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A Survey on a Persistent Greenish Bloom in the Comacchio Lagoons (Ferrara, Italy)

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The Comacchio lagoonal system has been affected for years by a persistent phytoplanktonic bloom which makes the waters greenish. Surveys carried out from April 1995 to February 1996 showed that this bloom was caused by a *Synechococcus* sp. and by an Eustigmatophycean belonging to the genus *Nannochloropsis*. However, while the *Synechococcus* sp. was mainly present in the summer, *Nannochloropsis* was always present with constant density values. Moreover, this species shows many ultrastructural features and a pigment composition similar to those of *Nannochloropsis* spp. and reproduces by autospores. The 18S rRNA gene sequence of this strain is identical to those of five strains of *Nannochloropsis gaditana*. In this paper, besides phytoplankton (including picophytoplankton), we also report the data of the main chemico-physical parameters. Among them, the salinity and nutrient values showed an anomalous seasonal trend.

Introduction

The Comacchio lagoons, Magnavacca (6160 ha), Fossa di Porto (2980 ha) and Campo (1670 ha), are situated north of the Reno River and are separated from the sea by a 2 km. wide spit. They are connected with the Adriatic Sea by the Logonovo and Gobbino channels. Magnavacca and Fossa di Porto lagoons are partly separated by the Boscoforte peninsula and communicate with the Campo valley by two drainage channels (Fig. 1). Consequently these three lagoons constitute a single system. They are famous for fishing and for eel breeding.

The water regime is regulated by the drains located in the Logonovo and Gobbino channels and in the Reno River: the first opens in spring and in autumn, the latter in late winter-early spring. In summer, where the rainfall is low and the temperature is high, these waters, which are shallow (0.5–1.5 metres), show a marked increase in temperature and salinity.

Up to the 1970s, both the environmental and the biological parameters were similar to those of other lagoons not directly affected by human activities (Cognetti *et al*. 1975). The phytoplankton was characterized by a high specific diversity, due to the presence of diatoms, and the lagoon bottoms were covered by phytobenthos (macroalgae and phanerogames) (Boni 1971, Ferrari *et al.* 1972). Over the last two decades there have been dramatic changes and at the present time, the Comacchio biocoenosis is completely altered and cannot be compared either with other lagoons of the Po Delta or other areas involved in eutrophication. Phytobenthos is completely absent, the waters are murky and always greenish in colour; the phytoplankton is represented almost exclusively by coccoid cyanobacteria and by *Chlorella* sp. (Boni *et al.* 1995). In a recent investigation, carried out in the summer and in autumn of 1993, Sorokin *et al.* (1996), finding a depletion of all kinds of zooplankton, mortality of the benthic fauna and the collapse of eel and mullet fisheries, asserted that this was an 'ecological catastrophe'. This situation was caused by the conflux of effluent waters and by the accumulation of un-consumed food introduced during intensive fish farming in operation from 1972 to 1993. According to Sorokin *et al.* (1996) the influx of these large amounts of organic matter may have resulted in the accumulation of sulphide in the upper sediment layer. This high sulphide content, over a period of years, could have caused the persistent bloom of coccoid picocyanobacteria, which could have lead to the lack of both zooplankton and bottom biocoenosis. The high sulphide content, easily oxidisable, could have also caused high oxygen consumption and consequently ipoxia and anoxia in these lagoons (Sorokin *et al.* 1996). The particular conditions were greatly worsened by the absence of good water exchanges. From 1968 to 1992, in fact, water exchange with the Reno River was blocked, while the exchanges with the sea were sometimes interrupted by the silting up of the Logonovo and Gobbino channels (Comacchio commune, unpublished data). As a consequence of this ecological situation, but above all due to the statement of Sorokin *et al.* (1996), the Regione Emilia Romagna has begun a programme for several years to monitor the chemico-physical and biological parameters (phytoplankton

and zooplankton) of the Comacchio lagoons. The present work is a part of this project and includes the results on the phytoplankton and the chemico-physical parameters obtained during the first year of this monitoring.

Materials and Methods

Sampling was carried out monthly from April 1995 to February 1996 at nine sampling stations (Fig. 1). On account of the shallow depths of these lagoons, we mixed two water samples, 5 L each, respectively collected from the surface and the bottom, using a Niskin bottle. Samples were collected to determine the concentration of nitrate, nitrite, ammonium, phosphate, chlorophyll-*a*, to count picophytoplankton and to identify and count nanoplanktonic and microplanktonic species. Temperature, salinity, pH and oxygen were measured using a Hydrolab Surveyor II multiparametric probe. Oxygen was also determined by the Winkler method. A Secchi disk was used to measure the transparency.

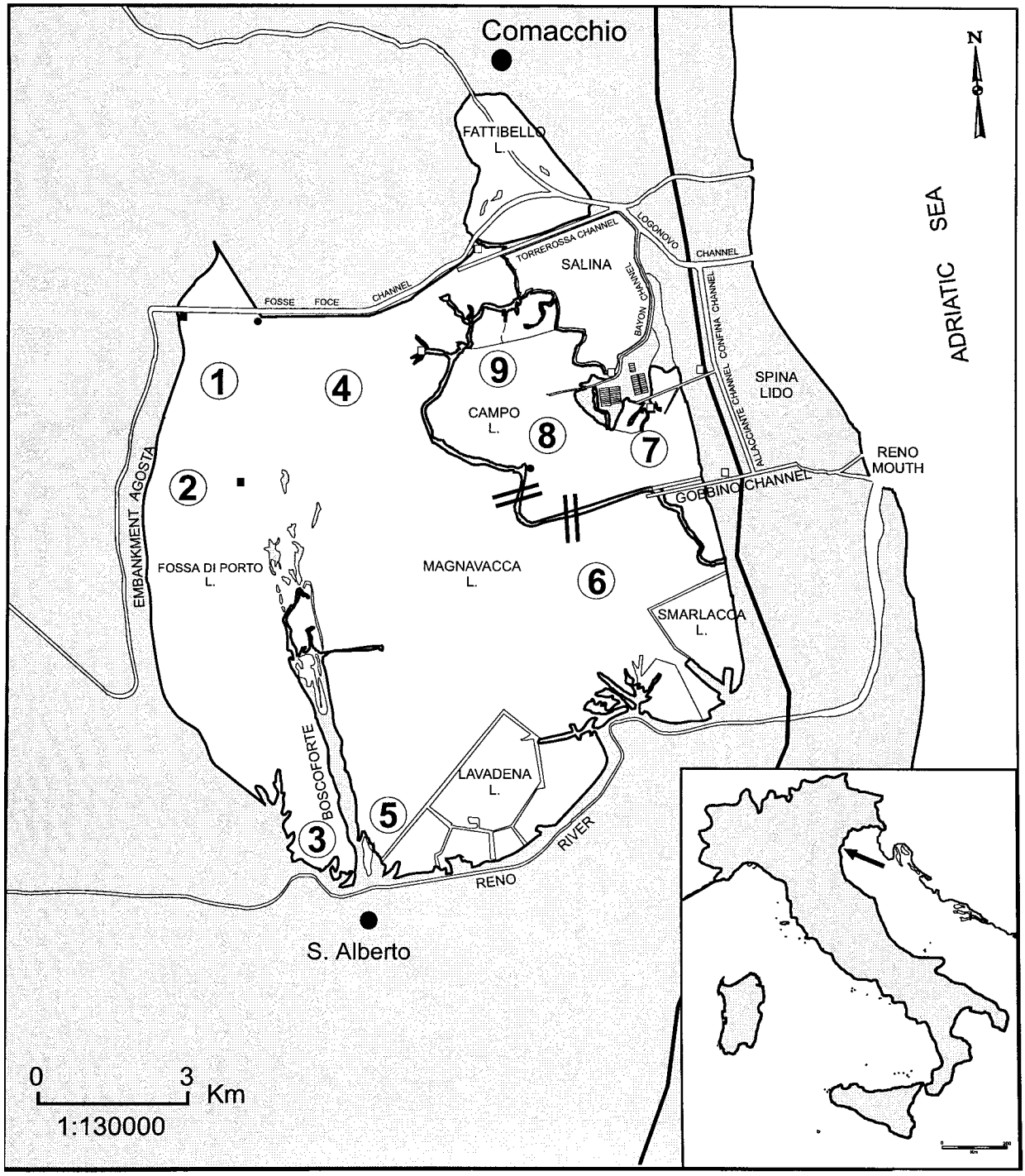


Fig. 1. The Comacchio lagoons showing the sampling stations (1−9).

Samples for nutrient analyses were filtered (Whatman GF/C) and subsequently analysed by the methodology of APHA (1981).

For the chlorophyll-*a* estimation, water samples were filtered through a GF/F filter with pores of 0.45 μm and photosynthetic pigments were estimated following the standard procedure of SCOR-UNESCO (1966) on 90% acetone extracts, using Jeffrey and Humphrey (1975) equations. The chlorophyll data were not corrected for phaeopigments.

The picophytoplanktonic forms were studied with a Bruker ACR-1500 flow cytometer (Troussellier *et al.* 1993, Andreoli *et al.* 1997) on samples preserved according to the method of Vaulot *et al.* (1989).

Phytoplankton > 2 μm, preserved with 1% final volume Lugol's solution in glass bottles, was identified, counted and sized using the Utermöhl method (1958). In particular, for each sample, 2−5 mL of water were allowed to settle for 24 h in a compound sedimentation chamber whose bottom was subsequently observed using a Zeiss inverted microscope. Sixty random areas of the chamber, the equivalent of about 0.6 mL of sample, were examined at 400 × magnification to count the more frequent forms; the entire chamber bottom was scanned at 200 × to enumerate the large and less frequent organisms.

Cell volumes were calculated by comparing cell shapes with appropriate geometric figures. Cell carbon biomass was estimated from cell volumes and cell abundance. The cell volume to carbon (μg C L^{-1}) relationships were calculated using the equations of Eppley *et al.* (1970). For the picophytoplankton the biovolumes were converted to carbon using a carbon conversion factor 470 fg C μm^{-3} (Verity *et al.* 1992).

For diatom nomenclature we followed Van Landingham (1967−1979) and for dinoflagellates and other planktonic marine flagellates the reference works were Dodge (1982) and Throndsen (1993) respectively.

Water samples were also used to set up cultures of the more representative species. The cultures led us to carry out further surveys on the pigment composition of these organisms and ultrastructural analysis using the transmission electron microscope (TEM).

Microalgae were grown in the Comacchio water, which had been previously filtered through a Millipore GF/F filter, sterilized and enriched with F/2 medium (Guillard 1975). The cultures were incubated at 20 °C with a 12:$\overline{12}$, L:$\overline{D}$ cycle (100 μmol m^{-2} s^{-1}). The pigment composition analysis was carried out using a spectrophotometer for the phycobiliprotein and an HPLC for the carotenoid contents.

The phycobiliprotein analyses were carried out on extracts obtained, after centrifugation, by grinding cell samples in a mortar, with quartz glass beads, in 50 mM phosphate buffer. The extract, was kept at 4 °C for 20 h, and subsequently centrifugated at 23000 *g* for 15 minutes at 4 °C. The pigment concentrations were calculated using the equations of Bennett and Bogorad (1973). For high performance liquid chromatographic (HPLC) analysis of chlorophylls and carotenoids, the pigments were extracted in 100% methanol buffered with 2% ammonium acetate using a liquid nitrogen-cooled Retsch Mixer Mill MM2000 homogenizer with agate pearls. After centrifugation, the methanol extract was filtered through Gelman nylon filters with pores of 0.47 μm. Methanol was used as the extraction solvent instead of acetone because it produces sharper peaks on the following HPLC analysis (Zapata and Garrido 1991). A volume of 20 μL was directly injected into the HPLC Beckman-system Gold Programmable Solvent (mod. 126). The reversed-phase column used was a Zorbax ODS-not blocked (Dupont), 25 cm in length and 4.6 mm in diameter. It was preceded by an Adsorbosphere C-18 pre-column. In accordance with Mantoura and Llewellyn (1983), the mobile phases used in the gradient elution consisted of a primary eluant (A) made up of a 10:10:80 mixture, by volume, of solution P:water:methanol, and a secondary eluant (B) made up of 30:70 ethylacetate:methanol, by volume. The ion-pairing reagent (solution P) was prepared from 1.5% (w/v) of tetrabutyl-ammonium acetate (Fluka, 95% purity) and 7.7% (w/v) of ammonium acetate. The flow rate was 1 mL min^{-1}, and the gradient system used is shown in Table I. Pigments were detected with a Diode Array Detector (mod. 168) at 436 nm.

The spectra and retention times of the various pigments of the microalga collected in the Comacchio lagoons were compared with absorption and chromatographic characteristics of the pigment extracts from cultures of *Pleurochloris meiringensis* Vischer (culture collection Göttingen, strain CCAP 860/3 = UTEX 311) and *Chlorella vulgaris* Beijerinck (culture collection Göttingen, strain CCAP 211/11N).

For transmission electron microscopy (TEM), cells were fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer (pH 6.9) for 3 h at approximately 18 °C. After washing in three changes of 0.1 M cacodylate buffer, specimens were postfixed in 1% OsO_4 for 2 h and dehydrated in a graded series of ethyl alcohols and propylene oxide. Staining of the samples with uranyl acetate was done while they were dehydrating with ethanol 75%. The samples were embedded in an Epon-Durcupan ACM mixture. The thin sections,

Table I. Analytical gradient protocol.

Time (min)	Solvents (%)		Gradient system
	A	B	
0	100	0	Injection
1	75	25	Linear
41	0	100	Linear
56	0	100	isocratic phase
60	100	0	re-equilibration

cut with a Reichert Ultracut S, were poststained with lead citrate and examined using a Hitachi HS9 microscope operating at 75 kV. Further observations were carried out using a Cambridge Stereoscan 260 Scanning electron microscope (SEM) on samples fixed with 3% glutaraldehyde. They were subsequently dehydrated in a graded series of ethyl alcohols, critical-point dried and gold coated.

For the *Nannochloropsis* strain, genomic DNA was isolated using a modification of the Sony and Murray method (1994) in which ethanol precipitation of DNA was replaced by isopropanol precipitation. The 18S rRNA gene was amplified from DNA extracts by PCR (Polymerase Chain Reaction), using the primers listed in Table II (oligo 1 with oligo 2; oligo 4 with oligo 5; oligo 6 with oligo 7), to give three overlapping fragments. Amplification conditions were: an initial denaturation step of 2 min at 95 °C followed by 45 sec at 95 °C (DNA denaturation), 30 sec at 58 °C (annealing), and 45 sec at 72 °C (extension) for 40 cycles with a final extension step of 10 min at 72 °C. The PCR products were run on 0.8−1% agarose gels, bands were excised and the DNA was extracted using the Jetsorb Gel Extraction Kit (Genomed GmbH, Germany). The PCR products were directly sequenced using the ABI PRISM Dye Terminator Cycle sequencing Core Kit (Perkin Elmer), covering the entire length of the gene in both directions, using the primers listed in Table II. Electrophoresis of sequencing reactions were completed on the ABI PRISM model 377, version 2.1.1 automated sequencer. The sequence of 18S rRNA for the *Nannochloropsis* of the Comacchio lagoons was aligned together with homologous gene sequences of other species of this genus, already available in GenBank. A multiple sequence alignment was obtained using the CLUSTALW computer program (Thompson *et al.*, 1994). This sequence of the 18S rRNA gene of *Nannochloropsis gaditana* has been deposited in GenBank and has the following accession number: AF133819. A living culture of the type has been deposited at the Sammlung von Algenkulturen at the University of Göttingen (SAG 2.99) and Provasoli-Guillard National Center for Culture of Marine Phytoplankton, West Boothbay Harbor, ME (CCMP 1894)

Results

Environmental parameters (Figs 2−3)

Throughout the research the water transparency was very low (26−30 cm) in all sampling stations, the water was slightly clearer in the winter with values up to 45 cm. The water temperature showed a seasonal trend, ranging from 3 °C in January to 28 °C in August. With regards to pH, the lowest values (7.9) were registered in winter, while the highest values (8.5−8.6) were measured in September and October. The salinity, after the progressive increase throughout the summer reached the highest levels (32−33‰) in November and December. Dissolved oxygen concentrations fluctuated from 6.53 ± 1.24 to 10.96 ± 1.8 mg L^{-1}. The lowest values (5 mg L^{-1}) were registered in August (Stations 2, 3, 5 and 6) and in November (Stations 1 and 5). The saturation percentage values were on average of 80−90% and showed oversaturation in summer-autumn (Fig. 2).

The trend of nutrient concentrations is shown in Figure 3. The N-nutrient concentrations were very low (nitrite and ammonium < 1 μmol L^{-1}, nitrate < 10 μmol L^{-1}) from May to November. However, these values increased throughout the winter reaching concentrations which were three or four times higher (Fig. 3). Orthophosphate concentrations were very low (< 1 μmol L^{-1}), except in the Campo lagoon in August and in November where they reached 1.49 ± 0.8 mg L^{-1} and 1.24 ± 0.9 mg L^{-1} respectively. In contrast, silicate amounts were low only in April and May (20 μmol L^{-1}); from June, in fact, they increased considerably until they reached 92.25 ± 4.09 μmol L^{-1} in October.

Phytoplankton (Figs 4−16)

Phytoplankton of the Comacchio lagoons was rich in picophytoplankton, which was only low in the winter months, December, January and February (Fig. 4). Flow cytometer analyses showed that over 90% of the phytoplankton was represented by red-fluorescing organisms (Fig. 5), which, after their isolation, were found to be phycocyanin-rich cyanobacteria (PCC) such as *Synechococcus*. This was also con-

Table II: List of primers used for PCR (* Numbers are relative to the nucleotide position for the 18S rRNA gene for *Nannochloropsis gaditana* (AF045040)).

Primer	Sequence		Nucleotide position* (5'→3')
Oligo 1	5'-CCTGCCAGTAGTCATACGCT-3'	Sense	+ 13 → + 32
Oligo 2	5'-TGCTTTCGCAGTAGTTCGTC-3'	Antisense	+ 948 → + 929
Oligo 3	5'-TTGGATGTGGTAGCCGTCTC-3'	Antisense	+ 417 → +398
Oligo 4	5'-GATTCCGGAGAGGGAGCCTG-3'	Sense	+ 377 → +397
Oligo 5	5'-CACCCATAGAATCAAGAAAG-3'	Antisense	+ 1269 → + 1250
Oligo 6	5'-CAGAGGTGAAATTCTTGGAT-3'	Sense	+ 901 → + 920
Oligo 7	5'-AAACCTTGTTACGACTTCAC-3'	Antisense	+ 1768 → + 1749
Oligo 8	5'-TCTGTGATGCCCTTAGATGT-3'	Sense	+ 1434 → + 1453

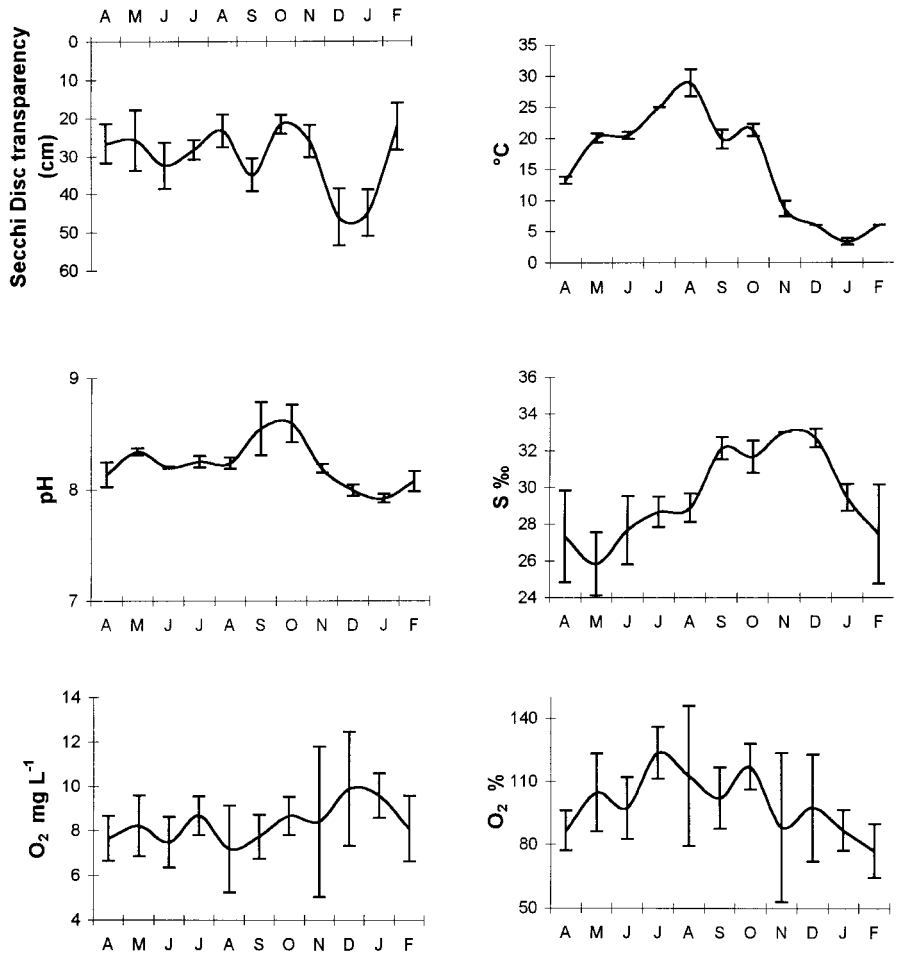


Fig. 2. Mean values of transparency, temperature, salinity, pH, dissolved oxygen and the percentage saturation of oxygen for the nine sampling stations (vertical bars are ± SD).

firmed by the phycobiliprotein analysis which showed that the organisms were richer in phycocyanin (0.086 mg mL^{-1}) than in phycoerythrin (0.0013 mg mL^{-1}) (Fig. 6).

The density values of the fraction > 2μm (nano- + microphytoplankton) were more uniform (500×10^3 cells mL^{-1}) than picophytoplankton in all sampling stations throughout the research (Fig. 4). The phytoplankton in this fraction was composed almost exclusively (98−99 %) of a coccoid nanoplanktonic species (2−5 μm in diameter) lacking flagella (Figs 7−8), which was responsible for the higher biomass values. The organic carbon content of the sample, in fact, was contributed mostly by this organism (Fig. 9).

The ultrastructure of this organism is simple, with cells consisting of one chloroplast without a pyrenoid, a mitochondrion with tubular cristae, a nucleus containing a nucleolus, and one or two vacuoles (Figs 10 and 12). In sections, the cell wall is electron-dense. The chloroplast lamellae consist of three adjacent thylakoids and no girdle lamella is present (Fig. 11). No starch was detected in the chloroplast or cytoplasm. The chloroplast and nuclear envelopes are confluent (Figs 10 and 12). In the cytoplasm, particularly of the autospores, lamellate vesicles are present (Figs 13−14). Moreover during the autospore production, the cell wall of the mother cell showed a plug-like structure (Fig. 13). High Performance Liquid Chromatography analyses, carried out comparing this microalga with *Chlorella vulgaris* (211/11N) and *Pleurochloris meiringensis* (860/3) showed that our unknown species lacked chlorophyll *c* and chlorophyll *b* and possessed violaxanthin as the major carotene pigment. The other pigments, according to the polarity decrease of individual pigments, were trans-neoxanthin, vaucheriaxanthin-ester, lutein, zeaxanthin, cantaxanthin, chlorophyll *a* and β-carotene (Fig. 15, Table III). In agreement with other authors (Antia *et al.* 1975, Hibberd 1981, Lubián 1982, Santos and Leedale 1995, Karlson *et al.* 1996, Jeffrey *et al.* 1997) all these features show that this microalga is an Eustigmatophycean of the genus *Nannochloropsis*. Its reproduction is by 2 or 4 autospores (Figs 13−14).

Comparing the 18S rRNA gene sequence of this strain with those of twenty one strains of *Nannochloropsis* obtained by Andersen *et al.* (1998) and with all other gene sequences of this genus reported in GenBank, it is evident that the Comacchio isolate is *N. gaditana* Lubián. From this comparison, both our

strain and the five *N. gaditana* ones reported by Andersen *et al.* (1998), show two nucleotide substitutions with the gene sequence AF067957 [in position 1 (A→C) with AF045040 and in position 119 (T→C) with AF045040 and our strain]. As reported in other similar studies (Medlin *et al.* 1991), this ambiguity may reflect misincorporation during the PCR reactions or variation between individual cells within the cultures.

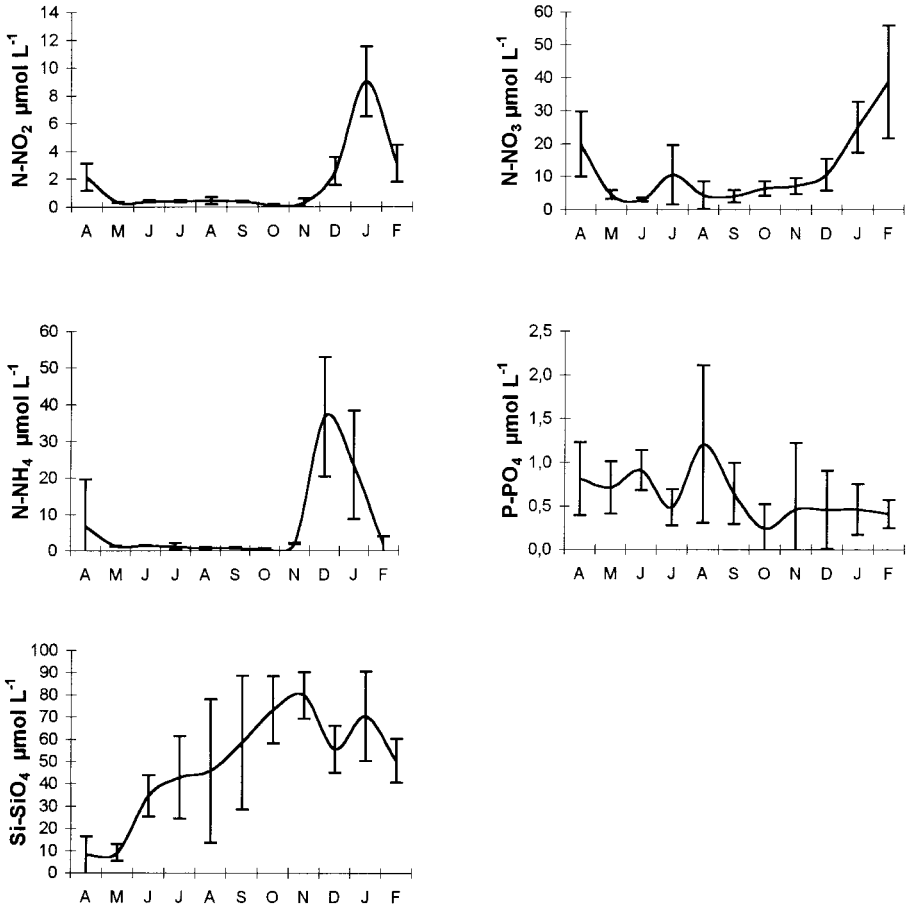


Fig. 3. Mean nutrient concentrations for the nine sampling stations (vertical bars are ± SD).

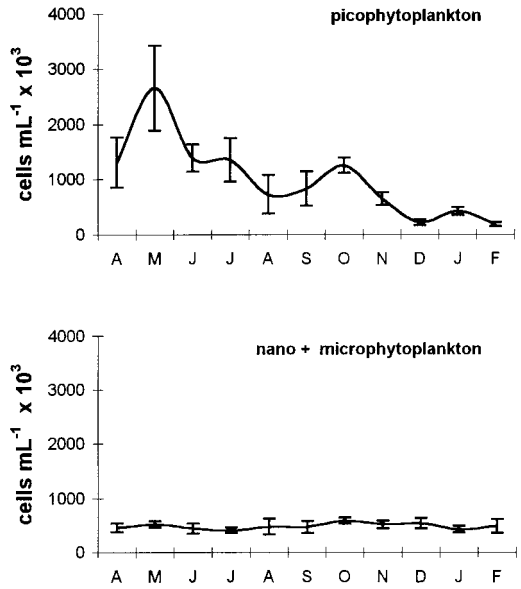


Fig. 4. Mean of picophytoplankton and nano + microphytoplankton densities (cells mL^{-1}) for the nine sampling stations (vertical bars are ± SD).

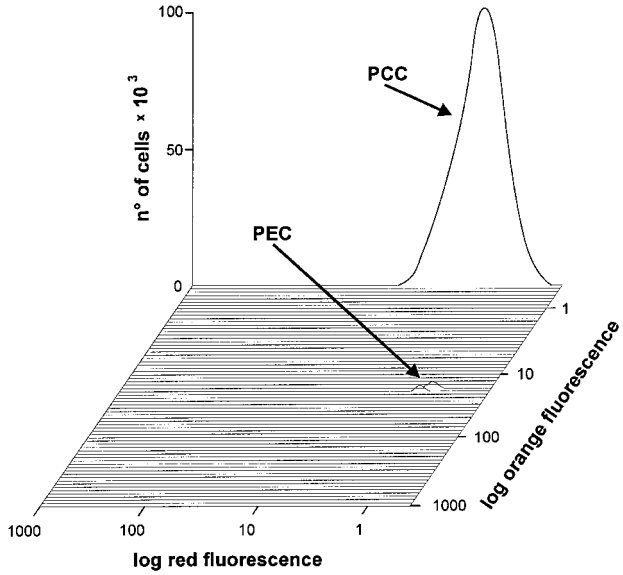
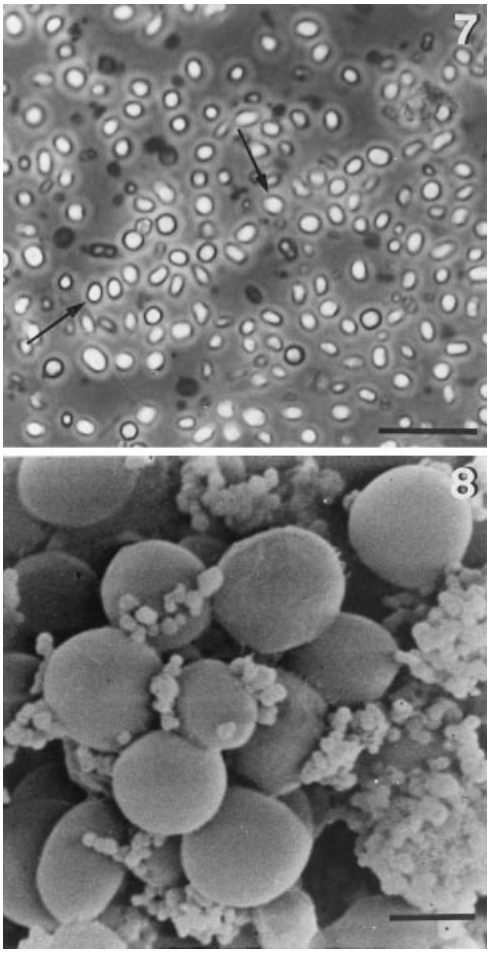


Fig. 5. Flow cytometry cytogram of the picophytoplankton fraction in May; PCC = phycocyanin-rich cyanobacteria, PEC = phycoerythrin-rich cyanobacteria.

Moreover, as reported by Lubián (1982), the cultures of our strain also become red-pigmented with ageing.

In addition to this eustigmatophycean, the phytoplanktonic fraction > 2 μm was represented by 82 taxa: 53 diatoms, 9 dinoflagellates, 1 cryptophycean, 2 chrysophyceans, 4 prasinophyceans, 3 euglenophyceans and 10 chlorophyceans. The contribution of these taxa to the primary production, however, was very low if compared with that of *Nannochloropsis gaditana* (Table IV). Some exceptions were registered in April by *Cyclotella* sp. ($6-15 \times 10^3$ cells mL^{-1}) and in June and in January by 'undetermined small flagellates' ($2-8 \times 10^3$ cells mL^{-1}) (Table IV). The presence of freshwater taxa, belonging to the Chlorophyceae, was recorded only in April, coinciding with the opening of the communication with the Reno River. The density values of these microorganisms, however, were very low (20 cell mL^{-1}) and limited to station 5, which was nearer to the opening to the river. In September, October, and November an unusual but low presence of *Oxyphysis oxytoxoides* Kofoid (Table IV) was noticed. This species, besides being the only species belonging to the Oxyphysaceae, is found very rarely both in the Mediterranean Sea and in the open ocean (Sournia 1986).

The monthly trend of the chlorophyll *a* concentrations is shown in Figure 16 and indicates that, in con-



Figs. 7–8. *Nannochloropsis gaditana*.
Fig. 7. The species dominating in a LM preparation, arrows, bar = 20 μm. Fig. 8. The coccoid cells in the SEM, bar = 2 μm.

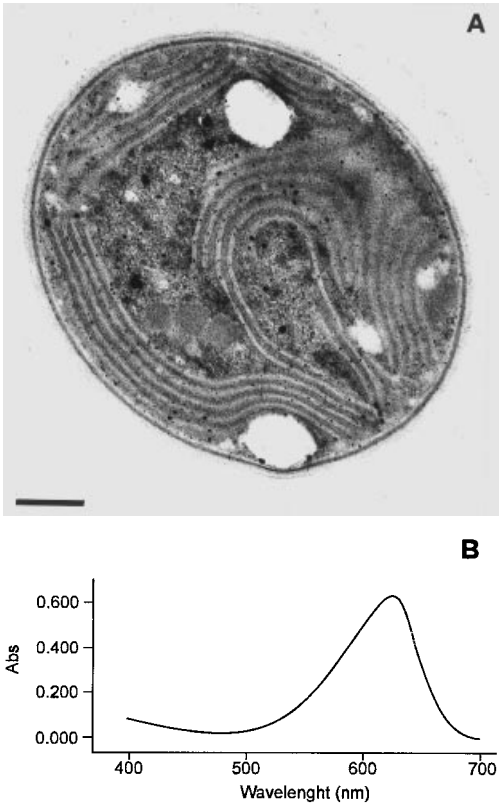


Fig. 6. *Synechococcus* sp. A. Ultrastructural features of a cell in thin section of embedded material, TEM graph, scale bar 0.5 μm. B. Absorption spectrum showing the prevalence of phycocyanin (615 nm) over phycoerythrin (562 nm).

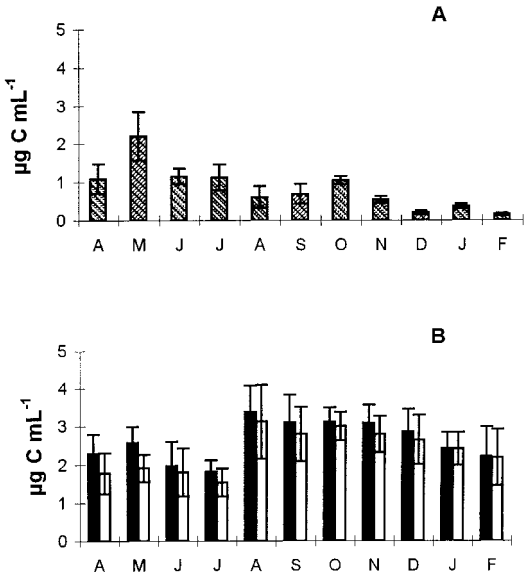
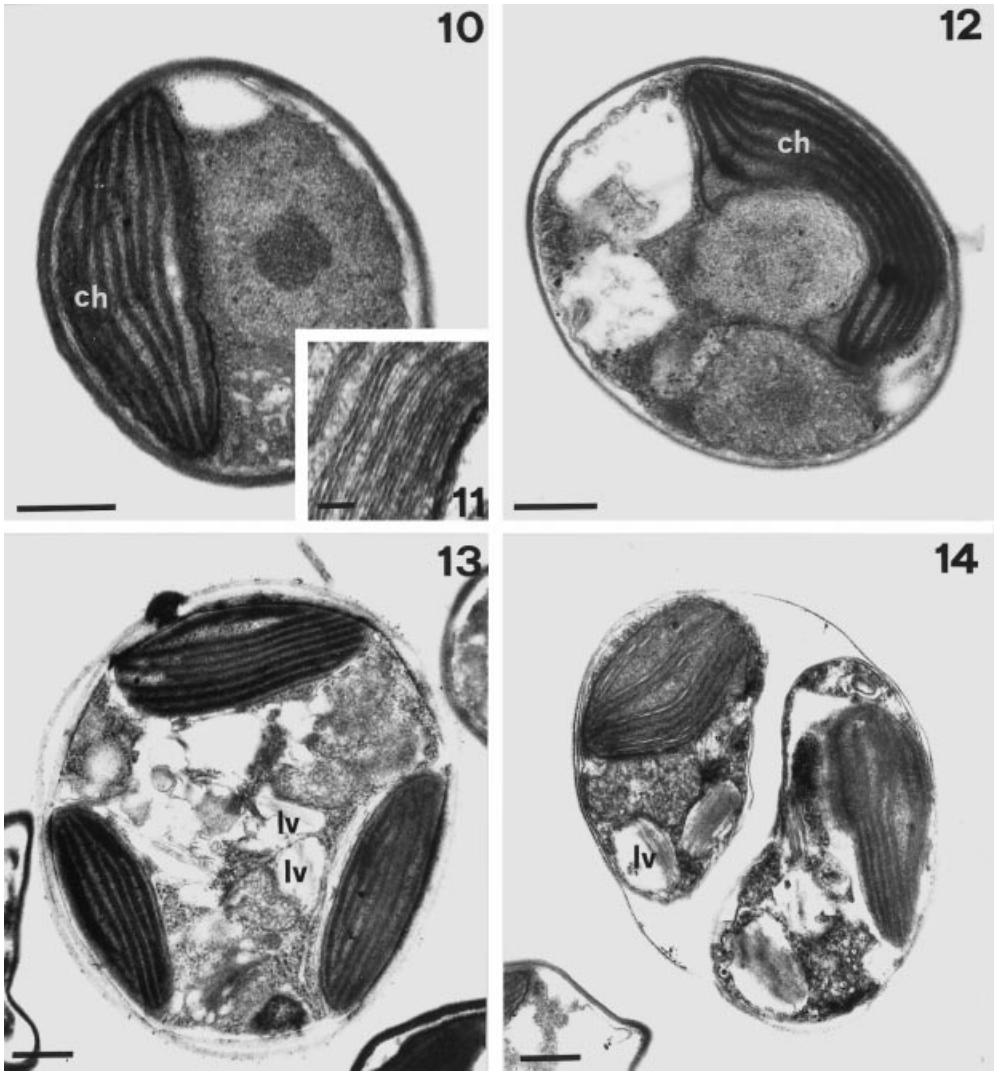


Fig. 9. A: biomass of picophytoplankton. B: biomass of nano + microphytoplankton (black bars) and *Nannochloropsis gaditana* (white bars). Mean values for stations 1–9, from April 1995 to February 1996 (vertical bars are ± SD).



Figs 10−14. Thin sections of *Nannochloropsis gaditana* from the Comacchio lagoons. Note a single chloroplast (ch) without starch and pyrenoid showing a structure including lamellae made up of three thylakoids (Figs 10−11). The nuclear envelope is connected to the chloroplast (Fig. 12). During the autospore production lamellate vesicles (lv) are present in the cytoplasm (Figs 13−14) (in Figure 11 bar = 0.1 μm, in the others, bars = 0.5 μm).

trast to density and biomass of the > 2 μm fraction (Figs 4 and 9), the period of higher primary production was summer-autumn (from August to November).

Discussion and Conclusions

In agreement with Boni *et al.* (1995) and Sorokin *et al.* (1996), our results confirm that the waters of the Comacchio lagoons are affected by a persistent phytoplanktonic bloom. However, in contrast to these authors, our surveys show that this bloom was mainly produced by *Nannochloropsis gaditana* and by *Synechococcus* sp., the first was always present whereas the second was only present from May to November. Our results also differ from those of Boni *et al.* (1995), in that we conclude that the principle nanoplanktonic organism is *Nannochloropsis gaditana* (Eustigmatophyceae) and not a *Chlorella* species. Species of *Nannochloropsis*, at the LM level may be mistaken for members of the Chlorophyceae e. g. *Chlorella* and *Nannochloris*. *Chlorella minutissima* (Fott *et* Nováková) (strain UTEX 2341), for example, has been recognized as a species of *Nannochloropsis* (Gladu *et al.* 1995) and five isolates, ascribed to the genus *Nannochloris*, were identified as *Nannochloropsis salina* Hibberd (Turner and Gowen 1984). Our results are clearly different from those of Sorokin *et al.* (1996). These authors found that 80−97% of the phytoplanktonic biomass was due to several species of picocyanobacteria of the genera *Coelosphaerium*, *Aphanothece* and *Synechococcus.* We observed that picophytoplankton was mainly represented by a *Synechococcus* sp. and its contribution to the phytoplanktonic biomass was lower than that produced by *Nannochloropsis gaditana* (Fig. 9). In contrast, with the disappearance of zooplankton and the high fish mortality observed by Sorokin *et al.*

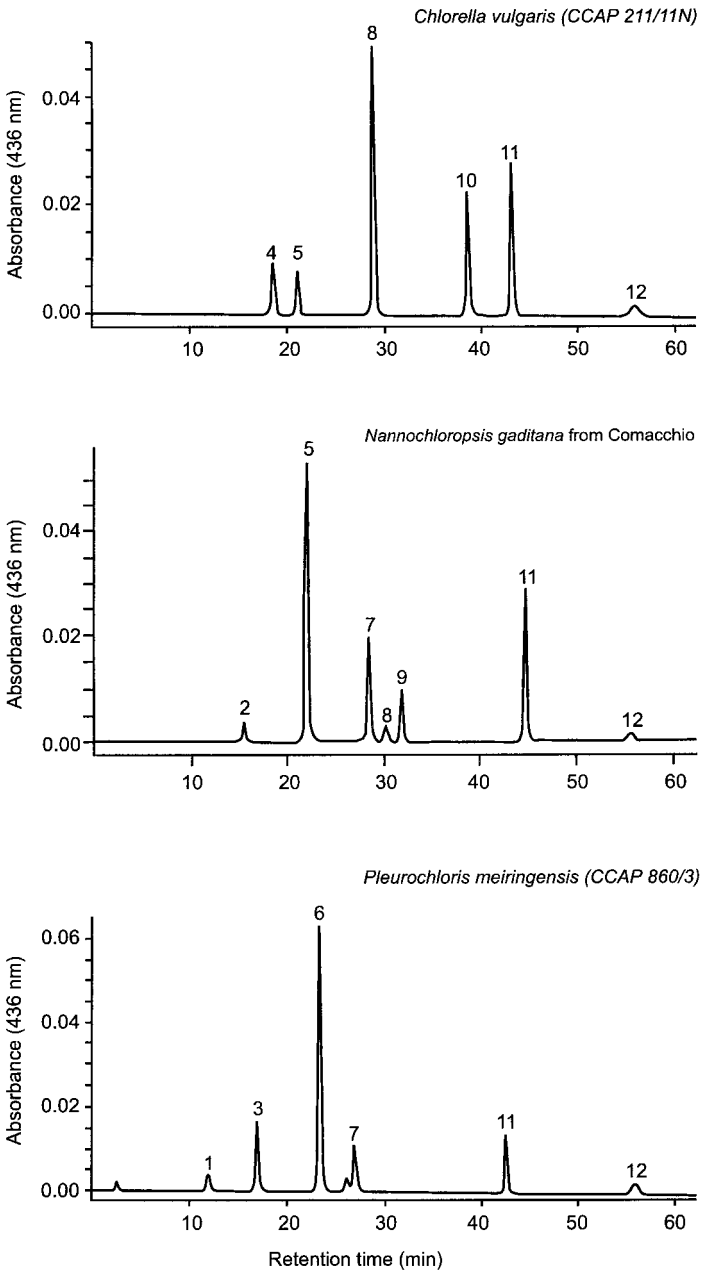


Fig. 15. HPLC analyses carried out comparing *Nannochloropsis gaditana* from Comacchio lagoons with *Chlorella vulgaris* and *Pleurochloris meiringensis*. For the identification of the different peaks see Table III.

(1996), the high density of *Nannochloropsis gaditana* was accompanied by the presence of a large autochtonous zooplankton population in 1997 (Ferrari, unpublished data) and, in recent years, by large amounts of *Engraulis encrasicholus* L. (anchovy) (585, 913 and 1000 tonn/year were caught in 1995, 1996 and 1997 respectively). Its presence, therefore, seems to be linked to the growth of these organisms. Species of the genus *Nannochloropsis* [*N. oculata* (Droop) Hibberd, *N. gaditana* Lubián, *N. granulata* Karlson *et* Potter and *N. salina* Hibberd], are in fact, used in aquaculture, in particular in the batch culture of *Brachionus* spp. (Mourente *et al.* 1990, Renaud *et al.* 1991, Gladu *et al.* 1995, Yúfera and Navarro 1995, Hagiwara *et al.* 1997). The negative effects of *Nannochloropsis gaditana* in the Comacchio lagoon, therefore, seem to be limited to apparent suppression of other microalgae and to the absence of macroalgae and phanerogams, as in mesocosm experiments carried out by Taylor *et al.* (1995).

Compared with other lagoons of the northern Adriatic Sea (Marchesoni 1954, Brunetti *et al.* 1977, Cioce *et al.* 1979, Marzocchi *et al.* 1980, Andreoli *et al.* 1994, 1997), the chemico-physical conditions observed here were very unusual. Only the temperature fluctuations of the waters, accentuated by the shallowness of this environment, showed the typical seasonal trend. The transparency of the water was very low (about 30 cm.) and uniform during all months. Moreover, in contrast to Sorokin *et al.* (1996), we

found that these waters were sometimes oversaturated or saturated with oxygen in summer. Among the nutrients, the N-compounds were low from spring to autumn but higher in winter; the phosphate concentrations were low but uniform during all seasons. Higher availability of all N-compounds in the winter, may be a result of the collapse of the *Synechococcus* sp. bloom and by an increased run off from the neighbouring fields caused by the higher rainfall in this period, the latter is confirmed by the lowering of the salinity values recorded in January and February.

Table III. Pigments identified in the different chromatograms with reference to peak number, elution orders and their visible absorption.

Peak n°	Pigment	Retention time (min)	Absorption
1	Chlorophyll *c2*	12.42	444
2	*trans*-Neoxanthin	14.67	421−440−469
3	Heteroxanthin	17.14	419−445−473
4	Neoxanthin	18.59	413−437−464
5	Violaxanthin	21.08	417−439−469
6	Diadinoxanthin	23.51	419−445−473
7	Vaucheriaxanthin ester	26.99	417−441−469
8	Lutein	28.84	421−445−473
9	Zeaxanthin	30.49	421−443−471
10	Chlorophyll *b*	38.52	465
11	Chlorophyll *a*	43.02	414−430
12	β-Carotene	55.35	(427)-449−475

The low amount of the N-compounds and the contemporary presence of two blooms could be compared with the ecological situation reported in Moriches Bay, Long Island (Ryther and Dunstan 1971). Even though this environment takes the wastes of the duck farms, it did not show high concentrations of nitrogen in any form, during dense phytoplankton blooms. Ryther and Dunstan (1971) hypothesised and subsequently confirmed with cultures of *Nannochloris atomus* Butcher (Chlorophyceae) that the growth of phytoplankton was due to the ability of microalgae to assimilate nitrogen in whatever form it left the duck farm, exhausting the element from the water in the tributaries before it could reach the bay. The phytoplankton represented a stable population that was able to persist for periods of time. In the Comacchio lagoon, the fish farm activities were stopped from 1994 and further surveys still being car-

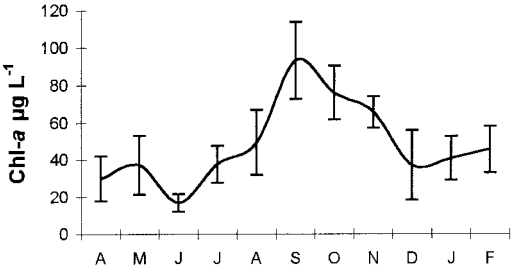


Fig. 16. Total chlorophyll *a* content, mean values for stations 1−9, through the period April 1995 to February 1996 (vertical bars are ± SD).

Table IV. List of the most important photosynthetic taxa > 2 µm found in the Comacchio lagoons during the sampling period. It includes their mean cell volume (µm^3), the season of their main presence (s) and their maximum (max), minimum (min), median (M) and mean (m) densities (cells mL^{-1}).

	µm^3	season / s	max	min	M	m
Diatoms						
Pennate diatoms	1714	all seasons	9880	6	99	1723
Chaetoceros spp.	809	winter	511	1	9.5	64
Cyclotella sp.	118	all seasons	15331	2	56	1163
Cylindrotheca closterium (Ehr.) Reimann et Lewin	748	summer	55	1	5	16
Nitzschia sp.	791	all seasons	100	2	14	19
Skeletonema costatum (Greville) Cleve	154	winter	170	1	5.5	21
Dinoflagellates						
Oxyphysis oxytoxoides Kofoid	3620	autumn	51	1	6	10
Undetermined dinoflagellates	664	all seasons	220	1	20.5	41
Others						
Calycomonas sp.	41	summer-autumn-winter	4996	1	425.5	568
Nannochloropsis gaditana Lubián	33	all seasons	680698	312412	476284	479552
Chlamydomonas sp.	66	spring-winter	4996	3	1731.5	867
Pyramimonas sp.	164	summer-autumn-winter	1022	2	35.5	86
Tetraselmis sp.	160	summer	170	1	3	40
Undetermined cryptophyceans	169	all seasons	6529	2	94.5	628
Undetermined phytoflagellates	128	all seasons	9766	5	2526.5	173
Undetermined taxa	250	winter	1362	5	63	63

ried out confirm the persistence of these two blooms (Andreoli, unpublished data). The high and constant presence of *Nannochloropsis gaditana*, is linked to the ammonium and organic nitrogen availability. In *Nannochloropsis oculata* (Droop) Hibberd, during N-deprivation and ammonium supplimentation, Flynn *et al.* (1993) found that growth continued after exhaustion of the N-source, thanks to its ability to make very rapid use of some amino-acids (in particular glutamate, glutamine and alanine) excreted. Turner and Gowen (1984), found that both this species and *N. salina* were able to utilize not only amino-acids but also urea and uric acid. In the Comacchio lagoon, therefore, the continuous presence of *Nannochloropsis gaditana* could be correlated with the turnover of the ammonium and organic compounds. They were probably the result of the zooplankton grazing and the high density of *Engraulis encrasicholus* L. (anchovy). The uniform level of orthophosphate throughout the year, however, may be explained, by the phosphatase activity of this microalga as observed by Lubián (1982) and Lubián *et al.* (1992).

It is probable that these waters are influenced by herbicides like 3-(3:4-dichlorophenyl)-1:1-dimethyl urea (DCMU) originating either from the neighbouring fields, or from the Po River which drains its waters into the sea north of this environment. While these compounds reduce the growth rate of some microalgae, they appear to have no effect on *Nannochloropsis* species. In this way, DCMU can be used as a selective tool to maintain a monoculture of these taxa (Gonen-Zurgil *et al.* 1996).

Identical 18S rRNA gene sequences of the Comacchio isolate and *Nannochloropsis gaditana*, found by Lubián (1982) in Cádiz Bay (Southern Spain), confirm that these two taxa are the same species. The production of two or four autospores is in agreement with the observations of Santos (1996) and the binary fission, observed in all *Nannochloropsis* species, could has been mistaken with the release of autospores, a form of vegetative reproduction known in other Eustigmatophyceae (Hibberd 1981).

The presence of *Nannochloropsis gaditana* in the Comacchio lagoons is a further contribution to the distribution and to the ecological role of *Nannochloropsis* species, found in supralittoral pools, open ocean and coastal waters (Droop 1955, Bourrelly 1957, Berland *et al.* 1970, Lubián *et al.* 1985, Karlson *et al.* 1996).

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