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SCIENCE DIRECT
Estuarine, Coastal and Shelf Science 64 (2005) 395–406
ESTUARINE COASTAL AND SHELF SCIENCE
www.elsevier.com/locate/ECSS

The distribution of sterols and organic-walled dinoflagellate cysts in surface sediments of the North-western Adriatic Sea (Italy)

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Received 15 November 2004; accepted 7 March 2005
Available online 26 April 2005

Abstract

The distributions of sterols and organic-walled dinoflagellate cysts (dinocysts) in five NW Adriatic Sea surface sediment samples were investigated. Samples are representative of areas differently influenced by freshwater inputs, mainly coming from the Po River. All the investigated samples exhibit the same suite of principal sterols, with cholest-5-en-3β-ol (cholesterol), 4α,23,24-trimethyl-5α-cholest-22*E*-en-3β-ol (dinosterol), 24-ethylcholest-5-en-3β-ol (sitosterol) and 24-methylcholesta-5,22*E*-dien-3β-ol (brassicasterol or epibrassicasterol) displaying the highest concentrations and relative abundances. The distribution of sterols in the samples is not related to their distance from the coast and/or with the C/N ratios and suggests a prevalent input of marine, autochthonous organic matter in the surface sediments. In particular, the high abundance of dinosterol underlines the importance of dinoflagellate productivity in this area and its contribution to the organic matter in sediments. However, absolute and relative abundances of dinosterol do not follow the trend observed for dinocyst concentrations in the investigated samples, with the exception of *Spiniferites* spp. cysts and cysts produced by *Gonyaulax* species.

Keywords: sterols; dinocysts; North Adriatic Sea; surface sediments

1. Introduction

The Northern Adriatic Sea is a seasonally stratified shallow basin (maximum depth about 70 m), which receives high freshwater input from 22 major and minor Italian rivers. These rivers carry large amounts of nutrients and allochthonous organic matter to the sea (e.g., Bortoluzzi et al., 1984; Provini et al., 1992; Pettine et al., 1998). High primary productivity characterizes the area (e.g., Vollenweider et al., 1992; Penna et al., 2004). Phytoplankton biomass and production are very variable both in space and time (Cabrini et al., 2002), since the dynamics of phytoplankton in coastal systems (Zingone et al., 1990) becomes even more complex in areas affected by large freshwater discharge such as the NW Adriatic Sea. Several authors (Vollenweider et al., 1992; Bernardi Aubry et al., 2004 for an overview) have studied phytoplankton distribution in the North Adriatic Sea. They show that the phytoplankton population is composed of hundreds of species, the majority of which are dinoflagellates, even if, as expected in nutrient-enriched systems, the community structure is dominated by diatoms over most of the year. Blooms frequently occur, especially close to the Italian coast,

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doi:10.1016/j.ecss.2005.03.005

due to the water circulation patterns, which confine the nutrient-enriched waters near the coast (Franco and Michelato, 1992; Zavatarelli et al., 1998). Blooms are produced by few species, such as the diatoms *Skeletonema costatum*, *Thalassiosira decipiens*, *Chaetoceros* spp., *Pseudo-nitzschia delicatissima*, and the dinoflagellates *Glenodinium lenticula*, *Prorocentrum* spp., *Lingulodinium polyedrum*, *Scrippsiella trochoidea*, *Gymnodinium* sp., *Gonyaulax fragilis* and *Alexandrium tamarense* (e.g., Boni et al., 1986; Vollenweider et al., 1992). Dinoflagellates are most abundant in June–July, once the spring bloom of diatoms has left relatively nutrient-poor conditions in the waters. Dinoflagellates in fact have lower nutritional requirements than diatoms (Thingstad and Sakshaug, 1990) and show a more thermophilic character (Bernardi Aubry et al., 2004).

In the North Adriatic Sea, the concentrations of particulate organic carbon (POC) and particulate organic matter (POM) depend mainly on the input of the Po River, the largest Italian river, and from the phytoplanktonic biomass (Gilmartin and Revelante, 1991). POC and POM show clear vertical and horizontal concentration gradients, which are linked to freshwater input and to the development of frontal systems. Surface waters and coastal waters usually have about 30–70% higher POC compared to open and deep waters, respectively (Matteucci and Frascari, 1997). Although the influence of external input on this basin is high, the cycling of organic matter and information on organic carbon sources are still poorly understood in the North Adriatic Sea. Due to their relatively good chemical stability and their taxonomically biased structures, sterols have been widely used as tracers for studying sources of organic matter in coastal areas (Grimalt et al., 1990; Yunker et al., 1995; Hudson et al., 2001; Pinturier-Geiss et al., 2002). In the Adriatic Sea, the distribution of sterols has been mainly investigated in biota, including marine molluscs (Piretti and Viviani, 1976, 1989; Piretti et al., 1982, 1987), zooplankton (Serrazanetti et al., 1982, 1992) and microalgae (Piretti et al., 1997). The few studies published on the distribution of sterols in Adriatic Sea particulate matter and sediments dealt principally with the origin of organic matter in estuarine and coastal lagoonal areas (Laurcillard and Saliot, 1993; Benfenati et al., 1994; Fattore et al., 1996).

Most of the investigations on marine productivity in the North Adriatic sediments deal with the analyses of foraminifera assemblages, geochemistry and total organic matter content (e.g., Bortoluzzi et al., 1984; Donnici and Serandrei Barbero, 2002; Duijnstee et al., 2004). Few studies consider the analyses of organic-walled dinoflagellate cyst (dinocyst) assemblages in this area as tools to reconstruct primary productivity in the sediments (Sangiorgi et al., 2001). Nevertheless, dinocysts preserved in sediments are the remains of a conspicuous group of primary producers especially in this basin (Vollenweider et al., 1992) and they have shown to be good indicators of (paleo-)productivity changes not only in this basin (Sangiorgi and Donders, 2004), but also in other geographical settings (e.g., Dale et al., 1999; Matsuoka, 1999). Frustules of diatoms, which are the main primary producers, are easily dissolved in the water column or in the sediments (Puškaric et al., 1990). Only about 15% of dinoflagellate species form cysts; the cyst–theca relationship for many of them is not known and also the mechanisms leading to cyst formation are not completely clear (Dale, 2001). Nevertheless, investigations of living phytoplankton are only marginally informative, since phytoplankton is highly variable in space and time.

The aim of this paper is to supply the first detailed information on the distribution of sterols in the North Adriatic Sea surface sediments in conjunction with the distribution of organic-walled dinocysts. The abundance of dinosterol and organic-walled dinocyst contents is compared to verify if a similar trend between these chemical and palynological indicators for dinoflagellates may exist.

2. Materials and methods

2.1. Sampling sites

Five surface sediment samples (0–1 cm depth) were collected by means of a box-corer in 1998, three along a transect south of the city of Chioggia, in the Po River Delta mouth area (CH64, CH55 and CH50), and two from offshore the city of Ancona (AN35 and AN33) (Fig. 1). Considering the sediment accumulation rates calculated for the area, which vary approximately between 2 and 14 mm $year^{-1}$ (e.g., Frignani and Langone, 1991; Langone et al., 1996; Hammond et al., 1999; Cattaneo et al., 2003), the surface samples analyzed should represent the last few years.

The box-corers were immediately sealed and transported to the laboratory where they were kept at 4 °C. Part of the top layer of each sample was subsequently used for dinocyst analyses and organic matter determination and the remainder stored at −15 °C for sterol analyses. Specific information on the samples is reported in Table 1.

2.2. C/N analyses

Total nitrogen (TN) and total organic carbon (TOC) were determined using a Fisons NA 1500 CHN analyzer. For the determination of organic carbon, dry samples were previously acidified with 1 M HCl in order to eliminate carbonates (Hedges and Stern, 1984). Standard deviation is less than 5% for TOC and less

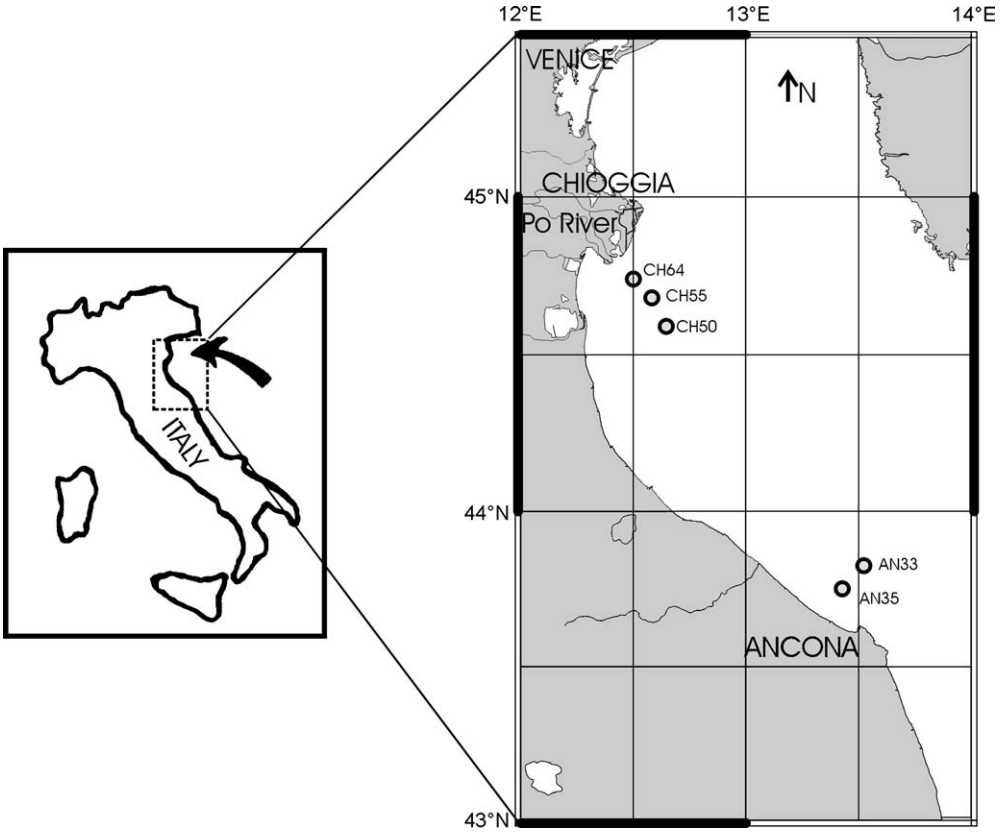

Fig. 1. Location of the surface sediment samples.

than 10% for TN. Carbonate content was determined by dry weight difference between the initial sample and the sample after acidification.

2.3. Determination of sterols

2.3.1. Sample treatment

About 3 g of wet sediment sample was extracted three times with a mixture of dichloromethane/methanol (DCM/MeOH) (3:1 v/v) (24 ml, 8 h under reflux). The organic extracts were collected, rinsed three times with 3 ml of sodium chloride solution (5%), dried over anhydrous sodium sulphate, and the solvent was removed by rotary evaporation. The residue was saponified by refluxing with 3 ml methanolic KOH (6% w/v) for 4 h. The solution was then diluted with 5 ml of water, acidified with 6 M HCl to about pH 2 and neutral/acidic lipids were subsequently extracted with 3×3 ml of *n*-hexane. The organic layers were combined, evaporated to dryness and the residue was dissolved again in 1 ml DCM and chromatographed on a solid phase extraction cartridge containing 1 g silica as stationary phase (Mega Bound Elute Si purchased from Superchrom). Two fractions were collected by elution with 15 ml *n*-hexane/DCM 1:1 v/v followed by elution with 15 ml DCM. The second fraction containing sterols was concentrated and treated with 0.5 ml *N*,*O*-bis(trimethylsilyl)trifluoracetamide (BSTFA) overnight in a desiccator for converting sterols into the corresponding trimethylsilyl (TMS) ethers. The solution was evaporated to dryness by nitrogen gas blow-down; the residue was dissolved in 50 μl DCM containing 5α-cholestane as internal standard (10 mg/l) and analyzed by GC–MS.

Table 1
Sample identification codes, depths of the overlying water column, sediment clay percentages, carbonates and organic carbon percentages, and C/N ratios in the sediment samples analyzed

Sample name	Water column depth (m)	% Fraction < 63 μm	CO_3%	C%	C/N
CH64	23	99.47	42	1.20	10.03
CH55	31	99.25	40	0.92	9.92
CH50	35	98.05	41	0.82	8.87
AN35	49	97.70	38	0.79	9.45
AN33	70	97.20	39	0.76	8.75

2.3.2. GC–MS analysis

GC–MS analyses were performed with a Varian 3400 gas chromatograph equipped with a septum programmable injector split/splitless (Varian 1078) coupled to a Saturn 2000 ion-trap mass spectrometer (Varian Analytical Instruments). Compounds were separated on a 30 m × 0.32 mm i.d., 0.25-μm film thickness, SPB5

capillary column (Supelco) with helium as carrier gas at a flow rate of 1.5 ml min^{-1}. The oven temperature was held at 50 °C for 1 min then programmed at 10 °C/min to 300 °C and held at 300 °C for 35 min. Injector temperatures were programmed from 250 °C to 300 °C at 100 °C/min and held in splitless mode for 1.5 min. Transfer line temperature was 280 °C and the ion trap set at 200 °C. Electron-impact mass spectra (electron energy 70 eV) between m/z 50 and 600 were recorded at 1 scan s^{-1}.

2.3.3. Quantitation

Sterol TMS-ethers identification was tentatively established by comparison with literature data on the distribution of sterols in marine sediments (Volkman, 1986; Bayona et al., 1989; Hudson et al., 2001), including data available for the Adriatic basin (Laurcillard and Saliot, 1993; Fattore et al., 1996), and on the interpretation of mass spectra. In particular, for sterols and steroidal ketones of dinoflagellates, comparison was made with GC–MS data reported by Harvey et al. (1988), Mansour et al. (1999), Leblond and Chapman (2002, 2004). The identification was confirmed by the analysis of TMS-ethers of pure standard for cholesterol, β-sitosterol and stigmasterol (Aldrich), and for dinosterol from the analysis of sterols extracted from the dinoflagellate *Prorocentrum micans* isolated from the North Adriatic and cultured in our laboratories (unpublished data). No attempt was made to distinguish between sterols epimers at C-24, or between sterol isomers for the configuration of the side chain double bonds. When the possibility of more than one stereoisomer is possible we indicate, for simplicity, the name of the sterol most commonly found in sediments.

Quantitation was accomplished by using the peak areas determined in the mass chromatograms using characteristic ions normalized to that of 5α-cholestane as internal standard (using ion fragments at m/z 217 + 357). Analysis of extracts without the addition of internal standard showed that cholestane was not present at detectable levels in sediments. The precision of the method was determined by triplicate analyses of sediment samples collected at site CH50. Percent standard deviations of calculated sterol concentrations ranged from 5% to 29%, 12% on average. Recovery of the overall procedure was determined by analysis of a wet sediment sample (about 3.5 g) spiked with 0.30 ml of a DCM solution containing 1500 ng cholestane. Recovery of cholestane was 85% ± 14% ($n = 3$). No sterols were detected in procedural blanks, performed following the overall procedure in the absence of sediment sample.

2.4. Dinocyst analyses

For quantitative dinocyst analyses, sediment samples were dried at 60 °C, weighed and treated with 10% HCl and 38% HF in five alternate and subsequent steps; decantation was carried out after each step. Samples were sieved over a 10-μm sieve and the residue was centrifuged (5 min; 2500 rpm) and concentrated to 1 ml. With a micropipette, sub-samples of a known volume (10–50 μl) of homogenized residue were placed on a microscope slide, embedded in glycerin jelly and sealed with paraffin wax. Whole slides were counted for dinocysts (up to 500 specimens in rich samples) using a light microscope at magnifications of 400×. Dinocyst taxonomy follows Williams et al. (1998).

3. Results and discussion

3.1. Bulk analyses

Percentages of organic carbon are reported in Table 1 along with carbonate contents and C/N ratios.

The three sites along the Chioggia transect exhibit organic carbon content around 1% similar to the values reported for surface sediments influenced by the Po river deposits (Giordani et al., 1992; Fabbri et al., 2001), whereas lower values are observed in the Ancona transect. The C/N atomic ratios range between 8.8 and 10.0 and values slightly decrease with increasing distance from the coast for both transects. This trend could be partly ascribed to a decreasing contribution of terrestrial organic matter as indicated by the analysis of pyrolytic and lipid molecular markers (Fabbri et al., 2005). Deviations from the classical Redfield ratio associated with plankton materials (C/N = 6.6) mainly reflect the different contribution of continental derived organic materials and the extent of diagenetic processes (Meyers, 1994, 1997). Giani et al. (2001) showed that C/N ratios in material collected from sediment traps are usually lower than in sediments, indicating a probable faster degradation of nitrogen containing components (protein, amino acids, etc.) with respect to other kind of organic compounds during sedimentation. Comparison between our data and data previously derived from Northern Adriatic sediments shows good agreement (e.g., Giani et al., 2001). C/N ratios determined in sediments along the Po estuary ranged between 4.7 and 12 at riverine and tidal sites and between 7 and 15 at marine sites (Martinotti et al., 1997).

3.2. Sterols

A typical total ion chromatogram in the time region of sterol TMS-ethers obtained from sediment samples is shown in Fig. 2. The structural attribution of the principal GC–MS peaks is presented in Table 2. Besides C_{28} and C_{30} fatty alcohols (peaks # 4 and 16) indicative of a terrigenous component (Fabbri et al., 2005), the most intense peaks are associated to cholest-5-en-3β-ol

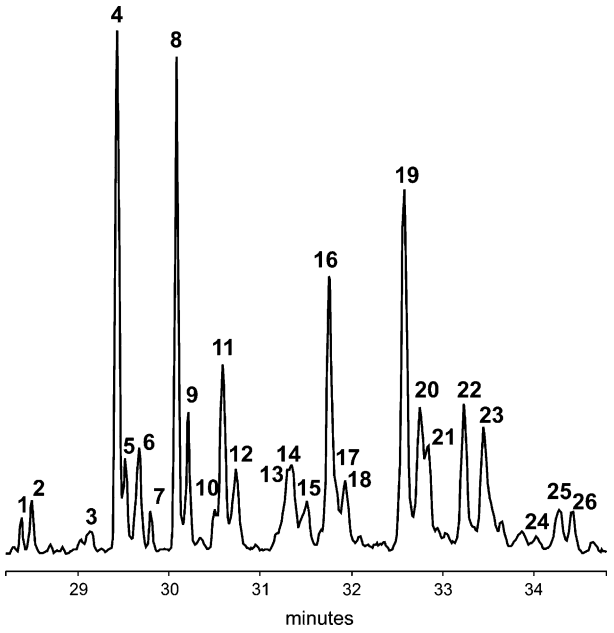

Fig. 2. Total ion GC–MS trace in the region of sterols obtained from the analysis of surface sediment from station CH50. Peak numbers refer to compounds listed in Table 2.

(peak # 8, cholesterol), 24-methylcholesta-5,22*E*-dien-3β-ol (peak # 11, brassicasterol or its epimer at C_{24}) and 24-ethylcholest-5-en-3β-ol (peak # 19, sitosterol). The corresponding 5α(H)-stanols produce quite intense signals (peaks # 9, 15 and 20), whereas 5β(H)-stanols (e.g., peak # 3, attributed to coprostanol) are identified at significant levels at sites closer to the coast probably as a result of sewage contamination (Fattore et al., 1996).

Although most of the sterols are represented by species with 27, 28 and 29 carbon atoms, the C_{30}-sterol dinosterol produces an intense peak (# 22) in the chromatograms obtained from all the investigated sediments. In addition to dinosterol, other minor 4-methylsterols typical of dinoflagellates (# 24, 25 (dinostanol) and 26), have been tentatively identified, confirming the contribution of dinoflagellates to the pool of sedimentary organic matter. However, the corresponding steroidal ketones (e.g., dinosterone and dinostanone) could not be revealed in the lipid fractions, although these compounds can be found in sediments along with 4-methylsterols (e.g., Bayona et al., 1989).

The $27\Delta^{5,22}$ sterols 27-*nor*-24-methylcholesta-5,22*E*-dien-3β-ol and cholesta-5,22*E*-dien-3β-ol (peaks # 5 and 6) have been already identified in estuarine sediments of the Adriatic Sea (Laurcillard and Saliot, 1993). There are also smaller peaks assigned to C_{26}-sterols (peaks #1 and 2 co-eluting with C_{27} alcohol).

Table 3 reports the concentrations normalized to organic carbon content of selected major plant sterols, while the corresponding percentage distributions are depicted in Fig. 3. All the investigated sites show

Table 2
Structural assignment of GC–MS peaks reported in Fig. 2

Peak #	Compound[a]	Symbol	*m*/*z*[b]
1	24-*nor*-Cholesta-5,22*E*-dien-3β-ol	$26\Delta^{5,22}$	352, *442*
2	*n*-C_{27}-alcohol + 24-*nor*-5α-cholest-22-en-3β-ol	$26\Delta^{22}$	*468*, *444*
3	5β-Cholestan-3β-ol	27Δ	370, *460*
4	*n*-C_{28}-alcohol		467, *482*
5	**27-*nor*-24-Methylcholesta-5,22*E*-dien-3β-ol**	**$27\Delta^{5,22}$**	**366**, *456*
6	**Cholesta-5,22*E*-dien-3β-ol**	**$27\Delta^{5,22}$**	**366**, *456*
7	5α-Cholest-22*E*-en-3β-ol	$27\Delta^{22}$	345, *458*
8	**Cholest-5-en-3β-ol**	**$27\Delta^{5}$**	**368**, *458*
9	**5α-Cholestan-3β-ol**	**27Δ**	**355**, *460*
10	*n*-C_{29}-alcohol		481, *496*
11	**24-Methylcholesta-5,22*E*-dien-3β-ol**	**$28\Delta^{5,22}$**	**380**, *470*
12	24-Methyl-5α-cholest-22-en-3β-ol	$28\Delta^{22}$	255, *472*
13	**24-Methylcholesta-5,24(28)-dien-3β-ol**	**$28\Delta^{5,24}$**	**386**, *470*
14	**24-Methylcholest-5-en-3β-ol**	**$28\Delta^{5}$**	***472***
15	**24-Methyl-5α-cholestan-3β-ol**	**28Δ**	**459**, *474*
16	*n*-C_{30}-alcohol		495, *510*
17	**24-Ethylcholesta-5,22*E*-dien-3β-ol**	**$29\Delta^{5,22}$**	**394**, *484*
18	24-Ethyl-5α-cholest-22-en-3β-ol	$29\Delta^{22}$	255, *486*
19	**24-Ethylcholest-5-en-3β-ol**	**$29\Delta^{5}$**	**396**, *486*
20	**24-Ethyl-5α-cholestan-3β-ol**	**29Δ**	**215**, *488*
21	**24-Ethylcholesta-5,24(28)-dien-3β-ol**	**$29\Delta^{5,24}$**	**386**, *484*
22	**4α,23,24-Trimethyl-5α-cholest-22-en-3β-ol**	**$30\Delta^{22}$**	**359**, *500*
23	Unknown (contaminant?)		441
24	4α,23,24-Trimethyl-5α-cholest-8(14)-en-3β-ol	$30\Delta^{8}$	410, *500*
25	4α,23,24-Trimethyl-5α-cholestan-3β-ol	30Δ	412, *502*
26	Structural isomer of 25	30Δ	412, *502*

[a] As trimethylsilyl-ethers. In bold sterols selected for quantitation (Table 3).
[b] Mass to charge ratio (*m*/*z*) of ions in mass spectra are reported in bold for quantitation ions, in italics for molecular ions.

a similar pattern in the sterol distribution, which is dominated by cholesterol, brassicasterol (or epibrassicasterol), sitosterol and dinosterol. The four sterols together represent between 69% and 74% of the total sterol content in the samples analyzed (Fig. 3).

Cholesterol is a major sterol in marine zooplankton and terrestrial fauna and it is present at high concentrations in fecal pellets (Volkman, 1986). Cholesterol can be abundant in trap materials, and is a minor component in sediments when fecal pellets have a minor influence on sediment composition (Yunker et al., 1995). Cholesterol is also widespread in marine algae and is the predominant sterol in some diatoms (Volkman, 1986; Barrett et al., 1995) and in some dinoflagellates including *Prorocentrum micans* (Volkman et al., 1999). High percentages of cholesterol have been registered in some *Gonyaulax* species, such as *Lingulodinium polyedrum* (formerly named *Gonyaulax polyedra*) involved in massive blooms (Alam et al., 1979). As its origin as animal or vegetal (as well as autochthonous or autochthonous) could be questionable in absence of other evidence (e.g., compound specific carbon isotopic ratio, Oldenburg et al., 2000), cholesterol is taken as

Table 3
Sterol concentrations (μg/g organic carbon) in surface sediments

Peak #	Compound	Common name[a]	Symbol	Site CH64	Site CH55	Site CH50	Site AN35	Site AN33
5	27-*nor*-24-Methylcholesta-5,22*E*-dien-3β-ol	Occelasterol	$27\Delta^{5,22}$	0.8	1.9	1.2	3.7	2.1
6	Cholesta-5,22*E*-dien-3β-ol	Dehydrocholesterol	$27\Delta^{5,22}$	6.3	6.5	0.91	7.1	3.3
8	Cholest-5-en-3β-ol	Cholesterol	$27\Delta^{5}$	44.2	29.4	13.4	27.9	12.1
9	5α-Cholestan-3β-ol	Cholestanol	27Δ	5.3	7.6	1.0	3.2	2.0
11	24-Methylcholesta-5,22*E*-dien-3β-ol	Brassicasterol (24β) or epibrassicasterol (24α)	$28\Delta^{5,22}$	7.5	10.1	4.9	9.4	4.5
13	24-Methylcholesta-5,24(28)-dien-3β-ol	24-Methylenecholesterol	$28\Delta^{5,24}$	3.4	5.8	1.6	3.2	1.2
14	24-Methylcholest-5-en-3β-ol	Campesterol	$28\Delta^{5}$	4.4	4.1	1.6	5.3	1.6
15	24-Methyl-5α-cholestan-3β-ol	Ergostanol	28Δ	1.5	2.7	0.4	0.3	0.3
17	24-Ethylcholesta-5,22*E*-dien-3β-ol	Stigmasterol	$29\Delta^{5,22}$	5.6	4.0	1.6	5.2	2.2
19	24-Ethylcholest-5-en-3β-ol	Sitosterol	$29\Delta^{5}$	20.0	33.2	4.7	4.8	5.4
20	24-Ethyl-5α-cholestan-3β-ol	Stigmastanol	29Δ	3.3	5.2	1.2	1.8	0.9
21	24-Ethylcholesta-5,24(28)-dien-3β-ol	Fucosterol	$29\Delta^{5,24}$	1.2	5.3	1.2	3.8	1.1
22	4α,23,24-Trimethyl-5α-cholest-22-en-3β-ol	Dinosterol	$30\Delta^{22}$	18.3	28.3	3.7	31.0	10.1

[a] The name of the probable most common isomer is reported. Peak numbers refer to Fig. 2 and Table 2.

a marker for marine plankton (Grimalt and Albaigés, 1990; Hudson et al., 2001) and probably indicates extensive grazing of primary marine biomass by zooplankton or benthic fauna. Cholesterol has been reported to be the predominant sterol in zooplankton collected in Adriatic Sea (Serrazanetti et al., 1992). In the samples analyzed, cholesterol is more concentrated in the coastal areas. Although the phyto-zooplankton relationships are not widely studied in the North Adriatic Sea, zooplankton and microzooplankton play a basic role in the dynamics of the phytoplankton populations because they control primary productivity with their predatory action and they supply nutrients, which stimulate algae growth (Cattani and Corni, 1992).

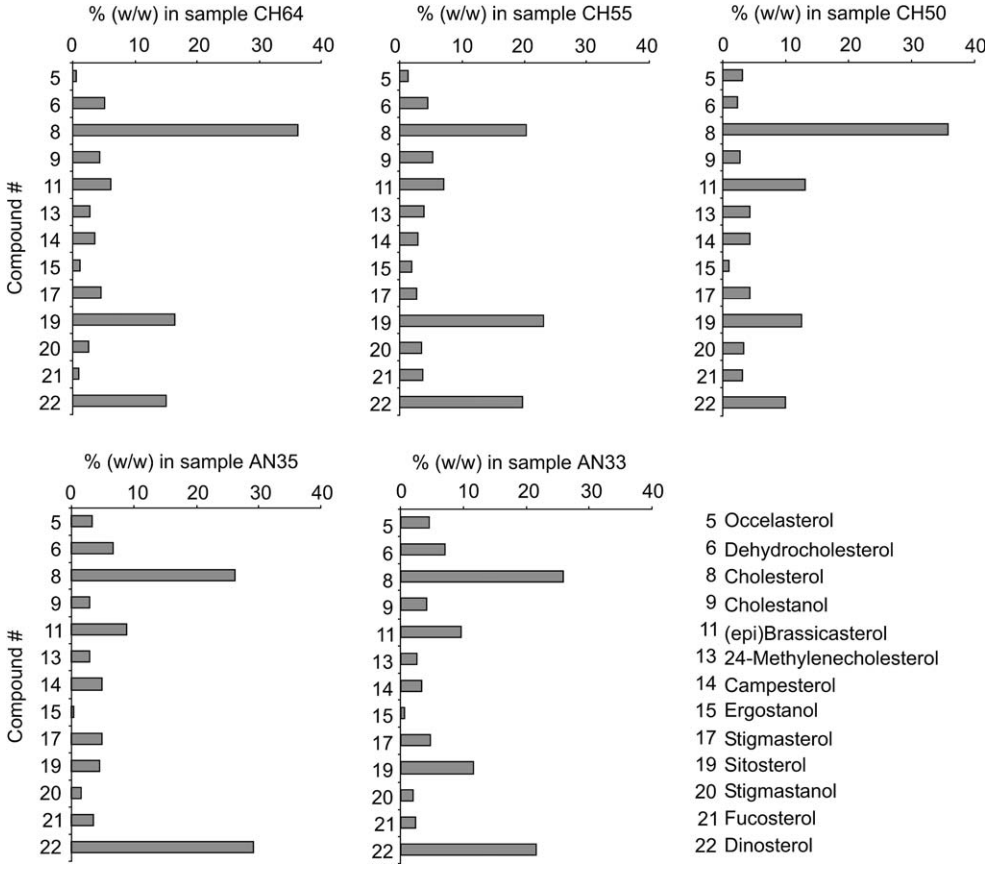

Fig. 3. Relative abundances (% w/w) of the sterols found in the surface sediment samples analyzed (numbers refer to peak # as listed in Table 2).

Microzooplankton blooms have been observed along the North Adriatic coast together with dinoflagellate blooms. High abundance of large tintinnids has been associated with red tide phenomena and in particular *Gonyaulax* blooms (Cattani and Corni, 1992 and references therein).

The 29Δ^5 sterol sitosterol has been used as a marker of allochthonous materials in estuarine environments (Laurcillard and Saliot, 1993; Mudge and Norris, 1997), due to its abundance in land plants. In our samples, sitosterol does not follow the trend expected to trace terrestrial material with decreasing abundance as the distance from the coast (or depth) increases. Sitosterol is more concentrated in the middle sample in the Chioggia transect and in the most offshore sample along the Ancona transect. A comparison with the distribution of molecular markers of terrestrial origin (lignin phenols, long chain fatty alcohols and *n*-alkanes) strongly suggests that 24-ethylcholest-5-en-3β-ol might be of marine origin in the investigated area (Fabbri et al., 2005). In fact, 24-ethylcholest-5-en-3β-ol can be the major sterols in many microalgae, and in marine sediments an autochthonous contribution of 24-ethylcholest-5-en-3β-ol is consistent with the fact that they are major components of some diatom species (Volkman, 1986; Barrett et al., 1995).

The C_{28}-sterol 24-methylcholesta-5,22*E*-dien-3β-ol (brassicasterol) is often related to diatoms, but it is also abundant in other algal groups (Volkman, 1986) including the dinoflagellate *Gymnodinium simplex* (Goad and Withers, 1982), and haptophytes (Volkman et al., 1998). It was detected in the water column after massive blooms of *Emiliania huxleyi* (Conte et al., 1995); in the Adriatic Sea coastal system, however, coccolithophorids are usually scarce (Totti et al., 2000; Bernardi Aubry et al., 2004).

In comparison to the above-discussed sterols, dinosterol is usually considered more taxonomically specific, being characteristic of dinoflagellates, although it can be a minor sterol (or even lacking) in some species (Robinson et al., 1984; Withers, 1987; Harvey et al., 1988; Volkman et al., 1993; Mansour et al., 1999; Leblond and Chapman, 2002). Dinosterol has been found as an abundant component in several cultured dinoflagellates collected in the Adriatic Sea such as *Prorocentrum micans*, *Lingulodinium polyedrum*, *Gymnodinium* sp. and, mostly, *Alexandrium tamarense* (Piretti et al., 1997). Its relatively high concentration in all the analyzed sediments, except in the offshore sample of the Chioggia transect, underlines the importance that this phytoplankton group has to the pool of organic matter in sediments. In the Chioggia transect the relative abundances of dinosterol are 15%, 20% and 10% at sites CH64, CH55 and CH50, respectively, while in the Ancona transect abundances are 29% and 22% at site AN35 and site AN33, respectively. Although the high abundance of dinosterol in sediments is not necessarily an indication that dinoflagellates are major phytoplankton constituents in the environment (De Leeuw et al., 1983), dinosterol has been found at low levels in sediments where dinoflagellates were not abundant algal species in surface waters (Yunker et al., 1995; Hudson et al., 2001).

The variety of sterols identified in the sediment extracts is the consequence of the different origin of the organic matter and the result of the richness of phytoplankton species in the NW Adriatic surface waters (e.g., Bernardi Aubry et al., 2004) as well as of the diversity of sterols occurring in many algal species (e.g., Volkman et al., 1998).

The original fingerprint of sterols in sediments can be modified by inter-related factors, such as bioturbation processes and oxic/anoxic conditions (Sun and Wakeham, 1999; Sun et al., 2002; Grossi et al., 2003), and other factors such as column water depth, sedimentation rates, grain-size (Sinninghe Damsté et al., 2002). All the investigated samples have comparable grain-size (Table 1), and are located in areas with high sedimentation rates (e.g., Frignani and Langone, 1991; Langone et al., 1996; Hammond et al., 1999; Cattaneo et al., 2003) and with similar oxygen conditions, where water stable stratification occurs seasonally especially in the open waters (Franco and Michelato, 1992). In fact, beside the complex dynamics of this basin, during periods of vertical stability, the boundary between coastal and open waters is placed along the 15 m isobath approximately (Franco and Michelato, 1992). In the analyzed samples, the concentration of sterols does not seem to show any particular trend with water column depth.

Altogether, the data obtained lead us to believe that the autochthonous/marine contribution to the total sterol concentrations is predominant over the allochthonous/terrestrial source. Nonetheless, a recent study has established that these sediments contain a strong component of terrigenous organic matter, which decreases seawards (Fabbri et al., 2005). Hence it is possible that a fraction of sterols might be ultimately derived from phytoplankton living in the eutrophic Po River.

3.3. Dinoflagellate cysts distribution

The most abundant dinocyst species or groups, which account for at least 81% of the assemblages, are the heterotrophs *Brigantedinium* spp. and *Selenopemphix quanta* (cysts of *Protoperidinium* spp. and *Protoperidinium conicum*, respectively), and the autotrophs *Lingulodinium machaerophorum* (cysts of *Lingulodinium polyedrum*), *Operculodinium centrocarpum* (cyst of *Protoceratium reticulatum*) and *Spiniferites* spp., mainly *Spiniferites membranaceus* and *Spiniferites ramosus* (cysts of *Gonyaulax* spp.). Heterotrophic dinoflagellates feed on diatoms and other organic matter (Jacobson and

Anderson, 1986), and usually the concentration and/or the proportion of cysts of this group in the assemblages is considered an indicator for primary productivity in coastal waters (e.g., Dale et al., 1999; Matsuoka, 1999; Sangiorgi and Donders, 2004). Nevertheless, heterotrophic dinocysts have shown to be very sensitive to oxygen content in the water column (e.g., Zonneveld et al., 2001). Therefore the occurrence of heterotrophic cysts in sediments can be affected by oxygen availability in waters. In the North Adriatic Sea about a half of the productivity in surface waters reaches the sea floor (Giordani et al., 1992) and anoxic or near anoxic events frequently occur in bottom waters, especially in late summer and autumn as a consequence of high downward organic fluxes, microbial decay and thermal stratification (Justic et al., 1987; Degobbis, 1989; Zavatarelli et al., 1998). In order to avoid interpretations biased by possible different oxygen concentrations of the overlying waters and/or sediments, a group of oxygen resistant cysts (including *Nematosphaeropsis labyrinthus*, *O. centrocarpum* and *Pentapharsodinium dalei*, according to Versteegh and Zonneveld, 2002) is also taken into consideration for discussion.

Concentrations of heterotrophic dinocysts, *Operculodinium centrocarpum*, total dinocysts and oxygen resistant cysts increase with the distance from the coast in the samples close to the Po River mouth, while they usually decrease seawards in the samples offshore Ancona (Fig. 4). The only exception is represented by the concentration of the cosmopolitan species *O. centrocarpum*, which increases with the distance from the coast also in the Ancona samples. In the Po River mouth offshore transect, the concentration of autotrophic dinocysts displays comparable values, being slightly more concentrated seawards. In the Ancona samples their concentration is higher closer to the coast. These trends may suggest that dinoflagellate productivity is, on average, higher in more open waters than in coastal areas, at least close to the Po River mouth area. It must be noted that, in areas where nutrients are not a limiting factor for phytoplankton growth, such as areas close to a freshwater nutrient-enriched input, diatoms and dinoflagellates prefer different environmental conditions. In general, in the North Adriatic Sea, diatoms are more concentrated close to the coast, whereas dinoflagellates prefer more transparent, calm and open waters, being more abundant offshore. Dinoflagellates have lower nutritional requirements than diatoms (Thingstad and Sakshaug, 1990) and show a more thermophilic character (Bernardi Aubry et al., 2004).

The concentration of *Lingulodinium machaerophorum* decreases seawards both in Chioggia and in Ancona samples. This species shows high affinities for stratified and nutrient-enriched coastal waters, where it often forms seasonal blooms (e.g., Marret and Zonneveld, 2003). Red tides caused by this species are frequent occurrences in the North Adriatic close to the Italian coast (e.g., Boni et al., 1986); therefore, the species distribution in the North Adriatic samples seems to be related to its environmental preferences. *Spiniferites* spp. (mainly represented by the coastal/neritic species *Spiniferites membranaceus* and *Spiniferites ramosus*) are more abundant in the middle sample along the Chioggia transect (CH55) and in the Ancona sample closer to the coast (AN35). In general, this group includes cysts produced by *Gonyaulax* spp., although *S. membranaceus* and *S. ramosus* are formed by distinctively different motile dinoflagellates (Lewis et al., 1999). The concentrations of cysts produced by dinoflagellates belonging to the genus *Gonyaulax* (*Spiniferites* spp., *Bitectatodinium*

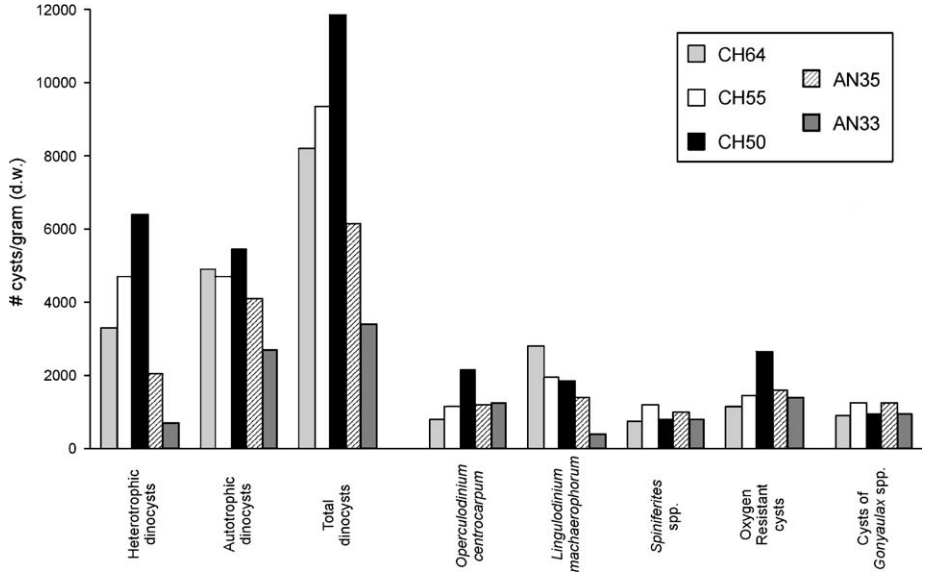

Fig. 4. Concentrations (# cysts/g sediment, dry weight) of the main dinoflagellate cyst groups or species in the sediment samples.

tepikiense and *Nematosphaeropsis labyrinthus*) are also reported in Fig. 4.

3.4. Comparing the distributions of dinocysts and dinosterol

Although a rigorous statistical analysis is not applicable due to the low sample size ($n = 5$), it is worth comparing the distribution of dinocysts and dinosterol in the investigated sediments. From Fig. 5, it is apparent that the absolute abundance of dinosterol in sediments does not parallel the concentrations of dinocysts, even when taking into account the potentially different oxygen levels and therefore the possible different degrees of cysts preservation in the sediments (i.e. the distribution of heterotrophic or oxygen resistant cysts). However, when the trends exhibited by more specific groups are considered, we can observe a certain degree of correspondence with the dinosterol distribution for the *Spiniferites* spp. and the dinocysts produced by *Gonyaulax* species. In fact, for these groups, the largest concentrations of cysts are found at sites CH55 and AN35, where dinosterol displays high values of both relative abundance and absolute concentration. This result can be considered in agreement with what stated by Marret and Scourse (2002), who found a positive exponential correlation ($R = 0.61$) between cysts concentration and dinosterol in samples from the Irish and Celtic Seas, where the *Spiniferites* species dominated the assemblages.

Such a general mismatching between dinosterol and dinocyst concentrations is though not completely surprising since only 15% of the living dinoflagellate produce a resting cyst (Dale, 2001), and also different dinoflagellate species produce more than one sterols and some do not produce dinosterol at all (Withers, 1987). Even dinoflagellates belonging to the same genus such as *Prorocentrum* spp. produce different sterol in different quantities (Volkman et al., 1999). Finally, in the North Adriatic Sea, not all the dinoflagellates commonly forming blooms, which could represent the major contribution to dinosterol in sediments, also form an organic-walled cyst. Therefore, a direct and clear relationship between dinocysts and dinosterol cannot be achieved and (paleo-)productivity interpretations using only dinosterol or dinocysts should be considered with caution, at least in such complex environments.

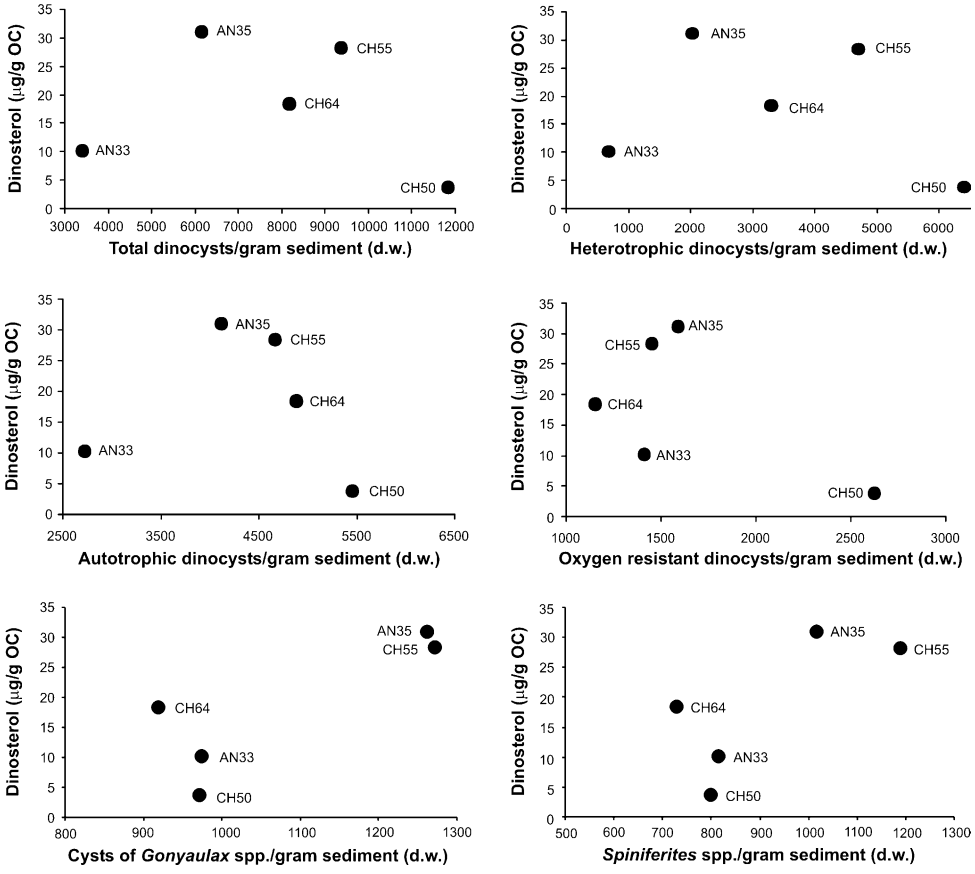

Fig. 5. Dinosterol concentration (µg/g organic carbon) versus concentrations of different dinocyst groups.

4. Conclusions

The sets of sterols found in the two different areas of the North Adriatic Sea sediments are fairly comparable and mainly represented by cholesterol, dinosterol, 24-methylcholesta-5,22*E*-dien-3β-ol ((epi)brassicasterol) and 24-ethylcholest-5-en-3β-ol (sitosterol). Their distribution found in the North Adriatic samples underlines a prevailing importance of marine, autochthonous organic matter to the sterol composition of surface sediments, although the terrigenous input is obviously not negligible in these samples (Fabbri et al., 2005).

Ruling out different post-depositional degradation of sterols in the different samples analyzed, cholesterol concentration shows a gradually decreasing trend from the coastal samples seawards possibly as a consequence of zooplankton grazing on primary producers. In fact, some species of microzooplankton feed mainly on *Gonyaulax* species when they generate coastal blooms and red tides (Cattani and Corni, 1992). The relatively high abundance of dinosterol (up to 29%) suggests the important contribution of dinoflagellates to the sediments organic pool. The relative and absolute concentration of dinosterol changes significantly in surface sediments of the investigated area, and the observed pattern is not clearly correlated with dinocyst trends. Discrepancy between dinosterol and dinocyst distributions has been reported in other sites (Pinturier-Geiss et al., 2002). Organic-walled dinocysts do not represent the remains of the entire living dinoflagellate assemblages and dinoflagellates do not produce only dinosterol, when they produce it at all. Therefore, careful conclusions should be drawn when considering dinosterol and/or total dinocyst concentrations as proxies for (paleo-)productivity interpretation and dinoflagellates occurrence.

Acknowledgements

The authors would like to thank Prof. Colantoni and his staff from Urbino University for providing the boat to collect samples during the oceanographical cruise PRISMA2 and Dr. Rossella Pistocchi for providing the culture of *Prorocentrum micans*. F. Sangiorgi acknowledges Dr. Henk Brinkhuis, Dr. Gert-Jan Reichart and the technicians from the Paleobotany/Palynology and Geochemistry laboratories, Utrecht University, The Netherlands, for assistance with palynological and geochemical analyses.

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