Highlights:

- The Sydney rock oyster microbiota is influenced by location and season.
- QX disease-resistance influences the Sydney rock oyster microbiota in winter.
- A shifting microbiota before the QX disease period could contribute to QX disease dynamics.

The Sydney rock oyster microbiota is influenced by location, season and genetics

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Abstract:

Queensland unknown (QX) disease is a significant cause of economic loss for the Sydney rock oyster (SRO) aquaculture industry. Evidence is emerging that QX disease is multi-factorial in nature, with a number of environmental and host factors contributing to disease dynamics. Efforts to mitigate the impacts of QX disease are primarily focused on breeding for disease resistance however, the mechanisms that drive disease resistance are poorly understood. One potential factor influencing disease resistance is the microbiota. To determine the influence of location, season and disease resistance on the SRO microbiota, we used 16S rRNA (V1 – V3 region) amplicon sequencing. The microbiota of six SRO families with two categorised as QX-resistant and four as QX-susceptible, deployed to two different locations (Port Stephens and Wallis Lake, NSW, Australia) and over two seasons (Austral summer and winter), were characterised. As expected, the SRO microbiota was distinct to the microbial community found in seawater. Further, the SRO microbiota was significantly influenced by location and season, with operational taxonomic units (OTUs) assigned to the *Candidatus Hepatoplasma* and *Endozoicomonas* genera identified as significant drivers of microbiota dissimilarity between locations and seasons. Disease resistance also significantly influenced the SRO microbiota but only at the winter time point which is before the typical QX disease period. Overall, OTUs assigned to the *Mycoplasma*, *Borrelia* and *Endozoicomonas* genera were over-represented in QX-resistant SRO microbiota, whereas members of the *Pseudoalteromonas*, *Vibrio*, and *Candidatus Hepatoplasma* genera were over-represented in QX-sensitive microbiota. These

findings confirm the influencing role of location and season on the microbiota structure as evidenced in other molluscan species, but also provide preliminary evidence that the microbiota assemblage before the QX disease period may be important for resistance to disease and may provide new avenues for managing SRO aquaculture in the future.

Keywords: Microbiota, Sydney rock oyster, QX disease, 16S rRNA, disease resistance

1. Introduction

The Sydney rock oyster (SRO; *Saccostrea glomerata*) is native to Australia, where it is one of the most intensively cultivated oyster species (O'Connor & Dove, 2009; Schrobback *et al.*, 2014). However, since the mid-1970's production of this species has been impacted by QX-disease, which can recurrently cause up to 90% mortality in affected estuaries (Department of Primary Industries, 2016; Nell, 2007; O'Connor & Dove, 2009; Peters & Raftos, 2003; Schrobback *et al.*, 2014). The aetiological agent for QX disease is a spore-forming protozoan parasite called *Marteilia sydneyi.* This parasite has an infection cycle that typically enters through the palps and gills in summer and ends in the oyster digestion gland, impacting nutrient uptake and ultimately causing starvation and death through autumn and into winter (Kleeman *et al.*, 2002; Nell, 2007; Wolf, 1979).

To mitigate the impacts of QX disease, the New South Wales Department of Primary Industries (NSW DPI) has led a selective breeding program using both mass selection methods and family based breeding that has greatly reduced SRO mortalities, with some families showing 85% survival through one cycle of disease (Dove *et al.*, 2020). There is evidence that increased levels of resistance in some families may be linked to higher activity of phenoloxidase, an enzyme thought to be involved in oyster defence mechanisms (Newton *et al.*, 2004), yet the full mechanism(s) for resistance remain unresolved.

The oyster microbiota is emerging as a factor in disease dynamics (King *et al.*, 2019a) and is an unexplored factor in SRO QX disease resistance. The potential protective role of the mollusc microbiota has been characterised previously, with some microbial members providing anti-pathogen activities (Offret *et al.*, 2019; Prado *et al.*, 2009). In other studies, the microbiota appears to contribute to disease dynamics, for the Pacific oyster it has been demonstrated that summer mortality in France is due to a progressive replacement of non-virulent commensal

vibrios with pathogenic vibrios indicating that microbiota dysbiosis precedes mortality (Lemire *et al.*, 2015). Similarly, Pacific oyster mortality syndrome is polymicrobial in nature with a recent study showing that the viral Ostreid Herpesvirus 1 (OsHV-1) suppresses Pacific oyster immunity, allowing opportunistic bacterial pathogens such as *Vibrio* species to thrive (de Lorgeril *et al.*, 2018). Interestingly, the microbiota of Pacific oyster families bred for resistance to OsHV-1 were significantly different to their disease-susceptible counterparts and had a significantly reduced abundance of *Vibrio* species (King *et al.*, 2019c). In SROs, only one study has investigated the QX-disease-affected microbiota by comparing the digestive gland of QX-infected and uninfected oysters (Green & Barnes, 2010). In QX-infected oysters, bacterial diversity was substantially reduced, with the microbiota dominated by a *Rickettsiales*-like operational taxonomic unit (OTU).

A first step in understanding the role of a microbiota in disease dynamics is characterising its composition and determining the factors that shape its structure. In previous studies in other oyster species, the oyster microbiota has been shown to be influenced by both environmental and host factors including location, temperature, infection state, season, genetics, life stage and resistance to disease (Green & Barnes, 2010; King *et al.*, 2012; King *et al.*, 2019b; King *et al.*, 2019c; Lokmer & Wegner, 2015; Lokmer *et al.*, 2016a). However, there is a paucity of studies examining the factors that influence the SRO microbiota assemblage. Therefore, to characterise the influence of location, season and disease-resistance (genetics) on the SRO microbiota, six SRO families with varying degrees of resistance to QX disease were deployed into two locations and sampled in the Austral summer and winter. Understanding the mechanism(s) that drive disease-resistance, including the potential contribution of the microbiota to disease, are imperative for the successful and sustainable management of SRO aquaculture.

2. Materials and methods

2.1. Experimental design and sampling

Fourty-four different *Saccostrea glomerata* families from the 2015 year class were deployed in the 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) estuaries in NSW, Australia. Wallis Lake is a wave-dominated barrier estuary whereas Port Stephens is a tide-dominated drowned valley estuary (Roy *et al.*, 2001). These

estuaries are approximately 70 km apart and are not affected by QX disease. These sites were selected to remove the influence of infection- or disease-state on the microbiota. For this study, six families from the 2015 class were selected according to their predicted level of resistance to QX disease using the Estimated Breeding Values (EBVs), which provides an estimation of how well families will perform for a particular trait and the likelihood of passing those traits to their progeny. As EBV is only a predictor, we selected six different families with a predicted range of QX disease resistance to ensure that we had sufficient oyster numbers for comparing the microbiota of oysters with differing QX disease resistance. Subsequent exposure of these families to QX disease at Lime Kiln Bar in the Georges river (33°59'19"S 151°03'21"E) demonstrated that four of the families exhibited ≤50% survival (characterised as QX-susceptible), while the other two families displayed >50% survival (QX-resistant; Table 1).

Five oysters per family were collected from each site in the 2017 Austral summer (January) and Austral winter (June), four and nine months after deployment respectively (120 oyster samples in total). Oysters were randomly collected by farmers from cultivation trays, placed into labelled plastic bags, transported to the laboratory on ice (3 - 4 hours) and stored whole in their shell at -80^{0}C for later processing. Because oyster leases could only be accessed by boat, seawater samples were collected from jetties (piers) approximately 800 metres away from the oyster leases. The jetties face the oyster leases and are suspended over water that are a few metres deep ensuring no sediment was suspended from the bottom during collection. Ten litres of surface seawater samples were collected and kept on ice during transport to the laboratory. Triplicate seawater samples of 2000 mL for each sampling time were filtered with Durapore Membrane Filters (0.22 µm pore size) for subsequent microbiota analyses. All filtered samples were frozen in liquid nitrogen upon collection in sterile 5 mL cryotubes and kept at -80^{0}C prior to analysis.

Table 1**:** 2015 year class Sydney rock oyster average family survival (n = 3, ± SD) following exposure to QX disease at Lime Kiln Bar, Georges river. Oysters were deployed to Lime Kiln Bar on 12 December 2016 and oyster survival was counted on 20 September 2017.

Family line	Average survival (%)
F25	59.67 ± 0.58
F22	55.33 ± 3.06
F18	19.67 ± 3.79
F03	3.33 ± 2.31
F32	2.67 ± 3.06
F37	0.67 ± 1.15

2.2. Measurement of environmental parameters, nutrients and chlorophyll a in seawater

Environmental parameters (temperature, oxygen, pH, and conductivity) were measured at jetties adjacent to the oyster leases using a WTW multiprobe meter (Multi 3430, Germany) at the time of oyster sample collections. For nutrient analysis, 50 mL triplicate seawater samples were syringe filtered through a 0.45 μm filter into 50 mL sterile falcon tubes, transported to the laboratory on ice, and frozen at -20^{0}C. Nutrient analysis (nitrite (NO_2^-), nitrate (NO_3^-), ammonia (NH_3) and phosphate (PO_4^{3-})) was performed by Envirolab Services Pty Ltd (Sydney, New South Wales, Australia). From the 10 L of seawater collected above, triplicate 200 mL aliquots were filtered through glass microfiber filters (0.7 μm pore size) and stored at -80^{0}C for subsequent chlorophyll-a analyses. Chlorophyll a was analysed based on a Spectrophotometric method described previously (Ritchie, 2006).

2.3. DNA extractions and 16S rRNA amplicon sequencing

DNA extractions commenced only after the last sample had been collected and frozen. Samples were randomly thawed in batches of 20 and all samples were processed using a single DNA extraction kit. Thawed oysters were washed under running tap water to remove debris. Using sterile instruments, each oyster was carefully opened using a shucking knife and the oyster flesh excised and placed onto a Petri dish. Approximately 25-50 mg of adductor muscle tissue was then excised using a sterile scalpel blade and placed into a 1.5 mL Eppendorf tube for subsequent DNA extraction using the Qiagen DNeasy Blood and Tissue DNA extraction Kit

(Qiagen, Germany), according to the manufacturer's instructions. Haemolymph is often used to study the oyster microbiota (Lokmer *et al.*, 2016a; Lokmer *et al.*, 2016b) but can be difficult to extract from small oysters and is not possible to extract once oysters have been frozen. To minimise variation, we decided to freeze oysters so they could be later processed together. Therefore, the adductor muscle was selected for microbiota analysis as it contains haemolymph sinuses thus allowing us to easily sample the haemolymph. This approach has been successfully used before (King *et al.*, 2019b; King *et al.*, 2019c). The instruments used to process the oysters, including the shucking knife, were cleaned, soaked in 1:15 bleach solution for 15 min and then rinsed with sterile Milli-Q water prior to use and between samples. DNA from filtered seawater samples were extracted using the PowerWater DNA Isolation Kit (MoBio, USA) according to the manufacturer's protocol.

The V1–V3 region of the 16S rRNA gene was amplified by PCR using the 27F (5′-AGAGTTTGATCMTGGCTCAG-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 30 cycles of 94°C for 30s, 50°C for 30s and 72°C for 30s and a final extension of 72°C for 10 min. Amplicons were sequenced using the Illumina MiSeq platform (2 × 300 bp) at the Ramaciotti Centre for Genomics (University of New South Wales, Sydney, Australia). Raw data files in FASTQ format were deposited in NCBI Sequence Read Archive (SRA) with the study accession number (SRP234946) under Bioproject number PRJNA593911.

2.4. Bioinformatics analyses

Demultiplexed paired-end reads were combined using FLASH (Magoč & Salzberg, 2011) and trimmed using Mothur (Schloss *et al.*, 2009) (Parameters: maxhomop = 5, maxambig = 0, minlength = 471, maxlength = 501). Fragments were clustered into operational taxonomic units (OTUs) at 97% sequence similarity, and chimeric and singleton sequences were identified and removed using VSEARCH (Rognes *et al.*, 2016). Taxonomic assignment of OTUs were performed in QIIME version 1.9.1 (Caporaso *et al.*, 2010) using the UCLUST algorithm (Edgar, 2010) against the SILVA v128 dataset (Quast *et al.*, 2013). Mitochondrial and chloroplast data were filtered out of the dataset and the remaining reads were rarefied to the same depth to remove the effect of sampling effort upon analysis. For beta diversity, the relative abundance of OTUs was calculated and all OTUs with a relative abundance below 0.1% were

filtered from the dataset. Alpha diversity indices, including species richness (Chao1), species evenness (Simpson) and species diversity (Shannon index) were calculated using QIIME (Caporaso *et al.*, 2010).

2.5. Statistical analyses

Alpha diversity metrics were compared between groups using a Kruskal-Wallis test. All beta diversity analyses were performed with a Bray-Curtis dissimilarity index. To easily visualise how samples related to one another and observe distance matrices between groups, non-metric multidimensional scaling analysis (nMDS) with three dimensions (3D) was used. Patterns elucidated by the 3D nMDS were statistically tested using a permutational multivariate analysis of variance (PERMANOVA) with 9999 permutations using transformed (square root(x)) data. To identify the OTUs driving the difference between the microbial assemblage at different locations or time points, SIMPER analysis was used. All alpha and beta diversity comparisons were performed in the PAST statistical environment (Hammer *et al.*, 2001). To determine whether the relative abundances of OTUs were significantly different between oyster groups with differing QX-resistance, a Welch's T-Test was performed using the STAMP (Statistical Analysis of Metagenomic Profiles) software package version 2.1.3 (Parks *et al.*, 2014). A file listing the relative abundance of all OTUs was used as input data along with a metadata file containing location, sampling time and QX-resistance group information. A Welch's T-Test with a p-value of <0.05 as a statistical cut-off was used. To visualise the significant difference in the relative abundance of OTUs between the QX-sensitive QX-and resistant groups at a single location at each sampling time, extended error bar plots with corrected p-values were produced.

3. Results

Following amplicon sequencing of the 132 samples (oysters and seawater), data were rarefied to 7,178 reads retaining a total of 753,690 reads from 105 samples (Supplementary Table 1). After data filtering, a total of 1,889 OTUs were observed across the entire dataset. Of these, 1,619 and 190 OTUs were unique to the oyster and seawater microbiota respectively, with only 80 OTUs found in both the oyster and seawater samples.

3.1. The SRO microbiota is distinct from the seawater microbiota

Across the entire dataset, species richness, evenness and diversity were higher in seawater samples relative to the SRO adductor muscle microbiota (Figure 1 and Supplementary Table 2). When grouping all SRO or seawater samples, an 3D nMDS analysis revealed that the composition of the SRO and seawater microbiota were distinct from one another (Supplementary Figure 1), with these differences confirmed as significantly different by PERMANOVA (F = 13.54, p = 0.0001). SIMPER analysis revealed a 99.1% dissimilarity between the SRO and seawater microbiota, with *Candidatus Hepatoplasma* genus (OTU 14887) and *Endozoicomonas* genus (OTU 3829) over-represented in SRO microbiota and driving 5.7% and 2.9% of the difference respectively (Figure 2 and Supplementary Table 3). In seawater, the *Candidatus Actinomarina* genus (OTU 22961) and NS5 marine group genus (OTU 5409) were over-represented, driving 4.2% and 3.6% of the difference respectively (Figure 2 and Supplementary Table 3).

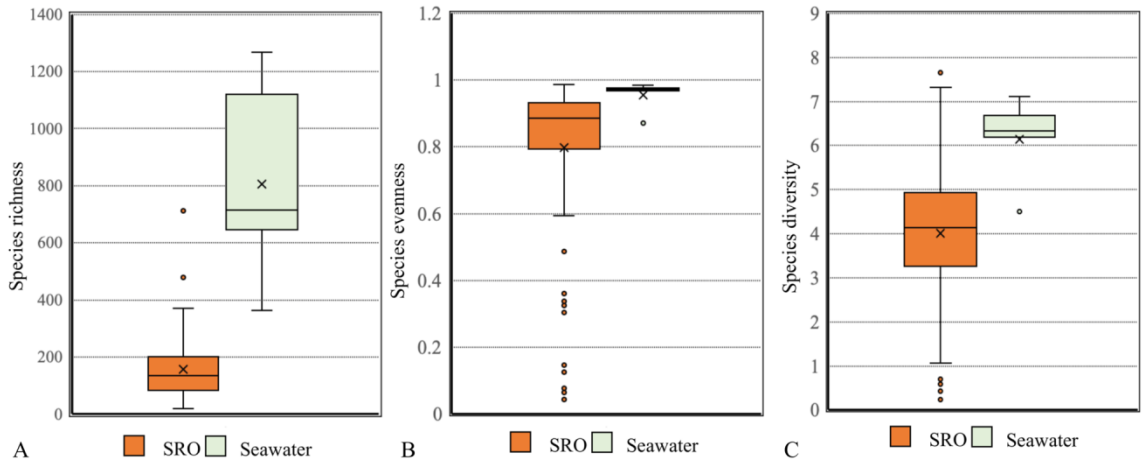

Figure 1: Box and whisker plot of species richness (A), evenness (B) and diversity (C) for SRO and seawater microbiota. The x in the box plot is the mean of the dataset.

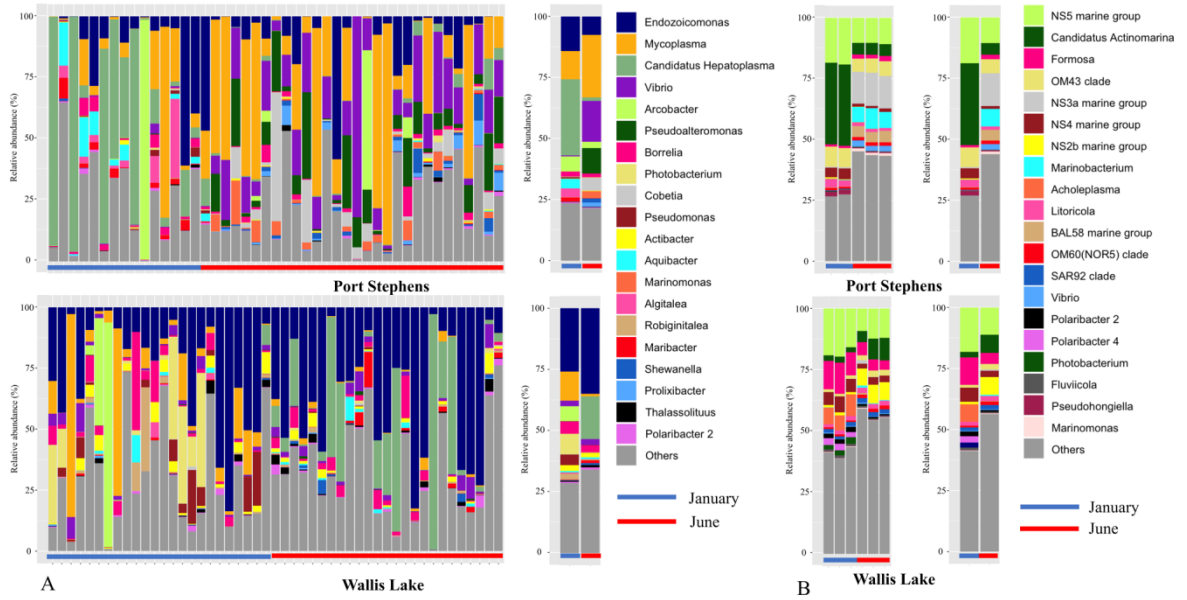

Figure 2: Microbiota composition of SRO (A) and seawater samples (B) in Port Stephens (upper panels) and Wallis Lake (lower panels) showing the top 20 dominant and remaining taxa in January (underlined by blue bar) and June (underlined by red bar). The right bars in each panel show the mean abundance of each taxon within each group. Data is summarised at the genus level.

3.2. Location is a factor shaping the SRO microbiota

Overall, Port Stephens had higher temperatures, pH and chlorophyll a at each time point, whereas Wallis Lake had higher levels of dissolved oxygen relative to Port Stephens. A rainfall event occurred during the June (winter) sampling at Port Stephens which likely explains the decrease in conductivity and increase in nutrients during this time point (Table 2).

When the total SRO microbiota deployed in Port Stephens and Wallis Lake were compared, species richness and diversity were statistically higher in Wallis Lake ($p = 0.029$ and $p = 0.007$ respectively, Supplementary Figure 2A and Supplementary Table 4). However, no statistical difference in alpha indices was observed when SRO microbiota from Port Stephens and Wallis Lake were independently compared in January and June (Supplementary Figure 2B and Supplementary Table 4).

Despite some overlap, a 3D nMDS plot showed that SRO microbiota clustered according to location (Figure 3A) and were significantly different according to site (PERMANOVA, F = 8.955, p = 0.0001). This effect of location was also evident within each season in January (Figure 3B and 3C; PERMANOVA, F = 5.117, p = 0.0001) and June (PERMANOVA, F = 11.81, p = 0.0001). Across the entire dataset, the SRO microbiota at Port Stephens and Wallis Lake were 90.5% dissimilar to one another. Similarly, in January and June, the SRO microbiota from the two sites were 90.3% and 91.9% dissimilar respectively. Interestingly, the main dissimilarity contributor, *Candidatus Hepatoplasma* genus (OTU 14887), was over-represented at Port Stephens in January contributing 17.7% to the dissimilarity between microbiota however, was over-represented at Wallis Lake in June contributing 9.6% of the microbiota dissimilarity (Supplementary Table 5). Additionally, a member of the *Endozoicomonas* genus (OTU 1831) was over-represented in Wallis Lake in both January and June contributing 3.0% and 6.4% respectively.

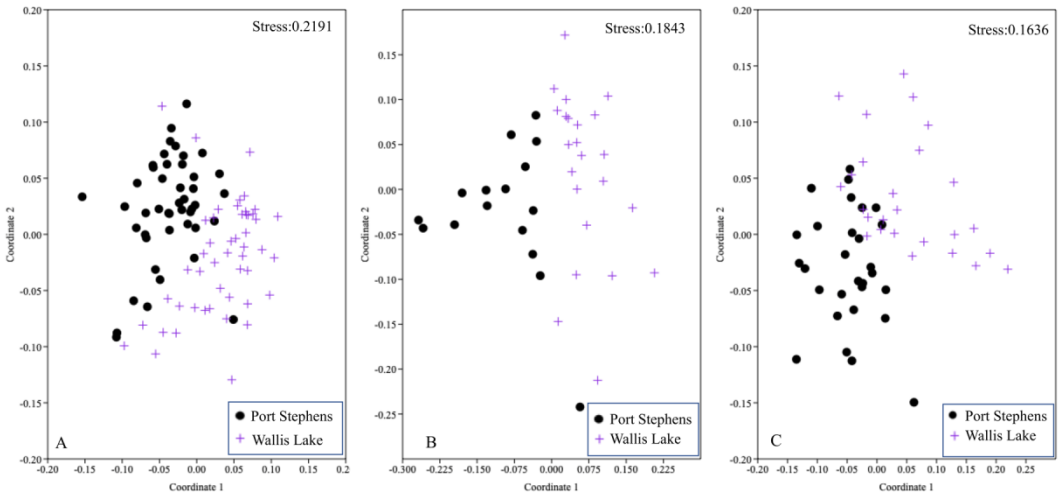

Figure 3: 3D nMDS plots of total SRO microbiota (A) and those from January (B) and June (C) show separation according to location.

Table 2: Environmental parameters in Port Stephens and Wallis Lake at time of sampling

Time	Temperature (^{0}C)	pH	DO (mg/L)	Conductivity (µS/cm)	NO_3 (mg/L)	NO_2^- (mg/L)	NH_3 (mg/L)	PO_4^{3-} (mg/L)	Chlorophyll a (µg/ml)	Rainfall*
Port Stephens										
January	27.8	8.0	8.18	53.3	<0.005	0.004 ± 0.0	0.012 ± 0.003	0.014 ± 0.003	11.41 ± 1.48	Rainfall 2 days before sampling (0.4 mm). Monthly total rainfall was 69.9mm
June	24	8.3	8.88	27.6	0.047 ± 0.01	<0.005	0.038 ± 0.001	<0.005	23.03 ± 3.13	Rainfall over 6 days including during sampling (average 23.65 mm/day). Monthly total rainfall was 315.1mm
Wallis Lake										
January	24	7.2	9.5	53.9	<0.005	0.004 ± 0.0	0.013 ± 0.004	0.007 ± 0.001	9.05 ± 0.62	Rainfall event 2 days before sampling (2.0 mm). Monthly total rainfall was 89.2mm
June	18.3	8.2	9.07	53.6	0.014 ± 0.014	<0.005	0.018 ± 0.001	<0.005	9.52 ± 0.57	Rainfall over 3 days before sampling (average 5.6 mm/day). Monthly total rainfall was 188.1mm

*Data obtained from (Bureau of Meteorology, 2019)

3.3. Season is a factor shaping the SRO microbiota

We next examined whether seasonality influenced the SRO microbiota within a given location. There were no statistical differences in alpha diversity in either Port Stephens or Wallis Lake (Supplementary Figure 2C and Supplementary Table 4). However, 3D nMDS plots revealed the SRO microbiota at both sites tended to cluster according to sampling time (Figure 4). This seasonal variability was more pronounced in Port Stephens (PERMANOVA, $F = 10.42$, $p = 0.0001$) than Wallis Lake (PERMANOVA, $F = 3.451$, $p = 0.0001$). At Wallis Lake, the SRO microbiota was 86.5% dissimilar with OTUs assigned as members of the *Endozoicomonas* genus (OTU 1831) and the *Candidatus Hepatoplasma* genus (OTU 14887) over-represented in January and June respectively, contributing 8.1% and 10.4% to the microbiota dissimilarity (Supplementary Table 6). At Port Stephens, there was 92.7% dissimilarity in SRO microbiota composition between seasons, with an OTU assigned to the *Candidatus Hepatoplasma* genus (OTU 14887) over-represented in January and contributing 16.8% to the dissimilarity. In June, OTUs assigned as *Vibrio* (OTU 2), *Mycoplasma* (OTU 14900) and *Pseudoalteromonas* (OTU 8917) were over-represented, contributing 6.6%, 5.6% and 5.0% to the dissimilarity between seasons respectively (Supplementary Table 6).

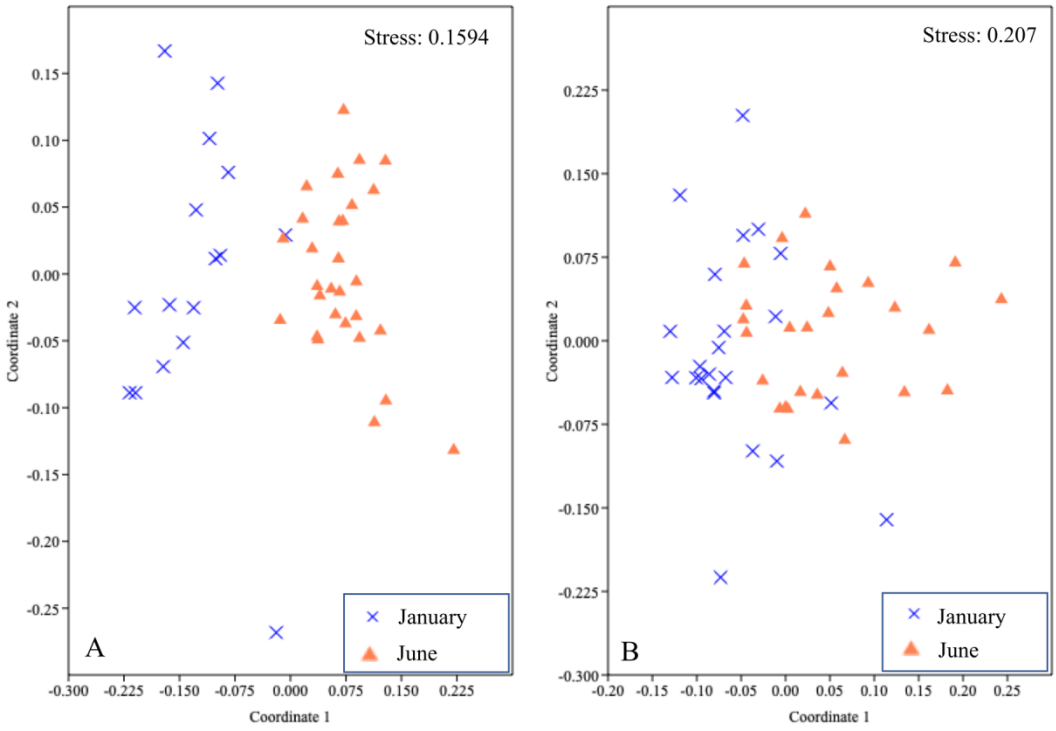

Figure 4: 3D nMDS plots of SRO microbiota in Port Stephens (A) and Wallis Lake (B) separating according to time of sampling.

3.4. The effect of QX-resistance on the SRO microbiota

Across times and sites, we analysed differences in the oyster microbiota between SROs with different levels of resistance to QX disease. Families were grouped as QX-sensitive if survival was ≤50% and QX-resistant if displayed >50% survival (Table 1). Species richness was higher in the QX-sensitive group at Port Stephens in January (Average: 74 ± 3.26 vs 143.38 ± 77.87, $p = 0.039$; Supplementary Table 7). No other significant differences in alpha diversity indices were observed between the QX groups in each location at each time point (Supplementary Table 7). PERMANOVA showed statistically significant differences in the microbiota structure of different QX-resistance groups only in June at both locations (Table 3).

At Port Stephens in June, SIMPER analysis revealed a 75.7% dissimilarity between the QX-sensitive and QX-resistant groups with two OTUs (OTU 12669 and OTU 14900) from the *Mycoplasma* genus over-represented in the QX-resistant group and contributing 9.6% and 9.2% to the microbiota dissimilarity. OTUs belonging to the *Pseudoalteromonas* (OTU 8917)

and *Vibrio* (OTU 2) genera were over-represented in the QX-sensitive group contributing 6.4% and 6.1% to the microbiota dissimilarity (Supplementary Table 8), while another OTU assigned to the *Vibrio* genus (OTU 1) was over-represented in the QX-resistant microbiota contributing 5.6% dissimilarity (Supplementary Table 8). Additionally, two *Mycoplasma* OTUs (OTU 12669 and OTU 14900) were over-represented in the QX-resistant group, contributing 9.6% and 9.2% to the microbiota dissimilarity. At Wallis Lake in June, SIMPER revealed 77.9% microbiota dissimilarity between the QX groups. A member assigned to the *Candidatus Hepatoplasma* genus (OTU 14887) was over-represented in the QX-sensitive group and contributed 15.86% of the microbiota dissimilarity, whereas 5 OTUs, all assigned to the *Endozoicomonas* genus (OTUs 1831, 3829, 6283, 3483 and 4530), were over-represented in the QX-resistant microbiota.

Table 3. PERMANOVA results comparing the microbiota of QX-sensitive (F03, F18, F32 and F37) and QX-resistant (F022 and F025) families at each location and time point.

	Port Stephens	**Wallis Lake**
January	F = 1.184, p = 0.2233	F = 1.1, p = 0.263
June	F = 1.562, p = 0.0491	F = 1.614, p = 0.0378

To further decipher beta diversity patterns between QX-resistant and -sensitive SRO's, STAMP with a Welch's T-Test was used. This analysis identified members of the *Vibrio* (OTU 2, p = 0.003) and *Colwellia* (OTU 3670, p = 0.028) genera with significantly higher relative abundance in the QX-sensitive group from Port Stephens in June (Figure 5A). In Wallis Lake, a member assigned as the *Thiohalocapsa* genus (OTU 11899) had a significantly higher relative abundance in QX-sensitive oysters (p = 0.025), whereas OTUs assigned to the *Borrelia* (OTU 651, p = 0.038) and *Endozoicomonas* (OTU 4530, p = 0.047) genera had a significantly higher relative abundance in QX-resistant oysters (Figure 5B).

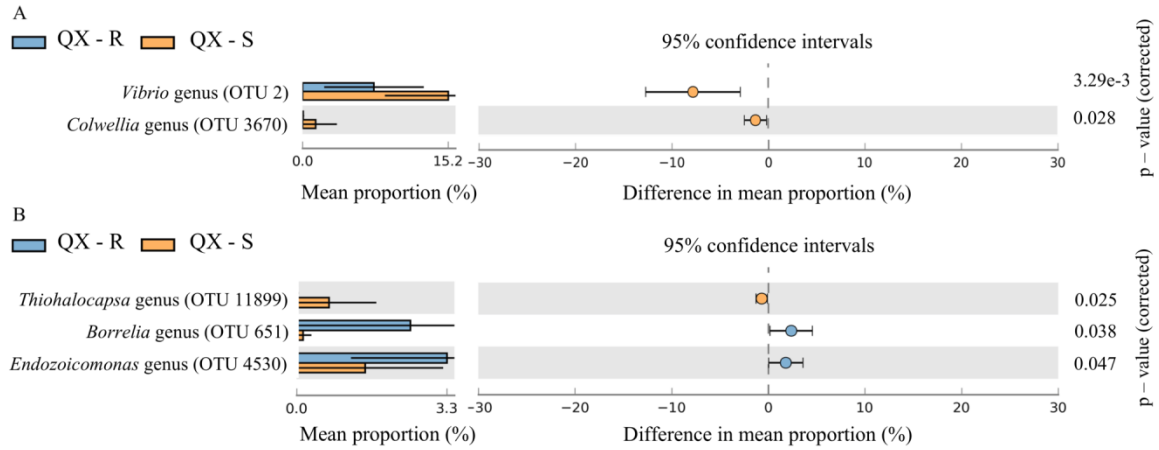

Figure 5: Extended error bar plots showing OTUs with a significant difference in relative abundance between the QX-sensitive (QX - S) and resistant groups (QX - R) at Port Stephens (A) and Wallis Lake (B) in June.

4. Discussion

This study investigated the influence of location, season and oyster genetics (QX-resistance) on shaping the SRO microbiota. Despite the filter-feeding nature of oysters, our results indicate that the SRO microbiota is highly distinct from the planktonic microbiota within the surrounding seawater. It is possible that part of the observed variation is due to the seawater samples being collected from jetties 800 m from the oyster leases however, it is unlikely that the main bacterial patterns in the seawater would substantially vary across this small distance. Additionally, it is also possible that a part of the observed variation is due to the use of different DNA extraction kits for the oysters and water samples. Nevertheless, the patterns we observed are consistent with previous studies on the microbiota of the Pacific oyster (Lokmer *et al.*, 2016a; Lokmer *et al.*, 2016b).

The microbiota varies between oyster tissues (King *et al.*, 2012; King *et al.*, 2020; Lokmer *et al.*, 2016b) however, some overlap is observed such as the genus *Mycoplasma* which is dominant in the adductor muscle, gill, stomach, digestive gland and haemolymph (Green & Barnes, 2010; King *et al.*, 2012; King *et al.*, 2019b; King *et al.*, 2020; Wegner *et al.*, 2013). Here, we elected to use the adductor muscle as it allows sampling of the circulatory haemolymph from the sinuses. Overall, the SRO microbiota was dominated by OTUs assigned to the *Candidatus Hepatoplasma*, *Endozoicomonas* and *Mycoplasma* genera. *Candidatus*

Hepatoplasma has been found associated with various marine organisms such as starfish (Nakagawa *et al.*, 2017), Norway lobsters (Meziti *et al.*, 2012), corals (van de Water *et al.*, 2018) and starlet sea anemones (Mortzfeld *et al.*, 2016). However, the function of this bacterium in marine organisms, including SROs, is unknown. *Mycoplasma* is consistently identified in healthy oysters including Eastern oysters, Pacific oyster and SROs (Green & Barnes, 2010; King *et al.*, 2012; King *et al.*, 2019b; King *et al.*, 2019c; Wegner *et al.*, 2013) suggesting that these bacteria are potentially important for oyster health. Members of the *Endozoicomonas* genus have been found to be associated with numerous marine organisms (Neave *et al.*, 2016) such as sponges (Nishijima *et al.*, 2013; Rua *et al.*, 2014) and corals (Bayer *et al.*, 2013; Ziegler *et al.*, 2016) with members of this genus previously shown to comprise a large proportion of the Indo-Pacific (Roterman *et al.*, 2015; Zurel *et al.*, 2011) and Black-Lipped pearl oyster (Dubé *et al.*, 2019) bacterial communities. In sponges and corals, these bacteria play a role in nitrogen and carbon recycling, provision of proteins to their hosts and production of antibiotics (Neave *et al.*, 2017; Nishijima *et al.*, 2013; Rua *et al.*, 2014) and may suggest a similar role in SROs.

4.1. The SRO microbiota is influenced by location

The same oyster families were deployed in Port Stephens and Wallis Lake reducing the influence of genetics as a confounding factor in our analyses and allowing us to investigate whether location or season influence the composition of the SRO microbiota. Consistent with previous studies that have characterised the influence of location on the oyster microbiota (King *et al.*, 2012; Ossai *et al.*, 2017; Roterman *et al.*, 2015; Trabal *et al.*, 2012; Zurel *et al.*, 2011), we observed that SRO microbiota was significantly different between two sites which are approximately 70 km apart and differ in estuarine type (Roy *et al.*, 2001). Data collected in this study identified higher chlorophyll a concentrations and temperature in Port Stephens relative to Wallis Lake. While both estuaries have similar percentages of agricultural land usage in their respective catchments (approximately 30%), Port Stephens has significantly higher sediment and nutrient inputs compared to Wallis Lake (Roper *et al.*, 2011). Given the higher nutrient and sediment loads at Port Stephens, these factors could explain the microbiota variability between the locations. A member of the *Endozoicomonas* genus (OTU 1831) was more abundant in Wallis Lake than in Port Stephens at both sampling times. In coral species, the anthropogenically influenced coral microbiota (*Pocillopora verrucosa* and *Acropora hemprichii*) was marked by a reduction of *Endozoicomonas* relative abundance (Ziegler *et al.*,

2016), suggesting that the lower relative abundance of this bacteria in SROs at Port Stephens could be related to the higher nutrient and sediment loads.

4.2. The SRO microbiota is influenced by season

In a number of marine organisms, including corals (Sharp *et al.*, 2017) and Pacific oysters (Pierce *et al.*, 2016; Zurel *et al.*, 2011), there is evidence for significant temporal heterogeneity in microbiota composition. Consistent with these findings, we observed a significant influence of season (summer versus winter) on the SRO microbiota for both locations. At Port Stephens, seasonal shifts in environmental conditions were dominated by changing temperature, chlorophyll a and conductivity, while at Wallis Lake, seasonal changes in environmental parameters were mostly driven by temperature and pH. Previous studies have characterised the influence of temperature on the oyster microbiota (Lokmer & Wegner, 2015; Pierce *et al.*, 2016) and salinity perturbations have also been observed to influence the oyster microbiota (del Refugio Castañeda Chávez *et al.*, 2005; Larsen *et al.*, 2013). Seasonal shifts in the SRO microbiota were characterised by changes in the relative abundance of several OTUs, including those assigned to the *Candidatus Hepatoplasma* and *Vibrio* genera. Interestingly, we observed inverse patterns for the relative abundance of an OTU assigned to the *Candidatus Hepatoplasma* genus (OTU 14887) between the two sampling sites. At Port Stephens, this OTU was significantly more abundant in summer, while at Wallis Lake, it was considerably more abundant in winter. The environmental data collected at the time suggests no similarities between the Port Stephens summer and Wallis Lake winter samples that could explain this pattern (conductivity was similar for these two sampling points but conductivity did not change between the Wallis Lake summer and winter sampling points) and this OTU was rare or absent in the seawater communities, therefore future studies should increase the suite of environmental parameters collected to explain these patterns. At both locations, a member of the *Vibrio* genus (OTU 2) had a higher relative abundance in winter than in summer. This pattern is interesting given that *Vibrio* typically exhibit preferences for warm water temperatures. However, some *Vibrio* species such as *Vibrio splendidus,* have elsewhere been found to be most abundant during winter and spring (Arias *et al.*, 1999; Pujalte *et al.*, 1999). It is also conceivable that other environmental factors, such as chlorophyll a or nutrient levels, underpinned the higher winter relative abundance of this *Vibrio* species (OTU 2).

4.3. The SRO microbiota is influenced by disease resistance

Oyster genetics have previously been shown to influence the Pacific oyster microbiota structure (King *et al.*, 2019c; Wegner *et al.*, 2013), with the microbiota of disease-resistant Pacific oysters showing a significantly different structure to disease-susceptible oysters (King *et al.*, 2019c). However, the influence of genetics on the Pacific oyster microbiota can be superseded by stress, such as temperature perturbations (Wegner *et al.*, 2013). In this study, we observed significant differences of the microbiota between QX-resistant and QX-susceptible oysters, but only in winter (June). This pattern suggests that there is a synergistic interaction of genetics and environmental drivers in shaping the SRO microbiota, which is consistent with previous studies in marine organisms such as Pacific oysters (Wegner *et al.*, 2013) and corals (Klaus *et al.*, 2005). While QX disease typically occurs between November to May (Bezemer *et al.*, 2006; Rubio *et al.*, 2013), infections by *M. sydneyi* that cause no mortality (Adlard & Wesche, 2005) have been observed between May to July (Rubio *et al.*, 2013), corresponding to the period where microbiota heterogeneity between resistance groups was observed in this study. This could indicate that the microbiota assemblage prior to the peak mortality period is important and could contribute to QX disease dynamics, although future studies should consider performing a temporal study to capture possible microbiota dynamics.

A previous study characterising the influence of disease-resistance on Pacific oyster microbiota identified disease-susceptible oysters as having a higher absolute abundance of *Vibrio* species (King *et al.*, 2019c). Interestingly, this pattern is consistent with observations made in this study, where at Port Stephens we observed an over-representation of an OTU assigned to the *Vibrio* genus (OTU 2) in QX-susceptible oysters. *Vibrio* species are commonly implicated as pathogens affecting marine molluscs such as clams, mussels and oysters (Paillard *et al.*, 2004; Travers *et al.*, 2015). For example, *Vibrio* species have a crucial role in summer mortalities of Pacific oysters (de Lorgeril *et al.*, 2018; Garnier *et al.*, 2007; King *et al.*, 2019b; Lemire *et al.*, 2015; Petton *et al.*, 2015; Saulnier *et al.*, 2010; Sugumar *et al.*, 1998) with a non-virulent *Vibrio* community replaced by a pathogenic one (Lemire *et al.*, 2015). Given their role in marine molluscs and other oyster diseases, investigating whether *Vibrio* species influence QX-disease dynamics would be of interest. At Wallis Lake, an OTU assigned to the *Endozoicomonas* genus (OTU 4530) was significantly over-represented in the QX-resistant oysters. *Endozoicomonas* bacteria have found to be associated with many marine organisms such as sponges, corals and oysters (Dubé *et al.*, 2019; Neave *et al.*, 2016; Roterman *et al.*, 2015; Zurel *et al.*, 2011). Given the importance of *Endozoicomonas* species in sponges and corals (Neave *et al.*, 2017;

Nishijima *et al.*, 2013; Rua *et al.*, 2014), future studies should investigate their potential role in QX-resistant oysters.

5. Conclusion

There is emerging evidence that the microbiota of benthic organisms, including oysters, are dynamic and driven by multiple factors, but the impact of location, season and genetics (disease resistance) on the SRO microbiota have not been reported previously. Understanding the factors that drive SRO microbiota composition are pivotal when deciphering the role of the microbiota during disease events, and to explain microbiota shifts prior to, or during, disease. However, this is currently hindered by a paucity of SRO microbiota studies. This study demonstrated that the SRO microbiota assemblage is influenced by location and season, which highlights the importance of performing temporal studies at individual locations as interpreting microbiota patterns from other locations or time points can lead to erroneous microbiota explanations. Further, breeding for QX disease resistance (genetics) was found to influence the SRO microbiota although this was only observed in the winter. This sampling time point is before the typical QX disease period, which may indicate that a microbiota shift could be a factor in QX disease dynamics. Overall, these data suggest that there is a synergistic interaction of genetics and environmental drivers in shaping the SRO microbiota.

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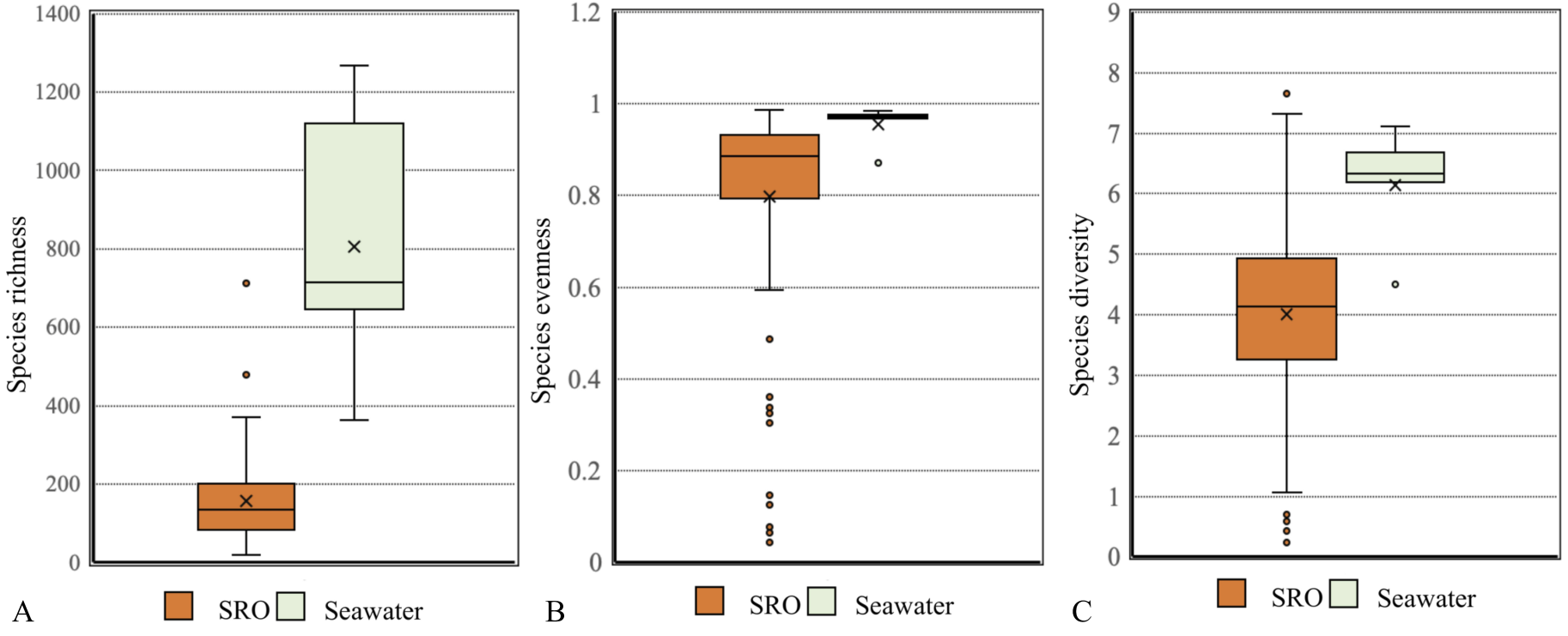
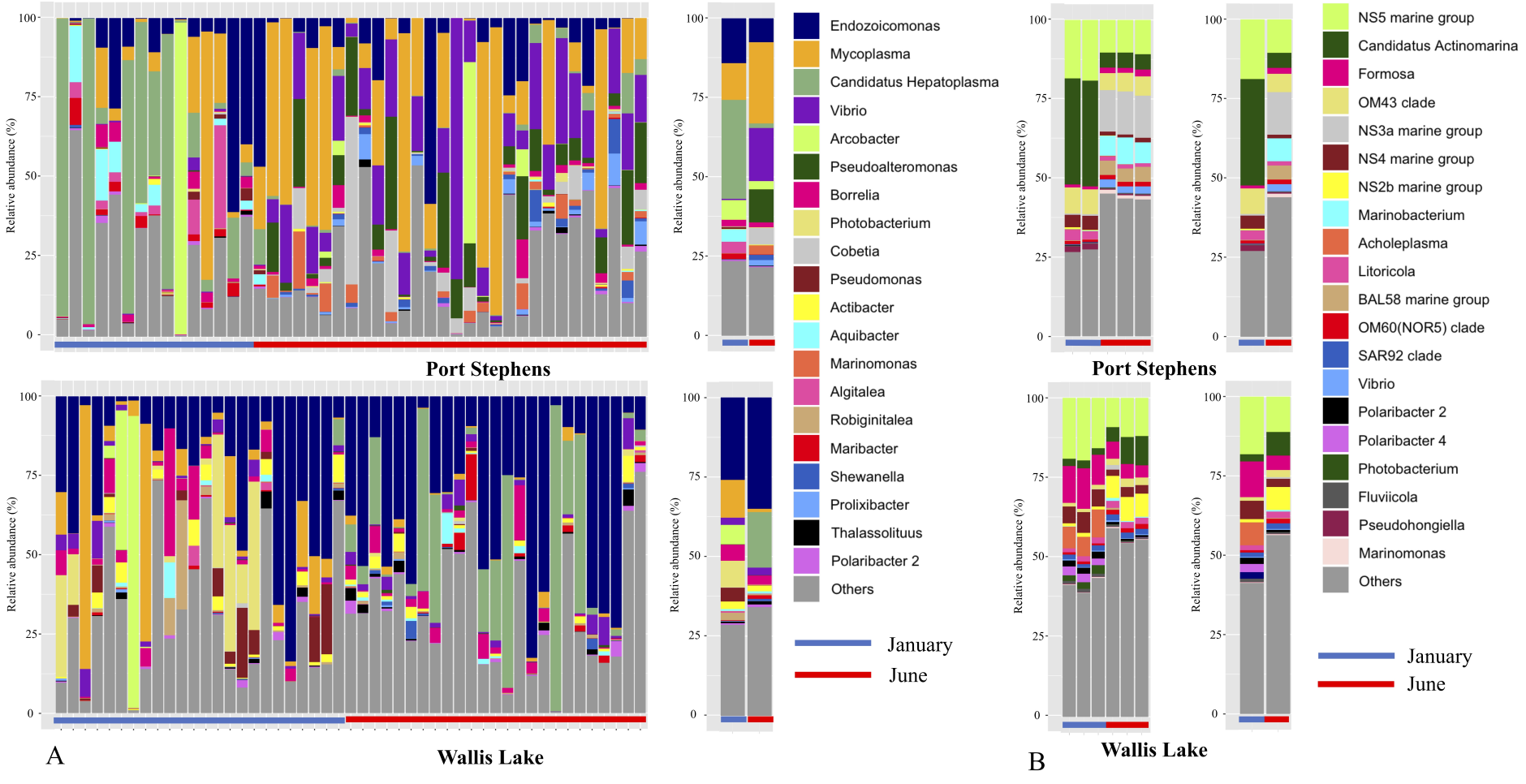
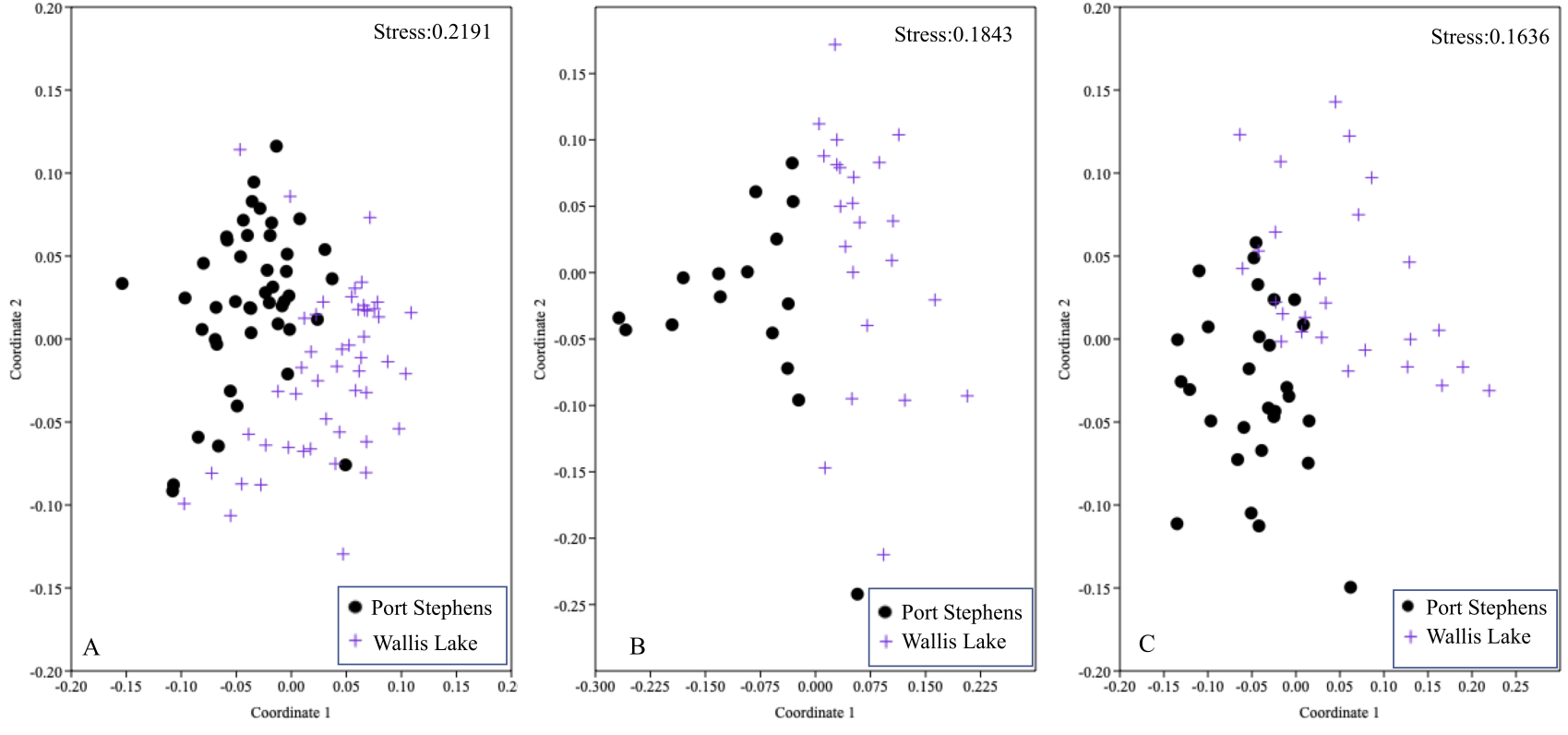
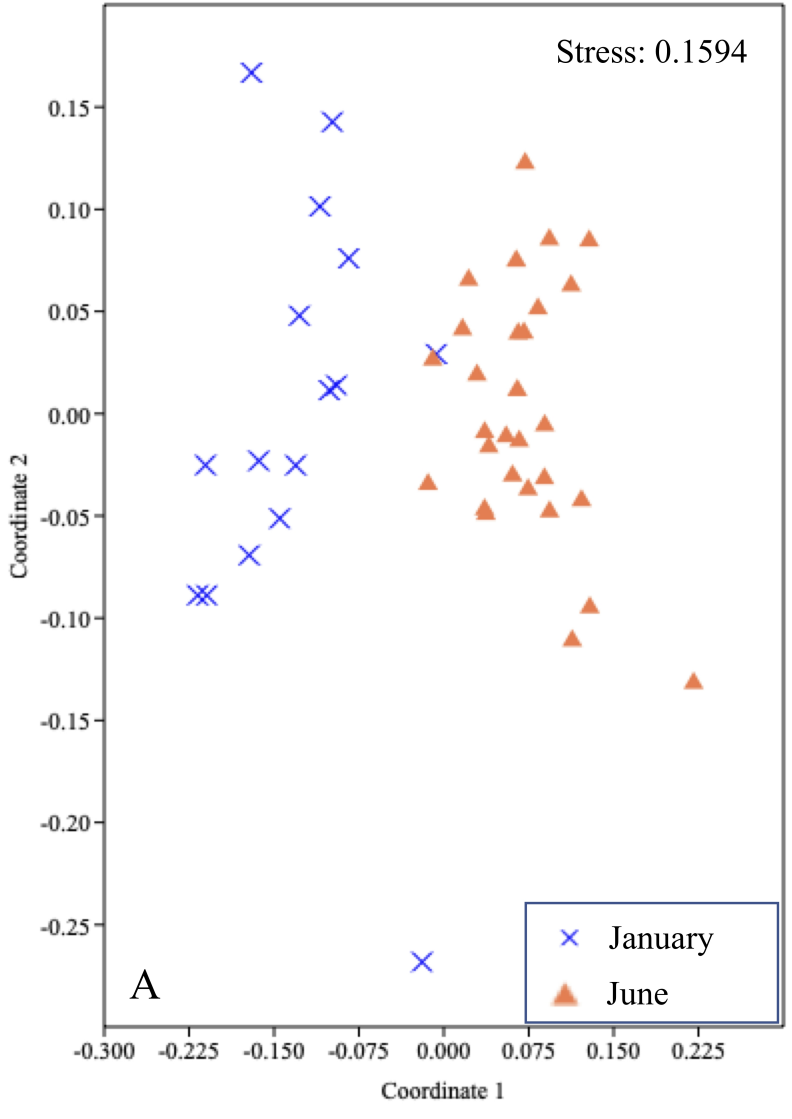

Species richness
1400
1200
1000
800
600
400
200
0
SRO
Seawater
A
Species evenness
1.2
1
0.8
0.6
0.4
0.2
0
SRO
Seawater
B
Species diversity
9
8
7
6
5
4
3
2
1
0
SRO
Seawater
C


Relative abundance (%)
100
75
50
25
0
Port Stephens
Wallis Lake
A
B
Endozoicomonas
Mycoplasma
Candidatus Hepatoplasma
Vibrio
Arcobacter
Pseudoalteromonas
Borrelia
Photobacterium
Cobetia
Pseudomonas
Actibacter
Aquibacter
Marinomonas
Algitalea
Robiginitalea
Maribacter
Shewanella
Prolixibacter
Thalassolituus
Polaribacter 2
Others
NS5 marine group
Candidatus Actinomarina
Formosa
OM43 clade
NS3a marine group
NS4 marine group
NS2b marine group
Marinobacterium
Acholeplasma
Litoricola
BAL58 marine group
OM60(NOR5) clade
SAR92 clade
Vibrio
Polaribacter 2
Polaribacter 4
Photobacterium
Fluviicola
Pseudohongiella
Marinomonas
Others
January
June


Stress:0.2191
Port Stephens
Wallis Lake
A
Coordinate 1
Coordinate 2
Stress:0.1843
Port Stephens
Wallis Lake
B
Coordinate 1
Coordinate 2
Stress:0.1636
Port Stephens
Wallis Lake
C
Coordinate 1
Coordinate 2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Coordinate 1
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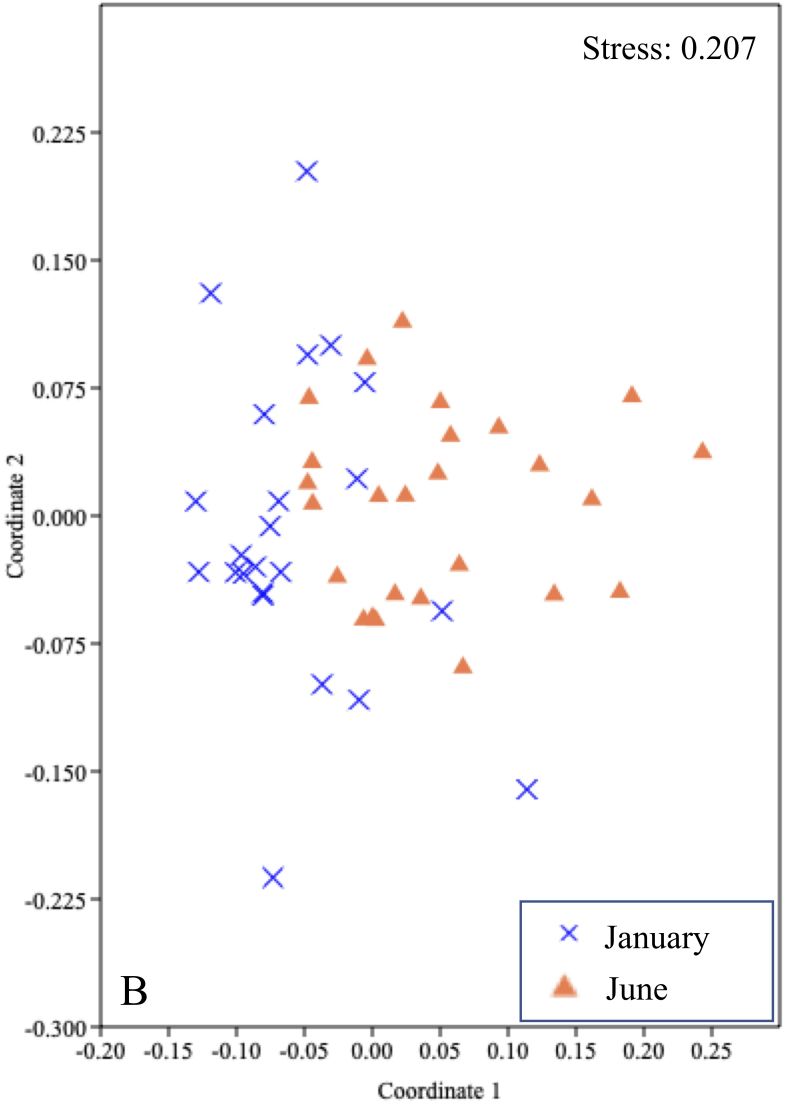
0.10
0.05
0.00
-0.05
-0.10
-0.15
-0.20
-0.25
-0.300
-0.225
-0.150
-0.075
0.000
0.075
0.150
0.225
January
June
A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Coordinate 2
Coordinate 1
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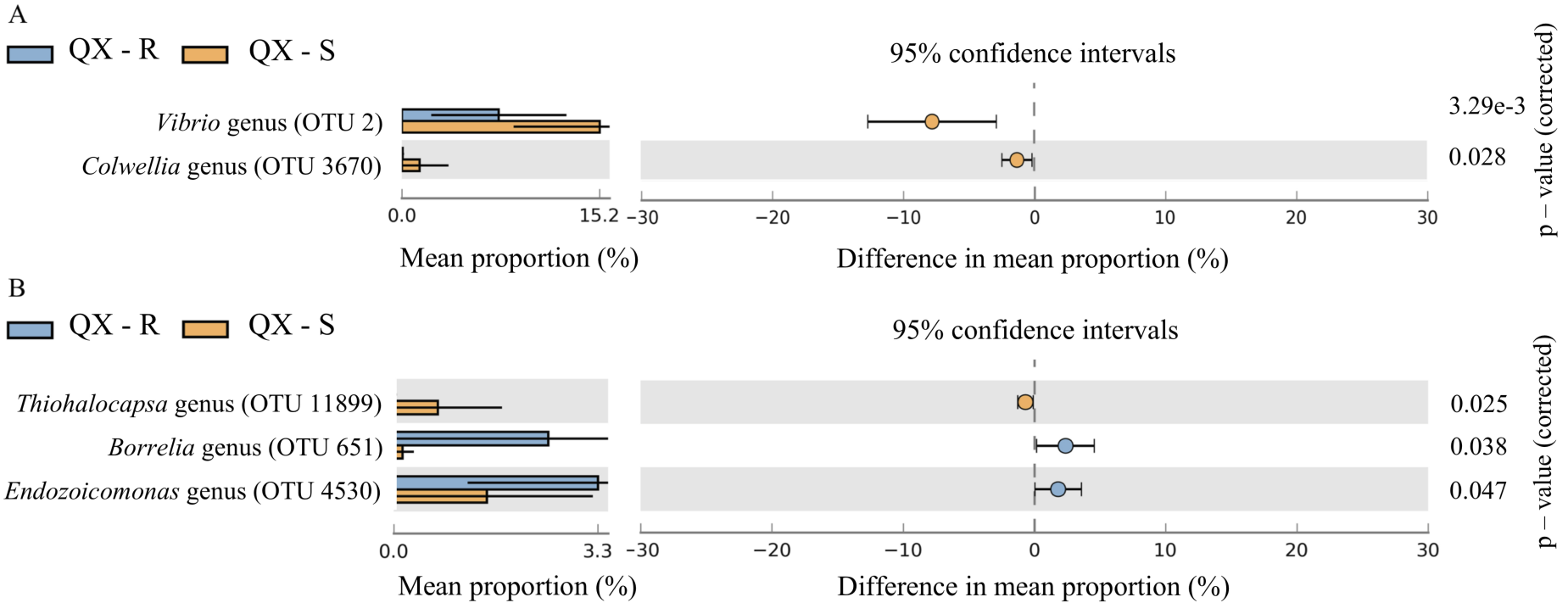
0.150
0.075
0.000
-0.075
-0.150
-0.225
-0.300
-0.20
-0.15
-0.10
-0.05
0.00
0.05
0.10
0.15
0.20
0.25
January
June
B


A
QX - R
QX - S
95% confidence intervals
Vibrio genus (OTU 2)
Colwellia genus (OTU 3670)
3.29e-3
0.028
p – value (corrected)
0.0
15.2
−30
−20
−10
0
10
20
30
Mean proportion (%)
Difference in mean proportion (%)
B
QX - R
QX - S
95% confidence intervals
Thiohalocapsa genus (OTU 11899)
Borrelia genus (OTU 651)
Endozoicomonas genus (OTU 4530)
0.025
0.038
0.047
p – value (corrected)
0.0
3.3
−30
−20
−10
0
10
20
30
Mean proportion (%)
Difference in mean proportion (%)

Declaration of interests

 The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Author Statement on roles

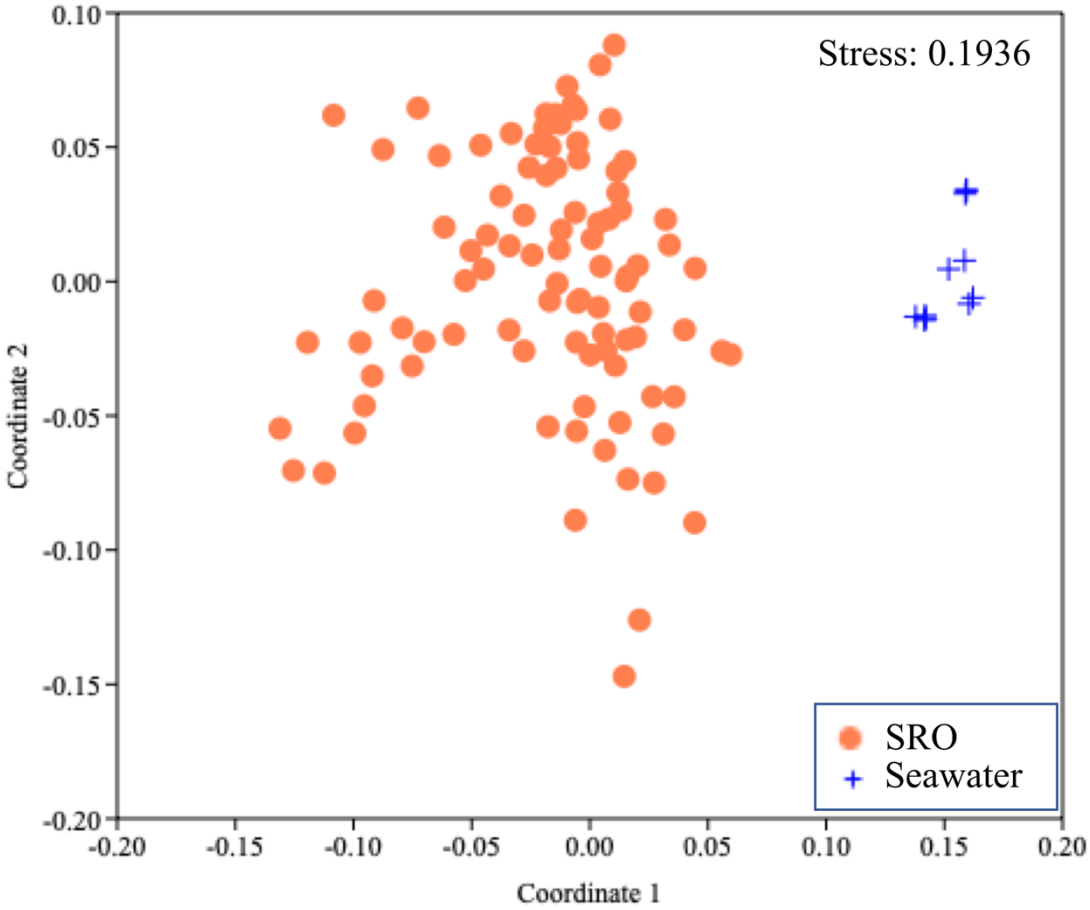
Viet Khue Nguyen: Formal analysis, Investigation, Writing – Original draft preparation, Visualization. **William L King:** Formal analysis, Writing – Original draft preparation. **Nachshon Siboni:** Investigation, Methodology, Writing – Reviewing and Editing. **Khandaker Rayhan Mahbub:** Investigation, Writing – Reviewing and Editing. **Michael Dove:** Methodology, Resources, Writing – Reviewing and Editing. **Wayne O'Connor:** Resources, Writing – Reviewing and Editing, Funding acquisition. **Justin R. Seymour:** Conceptualization, Writing – Original draft preparation, Project administration, Funding acquisition. **Maurizio Labbate:** Conceptualization, Writing – Original draft preparation, Project administration, Funding acquisition.

Supplementary Table 1: Remaining samples for each SRO family and seawater after rarefication to 7,178 reads.

Sample	Port Stephens		Wallis Lake	
	January	**June**	**January**	**June**
F18	3	5	5	4
F22	5	5	4	5
F25	2	5	4	5
F03	2	5	4	4
F37	3	5	3	5
F32	1	4	3	3
Seawater	2	3	3	3

Supplementary Table 2: Kruskal-Wallis test of alpha diversity indices between total SRO and total seawater microbiota, including species richness (Chao1), species evenness (Simpson) and species diversity (Shannon).

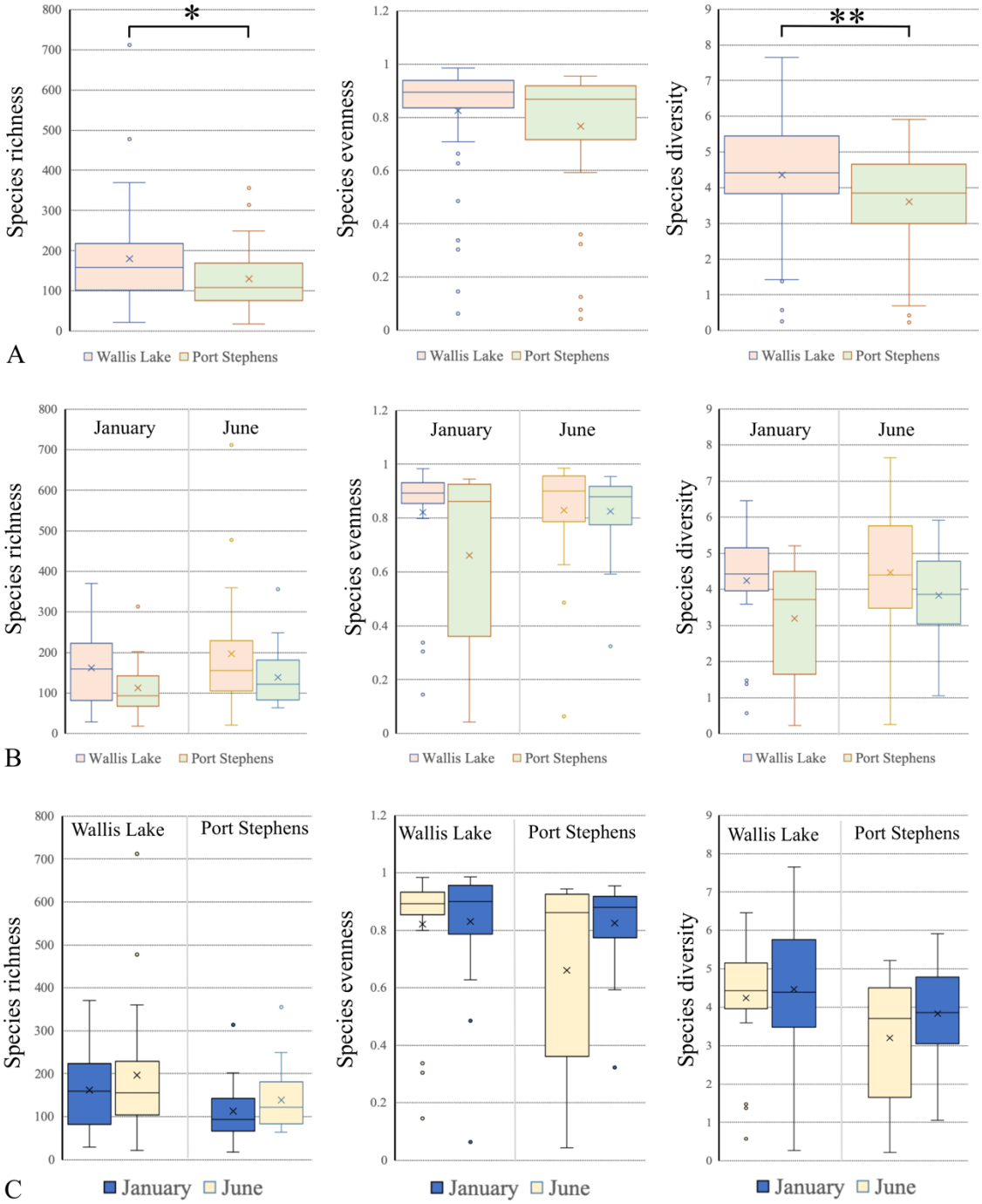
Comparison	H	p-value
Richness of SRO (n =94) vs seawater (n =11)	28.25	1.06E-07
Evenness of SRO (n =94) vs seawater (n =11)	15.64	7.65E-05
Diversity of SRO (n =94) vs seawater (n = 11)	20.06	7.52E-06



Supplementary Figure 1: 3D nMDS plot showing separation of the SRO and seawater microbiota samples.

Supplementary Table 3: SIMPER analysis comparing the SRO and seawater microbiota. The top 10 OTUs are displayed with their dissimilarity contribution and mean representation. Dissimilarity contribution is cumulative.

Taxon	Contrib. %	Mean SRO	Mean Water
Candidatus Hepatoplasma genus (OTU 14887)	5.736	10.6	0.0481
Candidatus Actinomarina genus (OTU 22961)	4.171	0.0467	7.86
NS5 marine group genus (OTU 5409)	3.641	0.0603	6.75
Endozoicomonas genus (OTU 3829)	2.917	5.38	0.0101
Oceanospirillales order (OTU 12673)	2.554	0.0655	4.82
Endozoicomonas genus (OTU 1831)	2.441	4.5	0
Vibrio genus (OTU 2)	2.255	4.07	0.485
Mycoplasma genus (OTU 14900)	1.885	3.48	0
OM43 clade genus (OTU 6156)	1.867	0.0424	3.47
Arcobacter genus (OTU 6697)	1.787	3.31	0



Supplementary Figure 2: Box and whisker plots of species richness, evenness and diversity of total SRO microbiota from Port Stephens and Wallis Lake (A), SRO microbiota from Port Stephens and Wallis Lake at each season (B) and SRO microbiota from January and June at each location (C). A single asterisk and two asterisks indicate a statistical significance of $p<0.05$ and $p<0.01$ respectively.

Supplementary Table 4: Kruskal-Wallis ANOVA test of alpha diversity indices between location and season including species richness (Chao1) species evenness (Simpson) and species diversity (Shannon).

Comparison	H	p-value
Location (January and June)		
Richness in Wallis Lake (n =49) vs Port Stephens (n =45)	4.768	0.02899
Evenness in Wallis Lake (n =49) vs Port Stephens (n =45)	3.769	0.05221
Diversity in Wallis Lake (n =49) vs Port Stephens (n =45)	7.199	0.007294
Location (January)		
Richness in Wallis Lake (n =23) vs Port Stephens (n =16)	2.935	0.08667
Evenness in Wallis Lake (n =23) vs Port Stephens (n =16)	3.134	0.07669
Diversity in Wallis Lake (n =23) vs Port Stephens (n =16)	3.551	0.05951
Location (June)		
Richness in Wallis Lake (n =26) vs Port Stephens (n =29)	2.251	0.1335
Evenness in Wallis Lake (n =26) vs Port Stephens (n =29)	1.201	0.2732
Diversity in Wallis Lake (n =26) vs Port Stephens (n =29)	3.254	0.07126
Season in Wallis Lake		
Richness in January (n =23) vs June (n =26)	0.2508	0.6165
Evenness in January (n =23) vs June (n =26)	0.006421	0.9361
Diversity in January (n =23) vs June (n =26)	0.04013	0.8412
Season in Port Stephens		
Richness in January (n =16) vs June (n =29)	2.6	0.1069
Evenness in January (n =16) vs June (n =29)	0.9918	0.3193
Diversity in January (n =16) vs June (n =29)	0.506	0.4769

Supplementary Table 5: SIMPER analysis of the SRO microbiota between Port Stephens and Wallis Lake. The top 10 OTUs are displayed with their dissimilarity contribution and mean representation. Dissimilarity contribution is cumulative.

Taxon	Contrib. %	Port Stephens mean	Wallis Lake mean
January and June			
Candidatus Hepatoplasma genus (OTU 14887)	10.05	12	9.27
Endozoicomonas genus (OTU 1831)	4.859	0.0341	8.6
Vibrio genus (OTU 2)	4.309	7.7	0.73
Endozoicomonas genus (OTU 3829)	3.961	3.75	6.88
Mycoplasma genus (OTU 14900)	3.919	6.81	0.423
Arcobacter genus (OTU 6697)	3.611	3.79	2.87
Pseudoalteromonas genus (OTU 8917)	3.323	5.88	0.077
Mycoplasma genus (OTU 12669)	2.896	5.04	0.119
Mycoplasma genus (OTU 14921)	2.865	2.69	3.2
Mycoplasma genus (OTU 14937)	2.69	3.81	1.6
January			
Candidatus Hepatoplasma genus (OTU 14887)	17.66	31.2	0
Arcobacter genus (OTU 6697)	6.468	6.15	6.12
Mycoplasma genus (OTU 14921)	5.095	4.66	6.3
Endozoicomonas genus (OTU 3829)	4.732	5.89	7.02
Endozoicomonas genus (OTU 1831)	3.028	0	5.35
Photobacterium genus (OTU 3)	2.826	0.0871	4.87
Endozoicomonas genus (OTU 6283)	2.761	3.23	4.13
Mycoplasma genus (OTU 14937)	2.737	2.88	3.35
Pseudomonas genus (OTU 12985)	2.304	0.589	3.97
Aquibacter genus (OTU 12017)	2.054	3.63	0.153
June			
Candidatus Hepatoplasma genus (OTU 14887)	9.6	1.43	17.5
Endozoicomonas genus (OTU 1831)	6.38	0.0528	11.5
Vibrio genus (OTU 2)	6.115	11.9	1.31
Mycoplasma genus (OTU 14900)	5.283	9.45	0.218
Pseudoalteromonas genus (OTU 8917)	5.051	9.12	0.111
Mycoplasma genus (OTU 12669)	4.38	7.82	0.0338
Endozoicomonas genus (OTU 3829)	3.424	2.56	6.76
Cobetia genus (OTU 2869)	2.916	5.22	0.00536
Mycoplasma genus (OTU 14937)	2.432	4.32	0.0595
Endozoicomonas genus (OTU 6283)	2.282	2.03	3.78

Supplementary Table 6: SIMPER analysis of the SRO microbiota between the two sampling times in Port Stephens and Wallis Lake. The top 10 OTUs are displayed with their dissimilarity contribution and mean representation. Dissimilarity contribution is cumulative.

Taxon	Contrib. %	Mean January	Mean June
Wallis Lake			
Candidatus Hepatoplasma genus (OTU 14887)	10.37	0	17.5
Endozoicomonas genus (OTU 1831)	8.142	5.35	11.5
Endozoicomonas genus (OTU 3829)	4.412	7.02	6.76
Mycoplasma genus (OTU 14921)	3.803	6.3	0.453
Arcobacter genus (OTU 6697)	3.623	6.12	0
Photobacterium genus (OTU 3)	2.96	4.87	0.0177
Endozoicomonas genus (OTU 6283)	2.756	4.13	3.78
Pseudomonas genus (OTU 12985)	2.382	3.97	0.0707
Mycoplasma genus (OTU 14937)	1.995	3.35	0.0595
Endozoicomonas genus (OTU 1993)	1.965	0	3.33
Port Stephens			
Candidatus Hepatoplasma genus (OTU 14887)	16.77	31.2	1.43
Vibrio genus (OTU 2)	6.575	0.00697	11.9
Mycoplasma genus (OTU 14900)	5.612	2.01	9.45
Pseudoalteromonas genus (OTU 8917)	5.008	0	9.12
Arcobacter genus (OTU 6697)	4.553	6.15	2.49
Mycoplasma genus (OTU 12669)	4.288	0	7.82
Endozoicomonas genus (OTU 3829)	3.401	5.89	2.56
Mycoplasma genus (OTU 14937)	3.171	2.88	4.32
Cobetia genus (OTU 2869)	2.854	0.0313	5.22
Mycoplasma genus (OTU 14921)	2.695	4.66	1.6

Supplementary Table 7: Kruskal-Wallis ANOVA test of alpha diversity indices between QX-sensitive and QX-resistant groups including species richness (Chao1), species evenness (Simpson) and species diversity (Shannon).

Comparison	H	p-value
Port Stephens in January		
Richness in QX-sensitive (n =9) vs QX-resistant (n =7)	4.26	0.039
Evenness in QX-sensitive (n =9) vs QX-resistant (n =7)	2.692	0.1009
Diversity in QX-sensitive (n =9) vs QX-resistant (n =7)	2.692	0.1009
Wallis Lake in January		
Richness in QX-sensitive (n =15) vs QX-resistant (n =8)	0.6003	0.4385
Evenness in QX-sensitive (n =15) vs QX-resistant (n =8)	0.0375	0.8465
Diversity in QX-sensitive (n =15) vs QX-resistant (n =8)	0.0375	0.8465
Port Stephens in June		
Richness in QX-sensitive (n =19) vs QX-resistant (n =10)	0.6086	0.4353
Evenness in QX-sensitive (n =19) vs QX-resistant (n =10)	1.771	0.1833
Diversity in QX-sensitive (n =19) vs QX-resistant (n =10)	1.895	0.1687
Wallis Lake in June		
Richness in QX-sensitive (n =16) vs QX-resistant (n =10)	2.669	0.1023
Evenness in QX-sensitive (n =16) vs QX-resistant (n =10)	0.1	0.718
Diversity in QX-sensitive (n =16) vs QX-resistant (n =10)	0.5444	0.4606

Supplementary Table 8: SIMPER analysis comparing the SRO microbiota of QX-sensitive and QX-resistant groups at Port Stephens and Wallis Lake in June. The top 10 OTUs are displayed with their dissimilarity contribution and mean representation. Dissimilarity contribution is cumulative.

Taxon	Contrib. %	Mean QX-resistant	Mean QX-sensitive
Port Stephens			
Mycoplasma genus (OTU 12669)	9.644	10.9	6.19
Mycoplasma genus (OTU 14900)	9.27	12.7	7.76
Pseudoalteromonas genus (OTU 8917)	6.394	7.59	9.92
Vibrio genus (OTU 2)	6.11	7.24	14.4
Vibrio (OTU 1)	5.662	8.42	0.0667
Mycoplasma genus (OTU 14937)	5.115	5.42	3.74
Cobetia genus (OTU 2869)	4.655	2.24	6.78
Arcobacter genus (OTU 6697)	4.591	6.57	0.345
Marinilabiaceae family (OTU 2173)	3.33	2.42	4.11
Endozoicomonas genus (OTU 6283)	2.644	3.69	1.15
Wallis Lake			
Candidatus Hepatoplasma genus (OTU 14887)	15.86	15.6	18.6
Endozoicomonas genus (OTU 1831)	9.846	11.8	11.3
Endozoicomonas genus (OTU 3829)	4.867	9.2	5.24
Endozoicomonas genus (OTU 1993)	3.495	1.89	4.23
Endozoicomonas genus (OTU 6283)	3.248	5.59	2.64
Gammaproteobacteria class (OTU 6670)	3.003	4.5	0.0679
Endozoicomonas genus (OTU 3483)	2.109	3.33	1.56
Flavobacteriaceae family (OTU 12808)	2.105	0.0111	3.16
Endozoicomonas genus (OTU 1949)	1.975	1.04	2.45
Endozoicomonas genus (OTU 4530)	1.769	3.25	1.4