Core Community Persistence Despite Dynamic Spatiotemporal Responses in the Associated Bacterial Communities of Farmed Pacific Oysters

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Core community persistence despite dynamic spatiotemporal responses in the associated bacterial communities of farmed Pacific oysters

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Abstract

A breakdown in host-bacteria relationships have been associated with the progression of a number of marine diseases and subsequent mortality events. For the Pacific oyster, *Crassostrea gigas,* Summer Mortality Syndrome (SMS) is one of the biggest constraints to growth of the sector and is set to expand into temperate systems as ocean temperatures rise. Currently, a lack of understanding of natural spatiotemporal dynamics of the host-bacteria relationship limits our ability to develop microbial-based monitoring approaches. Here, we characterised the associated bacterial community of C. *gigas,* at two Irish oyster farms, unaffected by SMS, over the course of a year. We found *C. gigas* harboured spatiotemporally variable bacterial communities that were distinct from bacterioplankton in surrounding seawater. Whilst the majority of bacteria-oyster associations were transient and highly variable, we observed clear patterns of stability in the form of a small core consisting of six persistent Amplicon Sequence Variants (ASVs). This core made up a disproportionately large contribution to sample abundance (34 ± 0.14 %), despite representing only 0.034% of species richness across the study, and have been associated with healthy oysters in other systems. Overall, our study demonstrates the consistent features of oyster bacterial communities across spatial and temporal scales and provides an ecologically meaningful baseline to track environmental change.

Introduction

Bacterial communities associated with animals and plants play an important role in mediating processes at the individual host and wider community and ecosystem scales [1–5]. In marine systems, the surfaces of host organisms are in direct contact with seawater, providing a constantly changing bacterial community [6]. As such, marine hosts often have thousands of bacteria associations that are extremely responsive across various spatial and temporal scales, which makes them challenging to understand [7]. Recent attempts to simplify this complexity have focussed around identifying signatures of stability through space and time [8–11]. Here, by identifying core stable taxa, it may be possible to define components of the community that are important for host function, whilst also facilitating comparisons across individuals, populations and ecological contexts [12, 13]. Such considerations are important for understanding and interpreting future disturbances and environmental changes [14, 15] and there is increasing interest in developing microbial indicators to assess coastal ecosystem health [16].

A range of stressors can disrupt host-bacterial relationships [10, 17–19], with wide-ranging implications for host performance, including enhanced susceptibility to pathogens [15]. Bivalve molluscs seem particularly vulnerable to stress-induced disease outbreaks, which often result in mass mortality events that can have serious and far-reaching ecological and economic ramifications [20, 21]. For example, Summer Mortality Syndrome (SMS) in the Pacific oyster, *Crassostrea gigas*, has become more common in recent decades and is an ever-increasing constraint to the expansion of the aquaculture sector [22, 23]. While the underlying mechanisms causing SMS are multifaceted and complex [24], the breakdown of the host-bacteria relationship has been frequently observed [23, 25–27]. However, microbial communities associated with oyster hosts are most often sampled when they are already in a state of dysbiosis, usually during a disease event [28]. This limits current ability to develop microbial-based monitoring approaches, as our understanding of natural seasonal dynamics (without periods of disease), are generally lacking (but see [29])

In the face of recent and projected ocean warming trends, a better understanding of host-bacteria relationships is even more pressing, given the increasing likelihood of disruption and detrimental host impacts [19, 27, 30, 31]. For *C. gigas*, warming can push previously safe areas into prevailing climates associated with SMS. Even in areas already affected, the additional stress burden imposed by more intense summer temperatures now elicits SMS with increasing frequency, intensity and without an obvious aetiological agent [27, 28, 32]. In the Northeast (NE) Atlantic, the Irish and Celtic Seas represent a transition zone for SMS (~ 19 °C summer SST). Mortality events here are infrequent but risk increase dramatically with decreasing latitude and increasing temperatures [33]. Therefore, warming trends and associated mortality events may threaten the expansion of the industry over the coming decades [34]. With this in mind, we characterised the host-bacterial relationship at two *C. gigas* farms in the Irish/Celtic Seas over the course of a year. In doing so, we aimed to i) establish important baselines against which dysbiosis can be measured and ii) examine patterns of spatiotemporal variability in bacterial communities to elucidate possible drivers of structure and richness.

Methods

Sampling Approach

Sampling took place at two commercial oyster farms in Ireland (Figure 1) (Bannow Bay and Cromane) in spring (March/May), summer (June/July/August) and winter (December) 2018. Increased sampling frequency was conducted during summer to capture shifts in bacterial communities during periods of thermal stress and potential dysbiosis. During each sampling event, eight oysters were randomly taken from trestles at low shore height and three one litre replicates of seawater were collected in sterile Nalgene bottles. Oysters and seawater were frozen at – 20 °C until DNA extraction.

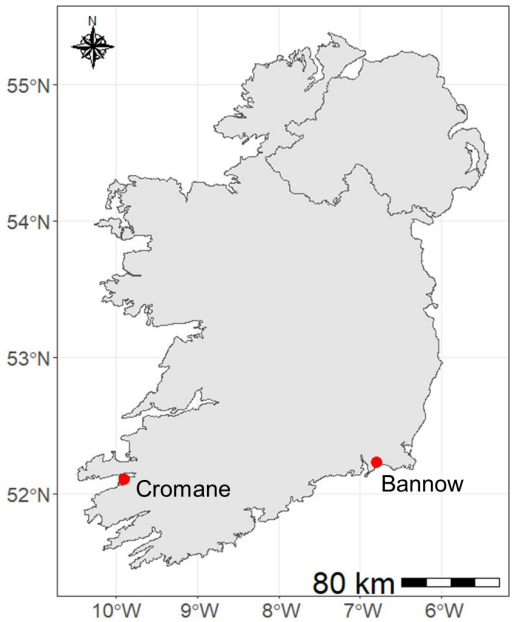

Figure 1. Locations of study sites for collection of seawater and Pacific oyster, *Crassostrea gigas*, samples in southeast and southwest Ireland

After defrosting, each oyster was shucked into a 50 ml falcon tube, mixed with equal volume (v:v) sterile artificial seawater and blended using a tissue homogeniser. Defrosted seawater was concentrated by filtering through a 0.22 µm nitrocellulose filter. DNA was extracted from one ml of oyster homogenate and whole seawater filters using Qiagen DNeasy Powersoil extraction kits, following the manufacturers instructions. DNA was then weighed using a qubit fluorimeter and re-suspended to 2 ng/µl.

Library preparation and sequencing of the V4 region of the 16S rDNA gene using primers (515f - GTGCCAGCMGCCGCGGTAA + 806r – GGACTACHVGGGTWTCTAAT) was conducted by StarSEQ (StarSEQ GmbH, Mainz, DE) following an optimised protocol of [35]. At least one negative PCR control was run on each plate and demonstrated runs were free from contamination.

Sequence processing

All processing and analysis was conducted in the r statistical environment. Paired-end reads were processed according to the BIOCONDUCTER workflow for microbiome data analysis [36]. Sequences were trimmed and truncated using the “filterAndTrim” function in DADA2 with the following parameters: truncLen, f= 240, r = 160; truncQ = 2; trimLeft, f = 20, r = 19, to

114 remove primers and low quality reads. Amplicon Sequence Variants (ASVs) were resolved
115 using DADA2 [36]. Chimeras (0.97% of sequences) were removed using the
116 “removeBimeraDenovo” function in DADA2. Sequence taxonomy was assigned using the
117 RDP naïve Bayesian classifier against the SILVA release 132 database [37] using the
118 “assignTaxonomy” function in DADA2. Sequence read counts, taxonomic assignments and
119 metadata were assembled as an object in the r package “”PHYLOSEQ” and was used in
120 downstream analysis [38]. Samples containing < 10,000 reads, taxa contributing < 0.01% of
121 the reads in the dataset and ASVs identified as mitochondria, chloroplast or Archaea were
122 then removed from the PHYLOSEQ object. Sequence counts were then expressed as relative
123 abundance (in proportion to the total sample count). Sequences are accessible through the
124 EMBL database (accession no. PRJEB52444). ASV table and metadata are available at
125 (https://figshare.com/s/b36ed8e1872f496d437a).

126 **Statistical Analysis**

127 After sequence processing three seawater samples had to be discarded from the dataset. Due
128 to this, replication was too low for some of the interaction terms between Sample Type, Month
129 and Season. As the focus of this study was to track the shifting oyster bacterial community
130 through time, we made initial comparisons between oyster and seawater samples as a single
131 dataset to determine overall differences between the two sample types. We then based
132 subsequent analyses on differences between sites and months solely on the oyster samples.
133 To account for differences in sequence depth between samples in alpha diversity estimates,
134 the dataset was rarefied to the minimum sample depth (12430 reads), using the
135 “rarefy_even_depth” function in PHYLOSEQ. Alpha diversity for each sample was estimated
136 through the Chao1 index [39] implemented through the “estimate_richness” function in
137 PHYLOSEQ. The Chao1 index estimates ASV richness, and the standard error surrounding
138 this estimate, based on the observed number of ASVs, the observed number of ASVs
139 occurring only once, and the observed number of ASVs occurring only twice [39]. Alpha
140 diversity was compared using a two-way Analysis of Variance (ANOVA). Model factors

141 consisted of Site (fixed factor; two levels: Bannow, Cromane) and Month (fixed factor; six
142 levels: March, May, June, July, August, December). Differences in community structure were
143 determined using PERMANOVA (Anderson, 2001) based on Bray-Curtis dissimilarity and
144 implemented through the "Adonis" function in the package "VEGAN" [40]. This analysis was
145 repeated across all taxonomic levels. *Post-hoc* pairwise comparisons were performed to
146 determine where the differences in community structure lay (at $p < 0.05$). Model design was
147 the same as that for alpha diversity. Differences in multivariate dispersion between
148 communities were examined using the "betadisper" function in "VEGAN". A similarity of
149 percentage (SIMPER) procedure was conducted to determine which taxa contributed the most
150 to any observed dissimilarities.

151 We defined core taxa at the ASV level and used a compositional dataset. There is no
152 consistent definition of a "core" in the literature with authors setting prevalence thresholds from
153 50 – 100 %. Here, we used a prevalence threshold of 80 % (of the total dataset with all months
154 and sites included).

155 Results

156 General Patterns

157 In total, we sampled bacterial communities from 95 oysters and 33 water samples, which
158 resulted in 4689972 paired end reads with an average coverage of 37519 reads per sample.
159 After processing, we identified 17533 ASVs from 70 Phyla, 188 Classes, 342 Orders, 659
160 Families and 1831 Genera. The most diverse phyla were Proteobacteria (aka
161 Pseudomonadota) (5153 ASVs), Planctomycetota (2805 ASVs), Bacteroidota (2607 ASVs),
162 Firmicutes (aka Bacillota) (1556 ASVs) and Verrucomicrobiota(1419 ASVs), which accounted
163 for 76% of all ASVs recorded. There were key differences in the phyla dominating the relative
164 abundances of the bacterial communities of oyster and seawater. Seawater samples were
165 dominated by Proetobacteria (52%) and Bacteroidota (23.2%) whereas oyster samples were

dominated by Firmicutes (32%), Proteobacteria (22.5%) and Spirochaetota (16.8%) (Figure 2).

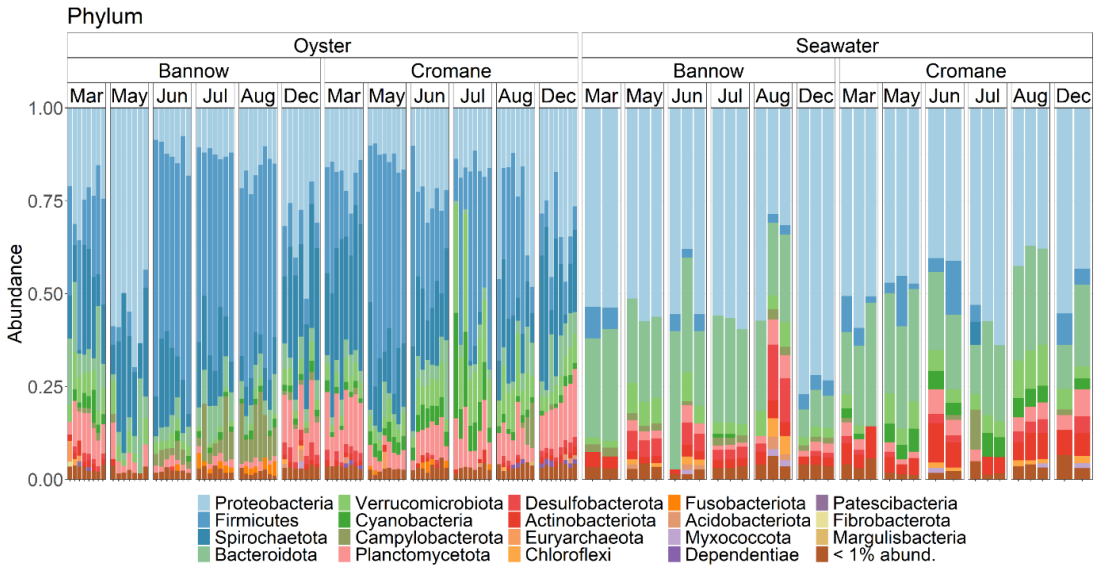

Figure 2. Relative abundance of bacterial phyla associated with the Pacific oyster, *Crassostrea gigas* and bacterioplankton (seawater) from two oyster farms (Cromane and Bannow Bay) in Ireland. Samples were taken over the course of one year (March – December 2018).

Alpha Diversity

Overall, alpha diversity (Chao1 index) ranged from 377 ± 15.4 in oysters to 413 ± 9.5 in seawater but there was no significant difference between the two sample types ($F_{(1, 123)} = 2.6$, $p = 0.14$). When oysters were analysed separately, alpha diversity differed by Month but there was no significant effect of Site or the interaction term (Table 1). *Post hoc* analysis showed that March (561 ± 27.9) and December (519 ± 24) were significantly greater than all other months (May - 258 ± 31, June - 311 ± 18, July - 309 ± 13 and August - 312 ± 23) but were similar to one another (Figure 3). Alpha diversity also differed significantly by Month in seawater samples ($F_{(5,27)} = 3.27$, $p = 0.02$). *Post hoc* analysis showed that the only significant difference was observed between March and May (Figure S1).

Table 1. Results of univariate (ANOVA) for alpha diversity and multivariate community structure (PERMANOVA) between Site, Month and their interaction.

	Alpha diversity (Chao1 index)			Multivariate structure (PERMANOVA)		
	df	F	p	df	Pseudo-F	p
Site	1	1.8	0.180	1	16.6	0.001
Month	5	30.2	<0.0001	5	8.1	0.001
Site*Month	5	1.6	0.17	5	7.5	0.001

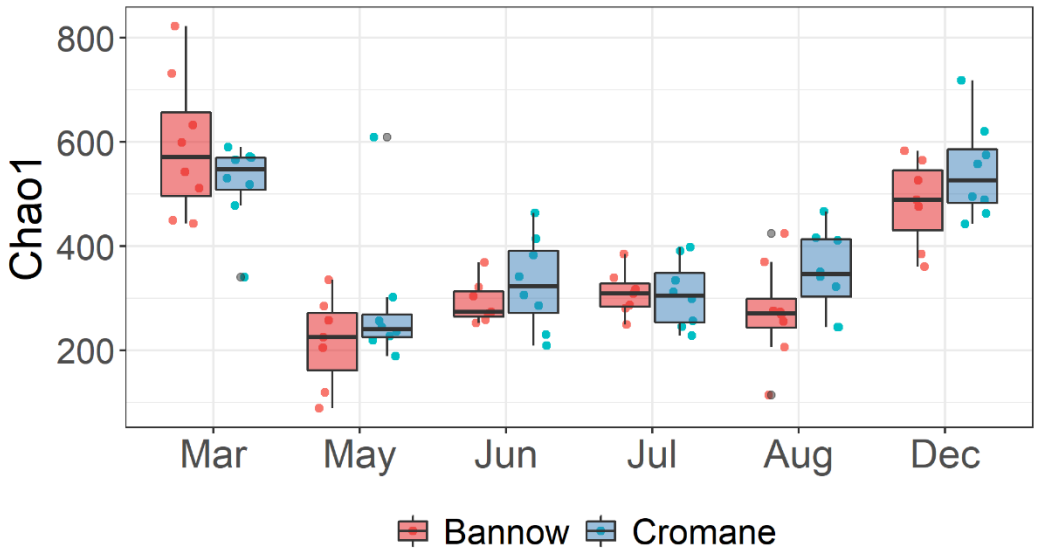

Figure 3. Box plots representing alpha diversity (Chao1 index) for bacterial communities associated with the oyster *Crassostrea gigas* from two oyster farms in Ireland. Site locations can be seen in Figure 1.

Shared ASVs

Out of the 17533 ASVs recorded across this study, 2014 ASVs (11.5%) were shared between seawater and oysters. The majority of ASVs were found in oysters, which hosted 12945 ASVs compared to 2574 ASVs recorded solely in seawater. Across the oyster samples, 2189 ASVs (15%) were shared between sites, and this was similar irrespective of the sampling month. Here, shared ASVs between sites ranged from 226 ASVs (7.6%) in August to 314 ASVs (12.8%) in June. A large component of the bacterial community was temporally very transient. Overall, only 275 ASVs (~ 2%) were found between all months and each month harboured a considerable unique portion of overall observed ASVs. This ranged from 1037 ASVs (7%) in June to 3625 ASVs (25.4 %) in March. In total, the number of ASVs that were only associated with a single sampling month accounted for 77.7% of all ASVs observed in oysters across the entire study.

Community Structure

Initial comparisons between oyster and seawater samples showed bacterial communities to be clearly differentiated (Figure S2), and further analysis focussed solely on oyster associated

205 communities. PERMDISP showed no significant differences in within-factor multivariate
206 dispersion for either Month ($F_{(5, 86)} = 2.24$, $p = 0.06$) or Site ($F_{(1, 90)} = 0.05$, $p = 0.81$). Bacterial
207 community structure exhibited a Month x Site interaction (Table 1) suggesting the magnitude
208 of difference between sites was not consistent between months or vice versa. This pattern
209 was evident when the dataset was aggregated to coarser taxonomic resolutions (Table S1).
210 *Post hoc* analysis showed all pairwise comparisons within this interaction term to be
211 significant. nMDS ordination showed a clear division between sites and differentiation between
212 March and December and all other months. SIMPER analysis revealed that this was largely
213 due to a shift in the most dominant taxa. In warmer months (May, June, July and August)
214 ASVs belonging to the genus *Mycoplasma* were far more abundant, while AS1 from the family
215 Spirochaetacae was consistently present in far lower abundance (Table S2). Together, these
216 ASVs accounted for up to 41% of observed dissimilarity between monthly comparisons. nMDS
217 ordination also showed communities at Bannow in May to be more structurally dissimilar than
218 those sampled in other warmer months (June, July and August), a pattern not observed at
219 Cromane. SIMPER analysis revealed that this was largely driven by a dominance (~ 45 %) of
220 ASV-7 from the genus *Pseudomonas* at Bannow during May.

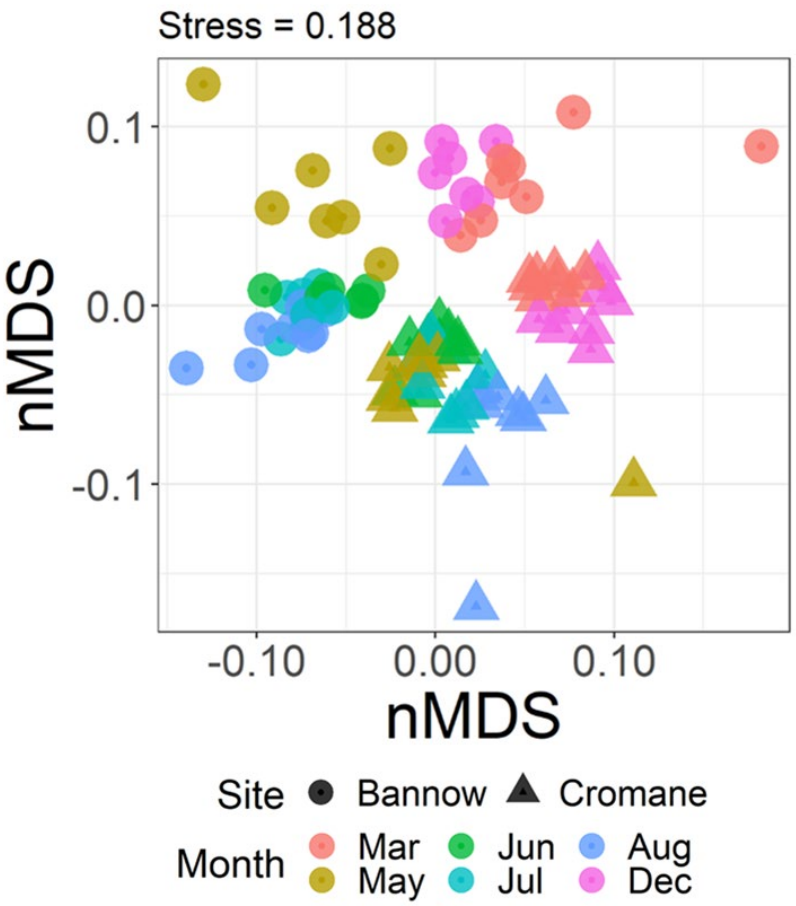

Figure 4. nMDS plots depicting the structure of bacterial communities associated with the Pacific oyster, *Crassostrea gigas* from oyster farms in Ireland. Data are based on Bray-Curtis similarity between untransformed relative abundance data.

Core community

We observed six ASVs that occurred in at least 80% of oyster samples: ASV1 (Family – Spirochaeyaceae), ASV2 & ASV4 (Genus - *Mycoplasma*), ASV22 (Genus - *Rubripirella*) ASV 37 (Family - Desulfocapsaceae) and ASV8 (Genus - *Synechoccus*) (Figure 5). Together, this 'core' contributed 34 ± 0.14 % to overall sample abundance, despite representing only 0.034 % of overall bacterial diversity found in oyster samples.

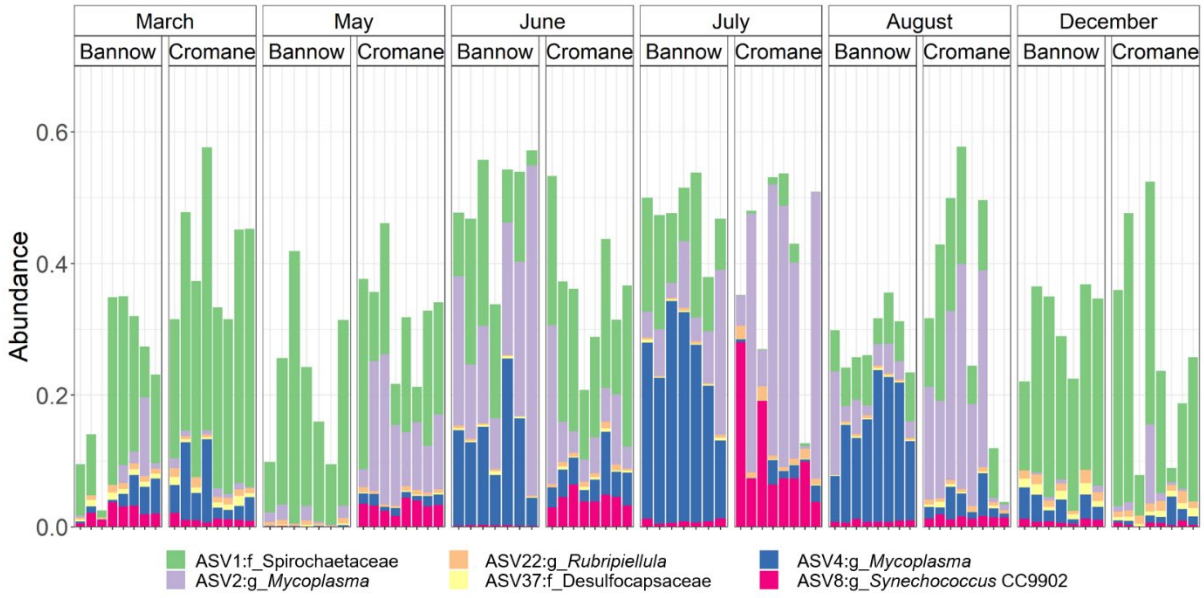

Figure 5. Relative abundance of the core bacteria community (defined at taxa present in > 80% of samples at a relative abundance > 0.1%) associated with the Pacific oyster, *Crassostrea* gigas. Abundance is expressed as proportion of entire sample.

236 ## Discussion

237 The host-bacteria relationship is important for the healthy functioning of benthic
238 organisms, but understanding the complex and dynamic nature of microbial communities
239 remains challenging. For the Pacific oyster, *Crassostrea gigas,* such understandings are
240 crucial given this species' vulnerability to disease outbreaks and subsequent dysbiosis of this
241 relationship. Here, we characterised the associated bacterial communities at two Irish, *C.*
242 *gigas* farms, over the course of a year. We found *C. gigas* harboured spatiotemporally variable
243 bacterial communities that were distinct from bacterioplankton in surrounding seawater.
244 However, despite high variation, we observed clear patterns of stability in the form of a small
245 core component that was persistent across space and time.

246 *Spatial structuring*

247 We found clear structuring between sites that was evident in every sampling period.
248 This is consistent with the spatial structuring observed in *C. gigas* [29] and Sydney Rock
249 Oyster, *Saccostrea glomerata* [41] in Australian farms and the eastern oyster, *Crassostrea*
250 *virginica,* across the east coast of the USA [42]. Whilst we do not have the necessary
251 environmental data to examine potential underlying mechanisms, a range of processes
252 operating over the geographic scale covered here (i.e. ~ 400 km) may be important drivers of
253 variability. These include deterministic factors such as temperature [43], pH [19], nutrient loads
254 [44] and sediment characteristics [45] or neutral processes (e.g. isolation) that facilitate
255 ecological drift [46]. The clear structuring between sites is in contrast to the high within-site
256 variability and small (albeit significant) site level differences observed between 'wild' *C. gigas*
257 populations in the north and south Wadden Sea [47]. Greater similarity open coast "wild"
258 Wadden Sea populations, compared to our estuarine sites, may be related to greater
259 connectivity, homogenising any selection pressures or drift at that spatial scale. Future studies
260 coupling high resolution *in situ* environmental data with microbial community structure are
261 needed to better understand mechanistic drivers of pattern.

262 *Temporal structuring*

263 The structure of bacterial communities associated with *C. gigas* varied markedly
264 across sampling periods. In particular, communities sampled in the cooler months (March and
265 December) were distinct and harboured greater diversity compared with those in the warmer
266 months (May, June, July and August). Patterns in alpha diversity mirrored the prevailing
267 patterns of the bacterioplankton (seawater), where greater mixing of the water column and
268 elevated coastal run off may elevate diversity in winter, as has been recorded in many
269 temperate seas [48–51]. The clear structuring between warm and cool months is similar to
270 that reported in the oysters, *C. virginica* [52] and *S. glomerata* [41]. A large proportion of
271 variation between months was consistently driven by ASV4 (genus - *Mycoplasma*) and ASV1
272 (family - *Spirochaetaceae*) that were also members of the core component (see below). These
273 taxa displayed clear seasonal dynamics with ASV4 overrepresented in warmer months and
274 ASV1 in cooler months. Differences between months was also driven by a transient
275 component of bacterial communities that was unique to any given sampling month. It is likely
276 that temporal structuring is driven by both stochastic effects associated with passively
277 acquiring ASVs from a dynamic and shifting planktonic community, as well as deterministic
278 processes imposed by the host on resident/core taxa.

279 *Core component*

280 Despite clear differences between site and sampling month, there was a small
281 temporally and spatially stable 'core', which contributed disproportionately to overall sample
282 abundance. Many of these core taxa have also been observed in *C. gigas* from other systems
283 [29, 47, 53]. For example, taxa from the Spirochaetaceae family were found across six
284 estuaries spanning ~ 500km along Australia's east coast and the genera's *Mycoplasma* and
285 *Synechococcus* were consistently found across 12 sites in Port Stevens estuary [28, 29].
286 Moreover, they have also been observed in other oyster species around the world [41, 44, 52,
287 54, 55] and may, therefore, represent part of a consistent core component for oysters
288 generally. Core taxa are hypothesised to be associated with a "healthy" microbiome and may

289 be critically important to the host [9, 11]. Whilst the role they play for function of the host
290 remains largely unknown, they are consistently associated with healthy (when compared to
291 diseased) individuals. Lasa et al. (2019) [26] compared healthy *C. gigas* to those symptomatic
292 with SMS during mortality events in populations across Europe. They found taxa within our
293 persistent core (*Mycoplasma*, *Synechococcus* and Spirochaetaceae) to dominate healthy
294 (non-infected) individuals, suggesting a role in oyster health and fitness. Similarly, a decrease
295 in *Mycoplasma* has been associated with infection of the protozoan, *Martelia sydneyi,* in the
296 Sydney rock oyster, *S. glomerata* [56] and a reduction in the wider class Mollicutes in eastern
297 oysters, *C. virginica* infected with the avleolate, *Perkinsus marinus* [55]. However, further
298 studies incorporating other hosts and a greater understanding of the functional profiles of
299 these core taxa is required before the ubiquity and utility of this core can be determined.

300 *Conclusion*

301 In summary, we identified stable and variable features of the host-bacteria relationship
302 of Pacific oysters, which with their extensive introduced distribution (> 50 countries) and
303 commercial dominance in many regions, are perhaps the world's most globalised bivalve.
304 Microbial communities are increasingly recognised for their role in mediating host resilience to
305 environmental perturbations and there is increasing interest in developing microbial indicators
306 to assess ecosystem health. Importantly, no mortalities associated with SMS were reported
307 by farms during the study, which means these communities represent "healthy" and "normal"
308 baselines. This represents a crucial first step towards identification of microbial indicators to
309 assess the health of oyster farms. Future studies may build upon this and document how the
310 breakdown of this relationship may impact host condition. This may lead to robust microbial
311 indicators in response to a range of climatic and local stressors.

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Author Contribution

NGK and SKM conceived the designed the study. JT conducted all laboratory work. RB and AA conducted all fieldwork. NGK lead the manuscript preparation and all authors contributed equally to subsequent edits. All authors read and approved the final manuscript

Data Accessibility

Sequences are accessible through the EMBL database (accession no. PRJEB52444). ASV table and metadata are available at (https://figshare.com/s/b36ed8e1872f496d437a)

Statements and Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical Declaration No approval of research ethics committees was required to accomplish the goals of this study.

337

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Supplementary Information

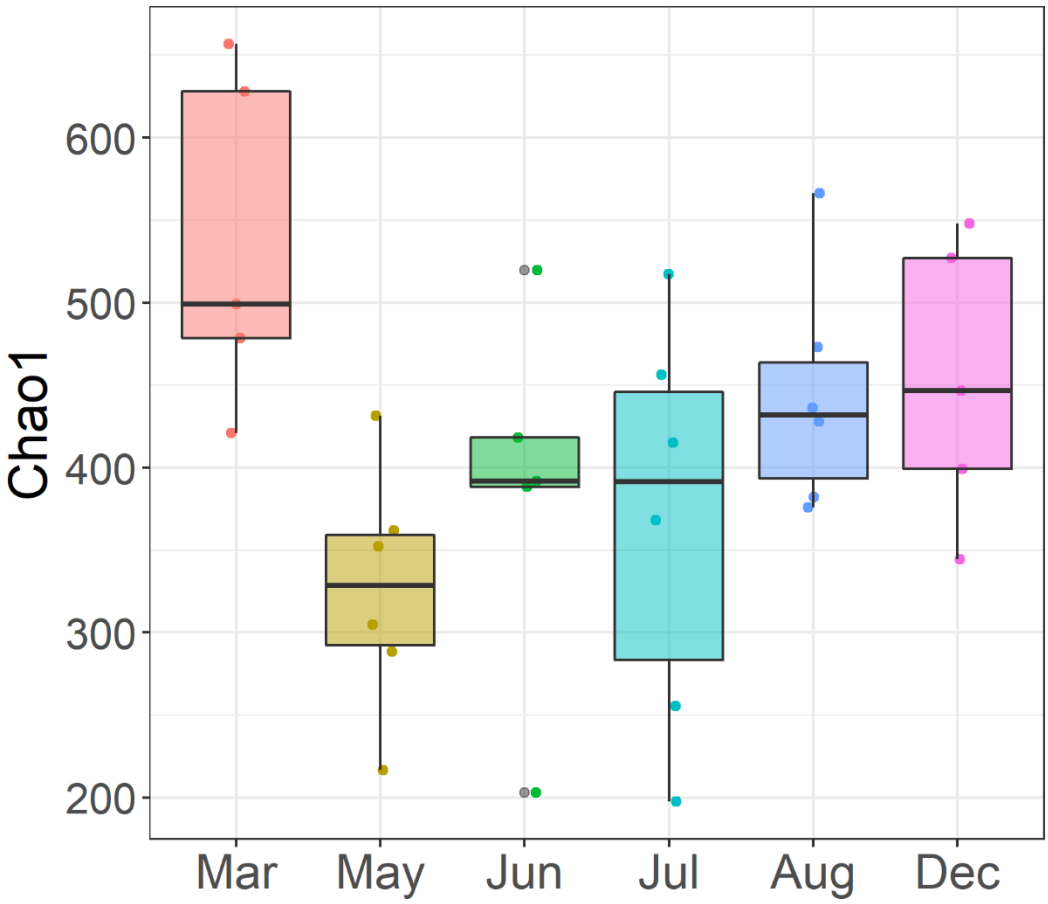

Figure S1. Box plots representing alpha diversity (Chao1 index) for bacterial communities of bacterioplankton from two *Crassostrea gigas* farms in Ireland. Site locations can be seen in Figure 1.

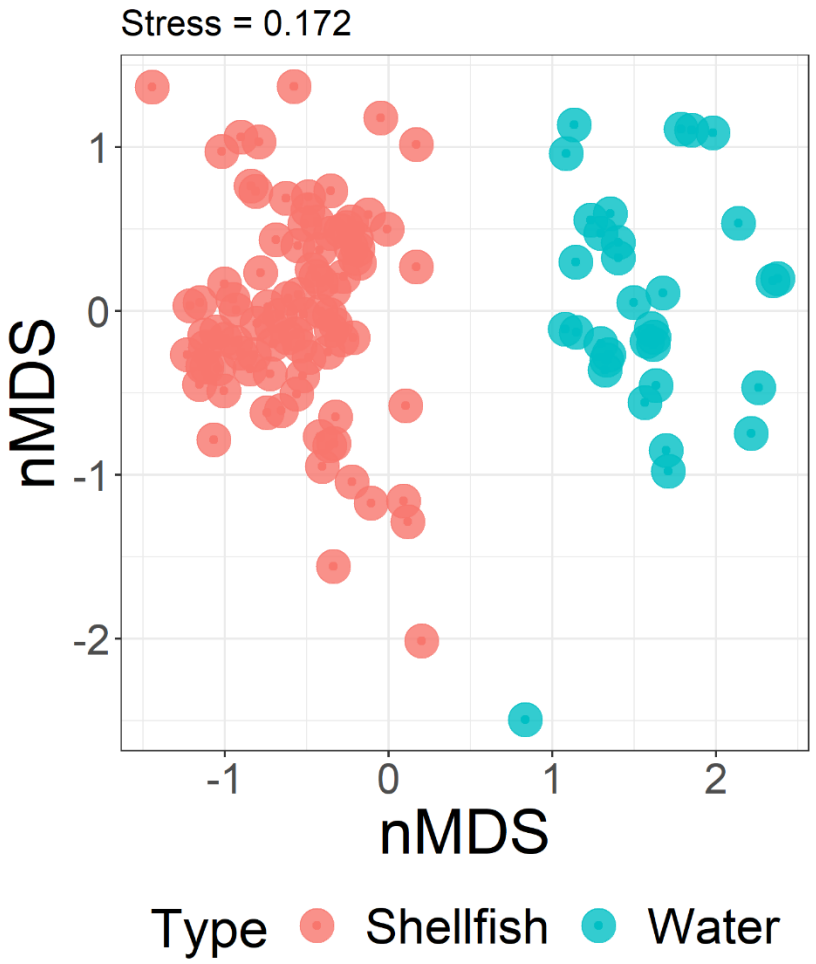

Figure S2. nMDS plots depicting Bray-Curtis dissimilarity between bacterial communities associated with the Pacific oyster, *Crassostrea gigas* and bacterioplankton. Sampling locations can be seen in Figure 1.

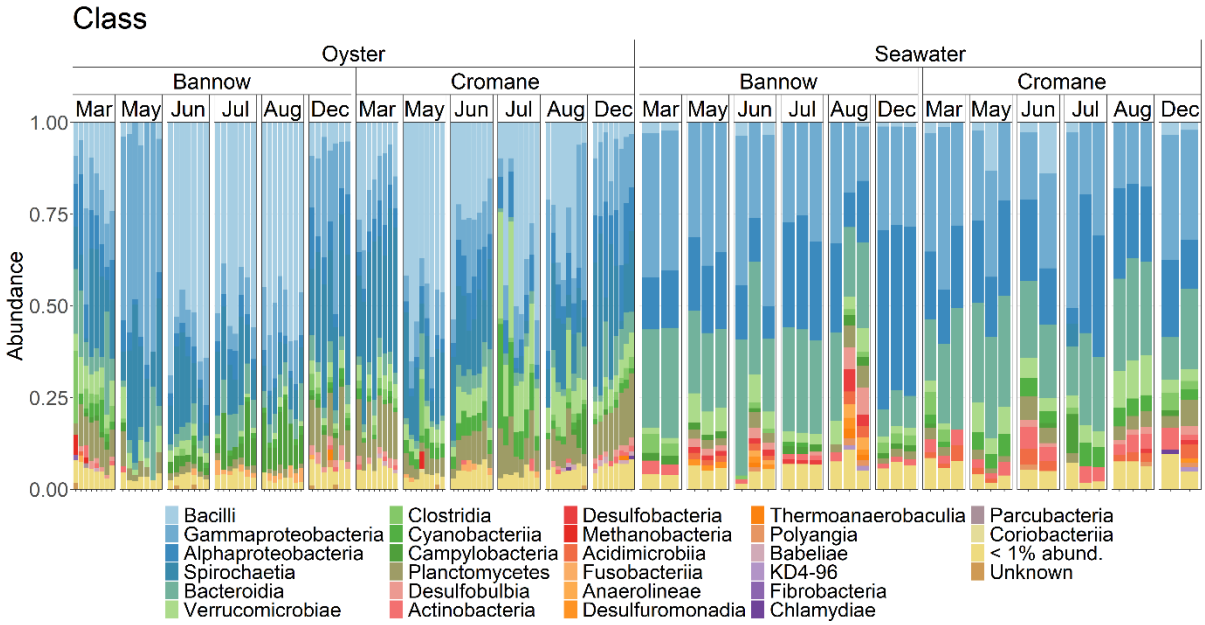

Figure S3. Relative abundance of bacterial classes associated with the Pacific oyster, *Crassostrea gigas* and bacterioplankton (seawater) from two oyster farms (Cromane and Bannow Bay) in Ireland. Samples were taken over the course of one year (March – December 2018). Locations of oyster farms can be seen in Figure 1.

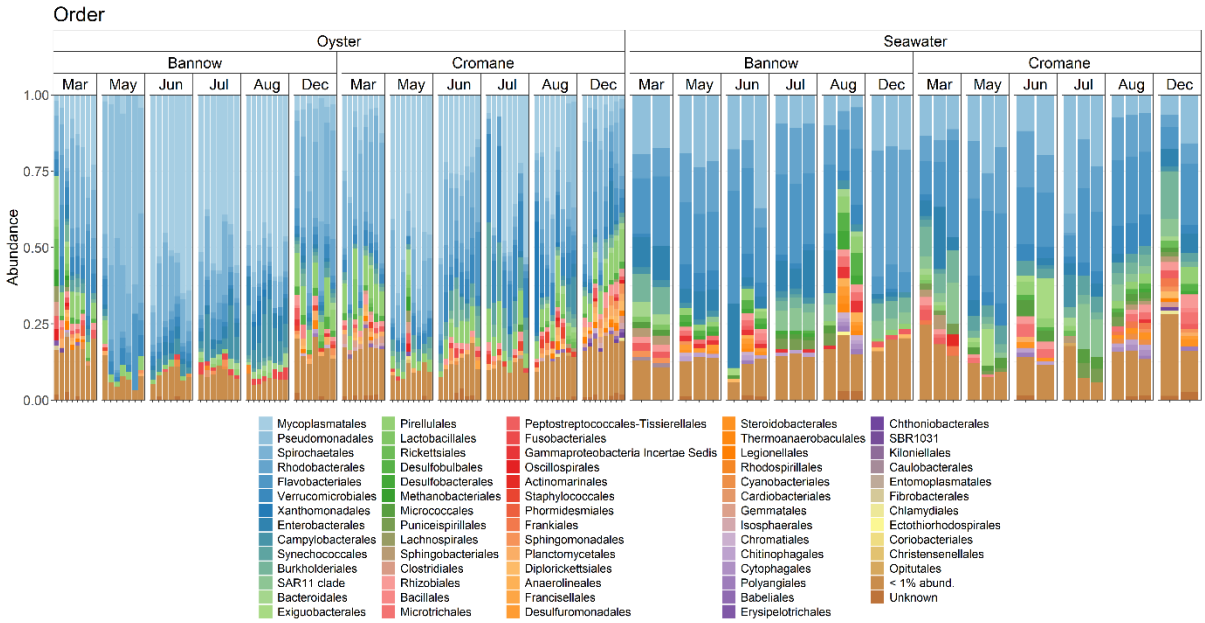

Figure S4. Relative abundance of bacterial orders associated with the Pacific oyster, *Crassostrea gigas* and bacterioplankton (seawater) from two oyster farms (Cromane and Bannow Bay) in Ireland. Samples were taken over the course of one year (March – December 2018). Locations of oyster farms can be seen in Figure 1.

554 **Table S1.** Results of multivariate community structure (PERMANOVA) between Sites and Sampling months in the
555 Pacific oyster *Crassostrea gigas*. Analysis is based on Bray-Curtis dissimilarity and was performed on data
556 agglomerated back to the taxonomic ranks of class, order and family.

		Phylum		Class		Order		Family	
	df	Pseudo-F	p	Pseudo-F	p	Pseudo-F	p	Pseudo-F	p
Site	1	15.6	<0.001	15.6	<0.001	16.2	<0.001	15.0	<0.001
Month	5	19.4	<0.001	17.9	<0.001	16.2	<0.001	15.8	<0.001
Site*Month	5	17.7	<0.001	14.3	<0.001	11.9	<0.001	11.8	<0.001

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558 **Table S2**. SIMPER analysis on significant pairwise comparisons identified by PERMANOVA analysis. Presented
559 taxa represent those that contributed to 50% of observed dissimilarity between comparisons. Letter represents
560 lowest resolved taxonomic rank C = Class, F = Family, G = Genus

Contrast	average	sd	ratio	ava	avb	cumsum	p	best_hit
December vs June								
	0.15	0.09	1.73	0.05	0.34	0.23	0.01	ASV2:g__*Mycoplasma*
	0.07	0.04	1.52	0.26	0.17	0.34	0.37	ASV1:f__Spirochaetaceae
	0.04	0.04	0.98	0.00	0.07	0.39	0.91	ASV5:f__Mycoplasmataceae
	0.03	0.05	0.56	0.06	0.01	0.44	0.04	ASV27:g__*Psychrobacter*
	0.01	0.02	0.61	0.03	0.02	0.46	0.04	ASV67:f__Rhodobacteraceae
	0.01	0.01	1.33	0.01	0.02	0.48	0.76	ASV8:g__*Synechococcus* CC9902
	0.01	0.01	0.75	0.01	0.02	0.50	0.16	ASV26:g__*Polynucleobacter*
December vs July								
	0.18	0.09	1.99	0.05	0.39	0.24	0.01	ASV2:g__*Mycoplasma*
	0.10	0.06	1.69	0.26	0.08	0.38	0.01	ASV1:f__Spirochaetaceae
	0.04	0.04	0.95	0.00	0.08	0.43	0.01	ASV20:g__*Roseibacillus*
	0.03	0.06	0.53	0.06	0.00	0.47	0.03	ASV27:g__*Psychrobacter*
December vs August								
	0.13	0.07	2.01	0.05	0.30	0.19	0.02	ASV2:g__*Mycoplasma*
	0.08	0.05	1.71	0.26	0.11	0.30	0.01	ASV1:f__Spirochaetaceae
	0.03	0.04	0.77	0.00	0.06	0.35	1.00	ASV5:f__Mycoplasmataceae
	0.03	0.05	0.54	0.06	0.01	0.39	0.05	ASV27:g__*Psychrobacter*
	0.02	0.04	0.38	0.00	0.03	0.41	0.01	ASV47:g__*Stenotrophomonas*
	0.01	0.02	0.84	0.00	0.03	0.43	0.01	ASV74:g__*Persicirhabdus*
	0.01	0.02	0.62	0.03	0.01	0.45	0.01	ASV67:f__Rhodobacteraceae
	0.01	0.02	0.70	0.01	0.02	0.47	0.01	ASV66:g__*Vibrio*
	0.01	0.02	0.76	0.00	0.03	0.49	0.07	ASV24:l*ekithochrous*
December vs March								
	0.07	0.05	1.39	0.26	0.24	0.13	0.28	ASV1:f__Spirochaetaceae
	0.03	0.04	0.98	0.00	0.07	0.20	0.91	ASV5:f__Mycoplasmataceae
	0.03	0.05	0.69	0.06	0.03	0.27	0.01	ASV27:g__*Psychrobacter*
	0.03	0.02	1.06	0.05	0.07	0.32	1.00	ASV2:g__*Mycoplasma*
	0.01	0.02	0.61	0.03	0.01	0.34	0.01	ASV67:f__Rhodobacteraceae
	0.01	0.01	0.72	0.02	0.00	0.36	0.01	ASV56:g__*Halomonas*
	0.01	0.02	0.31	0.01	0.00	0.37	0.42	ASV66:g__*Vibrio*
	0.01	0.01	1.08	0.01	0.01	0.38	0.83	ASV26:g__*Polynucleobacter*

0.01	0.02	0.32	0.00	0.01	0.39	0.28	ASV63:g__*Polaribacter*
0.01	0.00	1.25	0.01	0.02	0.40	1.00	ASV8:g__*Synechococcus* CC9902
0.01	0.01	0.39	0.00	0.01	0.41	0.22	ASV72:g__*Wolbachia*
0.01	0.00	1.26	0.01	0.01	0.42	0.02	ASV201:*algae*
0.00	0.00	1.21	0.02	0.02	0.43	0.98	ASV73:g__*Blastopirellula*
0.00	0.01	0.35	0.00	0.01	0.44	0.19	ASV111:g__*Streptococcus*
0.00	0.00	1.35	0.01	0.01	0.45	0.01	ASV328:f__Gemmataceae
0.00	0.01	0.64	0.01	0.00	0.46	0.02	ASV128:g__*Neptunomonas*
0.00	0.00	1.24	0.02	0.01	0.47	0.42	ASV37:f__Desulfocapsaceae
0.00	0.00	1.32	0.02	0.02	0.47	0.97	ASV123:f__Pirellulaceae
0.00	0.00	1.39	0.01	0.01	0.48	0.84	ASV244:f__DEV007
0.00	0.00	1.31	0.02	0.01	0.48	0.81	ASV22:g__*Rubripirellula*
0.00	0.01	0.57	0.00	0.01	0.49	0.09	ASV380:f__Flavobacteriaceae
0.00	0.00	1.36	0.01	0.01	0.50	0.08	ASV1663:g__*Legionella*
December vs May							
0.11	0.12	0.92	0.00	0.21	0.16	0.01	ASV7:g__*Pseudomonas*
0.08	0.08	0.97	0.00	0.16	0.28	0.01	ASV5:f__Mycoplasmataceae
0.07	0.05	1.50	0.26	0.19	0.39	0.21	ASV1:f__Spirochaetaceae
0.05	0.04	1.18	0.05	0.11	0.46	1.00	ASV2:g__*Mycoplasma*
June vs July							
0.10	0.07	1.42	0.34	0.39	0.20	0.50	ASV2:g__*Mycoplasma*
0.06	0.04	1.61	0.17	0.08	0.32	0.77	ASV1:f__Spirochaetaceae
0.04	0.04	0.96	0.01	0.08	0.39	0.01	ASV20:g__*Roseibacillus*
0.04	0.04	0.99	0.07	0.02	0.47	0.86	ASV5:f__Mycoplasmataceae
June vs August							
0.09	0.06	1.46	0.34	0.30	0.18	0.96	ASV2:g__*Mycoplasma*
0.04	0.03	1.56	0.17	0.11	0.26	1.00	ASV1:f__Spirochaetaceae
0.04	0.04	1.14	0.07	0.06	0.34	0.73	ASV5:f__Mycoplasmataceae
0.02	0.04	0.38	0.00	0.03	0.37	0.02	ASV47:g__*Stenotrophomonas*
0.01	0.01	0.94	0.01	0.03	0.40	0.01	ASV74:g__*Persicirhabdus*
0.01	0.02	0.78	0.00	0.03	0.43	0.04	ASV24:*lekithochrous*
0.01	0.03	0.38	0.00	0.02	0.45	0.06	ASV19:g__*Endozoicomonas*
0.01	0.01	1.81	0.02	0.01	0.47	0.69	ASV8:g__*Synechococcus* CC9902
0.01	0.01	0.92	0.01	0.02	0.49	0.01	ASV44:f__Helicobacteraceae
June vs March							
0.14	0.09	1.60	0.34	0.07	0.23	0.01	ASV2:g__*Mycoplasma*
0.06	0.04	1.49	0.17	0.24	0.34	0.52	ASV1:f__Spirochaetaceae
0.04	0.03	1.19	0.07	0.07	0.41	0.83	ASV5:f__Mycoplasmataceae
0.02	0.01	1.08	0.01	0.03	0.43	0.68	ASV27:g__*Psychrobacter*
0.01	0.02	0.73	0.02	0.01	0.45	0.09	ASV26:g__*Polynucleobacter*
0.01	0.01	1.66	0.02	0.02	0.47	0.70	ASV8:g__*Synechococcus* CC9902
0.01	0.00	1.60	0.01	0.02	0.48	0.05	ASV73:g__*Blastopirellula*
0.01	0.02	0.39	0.01	0.01	0.49	0.12	ASV63:g__*Polaribacter*
June vs May							
0.12	0.08	1.50	0.34	0.11	0.21	0.04	ASV2:g__*Mycoplasma*

0.11	0.12	0.92	0.00	0.21	0.39	0.01	ASV7:g__*Pseudomonas*
July vs August							
0.10	0.07	1.38	0.39	0.30	0.18	0.69	ASV2:g__*Mycoplasma*
0.04	0.03	1.53	0.08	0.11	0.25	1.00	ASV1:f__Spirochaetaceae
0.04	0.04	0.99	0.08	0.01	0.33	0.01	ASV20:g__*Roseibacillus*
0.03	0.04	0.82	0.02	0.06	0.39	0.86	ASV5:f__Mycoplasmataceae
0.03	0.04	0.76	0.06	0.01	0.44	0.01	ASV8:g__*Synechococcus* CC9902
0.02	0.02	0.88	0.02	0.03	0.47	0.01	ASV24:*lekithochrous*
July vs March							
0.17	0.08	2.00	0.39	0.07	0.24	0.01	ASV2:g__*Mycoplasma*
0.09	0.06	1.61	0.08	0.24	0.37	0.01	ASV1:f__Spirochaetaceae
0.04	0.04	0.96	0.08	0.00	0.42	0.01	ASV20:g__*Roseibacillus*
0.04	0.04	1.00	0.02	0.07	0.47	0.87	ASV5:f__Mycoplasmataceae
July vs May							
0.16	0.08	1.96	0.39	0.11	0.22	0.01	ASV2:g__*Mycoplasma*
0.11	0.11	0.93	0.01	0.21	0.37	0.01	ASV7:g__*Pseudomonas*
0.08	0.08	1.00	0.02	0.16	0.48	0.01	ASV5:f__Mycoplasmataceae
August vs March							
0.12	0.06	1.94	0.30	0.07	0.18	0.03	ASV2:g__*Mycoplasma*
0.08	0.05	1.56	0.11	0.24	0.30	0.02	ASV1:f__Spirochaetaceae
0.04	0.04	1.14	0.06	0.07	0.36	0.80	ASV5:f__Mycoplasmataceae
0.02	0.04	0.38	0.03	0.00	0.39	0.01	ASV47:g__*Stenotrophomonas*
0.01	0.01	1.07	0.01	0.03	0.41	0.65	ASV27:g__*Psychrobacter*
0.01	0.02	0.84	0.03	0.00	0.43	0.01	ASV74:g__*Persicirhabdus*
0.01	0.02	0.76	0.03	0.00	0.45	0.04	ASV24:*lekithochrous*
0.01	0.03	0.39	0.02	0.00	0.47	0.02	ASV19:g__*Endozoicomonas*
0.01	0.01	0.79	0.02	0.00	0.48	0.01	ASV44:f__Helicobacteraceae
0.01	0.01	1.10	0.02	0.00	0.49	0.27	ASV66:g__*Vibrio*
August vs May							
0.11	0.06	1.72	0.30	0.11	0.16	0.34	ASV2:g__*Mycoplasma*
0.11	0.12	0.91	0.00	0.21	0.32	0.01	ASV7:g__*Pseudomonas*
0.08	0.07	1.15	0.06	0.16	0.44	0.01	ASV5:f__Mycoplasmataceae
March vs May							
0.11	0.12	0.92	0.00	0.21	0.17	0.01	ASV7:g__*Pseudomonas*
0.08	0.06	1.22	0.07	0.16	0.30	0.01	ASV5:f__Mycoplasmataceae
0.07	0.05	1.45	0.24	0.19	0.41	0.29	ASV1:f__Spirochaetaceae
0.05	0.04	1.29	0.07	0.11	0.48	1.00	ASV2:g__*Mycoplasma*
Bannow vs Cromane							
0.11	0.08	1.38	0.25	0.18	0.17	0.01	ASV2:g__*Mycoplasma*
0.06	0.05	1.37	0.17	0.18	0.27	0.77	ASV1:f__Spirochaetaceae
0.05	0.05	0.86	0.05	0.07	0.35	0.73	ASV5:f__Mycoplasmataceae
0.04	0.08	0.45	0.07	0.00	0.41	0.02	ASV7:g__*Pseudomonas*
0.02	0.03	0.57	0.04	0.01	0.44	0.01	ASV27:g__*Psychrobacter*
0.02	0.02	0.65	0.01	0.04	0.47	0.01	ASV8:g__*Synechococcus* CC9902
0.01	0.03	0.49	0.00	0.03	0.49	0.04	ASV20:g__*Roseibacillus*