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Research Article

Distribution and sources of phytosterols in coastal and river sediments of south-central Chile

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ABSTRACT. Phytosterols are potential endocrine-disrupting compounds. Quantification of phytosterols was carried out in sediments from four coastal zones and two rivers in south-central Chile. Sterol concentrations were determined by capillary gas chromatography-mass spectrometry and the sources of sedimentary organic matter were determined using sterol ratios and lipid biomarkers. Total sterol concentrations (0.03 to 10.4 μg g^{-1}) were within the range reported for other marine ecosystems and the β-sitosterol concentration (0.01 to 2.01 μg g^{-1}) was lower than previously reported for the upwelling system off Peru. Some coastal stations adjacent to the rivers had β-sitosterol of terrestrial origin. High concentrations of β-sitosterol were also found in sediments from more oceanic stations, supporting the notion that this sterol can also be produced by phytoplankton. No differences in the sterol concentration between the coastal zones were found. However, significant differences were found between almost all coastal zones and both rivers, and between rivers. At the station level and using different biomarkers of the source of organic matter, some areas were found to have a clear terrestrial influence; whereby it is assumed that the source of the phytosterols (especially β-sitosterol) would be vascular plants. The BioBío River and its mouth have a wide variety of sterols and lipids and high levels of cholesterol and epicholestanol, which is possibly related to the presence of domestic effluents derived from large cities. No clear spatial pattern emerge between the location of pulp mill industries and β-sitosterol sediment concentration, with the exception of one station located in the Gulf of Arauco.

Keywords: phytosterols, biomarkers, endocrine disruption, pulp mill, Chile.

Distribución y fuentes de fitoesteroles en sedimentos costeros y de ríos del centro-sur de Chile

RESUMEN. Los fitoesteroles son potenciales disruptores endocrinos. Se cuantificaron fitoesteroles en sedimentos de cuatro zonas costeras y dos ríos en el centro-sur de Chile. Se determinó la concentración de esteroles utilizando cromatografía de gas con espectrómetro de masa y las fuentes de materia orgánica sedimentaria se determinaron utilizando proporciones de esteroles y biomarcadores lipídicos. Las concentraciones de esteroles totales (0,03 a 10,4 μg g^{-1}) se encuentran dentro del rango informado para otros ecosistemas marinos y la concentración de β-sitosterol (0,01 a 2,01 μg g^{-1}) fue menor que la previamente informada para el sistema de surgencia de Perú. Algunas estaciones costeras adyacentes a los ríos presentaron β-sitosterol de origen terrestre. Además se encontró una alta concentración de este compuesto en sedimentos de estaciones más oceánicas, confirmando que este esterol también puede ser producido por fitoplancton. Al considerar la concentración y presencia-ausencia de esteroles en sedimentos, no fue posible encontrar diferencias significativas entre las cuatro áreas costeras. Sin embargo, se obtuvo una clara diferencia entre las áreas costeras y los ríos, así como entre ambos ríos. Considerando todas las estaciones y utilizando diferentes biomarcadores del origen de la materia orgánica, se encontraron algunas áreas con clara influencia terrestre, donde se asume que la fuente de origen de los fitoesteroles (especialmente β-sitosterol) podrían ser las plantas vasculares. El río BioBío y su desembocadura poseen una amplia diversidad de esteroles y lípidos, y altos niveles de colesterol y epicholestanol, los que estarían posiblemente relacionados a la presencia de efluentes domésticos provenientes de grandes ciudades. No se observaron claros patrones espaciales entre la ubicación

de las industrias de celulosa y la concentración de β-sitosterol en sedimentos, excepto para una estación ubicada en el golfo de Arauco.

Palabras clave: fitoesteroles, biomarcadores, disrupción endocrina, industria de celulosa, Chile.

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INTRODUCTION

Sterols are important hormonal regulators of growth, respiration and reproduction in organisms, as well as important structural components of cell membranes (Gagosian & Nigrelli, 1979). The relatively high resistance of the sterol skeleton to degradation, after release into the environment, makes them valuable as tracers of the transport and transformation processes of biogenic material (Gagosian & Nigrelli, 1979) and as tracers for studying sources of organic matter in coastal areas (Lee & Wakeham, 1989; Yunker *et al*., 1995; Hudson *et al*., 2001).

In the marine environment, cholesterol and sterols with 27 carbons (C_{27}) are predominant in zooplankton, invertebrates and vertebrates, while phytoplankton and seaweeds may synthesize a wide range of sterols including large quantities of phytosterols, which may have 28 carbons (C_{28}), (*e.g.*, brassicasterol [24-methylcholesta-5,22E-dien-3β-ol], 24-methylene cholesterol, campesterol [24-methylcholest-5-en-3β-ol] (Huang & Meinschein, 1979; Volkman, 1986; Kerr & Baker, 1991), or 29 carbons (C_{29}) (mainly β-sitosterol [24-ethylcholest-5-en-3β-ol] (Huang & Meinschein, 1976, 1979; Volkman, 1986). These phytosterols are present as well in vascular plants; the most common are campesterol, β-sitosterol and stigmasterol [24-ethylcholesta-5,22-diene-3β-ol] (Volkman, 1986; Lahdelma & Oikari, 2006).

Phytoplankton and terrestrial phytosterols differ principally in the configuration of the methyl or ethyl group at the C_{24} position, with a 24α configuration in vascular plants and a 24β configuration in algal sterols (Hassett & Lee, 1977; Volkman, 1986). However, β-sitosterol may also originate from marine organisms such as microalgae and perhaps cyanobacteria (Volkman, 1986). This makes the differentiation of marine and terrestrial phytosterols difficult in coastal sediments. Therefore, when used as terrestrial biomarkers, different approaches have been utilized to improve their sensitivity, such as (i) the use of phytosterol ratios (*e.g.*, Laureillard & Saliot, 1993; Mudge & Norris, 1997; Curiale & Harrison, 2007), (ii) the use of percentages of C29, C28 and C27 sterols (Huang & Meinschein, 1979), and (iii) the determination of the presence of other biomarkers (*e.g.*, fatty acids, alkanes, fatty alcohols, triterpenoids (Volkman, 1986; Bayona *et al.,* 1989; Zimmerman & Canuel, 2001; Jeng *et al.*, 2003).

Rivers and atmospheric inputs are the main pathways for the entry of vascular plant phytosterols into the marine environment (Volkman *et al*., 1987). Nevertheless, other inputs of vascular plant phytosterols into coastal waters may come from upland forests, mangroves, oil palm, or coconut plantations (Ali *et al.*, 2009), waste waters (Quemeneur & Marty, 1994; Liu *et al*., 2010) and the pulp and paper mill industry (Owens, 1991; Sepúlveda *et al.*, 2003; Orrego *et al*., 2005a, 2009). Important research efforts has been undertaken to evaluate the impact of the discharge of pulp mill effluents on fish reproductive behavior (*e.g.*, Walker *et al*., 2002; Dubé *et al.*, 2008), because it has been shown that fishes exposed to wood-derived sterols present endocrine disruption (Mellanen *et al.*, 1996; MacLatchy *et al*., 1997; Nakari & Erkomaa, 2003).

The marine ecosystem off south-central Chile is a productive system with an average primary productivity of 2.5 g C m^{-2} day^{-1} (Daneri *et al.*, 2000; Montero *et al.*, 2007) and daily values as high as 9.9 g C m^{-2} day^{-1} (Fossing *et al.*, 1995), which are among the highest reported in the literature. The productivity is fuelled mostly by nutrient fertilization of surface ocean by wind-driven upwelling (Arcos & Navarro, 1986), although mixing events associated with Kelvin waves have also been postulated as an important factor in triggering and maintaining this productivity (Djurfeldt, 1989). The BioBío (36º50`S) and the Itata (36º23`S) rivers are the two major rivers of south-central Chile, with typical annual average runoff at their mouths of 900 and 300 m^3 s^{-1}, respectively (Quiñones & Montes, 2001). These rivers have dissimilar hydrological dynamics due to the differential effects of factors such as the total surface and shape of each basin (BioBío = 24360 km^2; Itata = 11385 km^2), river length (BioBío = 380 km; Itata = 230 km), nival influences, spatial and temporal dynamics of rainfall level and river slope (Santibañez & Uribe, 1993; Parra & Habit, 1998; Dussaillant, 2009). This region has approximately 1.9 million inhabitants and multiple industrial activities are present in the area. They include, among others, forestry, fishing, pulp mills, an oil refinery, fish meal industries and iron-steel production. As a result, the region's aquatic systems receive a substantial amount

of urban and industrial wastes. In fact, Bertin *et al*. (2011) recently showed remarkably high levels of 17α-ethinylestradiol in coastal sediments, enough to cause endocrine disorders in fish inhabiting some of the aquatic ecosystems of this region. In addition, several studies have shown endocrine-disrupting effects in caged rainbow trout located downstream from pulp mill discharges in the BioBío River (Orrego *et al.,* 2005a, 2006, 2009). There is an important production of cellulose in south-central Chile of about 3.32 million ton annually; accordingly, phytosterols derived from this activity are incorporated into aquatic systems. Up till now the possible presence of phytosterols in coastal sediments off south-central Chile and their sources remain unknown. We hypothesize that aquatic areas close to the emissions of the pulp mill companies should have higher concentrations of phytosterols of terrestrial origin in comparison to areas without their influence. Alternatively, in a system with very high primary productivity, such as the upwelling system off central-south Chile, phytoplankton could also be a significant source of phytosterols as seen in the upwelling system off Peru (Volkman *et al.*, 1987). We aim to establish the relative contribution of marine, terrestrial and anthropogenic sources to the sterols present in coastal and river sediments of south-central Chile.

MATERIALS AND METHODS

Study area

The study area is located off south-central Chile (35-39ºS), which is part of the Humboldt Current System. In this area upwelling displays strong seasonality (Brandhorst, 1971; Ahumada *et al*., 1983), with high primary production rates (*ca*. 19.9 g C m^{-2} d^{-1}) (Peterson *et al*., 1988, Daneri *et al*., 2000). The continental shelf and slope of this zone extend further offshore (reaching 50 km extension near 36°S) and are therefore favorable for sediment accumulation (Muñoz *et al.*, 2004). Several rivers drain this coastal region, mainly the BioBío and Itata, supplying terrestrial detritus to the adjacent sediments (Lamy *et al.*, 1998). Regarding the physicochemical characteristics of the coastal sediments off south-central Chile, they consist mainly of clayey-silty to silty-clayey mud (Schubert *et al.*, 2000), with fine-grained shelf sediments in very nearshore areas and sandy sediments on the outer shelf and uppermost slope (Lamy *et al.*, 1998).

Sampling

Sediment samples were collected from 25 stations located off the south-central coast of Chile (Fig. 1a). These stations were divided into four general zones: (i) Gulf of Arauco (Zone A, stations A1, A2, A4, A5, A6, A7, A8, A12 and A-Pl). This zone is influenced by the BioBío River, some small rivers (*e.g.*, Carampangue, Las Cruces), sewage treatment plants and pulp mill effluents; (ii) BioBío River canyon, off the BioBío River mouth (Zone B, stations B1, B2, B3, B4, B5, B6, B7, Fig. 1). Four pulp mill industries and several sewage treatment plants release their effluents into the river basin; (iii) coastal shelf adjacent to the Itata River mouth (Zone P, stations P3, P4, P5, P6, P7, P8, P9, Fig. 1). Only one pulp mill industry is located in this river basin; (iv) Coliumo Bay (Stations PE2-PE3), that receives the contribution of two small rivers (Pingueral, Villarrica) and sewage treatment plants.

In addition to the marine sampling sites, 14 river stations were sampled (Figs. 1b, 1c): 6 in the Itata River basin (I1, I2, I3, I4, I5, I6) and 8 in the BioBío River basin (BB-A, BB-B, BB-C, BB-E, BB-F, BB-G, BB-I, BB-J) (Fig. 1).

Sampling took place mainly in autumn (2008, 2009), except in the case of the BioBío River in which sampling was conducted in summer (2008). In some areas samples were also obtained during other seasons but, in order to avoid an interseasonal component, these data were not used in the spatial comparative analysis.

All sediment samples were extracted with a Van Veen grab. A subsample was obtained from the top 5 cm at the surface. The samples were stored at -20ºC until analysis. Environmental parameters (depth, temperature, oxygen and salinity) were measured at all sampling sites using a CTDO SAIV A/S model SD204.

Sediment granulometry, total organic matter and total organic carbon

Granulometry was determined using approximately 500 g of wet sediment, which was dried at 100ºC for 24 h. The sediment was grounded and sieved through 9 sieves (2 mm-45 μm) for 3 min each. The sediment that remained in each sieve was weighed and the percentages of different sediment fractions were calculated (Folk & Ward, 1957). Total organic matter was determined in sediment subsamples by weight loss upon ignition at 550ºC to constant weight (Dean, 1974). Total organic carbon was estimated from organic matter using the van Bemmelan conversion factors of 1.72 for coastal sediments (Beaudoin, 2003) and 2.5 for river sediments (Nelson & Sommers, 1996).

Determination of sterols and other lipids

Sediment was dried at 50°C in an oven for 36 h and homogenized by grinding in an agate mortar.

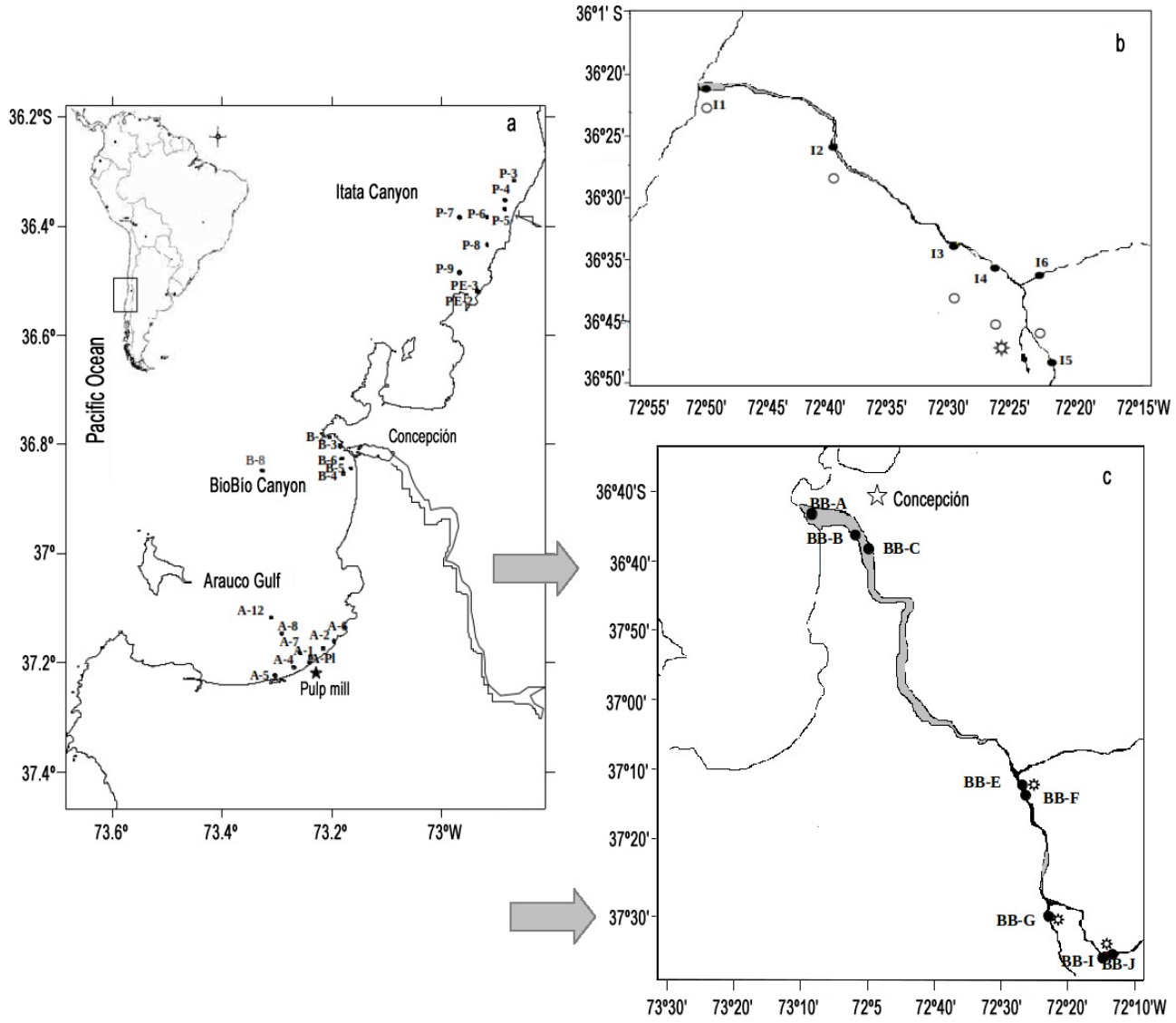

Figure 1 Sample stations location of a) coastal zones in south-central Chile, b) Itata River, and c) BioBío River, ✡pulp mill industries.

Particulate material more than 0.5 cm in size, *e.g.* fish bones, shells, worm-tubes and remains of worms were excluded from the sediments. Lipids were extracted according to Harvey (1994) and Lopez de Alda & Barceló (2001) with some modifications. Around 30 g of sediment was mixed with 30 mL dichloromethane: methanol (1:1) and sonicated for 10 min three times sequentially with fresh solvent to extract the lipids. The extracts were combined and evaporated to dryness under a stream of nitrogen gas, then re-dissolved into dichloromethane and purified according to Wakeham & Pease (1992). The extracts were filtered through a Pasteur pipette column filled with fiberglass and eluted with hexane and dichloromethane. Part of the hexane fraction (1 mL), was passed through an SPE aminopropyl cartridge. Three fractions were obtained: F1: eluted with hexane (for hydrocarbons); F2: eluted with hexane: dichloromethane (3:1) (for phthalates); F3: eluted with dichloromethane: acetate (9:1) (for alcohols, sterols and free fatty acids). The third fraction was concentrated and evaporated to dryness under a stream of nitrogen gas, treated with bis (trimethylsilyl) trifluoroacetamide (BSTFA) (Sigma Aldrich, Saint Louis, MO) heated at 70ºC for 30 min, and finally re-dissolved with dichloromethane. For determination of recovery, the internal standard utilized was 17β-estradiol (Sigma Aldrich).

The aim of this study was restricted to the concentration/distribution of sterols, however, some other components of the lipid fraction were distin-

guished in the chromatogram; these included some markers of terrestrial sources such as long chain fatty acids (>C_{22}), long chain alkanes (>C_{22}), long chain fatty alcohols and resin acids. The chromatogram results of these other components of the lipid fraction were only used to determine presence or absence of these compounds and not their concentrations.

GC-MS analysis

Analysis of the final extracts was performed by gas chromatography/mass spectrometry (GC/MS) using a Hewlett Packard 5890 GC coupled to an HP 5972 mass selective detector. The inlet was operated in the splitless mode with total and purge flows adjusted to 30 and 3 mL min^{-1}, respectively. The GC/MS was fitted with an HP-5 30 m x 0.25 mm (id) fused-silica capillary column (0.25 μm film), with the helium carrier gas flow rate through the column adjusted to 1 mL min^{-1}. A two-stage temperature program consisting of 50-120ºC at a rate of 10ºC min^{-1}, followed by a 3ºC min^{-1} rate to 275ºC was used in all separations. Sterol quantification was performed using tetracosane (Sigma Aldrich) as the internal injection standard. Structural identification of the compounds was determined by comparison of retention times with both internal and external standards (Sigma Aldrich) and mass spectral interpretation of the ion fragmentation (Smith *et al.,* 1982; Jones *et al.*, 1994). No attempt was made to distinguish between sterol epimers at C_{24}. Depending on the structure of the sterol, the detection limits ranged from 0.1 to 1.0 ng g^{-1}. Recovery of sterols was estimated to be 82%.

Sterol source

Distinguishing between vascular plant sterols and sterols derived from marine phytoplankton is a complex task because of the lack of resolution between the C-24 epimers of campesterol, β-sitosterol and stigmasterol with the analytical techniques used. However, indirect evidence may be obtained by correlation with other terrestrial source indicators. In this study, seven different approaches were used:

(1) Percentages of different sterols in each sediment sample, according to the number of carbons (C_{27}, C_{28} and C_{29}), differentiating marine from terrestrial sources (Huang & Meinschein, 1979).

(2) Three sterol source indices (SSI) were calculated to evaluate terrestrial organic matter input into the aquatic environment, using the ratio of stigmasterol/cholesterol, β-sitosterol/cholesterol and campesterol/cholesterol, with cholesterol as the assumed marine sterol (Mudge & Norris, 1997; Seguel *et al*., 2001; Fabbri *et al*., 2005; Ali *et al*., 2009).

(3) The ratios of the three principal terrestrial sterols campesterol, stigmasterol and β-sitosterol (Volkman, 1986), *i.e.*, 1:1.6:6.6, which is characteristic of sediments where most of the sterols are derived from higher plants (Volkman, 1986).

(4) The ratio between β-sitosterol and stigmasterol at each sampling station as a source proximity indicator (Laureillard & Saliot, 1993; Curiale & Harrison, 2007). This ratio increases with terrigenous organic content in the sediment.

(5) Use of other components present in the lipid fraction as indirect evidence of terrestrial influence such as long chain fatty acids (>C_{22}) that are utilized as indicators of land input (Volkman, 1986; Canuel *et al*., 1995; Zimmerman & Canuel, 2001).

(6) The presence of long chain alkanes (>C_{22}) and long chain fatty alcohols, considered terrestrial markers (Brassell *et al.*, 1980; Seguel *et al.*, 2001).

(7) Dehydroabietic acid (DHAA) was measured in all sediment samples as an indicator of the influence of pulp mill effluents (Volkman & Holdsworth, 1993; Johnsen *et al.*, 1995).

Statistical analysis

Associations between sampling stations and locations were explored using non-metric multidimensional scaling (n-MDS; Software PAST) based on the Bray-Curtis index using compound concentrations normalized to total organic carbon (Clarke, 1993a, 1993b). Data were standardized and transformed to the fourth root [√√(x+1)], before the Bray Curtis index calculation were done (Field *et al.*, 1982). Differences in compound compositions between five geographical zones (Itata River canyon, Gulf of Arauco, BioBío River canyon, Itata River and BioBío River) were tested using a one-way analysis of similarity (ANOSIM) (Clarke, 1993a, 1993b). To establish which compounds appear together, a Bray-Curtis cluster analysis was performed for both concentration data and presence-absence data. Pretreatment of the data was performed as described above.

One-way analyses of variance were performed to test significant differences between five geographical zones in the concentration of each of the following compounds: beta-sitosterol, cholesterol, brassicasterol and epicholestanol. A type III sum of square was used because the design is unbalanced. The assumptions of normality and homogeneity of variance were tested with the Shapiro-Wilks and Levene tests, respectively. When the assumptions of normality and homogeneity

of variance were violated the data was log transformed and if the problem remained a Kruskal-Wallis test was performed.

A Spearman correlation analysis was used to explore the relationships between environmental parameters and sterol concentration. In all cases, *P* values <0.05 were considered statistically significant. These analyses were performed with the statistical software Minitab 15.

RESULTS

Granulometry and sediment organic matter

Coastal sediments were sand dominated with different size distributions. Fine and very fine sand dominated BioBío Canyon and the coastal area adjacent to Itata Canyon, while in the Gulf of Arauco fine and medium sand sediments were found (Table 1). The rivers showed different sediment size distributions, with larger sizes in BioBío River sediments where coarse sand dominated almost all sample stations. The Itata River sediments were dominated by fine sands.

Organic matter ranged between 0.65 and 10.6% in coastal sediments, and between 0.7 and 21.5% in river sediments (Tables 1, 2). No relationship was found between organic matter and grain size.

Sterol concentration and differences among locations

Total sterol concentrations in marine sediments ranged from 0.03 to 10.4 μg g^{-1}, whereas in river sediments total sterol concentrations ranged from 0.04 to 4.12 μg g^{-1} (Table 3). The structures of the sterols identified ranged from C26-C30 with various levels of unsaturation and one steroid hormone (pregn-5-en-20-one) (Table 3). A total of 17 individual sterols were identified in marine sediments but only 10 were found in rivers (Fig. 2, Table 3), with a clear predominance of cholesterol (cholest-5-en-3β-ol) in marine stations (~60%, 0.06-6.73 μg g^{-1}) and lower content of this sterol in rivers (~23%, 0.02-0.60 μg g^{-1}) (Fig. 3, Table 3). In addition, a high correlation was found between cholesterol and total sterol concentration in all marine sediment samples (r^2 = 0.93, P < 0.001). The second most abundant sterol was β-sitosterol (24-ethylcholest-5-en-3β-ol), with concentrations ranging from 0.01 to 2.01 μg g^{-1} in marine sediments and from 0.01 to 2.33 μg g^{-1} in river sediments (Table 3). In the latter, there was a positive linear relation between β-sitosterol and total sterols (r^2 = 0.97, P < 0.001). Other sterols that appeared in lower concentrations were principally epicholestanol (5α-cholestan-3α-ol), brassicasterol (24-methylcholesta-5,22E-dien-3β-ol), cholesta-7,24-dien-3-ol and stigmasterol (24-ethylcholest-5,22-dien-3β-ol) (Figs. 2, 3, Table 3). Pregnenolone (pregn-5-en-20-one) was found at some stations in the BioBío River, Itata River, BioBío Canyon and Gulf of Arauco (Table 3). Coprostanol (5β-cholestan-3β-ol) was found at one station in BioBío Canyon (B4), one station in the BioBío River (BB-C) and two in the Itata River (I-1, I-2). Dinosterol (4,23,24-trimethylcholest-22E-en-3 β–ol) was only found at the more oceanic stations of the Gulf of Arauco (Fig. 2). Chalinasterol, fucosterol, and desmosterol were present only at some of the sampling stations and in very low concentrations (Figs. 2, 3, Table 3).

High variability was observed among the four studied marine zones in total sterol concentrations (CV = 124%) (Table 3). In terms of sterol composition, Coliumo Bay presented the lowest number of sterols of all marine sites sampled, whereas the Itata coastal zone had the highest number of sterols (Fig. 2). The low diversity of sterols in Coliumo Bay should be taken with caution since only two stations were sampled in this bay. To compare among sampling stations, disregarding the effect of the grain size, we normalized the concentration of sterols by the sediment organic carbon content (Jeng *et al.*, 2003). The ANOSIM analysis conducted on this data showed no differences among the four marine zones (Table 4), whereas significant differences in sterol composition and sterol presence/absence were observed between some coastal areas and the rivers, and also between the rivers (ANOSIM P < 0.05). These differences were also found in the quantity of cholesterol detected in the rivers; in the BioBío River cholesterol composed 27% of the total sterols, while in the Itata River this compound composed only the 11% of total sediment sterols. There were no significant differences between the five geographical zones comparing the median concentrations of individual sterols (β-sitosterol, cholesterol, brassicasterol and epicholestanol) using the Kruskal-Wallis test (β-sitosterol, H(4,34) = 2.493, P = 0.646; cholesterol, H(4,36) = 8.658, P = 0.070; brassicasterol, H(4,10) = 7.018, P = 0.135; epicolestanol, H(4,19) = 1.663, P = 0.798).

The highest β-sitosterol concentration was detected in Itata River sediment (I-2 = 2.33 μg g^{-1}) downstream from a pulp mill industry, although a station upstream from the pulp mill also showed high concentration of β-sitosterol (I-5 = 0.71 μg g^{-1}). Among the coastal stations, the highest concentration of β-sitosterol was found in station P-7 (2.07 μg g^{-1}) located 9.6 km west of the Itata River mouth. The greatest concentration of β-sitosterol normalized by organic carbon was found in station B-6 (20 μg gC_{org}^{-1}), off the mouth of the BioBío River.

Table 1. Marine study sites, bottom water characteristics and bulk sediment properties. TOC was estimated using the Van Bemmelen conversion factor of 1.72. TOM: total organic matter, TOC: total organic carbon, nm: not measured.

Collection date	Site	Abbreviation	Lat (S)	Long (W)	Depth (m)	Bottom temperature (ºC)	Bottom salinity	Bottom oxygen (mg L^{-1})	TOM (%)	TOC (%)	Grain size (ø)	Sediment type
03- Apr-2008	Coliumo Bay	PE-2	36°31'4.3"	72°56'8.4"					5.57	3.23	0	Very coarse sand
03- Apr-2008	Coliumo Bay	PE-3	36°31'8.7"	72°56'6.6"	11	12.00	33.50	5.50	0.65	0.40	1	Coarse sand
03- Apr-2008	Itata Canyon	P-3	36°19'0.4"	72°52'7.8"	61	11.16	34.37	0.93	10.19	5.92	3	Fine sand
03- Apr-2008	Itata Canyon	P-4	36°21'9.7"	72°53'4.2"	17	11.06	34.15	2.70	1.52	0.88	3	Fine sand
03- Apr-2008	Itata Canyon	P-5	36°22'7.7"	72°53'4.4"	16	11.16	34.17	2.68	1.14	0.66	3	Fine sand
03- Apr-2008	Itata Canyon	P-6	36°23'2"	72°55'4.5"	25	11.06	34.22	2.06	2.69	1.56	4	Very fine sand
03- Apr-2008	Itata Canyon	P-7	36°23'3.7"	72°58'3.2"	53	11.11	34.37	0.81	10.56	6.13	4	Very fine sand
03- Apr-2008	Itata Canyon	P-8	36°26'3"	72°55'2.8"	15	11.13	34.18	4.39	1.07	0.62	4	Very fine sand
03- Apr-2008	Itata Canyon	P-9	36°29'5.3"	72°58'30"	29	11.0	34.17	2.16	1.71	0.99	4	Very fine sand
13-Apr-2009	BioBio Canyon	B-2	36°47'55"	73°12'13"	26	11.52	34.49	2.42	2.40	1.40	4	Very fine sand
13-Apr-2009	BioBio Canyon	B-5	36°50'38"	73°09'55"	17	12.00	34.40	4.36	1.70	0.99	4	Very fine sand
13-Apr-2009	BioBio Canyon	B-6	36°49'32"	73°10'54"	164	10.26	34.53	0.25	2.07	1.20	3	Fine sand
13-Apr-2009	BioBio Canyon	B-3	36°48'57"	73°11'5.6"	18	12.54	34.37	5.49	1.67	0.97	3	Fine sand
13-Apr-2009	BioBio Canyon	B-8	36°50'52"	73°19'37"	173	10.77	34.57	0.02	nm	nm	nm	nm
13-Apr-2009	Gulf of Arauco	A-Pl	37°11'53"	73°14'28"	11	13.09	34.31	6.74	2.46	1.42	2	Medium sand
13-Apr-2009	Gulf of Arauco	A-1	37°11'23"	73°14'21"	15	13.85	34.25	8.05	2.61	1.51	2	Medium sand
13-Apr-2009	Gulf of Arauco	A-2	37°10'24"	73°12'55"	14	13.09	34.30	6.74	2.30	1.33	3	Fine sand
13-Apr-2009	Gulf of Arauco	A-4	37°12'26"	73°16'10"	15	12.74	34.35	4.05	2.6	1.51	2	Medium sand
13-Apr-2009	Gulf of Arauco	A-5	37°13'21"	73°18'12"	17	12.65	34.37	5.44	2.62	1.52	3	Fine sand
13-Apr-2009	Gulf of Arauco	A-6	37°09'34"	73°11'46"	13	11.94	34.47	2.62	2.26	1.31	2	Medium sand
13-Apr-2009	Gulf of Arauco	A-7	37°10'52"	73°15'31"	16	13.01	34.29	4.10	1.99	1.15	3	Fine sand
13-Apr-2009	Gulf of Arauco	A-8	37°08'46"	73°17'30"	33	11.88	34.47	0.54	6.47	3.75	3	Fine sand
13-Apr-2009	Gulf of Arauco	A-12	37°07'0.12"	73°18'35"	39	11.04	34.51	0.13	8.71	5.10	3	Fine sand

Table 2. River study sites, water characteristics and bulk sediment properties. TOC was estimated using a conversion factor of 2.5 (Nelson & Sommers, 1996). * Itata river data was obtained from Araneda *et al*. (2009) and Urrutia *et al*. (2009). TOM: total organic matter, TOC: total organic carbon, OD: dissolved oxygen, nm: not measured.

Collection date	Site	Abbre viation	Lat (S)	Long (W)	Depth (m)	DO*	Bottom temperature (ºC) *	Salinity	pH *	TOM (%)	TOC (%)	Grain size (ø)	Sediment type
07-May-2008	Itata River	I-1	36°23'27"	72°52'0.1"	<0.5	9.1	13.70	nm	7.88	nm	nm	nm	nm
07-May-2008	Itata River	I-2	36°27'9.3"	72°41'5.5"	<0.5	9.5	15.80	nm	8.42	nm	nm	nm	nm
07-May-2008	Itata River	I-3	36°35'7.1"	72°32'4.4"	<0.5	10.2	15.60	nm	8.44	1.63	0.65	3	Fine sand
07-May-2008	Itata River	I-4	36°37'5.2"	72°29'4.6"	<0.5	10.4	15.30	nm	8.20	1.13	0.45	3	Fine sand
07-May-2008	Itata River	I-5	36°45'5.4"	72°24'8.3"	<0.5	10.7	15.20	nm	8.05	21.51	8.60	3	Fine sand
07-May-2008	Itata River	I-6	36°38'3.3"	72°26'1.8"	<0.5	10.6	15.00	nm	8.73	0.68	0.27	-1	Very fine gravel
03-Dec- 2008	Biobío River	BB-A	36°48'25"	73°10'10"	nm	nm	nm	nm	nm	2.45	0.98	2	Medium sand
03-Dec- 2008	Biobío River	BB-B	36°50'36"	73°04'33"	31	7.98	22.22	0.04	7.21	1.00	0.40	1	Coarse sand
03-Dec- 2008	Biobío River	BB-C	36°50'49"	73°03'21"	nm	nm	nm	nm	nm	1.20	0.48	1	Coarse sand
03-Dec- 2008	Biobío River	BB-E	37°17'50"	72°42'59"	19	7.9	22.98	0.1	7.99	1.16	0.47	3	Fine sand
03-Dec- 2008	Biobío River	BB-F	37°18'1.7"	72°42'41"	35	9.8	25.26	0.02	7.78	0.72	0.29	1	Coarse sand
03-Dec- 2008	Biobío River	BB-G	37°30'33"	72°40'0.9"	15	7.8	20.95	0.06	7.08	6.47	2.59	1	Coarse sand
03-Dec- 2008	Biobío River	BB-I	37°34'59"	72°32'29"	13	10.2	19.49	0.02	7.06	6.40	2.56	1	Coarse sand
03-Dec- 2008	Biobío River	BB-J	37°34'49"	72°31'13"	21	10.5	21.35	0.02	7.22	nm	nm	nm	nm

Table 3. Concentrations (μg g^{-1}) of sterols in sediments of Coliumo Bay (PE), coastal shelf adjacent to the Itata River mouth (P), BioBío Canyon (B), Gulf of Arauco (A), BioBío River (BB) and Itata River (I) of central-southern Chile. nd: not detected.

	PE-2	PE-3	P-3	P-4	P-5	P-6	P-7	P-8	P-9	B-2	B-3	B-4	B-5	B-6	B-8
A. Beta sitosterol	0.925	0.264	0.337	0.180	0.021	nd	2.069	0.012	0.187	0.222	0.055	0.244	0.102	1.450	0.043
B. Stigmasterol	nd	nd	nd	nd	nd	nd	0.457	nd	nd	0.017	0.005	nd	0.019	0.154	nd
P. Fucosterol	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.066
Total C29 sterols	0.925	0.264	0.337	0.180	0.021	0.000	2.526	0.012	0.187	0.239	0.060	0.244	0.121	1.604	0.108
% C29 sterols	58.5	3.0	7.5	2.3	12.1	0.0	33.3	2.6	17.9	26.8	16.6	7.8	14.1	23.2	7.9
F. Cholesterol	0.655	6.732	2.785	6.158	0.143	0.624	4.071	0.373	0.686	0.380	0.226	2.649	0.489	2.540	0.434
D. Epicholestanol	nd	0.190	0.278	0.127	0.010	nd	0.900	0.023	nd	0.065	0.026	nd	0.064	0.494	0.064
G. Cholesta-7,24-dien-3-ol	nd	0.780	0.338	0.597	nd	nd	nd	0.025	0.048	0.031	nd	nd	0.032	nd	nd
I. Cholesta-4,6-dien-3-ol	nd	nd	nd	nd	nd	0.328	nd	nd	nd	nd	nd	nd	nd	nd	nd
S. Desmosterol	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.111	nd		0.027	nd	nd
T. Coprostanol	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.136	nd	nd	nd
Q. Gorgostenol	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Total C27 sterols	0.655	7.702	3.400	6.882	0.152	0.952	5.061	0.420	0.733	0.587	0.253	2.785	0.612	3.034	0.498
% C27 sterols	41.5	94.7	76.0	89.4	87.9	91.6	66.7	92.0	70.0	65.9	69.6	89.5	71.3	42.1	92.1
E. Campesterol	nd	nd	nd	nd	nd	nd	nd	0.024	nd	nd	0.018	nd	nd	nd	nd
C. Brassicasterol	nd	nd	0.737	0.554	nd	nd	nd	nd	0.042	0.045	nd	nd	nd	0.440	nd
H. Zymosterone	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.032	0.083	0.084	nd	nd
J. Dehydrocholesterol	nd	nd	nd	nd	nd	0.087	nd	nd	nd	nd	nd	nd	nd	nd	nd
N. Chalinasterol	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Total C28 sterols	nd	nd	0.737	0.554	nd	0.087	nd	0.024	0.042	0.045	0.050	0.083	0.084	0.440	nd
% C28 sterols	nd	nd	16.468	7.191	nd	8.402	nd	5.359	3.969	5.033	13.751	2.654	9.819	6.109	nd
M. 24-norcholesterol	nd	0.114	nd	0.083	nd	nd	nd	nd	0.086	nd	nd	nd	nd	nd	nd
% C26 sterols	nd	1.309	nd	1.076	nd	nd	nd	nd	8.187	nd	nd	nd	nd	nd	nd
O. Germanicol	nd	nd	nd	nd	nd	nd	0.187	nd	nd	nd	nd	nd	nd	nd	nd
R. Dinosterol	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.020	nd	nd	0.011	0.209	nd
Total C30 sterols	nd	nd	nd	nd	nd	nd	0.187	nd	nd	0.020	nd	nd	0.011	0.209	nd
% C30 sterols	nd	nd	nd	nd	nd	nd	2.4	nd	nd	2.2	nd	nd	1.2	2.9	nd
L. Pregenolone	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.030	1.849	0.018
% C21 sterols	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	3.53	25.68	nd
Total sterols	1.6	8.1	4.5	7.7	0.2	1.0	7.8	0.5	1.0	0.9	0.4	3.1	0.9	7.1	0.6

Continuation

	A-Pl	A-1	A-2	A-4	A-5	A-6	A-7	A-8	A-12	BB-A	BB-B	BB-C	BB-E	BB-F	BB-G
A. beta sitosterol	0.119	0.056	0.229	0.749	0.033	0.045	0.003	0.061	0.389	0.165	0.010	0.293	0.267	0.215	0.272
B. Stigmasterol	nd	nd	0.040	nd	nd	nd	nd	0.026	nd	0.028	nd	0.014	nd	0.058	0.014
P. Fucosterol	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Total C29 sterols	0.119	0.056	0.269	0.749	0.033	0.045	0.003	0.087	0.389	0.192	0.010	0.306	0.267	0.273	0.286
% C29 sterols	9.5	22.7	11.1	7.2	12.4	13.9	7.9	22.6	33.9	25.1	26.0	43.6	31.8	28.2	74.7
F. Cholesterol	0.830	0.130	1.643	7.728	0.185	0.211	0.025	0.108	0.056	0.393	0.028	0.220	0.157	0.532	0.070
D. Epicholestanol	nd	nd	nd	nd	nd	0.036	0.006	0.093	0.122	0.145	nd	0.108	0.414	nd	0.027
G. Cholesta-7,24-dien-3-ol	nd	nd	0.116	0.568	nd	0.018	nd	0.036	nd	nd	nd	nd	nd	nd	nd
I Cholesta-4,6-dien-3-ol	nd	0.012	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S. Desmosterol	nd	nd	0.132	0.268	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
T. Coprostanol	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.020	nd	nd	nd
Q. Gorgostenol	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Total C27 sterols	0.830	0.142	1.891	8.563	0.185	0.266	0.031	0.237	0.178	0.538	0.028	0.347	0.572	0.532	0.097
% C27 sterols	66.2	57.0	78.2	82.2	70.4	82.0	92.1	61.6	15.5	70.1	74.0	46.5	68.2	55.0	25.3
E. Campesterol	0.305	0.042	nd	nd	0.045	0.013	nd	0.022	nd	nd	nd	0.049	nd	nd	nd
C. Brassicasterol	nd	0.019	0.065	nd	nd	nd	nd	0.006	nd	nd	nd	nd	nd	nd	nd
H. Zymosterone	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
J. Dehydrocholesterol	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
N. Chalinasterol	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.037	nd	nd	nd	nd	nd
Total C28 sterols	0.305	0.062	0.065	nd	0.045	0.013	nd	0.028	nd	0.037	nd	0.049	nd	nd	nd
% C28 sterols	24.310	24.817	2.678	nd	17.152	4.1	nd	7.3	nd	4.8	nd	7.0	nd	nd	nd
M. 24-norcholesterol	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
% C26 sterols	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
O. Germanicol	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
R. Dinosterol	nd	nd	nd	0.040	nd	nd	nd	0.033	0.581	nd	nd	nd	nd	nd	nd
Total C30 sterols	nd	nd	nd	0.040	nd	nd	nd	0.033	0.581	nd	nd	nd	nd	nd	nd
% C30 sterols	nd	nd	nd	0.4	nd	nd	nd	8.5	50.6	nd	nd	nd	nd	nd	nd
L. Pregenolone	nd	nd	0.181	1.064	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.163	nd
% C21 sterols	nd	nd	7.47	10.22	nd	nd	nd	nd	nd	nd	nd	nd	nd	16.8	nd
Total sterols	1.25	0.26	2.41	10.42	0.26	0.32	0.03	0.38	1.15	0.77	0.04	0.70	0.84	0.97	0.38

Continuation

	BB-I	BB-J	I-1	I-2	I-3	I-4	I-5	I-6
A. beta sitosterol	0.101	0.112	nd	2.333	0.319	0.107	0.709	0.097
B. Stigmasterol	nd	0.027	nd	1.292	nd	0.075	0.128	nd
P. Fucosterol	Nd	nd	nd	nd	nd	nd	nd	nd
Total C29 sterols	0.101	0.139	nd	3.625	0.319	0.182	0.837	0.097
% C29 sterols	28.6	19.5	nd	85.7	36.4	38.7	58.0	37.9
F. Cholesterol	0.213	0.473	0.598	0.302	0.317	0.187	0.158	0.120
D. Epicholestanol	nd	nd	nd	nd	0.102	nd	nd	nd
G. Cholesta-7,24-dien-3-ol	nd	0.229	0.209	0.102	nd	0.413	nd	nd
I Cholesta-4,6-dien-3-ol	nd	nd	nd	nd	nd	nd	nd	nd
S. Desmosterol	nd	nd	nd	nd	nd	nd	nd	nd
T. Coprostanol	nd	nd	0.212	0.058	nd	nd	nd	nd
Q. Gorgostenol	nd	nd	nd	nd	nd	nd	nd	0.089
Total C27 sterols	0.213	0.703	1.019	0.462	0.419	0.600	0.158	0.209
% C27 sterols	60.4	66.1	100.0	13.4	47.7	61.3	39.6	42.9
E. Campesterol	0.039	0.035	nd	nd	nd	nd	nd	nd
C. Brassicasterol	nd	0.068	nd	nd	0.140	nd	nd	0.114
H. Zymosterone	nd	nd	nd	nd	nd	nd	nd	nd
J Dehydrocholesterol	nd	nd	nd	nd	nd	nd	nd	nd
N Chalinasterol	nd	nd	nd	nd	nd	nd	nd	nd
Total C28 sterols	0.039	0.103	nd	nd	0.140	nd	nd	0.114
% C28 sterols	11.0	14.4	nd	nd	16.0	nd	nd	43.5
M. 24-norcholesterol	nd	nd	nd	nd	nd	nd	nd	Nd
% C26 sterols	nd	nd	nd	nd	nd	nd	nd	Nd
O. Germanicol	nd	nd	nd	nd	nd	nd	0.036	Nd
R. Dinosterol	nd	nd	nd	nd	nd	nd	nd	Nd
Total C30 sterols	nd	nd	nd	nd	nd	nd	nd	Nd
% C30 sterols	nd	nd	nd	nd	nd	nd	2.5	Nd
L. Pregenolone	nd	nd	nd	0.036	nd	nd	nd	Nd
% C21 sterols	nd	nd	nd	0.862	nd	nd	nd	nd
Total sterols	0.353	0.945	1.019	4.123	0.879	0.783	1.031	0.419

A) 24-Ethylcholest-5-en-3β-ol, B) 24-Ethylcholest-5.22-dien-3β-ol, C) 24-Methylcholesta-5,22E-dien-3β-ol, D) 5α-cholestan-3α-ol, E) 24-Methylcholest-5-en-3β-ol, F) Cholest-5-en-3β-ol, G) Cholesta-7.24-dien-3-ol, H) Cholesta-8.24-dien-3-ol, I) Cholesta-4,6-dien-3-ol, J) Cholesta-5.22-dien-3-ol, L) pregn-5-en-20-one, M) 24-norcholesta-5.22E-dien-3β-ol, N) 24-Methylcholesta-5.24(28)-dien-3β-ol, P) 24-Ethylidencholest-5-ene-3β-ol, R) 4,23,24-trimethylcholest-22E-en-3β–ol, S) Cholesta-5,24-dien-3-ol, T) 5β-Cholestan-3β-ol.

Relationship of sterol concentration to distance from terrestrial influx and sediment organic matter content

No relationship was found between sediment organic matter and total sterol concentration (μg g^{-1}) in all sampling stations. However, when each sterol was related to the organic matter content a positive relationship was found between epicholestanol and organic matter in Gulf of Arauco sediments ($r^2 = 0.95$, $P = 0.021$), whereas no relationship was found for the other sampling zones ($P > 0.05$). Considering the environmental parameters, temperature, salinity and oxygen concentration, only epicholestanol showed a negative correlation with oxygen taking into account all sample stations (Table 5). In the Gulf of Arauco and in the coastal shelf adjacent to the Itata River mouth, a negative correlation was also observed between oxygen and the variables percent organic matter, depth and seaward distance ($P < 0.05$). Only epicholestanol showed positive patterns with offshore distance from the Carampange River and pulp mill effluent in the Gulf of Arauco ($r^2 = 0.92$, $P = 0.04$).

Terrestrial influence on sediment organic matter

Table 6 presents a summary of biomarker indicators of terrestrial influence. Some coastal stations had an important proportion of C_{29} sterols and a high β-sitosterol/cholesterol ratio (Table 6, Fig. 4a), such as B2, B-6, A-1, A-8, A-12, P-7 and PE-2, reflecting terrestrial sources. According to the SSI, stations PE-2, P-7, A-12, B-2 and B-6 had β-sitosterol as the most abundant phytosterol (Fig. 5a), whereas other stations

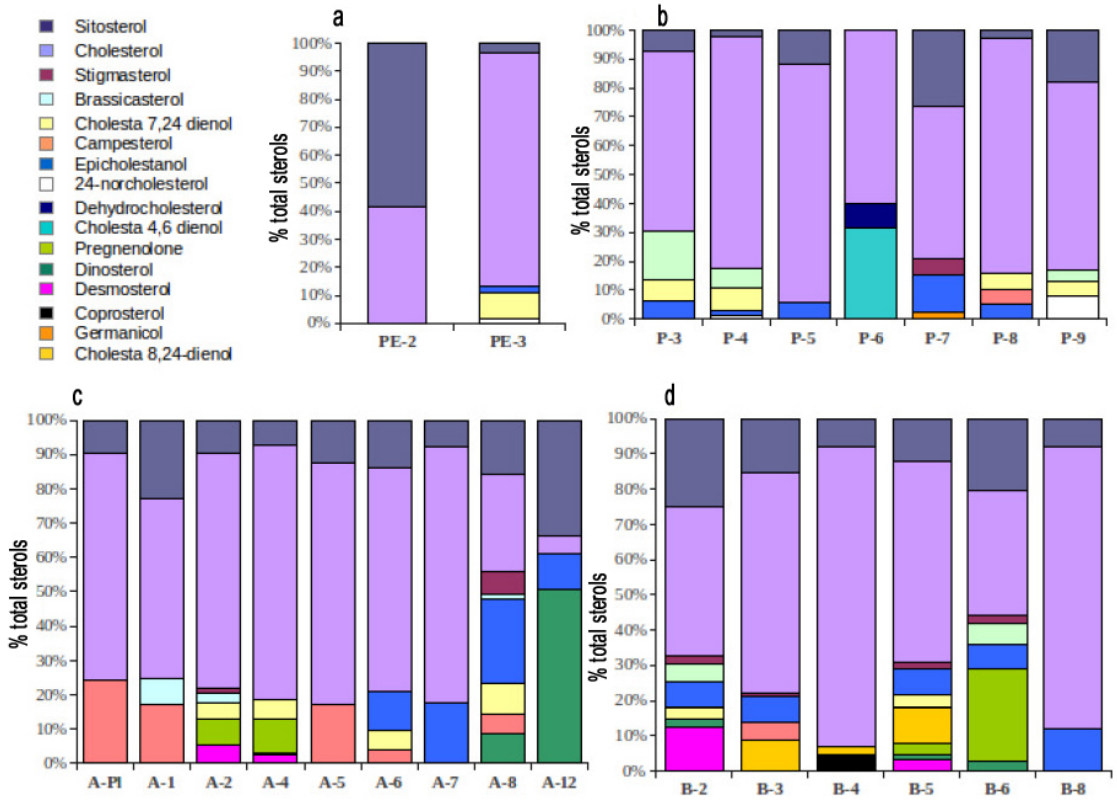

Figure 2. Percentages of sterols present at different marine sediment sampling stations. a) Coliumo Bay, b) coastal shelf adjacent to the Itata River mouth, c) Gulf of Arauco, and d) BioBío Canyon.

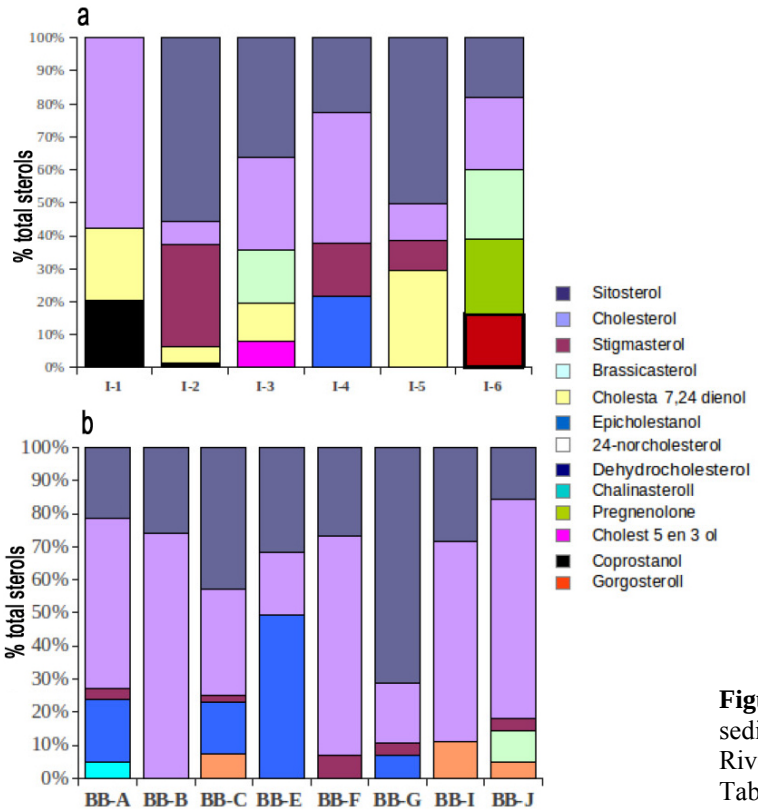

Figure 3. Percent sterols present at different river sediment sample stations. a) BioBío River, b) Itata River. Total concentration of sterols may be found in Table 2.

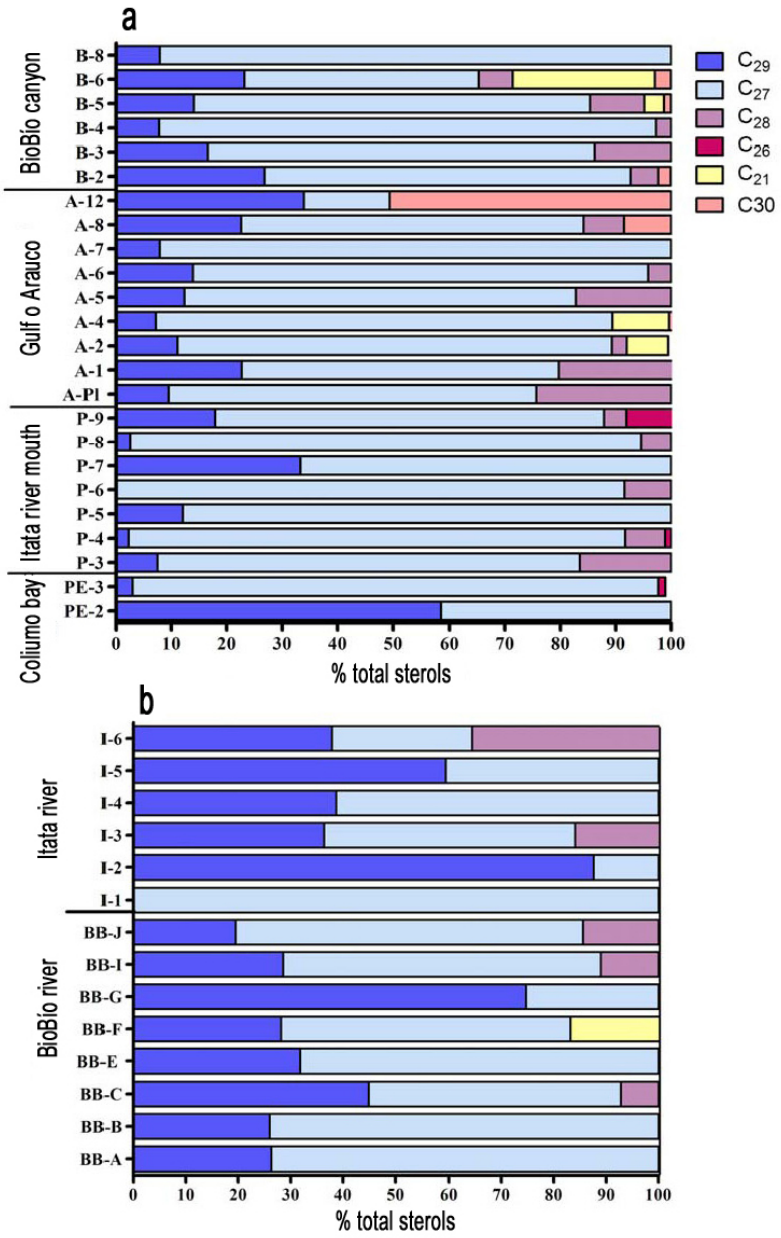

Figure 4. Sterol percentage classified according to carbon number, for a) coastal zones, b) rivers.

had the presence of the three vascular plant biomarkers (two in the Gulf of Arauco, A-1, A-8) (Fig. 5a). In almost all river stations there was a relevant presence of β-sitosterol (Fig. 5b), and therefore a high β-sitosterol/cholesterol ratio, indi-cating a terrestrial organic matter source. One BioBío River sample station (BB-J) showed a low β-sitosterol/cholesterol ratio (0.2), because of the relevant percentage of C_{28} sterols. In this study it was not possible to calculate the ratio proposed by Volkman (1986) between the principal vascular plant sterols, because of the absence of campesterol (24-methylcholest-5-en-3β-ol) in almost all sampling stations except for the Gulf of Arauco. However, a ratio between sitosterol and stigmasterol was calculated for all the stations that had both sterols, and a negative linear regression was obtained between this ratio and seaward distance (Fig. 6), indicating that at greater distance from the mouth of the river there is a higher concentration of stigmasterol, while sitosterol is more important closer to the coast.

No long chain fatty acids were found in the Itata River, but they were present in three sampling stations of the BioBío River (Fig. 7). The only fatty alcohol found was octacosanol (C_{28}), which is a compound classified as a terrestrial marker (Volkman, 1986; Bayona *et al.*, 1989; Jeng *et al.*, 2003). Octacosanol was found mainly in the BioBío River, BioBío

Table 4. ANOSIM results for differences in sterol concentration and diversity between all sample areas. For this analysis, data were normalized by organic carbon content in the sediment. Significant differences are in bold ($P < 0.05$).

	Itata shelf	Gulf of Arauco	BioBío canyon	Itata river	BioBío river
Coliumo Bay	R^2= -0.13 P = 0.636	R^2=-0.04 P = 0.543	R^2= 0.33 P = 0.19	R^2= -0.07 P = 0.658	R^2= 0.39 P = 0.182
Itata shelf		R^2= 0.05 P = 0.260	R^2= 0.18 P = 0.09	R^2=0.27 P = 0.055	R^2= 0.34 P = **0.014**
Gulf of Arauco			R^2= 0.07 P = 0.263	R^2= 0.28 P = **0.044**	R^2=0.12 P = 0.133
BioBío Canyon				R^2=0.36 P = **0.033**	R^2=0.36 P = **0.009**
Itata River					R^2= 0.353 P = **0.044**

Canyon and the continental shelf adjacent to the Itata River mouth. Only three long chain alkanes were detected (tetracontane, tetracosane, heptacosane) which were found in some marine and river sampling stations without revealing a spatial pattern, but showing some consistency with the presence of other terrestrial biomarkers (data not shown). Based on the number of terrestrial markers (Table 6) present in each sampling station, the BioBío Canyon is more subject to terrestrial influence than the other coastal areas, with the exception of one station situated in the coastal shelf adjacent to the Itata River mouth (P-7). The BioBío River sediments showed more terrestrial markers than those of the Itata River. Dinosterol was only found in three coastal stations localized in the Gulf of Arauco (A-4, A-8 y A-12).

Dehydroabietic acid (DHAA) was the main resin acid detected in the study zone (Table 6). DHAA was found in one station in the Gulf of Arauco (Table 6) and the highest presence of DHAA was found in the BioBío River canyon. On the coastal shelf adjacent to the Itata River mouth DHAA was present only in station P-7, which is a station located relatively far from shore, and which had the highest presence of terrestrial markers. The BioBío River also showed high abundance of resin acids at a station near Concepción, the largest city in south-central Chile.

The MDS (Fig. 8) segregated all compounds present in marine and river sediments according to their main source. The phytosterols were located between terrestrial and marine groups (Fig. 8), especially sitosterol and stigmasterol, showing the uncertain source of origin of both compounds, which may be of terrestrial origin in some areas and marine origin in others. The ANOSIM analysis showed significant differences between the terrestrial and marine groups ($P < 0.05$, Table 4).

Table 5. Correlation between environmental parameters and sterol concentration. The table only shows the significant correlations ($P < 0.05$).

	Valid	Spearman	t(N-2)	*P*-level
Depth & beta-sitosterol	23	0.456	2.347	0.028
Depth & epicholestanol	16	0.587	2.710	0.017
Oxygen & epicholestanol	16	-0.570	-2.600	0.021

DISCUSSION

Relative abundance of sterols in marine and river sediments in south-central Chile

In the study zone, considering total sterol concentration, marine sediments contained about 13% β-sitosterol and 60% cholesterol, while river sediments in general showed a higher percentage of β-sitosterol (28%) and lower cholesterol (22%). Clear differences were detected between the rivers, with the sediments of the BioBío River having more cholesterol and less β-sitosterol than the Itata River, which is probably due to the higher discharge of sewage effluents in the BioBío River coming from large cities situated in its basin (Bertin *et al.*, 2009). Generally, rivers have higher concentrations of β-sitosterol and lower cholesterol (Huang & Meinschein, 1976, 1979), as seen in the Itata River.

Regarding the marine environment, our results are consistent with most studies that demonstrate high concentrations of cholesterol in marine sediments and their increase with seaward distance (Huang &

Table 6. Summary of terrestrial influence biomarkers for marine and river sampling stations. The possible source of the phytosterols is assessed taking into account the percentage of C_{29} sterols as well as the sitosterol/cholesterol ratio (Canuel & Zimmerman, 1999). The criteria for classifying the source of organic matter as terrestrial was <25% C_{29} and a sitosterol/cholesterol ratio less than 0.5. Number of Terrestrial Biomarkers (NºTM) corresponds to the sum of LCFA, long chain alkanes and long chain alcohols. Disnosterol was used as a marine source biomarker. DHAA: Dehydroabietic acid, nd: not detected.

Sample	C27 (%)	C28 (%)	C29 (%)	Sitosterol/ cholesterol	Source	Dinosterol (%)	Nº of TM	Pulp mill markers DHAA
Coastal samples								
PE-2	41	0	59	1.41	Terrestrial	nd	nd	nd
PE-3	95	0	3	0.04	Marine	nd	nd	nd
P-3	76	17	8	0.12	Marine	nd	nd	nd
P-4	89	7	2	0.03	Marine	nd	nd	nd
P-5	88	0	12	0.15	Marine	nd	2	nd
P-6	92	8	0	0	Marine	nd	nd	nd
P-7	67	0	33	0.51	Terrestrial	nd	9	**x**
P-8	92	5	3	0.03	Marine	nd	2	nd
P-9	70	0	18	0.3	Marine	nd	nd	nd
A-Pl	66	24	10	0.14	Marine	nd	nd	nd
A-1	49	24	23	0.5	Terrestrial	nd	1	**x**
A-2	84	3	13	0.14	Marine	nd	1	nd
A-4	92	0	8	0.1	Marine	0.4	1	nd
A-5	70	17	12	0.18	Marine	nd	1	nd
A-6	82	4	14	0.21	Marine	nd	nd	nd
A-7	92	0	8	0.11	Marine	nd	1	nd
A-8	67	8	25	0.6	Terrestrial	0.03	2	nd
A-12	31	0	69	6.95	Terrestrial	0.6	2	nd
B-2	63	6	32	0.58	Terrestrial	nd	2	**x**
B-3	70	14	17	0.25	Marine	nd	3	**x**
B-4	89	3	8	0.09	Marine	nd	3	**x**
B-5	74	11	15	0.21	Marine	nd	5	**x**
B-6	60	9	32	0.57	Terrestrial	nd	6	**x**
B-8	92	0	8	0.1	Marine	nd	3	nd
River samples								
BB-A	74	0	26	0.35	Terrestrial	nd	2	nd
BB-B	74	0	26	0.35	Terrestrial	nd	2	nd
BB-C	48	7	45	0.94	Terrestrial	nd	2	**x**
BB-E	68	0	32	0.47	Terrestrial	nd	2	**x**
BB-F	66	0	34	0.52	Terrestrial	nd	3	nd
BB-G	25	0	75	3	Terrestrial	nd	6	**x**
BB-I	60	11	29	0.44	Terrestrial	nd	2	nd
BB-J	66	14	19	0.29		nd	1	nd
I-1	100	0	0	-	Marine	nd	1	**x**
I-2	12	0	88	7.3	Terrestrial	nd	1	nd
I-3	48	16	36	0.75	Terrestrial	nd	1	nd
I-4	61	0	39	0.64	Terrestrial	nd	1	**x**
I-5	41	0	59	1.44	Terrestrial	nd	3	nd
I-6	27	44	38	1.4	Terrestrial	nd	1	nd

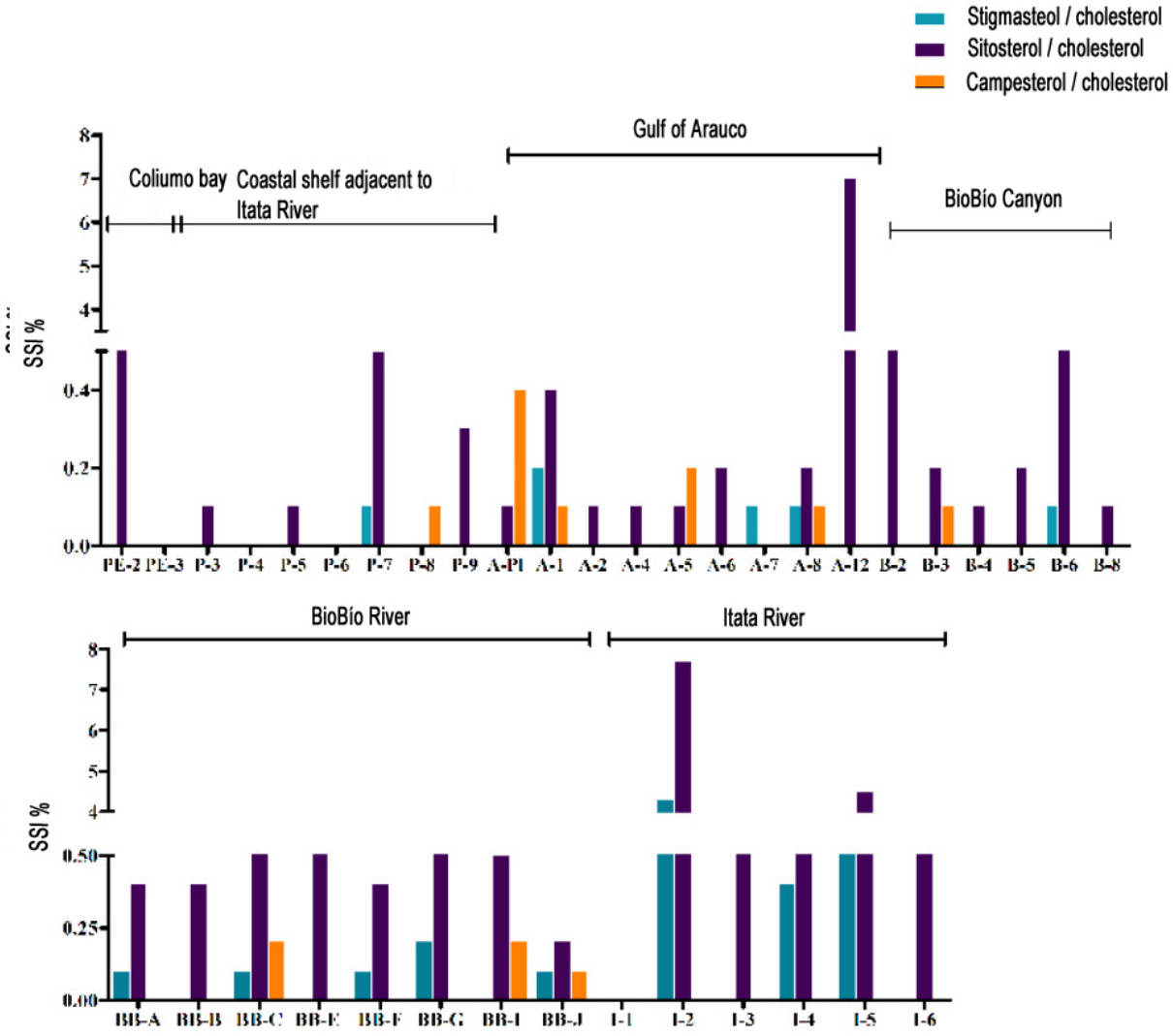

Figure 5. Sterol source index (SSI) for a) marine, and b) river sediments of south-central Chile.

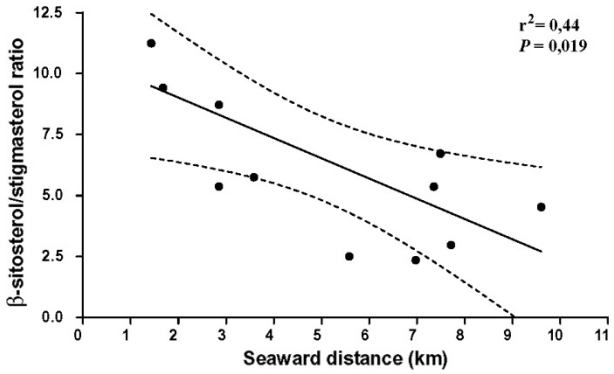

Figure 6. β-sitosterol/stigmasterol ratio versus seaward distance.

Meinschein, 1979; Lee & Wakeham, 1988). This cholesterol is likely to be originated mainly from zooplankton lipids, with a smaller contribution from phytoplankton lipids (Gagosian & Nigrelli, 1979; Gagosian *et al.*, 1983).

Total sterol concentrations obtained in the present study (0.03-10.4 μg g^{-1}) were compared with available data from around the world (Table 1, supplementary material). The concentrations found in the marine environment off Chile are within the range reported for offshore Australia and China by Jeng *et al.* (2003) and Jeng & Huh (2004). The concentrations observed off south-central Chile for stigmasterol, campesterol, β-sitosterol and cholesterol are similar to those reported by Volkman (1986) and Jeng & Huh (2004). However, for the upwelling region off Peru, Volkman *et al.* (1987) reported a β-sitosterol concentration near 12 μg g^{-1} for the first 0-2 cm of sediments, a value almost 5 times greater than the maximum concentration found off south-central Chile. According to

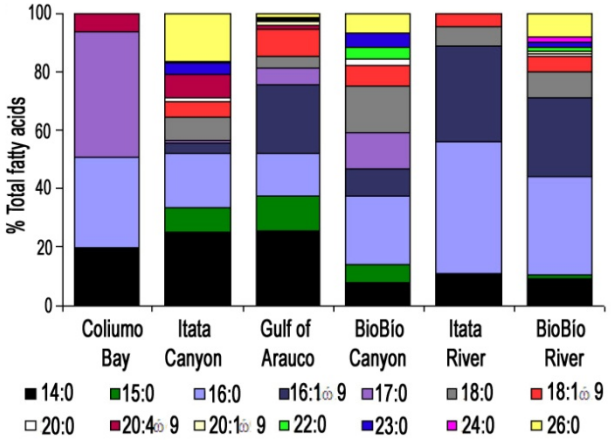

Figure 7. Percent of different fatty acids found in coastal zones and rivers of south-central Chile.

Volkman *et al.* (1987), only in one of the stations (the most coastal) the source of β-sitosterol was terrestrial, where the possible explanation could be the presence of a band of terrigenous mud that extended along the upper continental margin off Peru. However, the other stations sampled by Volkman *et al.* (1987) off Peru also showed high β-sitosterol concentrations that came from phytoplankton. A possible explanation for the difference in phytosterol concentration found off south-central Chile in comparison with the sediments off Peru is sediment granulometry, because in the latter case there were mostly muddy sediments, whereas off south-central Chile there was fine sand (Volkman *et al.,* 1987). It is known that finer sediments lead to greater retention of sterols (Reeves & Patton, 2005).

The highest β-sitosterol concentration was located in the sediments of the P-7 sample station (near the Itata River mouth) using the non-normalized data, while it was highest in B-6 sediments (at the BioBío River mouth) when data were normalized to organic carbon. This means that the high concentration of sterols detected in station P-7 was due to the higher organic content of sediments, and therefore this may be an accumulation zone, while station B-6 had a greater contribution of sterols, possibly coming from the BioBío River.

The lack of relationship between organic matter and sterol concentration is not coherent with other studies that found a positive relationship between them (Jeng *et al.*, 2003). This may be explained by the physical dynamics and geographical variation of this coastal zone, as well as by sediment bioturbation and the different degradation rates of the organic matter and specific biomarkers (Jeng *et al.*, 2003). This is also confirmed by the absence of a positive relationship between organic matter and sediment grain size, meaning that at the moment of sampling some stations had high organic matter content in relatively coarse sediment, as was the case in Coliumo Bay.

The distribution of some sterols and the phytosterol ratio (sitosterol/ stigmasterol) in the coastal zone were found to correlate with distance from the shore, especially in terms of distance from the river mouth (Fig.6). The case of epicholestanol is noteworthy, since it showed a positive relationship with distance from the coast and a negative relationship with oxygen content in the Gulf of Arauco and in the coastal shelf adjacent to the Itata River. Higher concentrations of epicholestanol were found in areas under low oxygen conditions, which are consistent with the fact that this sterol is derived from cholesterol only under special conditions, such as those prevailing in anoxic sediments rich in organic matter (Cordeiro *et al.*, 2008). Therefore the presence of epicholestanol, which made up almost 10% of total sterols, in coastal marine sediments off south-central Chile may indicate significant bacterial degradation of cholesterol in the hypoxic sediments (Cordeiro *et al.*, 2008) produced by the presence of the OMZ characteristics of the Humboldt Current System (Quiñones *et al.*, 2010).

Epicholestanol was found at almost all BioBío River sampling stations, whereas in the Itata river it was only found at one station (Table 2). The higher presence of epicholestanol in the sediments of the BioBío River is likely to be related to the higher influence of sewage from large cities, because epicholestanol is also considered to be a fecal marker (Cordeiro *et al.*, 2008).

Sources of phytosterols

The most relevant phytosterol found in the present study was β-sitosterol, which had higher relative concentration in river than in marine sediments. However, it is important to notice the high concentrations of β-sitosterol in sediments from oceanic stations (*e.g.*, A-12, Gulf of Arauco), which supports the notion that this sterol can also be of marine origin, and therefore cannot be utilized alone as a marker of terrestrial origin (Volkman, 1986). On the other hand brassicasterol, usually considered as a typical marine marker, was found in some river stations located quite far from the coast, which is consistent with reports that this sterol is also produced by freshwater phytoplankton (Fahl *et al.*, 2003).

The difficulty of using phytosterols alone as terrestrial markers was demonstrated by the high variability in their distribution and the lack of significant differences between the coastal areas

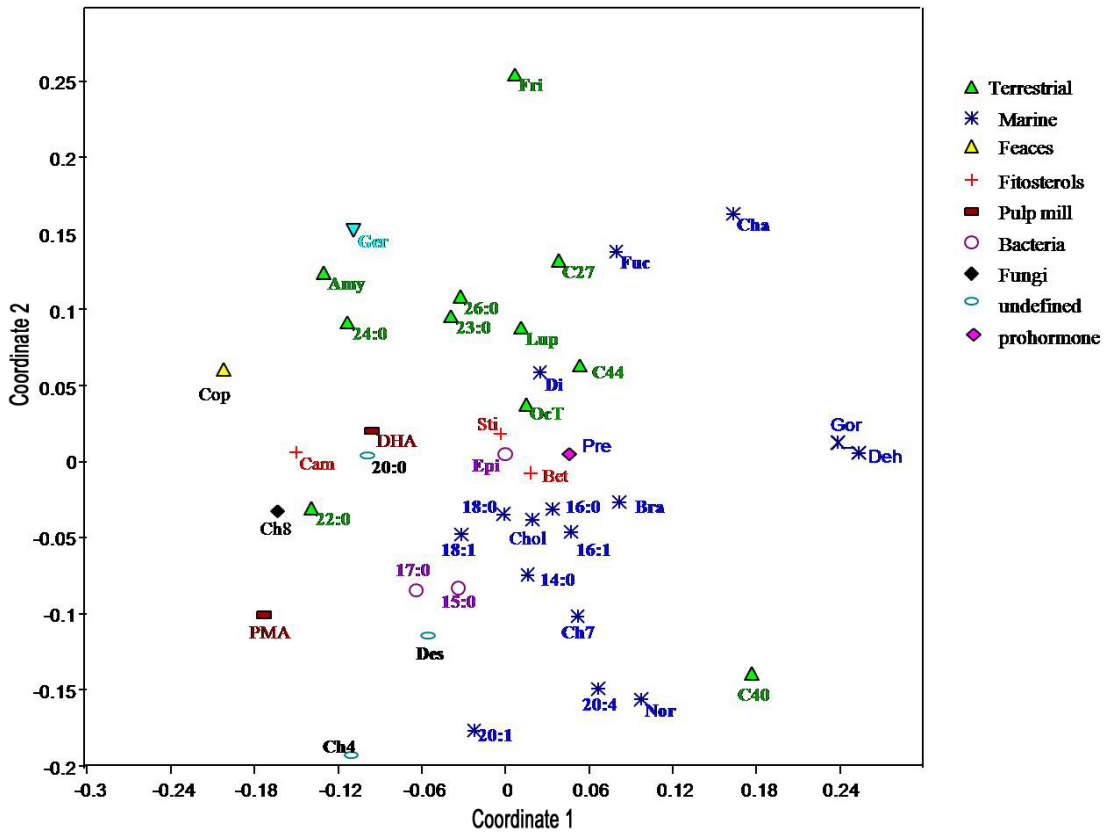

Figure 8. MDS for all lipid compounds present in marine and river sediments. Symbols represent the main source of origin of each compound (based on literature). Fatty acids: 14:0-26:0; Sterols: Bet: β-sitosterol, Sti: stigmasterol, Bra: brassicasterol, Epi: epicholestanol, Cam: campesterol, Chol: cholesterol, Ch7: Cholesta-7.24-dien-3-ol, Ch8: Zymosterone, Ch4: Cholesta-4.6-dien-3-ol, Deh: dehydrocholesterol, Pre: pregnenolone, Nor: 24-norcholesterol, Cha: chalinasterol, Fuc: fucosterol, Ger: germanicol, Gor: gorgostenol; Fatty alcohols: OcT: octacosanol; Alkanes : C_{44}: tetrateracontane; C_{40}: Tetracontane, C_{27}: Heptacosane; Triterpenes: Lup: lupanol, Amy: β-amyrine; Resin acids: DHA: dehydroabietic acid, PMA: pimaric acid.

(ANOSIM analysis) using only sterol concentration. However, it was possible to detect differences between most coastal zones and both rivers, which is possibly due to higher levels of phytosterols and different kinds of sterols present in river sediments.

Considering all the biomarkers of organic matter source, a clear difference was observed between the organic matter in marine and river sediments, with more sitosterol and terrestrial markers in river sediments and a transition zone at the river mouth, which is consistent with studies from other ecosystems (Huang & Meinschein, 1979; Li *et al.*, 1995; Hu *et al.*, 2009). Our results are consistent with the argument that it is more appropriate to use simultaneously different biomarkers to assess the origin of sediment organic matter (Huang & Meinschein, 1979; Volkman, 1986; Canuel *et al.*, 1995; Mudge & Norris, 1997; Seguel *et al.*, 2001; Zimmerman & Canuel, 2001; Curiale & Harrison, 2007; Ali *et al.*, 2009). Using these biomarkers, we found that some coastal stations with a high number of terrestrial markers (long chain fatty acids, long chain alkanes, long chain alcohols) also showed a high proportion of β-sitosterol (B-2, B-6 and P-7), suggesting that the source of this β-sitosterol is mainly terrestrial vascular plants. The high percentage of β-sitosterol present in the more oceanic stations of the Gulf of Arauco (A-8 and A-12) may came from phytoplankton, as indicated by the presence of dinosterol (dinoflagellate marker) in these sediments. A greater presence of terrestrial markers was also found in the BioBío Canyon, especially at station B-6 where high concentrations of β-sitosterol, stigmasterol and terrestrial markers were found. An important presence of dehidroabietic acid was also found in all the sampling stations of the BioBío Canyon except the most oceanic one (B-8), and even pimaric acid was present in one station (B-5), indicating probable deposition of higher plant resins

(Volkman & Holdsworth, 1993; Burns *et al.*, 2003). The greater diversity of compounds found in the BioBío Canyon, and especially land source biomarkers, is likely to be related to the larger and older contribution of anthropogenic activities to the continental shelf transported by this river in comparison to the Itata River.

It is interesting to note that the fewest number of land markers and the absence of long-chain fatty acids, typical of vascular plants, were found in the Itata River sediments. In contrast, sediments of the BioBío River contained higher presence of vascular plant markers such as fatty acids, which is probably due to the presence of pulp mill industries that have discharged their effluents for over 50 years into this river (Orrego *et al.,* 2005b). The pulp mill industry located on the Itata River started only 6 years ago, and includes state of the art environmental technology (*e.g.*, tertiary treatment) (CONAMA, 2010). The higher concentration of total sterols found in the Itata River sediments in comparison to the BioBío River, especially β-sitosterol and stigmasterol in the post-impact stations from a pulp mill, may be related to the greater biodegradation capacities of the microbial community inhabiting the BioBío River sediments (Karrasch *et al.*, 2006).

Our results highlight the differences between the two coastal areas adjacent to river mouths, with a high presence of terrestrial biomarkers in the sediments near the BioBío River mouth, while on the coastal shelf adjacent to the Itata River mouth the presence of terrestrial markers was greater in the more seaward stations. The latter is probably related to patterns of local circulation (Sobarzo & Bravo, 2009). It should be taken into account that the data were obtained during only one season; however, when sediments obtained during spring were analyzed (data not shown), the same trend was observed. A similar pattern was found in the Gulf of Arauco, where during autumn the more oceanic stations showed some terrestrial markers such as alkanes and LCFA, while during summer (data not shown), even resin acids were found in the more oceanic stations. This is likely to be related to the influence of the BioBío River plume in the Gulf, which for the period of the year under analysis tends to flow towards the south at the subsurface level (Parada *et al.*, 2001).

About half of the sampled stations had relative concentrations of β-sitosterol in the sediments at or above the range that may affect reproductive parameters of fish (*i.e.*, between 0.01 and 1.2 ppm β-sitosterol; MacLatchy & Van der Kraak, 1995; Mellanen *et al.,* 1999; Tremblay & Van der Kraak, 1999; Hewitt *et al.*, 2000). Some of these stations were associated with river mouths and pulp mill industries (B-6, P-7, A-1), but others were far from these influences (*e.g.*, A-12; Fig. 1). Since most fishes live in the water column and not within the sediment, the real impact of these concentrations in the sediments on the biota is not straightforward. Indeed it is known that in some places the sterol concentrations in water may be almost two orders of magnitude lower than those found in the sediments (Al-Farawati *et al.*, 2009). On the other hand, an emergent issue is the role that phytosterols present naturally in the environment may play at concentrations above the threshold of reproductive impairment, especially in zones where high concentrations of β-sitosterol are found in sediments such as those found off Peru (Volkmann, 1986), and where phytoplankton is an important source of these compounds.

In this study, no differences were found in sterol concentration between the geographical zones. However, at the station level and based on biomarkers of the source of organic matter, some areas have a clear terrestrial influence, suggesting that the source of phytosterols (especially β-sitosterol) is vascular plants. However, the presence of β-sitosterol of phyto-plankton origin is also a significant source in this highly productive coastal upwelling system. No clear spatial pattern emerges between the location of pulp mill industries and β-sitosterol sediment concen-tration, probably due to the high complexity of this coastal system produced by the interaction of river influence, marine currents, geomorphology, upwelling, OMZ, and very high primary production. The exception was provided by one station located in the Gulf of Arauco (A-1) which is closely located to a pulp mill emission. The presence of significant quantities of β-sitosterol of phytoplankton origin in the Humboldt Current System off Peru and Chile (Volkman, 1986 and this study, respectively) raises questions regarding the possible role of this compound in the life cycle of planktonic and benthic species in this eastern boundary current system.

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Supplementary material Tabla 1

Table 1. Sterol concentrations in aquatic systems worldwide (μg g^{-1}).

	Water type	Material	Total sterols	Cholesterol	Campesterol	Stigmasterol	β-sitosterol	References
Australia (intersticial)	marine	sediment	1.6	0.088	0.1232	0.1024	0.469	Volkman, (1986)
Antarctic lake	marine	sediment	1170	98.28	23.4	127.53	409.5	Volkman, (1986)
Perú coast (16-19 cm)	marine	sediment	9.4	1.21	0.658	0.9118	2.632	Volkman, (1986)
Perú coast (mean of first 5 cm)	marine	sediment	110.3	23.3	7.8	4.3	12.2	Volkman, (1987)
China coast	marine	sediment		0.165-1.35	0.041-0.360	0.051-0.522	0.107-0.809	Jeng & Huh, (2004)
Mean	marine	sediment		0.81022	0.203	0.315	0.536	Jeng & Huh, (2004)
China coast	marine	sediment	0.44-6.64		0.025-0.495	0.013-0.630	0.032-1.260	Jeng *et al.,* (2003)
Mean	marine	sediment	4.212		0.183	0.2811	0.59	Jeng *et al.,* (2003)
Open Atlantic Ocean	marine	sediment	6					Gargosian & Nigrelli, (1979)
Conwy Estuary (North Wales, UK)	estuary	sediment	2.8-124.5	0.1-42.8	0-1.8	0-6.7	0-4.1	Mudge & Norris, (1997)
Patos Lagoon, Brasil	marine	sediment		0.007-0.474	<DOL-0.511	< DOL-0.321		Martins *et al.,* (2007)
Antarctic lakes (Syowa oases)	marine	sediment	0.079-9.0	0.018-1.2	0.022-1.8	0.079-1.9	0.026-3.4	Matsumoto *et al.,* (1983)
Trinity bay (Canada)	marine	sediment	24- 44					Parrish *et al.,* (2000)
San Vicente Bay, Chile	marine	sediment		0.05-15.7			<0.01-8.2	Mudge & Seguel, (1999)
Mean				3.1			0.8	
Kuala Selangor (Malaysia)	estuary	sediment		3.19-2450.98	0.98-14.7	0.49-15.36	0.96-69.23	Ali *et al.,* (2009)
Derwent Estuary (Tasmania)	estuarine	sediment	24.1	5.88	1.5	0.756	3.4	Leeming & Nichols, (1998)
	more marine	sediment	1.1-3.7	0.217-0.973	0.129-0.304	0.056-0.158	0.127-0.295	Leeming & Nichols, (1998)
Patos Lagoon, Brasil	freshwater	sediment		0.004-0.1856	<DOL-0.122	< DOL-0.146	0.016-0.219	Martins *et al.,* (2007)
Coatzacoalco River México	freshwater	sediment				0.031-0.610	0.075-0.354	Cortes & Botello, (1988)
Ostion Lagoon, México	freshwater	sediment				0.014-1.169	0.002-0.159	Cortes & Botello, (1988)
Ria Formosa Lagoon, Portugal	freshwater	sediment	0.1-27.8					Mudge *et al.,* (1999)
Central -southern coast of Chile	marine	sediment	0.03-10.4	0.03-7.73	0.01-0.3	0-0.46	0.003-2.07	Present study
Mean			2.6	1.66	0.05	0.1	0.34	Present study
BioBio River (Chile)	freshwater	sediment	0.04-0.97	0.03-0.53	0.03-0.05	0.01-0.05	0.01-0.29	Present study
Mean			0.63	0.26	0.04	0.03	0.18	Present study
Itata River (Chile)	freshwater	sediment	0.3-4.1	0.12-0.6		0.07-1.3	0.1-2.3	Present study
Mean			1.4	0.3		0.5	0.7	Present study