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Article

Temporal and vertical variability of bacterioplankton composition in Chipana bay (21º20'S) in a coastal upwelling system of northern Chile: A fluorescence *in situ* hybridization approach

Variabilidad temporal y vertical del bacterioplancton en bahía Chipana (21º20'S) en un sistema de surgencia costera del norte de Chile: Una aproximación mediante hibridación *in situ* fluorescente

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Resumen.- Se estudió, mediante visualización y enumeración con la técnica de hibridación fluorescente *in situ* (FISH), el bacterioplancton en un sistema de surgencia asociado con una zona de mínimos de oxígeno (ZMO), en el Pacífico Sur Oriental. Se evaluaron 6 grupos taxonómicos diferentes (*Alfa*, *Beta*, *Gamma-proteobacteria*, *Cytophaga-flavobacterium* y el dominio *Archaea* y *Bacteria*). El análisis mostró una mayor abundancia del dominio *Bacteria* (20 a 68% de células hibridadas) sobre el dominio *Archaea* (2 a 18% de células hibridadas). Los grupos específicos mostraron que *Cytophaga-flavobacterium*, *Alfa-proteobacteria* y *Gamma-proteobacteria* son más abundantes en las capas superficiales. *Gamma-proteobacteria* también fue más abundante en la oxiclina profunda y *Beta-proteobacteria* es el grupo con las menores abundancias registradas. Se detectó a través de NMDS cambios en la distribución vertical de la comunidad del picoplancton en la columna de agua entre ZMO y la oxiclina. Este cambio fue producto de las transiciones de las abundancias de los grupos específicos *Cytophaga-flavobacterium* y *Gamma-proteobacteria* en las capas superficiales, producto de la baja concentración de clorofila-*a* causada por los períodos de relajación en la surgencia.

Palabras clave: Hibridación *in situ*, bacteria, arquea, Chipana, sistemas de surgencia, Chile

Abstract.- The bacterioplankton in the upwelling systems associated with oceanographic condition of the oxygen minimum zone (OMZ) of the Eastern tropical South Pacific was studied through visualization and enumeration with fluorescent *in situ* hybridization (FISH). Six different taxonomic groups were studied (*Alpha, Beta, Gamma-proteobacteria, Cytophaga-flavobacterium* and the domains *Archaea* and *Bacteria*). The analysis showed a greater predominance of the *Bacteria* domain (20 to 68% of hybridized cells) over *Archaea* (2 to 18% of hybridized cells). The specific groups showed that *Cytophaga-flavobacterium*, *Alpha-proteobacteria* and *Gamma-proteobacteria* are more abundant in the surface layer. *Gamma-proteobacteria* is also most abundant in the deep oxycline and, *Beta-proteobacteria* is the group with the lowest registered abundances. Changes in the vertical distribution of the bacterial community in the water column between OMZ and on oxycline were observed through NMDS. This change is a product of a shift in the abundances of the specific groups *Cytophaga-flavobacterium* and *Gamma-proteobacteria* in the surface layers, due to low concentration of chlorophyll-*a* caused by periods of relaxation in the upwelling.

Key words: Hybridization *in situ,* bacteria, archaea, upwelling systems, Chile

Introduction

The Eastern South Pacific (ESP) is one of the most productive regions of the world's ocean (Wyrtki 1966, Ryther 1969, Cushing 1990). This oceanic region belongs to the Humboldt Current Systems (HCS), which is characterized by events of coastal upwelling, driving nutrient-rich water into the surface layer of the ocean. Upwelling processes generate a high primary production in the coastal waters of northern Chile (Daneri *et al.* 2000) which causes a high production of herbivorous zooplankton throughout the year and a low diversity of the macrofauna (Escribano *et al.* 2009).

Due to a low ventilation and high primary production, the water column is characterized by a permanent oxygen minimum zone (OMZ), which is located between 100-500 m depth (Herrera & Escribano 2006, Palma *et al.* 2006). In addition, this OMZ is a permanent feature, forming ecosystems with unique characteristics where chemical

and redox gradients are intense, inducing vertical division and greater bacterial richness (Stevens & Ulloa 2008). Bacterioplankton is a major component of the biomass in oceanic oligotrophic systems (Azam *et al*. 1983, Cho & Azam 1988, Amann *et al*. 1990, 1991; Amann 1995, Binder *et al*. 1996, Carlson *et al*. 1996), and the role of these organisms in biogeochemical cycling of carbon, nitrogen, and sulfur is critical for the function of the ocean's ecosystem (Cole *et al*. 1988, Ducklow & Carlson 1992).

Another characteristic of the coastal upwelling systems with a permanent OMZ is the presence of certain biogeochemical processes, especially those associated with the nitrogen cycle, like denitrification and nitrite reduction (Graco *et al.* 2007). The bacterioplankton inhabiting the OMZ anaerobically breathes nitrate, generating a nitrite maximum, which is an important tracer of these areas (Codispoti *et al.* 1986, Castro-González *et al.* 2005). Silva *et al.* (2009) acknowledges this as a characteristic feature of northern Chile, there being a relative minimum of nitrate associated with a maximum of nitrite. Other important process in the nitrogen cycle is the anaerobic respiration of ammonium (anammox) (Thamdrup *et al.* 2006, Hamersley *et al.* 2007, Galán *et al.* 2009) and the dissimilative reduction of nitrate (NO_3) into ammonium (NH_4^+) (Lam *et al.* 2009)

The presence of an OMZ has a significative impact on the ecosystem, other studies in northern Chile showcase the occurrence of a community of bacterium and cyanobacterium in hypoxic conditions (Stevens & Ulloa 2008, Santander *et al.* 2017)

In the present study, the composition and abundance of the bacterioplankton community in Chipana bay are described using the fluorescence *in situ* hybridization (FISH) method (Delong *et al*. 1989, Amann *et al*. 1990, Glöckner *et al*. 1996). This technique permits the determination of abundance and cell morphologies or size, as well as detect linages of uncultured microbes in different environments (Llobet-Brossa *et al*. 1998, Anton *et al*. 1999, Glöckner *et al*. 2000, Pernthaler & Amann 2005, Fazi *et al*. 2007, Medina & Moraga 2016).

The information of this study is considered relevant to understand the composition of bacterioplankton in upwelling systems. Abundance and distribution are indicators of the limiting effect of nutrients, predation and the interaction among phytoplankton. By studying the abundance and phylogenetics of the microorganisms is possible to better understand the role of the biogeochemical processes, in the distribution and ecological strategy of the microorganisms in the water column.

Materials and methods

Field sampling

The study was carried out in the Chipana bay (21°20'S) upwelling ecosystem, located 120 km off Iquique in northern Chile (Fig. 1). The oceanographic and biological information used in this study was extracted from the database generated by the CENSOR program (Climate variability and El Niño Southern Oscillation: Implications for natural coastal resources and management). The research took place between January 2006 and November 2007. To describe the regional variability, the sea surface temperature anomaly was analyzed between 2006 and 2007. The data were obtained from the Chilean National Center of Hydrographic and Oceanographical Data (CENDHOC) of the Chilean Army's Oceanographic and Hydrographic Service (SHOA)[1].

For the purpose of estimating monthly upwelling intensity, the upwelling index was calculated using the wind information from Diego Aracena Airport at Iquique, supplied by the Chilean Meteorological Direction, using the following equation:

$$M_x = \frac{\tau_y}{f}$$

Where *Mx* is the Ekman Transport ($m^3\ s^{-1}\ km^{-1}$), *f* is the Coriolis coefficient and τ_y is the wind stress (Bowden 1983).

Oceanographic data in the station was obtained with a SBE19-CTDO profiler equipped with a Sea Bird SBE-43 oxygen sensor. Water samples were collected with 5L Niskin bottles at 1, 5, 10, 25, 50 and 75 m depth. Oxygen concentration was chemically analyzed by the modified Winkler method (Carpenter 1965); the Chlorophyll-*a* pigment concentrations were estimated using a TD-700 fluorometer (Turner Desing®). 250 ml duplicate samples were taken for each depth and were filtered through 0.7 µm pore size glass fiber filter, filters were extracted during 24 h in acetone (90% v/v) (Parsons *et al.* 1984).

[1]<http://www.shoa.cl>

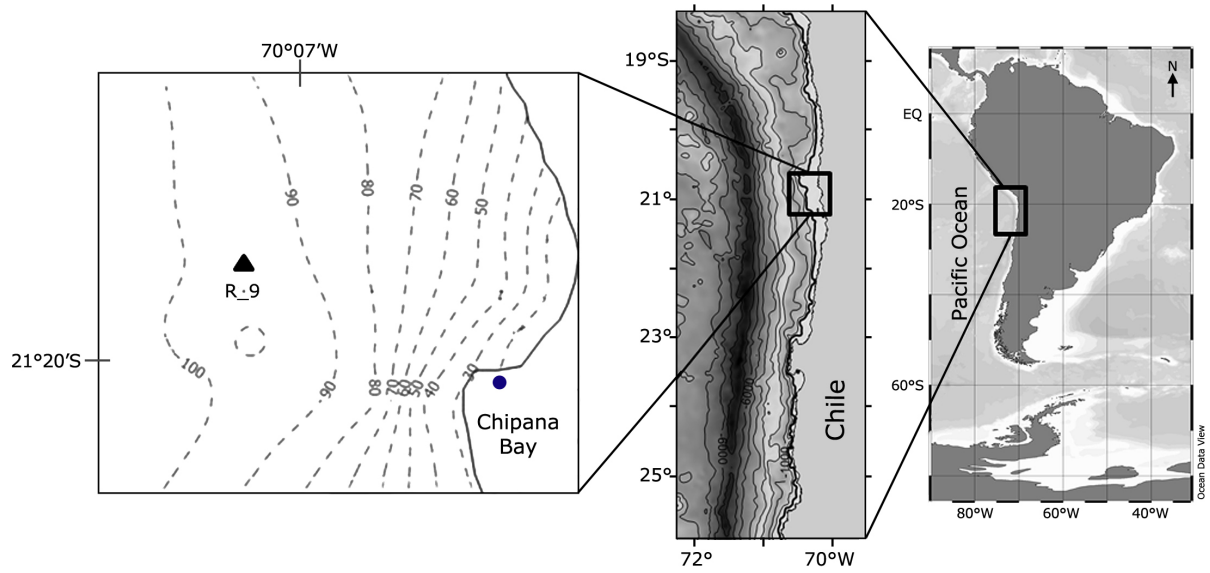

Figure 1. Sampling area off Chipana bay, northern Chile. The left image indicates the geographical position of the oceanographic station / Area de muestreo en bahía Chipana, norte de Chile. La imagen de la izquierda indica la posición de la estación oceanográfica

FISH, CY3-labeled oligonucleotide probes, staining and bacterial cell counts of samples

Samples of seawater were collected directly from Niskin bottles into sterile tubes and preserved in the darkness with Formalin (final concentration 1% v/v) at 4 ºC, 2 ml of each sample were filtered through a black polycarbonate filter (25 mm, 0.2 µm pore size).

Each filter was cut in 6 sections, at each section of the filter was hybridized with different probes, *Bacteria* domain (EUB338), specific bacterial group, *Alpha-proteobacteria* (ALF1b), *Beta-proteobacteria* (BET42a), *Gamma-proteobacteria* (GAM42a), *Cytophaga-flavobacterium* (CF319a) and *Archaea* (ARCH915). The probe sequences, hybridization conditions, and references are given in Table 1. The filter sections were placed on a glass slider and covered with 20 µl of hybridization solution containing 20 to 35% (p/v) formamide (depending on the experiment; see Table 1), a mixture [5M NaCl, 1M Tris-HCl (pH 7.2), 10% sodium dodecyl sulfate (SDS)] and 50 ng of the specific probe, and incubated at 46 °C for 2 h, in a equilibrated chamber. Probes BET42a and GAM42a were used with competitor oligonucleotides to optimize the hybridization conditions as previously described by Manz *et al.* (1992).

Afterwards, the filters sections were incubated in 10 ml of pre-warmed washing buffer at 48 ºC for 15 min in a sealed humid container. Hybridization buffer contained 5 mM EDTA, 0.01% SDS and variable concentration of NaCl). A formamide concentration of 35% was used 80 mM and 20% was used 0.225 mM of NaCl.

The filter sections were covered with 50 µl of DAPI solution, final concentration 72 mM, for 5 min at room temperature in the dark. Then, the filters were gently washed in 2 ml of 0,2 µm-filtered distilled water and ethanol, dried on blotting paper, and mounted on glass slides.

The hybridization and microscopy counts of hybridized and DAPI stained cells were performed as previously described by Medina & Moraga (2016). The filter sections were examined with a fluorescence microscope, equipped with a 50W high-pressure mercury bulb and specific filter sets [DAPI (Zeiss 01), Cy3 Chroma HQ 41007]. Each microscopic field was first viewed with the CY3 filter set before switching to the DAPI filter set, to avoid bleaching of CY3 during the DAPI examination. For each samples and probe, more than 500 DAPI-stained cells were counted, and the respective hybridized cells in 10 to 20 independent fields were counted. Standard deviations of counts ranged between 0.5 and 10%. The abundance of prokaryotic cells was performed using the protocol and algorithms proposed by Fry (1988).

Statistical analysis

The similarities of the samples were determined from a nonmetric multidimensional scaling analysis (NMDS) map based on a distance matrix calculated from the binary matrix constructed Bray-Curtis similarity. The similarity analyses comparing the sampling sites were performed by ANOSIM (Analysis of Similarity) (Clarke 1993). To avoid differences in the data distribution due to the sample size, proportions were transformed to log (x + 1) (Ramette 2007). NMDS analysis was performed with the software PRIMER version 6 (Clarke & Gorley 2005).

Results

Oceanographic setting

The weekly signal of the sea surface temperature (SST) showed the occurrence of a warm event (El Niño event) and a cold event (La Niña event). The warm event was detected in the winter of 2006 (June and July). Both were short-term events (approximately 2-4 months). During 2007 cold conditions prevailed, revealing the presence of La Niña event, which had an extension of six months (Fig. 2A). The wind persistently showed positive values, resulting in an upwelling index (UI) that revealed the prevalence of conditions that favor the occurrence of coastal upwelling during both, warm and cold events. The highest values of UI were detected in the spring-summer periods associated with wind velocities near 10 m s^{-1} (Fig. 2B).

The oceanographic conditions showed that the summers of 2006 and 2007 were related to the intensification of coastal upwelling events, which agrees with the UI values (Fig. 2B). On the other hand, the winter of 2006 was the warmest in the series, which is consistent with information from SST and coincident with the occurrence of a weak in 2006. The salinity displayed values between 34.3 and 35.1, with the prevalence of water with minimum salinity (< 34.7) revealing the presence of different water mases in the studied area, with a greater influence of Sub Antarctic Water (< 34.5 salinity, > 13 °C; > 2.0 ml DO L^{-1}), and a lesser one of Equatorial subsurface water (ESSW). The OMZ was always found between 20 and 50 m depth during the summer-spring and winter periods, respectively (Fig. 3C). In the periods when the OMZ was shallower, anoxic conditions were observed in the coastal margin, so there is direct evidence of the influence of offshore oceanographic conditions on the subtidal habitat. The chlorophyll-*a* showed

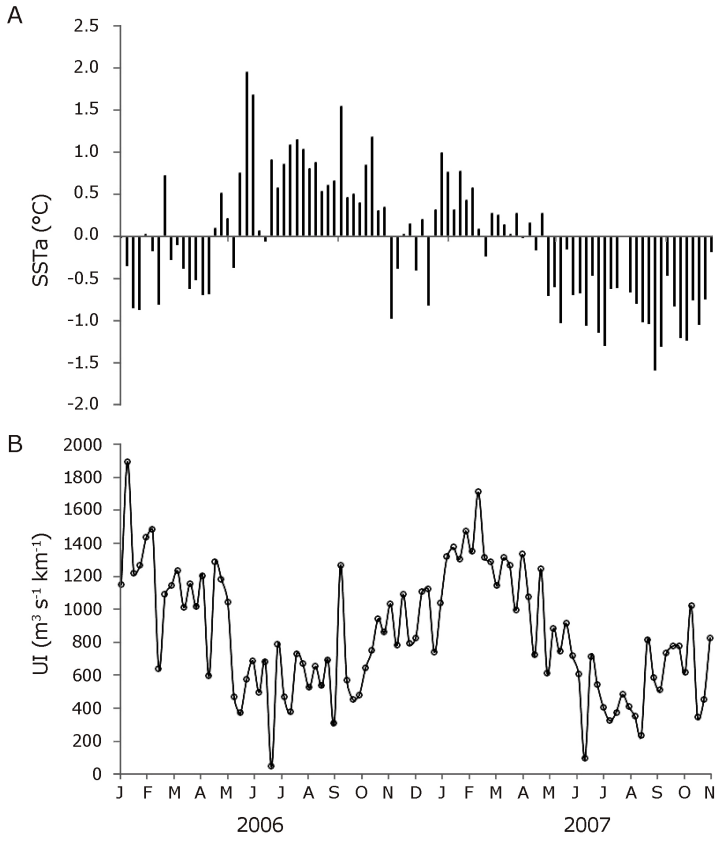

Figure 2. Temporal evolution of sea surface temperature anomaly (SSTa) and upwelling index (UI). Time series correspond to period between January 2006 and November 2007 / Evolución de la anomalía de la temperatura superficial del mar y el índice de surgencia. La serie de tiempo corresponde al periodo entre enero 2006 y noviembre 2007

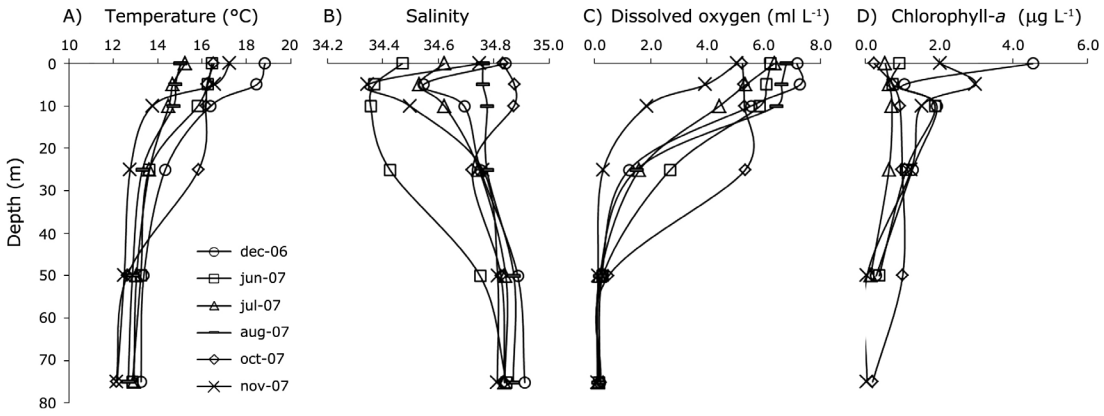

Figure 3. Oceanographic variables. A) Temperature, B) Salinity, C) Dissolved oxygen and D) Chlorophyll-*a*, in Chipana bay, northern Chile, during January 2006 and November 2007 / Variables oceanográficas. A) Temperatura, B) Salinidad, C) Oxígeno disuelto y D) Clorofila-*a*, en la bahía Chipana, norte de Chile, durante enero 2006 y noviembre 2007

changes during the studied period. In the course of 2007, the phytoplankton biomass declined to concentrations close to 1 µg L^{-1}, which was related to a cooling of the system due to La Niña event conditions that prevailed during the last part of the studied period. (Fig. 3)

Total cell count and domain-specific probing

The total DAPI cell counts found in our samples (10^6 to 10^7 cells ml^{-1}) were in the normal range reported for mesotrophic aquatic systems (Santander *et al.* 2017). In general, forms of cocci and bacilli symbionts and grouping were observed (Fig. 4). Standard deviations of counts ranged between 0.5 and 10%. The results are presented divided in two water columns, because the statistical analyzes showed differences in these two sections, between 0 to 10 m and 25 to 70 m depth.

Detection yields relative to EUB338 probe, present in most members of *Bacteria*, ranged from 9.3 x 10^5 to 2.2 x 10^6 cells ml^{-1} between 0 to 10 m, and 6.1x10^5 to 6.9 x 10^5 cells ml^{-1} between 25 to 70 m (Fig. 5). All examined samples showed bright hybridization signals and a clear distinction. Compared to the analysis of the probes used in this study, the percentages of hybridization for the *Bacteria* domain (EUB338) are low (between 20 to 68%). This is certainly due to the deficiency of the probe set used in this study. However, this can be improved by performing mixtures of probes for the *Bacteria* domain (Penthaler & Amann 2005).

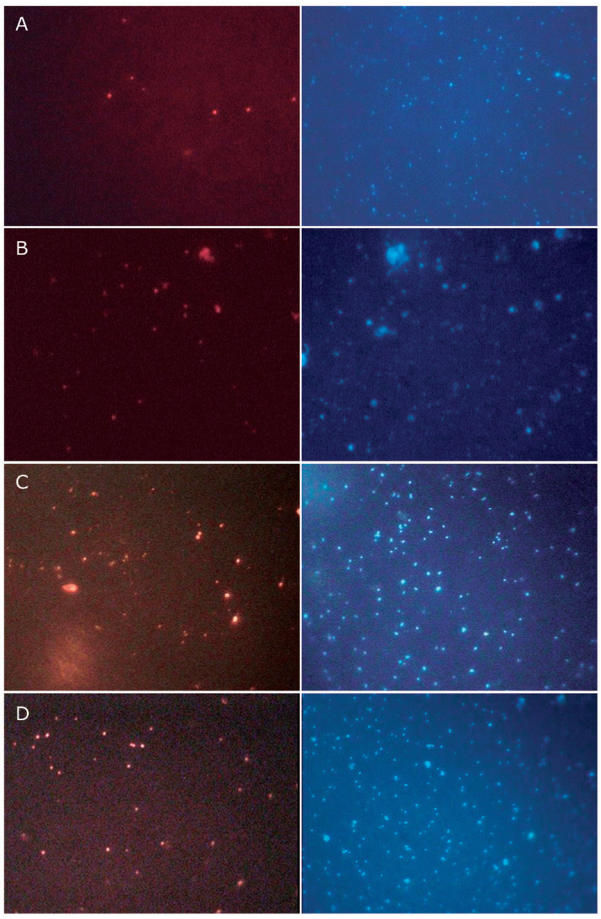

Figure 4. Abundance of bacterioplankton. Cell stained by DAPI (right) and by *in situ* hybridization with with CY3-labeled RNA-targeted oligonucleotide probes (left). A) Beta-proteobacteria, B) *Alfa-proteobacteria*, C) *Gamma-proteobacteria*, D) *Cytophaga-flavobacterium* / Abundancia del bacterioplancton. Células marcadas por DAPI (derecha) y por hibridación *in situ*, con sondas de oligonucleótidos dirigidas a ARN marcado con CY3 (izquierda)

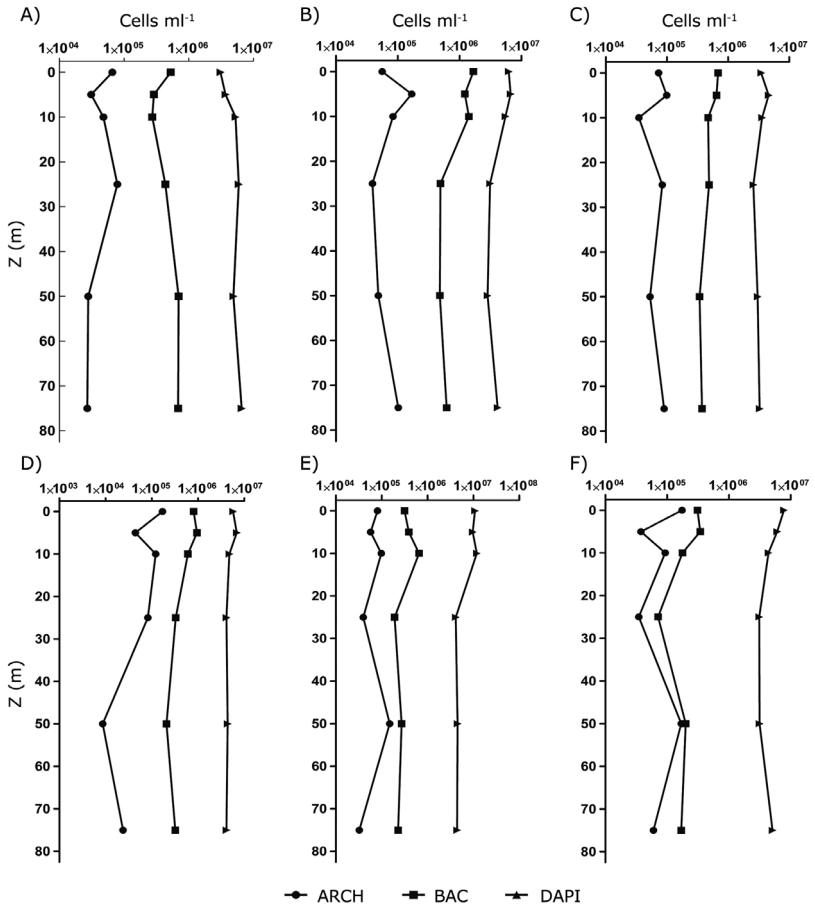

Figure 5. Vertical profiles of *Archaea* and *Bacteria* (ARCH, BAC) domain detected with FISH and DAPI cells detected in upwelling systems from Chipana bay. A) December, B) June, C) July, D) August, E) October, F) November / Perfiles verticales de los dominios *Archaea* y *Bacteria* (ARCH, BAC) detectadas con FISH, y células detectadas con DAPI en sistemas de surgencias de la bahía Chipana. A) diciembre, B) junio, C) julio, D) agosto, E) octubre, F) noviembre

Members of *Bacteria* were more abundant than members of *Archaea* in all samples. The specific counts with the oligonucleotide probe ARCH915 (*Archaea*) during the study period was of 2.1 to 18.3% of cells detected between 0 to 10 m and 4.3 to 16.9% of cells detected between 25 to 70 m depth, corresponding to the mean 5.3 $x10^4$ cells ml^{-1} (Table 2).

Alpha-proteobacteria dominated in the surface with 4.9 to 20.2% and deep layer with 9.3 to 21.5% of the total cells. Estimates of the relative abundance of *Gamma-proteobacteria* determined by FISH with the Gam42a probe indicated that they made up for 2.9 to 18.7% in the surface, and 4.4 to 23.6% in the layer below the oxycline of cells detected of the community.

The *Beta* subclass proteobacteria comprised small fractions of the bacterial communities as determined by FISH. In the Surface layer (0 to 10 m), it made up 3.4 to 10.5% of cells hybridization, and in the deep layer the count rounded between 3.6 to 9.3% of cells hybridization.

Members of the *Cytophaga-flavobacterium* group could be found in all examined marine samples. The relative abundance ranged from 6.7 to 27.9% cells detected (depth 0 to 10 m), and 7.5 to 13.3% cells detected (depth 25 to 70 m).

Table 2. Total DAPI count and specific FISH studies for months / Recuento total DAPI y FISH por mes de estudio

Depth (m)	Date	Total cell counts (cells m^{-1} 10^7) (mean ± SD)	Fraction (%) of total cell (mean ± SD) detected with probes				
			ALF968	BET42a	GAM42a	CF319a	Arch915
0-25	Dec-06	0.4 ± 0.2	13.5 ± 6.5	7.8 ± 3.1	10.9 ± 3,2	16.7 ± 2.3	6.5 ± 1.0
	Jun-07	0.4 ± 0.2	18.7 ± 9.1	10.5 ± 5.7	18.7 ± 4.3	27.9 ± 5.3	9.5 ± 2.2
	Jul-07	0.3 ± 0.03	20.2 ± 3.8	7.2 ± 1.1	16.1 ± 2.3	15.2 ± 3.1	12.7 ± 3.3
	Agu-07	0.6 ± 0.1	16.1 ± 1.4	6.2 ± 3.5	16.2 ± 2.2	15.7 ± 2.9	18.1 ± 3.6
	Oct-07	1.4 ± 2.0	4.9 ± 3.4	3.4 ± 1.4	2.9 ± 1.2	6.7 ± 0.8	2.6 ± 0.8
	Nov-07	0.7 ± 3.0	10.7 ± 4.5	7.0 ± 4.0	NA[b]	NA[b]	6.0 ± 2.5
50-70	Dec-06	0.5 ± 0.1	13.6 ± 4.4	8.5 ± 1.6	19.2 ± 0.9	13.3 ± 8.3	6.0 ± 3.3
	Jun-07	0.3 ± 0.05	15.9 ± 8.1	5.5 ± 3.3	20.6 ± 3.7	12.7 ± 8.7	16.9 ± 8.8
	Jul-07	0.2 ± 0.03	21.4 ± 10.4	7.0 ± 0.4	19.9 ± 2.6	8.2 ± 5.2	7.7 ± 3.2
	Agu-07	0.6 ± 0.4	21.5 ± 2.9	3.6 ± 1.5	23.6 ± 2.5	9.5 ± 1.6	4.9 ± 3.1
	Oct-07	0.5 ± 0.1	10.7 ± 9.2	8.5 ± 3.8	4.4 ± 1.0	7.5 ± 1.3	4.6 ± 4.1
	Nov-07	0.3 ± 0.07	9.3 ± 1.6	9.3 ± 5.2	NA[b]	NA[b]	10.7 ± 5.3

[a]Detection rate compared with DAPI. Mean and standard deviation were calculated by counting 20 fields in the section of the filter
[b]Unparsed

Spatial-temporal variability

The taxonomic composition of the prokaryotic component showed a low difference for the period under study where the value R statistical global is (0.357; $P < 0.01$); however, the NMDS shows that the month of October is different from the rest of the period (Fig. 6). This month was characterized by a general low abundance in the studied groups (see Table 2). This low prokaryotic abundance is contrasted with a low primary production suggested by a low chlorophyll-*a* concentration. The oceanographic conditions observed during the study period indicate a high variability, especially in the Surface layer above the thermocline. Moreover, differences were observed in relation to the depth with a global R (0.55; significance level of 0.1%). The NMDS clearly shows that the group of samples taken from the depths between 0-25 m, had the greatest abundances, while those taken between 50 and 75 m had the lowest abundances.

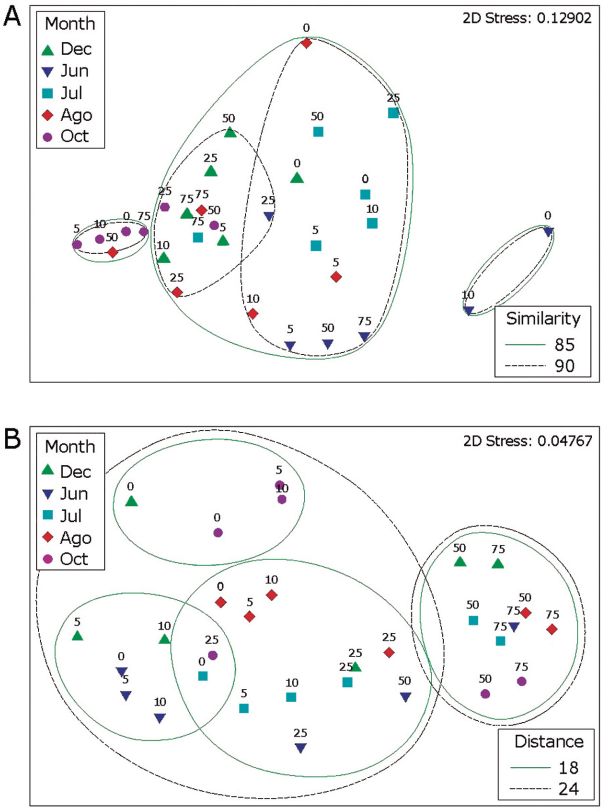

Figure 6. A) NMDS, temporary scale representation of the bacterioplankton abundances. Log transformation (X+1), Stress= 0.13 and Random 999 permutations. B) NMDS, vertical scale representation of the bacterioplankton abundances. Log transformation (X+1), Stress= 0.05 and Random 999 permutations / A) NMDS Representación a escala temporal de las abundancias del bacterioplancton. B) NMDS, representación vertical de las abundancias del bacterioplancton

Discussion

General observation of the Chipana bay microbial community

The visualization and enumeration of bacterial communities found in this study resemble those routinely found in a variety of pelagic marine environments using FISH methods (see *e.g.,* Glöckner *et al.* 1999, 2000; Penthaler & Amann 2005). The phylogenetic distribution determined in the study is consistent with previous works in Chipana bay (Delong *et al.* 1999, Stevens & Ulloa 2008).

The *Bacteria* domain showed a low abundance with the FISH technique (between 20 to 68%), the probe used, EUB338 have target site in 97% of bacterial 16S rRNA with sequences only 6 of 18 nucleotides are completely conserved (*E. coli* positions 338, 343, 346, 352, 354, 355). However, this deficiency of the probe can be improved by performing mixtures of probes for the *Bacteria* domain. (Penthaler & Amann 2005). It is suggested the use of the probes EUB338, EUB338 II, EUB338 III. The sequence variation type II and III are present within the candidate the Verrucomicrobia, and the Planctomycetales. Recent molecular analysis demonstrated a previously importance of these bacterial phyla for the OMZ ecosystems Stevens & Ulloa (2008).

Bacteria and the *Archaea* domain showed a similar condition to that indicated by Delong *et al.* (1999) and Stevens & Ulloa (2008), where the *Bacteria* domain was more abundant than *Archaea*.

This could be influenced by the convergence of low oxygen concentrations and a high presence of organic matter which modulate the abundance and structure of microbial communities associated to this environment. From the biogeochemical point of view, a series of studies made in the Eastern South Pacific, evaluate the impact of these areas upon the carbon cycle, the remineralization rates, the CO2 exchange (Chavez 2005, Paulmier 2005) and the nitrogen cycle (Daneri *et al.* 2000, Castro-González *et al.* 2005, Galan *et al.* 2009). Associated with the nitrogen cycle in the OMZ, processes like denitrification and nitrate reduction appear like key factors, being the nitrite an important tracer in this areas where the *Bacteria* domain could possibly be influenced by these conditions and by primary production associated to coastal upwelling, which is in turn, expressed in a major diversity and abundance of this domain in the studied area

The *Archaea* domain is characterized by being present in extreme environments, however, this group has been previously detected in marine environments, as in the deep plankton (Carlson *et al*. 1996, Karner *et al.* 2001) in the Antarctic sea when austral winter predominates and in spring when solar radiation is high. (Moore *et al.* 1998, Murray *et al.* 1999). Using the FISH technique, it was found a 3% abundance in the North Sea and a 2% in the Pacific Ocean (Giovannoni *et al.* 1996, Glöckner *et al.* 1996). Likewise, other studies with genomic techniques have detected the presence of the *Archaea* domain in various marine environments (Massana *et al.* 1997, Murray *et al.* 1998, Belmar *et al.* 2011).

The presence of *Archaea* has been described as an important fraction of the microbial component (Murray *et al.* 1998, Delong *et al.* 1999), and its presence contributes to the ecological processes in the picoplankton. The biogeochemical role is not yet well-known, however recent studies have revealed its relationship with the ammonia oxidation process by marine groups of *Crenarchaeota* (Beman *et al*. 2008, Church *et al*. 2010) and the oxidation processes of oxycline in the Pacific Ocean it is one of the main causes of a net source of N_2O to the atmosphere and contributes to the greenhouse effect (Belmar *et al*. 2011).

Phylogenetic group and their consequences on biochemical cycles

The relative abundance of specific bacterial groups (*Alpha*, *Beta*, *Gamma-proteobacteria* and *Cytophaga-flavobacterium*) was detected in all the samples studied. The distribution of bacterial groups in this study is similar to that found in other pelagic marine environments (Kirchman 2002, Stevens & Ulloa 2008).

Our results show that the *Cytophaga-flavobacterium*, *Alpha-proteobacteria* and *Gamma-proteobacteria* groups are important components of the bacterioplankton in the surface layers. *Cytophaga-flavobacterium* has been reported in different studies as an important group in the sea water (Manz *et al.* 1996, DeLong *et al.* 1999, Glöckner *et al.* 1999, Cottrell & Kirchman 2000, Stevens & Ulloa 2008). Moreover, it has also been described the relation between bacterioplankton and primary production and its exudates, in both fresh and marine waters (Kirchman & Rich 1997, Kirchman 2002). It has been demonstrated that bacterial abundance and production are positively related with phytoplankton primary production (Cole *et al.* 1993) and that bacterioplankton uses most of the proteins and amino acids coming from phytoplankton (Rosenstock & Simon 2001), continuing with the carbon flow in the environment and taking a part in the remineralization of organic matter in phytoplanktonic blooms, as reported by Grossart (1999).

The most typical *Alpha-proteobacteria* marine groups detected in other studies are the *Roseobacter* clade and the SAR11 clade (Field *et al.* 1997, Bouman *et al.* 2006, DeLong *et al.* 2006, Hill *et al.* 2010). Possibly, these clades are dominant in this environment, and its biogeochemical role is related to the sulfur cycle, as described by Stevens & Ulloa (2008).

The relative abundances of the *Gamma-proteobacteria* represent an important contribution to the bacterioplankton community and are constant in the surface (0 to 10 m) and deep layer (25 to 70 m). In other studies, a high abundance has been observed in the superficial layers of the *Gamma-proteobacteria* SAR86 clade, apparently, they interact with the primary producers in the photic zone (Stevens & Ulloa 2008).

The *Beta* subclass proteobacteria comprise a small fraction of the bacterial communities, these were detected in the order of 4% of hybridized cells in the Pacific Ocean (Glokner *et al*. 1999), in the South Pacific were detected 3% of the sequences from four clone libraries, all affiliated with *Methylophilaceae.* It is suggested that this group has an active role in methylotrophy (methane-oxidizing) (Stevens & Ulloa 2008). Furthermore, the HIMB624 strain is a planktonic marine bacterium within the family *Methylophilaceae* of the class *Beta-proteobacteria* isolated from coastal seawater of Oahu, Hawaii (Huggett *et al.* 2012). The upwelling of methane may be an indicator of the increase in abundance of the *Beta-proteobacteria* group, which may explain the higher relative abundances in this study.

Statistical abundance analyses

A vertical change in the structure of the microbial community was found in relation to the sampling depths, the NMDS clearly shows two clusters of samples at depths between 0-25 m and 50-75 m, with a global R= 0.55 and a significance level of 0.1% (see Fig. 3).

These results are consistent with the report for Stevens & Ulloa (2008), where a change in vertical distribution was observed in the bacterial community in the water column, among the OMZ, the more oxygenated surface and deep oxycline. In addition, observed exclusive groups of the OMZ (*Planctomycetes* and *Delta-proteobacteria*) and also observed a decrease in the abundances of *Cytophaga-flavobacterium* and an important contribution of the abundance of the *Gamma-proteobacteria* group (Stevens & Ulloa 2008).

Vertical distribution of bacterial communities in the oceanic water column has been reported to have a strong correlation to chlorophyll-*a* concentration with sequences affiliated to the SAR 406 and the SAR 202 clade (Giovannoni *et al.* 1996, Gordon & Giovannoni 1996). Depending on various environmental factors as light, (Moore *et al.* 1998) temperature, (Johnson *et al*. 2006), nutrients (Moore *et al.* 2005), chemocline (in gradients of sodium, dissolved manganese and nitrate content) (Bouman *et al.* 2006), and the redox potential. A close relationship was observed between chlorophyll-*a* concentration and bacterioplankton abundance.

In addition, a clear difference in abundances were observed during October 2007 (MDS). During this month, there was a decrease in abundance (see Fig. 6, Table 2). Likewise, during 2007 a strong La Niña even occurred, which coincides with the campaigns reported in this study, resulting in negative thermal anomalies (>1.5 °C), affecting mainly the phytoplankton, with low productivity and concentration of chlorophyll-*a*, corresponding to the results obtained in this work. These results indicate a clear correlation between the concentration of chlorophyll-*a* and the abundance of bacterioplankton.

However, the diversity observed is constant in all depths studied with the used methodology, which suggests that changes are based on abundances by specific groups and that they are affected by environmental factors and by primary production. These abundances and phylogenetic changes are directly related to the oceanographic characteristics. One of the most relevant gradients affecting the distribution of organisms is the shallow and intense OMZ, found in the water column and over the continental shelf, in addition, to the entrance of nutrients, especially the nitrogen by upwelling events (Lam *et al.* 2009), where a maximum secondary nitrite layer forms an intense chemical gradient (Castro-González *et al*. 2005, Morrison *et al*. 1999), this features could cause a biological barrier in the phylogenic of microorganisms.

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