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Future oysters CRC-P: Polymicrobial involvement in OsHV outbreaks (and other diseases)

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Executive Summary

The principal goal of this research was to provide a detailed characterisation of the *oyster microbiome* and identify links between specific features of the microbiome and oyster disease and mortality events. The conceptual framework for this work is based upon: (i) increasing evidence, across a broad range of species, that the nature of a host organism's microbiome exerts a fundamental control on host physiology and health, and (ii) the critical paucity in knowledge on the factors contributing to oyster health and the triggers for oyster mortality events and disease outbreaks. The research reported here involved a collaboration between the University of Technology Sydney (UTS) and the NSW Department of Primary Industries (DPI), whereby the UTS members of the team provided expertise in molecular microbial ecology and the DPI team members provided expertise and support in oyster physiology and ecology and aquaculture. The research involved a large-scale screening of the microbiomes of both Pacific Oysters and Sydney Rock Oysters using high-throughput DNA sequencing technologies, providing a characterisation of the microbial communities associated with oysters. The outcomes of this analysis revealed that for both Pacific Oysters and Sydney Rock Oysters, the oyster microbiome is remarkably variable among different oyster families, and over space and time, indicating that both intrinsic physiological features of the oyster host and environmental factors play a role in governing the oyster microbiome. Notably, despite this heterogeneity, a small sub-set of the microbiome was shown to be conserved across oysters within a species, pointing to the existence of a core group of microbes with intrinsic links to oyster ecology and condition. Similarly, a small group of microbes, including members of the *Vibrio* genus, were consistently associated with diseased or susceptible oysters, indicating a potentially antagonistic role of these microbes. These observations support the hypothesis that the oyster microbiome plays a role in defining oyster health, but also reveal substantial complexities related to the marked heterogeneity of the oyster microbiome over space and time. Appropriately considering this microbiome heterogeneity, while also sharpening focus on the few core microbiome members identified in this research, will be important requisites for future efforts hoping to employ the oyster microbiome for diagnostic purposes.

Background

During the last two decades a number of disease outbreaks have led to mass oyster mortalities and the closure of several oyster-harvesting regions, resulting in multi-million dollar losses. These outbreaks mirror a global pattern of increased aquaculture disease, with disease emergence potentially linked to environmental degradation (pollution) and climate change related processes, such as rising seawater temperature. Within NSW estuaries, multiple microbiological agents have

been implicated in oyster diseases, but a clear understanding of the ecological and environmental drivers of disease outbreaks has remained elusive. This means we cannot currently predict when outbreaks will occur, making it very difficult to manage infection events and develop strategies to mitigate future oyster disease events.

Across a wide-range of animal and plant systems, including several benthic marine organisms, there is growing evidence that the structure and function of a host organism's *microbiome* – the community of microorganisms living in prolonged association with the host macroorganism - plays a fundamental role in the physiology and health of the host and its susceptibility to disease. Shifts in a host organism's microbiome (dysbiosis) can either precede or follow measurable symptoms of syndromes and/or disease, with examples of both microbiome shifts causing disease or occurring in response to disease on-set.

There is a growing recognition for the potential importance of the microbiome in oyster health and physiology (Trabal et al., 2012; Wegner et al., 2013; Lemire et al., 2015; Lokmer and Wegner, 2015; Petton et al., 2015; Lokmer et al., 2016b; de Lorgeril et al., 2018; King et al., 2019b), with emerging evidence suggesting that the oyster microbiome might be directly related to oyster disease dynamics (Wegner et al., 2013; Lokmer and Wegner, 2015; de Lorgeril et al., 2018; Green et al., 2018; King et al., 2019a). However, the factors governing the structure of the oyster microbiome are very poorly resolved, with very little, to no, understanding of the inherent characteristics of a "healthy oyster microbiome" or the identity of core beneficial *vs* pathogenic microbes within the oyster microbiome. This lack of knowledge currently precludes the use of the oyster microbiome as a diagnostic marker for oyster health or disease status.

Aims/objectives

The three over-arching Objectives of this research were to:

1) Define microbial communities associated with oysters and identify potential microbial threats

2) Link changes in environmental conditions to shifts in the Oyster microbiome

3) Better understand the association between the oyster microbiome and disease

These Objectives gave rise to the following more specific Aims, which evolved as the project progressed:

Aim 1: Characterise the composition of the Pacific Oyster microbiome across diverse oyster families, including those exhibiting different levels of susceptibility to OsHV-1 μvar disease

Aim 2: Define the composition of the Sydney Rock Oyster microbiome across diverse oyster families, including breeding lines generated for resistance to QX disease, and examine spatial and temporal heterogeneity in microbiome structure

Aim 3: Examine spatial heterogeneity in Pacific Oyster microbiome structure at the individual oyster level and across regional-scales

Aim 4: Define the Sydney Rock Oyster microbiome associated with QX disease events

Aim 5: Measure temporal patterns in the Pacific Oyster Microbiome during the Summer OsHV-1 Mortality Period

Aim 6: Elucidate patterns in *Vibrio* community diversity and abundance within the microbiomes of oysters subject to disease and mortality events

Methodology

To address our Aims we focussed our research on two of the major commercial oyster species in Australia, the Pacific Oyster and Sydney Rock Oyster, with a focus on diseases affecting these species, namely OsHV-1 and QX disease respectively. Our approach involved a tiered characterisation of the oyster microbiome, which included:

(i) Characterising the "base-line" microbiome of Pacific Oysters and SRO

(ii) Examining variability in the oyster microbiome across diverse family/breeding lines, including families exhibiting differing levels of susceptibility to OsHV-1 and QX disease

(iii) Defining spatial and temporal variability in Pacific Oyster and SRO microbiomes across a continuum of scales, ranging from comparisons across different oyster tissues and between different estuaries.

(iv) Measuring patterns in Pacific Oyster and SRO microbiomes associated with disease outbreaks and mortality events

(v) Targeted screening of oysters for microbiome members putatively involved in oyster disease or mortality.

Throughout the course of this project we characterised the microbiomes associated with Pacific Oysters and SROs using 16S rRNA amplicon sequencing, which is currently the optimum approach for defining the diversity and composition of a microbiome. Briefly, this technique involves extraction of microbial DNA from oyster samples, amplification of the bacterial 16S rRNA gene and Illumina miSeq sequencing of the amplified DNA. This technique provides an inventory of the bacterial composition and diversity within a sample (a list of Operational Taxonomic Units; OTUs), allowing for inter-microbiome comparisons and the identification of specific discriminatory or indicator microorganisms. Using a suite of multidimensional statistical analyses we identified

patterns in oyster microbiome structure across environments, over time and between different oyster breeding lines. This approach allowed us to both identify members of the "core oyster microbiome" and organisms most responsible for the discrimination of different oyster microbiomes.

Results

The key findings of this research included:

- The identification of a small sub-set of "core members" of the oyster microbiome, including members of the *Spirochaetaceae* family that were conserved over a continuum of spatial and temporal scales, which may be indicative of key oyster-associates that play a role in oyster physiology and health;
- Significant heterogeneity in both the Pacific Oyster and SRO microbiomes over space and time, indicating that local environmental factors govern the structure of the oyster microbiome;
- Variability in the oyster microbiome across different oyster family-lines, and between different oyster tissue types (e.g. gill, mantle adductor muscle etc) indicating that intrinsic genetic and physiological features of the oyster host also govern microbiome structure;
- Sub-sets of the oyster microbiome that were differentially prevalent in Pacific Oyster and SRO family-lines with differing levels of susceptibility to OsHV-1 and QX disease respectively, indicating that certain members of the oyster microbiome may either facilitate or protect the oyster from infection

Implications and Recommendations for relevant stakeholders

The outcomes of this research indicate the highly dynamic nature of the Pacific Oyster and SRO microbiomes and in some cases point to a potentially significant role of the oyster microbiome in governing oyster health and susceptibility to disease. This, on the one hand, suggests that the oyster microbiome may have substantial utility as a new diagnostic measure of oyster health, but on the other hand, the inherent heterogeneity of the oyster microbiome observed here means that it may be difficult to identify and subsequently use universal community signatures or indicator organisms across oyster microbiomes originating from different environments or genetically dissimilar oyster stocks. We therefore suggest that while the incorporation of characterisation of the oyster microbiome into assessments of oyster condition has substantial promise, care should be taken to ensure that data is collected and interpreted using a context-specific (e.g. environment, oyster genetic stock) approach.

***Keywords*:** Microbiome; Oyster Disease; Pacific Oyster, Sydney Rock Oyster; Bacteria; Pathogen

Introduction

Oyster aquaculture contributes almost $100 million yr^{-1} to the Australian economy (FRDC 2013) and produces one of our nation's favourite seafood products. However, during the last two decades a number of disease outbreaks have led to mass oyster mortalities and the closure of several oyster-harvesting regions in NSW and Tasmania, resulting in multi-million dollar losses (Wilkie et al. 2013; Jenkins et al. 2013) . Within NSW estuaries, multiple agents have been implicated in oyster diseases (Jenkins et al. 2013), but a clear understanding of the ecological and environmental drivers of disease outbreaks has remained elusive. This means we cannot yet predict why, when or where outbreaks will occur, making it difficult to manage infection events and develop strategies to mitigate future oyster disease outbreaks.

The largest oyster industry in Australia targets the Pacific Oyster (FRDC 2013). Here, and elsewhere in the world where Pacific Oysters are harvested, oyster production has recently been heavily impacted by a number of significant mortality events, with a variety of environmental factors and putative pathogens implicated (Malham et al. 2009). Perhaps most notably, Pacific Oyster culture in several parts of the world has been decimated by the influence of Pacific Oyster Mortality Syndrome (POMS), which is associated with infection by the OsHV-1 virus (Ostreid herpesvirus 1), resulting in high (> 95%) rates of juvenile oyster mortality (Jenkins et al. 2013). While the influence of POMS has been most significant in Europe, recent infections in New Zealand (Keeling et al. 2014) and Australia (Jenkins et al. 2013) have had substantial impacts. An outbreak within the Hawkesbury River (NSW) in 2013 led to the loss of $6 million worth of oysters, while a February 2016 outbreak at several sites in southern and eastern Tasmania led to significant Pacific Oyster mortality, with associated costs exceeding $5.6 M. Notably, recent evidence indicates that POMS is a polymicrobial syndrome that is not only caused by the OsHV-1 virus, but potentially includes the involvement of bacteria from within the oyster microbiome (Petton et al. 2015).

The other major commercially harvested oyster species in NSW is the Sydney Rock Oyster (SRO: *Saccostrea glomerata*). This species also experiences substantial mortality events, which are associated with QX disease, the major disease impacting SRO culture in NSW and southern Queensland, periodically causing losses in oyster stock of nearly 100% in some areas (Peters and Raftos, 2003). QX disease was first reported in SROs cultured in Queensland in the late 1960s

(Wolf, 1972) and has subsequently been observed in several NSW estuaries (Raftos *et al.*, 2014). The pathogen responsible for QX disease is the spore-forming protozoan parasite *Marteilia sydneyi* (Perkins and Wolf, 1976; Nell, 2007), which enters the oysters digestive gland where it proliferates, ultimately leading to blockage of the digestive glands and oyster starvation.

A common feature across most oyster diseases is that, while a principle pathogen may have been identified, the mechanisms underpinning the onset of disease outbreaks are unclear, meaning that in many instances the oyster industry currently lacks the capacity to predict and prevent disease outbreaks. Often the pathogenic microbe can be present in the environment, and even persist within healthy oysters, without causing disease, until a specific environmental trigger catalyses a disease outbreak. The nature of this trigger is generally not defined, but water temperature, rainfall, eutrophication, algal blooms and shifts in the oyster microbiome have all been implicated as potential catalysts for disease outbreaks (reviewed in King et al. 2018). Detangling the causative mechanisms of disease from this complex "interactome" is not trivial – in particular, little information is known regarding the role of the oyster microbiome in disease protection or susceptibility.

The microbiome is generally defined as *the consortia of microorganisms living in sustained association with a host organism*. To date, the bulk of microbiome research has been focussed on humans, with shifting patterns in the composition of the human microbiome correlated with a number of disorders and diseases (Turnbaugh et al., 2006; Abraham and Cho, 2009; Heijtz et al., 2011). However, increasing evidence suggests that the microbiome fundamentally influences the fitness of a broad range of host organisms, including most animals and plants.

In many benthic marine organisms, the host microbiome is an important determinant of host health and physiology (Rosenberg et al., 2007; Tarnecki et al., 2017; Crump et al., 2018; Pita et al., 2018). However, the structure and influence of the microbiome hosted by many species is not static, with multiple factors including diet (Wilkes Walburn et al., 2019), location (Cúcio et al., 2016), and time (Kimes et al., 2013), driving significant biogeographical, seasonal and (within-site) inter-individual heterogeneity in microbiome composition. However, despite this apparent variability within microbiomes, core components of the microbiome are often conserved over large geographic scales (Ainsworth et al., 2015) and time periods (Aronson et al., 2017), implying an inherent coupling between the host organism and some members of its microbiome.

There is emerging evidence that the microbiome of an organism plays an essential role in maintaining host homeostasis and disease resistance *vs* susceptibility (Shin et al., 2011; Earley et al., 2015). For instance, in humans the microbiome maintains immune homeostasis through reduction of inflammation (Kelly et al., 2004), providing defence against microbial invasion (Fukuda et al., 2011), and assisting in nutrient degradation and uptake (Turnbaugh et al., 2009). On the other hand, imbalances in the microbiome, often termed *dysbiosis*, have been linked to chronic diseases (Frank et al., 2007). Indeed, across a broad range of organisms, the role of the microbiome in disease dynamics appears to be an important factor in the progression and severity of infection (Petton et al., 2015).

Within oysters, the microbiome can shift under different environmental conditions (Green and Barnes, 2010; Wegner et al., 2013; Lokmer and Wegner, 2015; Lokmer et al., 2016a; Lokmer et al., 2016b), between seasons (Pierce et al., 2016) and locations (Lokmer et al., 2016a; King et al. 2018). Despite this complexity, developing a clear understanding of the processes governing the structure of the oyster microbiome may be potentially very important, because there is evidence suggesting that the oyster microbiome might directly influence oyster disease dynamics (Wegner et al., 2013; Lokmer and Wegner, 2015; de Lorgeril et al., 2018; Green et al., 2018; King et al., 2019a).

Reduced mortality in antibiotic-treated specific-pathogen-free (SPF) oysters subsequently exposed to OsHV-1 suggests an important role for the oysters microbiome in disease protection (Petton et al., 2015). In fact, recent evidence indicates that POMS is a polymicrobial syndrome that is not only caused by the OsHV-1 virus, but includes the involvement of bacteria from the *Vibrio* genus (Petton et al. 2018). *Vibrio* species are responsible for disease in a variety of aquaculture industries (Chatterjee and Haldar 2012), and are recurrently implicated in oyster mortality events, but our understanding of the complex tripartite interaction involved in POMS outbreaks is incipient. Notably, it has also been demonstrated that the non-virulent *Vibrio* portion of the oyster microbiome progressively shifts towards a virulent population during the onset of summer mortality events in Pacific Oysters (Lemire et al., 2015). Within this context it is notable that *C. gigas* cultivated at sites experiencing a summer mortality outbreak in Port Stephens, NSW, had a significantly different microbiome structure, with a higher proportion of *Vibrio*, than specimens from sites unaffected by summer mortality (King et al., 2018). In SRO, there is evidence that QX

infection reduces the diversity of the SRO microbiome, with sequences with high homology to *Rickettsiale*-like prokaryotes highly elevated in infected oysters (Green and Barnes, 2010).

While this emerging evidence implies that the oyster microbiome may be intimately involved in polymicrobial infection dynamics (Petton et al., 2015; de Lorgeril et al., 2018), there are limited culture-independent studies examining the oyster microbiome without the confounding influence of disease (Lokmer et al., 2016b; Lokmer et al., 2016a). Identifying a 'healthy' baseline microbiome and defining the dynamics (e.g. physiological and environmental factors) that can shift the structure of the oyster microbiome is clearly an essential requisite when aiming to interpret the role of the oyster microbiome in disease. For instance, it is currently not understood how the oyster microbiome responds before, during and after an environmental disease outbreak. Similarly, it is currently not known whether differences in susceptibility to disease among genetically dissimilar oysters (e.g. family lines, including disease resistance bred lines) are reflected in differences in the oyster microbiome. Finally, across many model systems, it has become clear that disease is rarely a straightforward or stochastic consequence of a linear infection process, but is often the product of a suite of interacting factors, which collude to promote infection (Vidal et al. 2011). Rather than occurring in isolation, these factors are often linked via complex networks of interaction. Indeed, in oysters it is highly likely that disease outbreaks are ultimately a consequence of a shift or fracture in the interplay of specific environmental factors and biotic processes that act to maintain ecological balance. Developing a robust understanding of the interplay of these complex processes will be crucial for determining the influence of the oyster microbiome in either facilitating or preventing oyster disease, and is the principal focus of this project.

Objectives

Both internationally and within Australia, oyster aquaculture has been increasingly impacted by disease outbreaks and unexplained mortality events. Often the trigger for these costly events is undefined, but there is growing evidence that the oyster microbiome may play a significant role. However, we currently lack a thorough understanding of the nature and influence of the oyster microbiome within the context of Australian oyster aquaculture. Within this framework, the three major *Objectives* of this research were to:

1) Define microbial communities associated with oysters and identify threats

2) Link changes in environmental conditions to changing microbial communities

3) Better understand the association between microbial communities and disease

These Objectives gave rise to the following more specific Aims, which evolved as the project progressed:

Aim 1: Characterise the composition of the Pacific Oyster microbiome across diverse oyster families, including those exhibiting different levels of susceptibility to OsHV-1 μvar disease

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Aim 5: Measure temporal patterns in the Pacific Oyster Microbiome during the Summer OsHV-1 Mortality Period

Aim 6: Elucidate patterns in *Vibrio* community diversity and abundance within the microbiomes of oysters subject to disease and mortality events

Methods

To address the Objectives described above and deliver the proposed outcomes and outputs, this research was carried out within the context of several Aims, spanning studies on Pacific Oysters and Sydney Rock Oysters. Below we present the Methodology and Results within the context of these Aims.

<u>Aim 1:</u> Characterising the composition of the Pacific Oyster microbiome across diverse oyster families, including those exhibiting different levels of susceptibility to OsHV-1 disease

Rationale and Goals

Current understanding of the factors determining the nature of the Pacific Oyster microbiome and how it may influence, or be influenced by, diseases including OsHV-1 infections is profoundly limited. In order to address both **Objectives 1 and 3** and define microbial communities associated with Pacific Oysters and better understand the association between the oyster microbiome and disease, we carried out a large-scale characterization of the microbiome associated with 35 *C. gigas* families, incorporating oysters with different levels of susceptibility to OsHV-1 µvar disease. This approach provided an opportunity to both: (i) determine whether different *C. gigas* families harbour distinct microbial community assemblages and whether persistent bacterial taxa (core microbiome) are common across different families, and (ii) explore how breeding for resistance to OsHV-1 µvar affects the oyster microbiome.

Sources and sampling of C. gigas

Since the first OsHV-1 µvar outbreak in Australia in 2010, Australian Seafood Industries (ASI) has been breeding *C. gigas* families for OsHV-1 µvar disease resistance through field exposure. In 2016, ASI deployed thirty-five (n = 35) 5th generation families (5 consecutive years of bi-parental breeding) of juvenile *C. gigas* into three areas known to harbour the OsHV-1 virus, the Georges River (New South Wales, Australia; 34.035S, 151.145E), Pipe Clay Lagoon (Tasmania, Australia; 42.970S, 147.525E) and Pittwater (Tasmania, Australia; 42.802S, 147.509E) (Kube et al., 2018). Based on these field disease-exposure studies, expected breeding values (EBVs) were calculated by ASI. These EBVs provide an estimation of how well the oysters will perform for a particular trait and the likelihood of passing those traits to their progeny. For the purposes of this study,

families were classified into 'resistance groups' (RG) based on their OsHV-1 µvar disease resistance EBV. Families with an EBV greater than 0.6 were placed into RG1 (high disease-resistance), those with an EBV greater than 0.3 and less than 0.6 were placed into RG2 (medium disease-resistance), and families with an EBV less than 0.3 were placed into RG3 (low disease-resistance) (Table 1). The estimated heritability is the likelihood of the offspring demonstrating a particular trait, in this case OsHV-1 µvar disease resistance. Resistance is determined by the combination of many genes, since the stock used are derived from a number of genetically distinct families, each family differs in its resistance, and crosses between families differ.

In addition to disease-resistance, EBVs of other oyster traits were also provided by ASI. These traits include: meat condition, the ratio of wet meat to the total weight; depth index, the ratio of shell depth to shell length; shell length; oyster weight, including the oyster shell; and width index, the ratio of shell width to shell length. As EBV's are proprietary information, rather than providing absolute values for each index, we generated a 'rank' system to categorise families according to each index, with ranks of 1 being the highest (Table 1).

Table 1: Expected breeding value ranks for the studied oyster families including OsHV-1 µvar disease-resistance.

Family line	OsHV-1 µvar resistance	Resistance group (RG)	Meat condition	Depth index	Shell length	Oyster weight	Width index
F_01	8	RG2	22	6	28	7	7
F_02	25	RG3	20	1	29	10	10
F_03	6	RG2	17	18	21	13	13
F_07	16	RG2	6	4	34	1	1
F_10	26	RG3	16	10	32	5	5
F_11	24	RG3	11	7	31	6	6
F_15	29	RG3	6	4	34	1	1
F_16	31	RG3	3	14	11	23	23
F_19	17	RG2	17	18	21	13	13
F_20	28	RG3	4	20	9	13	13
F_23	15	RG2	13	28	19	19	19
F_25	20	RG2	6	21	19	20	20
F_26	32	RG3	12	8	25	22	22
F_27	22	RG3	25	2	27	17	17
F_29	7	RG2	27	31	2	35	35
F_30	18	RG2	23	30	3	33	33
F_35	34	RG3	1	27	10	8	8
F_36	10	RG2	9	24	17	24	24
F_37	12	RG2	4	9	30	11	11
F_39	27	RG3	2	15	17	9	9

F_40	11	RG2	17	12	26	21	21
F_43	19	RG2	20	10	21	28	28
F_51	23	RG3	14	28	13	12	12
F_61	30	RG3	14	35	1	34	34
F_62	33	RG3	24	24	7	17	17
F_65	13	RG2	30	31	3	30	30
F_66	1	RG1	30	31	3	30	30
F_67	9	RG2	30	31	3	30	30
F_68	3	RG1	30	16	15	25	25
F_69	5	RG1	30	16	15	25	25
F_72	2	RG1	35	21	13	16	16
F_77	4	RG1	26	24	8	25	25
F_80	21	RG3	29	23	12	29	29
F_84	14	RG2	28	2	33	3	3
F_86	35	RG3	10	13	24	4	4

Survival data was determined after deployment in three different OsHV-1 µvar positive estuaries across Australia. Expected breeding values (EBVs), including OsHV-1 µvar disease-resistance, are shown as a rank number out of 35.

For this microbiome study, the families were deployed into the Georges River (34.035S, 151.145E) on the 16th of August 2016 and sampled two months after deployment date. The two-month deployment time was the first opportunity to sample the deployed juvenile oysters and was sufficient time to ensure no evidence of disease or morbidity. Oysters were deployed in a resolvable incomplete block design to account for micro-geographic variation, whereby blocks were subsections of a replicate and there were three replicates for each family, with each family stocked into a subsection of the tray (Kube et al., 2018). Five oysters from each of the 35 families (total = 175 samples) were sampled and immediately placed on ice and transported to the laboratory where they were stored at -80 °C until further processing.

DNA extraction, sequencing and bioinformatics

The outer shell of the five sampled oysters was rinsed under running tap water to remove any remaining mud and debris. Defrosted oysters were then shucked with sterilised shucking knifes and approximately 25 mg of adductor muscle tissue was aseptically removed using sterile scalpel blades.

The Qiagen DNeasy blood and tissue kit (catalogue: 69506) was used to extract DNA samples, as per the manufacturer's instructions. Microbial community composition within samples was subsequently assessed using 16S rRNA amplicon sequencing, whereby the ribosomal 16S rRNA V1-V3 region was targeted using the 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 519R (5'-

GWATTACCGCGGCKGCTG-3') primer pair. The PCR cycling conditions were as follows: 95 °C for 3 min, 25 cycles of 95 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s, and a final extension at 72 °C for 5 min. Amplicons were sequenced using the Illumina MiSeq platform (2 x 300 bp) using standard approaches (Ramaciotti Centre for Genomics at the University of New South Wales, Sydney, Australia). Raw data files in FASTQ format were deposited in the NCBI Sequence Read Archive (SRA) under the Bioproject number PRJNA497763.

16S rRNA paired-end DNA sequences were joined using Flash (Magoc and Salzberg, 2011) and subsequently trimmed using Mothur (Schloss et al., 2009) (Parameters: maxhomop=5, maxambig=0, minlength=432, maxlength=506). The resulting fragments were clustered into operational taxonomic units (OTUs) at 97% sequence identity, and chimeric sequences were identified using vsearch (Rognes et al., 2016). Taxonomy was assigned in QIIME (Caporaso et al., 2010) using the uclust algorithm (Edgar, 2010) against the Silva v128 database. Mitochondrial and chloroplast data were filtered out of the dataset and the remaining data were rarefied to allow for even coverage across all samples. OTUs representing less than 0.1% relative abundance in an individual sample were also filtered from the dataset (Supplementary Table 1 and Supplementary Table 2 – Appendix).

Core microbiome analysis

To determine whether a core oyster microbiome could be characterised, we examined the microbiome of oysters at three different thresholds. First, for individual families, then for RGs, then for all samples together. A core OTU was defined as an OTU that was present in at least all but one replicate (to account for outliers) within a family. To achieve this, the panbiom.py script was used as detailed in Kahlke (2017). Briefly, the final biom file generated during the QIIME analysis was used in conjunction with a treatment file that identifies which samples are replicates within a family. The panbiom.py arguments were as follows: a replicate threshold of 1 (-r parameter) and an outlier threshold of 'x' (-x parameter). The –x parameter treats the replicate threshold value as an outlier threshold value, simply put, a core microbiome member can be absent in one replicate sample (indicated by –r = 1 and –x = x).

Quantitative PCR (qPCR)

Due to the potential role of *Vibrio* in OsHV-1 µvar disease dynamics (Segarra et al., 2010; Jenkins et al., 2013; Lemire et al., 2015; Petton et al., 2015; de Lorgeril et al., 2018), quantitative PCR (qPCR) was used to examine patterns in *Vibrio* abundance across the RGs. qPCR was performed using an epMotion 5075l Automated Liquid Handling System on a Bio-Rad CFX384 Touch Real-Time PCR Detection System with a six-point calibration curve and negative controls on every plate. The calibration curve was built from a known amount of amplicon DNA measured by Qubit, followed by a ten-fold dilution to fill out the calibration curve. All sample analyses were performed with three technical replicates, using the following reaction mixture: 2.5 µL iTaq Universal SYBR Green supermix, 0.4 µM of each forward and reverse primer, 1 µL of diluted (1:15) template DNA, and the remainder made up with water. To quantify abundance of the *Vibrio* community, the *Vibrio*-specific 16S rRNA primers Vib1-f (5'- GGCGTAAAGCGCATGCAGGT -3') and Vib2-r (5'- GAAATTCTACCCCCCTCTACAG -3') were used (Thompson et al., 2004; Vezzulli et al., 2011; Siboni et al., 2016). The qPCR cycling conditions were as follows: 95 °C for 3 minutes followed by 45 cycles of 95 °C for 15 seconds and 60 °C for 1 minute. The resulting data were normalised to both elution volume (200 µL) and tissue weight. A coefficient of variation (CV) was then calculated for the technical triplicates, and samples with CV > 10% were removed from the analysis. A melting curve was added to the end of every run to confirm the presence of a single PCR product.

Statistical analysis

Comparisons of alpha diversity were performed with a One-Way ANOVA followed by a Tukey's pairwise test. Normalised [square root (x)] data were used to compare community compositions using non-metric multidimensional scaling analysis (nMDS) with a Bray-Curtis similarity index. To determine significantly different microbial assemblage between families and RGs, and to compare qPCR data, a one-way PERMANOVA was used. To examine which OTUs contributed to differences between RGs, a SIMPER analysis with a Bray-Curtis similarity index was used. To define associations between breeding values and OTUs, breeding values were normalised (x-mean/standard deviation) and used within a canonical correspondence analysis (CCA). All analyses were performed using the PAST statistical software (Hammer et al., 2001). To determine whether an OTU was significantly elevated in a particular RG, the group_significance.py script using the default analysis (Kruskal Wallis ANOVA) was used in QIIME. To examine correlations

between EBVs, we performed a maximal information-based nonparametric exploration (MINE) analysis (Reshef et al., 2011).

<u>Aim 2:</u> Defining the composition of the Sydney Rock Oyster microbiome across diverse oyster families, including breeding lines generated for resistance to QX disease, and examining spatial and temporal heterogeneity in microbiome structure

Rationale and Goals

In parallel to Aim 1 (above), we also considered how the microbiome associated with Sydney Rock Oysters (SRO) differs according to both oyster genetics and disease resistance, allowing us to again address Objectives 1 and 3, this time within the context of the Sydney Rock Oyster. By also characterising SRO microbiome structure across different sites we also considered the influence of environmental factors in defining the oyster microbiome (Objective 2). Within this context we specifically aimed to answer the following questions: i) how does location affect the microbiome of genetically identical SROs? ii) how does time affect the microbiome of genetically identical SROs? iii) How does SROs bred for QX-resistance affect the microbiome?

Experimental Design

Sixty eight single pair-mated families were deployed within the low QX disease risk areas of Port Stephens (32°43'12.81''S 152°03'40.52''E) and Wallis Lake (32°11'21.3''S 152°29'09.7''E) in NSW (Nial, 2017). These estuaries have intrinsic environmental differences, with Port Stephens containing a muddy estuary bed and Wallis Lake, a sandy bed. On the basis of their QX-resistance estimated breeding value (EBV), which predicts the mean survival of progeny from a family when exposed to QX disease, 6 family lines were selected, consisting of 2 family lines categorized as either susceptible (<10% survival), resistant (>50% survival) or intermediately resistant (15-30% survival) (Table 2). Five oysters per family line were collected at both sites in January (summer season) and June (winter season) 2017 (4 and 9 months after deployment).

Table 2: SRO breeding family lines, predicted QX survival (%) and QX resistance classification for this study

No	Family line	Predicted QX survival (%)*	QX resistance level
1	2015025	72.5	Resistant
2	2015022	54	Resistant
3	2015018	27.5	Intermediate
4	2015003	18.6	Intermediate
5	2015032	-16.5	Susceptible
6	2015037	-13.9	Susceptible

* the percentage of oyster survival is based on estimated breeding value (EBV)

Oysters were randomly collected by farmers from cultivation trays and placed into a labelled plastic bag. Collected oysters were kept on ice for transport (2-3 h) to the laboratory and stored at -80 ^{0}C for later processing. As oyster leases could only be accessed by boat, water samples were collected from jetties approximately 800 meters away from the oyster leases. Water was collected at a depth of 10 to 20 cm below the surface and kept on ice for transport for subsequent nutrient and chlorophyll a analyses and DNA extraction.

Measurement of environmental parameters

Environmental parameters (temperature, oxygen, pH and conductivity) were measured using a WTW multiprobe meter (Multi 3430, Germany) at time of collection. For nutrient analysis, triplicate water samples were syringe filtered through a 0.45 μm filter into 50 mL clean falcon tubes and kept on ice during transport to the laboratory before being frozen at –20^0 C. Nutrient analysis (Nitrite (NO_2^-), Nitrate (NO_3^-), Ammonia (NH_3) and Phosphate (PO_4^{3-})) was carried out at the Envirolab Services Pty Ltd (Sydney, New South Wales, Australia) using standard methods (Rice, Bridgewater, American Public Health, American Water Works, & Water Environment, 2012). For chlorophyll a, triplicate water samples of 200 mL were filtered with Glass microfiber filters (0.7 μm pore size) and frozen at – 80^0 C prior to analysis. Chlorophyll a was analysed based on a Spectrophotometric method described previously (Ritchie, 2006).

DNA extractions from oysters and water samples

Frozen oysters were thawed and washed under running water to remove unattached material. Using sterile instruments, each oyster was carefully opened using a shucking knife and the oyster flesh placed into a Petri dish. The adductor muscle was carefully excised using a sterile scalpel blade and placed into a 1.5 mL Eppendorf tube. From each oyster, approximately 25-50 ng of the adductor muscle tissue was collected for subsequent DNA extraction using the Qiagen DNeasy Blood and Tissue DNA extraction Kit (Qiagen, Germany). For water samples, triplicate 2 L water samples were filtered through a 0.22 μm filter using peristatic pump and then stored at -80 ^{0}C. DNA from filtered water samples was extracted using the PowerWater DNA Isolation Kit (MoBio, USA). For all DNA samples purity and concentrations were measured using a Thermo Scientific™ NanoDrop™ One UV-Vis Spectrophotometer.

Extracted DNA was amplified using PCR targeting the ribosomal 16S rRNA V1–V3 region using the 27F (5′-AGAG TTTGATCMTGGCTCAG-3′) and 519R (5′- GWATTACCGCGGCKGCTG-3′) primer pair (Lane, 1991; Turner *et al.*, 1999). The PCR cycling conditions were as follows: 94°C for 2 min, followed by30cycles of 94°C for 30s, 50°C for 30s and 72°C for 30 s and a final extension at 72°C for 10 min. Amplicons were sequenced using the Illumina MiSeq platform (version 3; 2 × 300 bp) at the Ramaciotti Centre for Genomics (University of New South Wales, Sydney, Australia).

Bioinformatics

All forward and all reverse fastq reads were grouped together to create one file using the Linux command *cat*. Paired-end reads were combined using FLASH (Magoč & Salzberg, 2011) and filtered by length (471-501bp) and quality scores (sequences containing more than 6 homopolymers, ambiguous bases and an average score below 25 were removed) using Mothur (Schloss et al., 2009). Chimeric and singleton sequences were identified and removed using VSEARCH (Rognes, Flouri, Nichols, Quince, & Mahé, 2016). Taxonomic assignment of OTUs was performed using the RDP Classifier method (Wang, Garrity, Tiedje, & Cole, 2007) against the SILVA v128 dataset (Quast et al., 2013) with a confidence value of 0.8. Alignment was performed with PyNAST (Caporaso, Bittinger, et al., 2010) and was followed by filter alignment. All unassigned OTUs, or those identified as chloroplast or mitochondria were removed from the analysis. Sequences were then rarefied using QIIME (Caporaso, Kuczynski, et al., 2010) to the

same depth to remove the effect of sampling effort upon analysis. QIIME was also used to calculate alpha diversity (Chao1, Shannon and observed species index).

Statistical analyses

Differences in alpha diversity between groups were tested using nonparametric Kruskal–Wallis ANOVA. For beta diversity, the relative abundance of OTUs was calculated and normalized (square root (x)). Beta diversity analyses were performed with a Bray-Curtis dissimilarity index. Non-metric multidimensional scaling (nMDS) with three dimensions was used to visualize distance matrices between sample groups. Differences in the relative abundance of OTUs between sample types was tested using permutational multivariate analysis of variance (PERMANOVA) with 9999 permutations. The Similarity Percentages (SIMPER) test was used to quantify the dissimilarity of the microbiome between groups and identify the organisms most responsible for between group differences. PERMANOVA, SIMPER and nMDS statistical analyses were all performed using PAST (Hammer et al. 2001. To determine whether OTUs were significantly different between oyster groups, a Welch's t-test was performed using the STAMP software (Parks et al. 2014).

<u>Aim 3:</u> Examining spatial heterogeneity in Pacific Oyster microbiome structure at the individual oyster level and across regional-scales

Rationale and Goals

The factors driving the composition of the Pacific Oyster microbiome are poorly understood, with interpretations of the microbiome of disease-affected oysters further hindered by the limited understanding of how stable or variable the microbiome is in space and time. In other benthic organisms (e.g. corals and seagrasses), it has been demonstrated that the different tissues and organs of a host macroorganism can host discrete microbiomes within an individual host. At broader geographical scales, there is also evidence that the structure and function of microbiomes can be highly dynamic, and influenced by location, time, genetic characteristics of the host, and disease state factors. To define the baseline *C. gigas* microbiome (**Objective 1)** and in doing so consider the extent of microbiome heterogeneity between different oyster tissues (oyster microenvironments), and different regions, we examined the microbiome of four different oyster tissues across six different estuaries, spanning 4 degrees of latitude, along the eastern coastline of

Australia (New South Wales, Australia). Our principal goals were to understand the extent of heterogeneity versus conservation of the *C. gigas* microbiome across different spatial scales, including between different oyster microenvironments (tissue types) and across biogeographically disparate environments. Answering these questions will be essential for efforts to disentangle the role of the microbiome in oyster health, and potentially further resolve the complexity of oyster diseases.

Oyster collection sites and sampling

To examine the spatial heterogeneity of oyster microbiomes, *C. gigas* samples were collected from six oyster farms along the east coast of New South Wales (NSW), Australia (Figure 1; map), spanning a distance of approximately 470 kilometres. Starting from the southernmost location, the sampled environments included: The Wapengo Lagoon, Clyde River, Shoalhaven River (Crookhaven river), Georges River, Hawkesbury River, and Port Stephens. The Clyde River is the largest producer of *C. gigas,* representing 41 % of all oysters produced in NSW, followed by Port Stephens (27 %), the Hawkesbury River (9 %), and the Shoalhaven River (9 %) (DPI, 2019). All sampling locations are tide-dominated drowned valley estuaries (Roy et al., 2001), except for the Wapengo and Shoalhaven sites, which are wave-dominated barrier estuaries (Roy et al., 2001). Adult oysters were collected from each of these sites during a six-day period in August 2018. Samples were immediately frozen (-20 °C), transported to the laboratory in a portable freezer and stored at -20 °C prior to analysis.

Extraction of DNA from different Oyster Tissue Types

We examined the microbiome associated with four different oyster tissue types, including the mantle, gill, adductor muscle, and digestive gland (inclusive of digestive diverticula). Ten oyster samples from each location were rinsed under running tap water to remove any external debris and mud. Thawed oysters were then shucked using sterile shucking knives and placed in sterile petri dishes. Oysters were weighed and approximately 25 mg of each respective tissue was dissected and removed from each oyster sample with sterile scalpel blades. DNA was then extracted from the 240 individual tissue samples using the Qiagen DNeasy Blood and Tissue Kit (catalogue: 69506), as per the manufacturer's instructions.

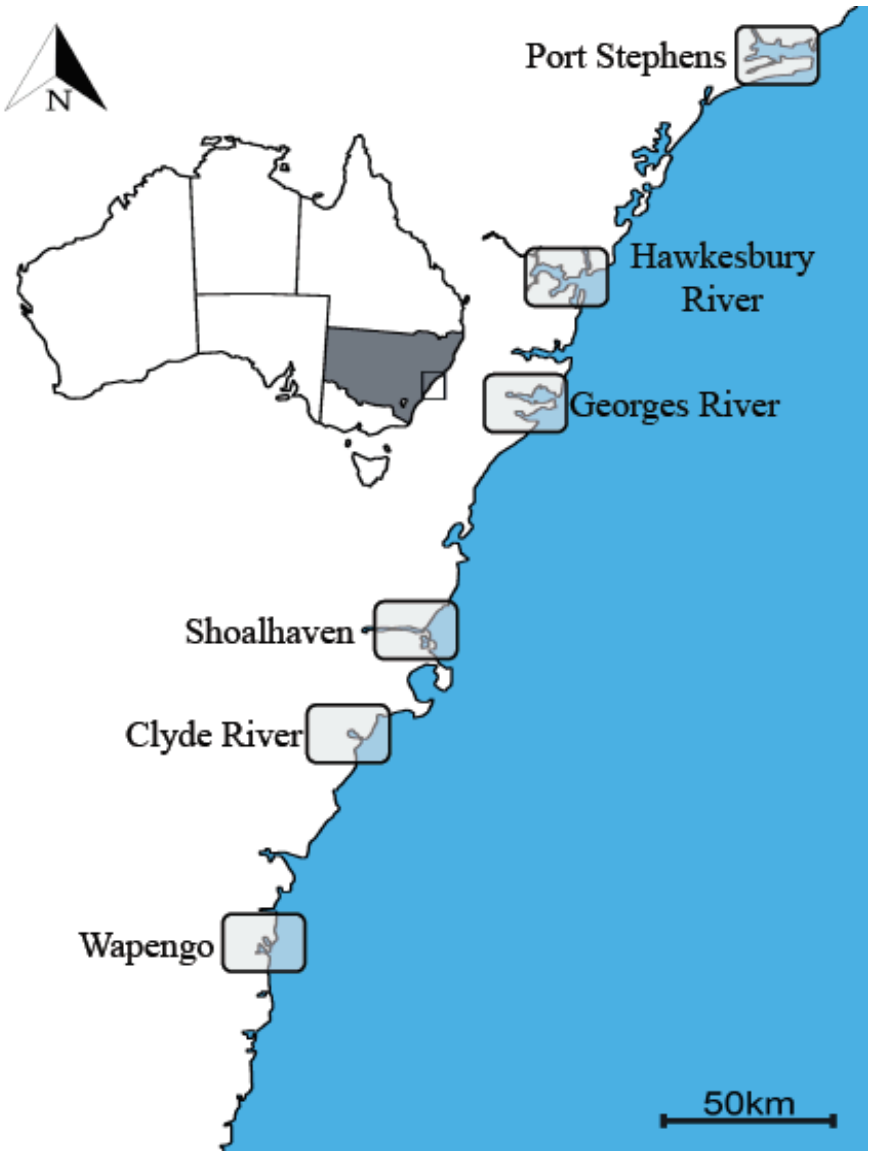


Figure 1: Sampling locations across New South Wales, Australia

Quantitative PCR

To provide an indication of the bacterial abundance within each sample, we employed a quantitative PCR (qPCR) assay to quantify total 16S rRNA gene copies. An epMotion 5075l Automated Liquid Handling System integrated with a Bio-Rad CFX384 Touch Real-Time PCR Detection System was used to perform the analysis. All analyses were performed on three technical replicates, with a standard curve and negative controls, using the following reaction mixture: 2.5 µL iTaq Universal SYBR Green supermix, 0.2 µL of each 10 µM forward and 10 µM reverse primer, 1 µL of template DNA, and 1.1 µL of sterile water. Bacterial abundance was quantified using the 16S rRNA specific primers BACT1369F (CGGTGAATACGTTCYCGG) and

PROK1492R (GGWTACCTTGTTACGACTT) (Suzuki et al., 2000). The qPCR cycling conditions were: 95 °C for 3 minutes followed by 45 cycles of 95 °C for 30 seconds, 55 °C for 30 seconds and 72 °C for 30 seconds. The resulting data were normalised to tissue weight. A coefficient of variation (CV) was then calculated for the technical triplicates, and where necessary, samples with CV > 2 % had a replicate removed from the analysis. A melting curve was added to the end of every run to confirm the presence of a single PCR product.

Oyster Microbiome Analysis

The microbial community composition of each oyster tissue was characterised with 16S rRNA amplicon sequencing, using the 341F (CCTACGGGNGGCWGCAG) and 805R (GACTACHVGGGTATCTAATCC) primer pair (Herlemann et al., 2011) targeting the V3-V4 region of the 16S rRNA gene. The PCR cycling conditions generating the 16S rRNA amplicons were as follows: 95 °C for 3 min, 25 cycles of 95 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s, and a final extension at 72 °C for 5 min. Sequencing was performed using the Illumina MiSeq platform at the Ramaciotti Centre for Genomics (University of New South Wales, Sydney, Australia). Raw data files in FASTQ format were deposited in the NCBI Sequence Read Archive (SRA).

Raw demultiplexed data was processed using the Quantitative Insights into Microbial Ecology (QIIME 2 version 2018.6.0) pipeline (Bolyen et al., 2018). Briefly, paired-ended 16S DNA sequences were imported using the 'qiime tools import' command. Sequences were then trimmed and denoised using DADA2 version 1.6, which also removes chimeras (Callahan et al., 2016). Taxonomy was then assigned at the single nucleotide level using the classify-consensus-vsearch qiime feature classifier (Pedregosa et al., 2011) against the Silva v132 database (Quast et al., 2013). Sequences identified at the single nucleotide threshold are henceforth denoted as ZOTUs (zero-radius OTUs). For those ZOTUs with poor taxonomic assignment, the representative sequence was blasted against the NCBI database. The dataset was further cleaned by removing ZOTUs with less than 400 reads and those identified as chloroplasts or mitochondria. Cleaned data were then rarefied at 8,100 reads per sample, corresponding to a threshold that permitted the inclusion of 5 or more replicate samples for every tissue type.

Core microbiome analysis

To determine whether a core microbiome was conserved for a given tissue type across all sampling environments, we used the panbiom.py analysis described in Kahlke (2017). The analysis was performed with the following parameters: abundance minimum of 0.0 (-m parameter) and a replicate threshold corresponding to 80% (-r parameter)). A core ZOTU was defined as a ZOTU present in 80 % of a given sample type to account for outliers.

Statistical analysis

Alpha diversity measures, including species diversity (Shannon's index), species evenness, and species richness (observed species) were calculated in the Qime 2 statistical environment and compared using a Kruskal-Wallis test. To compare community structure between sampling locations and tissue types, normalised data (square root (x)) were first compared using non-metric multidimensional scaling analysis (nMDS). Microbial assemblages were subsequently compared using a one-way PERMANOVA to elucidate significant microbiome patterns across tissue types and sampling locations. To identify which bacterial taxa were most responsible for driving the differences between locations and tissue types, a similarity percentage analysis (SIMPER) with a Bray-Curtis similarity index was used. Comparisons of 16S rRNA gene copies (16S rRNA qPCR) were first performed with a Kruskal-Wallis statistical test followed by a Mann-Whitney pairwise test. All beta diversity (nMDS, PERMANOVA, and SIMPER) and qPCR comparisons were performed in the PAST statistical environment (Hammer et al., 2001).

<u>Aim 4:</u> Defining the Sydney Rock Oyster microbiome associated with QX disease events

Rationale and Goals

There is evidence from other organisms that a host's microbiome can shift before, during and subsequent to disease events and that these microbiome changes can either cause or be caused by disease on-set. We propose that deciphering the role of the oyster mirobiome in disease events may provide clearer insights into the complex mechanisms behind infection. To provide a better understanding of the the association between microbial communities and disease, we examined the microbiome of SROs during a QX disease event, with the goals of answering the following questions: i) How does the microbiome of SRO change between before and during QX disease

events? ii) How does *M. sydneyi* infection affect the microbiome of SROs? iii) How does the stage of *M. sydneyi* infection affect the microbiome of SROs ? What is the relationship between environmental variables, the oyster microbiome and QX disease events?

Experimental design and sampling

A QX disease field challenge was performed in Georges River, NSW (33°59'19''S 151°03'21''E), a site that is considered at high risk for QX disease events (Nell and Perkins, 2006; Dove *et al.*, 2013). On the basis of their QX-resistance estimated breeding value (EBV), which predicts the mean survival of progeny from a family when exposed to QX disease, 4 family lines (including 2016032, 2016043, 2016048 and 2016067) categorized as intermediate QX disease resistance (20 – 50% survival) were selected for QX disease exposure.

Sporonts of the *M. sydneyi* parasite can be detected approximately 2 weeks after the first molecular detection (Peters and Raftos, 2003). Therefore, samples were collected every fortnight. Prior to the QX disease event, five oysters per family line were collected per sampling time, while during the QX disease event ten oysters per family line were collected. Oysters were randomly collected from cultivation trays and placed into labelled zip-lock bags. Collected oysters were kept on ice for transport to the laboratory (< 1hr) and stored at -80 ^{0}C for subsequent processing.

Measurement of environmental parameters, nutrients and chlorophyll a in water

Environmental parameters (temperature, oxygen, pH and conductivity) were measured using a WTW multiprobe meter (Multi 3430, Germany) at the time of sample collection. For nutrient analysis, triplicate water samples were syringe filtered through a 0.45 µm filter into 50 mL clean falcon tubes and kept on ice during transport to the laboratory, before being frozen at –20^{0} C. Nutrient analysis (Nitrite (NO_2^-), Nitrate (NO_3^-), Ammonia (NH_3) and Phosphate (PO_4^{3-})) was carried out at Envirolab Services Pty Ltd (Sydney, New South Wales, Australia) using standard methods (Rice et al., 2012). For chlorophyll *a* analysis, triplicate 200 mL water samples were filtered through Glass microfiber filters (0.7 µm pore size) and frozen at – 80^{0} C prior to analysis. Chlorophyll *a* was analysed based using Spectrophotometric methods (Ritchie, 2006).

DNA extractions

Frozen oysters were thawed and washed under running water to remove unattached material. Using sterile instruments, each oyster was carefully opened using a shucking knife and immediately

placed into a Petri dish. The adductor muscle was excised using a sterile scalpel blade and placed into a 1.5 mL Eppendorf tube. From each oyster, approximately 25-50 ng of the adductor muscle tissue was collected for subsequent DNA extraction using the Qiagen DNeasy Blood and Tissue DNA extraction Kit (Qiagen, Germany) according to the manufacturer's protocol. For water samples, DNA from filtered water samples was extracted using the PowerWater DNA Isolation Kit (MoBio, USA) according to the manufacturer's protocol.

Diagnosis of QX exposure and disease

Oysters were confirmed to be either infected or uninfected by *M. sydneyi* using PCR and the primers LEG1 (5'-CGATCTGTGTAGTCG- GATTCCGA) and PRO2 (5'-TCAAGGGACATC-CAACGGTC) (Adlard and Wilmer, 2003). The PCR reaction contained 1 µl DNA, 10 µl MangoMix, 1 µl LEG1 primer, 1µl PRO2 primer and 7 µl water. The PCR cycling conditions were as follows: 94 °C for 2 min, followed by 30 cycles of 94°C for 30s, 50°C for 30s and 72 °C for 30s and a final extension at 72 °C for 10 min. DNA purified from an oyster confirmed to be infected with *M. sydneyi* (provided by Dr Cheryl Jenkins - Elizabeth Macarthur Agricultural Institute) was used as a positive control. During the QX disease event, all oysters were confirmed to be either exposed or unexposed with sporulating *M. sydneyi* by employing the tissue imprint methods of Kleeman et al (2000) using the Rapid Diff kit (Australia Biostain Company) for staining.

16S rRNA amplicon sequencing

Extracted DNA was amplified using PCR targeting the ribosomal 16S rRNA V1–V3 region using the 27F (5′-AGAG TTTGATCMTGGCTCAG-3′) and 519R (5′- GWATTACCGCGGCKGCTG-3′) primer pair (Lane, 1991; Turner *et al.*, 1999). The PCR cycling conditions were as follows: 94°C for 2 min, followed by 30cycles of 94°C for 30s, 50°C for 30s and 72°C for 30 s and a final extension at 72°C for 10 min. Amplicons were sequenced using the Illumina MiSeq platform (version 3; 2 × 300 bp) at the Ramaciotti Centre for Genomics (University of New South Wales, Sydney, Australia).

Bioinformatic analysis

All forward and reverse fastq reads were grouped using the Linux command *cat*. Paired-end reads were combined using FLASH (Magoč and Salzberg, 2011) and filtered by length (shorter or longer than 471-500bp) and quality scores (sequences containing more than 6 homopolymers, containing any ambiguous bases and an average score below 25 were removed) using Mothur (Schloss *et al.*,

2009). Chimeric and singleton sequences were identified and removed using VSEARCH (Rognes *et al.*, 2016). Taxonomic assignment of OTUs was performed using the RDP Classifier (Wang *et al.*, 2007) against the SILVA v128 dataset (Quast *et al.*, 2013) with a confidence value of 0.8. Alignment was performed using PyNAST (Caporaso *et al.*, 2010) and was followed by filter alignment. All OTUs identified as chloroplast or mitochondria were removed from the analysis. Sequences were rarefied using QIIME 1.9.1 (Caporaso *et al.*, 2010) to the same depth to remove the effect of sampling effort upon analysis. The multiple_rarefactions.py was used in QIIME1.9.1, then rarefaction plots (Observed_species) were generated to determine the rarefaction depth. Alpha diversity indices (Chao1, Shannon and observed species index) were calculated using QIIME 1.9.1.

Statistical analyses

Differences in alpha diversity between groups were tested using nonparametric Kruskal–Wallis ANOVA tests. For beta diversity, the relative abundance of OTUs was calculated and normalized (square root (x)). Beta diversity analyses were performed with a Bray-Curtis dissimilarity index. Non-metric multidimensional scaling (nMDS) was used to visualize distance matrices between sample groups. Differences in the relative abundance of OTUs was tested using permutational multivariate analysis of variance (PERMANOVA) with 9999 permutations. The Similarity Percentages (SIMPER) test was used to identify the observed dissimilarity of the microbiome between groups. To determine whether OTUs were significantly different between oyster groups, Welch's t –test was performed using STAMP software (Parks *et al.*, 2014).

Mictools was used to identify significant correlations among variables (Donati et al., 2018). All parameters for the analyses were set to defaults with the false discovery rate (FDR) method used for multiple testing correction (Donati et al., 2018). Correlations between environmental parameters, microbiome and QX disease infection were identified based on the output of mictools with p values of < 0.05. A single heatmap was used to visualise the direction of the significant correlations between environmental variables, QX disease infection and dominant bacterial members (function heatmap.2 from the package gplots in Rstudio Version 1.1.463).

<u>Aim 5:</u> Measuring temporal patterns in the Pacific Oyster microbiome during the summer OsHV-1 mortality period

Rationale and Goals

In recent years, significant Pacific Oyster mortality events have occurred in Australian estuaries and in some cases have been linked to OsHV-1 outbreaks. However, the triggers for these outbreaks have remained elusive, with some circumstantial evidence pointing towards a synergistic influence of environmental factors (e.g. temperature). Within the context of Objectives 2 and 3, we established an experimental community of Pacific Oysters within Sydney Harbour and performed a time-series study to identify the existence of links between the oyster microbiome, environmental variability and oyster mortality.

Temporal study design and sampling

C. gigas samples were sourced from a commercial oyster farmer in Port Stephens, NSW. A total of 960 oysters, with a mean length of 20mm, were deployed at the Sydney Institute of Marine Science (SIMS) in two different experimental conditions. In the first condition, oysters were hung from oyster cultivation baskets (600mm long and 200mm wide) from a jetty, directly into the waters of Sydney Harbour. Three baskets were deployed from the jetty with a stocking density of 160 oysters per basket. In the second condition, three 500L high-flow through oyster-holding tanks were used. Each oyster holding tank held one oyster cultivation basket with a stocking density of 160 oysters per basket. High-flow through unfiltered seawater was pumped into these tanks from a continuous depth of 50cm.

Before transportation from the oyster farmer, 6 oysters were snap frozen and stored at -80°C. Upon arrival of the oysters to SIMS, they were immediately placed into filtered seawater and 10 oysters were snap frozen and stored at -80°C. Samples were taken at these time points to determine any effect of transportation stress on the bacterial community and the expression of host stress genes from the oysters.

Initially, 6 oysters were sampled weekly from each SIMS experimental treatment by collecting two oysters from each basket/tank (total of n = 12). On the fifth week of sampling, the number of sampled replicates per treatment was increased to 9 (n = 18). Collected oysters were immediately snap frozen in liquid nitrogen and stored at -80°C until processed. Oysters were gently removed

from the baskets each week and placed into a container filled with water from the same deployment condition to observe any mortalities that may have occurred.

Tissue sample processing

Snap frozen oysters were shucked using sterile knives and were removed from the shell using a sterile scalpel. This tissue was weighed and then placed in a pre-chilled stainless-steel mortar and pestle-like vessel. The tissue was then pulverised using a hydraulic shop press to crush the oyster tissues into powder. This powder was then stored -80°C until processed.

Measurement of environmental factors

At SIMS, temperature, pressure, dissolved oxygen, conductivity, pH, oxidative-reduction potential (ORP), turbidity and total suspended solids were measured weekly from both experimental conditions using a YSI ProDSS (catalogue: 626870-1). In addition, temperature and conductivity were measured every thirty minutes using an odyssey temperature and conductivity data logger (catalogue: ODYCT).

DNA extraction and sequencing

The Qiagen DNeasy blood and tissue kit (catalogue: 69506) was used to extract DNA from 25 mg of homogenised powdered tissue. DNA was extracted from seawater samples using the PowerWater DNA extraction kit. The microbial community composition of each oyster sample was characterised with 16S rRNA amplicon sequencing, using the 341F (CCTACGGGNGGCWGCAG) and 805R (GACTACHVGGGTATCTAATCC) primer pair (Herlemann et al., 2011) targeting the V3-V4 region of the 16S rRNA gene. The PCR cycling conditions generating the 16S rRNA amplicons were as follows: 95 °C for 3 min, 25 cycles of 95 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s, and a final extension at 72 °C for 5 min. Sequencing was performed using the Illumina MiSeq platform at the Ramaciotti Centre for Genomics (University of New South Wales, Sydney, Australia).

Raw demultiplexed data was processed using the Quantitative Insights into Microbial Ecology (QIIME 2 version 2018.6.0) pipeline (Bolyen et al., 2018). Briefly, paired-ended 16S DNA sequences were imported using the ‘qiime tools import’ command. Sequences were then trimmed and denoised using DADA2 version 1.6, which also removes chimeras (Callahan et al., 2016). Taxonomy was then assigned at the single nucleotide level using the classify-consensus-vsearch

qiime feature classifier (Pedregosa et al., 2011) against the Silva v132 database (Quast et al., 2013). Sequences identified at the single nucleotide threshold are henceforth denoted as ZOTUs (zero-radius OTUs). For those ZOTUs with poor taxonomic assignment, the representative sequence was blasted against the NCBI database. The dataset was further cleaned by removing ZOTUs with less than 400 reads and those identified as chloroplasts or mitochondria.

Statistical analysis

Alpha diversity measures, including species diversity (Shannon's index), species evenness, and species richness (observed species) were calculated in the Qime 2 statistical environment and compared using a Kruskal-Wallis test. To compare community structure between sampling times, normalised data (square root (x)) were first compared using non-metric multidimensional scaling analysis (nMDS). Microbial assemblages were subsequently compared using a one-way PERMANOVA to elucidate significant microbiome patterns across tissue types and sampling locations. To identify which bacterial taxa were responsible for driving the differences between times, a similarity percentage analysis (SIMPER) with a Bray-Curtis similarity index was used with data summarised at the genus level. All beta diversity (nMDS, PERMANOVA, and SIMPER) tests were performed in the PAST statistical environment (Hammer et al., 2001).

<u>Aim 6:</u> Elucidating patterns in *Vibrio* community diversity and abundance within the microbiomes of Pacific Oysters subject to disease and mortality events

Rationale and Goals: Results from our research (King et al. 2017; Green et al. 2018; and project sections detailed above) and others (Petton et al. 2015) have indicated some consistent patterns in the microbiomes of oysters during mortality and disease events. More specifically, bacteria within the *Vibrio* genus often become more prominent in compromised or diseased oysters. *Vibrio* are a group of gram-negative, marine bacteria that are ubiquitous members of microbial assemblages inhabiting estuaries and coastal environments (Thompson et al. 2004; Simidu and Tsukamoto 1985). They are also notable for their ecological associations with a number of marine organisms including but not limited to: bivalves, cephalopods, polychaetes, fish, corals, and algae (Lee and Ruby 1994; Nyholm and Nishiguchi 2008; Miyashiro and Ruby 2012; Ben-Haim and Rosenberg 2002; Lemire et al. 2015; Tout et al. 2015; Grisez et al. 1997; Raguenes et al. 1997; Hood and

Winter 1997) and there is circumstantial evidence for their involvement in infections in a variety of oyster species. However, a clear understanding of the role of these bacteria in Pacific Oyster disease is lacking, in large part because of difficulties in accurately characterising *Vibrio* diversity in natural samples. Due to the potential importance of *Vibrio* bacteria in oyster disease dynamics (Daniels and Shafaie 2000), it is imperative that a robust analytical assay is developed to improve the resolution of current sequencing technologies for this bacterial group (Jesser and Noble 2018). Within this context, we developed a new *Vibrio*-specific high-throughput sequencing assay and applied it to *Crassostrea gigas* samples associated with mortality events, in which *Vibrio* bacteria were implicated as a potential contributing factor.

Primer and Vibrio reference dataset construction

In order to develop a reference dataset to aid the design of a new set of degenerate primers targeting the *Vibrio hsp60* gene, 100 *Vibrio hsp60* coding sequences were collected from the NCBI repository and blasted against the NCBI nucleotide database (nt file). Sequences were extracted using extract_hitseqs_from_sequences.pl and both accession numbers and their respective taxonomy were then extracted using list_basta_taxa.py provided by BASTA v1.3.2.3 (Kahlke and Ralph, 2019). The blast output was filtered to retain taxa assigned to the *Vibrio* genus using the filter_basta_fasta.py script also provided by BASTA (Kahlke and Ralph, 2019) and genes assigned as *hsp60* and *groEL* were collected and added to our *Vibrio-hsp60* dataset. Both of these genes were chosen because they have previously been assigned as the same gene, but are annotated differently (Silvester et al., 2017). The *Vibrio-hsp60* data set was aligned with MAFFT (Katoh et al., 2017) using the einse –reorder option. The aligned untrimmed *hsp60* dataset was visualised using UGENE (Golosova et al., 2014) and highly conserved areas within the consensus sequence were chosen for primer construction. Primers were constructed using the Primer3Plus (Untergasser et al., 2007) software. The constructed degenerate primers were named Vib-hspF3-23 and Vib-hspR401-422 (Table 1), and their application resulted in the amplification of a 487 bp PCR product (Illumina adapters inclusive).

To ensure that the *Vibrio* reference dataset was constructed with accurately assigned *Vibrio* taxa and not partial *hsp60* reads (which could possibly be assigned to the wrong taxa), we constructed a reference dataset using *hsp60* sequences taken from whole genomes. First, all of the currently available complete *Vibrio* genomes were collected from the NCBI repository (185 genomes) and a BLAST database was constructed using these genomes. *Vibrio hsp60* sequences were compared

against this database and all hits at least 65 % similar to the query *hsp60* sequence and at least 400 base pairs long were extracted. BLAST hits were then visualised and trimmed to the primer locations in MEGA (version 7.0.26). To determine the coverage of *Vibrio* species in our dataset, we compared the taxa in this trimmed dataset against the listed *Vibrio* species in the NCBI taxonomy database. Where possible, *hsp60* sequences for missing *Vibrio* species were collected from incomplete whole genomes and added to the *Vibrio* reference dataset. This yielded a dataset comprising 106 different *Vibrio* species incorporating 284 *hsp60* sequences. In some instances, *hsp60* was found in both *Vibrio* chromosomes. Where known, the second copy of the gene was named as 'group2'.

Table 3: Primers used in this study. Underlined sequences are Illumina sequencing adapters

Primer name	Sequence (5'-3')	Source
Vib-hspF3-23	TCGTCGGCAGCGTCAGATGTGTATAAGAGAC AGGAACCCNATGGAYCTKAARCG	This study
Vib-hspR401-422	GTCTCGTGGGCTCGGAGATGTGTATAAGAGA CAGGCVATGATHARHAGHGRRCGNG	This study
16S 341F	AGAGTTTGATCMTGGCTCAG	(Herlemann et al., 2011)
16S 805R	GWATTACCGCGGCKGCTG	(Herlemann et al., 2011)
VF169	TCGTCGGCAGCGTCAGATGTGTATAAGAGAC AGGGATAACYATTGGAAACGATG	(Yong et al., 2006)
Vib-680R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGA CAGGAAATTCTACCCCCCTCTACAG	(Thompson et al., 2004)
Vib1-f	GGCGTAAAGCGCATGCAGGT	(Vezzulli et al., 2011)

Vib2-r	GAAATTCTACCCCCCTCTACAG	(Thompson et al., 2004)

Mock Vibrio community preparation

Ten *Vibrio* species, spanning five clades (Sawabe et al., 2013; Turner et al., 2018) and incorporating species that are relevant to both human health (Daniels and Shafaie, 2000) and aquaculture diseases (Luna-González et al., 2002; Bruto et al., 2017; Go et al., 2017) were grown overnight in LB20 broth (per litre: 10 g tryptone, 5 g yeast extract, 20 g NaCl), with shaking at 28°C. Bacterial cells were enumerated using a Beckman CytoFLEX flow cytometer and cell counts were diluted to a standardised concentration across all strains. Three different mock *Vibrio* communities were prepared by mixing the 10 *Vibrio* species in different dilution ratios (Table 2). DNA was then extracted from mock assemblages using the Qiagen DNeasy UltraClean Microbial Kit (catalogue: 12224-250) following the manufacturer's instructions.

Table 4: Composition of mock Vibrio communities generated in this study.

Vibrio species	Mock1 (%)	Mock2 (%)	Mock3 (%)
V. vulnificus	10	30	7.5
V. rotiferianus	10	5	7.5
V. sinaloensis	10	5	7.5
V. cholerae	10	30	7.5
V. campbellii	10	5	7.5
V. alginolyticus	10	5	7.5
V. diabolicus	10	5	7.5
V. harveyi	10	5	20
V. crassostreae	10	5	7.5
V. parahaemolyticus	10	5	20

Mock community PCR conditions and sequencing

DNA extracted from the mock bacterial communities was diluted to 10 ng μL^{-1} and used in a 50 μL PCR reaction volume as follows: 10 μL of 5 x Hi-Fi Buffer (Bioline), 5 μL of 10 mM dNTPs, 2 μL of high-fidelity Velocity polymerase (0.5 units μL^{-1}; Bioline), 2.5 μL of 10 μM forward primer (VF169 or Vib-hspF3-23), 2.5 μL of 10 μM reverse primer (Vib-680R or Vib-hspR401-422), 2 μL of DNA template (10 ng μL^{-1}), with the remaining volume made up with sterile water. The PCR mixture was then subjected to the following PCR conditions: one cycle of 98°C for 2 minutes, 30 cycles of 98°C for 30 seconds, 50°C for 30 seconds, and 72°C for 30 seconds, and a final extension time of 72°C for 10 minutes. PCR products were purified with a Bioline Isolate II PCR and Gel Kit (catalogue: BIO-52059) using the manufacturer's instructions. For 16S rRNA sequencing, extracted DNA was amplified with the following PCR conditions: 95°C for 3 minutes, 25 cycles of 95°C for 30 seconds, 55°C for 30 seconds and 72°C for 30 seconds, and a final extension at 72°C for 5 minutes.

Mock bacterial community amplicons were characterised on the Illumina MiSeq platform (Ramaciotti Centre for Genomics; Sydney, NSW, Australia) using the manufacturers guidelines, using three primer sets (Table 1): the universal 16S rRNA primers 341F and 805R (Herlemann et al., 2011); a previously published *Vibrio*-specific 16S rRNA primer pair, VF169 (Yong et al., 2006) and Vib-680R (Thompson et al., 2004; Siboni et al., 2016); and the *Vibrio*-specific *hsp60* primer pair designed in this study, Vib-hspF3-23 and Vib-hspR401-422.

Mock community sequence analysis

Bacterial 16S rRNA and *hsp60* sequencing reads for the mock communities were processed as outlined in Kahlke (2018). Briefly, paired-end DNA sequences were joined using FLASH (Magoč and Salzberg, 2011) and subsequently trimmed using Mothur (Schloss et al., 2009) (PARAMETERS: universal 16S - maxhomop=6, maxambig=0, qaverage=25, minlength=491, maxlength=501; *Vibrio*-specific 16S - maxhomop=6, maxambig=0, qaverage=25, minlength=533, maxlength=534; *hsp60* - maxhomop=6, maxambig=0, qaverage=25, minlength=420, maxlength=420). The resulting fragments were clustered at 97 % into operational taxonomic units (OTUs) and chimeric sequences were identified and removed using vsearch (Rognes et al., 2016). To assign taxonomy, QIIME (Caporaso et al., 2010) was used with the RDP classifier against either the Silva v128 database (for 16S rRNA analysed samples) or against our custom *Vibrio-hsp60*

reference dataset. Sequences were then rarefied to the same depth to remove the effect of sampling effort upon analysis.

Seawater collection, 16S rRNA sequencing and data analysis

To test the newly designed *Vibrio*-specific *hsp60* sequencing assay on seawater samples, water was collected from Sydney Harbour (33.839S, 151.254E) in the Austral summer. Seawater was filtered in triplicate through 0.22 μm membranes and the filters immediately snap frozen in liquid nitrogen. Microbial DNA was subsequently extracted from filters using a Qiagen DNeasy PowerWater kit (catalogue: 14900-100-NF) and sent to the Ramaciotti Centre for Genomics (University of New South Wales, Sydney, Australia) for 16S rRNA (341F and 805R) sequencing on the Illumina MiSeq platform.

Raw 16S rRNA demultiplexed paired-end DNA sequences were joined using Flash (Magoč and Salzberg, 2011) and trimmed with Mothur (Schloss et al., 2009) (PARAMETERS: maxhomop=6, maxambig=0, qaverage=25, minlength=441, maxlength=466). Fragments were then clustered into OTUs at 97 % and chimeric sequences were removed using vsearch (Rognes et al., 2016). QIIME (Caporaso et al., 2010) and the RDP classifier were then used to assign taxonomy against the Silva v128 database. Sequences were then rarefied.

Seawater hsp60 PCR conditions, sequencing and data analysis

DNA from seawater was amplified using the Vib-hspF3-23 and Vib-hspR401-422 primer pair with the Illumina adapters added to the primers (Table 1). The 30 μL PCR reaction mixture was as follows: 6 μL of 5 x Hi-Fi Buffer (Bioline), 3 μL of 10 mM dNTPs, 0.5 μL of high-fidelity Velocity polymerase (2 units μL^{-1}; Bioline), 1.5 μL of 20 μM forward primer, 1.5 μL of 20 μM reverse primer, 3.5 μL of template DNA, and the remainder (14 μL) made up with sterile water. The mixture was used with the following touchdown PCR conditions: one cycle of 98°C for 2 minutes, 5 cycles of 98°C for 30 seconds, 60°C for 30 seconds, and 72°C for 45 seconds, 21 cycles of 98°C for 30 seconds, 60°C for 30 seconds with a reduction of 0.5°C per cycle (60°C to 50°C), and 72°C for 45 seconds, 14 cycles of 98°C for 30 seconds, 50°C for 30 seconds, and 72°C for 45 seconds, and a final extension time of 72°C for 10 minutes. Amplicons were then purified by the Ramaciotti Centre for Genomics, and characterised on the Illumina MiSeq platform using the manufacturers guidelines.

As the *Vibrio*-specific *hsp60* primer pair was found to in some scenarios non-specifically amplify other taxa, a further cleaning step was added to the data analysis. In the first instance, pair-ended sequences were joined using FLASH (Magoč and Salzberg, 2011) and trimmed using mothur (Schloss et al., 2009) (PARAMETERS: maxhomop=5, maxambig=0, qaverage=25, minlength=420, maxlength=420). These fragments were then clustered at 97% similarity into operational taxonomic units (OTUs) and chimeric sequences were identified and removed using vsearch (Rognes et al., 2016) against the *Vibrio-hsp60* reference dataset. To remove reads not belonging to the *Vibrio* genera, a BLAST database was constructed using the cleaned OTU fasta file after removing chimeras. The *Vibrio-hsp60* reference dataset was then blasted against the cleaned OTU fasta file, and OTUs that were 90 % similar to sequences in the *Vibrio-hsp60* reference dataset, and over 400 bp in length, were retained. The best BLAST hit for each OTU was then extracted, therefore removing the possibility of retaining multiple BLAST hits for each OTU. This fasta file was then used to assign taxonomy against with the RDP classifier. Due to the large spread of reads per sample, data were not rarefied, reads were normalised to the number of reads per sample to produce the relative abundance of each taxa for each sample.

Quantitative PCR (qPCR)

To provide an indication of *Vibrio* abundance in each sample, a quantitative PCR (qPCR) assay was used to quantify the number of *Vibrio*-specific 16S rRNA gene copies in each sample using the *Vibrio*-specific 16S rRNA gene primers Vib1-f and Vib2-r (Table 1) (Thompson et al., 2004; Vezzulli et al., 2011; Siboni et al., 2016). qPCR was performed using an epMotion 5075l Automated Liquid Handling System on a Bio-Rad CFX384 Touch Real-Time PCR Detection System with a seven-point calibration curve and a negative control. The calibration curve was created from 10-fold dilutions of a known quantity of amplicon DNA, measured by a Qubit fluorometer. All sample analyses were performed with three technical replicates, using the following reaction mixture: 2.5 μL iTaq Universal SYBR Green supermix, 0.4 mM of each forward and reverse primer, 1 μL of template DNA (50ng μL^{-1}), with the remainder made up with water. The qPCR cycling conditions were as follows: 95°C for 3 min followed by 45 cycles of 95°C for 15 s and 60°C for 1 min. The resulting data were normalised to millilitres of collected water. A coefficient of variation (CV) was calculated for the technical triplicates, and where necessary,

samples with CV > 1 % had a replicate removed from the analysis. A melting curve was added to the end of every run to confirm the presence of a single PCR product.

Laboratory-induced oyster mortality event

The newly designed *Vibrio*-specific *hsp60* primer set was applied to examine patterns in *Vibrio* community diversity during a previously described laboratory-induced Pacific Oyster mortality event, where *Vibrio* species had previously been implicated as the cause of oyster mortality during a simulated marine heatwave (Green et al., 2019).

Briefly, triploid Pacific oyster (*C. gigas*) spat were collected from Port Stephens, New South Wales, Australia, prior to a forecasted marine heat wave. Spat were held in two different temperature conditions (low 20°C ± 1°C and high 25°C ± 1°C) with and without antibiotics (100 units/ml of penicillin and 0.1 mg/ml of streptomycin) and monitored for six days. Spat were placed in sterilised glass tanks, and UV and 5 μm filter sterilised seawater were added and replaced daily. Triplicate oyster spat were sampled on days 0, 3, 4, 5, and 6, and dead oysters were removed and frozen at -80 °C prior to processing. Cumulative mortality was 77.4 ± 10.7 % and 3.4 ± 5.9 % for the high and low temperature treatment respectively, with antibiotics in the high temperature treatment reducing the cumulative mortality to 4.3 ± 3.7 %. Mortalities were greatest between days three to five. For the purposes of this study, DNA extracted from spat exposed to the high and low temperature treatments from each sampling point, were amplified with the *Vibrio*-specific *hsp60* primer pair (Table 1). *Vibrio* dynamics were previously characterised within oyster tissues using a combination of culture-based approaches, quantitative PCR and 16S rRNA amplicon sequencing (Green et al., 2019). Oyster DNA were subject to *hsp60* PCR amplification, sequencing and data analysis, and qPCR, as described above for the seawater samples, with the exception of DNA being diluted to 50 ng μL^{-1} for the PCR conditions.

Statistical analyses

Comparisons of community compositions were performed using non-metric multidimensional scaling analysis (nMDS) with a Bray-Curtis dissimilarity index, using data normalised to the number of reads per sample, with reads less than 1 % relative abundance removed and then transformed (square root). Patterns observed in the nMDS analysis were statistically tested with a one-way PERMANOVA with 9999 permutations. To examine the similarity between each

characterised community to the mock community, similarity indices were calculated with a Bray-Curtis dissimilarity index using data that was filtered, transformed and with reads assigned to the second chromosome (group2) combined with their respective assigned species. To examine the contribution of individual *Vibrio* species to community dissimilarity, a SIMPER analysis with untransformed data was used with a Bray-Curtis dissimilarity index. To determine the relationship between *Vibrio*-specific 16S rRNA gene copies and the yield of *hsp60* reads from the QIIME analysis, an ordinary least squares linear regression was used. Spearman's rank correlation was used to examine relationships between *Vibrio* species (summarised at the species level) and oyster mortality. All statistical comparisons were performed in the PAST statistical environment (Hammer et al., 2001).

Results

<u>Aim 1:</u> Characterising the composition of the Pacific Oyster microbiome across diverse oyster families, including those exhibiting different levels of susceptibility to OsHV-1 disease

The C. gigas microbiome

Following data filtering and rarefication, a total of 3294 bacterial operational taxonomic units (OTUs) were observed across the entire dataset. Of these, 68.5% comprised < 1% of the total relative abundance. Conversely, across all samples and spanning all RGs, a single member of the *Pseudomonas* genus (OTU 2034) had the highest relative abundance, comprising 5.6% of the bacterial community. This was followed by OTUs matching an uncultured bacterium in the *Psychrobacter* genus (OTU 1488) and an uncultured bacterium in the *Mycoplasma* genus (OTU 3150), which represented 4.8% and 4% of the *C. gigas* microbiome across the whole dataset respectively (Figure 2).

Variability in the C. gigas microbiome across different resistance lines

To determine whether breeding for disease resistance shaped the *C. gigas* microbiome, the microbiome of oysters assigned to RGs were characterised and compared. Alpha diversity, quantified using Shannon's diversity index was significantly higher in the medium OsHV-1 resistance group (RG2) when compared to the low resistance group (RG3) ($F_{(1, 141)} = 6.8$, $p = 0.025$), but did not vary significantly when compared to the high resistance group (RG1) ($F_{(1, 93)} = 0.4$, $p = 0.51$). Species richness (Chao1) did not differ significantly between any of the RGs (RG1 vs RG2 - $F_{(1, 93)} = 0.03$, $p = 0.85$; RG1 vs RG3 - $F_{(1, 94)} = 1.3$, $p = 0.26$; RG2 vs RG3 - $F_{(1, 141)} = 1.08$, $p = 0.30$).

Comparisons of microbiome composition (beta diversity) across different RGs revealed that the microbiomes of RG1 and RG2 were both significantly different to the least disease resistant group, RG3 ($p = 0.019$ and $p = 0.0001$; $F_{(1, 94)} = 1.47$ and $F_{(1, 141)} = 2.93$ respectively). No significant difference was found between the microbiomes of RG1 and RG2 ($F_{(1, 93)} = 1.29$, $p = 0.055$). Statistical differences between RG2 and RG3 appeared to be stronger than those between RG1 and RG3, possibly due to more families being assigned to RG2, therefore potentially adding more microbiome variability to this group. No clear dissimilarity in the microbiome of the RGs

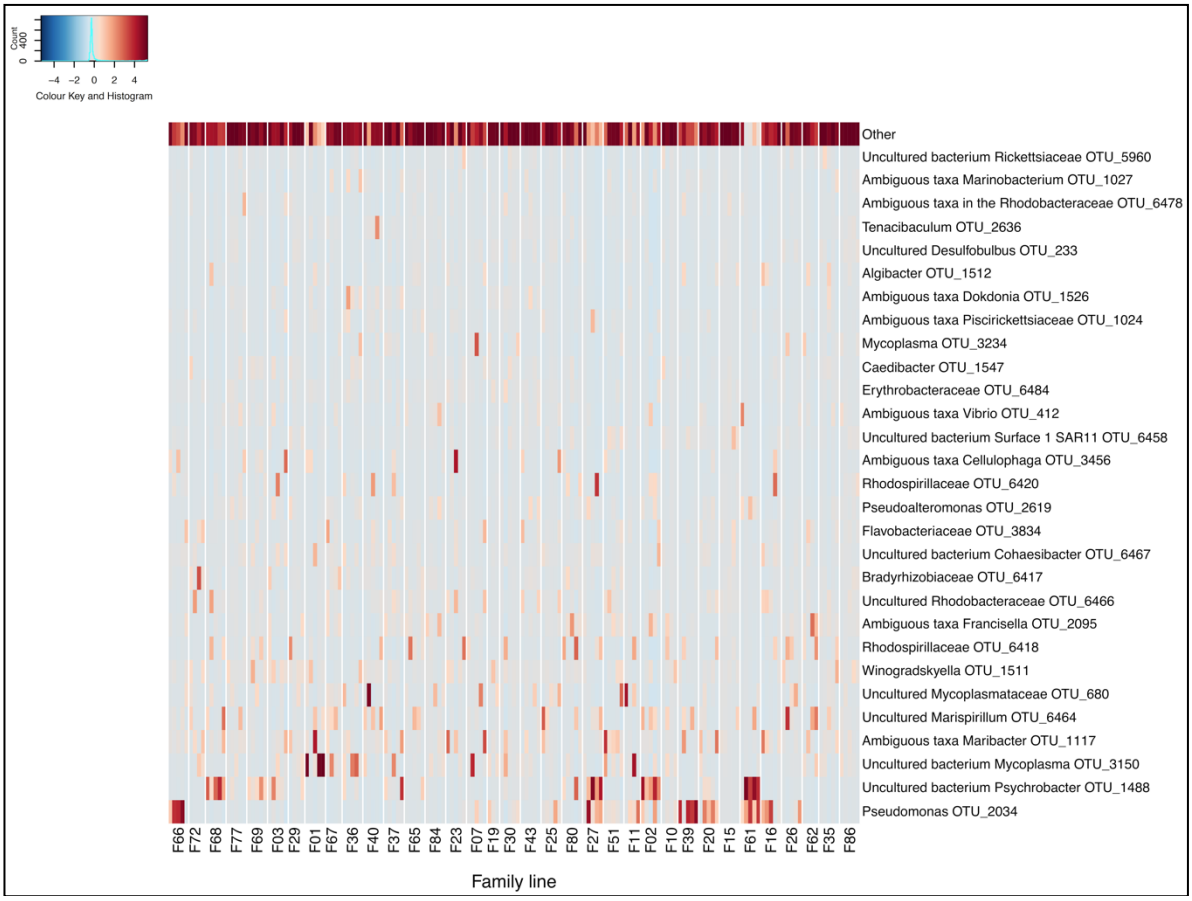


Figure 2: Heatmap of scaled OTU relative abundance for the 30 most abundant OTUs, as well as the remaining summed lowly abundant OTUs. Families ordered by OsHV-1 µvar disease-resistance. Heatmap was made using the R statistical environment using scaled data with the gplots and RColorBrewer packages (Neuwirth, 2014; Warnes et al., 2016; R_Core_Team, 2017).

was apparent in a 3D nMDS (Stress = 0.34), or a PCoA (Supplementary Figure 1 – Appendix). However, SIMPER comparisons showed that the composition of the microbiomes associated with RG1 and RG2 were 81.83% and 82.12% dissimilar to RG3 respectively (Supplementary Table 3; Supplementary Table 4 – Appendix).

As the RG with the lowest level of disease-resistance (RG3) was found to have a significantly different microbial assemblage to both RG2 and RG1, we examined which OTUs were responsible for driving the differences in microbiome structure between these groups (Figure 3). An OTU assigned to the *Pseudomonas* genus (OTU 2034; the most abundant OTU in the entire dataset) was over-represented in the RG3 microbiome relative to both RG1 ($H_{(1, 94)} = 7.6$, $p = 0.0058$) and RG2 ($H_{(1, 141)} = 15$, $p = 0.00011$). Conversely, an OTU assigned to the *Tenacibaculum* genus (OTU

2636) and two separate OTUs assigned to the *Dokdonia* genus (OTUs 2162 and 1526) were all significantly under-represented in RG3 (*Tenacibaculum* RG1 $H_{(1, 94)} = 4.5$, $p = 0.033$ and RG2 $H_{(1, 141)} = 15.2$, $p = 0.0056$; *Dokdonia* RG1 $H_{(1, 94)} = 7.7$, $p = 0.0001$ and RG2 $H_{(1, 141)} = 30.3$, $p < 0.0001$).

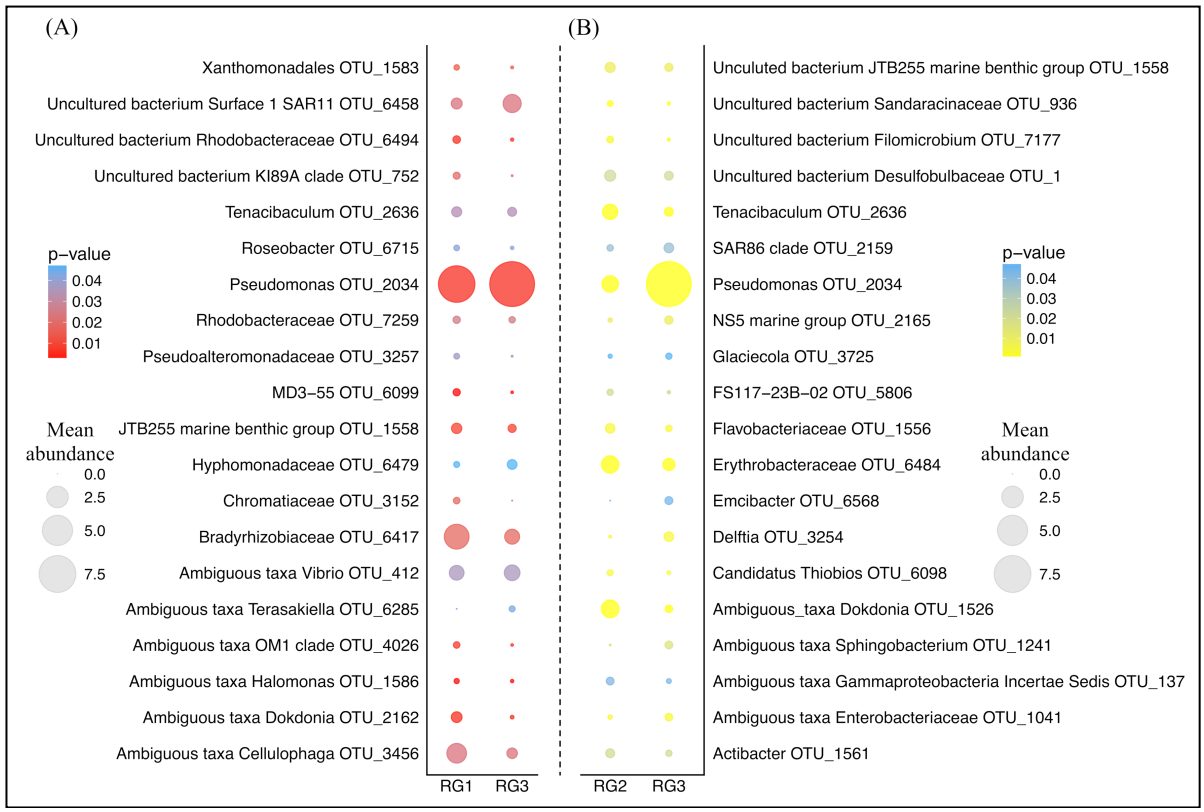


Figure 3: Bubble plot of group_significance.py analysis results using the default Kruskal-Wallis parameters. **(A)** represents the comparison between RG1 and RG3. **(B)** represents the comparison between RG2 and RG3. Colour represents the strength of the p-value. Size represents the mean relative abundance of that OTU across the whole resistance group (RG). OTUs assigned to the genus and species level were chosen, and those 20 most abundant from each RG are displayed.

Notably, a member of the *Vibrio* genus (OTU 412) was found to be significantly over-represented in the least disease-resistant group (RG3) relative to the most disease-resistant group (RG1) ($H_{(1, 94)} = 4.4$, $p = 0.036$). Due to the previously demonstrated importance of *Vibrio* species in OsHV-1 µvar infection (Segarra et al., 2010; Jenkins et al., 2013; de Lorgeril et al., 2018), we subsequently employed a *Vibrio*-specific 16S rRNA qPCR assay to compare total abundances of *Vibrio* across RGs. A significant elevation of *Vibrio* 16S rRNA gene copies was observed in RG3 compared to RG1 ($F_{(1, 94)} = 2.86$, $p = 0.027$) and RG2 ($F_{(1, 141)} = 3.25$, $p = 0.014$) (average of 179, 107 and 75 gene copies mg of $tissue^{-1}$ respectively; Supplementary Figure 2 – Appendix). Furthermore, OTUs

assigned to the *Vibrio* genera were significantly elevated in RG3 when compared to RG1 ($F_{(1, 94)} = 4.27$, $p = 0.011$), but not RG2 ($F_{(1, 141)} = 2.48$, $p = 0.07$). To determine the extent to which *Vibrio* OTUs were responsible for driving the differences between RG1 and RG3 microbiomes, OTUs assigned to the *Vibrio* genus were removed and the RG beta diversity comparison was re-performed. When doing this, we observed a slight weakening of the statistical comparison between RG1 and RG3, from ($F_{(1, 94)} = 1.47$, $p = 0.019$) to ($F_{(1, 94)} = 1.46$, $p = 0.024$), supporting the notion that disimilar abundance of Vibrios was a significant contributor to the differences in microbiomes between the microbiomes of oysters with differing levels of resistance to OsHV-1.

A CCA was used to highlight associations between specific OTUs, OsHV-1 μvar disease-resistance and EBVs of other traits (Figure 4). OTUs matching the *Cupriavidus* (OTU 2182) and *Psychrilyobacter* (OTU 5046) genera were closely coupled with disease-resistance, followed by a member of the *Tenacibaculum* (OTU 2153) genus and an uncultured bacterium in the *Frankiales* order (OTU 5180). While OTUs assigned to members of the *Photobacterium* (OTU 1063; OTU 654; OTU 1053), *Vibrio* (OTU 651; OTU 653) and *Aliivibrio* (OTU 1248) genera were negatively associated with disease-resistance, but strongly associated with meat condition. Furthermore, members of the *Streptococcus* (OTU 814) and *Roseovarius* (OTU 7180) genera were closely associated with depth and width index, and also negatively associated with disease-resistance. The community composition was largely influenced by the first axis, driven by growth related EBVs. A MINE analysis identified a negative correlation between disease resistance and width index ($p = 0.047$; linear regression = -0.34), and a positive correlation between disease resistance and oyster weight ($p = 0.038$; linear regression = 0.15). Shell length and depth index had the strongest negative correlation ($p = <0.001$; linear regression = -0.92), while oyster weight and shell length had the strongest positive correlation ($p = 0.002$; linear regression = 0.74; Supplementary Table 5 – Appendix).

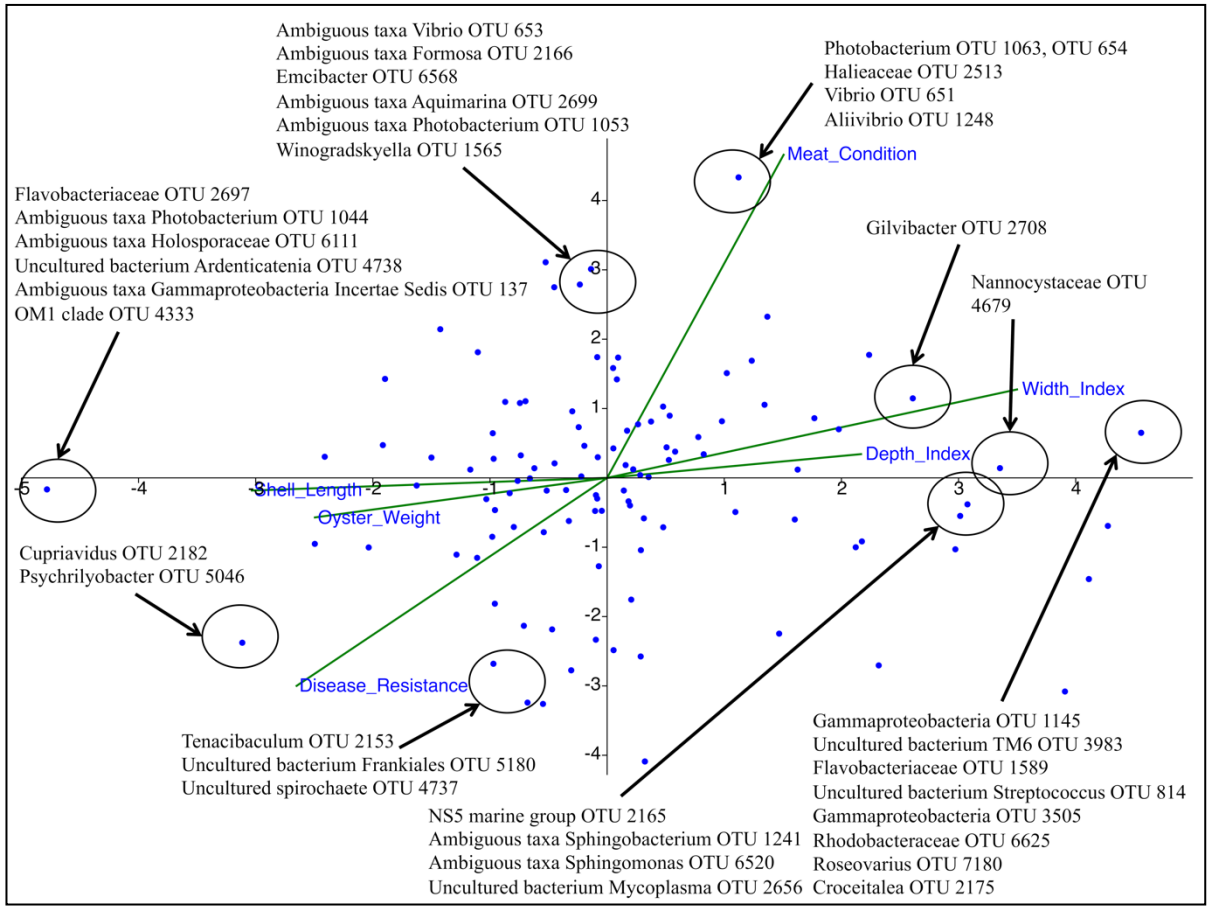


Figure 4: Canonical correspondence analysis plot using 3% relative abundance filtered data. *Cupriavidus*, *Psychrilyobacter*, *Tenacibaculum* and *Frankiales* were found to be strongly associated with OsHV-1 µvar disease-resistance, while OTUs assigned to the *Photobacterium*, *Vibrio* and *Aliivibrio* were negatively associated with OsHV-1 µvar disease-resistance. Axis 1 and 2 were able to significantly represent 53.2% of the data ($p = 0.001$ for both axes with 999 permutations).

Defining the core C. gigas microbiome across different resistance lines

Due to the dynamic nature of oyster microbiomes, identifying a core microbiome can provide insights into which members may be most responsible for driving the within-microbiome interactions and possibly playing important roles in shaping the over-all community composition. While we were unable to identify a universal core microbiome across all samples, analyses of individual families revealed that each family had a small core microbiome (9-109 OTUs), with many of these OTUs shared across families. Families 30 and 84, within RG2, shared the most core OTUs (4) (Supplementary Figure 3). In contrast, family 19 of RG2 had the most unique core OTUs (27), that is those core OTUs were not shared with any other family. To determine how many unique core OTUs were present in each oyster family (and therefore each RG), we compiled all of the core OTUs from the core analysis and removed duplicate bacteria. When doing this, a total of

9, 54 and 16 unique OTUs were assigned to RG1, RG2 and RG3 respectively (Table 5). When performing a separate core analysis on each RG as a whole, RG1 was comprised of two core members, a member of the *Winogradskyella* genus (OTU 1511) and a member of the *Bradyrhizobiaceae* family (OTU 6417). While, no core bacterial members were found for RG2 or RG3 microbiomes.

Table 5: Unique core bacterial members from individual oyster families organised according to their respective resistance groups (RG).

Resistance group	Combined unique core members from individual family lines	
RG1	Acinetobacter OTU_2667 Ambiguous taxa Cellulophaga OTU_3456 Brevundimonas OTU_6676 Ambiguous taxa Marinomonas OTU_1295 Ambiguous taxa Ilumatobacter OTU_4817	Rhodobacteraceae OTU_6650 Ambiguous taxa Marinomonas OTU_1295 Roseobacter OTU_6715 Rhodobacteraceae OTU_7212
RG2	Uncultured Salinimonas OTU_6618 Planctomycetaceae OTU_4123 Ambiguous taxa Gammaproteobacteria Incertae Sedis OTU_465 Uncultured bacterium Anaerolineaceae OTU_4681 Uncultured bacteria Gammaproteobacteria OTU_5902 Croceitalea OTU_2175 Uncultured bacterium Halanaerobiales ODP1230B8.23 OTU_4816 Oceanospirillaceae OTU_1577 Pseudoalteromonadaceae OTU_3257 Uncultured bacterium Ilumatobacter OTU_4687 Uncultured bacterium Acidobacteria Subgroup 21 OTU_5711 Rhodobacteraceae OTU_6481 Ambiguous taxa Acidobacteria Subgroup 9 OTU_7 Persicirhabdus OTU_866 Uncultured bacterium Ralstonia OTU_1255 Flavobacteriaceae OTU_1551 Ambiguous taxa Profundimonas OTU_1559	Sva0725 OTU_433 Uncultured bacterium OM1 clade OTU_4332 Uncultured bacterium Sva0996 marine group OTU_4477 Ambiguous taxa Ilumatobacter OTU_4840 Ambiguous taxa Sva0996 marine group OTU_4982 Uncultured bacterium Ardenticatenia OTU_5150 Rhodobiaceae OTU_6148 Rhodobacteraceae OTU_6485 Sphingomonadales OTU_6620 Marivita OTU_6626 Beijerinckiaceae OTU_6687 PAUC43f marine benthic group OTU_699 Anderseniella OTU_7187 Ambiguous taxa Sandaracinaceae OTU_1154 Ambiguous taxa Thiogranum OTU_1467 Ambiguous taxa Holophagae Subgroup 23 OTU_251 Candidatus Thiobios OTU_6098

	Ambiguous taxa JTB255 marine benthic group OTU_1588 JTB255 marine benthic group OTU_1642 Gilvibacter OTU_2167 Myxococcales OTU_238 Ambiguous taxa Flavobacteriaceae OTU_3100 Uncultured bacterium Pir4 lineage OTU_3294 Halieaceae OTU_3519 Uncultured bacterium OM1 clade OTU_4096 Planctomycetaceae OTU_4100 Ambiguous taxa OM1 clade OTU_4271 Planctomycetaceae OTU_4278	JTB255 marine benthic group OTU_1566 OM190 OTU_2018 Uncultured bacterium Holophagae Subgroup 23 OTU_240 Uncultured bacterium Belgica2005-10-ZG-3 OTU_25 Ambiguous taxa Roseibacillus OTU_2529 OM60(NOR5) clade OTU_3504 Uncultured bacterium Desulfobulbus OTU_404 Ambiguous taxa Sva0996 marine group OTU_4976 Ambiguous taxa Acidobacteria Subgroup 17 OTU_785
RG3	Rhizobiales OTU_6486 Uncultured bacterium Rickettsiaceae OTU_5903 Vibrionaceae OTU_655 Ambiguous taxa Sphingobacterium OTU_1241 Ambiguous taxa NS4 marine group OTU_2698 Pseudomonas OTU_3032 Gammaproteobacteria OTU_3505 Roseovarius OTU_7180 Uncultured bacterium Maribacter OTU_1486	Desulfobulbus OTU_235 Uncultured bacterium Emcibacter OTU_6286 Rhodobacteraceae OTU_7097 Mycoplasma OTU_3722 Uncultured bacterium Phyllobacteriaceae OTU_6619 Ruegeria OTU_6653 Uncultured bacterium Rhodobacteraceae OTU_7173

Each family was found to have a core microbial community, the displayed core OTUs are those not shared with any other family line. RG1 is the most disease RG, RG2 is an intermediate RG, and RG3 is the most disease susceptible group.

<u>Aim 2:</u> Defining the composition of the Sydney Rock Oyster microbiome across diverse oyster families, including breeding lines generated for resistance to QX disease, and examining spatial and temporal heterogeneity in microbiome structure

SRO and water microbiomes

16S rRNA sequencing data from 132 samples consisting of 120 oyster adductor tissue and 12 estuarine water samples was analyzed, with a total of 15,788,760 raw reads obtained. After rarefaction to 3,000 read per samples, we were left with 107 SRO and 12 estuarine water samples for downstream analyses.

Alpha diversity (Chao1, observed species and Shannon's Index) was calculated for oyster and estuarine water samples (Supplementary Table 6 – Appendix). All three measured diversity indices were significantly lower in oysters than in water samples (Kruskal – Wallis ANOVA, p = 4.25E -08, 2.74E- 07 and 1.53E–05 respectively).

An nMDS analysis revealed that the over-all composition of SRO and seawater microbiomes differed (Supplementary Figure 3 – Appendix), with this pattern confirmed by PERMANOVA (F = 12.95, p = 0.0001). We then used the SIMPER test to examine the dissimilarity of the microbiome composition between oysters and water samples, with the oyster microbiome found to be 96.4% dissimilar to the water microbiome (Supplementary Table 7 – Appendix). The top 10 OTUs contributing to this dissimilarity between SRO and water microbiomes, included *Candidatus Actinomarina* genus (OTU 22961), *NS5 marine group* genus (OTU 5409), *Oceanospirillales* order (OTU 12673), *Candidatus Hepatoplasma* genus (OTU 14887), *Endozoicomonas* genus (OTU 3829), *OM43 clade* genus (OTU 6156), *Litoricola* genus (OTU 5208), *Endozoicomonas* genus (OTU 6283), *SAR86 clade* family (OTU 12751) and *NS5 marine group* genus (OTU 10013). STAMP analysis indicated that 305 OTUs were significantly over-represented in the SRO microbiome relative to seawater, while 120 OTUs were over-represented in the seawater (Welch's t-test; p <0.05; Supplemental Table 8 – Appendix).

The SRO microbiome is influenced by location and season

All three measured alpha diversity indices were significantly lower in the SROs from Port Stephens than those from Wallis Lake (Kruskal – Wallis ANOVA, p = 0.02886, 0.01872 and 0.01568 respectively; Supplementary Table 9 – Appendix). A 3D nMDS analysis also showed that the SRO

microbiome composition was distinct between the two locations (Stress = 0.2059; Figure 5A), and between sampling times at each location (Stress = 0.1403 and 0.2114; Figure 5B& C). PERMANOVA confirmed that the microbiome of the oysters was significantly different between Port Stephens and Wallis Lake (F = 8.842, p = 0.0001). SIMPER analysis demonstrated that the SRO microbiome in Port Stephens was 85.58% dissimilar to the oyster microbiome in Wallis Lake and identified an OTU belonging to the *Candidatus Hepatoplasma* genus (OTU 14887) as the greatest contributor to the differences between the two locations followed by OTUs assigned to the *Endozoicomonas* (OTU 1831) and *Vibrio* (OTU 2) genera (Supplementary Table 10 – Appendix).

STAMP analysis indicated that 56 OTUs occurred in significantly higher abundance in the Port Stephens SRO microbiomes than in Wallis Lake, while 137 other OTUs were more abundant in Wallis Lake than in Port Stephens (Welch's t-test; p <0.05; Supplementary Table 11 – Appendix). Similarly to SIMPER, STAMP analysis indicated that OTUs assigned to *Vibrio* genus (OTU 2) and *Endozoicomonas* genus (OTU 1831) were most responsible for mirobiome differences between Port Stephens and Wallis Lake (Figure 6), with the *Vibrio* OTU in significantly higher relative abundance in Port Stephens than in Wallis Lake (Welch's t-test; p = 8.71E - 7; Figure 2), while the *Endozoicomonas* OTU was considerably more abundant in Wallis Lake than in Port Stephens (Welch's t-test; p = 1.40E - 4; Figure 6).

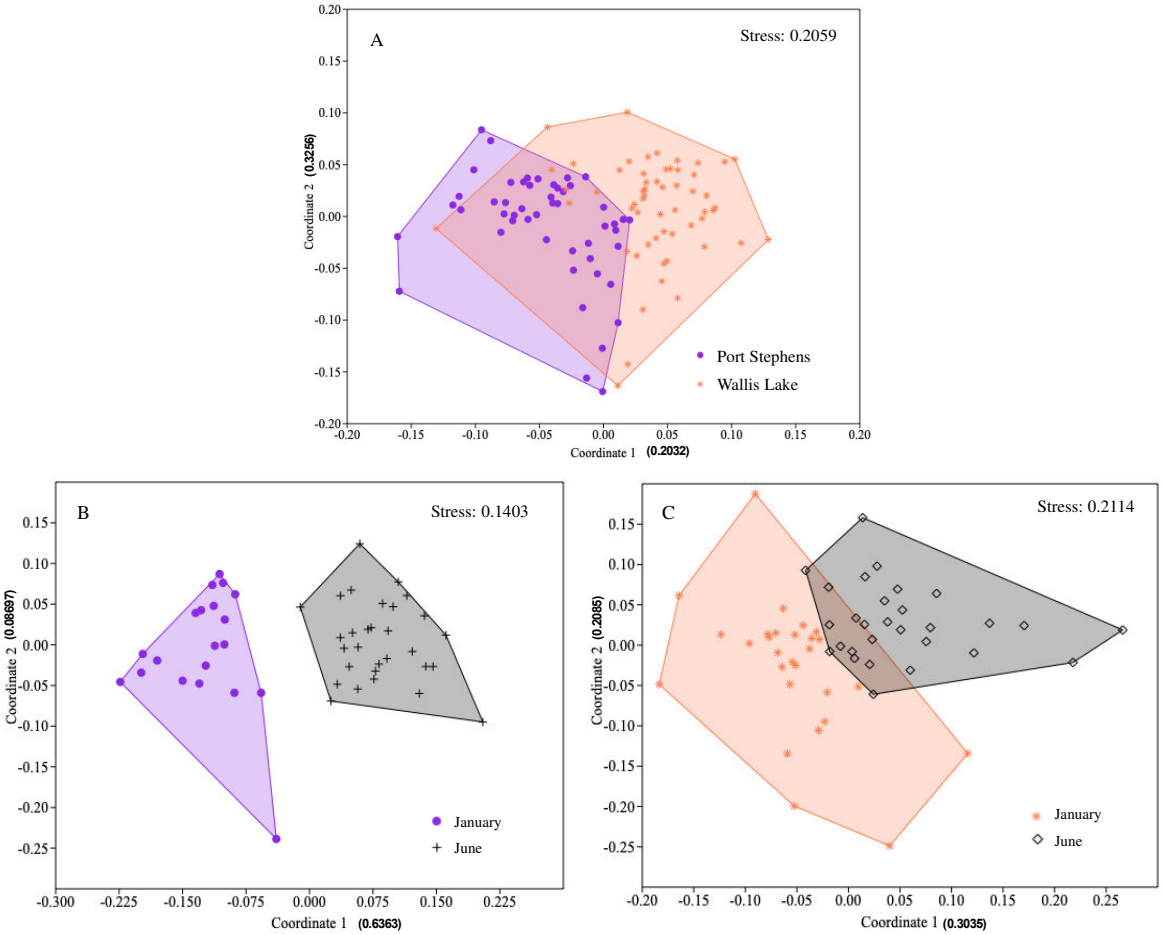


Figure 5: 3D nMDS plots showing the microbiome of SROs are grouped differently based on location as well as sampling time. A: Samples separate spatially based on the location, C: Samples in Port Stephens grouped separately based on the sampling time, D: Samples in Wallis Lake separate spatially based on the sampling time.

Table 6: Environmental variables in Port Stephens and Wallis Lake at two sampling times

Location & Time	Temperature (^{0}C)	pH	DO (mg/L)	Conductivity (µS/cm)	NH_3 (mg/L)	Chlorophyll a (µg/ml)	Notes
Pt. Stephens - January	27.8	8.0	8.18	53.3	0.012 ± 0.003	11.41 ± 1.48	Rainfall event 2 days before sampling (0.4 mm)
Pt. Stephens - June	24	8.3	8.88	27.6	0.038 ± 0.001	23.03 ± 3.13	Rainfall over 6 days including during sampling (average 23.65 mm/day)
Wallis Lake - January	24	7.2	9.5	53.9	0.013 ± 0.004	9.05 ± 0.62	Rainfall event 2 days before sampling (2.0 mm)
Wallis Lake - June	18.3	8.2	9.07	53.6	0.018 ± 0.001	9.52 ± 0.57	Rainfall over 3 days before sampling (average 5.6 mm/day)

* Daily rainfall data was downloaded from the Australian Bureau of Meteorology Website (http://www.bom.gov.au/climate/data/index.shtml)

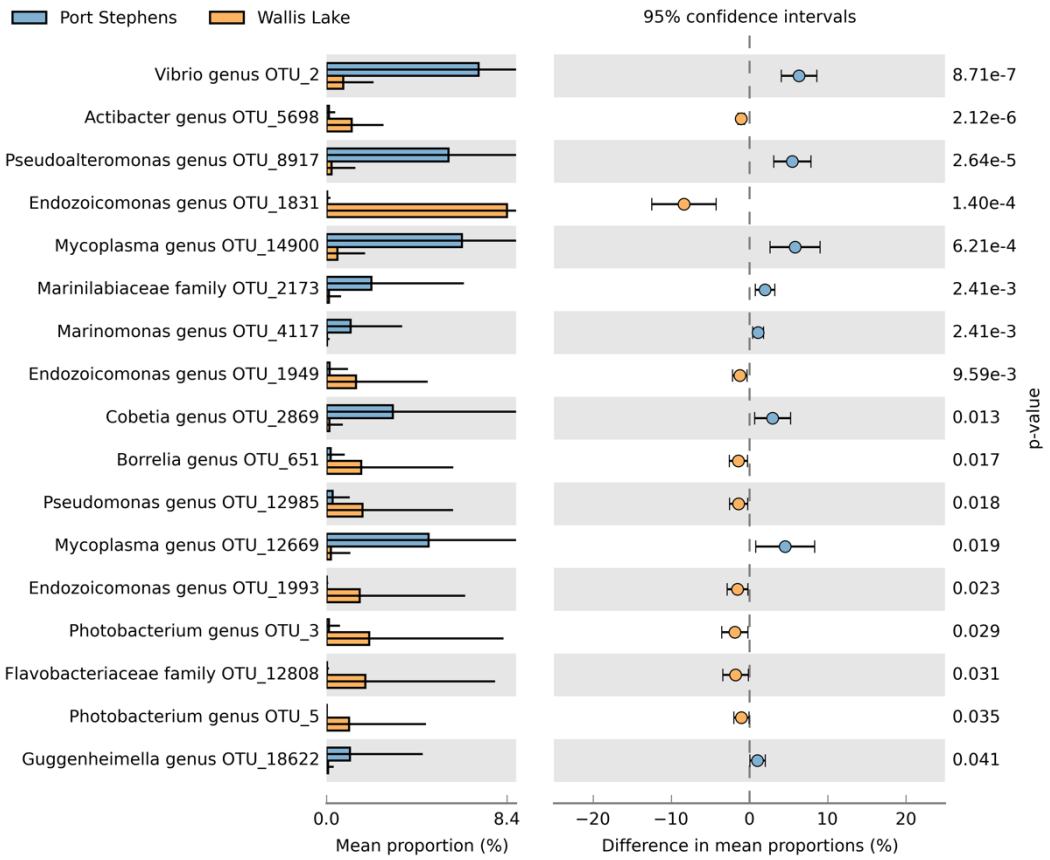


Figure 6: Extended error bar plot from STAMP showing OTUs differing significantly between Port Stephens (n = 50) and Wallis Lake (n = 57) with an effect size ≥ 1%.

As the SRO microbiomes differed significantly between locations, the analysis of temporal patterns in the composition of the SRO microbiomes was performed separately for each location. At Port Stephens, alpha diversity (Chao1, Observed species and Shannon index) did not differ between two sampling times (Kruskal – Wallis ANOVA, p = 0.2507, 0.6275 and 0.9054 respectively; Supplementary Table 12 – Appendix). For beta diversity, PERMANOVA showed that the microbiome of the oysters was significantly different between January and June (F = 10.92, p = 0.0001) and SIMPER confirmed that the oyster microbiome in January was 88.12% dissimilar to June. An OTU belonging to the *Candidatus Hepatoplasma* genus (OTU 14887) contributed the greatest difference between January and June and followed by an OTU from the *Vibrio* genus (OTU 2) (Supplementary Table 13 – Appendix). At Wallis Lake, similar to Port Stephens, alpha diversity (Chao1, Observed species and Shannon index) did not differ between the two sampling times

(Kruskal – Wallis ANOVA, p = 0.7375, 0.402 and 0.632 respectively; Supplementary Table 14 – Appendix). For beta diversity, PERMANOVA demonstrated that the microbiome of the oysters was significantly different between January and June (F = 3.62, p = 0.0001), although this result was not as strong as that observed at Port Stephens. SIMPER found the SRO microbiomes in January to be 81.86% dissimilar to those from June. Similarly to Port Stephens, an OTU belonging to *Candidatus Hepatoplasma* genus (OTU 14887) contributed the greatest difference between January and June followed by OTU assigned to *Endozoicomonas* genus (OTU 1831) (Supplementary Table 15 – Appendix).

At Port Stephens, STAMP analysis revealed that the relative abundance of 23 OTUs were significantly higher in January than in June and 65 OTUs were more abundant in June than in January (Welch's t-test; p <0.05; Supplementary Table 16 – Appendix). At Wallis Lake, the relative abundance of 18 OTUs was significantly higher in January than in June and 26 other OTUs were considerably more abundant in June than in January (Welch's t-test; p <0.05; Supplementary Table 17 – Appendix). Interestingly, at both Port Stephens and Wallis Lake, we observed an OTU assigned to the *Vibrio* genus (OTU 2) that was significantly more abundant in June than in January (Welch's t-test; p <0.05; Figure 7). An OTU belonging to the *Candidatus Hepatoplasma* genus (OTU 14887) shifted differently between the seasons at the two locations. At Port Stephens, it was more abundant in January than in June (Welch's t-test; p = 3.93E -3; Figure 7A) whereas at Wallis Lake it was more abundant in June than in January (Welch's t-test; p = 6.66E - 4; Figure 7B).

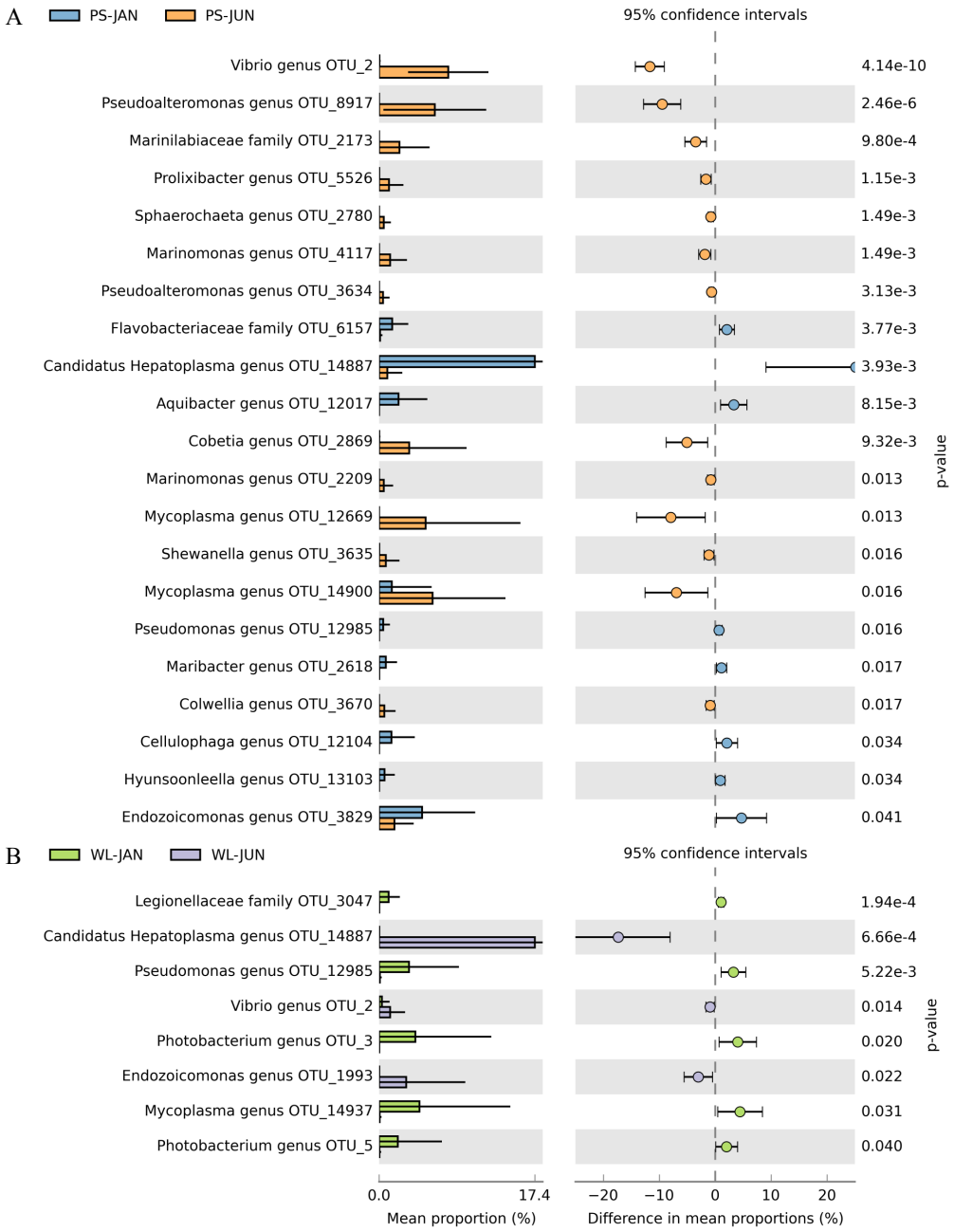


Figure 7: Extended error bar plot showing OTUs differing significantly between January and June in two locations with an effect size ≥ 0.5%. A. Port Stephens: January (n = 20) and June (n = 30), and B. Wallis Lake: January (n = 29) and June (n = 30).

SRO microbiomes are influenced by intrinsic resistance to QX disease

The SROs deployed at the two locations were genetically identical and categorised into three groups based on their resistance to QX disease (Table 6). Due to the separation of the SRO microbiome according to location and season, we compared the microbiome between three QX

resistance levels for each sampling time and location. Examining the bacterial communities at genus level showed that microbiomes of SRO were dominant by *Endozoicomonas, Candidatus Hepatoplas* and *Mycoplasma* (Figure 8), but that the composition of the microbiome differed among QX resistance groups. PERMANOVA confirmed that at Port Stephens, the resistant and intermediate groups were significantly different from the sensitive group in both January and in June ($p < 0.05$; Table 7); however, no significant difference was observed between the resistant and intermediate groups. At Wallis Lake in January, similar results were observed to Port Stephens, with the microbiome of the resistant and intermediate groups significantly different to the sensitive group ($p < 0.05$; Table 7). However, in June, the inverse pattern was observed, with only the resistant and intermediate groups exhibiting significant difference to one another ($F = 1.695$, $p = 0.011$; Table 7). SIMPER analyses indicated that during January at Port Stephens, the SRO microbiome associated with QX sensitive group was 77.42 and 78.52% dissimilar to the resistant and intermediate group respectively. In June, the dissimilarities between these groups decreased slightly to 73.61 and 70.05% (Supplementary Table 18 – Appendix). At Wallis Lake, during January, the SRO microbiome associated with the sensitive group was 79.5 and 79.6% dissimilar to the resistant and intermediate group respectively. In June, the SRO microbiome of the resistant group was 77.43% dissimilar to the intermediate group (Supplementary Table 19 – Appendix).

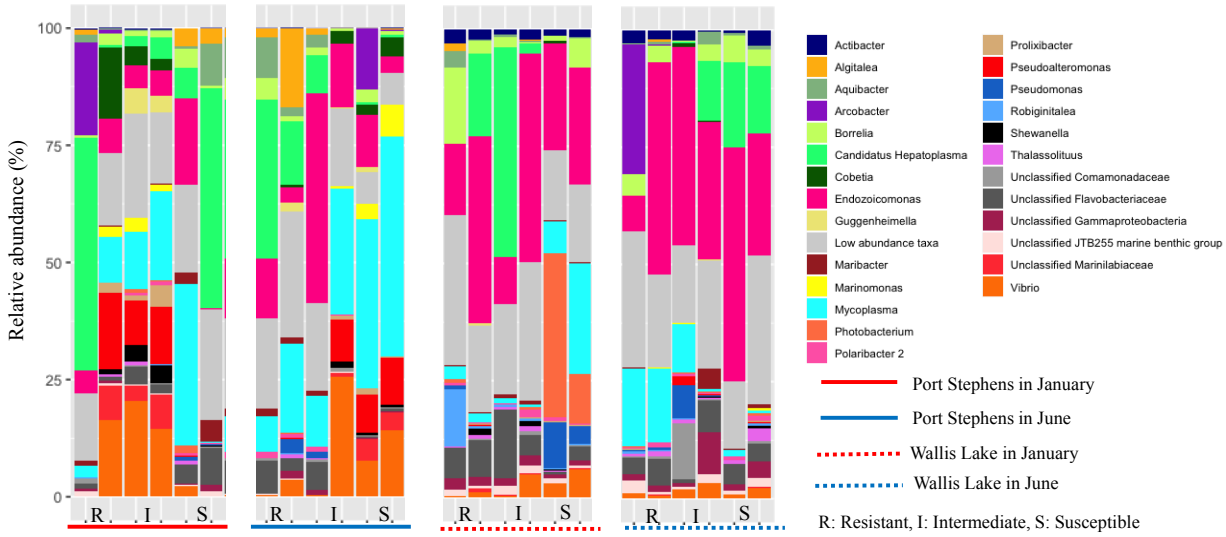


Figure 8: Microbiome composition (relative abundance) of QX resistance groups at genus level in two locations and two seasons. All OTU with <1% grouped as low abundance taxa

Table 7: PERMANOVA test to compare microbiome of the QX resistance groups in Port Stephens and Wallis Lake

Location/sampling time/ QX resistance groups		Port Stephens		Wallis Lake	
		Resistant	Intermediate	Resistant	Intermediate
January	Intermediate	F = 0.9758 *p* = 0.4556		F = 1.145 *p* = 0.1999	
	Susceptible	F = 1.832 *p* = 0.0071	F = 1.72 *p* = 0.0338	F = 1.415 *p* = 0.0208	F = 1.362 *p* = 0.0471
June	Intermediate	F = 1.341 *p* = 0.1155		F = 1.419 *p* = 0.0264	
	Susceptible	F = 1.939 *p* = 0.0032	F = 2.018 *p* = 0.001	F = 1.414 *p* = 0.0827	F = 1.11 *p* = 0.265

STAMP analyses indicated that at Port Stephens, differences in the composition of the microbiome of the resistant, intermediate and sensitive groups were mainly contributed to by different abundances of OTUs assigned as members of the *Endozoicomonas, Candidatus Hepatoplas, Mycoplasma* and *Vibrio* (Supplementary Figure 4 – Appendix). Interestingly, the relative abundances of these bacteria were significantly higher in the susceptible group, relative to both the intermediate and resistant groups (Welch's t-test; p = < 0.05; Supplementary Figure 4 - Appendix), with the exception of the OTU classified as a member of the *Mycoplasma* genus (OTU 14921), which was significantly more abundant in the intermediate than the susceptible group (Welch's t-test; p = 2.67E - 3; Supplementary Figure 4E - Appendix). At Wallis Lake, in January, the relative abundances of *Endozoicomonas* were substantially higher in the QX susceptible group (Welch's t-test; p < 0.05; Supplementary Figure 5A,B – Appendix). In June, 15 OTUs were considerably more abundant in the intermediate group (Welch's t-test; p < 0.05), while only one *Endozoicomonas* genus (OTU 3483) was significantly higher in the resistant than intermediate group (Welch's t-test; p = 0.033 ; Supplementary Figure 5C - Appendix).

<u>Aim 3:</u> Examining spatial heterogeneity in Pacific Oyster microbiome structure at the individual oyster level and across regional-scales

Patterns in Bacterial abundance inferred from 16S rRNA qPCR

Estimations of bacterial abundance, as determined with qPCR quantification of 16S rRNA gene copies, differed significantly between sampling locations ($H = 252$; $p < 0.0001$), with the highest number of 16S rRNA gene copies observed in oysters collected from the Shoalhaven and Wapengo sampling locations (Figure 9; Supplementary Table 20 – Appendix). 16S rRNA gene copies were also significantly different between oyster tissue types ($H = 133$; $p < 0.0001$), with the mantle tissue harbouring the greatest number of 16S rRNA gene copies per milligram of tissue, followed by the gill, adductor muscle and digestive gland (Supplementary Table 21 – Appendix) at all sites, except for the Shoalhaven site, where the mantle and gill tissues were not significantly different (Supplementary Table 22 – Appendix).

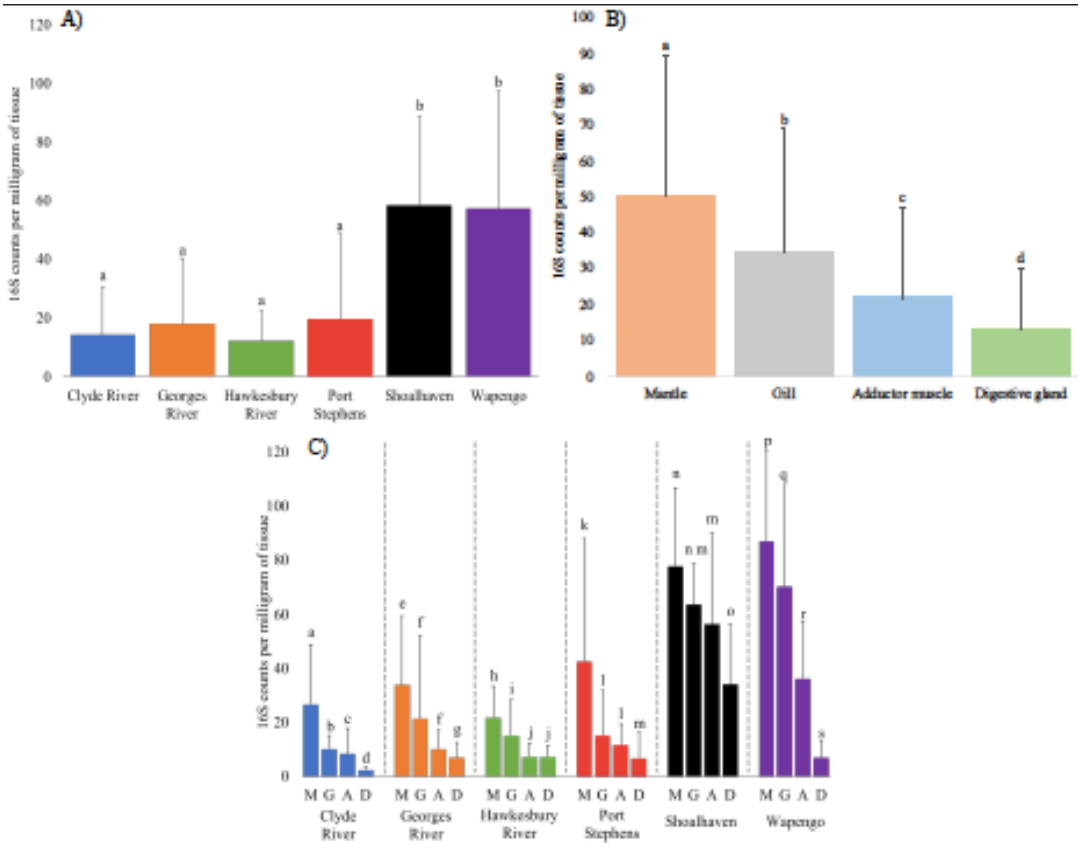


Figure 9: 16S rRNA qPCR to examine bacterial loads at each site and tissue type. Data are average 16S rRNA counts per milligram of tissue, with standard deviation. A) are 16S rRNA counts per location. B) are 16S rRNA counts per tissue type. C) are 16S rRNA counts for each tissue at each

location. Significant comparisons are denoted by different letters. Comparisons in C) were performed within locations.

Alpha diversity comparisons

Species richness, evenness, and diversity were all significantly different between sampling locations (richness H = 98, p = < 0.0001; evenness H = 32, p = < 0.0001; diversity H = 70, p = < 0.0001). Microbiomes from the Wapengo and Shoalhaven locations displayed the greatest levels of species richness and diversity (Supplementary Table 23 – Appendix), while microbiomes derived from the Wapengo, Shoalhaven, and Hawkesbury River locations had the greatest species evenness.

All measured Alpha diversity indices were also significantly different between tissue-types (richness H = 24, p = < 0.0001; evenness H = 17, p = 0.0008; diversity H = 28, p = < 0.0001). The digestive gland microbiome displayed the greatest levels of species richness (Supplementary Table 24 – Appendix). Species evenness was consistent across the gill, adductor muscle, and digestive gland, but was significantly lower in the mantle. Similarly, the mantle tissue had the lowest levels of species diversity, with highest diversity levels within the digestive gland and gill (Supplementary Table 25 – Appendix).

Geographic location and tissue type are significant determinants of the C. gigas microbiome

The structure of the *C. gigas* microbiome differed significantly according to both sampling location (F = 11; p = 0.0001) and the oyster tissue type (F = 13.6; p = 0.0001). However, despite these statistical differences, clear partitioning of the microbial assemblages was not evident when all data was included in an nMDS analysis (stress 0.28).

To further resolve the influence of tissue type or sampling environment on the oyster microbiome structure, we compared the microbiomes of different tissue types within, and between, sampling environments. Tissue-specific oyster microbiomes differed significantly to each other within all locations (Figure 10; Clyde River F = 4.5, p = 0.0001; Georges River F = 5, p = 0.0001; Hawkesbury River F = 3.6, p = 0.0001; Port Stephens F = 5.2, p = 0.0001; Shoalhaven F = 3.9, p = 0.0001; Wapengo F = 3.9, p = 0.0001). Notably, significant differences occurred in pairwise

comparisons between tissues at all sites, implying a strong tissue-type influence on the oyster microbiome.

When using data summarised at the genus level, uncultured *Spirochaetaceae* bacteria were the strongest driver of tissue-specific microbiome differences within sites (Figure 11), contributing 10.7 % to the dissimilarity between tissues (Table 3), primarily due to the over-representation of these bacteria in the mantle tissue. Members of the *Mycoplasma* and *Vulcaniibacterium* genera were responsible for 6.1 and 4.8 % of the dissimilarity contribution between tissues, primarily due to an overabundance of these genera in the digestive gland. Members of the *Spirochaetaceae* family and the *Margulisbacteria* phylum were responsible for 2.4 %, and 2.1 % of the microbiome variability between tissues respectively, predominantly due to their over-representation in the gill tissue. Members of the *Acidovorax* genus accounted for 4.5 % of the microbiome dissimilarity between tissues, and were most abundant in adductor muscle and digestive gland microbiomes. The *Polynucleobacter* genus accounted for 3.4 % of the dissimilarity contribution between tissue-types, and were most abundant in the mantle.

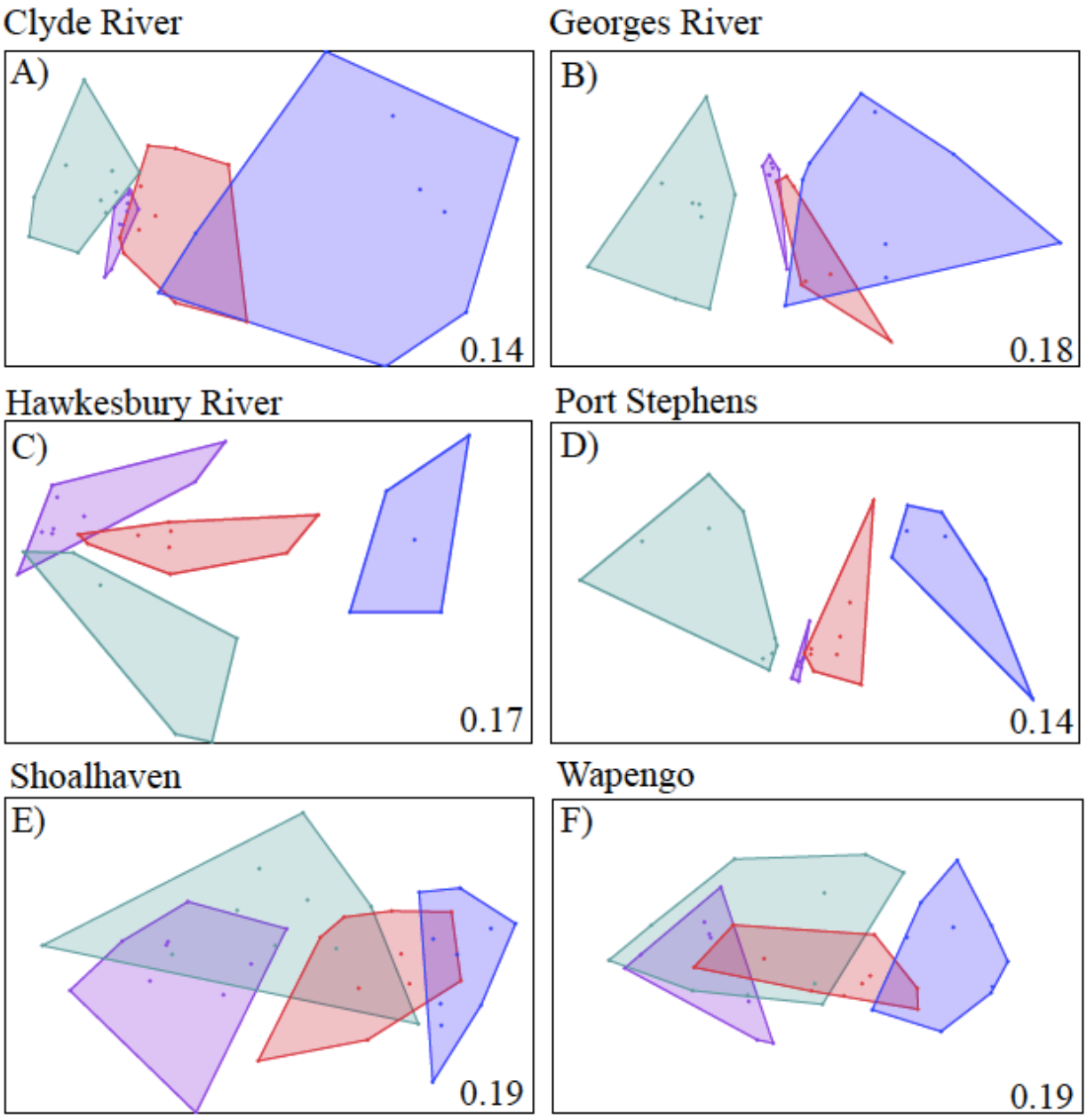


Figure 10: nMDS plots of oyster tissue-type microbiomes at individual locations. Mantle tissues are coloured purple, gill tissues are green, adductor muscle tissues are red, and digestive gland tissues are blue. Stress values are provided in the lower right corner.

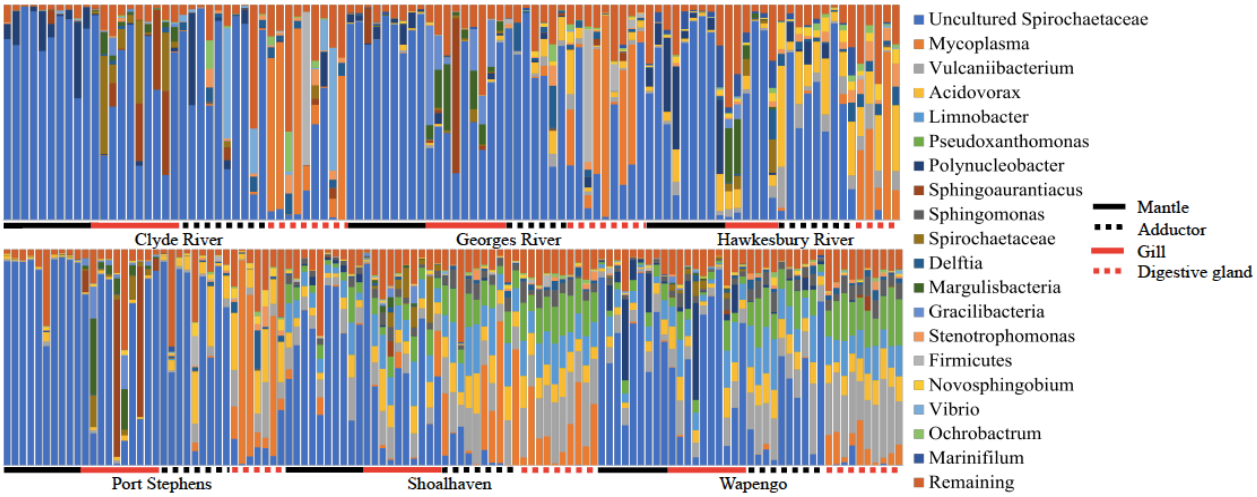


Figure 11: Summarised oyster microbiomes at the genus level, across six sampling locations and four sampled tissues. At each location, the tissues are ordered by mantle (black bar), gill (red bar), adductor muscle (black dashes), and digestive gland (red dashes). Top 20 summarised genera are shown, including the remaining genera (other).

Tissue-specific microbiomes also differed significantly between sampling locations (Figure 12; Mantle $F = 5.3$, $p = 0.0001$; Gill $F = 4$, $p = 0.0001$; Adductor muscle $F = 5.3$, $p = 0.0001$; Digestive gland $F = 4.4$, $p = 0.0001$), further confirming the regional-scale spatial variability of the *C. gigas* microbiome composition. However, when examining pairwise comparisons of specific tissues between individual locations, the mantle, gill, and digestive gland microbiomes from Wapengo were not significantly different to the same tissue types at the Shoalhaven site. These similarities are perhaps notable, given that the Wapengo and Shoalhaven sites are the only two sites characterised as wave-dominated estuaries. Pairwise comparisons of adductor muscle microbiomes from the tide-dominated estuaries were not significantly different between the Georges River site when compared to the Clyde River and Port Stephens sites. Further, the adductor muscle microbiome from the Hawkesbury River was not significantly different to that from Port Stephens. These data suggest a regional-scale influence on the oyster microbiome composition, though it is notable that these large-scale differences in microbiome structure were not as strong as the microenvironmental-scale, tissue-type influence.

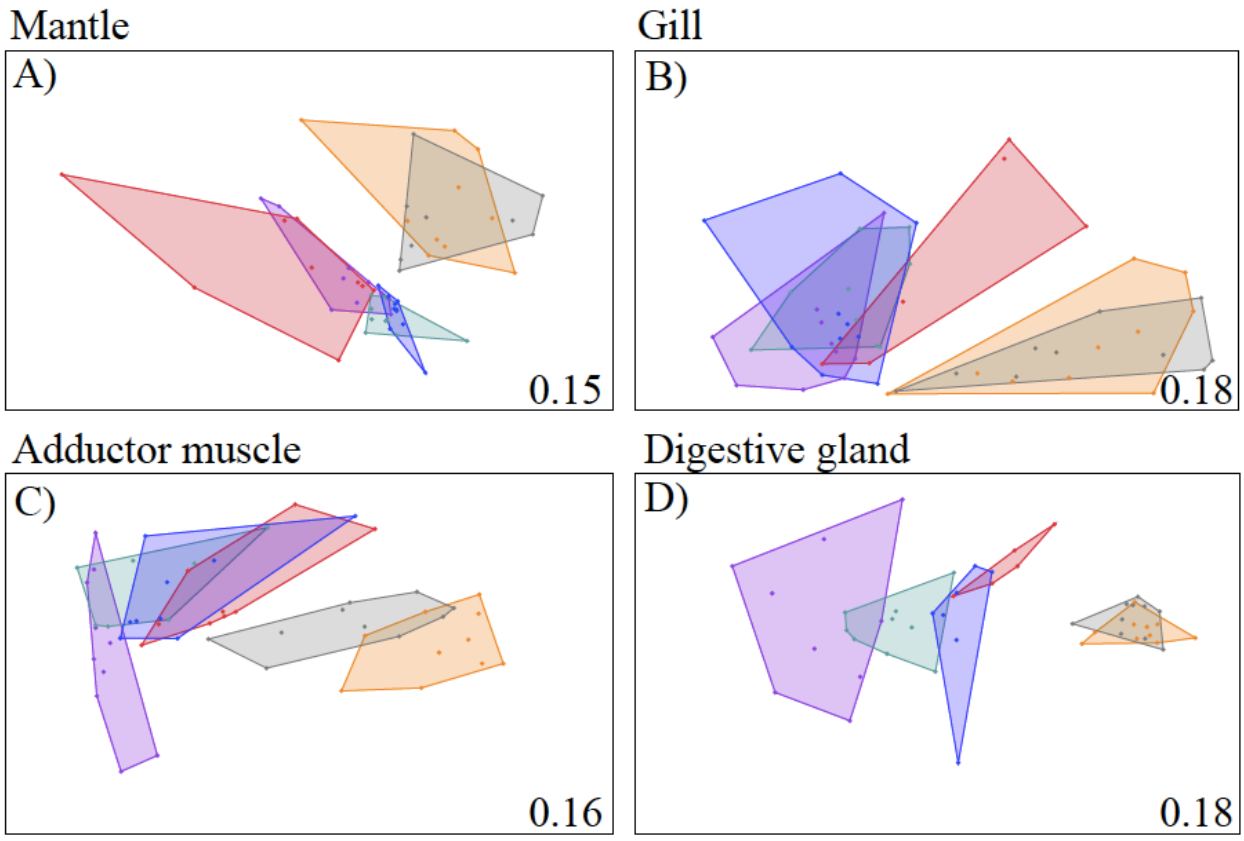


Figure 12: nMDS plots of oyster tissue-type microbiomes at different locations. Microbiomes are coloured as follows: Clyde River is purple, Hawkesbury River is red, Georges River is green, Port Stephens is blue, Shoalhaven is orange, and Wapengo is grey. Stress values are provided in the lower right corner.

Uncultured *Spirochaetaceae* bacteria contributed to the greatest dissimilarity between microbiomes from different sampling locations, accounting for 10 % of the variability between sites, largely due to a relative over-abundance of these bacteria in the Clyde River, Georges River, Hawkesbury River, and Port Stephens (Table 4). Bacteria assigned to the *Vulcaniibacterium* genus were responsible for 5.2 % of the microbiome variability between sites, driven by an over-representation in the Wapengo and Shoalhaven sampling locations. At these sites, members of the *Limnobacter* and *Pseudoxanthomonas* genera were also over-represented, contributing 4.5 and 4.1 % to the microbiome dissimilarities, while they were completely absent, or in low abundance, at the other four sampling locations. Bacteria assigned to the *Vibrio* genus were over-represented in the adductor muscle and digestive gland microbiomes at the Clyde River site, relative to all other locations, contributing 1.1 % of the dissimilarity between microbiomes. Members of the SAR11 clade contributed 1 % to the dissimilarity between sites, and were common across the Clyde River, Georges River, Hawkesbury River, and Port Stephens sites, but were almost completely absent in the Wapengo and Shoalhaven sites.

Conservation of the C. gigas core microbiome

As the structure of the oyster microbiome was governed by both the sampling location and tissue type, we sought to identify core microbiomes for (i) all of the tested oyster microbiomes (universal core microbiome), (ii) each sampling location, and (iii) each tissue type (Figure 6). When including all samples in the core analysis, several ZOTUs assigned to an uncultured *Spirochaetaceae* (ZOTUs 4655, 9fe1, e651, bb6f, 95f6, 0d03, 986e, and 9435) were characterised as members of the 'universal' core microbiome, whereby they were found in at least 80% of all tested samples, regardless of sampling location or tissue type.

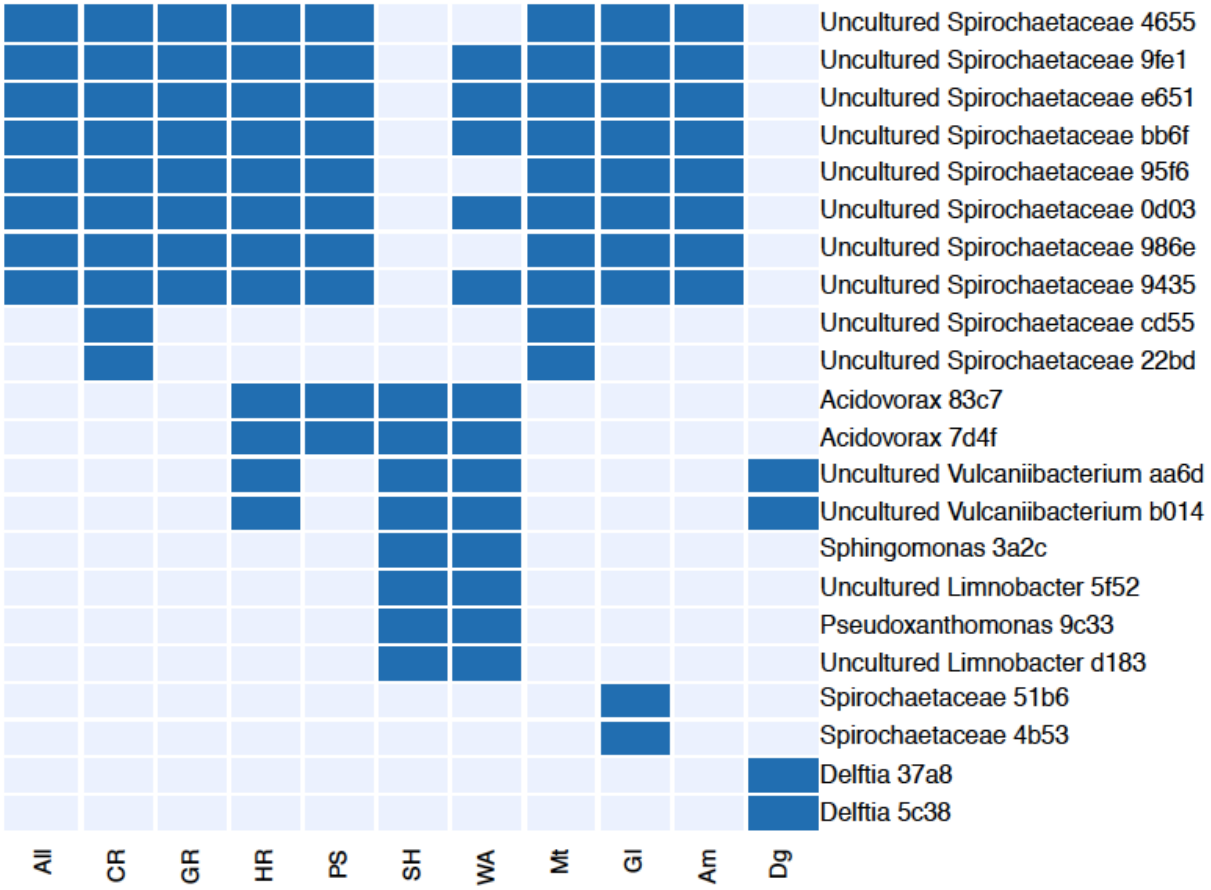


Figure 13: Presence/absence heatmap of taxa identified as the core microbiome. All = All samples were included in the analysis, CR = Clyde River, GR = Georges River, HR = Hawkesbury River, PS = Port Stephens, SH = Shoalhaven, and WA = Wapengo. Mt = mantle, Gl = gill, Am = adductor muscle, Dg = digestive gland.

The oyster microbiomes from the Wapengo and Shoalhaven sampling locations harboured a distinct core microbiome relative to the other sampling sites. This (Wapengo-Shoalhaven) core

microbiome was consistent across all tissue types, and included ZOTUs assigned to the *Acidovorax* (ZOTUs 83c7 and 7d4f), *Vulcaniibacterium* (ZOTUs aa6d and b014), *Pseudoxanthomonas* (ZOTU 9c33), *Limnobacter* (ZOTUs 5f52 and d183), and *Sphingomonas* (ZOTU 3a2c) genera.

Individual tissues were also found to harbour unique core bacteria. In addition to the *Spirochaetaceae* ZOTUs identified in the universal core microbiome, the mantle and gill tissues consisted of other uncultured *Spirochaetaceae* bacteria (ZOTUs cd55 and 22bd; ZOTUs 51b6 and 4b53 respectively). No additional core ZOTUs were identified in the adductor muscle microbiome. No core microbiome was identified for the digestive gland, however, slightly relaxing the core analysis parameters from 80% (present in 40/50 samples) to 78% (present in 39/50 samples) allowed for the inclusion of ZOTUs classified as members of the *Vulcaniibacterium* (ZOTUs aa6d and b014) and *Delftia* (ZOTUs 37a8 and 5c38) within the core microbiome of the digestive gland.

Aim 4: Defining the Sydney Rock Oyster microbiome associated with QX disease events

QX disease event confirmation

A QX disease event was recorded in February 2018. During this event, a total of 140 oysters were collected at four discrete sampling times, of which 62 oysters were found to be positive for *M. sydneyi* infection using the PCR method. Furthermore, mature sporonts were identified in 24 oysters using the cytology method (Figure 14), with these oysters confirmed as infected by *M. sydneyi* using PCR.

SRO microbiome characterization

A total of 10,882,690 raw reads of the 16S rRNA gene were obtained from 300 oyster samples collected during this study. After filtering, removal of chimeric, singletons, chloroplast, mitochondrial and unassigned sequences, the average number of sequences per samples was 6,575. A rarefaction plot (Observed species) was performed to a depth of 2,000 reads over 300 samples (Supplementary Figure 6A,B – Appendix), showing almost 210 curves (one curve per sample) reaching asymptote (Supplementary Figure 6C – Appendix). Furthermore, alpha diversity (observed species) was calculated for different depths (Supplementary Table 26 – Appendix). Comparisons indicated that the numbers of observed species were relatively consistent over a range sampling depths, from 2,000 to 3,000 (Kruskal–Wallis ANOVA test: $p > 0.05$; Supplementary

Table 27 – Appendix). Therefore, sequence data were rarefied to 2,000 reads, leaving 210 samples for further downstream analysis.

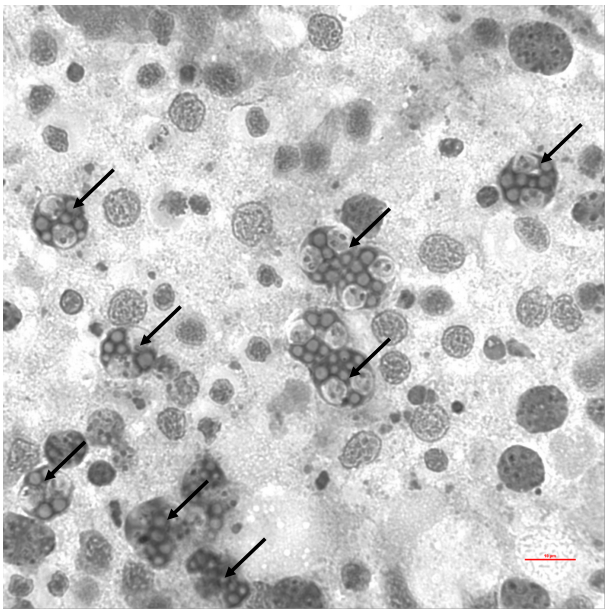


Figure 24: Tissue imprints of the digestive gland tissues of SRO infected with *M. sydneyi* showing mature sporonts (arrows). Scale bar, 10µm. Rapid diff kit stain.

The final OTU table contained 4,490 unique OTUs. At the genus level, these unique OTUs included 526 bacterial genera (82 uncultured bacteria and 215 unclassified genera), with the SRO microbiome dominated by *Mycoplasma,* followed by *Candidatus Hepatoplasma* and then *Arcobacter* (Figure 15).

SRO microbiome before and during QX disease event

Alpha diversity measures of SRO microbiomes were calculated for samples obtained from before and during the QX event (Supplementary Table 28 – Appendix). There was no significant difference in Chao1, the Observed species and Shannon's Index between samples collected before and during the QX disease event (Kruskal – Wallis ANOVA, p = 0.1022, 0.1221 and 0.9272 respectively). However, when beta diversity was characterised, bacterial community structure varied significantly between before and during the QX disease event (Figure 15; PERMANOVA F = 7.542, p = 0.0001). SIMPER tests indicated that the SRO microbiome before the QX disease event was 85.3% dissimilar to the oyster microbiome during the QX disease event, and demonstrated that an OTU belonging to the *Mycoplasma* genus (OTU 11355) contributed the most to the differences between the two groups, followed by an OTUS identified as a member of the

Candidatus Hepatoplasma genus (OTU 11357), and then an OTU assigend as a member of the *Acrobacter* genus (OTU 17190) (Supplementary Table 29 – Appendix).

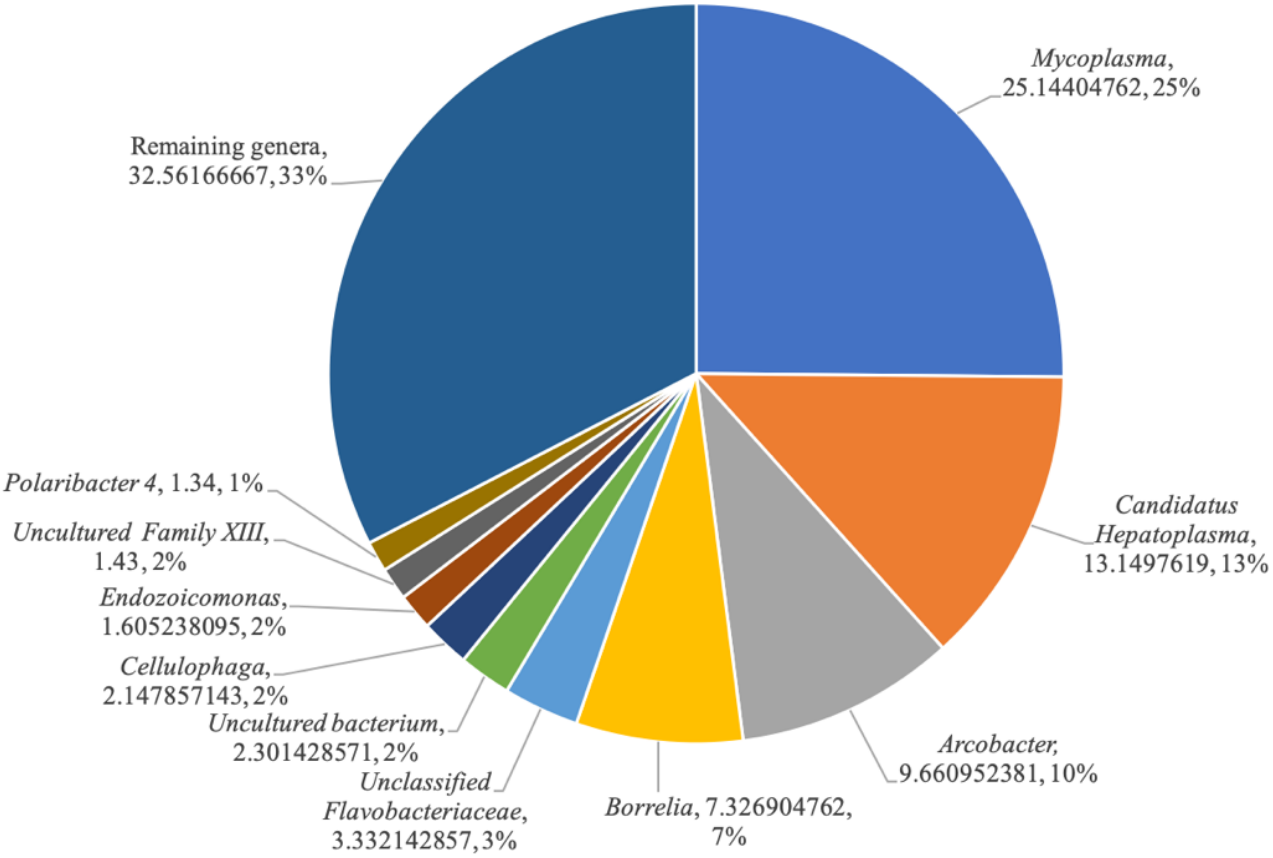


Figure 15: SRO bacterial community at the genus level. Top 10 dominant genera accounted for approximate 67% and remaining genera (516 genera) accounted only around 33%.

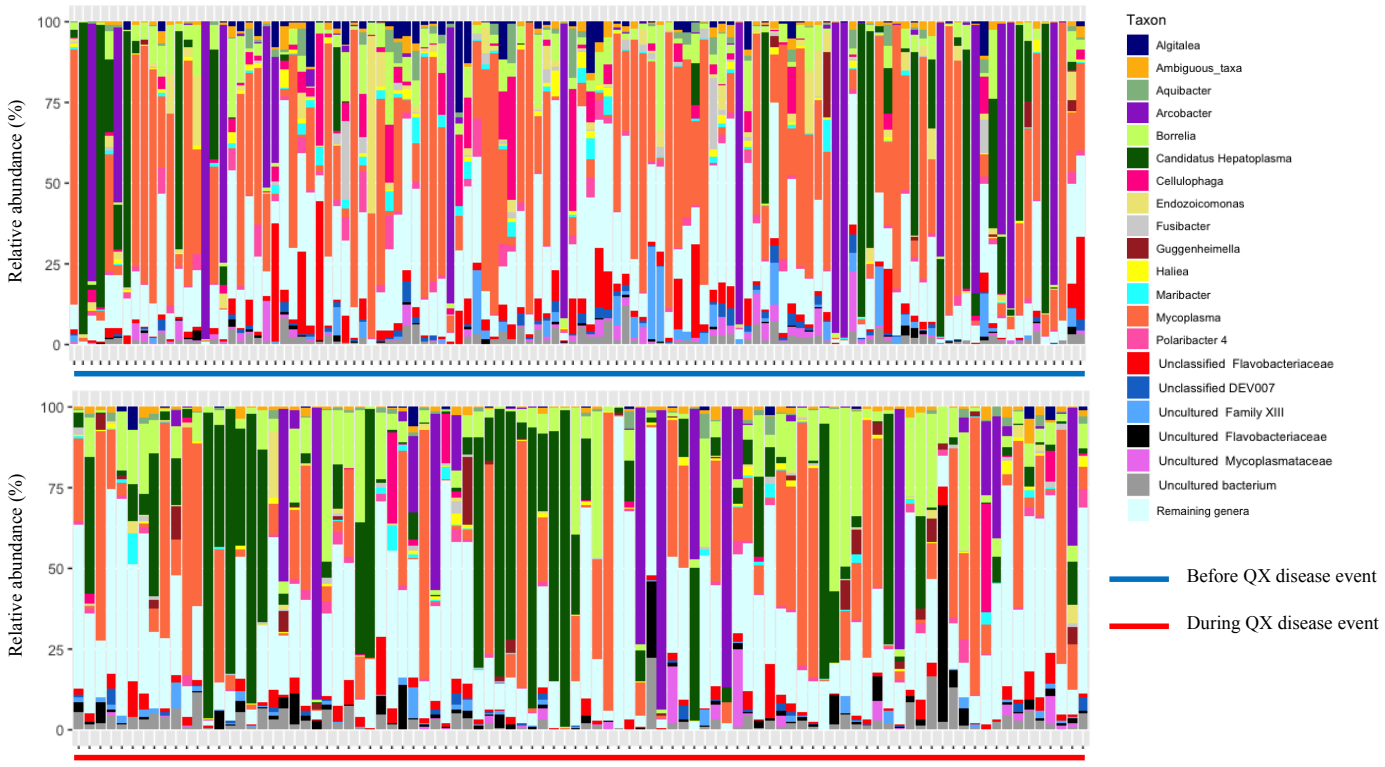


Figure 16: Bacterial community at the genus level in the SRO microbiome before and during the QX disease event. Top 20 genera displayed, with other genera grouped as 'remaining genera'.

Further statistical analysis to determine which OTUs were significantly different between before and the during QX disease event indicated that 80 OTUs occurred in significantly higher abundances during the QX disease event than prior to the event, while 140 other OTUs were more abundant before than during the QX disease event (Welch's t-test; p <0.05; Supplementary Table 30 – Appendix). According to this analysis, and in-line with the results of the SIMPER analysis, OTUs assigned to *Mycoplasma* genus (OTU 11355), and *Candidatus Hepatoplasma* genus (OTU 11357) were most responsible for driving differences in the SRO microbiome between before and during the QX disease event (Figure 5). The *Mycoplasma* genus OTU (OTU 11355) occurred in significantly higher relative abundance before the QX disease event than during it (Welch's t-test; p = 0.044; Figure 5), while the *Candidatus Hepatoplasma* genus OTU (OTU 11357) was considerably more abundant during than before the QX disease event (Welch's t-test; p = 0.024; Figure 5). Additionally, an OTU assigned as a member of the *Borrelia* genus (OTU 1) was significantly more abundant during the QX event compared to before the QX disease event (Welch's t-test; p = 7.10E - 4; Figure 18).

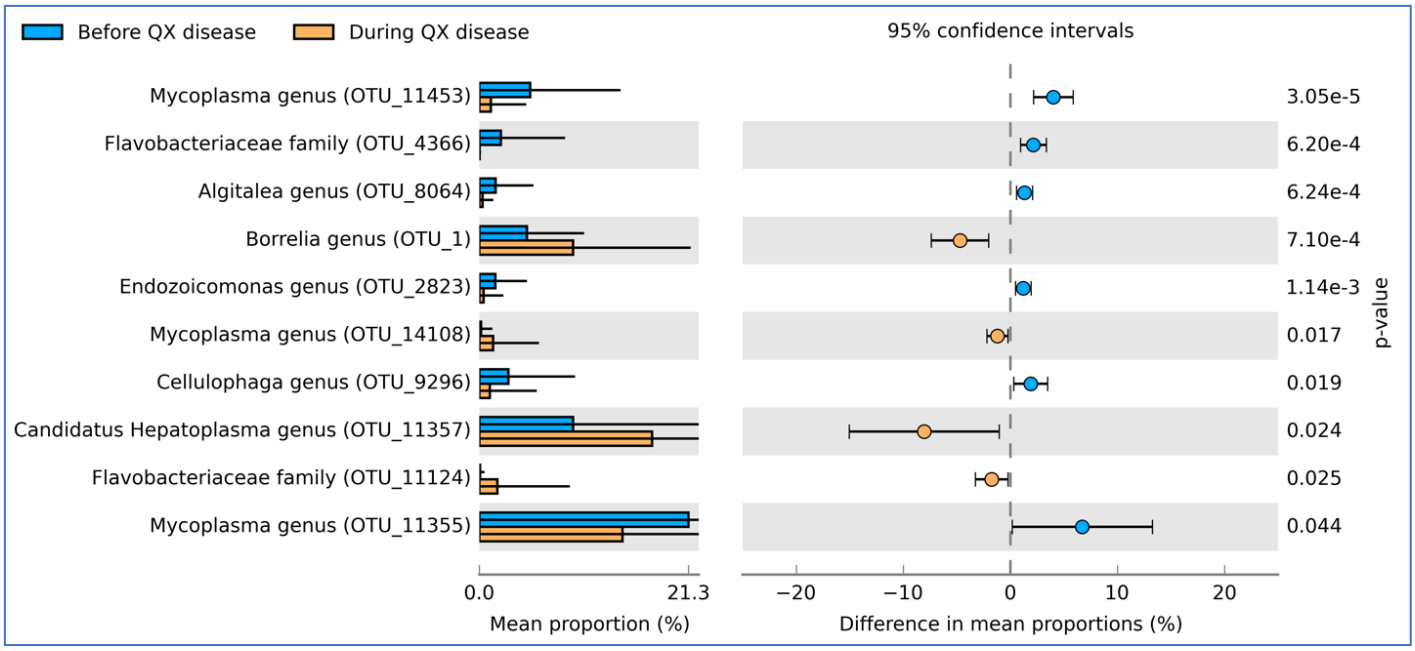


Figure 17: Extended error bar plot showing OTUs differing significantly between before and during the QX disease event with an effect size ≥ 1%.

Comparison the microbiome of SRO between uninfected and infected QX disease

Alpha diversity measures of the SRO microbiome were calculated for uninfected and QX infected SROs (Supplementary Table 31 – Appendix). There was no significant difference in Chao1, the Observed species and Shannon between uninfected and infected oysters (Kruskal – Wallis ANOVA, p = 0.3842, 0.6163 and 0.5154 respectively).

Although no clear dissimilarity in the microbiome between uninfected and QX infected oysters was apparent in a 3D nMDS (Stress = 0.2551; Supplementary Figure 7 – Appendix), PERMANOVA comparison of microbiome composition between SRO groups showed that microbiomes of QX infected oysters were significantly different to uninfected oysters (PERMANOVA; F = 2.074, p = 0.0001). SIMPER indicated that the microbiome of uninfected oysters was 85.1% dissimilar to the microbiome of QX disease infected oysters, with an OTU belonging to *Candidatus Hepatoplasma* genus (OTU 11357) contributing most to the difference between the two groups followed by OTUs assigned to the *Mycoplasma* genus (OTU 11355), and *Borrelia* genus (OTU 1) (Supplementary Table 32 – Appendix).

STAMP analysis confirmed the variation of microbiomes between uninfected and QX infected oysters was driven by 22 OTUs (Supplementary Table 33 – Appendix). This analysis also indicated

that OTUs assigned as members of the *Mycoplasma* genus (OTU 11355) and *Borrelia* genus (OTU 1) were responsible for the greatest differences in mean proportions (%) between the uninfected and QX disease infected groups (Figure 18), with the *Mycoplasma* genus OTU (OTU 11355) occuring in significantly higher relative abundance in uninfected samples (Welch's t-test; p = 0.032), while the *Borrelia* genus OTU (OTU 1) was significantly more abundant in the infected oysters (Welch's t-test; p = 2.75E -4).

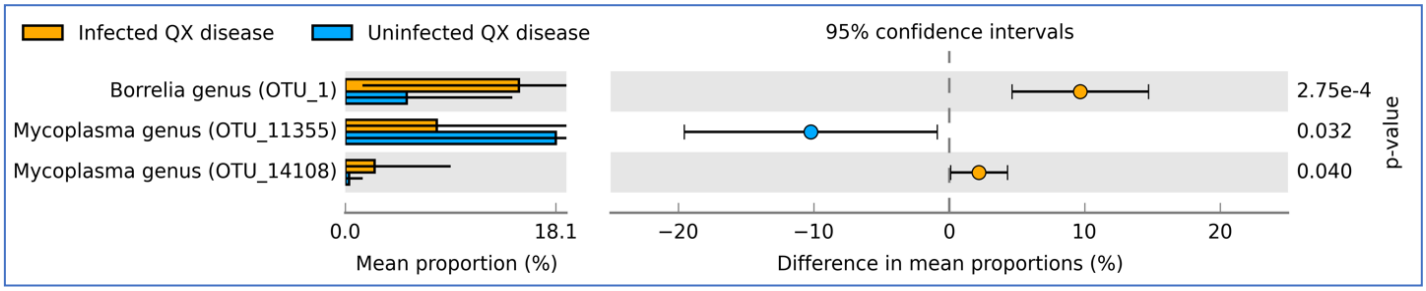


Figure 18: Extended error bar plot showing OTUs differing significantly between uninfected and QX infected oysters, with an effect size ≥ 1% .

As we observed significant differences in the microbiome composition between uninfected and QX infected oysters, we next sought to examine whether microbiome shifts were influenced by the parasite infection over time. Statistical analyses indicated that on the 13th of March, the relative abundances of 18 OTUs were significantly lower in infected than in uninfected oysters (Welch's t-test; p< 0.05; Supplementary Figure 8A – Appendix). On the 27th of March, an OTU belonging to the *Borrelia* genus (OTU 1) occurred in significantly higher relative abundance in QX infected oysters than in uninfected oysters (Welch's t-test; p = 0.018), while an OTU within the *Mycoplasma* genus (OTU 11355) occurred in significantly higher relative abundance in uninfected than in infected samples (Welch's t-test; p = 0.02; Supplementary Figure 8B – Appendix). On the 11th of April, the *Borrelia* genus OTU (OTU 1) remained in significantly higher relative abundance in the infected oysters (Welch's t-test; p = 2.61E - 3). In addition, an OTU assigned to *Mycoplasma* genus (OTU 11599) occurred in significantly higher abundance in QX infected than in uninfected oysters (Welch's t-test; p = 0.023; Supplementary Figure 3C). These results indicate that shifts in the microbiome were intially characterised by a decrease in abundances of many bacterial members, followed by an increase in the abundance of OTUs within the *Borrelia* genus (OTU 1) and *Mycoplasma* genus (OTU 11599).

Surprisingly, microbiomes of SRO did not differ between early and late QX parasite infection (PERMANOVA test; F = 0.9487, $p = 0.5516$). There was no significant difference in the relative abundance of any OTU between the early and late QX parasite infection groups.

Correlation between environmental variables, QX disease and microbiome

While we observed significant differences in the relative abundances of several bacterial members between oyster groups, some of these patterns were inconsistent over time, suggesting that besides the influence of the QX disease infection, microbiome shifts may have been influenced by other environmental factors (Table 8). Therefore, we sought to examine the existence of correlations between environmental variables, QX disease and the SRO microbiome. There were 331 significant correlations between environmental variables, QX disease and microbiome structure ($p < 0.05$; Supplementary Table 34 – Appendix). Similar to the results derived from STAMP analysis, mictools analysis revealed that an OTU within the *Borrelia* genus (OTU 1) exhibited the strongest positive correlation with QX disease (MICe = 0.223559; Pearson: R = 0.414967, p = 2.91E – 06), while an OTU classified as a member of the *Mycoplasma* genus (OTU 11355) displayed the strongest negative correlation with QX disease (MICe = 0.271212; Pearson: R = - 0.304624, p = 2.91E – 06; Supplementary Table 9). This Mycoplasma genus OTU (OTU 11355) exhibited positive correlations with pH and temperature (MICe = 0.197116 and 0.185596; Pearson: R = 0.087919 and 0.056111, p = 1.61E-03 and 5.52E-03 respectively; Supplementary Table 34 – Appendix). A *Candidatus Hepatoplasma* genus (OTU 11357) also exhibited significantly positive association with QX disease (MICe = 0.179022; Pearson: R = 0.267949, p = 8.98E-06), as well as water conductivity (MICe = 0.441425; Pearson: R = 0.346414, p = 2.91E – 06). Furthermore, multivariate multiple linear regression analysis confirmed that only the *Borrelia* genus (OTU 1) exhibited positive correlation with QX disease (p = 3.5337E – 8; Supplementary Table 35 – Appendix). We also identified significant positive correlations between environmental variables and QX disease using network analysis (Supplementary Table 35 – Appendix), with phosphate concentrations in the water column identified as the strongest positive correlate with QX disease (Mictools: MICe = 0.326157, PearsonR = 0.308414, p = 2.91E-06).

Table 8: Environmental variables at 12 sampling times

Time	pH	DO (mg/L)	Temp (^{0}C)	Conductivity (µS/cm)	Nitrate (mg/L)	Ammonia (mg/L)	Phosphate (mg/L)	Chlorophyll a (µg/ml)
8-Nov-17	9.8	8.72	19.9	50.5	0.0267 ± 0.0058	0.0193 ± 0.0085	0.0120 ± 0.0026	0.0331 ± 0.0125
21-Nov-17	7.9	8.9	20.3	32.9	0.0367 ± 0.0058	0.0270 ± 0.0082	0.0090 ± 0.0020	0.0295 ± 0.0065
5-Dec-17	7.76	7.43	23.8	49.9	0.1150 ± 0.0687	0.0287 ± 0.0045	0.0177 ± 0.0119	0.1383 ± 0.1500
15-Dec-17	9	7.37	26.4	33.8	0.0233 ± 0.0266	0.0230 ± 0.0070	0.0270 ± 0.0017	0.0237 ± 0.0063
3-Jan-18	8.09	7.33	26	35.3	0.0040 ± 0.000	0.0127 ± 0.0015	0.0263 ± 0.0032	0.0290 ± 0.0037
17-Jan-18	7.86	7.88	23.7	52.2	0.0047 ± 0.0012	0.0127 ± 0.0015	0.0273 ± 0.0035	0.0338 ± 0.0201
29-Jan-18	8.036	7.1	27.2	52.5	0.0053 ± 0.0023	0.0123 ± 0.0021	0.0383 ± 0.0047	0.0207 ± 0.0107
13-Feb-18	8.03	7.73	27.9	52.5	0.0057 ± 0.0015	0.0140 ± 0.0044	0.0377 ± 0.0049	0.0190 ± 0.0030
27-Feb-18	7.91	7.01	24	46	0.0127 ± 0.0150	0.0260 ± 0.0287	0.0503 ± 0.0087	0.0241 ± 0.0053
13-Mar-18	7.52	7.53	24.7	49.4	0.0267 ± 0.0058	0.0400 ± 0.0238	0.0443 ± 0.0140	0.0299 ± 0.0037
27-Mar-18	7.71	8.01	22.9	47.5	0.0113 ± 0.0081	0.0120 ± 0.0072	0.0350 ± 0.0026	0.0305 ± 0.0027
11-Apr-18	8.058	7.57	24.9	49.6	0.0300 ± 0.01	0.0120 ± 0.001	0.0340 ± 0.002	0.0243 ± 0.0020

<u>Aim 5:</u> Measuring temporal patterns in the Pacific Oyster microbiome during the summer OsHV-1 mortality period

During the 24-week study period a total of 290 oysters (211 living and 79 dead) were sampled from the experimental tanks at the Sydney Institute of Marine Science. During this period, changes in environmental conditions involved fluctuations in ammonia and phosphate levels, oxidative-reduction potential (ORP) and pH (Figures 20, 22). Minor fluctuations were also observed in oxygen saturation/percentage and nitrate, while temperature and salinity were relatively stable across the experiment (Figure 21).

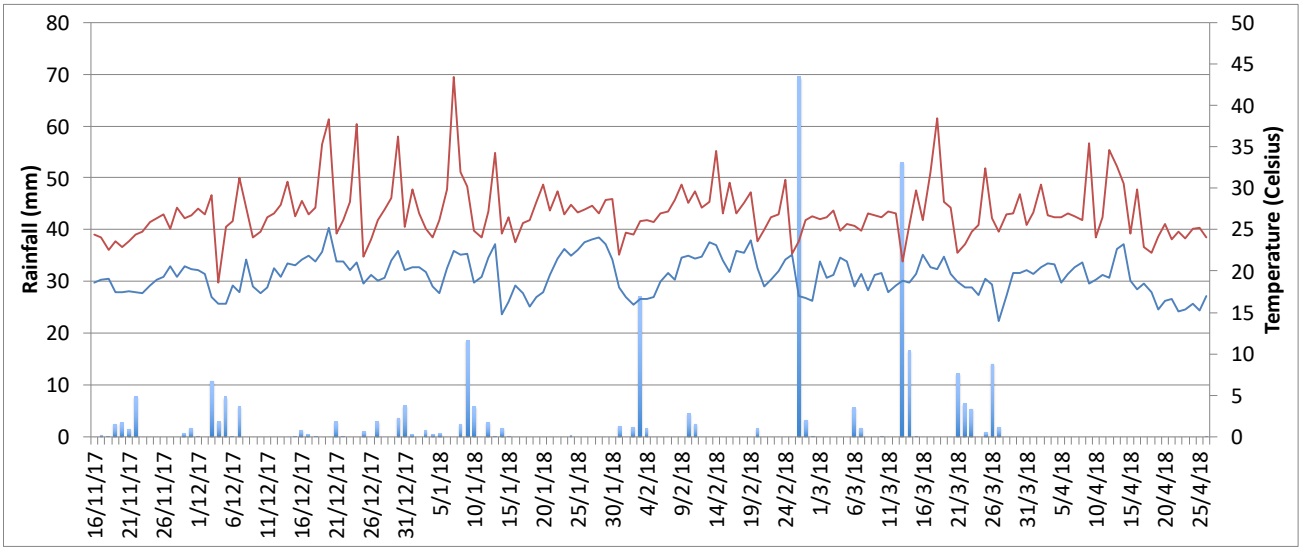


Figure 19: Rainfall (primary y-axis; blue columns) and minimum and maximum air temperature (secondary y-axis; blue and red line respectively) over the entire sampling period. Data collected from the bureau of meteorology from station 066062

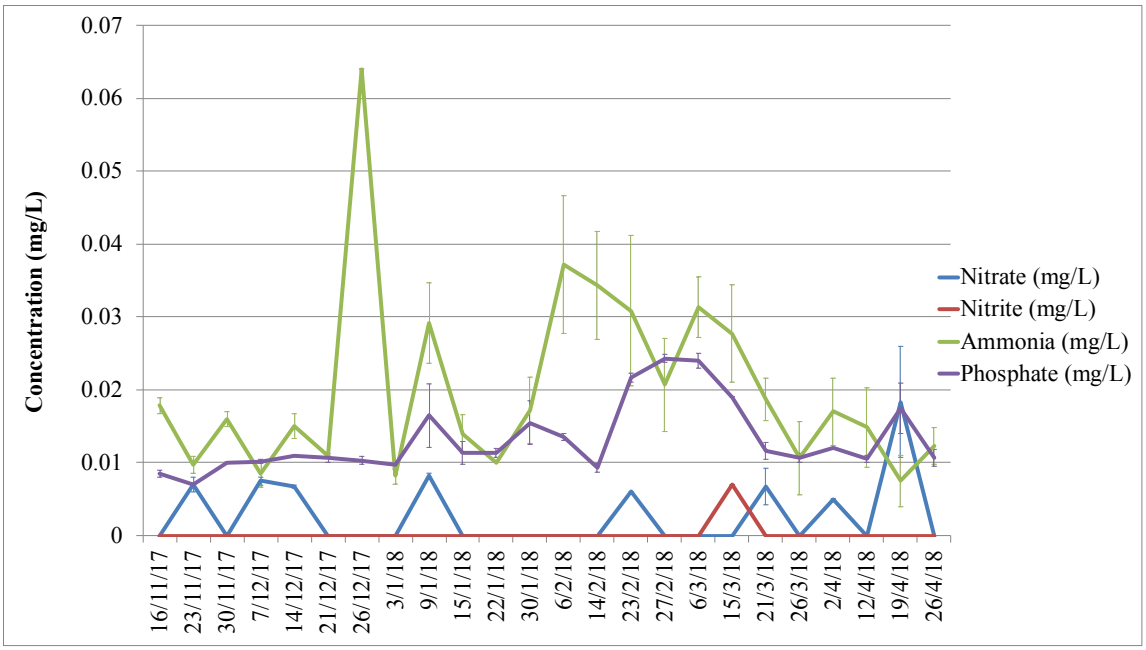


Figure 20: Changes in nutrient concentrations during the sampling period.

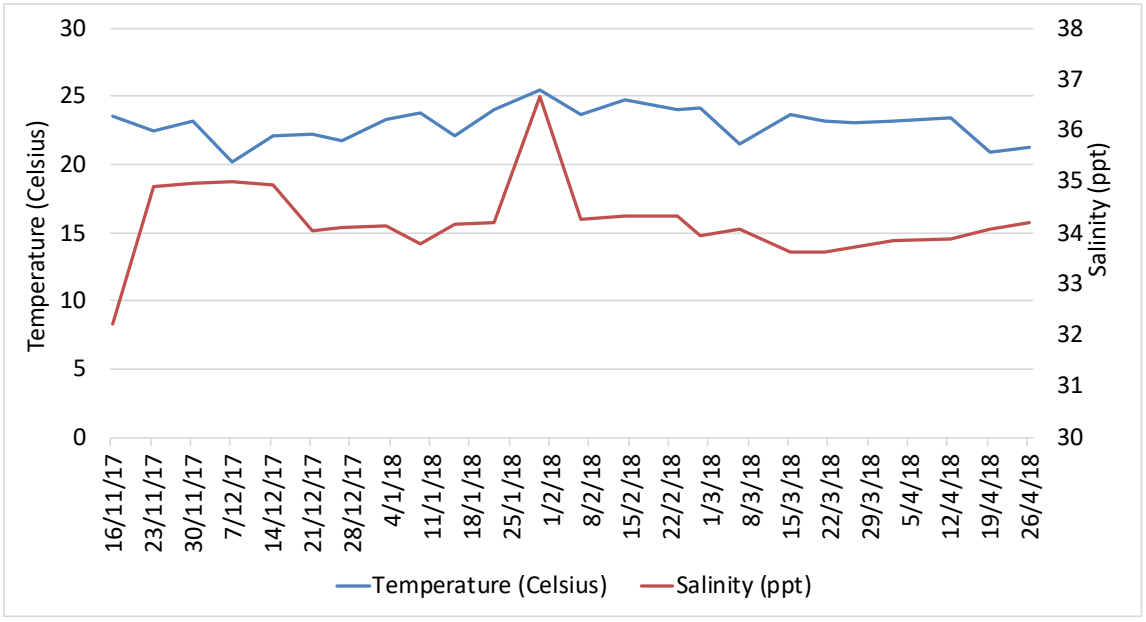


Figure 21: Temperature (primary y-axis) and salinity (secondary y-axis) measured from oyster tanks during the study period

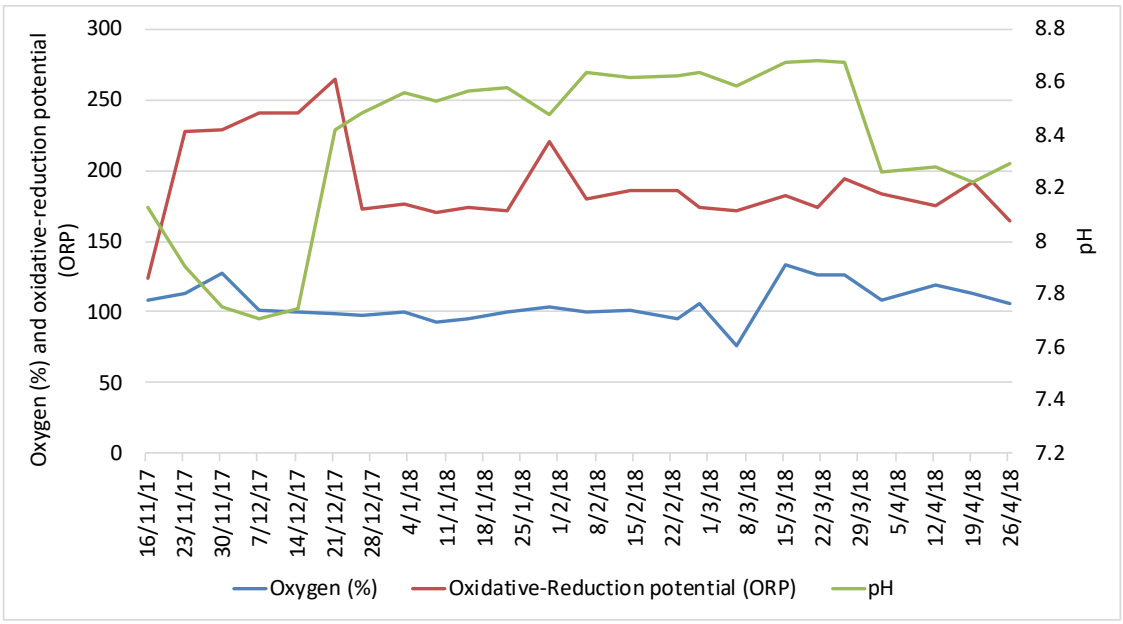


Figure 22: Oxygen (%) and oxidative-reduction potential (ORP) both on the primary y-axis. pH displayed on the secondary y-axis.

Oyster mortality was assessed throughout the sampling period according to visual inspection of individual oysters, with three clear phases of oyster mortality observed. During the first 12 weeks of the study, negligible levels of oyster mortality occurred, with the cumulative mortality < 5%. Between February 6 and March 15, a gradual increase in mortality was observed, with cumulative mortality reaching approximately 10% on 15/3/18. Following this period, cumulative oyster mortality rates rose sharply, reaching 50% by early April (Figure 23). Levels of oyster mortality were negatively correlated to salinity ($p = 0.019$; $r_s = -0.47$), but positively correlated to pH ($p = 0.0019$; $r_s = 0.61$) and phosphate ($p = 0.011$; $r_s = 0.51$).

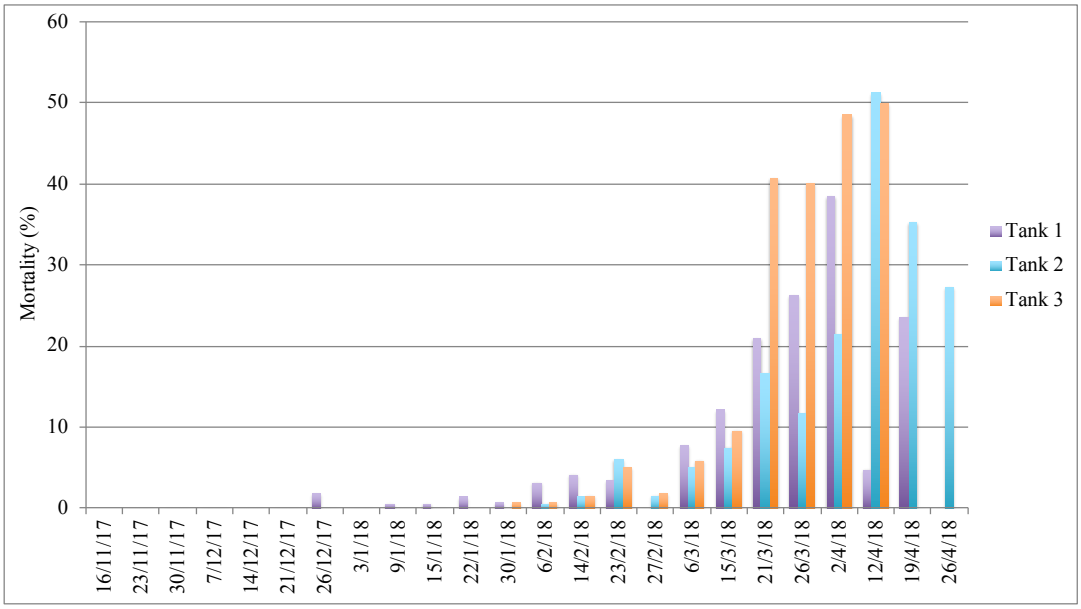


Figure 23: Cumulative oyster mortality during the 28 week experiment. Data is presented individually for each experimental enclosure (tank) and is calculated as the mortality of the proportion of living oysters from the previous week.

These three phases of negligible (16/11/17- 6/2/18), low (6/2/18-15/3/18) and high (15/3/18-26/4/18) oyster mortality rates, hereafter referred to groups 1, 2 and 3 (G1, G2 and G3 respectively), corresponded with shifts in the oyster microbiome. The over-all composition of the oyster microbiome progressively shifted between these three distinct periods, as illustrated by three discrete communities apparent in nMDS plots (Figure 24) and confirmed by statistical analyses (One-way PERMANOVA). Oyster microbiome structure during G1(16/11/17- 6/2/18) differed significantly to both G2 (6/2/18-15/3/18) [$p < 0.001$] and G3 (15/3/18-26/4/18) [$p < 0.001$], while microbiome structure during G2 also differed to that during G3 [$p < 0.001$].These shifts in oyster microbiome structure were reflected in changes in the relative abundance of several bacterial taxa during the course of the time-series.

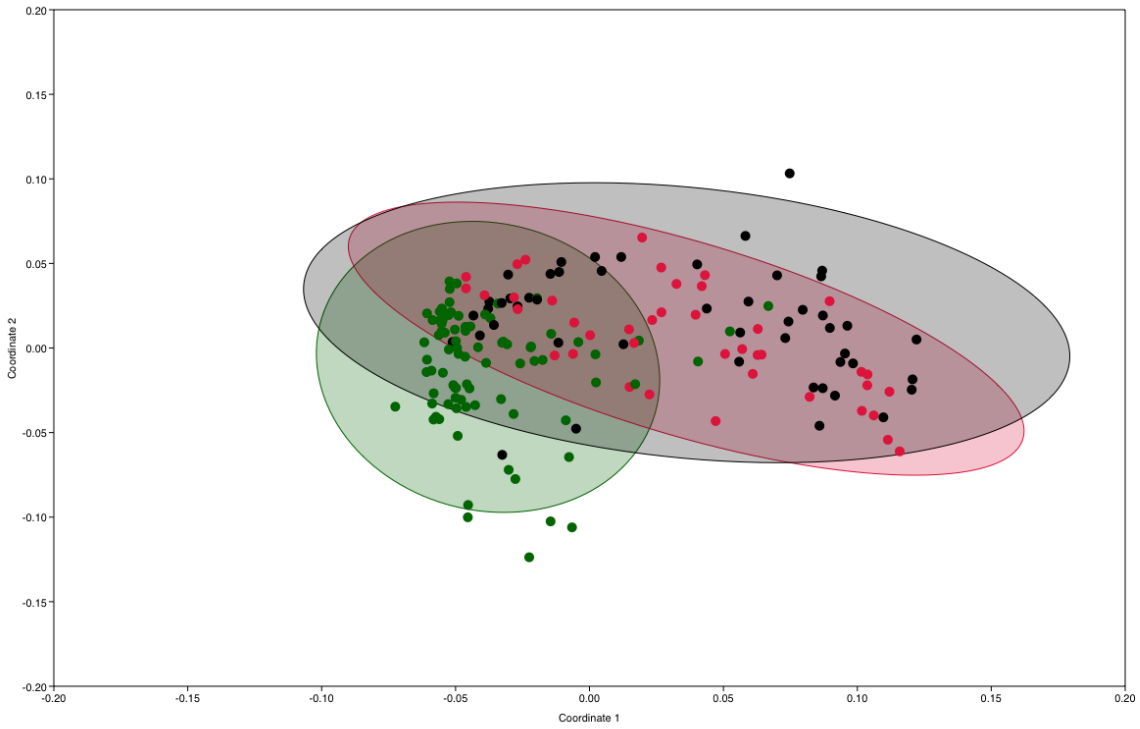


Figure 24: A Non-metric Multidimensional Scaling (nMDS) plot of Pacific Oyster microbiome structure during the course of the 24-week time-series. Green dots represent individual samples (oyster microbiomes) derived from the initial negligible mortality (G1) period, red dots represent the low mortality (G2 period) and black dots represent samples from the high mortality (G3) period. 95 % ellipses are shown

The temporal shifts in the oyster microbiome were reflected in changes in the relative abundance of bacterial OTUs (Figure 25). Although substantial week-to-week variability in the oyster microbiome was observed throughout the 28 week study period, substantial fidelity in oyster microbiome composition was observed across the three experimental enclosures (Figure 25). When examining data summarised at the genus level, the most abundant members of the first phase of the study (G1) were classified as members of the Spirochaetaceae (average of 21.5 % relative abundance across the three enclosures), Flavobacteraceae (6.1 %) and Mycoplasmadaceae (7.2 %). During G2, there was a sharp decline in the relative abundance of Spirochaetaceae (9 %) and a concomitant increase in Helicobacteraceae (8.8 %), Rhodobacteraceae (9.2 %) and Marinifilum (3.6 %). Finally during G3, when highest levels of oyster mortality occurred, there were notable increases in the relative abundance of members of the Vibrio (5.1 %), Colwellia (7.1 %), Tenacibaculum (4.6 %) and another group of Spirochaetaceae (Spirochaeta 2; 6.4 %).

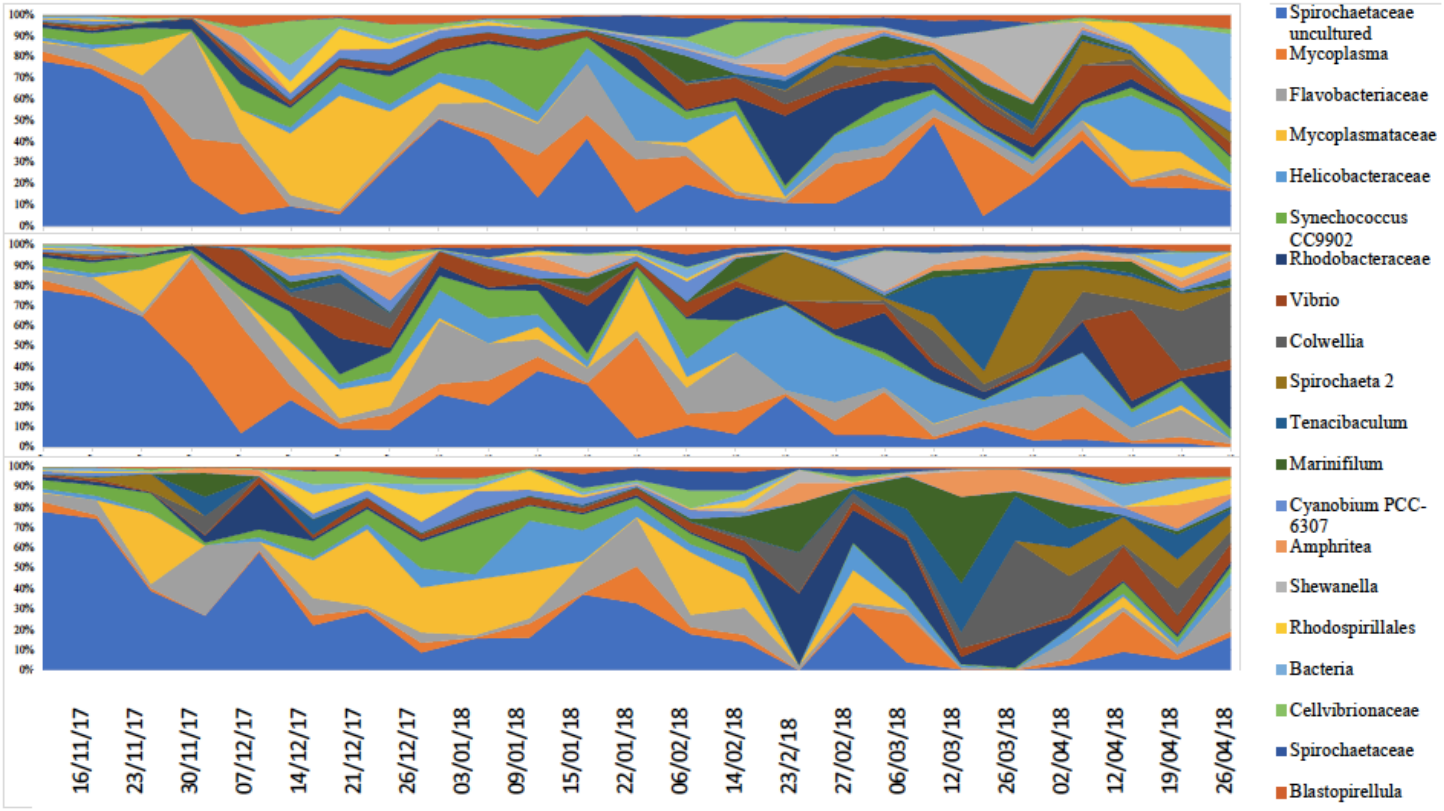


Figure 25: Patterns in the relative abundance of bacterial genera within the three experimental enclosures (Top = Tank 1; Middle = Tank 2; Botom = Tank 3) during the 24 week time-series study. Temporal patterns for the 20 most dominant genera are presented.

Beyond these qualitative shifts in the abundance of dominant microbiome members, statistical comparisons between the three stages of oyster mortality further revealed the principal drivers of the shifts in microbiome composition (Figure 26). The major determinants in the shift in oyster microbiome composition between the different phases of the mortality event were subtle shifts in the composition of the Spirochaeate community between several zOTUS occurring in different relative abundance during each stage (Figures 27 and 28). At a broader taxonomic level (e.g. genus), microbiome shifts between G1 and G2 were attributable to significant relative abundance decreases in uncultured members of the spirochaetaceae and mycoplasmataceae and significant increases in members assigned to the Rhodobacteraeae, Helicobacteraeae and Spirochaeta 2. The microbiome shift between G2 and G3 was also contributed to by a further relative abundance decreases in members assigned to uncultured Spirochaetaceae. But, was also characterised by significant increases in the relative abundances of OTUs classified as members of the Arcobacter,

Amphritea and Spirochaeta 2. When the microbiomes of oysters during the initial negligible mortality period were compared to oysters during the high mortality period, the principal drivers of the shift in microbiome composition were significant decreases in members assigned to uncultured Spirochaetaceae and Mycoplasmataceae and significant relative abundance increases to members of the Colwellia, Vibrio, Spirocheata 2 and the Tenacibaculum. When examining consistent patterns across the entire temporal study within the context of increasing mortality over time, we observed a progressive reduction in uncultured Spirochaetaceae members and a progressive increase in members of the Spirocheata 2.

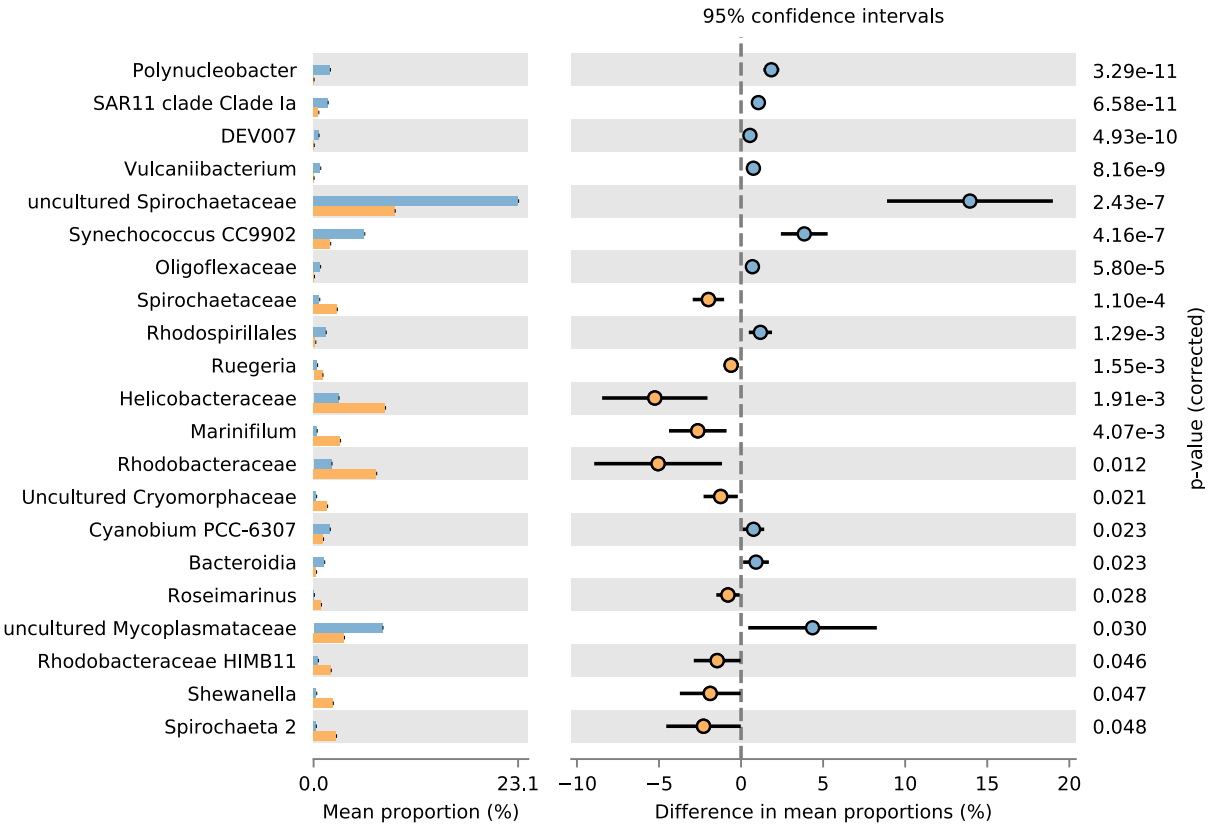


Figure 26: STAMP analysis of G1 versus G2. Data summarised at the genus level were used for the analysis. Displayed genera are significantly different (Welsh's two-sided t-test) and only those significantly different genera with an effect size greater than 0.5 are shown.

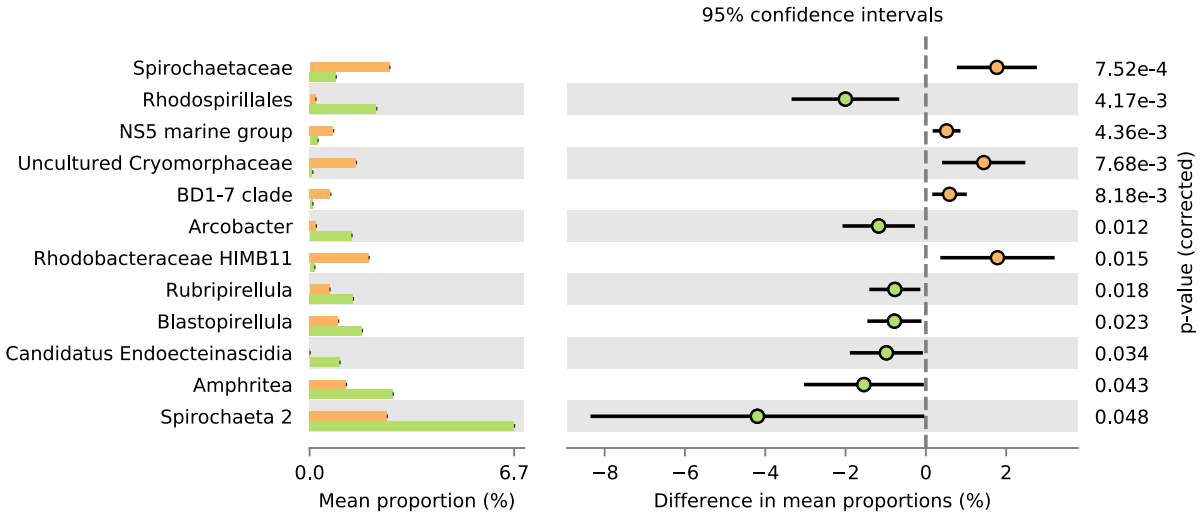


Figure 27: STAMP analysis of G2 versus G3. Data summarised at the genus level were used for the analysis. Displayed genera are significantly different (Welsh's two-sided t-test) and only those significantly different genera with an effect size greater than 0.5 are shown.

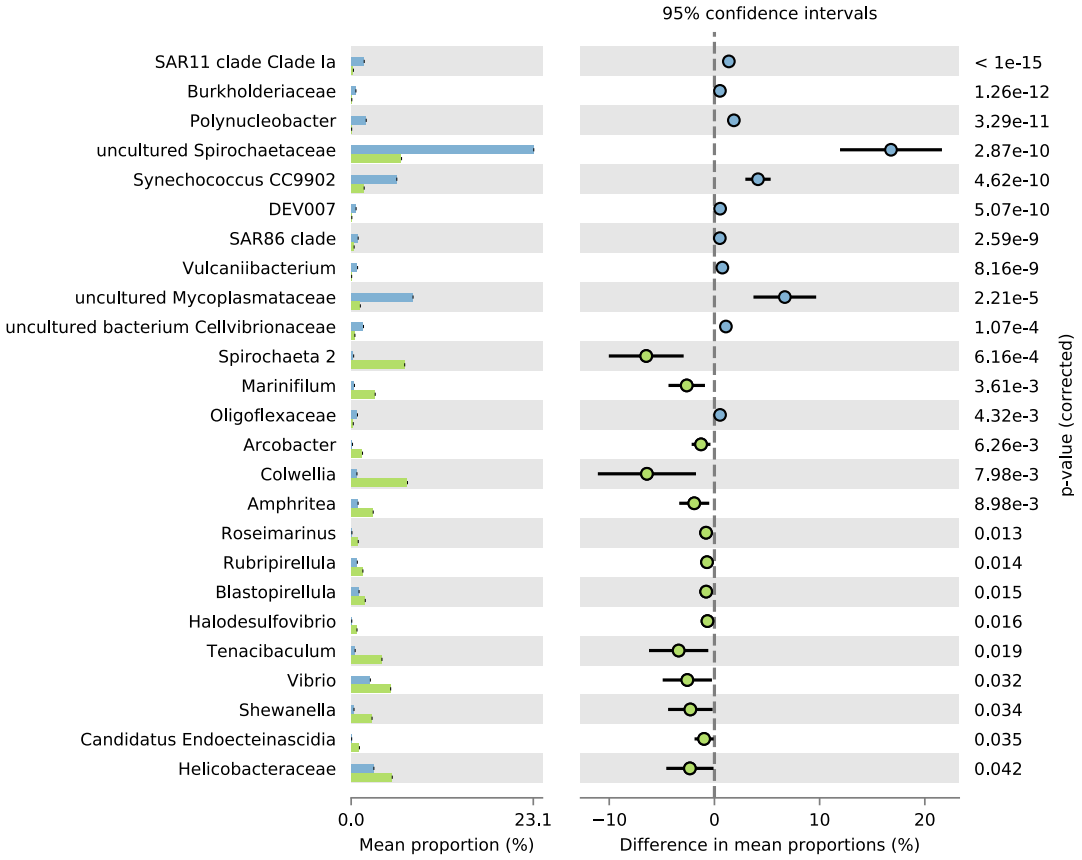


Figure 28: STAMP analysis of G1 versus G3. Data summarised at the genus level were used for the analysis. Displayed genera are significantly different (Welsh's two-sided t-test) and only those significantly different genera with an effect size greater than 0.5 are shown.

A significant increase in reads belonging to the *Vibrio* genus was observed between G1 to G3. As *Vibrio* species are often implicated in disease dynamics we quantified the numbers of *Vibrio*-specific 16S rRNA copies to estimate *Vibrio* abundance (Figure 29). *Vibrio* abundance was strongly positively correlated to mortality (Pearson's $p = 0.0095$ and $r = 0.57$; Spearman's $p = 0.03$ and $r_s = 0.45$). When comparing *Vibrio* abundance between all of the groups, a Kruskal-Wallis ANOVA identified significant differences in *Vibrio* abundance ($p < 0.0001$, $H = 120.4$). This difference primarily driven by an elevation of *Vibrio* abundance in G3 compared to G1 ($p < 0.0001$, $U = 1427$) and G2 ($p < 0.0001$, $U = 633$). In agreement with the STAMP analysis, no significant difference in *Vibrio* abundance were identified between G1 and G2 (Mann-Whitney pairwise $p = 0.53$; $U = 2613$). When considering mortality samples (dead oysters) as as single group (mean = 2702.9, Standard error = 871), these samples had the highest levels of *Vibrio* abundance compared to all other tested groups (all p-values < 0.0001; G1 U = 555; G2 U = 235; G3 U = 631).

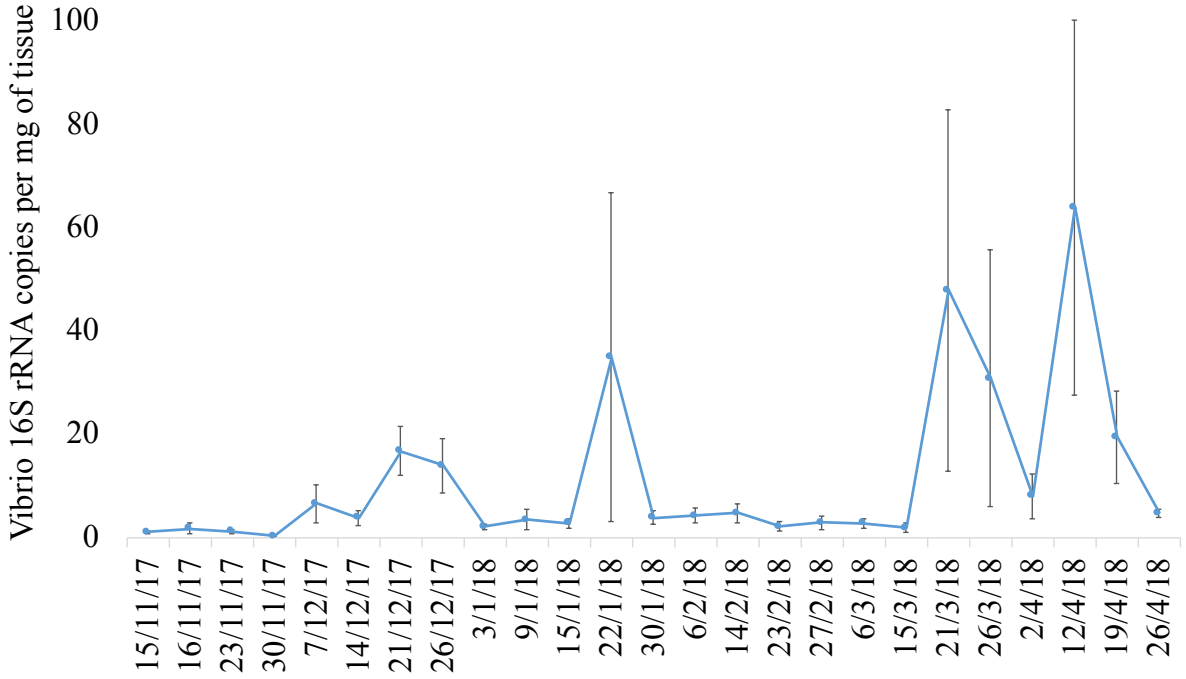


Figure 29: Vibrio-specific 16S rRNA qPCR. Displayed data is averaged across all three enclosures and the standard error is shown.

Network analysis identified numerous significant relationships between abiotic factors and mortality (Figure 30). In agreement with the previously performed Spearman's correlation, salinity was negatively correlated to mortality while pH and phosphate were positively correlated to

mortality. In addition, oxygen, temperature, rainfall, pressure and nitrate were positively correlated to mortality.

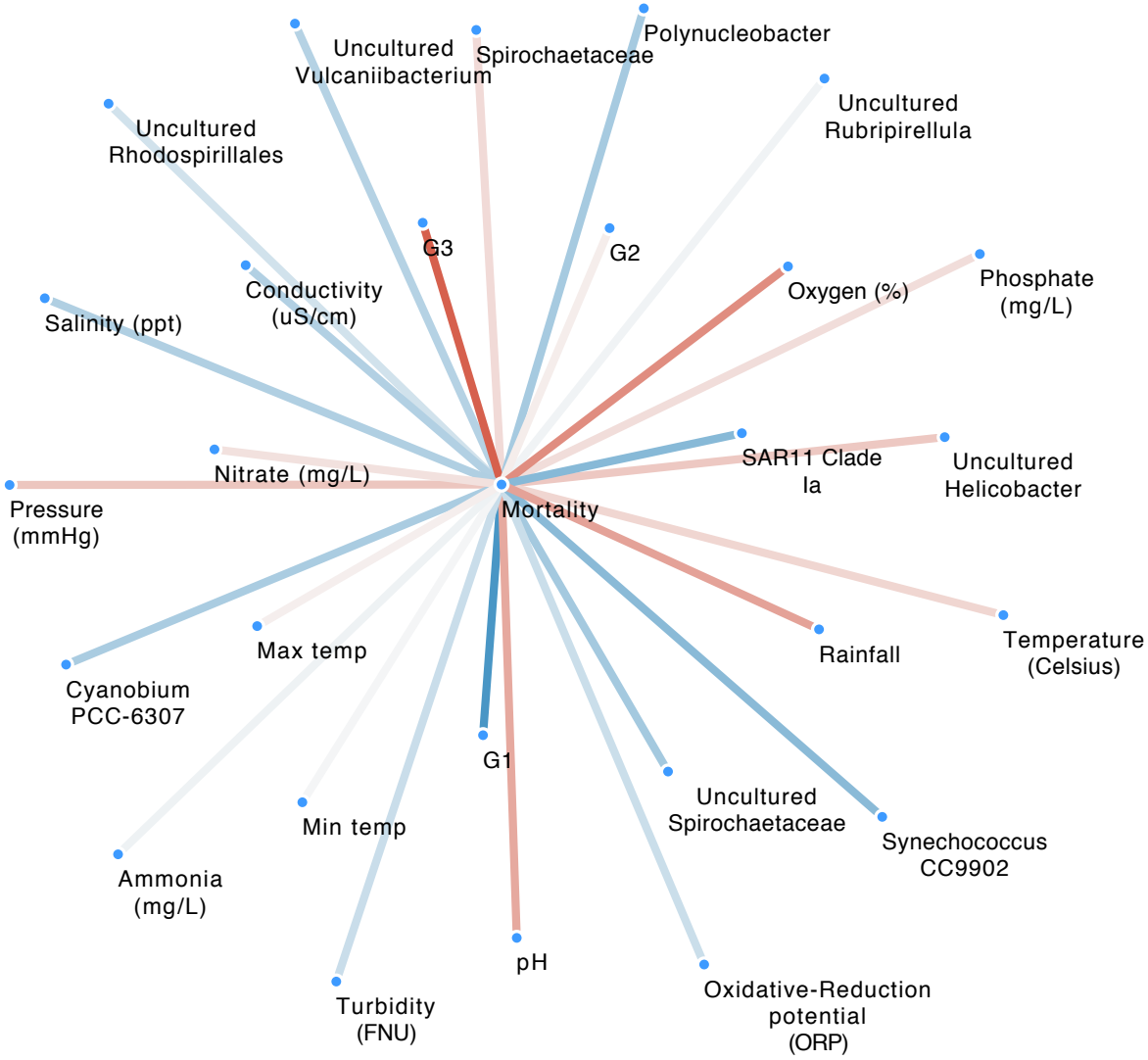


Figure 30: Network analysis identifying significant relationships between mortality, abiotic parameters and bacterial genera. 16S rRNA data were summarised at the species level and filtered to remove genera with less than 1 % relative abundance prior to analysis. Blue lines represent negative correlations. Red lines are positive correlations.

<u>Aim 6:</u> Elucidating patterns in *Vibrio* community diversity and abundance within the microbiomes of Pacific Oysters subject to disease and mortality events

Comparison of Vibrio mock community characterisation using 16S rRNA and hsp60

Mock *Vibrio* communities consisting of 10 different *Vibrio* species were characterised using the 16S rRNA, *Vibrio*-specific 16S rRNA and *Vibrio*-specific *hsp60* primer pairs followed by Illumina MiSeq sequencing of the amplicons. When examined on a non-metric multidimensional scaling analysis (nMDS), the *Vibrio* community structure defined by the *Vibrio*-specific *hsp60* assay clustered closer to the true mock community, with the true mock community sitting within the 95 % ellipses for each *Vibrio*-specific *hsp60* characterised community (Figure 31A-C). Comparatively, the compositions of the 16S rRNA, *Vibrio*-specific 16S rRNA, and *Vibrio*-specific *hsp60* characterised communities were on average 16, 25, and 77 % similar to the true mock community, respectively (Figure 31D).

The *Vibrio* community data derived from the traditional V3-V4 16S rRNA primer pair was poorly characterised beyond the genus level, with only one *Vibrio* species used in the mock community correctly identified (Figure 32). On average, reads were not defined beyond the *Vibrio* genus level 90, 74 and 89 % of the time in mock communities 1, 2 and 3 respectively. Of the sequences that were assigned beyond the *Vibrio* genus level, they were only assigned to *V. cholerae* and *V. azureus*. Notably, *V. azureus* was not part of the mock communities indicating not only imprecise, but incorrect taxonomic classification. *V. azureus* is closely related to the *V. harveyi* clade, for which it was probably incorrectly attributed (Yoshizawa et al., 2009). Reads assigned to *V. cholerae* were correctly assigned but were marginally under-represented when compared to the mock community (6 - 23 % for 16S rRNA; 7.5 - 30 % for the mock communities).

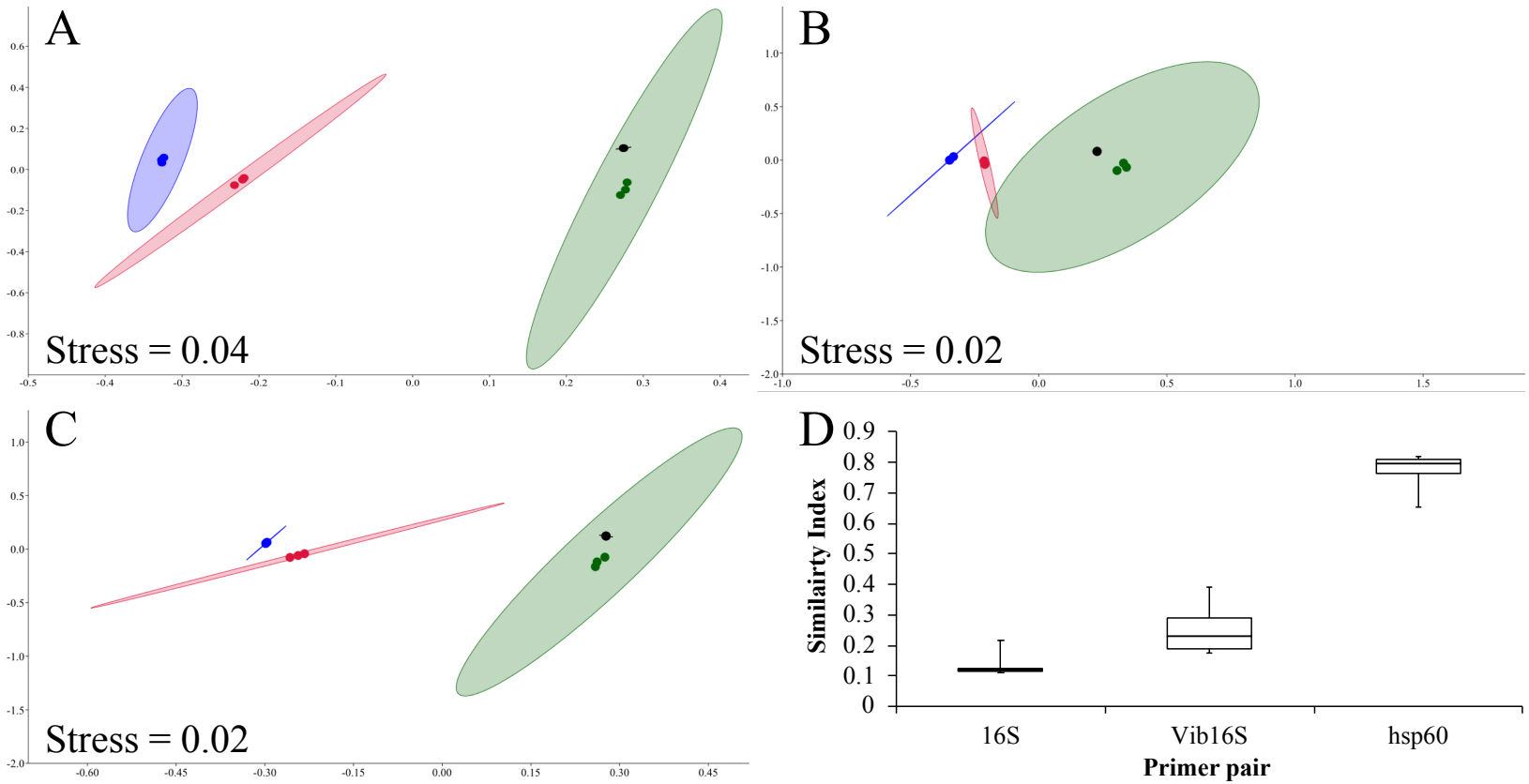


Figure 31: nMDS analysis of the 16S rRNA (blue dots), *Vibrio*-specific 16S rRNA (red dots) and *Vibrio*-specific *hsp60* (green dots) characterised mock communities, and the true mock community (black dots). Mock communities 1, 2 and 3 are panes A, B and C respectively. 95 % ellipses are shown. Panel D: Box and whisker plot of Bray-Curtis similarity comparisons of community composition compared to the true mock communities. Data for all three mock communities is combined. For species assigned across two taxonomic assignments (e.g. group 2), they were combined with their respective species for panel D.

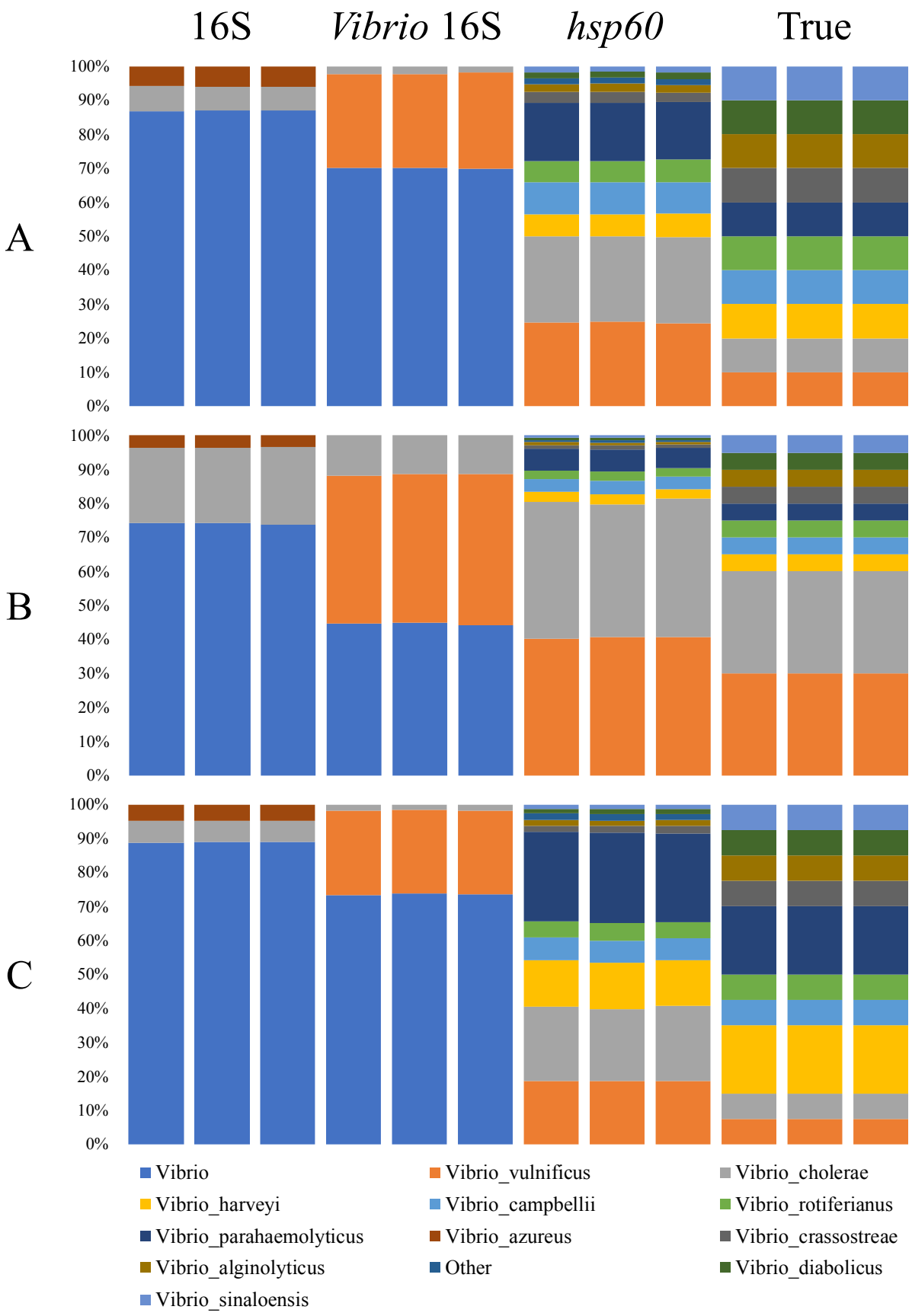

16S
Vibrio 16S
hsp60
True
A
B
C
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Vibrio
Vibrio_vulnificus
Vibrio_cholerae
Vibrio_harveyi
Vibrio_campbellii
Vibrio_rotiferianus
Vibrio_parahaemolyticus
Vibrio_azureus
Vibrio_crassostreae
Vibrio_alginolyticus
Other
Vibrio_diabolicus
Vibrio_sinaloensis

Figure 32: (Previous page): Comparison of amplicon sequenced phylogenetic markers for the *Vibrio* mock communities. Mock communities 1, 2, and 3 are A, B, and C respectively. Communities were characterised using 16S rRNA V3-V4 (Herlemann et al., 2011), *Vibrio*-specific 16S rRNA (Thompson et al., 2004; Yong et al., 2006), and *Vibrio*-specific *hsp60* primer pairs. The true mock community composition is also shown. Displayed data is relative abundance summarised at the species level. For reads assigned to the second chromosome (group2), they were combined with their respective species. Reads representing less than 1 % of the relative abundance were removed.

Similarly to the 16S rRNA characterised community composition, the data derived from the *Vibrio*-specific 16S rRNA sequencing assay was only able to identify two *Vibrio* species used in the mock community, with the majority of the reads not resolved beyond the genus level. Although correctly identified as *Vibrio*, the majority of sequences could not be assigned to the species level in 70, 45 and 74 % of the time in mock communities 1, 2 and 3 respectively, an improvement to those observed for the 16S rRNA characterised communities. For the remainder of the reads assigned beyond the genus level, they were correctly assigned to *V. vulnificus* and *V. cholerae*. Reads assigned to *V. vulnificus* were over-represented when compared to the mock community (25-44 % for *Vibrio*-specific 16S rRNA; 7.5-30 % for the mock communities), while reads assigned to *V. cholerae* were under-represented (2-12 % for *Vibrio*-specific 16S rRNA; 7.5-30 % for the mock communities).

Relative to both of the 16S rRNA based sequencing assays, the *Vibrio*-specific *hsp60* primer set identified the greatest number of species in the *Vibrio* community, with all of the species present in the mock community correctly identified by this assay. While all of the species were correctly identified, differences in the relative abundance of each species was observed when compared to the true mock community (Table 9). *V. campbellii* was the best represented species with each *Vibrio*-specific *hsp60* characterised mock community only showing a 1 % difference to the true mock community, while *V. sinaloensis* was the most under-represented species with differences of 4-8 % for the *Vibrio*-specific *hsp60* characterised communities. For *V. vulnificus* and *V. cholerae*, the only two correctly identified species in the 16S rRNA and *Vibrio*-specific 16S rRNA characterised communities, the *Vibrio*-specific *hsp60* assay provided marginally better representation for *V. vulnificus* (over-representation of 10-13 % compared to the mock community) compared to the *Vibrio*-specific 16S rRNA assay (over-representation of 14-18 %) and 16S rRNA assay (not identified). For *V. cholerae*, this species was under-represented in both the 16S rRNA (1-8 %) and *Vibrio*-specific 16S rRNA assays (6-20 %) compared to an over-representation in the

Vibrio-specific *hsp60* assay (9-14 %). The exaggeration of *V. vulnificus* and *V. cholerae* could possibly be due to a greater *hsp60* primer affinity to these species, or the presence of two copies of *hsp60* in the genomes of these bacteria (one copy was identified in each chromosome for these two species).

Notably, the *Vibrio*-specific *hsp60* sequencing assay also distinguished members of the *V. harveyi* clade, a tight phylogenetic group within the *Vibrio* genus (Sawabe et al., 2013; Urbanczyk et al., 2013), which has previously had numerous incorrect taxonomic assignments to species within this clade due to their close 16S rRNA genetic similarity (Lin et al., 2010; Sawabe et al., 2013; Urbanczyk et al., 2013). This clade includes *V. parahaemolyticus*, *V. alginolyticus*, *V. harveyi*, *V. campbellii*, *V. diabolocus* and *V. rotiferianus*, all of which were identified with the *Vibrio*-specific *hsp60* sequencing assay (Sawabe et al., 2013; Turner et al., 2018). Many of these species are important pathogens (Daniels and Shafaie, 2000; Luna-González et al., 2002; Go et al., 2017) and therefore accurately identifying their presence in environmental samples is an important requisite of a *Vibrio* specific assay of this type.

Previous attempts to perform *hsp60* amplicon sequencing have used universal *hsp60* primers and filtered the data for the assigned *Vibrio* sequences (Jesser and Noble, 2018). Only 0.5 % of the total *hsp60* data were assigned to *Vibrio* species in the previous study, compared to retaining 21.1 % of the data for the *Vibrio*-specific *hsp60* assay produced in this study. Further, the remaining 0.5 % included a significant number of unassigned *Vibrio* species (Jesser and Noble, 2018), attributable to poor *Vibrio* species representation in the *cpn60* database (Hill et al., 2004; Jesser and Noble, 2018). The *Vibrio* reference dataset produced in this study encompasses 106 different *Vibrio* species compared to only 63 unique species in the *cpn60* database (accessed August 2019). Therefore, the assay produced in this study delivered greater data yield compared to using universal *hsp60* primers and included more *Vibrio* species in the reference dataset.

Table 9: Relative abundance comparisons between the *Vibrio*-specific *hsp60* characterised mock communities and the true mock communities. Displayed 1 % filtered relative abundance is averaged across three replicates and in those cases where reads were assigned to the second chromosome (group 2), they were combined with their respective species. Relative abundance differences are also shown. 1, 2 and 3 represent mock communities 1, 2 and 3 respectively.

Taxa	hsp60_1	True_1	Difference_1	hsp60_2	True_2	Difference_2	hsp60_3	True_3	Difference_3
V. vulnificus	23.4	10	13.4	39.8	30	9.8	17.9	7.5	10.4
V. cholerae	23.9	10	13.9	39.2	30	9.2	20.9	7.5	13.4
V. parahaemolyticus	16.2	10	6.2	6.1	5	1.1	25.3	20	5.3
V. harveyi	6.4	10	-3.6	2.8	5	-2.2	13.1	20	-6.9
V. campbellii	8.8	10	-1.2	3.8	5	-1.2	6.2	7.5	-1.3
V. rotiferianus	6.0	10	-4.0	2.6	5	-2.4	4.7	7.5	-2.8
V. alginolyticus	7.4	10	-2.6	2.7	5	-2.3	5.7	7.5	-1.8
V. crassostreae	2.9	10	-7.1	1.1	5	-3.9	1.9	7.5	-5.6
Other	1.7	0	1.7	0.6	0	0.6	1.8	0	1.8
V. diabolicus	1.8	10	-8.2	0.8	5	-4.2	1.4	7.5	-6.1
V. sinaloensis	1.6	10	-8.4	0.6	5	-4.4	1.2	7.5	-6.3

Vibrio diversity in seawater

After confirming the utility of the *Vibrio*-specific *hsp60* sequencing assay using mock communities, this assay was used to characterise *Vibrio* diversity in seawater samples collected from Sydney Harbour, with the measured community composition compared to that derived from traditional 16S rRNA sequencing (Figure 33). Reads assigned to the *Vibrio* genus or *Vibrio* species only made up 0.13-0.17 % of the total bacterial community using 16S rRNA sequencing, with the majority (59-77 % relative abundance) of these reads not resolved beyond the *Vibrio* genus-level. In contrast, the proportion of the community assigned to the *Vibrio* genus when using the Vibrio-specific *hsp60* sequencing assay was only 1.4-1.7 %. Ten different *Vibrio* species were identified within the seawater samples using the *Vibrio*-specific *hsp60* sequencing assay, most of which were lowly abundant (1-4 % relative abundance) except for *V. azureus* (58-71 %) and *V. mediterranei* (10-29 %). In contrast, only three *Vibrio* species were identified using the 16S rRNA assay. Both assays identified the presence *of V. mediterranei,* with similar levels of relative abundance (16S rRNA: 19-34 %; *hsp60*: 10-29 %). The unique co-occurrence of *V. azureus* and *V. mediterranei* in seawater has been observed in a previous study (Amin et al., 2016) and may explain the co-dominance of these species in these *Vibrio* seawater communities.

Vibrio abundance determines assay efficacy

To determine assay efficiency, both seawater and oyster samples were used. As expected, samples with the greatest abundance of *Vibrio,* as determined using qPCR targeting *Vibrio* 16S rRNA gene copies, had the greatest number of *hsp60* reads (Supplementary Figure 1), with a significant relationship observed between *Vibrio* 16S rRNA gene copies and *hsp60* reads (R^2 = 0.87; p = 0.0001) (Figure 34). It is possible that the low number of *hsp60* reads in samples with low *Vibrio* biomass was due to non-specific amplification of *hsp60* sequences associated with other bacterial genera. However, when significant levels of *Vibrio* are present within a sample, this assay delivers substantial capacity to probe the diversity of the community.

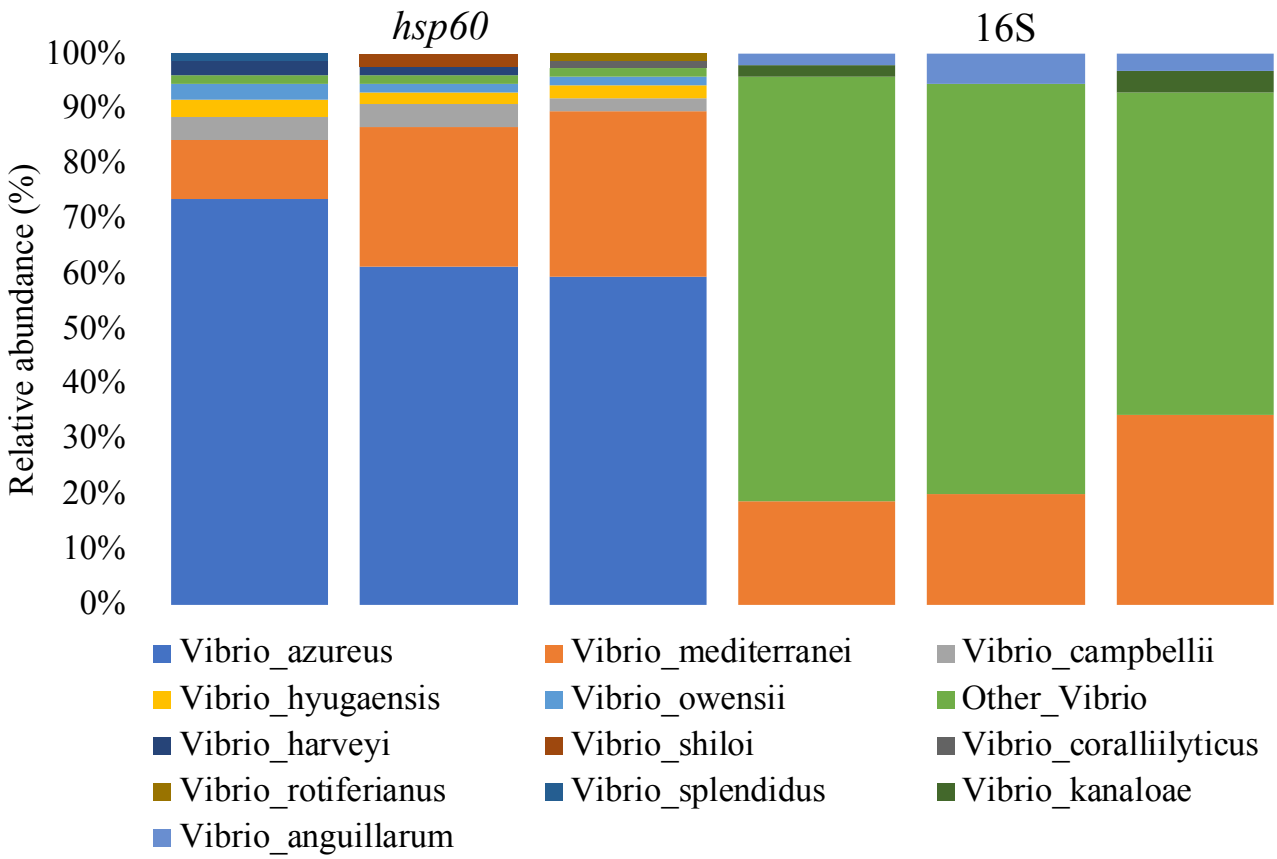


Figure 33: *Vibrio* diversity in seawater from Sydney Harbour. DNA were characterised with the *Vibrio*-specific *hsp60* and 16S rRNA V3-V4 (Watermann et al., 2008) primer sets. Displayed data is relative abundance summarised at the species level.

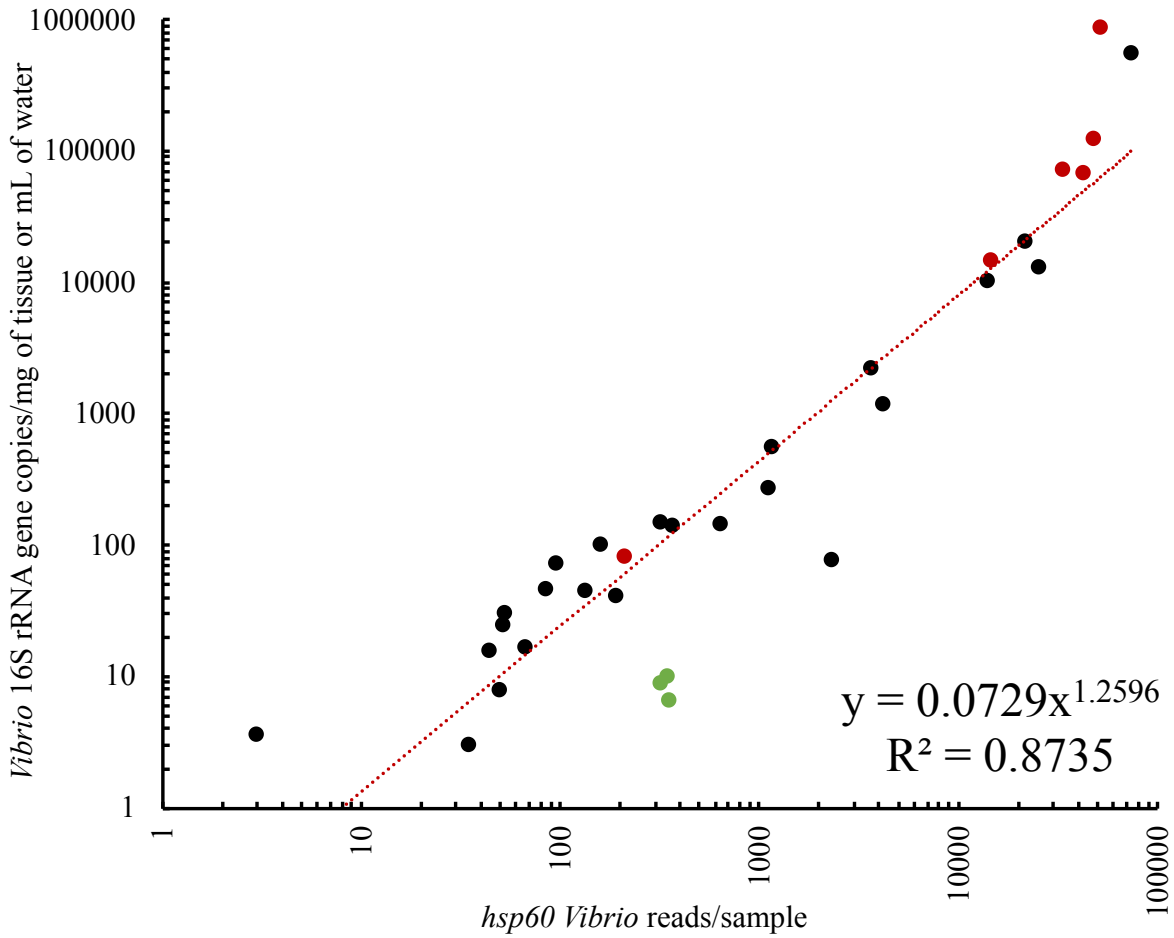


Figure 34: Ordinary least squares linear regression of *Vibrio* 16S rRNA gene copies and *hsp60* reads per sample. Black dots are oyster samples, red dots are oyster mortality samples and green dots are seawater samples. Both axes are logarithmic in scale.

Vibrio diversity during a laboratory induced oyster mortality event

After confirming the utility of the *Vibrio*-specific *hsp60* assay to track *Vibrio* community dynamics with high fidelity using a mock community and successfully applying it to characterise *Vibrio* diversity within natural seawater samples, it was next used to examine patterns in *Vibrio* diversity during a laboratory-induced oyster mortality. During this simulated heatwave event described in detail in Green et al. (2019), significant levels of oyster mortality were observed in oysters exposed to an increase in water temperature to 25°C (77.4 ± 10.7 %), relative to oysters maintained at ambient temperature levels at 20°C (3.4 ± 5.9 %). The *Vibrio*-specific *hsp60* assay was applied on

samples derived from this study, because previous analyses suggested that *Vibrio* were implicated and overly abundant in this mortality event (Green et al., 2019).

Using the *Vibrio*-specific *hsp60* sequencing assay, the *Vibrio* community composition associated with Pacific oysters was significantly different in accordance with differences in temperature ($F = 6.5$, $p = 0.0005$) and oyster mortality ($F = 14.8$, $p = 0.0003$ versus low temperature; $F = 4.4$, $p = 0.013$ versus high temperature). The 'baseline' *Vibrio* community (Figure 35) on the first day of the experiment (day zero), four days prior to significant mortalities, was distributed across nine different species, with *V. brasiliensis*, *V. chagasii*, *V. fortis*, and *V. harveyi* representing the dominant members of the *Vibrio* community with average relative abundances of 9, 20, 11, and 35 % respectively.

When comparing temperature treatments, the *Vibrio* communities were on average 56 % dissimilar to each other. In the low temperature treatment, *V. campbellii* and *V. chagasii* were the most prominent members, contributing 18 and 15 % to the community dissimilarity (21 and 17.6 % average relative abundance respectively) and were both negatively correlated to temperature ($r_s = -0.4$, $p = 0.04$; $r_s = -0.53$, $p = 0.008$, respectively) and mortality ($r_s = -0.45$, $p = 0.02$; $r_s = -0.52$, $p = 0.007$, respectively. While, *V. harveyi* dominated the *Vibrio* community in the high temperature treatments contributing 37 % to the community dissimilarity between temperature treatments and was positively correlated to temperature ($r_s = 0.52$, $p = 0.011$) and mortality ($r_s = 0.55$, $p = 0.006$). On days three, four, and five, *V. harveyi* comprised 73-75 % of the whole community, followed by a decrease in relative abundance (41 %) on day six. This pattern is consistent with the results of a *V. harveyi* specific qPCR assay performed on these samples in a previous study, where a significant increase in copies of the *V. harveyi* gyrase B gene was observed on days three, four and five, followed by a decrease on day six (Green et al., 2019).

Notably, a sharp increase in the relative abundance of *V. harveyi* was also observed in the low temperature treatment on days five (6 % on day four to 65 %) and six (68 %), which was again consistent with qPCR data (Green et al., 2019). Dead oyster samples collected on days four and five from the high temperature treatment were also completely dominated by *V. harveyi*, which represented 97 % and 96 % of the *Vibrio* community respectively. Low levels of oyster mortality

(2%) were observed in the low temperature treatment on day six (Green et al., 2019), which notably corresponded with an increase in the relative abundance of *V. harveyi* on the preceding day (6 to 65 % from days four to five). *V. harveyi* was previously implicated as the causative agent behind this mortality event (Green et al., 2019) and a previous study implicated *V. harveyi* as a causative agent for an unknown mass mortality outbreak from the same region the oysters were sourced from (Port Stephens) (Go et al., 2017). The data derived from the *Vibrio*-specific *hsp60* sequencing approach was able to unambiguously pinpoint the putative pathogen that increased in abundance prior to disease onset, as evidenced by previous culturing studies (Go et al., 2017; Green et al., 2019).

Temperature was strongly correlated to mortality ($r_s = 0.87$, $p = 0.0001$) and may have provided a selective advantage for *V. harveyi* allowing for an increase in the relative abundance of this species, effectively replacing the putative commensal *Vibrio* species (Lemire et al., 2015) and/or temperature may have acted as an immunosuppressant in the oysters allowing for a shift in the *Vibrio* community preceding disease (Lokmer and Wegner, 2015). Interestingly, the oysters on day six in the high temperature treatment had a decreased number of reads assigned to *V. harveyi* relative to the preceding days (75 to 45 % from days five to six). A possible explanation for this pattern is that a sub-population of surviving oysters exhibited higher tolerance to the elevated temperature conditions, avoided colonisation by *V. harveyi* and survived. These results indicate that temperature stressed oysters undergo a substantial shift in the composition of their *Vibrio* community, involving a dramatic increase in the relative abundance of *V. harveyi*, which precedes oyster mortality. Both the occurrence of elevated levels of the *V. harveyi* in oysters before and during mortality and the very high levels of *V. harveyi* in freshly deceased oysters further implicate this species in oyster mortality events, in agreement with previous studies (Saulnier et al., 2010; Segarra et al., 2010; Jenkins et al., 2013; Le Roux et al., 2016; Go et al., 2017).

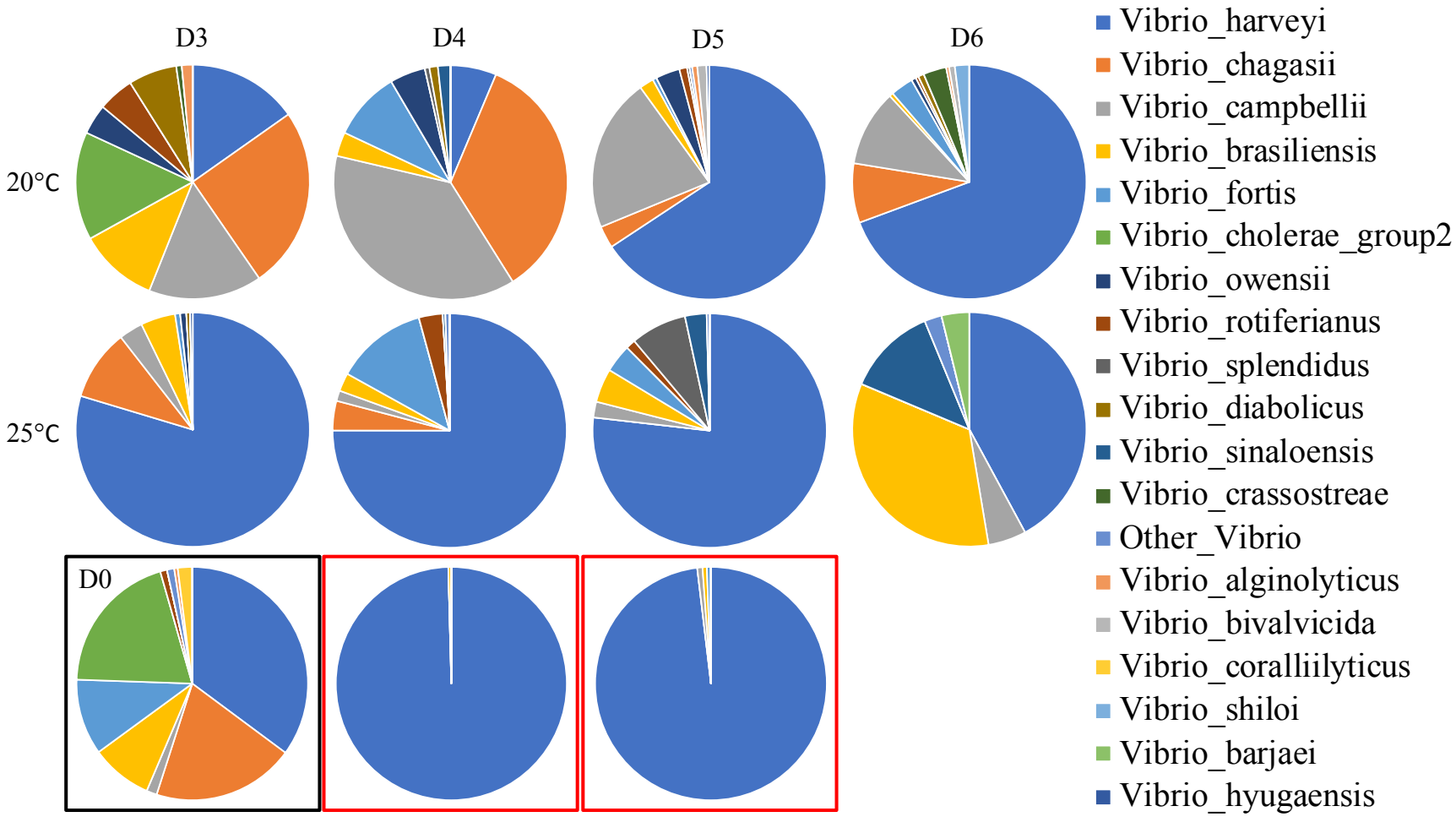


Figure 35: *Vibrio* community of *C. gigas* spat across six days and two temperature treatments. D0, D3, D4, D5, and D6 correspond to sampling days zero through to six. Communities are averaged across three biological replicates and summarised at the species level. Communities in a black box are day zero. Communities in red boxes are dead *C. gigas* spat from the high (25°C) temperature treatment, taken on days four and five respectively. Reads representing less than 1% of the relative abundance were removed.

Most standard approaches for examining *Vibrio* diversity are constrained by poor taxonomic resolution beyond the genus level. This is often a significant limitation because *Vibrio* species are often implicated in disease events among both natural populations of marine organisms (Kushmaro et al., 2001; Austin and Zhang, 2006; Rubio-Portillo et al., 2014) and commercially important aquaculture species, including several oyster species (Goarant et al., 2000; Becker et al., 2004; Frans et al., 2011; Geng et al., 2014; Vezzulli et al., 2015). Here, a *Vibrio*-specific *hsp60* sequencing assay was created using primers tailored to *Vibrio*-specific *hsp60* and used in combination with a custom-built *Vibrio* reference dataset including 106 *Vibrio* species. The sequencing assay was able to successfully identify every *Vibrio* species included within a mock community constructed with known dilutions of different *Vibrio* species. Despite an exaggeration in the relative abundance of some species, the *Vibrio*-specific *hsp60* sequencing assay provided superior taxonomic resolution when compared to conventional 16S rRNA sequencing methods. The *Vibrio*-specific *hsp60* sequencing assay was subsequently successfully applied to seawater samples providing better discrimination of *Vibrio* diversity compared to 16S rRNA amplicon sequencing approaches, highlighting its utility in seawater. Next, the sequencing assay was able to unambiguously identify the *Vibrio* species that increased in abundance during an oyster mortality event, pinpointing a putative pathogen involved in the deaths of oysters following a simulated marine heatwave. This *Vibrio*-specific *hsp60* sequencing assay offers the potential for high throughput characterisation of *Vibrio* diversity while retaining a highly specific degree of taxonomic resolution in environmental samples, important for dissecting species level community dynamics and their relationship with the environment or disease.

Discussion

<u>Aim 1:</u> Characterising the composition of the Pacific Oyster microbiome across diverse oyster families, including those exhibiting different levels of susceptibility to OsHV-1 disease

The principal goal of this study was to identify patterns in the *C. gigas* microbiome across 35 oyster families with differing levels of resistance to OsHV-1 µvar disease, with the objective of elucidating microbial taxa associated with disease resistance. Immunosuppression from OsHV-1 µvar infection allows opportunistic bacteria within the oyster's microbiome to induce bactericaemia, killing the host (Petton et al., 2015; de Lorgeril et al., 2018). Characterising these interactions and gaining insights into the oyster microbiome is essential to further understand the dynamic interplay between the microbiome, OsHV-1 µvar and disease.

A significant difference in the structure of the microbiome of oysters exhibiting different levels of resistance to OsHV-1 µvar disease was observed. Specifically, the microbiomes associated with the oysters showing the most resistance to OsHV-1 µvar disease (RG1) and moderately resistant oysters (RG2) were significantly different to the most disease susceptible (or least resistant) group (RG3). When considering disease resistance, we observed a strong negative association between the OsHV-1 µvar disease resistance of oyster hosts and the occurrence of OTUs assigned to the *Vibrio* (OTUs 651 and 653), *Photobacterium* (OTUs 1063, 654 and 1053), *Aliivibrio* (OTU 1248), *Streptococcus* (OTU 814) and *Roseovarius* (OTU 7180) genera, while on the other hand, the microbiomes of the most resistant families had an over-representation of OTUs assigned to the *Cupriavidus* (OTU 2182), *Psychrilyobacter* (OTU 5046) and *Tenacibaculum* (OTU 2153) genera.

The association between the occurrence of *Vibrio* and disease susceptibility was further supported by a significant elevation of an uncharacterised member of the *Vibrio* in RG3, and the results of a *Vibrio*-specific qPCR assay. These results are consistent with growing evidence implicating a role of the *Vibrio* community in oyster disease (Sugumar et al., 1998; Waechter et al., 2002; Garnier et al., 2007; Saulnier et al., 2010; Lemire et al., 2015; Petton et al., 2015; Green et al., 2018; King et al., 2018).

Specifically, there is previous evidence that prior to oyster disease onset, the native *Vibrio* community is replaced by pathogenic *Vibrio* species (Lemire et al., 2015). Further, in corals, small shifts in the *Vibrio* community are sufficient to shift the microbiome metabolism (Thurber et al., 2009). Our data provides a new perspective on this interaction, whereby the total load of *Vibrios* differed between disease susceptible and resistant oyster families. This is supported by a recent study, which demonstrated that the *Vibrio* load following OsHV-1 µvar infection was significantly higher in disease-susceptible oysters (de Lorgeril et al., 2018). An increased *Vibrio* community size may provide further potential for pathogenic species to replace benign colonisers. On the other hand, a higher background load of *Vibrio* may become important under periods of stress, such as with OsHV-1 µvar infection, resulting in duel infection, as has recently been described (de Lorgeril et al., 2018). This is also indirectly supported by a previous study which observed reduced mortality in OsHV-1 infected oysters that were treated with antibiotics (Petton et al., 2015).

Increases in the abundance of OTUs assigned to the *Photobacterium* genus, as were observed here, often co-occur with an increase in the *Vibrio* community in oyster microbiomes (Wegner et al., 2013; Lokmer and Wegner, 2015). While members assigned to this genus have been identified as pathogens of other aquatic organisms (Pedersen et al., 2009; Liu et al., 2016), to our knowledge, no species of *Photobacterium* has been identified as an oyster pathogen. Members of the *Streptococcus* and *Aliivibrio* genera are known pathogens of fish and crabs (Pappalardo and Boemare, 1982; Egidius et al., 1986; Creeper and Buller, 2006; Urbanczyk et al., 2007), while a member of the *Roseovarius* genus is the causative agent of roseovarius oyster disease (formally juvenile oyster disease) in *Crassostrea virginica* (Boettcher et al., 2005; Maloy et al., 2007), yet to our knowledge these genera have not been implicated in disease of *C. gigas* previously, despite being over-represented in the most disease susceptible oyster families.

On the other hand, a strong positive association was observed between levels of disease resistance and the occurrence of OTUs assigned to the *Cupriavidus* (OTU 2182), *Psychrilyobacter* (OTU 5046) and *Tenacibaculum* (OTU 2153). Currently, little is known about the role of these genera in oysters. *Cupriavidus* species are commonly

isolated from plants and soil (Cuadrado et al., 2010; Estrada-De Los Santos et al., 2014), but members of the *Psychrilyobacter* and *Tenacibaculum* have previously been observed in *C. gigas* microbiomes (Lee et al., 2009; Fernandez-Piquer et al., 2012; Wegner et al., 2013). *Psychrilyobacter* was observed in *C. gigas* microbiomes from Tasmania, Australia (Fernandez-Piquer et al., 2012), which is perhaps notable given that the oysters used in this study were initially sourced from Tasmania. In addition, we have previously identified an over-representation of a *Tenacibaculum* OTU in oyster microbiomes that were unaffected by a summer mortality outbreak (King et al., 2018).

As already stated, a significant elevation of OTUs belonging to the *Vibrio* and *Photobacterium* genera abundance in disease susceptible oysters has also been previously observed (de Lorgeril et al., 2018), supporting our findings. However, while we identified members of the *Psychrilyobacter* and *Tenacibaculum* genera to be associated with disease resistance, the same study (de Lorgeril et al., 2018) observed an increase in these same genera in an experimental infection experiment using disease susceptible oysters. Differences in bacterial taxa abundance and taxonomic assignment could be attributed to contrasting sequencing techniques and data analysis. For example, we used the V1-V3 hypervariable region, and clustered OTUs at the 97% identity level, compared to V3-V4 and having OTUs clustered at a 3-nucleotide difference threshold (de Lorgeril et al., 2018). Furthermore, this study deployed oysters to the field, while the aforementioned study carried out their experiments in tanks.

The oyster microbiome is dynamic in nature, changing in response to stressors such as disease, antibiotics, translocation, and heat (Wegner et al., 2013; Lokmer and Wegner, 2015; Lokmer et al., 2016b; de Lorgeril et al., 2018; Green et al., 2018; King et al., 2018). The microbiome assemblage can also be influenced by the oyster life stage, the genetics of the host oyster, and spatial location (Trabal et al., 2012; Wegner et al., 2013; Lokmer et al., 2016a; King et al., 2018). Because we only have one sampling point, our study would not capture the dynamic nature of the oyster microbiome, and thus the oyster microbiome could change before the onset of disease. To fully capture the importance of the taxa identified in this study, a temporal study in the field encompassing a disease outbreak would be needed. However, as disease outbreaks are often very sudden, capturing a disease outbreak in the environment can be difficult.

In addition to identifying OTUs that are over- or under-represented within the microbiomes of oysters with different levels of disease-resistance, another way to identify putatively important bacteria within the microbiome of a host organism involves the identification of "core" microbiome members (Ainsworth et al., 2015). Identifying which bacterial members are consistent and stable across microbial communities is important in unravelling the functional contribution of these core bacteria (Ainsworth et al., 2015). Notably, we could not define a universal core microbiome across all of the studied oyster families at the OTU level, suggesting significant heterogeneity in oyster microbiome structure, or possible differences in micro-geographic variation. However, we identified core microbiome members within each family microbiome, whereby a number of 'unique' core members often occurred exclusively in the core microbiome of a family. This is in accordance with previous observations that the composition of an oyster's microbiome is partially governed by oyster genetics, particularly for shaping the rare specialist bacterial community (<1% abundance) (Wegner et al., 2013), although we have no information pertaining to the genetic differentiation between the studied oyster families. However, when examining the core microbiome across all of the families comprising the most highly disease-resistant group (RG1), we identified two core members, which included OTUs classified as members of the *Winogradskyella* genus (OTU 1511) and *Bradyrhizobiaceae* family (OTU 6417). OTUs assigned to the *Bradyrhizobiaceae* family have previously been observed in oysters (Sakowski, 2015), however, due to the coarse taxonomic assignment of this OTU, it is unclear what potential role this member of the *Bradyrhizobiaceae* family might have. *Winogradskyella* species are commonly found in numerous marine organisms, including oysters (Valdenegro-Vega et al., 2013; Park et al., 2015; Lee et al., 2017; Schellenberg et al., 2017; Franco et al., 2018), and are known for their role in amoebic-induced fish gill diseases (Embar-Gopinath et al., 2005; Embar-Gopinath et al., 2006). However, it is uncertain what function(s) *Winogradskyella* species play in oysters. We currently know little about the potential role, if any, of these core microbiome members in resistance, but these observations provide candidate target organisms for focussed examinations of potential beneficial microbes within OsHV-1 μvar disease-resistance.

In conclusion, we found that the microbiome of *C. gigas* displays significantly different microbial assemblage structure according to oyster disease-resistance. This study provides insights into the *C. gigas* microbiome within the context of oysters bred for disease-resistance and highlights the potential involvement of the oyster microbiome in disease-resistance. Members of the *Vibrio*, *Photobacterium*, *Aliivibrio*, *Streptococcus* and *Roseovarius* genera were over-represented features of the microbiome of oysters with high OsHV-1 μvar disease susceptibility, which is consistent with previous studies implicating *Vibrio* in oyster disease dynamics. Furthermore, a significant elevation of *Vibrio* 16S rRNA gene copies in disease-susceptible oyster families could indicate a lack of immune response against *Vibrio* pathogens. However, further research is required to elucidate the role of these bacteria in oyster disease dynamics. Examination of 'core' bacteria identified species assigned to the *Winogradskyella* genus and *Bradyrhizobiaceae* family as core members of microbiomes assigned to RG1 and may also play a role in OsHV-1 μvar disease resistance. These results deliver evidence that the *C. gigas* microbiome differs between oysters with different levels of susceptibility to OsHV-1 μvar disease and identifies putative microbial determinants in disease onset and resistance.

<u>Aim 2:</u> Defining the composition of the Sydney Rock Oyster microbiome across diverse oyster families, including breeding lines generated for resistance to QX disease, and examining spatial and temporal heterogeneity in microbiome structure

In this study, we observed that the microbiome of genetically identical SROs is significantly affected by location, which is consistent with previous studies (King, Judd, Kuske, & Smith, 2012; Ossai et al., 2017; Roterman, Benayahu, Reshef, & Gophna, 2015; Trabal et al., 2012; Zurel, Benayahu, Or, Kovacs, & Gophna, 2011). Local environmental parameters likely underpin microbiome differences between our deployment sites, which are approximately 70 km apart. There is substantial variation in the inherent environmental properties of the two sampling locations, with Port Stephens containing a muddy bottom sediment, while Wallis Lake has a sandy bottom sediment. Our measurements also demonstrated, that at the times of sampling, chlorophyll-a concentrations were significantly higher in Port Stephens than Wallis Lake. Finally, oyster farming density at these two locations differs substantially, with a higher density of oysters grown in Wallis Lake.

Our characterisation of the SRO microbiome revelaed statistically significant differences in microbiome composition between Port Stephens and Wallis Lake, with the relative abundance of members of the *Vibrio* genus (OTU 2) significantly higher in Port Stephens. *Vibrio* are common members of oyster microbiomes (Cook et al., 2002; Ortigosa, Garay, & Pujalte, 1994; Paillard, Le Roux, & Borrego, 2004; Pruzzo, Gallo, & Canesi, 2005; Wendling et al., 2014), with some species identified as oyster pathogens (de Lorgeril et al., 2018; Paillard et al., 2004; Wendling et al., 2014). Several environmental factors promote *Vibrio* growth in the environment, in particular water temperature (Arias, Macián, Aznar, Garay, & Pujalte, 2003; Pujalte et al., 1999) and chlorophyll a levels (Wendling et al., 2014). Our measurements indicate that during the sampling periods, the average water temperatures at Port Stephens were warmer than Wallis Lake during the winter period, when this site also hosted substantially higher chlorophyll a concentrations. Notably, recent Summer Mortality outbreaks among Pacific Oysters in Port Stephens were circumstantially linked to elevated levels of Vibrio in oysters inhabiting high mortality regions (King et al., 2018).

The relative abundance of members of the *Pseudoalteromonas* genus (OTU 8917) were also substantially higher in Port Stephens than in Wallis Lake. *Pseudoalteromonas* are regularly isolated from benthic marine animals, including mussels, sponges (Holmström & Kjelleberg, 1999) and Pacific Oysters (Defer et al., 2013; Desriac et al., 2013; Garnier, Labreuche, Garcia, Robert, & Nicolas, 2007; Zarkasi & Nazari, 2018). *Pseudoalteromonas* species have been demonstrated to produce antibacterial compounds, which are active against a variety of target organisms (Defer et al., 2013; Desriac et al., 2013; Holmström & Kjelleberg, 1999; Richards et al., 2017). Members of the *Mycoplasma* genus (OTU 14900 and OTU 12669) also were considerably higher in the microbiomes of SROs within Port Stephens than those in Wallis Lake (Welch's t -test; p = 6.21E – 4 and 0.019 respectively). *Mycoplasma* have been elsewhere demonstrated to be abundant members of the microbiome of Eastern Oysters (King et al., 2012), Pacific Oysters (Wegner et al., 2013) and Sydney Rock Oysters (Green & Barnes, 2010). Notably, members of this group have been identified as shellfish pathogens, in species including *Patinopecten yessoensis* and *Cerastoderma edule* (Paillard et al., 2004). Oyster-associated *Mycoplasma* have previously been shown to have a preference for higher water temperatures (Wegner et al., 2013), which is

consistent with the warmer water temperatures recorded at Port Stephens during the time of sampling.

At Wallis Lake, the relative abundance of members of the *Endozoicomnas* genus (OTU 1831) was significantly higher in the SROs than in Port Stephens. *Endozoicomonas* are known as symbiotic bacteria of numerous marine hosts (Neave et al. 2016) with members of this genus dominating the bacterial community of corals (Roterman et al., 2015; Zurel et al., 2011). However, the functional role of these bacteria in oysters (if any) is poorly understood (Neave et al., 2016; Roterman et al., 2015; Zurel et al., 2011).

The SRO microbiome is affected by season

In addition to the differences between estuaries, we observed significant differences in the composition of the SRO microbiome between January (summer) and June (winter) at both sampling locations, potentially due to seasonal changes in environmental conditions (e.g. temperature and chlorophyll-a). It is noteworthy that during the winter sampling in Port Stephens, a substantial rainfall event, resulted in reduced salinity and increased NH_3.

At both locations, members of the *Vibrio* genus (OTU 2) occurred in higher relative abundances in winter than in summer. This patterns is interesting given that *Vibrio* typically exhibit preferences for warm water temperatures. However, some *Vibrio* species such as *V. splendidus,* have elsewhere been found to be most abundant during winter and spring (Arias et al., 2003; Pujalte et al., 1999). However, it is probable that other environmental factors, such as Chlorophyll-a or nutrient levels, underpinned the higher winter abundances of members of the *Vibrio* genus (OTU 2).

Interestingly, we observed inverse patterns in the relative abundance of an OTU belonging to the *Candidatus Hepatoplasma* genus (OTU 14887) between the two sampling locations. At Port Stephens, this OTU was significantly more abundant during summer than winter, while in Wallis Lake, it was considerably less abundant in summer than in winter. The *Candidatus Hepatoplasma* genus are considered a sister group to *Mycoplasma* (Leclercq, Dittmer, Bouchon, & Cordaux, 2014), which as noted above are commonly found in oysters (King et al., 2012; Wegner et al., 2013). However, the role of *Hepatoplasma* in the SRO microbiome is unknown.

The SRO microbiome is affected by QX-disease resistance

In addition to the spatial and temporal shifts in microbiome structure described above, the SRO microbiome also varied significantly according to the level of QX resistance among. At both sampling times at Port Stephens, the SRO microbiome differed significantly between the QX resistant and susceptible oysters, while in Wallis Lake, this pattern was only observed during summer. This indicates a potential synergistic interaction between oyster genetics and environmental factors in determining the structure of the SRO microbiome, which is consistent with patterns observed in Pacific Oysters (King et al., 2019; Wegner et al., 2013).

During both sampling times in Port Stephens, there was a higher relative abundance of *Endozoicomonas*, *Candidatus Hepatoplasma*, *Mycoplasma* and *Vibrio* genus in the microbiomes of QX susceptible SROs relative to SROs with intermediate resistance and high QX resistance. This finding is consistent with a previous study, which showed that *Vibrio* bacteria in Pacific Oysters were associated with low ostreid herpesvirus-1 microvariant (OsHV-1 µvar) disease resistance (King et al., 2019). Several *Vibrio* and *Mycoplasma* have been identified as (opportunistic) pathogens in shellfish (de Lorgeril et al., 2018; Paillard et al., 2004; Wendling et al., 2014), so the elevated abundance of bacteria from these groups in the microbiome of QX susceptible oysters is notable. During January at Wallis Lake, only a single OUT, identified as a member of the *Endozoicomonas* genus, occurred in higher relative abundance in QX susceptible oysters, while in June several OTUs, including members of the *Thalassolituus*, *Endozoicomonas*, *Aliivibrio* and *Flavobacteriaceae* occurred in significantly higher abundances in the intermediate resistance group.

In summary this study has demonstrated that the SRO microbiome is highly dynamic in space and time and among oysters with differing levels of resistance to QX disease. Our data indicate a synergistic interaction of oyster genetics and environmental drivers in shaping the SRO microbiome, with the highly dynamic nature of the microbiome perhaps suggestive of an intimate oyster-microbiome ecological relationship. Our analysis revealed key groups of bacteria responsible for defining patterns in the SRO microbiome, with some groups of known oyster associates, including *Vibrio*,

Endozoicomonas and *Mycoplasma* consistently emerging as key determinants of microbiome structure.

<u>Aim 3:</u> Examining spatial heterogeneity in Pacific Oyster microbiome structure at the individual oyster level and across regional-scales

The principal goal of this component of the study was to elucidate patterns in the *C. gigas* microbiome across different environments and oyster tissue-types, with the objectives of identifying taxa innately tied to oyster tissue-type, understanding how the *C. gigas* microbiome varies spatially, and to reveal the existence of a core oyster microbiome. Oyster microbiomes were found to significantly differ according to both environment and tissue-type, with tissue-type within a location driving the greatest heterogeneity in microbiome composition. Geographic location has previously been found to influence the haemolymph, mantle, gill (Lokmer et al., 2016b; Lokmer et al., 2016a), and disease-affected adductor muscle microbiomes (King et al., 2019a). Consistent with these studies, we observed a significant effect of location on the oyster microbiome. However, microbiome similarities between the mantle, gill, and digestive gland microbiomes from Shoalhaven and Wapengo locations (wave-dominated estuaries), and between the adductor muscle microbiomes at the remainder of the sampling locations (tide-dominated estuaries) over large geographic distances, suggests that geographic location alone is not responsible for driving differences in microbiome heterogeneity. These data suggest that estuary-type can also influence the microbiome composition, and should be considered when examining patterns in microbiome heterogeneity between individuals. The oyster microbiome assemblage was also influenced by the oyster tissue, with each tissue harbouring a unique microbial consortia, as previously observed (Lokmer et al., 2016a). This pattern was observed for all pairwise comparisons within all locations, suggesting that tissue-type is a stronger driver of microbiome composition than geographic location.

Estuary properties and their potential influence on the oyster microbiome

Similarities between the microbiomes from the Wapengo and Shoalhaven sites were surprising, given the distance between sampling sites (approximately 200 km). These two sites shared a core microbiome not observed in any other sampling locations, and displayed no significant microbiome differences between the mantle, gill, and digestive gland microbiomes. Members of the *Vulcaniibacterium*, *Limnobacter* and

Pseudoxanthomonas genera represented the predominate taxa driving the differences between the Wapengo and Shoalhaven sites and the four other sampling locations.

The Shoalhaven site has a catchment size of 7,500 km^2 (Roy et al., 2001), with approximately 35 % of the catchment used for agricultural purposes (OceanWatch-Australia, 2017). In contrast, Wapengo has a significantly smaller catchment of 73 km^2 (Roy et al., 2001), and a similar level of agricultural usage at 20 % (OceanWatch-Australia, 2010). Both locations have a high proportion of forest/undisturbed area, with approximately 50 % of the catchment at the Shoalhaven site and 70 % at the Wapengo site (OceanWatch-Australia, 2010, 2017). Further, both sampling locations are shallow wave-dominated estuaries (Roy et al., 2001). As both estuaries are shallow, it is possible that wave action resuspends particulate matter from the sediment, or water-side soil, into the water column, which is then consumed by the oysters. This could explain the higher abundance of soil associated microbes (i.e. *Vulcaniibacterium* and *Pseudoxanthomonas* bacteria) (Yoo et al., 2007; Young et al., 2007; Wei et al., 2012; Yu et al., 2013) in the Pacific Oyster microbiome at these sites, compared to the other tide-dominated locations, and may explain the similarities in microbiome composition between the Shoalhaven and Wapengo locations. Future studies should aim to characterise the involvement of wave-action and resuspended sediment particulate matter on the oyster microbiome, and whether carry-over from taxa in the soil have implications for oyster health.

Of the sampled locations, the Clyde River represents the most 'pristine' environment (Rubio et al., 2008). The Clyde River catchment spans an area of 1,791 km^2 (Roy et al., 2001), of which, 95 % consists of forest/undisturbed area and 4 % is agricultural/rural usage (Cavanagh et al., 2004). Previous studies comparing the Shoalhaven and Clyde River identified that oysters grown in the Shoalhaven grew approximately 27 % faster than their counterparts in the Clyde River (Rubio et al., 2008). Accordingly, the oysters sampled in this study from the Clyde River were among the smallest of all sites. Increased growth rates in the Shoalhaven were attributed to increased nutrient loads and on average, higher water temperature (Rubio et al., 2008). Microbiomes from the Clyde River were dominated by uncultured *Spirochaetaceae* bacteria, and the adductor muscle and digestive gland microbiomes at this site were markedly over-represented by *Vibrio* bacteria when compared to all other locations. *Vibrio* bacteria are important

contributors to *C. gigas* diseases (Lemire et al., 2015; Bruto et al., 2017; de Lorgeril et al., 2018), and we have previously identified an oyster genetic element to this interplay, with higher *Vibrio* bacterial loads in disease-susceptible oysters (King et al., 2019b). Given the reduced growth rate and lower nutrient loads in the Clyde River, these could act as a stressor on the oyster allowing *Vibrio* bacteria to colonise and proliferate.

Oyster tissue microbiome heterogeneity

Given the conservation of microbiomes associated with specific tissues across geographically discrete locations, it is likely that the type of oyster tissue is a stronger driver of microbiome composition than geographic location. Several ZOTUs were most responsible for driving the differences between tissue-types and may be important in tissue-specific processes. Of these, ZOTUs classified as members of the *Mycoplasma* and *Vulcaniibacterium* genera were over-represented in the digestive gland. Mycoplasma are commonly identified in the oyster digestive system (Green and Barnes, 2010; King et al., 2012), but the *Vulcaniibacterium* genus is a newly described group, and only includes two species (Yu et al., 2013). Members of the *Spirochaetaceae* family and the *Margulisbacteria* phylum were over-represented in the gill. While we observed a strong connection between spirochaete bacteria and the gill microbiome, there are conflicting reports with previous studies often observing these bacteria in the oyster digestive gland (Green and Barnes, 2010), oyster homogenates (Fernandez-Piquer et al., 2012), or the adductor muscle (King et al., 2019a; King et al., 2019b). This is likely due to the high taxonomic classification of the *Spirochaetaceae* family, as it could represent a diverse range of different oyster-associated microbes. Furthermore, little is known about the *Margulisbacteria* phylum, however, a previous study observed attachment of a *Margulisbacteria* bacteria to an ectosymbiotic spirochaete bacteria in termite guts (Utami et al., 2019), which may explain their co-dominance with bacteria assigned to the *Spirochaetaceae* family in the oyster gill microbiome. Bacteria assigned to the *Polynucleobacter* genus and an uncultured *Spirochaetaceae* were over-represented in the mantle. *Polynucleobacter* species have previously been observed in oyster homogenate microbiomes (Fernandez-Piquer et al., 2012), this genus contains both obligate endosymbionts of ciliates (Heckmann and Schmidt, 1987; Vannini et al., 2005) and planktonic bacteria (Hahn et al., 2010). Finally, members of the *Acidovorax* genus were over-represented in the adductor muscle and digestive gland microbiomes. Members of the *Acidovorax* have been

isolated from a diverse range of environments including soil (Chaudhary and Kim, 2018), water (Pal et al., 2018), and from cyanobacterial blooms (Chun et al., 2017).

Conservation of Spirochaete ZOTUs across sampling environments and tissue types

Despite the significant heterogeneity in the oyster microbiome across environments and tissue types, we surprisingly identified core taxa associated with *all* locations and tissue types. Several ZOTUs, classified as *Spirochaetaceae* bacteria were consistent members of the *C. gigas* core microbiome across all sites and tissues. These uncultured spirochaete bacteria have previously been identified in *C. gigas* in Tasmania, Australia (Fernandez-Piquer et al., 2012), as well as in *C. gigas* in Germany and the Netherlands (Lokmer et al., 2016b), and in *Saccostrea glomerata* in Queensland, Australia (Green and Barnes, 2010), indicating a very wide geographical distribution of these core oyster associates. Furthermore, we previously identified these bacteria as members of the core microbiome in Port Stephens oyster microbiomes (OTUs 32677 and 24319 in (King et al., 2019a), although these organisms were assigned as members of the *Brachyspiraceae* family. This discrepancy is likely attributed to previously using the Greengenes database for taxonomy assignment, as opposed to the SILVA database in this study. We also previously found members of this group to be linked to disease resistance (OTU 4737 in (King et al., 2019b). Besides the presence of *Spirochaetaceae* in numerous different oyster microbiome datasets across different countries and locations within Australia, little is known about these bacteria. Future studies should attempt to further phylogenetically characterise these bacteria and identify their potential functional roles within *C. gigas*.

Emerging evidence suggests that the oyster microbiome is dynamic, shaped by a range of broad- and individual-scale processes, however, elements such as wave-action have yet to be considered as influencing the microbiome. Our analysis revealed that the structure of the *C. gigas* microbial assemblage is governed by both geographic location and tissue type, with microbiomes derived from wave-dominated estuaries exhibiting similar microbiome assemblages despite large geographic separation, with a predominance of soil/particulate-associated bacteria within these microbiomes. Given the dynamic nature of oyster microbiomes, our understanding of whether the oyster microbiome has conserved elements across regions or microenvironments, is lacking. We revealed a core microbiome within individual tissue-types, and a universal core

microbiome consisting of uncultured *Spirochaetaceae* bacteria, as conserved across all sampling locations and tissue types, this finding was strengthened by the presence of this microbe in other previously published oyster microbiome datasets. Due to the dynamic nature of the microbiome, and the strong effect of location and tissue-type on the oyster microbiome, it is important to avoid extrapolation of interpretations of the disease-affected microbiome to oyster microbiomes characterised in different locations or tissues. Instead, studies should aim to characterise the healthy microbiomes of oysters for those locations where oysters are grown, in an effort to build a healthy microbiome profile to use as a reference during disease.

<u>Aim 4:</u> Defining the Sydney Rock Oyster microbiome associated with QX disease events

QX Disease has had substantial impacts on the cultivation of Sydney Rock Oysters in a number of estuaries along the east coast of Australia. While the parasite responsible for QX disease, *M. sydneyi*, has been known for many years, with its mode of infection well documented and characterised, a number of questions around the environmental determinants of infection and the influence of oyster physiology on infection dynamics remain unanswered. Here, following on from the outcomes of Aim 2, where we demonstrated that SRO with different levels of resistance to QX have different microbiome composition, we examined the potential role of the SRO microbiome during QX infection events and compared the microbiome of QX infected and uninfected oysters.

A significant QX event occurred during the course of this study (February 2018), providing us with an excellent opportunity to compare both the microbiomes of (i) oyster communities before and during the QX disease event and (ii) QX infected and uninfected oysters (as determined by PCR and cytology). Surprisingly consistent patterns emerged from these two approaches, with potentially key members of the oyster microbiome within QX disease dynamics identified using a suite of different analytical approaches.

Both pre- QX event SRO communities and uninfected oysters were discriminated from other oysters by an over-representation of a single OTU from the *Mycoplasma* genus.

Notably, bacteria from this genus were also shown to be a key component of the SRO microbiome during Aim 2 and in previous research (Green & Barnes, 2010), implying a potentially important ecological link between this group and SROs. The significant decrease in relative importance of these bacteria both in oyster communities during periods of QX disease and within confirmed QX disease individuals suggests that QX disease has a fundamental effect on the microbiome of SROs.

In addition to a significant drop in the relative importance of members of the *Mycoplasma,* the microbiome of oysters during periods of QX impact was characterised by a relative increase in the occurrence of bacteria from the *Hepatoplasma* and *Borrelia* genera, while shifts in the microbiome of QX infected SROs were principally driven by a relative increase in a *Borrelia* OTU. *Borrelia* belong to the Spirochaete phylum, which notably includes organisms implicated as causative agents in Pearl Oyster disease (Matsuyama et al. 2017). However, whether the observed increase in Borrelia in QX positive oysters is a consequence of an opportunistic secondary infection by this organism or a general shift in the microbiome associated with a change in oyster physiology or metabolism requires further investigation.

Using clone library approaches, Green and Barnes (2010) observed similar shifts in the microbiome of QX infected SROs to those observed here, including a decrease in the occurrence of *Mycoplasma* in QX infected oysters. They suggested that changes in the microbiome of QX positive oysters may be linked to the cessation of feeding known to occur within QX disease. The significantly different microbiome structure of QX infected oysters observed in our study indeed implies a substantial shift in the nature of interaction between SROs and their associated microbiota, suggestive of a fundamental change in over-all oyster physiology and health. To what extent, if any, this shift precedes or underpins the ultimate demise of QX infected oysters represents an interesting angle for future research, particularly when considered through the lens of potential probiotic therapies or early warning strategies.

<u>Aim 5:</u> Measuring temporal patterns in the Pacific Oyster microbiome during the summer OsHV-1 mortality period

Major oyster mortality events have become a recurrent feature of oyster cultivation. In particular, disease outbreaks have heavily impacted Pacific Oyster stocks in Australia and globally. Pacific Oyster mortalities frequently occur during the summer months, with "summer mortality" often used as an umbrella term to encompass mortalities resulting from viral and/or bacterial infections often precipitated by environmental stressors (Friedman et al. 2005; Garnier et al. 2007; Malham et al. 2009). In recent years, mass mortalities of Pacific Oysters have been attributed to infection by the ostreid herpesvirus (OsHV-1) or its micro-variant (OsHV-1 µvar), which affects oyster larvae, spat or juveniles (Friedman et al. 2005; Segarra et al. 2010; Mortensen et al. 2016). In other instances of *C. gigas* summer mortality, there is emerging evidence that bacteria may also play a role with several members of the *Vibrio* genus implicated as potential disease-causing agents (Jeffries 1982; Waechter et al. 2002; Garnier et al. 2007). There is also strong evidence that Pacific Oyster mortality events are not the product of a simple interaction between the oyster and a single pathogen, but are the product of a complex interplay between multiple biotic and environmental factors (King et al. 2019). However, given the regularly sudden and difficult to forecast nature of oyster mortality events, it has often proven difficult to isolate the relative contribution of these interacting factors. Here we attempted to overcome this difficulty by performing a long-term, high temporal resolution study of oyster mortality and its links to the oyster microbiome over the course of a summer in NSW.

To allow ready access to oysters and to facilitate sampling and measurement of environmental parameters, we performed this study in experimental enclosures at the Sydney Institute of Marine Sciences. During the 24 week period of this study, significant levels of Pacific Oyster mortality occurred during the latter stages of the study in late summer/early Autumn 2018. Oyster mortality was significantly correlated with decreased salinity and elevated phosphate levels, with shifts in these parameters at times driven by rainfall events. Drops in water salinity and elevated inorganic nutrient levels have elsewhere been implicated in oyster mortality events (reviewed in King et al. 2019) and may have played a role here, but the shifts in salinity and phosphate observed during the study period were relatively modest and not outside of the range likely to be experienced by oysters in the environment. Another potential

explanation for the increasing levels of observed oyster mortality is an interactive influence of this environmental variability and microbial pathogens.

The Ostreid herpesvirus (OsHV-1) has been implicated as the principal pathogen responsible for mass mortalities of Pacific Oysters during the last decade. Several substantial Pacific Oyster mortality events in NSW and Tasmania, since 2013 and 2016 respectively, are believed to have been caused by OsHV-1 infections. Quantification of the OsHV-1 virus in oyster samples derived from this study are currently pending, but given the nature of the mortality event, which was characterised by a gradual increase in oyster mortality over the summer, rather than the sudden and intense mortality typical of an OsHV-1 outbreak, along with the low levels of OsHV-1 occurrence in NSW Pacific Oysters during the summer of 2017/2018, it is perhaps likely that the OsHV-1 virus played a limited role in this mortality event. Previously, we have demonstrated the potential role of the bacterial-component of the Pacific Oyster microbiome during a summer mortality event (King et al. 2018), but that work only involved comparisons of oysters across impacted and un-impacted regions of Pt Stephens estuary from a single time-point. The current study has allowed us to examine the extent to which the oyster microbiome changes over time and in concert with shifting oyster mortality levels.

A clear shift in the oyster microbiome occurred during the course of the 24 week study, with statistically discrete microbiome signatures occurring during the three phases of oyster mortality observed. Our microbiome approach allowed us to identify specific bacterial taxa that shifted in abundance immediately prior to and during the periods of significant oyster mortality, with some key "suspects" emerging. Specifically, we observed a subtle, yet potentially important change in the relative abundance of *Spirochaete* zOTUs. This often involved a switch in the abundance of very closely related bacteria within this group, but is notable given that our preceding research (e.g. Aim 3 above) consistently highlighted members of the *Spirochaetaceae* as constantly present members of the Pacific Oyster microbiome, and a potential signature of a 'healthy' oyster microbiome. Shifts in the relative occurrence of very closely related bacterial taxa within the oyster microbiome have elsewhere been shown to have significant health effects for the host (Lemire et al. 2015), so these subtle changes in the Pacific Oyster microbiome may have relevance within the context of the mortality

observed here. These results support the findings of Aim 3 (above) in suggesting that members of the *Spirochaetaceae* represent potentially important targets for more focussed interrogations of the Pacific Oyster microbiome in the future.

Another prominent feature of the shifts in Pacific Oyster microbiome observed during this temporal study involved increases in the relative abundance of *Vibrio* sequences during the latter stages of the study, when oyster mortality levels became elevated. This pattern was subsequently confirmed by quantitative PCR targeting *Vibrio* abundance. Similarly to the correspondence between the patterns in *Spirochaetaceae* observed here and in Aim 3 (above), this increase in the prominence of *Vibrio* is in-line with earlier observations in this project (Aims 1 and 3) and elsewhere in the literature, implicating *Vibrio* as key agents in Pacific Oyster health and mortality. Our results reveal a substantial increase in *Vibrio* occurrence during periods when oyster mortality was highest. This could be explained by one of two dynamics: (i) *Vibrio* are the causative agents of the oyster mortality, or (ii) *Vibrio* increased in abundance in compromised or dying oysters, or potentially a combination of both i and ii. To isolate which of these scenarios occurred here, further manipulation experiments using isolates of the *Vibrio* strains potentially involved are required. However, before taking that step a more precise identification of the specific *Vibrio* species involved is required, which cannot be provided by standard 16S rRNA-based microbiome approaches. As a consequence, in Aim 6 we have developed a new *Vibrio*-specific sequencing assay that will allow for this type of information to be acquired more precisely.

In summary, we identified a clear relationship between increasing levels of Pacific Oyster mortality observed over the course of 24 week summer period and a marked shift in the oyster microbiome. Three discrete temporal phases, categorised according to differing levels of oyster mortality, were characterised by distinct microbiome structure. The main determinants of shifting microbiome composition involved changes in the relative abundance of closely related *Spirochaetaceae* zOTUs and an increase in the prevalence of *Vibrio* during the high oyster mortality period. These shifts are notable given the prominent status of these bacteria identified in the research conducted as part of Aims 1 and 3 (above). Although it is currently difficult to discriminate a causative role from these correlative patterns, these results further highlight the

potential importance of these bacterial groups within oyster health and disease dynamics.

Conclusions

The principal goal of this research was to elucidate the role of the oyster microbiome in the health and disease susceptibility or resistance of two of Australia's major commercially harvested oyster species, the Pacific Oyster (*C. gigas*) and Sydney Rock Oyster (*S. glomerate)*. The two major rationale underpinning this work were that: (i) oyster cultivation has recently been heavily impacted by disease outbreaks and other unexplained mortality events, with the causative agent behind the oyster mortality often undefined, and (ii) substantial emerging evidence from a wide range of other organisms indicates that a host organism's microbiome can play a significant role in governing host health and disease susceptibility. Using high throughput amplicon sequencing approaches, we considered how the composition of the oyster microbiome varied over space, time, between different oyster genetic lines and among oysters exhibiting different levels of disease tolerance and exposure. Across several discrete studies, we found that both the Pacific Oyster and SRO microbiome is highly variable with time and location, with marked shifts in the oyster microbiome apparent between different estuaries, different oyster tissues and seasonally. This high level of heterogeneity in the base-line oyster microbiome was often more pronounced than has been described in other benthic marine organisms (e.g. corals, seaweeds) and may be a reflection of the large quantities of seawater that are filtered through each oyster per day. These patterns indicate that care must be taken to avoid any presumption of a 'universal oyster microbiome' when incorporating microbiological measurements into studies of oyster ecology or disease dynamics.

While the over-all composition of the oyster microbiome was highly variable, in both Pacific Oysters and SROs we found evidence for the existence of specific 'core microbiome members' that were conserved across oyster specimens collected from geographically disparate sites and over seasonal time-scales. While sometimes only making up a small proportion of the entire microbiome, these core microbiome members, defined here as organisms found in all specimens of a given species or tissue

type, may represent important microbial markers or indicators for oyster health or disease. For instance, within Pacific Oysters, we found that bacteria within the *Spirochaetaceae* were an omnipresent feature of the oyster microbiome, even among oysters collected from estuaries separated by hundreds of kilometres. While little is currently known about the potential (positive or negative) influence that these bacteria have on oyster health, their persistent occurrence points to a potentially important ecological relationship with the Pacific Oyster that we argue warrants further investigation in the future.

While members of the *Spirochaetaceae* were persistently associated with 'healthy' oysters, we also found certain bacterial taxa that exhibited consistent relationships with either disease susceptible or impacted oysters. The most notable of these were members of the *Vibrio* genus. This observation is consistent with patterns emerging from other regions and studies, that indicate that members of this group may either be primary oyster pathogens, participants in polymicrobial infections or opportunistic colonisers of compromised oyster hosts. Notably, we observed increases in the relative abundance of *Vibrios* in both oyster populations subject to disease or mortality events and within oyster family-lines with elevated susceptibility to diseases, such as OsHV-1. While further studies are required to determine how *Vibrios* contribute to oyster mortality or disease susceptibility, our results shine a further light on the potential importance of this group of bacteria in undermining oyster cultivation efforts. To provide better capacity to study and/or monitor for this group of bacteria, we developed a new method for more precisely characterising *Vibrio* diversity in oyster tissues (Aim 6), which we believe will provide a valuable tool for future work in this area.

Unfortunately (or fortunately from the oyster industry perspective), during the period of this study there was not a significant OsHV-1 mortality event at the sites that we conducted sampling. This made it difficult to make clear and direct links between OsHV-1 induced mortality and the Pacific Oyster microbiome. However, evidence from this research indicated clear differences in the microbiome of Pacific Oyster family lines with differing levels of susceptibility to OsHV-1, as well as specific microbiome features (e.g. higher relative loads of *Vibrio*) in oysters experiencing other causes of mortality.

Cumulatively, the work presented in this project delivers an important foundation for the future incorporation of microbiome measurements into the study of oyster ecology and disease. While our research did not, in either the case of Pacific Oysters of SROs, identify a clear 'smoking gun' for *causes* of oyster disease or mortality, we identified several aspects of the oyster microbiome that both warrant deeper investigation and are indicative of potential microbial markers for oyster health status. An important conclusion derived from our findings is that future research examining the role of the oyster microbiome should avoid using over-all microbiome signatures as diagnostic markers (due to the inherent heterogeneity in oyster microbiome structure) and instead focus on specific microbial indicators, such as those that were shown here to be affiliated with healthy (e.g. *Spirochaetaceae*) and compromised oysters (e.g. *Vibrio*) here.

Implications & Recommendations

This research provided one of the first comprehensive steps towards defining the role of the oyster microbiome in governing the health and disease susceptibility of commercially important oyster species in Australia. Of particular note, our results indicate that the oyster microbiome is highly variable, with its composition determined by environmental factors, oyster genetics and oyster disease resistance. We argue that this dynamic nature of the microbiome implies it is closely coupled with the ecology of the oyster and may hence influence oyster health status. Therefore, we suggest that the oyster microbiome has potential utility as a diagnostic tool within studies of oyster ecology and disease dynamics. However, the highly heterogenous nature of the oyster microbiome suggest that it will be difficult, or perhaps impossible, to define a universal oyster microbiome across different ecosystems and/or over time, and we suggest that this needs to be carefully considered within any efforts to employ the oyster microbiome diagnostically. Within this context, we recommend that future studies, or management practices, that employ measurements of the oyster microbiome should use caution when designing sampling regimes and interpreting microbiome data. Specifically:

(i) characterisation of oyster microbiomes across different environments or seasons should always carefully consider the inherent variability between locations and time when interpreting patterns in microbiome structure among different treatments or oyster conditions.

(ii) Examination of shifts in the abundance of specific 'indicator microbes' within the core oyster microbiome are often likely to be more instructive than comparisons of whole microbiome structure.

Further development

While this research has provided an important foundation for future efforts to employ the oyster microbiome to study oyster ecology or manage oyster culture activities, it was not possible to make any direct links between OsHV-1 disease outbreaks and the nature of the oyster microbiome. This is because during the time of this research there was not significant OsHV-1 related mortality at the sites that sampling was conducted. Indeed, throughout the project period, the impact of OsHV-1 was lower throughout Australian oyster growing regions than in preceding years. However, our results are suggestive of significant differences in the microbiome of oysters with different levels of resistance to OsHV-1, indirectly pointing to a potential role of the oyster microbiome in the impact of OsHV-1. Therefore, we suggest that future efforts should focus on aiming to characterise patterns in the Pacific Oyster microbiome during major OsHV-1 outbreaks to develop a more definitive understanding of how features of the oyster microbiome may augment or buffer OsHV-1 mortality.

Extension and Adoption

The outcomes of this project have been communicated to the wider research community and Australian oyster industry via the following presentations:

Seymour, J.R., Labbate, M, King, W., Siboni, N., Dove, M., O'Connor, W. (2019) The oyster microbiome and its potential role in Pacific Oyster and Sydney Rock Oyster diseases. Presentation at the Shellfish Futures Tasmanian Oyster Industry Meeting, Orford, Tasmania, August 2019.

Labbate, M, King, W., Siboni, N., Dove, M., O'Connor, W., Seymour, J.R. (2019) The oyster microbiome and its potential role in Pacific Oyster and Sydney Rock Oyster diseases. Presentation at the Oysters South Australia Meeting, August 2019.

Siboni, N., Labbate, M, King, W., Dove, M., O'Connor, W., Seymour, J.R., (2019) The oyster microbiome and its potential role in Pacific Oyster and Sydney Rock Oyster diseases. Presentation at the New South Wales Oyster Conference, Forster, SNW 2019.

Nguyen, K.V., King, W., Siboni, N., Mahbub, K.R., Dove, M., O'Connor, W., Seymour, J.R., Labbate, M. (2019) The Sydney Rock oyster microbiome is influenced by local environmental parameters and QX disease resistance. The 3rd International conference on Fish and Shellfish Immunology, 16 – 20th June, 2019, Las Palmas De Gran Canaria, Spain

Labbate, M., King, W.L., Siboni, N., Williams, N., Kahlke, T., Jenkins, C., Dove, M., O'Connor, W., Seymour, J.R. (2018) Is there a role for microbiomes in oyster mortality events? The Australian Shellfish Quality Assurance Program Meeting, UTS

King, W.L., Siboni, N., Williams, N., Kahlke, T., Jenkins, C., Dove, M., O'Connor, W., Seymour, J.R., and Labbate, M. (2018) Insights into the influence of the pacific oyster microbiome in oyster disease and resistance. International Society for Microbial Ecology (ISME), 17th International Symposium on Microbial Ecology 2018

King, W.L., Siboni, N., Williams, N., Kahlke, T., Jenkins, C., Dove, M., O'Connor, W., Seymour, J.R., and Labbate, M. (2018) Characterisation of the Pacific Oyster microbiome during a summer mortality event. 110th Annual Meeting of the National Shellfisheries Association

King, W.L., Siboni, N., Williams, N., Kahlke, T., Jenkins, C., Dove, M., O'Connor, W., Seymour, J.R., and Labbate, M. (2018) Polymicrobial involvement in OsHV-1 outbreaks (and other diseases). Oysters Australia Research and Development Meeting

King, W.L., Siboni, N., Williams, N., Kahlke, T., Jenkins, C., Dove, M., O'Connor, W., Seymour, J.R., and Labbate, M. (2017) Elucidating links between the Pacific Oyster microbiome and summer mortality events. 4th FRDC Australasian Aquatic Animal Health & Biosecurity Scientific Conference

King, W.L., Siboni, N., Williams, N., Kahlke, T., Jenkins, C., Dove, M., O'Connor, W., Seymour, J.R., and Labbate, M. (2017) Microbiome investigations in a Pacific Oyster summer mortality outbreak in Port Stephens, New South Wales. Australian Microbial Ecology Conference (AUSME)

King, W.L., Siboni, N., Williams, N., Kahlke, T., Jenkins, C., Dove, M., O'Connor, W., Seymour, J.R., and Labbate, M. (2016) The effect of microbiological and environmental factors on a summer mortality event in Pacific Oysters. Australian Shellfish Quality Assurance Advisory Committee conference (ASQAAC)

Project materials developed

Published papers based on the outcomes of this research:

King, W.L., Siboni, N., Williams, N., Kahlke, T., Jenkins, C., Dove, M., O'Connor, W., Seymour, J.R., and Labbate, M. (2019). Variability in the composition of Pacific Oyster microbiomes across oyster family lines exhibiting different levels of susceptibility to OsHV-1 µvar disease. Frontiers in Microbiology 10, 473.

King, W.L., Jenkins, C., Seymour, J.R., and Labbate, M. (2019). Oyster disease in a changing environment: decrypting the link between pathogen, microbiome and environment. Marine Environmental Research 143, 124-140.

Green, T.J., Siboni. N., King, W.L., Labbate, M., Seymour, J.R., and Raftos, D. (2019). Simulated marine heat wave alters abundance and structure of Vibrio populations associated with the Pacific oyster resulting in a mass mortality event, Microbial Ecology, 77, 736-747.

King, W.L., Siboni, N., Kahlke, T., Dove, M., O'Connor, W., Mahbub, K.R., Jenkins, C., Seymour, J.R., and Labbate, M. Multiscale heterogeneity in the Crassostrea gigas microbiome. Currently under review.

Appendices

Supplementary Figures and Tables

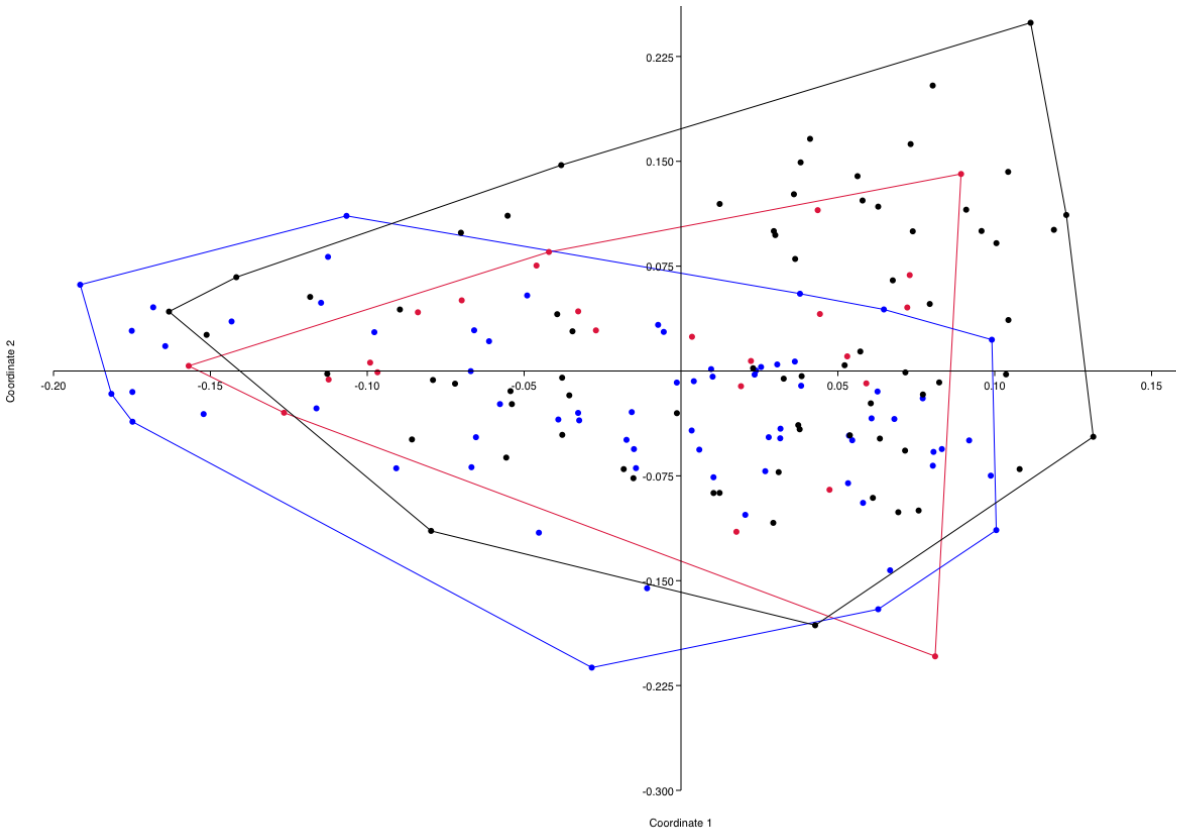
Supplementary Table 1 Information pertaining to the data analysis, including the number of reads before and after cleaning, and rarefaction at 6000 reads per sample.

Information	Read/Observation number
Reads after trimming	4847965
Reads after cleaning	3961808
Observations (OTUs) after cleaning	4188
Samples before rarefaction	175
Reads after rarefaction	1002000
Observations (OTUs) after rarefaction	4188
Reads after filtering below 0.1% prevalence	984544
Observations (OTUs) after 0.1% filtration	3294
Samples after rarefaction	167

Supplementary Table 2 Number of reads per sample before rarefication

F29_05: 731.0	F30_01: 10205.0	F43_04: 15877.0	F77_03: 20678.0	F84_01: 24949.0	F02_02: 36633.0
F72_05: 1880.0	F86_05: 10215.0	F51_03: 15919.0	F36_05: 20746.0	F15_01: 25025.0	F40_03: 37209.0
F10_01: 2033.0	F80_05: 10223.0	F26_05: 15990.0	F66_02: 20872.0	F07_01: 25612.0	F20_04: 37217.0
F67_05: 2061.0	F43_03: 10302.0	F35_02: 16359.0	F11_03: 21110.0	F20_01: 25728.0	F39_04: 37323.0
F19_01: 4128.0	F07_02: 10462.0	F23_03: 16468.0	F36_02: 21131.0	F25_01: 25940.0	F16_02: 37486.0
F19_03: 4587.0	F15_05: 10583.0	F11_05: 16469.0	F69_01: 21272.0	F29_02: 26149.0	F40_05: 37565.0
F62_01: 4597.0	F35_01: 10795.0	F62_05: 16607.0	F07_03: 21634.0	F72_01: 26169.0	F03_03: 37590.0
F11_02: 5063.0	F37_04: 11090.0	F37_01: 17028.0	F51_02: 21682.0	F27_05: 26187.0	F161_03: 37681.0
F16_04: 6230.0	F86_04: 11283.0	F68_05: 17041.0	F23_04: 21743.0	F03_04: 26265.0	F161_05: 37814.0
F23_05: 6568.0	F43_05: 11385.0	F02_03: 17135.0	F66_05: 21827.0	F43_01: 26583.0	F20_03: 39270.0
F16_05: 6577.0	F66_01: 11654.0	F07_04: 17364.0	F02_04: 21901.0	F69_03: 26794.0	F02_01: 39548.0
F67_04: 6993.0	F86_03: 11756.0	F02_05: 17365.0	F25_02: 21910.0	F16_01: 27004.0	F161_02: 41347.0
F10_03: 7114.0	F15_03: 12194.0	F65_02: 17554.0	F62_03: 22024.0	F26_01: 27084.0	F39_05: 41547.0
F65_04: 7295.0	F80_04: 12287.0	F69_04: 17596.0	F25_04: 22134.0	F37_05: 27097.0	F36_01: 43010.0
F27_01: 7373.0	F03_05: 12362.0	F35_03: 17972.0	F27_03: 22176.0	F161_01: 28055.0	F161_04: 43782.0
F29_01: 7538.0	F68_04: 12491.0	F15_02: 17985.0	F84_03: 22216.0	F16_03: 28125.0	F01_02: 46130.0
F86_01: 7590.0	F77_01: 12839.0	F30_05: 18074.0	F20_05: 22284.0	F77_05: 28252.0	F36_04: 46154.0
F30_03: 7702.0	F26_03: 12946.0	F68_01: 18220.0	F77_04: 22291.0	F26_02: 28352.0	F03_01: 46315.0
F15_04: 7736.0	F19_04: 13207.0	F11_04: 18400.0	F39_03: 22562.0	F66_04: 28784.0	F40_01: 46664.0
F10_02: 7774.0	F23_02: 13661.0	F84_05: 18593.0	F10_05: 22582.0	F39_01: 29096.0	F43_02: 46703.0
F19_05: 8045.0	F72_04: 14037.0	F36_03: 18782.0	F86_02: 22626.0	F51_01: 29221.0	F27_04: 47122.0
F62_02: 8424.0	F07_05: 14103.0	F65_03: 19453.0	F62_04: 22643.0	F72_03: 29503.0	F01_03: 48356.0
F29_04: 8540.0	F23_01: 14296.0	F69_02: 19735.0	F25_03: 22824.0	F35_05: 30062.0	F01_05: 51053.0
F37_02: 8847.0	F67_03: 14395.0	F84_02: 19880.0	F80_01: 22928.0	F72_02: 30859.0	F29_03: 52418.0
F65_05: 9150.0	F30_04: 14551.0	F77_02: 19980.0	F68_03: 23996.0	F26_04: 30959.0	F40_04: 55924.0
F65_01: 9311.0	F80_02: 14566.0	F69_05: 20120.0	F66_03: 24114.0	F11_01: 31146.0	F03_02: 57403.0
F10_04: 9389.0	F80_03: 15462.0	F51_04: 20441.0	F68_02: 24349.0	F35_04: 32978.0	F27_02: 58607.0
F37_03: 9452.0	F51_05: 15730.0	F67_01: 20468.0	F19_02: 24435.0	F39_02: 33203.0	F40_02: 69726.0

F67_02: 9781.0 F84_04: 15775.0 F25_05: 20541.0 F20_02: 24597.0 F30_02: 35308.0 F01_04: 77185.0
F01_01: 78470.0



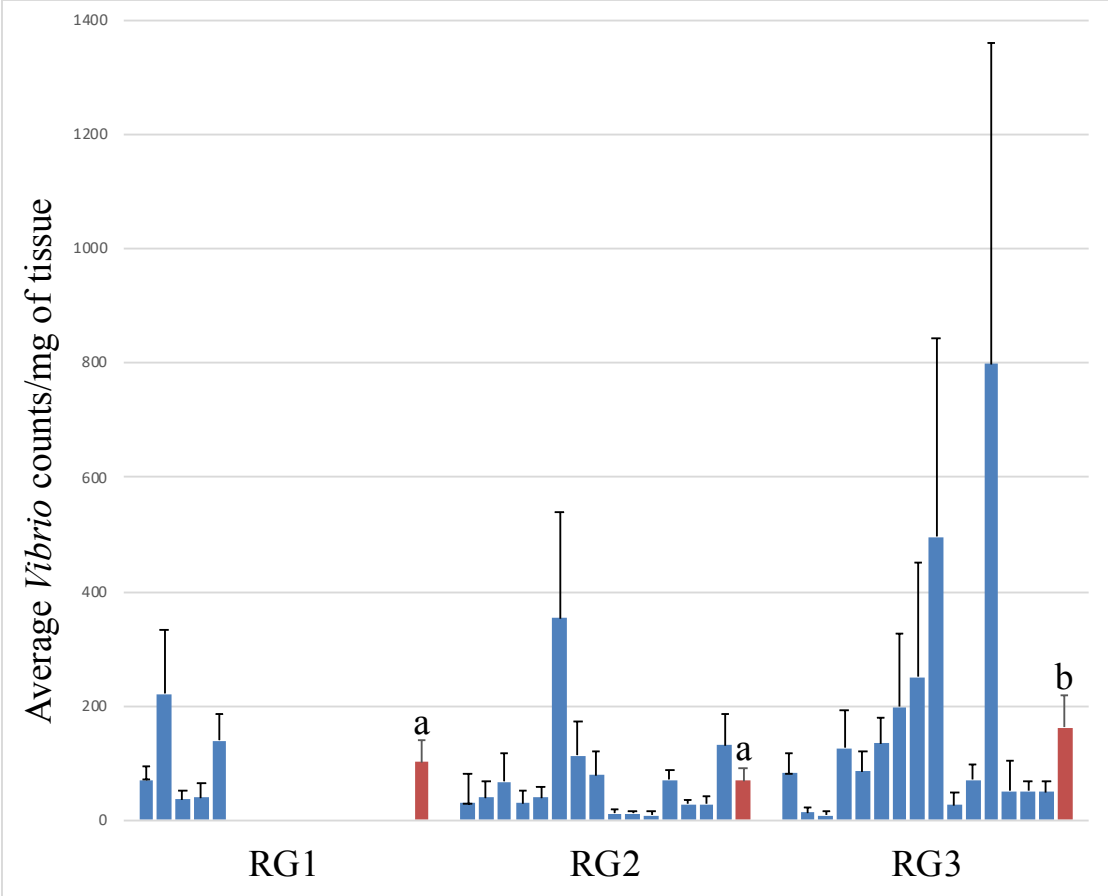
Supplementary Figure 1 Principal Coordinates Analysis (PCoA) of oyster microbiomes with a Bray-Curtis similarity index. Blue dots are resistance group 1 (RG1) microbiomes, red dots are resistance group 2 (RG2) microbiomes, and black dots are resistance group 3 (RG3) microbiomes. Axes 1 and 2 represent 10.7% and 6.4% of the data respectively. Transformation exponent c = 6.

Supplementary Table 3 SIMPER analysis of resistance group 1 (RG1) microbiomes compared to resistance group 3 (RG3) microbiomes. The top 10 OTUs are displayed with their dissimilarity contribution and transformed mean representation. Dissimilarity contribution is cumulative.

OTU	Dissimilarity (%)	RG1 mean	RG3 mean
Pseudomonas 2034	2.204	1.02	2.05
Uncultured bacterium *Psychrobacter* 1488	2.001	1.81	1.35
Uncultured bacterium *Marispirillum* 6464	1.267	1.03	1.13
Ambiguous taxa *Maribacter* 1117	1.16	1.28	1.29
Rhodospirillaceae 6418	1.062	0.709	0.926
Winogradskyella 1511	0.9522	1.42	1.1
Uncultured bacterium *Mycoplasma* 3150	0.9466	0.598	0.891
Uncultured bacterium *Rhodobacteraceae* 6466	0.9147	0.977	0.903
Uncultured bacterium *Mycoplasmataceae* 680	0.8714	0.759	0.68
Ambiguous taxa *Francisella* 2095	0.8521	0.766	1.14

Supplementary Table 4 SIMPER analysis of resistance group 2 (RG2) microbiomes compared to resistance group 3 (RG3) microbiomes. The top 10 OTUs are displayed with their dissimilarity contribution and transformed mean representation. Dissimilarity contribution is cumulative.

OTU	Dissimilarity (%)	RG2 mean	RG3 mean
Pseudomonas 2034	1.861	0.535	2.05
Uncultured bacterium *Mycoplasma* 3150	1.547	1.63	0.891
Uncultured bacterium *Psychrobacter* 1488	1.501	0.634	1.35
Ambiguous taxa *Maribacter* 1117	1.263	1.37	1.29
Uncultured bacterium *Rhodobacteraceae* 6466	1.196	0.965	1.13
Rhodospirillaceae 6418	1.083	0.721	0.926
Uncultured bacterium *Mycoplasmataceae* 680	1.07	1.09	0.68
Ambiguous taxa *Francisella* 2095	0.9425	0.991	1.14
Winogradskyella 1511	0.8938	1.33	1.1
Uncultured bacterium *Rhodobacteraceae* 6466	0.841	0.872	0.903



Supplementary Figure 2 The total *Vibrio* load determined with a *Vibrio* specific 16S qPCR assay. Blue columns represent the average *Vibrio* load in a family, while red columns represent the average *Vibrio* load for the resistance group (RG). Error bars are standard error. Statistical comparisons between resistance groups are displayed on the resistance group mean values.

Supplementary Table 5 Maximal information-based nonparametric exploration (MINE) analysis of expected breeding values (EBVs).

X variable	Y variable	MIC	Linear regression	p-value
Shell length	Depth index	0.81363	-0.9193912	<0.001
Oyster weight	Shell length	0.59185	0.74213886	0.002
Width index	Meat condition	0.54716	0.44662884	0.006
Width index	Depth index	0.54643	0.7001985	0.006
Width index	Shell length	0.54168	-0.8329716	0.007
Oyster weight	Meat condition	0.49895	-0.3160977	0.02
Disease resistance	Oyster weight	0.45763	0.1452698	0.04
Shell length	Meat condition	0.45451	-0.2803066	0.04
Oyster weight	Width index	0.45087	-0.5962815	0.04
Disease resistance	Width index	0.44843	-0.3397912	0.05

Supplementary Table 6: Alpha diversity (Chao1, Observed species and Shannon) for SROs and estuarine waters

Sample	Chao1	Observed species	Shannon
Oyster (n = 107)	142.144 ± 95.466	112.963 ± 74.471	4.068 ± 1.432
Water (n = 12)	591.453 ± 270.276	324.25 ± 126.296	5.943 ± 0.888

Supplementary Table 7: Top 10 OTUs significantly different between oysters (n= 107) and water samples (n= 12) (Welch's t-test; $p < 0.05$) as identified by SIMPER.

Taxon	Average dissimilarity	% Contribution
Candidatus Actinomarina genus OTU_22961	1.494	1.55
NS5 marine group genus OTU_5409	1.391	1.443
Oceanospirillales order OTU_12673	1.118	1.16
Candidatus Hepatoplasma genus OTU_14887	1.071	1.111
Endozoicomonas genus OTU_3829	0.9972	1.035
OM43 clade genus OTU_6156	0.9713	1.008
Litoricola genus OTU_5208	0.765	0.7936
Endozoicomonas genus OTU_6283	0.7573	0.7857
SAR86 clade family OTU_12751	0.7127	0.7394
NS5 marine group genus OTU_10013	0.7073	0.7338

Supplementary Table 8: OTUs significantly different between oysters and water samples (Welch's t-test; p <0.05) as identified by STAMP.

Taxonomy	Oyster (n =107)		Water (n =12)		p-values
	Mean (%)	Std. dev. (%)	Mean (%)	Std. dev. (%)	
Endozoicomonas genus OTU_3829	5.76199377	7.05487532	0.04166667	0.09341762	2.88E-13
Endozoicomonas genus OTU_6283	3.57725857	4.85413527	0.01944444	0.03716413	1.64E-11
Endozoicomonas genus OTU_4530	1.92336449	2.61761433	0.01111111	0.02078699	1.86E-11
Endozoicomonas genus OTU_3483	1.89906542	2.728955	0	0	1.08E-10
Endozoicomonas genus OTU_6587	0.72959502	1.18342944	0.00555556	0.0124226	7.02E-09
Endozoicomonas genus OTU_4405	0.61183801	1.01504009	0	0	1.07E-08
Borrelia genus OTU_120	1.25233645	2.07452587	0.00277778	0.00921285	1.10E-08
Polaribacter 2 genus OTU_13000	0.38068536	0.64067782	0	0	1.62E-08
Actibacter genus OTU_5698	0.67819315	1.22488139	0.01944444	0.03179312	2.39E-07
Borrelia genus OTU_647	0.58099689	1.09298825	0	0	2.99E-07
Endozoicomonas genus OTU_3903	0.01838006	0.03566128	0	0	6.18E-07
Thalassolituus genus OTU_17143	0.54299065	1.08821698	0.00555556	0.0124226	1.61E-06
Litoricola genus OTU_5208	0.02056075	0.09053262	1.95277778	0.69474993	1.62E-06
Vibrio genus OTU_2	3.72741433	6.28682351	0.48611111	0.76041635	2.34E-06
Flavobacteriaceae family OTU_15116	0.03271028	0.18257312	0.96666667	0.36029823	2.60E-06
Gammaproteobacteria class OTU_5209	0.3211838	0.64018093	0.01666667	0.02151657	3.82E-06
Candidatus Hepatoplasma genus OTU_14887	10.0682243	21.8776288	0.08888889	0.21998878	8.04E-06
Sandaracinaceae family OTU_2218	0.07227414	0.15042246	0.00277778	0.00921285	8.30E-06
SAR86 clade family OTU_12751	0.01028037	0.04959048	1.84166667	0.79420645	9.97E-06
NS5 marine group genus OTU_10072	0.00031153	0.00320736	0.29166667	0.12918907	1.23E-05
NS9 marine group family OTU_12573	0.00654206	0.03958226	0.21111111	0.09262962	1.33E-05

Cellvibrionales order OTU_20446	0.02087227	0.04747484	0	0	1.57E-05
OM60(NOR5) clade genus OTU_17168	0.00529595	0.03445151	0.26111111	0.11928284	1.86E-05
Desulfovibrio genus OTU_8837	0.04392523	0.10216739	0	0	2.33E-05
Pseudoalteromonas genus OTU_8917	2.78286604	6.2985046	0.08611111	0.14622303	2.60E-05
Thiogranum genus OTU_5212	0.12274143	0.2681655	0.00833333	0.01443376	3.25E-05
Gammaproteobacteria class OTU_6160	0.15264798	0.36286724	0	0	3.38E-05
Fluviicola genus OTU_3103	0.00342679	0.01929956	0.15277778	0.07632573	4.35E-05
JTB255 marine benthic group family OTU_6360	0.13831776	0.31168999	0.01111111	0.02078699	7.39E-05
Mycoplasma genus OTU_14937	3.00623053	7.58616291	0.00555556	0.0124226	9.00E-05
Bacillus genus OTU_10	0.13894081	0.35133586	0	0	9.02E-05
Mycoplasma genus OTU_14900	3.22429907	8.1668613	0.00277778	0.00921285	9.38E-05
Endozoicomonas genus OTU_5804	0.05202492	0.10493311	0.00555556	0.01842569	0.00012279
Aliivibrio genus OTU_9	0.15264798	0.38685669	0.00277778	0.00921285	0.00012674
Cryomorphaceae family OTU_15039	0.0105919	0.05025338	1.74722222	1.01811903	0.0001471
NS5 marine group genus OTU_5409	0.11619938	0.34125675	7.26944445	4.24315262	0.00016207
Vibrio genus OTU_514	0.03676013	0.09698836	0	0	0.00016773
Vibrio genus OTU_152	0.12429907	0.32129573	0.00277778	0.00921285	0.00018116
Endozoicomonas genus OTU_1831	4.50529595	11.9719846	0.00833333	0.02763854	0.00019026
OM1 clade family OTU_22479	0.03457944	0.09282966	0	0	0.00021338
Family XIII family OTU_15833	0.11246106	0.30279156	0	0	0.00022209
Flavobacteriaceae family OTU_6157	1.05420561	2.84400418	0	0	0.00022818
Flavobacteriaceae family OTU_6214	0.10062305	0.24600574	0.00833333	0.01443376	0.00023586
Vibrio genus OTU_8	0.42056075	0.86156713	0.08611111	0.09949719	0.00026524
Gammaproteobacteria class OTU_18201	0.00186916	0.01354334	0.28611111	0.1848214	0.00034322
Pseudoalteromonas genus OTU_16987	0.06604361	0.16513196	0.00555556	0.0124226	0.00036649

NS4 marine group genus OTU_12242	0.03987539	0.25008573	1.16666667	0.74261799	0.00037317
Maribacter genus OTU_2618	0.40965732	1.15004185	0	0	0.00038501
Rubripirellula genus OTU_21007	0.03084112	0.0867913	0	0	0.00039704
Halieaceae family OTU_16709	0.10280374	0.27153353	0.00555556	0.0124226	0.00040204
Haliea genus OTU_14854	0.0728972	0.17697885	0.00833333	0.01443376	0.00040777
Oceanospirillales order OTU_12673	0.08504673	0.3199245	4.70277778	3.06900449	0.00040777
Escherichia-Shigella genus OTU_3637	0.16666667	0.47030182	0	0	0.00041092
Formosa genus OTU_9479	0.13800623	0.57802343	1.88055556	1.17429714	0.00043529
HOC36 order OTU_12864	0.02772586	0.07939098	0	0	0.0004931
Legionellaceae family OTU_3047	0.28161994	0.79291035	0.00555556	0.0124226	0.00051845
OM60(NOR5) clade genus OTU_17125	0.09906542	0.28512656	0	0	0.00052509
Microbacteriaceae family OTU_22916	0.00841122	0.0515781	0.44722222	0.30137289	0.00052521
Parahaliea genus OTU_14860	0.06947041	0.20186676	0	0	0.00058943
JL-ETNP-Y6 family OTU_6175	0.00218069	0.01721589	0.23888889	0.16545859	0.00060358
Marinimicrobia (SAR406 clade) phylum OTU_730	0.00373832	0.02247745	1.30555556	0.91488042	0.00063004
JTB255 marine benthic group family OTU_6162	0.06728972	0.16694564	0.00833333	0.01443376	0.00063548
Acholeplasma genus OTU_21024	0	0	0.02222222	0.01571348	0.00066031
Flavobacteriaceae family OTU_10765	0.01214953	0.11259172	0.31111111	0.21271322	0.00066517
Endozoicomonas genus OTU_6729	0.17663551	0.51886959	0	0	0.00067068
Endozoicomonas genus OTU_12497	0.00903427	0.0267949	0	0	0.00075054
Haliea genus OTU_17133	0.04174455	0.12414555	0	0	0.00077435
Gammaproteobacteria class OTU_3922	0.01090343	0.03247355	0	0	0.00078753
Halieaceae family OTU_14855	0.08909657	0.25617101	0.00277778	0.00921285	0.00080551
Flavobacteriaceae family OTU_3496	0.10529595	0.25185293	0.01944444	0.01643356	0.00081652
Actibacter genus OTU_6184	0.16137072	0.4240229	0.01666667	0.02886751	0.00082126

Flavobacteriaceae family OTU_14735	0.0046729	0.0140083	0	0	0.00084861
Gammaproteobacteria Incertae Sedis order OTU_20174	0.00560748	0.02827178	0.175	0.12481468	0.00089357
Myxococcales order OTU_2220	0.0199377	0.0600899	0	0	0.00090178
NS5 marine group genus OTU_10092	0	0	0.13055556	0.09761824	0.00100214
Flavobacteriaceae family OTU_10983	0.02928349	0.11526396	0.88611111	0.64196722	0.00101338
Marinilabiaceae family OTU_2173	1.03364486	3.14015793	0.00277778	0.00921285	0.00101625
Cellvibrionales order OTU_17179	0.09376947	0.28613262	0	0	0.00103563
Maribacter genus OTU_6371	0.10249221	0.30334586	0.00277778	0.00921285	0.00104594
Haliea genus OTU_17147	0.02087227	0.06421074	0	0	0.00113239
Roseibacillus genus OTU_11900	0.09096573	0.28005971	0	0	0.00114195
Aeromonas genus OTU_3790	0.14890966	0.41924914	0.01111111	0.0248452	0.00118353
Prolixibacter genus OTU_5526	0.52429907	1.49372221	0.04444444	0.03928371	0.00132766
Mycoplasma genus OTU_14921	2.96074766	9.24590877	0	0	0.00133081
Endozoicomonas genus OTU_14698	0.00809969	0.02530853	0	0	0.00133899
Gammaproteobacteria class OTU_17277	0.02305296	0.09552948	0.74166667	0.5596171	0.00134188
Rhodothermaceae family OTU_16054	0.02616822	0.08241274	0	0	0.00145509
NS5 marine group genus OTU_10013	0.02990654	0.18251358	1.825	1.41422175	0.00145913
Rhodopirellula genus OTU_21032	0.02024922	0.06408668	0	0	0.0015319
MB11C04 marine group order OTU_11968	0	0	0.1	0.07934921	0.00153711
JTB255 marine benthic group family OTU_6164	0.1105919	0.29965706	0.01388889	0.02133652	0.00154571
SAR86 clade family OTU_20168	0.00685358	0.05289899	0.49722222	0.39026542	0.00156756
Sphaerochaeta genus OTU_2780	0.23239875	0.73722358	0	0	0.00156917
Pseudomonas genus OTU_12985	1.02523365	3.21308928	0.01388889	0.03716413	0.00160348
DEV007 family OTU_10048	0.02554517	0.08121347	0	0	0.00160521

KI89A clade order OTU_15846	0.00093458	0.00962208	0.125	0.09918651	0.00161935
Desulfuromusa genus OTU_20170	0.02336449	0.07435635	0	0	0.00162216
Desulfovibrio genus OTU_6212	0.03146417	0.10034538	0	0	0.00165786
OM43 clade genus OTU_6156	0.06386293	0.20472718	3.78055556	2.98373549	0.00166772
Planctomycetaceae family OTU_21014	0.04174455	0.1216103	0.00277778	0.00921285	0.0017146
Cryomorphaceae family OTU_9135	0.00716511	0.03813624	1.30277778	1.04778941	0.00175603
NS9 marine group family OTU_10075	0.00093458	0.00714476	0.27222222	0.22061922	0.00182524
SAR86 clade family OTU_12769	0.00031153	0.00320736	0.24722222	0.20159472	0.00187626
Sandaracinaceae family OTU_3649	0.05327103	0.17238537	0	0	0.00192183
Algibacter genus OTU_5363	0.3470405	1.12538862	0	0	0.00196266
Endozoicomonas genus OTU_1949	0.79657321	2.58804639	0.00277778	0.00921285	0.00207176
Borrelia genus OTU_7	0.13956386	0.45643275	0	0	0.00213464
Marinicella genus OTU_2208	0.12211838	0.39981678	0	0	0.00215777
OM1 clade family OTU_22480	0.03613707	0.11849888	0	0	0.00219122
Microbulbifer genus OTU_17121	0.10778816	0.35477984	0	0	0.00227283
KI89A clade order OTU_18222	0.00031153	0.00320736	0.08888889	0.07494854	0.00239378
NS4 marine group genus OTU_11901	0.0199377	0.12252618	1.1	0.9246621	0.00258842
OM60(NOR5) clade genus OTU_18064	0.00404984	0.01971251	0.12777778	0.10613874	0.00262205
Cryomorphaceae family OTU_15643	0.00031153	0.00320736	0.09722222	0.08328702	0.00265712
Run-SP154 order OTU_11910	0.0376947	0.11357351	0.00277778	0.00921285	0.00267363
NS5 marine group genus OTU_12187	0.00031153	0.00320736	0.05555556	0.0477907	0.00277539
Oceanospirillales order OTU_3644	0.00155763	0.01057356	0.32222222	0.279329	0.00290559
Marinomonas genus OTU_4117	0.53800623	1.73695075	0.025	0.04538926	0.00305653
Milano-WF1B-44 class OTU_6171	0.07133956	0.2423684	0	0	0.00306858
Aquibacter genus OTU_12017	0.77071651	2.61717988	0.00277778	0.00921285	0.00315994

Borrelia genus OTU_651	0.95140187	3.24856476	0	0	0.00321351
OM182 clade family OTU_10024	0.00155763	0.00838232	0.24722222	0.21792725	0.00327317
Halieaceae family OTU_17123	0.02679128	0.11241143	1.40833333	1.2261899	0.00328386
NS11-12 marine group family OTU_10223	0.00062305	0.00451445	0.07777778	0.06849349	0.00328863
Halioglobus genus OTU_17131	0.08504673	0.27302519	0.00555556	0.0124226	0.00367865
Thiohalocapsa genus OTU_11899	0.15233645	0.53253664	0	0	0.00396826
Bacteroidetes BD2-2 class OTU_15999	0.04361371	0.1529503	0	0	0.00408047
JTB255 marine benthic group family OTU_6200	0.02647975	0.09303226	0	0	0.00414597
Planctomycetaceae family OTU_19991	0.18442368	0.38535813	0.05277778	0.08103307	0.00424611
PAUC43f marine benthic group class OTU_130	0.02679128	0.09465895	0	0	0.00435374
Vibrio genus OTU_135	0.02398754	0.08486681	0	0	0.00440451
Hyunsoonleella genus OTU_13103	0.24953271	0.88362705	0	0	0.00443862
NS5 marine group genus OTU_11798	0.00249221	0.0226495	0.40833333	0.37813504	0.00447585
Pseudohaliea genus OTU_17191	0.01806854	0.06417143	0	0	0.00455201
Endozoicomonas genus OTU_3803	0.01308411	0.04664374	0	0	0.00470012
Psychromonas genus OTU_3657	0.01931464	0.06896918	0	0	0.00476667
Pseudoalteromonas genus OTU_15862	0.03862928	0.13816401	0	0	0.00483309
NS5 marine group genus OTU_10078	0	0	0.11388889	0.107547	0.00486472
Formosa genus OTU_10280	0.00218069	0.01460524	0.07222222	0.06643478	0.00500359
Planctomyces genus OTU_10035	0.01682243	0.06069412	0	0	0.0051993
Marinifilum genus OTU_2242	0.01931464	0.06986672	0	0	0.00531254
Burkholderia-Paraburkholderia genus OTU_10015	0.06978193	0.20545685	0.00833333	0.02763854	0.00532873
Sphaerochaeta genus OTU_3805	0.00903427	0.03272659	0	0	0.00537617
Mycoplasma genus OTU_18298	0.00903427	0.03272659	0	0	0.00537617
OM60(NOR5) clade genus OTU_6179	0.00186916	0.01191156	0.56388889	0.54082556	0.00546078

OM60(NOR5) clade genus OTU_17145	0.01931464	0.07016334	0	0	0.00550184
Aliivibrio genus OTU_428	0.05264798	0.16701132	0.00555556	0.0124226	0.00552062
Moritella genus OTU_2212	0.10623053	0.38712753	0	0	0.00564742
Endozoicomonas genus OTU_8033	0.01775701	0.06482147	0	0	0.00572713
Fluviicola genus OTU_3642	0.00186916	0.01354334	0.26666667	0.25819889	0.00591504
Vibrio genus OTU_28	0.08816199	0.28981508	0.00833333	0.01443376	0.00598778
Flavobacteriaceae family OTU_10291	0	0	0.03888889	0.03808697	0.00607282
Planctomyces genus OTU_21011	0.05420561	0.19971545	0	0	0.00617301
Gammaproteobacteria Incertae Sedis order OTU_20185	0.00062305	0.00641472	0.025	0.02405626	0.00636963
Gammaproteobacteria class OTU_2787	0.0305296	0.11302703	0	0	0.00641595
Cryomorphaceae family OTU_9738	0.00031153	0.00320736	0.04444445	0.04374449	0.00652394
Endozoicomonas genus OTU_4728	0.00654206	0.02430306	0	0	0.00659281
SAR86 clade family OTU_20169	0.00124611	0.01282945	0.27222222	0.26937284	0.00663648
CS-B046 order OTU_11932	0.03364486	0.12509563	0	0	0.00663827
Aliivibrio genus OTU_3636	0.19439252	0.69998496	0.00555556	0.01842569	0.0066397
Legionellaceae family OTU_1781	0.03239875	0.12087067	0	0	0.00681791
NS9 marine group family OTU_10675	0	0	0.01666667	0.01666667	0.0068723
Balneola genus OTU_8852	0	0	0.14444444	0.14487117	0.00699242
Rhodopirellula genus OTU_21456	0.01028037	0.03851102	0	0	0.00704109
Endozoicomonas genus OTU_1685	0.00342679	0.01283701	0	0	0.00704109
Vibrio genus OTU_2647	0.02398754	0.08997454	0	0	0.00711205
JTB255 marine benthic group family OTU_3130	0.03489097	0.11728879	0.00277778	0.00921285	0.00715114
Pseudoalteromonas genus OTU_3634	0.19345794	0.65778254	0.01666667	0.03191424	0.00724373
Cobetia genus OTU_2869	1.51495327	5.67526969	0.00555556	0.0124226	0.00724819

Candidatus Actinomarina genus OTU_22915	0.00529595	0.02045664	2.01666667	2.03075885	0.0072695
Cryomorphaceae family OTU_9165	0.01775701	0.10355245	1.225	1.22104426	0.00734713
Vibrio genus OTU_13	0.07663551	0.26566068	0.00555556	0.0124226	0.00745842
Blastopirellula genus OTU_20194	0.01370717	0.05175092	0	0	0.00748157
Shewanella genus OTU_3635	0.39283489	1.3714719	0.02777778	0.0487498	0.00752053
Actibacter genus OTU_14402	0.00218069	0.00824222	0	0	0.00754564
Cryomorphaceae family OTU_14896	0.00093458	0.00550266	0.36388889	0.37029976	0.00772384
Gilvibacter genus OTU_12138	0.07040498	0.26718513	0	0	0.00778363
Bacillus genus OTU_731	0.0317757	0.1207783	0	0	0.00787792
Cryomorphaceae family OTU_8975	0.00996885	0.0618535	2.56111111	2.61490859	0.00793393
Cryomorphaceae family OTU_12315	0.00124611	0.00779439	1.06944445	1.09902805	0.00810718
Porphyromonadaceae family OTU_5211	0.15358256	0.58657102	0	0	0.00817043
OM182 clade family OTU_8848	0.00062305	0.00451445	0.06666667	0.06804138	0.00817168
Hydrogenophilaceae family OTU_6231	0.00031153	0.00320736	0.24166667	0.24986107	0.00839897
Tenacibaculum genus OTU_15085	0.31588785	1.2139236	0	0	0.00855866
JTB255 marine benthic group family OTU_6174	0.07507788	0.2646769	0.00555556	0.0124226	0.00858677
Sandaracinaceae family OTU_1560	0.02990654	0.10056276	0.00277778	0.00921285	0.00863216
Actibacter genus OTU_5659	0.0470405	0.1680598	0.00277778	0.00921285	0.00864349
Fusibacter genus OTU_18286	0.04143302	0.15988321	0	0	0.00882702
Endozoicomonas genus OTU_7863	0.06542056	0.2528724	0	0	0.00893796
Cryomorphaceae family OTU_15338	0.00031153	0.00320736	0.04166667	0.04330127	0.00895993
Endozoicomonas genus OTU_6187	0.04330218	0.16801418	0	0	0.00919219
Mycoplasma genus OTU_3843	0.00809969	0.03184892	0	0	0.01012916
Rhodopirellula genus OTU_21010	0.05109034	0.20167099	0	0	0.01041392
Flavobacteriaceae family OTU_13122	0	0	0.02777778	0.02991758	0.01048236

Polaribacter 2 genus OTU_7709	0.12959502	0.51214385	0	0	0.01049976
OM1 clade family OTU_22477	0.01495327	0.05928505	0	0	0.0107444
Flavobacteriaceae family OTU_14459	0.00249221	0.00988084	0	0	0.0107444
JTB255 marine benthic group family OTU_6234	0.01339564	0.05311869	0	0	0.01075756
Mycoplasma genus OTU_12669	2.33302181	9.25848772	0	0	0.01081698
Actibacter genus OTU_8128	0.09065421	0.36021784	0	0	0.01091545
Aquibacter genus OTU_9355	0.04579439	0.18203439	0	0	0.01094461
Hydrogenophilaceae family OTU_12809	0	0	0.04166667	0.04538926	0.01115432
Cryomorphaceae family OTU_10033	0.00062305	0.00641472	0.31944445	0.3475998	0.01120601
Flammeovirga genus OTU_14853	0.09626168	0.37214338	0.00277778	0.00921285	0.01127296
NS5 marine group genus OTU_11008	0	0	0.01944444	0.02133652	0.01160347
JTB255 marine benthic group family OTU_3562	0.06261682	0.25100878	0	0	0.01161067
Polaribacter 2 genus OTU_6718	0.00342679	0.01638996	0.63888889	0.69918383	0.01177426
Shewanella genus OTU_1350	0.12461059	0.48867414	0.00277778	0.00921285	0.01178834
Blastopirellula genus OTU_20180	0.01090343	0.0438954	0	0	0.01196064
Flavobacteriaceae family OTU_12782	0.00062305	0.00641472	0.21111111	0.23227272	0.01196096
Planococcaceae family OTU_1218	0.03956386	0.15931648	0	0	0.01198086
Desulforhopalus genus OTU_1780	0.03956386	0.15931648	0	0	0.01198086
Endozoicomonas genus OTU_6024	0.02492212	0.10068235	0	0	0.01225134
SAR86 clade family OTU_11902	0.00062305	0.00641472	0.44722222	0.49748596	0.01257856
Flavobacteriaceae family OTU_7686	0.005919	0.02403402	0	0	0.01268553
Mycoplasmataceae family OTU_869	0.10778816	0.43781719	0	0	0.0127142
Flavobacteriaceae family OTU_6249	0.01869159	0.07614513	0	0	0.01297022
Borrelia genus OTU_536	0.01277259	0.05208924	0	0	0.0130663
Blastopirellula genus OTU_19733	0.00778816	0.03176348	0	0	0.01307117

Sandaracinaceae family OTU_1539	0.02398754	0.09793235	0	0	0.0131625
Pir4 lineage genus OTU_16082	0.01121495	0.04578698	0	0	0.01316332
Microbacteriaceae family OTU_22937	0.00280374	0.02044241	0.06666667	0.07200823	0.01349083
JTB255 marine benthic group family OTU_6260	0.02616822	0.07516379	0.00555556	0.0124226	0.01357794
Lachnospiraceae family OTU_18338	0.00747664	0.03067547	0	0	0.01360696
Flavobacteriaceae family OTU_12763	0.03239875	0.13297912	0	0	0.01364268
Planctomyces genus OTU_22498	0.01090343	0.04483168	0	0	0.01380462
DEV007 family OTU_8903	0.00716511	0.02954414	0	0	0.01406661
Actibacter genus OTU_8450	0.01370717	0.05654527	0	0	0.01410969
Flavobacteriaceae family OTU_6173	0.0271028	0.11201272	0	0	0.01428391
Bacillus genus OTU_736	0.01246106	0.05166458	0	0	0.01458755
Flavobacteriaceae family OTU_10447	0.00062305	0.00451445	0.08611111	0.0985622	0.01506286
Marinomonas genus OTU_2209	0.22679128	0.9213228	0.00555556	0.0124226	0.01509648
PAUC43f marine benthic group class OTU_598	0.02305296	0.07990275	0.00277778	0.00921285	0.01537453
Pir4 lineage genus OTU_10470	0.00560748	0.02345362	0	0	0.01544638
Gammaproteobacteria class OTU_18396	0.00186916	0.0163247	0.02777778	0.02991758	0.0155557
Alphaproteobacteria class OTU_20232	0.00031153	0.00320736	0.03333333	0.03849002	0.01593473
OM190 class OTU_1791	0.0211838	0.08923263	0	0	0.01616794
Ralstonia genus OTU_3668	0.01214953	0.05123451	0	0	0.01628345
Mycoplasma genus OTU_18288	0.00934579	0.03941272	0	0	0.01628757
Cryomorphaceae family OTU_15045	0.00124611	0.00779439	0.05833333	0.06684005	0.016311
Photobacterium genus OTU_3	1.11557632	4.6804299	0.00833333	0.01443376	0.01653859
Psychrobacter genus OTU_6015	0.02429907	0.10287357	0	0	0.01669741
JTB255 marine benthic group family OTU_6195	0.01931464	0.08203504	0	0	0.0170414
JTB255 marine benthic group family OTU_6273	0.03115265	0.08650008	0.00833333	0.01443376	0.01768917

Hyunsoonleella genus OTU_18735	0.01090343	0.04664791	0	0	0.01783678
Colwellia genus OTU_3670	0.25358256	1.09021664	0	0	0.01838709
JTB215 family OTU_19717	0.03426791	0.14742215	0	0	0.01846097
Flavobacteriaceae family OTU_13171	0.00529595	0.02285424	0	0	0.01881749
DEV007 family OTU_10294	0.00404984	0.01747883	0	0	0.0188311
Pseudohongiella genus OTU_6178	0.00249221	0.01088115	0.225	0.26878499	0.01904262
E01-9C-26 marine group order OTU_2222	0.02242991	0.09718528	0	0	0.01928783
Flavobacteriaceae family OTU_13485	0.07694704	0.33343959	0	0	0.01930199
Endozoicomonas genus OTU_16507	0.00280374	0.01216151	0	0	0.01941747
Parahaliea genus OTU_17146	0.04859813	0.2118151	0	0	0.01999225
Actibacter genus OTU_6203	0.03613707	0.15761208	0	0	0.02007562
Vibrio genus OTU_4046	0.00716511	0.03125218	0	0	0.02008147
BD7-8 marine group order OTU_6721	0.00778816	0.03397496	0	0	0.02010003
NS5 marine group genus OTU_10211	0	0	0.03333333	0.04082483	0.02036307
Algibacter genus OTU_12779	0.04205608	0.18412243	0	0	0.0205375
Endozoicomonas genus OTU_2993	0.08442368	0.37035297	0	0	0.02078642
Cryomorphaceae family OTU_5247	0	0	0.025	0.03080705	0.02097474
Flavobacteriaceae family OTU_6334	0.00031153	0.00320736	0.09444444	0.11613636	0.02109639
Guggenheimella genus OTU_18622	0.54267913	2.38716625	0	0	0.02112836
Blastopirellula genus OTU_19192	0.00529595	0.02330418	0	0	0.02117193
Pseudoalteromonas genus OTU_16106	0.02959502	0.09883297	0.00555556	0.0124226	0.02138897
NS5 marine group genus OTU_10101	0.00093458	0.00962208	0.08055556	0.09856219	0.02145469
BD7-8 marine group order OTU_11824	0.00778816	0.03458084	0	0	0.02232765
Vibrio genus OTU_17	0.09252336	0.31640203	0.01944444	0.02530676	0.02278917
Endozoicomonas genus OTU_3999	0.00404984	0.01806316	0	0	0.0229219

Rhodopirellula genus OTU_8911	0.00498442	0.02223435	0	0	0.02293846
Blastopirellula genus OTU_20175	0.01869159	0.08355765	0	0	0.02322537
Haliea genus OTU_17137	0.04080997	0.13202697	0.00833333	0.0198373	0.02365601
Candidatus Actinomarina genus OTU_22961	0.10155763	0.31080852	9.56666667	11.9879106	0.02388728
JTB255 marine benthic group family OTU_8828	0.01183801	0.05319537	0	0	0.02393125
Endozoicomonas genus OTU_1993	0.83115265	3.6759731	0.01388889	0.01643356	0.02407523
NS9 marine group family OTU_10047	0	0	0.08333333	0.10584755	0.02420716
Marinomonas genus OTU_2274	0.00872274	0.03929188	0	0	0.02426644
NS4 marine group genus OTU_14248	0	0	0.02222222	0.02832789	0.02461598
Halieaceae family OTU_17159	0.01090343	0.04924681	0	0	0.02464069
Planctomyces genus OTU_22969	0.00311527	0.01407052	0	0	0.02464069
Desulfobulbaceae family OTU_19246	0.00155763	0.00703526	0	0	0.02464069
Winogradskyella genus OTU_8005	0.00155763	0.00703526	0	0	0.02464069
Marinomonas genus OTU_2558	0.00155763	0.00703526	0	0	0.02464069
Vibrio genus OTU_482	0.00155763	0.00703526	0	0	0.02464069
Endozoicomonas genus OTU_5023	0.00155763	0.00703526	0	0	0.02464069
Candidatus Actinomarina genus OTU_22984	0.00124611	0.01011382	0.12777778	0.1614938	0.0247645
Haliea genus OTU_17127	0.04267913	0.19338996	0	0	0.02509673
Rhodopirellula genus OTU_22060	0.01900312	0.08612003	0	0	0.02511668
Milano-WF1B-44 class OTU_14876	0.02274143	0.10307808	0	0	0.02513931
OM43 clade genus OTU_6395	0.00062305	0.00451445	0.12777778	0.16320479	0.02540938
Bacteroidetes VC2.1 Bac22 class OTU_17172	0.00934579	0.04245684	0	0	0.02546263
SAR92 clade genus OTU_17153	0.00062305	0.00451445	0.55555556	0.7145749	0.02578903
ML635J-21 class OTU_21049	0	0	0.01666667	0.02151657	0.02609468
NS4 marine group genus OTU_12191	0	0	0.01666667	0.02151657	0.02609468

Vibrio genus OTU_342	0.00436137	0.01990847	0	0	0.02615751
Actibacter genus OTU_8301	0.06510903	0.29745836	0	0	0.02628232
Mycoplasma genus OTU_18257	0.21682243	0.99067693	0	0	0.02629682
Mycoplasma genus OTU_18534	0.01308411	0.05989902	0	0	0.02658434
Hydrogenophilaceae family OTU_13183	0	0	0.03888889	0.05061352	0.02707557
KI89A clade order OTU_12908	0.00342679	0.01574365	0	0	0.02711311
Gammaproteobacteria class OTU_6670	0.62024922	2.83699326	0.00277778	0.00921285	0.02712847
Halioglobus genus OTU_17217	0.005919	0.02727236	0	0	0.02754922
CA002 family OTU_12	0.0529595	0.24435276	0	0	0.02775892
Pir4 lineage genus OTU_22088	0.00778816	0.03605103	0	0	0.02825647
Roseibacillus genus OTU_11909	0.01962617	0.09102546	0	0	0.0285576
OM1 clade family OTU_22490	0.00903427	0.04190929	0	0	0.0285898
NS5 marine group genus OTU_12525	0.00218069	0.01947975	0.12777778	0.16545859	0.02863496
MSBL9 order OTU_10157	0.00529595	0.02460468	0	0	0.02882554
Xanthomonadales order OTU_14889	0.00965732	0.04488577	0	0	0.02888977
Gammaproteobacteria class OTU_18101	0.00031153	0.00320736	0.01944444	0.02530676	0.02916013
Flavobacteriaceae family OTU_12808	0.9682243	4.51134806	0	0	0.02928053
Haliea genus OTU_15124	0.01619938	0.07548589	0	0	0.02929405
Winogradskyella genus OTU_6190	0.03582555	0.16705257	0	0	0.02940083
NS5 marine group genus OTU_10332	0.00280374	0.0228415	0.13611111	0.17714732	0.02977289
Flammeovirgaceae family OTU_12784	0.02866044	0.1010278	0.00555556	0.0124226	0.02981308
OM182 clade family OTU_5988	0	0	0.03611111	0.04803227	0.02985081
Cryomorphaceae family OTU_15109	0.00031153	0.00320736	0.09722222	0.12942775	0.03039329
Marinobacter genus OTU_6206	0.0411215	0.19294835	0	0	0.03040228
Muricauda genus OTU_10134	0.00809969	0.03808531	0	0	0.03074613

NS2b marine group genus OTU_12883	0	0	0.08333333	0.1118034	0.0310075
Planococcus genus OTU_1244	0.00716511	0.03380601	0	0	0.03130741
OM1 clade family OTU_20182	0.00903427	0.04264614	0	0	0.0313897
Cryomorphaceae family OTU_12844	0	0	0.04444444	0.05983517	0.03148108
OM43 clade genus OTU_9153	0	0	0.025	0.03367877	0.03156901
Flavobacteriaceae family OTU_14881	0.01401869	0.09943113	0.12222222	0.14423061	0.0316263
Rhodothermaceae family OTU_15707	0.00498442	0.02359389	0	0	0.03184926
Coxiella genus OTU_6267	0.00249221	0.01179695	0	0	0.03184926
Actibacter genus OTU_8715	0.00249221	0.01179695	0	0	0.03184926
Pir4 lineage genus OTU_16160	0.00249221	0.01179695	0	0	0.03184926
SPOTSOCT00m83 class OTU_6243	0.01588785	0.07534563	0	0	0.03216195
Mycoplasma genus OTU_15400	0.53021807	2.51637434	0	0	0.03228936
Rhodopirellula genus OTU_21022	0.00373832	0.01784152	0	0	0.03324642
Granulosicoccus genus OTU_86	0.00186916	0.00892076	0	0	0.03324642
Flavobacteriaceae family OTU_13345	0.00186916	0.00892076	0	0	0.03324642
Legionella genus OTU_16227	0.00186916	0.00892076	0	0	0.03324642
Legionella genus OTU_23158	0.00186916	0.00892076	0	0	0.03324642
PAUC43f marine benthic group class OTU_559	0.00685358	0.03277993	0	0	0.03361883
Fluviicola genus OTU_10058	0	0	0.07777778	0.10657403	0.03397833
Photobacterium genus OTU_5	0.56137072	2.67717226	0.00277778	0.00921285	0.03398571
NS2b marine group genus OTU_10820	0.00436137	0.02236491	1.18333333	1.61821232	0.03422657
Alteromonadaceae family OTU_12804	0.00623053	0.02992925	0	0	0.03437658
Planctomycetaceae family OTU_21455	0.00623053	0.02992925	0	0	0.03437658
Endozoicomonas genus OTU_6456	0	0	0.05277778	0.07259519	0.03453883
Pir4 lineage genus OTU_16036	0.00965732	0.046477	0	0	0.03470669

Mycoplasma genus OTU_18779	0.02149533	0.10354589	0	0	0.03487343
Blastopirellula genus OTU_20192	0.00498442	0.02402998	0	0	0.03501696
NS4 marine group genus OTU_14677	0	0	0.03611111	0.04992278	0.03529214
JTB255 marine benthic group family OTU_6344	0.00809969	0.03916075	0	0	0.03553086
Cellulophaga genus OTU_12104	0.394081	1.90609676	0	0	0.03560428
Marinobacterium genus OTU_6209	0.00841122	0.04078499	0	0	0.03605597
Marinilabiaceae family OTU_10021	0.05669782	0.27597519	0	0	0.03675708
Portibacter genus OTU_14916	0.00404984	0.01971251	0	0	0.03675708
HOC36 order OTU_11945	0.00404984	0.01971251	0	0	0.03675709
Desulfopila genus OTU_2813	0.00685358	0.03340749	0	0	0.03702202
Sandaracinaceae family OTU_3733	0.01464175	0.07164838	0	0	0.03774644
OM60(NOR5) clade genus OTU_15837	0.01838006	0.07107448	0.00277778	0.00921285	0.03822472
Lentimicrobiaceae family OTU_16032	0.00996885	0.0489166	0	0	0.03826633
Pir4 lineage genus OTU_22081	0.03208723	0.14112184	0.00277778	0.00921285	0.03834193
Pir4 lineage genus OTU_20216	0.00436137	0.02141617	0	0	0.03840056
SAR86 clade family OTU_22885	0	0	0.01111111	0.01571348	0.03881409
SAR86 clade family OTU_22595	0	0	0.01111111	0.01571348	0.03881409
TM6 (Dependentiae) phylum OTU_17300	0	0	0.01111111	0.01571348	0.03881409
SAR86 clade family OTU_16365	0	0	0.02222222	0.03142697	0.03881409
Endozoicomonas genus OTU_915	0.00872274	0.0430741	0	0	0.03947862
Lentimicrobiaceae family OTU_16001	0.01370717	0.06789419	0	0	0.04006978
Halieaceae family OTU_17173	0.00809969	0.04020744	0	0	0.04049962
Arcobacter genus OTU_6697	2.89688474	14.3892473	0	0	0.04062138
Pseudoalteromonas genus OTU_2288	0.00311527	0.01547631	0	0	0.04065118
Hydrogenophilaceae family OTU_12858	0.00062305	0.00451445	0.075	0.10639288	0.04068677

Formosa genus OTU_10119	0	0	0.03611111	0.05174427	0.04096595
Formosa genus OTU_13143	0	0	0.03611111	0.05174427	0.04096595
Marinoscillum genus OTU_15988	0.00249221	0.0226495	0.26111111	0.37064342	0.04101005
OM60(NOR5) clade genus OTU_17152	0.01277259	0.06358041	0	0	0.04104801
Owenweeksia genus OTU_18290	0.01183801	0.05893699	0	0	0.04107764
Sva0071 order OTU_11918	0.01433022	0.0714938	0	0	0.04149293
Roseivirga genus OTU_17281	0.01121495	0.0559899	0	0	0.04162946
MB11C04 marine group order OTU_12031	0	0	0.03055556	0.04400828	0.04182695
Marinimicrobia (SAR406 clade) phylum OTU_969	0	0	0.03055556	0.04400828	0.04182695
NS4 marine group genus OTU_6182	0.00404984	0.02484021	0.88611111	1.27260729	0.04212257
Planctomyces genus OTU_22491	0.01246106	0.06240646	0	0	0.04226027
Candidatus Actinomarina genus OTU_21209	0.00031153	0.00320736	0.03611111	0.05174427	0.04244751
Mycoplasma genus OTU_18263	0.02990654	0.14996951	0	0	0.04252237
Candidatus Amoebophilus genus OTU_14890	0.0046729	0.02343706	0	0	0.04255985
CA002 family OTU_539	0.00965732	0.04844612	0	0	0.04259978
Pseudohongiella genus OTU_6185	0	0	0.23333333	0.33774854	0.04268093
Flexithrix genus OTU_14857	0.0411215	0.19072902	0.00277778	0.00921285	0.04304253
Arenicella genus OTU_11903	0.07819315	0.39332707	0	0	0.0431553
NS2b marine group genus OTU_12895	0.00093458	0.00550266	0.71944445	1.04389392	0.04332067
Marinilabiaceae family OTU_13177	0.005919	0.0298188	0	0	0.04346561
SAR86 clade family OTU_19911	0.00031153	0.00320736	0.03055556	0.04400828	0.04361311
Mesoflavibacter genus OTU_16057	0	0	0.02777778	0.04044506	0.04369958
Rhodopirellula genus OTU_21362	0.03676013	0.1691058	0.00277778	0.00921285	0.0437212
Arenicellaceae family OTU_11919	0.02803738	0.1420916	0	0	0.04470373
KI89A clade order OTU_15181	0.01308411	0.06631637	0	0	0.04472572

Robiginitalea genus OTU_8854	0.01433022	0.07264648	0	0	0.04476689
Cryomorphaceae family OTU_8884	0	0	0.04166667	0.06104795	0.04480224
Flavobacteriaceae family OTU_6194	0.02679128	0.13591269	0	0	0.04491425
Pseudoalteromonas genus OTU_16438	0.00249221	0.01264659	0	0	0.04497333
Arcobacter genus OTU_22541	0.00124611	0.0063233	0	0	0.04497333
Endozoicomonas genus OTU_8727	0.00124611	0.0063233	0	0	0.04497333
Vibrio genus OTU_185	0.00124611	0.0063233	0	0	0.04497333
Fusibacter genus OTU_19147	0.01028037	0.05224179	0	0	0.04527453
Formosa genus OTU_5375	0.02274143	0.18654636	1.45277778	2.10253191	0.04546176
Flavicella genus OTU_10086	0	0	0.03333333	0.04906534	0.04563291
Mycoplasma genus OTU_18251	0.36697819	1.85971388	0.00277778	0.00921285	0.04632584
Nitrosomonas genus OTU_10148	0.02149533	0.10977673	0	0	0.04633158
Comamonadaceae family OTU_19412	0	0	0.01944445	0.02873356	0.04634165
OM43 clade genus OTU_10256	0	0	0.04444445	0.06573422	0.04650141
Marinobacterium genus OTU_3640	0.01121495	0.06379223	0.99444445	1.45747329	0.04692064
HOC36 order OTU_1228	0.00996885	0.0511985	0	0	0.04754912
Flammeovirgaceae family OTU_17130	0.05046729	0.25969726	0	0	0.04797444
Gammaproteobacteria class OTU_5911	0.02149533	0.07238015	0.00555556	0.0124226	0.04814291
SS1-B-06-26 family OTU_6378	0.00778816	0.0401398	0	0	0.04831984
Bythopirellula genus OTU_16095	0.005919	0.03050734	0	0	0.0483277
Bythopirellula genus OTU_16084	0.005919	0.03050734	0	0	0.0483277
Mycoplasma genus OTU_18633	0.03489097	0.18013397	0	0	0.04869719
Vibrio genus OTU_55	0.00716511	0.03703105	0	0	0.04893222
Endozoicomonas genus OTU_5001	0.00280374	0.01449854	0	0	0.04905675
OM1 clade family OTU_22485	0.00934579	0.04840003	0	0	0.04938641

Fusibacter genus OTU_18356	0.00311527	0.01613334	0	0	0.04938641
BAL58 marine group genus OTU_12893	0.00062305	0.00451445	0.04722222	0.0699978	0.04941581
Photobacterium genus OTU_22	0.04984424	0.25821392	0	0	0.049456
Halomonas genus OTU_6204	0.0411215	0.21319794	0	0	0.04963597
Polaribacter 4 genus OTU_12840	0.00654206	0.03393208	0	0	0.04972996
Seonamhaeicola genus OTU_16012	0.00965732	0.05013157	0	0	0.0499152

Supplementary Table 9: Alpha diversity (Chao1, Observed species and Shannon) for SROs in Port Stephens and Wallis Lake

Location	**Chao1**	**Observed species**
Port Stephens (n = 50)	121.078667 ± 66.9134796	94.84 ± 52.2397769
Wallis Lake (n = 57)	160.622554 ± 112.203511	128.859649 ± 86.9444483

Supplementary Table 10: Top 10 OTUs contributing to the difference between the SRO microbiomes from Port Stephens (n = 50) and Wallis Lake (n= 57) as identified by SIMPER.

Taxon	Average dissimilarity	% Contribution
Candidatus Hepatoplasma genus OTU_14887	2.262	2.625
Endozoicomonas genus OTU_1831	1.757	2.039
Vibrio genus OTU_2	1.475	1.712
Endozoicomonas genus OTU_3829	1.403	1.628
Pseudoalteromonas genus OTU_8917	1.321	1.533
Mycoplasma genus OTU_14900	1.269	1.473
Endozoicomonas genus OTU_6283	1.167	1.354
Mycoplasma genus OTU_14937	1.14	1.323
Mycoplasma genus OTU_14921	1.114	1.292
Endozoicomonas genus OTU_3483	0.8907	1.034

Supplementary Table 6: OTUs significantly different between Port Stephens and Wallis Lake (Welch's t-test; $p < 0.05$) as identified by STAMP

Taxonomy	**Port Stephens (n = 50)**		**Wallis Lake (n = 57)**		**p-values**
	Mean (%)	**Std. dev. (%)**	**Mean (%)**	**Std. dev. (%)**	
Vibrio genus OTU_2	7.094666667	7.810475473	0.773684211	1.418490217	8.71E-07
Mycoplasma genus OTU_14900	6.322666667	11.08210858	0.506432749	1.290638638	0.000620696
Pseudoalteromonas genus OTU_8917	5.697333333	8.217460651	0.226315789	1.117846315	2.64E-05
Mycoplasma genus OTU_12669	4.760666667	13.09322079	0.203508772	0.910148205	0.018718276
Cobetia genus OTU_2869	3.089333334	7.989483199	0.133918129	0.622529933	0.012812257
Marinilabiaceae family OTU_2173	2.091333333	4.317019599	0.105847953	0.565827477	0.002407941
Marinomonas genus OTU_4117	1.124666667	2.407403488	0.023391813	0.113591881	0.002413861
Guggenheimella genus OTU_18622	1.086	3.399634556	0.066081871	0.270260233	0.041372564

Prolixibacter genus OTU_5526	1.013333333	2.06820373	0.095321637	0.20599576	0.003236766
Cellulophaga genus OTU_12104	0.838	2.721168131	0.004678363	0.018138085	0.037052792
Tenacibaculum genus OTU_15085	0.658666667	1.71116023	0.015204678	0.065507518	0.011353213
Shewanella genus OTU_3635	0.7	1.887220178	0.123391813	0.501302977	0.042613592
Colwellia genus OTU_3670	0.538666667	1.546154513	0.003508772	0.018400155	0.01914673
Marinomonas genus OTU_2209	0.474666667	1.303611394	0.009356725	0.039368497	0.015906007
Sphaerochaeta genus OTU_2780	0.476666667	1.021246515	0.018128655	0.08448218	0.00289788
Pseudoalteromonas genus OTU_3634	0.399333333	0.917229888	0.012865497	0.066594811	0.004943409
Porphyromonadaceae family OTU_5211	0.294	0.826066718	0.030409357	0.121761071	0.031432921
Aeromonas genus OTU_3790	0.286	0.55144618	0.028654971	0.17960739	0.002775409
Aliivibrio genus OTU_9	0.264	0.506878903	0.05497076	0.187472283	0.008321645
Mycoplasmataceae family OTU_869	0.215333333	0.621046071	0.013450292	0.049504228	0.027645264
Vibrio genus OTU_152	0.208	0.398082069	0.050877193	0.207942231	0.01539528
Flammeovirga genus OTU_14853	0.178666667	0.526149958	0.023976608	0.077170828	0.046672419
Vibrio genus OTU_13	0.150666667	0.372513385	0.011695906	0.041647939	0.012305594
Pseudoalteromonas genus OTU_16987	0.134666667	0.220500441	0.005847953	0.028009193	0.000170712
JTB255 marine benthic group family OTU_3562	0.13	0.354604631	0.003508772	0.022237606	0.016082347
Marinilabiaceae family OTU_10021	0.117333333	0.394531508	0.003508772	0.019430699	0.04912522
CA002 family OTU_12	0.112666667	0.347939331	0.000584795	0.004376207	0.028650319
Flammeovirgaceae family OTU_17130	0.108	0.371636621	0	0	0.04735825
Aliivibrio genus OTU_428	0.099333333	0.23146778	0.011695906	0.042113385	0.011724901
Fusibacter genus OTU_18286	0.088	0.224970121	0.000584795	0.004376207	0.009017966
Shewanella genus OTU_3645	0.087333333	0.286813141	0.001754386	0.007443229	0.042008272
Pseudoalteromonas genus OTU_12646	0.085333333	0.230473908	0.003508772	0.022237606	0.016767023
Planococcaceae family OTU_1218	0.082666667	0.22501753	0.001754386	0.013128622	0.015276632
Pseudoalteromonas genus OTU_15862	0.08	0.193677854	0.002339181	0.010558754	0.007217407
JTB215 family OTU_19717	0.068666667	0.209487205	0.004093567	0.018804602	0.036435873

Flavobacteriaceae family OTU_11913	0.064	0.209214404	0	0	0.037245407
Pseudoalteromonas genus OTU_16106	0.061333333	0.137656577	0.001754386	0.007443229	0.003935149
Vibrio genus OTU_514	0.066666667	0.130469239	0.010526316	0.035407896	0.005063295
Rhodothermaceae family OTU_16054	0.055333333	0.113648581	0.000584795	0.004376207	0.001470664
Vibrio genus OTU_135	0.051333333	0.11836103	0	0	0.003834043
Mycoplasma genus OTU_18779	0.046	0.147706917	0	0	0.034091372
Psychrobacter genus OTU_6015	0.048	0.145626615	0.003508772	0.018400155	0.038617382
Vibrio genus OTU_2647	0.046	0.125590162	0.004678363	0.023725688	0.027505589
Marinomonas genus OTU_3726	0.037333333	0.1217721	0.000584795	0.004376207	0.039861745
Marinifilum genus OTU_2242	0.038	0.097527204	0.002923977	0.015648056	0.016072923
Mycoplasma genus OTU_18534	0.028	0.085208242	0	0	0.025732191
Mycoplasma genus OTU_18288	0.02	0.055777335	0	0	0.015426247
Planctomyces genus OTU_22498	0.020666667	0.062499778	0.002339181	0.013764447	0.049466439
Marinomonas genus OTU_2274	0.018	0.055860939	0.000584795	0.004376207	0.034315272
Sphaerochaeta genus OTU_3805	0.018	0.045318625	0.001169591	0.008752415	0.013460688
Vibrio genus OTU_4046	0.015333333	0.044327067	0	0	0.019209156
Marinilabiaceae family OTU_13177	0.012666667	0.042630193	0	0	0.042787112
Portibacter genus OTU_14916	0.008666667	0.028134597	0	0	0.035995607
Vibrio genus OTU_482	0.003333333	0.01	0	0	0.023778466
Arcobacter genus OTU_22541	0.002666667	0.009043107	0	0	0.044315177
Vibrio genus OTU_185	0.002666667	0.009043107	0	0	0.044315177
Desulfobulbaceae family OTU_19246	0	0	0.002923977	0.009429541	0.023982787
Legionella genus OTU_23158	0	0	0.003508772	0.011984738	0.032637746
Legionella genus OTU_16227	0	0	0.003508772	0.011984738	0.032637746
Actibacter genus OTU_8715	0	0	0.004678363	0.015843529	0.031230354
Planctomyces genus OTU_22969	0	0	0.005847953	0.018859082	0.023982787
KI89A clade order OTU_12908	0	0	0.006432749	0.021117509	0.026465598

Rhodopirellula genus OTU_21022	0	0	0.007017544	0.023969475	0.032637745
HOC36 order OTU_11945	0	0	0.007602339	0.026503554	0.036177007
DEV007 family OTU_10294	0	0	0.007602339	0.023377188	0.018165877
NS2b marine group genus OTU_10820	0	0	0.008187135	0.030126893	0.046737715
Candidatus Amoebophilus genus OTU_14890	0	0	0.00877193	0.031546442	0.042034316
Rhodopirellula genus OTU_8911	0	0	0.009356725	0.029784401	0.022275653
Pir4 lineage genus OTU_10470	0	0	0.010526316	0.03131796	0.01479232
Marinomonas genus OTU_6571	0	0	0.011111111	0.039654125	0.040537449
Bythopirellula genus OTU_16095	0	0	0.011111111	0.041102417	0.047863012
Halioglobus genus OTU_17217	0	0	0.011111111	0.036585947	0.026903895
Endozoicomonas genus OTU_4728	0	0	0.012280702	0.032222169	0.00607311
PAUC43f marine benthic group class OTU_559	0	0	0.012865497	0.044042504	0.033013
BD7-8 marine group order OTU_11824	0.000666667	0.004666667	0.014035088	0.046283881	0.035871929
DEV007 family OTU_8903	0	0	0.013450292	0.039420584	0.013421602
Blastopirellula genus OTU_19733	0	0	0.014619883	0.042356302	0.012434688
SS1-B-06-26 family OTU_6378	0	0	0.014619883	0.054080131	0.047855063
Endozoicomonas genus OTU_14698	0	0	0.015204678	0.033081019	0.001107457
Halieaceae family OTU_17173	0	0	0.015204678	0.054099099	0.039953829
Muricauda genus OTU_10134	0	0	0.015204678	0.051135327	0.030119681
Endozoicomonas genus OTU_915	0	0	0.016374269	0.057944925	0.038923139
Flavobacteriaceae family OTU_10151	0	0	0.016959064	0.054477066	0.023458311
OM1 clade family OTU_22485	0	0	0.01754386	0.065219755	0.048933438
Bacteroidetes VC2.1 Bac22 class OTU_17172	0	0	0.01754386	0.056920746	0.024807783
Cellvibrionales order OTU_20446	0.011333333	0.02952212	0.029239766	0.057589722	0.044375936
Xanthomonadales order OTU_14889	0	0	0.018128655	0.060236757	0.028251786
OM60(NOR5) clade genus OTU_17209	0	0	0.018128655	0.059912274	0.027443565
Lentimicrobiaceae family OTU_16032	0	0	0.01871345	0.065788824	0.037699661

HOC36 order OTU_1228	0	0	0.01871345	0.068971148	0.047075915
Lentimicrobiaceae family OTU_16048	0	0	0.019298246	0.050705498	0.006139598
Blastopirellula genus OTU_20180	0	0	0.020467836	0.058491227	0.01133592
Halieaceae family OTU_17159	0	0	0.020467836	0.066006789	0.023982787
Planctomycetaceae family OTU_21020	0.001333333	0.009333333	0.022222222	0.070180312	0.031400091
JTB255 marine benthic group family OTU_8828	0.000666667	0.004666667	0.021637427	0.071325853	0.03229744
Pir4 lineage genus OTU_16082	0	0	0.021052632	0.061060025	0.012525969
Roseivirga genus OTU_17281	0	0	0.021052632	0.075350145	0.04109467
Endozoicomonas genus OTU_3803	0.000666667	0.004666667	0.023976608	0.061734003	0.006668888
Pseudohaliea genus OTU_17191	0.005333333	0.021457969	0.029239766	0.084019439	0.044041258
OM1 clade family OTU_22477	0.002	0.014	0.026315789	0.078419286	0.026220221
JTB255 marine benthic group family OTU_6234	0	0	0.025146199	0.070719141	0.010148661
Rhodopirellula genus OTU_21032	0.006666667	0.031269438	0.032163743	0.080921812	0.032485006
Actibacter genus OTU_8450	0	0	0.025730994	0.075449929	0.013464353
Planctomyces genus OTU_21445	0	0	0.026315789	0.082063267	0.019758342
Haliea genus OTU_17147	0.006666667	0.024944383	0.033333333	0.082834766	0.024964609
Robiginitalea genus OTU_8854	0	0	0.026900585	0.097820019	0.044263986
JTB255 marine benthic group family OTU_6186	0.000666667	0.004666667	0.028654971	0.102681133	0.046327535
OM60(NOR5) clade genus OTU_15837	0.003333333	0.019148542	0.031578947	0.093746174	0.031376956
Endozoicomonas genus OTU_8033	0.002	0.01034945	0.031578947	0.085935071	0.013282649
Haliea genus OTU_17139	0.003333333	0.019148542	0.033333333	0.10832771	0.046135274
Gammaproteobacteria class OTU_5911	0.005333333	0.028565714	0.035672515	0.093212045	0.023668679
Thiogranum genus OTU_5220	0	0	0.030994152	0.095892168	0.018849281
Planctomyces genus OTU_10035	0	0	0.031578947	0.080306686	0.00472724
Sandaracinaceae family OTU_3763	0	0	0.032748538	0.08411707	0.005128125
Flavobacteriaceae family OTU_6249	0.000666667	0.004666667	0.034502924	0.101636687	0.015821651
Blastopirellula genus OTU_20175	0	0	0.035087719	0.111942106	0.022563244

Roseibacillus genus OTU_11909	0.000666667	0.004666667	0.03625731	0.122240408	0.033699379
OM60(NOR5) clade genus OTU_17164	0	0	0.035672515	0.113573816	0.022299041
Rhodopirellula genus OTU_22060	0	0	0.035672515	0.115446358	0.02446049
OM60(NOR5) clade genus OTU_17145	0	0	0.03625731	0.092881257	0.005018373
Chromatiaceae family OTU_14895	0	0	0.03625731	0.119824547	0.027443565
Myxococcales order OTU_2220	0	0	0.037426901	0.078253393	0.000720699
E01-9C-26 marine group order OTU_2222	0.001333333	0.006531973	0.040935673	0.130229598	0.026900037
PAUC43f marine benthic group class OTU_598	0.001333333	0.006531973	0.042105263	0.105691138	0.005601092
HOC36 order OTU_12864	0.005333333	0.016812694	0.047368421	0.103721656	0.004076655
Milano-WF1B-44 class OTU_14876	0	0	0.042690058	0.138180093	0.024483207
Thiotrichaceae family OTU_6265	0.004	0.023701852	0.049707602	0.138232057	0.017945572
Planctomycetaceae family OTU_21014	0.017333333	0.044342104	0.063157895	0.158290602	0.041863866
DEV007 family OTU_10048	0	0	0.047953216	0.106333094	0.001347289
JTB255 marine benthic group family OTU_6200	0.000666667	0.004666667	0.049122807	0.123007355	0.00468501
PAUC43f marine benthic group class OTU_130	0.000666667	0.004666667	0.049707602	0.125209035	0.004909785
Desulfovibrio genus OTU_6212	0.005333333	0.021457969	0.054385965	0.131808766	0.008021728
JTB255 marine benthic group family OTU_3130	0.008	0.032359096	0.058479532	0.153995608	0.019754437
Flammeovirgaceae family OTU_12784	0.000666667	0.004666667	0.053216374	0.133604935	0.004740723
Gammaproteobacteria class OTU_2787	0.002	0.01034945	0.055555556	0.150156946	0.010097844
Flexithrix genus OTU_17150	0	0	0.057309942	0.180075183	0.020660573
Pir4 lineage genus OTU_22081	0.001333333	0.009333333	0.059064327	0.189079801	0.026309178
CS-B046 order OTU_11932	0.002666667	0.013063945	0.060818713	0.166271204	0.011615441
OM1 clade family OTU_22480	0.002	0.014	0.066081871	0.15578403	0.003330597
Actibacter genus OTU_6203	0.000666667	0.004666667	0.067251462	0.211048735	0.021765242
Rhodopirellula genus OTU_21362	0.000666667	0.004666667	0.068421053	0.226974426	0.029537632
Pseudomonas genus OTU_12790	0.012666667	0.041036569	0.080701754	0.215296828	0.023888906
Run-SP154 order OTU_11910	0.001333333	0.009333333	0.069590643	0.14818995	0.001103156

Desulforhopalus genus OTU_1780	0.000666667	0.004666667	0.073684211	0.212452205	0.012814189
Flexithrix genus OTU_14857	0.001333333	0.006531973	0.076023392	0.256209769	0.033414788
Haliea genus OTU_17127	0.002	0.014	0.078362573	0.259440937	0.032000847
Haliea genus OTU_17137	0	0	0.076608187	0.173144849	0.001631122
Marinobacter genus OTU_6206	0	0	0.077192982	0.259039905	0.029773583
Haliea genus OTU_17133	0	0	0.078362573	0.161437406	0.000610214
Parahaliea genus OTU_17146	0.006	0.042	0.085964912	0.282287189	0.040622952
Bacteroidetes BD2-2 class OTU_15999	0	0	0.081871345	0.201946684	0.003657011
Parahaliea genus OTU_14860	0.025333333	0.145382255	0.108187134	0.233982447	0.029698063
Actibacter genus OTU_5659	0	0	0.088304094	0.222206832	0.004332081
Rhodopirellula genus OTU_21010	0.004	0.028	0.092397661	0.268343961	0.017360138
Sandaracinaceae family OTU_3649	0.002666667	0.011234866	0.097660819	0.226840289	0.002774155
Winogradskyella genus OTU_6166	0	0	0.097660819	0.3405568	0.036223348
Flavobacteriaceae family OTU_6214	0.042666667	0.132839084	0.151461988	0.304294805	0.017619687
Sandaracinaceae family OTU_2218	0.013333333	0.048074017	0.123976608	0.186352533	6.27E-05
JTB255 marine benthic group family OTU_6162	0.008	0.035627705	0.119298246	0.213112767	0.00029359
Haliea genus OTU_14854	0.007333333	0.030029615	0.130409357	0.225670386	0.000158188
Family XIII family OTU_15833	0.043333333	0.176162804	0.173099415	0.370155575	0.021799466
Milano-WF1B-44 class OTU_6171	0.000666667	0.004666667	0.133333333	0.31941711	0.002959457
JTB255 marine benthic group family OTU_6174	0.000666667	0.004666667	0.140350877	0.349811246	0.00416374
Endozoicomonas genus OTU_2993	0.006	0.042	0.153216374	0.495785735	0.030922674
Halioglobus genus OTU_17131	0.000666667	0.004666667	0.159064328	0.358033265	0.001633863
Halieaceae family OTU_14855	0.001333333	0.009333333	0.166081871	0.332307851	0.000479887
Roseibacillus genus OTU_11900	0.002666667	0.009043107	0.168421053	0.366502943	0.001312372
Microbulbifer genus OTU_17121	0.017333333	0.057053581	0.187134503	0.468989806	0.00940141
Actibacter genus OTU_8128	0	0	0.170175439	0.479631283	0.010304275
Halieaceae family OTU_16709	0.012	0.035752234	0.18245614	0.351721207	0.000653874

JTB255 marine benthic group family OTU_6164	0.013333333	0.044221664	0.195906433	0.388934175	0.00094115
OM60(NOR5) clade genus OTU_17125	0	0	0.185964912	0.369391648	0.000398342
Thiogranum genus OTU_5212	0.023333333	0.07490735	0.20994152	0.337342262	0.000155601
JTB255 marine benthic group family OTU_6360	0.02	0.056960025	0.242105263	0.39556638	0.000107901
Actibacter genus OTU_6184	0.029333333	0.113654447	0.277192982	0.545409977	0.001519529
Gammaproteobacteria class OTU_6160	0.012	0.053649065	0.276023392	0.460517312	7.70E-05
Endozoicomonas genus OTU_6729	0.028	0.172994541	0.307017544	0.66540046	0.003584185
Thiohalocapsa genus OTU_11899	0	0	0.285964912	0.702958032	0.003550316
Thalassolituus genus OTU_17143	0.270666667	0.540026337	0.781871345	1.358333892	0.01145927
Gammaproteobacteria class OTU_5209	0.046	0.121085828	0.562573099	0.794843768	1.12E-05
Legionellaceae family OTU_3047	0.005333333	0.023437861	0.523976608	1.026657446	0.000383141
Endozoicomonas genus OTU_6587	0.435333333	1.012333059	0.987719298	1.259948815	0.014400915
Algibacter genus OTU_5363	0.040666667	0.284666667	0.615789474	1.466907281	0.005595957
Borrelia genus OTU_647	0.222	0.375446993	0.895906433	1.380822003	0.000824856
Photobacterium genus OTU_5	0.008	0.038620662	1.046783626	3.598439448	0.035058142
Actibacter genus OTU_5698	0.111333333	0.292317179	1.175438597	1.487393764	2.12E-06
Endozoicomonas genus OTU_1949	0.136666667	0.858221676	1.375438597	3.348168894	0.009586512
Pseudomonas genus OTU_12985	0.280666667	0.804075453	1.678362573	4.230841424	0.018411224
Borrelia genus OTU_651	0.189333333	0.661326613	1.619883041	4.297720778	0.016931285
Endozoicomonas genus OTU_1993	0.012	0.071105555	1.549707602	4.925112175	0.023086977
Flavobacteriaceae family OTU_12808	0.015333333	0.09825364	1.804093567	6.058174991	0.031264012

Supplementary Table 12: Alpha diversity (Chao1, Observed species and Shannon) for SROs in Port Stephens in two sampling times

Sampling time	**Chao1**	**Observed species**	**Shannon**
January (n = 20)	112.342424 ± 73.9134775	92.75 ± 57.0086559	3.55345452 ± 1.71306164
June (n = 30)	126.902829 ± 62.4357236	96.2333333 ± 49.7668241	3.84893344 ± 1.03875543

Supplementary Table 13: Top 10 OTUs contributing to the difference between the SRO microbiomes in January (n= 20) and June (n= 30) at Port Stephens as identified by SIMPER.

Taxon	Average dissimilarity	% Contribution
Candidatus Hepatoplasma genus OTU_14887	3.519	3.993
Vibrio genus OTU_2	2.776	3.15
Pseudoalteromonas genus OTU_8917	2.516	2.855
Mycoplasma genus OTU_14900	2.001	2.271
Cobetia genus OTU_2869	1.525	1.731
Endozoicomonas genus OTU_3829	1.517	1.722
Mycoplasma genus OTU_12669	1.454	1.651
Mycoplasma genus OTU_14937	1.388	1.575
Mycoplasma genus OTU_14921	1.217	1.381
Endozoicomonas genus OTU_6283	1.213	1.377

Supplementary Table 14: Alpha diversity (Chao1, Observed species and Shannon) for SROs in Wallis Lake in two sampling times

Sampling time	**Chao1**	**Observed species**	**Shannon**
January (n = 50)	148.608752 ± 75.5505276	114.678571 ± 59.701761	4.2194837 ± 1.31993987
June (n = 57)	172.222087 ± 139.253512	142.551724 ± 106.237465	4.50197369 ± 1.58585561

Supplementary Table 15: Top 10 OTUs contributing to the difference between the SRO microbiomes in January (n= 29) and June (n= 30) at Wallis Lake as identified by SIMPER.

Taxon	Average dissimilarity	% Contribution
Candidatus Hepatoplasma genus OTU_14887	2.562	3.129
Endozoicomonas genus OTU_1831	1.881	2.297
Endozoicomonas genus OTU_3829	1.226	1.498
Mycoplasma genus OTU_14921	1.119	1.368
Endozoicomonas genus OTU_6283	1.028	1.256
Mycoplasma genus OTU_14937	0.9543	1.166
Pseudomonas genus OTU_12985	0.824	1.007
Endozoicomonas genus OTU_3483	0.7892	0.9641
Endozoicomonas genus OTU_4530	0.7547	0.9219
Borrelia genus OTU_651	0.7296	0.8913

Supplementary Table 16: OTUs significantly different between January and June at Port Stephens (Welch's t-test; $p < 0.05$) as identified by STAMP.

Taxonomy	January (n = 20)		June (n = 30)		p-values
	Mean (%)	Std. dev. (%)	Mean (%)	Std. dev. (%)	
Vibrio genus OTU_2	0.07333333	0.18184242	11.7755556	6.84643694	4.14E-10
Pseudoalteromonas genus OTU_8917	0.00333333	0.01452966	9.49333333	8.74758549	2.46E-06
Pseudoalteromonas genus OTU_16987	0	0	0.22444444	0.24674673	3.36E-05
Aliivibrio genus OTU_9	0	0	0.44	0.5922587	0.00039926
Vibrio genus OTU_152	0.00166667	0.00726483	0.34555556	0.46559268	0.00042567
Aeromonas genus OTU_3790	0.00166667	0.00726483	0.47555556	0.64572287	0.00045579
Marinilabiaceae family OTU_2173	0	0	3.48555556	5.11873598	0.00098005
Shewanella genus OTU_1350	0	0	0.21222222	0.31487603	0.00108265
Prolixibacter genus OTU_5526	0.025	0.0947951	1.67222222	2.45719026	0.00114717
Sphaerochaeta genus OTU_2780	0	0	0.79444444	1.21892738	0.00148559
Marinomonas genus OTU_4117	0.00166667	0.00726483	1.87333333	2.87367876	0.00149499
Rhodothermaceae family OTU_16054	0.12833333	0.15102428	0.00666667	0.02	0.00237708
Bacillus genus OTU_10	0	0	0.31111111	0.51433764	0.00286485
Vibrio genus OTU_135	0	0	0.08555556	0.14290202	0.00312004
Pseudoalteromonas genus OTU_3634	0.00166667	0.00726483	0.66444444	1.10744729	0.00313039
Aliivibrio genus OTU_428	0	0	0.16555556	0.27987872	0.00344387
Flavobacteriaceae family OTU_6157	2.23333333	2.74899982	0.14444444	0.44207703	0.00377152
Candidatus Hepatoplasma genus OTU_14887	26.545	33.3748879	1.4	2.56279651	0.00392881
Pseudoalteromonas genus OTU_15862	0	0	0.13333333	0.23538778	0.00484766
Desulfovibrio genus OTU_8837	0	0	0.08444444	0.14950123	0.00495362

Vibrio genus OTU_13	0	0	0.25111111	0.45393207	0.00579428
Desulfuromusa genus OTU_20170	0	0	0.05333333	0.09760085	0.0063413
Sphaerochaeta genus OTU_3805	0	0	0.03	0.05534404	0.00672214
Fusibacter genus OTU_18286	0.21833333	0.31331117	0.00111111	0.00598352	0.00701323
Marinifilum genus OTU_2242	0	0	0.06333333	0.11936561	0.00782568
Aquibacter genus OTU_12017	3.31333333	4.88946032	0	0	0.00815325
Cobetia genus OTU_2869	0.04666667	0.16069294	5.11777778	9.8021794	0.00931862
Maribacter genus OTU_6371	0.37166667	0.54234829	0.01888889	0.07387845	0.01080739
Pseudoalteromonas genus OTU_12646	0	0	0.14222222	0.28361859	0.01143848
Planococcaceae family OTU_1218	0	0	0.13777778	0.27711922	0.01208488
Vibrio genus OTU_2647	0	0	0.07666667	0.15471599	0.01234276
Marinomonas genus OTU_2209	0	0	0.79111111	1.60685876	0.01285812
Mycoplasma genus OTU_12669	0.00166667	0.00726483	7.93333333	16.1417517	0.0130164
Porphyromonadaceae family OTU_5211	0	0	0.49	1.02042656	0.0150029
Shewanella genus OTU_3635	0.03166667	0.1380318	1.14555556	2.32959197	0.01558271
Mycoplasma genus OTU_14900	2.15666667	6.79051708	9.1	12.4363922	0.01629507
Pseudomonas genus OTU_12985	0.69833333	1.15125415	0.00222222	0.01196703	0.01629736
Maribacter genus OTU_2618	1.16	1.88569468	0.02444444	0.08341478	0.01671088
Gammaproteobacteria class OTU_3922	0	0	0.00777778	0.01651785	0.01686916
Colwellia genus OTU_3670	0	0	0.89777778	1.91361464	0.01723576
Endozoicomonas genus OTU_1685	0	0	0.01	0.02134375	0.01737236
Vibrio genus OTU_4046	0	0	0.02555556	0.05489609	0.01803388
Bacillus genus OTU_736	0	0	0.03333333	0.07252075	0.01940227
Pseudoalteromonas genus OTU_3490	0	0	0.03222222	0.07017615	0.0195171
Vibrio genus OTU_8	0.085	0.32599847	0.38555556	0.53890722	0.02006382

Flammeovirga genus OTU_14853	0	0	0.29777778	0.65262622	0.02022597
Planctomyces genus OTU_22498	0.05166667	0.09035424	0	0	0.02208576
Polaribacter 2 genus OTU_7709	0	0	0.10555556	0.23508601	0.02211713
Vibrio genus OTU_482	0	0	0.00555556	0.0124226	0.02260838
Candidatus Actinomarina genus OTU_22961	0.15833333	0.26201675	0.00888889	0.02712568	0.0226524
Psychrobacter genus OTU_6015	0	0	0.08	0.1810668	0.02414367
Moritella genus OTU_2212	0	0	0.21888889	0.49663187	0.02446239
CA002 family OTU_12	0.28166667	0.5050275	0	0	0.02512418
Legionellaceae family OTU_1781	0.12	0.2171533	0	0	0.02632138
Marinomonas genus OTU_2274	0	0	0.03	0.06957543	0.02745781
Mycoplasma genus OTU_3843	0	0	0.02111111	0.04905276	0.02772666
Bacillus genus OTU_731	0	0	0.08111111	0.18947947	0.02850292
Vibrio genus OTU_55	0	0	0.01444444	0.03408414	0.03000046
Aquibacter genus OTU_9355	0.19833333	0.36915444	0	0	0.03023415
Pseudomonas genus OTU_12790	0.03166667	0.0600694	0	0	0.03309658
Portibacter genus OTU_14916	0.02166667	0.04119736	0	0	0.0334662
Cellulophaga genus OTU_12104	2.095	3.98478042	0	0	0.03351798
Hyunsoonleella genus OTU_13103	0.91833333	1.74357471	0.00222222	0.00831479	0.03361737
Borrelia genus OTU_536	0.05166667	0.09858724	0	0	0.03402379
Flavobacteriaceae family OTU_11913	0.16	0.3067029	0	0	0.03475647
Vibrio genus OTU_514	0.02333333	0.08171767	0.09555556	0.14775689	0.03484676
Tenacibaculum genus OTU_6227	0	0	0.01	0.02457038	0.03657796
Shewanella genus OTU_3645	0	0	0.14555556	0.35864801	0.03707205
Polaribacter 2 genus OTU_13000	0.51166667	0.58254947	0.19	0.32988775	0.03749057
Microbulbifer genus OTU_17121	0	0	0.02888889	0.07135375	0.03749674

JTB215 family OTU_19717	0.005	0.02179449	0.11111111	0.26138284	0.03777947
Bacillus genus OTU_747	0	0	0.02111111	0.05269291	0.03938428
Mycoplasma genus OTU_18779	0.00166667	0.00726483	0.07555556	0.18477881	0.03991243
Flavobacteriaceae family OTU_15116	0.07	0.12688578	0.00555556	0.01940472	0.04022887
Endozoicomonas genus OTU_3829	7.31833333	9.03946578	2.61777778	3.28327674	0.04052892
Endozoicomonas genus OTU_3483	2.54333333	3.61374075	0.67111111	1.28209127	0.04094876
Marinilabiaceae family OTU_10021	0	0	0.19555556	0.49409351	0.04165937
Marinilabiaceae family OTU_13177	0	0	0.02111111	0.05339117	0.04184315
Alteromonadaceae family OTU_12804	0	0	0.02111111	0.05339117	0.04184315
Vibrio genus OTU_185	0	0	0.00444444	0.01133115	0.04339742
Marinomonas genus OTU_2558	0	0	0.00444444	0.01133115	0.04339742
Arcobacter genus OTU_22541	0	0	0.00444444	0.01133115	0.04339742
Flavobacteriaceae family OTU_13485	0	0	0.05444444	0.13943891	0.04429109
Marinomonas genus OTU_3726	0.00166667	0.00726483	0.06111111	0.1525301	0.0449461
Planococcus genus OTU_1244	0	0	0.01777778	0.04613453	0.04694886
Mycoplasma genus OTU_18534	0.00333333	0.01	0.04444444	0.10657403	0.04787522
Sandaracinaceae family OTU_2854	0	0	0.03	0.07857528	0.04887679
Mycoplasma genus OTU_18633	0	0	0.12333333	0.32376832	0.04935947

Supplementary Table 17: OTUs significantly different between January and June at Wallis Lake (Welch's t-test; $p < 0.05$) as identified by STAMP.

Taxonomy	January		June		p-values
	Mean (%)	Std. dev. (%)	Mean (%)	Std. dev. (%)	
Mycoplasma genus OTU_14937	4.50357143	10.1317955	0.05862069	0.21878368	0.03077788
Photobacterium genus OTU_3	4.04285714	8.46369397	0.0183908	0.06347909	0.02008876
Pseudomonas genus OTU_12985	3.33571429	5.56688714	0.07816092	0.22011419	0.00521913
Photobacterium genus OTU_5	2.08452381	4.91988581	0.04482759	0.1916406	0.04041538
Legionellaceae family OTU_3047	1.05595238	1.26013521	0.01034483	0.0382605	0.00019363
Roseibacillus genus OTU_11900	0.34285714	0.46221148	0	0	0.00064975
Candidatus Actinomarina genus OTU_22961	0.26071429	0.52190536	0.0045977	0.01902637	0.01681046
Pseudomonas genus OTU_12790	0.16309524	0.28456864	0.00114943	0.00608219	0.0063881
Flexithrix genus OTU_17150	0.10833333	0.24601991	0.00804598	0.01888698	0.04395762
DEV007 family OTU_10048	0.09285714	0.13667164	0.0045977	0.01902637	0.00247847
Roseibacillus genus OTU_11909	0.07380952	0.16627505	0	0	0.02898624
Planctomyces genus OTU_10035	0.06428571	0.10500513	0	0	0.0036685
Delftia genus OTU_15997	0.05238095	0.12953781	0	0	0.04509875
DEV007 family OTU_8903	0.02738095	0.05274495	0	0	0.01189332
Marinomonas genus OTU_6571	0.02261905	0.05422877	0	0	0.03919661
Vibrio genus OTU_342	0.01309524	0.03253726	0	0	0.0460436
Legionella genus OTU_16227	0.00714286	0.01632299	0	0	0.03114393
Legionella genus OTU_23158	0.00714286	0.01632299	0	0	0.03114393
Vibrio genus OTU_157	0	0	0.0045977	0.01149425	0.04331307
Endozoicomonas genus OTU_4728	0.00119048	0.0061859	0.02298851	0.04207587	0.01110662

Planctomycetaceae family OTU_21455	0	0	0.02183908	0.05415733	0.0417565
BD7-8 marine group order OTU_6721	0	0	0.02643678	0.06025445	0.02774888
Gammaproteobacteria class OTU_3922	0.00238095	0.00858465	0.02988506	0.05490837	0.01384709
BD7-8 marine group order OTU_11824	0	0	0.02758621	0.06194112	0.02566983
Blastopirellula genus OTU_20194	0	0	0.03448276	0.08045977	0.03125186
Psychromonas genus OTU_3657	0	0	0.03908046	0.09511798	0.03830781
JTB255 marine benthic group family OTU_6234	0.0047619	0.01934295	0.04482759	0.09316728	0.0335203
Roseivirga genus OTU_17281	0	0	0.04137931	0.10157955	0.03986281
Flammeovirga genus OTU_14853	0.00119048	0.0061859	0.04597701	0.10335884	0.02979287
Myxococcales order OTU_2220	0.01309524	0.04303864	0.06091954	0.09551994	0.02084148
Rhodopirellula genus OTU_21032	0.00714286	0.02246186	0.05632184	0.10580958	0.0224713
Sandaracinaceae family OTU_3763	0.00595238	0.02191899	0.05862069	0.10991295	0.01870142
PAUC43f marine benthic group class OTU_598	0.01309524	0.03709628	0.07011494	0.13795019	0.04277275
Gammaproteobacteria class OTU_5911	0.0047619	0.01467718	0.06551724	0.1227035	0.01452
Thiogranum genus OTU_5220	0	0	0.06091954	0.12747743	0.01736398
Gammaproteobacteria class OTU_2787	0.01547619	0.05806547	0.09425287	0.19496909	0.04877013
Planctomycetaceae family OTU_21014	0.01547619	0.03616776	0.1091954	0.20897424	0.02636484
Sandaracinaceae family OTU_2218	0.06785714	0.12550917	0.17816092	0.2169584	0.02503926
Marinobacter genus OTU_6206	0.00119048	0.0061859	0.15057471	0.34769307	0.03088477
Parahaliea genus OTU_14860	0.03095238	0.07555539	0.18275862	0.30128978	0.01462227
Winogradskyella genus OTU_6166	0.00357143	0.01855769	0.18850575	0.45915764	0.04211548
Vibrio genus OTU_2	0.3047619	0.86994409	1.22643678	1.67536533	0.01365412
Endozoicomonas genus OTU_1993	0.01428571	0.03926767	3.03218391	6.57279009	0.02178441
Candidatus Hepatoplasma genus OTU_14887	0.02261905	0.03456484	17.3712644	23.9846386	0.00066609

Supplementary Table 18: Top 10 OTUs contributing to the difference between the SRO microbiomes in QX resistance groups in Port Stephens as identified by SIMPER.

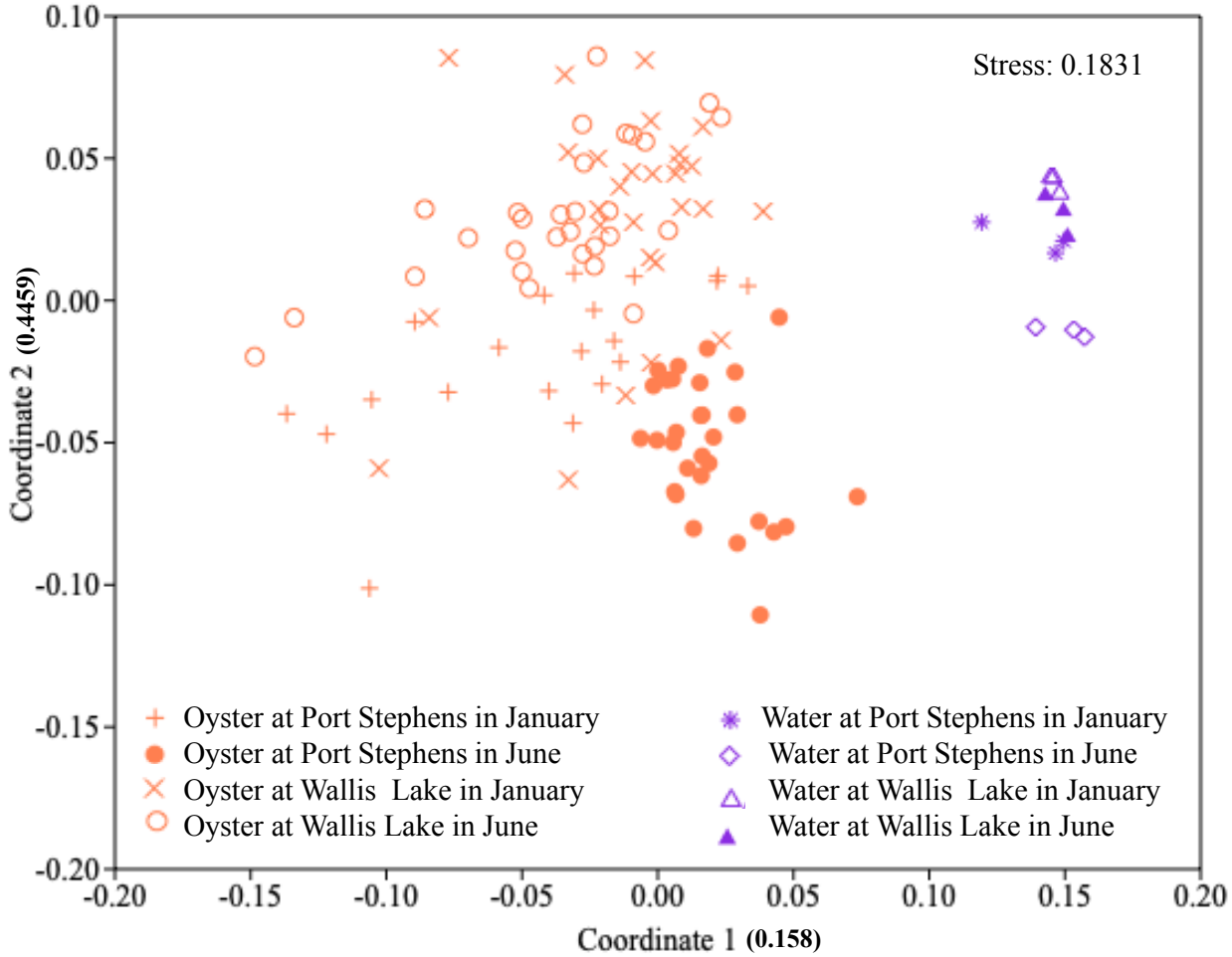
Taxon	Average dissimilarity	% Contribution
Resistant vs susceptible group in January (Overall average dissimilarity: 77.42%)		
Candidatus Hepatoplasma genus OTU_14887	2.871	3.709
Endozoicomonas genus OTU_3829	2.178	2.813
Endozoicomonas genus OTU_6283	1.582	2.043
Arcobacter genus OTU_6697	1.573	2.032
Mycoplasma genus OTU_14921	1.465	1.892
Endozoicomonas genus OTU_4530	1.215	1.569
Algitalea genus OTU_10007	1.189	1.536
Endozoicomonas genus OTU_3483	1.141	1.474
Mycoplasma genus OTU_14937	1.101	1.422
Mycoplasma genus OTU_14900	0.9998	1.291
Intermediate vs susceptible group in January(Overall average dissimilarity: 78.52%)		
Candidatus Hepatoplasma genus OTU_14887	3.814	4.857
Endozoicomonas genus OTU_3829	2.371	3.02
Endozoicomonas genus OTU_6283	1.697	2.161
Mycoplasma genus OTU_14937	1.466	1.867
Endozoicomonas genus OTU_4530	1.33	1.693
Aquibacter genus OTU_12017	1.309	1.667
Algitalea genus OTU_10007	1.264	1.61
Cellulophaga genus OTU_12104	1.263	1.608
Endozoicomonas genus OTU_3483	1.118	1.423
Mycoplasma genus OTU_14921	1.062	1.353
Resistant vs susceptible group in June (Overall average dissimilarity: 73.61%)		
Mycoplasma genus OTU_14900	2.132	2.897

Mycoplasma genus OTU_12669	1.768	2.402
Mycoplasma genus OTU_14937	1.629	2.213
Vibrio genus OTU_2	1.51	2.051
Pseudoalteromonas genus OTU_8917	1.374	1.867
Vibrio genus OTU_1	1.282	1.741
Marinilabiaceae family OTU_2173	1.207	1.639
Endozoicomonas genus OTU_3829	1.109	1.506
Guggenheimella genus OTU_18622	1.08	1.467
Endozoicomonas genus OTU_6283	1.042	1.415
Intermediate vs susceptible group In June (Overall average dissimilarity: 70.05%)		
Mycoplasma genus OTU_14900	1.845	2.616
Mycoplasma genus OTU_12669	1.766	2.504
Cobetia genus OTU_2869	1.614	2.29
Pseudoalteromonas genus OTU_8917	1.345	1.908
Mycoplasma genus OTU_14937	1.319	1.871
Mycoplasma genus OTU_14921	1.271	1.803
Guggenheimella genus OTU_18622	1.171	1.661
Marinilabiaceae family OTU_2173	1.094	1.552
Tenacibaculum genus OTU_15085	0.993	1.408
Candidatus Hepatoplasma genus OTU_14887	0.933	1.323

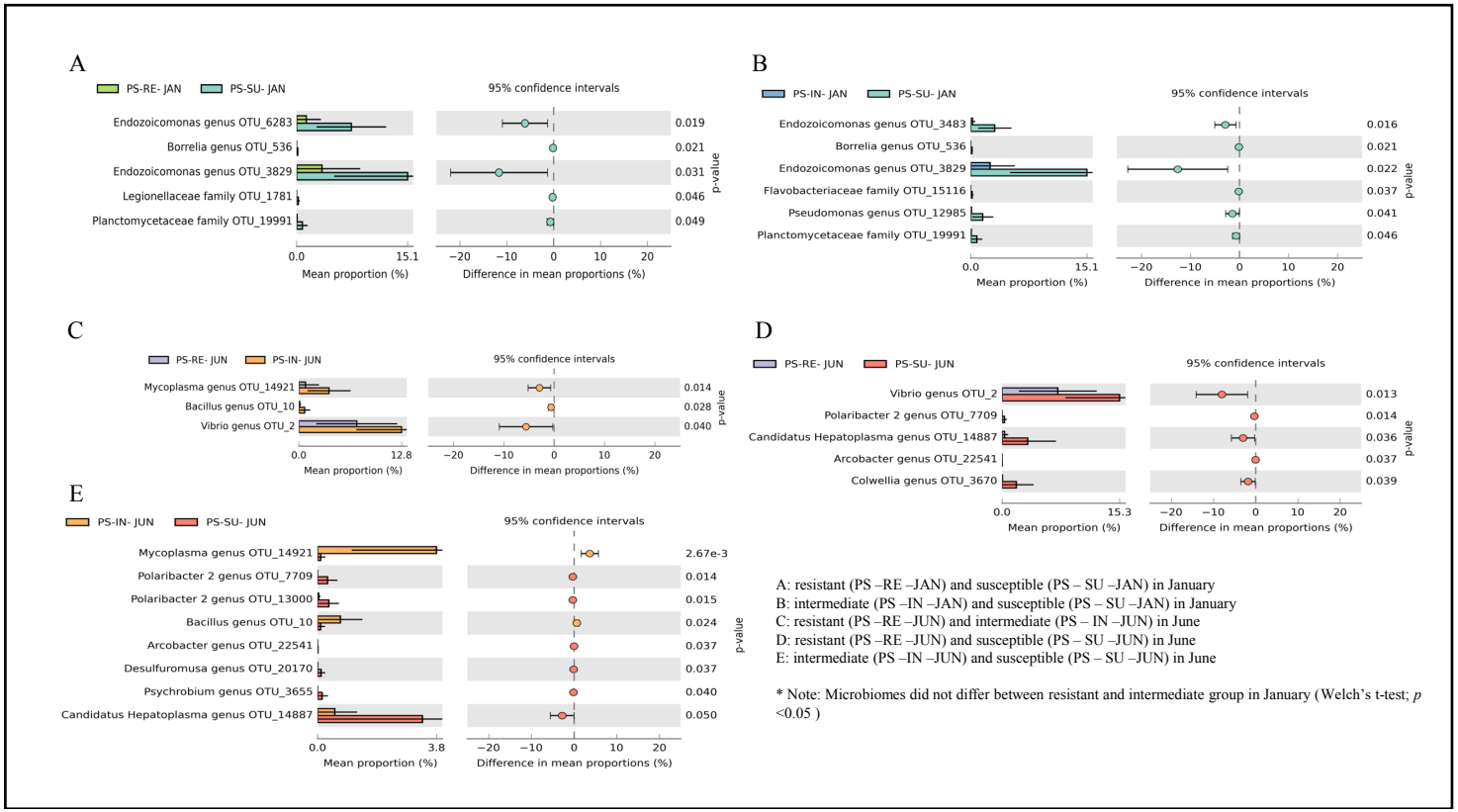
Supplementary Table 19: Top 10 OTUs contributing to the difference between the microbiomes of QX resistance groups in Wallis Lake as identified by SIMPER.

Taxon	Average dissimilarity	% Contribution
Resistant vs susceptible group in January (Overall average dissimilarity: 79.5%)		
Photobacterium genus OTU_3	1.634	2.056
Endozoicomonas genus OTU_1831	1.538	1.934
Endozoicomonas genus OTU_3829	1.414	1.778
Borrelia genus OTU_651	1.393	1.753
Pseudomonas genus OTU_12985	1.279	1.608
Mycoplasma genus OTU_14937	1.249	1.571
Endozoicomonas genus OTU_6283	1.117	1.405
Robiginitalea genus OTU_10008	0.9825	1.236
Photobacterium genus OTU_5	0.9114	1.146
Comamonadaceae family OTU_18670	0.9027	1.136
Intermediate vs susceptible group in January (Overall average dissimilarity: 79.55%)		
Mycoplasma genus OTU_14921	2.098	2.637
Arcobacter genus OTU_6697	1.841	2.315
Endozoicomonas genus OTU_3829	1.682	2.114
Mycoplasma genus OTU_14937	1.601	2.012
Endozoicomonas genus OTU_1831	1.586	1.993
Endozoicomonas genus OTU_6283	1.523	1.914
Pseudomonas genus OTU_12985	1.06	1.333
Endozoicomonas genus OTU_4530	1.006	1.265
Endozoicomonas genus OTU_3483	0.9405	1.182
Comamonadaceae family OTU_18670	0.8647	1.087
Resistant vs intermediate group in June (Overall average dissimilarity: 77.43%)		
Candidatus Hepatoplasma genus OTU_14887	2.064	2.666

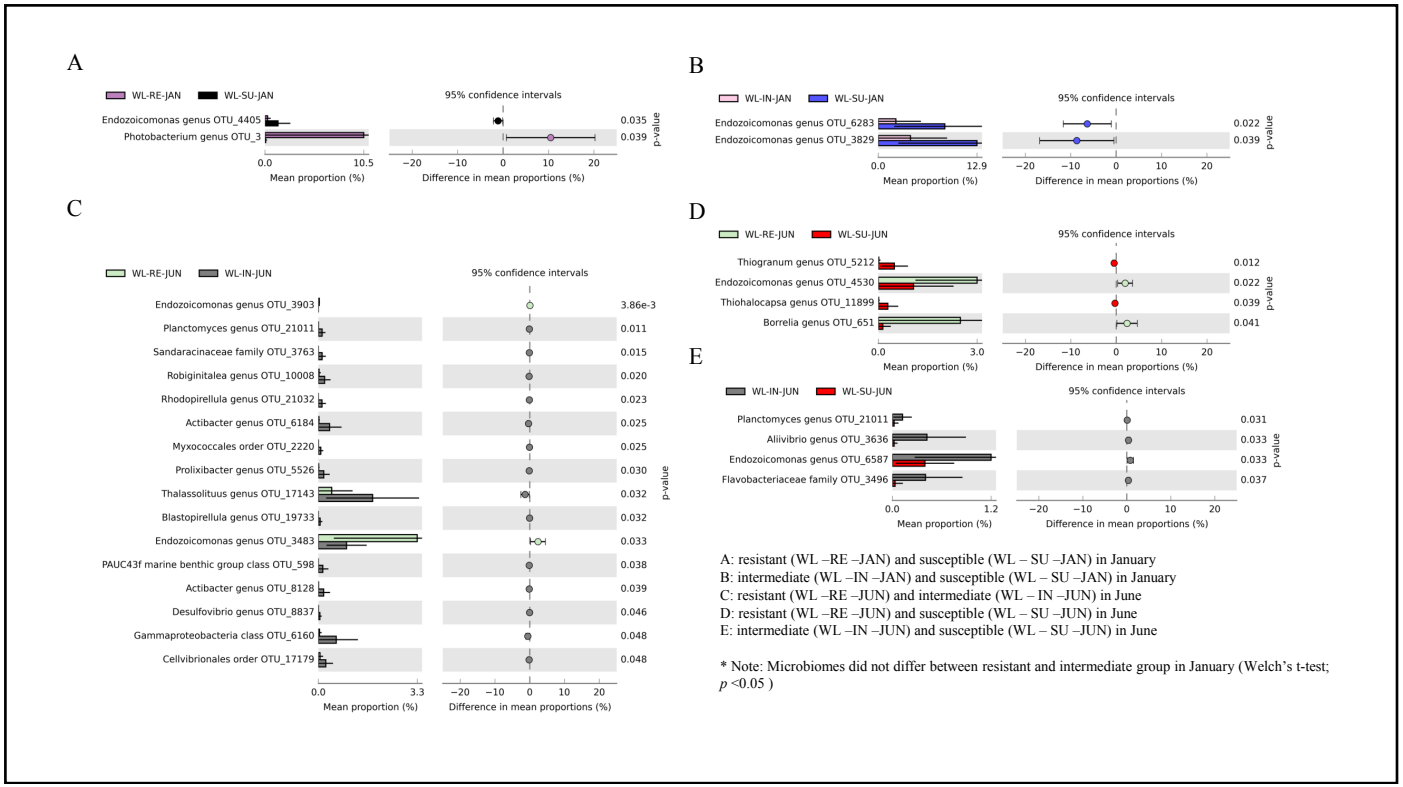
Endozoicomonas genus OTU_1831	1.489	1.923
Endozoicomonas genus OTU_3829	1.11	1.434
Endozoicomonas genus OTU_1993	0.9879	1.276
Endozoicomonas genus OTU_6283	0.8791	1.135
Endozoicomonas genus OTU_1949	0.864	1.116
Gammaproteobacteria class OTU_6670	0.7923	1.023
Endozoicomonas genus OTU_3483	0.7302	0.9431
Flavobacteriaceae family OTU_12808	0.6755	0.8724
Endozoicomonas genus OTU_4530	0.6646	0.8584



Supplementary Figure 3: nMDS plot showing the microbiome of oyster and estuarine water sampl



Supplementary Figure 4: Extended error bar plot showing OTUs differing significantly between QX resistance groups in Port Stephens in January and June



A: resistant (WL –RE –JAN) and susceptible (WL – SU –JAN) in January
B: intermediate (WL –IN –JAN) and susceptible (WL – SU –JAN) in January
C: resistant (WL –RE –JUN) and intermediate (WL – IN –JUN) in June
D: resistant (WL –RE –JUN) and susceptible (WL – SU –JUN) in June
E: intermediate (WL –IN –JUN) and susceptible (WL – SU –JUN) in June

* Note: Microbiomes did not differ between resistant and intermediate group in January (Welch's t-test; $p < 0.05$)

Supplementary Figure 5: Extended error bar plot showing OTUs differing significantly between QX resistance groups in Wallis Lake in January and June

Supplementary Table 20: Mann-Whitney pairwise comparisons between sampling locations for bacterial loads as determined by a 16S rRNA-specific qPCR assay.

Location	Clyde river	Georges river	Hawkesbury river	Port Stephens	Shoalhaven
Georges river	0.12 (3823)				
Hawkesbury river	0.63 (4190)	0.15 (4829)			
Port Stephens	0.15 (3648)	0.86 (5123)	0.073 (4397)		
Shoalhaven	<0.001 (734)	<0.001 (1290)	<0.001 (603)	<0.001 (1264)	
Wapengo	<0.001 (1171)	<0.001 (1750)	<0.001 (1238)	<0.001 (1718)	0.34 (5360)

Supplementary Table 21: Mann-Whitney pairwise comparisons between tissue types for bacterial loads as determined by a 16S rRNA-specific qPCR assay.

Tissue type	Mantle	Gill	Adductor muscle
Gill	<0.001 (8904)		
Adductor muscle	<0.001 (6871)	<0.001 (>10000)	
Digestive gland	<0.001 (2775)	<0.001 (4433)	<0.001 (7359)

Supplementary Table 22: Mann-Whitney pairwise comparisons within sampling locations for bacterial loads as determined by a 16S rRNA-specific qPCR assay.

Clyde River	Comparison	p-value (U-value)	Georges River	Comparison	p-value (U-value)
Adductor muscle	Digestive gland	0.0081 (26)	Adductor muscle	Digestive gland	0.04 (253)
	Gill	0.0047 (170)		Gill	0.19 (258)
	Mantle	<0.001 (159)		Mantle	<0.001 (77)
Digestive gland	Gill	<0.001 (1)	Digestive gland	Gill	0.009 (152)
	Mantle	<0.001 (9)		Mantle	<0.001 (34)
Gill	Mantle	0.0047 (156)	Gill	Mantle	0.0016 (146)
Hawkesbury River	Comparison	p-value (U-value)	Port Stephens	Comparison	p-value (U-value)
Adductor muscle	Digestive gland	0.79 (402)	Adductor muscle	Digestive gland	<0.001 (105)
	Gill	0.0063 (213)		Gill	0.87 (338)
	Mantle	<0.001 (81)		Mantle	<0.001 (176)
Digestive gland	Gill	0.0095 (204)	Digestive gland	Gill	<0.001 (85)
	Mantle	<0.001 (78)		Mantle	<0.001 (52)
Gill	Mantle	0.045 (171)	Gill	Mantle	0.0011 (158)
Shoalhaven	Comparison	p-value (U-value)	Wapengo	Comparison	p-value (U-value)
Adductor muscle	Digestive gland	0.0074 (237)	Adductor muscle	Digestive gland	<0.001 (7)
	Gill	0.25 (358)		Gill	<0.001 (156)
	Mantle	0.0083 (271)		Mantle	<0.001 (76)
Digestive gland	Gill	<0.001 (115)	Digestive gland	Gill	<0.001 (2)
	Mantle	<0.001 (81)		Mantle	<0.001 (0)
Gill	Mantle	0.077 (318)	Gill	Mantle	0.033 (283)

Supplementary Table 23: Kruskal-Wallis comparisons between sampling locations. Results are presented in the following order; Shannon's diversity index, species evenness, and observed species (species richness). Significant results are also presented with a H-value.

Location	Clyde river	Georges river	Hawkesbury river	Port Stephens	Shoalhaven
Georges river	0.89 0.36 0.23				
Hawkesbury river	0.001 (10.8) 0.03 (4.5) <0.001 (12.3)	0.001 (10.2) 0.003 (8.5) 0.016 (5.8)			
Port Stephens	0.55 0.06 0.041 (4.2)	0.61 0.18 0.40	0.002 (9.6) <0.001 (11.7) 0.062		
Shoalhaven	<0.001 (29.9) 0.022 (5.2) <0.001 (45.5)	<0.001 (26.3) <0.001 (12.9) <0.001 (33.6)	0.07 0.48 <0.001 (23.2)	<0.001 (21.1) <0.001 (10.9) <0.001 (31.9)	
Wapengo	<0.001 (34.6) 0.006 (7.3) <0.001 (43.7)	<0.001 (30.7) <0.001 (16.7) <0.001 (32)	0.036 (4.4) 0.73 <0.001 (20.3)	<0.001 (24.1) <0.001 (13.9) <0.001 (30)	0.93 0.71 0.92

Supplementary Table 24: Kruskal-Wallis comparisons between tissue types. Results are presented in the following order; Shannon's diversity index, species evenness, and observed species (species richness). Significant results are also presented with a H-value.

Tissue type	Mantle	Gill	Adductor muscle
Gill	<0.001 (15.1) <0.001 (12.1) 0.004 (8.2)		
Adductor muscle	0.003 (9.1) 0.002 (9.3) 0.18	0.41 0.63 0.31	
Digestive gland	<0.001 (23.5) <0.001 (11) <0.001 (21.8)	0.16 0.72 0.009 (6.9)	0.03 (4.7) 0.52 0.004 (8.2)

Supplementary Table 26: Observed species for SRO in different sequence depths

Sequences per sample	Number of sample remaining	Observed species
1,000	262	87.98854962 ± 56.93065506
1,500	230	101.5347826 ± 65.89787062
2,000	210	111.3619048 ± 72.91046142
2,500	194	119.4845361 ± 119.4845361
3,000	183	124.284153 ± 83.18380363

Supplementary Table 17: Comparison observed species for SRO in different sequence depths (Kruskal-Wallis ANOVA test)

Comparison	H (chi2)	Hc (tie corrected)	p value
1,000 vs 1,500 sequences per sample	4.795	4.796	0.02853
1,000 vs 2,00 sequences per sample	11.82	11.82	0.0005865
1,000 vs 2,500 sequences per sample	11.71	11.71	2.57E -05
1,000 vs 3,000 sequences per sample	21.29	21.29	3.95E - 06
1,500 vs 2,000 sequences per sample	1.671	1.671	0.1962
1,500 vs 2,500 sequences per sample	4.496	4.496	0.03397
1,500 vs 3,000 sequences per sample	6.641	6.642	0.00996
2,000 vs 2,500 sequences per sample	0.7463	0.7464	0.3876
2,000 vs 3,000 sequences per sample	1.751	1.751	0.1857

Supplementary Table 28: Alpha diversity (Chao1, Observed species and Shannon) for SRO in before and during the QX disease event

Sample	Chao1	Observed species	Shannon
Before QX disease event (n = 16)	148.251994 ± 93.312472	116.732759 ± 72.6392345	3.58953578 ± 1.73200282
During the QX disease event (n = 36)	137.508816 ± 104.438213	104.734043 ± 73.085776	3.54434796 ± 1.59336403

Supplementary Table 29: Top 10 OTUs displayed their dissimilarity and distribution to the difference between microbiomes in before (n= 116) and during the QX disease event (n= 94) as identified by SIMPER. Overall dissimilarity is 85.27%

Taxon	Av. dissim	Contribution (%)
Mycoplasma genus (OTU_11355)	3.04	3.565
Candidatus Hepatoplasma genus (OTU_11357)	3.033	3.557
Arcobacter genus (OTU_17190)	2.253	2.642
Borrelia genus (OTU_1)	1.503	1.763
Mycoplasma genus (OTU_11453)	1.261	1.479
Cellulophaga genus (OTU_9296)	0.9435	1.106
Endozoicomonas genus (OTU_2823)	0.7161	0.8397
Mycoplasmataceae family (OTU_376)	0.69	0.8092
Polaribacter 4 genus (OTU_9546)	0.6893	0.8084
Mycoplasma genus (OTU_11533)	0.6698	0.7855

Supplementary Table 30: OTUs significantly different between before and during the QX disease event (Welch's t-test; p <0.05) as identified by STAMP.

Taxon	Before QX disease event		During the QX disease event		p-values
	Mean (%)	dev. (%)	Mean (%)	std. dev (%)	
Vibrio genus (OTU_3226)	0.30775862	0.69495111	0	0	5.94E-06
Mycoplasma genus (OTU_11453)	5.19181034	9.19294131	1.17446809	3.6050035	3.05E-05
Winogradskyella genus (OTU_12137)	0.00086207	0.00924466	0.19840426	0.43907859	3.65E-05
Flavobacteriaceae family (OTU_5043)	0.02284483	0.10471477	0.21861702	0.48881011	0.0002554
Maribacter genus (OTU_2121)	0.88448276	1.70080374	0.23138298	0.81020696	0.00036192
Flavobacteriaceae family (OTU_4366)	2.17974138	6.54020384	0.03244681	0.15380069	0.00061985
Algitalea genus (OTU_8064)	1.64396552	3.86863579	0.31755319	1.11574103	0.00062435
Borrelia genus (OTU_1)	4.85948276	5.81212286	9.56117021	11.9757116	0.00070974
Mycoplasma genus (OTU_14055)	0.15086207	0.32536338	0.0356383	0.14778909	0.00087198
Ambiguous_taxa genus (OTU_15557)	0.31637931	0.50304143	0.95212766	1.73382977	0.00088649
Desulfopila genus (OTU_2533)	0.15948276	0.4935757	0.00478723	0.01796739	0.00106318
Endozoicomonas genus (OTU_2823)	1.63232759	3.2201966	0.42553191	2.01002467	0.00114266
Bythopirellula genus (OTU_12195)	0.02025862	0.05980516	0.00159574	0.00878868	0.00125918
Halieaceae family (OTU_13130)	0.04181034	0.13178875	0.00106383	0.0102592	0.00126917
Cytophagaceae family (OTU_10166)	0.00818966	0.02451597	0.00053191	0.0051296	0.0014177
Uncultured bacterium genus (OTU_113)	0.90775862	1.34129703	0.40638298	0.92968864	0.0017316
PAUC43f marine benthic group class (OTU_16)	0.05689655	0.12245628	0.01648936	0.05427878	0.00179317
Flavobacteriaceae family (OTU_6960)	0.0112069	0.03776413	0	0	0.00187866
Legionella genus (OTU_3223)	0.20517241	0.66314231	0.00797872	0.05520908	0.00191387
Ambiguous_taxa genus (OTU_4769)	0.15818966	0.29316524	0.05691489	0.16576725	0.00198769
Halioglobus genus (OTU_12641)	0.11293103	0.25873704	0.02978723	0.10602066	0.00204129
Bacillus genus (OTU_46)	0.01896552	0.06004853	0.00106383	0.0102592	0.00210966

Legionella genus (OTU_5020)	0.13362069	0.44435035	0.0037234	0.02216879	0.00221242
Aureispira genus (OTU_9355)	0.01077586	0.0458662	0.06968085	0.17658373	0.00225522
Bacillus genus (OTU_848)	0.025	0.07146858	0.0037234	0.01669399	0.00244754
Flavobacteriaceae family (OTU_11405)	0.01163793	0.03496165	0.00106383	0.0102592	0.00246946
Aquibacter genus (OTU_5525)	1.06163793	2.41030794	0.30797872	0.95706508	0.00254392
Persicirhabdus genus (OTU_9356)	0.21336207	0.59543369	0.03776596	0.12978832	0.00258454
Photobacterium genus (OTU_2036)	0.18491379	0.6442792	0	0	0.00260719
Algitalea genus (OTU_7154)	0.01508621	0.04834316	0.00106383	0.00721524	0.00265375
Rubripirellula genus (OTU_16571)	0.05775862	0.16733245	0.00904255	0.03417941	0.00283491
Flavobacteriaceae family (OTU_8256)	0.0137931	0.04894852	0	0	0.00309736
Bacillus genus (OTU_937)	0.00775862	0.02508902	0.00053191	0.0051296	0.00313324
Mycoplasmataceae family (OTU_538)	0.03362069	0.11592504	0.00106383	0.0102592	0.00332631
Uncultured bacterium genus (OTU_3233)	0	0	0.05425532	0.17407194	0.00340546
endosymbionts genus (OTU_3239)	0.00732759	0.05770552	0.10585106	0.31317322	0.00349442
Blastopirellula genus (OTU_15942)	0.02543103	0.07864578	0.00319149	0.01757735	0.00385583
Flavobacteriaceae family (OTU_12138)	0.10043103	0.27757382	0.01914894	0.11135203	0.00469887
Halieaceae family (OTU_13060)	0.04008621	0.13150649	0.00425532	0.02381173	0.00490701
Desulfovibrio genus (OTU_6923)	0.00301724	0.02481726	0.14574468	0.47734079	0.0049262
Coxiella genus (OTU_5040)	0.06982759	0.26145146	0	0	0.00497249
Ambiguous_taxa genus (OTU_16251)	0	0	0.05425532	0.18213561	0.00504165
Thalassotalea genus (OTU_2093)	0.00258621	0.01445091	0.05159574	0.16429283	0.00510094
Mycoplasma genus (OTU_14021)	0.23275862	0.75089325	0.02765957	0.16672965	0.00518773
Halieaceae family (OTU_13141)	0.04137931	0.12650403	0.00691489	0.02689723	0.00520246
Haliea genus (OTU_12961)	0.17543103	0.31965065	0.07925532	0.16018765	0.00537644
Planctomycetaceae family (OTU_16573)	0.05818966	0.14664994	0.01542553	0.06400906	0.00550262
OM60(NOR5) clade genus (OTU_13093)	0.03060345	0.08996445	0.00585106	0.02615053	0.00571458
Microbulbifer genus (OTU_11346)	0.25387931	0.4428625	0.11648936	0.26117455	0.00594337

Actibacter genus (OTU_2932)	0.19267241	0.31143815	0.09521277	0.19516348	0.00645306
Polaribacter 4 genus (OTU_9546)	1.6125	2.41101884	0.88989362	1.32057329	0.00664949
Planctomyces genus (OTU_7033)	0.00387931	0.02555493	0.07765957	0.25595223	0.00677336
Sandaracinaceae family (OTU_1434)	0.02974138	0.07398114	0.00851064	0.033926	0.00677474
Anaerolineaceae family (OTU_16580)	0.03965517	0.1216831	0.00744681	0.03338799	0.00748921
Lentimicrobiaceae family (OTU_12177)	0.01163793	0.04372611	0.00053191	0.0051296	0.00792617
Ulvibacter genus (OTU_14044)	0.02758621	0.09499202	0.00265957	0.02564801	0.00792835
Uncultured bacterium genus (OTU_1704)	0.01465517	0.05836022	0	0	0.00814196
Ulvibacter genus (OTU_8049)	0.00258621	0.02773398	0.05425532	0.18271877	0.00814443
Candidatus Fritschea genus (OTU_2064)	0	0	0.00851064	0.03063017	0.00872034
Haliea genus (OTU_13050)	0.10258621	0.24816958	0.0356383	0.09637646	0.00873652
Microbulbifer genus (OTU_13047)	0.28491379	0.41326183	0.15904255	0.27675578	0.00947216
Thiogranum genus (OTU_4314)	0.08836207	0.19667032	0.03457447	0.08873418	0.00956429
Bacillus genus (OTU_873)	0.00689655	0.02533955	0.00053191	0.0051296	0.00965729
Ambiguous_taxa genus (OTU_17409)	0.01551724	0.0543035	0.00159574	0.0153888	0.0097256
Flavobacteriaceae family (OTU_4504)	0.175	0.31870563	0.08138298	0.19691558	0.0101402
Legionellaceae family (OTU_3288)	0.01465517	0.06017834	0	0	0.01021483
NS5 marine group genus (OTU_4655)	0.01939655	0.07734037	0.00053191	0.0051296	0.01028417
Rubrivirga genus (OTU_9407)	0.01206897	0.04717022	0.00053191	0.0051296	0.01039742
Bacillus genus (OTU_908)	0.00689655	0.02853956	0	0	0.0107972
Pirellula genus (OTU_18170)	0.03491379	0.10611067	0.00744681	0.03856291	0.01102761
Gammaproteobacteria class (OTU_16574)	0.0125	0.04093466	0.00212766	0.01245181	0.01108775
Mycoplasma genus (OTU_14678)	0.0012931	0.01029983	0.23510638	0.87812753	0.01183426
Uncultured bacterium genus (OTU_9408)	0.02284483	0.08024538	0.00319149	0.01757735	0.01188754
Flammeovirgaceae family (OTU_11350)	0.00474138	0.02365197	0.07606383	0.26734796	0.01190994
Ambiguous_taxa genus (OTU_15913)	0.02672414	0.07294041	0.00797872	0.02758275	0.01206364
Rhodopirellula genus (OTU_16576)	0.02887931	0.08177736	0.00797872	0.03120215	0.01264826

Prolixibacter genus (OTU_4662)	0.03275862	0.12967191	0.78670213	2.85800644	0.01267369
Roseibacillus genus (OTU_9369)	0.00172414	0.01124	0.03989362	0.14468965	0.0128007
Desulfovibrio genus (OTU_5030)	0.05172414	0.22369651	0.20265957	0.54094908	0.01299293
Sandaracinaceae family (OTU_2040)	0.05387931	0.13867459	0.01755319	0.06345858	0.01324556
Ulvibacter genus (OTU_10045)	0.0262931	0.12636517	0.1212766	0.34644773	0.01340773
Bacillus genus (OTU_839)	0.00905172	0.03321763	0.00106383	0.00721524	0.01343704
Sandaracinaceae family (OTU_3236)	0.12068966	0.23717709	0.05691489	0.12432821	0.01363356
Cellulophaga genus (OTU_12581)	0.00258621	0.01107348	0	0	0.0136624
Desulfobulbaceae family (OTU_2584)	0.01637931	0.07033659	0	0	0.01393118
BD1-7 clade genus (OTU_13049)	0.02931034	0.0866197	0.10212766	0.27043256	0.01409425
Planctomyces genus (OTU_17752)	0.02327586	0.09791601	0.00053191	0.0051296	0.01431881
Flavobacteriaceae family (OTU_9596)	0.02284483	0.05662651	0.00851064	0.02378796	0.01497113
Maribacter genus (OTU_5047)	0.01594828	0.10267943	0.1393617	0.47618119	0.01586987
Phaeocystidibacter genus (OTU_10140)	0.01163793	0.05100615	0	0	0.01592547
Flavobacteriaceae family (OTU_5038)	0.22327586	0.38019001	0.11648936	0.2541557	0.01652675
Mycoplasma genus (OTU_14108)	0.17801724	1.14467073	1.39095745	4.69106758	0.0166042
Algitalea genus (OTU_8324)	0.0125	0.05527166	0	0	0.01685342
Winogradskyella genus (OTU_5135)	0.02801724	0.12392448	0	0	0.01688773
Uncultured bacterium genus (OTU_9425)	0.03965517	0.11643358	0.01117021	0.04445549	0.01691202
Uncultured bacterium genus (OTU_12134)	0.05603448	0.34007829	0.56968085	2.01825733	0.01708206
Endozoicomonas genus (OTU_8976)	0.16293103	0.57588465	0.02553191	0.1871887	0.01738736
Gammaproteobacteria class (OTU_4316)	0.05172414	0.14322803	0.01648936	0.05852273	0.01746766
Ambiguous_taxa genus (OTU_18163)	0.01810345	0.07263411	0.00159574	0.0114207	0.01786971
Legionellaceae family (OTU_1692)	0.03189655	0.13218922	0.00212766	0.01443049	0.01807605
Ambiguous_taxa genus (OTU_17405)	0.0375	0.08829686	0.01382979	0.05379445	0.01827989
Tenacibaculum genus (OTU_7777)	0.00646552	0.04127816	0.04946809	0.16924342	0.01852016
Microbulbifer genus (OTU_11950)	0.00172414	0.01848932	0.17074468	0.68059773	0.01866064

SS1-B-06-26 family (OTU_5136)	0.01896552	0.06968376	0.00265957	0.02109631	0.01874434
Cellulophaga genus (OTU_9296)	2.96724138	6.78662828	1.06914894	4.76487652	0.01901408
HOC36 order (OTU_11349)	0.05689655	0.11120356	0.02553191	0.08113054	0.0197792
OM60(NOR5) clade genus (OTU_13072)	0.1262931	0.29172947	0.05	0.17166271	0.01996172
E01-9C-26 marine group order (OTU_2037)	0.04741379	0.11387471	0.01755319	0.06791243	0.02012373
Deferrisoma genus (OTU_16302)	0.00172414	0.01457891	0.01914894	0.06999483	0.02024123
Cellulophaga genus (OTU_12572)	0.00344828	0.01570764	0	0	0.02025974
Mycoplasma genus (OTU_12128)	0.50603448	2.04681371	0.05319149	0.27760454	0.0206115
JTB255 marine benthic group family (OTU_6616)	0.31422414	0.40597934	0.20265957	0.28465797	0.02110914
Thiotrichaceae family (OTU_3229)	0.025	0.14421308	0.09308511	0.25070126	0.02143082
Lutibacter genus (OTU_5705)	0.05948276	0.24458919	0.29574468	0.95284176	0.02176204
Winogradskyella genus (OTU_5121)	0.02284483	0.07292895	0.11382979	0.37177008	0.02216005
Gammaproteobacteria class (OTU_9353)	0.00474138	0.02934519	0.22819149	0.92709104	0.02233368
Hyunsoonleella genus (OTU_15532)	0.0012931	0.01386699	0.03244681	0.12958761	0.02319067
Ambiguous_taxa genus (OTU_5041)	0.0762931	0.14057474	0.03670213	0.10992636	0.02367895
Candidatus Hepatoplasma genus (OTU_11357)	9.54439655	22.70408	17.5989362	27.4421277	0.02433702
Flammeovirga genus (OTU_8427)	0.00086207	0.00650848	0.02606383	0.10612335	0.02447082
Peredibacter genus (OTU_8037)	0	0	0.00531915	0.02244151	0.02453938
Coxiella genus (OTU_1113)	0.01982759	0.09351362	0	0	0.02483627
Halieaceae family (OTU_13168)	0.00905172	0.03690572	0.00106383	0.00721524	0.02502917
Flavobacteriaceae family (OTU_11124)	0.08232759	0.44184441	1.8287234	7.39308363	0.02520614
Ambiguous_taxa genus (OTU_5243)	0.01206897	0.05709214	0	0	0.02526208
Polaribacter 2 genus (OTU_8034)	0	0	0.06542553	0.27772122	0.02540071
Lutibacter genus (OTU_5017)	0.09913793	0.6348358	0.34840426	0.89971781	0.02541406
Uncultured bacterium genus (OTU_542)	0.00818966	0.03880986	0	0	0.02551651
Ambiguous_taxa genus (OTU_17413)	0.00689655	0.02533955	0.00106383	0.0102592	0.02577294
Planctomycetaceae family (OTU_16595)	0.00775862	0.03386296	0.00053191	0.0051296	0.02580903

Oleiphilus genus (OTU_12153)	0.00301724	0.02000947	0.03882979	0.15161441	0.02595963
Muricauda genus (OTU_8104)	0.00043103	0.00462233	0.01595745	0.06612881	0.02615095
SS1-B-06-26 family (OTU_5168)	0.01508621	0.05916802	0.00212766	0.01616881	0.02624142
Aquimarina genus (OTU_8011)	0.06508621	0.2390913	0.01170213	0.07970208	0.02627014
Uncultured bacterium genus (OTU_9)	0.03448276	0.13413416	0.00531915	0.03457038	0.02665383
HOC36 order (OTU_9389)	0.00517241	0.02489574	0	0	0.02782514
Flavobacteriaceae family (OTU_9360)	0.00818966	0.04401409	0.09893617	0.38996581	0.02792321
Aquimarina genus (OTU_7356)	0.02327586	0.08988283	0.25638298	1.0036697	0.02794711
Planctomyces genus (OTU_5077)	0.01853448	0.05273967	0.00638298	0.02444495	0.02943154
Uncultured planctomycete genus (OTU_6928)	0.00172414	0.01301696	0.04255319	0.17804456	0.0297746
Desulforhopalus genus (OTU_2853)	0.1125	0.43121096	0.02234043	0.07976595	0.02992764
Anaerolineaceae family (OTU_17270)	0	0	0.00425532	0.01882107	0.03175421
Cryomorphaceae family (OTU_5037)	0.00991379	0.05220146	0.04734043	0.15934824	0.03196377
Ambiguous_taxa genus (OTU_2912)	0.0137931	0.06283058	0.15319149	0.61569924	0.032182
Guggenheimella genus (OTU_14367)	0.33448276	2.04352862	1.10744681	2.92208172	0.0322982
Robiginitalea genus (OTU_12180)	0.00948276	0.04399931	0.00053191	0.0051296	0.03250776
Rhodothermaceae family (OTU_12220)	0.11293103	0.45647264	0.02021277	0.05275352	0.03274666
Ambiguous_taxa genus (OTU_16585)	0.03189655	0.09900291	0.00851064	0.05537027	0.03275793
Tenacibaculum genus (OTU_12372)	0.00258621	0.01287343	0	0	0.03329842
OM1 clade family (OTU_17749)	0.00948276	0.03210556	0.00212766	0.01616881	0.03343955
Ambiguous_taxa genus (OTU_5648)	0.02112069	0.07373966	0.00531915	0.02575259	0.03381852
Ambiguous_taxa genus (OTU_5149)	0.00086207	0.00924466	0.02021277	0.0863932	0.03409446
Bacillus genus (OTU_10530)	0.00301724	0.01509852	0	0	0.03421957
Nitrosomonadaceae family (OTU_10926)	0.00301724	0.01509852	0	0	0.03421957
Marinicella genus (OTU_2038)	0.01939655	0.11499092	0.09095745	0.30500809	0.0342494
Microbulbifer genus (OTU_12021)	0.00086207	0.00924466	0.03404255	0.14879172	0.03436344
Ambiguous_taxa genus (OTU_9362)	0	0	0.00851064	0.03834217	0.03492573

Halieaceae family (OTU_13056)	0.0762931	0.16641002	0.03617021	0.105034	0.03557054
Flavobacterium genus (OTU_14050)	0	0	0.01968085	0.08909058	0.03578088
Flavobacteriaceae family (OTU_5142)	0.0012931	0.01029983	0.01170213	0.04637127	0.03622945
Halieaceae family (OTU_13110)	0.00818966	0.04149365	0	0	0.03645242
Longispora genus (OTU_14329)	0.00560345	0.02844501	0	0	0.03680656
Planctomyces genus (OTU_6975)	0.00560345	0.02844501	0	0	0.03680656
Mycoplasma genus (OTU_11599)	0.01767241	0.15801221	0.89202128	3.99760103	0.03772139
Uncultured Acidobacteria bacterium genus (OTU_14989)	0.01336207	0.06834174	0	0	0.03821214
Peredibacter genus (OTU_10082)	0	0	0.02180851	0.10014843	0.0384343
Bythopirellula genus (OTU_12217)	0.00517241	0.02309959	0.00053191	0.0051296	0.03844743
Pir4 lineage genus (OTU_18272)	0.00603448	0.0309025	0	0	0.03844875
Flavobacteriaceae family (OTU_5347)	0.00344828	0.0234191	0.0287234	0.11427136	0.03846951
JTB255 marine benthic group family (OTU_6177)	0.00862069	0.0441763	0	0	0.03857761
Limnobacter genus (OTU_5382)	0	0	0.02659574	0.12262686	0.03920473
Ambiguous_taxa genus (OTU_8243)	0.00043103	0.00462233	0.0143617	0.06425611	0.03965906
Coxiella genus (OTU_2042)	0.00086207	0.00924466	0.01755319	0.07708363	0.04057855
endosymbionts genus (OTU_3253)	0.00086207	0.00650848	0.01808511	0.07984395	0.04075174
Pir4 lineage genus (OTU_14948)	0	0	0.01329787	0.06196067	0.04125366
Photobacterium genus (OTU_69)	0.01724138	0.05457652	0.00425532	0.03621712	0.04135486
Desulfuromusa genus (OTU_15908)	0.0375	0.23331152	0.19148936	0.68845488	0.04142745
Uncultured bacterium genus (OTU_14098)	0.00689655	0.03589769	0	0	0.04163357
Winogradskyella genus (OTU_8067)	0.00086207	0.00924466	0.0356383	0.16236653	0.04190422
Pir4 lineage genus (OTU_8121)	0.00646552	0.0304635	0.00053191	0.0051296	0.04218553
Uncultured bacterium genus (OTU_3242)	0	0	0.04946809	0.23183985	0.04241949
Cellvibrionales order (OTU_13089)	0.00775862	0.03748761	0.00053191	0.0051296	0.04315999
Blastopirellula genus (OTU_15542)	0.01724138	0.06502766	0.00425532	0.01882107	0.04339989
Uncultured planctomycete genus (OTU_7500)	0.00862069	0.0446615	0.05478723	0.21388866	0.04341571

Fusibacter genus (OTU_15110)	0.00086207	0.00924466	0.03404255	0.15645895	0.04394083
Flavobacteriaceae family (OTU_10094)	0.08275862	0.19784436	0.0393617	0.10489923	0.04419823
Mycoplasma genus (OTU_11355)	21.287931	24.7377683	14.5691489	23.0114485	0.04423379
Ambiguous_taxa genus (OTU_5075)	0.00387931	0.02947154	0.02446809	0.09389466	0.0442934
Pir4 lineage genus (OTU_12188)	0.00517241	0.03497494	0.03776596	0.15118084	0.04441545
Coxiella genus (OTU_4374)	0.00172414	0.00912328	0	0	0.04501585
Planctomyces genus (OTU_18325)	0.00172414	0.00912328	0	0	0.04501585
Uncultured bacterium genus (OTU_13253)	0.00172414	0.00912328	0	0	0.04501585
OM60(NOR5) clade genus (OTU_13305)	0.00344828	0.01824656	0	0	0.04501585
Aquimarina genus (OTU_10032)	0.02586207	0.22432684	0.2856383	1.21809544	0.04516636
Uncultured bacterium genus (OTU_11361)	0.01896552	0.10057061	0	0	0.04546739
Gammaproteobacteria class (OTU_3448)	0.00431034	0.03319805	0.0212766	0.07526173	0.0454885
Aquibacter genus (OTU_11420)	0.01293103	0.06858191	0	0	0.04550155
Salegentibacter genus (OTU_4470)	0.14612069	0.50655832	0.0393617	0.23268465	0.0457359
Coxiella genus (OTU_2405)	0.00517241	0.02401446	0.00053191	0.0051296	0.04588024
Lentimicrobiaceae family (OTU_12267)	0.00948276	0.05039234	0	0	0.04591989
Ambiguous_taxa genus (OTU_11354)	0.02155172	0.07577876	0.00638298	0.02550974	0.04623542
Guggenheimella genus (OTU_14395)	0	0	0.00797872	0.03810902	0.04636099
JTB255 marine benthic group family (OTU_5070)	0.02327586	0.08341603	0.00691489	0.02374629	0.04689809
Fabibacter genus (OTU_13272)	0.00560345	0.02992199	0	0	0.04696224
Ambiguous_taxa genus (OTU_2545)	0.02715517	0.13186204	0.00212766	0.02051841	0.047116
Bacillus genus (OTU_626)	0.00862069	0.04608644	0	0	0.04720999
PeM15 order (OTU_18205)	0.00603448	0.03226718	0	0	0.04725499
Vibrio genus (OTU_5114)	0.00172414	0.01457891	0.04308511	0.19821022	0.04752192
Vibrio genus (OTU_193)	0.00086207	0.00650848	0.00478723	0.01796739	0.04755944
Planctomycetaceae family (OTU_18234)	0.0112069	0.05689002	0.00053191	0.0051296	0.04756984
Lutibacter genus (OTU_5113)	0.00043103	0.00462233	0.22712766	1.08898413	0.04759538

Enterovibrio genus (OTU_3354)	0	0	0.00585106	0.02811109	0.04762709
Desulfobulbaceae family (OTU_2548)	0	0	0.0212766	0.10223288	0.04764998
Chitinophagaceae family (OTU_12311)	0	0	0.00851064	0.04102302	0.04834338
CS-B046 order (OTU_9386)	0.00043103	0.00462233	0.02978723	0.14148136	0.0483997
Uncultured gamma proteobacterium genus (OTU_2053)	0.00172414	0.01457891	0.01968085	0.08574395	0.04868358
Polaribacter 4 genus (OTU_10561)	0.00603448	0.02192775	0.00159574	0.00878868	0.04913333
Hahella genus (OTU_12159)	0.00560345	0.04343622	0.03457447	0.13488107	0.04914764
Pir4 lineage genus (OTU_12439)	0.01637931	0.07362998	0.00212766	0.02051841	0.04941139
Ambiguous_taxa genus (OTU_8097)	0.00086207	0.00924466	0.01489362	0.06756785	0.04970235

Supplementary Table 31: Alpha diversity (Chao1, Observed species and Shannon) for uninfected and infected QX disease oysters

Sample	Chao1	Observed species	Shannon
Uninfected QX disease oysters (n = 43)	146.627113 ± 111.916309	109.627907 ± 79.4831588	3.37605273 ± 1.70765431
Infected QX disease oysters (n = 42)	121.431704 ± 97.1604982	95.047619 ± 65.7482087	3.68728748 ± 1.41280249

Supplementary Table 32: Top 10 OTUs displayed their dissimilarity and distribution to the difference between microbiomes in uninfected (n= 43) and infected QX disease oysters (n= 42) as identified by SIMPER. Overall dissimilarity is 85.1%

Taxon	Av. dissim	Contrib. %
Candidatus Hepatoplasma genus (OTU_11357)	3.285	3.86
Mycoplasma genus (OTU_11355)	2.754	3.236
Borrelia genus (OTU_1)	2.075	2.438
Arcobacter genus (OTU_17190)	1.898	2.23
Flavobacteriaceae family (OTU_11124)	0.9165	1.077
Guggenheimella genus (OTU_14367)	0.744	0.8743
Mycoplasma genus (OTU_14108)	0.7061	0.8298
Mycoplasma genus (OTU_11453)	0.6995	0.822
Ambiguous_taxa genus (OTU_15557)	0.6428	0.7554
Mycoplasma genus (OTU_11599)	0.629	0.7392

Supplementary Table 33: OTUs significantly different between uninfected (n = 43) and infected QX disease oysters (n = 42) (Welch's t-test; p <0.05) as identified by STAMP.

Taxon	Uninfected QX disease oysters		Infected QX disease oysters		p-values
	Mean (%)	std. dev (%)	Mean (%)	std. dev (%)	
Borrelia genus (OTU_1)	5.26627907	9.09164401	14.925	13.4400089	0.00027546
Thalassolituus genus (OTU_11004)	0.3872093	0.71723735	0.04642857	0.12169089	0.00401231
Ambiguous_taxa genus (OTU_1700)	0.03837209	0.08815763	0.00238095	0.01064794	0.01189133
Uncultured bacterium genus (OTU_3233)	0.10581395	0.24333184	0.01190476	0.04604543	0.01794709
Coxiella genus (OTU_2108)	0.00581395	0.01602796	0	0	0.02350235
Rhodopirellula genus (OTU_15867)	0.28255814	0.53612344	0.08333333	0.20431846	0.0287404
Ambiguous_taxa genus (OTU_17405)	0.01627907	0.04665675	0	0	0.0289863
Mycoplasma genus (OTU_11355)	18.1046512	24.9487307	7.87142857	17.1489315	0.03225234
OM60(NOR5) clade genus (OTU_13093)	0.0127907	0.03749887	0	0	0.0325676
Pricia genus (OTU_6854)	0	0	0.00833333	0.02419334	0.03308027
Uncultured bacterium genus (OTU_113)	0.47674419	1.05060054	0.11190476	0.27920157	0.03471853
Planctomyces genus (OTU_17455)	0.02093023	0.0621631	0	0	0.03475036
SAR86 clade family (OTU_7230)	0.03023256	0.08966315	0.18214286	0.44944694	0.03936029
Flammeovirga genus (OTU_8427)	0.05348837	0.1515023	0.00357143	0.01687791	0.03964448
Uncultured bacterium genus (OTU_12134)	0.15116279	0.39700746	1.1202381	2.89883104	0.03977651
Mycoplasma genus (OTU_14108)	0.31976744	1.17052659	2.51071429	6.53875385	0.04039318
Uncultured bacterium genus (OTU_13090)	0.01744186	0.05379085	0	0	0.04164526
Lutibacter genus (OTU_6937)	0.01976744	0.06113228	0	0	0.04218634
Cryomorphaceae family (OTU_8761)	0.00465116	0.01452325	0	0	0.04410108
Uncultured bacterium genus (OTU_7017)	0.00465116	0.01452325	0	0	0.04410108
Flavobacteriaceae family (OTU_5142)	0.01744186	0.05486105	0	0	0.04558931
JTB255 marine benthic group family (OTU_5026)	0.06511628	0.12737734	0.15595238	0.25985398	0.04850037

Supplementary Table 34: Significant correlations between environmental variables, QX disease and OTUs ($p < 0.05$) as identified by Mictools.

Environmental variable and QX disease	Taxon	p value	PearsonR	MICe
pH	Borrelia genus (OTU_1)	2.69E-02	-0.04121	0.158981
pH	Mycoplasma genus (OTU_11355)	1.61E-03	0.087919	0.197116
pH	Polaribacter 4 genus (OTU_9546)	1.21E-03	0.231972	0.211944
pH	Flavobacteriaceae family (OTU_9833)	7.47E-03	0.219177	0.132492
pH	Candidatus Hepatoplasma genus (OTU_11357)	2.91E-06	-0.315164	0.45664
pH	JTB255 marine benthic group family (OTU_6616)	7.36E-03	0.211023	0.137181
pH	Mycoplasma genus (OTU_11453)	2.39E-03	0.163292	0.160077
pH	Uncultured bacterium genus (OTU_113)	2.26E-05	0.291304	0.254146
pH	Ambiguous_taxa genus (OTU_15557)	4.12E-04	-0.069379	0.192714
pH	Haliea genus (OTU_8857)	1.78E-03	0.259383	0.140876
pH	Comamonadaceae family (OTU_14443)	2.68E-02	0.032526	0.141275
pH	Mycoplasmataceae family (OTU_376)	5.65E-06	0.273712	0.223775
pH	Microbulbifer genus (OTU_13047)	2.67E-02	0.164338	0.18917
pH	JTB255 marine benthic group family (OTU_5026)	1.16E-03	0.223971	0.137918
pH	Rhodopirellula genus (OTU_15867)	3.37E-02	0.038391	0.195688
pH	Maribacter genus (OTU_2121)	6.95E-05	0.143716	0.214758
pH	Arcobacter genus (OTU_17190)	2.12E-02	-0.038556	0.18267
pH	Microbulbifer genus (OTU_11346)	1.48E-04	0.239913	0.161104
pH	Flavobacteriaceae family (OTU_5038)	4.06E-03	0.199346	0.144666
pH	Flavobacteriaceae family (OTU_4460)	8.66E-03	0.116235	0.146963
pH	Endozoicomonas genus (OTU_2823)	1.13E-04	0.048899	0.164936
pH	Cellulophaga genus (OTU_9296)	8.98E-06	0.298293	0.288598
pH	Haliea genus (OTU_12961)	2.88E-02	0.149851	0.122972
pH	Actibacter genus (OTU_2932)	6.51E-03	0.153907	0.131145

pH	Uncultured bacterium genus (OTU_5533)	7.26E-03	0.155231	0.101984
pH	Actibacter genus (OTU_5023)	1.67E-02	0.192716	0.101839
pH	Actibacter genus (OTU_2972)	2.46E-02	0.203091	0.121648
pH	Flavobacteriaceae family (OTU_4504)	2.79E-03	0.211646	0.137708
pH	JTB255 marine benthic group family (OTU_5117)	2.53E-02	0.089744	0.1026
pH	Algitalea genus (OTU_8064)	2.42E-05	0.390892	0.256296
pH	Flavobacteriaceae family (OTU_2707)	4.36E-02	0.012616	0.145071
pH	Piscirickettsiaceae family (OTU_1817)	3.26E-04	0.182489	0.204691
pH	Family XIII family (OTU_11608)	1.19E-02	0.005406	0.16732
pH	Aquibacter genus (OTU_5525)	2.20E-03	0.270526	0.176488
pH	Ambiguous_taxa genus (OTU_4769)	1.61E-04	0.458997	0.180262
pH	Sandaracinaceae family (OTU_3236)	2.65E-03	0.262074	0.132522
pH	Pricia genus (OTU_5718)	2.94E-02	-0.007115	0.1221
pH	Marinifilum genus (OTU_8076)	1.23E-04	-0.056589	0.177785
DO	Borrelia genus (OTU_1)	1.52E-02	0.061311	0.161454
DO	Mycoplasma genus (OTU_11355)	4.62E-03	-0.037136	0.17187
DO	Polaribacter 4 genus (OTU_9546)	1.17E-02	0.207362	0.204934
DO	Flavobacteriaceae family (OTU_9833)	9.37E-03	0.109829	0.138205
DO	Candidatus Hepatoplasma genus (OTU_11357)	3.12E-05	-0.13277	0.456351
DO	JTB255 marine benthic group family (OTU_6616)	2.28E-02	0.129353	0.130102
DO	Mycoplasma genus (OTU_11453)	1.02E-03	0.018969	0.148309
DO	Uncultured bacterium genus (OTU_113)	2.91E-06	0.06982	0.254146
DO	Ambiguous_taxa genus (OTU_15557)	6.60E-04	0.031496	0.212239
DO	Haliea genus (OTU_8857)	6.52E-04	0.153349	0.209426
DO	Comamonadaceae family (OTU_14443)	2.53E-02	-0.052788	0.121285
DO	Mycoplasmataceae family (OTU_376)	8.68E-04	-0.007696	0.206751
DO	Microbulbifer genus (OTU_13047)	8.66E-03	0.158848	0.158006

DO	JTB255 marine benthic group family (OTU_5026)	9.98E-03	-0.03112	0.118053
DO	Rhodopirellula genus (OTU_15867)	7.95E-06	-0.272839	0.195688
DO	Maribacter genus (OTU_2121)	1.06E-04	0.298613	0.226139
DO	Arcobacter genus (OTU_17190)	3.01E-02	-0.054401	0.17022
DO	Microbulbifer genus (OTU_11346)	5.64E-04	0.056726	0.161104
DO	Flavobacteriaceae family (OTU_5038)	2.46E-02	-0.010362	0.133726
DO	Flavobacteriaceae family (OTU_4460)	1.67E-02	-0.06537	0.168267
DO	Endozoicomonas genus (OTU_2823)	1.00E-02	-0.039956	0.146739
DO	Cellulophaga genus (OTU_9296)	2.91E-06	0.385629	0.391276
DO	Haliea genus (OTU_12961)	1.20E-02	0.042583	0.110103
DO	Actibacter genus (OTU_2932)	1.67E-02	0.058485	0.108871
DO	Uncultured bacterium genus (OTU_5533)	2.10E-02	-0.010391	0.121527
DO	Actibacter genus (OTU_5023)	3.83E-02	0.0204	0.103442
DO	Mycoplasma genus (OTU_11533)	5.18E-03	0.118863	0.146522
DO	Actibacter genus (OTU_2972)	6.36E-03	0.148223	0.124467
DO	Flavobacteriaceae family (OTU_4504)	9.60E-03	0.109752	0.126362
DO	Algitalea genus (OTU_8064)	2.91E-06	0.449276	0.274066
DO	Flavobacteriaceae family (OTU_2707)	3.14E-02	0.023209	0.153073
DO	Piscirickettsiaceae family (OTU_1817)	1.26E-05	0.345608	0.242581
DO	Family XIII family (OTU_11608)	3.71E-04	-0.201105	0.183326
DO	Aquibacter genus (OTU_5525)	3.28E-04	0.295025	0.184195
DO	Thalassolituus genus (OTU_11004)	2.40E-02	-0.066504	0.127322
DO	Ambiguous_taxa genus (OTU_4769)	8.98E-06	0.275111	0.180262
DO	Sandaracinaceae family (OTU_3236)	1.28E-02	0.073634	0.12112
DO	Pricia genus (OTU_5718)	2.27E-02	0.108002	0.109647
DO	Marinifilum genus (OTU_8076)	7.65E-05	-0.101099	0.177785
DO	Rhodopirellula genus (OTU_16569)	3.40E-02	0.075886	0.102887

DO	Gammaproteobacteria class (OTU_2554)	3.78E-02	0.130544	0.088451
Temperature	Borrelia genus (OTU_1)	2.82E-02	-0.060873	0.161454
Temperature	Mycoplasma genus (OTU_11355)	5.52E-03	0.056111	0.185596
Temperature	Polaribacter 4 genus (OTU_9546)	2.65E-03	-0.232815	0.204934
Temperature	Flavobacteriaceae family (OTU_9833)	4.06E-03	-0.134393	0.138205
Temperature	Candidatus Hepatoplasma genus (OTU_11357)	1.08E-04	0.149766	0.45664
Temperature	JTB255 marine benthic group family (OTU_6616)	1.52E-02	-0.19335	0.130102
Temperature	Mycoplasma genus (OTU_11453)	3.56E-04	0.074629	0.150436
Temperature	Uncultured bacterium genus (OTU_113)	8.21E-04	-0.180109	0.254146
Temperature	Ambiguous_taxa genus (OTU_15557)	1.86E-04	-0.173906	0.212239
Temperature	Haliea genus (OTU_8857)	4.34E-04	-0.218138	0.209426
Temperature	Comamonadaceae family (OTU_14443)	3.92E-02	0.073083	0.124852
Temperature	Mycoplasmataceae family (OTU_376)	3.75E-04	0.03083	0.206751
Temperature	Microbulbifer genus (OTU_13047)	9.66E-03	-0.207684	0.158006
Temperature	JTB255 marine benthic group family (OTU_5026)	1.07E-02	-0.002852	0.119666
Temperature	Rhodopirellula genus (OTU_15867)	4.95E-03	0.190141	0.195688
Temperature	Maribacter genus (OTU_2121)	3.12E-05	-0.302662	0.226139
Temperature	Arcobacter genus (OTU_17190)	2.94E-02	0.055427	0.166545
Temperature	Microbulbifer genus (OTU_11346)	3.22E-03	-0.166235	0.190092
Temperature	Flavobacteriaceae family (OTU_5038)	2.82E-02	-0.005065	0.141461
Temperature	Flavobacteriaceae family (OTU_4460)	4.40E-02	0.04377	0.166337
Temperature	Endozoicomonas genus (OTU_2823)	2.04E-03	0.126026	0.144679
Temperature	Cellulophaga genus (OTU_9296)	2.91E-06	-0.383376	0.391276
Temperature	Haliea genus (OTU_12961)	1.57E-02	-0.120398	0.119232
Temperature	Actibacter genus (OTU_2932)	2.69E-02	-0.048911	0.113804
Temperature	Mycoplasma genus (OTU_11533)	8.97E-04	-0.181236	0.134311
Temperature	Actibacter genus (OTU_2972)	5.78E-03	-0.203477	0.110917

Temperature	Flavobacteriaceae family (OTU_4504)	5.58E-03	-0.151653	0.142178
Temperature	Algitalea genus (OTU_8064)	2.91E-06	-0.426815	0.274066
Temperature	Flavobacteriaceae family (OTU_2707)	3.50E-02	-0.085741	0.152588
Temperature	Piscirickettsiaceae family (OTU_1817)	8.98E-06	-0.330286	0.242581
Temperature	Family XIII family (OTU_11608)	2.37E-04	0.206189	0.148141
Temperature	Aquibacter genus (OTU_5525)	9.31E-05	-0.295333	0.184195
Temperature	Thalassolituus genus (OTU_11004)	2.36E-02	0.104114	0.117327
Temperature	Ambiguous_taxa genus (OTU_4769)	7.95E-06	-0.292421	0.180262
Temperature	Sandaracinaceae family (OTU_3236)	2.02E-02	-0.172749	0.127016
Temperature	Pricia genus (OTU_5718)	3.10E-02	-0.09147	0.108286
Temperature	Marinifilum genus (OTU_8076)	5.16E-05	0.100914	0.177785
Temperature	Rhodopirellula genus (OTU_16569)	3.17E-02	-0.084497	0.11287
Temperature	Gammaproteobacteria class (OTU_2554)	3.95E-03	-0.20559	0.090065
Conductivity	Borrelia genus (OTU_1)	2.39E-03	-0.002694	0.150088
Conductivity	Mycoplasma genus (OTU_11355)	3.47E-03	-0.094146	0.187372
Conductivity	Polaribacter 4 genus (OTU_9546)	2.35E-02	-0.093457	0.175079
Conductivity	Flavobacteriaceae family (OTU_9833)	2.88E-02	-0.000166	0.167434
Conductivity	Candidatus Hepatoplasma genus (OTU_11357)	2.91E-06	0.346414	0.441425
Conductivity	JTB255 marine benthic group family (OTU_6616)	2.68E-02	-0.01985	0.121391
Conductivity	Mycoplasma genus (OTU_11453)	1.38E-03	-0.251668	0.15995
Conductivity	Uncultured bacterium genus (OTU_113)	2.42E-05	-0.259675	0.245196
Conductivity	Ambiguous_taxa genus (OTU_15557)	7.95E-06	-0.017528	0.179208
Conductivity	Haliea genus (OTU_8857)	3.85E-02	-0.066915	0.136024
Conductivity	Comamonadaceae family (OTU_14443)	1.57E-02	-0.023164	0.131318
Conductivity	Mycoplasmataceae family (OTU_376)	8.28E-05	-0.153588	0.19613
Conductivity	Microbulbifer genus (OTU_13047)	7.36E-03	-0.143537	0.150295
Conductivity	JTB255 marine benthic group family (OTU_5026)	1.19E-02	0.050296	0.113031

Conductivity	Rhodopirellula genus (OTU_15867)	2.56E-03	-0.16735	0.181812
Conductivity	Maribacter genus (OTU_2121)	2.45E-03	-0.256844	0.209746
Conductivity	Arcobacter genus (OTU_17190)	3.40E-02	0.06561	0.154803
Conductivity	Microbulbifer genus (OTU_11346)	4.19E-03	-0.058395	0.160569
Conductivity	Flavobacteriaceae family (OTU_5038)	2.84E-02	-0.001452	0.124887
Conductivity	Flavobacteriaceae family (OTU_4460)	1.73E-02	-0.157888	0.149601
Conductivity	Endozoicomonas genus (OTU_2823)	1.28E-04	-0.049428	0.201229
Conductivity	Cellulophaga genus (OTU_9296)	4.23E-05	-0.178147	0.278671
Conductivity	Haliea genus (OTU_12961)	3.49E-02	0.04095	0.110813
Conductivity	Actibacter genus (OTU_2932)	3.71E-03	-0.063818	0.146743
Conductivity	Uncultured bacterium genus (OTU_5533)	4.47E-02	-0.152548	0.091265
Conductivity	Actibacter genus (OTU_5023)	4.64E-02	-0.036918	0.095461
Conductivity	Actibacter genus (OTU_2972)	2.14E-02	-0.074733	0.100893
Conductivity	Flavobacteriaceae family (OTU_4504)	2.53E-02	-0.024716	0.135411
Conductivity	JTB255 marine benthic group family (OTU_5117)	3.17E-02	0.053653	0.08978
Conductivity	Algitalea genus (OTU_8064)	2.26E-05	-0.236667	0.244833
Conductivity	Flavobacteriaceae family (OTU_2707)	8.45E-03	-0.010544	0.174693
Conductivity	Piscirickettsiaceae family (OTU_1817)	1.49E-03	-0.142465	0.198462
Conductivity	Family XIII family (OTU_11608)	1.86E-02	-0.189838	0.158773
Conductivity	Aquibacter genus (OTU_5525)	4.83E-03	-0.190134	0.170259
Conductivity	Ambiguous_taxa genus (OTU_4769)	6.39E-04	-0.040606	0.180141
Conductivity	Sandaracinaceae family (OTU_3236)	4.84E-03	-0.050885	0.117644
Conductivity	Pricia genus (OTU_5718)	6.23E-04	-0.087254	0.179461
Conductivity	Marinifilum genus (OTU_8076)	3.20E-04	-0.045976	0.177663
Conductivity	Rhodopirellula genus (OTU_16569)	2.43E-02	-0.080976	0.106161
Conductivity	Gammaproteobacteria class (OTU_2554)	1.13E-02	-0.007264	0.087841
Nitrate	Borrelia genus (OTU_1)	4.37E-04	0.069378	0.170839

Nitrate	Mycoplasma genus (OTU_11355)	1.90E-02	0.013523	0.159238
Nitrate	Polaribacter 4 genus (OTU_9546)	2.83E-03	0.137132	0.168402
Nitrate	Flavobacteriaceae family (OTU_9833)	3.12E-03	0.135076	0.13837
Nitrate	Candidatus Hepatoplasma genus (OTU_11357)	2.08E-05	-0.298873	0.366627
Nitrate	JTB255 marine benthic group family (OTU_6616)	2.51E-02	0.117541	0.137707
Nitrate	Mycoplasma genus (OTU_11453)	4.98E-02	-0.022339	0.135538
Nitrate	Uncultured bacterium genus (OTU_113)	3.06E-02	0.124415	0.203657
Nitrate	Ambiguous_taxa genus (OTU_15557)	5.50E-04	0.09778	0.17423
Nitrate	Haliea genus (OTU_8857)	3.69E-02	0.136665	0.126024
Nitrate	Comamonadaceae family (OTU_14443)	2.50E-02	0.103233	0.128579
Nitrate	Mycoplasmataceae family (OTU_376)	1.83E-02	-0.081277	0.146807
Nitrate	Microbulbifer genus (OTU_13047)	4.20E-02	0.163479	0.141676
Nitrate	JTB255 marine benthic group family (OTU_5026)	4.43E-02	0.101047	0.1138
Nitrate	Rhodopirellula genus (OTU_15867)	1.45E-03	0.113374	0.179453
Nitrate	Maribacter genus (OTU_2121)	8.98E-06	0.346585	0.198604
Nitrate	Arcobacter genus (OTU_17190)	2.15E-02	-0.108485	0.165764
Nitrate	Microbulbifer genus (OTU_11346)	2.82E-02	0.206079	0.150924
Nitrate	Flavobacteriaceae family (OTU_5038)	2.27E-02	0.078335	0.107371
Nitrate	Flavobacteriaceae family (OTU_4460)	7.85E-03	0.200472	0.122621
Nitrate	Endozoicomonas genus (OTU_2823)	4.12E-04	-0.020031	0.216369
Nitrate	Cellulophaga genus (OTU_9296)	8.98E-06	0.276253	0.205216
Nitrate	Haliea genus (OTU_12961)	2.15E-02	0.298765	0.120096
Nitrate	Actibacter genus (OTU_2932)	4.74E-02	0.038542	0.11038
Nitrate	Actibacter genus (OTU_2972)	4.19E-03	0.110726	0.099073
Nitrate	Flavobacteriaceae family (OTU_4504)	4.90E-03	0.21231	0.135896
Nitrate	JTB255 marine benthic group family (OTU_5117)	4.60E-02	0.126034	0.116777
Nitrate	Algitalea genus (OTU_8064)	8.98E-06	0.186012	0.189909

Nitrate	Flavobacteriaceae family (OTU_2707)	3.38E-03	0.188604	0.210797
Nitrate	Piscirickettsiaceae family (OTU_1817)	8.98E-06	0.198851	0.192348
Nitrate	Aquibacter genus (OTU_5525)	4.36E-04	0.23072	0.15045
Nitrate	Thalassolituus genus (OTU_11004)	3.34E-02	-0.09337	0.097365
Nitrate	Ambiguous_taxa genus (OTU_4769)	8.03E-03	0.064585	0.109785
Nitrate	Sandaracinaceae family (OTU_3236)	1.07E-03	0.248074	0.101911
Nitrate	Marinifilum genus (OTU_8076)	8.07E-03	-0.047691	0.110296
Nitrate	Gammaproteobacteria class (OTU_2554)	4.74E-02	0.17217	0.083562
Ammonia	Borrelia genus (OTU_1)	1.17E-02	0.081732	0.158981
Ammonia	Mycoplasma genus (OTU_11355)	4.45E-03	-0.120097	0.184401
Ammonia	Polaribacter 4 genus (OTU_9546)	1.90E-03	0.079533	0.140088
Ammonia	Flavobacteriaceae family (OTU_9833)	7.82E-03	0.066959	0.126236
Ammonia	Candidatus Hepatoplasma genus (OTU_11357)	2.91E-06	-0.087717	0.401385
Ammonia	JTB255 marine benthic group family (OTU_6616)	7.85E-04	0.044926	0.176253
Ammonia	Mycoplasma genus (OTU_11453)	5.67E-04	0.019827	0.159336
Ammonia	Uncultured bacterium genus (OTU_113)	7.95E-06	0.089001	0.184026
Ammonia	Ambiguous_taxa genus (OTU_15557)	2.38E-04	0.049241	0.166893
Ammonia	Haliea genus (OTU_8857)	4.90E-03	0.035052	0.124142
Ammonia	Comamonadaceae family (OTU_14443)	2.49E-02	0.004954	0.135996
Ammonia	Mycoplasmataceae family (OTU_376)	1.26E-05	-0.206891	0.187244
Ammonia	Microbulbifer genus (OTU_13047)	3.06E-02	-0.00897	0.14729
Ammonia	JTB255 marine benthic group family (OTU_5026)	2.04E-02	-0.026935	0.122395
Ammonia	Rhodopirellula genus (OTU_15867)	7.67E-03	0.122132	0.156758
Ammonia	Maribacter genus (OTU_2121)	2.91E-06	0.174217	0.214447
Ammonia	Arcobacter genus (OTU_17190)	2.35E-02	-0.052524	0.135077
Ammonia	Microbulbifer genus (OTU_11346)	1.26E-03	-0.055356	0.153685
Ammonia	Flavobacteriaceae family (OTU_5038)	3.87E-03	0.044319	0.126659

Ammonia	Flavobacteriaceae family (OTU_4460)	1.25E-02	0.118005	0.10345
Ammonia	Endozoicomonas genus (OTU_2823)	1.72E-04	-0.097522	0.141528
Ammonia	Cellulophaga genus (OTU_9296)	2.91E-06	0.148506	0.280014
Ammonia	Haliea genus (OTU_12961)	1.07E-02	0.074752	0.113563
Ammonia	Actibacter genus (OTU_2932)	9.82E-03	-0.102885	0.161247
Ammonia	Uncultured bacterium genus (OTU_5533)	4.72E-02	0.023309	0.104054
Ammonia	Actibacter genus (OTU_5023)	3.91E-02	-0.05065	0.114273
Ammonia	Actibacter genus (OTU_2972)	5.09E-04	0.134082	0.123389
Ammonia	Flavobacteriaceae family (OTU_4504)	1.11E-04	0.145063	0.143759
Ammonia	Algitalea genus (OTU_8064)	2.91E-06	0.112415	0.244271
Ammonia	Flavobacteriaceae family (OTU_2707)	1.05E-02	0.117417	0.149331
Ammonia	Piscirickettsiaceae family (OTU_1817)	6.60E-04	0.051641	0.192666
Ammonia	Family XIII family (OTU_11608)	2.42E-02	0.107042	0.162107
Ammonia	Aquibacter genus (OTU_5525)	5.33E-04	0.053523	0.165465
Ammonia	Ambiguous_taxa genus (OTU_4769)	4.31E-04	-0.052466	0.141064
Ammonia	Sandaracinaceae family (OTU_3236)	4.19E-03	0.054596	0.11804
Ammonia	Marinifilum genus (OTU_8076)	2.22E-03	0.033037	0.175192
Phosphate	Borrelia genus (OTU_1)	2.32E-02	0.042679	0.15732
Phosphate	Mycoplasma genus (OTU_11355)	3.11E-03	-0.108032	0.219084
Phosphate	Polaribacter 4 genus (OTU_9546)	3.83E-04	-0.256536	0.204934
Phosphate	Flavobacteriaceae family (OTU_9833)	4.34E-03	-0.158927	0.163769
Phosphate	Candidatus Hepatoplasma genus (OTU_11357)	2.91E-06	0.393052	0.48107
Phosphate	JTB255 marine benthic group family (OTU_6616)	1.66E-04	-0.21849	0.160726
Phosphate	Mycoplasma genus (OTU_11453)	2.62E-04	-0.164611	0.162776
Phosphate	Uncultured bacterium genus (OTU_113)	2.91E-06	-0.260046	0.291649
Phosphate	Ambiguous_taxa genus (OTU_15557)	3.12E-05	0.050262	0.234268
Phosphate	Haliea genus (OTU_8857)	1.41E-04	-0.232289	0.209426

Phosphate	Comamonadaceae family (OTU_14443)	3.62E-02	-0.037428	0.133189
Phosphate	Mycoplasmataceae family (OTU_376)	1.26E-05	-0.13922	0.206751
Phosphate	Microbulbifer genus (OTU_13047)	2.67E-04	-0.270119	0.158006
Phosphate	JTB255 marine benthic group family (OTU_5026)	8.21E-03	-0.092798	0.122792
Phosphate	Rhodopirellula genus (OTU_15867)	3.46E-03	0.101137	0.195688
Phosphate	Maribacter genus (OTU_2121)	2.91E-06	-0.43706	0.235612
Phosphate	Arcobacter genus (OTU_17190)	1.62E-02	0.073232	0.153908
Phosphate	Microbulbifer genus (OTU_11346)	1.11E-05	-0.254334	0.214767
Phosphate	Flavobacteriaceae family (OTU_5038)	5.58E-03	-0.108007	0.117226
Phosphate	Flavobacteriaceae family (OTU_4460)	1.94E-02	-0.096831	0.149561
Phosphate	Endozoicomonas genus (OTU_2823)	8.03E-04	-0.117708	0.190627
Phosphate	Cellulophaga genus (OTU_9296)	2.91E-06	-0.459335	0.391276
Phosphate	Haliea genus (OTU_12961)	1.52E-03	-0.193451	0.120265
Phosphate	Actibacter genus (OTU_2932)	1.64E-02	-0.165323	0.128997
Phosphate	Uncultured bacterium genus (OTU_5533)	1.96E-03	-0.148954	0.113917
Phosphate	Actibacter genus (OTU_5023)	4.36E-02	-0.080049	0.103716
Phosphate	Mycoplasma genus (OTU_11533)	2.09E-03	-0.214984	0.139816
Phosphate	Actibacter genus (OTU_2972)	4.19E-04	-0.174182	0.129079
Phosphate	Flavobacteriaceae family (OTU_4504)	3.77E-04	-0.204514	0.135377
Phosphate	JTB255 marine benthic group family (OTU_5117)	4.43E-02	-0.038189	0.108283
Phosphate	Algitalea genus (OTU_8064)	2.91E-06	-0.479084	0.27956
Phosphate	Flavobacteriaceae family (OTU_2707)	4.76E-02	-0.052917	0.148133
Phosphate	Piscirickettsiaceae family (OTU_1817)	2.91E-06	-0.35973	0.247267
Phosphate	Family XIII family (OTU_11608)	6.73E-03	0.06653	0.161895
Phosphate	Aquibacter genus (OTU_5525)	7.95E-06	-0.384399	0.209119
Phosphate	Thalassolituus genus (OTU_11004)	5.04E-03	0.1876	0.141312
Phosphate	Ambiguous_taxa genus (OTU_4769)	8.98E-06	-0.318732	0.180262

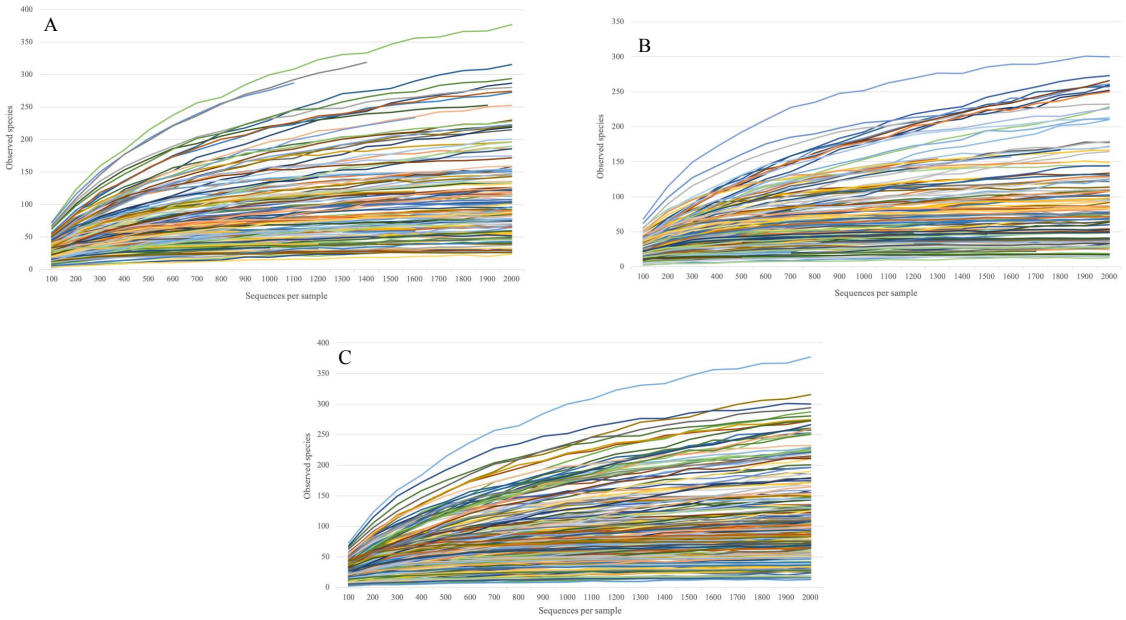
Phosphate	Sandaracinaceae family (OTU_3236)	1.28E-04	-0.236475	0.140468
Phosphate	Pricia genus (OTU_5718)	1.92E-02	-0.064347	0.159743
Phosphate	Marinifilum genus (OTU_8076)	7.95E-06	0.122024	0.177785
Phosphate	Rhodopirellula genus (OTU_16569)	2.54E-02	-0.075073	0.108319
Phosphate	Gammaproteobacteria class (OTU_2554)	6.19E-03	-0.179333	0.090065
Chlorophyll-a	Mycoplasma genus (OTU_11355)	7.13E-03	0.048395	0.201104
Chlorophyll-a	Polaribacter 4 genus (OTU_9546)	2.19E-02	0.073239	0.159792
Chlorophyll-a	Flavobacteriaceae family (OTU_9833)	2.83E-02	0.087526	0.139339
Chlorophyll-a	Candidatus Hepatoplasma genus (OTU_11357)	1.84E-04	-0.208409	0.45664
Chlorophyll-a	JTB255 marine benthic group family (OTU_6616)	1.61E-02	0.11323	0.147347
Chlorophyll-a	Mycoplasma genus (OTU_11453)	8.13E-04	-0.056252	0.145091
Chlorophyll-a	Uncultured bacterium genus (OTU_113)	9.68E-03	0.143357	0.254146
Chlorophyll-a	Ambiguous_taxa genus (OTU_15557)	1.26E-03	0.115891	0.180515
Chlorophyll-a	Haliea genus (OTU_8857)	1.44E-02	0.129541	0.126739
Chlorophyll-a	Comamonadaceae family (OTU_14443)	8.79E-03	0.082603	0.13066
Chlorophyll-a	Mycoplasmataceae family (OTU_376)	3.20E-04	-0.016821	0.206751
Chlorophyll-a	JTB255 marine benthic group family (OTU_5026)	3.46E-02	0.103104	0.130233
Chlorophyll-a	Rhodopirellula genus (OTU_15867)	1.10E-03	0.128117	0.195688
Chlorophyll-a	Maribacter genus (OTU_2121)	1.41E-03	0.266657	0.214758
Chlorophyll-a	Microbulbifer genus (OTU_11346)	2.70E-03	0.212445	0.172464
Chlorophyll-a	Flavobacteriaceae family (OTU_5038)	3.37E-02	0.096691	0.131097
Chlorophyll-a	Flavobacteriaceae family (OTU_4460)	4.93E-02	0.149614	0.12889
Chlorophyll-a	Endozoicomonas genus (OTU_2823)	1.13E-02	0.046565	0.154421
Chlorophyll-a	Cellulophaga genus (OTU_9296)	4.02E-03	0.17268	0.288598
Chlorophyll-a	Haliea genus (OTU_12961)	6.57E-03	0.325989	0.119232
Chlorophyll-a	Actibacter genus (OTU_5023)	3.27E-02	0.007233	0.105281
Chlorophyll-a	Mycoplasma genus (OTU_11533)	8.21E-03	0.225956	0.12661

Chlorophyll-a	Flavobacteriaceae family (OTU_4504)	1.34E-02	0.197672	0.123114
Chlorophyll-a	JTB255 marine benthic group family (OTU_5117)	2.28E-02	0.112469	0.103054
Chlorophyll-a	Algitalea genus (OTU_8064)	5.25E-03	0.074013	0.256296
Chlorophyll-a	Piscirickettsiaceae family (OTU_1817)	4.28E-03	0.10475	0.204691
Chlorophyll-a	Family XIII family (OTU_11608)	1.64E-02	-0.036332	0.164806
Chlorophyll-a	Aquibacter genus (OTU_5525)	3.94E-02	0.159042	0.176488
Chlorophyll-a	Thalassolituus genus (OTU_11004)	2.25E-02	-0.124456	0.116837
Chlorophyll-a	Ambiguous_taxa genus (OTU_4769)	6.62E-03	0.034744	0.180262
Chlorophyll-a	Sandaracinaceae family (OTU_3236)	3.19E-03	0.229578	0.127016
Chlorophyll-a	Pricia genus (OTU_5718)	4.55E-02	-0.014669	0.131725
Chlorophyll-a	Marinifilum genus (OTU_8076)	1.96E-03	-0.049909	0.177785
Chlorophyll-a	Rhodopirellula genus (OTU_16569)	1.64E-02	0.050606	0.099572
Chlorophyll-a	Gammaproteobacteria class (OTU_2554)	1.88E-03	0.172341	0.100724
QX disease	Borrelia genus (OTU_1)	2.91E-06	0.414967	0.223559
QX disease	Mycoplasma genus (OTU_11355)	2.91E-06	-0.304624	0.271212
QX disease	Polaribacter 4 genus (OTU_9546)	4.37E-02	-0.139269	0.127814
QX disease	Flavobacteriaceae family (OTU_9833)	4.49E-02	-0.106457	0.094751
QX disease	Candidatus Hepatoplasma genus (OTU_11357)	8.98E-06	0.267949	0.179022
QX disease	JTB255 marine benthic group family (OTU_6616)	1.86E-03	-0.186235	0.125694
QX disease	Mycoplasma genus (OTU_11453)	1.86E-04	-0.224574	0.134906
QX disease	Uncultured bacterium genus (OTU_113)	2.91E-06	-0.279391	0.184362
QX disease	Haliea genus (OTU_8857)	2.18E-02	-0.158428	0.104126
QX disease	Mycoplasmataceae family (OTU_376)	2.91E-06	-0.231367	0.172367
QX disease	Rhodopirellula genus (OTU_15867)	5.96E-04	-0.164268	0.105636
QX disease	Arcobacter genus (OTU_17190)	2.22E-02	-0.086863	0.106156
QX disease	Microbulbifer genus (OTU_11346)	4.71E-02	-0.103603	0.12557
QX disease	Flavobacteriaceae family (OTU_4460)	1.64E-02	-0.096858	0.10108

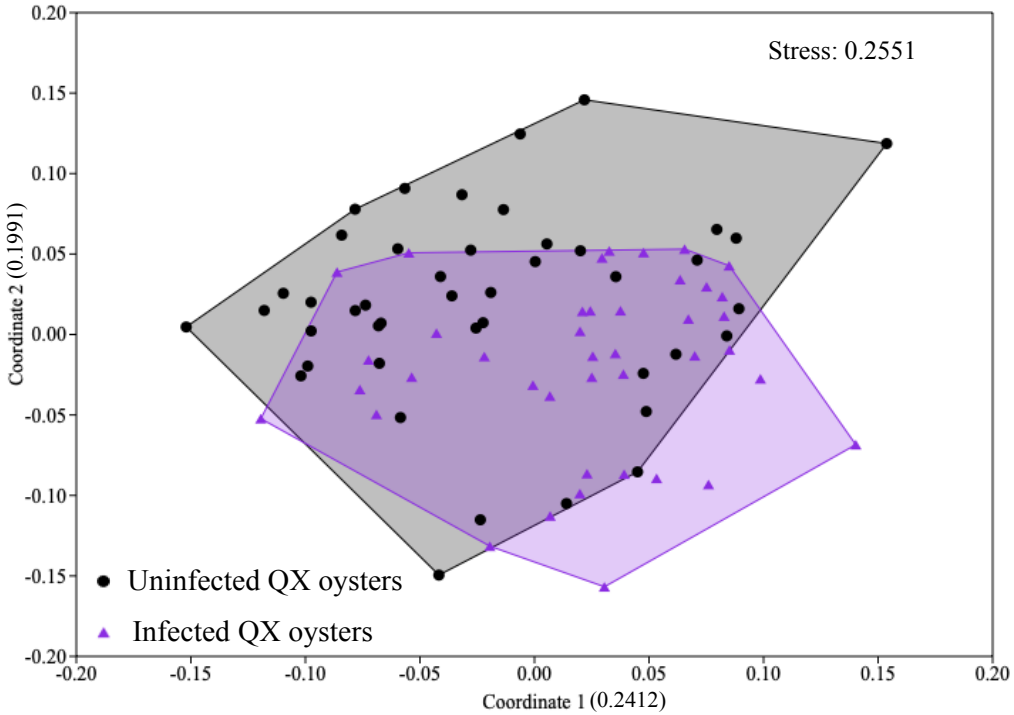
QX disease	Endozoicomonas genus (OTU_2823)	1.20E-04	-0.215718	0.129529
QX disease	Cellulophaga genus (OTU_9296)	1.52E-04	-0.160761	0.122339
QX disease	Haliea genus (OTU_12961)	3.85E-03	-0.169034	0.073572
QX disease	Flavobacteriaceae family (OTU_4504)	2.35E-03	-0.169631	0.098016
QX disease	Thalassolituus genus (OTU_11004)	1.45E-02	-0.150624	0.065233
QX disease	Sandaracinaceae family (OTU_3236)	4.81E-02	-0.113178	0.069339
QX disease	Pricia genus (OTU_5718)	2.22E-02	0.209543	0.117275
QX disease	Marinifilum genus (OTU_8076)	3.09E-03	0.046691	0.062685
QX disease	Rhodopirellula genus (OTU_16569)	2.17E-02	-0.091322	0.101354
QX disease	Gammaproteobacteria class (OTU_2554)	3.19E-02	-0.056123	0.067314

Supplementary Table 35: Significant correlations between environmental variables and QX disease ($p < 0.05$) as identified by Mictools

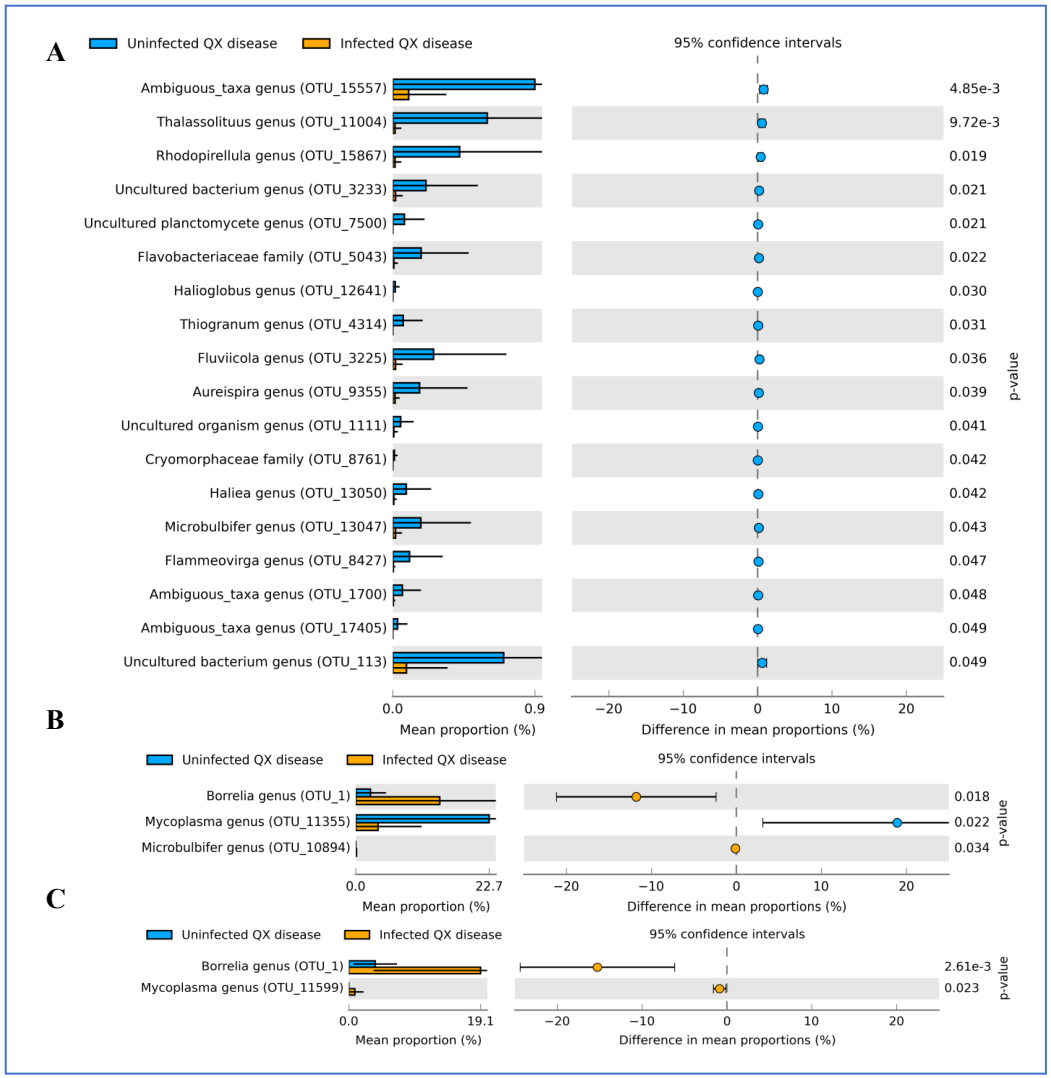
Environmental variables	TICePVal	PearsonR	MICe
pH	2.91E-06	-0.253382	0.326157
Oxygen	2.91E-06	-0.07594	0.326157
Temperature	2.26E-05	0.037423	0.326157
Conductivity	2.91E-06	0.175343	0.326157
Nitrate	3.96E-04	-0.048619	0.274186
Ammonia	2.91E-06	0.010573	0.322509
Phosphate	2.91E-06	0.308414	0.326157
Chlorophyll-a	3.12E-05	-0.128742	0.326157



Supplementary Figure 6: Rarefaction plots for observed species. A: Rarefaction plots of 150 samples. A: Rarefaction plots of 150 other samples. C Rarefaction plots of 210 samples (one curve per sample) showing almost curves reaching asymptote at the cut-off 2000 reads.



Supplementary Figure 7: 3D nMDS plot showing microbiome of uninfected and infected QX oysters shared spatially.



Supplementary Figure 8: Extended error bar plots showing OTUs differing significantly between uninfected and infected QX disease group over three sampling times . A: 13th March, B: 27th March and C: 11th April 2018.

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