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Dynamics of the picoplankton community from coastal waters to the open sea in the Central Adriatic

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Abstract

Flow cytometry was used to describe seasonal cycles of *Prochlorococcus* (Prochl), *Synechococcus* (Syn), picoeukaryotes and heterotrophic bacteria in the central Adriatic Sea along the trophic gradient from January to December 2010. All picoplankton parameters decreased from eutrophic to oligotrophic areas, while the biomass ratio of bacterial to autotrophic picoplankton showed an increase along the trophic gradient. Bacterial biomass ranged from 5.28 to 21.20 µg C l^{-1}. Increased values were present during warmer seasons with the domination of the low nucleic acid (LNA) group of bacteria. The high nucleic acid (HNA) bacterial group dominated during winter and spring. Bacterial production ranged from 0.09 -0.45 × 10^4 cells ml^{-1} h^{-1} . At coastal stations, increased production was present during the winter and spring and was more or less uniform at open sea stations. The biomass of Syn and Prochl ranged from 0.16 to 11.47 µg C^{-1} l^{-1} and from 0.01 to 3.08 µg C l^{-1}, respectively. They were elevated during the summer and the autumn at coastal stations and during late winter at the open sea. Syn biomass always dominated over Prochl, with 61.6-97.2% participation in the biomass of cyanobacteria. The biomass of picoeukaryotes ranged from 1.21 to 21.85 µg C l^{-1} and was the highest during the winter. Their biomass notably prevailed in autotrophic picoplankton (APP) biomass over that of picocyanobacteria during the whole year. Autotrophic components (Prochl, Syn and picoeukaryotes) made a greater contribution to picoplankton biomass in mesotrophic and eutrophic areas, while heterotrophic bacteria became more important under oligotrophic conditions.

Keywords: *Prochlorococcus, Synechococcus,* picoeukaryotes, picophytoplankton, HNA bacteria, LNA bacteria, Adriatic Sea.

Introduction

Prochlorococcus (Prochl), *Synechococcus* (Syn), picoeukaryotes and heterotrophic bacteria represent the smallest size-class of picoplankton (cells 0.2–2 µm). Their importance as major contributors of biomass and primary production makes them an essential component for understanding the food web dynamics and carbon cycle in marine systems (Li, 1994; Partensky *et al*., 1996; Grob *et al*., 2007). The autotrophic component of the picoplankton community includes cyanobacteria of the genera *Synechococcus* and *Prochlorococcus* and small eukaryotic cells of diverse taxa, picoeukaryotes. These tiny primary producers tend to dominate the photosynthetic biomass and primary production in oligotrophic waters like the Mediterranean Sea (Li, 1998; Zubkov *et al*., 2000; Li & Harrison, 2001). The eukaryotic component of picoplankton, picoeukaryotes, can contribute significantly to biomass and productivity in a wide variety of aquatic environments, even when present at lower abundances than cyanobacteria. This is due to their bigger size and higher intracellular chlorophyll *a* (Chl *a*) and carbon content than of cyanobacteria. Picoeukaryotes are consumed by grazers, thus forming a link to higher trophic levels, which has variety of implications for the fate of their fixed carbon (Li, 1994; Partensky *et al*., 1996; Blanchot *et al*., 2001).

The heterotrophic component of the picoplankton community, heterotrophic bacteria, contributes to a larger percentage to total plankton biomass, acting not only as decomposers of organic matter but also as important producers of new biomass. Heterotrophic bacteria often consume 10-50% of total primary production (Stockner, 1988; Fuhrman, 1992) and through grazing by flagellates and ciliates their biomass becomes available at higher trophic levels. Therefore, heterotrophic bacteria, as a part of the picoplankton community, undoubtedly play an important role in carbon flow through marine system.

Extensive literature is available concerning picoplankton community distribution and dynamics in the Adriatic Sea (Aubry *et al*., 2006; Paoli *et al*., 2007; Pugnetti *et al*., 2008; Šolić *et al*., 2008; Vilibić & Šantić,

2008; Šantić *et al*., 2011, 2012a, b). However, information about picoeukaryotes is extremely scarce and only one study has been published to date concerning their distribution in the central Adriatic (Ninčević Gladan *et al*., 2006). Papers about picoeukaryotes in the Adriatic Sea have shown the importance of local patterns (Radić *et al*., 2009; Viličić *et al*., 2009; Šilović *et al*., 2011) and their seasonality has been described by Ninčević Gladan *et al*. (2006). However, this study is the first to describe seasonal cycles of Syn, Prochl, picoeukaryotes and heterotrophic bacteria simultaneously in the central Adriatic Sea, along the trophic gradient. Moreover, information about the picoeukaryotic community in the central Adriatic Sea based on flow cytometry is reported for the first time. The aim of this study is to describe the population dynamics of major picoplankton groups (heterotrophic bacteria, Prochl, Syn and picoeukaryotes) and identify the factors responsible for the observed distributions. Our results highlight the importance of picoeukaryotes in these waters.

Materials and Methods

Study area

The Adriatic Sea is the northernmost basin in the Mediterranean, 800 km long and 200–250 km wide. Bathymetry divides the basin into three parts; a broad northern Adriatic shelf with an average depth of 40 m, and the central Adriatic with depressions as deep as 280 m, connected to the southern Adriatic circular basin over the Palagruža Sill. The coastal area investigated is located in the central Adriatic basin (Fig. 1) covering the coastal zone partly under the influence of the karstic river Jadro. In the open sea, samples were collected from station CA009, located near the island of Vis, and from the open sea (station CA011).

Sampling

Samples were taken at monthly intervals from January 2010 to December 2010 on board the RV Bios using Niskin bottles (5 l), along the transect in the central Adriatic. The transect followed the trophic gradient, with decreasing influence of the Jadro river toward the open sea. Samples were collected at several depths between the surface and the bottom (5 m to 10 m intervals for the upper 50 m, and 75 m and 100 m). At station CA011 no sampling was carried out during autumn.

Environmental variables

A SeaBird 25 CTD profiler recorded temperature and salinity data. Nutrient concentrations (NO_3^-, NO_2^-, NH_4^+, total dissolved inorganic nitrate (TIN) and soluble reactive phosphate (SRP)) were determined using the modified auto analyser method by Grasshof (1976).

Chl *a* concentrations were determined with the fluorometric method according to Strickland & Parsons (1972). Samples were filtered through a glass microfiber filter (Whatman GF/F) and then frozen until analysis. Chl *a* was extracted in 90% acetone and fluorescence was measured using a TURNER TD-700 fluorometer.

Flow cytometry analysis of the picoplankton community

Abundance of Syn, Prochl, picoeukaryotes and heterotrophic bacteria was determined using flow cytometry (Marie *et al*., 1997). For autotrophic cell counts, 2 ml samples were preserved in 0.5% glutaraldehyde, frozen at -80 °C and stored until analysis (5–10 days). Samples for heterotrophic bacteria were preserved in 2% formaldehyde and stored at 4 °C until analysis (5–10 days). 1 ml samples were stained with SybrGreen I and analysed on the Beckman Coulter EPICS XL-MCL (high flow rate from 1 to 1.2 $\mu l\ s^{-1}$). To standardise fluorescence intensity of cells, 1µm yellow-green beads were added (Level-III Epics Division of Coulter Corporation Hialeah, Florida). Two groups of bacteria were distinguished according to their relative green fluorescence as a proxy for nucleic acid content (Jochem, 2001), referred to as the high nucleic acid (HNA) and the low nucleic acid bacteria (LNA), and light scattering.

Autotrophic cells were separated into two groups, cyanobacteria (Syn and Prochl) and picoeukaryotes, which were distinguished according to light scattering (red emis-

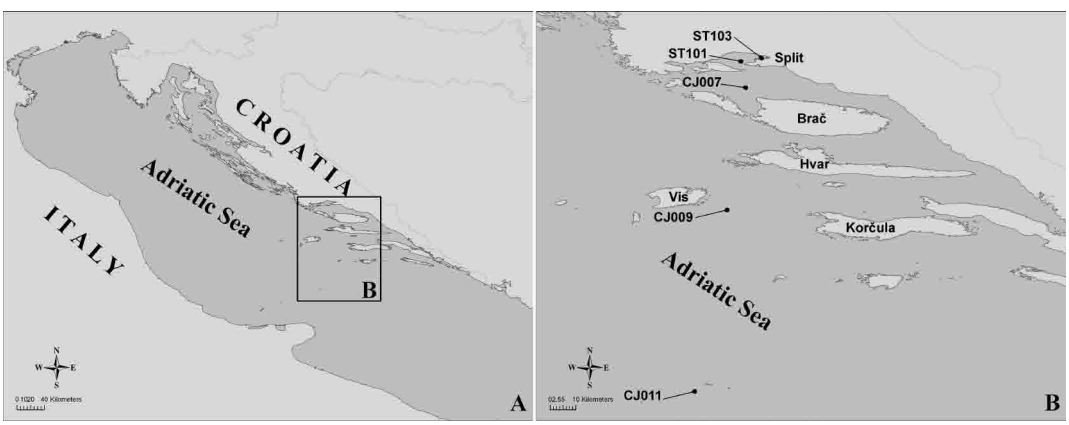

Fig. 1: Study area with sampling stations.

sion of cellular chlorophyll content and orange emission of phycoerythrin-rich cells). The biomass of Syn, Prochl, picoeukaryotes and heterotrophic bacteria was calculated using the following volume-to-carbon conversion factors: 255 fg C cell^{-1} for Syn (Buitenhuis *et al*., 2012), 36 fg C cell^{-1} for Prochl (Buitenhuis *et al*., 2012), 2590 fg C cell^{-1} for picoeukaryotes (Buitenhuis *et al*., 2012) and 20 fg C cell^{-1} for heterotrophic bacteria (Lee & Fuhrman, 1987).

Bacterial production

Bacterial production was measured from DNA synthesis, based on incorporation rates of ^{3}H-thymidine (Fuhrman & Azam, 1982). Conversion factors (CF) for bacterial production were calculated from bacterial cell numbers and ^{3}H-thymidine incorporation during bacterial growth in 1 µm pre-filtered seawater (Riemann *et al*., 1987): CF = (N_2-N_1) / ^{3}H; where N_1 and N_2 are the numbers of bacteria at the beginning and the end of the experiment and ^{3}H is the integrated ^{3}H-thymidine incorporation rate during the experiment.

Heterotrophic nanoflagellates

The number of heterotrophic nanoflagellates (HNF) was estimated using epifluorescence microscopy. Samples were stained with 4'-6-diamidino-2-phenylindole (DAPI) for 10 minutes and filtered through 0.8 µm black polycarbonate filters (Milipore, Ireland). Microscope slides were examined under UV light at a magnification of 1,000X (Porter & Feig, 1980).

Results

Environmental parameters

Mean seasonal values of temperature, salinity and nutrients are presented in Table 1.

A general trend of sharp Chl *a* decrease from the inshore station towards the open sea was noted. Chl *a* concentrations ranged from 0.29-2.99 mg m^{-3} (average 1.35 mg m^{-3}) at coastal stations ST103 and ST101, to 0.01-0.76 mg m^{-3} (average 0.25 mg m^{-3}) at open sea stations CA007, CA009 and CA011. The seasonal pattern of Chl *a* distribution was similar for all stations and was characterized by higher values in March, April and December and lower values during the summer. At coastal stations ST103 and ST101, the winter Chl *a* increase was recorded only in the surface layer (from 0-5m). At open sea stations, the winter Chl *a* increase was noted from the surface to the bottom (0-100m).

Autotrophic picoplankton

Autotrophic picoplankton (APP) biomass decreased from the coast towards the open sea, following the same pattern as that observed for Chl *a*. However, the contribution of APP biomass to total phytoplankton biomass did

Table 1. Mean values and standard deviations (±) of environmental parameters (temperature (°C), salinity (S), nitrate (NO_3^-), nitrite (NO_2^-), ammonium ion (NH_4^+), total dissolved inorganic nitrate (TIN) and soluble reactive phosphate (SRP)) during different seasons.

	T (°C)	S	NO_3^- (µM)	NO_2^- (µM)	NH_4^+ (µM)	TIN (µM)	SRP (µM)
Winter							
ST103	12.87±0.83	34.5±2.08	2.75±1.69	0.28±0.18	0.65±0.35	3.68±1.59	0.04±0.02
ST104	12.61±0.88	35.32±2.80	2.16±1.11	0.28±0.17	0.80±0.50	3.24±1.14	0.05±0.03
CA007	12.97±0.49	36.34±1.69	1.28±0.61	0.21±0.10	0.60±0.34	2.09±0.80	0.06±0.06
CA009	13.51±0.33	37.93±0.45	0.77±0.86	0.15±0.09	0.34±0.23	1.26±0.83	0.04±0.03
CA011	13.72±0.13	38.27±0.10	1.05±0.63	0.17±0.11	0.58±0.33	1.79±0.53	0.06±0.04
Spring							
ST103	21.22±4.35	34.30±2.89	0.78±0.01	0.14±0.04	1.01±0.05	1.93±0.08	0.13±0.04
ST104	19.11±4.57	35.63±2.68	0.68±0.70	0.15±0.18	0.64±0.18	1.,48±1.20	0.08±0.01
CA007	18.14±3.71	36.71±1.51	0.61±1.01	0.06±0.12	0.67±0.30	1.34±1.29	0.03±0.01
CA009	17.94±352	38.19±0.33	1.02±1.36	0.03±0.02	0.53±0.38	1.59±1.25	0.05±0.01
CA011	17.92±3.90	38.11±0.45	0.61±0.55	0.01±0.02	0.39±0.22	1.02±0.77	0.02±0.01
Summer							
ST103	19.04±4.59	34.40±8.28	1.42±1.82	0.11±0.04	1.47±1.29	3.00±2.98	0.07±0.04
ST104	19.04±2.72	37.29±0.67	1.08±1.44	0.09±0.06	1.90±2.69	3.00±3.98	0.07±0.03
CA007	18.60±3.05	37.72±0.77	0.43±0.65	0.11±0.11	0.,73±0.53	1.27±0.86	0.07±0.03
CA009	18.54±3.45	38.31±0.30	0.58±0.63	0.08±0.06	0.62±0.42	1.29±0.77	0.05±0.03
CA011	19.53±4.62	38.25±0.26	0.48±0.62	0.05±0.02	0.34±0.17	0.86±0.74	0.08±0.04
Autumn							
ST103	17.42±0.43	34.88±1.32	1.84±0.94	0.16±0.05	1.06±0.26	3.05±0.85	0.06±0.02
ST104	17.44±0.68	35.38±1.78	2.08±0.94	0.33±0.50	1.54±0.73	3.95±0.73	0.05±0.01
CA007	17.95±0.44	37.59±0.37	0.60±0.13	0.20±0.13	0.64±0.29	1.44±0.38	0.06±0.01
CA009	18.27±1.14	38.07±0.25	0.31±0.36	0.10±0.03	0.29±0.08	6.63±0.13	0.06±0.02

not show any trend along the trophic gradient, being the highest at ST103 and the lowest at station ST101 nearby. We observed a similar seasonal trend of APP and Chl *a* for open sea stations CA009 and CA011, characterized by highest values in February, March and April and the lowest in June, July and August. At station CA009, both Syn and Prochl followed the distribution of Chl *a* while at station CA011 only picoeukaryotes showed a seasonal distribution similar to that of Chl *a*.

Average monthly values of Syn and Prochl biomass ranged from 0.16 to 11.47 µg C l^{-1} and from 0.01 to 3.08 µg C l^{-1}, respectively. The highest biomass of both cyanobacteria was recorded at station ST101 (24.83 µg C l^{-1} for Syn and 5.97 µg C l^{-1} for Prochl). Syn biomass dominated over Prochl during the whole year, with 61.6-97.2% participation in the biomass of cyanobacteria. There was no seasonality in Syn domination at either of the stations but we observed a decrease in Syn contribution to cyanobacterial biomass towards the open sea. At the open sea station CA011, Syn biomass levels were much lower (0.25-0.64 µg C l^{-1}). The lowest Syn biomass was present during spring. Prochl also showed the strong gradient of biomass decrease from the coast to the open sea with the exception of station ST101. Its seasonal distribution generally followed the distribution of Syn with peaks in the summer and the autumn at stations ST103 and ST101 and during the late winter at the stations further from the coast (CA007, CA009 and CA011).

The biomass of picoeukaryotes ranged from 1.21 to 21.85 µg C l^{-1} with a clear trend of biomass decrease towards the open sea. A statistically significant difference in picoeukaryotic biomass between seasons was noted (ANOVA, F = 4.798: P < 0.007), with the highest biomass always present during the winter.

Differences in the composition of the APP community between stations were clearly evident (ANOVA, F=5.045, p=0.0006 for Syn; F=5.732, p=0.0036 for Prochl and F=9.735; p=0.002 for picoeukaryotes). Picoeukaryotes notably prevailed over picocyanobacteria in APP especially during the winter and spring, when they accounted for 59-93% of APP biomass. Cyanobacteria dominated in APP only at station ST101 during the summer and the autumn when they made up between 65% and 72% of APP biomass.

Heterotrophic picoplankton

The average biomass of heterotrophic bacteria, integrated from the surface to the bottom layer ranged from 5.28 to 21.20 µg C l^{-1}. The seasonal distribution showed strong variability and these differences were statistically significant (ANOVA, F = 4.971; p < 0.0005). Higher biomass (9.27±3.41 µg C l^{-1}) was present during the summer at all stations except for coastal station ST103. At station ST103, the maximum value of 21.21±6.27 µg C l^{-1} was observed during the spring. The lowest bacterial biomass occurred in the late winter at stations closer to the coast (ST103, ST101 and CA007) and in the late summer at open sea stations (CA009 and CA011).

There was the strong gradient of biomass decrease from the coast towards the open sea (Fig. 2). During all seasons, highest biomass was present at station ST103 (16.02±3.82 µg C l^{-1}). At the open sea station CA011, val-

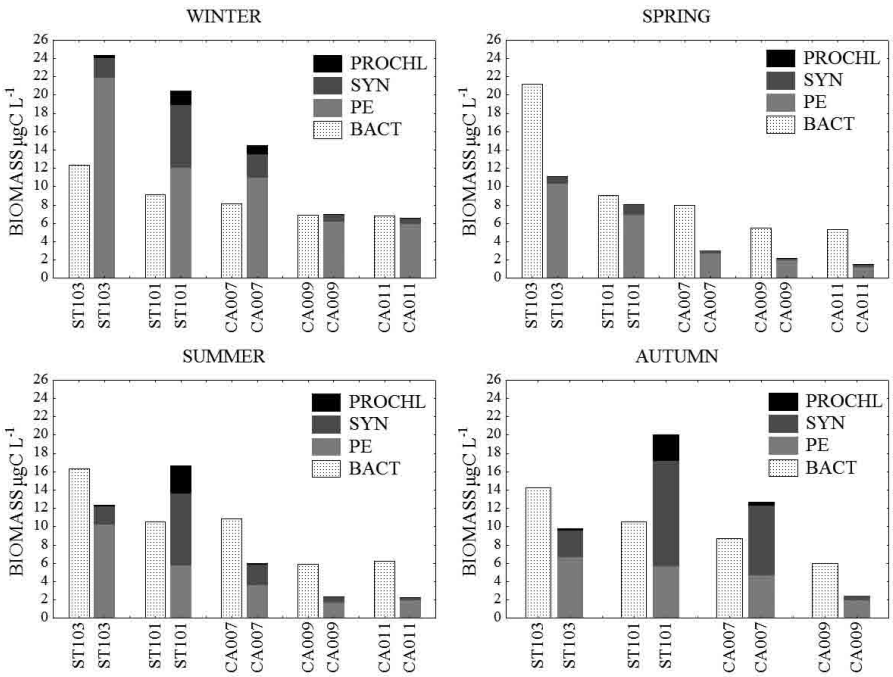

Fig. 2: Biomass of different groups of picoplankton along the trophic gradient during four different seasons: total heterotrophic bacteria (BACT) picoeukaryotes (PE); *Synechococcus* (SYN) and *Prochlorococcus* (PROCHL).

ues were much lower (6.13±0.78 µg C l^{-1}). The differences in terms of bacterial biomass between stations were statistically significant (ANOVA, F = 25.34; $p < 0.0001$).

The average percentage of HNA bacteria ranged from 34.03 to 69.68%. Different temporal patterns were found for the HNA and also, therefore, for the LNA bacterial group. The seasonal distribution showed a prevalence of the HNA group during the winter and the spring season and dominance of LNA bacteria during the summer and the autumn (Table 2). Differences in proportions of HNA and LNA groups between seasons were statistically significant (ANOVA, F = 98.01; $p < 0.0001$). Highest proportions of HNA were found at station ST103, with decreasing values towards the open sea as shown in Table 2. Differences in the percentage of HNA bacteria at investigated stations were also statistically significant (ANOVA, F = 55.90; $p < 0.0001$).

Bacterial production, such as Chl *a* and bacterial biomass abundance, was generally the highest at the station nearest to the coast (ST103) decreasing towards the open sea. Average values of bacterial production during different seasons ranged from 0.09×10^4 cells ml^{-1} h^{-1} at CA011 to 0.45×10^4 cells ml^{-1} h^{-1} at ST103 (Table 2). At coastal stations ST103 and ST101, increased production was noted during the winter and the spring, while at stations distant from the coast values were more or less uniform during the whole year. Differences in bacterial production between stations were statistically significant (ANOVA, F = 14.26; $p < 0.0001$).

Factors influencing the picoplankton community

Picoplankton biomass notably decreased towards the open sea to a different extent for the various organisms. The relative contribution of autotrophic picoplankton to total picoplankton biomass considerably changed according to season and along the trophic gradient. In general, autotrophic components made a greater contribution to picoplankton biomass in mesotrophic and eutrophic areas, while heterotrophic bacteria biomass became more important under oligotrophic conditions.

The ratio between heterotrophic and autotrophic picoplankton biomass was highly variable and dependant on the season and station. There was the common trend of a ratio increase towards the open sea (Table 2). Bacterial biomass tended to be lower than autotrophic biomass only during the winter at stations near the coast (ST101, ST103 and CA007) when the ratio was around 0.5. During other seasons, the ratio increade significantly for all

Table 2. Bacterial production (BP), % high nucleic acid (HNA) bacteria and ratio between heterotrophic and autotrophic picoplankton biomass during different seasons. Average values and standard deviations (±) are presented.

	ST103	ST101	CA007	CA009	CA011
			Winter		
BP	0.27 ± 0.16	0.25 ± 0.08	0.13 ± 0.04	0.09 ± 0.03	0.09 ± 0.03
HNA	69.68 ± 3.48	62.77 ± 5.48	58.56 ± 2.17	55.42 ± 4.28	58.25 ± 1.07
Heterotrophic vs. Autotrophic Biomass	0.50	0.45	0.56	0.99	1.05
			Spring		
BP	0.45 ± 0.25	0.16 ± 0.04	0.11 ± 0.02	0.13 ± 0.05	0.10 ± 0.03
HNA (%)	66.37 ± 1.88	56.36 ± 0.66	53.80 ± 2.39	52.36 ± 2.05	54.10 ± 4.46
Heterotrophic vs. Autotrophic Biomass	1.91	1.12	2.64	2.56	3.54
			Summer		
BP	0.20 ± 0.104	0.15 ± 0.05	0.13 ± 0.04	0.10 ± 0.02	0.12 ± 0.03
HNA (%)	48.08 ± 9.31	40.10 ± 8.05	37.82 ± 8.25	38.26 ± 11.03	34.93 ± 2.17
Heterotrophic vs. Autotrophic Biomass	1.32	0.78	1.81	2.54	2.75
			Autumn		
BP	0.27 ± 0.19	0.24 ± 0.04	0.15 ± 0.03	0.12 ± 0.04	
HNA (%)	49.73 ± 9.00	42.46 ± 6.44	38.18 ± 2.59	34.03 ± 1.48	
Heterotrophic vs. Autotrophic Biomass	1.46	0.52	0.68	2.41	

stations, reaching a peak of 3.5 during the spring at station CA011. During warmer seasons as well as towards the open sea, autotrophic biomass was much lower than bacteria biomass.

Results of Pearson's correlation analysis between environmental parameters and picoplankton are presented in Table 3. Relationships were tested during two contrasting periods of the year considering the temperature of the water column (isothermal and stratified period). The analysis revealed distinct correlation patterns between variables tested during two periods.

Salinity seemed to be the most important environmental factor for picoplankton during both periods, exhibiting significant negative correlations with all biological variables tested. Temperature displayed a significant correlation only to the biomass of bacteria and Syn and to HNA abundances and during the isothermal period. Among nutrients, nitrates showed various significant correlations with the picoplankton community during the isothermal period, while nitrites were more important under stratified conditions. NH_4^+ significantly correlated to Syn biomass, bacterial production and LNA abundances during the isothermal period.

Analysis of relationships within the plankton community also revealed interesting associations. During the stratification period, both heterotrophic and autotrophic picoplankton showed a very strong relationship with Chl *a* distribution. However, under isothermal conditions, biomass of bacteria, Syn and Prochl did not show a significant correlation with Chl *a*. HNF was significantly correlated to picoeukaryotes, bacterial production, HNA and LNA during both periods. Hoverer, correlations between HNF and bacterial biomasses and between HNF and biomasses of cyanobacteria were weak or not statistically significant. During the thermally stratified period, the positive relationship between picoeukaryotes and bacteria was identified (Fig. 3) as well as between Syn and LNA bacteria (Fig. 3), and also between bacterial production and bacterial biomass (Fig. 4). During the isothermal period, bacterial production exhibited a very strong correlation with HNA bacteria (Fig. 5).

Discussion

Previous research work has described the central Adriatic as the area with a trophic gradient, from eutrophic coastal waters to the oligotrophic open sea (Krstulović *et al.,* 1995*,* 1997; Ninčević-Gladan *et al*., 2006; Šolić *et al*., 2009). Due to contrasting hydrological conditions in these areas, apparent differences in the picoplankton community structure and distribution are expected.

We observed the trend of biomass decrease from the coast towards the open sea for all members of the picoplankton community. This pattern has already been described by many authors that conducted their research in this area (Šolić *et al*., 2008, 2009; Šantić *et al*., 2012a, b) but also in the Mediterranean (Gasol *et al*., 1999) and in the Pacific (Grob *et al*., 2007).

Within cyanobacteria populations, Syn dominated over Prochl biomass, a phenomenon that has already been established for P-depleted environments (Martiny *et al*., 2009; Llabrés *et al*. 2010). In this survey, N:P ratios of inorganic nutrients ranged from 6 to 1407 (average 80) during the isothermal period, and from 3 to 183 (average 29) during the period of stratification, suggesting that this area is phosphorus limited during most of the year, as reported previously (Ninčević Gladan *et al*., 2006). Due to the high affinity for inorganic P and higher phosphate uptake rates, Syn hold the advantage over the genus Prochl and thrive in P-depleted environments, as reported recently (Moutin *et al*., 2002; Martiny *et al*.,

Table 3. Pearson correlations between biotic and environmental parameters during periods of isothermal and stratified water columns: temperature (°C), salinity (S), nitrate (NO_3^-), nitrite (NO_2^-), ammonium ion (NH_4^+), soluble reactive phosphate (SRP), chlorophyll *a* (chl *a*), heterotrophic nanoflagellates (HNF) abundance, bacterial production (BP), high nucleic acid bacteria (HNA), low nucleic acid bacteria (LNA). Statistically significant correlations are presented in bold ($p < 0.05$) and underlined ($p < 0.001$)

	T	S	NO_3^-	NO_2^-	NH_4^+	SRP	chl*a*	HNF
Isothermal period								
Bacterial biomass	**0.27**	**0.26**	-0.14	-0.14	-0.17	-0.09	-0.17	-0.11
Prochlorococcus biomass	-0.16	**-0.33**	0.17	-0.06	0.10	-0.21	0.23	0.16
Synecococcus biomass	**0.39**	-0.17	**0.29**	0.01	**0.56**	0.16	0.08	0.08
Picoeucariotes biomass	-0.17	**-0.38**	**0.22**	-0.08	**0.23**	-0.13	**0.44**	**-0.28**
BP	-0.05	**-0.62**	**0.47**	0.09	**0.27**	0.08	**0.60**	**0.31**
HNA abundance	**-0.35**	**-0.70**	**0.53**	**0.24**	0.12	-0.07	**0.65**	**0.36**
LNA abundance	**0.31**	**-0.41**	**0.33**	0.06	**0.42**	0.15	**0.37**	0.10
Stratified period								
Bacteria biomass	0.13	**-0.48**	0.02	**0.24**	0.12	**0.36**	**0.69**	0.04

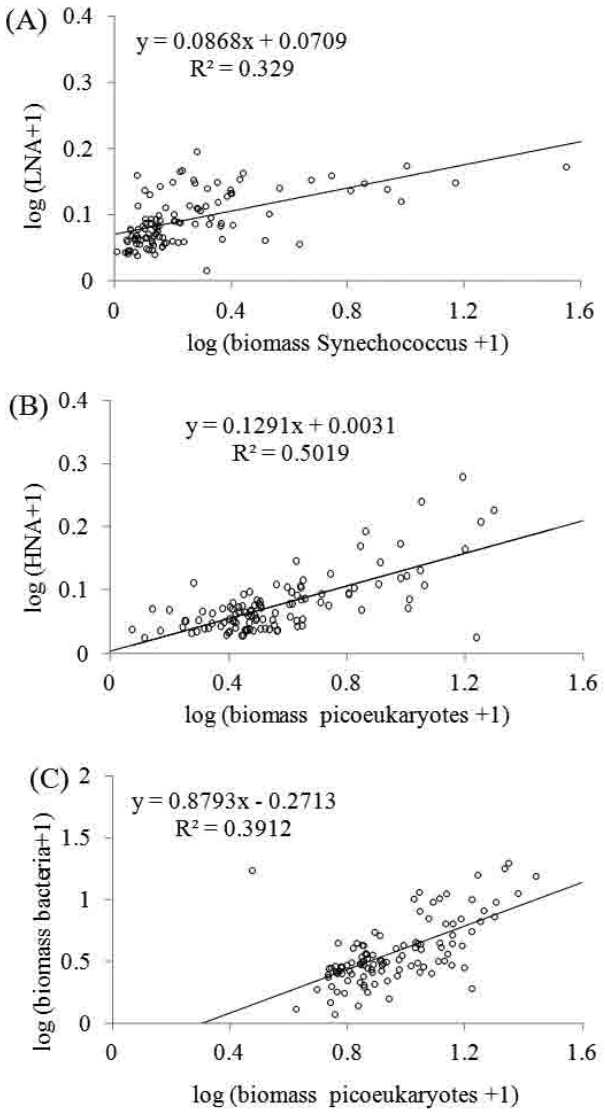

Fig. 3: Relationships between different groups of picoplankton members under thermally stratified conditions: (A) relationship between *Synechococcus* biomass and abundances of low nucleic acid bacteria (LNA); (B) relationship between picoeukaryote biomass and abundances of high nucleic acid bacteria (HNA); (C) relationship between picoeukaryote biomass and biomass of total heterotrophic bacteria.

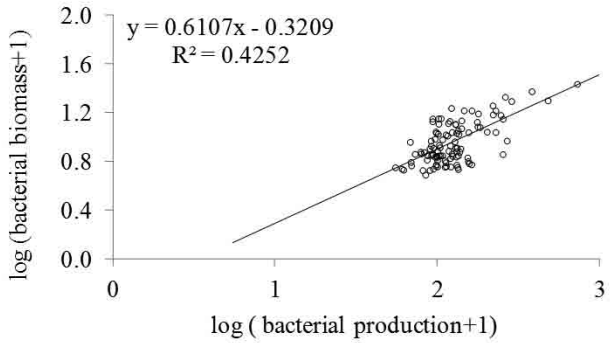

Fig. 4: The relationship between bacterial production and bacterial biomass under thermally stratified conditions.

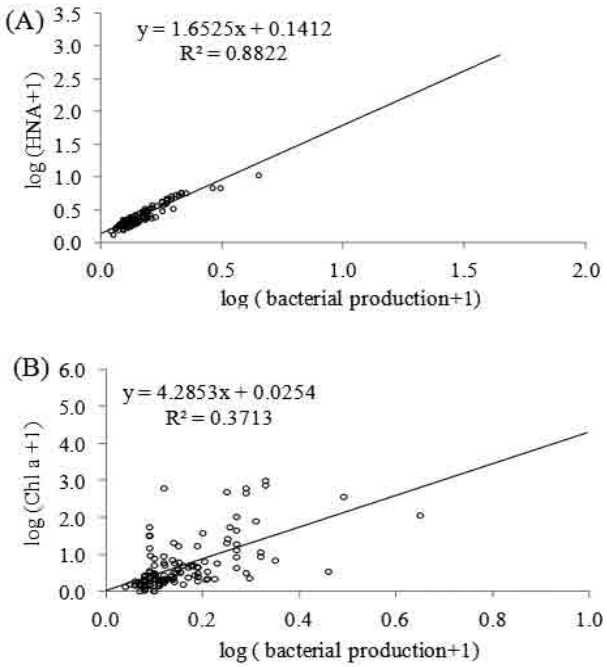

Fig. 5: Relationships between bacterial production and (A) abundances of high nucleic acid bacteria (HNA) and (B) chlorophyll *a* (Chl *a*) during the isothermal period.

2009). The same pattern of distribution (increasing from oligo- to eutrophic conditions) of both cyanobacterial groups is a feature not commonly found in marine environments. Prochl typically show an opposite pattern compared to the distribution of Syn along the trophic gradient and usually become a less important component of the picoplankton community from oligo- to eutrophic conditions (Partensky *et al*., 1996, Zubkov *et al*., 2000; Calvo-Diaz & Moran, 2006). In the central Adriatic Sea, however, its contribution to picoplanktonic biomass is much larger in coastal eutrophic waters. This is a characteristic that is typical of the central Adriatic (Šantić *et al*., 2011, 2012b) given that, in northern and southern Adriatic, cyanobacteria are distributed uniformly along the trophic gradient (Radić *et al*., 2009; Šilović *et al*., 2012). The trend of cyanobacterial biomass decrease towards the open oligotrophic sea has been observed by authors in other oceanic regions (Zubkov *et al*.., 2000 in the Atlantic Ocean, Grob *et al*., 2007 in the South Pacific Ocean and Calvo-Diaz & Moran, 2006 in the Bay of Biscay).

This is the first field study on autotrophic picoeukaryotes in the central Adriatic Sea and the results highlight their importance in the picoplanktonic community of this area. The average annual picoeukaryotic biomass of 9.77 μgCL^{-1} is consistent with values for the North Temperate Zone (Buitenhuis *et al*., 2012), showing their greatest contribution to APP biomass than either or both genera of cyanobacteria, especially in the coastal zone. The importance of picoeukaryotes has also been recorded for the northern Adriatic (Radić *et al*., 2009) and for

other coastal areas (Worden *et al*., 2004; Sherr *et al*., 2005; Grob *et al*., 2007; Buitenhuis *et al*., 2012). Their higher biomass in coastal waters than in the oligotrophic open sea is governed by their preference for the less stable water column and shallower nutricline, which allows the injection of nutrients into the surface, thus promoting their growth (Partensky *et al*., 1996; Shalapyonok *et al*., 2001). Picoeukaryotes also exhibited a strong seasonal pattern with noteworthy higher biomass values during the winter. During that period, their biomass was notably higher than even that the biomass of heterotrophic bacteria, especially in the coastal area. The same seasonal pattern has been observed in the eastern South Pacific (Grob *et al*., 2007), the North South China Sea (Liu *et al*., 2007) or in the Sargasso Sea (DuRand *et al*., 2001). The winter "bloom" of picoeukaryotes coincided with high nitrate concentration in the water column, especially in the coastal area. This finding is consistent with the fact that picoeukaryotes are highly successful in environments with elevated nitrate levels (DuRand *et al*., 2001; Shalapyonok *et al*., 2001; Radić *et al*., 2009) since larger cells and autrophs have a stronger response than heterotrophs to high nutrient availability (Duarte *et al*., 2000).

The seasonal distribution pattern of bacterial biomass and bacterial production found during this study is in agreement with the results of previous research in this area (Šolić *et al*., 2008, 2009). HNA cells constituted the main fraction of the bacterial community, coinciding with periods of higher bacterial production and higher Chl *a* concentrations. This result supports the view of Lebaron *et al*. (2001), Bouvier & del Giorgio (2002), Bouvier *et al*. (2007) and many others that most active cells belong to the HNA subpopulation. Moreover, Sherr *et al*. (2006) found that HNA bacteria represent only a minor fraction of active bacteria when phytoplankton biomass is low, a situation found during the summer at open sea stations.

The annual biomass ratio of bacterial to autotrophic picoplankton was on average >1, which is consistent with the survey carried out in Biscay Bay (Calvo-Diaz & Morán, 2006) and in oligotrophic regions with low chlorophyll levels (Li & Harrison, 2001). The ratio was higher during warmer seasons in oligotrophic waters stations, while values <1 were recorded during the winter and at coastal sites. This is due to the fact that bacterial biomass tends to increase more slowly than phytoplankton biomass along the trophic gradient (Cole *et al*., 1988; Sanders *et al*., 1992). These results show that within the picoplankton community the autotrophic part makes a greater contribution to total picoplankton biomass in mesotrophic or relatively eutrophic areas, while heterotrophic bacteria become more important under oligotrophic conditions by contributing to the carbon cycle through the microbial loop (Azam *et al*., 1983). Our results show the prevalence of autotrophic biomass in total picoplankton biomass and an increase in the biomass ratio from the coast toward the open sea region, which indicates the importance of autotrophic picoplankton in coastal estuarine systems and ecosystems (Vaquer *et al*., 1996; Ning *et al*., 2000; Murrel & Lores, 2004). According to the research cited, there is evidence that picoautotrophic phytoplankton biomass could be significant in carbon export at higher trophic levels (Barber, 2007; Buesseler *et al*., 2007). Our results show the importance of the picoplankton community not only under stratified, oligotrophic conditions (Li *et al*., 1993; Li & Harrison, 2001), but also in well mixed waters (Calvo-Diaz & Morán, 2006) during the year.

Relationships among picoplankton members and different environmental factors were found to differ during two contrasting periods. The only environmental factor that showed significant a correlation with all picoplanktonic groups during both periods was salinity. Negative relationships between biomass and salinity for all 4 picoplanktonic groups, such as the ones found here, have already been observed along a marked salinity gradient for salinities higher than 23.5 (Jochem 2003; Grob *et al*., 2007), although this is not always the case. The results show that salinity is an important parameter describing the habitat of the picoplanktonic community in the central Adriatic.

Significant positive relationships between bacterial biomass and bacterial production as well as between bacterial parameters and Chl *a* during the stratified period indicate that the ecosystem responds to higher substrate supply by accumulating bacterial biomass, which is consistent with Moran *et al*. (2010). The results indicate that the bacterial population could be bottom-up controlled during warm periods, which is in agreement with other seasonal studies (e.g. Moran *et al*., 2010) but contrary to previous investigations conducted in the central Adriatic (Šolić *et al*., 2009), where bottom-up control was dominant during the colder period. Two bacterial subpopulations responded differently to Chl *a* in different temperature regimes. HNA bacterial abundance was significantly correlated with Chl *a* during both periods with similar correlation coefficients. However, LNA yielded a stronger correlation with Chl *a* during the warm period and a rather weak one during cold months. High values of HNA from the winter to the early spring and its stronger dependence on Chl *a* concentrations would reflect the direct dependence of HNA cells on dissolved primary production, as suggested by Scharek & Latasa (2007) and Moran *et al*. (2010). LNA domination during the warmer period when dissolved nutrients are scarce as well as in the oligotrophic open sea reflects their successful adaptation to nutrient-poor conditions (Morris *et al*., 2002; Mary *et al*., 2006) when the microbial loop in marine ecosystem and regeneration processes become dominant.

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