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Latitudinal Dynamics of *Vibrio* along the Eastern Coastline of Australia

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Abstract: The marine genus of bacteria, *Vibrio*, includes several significant human and animal pathogens, highlighting the importance of defining the factors that govern their occurrence in the environment. To determine what controls large-scale spatial patterns among this genus, we examined the abundance and diversity of *Vibrio* communities along a 4000 km latitudinal gradient spanning the Australian coast. We used a *Vibrio*-specific amplicon sequencing assay to define *Vibrio* community diversity, as well as quantitative PCR and digital droplet PCR to identify patterns in the abundances of the human pathogens *V. cholera*, *V. parahaemolyticus* and *V. vulnificus*. The *hsp60* amplicon sequencing analysis revealed significant differences in the composition of tropical and temperate *Vibrio* communities. Over 50% of *Vibrio* species detected, including the human pathogens *V. parahaemolyticus* and *V. vulnificus*, displayed significant correlations with either temperature, salinity, or both, as well as different species of phytoplankton. High levels of *V. parahaemolyticus* and *V. vulnificus* were detected in the tropical site at Darwin and the subtropical Gold Coast site, along with high levels of *V. parahaemolyticus* at the subtropical Sydney site. This study has revealed the key ecological determinants and latitudinal patterns in the abundance and diversity of coastal *Vibrio* communities, including insights into the distribution of human pathogens, within a region experiencing significant ecological shifts due to climate change.

Keywords: *Vibrio* ecology; *Vibrio* pathogens; microbial ecology; water quality

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1. Introduction

Understanding the distributional dynamics of a species or a community of organisms is pivotal to understanding their ecology, and how they may respond to environmental perturbations [1]. Across a wide range of organisms, species diversity and abundance patterns have been shown to be variable across latitudinal gradients, with many terrestrial and aquatic organisms displaying highest diversity levels at low latitudes [2–4]. Importantly, there is also growing evidence showing that latitudinal range shifts in multiple species are occurring as a consequence of climate change [5].

Latitudinal gradients have been shown to exist among microorganisms in both terrestrial [6] and aquatic environments [7,8], with two ecological processes often used to explain these patterns. The first of these is that diversity and productivity increase together because larger numbers of organisms are supported by larger rates of resource supply [9]. The second is that temperature and diversity increase together, as temperature impacts biological kinetics and therefore the rate of ecological processes such as speciation, ultimately resulting in an increase in diversity [10,11].

Previous studies (e.g., [12,13]) have demonstrated that marine bacterial species richness is correlated with temperature and inversely correlated with latitude. In addition to shaping the diversity of entire bacterial communities, latitude and temperature have

been shown to be critical determinants of the distributions of keystone marine bacteria, including numerically abundant groups such as the *SAR11* clade [11] and dominant primary producers, including the *Cyanobacteria* genera *Synechococcus* and *Prochlorococcus* [14].

Within coastal and estuarine ecosystems, members of the *Vibrio* genus, which can be particle associated [15], or free-living [16], are notable members of natural bacterial assemblages because of their often-important ecological relationships with marine animals, which can span mutualistic [17] to parasitic interactions with a wide diversity of species, including fish [18], corals [19], and shellfish [20]. In addition to impacting natural marine communities, diseases caused by *Vibrio* species are also particularly detrimental to the aquaculture sector, where they globally cause hundreds of millions of dollars in losses across a broad range of industries such as a USD 44 billion production loss to the shrimp industry between 2010 and 2016 [21], a USD 7.4 million production loss to the Malaysian seabass industry in the 1990s [22] and a substantial impact on the salmon industry in the 1990s [23].

In addition to impacting marine species and ecosystems, several *Vibrio* species are notable human pathogens. For example, *V. cholerae* causes the severe gastrointestinal disease cholera, which globally results in over 2.8 million cases and up to 95,000 deaths per year [24]. *V. parahaemolyticus* is a major cause of seafood poisoning, causing severe and sometimes fatal gastrointestinal disease in up to 30,000 people per year [25]. Infections by *V. vulnificus* can follow consumption of seafood as well, but can also result from direct exposure to seawater, and can be particularly dangerous, with up to a 50% mortality rate among infected individuals [26]. Notably, there are indications that the global distributions and seasonal dynamics of some of these pathogenic *Vibrio* species are shifting as a consequence of climate change [27–29], with evidence for poleward latitudinal range shifts [28].

A suite of environmental factors have been demonstrated to influence *Vibrio* abundance and diversity. *Vibrio* abundance has been shown to increase with nutrients such as phosphate [30], but in contrast decrease with increasing levels of nitrogen, dissolved oxygen, and pH [31]. The most widely observed determinants of *Vibrio* abundance, however, are salinity, temperature, and chlorophyll [29,30,32–37], with moderate salinity levels and elevated temperatures often leading to increased *Vibrio* abundances [32,37–40]. However, the environmental determinants and optimum conditions vary across *Vibrio* species [40]. For instance, *V. cholerae*, *V. vulnificus* and *V. parahaemolyticus* are generally more abundant within warm waters where the temperature exceeds 20 °C and salinity is below 10 ppt [40]. This is particularly relevant as climate change is increasing sea surface temperatures (SST), as well as increasing the frequency of severe rainfall events [41], which can result in decreased salinity in coastal environments. Together, these shifting environmental conditions have the potential to create ideal conditions for *Vibrio* blooms in the environment.

In light of the substantial environmental and human health importance of *Vibrio* species, we aimed to determine the patterns of abundance and diversity of the *Vibrio* community during the Australian summer using the high throughput *hsp60* gene amplicon sequencing assay to characterise the *Vibrio* community across a large latitudinal transect spanning the entire eastern coastline of Australia, one of the largest latitudinal *Vibrio* ecology gradients studied to date. Previous studies have detected high levels of *Vibrio*, including the human pathogens *V. cholerae*, *V. vulnificus* and *V. parahaemolyticus* in tropical [42] and subtropical [43] Australian environments during summer months when water temperature is highest, but neither of these studies have investigated *Vibrio* ecology on such a large scale. This is particularly relevant within Australia, where there is evidence that climate change is increasing seawater temperatures more rapidly than in any other part of the southern hemisphere [44], making this region a climate change hotspot, and a potential location for substantial increases in the abundance of pathogenic *Vibrio* species.

2. Methods

2.1. Sampling Locations and Protocol

Water samples were collected once each from 28 coastal sites along a latitudinal gradient spanning Australia's eastern coast, from Darwin (12°22′42.8″ S, 130°50′30.8″ E)

to Hobart (42°51′5.7″ S, 147°31′16.2″ E) (Figure 1, Supplementary Table S1) in a sampling campaign conducted during the Australian summer (December 2017–January 2018) over a period of 48 days. To consider the impacts of salinity and the common occurrence of *Vibrio* in estuarine environments, at each location, both a river (R-Site) and a beach (B-Site) sample were collected, except for at Rockhampton (23°15′17.8″ S, 150°49′44.5″ E), where both samples were taken from the beach due to a lack of river access at the time of sampling, and Bundaberg (24°45′41.8″ S, 152°23′14.6″ E), where both samples were taken from the river due to no beach access at the time of sampling.

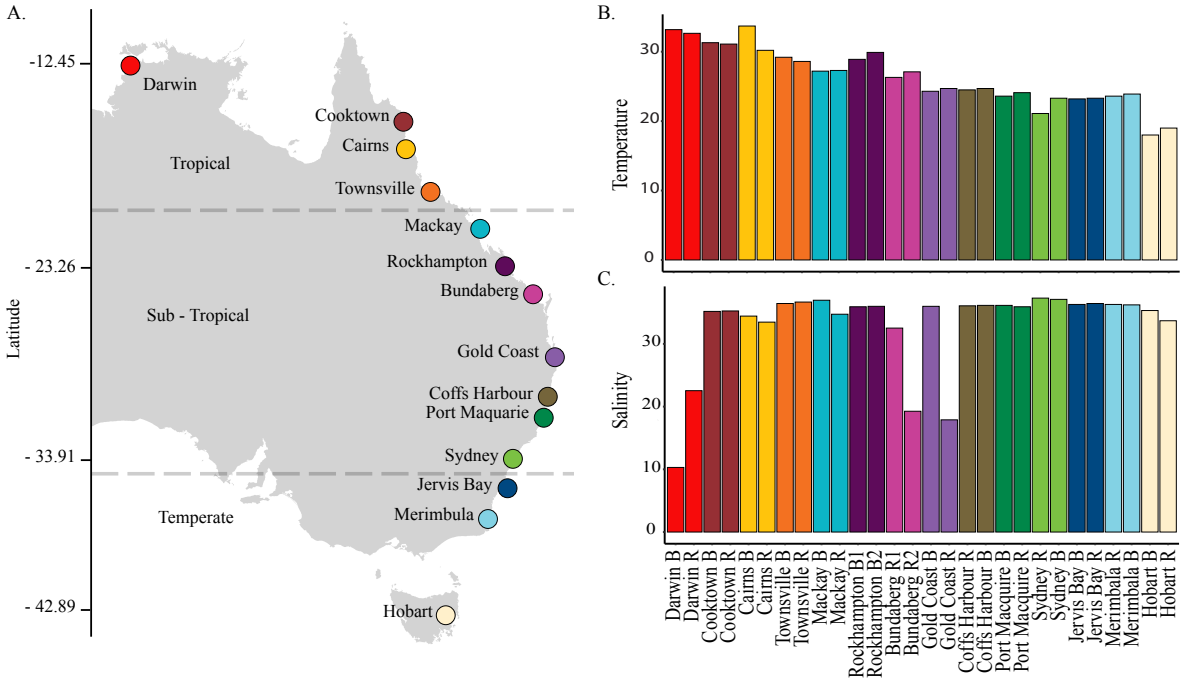

Figure 1. (**A**) Map of sampling locations. At each location, a sample was taken at the beach and in the river with the exception of Rockhampton where both samples were beach sites and Bundaberg where both sites were river sites. (**B**) Water temperature at each site in °C. (**C**) Salinity at each site in ppt.

At each sampling site, 10 L of water was collected from the surface in tinted, plastic drums, which had been pre-rinsed with bleach and then miliQ water and then rinsed three times on site prior to sampling. Physiochemical parameters including temperature, dissolved oxygen, salinity and pH were measured in situ using a WTW multiprobe meter (Multi3430, Wertheim, Germany). Triplicate water samples were filtered through 0.22 µm pore size, 47 mm diameter filters (Millipore, DURAPORE PVDF. 22UM WH PL) using a peristaltic pump within 3 h of collection, with the volume of sample filtered recorded to allow for normalisation of gene copy numbers. Filters were immediately placed in dry ice, where they remained for the duration of the sampling trip. Upon returning to the lab, samples were stored at −80 °C until processing.

2.2. DNA Extraction

Microbial DNA was extracted from filters using the PowerWater DNA isolation Kit (QIAGEN) in accordance with the manufacturer's instructions. DNA quantity and purity were then evaluated using a Nanodrop-1000 Spectrophotometer.

2.3. Quantitative PCR (qPCR)

Total bacterial community abundance, as well as the abundance of the total *Vibrio* community were measured using quantitative PCR (qPCR). Total bacterial abundance was quantified using the BACT1369F and PROK1492R 16S primer pair [45], while total

Vibrio community abundance was quantified using the primer pair Vib1-f and Vib2-r [46], which targets the *Vibrio*-specific 16S rRNA gene. To quantify levels of *V. parahaemolyticus* we targeted the thermostable direct hemolysin (tdhS) gene [47] and to quantify levels of *V. vulnificus*, we targeted the *V. vulnificus* hemolysin (*vvhA*) gene [43]. To quantify *V. cholerae*, we targeted the outer-membrane protein *ompW* gene [48]. Additionally, we tested for toxigenic *V. cholerae*, targeting the enterotoxin gene *ctxA* [49]. For further qPCR assay details see Supplementary Table S2.

All qPCR analyses were performed on the BIO-RAD CFX384 Touch™ Real-Time PCR Detection System™, with gene copies calculated within each sample by plotting against their respective standard curve using BIO-RAD's CFX MAESTRO™ software version 1.1. Each qPCR analysis was run in triplicate, with all assays set-up in 5 µL reaction volumes that consisted of 2.5 µL BIO-RAD iTaq Universal SYBR® Green Supermix, 1.1 µL nuclease free water, 0.2 µL of each forward and reverse primer, and 1 µL of diluted (1:20) DNA template, with the exception of the ctxA assay, which consisted of 2.5 µL BIO-RAD iTaq Universal PROBES Supermix, 1 µL nuclease free water, 0.2 µL of each forward and reverse primer, 0.1 µL of probe and 1 µL of diluted (1:20) DNA template. Calibration curves were run with every plate and for each SYBR-based assay specificity was confirmed with a melt curve. Plate preparation was conducted using an epMotion® 5075 I Automated Liquid Handling System.

2.4. Digital Droplet Analysis

The abundance of *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus* was initially determined via qPCR. While we detected no *V. cholerae*, we did detect *V. parahaemolyticus* and *V. vulnificus*, however these levels were very low and significant variation between technical replicates was observed. Digital droplet PCR (ddPCR) has increased precision when quantifying low copy numbers of a gene [50], and so, to quantify *V. parahaemolyticus* and *V. vulnificus* more precisely we used ddPCR. All ddPCR analyses were performed using the Bio-Rad QX200 digital droplet PCR instrument. For assay details see Supplementary Table S2. The ddPCR mix used comprised of 12.5 µL of EvaGreen Supermix (Bio-Rad), 0.2 µL of forward and reverse primers, 1 µL of diluted DNA template and nuclease free water up to 25 µL, with 20 µL then being used for droplet generation. The PCR reaction was performed on a C1000 Touch Thermal Cycler with 96-Deep Well reaction module (Bio-Rad) using the evergreen protocol with a single modification of the annealing/extension step, which was adjusted to 56 °C for *V. vulnificus vvhA* to suit the Tm of this primer set. The droplets were read with a QX200 Droplet Reader and QuantaSoft software version 1.7.4 (Bio-Rad). A single threshold was applied on all samples and negative controls were used, with the absolute number of gene copies converted to gene copies per litre.

2.5. 16S rRNA Gene Amplicon Sequencing

To characterise the total bacterial community composition in samples, the V3–V4 region of the bacterial 16S rRNA gene was amplified using the 341f/805r primer pair [51], with the following cycling conditions: 95 °C for 3 min followed by 25 cycles of: 95 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s, and then 72 °C for 5 min with a final hold at 4 °C [52]. PCR amplicons were then sequenced using the Illumina MiSeq platform (300 bp paired-end analysis at the Ramaciotti Institute of Genomics, University of New South Wales, Sydney, NSW, Australia).

The DADA2 pipeline [53] was used to process the paired R1 and R2 fastq reads. Reads with 'N' bases were removed and primers were truncated using cutadapt [54]. Low quality terminal ends were removed by trimming reads (trunc (R1 = 280; R2 = 250)). To produce the highest number of merged reads after learning error rate and removing chimeric sequences, we used the dada2 removeBimeraDenovo program at the default threshold stringent minFoldParentOverAbundance = 1. Amplicon sequencing variants (ASVs) were annotated against the SILVA v138 database with a 50% probability cut-off. The ASV table was subsequently filtered to remove ASVs not assigned as kingdom bacteria, as well as

any ASVs classified as chloroplast or mitochondria. The chloroplast data was removed and then ASVs were annotated using the PR2 version 4.14.0 [55] database with a 50% probability cut-off and then joined back to the bacteria dataset. Finally, the dataset was rarefied to 30,000 reads using vegan [56]. Sequences are available in the NCBI database (ID-PRJNA776096). For 16S pipeline script please see: (https://github.com/Nwilliams96/Australian_Vibrio_Latitude_Project.git, accessed on 21 May 2022).

2.6. hsp60 Sequencing

To quantify the *Vibrio* community with greater species-level precision than is delivered by 16S rRNA gene amplicon sequencing, we employed a *Vibrio*-specific *hsp60* gene amplicon sequencing assay, using the Vib-shpF3-23 and Vib-hspR401-422 primer pairing [57]. DNA was diluted 1:10 and 3 µL was used in a 30 µL PCR reaction volume comprising of 6 µL of 5 x Hi-Fi Buffer (Meridian Bioscience, Cincinnati, OH, United States), 3 µL of 10 mM dNTPs, 0.5 µL of high-fidelity velocity polymerase, 3 µL of 10 µM forward primer (Vib-shpF3-23), and 3 µL of 10 µM reverse primer (Vib-hspR401-422). Amplification was performed using the following PCR cycling conditions: one cycle of 98 °C for 2 min, 5 cycles of 98 °C for 30 s, 60 °C for 30 s, and 72 °C for 45 s, 21 cycles of 98 °C for 30 s, 60 °C for 30 s with a reduction of 0.5 °C per cycle (60 °C to 50 °C), and 72 °C for 45 s, 16 cycles of 98 °C for 30 s, 50 °C for 30 s, and 72 °C for 45 s, and a final extension time of 72 °C for 10 min. Amplicons were subsequently sequenced using the Illumina MiSeq platform (300 bp paired-end analysis at the Ramaciotti Institute of Genomics, University of New South Wales, Sydney, NSW, Australia).

As described in King et al. (2019), paired-end sequences were joined using FLASH [58] and trimmed using Mothur [59]. These fragments were then clustered, and chimeric sequences were removed using vsearch [60]. Cleaned sequences were BLASTed against a *Vibrio-hsp60* reference dataset, discarding non-*Vibrio* sequences. The fasta file obtained was used to assign operational taxonomic units (OTUs) against the custom *Vibrio-hsp60* reference dataset with the RDP classifier. As there was a large spread of sequences per sample, the data was not rarefied, but the sequences were normalised to the number of sequences per sample to provide the relative abundance for each taxon for each sample. For hsp60 pipeline script please see (https://github.com/Nwilliams96/Australian_Vibrio_Latitude_Project.git, accessed on 21 May 2022).

2.7. Statistical Analysis

To test for differences in abiotic variables, qPCR and ddPCR data, between sampling sites and climatic zones, Kruskal–Wallis tests were used, followed by Mann–Whitney pairwise comparisons with Bonferroni corrected *p*-values. Correlations between qPCR data, ddPCR data and abiotic variables were determined using Spearman's RS, with Bonferroni corrected p values. These statistics were performed in Past Version 4 [61].

To test for differences in microbial community composition (16S rRNA data) and *Vibrio* community composition (*hsp60* data) between samples, as well as differences in alpha diversity measures in both data sets, we used the adonis function from Vegan [56] and the pairwise.adonis function from the PairwiseAdonis [62] R package. To determine how each sample within the 16S rRNA dataset were related to each other, we used the similarity profile routine test (SIMPROF) in the r package 'clustsig' [63]. For the 16S rRNA gene sequencing dataset, we found that the samples clustered into two significantly different groups and so ran the multipatt function in the indicspecies r package [64] to determine indicator ASVs within each group. Additionally, we used the correlation analysis package MicTools [65] to test for potential correlations between chloroplasts, the *Vibrio* community and abiotic variables, only accepting *p*-values < 0.05 and using Spearmans rs values for analysis.

3. Results

3.1. Environmental Conditions

Across the latitudinal gradient examined in this study, water temperatures (Figure 1B) varied significantly [$p < 0.01$] between tropical (12°22′42.8″ S–19°16′29.3″ S), subtrop-

ical (21°06′37.6″ S–33°91′00″ S), and temperate (35°00′46.0″ S–42°53′20.9″ S) climatic zones (mean: 31.25 ± 1.85 °C, 26 ± 2.38 °C, and 21 ± 2.61 °C, respectively). In contrast, salinity (Figure 1C) remained statistically indistinguishable between these zones (average 30.53 ± 9.36 ppt, 32.79 ± 6.40 ppt, and 35.70 ± 1.07 ppt, respectively). Of note however, four sites had significantly lower [$p < 0.01$] salinity than the mean salinity of the dataset (Darwin river: 22.54 ppt, Darwin beach: 10.30 ppt, Bundaberg river site 2: 19.26 ppt and Gold Coast river: 17.90 ppt), which coincided with rainfall events in the 3 days prior to sampling (Darwin: 24 mm, Bundaberg: 3.2 mm and Gold Coast: 17.4 mm). The Hobart sites had significantly lower [$p < 0.01$] dissolved oxygen levels than the other sites (Hobart beach: 9.48%, Hobart river: 10.01%), and were in fact 10-times lower than the mean dissolved oxygen observed along the latitudinal gradient. The pH within samples did not vary significantly between climatic zones.

3.2. Quantification of the Total Bacterial Community

Along the Eastern Coast of Australia, there were no correlations observed between levels of the 16S rRNA gene, used here as a proxy measure for bacterial biomass, and any of the environmental variables measured. The highest levels of 16S rRNA gene copies were observed within the subtropical region [mean: $1.69 \times 10^{11} \pm 1.24 \times 10^{11}$ copies/L, n = 24], where levels were 61- and 68-times higher than levels detected within the tropical [mean: $1.05 \times 10^{11} \pm 7.55 \times 10^{10}$ copies/L, n = 43] and temperate [mean: $1.00 \times 10^{11} \pm 5.58 \times 10^{10}$ copies/L, n = 18] regions. Average levels of the 16S rRNA gene did not statistically differ [$p > 0.05$] between beach [mean: $1.39 \times 10^{11} \pm 1.12 \times 10^{11}$ copies/L, n = 42] and river [mean: $1.56 \times 10^{11} \pm 1.40 \times 10^{11}$ copies/L, n = 42] sites along the latitudinal gradient. However, the beach sites within the subtropical region displayed significantly higher [$p < 0.01$] levels of the 16S rRNA gene than the beach sites within the tropical and temperate regions. This pattern was not true of river sites, whereby across regions there was no statistically distinguishable difference.

3.3. Bacterial Community Analysis

Across the dataset, the 16S rRNA gene amplicon sequencing analysis revealed a total of 67, 218 unique bacterial ASVs. Both alpha diversity [$F = 93.63$, $p < 0.01$] and community composition [$F = 104.7$, $p < 0.01$] were significantly different between sites across the entire dataset. When beach and river sites were pooled, the most diverse climatic zone was the temperate zone, with both Shannons [$F = 6.6$, $p < 0.05$] and Simpson diversity [$F = 16.9$, $p < 0.01$] indexes within this zone being significantly higher than within the tropical zone. Simpsons diversity index was also significantly higher [$F = 6.2$, $p < 0.05$] within the temperate climatic zone than within the subtropical zone. When dividing the samples into river and beach sites, diversity did not differ significantly between the river sites within any of the climatic zones along the latitudinal gradient, with no significant difference in Chao1, Simpson or Shannons diversity index. In contrast, within the beach sites, Chao1 was significantly lower within the temperate climatic zone compared to the subtropical climatic zone [$F = 8.39$, $p < 0.01$].

To identify patterns in similarity among bacterial assemblages, we performed a SIMPROF analysis, which revealed a bifurcation of samples into two major groups that were significantly different [$F = 21.472$, $p < 0.01$] (Figure 2). The first group contained samples from only above a latitude of 30.29° S (the Sydney Beach site was an exception to this and clustered with these northern samples) and the second group contained samples from only below 30.29° S.

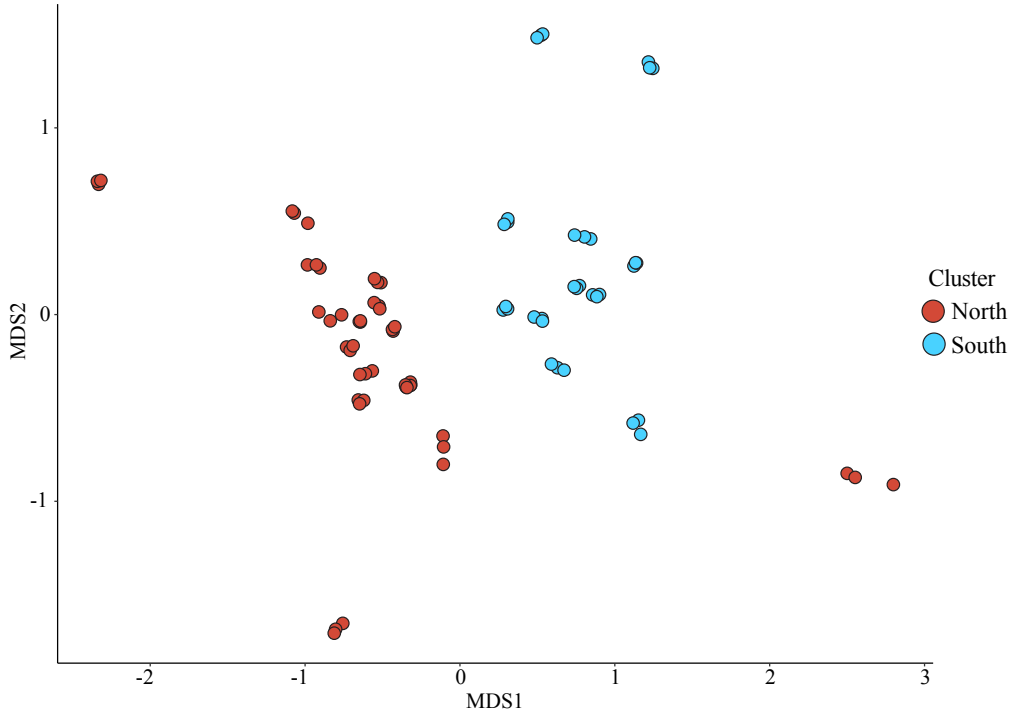

Figure 2. NMDS (stress = 0.1269098) showing the results of a SIMPROF (coloured categories) and the bacterial community composition similarities between locations. This cut-off occurred at latitude 30.29° S.

To determine what bacterial ASVs may be indicators of both the northern and southern communities, we used the multipatt function within the indicspecies R package. This analysis revealed 663 ASVs as indicators of the southern community, with the most abundant of these including a *Rubritaleaceae* ASV, a *Flavobacteriaceae* ASV and a *Cyanobiaceae* ASV, which contributed to 2.8%, 2.4% and 2.3% to the relative abundance of the southern community, respectively. There were also 464 ASVs identified as indicators of the northern community, with the most abundant of these consisting of a *Rhodobacteraceae* ASV and two *Actinomarinaceae* ASVs, contributing 1.4%, 1.6% and 0.8% to the relative abundance of the northern community, respectively. Of note, within the northern community indicator ASVs, 11 *Vibrio* spp. were identified. These *Vibrio* spp. included seven unassigned *Vibrio* spp., *V. brasiliensis*, *V. maritimus*, *V. harveyi* and *V. sinaloensis*.

3.4. Quantification of the Total Vibrio Community

Vibrio were detected via qPCR at all 28 sites sampled along the Australian eastern coastline (Figure 3A,B). Along the latitudinal gradient we observed no correlations between total *Vibrio* levels and any of the environmental variables.

The highest levels of total *Vibrio* occurred within the subtropical climatic zone [mean: $1.00 \times 10^7 \pm 9.29 \times 10^6$ copies/L, n = 41], where levels were 14-times higher than those detected within the tropical climatic zone [mean: $8.81 \times 10^6 \pm 1.13 \times 10^7$ copies/L, n = 22] and 184-times higher than the temperate climatic zone [mean: $3.54 \times 10^6 \pm 2.33 \times 10^6$ copies/L, n = 17]. However, in contrast to this overall pattern, the highest levels of *Vibrio* were detected within the Darwin river [mean: $3.05 \times 10^7 \pm 8.93 \times 10^6$ copies/L, n = 3] and Darwin beach [mean: $2.47 \times 10^7 \pm 1.30 \times 10^7$ copies/L] sites, where levels were significantly higher [$p < 0.01$] than any other site. Moreover, there were only three other regions (Rockhampton, Gold Coast and Sydney, Australia) (Figure 3A,B), which had total *Vibrio* levels exceeding 1×10^7 copies/L. The sites within these regions included the Rockhampton beach site 1 [mean: $2.13 \times 10^7 \pm 2.40 \times 10^6$ copies/L, n = 3], Rockhampton beach site 2 [mean: $1.25 \times 10^7 \pm 4.81 \times 10^5$ copies/L, n = 3], the Gold Coast beach, Gold Coast river, Sydney beach and Sydney river sites, all of which were significantly higher [$p < 0.05$] than any other site (excluding the Darwin sites) along the latitudinal gradient.

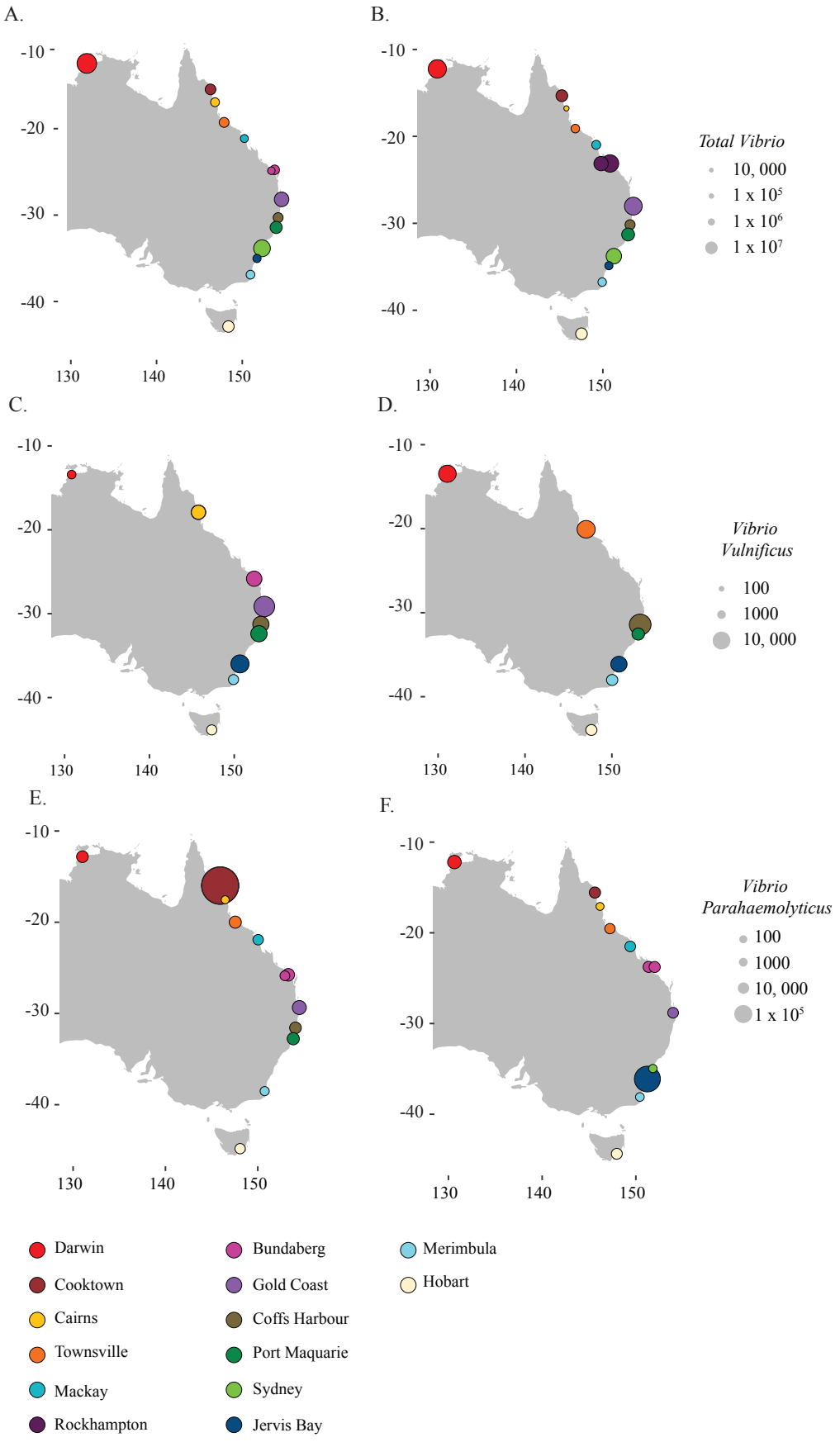

Figure 3. Results from (**A**,**B**) Total *Vibrio* qPCR, (**C**,**D**) *Vibrio vulnificus* ddPCR, (**E**,**F**) *Vibrio parahaemolyticus* ddPCR. (**A**,**C**,**E**) are river sites, while (**B**,**D**,**F**) are beach sites. Size scale represents copies/L of each assay.

Across the entire dataset, average *Vibrio* levels were 37 times higher at the beach [mean: $9.57 \times 10^6 \pm 9.31 \times 10^6$ copies/L, n = 41] sites compared to river sites [mean: $7.01 \times 10^6 \pm 9.11 \times 10^6$ copies/L, n = 31], which was also true within the subtropical and temperate climatic zones, [subtropical beach mean: $1.25 \times 10^7 \pm 8.53 \times 10^6$ copies/L, n = 21; temperate beach mean: $3.41 \times 10^6 \pm 2.42 \times 10^6$ copies/L, n = 9], which were characterised by significantly higher [$p < 0.01$] *Vibrio* abundances than the river sites [subtropical mean: $7.46 \times 10^6 \pm 9.55 \times 10^6$ copies/L, n = 20, temperate mean: $3.67 \times 10^6 \pm 2.39 \times 10^6$ copies/L, n = 8].

3.5. Vibrio Diversity

Vibrio-specific *hsp60* gene amplicon sequencing identified the occurrence of 59 unique *Vibrio* species across the entire dataset, with the most abundant species including *V. campbellii* and *V. harveyi*, which were detected in 93% and 89% of samples, respectively (Figure 4). All sites had a significantly different [F = 7.5, $p < 0.001$] Shannon's diversity and a significantly different [F = 7.15, $p < 0.001$] community composition. No significant differences were observed in *Vibrio* diversity between tropical, subtropical and temperate zones, nor did any of the diversity indices correlate with environmental variables. There was, however, a significant difference [F = 4.5, $p < 0.01$] in *Vibrio* community composition between the tropical and temperate climatic zones, which was driven by *V. campbellii* and *V. harveyi*, accounting for 24% and 6% of the dissimilarity between these two regions, respectively. *Vibrio* community composition did not differ between river and beach sites, nor was there a significant difference between tropical and subtropical, or subtropical and temperate climatic zones.

Among the most abundant *Vibrio* species, the relative abundance of *V. harveyi* (mean relative abundance = 7.69%, n = 78) was positively correlated with temperature, DO% and latitude [r_s = 0.38, 0.08 and 0.38, $p < 0.01$], and negatively correlated with salinity and pH [$r_s = -0.43$ and -0.54, $p < 0.01$]. The relative abundance of *V. harveyi* was also positively correlated [r_s = 0.03, $p < 0.05$] with the relative abundance of one phytoplankton ASV belonging to the genus *Tetraselmis*. The relative abundance of *V. campbellii* (mean relative abundance = 34.94%, n = 78) was positively correlated with temperature and latitude [r_s = 0.18 and 0.10, $p < 0.01$] and negatively correlated with DO%, salinity and pH [$r_s = -0.10$. -0.32 and -0.46, $p < 0.01$]. *V. campbellii* relative abundance was also positively correlated with 17 phytoplankton ASVs, which were mostly members of the *Bacillariophyta* family. The strongest correlation [r_s = 0.1] with an ASV from the *Phaeocystis* genera.

Among known human pathogens, *V. parahaemolyticus* OTUs were detected in 78% (n = 22/24) of samples, with their relative abundance positively correlated with latitude [r_s = 0.26, $p < 0.01$] and negatively correlated with salinity and pH [$r_s = -0.71$ and -0.52, $p < 0.01$] (Figure 5). *V. parahaemolyticus* relative abundance was also significantly correlated with two *Raphid-Pennate* ASVs [r_s = 0.28 and 0.12, $p < 0.05$], an *Arcocellulus* ASV [r_s = 0.09, $p < 0.05$], two *Olisthodiscus* ASVs [r_s = 0.09 and 0.02, $p < 0.05$], a *Teleaulax* ASV [r_s = 0.09, $p < 0.05$] and a *Marsupiomonas* ASV [r_s = 0.09, $p < 0.05$]. *V. vulnificus* OTUs were recorded at three sites, namely the tropical environments at Bundaberg R2, Darwin B and Darwin R, where the relative abundance of this pathogen displayed a positive correlation to temperature, latitude and DO% [r_s = 0.06, 0.06 and 0.07, $p < 0.01$] while displaying a negative correlation to salinity [$r_s = -0.47$, $p < 0.01$].

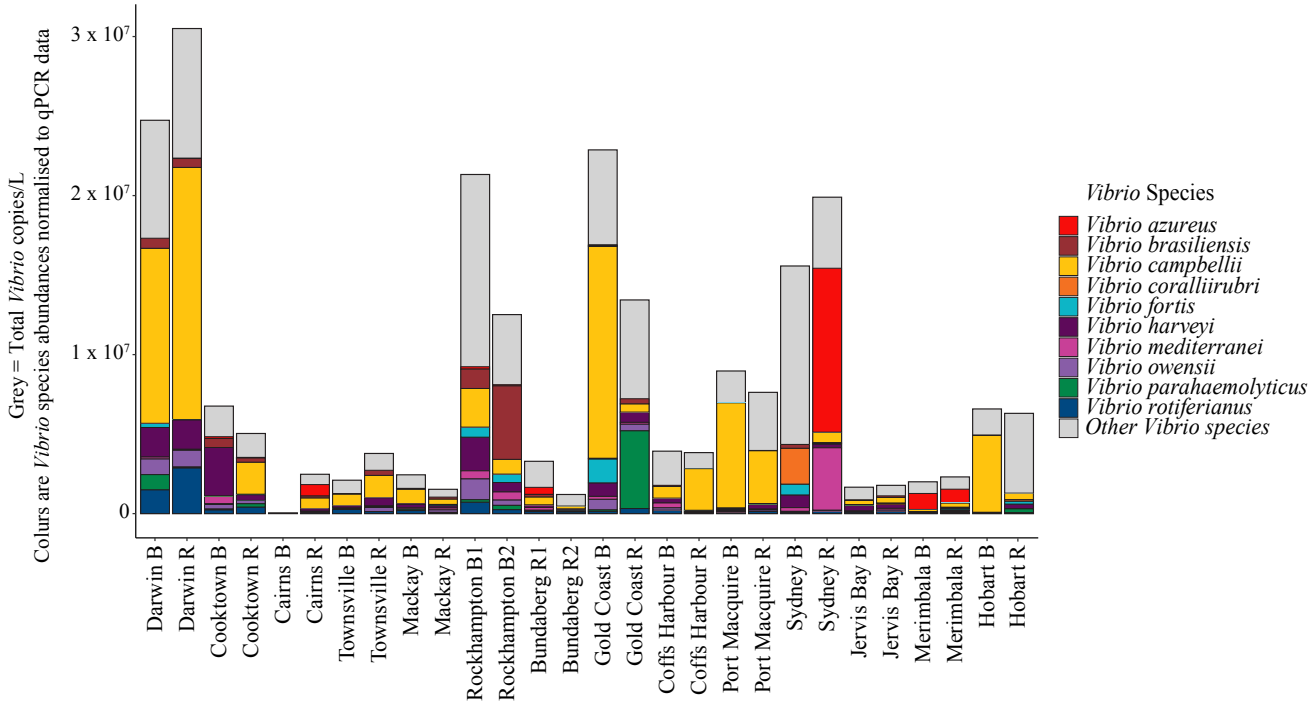

Figure 4. Stacked bar-plot of the top 10 most abundant *Vibrio* species from the *hsp60* gene amplicon sequencing data along the East Coast of Australia. Data is normalised to the total *Vibrio* qPCR data (grey).

3.6. Potentially Pathogenic Vibrio Species: Vibrio Cholerae, Vibrio parahaemolyticus and Vibrio vulnificus

V. cholerae was not detected in any sample using qPCR, which is consistent with the results from the *hsp60* sequencing analyses where this species was also not detected. *V. parahaemolyticus* was detected in 79% (22/28) of samples, with the highest levels detected at the Cooktown river site (15°27′37.8″ S, 145°14′57.6″ E) [mean: $2.56 \times 10^5 \pm 4.43 \times 10^5$ copies/L, n = 3] and the Jervis Bay beach site (35°00′46.0″ S, 150°41′38.5″ E) [mean: $9.5 \times 10^4 \pm 1.65 \times 10^5$ copies/L, n = 3]. On average, the tropical climatic zone had the highest levels of *V. parahaemolyticus* [mean: $4.59 \times 10^4 \pm 1.66 \times 10^5$ copies/L, n = 21], where they were 310-times higher than levels within the subtropical climatic zone and 1814-times and significantly [$p < 0.05$] higher than levels within the temperate climate zone (Figure 3E,F). *V. vulnificus* was detected in 57% (16/28) of samples, with the highest levels detected at the Coffs Harbour beach site [mean: $1.34 \times 10^4 \pm 2.61 \times 10^3$ copies/L, n = 2] and the Gold Coast river site [mean: $1.26 \times 10^4 \pm 1.60 \times 10^4$ copies/L, n = 3]. On average, the subtropical climatic zone had the highest levels of *V. vulnificus* [mean: $3.65 \times 10^3 \pm 6.22 \times 10^3$ copies/L, n = 34], where they were 26 times higher than levels within the tropical climatic zone [mean: $2.90 \times 10^3 \pm 4.13 \times 10^3$ copies/L, n = 22] and 44 times higher than levels within the temperate climatic zone [mean: $2.54 \times 10^3 \pm 3.03 \times 10^3$ copies/L, n = 23] (Figure 3C,D). In contrast to the patterns seen in the *hsp60* gene amplicon sequencing data, no significant correlations were observed between *V. vulnificus* or *V. parahaemolyticus* and any measured environmental variables. However, this may be due to the limited proportion of samples that these organisms were detected in, and it is notable that both organisms occurred in greatest abundance in warm water tropical and subtropical environments, such as Cooktown and the Gold Coast.

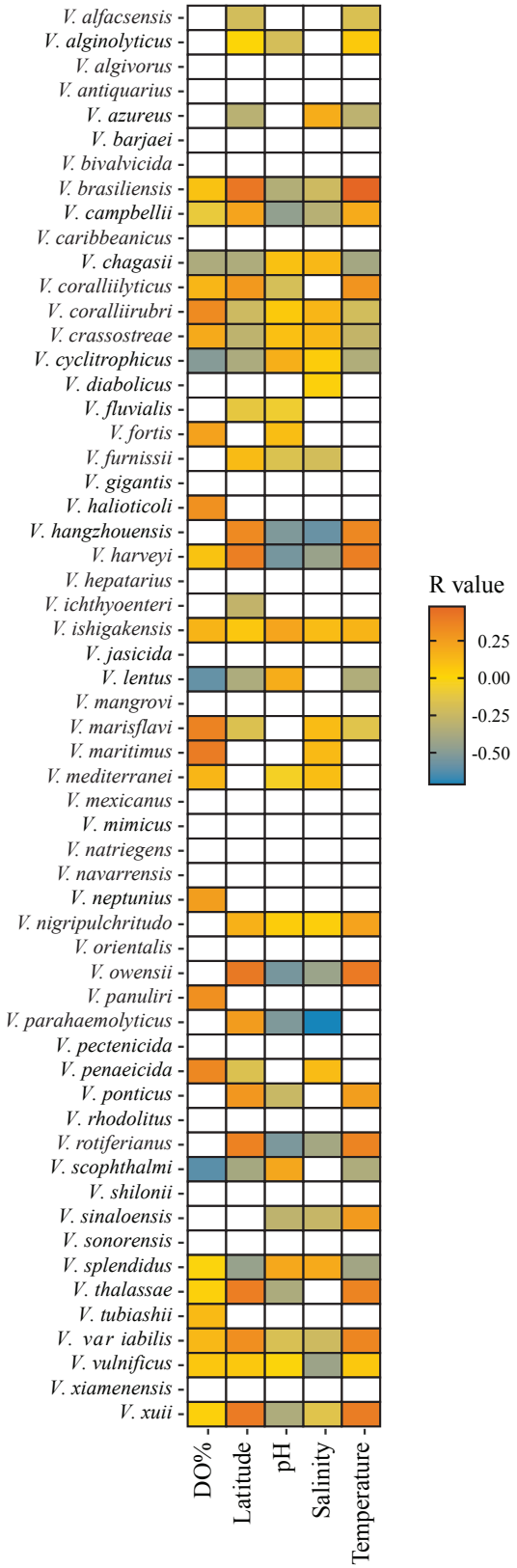

Figure 5. Heatmap displaying the correlations from MicTools analysis between environmental variables and *Vibrio* species from *hsp60* dataset. Each cell is either coloured or white, with coloured cells representing pearsonR R value and white cells representing no significant correlation.

4. Discussion

The principal goal of this study was to characterise patterns in the diversity and abundance of *Vibrio* species, along a large latitudinal gradient spanning the eastern coastline of Australia, which is a region that has been identified as a climate change hotspot [44]. Along this transect, we observed the highest levels of *Vibrio* bacteria in Darwin, the northernmost and warmest site sampled, as well as positive correlations between both pathogenic and non-pathogenic *Vibrio* species with temperature, salinity and phytoplankton ASVs.

Bacterial communities are often governed by differing environmental variables such as temperature [66] along latitudinal gradients. Previous studies have demonstrated that some bacterial populations change with latitude along the eastern coastline of Australia, including poleward increases in the abundance of members of the *Marine Roseobacter Group* [67], and latitudinal shifts in the abundance of aerobic anoxygenic phototrophic bacteria [49]. In this study, we observed a notable bifurcation of the total bacterial community into two significantly different groups that separated at approximately 30° S, into a northern and southern group of samples. Upon examination of the taxa responsible for this split, 11 *Vibrio* ASVs were defined as indicators of the northern community, which is consistent with prior observations that many *Vibrio* species show a preference for warmer waters [30,32–34,36,37,39,68,69].

4.1. Latitudinal Trends in Vibrio Abundance and Diversity

Vibrio often exist in a higher abundance at low latitudes where water temperature is high [42,70]. We observed the greatest levels of *Vibrio* spp. in Darwin, the lowest latitude at which we sampled, as well as high levels in Rockhampton, the Gold Coast and in Sydney. Not only were these sites amongst the sites with the warmest temperatures (Figure 1B), but two of these sites, Darwin and the Gold Coast River site, also had the lowest salinities. This finding aligns with other studies [37,39,68,69], which have also found a higher abundance of *Vibrio* bacteria at low salinities and high temperatures. While not examined in this study, in a recent study, *Vibrio* spp. were discovered to form biofilms on microplastic particles and, their occurrence on these microplastics was correlated to proximity to major cities [71], which may explain high levels of *Vibrio* bacteria at these locations, which are all highly urbanised. Another potential reason for these differences may be due to temporal changes, which were not accounted for in this study, with each site being sampled once within a period of 48 days during the Australian summer.

We observed a significant difference in community composition between all sites and between the tropical and temperate regions of the coastline. These differences were largely driven by heterogeneity in the relative abundance of *V. campbellii*, *V. azureus* and *V. harveyi* OTUs, whereby *V. campbellii* and *V. harveyi* OTUs were more abundant in the tropical sites and *V. azureus* OTUs were more abundant at the temperate sites. Notably, strains of all of these species are known marine pathogens, infecting shrimp [72], oysters [73], and fish species [18]. These patterns show some similarities to those observed in other large-scale examinations of *Vibrio* diversity, where *V. campbellii* and *V. harveyi* were also amongst the most abundant *Vibrio* species isolated from corals, fireworms, soil and water, along the tropical coast of Brazil [74]. Additionally, *V. campbellii*, *V. azureus* and *V. harveyi* OTUs displayed positive correlations with specific phytoplankton groups identified in the 16S rRNA gene amplicon sequencing analysis. These observations point towards a potentially important role of phytoplankton in defining the abundance of some *Vibrio* species, which is consistent with previous observations (e.g., [35]) that have observed strong correlations between *Vibrio* and chlorophyll a. In this case, it is possible that species—specific relationships underpin this pattern, which has been observed before during a yearlong time-series, where an unknown *Vibrio* species increased from baseline relative abundance of 0–2% up to 54%, closely following an increase in the relative abundance of the diatom *Chaetoceros compressus* [66].

Of the human pathogens detected within the community data, a *V. vulnificus* OTU was detected at the northernmost sampling location Darwin, where water temperatures

were <32 °C, as well as the Bundaberg river 2 site. *V. parahaemolyticus* OTUs occurred within 78% (22/28) of sites but were also most abundant in Darwin. *V. vulnificus* OTUs levels displayed a significant, albeit weak, correlation with temperature across the transect, but a much stronger negative correlation to salinity, which is consistent with previous observations [31,37]. *V. parahaemolyticus* was correlated positively with phytoplankton ASVs including a *Raphid-Pennate* ASV, an *Arcocellulus* ASV, two *Olisthodiscus* ASVs, a *Teleaulax* ASV and a *Marsupiomonas* ASV. Whilst *V. parahaemolyticus* has previously been shown to display ecological interactions with phytoplankton [75], to our knowledge this is the first evidence for links between *V. parahaemolyticus* and these specific phytoplankton taxa.

4.2. Latitudinal Patterns in Vibrio Pathogens

Some strains of *V. cholerae* cause cholera, a disease responsible for ~100,000 deaths annually [24]. However, non-cholera causing *V. cholerae* have also been implicated in milder forms of gastroenteritis, otitis and life-threatening necrotizing fasciitis [76]. However, neither environmental nor toxigenic strains of *V. cholerae* were detected in any samples in this study. This is in contrast to recent studies that have detected low levels (101–103 copies/mL) of non-toxigenic *V. cholerae* in some of the Australian coastal environments examined here, including Darwin [42] and Sydney Harbour [43]. Seasonal differences in the timing of sampling may explain these divergent observations.

V. parahaemolyticus can cause foodborne disease and wound infections [25]. The highest levels of *V. parahaemolyticus* were detected in the tropical regions of the east Australian coast, where levels of this species were an order of magnitude higher than those observed in the temperate regions. The highest levels of *V. parahaemolyticus* were observed in Cooktown, where abundances reached levels similar to those recorded by Padovan et al. (2021), in the tropical waters around the Darwin area. However, it is notable that very high levels of *V. parahaemolyticus* were also observed in temperate samples, as far south as Jervis Bay (35°00′46.0″ S, 150°41′38.5″ E).

V. vulnificus causes foodborne disease and severe wound infections, which can often result high rates of mortality [77]. The abundance of *V. vulnificus* was not restricted to tropical waters, with levels 20 and 30 times higher in the subtropical sites compared to the tropical and temperate sites, respectively. The highest levels of *V. vulnificus* were recorded at the Coffs Harbour beach (30°18′17.4″ S, 153°08′34.9″ E) and Gold Coast river (28°10′44.1″ S, 153°32′30.1″ E) sites. Notably, these levels were similar to levels previously recorded in tropical northern Australian waters [42].

4.3. Implications of High Levels of Vibrio Bacteria along Australia's East Coast

This is the first study to examine the spatial dynamics of *Vibrio* abundance and diversity along Australia's eastern coast and has revealed levels of *Vibrio* to be higher in the tropical and subtropical sites compared to the temperate sites, with the highest levels in Darwin, the northernmost site, which was characterised by the highest water temperature and lowest salinity. Additionally, we observed multiple *Vibrio* species that displayed significant correlations with temperature, salinity and phytoplankton ASVs.

Notably, *Vibrio* abundances were not always most strongly driven by temperature, which is contrast to numerous previous studies [30,32–34,36,37,39,68,69]. Models of future SST scenarios predict 38,000 km of new coastal areas by 2100 being suitable for *Vibrio* growth as a result of climate change [28], as well as an increase in *Vibrio* cases of 1.93 times for every 1 °C increase in SST [78]. This is particularly concerning for Australia, whereby the SST is predicted to rise 1.5–3 °C by 2070, translating to a 2.9–5.8 times increase in the abundance of *Vibrio* spp. in Australian waters. While the highest levels of total *Vibrio* were indeed detected in the very warm water tropical sites in Darwin, it is notable that we also observed high levels in higher latitude locations, including sub-tropical sites at Gold Coast and in Sydney. Notably these are two very densely populated coastal areas, where high levels of recreational use of waterways occurs, highlighting potential human health hazard through the exposure to these pathogens [79].

5. Conclusions

The study of ecological patterns in the abundance and diversity of organisms is essential for developing an understanding of where and when they are abundant and for predicting distributions in future scenarios. We observed significant differences in both the entire bacterial and *Vibrio* communities between the northern and southern sites. Across this transect, both pathogenic and non-pathogenic *Vibrio* species correlated positively with temperature and negatively with salinity. Interestingly, we also observed correlations between several pathogenic *Vibrio* species and specific phytoplankton species, highlighting ecological relationships with phytoplankton as a potentially important, but often overlooked, determinant of *Vibrio* spatial and temporal dynamics. During the summer period that this study was performed, we detected pathogenic *Vibrio* species not only in warm tropical waters, but also southerly latitudes, including the highly urbanised environments, where opportunities for human exposure are significant. Given that ocean temperatures are rising [80] and severe rainfall events are increasing [41] as a consequence of climate change, many coastal environments may become more ideal habitats for pathogenic *Vibrio* species, indicating a potentially heightened human health risk.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/w14162510/s1, Table S1. List of locations sampled, detailing date, latitude, longitude, sample type and Koppen-Classification. Table S2. Primers used in study.

Author Contributions: J.R.S., N.S. and V.B. designed the study. N.L.R.W. and V.B. took all samples. qPCR was performed by N.L.R.W. ddPCR and hsp60 PCR were performed by N.L.R.W., N.S., A.B. and N.L.R.W. performed the 16S rRNA gene sequencing analysis. N.L.R.W. and W.L.K. performed the hsp60 gene sequencing analysis. N.L.R.W. and J.R.S. prepared and wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: All abiotic, qPCR and ddPCR data are available in the Supplementary Materials. Sequences are available in the NCBI database (ID—PRJNA776096). Scripts are available at (https://github.com/Nwilliams96/Australian_Vibrio_Latitude_Project.git, accessed on 24 May 2022).

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