viruses

Krasnov 2011
Skjseol 2011
LeBlanc 2010

Published signatures
Union (532 features)

Exploration Analysis
Gene Shaving, Sparse Independent PCA

Purcell 2011
Miller 2007

VDD 44 biomarker panel
Molecular biomarkers – early disease detection

Molecular biomarkers identify development of viral disease state

Time Course Plot for 45 samples (injected-headKidney) and 39 features from IHNV Sockeye (data scaled)

standardized log2 expression ratios

control_day1 day1 day2 day4 day6 day8 day10 day12 day14

-2 -1 0 1 2

IHNV Infection challenge

Disease…………………mortality

PCA for 52 samples and 40 features

MGL Jaundice Chinook Biomarker

PC1

PC2

Combined Indice Score 0

Combined Indice Score 2 or 3

Viral jaundice

Healthy

Farmed Chinook

Miller et al. In Review
Conservation Physiology
Molecular biomarkers – early disease detection

Molecular biomarkers identify differentiating 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.

PCA for 17 training samples used to predict 40 All Diseases samples (MGL Pacific Audit Biomarker)

- High PRV
- High PRV and Jaundice
- Novel viruses
- Viral 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
• 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

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Questions?

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Fluidigm
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

Molecular biomarkers – early disease detection
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 HSNI 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 Distribution of Atlantic salmon audits

Number of Samples by Disease and Train/Test Category (Atlantic Salmon Audit Data) (excluding 144 test samples in 'Unknown' disease category)
Diagnostic Distribution of Chinook salmon audits

Number of Samples by Disease and Train/Test Category (Pacific Salmon Audit Data)