Relationship of aquatic environmental factors with the abundance of *Vibrio cholerae, Vibrio parahaemolyticus, Vibrio mimicus* and *Vibrio vulnificus* in the coastal area of Guaymas, Sonora, Mexico

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ABSTRACT

Members of the genus *Vibrio* are common in aquatic environments. Among them are *V. cholerae*, *V. vulnificus*, *V. parahaemolyticus* and *V. mimicus*. Several studies have shown that environmental factors, such as temperature, salinity, and dissolved oxygen, are involved in their epidemiology. Therefore, the main objective of this study is to determine if there is a correlation between the presence/amount of *V. cholerae*, *V, vulnificus*, *V. parahaemolyticus* and *V. mimicus* and the environmental conditions of the seawater off the coast of Guaymas, México. Quantification of all four pathogenic bacteria was performed using the most probable number method, and suspected colonies were identified by polymerase chain reaction (PCR). Correlations were found using principal component analysis. *V. parahaemolyticus* was the most abundant and widely distributed bacteria, followed by *V. vulnificus*, *V. mimicus* and *V. cholerae*. Positive correlations between *V. parahaemolyticus*, *V. vulnificus* and *V. mimicus* with temperature, salinity, electric conductivity, and total dissolved solids were found. The abundance of *V. cholerae* was mainly affected by the sampling site and not by physicochemical parameters.

Key words | environmental factors, *V. cholerae*, *V. mimicus*, *V. parahaemolyticus*, *V. vulnificus*

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INTRODUCTION

Vibrio species naturally reside in aquatic and marine environments. They are found in waters that are deep and coastal, as well as temperate and tropical waters. They are present in fish and seafood products, along with birds and herbivores from the surrounding coastal areas (Thompson *et al.* 2004; Urakawa & Rivera 2006). *Vibrio cholerae*, *V. vulnificus*, *V. parahaemolyticus*, and more recently *V. mimicus*, have been associated with gastrointestinal and skin infections, as well as septicemia (Ottaviani *et al.* 2003, 2005; Austin 2010). Infections are normally associated with ingestion of contaminated seafood and raw molluscan shellfish (Rowe-Magnus *et al.* 2006).

Seasonal fluctuations in environmental conditions have been well documented for *Vibrio* species (FAO/WHO 2005a). Their abundance spikes during the warm season due to the high water temperature (Pfeffer *et al.* 2003). In tropical and semi-desertic areas with stable temperatures throughout the year, *Vibrio* prevalence is mainly affected by the levels of organic material and the dry season (Deepanjali *et al.* 2005). Biofilm formation, or association with other marine organisms, has been suggested as a survival mechanism (Leyton & Riquelme 2008) used during unfavorable conditions (Lipp *et al.* 2002). These organisms have been reported to colonize crustacean chitin, algae,

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phytoplankton, copepods, and aquatic plant roots (Fernández & Alonso 2009).

The effect of physicochemical environmental factors has been widely studied for pathogenic *Vibrio* species, especially for *V. cholerae*. The potential significance of environmental factors on the dynamics of the disease caused by *V. cholerae* has been reported in several studies, with temperature and salinity being the major factors affecting its ecology (Pardio-Sedas 2007). Generally, *Vibrio* species are more common in warm waters, particularly when the water temperature is higher than 17 °C (Thompson *et al.* 2004). Additionally, a positive correlation between *V. cholerae* and salinity has been reported (Huq *et al.* 2005). Such environmental factors, climate change and climate variability affect growth and reproduction in the marine environment (Gerba 2009).

Most *Vibrio* species isolated from marine environments are non or low virulent strains (Farfán *et al.* 2000; Cabrera-García *et al.* 2004; Faruque *et al.* 2004), but they are also capable of producing human infection. As an example, *V. cholerae* O1 and O139 are recognized as toxigenic and are associated with endemic cholera, but non-O1/non-O139 serogroups can also cause human illness, and produce gastroenteritis and or extraintestinal infections (Calduch Broseta *et al.* 2003; González Fraga *et al.* 2009). During 2003–2005, thirty-four isolates of *V. cholerae* non-O1 and non-O139 were found in diarrhea stool samples in Argentina. None of the isolates showed the cholera toxin gen (*ctx*A) or thermostable toxin gen (*stn/stp*), but all included the *tox*R gen (González Fraga *et al.* 2009). Virulence factors can be horizontally transferred in the aquatic environment (Zo *et al.* 2002; Chun *et al.* 2009) and it has been suggested that 'each *V. cholerae* cell would have the same probability of being transformed into a toxigenic strain' (Farfán *et al.* 2000). Although the mechanisms involved in the emergence of new toxigenic strains have not been completely elucidated, it has been suggested that genetic changes and natural selection affected by environmental factors could be involved in this process (Boyd *et al.* 2000; Vora *et al.* 2005).

The complex ecology of pathogenic *Vibrio* species has been reported to impact their occurrence and abundance in the marine environment and has been associated with its potential to cause human infection (Gerba 2009). It has also been suggested that bacteria–bacteria interactions are specific and regulate the proliferation of some species in the aquatic environment (Sotomayor & Balcázar 2003). The interaction of bacteria with the environment and the environmental effects on their role in disease transmission are important research topics with implications in the epidemiology of pathogenic *Vibrio* species such as *V. cholerae*, *V. parahaemolyticus*, *V. vulnificus* and *V. mimicus*.

In Mexico, only *V. cholerae* is under continuous surveillance by health authorities, and just human positive isolates of *V. cholerae* O1 and O139 are considered for statistics purposes (NOM-016-SSA2-1994). However, other *Vibrio* species have been involved in well-documented cases and outbreaks (Porras-Cortés *et al.* 1994; Castañón-González *et al.* 2000; Cornejo-Juárez *et al.* 2000; Cabrera-García *et al.* 2004; Cabanillas-Beltrán *et al.* 2006). Official statistics consider only nine sporadic cholera cases in the last 12 years (2000–2012) (CoNaVe 2012), but local health authorities recognize an annual average of suspected cholera cases between 3,000 and 4,000, with a minimal positive cases (El Heraldo de Tabasco 2011); probably caused for other *Vibrio* species, including *V. cholerae* non-O1 and non-O139. Therefore, understanding the behavior or abundance pattern of potentially pathogenic *Vibrio* species in recreational or fishing activities seawater is important, especially when the consumption of raw or not fully cooked seafood is a common practice in the region.

METHODS

Study area and sampling

Guaymas is located in the southwest part of the Estate of Sonora in the northwestern Mexico. The study area is located in the surrounding coastal waters of Guaymas in the Gulf of California (Figure 1). The climate of the region is hot and dry with rainy summers (<100 mm). The main human activities that take place are fishing, fishing industry, commerce and tourism.

Water samples were collected monthly at high tide from February to August 2011. Samples were collected at six different points along the coastal area of Guaymas, Sonora Mexico (Figure 1), covering all six points on a 2-day basis.

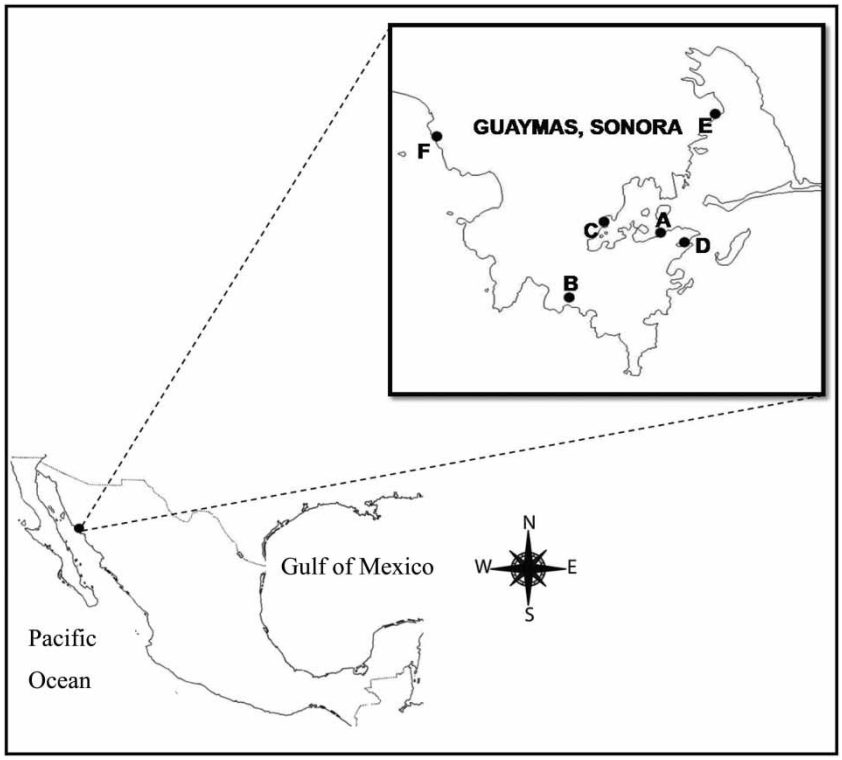

Figure 1 | Sampling area (Guaymas, Sonora, Mexico coastal area); pinpoints represent each sampling site.

Inside (A, C and E) and outside (B, D and F) bay points were considered, covering the main human activities. The temperature (T-°C), salinity (S-psu), conductivity (C-mS/cm), pH, total dissolved solids (TDS–g/L), dissolved oxygen (DO–mg/L), REDOX potential (mV) of the water body and the barometric pressure (P–mmHg) were determined *in situ* (YSI 556). Samples were aseptically collected in sterile plastic bags and transported to the laboratory in insulated boxes to maintain low temperature. Samples were processed within 4 h of collection.

Bacteriological and polymerase chain reaction (PCR) analysis

Samples were analyzed based on the Bacteriological Analytical Manual (Kaysner & DePaola 2004) for the isolation of potential *V. cholerae, V. parahaemolyticus, V. vulnificus* and *V. mimicus.* Control strains were acquired from the Collection of Aquatic Important Microorganisms (CAIM, www.ciad.mx/caim); CAIM 1409 – *V. cholerae*, CAIM 320^{T} – *V. parahaemolyticus*, CAIM 602^{T} – *V. mimicus,* and CAIM 612 – *V. vulnificus.* Samples (50 mL) were diluted with 450 mL of PBS (phosphate buffered saline), and additional tenfold dilution (up to 10^{-6}) were prepared. Three-tubes with 2X alkaline peptone water (APW) were inoculated with 1 mL of the first dilution and three-tubes of APW with the six ten-fold dilution. All tubes were incubated partial (6–8 h) and complete (24 h) at 35–37 °C before transferring to TCBS (thiosulfate-citrate-bile salts-sucrose, Difco^{TM}, Becton, Dickinson and Company, Sparks, MD, USA) agar plates and incubated at 35–37 °C overnight. All yellow and green isolated colonies from each plate were selected recording the tube number and dilution. Selected colonies were inoculated onto trypticase soy agar, and the resultant pure, isolated colonies were used for the oxidase test. Only oxidase positive isolates were used for the multiplex PCR identification. The most probable number (MPN) for each *Vibrio* species was calculated based on the PCR identification considering the original dilution and tube number.

For PCR identification, pure oxidase positive colonies and control strains were grown overnight in APW, 1 mL was boiled and centrifuged, and finally 5 μL of supernatant

was used as crude DNA template (Blackstone *et al.* 2003). PCR amplification was performed in the Gene Amp PCR System 9700 thermal cycler using a final reaction volume of 50 μL (5 μL of crude DNA template, 10 μL of buffer 5X (Colorless Go Taq Promega), 1 μL of dNTP mix 10 mM (Promega), 3 μL of 25 mM $MgCl_2$ (Promega), 1 μL of each primer at 100 pM (Invitrogen) and 0.4 μL of *Taq* polymerase (5 U/μL Go Taq Flexi Promega). Target sequences, amplicon size and primers sequences used in this study are listed in Table 1. These primers have been previously reported as species-specific and are suggested for the identification of *V. cholerae* (Nandi *et al.* 2000), *V. parahaemolyticus* (Bej *et al.* 1999), *V. mimicus* (Shi *et al.* 2000), and *V. vulnificus* (Wang *et al.* 1997).

Two multiplex PCR reactions were performed, one for *V. parahaemolyticus–V. mimicus* and the second for *V. cholerae–V. vulnificus*. Both reactions were conducted using the same temperature profile: 95 °C–2 min; 35 cycles: 95 °C–45 s, 54 °C–45 s, 72 °C–45 s; and finally 72 °C–5 min. The PCR fragments were visualized by gel electrophoresis in 1.5% agarose gel (Sigma-Aldrich) (50 min, 100 V). All yellow and green oxidase positive not identified by PCR as any of the four target *Vibrio* species were marked as unidentified isolates and were considered 'presumptive *Vibrio* spp.' for the purposes of this study.

Statistical analysis

Environmental factors, sample site and bacterial population (Log MPN) of presumptive *Vibrio* spp., *V. parahaemolyticus*, *V. vulnificus*, *V. cholerae* and *V. mimicus* were used for PCA analysis (principal component analysis) using JMP 9.0.2 (2010 SAS Institute Inc.). A regression analysis (Model XY) including the first four principal components and the *Vibrio* species populations (Log MPN/mL) was performed.

RESULTS AND DISCUSSION

Physicochemical parameters

The temperature (T), electrical conductivity (EC) and barometric pressure (Bar Press) observed in the coastal zone of Guaymas, Mexico each showed seasonal variation with minimal fluctuations between sites at the same time. Temperature varied from 14.4 °C in February to 33.7 °C and 32.5 °C in July and August, respectively. EC show the lowest values from February to May and gradual increases from 44.5 up to 65.8 ms/CM in July (Figure 2).

A high variation between sampling sites was observed for DO and REDOX potential (ORP). DO varied from 4.7 to 15.5 mg/L, showing the higher values in May, whereas the ORP values always corresponded to aerobic conditions, varying from 73.7 to 301.5 mV (Figure 2). Stable measures with minimal differences between sites and sampling period were observed for pH (7.9–8.4), salinity (S) (29.6–37.9 psu), and TDS (29.7–37.2), which agrees with previous reports for S, pH in the Guaymas area (Roden 1958; García-Rico *et al.* 2011).

Normal temperature variations were found for the study area, in which annual average values ranging from 16 to

Table 1 | List of species-specific primers and target genes used for each species including amplicon size (pb) and reference

Species	Target gen	Amplicon size (pb)	Primer sequences	Reference
V. cholerae	*omp*W (outer membrane protein)	588	*F* caccaagaaggtgactttattgtg *R* gaacttataaccacccgcg	Nandi *et al.* (2000)
V. parahaemolyticus	*tl (*thermolabile hemolysin*)*	450	*F* aaagcggattatgcagaagcactg *R* gctactttctagcattttctctgc	Bej *et al.* (1999)
V. mimicus	*Vmh* (hemolysin)	389	*F* ggtagccatcagtcttatcacg *R* atcgtgtcccaatacttcaccg	Shi *et al.* (2000)
V. vulnificus	*Vvh* (cytolysin gene)	383	*F* ctcactggggcagtggct *R* ccagccgttaaccgaacca	Wang *et al.* (1997)

F, forward.
R, reverse.

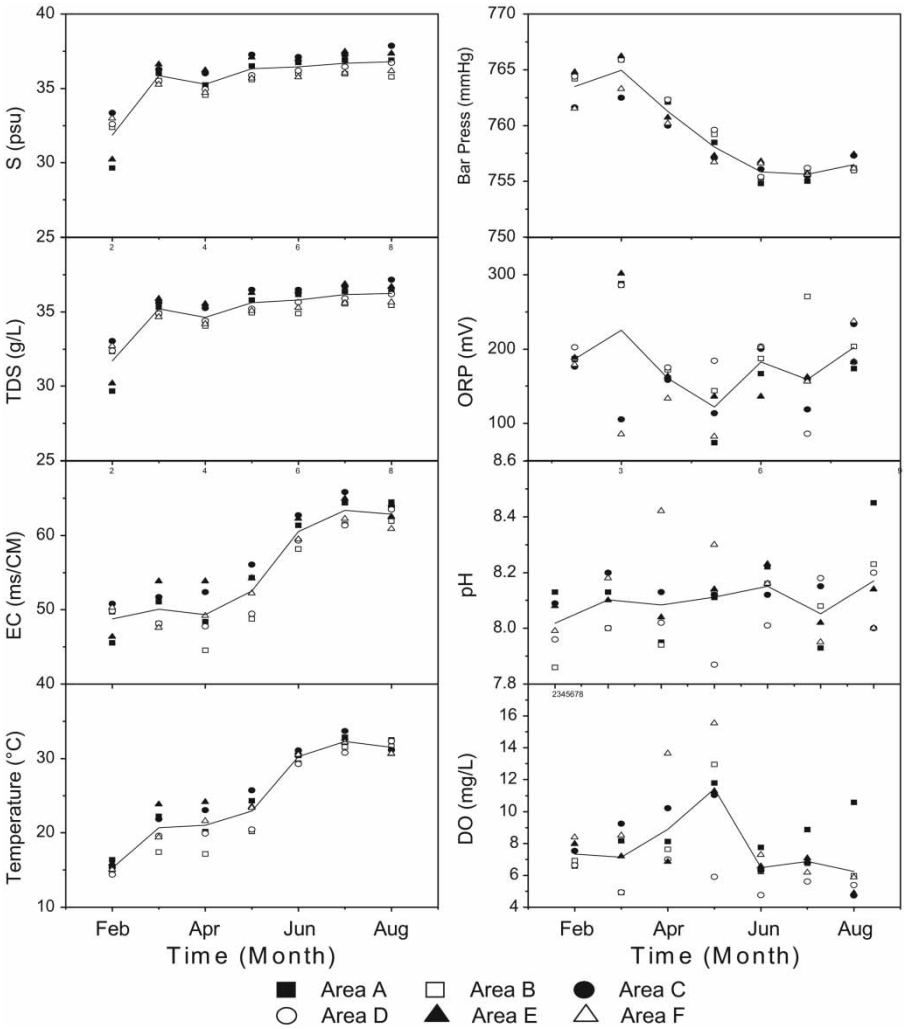

Figure 2 | Physicochemical parameters by area and sampling time. Temperature (°C), electrical conductivity (EC-mS/cm), total dissolved solids (TDS-g/L), salinity (S-psu), dissolved oxygen (DO-mg/L), pH, REDOX-ORP (ORP-mV) and barometric pressure (Bar Press-mmHg) from February to August, 2011 for all sampling areas (A to F). Line is constructed by the arithmetic mean of the parameter for all areas at the same sampling time.

32 °C have been reported (Roden 1958). The DO fluctuations were also similar to those previously reported by García-Rico *et al.* (2011). Because dissolved oxygen fluctuations are a result of water turbulence, temperature, photosynthetic organisms and sediments, as well as their oxygen consumption and organic matter oxidation, the negative correlation found between DO and ORP was expected. A positive correlation was found between T and DO, TDS and S, and a negative correlation with P was found. Correlations between DO, pH and ORP were also observed. The sampling site did not correlate with any of the measured parameters because similar conditions were found at the same time at different sampling sites. In general, physicochemical parameters showed normal variations in the study area; therefore, they could not atypically affect the *Vibrio* population in this study.

Vibrio species

A total 1,295 isolates of suspected *Vibrio* species were isolated from seawater samples. Most of the isolates were unidentified ('Presumptive *Vibrio* spp.' – 62%), only 31% corresponds to *V. parahaemolyticus*, 5% to *V. vulnificus*, while *V. cholerae* and *V. mimicus* represented less than 1% of the isolates. *V. parahaemolyticus* was the most abundant identified bacteria at all points, with a

Table 2 | Maximum and minimum bacterial load (MPN/mL) found at each sampling site for presumptive *Vibrio* spp., *V parahaemolyticus* (*Vp*), *V. vulnificus* (*Vv*), *V. cholerae Vc* and *V. mimicus* (*Vm*)

Sampling site	*Vibrio* spp. MPN/mL	*Vp* MPN/mL	*Vv* MPN/mL	*Vc* MPN/mL	*Vm* MPN/mL
A	23–2,400	ND–240	ND–3.6	< 0.3	ND–7.3
B	< 0.3–240	ND–9.3	ND–3.6	ND–0.36	> 0.3
C	1.5–2,400	ND–2,400	ND–4.3	ND	ND–0.3
D	3.6–2400	ND–430	ND–4.3	ND–0.36	ND
E	23–9,300	ND–93	ND–3.6	ND–0.36	ND–0.36
F	3.6–240	ND–240	ND–0.3	ND–3.6	ND

ND, non-detected; <0.3, non-detected by MPN method, but isolated by direct plating.

maximum value of 2,400 MPN/mL in sampling site C. The other identified *Vibrio* species were much less abundant with a higher value of 7.3 MPN/mL for *V. mimicus* (sampling site A), 4.3 MPN/mL for *V. vulnificus* (sampling sites C and D) and 3.6 MPN/mL for *V. cholerae* (sampling site F) (Table 2). Similar situations have been reported in Europe and the Americas, where *V. parahaemolyticus* predominates over *V. cholerae* and *V. vulnificus* (Deter *et al.* 2010). This is different from African countries such as Nigeria, where *V. cholerae* is the most abundant and frequently isolated *Vibrio* species (Abedayo-Tayo *et al.* 2011).

Presumptive *Vibrio* spp. were distributed consistently at all times and sampling areas, showing similar abundance patterns, except in sampling area C. Their abundance was most influenced by TDS, S and abundance of *V. parahaemolyticus* (Figure 3), than any other parameter. *V. parahaemolyticus* and *V. vulnificus* were distributed among all sampling sites, while *V. cholerae* and *V. mimicus* were found at just five and four sampling sites, respectively (Table 2). Minimal and maximum value for each physicochemical parameter at which *V. parahaemolyticus*, *V. vulnificus*, *V. cholerae* and *V. mimicus* were found are presented in Table 3. The presence of all four *Vibrio* species in water and marine products has been well documented worldwide (Noriega-Orozco *et al.* 2007; Collin & Rehnstam-Holm 2011; Gavilán & Martínez-Urtaza 2011). Their presence in waters surrounding Guaymas needs more attention, because at three of the sampling sites the extraction of products intended for human consumption is common. Although, detection of virulence factors was not part of this study, it is important to note that *V. parahaemolyticus* was the most abundant *Vibrio* species found, and local health authorities have detected an increase of diarrhea cases associated with the consumption of raw shrimp and *V. parahaemolyticus* (Benitez Cabrera 2012). Also, from 2005 to 2008 the health authorities in Mexico confirmed 547 cases of gastroenteritis caused by *V. parahaemolyticus* (Flores Sosa 2010).

Temperature

'Presumptive *Vibrio* spp.', *V. parahaemolyticus*, *V. vulnificus* and *V. cholerae* were isolated over a wide range of temperatures (Table 3, Figure 4), coinciding with other studies where seasonality and high temperature promote the growth of these bacteria (DePaola *et al.* 2000; Pfeffer *et al.* 2003; Blackwell & Oliver 2008; Sudhanandh *et al.* 2010). In contrast, *V. mimicus* was isolated at high temperatures within a small range (30.4–32.9 °C) (Table 3), although this bacteria has been reported at much larger intervals (10–33 °C) (Chowdhury *et al.* 1989). It is important to note that although the highest densities of *V. vulnificus* and *V. cholerae* were found at relatively low temperatures (15.8 and 19.4 °C, respectively), they were more frequently isolated at approximately 30 °C (Figure 4). This behavior could be explained as the result of a combination of various factors such as salinity and is not limited to the effect of temperature. For example, low temperatures coincide with the lowest salinity values at all sampling sites, and *V. cholerae* prefers moderate or low salinity levels (Singleton *et al.* 1982). A more complex relation between *V. vulnificus* abundance and salinity has been reported, showing that high salinity values (>30 ppt) rarely show high bacteria counts, independently of temperature, but they are common at moderate (<30 ppt) salinity values (FAO/WHO 2005b). Generally, *V. parahaemolyticus* and *V. vulnificus* were found at a wider ranges of temperature, EC, and DO, while *V. cholerae* and *V. mimicus* were found at the smallest ranges, but at high salinity values (Figure 4).

Salinity

The combined effect of temperature and salinity influence the density of *V. parahaemolyticus*, *V. cholerae* and *V. mimicus*, because the highest isolation frequency was limited by

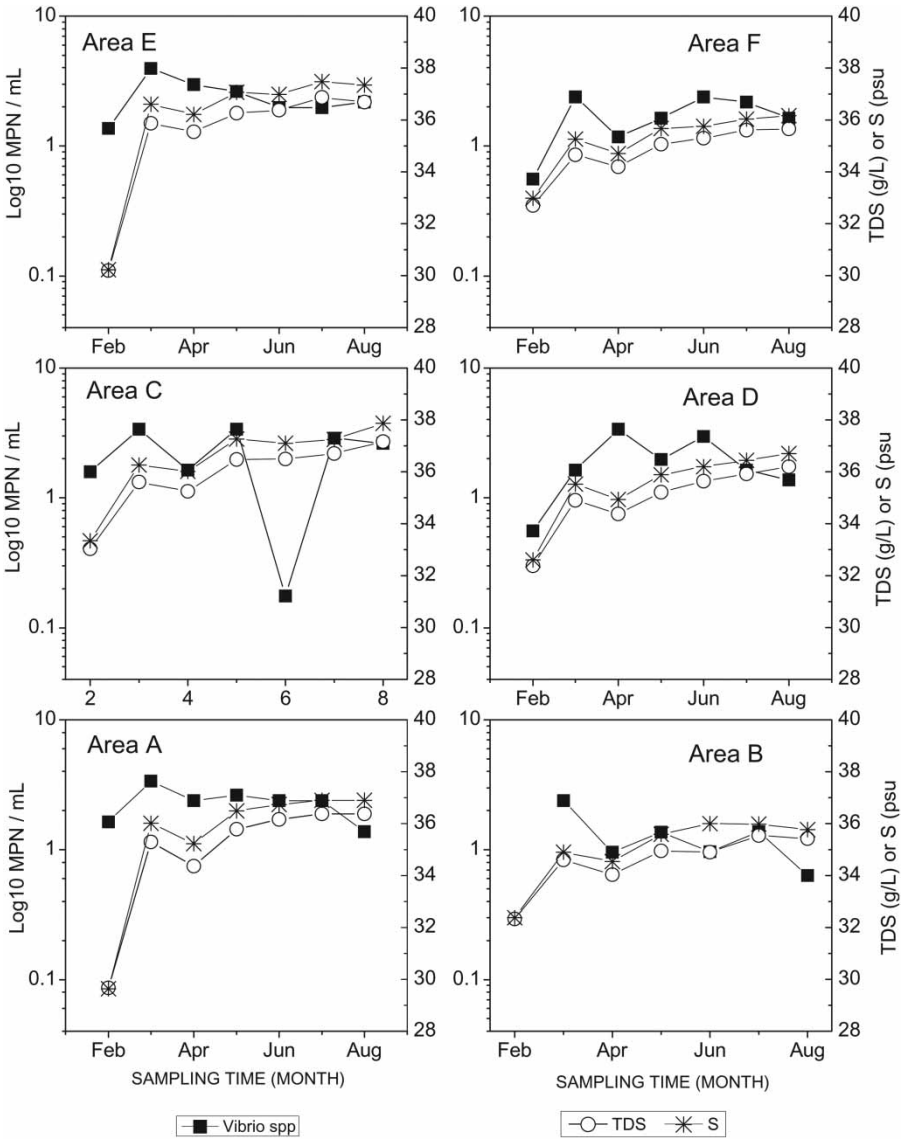

Figure 3 | *Vibrio* spp., total dissolved solids (TDS-g/L) and salinity (S-psu) variation from February to August 2011 by sampling area (A to F).

salinity. Recently, Caburlotto *et al.* (2012) found that temperature and salinity had a great influence on *Vibrio* populations, reporting a greater salinity range (23.7–35 psu) than those found in this study. Additionally, *V. cholerae* has been reported at salinity values up to 35 psu (Singleton *et al.* 1982), while *V. parahaemolyticus* has been found between 30.9 and 36.2 psu (Martinez-Urtaza *et al.* 2008). *V. vulnificus* has been reported from 30 to 38 psu (Kaspar & Tamplint 1993), which is similar to the conditions found in this study. Contrary to our findings, *V. mimicus* was isolated in brackish water at maximum salinity values of 21.5 psu in Veracruz, Mexico (González-Vázquez 2006). In this study, *V. mimicus* was most frequently isolated at high temperatures, and high, stable salinity values. This supports the notion that more than one factor limits the *V. mimicus* density.

Dissolved oxygen

Water DO values vary from 4.7 to 15.5 mg/L in the study area, but any targeted *Vibrio* specie was isolated from waters containing the highest values (Table 3). *V. parahaemolyticus* displayed the highest range (4.7–13.6 mg/L) and

Table 3 | Range of physicochemical parameters within *V. parahaemolyticus* (*Vp*), *V. vulnificus* (*Vv*), *V. cholerae* (*Vc*) and *V. mimicus* (*Vm*) were isolated, compared with the maximum and minimum values found at seawater during the sampling period

Parameter	*Vp*	*Vv*	*Vc*	*Vm*	Water
T	19.4–33.7	15.8–33.7	19.4–32.3	30.4–32.9	14.8–33.7
EC	47.6–65.9	46.4–65.8	47.6–63.5	61.3–64.4	44.5–65.8
TDS	34.2–37.2	30.2–37.2	34.7–36.7	35.5–37.2	29.3–37.2
S	34.7–37.9	30.2–37.9	35.3–37.3	36.0–37.9	29.6–37.9
DO	4.7–13.6	4.7–10.6	4.9–8.5	4.7–8.9	4.7–15.5
pH	7.9–8.5	8–8.5	8–8.2	7.9–8.2	7.9–8.8
ORP	73.8–301.5	85.9–270.9	85.7–288.1	158.5–270.9	73.8–301.5
P	754.8–766.2	755.3–764.8	754.8–765.9	754.8–757.4	754.8–766.2

T, temperature (°C).
EC, electrical conductivity (mS/cm).
TDS, total dissolved solids (g/L).
S, salinity (psu).
DO, dissolved oxygen (mg/L).
pH, ORP, REDOX potential (mV).
P, barometric pressure (mmHg).

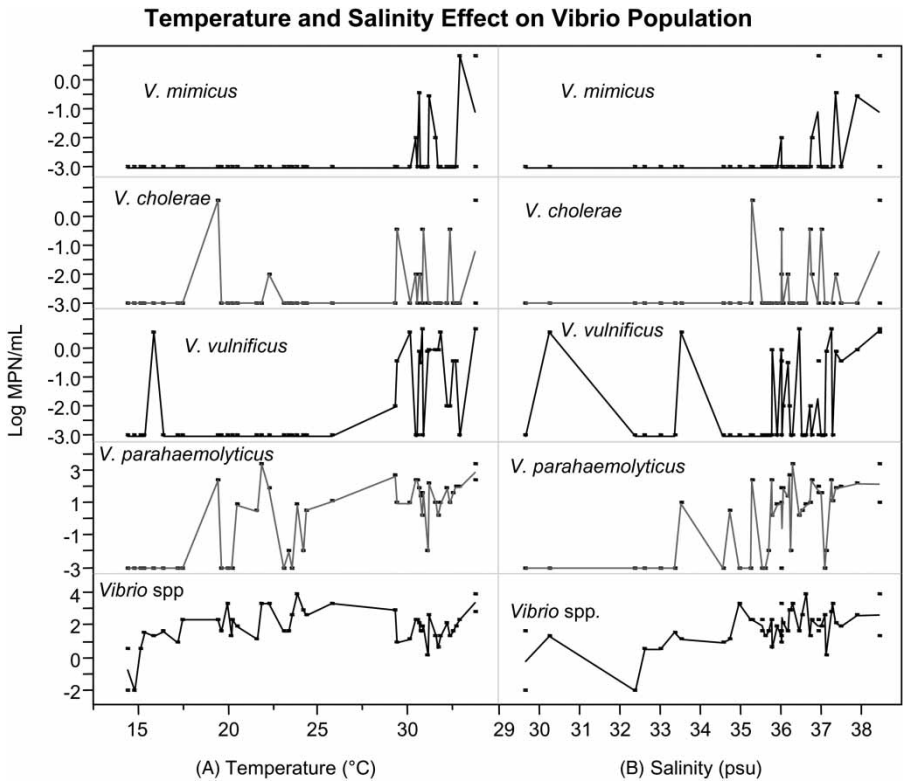

Figure 4 | Bacterial variation (Log MPN/mL) related to temperature (A) and salinity (B) of presumptive *Vibrio* spp., *V. parahaemolyticus, V. vulnificus, V. cholerae* and *V. mimicus*.

V. cholerae and *V. mimicus* the lowest (4.9–8.5 and 4.7–8.9 mg/L, respectively). Similar values have been reported for shrimp pond water, and DO was generally associated with bacterial diversity changes (Noriega-Orozco *et al.* 2007). Various studies have shown a wide variety of intervals for *V. cholerae* (0–12.5 mg/L) (Kaper *et al.* 1979; Aulet *et al.* 2007), *V. parahaemolyticus* (4.6–14.6 mg/mL) (Sarkar *et al.* 1985) and *V. vulnificus* (2.5–9.2 mg/L) (Oliver *et al.* 1983). In this study, DO only correlated with other chemical parameters, without showing any effect on the presence of *Vibrio* species.

REDOX potential (ORP)

V. parahaemolyticus was isolated from water covering the entire observed ORP range (73.8–301.5 mV), while *V. mimicus* was found in the smallest range (158.5–270.9 mV). There was, however, no correlation between this parameter and any of the four pathogenic species covered in this study. There are no previous reports that associate ORP with *Vibrio* species, but their facultative nature is well known.

Electrical conductivity (EC)

Electrical conductivity highly correlates to *V. parahaemolyticus*, *V. vulnificus* ($P < 0.0001$) and *V. mimicus* ($P < 0.0092$) population levels. Different values have been reported for seawater from which *V. parahaemolyticus* was isolated (31.8–45.1 mS/cm) (Yamamoto *et al.* 1982), as well as for *V. cholerae* (Locascio de Mitrovich *et al.* 2010).

Total dissolved solids (TDS)

TDS values were significant only for V. *parahaemolyticus* ($P < 0.0001$), and 'presumptive *Vibrio* spp.'. The influence of TDS has been previously reported for the total *Vibrio* population (Hasan *et al.* 2011).

Barometric pressure (P)

We found a negative correlation between this parameter, *V. parahaemolyticus, V. vulnificus* and environmental factors (T, S, SE, TDS and ORP). Recently, barometric pressure has been incorporated into predictive models for *Vibrio* populations and has been associated with wind and water surface temperature changes (Paz & Broza 2007; Constantin de Magny *et al.* 2009), more than bacterial population.

In general, environmental conditions found at the sampling sites support and promote the growth of *V. parahaemolyticus*, *V. vulnificus*, *V. cholerae* and *V. mimicus*. However, the abundance of *V. parahaemolyticus* was more influenced by environmental parameters than any other *Vibrio* species, especially T, S, TDS and CE ($P < 0.001$), than that of the other species. *V. cholerae, V. vulnificus* and *V. mimicus* were found in very low numbers.

Principal component analysis (PCA)

Most of the data variance (72.8%) was explained with the first four principal components (Prin1 to Prin4, Table 4). Clear relationships between some of the physicochemical parameters and the Log MPN/mL of the *Vibrio* species (Prin1) were observed (Table 4). T, EC, TDS and S influenced all of the *Vibrio* species included in this study, except *V. cholerae,* and their populations were inversely proportional to barometric pressure changes (Prin1). *V. parahaemolyticus* was the species most affected by environmental factors, followed

Table 4 | Correlation matrix for the first four principal components (Prin1 to Prin4) which explains 72.866% of the variance

Loading matrix Variable	Prin1	Prin2	Prin3	Prin4
Sampling site	0.04211	0.21148	– 0.0946	**0.60662**
Log Vspp	0.31958	0.19818	**0.76837**	0.13001
Log Vp	**0.79126**	0.03996	0.15724	0.24175
Log Vv	**0.51005**	– 0.3706	– **0.56179**	– 0.01835
Log Vc	0.24465	0.13368	– 0.11215	**0.69704**
Log Vm	**0.40629**	– 0.34069	0.19765	– 0.36975
T (°C)	**0.9622**	– 0.09208	– 0.12212	– 0.02897
CE (mS/cm)	**0.92365**	– 0.18551	– 0.20648	– 0.06635
TDS (g/L)	**0.87117**	0.08266	0.31482	– 0.04764
S (ppt)	**0.84953**	0.10494	0.34532	– 0.03879
DO (mg/L)	– 0.13734	**0.84692**	0.01274	– 0.37392
pH	0.32629	**0.64156**	– 0.27302	0.08184
ORP (mV)	– 0.13531	– **0.74069**	0.23522	0.14791
P (mmHg)	– **0.83037**	– 0.11613	0.32403	0.19721

by *V. vulnificus* and *V. mimicus*. Previous reports have correlated free living *Vibrio* abundance with temperature and salinity (Turner *et al.* 2009).

Additionally, DO, pH and ORP did not correlate with the bacterial density of any species, but a positive correlation between DO and pH and a negative correlation with ORP were found (Prin2). This is contrary to a previous study where a correlation between pH and *V. vulnificus* was documented (Blackwell & Oliver 2008).

The abundance of *V. cholerae* was mainly associated with the sampling site, rather than environmental factors (Prin4), despite the fact that the relationship of this bacterium with temperature has been widely documented. Nevertheless, it is important to highlight that while there is no doubt temperature affects the abundance of this bacteria, it can also be indirectly linked to geographical areas. In this manner, the presence of *V. cholerae* has always been associated with high temperatures, but is found in low densities in Europe and high densities in African or Asian countries such as India and Nigeria (Sudhanandh *et al.* 2010; Abedayo-Tayo *et al.* 2011). Officially, cholera is still occurring at an epidemic level in Mexico, and the need for surveillance to detect endemicity is recognized (Sepúlveda *et al.* 2006). This work confirms that *V. cholerae* can be considered well established in the five points from which it was isolated. For that reason, it would be advisable to conduct regular surveillance in the area, especially where products intended for human consumption are extracted.

Statistical analysis (PCA and lineal model) satisfactorily explains the abundance of the *Vibrio* species included in this study ($P < 0.0001$), showing strong correlations between presumptive *Vibrio* spp., *V. vulnificus* ($P < 0.0001$), *V. parahaemolyticus* and *V. vulnificus* and environmental conditions (T, CE, TDS, S and P) ($P < 0.001$). *V. cholerae* and *V. mimicus* displayed a correlation with both sampling site ($P < 0.001$ and 0.0042, respectively) and environmental conditions ($P < 0.0281$ and <0.0019, respectively).

Even though the parameters considered in this study affected *Vibrio* abundance to some degree, *V. parahaemolyticus* showed a more predictable model. It is likely that other parameters not considered in this study also affected *V. cholerae* and *V. mimicus*.

CONCLUSIONS

Variations in seawater environmental factors observed in the study area support the survival and growth of the clinical concern *Vibrio* species included in this study. *V. parahaemolyticus* was the most abundant and widely distributed among the sampling sites, followed by *V. vulnificus, V. mimicus* and *V. cholerae*. Despite *V. mimicus* being the least widely distributed, it was still slightly more abundant than *V. vulnificus* and *V. cholerae*.

The presence of *V. parahaemolyticus* and *V. vulnificus* correlated positively with temperature, salinity, electrical conductivity and total dissolved oxygen. On the contrary, the abundance of *V. cholerae* did not significantly correlate with the environmental factors included in this study. It was only affected by sampling site. *V. mimicus* showed a weak but significant correlation with sampling site and environmental parameters. Although the parameters considered in this study explained bacterial concentration of all four *Vibrio* species to some extent, variations in the *V. parahaemolyticus* population can be more reliably predicted based on physicochemical parameters. *V. cholerae*, and *V. mimicus* may have been affected by other parameters not considered in this study. In the case of *V. vulnificus, V. cholerae* and *V. mimicus* would also be important to extend the data series, not only in time, but also geographically in order to cover more environment conditions.

We can conclude that *V. parahaemolyticus*, *V. vulnificus, V. cholerae* and *V. mimicus* are present in the waters surrounding Guaymas, Sonora, Mexico. Moreover, even though *V. cholerae* is not officially considered endemic in Mexico, it has established a viable population in part of the study area. Surveillance of *Vibrio* species is advisable, especially in those areas used to procure products for human consumption. The presence of viable cells of all four *Vibrio* species, included in this study, by themselves represents a likely public health risk because, at least three of the sampling sites are local fishing areas. The products of which are intended for human consumption and the probability to human infection is also present in non-pathogenic strains. However, we recognize and strongly suggest the need to conduct further studies that extend beyond the study area and consider virulence factors, and

other biotic and abiotic factors associated with *Vibrio* species for a better understanding of their behavior.

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