Seasonal seawater temperature as the major determinant for populations of culturable bacteria in the sediments of an intact mangrove in an arid region

Barbara Gonzalez-Acosta[1,2,3], Yoav Bashan[1], Norma Y. Hernandez-Saavedra[1], Felipe Ascencio[1] & Gustavo De la Cruz-Agüero[2,3]

[1]Centro de Investigaciones Biológicas del Noroeste (CIBNOR); [2]Centro Interdisciplinario de Ciencias Marinas (CICIMAR-IPN), La Paz, Mexico; and [3]Fellowship COFAA, Mexico City, Mexico

Correspondence: Yoav Bashan, Environmental Microbiology Group, Centro de Investigaciones Biológicas del Noroeste (CIBNOR), Mar Bermejo 195, Col. Playa Palo de Santa Rita, La Paz, B.C.S. 23090, Mexico. Tel.: +52 612 1238484 ext. 3668; fax: +52 612 1254710; e-mail: bashan@cibnor.mx

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Abstract

Mangroves are highly productive marine ecosystems where bacteria (culturable and nonculturable) actively participate in biomineralization of organic matter and biotransformation of minerals. This study explores spatial and seasonal fluctuations of culturable heterotrophic bacteria and *Vibrio* spp. in the sediments of an intact mangrove ecosystem and determines the dominant environmental factors that govern these fluctuations. Sediment samples were collected monthly from three stations in the mangroves of Laguna de Balandra, Baja California Sur, Mexico, through an annual cycle. Physicochemical parameters included seawater temperature, salinity, and concentration of dissolved oxygen. Viable counts of culturable heterotrophic bacteria and *Vibrio* spp. were made. In one sample (March 2003), nutrient concentrations (ammonium, nitrites, nitrates, and phosphates), organic matter, pH and sediment texture were also determined. General cluster analyses, analysis of variance of specific variables, and several principal component analyses demonstrated that seawater temperature is the principal determinant of seasonal distribution of culturable heterotrophic bacteria and *Vibrio* spp. in mangrove sediments. Soil texture, concentration of nutrients, and organic matter concentration contribute to heterogenicity to a lesser extent. A large spatial variation in bacterial populations was observed over short distances (~1 m) in sampling areas within the same site. These analyses show that the culturable bacterial distribution in sediments of mangroves has high spatial and temporal heterogeneity.

Introduction

Mangroves are large or small tropical and subtropical tidal zone ecosystems that are highly productive. They offer protected breeding and growing environments for a wide range of coastal and offshore marine organisms; many of which are economically and ecologically important for tropical marine areas. Mangroves are important refuges for resident and migratory birds, and are a major stabilizing feature of coastlines (Lacerda *et al.*, 1993; Rönnbäck, 1999; Primavera *et al.*, 2004).

Mangroves provide leaves and wood that are degraded primarily by a large variety of microorganisms and later by higher organisms residing in the ecosystem (Alongi *et al.*, 1989; Holguin *et al.*, 2001). The major product of the general recycling of organic matter by microorganisms is detritus; organic matter in the process of decomposition. This detritus is rich in energy and protein, and contains large microbial populations (for review, see Holguin *et al.*, 2001). Bacteria are the major participants in the nitrogen and phosphorus cycles in mangroves (Toledo *et al.*, 1995; Vazquez *et al.*, 2000; Rojas *et al.*, 2001); however, information about nutrient dynamics and energy flow in mangroves is limited.

Bacterial activity is responsible for most of the carbon recycling in mangrove sediments. Some authors believe that tree root exudates fuel many bacterial activities in mangrove sediments (Alongi *et al.*, 1993; Alongi, 1994). Evidence suggests that there is a close relationship between heterotophic microorganisms, nutrients, trees and marine organisms that controls the mechanisms of nutrient recycling in mangroves. This recycling preserves most of the necessary nutrients for the natural sustainability of these ecosystems (Holguin *et al.*, 1992; Alongi *et al.*, 1993; Alongi, 1994;

Toledo *et al.*, 1995; Bano *et al.*, 1997; Vazquez *et al.*, 2000; Holguin *et al.*, 2001). A component of the heterotophic bacteria is the genus *Vibrio* spp., one of the most common bacterial genera in marine environments. Some species may cause infections in humans and aquaculture resources (Baross & Liston, 1970; Kaneko & Colwell, 1973; Høi *et al.*, 1998). Physicochemical factors determine to a great extent the growth rate and biomass production of heterotrophic bacteria and *Vibrio* spp. in intertidal sediments in tropical zones (Alongi, 1988).

The mangrove of Balandra is a protected and almost intact arid zone ecosystem. It is some distance from the urbanized area of the city of La Paz. The beaches of Balandra, but not the mangrove, are used solely for recreation. These mangroves were the site of several microbial ecological studies, which showed that N_2-fixation is a major bacterial activity (Holguin *et al.*, 1992; Toledo *et al.*, 1995; Rojas *et al.*, 2001). Where black mangrove (*Avicennia germinans*) are present, N_2-fixing and non-N_2-fixing cyanobacteria, diatoms, microalgae and, to a lesser degree, bacteria densely colonize the surface of pneumatophores (aerial roots). Light and seawater temperature are the main environmental factors controlling N_2-fixing activity in pneumatophores (Toledo *et al.*, 1995). A significant microbial activity in mangroves is the solubilization of phosphate. Many species of phosphate-solubilizing rhizosphere bacteria associated with black mangrove roots were found. The mechanism responsible for P solubilization probably involves the production of several organic acids (Vazquez *et al.*, 2000). P-solubilizing and N_2-fixing bacteria from this mangrove had synergistic effects on each other (Rojas *et al.*, 2001), and promoted the growth of *Salicornia bigelovii*, an oil-seed plant that also resides in mangroves (Bashan *et al.*, 2000). The sediment bacteria produced methane; however, the ecosystem does not emit methane as long as it is not disturbed (Giani *et al.*, 1996; Strangmann *et al.*, 1999).

Holguin *et al.* (2001) reviewed the pivotal role of bacterial communities in the biomineralization of organic matter in sediments and in the maintenance of the food chain of mangrove systems. They proposed an investigation into the role of bacteria in the primary productivity of these ecosystems. In this context, the objective of this study was to explore spatial and seasonal fluctuation of heterotrophic bacteria and *Vibrio* spp. in the sediments over an annual cycle and to evaluate the influence of physicochemical and nutritional factors on their populations.

Materials and methods

Sampling site

The mangrove at Balandra is located 25 km north of the city of La Paz, Baja California Sur, Mexico. Its geographic, geologic, sedimentologic, and floristic character were previously described (Pedrin-Avilés *et al.*, 1990; Holguin *et al.*, 1992; Giani *et al.*, 1996; Toledo *et al.*, 2001). It is an undisturbed arid zone mangrove ecosystem receiving no fresh water apart from rain (average 175 mm year^{-1}). Samples were collected monthly from April 2002 to March 2003 at three stations in the tidal zone where black mangrove [*A. germinans* (L.) Stern] grow. The locations of the stations were determined by use of the global positioning system (Fig. 1).

Determination of environmental parameters

Between 08:00 and 11:00 hours, seawater temperature, salinity, and dissolved oxygen concentration were recorded *in situ* in the surface water above the bottom sediments with a meter (sensor Model 58, YSI, Cincinnati, OH).

Collection of samples of sediment

Sediment cores were collected monthly over an annual cycle, using a hand-held stainless steel core sampler (3.2 cm diameter, 20 cm long). The cores were packed in capped polycarbonate tubes (US Plastics Corp, Lima, OH). Immediately after sampling, the cores were stored on ice in ice chests and transported to the laboratory within 3 h. They were immediately processed at the laboratory. Three replicate sediment cores were taken from three stations in the mangrove. The distance between sampling areas within each site was 1 m. Samples were processed in triplicate for all analyses. Bacteria were counted (CFU) from the upper 3 cm of each sediment core. Apart from these, samples of sediment collected in March 2003 were collected directly with wide polycarbonate tubes (10 cm diameter). These samples were used to determine ammonium, nitrites, nitrates, phosphates, organic matter, pH and texture. As in bacterial sampling, only the upper 3 cm of each core was used. These samples were placed in plastic bags and frozen at $-20\,^{\circ}$C until analysis.

Quantification of culturable aerobic heterotrophic bacteria and *Vibrio* spp.

Sediment samples of 10 g were homogenized with 90 mL 0.08 M PBS at pH 7.5 using a vortex mixer. Serial decimal dilutions were then made in the PBS mixture. Between each dilution, the samples were mixed thoroughly on a vortex mixer at the highest speed to release bacterial cells from sediment particles. Culturable heterotrophic bacteria were counted using a conventional plate count method on Marine Agar 2216 (Difco^{TM}, Becton, Dickenson and Co., Franklin Lakes, NJ). Plates were incubated for 5–7 days at $25 \pm 2\,^{\circ}$C. All colonies were counted and the bacterial

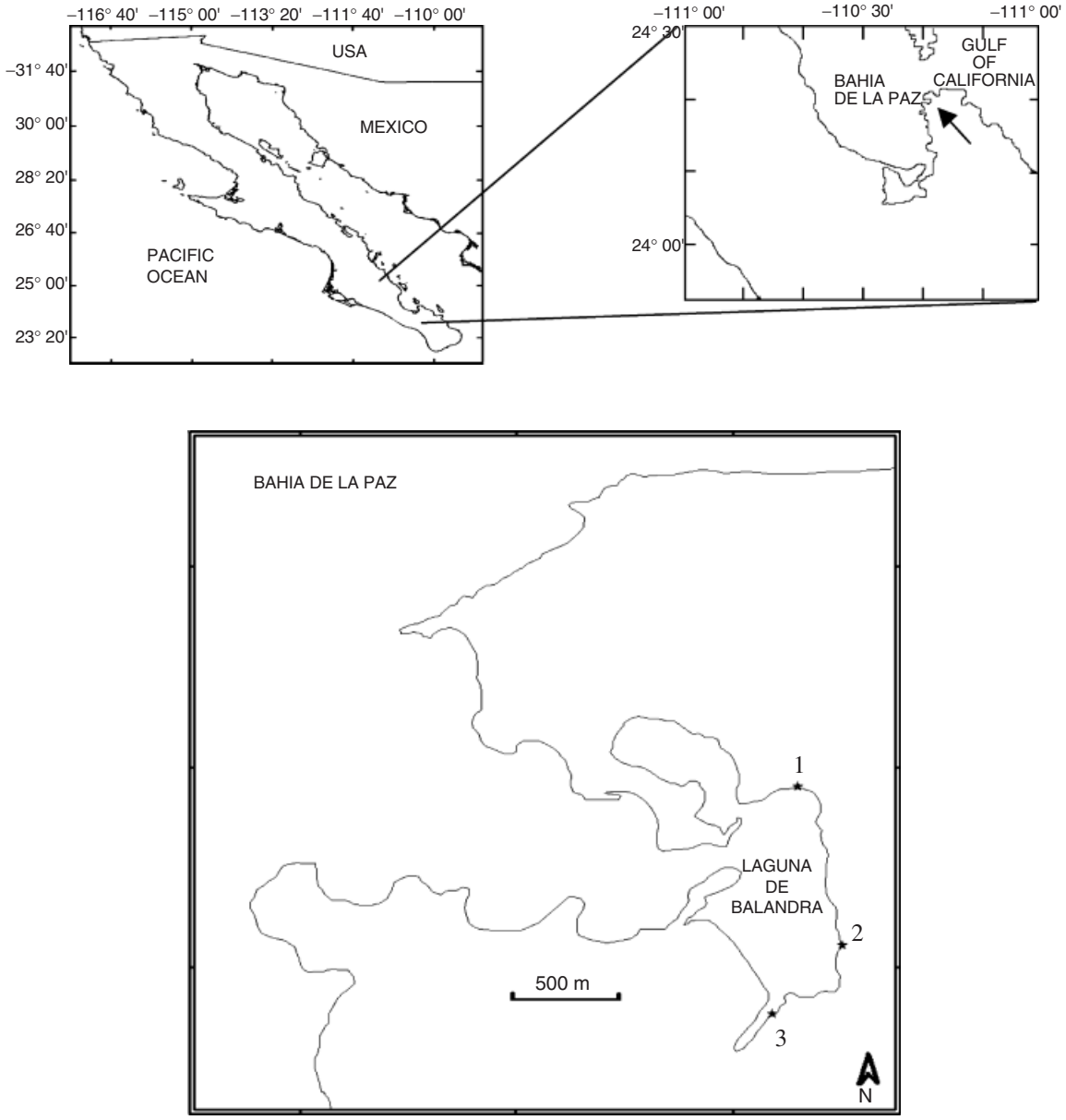

Fig. 1. Location of sampling stations in the mangroves of the lagoon at Balandra.

populations were expressed as CFU per g sediment fresh weight. Fresh weight is defined as sediment at seawater holding capacity. *Vibrio* spp. were similarly counted, but on TCBS agar medium (Difco^{TM}) incubated for 24–48 h (APHA, AWWA, WPCF, 1992). TCBS agar medium is the standard medium for counting vibrios from seawater samples (Oliver, 2003) and is the legal medium for evaluation of vibrio microorganisms that are of concern in public health in the USA (U.S. Food and Drug Administration, 2001) and Mexico (NORMA, 1994). The recovery of the medium for vibrios was between 61% (Pfeffer & Oliver, 2003) and 83% (Bolinches *et al.*, 1988). In a preliminary study, the developing colonies were identified as *Vibrio* spp. on TCBS agar medium using an analytical kit (BD BBL Crystal^{TM} Identification Systems, enteric/nonfermented ID Kit; Becton, Dickinson and Co.) that analyzes 15 biochemical reactions. The recovery capacity of the medium for *Vibrio* spp. was 80%. The data presented in this study reflect this percentage of recovery.

Sediment parameters analyses

Physicochemical analyses were made using 100 g sediment samples at the Soil Science Service Laboratory at CIBNOR,

carrying out the following determinations: pH according to Jackson (1976); organic matter according to Walkley & Black (1934); sediment texture according to Folk (1964); N-NO_2^- and N-NO_3^- according to Strickland & Parsons (1972); N-NH_4^+ according to Solorzano (1969); and P-PO_4^{-3} according to Jackson (1976)). Data were calculated on the basis of sediment dry weight.

Experimental design and data analysis

The culturable bacterial populations (heterotrophic and *Vibrio* spp.) were determined by plating three replicates of each dilution. The CFU per g was calculated from three plates with the same dilution. Although data in CFU per g were log-transformed, the requirements of normality and homogeneity of variances were not met. Therefore, a rank-transformation analysis was chosen to establish whether there were significant differences between months and between sampling sites according accepted statistical methods (Zar, 1999). Differences in seawater temperature, dissolved oxygen, and salinity (between the hot and the warm seasons) were determined by one-way analysis of variance (ANOVA). Linear correlations between bacterial populations and environmental parameters were also calculated for all sites (Sokal & Rohlf, 1981).The results were summarized in three data matrices: environmental parameters, physicochemical parameters, and bacterial populations for either heterotrophic bacteria or *Vibrio* spp. Ordination of data matrices for environmental and physicochemical parameters and for bacterial populations was done by Principal Component Analysis (PCA). All regular analyses used STATISTICA™ software (StatSoft Inc., Tulsa, OK). Community analyses were done using ANACOM software (De la Cruz-Agüero, 1994).

Results

Environmental parameters

The highest seawater temperature was recorded in August (31.0 °C) and the lowest in January (20.1 °C). Significant differences between the hot season (May to October) and the warm season (November to April) were found ($P \leq 0.001$, ANOVA). Although high variability in the dissolved oxygen pattern was observed, significant differences were found in the dissolved oxygen concentrations between hot and warm seasons ($P \leq 0.001$, ANOVA). There were similar fluctuations in the salinity at Stations 1 and 3 during the year, but the salinity at Station 2 was consistently higher. Salinity was lowest in Stations 1 and 3 during August because rain associated with hurricanes brought considerable freshwater runoff. Otherwise, no significant differences in salinity between the summer and winter seasons were found ($P \leq 0.05$, ANOVA) (Fig. 2).

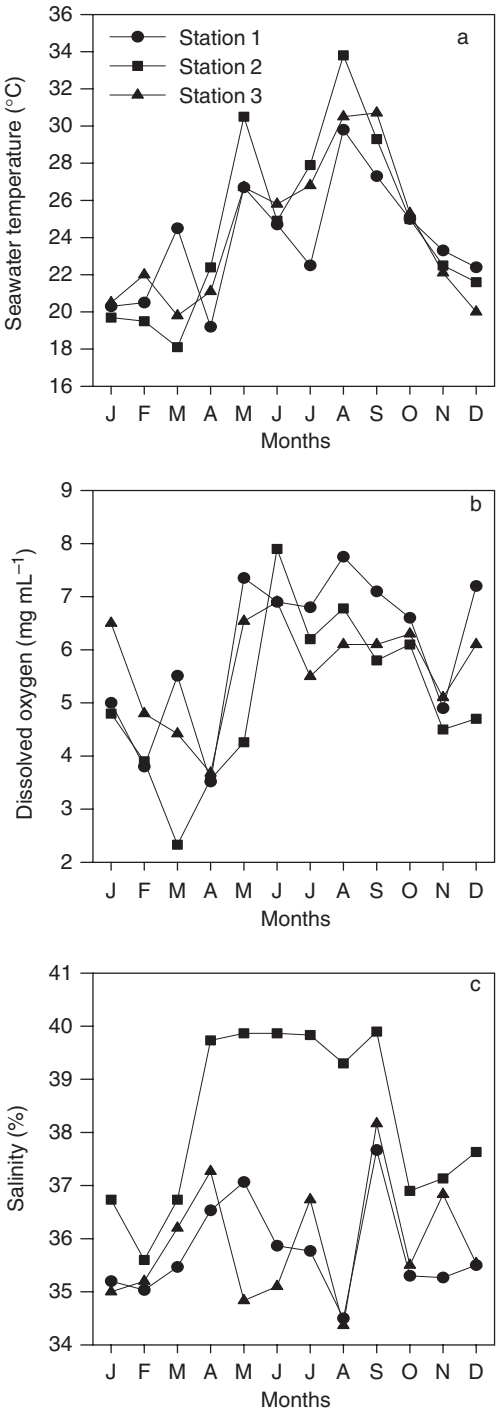

Fig. 2. Seasonal fluctuations of physicochemical parameters: (a) seawater temperature, (b) dissolved oxygen, and (c) salinity. $n = 3$ replicates per station and three samples from each replicate.

Temporal and spatial variation of heterotrophic bacteria

The yearly mean population of heterotrophic bacteria was $1.64 \pm 1.4 \times 10^7$ (expressed as CFU per g sediment fresh

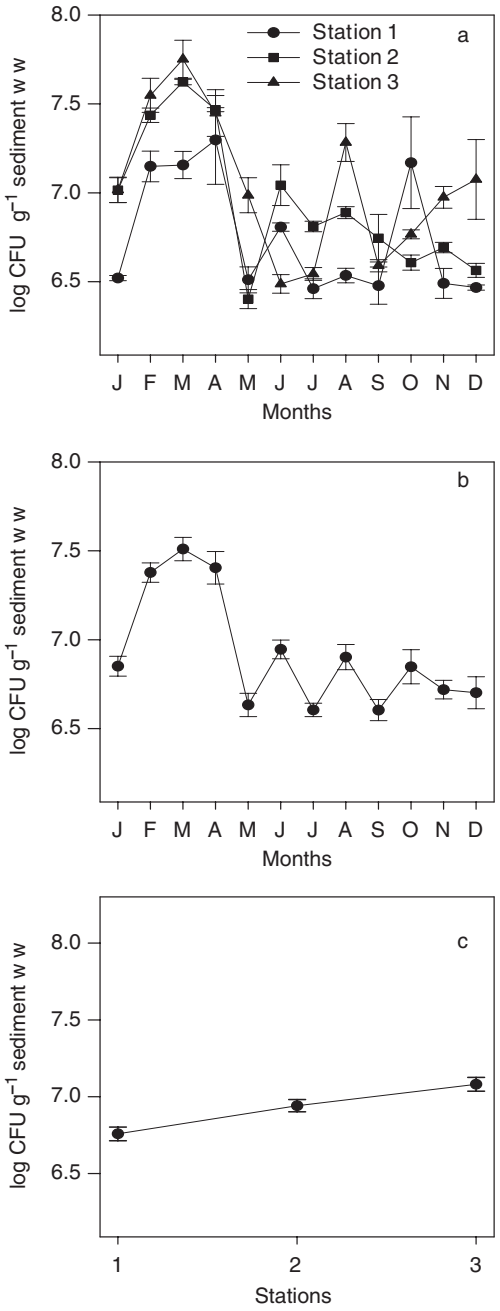

Fig. 3. Temporal and spatial fluctuations of populations of culturable heterotrophic bacteria in the sediment of mangroves. (a) Temporal fluctuations per station, (b) seasonal variation; (c) spatial seasonal variation per station. $n = 3$ replicates per station and three samples from each replicate. Bar represents standard error; ww, wet weight.

weight) with a minimum population of 1.5×10^6 and a maximum population of 1.57×10^8. Each station showed different seasonal patterns of bacterial abundance (Fig. 3a). The maximum bacterial abundance occurred during the warm season and was reduced in the hot season (Fig. 3b). Smaller populations were always recorded in Station 1 than in the other stations. Stations 2 and 3 had larger but similar populations (Fig. 3c). The rank-transformation analysis (Table 1) carried out for these bacteria showed that there is a significant difference in bacterial abundance between months and stations ($P \leq 0.001$). Dissolved oxygen concentrations and bacterial abundance showed a negative linear correlation ($r = -0.78$; $y = 8.133 - 0.218x$, $P \leq 0.05$). No correlations were found between seawater temperature, salinity, and bacterial abundance ($P \leq 0.05$).

Table 1. Rank-transformation analysis of heterotrophic bacteria

Source of variation	d.f.	SS	MS	*F*
Months	11	1 209 884.41	109 989.49	240.86***
Stations	2	2 221 430.53	111 074.77	243.23***
Error	310	1 401 563.80	456.66	
Total	323	2 833 591.74		

***$P \leq 0.001$.

Seasonal and area variations of *Vibrio* spp.

The yearly mean population abundance of *Vibrio* spp. was $2.12 \pm 1.8 \times 10^4$ with a minimum population of 7×10^2 and a maximum population of 2.36×10^5 CFU g^{-1} sediment fresh weight. *Vibrio* spp. showed very similar temporal patterns of abundance at the three stations (Fig. 4a). The maximum abundance occurred during the hot season and was lower in the warm season (Fig. 4b). Stations 1 and 2 had very similar populations of *Vibrio* spp. The largest population was found at Station 3 (Fig. 4c). The rank-transformation analysis (Table 2) for this group of bacteria showed that there were significant differences between the months and stations ($P \leq 0.001$). Seawater temperature and bacterial population showed a positive linear correlation ($r = 0.6$; $y = 3.165 + 0.0354x$, $P \leq 0.05$). No linear correlation was found between either dissolved oxygen concentration ($r = 0.035$) or salinity ($r = 0.54$) and bacterial abundance ($P \leq 0.05$).

Principal component analyses

Ranking of stations regarding sampling and experimental replicates by PCA showed that, for heterotrophic bacteria (Fig. 5a), Station 1 was distinct from Stations 2 and 3. Additionally, Stations 2 and 3 showed a similar distribution of bacteria, except for one set of replicates of Station 3. Ranking of months by the abundance of heterotrophic bacteria showed seasonal variations (Fig. 5b). Spatial ranking of stations related to sampling and experimental replicates by PCA for *Vibrio* spp. was calculated (Fig. 5c). The variability among replicates was low and the location of the stations dictated a characteristic pattern because the stations presented a low variability amongst themselves. Ranking of

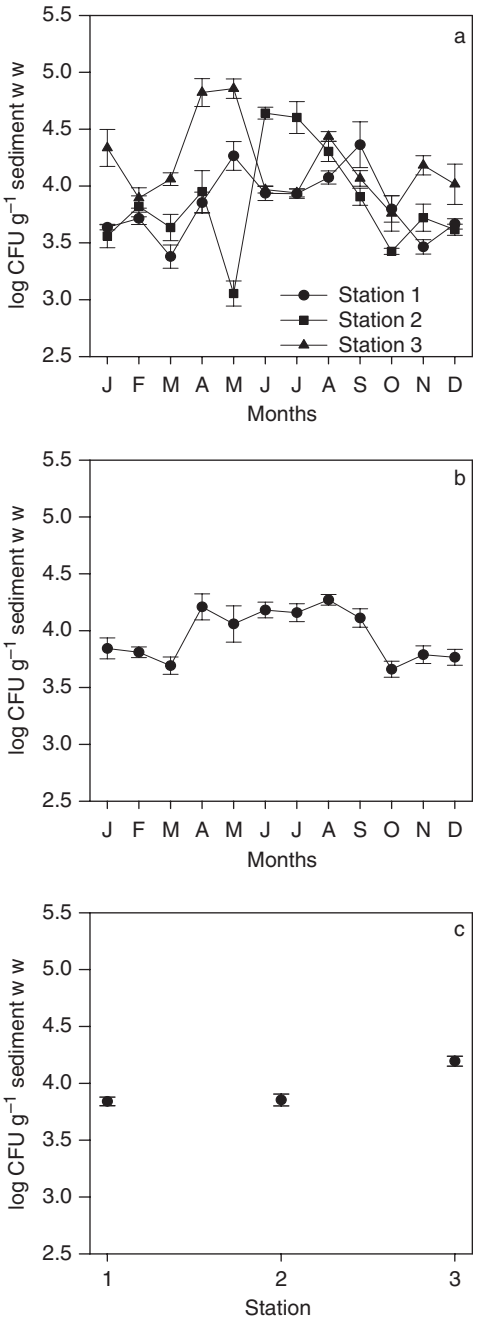

Fig. 4. Temporal and spatial fluctuations of populations of *Vibrio* spp. in the sediment of mangroves. (a) Temporal fluctuations per station; (b) mean of three sites over the seasonal sampling; (c) mean of seasonal data per station. $n = 3$ replicates per station and three samples from each replicate. Bar represents standard error.

months by abundance of *Vibrio* spp. showed seasonal variations (Fig. 5d). In both cases, they were supported by the first principal component, which accounted for 99.8% and 99.0% of the variance in the data for heterotrophic bacteria and for *Vibrio* spp., respectively (Figs 5b and d).

Table 2. Rank-transformation analysis of *Vibrio* sp.

Source of variation	d.f.	SS	MS	*F*
Months	11	565 493.20	51 408.47	8.37***
Stations	2	280 009.85	140 004.93	22.79***
Error	310	1 904 714.09	6142.50	
Total	323	2 749 677.14		

***$P \leq 0.001$.

Ranking environmental variables of the sediments by PCA showed monthly data divided in two groups related to seawater temperature (Fig. 6a). The seasons can be clearly differentiated: the hot season included May through October and the warm season November through April. Seawater temperature (PCA 1) accounted for 58% of the variance in the data, salinity (PCA 2) for 34%, and dissolved oxygen concentration (PCA 3) for only 8%. This distribution in PCA variables showed gradient patterns related to seawater temperature and salinity during the year, which indirectly explains the variation of bacterial abundance.

Sediment parameters

Principal Component Analysis (PCA) of the parameters of the sediment (Table 3) was used to evaluate the effect of these sediment parameters on the distribution of bacteria at each station and each replicate (Table 4). This analysis attempted to explain the variations found among replicates from the same station (Fig. 6b). Clay is the main factor determining the distribution for PCA 1, N-NH_4^+ the main factor for PCA 2, and pH the main factor for PCA 3. Component 1 accounted for 39% of the variance in the data, Component 2 for 31%, and Component 3 for 16.5%. Although eigenvalues showed that Component 1 has the greatest weight in the distribution, the effects of P-PO_4^{-3} and N-NO_3^- should also be considered, as eigenvalues of these parameters are very similar to those of clay. These three components accounted for 86.71% of the variance detected (Table 4).

Discussion

The abundance of culturable heterotrophic bacteria and *Vibrio* spp. in the arid mangroves of the lagoon at Balandra showed a characteristic pattern of distribution during the year-long sampling. We assume that this is a regular pattern because there were no measurable meteorological changes in the area compared to previous years (CIBNOR meteorological station; http://www.cibnor.mx/meteo, accessed 26 April 2005). Several types of statistical analysis of bacterial abundance and environmental parameters demonstrated that the temperature of seawater above the sediment negatively affected the abundance of culturable heterotrophic

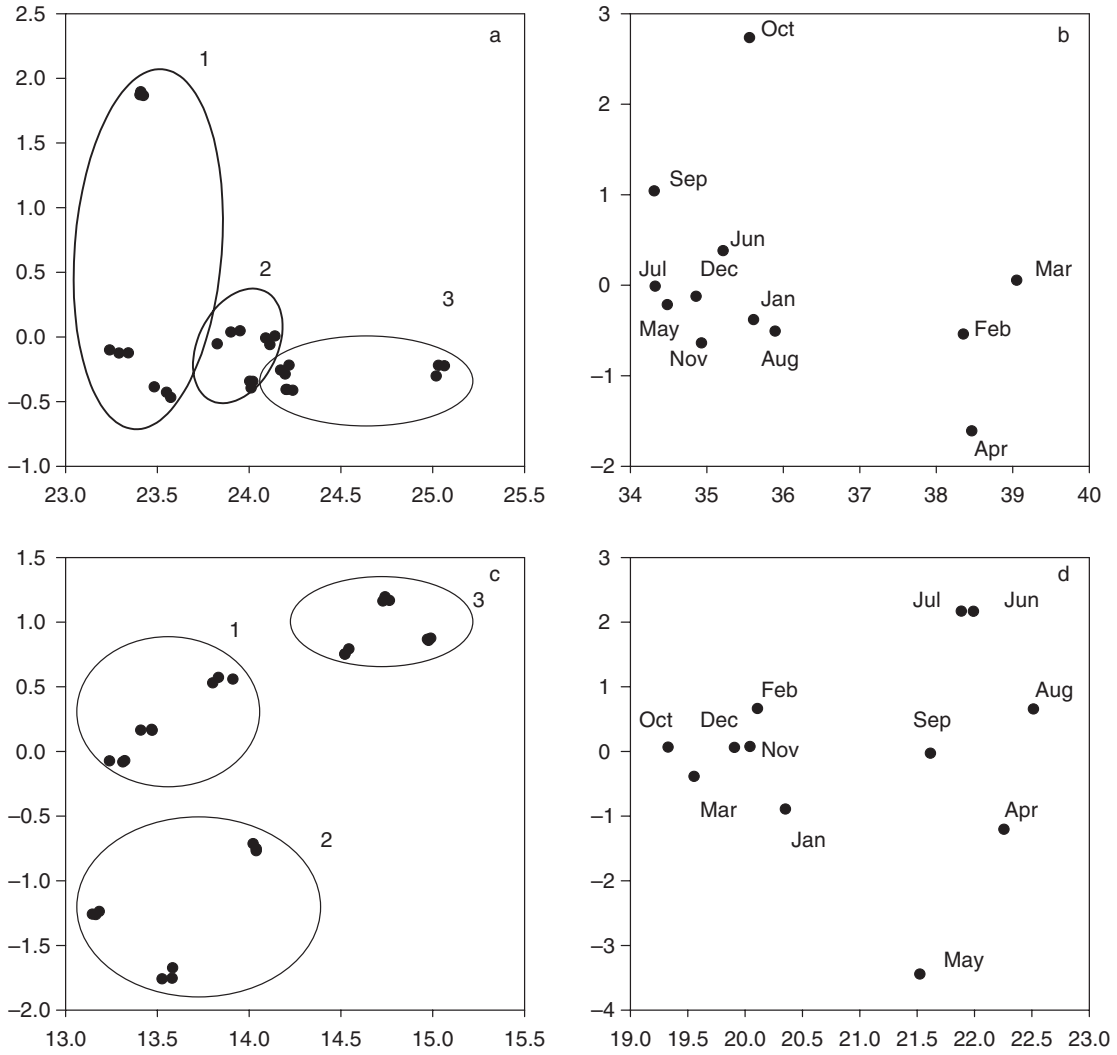

Fig. 5. Principal components plots. (a) Spatial distribution of culturable heterotrophic bacteria; (b) temporal distribution of heterotrophic bacteria per month; (c) Spatial distribution of *Vibrio* spp.; (d) temporal distribution of *Vibrio* spp. per month. Large circles represent stations and solid circles (•) represent experimental replicates. Numbers 1–3 in (a) and (c) are the sampling station numbers. $n = 3$ replicates per station and three samples from each replicate.

bacteria in the sediment. When seawater temperature increased, heterotrophic bacterial populations decreased. No other single parameter had a more drastic effect on the abundance of the heterotrophic bacteria. Conversely, seawater temperature was positively correlated with abundance of *Vibrio* spp. Fluctuations in bacterial populations in these arid zone mangroves resemble the trend of seasonal bacterial variations – with significantly higher concentrations in the wet than in the dry season – reported for tropical mangroves in Australia that receive inputs of fresh water, but with higher bacterial populations than found in tropical mangroves (Alongi, 1988). The hot season in Baja California, Mexico, is also the wet season.

A plausible explanation connects temperature, the level of dissolved oxygen and the abundance of culturable bacteria. Normally, the level of dissolved oxygen is lower in warm seawater. However, the levels of oxygen in these arid mangroves are unique. Hot summers (seawater temperature 27–31 °C) enhance several indices of productivity and respiration levels of these ecosystems. High water temperatures in these arid mangroves support a massive proliferation of algae and microalgae in these waters [Toledo *et al.*, 1995 (Laguna Balandra); Jimenez-Quiroz, C., 1991; Holguin *et al.* 2005, unpublished data (concerning arid mangroves in Ensenada de La Paz, 25 km southern of Laguna de Balandra)], enriching the seawater with dissolved oxygen (this study). The heterotrophic community of the mangrove contains a large population of N_2-fixing bacteria (Holguin *et al.*, 1992; Toledo *et al.*, 1995) that might be suppressed because of the oxygen produced. This would partially

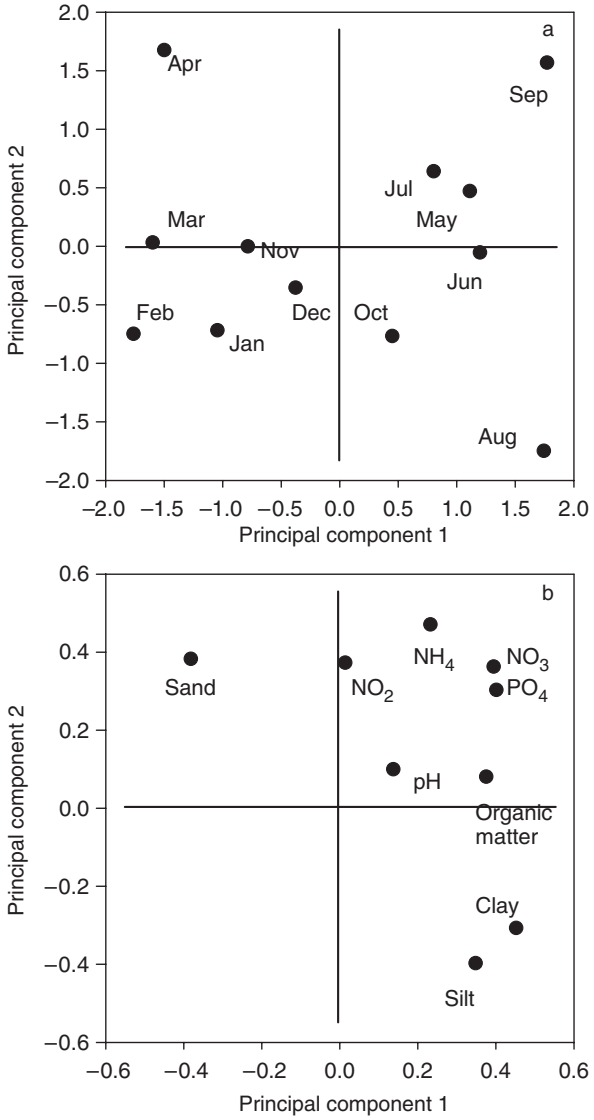

Fig. 6. (a) Principal component plot of the three environmental parameters (seawater temperature, dissolved oxygen, and salinity). (b) Principal component plot of sediment parameters.

Table 4. Principal component analysis of sediment parameters

Statistical component	Variance (%)	Cumulative (%)
I (clay)	39.12	39.12
II (NH_4^+)	31.04	70.16
III (pH)	16.55	86.71
Total		86.71

explain the relative decrease in the population of heterotrophic bacteria generally detected in this study. Vibrio organisms are immune to increased oxygen level; therefore, higher temperatures support denser populations of this group, as was the case here.

Although bacteria play a central role in marine sediments, there is insufficient information regarding bacteria in mangrove sediments. To evaluate the bacteria population in sediments, a comparison may be made with similar sediments and with terrestrial soil fractions resembling sediments as the physicochemical characteristics found in these soil environments are similar (Ransom *et al*., 1999). Several studies have demonstrated that microbial biomass was mainly concentrated on the silt and clay fractions and those have a controlling role related to populations (Bashan *et al*., 1995; Mendes & Bottomley, 1998; Ransom *et al*., 1999; Ranjard *et al*., 2000a, b; Sessitsch *et al*., 2001). The quantity of clay, silt, nitrogen, and water holding capacity of soils were the crucial factors determining the survival of *Azospirillum brasilense*, an important soil- and rhizosphere-dwelling bacterium (Bashan *et al*., 1995; Bashan & Vazquez, 2000; Chotte *et al*., 2002). The spatial distribution of microorganisms in a silty-loam soil was determined mainly by the placement of clay and organic carbon (Van Gestel *et al*., 1996). These colloids may provide beneficial functions regarding survival and nutrition to microorganisms closely adhering to their surfaces, modifying cell metabolism due to charged surfaces (Ransom *et al*., 1999), or protecting them from protozoan grazing and desiccation (England *et al*., 1993).

Table 3. Characteristics of sediment samples obtained in March 2003

		Variable								
Station	Sampling within the station	pH	Organic matter ($\mu g\ g^{-1}$)	PO_4^{-3} ($\mu g\ g^{-1}$)	NO_3^- ($\mu g\ g^{-1}$)	NO_2^- ($\mu g\ g^{-1}$)	NH_4^- (%)	Sand (%)	Silt (%)	Clay (%)
1	1	5.27	9.41	9.0	0.28	0.01	8.2	74.5	10.4	15.1
	2	5.47	3.70	3.9	0.23	0.01	11.6	82.1	6.2	11.7
	3	6.18	4.29	10.9	0.34	0.07	23.2	79.4	7.9	12.8
2	1	5.46	3.64	4.6	0.21	0.02	10.2	59.1	23.0	17.8
	2	6.11	10.24	17.2	0.43	0.02	23.3	60.2	20.7	19.1
	3	5.85	6.64	5.9	0.27	0.03	8.0	61.7	21.4	16.9
3	1	5.71	3.87	4.4	0.22	0.02	8.8	66.9	17.4	15.7
	2	5.69	5.82	4.7	0.24	0.04	17.4	69.6	16.1	14.4
	3	6.05	4.63	10.5	0.21	0.01	12.4	70.4	13.9	15.8

$n = 3$.

Soil particle size not only affects the bacterial biomass, but also determines the structure of these communities. Soils composed predominantly of clay and fine silt particles showed a greater diversity of bacteria than soils with large particles (Sessitsch *et al*., 2001). In intertidal muddy sediment, bacterial abundance was greater by two orders of magnitude than in sandy sediments (Montagna, 1982). Similarly, the abundance of bacterial populations in marine sediments near a tropical mangrove forest showed significant differences seasonally and spatially, higher densities being correlated with the smaller particle size of the sediments (Alongi *et al*., 1989). These findings suggest the reason that Station 1, having a higher percentage of sand, had the smallest bacteria populations. Stations 2 and 3, having more silt and clay, had higher populations of bacteria throughout the year. The relatively higher salinity at Station 2 can be explained by the unique characteristics of the location, a wide mud flat in front of the trees with only occasional flooding during very high tides. The mud flat has a salt crust during lower tides. It is plausible that when another high tide washes out the salt, the relative salinity increases. This hypothesis is supported by the fact that there are fewer and shorter trees in the area (unpublished data). A lower tree canopy in arid climate mangroves is usually associated with high salinity (Cintrón *et al*., 1978; Arreola-Lizárraga *et al*., 2004; Gonzalez-Zamorano, P., 2002; Holguin *et al*. 2005; unpublished data). Our samples also exhibited spatial variations of large heterogeneity among replicates over short distances. PCA clearly showed great variability in the abundance of culturable heterotrophic bacteria and *Vibrio* spp. between stations and among replicates.

Nutrients in the sediments contributed to the variability and distribution of the bacteria. Our nutrient analyses showed low concentrations of nitrogen and phosphorus in sediments. Nitrogen was mainly present as ammonium and only low concentrations of nitrites and nitrates were detected. These results confirm previous analyses of mangrove sediments (Boto & Wellington, 1984; Holguin *et al*., 1992). It seems reasonable to conclude that nutritional elements contribute to spatial variability.

In summary, our analyses showed seasonal and spatial fluctuations of culturable heterotrophic bacteria and *Vibrio* spp. in sediments of a mangrove in an arid climatic region. The main factor contributing to seasonal fluctuations of bacteria biomass was seawater temperature. Spatial variations were possibly related to several sediment conditions, including amount of clay, concentration of ammonium, and pH.

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