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# Archaeal and bacterial assemblages in the Oxygen Minimum Zone of the upwelling ecosystem off Central Chile as determined by organic biomarkers

## Ensamblajes de arqueas y bacterias en la Zona de Mínimo Oxígeno del ecosistema de surgencia de Chile central determinados mediante biomarcadores orgánicos

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### ABSTRACT

Organic biomarkers were used to investigate the influence of seasonal changes in oxygenation and water chemistry on the distribution of archaea and bacteria in the water column and surface sediments of the continental shelf off central Chile (*ca.* 36°S), an area influenced by seasonal upwelling and the development of an oxygen minimum zone. We were interested in establishing if occurrence of archaea and bacteria responds to oxygenation and water chemistry for which we analyzed archaeal isoprenoid (i) and bacterial branched (br) glycerol dialkyl glycerol tetraethers (GDGTs). Our results combined with molecular data from a year round observational program at the same sampling site and depths indicatives the occurrence and dominance of the marine pelagic group Thaumarchaeota. Changes in the distribution of iGDGTs might be explained by (i) the presence of archaeal populations in sub-oxic waters, phylogenetically different from those in surface water, (ii) changes in the relative contribution of Euryarchaeota with depth, and (iii) a relationship between Thaumarchaeota and environmental factors other than temperature. Branched GDGTs were more abundant in the upper, oxic layer during the non-upwelling season, may be a result of higher river runoff, whereas their diversity was higher within sub-oxic waters. Our results indicate a vertical segregation of iGDGTs and brGDGTs, with predominance of archaeal biomarkers during the low productivity season.

**KEYWORDS:** Glycerol dialkyl glycerol tetraethers (GDGTs), archaea, bacteria, oxygen minimum zone, upwelling, Chile.

### RESUMEN

Se utilizaron biomarcadores orgánicos en para investigar la influencia de cambios estacionales en los niveles de oxigenación y la química del agua sobre la distribución de arqueas y bacterias en la columna de agua y los sedimentos superficiales de la plataforma continental frente a Chile central, un área influenciada por surgencia estacional asociada al desarrollo de una zona de mínimo oxígeno. Nuestro interés es establecer si la ocurrencia de arquea y bacteria responde a la oxigenación y química del agua para lo cual analizamos glicerol dialquil glicerol tetra-éteres (GDGTs) isoprenoides arqueanos (i) y ramificados bacterianos (r). Nuestros resultados, combinados con datos moleculares de observaciones durante un año en el mismo lugar y profundidades del sitio de estudio indican la presencia y dominancia del grupo arqueano marino-pelágico Thaumarchaeota. Los cambios observados en la distribución de iGDGTs podrían explicarse por (i) la presencia de poblaciones de arqueas marinas en la capa de agua sub-óxica, filogenéticamente diferentes a las de aguas superficiales, (ii) cambio en la contribución relativa de Euryarchaeota con profundidad, y (iii) una relación entre Thaumarchaeota y factores ambientales distintos a la temperatura. Los GDGTs ramificados fueron más abundantes en la capa óxica superior durante el periodo de no-surgencia, tal vez influenciado por la alta descarga de ríos, mientras que su diversidad fue más alta en el agua sub-óxica. Nuestros resultados indican una segregación vertical de los GDGTs isoprenoides y ramificados, con el predominio de biomarcadores arqueanos durante el periodo de baja productividad.

**PALABRAS CLAVE:** Glicerol dialquil glicerol tetraéteres (GDGTs), arquea, bacteria, zona de mínimo de oxígeno, surgencia, Chile.

## INTRODUCTION

Isoprenoid (i) and branched (br) glycerol dialkyl glycerol tetraethers (GDGTs) are cell membrane lipids diagnostic of archaea and bacteria, respectively (Langworthy *et al.* 1983; Hoefs *et al.* 1997; Hopmans *et al.* 2004; Zhou *et al.* 2011; Fietz *et al.* 2012). These biomarkers have been increasingly used to investigate microbial diversity, biogeochemistry, and terrestrial input into contemporaneous and ancient aquatic systems, as well as for reconstructions of past ocean temperatures by using the TEX<sub>86</sub> index (Kuypers *et al.* 2001; Schouten *et al.* 2002; Blumenberg *et al.* 2004; Hopmans *et al.* 2004; Herfort *et al.* 2006; Kim *et al.* 2010; Schouten *et al.* 2013; Pearson & Ingalls 2013). Archaeal iGDGTs are ubiquitous in marine and freshwater and sediments, as well as in soils (Schouten *et al.* 2000; Wuchter *et al.* 2005; Sinninghe Damsté *et al.* 2012). In the marine realm, GDGTs have been suggested to derive mostly from planktonic Thaumarchaeota (De Long *et al.* 1998; Schouten *et al.* 2000; Sinninghe Damsté *et al.* 2002a, 2002b; Wuchter *et al.* 2005; Turich *et al.* 2007; Pitcher *et al.* 2011a), although a contribution from planktonic euryarchaeota and benthic archaea cannot be excluded (Pearson & Ingalls 2013; Lincoln *et al.* 2014). Core archaeal lipids comprise a diverse group of compounds, including dialkyl glycerol diethers (DGDs), isoprenoid glycerol dialkanol diethers (iGDDs) and iGDGTs containing between 0 and up to 8 cyclopentane moieties and, in the case of crenarchaeol, four pentacyclic moieties and one cyclohexane moiety (Langworthy *et al.* 1972; Kates 1992; Gambacorta *et al.* 1994; Schleper *et al.* 1995; De Rosa 1996; Swain *et al.* 1997; Shimada *et al.* 2002; Macalady *et al.* 2004; Schouten *et al.* 2007; Liu *et al.* 2012; Schouten *et al.* 2013).

The most common iGDGTs in marine environments are the acyclic GDGT-0 and crenarchaeol (Nishihara *et al.* 1987; Schouten *et al.* 2000; Sinninghe Damsté *et al.* 2000; Turich *et al.* 2007). Although GDGTs 1-4 have been found in enrichment cultures and pure cultures of marine archaea (Wuchter *et al.* 2004; De la Torre *et al.* 2008; Schouten *et al.* 2008; Pitcher *et al.* 2010), they comprise a minor proportion of the GDGT pool found in marine particulate matter and sediments (Schouten *et al.* 2000; Schouten *et al.* 2002; Pitcher *et al.* 2011b).

Archaea were originally thought to inhabit extreme environments such as those characterized by high salinity, high temperature, or anoxia (Woese *et al.* 1978; De Rosa & Gambacorta 1988; De Long 2003). However, non-extremophilic archaea are ubiquitous and abundant in oceanic (Fuhrman 1992; DeLong *et al.* 1994; Murray *et al.* 1999; Karner *et al.* 2001) and coastal (DeLong 1992; Murray *et al.* 1998a; Pernthaler *et al.* 2002; Levipan *et al.* 2007a; Quiñones *et al.* 2009) regions. The distribution of

genes markers and membrane lipids indicates that marine Thaumarchaeota (formerly Marine Group 1 Crenarchaeota, Brochier-Armanet *et al.* 2008) are ubiquitous in both epipelagic and mesopelagic areas of the water column, although they are more abundant in subsurface water near the base of the photic zone (Sinninghe Damsté *et al.* 2002a, 2002b; Herndl *et al.* 2005; Wuchter *et al.* 2005; Levipan *et al.* 2007a; 2007b; Turich *et al.* 2007; Quiñones *et al.* 2009; Pitcher *et al.* 2011b). Euryarchaeota also occur in surface, mesopelagic and deep waters, although generally in lower abundances compared to Thaumarchaeota (Massana *et al.* 1997; Lopez-Garcia *et al.* 2001; Bano *et al.* 2004; Moreira *et al.* 2004).

Since brGDGTs have been shown to be ubiquitous in peat and soil (Schouten *et al.* 2000; Sinninghe Damsté *et al.* 2000; Hopmans *et al.* 2004; Weijers *et al.* 2006, 2007; Huguet *et al.* 2010a) as well as in marine and lacustrine environments influenced by terrestrial input (Schouten *et al.* 2007; Belicka & Harvey 2009; Blaga *et al.* 2009; Kim *et al.* 2009; Powers *et al.* 2010), it is thought that soil bacteria synthesize brGDGTs. However only one brGDGT (brGDGT-I; Fig. 3i) has been identified in two cultures of anaerobic acidobacteria (Sinninghe Damsté *et al.* 2011). Since high concentrations of brGDGTs are found in sub-oxic-anoxic aquatic environments, it is likely that they are also synthesized by other groups of bacteria (Sinninghe Damsté *et al.* 2011).

iGDGTs are more abundant in sub-surface oxic water (Huguet *et al.* 2007; Ingalls *et al.* 2012), as well as in sub-oxic water, such as the Arabian Sea (Sinninghe Damsté *et al.* 2002a, 2002b), Black Sea (King *et al.* 1998; Wakeham *et al.* 2007), Cariaco Basin (Wakeham *et al.* 2004, 2012), and the eastern tropical North Pacific (Xie 2013). In these areas, oxygen minimum zones are characterized by dissolved oxygen concentrations lower than 22  $\mu\text{mol L}^{-1}$  (sub-oxic conditions) (Helly & Levin 2004). Notably, the distribution of iGDGTs in sub-oxic waters exhibit an elevated contribution of GDGT-2 and -3, thereby yielding TEX<sub>86</sub>-derived temperature values that largely offset *in situ* temperature (Schouten *et al.* 2012a, 2012b; Xie 2013). Most studies suggest that pelagic Thaumarchaeota are the most likely biological source of iGDGTs in epipelagic and mesopelagic environments, including sub-oxic settings (DeLong *et al.* 1998; Sinninghe Damsté *et al.* 2002a, 2002b; Francis *et al.* 2005; Herndl *et al.* 2005; Wuchter *et al.* 2005; Pitcher *et al.* 2011b; Schouten *et al.* 2012a). However, the potential contribution of euryarchaeota, as well as the role of environmental variables other than temperature on the relative contribution of different iGDGTs, remains controversial. The distribution of iGDGTs in sub-oxic environments suggests that facultative anaerobic organisms involved in the cycling of C and N are potential sources.

However, few environmental studies have investigated the spatial and temporal variability of iGDGTs and brGDGTs in settings affected by seasonal variations in O<sub>2</sub> concentration (e.g. Lengger *et al.* 2012; Schouten *et al.* 2012b).

The eastern tropical South Pacific is characterized by a prominent, intermediate-water oxygen minimum zone, maintained by the combined effect of poorly ventilated waters and high microbial respiration of settling organic matter (Pantoja *et al.* 2004). Intense biological respiration results from the upwelling of nutrient-rich, oxygen depleted equatorial sub-surface water that enhances biological productivity in surface water (Kamykowski & Zentara 1990; Helly & Levin 2004). The coastal area off central Chile (*ca.* 36 °S), in the southernmost area of intense seasonal upwelling, is one of the most productive areas of the oceans (1-20 g C m<sup>-2</sup> d<sup>-1</sup>; Montero *et al.* 2007). In this area, fertilization of surface waters occurs seasonally and is driven by an anti-cyclonic atmospheric circulation regime favoring southwesterly winds, leading to the upwelling of equatorial sub-surface water during austral spring-summer (Sobarzo *et al.* 2007). Consequently, a seasonal oxygen minimum zone develops over the continental shelf off central Chile during spring and summer, expanding from the water-sediment interface up to the photic zone (Ahumada & Chuecas 1979; Sobarzo *et al.* 2007). This markedly seasonal oceanographic variability allows the study of spatial and temporal changes in the distribution, composition and abundance of iGDGTs and brGDGTs under varying regimes of nutrient content, productivity and oxygenation.

We investigated the distribution of these two lipid classes in oxic surface and seasonally sub-oxic sub-surface waters and surface sediments during the upwelling and non-upwelling seasons off the coast of Concepción, Chile. The goal was to use these two contrasting conditions to assess if the occurrence of archaea and bacteria responds to oxygenation and water chemistry and reflects on vertical and seasonal patterns of the prokaryotic assemblage, and to evaluate whether the GDGT biomarker signal is imprinted in surface sediments. In order to independently assess Archaeal diversity and abundance, we analyzed PCR-DGGE and 16S rRNA dot blot hybridization in surface and subsurface water samples collected from the study site.

## METHODS

### SAMPLING

The study area (Station 18; 36°30.8'S 73°7'W) is *ca.* 18 nautical miles from the coastline off Concepción, with a depth of 90 m (Fig. 1). Station 18 is the site of the Oceanographic Time Series maintained by the Center for Oceanographic Research in the eastern South Pacific at University of Concepción (COPAS Center; [www.copas.udec.cl/eng/research/serie](http://www.copas.udec.cl/eng/research/serie)). The sampling cruises were supported in the framework of the Moore Foundation project “Microbial Initiative in Low Oxygen off Concepción and Oregon (MILOCO; [http://mi\\_loco.coas.oregonstate.edu](http://mi_loco.coas.oregonstate.edu)) and the COPAS Center.

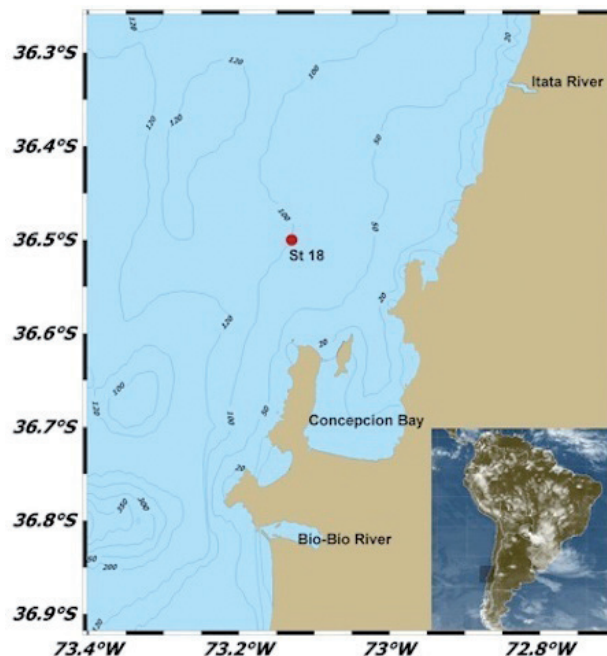


FIGURE 1. Location of the study site in the upwelling area off Concepción in Central Chile (36°S 73°W).

FIGURA 1. Ubicación del sitio de estudio en el area de surgencia de Chile Central (36°S 73°W).

Water samples were collected onboard R/V Kay-Kay II in September 2009 (upwelling season) and June 2010 (non-upwelling season), from water masses of contrasting redox conditions. *Ca.* 100 L of seawater were collected at 10 (oxic-surface layer) and 80 m (seasonal sub-oxic sub-surface layer) depth using a rosette equipped with Niskin bottles. Samples were transferred to darkened carboys and filtered onshore through pre-combusted (450 °C, 4 h) glass fiber filters (0.7 µm, Millipore) with a peristaltic pump. Ancillary

data including temperature, salinity, chlorophyll, and O<sub>2</sub> and nutrient concentrations were collected in the whole water column (Fig. 2).

Additionally, a 25 -cm -long sediment core was collected at the same site in February 2009 (summer upwelling season) using a GOMEX BOX corer. In this study, we analyzed the 0-0.25 cm section.

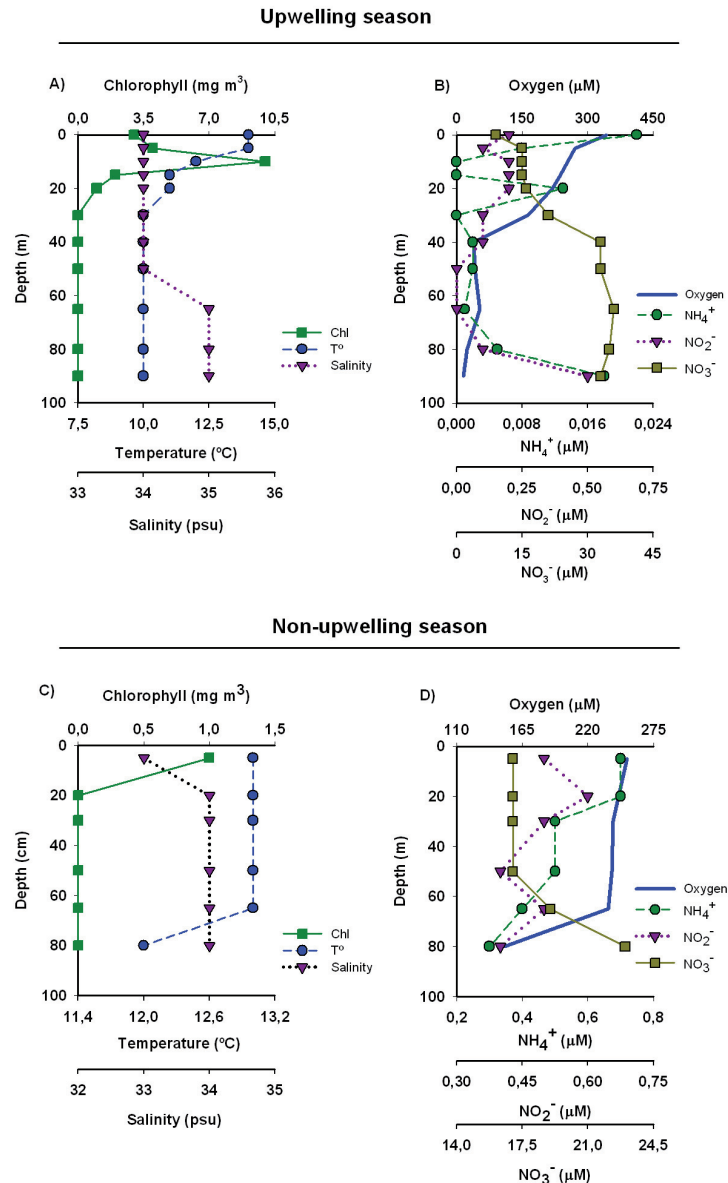


FIGURE 2. Vertical distribution of chlorophyll, temperature, salinity, oxygen, ammonium, nitrite and nitrate during upwelling (September 2009; a-b) and non-upwelling (June 2010; c-d) seasons. Information of the COPAS Center Time Series Oceanographic Station 18 is available at [www.copas.udec.cl/eng/research/serie/](http://www.copas.udec.cl/eng/research/serie/).

FIGURA 2. Distribución vertical de clorofila, temperatura, salinidad, oxígeno, amonio, nitrito y nitrato durante las estaciones de surgencia (Septiembre 2009; a-b) no-surgencia (Junio 2010; c-d). La información de la Serie de Tiempo de la Estación 18 del Centro COPAS está disponible en: [www.copas.udec.cl/esp/investigacion/serie/](http://www.copas.udec.cl/esp/investigacion/serie/).

For PCR-DGGE analyses, we collected water samples at 10 and 80 m of depth in January (upwelling season; 2006) and June (non-upwelling season; 2006), and in January, February, March, April, June, July, August, September, October, November 2006 for 16S rRNA dot blot hybridization.

Samples (500 mL) were pre-filtered through 25 µm and concentrated by vacuum filtration (<10 cm Hg) on cellulose ester filters (pore size 0.22 µm; GSWP04700; Millipore). Genomic DNA was extracted directly from the thawed filters using the PowerSoil™ DNA Isolation Kit (MoBio Laboratories, USA). Genomic DNA extracts were stored at -20°C until further PCR-DGGE analyses.

Seven liters of seawater pre-filtered through 25 µm and concentrated by vacuum filtration (< 10 cm Hg) using filters of cellulose ester (pore size 0.22 µm; GSWP04700, Millipore).

#### LIPID EXTRACTION AND ANALYSIS

Filters containing particulate organic matter were sequentially extracted by ultrasonication (3x) with methanol, dichloromethane/methanol (1:1, vol:vol) and dichloromethane. Lipid extracts were concentrated using a rotary evaporator and dried over a small Pasteur pipette filled with combusted glass wool and anhydrous Na<sub>2</sub>SO<sub>4</sub>. Lipids were separated into a non-polar and a polar fractions using a Pasteur pipette filled with activated Al<sub>2</sub>O<sub>3</sub>, after elution with hexane/dichloromethane (9:1, vol:vol) and dichloromethane and methanol(1:1 vol:vol), respectively. For GDGT analysis, an aliquot of the polar fraction was dissolved in hexane/propanol (99:1, vol:vol) and filtered through a 0.45 µm PFTE filter. Samples were analyzed using high performance liquid chromatography-mass spectrometry (HPLC-MS), following Hopmans et al. (2000). The HPLC-MS system comprised a 1200 Series HPLC system (Agilent Technologies) equipped with an auto-sampler and a binary pump linked to a Q-TOF 6520 mass spectrometer via an atmospheric pressure chemical ionization interface (Agilent Technologies). Samples were dissolved in 200 µl hexane/isopropanol (99:1, vol:vol). The iGDGTs and brGDGTs were separated using a Prevail Cyano column (2.1 x 150 mm, 3 mm; Grace, Deerfield, IL, USA) maintained at 35 °C, at a flow rate of 0.25 ml min<sup>-1</sup>. The elution program was: 5 min 100% eluent A (hexane/isopropanol, 99:1, vol:vol) in 20 min, followed by a linear gradient to 100% eluent B (hexane/isopropanol, 90:10 vol:vol) at 35 min, and then held at 100% eluent B for 5 min. The column was re-equilibrated with 100% A at a 0.6 ml min<sup>-1</sup> for 5 min between injections. Semi quantification of core GDGTs was achieved by co-injection of samples with a C<sub>46</sub> GDGT standard (Huguet et al. 2010b). Results are given as GDGTs concentration ± analytical error.

The iGDGTs and brGDGTs (Fig. 3) were identified from their characteristic [M + H]<sup>+</sup> ions: GDGT-0 (*m/z* 1302), GDGT-1 (*m/z* 1300), GDGT-2 (*m/z* 1298), GDGT-3 (*m/z* 1296), GDGT-4 (*m/z* 1294), crenarchaeol (Cren) and crenarchaeol regio-isomer (Cren') (*m/z* 1292), brGDGT-III (*m/z* 1050); brGDGT-IIIb (*m/z* 1048); brGDGT-IIIc (*m/z* 1046); brGDGT-II (*m/z* 1036), brGDGT-IIc (*m/z* 1032); brGDGT-I (*m/z* 1022), brGDGT-IIb (*m/z* 1034), brGDGT-Ib (*m/z* 1020), brGDGT-Ic (*m/z*1018).

TEX<sub>86</sub> and TEX<sub>86</sub><sup>L</sup> indices for suspended particulate matter (SPM) and surface sediments were calculated as described by Schouten et al. (2002) and Kim et al. (2010):

$$TEX_{86} = (GDGT-2 + GDGT-3 + Cren') / (GDGT-1 + GDGT-2 + GDGT-3 + Cren') \quad (1)$$

$$TEX_{86}^L = \log [(GDGT-2) / (GDGT-1 + GDGT-2 + GDGT-3)] \quad (2)$$

Additionally water column TEX<sub>86</sub> values were converted to temperature according to Wuchter et al. (2005):

$$TEX_{86} = 0.017 \times T + 0.29 \quad (3)$$

Sedimentary TEX<sub>86</sub> and TEX<sub>86</sub><sup>L</sup> values were converted to temperatures ± (proxy residual error + analytical error) according to Kim et al. (2008):

$$SST = 56.2 \times TEX_{86} - 10.8 \quad (4)$$

and Kim et al. (2010):

$$SST = 67.5 \times (TEX_{86}^L) + 46.9 \quad (5)$$

In order to assess the overall temporal and spatial variations in iGDGTs we calculated the Ring Index (RI; weighted average number of cyclopentane rings in GDGTs) according to Liu et al. (2011):

Ring Index =

$$[(\% GDGT-1) + 2(\%GDGT-2) + 3(\%GDGT-3) + 5(\%Crenarchaeol+Cren')]/100 \quad (6)$$

Assuming that the average number of cyclopentane rings in the GDGT pool increases with growth temperature (Schouten et al. 2002; Wuchter et al. 2004), we could expect a positive relationship between RI and TEX<sub>86</sub> values.

The GDGT-2/GDGT-3 ratio was calculated according to Taylor et al. (2013) in order to evaluate the contribution from archaeal community inhabiting deeper water column during both upwelling and non-upwelling contrasting conditions.

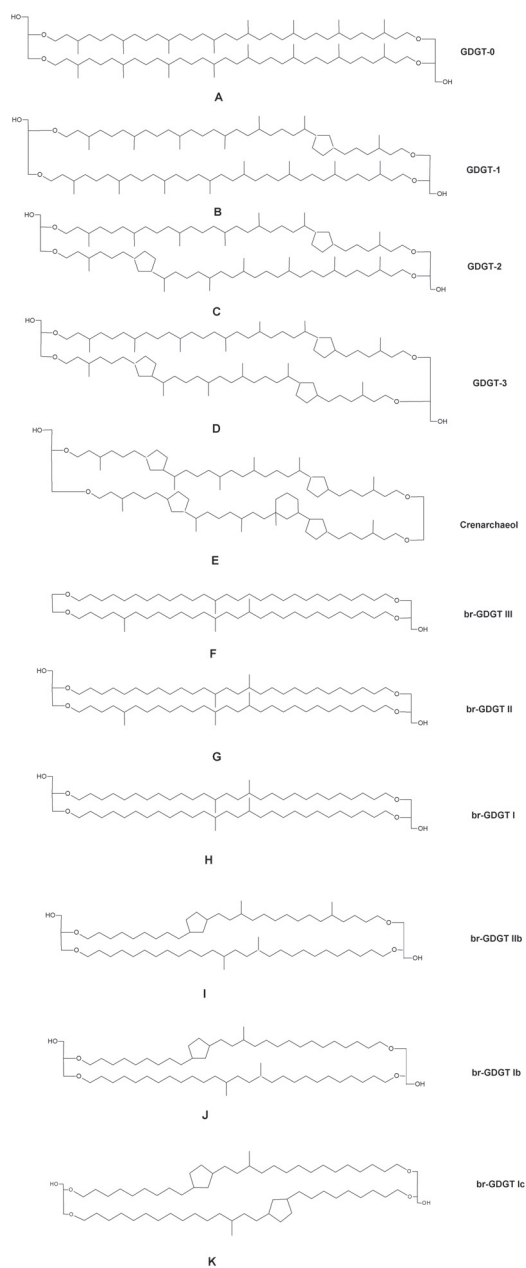


FIGURE 3. Molecular structure of isoprenoid and branched GDGTs mentioned in this study. a: GDGT-0; b, GDGT-1; c, GDGT-2; d, GDGT-3; e, crenarchaeol; f, brGDGT-III; g, brGDGT-II; h, brGDGT-I; i, brGDGT-IIb; j, brGDGT-Ib; k, brGDGT-Ic. Numbers 0-3 represents isoprenoid GDGTs with 0 to 3 pentacyclic rings. Roman numbers III, II, I, IIb, Ib and Ic indicate branched GDGTs with different numbers of methyl groups and pentacyclic rings.

FIGURA 3. Estructura molecular de GDGTs isoprenoides y ramificados mencionados en el presente estudio. a: GDGT-0; b, GDGT-1; c, GDGT-2; d, GDGT-3; e, crenarchaeol; f, brGDGT-III; g, brGDGT-II; h, brGDGT-I; i, brGDGT-IIb; j, brGDGT-Ib; k, brGDGT-Ic. Los números 0-3 representan GDGTs isoprenoides con 0 y 3 anillos penta-cíclicos. Los números romanos III, II, I y Ic indican GDGTs ramificados con diferente número de grupos metilos y anillos penta-cíclicos.

The GDGT-2/crenarchaeol ratio was calculated according to Weijers *et al.* (2011) in order to evaluate the contribution from Euryarchaeota during both upwelling and non-upwelling conditions.

#### DNA EXTRACTION FROM SEAWATER AND PCR AMPLIFICATION OF ARCHAEAL 16S rDNA FRAGMENT

PCR (50  $\mu$ L) contained 65 ng of template DNA and 5x GoTaq flexi buffer (1x; Promega, USA), deoxynucleotide mix (200  $\mu$ mol L<sup>-1</sup>), MgCl<sub>2</sub> solution (3.5 mmol L<sup>-1</sup>), primers forward and reverse (1  $\mu$ mol L<sup>-1</sup> each one), and GoTaq polymerase (1.25 U; Promega). Amplicons (580 bp) suitable for subsequent DGGE were obtained with the primer combination 344f-GC (Raskin *et al.* 1994) and 927r (Kormas *et al.* 2003) and their respective sequences (5' to 3') are CCGCGCGCGGGCGGGGCGGGGGCCCTACGGGGYGCASCAGGCG and CCCGCCAATTCCTTAAAGTTT. The PCR were performed using a T-personal thermocycler (Biometra, Göttingen, Germany) and the program described elsewhere (Levipan *et al.* 2012).

#### DENATURING GRADIENT GEL ELECTROPHORESIS (DGGE)

Gels were cast using a DCode™ Universal Mutation Detection System (Bio-Rad Laboratories, USA) as in Levipan *et al.* (2012). Reliable individual bands were identified using the “Peak Height Threshold” command of the 1 Dscan EX software (v.3.0), which marks the level above which a peak density was considered to be a true band. Gel images were analyzed by assigning numbers to each of the bands (Operational Taxonomic Units = OTUs) on the gel; these were then scored as present (score=1) or absent (score=0). The scoring of banding patterns resulted in a binary matrix containing presence/absence data, which were converted into a similarity matrix using the Jaccard coefficient of proximity and the PAST software (version 3.01).

#### RNA EXTRACTION FROM SEAWATER AND DOT BLOT HYBRIDIZATIONS

RNA was extracted from filters as in Summers (1970) with modifications (Levipan *et al.* 2007a; Quiñones *et al.* 2009). Extracts were loaded onto nitrocellulose membranes for nucleic acids (Hybond-N; Amersham Bio-Sciences, UK) using a dot blotting apparatus (Bio-Rad, Hercules, CA, USA). Membrane hybridizations analysis was carried out at 44°C by using 5' end digoxigenin-labeled probes (Thermo Biosciences) and the protocol of Raskin *et al.* (1994). Probes (5' to 3') EUB338 (5'-GCTGCCTCCCGTAGGAGT; Amann *et al.* 1990) and ARCH915 (5'-GTCCTCCCCCGCCAATTCCT; Stahl & Amann 1991), allowed to determine the prokaryote 16S rRNA concentration. The EURY498 (5'-CTTGCCRCGCCCT; Burggraf *et al.* 1994) and MS1414 (5'-CTCACCCATACCTCACTCGGG; Raskin *et al.* 1994) probes were used to detect most Euryarchaeota and some methanogens, respectively.

## RESULTS

### OCEANOGRAPHIC CONDITIONS

During the upwelling season (September 2009), surface temperature ranged between 11 and 14 °C, with a thermocline located between 10 and 30 m, whereas bottom temperature was *ca.* 10 °C (Fig. 2a). Salinities of *ca.* 34 and 35 psu were found above and below 60 m, respectively (Fig. 2a). Chlorophyll concentration peaked at 10 m (10 mg m<sup>-3</sup>; Fig. 2a) and then rapidly decreased with depth. O<sub>2</sub> concentration varied between 271 and 343 μmol L<sup>-1</sup> in the top 5 m, with an oxycline between 5 and 40 m and dropped to 16 μmol L<sup>-1</sup> (sub-oxic conditions) in bottom water (Fig. 2b). The presence of a shallow oxycline, coupled with high salinity and low O<sub>2</sub> concentration through part of the water column suggests the presence of upwelled equatorial sub-surface water during this period (Brandshost 1971; Sobarzo *et al.* 2007). Under these conditions, NH<sub>4</sub><sup>+</sup> concentration was < 0.02 μmol L<sup>-1</sup> with maxima at 20 m and in bottom water (Fig. 2b). NO<sub>2</sub><sup>-</sup> concentration was < 0.5 μmol L<sup>-1</sup>, with minimum values at 50-60 m, and a maximum concentration in bottom water. NO<sub>3</sub><sup>-</sup> concentration up to 35 μmol L<sup>-1</sup> was detected. NO<sub>3</sub><sup>-</sup> was not fully exhausted in surface water and increased with depth (Fig. 2b).

During the non-upwelling season (June 2010), temperature was homogeneous around 13 °C at the top 60 m and dropped to 12 °C near the bottom (Fig. 2c). Salinity was < 33 psu at the surface and increased to 34 psu below 20 m (Fig. 2c). Chlorophyll concentration was highest (1 mg m<sup>-3</sup>) at the surface, and was not detected below 20 m (Fig. 2c). O<sub>2</sub> concentration was > 237 μmol L<sup>-1</sup> in the upper 65 m and dropped to 150 μmol L<sup>-1</sup> below 80 m (Fig. 2d). The occurrence of a less saline, warmer, well oxygenated, and less stratified water column compared with the upwelling season indicates the presence of Intermediate Antarctic water (Brandhorst 1971) and a well-mixed water column during this period. NH<sub>4</sub><sup>+</sup> concentration was higher than during the upwelling season and decreased from 0.7 μmol L<sup>-1</sup> at the surface to 0.3 μmol L<sup>-1</sup> in bottom water. NO<sub>3</sub><sup>-</sup> varied between 17 and 25 μmol L<sup>-1</sup> from the surface to bottom water, whereas a general decrease in NO<sub>2</sub><sup>-</sup> concentration with depth was observed (Fig. 2d).

SPATIAL AND TEMPORAL VARIATION OF iGDGTs AND BRGDGTs  
GDGT-0 and crenarchaeol were the dominant iGDGTs during both seasons and water depths (Fig. 4a, 4b). During the upwelling season, only GDGT-0 (49%), crenarchaeol (50%) and Cren' (0.7%) were detected in surface waters, whereas GDGT-0 (39%), crenarchaeol (52%) and GDGT-1 (5%), GDGT-2 (4%), GDGT-3 (0.4%), and Cren' (0.7%) were found in subsurface sub-oxic water (Fig. 4a). During the non-upwelling season, only GDGT-0 (49%), GDGT-1 (3%), crenarchaeol (48%) and Cren' (0.1%) were detected in surface waters, whereas GDGT-0 (46%), GDGT-1 (3%),

GDGT-2 (2%), GDGT-3 (1%), crenarchaeol (48%) and Cren' (0.3%) occurred in subsurface water (Fig. 4b). The degree of cyclization of iGDGTs, expressed as the Ring Index, was higher in the subsurface water during upwelling conditions (*ca.* 3) than during the non-upwelling season (*ca.* 2) (Table 1). The GDGT-2/GDGT-3 ratio in subsurface water was higher during upwelling (*ca.* 9) than non-upwelling (*ca.* 4) conditions (Table 1). The GDGT-2/crenarchaeol ratio was higher in sub-surface water during upwelling (0.2; Table 1) compared to non-upwelling (0.04; Table 1). In both seasons, archaeal GDGTs were more abundant in subsurface water (80 m), whereas bacterial GDGTs were more abundant in surface water (10 m; Fig. 5a, 5b). During the upwelling season, the concentration of iGDGTs was 0.43 ± 0.08 ng L<sup>-1</sup> in surface water and of 0.91 ± 0.2 ng L<sup>-1</sup> in subsurface water (Fig. 4a). During the non-upwelling season, the concentration of iGDGTs was 2.7 ± 0.5 ng L<sup>-1</sup> in surface water and 36.3 ± 7.2 ng L<sup>-1</sup> in subsurface water (Fig. 4b).

During the upwelling season, only brGDGT-I was found in surface (0.08 ± 0.02 ng L<sup>-1</sup>) and sub-surface (0.07 ± 0.01 ng L<sup>-1</sup>) waters (Fig. 5a), while during non-upwelling conditions, we detected brGDGT-Ic (0.3 ± 0.06 ng L<sup>-1</sup>), brGDGT-I (0.2 ± 0.04 ng L<sup>-1</sup>), and brGDGT-II (0.1 ± 0.02 ng L<sup>-1</sup>) in surface, and brGDGT-III (0.1 ± 0.02 ng L<sup>-1</sup>) in sub-surface waters (Fig. 5b).

Sedimentary iGDGTs were dominated by GDGT-0 (53%) and crenarchaeol (34 %) followed by GDGT-1 (7%), GDGT-2 (4%), GDGT-3 (1%) and Cren' (2%) (Fig. 6a), and their total concentration was of 28.4 ± 5.7 μg (g dry wt.)<sup>-1</sup> (Fig. 6b). The GDGT-2/GDGT-3 ratio in surface sediment resembled that of subsurface water during the non-upwelling season (*ca.* 4, Table 1).

The distribution of sedimentary brGDGTs was dominated by GDGT-III (46 %), GDGT-I (23%) and GDGT-II (14%). Pentacyclic brGDGTs Iib (8%), Ib (6%), and Ic (2%) were minor components (Fig. 6b). Their total concentration was 0.6 ± 0.1 μg (g dry wt.)<sup>-1</sup>.

### TEX<sub>86</sub>-DERIVED TEMPERATURES

We compared TEX<sub>86</sub>- and TEX<sub>86</sub><sup>L</sup>-derived temperatures from particulate matter and surface sediments, with *in situ* temperature. However, due to the absence of GDGTs -2 and -3 from surface water, temperatures were only calculated for subsurface water. During the upwelling and non-upwelling seasons, TEX<sub>86</sub> converted to temperature using eq. 3 (12 ± 2 °C and 14 ± 3 °C, respectively) were about 2 °C higher than *in situ* temperatures (Table 1). In surface sediments, values of 17 ± 5 °C and 15 ± 7 °C; were obtained using TEX<sub>86</sub> and TEX<sub>86</sub><sup>L</sup> converted to temperatures using equations 4 and 5, respectively, being 5 and 3 °C higher than the reported annual mean SST in the study site (12 °C; Table 1).



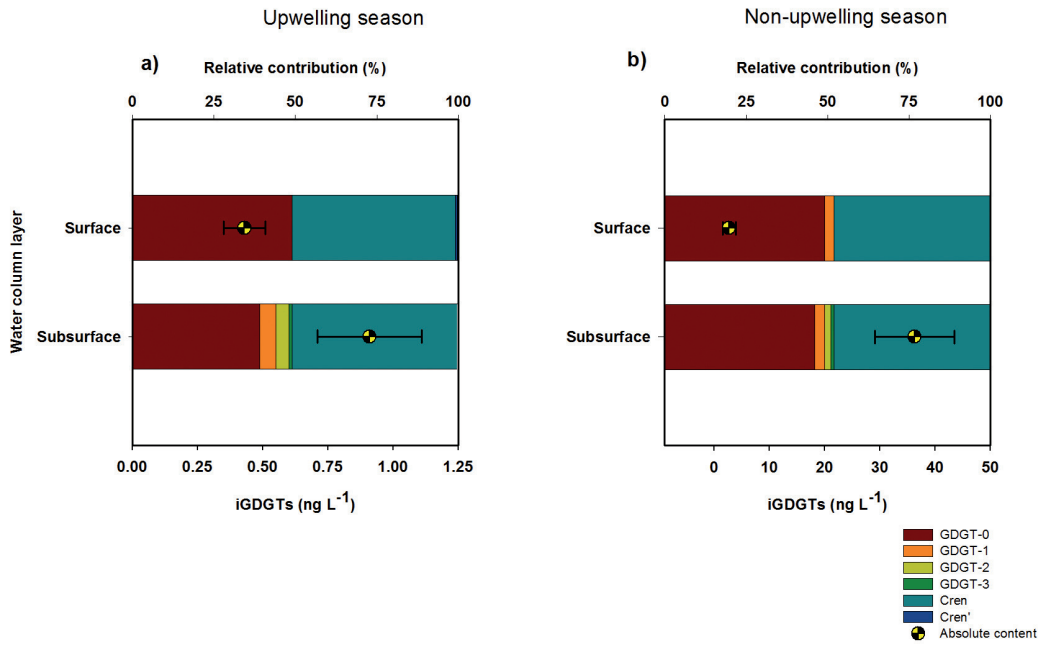


FIGURE 4. Relative and absolute concentrations of isoprenoid GDGTs during the (a) upwelling and (b) non-upwelling seasons.

FIGURA 4. Concentraciones relativas y absolutas de GDGTs isoprenoides durante las estaciones de (a) surgencia y (b) no-surgencia.

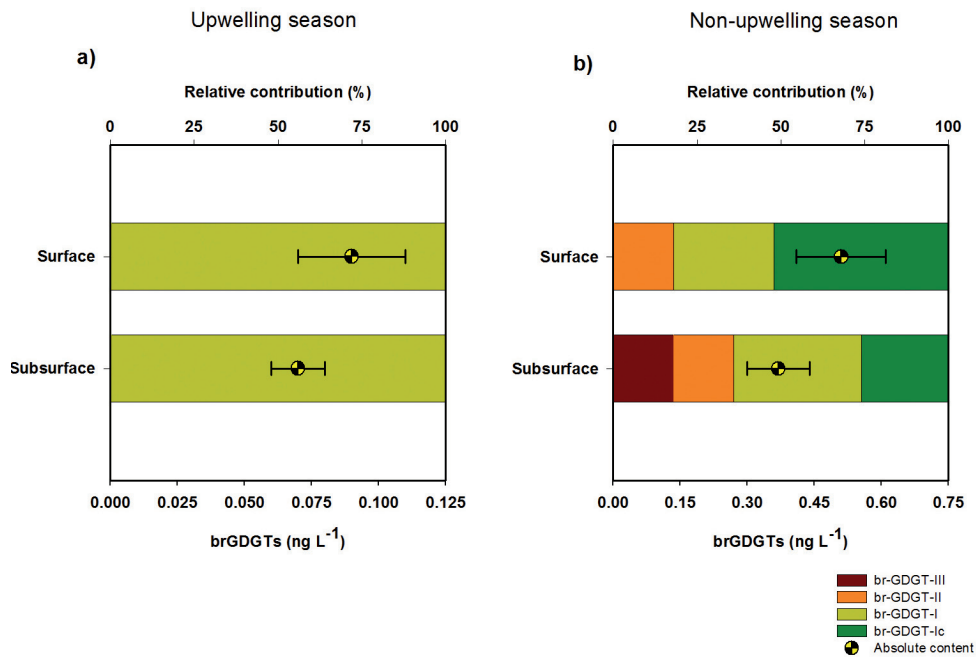


FIGURE 5. Relative and absolute concentrations of branched GDGTs during the (a) upwelling and (b) non-upwelling seasons.

FIGURA 5. Concentraciones relativas y absolutas de GDGTs ramificados durante las estaciones de (a) surgencia y (b) no-surgencia.

TABLE 1. Comparison between *in situ*  $TEX_{86}^L$  and  $TEX_{86}$  derived temperature, the Ring Index, and the GDGT-2/ GDGT-3 ratio and GDGT-2/crenarchaeol in suspended particles and surface sediments during upwelling and non-upwelling seasons off Concepción (36°S). iGDGTs 2 and 3 were under detection limit in the surface layer.

TABLE 1. Comparación entre temperaturas derivadas *in situ* desde  $TEX_{86}^L$  el índice de Anillos y las razones GDGT-2/GDGT-3 y el GDGT-2/crenarchaeol en partículas suspendidas y sedimento superficial durante las estaciones de surgencia y no-surgencia frente a Concepción (36°S). iGDGTs 2 y 3 estuvieron bajo el límite de detección en la capa superficial.

UPWELLING						
In situ T (°C)	SPM- $TEX_{86}$	SPM- $TEX_{86}^{-1}T$ (°C)	$TEX_{86}^L$	SPM- $TEX_{86}^{L,3}T$ (°C)	Ring Index <sup>4</sup>	(GDGT-2/GDGT-3) <sup>5</sup> (GDGT-2/Crenarchaeol) <sup>6</sup>
Surface	ND	ND	ND	ND	ND	ND
Subsurface	0.49	12 ± 2	-0.38	20 ± 3	3	9 0.2
Non-Upwelling						
In situ T (°C)	SPM- $TEX_{86}$	SPM- $TEX_{86}^{-1}T$ (°C)	$TEX_{86}^L$	SPM- $TEX_{86}^{L,3}T$ (°C)	Ring Index <sup>4</sup>	(GDGT-2/GDGT-3) <sup>5</sup> (GDGT-2/Crenarchaeol) <sup>6</sup>
Surface	ND	ND	ND	ND	ND	ND
Subsurface	0.52	14 ± 3	-0.46	16 ± 4	2	4 0.1
Surface sediment						
In situ	$TEX_{86}$	$TEX_{86}^L$	$TEX_{86}^L T^2$ (°C)	$TEX_{86}^L T^3$ (°C)	Ring Index <sup>4</sup>	(GDGT-2/GDGT-3) <sup>5</sup> (GDGT-2/Crenarchaeol) <sup>6</sup>
ND	0.5	-0.5	17 ± 5	15 ± 7	2	4 0.1

1: Temperatures obtained using  $TEX_{86}$  and temperature relationship from Wuchter et al. (2005):  $TEX_{86} = 0.017 * T + 0.29$

2: Temperatures obtained using  $TEX_{86}$  and temperature relationship from Kim et al. (2008):  $SST = 56.2 * TEX_{86} - 10.8$

3: Temperatures obtained using  $TEX_{86}^L$  and temperature relationship from Kim et al. (2010):  $67.5 * TEX_{86}^L + 46.9$

4: According to Liu et al. (2011b)

5: According to Tylor et al. (2013)

6: According to Weijers et al. (2007)

ND: Not determined

TABLE 2. Similarity matrix using the Jaccard coefficient of proximity for DGGE banding patterns of archaeal 16S rDNA PCR amplicon obtained from surface and sub-surface waters off Concepción (36°S) during upwelling (January 2006) and non-upwelling (June 2006) seasons.

TABLE 2. Matriz de similitud usando el coeficiente de proximidad de Jaccard para los patrones de bandas de EGGD archaeal 16S rDNA PCR amplificados obtenidos de aguas superficiales y sub-superficiales de la zona de estudio frente a Concepción (36°S) en época de surgencia (Enero 2006) y no surgencia (Junio 2006).

	January 10m	June 10 m	January 80m	June 80 m
January 10m	1	0.5	0.6	0.4
June 10 m	0.5	1	0.6	0.8
January 80m	0.6	0.6	1	0.8
June 80 m	0.4	0.8	0.8	1

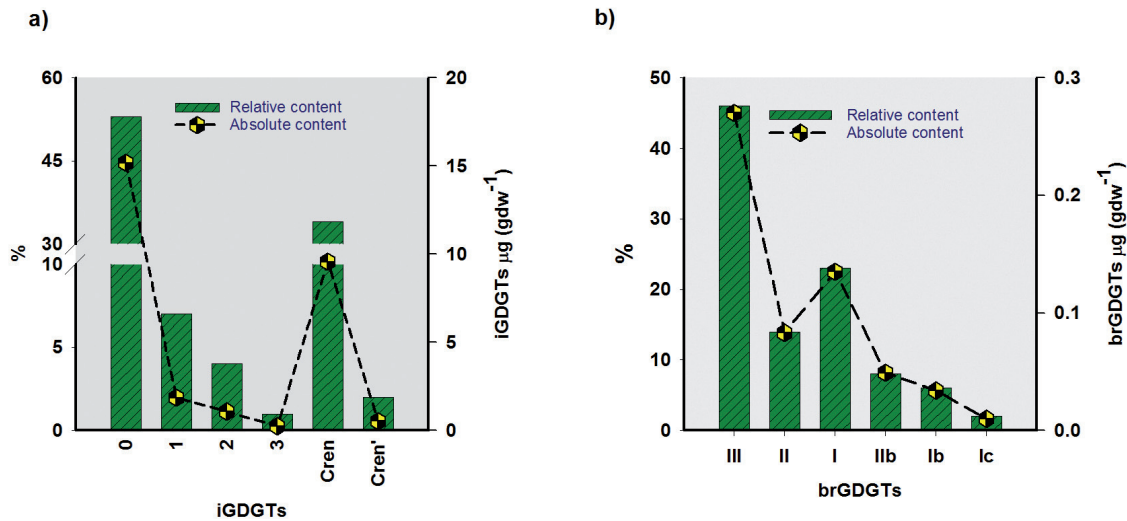


FIGURE 6. Relative contribution and absolute concentration of (a) isoprenoid and (b) branched GDGTs in a top-core sediment sample at Station 18.

FIGURA 6. Contribución relativa y concentración absoluta de los GDGTs (a) isoprenoides y (b) ramificados en una muestra superficial de un testigo de sedimento en la Estación 18.

VERTICAL AND SEASONAL VARIABILITY IN THE RELATIVE ABUNDANCE OF ARCHAEAL RIBOTYPES IN THE STUDY SITE

At the subsurface suboxic water (80 m depth), the relative contribution of Archaea ranged between ~ 5 and 38 %, and was the highest during the austral summer-spring, specifically in February and September (Fig. 7). The euryarchaeal relative abundance ranged from 0.1 to 5% (see EURY498 in Fig. 7), and a lower contribution of methanogens was detectable only during the January (0.9%) and February (3.9%) months.

In addition, an interesting secondary peak in the relative abundance of Archaea was observed in the winter season at 10 (July) and 80 m (June) of depth (Fig. 7).

VERTICAL AND SEASONAL SHIFTS IN THE COMPOSITION OF THE ARCHAEAL COMMUNITY

The DGGE profiles showed inter and intra-seasonal changes in the epipelagic archaeal communities off Concepción. During summer (upwelling), surface water showed 3 main archaeal ribotypes (OTUs), while in the subsurface water we detected 5 dominant archaeal ribotypes (Fig. 7). Both depths share 60% of the OTUs (Table 2).

During winter (non-upwelling) there were 3 dominant archaeal ribotypes in surface water and 4 in subsurface water yielding a higher percent of similitude in both depths (75%; Fig. 7; Table 2).

A marked inter-seasonal change in the archaeal community composition was more evident in surface than sub-surface water. In surface water, the similitude between summer and winter was 50%, while in sub-surface water the similitude was 80% (Table 2).

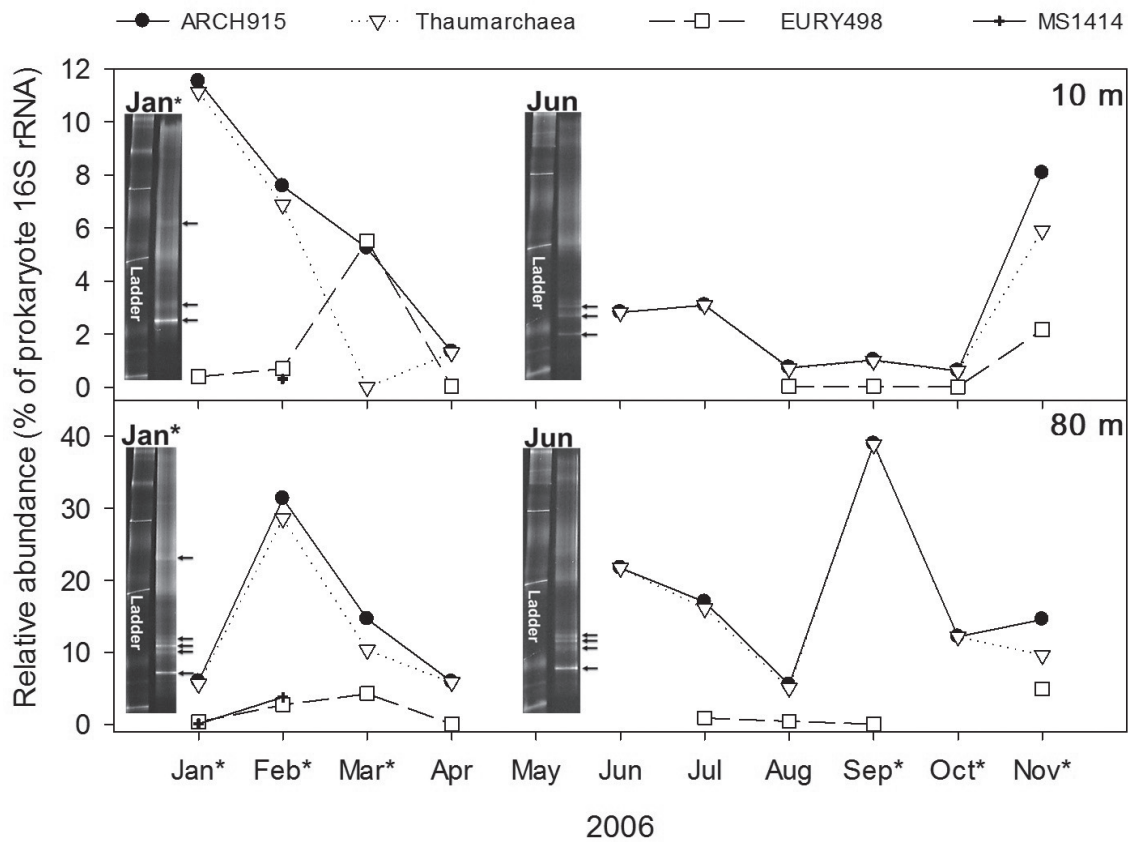


FIGURE 7. Abundance of total archaeal ribotypes (ARCH915, domain-specific probe), most crenarchaeota (CREN499, able to recognize representatives of thaumarchaeota initially classified as ‘Marine Group 1 crenarchaeota’), most euryarchaeota (EURY498) and some versatile methanogens (MS1414) at 10 and 80 m depth in the st.18 as determined by quantitative dot-blot 16S rRNA hybridizations. The abundances are expressed as a percentage of prokaryote rRNA that was determined by adding archaeal rRNA plus (ARCH915) bacterial one (EUB338). Months (2006) marked with asterisks show the upwelling-favorable period in the study area. No samples were available in May. Other gaps correspond to dates when the target groups were below detection limit. A comparative denaturing gradient gel electrophoresis (DGGE) profile analysis of archaeal 16S rDNA gene is shown for January and June (2006) at both depths. Arrows show bands that were reliably selected for similarity analysis by using the 1Dscan EX software.

FIGURA 7. Abundancias totales de lo ribo-tipos arqueanos (prueba del dominio-específico ARCH915), la mayoría de las crenarchaeotas (CREN499, capaz de reconocer representantes de thaumarchaeota, inicialmente clasificados como “Grupo Marino 1 crenarchaeota”), la mayoría de las euryarchaeotas (EURY498) y algunos metanógenos versátiles (MS1414) a 10 y 80 m de profundidad en la est. 18 determinados mediante hibridaciones cuantitativas de dot-blot 16S rARN. Las abundancias son expresadas como el porcentaje del rARN procarionte total, el cual fue determinado por la adición del rARN arqueano (ARCH915) más uno bacteriano (EUB338). Los meses etiquetados con asteriscos muestran el periodo favorable de surgencia en el área de estudio (2006). No hubieron muestras disponibles en Mayo. Los otros espacios vacíos corresponden a las fechas cuando los grupos objetivos estuvieron bajo el límite de detección. Un análisis de perfil comparativo de electroforesis en gel con gradiente de desnaturalización de genes 16S rADN arqueanos son mostrados en Enero y Junio (2006) para ambas profundidades. Las flechas muestran las bandas que fueron seleccionadas confiablemente para el análisis de similitud utilizando el programa 1Dscan EX.

## DISCUSSION

### SPATIAL AND TEMPORAL VARIABILITY OF ARCHAEOAL GDGTs

IGDGTs concentrations were higher in the deeper sub-surface water compared to surface water during upwelling and non-upwelling seasons (Figs. 4 a, b). The iGDGTs depth distribution in our study site showed a similar pattern to those found at the equatorial Pacific, northeast Pacific, Santa Monica Basin (Wuchter *et al.* 2005), the Arabian Sea (Sinninghe Damsté *et al.* 2002a), and the Cariaco Basin (Wakeham *et al.* 2004). The seasonal contrast in the iGDGTs abundance observed off Concepción is also consistent with previous reports. A higher abundance of iGDGTs during winter in the North Sea was reported by Wuchter *et al.* (2005) and Herfort *et al.* (2006), suggesting that Thaumarchaeota thrive in winter due to lack of competition with phytoplankton for NH<sub>3</sub> (Yamamoto *et al.* 2012). Similarly, we found the highest abundance of core iGDGTs in suspended particulate matter collected in austral-winter (June 2010, Fig. 4b) when primary production was the lowest (Fig. 2c). Consequently, one could infer that maximum production of iGDGTs off Concepción could correspond to a seasonal bloom of Thaumarchaeota during austral-winter season. This conclusion must be taken with caution, although not completely discarded, since recent evidence has shown that intact iGDGTs are present in the free-living particle size fraction 0.2-0.7 µm, and core iGDGTs are enriched in suspended particles (0.7-60 µm) and aggregates > 60 µm, implying that archaeal biomass quickly becomes attached to particles once organisms are dead or dying (Ingalls *et al.* 2012). Since turnover time of archaeal cells in the water column is on the order of days (Jones *et al.* 1996), free core iGDGTs we measured must have derived from living or recently dead archaea inhabiting the water column during winter.

The observed distribution of iGDGTs resembles what is described for cold areas where the iGDGTs distribution is dominated by iGDGT-0 (Fig. 3a; 4a, b) and crenarchaeol (Fig. 3e; 4a, b) (Wuchter *et al.* 2005). However, an increase of 1-3 cyclopentane-containing iGDGTs was observed during the upwelling season, contrary to the expected increase cyclization with temperature, concluded from environmental observations (De Rosa *et al.* 1986; Uda *et al.* 2001; Macalady *et al.* 2004; Boyd *et al.* 2011), and cultures of marine Thaumarchaeota (Wuchter *et al.* 2004; Schouten *et al.* 2007) and thermophilic Archaea (Gliozzi *et al.* 1983; Ward *et al.* 1985; De Rosa & Gambacorta 1988; Uda *et al.* 2001). The addition of pentacyclic rings in the trans-membrane portions of the lipids imply an enhanced membrane packing and reduced fluidity (Benvegnu *et al.* 2008) resulting in a molecular adaptive advantage to Archaea thriving in warmer and thermophilic environments, which suggest that the temperature is not the only environmental variable controlling the iGDGTs distribution

in the study site. Variations in the distribution of iGDGTs in marine environments could also respond to nutrient regime and energy stress, as well as to variation in the relative contribution of Thaumarchaeota and Euryarchaeota (Pearson & Ingalls 2013). Possible explanations for this observation are discussed below.

We also observed a higher RI and a larger contribution of GDGT-2 during upwelling, when temperature was generally lower, than during the non-upwelling season (Table 1, Fig. 2a, 2b). When water column TEX<sub>86</sub>-derived temperature increases, RI values decreased, contrary to the expected positive relationship between these two parameters when water temperature is the main factor controlling the composition and distribution of iGDGTs (Table 1). A similar decoupling has been observed in waters associated with gas hydrates where iGDGTs are derived from methanotrophic archaea (Zhang *et al.* 2011). This evidence suggests that factors other than temperature might control the distribution of iGDGTs in the seasonal upwelling system off Concepción, and consequently TEX<sub>86</sub>-derived temperature values. This notion is supported by studies indicating that pelagic Thaumarchaeotal communities vary seasonally in response to oceanographic variability including nutrient concentration and O<sub>2</sub> content (Massana *et al.* 1997; Murray *et al.* 1998a, 1998b; Wuchter *et al.* 2004; Herfort *et al.* 2007; Pitcher *et al.* 2011b; Bale *et al.* 2013). Since it has been suggested that not all planktonic Thaumarchaeota are strict autotrophs (Ouverney & Fuhrman 2000; Ingalls *et al.* 2006; Pearson & Ingalls 2013), metabolic diversity might also control the number of cyclic moieties due to modification of membrane lipid associated to episodic or chronic energy stress (van de Vossenberg *et al.* 1998; Mathai *et al.* 2001; Valentine 2007; Pearson & Ingalls 2013).

We observed that crenarchaeol increased its relative abundance during the upwelling season, mainly within oxygen-deficient waters, concomitant with the intrusion of nutrient-rich, high salinity and low temperature Equatorial Sub-Surface Waters (Figs. 2a, 4a). Crenarchaeol has been isolated in the non-thermophilic group-I crenarchaeota, a subgroup of archaea occurring in seawater and lakes as well as in soils (Sinninghe Damsté *et al.* 2002b; Koga & Morii 2005; Schouten *et al.* 2007; Weijers *et al.* 2007). Recently, Lincoln *et al.* (2014) reported that MG-II Euryarchaeota are a major source of iGDGTs particularly crenarchaeol in shallow and intermediate waters of North Pacific Subtropical Gyre. It has been suggested that the formation of a cyclohexane ring was an adaptive response of crenarchaeota to relatively low temperature environment in which this group evolved (Sinninghe Damsté *et al.* 2002b). The increased relative abundance of crenarchaeol could indicate a relationship between the decreased water column temperatures observed during upwelling. Alternatively, the coincident increase

in the relative abundance of crenarchaeol and high ratios GDGT-2/GDGT-3 and GDGT-2/crenarchaeol during upwelling may be the result of an increase of Euryarchaeota in the study site (Table 1; Fig. 7).

Increase in GDGT-2 (Table 1, Fig. 2) has previously been reported in subsurface, sub-oxic water in the Arabian Sea (Schouten *et al.* 2012), and the eastern tropical North Pacific (Xie 2013), and South Pacific (Sepúlveda *et al.* 2013). A recent revision of global data sets of suspended organic matter indicates that GDGT-2/GDGT-3 increases with water depth, particularly in water columns affected by O<sub>2</sub>-deficiency (Taylor *et al.* 2013), suggesting that GDGT-derived temperature must therefore consider export dynamics and water depth, whereas a growing body of information suggests that O<sub>2</sub> concentrations should also be taken into account. However, the exact mechanism behind the increased proportion of GDGT-2 in subsurface waters, especially in sub-oxic areas, remains poorly understood. Although our biomarker data does not allow us to gather further insights into the responsible mechanisms, molecular data support three possible explanations (i) phylogenetically different thaumarchaeotal populations (ii) changes in the relative contribution of Euryarchaeota with depth; (iii) a relationship between archaea and environmental factors other than temperature (*e.g.* O<sub>2</sub>, nutrients and pH).

DGGE profiles at the study site support the first mechanism, since we have observed that the dominant archaeal ribotypes of surface water were different from those found in the subsurface water, especially during summer, when subsurface sub-oxic conditions prevailed (Fig. 7, Table 2). In the subsurface water, a higher number of archaeal OTUs were observed during summer and winter, compared with surface water (Fig.7). This segregation agrees previous reports of Levipan *et al.* (2012), who described two different archaeal communities inhabiting surface and sub-surface coastal waters off Concepción, and the finding of high diversity of ammonia oxidizing archaea in sub-oxic water off Concepción (Molina *et al.* 2010). However, the role of upwelling in transporting deeper archaea to surface waters can not be neglected as suggested by Santoro *et al.* (2010) who found gene copies of deep-water archaea in surface waters of the coastal upwelling region off California.

16S rRNA hybridizations data revealed seasonal and vertical changes in the euryarchaeotal and methanogens, with the highest contribution during austral summer, supporting our second explanation (changes in the relative contribution of Euryarchaeota with depth) for the increased proportion of GDGT-2 (Table 1) in sub-surface waters. Similarly, Levipan *et al.* (2007b) found that abundance and occurrence of methylotrophic methanogenic archaea was the highest and almost exclusively during active upwelling

(austral spring-summer) agreeing with values of the index GDGT-2/crenarchaeol reported here (Table 1). The latter is consistent with previous finding in other marine ecosystems such as the North Sea (van der Maarel *et al.* 1999).

Distribution of archaea and iGDGTs can also be influenced by water chemistry and associated biological production at the study site. The highest abundance of iGDGTs during the non-upwelling season when lower photosynthetic production is verified (Fig. 4b) could be the result of ecological decoupling between phytoplankton and pelagic marine archaea. Wuchter *et al.* (2005) and Herfort *et al.* (2007) found high abundance of iGDGTs in winter in the North Sea. The underlying mechanism appears to be competition for nutrients since abundance of intact iGDGTs, and Thaumarchaeota 16 rRNA genes and amoA genes showed a seasonal cycle with a maximum during winter in the North Sea (Pitcher *et al.* 2011a). In the study area, Molina *et al.* (2010) found that ammonia-oxidizing archaea community changed according to the oxygen content in waters of eastern South Pacific. Turich *et al.* (2007) reported variability in iGDGTs composition in the water column at different oceanographic settings suggesting that changes in archaeal ecology, nutrient regimes and oceanographic conditions can potentially iGDGTs composition. Even though, we detect the pattern, our data set does not allow disentangling whether one or more variables control iGDGTs variability at the study site.

#### 4.2. ECOLOGICAL SIGNIFICANCE OF TEMPORAL AND VERTICAL DISTRIBUTION OF iGDGTs

The distribution of iGDGTs in the water column off Concepción resembles the characteristic signature found in marine planktonic Thaumarchaeota (Sinninghe Damsté *et al.* 2002a, 2002b; De la Torre *et al.* 2008; Schouten *et al.* 2008). Our biomarker data shown a clear seasonal pattern, with enhanced abundance during the non-upwelling season (austral autumn-winter), particularly in subsurface water coinciding with the molecular data that showed a secondary peak in the archaeal abundance during winter (*e.g.*, June-July) (Fig. 6b, Fig.7). In agreement with this result, Levipan *et al.* (2007a) and Quiñones *et al.* (2009) reported that marine Archaea comprise a significant fraction of the planktonic prokaryotic community in subsurface sub-oxic waters (*ca.* 50% of total prokaryote community), where Thaumarchaeota was the dominant group. This seasonal pattern in Thaumarchaeota abundance is consistent with other coastal settings (Wuchter 2006; Wuchter *et al.* 2006; Herfort *et al.* 2007; Pitcher *et al.* 2011b). Thaumarchaeota are more prominent during winter following phytoplankton blooms, and are negatively correlated with chlorophyll concentration (Murray *et al.* 1998b). In the North Sea, Thaumarchaeota were less abundant when large phytoplankton (> 3 µm) dominated the algal population, even in the presence of

favorable nutrient concentrations (Herfort *et al.* 2007). Thus, it has been hypothesized that nutrient concentration, together with phytoplanktonic biomass and community structure, can control the population of marine Thaumarchaeota (Herfort *et al.* 2007). In the study area, a phytoplankton assemblage dominated by large diatoms ( $> 3 \mu\text{m}$ ) along with high chlorophyll concentration is typically found during the upwelling season (Montero *et al.* 2007). Conversely, during the non-upwelling season chlorophyll concentration is *ca.* one order of magnitude lower than during the upwelling season, whereas  $\text{NH}_4^+$  and  $\text{NO}_2^-$  were higher (Fig. 2).

In marine sub-oxic waters, the occurrence of  $\text{NH}_4^+$ -oxidizing archaea is well correlated with crenarchaeol concentrations (De Long *et al.* 1998; Schouten *et al.* 2000). A previous study from the same site (Station 18) indicates that most of the archaeal *amoA* gen belongs to the uncultured cluster A, when sub-oxic conditions and high  $\text{NH}_4^+$  concentration prevail (Molina *et al.* 2010).  $\text{NH}_4^+$  concentrations were in average 42 times higher during the non-upwelling period than during upwelling (Fig. 2b, d), yielding a greater ( $\text{NH}_4^+ + \text{NO}_2^-$ ) to P ratio during winter (13 vs. 10 during upwelling) when higher abundance of iGDGTs (Fig. 4) occurs, suggesting that Thaumarchaeota abundance depends on  $\text{NH}_4^+$  availability in the water column of the study site.

The ecological role of Thaumarchaeota in the marine N cycle has been shown by the co-occurrence of crenarchaeol and  $\text{NO}_2^-$  maxima (Massana *et al.* 1997; Murray *et al.* 1998a, 1998b; Sinninghe Damsté *et al.* 2002a) as well as archaeal  $\text{NH}_3$  oxidation genes (*amoA*) (Francis *et al.* 2005; Hallam *et al.* 2006; Wuchter *et al.* 2006). Our results support previous molecular data obtained from samples of the same site (Levipan *et al.* 2007a; Molina *et al.* 2010) that have detected a role of pelagic  $\text{NH}_3$  oxidizing Archaea in N cycling in waters of the eastern South Pacific.

#### SPATIAL AND TEMPORAL VARIABILITY OF BACTERIAL GDGTs

The highest concentration and diversity of brGDGTs were found during the non-upwelling season in surface and subsurface waters (Fig. 5b), consistent with enhanced terrestrial input from rivers Itata and Biobio during austral winter, based on their terrestrial biological source (Hopmans *et al.* 2004; Weijers *et al.* 2006). However, both the water column vertical distribution as well as the large seasonal differences in diversity (Fig. 5 a, b) suggest that *in situ* production cannot be entirely ruled out. Previous studies have demonstrated *in situ* production in lakes and fjords based on the differences in the degree of methylation and cyclization in soils and the water column (Peterse *et al.* 2009; Tierney & Russel 2009; Tierney *et al.* 2010).

Although the effect of diagenesis is not well constrained, their stability under oxic and suboxic conditions in the

water column cannot be excluded as a control of brGDGT abundance and distribution. Tierney *et al.* (2012) found a higher abundance of methylated brGDGTs in a seasonally anoxic and eutrophic suburban lake compared with deeper layers of sediments that were deposited under oxygenated conditions. Similarly, Bechtel *et al.* (2010) observed that anoxic lakes contained preferentially more methylated over cyclized brGDGTs than oxic lakes. In the seasonal upwelling system off Concepción, the absolute predominance of methylated brGDGTs during upwelling conditions (Fig. 4C) could be reflecting the loss of cyclized brGDGTs as result of the exposure to oxygen during austral winter. Alternatively, it could reflect that the organisms synthesizing brGDGTs are sensitive to variations in water column redox, modifying their brGDGTs lipid composition as response to the environmental redox changes of the study site, or by seasonal changes of bacterial producer brGDGTs community structure with changes in the whole oceanographic conditions of water column off Concepción. These uncertainties remain unconstrained at the moment.

## CONCLUSIONS

A seasonal pattern in the distribution and composition of i and brGDGTs was found in the upwelling ecosystem off Concepción where an Oxygen Minimum Zone develops during austral summer. This pattern reflects the distribution of archaea –traumarchaeota in the area as compared with a year round observation of rDNA and rRNA molecular data. The fractional abundances of iGDGTs showed that Euryarchaeota was most prominent during upwelling conditions. The highest abundance and diversity of iGDGTs occurred in sub-surface water during non-upwelling conditions. During upwelling conditions, a higher relative contribution of GDGT-2 was found in sub-surface, sub-oxic water, leading to discrepancies between  $\text{TEX}_{86}$ -derived and *in situ* temperatures. Similarly,  $\text{TEX}_{86}$ -derived temperatures from surface sediments yielded values that exceeded seasonal and year averages in surface and subsurface water. Additionally, the distribution of iGDGTs in surface sediments over the continental shelf off Concepción might be biased by the seasonal input of iGDGTs from Euryarchaeota during the upwelling season as well as by soil archaea during the low productivity season in austral fall-winter. The highest abundance of brGDGTs occurred during non-upwelling conditions (austral fall-winter), particularly in surface water.

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