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

ŠANTIĆ D. Institute of Oceanography and Fisheries, P. O. BOX 500, Split, Croatia
ŠESTANOVIĆ S. Institute of Oceanography and Fisheries, P. O. BOX 500, Split
ŠOLIĆ M. Institute of Oceanography and Fisheries, P. O. BOX 500, Split
KRSTULOVIĆ N. Institute of Oceanography and Fisheries, P. O. BOX 500, Split
KUŠPILIĆ G. Institute of Oceanography and Fisheries, P. O. BOX 500, Split
ORDULJ M. University Department of Marine Studies, University of Split, Livanska 5/III, Split
GLADAN Ž. Institute of Oceanography and Fisheries, P. O. BOX 500, Split

http://dx.doi.org/10.12681/mms.701

Copyright © 2014

To cite this article:

ŠANTIĆ, D., ŠESTANOVIĆ, S., ŠOLIĆ, M., KRSTULOVIĆ, N., KUŠPILIĆ, G., ORDULJ, M., & GLADAN, Ž. (2013). Dynamics of picoplankton community from coastal waters to the open sea in the Central Adriatic. Mediterranean Marine Science, 15(1), 179-188. doi:http://dx.doi.org/10.12681/mms.701
Dynamics of the picoplankton community from coastal waters to the open sea in the Central Adriatic

D. Šantić1, S. Šestanović2, M. Šolić1, N. Krstulović1, G. Kušpilić1, M. Orduljić1 and Ž. Ninčević Gladan1

1 Institute of Oceanography and Fisheries, P. O. BOX 500, Split, Croatia
2 University Department of Marine Studies, University of Split, Livanjska 5/III, Split, Croatia

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 × 104 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 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; Blanchet 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, 2010; Ž. Ninčević Gladan et al., 2012).
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 Sea (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 µl 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-
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 (Porier & Feig, 1980).

**Results**

**Environmental parameters**

Mean seasonal values of temperature, salinity and nutrients are presented in Table 1. A general trend of sharp Chl \(\alpha\) decrease from the inshore station towards the open sea was noted. Chl \(\alpha\) 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 \(\alpha\) 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 \(\alpha\) increase was recorded only in the surface layer (from 0–5m). At open sea stations, the winter Chl \(\alpha\) 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 \(\alpha\). However, the contribution of APP biomass to total phytoplankton biomass did...
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 \(\alpha\) 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 \(\alpha\) while at station CA011 only picoeukaryotes showed a seasonal distribution similar to that of Chl \(\alpha\).

Average monthly values of Syn and Prochl biomass ranged from 0.16 to 11.47 \(\mu\)g C l\(^{-1}\) and from 0.01 to 3.08 \(\mu\)g C l\(^{-1}\), respectively. The highest biomass of both cyanobacteria was recorded at station ST101 (24.83 \(\mu\)g C l\(^{-1}\) for Syn and 5.97 \(\mu\)g 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 \(\mu\)g 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 \(\mu\)g 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 \(\mu\)g 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 \(\mu\)g 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 \(\mu\)g 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 \(\mu\)g C l\(^{-1}\)). At the open sea station CA011, val-
ues were much lower (6.13 ± 0.78 μg C l⁻¹). The differenc-
es 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 bacter-

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 × 10⁴ cells ml⁻¹ h⁻¹ at CA011 to 0.45 × 10⁴ cells ml⁻¹ h⁻¹ at ST103 (Table 2). At coastal stations ST103 and ST101, increased produc-
tion 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 pico-
plankton 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). Bacte-
rial 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 increase significantly for all

Table 2. Bacterial production (BP), % high nucleic acid (HNA) bacteria and ratio between heterotrophic and autotrophic pico-
plankton 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 | 2.45 | 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. NH₄⁺ 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. However, 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; Santić 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 (NO₃⁻), nitrite (NO₂⁻), ammonium ion (NH₄⁺), 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   | NO₃⁻| NO₂⁻| NH₄⁺| SRP | chl a|
| Isothermal period |     |     |     |     |     |     |     |
| Bacterial biomass | 0.27| -0.26| -0.14| -0.14| -0.17| -0.09| -0.17|
| Prochlorococcus biomass | -0.16| -0.33| 0.17| -0.06| 0.10| -0.21| 0.23|
| Synecococcus biomass | 0.39| -0.17| 0.29| 0.01| 0.56| 0.16| 0.08|
| Picoeucariotes biomass | -0.17| -0.38| 0.22| -0.08| 0.23| -0.13| 0.44|
| HNF | -0.05| -0.62| 0.47| 0.09| 0.27| 0.08| 0.60|
| BP | -0.35| -0.70| 0.53| 0.24| 0.12| -0.07| 0.65|
| LNA abundance | 0.31| -0.41| 0.33| 0.06| 0.42| 0.15| 0.37|
| Stratified period | | | | | | | |
| Bacteria biomass | 0.13| -0.48| 0.02| 0.24| 0.12| 0.36| 0.69|
| HNF | | | | | | | |

Medit. Mar. Sci., 15/1, 2014, 179-188
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; Radič et al., 2009) since larger cells and autotrophs 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), Bouveri & del Giorgio (2002), Bouveri 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 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.

Acknowledgements

This research was supported by the Croatian Ministry of Science, Education and Sports as part of research
program “Role of plankton communities in the energy and matter flow in the Adriatic Sea” (Project no. 001-0013077-0845)

References

Aubry, B.F., Acri, F., Bastianini, M., Pugnotti, A., Socal G., 2006. Picophytoplankton contribution to the phytoplankton community structure in the gulf of Venice (NW Adriatic sea). International Review of Hydrobiology, 91 (1), 51-70.

Azam, F., Fenchel, T., Field, K.G., Gray, K.S., Meyer-Reil, L.A. et al., 1983. The ecological role of water-column microbes in the sea. Marine Ecology Progress Series, 10, 257-263.

Barber, R.T., 2007. Picoplankton do some heavy lifting. Science, 315, 777-778.

Blanchot, J., Andre, J.M., Navarette, C., Neveux, J., Radenac, M.H., 2001. Picophytoplankton in the equatorial Pacific: Vertical distributions in the warm pool and in high nutrient low chlorophyll conditions. Deep-Sea Research I, 48 (1), 297-314.

Bouvier, T.C., Del Giorgio P.A., 2002. Compositional changes in free-living bacterial communities along a salinity gradient in two temperate estuaries. Limnology and Oceanography, 47(2), 453-470.

Bouvier, T., Del Giorgio, P., Gasol, J., 2007. A comparative study of the cytometric characteristics of high and low nucleic acid bacterioplankton cells from different aquatic ecosystems. Environmental Microbiology, 9, 2050-2066.

Buitenbuis, E.T., Li, W.K.W., Vaulot, D., Lomas, M.W., Landry, M.R., et al., 2012. Picophytoplankton biomass distribution in the global ocean. Earth System Science Data, 4, 37-46.

Buesseler, K.O., Lamborg, C.H., Boyd, P.W., Lam, P.J., Trull, T.W., et al., 2007. Revisiting carbon flux through the ocean’s twilight zone. Science, 316, 567-570.

Calvo-Diaz, A., Moran, X.A., 2006. Seasonal dynamics of picophytoplankton in shelf waters of the southern Bay of Biscay. Aquatic Microbial Ecology, 42, 159-174.

Cole, J.J., Findlay, S.E.G., Pace, M.L., 1988. Bacterial production in fresh and saltwater ecosystems: a cross-system overview. Marine Ecology Progress Series, 43, 1-10.

Duarte, C.M., Goñi, S., Gasol, J.M., Vaqué, D., Vázquez-Dominguez, E., 2000. Effect of nutrient supply on the biomass structure of planktonic communities: an experimental test on a Mediterranean coastal community. Marine Ecology Progress Series, 206, 87-95.

Dugand M.D., Olson R.J., Chisholm S.W., 2001. Phytoplankton population dynamics at the Bermuda Atlantic Time-series station in the Sargasso Sea. Deep-Sea Research II, 48, 1983–2003.

Fuhrman, J.A., 1992. Bacterioplankton roles in cycling on organic matter. The microbial food web. p. 361-383. In: Primary productivity and biogeochemical cycles in the sea. Falkowski, P., Woodhead, A.D. (Eds). Plenum Press, New York.

Fuhrman, J.A., Azam, F., 1982. Thymidine incorporation as a measure of heterotrophic bacterioplankton production in marine surface waters. Marine Biology, 66, 109-120.

Gao, J.M., Zawel, J., Peters, F., Fuhrman, J.A., Hagström, Å., 1999. Significance of Size and Nucleic Acid content Heterogeneity as measured by Flow Cytometry in Natural Planktonic Bacteria. Applied and Environmental Microbiology, 65, 4475-4483.

Grasshoff, K., 1976. Methods of Seawater Analysis. Verlag Chemie, Weinheim, 317 pp.

Groh, C., Ulloa, O., Li, W.K.W., Alarcón, G., Fukasawa, M. et al., 2007. Picoplankton abundance and biomass across the eastern South Pacific Ocean along latitude 32.5°S. Marine Ecology Progress Series, 332, 53-62.

Jochem, F.J., 2001. Morphology and DNA content of bacterioplankton in the northern Gulf of Mexico: analysis by epifluorescence microscopy and flow cytometry. Aquatic Microbial Ecology, 25, 179-194.

Jochem, F.J., 2003. Photo- and heterotrophic pico- and nanoplankton in the Mississippi River plume: distribution and grazing activity. Journal of Plankton Research 25, 1201-1214.

Krstulović, N., Pucher-Petković, T., Šolić, M., 1995. The relation between bacterioplankton and phytoplankton in the mid Adriatic Sea. Aquatic Microbial Ecology, 9, 41-45.

Krstulović, N., Šolić, M., Marasović, I., 1997. Relationship between bacteria, phytoplankton and heterotrophic nanoflagellates along the trophic gradient. Helgoländer Meeresuntersuchungen, 51 (4), 433-443.

Lebaron, P., Servais, P., Agogué, H., Courties, J., Jouys, F. 2001. Does the high nucleic-acid content of individual bacterial cells allow to discriminate active cells in aquatic systems? Applied Environmental Microbiology, 67, 1775-1782.

Lee, S., Fuhrman, J.A., 1987. Relationships between biovolume and biomass of naturally derived marine bacterioplankton. Applied Environmental Microbiology, 53 (6), 1298-1303.

Li, W.K.W., 1994. Primary production of prochlorophytes, cyanobacteria, and eukaryotic ultraplankton: Measurements from flow cytometric sorting. Limnology and Oceanography, 39 (1), 169-175.

Li, W.K.W., 1998. Annual average abundance of heterotrophic bacteria and Synechococcus in surface ocean waters. Limnology and Oceanography, 43 (7), 1746-1753.

Li, W.K.W., Harrison, W.G., 2001. Chlorophyll, bacteria and picophytoplankton in ecological provinces of the North Atlantic. Deep-Sea Research II, 48 (10), 2271-2293.

Li, W.K.W., Zohary, T., Yacobi, Y.Z., Wood, A.M., 1993. Ultraphytoplankton in the eastern Mediterranean Sea: Towards deriving phytoplankton biomass from flow cytometric measurements of abundance fluorescence and light scatter. Marine Ecology Progress Series, 102, 79-87.

Li, H., Chang, J., Tseng, C.M., Wei, L.S., Liu, K.K., 2007. Seasonal variability of picoplankton in the Northern South China Sea at the SEATS station. Deep-Sea Research II, 54 (14-15), 1602-1616.

Llabrés, M., Agustí, S., Alonso-Laita, P., Hendli, G.J., 2010. Synechococcus and Prochlorococcus cell death induced by UV radiation and the penetration of lethal UVR in the Mediterranean Sea. Marine Ecology Progress Series, 399, 27-37.

Mance, D., Partensky, F., Jacquet, S., Vaaulot, D., 1997. Environon in the mid Adriatic Sea. Aquatic Microbial Ecology, 9, 41-45.

Marin, I., Partensky, F., Jacquet, S., Vaulot, D., 1997. Environon in the mid Adriatic Sea. Aquatic Microbial Ecology, 9, 41-45.

Martiny, A.C., Huang, Y., Li, W., 2009. Occurrence of phosphate acquisition genes in Prochlorococcus cells from different ocean regions. Environmental Microbiology, 11(6), 1340-1347.

Mary, I., Heywood, J.L., Fuchs, B.M., Amann, R., Burkhill, P.H. et al., 2006. SAR11 dominance among metabolically active low nucleic acid bacterioplankton in surface waters along an
Atlantic meridional transect. Aquatic Microbial Ecology, 45 (2), 107-113.

Moran, X.A.G., Calvo-Diaz, A., Ducklow, H.W., 2010. Total and phytoplankton mediated bottom-up control of bacterioplankton change with temperature in NE Atlantic shelf waters. Aquatic Microbial Ecology, 58 (2), 229-239.

Morriss, R.M., Rappe, M.S., Connon, S.A., Vergin, K.L., Siebold, W.A., et al., 2002. SAR11 clade dominates ocean surface bacterioplankton communities. Nature, 420, 802-805.

Moutin, T., Thingstad, T.F., Van Wambeke, F., Marie, D., Slawyk, G. et al., 2002. Does competition for nanomolar phosphate supply explain the predominance of the cyanobacterium Synechococcus? Limnology and Oceanography, 47, 1562-1567.

Murrell, M.C., Loes, E.M. 2004. Phytoplankton and zooplankton sequence of carbon fluxes in a subtropical estuary: importance of cyanobacteria. Journal of Plankton Research, 26 (3), 371-382.

Ninčević Gladan, Ž., Marasović, I., Kušpilić, G., Krstulović, N., Šolić, M., 2006 Abundance and composition of picoplankton in the mid Adriatic Sea. Acta Adriatica, 47 (2), 127-140.

Ning, X., Cloern, J.E., Cole, B.E., 2000. Spatial and temporal variability of picocyanobacteria Synechococcus sp. in San Francisco Bay. Limnology and Oceanography, 45 (3), 695-702.

Paoli, A., Celussi, M., Valeri, A., Larato, C., Bussani, A. et al., 2007. Picocyanobacteria in Adriatic transitional environments. Estuaries, Coastal and Shelf Science, 75 (1-2), 13-20.

Partensky, F., Blanchot, J., Lantoine, F., Neveux, J., Marie, D., 1996. Vertical structure of picophytoplankton at different trophic sites in the northeastern Atlantic Ocean. Deep-Sea Research I, 43 (8), 1191-1213.

Porter, K.G., Feig, Y.S., 1980. The use of DAPI for identifying and counting aquatic microflora. Limnology and Oceanography, 25 (5), 943-948.

Pugnetti, A., Bazzoni, A.M., Beran, A., Aubry, F.B., Camatti, E. et al., 2008. Changes in biomass structure and trophic status of the plankton communities in a highly dynamic ecosystem (Gulf of Venice, Northern Adriatic Sea). Marine Ecology, 32 (3), 367-374.

Radić, T., Šilović, T., Šantić, D., Fuks, D., Mičić, M., 2009. Preliminary flow cytometric analyses of hototrophic pico-and nanoplankton communities in the Northern Adriatic. Fresenius Environmental Bulletin, 18 (5a), 715-724.

Riemann, B., Bjornsen, P.K., Newell, S., Fallon, R., 1987. Calculation of cell production of coastal marine bacteria based on measured incorporation of 3H thymidine. Limnology and Oceanography, 32, 471-476.

Sanders, R.W., Caron, D.A., Berninger U.G., 1992. Relationships between bacteria and heterotrophic nanoplankton in marine and fresh waters: an inter-ecosystem comparison. Marine Ecology Progress Series, 86, 1-14.

Scharf, R., Lynne, M., 2007. Growing, grazing and carbon flux of high and low nucleic acid bacteria differ in surface and deep chlorophyll maximum layers in the NW Mediterranean Sea. Aquatic Microbial Ecology, 46 (2), 153-161.

Shalapyonok, A., Olson, R.J., Shalapyonok, L.S., 2001. Arabian Sea phytoplankton during South West and Northeast Monsoons 1995: Composition, size structure and biomass from individual cell wall properties measured by flow cytometry. Deep-Sea Research II, 48 (6-7), 1231-1261.

Sherr, E.B., Sherr, B.F., Wheeler, P.A., 2005. Distribution of coccolid cyanobacteria and small eukaryotic phytoplankton in the upwelling ecosystem off the Oregon coast during 2001 and 2002. Deep-Sea Research II, 52 (1-2), 317-330.

Sherr, E.B., Sherr, B.F., Longnecker, K., 2006. Distribution of bacterial abundance and cell-specific nucleic acid content in the Northeast Pacific Ocean. Deep-Sea Research I, 53, 713-725.

Stockner, J.G., 1988. Phototropic picoplankton: an overview from marine and freshwater ecosystems. Limnology and Oceanography, 33 (4, pt2), 765-775.

Strickland, J.D.H., Parsons, T.R., 1972. A practical Handbook of seawater analysis. Bulletin Fisheries Research Board of Canada, 167, 310 pp.

Šantić, D., Kristulović, N., Solić, M., Kušpilić, G., 2011. Distribution of Synechococcus and Prochlorococcus in the central Adriatic Sea. Acta Adriatica, 52 (1), 101-114.

Šantić, D., Kristulović, N., Solić, M., Ordalj, M., Kušpilić, G., 2012a. Dynamics of prokaryotic picoplankton community in the central and southern Adriatic Sea (Croatia). Helgoland Marine Research, DOI 10.1007/s10152-012-0336-x.

Šantić, D., Kristulović, N., Solić, M., Kušpilić, G., 2012b. HNA and LNA bacteria in relation to the activity of heterotrophic bacteria. Acta Adriatica, 53 (1), 25-40.

Šilović, T., Bosak, S., Jakšić, Ž., Fuks, D., 2012. Seasonal dynamics of the autotrophic community in the Lim bay (NE Adriatic sea). Acta Adriatica, 53 (1), 41-56.

Šilović, T., Ljubešić, Z., Mihanović, H., Olujić, G., Terzić, S. et al., 2011. Picoplankton composition related to thermal haline circulation: The Albanian boundary zone (southern Adriatic) in late spring. Estuarine, Coastal and Shelf Science, 91 (4), 519-525.

Šolić, M., Kristulović, N., Vilibić, I., Bojanić, N., Kušpilić, G. et al., 2009. Variability in the bottom-up and top-down control of bacteria on trophic and temporal scale in the middle Adriatic Sea. Aquatic Microbial Ecology, 58 (1), 15-29.

Šolić, M., Kristulović, N., Vilibić, I., Kušpilić, G., Sestanović, S. et al., 2008. The role of water mass dynamics in controlling bacterial abundance and production in the middle Adriatic Sea. Marine Environmental Research, 65 (5), 388-404.

Vaquer, A., Troussellier, M., Courties, C., Ribentrop, B., 1996. Standing stock and dynamics of picoplankton in the Thau lagoon (Northwest Mediterranean coast). Limnology and Oceanography, 41 (8), 1821-1829.

Vilibić, I., Šantić, D., 2008. Deep water ventilation traced by Synechococcus cyanobacteria. Ocean Dynamics, 58 (2), 119-125.

Vilibić, I., Kuzmić, M., Bosak, S., Šilović, T., Hrustić, E. et al., 2009. Distribution of phytoplankton along the thermal haline gradient in the north-eastern Adriatic channel: winter aspect. Oceanologya, 51 (4), 495-513.

Worden, A.Z., Nolan, J.K., Palenik, B., 2004. Assessing the dynamics and ecology of marine picophytoplankton: The importance of the eukaryotic component. Limnology and Oceanography, 49 (1), 168-179.

Zubkov, M.V., Sleigh, M.A., Burkhill, P.H., Leakey, R.J.G., 2000. Picoplankton community structure on the Atlantic Meridional Transect: A comparison between seasons. Progress in Oceanography, 45 (3-4), 369-386.