

Dinosterols or dinocysts to estimate dinoflagellate contributions to marine sedimentary organic matter?

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Abstract

Dinosterol (4 α ,23,24-trimethyl-5 α -cholest-22E-en-3 β -ol) is frequently used as an alternative to dinoflagellate cyst (dinocyst) counting in paleoceanography to assess dinoflagellate inputs to marine sediments. However, recent studies have shown poor correlation between these two proxies in continental-margin sediments. We reevaluated the relationship and expanded it to include a suite of biogeochemical transformation products of the parent dinosterol (dinosterone, dinostanone, and dinostanol). These dinoflagellate-specific 4 α ,23,24-trimethyl steroidal species ($\Sigma_{\text{dinosterol}}$) are compared to dinocyst counts in sediments from the western Mexican margin (375–3,500 m). Samples were taken from subsurface (3–6 cm) and down core (16–27 cm) to reflect widely contrasting organic carbon content and redox conditions. A strong correlation was found between the sum of all dinoflagellate-derived sterols, $\Sigma_{\text{dinosterols}}$, and total dinocyst counts, highlighting the importance of including diagenetic alteration products of the parent molecule when exploiting organic biomarkers in paleoceanographic studies. In low-energy environments and for well-preserved samples, such as those studied in this work, both methods provide robust, internally consistent data, suggesting that when diagenetic transformation products of dinosterol are taken into account, gas chromatography and optical microscopy could be used interchangeably to estimate dinoflagellate inputs to marine sediments.

Extractable lipids comprise a very small fraction of molecularly characterizable sedimentary organic matter (Hedges et al. 2000). Owing to their structural diversity and specificity, an increasing number of lipids are being used as biological markers for indicating organic matter sources and reaction pathways (Wakeham and Lee 1993; Nash et al. 2005), and as diagnostic tools to probe variations in paleoecological (e.g., Brassell 1993) and environmental conditions (Sikes et al. 2005). Sterols in particular have

attracted much attention and have been exploited as source markers for marine particulate and sedimentary organic matter (Nash et al. 2005; Volkman 2005). Their broad diversity, biosynthetic specificity, and good stability toward diagenetic reworking in marine sediments make sterols excellent indicators for sedimentary contributions of their specific biological sources (Volkman 1986; Hudson et al. 2001; Nash et al. 2005).

Sterols are ubiquitous components of eukaryotic cell membranes and represent a significant fraction of the lipid pool in marine algae (Jones et al. 1994). Composed of a skeleton of four fused rings, sterols vary in the number and position of double bonds, side chains and/or ring methylations, as well as in their stereochemistry (Volkman 1986). A subset of the sterol pool, the 4 α ,23,24-trimethyl sterols (and particularly dinosterol, Table 1), have been proposed as dinoflagellate tracers for sedimentary organic matter (Withers 1987; Volkman et al. 1999) since they are not synthesized to a significant extent by other organisms (Volkman et al. 1993).

Dinocysts have also proved useful as a proxy for the dinoflagellate contribution to sedimentary organic matter. They consist of organic-walled capsules composed of a highly refractory aliphatic biomacropolymer (*dinosporin*, Kokinos et al. 1998). Dinocysts protect the cell during the dormancy, or “hypozygote” phase, of many dinoflagellate

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Table 1. Sample location, water-column depth, and bulk data for the western Mexican margin sediments used in this study.

Station	Coordinates (latitude, longitude)	Water depth (m)	Sediment depth (cm)	Organic carbon* (%)	Atomic C:N ratio*
300	25°19.7'N 112°46.0'W	387	3–6 16–27	6.39±0.03 7.49±0.07	10.22±0.03 10.40±0.07
306	22°43.2'N 106°28.9'W	375	3–6 16–27	7.70±0.13 7.86±0.05	10.12±0.07 10.50±0.02
305	22°11.4'N 107°19.0'W	2,990	3–4 19–22	3.27±0.02 3.20±0.09	9.73±0.04 11.62±0.01
304	21°34.3'N 108°18.5'W	3,070	3–4 16–19	2.38±0.01 2.30±0.01	9.10±0.07 9.96±0.16
310	19°06.8'N 106°14.6'W	3,500	3–4 16–19	1.96±0.02 1.87±0.04	9.21±0.01 9.27±0.13

* Number of replicates is 3–4.

species (Head 1996). Dinocysts are generally well preserved in marine sediments and are thus particularly useful for reconstructing paleoenvironmental variables and processes (Stickley et al. 2004; de Vernal et al. 2005). The assemblages, or relative proportions of cysts from an array of dinoflagellate species in a sample, constitute useful indicators of physical and chemical conditions such as temperature, salinity, seasonality, or sea-ice coverage in the recent past ocean (e.g., de Vernal et al. 2005).

If dinosterol and dinocysts are specific to dinoflagellates and similarly resistant toward degradation and alteration, their abundances in fresh sedimentary deposits should be correlated, making the cyst-counting approach using optical microscopy (Rochon et al. 1999; de Vernal et al. 2005) and the gas chromatography (GC)-based dinosterol biomarker method (Jones et al. 1994) interchangeable. While early studies were promising (Djerassi 1981), recent reports have demonstrated nonlinear (Marret and Scourse 2003) or no significant correlations (Sangiorgi et al. 2005) between total dinocyst counts and dinosterol concentrations in ocean-margin sediments.

Several reasons could explain these findings. Ecological and population dynamics can affect source material because: (1) dinocysts recovered in sediments represent a fraction of the original dinoflagellate populations since only ~15% of species produce fossilizable organic-walled cysts (e.g., Head 1996); (2) while dinosterol is produced almost exclusively by dinoflagellates (Volkman et al. 1993), it is not the major constituent of the total sterol pool in all dinoflagellate species (Leblond and Chapman 2002); (3) while dinoflagellates synthesize or contain dinosterol at any stage of their biological cycle, dinocysts are only formed during their dormancy phase (Head 1996); and (4) variations in dinoflagellate populations over time and/or geographical locations, or changes in growth conditions of individual species in the surface ocean, can result in a decoupling between dinocyst and dinosterol inputs to sediments. Postdepositional biogeochemical processes also act as decoupling agents since: (5) cysts from heterotrophic dinoflagellates have been shown to be less resistant to degradation under oxic conditions than cysts from autotrophic species (Zonneveld et al. 2001); (6) although the steroidal skeleton is very robust and survives many

biogeochemical transformations (Wakeham 1989), sterols are subject to several transformations following deposition to sediments, most notably the oxidation of the alcohol in position C3 to form a ketone, and the reductive hydrogenation of unsaturation(s) present in the B-ring of the sterol backbone and/or side chain, forming stenones, stanones, and stanols; and (7) because the major fraction of sedimentary organic matter, including the sterol pool, is bound to mineral surfaces (Keil et al. 1994), whereas intact dinocysts are present as organic particles of low density, hydrodynamic sorting could lead to density-based fractionation of dinosterol and dinocysts (Mollenhauer et al. 2006), particularly in high-energy environments.

The weak correlations between sedimentary dinosterol concentrations and dinocyst counts reported by Marret and Scourse (2003) and Sangiorgi et al. (2005) might for example be explained by the fact that only dinosterol was quantified in these studies; since all 4 α ,23,24-trimethyl steroidal species found in sediments originate from dinoflagellates either through direct synthesis by dinoflagellates (Leblond and Chapman 2002), or following the biogeochemical alteration of dinosterol in oxic and anoxic sediments (Sun and Wakeham 1994), the complete suite of 4 α ,23,24-trimethyl sterols (e.g., dinosterol, dinostanol, dinosterone, and dinostanone, henceforth represented by $\Sigma_{\text{dinosterols}}$) should thus be quantified when comparing the steroidal biomarker approach to dinocyst counts.

The main goals of the work reported in this article are thus to revisit the relationship between the dinoflagellate-specific sterols ($\Sigma_{\text{dinosterols}}$) and total dinocyst counts, and to reassess whether these sterols can be used as an alternate proxy for the contribution of dinoflagellates to sedimentary organic carbon (OC). The site selected for this study extends over a small geographical area (~400 km × 200 km; see Fig. 1), and is characterized by high total organic carbon concentrations (2–8 wt% OC) and contrasting sedimentary redox conditions (from fully oxic to sulfidic). The distribution of 23 sterols was determined in near-surface and deep layers of five sediment cores using lipid extraction and gas chromatography analysis, and the total concentration found for $\Sigma_{\text{dinosterols}}$ was compared to total dinocyst counts obtained from the same samples using the traditional optical microscopy method.

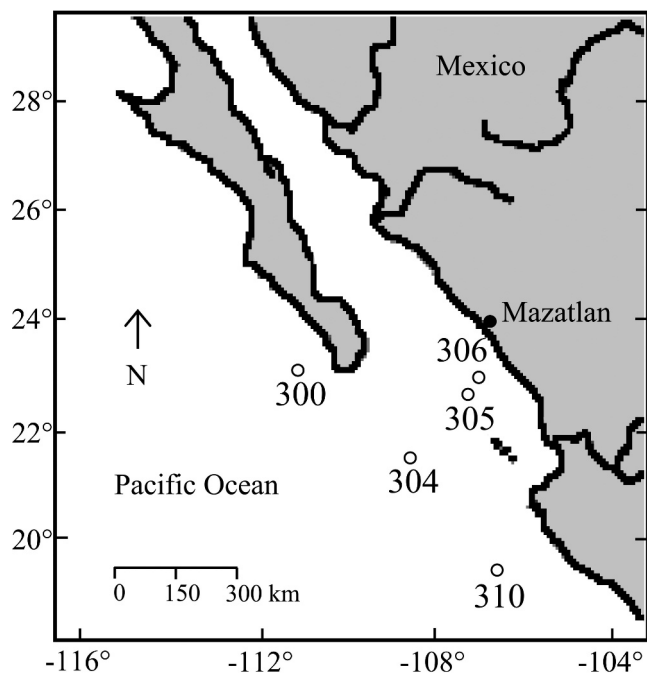


Fig. 1. Sampling area showing the five western Mexican margin stations selected for this study.

Materials and methods

Sampling sites—Sediment samples were collected using a multicorer at five stations on the western Mexican margin in the Pacific Ocean (Fig. 1) during the February 1999 RV *New Horizon* cruise. These samples covered a wide range of redox conditions and organic carbon contents; sediments from Stas. 300 and 306 accumulate under suboxic bottom water and are sulfidic below 15 cm, while the pore-water dissolved oxygen and nitrate concentrations in sediments from Stas. 305, 304, and 310 reach undetectable levels at depths of 0.6 and 2.5 cm, 0.6 and ~8 cm, and 0.9 and ~9 cm, respectively. Deep sediments (15 cm to ~30 cm, maximum sampling depth) at these latter three stations are anoxic but not sulfidic (the assessment of redox conditions for the studied samples is based on unpublished dissolved O_2 , NO_3^- , Mn^{2+} , Fe^{2+} , and HS^- profiles measured during the sampling cruise using microelectrodes and spectrophotometric methods).

Each core was subsampled below the surface (3–6 cm at Stas. 300 and 306; 3–4 cm, Stas. 304, 305, and 310) and at a depth (16–27 cm at Stas. 300 and 306; 16–19 cm at Stas. 304 and 310; 19–22 cm at Sta. 305) where the sediment is diagenetically stabilized. Sample availability kept us from using the same depths at all stations; we estimate however that these differences are negligible with respect to the main goal of this study, i.e., the comparison between the abundances of dinoflagellate-specific sterols and total dinocysts counts. We decided not to use surface sediments (0–2 cm) because the degradation rates of sterol and other lipids can be as much as one order of magnitude higher close to the sediment–water interface compared to deeper in the sediment (Canuel and Martens 1996). Surface sediment layers might also contain significant amounts of

dinoflagellate cells (or cell remains) that are enriched in dinoflagellate-derived sterols. At an average sediment accumulation rate of $\sim 10 \text{ mg cm}^{-2} \text{ yr}^{-1}$ in the investigated area (Hartnett et al. 1998), the two selected depth intervals correspond to ages of roughly 750–1,500 yr, and 4,000–6,750 yr B.P., respectively (not taking into account the effect of bioturbation in cores sampled under oxic water columns, and assuming constant sedimentation rates at each site). Upon collection, all samples were sliced in a N_2 -purged glove box, centrifuged to remove pore water, and stored frozen at -80°C until analysis. Each sample was then thawed, homogenized, and split in two parts for sterol analysis and dinocyst counting.

Sample treatment—All solvents used were high-pressure liquid chromatography (HPLC)-grade. Approximately 5 g of dry sediment was extracted for total lipids. The samples were sonicated for 30 min and extracted three times with 200 mL of a 2:1 dichloromethane:methanol solution. The organic phase was collected and vacuum-filtered on precombusted Whatman glass fiber (model GF/F) filters to remove residual particles from the organic phase.

The extracts were taken to dryness by roto-evaporation, recovered in chloroform, and passed through an activated copper column to remove elemental sulfur. Saponification of the eluant was carried out with 25 mL of methanolic KOH (0.5 mol L^{-1}) at pH 14 followed by the addition of 5 mL of distilled water. The mixture was refluxed for two hours, at which point 10 mL of a 5% NaCl solution was added and the product extracted three times with 30 mL of dichloromethane. Extracts of individual samples were combined and dried over anhydrous sodium sulfate.

Polar lipids, including sterols, were isolated from non-polar lipids by long column silica gel chromatography. A 25-cm glass column was filled with ~7 g of hexane-rinsed deactivated silica (60–100 mesh). Straight-chain and branched hydrocarbons eluted first with hexane and a 1:1 solution of dichloromethane/methanol, respectively. Polar lipids were then recovered with a 10% solution of methanol in dichloromethane and transformed into their trimethylsilyl derivatives using a 1:1 solution of N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and pyridine (70°C , 2 h). The solution was evaporated to dryness under a stream of N_2 , and derivatized polar lipids were dissolved in 200 μL of chloroform for analysis by gas chromatography-mass spectrometry (GC-MS) or gas chromatography with flame ionization (GC-FID).

Analysis—Mass spectral identification of sterols was achieved using a Varian Saturn CP-3800/2200 ion trap GC-MS. The instrument was fitted with a split/splitless injector set at 320°C and a CP-Sil 8 CB Low-Bleed MS column (30 m \times 0.25 mm internal diameter (i.d.), film thickness = $0.25 \mu\text{m}$, Varian Inc.). Helium was used as the carrier gas with a constant flow rate of 1.3 mL min^{-1} . The initial oven temperature was set at 45°C and ramped to 255°C at a rate of $30^\circ\text{C min}^{-1}$, then held for 10 min. Three additional ramps followed (to 257°C at 1°C min^{-1} , 271°C at 4°C min^{-1} , and finally to 310°C at $20^\circ\text{C min}^{-1}$) and the oven held at 310°C for 10 min. Electron-impact mass spectra

(70eV) were recorded between 50 and 600 m/z at a rate of 1 scan s^{-1} .

An Agilent GC-FID model 6890N fitted with the same column as above but with a 0.51 mm i.d., and a Gerstel Cooled Injection System CIS-3, was used for quantification. A constant carrier gas (helium) flow rate of 2 mL min^{-1} and an inlet split ratio of 50:1 were used throughout. The initial oven temperature was set at 45°C and increased to 281°C at 30°C min^{-1} , followed by a 4°C min^{-1} ramp to 310°C, where it was held for 10 min. The FID was set at 315°C and the injector temperature was initially programmed at 65°C (1 min), and then ramped to 305°C at 1°C s^{-1} , where it was held for 10 min.

Identification and quantification—International Union of Pure and Applied Chemistry (IUPAC) nomenclature and abbreviations for all sterols identified in this study are listed in Table 2. Standard mixtures of sterols (Aldrich) used in this work contained: cholest-5-en-3 β -ol (cholesterol, or 27 Δ^5), 5 α -cholestan-3 β -ol (dihydrocholesterol, or 27 Δ^0), 24-methylcholesta-5,22 E -trien-3 β -ol (ergosterol, or 28 $\Delta^{5,7,22}$), 24-ethylcholesta-5,22 E -dien-3 β -ol (stigmasterol, or 29 $\Delta^{5,22}$), 24-ethylcholest-5-en-3 β -ol (sitosterol, or 29 Δ^5), 24-methylcholesta-5-en-3 β -ol (campesterol, or 28 Δ^5), and 24-methyl-5 α -cholest-22 E -en-3 β -ol (*epi*-brassicastanol, or 28 Δ^{22}). Identification of the other sterols found in sediments is based on mass spectral fragmentation patterns and/or sedimentary sterol chromatographic data published elsewhere (Volkman 1986; Volkman et al. 1998; Leblond and Chapman 2002).

Sterol quantification was achieved through comparison of peak areas (GC-FID trace) with those obtained for a series of standard solutions (25–250 $\mu g mL^{-1}$) containing the sterols listed above. Typical correlation coefficients were above 0.98, and the absolute limit of detection (LOD) was calculated as 25 ng for an injection volume of 1 μL . The response factors of sterols for which no commercial standards are available were calculated by averaging the areas of the closest neighboring standard sterols. The method was validated (linearity, detection limits, and reproducibility) using either standard sterol mixtures of known concentrations or spiked and natural samples. Reproducibility of the overall procedure (extraction, purification, derivatization, and GC analysis) was determined by analyzing three aliquots of natural and spiked (100 mg L^{-1} equivalent of the standard sterol mixture in the final solution) sediments collected in the Saguenay Fjord (Quebec, Canada) in May 2002. The average relative standard deviation was $\pm 15\%$. There was no carryover of sterols detected in procedural blanks at the end of each run. Due to limited sample availability and the large quantity of sediments required for the accurate quantification of sterols and dinocysts (~ 5 g each), each Mexican margin sediment sample could be analyzed only once per selected depth.

Analysis of dinocysts—Dinocysts were counted following standardized method detailed in Rochon et al. (1999). Briefly, ~ 5 g of wet sediment was spiked with a capsule of lycopode spores (*Lycopodium clavatum*, 10,679 spores per capsule). Each sample was passed through two stacked

sieves (120 μm and 10 μm), and the fraction between 120 μm and 10 μm was recovered and centrifuged at 2,000 rpm ($\sim 1,200$ g) for 10 min. The supernatant was removed and the sample was treated with ~ 2 mL of 10% HCl (20 min at 50–60°C). The HCl supernatant was removed by centrifugation and ~ 2 mL of 49% HF was added and left to react overnight at room temperature. HF supernatant was removed by centrifugation and samples were again treated with about 2 mL of 10% HCl, sieved once more to collect the 10–120 μm fraction, and centrifuged at 2,000 rpm for 10 min to recover the pellet. Subsamples of the pellet were mounted on microscopic slides embedded in glycerine jelly. Depending on the cyst density, a few lines to the entire slide area (22 mm \times 45 mm) was scanned using an optical microscope with a magnification power of $\times 250$ –1,250 to identify and count dinocysts, *Lycopodium* marker spores, and other palynomorphs. A minimum of 200 dinocysts were counted in each sample for further calculation of species percentages, except in sample 310 for which the total counts were low.

Results and discussion

Sterols fingerprinting and abundances—The bulk characteristics of sediments from the Mexican margin are shown in Table 1. OC concentrations are highest in the oxygen minimum zone at Stas. 300 and 306 (6.39 ± 0.03 wt% and 7.86 ± 0.05 wt% OC, respectively), and decrease sharply with increasing water-column depth and increasing oxygen exposure time (Hartnett et al. 1998; Gélinas et al. 2001) to less than 2 wt% OC at Sta. 310. Little variation in OC concentrations down core characterize each site, except Sta. 300 where there is a slight increase from 6.39 ± 0.03 wt% to 7.49 ± 0.07 wt% OC. Atomic C:N ratios at all sites suggest a marine source for sedimentary organic matter, in agreement with the findings of Dickens et al. (2006). C:N ratios generally increase down core, consistent with a slow diagenetic transformation of the organic matter. The increase is statistically significant except at Sta. 310.

Individual sterols were analyzed in the five cores studied in this work. Because all sterols are characterized by the fused 4-ring steroidal skeleton and a side chain of 8–10 carbon atoms, they elute within a narrow time window during GC analysis, i.e., the “sterol window.” More than 35 peaks appeared in most samples within the sterol window, of which 23 were identified either through GC-MS analysis or comparison with literature data (Fig. 2; Table 2). No further attempt was made at identifying the remaining peaks. Three sterols accounted for ~ 63 –87% (average of $75.8 \pm 7.6\%$) of the total sterol pool in these samples, namely, 27 Δ^5 (cholesterol, peak No. 6), 28 $\Delta^{5,22}$ (*epi*-brassicasterol, peak No. 9), 28 Δ^5 (campesterol, peak No. 12) and 29 $\Delta^{5,22}$ (stigmasterol, peak No. 14) (Fig. 3; Table 3) (The sum of the concentrations of all identified sterols, in mg sterols g^{-1} OC, is given at the bottom of Table 3.) The 5 α (H)-stanol counterparts of the above sterols (27 Δ^0 , cholestanol, peak No. 7, 28 Δ^{22} , *epi*-brassicastanol, peak No. 10, 28 Δ^0 , campestanol, peak No. 13, and 29 Δ^{22} , stigmastanol, peak No. 15, respectively) also

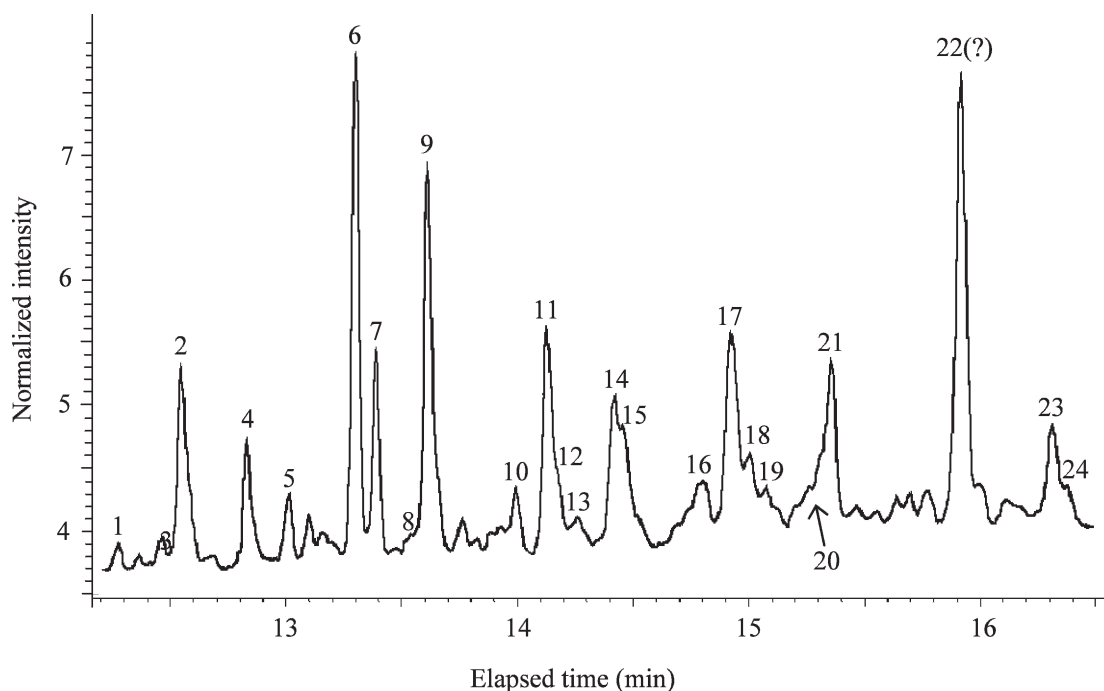


Fig. 2. Partial GC-MS chromatogram showing the elution window for sterol trimethylsilyl (TMS)-ethers extracted from a typical sample. Numbers correspond to compounds listed in Table 2.

generated quantifiable signals, representing between 6.0% and 17.4% of the total sterol pool (average of $10.9 \pm 4.2\%$). The relative abundance of $28A^{5,22}$ (*epi*-brassicasterol), a widely used biomarker for diatoms and other algae (Volkman 1986), is high in most samples suggesting mostly

autochthonous, marine-derived organic matter in these sediments originating from diatom-dominated primary productivity in the surface waters. A small contribution of terrigenous organic matter is also suggested by the presence of the $29A^{5,22}$ (stigmasterol) and $29A^5$ (sitosterol)

Table 2. Compound assignment for GC-MS peaks reported in Fig. 2.

Peak	Compound	Common name	Abbreviation
1	5 β -cholestan-3 β -ol	Coprostanol	5 β -27A ⁰
2	<i>n</i> -C28-alcohol	—	—
3	27- <i>nor</i> -24-methylcholesta-5,22 <i>E</i> -dien-3 β -ol	Ocellasterol	27- <i>nor</i> -27A ^{5,22}
4	Cholesta-5,22 <i>E</i> -dien-3 β -ol	Dehydrocholesterol	27A ^{5,22}
5	5 α -cholest-22 <i>E</i> -en-3 β -ol	Dehydrocholestanol	27A ²²
6	Cholest-5-en-3 β -ol	Cholesterol	27A ⁵
7	5 α -cholestan-3 β -ol	Cholestanol	27A ⁰
8	<i>n</i> -C29-alcohol	—	—
9	24-methylcholesta-5,22 <i>E</i> -dien-3 β -ol	<i>epi</i> -brassicasterol	28A ^{5,22}
10	24-methyl-5 α -cholest-22 <i>E</i> -en-3 β -ol	<i>epi</i> -brassicastanol	28A ²²
11	24-methylcholesta-5,24(28)-dien-3 β -ol	24-methylenecholesterol	28A ^{5,24(28)}
12	24-methylcholest-5-en-3 β -ol	Campesterol	28A ⁵
13	24-methyl-5 α -cholestan-3 β -ol	Campestanol	28A ⁰
14	24-ethylcholesta-5,22 <i>E</i> -dien-3 β -ol	Stigmasterol	29A ^{5,22}
15	24-ethyl-5 α -cholest-22-en-3 β -ol	Stigmastanol	29A ²²
16	4 α ,23,24-trimethyl-5 α -cholest-22-en-3-one	Dinosterone	3-one-30A ²²
17	24-ethylcholest-5-en-3 β -ol	Sitosterol	29A ⁵
18	24-ethyl-5 α -cholestan-3 β -ol	Sitostanol	29A ⁰
19	24-ethylcholesta-5,24(28)-dien-3 β -ol	Fucosterol	29A ^{5,24(28)}
20	4 α ,23,24-trimethyl-5 α -cholestan-3-one	Dinostanone	3-one-30A ⁰
21	4 α ,23,24-trimethyl-5 α -cholest-22 <i>E</i> -en-3 β -ol	Dinosterol	30A ²²
22	Unidentified	—	—
23	4 α ,23,24-trimethyl-5 α -cholest-8(14)-en-3 β -ol (?)*	—	30A ⁸⁽¹⁴⁾ (?)
24	4 α ,23,24-trimethyl-5 α -cholestan-3 β -ol	Dinostanol	30A ⁰

* Identification uncertain.

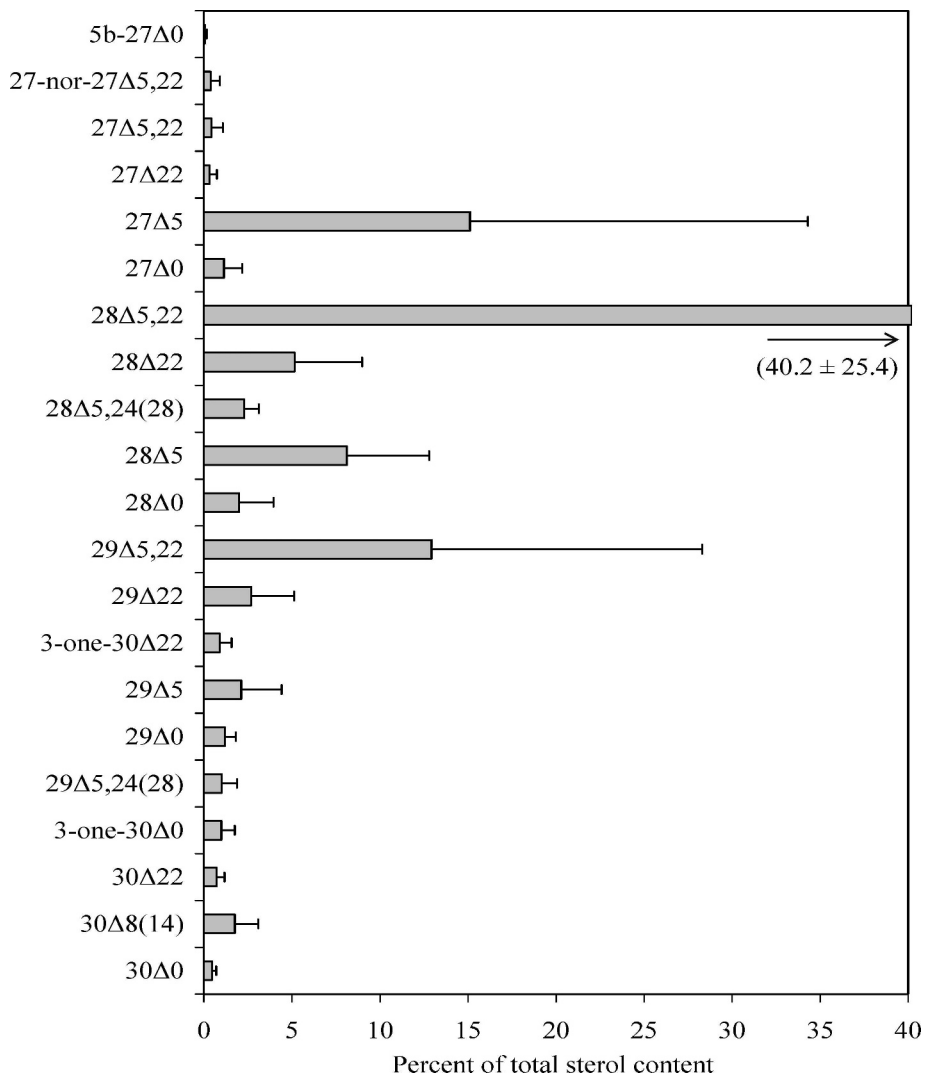


Fig. 3. Mean abundance and standard deviation of the identified sterols in the studied samples ($n = 10$). The individual contributions are expressed in terms of percentage of the sum of the 23 identified sterols shown in Table 2.

sterols in all the sediments. Sitosterol is the major sterol compound in higher plants (Volkman 1986), although it is also found in some diatoms and other microalgae (Volkman et al. 1998). Note that the individual sterol contributions to the total sterol pool at Sta. 310 are markedly different from those measured at the other four stations. As this sampling station is located more than 200 km southeast of the main inshore-offshore transect explored in this work (Stas. 306, 305, and 304; Fig. 1), this result likely reflects differences in sedimentary organic matter composition, and by extension organic matter sources, at this site compared to the other four.

In the majority of the investigated samples, the peak for the C_{30} -sterol $30\Delta^{22}$ (dinosterol, peak No. 21) was easily detected, indicating a significant contribution of dinoflagellates to these sediments. Dinosterol is the most utilized biomarker for dinoflagellates even though it is a minor component in some dinoflagellate species and completely absent in others (Withers 1987; Volkman et al. 1993;

Leblond and Chapman 2002). Other dinoflagellate steroidal species present are the corresponding stanol, $30\Delta^0$ (dinostanol, peak No. 24), the $4\alpha,23,24$ -trimethyl steroidal ketones, 3-one- $30\Delta^{22}$ and 3-one- $30\Delta^0$ (dinosterone, peak No. 16, and dinostanone, peak No. 20, respectively). Another $4\alpha,23,24$ -trimethyl sterol, tentatively identified as $30\Delta^{8(14)}$ (peak No. 23), is also present but more work is needed to confirm its structure. These minor components are all indicative of dinoflagellate inputs to the sediments as well.

Similar sterol concentration trends were found for most stations, with a higher proportion of OC that can be identified as sterol carbon at 3–6 cm than at depth (Table 3), suggesting slow degradation or alteration of sterols with time. The only exception is Sta. 304, where the sum of the relative abundances for the identified sterols is slightly higher at a depth of 16–19 cm than at 3–4 cm (1.56 mg sterols g^{-1} OC vs. 2.03 mg sterols g^{-1} OC, respectively). However, this result can be explained by the

Table 3. Sterol concentrations (in percent of total sterol content) in the samples.

Peak	Sterol	Station300	Station306	Station305	Station304	Station310	Average±SD
1	5β-27Δ ⁰	0.0*	0.0	0.0	0.1	0.0	0.02±0.05
		0.0*	0.0	0.3	0.2	0.0	0.09±0.14
3	27- <i>nor</i> -27Δ ^{5,22}	0.2	0.1	1.2	0.0	0.0	0.31±0.52
		0.4	0.1	1.4	0.0	0.3	0.45±0.58
4	27Δ ^{5,22}	0.1	0.1	0.4	0.5	0.0	0.21±0.22
		0.0	0.1	2.1	0.9	0.1	0.63±0.89
5	27Δ ²²	0.1	0.0	0.3	0.4	0.1	0.18±0.16
		0.1	0.1	1.4	0.4	0.2	0.45±0.58
6	27Δ ⁵	8.6	9.2	25.9	9.0	16.8	13.9±7.5
		4.0	4.6	3.9	3.3	65.9	16.4±27.7
7	27Δ ⁰	1.2	1.0	1.2	3.5	0.0	1.39±1.30
		0.4	0.7	2.0	1.3	0.0	0.87±0.77
9	28Δ ^{5,22}	60.8	64.5	28.9	38.5	0.0	38.5±26.2
		32.3	51.0	52.9	73.1	0.0	41.9±27.5
10	28Δ ²²	4.7	5.9	9.5	3.5	3.2	5.34±2.54
		0.0	6.6	12.9	4.2	1.2	4.97±5.15
11	28Δ ^{5,24(28)}	1.8	2.2	1.7	1.0	3.1	1.97±0.76
		2.4	3.7	3.2	1.5	2.3	2.62±0.85
12	28Δ ⁵	5.8	2.5	8.9	10.7	14.7	8.53±4.65
		9.7	5.5	3.4	4.0	16.0	7.70±5.22
13	28Δ ⁰	1.3	0.2	1.1	0.8	5.6	1.79±2.16
		4.4	2.2	0.1	0.3	3.9	2.20±1.97
14	29Δ ^{5,22}	4.1	3.3	3.3	12.3	46.6	13.9±18.7
		35.0	12.5	3.2	3.5	5.4	11.9±13.4
15	29Δ ²²	1.0	1.0	3.2	2.0	8.6	3.12±3.20
		4.7	3.0	1.8	0.7	0.9	2.20±1.65
16	3-one-30Δ ²²	1.4	1.5	0.9	0.7	0.0	0.91±0.61
		0.4	1.6	1.9	0.4	0.1	0.89±0.79
17	29Δ ⁵	1.0	1.4	3.5	8.2	0.4	2.91±3.17
		1.1	1.3	1.4	2.0	0.8	1.34±0.43
18	29Δ ⁰	1.8	0.9	1.6	2.4	0.4	1.40±0.78
		0.8	1.1	0.7	0.7	1.5	0.97±0.35
19	29Δ ^{5,24(28)}	1.4	0.8	2.9	1.6	0.2	1.36±1.02
		0.7	1.6	0.2	0.4	0.3	0.65±0.58
20	3-one-30Δ ⁰	1.7	2.7	1.0	0.4	0.1	1.17±1.05
		0.3	0.9	1.2	1.1	0.6	0.81±0.37
21	30Δ ²²	0.6	0.8	0.9	1.0	0.1	0.67±0.34
		0.4	0.6	1.7	0.9	0.2	0.78±0.58
23	30Δ ⁸⁽¹⁴⁾ (?)	2.0	1.4	3.4	2.7	0.0	1.89±1.29
		2.7	2.3	3.1	0.0	0.0	1.62±1.50
24	30Δ ⁰	0.4	0.5	0.5	0.7	0.1	0.43±0.22
		0.2	0.4	1.0	0.5	0.2	0.45±0.32
Total	(mg sterols g ⁻¹ OC)	1.66	2.11	2.45	1.56	3.67	2.29±0.85
		0.49	1.01	0.43	2.03	1.60	1.11±0.70

* Top value is for depth 3–6 cm; bottom value is for depth 16–27 cm, 16–19 cm, or 19–22 cm (see Table 1).

Table 4. Individual and summed stanol:stenol ratios in the samples.

Ratio	Station300	Station306	Station305	Station304	Station310	Average† (per depth)	Average† (all samples)
27A ⁰ :27A ⁵	0.14*	0.11	0.05	0.39	—	0.17±0.15	0.23±0.17
	0.11*	0.14	0.50	0.38	—	0.28±0.19	
28A ²² :28A ^{5,22}	0.08	0.09	0.33	0.09	—	0.15±0.12	0.15±0.10
	—	0.13	0.24	0.26	—	0.14±0.09	
28A ⁰ :28A ⁵	0.22	0.07	0.12	0.08	0.38	0.17±0.13	0.21±0.16
	0.45	0.41	0.04	0.08	0.25	0.24±0.19	
29A ²² :29A ^{5,22}	0.25	0.29	0.98	0.16	0.19	0.37±0.34	0.31±0.26
	0.13	0.24	0.55	0.20	0.17	0.26±0.17	
29A ⁰ :29A ⁵	1.74	0.62	0.45	0.29	0.96	0.81±0.58	0.83±0.54
	0.68	0.85	0.51	0.37	1.79	0.84±0.56	
30A ⁰ :30A ²²	0.77	0.61	0.54	0.70	0.51	0.63±0.11	0.61±0.09
	0.59	0.71	0.56	0.51	0.64	0.60±0.08	
Σ _{stanols} :Σ _{stenols}	0.13	0.12	0.24	0.17	0.23	0.18±0.06	0.17±0.07
	0.13	0.19	0.29	0.09	0.09	0.16±0.08	

* Top value is for depth 3–6 cm; bottom value is for depth 16–27 cm, 16–19 cm, or 19–22 cm (see Table 1).

† Average ± standard deviation.

extremely high concentration of 28A^{5,22} (*epi*-brassicasterol) found at depth in that sample (1.4 mg g⁻¹ OC, which accounts for almost 74% of the total sterol pool). Except in a few cases, the differences in sterol abundances between the 3–6 cm and the 14–27 cm layers are within a factor of 10, with the majority within a factor of five.

Previous work has shown that stenols, i.e., the A⁵-sterols, are bioconverted following deposition to marine sediments (Volkman 1986; Sun and Wakeham 1998). In anoxic settings, the unsaturation in position C5 of the A⁵-sterols is reductively biohydrogenated to form the A⁰-stanols, while oxic settings promote the oxidation of the hydroxyl group in position C3 of stenols and stanols to the corresponding stenones (also named sterones) and stanones. Steroidal ketones (the 3-one-A⁵ and 3-one-A^{5,22} sterols) are also reduced to the corresponding alcohols in anoxic sediments. Although the major sterols synthesized by algae are stenols, stanols and stenones can also directly derive from specific sources of algae (Volkman et al. 1998; Arzayus and Canuel 2004). Stanol:stenol ratios have however often been used as an indicator of the diagenetic status, or “freshness,” of organic matter in marine sediments (Li et al. 1995; Arzayus and Canuel 2004).

In our samples, which cover the higher end of OC concentrations found in the world ocean, very few discernible trends were found in the stanol:stenol ratios for any individual stanol-stanol pair (Table 4). This is true whether comparing intracore (subsurface vs. at depth) or intercore (subsurface or at depth for all cores) variations in the ratios. Such patchiness in sterol concentrations (Table 3) and stanol:stenol ratios (Table 4) has been reported for a similar coastal environment (oxygen minimum zone at the Oman Margin, Arabian Sea; Smallwood and Wolff 2000) and was attributed to variability of inputs and/or physical and biological reworking in the sediments. No evidence for stenol hydrogenation to the corresponding stanol was found at another coastal site where relatively high organic matter content and reducing sedimentary conditions should have supported stenol reduction (Arzayus and Canuel 2004). Alternatively,

varying inputs from algae that specifically produce stanols could also mask the diagenetic signal. Average ratios (all samples, $n = 10$) for the C₂₇ and C₂₈ sterols (27A⁰:27A⁵, 28A²²:28A^{5,22}, and 28A⁰:28A⁵) were generally lower than for the C₂₉ and C₃₀ sterols (29A²²:29A^{5,22}, 29A⁰:28A⁵, and 30A⁰:30A²²), with values ranging between 0.17 and 0.23 versus a range of 0.31 to 0.83, respectively (Table 4). These differences could either reflect faster biohydrogenation kinetics for the heavier sterols, or more likely, the effect of aging of a fraction of the sedimentary organic matter in an intermediate reservoir between the site of production and the sediments: for the most part C₂₇ and C₂₈ sterols originate from local marine sources whereas the C₂₉ sterols are terrestrial biosynthetic products and are thus subject to aging for an unknown amount of time in soils and/or river beds prior to deposition to the sediments. Noteworthy, the within- and between-core variability in the dinostanol: dinostenol (30A⁰:30A²²) ratios is strikingly lower than that of the other stanol:stenol pairs (Table 4). This result could be explained by the fact that these two sterols originate from only one local source (dinoflagellates from the surface ocean) while the other stanol:stenol pairs either are ubiquitous or are synthesized by distant source organisms. The overall stanols:stenols ratios (sum of all stanols divided by the sum of all stenols, or Σ_{stanols}:Σ_{stenols}) are modulated by the ratios calculated for the more abundant C₂₇ and C₂₈ sterols and thus lie at the lower end of the range found for individual stanol:stenol pairs, i.e., 0.09–0.29 (average of 0.17 ± 0.07, Table 4).

The above data suggests that the marine organic matter at these sites is relatively well preserved and has not been extensively altered by biological and physical reworking, in agreement with previous work carried out in the area (Gélinas et al. 2001; Dickens et al. 2006). The absence of marked trends between stations suggests that redox conditions do not constitute a major constraint on sterol preservation/alteration in this environment over a timescale of a few thousands of years. While surprising at first glance, this conclusion can be explained by the fact that surface sediments, where degradation rates for the different classes

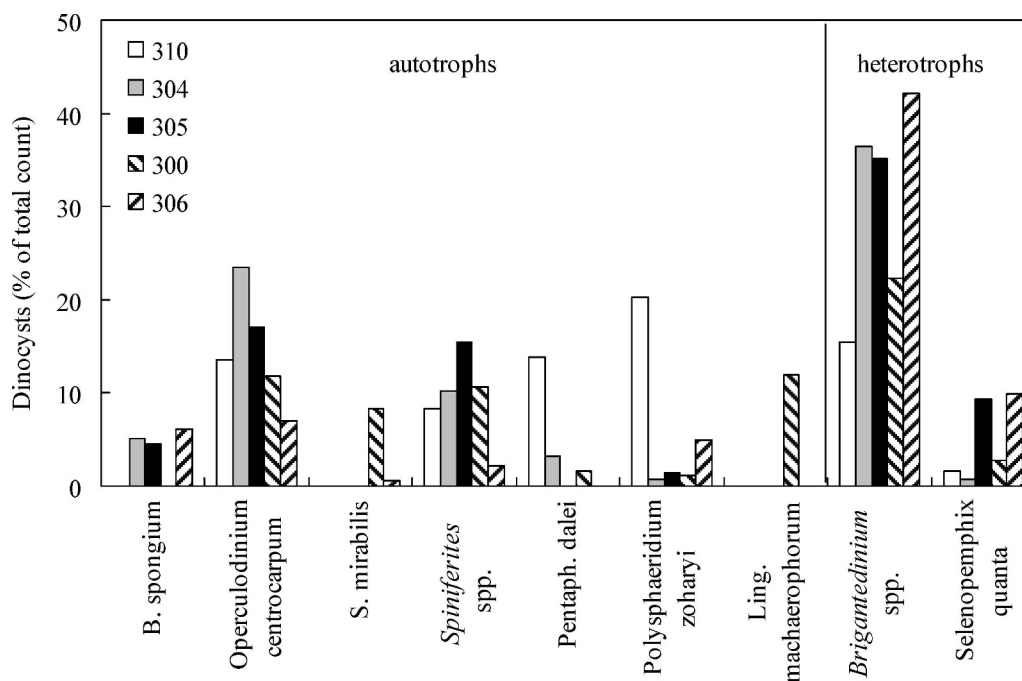


Fig. 4. Relative abundances (% of total dinocyst counts) of the major dinoflagellate species found in the sediments from Stas. 300 to 310. The sum of the contributions for the nine species shown in this graph varies between 71% and 83% of the total dinocyst counts.

of lipids can be as much as one order of magnitude higher than deeper in the sediment (Canuel and Martens 1996), were not analyzed in this work.

Dinocysts counts

Counting of dinocysts is tedious and time consuming. Trained technicians are usually able to process only a few samples per day. The major difficulty lies in the identification and counting of cysts from individual dinoflagellate species, from which quantitative paleoceanographical reconstructions can be made (see for example de Vernal et al. 2005). Thirty-four species of dinocysts were identified in the samples, with fairly uniform relative proportions of each species at the different sampling sites. The most abundant dinocysts were those of the heterotrophic taxa *Brigantedinium* spp. and *Selenopemphix quanta*. These are cysts of *Protoperidinium* spp. and *Protoperidinium conicum*, respectively. The most abundant autotrophic dinocyst species were *Operculodinium centrocarpum* (cyst of *Protoperaetium reticulatum*), *Spiniferites* spp. (cysts of *Gonyaulax* spp.), *Bitectatodinium spongium* (unknown, but probably belonging to *Gonyaulax* sp.), *Lingulodinium machaerophorum* (cyst of *Lingulodinium polyedrum*), cysts of *Pentapharsodinium dalei*, and *Polysphaeridium zoharyi* (cyst of *Pyrodinium bahamense*). The above species contributed between 70% and 83% of the total dinocysts counts in these samples (Fig. 4). Percentages of cysts from total heterotrophic and total autotrophic species varied between 21–56% and 17–52%, respectively, with generally, but not systematically, a dominance of autotrophic species in offshore samples. As could be expected from sampling locations, the

main differences in the relative contributions of each species are found for Stas. 300 and 310, which are located ~200 km north and 200 km south, respectively, of the inshore–offshore transect formed by Stas. 306–305–304. Some species are found in significant amounts only at Sta. 300 (*S. mirabilis* and *Lingulodinium machaerophorum*) or at Sta. 310 (*Pentapharsodinium dalei* and *Polysphaeridium zoharyi*), while others are less abundant at these two stations than at Stas. 304, 305, and 306 (*Bitectatodinium spongium* and *Brigantedinium* spp.).

Comparison of $\Sigma_{\text{dinosterols}}$ and dinocysts distributions

Two recent studies report very weak or non-linear correlations between total dinocyst counts and $30A^{22}$ (dinosterol) concentrations for sediments from the Adriatic (Sangiorgi et al. 2005), Celtic and Irish (Marret and Scourse 2003) seas. Sediments from these two studies were collected in shallow (≤ 100 m), highly dynamic environments, with the former taking place close to the mouth of the Po River resulting in a high contribution of terrestrially derived organic matter. Three potentially major problems might have affected the authors' conclusions: (1) by measuring only the $30A^{22}$ sterol rather than the complete suite of $4\alpha,23,24$ -sterols, biogeochemical alteration of sterols to stanols, stenones and/or stanones was assumed to be negligible in the water column and in recent marine sediments, (2) hydrodynamic sorting, which is particularly important at shallow depths and in the vicinity of large river mouths, could have resulted in the decoupling of mineral particle-attached $4\alpha,23,24$ -sterols and the organic dinocysts in the environments under study, and (3) surface

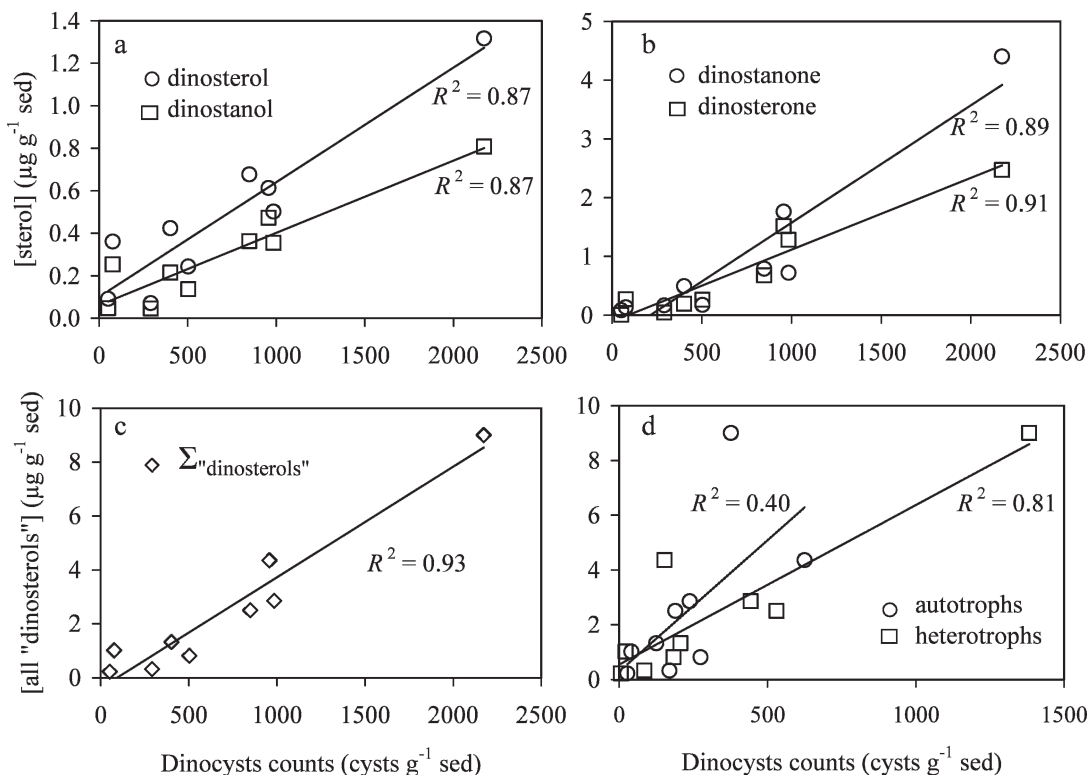


Fig. 5. Correlation between sterol concentrations ($\mu\text{g g}^{-1}$ dry weight sediment) and dinocyst counts (cysts g^{-1} dry weight sediment). (a) Dinosterol and dinostanol; (b) dinosterone and dinostanone; (c) the summed concentration for dinosterol, dinostanol, dinosterone, and dinostanone plotted as a function of the total dinocysts counts; and (d) the summed concentration plotted as a function of total dinocysts from heterotrophic and autotrophic species.

sediments might contain a significant amount of dinosterol comprised in relatively fresh dinoflagellate-derived material that is for the most part rapidly degraded in the very early stages of diagenesis. Moreover, by normalizing dinosterol concentrations and total dinocyst counts to a different reference (μg of dinosterol per gram of organic carbon and total dinocysts per gram of dry sediment, respectively), Sangiorgi et al. (2005) have artificially amplified the effect of mineral dilution and hydrodynamic sorting on the dinocyst-dinosterol correlation. With the exception of one sample (CH50, a possible outlier), the recalculated best fit for the regression between dinocyst counts and dinosterol concentrations for all the Adriatic Sea samples is linear with a correlation coefficient that increases from 0.38 to 0.78 when normalizing both parameters to grams of dry sediment. Similarly, the correlation coefficient found in our study decreases from 0.87 to 0.41 when dinocysts counts are normalized to grams of sediment while dinosterol concentrations are normalized to mg of organic carbon.

To revisit the relationship between dinocysts and dinoflagellate-specific sterols in sediments while minimizing the effect of diagenetic alteration of sterols or hydrodynamic sorting of particles, we measured the concentrations of all the positively identified $4\alpha,23,24$ -sterols ($\Sigma_{\text{dinosterols}}$, see above) in a series of samples from a small geographical area on the western Mexican margin near Mazatlan (Fig. 1). These sampling sites lie at water-column depths ranging from 375 m to 3,500 m and are characterized by

high organic carbon concentrations (2.0–7.9 wt%), variable bottom water redox conditions and low terrestrial organic matter inputs owing to the absence of any rivers with a large water discharge in the area. It is noteworthy that the correlations reported here were obtained using organic matter that has aged in the sediment for a minimum of ~ 750 yr to avoid working with highly reactive surface sediment samples for which sterol degradation rates can be as much as two orders of magnitude higher than deeper in the sediment (Canuel and Martens 1996), and in which the high abundance of fresh and labile organic matter, including dinoflagellate-derived material, can result in the decoupling of the dinoflagellate-dinocyst relationship.

We found positive linear relationships between total dinocyst counts and the concentrations of each individual $4\alpha,23,24$ -trimethylsterol ($30A^0$, $30A^{22}$, 3-one- $30A^0$, 3-one- $30A^{22}$, all normalized to total grams of sediment), with correlation coefficients ranging between 0.87 and 0.91 (Fig. 5a,b). Such strong relationships confirm the previously reported common origin for these $4\alpha,23,24$ -sterols (Leblond and Chapman 2002), either through direct synthesis by dinoflagellates or following the biogeochemical alteration of the predominant $30A^{22}$ parent sterol. They also suggest the differences in redox conditions of bottom waters and surficial sediments between the sampling stations studied here are not large enough to result in measurable differences in the alteration patterns of the $30A^{22}$ sterol, most likely because the analyzed samples have

aged for a minimum of ~ 750 yr in the sediment and were bathed in suboxic or anoxic pore water at the time of collection. Arzayus and Canuel (2004) also reported similar apparent degradation rate constants for stenols and stanols in contrasting deposition regimes. They suggest that sterols and other simple lipids are part of a labile sedimentary organic matter pool that is degraded at comparable rates in oxic and anoxic environments. Significantly important differences could however arise when studying highly reactive surface sediments in which the unsaturated alcohol form is most abundant. Noteworthy, the ketone forms (3-one- $30\Delta^0$ and 3-one- $30\Delta^{22}$) are abundant in all samples, suggesting either that these sterones are directly biosynthesized by dinoflagellates, or that oxidation of the hydroxyl group in position C3 occurs rapidly in the oxic surface waters and are relatively stable even under suboxic and anoxic conditions.

The correlation coefficient increases to a value of 0.93 when plotting $\Sigma_{\text{dinosterols}}$ against total dinocysts counts (Fig. 5c). The increase in correlation suggests that all dinosterol species should be quantified when assessing dinoflagellate inputs to sediments, particularly when comparing samples that have resided in sedimentary regimes with contrasting redox conditions. Although the correlation coefficient falls to 0.77 when the near-surface sample from Sta. 306 (highest $\Sigma_{\text{dinosterols}}$ concentrations and dinocysts counts) is excluded from the regression, the slope remains about the same (0.0041 vs. 0.0033, respectively), suggesting that the data point is not an outlier despite the very high sterol and dinocyst abundances measured in the sample. Importantly, measuring $\Sigma_{\text{dinosterols}}$ concentrations rather than just $30\Delta^{22}$ (dinosterol), or any other individual $4\alpha,23,24$ -steroidal species, reduces the scatter of the data for samples with low dinocyst counts and sterol abundances, which likely is the case for most marine sediments accumulating under oxic bottom water conditions. Although the differences are not statistically significant in some cases owing in part to the small data set considered here, the correlation coefficient between dinocysts counts and any individual dinoflagellate-derived species falls to a range of 0.61 to 0.64 (compared to 0.77 for $\Sigma_{\text{dinosterols}}$) when neglecting the near-surface sample from Sta. 306 (data not shown). The negative intercept on the y -axis, although again not statistically significant, suggests that dinocysts are slightly better preserved than sterols in this environment, in agreement with the more refractory nature of highly cross-linked, macromolecular aliphatic dinosporin compared to simple sterols (Kokinos et al. 1998; Gélinas et al. 2001).

Some studies suggested that cysts from autotrophic and heterotrophic dinoflagellates have contrasting susceptibilities to degradation in oxic environments, with higher degradation kinetics for heterotrophic species on a time scale of $\sim 10,000$ yr (Zonneveld et al. 2001). Our results do not reflect this trend, either when looking at the dinocyst counts for the most abundant dinoflagellate species found in the studied area (Fig. 4), or when plotting total dinocyst counts of heterotrophic (or autotrophic) dinoflagellates species against any individual $4\alpha,23,24$ -sterol, the reduced form of dinosterol (dinostanol, or $30\Delta^0$), or their oxidized

forms (dinosterone and dinostanone, or 3-one- $30\Delta^{22}$ and 3-one- $30\Delta^0$, respectively). Although the correlation found between the total dinocyst counts for heterotrophic species and $\Sigma_{\text{dinosterols}}$ is stronger than for dinocysts of autotrophic dinoflagellates ($r^2 = 0.81$ vs. 0.40, respectively; Fig. 5d), it decreases to only 0.27 when Sta. 306 is excluded from the regression. The large variations in dissolved O_2 concentrations in bottom water and surface sediment pore water thus do not appear to play a major role in the preservation of autotrophic versus heterotrophic cysts in this system, possibly because the time of exposure to oxygen of our samples (maximum of a few thousand years, Hartnett et al. 1998; Gélinas et al. 2001) was much shorter than in the study of Zonneveld et al. (2001). It is clear however that both heterotrophic and autotrophic dinoflagellates contribute to the $4\alpha,23,24$ -sterols pool, and that none of the individual $4\alpha,23,24$ -sterols can be specifically linked to heterotrophic or autotrophic dinoflagellate synthesis (data not shown).

The results obtained in this study suggest that $\Sigma_{\text{dinosterols}}$ is a better proxy than $30\Delta^{22}$ (dinosterol) alone for estimating variations in dinoflagellate inputs to diagenetically stabilized marine sediments, expanding on early studies reporting on the specificity of these molecules for dinoflagellate organisms (Djerassi 1981). Although the improvement is not statistically significant in this case most likely because a large fraction of the possible alteration products of dinosterone (dinostanol, dinostanone, and dinosterone, or $30\Delta^0$, 3-one- $30\Delta^0$, and 3-one- $30\Delta^{22}$ respectively) are directly derived from dinoflagellates, it could become much more significant at longer time scales when alteration products of the dinoflagellate-derived sterols become proportionally more important. Our work also suggests the two independent methods for counting dinocysts and measuring $\Sigma_{\text{dinosterols}}$ provide internally consistent data. Such internal consistency suggests that both methods provide robust, semi-quantitative proxies for the total contribution of dinoflagellates to marine sediments, at least from environments such as the western Mexican margin where hydrodynamic sorting effects are limited, and over time scales of a few thousands of years. In cases where detailed dinocyst assemblage information is not needed, the more commonly available and less tedious GC-based method could thus replace the microscopy-based cyst counting method used in paleoecological laboratories. More work should be undertaken to estimate the sensitivity of the dinocysts- $\Sigma_{\text{dinosterols}}$ relationship to longer time scales and different geographical areas, and to determine which one of the two proxies provides better estimates of dinoflagellate contributions to sedimentary organic matter in these conditions.

Although promising, the relationship between dinoflagellate inputs and $\Sigma_{\text{dinosterols}}$ or total dinocyst counts is most likely not universal and should thus be used with caution. While some of the scatter in the data (Fig. 5c) could originate from the measurement uncertainties affecting both methods, we speculate that it is mostly stemming from hydrodynamic effects that lead to focusing of particles of contrasting densities (Mollenhauer et al. 2006). As dinoflagellate cells and dinocysts are both composed only

of organic materials of very similar densities, little differential sorting of sterols and dinocysts through lateral transport is probably occurring in the water column (both likely have very similar sinking rates). However, upon reaching the sediment surface, the more polar sterol-associated cell membranes (compared to dinocyst surfaces) bind more intimately to mineral surfaces than dinocysts and become anchored to high density particles. Dinocysts on the other hand likely remain free particles of lower density, and their high molecular weight, highly cross-linked, aliphatic matrix makes them highly resistant to degradation, particularly in anoxic environment (Kokinos et al. 1998; Gélinais et al. 2001; Arzayus and Canuel 2004). Resuspension of sediments can therefore influence depositional patterns and lead to the spatial and, possibly, temporal decoupling of dinocysts and 4 α ,23,24-sterols (Mollenhauer et al. 2006), particularly in high-energy environments such as ocean margins and slopes, or in the vicinity of river mouths. Only the coupled compound-specific radiocarbon analysis of 4 α ,23,24-sterols and dinocysts from individual samples would allow testing this hypothesis. If proved valid, it would imply that the dinoflagellate-associated $\Sigma_{\text{dinosterols}}$ constitutes a better proxy than total dinocyst counts for estimating dinoflagellate inputs to marine sediments.

Our data also re-emphasize the importance of considering diagenetic alteration products when exploiting molecular biomarkers for apportioning organic matter (OM) sources or monitoring reaction histories and pathways in recent and ancient sedimentary settings. Contrary to bulk proxies, molecular biomarker analyses are highly specific to the chemical composition and stereochemistry of the studied compound; the most subtle chemical alteration often is sufficient to shift the resulting product away from the narrow analytical window specific to the parent biomarker. Such was the case in this study, although to a limited extent, in which a slightly better correlation was found between dinocyst counts and the sum of all 4 α ,23,24-sterols than when only considering the 30 Δ ²² sterol (dinosterol), and, more importantly, in which the scatter of the data was reduced for samples with the lowest dinocyst and sterol abundances. Where diagenetic effects are much greater than for our series of samples (for example in samples containing even lower amounts of highly degraded organic matter [<2 wt%], such as in pelagic environments, or in ancient paleoenvironments), the measurement of $\Sigma_{\text{dinosterols}}$ rather than dinosterol alone would be even more critical. More work would be needed to confirm these hypotheses but the analysis of 4 α ,23,24-sterols provides an independent new perspective on dinoflagellate inputs to marine sediments, with the potential for an enhanced comprehension of the potential factors affecting the accuracy of the dinocyst records in marine sediments.

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