Multi-species quantitation with eDNA – is it possible?

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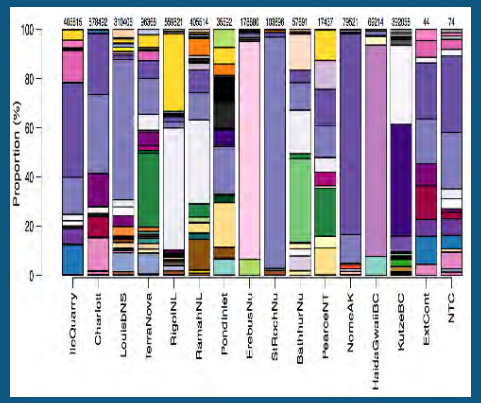
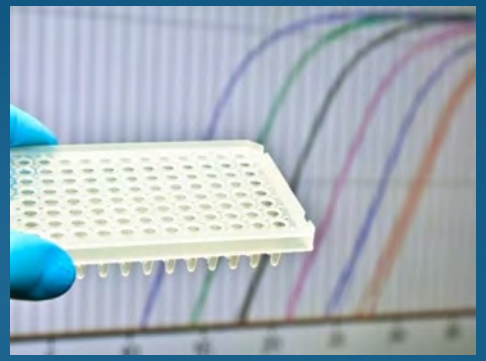
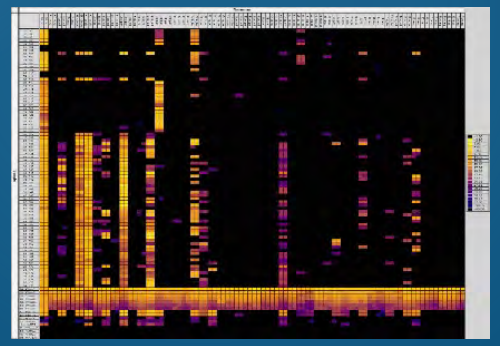
PICES 2019

Fisheries and Oceans Canada

Environmental DNA







1: Species biodiversity: metabarcoding and high throughput sequencing

- Ecosystem monitoring
- Zooplankton assemblages
- Diet analyses (scats or stomach contents)
- Industrial bioremediation

2: Single species quantitation or presence/absence: qPCR

- Invasive species assessments
- Species at risk
- Species surveys—occupancy models, range expansion, abundance estimates

3: Multi-species quantitation: microfluidics qPCR

- Ecosystem-based stock assessments
- Diet quantitation
- Infectious agent distribution and spread; transmission risk
- Salmonsphere--quantitating salmon, their predators, prey, competitors

eDNA quantitation in the salmonsphere

- Salmon occupy freshwater and saltwater ecosystems
  - Freshwater: rivers, lakes
  - Estuaries
  - Marine: coastal and offshore
- Stock assessments use different methods to assess presence, abundance, biomass, catch per unit effort
  - Freshwater—electrofishing, hydroacoustics, beach seining, trawl and gillnet sampling, fence enumeration
  - Ocean—trawl and gill net sampling, hydroacoustics
- Program establishing eDNA methods for relative quantitation of the salmonosphere, including:
  - Biological agents negatively impacting salmon: Pathogens and harmful algae
  - Salmon species
  - Marine fish competitors/prey
  - Planktonic prey
  - Predators

Literature on eDNA quantitation

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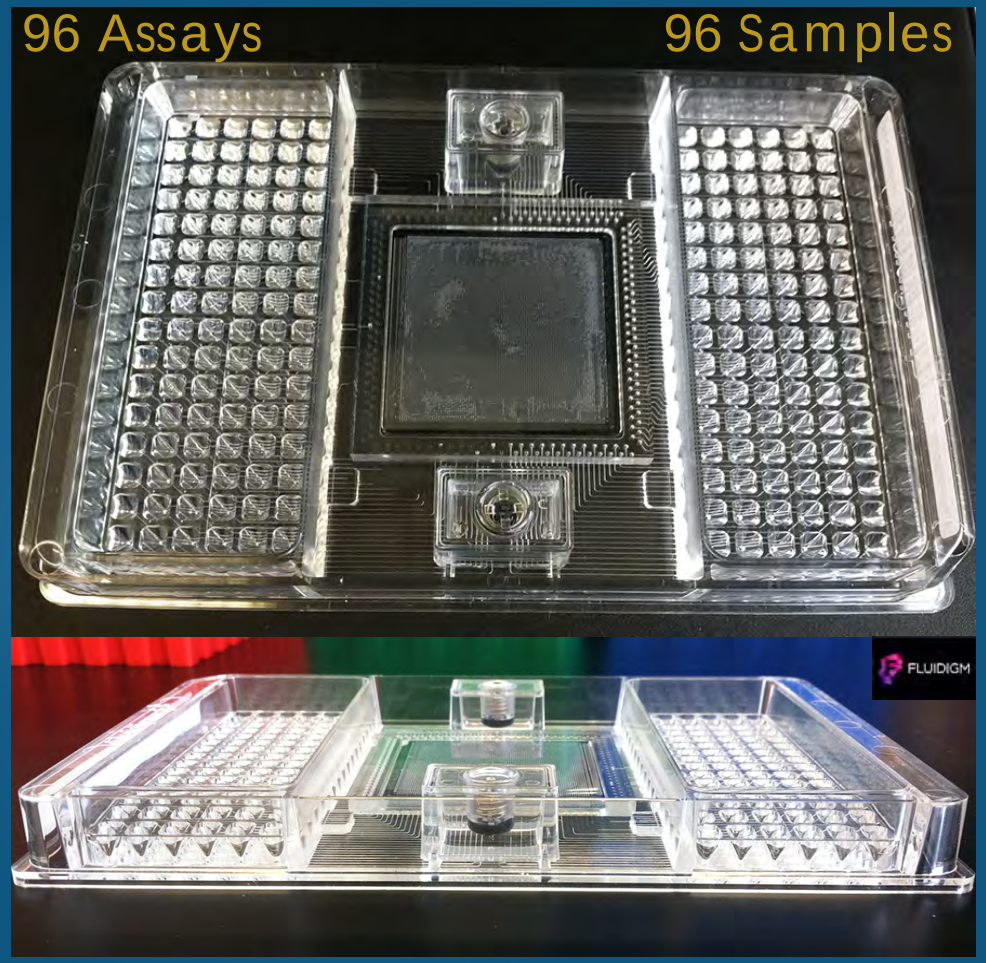
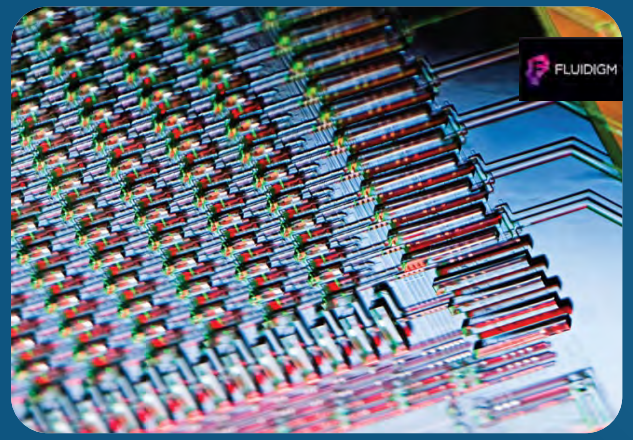
Multi-species quantitation:

Fluidigm BioMark™ Microfluidics System:

High Throughput qRT-PCR



Images: web.biosci.utexas.edu



96x96 Dynamic Array
= 9,216 reactions / run

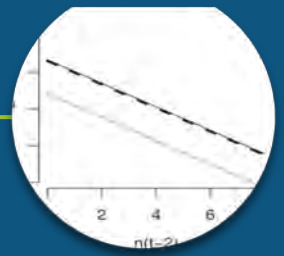
Proven method for simultaneous quantification of 47 salmon pathogens from tissue samples

→ applications to eDNA?

Quantitating Pathogens: Salmon farms and pathogen spillover

Sustained farm transmission

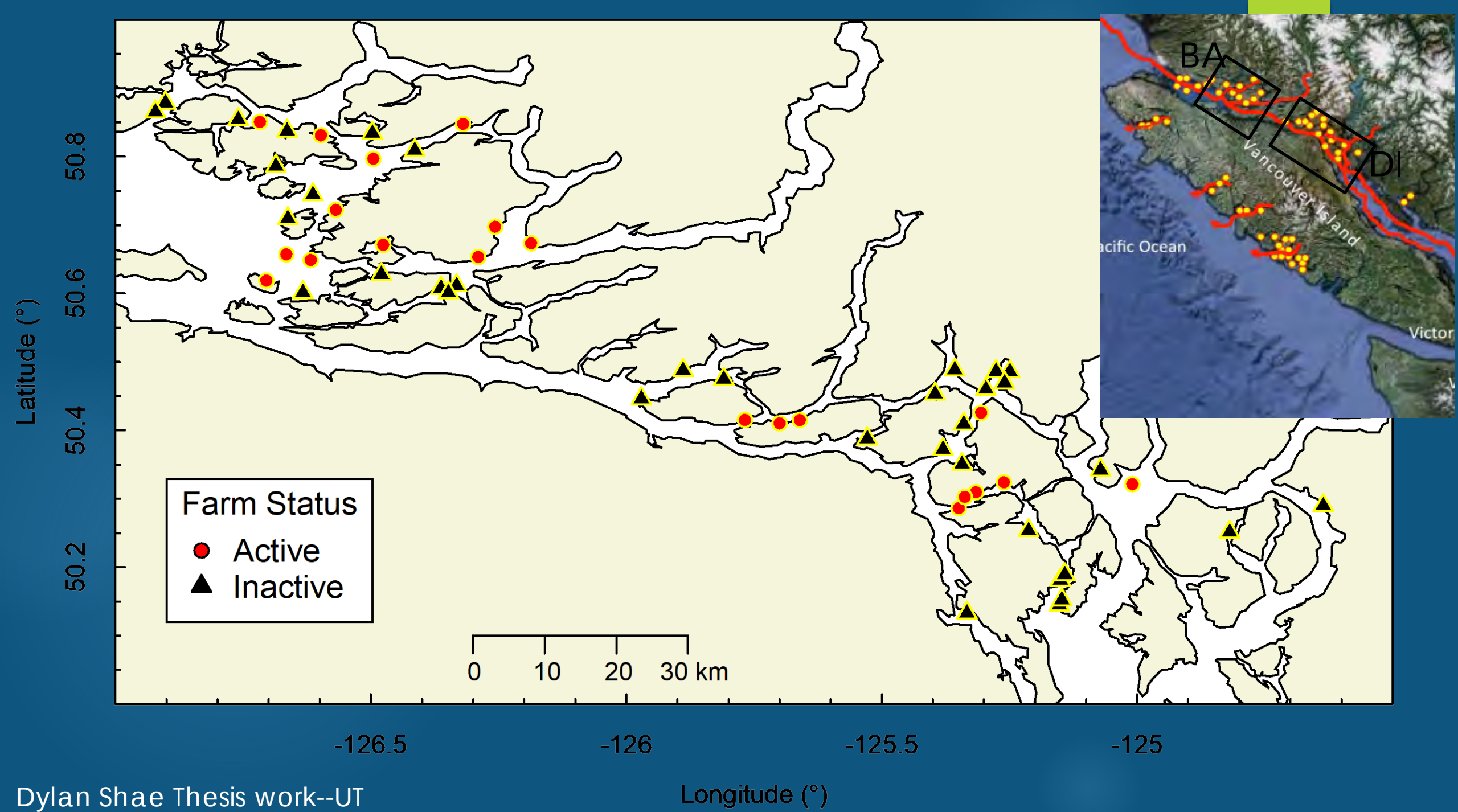
Density independent transmission in wild hosts



Disease-induced declines (Krkosek et al., 2007)

Dylan Shae Thesis work--UT

Aerial Photo: Sea Shepherd
Farm Pen Photo: George Quocksister Ju



Dylan Shae Thesis work--UT

Herring are attracted to active farm sites

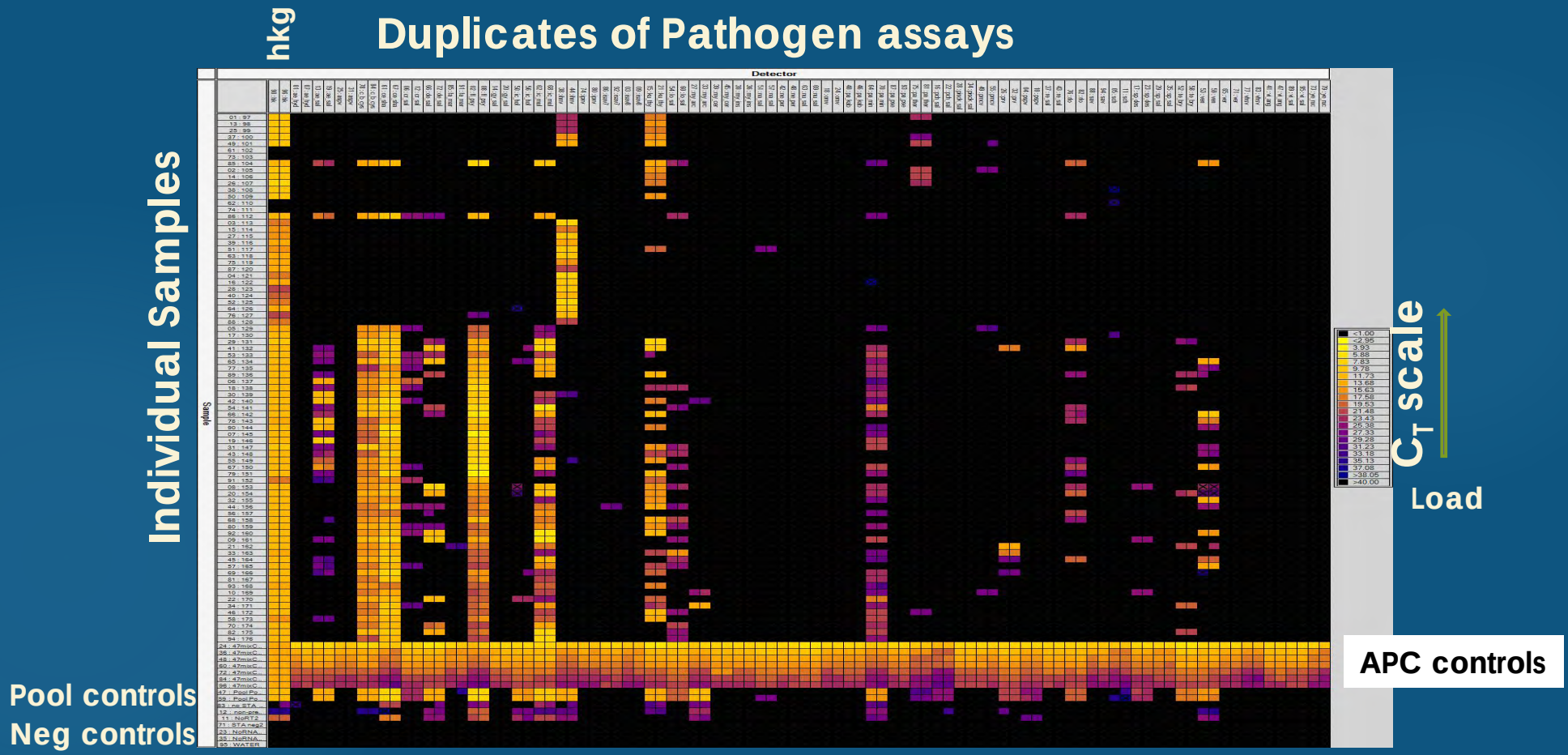
Photo:Simon Ager

Dylan Shae Thesis work--UT

Photo: George Quocksister Junior

Fluidigm BioMark™:

High Throughput Pathogen monitoring



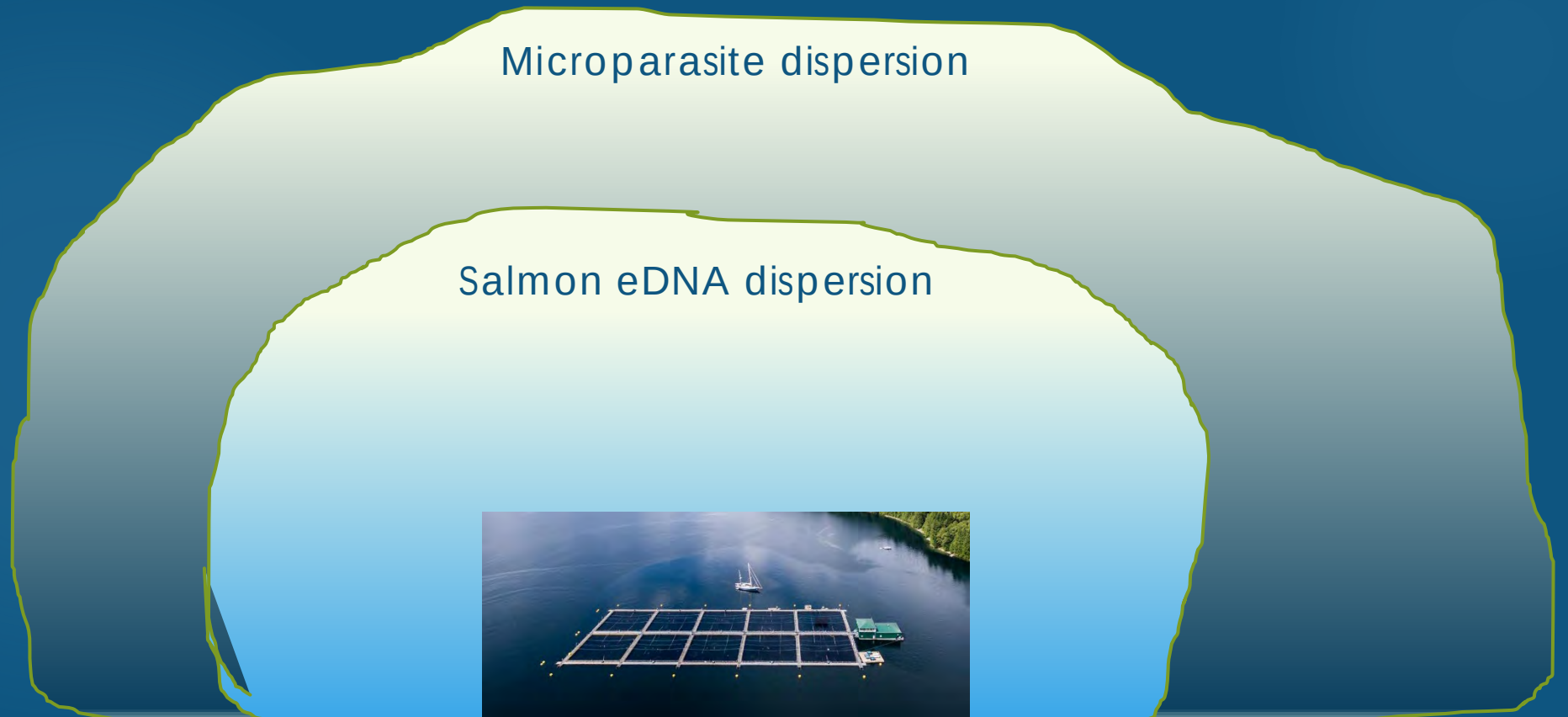
Pathogen Detections (2017)

Agent	Classification	Pathology	Detections	
			Active	Fallowed
Flavobacterium psychrophilium	Bacteria	coldwater disease	2	2
Moritella viscosa	Bacteria	winter ulcer	9	3
Piscirickettsia salmonis	Bacteria	piscirickettsiosis	13	5
Uncultured Chlamydia spp.	Bacteria	gill chlamydia	19	33
Tenacibaculum maritimum	Bacteria	tenacibaculosis	2	0
Vibrio anguillarum	Bacteria	vibriosis	0	1
Vibrio salmonicida	Bacteria	anemia	3	1
Yersinia ruckeri	Bacteria	enteric redmouth disease	1	1
Erythrocytic necrosis virus	dsDNA virus	erythrocytic necrosis	8	8
Newly characterized RNA Virus	dsRNA virus	Unknown	2	0
Viral encephalopathy and retinopathy	ssRNA (+sense) virus	viral nervous necrosis	1	0
Facilispora margolisi	Microsporidian parasite	various symptoms	1	0
Paranucleospora theridion	Microsporidian parasite	proliferative gill disease	6	8
Ichthyophonus hoferi	Protozoan parasite	muscle lesions	1	0

Consistency of results between years

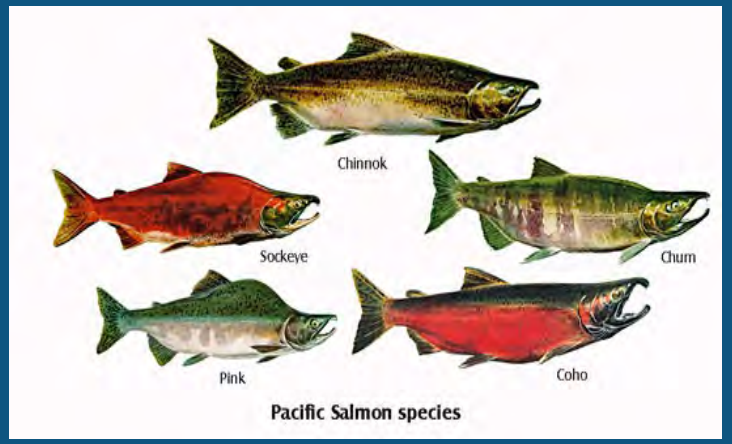
Biosecurity Monitoring: Next Steps

- Assess dispersion of pathogens away from salmon farms
- Use stationary biomass of non-endemic Atlantic salmon to assess coastal dispersion of fish eDNA
  - Given differences in longevity/degradation (days to weeks vs <1 day), expect that microparasite dispersion is considerably greater than fish eDNA



eDNA: “Salmonsphere”

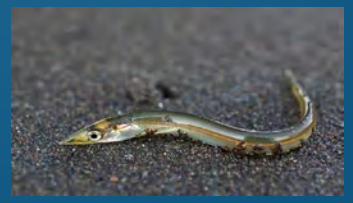
Species-Specific Assays to Fishes









14 assays for 13 species validated on the fluidigm

- Salmon: Atlantic (2), Chinook, chum, coho, sockeye, pink
- Marine fishes: anchovy, eulachon, herring, pollock, sandlance, stickleback, surf smelt
- Under Development
  - Trout: steelhead, cut throat, bull trout
  - Marine fishes: lamprey, Pacific cod, dolly varden

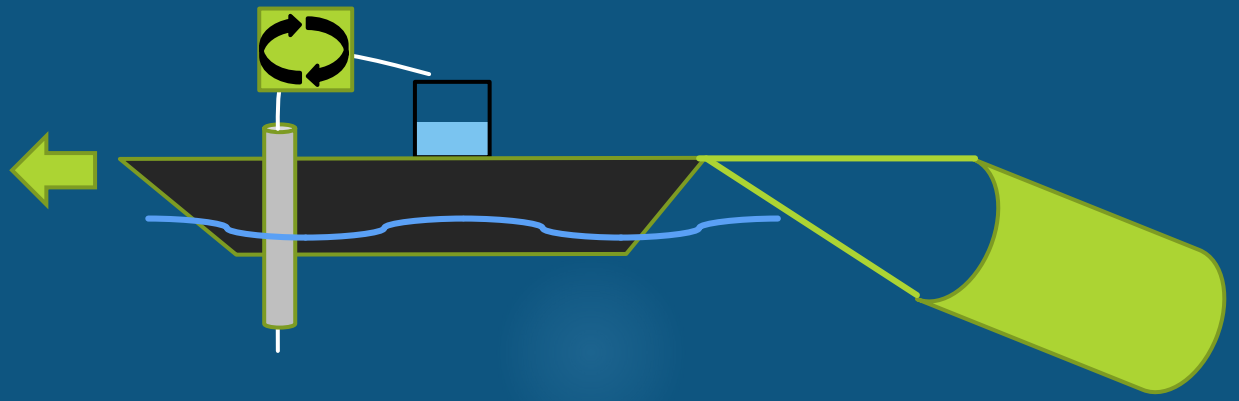




eDNA: “Salmonsphere”

Straight of Georgia

- eDNA sampling integrated with existing research stock assessment cruises
- Trawl Sampling in transects over ~1-3 km (C. Neville)
- eDNA point sampling (2017/18)
- eDNA transect collection (2019)
- Filtration (2 litres – 0.22 Sterivex filter)

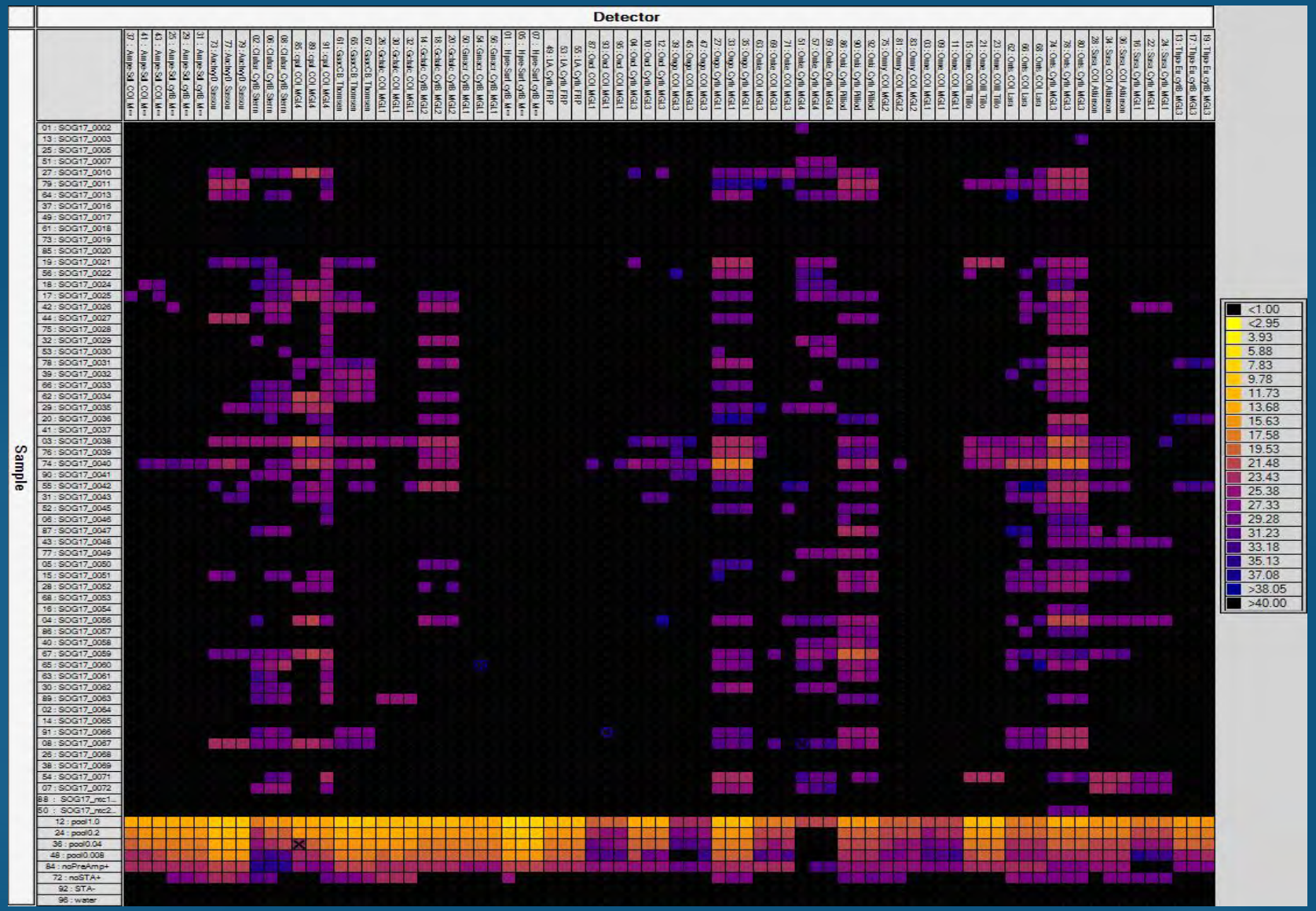


eDNA: “Salmonsphere”

Straight of Georgia stock assessment

- Samples processed
  - 2017: 60 (point source)
  - 2018: 29 (point source)
  - 2019: 44 (mostly transect)

Fluidigm Heat Map: fish eDNA



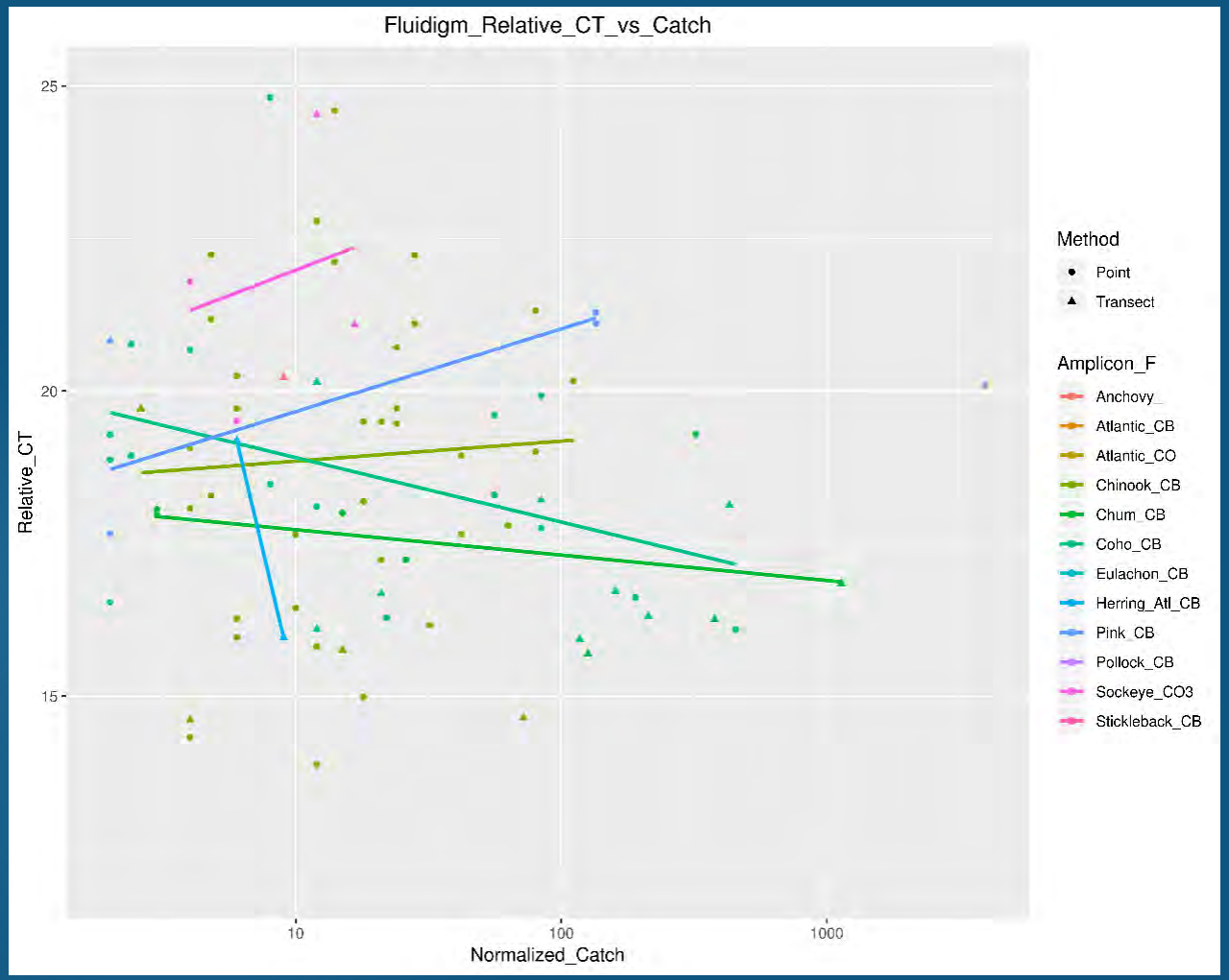
eDNA processing and detections

Species	Assay	# of Detections
SALMON		
Atlantic salmon	Sasa_Cytb_MGL1	13
Atlantic salmon	Sasa_COI_Atkinson	8
Chinook	Onts_Cytb_MGL3	48
Chum	Onke_Cytb_MGL4	16
Coho	Onki_Cytb_Pilliod	39
Sockeye	Onne_COIII_Tillo	12
Pink	Ongo_Cytb_MGL1	6
MARINE FISHES		
Anchovy	AnchvyD_Sassou	20
Eulachon	Thpa-Eu_cytB_MGL3	4
Herring	Cluhar_CytB_Sterrn	56
Pollock	Gchalc_CytB_MGL2	3
Sandlance	Ampe-Sdl_COI_MGL6	5
Stickleback	GaacCB_Thomsen	13
Surfsmelt	Hpre-Surf_cytB_MGL3	0

- Sample processing details
  - Dneasy PowerWater Sterivex kit for DNA isolation
  - Control samples of all species pooled and diluted to evaluate efficiencies
  - 9 blank field replicates (undetectable DNA, only one very weak positive)
  - No biological replicates run (yet)
- Linkages between trawl and eDNA overall (all detections vs only triplicate detections)
  - eDNA only: 65% vs 33%
  - Trawl only: 15% vs 33%
    - Note low DNA conc samples not removed
  - Co-detection: 17% vs 33%

eDNA: “Salmonsphere”

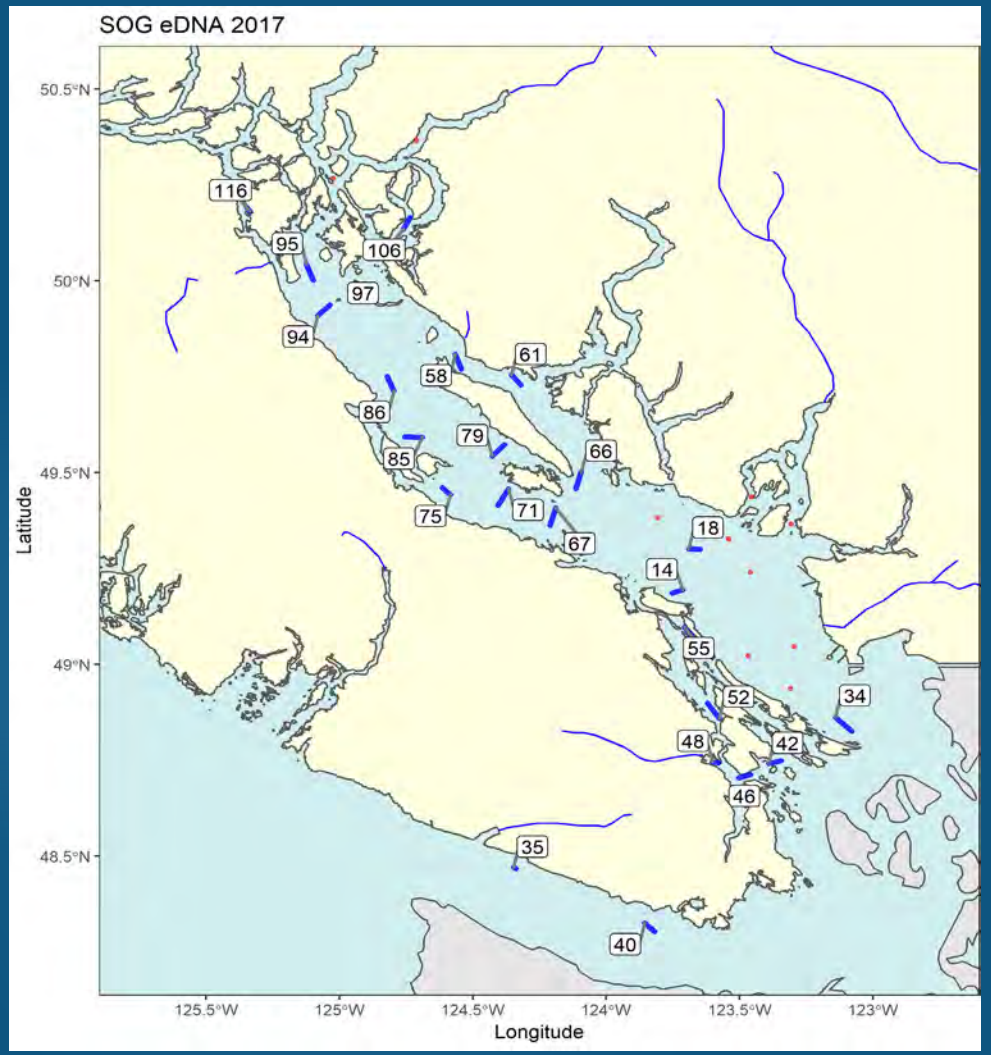
Straight of Georgia



- Positive trend for sockeye and pink salmon
- Eulachon negative trend
- Insufficient data to run transect alone

eDNA: “Salmonsphere”

Straight of Georgia stock assessment

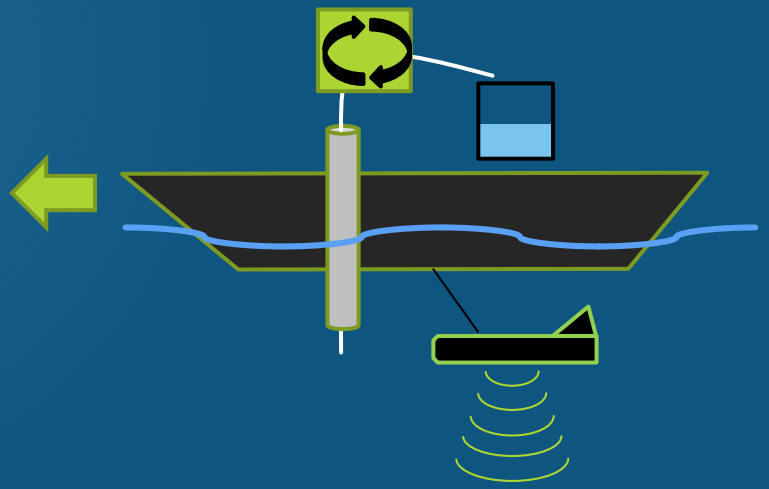


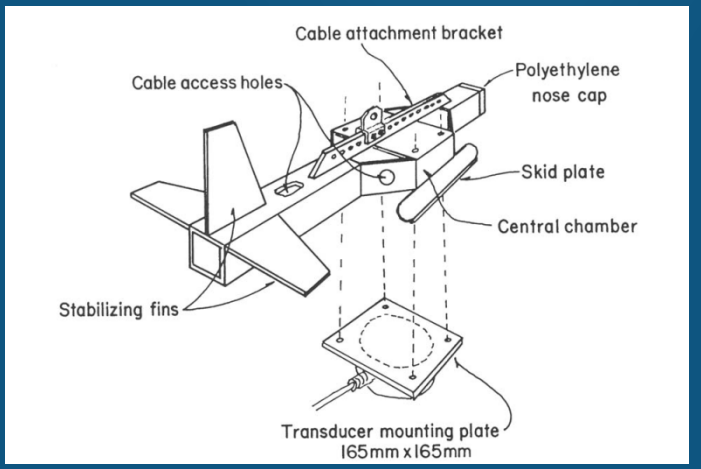
- Stochastic factors not currently accounted for:
  - Variable trawl depths sampled – 0 to 60 meters
  - Variable seasons – summer vs fall
  - Variable transect lengths – 13-30 min (approx. 1-3 km)
- eDNA dispersion scale: Direct correlation within point samples and transects vs more broadly within oceanographically defined “regions”

eDNA characterization of the “Salmonsphere”

Interior lakes juvenile assessment

- DFO Freshwater Ecosystems Section (D Selbie)
- Species, density, and biomass of pelagic fish assemblages in juvenile sockeye salmon (*Oncorhynchus nerka*) rearing lakes
- Night time hydroacoustic surveys
  - **More objective biomass estimation than trawl survey**
- eDNA survey along transect

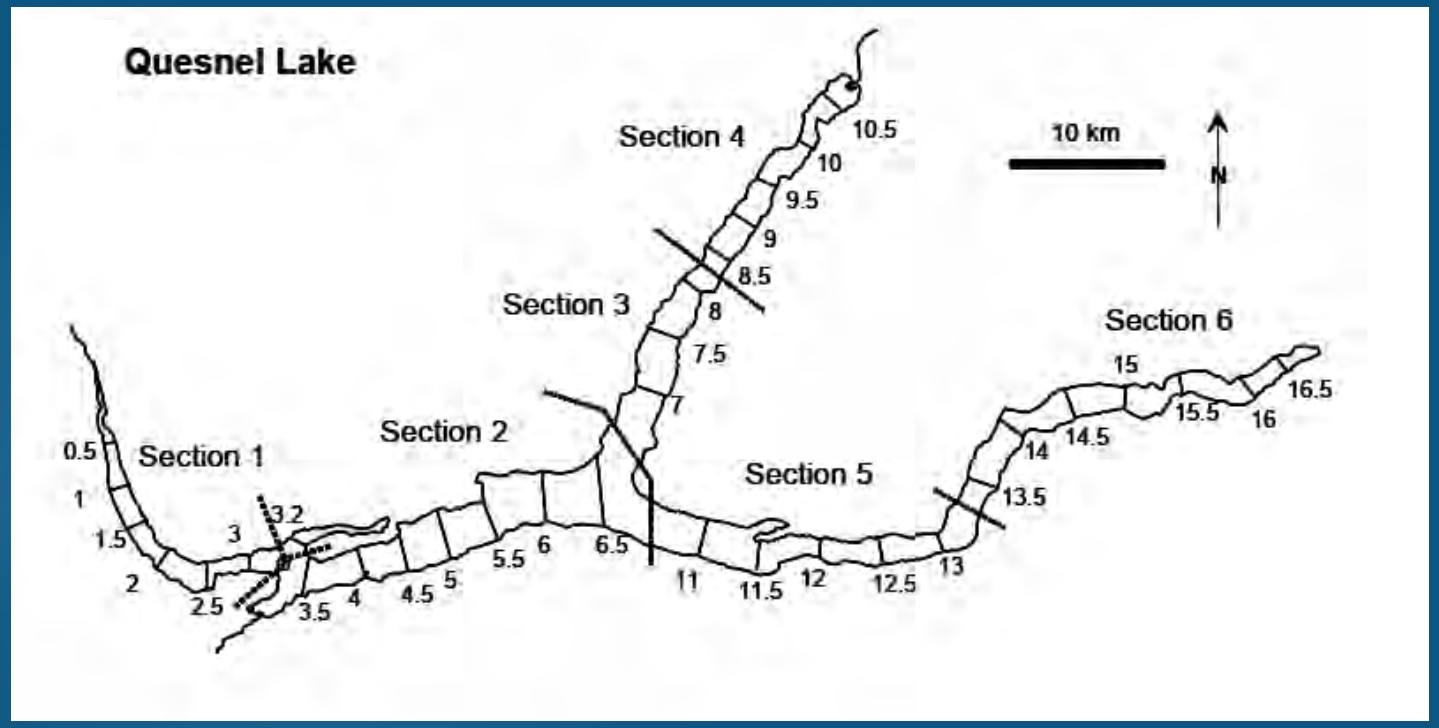




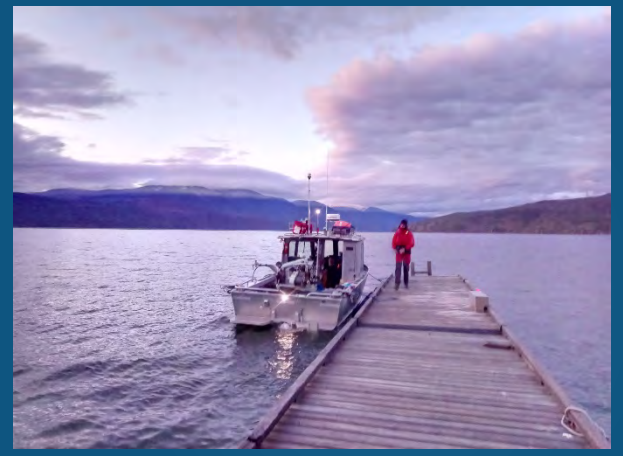
Acoustic transducer deployed from boat during hydroacoustic surveys

eDNA characterization of the “Salmonsphere”

Interior lakes juvenile assessment: Quesnel lake



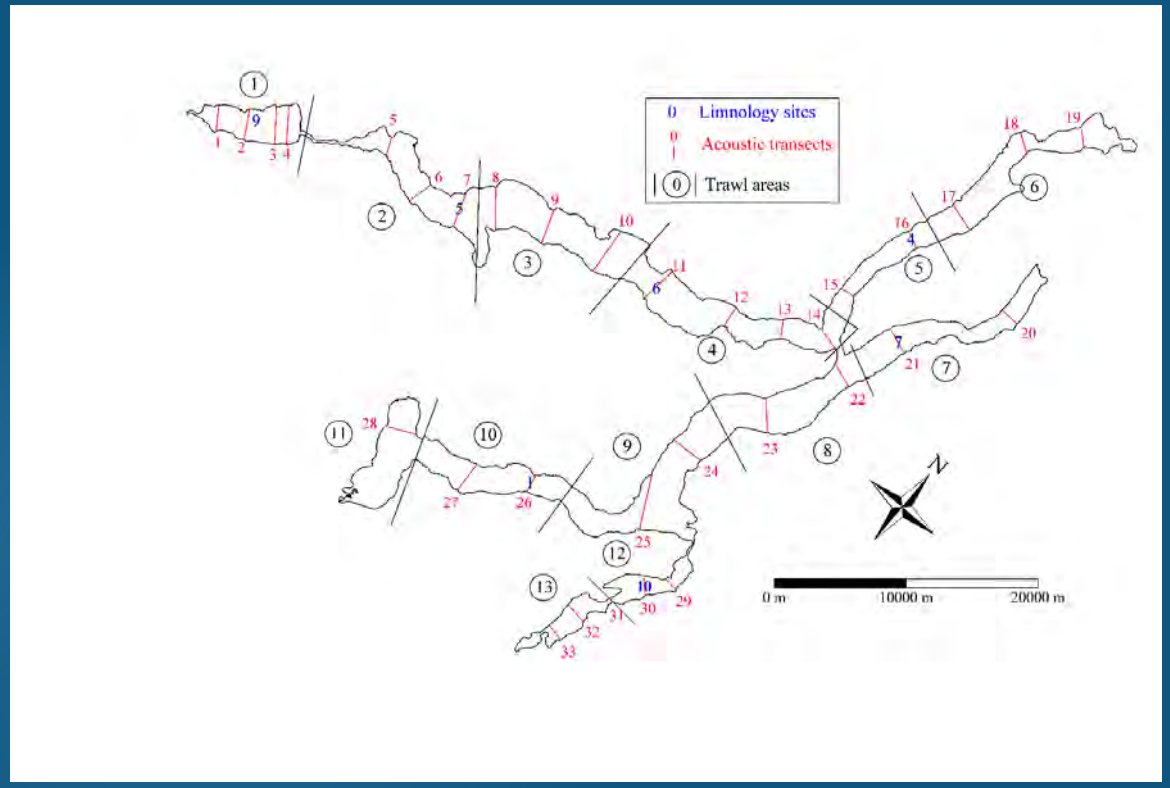
Quesnel Lake, BC, October 2019

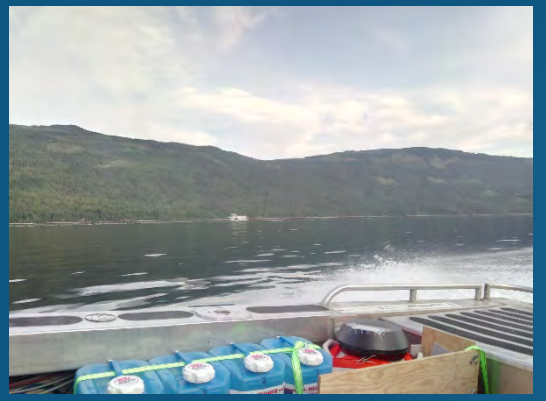


Crew heading out to night time survey and sample processing

eDNA characterization of the “Salmonsphere”

Interior lakes juvenile assessment: Shuswap Lake





Shuswap Lake, BC, August 2019 nighttime surveys

eDNA

Summary

- Simultaneous detections of 19 viruses, bacteria, and micorparasites show higher diversities close to farms, consistent with agent distributions on farms
- Species-specific assays to 13 salmon and marine fish species developed for simultaneous quantitation
- Field sampling methods to work alongside stock assessment activities developed, but stochasticity in traditional methods (e.g. depth and transect lengths sampled) may impact our ability to correlate with eDNA
- Future directions:
  - Increase sample sizes and include biological replicates
  - develop models that explore relationships between quantitations with depth, point vs transect sampling vs oceanographically-defined regions
  - Evaluate eDNA quantitation and dispersion in coastal marine environment using stationary biomass of farmed Atlantic salmon
  - Combine hydroacoustic models with established degradation rates of salmon eDNA in the laboratory to model the spatial resolution for eDNA quantitation of fish in the coastal margin