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# Spatial and temporal variability of planktonic archaeal abundance in the Humboldt Current System off Chile

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ABSTRACT

The latest advances in the field of microbial ecology have shown that planktonic Archaea are one of the most abundant unicellular microorganisms of the oceans. However, no information is available on the contribution this group makes to the prokaryote assemblages that inhabit the eastern South Pacific Ocean. Here, we describe the relative abundance and vertical distribution of planktonic Archaea off northern and central-southern Chile. Data come from several cruises and a 45-month time series at a station located on the shelf off central-southern Chile. Both the taxonomic composition of the prokaryote community and its relative abundance were determined using quantitative dot blot 16S-rRNA hybridizations. Total Archaea in central-southern Chile made up 6–87% of the prokaryote rRNA in the water column and did not present evidence of any seasonal pattern. Crenarchaea were the most abundant archaeal group at this site and were significantly associated with the ammonium concentration ($r^2 = 0.16$, $p = 0.0003$, $n = 80$). Archaeal abundance in the time series was usually greater in the deeper layer ($>50$ m), with contributions reaching up to ~90% of the prokaryote rRNA on certain occasions, and decreasing towards the surface. Important increments in the relative abundance of total Archaea were observed on given dates at the surface of the time-series station off central-southern Chile. Off northern Chile, total Archaea normally contributed from ~10% to 50% of the prokaryote rRNA found between 10 and 1000 m, and were generally important in the mesopelagic realm. Our results indicate that Archaea constitute an important fraction of the prokaryote assemblage in the water column of the Humboldt Current System, especially in the oxygen minimum zone.



## 1. Introduction

The continental shelf located between central-southern and northern Chile is part of one of the most productive marine ecosystems of the world (Daneri et al., 2000; Troncoso et al., 2003). This shelf is characterized by the presence of a subsurface low-oxygen water mass (Equatorial Subsurface Water, ESSW), geographically different upwelling zones, and the important influence of interannual variations due to El Niño Southern Oscillation (ENSO). An oxygen minimum zone (OMZ) that is associated with ESSW acts as an important regulator for both aerobic respiration (Eissler and Quiñones, 1999; González and Quiñones, 2002) and pelagic biota distribution (Boyd et al., 1980; Morales et al., 1996; Escribano and Hidalgo, 2000; González and Quiñones, 2000).

It is widely known that prokaryotes make up a major portion of the biomass in marine planktonic environments (Cole et al., 1988), playing a key role in the carbon flux as reported for the Humboldt Current System (HCS) (Troncoso et al., 2003; Cuevas et al., 2004). Molecular biology techniques have revealed that Archaea are abundant and distributed virtually in all environments (e.g., oceanic, freshwater, soil; for review, see Chaban et al., 2006). Indeed, uncultured Archaea are one of the most abundant unicellular groups in the ocean, especially in the mesopelagic zone (Massana et al., 1997, 2000; Fuhrman and Ouverney, 1998; Karner et al., 2001). In this context, Karner et al. (2001) suggest that the global oceans harbor approximately $1.3 \times 10^{28}$ archaeal cells, which is roughly equivalent to 42% of the global abundance of bacteria in the oceans.

Three major phyla have been proposed as constituting the Archaea domain although only two are presently accepted (Boone and Castenholz, 2001): Crenarchaea and Euryarchaea (Woese and Olsen, 1986). The third phylum (candidate), called Korarchaea, is a more distant and deeper branch of Archaea whose existence has been suggested based only on DNA sequences from environmental surveys (Barns et al., 1994). A fourth group, the Nanoarchaea, have

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been proposed as a new archaeal phylum (Huber et al., 2002, 2003). However, as in the case of Korarchaea, its position in the archaeal tree is controversial (Robertson et al., 2005).

The 16S-rDNA sequences from picoplanktonic Archaea can be classified in four marine groups: marine group I falls within the Crenarchaea whereas marine groups II, III, and IV fall within the Euryarchaea (Giovannoni and Stingl, 2005; Schleper et al., 2005). In marine temperate regions, marine group I Crenarchaeota dominates at depths $\geqslant$75 m, depending on the site studied, and Euryarchaea (group II) can be important in shallow waters (Massana et al., 1997; DeLong et al., 1999; Murray et al., 1999a; Massana et al., 2000; Pernthaler et al., 2002). Nonetheless, marine group I frequently represents the bulk fraction throughout the water column (Massana et al., 1998; Murray et al., 1998, 1999b; Karner et al., 2001).

To the best of our knowledge, no information is currently available regarding the contribution of planktonic Archaea to the prokaryotic assemblages inhabiting the eastern South Pacific Ocean. Here, we describe the vertical distribution of planktonic Archaea and its temporal fluctuations using vertical profiles of relative abundance obtained during several cruises off Chile and a nearly 4-year-long time series at a shelf station that is sampled monthly.

## 2. Material and methods

### 2.1. Field sampling and environmental variables

The study was conducted at two upwelling sites in the HCS off Chile and the data comes from several cruises and a 45-month time series at a station located off central-southern Chile (Fig. 1, Table 1). Water samples of at least 7 L were collected off central-southern Chile onboard the R/V *Kay-Kay* (University of Concepción) and off northern Chile onboard the R/V *Vidal Gormaz* (Chilean Navy). The samples were collected at different depths with Niskin bottles and kept onboard (at *in situ* surface temperature and in complete darkness) in carboys pre-washed with 10 N HCl until arrival at the marine coastal laboratory. These samples were used to analyze the taxonomic composition at the domain level by quantitative dot blot hybridization.

Water samples were also collected for analyses of phosphate, silicate, nitrite, nitrate, ammonium, dissolved oxygen, and chlorophyll. Temperature, salinity, and oxygen profiles were taken with a CTDO (model SBE-19, Sea-Bird Electronics Inc.). $PO_4$, $SiO_4$, $NO_2$-N, and $NO_3$-N were determined using an ALPKEM Flow-Solution IV autoanalyzer (OI Analytical, College Station) according to Strickland and Parsons (1972). $NH_4$-N was determined as described by Holmes et al. (1999) using a Turner Designs fluorometer (model 10-AU). The oxygen measurements taken with the CTDO were calibrated using dissolved oxygen determinations by the Winkler method (Carpenter, 1965) in discreet samples. Phytoplanktonic standing stock was estimated through chlorophyll-*a* (Chl-*a*) measurements following Holm-Hansen et al. (1965).

### 2.2. Dot blot hybridizations of rRNA

RNase-inactivation was achieved by treating all solutions with diethylpyrocarbonate (DEPC, Sigma Chemical Co.) as described by Sambrook et al. (1989). The seawater was pre-filtered through 25 μm and concentrated by vacuum filtration ($<$10 cm Hg) using cellulose ester filters (pore size 0.22 μm; GSWP04700, Millipore Corp.). Subsequently, the microorganisms retained on the filters were resuspended in pre-filtered (0.22 μm; GSWP04700, Millipore Corp.) seawater containing Tween 20 (final concentration

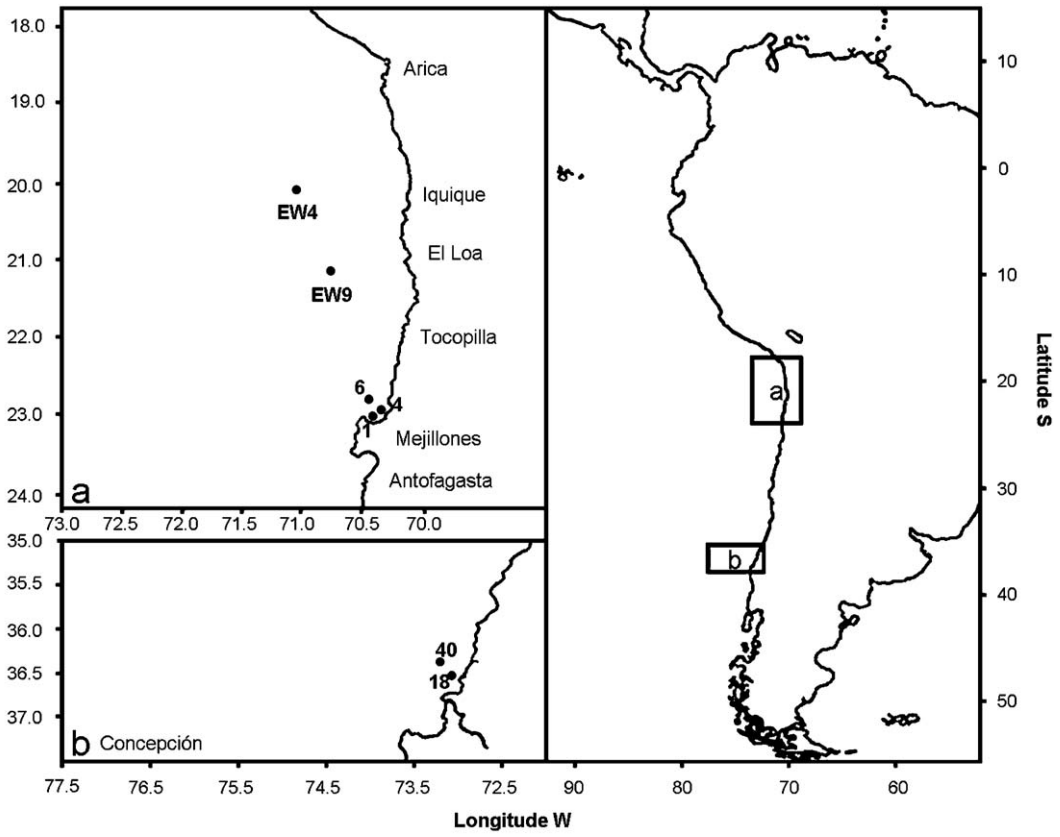


**Fig. 1.** Study area in the eastern South Pacific. Sampling stations are indicated by black dots.