Oyster Microbiome and its potential role in Sydney Rock Oyster and Pacific Oyster diseases

Nachshon Siboni

UTS: Viet Khue Nguyen, William L King, Nachshon Siboni, Maurizio Labbate, Justin Seymour

DPI: Michael Dove, Wayne O'Connor, Cheryl Jenkins, Jeffrey Go





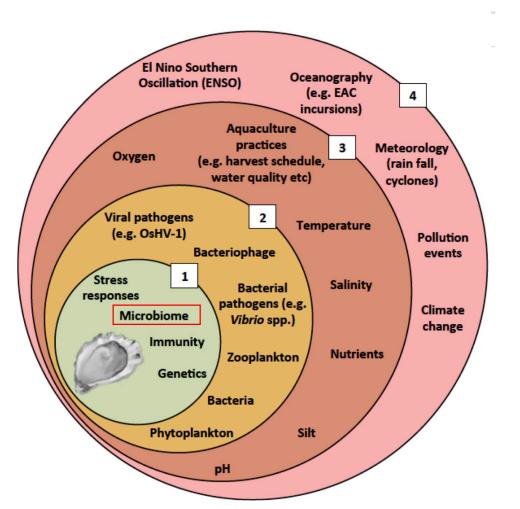


QX-disease

The Sydney Morning Herald

NATIONAL

Sydney rock oysters face wipeout from parasite



POMS – OsHV-1

Hawkesbury River oyster farming industry under threat from Pacific Oyster Mortality Syndrome

By Mohamed Taha

Updated Mon 28 Jul 2014, 3:15pm

Oyster farmers on the Hawkesbury River on the NSW Central Coast are fighting to save the crippled industry as an outbreak of disease destroys oysters.

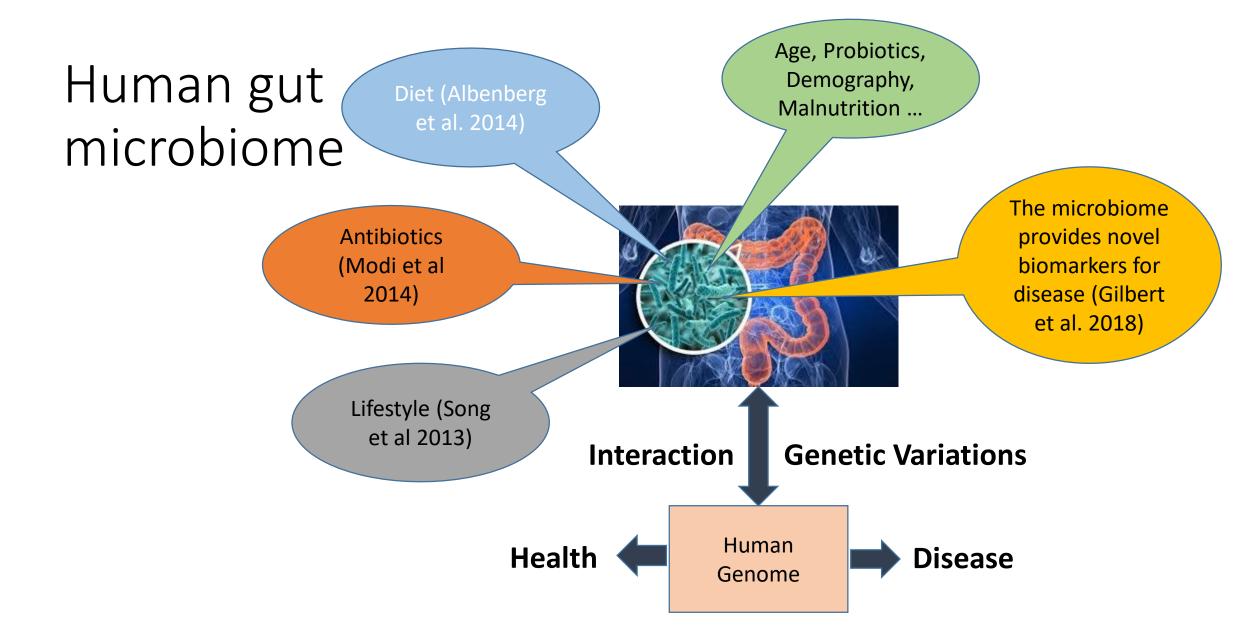
Pacific Oyster Mortality Syndrome disease found in Tasmania for first time

By Alex Blucher

Updated Tue 2 Feb 2016, 7:41pm

An oyster disease capable of wiping out the Tasmanian industry has been found in the state for the first time.





Oyster Microbiome – what was known in relate to QX and POMS



SRO; Saccostrea glomerata

• SRO digestive gland microbiota is changed following infection by spore forming protozoan parasite, *Marteilia sydneyi* (Green and Barnes 2010).



PO; Crassostrea gigas

 Mortalities are reduced when oysters infected with OsHV-1 are treated with antibiotics (Petton et al. 2015)

• OsHV-1 infection leads to immune-suppression allowing bacteria to cause disease (De longeril et al. 2018)

Hypotheses

Oyster Microbiome is involved in disease. Understanding the process that change oyster microbiome can help us to identified important triggers for those diseases.

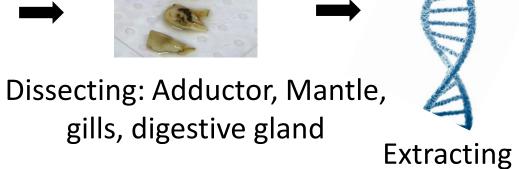
Aims

- Characterise SRO and PO microbiome composition across family lines with different level of QX and OsHV-1 disease resistance
- Characterise SRO and PO microbiome composition before and during QX and OsHV-1 disease event
- Identified bacterial groups that can act as a potential marker for disease onset

Methods







DNA



DNA sequencing (16S rRNA gene) to view the microbiome

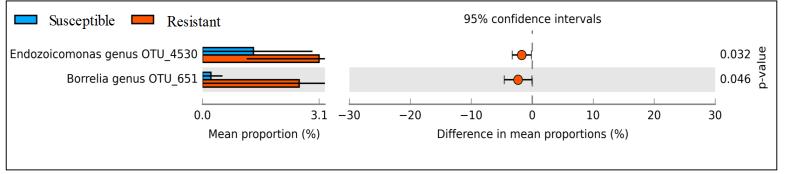
↓data analysis

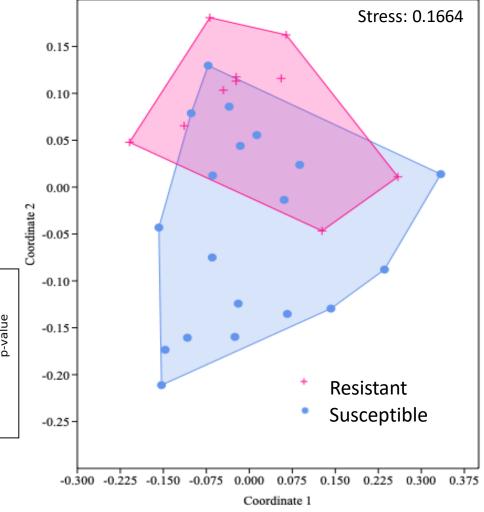
SRO-microbiomes separation according to QX resistance

NMDS - Non-metric multidimensional scaling

Wallis Lake: 6 family lines

- 4 susceptible (≤ 50% survival)
- 2 resistant (>50% survival)
- 5 oysters/family line/sampling time





PERMANOVA: F = 1.663, p = 0.0215STAMP analysis – Wallis Lake

PO - Microbiome composition differs according to disease-resistance family line

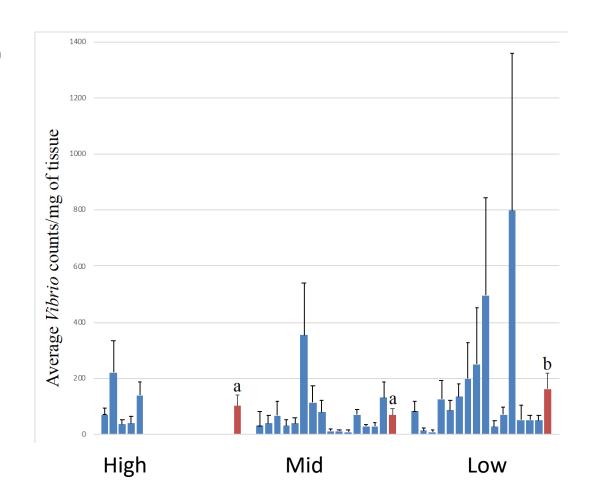
George river: 35 family lines

- 15 susceptible = low resistance (≤ 30% survival)
- 15 mid resistant (30% < survival < 60%)
- 5 high resistant (60% ≤ survival)
- 5 oysters/family line/sampling time

For example

TOT CAUTIFIC		
Family	POMS	Resistance
line	survival (%)	group (RG)
F_66	82.6	High
F_84	39.1	Med
F_86	5.2	Low

Association analysis
Tenacibaculum sp.
<i>Vibio</i> sp.



High vs. Low - p = 0.0281; F = 1.47 Med vs. Low - p = 0.0001; F = 2.93

Microbiome shift during QX outbreak in George river

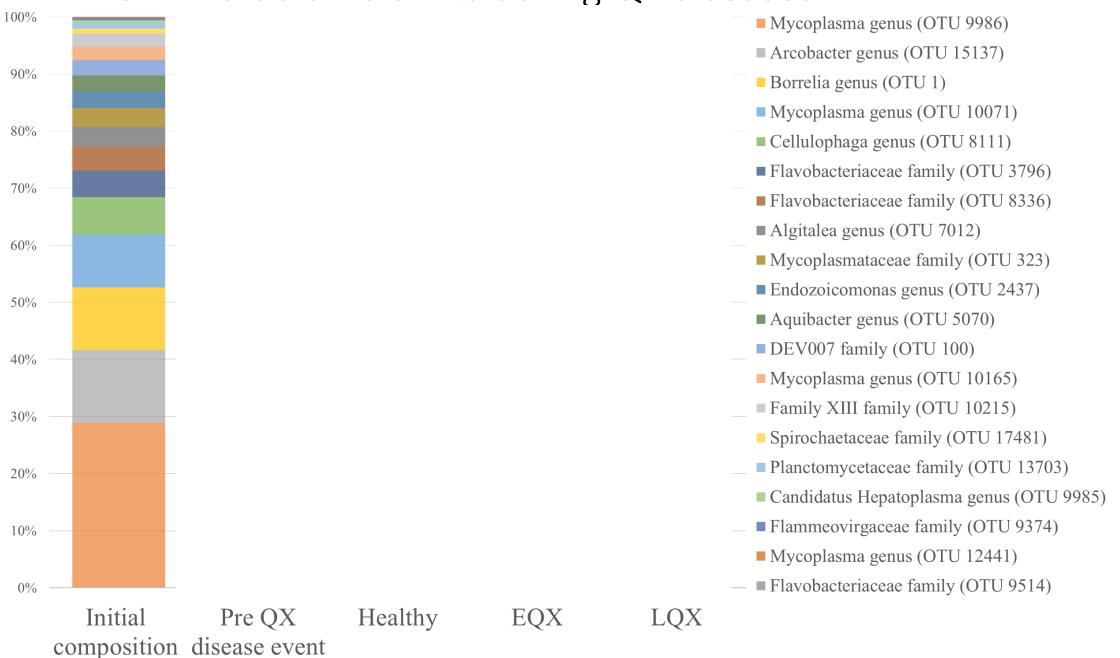
- 3 mid resistance family lines
 50% survival rate
- Pre QX disease event: 5 oysters/family line/fortnight
- During QX disease event: 10 oysters/family line/fortnight



Samples were tested for QX using Cytology and PCR on the ITS1 region



PRO - Microbiome shifts during QX diseases



PRO - Microbiome shifts during QX diseases 100% ■ Mycoplasma genus (OTU 9986) ■ Arcobacter genus (OTU 15137) 90% ■ Borrelia genus (OTU 1) ■ Mycoplasma genus (OTU 10071) 80% ■ Cellulophaga genus (OTU 8111) ■ Flavobacteriaceae family (OTU 3796) 70% ■ Flavobacteriaceae family (OTU 8336) ■ Algitalea genus (OTU 7012) 60% ■ Mycoplasmataceae family (OTU 323) ■ Endozoicomonas genus (OTU 2437) 50% ■ Aquibacter genus (OTU 5070) ■ DEV007 family (OTU 100) 40% ■ Mycoplasma genus (OTU 10165) ■ Family XIII family (OTU 10215) 30% ■ Spirochaetaceae family (OTU 17481) ■ Planctomycetaceae family (OTU 13703) 20% ■ Candidatus Hepatoplasma genus (OTU 9985) ■ Flammeovirgaceae family (OTU 9374) 10% ■ Mycoplasma genus (OTU 12441) ■ Flavobacteriaceae family (OTU 9514) 0%**Initial** Pre QX Healthy LQX EQX composition disease event

PO - Longitudinal study:

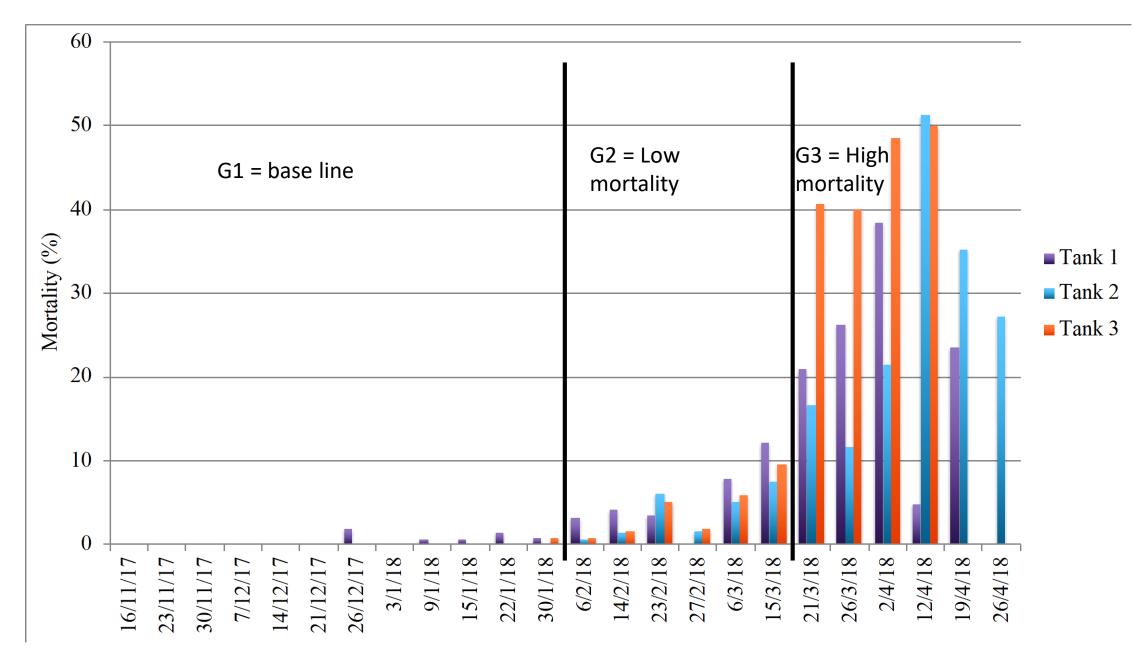
- 28 weeks of weekly sampling
- 424 oysters sampled
- 283 dead oysters



Mortality in three separate tanks shown mortality as a proportion of the previous week

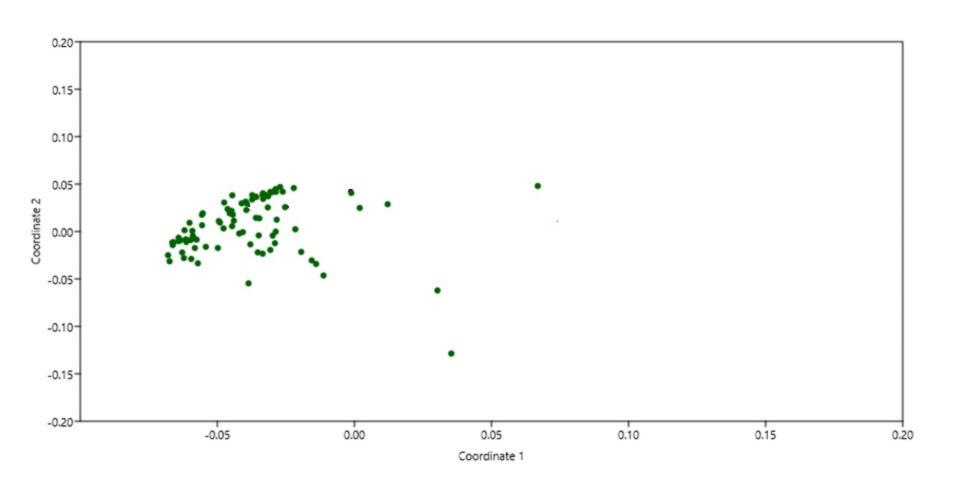


■ Tank 1 ■ Tank 2 ■ Tank 3



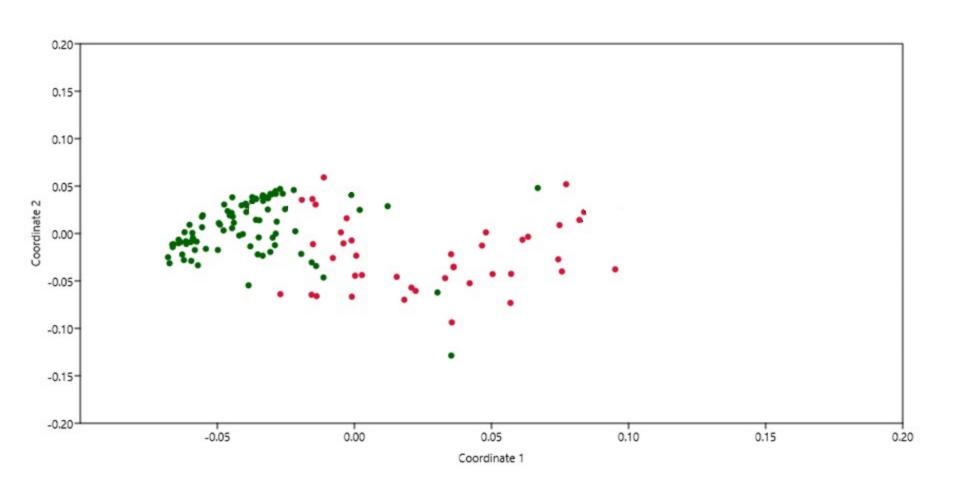
For comparisons between different mortality thresholds, samples were split into three groups.

G2 begins when all three tanks have a mortality. G3 starts when all three tanks are >10% mortality



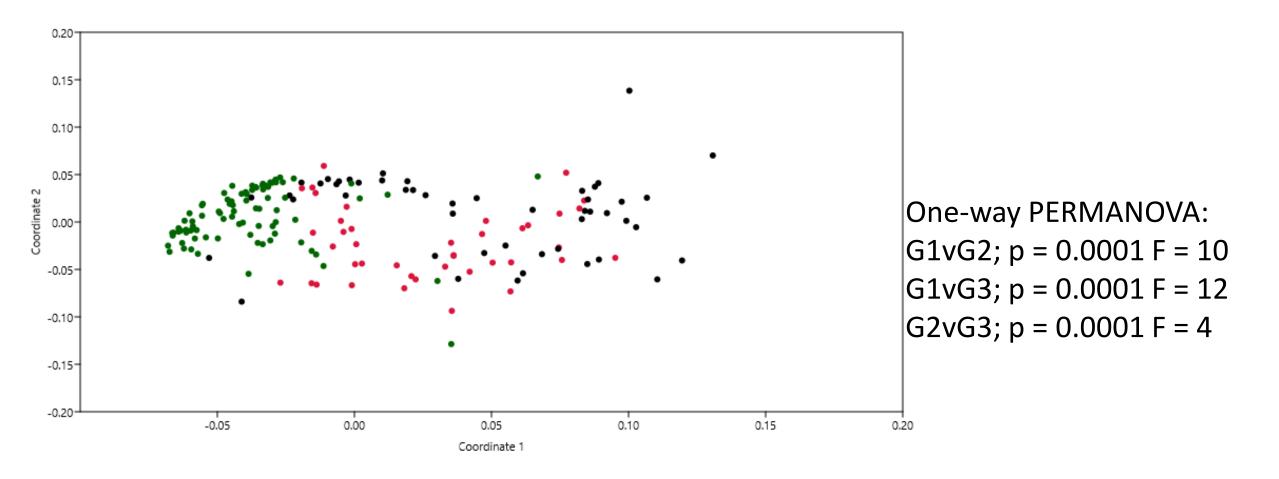


nMDS with 95% ellipses at the zOTU level for: baseline (G1; green), low mortality (G2; red) and high mortality (G3; black). Stress = 0.26

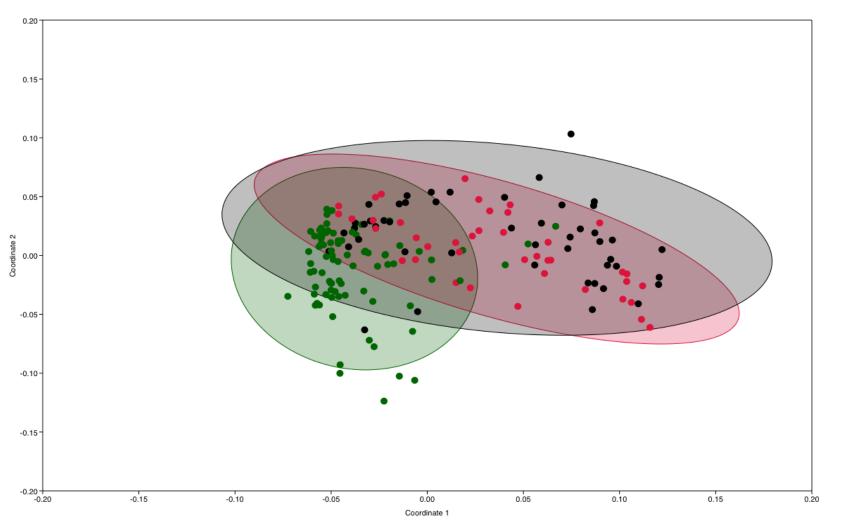




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One-way PERMANOVA:

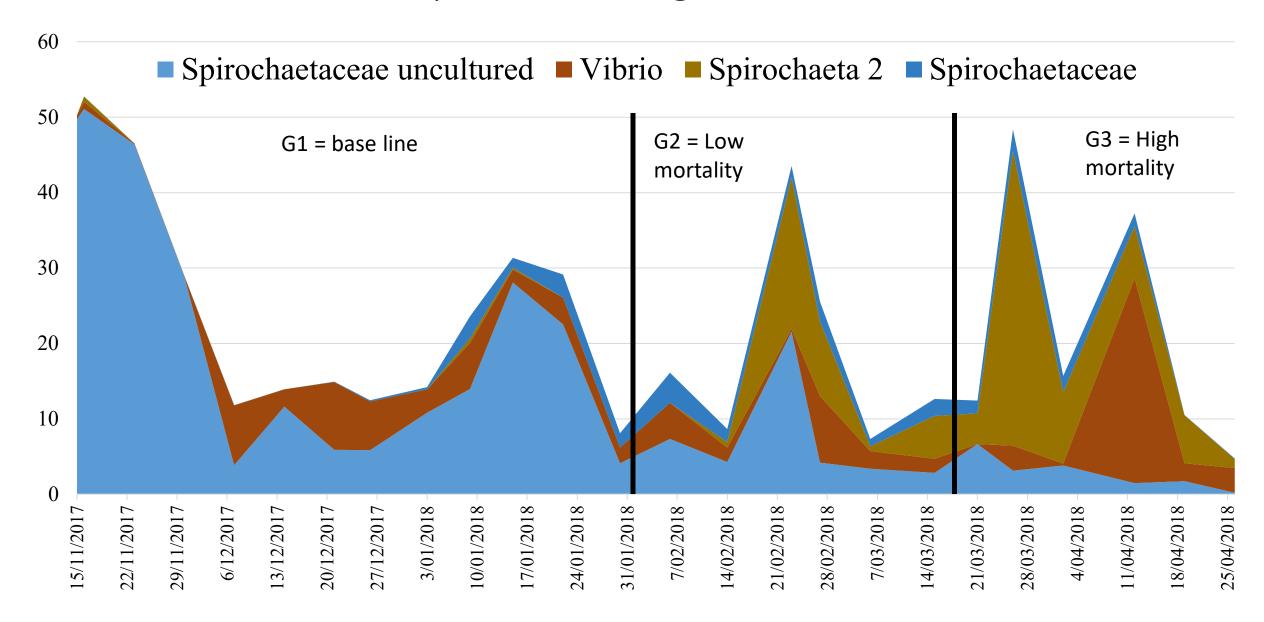
G1vG2; p = 0.0001 F = 10

G1vG3; p = 0.0001 F = 12

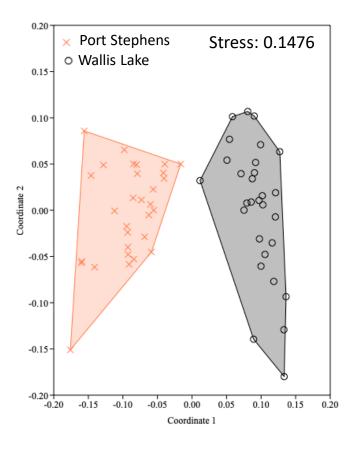
G2vG3; p = 0.0001 F = 4

nMDS with 95% ellipses at the zOTU level for: baseline (G1; green), low mortality (G2; red) and high mortality (G3; black). Stress = 0.26

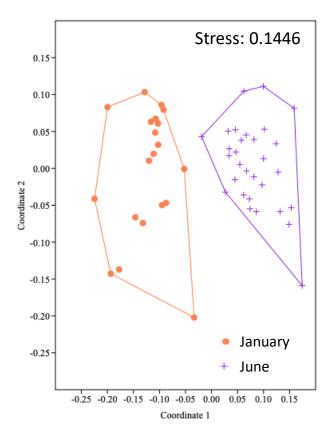
Beta diversity shifts at the genus level for Tanks 2



SRO microbiomes separate according to geographic location and season



PERMANOVA: F = 12.69, p = 0.0001



Port Stephens

PERMANOVA: F = 11.36, p = 0.0001

SIMPER: 88.58% dissimilarity

Summary

- Disease resistance (genetics) influences microbiome composition
 - Vibrio abundance higher in disease susceptible oysters
- Potential to use the microbiome as a marker for disease onset
 - Shift in the spirochete community
- Microbiome is dynamic, shifts in response to:
 - Location
 - Season
 - Oyster tissue type

Acknowledgements



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Wayne O'Connor







Jeffrey Go



Michael Dove





