

# Reconstructing 150 years of eutrophication in the north-western Adriatic Sea (Italy) using dinoflagellate cysts, pollen and spores

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## Abstract

Recent trends in the trophic conditions of the north-western Adriatic Sea have been evaluated using organic-walled dinoflagellate cysts (dinocysts), pollen and spores. Palynological analyses performed on a sediment core covering the period AD 1830–1990 have revealed a progressive increase in eutrophication beginning in the 20th century. The first signal of a change seems to occur earlier in the terrestrial ecosystem and later in the marine realm. Pollen data indicate that the source of enhanced nutrient loading to the North Adriatic Sea, which partly resulted from increased wetland reclamation and forest clearance, began at about AD 1910. The clear shift in the relative abundance of the dinocyst *Lingulodinium machaerophorum* at AD 1930 suggests an increase in eutrophication. Dinocyst assemblages point to stressful conditions from AD 1960, reaching a maximum at about AD 1978. Subsequently eutrophication levels decrease, although dinocyst diversity suggests that the ecosystem has not completely recovered. © 2004 Elsevier Ltd. All rights reserved.

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## 1. Introduction

The Northern Adriatic Sea is the northernmost area of the Mediterranean Sea. It is characterised by shallow waters (70 m maximum depth) and considerable freshwater input mainly coming from the Po River, which is the largest Italian river. Water circulation is dominated by a counter-clockwise flow, induced by thermohaline factors and Coriolis acceleration, which confines the nutrient-enriched freshwaters to the western coastal regions (Franco et al., 1982; Franco and Michelato, 1992; Zavatarelli et al., 1998). Benthic flux measurements indicate that recycled nutrients from the sea bottom may be comparable to the input of nutrients from the Po River (Giordani et al., 1992). The Po River represents a primary source of nutrients to the Adriatic Sea (e.g., Bortoluzzi et al., 1984; Degobbis and Gilmartin, 1990; Provini et al., 1992). Consequently, massive diatom and dinoflagellate blooms, “red tides” sometimes associated

with toxicity episodes, are well known along the north-western Adriatic coast (Boni et al., 1983; Boni and Annibaldi, 1984; Fonda-Umani, 1985; Boni et al., 1986, 1992, 2000). The term eutrophication refers to an excessive enrichment of waters with nutrients, and its associated adverse biological effects (European Environmental Agency, 1994), or alternatively, to the increased rate of organic matter supply to an ecosystem (Nixon, 1995). Cultural eutrophication, which results from human activity, may negatively affect marine ecosystems, increasing the occurrence of massive benthos and fish mortality, loss of diversity, poisoning episodes which also cause human illness, and mucilage production (e.g., Smayda, 1990; Viviani, 1992; Vollenweider et al., 1992; Cattani and Vitali, 1995; Degobbis et al., 1995). Anthropogenic nutrient loading of rivers entering the north-western Adriatic Sea has considerably increased nutrient input to the system during the late 20th century, especially between 1968 and 1980 (Marchetti et al., 1989). A major fraction 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

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as a consequence of high downward organic fluxes, microbial decay and thermal stratification (Justic, 1987; Justic et al., 1987; Degobbis, 1989; Zavatarelli et al., 1998).

This study combines, for the first time, marine and terrestrial proxies to reconstruct (cultural) eutrophication in the Adriatic Sea during the last ~150 years. It aims to discriminate between the pre-industrial state of the basin and the consequences of enhanced anthropogenic impact in the marine and terrestrial environments. The marine and the terrestrial signals are derived from palynological (organic-walled dinoflagellate cysts, hereafter dinocysts, pollen and spores assemblages) analyses of a well-dated sediment core from the north-western Adriatic Sea. This paper also represents a completion of work by Puškaric et al. (1990) and Barmawidjaja et al. (1995), in which phytoplankton remains (diatoms, calcareous nannoplankton and silicoflagellates) and benthic foraminifers, respectively, from the same core were investigated.

### 1.1. Productivity indicators

Chlorophyll-*a* trends in the North Adriatic Sea follow a well-defined seasonal cycle with highest phytoplanktonic biomass in winter, a progressive decrease through spring and summer and an increase in autumn (Zavatarelli et al., 1998). During winter and spring, diatoms prevail, whereas in summer and autumn dinoflagellates and nanoflagellates are dominant (Socal et al., 1992; Vollenweider et al., 1992; Acri et al., 2000). Dinoflagellates and diatoms are important members of eukaryotic phytoplankton in the North Adriatic Sea, with the latter representing the main component (e.g., Alberighi et al., 1997). Nevertheless, diatom siliceous skeletons are not well preserved and poorly silicified diatoms may be rapidly dissolved (Bidle and Azam, 1999). The total absence of remains of diatoms with very fragile frustules (e.g., *Chaetoceros* spp. and *Skeletonema* spp.), which are abundant in surface waters, in the three cores from the North Adriatic Sea is an indication of opal dissolution in this area (Puškaric et al., 1990). Some dinoflagellate species produce organic-walled cysts (dinocysts) as a part of their life cycle (Head, 1996). Dinoflagellate assemblages consist of autotrophic, mixotrophic and heterotrophic species. Among cysts forming dinoflagellates, Gonyaulacoids and Gymnodinioids (except for *Polykrikos*) are autotrophic, whereas Proto-peridinioids are generally heterotrophic. Since heterotrophs prey on both solitary and colonial diatoms (Jacobson and Anderson, 1986; Gaines and Elbrächter, 1987), the proportion of their cysts in the assemblages has been used as a proxy for tracing eutrophication in coastal waters (e.g., Thorsen and Dale, 1997; Matsuoka, 1999), although the extent to which cyst production reflects total primary productivity is at present still not

completely known. Some works suggest that cysts are also potential indicators of anthropogenic impact, reflecting both eutrophication and industrial pollution (Dale and Fjellså, 1994; Sætre et al., 1997; Thorsen and Dale, 1997; Dale et al., 1999; Dale, 2001a; Sangiorgi et al., 2001a). A few studies on the distribution of dinocysts in surface and recent sediments are available for the Adriatic Sea (Nichetto et al., 1995; Belmonte et al., 1998; Rubino et al., 2000; Sangiorgi et al., 2001b) but they do not span long time periods.

### 1.2. Vegetation and land use

Land reclamation, predominantly carried out in the Po River catchment area between the end of the 19th century and the 1970s, allowed cultivation of several thousand hectares of previously swampy lands. At present, agriculture is one of the main activities in the Po plain and it negatively influences quality and quantity of waters as a consequence of the use of fertilisers, pollution caused by pesticides, and water withdrawal for irrigation (Cencini, 1998).

The main vegetation belts in the Po catchment area are controlled by differences in altitude. Presently, the Po plain is characterised by large stands of common oak (*Quercus robur*), hornbeam (*Carpinus betulus*), and ash (*Fraxinus excelsior*). Formations of black alder (*Alnus glutinosa*), white poplar (*Populus alba*), and willow (*Salix* spp.) occur at lakeshores and riverbanks. Anthropogenic influence is evident from the presence of cultivated land. Main cultivation consists of wheat, corn, sugar beet, lucerne, sunflowers, soybean, rice, and horticulture in general. Poplar-woods, vineyards, orchards and arboreal cultures are widespread (Pignatti, 1979; Ferrari, 1997). Coastal pinewoods consist of *Pinus maritimus* and *Pinus pinaster*. The Po plain is lined to the north and the south by a belt of mixed oak–pine forest (*Pinus sylvestris*). In the lower Alps and in the Apennines, a mesophilous deciduous forest occurs. This is dominated by beech (*Fagus sylvatica*) and silver fir (*Abies alba*) together with cultivated conifer woods and grass- and pasturelands. Evergreen coniferous forests (with ericaceous undergrowth) dominate the high Alps, where Norway spruce (*Picea excelsa*) and larch (*Larix decidua*) prevail (Pignatti, 1979; Ferrari, 1997).

## 2. Material and methods

### 2.1. Sampling, processing, counting and age-model

A total of 21 sediment samples were analysed from a 57-cm-long core (core 108) collected in 1989 in the North Adriatic Sea (44°45.4'N, 12°45.0'E; 32 m water depth; Fig. 1), in an area of active clay deposition (Brambati et al., 1983). Each sample (about 8 g dry

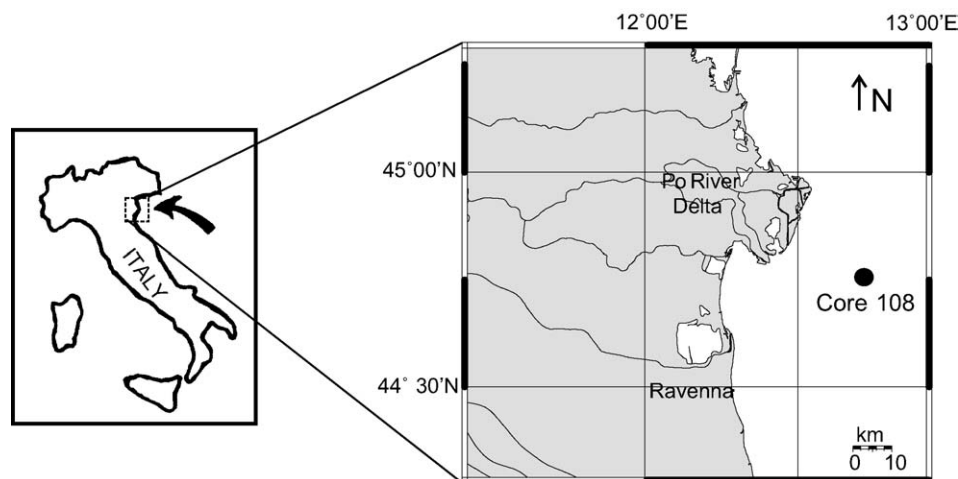


Fig. 1. Location of the studied core 108.

weight) was treated with a palynological method for qualitative preparation (consisting of five subsequent steps of HCl 10% and HF 38%), and then sieved to retain the  $>10\ \mu\text{m}$  fraction. The residue was mounted on microscope slides and analysed for organic-walled dinocysts, pollen and spores. An average of 230 dinocysts and a minimum of 300 pollen grains were identified and counted from each sample to determine species percentages. Dinocyst taxonomy follows Williams et al. (1998) and Rochon et al. (1999), whereas the ecological significance of each species is generally in accord with Marret and Zonneveld (2003). Pollen and spore identification was based on Moore et al. (1991), Reille (1992) and the reference collection of the Laboratory of Palaeobotany and Palynology, Utrecht, The Netherlands.

The same age-model and sedimentation rate ( $\sim 0.35\ \text{cm/year}$ , based on  $^{137}\text{Cs}$  and  $^{210}\text{Pb}$  analysis of this core) were used as in Puškaric et al. (1990) and in Barmawidjaja et al. (1995).

## 2.2. Numerical analysis

To identify statistically significant dinocyst and pollen zones in the record, a stratigraphically constrained cluster analysis was performed. This classification technique is used to determine relationships between samples in a stratigraphic record based on the relative abundance of species. Samples are linked into clusters based on their (dis-) similarity to each other. The samples were numerically zoned using optimal sum-of-squares partitioning of Birks and Gordon (1985) as implemented in the program ZONE (Lotter and Juggins, 1991). The number of significant stratigraphical zones was assessed using the broken stick model (Bennett, 1996). Numerical zonation was performed on pollen percentages both including and excluding conifer pollen from the total pollen sum. Results were compared

and the impact of typically over-represented *Pinus* pollen (Erdtman, 1969) on the zonation results was determined. Dinocyst and pollen percentages were calculated with the program Tilia 2.0.b.4 and the results plotted (together with the cluster analysis output) using TiliaGraph 2.0.b.5. To plot the pollen diagram, taxa with abundances lower than 2% of the total pollen sum and/or with less than five occurrences were omitted. A diversity index was determined using rarefaction analysis to normalise the number of different palynomorphs encountered in each sample (Birks and Line, 1992). This method calculates the diversity that would be expected if identical counting sums were used in every sample.

## 3. Results

### 3.1. Dinocyst assemblages

The most abundant dinocyst species, plotted versus both depth (cm) and age (years AD) are shown in Fig. 2, together with the Heterotrophic/Autotrophic ratio curve, the diversity index of each assemblage and the number of counted specimens in each sample. Thirty-six dinocyst species were identified (Appendix 1). A minimum of 18 species was recovered in the lowermost sample (56.5 cm, AD 1832) and a maximum of 26 species occurred in the samples between 10.5 and 12.5 cm (AD 1954–1960). Among the Protoperidinioid heterotrophic cysts, *Brigantedinium* spp. and *Selenopemphix quanta* (grouping cyst of *Protoperidinium conicum* and cyst of *Protoperidinium nudum*) are the most abundant. Both taxa are generally associated with upwelling and/or nutrient-enriched waters (e.g., Rochon et al., 1999 and references therein) but the latter has shown a positive correlation with the concentration of trace metals (Zn, Pb) in sediments indicative of a

**Station 108, North Adriatic Sea**  
 Percentage dinoflagellate cysts (selected taxa)

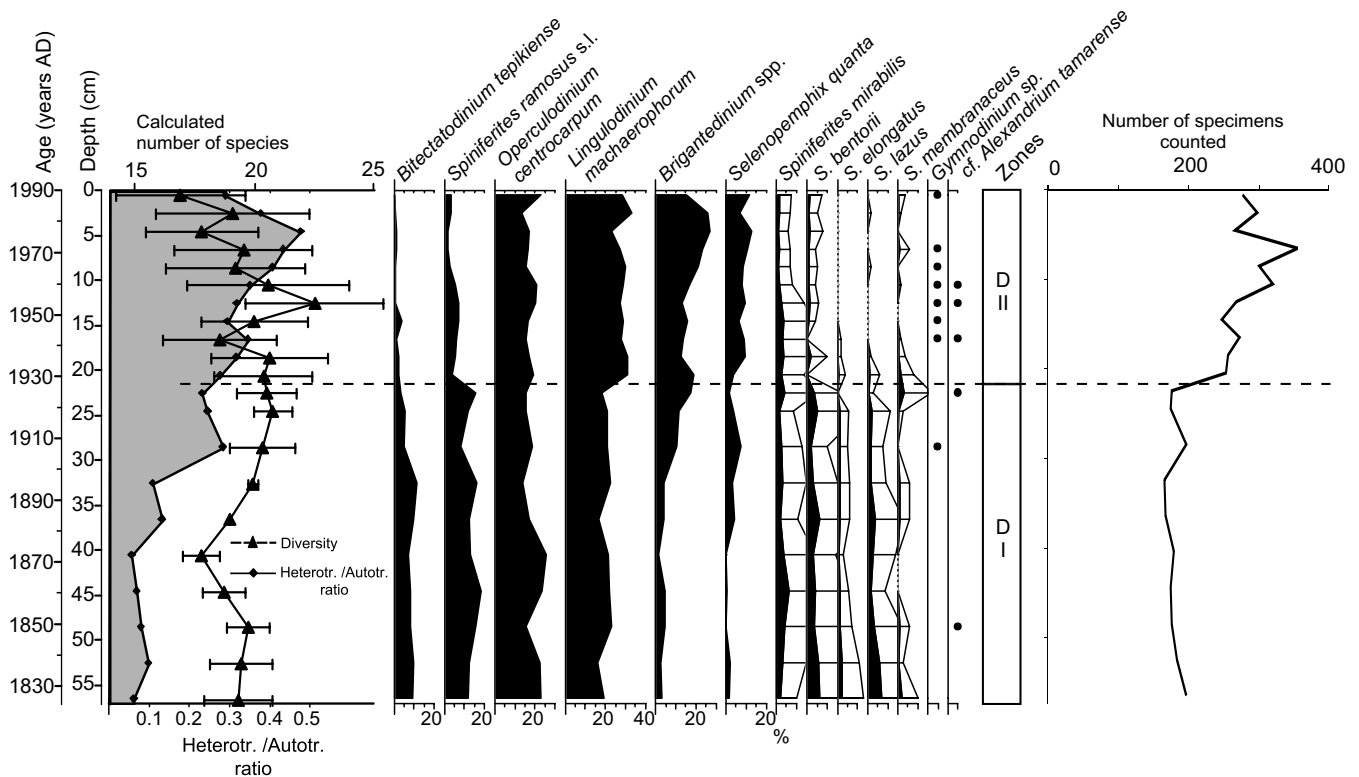


Fig. 2. Relative abundances of the most representative dinoflagellate cysts in core 108 (Northern Adriatic Sea) plotted against time (years AD) and depth (cm). Closed circles (●) indicate species presence. The graph on the left side shows Heterotrophic/Autotrophic dinocyst ratio (◆, closed curve) and the calculated diversity of each assemblage (▲, with error bars). The graph on the right shows the number of specimens counted in each sample. Results of optimal sum-of-squares partitioning are given as statistically significant dinocyst zones D-I and D-II.

polluted environment (e.g., Sætre et al., 1997; Sangiorgi et al., 2001a). The relative abundance of *Brigantedinium* spp. is lower than 5% in the lower part of the core (up to 32.5 cm, AD 1900), then reaches values of ~20% in the middle part (between 28.5 and 10.5 cm, AD 1910–1960) and later increases further to nearly 30% at 4.5 cm (AD 1978). In the top sample, *Brigantedinium* spp. decreases again to 16%. *S. quanta* relative abundance displays generally low values in the lower part of the core (up to 20.5 cm, AD 1930), whereas in the upper part of the core it increases threefold. An exception is the sample at 28.5 cm (AD 1910) where the relative abundance of this species is as high as in the upper part of the core. Protoperidinioid heterotrophic cysts progressively increase throughout the core, reaching maximum values of about 40–50% between 8.5 and 2.5 cm (AD 1966–1984). *Operculodinium centrocarpum* (cyst of *Protocera-tium reticulatum*), the most cosmopolitan species known today (Dale, 2001b; Marret and Zonneveld, 2003 and references therein), is present throughout the core. Its relative abundance does not display any particular trends and ranges from a minimum of 13% to a maximum value of 25% at 40.5 cm (AD 1875). *Lingulodinium machaerophorum* (cyst of *Lingulodinium polyedrum*) is

also well represented and it markedly increases in the upper part of the core, starting from 20.5 cm (AD 1930) and reaching a maximum abundance (~33%) at 2.5 cm (AD 1984). *L. machaerophorum* is a typical coastal species, and its high occurrence is generally indicative of warm eutrophic waters. It is also very abundant in estuaries, in association with high nutrients and, possibly, low salinity (Dale and Fjellså, 1994; Thorsen et al., 1995; Dale, 1996, 2001a). Among the genus *Spiniferites*, the cosmopolitan species *Spiniferites ramosus* and *S. bulloides* (grouped as *S. ramosus* s.l., sensu lato) are the most abundant. Its relative abundance decreases by a factor of more than two in the upper part of the core (from 20.5 cm, AD 1930). *Spiniferites elongatus* (a cold-temperate water species) and *S. bentorii* and *S. lazus* (temperate coastal species) display a marked decreasing trend towards the top of the core, while the warm water indicator *S. mirabilis* and the cold-temperate water species *S. membranaceus* (e.g., Thorsen et al., 1995; Dale, 2001b) have fairly stable values throughout the core. *Bityectatodinium tepikiense* shows fairly constant values (9–11%) up to 32.5 cm (AD 1900), and a decreasing trend towards the top of the core. Finally, we found some *Gymnodinium* sp. cysts (~1%) resembling

those of the toxic *Gymnodinium catenatum* but smaller, about 20  $\mu\text{m}$ , the same morphotypes described in Rubino et al. (2000) for this area. Probably, the *Gymnodinium* sp. cysts are those of *Gymnodinium nolleri*, a non-toxic species (Ellegard and Moestrup, 1999). Some cf. *Alexandrium tamarense*-type cysts (the type described in Rochon et al., 1999) have been found, mainly in the upper part of the core. The Heterotrophic/Autotrophic ratio curve, in which Protoperidinioids account for almost all the heterotrophic species since *Polykrikos* never exceed 0.5%, shows low values until 32.5 cm (AD 1900), a progressive, marked increase up to 4.5 cm (AD 1978) and a shift towards relatively lower values in the topmost two samples. The Heterotrophic/Autotrophic ratio can be considered as a synthetic proxy to detect eutrophication trends (e.g., Matsuoka, 1999).

Based on the optimal sum-of-squares partitioning, two significant dinocyst zones can be identified (Fig. 2, D-I and D-II). The boundary between these zones lies between 20.5 and 22.5 cm (AD 1924 and AD 1930, respectively).

### 3.2. Pollen assemblages

A total of 67 plant taxa were identified in the assemblage (Appendix 2). Relative pollen and spore abundances of the most abundant taxa are plotted in Fig. 3. The cluster analysis showed the spectra to be highly

influenced by the high *Pinus* abundances; optimal sum-of-squares partitioning only with conifers inside the pollen sum produced an inconsistent zone boundary. Repeated analysis excluding conifer pollen from the total pollen sum provided the most consistent zonation without simply creating a zone boundary between samples where the largest decrease of *Pinus* pollen occurs. Two significant (at the 95% significance level) stratigraphic zones were identified in the cluster analysis, marked P-I and P-II (Fig. 3, results from cluster analysis with conifers out of pollen sum). The zone boundary is situated between 28.5 and 32.5 cm from the core top (between AD 1900 and AD 1910). *Pinus*, *Quercus robur* type and Poaceae pollen are continuously dominant throughout the core; although no directional change in calculated diversity occurs, there is significant variation between samples, so that total calculated diversity varies between 38 and 29 morphotypes (Fig. 3, Appendix 2). *Q. robur* type is nearly constant, averaging about 15% of total abundance throughout the core. Although the record is dominated by arboreal pollen (AP, varying between 65 and 75%), a large number of herb taxa occur. The non-arboreal pollen (NAP) shifts dominance from a relatively fern-rich assemblage (30–40% of total NAP in zone P-I) with the presence of some aquatic taxa (*Typha augustifolia*, *T. latifolia*, *Nymphaea* spp. and Cyperaceae) around AD 1850 (zone P-I) towards a more herb (notably Chenopodiaceae, *Plantago lanceolata* and

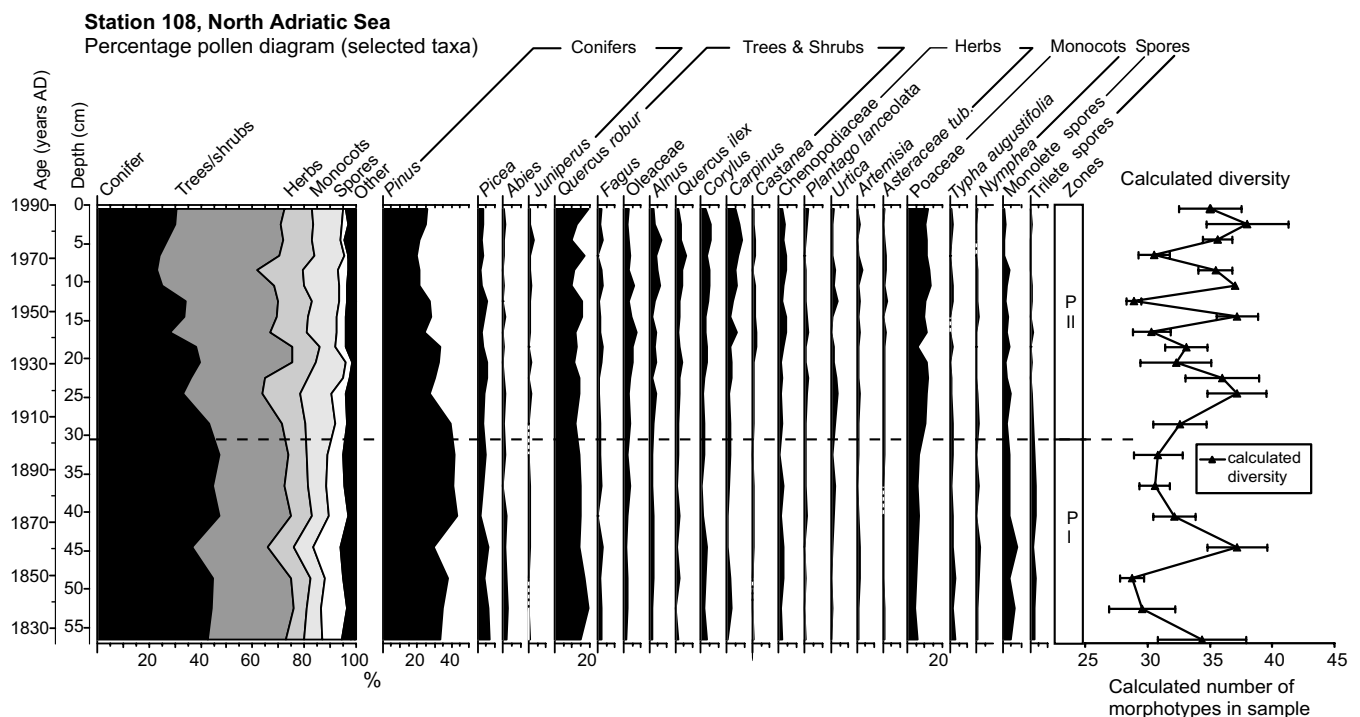


Fig. 3. Relative abundances of selected pollen and spores in core 108 (Northern Adriatic Sea) plotted against time (years AD) and depth (cm). Taxa with a maximum abundance lower than 2% of the total pollen sum and/or with less than five occurrences were omitted. All species were included in the pollen sum for calculation of the group abundances, left side of the figure. Graph on the right side shows calculated diversity of each assemblage (▲, with error bars). Results of optimal sum-of-squares partitioning are given as statistically significant dinocyst zones P-I and P-II.

*Urtica* spp.) and grass-rich assemblage (spores 5–10% of total NAP) around AD 1980. The major shift occurs between AD 1890 and AD 1910. During this same period, conifer (mainly *Pinus*) abundance starts to decline from 60% (of total AP), reaching a minimum of 35% at AD 1965 followed by a slight increase. The decline is initially in favour of herbs (at the zone boundary), followed by small increases in deciduous taxa (Oleaceae, *Alnus*, *Quercus ilex*, *Corylus* and *Carpinus*). Upland taxa such as *Fagus* and particularly *Picea* show no clear trend in abundance, although *Abies* is slightly more abundant in the period before AD 1880 than later.

#### 4. Discussion

##### 4.1. Dinocysts assemblages

In general, the dinocyst assemblages in core 108 are indicative of a coastal water environment, with cosmopolitan species, such as *Spiniferites ramosus* s.l. and *Operculodinium centrocarpum*, very well represented. *O. centrocarpum* is the cyst of the yessotoxin-producing *Protoceratium reticulatum* (Satake et al., 1997), which has been found at high cell concentrations in the North Adriatic waters during episodes of toxicity in the summers of 1997, 1998 and 1999 (Boni et al., 2000).

The statistical analysis of the dinocyst results indicates two main zones. The lower dinocyst zone (D-I) represents the period between AD 1830 and AD 1924. One of the most interesting features is the highest occurrence of the two species *Spiniferites elongatus* and *Bitectatodinium tepikiense* in the lowermost part of the core (before AD 1900). Both species are considered indicators of temperate-cold sea surface temperatures (SSTs), and *B. tepikiense* has affinities for nutrient-enriched, stratified surface waters characterised by large seasonal temperature contrast and low salinity (e.g., Mudie, 1992; Rochon et al., 1999). Their high occurrences in the lower part of the record and their progressive decreases concur with the end of the Little Ice Age, a colder and very humid period, which had large impact on the environmental evolution of the Po area and on the coastline configuration (e.g., Marabini, 1996).

The shift towards higher values of the Heterotrophic/Autotrophic ratio between AD 1900 and 1910, mainly due to an increase in *Brigantedinium* spp., and a peak in *Selenopemphix quanta*, points to enhanced cultural eutrophication. This conclusion is supported by evidence of an increase in oxygen supersaturation in surface waters starting from 1911 to 1913 (Justic et al., 1987) and by the record of benthic foraminifera analysed in the same core (Barmawidjaja et al., 1995), which shows a simultaneous increase in nutrient load and stressful conditions (eutrophication, pollution and

low oxygen content) on the benthic system. The peak in *S. quanta* (8% of the assemblage at AD 1910) suggests a temporary enhancement of heavy metal pollution (Sætre et al., 1997; Sangiorgi et al., 2001a), possibly related to the first phase of acceleration in the industrialisation process in the northern Italy, which started about 1902 and reached its acme around 1911 (Cafagna, 1989). The calculated diversity in the assemblages shows little variability, from a minimum number of 17 to a maximum of 22 species (Fig. 2).

The second dinocyst zone (D-II) covers the period AD 1930–1989. During this period, a progressive shift towards higher Heterotrophic/Autotrophic ratio can be observed, reaching the highest values between AD 1966 and AD 1978. This trend is in agreement with the results obtained from the same core by Puškaric et al. (1990). They recorded an increase in phytoplankton remains (diatoms, calcareous nannoplankton, silicoflagellates) during the years 1950–1990, in correlation with the post-war increase of phosphorus load originating from human activity and with the estimated doubling of nutrient load carried by the Po River between 1968 and 1980 (Marchetti et al., 1989). In addition, oxygen supersaturation in surface waters (Justic et al., 1987) was coupled with anoxia in bottom waters during the period between 1955/1956 and 1972/1982. Episodes of severe anoxia were also recorded in 1977 and 1988 (Degobbi et al., 1979; Degobbi, 1989). These periods correspond to the major increase in Heterotrophic/Autotrophic ratio (and to the highest occurrence of *Brigantedinium* spp.). Since Protoperidinioid cysts are considered to be very sensitive to oxygen levels (e.g., Zonneveld et al., 2001; Reichart and Brinkhuis, 2003), the shift could represent both increased eutrophication and increased cyst preservation. Nevertheless, the Heterotrophic/Autotrophic ratio curve found here shows a decreasing trend in the uppermost part of the core (AD 1984–AD 1989), although episodes of anoxia and years without winter vertical mixing are still a common feature of this area (Socal et al., 2001; Bastianini, 2002). The Heterotrophic/Autotrophic trend corresponds to recorded decreases in phosphorus loading, beginning in the middle 1980s, after regulations that reduced phosphate contents in detergents were imposed (e.g., Decreto Legis 30 Dicembre 1981, n. 801; Ivancic and Degobbi, 1998). Phosphorus is in fact the factor limiting phytoplankton growth in the North Adriatic Sea (Zavatarelli et al., 1998).

At the boundary between dinocyst zones D-I and D-II, about AD 1930, the relative abundance of *Lingulodinium machaerophorum* shows a clear increase. Considering this species as a proxy for eutrophication (according to Dale et al., 1999), support can be given to the hypothesis that the real change in nutrient content in the North Adriatic system occurred around this time, as a consequence of modern farming methods, increase

of industrialisation, and urbanisation (Barmawidjaja et al., 1995). *Lingulodinium polyedrum* is a very common species in the North Adriatic coastal environment (Nichetto et al., 1995; Belmonte et al., 1998; Rubino et al., 2000) where it blooms in late summer and has caused red tides in recent years, particularly between 1977 and 1985 (Boni et al., 1986; Fonda-Umani et al., 1991). Analyses of surface sediments along a transect in the North Adriatic Sea offshore Ravenna in spring 1997 have recovered numerous *L. machaerophorum* cysts (both empty and full). Their percentage in the assemblages varied between 30% and 50% and their concentration reached 4200 cysts/g dry sediment, decreasing seawards (Sangiorgi et al., 2001a). During late summer of the same year, a bloom of *L. polyedrum* occurred in the same coastal area (Sangiorgi, pers. comm.).

Cysts of the potentially toxic cf. *Alexandrium tamarense* and *Gymnodinium* sp. are more common in samples from the dinocyst zone D-II than in samples from zone D-I. In 1984, a conspicuous bloom of *Gymnodinium* sp. occurred along the north-western Adriatic Sea coast, to an extent never seen before or since (Vollenweider et al., 1992). The last and the highest occurrence of *Gymnodinium* sp. cysts in the record exactly corresponds to AD 1984. The cyst of *Gymnodinium* sp. could be that of *Gymnodinium nolleri*, a non-toxic species not always causing red tides (Ellegard and Moestrup, 1999). In fact, no poisoning effect was detected after that bloom (Boni, pers. comm.).

In the upper part of zone D-II, *Bitectatodinium tepikiense* markedly decreases and it disappears in the top sample. This trend seems to indicate a reduced seasonal temperature contrast in the surface waters. At present, sea surface temperatures (SSTs) in the North Adriatic display a strong seasonal contrast, with winter average SST of  $\sim 9$  °C and summer average SST of  $\sim 20.5$  °C (Zavatarelli et al., 1998). Nevertheless, temperature data collected since 1970 have shown a relevant increase in SST in all seasons, with the highest positive temperature anomalies in spring and autumn (Bastianini, 2002). The trend of this species may therefore indicate a reduced temperature contrast between the periods (mainly spring–summer and summer–autumn, Fonda-Umani et al., 1991; Fonda-Umani, 1996) during which dinoflagellates bloom in the area. Finally, calculated diversity in zone D-II shows high variability with the lowest number in the sample correspondent to AD 1978. A drop in diversity points to increased stressful environmental conditions (eutrophication, pollution and high oxygen index values, indicative of dysoxia or anoxia) caused by intensified anthropogenic disturbance.

#### 4.2. Pollen assemblages

Pollen and spore diversity, notably herb and deciduous tree taxa, indicates substantial pollen input

through water transport. Taxa producing relatively few and large pollen (i.e. *Centaurea*, *Hedera* and Caryophyllaceae) (Erdtman, 1969; Andersen, 1970) are present throughout the core. Such large, thick-walled and sculptured pollen grains do not travel large distances by air (Whitehead, 1983). The Po River is an obvious source of terrestrial runoff, including pollen and spores. Therefore, the Po catchment area is likely to be a major source of the pollen and spores in the core. Changes in arboreal pollen (AP) are mainly due to changes in the relative abundance of *Pinus* (due to forest clearance) thereby increasing the relative abundances of deciduous tree taxa in the record. *Pinus* pollen, however, are largely wind-transported (Whitehead, 1983), and although the influence of Po runoff should not be underestimated, a clear source of *Pinus* influx is difficult to determine since wind patterns play an important role in the transport of this pollen. Total decrease in *Pinus* from the zone boundary towards its minimum around AD 1970 is sufficiently large ( $\sim 20\%$ ) to indicate some change in forest composition in the Po catchment area, analogous to that described in Willard et al. (2003).

The main shift in non-arboreal pollen (NAP) is caused by the increase of Poaceae (grass) pollen, indicating an enlargement of open vegetation (i.e. pasture- or meadow land) starting at about 1890. This rise is accompanied by small increases in anthropogenic indicators (Chenopodiaceae, *Plantago lanceolata*, *Rumex* spp. and *Urtica* spp.) associated with dry cultivated areas and high-nutrient input (Behre, 1986). The fern assemblage declines over the same period, indicating a decrease in swampland area in the Po catchment (supported by the decline in aquatic taxa). Large areas in the Po delta were converted from swamp into pastureland predominantly from the end of the 19th century to 1970 (Cencini, 1998). The pollen signal reflects the entire Po catchment area, and the effects of local changes are probably muted. This effect is indicated by the stability in the pollen assemblages and by the minor changes in species diversity. Nonetheless, the Po delta area is where the most prominent change in land use occurred and thus this is the most probable source of pollen and spore assemblage changes. Notably, between AD 1890 and AD 1910, non-arboreal pollen changed from a relatively high proportion of humid taxa towards more dry land and anthropogenic-indicating taxa (Poaceae, Chenopodiaceae, *Plantago lanceolata*, *Rumex* spp. and *Urtica* spp.). This is followed by an increase in deciduous tree taxa after 20–30 years (between AD 1920 and AD 1950). This succession is in line with the land-reclamation activities of the early 20th century in the coastal Po delta area. Any decrease in conifers within the Po catchment would however create the same trend in tree pollen abundances. The exact cause of the increase in deciduous taxa cannot therefore be determined.

## 5. Conclusions

The Heterotrophic/Autotrophic dinocyst points to a progressive enhancement of (cultural) eutrophication in the north-western Adriatic Sea during the 20th century, particularly marked between AD 1930 and AD 1978. This change is accompanied by a clear increase at AD 1930 in the relative abundance of *Lingulodinium machaerophorum*, related to nutrient-enriched coastal waters. Although the Heterotrophic/Autotrophic ratio can be affected by changes in cyst preservation, it displays a clear decrease after AD 1978 while the oxygen index in bottom waters indicative of dysoxic or anoxic episodes increases (Duijnsteet al., in press and references therein). This can be interpreted as a positive response of the marine environment to a changed Italian environmental policy after 1980. These observations support the validity of the ratio as a proxy for tracing changes in the trophic state of the area. Increased stressful conditions, as derived by dinocyst diversity can be identified after AD 1960. From this time to AD 1978, dinocyst diversity decreases with the continuous increase of nutrient load into the North Adriatic Sea and, although eutrophic levels started to drop at about AD 1980, the marine ecosystem does not seem to have recovered yet. The highest value of the Heterotrophic/Autotrophic dinocyst ratio, together with low dinocyst diversity at AD 1978, leads to the conclusion that the most stressful conditions in the marine environment occurred around that time.

The pollen data show that the source of the increased nutrient loading of the North Adriatic can be partly attributed to the increase in land made suitable for agriculture starting at about AD 1890 as indicated by a succession from abundant ferns towards dry-land herbs, and culminating in increased abundance of deciduous taxa at about AD 1950. Historically known intensification (e.g., increased use of fertilisers) of agriculture is less apparent from the pollen data, although high nutrient indicating pollen taxa do occur increasingly from AD 1910 onwards.

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## Appendix 1

(A) Dinocyst in the assemblages	(B) Dinocyst diversity		
	Depth (cm)	Calculated diversity	Species richness
Gonyaulacoids			
cf. <i>Alexandrium tamarense</i> type	0.5	16.95	21
<i>Ataxiodinium choane</i>	2.5	19.12	24
<i>Bitectatodinium tepikiense</i>	4.5	17.84	20
<i>Lingulodinium machaerophorum</i>	6.5	19.60	23
<i>Operculodinium centrocarpum</i> sensu Wall and Dale, 1966	8.5	19.26	23
<i>Operculodinium centrocarpum</i> var. <i>truncatum</i> sensu Mudie, 1992	10.5	20.61	26
<i>Operculodinium israelianum</i>	12.5	22.59	26
<i>Pyxidinosia reticulata</i>	14.5	20.06	23
<i>Spiniferites</i> spp.	16.5	18.60	22
<i>Spiniferites bentorii</i>	18.5	20.67	23
<i>Spiniferites belerius</i>	20.5	20.43	22
<i>Spiniferites bulloides</i> (grouped with <i>S. ramosus</i> )	22.5	20.55	21
<i>Spiniferites delicatus</i> (grouped with <i>S. ramosus</i> )	24.5	20.82	21
<i>Spiniferites elongatus</i>	28.5	20.41	21
<i>Spiniferites hyperacanthus</i> (grouped with <i>S. mirabilis</i> )	32.5	19.99	20
<i>Spiniferites lazus</i>	36.5	19.00	19
<i>Spiniferites membranaceus</i>	40.5	17.82	19
<i>Spiniferites mirabilis</i>	44.5	18.77	19
<i>Spiniferites ramosus</i>	48.5	19.79	21
	52.5	19.48	20
	56.5	19.36	18
Gymnodinioids			
<i>Gymnodinium</i> sp.			
<i>Polykrikos schwartzii</i>			
Protoperidinioids			
<i>Brigantedinium</i> spp.			
<i>Brigantedinium cariacense</i> (grouped with <i>Brigantedinium</i> spp.)			
<i>Brigantedinium simplex</i> (grouped with <i>Brigantedinium</i> spp.)			
<i>Echinidinium</i> spp.			
<i>Lejeunecysta</i> spp.			
<i>Pentapharsodinium dalei</i>			
Cyst of <i>Protoperidinium nudum</i> (grouped with <i>Selenopemphix quanta</i> )			
Cyst of <i>Protoperidinium stellatum</i>			
<i>Quinquecuspis concreta</i>			
<i>Selenopemphix nephroides</i>			
<i>Selenopemphix quanta</i>			
<i>Trinovantedinium applanatum</i>			
<i>Votadinium calvum</i>			
<i>Votadinium spinosum</i>			
<i>Xandarodinium xanthum</i>			
Not determined			
Cyst 1 as Cyst type X (Pospelova et al., 2002)			



## Appendix 2

## (A) Pollen and spores in assemblage

Arboreal pollen (AP)	Non-arboreal pollen (NAP)	
Conifers	Herbs	
<i>Pinus</i> spp.	Alismataceae	
<i>Abies</i> spp.	Apiaceae	
<i>Picea</i> spp.	<i>Artemisia</i> spp.	
<i>Juniperus</i> spp. Type	Asteraceae tubuliflora	
	Asteraceae liguliflora	
Deciduous trees and shrubs	Brassicaceae	
<i>Alnus glutinosa</i>	<i>Cannabis/Humulus</i> type	
<i>Betula</i> spp.	Caryophyllaceae	
<i>Carpinus/Ostrya</i> type	<i>Centaurea cyanus</i>	
<i>Castanea</i> spp.	Chenopodiaceae	
<i>Corylus avellana</i>	<i>Ephedra</i> spp.	
<i>Fagus sylvatica</i>	Fabaceae	
<i>Fraxinus excelsior</i>	<i>Filipendula vulgaris</i>	
<i>Juglans</i> spp.	<i>Galium</i> spp.	
Oleaceae	Gentianaceae	
<i>Platanus</i> spp.	<i>Hedera helix</i>	
<i>Populus</i> spp.	<i>Helianthemum</i> spp.	
<i>Quercus robur</i>	<i>Hippophae rhamnoides</i>	
<i>Quercus ilex</i>	<i>Hypericum</i> spp.	
<i>Salix</i> spp.	<i>Linum</i> spp.	
<i>Sambucus nigra</i>	<i>Lysimachia</i> type	
<i>Tilia</i> spp.	<i>Plantago lanceolata</i>	
<i>Ulmus glabra</i>	<i>Plantago major</i>	
<i>Vitis</i> spp.	<i>Plantago maritima</i>	
	<i>Polygonum persicaria</i> type	
Monocots (grasses)	Ranunculaceae	
Poaceae	Rhamnaceae	
Cyperaceae	Rosaceae	
Cerealia	<i>Rumex</i> spp.	
	Rutaceae	
Heath	<i>Saxifraga stellaris</i> type	
Ericaceae	<i>Scophulariaceae</i>	
	Solanaceae	
	<i>Urtica</i> spp.	
Aquatic taxa		
<i>Myriophyllum spicatum</i>		
<i>Nymphaea</i> spp.		
<i>Typha augustifolia</i>		
<i>Typha latifolia</i>		
Ferns		
<i>Polypodium vulgare</i>		
Monolete spores		
Trilete spores		
(B) Pollen and spore diversity		
Depth (cm)	Number of morphotypes	Calculated diversity
0.5	40	35.04
2.5	44	38.02
4.5	39	35.61
6.5	35	30.54
8.5	38	35.47
10.5	39	37.00
12.5	31	28.91
14.5	40	37.20
16.5	34	30.33
18.5	36	33.15
20.5	37	32.27
22.5	41	35.99
24.5	41	37.18
28.5	36	32.61
32.5	35	30.86
36.5	34	30.59
40.5	37	32.14
44.5	42	37.20
48.5	31	28.74
52.5	33	29.57
56.5	42	34.37

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