

Epidemiological investigation of Tenacibaculosis in farmed salmon in BC: what we know and our proposed approach



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Overview

- Background/Introduction
- Objectives/Hypothesis
- Study design/Data/Methods
- Environmental factors
- Host factors
- Microbiome
- Conclusion and Future Directions



Picture Source: Barry Milligan



Background

- Yellow Mouth (YM) / Tenacibaculosis
 - Emergent ulcerative disease of marine fish
 - Outbreak post seawater entry
 - Yellow plaques on the mouth
 - First peak 1-2 weeks post sea water entry
 - Second peak 3-4 months at sea
 - High mortalities (up to 40% of cumulative mortality)
 - Major cause of economic loss
 - *Tenacibaculum maritimum*, G-ve bacterium
 - *Likely multifactorial?*
 - *Predisposing conditions*



Background

- Marine diseases increasing in emergence, severity, and prevalence
 - Due to changing ecosystem
- Some are opportunistic
 - pronounced shifts in microbial community structures “dysbiosis”
- Can expect similar diseases to emerge,
 - such as ulcer disease on the west coast



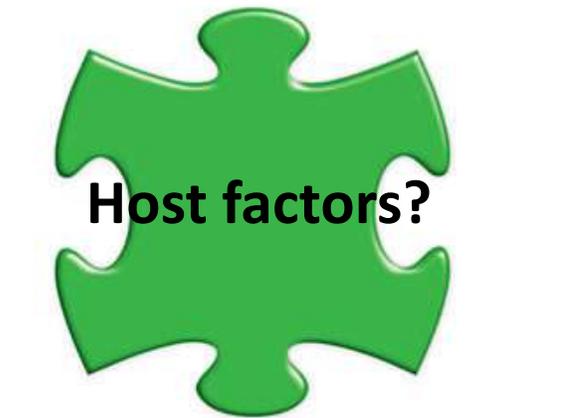
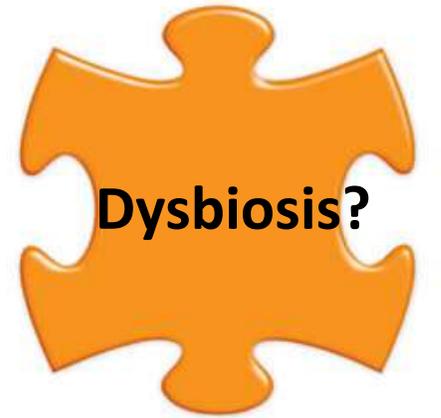
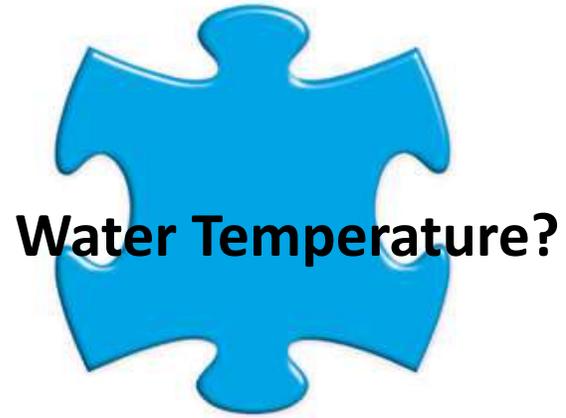
Component- Causal Model

- Sufficient cause
 - Enough to produce the disease
 - Can be a single cause (etiology) or a combination of component causes
- Necessary cause
 - Always needed for disease occurrence but not sufficient alone
- Component cause
 - One of a number of factors that, in combination, constitute a sufficient cause



Component-cause Model of Tenacibaculosis

- Tenacibaculosis puzzle
- Component -Causal model
 - Sufficient cause
 - Is there a single sufficient cause?
 - Necessary cause
 - *T. maritimum*?
 - Component cause



Hypotheses/Objectives

Hypotheses:

- *Tenacibaculum* spp. are present on the farms as opportunistic bacteria
- Outbreaks result from dysbiosis in the microbial community of affected fish

Objective:

- To elucidate the interactions between host, YM pathogen(s), and environment. Specifically,
 - Investigate the potential environmental sources of the causative agent(s) using PCR screening and 16S microbiome
 - Assess the association of environmental factors with outbreaks in BC
 - Evaluate profile differences in microbial communities between healthy and clinical YM fish



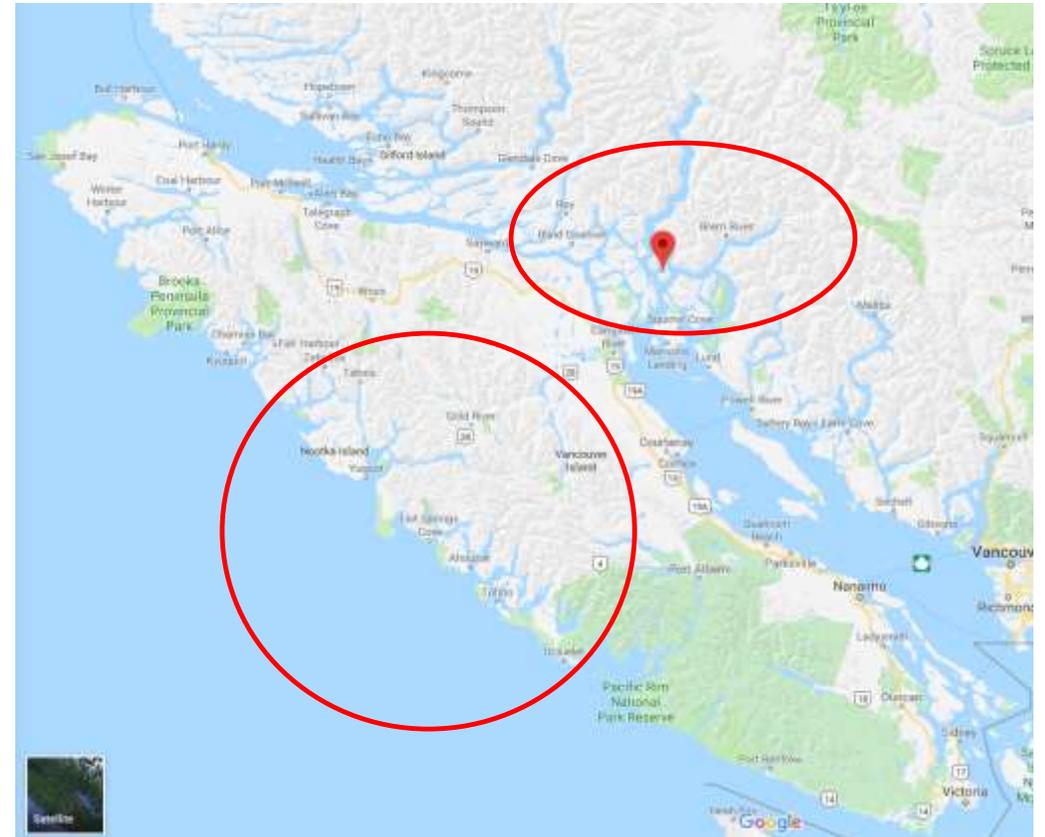
Study design

- Prospective study at selected farm sites with history of YM
 - Screening for environmental sources of *Tenacibaculum* pre-stocking, during outbreak and post-outbreak
 - Individual fish samples from healthy and clinical fish during outbreak
 - Individual fish samples from post-outbreak



Data

- Four Atlantic salmon production sites
 - Well smoltified, with and without initial outbreak
- Variables
 - Daily mortality (Yellow Mouth) %
 - Temperature
 - Salinity
 - Dissolved oxygen
 - Plankton count and index
- Additional data on individual fish from 2 sites
 - 20 healthy and 20 clinical fish with Yellow Mouth
 - Weight, length, body condition,
 - PCR test results and microbiome

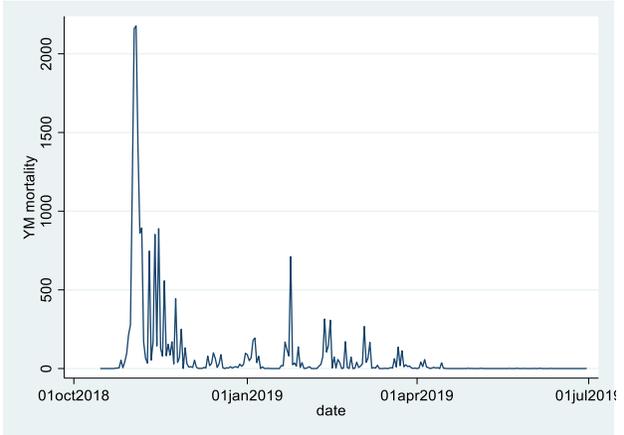
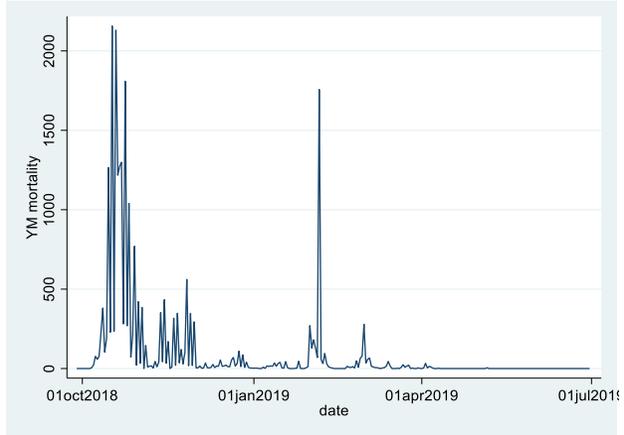
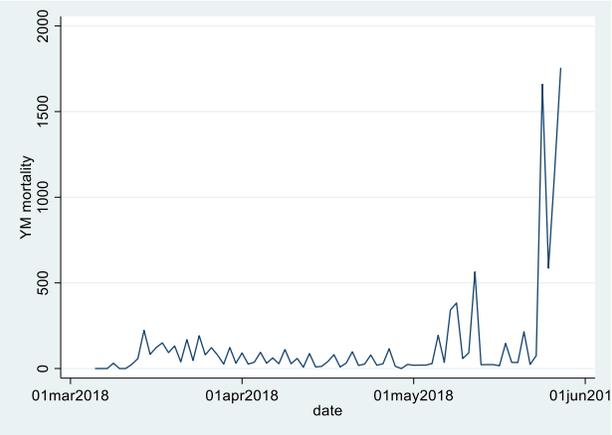
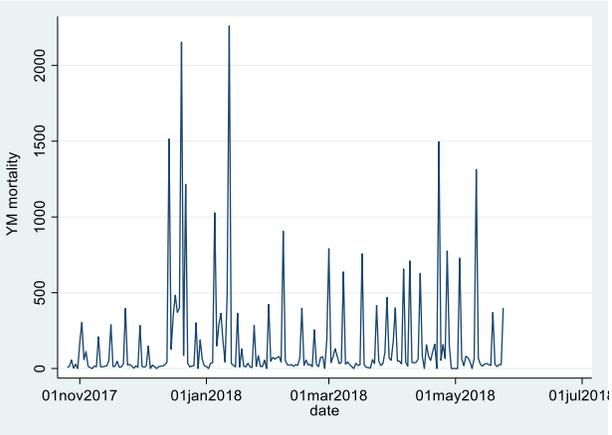
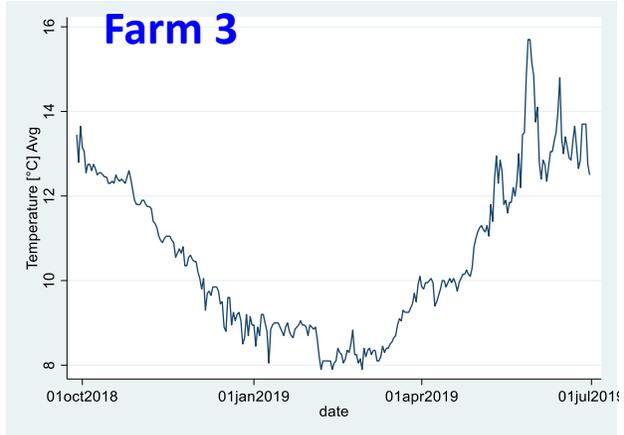
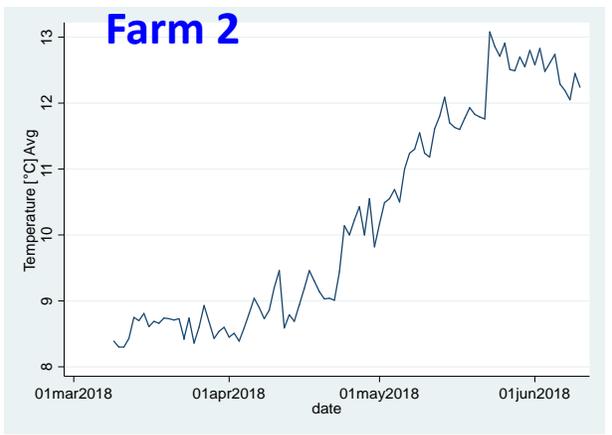


Methods

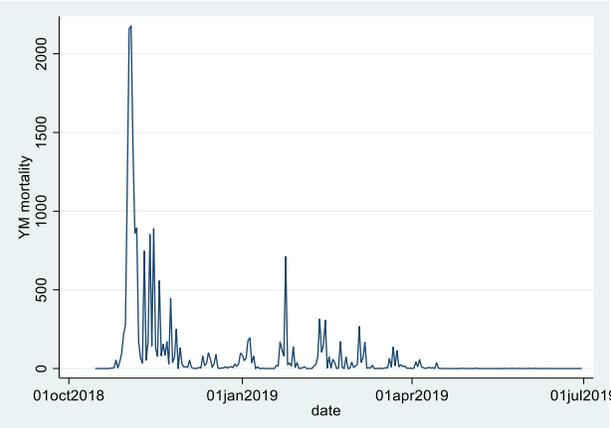
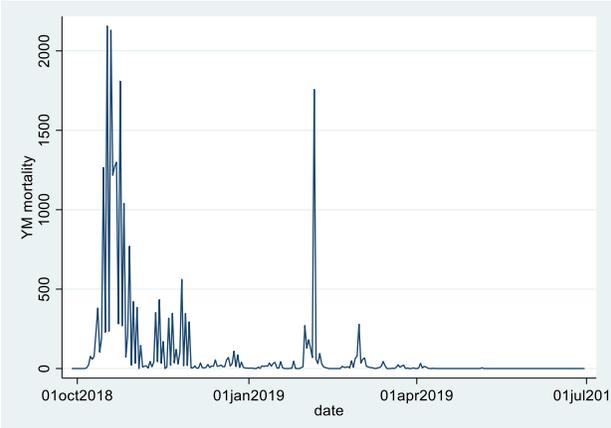
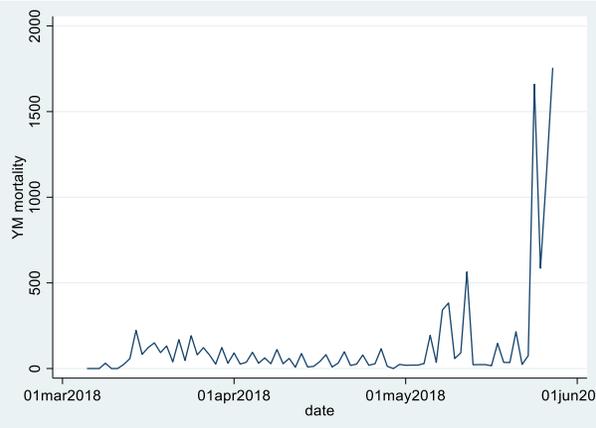
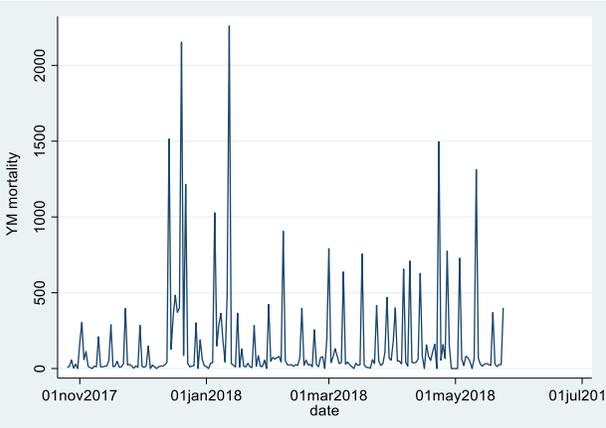
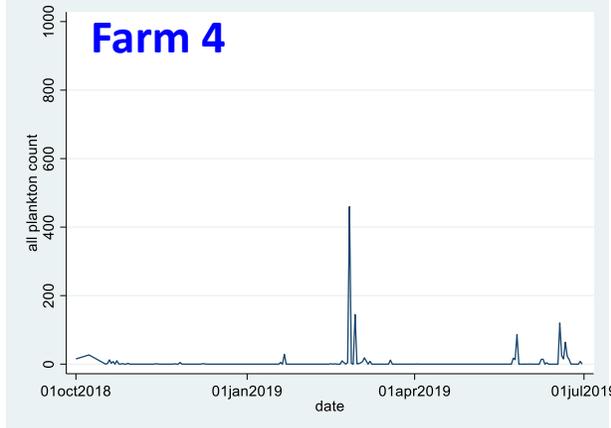
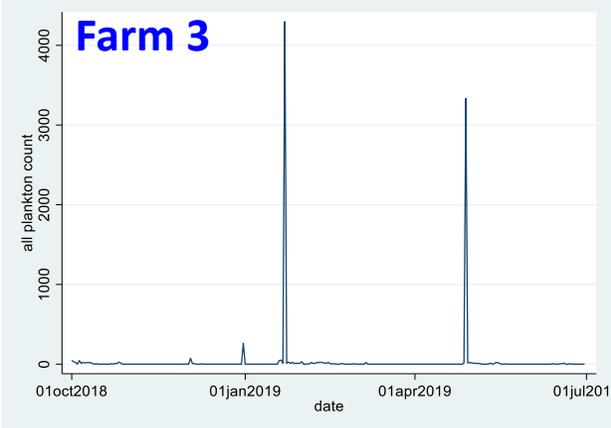
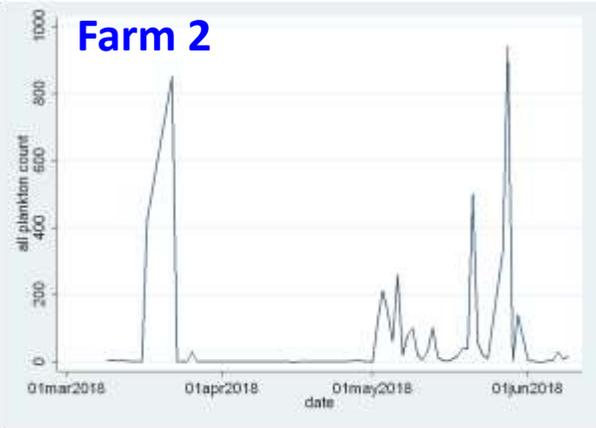
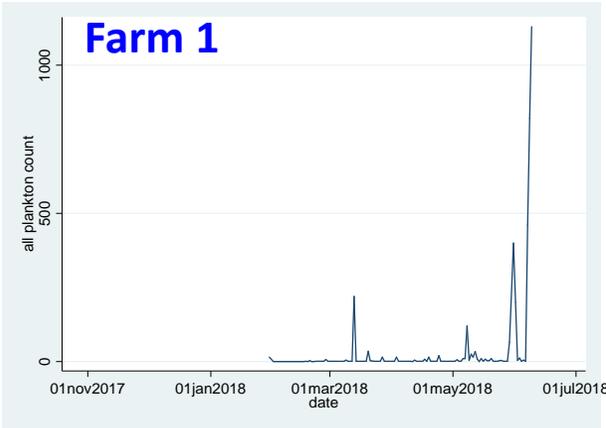
- Exploratory analysis
 - Time series plots
- Two stage time-series regression with lag effects for environmental factors
 - Such as 1-14 days moving average, 8-14days moving average, 1-7 days moving average etc.
- Association between weight, length and body condition score with clinical cases



Average daily temperature and YM mortality



All plankton count and YM mortality



Preliminary time series analysis results

- Lag effect of temperature (moving average 1-14 days before) is associated with YM related mortality
- Not enough evidence of association with harmful planktons and any planktons count with YM related mortality
- Some evidence that dissolved oxygen may also be associated with YM related outbreaks



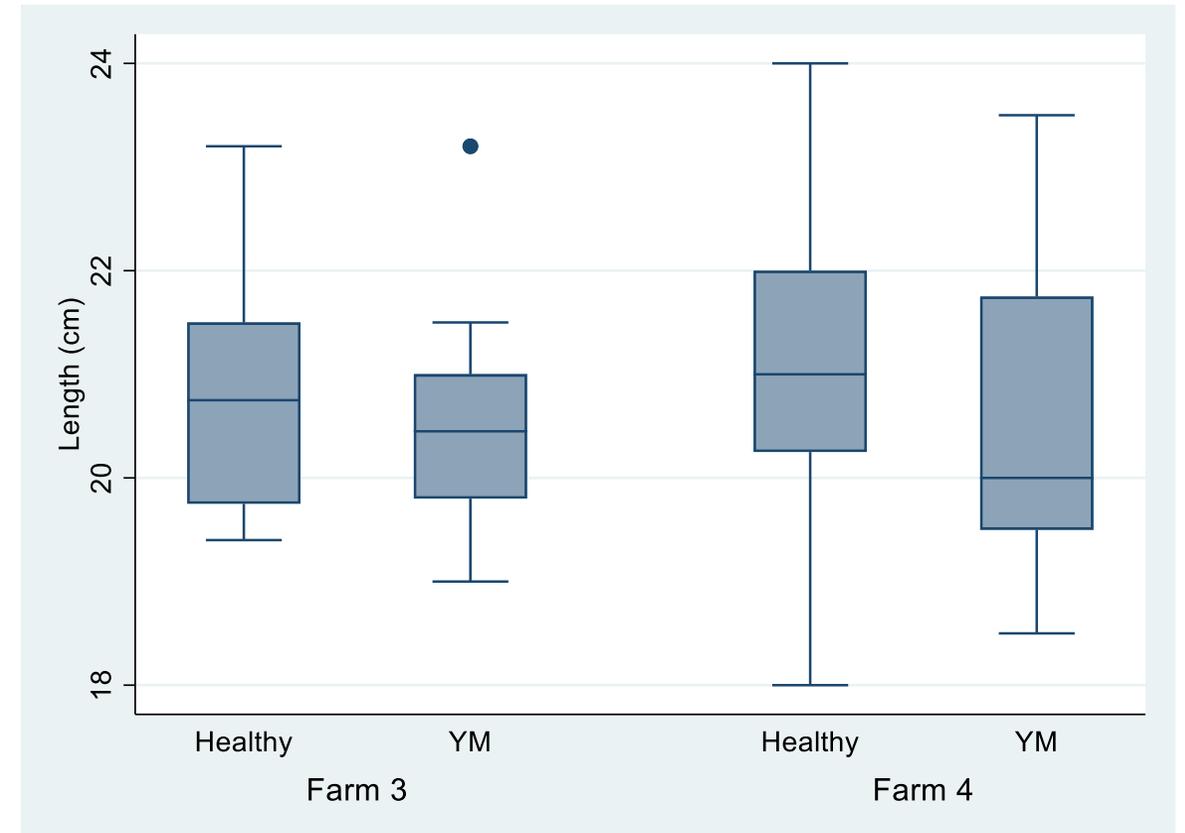
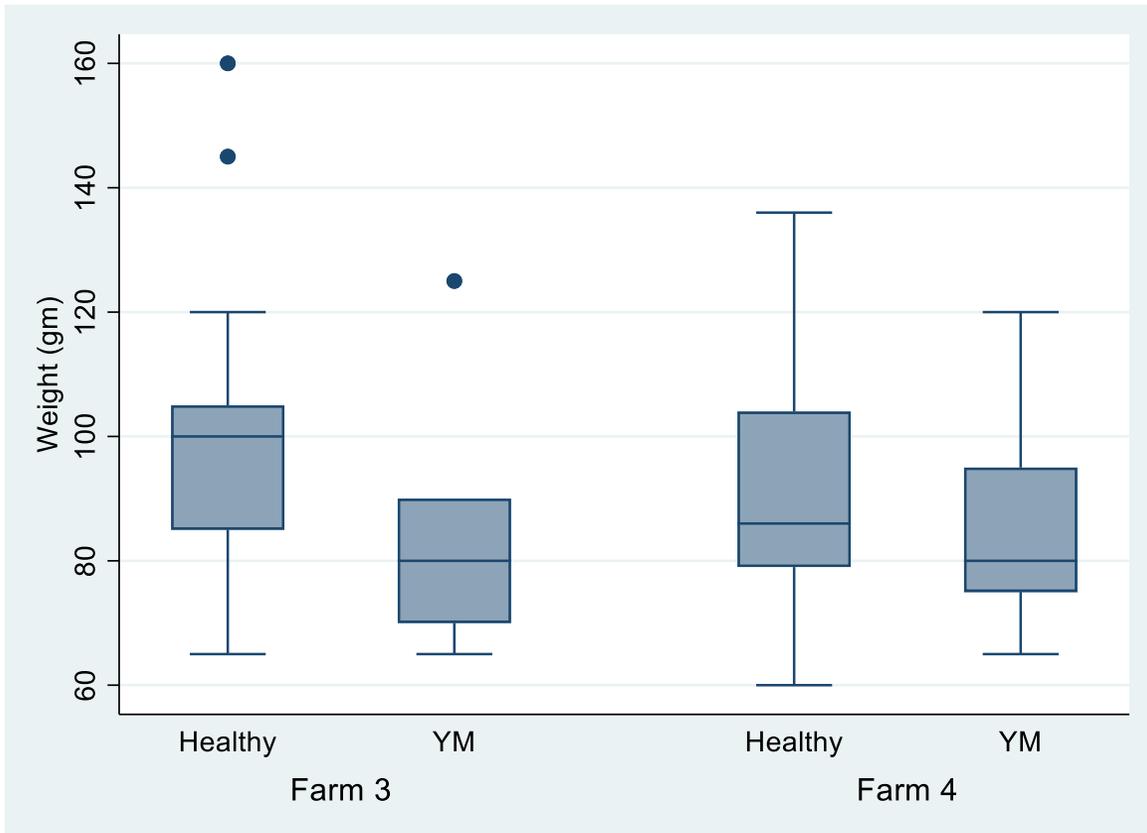
Individual fish level characteristics and their association with clinical YM cases

- PCR results for *Tenacibaculum spp* (using universal primers)
 - Individual fish samples

	Healthy Fish	Clinical YM fish
PCR Positive	1	25
PCR Negative	39	15



Individual fish level characteristics and their association with clinical YM cases

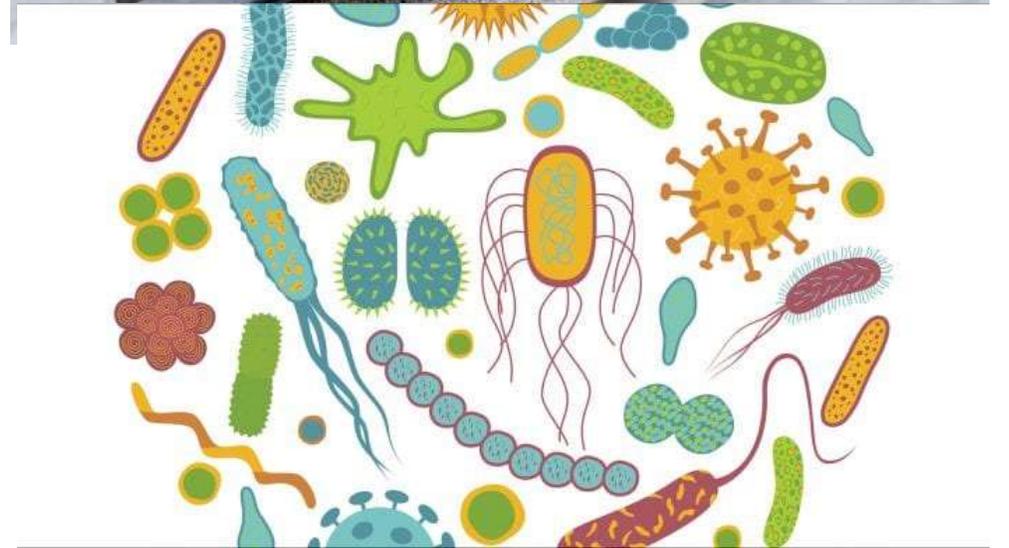


Weight significantly different in Farm 3 between healthy and clinical fish



Investigation of microbiome during Yellow Mouth outbreak

James Wynne, Krishna Thakur, Joel Slinger, Ahmed Siah |



The microbiome of Yellow Mouth



Samples taken from farm site:

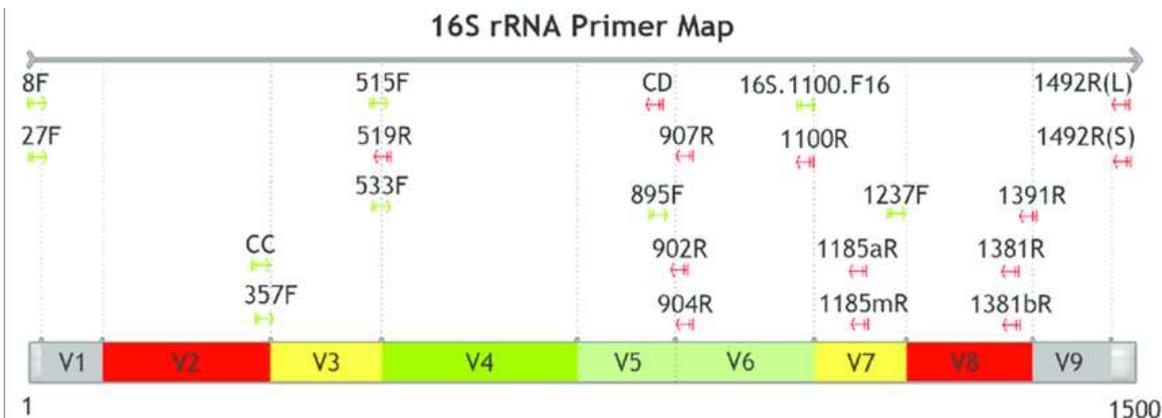
Fish

- The microbiome is the collection of all (theoretically) microbial organisms within a sample
- For bacteria we interrogate the conserved 16S ribosomal gene
- Amplify and sequence the hypervariable region V123

- 20 x swab without Yellow Mouth, P0596, Millar Channel (HF: Healthy Fish)
- 20 x swab with Yellow Mouth, P0596, Millar Channel (MP: Yellow Mouth Plaque)
- 20 x swab post outbreak, R0266, Millar Channel

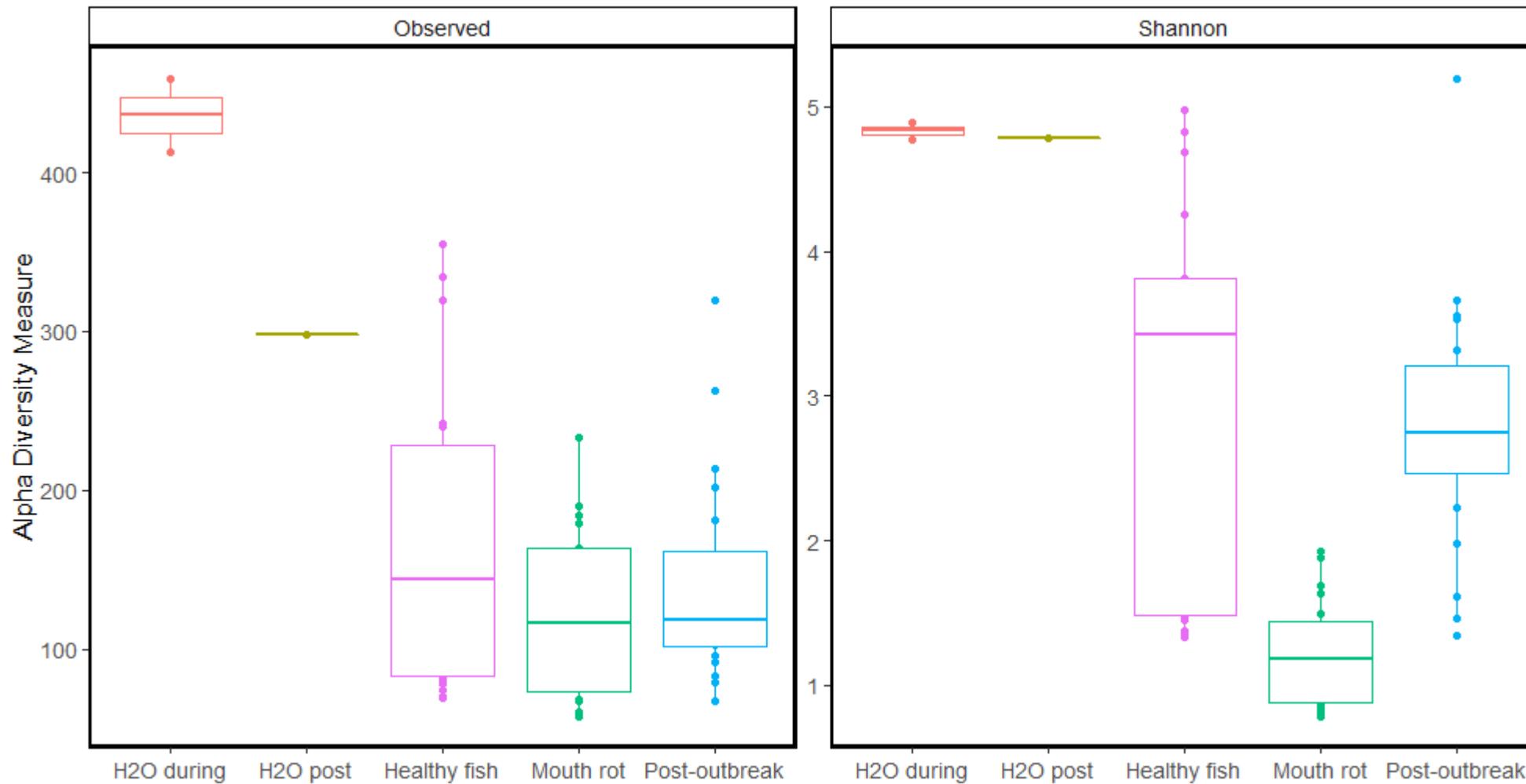
Water

- 1 x Sterivex filter, Swab Billet#1 Yellow Mouth outbreak collected Oct.12, 2018 ,Millar Channel
- 1 x Sterivex filter ,Swab Billet#2 Yellow Mouth outbreak Sept. 2018, Millar Channel
- 1 x Sterivex filtered plankton tow, post outbreak, R0266 Millar Channel

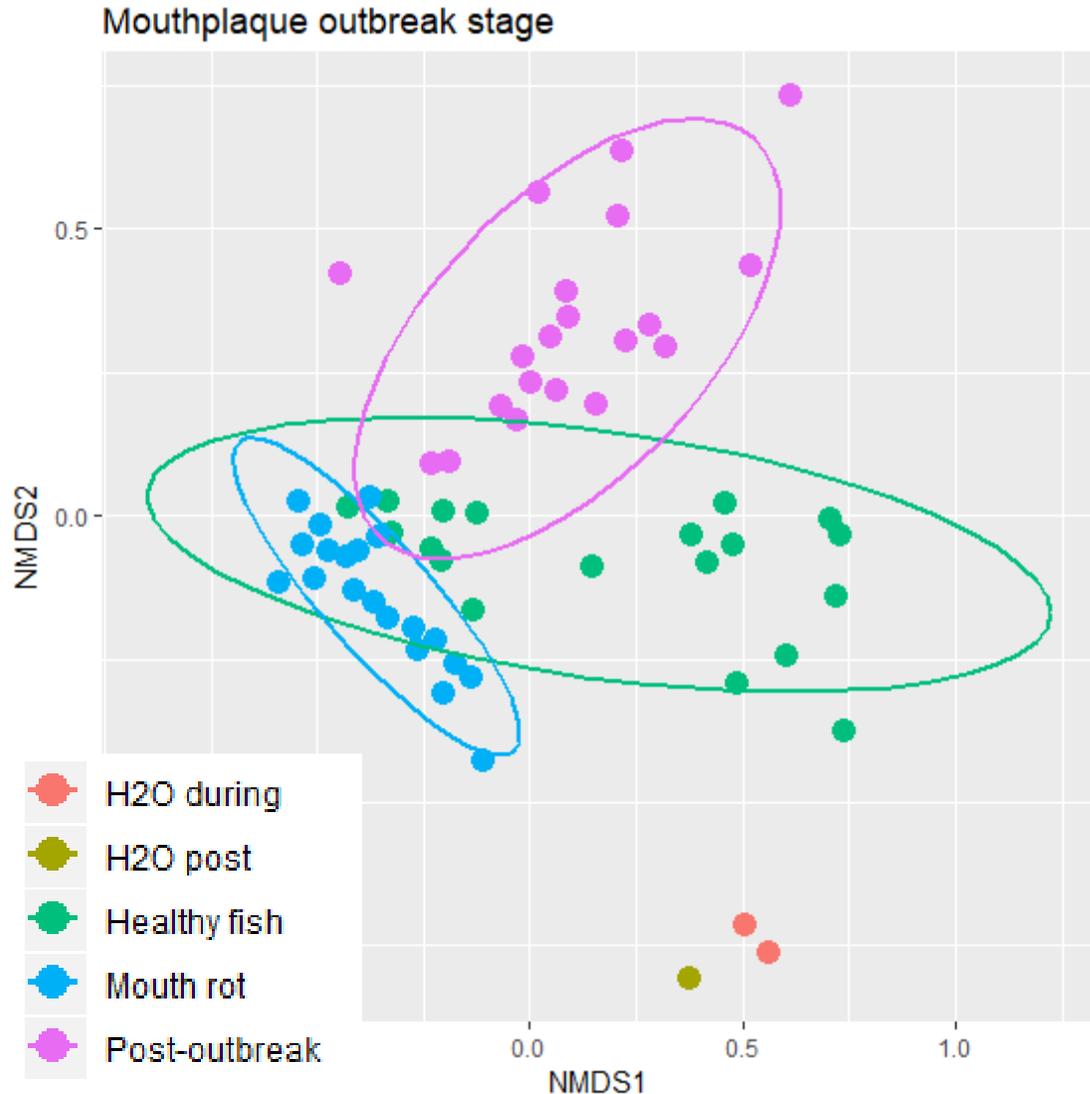




Sample richness and diversity



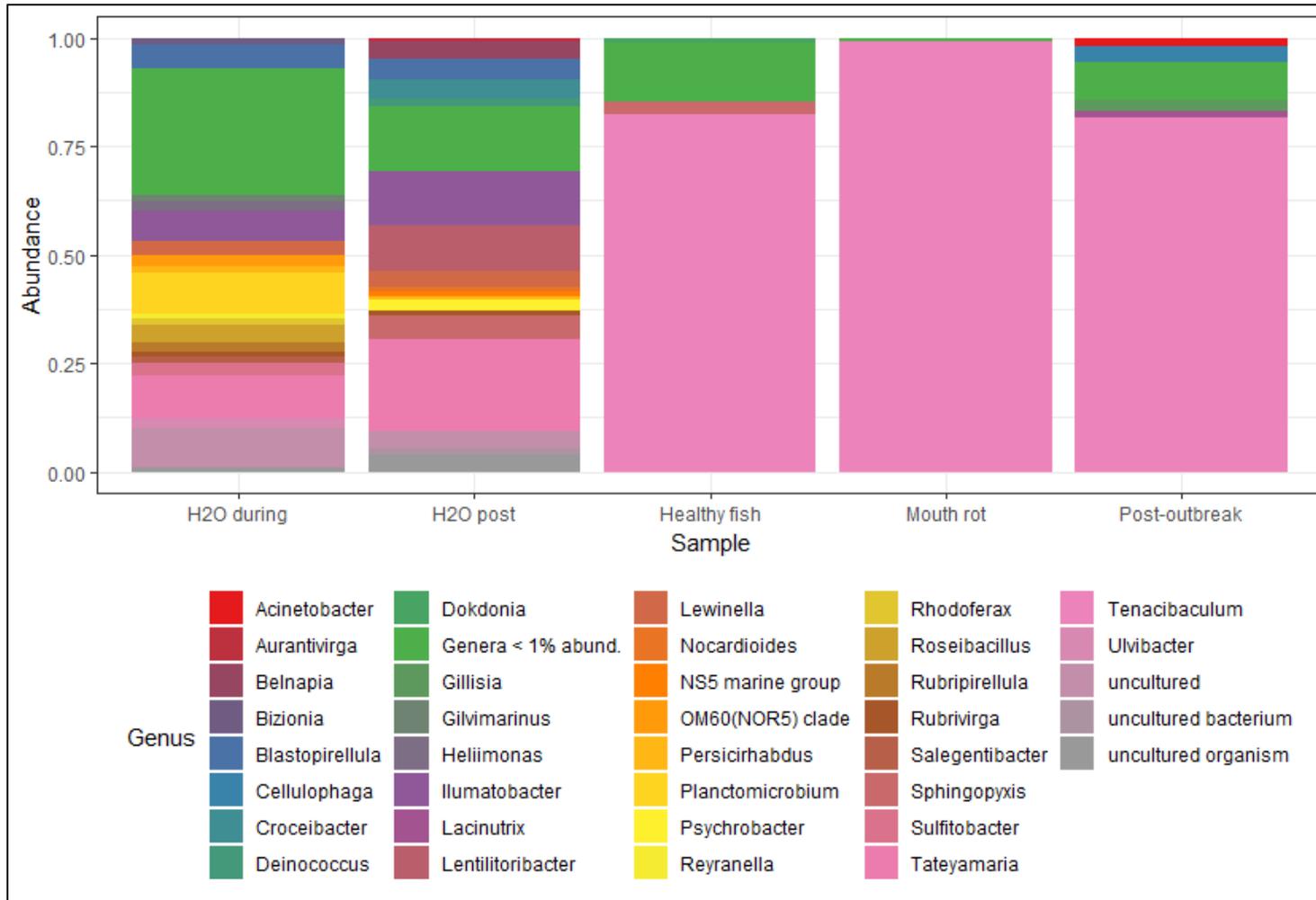
Between sample community variance



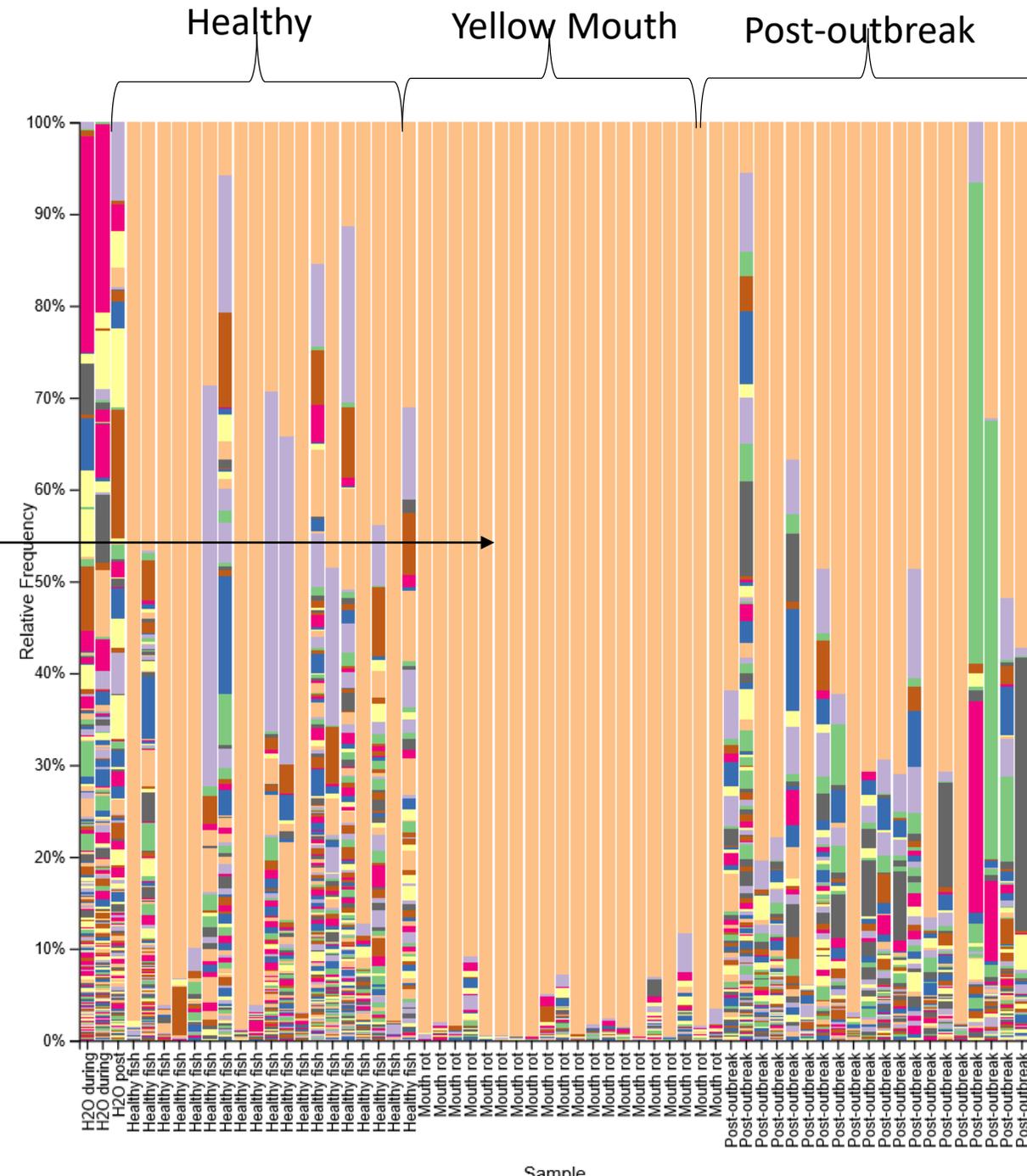
Key points

- Healthy fish don't all have the same microbiomes
- Yellow Mouth fish all have very similar microbiomes
- Recovered fish have a different microbiome
 - But it doesn't return to 'healthy'
- Water samples during and post outbreak are similar

Taxonomic abundance (samples grouped)



Microbial communities (species level) per sample



T. maritimum



Conclusions



- Yellow Mouth was characterized by a massive dysbiosis dominated by *T. maritimum*.
- Healthy fish also have very high loads of *T. maritimum* (sometimes as dominant as the diseased fish).
- The post-outbreak fish had a more diverse community than diseased fish, but this community differed from the healthy fish.
- Water samples had the most diversity compared to fish samples.



Thank you

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Overall conclusions

- We detected *Tenacibaculum* spp from the farm environment, pre-stocking, during and post outbreak as well as from healthy fish confirming our hypothesis that *Tenacibaculum* is an opportunistic bacteria
- We have also been able to demonstrate remarkable dysbiosis in microbe community in clinical YM fish
- Our preliminary findings suggest likely environmental role in Tenacibaculosis outbreaks in BC, need outbreak data from different farms to conclusively assess that relationship
- Need more data from both healthy and clinical YM fish to identify risk factors/component causes for clinical YM



Thank you

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