Changes in the Abundance and Species Composition of Phytoplankton in the Last 150 Years in the Southern Black Sea

Erik Askov Mousing · Thorbjørn Joest Andersen ·
Marianne Ellegaard

Abstract Changes in total abundance and in species composition of phytoplankton in the last 150 years in the southern Black Sea were investigated through a paleoecological study of two sediment cores. The results show changes in the species composition and a marked increase in total abundance of siliceous protists after around 1960. In core 22 (42°13.534′ N/36°29.555′ E), the dominating species before 1960 were *Cyclotella choctawhatcheeana* and *Thalassiosira oestrupii*. In core 25 (42°6.212′ N/36°37.460′ E), the dominating species before 1960 were *T. oestrupii*, *Cyclotella meneghiniana*, *C. choctawhatcheeana*, and *Pseudosolenia* cf. *calcar-avis*. Core 22 was located in closer proximity to the rim current than core 25, and the differences in total abundance between the cores could be related to differences in local nutrient loading prior to 1960. After around 1960, both cores changed to a community dominated by *C. choctawhatcheeana*. The changes in total abundance and species composition after around 1960 could be related to the increased nutrient loading from the Danube River into the rim current after the late 1960s. The results also showed changes in both total abundance and in species composition of dinoflagellate cysts. The dominating dinoflagellate cysts recorded were *Lingulodinium polyedrum*, *Polykrikos schwartzii*, and *Spiniferites* spp.

Keywords Black Sea · Paleoecology · Eutrophication · Diatoms · Dinoflagellate cysts

Introduction

Since the latter part of the 1960s, the Black Sea has been subject to nutrient enrichment, mainly due to discharge into the north-western shelf region of domestic and agricultural waste from rivers (Oguz 2005). This has transformed the Black Sea from a low production system prior to the 1960s to one that is highly eutrophied (Oguz 2005; Mee 1992). This has resulted in a number of effects on its coastal ecosystems, i.e., loss of shallow water macrophytes (Bologa 1987), changes in phytoplankton biomass, composition and bloom intensity (Bodeanu 1993), a shift in the mesozooplankton community towards species of lower food value for higher trophic levels (Kideys et al. 2000), an enormous increase in organic matter sedimentation resulting in increased hypoxia, and a large decrease in benthic invertebrate abundance and diversity (Konsulov et al. 1998; Zaitsev 1994).

The Black Sea (Fig. 1) is an inland sea located between latitudes 40°55′ and 46°32′ N and longitudes 27°24′ and 41°42′ E and is connected to the Mediterranean Sea through the Bosporus strait and the Marmara Sea. It covers approximately 423,000 km² and has an average depth of 1,262 m. The general circulation is wind driven and features two cyclonic central gyres and a cyclonic rim current closely following the continental slope with a current speed of ca. 25 cm s⁻¹ (Murray et al. 2007; Neuman 1942). The sea functions as a two-layer

E. A. Mousing (✉)
Center for Macroecology, Evolution and Climate,
Department of Biology, University of Copenhagen,
Universitetsparken 15, 2100 Copenhagen, Denmark
e-mail: eamousing@bio.ku.dk

M. Ellegaard
Marine Biological Section, Department of Biology,
University of Copenhagen,
Øster Farimagsgade 2D, 1353 Copenhagen, Denmark

T. J. Andersen
Department of Geography and Geology,
University of Copenhagen,
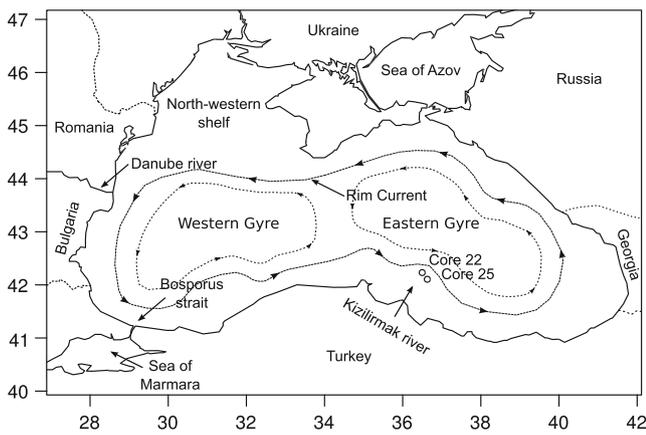Øster Voldgade 10, 1350 Copenhagen, Denmark

Fig. 1 Map of the Black Sea including the position of the cores and the approximate position of the rim current and the two central gyres

system featuring estuarine-like circulation with freshwater input to the surface layer and saline and warm water inflow through the Bosporus strait to the deep water layers (Konovalov et al. 2005; Murray et al. 2007).

The salinity differences causes a brackish surface layer (salinity 18 in the central Black Sea) to be positioned on top of a more saline bottom layer (salinity, 22.3). This supports a strong, permanent pycnocline positioned at 50–200 m depth ($\sigma=16$) (Murray et al. 2007). The pycnocline limits water mixing between the upper and lower layers and effectively minimizes the downward flux of oxygen, resulting in permanently anoxic conditions beneath the pycnocline (Konovalov and Murray 2001; Murray et al. 2007). Furthermore, in the sub-oxic transition zone, the upwards flux of labile nitrogen and phosphorous are totally consumed or removed by either anammox/denitrification processes or MnO_2/FeOOH precipitation and sinking, rendering deep sea nutrients inaccessible for new production in the euphotic layer (Murray et al. 1995; Murray et al. 2005; Kuypers et al. 2003).

The north-western shelf receives 87 % of the total riverine input to the Black Sea with the Danube River being the main contributor, i.e., supplying more than 50–70 % of the total input (Sur et al. 1996; Zaitsev and Mamaev 1997). The rivers on the north-western shelf with the Danube River are also important contributors to the nutrient loading here. Mee (1992) reported an annual introduction of 60,000 t of total phosphorous and 340,000 t of inorganic nitrogen from the Danube River to the north-western shelf. Medians of the mean winter concentration in Romanian coastal waters from 1960 to 1995 show an 18-fold increase in phosphate after 1971, a sixfold increase in nitrate after 1980 and a decrease in silicate to one third of the concentration prior to 1975 (Cociasu et al. 1996). The decrease in silicate after 1980 was caused by the construction of several dams along the Danube River in the 1970s which led to intense diatom blooms in the

dam reservoirs and resulted in a decrease in the silicate input to the Black Sea (Cociasu et al. 1996; Humborg et al. 1997).

Nutrient changes in the Danube River can, potentially, influence the entire Black Sea through lateral transport in the rim current (Sur et al. 1996). Sur et al. (1996, and references within) reported that the eutrophication started on the north-western shelf and progressed south along the western shelf. Export and import of nutrients through the Bosporus strait are roughly in balance (Polat and Tugrul 1995). However, during exceptionally cold years, a temperature-induced decrease in phytoplankton growth rate (Eppley 1972), can result in a significant fraction of the nutrients from the north-western shelf reaching the Strait without being consumed in photosynthesis. This can contribute to new primary production (sensu Dugdale and Goering 1967) in the Marmara Sea in the following spring (Polat and Tugrul 1995). For the open Black Sea, Yunev et al. (2005) reported a gradual increase in the nitrate concentration from the late 1960s until the start of the 1990s, i.e., the period corresponding with the increased nutrient input from the Danube River. Both the north-western shelf and the open Black Sea showed decreasing nutrient levels after 1992, apparently resulting from a decrease in anthropogenic pressure due to the economic transition in Central Europe after the fall of the Soviet Union (Eker-Develi and Kideys 2003; Yunev et al. 2005).

The succession, intensity, frequency, and extent of phytoplankton blooms were modified over the entire basin as a result of the above-described changes in nutrient loading (Oguz 2005). On the north-western shelf, for example, Cociasu et al. (1996) reported a dramatic increase in phytoplankton cell densities in Romanian coastal waters and an increase in the number of blooms from 12 in 1960–1970 to 42 in 1970–1980. Along the western shore, the dominating bloom-forming group changed from being diatoms to dinoflagellates after 1970, and summer blooms occurred frequently in addition to spring blooms (Bodeanu 1993). In the open Black Sea, increased chlorophyll *a* concentrations were observed after 1988, with signs of decrease after 1992. Thus, an increase in chlorophyll *a* was observed later in the open Black Sea compared with the north-western shelf and was believed to be related to the increased nutrient loading (Yunev et al. 2002).

Long-term studies with good spatial coverage of phytoplankton species compositions are not available but there are records of bloom forming species from the north-western shelf (Cociasu et al. 1996). Throughout the 1960s, the bloom forming species were the diatoms were identified as being *Skeletonema costatum*, *Pseudo-nitzschia delicatissima*, *Leptocylindrus danicus*, and the dinoflagellate *Prorocentrum cordatum*. After the mid-1970s, the intensity and frequency of blooms increased with *S. costatum* and *P. cordatum* being

extremely abundant. Humborg et al. (1997) reported an in-crease in diatom blooms by a factor of 2.5 after 1970 with a concomitant increase in non-diatom blooms by a factor of 6. They suggested that the decrease in silicate was the primary cause of the shift to non-diatom species. The pattern was supported by Eker-Develi and Kideys (2003) who reported for the southern Black Sea that dinoflagellates constituted a relatively high proportion of the total phytoplankton species composition compared with observations made before the 1960s.

Thus, there are numerous reports documenting the effects of recent anthropogenic nutrient loading on chlorophyll *a* concentrations and phytoplankton distributions in the north-ern and central parts of the Black Sea. However, there are few reports on the effects on the southern parts of the Black Sea and almost none on the effects of the increased nutrient loading on species compositions and distributions.

When working with plankton samples, good temporal coverage is often not achievable as sampling is labor inten-sive. Moreover, studies of plankton samples cannot be col-lected back through time. Thus, in order to effectively assess the effects of ecosystem change on the phytoplankton com-munity, other methods need to be employed. Paleoecologi-cal methods (i.e., the study of past ecosystems based on biological and chemical indicators stored in the sediment of the sea floor) constitute a valuable approach for identifying ecosystem change through time. The purpose of this study was to examine long-term changes in phytoplankton com-munity composition using paleoecological methods and to identify possible correlations between these changes and the changing nutrient loading of the Black Sea.

Methods

General Preparations and Dating of the Cores

Two sediment cores collected in the southern Black Sea on board the RV Meteor (cruise M72-3b) in spring 2007 (Fig. 1) were used in this study. Core 22-MUC-1 (referred to through-out as core 22) was collected at 842 m depth with a multiple corer (Black et al. 2002) at 42°13.534′ N/36°29.555′ E. Core 25-MUC-2 (referred to throughout as core 25) was collected at 418 m depth with a multiple corer at 42°6.212′ N/36°37.460′ E. The cores were stored for 1.5 years at 4 °C in the dark before being used in this study. The uppermost 10 cm of the cores were subsampled in the middle of the cores using u-channels (dimension, 10×4×2.4 cm). The samples were wrapped in plastic and sent by mail from Potsdam, Germany to Copenhagen, Denmark. When received, the sediment cores were transferred from the u-channels to a cutting board and cut into samples of 0.5 cm width (0.5 cm sediment depth). The samples were then freeze dried (Heto FD5-66) for 96 h and

subsequently stored in re-sealable plastic bags at room tem-perature. Both cores were dated using ^{210}Pb dating. Approx-imately 1 g of freeze-dried material from each layer was used. The samples were analyzed for the activity of ^{210}Pb, ^{226}Ra, and ^{137}Cs via gamma spectrometry. The measurements were carried out on a Canberra low-background germanium well detector. ^{210}Pb was measured via its gamma peak at 46.5 keV, ^{226}Ra via the granddaughter ^{214}Pb which peaks at 295 and 352 keV, and ^{137}Cs via its peak at 661 keV. Afterwards, CRS modeling (Constant Rate of Supply, Appleby 2001) was ap-plied to date the individual samples.

Preparation of the Siliceous Protist Samples

Samples for enumerating siliceous protists were prepared following the method of Renberg (1990) with some modi-fications. For each sediment layer, approximately 0.03 g of freeze-dried material was weighed and transferred to a cen-trifuge glass with a metal spatula. To oxidize organic matter in the sediment, 1.0 ml hydrogen peroxide (H_2O_2) 35 % was added. The centrifuge glass was kept for a minimum of 1 h at room temperature to prevent a violent initial reaction and subsequently placed in a water bath (80 °C). After 1 h, 2.0 ml of H_2O_2 (35 %) were added. After a total of 3 h, the sample was removed from the bath. To remove salts soluble in hydrochloric acid (HCl), two drops of HCl (10 %) were added and the suspension was mixed for 30 s on a vortex mixer. Distilled water was then added, and the sus-pension was centrifuged for 10 min at 3,500 rpm. After-wards, the sample was washed four times as follows: the supernatant was removed using a pipette, distilled water was added and the sediment was re-suspended on a vortex mixer for 30 s, and, finally, centrifuged for 10 min at 3,500 rpm. In the last wash, two drops of ammonium solution (NH_4) 10 % were added to remove clay particles. The cleaned sample was transferred to a glass container and distilled water was added to a total of 10.0 ml suspension. Permanent slides were produced using Naphrax as the mounting material. A total of 0.50-ml suspension containing microspheres at 20,500 microspheres ml^{-1} was added to each sample to allow concentrations to be calculated.

Preparation of the Dinoflagellate Cysts Samples

Samples for enumerating dinoflagellate cysts were prepared following the method initially described by Bolch (1997) with some modifications. About 0.3 g of freeze-dried sediment from each layer was weighed and transferred to a centrifuge glass. The sediment was suspended in distilled water, placed in an ultrasonic bath for 2 min, and size fractionated by sieving through 2 meshes in succession (100 and 25 μm). The sedi-ment was then transferred to a centrifuge glass, placed in an ultrasonic bath for 2 min and sieved again. After size

fractionation, the dinoflagellate cysts were separated from the sediment using heavy liquid. Sodium metatungstate solution with a density of 2.1 g ml^{-1} was produced by dissolving 50 g sodium metatungstate monohydrate (3Na$_2$WO$_4$9WO$_3$H$_2$O) in 25 ml distilled water. The exact density was achieved by adding distilled water while using a hydrometer. All samples were prepared using the following procedure: 2 ml sodium metatungstate solution was added to a centrifuge glass; 7 ml fractionated sediment suspension was carefully added onto the sodium metatungstate layer, and the sample was centrifuged for 10 min at 3,000 rpm. After centrifugation, the transition layer containing the cysts and, afterwards, the water phase were removed with a pipette and transferred to a centrifuge glass. Distilled water was added and the sample was centrifuged for 10 min at 3,000 rpm. The supernatant was removed and the pellet re-suspended with distilled water to an appropriate concentration. Permanent slides were produced using glycerol gelatin sealed with paraffin wax as the mounting material.

Microscopy and Calculations

Literature used for species identification was Krammer and Lange-Bertalot (1986, 1988, 1991, 2004), Hasle and Syvertsen (1996), Throndsen et al. (2003), and Matsuoka and Fukuyo (2000). The siliceous protists were identified and counted using phase contrast at ×1,000 on an Olympus BH-2 light microscope. The dinoflagellate cysts were identified and counted using differential interference contrast at ×400 on an Olympus BX51 light microscope. In all samples containing siliceous protists, at least 200 microfossils were counted. In the samples from core 22 containing dinoflagellate cysts, only the two top samples contained more than 200 cysts and the abundance and species composition for the four lower samples were, therefore, based on fewer than 200 cysts with the least abundant sample containing 122 cysts. In core 25, for determination of the species composition, at least 200 dinoflagellate cysts were counted in each sample. For total abundance, the entire sample was counted.

The abundance of siliceous protists was calculated by relating the number of counted microfossils to the number of microspheres. Before calculating the abundance of diatoms, the number of counted valves was divided by 2 as two valves make up one cell. The abundance of siliceous protists was then calculated using Eq. (1):

$$C_{\mathrm{p}} = \frac{P}{C_{\mathrm{s}} \times \left(\frac{m}{C_m}\right)} \tag{1}$$

Where C_{p} is the concentration of protists per gram dry sediment, P is the count of protists in the sample, C_{s} is the concentration of dry sediment in the sample in grams per milliliter, m is the count of microspheres, and C_m is the concentration of microspheres per milliliter.

The concentration of dinoflagellate cysts was calculated using Eq. (2):

$$C_{\mathrm{d}} = \frac{d}{W_{\mathrm{s}}} \tag{2}$$

Where C_{d} is the concentration of dinoflagellate cysts per gram dry sediment, d is the count of dinoflagellate cysts in the sample, and W_{s} is the weight of processed dry sediment in grams.

Statistics and Data Handling

Patterns in the species composition were investigated using constrained hierarchical clustering analysis using the "coniss" agglomeration method (Gordon and Birks 1972). Analyses were done with the free software R (R Development Core Team 2011) using the package "rioja" version 0.5-6 (Juggins 2009). The number of samples in each core was not sufficient to test for the number of significant zones (e.g., with the broken stick model (Bennett 1996)) and the number of zones were, therefore, determined qualitatively by comparing the clustering analysis with the biological proxies.

In order to investigate a possible link between the nutrient enrichment on the north-western shelf and the abundances in the cores, total abundance of siliceous protists and dinoflagellate cysts were plotted against the mean phosphate winter concentration from Romanian coastal waters. Phosphate was chosen because data exist for the entire period 1960 to 1992. Nutrient data were extracted from Cociasu et al. (1996) using Datathief III version 1.6 (Tummers 2006). For each core, the phosphate data were split into time periods corresponding to sample dates and the mean was calculated to represent the nutrient conditions for the period as a whole. For core 22, nutrient data were not available for the sample dated to around 1943. As there was no change in the phosphate concentration from 1960 to 1965 (Cociasu et al. 1996) (Fig. 4) and there is no evidence for significant changes in the nutrient concentrations before the 1960s, the phosphate concentration used for this sample was estimated as the mean of the years 1960–1965. The correlations were, thereafter, investigated using Pearsons correlation coefficient and with general linear modeling using ordinary least square estimation.

Results

Chronology of the Cores

When the measurements of the activity of ^{210}Pb and ^{137}Cs and the profiles of the biological proxies were aligned, the patterns suggested that the upper 0.5 cm of core 22 was missing (Fig. 2). That is, when core 22 was moved down
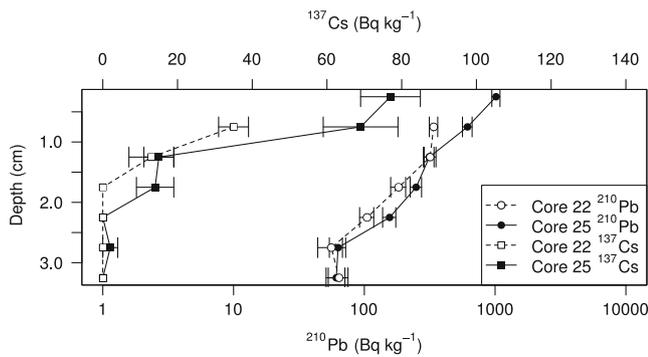
Fig. 2 Activity of ^{210}Pb and ^{137}Cs in relation to depth in cores 22 and 25 where the depth of core 22 is offset by 0.5 cm in relation to core 25

0.5 cm in relation to core 25, the calculated dates and the total abundance and species composition in both cores became synchronized. Assuming that both cores represent the same general area, we recalculated the chronology for core 22 with this 0.5 cm offset. The results of CRS modeling of cores 22 and 25 are given in Table 1. The chronology showed a rapid decrease in sediment age in relation to depth with 100 years of age being reached at about 3 cm depth. This is in agreement with Ross et al. (1970) who estimated the sedimentation rate of the same area to 10–30 cm/1,000 years.

The calculated flux of unsupported ^{210}Pb in core 25 was 142 Bq m^{-2} year^{-1} which is about twice the estimated local atmospheric supply (Appleby 2001). This indicates that the site is subject to sediment focusing (Davis and Ford 1982) and shows that the site is a net sink for suspended material. Calculation of the chronology using a Constant Initial Concentration model (Appleby 2001) gave an essentially similar

result and the chronologies were, therefore, considered as being reliable and showing low errors on the dates. This, together with the sediment focusing and the similar chronologies in the two cores, indicate that the cores are representative for the area. It is, therefore, reasonable to interpret changes in the total abundance and in the species composition in the cores as being a reflection of changes occurring in the water column, bearing in mind that not all species groups are preserved.

General Observations

Twenty-four taxa of siliceous protists could be successfully distinguished in the cores. Of these, 16 could be identified to the species level (Table 2). The rest were determined to the highest possible taxonomic level which, in most cases, was genus. In all samples, taxa identified to species level accounted for more than 90 % of the observed total abundance. In the case of the dinoflagellate cysts, 18 taxa were identified, 14 to the species level. In all samples, taxa identified to species level accounted for more than 90 % of the total abundance, except for the uppermost sample in core 25 (dated to ca. year 2000) where *Spiniferites* sp. 1 comprised >60 % of the total abundance.

Temporal Changes in the Total Abundance

The total abundance of diatoms (Fig. 3a) increased in both cores after around 1960 with a fourfold increase in core 22 and a threefold increase in core 25. The total abundance of

Table 1 Results from CRS-modeling of core 22 and 25 where core 22 has been calculated with a depth offset of 0.5 cm in relation to core 25

Depth (cm)	Age (years)	Age error (years)	Date (year)	Accumulation rate (kg m^{-2} year^{-1})	Accumulation rate error (kg m^{-2} year^{-1})
Core 22					
0	–	–	2008	–	–
0.75	24	3	1984	0.12	0.01
1.25	41	4	1967	0.12	0.02
1.75	65	6	1943	0.09	0.02
2.25	91	8	1917	0.08	0.02
2.75	116	13	1892	0.08	0.04
3.25	149	23	1859	0.06	0.04
Core 25					
0	–	0	2008	–	–
0.25	8	2	2000	0.13	0.01
0.75	23	2	1985	0.11	0.01
1.25	38	3	1970	0.13	0.01
1.75	54	4	1954	0.14	0.02
2.25	75	5	1933	0.11	0.02
2.75	95	6	1912	0.11	0.03
3.25	112	5	1896	0.13	0.02

Table 2 Species check list

Species	Core 22	Core 25
Dinoflagellate cysts		
Order Centrales		
Asteromphalus sp. 1 Ehrenberg	X	X
Coscinodiscus radiatus Ehrenberg	X	X
Coscinodiscus sp. 1 Ehrenberg	X	X
Cyclotella choctawhatcheeana Prasad (synonym *Cyclotella caspia* Grunow)	X	X
Cyclotella meneghiniana Kützing	X	X
Thalassiosira eccentrica (Ehrenberg) Cleve	X	X
Thalassiosira oestrupii (Ostenfeld) Hasle	X	X
Thalassiosira spp. Cleve	X	X
Order Pennales		
Achnanthes brevipes Agardh	X	
Cocconeis scutellum Ehrenberg	X	X
Cymbella affinis Kützing	X	
Diploneis sp. 1 Ehrenberg ex Cleve	X	X
Fragilaria capucina Desmazières	X	
Fragilaria spp. Lyngbye	X	X
Lioloma pacificum (Cupp) Hasle		X
Navicula spp. Bory de Saint-Vincent	X	X
Nitzschia spp. Hassall	X	X
Pseudo-nitzschia spp. Peragallo	X	
Pseudosolenia cf. *calcar-avis* (Schultze) Sundström	X	X
Thalassionema frauenfeldii (Grunow) Hallegraeff	X	X
Thalassionema nitzschioides (Grunow) Mereschkowsky	X	X
Silicoflagellates		
Dictyocha octonaria Ehrenberg	X	X
Dictyocha speculum Ehrenberg	X	X
Heterotrophic flagellates		
Hermesinum adriaticum Zacharias	X	X
Dinoflagellate cysts		
Order Gonyaulacales		
Lingulodinium polyedrum (Stein) Dodge	X	X
Protoceratium reticulatum (Claparède et Lachmann) Bütschli	X	X
Spiniferites bentorii (Ross) Wall et Dale	X	X
Spiniferites bulloideus (Deflandre et Cookson) Sargeant	X	X
Spiniferites delicatus Reid	X	X
Spiniferites mirabilis (Rossignol) Sargeant	X	X
Spiniferites ramosus (Ehrenberg) Mantell	X	X
Spiniferites sp. 1 Mantell	X	X
Order Gymnodiniales		
Cochlodinium sp. 1 Schütt	X	X
Cochlodinium sp. 2 Schütt	X	X
Polykrikos schwartzii Bütschli	X	X
Order Peridiniales		
Echinidinium delicatum Zonneveld	X	X
Pentapharsodinium dalei Indelicato et Loeblich III	X	X
Protoperidinium conicum (Gran) Balech	X	X

Table 2 (continued)

Species	Core 22	Core 25
Dinoflagellate cysts		
Protoperidinium oblogum (Aurivillius) Parke et Dodge	X	X
Protoperidinium sp. 1 Bergh	X	X
Scrippsiella trochoidea (Stein) Balech ex Loeblich III	X	X
Stelladinium stellatum (Wall et Dale) Reid	X	X

silicoflagellates (Fig. 3b) in core 22 increased threefold in the period around 1943–1984 compared with 1859–1917. In core 25, the total abundance of silicoflagellates decreased around 1970 and subsequently increased in the period around 1985–2000 to the same abundance as was observed before 1970. The total abundance of the ebridian flagellate *Hermesinum adriaticum* (Fig. 3c) increased in both cores around 1970 with a slight increase in core 22 and a threefold increase in core 25 in the period 1970–2000 compared with 1913–1954. The total abundances of dinoflagellate cysts (Fig. 3d) in both cores were relatively stable until around 1985 with a slight increase occurring during the period. An exception, however, was noted in 2000 for core 25, where the total abundance of dinoflagellate cysts increased eightfold compared with the period 1896–1985. Before 1960, the total abundance of microfossils was consistently lower in core 22 than in core 25.
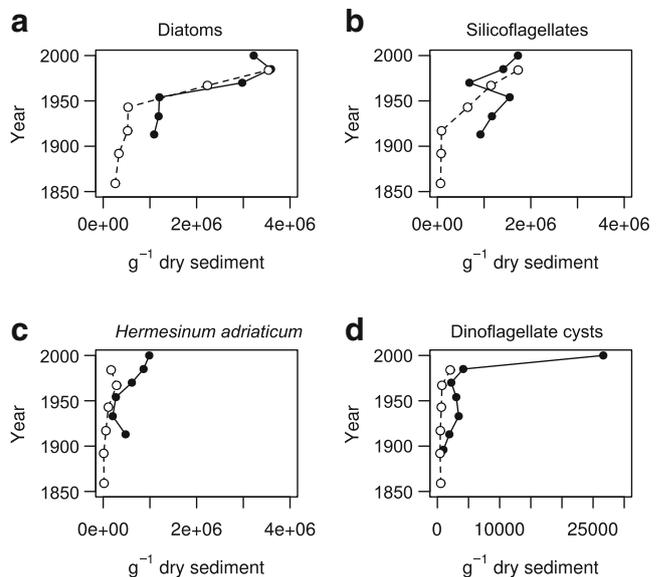

Fig. 3 Changes with time in the total abundance of microfossils in cores 22 (*open circles*) and 25 (*closed circles*): **a** diatoms, **b** silicoflagellates, **c** *H. adriaticum*, **d** dinoflagellate cysts

Correlating Total Abundances with Nutrient Enrichment

The total abundances of both siliceous protists (core 22, *r*= 0.96; core 25, *r*=0.99) and dinoflagellate cysts (core 22, *r*= 0.97; core 25, *r*=0.70) were positively correlated with the mean phosphate winter concentration on the Romanian coast (Table 3; Fig. 4a, b). For the siliceous protists, there was a clear linear relationship with the slope being more steep for core 22. For the dinoflagellate cysts, the relationship was less clear but both showed the same positive trend.

Temporal Changes in the Relative Abundance of Siliceous Protists

Constrained clustering analysis for both cores (Figs. 5 and 6) showed two zones with a lower, older zone from before ca. 1960 and an upper, recent zone from around 1960 to 2000. This indicates that a significant change in the species composition of siliceous protists occurred after around 1960. General trends in the species composition were similar in both cores, with a decrease in the relative abundance of *Cyclotella meneghiniana* and an increase in the relative abundance of *Thalassionema frauenfeldii* and *Thalassionema nitzschioides*. *Cyclotella choctawhatcheeana* and *Thalassiosira oestrupii* both showed shift with *C. choctawhatcheeana* peaking after around 1960 whereas *T. oestrupii* dominated in the intermediate period from around 1920 to 1960. In the most recent sediment, i.e., that from around 2000 (data from core 25), the species composition shifted to be dominated by *Pseudosolenia* cf. *calcar-avis* and *T. oestrupii*.

Temporal Changes in Relative Abundance of Dinoflagellate Cysts

Constrained clustering analysis yielded different results for the two cores. In core 22 (Fig. 7), two zones were identified with a lower, older zone from around 1859 to 1910 and an upper, recent zone from around 1910 to 1985. The dominating species were *Lingulodinium polyedrum*, *Polykrikos schwartzii*, and *Spiniferites* spp., and the general trends in the species
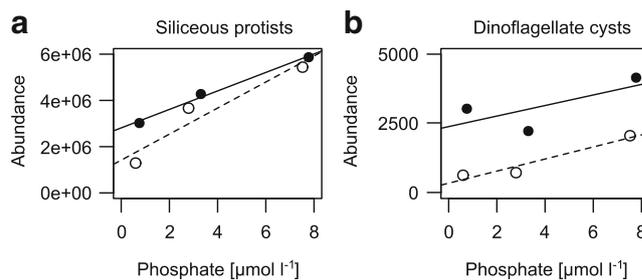
Fig. 4 Total abundances of **a** siliceous protists and **b** dinoflagellate cysts vs. the mean phosphate winter concentration in Romanian waters (Cociasu et al. 1996) for cores 22 (*open circles*) and 25 (*closed circles*)

composition showed an increase in the relative abundance of *L. polyedrum* and a decrease in *Pentapharsodinium dalei* and *P. schwartzii*. Concentrations of *Spiniferites* spp. were relatively stable over the entire period.

In core 25 (Fig. 8), three zones were identified with a lower, older zone from around 1896 to 1945, an intermediate zone from around 1945 to 1990 and an upper, recent zone comprising only one sample from around 1990 to 2000. The dominating species were *L. polyedrum*, *P. schwartzii*, *Spiniferites* sp. 1, and *Spiniferites* spp. and the general trends showed a decrease in the relative abundance of *L. polyedrum* and *P. schwartzii*. *Spiniferites* sp. 1 was almost nonexistent before around 1990 but increased to constitute more than 60 % of the total abundance by around 2000.

Discussion

The total abundance of siliceous protists generally increased after 1960 in both cores corresponding with the increasing nutrient load into the rim current in the late 1960s (Bodeanu 1993; Yunev et al. 2002; Zaitsev and Alexandrov 1997). Although total abundance is not a direct measure of biomass, the increase in total abundance in both cores is very pronounced suggesting a marked increase in phytoplankton biomass in the southern Black Sea after around 1960. Assuming a current speed of 25 cm s^{-1} (Neuman 1942), it would take nutrients introduced from the Danube River about 1.7 months

Table 3 Descriptive statistics of the winter phosphate concentrations extracted from Cociasu et al. (1996)

Date (year)	Number of observations	Mean phosphate winter concentration (µmol l^{-1})	Standard deviation (µmol l^{-1})	Standard error (µmol l^{-1})
Core 22				
1962	26	0.60	0.66	1.13
1967	70	2.79	4.85	0.58
1984	58	7.52	3.95	0.52
Core 25				
1961	14	0.75	0.85	0.23
1970	60	3.30	5.12	0.66
1985	56	7.78	3.94	0.53

Fig. 5 Changes with time in the relative abundance of diatoms in core 22 (*left*) including the results from a constrained cluster analysis (*right*) and the position of the zones assuming two zones (*dashed line*)
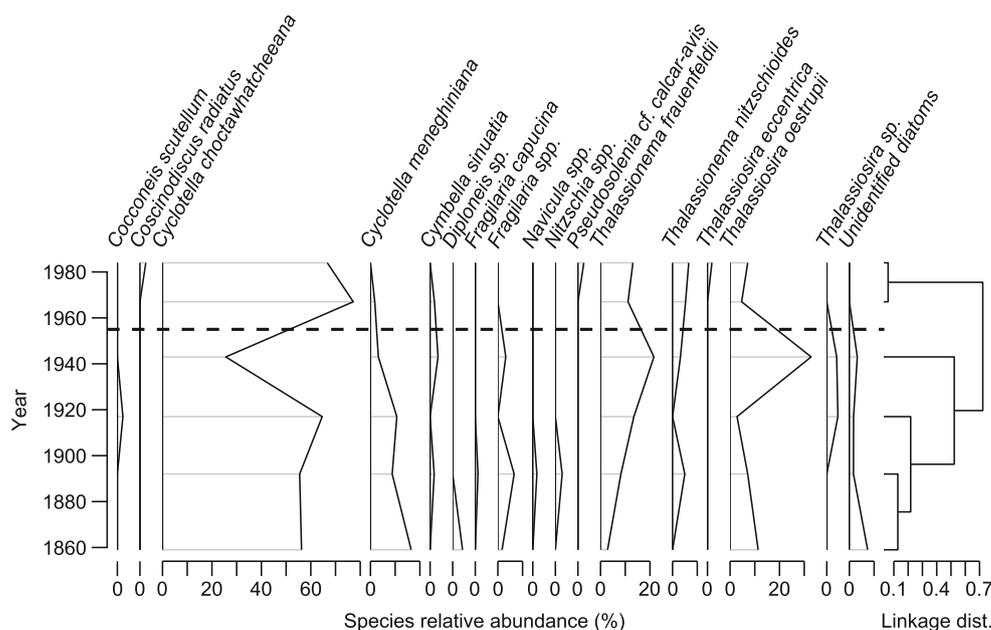
to be transported the ca. 1,100 km to the location of the cores. During winter, this time window could allow for nutrient to be transported this far without being consumed by photosynthetic processes, similar to the patterns observed in the Marmara Sea (Polat and Tugrul 1995).

In addition, as the upward flux of nutrients from the deep sea is consumed in the suboxic layer (Murray et al. 1995; Murray et al. 2005; Kuypers et al. 2003), the only supply of nutrients to the surface layer must be lateral transport from either local or regional sources. Correlating the total abundance of siliceous protists and dinoflagellate cysts with mean winter phosphate concentrations showed a clear linear relationship, suggesting that eutrophication in the southern

Black Sea may be influenced by the nutrient enrichment on the north-western shelf. The pattern supports the general picture that increasing nutrient enrichment, especially from the Danube River, has caused increasing eutrophication on the north-western shelf (Cociasu et al. 1996), on the western coast (Sur et al. 1996), in the open Black Sea (Yunev et al. 2002), and in the Marmara Sea—after especially cold winters (Polat and Tugrul 1995).

The total abundance of diatoms and silicoflagellates changed from being markedly different in the two cores before around 1960 to being similar after around 1960. Before around 1960, the Black Sea was an oligotrophic system (Oguz 2005; Mee 1992) and we hypothesize that

Fig. 6 Changes with time in the relative abundance of diatoms in core 25 (*left*) including the results from a constrained cluster analysis (*right*) and the position of the zones assuming two zones (*dashed line*)
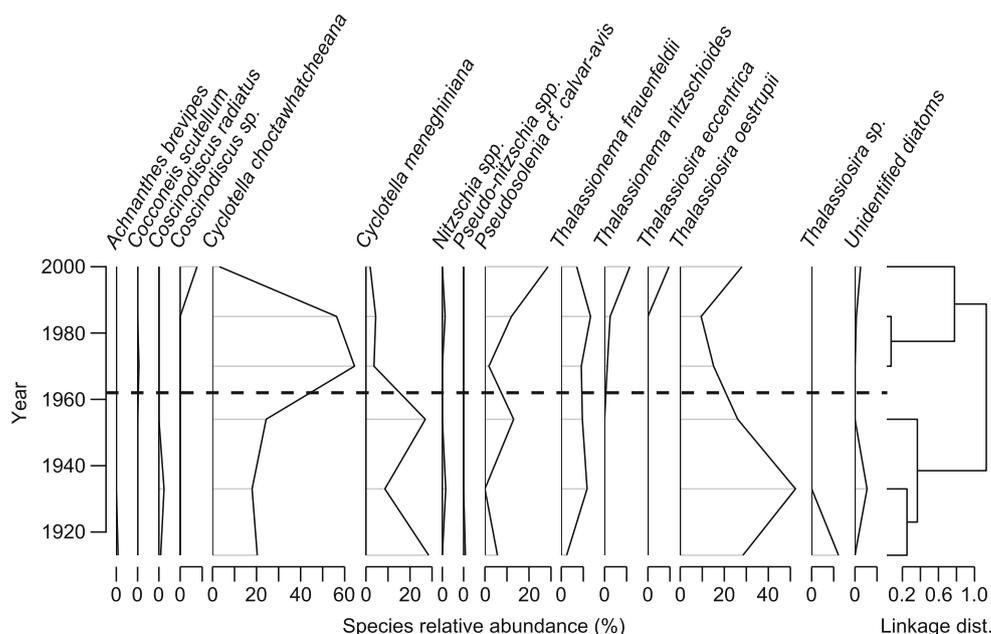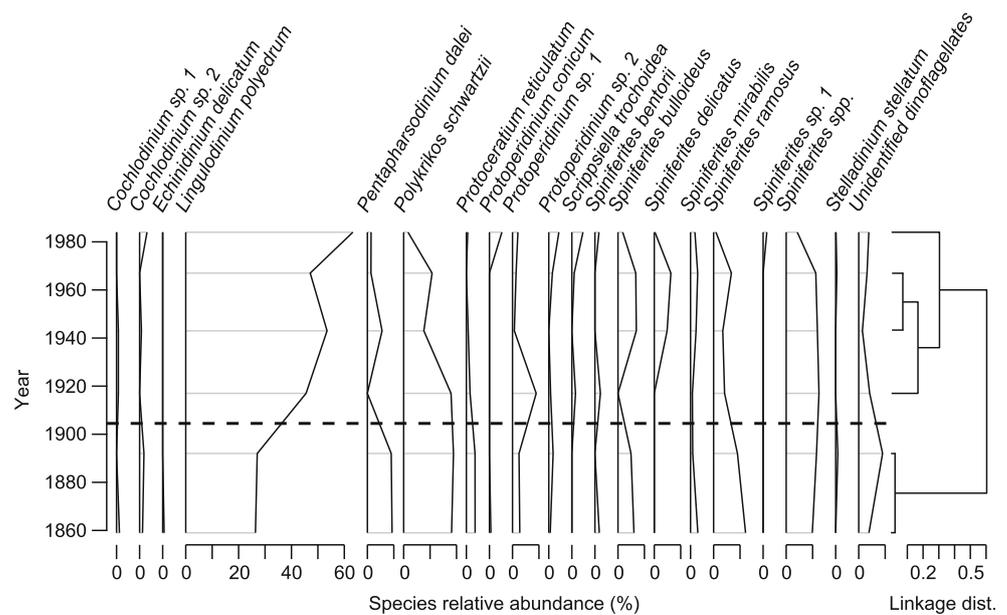
Fig. 7 Changes with time in the relative abundance of dinoflagellate cysts in core 22 (*left*) including the results from a constrained cluster analysis (*right*) and the position of the zones assuming two zones (*dashed line*)


nutrient transport in the rim current was low or nonexistent. If this assumption holds, then primary production in the southern Black Sea at that time would likely have been controlled by local sources only (e.g., nutrient loading from the Kizilirmak River). The rim current closely follows the continental slope, thus limiting transport of water and material beyond the slope across the flow (Sur et al. 1996). This effectively shields the open Black Sea from the polluted coastal areas (Vinogradov et al. 1992 in Yunev et al. 2002; Oguz et al. 1993). Although the two cores are located at approximately the same distance from shore, the water depth where core 22 was retrieved is twice as deep as that above core 25 (842 vs. 418 m). Thus, core 22 was taken further out in the rim current than core 25 and this,

presumably would mean limited local nutrient loading to the waters above core 22. This assumed difference in local nutrient loading could explain the differences observed in species abundance in the cores before around 1960. After around 1960, the hypothesized increase in nutrient loading from the north-western shelf through the rim current would increase the amount of nutrients being transported to the southern Black Sea. This would support higher production at both sites, making the local nutrient contribution relatively less important and explain the change to similar diatom and silicoflagellate total abundances after around 1960.

Nutrients are considered to be one of the major forcing factors in determining phytoplankton species compositions and succession (Smayda and Reynolds 2003). The niche

Fig. 8 Changes with time in the relative abundance of dinoflagellate cysts in core 25 (*left*) including the results from a constrained cluster analysis (*right*) and the position of the zones assuming three zones (*dashed line*)
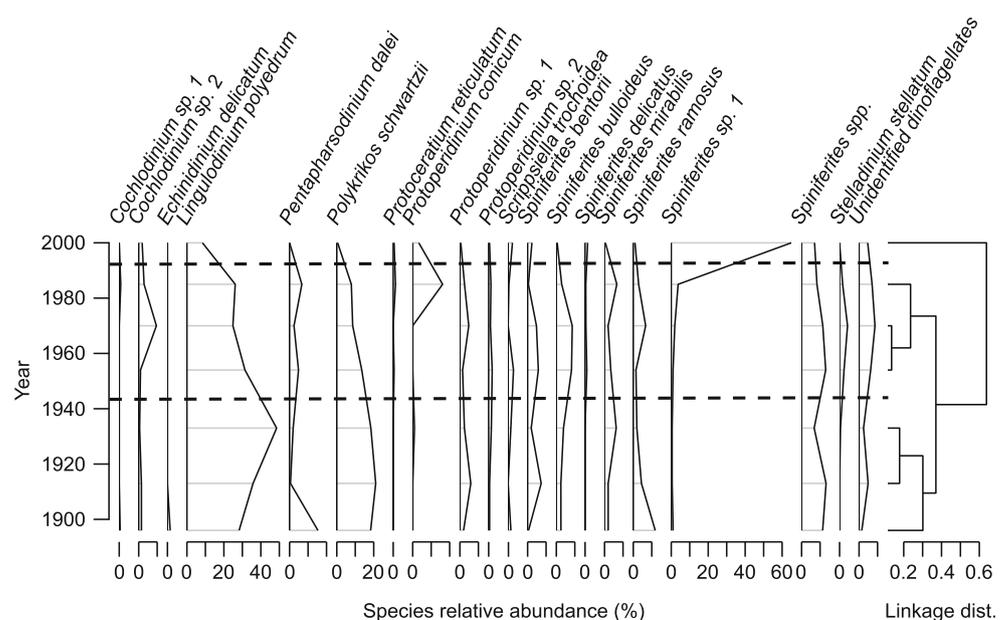
concept suggests that each species is adapted to a certain range of physical and chemical factors, in which it has optimal growth conditions. This knowledge can be used to nominate indicator species as representatives for different environmental conditions. Studies identifying diatoms as indicator species in marine environments are few and mainly restricted to the Baltic Sea. Weckström and Juggins (2005) identified *Cyclotella atomus*, *C. meneghiniana*, and *Thalassiosira pseudonana* as good indicators of highly elevated nutrient concentrations. Jaanus et al. (2009) identified *C. choctawhatcheeana* and *Cylindrotheca closterium* as good indicators of elevated levels of total phosphorous, whereas *S. costatum* was a good indicator for elevated levels of total nitrogen. Tilman et al. (1982) identified *Cyclotella* spp. as being good competitors compared with larger diatoms under silicate limitation.

The results from core 25 show a relatively stable abundance of *C. choctawhatcheeana* from the beginning of the 1900s to around 1950, where after this species was increasingly dominant. In core 22, the pattern is less evident, but the relative abundance of *C. choctawhatceheeana* increases in a similar manner to core 25 after around 1940. In the periods of lower relative abundances of *C. choctawhatcheeana* (i.e., around 1920–1960 in core 25 and in the sample dated at about 1940 in core 22), the species composition was dominated by the larger species, *T. oestrupii* and *T. frauenfeldii*. The shift after around 1960, where *C. choctawhatcheeana* exhibits its highest level of dominance accompanied by a several-fold increase in total abundance, is in good agreement with the increased nutrient loading into the rim current. According to the resource competition theory, a small diffusion boundary layer and a large surface-to-volume ratio gives smaller species an advantage relative to larger species at low nutrient concentrations (Kiørboe 1993; Raven 1986; Tilman et al. 1982). Therefore, in a period following nutrient enrichment, larger species can be expected to increase in abundance. However, in the Black Sea, the increase in nitrogen and phosphate was accompanied by a decrease in silicate (Cociasu et al. 1996; Konovalov et al. 1999). Tugrul et al. (1992) suggested that this was a limiting factor for the growth of diatoms, despite the generally large increase in nutrient availability. *C. choctawhatcheeana* has previously been reported to be an indicator of both phosphate enrichment (Jaanus et al. 2009) and, due to its size, silicate limitation (Tilman et al. 1982). Thus, the relative increase of *C. choctawhatcheeana* after the 1960s suggests a decrease in the availability of silicate in the southern Black Sea and adds to the evidence that the increased eutrophication in the southern Black Sea was influenced by nutrient transported from the north-western shelf.

The nutrient loading into the rim current decreased after 1993 (Eker-Develi and Kideys 2003) which resulted in general improvement in water quality (Konovalov et al. 1999) and a decline in chlorophyll *a* after 1993 in the open Black Sea

(Yunev et al. 2002). In the present study, there are only data from after 1993 from core 25. The abundance and species composition from this sample, however, show very little evidence of decreasing eutrophication. The results from around 2000 show a slight decrease in total abundance of diatoms compared to around 1985, but the total abundance is still very high compared with the years before 1970. Furthermore, the species composition of diatoms changed dramatically around 2000 with *C. choctawhatcheeana* almost disappearing and being replaced by *P.* cf. *calcar-avis*, *T. nitzschioides*, *Thalassiosira eccentric* and *T. oestrupii*. Although, the decrease in *C. choctawhatcheeana* with a concomitant increase in *T. oestrupii* results in the species composition resembling the species composition before 1970, the appearance of *T. nitzchioides* and *T. eccentrica* which are not present in the cores before 1970 makes this argument much less convincing.

Only a few studies have investigated ebridians as paleoecological indicator species. Studies in the Baltic Sea have identified *Ebria tripartita* as a potential indicator for elevated nutrient conditions (Korhola and Grönlund 1999). The ebrid, *H. adriaticum*, identified in the present study, has not earlier been identified as a paleoecological indicator. Osawa et al. (2005) reported that the ebridian species composition in the central Black Sea changed from being dominated by *H. adriaticum* in 1989 to being dominated by *E. tripartita* in 1991. They speculated that this was caused by increased nutrient loading. In the present study, however, a change from *H. adriaticum* to *E. tripartita* was not observed and *H. adriaticum* remained the dominant ebridian in the entire period investigated.

With regard to dinoflagellate cysts, both cores were dominated by *L. polyedrum* and, to a lesser extent, *P. schwartzii* and several species of the genus *Spiniferites*. Both *L. polyedrum* and *P. schwartzii* have been identified as indicators of eutrophication (Dale 1996; Ellegaard et al. 2006; Matsuoka 1999). There was a steady increase in relative abundance of *L. polyedrum* in core 22, but no consistent increase in core 25. *L. polyedrum* has also been identified as an indicator of brackish water (Morzadec-Kerfourn 1976) and this could also explain the success of this species in the Black Sea. The large increase in total abundance around 2000 was mainly caused by the unidentified species *Spiniferites* sp. 1. Bat et al. (2007) investigated the species distribution of phytoplankton in the southern Black Sea in 1999–2000 but none of the important dinoflagellates reported produce cysts resembling *Spiniferites* sp. 1. Without knowing the biology of *Spiniferites* sp. 1, it is not possible to identify the underlying processes leading to changes in the abundance of this species.

Correlation does not necessarily imply causation and it is impossible to rule out that other factors than nutrients transported from the north-western shelf could significantly affect the species composition in the southern Black Sea.

Some authors have argued that increased water use and damming might result in less freshwater input and that this, in turn would lead to an increase in salinity (e.g., Tolmazin 1985). This might explain the patterns in the occurrence of salinity sensitive species such as *C. meneghiniana* and *L. polyedrum*. There is, however, no clear evidence of salinity change during the period 1960–1995 (Konovalov and Murray 2001).

The Black Sea has also experienced changes in temperature with a decrease in average temperature of about 0.75 °C after the mid-1980s. The effect of temperature on phytoplankton distributions and phytoplankton size structure is poorly understood but both mesocosm, and large-scale datasets show a pattern of decreasing size with increasing temperature (e.g., Yvon-Durocher et al. 2011; Hilligsøe et al. 2011). The only significant change in our data dating after the mid-1980s indicates a shift from a small species (*C. choctawhatcheeana*) to larger species (*T. oestrupii*, *P.* cf. *calcar-avis*, etc.) and changes in temperature might, therefore, play a role in recent changes in species composition in the southern Black Sea.

It is also important to note that paleoecological investigations are limited to the species that are preserved. Diatoms and dinoflagellate cysts generally preserve well after they die/produce cysts and it has been shown for dinoflagellates that total abundance in the sediment reflects total abundance of cysts in the water column (Dale 1996). The species composition in the water column, however, can be very different from the species composition in the sediment because some species are not well preserved (e.g., cyanobacteria, green algae, etc.) or are destroyed during preparation (e.g., the Coccolithophore *Emiliania huxleyi* which is very common in the Black Sea). In this study, we approach the limitation set by the paleoecological method by using indicator species for which surface studies have revealed a correlation between the relative success of the species and some ecological factor.

Conclusions

In the present study, changes in the phytoplankton species composition over the last 150 years in the Black Sea have been investigated. Both total abundance and species composition of siliceous protists shifted after around 1960 consistent with this being a response to increasing nutrient loading. The timing of this shift corresponded with the increasing nutrient loading into the rim current after the late 1960s facilitated by discharge of nutrient enriched water from the Danube River in the north-western part of the Black Sea. In addition, the construction of several dams on the Danube River affected the nutrient composition in the Black Sea water where silicate became relatively less

abundant. The relative dominance of *C. choctawhatcheeana* after around 1960 could be related to both the general increase in nutrient loading and the change in nutrient composition. The southern Black Sea thus shows a eutrophication pattern similar to the north-western, western and the open Black Sea. This suggests that nutrient enriched river discharge can affect ecosystems far from the position of the river estuary. Other proxies (dinoflagellate cysts and ebridians) showed a different pattern in this period. These may be controlled by other factors than nutrient availability (i.e., salinity, temperature, etc.) in this system.

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