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Seasonal distribution of plankton diatoms in Lim Bay, northeastern Adriatic Sea

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The seasonal distribution of planktonic diatoms is presented in relation to the hydrographic and chemical parameters in Lim Bay, a 10 km long fjord-like embayment situated in the north east Adriatic Sea. Water samplings for physicochemical and biological variables were conducted from March 2002 to November 2007 at three stations along the bay. The phytoplankton was dominated by diatoms throughout the year, with the minimum species diversity in summer and the maximum in autumn. A total of 100 diatoms were identified and classified according to their seasonal occurrence in the plankton; some were consistently present throughout the whole year, but a large fraction showed a distinct seasonal cycle. The inner two stations differed significantly in terms of phytoplankton biomass and nutrient content from the outer station, indicating a higher nutrient input in the inner part. The majority of the dominant diatoms recorded in this study prefer nutrient-enriched conditions. Due to the anticipated increase of human impact in the area, this study can serve as a base for future environmental studies in Lim Bay.

Keywords: Diatom, phytoplankton, seasonality, Lim Bay, Adriatic Sea

Introduction

The Adriatic Sea is the northernmost basin in the Mediterranean, with a length of 800 km and a width of 200–250 km. It may be divided into three parts according to the bathymetry and latitude: the northern Adriatic basin with an average depth of 40 m, the middle Adriatic with depressions up to 280 m and the southern Adriatic circular basin with depths of up to 1230 m. The circulation of the Adriatic is cyclonic due to the considerable freshwater input from the northern Adriatic rivers, which generates the south-easterly outflowing West Adriatic Current (ORLIĆ et al. 2007). Thus the nutrient enriched waters reach the middle and south Adriatic basin. The eastern Adriatic region is exposed to the East Adriatic Current which brings highly saline and low-nutrient waters from the Ionian and Levantine Seas (ORLIĆ et al. 2007). These hydrographical features combine to make

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the system quite heterogeneous with across-shelf and longitudinal trophic gradient resulting in the asymmetric distribution of the phytoplankton composition, abundance and biomass (POLIMENE et al. 2007). The literature concerning phytoplankton distribution and dynamics of the northern (REVELANTE and GILMARTIN 1976, 1980; REVELANTE et al. 1984), middle (CAROPPO et al. 1999, TOTTI et al. 2000) and southern (VILIČIĆ et al. 1995) Adriatic is wide. Each of these studies stresses the influence of above-mentioned environmental forcings on the spatial and temporal high variability of phytoplankton biomass and qualitative distribution.

The Po River discharge and the meteorological forcing factors are the main components triggering the alternation of stratification and mixing of the water column and that strongly affect the phytoplankton annual dynamics in the northern Adriatic (REVELANTE et GILMARTIN 1976). Diatoms dominate the phytoplankton assemblages (both microplankton and nanoplankton fractions) over most of the year while flagellates dominate only in oligotrophic conditions, in June–July (BERNARDI AUBRY et al. 2004).

Diatom dominance is often described in connection with the appearance of large mucilaginous aggregates that often occur during late spring/early summer in the northern basin (TOTTI et al. 2005). The exact role of diatoms remains elusive, but some studies indicate the importance of the intensive production of extracellular polysaccharide by several diatom species (e.g. *Skeletonema marinoi*, *Chaetoceros* spp., *Ceratoneis closterium*) commonly found during mucilage occurrences (TOTTI et al. 2005). Some toxin-producing diatom species of the genus *Pseudo-nitzschia* have been described from different parts of the Adriatic Sea. Diatoms belonging to the genus *Pseudo-nitzschia* are present the entire year and are generally considered to be dominant in the phytoplankton of the Adriatic Sea (VILIČIĆ et al. 1995, 2009), but the actual species composition and species succession remain to be elucidated. LUNDHOLM et al. (2003) reported the occurrence of *P. calliantha* in samples from the northern and middle part, BURIĆ et al. (2008) described *P. calliantha* from the middle part and CARROPO et al. (2005) identified *P. calliantha* and *P. delicatissima* from the southern part of the basin.

The first report on the taxonomic composition of phytoplankton from the north east Adriatic Sea (REVELANTE 1985) has been recently supplemented with records from the middle and southern Adriatic (VILIČIĆ et al. 2002). A total of 829 phytoplankton taxa were recorded for the eastern Adriatic Sea; these taxa comprise 328 pennate diatoms and 179 centric diatoms, 220 dinoflagellates, 94 prymnesiophytes, 3 chrysophytes, 1 euglenophyte, 2 cryptophytes, 1 raphidophyte and 1 chlorophyte.

Diatoms represent the dominant taxonomic group of the northern Adriatic phytoplankton assemblages throughout most of the year (VILIČIĆ et al. 2009), and the aim of this study was to assess their seasonal distribution in Lim Bay, in the north east part of the Adriatic Sea.

Study area

Lim Bay is a fjord-like embayment, 11 km long and 0.5 km wide, located on the west side of the Istrian peninsula in the north east Adriatic Sea (Fig. 1.). The maximum depth is about 33 m in the outer part while the inner part is about 17 m deep. Many effluents charac-

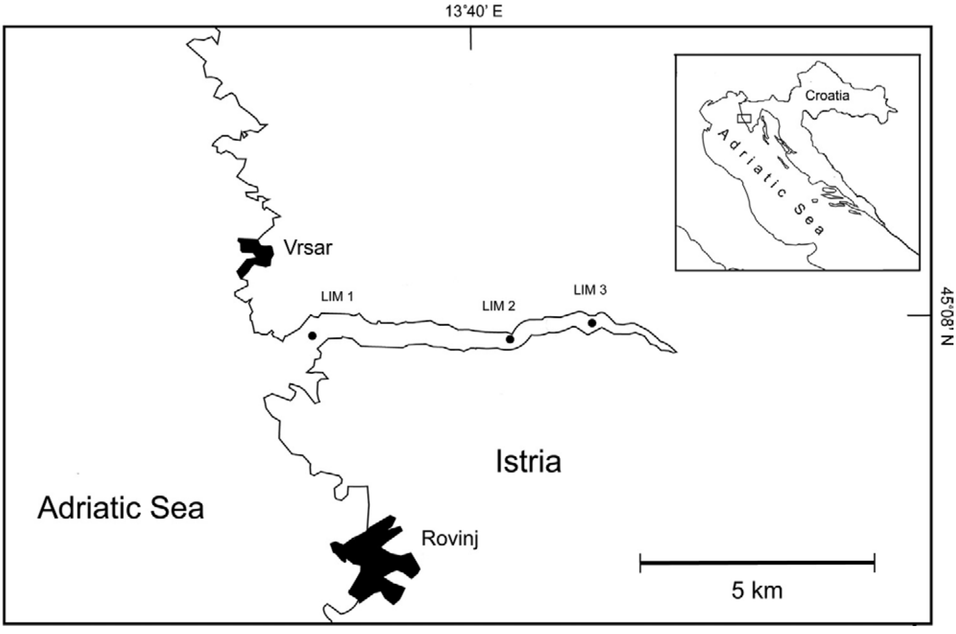

Fig. 1. Location of the sampling stations in Lim Bay, north east Adriatic Sea, visited from March 2002 to November 2007.

teristic of the karstic region and a number of underwater freshwater springs, especially in the inner part, contribute to the productivity of Lim Bay. Freshwater inflow becomes important during heavy rainfall periods, which are usually in the period from September to December and in April. (PENZAR et al. 2001). Some shellfish and fish farming operations are to be found in the inner and middle parts of the bay (KRAJNOVIĆ-OZRETIĆ et al. 2001) which is also known for providing a good spawning ground as well as a hiding place for many commercial fish (HULJEV and STROHAL 1983). The climate is classified as moderate Mediterranean, with an annual precipitation averaging 873 mm (GAJIĆ-ČAPKA et al. 2003).

Materials and methods

Sampling was carried out five times from March to August 2002 and seven times yearly from February 2003 to November 2007 at three stations located along Lim Bay (Tab. 1). In all, 414 samples were collected and analyzed throughout the study period. Water samples were collected with 5 L Niskin bottles at stations LIM1 and LIM2 at five (surface, 5m, 10m, 20 m and 2 m above bottom) or at station LIM3 at four depths (surface, 5 m, 10 m and 2 m above bottom). At each station, water temperature and salinity were measured using a CTD probe (Seabird, USA). Dissolved oxygen concentration was determined by the Winkler titration method (PARSONS et al. 1984), while the saturation percent of dissolved oxygen in each water sample was calculated (from the quotient between the measured oxygen concentration and the oxygen solubility) following the Benson and Krause equation (UNESCO 1986).

Tab. 1. Distribution of sampling during 2002–2007. The number of samples collected for each period is indicated. Total number of samples is 414.

	Month											
Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2002			9	6	6	6		6				
2003		12	12			12	11	10	12	10		
2004		11			11		11	12	11	11		9
2005		11	12		11	8	3		10		11	
2006	10		12		12	10	13	12			12	
2007			13		13	7	11	10		12	13	

Subsamples for the determination of nitrate (NO_3), phosphate (PO_4) and silicic acid ($Si(OH)_4$) were measured using a Shimadzu UV-Mini 1240 spectrophotometer (Ivančić and Degobbis 1984, Parsons et al. 1984). Subsamples for the determination of chlorophyll (chl) *a* were filtered onto Whatman GF/C filters. Following a 3 hour extraction in 90% acetone (at dark, with grinding), chl *a* concentrations were determined on a Turner TD-700 fluorometer (Parsons et al. 1984).

For the enumeration of phytoplankton cells, 150 mL samples were preserved with 2% (final concentration) disodium tetraborate (borax) buffered formaldehyde, as well as additional net samples for qualitative analyses. Cells were identified and enumerated using an inverted microscope (Zeiss Axiovert 200) operating with phase contrast and brightfield optics (Utermöhl 1958, Lund et al. 1958, Viličić et al. 2008). We used the following references for phytoplankton identification: Hustedt 1959, Ricard 1987, Hasle and Syversten 1997, Round et al 1990, Bérard-Therriault et al. 1999, Horner 2002, Throndsen et al. 2007.

The data set represents presence/absence observations combined from the net and bottle samples as well as abundance data from the bottle samples. Our monthly observations of diatom flora were combined into seasonal categories as follows

A (autumn): October, November
W (winter): December, January
W/S (winter/spring transition): February, March
S (spring): April, May
S/SU (spring/summer transition): June
SU (summer): July, August
SU/A (summer/autumn transition): September

The categories were defined by taking into consideration local meteorological variables (monitored by the Croatian Meteorological and Hydrological Service DHMZ, available at http:// meteo.hr)

Two diversity indices were calculated using the statistical program PRIMER V5. These were the Margalef species richness index (d) (Margalef 1968), which represents a measure of the number of different species (S) present in the sample, making some allowance for the number of individuals (N), which was calculated using the formula: d = (S-1)/log

(N) and the Shannon-Wiener (H') diversity index (SHANNON and WEAVER 1963) using the formula $H' = \Sigma_i p^i \ln (p^i)$ where p_i is the proportion of abundance of species.

Environmental and biological data were analysed by one-way ANOVAs with post-hoc Bonferroni tests using the program Statistica, version 6.0. A logarithmic transformation (log (x+1)) was used on nutrient and diatom abundance data to obtain the normal distribution.

Results

The range of variation in the environmental and biological variables measured in Lim Bay over the six-year period is listed in table 2. Salinity and temperature profiles (Fig. 2A, B) indicate a vertical instability of the water column most of the year, except in summer, which is characterized by a shallow thermocline in the 5–10 m layer. Salinity was rather constant, but with some occasional low values (< 30) recorded in the surface layer (0–5 m) of the inner stations in the period February to May, due to winter and early spring precipitation events.

Tab. 2. Descriptive statistics of physical, chemical and biological variables measured in the water column at three stations of Lim Bay between March 2002 and November 2007. n: number of observations. n.d.: not detected.

	Min	Mean	Max	SD	n
Temperature (°C)	7.8	17.3	27.6	5.3	519
Salinity (psu)	23.2	37.4	38.5	1.4	519
Oxygen (%)	41	99	135	13	519
Total phytoplankton (cells L^{-1})	160	9.38×10^4	245×10^4	20.5×10^4	414
Diatoms (cells L^{-1})	40	8.44×10^4	243×10^4	20.2×10^4	414
Chlorophyll *a* (µg L^{-1})	0.19	1.04	9.55	0.93	492
NO_3 (µmol L^{-1})	0.07	2.14	30.10	2.72	519
PO_4 (µmol L^{-1})	n.d.	0.06	0.60	0.08	519
$Si(OH)_4$ (µmol L^{-1})	0.02	4.63	40.07	4.58	515

Mean phosphate concentrations were at least two times higher at the inner two stations than at the outer one and showed a slight increase from late spring to autumn (Fig. 3A). Mean nitrate and silicic acid concentrations were lower during the summer stratification period (Fig. 3B, D). The seasonal distribution of diatom abundances and chlorophyll *a* concentrations showed a slight increase during the summer and autumn period in all three stations (Fig. 4). One-way ANOVA with post-hoc Bonferroni test run on nutrient (phosphate, nitrate, silicid acid) and chlorophyll a data showed a statistically significant difference ($p < 0.001$) between the two inner (LIM2, LIM3) and the outer station (LIM1). The same test run on diatom abundance data showed significant difference between the middle (LIM2) and other two (LIM1, LIM3) stations ($p < 0.001$).

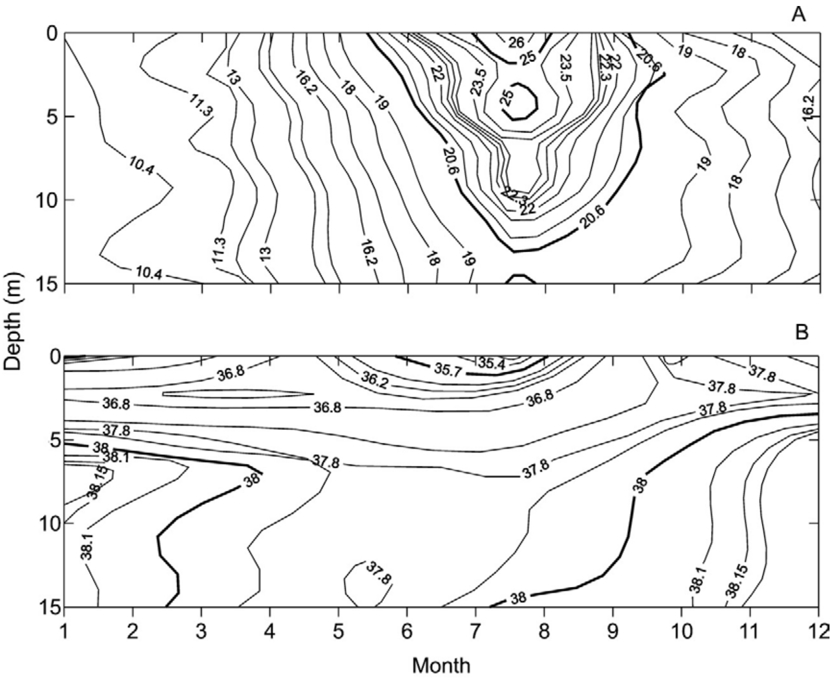

Fig. 2. Distribution of temperature (A) and salinity (B) at station LIM3 in Lim Bay in the period March 2002 to November 2007.

A total of 100 diatom taxa were identified from Lim Bay (Tab. 3) together with seasonal distribution and qualitative occurrence. Although the majority of taxa are marine planktonic diatoms, there are several benthic species that occurred regularly or occasionally. Eleven taxa are characterized as dominant with maximum abundance greater than 10^5 cells L^{-1} and occurrence greater than 20% in the samples: *Cerataulina pelagica*, *Chaetoceros socialis*, *Chaetoceros* sp., *Dactyliosolen fragilissimus*, *Guinardia striata*, *Leptocylindrus danicus, Nitzschia longissima*, *Proboscia alata, Pseudo-nitzschia* spp., *Rhizosolenia imbricata* and *Thalassionema nitzschioides.*

Some diatoms showed no seasonal variation but were consistently present throughout the year: *Ceratoneis closterium*, *Chaetoceros* sp., *Coscinodiscus* spp., *Dactyliosolen fragilissimus*, *Diploneis bombus*, *Guinardia flaccida*, *G. striata*, *Hemiaulus hauckii*, *Pleurosigma* sp., *Pseudosolenia calcar-avis*, *Rhizosolenia imbricata*, *Rhizosolenia* sp. and *Thalassiosira* spp. A large fraction of the diatoms recorded showed a distinct seasonal cycle, defined by their presence in one season only or all year round but with a most frequent occurrence at a given season. The most frequent occurrence defined as more than three observations for a given seasonal category is sometimes, but not always, accompanied by the highest abundance (Tab. 3).

Both the species richness (d) and biodiversity index (H') showed high values in autumn as opposed to low summer values although phytoplankton cell abundances were similar (Figs. 4A, 5). The high diversity recorded in autumn is mainly explained by the high number of different species of the planktonic diatoms *Chaetoceros* and *Bacteriastrum*.

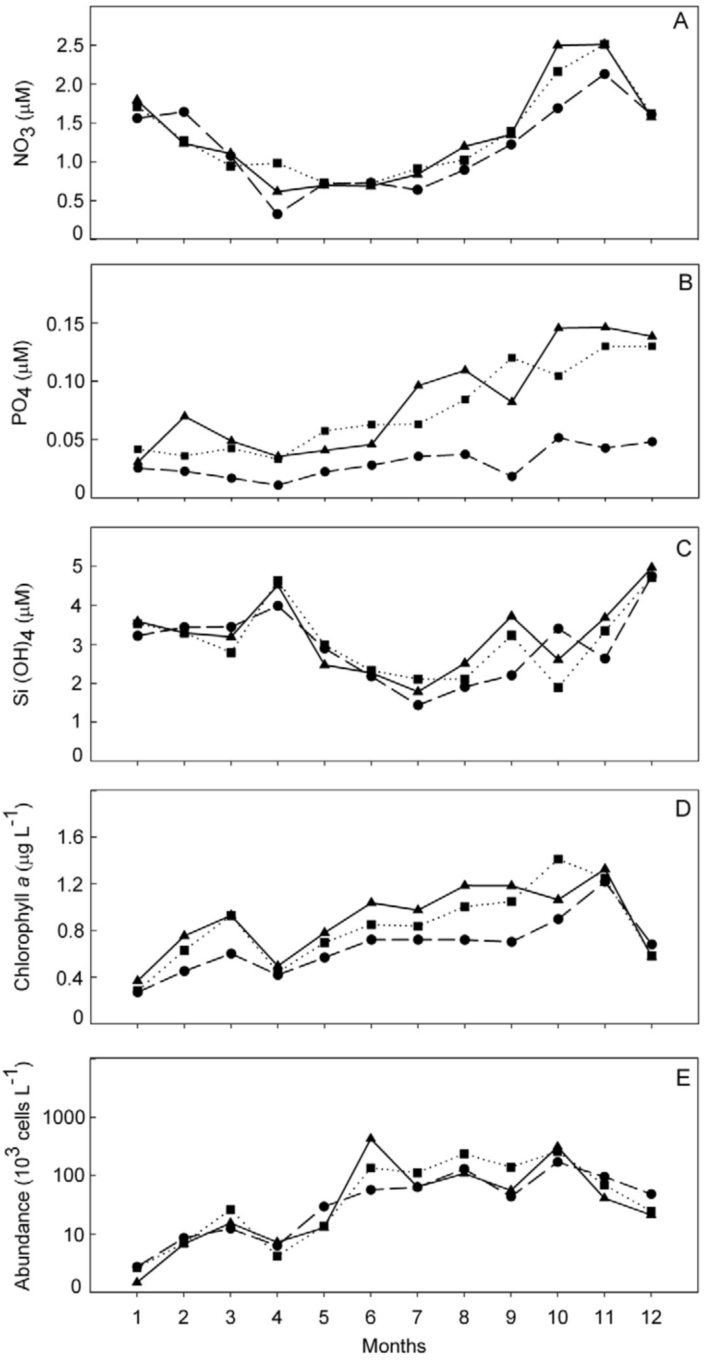

Fig. 3. Annual distribution of nitrate (NO_3) (A), phosphate (PO_4) (B) and silicic acid ($Si(OH)_4$) (C), chlorophyll *a* (D) and diatom abundance (E) at three stations in Lim Bay from March 2002 to November 2007, at stations LIM1 (•), LIM2 (■) and LIM3 (▲). Data are presented as the monthly average obtained from the entire data set, clustered for each month per each station.

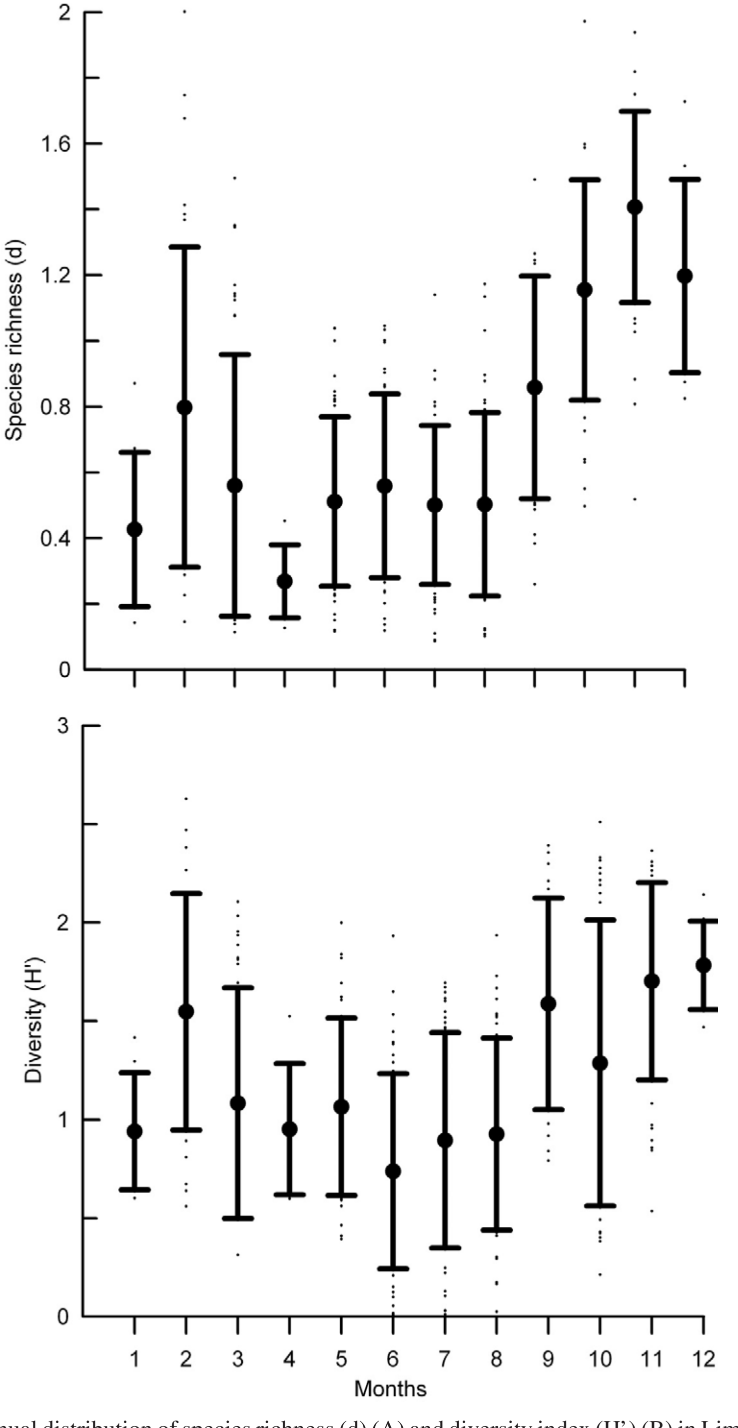

Fig. 4. Annual distribution of species richness (d) (A) and diversity index (H') (B) in Lim Bay from March 2002 to November 2007.

Tab. 3. List of planktonic diatoms recorded at three stations in Lim Bay between March 2002 and November 2007. Information on seasonality follows after each species name. W: winter; W/S: winter/spring; S: spring; S/SU: spring/summer; SU: summer; SU/A: summer/autumn; A: autumn; bold character indicates the most frequent occurrence, sometimes accompanied by highest cell abundance. only name of the month indicates single occurrence, not in all months within defined category.

Actinocyclus spp.	Mar, Nov
Actinoptychus splendens (Shadbolt) Ralfs	Nov
Amphora spp.	Aug, Oct
Amphiprora spp.	Feb, Nov
Asterionellopsis glacialis (Castracane) Round	**SU/A, A**, Mar, Dec
Asteromphalus flabellatus (Brébisson) Greville	SU/A
A. heptactis (Brébisson) Ralfs	S/SU, Aug
Bacillaria paxillifera (O.F. Müller) Hendey	S/SU, SU/A, Oct
Bacteriastrum delicatulum Cleve	All year, **S, SU/A**, **A**
B. elongatum Cleve	Nov
B. hyalinum Lauder	Jan, Mar, **SU/A, A**
B. mediterraneum Pavillard	W/S, SU, **A**
Bacteriastrum spp.	Mar, SU, SU/A**,** A
Biddulphia titiana Grunow	Nov
Cerataulina pelagica (Cleve) Hendey	All year, **S/SU**, **SU**
Ceratoneis closterium Ehrenberg	All year
Chaetoceros affinis Lauder	W/S, **SU/A, A**, May, Dec
C. anastomosans Grunow	SU
C. brevis Schutt	Mar, SU
C. compressus Lauder	Mar, May, **Jul, SU/A, A**
C. convolutus Castracane	Feb, Aug, SU/A, Oct
C. costatus Pavillard	W/S, Aug, A, **Dec**
C. curvisetus Cleve	All year, **Mar, Aug**
C. danicus Cleve	All year, **SU/A, A**
C. decipiens Cleve	All year, **SU/A, A**
C. densus (Cleve) Cleve	Mar, Dec
C. didymus Ehrenberg	Feb, Nov
C. diversus Cleve	**May**, Aug, **SU/A**, Nov
C. eibenii (Grunow) Meunier	Jan, A
C. lauderi Ralfs	SU, **Nov**
C. lorenzianus Grunow	May, **S/SU**, Jul, SU/A, A
C. perpusillus Cleve	SU/A
C. peruvianus Brightwell	Nov
C. rostratus Lauder	**Jul**, Aug, **A**
C. simplex Ostenfeld	**May**, **S/SU**, Nov

Tab. 3. continued

C. socialis Lauder	All year, **SU**
Chaetoceros spp.	All year
C. tenuissimus Meunier	SU/A, A
C. throndsenii Marino, Montresor et Zingone	S/SU
C. tortissimus Gran	SU/A
C. vixvisibilis Schiller	May, S/SU, SU, **Oct**
Cocconeis scutellum Ehrenberg	SU, Nov
C. placentula Ehrenberg	S/SU
Coscinodiscus granii Gough	Nov
C. perforatus Ehrenberg	Nov
Coscinodiscus spp.	All year
Cyclotella spp.	All year, **S/SU, SU**
Dactyliosolen blavyanus (Peragallo) Hasle	May, Nov
D. fragilissimus (Bergon) Hasle	All year
D. phuketensis (Sundström) Hasle	Jan, **W/S**
Detonula pumila (Castracane) Gran	W, W/S, Nov
Diploneis bombus Ehrenberg	All year
D. crabro Ehrenberg	SU, SU/A, A
Ditylum brightwellii (West) Grunow	W/S, Nov
Eucampia cornuta (Cleve) Grunow	Mar, Aug, A
E. zodiacus Ehrenberg	Jan, Feb
Grammatophora sp.	Oct
Guinardia delicatula (Cleve) Hasle	SU/A
Guinardia flaccida (Castracane) Peragallo	All year
G. striata (Stolterfoth) Hasle	All year
Gyrosigma balticum (Ehrenberg) Rabenhorst	Feb, Nov
Hemiaulus hauckii Grunow	All year
H. sinensis Greville	W/S, S/SU, SU, A
Lithodesmium undulatum Ehrenberg	Jan
Lauderia annulata Cleve	Jan, Feb, Aug
Leptocylindrus adriaticus Schroeder	Feb
L. danicus Cleve	W/S, May, **Jun**, Aug
L. mediterraneus (H. Peragallo) Hasle	Mar, Aug, A
Licmophora spp.	W/S, S
Lioloma pacificum (Cupp) Hasle	Jul, **A**, Dec
Lyrella lyra(Ehrenberg) Karayeva	Aug
Neocalyptrella robusta (Norman) Hernández-Becerril et Meave	W, W/S, May, Nov
Navicula spp.	SU, A
Nitzschia incerta (Grunow) M. Peragallo	Aug

Tab. 3. continued

Nitzschia longissima (Brébisson) Ralfs	All year, **S/SU**, **SU**
Nitzschia spp.	S/SU, SU/A, Oct
Odontella mobiliensis (Bailey) Grunow	Nov
Opephora marina (Gregory) Petit	Mar, Oct
Paralia sulcata (Ehrenberg) Cleve	All year, **SU/A**
Pleurosigma angulatum (Quekett) Smith	SU, SU/A, A
P. normanii Ralfs	Aug
Pleurosigma spp.	All year
Proboscia alata (Brightwell) Sundström	All year, **Jun**
P. indica (H. Peragallo) Hernández-Becerril	May
Psammodictyon panduriforme (Gregory) Mann	Aug, A
Pseudo-nitzschia spp.	All year, **SU/A, Aug, Oct**
Pseudosolenia calcar-avis (Schultze) Sundström	All year
Rhizosolenia hebetata Bailey	S/SU, SU
R. imbricata Brightwell	All year
Rhizosolenia spp.	All year
Skeletonema marinoi Sarno et Zingone	W, W/S, Aug, SU/A, **A**
Striatella unipunctata (Lyngbye) Agardh	Mar, S, S/SU, SU
Synedra toxoneides Castracane	Jul
Thalassionema nitzschioides (Grunow) Mereschkowsky	All year**, SU/A, A**
T. frauenfeldii (Grunow) Hallegraeff	Dec, SU, **SU/A**, **A**
Thalassiosira eccentrica (Ehrenberg) Cleve	Feb
T. rotula Meunier	Feb
Thalassiosira spp.	All year
Thalassiothrix longissima Cleve et Grunow	Oct
Toxarium undulatum Bailey	S/SU

Discussion

The northern Adriatic basin is a complex system in which the spatial distribution and seasonal variability of phytoplankton are mainly driven by the Po River discharge, coupled with the stratification/mixing regime and circulation (Revelante and Gilmartin 1976). Lim Bay is a semi-enclosed area without any strong freshwater source, unlike the nearby Gulf of Trieste, which has a strong seasonal pattern related to riverine nutrient input (Malej et al. 1995). The freshwater input from underwater springs was not very prominent, which can be seen on the salinity profile (Fig. 2). The minimum salinity values were recorded in the surface layer at the inner stations due to the winter and early spring precipitation events.

Nutrients in Lim Bay are generally low, the results corroborating those obtained from the adjacent northern Adriatic basin (Degobbis et al. 2005, Totti et al. 2005). No major unusual patterns in the seasonal distribution of nutrients were noticed during the seven years investigated. The only exception is the maximum phosphate concentration of 0.60 μM, recorded in the middle part of the bay (station LIM2), and the generally higher concentrations of this nutrient during the summer and autumn months (> 0.3 μM) in the inner part, which could be possibly related to the influence of fish farms. The high concentrations of nitrate and silicic acid could be attributed to freshwater inputs during the precipitation season. The high availability of three major nutrients could contribute to high phytoplankton biomass values, especially in the period from July to November; this could be seen in the seasonal chlorophyll *a* distribution (Fig. 4B). In summer, the nutrient input from the northern Adriatic basin could also be possible after the formation of the Istrian Coastal Countercurrent (ICCC), the surface current that usually appears along the Istrian coastal line and runs counter to the general Adriatic-wide cyclonic flow (SUPIĆ et al. 2000).

The seasonal distribution of chlorophyll *a* in the northeast Adriatic Sea in the last decade (1993–2000) shows a bimodal pattern with a more pronounced maximum in autumn than in spring, which may be attributed to hydrological changes in the Po River (SUPIĆ et al. 2006, VILIČIĆ et al. 2009). This trend was in accord with the results from this study. A detailed taxonomic analysis showed a more or less similar composition of plankton diatoms recorded in both Lim Bay and north east Adriatic Sea, with some differences. Some benthic pennate diatoms, such as *Cocconeis* spp., *Licmophora* sp., *Lyrella lyra* and *Psammodictyon panduriforme*, were probably released from the bottom by turbulence in the water column and no apparent seasonal distributional pattern was detected. The majority of the dominant diatoms in Lim Bay i.e. *Cerataulina pelagica*, *Chaetoceros socialis*, *Dactyliosolen fragilissimus*, *Guinardia striata*, *Leptocylindrus danicus, Nitzschia longissima*, *Pseudo-nitzschia* spp., *Rhizosolenia imbricata* and *Thalassionema nitzschioides,* showed a preference for nutrient-enriched conditions in the Adriatic Sea (REVELANTE and GILMARTIN 1980, 1985, PUCHER-PETKOVIĆ and MARASOVIĆ 1980), which agrees well with the classification of species according to their eutrophication level preferences (YAMADA et al. 1980).

Comparison of Lim Bay with other, similar, systems in the eastrern-central Adriatic Sea, characterized as moderately eutrophic environments, Novigrad Sea and Šibenik harbour (BURIĆ et al. 2005, CETINIĆ et al. 2006) showed that the same plankton diatom taxa were frequent, but not equally abundant. High nutrient levels in the Novigrad Sea, the lower part of the oligotrophic Zrmanja estuary, were of natural origin (BURIĆ et al. 2005) while in Šibenik harbour, in the lower part of the Krka estuary, a higher phosphate content indicated a more prominent anthropogenic influence (CETINIĆ et al. 2006). The composition of dominant species during the summer-autumn period in Lim Bay was more similar to that of Šibenik harbour than that of Novigrad Sea. The eutrophic centric diatom *Skeletonema marinoi* occurs most of the year in Lim Bay, being found in 18% of samples, with a maximum abundance of 1.45 x10^5 cells L^{-1} (recorded November 2005) but with decreasing abundance in the adjacent oligotrophic coastal northeast Adriatic Sea (VILIČIĆ et al. 2009). In Šibenik harbour it was recorded as one of the dominant species, while in Novigrad Sea it was not detected at all. Its presence in Lim Bay could be one of the indicators of the higher anthropogenic influence in this area.

Although our data set covers a relatively short time interval and it is more restricted than some other studies, it brings new information on the composition and average yearly fluctuation of the diatom flora. It can serve as a base for future studies in Lim Bay where an increase of the human impact can be anticipated.

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