Dissolved Methane Distribution in the Reloncaví Fjord and Adjacent Marine System During Austral Winter (41°–43° S)

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Abstract Within the earth’s atmosphere, methane (CH4) is one of the most important absorbers of infrared energy. It is recognized that coastal areas contribute higher amounts of CH4 emission; however, there is a lack of accurate estimates for these areas. This is particularly evident within the extensive northern fjord region of Chilean Patagonia, which has one of the highest freshwater runoffs in the world. Oceanographic and biogeochemical variables were analyzed between the Reloncaví fjord (41° S) and the Interior Sea of Chiloé (ISC) (43° S), during the 2013 austral winter. Freshwater runoff into the fjord influences salinity distribution, which clearly delimits the surface (<5 m depth) and subsurface layers (>5 m depth), and also separates the estuarine area from the marine area. In the estuary, the highest CH4 levels are generally observed in the cold and brackish nutrient-depleted surface waters (N- and P-depleted), ranging from 16.97 to 151.4 nM (mean ± SD 52.20 ± 46.49), equivalent to 640–4537% saturation except for the case of Si(OH)4. Conversely, subsurface waters have lower CH4 levels, fluctuating from 14.3 to 29.6 nM (mean ± SD 22.75 ± 4.36 nM) or 552–1087% saturation. A significant negative correlation was observed between salinity and CH4, and a positive correlation between Si(OH)4 and CH4, suggesting that some of the CH4 in estuarine water is due to continental runoff. Furthermore, the accumulation of seston and/or plankton at the pycnocline may potentially generate the accumulation of CH4 via microbial processes, as observed in estuarine waters. By contrast, the marine area (the ISC), which is predominantly made up of modified subantarctic water, has a relatively homogenous CH4 distribution (mean ± SD 9.84 ± 6.20 nM). In comparison with other estuaries, the Reloncaví fjord is a moderate source of CH4 to the atmosphere, with effluxes ranging from 23.9 to 136 μmol m⁻² day⁻¹. This is almost double the levels observed in the ISC, which ranges from 22.2 to 46.6 μmol m⁻² day⁻¹. Considering that Chilean Patagonia has numerous other fjord systems that are geomorphologically alike, and in some cases have much greater freshwater discharge, this study highlights their potential to be a significant natural source of this greenhouse gas.

Keywords Methane · Fjords · North Chilean Patagonia

Introduction

Methane (CH4) is an important atmospheric trace gas because it both directly (due to its stronger greenhouse effect) and indirectly (due to chemical reactivity) influences the Earth’s climate (Wuebbles and Hayhoe 2002). Indeed, CH4 has important effect on both tropospheric and stratospheric chemistry, significantly affecting levels of the ozone, water vapor, the hydroxyl radical, and numerous other compounds (Wuebbles and Hayhoe 2002). CH4 concentration has doubled since the beginning of the industrial era, and the cause of this rise is believed to be due to anthropogenic sources (Bousquet et al. 2006). Estimating the contribution of different sources of emissions to the global CH4 budget, such as terrestrial (soils) and marine ecosystems (oceans, estuaries), and also the relative contribution of anthropogenic CH4 production (combustion of fossil fuels, forest burning, cattle rearing, rice fields) remains uncertain (Kirschke et al. 2013).
CH₄ emissions into the atmosphere vary between different natural sources. The world’s oceans seem to play a modest role in the global atmospheric CH₄ budget (Matthews 1994); however, this role may fluctuate over both spatial and temporal scales, and the extent of this variation remains unclear (Bates et al. 1996). Within aquatic systems, estimates of coastal CH₄ content and fluxes are uncertain due to the lack of an adequate existing database (IPCC 2013). Estuaries and continental shelves appear to be responsible for ca. 75% of the oceanic CH₄ emissions (Bange et al. 1994; Borges and Abril 2011), due to the fact that these highly productive areas favor the benthic–pelagic coupling, and the underlying sediments emit large quantities of CH₄ (Borges et al. 2016).

The content and rate of exchange of dissolved CH₄ with the atmosphere is determined by the balance between production and consumption processes, which in turn are regulated by environmental conditions, principally by organic matter availability and oxygen concentration (Wolfe 1971). CH₄ is generally formed by methanogens during anaerobic organic matter degradation (Reeburgh 2007), or by methyloths (Sowers and Ferry 1983; Sun et al. 2011). CH₄ formation via methylothyrophy occurs by transformations and cycling of methyl compounds, mediated by bacterioplankton, such as methylphosphonate (MPn) (Karl et al. 2008), dimethylsulphoniopropionate (DMSP) (Damm et al. 2010), and dimethyl sulfide (DMS) (Florez-Leiva et al. 2013). In addition, CH₄ can be consumed (oxidized) via aerobic methanothrophy (Hanson and Hanson 1996), or when O₂ is exhausted, NO₃⁻ and SO₄²⁻ act as electron acceptors (Valentine 2011).

The southern coast of Chile, from the latitudes of the Reloncavi fjord (41° 40’ S) to Cape Horn (55° S), covers an area of approximately 241,000 km² and is characterized by an extensive coastline composed of numerous islands, fjords, sounds, basins, and gulfs (Lange 2012). Strong seasonal changes in climate variables (e.g., solar radiation, wind, and precipitation), as well as different physical regimes (mixing and circulation in the water column), influence biogeochemical dynamics and also provoke complex marine–terrestrial–atmospheric interactions. This results in a marked seasonality of biological production, which generally peaks during spring–summer (González et al. 2010; Montero et al. 2011). This is the first study of CH₄ distribution in northern Chilean Patagonia. This region is a quasi-uninhabited area with minimum human activity, thus minimal influences of eutrophication are expected. However, due to the expansion of intense aquaculture activities, some signals of perturbations have been observed in aquatic systems in the region (Iriarte et al. 2010). Oceanographically, this region could be considered as a transitional marine system, influenced by nutrient-rich and high-salinity oceanic subsurface waters, and nutrient-poor and low-salinity surface freshwater (Valle-Levinson et al. 2007). The main objective of the present study is to describe for the first time the CH₄ distribution in the region, and to determine the relative contribution of estuarine and marine sources of CH₄ under the influence of a strong freshwater runoff, during the winter season in northern Chilean Patagonia.

**Methods**

**Characteristics of Study Sites**

The study area comprises the area from the Reloncavi fjord (41° 40’ S) to the Guafo mouth (43° 30’ S) (Fig. 1). The Reloncavi fjord is one of the most widely studied fjords in Chile (e.g., Castillo et al. 2016). It is 55 km long and 2–3 km wide and receives freshwater discharge from the Petrohué (annual mean ∼ 280 m³ s⁻¹) and the Puelo River (annual mean ∼ 650 m³ s⁻¹). The Puelo River is the main supplier of freshwater to the system, with a mixed hydrological regime, presenting larger contributions from rain in the austral winter and snow melt in the austral spring (León-Muñoz et al. 2013). The Reloncavi fjord opens into the Reloncavi Sound, which is ∼450 m deep and is characterized by the absence of a sill at the mouth; the resulting depth of the system enables conditions for recirculation. This sound is connected within the Inner Sea of Chiloé (ISC), which includes the Ancud and Corcovado Gulf. The Corcovado Gulf is connected with the adjacent Pacific Ocean through a wide entrance, the Guafo mouth, through the Penas channel (to the South of Chiloé Island), while the Ancud Gulf is connected to the Pacific through the Chacao channel (north of Chiloé Island) (Fig. 1). In northern Patagonia, water circulation essentially follows a two-layer estuarine flow pattern (Sievers and Silva 2008); brackish water moves out superficially, and marine water enters predominantly through a subsuperficial layer in the Guafo mouth. Occasionally, wind-associated (Cáceres et al. 2002) and/or non-linear tidal effects (Valle-Levinson et al. 2007) can generate a three-layer circulation pattern, which consists of a thin surface layer flowing ocean-ward, a thick intermediate layer flowing inward, and a narrow bottom layer flowing ocean-ward.

**Sampling**

Samples were collected during the CIMAR 19 cruise (Cruceros de Investigación Marina CONA) at 15 stations along the Reloncavi fjord and the interior sea of Chiloé. The cruise took place from July 4 to July 17, 2013, on board the R.V. Cabo de Hornos. Continuous vertical profiles of temperature (T°C), salinity (PSU), density (kg m⁻³) (as derived variable, Sigma-t), and dissolved O₂ (μM) were obtained using a conductivity, temperature, and depth (CTD) sensor. Seawater samples were collected using a SBE 911 plus CTD unit (Sea-Bird Scientific) mounted on a 12-bottle rosette. The measurement of suspended particle concentrations and size was
carried out with a LISST-25X (Sequoia Scientific Inc). This instrument functions through laser diffraction; when the laser beam encounters a particle, it scatters in a manner that is proportional to the particle’s cross-sectional area; the LISST-25X is suitable for operations within a concentration range of approximately 1 to 2000 mg L$^{-1}$. Within this range, the sensor is capable of calibrating sediment concentration regardless of particle color or size.

Water samples for CH$_4$ and nutrients (sampled in this consecutive order) were obtained from 10 depths (0, 5, 10, 15, 25, 40, 50, 75, 100, and 200 m depth). Water samples for CH$_4$ (triplicate) for each depth (one Niskin bottle per depth) were taken in 20-mL
glass vials and poisoned with HgCl₂ (0.1 mL of saturated HgCl₂ solution per vial). Subsequently, the vials were sealed with a butyl rubber septum and an aluminum cap to ensure gastight conditions, avoiding bubble formation, and stored in darkness at room temperature for 1 month, until laboratory analysis. Nutrient samples (NO₃⁻, NO₂⁻, PO₄³⁻, and Si(OH)₄) were taken using 50-mL syringes directly connected to the spigot of the Niskin bottle at each sample depth. Duplicate samples were collected and drawn through a 0.75-μm glass fiber filter (GFF) adapted to the syringe, and then stored for 1 week until analysis in the laboratory.

### Chemical Analysis

CH₄ was analyzed via the generation of a 5-mL ultra-pure helium headspace into the vial using a gastight syringe. Subsequently, the gas and liquid phases were equilibrated within the vial at 40 °C. The procedure of equilibration entails the creation of a headspace, and the withdrawal of a seawater sample through a septum pierced with a double-needle liquid-transfer/gas-purge (5 mL helium headspace and 15 mL of seawater). As the sample is withdrawn through one needle, gas (helium) enters through the other needle, which acts as a gas port and forms the headspace at atmospheric pressure. Subsequently, CH₄ was quantified in the vial headspace Sc. CH₄ was analyzed via the generation of a 5-mL ultra-pure helium headspace into the vial using a gastight syringe. Subsequently, the gas and liquid phases were equilibrated within the vial at 40 °C. The procedure of equilibration entails the creation of a headspace, and the withdrawal of a seawater sample through a septum pierced with a double-needle liquid-transfer/gas-purge (5 mL helium headspace and 15 mL of seawater). As the sample is withdrawn through one needle, gas (helium) enters through the other needle, which acts as a gas port and forms the headspace at atmospheric pressure.

### Data Analysis

Saturation percentages of CH₄ were calculated from the measured CH₄ concentrations and using the estimations of atmospheric gas concentrations provided by NOAA (ftp://aftp.cmdl.noaa.gov/products/trends/ch4/ch4_mm_gl.txt), based on in situ temperature and salinity records, and relating this to the equivalent CH₄ solubility parameterization (Wiesenburg and GuinassoJr 1979). Air–sea CH₄ exchange fluxes \( F_{\text{air}} \) (in μmol day\(^{-1}\) m\(^{-2}\)) were estimated from the following equation:

\[
F_{\text{air}} = k_w(C_w - C_{\text{sat}})
\]

where \( k_w \) (cm s\(^{-1}\)) is the gas transfer velocity depending on wind speed, \( C_w \) is the CH₄ concentration (nM), and \( C_{\text{sat}} \) is the CH₄ concentration in equilibrium to the atmospheric concentration, according to the solubility parameterization of Weiss and Price (1980), and assuming that the atmospheric CH₄ concentration is 1803 ppb (IPCC 2013). \( k_w \) was calculated using the parameterization of Nightingale et al. (2000):

\[
k_w = \left(9.25 \times 10^{-7}u + 6.17 \times 10^{-7}u^2\right) \left(\frac{\text{Sc}}{600}\right)^{-0.5}
\]

where \( u \) is the wind speed (cm s\(^{-1}\)) and Sc is the Schmidt number for N₂O. The Schmidt number relates the relationship between viscosity and the diffusion coefficient of CH₄ in water, depending on the temperature and salinity of the seawater. For N₂O, the Schmidt number as a function of temperature (°C) is given by Wanninkhof (1992):

\[
\text{Sc} = 230.1 - 151.15 \times T + 4.7364 \times T^2 - 0.059431 \times T^3
\]

Nightingale’s parameterization shows a dependence between those of Liss and Merlivat (1986) and Wanninkhof (1992) and reduces the uncertainty of other parameterizations. The wind speed data was measured on board and normalized to 10 m height using the relationship of Garratt (1977). Wind speed was estimated as a moving 7-day average prior to the sampling period in order to smooth out short-term fluctuations and highlight longer-term trends. In the case of rivers and fjords, if the drag and current conditions are sufficiently high or exceed an order of magnitude of wind (>10 m s\(^{-1}\) in the fjord valley), surface currents must also be considered. In this case, it is not necessary to consider superficial currents, which were reported to be as low as 5 cm s\(^{-1}\) (even at maximum tidal currents; Valle-Levinson et al. 2007). Thus, surface current was not included in the estimation of gas transfer velocity \( k_w \), as was the case for estimates of N₂O fluxes (Yevenes et al. 2016); however, this may underestimate N₂O fluxes in the Reloncavi fjord. The mixed layer depth was calculated using a potential density-based criterion, defining it as the shallowest depth at which density increased by 0.02 kg m\(^{-3}\) from the sea surface value (Kara et al. 2003). Stratification was estimated using the Brunt–Väisälä frequency, which is used as a parameter to quantify the significance of stability and stratification in the water column.

In order to evaluate the variation between the two areas (estuarine and marine), the non-parametric multidimensional scaling (MDS) calculations were applied to physicochemical variables between 0 and 200 m depth (data was transformed to Log: X + 1), prior to the application of the Euclidian dissimilarity index. Then, an analysis of similarities (ANOSIM) was applied, with permutation of 9999 to determine statistical differences with a significance level of \( p < 0.05 \), testing the null hypothesis of similarity between the areas. In addition,
Spearman correlations (Rho) for salinity, CH₄, nutrients, and particle variation in surface (>10 m depth) and subsurface (10–200 m depth) waters were used, due to the fact that some variables behaved as non-normal data. The threshold value for statistical significance was set as \( p < 0.05 \). Data were processed using Primer 6.0 and SigmaStat 4.0. Spatial data were plotted using the Ocean Data View program 4.0 and SigmaPlot 10.0.

**Results**

**Spatial Distribution of Physical and Biogeochemical Variables**

Physical and biogeochemical variables are summarized in Table 1. The study area was divided into two layers, according to the vertical structure of the water column, i.e., two distinct layers: from 0 to <5 m (surface layer), and from 10 to 200 m (subsurface layer). Also, two sections were allocated depending on the relative influence of fresh vs. marine water (according to temperature, salinity, and density). The sections comprise the Reloncaví fjord and the sound (Sts. 7b, 7c, 6, 5, 4, 3, 8, and 9; hereafter the estuarine area), and the ISC comprise the Ancud and Corcovado Gulf (Sts. 14, 16, 20, 21, 32, 33, 36, 38; hereafter referred to as marine area). This division was previously established for the study area by Yevenes et al. (2016), taking into account the distribution of temperature, salinity, and Sigma-t. These spatial patterns were validated by multidimensional scaling (MDS) analysis applied to physicochemical (i.e., T°C, S, Sigma-t, and stability) and biogeochemical variables, including dissolved CH₄, particles, and nutrients (data not shown).

There is a clear separation between the outflow of colder and brackish surface water and the inflow of more saline and warmer subsurface water, which affects the stability of the water column. The stability, or Brunt–Väisälä frequency, is presented in Fig. 2a and shows a fluctuation from 0.1 to 115 cycle h⁻¹, which peaks in the surface water (predominantly between 5 and 10 m depth) and then decreases to relatively steady values in the subsurface layer (0.1 to 10 cycles h⁻¹). The maximum frequency marked a notable stratification in the surface layer, which creates a strong shear stress that may facilitate drag and the accumulation of particles (Fig. 2b). Particle concentration decreased exponentially with depth, with the highest values at the surface in the estuarine area, where the particle concentration reached up to 424.6 mg L⁻¹. Also, the largest particle sizes were registered in the surface layer, measuring from 0.02 to 4308 μm (Fig. 2c); conversely in subsurface waters, the concentration and size were one order of magnitude less than in the subsurface layer. In the marine area, both the concentration and size of particles in the surface layer were lower than in the estuarine area (mean ± SD 56.64 ± 56.08 mg L⁻¹ and 13.19 ± 15.78 μm, respectively), and the concentration and size reduced exponentially with depth. In the estuarine area, the particle size ratio between the surface and subsurface layers showed a 771-fold variation, whereas in the marine area the ratio was 3-fold, suggesting that in the estuarine area the particles have a continental origin.

Figure 3 shows the cross section distribution of CH₄ (nM) (Fig. 3a) along with dissolved O₂ (μM) (Fig. 3b) and Si(OH)₄ (μM) (Fig. 3c). Dissolved CH₄ showed a concentration range from 7.65 to 151.4 nM, following similar distributions to those observed for brackish water and particles. A maximum CH₄ concentration of 151.1 nM, equivalent to 1403% saturation, was found superficially (1 m depth) at St. 7b (located in the downstream sections of the Petrohué River) and at the following stations that are under riverine influence; towards the Reloncaví Sound and the ISC, CH₄ levels decreased. Marked differences in CH₄ content were also observed between the surface and subsurface layers, with mean ± SD values of 52.20 ± 46.49 and 22.75 ± 4.36 nM, respectively (Table 1). In the ISC, CH₄ showed a more uniform distribution in both the surface and subsurface layers, with mean ± SD values of 19.84 ± 6.20 and 18.18 ± 6.08 nM, respectively.

CH₄ fluxes across the air–sea interface, estimated from surface CH₄ levels and wind parameterization, ranged from 22.2 to 136 μmol m⁻² day⁻¹. As expected, the estuarine area had higher levels of CH₄ exchange to the atmosphere, ranging from 23.9 to 136 μmol m⁻² day⁻¹. There was a decrease in the CH₄ emissions towards the marine environment, with a narrower extent of efflux, from 22.2 to 46.6 μmol m⁻² day⁻¹.

The dissolved O₂ fluctuated between 128 and 379 μM, with the lowest concentrations in the subsurface layer of the estuary and gradually and lightly increasing towards the ISC (Gulf of Corcovado) (Fig. 3b). Within the ISC, O₂ distribution was homogenous throughout the water column, with values of around 250 μM. NO₃ and PO₄³⁻ distributions have been reported by Yevenes et al. (2016) during the same period; however, there are no records for Si(OH)₄. The latter ranged from 13.2 to 111 μM, with the highest concentrations observed in the surface water of the Reloncaví fjord, averaging 61.8 ± 38.8 μM, which contrasts with the lowest values in surface waters, observed in the ISC (mean ± SD 22.49 ± 7.54 μM) (Fig. 3c).

Table 2 shows Spearman correlations for variables that could explain CH₄ distribution. In surface waters, a significant negative relationship was found between salinity vs. CH₄ \( (p < 0.00) \) and salinity vs. Si(OH)₄ \( (p < 0.00) \), verifying that during winter, freshwaters have a high CH₄ and Si(OH)₄ content; this relationship was not significant in marine water. Remarkably, CH₄ levels in the surface of the estuary showed a positive correlation with particle size \( (p < 0.00) \) and
Table 1  Average of environmental variables measured in surface and subsurface layers in the estuarine and marine areas, including standard deviation values as well its range

| Zone          | Depth (m) | Variables | T (°C) | Sal (PSU) | Sigma-t | O₂ (µM) | Particle size (µm) | Particle concentration (mg/L) | Si(OH)₄ (µM) | CH₄ (nM) | % Sat CH₄ |
|---------------|-----------|-----------|--------|-----------|---------|---------|-------------------|-----------------------------|-------------|---------|-----------|
| **Estuarine** | 0–5 m     | Mean ± std. dev | 10.20 ± 0.57 | 24.12 ± 10.78 | 18.22 ± 8.53 | 265.37 ± 39.63 | 509.55 ± 977.13 | 189.56 ± 138.52 | 58.44 ± 39.35 | 52.20 ± 46.49 | 1735.98 ± 1403.09 |
|               | Min–max   | n         | 9.10–10.96 | 2.53–32.16 | 1.11–24.59 | 207.60–379.12 | 0.36–4308.31 | 5.92–424.64 | 14.61–111.72 | 16.97–151.43 | 639.65–4536.99 |
|               | Mean ± std. dev | n         | 10.37–11.28 | 26.65–33.03 | 20.13–25.17 | 127.34–279.72 | 0.05–58.28 | 0.58–361.68 | 18.01–62.46 | 14.34–29.62 | 552.45–1086.82 |
| **Marine**    | 0–5 m     | Mean ± std. dev | 10.39 ± 0.19 | 31.97 ± 0.54 | 24.47 ± 0.44 | 258.16 ± 14.47 | 13.19 ± 15.78 | 56.46 ± 56.08 | 22.49 ± 7.51 | 19.84 ± 6.20 | 742.80 ± 232.14 |
|               | Min–max   | n         | 10.11–10.74 | 30.82–32.77 | 23.55–25.14 | 239.40–319.27 | 0.38–47.87 | 4.78–242.60 | 15.48–41.36 | 7.65–32.64 | 284.85–1222.54 |
|               | Mean ± std. dev | n         | 10.58 ± 10.47 | 32.71 ± 32.76 | 25.03 ± 25.04 | 243.50 ± 254.96 | 4.16 ± 0.65 | 16.65 ± 13.14 | 23.64 ± 22.50 | 18.18 ± 6.08 | 682.54 ± 227.13 |
|               | Min–max   | n         | 10.08–11.22 | 31.14–33.20 | 23.79–25.51 | 205.12–272.20 | 0.02–48.58 | 3.33–213.49 | 13.19–99.35 | 8.98–28.51 | 344.83–1070.26 |

Note: All values are averages with standard deviation.
concentration ($p < 0.00$) (Table 2). However, these correlations were found neither in the subsurface water of the estuarine area nor in the marine area.

**Vertical Distribution of Gases and Nutrients**

Figure 4 presents typical particle (concentration and size), nutrient (Si(OH)$_4$ and NO$_3^-$), and gas (O$_2$ and CH$_4$) profiles from selected stations. We selected a station from the Reloncavi fjord (St. 6; Fig. 4a), in the Reloncavi Sound (St. 9, Fig. 4b), and in the northern (St. 20; Fig. 4c) and southern sections of the ISC (St. 38 close to Guao mouth). The vertical distribution of biogeochemical variables was clearly in line with the double-layer structure in the estuarine area, which in turn was predominantly controlled by the physical structure of the estuary. This structure was influenced by river discharge, characteristically low in NO$_3^-$ but high in O$_2$, CH$_4$, particles, and Si(OH)$_4$ load (Fig. 4a, b). Conversely, in the ISC, profiles are smoother (Fig. 4c, d) and mainly influenced by the entry of oceanic water masses through the Guao mouth, such a subantarctic water (SAAW). Additionally, a deep equatorial water mass (ESSW) rich in NO$_3^-$ and relatively lower O$_2$ was observed.

**Discussion**

**Hydrographic Setting**

The Patagonian fjord region is composed by a large number of fjords, sounds, and basins and is subjected to strong seasonal climatic changes (e.g., solar radiation, wind, and precipitation) as well as different physical regimes (mixing and/or stability of the water column), imposing an external influence on biogeochemical cycles. There is a notably high annual rainfall in the fjord region (1000–3500 m$^3$ s$^{-1}$) (Sievers and Silva 2008). Although studies of dissolved CH$_4$ distribution in adjacent ocean water (offshore Chiloé Island, 41°50′S) are currently not available, some existing data (Farías non-published data) indicate that CH$_4$ levels in SAAW are approximately 5–10 nM (Fig. 3a). Thus, CH$_4$ concentrations over the entire water column in the ISC are lightly enriched in CH$_4$ relative to the adjacent open ocean. These results may be due to circulation (longer water residence time) and/or mixing with estuarine water. Some in situ CH$_4$ production may be occurring, given that the ISC is shallower and receives more organic matter from continental runoff and salmon aquaculture activities (Iriarte et al. 2010) compared to the open sea.

CH$_4$ exchange across the air–sea interface reflects the CH$_4$ content in the surface layer. The highest CH$_4$ effluxes were observed in the upstream stations of the Reloncavi fjord, and the emissions decreased as salinity increased. In comparison with other estuaries and coastal areas, the Reloncavi fjord is a moderate area source of CH$_4$, ranging from 23.9 to 136 μmol m$^{-2}$ day$^{-1}$. The fluxes were two times greater than effluxes from open waters (Holmes et al. 2000) and from continental shelves (Bange 2006; US EPA 2010). This range is very modest compared to rivers where maximum values are up to several micromolars (Stanley et al. 2016; Borges et al. 2015a, 2015b). There are no existing studies of seasonal behavior for this area, but estimated fluxes in winter were lower than those registered in the eutrophic inner estuaries of Northern Europe (Bange 2006). In addition, if the results from this study can be extrapolated to other estuarine systems within the northern Patagonia Region, it would signify that this region represents a large natural source of CH$_4$ (Middelburg et al. 2002; Borges and Abril 2011). It is important to note that due to the remote region of the study sites, the seasonal behavior is not fully understood; however, it is clear that temperature and biological productivity increases in the summer (due to greater solar radiation), which may in turn increase the in situ production of methane.

**Autochthonous Methane Origin in the Reloncavi Fjord**

The origin of autochthonous CH$_4$ is thought to be a result of in situ production via methanogenesis within anoxic sediments
identified a wide range of particle sizes (0.02 to 4302 μm), owing to larger-sized particles (>300 μm) in surface waters within the upstream stations of the Reloncaví fjord (Fig. 2c).

This suggests that particles are not only of marine origin, but they are also input from soils as a result of precipitation. Previous studies indicate that surface sediments of the Reloncaví, Puyuhuapi, and Aysën fjords have high organic carbon contents (i.e., >3%; Silva et al. 2011), with significant inputs by major rivers (i.e., Puelo, Cisnes, and Aysën rivers). The allochthonous carbon content in the surface sediments of these fjords (estimated with δ13C) ranges between 50 and 90%, decreasing to less than 10% towards the oceanic area (Silva et al. 2011).

In most permanent and seasonally stratified oceanic waters, the maximum CH4 concentration (Figs. 3a and 4) is consistently observed to coincide with increased particle accumulation at pycnoclines (Sieburth and Donaghy 1993). Thus, it is believed that these particles may act as anoxic microenvironments, as they are chemical hotspots providing both organic and inorganic substrates to sustain the anaerobic metabolism of pelagic microbiota (Ploug et al. 1997). Indeed, both autochthonous (plankton) and/or allochthonous (seston) particles can be colonized by decomposers (heterotrophs), which include a proportion of methanogens. Sieburth and Donaghy (1993) reported the existence of methanogenic bacterial consortia that can use methylated amines to create reduced micro-niches in oxygenated seawater and produce both CH4 and HS- in the upper ocean. Methanogenic substrates such as monomethylamine and trimethylamine, which are constituents of diatoms, dinoflagellates, and flagellate phytoplankton species, could be involved in CH4 production during grazing by copepods (De Angelis and Lee 1994). In addition, methylotrophic methanogens that use the products generated from the degradation of methylated amine in microalgae could be responsible for the production of CH4 (Oremland et al. 1982; King et al. 1983; King 1984). Recently, this mechanism has been described using substrates as DMSP (Damm et al. 2010) and also DMS (Florez-Leiva et al. 2013).

CH4 content and emissions in aquatic systems reflect the characteristics of the surrounding catchment area, such as topography, soil type, and texture, as well as land use and other anthropogenic activities (Jones and Mulholland 1998), and also the features of the adjacent marine system, such as tidal and wave movements and geomorphology (US EPA 2010). In general, higher CH4 content and effluxes have been found in streams and rivers surrounded by peatlands and flooded forests (Jones and Mulholland 1998; Hope et al. 2001; Borges et al. 2015a, 2015b) compared to those with adjacent marine systems. The highest levels of CH4 measured in this study were located superficially at a salinity of less than 5, at upstream stations (Fig. 3a; Yevenes et al. 2016), with a Si(OH)4 of 109 μM, and a particle concentration and size of 500 mg L−1 and 300 μm, respectively (Figs. 3c and 2b, c).
Also, a positive correlation was observed between Si(OH)\textsubscript{4} and salinity in the estuarine area (Table 2), suggesting that water from continental runoff contributes high levels of Si(OH)\textsubscript{4} into the Reloncaví fjord. Indeed, northern Chilean Patagonia is characterized by andosol-type soil, which affects dissolved Si(OH)\textsubscript{4} concentrations in the river system and potentially explains the exceptionally high regional rates of biogenic Si(OH)\textsubscript{4} production (Vandekerkhove et al. 2016). In addition, Si(OH)\textsubscript{4} is well correlated with both particle concentration and dissolved CH\textsubscript{4} (Table 2), indicating that the majority of the particles suspended in the water column originate from continental runoff.

The extent of supply coming from continental runoff (both laterally and along the systems) depends on the vegetation type and adjacent soils; both factors stimulate the production of microbial CH\textsubscript{4}. Plants mediate CH\textsubscript{4} production as they constitute C substrates as products of photosynthesis, derived from living plants and/or labile organic carbon (Ding et al. 2002, 2005; Tsuruta and Li 2003). CH\textsubscript{4} is accumulated in the interstices of soils and then transported to the atmosphere by plants through the parenchyma system or by lateral diffusion and runoff from saturated soils. Thus, soils may be important in determining CH\textsubscript{4} levels in rivers and estuarine systems (De Angelis and Miller 1987; Sansone et al. 1999). As previously mentioned, Chile has one of the highest global incidences of peatlands, dominated by Sphagnum magellanicum, (Joosten and Clarke 2002). Although Chile lacks a detailed register of peatlands, they are predominately distributed from the Los Lagos (41° S) to the Magellan (55° S) regions. It should also be noted that they are being exploited as carbon energy source (Valdés-Barrera et al. 2012). CH\textsubscript{4} is released from peat columns as a result of recently fixed (young) carbon, and production rates can be substantially stimulated through addition of labile organic carbon at a low pH (Lai 2009), as demonstrated in these soils by Filipová et al. (2010).

It is also important to consider the type of land use; however, it remains uncertain if land use has significant influence on CH\textsubscript{4} levels in rivers. For example, where land use is mainly forest and/or fertilized agriculture, no systematic differences were observed in CH\textsubscript{4} content (US EPA 2010). In general, nitrogen fertilizers are not extensively used on Patagonian soils (non-agricultural activities), therefore the N and P nutrient load in the study area should be very low in comparison to rivers and estuaries in the Northern Hemisphere, many of which are subjected to increasing levels of organic matter input and nutrient enrichment (Bange 2006). In addition, within the study area, the Los Lagos region, there is a low–moderate level of cattle rearing, evident from low levels of

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**Table 2** Spearman correlations of the same variables measured in both surface and subsurface layers, including the estuarine and marine area

|                  | Sal (PSU) | Particle size (μm) | Particle concentration (mg/L) | Si(OH)\textsubscript{4} (μM) |
|------------------|-----------|--------------------|-------------------------------|----------------------------|
|                  | Rho       | p      | n | Rho | p      | n | Rho | p      | n | Rho | p      | n |
| CH\textsubscript{4} (nM) estuarine 0–5 m | −0.97* | 0.00 | 6 | 0.77* | 0.00 | 13 | 0.74* | 0.00 | 13 | 0.86* | 0.00 | 14 |
| CH\textsubscript{4} (nM) estuarine 10–200 m | −0.46 | 0.06 | 17 | 0.16 | 0.55 | 16 | 0.45 | 0.08 | 16 | −0.37 | 0.12 | 18 |
| CH\textsubscript{4} (nM) marine 0–5 m | −0.28 | 0.59 | 6 | −0.54 | 0.08 | 11 | −0.38 | 0.24 | 13 | 0.16 | 0.61 | 12 |
| CH\textsubscript{4} (nM) marine 10–200 m | −0.29 | 0.26 | 17 | 0.03 | 0.90 | 15 | 0.11 | 0.70 | 15 | 0.03 | 0.90 | 15 |
| Si(OH)\textsubscript{4} (μM) estuarine 0–5 m | −0.79* | 0.00 | 14 | 0.83* | 0.00 | 13 | 0.76* | 0.00 | 13 | − | − | − |
| Si(OH)\textsubscript{4} (μM) estuarine 10–200 m | −0.19 | 0.46 | 18 | 0.56* | 0.02 | 16 | 0.16 | 0.56 | 16 | − | − | − |
| Si(OH)\textsubscript{4} (μM) marine 0–5 m | −0.27 | 0.39 | 12 | 0.70* | 0.02 | 11 | 0.72* | 0.01 | 11 | − | − | − |
| Si(OH)\textsubscript{4} (μM) marine 10–200 m | −0.37 | 0.14 | 17 | 0.34 | 0.22 | 15 | 0.06 | 0.80 | 15 | − | − | − |

* Denotes significant correlations
observed ammonia emission (Martinez-Lagos et al. 2010), which is directly related to human activities.

Northern Chilean Patagonia has a low impact from urban settlements. However, the city of Puerto Montt, located at the
northern end of the Reloncavi Sound, is currently undergoing fast demographic growth, associated with the development of economic activities, especially with industries utilizing the natural resources that are locally available (predominantly aquaculture activities; Subpesca 2016). Thus, high levels of by-products from salmon and shellfish farming (surplus feed and/or feces) might affect the O₂ content in stations located downstream of the estuary as a result of organic matter degradation in water and surface sediments (Iriarte et al. 2010; Tapia and Giglio 2010), along with a long water flushing time (Valle-Levinson et al. 2007), which favors the accumulation of larger-sized particles that apparently stimulate additional O₂ consumption.

Aquaculture activities lead to an increased input of nitrogenous compounds (mainly ammonium and urea) and also result in feed additions (particles) below coastal salmon farms; however, this does not seem to stimulate CH₄ production. Surprisingly, in the Reloncavi Sound, CH₄ undergoes increased consumption relative to the surrounding water column (contrary to expected results of CH₄ accumulation), suggesting that CH₄ depletion is due to methanotrophy (Fig. 3a). This is contrary to the observed results for N₂O, where aquaculture favors N₂O production processes, such as nitrification (Yevenes et al. 2016), also including seasonal phytoplankton blooms and the growth of harmful algal blooms (Arzul et al. 1999; Iriarte et al. 2005).

Conclusions

The station at the head of the Reloncavi fjord had a high CH₄ content and efflux; also, the relationship between CH₄, salinity, and Si(OH)₄ indicates that most of the CH₄ comes from continental runoff. With regards to the in situ CH₄ production, it appears that the sediments are not an important source; however, some CH₄ accumulation was observed in the pycnoclines of the estuarine zone, where a particle accumulation also occurs. This pattern indicates the presence of aerobic methanogenesis processes. The marine area associated with the ISC has increased the CH₄ content compared to the offshore ocean; however, this is comparatively low with respect to estuarine waters, suggesting that CH₄ content in the ISC responds to mixing with freshwater rich in CH₄, along with some in situ production, with a longer water residence time in the ISC. Moreover, signals of increased eutrophic processes are observed from human activities; however, there is no evidence that this leads to an increase in the generation of CH₄.

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