

© <2020>. This manuscript version is made available under the CC-BY-NC-ND 4.0 license  
<http://creativecommons.org/licenses/by-nc-nd/4.0/>  
The definitive publisher version is available online at [https://doi.org/  
10.1016/j.aquaculture.2020.735472](https://doi.org/10.1016/j.aquaculture.2020.735472)

## 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

Viet Khue Nguyen<sup>1,2</sup>, William L King<sup>1,2†</sup>, Nachshon Siboni<sup>2</sup>, Khandaker Rayhan Mahbub<sup>1</sup>, Michael Dove<sup>3</sup>, Wayne O'Connor<sup>3</sup>, Justin R. Seymour<sup>2</sup>, Maurizio Labbate<sup>1\*</sup>

<sup>1</sup>School of Life Sciences, University of Technology Sydney, Sydney, NSW, Australia

<sup>2</sup>Climate Change Cluster, University of Technology Sydney, Sydney, NSW, Australia

<sup>3</sup>NSW Department of Primary Industries, Port Stephens Fisheries Institute, Port Stephens, NSW, Australia

†Current address: Department of Plant Pathology and Environmental Microbiology, The Pennsylvania State University, University Park, PA, USA

\*Corresponding author: Maurizio Labbate. Email: [maurizio.labbate@uts.edu.au](mailto:maurizio.labbate@uts.edu.au)

## 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

60  
61  
62 34 findings confirm the influencing role of location and season on the microbiota structure as  
63  
64 35 evidenced in other molluscan species, but also provide preliminary evidence that the microbiota  
65  
66 36 assemblage before the QX disease period may be important for resistance to disease and may  
67  
68 37 provide new avenues for managing SRO aquaculture in the future.  
69

70 39 **Keywords:** Microbiota, Sydney rock oyster, QX disease, 16S rRNA, disease resistance  
71

## 72 40

### 73 41 **1. Introduction**

#### 74 42

75  
76 43 The Sydney rock oyster (SRO; *Saccostrea glomerata*) is native to Australia, where it is one of  
77  
78 44 the most intensively cultivated oyster species (O'Connor & Dove, 2009; Schrobback *et al.*,  
79  
80 45 2014). However, since the mid-1970's production of this species has been impacted by QX-  
81  
82 46 disease, which can recurrently cause up to 90% mortality in affected estuaries (Department of  
83  
84 47 Primary Industries, 2016; Nell, 2007; O'Connor & Dove, 2009; Peters & Raftos, 2003;  
85  
86 48 Schrobback *et al.*, 2014). The aetiological agent for QX disease is a spore-forming protozoan  
87  
88 49 parasite called *Marteilia sydneyi*. This parasite has an infection cycle that typically enters  
89  
90 50 through the palps and gills in summer and ends in the oyster digestion gland, impacting nutrient  
91  
92 51 uptake and ultimately causing starvation and death through autumn and into winter (Kleeman  
93  
94 52 *et al.*, 2002; Nell, 2007; Wolf, 1979).  
95

96 53

97 54 To mitigate the impacts of QX disease, the New South Wales Department of Primary Industries  
98  
99 55 (NSW DPI) has led a selective breeding program using both mass selection methods and family  
100  
101 56 based breeding that has greatly reduced SRO mortalities, with some families showing 85%  
102  
103 57 survival through one cycle of disease (Dove *et al.*, 2020). There is evidence that increased  
104  
105 58 levels of resistance in some families may be linked to higher activity of phenoloxidase, an  
106  
107 59 enzyme thought to be involved in oyster defence mechanisms (Newton *et al.*, 2004), yet the  
108  
109 60 full mechanism(s) for resistance remain unresolved.  
110

111 61

112 62 The oyster microbiota is emerging as a factor in disease dynamics (King *et al.*, 2019a) and is  
113  
114 63 an unexplored factor in SRO QX disease resistance. The potential protective role of the mollusc  
115  
116 64 microbiota has been characterised previously, with some microbial members providing anti-  
117  
118 65 pathogen activities (Offret *et al.*, 2019; Prado *et al.*, 2009). In other studies, the microbiota  
119  
120 66 appears to contribute to disease dynamics, for the Pacific oyster it has been demonstrated that  
121  
122 67 summer mortality in France is due to a progressive replacement of non-virulent commensal

119  
120  
121 68 vibrios with pathogenic vibrios indicating that microbiota dysbiosis precedes mortality (Lemire  
122 *et al.*, 2015). Similarly, Pacific oyster mortality syndrome is polymicrobial in nature with a  
123 69 *et al.*, 2015). Similarly, Pacific oyster mortality syndrome is polymicrobial in nature with a  
124 70 recent study showing that the viral Ostreid Herpesvirus 1 (OsHV-1) suppresses Pacific oyster  
125 71 immunity, allowing opportunistic bacterial pathogens such as *Vibrio* species to thrive (de  
126 72 Lorgeril *et al.*, 2018). Interestingly, the microbiota of Pacific oyster families bred for resistance  
127 73 to OsHV-1 were significantly different to their disease-susceptible counterparts and had a  
128 74 significantly reduced abundance of *Vibrio* species (King *et al.*, 2019c). In SROs, only one study  
129 75 has investigated the QX-disease-affected microbiota by comparing the digestive gland of QX-  
130 76 infected and uninfected oysters (Green & Barnes, 2010). In QX-infected oysters, bacterial  
131 77 diversity was substantially reduced, with the microbiota dominated by a *Rickettsiales*-like  
132 78 operational taxonomic unit (OTU).  
133 79

140 80 A first step in understanding the role of a microbiota in disease dynamics is characterising its  
141 81 composition and determining the factors that shape its structure. In previous studies in other  
142 82 oyster species, the oyster microbiota has been shown to be influenced by both environmental  
143 83 and host factors including location, temperature, infection state, season, genetics, life stage and  
144 84 resistance to disease (Green & Barnes, 2010; King *et al.*, 2012; King *et al.*, 2019b; King *et al.*,  
145 85 2019c; Lokmer & Wegner, 2015; Lokmer *et al.*, 2016a). However, there is a paucity of studies  
146 86 examining the factors that influence the SRO microbiota assemblage. Therefore, to characterise  
147 87 the influence of location, season and disease-resistance (genetics) on the SRO microbiota, six  
148 88 SRO families with varying degrees of resistance to QX disease were deployed into two  
149 89 locations and sampled in the Austral summer and winter. Understanding the mechanism(s) that  
150 90 drive disease-resistance, including the potential contribution of the microbiota to disease, are  
151 91 imperative for the successful and sustainable management of SRO aquaculture.  
152 92

## 161 93 **2. Materials and methods**

### 163 94 164 95 **2.1. Experimental design and sampling**

165 96  
166 97 Forty-four different *Saccostrea glomerata* families from the 2015 year class were deployed  
167 98 in the Port Stephens (32°43'12.81"S 152°03'40.52"E) and Wallis Lake (32°11'21.3"S  
168 99 152°29'09.7"E) estuaries in NSW, Australia. Wallis Lake is a wave-dominated barrier estuary  
169 100 whereas Port Stephens is a tide-dominated drowned valley estuary (Roy *et al.*, 2001). These  
170  
171  
172  
173  
174  
175  
176  
177

178  
179  
180  
181  
182  
183  
184  
185  
186  
187  
188  
189  
190  
191  
192  
193  
194  
195  
196  
197  
198  
199  
200  
201  
202  
203  
204  
205  
206  
207  
208  
209  
210  
211  
212  
213  
214  
215  
216  
217  
218  
219  
220  
221  
222  
223  
224  
225  
226  
227  
228  
229  
230  
231  
232  
233  
234  
235  
236

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

112  
113 Five oysters per family were collected from each site in the 2017 Austral summer (January)  
114 and Austral winter (June), four and nine months after deployment respectively (120 oyster  
115 samples in total). Oysters were randomly collected by farmers from cultivation trays, placed  
116 into labelled plastic bags, transported to the laboratory on ice (3 - 4 hours) and stored whole in  
117 their shell at  $-80^{\circ}\text{C}$  for later processing. Because oyster leases could only be accessed by boat,  
118 seawater samples were collected from jetties (piers) approximately 800 metres away from the  
119 oyster leases. The jetties face the oyster leases and are suspended over water that are a few  
120 metres deep ensuring no sediment was suspended from the bottom during collection. Ten litres  
121 of surface seawater samples were collected and kept on ice during transport to the laboratory.  
122 Triplicate seawater samples of 2000 mL for each sampling time were filtered with Durapore  
123 Membrane Filters (0.22  $\mu\text{m}$  pore size) for subsequent microbiota analyses. All filtered samples  
124 were frozen in liquid nitrogen upon collection in sterile 5 mL cryotubes and kept at  $-80^{\circ}\text{C}$  prior  
125 to analysis.

126

237  
238  
239 127 Table 1: 2015 year class Sydney rock oyster average family survival (n = 3, ± SD) following  
240  
241 128 exposure to QX disease at Lime Kiln Bar, Georges river. Oysters were deployed to Lime Kiln  
242  
243 129 Bar on 12 December 2016 and oyster survival was counted on 20 September 2017.  
244

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

256 130  
257  
258 131 **2.2. Measurement of environmental parameters, nutrients and chlorophyll a in seawater**  
259  
260 132

261 133 Environmental parameters (temperature, oxygen, pH, and conductivity) were measured at  
262  
263 134 jetties adjacent to the oyster leases using a WTW multiprobe meter (Multi 3430, Germany) at  
264  
265 135 the time of oyster sample collections. For nutrient analysis, 50 mL triplicate seawater samples  
266  
267 136 were syringe filtered through a 0.45 µm filter into 50 mL sterile falcon tubes, transported to  
268  
269 137 the laboratory on ice, and frozen at -20°C. Nutrient analysis (nitrite (NO<sub>2</sub><sup>-</sup>), nitrate (NO<sub>3</sub><sup>-</sup>),  
270  
271 138 ammonia (NH<sub>3</sub>) and phosphate (PO<sub>4</sub><sup>3-</sup>)) was performed by Envirolab Services Pty Ltd (Sydney,  
272  
273 140 New South Wales, Australia). From the 10 L of seawater collected above, triplicate 200 mL  
274  
275 141 aliquots were filtered through glass microfiber filters (0.7 µm pore size) and stored at -80°C  
276  
277 142 for subsequent chlorophyll-a analyses. Chlorophyll a was analysed based on a  
278  
279 143 Spectrophotometric method described previously (Ritchie, 2006).  
280

280 144  
281 145  
282 146 **2.3. DNA extractions and 16S rRNA amplicon sequencing**  
283  
284 147

285 148 DNA extractions commenced only after the last sample had been collected and frozen. Samples  
286  
287 149 were randomly thawed in batches of 20 and all samples were processed using a single DNA  
288  
289 150 extraction kit. Thawed oysters were washed under running tap water to remove debris. Using  
290  
291 151 sterile instruments, each oyster was carefully opened using a shucking knife and the oyster  
292  
293 152 flesh excised and placed onto a Petri dish. Approximately 25-50 mg of adductor muscle tissue  
294  
295

296  
297  
298 153 (Qiagen, Germany), according to the manufacturer's instructions. Haemolymph is often used  
299  
300 154 to study the oyster microbiota (Lokmer *et al.*, 2016a; Lokmer *et al.*, 2016b) but can be difficult  
301  
302 155 to extract from small oysters and is not possible to extract once oysters have been frozen. To  
303  
304 156 minimise variation, we decided to freeze oysters so they could be later processed together.  
305  
306 157 Therefore, the adductor muscle was selected for microbiota analysis as it contains haemolymph  
307  
308 158 sinuses thus allowing us to easily sample the haemolymph. This approach has been successfully  
309  
310 159 used before (King *et al.*, 2019b; King *et al.*, 2019c). The instruments used to process the  
311  
312 160 oysters, including the shucking knife, were cleaned, soaked in 1:15 bleach solution for 15 min  
313  
314 161 and then rinsed with sterile Milli-Q water prior to use and between samples. DNA from filtered  
315  
316 162 seawater samples were extracted using the PowerWater DNA Isolation Kit (MoBio, USA)  
317  
318 163 according to the manufacturer's protocol.  
319  
320 164

317 165 The V1–V3 region of the 16S rRNA gene was amplified by PCR using the 27F (5'-  
318  
319 166 AGAGTTTGATCMTGGCTCAG-3') and 519R (5'- GWATTACCGCGGCKGCTG-3')  
320  
321 167 primer pair (Lane, 1991; Turner *et al.*, 1999). The PCR cycling conditions were as follows:  
322  
323 168 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  
324  
325 169 final extension of 72°C for 10 min. Amplicons were sequenced using the Illumina MiSeq  
326  
327 170 platform (2 × 300 bp) at the Ramaciotti Centre for Genomics (University of New South Wales,  
328  
329 171 Sydney, Australia). Raw data files in FASTQ format were deposited in NCBI Sequence Read  
330  
331 172 Archive (SRA) with the study accession number (SRP234946) under Bioproject number  
332  
333 173 PRJNA593911.  
334  
335 174

#### 333 175 **2.4. Bioinformatics analyses**

336 176 Demultiplexed paired-end reads were combined using FLASH (Magoč & Salzberg, 2011) and  
337  
338 177 trimmed using Mothur (Schloss *et al.*, 2009) (Parameters: maxhomop = 5, maxambig = 0,  
339  
340 178 minlength = 471, maxlength = 501). Fragments were clustered into operational taxonomic units  
341  
342 179 (OTUs) at 97% sequence similarity, and chimeric and singleton sequences were identified and  
343  
344 180 removed using VSEARCH (Rognes *et al.*, 2016). Taxonomic assignment of OTUs were  
345  
346 181 performed in QIIME version 1.9.1 (Caporaso *et al.*, 2010) using the UCLUST algorithm  
347  
348 182 (Edgar, 2010) against the SILVA v128 dataset (Quast *et al.*, 2013). Mitochondrial and  
349  
350 183 chloroplast data were filtered out of the dataset and the remaining reads were rarefied to the  
351  
352 184 same depth to remove the effect of sampling effort upon analysis. For beta diversity, the relative  
353  
354 185 abundance of OTUs was calculated and all OTUs with a relative abundance below 0.1% were



355  
356  
357 186 filtered from the dataset. Alpha diversity indices, including species richness (Chao1), species  
358  
359 187 evenness (Simpson) and species diversity (Shannon index) were calculated using QIIME  
360  
361 188 (Caporaso *et al.*, 2010).  
362

## 363 189 **2.5. Statistical analyses**

364  
365 190

366  
367 191 Alpha diversity metrics were compared between groups using a Kruskal-Wallis test. All beta  
368  
369 192 diversity analyses were performed with a Bray-Curtis dissimilarity index. To easily visualise  
370  
371 193 how samples related to one another and observe distance matrices between groups, non-metric  
372 194 multidimensional scaling analysis (nMDS) with three dimensions (3D) was used. Patterns  
373  
374 195 elucidated by the 3D nMDS were statistically tested using a permutational multivariate analysis  
375 196 of variance (PERMANOVA) with 9999 permutations using transformed (square root(x)) data.  
376  
377 197 To identify the OTUs driving the difference between the microbial assemblage at different  
378  
379 198 locations or time points, SIMPER analysis was used. All alpha and beta diversity comparisons  
380 199 were performed in the PAST statistical environment (Hammer *et al.*, 2001). To determine  
381  
382 200 whether the relative abundances of OTUs were significantly different between oyster groups  
383  
384 201 with differing QX-resistance, a Welch's T-Test was performed using the STAMP (Statistical  
385 202 Analysis of Metagenomic Profiles) software package version 2.1.3 (Parks *et al.*, 2014). A file  
386  
387 203 listing the relative abundance of all OTUs was used as input data along with a metadata file  
388 204 containing location, sampling time and QX-resistance group information. A Welch's T-Test  
389  
390 205 with a p-value of <0.05 as a statistical cut-off was used. To visualise the significant difference  
391 206 in the relative abundance of OTUs between the QX-sensitive QX-and resistant groups at a  
392  
393 207 single location at each sampling time, extended error bar plots with corrected p-values were  
394  
395 208 produced.  
396

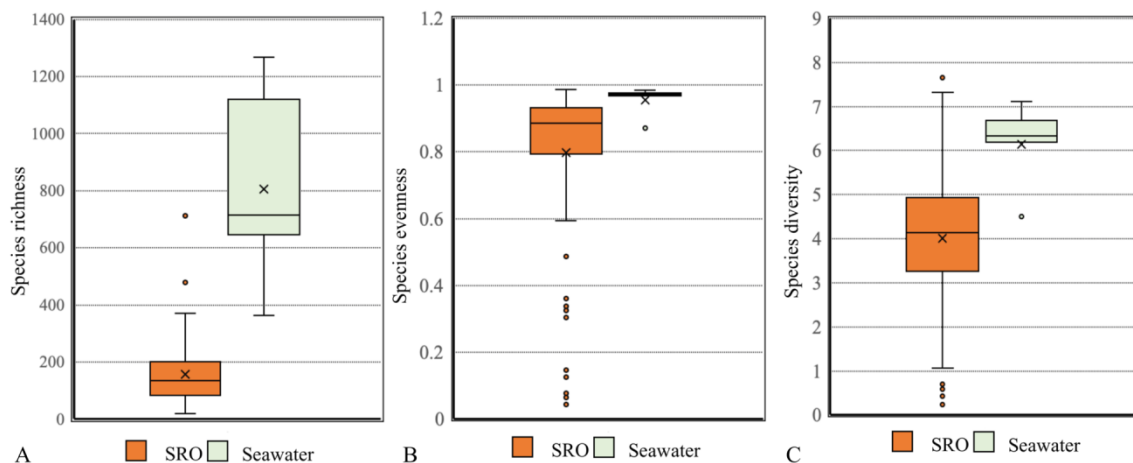
## 397 209 **3. Results**

398  
399 210

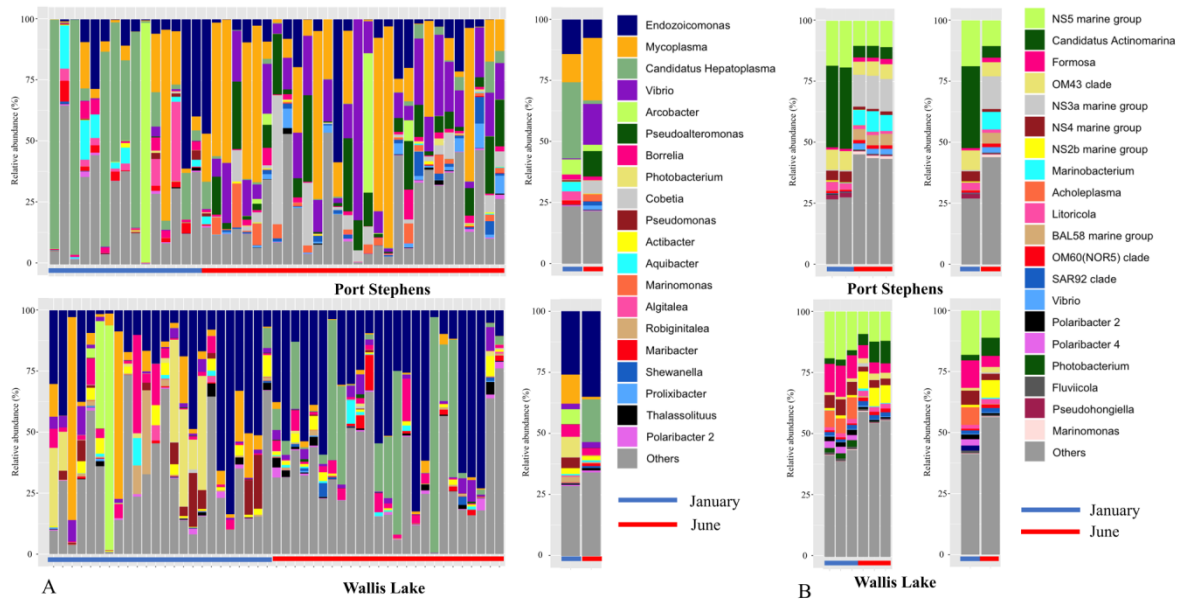
400  
401 211 Following amplicon sequencing of the 132 samples (oysters and seawater), data were rarefied  
402 212 to 7,178 reads retaining a total of 753,690 reads from 105 samples (Supplementary Table 1).  
403  
404 213 After data filtering, a total of 1,889 OTUs were observed across the entire dataset. Of these,  
405  
406 214 1,619 and 190 OTUs were unique to the oyster and seawater microbiota respectively, with only  
407 215 80 OTUs found in both the oyster and seawater samples.  
408  
409 216

414  
415  
416 217 **3.1. The SRO microbiota is distinct from the seawater microbiota**  
417  
418 218

419 219 Across the entire dataset, species richness, evenness and diversity were higher in seawater  
420 220 samples relative to the SRO adductor muscle microbiota (Figure 1 and Supplementary Table  
421 221 2). When grouping all SRO or seawater samples, a 3D nMDS analysis revealed that the  
422 222 composition of the SRO and seawater microbiota were distinct from one another  
423 223 (Supplementary Figure 1), with these differences confirmed as significantly different by  
424 224 PERMANOVA ( $F = 13.54, p = 0.0001$ ). SIMPER analysis revealed a 99.1% dissimilarity  
425 225 between the SRO and seawater microbiota, with *Candidatus Hepatoplasma* genus (OTU  
426 226 14887) and *Endozoicomonas* genus (OTU 3829) over-represented in SRO microbiota and  
427 227 driving 5.7% and 2.9% of the difference respectively (Figure 2 and Supplementary Table 3).  
428 228 In seawater, the *Candidatus Actinomarina* genus (OTU 22961) and NS5 marine group genus  
429 229 (OTU 5409) were over-represented, driving 4.2% and 3.6% of the difference respectively  
430 230 (Figure 2 and Supplementary Table 3).



231  
232 Figure 1: Box and whisker plot of species richness (A), evenness (B) and diversity (C) for SRO  
233 and seawater microbiota. The x in the box plot is the mean of the dataset.



234

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

240

### 241 3.2. Location is a factor shaping the SRO microbiota

242

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

247

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

254

526

527

528

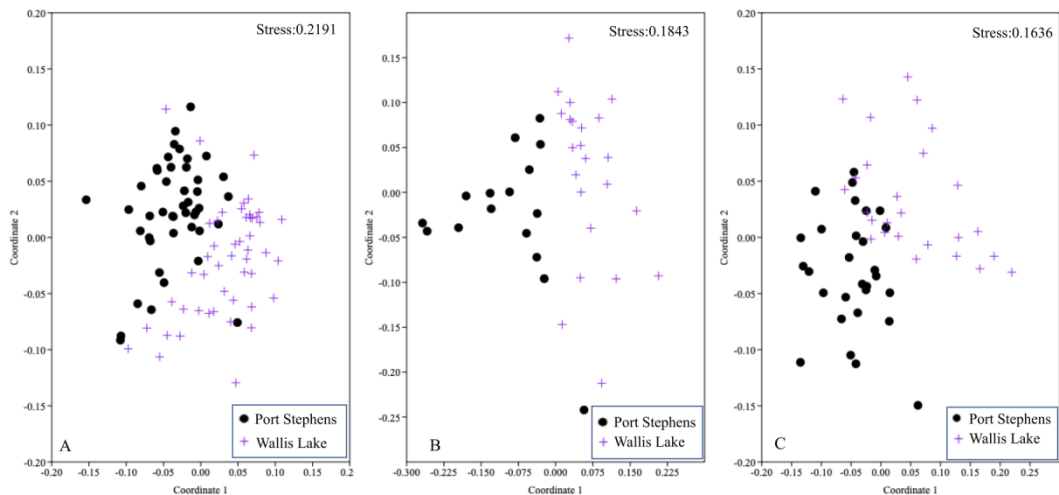
529

530

531

532  
533  
534  
535  
536  
537  
538  
539  
540  
541  
542  
543  
544  
545  
546  
547  
548  
549  
550  
551  
552  
553  
554  
555  
556  
557  
558  
559  
560  
561  
562  
563  
564  
565  
566  
567  
568  
569  
570  
571  
572  
573  
574  
575  
576  
577  
578  
579  
580  
581  
582  
583  
584  
585  
586  
587  
588  
589  
590

255 Despite some overlap, a 3D nMDS plot showed that SRO microbiota clustered according to  
256 location (Figure 3A) and were significantly different according to site (PERMANOVA,  $F =$   
257  $8.955$ ,  $p = 0.0001$ ). This effect of location was also evident within each season in January  
258 (Figure 3B and 3C; PERMANOVA,  $F = 5.117$ ,  $p = 0.0001$ ) and June (PERMANOVA,  $F =$   
259  $11.81$ ,  $p = 0.0001$ ). Across the entire dataset, the SRO microbiota at Port Stephens and Wallis  
260 Lake were 90.5% dissimilar to one another. Similarly, in January and June, the SRO microbiota  
261 from the two sites were 90.3% and 91.9% dissimilar respectively. Interestingly, the main  
262 dissimilarity contributor, *Candidatus Hepatoplasma* genus (OTU 14887), was over-  
263 represented at Port Stephens in January contributing 17.7% to the dissimilarity between  
264 microbiota however, was over-represented at Wallis Lake in June contributing 9.6% of the  
265 microbiota dissimilarity (Supplementary Table 5). Additionally, a member of the  
266 *Endozoicomonas* genus (OTU 1831) was over-represented in Wallis Lake in both January and  
267 June contributing 3.0% and 6.4% respectively.



268  
269 Figure 3: 3D nMDS plots of total SRO microbiota (A) and those from January (B) and June  
270 (C) show separation according to location.

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

Time	Temperature (°C)	pH	DO (mg/L)	Conductivity (µS/cm)	NO <sub>3</sub> (mg/L)	NO <sub>2</sub> <sup>-</sup> (mg/L)	NH <sub>3</sub> (mg/L)	PO <sub>4</sub> <sup>3-</sup> (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

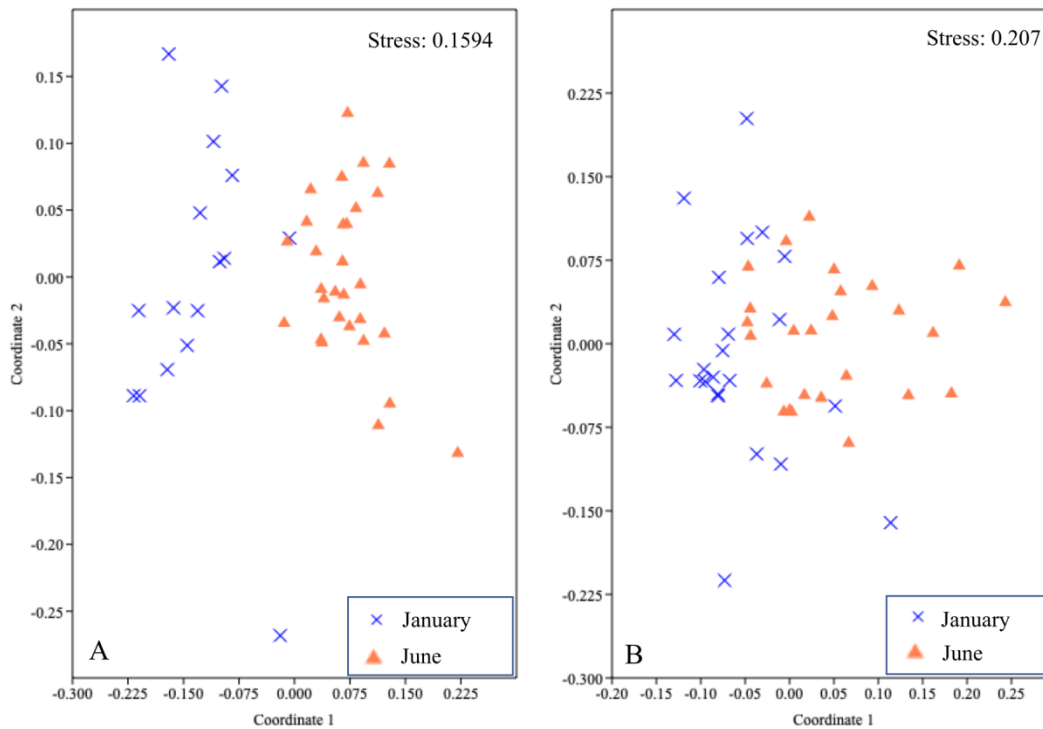
\*Data obtained from (Bureau of Meteorology, 2019)

632  
633  
634  
635  
636  
637  
638  
639  
640  
641  
642  
643  
644  
645  
646  
647  
648  
649  
650  
651  
652  
653  
654  
655  
656  
657  
658  
659  
660  
661  
662  
663  
664  
665  
666  
667  
668  
669  
670  
671  
672  
673  
674  
675  
676  
677  
678  
679  
680  
681  
682  
683  
684  
685  
686  
687  
688  
689  
690  
691

275 **3.3. Season is a factor shaping the SRO microbiota**

276  
277  
278  
279  
280  
281  
282  
283  
284  
285  
286  
287  
288  
289  
290  
291  
292

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).



293

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

296 **3.4. The effect of QX-resistance on the SRO microbiota**

297

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

306

307 At Port Stephens in June, SIMPER analysis revealed a 75.7% dissimilarity between the QX-  
 308 sensitive and QX-resistant groups with two OTUs (OTU 12669 and OTU 14900) from the  
 309 *Mycoplasma* genus over-represented in the QX-resistant group and contributing 9.6% and  
 310 9.2% to the microbiota dissimilarity. OTUs belonging to the *Pseudoalteromonas* (OTU 8917)

692  
693  
694  
695  
696  
697  
698  
699  
700  
701  
702  
703  
704  
705  
706  
707  
708  
709  
710  
711  
712  
713  
714  
715  
716  
717  
718  
719  
720  
721  
722  
723  
724  
725  
726  
727  
728  
729  
730  
731  
732  
733  
734  
735  
736  
737  
738  
739  
740  
741  
742  
743  
744  
745  
746  
747  
748  
749  
750  
751

752  
753  
754  
755  
756  
757  
758  
759  
760  
761  
762  
763  
764  
765  
766  
767  
768  
769  
770  
771  
772  
773  
774  
775  
776  
777  
778  
779  
780  
781  
782  
783  
784  
785  
786  
787  
788  
789  
790  
791  
792  
793  
794  
795  
796  
797  
798  
799  
800  
801  
802  
803  
804  
805  
806  
807  
808  
809  
810  
811

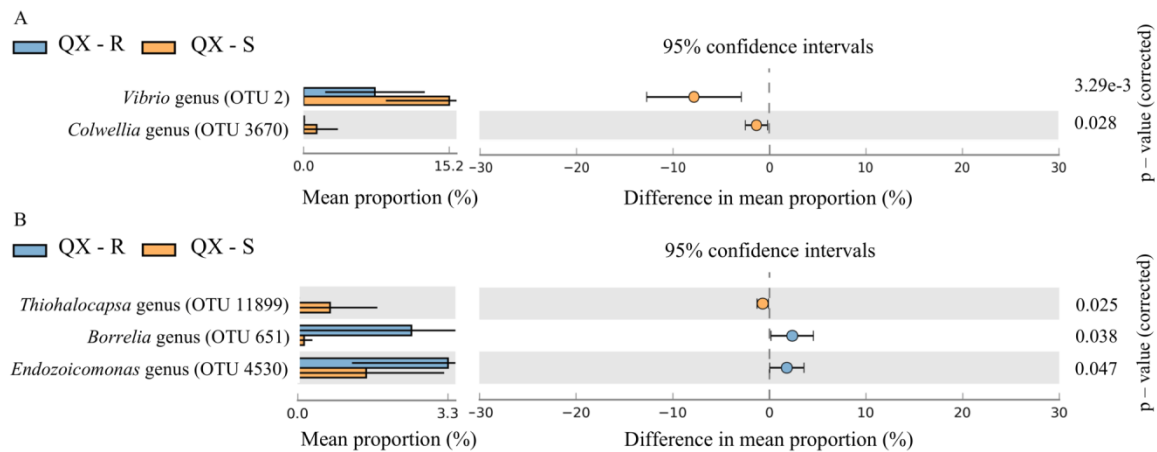
311 and *Vibrio* (OTU 2) genera were over-represented in the QX-sensitive group contributing 6.4%  
 312 and 6.1% to the microbiota dissimilarity (Supplementary Table 8), while another OTU assigned  
 313 to the *Vibrio* genus (OTU 1) was over-represented in the QX-resistant microbiota contributing  
 314 5.6% dissimilarity (Supplementary Table 8). Additionally, two *Mycoplasma* OTUs (OTU  
 315 12669 and OTU 14900) were over-represented in the QX-resistant group, contributing 9.6%  
 316 and 9.2% to the microbiota dissimilarity. At Wallis Lake in June, SIMPER revealed 77.9%  
 317 microbiota dissimilarity between the QX groups. A member assigned to the *Candidatus*  
 318 *Hepatoplasma* genus (OTU 14887) was over-represented in the QX-sensitive group and  
 319 contributed 15.86% of the microbiota dissimilarity, whereas 5 OTUs, all assigned to the  
 320 *Endozoicomonas* genus (OTUs 1831, 3829, 6283, 3483 and 4530), were over-represented in  
 321 the QX-resistant microbiota.

322  
 323 Table 3. PERMANOVA results comparing the microbiota of QX-sensitive (F03, F18, F32  
 324 and F37) and QX-resistant (F022 and F025) families at each location and time point.  
 325

	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

326  
 327 To further decipher beta diversity patterns between QX-resistant and -sensitive SRO's,  
 328 STAMP with a Welch's T-Test was used. This analysis identified members of the *Vibrio* (OTU  
 329 2, p = 0.003) and *Colwellia* (OTU 3670, p = 0.028) genera with significantly higher relative  
 330 abundance in the QX-sensitive group from Port Stephens in June (Figure 5A). In Wallis Lake,  
 331 a member assigned as the *Thiohalocapsa* genus (OTU 11899) had a significantly higher  
 332 relative abundance in QX-sensitive oysters (p = 0.025), whereas OTUs assigned to the *Borrelia*  
 333 (OTU 651, p = 0.038) and *Endozoicomonas* (OTU 4530, p = 0.047) genera had a significantly  
 334 higher relative abundance in QX-resistant oysters (Figure 5B).





335

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

339 **4. Discussion**

340  
 341 This study investigated the influence of location, season and oyster genetics (QX-resistance)  
 342 on shaping the SRO microbiota. Despite the filter-feeding nature of oysters, our results indicate  
 343 that the SRO microbiota is highly distinct from the planktonic microbiota within the  
 344 surrounding seawater. It is possible that part of the observed variation is due to the seawater  
 345 samples being collected from jetties 800 m from the oyster leases however, it is unlikely that  
 346 the main bacterial patterns in the seawater would substantially vary across this small distance.  
 347 Additionally, it is also possible that a part of the observed variation is due to the use of different  
 348 DNA extraction kits for the oysters and water samples. Nevertheless, the patterns we observed  
 349 are consistent with previous studies on the microbiota of the Pacific oyster (Lokmer *et al.*,  
 350 2016a; Lokmer *et al.*, 2016b).

351  
 352 The microbiota varies between oyster tissues (King *et al.*, 2012; King *et al.*, 2020; Lokmer *et*  
 353 *al.*, 2016b) however, some overlap is observed such as the genus *Mycoplasma* which is  
 354 dominant in the adductor muscle, gill, stomach, digestive gland and haemolymph (Green &  
 355 Barnes, 2010; King *et al.*, 2012; King *et al.*, 2019b; King *et al.*, 2020; Wegner *et al.*, 2013).  
 356 Here, we elected to use the adductor muscle as it allows sampling of the circulatory  
 357 haemolymph from the sinuses. Overall, the SRO microbiota was dominated by OTUs assigned  
 358 to the *Candidatus Hepatoplasma*, *Endozoicomonas* and *Mycoplasma* genera. *Candidatus*

872  
873  
874  
875  
876  
877  
878  
879  
880  
881  
882  
883  
884  
885  
886  
887  
888  
889  
890  
891  
892  
893  
894  
895  
896  
897  
898  
899  
900  
901  
902  
903  
904  
905  
906  
907  
908  
909  
910  
911  
912  
913  
914  
915  
916  
917  
918  
919  
920  
921  
922  
923  
924  
925  
926  
927  
928  
929  
930  
931

359 *Hepatoplasma* has been found associated with various marine organisms such as starfish  
360 (Nakagawa *et al.*, 2017), Norway lobsters (Meziti *et al.*, 2012), corals (van de Water *et al.*,  
361 2018) and starlet sea anemones (Mortzfeld *et al.*, 2016). However, the function of this  
362 bacterium in marine organisms, including SROs, is unknown. *Mycoplasma* is consistently  
363 identified in healthy oysters including Eastern oysters, Pacific oyster and SROs (Green &  
364 Barnes, 2010; King *et al.*, 2012; King *et al.*, 2019b; King *et al.*, 2019c; Wegner *et al.*, 2013)  
365 suggesting that these bacteria are potentially important for oyster health. Members of the  
366 *Endozoicomonas* genus have been found to be associated with numerous marine organisms  
367 (Neave *et al.*, 2016) such as sponges (Nishijima *et al.*, 2013; Rua *et al.*, 2014) and corals (Bayer  
368 *et al.*, 2013; Ziegler *et al.*, 2016) with members of this genus previously shown to comprise a  
369 large proportion of the Indo-Pacific (Roterman *et al.*, 2015; Zurel *et al.*, 2011) and Black-  
370 Lipped pearl oyster (Dubé *et al.*, 2019) bacterial communities. In sponges and corals, these  
371 bacteria play a role in nitrogen and carbon recycling, provision of proteins to their hosts and  
372 production of antibiotics (Neave *et al.*, 2017; Nishijima *et al.*, 2013; Rua *et al.*, 2014) and may  
373 suggest a similar role in SROs.

374

#### 375 **4.1. The SRO microbiota is influenced by location**

376

377 The same oyster families were deployed in Port Stephens and Wallis Lake reducing the  
378 influence of genetics as a confounding factor in our analyses and allowing us to investigate  
379 whether location or season influence the composition of the SRO microbiota. Consistent with  
380 previous studies that have characterised the influence of location on the oyster microbiota  
381 (King *et al.*, 2012; Ossai *et al.*, 2017; Roterman *et al.*, 2015; Trabal *et al.*, 2012; Zurel *et al.*,  
382 2011), we observed that SRO microbiota was significantly different between two sites which  
383 are approximately 70 km apart and differ in estuarine type (Roy *et al.*, 2001). Data collected in  
384 this study identified higher chlorophyll a concentrations and temperature in Port Stephens  
385 relative to Wallis Lake. While both estuaries have similar percentages of agricultural land  
386 usage in their respective catchments (approximately 30%), Port Stephens has significantly  
387 higher sediment and nutrient inputs compared to Wallis Lake (Roper *et al.*, 2011). Given the  
388 higher nutrient and sediment loads at Port Stephens, these factors could explain the microbiota  
389 variability between the locations. A member of the *Endozoicomonas* genus (OTU 1831) was  
390 more abundant in Wallis Lake than in Port Stephens at both sampling times. In coral species,  
391 the anthropogenically influenced coral microbiota (*Pocillopora verrucosa* and *Acropora*  
392 *hemprichii*) was marked by a reduction of *Endozoicomonas* relative abundance (Ziegler *et al.*,

932  
933  
934 393 2016), suggesting that the lower relative abundance of this bacteria in SROs at Port Stephens  
935  
936 394 could be related to the higher nutrient and sediment loads.  
937  
938 395

#### 939 396 **4.2. The SRO microbiota is influenced by season**

940  
941 397  
942 398 In a number of marine organisms, including corals (Sharp *et al.*, 2017) and Pacific oysters  
943 399 (Pierce *et al.*, 2016; Zurel *et al.*, 2011), there is evidence for significant temporal heterogeneity  
944 400 in microbiota composition. Consistent with these findings, we observed a significant influence  
945 401 of season (summer versus winter) on the SRO microbiota for both locations. At Port Stephens,  
946 402 seasonal shifts in environmental conditions were dominated by changing temperature,  
947 403 chlorophyll a and conductivity, while at Wallis Lake, seasonal changes in environmental  
948 404 parameters were mostly driven by temperature and pH. Previous studies have characterised the  
949 405 influence of temperature on the oyster microbiota (Lokmer & Wegner, 2015; Pierce *et al.*,  
950 406 2016) and salinity perturbations have also been observed to influence the oyster microbiota  
951 407 (del Refugio Castañeda Chávez *et al.*, 2005; Larsen *et al.*, 2013). Seasonal shifts in the SRO  
952 408 microbiota were characterised by changes in the relative abundance of several OTUs, including  
953 409 those assigned to the *Candidatus Hepatoplasma* and *Vibrio* genera. Interestingly, we observed  
954 410 inverse patterns for the relative abundance of an OTU assigned to the *Candidatus*  
955 411 *Hepatoplasma* genus (OTU 14887) between the two sampling sites. At Port Stephens, this  
956 412 OTU was significantly more abundant in summer, while at Wallis Lake, it was considerably  
957 413 more abundant in winter. The environmental data collected at the time suggests no similarities  
958 414 between the Port Stephens summer and Wallis Lake winter samples that could explain this  
959 415 pattern (conductivity was similar for these two sampling points but conductivity did not change  
960 416 between the Wallis Lake summer and winter sampling points) and this OTU was rare or absent  
961 417 in the seawater communities, therefore future studies should increase the suite of  
962 418 environmental parameters collected to explain these patterns. At both locations, a member of  
963 419 the *Vibrio* genus (OTU 2) had a higher relative abundance in winter than in summer. This  
964 420 pattern is interesting given that *Vibrio* typically exhibit preferences for warm water  
965 421 temperatures. However, some *Vibrio* species such as *Vibrio splendidus*, have elsewhere been  
966 422 found to be most abundant during winter and spring (Arias *et al.*, 1999; Pujalte *et al.*, 1999). It  
967 423 is also conceivable that other environmental factors, such as chlorophyll a or nutrient levels,  
968 424 underpinned the higher winter relative abundance of this *Vibrio* species (OTU 2).  
969 425

#### 987 426 **4.3. The SRO microbiota is influenced by disease resistance**

988  
989  
990  
991

992  
993  
994  
995  
996  
997  
998  
999  
1000  
1001  
1002  
1003  
1004  
1005  
1006  
1007  
1008  
1009  
1010  
1011  
1012  
1013  
1014  
1015  
1016  
1017  
1018  
1019  
1020  
1021  
1022  
1023  
1024  
1025  
1026  
1027  
1028  
1029  
1030  
1031  
1032  
1033  
1034  
1035  
1036  
1037  
1038  
1039  
1040  
1041  
1042  
1043  
1044  
1045  
1046  
1047  
1048  
1049  
1050  
1051

427

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.

444

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;

1052  
1053  
1054 461 Nishijima *et al.*, 2013; Rua *et al.*, 2014), future studies should investigate their potential role  
1055  
1056 462 in QX-resistant oysters.  
1057

## 1058 463 **5. Conclusion**

1061 464 There is emerging evidence that the microbiota of benthic organisms, including oysters, are  
1062  
1063 465 dynamic and driven by multiple factors, but the impact of location, season and genetics (disease  
1064  
1065 466 resistance) on the SRO microbiota have not been reported previously. Understanding the  
1066  
1067 467 factors that drive SRO microbiota composition are pivotal when deciphering the role of the  
1068  
1069 468 microbiota during disease events, and to explain microbiota shifts prior to, or during, disease.  
1070  
1071 469 However, this is currently hindered by a paucity of SRO microbiota studies. This study  
1072  
1073 470 demonstrated that the SRO microbiota assemblage is influenced by location and season, which  
1074  
1075 471 highlights the importance of performing temporal studies at individual locations as interpreting  
1076  
1077 472 microbiota patterns from other locations or time points can lead to erroneous microbiota  
1078  
1079 473 explanations. Further, breeding for QX disease resistance (genetics) was found to influence the  
1080  
1081 474 SRO microbiota although this was only observed in the winter. This sampling time point is  
1082  
1083 475 before the typical QX disease period, which may indicate that a microbiota shift could be a  
1084  
1085 476 factor in QX disease dynamics. Overall, these data suggest that there is a synergistic interaction  
1086  
1087 477 of genetics and environmental drivers in shaping the SRO microbiota.  
1088

## 1084 478 **Acknowledgements**

1085 479  
1086  
1087  
1088  
1089 480 This research was supported by an Australian Research Council Linkage Project  
1090  
1091 481 (LP160101785), a Cooperative Research Centre Project (CRC-P 2016-805; Future Oysters),  
1092  
1093 482 led by the Australian Seafood Industry Pty Ltd in partnership with a number of Australian  
1094  
1095 483 research organisations and, Ausgem, a research partnership initiated between the University of  
1096  
1097 484 Technology Sydney and the New South Wales Department of Primary Industries. VKN was  
1098  
1099 485 supported by a University of Technology, Sydney – Vietnam International Education  
1100  
1101 486 Development (UTS - VIED) Scholarship.

## 1101 487 **References**

1102 488  
1103  
1104 489 Adlard, R. D., & Wesche, S. (2005). *Aquatic Animal Health Subprogram: Development of a*  
1105  
1106 490 *disease zoning policy for *Marteilia sydneyi* to support sustainable production, health*  
1107  
1108  
1109  
1110  
1111

1112  
1113  
1114 491 *certification and trade in the Sydney rock oyster*. Retrieved from  
1115  
1116 492 <http://www.frdc.com.au/Archived-Reports/FRDC%20Projects/2001-214-DLD.pdf>  
1117  
1118 493 Arias, C. R., Macian, M. C., Aznar, R., Garay, E., & Pujalte, M. J. (1999). Low incidence of  
1119 494 *Vibrio vulnificus* among *Vibrio* isolates from sea water and shellfish of the western  
1120  
1121 495 Mediterranean coast. *J. Appl. Microbiol.*, 86(1), 125-134. doi:10.1046/j.1365-  
1122 496 2672.1999.00641.x  
1123  
1124 497 Bayer, T., Neave, M. J., Alsheikh-Hussain, A., Aranda, M., Yum, L. K., Mincer, T., Hughen,  
1125 498 K., Apprill, A., & Voolstra, C. R. (2013). The microbiome of the Red sea coral  
1126  
1127 499 *Stylophora pistillata* is dominated by tissue-associated *Endozoicomonas* bacteria. *Appl.*  
1128  
1129 500 *Environ. Microbiol.*, 79(15), 4759. doi:10.1128/AEM.00695-13  
1130  
1131 501 Bezemer, B., Butt, D., Nell, J., Adlard, R., & Raftos, D. (2006). Breeding for QX disease  
1132 502 resistance negatively selects one form of the defensive enzyme, phenoloxidase, in  
1133  
1134 503 Sydney rock oysters. *Fish Shellfish Immun.*, 20(4), 627-636.  
1135 504 doi:10.1016/j.fsi.2005.08.007  
1136  
1137 505 Bureau of Meteorology. (2019). Daily Rainfall. Climate Data Online. Retrieved from  
1138 506 <http://www.bom.gov.au/climate/data/index.shtml>.  
1139  
1140 507 <http://www.bom.gov.au/climate/data/index.shtml>  
1141  
1142 508 Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K.,  
1143 509 Fierer, N., Peña, A. G., Goodrich, J. K., Gordon, J. I., et al. (2010). QIIME allows  
1144  
1145 510 analysis of high-throughput community sequencing data. *Nat. Methods*, 7(5), 335-336.  
1146 511 doi:10.1038/nmeth.f.303  
1147  
1148 512 de Lorgeril, J., Lucasson, A., Petton, B., Toulza, E., Montagnani, C., Clerissi, C., Vidal-Dupiol,  
1149 513 J., Chaparro, C., Galinier, R., Escoubas, J.-M., et al. (2018). Immune-suppression by  
1150  
1151 514 OsHV-1 viral infection causes fatal bacteraemia in Pacific oysters. *Nat. Commun.*, 9(1),  
1152 515 4215. doi:10.1038/s41467-018-06659-3  
1153  
1154 516 del Refugio Castañeda Chávez, M., Sedas, V. P., Orrantia Borunda, E., & Reynoso, F. L.  
1155 517 (2005). Influence of water temperature and salinity on seasonal occurrences of *Vibrio*  
1156 518 *cholerae* and enteric bacteria in oyster-producing areas of Veracruz, México. *Mar.*  
1157 519 *Pollut. Bull.*, 50(12), 1641-1648. doi:10.1016/j.marpolbul.2005.06.036  
1160  
1161 520 Department of Primary Industries. (2016). *NSW oyster industry sustainable aquaculture*  
1162 521 *strategy*. NSW: Department of Primary Industries Retrieved from  
1163  
1164 522 [https://www.dpi.nsw.gov.au/\\_\\_data/assets/pdf\\_file/0006/638250/NSW-oyster-](https://www.dpi.nsw.gov.au/__data/assets/pdf_file/0006/638250/NSW-oyster-)  
1165 523 [industry-sustainable-aquaculture-strategy-2016.pdf](https://www.dpi.nsw.gov.au/__data/assets/pdf_file/0006/638250/NSW-oyster-industry-sustainable-aquaculture-strategy-2016.pdf)  
1166  
1167  
1168  
1169  
1170  
1171

1172  
1173  
1174 524 Dove, M., Kube, P., Wilkie, E., Cumbo, V., Raftos, D., O'Connor, W., & New South Wales  
1175 Department of Primary Industries (Fisheries). (2020). *Accelerated Sydney rock oyster*  
1176 525 *(SRO) breeding research. Future oysters CRC-P Project 2016-802*. Retrieved from  
1177 526  
1178 *Taylors Beach*:  
1179 527  
1180  
1181 528 Dubé, C. E., Ky, C.-L., & Planes, S. (2019). Microbiome of the Black-lipped pearl oyster  
1182 529 *Pinctada margaritifera*, a multi-tissue description with functional profiling. *Front.*  
1183 530 *Microbiol.*, *10*(1548). doi:10.3389/fmicb.2019.01548  
1184  
1185 531 Edgar, R. C. (2010). Search and clustering orders of magnitude faster than BLAST.  
1186 532 *Bioinformatics*, *26*(19), 2460-2461. doi:10.1093/bioinformatics/btq461  
1187  
1188 533 Garnier, M., Labreuche, Y., Garcia, C., Robert, M., & Nicolas, J.-L. (2007). Evidence for the  
1189 534 involvement of pathogenic bacteria in summer mortalities of the Pacific oyster  
1190 *Crassostrea gigas*. *Microb. Ecol.*, *53*(2), 187-196. doi:10.1007/s00248-006-9061-9  
1191 535  
1192 536 Green, T. J., & Barnes, A. C. (2010). Bacterial diversity of the digestive gland of Sydney rock  
1193 537 oysters, *Saccostrea glomerata* infected with the paramyxean parasite, *Marteilia*  
1194 538 *sydneyi*. *J. Appl. Microbiol.*, *109*(2), 613-622. doi:10.1111/j.1365-2672.2010.04687.x  
1195  
1196 539 Hammer, Øyvind, Harper, David, A. T., & Paul, D. R. (2001). Past: Paleontological statistics  
1197 540 software package for education and data analysis. *Palaeontol. Electronica*, *4*(1), 9.  
1200 541 Retrieved from [https://palaeo-electronica.org/2001\\_1/past/past.pdf](https://palaeo-electronica.org/2001_1/past/past.pdf)  
1201  
1202 542 King, G. M., Judd, C., Kuske, C. R., & Smith, C. (2012). Analysis of stomach and gut  
1203 543 microbiomes of the Eastern oyster (*Crassostrea virginica*) from coastal Louisiana,  
1204 544 USA. *PLoS One*, *7*(12), e51475. doi:10.1371/journal.pone.0051475  
1205  
1206 545 King, W. L., Jenkins, C., Seymour, J. R., & Labbate, M. (2019a). Oyster disease in a changing  
1207 546 environment: Decrypting the link between pathogen, microbiome and environment.  
1208 547 *Mar. Environ. Res.*, *143*, 124-140. doi:10.1016/j.marenvres.2018.11.007  
1209  
1210 548 King, W. L., Jenkins, C., Go, J., Siboni, N., Seymour, J. R., & Labbate, M. (2019b).  
1211 549 Characterisation of the Pacific oyster microbiome during a summer mortality event.  
1212 550 *Microbiol. Ecol.*, *77*(2), 502-512. doi:10.1007/s00248-018-1226-9  
1213  
1214 551 King, W. L., Siboni, N., Williams, N. L. R., Kahlke, T., Nguyen, K. V., Jenkins, C., Dove, M.,  
1215 552 O'Connor, W., Seymour, J. R., & Labbate, M. (2019c). Variability in the composition  
1216 553 of Pacific oyster microbiomes across oyster families exhibiting different levels of  
1217 554 susceptibility to OsHV-1  $\mu$ var disease. *Front. Microbiol.*, *10*, 473-473.  
1218 555 doi:10.3389/fmicb.2019.00473  
1219  
1220 556 King, W. L., Siboni, N., Kahlke, T., Dove, M., O'Connor, W., Mahbub, K. R., Jenkins, C.,  
1221 557 Seymour, J. R., & Labbate, M. (2020). Regional and oyster microenvironmental scale  
1222  
1223  
1224  
1225  
1226  
1227  
1228  
1229  
1230  
1231

1232  
1233  
1234  
1235  
1236  
1237  
1238  
1239  
1240  
1241  
1242  
1243  
1244  
1245  
1246  
1247  
1248  
1249  
1250  
1251  
1252  
1253  
1254  
1255  
1256  
1257  
1258  
1259  
1260  
1261  
1262  
1263  
1264  
1265  
1266  
1267  
1268  
1269  
1270  
1271  
1272  
1273  
1274  
1275  
1276  
1277  
1278  
1279  
1280  
1281  
1282  
1283  
1284  
1285  
1286  
1287  
1288  
1289  
1290  
1291

558 heterogeneity in the Pacific oyster bacterial community. *FEMS Microbiol. Ecol.*  
doi:10.1093/femsec/fiaa054

560 Klaus, J. S., Frias-Lopez, J., Bonheyo, G. T., Heikoop, J. M., & Fouke, B. W. (2005). Bacterial  
561 communities inhabiting the healthy tissues of two Caribbean reef corals: interspecific  
and spatial variation. *Coral Reefs*, 24(1), 129-137. doi:10.1007/s00338-004-0447-1

563 Kleeman, S. N., Adlard, R. D., & Lester, R. J. G. (2002). Detection of the initial infective stages  
564 of the protozoan parasite *Marteilia sydneyi* in *Saccostrea glomerata* and their  
565 development through to sporogenesis. *Int. J. Parasitol.*, 32(6), 767-784.  
doi:http://dx.doi.org/10.1016/S0020-7519(02)00025-5

567 Lane, D. J. (1991). 16S/23S rRNA Sequencing. In Stackebrandt, E & Goodfellow, M (Eds.),  
568 *Nucleic Acid Techniques in Bacterial Systematics* (pp. 115-147). West Sussex: John  
569 Wiley & Sons Ltd.

570 Larsen, A. M., Scott Rikard, F., Walton, W. C., & Arias, C. R. (2013). Effective reduction of  
571 *Vibrio vulnificus* in the Eastern oyster (*Crassostrea virginica*) using high salinity  
572 depuration. *Food Microbiol.*, 34(1), 118-122. doi:10.1016/j.fm.2012.11.009

573 Lemire, A., Goudenege, D., Versigny, T., Petton, B., Calteau, A., Labreuche, Y., & Le Roux,  
574 F. (2015). Populations, not clones, are the unit of vibrio pathogenesis in naturally  
575 infected oysters. *ISME J.*, 9(7), 1523-1531. doi:10.1038/ismej.2014.233

576 Lokmer, A., & Wegner, M. (2015). Hemolymph microbiome of Pacific oysters in response to  
577 temperature, temperature stress and infection. *ISME J.*, 9(3), 670-682.  
578 doi:10.1038/ismej.2014.160

579 Lokmer, A., Goedknecht, M. A., Thielges, D. W., Fiorentino, D., Kuenzel, S., Baines, J. F., &  
580 Wegner, K. M. (2016a). Spatial and temporal dynamics of Pacific oyster hemolymph  
581 microbiota across multiple scales. *Front. Microbiol.*, 7, 1367.  
582 doi:10.3389/fmicb.2016.01367

583 Lokmer, A., Kuenzel, S., Baines, J. F., & Wegner, K. M. (2016b). The role of tissue-specific  
584 microbiota in initial establishment success of Pacific oysters. *Environ. Microbiol.*,  
585 18(3), 970-987. doi:10.1111/1462-2920.13163

586 Magoč, T., & Salzberg, S. L. (2011). FLASH: fast length adjustment of short reads to improve  
587 genome assemblies. *Bioinformatics*, 27(21), 2957-2963.  
588 doi:10.1093/bioinformatics/btr507

589 Meziti, A., Mente, E., & Kormas, K. A. (2012). Gut bacteria associated with different diets in  
590 reared *Nephrops norvegicus*. *Syst. Appl. Microbiol.*, 35(7), 473-482.  
591 doi:10.1016/j.syapm.2012.07.004



1292  
1293  
1294  
1295  
1296  
1297  
1298  
1299  
1300  
1301  
1302  
1303  
1304  
1305  
1306  
1307  
1308  
1309  
1310  
1311  
1312  
1313  
1314  
1315  
1316  
1317  
1318  
1319  
1320  
1321  
1322  
1323  
1324  
1325  
1326  
1327  
1328  
1329  
1330  
1331  
1332  
1333  
1334  
1335  
1336  
1337  
1338  
1339  
1340  
1341  
1342  
1343  
1344  
1345  
1346  
1347  
1348  
1349  
1350  
1351

- 592 Mortzfeld, B. M., Urbanski, S., Reitzel, A. M., Künzel, S., Technau, U., & Fraune, S. (2016).  
593 Response of bacterial colonization in *Nematostella vectensis* to development,  
594 environment and biogeography. *Environ. Microbiol.*, *18*(6), 1764-1781.  
595 doi:10.1111/1462-2920.12926
- 596 Nakagawa, S., Saito, H., Tame, A., Hirai, M., Yamaguchi, H., Sunata, T., Aida, M., Muto, H.,  
597 Sawayama, S., & Takaki, Y. (2017). Microbiota in the coelomic fluid of two common  
598 coastal starfish species and characterization of an abundant *Helicobacter*-related taxon.  
599 *Sci. Rep.*, *7*, 8764. doi:10.1038/s41598-017-09355-2
- 600 Neave, M. J., Apprill, A., Ferrier-Pagès, C., & Voolstra, C. R. (2016). Diversity and function  
601 of prevalent symbiotic marine bacteria in the genus *Endozoicomonas*. *Appl. Microbiol.*  
602 *Biotechnol.*, *100*(19), 8315-8324. doi:10.1007/s00253-016-7777-0
- 603 Neave, M. J., Michell, C. T., Apprill, A., & Voolstra, C. R. (2017). *Endozoicomonas* genomes  
604 reveal functional adaptation and plasticity in bacterial strains symbiotically associated  
605 with diverse marine hosts. *Sci. Rep.*, *7*, 40579. doi:10.1038/srep40579
- 606 Nell, J. (2007). *Diseases of Sydney rock oysters*. NSW, Australia: NSW Department of Primary  
607 Industries, Retrieved from [https://marine-aquaculture.extension.org/wp-](https://marine-aquaculture.extension.org/wp-content/uploads/2019/05/Diseases-of-Sydney-Rock-Oysters.pdf)  
608 [content/uploads/2019/05/Diseases-of-Sydney-Rock-Oysters.pdf](https://marine-aquaculture.extension.org/wp-content/uploads/2019/05/Diseases-of-Sydney-Rock-Oysters.pdf)
- 609 Newton, K., Peters, R., & Raftos, D. (2004). Phenoloxidase and QX disease resistance in  
610 Sydney rock oysters (*Saccostrea glomerata*). *Dev. Comp. Immunol.*, *28*(6), 565-569.  
611 doi:10.1016/j.dci.2003.10.004
- 612 Nishijima, M., Adachi, K., Katsuta, A., Shizuri, Y., & Yamasato, K. (2013). *Endozoicomonas*  
613 *numazuensis* sp. nov., a gammaproteobacterium isolated from marine sponges, and  
614 emended description of the genus *Endozoicomonas* Kurahashi and Yokota 2007. *Int. J.*  
615 *Syst. Evol. Microbiol.*, *63*(Pt 2), 709-714. doi:10.1099/ijs.0.042077-0
- 616 O'Connor, W., & Dove, M. (2009). The changing face of oyster culture in New South Wales,  
617 Australia. *J. Shellfish Res.*, *28*, 803-811.
- 618 Offret, C., Rochard, V., Laguerre, H., Mounier, J., Huchette, S., Brillet, B., Le Chevalier, P.,  
619 & Fleury, Y. (2019). Protective efficacy of a *Pseudoalteromonas* strain in European  
620 abalone, *Haliotis tuberculata*, infected with *Vibrio harveyi* ORM4. *Probiotics*  
621 *Antimicro.*, *11*(1), 239-247. doi:10.1007/s12602-018-9389-8
- 622 Ossai, S., Ramachandran, P., Ottesen, A., Reed, E., DePaola, A., & Parveen, S. (2017).  
623 Microbiomes of American oysters (*Crassostrea virginica*) harvested from two sites in  
624 the Chesapeake bay. *Genome Announc.*, *5*(30), e00729-00717.  
625 doi:10.1128/genomeA.00729-17

1352  
1353  
1354  
1355  
1356  
1357  
1358  
1359  
1360  
1361  
1362  
1363  
1364  
1365  
1366  
1367  
1368  
1369  
1370  
1371  
1372  
1373  
1374  
1375  
1376  
1377  
1378  
1379  
1380  
1381  
1382  
1383  
1384  
1385  
1386  
1387  
1388  
1389  
1390  
1391  
1392  
1393  
1394  
1395  
1396  
1397  
1398  
1399  
1400  
1401  
1402  
1403  
1404  
1405  
1406  
1407  
1408  
1409  
1410  
1411

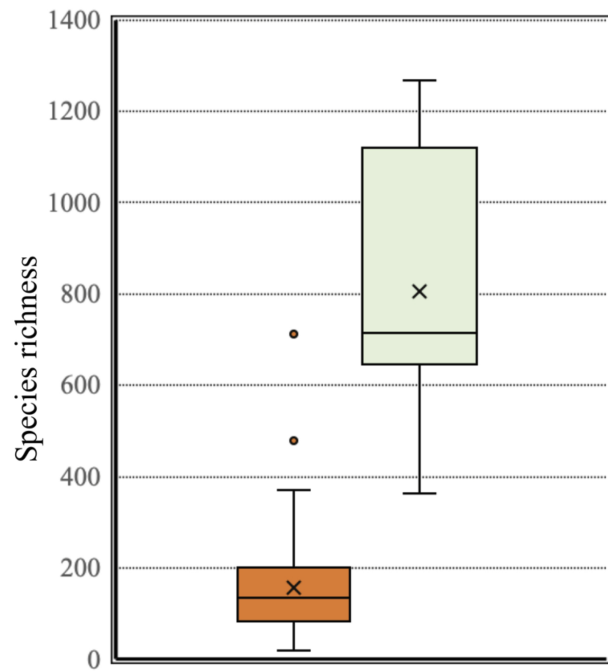
- 626 Paillard, C., Le Roux, F., & Borrego, J. J. (2004). Bacterial disease in marine bivalves, a review  
627 of recent studies: trends and evolution. *Aquat. Living Resour.*, 17(04), 477-498.
- 628 Parks, D. H., Tyson, G. W., Hugenholtz, P., & Beiko, R. G. (2014). STAMP: statistical analysis  
629 of taxonomic and functional profiles. *Bioinformatics*, 30(21), 3123-3124.  
630 doi:10.1093/bioinformatics/btu494
- 631 Peters, R., & Raftos, D. A. (2003). The role of phenoloxidase suppression in QX disease  
632 outbreaks among Sydney rock oysters (*Saccostrea glomerata*). *Aquaculture*, 223(1),  
633 29-39. doi:10.1016/S0044-8486(03)00169-8
- 634 Petton, B., Bruto, M., James, A., Labreuche, Y., Alunno-Bruscia, M., & Le Roux, F. (2015).  
635 *Crassostrea gigas* mortality in France: the usual suspect, a herpes virus, may not be the  
636 killer in this polymicrobial opportunistic disease. *Front. Microbiol.*, 6, 686.  
637 doi:10.3389/fmicb.2015.00686
- 638 Pierce, M. L., Ward, J. E., Holohan, B. A., Zhao, X., & Hicks, R. E. (2016). The influence of  
639 site and season on the gut and pallial fluid microbial communities of the eastern oyster,  
640 *Crassostrea virginica* (Bivalvia, Ostreidae): community-level physiological profiling  
641 and genetic structure. *Hydrobiologia*, 765(1), 97-113.
- 642 Prado, S., Montes, J., Romalde, J. L., & Barja, J. L. (2009). Inhibitory activity of *Phaeobacter*  
643 strains against aquaculture pathogenic bacteria. *Int. Microbiol.*, 12(2), 107-114.
- 644 Pujalte, M., Ortigosa, M., Macián, M., & Garay, E. (1999). Aerobic and facultative anaerobic  
645 heterotrophic bacteria associated to Mediterranean oysters and seawater. *Int.*  
646 *Microbiol.*, 2(4), 259 -266.
- 647 Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., & Glöckner,  
648 F. O. (2013). The SILVA ribosomal RNA gene database project: improved data  
649 processing and web-based tools. *Nucleic Acids Res.*, 41(Database issue), D590-D596.  
650 doi:10.1093/nar/gks1219
- 651 Ritchie, R. J. (2006). Consistent sets of spectrophotometric chlorophyll equations for acetone,  
652 methanol and ethanol solvents. *Photosyn. Res.*, 89(1), 27-41. doi:10.1007/s11120-006-  
653 9065-9
- 654 Rognes, T., Flouri, T., Nichols, B., Quince, C., & Mahé, F. (2016). VSEARCH: a versatile  
655 open source tool for metagenomics. *PeerJ*, 4, e2584. doi:10.7717/peerj.2584
- 656 Roper, T., Creese, B., Scanes, P., Stephens, K., Williams, R., Dela-Cruz, J., Coade, G., Coates,  
657 B., & Fraser, M. (2011). *Assessing the condition of estuaries and coastal lake*  
658 *ecosystems in NSW*. Retrieved from  
659 <https://www.environment.nsw.gov.au/resources/soc/20110717EstuariesTRS.pdf>

1412  
1413  
1414  
1415  
1416  
1417  
1418  
1419  
1420  
1421  
1422  
1423  
1424  
1425  
1426  
1427  
1428  
1429  
1430  
1431  
1432  
1433  
1434  
1435  
1436  
1437  
1438  
1439  
1440  
1441  
1442  
1443  
1444  
1445  
1446  
1447  
1448  
1449  
1450  
1451  
1452  
1453  
1454  
1455  
1456  
1457  
1458  
1459  
1460  
1461  
1462  
1463  
1464  
1465  
1466  
1467  
1468  
1469  
1470  
1471

- 660 Roterman, Y. R., Benayahu, Y., Reshef, L., & Gophna, U. (2015). The gill microbiota of  
661 invasive and indigenous *Spondylus* oysters from the Mediterranean Sea and northern  
662 Red Sea. *Environ. Microbiol. Rep.*, 7(6), 860-867. doi:10.1111/1758-2229.12315
- 663 Roy, P. S., Williams, R. J., Jones, A. R., Yassini, I., Gibbs, P. J., Coates, B., West, R. J., Scanes,  
664 P. R., Hudson, J. P., & Nichol, S. (2001). Structure and function of South-east  
665 Australian estuaries. *Estuarine, Coastal and Shelf Science*, 53(3), 351-384.  
666 doi:10.1006/ecss.2001.0796
- 667 Rua, C. P. J., Trindade-Silva, A. E., Appolinario, L. R., Venas, T. M., Garcia, G. D., Carvalho,  
668 L. S., Lima, A., Kruger, R., Pereira, R. C., Berlinck, R. G. S., et al. (2014). Diversity  
669 and antimicrobial potential of culturable heterotrophic bacteria associated with the  
670 endemic marine sponge *Arenosclera brasiliensis*. *PeerJ*, 2, e419-e419.  
671 doi:10.7717/peerj.419
- 672 Rubio, A., Frances, J., Coad, P., Stubbs, J., & Guise, K. (2013). The onset and termination of  
673 the Qx disease window of infection in Sydney rock oyster (*Saccostrea glomerata*)  
674 cultivated in the Hawkesbury river, NSW, Australia. *J. Shellfish Res.*, 32(2), 483-496.  
675 doi:10.2983/035.032.0228
- 676 Saulnier, D., De Decker, S., Haffner, P., Cobret, L., Robert, M., & Garcia, C. (2010). A large-  
677 scale epidemiological study to identify bacteria pathogenic to Pacific oyster  
678 *Crassostrea gigas* and correlation between virulence and metalloprotease-like activity.  
679 *Microb. Ecol.*, 59(4), 787-798. doi:10.1007/s00248-009-9620-y
- 680 Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B.,  
681 Lesniewski, R. A., Oakley, B. B., Parks, D. H., Robinson, C. J., et al. (2009).  
682 Introducing mothur: Open-source, platform-independent, community-supported  
683 software for describing and comparing microbial communities. *Appl. Environ.*  
684 *Microbiol.*, 75(23), 7537-7541. doi:10.1128/AEM.01541-09
- 685 Schrobback, P., Pascoe, S., & Coglan, L. (2014). History, status and future of Australia's native  
686 Sydney rock oyster industry. *Aquat. Living Resour.*, 27(3-4), 153-165.  
687 doi:10.1051/alr/2014011
- 688 Sharp, K. H., Pratte, Z. A., Kerwin, A. H., Rotjan, R. D., & Stewart, F. J. (2017). Season, but  
689 not symbiont state, drives microbiome structure in the temperate coral *Astrangia*  
690 *poculata*. *Microbiome*, 5(1), 120. doi:10.1186/s40168-017-0329-8
- 691 Sugumar, G., Nakai, T., Hirata, Y., Matsubara, D., & Muroga, K. (1998). *Vibrio splendidus*  
692 biovar II as the causative agent of bacillary necrosis of Japanese oyster *Crassostrea*  
693 *gigas* larvae. *Dis. Aquat. Organ.*, 33(2), 111-118. doi:10.3354/dao033111

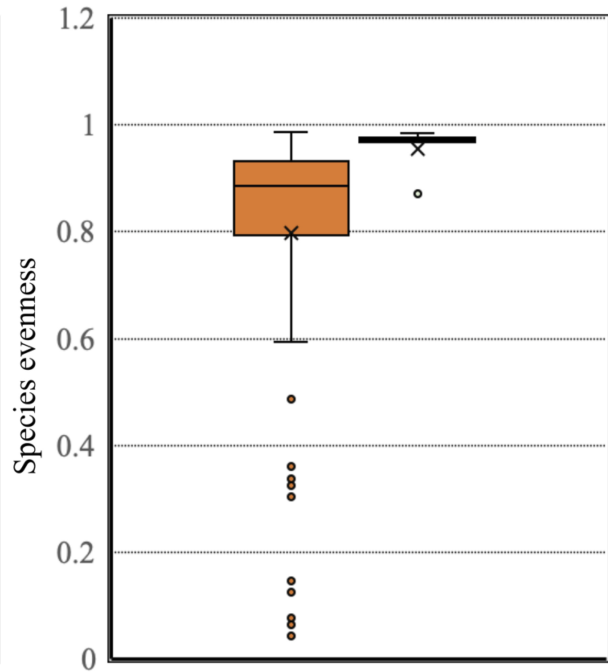
1472  
1473  
1474  
1475  
1476  
1477  
1478  
1479  
1480  
1481  
1482  
1483  
1484  
1485  
1486  
1487  
1488  
1489  
1490  
1491  
1492  
1493  
1494  
1495  
1496  
1497  
1498  
1499  
1500  
1501  
1502  
1503  
1504  
1505  
1506  
1507  
1508  
1509  
1510  
1511  
1512  
1513  
1514  
1515  
1516  
1517  
1518  
1519  
1520  
1521  
1522  
1523  
1524  
1525  
1526  
1527  
1528  
1529  
1530  
1531

- 694 Trabal, N., Mazón-Suástegui, J. M., Vázquez-Juárez, R., Asencio-Valle, F., Morales-  
695 Bojórquez, E., & Romero, J. (2012). Molecular analysis of bacterial microbiota  
696 associated with oysters (*Crassostrea gigas* and *Crassostrea corteziensis*) in different  
697 growth phases at two cultivation sites. *Microb. Ecol.*, *64*(2), 555-569.  
698 doi:10.1007/s00248-012-0039-5
- 699 Travers, M.-A., Boettcher Miller, K., Roque, A., & Friedman, C. S. (2015). Bacterial diseases  
700 in marine bivalves. *J. Invertebr. Pathol.*, *131*, 11-31. doi:10.1016/j.jip.2015.07.010
- 701 Turner, S., Pryer, K. M., Miao, V. P. W., & Palmer, J. D. (1999). Investigating deep  
702 phylogenetic relationships among Cyanobacteria and Plastids by small subunit rRNA  
703 sequence analysis. *J. Eukaryot. Microbiol.*, *46*(4), 327-338. doi:10.1111/j.1550-  
704 7408.1999.tb04612.x
- 705 van de Water, J. A. J. M., Voolstra, C. R., Rottier, C., Cocito, S., Peirano, A., Allemand, D., &  
706 Ferrier-Pagès, C. (2018). Seasonal stability in the microbiomes of temperate gorgonians  
707 and the Red coral *Corallium rubrum* across the Mediterranean sea. *Microb. Ecol.*,  
708 *75*(1), 274-288. doi:10.1007/s00248-017-1006-y
- 709 Wegner, K. M., Volkenborn, N., Peter, H., & Eiler, A. (2013). Disturbance induced decoupling  
710 between host genetics and composition of the associated microbiome. *BMC microbiol.*,  
711 *13*(1), 252.
- 712 Wolf, P. H. (1979). Life cycle and ecology of *Marteilia sydneyi* in the Australian oyster,  
713 *Crassostrea commercialis*. *Mar. Fish. Rev.*, *41*(1-2), 70 - 72.
- 714 Ziegler, M., Roik, A., Porter, A., Zubier, K., Mudarris, M. S., Ormond, R., & Voolstra, C. R.  
715 (2016). Coral microbial community dynamics in response to anthropogenic impacts  
716 near a major city in the central Red Sea. *Mar. Pollut. Bull.*, *105*(2), 629-640.  
717 doi:10.1016/j.marpolbul.2015.12.045
- 718 Zurel, D., Benayahu, Y., Or, A., Kovacs, A., & Gophna, U. (2011). Composition and dynamics  
719 of the gill microbiota of an invasive Indo-Pacific oyster in the eastern Mediterranean  
720 Sea. *Environ. Microbiol.*, *13*(6), 1467-1476. doi:10.1111/j.1462-2920.2011.02448.x



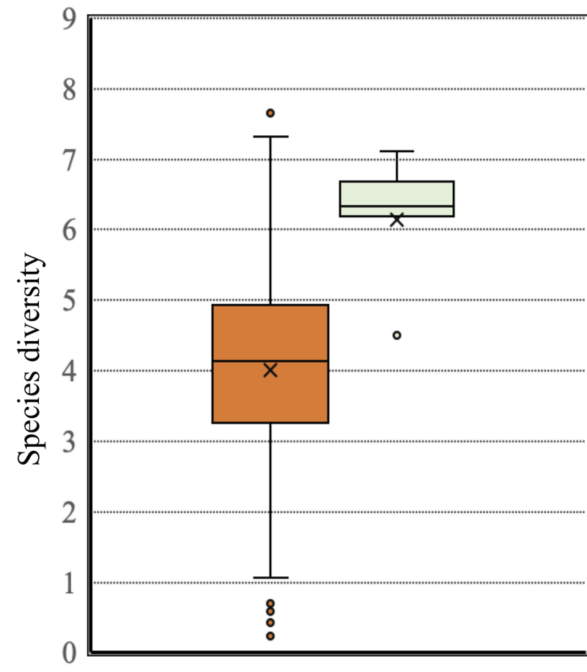
A

■ SRO ■ Seawater



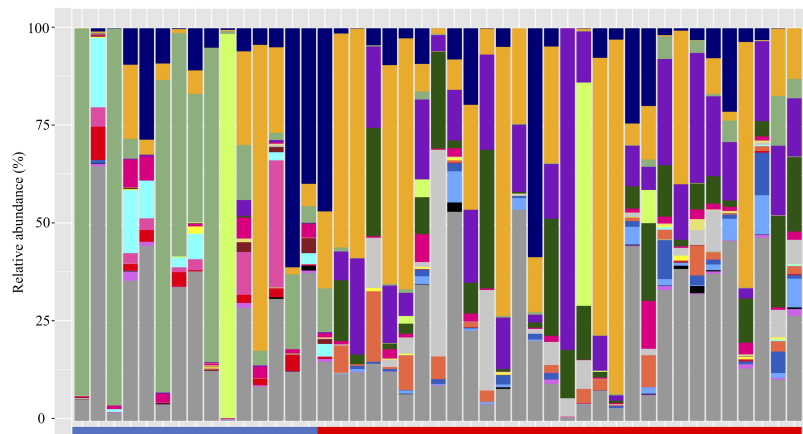
B

■ SRO ■ Seawater

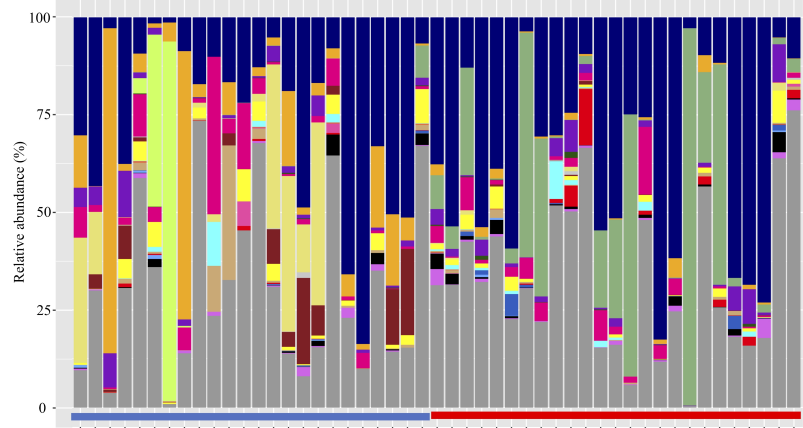


C

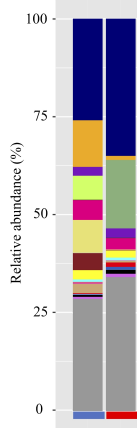
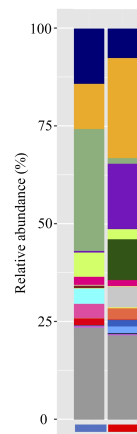
■ SRO ■ Seawater



Port Stephens

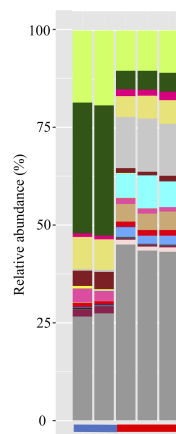


Wallis Lake

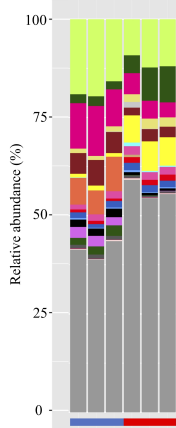


- Endozoicomonas
- Mycoplasma
- Candidatus Hepatoplasma
- Vibrio
- Arcobacter
- Pseudoalteromonas
- Borrelia
- Photobacterium
- Cobetia
- Pseudomonas
- Actibacter
- Aquibacter
- Marinomonas
- Algitalea
- Robiginitalea
- Maribacter
- Shewanella
- Prolixibacter
- Thalassolituus
- Polaribacter 2
- Others

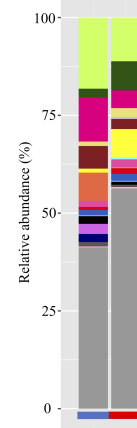
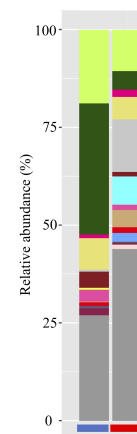
— January  
— June



Port Stephens



Wallis Lake

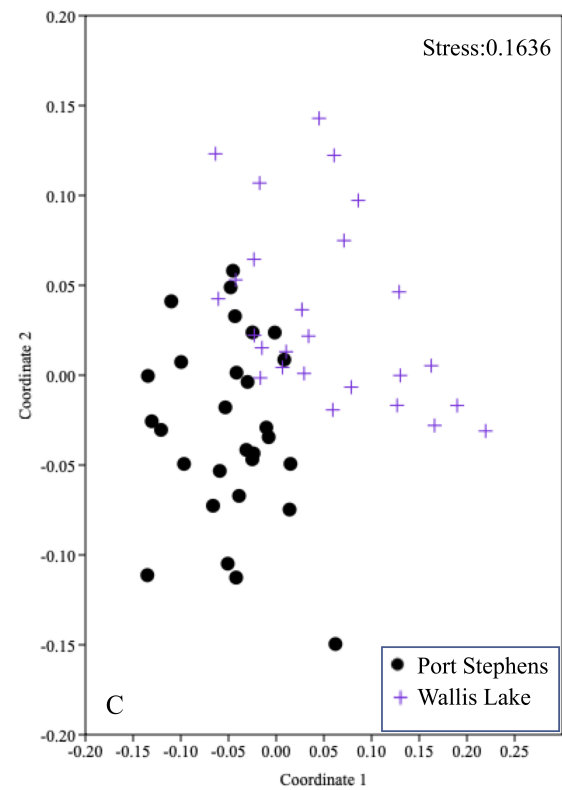
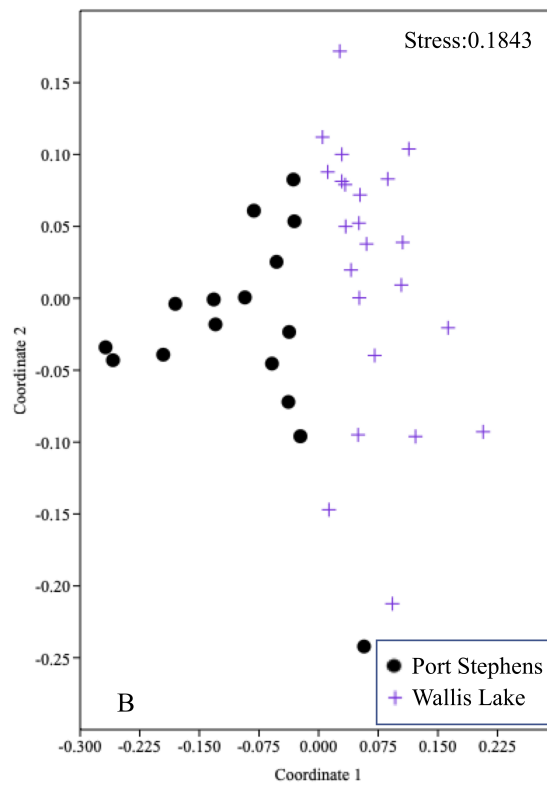
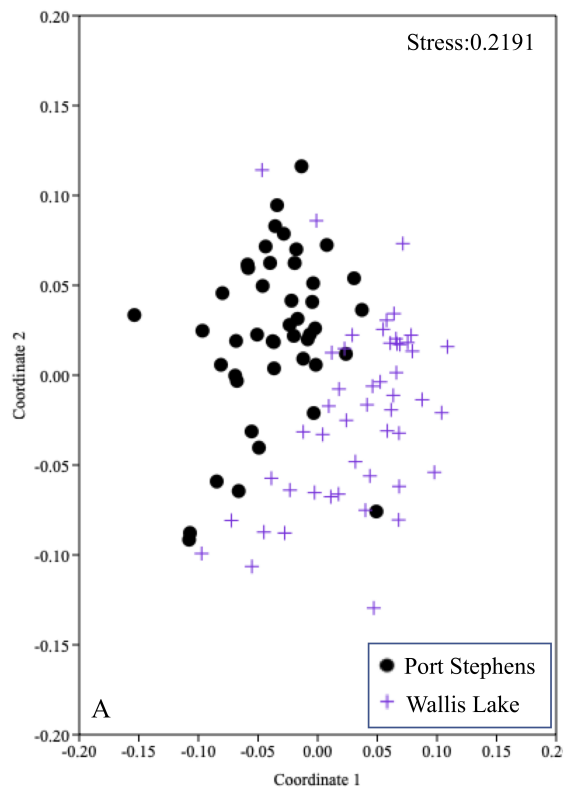


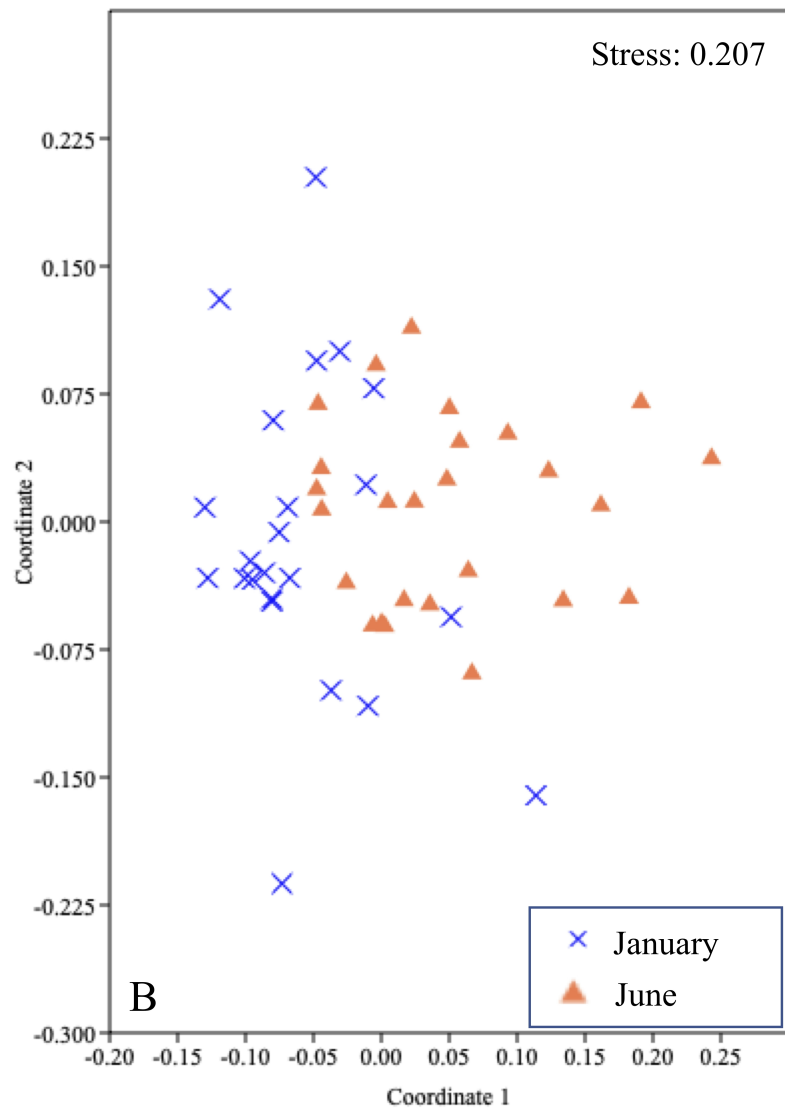
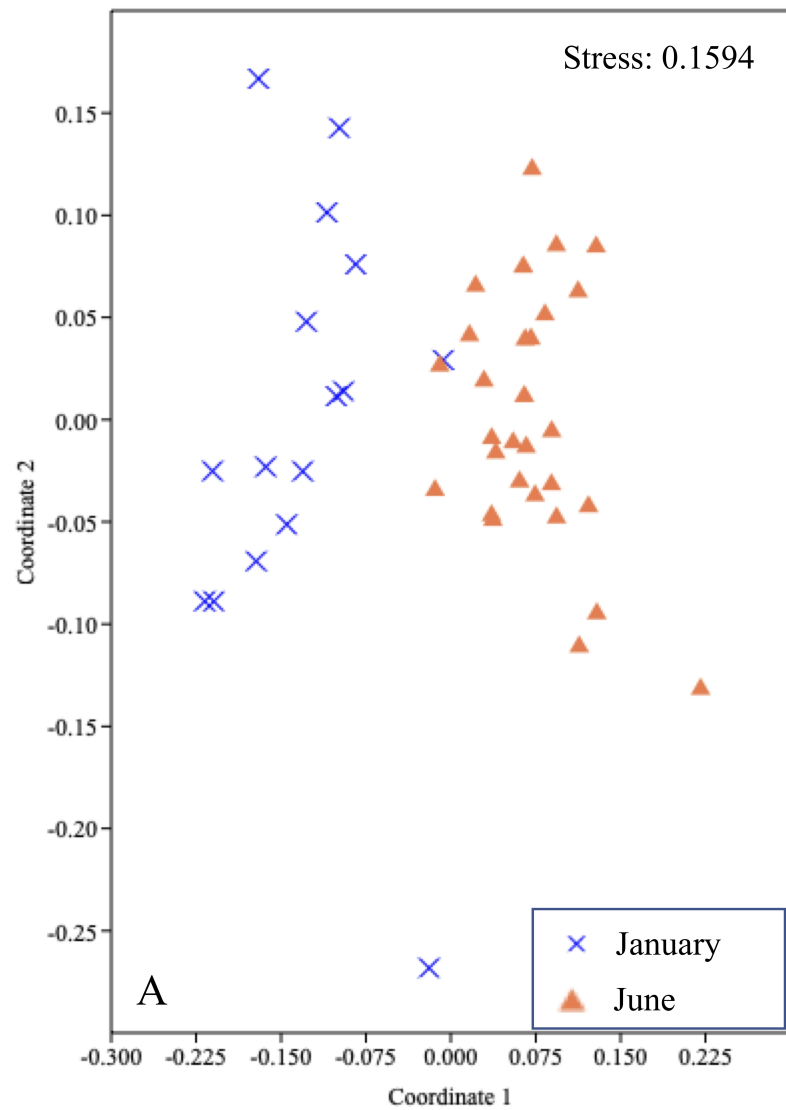
- NS5 marine group
- Candidatus Actinomarina
- Formosa
- OM43 clade
- NS3a marine group
- NS4 marine group
- NS2b marine group
- Marinobacterium
- Acholeplasma
- Litoricola
- BAL58 marine group
- OM60(NOR5) clade
- SAR92 clade
- Vibrio
- Polaribacter 2
- Polaribacter 4
- Photobacterium
- Fluviicola
- Pseudohongiella
- Marinomonas
- Others

— January  
— June

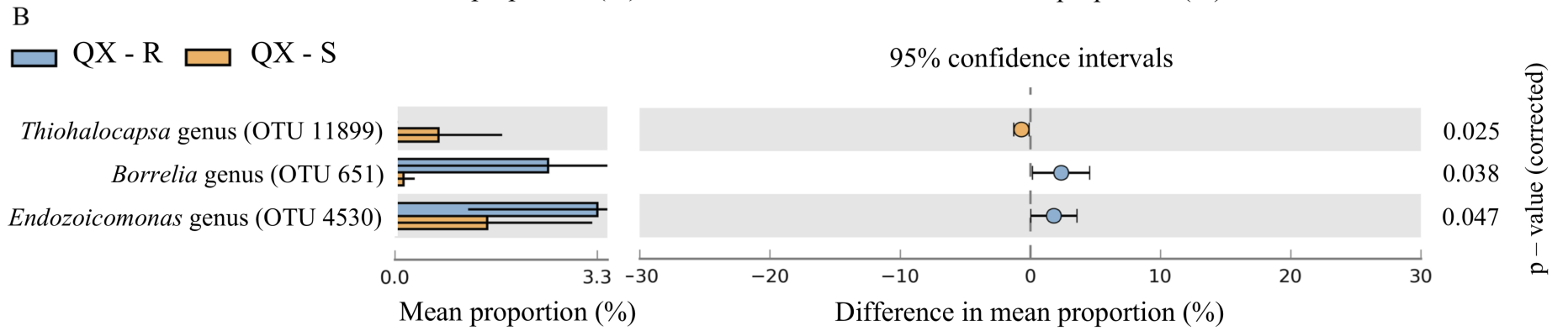
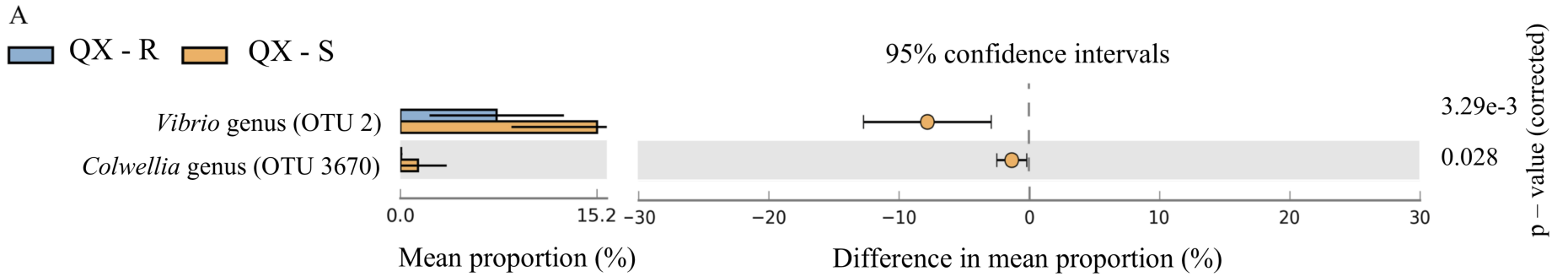
A

B









## 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

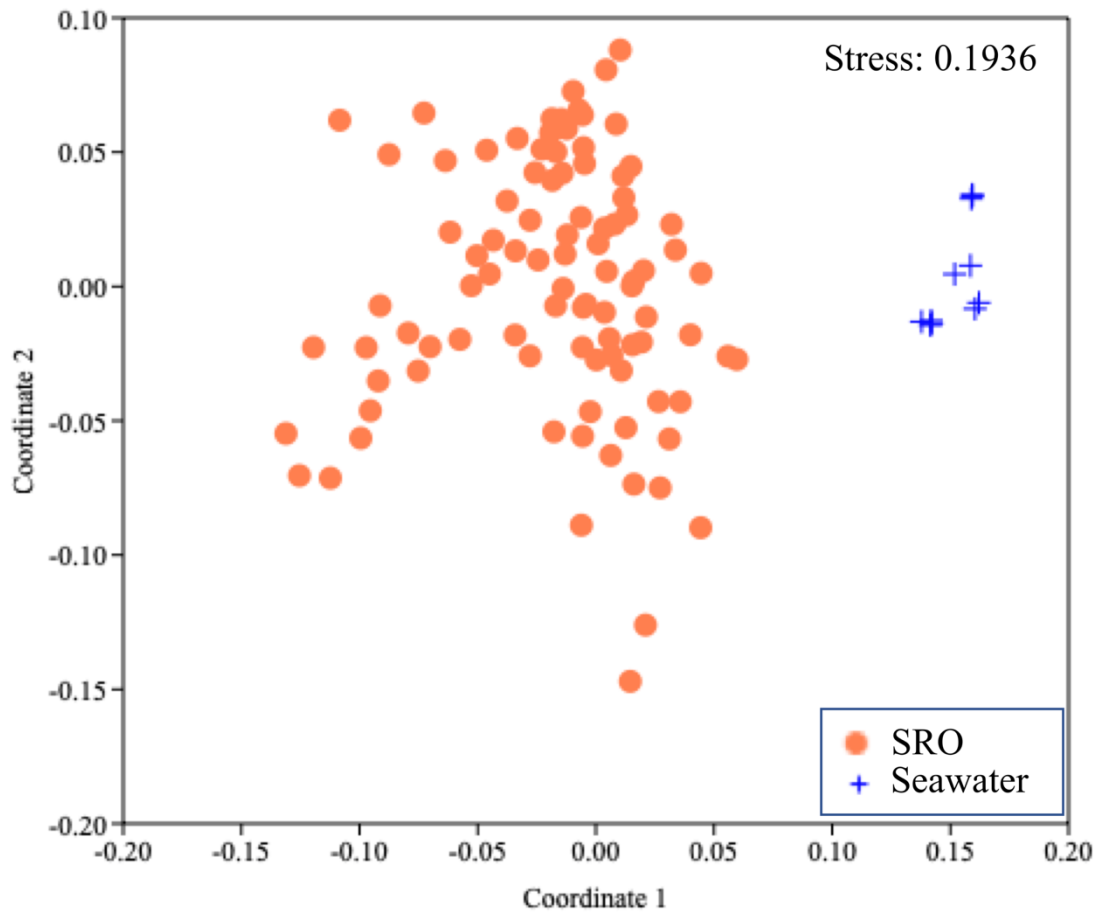
**Viet Khue Nguyen:** Formal analysis, Investigation, Writing – Original draft preparation, Visualization.  
**William L King:** Formal analysis, Writing – Original draft preparation. **Nachshon Siboni:** Investigation, Methodology, Writing – Reviewing and Editing. **Khandaker Rayhan Mahbub:** Investigation, 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).

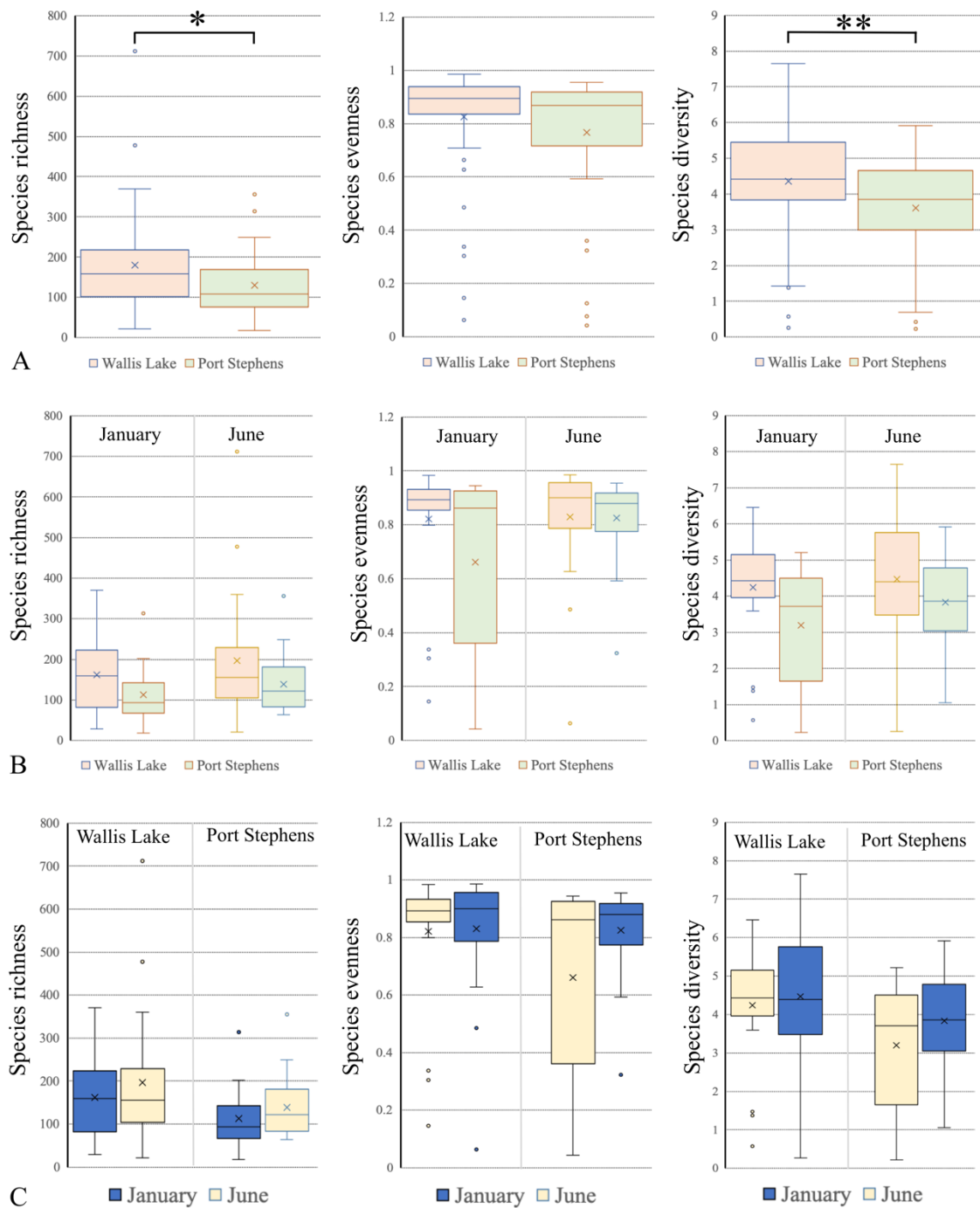
<b>Comparison</b>	<b>H</b>	<b>p-value</b>
Richness of SRO (n =94) vs seawater (n =11)	28.25	1.06E-07
Evenness of SRO (n =94) vs seawater (n =11)	15.64	7.65E-05
Diversity of SRO (n =94) vs seawater (n = 11)	20.06	7.52E-06



**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.

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

<b>Comparison</b>	<b>H</b>	<b>p-value</b>
<b>Location (January and June)</b>		
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
<b>Location (January)</b>		
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
<b>Location (June)</b>		
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
<b>Season in Wallis Lake</b>		
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
<b>Season in Port Stephens</b>		
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
<b>January and June</b>			
<i>Candidatus Hepatoplasma</i> genus (OTU 14887)	10.05	12	9.27
<i>Endozoicomonas</i> genus (OTU 1831)	4.859	0.0341	8.6
<i>Vibrio</i> genus (OTU 2)	4.309	7.7	0.73
<i>Endozoicomonas</i> genus (OTU 3829)	3.961	3.75	6.88
<i>Mycoplasma</i> genus (OTU 14900)	3.919	6.81	0.423
<i>Arcobacter</i> genus (OTU 6697)	3.611	3.79	2.87
<i>Pseudoalteromonas</i> genus (OTU 8917)	3.323	5.88	0.077
<i>Mycoplasma</i> genus (OTU 12669)	2.896	5.04	0.119
<i>Mycoplasma</i> genus (OTU 14921)	2.865	2.69	3.2
<i>Mycoplasma</i> genus (OTU 14937)	2.69	3.81	1.6
<b>January</b>			
<i>Candidatus Hepatoplasma</i> genus (OTU 14887)	17.66	31.2	0
<i>Arcobacter</i> genus (OTU 6697)	6.468	6.15	6.12
<i>Mycoplasma</i> genus (OTU 14921)	5.095	4.66	6.3
<i>Endozoicomonas</i> genus (OTU 3829)	4.732	5.89	7.02
<i>Endozoicomonas</i> genus (OTU 1831)	3.028	0	5.35
<i>Photobacterium</i> genus (OTU 3)	2.826	0.0871	4.87
<i>Endozoicomonas</i> genus (OTU 6283)	2.761	3.23	4.13
<i>Mycoplasma</i> genus (OTU 14937)	2.737	2.88	3.35
<i>Pseudomonas</i> genus (OTU 12985)	2.304	0.589	3.97
<i>Aquibacter</i> genus (OTU 12017)	2.054	3.63	0.153
<b>June</b>			
<i>Candidatus Hepatoplasma</i> genus (OTU 14887)	9.6	1.43	17.5
<i>Endozoicomonas</i> genus (OTU 1831)	6.38	0.0528	11.5
<i>Vibrio</i> genus (OTU 2)	6.115	11.9	1.31
<i>Mycoplasma</i> genus (OTU 14900)	5.283	9.45	0.218
<i>Pseudoalteromonas</i> genus (OTU 8917)	5.051	9.12	0.111
<i>Mycoplasma</i> genus (OTU 12669)	4.38	7.82	0.0338
<i>Endozoicomonas</i> genus (OTU 3829)	3.424	2.56	6.76
<i>Cobetia</i> genus (OTU 2869)	2.916	5.22	0.00536
<i>Mycoplasma</i> genus (OTU 14937)	2.432	4.32	0.0595
<i>Endozoicomonas</i> 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
<b>Wallis Lake</b>			
<i>Candidatus Hepatoplasma</i> genus (OTU 14887)	10.37	0	17.5
<i>Endozoicomonas</i> genus (OTU 1831)	8.142	5.35	11.5
<i>Endozoicomonas</i> genus (OTU 3829)	4.412	7.02	6.76
<i>Mycoplasma</i> genus (OTU 14921)	3.803	6.3	0.453
<i>Arcobacter</i> genus (OTU 6697)	3.623	6.12	0
<i>Photobacterium</i> genus (OTU 3)	2.96	4.87	0.0177
<i>Endozoicomonas</i> genus (OTU 6283)	2.756	4.13	3.78
<i>Pseudomonas</i> genus (OTU 12985)	2.382	3.97	0.0707
<i>Mycoplasma</i> genus (OTU 14937)	1.995	3.35	0.0595
<i>Endozoicomonas</i> genus (OTU 1993)	1.965	0	3.33
<b>Port Stephens</b>			
<i>Candidatus Hepatoplasma</i> genus (OTU 14887)	16.77	31.2	1.43
<i>Vibrio</i> genus (OTU 2)	6.575	0.00697	11.9
<i>Mycoplasma</i> genus (OTU 14900)	5.612	2.01	9.45
<i>Pseudoalteromonas</i> genus (OTU 8917)	5.008	0	9.12
<i>Arcobacter</i> genus (OTU 6697)	4.553	6.15	2.49
<i>Mycoplasma</i> genus (OTU 12669)	4.288	0	7.82
<i>Endozoicomonas</i> genus (OTU 3829)	3.401	5.89	2.56
<i>Mycoplasma</i> genus (OTU 14937)	3.171	2.88	4.32
<i>Cobetia</i> genus (OTU 2869)	2.854	0.0313	5.22
<i>Mycoplasma</i> 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).

<b>Comparison</b>	<b>H</b>	<b>p-value</b>
<b>Port Stephens in January</b>		
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
<b>Wallis Lake in January</b>		
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
<b>Port Stephens in June</b>		
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
<b>Wallis Lake in June</b>		
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.

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