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Dynamics of prokaryotic community in Boka Kotorska Bay (South-eastern Adriatic Sea)

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The dynamics of the prokaryotic picoplankton community were studied at six stations of three embayments in Boka Kotorska Bay from January 2010 to January 2011. The abundance of non-pigmented bacteria, high nucleic acid content (HNA) bacteria, low nucleic acid content (LNA) bacteria, bacterial production and heterotrophic nanoflagellates was determined, as well as the chlorophyll a, physical and chemical factors of the water column. It seems that freshwater input has the ability to control bacterial abundance as well as the proportion of HNA cells in bacterial community and thus the abundance of HNF cells. During the warmer seasons, in the investigated area, which is mainly oligotrophic, we found an increase in values and domination of the LNA group in the bacterial population. The dominance of the HNA group was found only during the colder seasons. Weak coupling between bacteria and HNF suggests that predation (top down control) is not dominant in controling of bacterial abundance in studied area.

Key words: heterotrophic bacteria, HNA, LNA, bacterial production, heterotrophic nanoflagellates, Boka Kotorska Bay

INTRODUCTION

Picoplankton is the dominant component of the planktonic food webs in a large number of marine ecosystems (CHRISTAKI et al., 2001). Non-pigmented bacteria are generally the most abundant component of the 0.2–2 μm size fraction and a major pathway in the flux of material and energy in pelagic marine ecosystems (AZAM & CHO, 1987; GASOL *et al.*, 1997).

The introduction of flow cytometry into the analysis of prokaryotic community (LI *et al.*, 1995; MARIE *et al.*, 1997) enables discovery of bacterial groups based on different contents of DNA and different amounts of fluorescence: a high nucleic acid content group with a high amount of fluorescence (HNA) and a group with low nucleic acid and low amount of fluorescence (LNA) content (GASOL & MORÁN, 1999; GASOL *et al.*, 1999). The general conclusion from the literature is that HNA bacteria are larger and more active on a cell basis than LNA bacteria, but the contri-

bution of LNA cells to total bacterial production is still a subject of much debate (LONGNECKER *et al.*, 2005; MORÁN *et al.*, 2010). Among bacterial predators, heterotrophic nanoflagellates (HNF) have been identified as a major source of bacterial mortality in aquatic ecosystems (CHRISTAKI *et al.*, 2001; ŠOLIĆ & KRSTULOVIĆ, 1994), but predation pressure was found to be dependent on the trophic state of the studied area (KRSTULOVIĆ *et al.*, 1997; ŠOLIĆ *et al.*, 1998, 2001).

The aim of the present study was to describe the seasonal cycles and areal distributions of total bacterial abundances and HNA and LNA bacterial groups. Furthermore, it aimed to indicate the role of HNA and LNA bacteria in bacterial activity and to identify the role of heterotrophic nanoflagellates in controlling the bacterial population in the area of Boka Kotorska Bay (southern Adriatic Sea, Montenegro). The accurate determination of picoplankton abundance, its activity and relationship with HNF is thus essential for understanding the functioning of aquatic ecosystems such as a semi-enclosed bay.

MATERIAL AND METHODS

Study area

Boka Kotorska Bay is a relatively large (87 km^2) semi-enclosed karstic bay situated in the south-east part of the Adriatic Sea. It is formed by three embayments where freshwater input can strongly modify temperature, salinity and current patterns. Karst is present elsewhere, particularly in Kotor Bay, where subaerial and submarine springs, among them Sopot and Ljuta, can reach peak discharges as large as 200 m^3/s in a very short time. Slow circulation of seawater is another very important characteristic of this area (RADULOVIĆ, 2006) with surface outflow circulation and bottom inflow. According to hydrodynamic processes in Boka Kotorska Bay, BELLAFIORE *et al.* (2011) classified Kotor Bay differently from the outermost area, Tivat and Herceg Novi Bay. This generally indicates a slow rate of water exchange between Kotor Bay and the open sea reaching a residence time (RT) of around 70 days during the period of minimum freshwater discharge. The other interesting characteristic is the anticyclone circulation in the central bay – Tivat Bay (BELLAFIORE *et al.*, 2011) – which has RT values of 25 days at the surface and 15 days in the deep layers. Some areas near to the open sea maintain RT values of 5 days at the surface and lower values in the deep layers (BELLAFIORE *et al.,* 2011). From the along-channel section in the Verige Strait the presence of a sill controls the bottom water exchange between Kotor Bay and Tivat Bay.

Sampling

Sampling was carried out monthly from January 2010 to January 2011. Niskin bottles (5 L) were used for sampling between the surface and the near bottom layer at six stations in three embayments: Kotor Bay (A), Tivat Bay (B) and Herceg Novi Bay (C) (Fig. 1). Samples were collected from a small ship at six depths: at the surface, 2 m, 5 m, 10 m, 20 m and 30 m for coastal stations and at the surface, 5 m, 10 m, 20 m, 30 m and 40 m depths at central bay stations. For bacterial production, samples were taken seasonally at the innermost part of Boka Kotorska Bay (OS1) and at central bay stations (C1, C2, C3).

Temperature and salinity were recorded

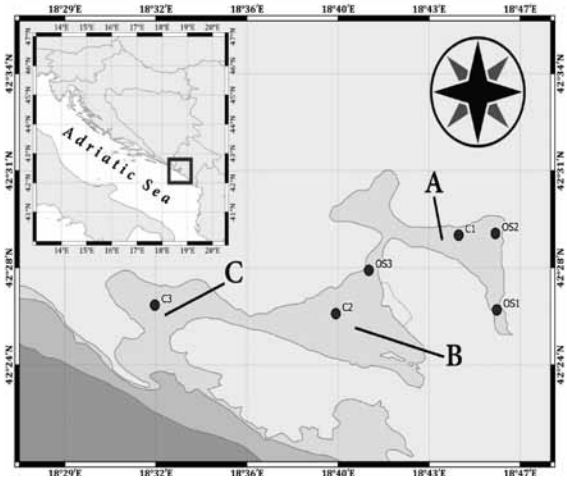

Fig. 1. Study area with sampling stations in the Bay of Kotor (Embayment A) the Bay of Tivat (embayment B) and the Bay of Herceg Novi (embayment C)

in situ with a universal meter (Multiline P4; WTW). Chl *a* was determined as absorbance with a Parkin/Elmer UV/VIS spectrophotometer and calculated according to JEFFREY *et al.* (1997).

Flow cytometry

Abundances of non-pigmented bacteria were determined using flow cytometry (MARIE *et al.*, 1997). For flow cytometry counts of bacteria samples were preserved in 2% formaldehyde and stored at 4 ºC until analysis. Samples of 1 ml were stained with SybrGreen I and without replicas were analysed on a Beckman Coulter EPICS XL-MCL with a high flow rate of between 1 and 1.2 μL sec^{-1}, and the analysed volume was calculated by acquisition time. To standardize the fluorescence intensity of the cells, 1.0 μm 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 (LNA) bacteria and light scattering.

Bacterial cell 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.

Samples for heterotrophic nanoflagellate (HNF) cell counts were preserved with formaldehyde that was filtered in 0.2 μm polycarbonate membranes to a 2% final concentration. In the laboratory samples were stained with 4′-6-diamidino-2-phenylindole (DAPI) for 10 min and filtered through polycarbonate filters with 0.8 μm pore diameters (Millipore, Ireland). The number of HNF was estimated using epifluorescence microscopy. Microscope slides were examined with an Olympus microscope under UV light at a magnification of 1,000 X (PORTER & FEIG, 1980).

Methods for determination of regulation mechanisms of non-pigmented bacteria

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 between bacterial and HNF abundance. The graph includes an empirically determined maximum attainable abundance (MAA) line and a mean realized abundance (MRA) according to the framework proposed by GASOL (1994). 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 bacteria. The point that lies well below the MRA line indicates conditions when bacterial abundance was not controlled by HNF grazing. Therefore, D values (distances between the maximum and realized HNF abundances at different bacterial concentrations) represent a good indicator of the importance of HNF predators in controlling bacterial abundance. 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.

Differences in each dependent variable of the data set were established using the analysis of variance (ANOVA) technique and considered for factor F1 – embayments (A *versus* B *versus* C). All variables were logarithmically transformed to comply with the assumptions of ANOVA. The relationship between abiotic and biotic factors and the abundance of heterotrophic prokaryotes were determined using Pearson's rank correlation index.

Principal component analysis (PCA) was applied to the multivariate chemical, physical and biological data set. This multivariate analysis rotates a cloud of data points such that the maximum variability is visible to identify the

most important gradient over the study period. Varimax rotation was applied to optimize the interpretation of the PCA results. Analyses were performed with STATISTICA software.

RESULTS AND DISCUSSION

During the investigation period the water temperature showed a regular seasonal fluctuation without marked differences among stations and embayments, ranging at the surface from 12.3 ± 1.5 in winter to 24.8 ± 2.6 in summer. The thermal stratification of the water column began in June and lasted until September when the isothermal period began. The exception was the coastal station OS1 where thermal stratification lasted until October due to the influence of the river Škurda. A strong vertical salinity gradient with a flow of fresh water at the surface is seen in the inner part of the bay, particularly for the station OS2 where the salinity varied from 0.6 to 36.4 at the surface. The lowest values and widest ranges of temperature and salinity in Kotor Bay were recorded mostly due to the influence of the river Škurda and the streams Ljuta and Sopot. Applying PCA analysis to the temperature and salinity in Kotor Bay we have separated the surface and subsurface layer (Table 1). Changes in temperature and salinity in the surface layer are connected ($r=0.60$, $p< 0.001$), suggesting a significant effect of freshwater inflow. According to the salinity, Tivat Bay is also characterized by two layers, but the surface layer is deeper than in Kotor Bay, reaching up to 10 m in depth (Table 2). Herceg Novi Bay, according to salinity, has three layers: the surface, subsurface and bottom layer at 40 m depth (Table 3).

In previous studies changes in physical, chemical and biological parameters were mainly dependent on natural factors such as the pattern of rainfall, and according to the Chl *a* and nutrient concentration it can be described as oligo-mesotrophic (KRIVOKAPIĆ *et al.*, 2011). The obtained values of chlorophyll *a* in this study were in a range from 0.07 to 3.51 chl *a* mg/m^3 with a mean value of 0.93 ± 0.45 mg chl *a*/m^3, which describes the investigated area according to KRSTULOVIĆ *et al.* (1998) as a "moderate" chlorophyll area.

Decreasing values of chlorophyll *a* were determined from January to May with minimum values in June, with the highest average concentration at coastal station OS1 of 2.38 mg/m^3 of chlorophyll *a* (Fig. 2).

Table 1. Results of principal component analysis applied to the salinity (a) and temperature (b) at different stations and different depths in the Bay of Kotor. Varimax rotation was applied to optimize the interpretation of the results. Factor F1 describes the surface layer and factor F2 describes the subsurface layer

a)			b)		
Variable	**F1**	**F2**	**Variable**	**F1**	**F2**
C1 0m	**0.833782**	-0.003308	C1 0m	0.218327	**0.957619**
C1 5m	0.110737	**0.959398**	C1 5m	0.503144	**0.830302**
C1 10m	-0.234367	**0.816193**	C1 10m	**0.957907**	0.205126
C1 20m	-0.141729	**0.870871**	C1 20m	**0.983261**	0.142325
C1 30m	0.184515	0.176787	C1 30m	**0.944567**	0.253749
OS1 0m	**0.944741**	-0.083058	OS1 0m	0.171852	**0.975449**
OS1 5m	0.667702	0.506692	OS1 5m	0.676020	**0.704044**
OS1 10m	0.291439	**0.892300**	OS1 10m	**0.859156**	0.463676
OS1 20m	0.647549	0.450300	OS1 20m	**0.811242**	0.310199
OS2 0m	**0.821390**	-0.193609	OS2 0m	0.136750	**0.951401**
OS2 5m	0.277974	0.334474	OS2 5m	0.589939	**0.793250**
OS2 10m	**0.760747**	0.384384	OS2 10m	**0.890983**	0.373324
OS2 20m	0.499584	0.495233	OS2 20m	**0.825899**	0.310500
Expl.Var	4.239406	4.181265	**Expl.Var**	6.802899	5.261745
Prp.Totl	0.326108	0.321636	**Prp.Totl**	0.523300	0.404750

Table 2. Results of principal component analysis applied to the salinity (a) and temperature (b) at different stations and different depths in the Bay of Tivat. Varimax rotation was applied to optimize the interpretation of the results. Factor F1 for salinity describes the surface layer and factor F2 describes the subsurface layer. For temperature, factor F1 describes the subsurface layer and factor F2 describes the surface layer

a)			b)		
Variable	**Factor 1**	**Factor 2**	**Variable**	**Factor 1**	**Factor 2**
OS3 0m	**0,868628**	-0,249661	OS3 0m	0,186179	**0,975901**
OS3 5m	**0,863220**	0,198300	OS3 5m	0,500050	**0,834327**
OS3 10m	**0,618106**	0,372749	OS3 10m	**0,807056**	0,583801
OS3 20m	0,212894	**0,754548**	OS3 20m	**0,960338**	0,241344
OS3 30m	-0,269041	**0,598880**	OS3 30m	**0,968796**	0,213546
C2 0m	**0,930439**	-0,066441	C2 0m	0,144042	**0,977825**
C2 5m	**0,909603**	0,083886	C2 5m	**0,718328**	0,624540
C2 10m	**0,934700**	-0,013916	C2 10m	**0,904543**	0,392634
C2 20m	0,235048	**0,751383**	C2 20m	**0,963811**	0,226473
C2 30m	-0,243161	**0,851055**	C2 30m	**0,975755**	0,149904
Expl.Var	4,680557	2,469109	**Expl.Var**	6,032837	3,667271
Prp.Totl	0,468056	0,246911	**Prp.Totl**	0,603284	0,366727

Table 3. Results of principal component analysis applied to the salinity a) and temperature b)) at different depths in the Bay of Herceg Novi. Varimax rotation was applied to optimize the interpretation of the results. For salinity, factor F1 describes the subsurface layer, factor F2 describes the surface layer and factor F3 describes the bottom layer. For temperature, factor F1 describes the subsurface layer and factor F2 describes the surface layer

a)				b)		
Variable	**Factor 1**	**Factor 2**	**Factor 3**	**Variable**	**Factor 1**	**Factor 2**
C3 0m	-0.115678	**0.971069**	0.064892	C3 0m	0,078015	**0,994416**
C3 5m	0.604056	**0.622350**	-0.431390	C3 5m	0,352742	**0,927807**
C3 10m	**0.634688**	**0.755505**	0.138861	C3 10m	**0,750054**	0,634076
C3 20m	**0.965337**	-0.112287	0.028494	C3 20m	**0,928174**	0,348961
C3 30m	**0.946482**	0.217547	-0.010362	C3 30m	**0,981154**	0,117140
C3 40m	-0.035252	-0.070091	**-0.989599**	C3 40m	**0,938904**	0,167574
Expl.Var	2.610039	1.965930	1.189817	**Expl.Var**	3,398804	2,415318
Prp.Totl	0.435007	0.327655	0.198303	**Prp.Totl**	0,566467	0,402553

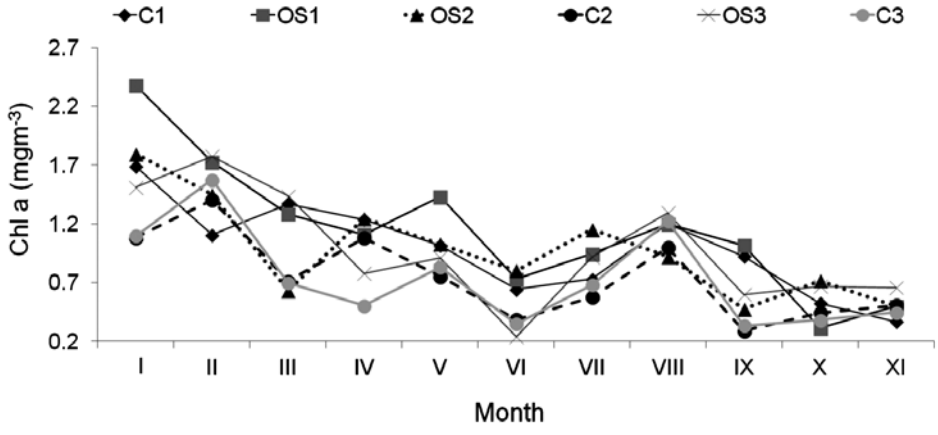

Fig. 2. Mean monthly value of chlorophyll a concentration at coastal and central bay stations

Abundance of non-pigmented bacteria and percentage of HNA and LNA bacteria in non-pigmented bacteria community

The average monthly abundance of non-pigmented bacteria, obtained as the average value from the surface to the bottom layers, ranged from 0.5 x 10^5 to 1.99 x 10^6 cells mL^{-1} (mean value = 0.62 ± 0.32 x 10^6 cells mL^{-1}). The seasonal distribution of non-pigmented bacteria in all the investigated stations has shown an increased abundance during warmer seasons with maximum abundance in August and lower abundance during the colder seasons, which confirms previous reports for coastal areas on the central and southern Adriatic Sea (ŠANTIĆ *et al.*, 2013; ŠOLIĆ *et al.*, 2001) (Fig. 3).

The outcome of the ANOVA carried out for testing the null hypothesis of equality among embayments for all physical and biological parameters is reported in Table 4. Differences among embayments were observed for all the considered variables except temperature and bacterial production.

The relationships among embayments in terms of bacterial abundance are analysed using PCA analysis. Only principal factors (PF) with eigenvalues > 1 are considered. In terms of bacterial abundance, PCA analysis shows a three-layer structure in the Bay of Kotor – subsurface, surface and bottom layer described by factors F1, F2 and F3 (31%, 22% and 25%, respectively) (Table 5). The surface layer by bacterial abundance coincides with the surface layer by salinity. The subsurface layer spreads from 5 m to 10 m and the bottom layer lies at 20 m for coastal stations and from 20 m to 30 m for the deeper central bay station. According to current patterns (BELLAFIORE *et al.*, 2011), there is a three-layer structure in Tivat Bay that does not maintain estuarine flow but registers an inflow in the bay, both from the surface and the bottom layers. According to PCA analysis of bacterial abundance, the water column is stratified into two layers (F1 = 47% and F2 = 34%). The surface layer for station OS3 extends to 10 m, which is coincident with salinity of surface layer (Table 6) due to the strong influence of surface

Table 4. Results of the ANOVA (F-test and P) on surface and subsurface values of chemical, physical and biological parameters among embayments

Variable	Layers	F	P
Temperature	Surface	0.49	n.s.
	Subsurface	0.09	n.s.
Salinity	Surface	17.55	***
	Subsurface	9.02	***
Chlorophyll *a*	Surface	0.52	n.s.
	Subsurface	7.78	***
Bacterial abundance	Surface	0.06	n.s.
	Subsurface	35.37	***
HNF	Surface	1.63	n.s.
	Subsurface	3.37	*
Bacterial production	Surface	0.53	n.s.
	Subsurface	1.49	n.s.

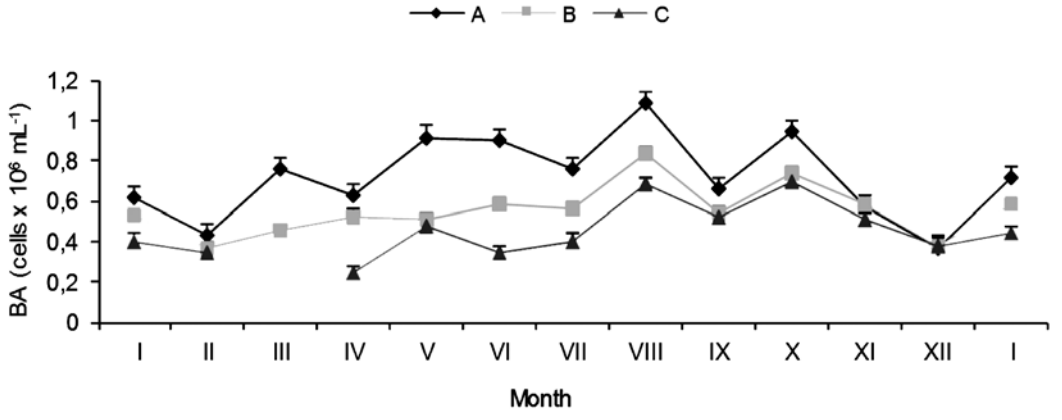

Fig. 3. Seasonal fluctuation of bacterial abundance (BA) the Bay of Kotor (A), the Bay of Tivat (B) and the Bay of Herceg Novi (C). Average values (line) and positive standard error (bars) are presented

Table 5. Results of principal component analysis applied to the bacterial abundance in the Bay of Kotor. Varimax rotation was applied to optimize the interpretation of the results. F1 describes the subsurface layer, factor F2 describes the surface layer and factor F3 describes the bottom layer.

Variable	Factor 1	Factor 2	Factor 3
C1 0m	0,151854	**0,920452**	-0,098605
C1 5m	**0,851187**	0,224631	0,367050
C1 10m	0,557424	-0,257040	0,688173
C1 20m	0,416856	-0,181760	**0,838962**
C1 30m	-0,107595	0,327398	**0,717237**
OS1 0m	0,086778	**0,946042**	0,154948
OS1 5m	**0,829757**	0,196751	-0,143669
OS1 10m	**0,924771**	-0,177527	-0,048882
OS1 20m	0,142348	0,120279	**0,739660**
OS2 0m	-0,040134	**0,790047**	-0,097297
OS2 5m	**0,809156**	0,387227	0,161926
OS2 10m	**0,764937**	-0,015697	0,438465
OS2 20m	-0,163968	0,099997	**0,755065**
Expl.Var	4,083496	2,868041	3,228514
Prp.Totl	0,314115	0,220619	0,248347

Table 6. Results of principal component analysis applied to the bacterial abundance in the Bay of Tivat. Varimax rotation was applied to optimize the interpretation of the results. F1 describes the surface layer and F2 describes the subsurface layer

Variable	Factor 1	Factor 2
OS3 0m	**0,976320**	-0,096647
OS3 5m	**0,903926**	0,069171
OS3 10m	**0,771852**	0,496431
OS3 20m	0,592973	0,522679
OS3 30m3	0,593019	**0,739373**
C2 0m	**0,748918**	-0,414977
C2 5m	**0,844495**	0,300913
C2 10m	-0,065189	**0,938361**
C2 20m	-0,466491	**0,756477**
C2 30m	0,347073	**0,786364**
Expl.Var	4,685702	3,414337
Prp.Totl	0,468570	0,341434

Table 7. Results of principal component analysis applied to the bacterial abundance in the Bay of Herceg Novi. Varimax rotation was applied to optimize the interpretation of the results. F1 describes the subsurface layer and F2 describes the surface and bottom layers

Variable	Factor 1	Factor 2
C3 0m	0,137270	**0,936822**
C3 5m	**0,817917**	0,535234
C3 10m	**0,945698**	0,101586
C3 20m	**0,965092**	-0,185316
C3 30m	**0,719654**	0,379606
C3 40m	-0,010412	**0,965183**
Expl.Var	3,031590	2,284451
Prp.Totl	0,505265	0,380742

outflow circulation through the Verige Channel. In Herceg Novi Bay there is a two-layer structure (Table 7). Factor F1 describes the surface and bottom layer (38%) and factor F2 describes the subsurface layer (51%).

Besides total bacterial abundance, the percentage of HNA bacteria also increased along the oligotrophic-mesotrophic gradient (Fig. 4A, C), and different temporal patterns were found for HNA and therefore for LNA bacterial groups. Seasonal distribution showed the prevalence of the HNA group only during the first three months of 2010 and the prevalence of LNA bacteria, or almost equal proportions of the LNA and HNA groups in the bacterial community, during the rest of the investigated time (Fig. 5). Comparing all the stations with the annual average, variations in the proportion of HNA bacteria were the most pronounced at coastal station OS1 – Kotor Bay, which is under the strongest influence of the river Škurda. For other stations the annual average showed a percentage of HNA bacteria of less than 50% (Fig. 4C). Thus, our findings are consistent with other research that found predominance of the LNA

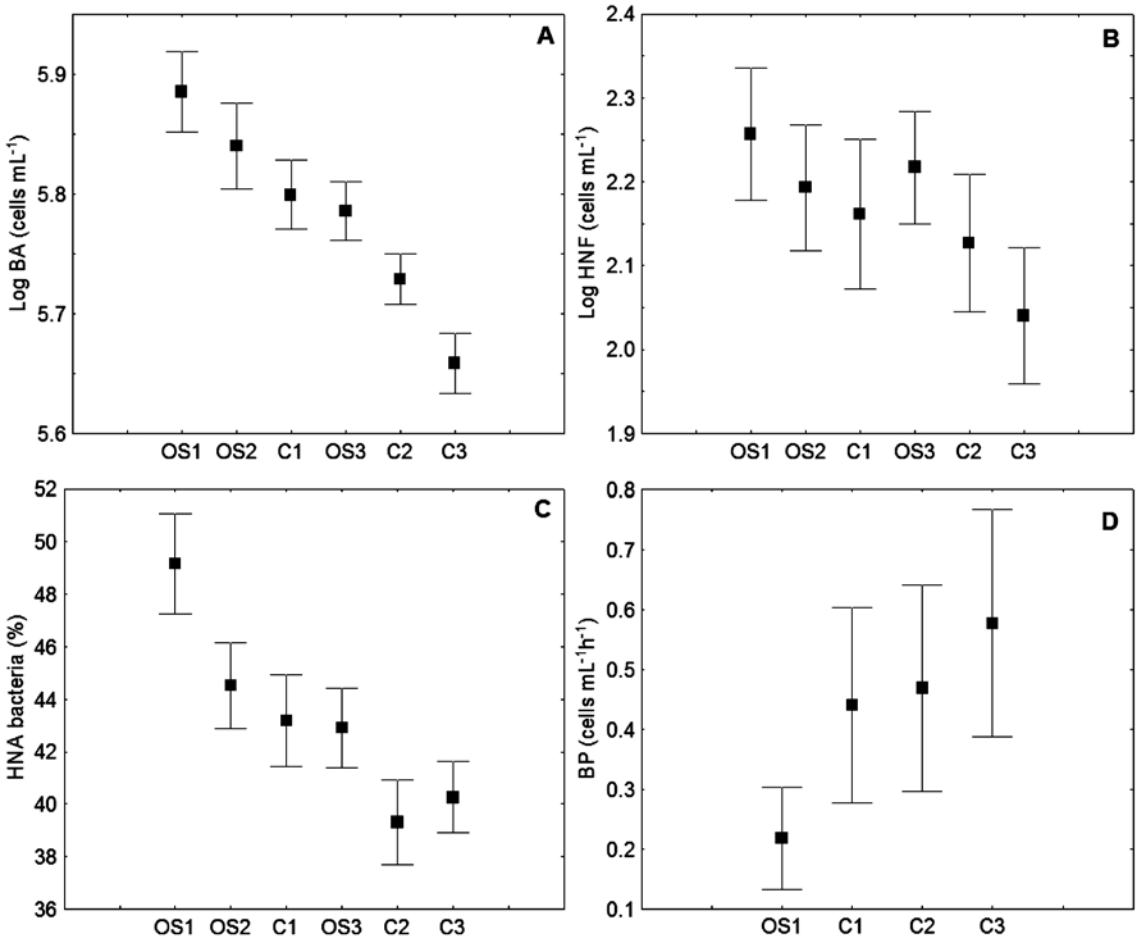

Fig. 4. Mean values (± SE) of bacterial abundance (BA) (A),) heterotrophic nanoflagellate (HNF) (B abundance, percentage of high nucleic acid (HNA) bacteria(C), and bacterial production (BP) (D) at different sampling stations for the period 2010–2011

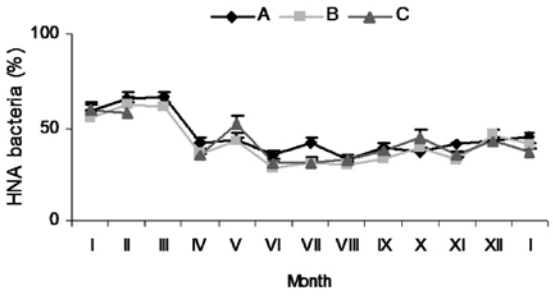

Fig. 5. Seasonal fluctuation of HNA bacteria in the Bay of Kotor (A), the Bay of Tivat (B) and the Bay of Herceg Novi (C)

group over HNA for other oligotrophic areas such as the Celtic Sea, Gulf of Mexico and open Adriatic Sea (JOCHEM *et al.*, 2004.; ŠANTIĆ *et al.*, 2013; ZUBKOV *et al.*, 2001.), as well as dominance of the HNA group during the colder seasons (CALVO-DÍAZ & MORÁN, 2006; ŠANTIĆ *et al.*, 2012)

Although the proportion of HNA bacteria, regarded as the indicator of the activity structure of the bacterial community (LEBARON *et al.*, 2001; SERVAIS *et al.*, 1999), was highest in most inner parts of the bay in station OS1 – Kotor Bay, the highest bacterial production was found in embayments directly connected to the open sea (Fig. 4C–D). The average monthly values of bacterial production in Boka Kotorska Bay ranged from 0.05 x 10^4 to 1.93 x 10^4 cells mL^{-1} h^{-1} with the maximum at the bottom in Herceg Novi Bay (3.85 x 10^4 cells mL^{-1} h^{-1}). Seasonal distribution shows increased bacterial productivity during the autumn and for Herceg Novi Bay during the spring and autumn (Fig. 6), which is consistent with data obtained for the central and southern Adriatic Sea (ŠANTIĆ *et al.*, 2013).

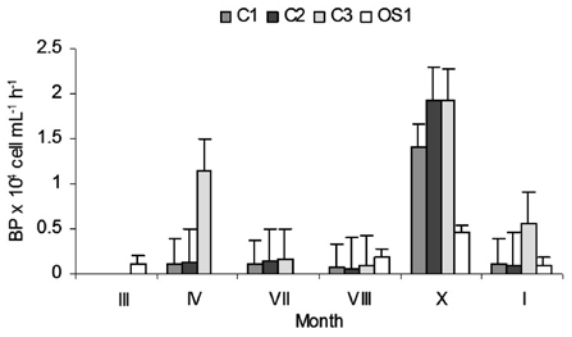

Fig. 6. Seasonal fluctuation of bacterial production (BP). Average values (column, line with markers) and positive standard error (bars) are presented

Bottom-up and top-down control of bacteria

The relationship of HNF as the most important bacterial predators with bacteria we described by distance points (D) obtained by the GASOL (1994) model. Thus lower D values show higher predation pressure on the bacterial population. All pooled data showed that the average value of D increased with increasing bacterial abundance (Fig. 7A–B).. That uncoupling between bacteria and HNF suggests the existence of strong grazing pressure on HNF by

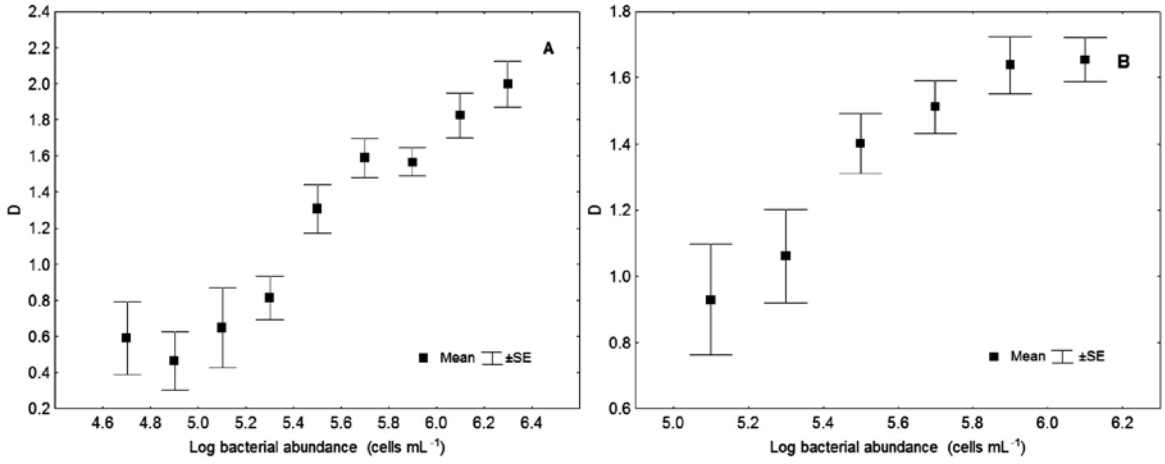

Fig. 7. Relationship between D values (the difference between the maximum and realized heterotrophic nanoflagellate (HNF) abundance at each bacterial concentration) and bacterial abundance (log values grouped in equidistant discrete categories) the Bay of Kotor(A), the Bay of Tivat(B) and the Bay of Herceg Novi

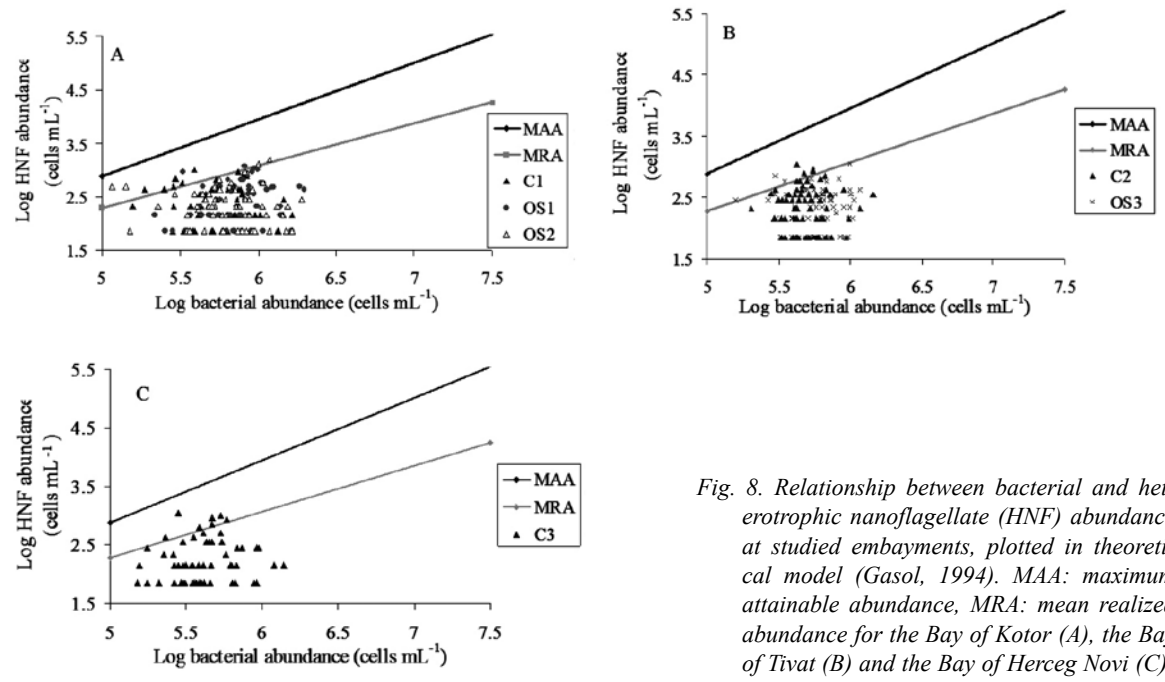

Fig. 8. Relationship between bacterial and heterotrophic nanoflagellate (HNF) abundance at studied embayments, plotted in theoretical model (Gasol, 1994). MAA: maximum attainable abundance, MRA: mean realized abundance for the Bay of Kotor (A), the Bay of Tivat (B) and the Bay of Herceg Novi (C)

ciliates. Similar results were found for the middle Adriatic Sea (ŠOLIĆ *et al.*, 2009). Looking at Fig. 8 (A–C), we can find points that fall close to and below the MRA line for all the studied embayments. Previous research of oligotrophic ecosystems in the Adriatic Sea showed that bacterial abundance is mostly controlled by the supply of nutrients (ŠANTIĆ *et al.*, 2013). It seems that freshwater input has the ability to control bacterial abundance as well as the proportion of HNA cells in the bacterial community and thus the abundance of HNF cells. In terms of the organic matter in Boka Kotorska Bay (DAUTOVIĆ *et al.*, 2012), freshwater inputs that are relatively poor in dissolved organic carbon have the ability to purify the system. Due to the high reactivity of the organic matter present in the area DAUTOVIĆ *et al*. (2012) suggest that organic matter could be removed from the water column fast enough to prevent major organic matter accumulation.

Based on the results obtained for bacterial abundance and mean D values, it seems that the studied area could be considered as oligotrophic with domination of BU control.

Influencing factors on bacteria

In the investigated area, we found statistically significant correlations between bacterial abundance and temperature ($r= 0.43$, $p < 0.001$). Also, we conclude that temperature and salinity were important factors in controlling bacterial abundance, and that temperature and the freshwater substrate supply acted synergistically, or the substrate supply itself was a highly temperature-dependent seasonal phenomenon. During the investigated period, a positive correlation between bacterial abundance and concentration of chlorophyll *a* was determined for warmer seasons ($r=0.47$, $p<0.01$), which implied that phytoplankton-derived substrates supply also has an impact in controlling bacterial abundance. This is in accordance with the previous research for the Adriatic Sea (KRSTULOVIĆ *et al.*, 1997; ŠANTIĆ *et al.*, 2013; ŠESTANOVIĆ *et al.*, 2004). Comparing all the data for the surface layers (0 m – 5 m), where the temperature and salinity fluctuation was the highest, we found a significant relationship between LNA cell abundance and temperature and salinity ($r = 0.58$, $r = 0.55$ respectively for $p< 0.001$). We also found a weak but significant correlation between bacterial production and the abundance of HNA cells ($r= 0.23$, $p <0.05$).

CONCLUSIONS

According to data obtained for bacterial density with a mean value of $0.62 \pm 0.22 \times 10^6$ cells mL^{-1}, which is consistent with data already published for the Adriatic and Mediterranean Sea, we can conclude that the trophic state in Boka Kotorska Bay is mainly oligotrophic. The present study showed a tendency for bacteria to be BU controlled in all three embayments. The predominance of the LNA group over the HNA group in the bacterial community during longer periods of the year and the prevalence of the HNA group during colder seasons are consistent with previous reports for other oligotrophic areas.

However, important factors that were not monitored in this study are the role of mixotrophic flagellates with their contribution to total flagellate grazing on bacteria, particularly under nutrient-limited conditions, and grazing pressure on HNF by ciliates, which should be the subject of our future research.

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Dinamika prokariotske zajednice u Bokokotorskom zaljevu (Južni Jadran)

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SAŽETAK

Raspodjela i aktivnost prokariotskog pikoplanktona u crnogorskom dijelu južnog Jadrana je studirana na šest postaja Bokokotorskog zaljeva u razdoblju od siječnja 2010. do siječnja 2011. Istraživana je brojnost nepigmentiranih bakterija, udjeli bakterija s visokim (HNA) i niskim (LNA) sadržajem nukleinskih kiselina, bakterijska proizvodnja, heterotrofni nanoflagelati (HNF) te klorofil *a* i fizikalno kemijski čimbenici. Utvrđeno je da unos slatke vode u istraživano područje ima utjecaja na bakterijsku brojnost, na udjele HNA I LNA grupa bakterija kao i na brojnost HNF-a. U toplijem razdoblju godine u istraživanom je području utvrđen porast i prevladavanje LNA bakterija dok je prevladavanje HNA bakterija utvrđeno u hladnijem dijelu godine. Slaba povezanost između bakterija i HNF ukazuje da predacija nije prevladavajući mehanizam kontrole bakterijske abundancije u istraživanom području.

Ključne riječi: heterotrofne bakterije, HNA, LNA, bakterijska proizvodnja, heterotrofni nanoflagelati, Bokokotorski zaljev