Phytoplankton response to high salinity and nutrient limitation in the eastern Adriatic marine lakes

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SUMMARY: Phytoplankton and physical-chemical parameters were investigated for the first time in the only natural hyperhaline marine lakes (salinity >40) along Croatia's Adriatic coast, Mala Solina and Velika Solina. Two periods were recognized during the one-year investigation: one euhaline-mesotrophic from December to May and one hyperhaline-eutrophic from June to November. Nutrient limitation appears to have been important in defining the lakes' seasonal phytoplankton composition. Phosphate was most likely limiting from October to December, silicate from January to April, and nitrogen from June to September when nitrate was depleted. Diatoms were most abundant in November to January, when temperature and salinity were low and nitrate and ammonium were high. They collapsed in March when silicate was depleted. *Amphora*, *Navicula*, and other naviculoid diatoms were the most frequent genera. *Nitzschia longissima* was the most abundant species. Dinoflagellate dominance began in June in Mala Solina and in March in Velika Solina. It continued while temperature, salinity, phosphate, and silicate were high. *Oxyrrhis marina* was the most abundant dinoflagellate (3.2×10^6 cells L^{-1}). Nanophytoplankton was the dominant size fraction. Chroococcoid cyanobacteria were most abundant from May to October, reaching 2.9×10^7 cells L^{-1} in July. Both nanophytoplankton and small microphytoplankton, such as *Oxyrrhis*, *Scrippsiella*, and *Tetraselmis*, were most abundant under hyperhaline, N-depleted conditions. Toxic and harmful taxa (e.g. *Alexandrium*, *Dinophysis*), expanding in Mediterranean waters, were not recorded in the lakes.

Keywords: phytoplankton, hydrography, nutrients, eutrophication, marine lakes, Adriatic Sea.

RESUMEN: Respuesta del fitoplancton a la limitación de nutrientes y salinidad elevada en lagunas hipersalinas en el Adriático oriental. – El fitoplancton y diversos parámetros físico-químicos fueron estudiados por primera vez en dos lagunas hiperhalinas naturales (salinidad >40) de la costa de Croacia, Mala Solina y Velika Solina. El estudio se llevó a cabo durante 1 año, y pudieron distinguirse dos períodos: uno euhalino - mesotrófico, de diciembre a mayo, y el otro hiperhalino - eutrófico, de junio a noviembre. La limitación por nutrientes resultó ser un factor determinante para las variaciones estacionales en la composición del fitoplancton de las lagunas. El fosfato fue limitante de octubre a diciembre, el silicato de enero a abril, y el nitrógeno de junio a septiembre, una vez consumido el nitrato. Las diatomeas fueron especialmente abundantes entre noviembre y enero, cuando la temperatura y la salinidad eran bajas y la concentración de nitrato y amonio eran altas. El número de diatomeas disminuyó en marzo, tras el agotamiento del silicato. *Amphora*, *Navicula* y otras diatomeas naviculares fueron el género más frecuente, y *Nitzschia longissima* fue la especie más abundante. La dominancia de dinoflagelados comenzó en junio en Mala Solina y en marzo en Velika Solina, y se mantuvo mientras la temperatura, la salinidad, y las concentraciones de fosfato y silicato fueron altas. *Oxyrrhis marina* fue el dinoflagelado más abundante (3.2×10^6 células L^{-1}). El nanofitoplancton fue la fracción de tamaño dominante en la comunidad. Las cianobacterias Chroococcales fueron muy abundantes de mayo a octubre, alcanzando concentraciones de 2.9×10^7 células L^{-1} en julio. El nanofitoplancton y el microfitoplancton más pequeño, como *Oxyrrhis*, *Scrippsiella*, o *Tetraselmis*, fueron particularmente abundantes en condiciones hiperhalinas y pobres en nitrógeno. Taxones tóxicos y nocivos (por ejemplo *Alexandrium*, *Dinophysis*), expandiéndose en las aguas del Mediterráneo, no han sido detectados en los lagos.

Palabras clave: fitoplancton, hidrografía, nutrientes, eutrofización, lagunas costeras, mar Adriático.

INTRODUCTION

Wide seasonal variation of physical and chemical parameters in temperate coastal ecosystems influences biological processes (e.g. growth and grazing rates) that affect phytoplankton abundance (Smayda, 1980; Reynolds, 1997; Longhurst, 1998). Pelagic production in these systems is stimulated by nutrient inputs from the watershed (Kemp and Boynton, 1984) and coupled to benthic production (Johnson and Wiederholm, 1992; Miller-Way and Twilley, 1996). Regardless of their proximate source, nutrients that support phytoplankton growth may be characterized broadly in terms of the atomic ratios of inorganic nitrogen, phosphorus and, in the case of diatoms, silicon (N:P:Si) (Redfield *et al.*, 1963; Brzezinski, 1985). Changes in nutrient supply are often reflected in their ratios (Yin *et al.*, 2001). Significant deviations from the typical ratio may signal nutrient-limitation (Dorth and Whitledge, 1992).

Small, well-defined natural systems, such as the marine lakes of Croatia's Adriatic coast, offer an opportunity to study nutrient-phytoplankton interactions without many of the complications encountered in the open ocean and on a much larger scale than is practical in the laboratory. Furthermore, because the Croatian lakes span a range of hydrographic and trophic conditions, the response of phytoplankton assemblages to considerably different nutrient environments may be compared. According to the Venice System (Anonymous, 1959), most of the marine lakes in question are euhaline (salinity 30-40) and slightly or only moderately eutrophic (Jasprica *et al.*, 1995; Carić and Jasprica, 2005; Čalić *et al.*, 2007). They typically support higher biomass but lower diversity than is found in adjacent Adriatic coastal waters (Kršinić *et al.*, 2000). In particular, phytoplankton diversity is generally lower and diatoms are the dominant group. Plankton studies in these lakes have started only recently (*cf.* Jasprica and Carić, 2001) and much remains to be investigated. These lakes include two hyperhaline (salinity >40) lakes on the central Adriatic coast, Mala ("Little") Solina and Velika ("Big") Solina (Fig. 1).

Phytoplankton studies in Mediterranean hyperhaline environments are relatively scarce, but it is clear that salinity generally determines the distribution of taxa and that other factors—such as predation, competition, nutrients and prey availability—play a major role in controlling seasonal patterns (Elloumi *et al.*, 2009). One such study in two Tunisian lagoons of very different salinities (40 and 90), in which diatoms and dinoflagellates predominated, observed lower abundance, species richness and diversity at higher salinity (Ayadi *et al.*, 2004). The present work contributes to the understanding of phytoplankton cycles in hyperhaline environments of the Mediterranean coast by reporting the first data on phytoplankton composition and seasonal distribution in two hyperhaline Adriatic ecosystems. Particular attention has been paid to interpreting the importance of key environmental variables,
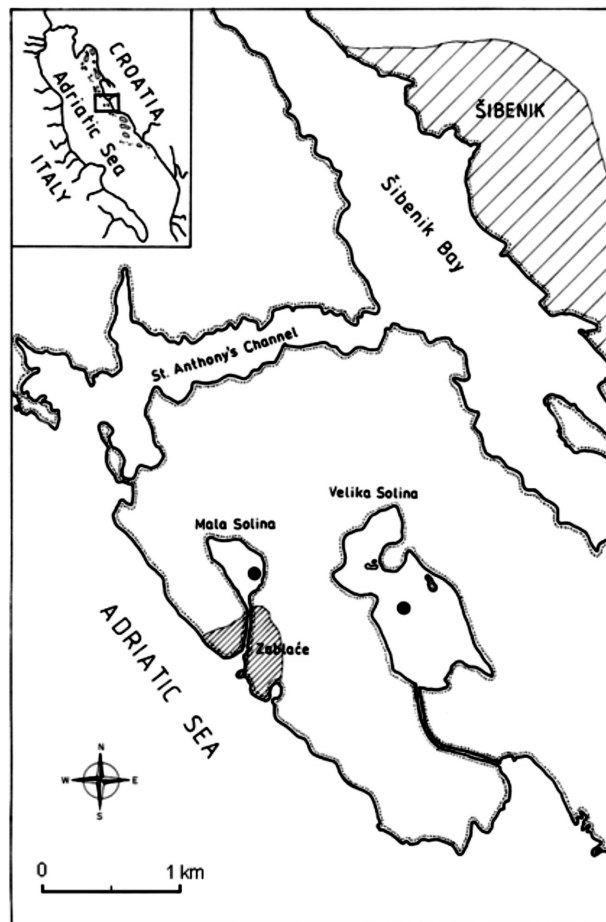

FIG. 1. – Geographic location of Mala Solina and Velika Solina with sampling sites.

particularly nutrient ratios, as predictors of phytoplankton abundance and structure.

Study area

Mala Solina (43°42'43.54"N, 15°52'0.89"E) and Velika Solina (43°42'31.71"N, 15°52'59.99"E) are hyperhaline marine lakes located along the Republic of Croatia's central Adriatic coast. The region has a typical Mediterranean climate of warm, dry summers and mild, rainy winters (Blondel and Aronson 1999). Average annual air temperature is 15.3°C and precipitation averages 789.8 mm yr^{-1} (data from the nearby Šibenik station for 1961-2000, Croatian Meteorological and Hydrological Service). The lakes are about 9 kilometres from the town of Šibenik (37060 in 2001) and near the village of Zablaće (Fig. 1). Zablaće, situated around Mala Solina, has 500 year-round inhabitants, but this number increases several-fold in summer. Mala Solina is thus exposed to a seasonal pattern of anthropogenic influence, especially discharge of untreated domestic waste.

Both lakes are very shallow, with a maximum depth of 1 m in Mala Solina and 1.5 m in Velika Solina. Mala Solina has a surface area of 15 ha and

a volume of 60000 m^3; Velika Solina has a surface area of 90 ha and a volume of 810000 m^3. The bottoms of both are partly covered with dense meadows of *Cymodocea nodosa*. Salt-marsh vegetation of the *Sarcocornetea fruticosi* vegetation class is distributed around each. These low, marshy coasts are a rare habitat of ecological value and are listed in Annexes of the EU Directive on the Conservation of natural habitats and of wild fauna and flora (Council Directive 92/43/EEC, 1992). Each lake has a narrow channel that connects it to the Adriatic, whose average sea level is 59.1 cm (1955-2004) at the nearest coastal station (Domijan, 2006). The channel that links Mala Solina to the sea is 200 m long and 1-2 m wide, and has a maximum depth of 30 cm; the one that links Velika Solina to the sea is 1 km long and 2-4 m wide, and has a maximum depth of 2 m. Projects are underway as of this writing to enlarge these channels to increase water exchange that, along with seasonal wind patterns, drives circulation in the lakes. North winds generally dominate from January to March, in June, and again from August to October; southwest winds prevail the rest of the year (Meteorological and Hydrological Service of Croatia).

MATERIALS AND METHODS

Sampling and laboratory procedures

Physical-chemical parameters and phytoplankton were collected monthly from November 1999 to October 2000, except for February 2000. The sampling station was situated at the deepest part of each lake: 10 m from shore for Mala Solina and 20 m from shore for Velika Solina. Samples were taken at mid-day with a 5-L Niskin bottle at 0.5 m, a depth midway between the surface and bottom. Transparency was measured with a 30-cm diameter Secchi disc. Temperature, salinity, density (expressed as sigma-t), dissolved oxygen, and nutrients (nitrate NO_3, nitrite NO_2, ammonium NH_4, phosphate PO_4, and silicate SiO_4,) were determined by standard oceanographic methods (Strickland and Parsons, 1972). Oxygen saturation ($O_2/O_2´$) was calculated from the solubility of oxygen in seawater as a function of temperature and salinity (Weiss, 1970; UNESCO, 1973).

Trophic status was characterized by the TRIX index, commonly used to classify coastal marine areas in the Mediterranean (Vollenweider *et al*., 1998, Karydis, 2009):

$$TRIX = (log\ [ChA \times D\%O \times DIN \times TP] + k\ )\ /\ m$$

Each of the four factors in the argument of the logarithm represents a variable understood to reflect trophic state: *ChA* = chlorophyll *a* concentration [μg L^{-1}], *D%O* = dissolved oxygen [absolute % deviation from saturation], dissolved inorganic nitrogen DIN = (NO_3+NO_2+NH_4) [μg L^{-1}], and TP = total phosphorus

[μg L^{-1}]. The parameters k = 1.5 and m = 1.2 set the range of the TRIX scale from 0 to 10 (0-4 oligotrophic, 4-5 mesotrophic, 5-6 eutrophic, 6-10 extremely eutrophic).

Chlorophyll *a* (Chl *a*) was determined from 500-mL sub-samples filtered through Whatman GF/F glass-fibre filters stored at -20°C. These were homogenized and extracted in 90% acetone for 24 h at room temperature (Holm-Hansen *et al*., 1965). Samples were analyzed fluorometrically with a Turner TD-700 Laboratory Fluorometer (Sunnyvale, CA) calibrated with pure Chl *a* (Sigma).

Phytoplankton samples were preserved in neutralized formalin (2% final concentration) and viewed with an Olympus IX-71 inverted microscope equipped with bright-field and phase contrasts (Utermöhl, 1958). Sub-samples (50 mL) were settled for 24 hours in counting chambers before analysis. Counts of microphytoplankton (MICRO >20 μm,) were done at 200× in 2-4 transects made across the central part of the counting chamber base plate and at 100× on the remainder of the chamber base plate. This fraction was divided into five groups: BACI (Heterokontophyta, Bacillariophyceae), diatoms; DINO (Dinophyta, Dinophyceae), dinoflagellates; PRYM (Prymnesiophyta, Prymnesiophyceae), coccolithophorids; CHLO (Chlorophyta, Prasinophyceae), green algae; and OTHER phytoplankton (filamentous cyanobacteria, Euglenophyceae and unidentified spherical cells). Whenever possible, identification was taken to the species or genus level using standard taxonomic guides (Kofoid and Swezy, 1921; Hustedt, 1930; Schiller, 1933, 1937; Cupp, 1943; Sournia, 1986; Tomas, 1993, 1996; Bérard-Therriault *et al*., 1999; Horner, 2002). The nomenclature of higher taxa (phyla, classes) follows Lee (1999). Nanophytoplankton (NANO, cells 2-20 μm) was counted in 30 randomly selected fields-of-view at 400×.

Species richness and similarity indices

Microphytoplankton species associations between the lakes were compared with Jaccard's similarity index (Jaccard, 1908), which is based on the presence/absence of a species rather than on its actual numbers:

$$JI = 100\ [a\ /\ (a + b + c)]$$

where *a* is the number of species present in both lakes; *b* is the number in lake 1 but not lake 2; and *c* is the number in lake 2 but not lake 1. Double absences were not considered.

Margalef's index (Margalef, 1965) was used to characterize species richness:

$$D = S - 1/\ log\ N$$

where *S* is the number of species and *N* the number of individuals counted to obtain *S* species.

Statistical analyses

Pearson product-moment correlations were calculated between physical-chemical parameters and phytoplankton species abundance. Ordination was performed using non-metric multidimensional scaling (MDS) based on the Bray-Curtis similarity matrix (Clarke and Warwick, 2001). MDS was applied to define the similarity of sampling dates with the respect to phytoplankton composition. ANOSIM randomization was used to test for significant differences in phytoplankton community structure. The dissimilarity percentage program (SIMPER) was used to identify the species making the greatest contribution to differences between clusters observed in the MDS plot. Statistical analyses were performed with STATISTICA (StatSoft Inc., Tulsa, OK) and PRIMER v5 software (Clarke and Gorley, 2001).

RESULTS

Environmental conditions

Secchi disc transparency ceased down to the bottom of lakes throughout the year. Water temperature (Fig. 2) ranged from 3.9°C (January) to 33°C (June) in Mala
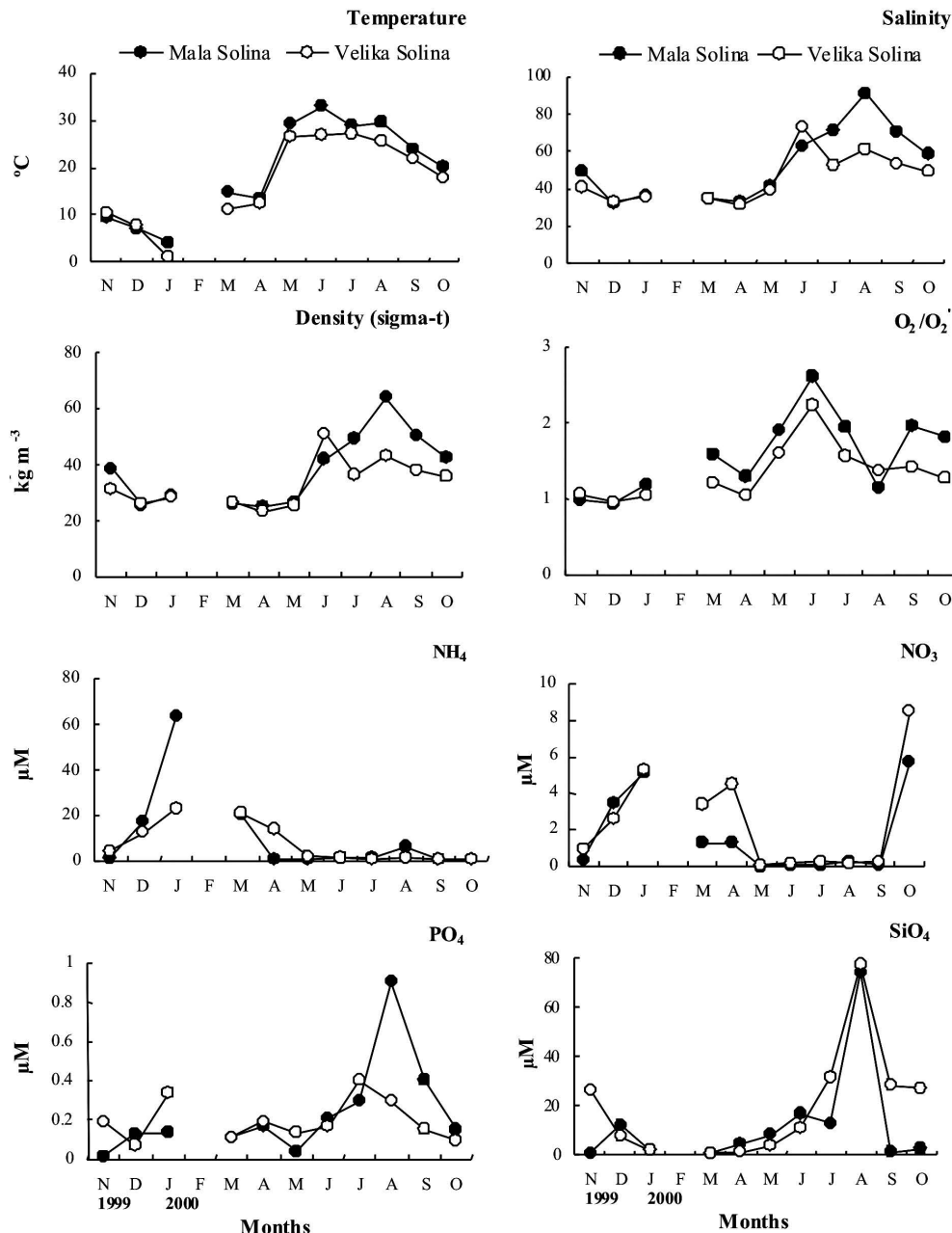

Fig. 2. – Monthly distribution of physical-chemical parameters in Mala Solina and Velika Solina.
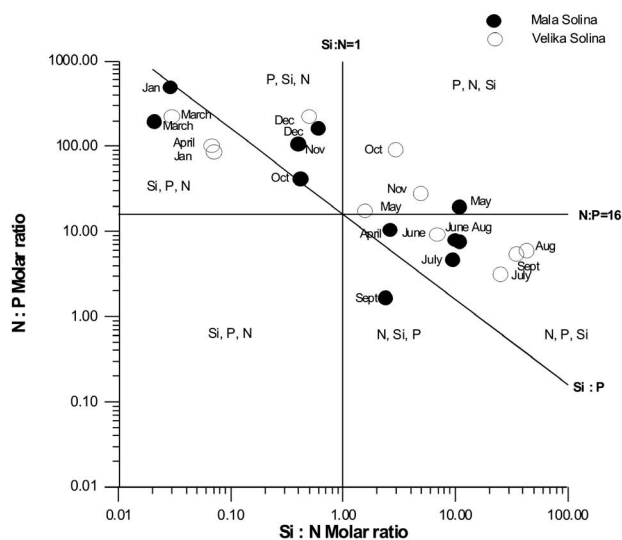
Fig. 3. – Si:N:P [SiO_4 : (NO_3+NO_2+NH_4) : PO_4] molar ratios in Mala Solina and Velika Solina. Molar quotients between in situ concentrations of potentially limiting nutrients are delimited by Si:N = 1, N:P = 16, and Si:P = 16 lines. Lines define six different areas within the plot, each characterized by a potentially limiting nutrient in order of priority (Rocha et al., 2002).
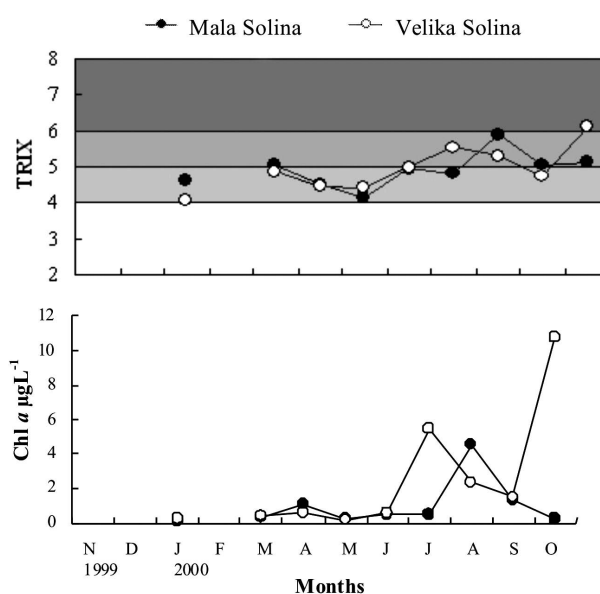

Fig. 4. – Chlorophyll a concentration (Chl a) and TRIX units (0-4 oligotrophic, 4-5 mesotrophic, 5-6 eutrophic, 6-10 extremely eutrophic) in Mala Solina and Velika Solina during the 1-year investigation.

Solina and from 0.9°C (January) to 27.3°C (July) in Velika Solina. In both lakes it rose steadily from March to May, remained fairly constant in August, and began to decrease in September. Salinity (Fig. 2) varied from 32.5 (December) to 90.5 (August) in Mala Solina; it was lower in Velika Solina, varying from 30.9 (April) to 72.55 (June). It increased in both lakes starting in May and was high from June to October. Seasonal density changes tracked salinity, ranging from 25.1 to 64.1 kg m^{-3} in Mala Solina and 23.4 to 51.2 kg m^{-3} in Velika Solina. Dissolved oxygen ranged from 3.69 to 9.28 ml L^{-1} and from 5.54 to 8.24 ml L^{-1} and with saturation (O_2/O_2') it ranged from 0.93 to 2.6 and 0.96 to 2.23, respectively, in Mala and Velika Solina.

Nitrate ranged from 0.03 (May) to 5.70 (October) μM in Mala Solina and from 0.08 (May) to 8.53 (October) μM in Velika Solina. Higher values were found from December to April. Ammonium varied from 0.46 (April) to 63.49 (January) μM in Mala Solina and from 0.52 (September) to 23.23 (January) μM in Velika Solina. Ammonium was very high from December to April, with Mala Solina exhibiting a particularly high value (6.53 μM) in August. Phosphate (PO_4) ranged from 0.02 (November) to 0.9 (August) μM in Mala Solina and from 0.07 (December) to 0.4 (July) μM in Velika Solina. Mala Solina's values were high from July to September. Silicate (SiO_4) ranged from 0.44 (March) to 74.01 (August) μM in Mala Solina and from 0.73 (March) to 77.41 (August) μM in Velika Solina. High values of around 30 μM, however, were found only in Velika Solina and in November, July, September, and October. Molar quotients of potentially limiting nutrients are shown in Figure 3. PO_4 was most likely limiting from October to December, SiO_4 from

January to April, and N from June to September, a period of NO_3 depletion. Trophic status was 4-5 TRIX units until May and 5-6 from June to October (Fig. 4). Chl a ranged from 0.08 (January) to 4.51 μg L^{-1} (August) in Mala Solina and from 0.08 (January) to 10.71 μg L^{-1} (October) in Velika Solina (Fig. 4).

Phytoplankton

Microphytoplankton (MICRO) varied from 1.3 x 10^3 to 5.1 x 10^6 cells L^{-1} in Mala Solina and from 1.2 x 10^3 to 2.6 x 10^5 cells L^{-1} in Velika Solina (Fig. 5). The highest abundances were in summer: August in Mala Solina and July in Velika Solina. The lowest were in January in Mala Solina and March in Velika Solina. Monthly variations of MICRO and their percentage of total abundance (Fig. 6) show that dinoflagellates were the most abundant group and largely responsible for the MICRO peak. Dinoflagellates dominated MICRO in November and again from June to October in Mala Solina (76.8-99.5%). In Velika Solina, dinoflagellates dominated from March to October (77.5-98.5%), with the exception of June. Maximum diatom abundance in Mala Solina (1.17 × 10^6 cells L^{-1}, August) occurred two months later and was about two orders of magnitude higher than in Velika Solina (3.49 × 10^4 cells L^{-1}, June). Diatoms were the most abundant MICRO in Mala Solina in January (70%) and in Velika Solina from November to January (81-91%). The percentage of coccolithophorids (PRYM) in the MICRO fraction (Fig. 6) was less than 1% throughout the year, except in Mala Solina in December (44%). Green algae were found only in Mala Solina from July to September and
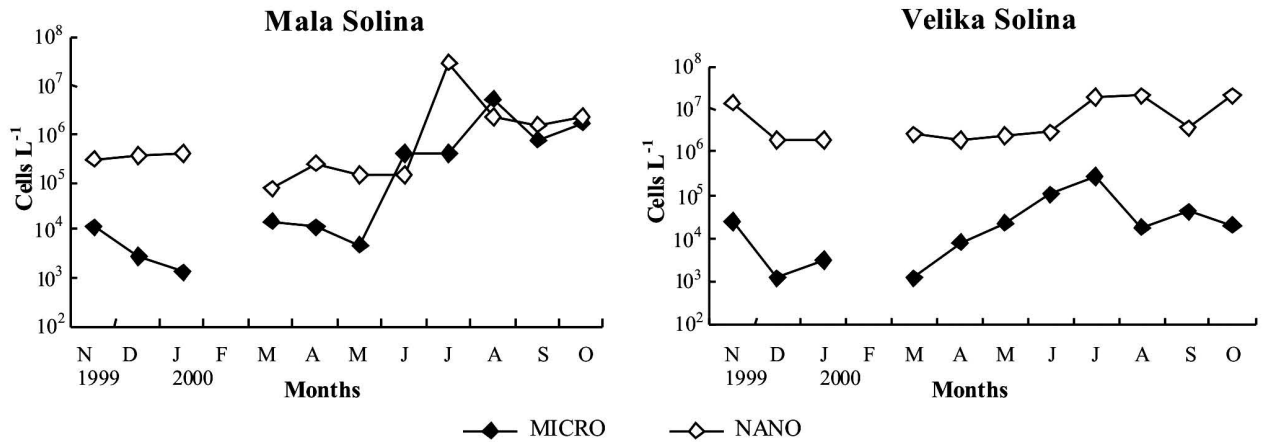
FIG. 5. – Monthly distribution of microphytoplakton (MICRO) and nanophytoplankton (NANO) abundances in Mala Solina and Velika Solina.
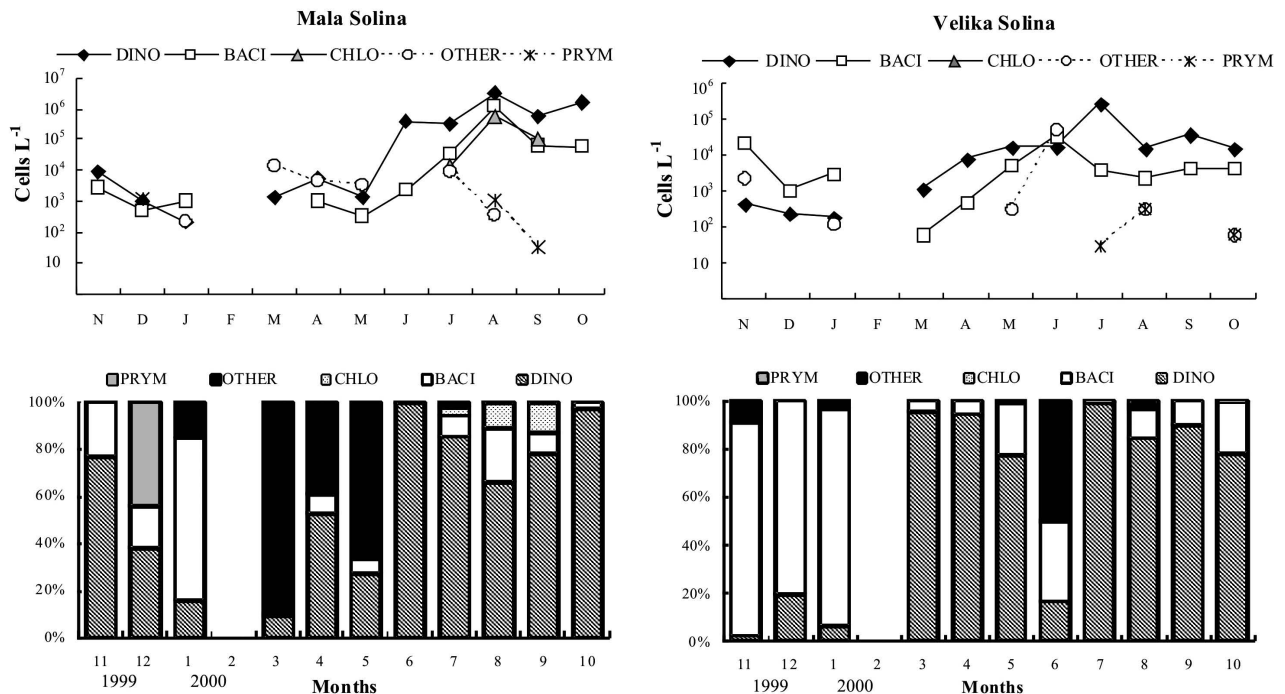

FIG. 6. – Monthly variations and relative contribution of different taxonomic groups to microphytoplankton abundance. DINO, Dinophyceae; BACI, Bacillariophyceae; CHLO, Micromonadophyceae; OTHER, Cyanophyceae and unidentified spherical cells; PRYM, Prymnesiophyceae.

ranged from 1.4×10^4 to 5.89×10^5 cells L^{-1}. The group designated OTHER contained mainly unidentified spherical cells of 20-30 μm (39-90%) in Mala Solina from March to May and filamentous cyanobacteria (50%) in Velika Solina in June.

Nanophytoplankton (NANO) abundances varied over almost three orders of magnitude, from 7.0×10^4 to 3.0×10^7 cells L^{-1}, in Mala Solina and over a much smaller range, from 1.8×10^6 to 2.2×10^7 cells L^{-1}, in Velika Solina (Fig. 5). The highest abundances were in July and August in Mala and Velika Solina, respectively; the lowest occurred in March in Mala Solina and in January in Velika Solina. Chroococoid cyanobacteria dominated NANO in Mala Solina in July

(98%, 2.9×10^7 cells L^{-1}) but were not found in Velika Solina. NANO cells smaller than 10 μm prevailed in both lakes during the rest of the year.

Altogether, 65 taxa (38 genera) of MICRO were identified (Table 1). Most of them were true planktonic (37 taxa or 57%). The vast majority of MICRO were either diatoms (34 taxa) or dinoflagellates (26 taxa). Mala Solina had 31 MICRO taxa and Velika Solina had 50. MDS analyses identified two phytoplankton communities: euhaline-mesotrophic and hyperhaline-eutrophic (Fig. 7). According to SIMPER analysis, *Oxyrrhis marina* (48.45%), chroccocoid cyanobacteria (27.48%), and *Prorocentrum scutellum* (8.36%) contributed most to variance between groups in Mala Solina, whereas

TABLE 1. – List of microphytoplankton taxa and monthly distribution of their abundance in Mala Solina (M) and Velika Solina (V) during the one-year investigation. Abundances are presented as follows: $1 = 10\text{-}10^2$; $2 = 10^2\text{-}10^3$; $3 = 10^3\text{-}10^4$; $4 = 10^4\text{-}10^5$; $5 = 10^5\text{-}10^6$; $6 = 10^6\text{-}10^7$ cells L^{-1}.

Years: 1999 = months 11–12; 2000 = months 1–10.

Taxon	11 M	11 V	12 M	12 V	1 M	1 V	3 M	3 V	4 M	4 V	5 M	5 V	6 M	6 V	7 M	7 V	8 M	8 V	9 M	9 V	10 M	10 V
Chlorophyta, Prasinophyceae																						
Tetraselmis sp.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	4	.	5	.	4	.	.	.
Primnesiophyta, Primnesiophyceae																						
Calyptrosphaera oblonga Lohmann	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	3	.	.	.	.	.
Michaelsarsia adriaticus (Schiller)Manton, Bremer et Oates	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.
Unidentified coccolithophoridae >20 μm	.	.	3	.	.	.	.	.	.	.	.	.	.	.	1	.	2	.	.	.	.	1
Heterokontophyta, Bacillariophyceae																						
Amphora ostrearia Brébisson	.	.	.	.	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Amphora sp.	3	.	.	.	.	2	.	.	1	2	.	.	3	2	4	2	4	2	4	2	4	2
Campylodiscus sp.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
Chaetoceros compressus Lauder	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Chaetoceros tortissimus Gran	.	.	.	.	.	.	.	.	.	.	.	.	3	.	.	.	.	.	.	.	.	.
Chaetoceros sp.	.	.	.	.	.	.	.	.	.	.	3	.	.	.	.	.	.	.	.	.	.	.
Coscinodiscus sp	2	.	.	.	.	.	.	.	2	.	.	.	.	.	.	.	.	.	.	.	.	.
Cyclotella sp.	.	3	.	.	.	2	.	.	.	2	.	3	.	3	.	.	.	.	.	.	.	.
Dactyliosolen blavyanus (Peragallo) Hasle	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2	.	.	.	.	.
Diploneis bombus (Ehrenberg) Cleve	.	.	.	.	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Diploneis sp.	.	.	.	.	2	.	.	.	.	.	.	.	.	1	.	.	2	.	.	.	.	.
Eunotia sp.	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Gyrosigma sp.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Licmophora flabellata C.Agardh	2	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Lyrella lyra (Ehrenberg) Karajeva	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.
Lyrella sp.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2
Mastogloia angulata Lewis	.	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Navicula sp.	.	3	.	.	2	2	.	1	.	2	.	.	2	.	2	.	3	.	3	.	3	.
Naviculoid cells	2	3	2	2	2	2	1	1	.	2	.	.	2	.	.	.	2	.	2	.	2	2
Nitzschia incerta (Grunow)M.Peragallo	.	1	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.
Nitzschia longissima (Brébisson) Ralfs	1	2	.	2	.	.	.	.	.	.	.	.	2	.	.	.	6	.	4	2	.	.
Nitzschia sp.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.
Pleurosigma angulatum (Quekett) W.Smith	.	.	2	.	1	.	.	.	.	.	.	.	2	2	1	.	1	.	.	.	.	.
Pleurosigma elongatum W.Smith	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Pleurosigma formosum W.Smith	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Pleurosigma sp.	.	.	.	.	.	.	.	.	.	.	.	.	2	.	.	.	.	.	2	.	.	.
Pseudo-nitzschia spp.	.	.	.	.	.	.	.	.	.	.	.	.	4	.	.	.	.	.	.	.	.	.
Rhabdonema sp.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.
Rhizosolenia sp.	.	.	.	.	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Surirella fastuosa Ehrenberg	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Synedra fulgens (Greville) W.Smith	.	.	2	.	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Synedra sp.	.	2	.	1	.	.	.	.	.	.	.	.	2	.	3	.	.	.	.	.	.	.
Thalassionema nitzschioides (Grunow)Mereschkowsky	.	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2
Tropidoneis sp.	.	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Ulnaria acus (Kützing)M.Aboal	.	3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Unidentified diatoms	2	.	.	.	2	2	.	1	.	.	.	.	2	.	.	.	.	.	.	.	2	2
Dinophyta, Dinophyceae																						
Diplopsalis complex	.	.	.	.	.	.	.	.	.	.	.	.	2	.	.	.	.	.	.	.	.	.
Gonyaulax digitale (Pouchet) Kofoid	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2	.	.	.	.	.	.
Gonyaulax hyalina Ostenfeld et Schmidt	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	5	.	.	.	.	.
Gonyaulax sp.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2	.	.	.	4	.	3	.
Gymnodinium simplex (Lohmann) Kofoid et Swezy.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2	.	.
Gymnodinium sanguineum K.Hirasaka	.	.	.	.	.	.	.	.	.	.	2	.	2	.	.	.	.	.	.	.	.	.
Gymnodinium spp.	.	.	.	.	.	.	.	.	.	.	.	2	.	2	.	.	.	.	.	.	.	.
Gymnodinioid cells	.	.	1	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	2
Gyrodinium spp.	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Neoceratium fusus (Ehrenberg) F.Gomez, D.Moreira et P.Lopez-Garcia	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2	2	.	.
Oxyrrhis marina Dujardin	.	.	.	.	.	.	.	.	.	.	.	.	5	.	5	3	6	5	5	3	6	.
Oxytoxum sphaeroideum Stein	.	.	.	.	.	.	.	.	.	.	.	.	.	2	.	.	.	.	.	.	.	.
Prorocentrum compressum (Bailey) Abé ex Dodge	.	.	.	.	.	.	.	.	.	.	.	.	.	2	.	.	.	.	.	.	.	.
Prorocentrum maculosum M.A.Faust	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	3	.
Prorocentrum micans Ehrenberg	.	2	2	1	.	.	2	.	1	.	.	.	2	2	.	.	.	.	.	.	.	2
Prorocentrum minimum (Pavillard) Schiller	.	.	.	1	.	2	.	.	.	.	.	.	.	2	.	.	.	.	.	.	.	.
Prorocentrum scutellum Schröder	3	2	2	1	.	.	3	2	3	3	2	4	2	3	2	2	2	.	2	.	4	4
Prorocentrum triestinum Schiller	.	.	.	.	.	.	.	.	3	.	.	.	2	.	.	.	.	.	3	1	.	.
Prorocentrum sp.	.	.	.	.	.	.	.	.	2	.	.	.	2	.	.	.	.	.	.	.	.	2
Protoperidinium brochii (Kofoid et Swezy) Balech	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2	.	.	1

TABLE 1 (cont.). – List of microphytoplankton taxa and monthly distribution of their abundance in Mala Solina (M) and Velika Solina (V) during the one-year investigation. Abundances are presented as follows: 1 = 10-10^2; 2 = 10^2-10^3; 3 = 10^3-10^4; 4 = 10^4-10^5; 5 = 10^5-10^6; 6 = 10^6-10^7 cells L^{-1}.

| Years	1999				2000																			
Months	11		12		1		3		4		5		6		7		8		9		10			
Stations	M	V	M	V	M	V	M	V	M	V	M	V	M	V	M	V	M	V	M	V	M	V		
Protoperidinium conicum (Gran) Balech	.	.	1	.	.	.	.	.	1	.	.	.	.	.	2	.	.	2	.	.	.	.		
Protoperidinium depressum (Bailey) Balech	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2	.	.	.	.	.	.	.		
Protoperidinium leonis (Pavillard) Balech	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2		
Protoperidinium tuba (Schiller) Balech	.	.	.	.	.	.	.	.	.	.	.	.	2	.	.	.	.	.	.	.	.	.		
Protoperidinium sp.	.	.	.	.	.	.	.	.	.	.	2	.	.	.	.	.	.	.	.	.	.	1		
Pseliodinium vaubanii Sournia	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.		
Scrippsiella trochoidea (Stein) Balech ex Loeblich III	2	.	.	.	2	.	.	2	2	3	.	3	.	3	2	3	.	.	.	3	3	3		
Unidentified dinoflagellates	.	.	2	.	2	1	.	1	.	2	2	2	.	3	2	.	.	.	.	2	3	2		
Others																								
Cyanobacteria, Cyanophyceae																								
Lyngbia sp.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2	.	.	.	.	.		
Oscillatoria sp.	.	3	.	.	.	.	.	.	.	.	.	.	.	3	.	.	.	.	.	.	.	.		
Unidentified cyanobacteria >20 µm	.	.	.	.	.	.	.	.	.	.	.	.	4	.	.	.	.	.	.	.	.	.		
Unidentified spherical cells >20 µm	.	.	.	.	2	2	4	.	3	.	3	2	.	2	.	.	.	2	.	.	.	1		
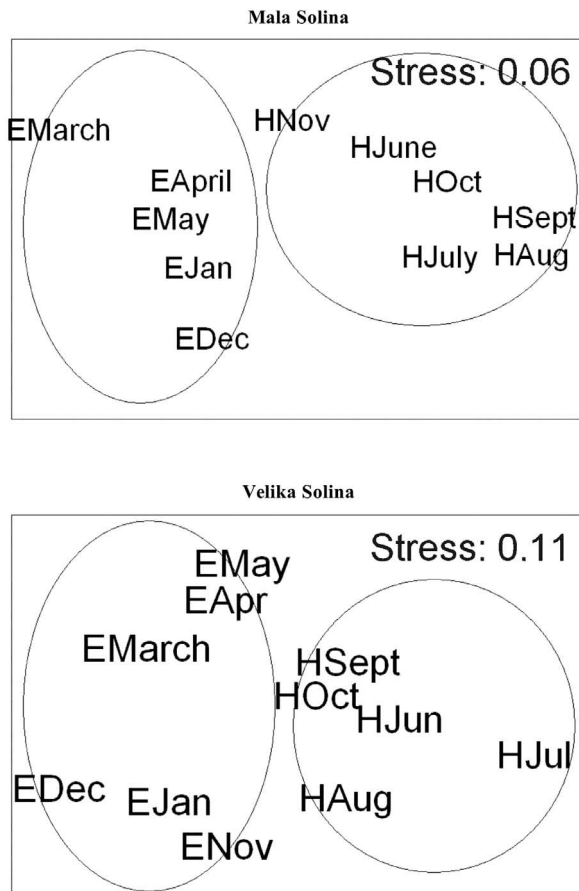
Fig. 7. – MDS ordination of phytoplankton composition and abundance based on the Bray-Curtis similarity matrix in Mala Solina (stress value = 0.06, ANOSIM, p < 0.01) and Velika Solina (stress value = 0.11, ANOSIM, p < 0.05). Symbols E (euhaline) and H (hyperhaline) refer to the two groups of significantly different phytoplankton communities.

Fig. 8. – Margalef's species richness index in Mala Solina and Velika Solina, and Jaccard's similarity index between the lakes' microphytoplankton.

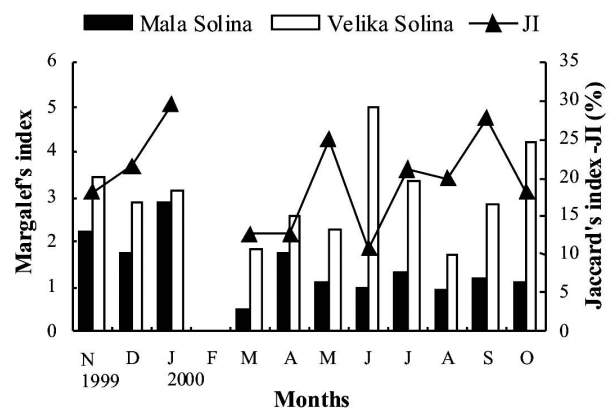*Oxyrrhis marina* (40.55%), *Prorocentrum scutellum* (24.13%), and *Scrippsiella trochoidea* (10.18%) contributed most to variance in Velika Solina. The most abundant MICRO species in both lakes was *Oxyrrhis marina* (Table 1). Intense development of this species was found in August in both Mala (3.2×10^6 cells L^{-1}) and Velika Solina (2.5×10^5 cells L^{-1}), and in October (1.6×10^6 cells L^{-1}) only in Mala Solina. *Nitzschia longissima* and *Gonyaulax hyalina* also attained high abundance (10^5 to 10^6 cells L^{-1}) in Mala Solina in August. The following taxa exceeded 10^3 cells L^{-1} in Mala Solina: *Amphora* sp., *Gonyaulax* sp., *Prorocentrum scutellum*, *Prorocentrum triestinum*, *Tetraselmis* sp., and *Scrippsiella trochoidea*. MICRO taxa in Velika Solina at more than 10^3 cells L^{-1} were *Chaetoceros tortissimus*, *Cyclotella* sp., *Navicula* sp., *Pseudo-nitzschia* spp., *Synedra* sp., small naviculoid cells, *Prorocentrum scutellum*, and *Scrippsiella trochoidea*. *Oxyrrhis marina*, *Tetraselmis* sp., and *Nitzschia longissima* were positively correlated with salinity (P<0.05), NH$_4$ (P<0.001), and PO$_4$ (P<0.001). *Amphora* sp., *Navicula* sp., *Synedra* sp., and small naviculoid cells were positively correlated with PO$_4$ (P<0.01). No other correlations between phytoplankton taxa and environmental variables were statistically significant.

MICRO species similarity between lakes was relatively low. Jaccard's index (Fig. 8) varied from 10.7% to 29.4%, with the highest in January and the lowest in June. Margalef's species richness index (Fig. 8) varied from 0.47 to 2.87 in Mala and from 2.27 to 5.37 in Velika Solina. The highest value in Mala Solina was in January and that in Velika Solina was in June. Species richness generally was higher in Velika Solina throughout the year.

DISCUSSION

Despite the monthly sampling design, this study provides the first information on phytoplankton structure in hyperhaline marine lakes on the eastern Adriatic coast. The annual cycle of phytoplankton in Mala Solina and Velika Solina during this study was characterized by two periods: a euhaline and mesotrophic period lasting from December to May; and a hyperhaline and eutrophic period lasting from June to November. According to the annual distribution of microphytoplankton abundance, both lakes are moderately eutrophic (Viličić, 1989). During their euhaline period, the lakes do not differ substantially in their hydrographic and chemical characteristics from other euhaline marine lakes along the Croatian coast (Carić and Jasprica, 1998; Ciglenečki et al., 2005; Čalić et al., 2007). During the hyperhaline period, however, in addition to elevated salinity, the lakes also have significantly higher temperatures, oxygen saturations and concentrations of SiO_4 and NH_4, but lower NO_3 and PO_4 than coastal lakes of a similar trophic status (Morais et al., 2003).

Seasonal temperature variations in the lakes are typical for the Mediterranean (Blondel and Aronson, 1999; Cushman-Roisin et al., 2007). The summer salinity increase results from the combination of high temperature, low precipitation, and the lakes' high surface-to-volume ratios (3 in Mala Solina and 1.5 in Velika Solina). Temperatures in 2000, in fact, were much higher than the 3-decade (1961-1990) average (Nikolić, 2000). According to Bonacci (1996), potential evaporation in this region is 1200-1300 mm per year, with the maximum in the order of 200 mm per month in July and August.

There was an annual peak of MICRO and NANO fractions in each lake. Mala Solina had a higher NANO peak (1.7 times higher than that in Velika Solina) and a much higher MICRO peak (19.3 times higher). These peaks occurred in summer, when temperature and salinity were high, silicate and phosphate reached their annual maxima, and ammonium was slightly elevated. Both PO_4 and NH_4 were higher in Mala Solina. One phytoplankton peak was also found in some slightly polluted Greek hyperhaline (41-60) lagoons in late winter/early spring (Nicolaidou et al., 2005). In contrast, other parts of the coastal Adriatic have been shown to experience not one but two annual MICRO peaks, most often between March and September (Jasprica and Carić, 1997; Carić and Jasprica, 2005). Some

areas of the Mediterranean even have four peaks: one such area is the eutrophic Bay of Iskenderun (Polat et al., 2000), where the two strongest peaks (August and October) occur when temperature (29.06°C) and salinity (39.24) are highest. Differences in phytoplankton annual peaks can be explained by environmental conditions, especially salinity and nutrient concentrations in particular ecosystems.

Diatoms and dinoflagellates were the major MICRO components. The diatom maximum was in August but—especially in Velika Solina—diatoms dominated from November to January, a period during which temperature and salinity were low but NO_3 and especially NH_4 were high. Silicate varied between 2 and 26 μM from November to January. This is above the half-saturation constant typical for coastal diatoms (Fisher et al., 1988). Diatoms thus should not have been silicate-limited during that period (Carlsson and Granéli, 1999; Granéli et al., 1999). Diatoms commonly enjoy an advantage when NO_3 is high (Bode and Dorch, 1996), as is generally the case in the coastal southern Adriatic (Jasprica and Carić, 2001). In a restricted Tunisian lagoon (salinity 32-40) diatoms dominated in spring and summer and also whenever nitrate was high (Sakka Hlaili et al., 2007). Decline of the Si:N ratio from January to March (Fig. 3), with available concentrations of SiO_4 falling below the level of reported half-saturation constants (Rocha et al., 2002 and references therein), may have been responsible for limiting diatoms and encouraging the shift to non-siliceous flagellates.

Anthropogenic effects are probably responsible for the higher PO_4 levels from June to September and the elevated NH_4 concentration of August. This period—the tourist season—is accompanied by a marked increase in waste discharge. Higher temperatures during this period can also be expected to increase the remineralization rate of organic nitrogen and phosphate in sediments, followed by the release of NH_4 and PO_4 into the overlying water column. The concentration of SiO_4 increased with the onset of higher temperatures and salinities in May and reached its annual maximum in August. The observed peak may result from more rapid regeneration under these warm conditions (Yamada and D'Elia, 1984). Maximum diatom abundance was related to peak silicate; both diatoms and silicate subsequently declined in September and October. The influence of the adjacent coastal water was high in October, as attested by the similarity of nutrient concentrations in both the sea and lakes at that time. NO_3, SiO_4, and PO_4 in the sea were, respectively, 15, 18, and 0.01 μM (Olujić, 2007).

The highest contribution of dinoflagellates to MICRO was mostly from June to October in Mala Solina, and from March to October in Velika Solina. Their dominance coincided with higher temperature, salinity, PO_4 and SiO_4, but lower NO_3. Nitrate was markedly below the half-saturation constant concentration typical of coastal phytoplankton (Fisher et al., 1988; Sommer, 1994). Nitrogen appeared to be limiting from

May to September (Fig. 3), which is consistent with the findings of Sakka Hlaili *et al*. (2006) that document the greatest increase in dinoflagellates under PO_4 enrichment. PO_4 decreased after the dinoflagellate maximum and was most likely limiting from October to December. Abid *et al*. (2008) found that growing dinoflagellates reduced phosphate concentrations. The alternating dominance of diatoms and dinoflagellates is consistent with the general theme of Margalef's mandala (Margalef, 1983). A transition from diatom to dinoflagellate dominance coincided with a shift from P- to Si-limitation in both lakes, which agrees with the results of Escaravage *et al*. (1996).

Filamentous cyanobacteria were found only in Velika Solina (June), where they made up 50% of MICRO. This is probably a result of their greater temperature tolerance, which exceeds that of most eukaryotic algae (Steiner *et al*., 1971).

Phytoplankton structure and seasonality were largely similar to those of comparable habitats in the Mediterranean (Abid *et al*., 2008; Ayadi *et al*., 2004; Sakka Hlaili and El Grami, 2004; Pavlova *et al*., 1998). As in the present study, diatoms, dinoflagellates and autotrophic flagellates were the main phytoplankton in these isolated and more-or-less hyperhaline ecosystems. Diatoms developed mostly in autumn-winter and dinoflagellates—especially *Oxyrrhis*, *Scrippsiella*, *Gonyaulax* and *Prorocentrum*—dominated in summer.

Despite broad similarity in the physical-chemical conditions to which the communities of the two lakes were exposed, each exhibited interesting differences. From the standpoint of structure, the similarity of MICRO species between lakes, as measured by the Jaccard index, was relatively low. Furthermore, Margalef's index was significantly higher in Velika than in Mala Solina. This may be related to Velika Solina's more extensive connection with the sea. Variations in important abiotic parameters not measured in this study (e.g. trace elements and dissolved organic matter), can be expected to affect phytoplankton diversity in these lakes.

The dinoflagellate *Oxyrrhis marina* was the most abundant MICRO species in this study and was found only during the hyperhaline period (summer–autumn). At the Kalloni saltworks (NE Aegean Sea, Greece) *Oxyrrhis marina* was an abundant species in summer and autumn along the salinity gradient 47-72 (Evagelopoulos *et al*., 2007, 2009). Additionally, among three ponds of increasing salinity in the Sfax solar saltern (Tunisia), this species was the most abundant in the pond, with a salinity of 78.7±8.8 and temperature of 23.7±7.1 (Abid *et al*., 2008). Presence of this widely distributed species (Schiller, 1933; Tomas, 1996), was noted in other hyperhaline environments in the Mediterranean Sea and Black Sea (Ayadi *et al*., 2004; Pavlova *et al*., 1998; Quintana and Moreno-Amich, 2002). The higher abundance of *O. marina* in Mala Solina in comparison with Velika Solina can be explained by the presence of chroococoid cyanobacteria and small MICRO taxa

(e.g. green algae and small diatoms). These organisms have been shown to be a major prey type of *O. marina* (Goldman *et al*., 1989; Hansen, 1991; Schumann *et al*., 1994). In our study, *Tetraselmis* sp. was present only in Mala Solina and the high abundance of this taxon may be an important source of prey for *O. marina*. In laboratory conditions, Hansen *et al*. (1996) reported grazing on *Tetraselmis* spp. by *O. marina* as they were available at concentrations ≥20 cells ml^{-1} in preference to the smaller algae. Among dinoflagellates, the gymnodinioids, peridinians, and prorocentroids were found in abundance in the lakes. This agrees with the "life-form" concept of eutrophic coastal waters developed by Smayda and Reynolds (2001), according to which small- to intermediate-sized dinoflagellates predominate relatively shallow, nutrient-enriched, nearshore environments.

The most frequent diatom genera were *Amphora* sp., *Navicula* sp., and *Navicula*-like cells. The most abundant species, *Nitzschia longissima*, is commonly found in blooms in eastern Adriatic marine lakes (Jasprica and Carić, 2003; Jasprica *et al*., 2005). *Amphora* and *Navicula* include species with very broad ecological tolerance (Raghukumar and Anil, 2003; Patil and Anil, 2005), but their role could not be evaluated properly in the present study because species-level identification was not always possible. The presence of diatoms commonly found in blooms—*Chaetoceros*, *Nitzschia*, and *Pseudo-nitzschia*—suggests the suitability of the high-nutrient environment of the lakes for such "eutrophic" species (Totti *et al*., 2000). Genera common in other Adriatic marine lakes of similar trophic status—*Leptocylindrus*, *Hemiaulus*, and *Guinardia*—were absent in our lakes. This seems to be more related to the stability/variability pattern than to the trophic status of the shallow ecosystem (Margalef, 1978).

In the present study, the results clearly showed a high contribution of tychoplanktonic taxa from benthos communities (43%), particularly diatoms. Tychoplanktonic taxa were mainly represented by *Amphora*, *Navicula*, *Niztschia*, and naviculoid cells. The same benthic taxa have been found in a shallow Venetian lagoon (Facca and Sfriso, 2007). Schreurs (1992) stressed the features of the lake bottom and the location of the lakes in a windy place as an important factors for appearance of filamentous *Cyanophyceae* (*Oscillatoriales*) in the water column. The importance of wind-induced resuspension of bottom sediments on phytoplankton is well recognized in shallow ecosystems (McQuoid and Godhe, 2004; Bonilla *et al*., 2005), and the shallowness of these lakes certainly makes them susceptible to sediment resuspension. Submerged macrophytes may also have an effect on the phytoplankton taxonomic composition. The leaves of the seagrass (*Cymodocea*) are an available substratum for numerous epiphytic microalgae (*cf.* Borowitzka *et al*., 2006).

Toxic/harmful taxa (e.g. *Alexandrium*, *Dinophysis*), expanding in Mediterranean waters, were not recorded in the lakes (Bravo *et al*., 2008; Ignatiades and Gotsis-

Skretas, 2010). Among toxic species, *Prorocentrum minimum* was occasionally presented only in Velika Solina under widely varying salinity and temperatures. According to Heil *et al*. (2005), this species blooms at high temperatures and low to moderate salinities. In the present study, there were more than one *Pseudonitzschia* species in the diatom assemblage. Along *Pseudo-nitzschia*, some other taxa have not been determined at a species level. A detailed taxonomic investigation must be undertaken for a better understanding ecology of the phytoplankton species in the lakes.

The NANO-dominated phytoplankton consisted mostly of phytoflagellates measuring less than 10 μm, as is generally the case throughout the Adriatic (Saracino and Rubino, 2006). Chroococoid cyanobacteria were the major NANO from May to October, reaching 2.9×10^7 cells L^{-1} in July, the period of N limitation. They sometimes dominate throughout the year but they are typically most abundant in summer (Schumann and Karsten, 2006) when they respond to higher temperatures with a growth rate faster than that of either diatoms or green algae (Andersson *et al*., 1994). The high summer temperatures in the lakes (as high as 27°C) may thus explain this pattern of cyanobacteria dominance. The lakes were eutrophic in summer when temperature and salinity were high and NO_3 and the Redfield ratio (DIN/PO$_4$=2-9) were low. The same conditions were reported for Quinta do Lago, where a long residence time and nitrogen limitation may have favoured the dominance of cyanobacteria (Morais *et al*., 2003). Cyanobacteria may also bloom in late spring and summer when high water temperatures and low DIN/PO$_4$ favour their development (Rocha *et al*., 2002).

Nanophytoplankton and small microphytoplankton (*Oxyrrhis*, *Scrippsiella*, and *Tetraselmis*) with lower half-saturation constants (Eppley *et al*., 1969) and higher surface-to-volume ratios (Morel *et al*., 1991) dominated when salinity was high and N was limiting. Reynolds (2006 and references therein) argues that a high surface-to-volume ratio favours nutrient acquisition and Irwin *et al*. (2006) predicts an increase in the abundance of larger cells with increasing nutrient availability.

Zooplankton grazing cannot be ignored as a factor in the lakes' phytoplankton patterns. Sakka Hlaili *et al*. (2007) reported the highest grazing impact on larger algae during the period of diatom abundance, but as zooplankton were not analyzed in this study, this and related topics must remain a subject for future investigation.

In summary, this study adds to the base of information on the structure and function of phytoplankton in two hyperhaline Mediterranean lakes. Of particular note, dense populations of heterotrophic dinoflagellate *Oxyrrhis marina* and a lack of common coastal diatom species distinguished these systems from those of other marine lakes in the region. The results will prove useful in framing the direction of future research and in establishing the long-term ecological trends of these particular lakes, a subject of increasing importance as the rapid development along the Croatian coast emphasizes the need for rational management of the natural resources, the seasonal tourism industry and the year-around economies of many coastal communities. For this purpose, a complex ecological investigation was started in January 2010.

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