Occurrence and dynamics of potentially pathogenic vibrios in the wet-dry tropics of northern Australia

Padovan, Anna; Siboni, Nachshon; Kaestli, Mirjam; King, William L.; Seymour, Justin R.; Gibb, Karen

Published in:
Marine Environmental Research

DOI:
10.1016/j.marenvres.2021.105405

Published: 01/07/2021

Document Version
Publisher's PDF, also known as Version of record

Marine Environmental Research 169 (2021) 105405

Contents lists available at ScienceDirect

Marine Environmental Research

journal homepage: www.elsevier.com/locate/marenvrev

Occurrence and dynamics of potentially pathogenic *vibrios* in the wet-dry tropics of northern Australia

Anna Padovan [a,*], Nachshon Siboni [b], Mirjam Kaestli [a], William L. King [b,c,1], Justin R. Seymour [b], Karen Gibb [a]

[a] Research Institute for the Environment and Livelihoods, Charles Darwin University, Darwin, NT, Australia
[b] Climate Change Cluster, University of Technology Sydney, Ultimo, NSW, Australia
[c] The School of Life Sciences, University of Technology Sydney, Ultimo, NSW, Australia

ARTICLE INFO

Keywords:
Vibrio
Tropics
hsp60
qPCR
Vibrio parahaemolyticus
Vibrio vulnificus
Vibrio cholerae

ABSTRACT

Bacteria from the *Vibrio* genus are a ubiquitous component of coastal and estuarine ecosystems with several pathogenic *Vibrio* species displaying preferences for warm tropical waters. We studied the spatial and temporal abundance of three key human potential pathogens *V. parahaemolyticus*, *V. cholerae* and *V. vulnificus* in northern tropical Australia, over the wet and dry seasons, to identify environmental parameters influencing their abundance. Quantitative PCR (qPCR) analysis revealed that *V. parahaemolyticus* occurred more frequently and in higher abundance than *V. cholerae* and *V. vulnificus* across all locations examined. All three species were more abundant during the wet season, with *V. parahaemolyticus* abundance correlated to temperature and conductivity, whereas nutrient concentrations and turbidity best explained *V. vulnificus* abundance. In addition to these targeted qPCR analyses, we assessed the composition and dynamics of the entire *Vibrio* community using *hsp60* amplicon sequencing. Using this approach, 42 *Vibrio* species were identified, including a number of other pathogenic species such as *V. alginolyticus*, *V. mimicus* and *V. fluvialis*. The *Vibrio* community was more diverse in the wet season, with temperature and dissolved oxygen as the key factors governing community composition. Seasonal differences were primarily driven by a greater abundance of *V. parahaemolyticus* and *V. vulnificus* during the wet season, while spatial differences were driven by different abundances of *V. harveyi, V. campbellii*, *V. cholerae* and *V. navarrensis*. When we related the abundance of *Vibrio* to other bacterial taxa, defined using 16S rRNA gene amplicon sequencing, *V. parahaemolyticus* was negatively correlated to several taxa, including members of the Rickettsiales and Saccharimonadales, while *V. vulnificus* was negatively correlated to Rhodobacteriaceae and Cyanobiaceae. In contrast, *V. alginolyticus, V. harveyi* and *V. mediterranei* were all positively correlated to Cyanobacteria. These observations highlight the dynamic nature of *Vibrio* communities and expands current understanding of the processes governing the occurrence of potentially pathogenic *Vibrio* spp. in tropical coastal ecosystems.

1. Introduction

Vibrios are gram-negative γ-proteobacteria that are ubiquitous members of coastal and estuarine microbiomes. They are metabolically diverse, with different species contributing to nutrient cycling (Crieminger et al., 2007), and the degradation of chitin (Giubergia et al., 2017) and hydrocarbons (Walker and Colwell, 1976). *Vibrio* spp. are, however, particularly notable for developing ecological associations with diverse marine animals and plants, with several pathogenic species responsible for causing disease in a wide variety of marine animals, including fish, cetaceans, crustaceans, echinoderms, and corals (Thompson et al., 2004). This genus also includes several human pathogens, including *Vibrio parahaemolyticus*, *V. vulnificus* and *V. cholerae,* which cause skin and tissue infections from seawater exposure and gastroenteritis from raw or undercooked seafood consumption (Feldhusen 2000; Oliver 2005; Jones and Oliver 2009; Baker-Austin et al., 2018).

Many studies have identified water temperature and salinity as important factors in determining the abundance of the key human

* Corresponding author.
E-mail address: anna.padovan@cdu.edu.au (A. Padovan).
[1] Present address: William L. King, Department of Plant Pathology and Environmental Microbiology, The Pennsylvania State University, USA.

https://doi.org/10.1016/j.marenvres.2021.105405
Received 14 December 2020; Received in revised form 24 June 2021; Accepted 27 June 2021
Available online 29 June 2021

pathogens *V. parahaemolyticus, V. vulnificus* and *V. cholerae* in temperate regions (Jones and Oliver 2009; Wong et al., 2019; Vijayan and Lee 2014). This has also been reported in some tropical regions (Bockemühl and Triemer 1974; Machado and Bordalo 2016; Rivas-Montaño et al., 2018), but when water temperatures are relatively stable year-round, other factors such as chlorophyll *a* (Wong et al., 2019), phytoplankton biomass (Asplund et al., 2011), inorganic phosphate (Gregoracci et al., 2012) and copepod abundance (Rehnstam-Holm et al., 2013) gain significance in driving *Vibrio* community diversity and composition. As outlined by Takemura et al. (2014), significant relationships of the genus *Vibrio* to a single environmental variable may not apply to all *Vibrio* species, and these relationships may also depend on the range of the environmental variables examined.

The coastline of northern Australia is sparsely populated by humans, yet vibrioses of humans have previously been reported ((Heath et al., 2001)Ralph and Currie 2007). In a study concerning *Vibrio* species isolated from patients at public hospitals in the Northern Territory who presented water associated skin and soft tissue infections, vibrios were implicated in 15% of the cases (McAuliffe et al., 2015). Seven *Vibrio* species were identified and the most common were *V. parahaemolyticus*, *V. alginolyticus*, and *V. vulnificus*. Over the study period, the highest incidence of *Vibrio* infections was in April (10 cases), which is towards the end of the wet season. Notably, the wet season coincides with the highest incidence of *V. parahaemolyticus* and *V. vulnificus* in Darwin Harbour (NT) wild harvest marine species, such as gastropod snails (Padovan et al., 2019). However, the specific environmental drivers behind this increased incidence of infection and occurrence of pathogens are still undefined. Run-off from heavy rain during the wet season in northern Australia reduces coastal seawater conductivity and early rainfall events can also increase nutrient loads in coastal areas (McKinnon et al., 2006), however, the knowledge about the impact of these events on *Vibrio* communities is scant.

Increasing *Vibrio* infections in temperate regions are linked to changing climate (Baker-Austin et al., 2016, 2017; Logar-Henderson et al., 2019; Ford et al., 2020; Froelich and Daines 2020). The impact of temperature rise in tropical regions is not well understood, however, microbial communities in shallow coastal waters would face more rapid change compared to deeper oceans (Brierley and Kingsford 2009). Severe weather events leading to increased rainfall and sediment resuspension can increase *Vibrio* growth and provide greater exposure opportunities to these potential pathogens (Morantz 2005).

Little is known about *Vibrio* ecology within tropical Australian waters or, more specifically, what environmental factors most strongly govern *Vibrio* abundance in this region. Aquaculture in northern Australia is estimated at AUD $212 million and Australia's National Aquaculture Strategy aims to increase Australian aquaculture to $2 billion/year by 2027 (DAWR, 2017). A key component of this increase is expanding aquaculture in northern Australia including tropical rock oyster aquaculture (Fleming 2015; Nowland et al., 2018). To ensure safe consumption, knowledge of the ecology of potentially pathogenic *Vibrio* spp. is needed for this region, to identify potentially high-risk periods for both food safety and water contact.

The aims of this study were to (1) assess the spatial and temporal abundance of significant *Vibrio* species and overall *Vibrio* diversity, and (2) understand the role of key environmental parameters in *Vibrio* dynamics to ensure public health in northern tropical Australia. Given their preference for warmer waters, we hypothesized that the abundance and diversity of *Vibrio* species would be higher than in temperate regions. We also predicted that due to narrower temperature ranges in tropical compared to temperate waters, other factors such as nutrients, conductivity and dissolved oxygen would gain a pivotal role influencing the abundance of pathogenic *Vibrio* species more so than in temperate regions.

2. Results

2.1. Environmental parameters

Sampling was conducted at three sites, Buffalo Creek, Ludmilla Creek and Rapid Creek, located in Darwin Harbour (Fig. 1). Environmental conditions at each site are summarized in Table S1. Water temperature varied by 7.8 °C being higher in March (31.1 °C ± 0.5) compared to June (25.0 °C ± 0.5) (Fig. 2). DO ranged from 4.7 to 9.4 mg/L and was higher in August compared to March ($P = 0.045$). On average, conductivity was lowest in February (24.6 mS/cm), increasing over the following drier months to an average of 59.0 mS/cm in June (Fig. 2). pH ranged from 7.2 to 8.0, and TN and TP were highly variable, with no significant differences between seasons ($P > 0.05$).

TP and TN were higher in Buffalo Creek, due to sewage discharge, compared to Rapid Creek and Ludmilla Creek, but this was not significant ($P = 0.063$). Turbidity and chl*a* did not vary significantly between sites or seasons but there was a particularly high measurement of both these parameters measured in Buffalo Creek in August. Notably, TN and TP were also high in Buffalo Creek at this time.

2.2 Abundance of the potential human pathogens *V. parahaemolyticus, V. vulnificus and V. cholerae*

All three potential human pathogen species examined were detected by qPCR (Fig. 2). *V. parahaemolyticus* was detected most frequently (98%; 62/63 samples), with abundances ranging from 0 to 553 copies/ml. *V. vulnificus* was detected in 65% (41/63) of the samples with concentrations between 0 and 88 copies/ml, and *V. cholerae* was the least frequently *Vibrio* species detected (14%; 9/63 samples) with abundances ranging from 0 to 465 copies/ml. *V. parahaemolyticus* and *V. cholerae* were more abundant in the wet season compared to the dry season ($P < 0.0001$ for *V. parahaemolyticus*; $P = 0.0444$ for *V. cholerae*), but no seasonal difference in abundance was observed for *V. vulnificus* ($P = 0.387$). Buffalo Creek had the highest concentrations of all three potentially pathogenic *Vibrio* species. *V. parahaemolyticus* and *V. cholerae* were screened for virulence genes (*trh* and *tdh* for *V. parahaemolyticus* and *ctxA* for *V. cholerae*) but none were detected by qPCR.

2.3. Environmental drivers of potentially pathogenic Vibrio abundance

Analysis of the relationship between *V. vulnificus* and measured environmental variables accounting for sites and month indicated positive associations for TN ($P = 0.006$), TP ($P = 0.007$), and turbidity ($P = 0.006$) (Figure S1; Table S2). *V. parahaemolyticus* abundance was positively associated with conductivity ($P = 0.003$) and temperature ($P < 0.001$) (Figure S2; Table S3). There was a significant interaction between temperature and DO i.e. the higher the temperature and the less DO in the water, the higher the *V. parahaemolyticus* levels.

2.4. Patterns in Vibrio community composition

Forty two *Vibrio* species were identified using amplicon sequencing targeting the *hsp60* gene. ASVs classified as potential human pathogens *V. alginolyticus*, *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus* were detected (Fig. 3). The relative abundances of *V. alginolyticus* (22%), *V. cholerae* (38%) and *V. parahaemolyticus* (30%) were highest in Buffalo Creek, in the wet season. The highest relative abundance of *V. vulnificus* (54%) was observed at Rapid Creek in January. Across the entire dataset, *V. campbellii* was the most abundant ASV (20%), followed by *V. parahaemolyticus* (9.1%), *V. harveyi* (7.2%), *V. vulnificus* (8.3%) and *V. natriegens* (6.2%) (Fig. 3). The overall relative abundance of *V. cholerae* was 1.6%. Several other species that have been isolated from patients infected in northern Australia were also identified, namely *V. fluvialis*, *V. alginolyticus* and *V. mimicus*.

V. harveyi was the main *Vibrio* species contributing to the

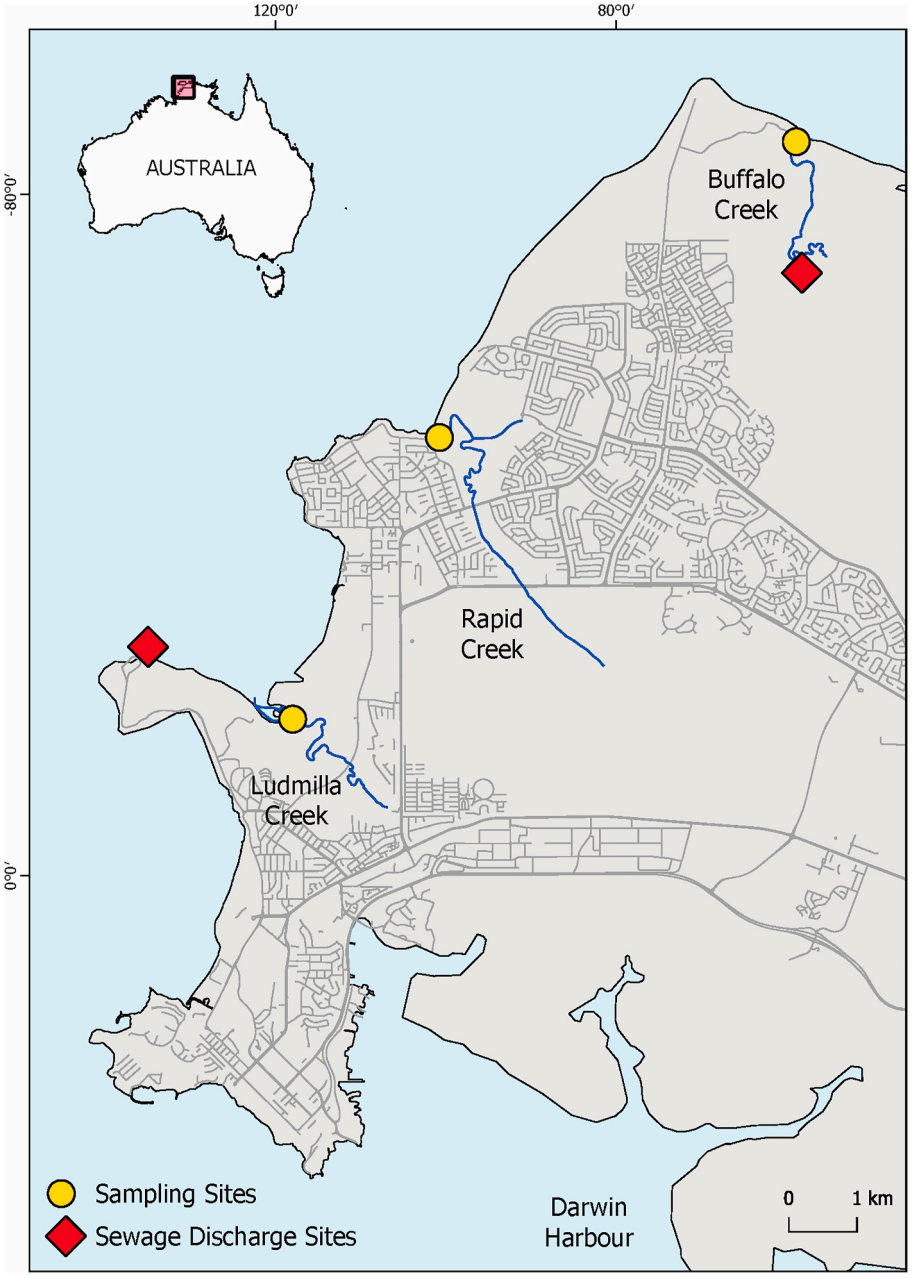

Fig. 1. Sampling locations in Darwin Harbour, Northern Territory, Australia.

discrimination between seasons, with highest relative abundance in the dry season. *V. parahaemolyticus* was also responsible for driving a discrimination between seasons, but unlike *V. harveyi*, was more abundant in the wet season (Figure S3). The *Vibrio* communities of Buffalo Creek were the most different while the communities of Ludmilla Creek clustered within the Rapid Creek communities (Figure S4). Buffalo Creek had the higher *Vibrio* diversity, contributing to the spatial differences detected, particularly due to the *V. cholerae* detection (Figure S4B).

To examine the association of the *hsp60 Vibrio* community with environmental variables RDA analysis was performed (Fig. 4). The final model had an adjusted R^2 of 25% with predictors TN (log transformed, Pseudo-F(1) 10.1, P = 0.001), temperature (Pseudo-F(1) 7.1, P = 0.001), and conductivity (Pseudo-F(1) 2.8, P = 0.020). The seasonal variations were more pronounced than the spatial variations. A positive correlation between TN and *V. vulnificus*, and *V. cholerae* could be observed, while *V. parahaemolyticus* was positively correlated with water temperature and wet season samples, and *V. campbellii* were more abundant in the dry season (Fig. 4). Morover, the *Vibrio* community composition was more diverse in the wet season compared to the dry season for all three creeks.

2.5. Bacterial 16S rRNA community and its relationships with Vibrio hsp60 community

Eleven *Vibrio* species were identified using 16S rRNA amplicon sequencing with no species-level resolution. Only 7 out of 62 samples contained *Vibrio* 16S rRNA sequences, thus supporting the use of *hsp60* sequence data to better identify and discriminate *Vibrio* species. The most abundant bacterial families were Rhodobacteraceae, Cyanobiaceae and Phormidaceae (Figure S5).

A sparse DIABLO analysis was conducted to select for *Vibrio hsp60* and 16S rRNA ASVs correlated with each other and which could discriminate between seasons. *V. vulnificus* was negatively correlated with various ASVs of the families Rhodobacteraceae and Cyanobiaceae,

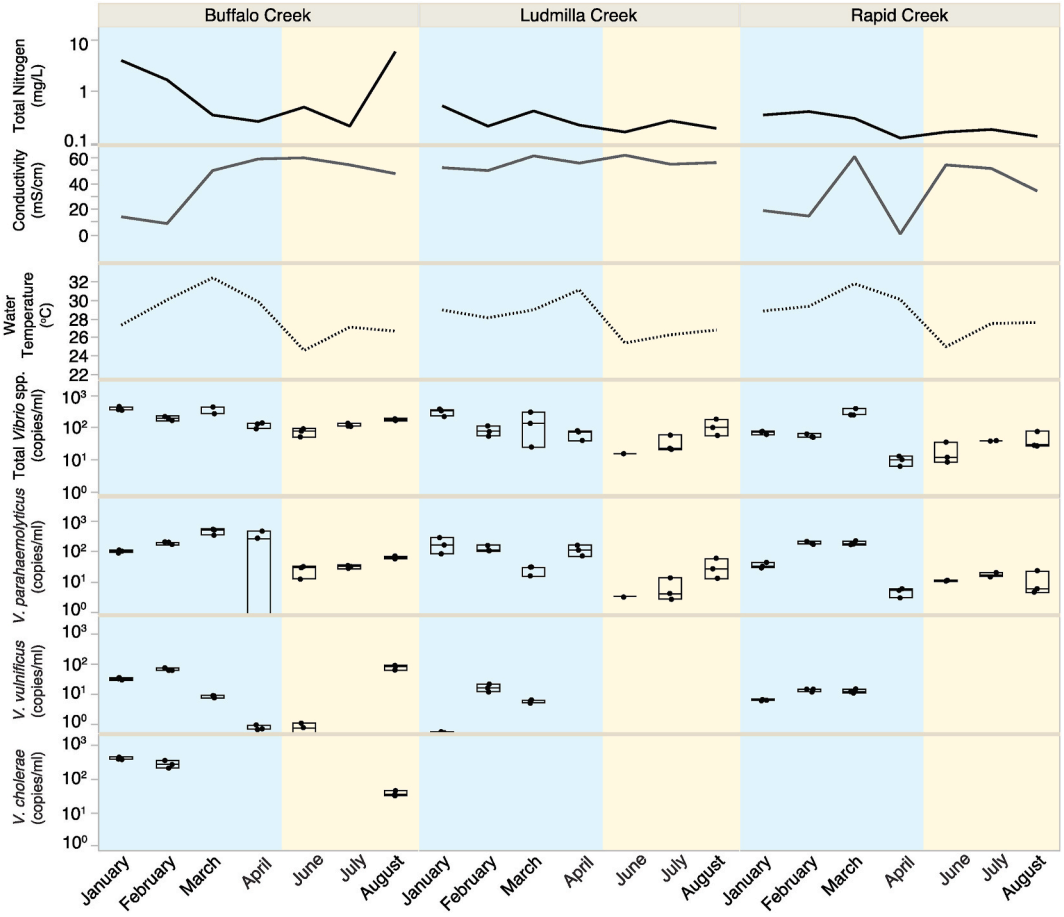

Fig. 2. Key environmental parameters (water temperature, conductivity and total nitrogen) and abundance of *V. parahaemolyticus*, *V. vulnificus*, *V. cholerae* and total *Vibrio* spp. (copies/ml) in creek water samples by qPCR. Shading indicates wet season (blue) and dry season (yellow).

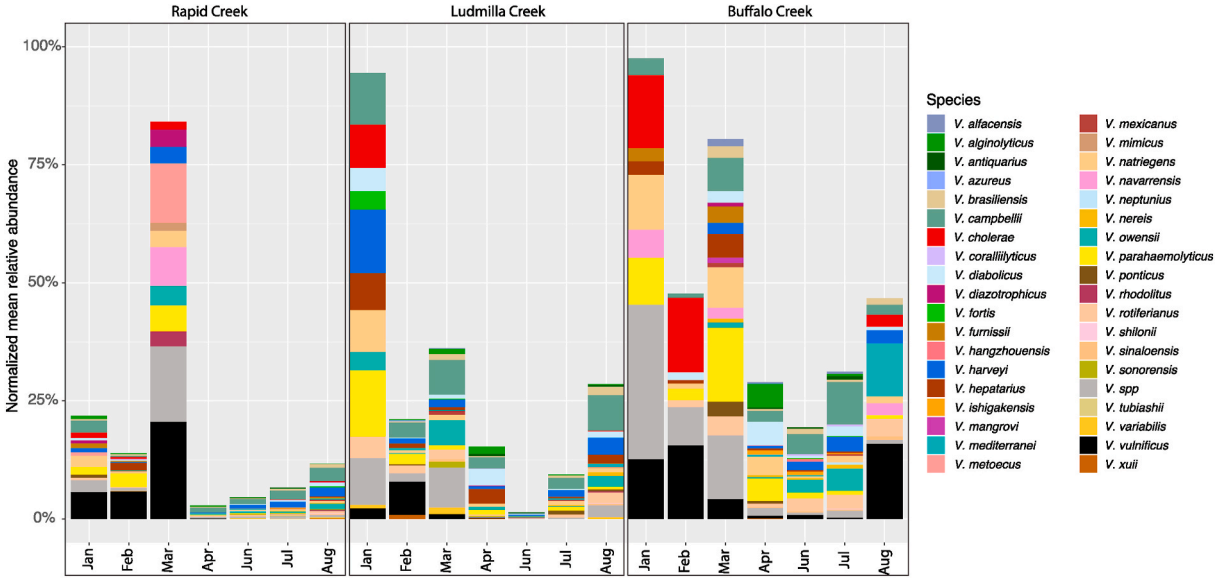

Fig. 3. Relative normalized abundance of *Vibrio* species in Darwin Harbour creek samples. Relative sequence abundance was normalized against *Vibrio* 16S rRNA qPCR abundance.

while *V. parahaemolyticus* was negatively correlated with Rickettsiales. *V. alginolyticus*, *V. harveyi* and *V. mediterranei* were positively correlated with various Cyanobacteria (Fig. 5).

3. Discussion

Vibrio species are widespread in warm estuarine waters and include several potential human pathogens. Identifying the major *Vibrio* species

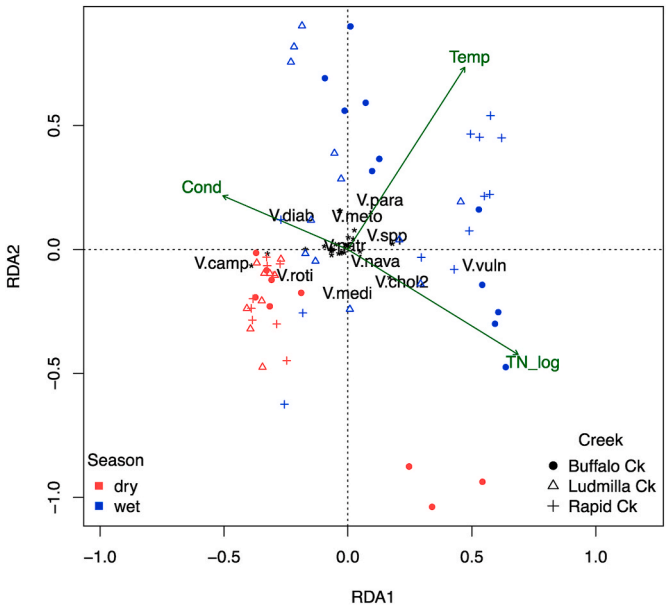

Fig. 4. RDA correlation biplot of the *hsp60 Vibrio* community and environmental variables. Type II scaling was used with angles between vectors including species reflecting their linear correlation. The RDA explained 32% of the *hsp60* variance of which 74% was explained by the first RDA axis and 15% by the second. To avoid overlapping text, the names of less abundant species were replaced with a star if close to a more abundant species (orditorp function). Species are *V. diabolicus* (V.diab), *V. campbellii* (V.camp), *V. rotiferianus* (V.roti), *V. mediterranei* (V.medi), *V. natriegens* (V.natri), *V. nereis* (V.nere), *V. cholerae* ASV2 (V.chol2), *V. parahaemolyticus* (V.para), *V. vulnificus* (V.vuln) and *Vibrio* other (V.spp).

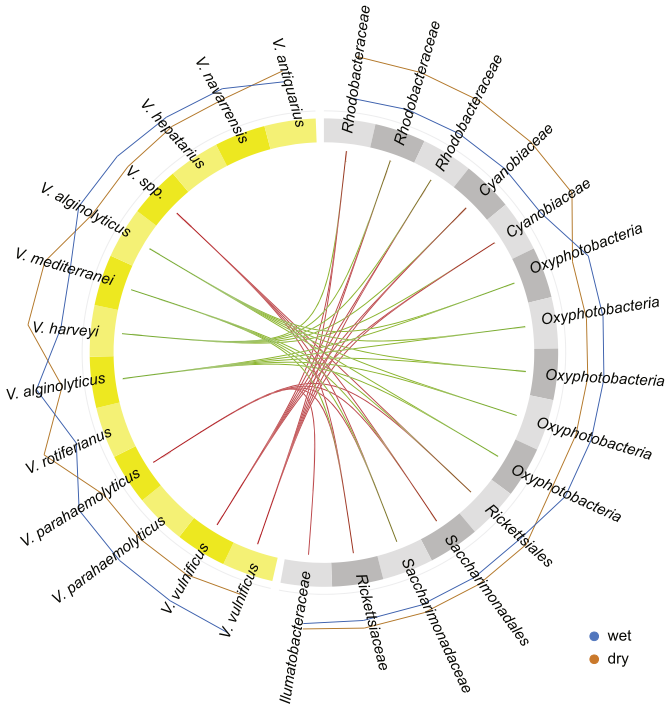

Fig. 5. CIRCOS plot based on sparse Diablo analysis showing ASVs from *Vibrio hsp60* sequences and 16S rRNA which were correlated with each other and which discriminated between seasons. Correlation cut-off is 0.6. Red lines show negative correlations and green lines show positive correlations. No lines mean a correlation of less than 0.6. Outer circles signify relative abundance in the dry season (——) and wet season (——).

and factors, that drive their abundance in the environment, is critical for assessing risk to human health. Across the tropical northern Australian sites examined here, potential human pathogens *V. parahaemolyticus*, *V. vulnificus* and *V. cholerae* were more abundant in the wet season corroborating previous studies (Gregoracci et al., 2012; Machado and Bordalo 2016; Wong et al., 2019). Moreover, although different methods are used for estimation, the abundance of *V. parahaemolyticus* was higher than for *V. vulnificus* and *V. cholerae*, which is also similar to other reports (Blackwell and Oliver 2008; Cantet et al., 2013; Hackbusch et al., 2020).

V. vulnificus abundance was positively associated with TN, TP, and turbidity, which is in contrast to many previous studies, which have identified temperature as the most significant driver of *V. vulnificus* variance (Blackwell and Oliver 2008; Johnson et al., 2010; Nigro et al., 2011). When water temperature range is optimal for *V. vulnificus* growth, turbidity, TN and TP gain relevance as factors explaining the abundance of *V. vulnificus*, suggesting an association with phytoplankton or suspended particles (Hughes et al., 1978; Blackwell and Oliver 2008). These data suggest that *V. vulnificus* and *V. cholerae* abundance could be influenced by environmental factors that are often associated with coastal run-off, including turbidity and inorganic nutrients, in some tropical habitats. The inability to detect certain *Vibrio* species under stressful conditions such as nutrient starvation could be due to cells entering a viable but not culturable (VBNC) state (Oliver et al., 2010). Therefore, sensitive and specific detection of potentially pathogenic *Vibrio* species including those in the VBNC state is important when addressing public health risks.

V. parahaemolyticus was detected more frequently than both *V. cholerae* or *V. vulnificus*. Given the strong, positive correlation to temperature reported in the literature for studies in temperate climates (DePaola et al., 1990; Blackwell and Oliver 2008; Julie et al., 2010) with a temperature range centred around 29 °C (Takemura et al., 2014), we had predicted that *V. parahaemolyticus* would be abundant along tropical coastlines. However, it is notable that even within constantly warm tropical waters, temperature still significantly influenced *V. parahaemolyticus* variance. This is consistent with some studies in the tropics showing significant positive correlations between temperature and *V. parahaemolyticus* (Machado and Bordalo 2016) but not when salinity was positively correlated with *V. parahaemolyticus* abundance (Rivas-Montaño et al., 2018). Conductivity, which is influenced by rainfall, was also a significant determinant of *V. parahaemolyticus* abundance, even though *V. parahaemolyticus* is known to tolerate a wide salinity range (Zimmerman et al., 2007).

In this study non toxigenic strains of *V. cholerae* were detected in 14% of samples, all from Buffalo Creek. Although, the reason for the restriction of *V. cholerae* to Buffalo Creek is unclear, this site is subject to the release of treated effluent upstream, which may favour conditions for the growth of environmental *V. cholerae* strains, either directly through dissolved nutrients or reduced salinity, or indirectly via increased phytoplankton biomass (Worden et al., 2006). *V. cholerae* abundance was influenced by temperature and sewage in the Bay of Bengal (Kopprio et al., 2020), while algal abundance and salinity were correlated with *V. cholerae* in Ecuador (Ryan et al., 2018). Future studies coupling *Vibrio* measurements with indicators for faecal contamination could provide more direct evidence for a link between sewage contamination and *Vibrio* dynamics within this environment.

The composition of the *Vibrio* community was far better resolved at the species level using a vibrio-specific amplicon sequencing approach targeting the *hsp60* gene (King et al., 2019) than standard 16S rRNA amplicon sequencing, with 42 *Vibrio* species identified, relative to 11 species using 16S rRNA sequencing. Several dominant *Vibrio* species were identified in this study depending on site and time of year. *V. campbellii* was the most abundant *Vibrio* species detected across the entire data-set, while *V. vulnificus* was the most abundant at all sampling sites. In other tropical studies, using different approaches to assess diversity, between 16 and 27 *Vibrio* species were identified with a range of

dominating species such as *V. harveyi*, *V. alginolyticus*, *V. brasiliensis*, *V. owensii*, *V. communis* and *V. rotiferianus* (Gregoracci et al., 2012; Vijayan and Lee 2014; Chimetto Tonon et al., 2015; Ortiz-Carrillo et al., 2015; Wong et al., 2019). In temperate regions, up to 22 *Vibrio* species have been identified (Zehr 2014; Amin et al., 2016; Jesser and Noble 2018; King et al., 2019; Liang et al., 2019; Wang et al., 2020).

The *Vibrio* community was more diverse in the wet season compared to the dry season across all three creeks, perhaps due to the more dynamic physicochemical conditions during the wet season (Williams et al., 2006). During the wet season, Darwin Harbour estuarine water is typically characterized by salinity gradients and higher nutrient loads from storms and run-off compared to the dry season (McKinnon et al., 2006; Williams et al., 2006). Moreover, recent evidence also highlights the importance of monsoon driven waves/wind driving sediment resuspension over and above tidal effects (Andutta et al., 2019). As *Vibrio* spp. commonly attach to solid particles (Lyons et al., 2007; Froelich et al., 2013), sediment resuspension may be a major factor determining shifts in *Vibrio* abundance in the water column within this environment, and warrants closer examination.

The *hsp60* amplicon sequencing assay confirmed the presence and provided the relative contribution of the three major human seafood pathogens, *V. parahaemolyticus*, *V. vulnificus* and *V. cholerae*. In addition, analysis of the whole *Vibrio* community was also useful for detecting other potential human pathogens associated with skin and tissue infections, such as *V. fluvialis*, *V. alginolyticus* and *V. mimicus*, and potential pathogens of other vertebrates and invertebrates such as *V. fortis*, *V. coralliilyticus, V. alginolyticus*, and *V. harveyi*. Thus, this new *Vibrio*-centric sequencing approach can be successfully used to screen recreational waters, aquaculture waters or wild fish/shellfish stock to identify dominant species which can then be more specifically targeted and quantified using other tools such as qPCR.

Seasonal differences in *V. parahaemolyticus*, *V. vulnificus* and *V. cholerae* were measured in the *hsp60* data, and were not only similar to findings based on qPCR data, but also allowed the dynamics of other species to be assessed. *V. parahaemolyticus* and *V. vulnificus* were abundant in the wet season, while *V. harveyi*, *V. campbellii* and other *Vibrio* species were abundant in the dry season. Differences in the *Vibrio* community between creeks were also observed with *V. cholerae* abundant in Buffalo Creek, while *V. harveyi* and *V. campbellii* were more relatively abundant in Rapid Creek and Ludmilla Creek, respectively. Precipitation and associated run-off leading to lower conductivity, higher nutrient loads and resuspension/turbidity, were the major variables influencing *Vibrio* community diversity between the wet and dry season with greater *Vibrio* diversity observed in the wet season. More frequent sampling in the wet season when physicochemical parameters are more variable may better resolve environmental drivers of *Vibrio* community composition and diversity during this time. Increased sampling frequency would also capture bloom events (Gilbert et al., 2011; Vijayan and Lee 2014) and community responses to storm events and heatwaves which may be missed with monthly sampling.

While storm events lead to the input of nutrients to Darwin Harbour (Skinner et al., 2009), the harbour is otherwise nitrogen limited (Burford et al., 2008). The greatest source of nitrogen into the estuary is from the open ocean with incoming tides (Burford et al., 2008), while terrestrial sources represent only 5–10% of the total nitrogen input and are associated with wet season rainfall (Fortune et al., 2020). However, between 2007 and 2017, terrestrial inputs from riverine, watershed and point sources have increased by 40%, as a consequence of increasing urbanisation (Fortune et al., 2020). Further pressure from anthropogenic processes is expected to lead to continued enrichment of coastal waters. Our work has shown that *V. vulnificus* responds to increased nitrogen levels. According to the RDA, *V. cholerae* and *V. parahaemolyticus* also responded to TN, although to a lesser extent, so we anticipate greater risk to human health from *V. vulnificus* with increased coastal eutrophication, particularly in the tropics where temperature does not have a growth-limiting effect. Algal blooms may also provide a resource for *Vibrio* species capable of feeding on algal polysaccharide exudates (Worden et al., 2006; Gilbert et al., 2011). In areas such as Buffalo Creek receiving treated effluent, the impact of algae on *Vibrio* populations needs to be further explored.

Microbial communities identified from 16S rRNA sequences showed a dominance of Rhodobacteraceae and Cyanobiaceae in the creek water, with Phormidiaceae dominating some Buffalo Creek samples. There were negative correlations of both *V. vulnificus* and *V. parahaemolyticus* to Cyanobacteria, while *V. alginolyticus* and *V. harveyi* were positively associated with Cyanobacteria. Notably, a previous study identified a negative correlation between *V. vulnificus* and Cyanobacteria in North Carolina seawater, but, in contrast to our data, a positive correlation between *V. parahaemolyticus* and Cyanobacteria was found (Jesser and Noble 2018). The relationship of particular *Vibrio* species with certain cyanobacteria or phytoplankton species should be further investigated especially if algal blooms enhance *Vibrio* growth and survival (Greenfield et al., 2017). Greater resolution of algal taxa, than that provided by short sequence reads, is needed to better understand the significance of these associations as correlations drawn at higher taxa levels may not adequately explain *Vibrio* species ecology (Main et al., 2015).

By combining qPCR and Vibrio-specific sequencing approaches this work has expanded our knowledge of *Vibrio* seasonality in the wet-dry tropics of northern Australia. All three tested potential human pathogens were more abundant during the wet season, with *V. parahaemolyticus* abundance correlated to temperature, whereas nutrients and turbidity best explained *V. vulnificus* variance. Taken together, these results allow us to predict high risk periods for vibriosis. We propose that tropical estuaries, which experience year-round temperature ranges that are ideal for *Vibrio* growth, as well as often elevated turbidity and inorganic nutrients, potentially require ongoing *Vibrio* surveillance programs to safeguard human health. Furthermore, given that the implications of *Vibrio* occurrence on aquaculture activities are less understood within this region than other tropical locations where intensive aquaculture activities occur, *Vibrio* monitoring should also be a high priority in any developing or existing aquaculture enterprise in northern Australian waters.

4. Materials and methods

4.1. Sites and sampling

Three tidal creek sites in Darwin Harbour, northern Australia (12°E, 130°S) were selected as sampling sites because of their use for recreational fishing and boat launching, as well as seafood harvesting (Fig. 1). Treated wastewater and wet season stormwater are discharged into the upper reaches of Buffalo Creek, approximately 7 km from the sampling site. The Ludmilla Creek site is approximately 2 km from an offshore wastewater outfall, but can be influenced by sewage overflow during high inflow events in the wet season. Rapid Creek is an urban creek with several storm water inputs. Samples were collected between January and August of 2018.

Water samples were collected in 1 L sterile bottles in triplicate and stored in an ice box for microbial analyses. Samples for TN and TP were collected in 250 ml HCl washed nutrient bottles, while 1 L amber bottles were used to collect samples for chlorophyll *a* analysis. Dissolved oxygen (DO), temperature, conductivity and pH were measured using a HYDROLAB® Quanta® water quality instrument. Nutrient samples were stored frozen prior to analysis.

Total phosphorus was measured by Kjeldahl digestion (reporting limit 0.003 mg/L) and total nitrogen was measured by persulphate digestion (reporting limit 0.02 mg/L) (Forensic and Health Services, Qld Govt). Chlorophyll *a* was measured by fluorometric detection adapted from the Trilogy® Laboratory Fluorometer (Turner Designs, San Jose, CA, USA) and standard acetone extraction methods (APHA, 2005), following filtration onto glass fiber filters. Calibrations were performed using stock concentrations of chlorophyll *a* (Sigma).

4.2. DNA extraction

Between 200 ml and 500 ml of seawater was filtered onto 0.45 μm filters using a Sartorius manifold. Volumes varied to accommodate the amount of particulate matter in the water. DNA was extracted using the FastDNA Spin kit for Soil (MP Biomedicals). DNA quality and quantity were measured using a NanoDrop™ (ThermoFisher Scientific).

4.3. Quantitative PCR

Standard curves for each pathogen were generated using DNA dilutions of pure *Vibrio* cultures: *V. vulnificus* ATCC® 27562 and *V. parahaemolyticus* ATCC® 17802 ($toxR^+/tlh^+/trh^+$) (Microbiologics, Minnesota, USA), *V. parahaemolyticus* ATCC® 43996 ($toxR^+/tlh^+/tdh^+$) (Jeremy Carson, Dept. Primary Industries, Parks, Water & Environment, Tasmanian Government), and *Vibrio cholerae* (clinical, non-pandemic isolate from Robert Baird, Royal Darwin Hospital, Pathology Laboratory). *Vibrio* cultures were grown overnight in alkaline peptide water (APW) at 35 °C and then serially diluted 10-fold. *Vibrio* concentrations were measured by plating onto tryptic soy agar (TSA) plates. A one ml portion of the undiluted overnight culture was boiled for 10 min, placed on ice for 10 min and then centrifuged for 1 min to obtain a crude DNA lysate template for PCR.

TaqMan probes were used for the quantification of *V. parahaemolyticus*, *V. vulnificus* and *V. cholerae* genes (PerfeCTa® qPCR ToughMix®). A SYBR Green assay was used for the quantification of total *Vibrio* spp (PerfeCTa® SYBR® Green SuperMix). Primers and probes for *V. parahaemolyticus*, *V. vulnificus* and *V. cholerae* were purchased from Integrated DNA Technologies (Table S4). Real time PCR was performed using a Rotor-Gene Q (Qiagen, Australia). Reaction and cycling conditions, primer and probe concentrations were performed as described in the literature (Table S4), and detailed in the supplementary material. Data was imported in JMP 12.0.1 (SAS Institute Inc, Buckinghamshire, UK) for plotting and further analysis. For quantification of environmental samples, 2 μl of DNA were used in a 20 μl reaction for each qPCR assay.

For quantification of environmental samples, 2 μl of DNA was used in each qPCR assay. Negative controls consisting of DNA extraction blanks (filter only) and qPCR blanks (no DNA added) were included in each run. Ct values of environmental samples were compared to the relevant standard curve for quantification, taking into account template volume, dilutions and seawater volume initially filtered.

4.4. Statistical analyses

Negative binomial models were conducted in R v4.0 and Stata/IC v15 (www.stata.com) to compare spatial and temporal *Vibrio* qPCR abundance, and to associate *V. parahaemolyticus* and *V. vulnificus* qPCR abundance data with environmental variables (TN, TP, temp, DO, conductivity or ln(turbidity+1)) while accounting for site and month. Coefficient estimates and P values, model AIC and pseudo-R^2 (McFadden) were calculated.

4.5. Amplicon sequencing

DNA was amplified using (a) 16S rRNA primers Bakt_341F and Bakt_805R which amplify the V3–V4 region (Herlemann et al., 2011) and (b) the *Vibrio*-centric *hsp60* primers Vib-hspF3-23 and Vib-hspR401-422, as previously described (King et al., 2019). Amplicons were sequenced using the Illumina MiSeq platform according to the manufacturer's guidelines (Ramaciotti Centre for Genomics). Raw data files in FASTQ format were deposited in NCBI Sequence Read Archive (SRA) under Bioproject number PRJNA616394.

Details of amplicon processing are given in the supplementary material. *Hsp60* and 16S rRNA gene sequences were analysed using the PhyloSeq package (McMurdie and Holmes 2013) in R (version 3.6.0 2017-06-30; Copyright (C) 2017 The R Foundation for Statistical Computing), to create abundance plots and rarefaction curves. The relative abundance of *Vibrio* species per sample were based on non-rarefied *hsp60* counts and normalized by total *Vibrio* qPCR values. For 16S rRNA gene sequences, all samples were rarefied to 10,160 sequences.

5. Sparse partial least squares discriminant analysis (sPLS-DA)

The relative abundance of all 42 ASVs (each summed to more than 0.01% of all ASVs) in the *hsp60* data was calculated and a CLR (logratio) transformation performed to account for the compositional nature of the data. Sparse Partial Least Squares Discriminant Analysis (sPLS-DA) was performed using the R package MixOmics (Lê Cao et al., 2009) as per http://mixomics.org/methods/pls-da/ to select the most discriminant ASVs for groups of interest. It is a particularly powerful method as highly correlated ASVs are predominantly selected with noisy ASVs excluded.

A sparse DIABLO analysis (Singh et al., 2019) was also conducted in MixOmics on the *hsp60* and 16S rRNA sequence data to tease out highly correlated ASVs which are able to discriminate between seasons. Results were visualized using CIRCOS (Krzywinski et al., 2009) and a correlation cut-off of 0.6.

A redundancy analysis (RDA) was performed on the Hellinger transformed *hsp60* sequences and normalized environmental data using the vegan package in R. ASVs which occurred in less than 4 samples were excluded (remaining 37 ASVs). TN was natural log transformed. TP and turbidity were excluded due to the high correlation with TN and for the latter, an extreme outlier. DO was excluded due to a negative correlation with temperature. The RDA model was tested for multicollinearity (variance inflation factors for all constrains were below 2), and only environmental variables significantly contributing to the model fit ($P < 0.05$) with higher scores for the first two RDA axes were included.

CRediT authorship contribution statement

Anna Padovan: Conceptualization, Validation, Investigation, Writing – original draft, Writing – review & editing. **Nachshon Siboni:** Validation, Resources, Data curation, Writing – review & editing. **Mirjam Kaestli:** Formal analysis, Data curation, Writing – review & editing, Visualization. **William L. King:** Methodology, Validation, Writing – review & editing. **Justin R. Seymour:** Resources, Writing – review & editing, Funding acquisition. **Karen Gibb:** Conceptualization, Resources, Writing – review & editing, Funding acquisition.

Declaration of competing interest

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.

Acknowledgements

This research was supported by Charles Darwin University internal grant and an Australian Research Council Linkage grant (LP 160101795) to JRS. We thank Zarah Tinning and Dion Lambrinidis for technical support.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marenvres.2021.105405.

References

Amin, A.K.M.R., Feng, G., Al-saari, N., Meirelles, P.M., Yamazaki, Y., Mino, S., Thompson, F.L., Sawabe, T., Sawabe, T., 2016. The first temporal and spatial assessment of *Vibrio* diversity of the surrounding seawater of coral reefs in Ishigaki, Japan. Front. Microbiol. 7, 90–12.

Andutta, F.P., Patterson, R.G., Wang, X.H., 2019. Monsoon driven waves superpose the effect from macro-tidal currents on sediment resuspension and distribution. Estuar. Coast Shelf Sci. 223, 85–93.

APHA, 2005. Standard Methods for the Examination of Water and Waste Water, 21st. American Public Health Association, Washington.

Asplund, M.E., Rehnstam-Holm, A.-S., Atnur, V., Raghunath, P., Saravanan, V., Härnström, K., Collin, B., Karunasagar, I., Godhe, A., 2011. Water column dynamics of *Vibrio* in relation to phytoplankton community composition and environmental conditions in a tropical coastal area. Environ. Microbiol. 13, 2738–2751.

Baker-Austin, C., Trinanes, J.A., Salmenlinna, S., Löfdahl, M., Siitonen, A., Taylor, N.G. H., Martinez-Urtaza, J., 2016. Heat wave–associated vibriosis, Sweden and Finland, 2014. Emerg. Infect. Dis. 22, 1216–1220.

Baker-Austin, C., Oliver, J.D., Alam, M., Ali, A., Waldor, M.K., Qadri, F., Martinez-Urtaza, J., 2018. *Vibrio* spp. infections. Nat. Rev. Dis. Prim. 1–20.

Baker-Austin, C., Trinanes, J., González-Escalona, N., Martinez-Urtaza, J., 2017. Non-cholera vibrios: the microbial barometer of climate change. Trends Microbiol. 25, 76–84.

Blackwell, K.D., Oliver, J.D., 2008. The ecology of *Vibrio vulnificus, Vibrio cholerae*, and *Vibrio parahaemolyticus* in North Carolina Estuaries. J. Microbiol. 46, 146–153, 3rd ed.

Bockemühl, J., Triemer, A., 1974. Ecology and epidemiology of *Vibrio parahaemolyticus* on the coast of Togo*. Bull. World Health Organ. 1–8.

Brierley, A.S., Kingsford, M.J., 2009. Impacts of climate change on marine organisms and ecosystems review. Curr. Biol. 19, R602–R614.

Burford, M.A., Alongi, D.M., McKinnon, A.D., Trott, L.A., 2008. Primary production and nutrients in a tropical macrotidal estuary, Darwin Harbour, Australia. Estuar. Coast Shelf Sci. 79, 440–448.

Cantet, Franck, Hervio-Heath, Dominique, Caro, Audrey, Le Mennec, Cecile, Monteil, Caroline, Quemere, Catherine, Jolivet-Gougeon, Anne, Colwell, Rita, Monfort, Patrick, 2013. Quantification of *Vibrio parahaemolyticus*, *Vibrio vulnificus* and *Vibrio cholerae* in French Mediterranean coastal lagoons. Res. Microbiol. 164, 867–874. https://doi.org/10.1016/j.resmic.2013.06.005.

Chimetto Tonon, L.A., Silva, B.S., Moreira, A.P., Valle, C., Alves Jr., N., Cavalcanti, G., Garcia, G., Lopes, R.M., Francini-Filho, R.B., de Moura, R.L., Thompson, C.C., Thompson, F.L., 2015. Diversity and ecological structure of vibrios in benthic and pelagic habitats along a latitudinal gradient in the Southwest Atlantic Ocean. PeerJ 3, e741. https://doi.org/10.7717/peerj.741. PMID: 25699199; PMCID: PMC4327252.

Criminger, J.D., Hazen, T.H., Sobecky, P.A., Lovell, C.R., 2007. Nitrogen fixation by *Vibrio parahaemolyticus* and its implications for a new ecological niche. Appl. Environ. Microbiol. 73, 5959–5961.

DePaola, A., Hopkins, L.H., Peeler, J.T., Wentz, B., McPhearson, R.M., 1990. Incidence of *Vibrio parahaemolyticus* in U.S. coastal waters and oysters. Appl. Environ. Microbiol. 56, 2299–2302.

Department of Agriculture and Water Resources. 2017. National aquaculture Strategy. Canberra, August. CC BY 4.0.

Feldhusen, F., 2000. The role of seafood in bacterial foodborne diseases. Microb. Infect. 2, 1651–1660.

Fleming, A.E., 2015. Improving business investment confidence in culture-aligned Indigenous economies in remote Australian communities: a business support framework to better inform government programs. IIPJ 6, 175–38.

Ford, C.L., Powell, A., Lau, D.Y.L., Turner, A.D., Rapkova, M.D., Martinez-Urtaza, J., Baker-Austin, C., 2020. Isolation and characterization of potentially pathogenic *Vibrio* 3 species in a temperate, higher latitude hotspot. Environ. Microbiol. Rep. 12, 424–434.

Fortune, J., Butler, E.C.V., Gibb, K., 2020. A decade of nitrogen inputs to a tropical macrotidal estuary of Northern Australia, Darwin Harbour. Reg. Stud. Mar. Sci. 36, 101275.

Froelich, B., Ayrapetyan, M., Oliver, J.D., 2013. Integration of *Vibrio vulnificus* into marine aggregates and its subsequent uptake by *Crassostrea virginica* oysters. Appl. Environ. Microbiol. 79, 1454–1458 third ed.

Froelich, B.A., Daines, D.A., 2020. In hot water: effects of climate change on Vibrio–human interactions. Environ. Microbiol. 22, 4101–4111.

Gilbert, J.A., Steele, J.A., Caporaso, J.G., ck, L.S.U., Reeder, J., Ben Temperton, Huse, S., McHardy, A.C., Knight, R., Joint, I., Somerfield, P., Fuhrman, J.A., Field, D., 2011. Defining seasonal marine microbial community dynamics. ISME J. 6, 298–308.

Giubergia, S., Phippen, C., Nielsen, K.F., Gram, L., 2017. Growth on chitin impacts the transcriptome and metabolite profiles of antibiotic-producing *Vibrio coralliilyticus* S2052 and *Photobacterium galatheae* S2753. mSystems 2, 387–12.

Greenfield, D.I., Gooch Moore, J., Stewart, J.R., Hilborn, E.D., George, B.J., Li, Q., et al., 2017. Temporal and environmental factors driving *Vibrio vulnificus* and *V. parahaemolyticus* populations and their associations with harmful algal blooms in South Carolina detention ponds and receiving tidal creeks. GeoHealth 1, 306–317. https://doi.org/10.1002/2017GH000094.

Gregoracci, G.B., Nascimento, J.R., Cabral, A.S., Paranhos, R., Valentin, J.L., Thompson, C.C., Thompson, F.L., 2012. Structuring of bacterioplankton diversity in a large tropical bay. PloS One 7, e31408–e31411.

Hackbusch, S., Wichels, A., Gimenez, L., pke, H.D., Gerdts, G., 2020. Potentially human pathogenic *Vibrio* spp. in a coastal transect: occurrence and multiple virulence factors. Sci. Total Environ. 707, 136113.

Heath, Christopher, Garrow, Stuart, Golledge, Clayton, 2001. Non-O1 *Vibrio cholerae*: a fatal caue of sepsis in northern Australia. Med. J. Aust. 174, 480–481. https://doi.org/10.5694/j.1326-5377.2001.tb143383.x.

Herlemann, D.P., Labrenz, M., Jürgens, K., Bertilsson, S., Waniek, J.J., Andersson, A.F., 2011. Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea. ISME J. 5, 1571–1579.

Hughes, J.M., Hollis, D.G., Gangarosa, E.J., Weaver, R.E., 1978. Non-cholera vibrio infections in the United States. Clinical, epidemiologic, and laboratory features. Ann. Intern. Med. 88, 602–606.

Jesser, K.J., Noble, R.T., 2018. *Vibrio* ecology in the Neuse River Estuary, North Carolina, characterized by next-generation amplicon sequencing of the gene encoding heat shock protein 60 (*hsp60*). Appl. Environ. Microbiol. 84 e00333–18–21.

Johnson, C.N., Flowers, A.R., Noriea III, N.F., Zimmerman, A.M., Bowers, J.C., DePaola, A., Grimes, D.J., 2010. Relationships between environmental factors and pathogenic vibrios in the Northern Gulf of Mexico. Appl. Environ. Microbiol. 76, 7076–7084.

Jones, M.K., Oliver, J.D., 2009. *Vibrio vulnificus*: disease and pathogenesis. IAI 77, 1723–1733.

Julie, D., Solen, L., Antoine, V., Jaufrey, C., Annick, D., Dominique, H.-H., 2010. Ecology of pathogenic and non-pathogenic *Vibrio parahaemolyticus* on the French Atlantic coast. Effects of temperature, salinity, turbidity and chlorophyll a. Environ. Microbiol. 12, 929–937.

King, W.L., Siboni, N., Kahlke, T., Green, T.J., Labbate, M., Seymour, J.R., 2019. A new high throughput sequencing assay for characterizing the diversity of natural *Vibrio* communities and its application to a Pacific oyster mortality event. Front. Microbiol. 10, 1–13.

Kopprio, G.A., Neogi, S.B., Rashid, H., Alonso, C., Yamasaki, S., Koch, B.P., Gärdes, A., Lara, R.J., 2020. *Vibrio* and bacterial communities across a pollution gradient in the Bay of Bengal: unraveling their biogeochemical drivers. Front. Microbiol. 11, 594.

Krzywinski, M., Schein, J., Birol, I., Connors, J., Gascoyne, R., Horsman, D., Jones, S.J., Marra, M.A., 2009. Circos: an information aesthetic for comparative genomics. Genome Res. 19, 1639–1645.

Lê Cao, K.-A., Martin, P.G., Robert-Granié, C., Besse, P., 2009. Sparse canonical methods for biological data integration: application to a cross-platform study. BMC Bioinf. 10, 321–17.

Liang, J., Liu, J., Wang, X., Lin, H., Liu, J., Zhou, S., Sun, H., Zhang, X.-H., 2019. Spatiotemporal dynamics of free-living and particle-associated Vibrio communities in the northern Chinese marginal seas. Appl. Environ. Microbiol. 85, 4103–4116.

Logar-Henderson, C., Ling, R., Tuite, A.R., Fisman, D.N., 2019. Effects of large-scale oceanic phenomena on non-cholera vibriosis incidence in the United States: implications for climate change. Epidemiol. Infect. 147, e243. https://doi.org/10.1017/S0950268819001316.

Lyons, M.M., Lau, Y.-T., Carden, W.E., Ward, J.E., Roberts, S.B., Smolowitz, R., Vallino, J., Allam, B., 2007. Characteristics of marine aggregates in shallow-water ecosystems: implications for disease ecology. EcoHealth 4, 406–420.

Machado, A., Bordalo, A.A., 2016. Detection and quantification of *Vibrio cholerae, Vibrio parahaemolyticus*, and *Vibrio vulnificus* in coastal waters of Guinea-Bissau (west Africa). EcoHealth 13, 339–349.

Main, C.R., Salvitti, L.R., Whereat, E.B., Coyne, K.J., 2015. Community-level and species-specific associations between phytoplankton and particle-associated *Vibrio* species in Delaware's inland bays. Appl. Environ. Microbiol. 81, 5703–5713.

McAuliffe, G.N., Baird, R.W., Hennessy, J., 2015. Relative frequency, characteristics, and antimicrobial susceptibility patterns of *Vibrio* spp., *Aeromonas* spp., *Chromobacterium violaceum*, and *Shewanella* spp. in the Northern Territory of Australia, 2000–2013. Am. J. Trop. Med. Hyg. 92, 605–610.

McKinnon, A.D., Smit, N., Townsend, S., Duggan, S., 2006. Darwin harbour: water quality and ecosystem structure on a tropical harbour in the early stages of urban development. In: The Environment in Asia Pacific Harbours. Springer, Netherlands, pp. 433–459.

McMurdie, P.J., Holmes, S., 2013. phyloseq: an R Package for reproducible interactive analysis and graphics of microbiome census data. PloS One 8 e61217–11.

Morantz, C., 2005. CDC reports on illnesses in Hurricane Katrina evacuees and relief workers. Am. Fam. Physician 72 (10), 2132.

Nigro, O.D., Hou, A., Vithanage, G., Fujioka, R.S., Steward, G.F., 2011. Temporal and spatial variability in culturable pathogenic *Vibrio* spp. in Lake Pontchartrain, Louisiana, following hurricanes Katrina and Rita. Appl. Environ. Microbiol. 77, 5384–5393 first ed.

Nowland, S.J., O'Connor, W., Southgate, P.C., 2018. Embryonic, larval, and early postlarval development of the tropical black-lip rock oyster *Saccostrea echinata*. J. Shellfish Res. 37, 73–77.

Oliver, J.D., 2005. Wound infections caused by *Vibrio vulnificus* and other marine bacteria. Epidemiol. Infect. 133, 383–391.

Oliver, J.D., 2010. Recent findings on the viable but nonculturable state in pathogenic bacteria. FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Rev. 34, 415–425.

Ortiz-Carrillo, Neyi, Eloisa E-G, Marcela, Z.-M., Rafael, R.-H., 2015. Diversity of *Vibrio* spp in Karstic coastal Marshes in the Yucatan Peninsula. PloS One 1–14.

Padovan, A., Kennedy, K., Rose, D., Gibb, K., 2019. Microbial quality of wild shellfish in a tropical estuary subject to treated effluent discharge. Environ. Res. 108921.

Ralph, A., Currie, B.J., 2007. *Vibrio vulnificus* and *V. parahaemolyticus* necrotising fasciitis in fishermen visiting an estuarine tropical northern Australian location. J. Infect. 54, e111–e114.

Rehnstam-Holm, A.S., Atnur, V., Godhe, A., 2013. Defining the niche of *Vibrio parahaemolyticus* during pre- and post-monsoon seasons in the coastal Arabian Sea. Microb. Ecol. 67, 57–65 second ed.

Rivas-Montaño, A.M., Luis-Villasenor, I.E., Pina-Valdez, P., Gomez-Gil, B., Lizarraga-Partida, M.L., 2018. Spatiotemporal distribution of *Vibrio parahaemolyticus* in

relation to environmental parameters in a coastal lagoon on the Pacific coast of northwestern Mexico. Cienc. Mar. 44, 141–153.
Ryan, S., Stewart-Ibarra, A., Ordóñez-Enireb, E., Chu, W., Finkelstein, J., King, C., Escobar, L., Lupone, C., Heras, F., Tauzer, E., Waggoner, E., James, T., Cárdenas, W., Polhemus, M., 2018. Spatiotemporal variation in environmental *Vibrio cholerae* in an estuary in southern Coastal Ecuador. Int. J. Environ. Res. Publ. Health 15, 486–13.
Singh, A., Shannon, C.P., Gautier, B., Rohart, F., Vacher, M., Tebbutt, S.J., Lê Cao, K.-A., 2019. DIABLO: an integrative approach for identifying key molecular drivers from multi-omics assays. Bioinformatics 35, 3055–3062.
Skinner, L., Townsend, S., Fortune, J., 2009. The impact of urban land use on total pollutant loads entering Darwin Harbour. In: Report 06/2008DDepartment of Natural Resources, Environment, the Arts and Sport.
Takemura, A.F., Chien, D.M., Polz, M.F., 2014. Associations and dynamics of Vibrionaceae in the environment, from the genus to the population level. Front. Microbiol. 5, 38.
Thompson, F.L., Iida, T., Swings, J., 2004. Biodiversity of vibrios. MMBR (Microbiol. Mol. Biol. Rev.) 68, 403–431.
Vijayan, M., Lee, C.W., 2014. Seasonality and diversity of culturable vibrios in tropical coastal waters. BMS 90, 599–610.
Wang, X., Liu, J., Liang, J., Sun, H., Zhang, X.-H., 2020. Spatiotemporal dynamics of the total and active *Vibrio* spp. populations throughout the Changjiang estuary in China. Environ. Microbiol. 46, 480–18.
Walker, J.D., Colwell, R.R., 1976. Enumeration of petroleum-degrading microorganisms. Appl. Environ. Microbiol. 31, 198–207.
Williams, D., Wolanski, E., Spagnol, S., 2006. Hydrodynamics of Darwin harbour, pp. 461–476. In: Wolanski, E. (Ed.), The Environment in Asia Pacific Harbours. Springer-Verlag, Berlin/Heidelberg.
Wong, Y.Y., Lee, C.W., Bong, C.W., Lim, J.H., Narayanan, K., Sim, E.U.H., 2019. Environmental control of *Vibrio* spp. abundance and community structure in tropical waters. FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Ecol. 95, 403 third ed.
Worden, A.Z., Seidel, M., Smriga, S., Wick, A., Malfatti, F., Bartlett, D., Azam, F., 2006. Trophic regulation of *Vibrio cholerae* in coastal marine waters. Environ. Microbiol. 8, 21–29.
Zehr JP. 2014. Vibrio Diversity and Dynamics in the Monterey Bay Upwelling Region 1–9.
Zimmerman, A.M., DePaola, A., Bowers, J.C., Krantz, J.A., Nordstrom, J.L., Johnson, C. N., Grimes, D.J., 2007. Variability of total and pathogenic *Vibrio parahaemolyticus* densities in Northern Gulf of Mexico water and oysters. Appl. Environ. Microbiol. 73, 7589–7596.