

## Abundance and composition of picoplankton in the mid Adriatic Sea

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*The abundance and structure of the picoplankton community were studied at two stations, one in coastal waters and one in offshore waters, of the middle Adriatic from December 1996 to June 1998. The abundance of prokaryotic Synechococcus cells, eukaryotic autotrophic picoplankton, and heterotrophic nanoflagellates (HNF) was determined, as were the chlorophyll a, physical and chemical factors, and nutrients of the water. Synechococcus dominated the picoplankton abundance by 96%. In offshore waters, the abundance of Synechococcus was influenced by nutrient availability and HNF abundance more than by temperature. In coastal waters, where nutrients were not a limiting factor, temperature had greater influence. Picophytoplankton contributed more to the total phytoplankton biomass in offshore (31%) than in coastal (9%) waters.*

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**Key words:** *Synechococcus*, eukaryotic picoplankton, heterotrophic nanoflagellates, Adriatic Sea

### INTRODUCTION

Picoplankton was originally believed to comprise almost exclusively heterotrophs. However, research in the last two decades has shown that the picoplankton size classification contains a significant amount of photosynthetically-active components such as minute chroococoid cyanobacteria (JOHNSON & SIEBURTH, 1979; WATERBURY *et al.*, 1979) and eukaryotic algae (MURPHY & HAUGEN, 1985; KUOSA, 1988). The photosynthetic picoplankton are a major contributor to the primary production rate of the overall plankton community and to the chlorophyll *a* biomass in the oligotrophic open sea (GOLDMAN

*et al.*, 1979; LI *et al.*, 1983; BERMAN *et al.*, 1984; HERBLAND *et al.*, 1985; NINČEVIĆ & MARASOVIĆ, 1998). It has been suggested that this size class is of less importance in the more eutrophic coastal areas (SØNDERGAARD *et al.*, 1991).

The size of phytoplankton cells is an important ecological variable that determines the length of the food chain and effectiveness of energy transfer to higher trophic levels. In oligotrophic areas where picoplankton are the dominant fraction in the phytoplankton community, the microbial loop is the most important way for circulating matter and energy through the ecosystem (POMEROY, 1974; AZAM *et al.*, 1983).

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All small unicellular cyanobacteria with ovoid to cylindrical cells that reproduce by binary transverse fission in a single plane and lack sheaths belong to the genus *Synechococcus* (RIPPKA *et al.*, 1979). The picoplanktonic marine cyanobacterium *Synechococcus* is found almost everywhere in the upper ocean and seas.

One goal of this paper was to establish whether prokaryotic or eukaryotic autotrophic picoplankton are the most abundant in the coastal and open Adriatic Sea. A second goal was to determine ecological factors that influence the distribution and abundance of *Synechococcus* in the coastal and open Adriatic Sea and how much the picoplankton community contributes to the overall phytoplankton chlorophyll *a* biomass.

## MATERIALS AND METHODS

Samples were taken at two stations in the middle Adriatic: a coastal station (no. 3) and an open station (no. 9; Fig. 1). Station 3 is located in the eastern part of the semi-closed Kaštela Bay with an average depth of 23 m. The

bay communicates with the adjacent channel through an inlet 1.8 km wide and 40 m deep. The Jadro River discharges into the eastern part of the bay, which also receives large quantities of untreated municipal and industrial effluents. Significant changes in the last decade indicate that eutrophication is taking place as a result of direct land influences (PUCHER-PETKOVIĆ & MARASOVIĆ, 1989). Station 9 is located 4 km southeast of an island in the offshore waters of the middle Adriatic. The station is 107 m deep and has a sandy bottom. Because of its distance from land influences, hydrographic parameters oscillate much less than in the coastal waters (BULJAN & ZORE-ARMANDA, 1979). Station 9 is characterized by vertical homogeneity during cold seasons and stratification during warm.

Water samples were taken monthly from December 1996 to June 1998 at depths of 0, 5, 10, and 18 m at station 3 and 0, 5, 10, 20, 30, 50, 75, and 100 m at station 9. Seawater temperature and salinity were recorded *in situ* by a CTD system IDRONAUT OS 316. Sea water density was calculated from temperature and

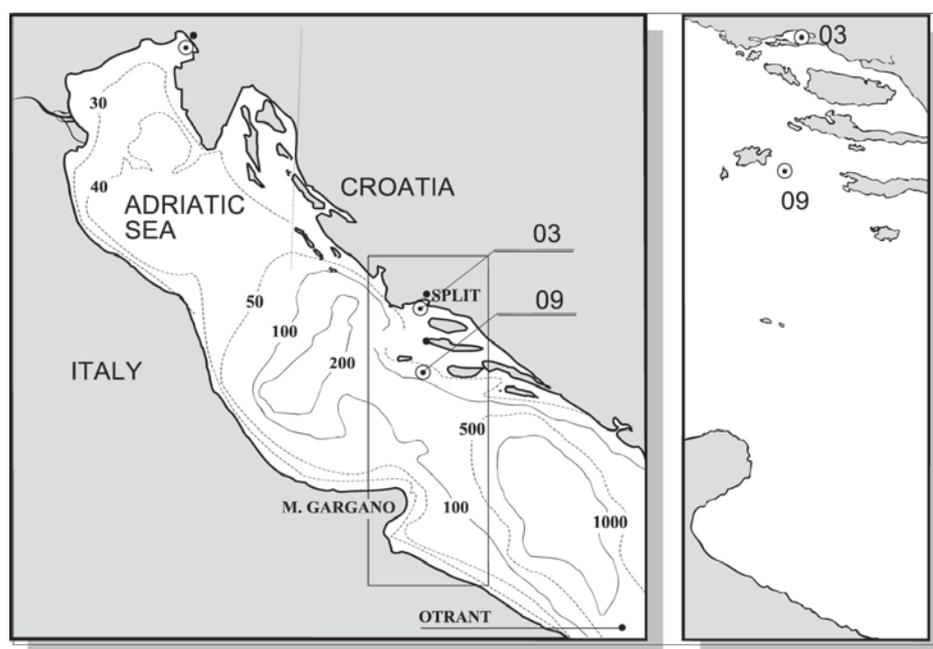


Fig. 1. Study area with sampling stations

salinity data and shown as  $\sigma_t$ , which represents the reduced form of the specific gravity anomaly of sea water (1-specific gravity anomaly). Transparency, was determined with a Secchi disc. Nutrient concentrations were determined immediately after sampling on board the R.V. Bios with a Technicon AutoAnalyzer II system (Bran&Luebbe, Germany) using modified automated methods according to GRASSHOFF (1976). Chlorophyll *a* concentrations were determined fluorometrically from 90% acetone extracts (STRICKLAND & PARSONS, 1972) with a Turner TD 700 laboratory fluorometer.

Seawater samples (15 ml) were filtered through 0.2  $\mu\text{m}$  Nucleopore black filters placed on a slide using oil, covered with cover slips, and examined through an oil immersion lens. Picophytoplankton (<2  $\mu\text{m}$ ) were counted using epifluorescence microscopy (WATERBURY *et al.*, 1979; MAUGERI *et al.*, 1990) under 1000x magnification. The samples were exposed to blue light under which phycoerythrin containing cyanobacteria appeared yellow and chlorophyll *a* containing eukaryotic picoplankton appeared red. The number of heterotrophic nanoflagellates (HNF) was estimated using epifluorescence microscopy and the proflavine staining technique (HAAS, 1982).

## RESULTS

### Physical and chemical water parameters

The temperature at station 3 ranged from 11.4°C in January 1997 to 26.5°C in June 1998 (Fig. 2). Thermal stratification formed in May and lasted until September when the isothermal period began. Station 3 is in a shallow area and the water column heats easily to generate the thermocline observed in July 1997. Inverse thermal stratification was recorded during the winter (December 1996 and January 1997). Low salinity due to river discharge was recorded in December 1996 and January 1997 (31.7 and 30.2, respectively). The highest salinity (38.3) was in December 1997 in the bottom layer. The vertical salinity gradient usually was between the

surface and 5 m. Homogeneous vertical densities occurred in March and September 1997 and in January-March 1998. Density stratification was caused by temperature stratification during warm periods and river discharge in cold. Secchi depth ranged 2-7 m, but mostly remained around 6 m. The highest transparency was in winter while the lowest was in August 1997.

The temperature at station 9 ranged from 13.5°C in March 1997 and 1998 to 24.0°C in August 1997 (Fig. 3). Thermal stratification formed in May and lasted throughout the warm period. The thermocline occurred between 20 and 30 m. Salinity ranged 37.56-38.78. High salinity in September 1997 (>38.55) indicated water advection from the Mediterranean. There was no evidence of halocline persistence. The homogeneous vertical density structure was broken with the heating of the sea water in spring. Due to temperature stratification, the pycnocline occurred at 20-30 m. Secchi ranged 15-28 m and was lowest in spring.

### Nutrients

Inorganic nitrogen (N) concentrations at station 3 ranged 0.22-9.82  $\mu\text{mol l}^{-1}$  (Fig. 4). The highest concentrations were recorded in September 1997 due to the vertical mixing of the water column after the summer phytoplankton bloom and in December 1996 due to river discharge. Nitrate ( $\text{NO}_3^-$ ) dominated the total dissolved inorganic nitrogen when the river influx was strongest (Fig. 5). During the spring, ammonium ( $\text{NH}_4^+$ ) dominated, probably due to zooplankton excretion. Phosphate ranged 0.03-0.41  $\mu\text{mol/l}$  (Fig. 6). The highest concentrations were recorded in August in the samples from 5 and 10 m and in December 1997 in the surface layer. N:P ratios ranged 2-206 with an average of 27, suggesting that this area is primarily phosphorus limited.

Inorganic nitrogen (N) concentrations at station 9 ranged 0.20-6.21  $\mu\text{mol/l}$  (Fig. 7). The highest concentrations were recorded in May 1997 and January 1998 at 75 and 100 m. During the period of stratification, the water column (down to 50 m) was depleted of nitrogen. Nitrate

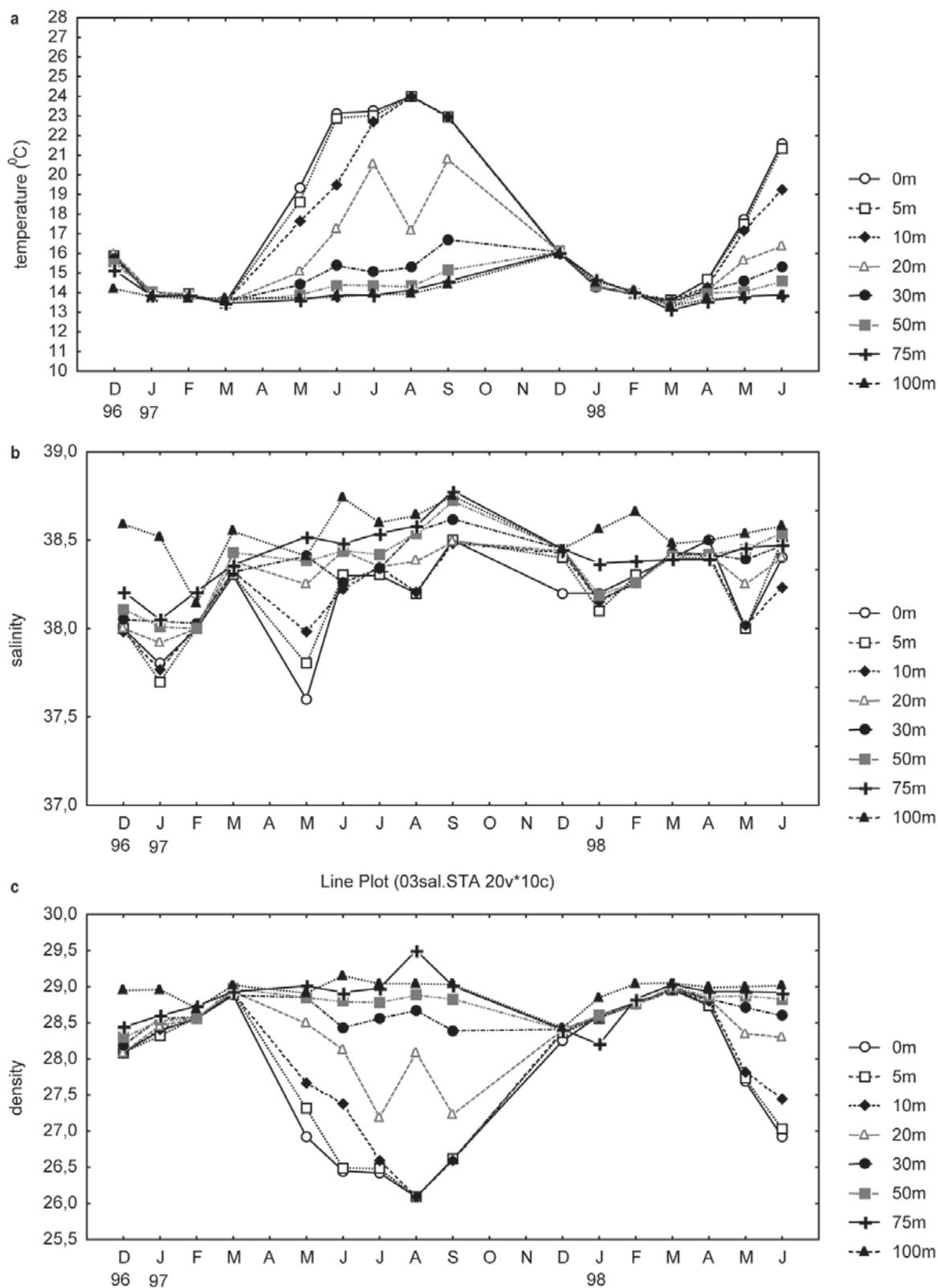


Fig. 2. Temperature (°C), salinity and density ( $\sigma$ ) at station 3

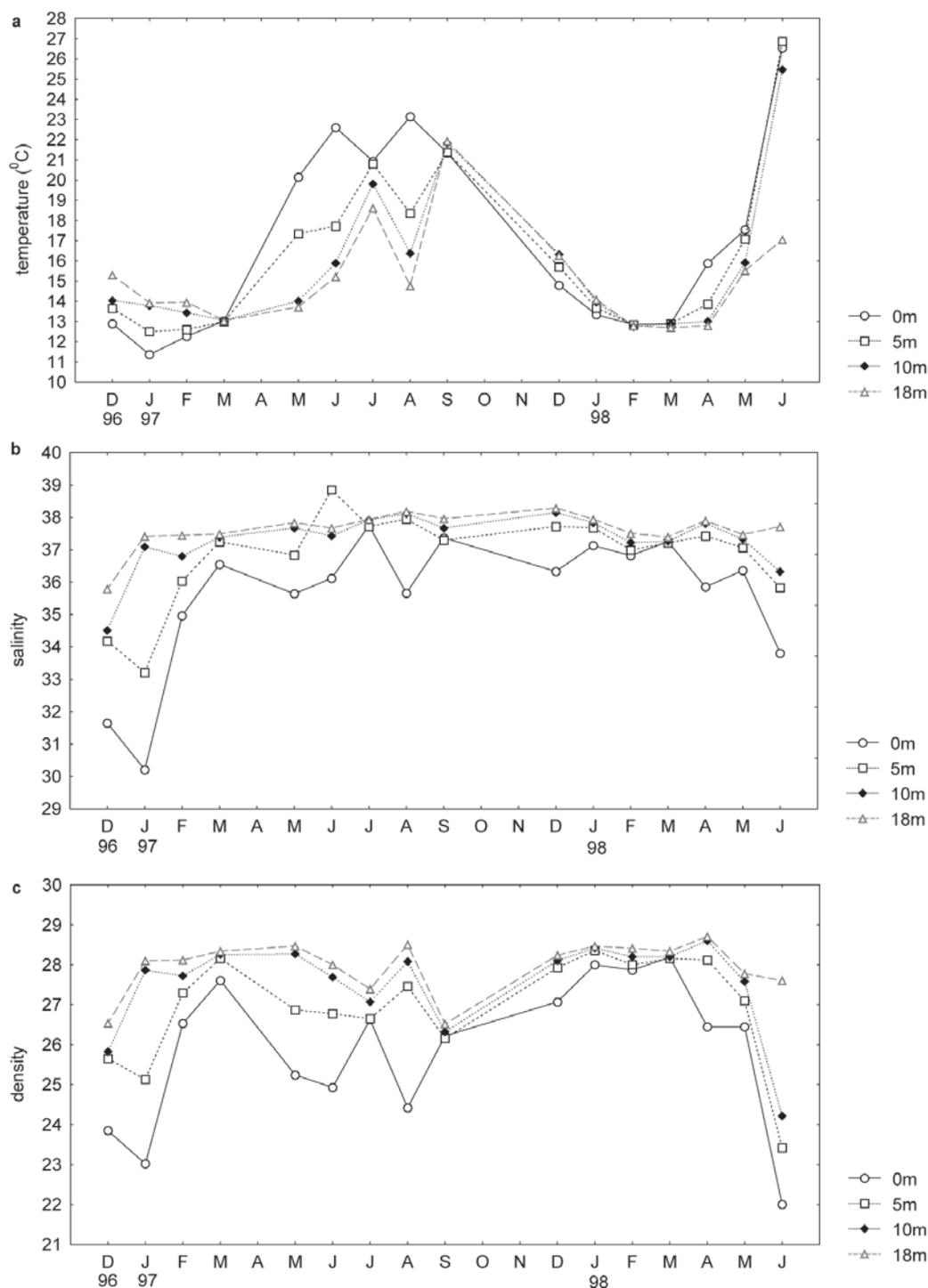


Fig. 3. Temperature (°C), salinity and density ( $\sigma_t$ ) at station 9

was the main source of nitrogen except in spring when ammonium prevailed (Fig. 8). Phosphates ranged 0.080-0.203  $\mu\text{mol l}^{-1}$  with a maximum in May 1998 at 75 m (Fig. 9). In the surface

layer, the highest values were recorded in the winter. N:P ratios ranged 3-234 with an average value of 38, suggesting that this area is mostly phosphorus limited.

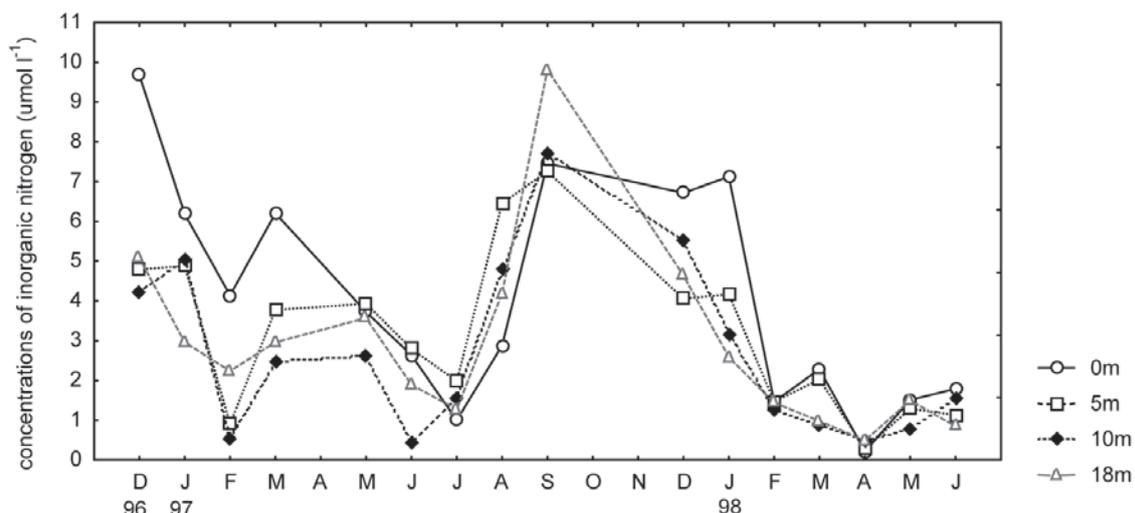


Fig. 4. Inorganic nitrogen concentrations ( $\mu\text{mol/l}$ ) at station 3

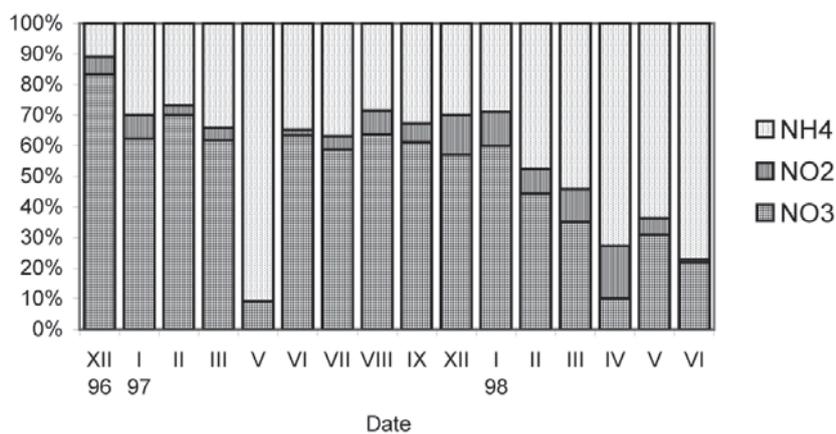


Fig. 5. Proportions of  $\text{NH}_4^+$ ,  $\text{NO}_2^-$ , and  $\text{NO}_3^-$  in the total inorganic nitrogen at station 3

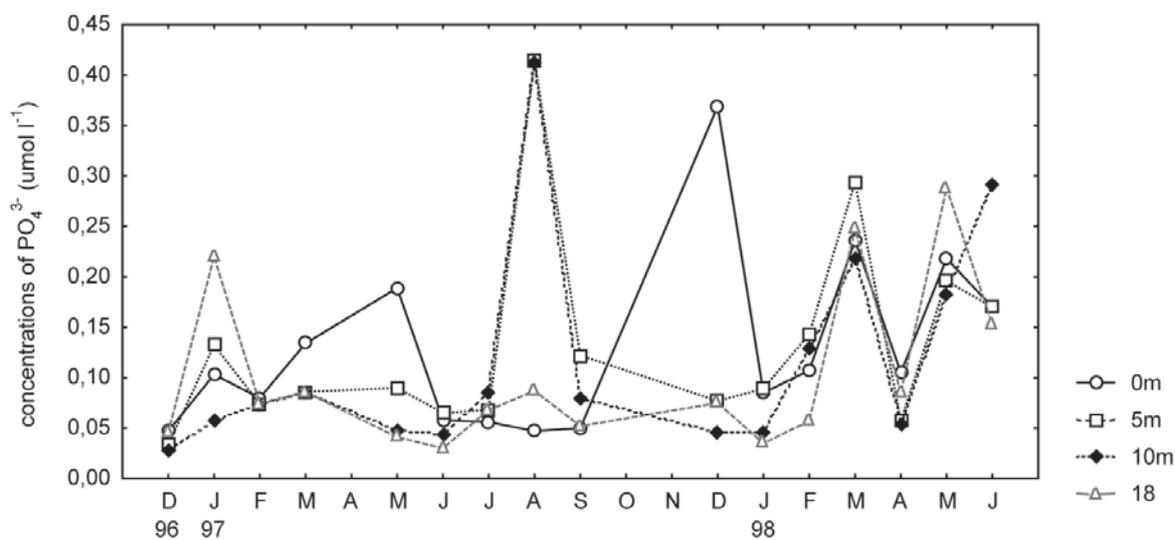


Fig. 6.  $\text{PO}_4^{3-}$  concentrations ( $\mu\text{mol/l}$ ) at station 3

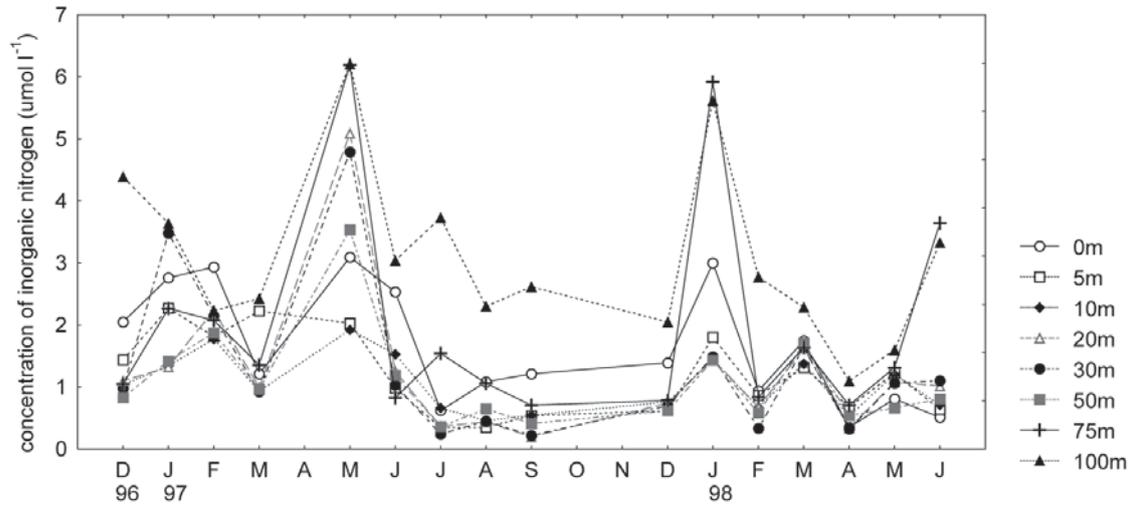


Fig. 7. Inorganic nitrogen concentrations ( $\mu\text{mol/l}$ ) at station 9

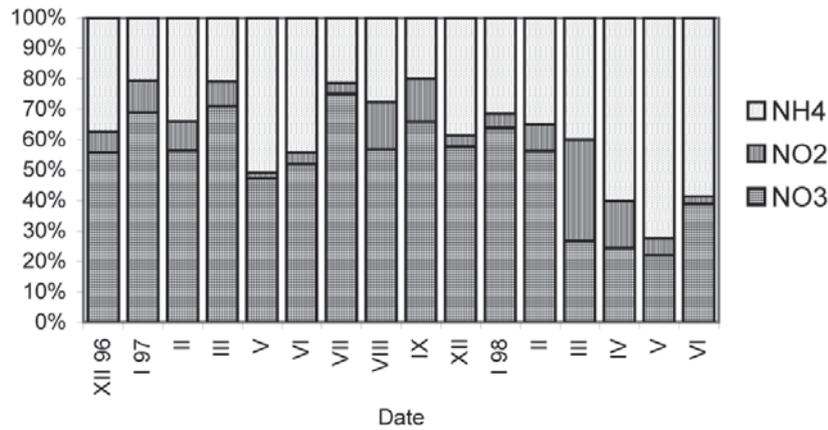


Fig. 8. Proportions of  $\text{NH}_4^+$ ,  $\text{NO}_2^-$ , and  $\text{NO}_3^-$  in the total inorganic nitrogen at station 9

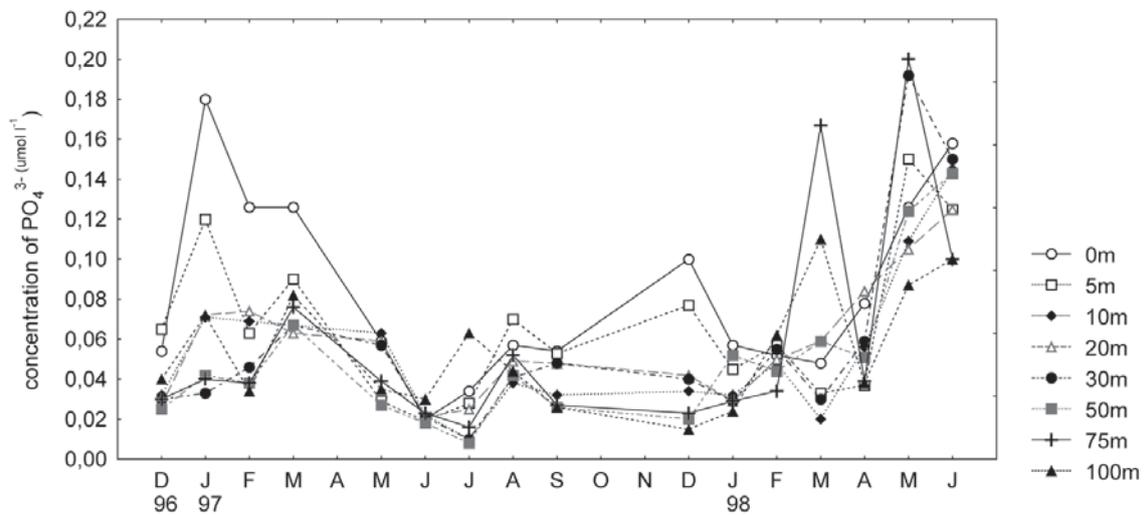


Fig. 9.  $\text{PO}_4^{3-}$  concentrations ( $\mu\text{mol/l}$ ) at station 9

### Abundance of *Synechococcus*

At station 3, *Synechococcus* ranged 1.9-62 x 10<sup>6</sup> cells l<sup>-1</sup> (Fig. 10). Higher abundance was observed in winter and summer, but in winter the higher abundance was in the surface layer while in summer it was in the deeper layers. The correlation between *Synechococcus* and temperature was  $r = 0.45$  and  $p = 0.0003$  ( $n = 60$ ). The abundance of cyanobacteria increased proportionally with the temperature. In the surface layer, there was no correlation between *Synechococcus* and temperature (Table 1) but there was a significant correlation between *Synechococcus* abundance and phosphate concentration ( $r = 0.57$ ,  $p < 0.05$ ,  $n = 15$ ). *Synechococcus* abundance was highest in June 1998 at 10 m when the river inflow was strong (salinity 33.8 at the surface and 36.3 at 10 m) and the phosphate concentration was high (0.29  $\mu\text{mol l}^{-1}$ ). There were no significant differences between periods of vertical mixing and of stratification at station 3.

Table 1. Correlation between cyanobacteria abundance and sea temperature at station 3 ( $n = 15$ )

Depth (m)	$r$	$P$
0	0.10	not significant
5	0.57	<0.05
10	0.89	<0.0001
18	0.68	<0.05

At station 9, *Synechococcus* abundance ranged from non-detectable to 48 x 10<sup>6</sup>l<sup>-1</sup>, with a maximum in May 1998 at 50 m (Fig. 11). The correlation with temperature was negative ( $r = -0.37$ ,  $p < 0.0001$ ,  $n = 111$ ) and the highest *Synechococcus* density occurred at 14-15°C. There were significant differences in abundance ( $p < 0.05$ ) between periods of temperature stratification (20 x 10<sup>6</sup> cells l<sup>-1</sup>) and salinity stratification (11 x 10<sup>6</sup> cells l<sup>-1</sup>). Abundance was significantly higher ( $p < 0.0001$ ) during periods of vertical mixing (15 x 10<sup>6</sup> cells l<sup>-1</sup>) than during stratification (7.1 x 10<sup>6</sup> cells l<sup>-1</sup>). There was a negative correlation between *Synechococcus* abundance and density difference between the surface and bottom layers ( $r = -0.626$ ,  $p < 0.05$ ,  $n = 14$ ).

There were no significant differences between *Synechococcus* abundance at stations 3 and 9 according to the  $t$  test for data collected at the same time (Table 2). The vertical distribution of cyanobacteria at station 3 was uniform with the exception of the bottom layer where it was somewhat lower during mixing periods and somewhat higher during stratification periods. Abundance at station 9 was homogenous throughout the water column during mixing periods and significantly higher ( $p < 0.05$ ) in the layer from 50 to 75 m during temperature stratification periods.

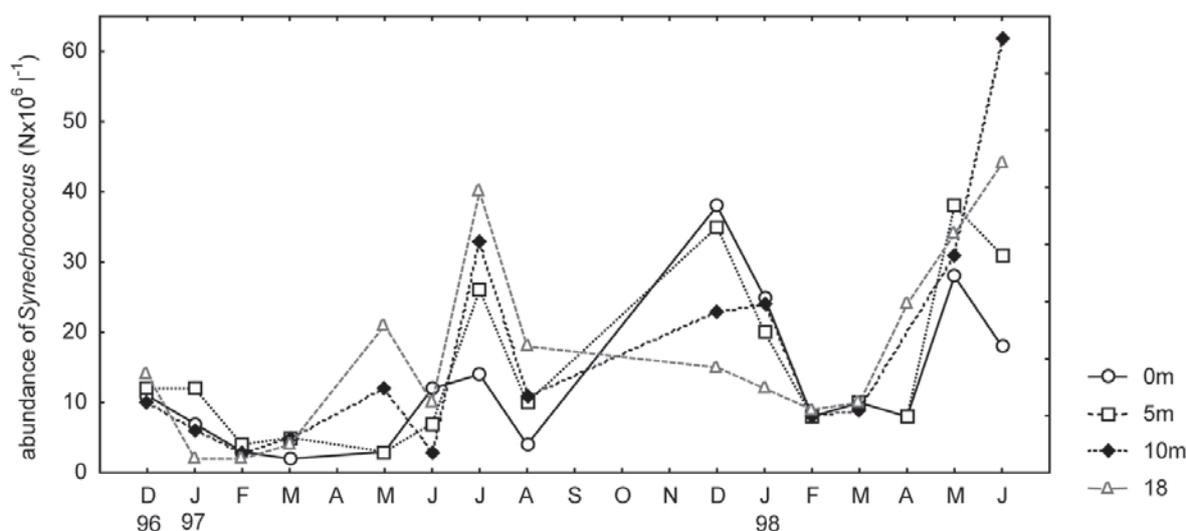


Fig. 10. Abundance of *Synechococcus* (10<sup>6</sup> l<sup>-1</sup>) at station 3

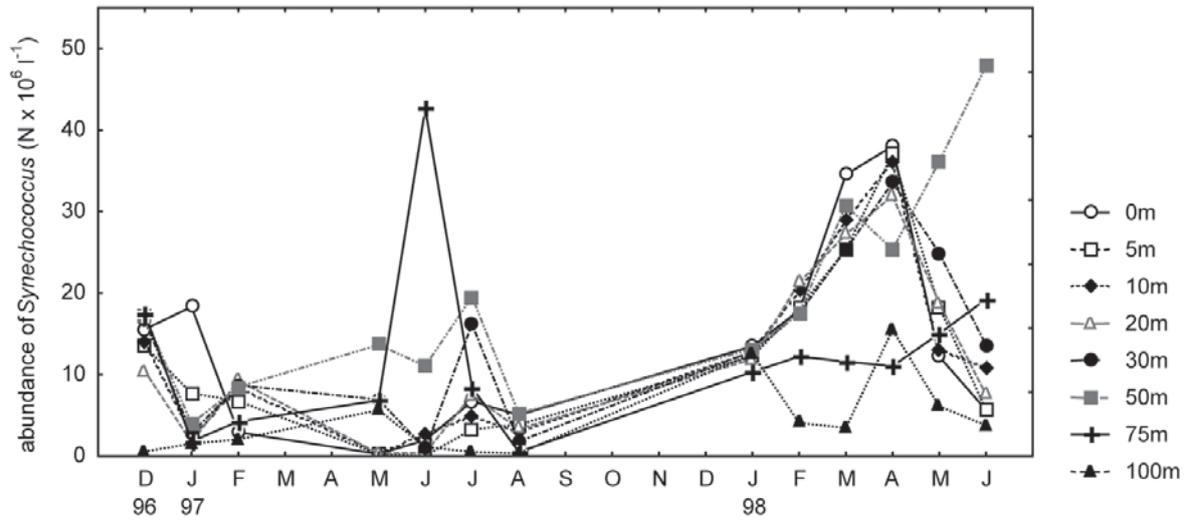


Fig. 11. Abundance of *Synechococcus* ( $10^6 l^{-1}$ ) at station 9

Table 2. Cyanobacteria abundance (cells  $\times 10^6 l^{-1}$ ) during mixing and stratification periods

Depth (m)	Period	
	Mixing	Stratification
Station 3		
0	10	13
5	10	16
10	11	19
18	8.2	20
Station 9		
0	19	3.3
5	17	2.7
10	17	3.6
20	17	3.2
30	18	6.6
50	19	19*
75	10	15*
100	5.7	2.7

\* significantly different from other values in the column ( $p < 0.05$ )

#### Abundance of autotrophic eukaryotic picoplankton

Abundance of eukaryotic picoplankton ranged from non-detectable to  $6.0 \times 10^6 l^{-1}$  (mean  $7.2 \times 10^5 l^{-1}$ ) at station 3. There was no correlation between the abundance of eukaryotic picoplankton and temperature. Eukaryotic

picoplankton abundance did not vary according to season. Abundance was greater in the surface layer during salinity stratification but greater in the bottom layer during temperature stratification. Abundance ranged from non-detectable to  $12 \times 10^6 l^{-1}$  (mean  $0.37 \times 10^6 l^{-1}$ ) at station 9. Also at this station, abundance did not vary according to season. There were no significant differences between the two stations using data obtained at the same time. Eukaryotic picoplankton represented about 4% of the total picoplankton at both stations.

#### Picoplankton contribution to community biomass

At station 3, the mean total chlorophyll *a* biomass was  $2.70 \text{ mg/m}^3$  and picoplankton chlorophyll *a* biomass  $0.24 \text{ mg/m}^3$ , about 9% of the total chlorophyll *a* biomass. At station 9, the mean total chlorophyll *a* biomass was  $0.27 \text{ mg/m}^3$  and the picoplankton chlorophyll *a* biomass was  $0.10 \text{ mg/m}^3$ , about 31% of the total.

#### Heterotrophic nanoflagellates (HNF)

The abundance of HNF ranged  $0.56\text{-}40 \times 10^6 l^{-1}$  (mean  $5.8 \times 10^6 l^{-1}$ ) at station 3 and from non-detectable to  $4.2 \times 10^6 l^{-1}$  (mean  $1.2 \times 10^6 l^{-1}$ ) at station 9.

## DISCUSSION

Many studies confirmed that *Synechococcus* prefer warm water (GLOVER *et al.*, 1985; MURPHY & HAUGEN, 1985; EL HAG & FOGG, 1986; WATERBURY *et al.*, 1986; JOCHEM, 1988; ODATE, 1989; IRIARTE & PURDIE, 1994; VANUCCI *et al.*, 1994; CHANG *et al.*, 1996). We obtained conflicting correlations between *Synechococcus* abundance and seawater temperature; the correlation was positive at station 3 and negative at station 9. The influence of temperature on the seasonal distribution of *Synechococcus* is unclear, suggesting that other factors influence their distribution. According to LI (1998), *Synechococcus* abundance increases with temperature until 14°C while there is no correlation above 14°C. We obtained a similar result at station 3 where the abundance of *Synechococcus* increased with temperature until around 17°C, after which we found no significant influence of temperature on *Synechococcus* abundance. At station 9 the highest abundance of *Synechococcus* was at 14-15°C and other factors, such as nutrients, light intensity, and reduced predation, had more influence than temperature.

Although there was no correlation between *Synechococcus* abundance and temperature in the surface layer at station 3, there was between *Synechococcus* abundance and phosphate concentration ( $r = 0.57$ ,  $p < 0.05$ ;  $n = 15$ ). The highest *Synechococcus* concentration at

station 3 coincided with the highest phosphate concentration, suggesting that phosphate limits *Synechococcus* abundance in coastal Adriatic waters.

Besides low nutrient concentrations, HNF abundance may limit *Synechococcus* abundance during warm periods (Fig. 12). HNF are the most important bacteria predators and, thereby, control bacteria abundance (SOROKIN, 1977; ANDERSON & FENCHEL, 1985; GALVAO, 1990). According to GOLDMAN (1988), THINGSTAD & SAKSHAUG (1990), and SØNDERGAARD *et al.* (1991), picophytoplankton are controlled by heterotrophic predation more than microphytoplankton.

According to EL HAG & FOGG (1986), the highest *Synechococcus* concentrations occur suddenly, suggesting that although temperature may exert an influence, it is unlikely that a direct effect of temperature on the physiology of *Synechococcus* is the only factor that determines variations in its distribution and abundance. Contrary to authors who established a positive relationship between *Synechococcus* abundance and temperature, some authors reported high levels of *Synechococcus* in low seawater temperature, e.g., in the Arctic below 0°C (SMITH *et al.*, 1985) and in Boothbay Harbor (Maine, USA) during winter.

Apart from the positive relationship with temperature, maximum concentrations of *Synechococcus* during warm periods can be

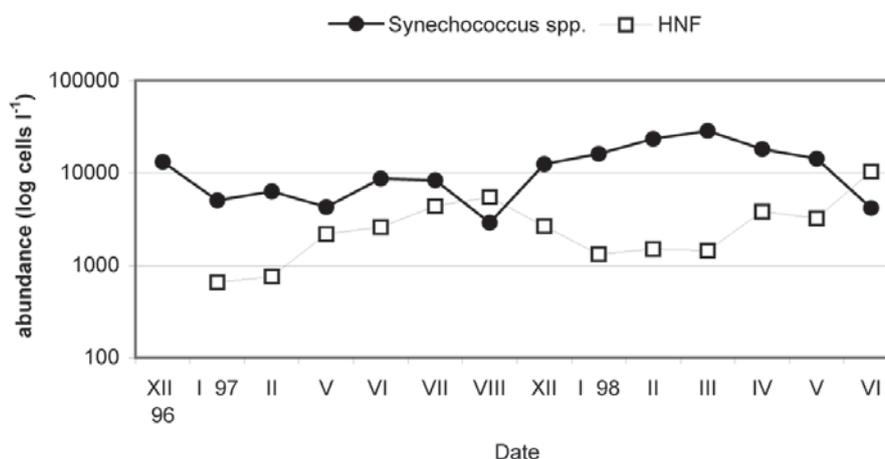


Fig. 12. Log average abundance of *Synechococcus* and HNF at station 9

explained by an imbalance between growth rate and predation rate. Temperature enhancement of growth rates in picocyanobacteria can outpace nanoheterotrophic grazing rates and/or increased food concentration for nanoflagellates (i.e., increased bacterial concentration in summer), resulting in reduced grazing pressure on picocyanobacteria. Further, nanoflagellates themselves are probably under greater grazing pressure by microzooplankton during summer (McMANUS & FUHRMAN, 1990).

The average abundance of *Synechococcus* spp. did not significantly differ between coastal (station 3) and open sea (station 9) waters. This result shows that *Synechococcus* abundance is not a good parameter for determining the eutrophication status of an area. Eukaryotic picoplankton is a much more heterogeneous group than prokaryotes and it includes many species of *Chlorophyceae*, *Prasinophyceae*, and *Chrysophyceae* (STOCKNER & ANTIA, 1986). Their concentration in seawater is often about  $10^6$  cells  $l^{-1}$  and generally an order of magnitude less than the picocyanobacterial abundance (MURPHY & HAUGEN, 1985). In coastal waters and estuaries they can generate blooms, reaching  $10^9$  cells  $l^{-1}$  (WILHELM *et al.*, 1982; HARGRAVES *et al.*, 1989). Such blooms can occur in oceanic waters (LI & WOOD, 1988) but cannot be sustained for long. We recorded low abundance of eukaryotic picoplankton in coastal ( $0.72 \times 10^6$   $l^{-1}$ ) and offshore ( $0.37 \times 10^6$   $l^{-1}$ ) Adriatic waters with a maximum abundance in winter and spring. The few data on the seasonal distribution of eukaryotic picoplankton indicate that abundance is maximum during summer in Southampton (UK) waters (IRIARTE & PURDIE, 1994) and Funaka Bay (Japan) waters (ODATE, 1989) while no seasonal pattern was recorded in the Baltic Sea (KUOSA, 1991).

In both offshore and coastal mid Adriatic waters, eukaryotic picoplankton contributed only 4% to the total picoplankton abundance while prokaryotic *Synechococcus* contributed 96%. Eukaryotic picoplankton also contributes much less to the total picoplankton abundance than picocyanobacteria (which contribute over 90%) in coastal waters of the northern Adriatic (VANUCCI *et al.*, 1994). TAKAHASHI *et al.* (1985) emphasized the dominance of prokaryotic picocyanobacteria in the total picoplankton biomass. On the other hand, the importance of eukaryotic picoplankton was emphasized in the upwelling area of New Zealand, with an increase in the ratio of prokaryotic to eukaryotic picoplankton cells corresponding to the distance from land (HALL & VINCENT, 1990).

The contribution of picoplankton to the total phytoplankton chlorophyll *a* biomass was higher in offshore than in coastal waters, but not as high as in the oligotrophic waters of Hawaii (TAKAHASHI & BIENGANG, 1983) or in the Gulf of Maine and the Mediterranean Sea (STOCKNER & ANTIA, 1986) where picoplankton contribute 60–90% of total phytoplankton biomass. The ratio of picoplankton chlorophyll *a* to net phytoplankton chlorophyll *a* can be used as an indicator of oligotrophic conditions (GOMES *et al.*, 1992).

In conclusion, the abundance of *Synechococcus* in the Adriatic Sea is influenced more by nutrient availability and HNF abundance than temperature. Seasonal distribution in offshore waters is strongly related to stability of the water column. There is a significantly higher abundance during mixing periods than during stratification when most of the *Synechococcus* population is located in deeper layers with higher nutrient concentrations. In coastal waters, phosphate limited the development of *Synechococcus*.

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## Brojnost i sastav pikoplanktonske zajednice u srednjem Jadranu

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### SAŽETAK

Brojnost i struktura pikoplanktonske zajednice istraživana je na dvjema postajama smještenim u obalnim i otvorenim vodama srednjeg Jadrana u razdoblju od prosinca 1996 do lipnja 1998. Istraživanjem je obuhvaćena brojnost cijanobakterija *Synechococcus*, eukariotskog pikoplanktona i heterotrofnih nanoflagelata (HNF), koncentracija klorofila *a* kao i fizikalno kemijski parametri (slanost, temperatura, hranjive soli). Rezultati istraživanja pokazali su da u pikofitoplanktonskoj zajednici dominiraju cijanobakterije *Synechococcus* (96%). Brojnost cijanobakterija u otvorenim vodama najvećim je dijelom uvjetovana raspoloživom koncentracijom hranjivih soli kao i veličinom populacije HNF. Temperatura ima veći utjecaj na brojnost cijanobakterija u obalnim vodama gdje hranjive soli nisu ograničavajući factor. Pikofitoplankton znatno više dobrinosi ukupnoj fitoplanktonskoj biomasi u otvorenim vodama (31%) nego u obalnim vodama (9%).

**Ključne riječi:** Jadransko more, *Synechococcus*, eukariotski pikoplankton, heterotrofni nanoflagelati