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Application of Gas Chromatography in a Study of Marine Biogeochemistry

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1. Introduction

A goal of marine biogeochemistry is to characterize the chemical composition of particulate organic matter (POM) in the oceanic water column in order to evaluate sources, reactivity and the potential for preservation of POM in the sedimentary environment, given its importance in biogeochemical cycles in the ocean. Organic matter transformation has traditionally been measured as changes in the concentration of organic matter (determined as organic carbon) at different depths (e.g., Gundersen et al., 1998; Wakeham, 1995). Nevertheless, studies of the transformation of specific compounds provide more precise information regarding degradation mechanisms (Abramson et al., 2010; Lee et al., 2004; Pantoja & Lee, 2003). Some of the specific compounds that have been investigated are amino acids (e.g., Abramson et al., 2010; Pantoja & Lee, 2003**)**, carbohydrates (e.g., Çoban-Yildiz et al., 2000), and many lipid classes (Burns et al., 2003; Galois et al., 1996; Minor et al., 2003; Parrish et al., 2000; Sheridan et al., 2002; Sun et al., 1997; Treignier et al., 2006; Wakeham, 1995; Wakeham et al., 1997, 2002).

Lipids could be efficiently characterized with standard chromatographic techniques such as the gas chromatography-mass spectrometry techniques. Although lipids may be a small fraction of the organic matter in plankton (about 15 %) (Wakeham et al., 2000), their composition has been extensively studied to learn about the sources, fluxes, and alterations of organic matter in the water column and sediments (e.g., Grossi et al., 2001; Muri et al., 2004;; Treignier et al., 2006; Wakeham, 1995). Lipids are suitable for such studies because they can be source-specific and, as such, are appropriate "biomarkers" (Pantoja & Wakeham, 2000; Pazdro et al., 2001; Volkman & Tanoue, 2002).

In this chapter, we will present the results of our research carried out in the upwelling system off Antofagasta, northern Chile. The Antofagasta coastal zone (≈23^{o}S) is part of the

Humboldt Current System (HCS) and is characterized by high biological production (Daneri et al., 2000), which is followed by a high export of organic matter (González et al., 2000, 2009) and its preservation in the sediments. The quantity and quality of organic matter preserved in the sediments is controlled by production at the sea surface and decomposition processes during transport in the water column (Lee et al., 2004). In highly productive environments, such as HCS, we would expect to find a clear imprint of marine processes in the distribution of organic molecules in the water column, with a minor contribution of terrestrial biomarkers. Thus, we determined the distribution pattern of free alcohols, free fatty acids, and sterols with the goal of assessing the molecular distribution of lipids in the water column to evaluate sources and reactivity and to infer their potential for preservation in the sedimentary environment. This study of marine biogeochemistry represents an example of gas chromatography – mass spectrometry application.

2. Methods

The investigation was carried out off Antofagasta in northern Chile, at two stations: one coastal (23º16'S; 70º40.3'W) and one oceanic (23º06.9'S; 71º58'W) (Fig. 1). Sampling was carried out during the *FluMO* (*Fluxes of Organic Matter*) cruise on board the RV Abate Molina, from April 18 to April 25, 2001. Suspended particulate organic matter was collected from the oxygenated surface layer, the Oxygen Minimum Zone (OMZ), and the oxygenated deep layer using a rosette equipped with Niskin bottles and a CTDO. Between 120 and 150 L of water were filtered through a 90 mm diameter borosilicate filter (nominal pore size 0.7 µm) precombusted at 450°C for 4 hours. Filters were kept frozen (-20 ºC) until analysis. Vertical profiles of dissolved O_2 were determined with the CTDO sensor, calibrated using the Winkler method, as published elsewhere (Pantoja et al., 2009).

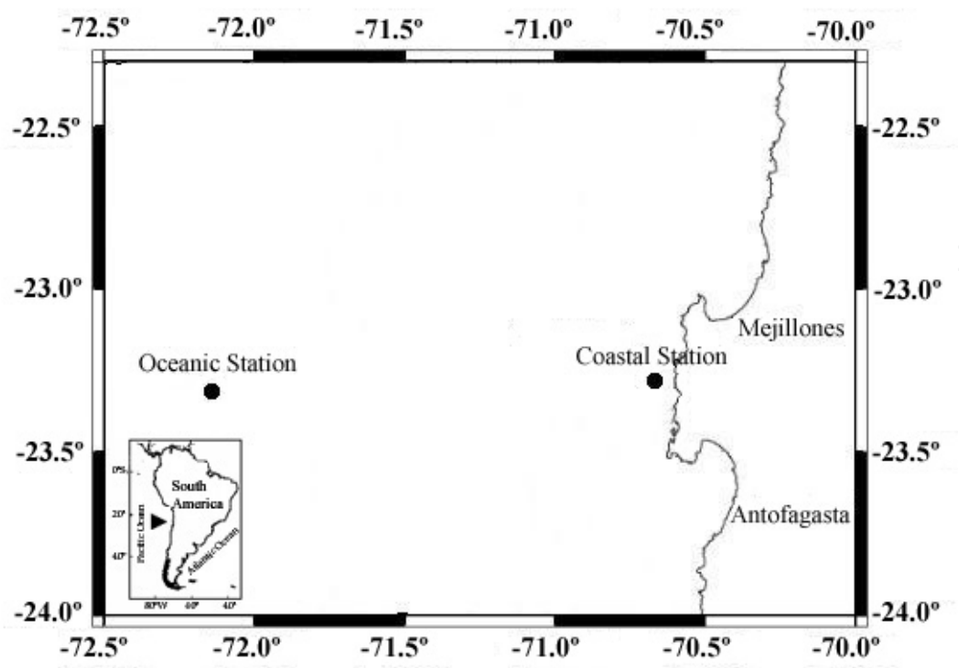

Fig. 1. Location of the sampling stations.

Filters were sub-sampled for determination of particulate organic carbon content, which was calculated as the difference between total carbon and inorganic carbon. Total carbon was analyzed by combustion in a Leco CS-444 instrument, and carbonate was determined by acidification using a UIC coulometer.

Filters were ultrasonically extracted with a solvent mixture of dichloromethane/methanol (2:1 v:v) for 30 minutes in centrifuge tubes (three times). After centrifugation, the combined supernatant was extracted three times with dichloromethane (30 mL each). The combined extracts were dried overnight with anhydrous sodium sulfate, concentrated in a rotary evaporator, and dried with nitrogen gas.

The total extract was dissolved in a small amount of dichloromethane, and 5α-androstan-3β-ol was added as an internal standard. The hexane-insoluble fraction (asphaltenes) was precipitated by adding an excess amount of n-hexane. After filtration, the asphaltene-free extract was separated into three fractions by liquid chromatography over an SiO_2 column (silica gel 60 Merck, activated with 5% water): Fraction 1 containing aliphatic and aromatic hydrocarbons was eluted with 7 mL hexane/dichloromethane (9:1); fraction 2 containing phthalates was eluted with 5 mL hexane/dichloromethane (1:1); and fraction 3 containing alcohols, sterols, stanols, and free fatty acids was eluted with dichloromethane/methanol (9:1). The third fraction was derivatized with N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) to form trimethylsilyl (TMS) ethers of alcohols, sterols, and and TMS esters of fatty acids.

Compounds eluted in the third fraction were analyzed on a Hewlett-Packard 5890 Series II gas chromatograph equipped with a DB5-HT high temperature fused silica capillary column (30 m length; 0.25 mm ID; 0.1 μm film thickness), a flame ionization detector, and a Gerstel KAS3 cold injection system. Helium was used as the carrier gas. Separations were achieved using a temperature program of 4°C/min from 60°C to 380°C. Selected samples were analyzed by gas chromatography-mass spectrometry (GC-MS) using a Finnigan MAT SSQ 710B mass spectrometer equipped with the same type of GC column and using helium as the carrier gas. Mass spectra were acquired over the range of *m/z* 50-650 at a rate of 1 scan/s, with an ionization electron energy of 70 eV. The temperature was programmed from 60°C to 350°C at a rate of 3°C/min.

Most extracts were analyzed by GC-MS for compound identification based on the elution order and a comparison of the mass spectral pattern with published data. Lipid quantification was based on response factors derived from the internal standard (5α-Androstan-3β-ol).

A Principal Component Analysis (PCA) was performed in order to determine the distribution patterns of biomarkers in the water column and to identify lipid sources and lability. For this, we used 44 lipid compounds present in nearly all samples, combined with the oxygen concentration, organic carbon content, and water depth. A correlation matrix was set up with the composition of individual compounds relative to total lipids in each sample, normalized according to:

$$n_{ik} = \frac{(x_{ik} - x)}{S_i}$$

where x is the relative percent of compound (i) in sample (k), and S is the variance (Reemtsma & Ittekkot, 1992).

The PCA was performed using STATISTICA software v. 99. Principal components were calculated from the correlation matrix and treated by Varimax rotation in order to maximize the load of each variable on one factor (Reemtsma & Ittekkot, 1992).

3. Results and discussion

In this study, 44 lipid compounds were identified and quantified from the following classes: alcohols (C_{14} to C_{32}), fatty acids (C_{14} to C_{21}), and sterols (C_{26} to C_{30}); these represented between 0.8 and 0.04% of total organic carbon (Table 1). Alcohols constituted the greatest percentage of the three lipid classes analyzed at both stations (45% at the coastal station and 37% at the oceanic station). Phytol, which is released by the chlorophyll *a* molecule through enzymatic hydrolysis when zooplankton consumes phytoplankton, was the most abundant alcohol (Table 2). In the laboratory, phytol release was achieved through saponification (Wakeham et al., 2002). In the present study, phytol is used as a biomarker for the decay of chlorophyll *a*, as the samples were not saponified prior to GC-MS analysis.

Station	Depth (m)	C_{org} (µg C_{org}/L)	% C_{org}	% Total Lipids		
			Total Lipids	*n*-Alcohols	Fatty acids	Sterols
Oceanic	100	122	0.22	38	30	32
	220	66	0.22	43	31	26
	1000	91	0.04	31	46	21
Average				37	36	26
Coastal	20	177	0.78	55	15	30
	250	76	0.52	54	27	20
	300	68	0.29	42	34	24
	800	93	0.04	29	29	42
Average				45	26	29

Table 1. Organic carbon (C_{org}), and percent of lipid concentrations to C_{org} in particulate organic matter.

The total lipid concentration (alcohols + fatty acids + sterols) was five times greater at the coastal than at the oceanic station (Figs. 2a, b). At both stations, the concentration of total lipids was highest in the samples from shallowest water depth, reaching values of 1.97 µg/L at the coastal station (20 m) and 0.20 µg/L at the oceanic station (100 m). This agrees with the greater primary production measured at the coastal station (between 1.3 and 3.3 g C/m² d) as compared with that at the oceanic station (between 0.27 and 0.30 g C/m² d) (Daneri and Pantoja, unpublished results). The total lipid concentrations decreased with depth. At the coastal station, the decrease was more significant, reaching values two orders of magnitude lower (0.04 µg/L) in the deepest sample (800 m). At the oceanic station, the decrease was less significant, dropping only to one half (0.11 µg/L) at 1000 m depth (Figs. 2a, b).

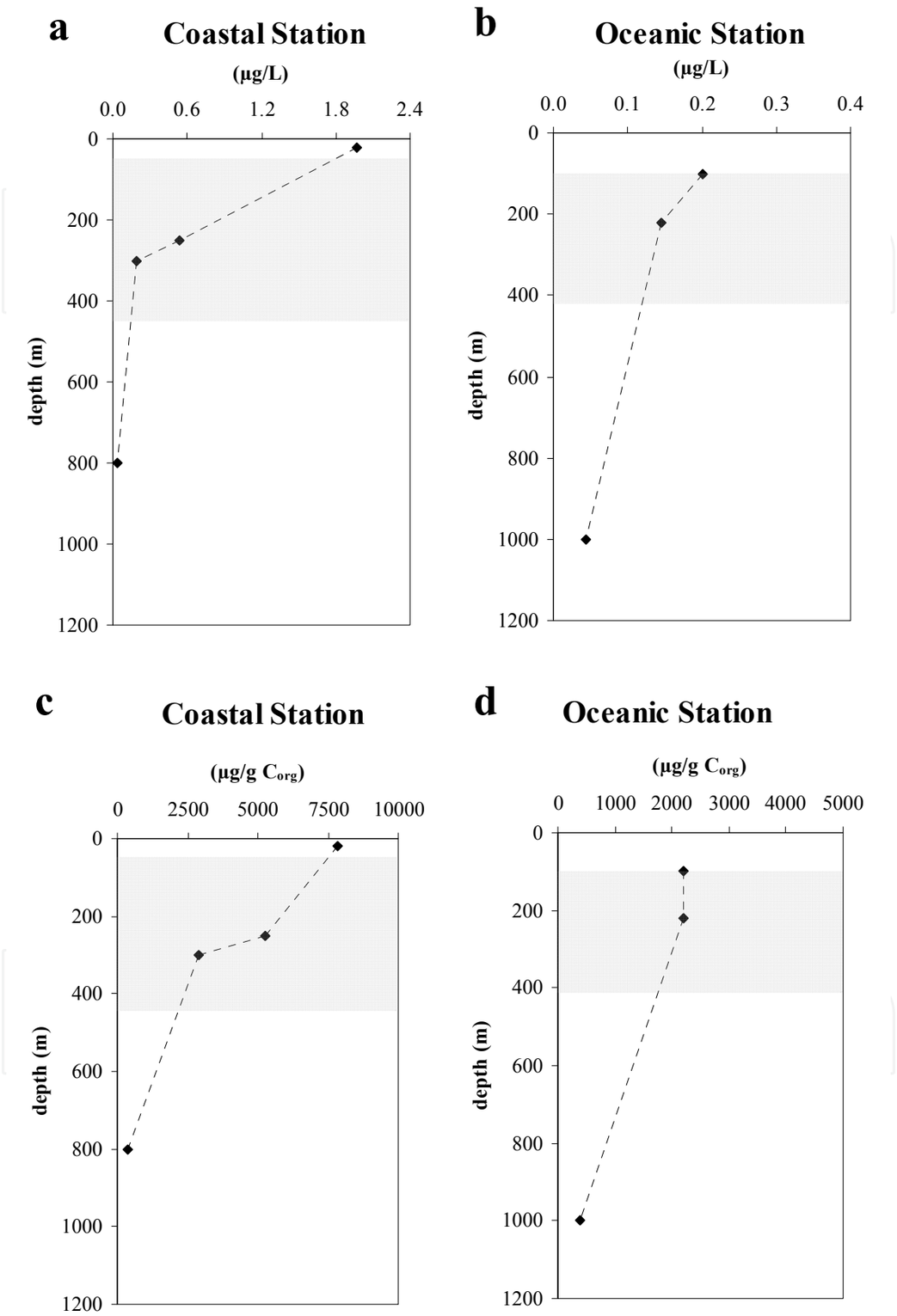

Fig. 2. Total lipid concentrations, and lipid/Corg ratios in particulate organic matter. Shaded areas represent the Oxygen Minimum Zone ([O2] < 22.5 µM).

Most of the particulate organic matter in the ocean is biosynthesized at the surface by photoautotrophic plankton via photosynthetic fixation of carbon (Lee et al., 2004). Although a lesser percentage could be biosynthesized by the microbial activity in the deeper ocean layers, the changes observed in the vertical concentrations (Table 2, Figs. 2a, b) mainly reflect the breakdown of the lipid molecules in transit to the seafloor. When the lipid concentration is normalized to the organic carbon content (Figs. 2c, d), it becomes obvious that the proportion of lipid components decreases in relation to the total organic carbon

Compound	Coastal Station				Oceanic Station		
	20 m	250 m	300 m	800 m	100 m	220 m	1000 m
Sterols							
A = 27-*nor*-24-Methylcholesta-5,22E-dien-3β-ol	3.43	2.24	2.07	nd	1.95	4.86	5.22
B = 27-*nor*-24-Methyl-5α-cholest-22E-en-3β-ol	4.5	nd	nd	nd	nd	nd	nd
C = Cholesta-5,22E-dien-3β-ol	4.58	0.93	nd	nd	1.06	1.28	1.93
D = 5α-Cholest-22E-en-3β-ol	4.07	0.62	nd				
E = Cholest-5-en-3β-ol	71.9	7.24	5.87	nd	nd	0.57	nd
F = 5α-Cholestan-3β-ol	8.70	2.45	4.01	7.24	5.86	6.76	5.64
G = 24-Methylcholesta-5,22(E)-dien-3β-ol	50.8	8.08	11.3	0.89	1.21	1.34	0.90
				nd	3.69	3.52	4.67
H = 24-Methyl-5α-cholest-en-3β-ol	4.63	1.26	0.55	nd	nd	0.82	1.28
I = 24-Methyl-5α-cholest-22E-dien-3β-ol	5.01						
		6.71	0.64	nd	2.49	0.78	nd
J = 24-Methylcholesta-5,24(28)-dien-3β-ol	107	14.4	3.41				
				0.75	8.00	nd	3.95
K = 24-Methyl-5α-cholest-24(28)-en-3β-ol	2.32	0.60	nd	nd	nd	nd	nd
L = 24-Methylcholest-5-en-3β-ol	59.4	6.84	0.88	0.93	nd	nd	nd
M = 23,24-Dimethylcholesta-5,22E-dien-3β-ol	6.29	0.92	nd	nd	0.79	nd	1.27
N = 24-Ethyl-5α-cholest-22E-en-3β-ol	16.1						
O = 23,24-Dimethylcholest-5-en-3β-ol		4.40	5.24	1.20	1.07	1.49	2.32
	2.37	1.32	0.84	nd	nd	nd	nd
P = 24-Ethylcholest-5-en-3β-ol	22.3						
Q = 24-Ethyl-5α-cholestan-3β-ol	4.12	3.98	2.62	nd	2.99	1.98	1.68
R = 4α,23,24-Trimethyl-5α-cholest-22E-en-3β-ol	31.8	4.55	4.34	5.03	0.910	nd	5.94
		9.04	1.52	nd	3.07	3.24	3.29
S = 4α,23,24-Trimethyl-5α-cholestan-3β-ol	3.68	1.01	0.91	nd	6.66	nd	3.64
Alcohols							
nC_{14}-OH	20.6	4.53	4.37	1.13	1.45	1.26	0.73
nC_{16}-OH	11.5	6.17	8.08	2.04	3.79	5.84	6.20
nC_{17}-OH	4.96	1.88	1.53	nd	nd	0.900	1.47
nC_{18}-OH	31.5	10.0	13.1	3.12	6.27	13.2	10.8
Phytol	580	143	10.5	0.70	5.69	1.65	2.95
nC_{19}-OH	3.16	2.13	1.65	nd	nd	0.79	0.98
nC_{20}-OH	13.5	3.93	6.19	1.94	1.80	3.66	2.49
nC_{21}-OH	6.74	1.59	0.87	nd	3.12	1.66	4.52
nC_{22}-OH	34.8	14.2	17.0	1.70	6.40	21.2	22.8
nC_{23}-OH	nd	nd	nd	nd	1.08	0.780	nd

nC_{24}-OH	19.0	6.31	5.91	0.580	2.17	3.69	5.06
n$C2_6$-OH	18.5	4.77	5.80	nd	1.33	2.49	3.49
nC_{28}-OH	8.43	7.86	nd	nd	2.78	2.83	3.63
nC_{30}-OH	4.18	1.73	nd	nd	2.90	0.950	nd
nC_{32}-OH	4.71	2.50	0.80	nd	1.33	0.740	0.590
(ΣC_{14}–C_{20})/ (ΣC_{22}–C_{32})	1.03	0.811	1.21	3.61	0.914	0.916	0.695
(ΣoddC_{15}–C_{19})/ (ΣevenC_{16}–C_{20})	0.119	0.152	0.089	0.0	0.137	0.075	0.126
Fatty Acids							
$C_{14:0}$	24.6	23.3	16.8	2.14	4.22	5.87	9.16
i-$C_{15:0}$	3.92	1.80	1.97	nd	1.29	2.86	2.72
ai-$C_{15:0}$	4.72	3.30	2.78	nd	1.61	3.10	3.37
$C_{16:1}$	20.0	12.4	7.10	nd		1.33	2.93
$C_{16:0}$	82.4	38.5	14.2	4.89	12.4	17.4	38.9
i-$C_{17:0}$	4.80	2.35	nd	nd	1.82	nd	1.01
ai-$C_{17:0}$	3.30	nd	nd	nd		nd	nd
$C_{18:1}$	28.7	11.9	16.4	1.60	5.16	5.53	15.0
$C_{18:0}$	28.2	6.14	14.8	2.38	4.59	9.71	18.5
$C_{21:0}$	7.84	5.92	4.14	nd	1.60	nd	1.18

Table 2. Concentration of lipids (ng L^{-1}) identified in suspended particulate organic matter at the two sampling stations off Antofagasta. nd = not detected.

(C_{org}). This is interpreted to show that the lipid molecules analyzed in this study are more labile than other components representing the bulk of the organic matter.

At the coastal station, phytol and the sterols were the most abundant compounds at the surface (20 m) and had the greatest decrease in concentration with increasing depth (Table 2, Fig. 3a). At the oceanic station, the shallowest sample was collected at the oxycline (100 m), which is the transition zone between the well-oxygenated surface layer and the deep layer, where oxygen concentrations fall below 22.5 µM (suboxic conditions). At this depth, the most abundant compounds were the fatty acids; their concentration decreased to 50% at 1000 m depth (Fig. 3b).

The vertical distribution of the concentration of each lipid class normalized to C_{org} indicates, on the one hand, the relative importance of each class, and on the other, the preferential consumption of some compound types with respect to others (Lee et al., 2004). At the coastal station, the most abundant compounds at 20 m were phytol (3.3×10^3 µg/g C_{org}) and the sterols (2.3×10^3 µg/g C_{org}), but within the Oxygen Minimum Zone, the fatty acids (1.4×10^3

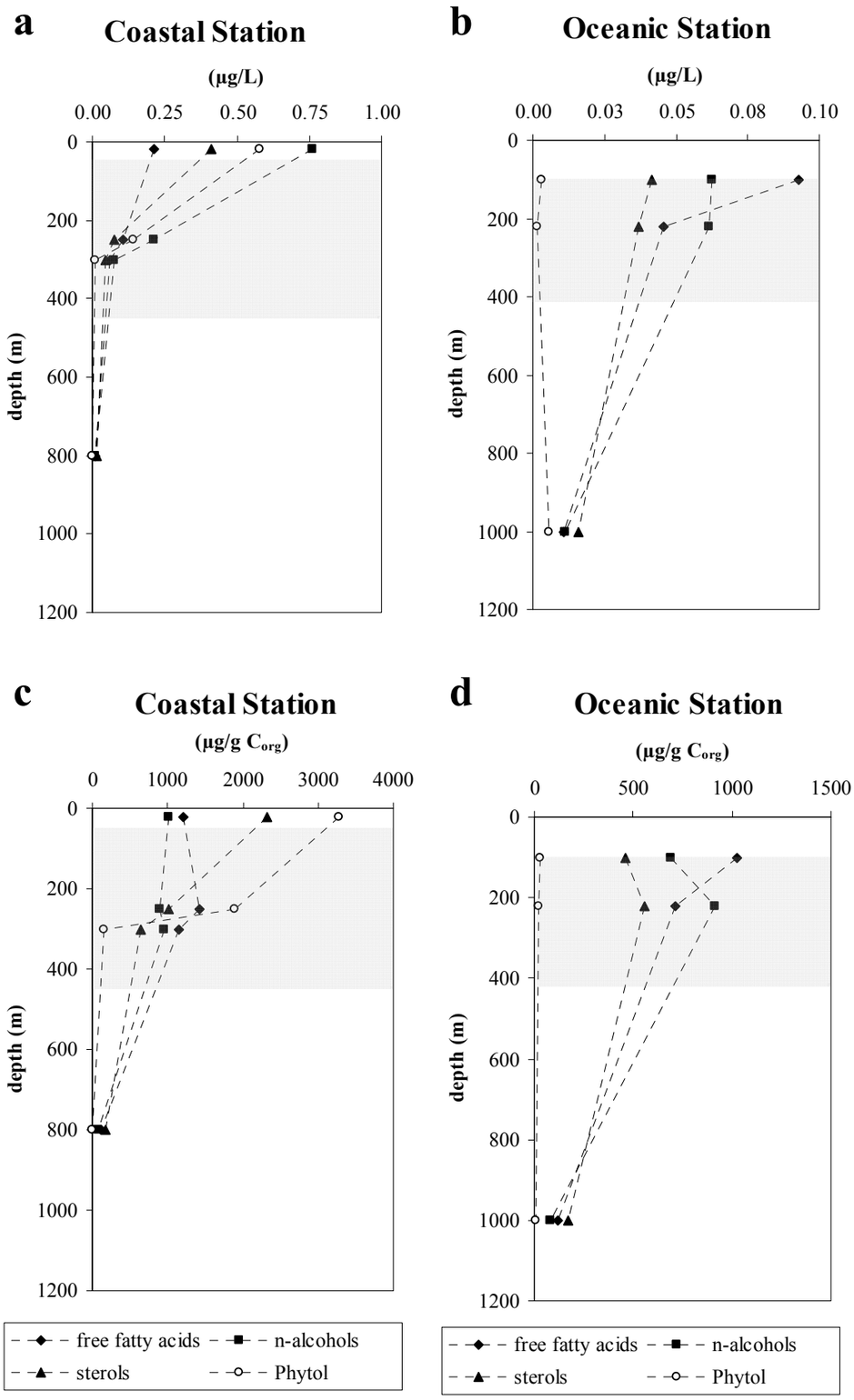

Fig. 3. Lipid class concentrations, and lipid classes/Corg ratios in particulate organic matter. Shaded areas represent the Oxygen Minimum Zone ([O2] < 22.5 µM).

µg/g C_{org}) and *n*- alkanols (0.95 x 10^3 µg/g C_{org}) were more abundant (Table 2, Fig. 3c). If we consider photosynthetic production to be the main source for the formation of organic matter at the ocean surface, our results show that phytol and sterols are preferentially consumed in the water column, as they disappear more quickly than C_{org} with increasing depth. At the coastal station, the fatty acids are the most abundant lipids at 100 m depth (1.0 x 10^3 µg/g C_{org}), but in the Oxygen Minimum Zone, the *n*- alkanols (0.9 x 10^3 µg/g C_{org}) and sterols (0.5 x 10^3 µg/g C_{org}) are more abundant than the fatty acids (Table 2, Fig. 3c). At the oceanic station, the compounds that disappear most quickly with depth are the fatty acids (Table 2, Fig. 3d), meaning that these are the most labile compounds in this environment.

3.1 Sterols

At the coastal station, the sterols represented 29% of all three lipid classes analyzed (Table 1). The surface sample (20 m) was dominated by 24-methylcholesta-5,24(28)-dien-3β-ol (24-methylenecholesterol), cholest-5-en-3β-ol (cholesterol), 24-methylcholest-5-en-3β-ol, and 24-methylcholesta-5,22(E)-dien-3β-ol (diatomsterol) (Table 2, Fig. 4a). The sterols 24-methylenecholesterol and diatomsterol are phytoplankton biomarkers (known as phytosterols) (Burns et al., 2003; Volkman et al., 1998; Wakeham et al., 1997), fundamentally diatom biomarkers (Burns et al., 2003; Parrish et al., 2000), and cholesterol is a biomarker of both phytoplankton and zooplankton (Burns et al., 2003). Although less abundant than the diatom biomarkers, some sterols (4α,23,24-trimethyl-5α-cholest-22E-en-3β-ol and 23,24-dimethylcholesta-5,22E-dien-3β-ol), biomarkers of dinoflagellates, were detected (Fig. 4a) (Burns et al., 2003; Volkman et al., 1998; Wakeham et al., 2002).

A general decrease in the relative abundance of phytosterols was observed within the Oxygen Minimum Zone (sampling depths 250 and 300 m). In this area, the phytosterols 24-methylenecholesterol, diatomsterol, cholesterol, 24-methylcholest-5-en-3β-ol, and 4α,23,24-trimethyl-5α-cholest-22E-en-3β-ol were equally predominant (Fig. 4a). An increase in the abundance of some stanols such as 5α-Cholestan-3β-ol, 24-methyl-5α(H)-cholest-22E-dien-3β-ol, and 24-ethyl-5α(H)-cholest-22E-en-3β-ol was also evident. Wakeham, 1989 sugested these compounds are produced by the bacterial hydrogenation of stenols under suboxic conditions, for which their presence has been recognized as the result of diagenetic transformation (Parrish et al., 2000; Volkman et al., 1998; Wakeham et al., 2007).

In the deepest sample (800 m), the relative abundance of cholesterol, 24-methylcholest-5-en-3β-ol, 24-ethyl-5α-cholestan-3β-ol, 24-ethyl-5α(H)-cholest-22E-en-3β-ol, and 5α-cholestan-3β-ol increased to different extents compared to the Oxygen Minimum Zone and partly also to the surface waters (Fig. 4a). At this depth, cholesterol was the most common sterol and, although common in phytoplankton, the principal source of this compound is zooplankton.

At the oceanic station, sterols represented 26% of the lipids on average (Table 1). The relative abundance pattern was different from that at the coastal station (Fig. 4b). At 100 m depth (oxycline), the most abundant sterols were 24-methylcholesta-5,24(28)-dien-3β-ol, cholesterol, and diatomsterol. At this station, a high proportion of the A-ring stanols 24-methyl-5α-cholest-24(28)-en-3β-ol, and 24-ethyl-5α-cholestan-3β-ol was also observed; these are used as indicators of bacterial decay of organic matter under suboxic conditions (Wakeham & Ertel, 1987).

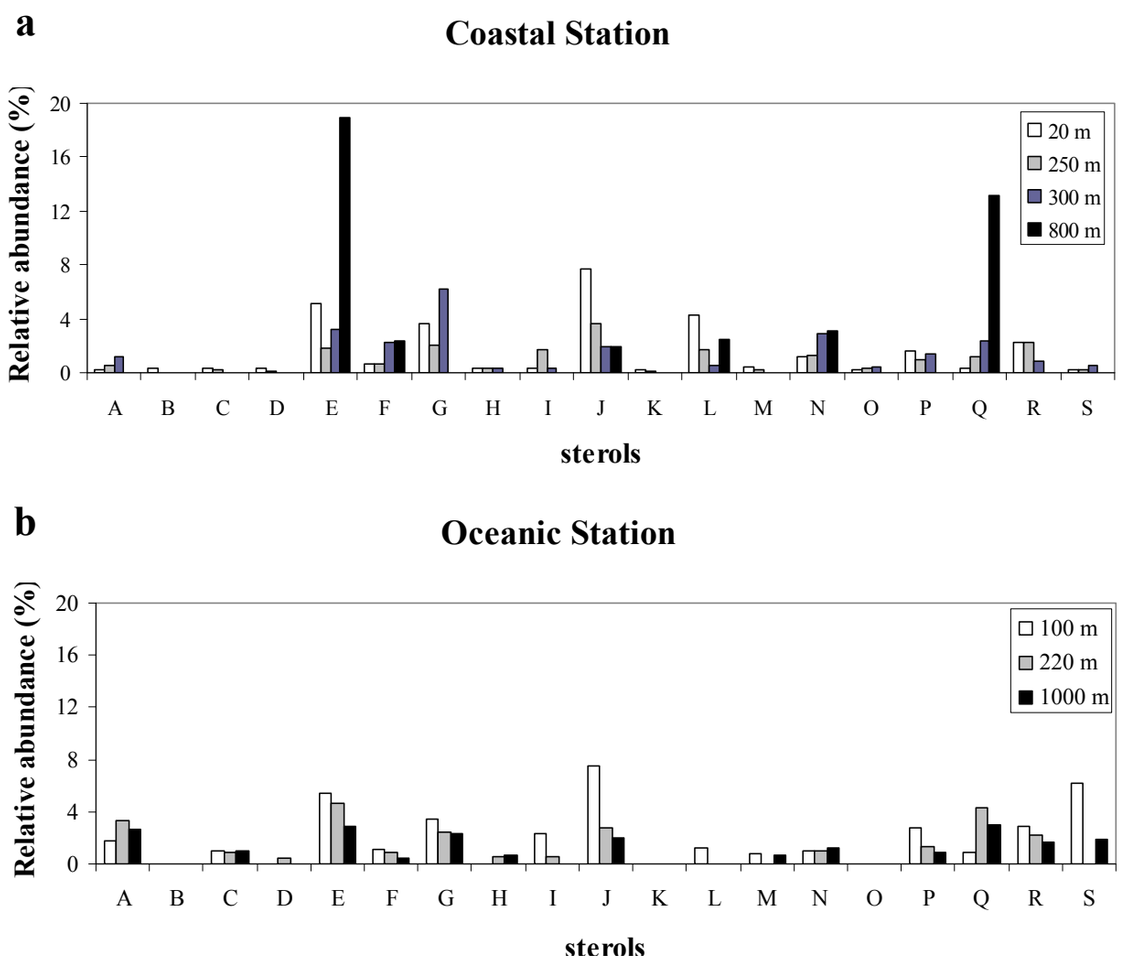

Fig. 4. Distribution of sterols in particulate organic matter. Letter codes are defined in Table 2.

Within the Oxygen Minimum Zone (220 m), the relative abundance of the majority of the sterols identified decreased (Fig. 4b), except for 27-*nor*-24-methylcholesta-5,22E-dien-3β-ol and 24-ethyl-5α-cholestan-3β-ol. The sterol 27-*nor*-24-methylcholesta-5,22E-dien-3β-ol, although commonly reported in sediments, has also been found in some dinoflagellates. Unlike at the coastal station, 24-ethyl-5α-cholestan-3β-ol was the only stanol that increased within the Oxygen Minimum Zone (Fig. 4b).

In spite of their different profiles in terms of relative sterol abundance, biomarkers for the diatoms, i.e. 24-methylenecholesterol and diatomsterol, were predominant at both stations (Fig. 4a, b). This result is not surprising, since studies performed along the northern coast of Chile off Antofagasta have shown that the phytoplankton assemblages in this area are more dominated by diatoms than by dinoflagellates (González et al., 2000; Iriarte y González, 2004; Iriarte et al., 2000).

3.2 Alcohols

The *n*-alkanols are formed by hydrolysis of esterified alcohols and are derived from a large variety of marine organisms (phytoplankton, zooplankton, bacteria) or higher plants

(Treignier et al., 2006; Jeng et al., 2003). In the Antofagasta area, the identified *n*-alkanols represented, on average, 45% of the total lipids at the coastal station and 37% at the oceanic station (Table 1). At both stations, 16 *n*-alkanols were identified in the range between nC$_{14}$OH and nC$_{32}$OH (Table 2), with a predominance of even-carbon-numbered chains, i.e. nC$_{16}$OH, nC$_{18}$OH, nC$_{20}$OH, and nC$_{22}$OH; of these, nC$_{22}$OH was the most abundant alkanol (Fig. 5). The short-chain *n*-alkanols (≤C$_{20}$) have a marine origin (phytoplankton, zooplankton, bacteria), whereas the long-chain alkanols (≥C$_{22}$) are attributed to terrestrial plants (Treignier et al., 2006; Wakeham, 2000; Jeng et al., 2003). Nonetheless, nC$_{22}$OH is also attributed to occur in cyanobacteria. For example, studies carried out in the Baltic Sea found that nC$_{22}$OH was the most abundant *n*-alkanol in samples containing principally cyanobacteria (Volkman et al., 1998). Given the very low contribution from terrestrial sources in the arid zone off Antofagasta, we attribute the presence of nC$_{22}$OH to cyanobacteria.

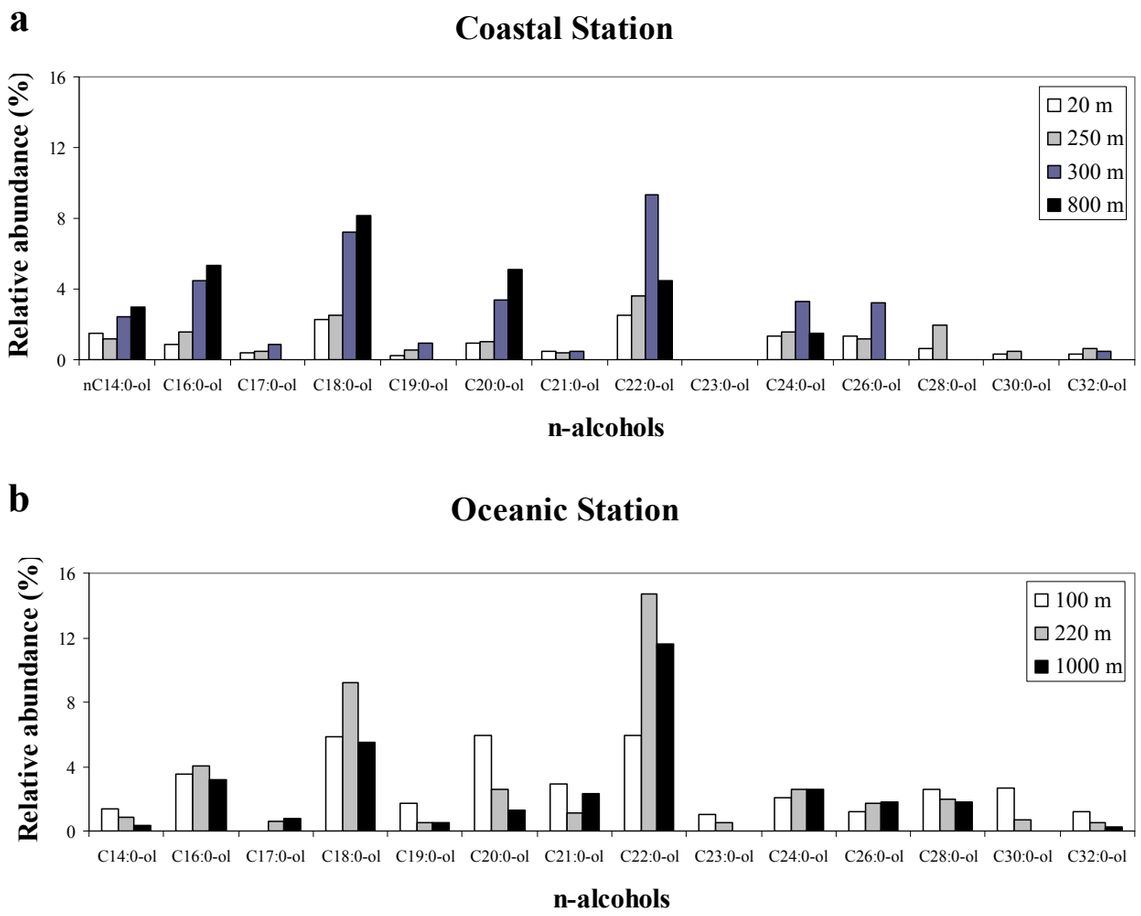

Fig. 5. Distribution of n-alkanols in particulate organic matter.

In order to determine the sources of the *n*-alkanols in aquatic environments, Fukushima and Ishiwatari developed a parameter that is based on the ratio of the sum of short-chain *n*-alkanols (Σ [nC$_{14}$OH–nC$_{20}$OH]) over the sum of long-chain *n*-alkanols (Σ [nC$_{22}$OH–nC$_{30}$OH])

(Treignier et al., 2006). Values >1 reveal a predominance of short-chain compounds and values <1 a predominance of long-chain compounds (Treignier et al., 2006). The values calculated for our samples at each depth level show that ratios >1 occur in the coastal zone (except at 250 m depth) and in the oceanic zone, indicating that the *n*-alkanols are primarily planktonic in origin (Table 2). We feel that the dominance of the $n C_{22}OH$ alcohol found at all depths at the oceanic station (Fig. 5b) should be attributed to cyanobacteria, as found for other ecosystems (Volkman et al., 1998).

The isoprenoid alcohol phytol (3,7,11,15-tetramethylhexadec-2-en-1-ol) was also found in all samples. This compound occurred at high concentrations (0.58 µg/g) in the coastal zone at 20 m depth and its concentration decreased rapidly with increasing water depth until values three orders of magnitude lower (0.001 µg/g) were reached at 800 m depth (Fig. 3a). At the oceanic station, the phytol concentration was greatest at 100 m (0.006 µg/g) and, as at the coastal station, decreased with depth until, at 1000 m, the compound was no longer detected (Fig. 3b). Phytol is a biomarker of phytoplankton consumed by zooplankton. It is released from chlorophyll *a* through enzymatic hydrolysis when zooplankton grazes on phytoplankton (Sheridan et al., 2002). As explained earlier, primary production is higher at the coastal than at the oceanic station, so that we expect greater grazing activity in the former environment as well. The abundance of metazoans (or zooplankton) was three times greater (2189 ± 1888 ind/m^3) in the surface layer (0-50 m) at the coastal station than at the oceanic station (625 ± 184 ind/m^3) (Zenteno-Devaud, unpublished results), which is supporting evidence for the phytol profiles observed. On the other hand, phytol is a fairly labile molecule, since it practically disappears in the deepest zones at both stations (800 and 1000 m) (Figs. 3a, b).

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3.3 Fatty acids

The free fatty acids represented 26% of the total lipids at the coastal station and 36% at the oceanic station (Table 1). As with the alcohols, the most abundant fatty acids were straight-chain compounds, which represented more than 65% of fatty acids at all depths, followed in abundance by unsaturated and branched (especially iso + anteiso) fatty acids, respectively

(Fig. 6). The straight-chain fatty acids C_{14}, C_{16}, and C_{18} come from mixed planktonic sources of phytoplankton, zooplankton (Canuel & Zimmerman, 1999; Wakeham , 1995), and bacteria (Gong & Hollander, 1997). In cyanobacteria, fatty acids are dominated by the C_{16} straight-chain acid (Wakeham, 1995), whereas diatoms biosynthesize large amounts of the 16:1ω7 acid (Wakeham et al., 2007). The absence of long-chain fatty acids (>24 carbon atoms) is indicative of the very low continental contribution in the northern zone of Chile (absence of important rivers), since the long-chain fatty acids are terrestrial higher plant markers (Derieux et al., 1998; Parrish et al., 2000).

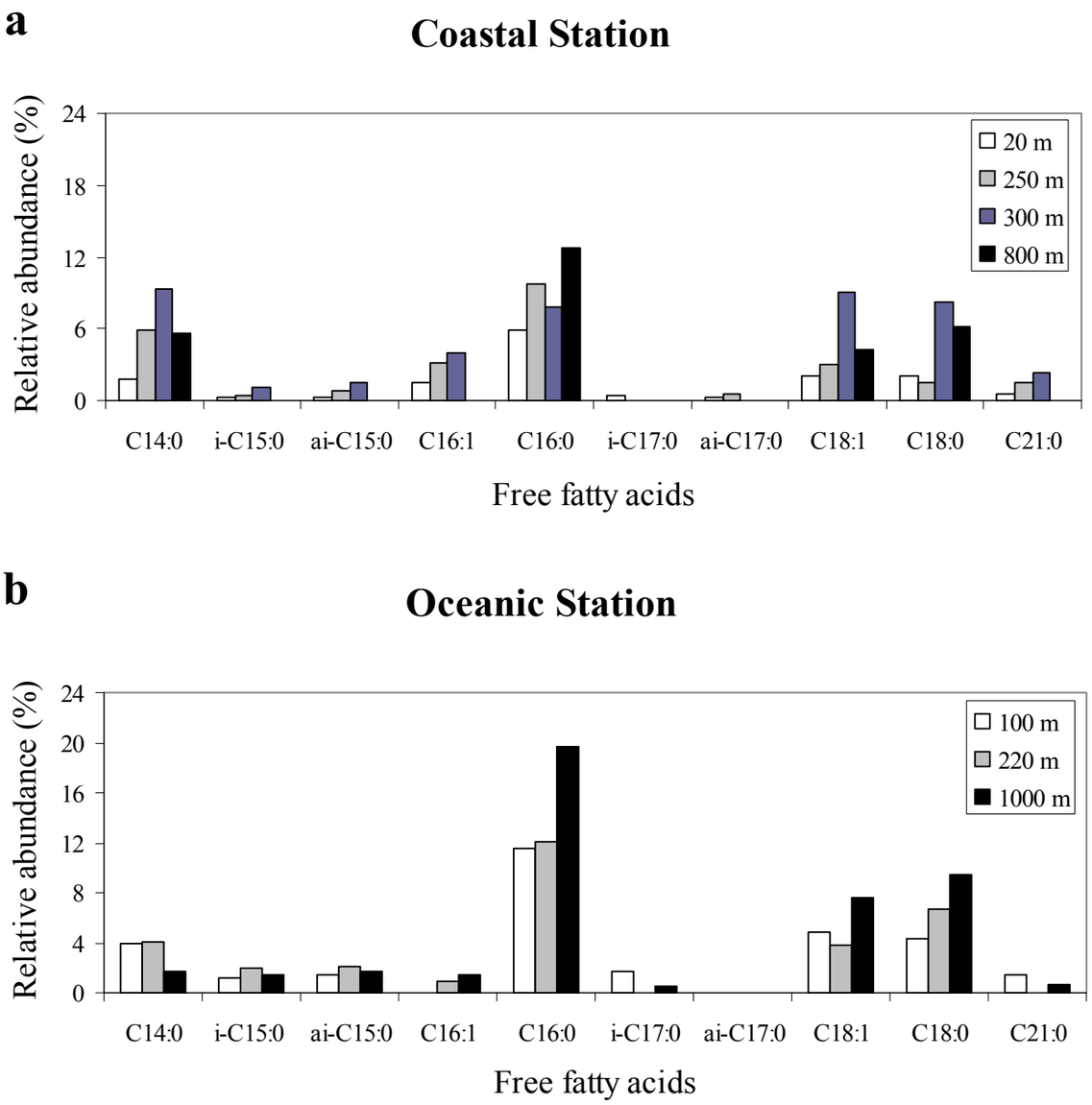

Fig. 6. Distribution of fatty acids in particulate organic matter.

In natural systems, fatty acids are found free or associated with other compounds through ester bonds. The associated fatty acids are more abundant (S.D. Killops & V.J. Killops, 1994; Pazdro et al., 2001) and mainly occur as constituents of phospholipids which are integral constituents of the membranes of all living organisms (Petsch et al., 2003, Wakeham et al., 2007). The relative abundance of free fatty acids analyzed in this work can be interpreted as the result of reworking of the organic matter of decayed organisms in the water column, more than as specific indicators of organism abundance (Pazdro et al., 2001; Wakeham, 1995). Thus, the greater relative abundance of saturated fatty acids over monounsaturated fatty acids (Fig. 6) and the absence of polyunsaturated fatty acids can be interpreted as the result of the increasing metabolization of organic matter with depth since the monounsaturated and polyunsaturated fatty acids are more susceptible to breakdown than the saturated fatty acids (Derieux et al., 1998; Galois et al., 1996; Pazdro et al., 2001).

The relative abundance of *iso*- and *anteiso*-$C_{15:0}$ was greater within the Oxygen Minimum Zone, as compared to surface and deep samples at both the coastal (250 – 300 m) and oceanic (220 m) stations (Figs. 6a, b). Considering that *i* and *ai*-$C_{15:0}$ fatty acids are markers of bacteria and that they are commonly used as indicators of intense reworking of organic matter (Pazdro et al., 2001; Wakeham, 2007), their occurrence within the Oxygen Minimum Zone can be interpreted as pointing to significant bacterial activity on the organic matter in this layer.

3.4 Principal Component Analysis

Principal component analysis (PCA) has become a powerful tool for reducing the quantity of variables and uncovering tendencies in data series having numerous entries. This analysis allows a simple graphic representation of the degree of likeness existing within a data group, thereby establishing the main characteristics of the group of variables in a series of samples, reducing it to a smaller set of derived variables (principal components). More information can often be extracted from these principal components than from the original variables.

Application of PCA to our data set showed that the three first components explained a large proportion of the total variance (75 %). Component I explained 32% of the variance, Component II 26%, and Component III 17% (Table 3). Figure 7 shows the plane that represents the first two components. For Factor I, to which the variables were most positively loaded (Table 3), comprised the sterols 24-ethyl-5α-cholest-22E-en-3β-ol, 24-ethyl-5α-cholestan-3β-ol, and 5α-cholestan-3β-ol; the C_{20}, C_{14}, and C_{16} *n*-alkanols; and the C_{14} fatty acid (in the hatched oval, Fig. 7), which suggests that these variables are related to each other. The *n*-alkanols, fatty acids, and two of the stenols, precursors of the stanols 24-ethylcolesta-5,22E-dien-3β-o1 and cholest-5-en-3β-ol, which loaded positively to Component I, were derived from phytoplanktonic organisms. On the other hand, free alcohols and free fatty acids are considered lipid decay indicators (since most of these compounds are found linked to other molecules (Galois et al., 1996; Gong & Hollander, 1997; Parrish et al., 2000); the same applies to the increase of the relative abundance of straight-chain fatty acids at the expense of their unsaturated counterparts (Wakeham, 1995) and the presence of stanols that are produced by the microbial reduction of their stenol analogues under suboxic conditions (Minor et al., 2003; Wakeham, 1995). These results lead us to conclude that the substances in Component I represented highly degraded organic matter of planktonic origin.

Variable	Factor 1	Factor 2	Factor 3
N = 24-Ethyl-5α-cholest-22E-en-3β-ol	**0.905**	0.093	-0.385
nC_{20}OH	**0.833**	-0.102	-0.216
Q = 24-Ethyl-5α-cholestan-3β-ol	**0.782**	-0.036	0.168
F = 5α-Cholestan-3β-ol	**0.750**	-0.001	-0.545
nC_{14}OH	**0.624**	0.476	-0.547
nC_{16}OH	**0.614**	-0.547	-0.273
$C_{14:0}$	**0.600**	-0.317	-0.612
E = Cholest-5-en-3β-ol	**0.600**	0.378	0.002
R = 4α,23,24-Trimethyl-5α-cholest-22E-en-3β-ol	**-0.964**	0.018	0.097
P = 24-Ethylcholest-5-en-3β-ol	**-0.823**	-0.061	-0.442
nC_{28}OH	**-0.807**	-0.374	0.130
nC_{30}OH	**-0.710**	0.026	-0.517
Σ *i*+*ai*(C_{15}-C_{17})	**-0.737**	-0.504	-0.3598
J = 24-Methylcholesta-5,24(28)-dien-3β-ol	**-0.758**	0.553	-0.102
M = 23,24-Dimethylcholesta-5,22E-dien-3β-ol	**-0.761**	-0.007	0.014
nC_{32}OH	**-0.641**	0.191	-0.719
L = 24-Methylcholest-5-en-3β-ol	-0.029	**0.964**	0.115
Phytol	-0.305	**0.823**	0.135
K = 24-Methyl-5α-cholest-24(28)-en-3β-ol	-0.285	**0.805**	0.223
Oxygen	-0.165	**0.783**	0.117
B = 27-*nor*-24-Methyl-5α-cholest-22E-en-3β-ol	-0.243	**0.718**	0.316
nC_{22}OH	0.053	**-0.904**	0.237
A = 27-*nor*-24-Methylcholesta-5,22E-dien-3β-ol	-0.347	**-0.865**	0.272
$C_{18:0}$	0.446	**-0.812**	0.023
nC_{24}OH	0.172	**-0.785**	-0.301
H = 24-Methyl-5α-cholest-en-3β-ol	-0.100	-0.460	**0.616**
$C_{21:0}$	-0.155	-0.006	**-0.838**
$C_{16:1}$	-0.645	-0.109	**-0.713**
Eigenvalues	**13.337**	**11.057**	**7.115**
% total variance	**32**	**26**	**17**

Table 3. Loading scores for the three factors explaining 75% of the variance in particulate organic matter.

On the other hand, the variables that were negatively loaded to Component I (in the dark grey oval, Fig. 7) were the sterols 4□,23,24-trimethyl-5α-cholest-22E-en-3β-ol, 24-ethylcholest-5-en-3β-ol, 24-methylcholesta-5,24(28)-dien-3β-ol, and 23,24-dimethylcholesta-5,22E-dien-3β-ol; the C_{28}, C_{30}, and C_{32} *n*-alkanols; and the *i- and ai*-C_{15} and -C_{17} (Σ *i*+*ai*(C15-C17)) bacterial fatty acid markers (Table 3). The long-chain *n*-alkanols and 24-ethylcholest-5-en-3β-ol are usually considered biomarkers of terrestrial higher plants (Parrish et al., 2000; Treignier et al., 2006; Hinrichs et al., 1999), but 24-ethylcholest-5-en-3β-ol in highly

productive marine ecosystems can also arise from planktonic sources. Nonetheless, since the PCA showed this sterol to be highly related to terrestrial biomarkers such as long-chain *n*-alcohols, this compound in the investigated marine environment appears to be more terrestrial than planktonic in origin.

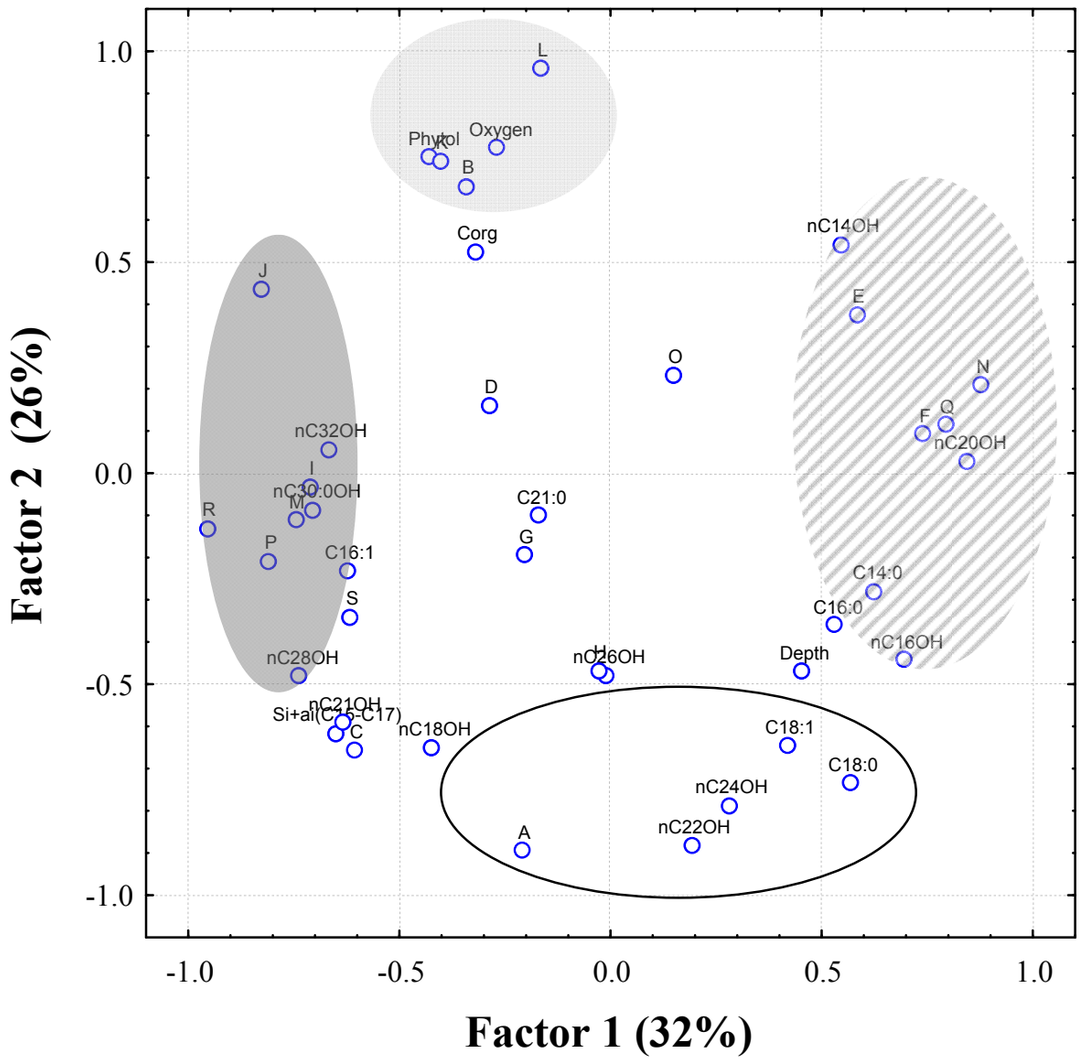

Fig. 7. Compound loadings on Factor 1 vs. Factor 2 for the principal component analysis of lipids in particulate organic matter. The hatched oval shows components with positive loadings on Factor I, the dark grey oval shows components with negative loadings on Factor I, the light gray oval shows components with positive loadings on Factor II, and the white oval shows components with negative loadings on Factor II.

For Factor II, the positively loaded variables were 24-methylcholest-5-en-3β-ol, 24-methyl-5α-cholest-24(28)-en-3β-ol, and 27-*nor*-24-methyl-5α-cholest-22E-en-3β-ol, phytol and the oxygen concentration (shown in the light gray oval, Fig. 7; Table 3). This Component is represented by diatom biomarkers. Phytol is the indirect indicator of phytoplankton, being released from the chlorophyll molecule, and the three sterols are bioindicators of diatoms.

These molecules, in turn, are directly related to the oxygen concentration, that is, as the oxygen concentration drops in the water mass, the concentration of these compounds decreases, which means that they are effectively degraded under suboxic conditions; this is supported by the disappearance of these biomarkers below the OMZ (Figs. 3 and 4).

The variables that were negatively loaded for Factor II (in the oval, Fig. 7) were the C_{22} and C_{24} *n*-alkanols; the $C_{18:0}$ fatty acid; and the sterol 27-*nor*-24-methylcholesta-5,22E-dien-3β-ol (Table 3). Both the C_{18} fatty acid and 27-*nor*-24-methylcholesta-5,22E-dien-3β-ol are planktonic in origin, the sterol was reported to be a bioindicator of some flagellates (Wakeham**,** 1995). The alkanols >C_{22} have been reported to be biomarkers of higher plants; nonetheless, there is uncertainty with respect to *n*C_{22}-OH, since it can also be derived from cyanobacteria (Volkman et al., 1998). In Figure 7, this alcohol is observed to be more related to planktonic biomarkers than to compounds of terrestrial origin, so we consider *n*-alkanol in the Antofagasta area to be derived from cyanobacteria.

PCA allowed the separation of planktonic biomarkers (positively charged in Component I) from terrestrial biomarkers (negatively charged in Component I). Moreover, it showed phytol, a biomarker of grazing activity, to be closely correlated with the oxygen concentration in the water column, as it was more abundant at the surface where the oxygen concentration was higher.

4. Conclusions

The analysis of individual compounds within each of several lipid classes (alcohols, fatty acids, and sterols) allowed us to determine that phytoplankton, mainly diatoms, are the main sources of lipids in the suspended particulate organic matter in the Antofagasta area. Continental contributions are scarce, as seen in the low concentrations of terrestrial biomarkers, e.g., the sterol 24-ethylcholest-5-en-3β-ol and the long-chain *n*-alkanols (>C_{22}), as well as in the absence of long-chain fatty acids.

On the other hand, the analysis of these lipid classes showed that, as depth increased, the particles were intensely degraded. The rapid disappearance of labile molecules such as phytol, the increase in *n*-alkanols and straight-chain fatty acids, the decrease of unsaturated fatty acids, and the increase of bacterial biomarkers all indicate increased microbial activity on the lipid molecules.

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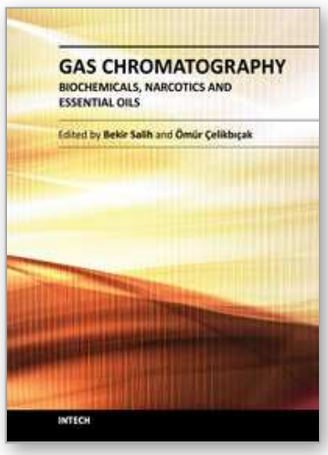

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Gas Chromatography involves the study of various vaporizable molecules in chemistry and the other related research fields. This analytical method has a number of features and advantages that make it an extremely valuable tool for the identification, quantification and structural elucidation of organic molecules. This book provides detailed gas chromatography information to applications of biochemicals, narcotics and essential oils. The details of the applications were briefly handled by the authors to increase their comprehensibility and feasibility. This guide should be certainly valuable to the novice, as well as to the experienced gas chromatography user who may not have the enough experience about the specific applications covered in this book. We believe this book will prove useful in most laboratories where modern gas chromatography is practiced.

How to reference
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