

Dynamics of prokaryotic picoplankton community in the central and southern Adriatic Sea (Croatia)

Danijela Šantić · Nada Krstulović · Mladen Šolić ·
Marin Ordulj · Grozdan Kušpilić

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Abstract This paper addresses the dynamics of the prokaryotic picoplankton community in the coastal and open sea areas of the central Adriatic and in the coastal area of the southern Adriatic. This involved the study, from January to December 2005, of bacteria (total number of non-pigmented bacteria; high nucleic acid content (HNA) bacteria; low nucleic acid content (LNA) bacteria), cyanobacteria (*Synechococcus* and *Prochlorococcus*) and heterotrophic nanoflagellates. During the warmer seasons, in the mainly oligotrophic area under investigation into the Adriatic Sea, bacterial densities and bacterial production have shown an increase in values and domination of the LNA group of the bacterial population. In contrast, in those areas influenced by karstic rivers, the domination of HNA bacteria in total abundance of non-pigmented bacteria and high values of bacterial production was estimated throughout the investigated period. Our results show the importance of both HNA and LNA bacterial groups in the total bacterial activity throughout the investigated area. The biomass of bacteria was mostly predominant in the prokaryotic community, while within the autotrophic community *Synechococcus* biomass mostly predominated. During the warmer seasons, an increase in autotrophic biomass was observed in relation to non-pigmented biomass. The importance of predation in controlling bacteria

by heterotrophic nanoflagellates was pronounced during the warmer period and in the coastal areas.

Keywords *Synechococcus* · *Prochlorococcus* · Heterotrophic bacteria · HNA · LNA · Adriatic Sea

Introduction

Non-pigmented bacteria and photosynthetic picoplankton are ubiquitous and represent numerous and active components of the marine ecosystem. Bacteria play an important role through their assimilation of dissolved organic matter (Cole et al. 1988) and by the decomposition of organic matter and through the transformation of inorganic compounds into forms suitable for primary producers (Ducklow et al. 1986). Photosynthetic picoplankton appears to be an essential component of marine ecosystems and one of their representatives, cyanobacteria *Synechococcus* (Waterbury et al. 1979) and *Prochlorococcus* (Chisholm et al. 1988) significantly contribute to phytoplankton biomass, especially in oligotrophic areas like the Mediterranean Sea (Magazzù and Decembri 1995; Agawin and Agustí 1997). In addition, the application of flow cytometry has expanded the knowledge of the microbial community members and has permitted discrimination within cyanobacterial cells (*Synechococcus* and *Prochlorococcus*) and non-pigmented bacterial cells based on different deoxyribonucleic acid content (Olson et al. 1993; Gasol and Del Giorgio 2000). There is much literature concerning the distribution and dynamics of non-pigmented cells within the investigated area of the Adriatic Sea. A few reports of *Synechococcus* and *Prochlorococcus* exist (Fuks et al. 2005; Ninčević et al. 2006; Radić et al. 2009; Šilović et al. 2011; Šantić et al. 2011), but simultaneous observations of *Synechococcus*,

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D. Šantić (✉) · N. Krstulović · M. Šolić · G. Kušpilić
Institute of Oceanography and Fisheries,
P. O. Box 500, Split, Croatia
e-mail: segvic@izor.hr

M. Ordulj
University Department of Marine Studies,
University of Split, Livnjska 5/III, Split, Croatia

Prochlorococcus and non-pigmented bacteria are absent. The objectives of this study were to describe the seasonal cycles of non-pigmented prokaryotes, indicate the possible role of HNA and LNA bacteria in bacterial activity in the investigated areas, show the relationship between biomasses of autotrophic and non-pigmented prokaryotes and identify the role of heterotrophic nanoflagellates in controlling the bacterial population.

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, the central Adriatic with depressions as deep as 280 m, and connected to the southern Adriatic circular basin over the Palagruža Sill. The coastal area investigated is located in the central and southern Adriatic basin, covering the coastal zone from Zadar to Dubrovnik and is partly under the influence of the karstic rivers: Krka (Šibenik area), Jadro (Kaštela Bay), Žrnovnica, Cetina (Split area), Neretva (Ploče area) and Trebišnjica (Dubrovnik area). In the open sea, samples were collected from station CA001 located in the Jabuka Pit, CA003 located in the open sea area of Šibenik and at station CA009 located near the island of Vis (Fig. 1).

Sampling

Sampling was mostly at monthly intervals from January 2005 to December 2005 on RV Bios using Niskin bottles (5 l). Samples were collected at several depths between the surface and the bottom (at 5- to 10-m intervals for the upper 50 m and at 75, 100, 168, 200 and 260 m at deepest station). A SeaBird 25 CTD profiler recorded temperature and salinity data. Nutrient concentrations (NO_3^- , NO_2^- , NH_4^+ , total dissolved inorganic nitrate/DIN and soluble reactive phosphate/SRP) were determined using the auto-analyser modified method by Grasshof (1976).

Flow cytometry

Abundances of *Synechococcus*, *Prochlorococcus* and non-pigmented bacteria were determined using flow cytometry (Marie et al. 1997). For flow cytometry counts of autotrophic cells, 2 ml of preserved samples in 0.5 % glutaraldehyde was frozen at -80°C and stored until analysis (5–10 days), while samples for bacteria were preserved in 2 % formaldehyde and stored at 4°C until analysis (5–10 days).

Abundances of SybrGreen I-stained non-pigmented bacteria were determined using flow cytometry (Marie et al. 1997). Samples of 1 ml without replicas were analysed on a Beckman Coulter EPICS XL-MCL with a high flow rate from 1 to $1.2\ \mu\text{L}\ \text{s}^{-1}$, and the analysed volume was calculated by acquisition time. To standardise the fluorescence intensity of the cells, $1.0\text{-}\mu\text{m}$ 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 the nucleic acid content (Jochem 2001), referred to as high nucleic acid (HNA) and low nucleic acid bacteria (LNA) and light scattering.

Autotrophic cells were separated into two groups of cyanobacteria (*Synechococcus* and *Prochlorococcus*) and were distinguished according to light scattering, red emission of cellular chlorophyll content and orange emission of phycoerythrin-rich cells. Biomasses of *Synechococcus*, *Prochlorococcus* and heterotrophic bacteria were calculated by using the following volume-to-carbon conversion factors: $250\ \text{fg}\ \text{C}\ \text{cell}^{-1}$ for *Synechococcus*, $53\ \text{fg}\ \text{C}\ \text{cell}^{-1}$ for *Prochlorococcus* and $20\ \text{fg}\ \text{C}\ \text{cell}^{-1}$ for non-pigmented bacteria (Zhang et al. 2008).

Bacterial cell production was measured from DNA synthesis based on incorporation rates of ^3H -thymidine (Fuhrman and Azam 1982). Conversion factors (CF) for bacterial production were calculated from bacterial cell numbers and ^3H -thymidine incorporation during bacterial growth in $1\text{-}\mu\text{m}$ pre-filtered seawater (Riemann et al. 1987): $\text{CF} = (N_2 - N_1) / ^3\text{H}$ where N_1 and N_2 are the numbers of bacteria at the beginning and the end of the experiment, and ^3H is the integrated ^3H -thymidine incorporation rate during the experiment. The number of heterotrophic nanoflagellates (HNF) was estimated using epifluorescence microscopy. Samples were stained with 4'-6-diamidino-2-phenylindole (DAPI) for 10 min and filtered through polycarbonate filters with $0.8\ \mu\text{m}$ pore diameters (Milipore, Ireland). Microscope slides were examined with an Olympus microscope under UV light at a magnification of 1,000X (Porter and Feig 1980).

Methods for determination of regulation mechanisms in the prokaryotic community

Relationships between abiotic and biotic factors and the abundance of autotrophic and heterotrophic prokaryotes were determined using the Pearson's rank correlation index.

To examine the regulation of bacteria by predation, data were analysed using an empirical model (Gasol 1994). The simultaneous observations of the abundance of bacteria and heterotrophic nanoflagellates (HNF) are plotted on a log–log graph, which provides information about coupling

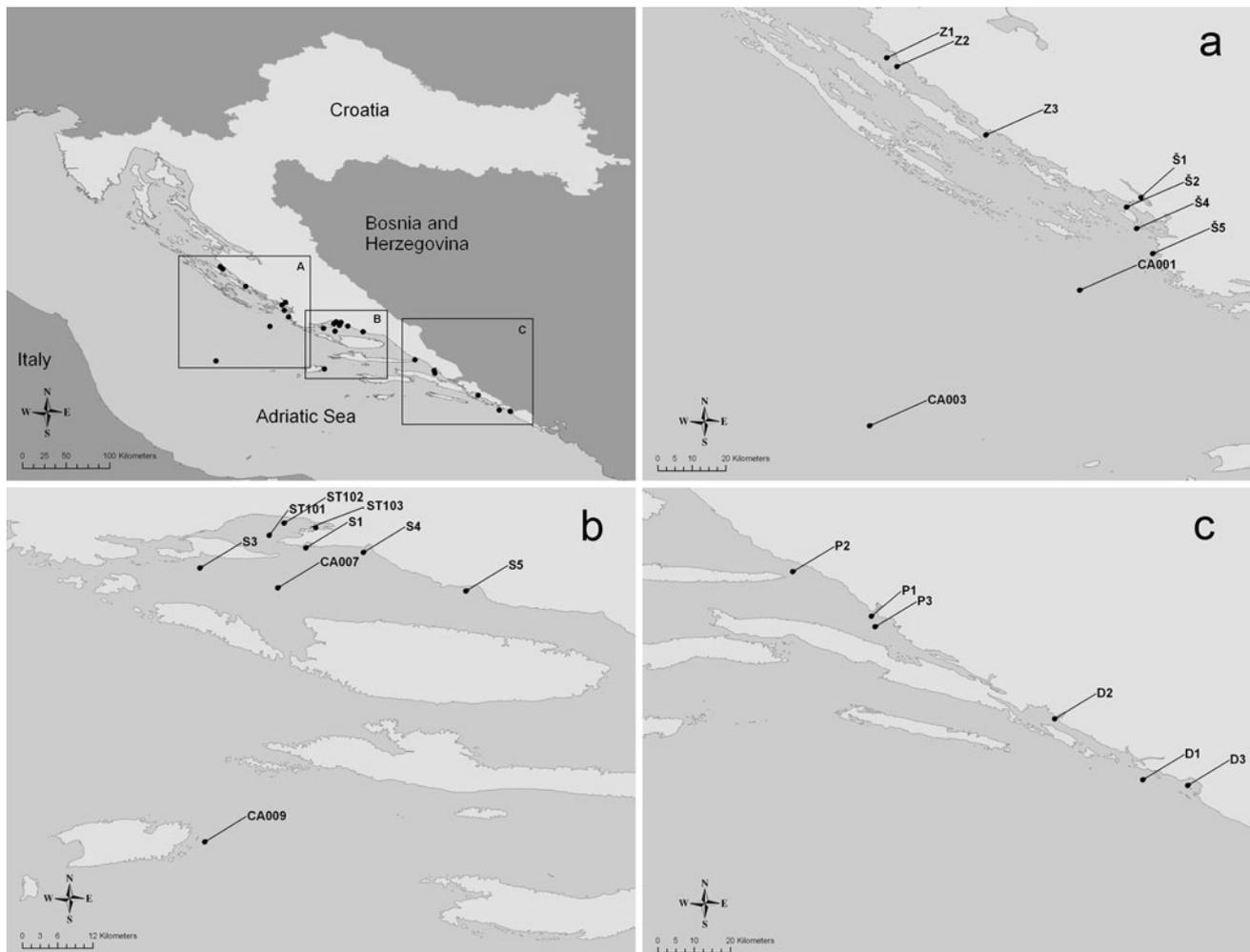


Fig. 1 Study area with sampling stations. **a** Coastal areas: Zadar (Z1–Z3), Šibenik (Š1–Š5) and open sea area (CA001, CA003) **b** coastal areas: Kaštela Bay (ST101–ST103), Split (S1–S5, CA007) and open sea area (CA009) **c** coastal areas: Ploče (P1–P3), Dubrovnik (D1–D3)

between bacterial and HNF abundance. The graph includes an empirically determined maximum attainable abundance (MAA) line (based on a large database from a variety of systems) depicting the HNF abundance that could be attained at a given bacterial abundance ($\max \log \text{HNF} = -2.47 + 1.07 \log \text{bacterial abundance}$) and a mean realised abundance (MRA) line. The points close to the MAA line indicate strong coupling between the bacteria and HNF, which according to Gasol (1994), could be interpreted as strong predation on the bacteria. The points that lie well below the MRA line indicate conditions when bacterial abundance was not controlled by HNF grazing. Therefore, distance (D) values (differences between the maximum and realised HNF abundances at different bacterial concentrations) represent a good indicator of the importance of HNF predators in controlling bacterial abundance. The distance (D) between the maximum and actual measured HNF abundance, for each bacterial abundance value, shows the

degree of uncoupling between bacteria and their predators and is surrogate for the grazing pressure of HNF on bacteria. Low D values mean strong coupling between the abundance of bacteria and HNF, while high D values mean no or low coupling between bacteria and HNF.

Results

Physicochemical conditions of seawater

In the investigated areas, an isothermal period was obtained from January to April and again in December, while thermal stratification of the water column began in May and lasted until September (Table 1). The lowest values and widest ranges of temperature and salinity were recorded in the areas of Šibenik, Kaštela Bay, Split and Ploče, mostly due to the influence of the river Krka and to a lesser

Table 1 Mean monthly value with standard deviation (\pm) of temperature ($^{\circ}\text{C}$), salinity, nutrient concentrations (μM) and chlorophyll a (mg m^{-3}) at coastal and open sea area

Complete data set	Month												
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	
Coastal sea $N = 370$													
Open sea $N = 148$													
Temperature ($^{\circ}\text{C}$)	13.47 \pm 1.59	11.16 \pm 0.57	10.61 \pm 0.86	13.30 \pm 1.17	17.18 \pm 1.93	18.72 \pm 2.74	19.30 \pm 3.42	19.30 \pm 3.42	21.27 \pm 1.55	20.28 \pm 1.82	17.55 \pm 1.74	15.10 \pm 1.30	15.10 \pm 1.30
Salinity	14.86 \pm 0.29	13.38 \pm 0.52	13.50 \pm 0.24	15.22 \pm 0.80	16.67 \pm 1.97	16.78 \pm 3.72	17.81 \pm 4.59	17.44 \pm 3.75	17.44 \pm 3.75	18.41 \pm 1.69	18.02 \pm 1.35	15.72 \pm 0.33	15.72 \pm 0.33
Coastal sea	37.11 \pm 4.02	37.71 \pm 0.69	37.35 \pm 1.07	37.16 \pm 0.86	36.73 \pm 3.63	37.26 \pm 3.28	37.40 \pm 2.24	37.40 \pm 2.24	37.78 \pm 1.77	38.03 \pm 0.90	37.24 \pm 4.14	36.09 \pm 6.36	36.09 \pm 6.36
Open sea	38.58 \pm 0.26	38.65 \pm 0.22	38.71 \pm 0.01	38.19 \pm 0.71	38.55 \pm 0.36	38.68 \pm 0.19	38.59 \pm 0.34	38.67 \pm 0.34	38.78 \pm 0.15	38.65 \pm 0.10	38.66 \pm 0.07	38.54 \pm 0.12	38.54 \pm 0.12
Nitrate (μM)	3.53 \pm 6.88	1.04 \pm 0.60	1.35 \pm 0.71	0.36 \pm 0.29	0.74 \pm 2.85	0.40 \pm 1.13	0.64 \pm 1.08	0.92 \pm 1.47	0.82 \pm 1.63	1.00 \pm 2.10	2.24 \pm 5.87	4.08 \pm 9.58	4.08 \pm 9.58
Open sea	2.03 \pm 0.47	1.30 \pm 0.81	0.71 \pm 0.19	0.32 \pm 0.37	0.64 \pm 0.63	0.30 \pm 0.59	0.49 \pm 0.26	0.95 \pm 0.72	0.77 \pm 0.78	0.46 \pm 0.65	0.35 \pm 0.22	0.47 \pm 0.19	0.47 \pm 0.19
Nitrite (μM)	0.29 \pm 0.18	0.37 \pm 0.24	0.16 \pm 0.10	0.11 \pm 0.07	0.10 \pm 0.06	0.05 \pm 0.03	0.04 \pm 0.05	0.07 \pm 0.10	0.10 \pm 0.17	0.09 \pm 0.19	0.16 \pm 0.17	0.25 \pm 0.15	0.25 \pm 0.15
Open sea	0.19 \pm 0.12	0.21 \pm 0.09	0.16 \pm 0.05	0.13 \pm 0.06	0.10 \pm 0.02	0.09 \pm 0.02	0.10 \pm 0.03	0.07 \pm 0.06	0.05 \pm 0.03	0.05 \pm 0.05	0.06 \pm 0.04	0.13 \pm 0.02	0.13 \pm 0.02
Ammonium (μM)	1.12 \pm 0.54	0.80 \pm 0.35	0.68 \pm 0.22	1.27 \pm 0.27	1.62 \pm 2.89	0.59 \pm 0.28	0.69 \pm 0.72	2.05 \pm 3.75	0.79 \pm 0.52	0.68 \pm 0.33	5.06 \pm 9.37	1.18 \pm 0.73	1.18 \pm 0.73
Open sea	1.25 \pm 0.33	0.95 \pm 0.41	0.79 \pm 0.32	1.58 \pm 0.32	1.17 \pm 0.65	0.57 \pm 0.35	0.60 \pm 0.45	0.56 \pm 0.45	0.43 \pm 0.32	0.65 \pm 0.12	0.41 \pm 0.18	0.91 \pm 0.39	0.91 \pm 0.39
Soluble reactive phosphate-SRP (μM)	0.04 \pm 0.03	0.05 \pm 0.02	0.04 \pm 0.01	0.07 \pm 0.02	0.05 \pm 0.05	0.07 \pm 0.03	0.07 \pm 0.02	0.08 \pm 0.19	0.06 \pm 0.09	0.05 \pm 0.06	0.06 \pm 0.06	0.04 \pm 0.03	0.04 \pm 0.03
Open sea	0.03 \pm 0.02	0.03 \pm 0.01	0.03 \pm 0.01	0.05 \pm 0.01	0.07 \pm 0.01	0.07 \pm 0.02	0.03 \pm 0.02	0.04 \pm 0.02	0.10 \pm 0.45	0.06 \pm 0.06	0.16 \pm 0.07	0.03 \pm 0.01	0.03 \pm 0.01
Chl a (mg m^{-3})	0.44 \pm 0.35	0.39 \pm 0.26	0.38 \pm 0.14	0.55 \pm 0.40	0.35 \pm 0.69	0.27 \pm 0.43	0.20 \pm 0.25	0.26 \pm 0.27	0.75 \pm 0.73	0.74 \pm 0.74	0.73 \pm 0.58	0.52 \pm 0.59	0.52 \pm 0.59
Open sea	0.08 \pm 0.01	0.10 \pm 0.03	0.13 \pm 0.02	0.14 \pm 0.04	0.06 \pm 0.03	0.05 \pm 0.06	0.06 \pm 0.05	0.09 \pm 0.07	0.11 \pm 0.08	0.18 \pm 0.05	0.20 \pm 0.07	0.19 \pm 0.04	0.19 \pm 0.04

extent, the rivers Jadro, Žrnovnica, Cetina and Neretva (Morović et al. 2006). Nitrates and nitrites in the investigated areas showed increased values in January to February and in December. Values of soluble reactive phosphates at the coastal sea stations were uniform during the isothermal and stratified periods. The highest concentration of ammonium in the coastal areas was detected in November, but high values were also recorded in May and August. In the open sea areas, slightly increased values of ammonium were detected in the period from April to May. Increased values of chlorophyll a were determined in the period from September to December with higher concentrations in coastal areas compared with the open sea (Table 1).

Abundance of non-pigmented bacteria

Average monthly abundance of bacteria, obtained as the average value from the surface to the bottom layers of the investigated stations, ranged from 0.24×10^6 to 1.3×10^6 cells mL^{-1} in the central and southern coastal areas and in the open sea ranged from 0.23×10^6 to 0.63×10^6 cells mL^{-1} (Fig. 2). Seasonal distribution of non-pigmented bacteria in the coastal areas has shown an increased abundance during the warmer seasons and lower abundance during the colder seasons, with pronounced variations in bacterial densities in relation to open sea area (Fig. 2). Comparing all investigated areas, the highest numbers of non-pigmented bacteria were found in the Kaštela Bay area (Table 2).

Percentage of HNA bacteria in the community of non-pigmented bacteria

The average monthly percentage of HNA bacteria in the central and southern coastal areas ranged from 26 to 83 %, while in the open sea HNA ranged from 28 to 65 %. Different temporal patterns were found for HNA and therefore for LNA bacterial groups. Seasonal distribution mostly showed the prevalence of the HNA group during the colder seasons and a prevalence of LNA bacteria, or equal proportions of the LNA and HNA groups, in the bacterial community during spring and summer. However, a prevalence of the HNA group in the bacterial community during all four seasons was found in the coastal areas directly influenced by rivers (Fig. 3), specifically in the Kaštela Bay (Jadro) and Šibenik areas (Krka). Comparing the investigated areas with the annual average, variations in the proportion of HNA bacteria were mostly more pronounced in the coastal sea areas compared with the open sea area. The annual average at the open sea stations CA001 and CA003 showed percentages of HNA bacteria less than 50 % (Table 2).

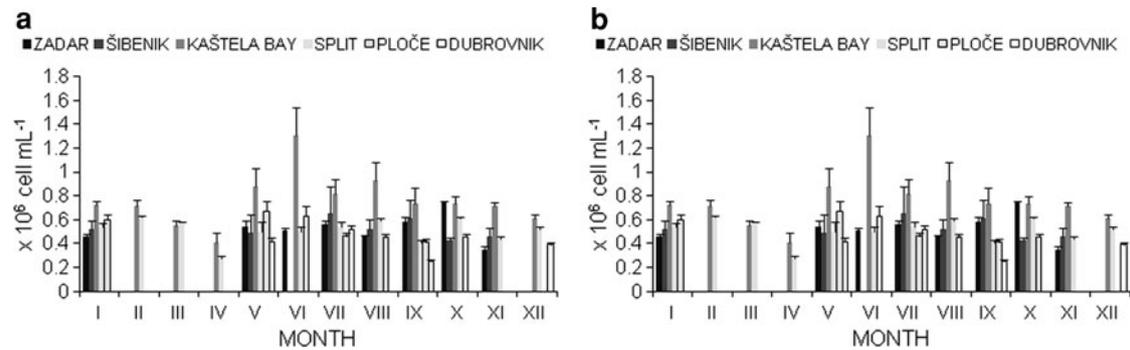


Fig. 2 Seasonal fluctuations of non-pigmented bacteria. **a** Coastal areas **b** open sea station. Average values (column, *line with markers*) and positive standard error (*bars*) are presented. The missing sampling points show that there was no sampling

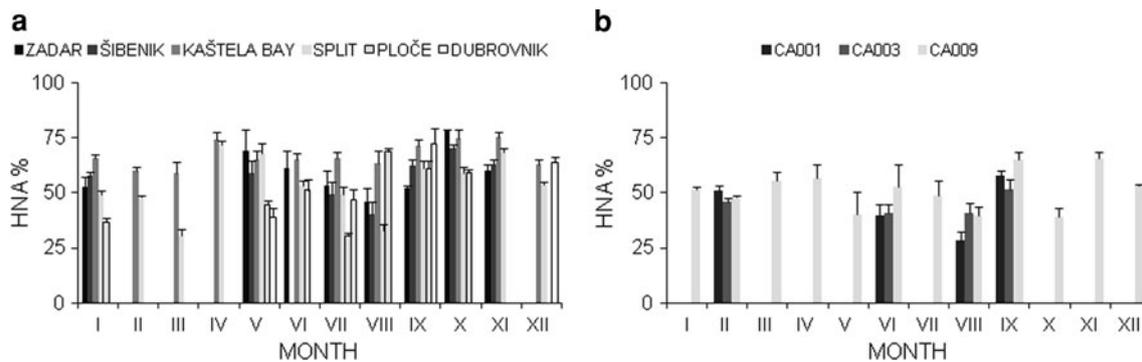


Fig. 3 Seasonal fluctuations of HNA %. **a** Coastal areas **b** open sea stations. Average values (column, *line with markers*) and positive standard error (*bars*) are presented. The missing sampling points show that there was no sampling

Relationship of bacterial production and the contribution of HNA bacterial group

The average monthly values of bacterial production in the central and southern coastal areas ranged from 0.03×10^4 to 2.8×10^4 cells $\text{mL}^{-1}\text{h}^{-1}$ and in the open sea ranged from 0.06×10^4 to 2.71×10^4 cells $\text{mL}^{-1}\text{h}^{-1}$ (Fig. 4). Seasonal distribution of bacterial production in the coastal areas from Zadar to Split shows increased bacterial productivity during the summer. The second highest values were found in the area of Šibenik during the spring and in the area of Split during the winter. In the area of Ploče, high values of bacterial production were found in January and December. In the southern coastal region, increased bacterial production was determined during the seasons of spring and autumn. Distribution at the open sea stations CA001 and CA003 showed increased bacterial productivity in the spring and at CA009, during winter and autumn (Fig. 4).

Comparing the investigated areas with the annual average, variations in bacterial production in the coastal and open sea areas were more pronounced in the area influenced by rivers Krka (Šibenik area) and less pronounced in the southern part of coastal sea (Table 2).

In order to examine bacterial activity, this study observed bacterial productivity in relation to seasonal distribution of

the HNA and LNA bacterial groups. The analysis of these two bacterial groups in relation to bacterial productivity in the coastal and open sea regions showed a simultaneous increase in the percentage of the LNA group and bacterial production (Figs. 3, 4). However, exceptions with a prevalence of the HNA group in the bacterial community during the highest values of bacterial productivity were found in those areas influenced by the Krka and Jadro Rivers (Figs. 3, 4). A comparison of all of the investigated areas of the one-year survey showed that the HNA bacterial group was dominant in the bacterial population in coastal areas over values of bacterial production ranging from 0.27×10^4 to 0.89×10^4 cells $\text{mL}^{-1}\text{h}^{-1}$. However, in the open sea areas, the LNA bacterial group was mostly prevalent in the bacterial population over values of bacterial production ranging from 0.53×10^4 to 0.89×10^4 cells $\text{mL}^{-1}\text{h}^{-1}$ (Table 2).

Biomass of non-pigmented bacteria in relation to biomasses of *Synechococcus* and *Prochlorococcus*

The biomass of the prokaryotic community throughout the studied area was mainly dominated by non-pigmented prokaryotes, except during the spring in the Dubrovnik area and during winter at the open sea station CA003, when the

Table 2 Abundance of non-pigmented bacteria, % HNA and bacterial production at coastal areas Zadar, Šibenik, Kaštela Bay, Split, Ploče, Dubrovnik and open sea stations (CA001, CA003 and CA009)

	Zadar	Šibenik	Kaštela Bay	Split	Ploče	Dubrovnik	CA001	CA003	CA009
Non-pigmented bacteria ($\times 10^6$ cell ml^{-1})	0.52 \pm 0.14	0.54 \pm 0.49	0.77 \pm 0.42	0.51 \pm 0.18	0.55 \pm 0.23	0.40 \pm 0.13	0.35 \pm 0.11	0.36 \pm 0.09	0.36 \pm 0.12
Bacterial production ($\times 10^4$ cell ml^{-1} h^{-1})	0.73 \pm 0.71	0.89 \pm 1.46	0.75 \pm 0.77	0.60 \pm 0.80	0.26 \pm 0.20	0.27 \pm 0.28	0.78 \pm 1.00	0.89 \pm 1.10	0.53 \pm 0.58
HNA %	58.7 \pm 18.68	56.70 \pm 17.46	66.77 \pm 12.54	54.42 \pm 17.76	50.33 \pm 15.17	56.51 \pm 21.56	44.38 \pm 14.66	44.95 \pm 11.51	51.45 \pm 16.79

Average annual values and standard deviation (\pm) are presented

biomass of autotrophic prokaryotes predominated over non-pigmented prokaryotes (Fig. 5). Average seasonal values of non-pigmented prokaryotic biomass in the coastal area ranged from 7.87 to 22.27 $\mu\text{gC L}^{-1}$ and in the open sea from 4.60 to 9.60 $\mu\text{gC L}^{-1}$. The values of the biomasses as well as their abundances showed seasonality, mostly demonstrating higher values during the warmer seasons (Fig. 5).

Influencing factors on prokaryotic picoplankton

In the investigated area, correlations between bacterial parameters with temperature and inorganic nutrients were weak or not statistically significant (Table 3). The relationship between HNF as a main predator of bacteria and the bacteria was described by distance points (D) obtained by the Gasol model (1994). Thus, lower D values show higher predation pressure and vice versa. Results indicate that the impact of HNF was more pronounced during the warmer season (Fig. 6). Comparing coastal and open sea areas, the strongest associations between HNF and the bacteria were found in the coastal areas of Šibenik, Ploče and Dubrovnik (Fig. 6).

In addition to a predator–prey relationship, other relationships within the prokaryotic community in the study area were also identified. In both thermal conditions of the water column, bacterial abundance was positively correlated with *Prochlorococcus* abundance (Table 3). During colder periods, in the coastal sea areas, a positive correlation between bacterial abundance and concentration of chlorophyll *a* (chl *a*) was determined. During warmer periods, in the open sea areas, a negative correlation between bacterial production and concentration of chl *a* was observed.

Discussion

The abundance of non-pigmented bacteria in most parts of the study area was lower than 10^6 cells mL^{-1} . According to bacterial density, the investigated area of the Adriatic Sea is mainly oligotrophic (Cotner and Biddanda 2002). The exceptions are those areas under direct influence of the Rivers Krka (Šibenik area) and Jadro (Kaštela Bay), where abundances of non-pigmented bacteria were more than 10^6 cells ml^{-1} . The obtained values of chlorophyll *a* were in a range from 0.02 to 4.73 chl *a* m^{-3} with the highest values in Kaštela Bay (1.53 chl *a* m^{-3}) and the Šibenik area (4.73 chl *a* m^{-3}), which describes the investigated area as mostly oligotrophic with mesotrophic and eutrophic parts (Marasović et al. 2006). During the investigation, seasonal distribution in the bacterial community in the coastal areas was determined with maxima in the spring–summer period

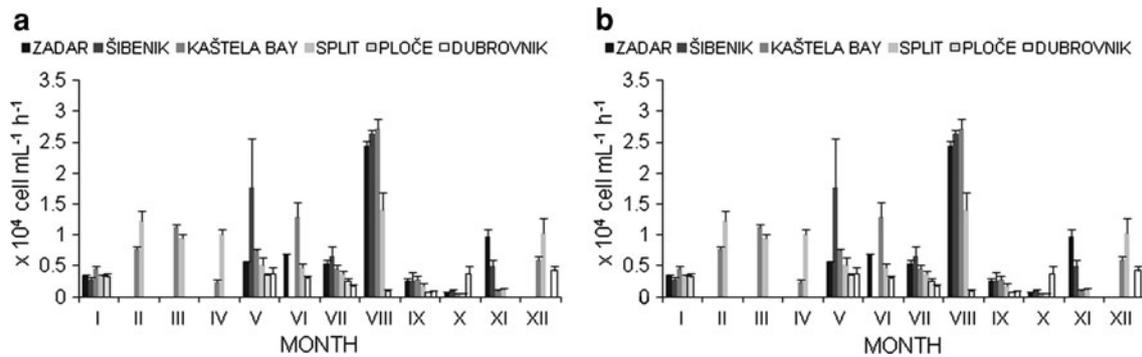


Fig. 4 Seasonal fluctuations of bacterial production. **a** Coastal areas **b** open sea stations. Average values (column, *line with markers*) and positive standard error (*bars*) are presented. The missing sampling points show that there was no sampling

and minima during the winter, which confirms previous reports on the central Adriatic (Krstulović 1992; Šolić et al. 2001). Seasonal distribution of non-pigmented bacteria at the open sea stations was not as pronounced as the seasonal distributions of bacteria in the Bay of Biscay (Calvo-Díaz and Morán 2006).

Generally, within the bacterial community, the HNA group predominated in eutrophic and mesotrophic areas, whilst the LNA group predominated in oligotrophic environments (Zubkov et al. 2004; Morán et al. 2007). The predominance of the LNA group in oligotrophic conditions can be explained by the high surface area to volume ratio of the cell (Button 1998; Jochem et al. 2004), which facilitates successful survival in poor conditions. However, the prevalence of an individual bacterial group in different environmental conditions could be the result of different life strategies. Members of the HNA bacterial group with large genomes are able to cope with a wide variety of environmental conditions, whilst the LNA group with a small genome occupy narrow ecological niches (Schattenhofer et al. 2011). In this paper, the domination of the HNA bacterial group in the bacterial community was established in areas that have a high trophic level and which are directly influenced by rivers. Thus, our finding is consistent with other research that found that rivers directly influence the dominance of the HNA over the LNA group in eutrophic areas (Li et al. 1995; Šolić et al. 2009). Furthermore, the annual average of the investigated oligotrophic open sea stations showed percentages of HNA bacteria below 50 %. This is in accordance with the predominance of the LNA group over HNA, which has also been established for other oligotrophic areas, such as the Celtic Sea, Gulf of Mexico and the Atlantic (Zubkov et al. 2001; Jochem et al. 2004; Andrade et al. 2007).

The seasonal distribution in all investigated oligotrophic areas showed the dominance of the LNA group in the bacterial community mostly during the summer season and dominance of the HNA group during the colder seasons, as described previously in the literature (Grégori et al. 2001;

Calvo-Díaz and Morán 2006). The domination of LNA cells during the warmer seasons can be attributed to selective grazing (Šimek and Chrzanowski 1992; Jürgens and Matz 2002), or better adaptation to oligotrophic conditions (Kjelleberg et al. 1993).

Historically, it was thought that the HNA bacteria were the active part of the microbial community, and the LNA were the inactive or dead part (Zweifel and Hagström 1995; Gasol and Morán 1999), and recent studies have confirmed the importance of the activity of the HNA group in the NW Mediterranean Sea (Mével et al. 2008). However, a number of papers have shown that the LNA group also contributes to the activity of bacterioplankton in the marine ecosystem (Grégori et al. 2001; Li and Dickie 2001; Zubkov et al. 2001; Jochem et al. 2004). Some authors have also shown that the LNA group successfully consumes nutrients and amino acids (Zubkov et al. 2006; Mary et al. 2006) and demonstrates a high-specific growth rate that can exceed the growth rate of HNA bacteria in oligotrophic conditions (Longnecker et al. 2005; Mary et al. 2006). In this study, bacterial activity was observed through seasonal distribution of bacterial productivity in relation to the percentage of the HNA and LNA bacterial groups. At the same time, it was determined that LNA values were higher than 50 % with increased values of bacterial production, which is consistent with previous results from the Atlantic (Morán et al. 2002, 2004). However, in the areas directly influenced by karstic rivers, the HNA bacterial group was dominant throughout the study as well as in areas directly influenced by inflowing rivers (Casotti et al. 2000; Jochem et al. 2004) during all four seasons, as reported by Longnecker et al. (2006). Our results indicate the relative importance of both bacterial groups in bacterial production and activity.

Data regarding abundance and seasonal distribution of *Synechococcus* and *Prochlorococcus* for the same period and same investigated stations have been previously published (Šantić et al. 2011). This paper showed the relationship between biomasses of autotrophic and

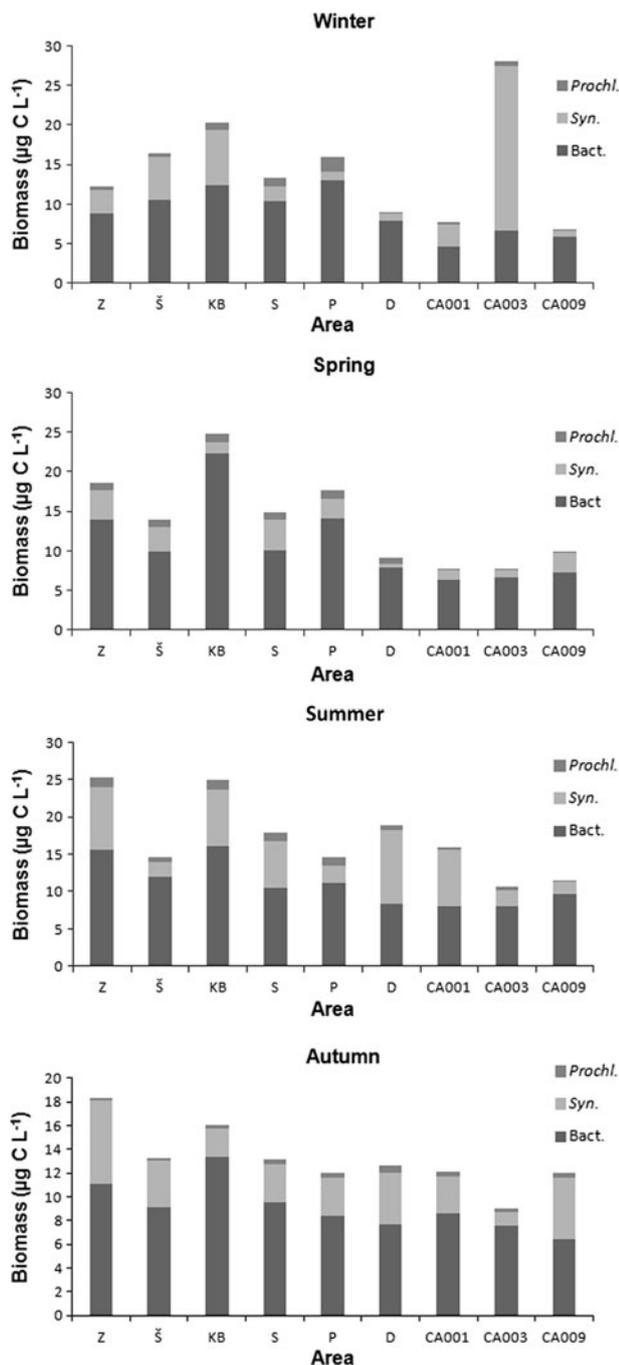


Fig. 5 Seasonal fluctuations of *Synechococcus*, *Prochlorococcus* and bacterial biomasses. Coastal areas: Zadar (Z), Šibenik (Š), Kaštela Bay (KB), Split (S), Ploče (P), Dubrovnik (D) and open sea stations (CA001, CA003, and CA009). Average values (stacked column) are presented

non-pigmented prokaryotes. Analysis of biomass in the prokaryotic community usually shows an increase of autotrophic picoplankton biomass in investigated sites, as well as in some research areas of the Mediterranean Sea, during the warmer season (Agawin et al. 2000). In relation to coastal areas, an increase of autotrophic prokaryotic

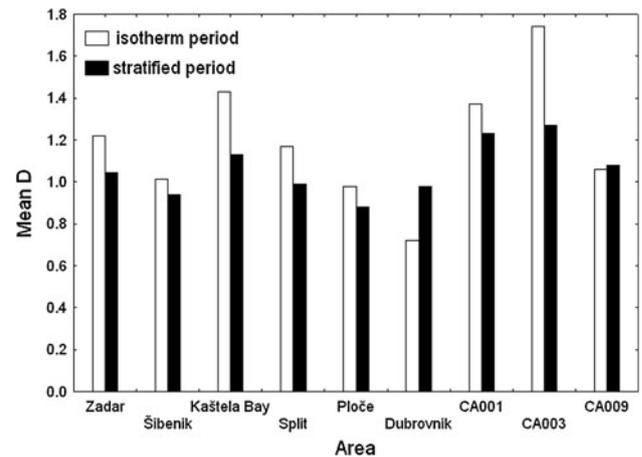


Fig. 6 Relationship between HNF and heterotrophic bacteria abundance according to D values

biomass was found in the open sea as well as in the Mediterranean Sea (Magazzù and Decembri 1995). Within the autotrophic fraction, it was found that *Synechococcus* biomass as well as abundance predominated over *Prochlorococcus*, as reported in the literature for the summer period in the surface layers of the Mediterranean Sea (Llabrés et al. 2010). This can be compared with data from the Coral Sea (Crosbie and Furnas 2001), Pacific and the Atlantic (Zhang et al. 2008). However, our results are contrary to those from different regions of the Mediterranean Sea (Mella-Flores et al. 2011), where abundances of *Prochlorococcus* mostly predominated over *Synechococcus*. The difference in domination of *Synechococcus* over the *Prochlorococcus* densities in the investigated areas of the Adriatic Sea could be explained by the fact that generally genus *Synechococcus* is known as a eurythermal organism (Waterbury et al. 1986; Shapiro and Haugen 1988; Neuer 1992). It is adapted on water-column mixing (Lindell and Post 1995) and is able to consume different types of nitrogen and phosphorus sources, which possibly gives it an advantage over genus *Prochlorococcus* in the coastal study area of the Adriatic Sea.

Our results show that predation has an important role in the observed seasonal and spatial distributions of non-pigmented bacteria. We identified changes in the degree of predation as a type of control on the seasonal scale, in the coastal areas where bacteria were more controlled by HNF during the warmer period, as also reported by Šolić et al. (2009). On the spatial scale, our results show the strongest correlation between bacteria and HNF in the coastal area, especially in the region of Šibenik and Ploče, which is described as the area richest in nutrients (Cetinić et al. 2006; Svendsen et al. 2007; Šolić et al. 2008). Our obtained results agree with Hobbie and Cole (1984) who highlighted the impact of predation by HNF in nutrient-rich environments.

Table 3 Pearson's correlations between the nutrient concentrations, chlorophyll *a*, *Prochlorococcus* abundance and the abundance of non-pigmented bacteria (B) and bacterial production (BP) during isothermal and stratified water column period

Complete data set	Temperature (°C)	Salinity	Nitrate (μM)	Nitrite (μM)	Ammonium (μM)	SRP* (μM)	<i>Prochlorococcus</i> (×10 ³ cells mL ⁻¹)	Chl <i>a</i> (mg m ⁻³)
Coastal sea, <i>N</i> = 131, <i>P</i> < 0.05 (isothermal period)								
Pearson's correlation with B	0.24	-0.22	0.09	-0.07	0.05	-0.23	0.22	0.87
Pearson's correlation with BP	0.09	0.06	-0.22	-0.22	0.06	0.09	-0.37	-0.26
Coastal sea, <i>N</i> = 239, <i>P</i> < 0.05 (stratified period)								
Pearson's correlation with B	0.12	0.01	-0.14	-0.26	-0.30	0.05	0.31	-0.03
Pearson's correlation with BP	-0.01	0.01	0.02	0.14	0.05	0.04	-0.15	0.04
Open sea, <i>N</i> = 45, <i>P</i> < 0.05 (isothermal period)								
Pearson correlation with B	0.19	-0.13	-0.26	0.22	0.10	-0.10	0.51	0.21
Pearson's correlation with BP	0.44	-0.06	-0.53	-0.26	-0.04	0.09	-0.14	0.68
Open sea, <i>N</i> = 103, <i>P</i> < 0.05 (stratified period)								
Pearson's correlation with B	0.14	-0.18	-0.32	-0.02	0.22	-0.27	0.29	-0.08
Pearson's correlation with BP	-0.12	-0.06	-0.27	0.07	-0.04	-0.07	-0.02	-0.38

Statistically significant correlations are presented bold

* SRP soluble reactive phosphate

In oligotrophic ecosystems, bacterial abundance is mostly controlled by the supply of nutrients (Cole et al. 1988; Ducklow 1992). According to the literature, the correlation between bacteria and phytoplankton excretion, like food source, is well known (Carlson et al. 1996; Kirchman and Rich 1997). Similar correlations with the concentration of chlorophyll *a* determined in this paper have also been reported in previous papers for the oligotrophic central Adriatic (Krstulović et al. 1997; Šestanović et al. 2004). The very weak relationship between bacteria and inorganic nutrients obtained in this paper could be explained by the fact that, during the investigated period, the warmer and more saline Levantine Intermediate Water influenced the deep waters in the eastern middle Adriatic Sea. This brings higher concentrations of inorganic nutrients (Šolić et al. 2008), and therefore, bacterial expansion was not limited by low concentrations of inorganic nutrients.

Conclusions

The proportions of HNA bacteria in total abundance of non-pigmented bacteria were highest in those areas with the highest bacterial production (in the areas influenced by karstic rivers) and the higher bacterial densities, during all investigated seasons. In oligotrophic areas, the LNA group dominated for a period of high bacterial production, indicating the importance of both bacterial groups in bacterial production and activity. The biomass of non-pigmented prokaryotes was mostly predominant in the prokaryotic community. Within the autotrophic community, *Synechococcus* biomass mostly predominated. Predation of

heterotrophic bacteria by HNF was pronounced during the warmer period in the coastal areas.

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References

- Agawin NSR, Agustí S (1997) Abundance, frequency of dividing cells and growth rates of *Synechococcus* sp. (cyanobacteria) in the stratified Northwest Mediterranean Sea. *J Plankton Res* 19:1599–1615
- Agawin NSR, Duarte CM, Agustí S (2000) Nutrient and temperature control of the contribution of picoplankton to phytoplankton biomass and production. *Limnol Oceanogr* 45:591–600
- Andrade L, Gonzales AM, Rezende CE, Suzuki M, Valentin JL, Paranhos R (2007) Distribution of HNA and LNA bacterial groups in the Southwest Atlantic Ocean. *Braz J Microbiol* 38:330–336
- Button DK (1998) Nutrient uptake by microorganisms according to kinetic parameters from theory as related to cytoarchitecture. *Microbiol Mol Biol Rev* 62:636–645
- Calvo-Díaz A, Morán XAG (2006) Seasonal dynamics of picoplankton in shelf waters of the southern Bay of Biscay. *Aquat Microb Ecol* 42:159–174
- Carlson CA, Ducklow HW, Sleeter TD (1996) Stocks and dynamics of bacterioplankton in the northwestern Sargasso Sea. *Deep-Sea Res II* 4:491–516
- Casotti R, Brunet C, Aronne B, D'Alcalà MR (2000) Mesoscale features of phytoplankton and planktonic bacteria in a coastal area as induced by external water masses. *Mar Ecol Prog Ser* 195:15–27
- Cetinić I, Viličić D, Burić Z, Olujić G (2006) Phytoplankton seasonality in a highly stratified karstic estuary (Krka, Adriatic Sea). *Hydrobiologia* 555:31–40

- Chisholm SW, Olson RJ, Zettler ER, Waterbury JB, Goericke R, Welschmeyer N (1988) A novel free-living prochlorophyte occurs at high cell concentrations in the oceanic euphotic zone. *Nature* 334:340–343
- Cole JJ, Findlay S, Pace ML (1988) Bacterial production in fresh and saltwater ecosystems: a cross-system overview. *Mar Ecol Prog Ser* 43:1–10
- Cotner JB, Biddanda BA (2002) Small players, large role: microbial influence on auto-heterotrophic coupling and biogeochemical processes in aquatic ecosystems. *Ecosystems* 5:105–121
- Crosbie ND, Furnas MJ (2001) Abundance, distribution and flow-cytometric characterization of picophytoprokarote populations in central (17°S) and southern (20°S) shelf waters of the Great Barrier Reef. *J Plankton Res* 23:809–828
- Ducklow HW (1992) Factors regulating bottom-up control of bacterial biomass in open ocean plankton communities. *Archiv für Hydrobiologie, Organ der Internationalen Vereinigung für Theoretische und Angewandte Limnologie, Beihefte. Ergebnisse der Limnologie* 37:207–217
- Ducklow HW, Purdie DA, Williams PJL, Davis JM (1986) Bacterioplankton: a sink for carbon in a coastal marine plankton community. *Science* 232:865–867
- Fuhrman JA, Azam F (1982) Thymidine incorporation as a measure of heterotrophic bacterioplankton production in marine surface waters. *Mar Biol* 66:109–120
- Fuks D, Radić J, Radić T, Najdek M, Blažina M, Degobbi D, Smolaka N (2005) Relationships between heterotrophic bacteria and cyanobacteria in the northern Adriatic in relation to the mucilage phenomenon. *Sci Total Environ* 353:178–188
- Gasol JM (1994) A framework for the assessment of top-down vs. bottom-up control of heterotrophic nanoflagellates abundance. *Mar Ecol Prog Ser* 113:291–300
- Gasol JM, Del Giorgio PA (2000) Using flow cytometry for counting natural planktonic bacteria and understanding the structure of planktonic bacterial communities. *Sci Mar* 64:197–224
- Gasol JM, Morán XAG (1999) Effects of filtration on bacterial activity and picoplankton community structure as assessed by flow cytometry. *Aquat Microb Ecol* 16:251–264
- Grasshof K (1976) *Methods of seawater analysis*. Verlag Chemie, Weinheim
- Grégori G, Citterio S, Ghiani A, Labra M, Scorbati SB, Denis M (2001) Resolution of viable and membrane-compromised bacteria in fresh water and marine waters based on analytical flow cytometry and nucleic acid double staining. *Appl Environ Microbiol* 67:4662–4670
- Hobbie JE, Cole JJ (1984) Response of a detrital foodweb to eutrophication. *B Mar Sci* 35:357–363
- Jochem FJ (2001) Morphology and DNA content of bacterioplankton in the northern Gulf of Mexico: analysis by epifluorescence microscopy and flow cytometry. *Aquat Microb Ecol* 25:179–194
- Jochem FJ, Lavrentyev PJ, First MR (2004) Growth and grazing rates of bacteria groups with different apparent DNA content in the Gulf of Mexico. *Mar Biol* 145:1213–1225
- Jürgens K, Matz C (2002) Predation as a shaping force for the phenotypic and genotypic composition of planktonic bacteria. *Antonie Leeuwenhoek* 81:413–434
- Kirchman DL, Rich JH (1997) Regulation of bacterial growth rates by dissolved organic carbon and temperature in the equatorial Pacific Ocean. *Microb Ecol* 33:11–20
- Kjelleberg S, Albertson N, Flaerdh K, Holmquist L, Jouper-Jaan A, Marouga R, Oestling J, Svenblad B, Weichart D (1993) How do nondifferentiating bacteria adapt to starvation? *Antonie Leeuwenhoek* 63:333–341
- Krstulović N (1992) Bacterial biomass and production rates in the central Adriatic. *Acta Adriat* 33:49–65
- Krstulović N, Šolić M, Marasović I (1997) Relationship between bacteria, phytoplankton and heterotrophic nanoflagellates along the trophic gradient. *Helgol Mar Res* 51:433–443
- Li WKW, Dickie PM (2001) Monitoring phytoplankton, bacterioplankton and virioplankton in a coastal inlet (Bedford Basin) by flow cytometry. *Cytometry* 44:236–246
- Li WKW, Jellett JF, Dickie PM (1995) DNA distribution in planktonic bacteria stained with TOTO or TO-PRO. *Limnol Oceanogr* 40:1485–1495
- Lindell D, Post AF (1995) Ultraphytoplankton succession is triggered by deep winter mixing in the Gulf of Aqaba (Eilat), Red Sea. *Limnol Oceanogr* 40:1130–1141
- Llabres M, Agustí S, Alonso-Laita P, Herndl GJ (2010) *Synechococcus* and *Prochlorococcus* cell death induced by UV radiation and the penetration of lethal UVR in the Mediterranean Sea. *Mar Ecol Prog Ser* 399:27–37
- Longnecker K, Sherr BF, Sherr EB (2005) Activity and phylogenetic diversity of bacterial cells with high and low nucleic acid content and electron transport system activity in an upwelling ecosystem. *Appl Environ Microb* 71:7737–7749
- Longnecker K, Sherr BF, Sherr EB (2006) Variation in cell-specific rates of leucine and thymidine incorporation by marine bacteria with high and with low nucleic acid content off the Oregon coast. *Aquat Microb Ecol* 43:113–125
- Magazzù G, Decembri F (1995) Primary production, biomass and abundance of phototrophic picoplankton in the Mediterranean Sea: a review. *Aquat Microb Ecol* 9:97–104
- Marasović I, Ninčević-Gladan Ž, skejić S, Bužančić M (2006) *Biološke osobine u Kušpilić G i sur. Kontrola kakvoće obalnog mora (Projekt Vir-Konavle 2005)*. Studije i elaborati Instituta za oceanografiju i ribarstvo, Split, pp 68–81
- Marie D, Partensky F, Jacquet S, Vault D (1997) Enumeration and cell cycle analysis of natural populations of marine picoplankton by flow cytometry using the nucleic acid stain SYBR Green I. *Appl Environ Microb* 63:186–193
- Mary I, Heywood JL, Fuchs BM, Amann R, Burkill PH, Tarran GA, Zubkov MV (2006) SAR11 dominance among metabolically active low nucleic acid bacterioplankton in surface waters along an Atlantic meridional transect. *Aquat Microb Ecol* 45:107–113
- Mella-Flores D, Mazard S, Humily F et al (2011) Is the distribution of *Prochlorococcus* and *Synechococcus* ecotypes in the Mediterranean Sea affected by global warming? *Biogeosciences* 8:2785–2804
- Mével G, Vernet M, Goutx M, Ghiglione JF (2008) Seasonal to hour variation scales in abundance and production of total and particle-attached bacteria in the open NW Mediterranean Sea (0–1000 m). *Biogeosciences* 5:1573–1586
- Morán XAG, Gasol JM, Pedrós-Alió C, Estrada M (2002) Partitioning of phytoplanktonic organic carbon production and bacterial production along a coastal-offshore gradient in the NE Atlantic during different hydrographic regimes. *Aquat Microb Ecol* 29:239–252
- Morán XAG, Fernández E, Pérez V (2004) Size-fractionated primary production, bacterial production and net community production in subtropical and tropical domains of the oligotrophic NE Atlantic in autumn. *Mar Ecol Prog Ser* 274:17–29
- Morán XAG, Bode A, Suárez LÁ, Nogueira E (2007) Assessing the relevance of nucleic acid content as an indicator of marine bacterial activity. *Aquat Microb Ecol* 46:141–152
- Morović M i sur (2006) *Fizikalne i kemijske osobine u Kušpilić G i sur. Kontrola kakvoće obalnog mora (Projekt Vir-Konavle 2005)*. Studije i elaborati Instituta za oceanografiju i ribarstvo, Split, pp 29–46
- Neuer S (1992) Growth dynamics of marine *Synechococcus* spp in the Gulf of Alaska. *Mar Ecol Prog Ser* 83:251–262

- Ninčević Gladan Ž, Marasović I, Kušpilić G, Krstulović N, Šolić M, Šestanović S (2006) Abundance and composition of picoplankton in the mid Adriatic Sea. *Acta Adriatic* 47:127–140
- Olson RJ, Zettler ER, DuRand MD (1993) Handbook of methods in aquatic microbial ecology. In: Kemp PF, Sherr BF, Sherr EV, Cole JJ (eds) *Phytoplankton analysis using flow cytometry*. Lewis Publishers, Boca Raton
- Porter KG, Feig YS (1980) The use of DAPI for identifying and counting aquatic microflora. *Limnol Oceanogr* 25:943–948
- Radić T, Šilović T, Šantić D, Fuks D, Mičić M (2009) Preliminary flow cytometric analyses of phototrophic pico- and nanoplankton communities in the Northern Adriatic. *Fresenius Environ Bull* 18:715–724
- Riemann B, Bjorsen PK, Newell S, Fallon R (1987) Calculation of cell production of coastal marine bacteria based on measured incorporation of (H)thymidine. *Limnol Oceanogr* 32:471–476
- Šantić D, Krstulović N, Šolić M, Kušpilić G (2011) Distribution of *Synechococcus* and *Prochlorococcus* in the central Adriatic Sea. *Acta Adriatic* 52:101–114
- Schattenhofer M, Wulf J, Kostadinov I, Glöckner FO, Zubkov MV, Fuchs BM (2011) Phylogenetic characterisation of picoplanktonic populations with high and low nucleic acid content in the North Atlantic Ocean. *Syst Appl Microbiol* 34:470–475
- Šestanović S, Šolić M, Krstulović N, Ninčević Ž (2004) Seasonal and vertical distribution of planktonic bacteria and heterotrophic nanoflagellates in the middle Adriatic Sea. *Helgol Mar Res* 58:83–92
- Shapiro LP, Haugen EM (1988) Seasonal distribution and tolerance of *Synechococcus* in Boothbay harbor. *Maine Estuar Coast Shelf Sci* 26:517–525
- Šilović T, Ljubešić Z, Mihanović H, Olujić G, Terzić S, Jakšić Ž, Viličić D (2011) Picoplankton composition related to thermohaline circulation: the Albanian boundary zone (southern Adriatic) in late spring. *Estuar Coast Shelf Sci* 91:519–525
- Šimek K, Chrzanowski TH (1992) Direct and indirect evidence of the size selective grazing on pelagic bacteria by freshwater nanoflagellates. *Appl Environ Microb* 58:3715–3720
- Šolić M, Krstulović N, Šestanović S (2001) The roles of predation, substrate supply and temperature in controlling bacterial abundance: interaction between spatial and seasonal scale. *Acta Adriatic* 42:35–48
- Šolić M, Krstulović N, Vilibić I, Kušpilić G, Šestanović S, Šantić D, Ordulj M (2008) The role of water mass dynamics in controlling bacterial abundance and production in the middle Adriatic Sea. *Mar Environ Res* 65:388–404
- Šolić M, Krstulović N, Vilibić I, Bojanić N, Kušpilić G, Šestanović S, Šantić D, Ordulj M (2009) Variability in the bottom-up and top-down control of bacteria on trophic and temporal scale in the middle Adriatic Sea. *Aquat Microb Ecol* 58:15–29
- Svensen C, Viličić D, Wassmann P, Arashkevich E, Ratkova T (2007) Plankton distribution and vertical flux of biogenic matter during high summer stratification in the Krka estuary (Eastern Adriatic). *Estuar Coast Shelf Sci* 71:381–390
- Waterbury JB, Watson S, Guillard RRL, Brand LE (1979) Widespread occurrence of a unicellular, marine, planktonic, cyanobacterium. *Nature* 277:293–294
- Waterbury JB, Watson S, Valois FW, Franks DG (1986) Biological and ecological characterization of the marine unicellular cyanobacterium *Synechococcus*. *Can J Fish Aquat Sci* 214:71–120
- Zhang Y, Jiao NZ, Hong N (2008) Comparative study of picoplankton biomass and community structure in different provinces from subarctic to subtropical oceans. *Deep-Sea Res II* 55:1605–1614
- Zubkov MV, Fuchs BM, Burkill PH, Amann R (2001) Comparison of cellular and biomass specific activities of dominant bacterioplankton groups in stratified waters of the Celtic Sea. *Appl Environ Microb* 67:5210–5218
- Zubkov MV, Allen JI, Fuchs BM (2004) Coexistence of dominant groups in marine bacterioplankton community—a combination of experimental and modelling approaches. *J Mar Biol Assoc UK* 84:519–529
- Zubkov MV, Tarran GA, Burkill PH (2006) Bacterioplankton of low and high DNA content in the suboxic waters of the Arabian Sea and the Gulf of Oman: abundance and amino acid uptake. *Aquat Microb Ecol* 43:23–32
- Zweifel UL, Hagström Å (1995) Total counts of marine bacteria include a large fraction of non-nucleoid-containing bacteria (ghosts). *Appl Environ Microb* 61:2180–2185