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

Ensambles 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 indicate 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, possibly influenced by the high 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 gliceroles dialquilo glicerol tetra-eteres (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 y bacterias 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 oxicia superior durante el periodo de no-surgencia, tal vez influenciado por la alta descarga de rios, 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 dialquilo glicerol tetraeteres (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; Hopmans 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$_{86}$ 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 Thaumarcheota (De Long et al. 1999; 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 dialkani diethers (iGDGDs) 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. 1997; 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; Leipan et al. 2007a; Quiñones et al. 2009) regions. The distribution of genes markers and membrane lipids indicates that marine Thaumarcheota (formerly Marine Group I 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; Leipan 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 Thaumarcheota (Massana et al. 1997; Lopez- García 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. 3) 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 Pacific (Xie 2013). In these areas, oxygen minimum zones are characterized by dissolved oxygen concentrations lower than 22 μmol 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$_{86}$-derived temperature values that largely offset in situ temperature (Schouten et al. 2012a, 2012b; Xie 2013). Most studies suggest that pelagic Thaumarcheota 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 O2 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⁻² d⁻¹; 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). 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) and the COPAS Center.
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 O₂ 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/.

**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/.
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. Lipids were separated into a non-polar and a polar fractions using a Pasteur pipette filled with activated Al₂O₃, 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/isopropanol (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 (Fig. 3) were identified from their characteristic [M + H]⁺ 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 (m/z 1292), brGDGT-III (m/z 1050), brGDGT-IIIb (m/z 1048), brGDGT-IIc (m/z 1046); brGDGT-II (m/z 1036), brGDGT-IIb (m/z 1032); brGDGT-I (m/z 1022), brGDGT-IIb (m/z 1034), brGDGT-Ib (m/z 1020), brGDGT-Ic (m/z 1018).

TEXₘₙ and TEXₘₙ indices for suspended particulate matter (SPM) and surface sediments were calculated as described by Schouten et al. (2002) and Kim et al. (2010):

\[
\text{TEX}_{\text{mk}} = \frac{(\text{GDGT-2} + \text{GDGT-3} + \text{Cren'})}{(\text{GDGT-1} + \text{GDGT-2} + \text{Cren'})} \quad (1)
\]

\[
\text{TEX}_{\text{m}} = \log \left[ \frac{(\text{GDGT-2})}{(\text{GDGT-1} + \text{GDGT-2} + \text{GDGT-3})} \right] \quad (2)
\]

Additionally water column TEXₘₙ values were converted to temperature according to Wuchter et al. (2005):

\[
\text{TEX}_{\text{mk}} = 0.017 \times T + 0.29 \quad (3)
\]

Sedimentary TEXₘₙ and TEXₘₙ values were converted to temperatures ± (proxy residual error + analytical error) according to Kim et al. (2008):

\[
\text{SST} = 56.2 \times \text{TEX}_{\text{mk}} - 10.8 \quad (4)
\]

and Kim et al. (2010):

\[
\text{SST} = 67.5 \times (\text{TEX}_{\text{mk}}) + 46.9 \quad (5)
\]

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):

\[
\text{Ring Index} = \left( \frac{(% \text{GDGT-1}) + 2(% \text{GDGT-2}) + 3(% \text{GDGT-3}) + 5(% \text{Crenarchaeol}+\text{Cren'})}{100} \right) \quad (6)
\]

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 TEXₘₙ 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.
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 μL) contained 65 ng of template DNA and 5x GoTaq flexi buffer (1x; Promega, USA), deoxynucleotide mix (200 μmol L⁻¹), MgCl₂ solution (3.5 mmol L⁻¹), primers forward and reverse (1 μmol L⁻¹ 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’): CCGGCCTCCCGTAGGAGT; Amann et al. 1990) and ARCH915 (5’-GTCCTCCCCCGCCAATTCCT; Stahl & Amann 1991), allowed to determine the prokaryote 16S rRNA concentration. The EURY498 (5’-CTTGCCRCRGCCGCG; Burggraf et al. 1994) and MS1414 (5’-CTACCCATTACCTACCTCGGG; Raskin et al. 1994) probes were used to detect most Euryarchaeota and some methanogens, respectively.

**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 Biosciences, UK) using a dot blotting apparatus (Bio-Rad, Hercules, CA, USA). Membrane hybridizations analysis was carried out at 44ºC by using 5’ end digoxigenin-labeled probes (Thermo Biosciences) and the protocol of Raskin et al. (1994). Probes (5’ to 3’) EUB338 (5’-GCTGCTTCAGGGAGT; Amann et al. 1990) and ARCH915 (5’-GTCCTCCCGCCAATTCCT; Stahl & Amann 1991), allowed to determine the prokaryote 16S rRNA concentration. The EURY498 (5’-CTTGCCRCRGCCGCG; Burggraf et al. 1994) and MS1414 (5’-CTACCCATTACCTACCTCGGG; 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 ºC, with a thermocline located between 10 and 30 m, whereas bottom temperature was ca.10 ºC (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⁻²; Fig. 2a) and then rapidly decreased with depth. O₂ concentration varied between 271 and 343 μmol L⁻¹ in the top 5 m, with an oxycline between 5 and 40 m and dropped to 16 μmol L⁻¹ (sub-oxic conditions) in bottom water (Fig. 2b). The presence of a shallow oxycline, coupled with high salinity and low O₂ 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, NH₄⁺ concentration was < 0.02 μmol L⁻¹ with maxima at 20 m and in bottom water (Fig. 2b). NO₃⁻ concentration was < 0.5 μmol L⁻¹, with minimum values at 50-60 m, and a maximum concentration in bottom water. NO₂⁻ concentration up to 35 μmol L⁻¹ was detected. NO₃⁻ 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 ºC at the top 60 m and dropped to 12 ºC 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⁻²) at the surface, and was not detected below 20 m (Fig. 2c). O₂ concentration was > 237 μmol L⁻¹ in the upper 65 m and dropped to 150 μmol L⁻¹ 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. NH₄⁺ concentration was higher than during the upwelling season and decreased from 0.7 μmol L⁻¹ at the surface to 0.3 μmol L⁻¹ in bottom water. NO₂⁻ varied between 17 and 25 μmol L⁻¹ from the surface to bottom water, whereas a general decrease in NO₃⁻ 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⁻¹ in surface water and of 0.91 ± 0.2 ng L⁻¹ in subsurface water (Fig. 4a). During the non-upwelling season, the concentration of iGDGTs was 2.7 ± 0.5 ng L⁻¹ in surface water and 36.3 ± 7.2 ng L⁻¹ in subsurface water (Fig. 4b).

During the upwelling season, only brGDGT-I was found in surface (0.08 ± 0.02 ng L⁻¹) and sub-surface (0.07 ± 0.01 ng L⁻¹) waters (Fig. 5a), while during non-upwelling conditions, we detected brGDGT-Ic (0.3 ± 0.06 ng L⁻¹), brGDGT-I (0.2 ± 0.04 ng L⁻¹), and brGDGT-II (0.1 ± 0.02 ng L⁻¹) in surface, and brGDGT-III (0.1 ± 0.02 ng L⁻¹) 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.)⁻¹ (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%). Pentaacyclic brGDGTs Ib (8%), Ib (6%), and Ic (2%) were minor components (Fig. 6b). Their total concentration was 0.6 ± 0.1 μg (g dry wt.)⁻¹.

TEXₑ⁻derived temperatures

We compared TXₑ⁻ and TEXₑ⁻⁻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, TXₑ⁻ converted to temperature using eq. 3 (12 ± 2 ºC and 14 ± 3 ºC, respectively) were about 2 ºC higher than in situ temperatures (Table 1). In surface sediments, values of 17 ± 5 ºC and 15 ± 7 ºC were obtained using TXₑ⁻ and TXₑ⁻⁻¹ converted to temperatures using equations 4 and 5, respectively, being 5 and 3 ºC higher than the reported annual mean SST in the study site (12 ºC; Table 1).
**Figure 4.** Relative and absolute concentrations of isoprenoid GDGTs during the (a) upwelling and (b) non-upwelling seasons.

**Figure 5.** Relative and absolute concentrations of branched GDGTs during the (a) upwelling and (b) non-upwelling seasons.
|                | Upwelling                                                                 |
|----------------|---------------------------------------------------------------------------|
|                | **Surface** | Subsurface |                | Surface | Subsurface |
| **In situ T°(C)** | 12          | ND         | ND             | 13      | ND         |
| **SPM-TEX$_{86}$** | ND          | ND         | 12 ± 2         | ND      | 14 ± 3     |
| **SPM-TEX$_{86}$-T° (°C)** | ND          | ND         | -0.38          | ND      | 16 ± 4     |
| **TEX$_{86}$** | ND          | ND         | 20 ± 3         | ND      | 2         |
| **TEX$_{86}$-T° (°C)** | ND          | ND         | 3              | ND      | 4         |
| **Ring Index** | ND          | ND         | ND             | ND      | ND         |
| **(GDGT-2/GDGT-3)** | ND          | ND         | ND             | ND      | ND         |
| **(GDGT-2/Crenarchaeol)** | ND          | ND         | ND             | ND      | ND         |
| **Surface sediment** | **In situ TEX$_{86}$** | **TEX$_{86}$-T° (°C)** | **TEX$_{86}$-T° (°C)** | **Ring Index** | **(GDGT-2/GDGT-3)** | **(GDGT-2/Crenarchaeol)** |
|                | ND          | 0.5        | -0.5           | 15 ± 7  | 2          |
| 1: Temperatures obtained using TEX$_{86}$ and temperature relationship from Wuchter et al. (2005): TEX$_{86} = 0.017 \times T + 0.29$
| 2: Temperatures obtained using TEX$_{86}$ and temperature relationship from Kim et al. (2008): SST = 56.2 \times TEX$_{86} + 10.8$
| 3: Temperatures obtained using TEX$_{36}$ and temperature relationship from Kim et al. (2010): 67.5 \times TEX$_{36} + 46.9$
| 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.

**TABLA 2.** Matriz de similitud usando el coeficiente de proximidad de Jaccard para los patrones de bandas de EGGD archaical 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 80 m | June 80 m |
|----------------|-------------|-----------|--------------|-----------|
| **January 10m** | 1           | 0.5       | 0.6          | 0.4       |
| **June 10 m**   | 0.5         | 1         | 0.6          | 0.8       |
| **January 80 m**| 0.6         | 0.6       | 1            | 0.8       |
| **June 80 m**   | 0.4         | 0.8       | 0.8          | 1         |
VERTICAL AND SEASONAL VARIABILITY IN THE RELATIVE ABUNDANCE OF ARCHAEOAL 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 ARCHAEOAL 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 archaeal GDGTs

iGDGTs concentrations were higher in the deeper subsurface 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 NH₃ (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. 3c; 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 an 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 TEX₈₆-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 TEX₈₆-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 O₂ 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 O₂-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 O₂ 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. O₂, 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 16S 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 μm) 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 NH₄⁺ and NO₂⁻ were higher (Fig. 2).

In marine sub-oxic waters, the occurrence of NH₄⁺-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 gene belongs to the uncultured cluster A, when sub-oxic conditions and high NH₄⁺ concentration prevail (Molina et al. 2010). NH₄⁺ concentrations were in average 42 times higher during the non-upwelling period than during upwelling (Fig. 2b, d), yielding a greater (NH₄⁺ + NO₂⁻) to P ratio during winter (13 vs. 10 during upwelling) when higher abundance of iGDGTs (Fig. 4) occurs, suggesting that Thaumarchaeota abundance depends on NH₄⁺ 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 NO₂⁻ maxima (Massana et al. 1997; Murray et al. 1998a, 1998b; Sinninghe Damsté et al. 2002a) as well as archaeal NH₄⁺ 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 (Levipp et al. 2007a; Molina et al. 2010) that have detected a role of pelagic NH₄⁺ 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. 5a, 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 -thaumarcheota 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 TEX₈⁶- and in situ temperatures. Similarly, TEX₈⁶- 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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