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HNA and LNA bacteria in relation to the activity of heterotrophic bacteria

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A one-year survey of HNA and LNA heterotrophic bacteria abundance and its relation to bacterial production was conducted in the middle and southern Adriatic. The average abundance of heterotrophic bacteria in the central and southern coastal areas of the eastern coast of the Adriatic Sea ranged from 0.24×106 to 2.39×106 cells mL-1 and in the open sea area ranged from 0.23×106 to 0.63×106 cells mL-1. Elevated average abundances were found in the coastal area of Šibenik and in Kaštela Bay. The average monthly percentage of HNA bacteria in the central and southern coastal areas investigated ranged from 21.48 to 93.18%, while in the open sea HNA ranged from 28.62 to 65.62%. The seasonal distribution in the coastal areas mostly showed simultaneously the prevalence of LNA bacteria in the bacterial community and high values of bacterial production, respectively, during spring and summer. The prevalence of the HNA group in the bacterial community was found during colder seasons together with low values of bacterial production. Exceptions are identified in the areas influenced by rivers with the highest bacterial production, where HNA bacteria were dominant in the microbial community during all four seasons. On the open sea during the periods with the highest values of bacterial production, when viewed seasonally and vertically, the water column was dominated by LNA bacteria indicating the importance of both groups of bacteria in the marine ecosystems of the waters studied.

Key words: heterotrophic bacteria, HNA, LNA, bacterial production, Adriatic Sea

INTRODUCTION

Heterotrophic bacteria are significant components of prokaryotic communities in marine ecosystems (AZAM & HODSON, 1977). Methods developed in last few decades for studying microorganisms, including optical, radioactive marking methods and molecular techniques, have shown that many of the methods previously used actually ignored most of the microbial biomass, activity and diversity. During the 1990s flow cytometry was introduced in oceanography (DARZYNKIEWICZ & CRISSMAN, 1990; ALLMAN *et al*., 1993; FOUCHET *et al*., 1993; TROUSSELLIER *et al*. 1993; SHAPIRO, 1995; DAVEY & KELL, 1996; PORTER *et al*., 1997; COLLIER & CAMPBELL, 1999). With its fast and precise analysis, the use of flow cytometry in marine microbiology has resulted in the discovery of several groups of bacterioplankton on the basis

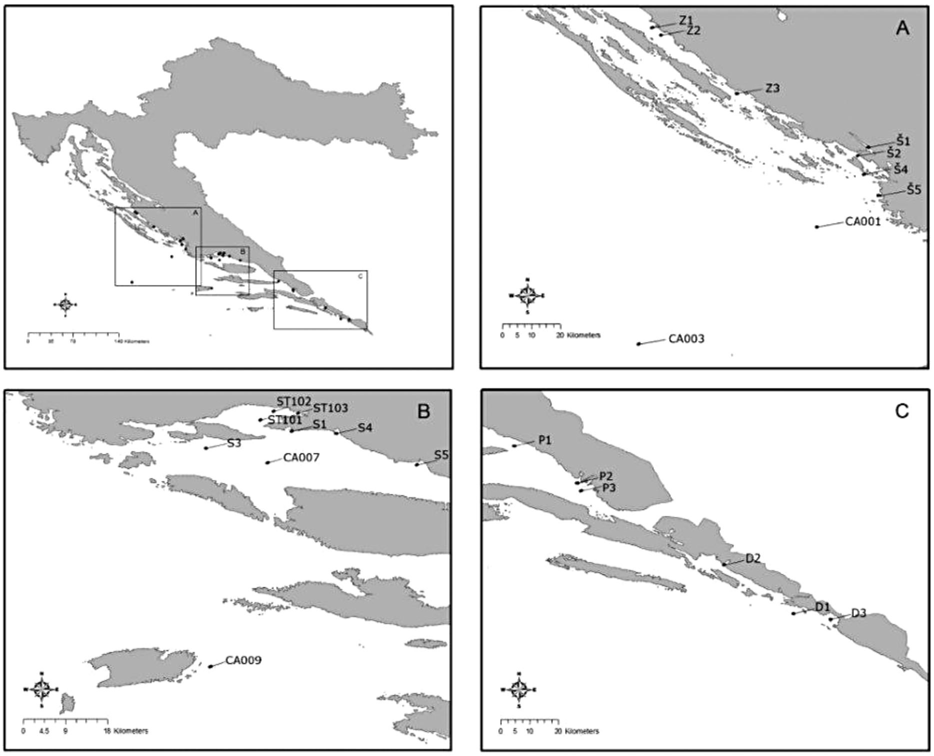

Fig. 1. Study area with sampling stations. A) Coastal areas: Zadar (Z1-Z3), Šibenik (Š1-Š5); B) coastal areas: Kaštela Bay (ST101-ST103), Split (S1-S5, CA007); C) coastal areas: Ploče (P1-P3), Dubrovnik (D1-D3)

of different proportions of deoxyribonucleic acid (LI *et al.,* 1995; MARIE *et al.,* 1997). Groups with more DNA are called high nucleic acid content (HNA) bacterial groups, whereas groups with a small amount of DNA are called low nucleic acid content (LNA) groups of bacteria (GASOL & MORÀN, 1999; GASOL *et al.,* 1999). The HNA bacteria were considered as an active and dynamic part of the community, while LNA cells were considered inactive, dead cell fragments (GASOL & MORÀN, 1999; GASOL *et al.,* 1999). Recent research showed that the HNA bacteria comprise an active group of heterotrophic bacteria in different marine ecosystems (GASOL & DEL GIORGIO, 2000); furthermore, in oligotrophic environments, LNA bacteria may represent the most active and predominating bacterioplankton community (ZUBKOV *et al.*, 2001; JOCHEM *et al.*, 2004; LONGNECKER *et al.*, 2005).

The aim of this study was to describe the seasonal cycles of bacterial abundance, variations in the distribution of HNA and LNA bacterial groups, HNA and LNA bacterial gruops in relation to bacterial activity and to identify the relevant physico-chemical and biological factors responsible for the observed seasonal and spatial distributions in the central and southern parts of the Adriatic Sea.

MATERIAL AND METHODS

Water samples for cell and nutrient analyses were taken at monthly intervals from January to December 2005. Samples were taken from two to eight different depths (0 m - 260 m) between the surface and the near-bottom layer of 21 stations located along the eastern coast of the central and southern Adriatic Sea and three stations (CA001, CA003, CA009) located in the open central Adriatic Sea (Fig. 1). Niskin bottles (5 L) were used for sampling. Temperature and salinity were also recorded using a SeaBird 25 CTD profiler. Nutrient concentrations (NO_3^-, NO_2^-, NH_4^+, total dissolved inorganic nitrate/DIN and soluble reactive phosphate/SRP) were determined from 500-mL samples using a modified autoanalyser method by GRASSHOF (1976). Chlorophyll *a* (Chl *a*) was determined from 500-mL sub-samples filtered through Whatman GF/F glass-fibre filters stored at -20°C. These were homogenized and extracted in 90% acetone. Samples were analyzed fluorometrically with a Turner TD-700 Laboratory Fluorometer calibrated with pure Chl *a* (Sigma) (STRICKLAND & PARSONS, 1972).

Abundances of SybrGreen I-stained heterotrophic bacteria were determined using flow cytometry (MARIE *et al.,* 1997). Samples were analysed on a Beckman Coulter EPICS XL-MCL with a high flow rate from 1 to 1.2 µL s^{-1}, and the analyzed volume was calculate by acquisition time. 1.0-µm yellow-green beads were added to standardize the fluorescence intensity of the cells (Level-III Epics Division of Coulter Corporation Hialeah, Florida). Two groups of heterotrophic bacteria were distinguished according to their relative green fluorescence as a proxy for the nucleic acid content, referred to as high nucleic acid (HNA) and low nucleic acid bacteria (LNA). Bacterial cell production was measured from DNA synthesis based on incorporation rates of ^{3}H-thymidine (FUHRMAN & AZAM, 1982). Conversion factors 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): $CF=(N_2-N_1)/^3H$, where N_1 and N_2 are the numbers of bacteria at the beginning and end of the experiment, and ^{3}H is the integrated ^{3}H-thymidine incorporation rate during the experiment.

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

RESULTS AND DISCUSSION

The average monthly abundance of heterotrophic bacteria in the coastal area ranged from 0.21×10^6 cells mL^{-1} to 2.39×10^6 cells mL^{-1} and in the open sea areas ranged from 0.23×10^6 cells mL^{-1} to 0.63×10^6 cells mL^{-1} (Fig. 2 - Fig. 8), and was similar to previous values reported for the coastal and open Adriatic Sea (KRSTULOVIĆ *et al.,* 1995; KRSTULOVIĆ *et al.,* 1997; KRSTULOVIĆ & ŠOLIĆ, 2006; ŠOLIĆ *et al.,* 2009). Abundances of heterotrophic bacteria of less than 1×10^6 cells mL^{-1} are considered to be typical for oligotrophic seas (COTNER & BIDDANDA, 2002) and the values of heterotrophic bacteria in most parts of the study area were lower than 10^6 cells mL^{-1}, so we can conclude that the area studied in the Adriatic Sea showed the characteristics of an oligotrophic area. Exceptions were found in areas under the direct influence of karstic rivers (Šibenik area, Ploče area and Kaštela Bay), where the abundance of heterotrophic bacteria was higher than 10^6 cells mL^{-1}.

In this paper, the seasonality of the bacterial community in coastal areas was determined, mostly with abundance maxima in the spring-summer period and minima during the winter (Fig. 2 - Fig. 7) as found in previous reports on the central Adriatic (KRSTULOVIĆ, 1992; ŠOLIĆ *et al.,* 2001). An exception was found in the Zadar area where an increased abundance was determined in November. Another exception was found in the Ploče area, where an increased bacterial abundance was determined during the winter and spring-summer periods due to the enrichment of this area by the inflow of the river Neretva during this period (ŠOLIĆ *et al.,* 2008). The seasonal distribution at the open sea station (CA 009) showed high values during the summer (Fig. 8), as was similarly reported by KRSTULOVIĆ (1992). The seasonal distributions at deeper open sea stations (CA 001 and CA 003) were not significantly pronounced (Fig. 8), and similar to previous findings in the open sea of the Bay of Biscay (VALENCIA *et al.,* 2003; CALVO-DÍAZ & MORÁN, 2006).

The average monthly percentage of HNA bacteria in the heterotrophic bacterial population in the coastal area ranged from 21.48% to 93.18%, while at the open sea stations ranged from 28.62% to 65.62% (Fig. 2 - Fig. 7). According to literature data, similar ranges were determined in the Bay of Biscay and the percentage of HNA bacteria was found to be, on

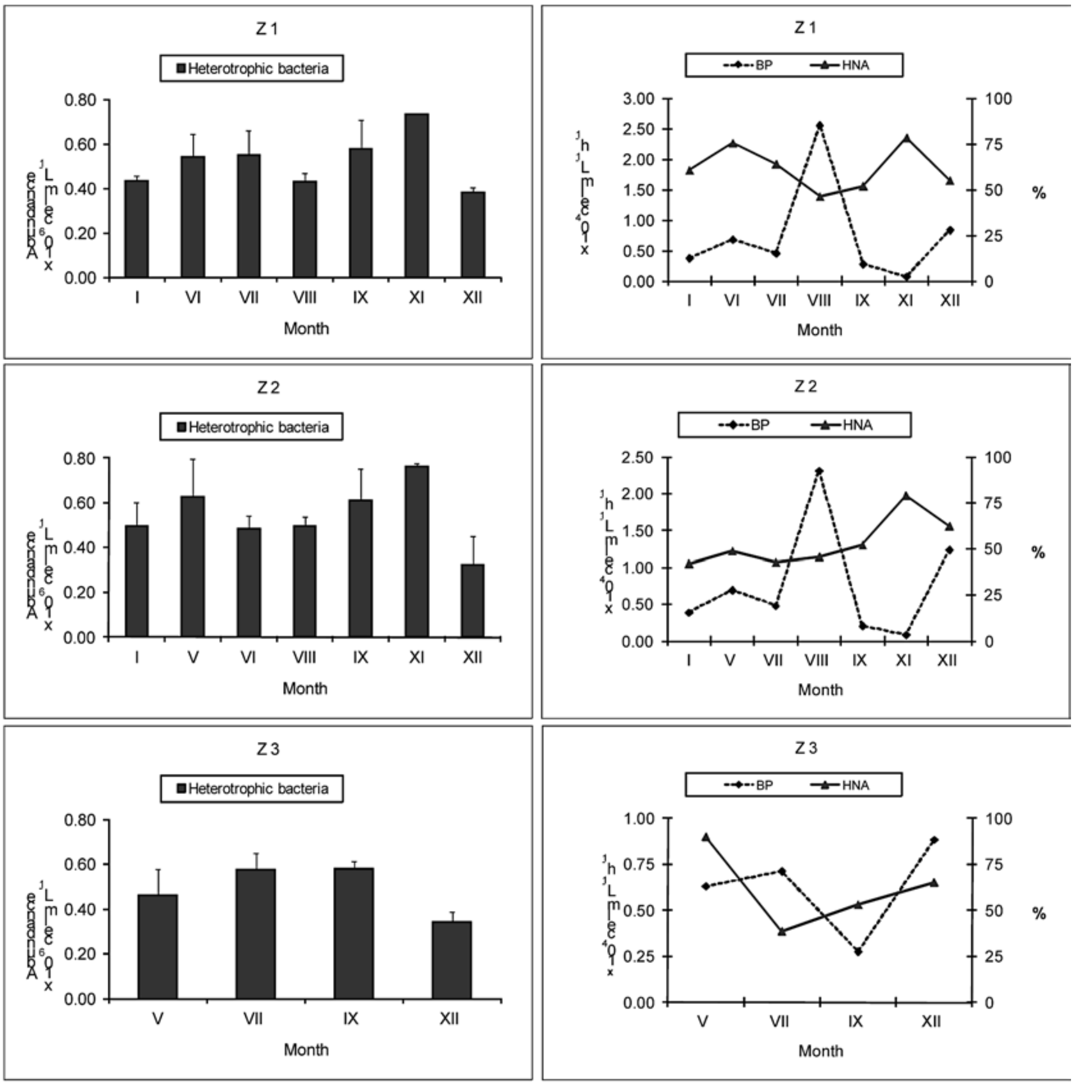

Fig. 2. Seasonal fluctuations of heterotrophic bacteria, HNA bacteria and bacterial production in the area of Zadar. Average values (column) and positive standard deviation (bars) are presented

average, 31% to 91% (CALVO-DÍAZ *et al.,* 2004; CALVO-DÍAZ & MORÁN, 2006; MORÁN *et al.,* 2007), while in the Atlantic Ocean (LI *et al.* 1995) and the north-western Mediterranean (LI *et al.,* 1995; VAQUÉ *et al.,* 2001) it was up to 90%.

The seasonal distribution at the coastal areas investigated, from Zadar to Dubrovnik, showed that the LNA group was dominant in the bacterial community during the summer season and that the HNA group was dominant during colder seasons, as previously described in the literature (GREGORI *et al.,* 2001; LI & DICKIE; 2001; CALVO-DÍAZ & MORÁN, 2006). The prevalence of HNA bacteria throughout all four seasons in these areas was influenced by the Krka and Jadro rivers (Fig. 2 - Fig. 7). However, in the areas of Split and Ploče, LNA bacteria were dominant in the water column during the colder seasons. The area of Ploče is under a significant influence of the river Neretva during the cold season (ŠOLIĆ *et al.,* 2008), whereas the wider area of Split is under the influence of the Žrnovnica and Cetina rivers, and are thus enriched with nutrients. According to data in the literature, although rare, the prevalence of LNA bacteria in environments rich in nutrients is known (ANDRADE *et al.,* 2007).

The seasonal distribution of both bacterial groups at the open sea stations investigated

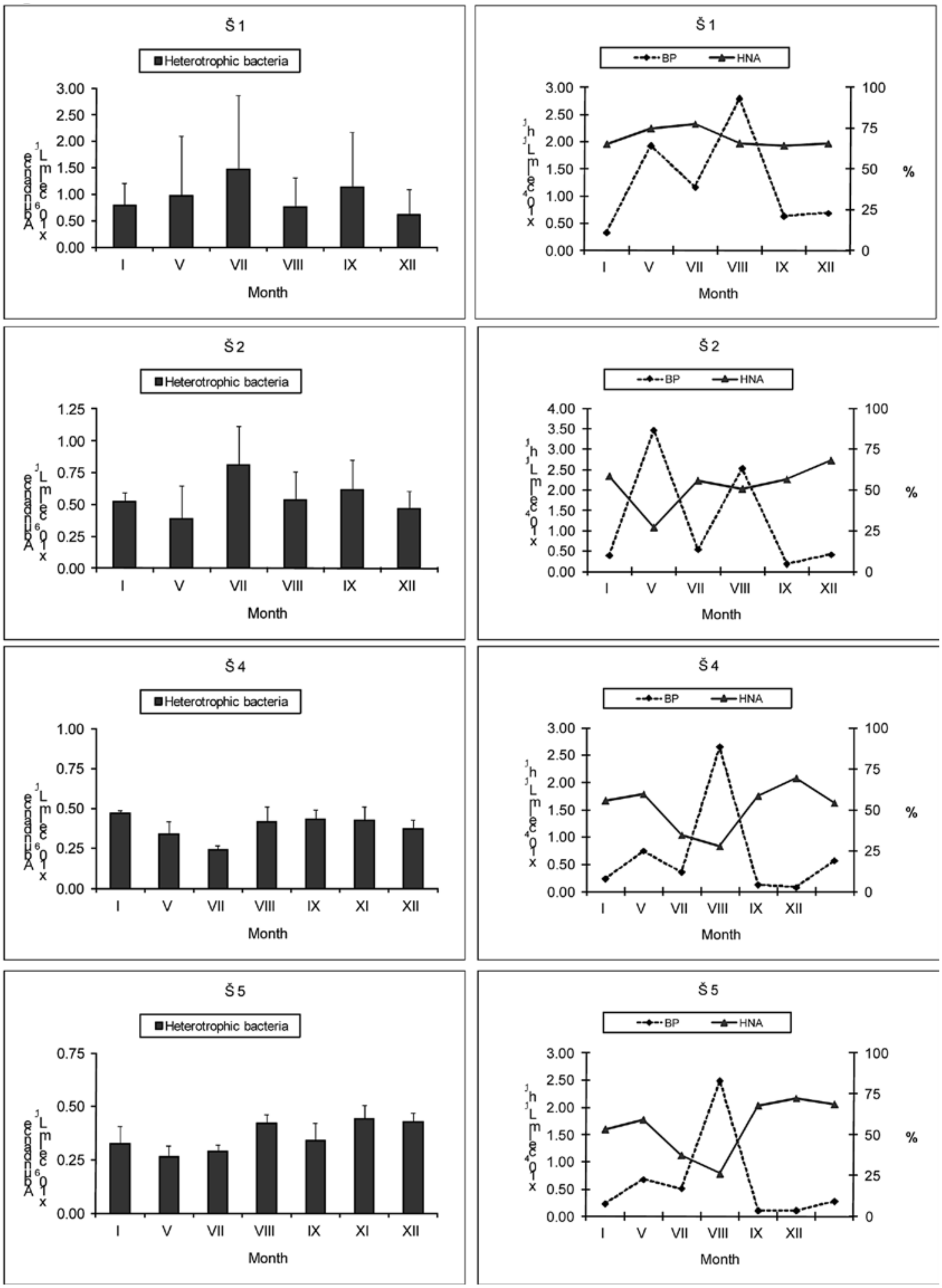

Fig. 3. Seasonal fluctuations of heterotrophic bacteria, HNA bacteria and bacterial production in the area of Šibenik. Average values (column) and positive standard deviation (bars) are presented

showed the prevalence of HNA during the spring, autumn and winter, whereas LNA bacteria were prevalent in the heterotrophic prokaryote population during summer, as found in a previous study ot offshore stations in the Pacific (GROB *et al*., 2007).

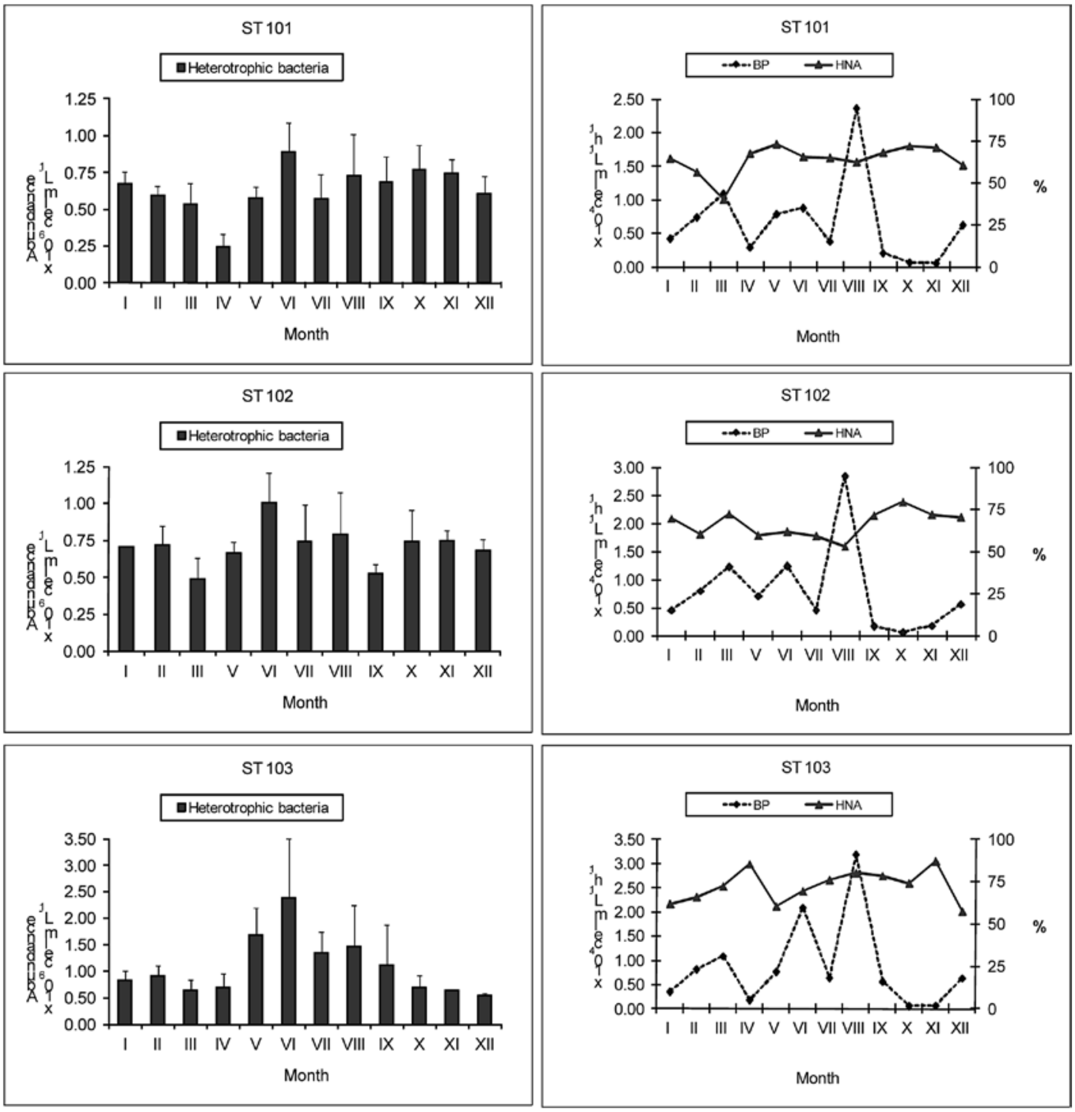

Fig. 4. Seasonal fluctuations of heterotrophic bacteria, HNA bacteria and bacterial production in Kaštela Bay. Average values (column) and positive standard deviation (bars) are presented

Data in the literature show that the contribution of HNA bacteria to the heterotrophic bacteria population changes seasonally and that the percentages of HNA bacteria in different areas vary. The most likely explanations for the seasonal changes and varying proportions in both groups is a positive relationship with the phytoplankton biomass (LI *et al.,* 1995; SHERR *et al.,* 2006), an association between the HNA group and changes in temperature, salinity (CALVO-DÍAZ & MORÁN, 2006) and allochthonous nutrients (ANDRADE *et al.,* 2007), and a positive correlation between the LNA groups and high concentrations of nitrate (ANDRADE *et al.,* 2007). Research results obtained for the Bay of Biscay (CALVO-DÍAZ & MORÁN, 2006) found that both groups of bacteria have equal responses to changes in temperature, salinity and concentrations of chlorophyll *a*. In this paper, the results obtained from the coastal and open sea areas showed statistically significant correlation between HNA bacteria and changes in concentrations of chlorophyll *a*, but weak correlations between both groups of bacteria and changes in temperature, salinity and inorganic nutrients, and weak correlations between LNA bacteria and concentrations of chlorophyll *a* (Table 1), similar to previous

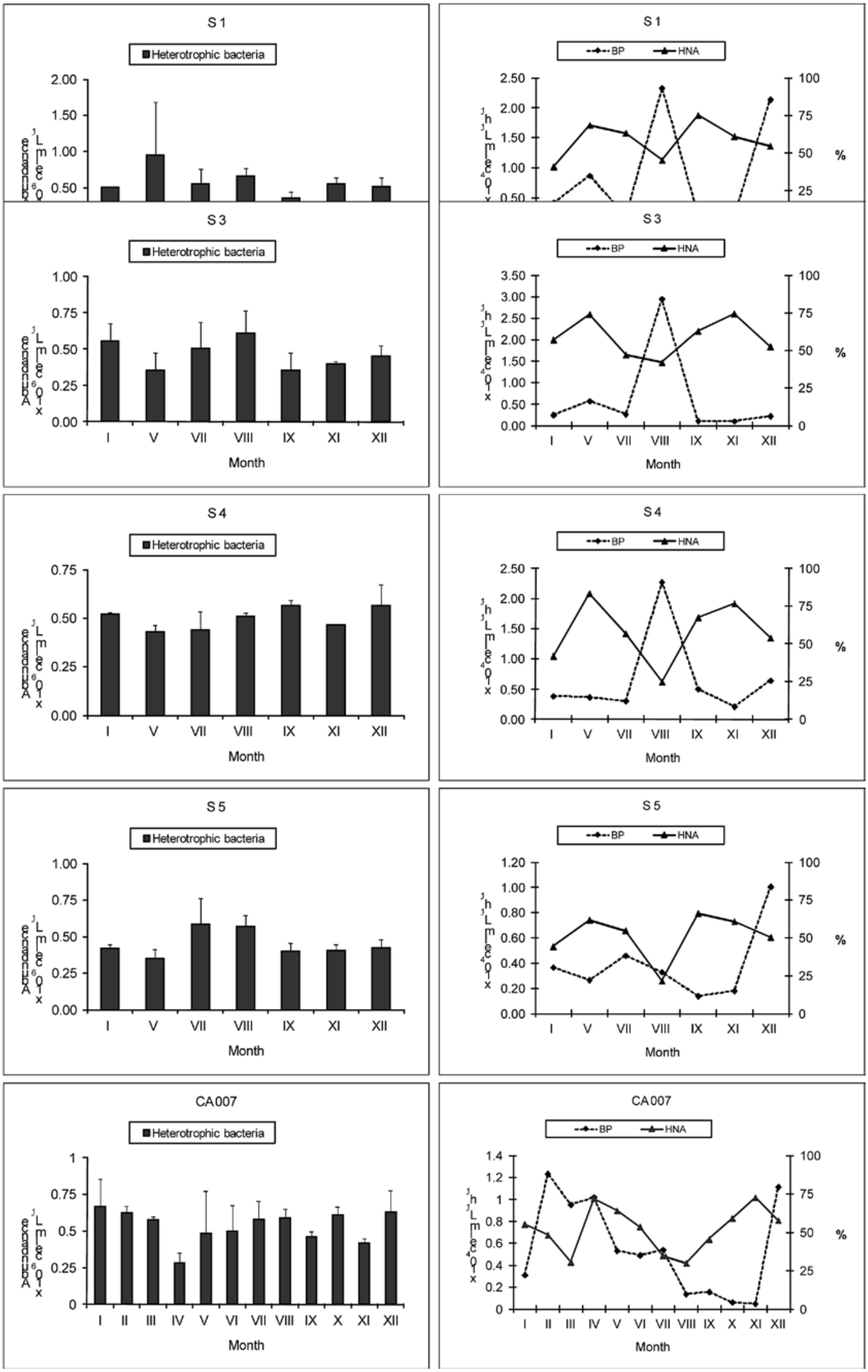

Fig. 5. Seasonal fluctuations of heterotrophic bacteria, HNA bacteria and bacterial production in the area of Split. Average values (column) and positive standard deviation (bars) are presented

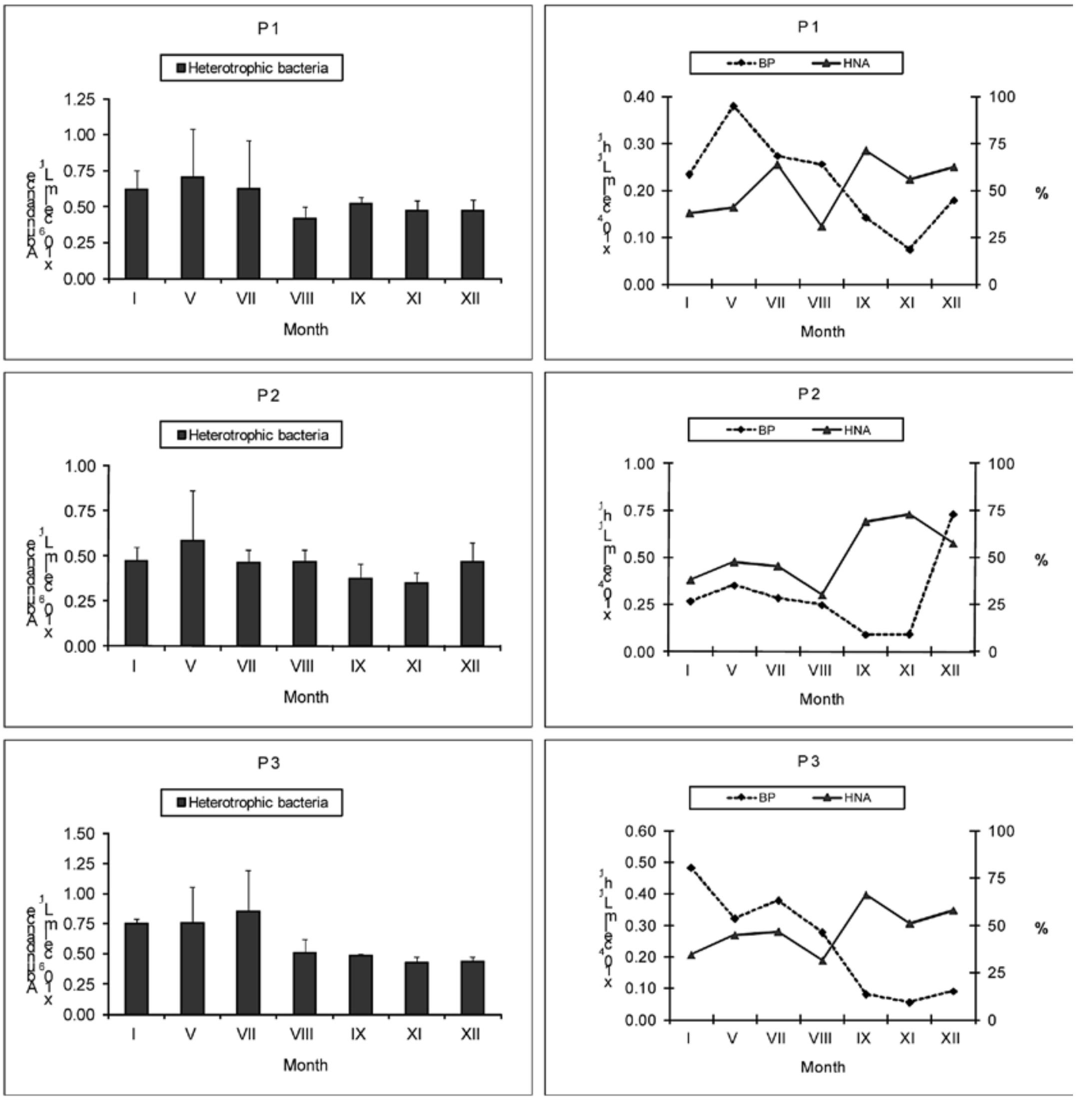

Fig. 6. Seasonal fluctuations of heterotrophic bacteria, HNA bacteria and bacterial production in the area of Ploče. Average values (column) and positive standard deviation (bars) are presented

research results in this area during the 2002-2006 period (ŠOLIĆ *et al*., 2009).

The present study found a significantly greater range of bacterial production values in the coastal Adriatic Sea, from 0.03×10^4 cells mL^{-1} h^{-1} to 6.28×10^4 cells mL^{-1} h^{-1} (Fig. 2 - Fig. 7), whereas earlier studies established bacterial production values in the middle of the Adriatic Sea from 0.84×10^4 cells mL^{-1} $h^{-1}$to 2.56×10^4 cells mL^{-1} h^{-1} (KRSTULOVIĆ, 1992; ŠOLIĆ & KRSTULOVIĆ, 1994). However, it should be noted that this work included the eutrophic area of Šibenik (up to 4.73 mg Chl *a* m^{-3}; MARASOVIĆ *et al.,* 2006) and the oligotrophic area of Dubrovnik (up to 0.6 mg Chl *a* m^{-3}; MARASOVIĆ *et al*., 2006), where the lowest values of bacterial production were found. Bacterial production in the central Adriatic open sea area was 0.06×10^4 cells mL^{-1} h^{-1} to 2.71×10^4 cells mL^{-1} h^{-1} (Fig. 8), and these values were very similar to values previously reported in the oligotrophic areas of the central Adriatic (KRSTULOVIĆ *et al.,* 1995). The same authors (KRSTULOVIĆ *et al.,* 1997) also found that the bacterial production value increases with the increasing trophic status of marine ecosystems. In this paper, the highest bacterial production

Complete dataset	Temperature °C	Salinity	Nitrate µM	Nitrite µM	Ammonia µM	SRP µM	Chl *a* mg m^{-3}
Coastal sea , N=355 P<0.05, N_{Chla} = 311							
Minimum	6.33	4.42	0	0	0.04	0	0.03
Maximum	26.93	38.87	53.86	1.05	27.30	1.50	4.73
Arithmetic Mean	17.51	37.47	1.24	0.12	1.34	0.06	0.37
Pearson correlation with HB	0.34	-0.30	0.16	n.s	0.10	n.s	0.51
Pearson correlation with HNA	0.18	-0.29	0.18	0.11	n.s	n.s	0.48
Pearson correlation with LNA	n.s	n.s	n.s	0.13	n.s	n.s	n.s
Pearson correlation with BP	n.s	n.s	n.s	-0.15	n.s	n.s	n.s
Open sea, N=148 P<0.05, N_{Chla} = 148							
Minimum	10.38	36.37	0	0	0.04	0	0.002
Maximum	23.96	38.94	3.11	0.44	2.28	0.28	0.28
Arithmetic Mean	16.60	38.60	0.79	0.11	0.80	0.06	0.11
Pearson correlation with HB	n.s	n.s	n.s	n.s	n.s	n.s	n.s
Pearson correlation with HNA	-0.20	n.s	n.s	n.s	n.s	n.s	0.18
Pearson correlation with LNA	n.s	n.s	n.s	n.s	n.s	-0.19	n.s
Pearson correlation with BP	-0.16	n.s	-0.23	n.s	n.s	n.s	n.s

Table 1. Abiotic and biotic factors: Ranges of abiotic and biotic factors measured and Pearson correlation coefficient between the given factors and the abundance and production of heterotrophic bacteria

values in the central Adriatic were found in the Šibenik area (Š 1 and Š 2) and it was noted that these high values could possibly have been due to the inflow of the river Krka. One significant indicator of the enrichment of waters in this study was the increased level of chlorophyll *a* in the Šibenik area (up to 4.73 mg Chl *a* m^{-3}; MARASOVIĆ *et al.*, 2006).

The seasonal distributions of bacterial production in this research study showed high values during the summer and significantly lower values during the winter (Fig. 2 - Fig. 8) which correspond with the results of previous studies (KRSTULOVIĆ, 1992; ŠOLIĆ & KRSTULOVIĆ, 1994) in the Adriatic Sea. In this paper, almost equal bacterial production values were found during the summer maximum and winter minimum in Kaštela Bay and in the area near the island Vis (CA 009), which is in contrast to the results of KRSTULOVIĆ (1992) who found extremely high bacterial production values in Kaštela Bay. This difference can be explained by the fact that Kaštela Bay was a highly eutrophic area until 2004; after this period, the input of nutrients and organic matter into this bay was reduced (KRSTULOVIĆ *et al.,* 1995; ŠOLIĆ *et al.,* 2010).

Attention should be drawn to the fact that unusually high bacterial production values were found in the areas of Zadar, Split, Ploče, Dubrovnik and the offshore island of Vis (Stončica) in December. The most likely explanations for these high values in December are the high seawater temperatures (14-18°C in Zadar), the availability of inorganic nutrients (in Zadar, the concentration of ammonium ions was > 1.5 mmol L^{-1}, off Split the average concentration of orthophosphate was 0.8 mmol L^{-1}, and in the area of Ploče and Dubrovnik the concentration of inorganic nitrogen was > 1 µmol L^{-1}), and the greater phytoplankton biomass during autumn and winter compared to the previous season (in the area of Split and Ploče, and autumn in Dubrovnik; MARASOVIĆ *et al.*, 2006).

Our results showed pronounced seasonal dynamics in the coastal areas compared to offshore (Fig. 2 - Fig. 8), which coincides with the seasonal variations found in open and coastal regions of the northern Adriatic Sea (DEL NEGRO *et al.,* 2008). As found by ŠOLIĆ *et al.*, (2008), the values of bacterial

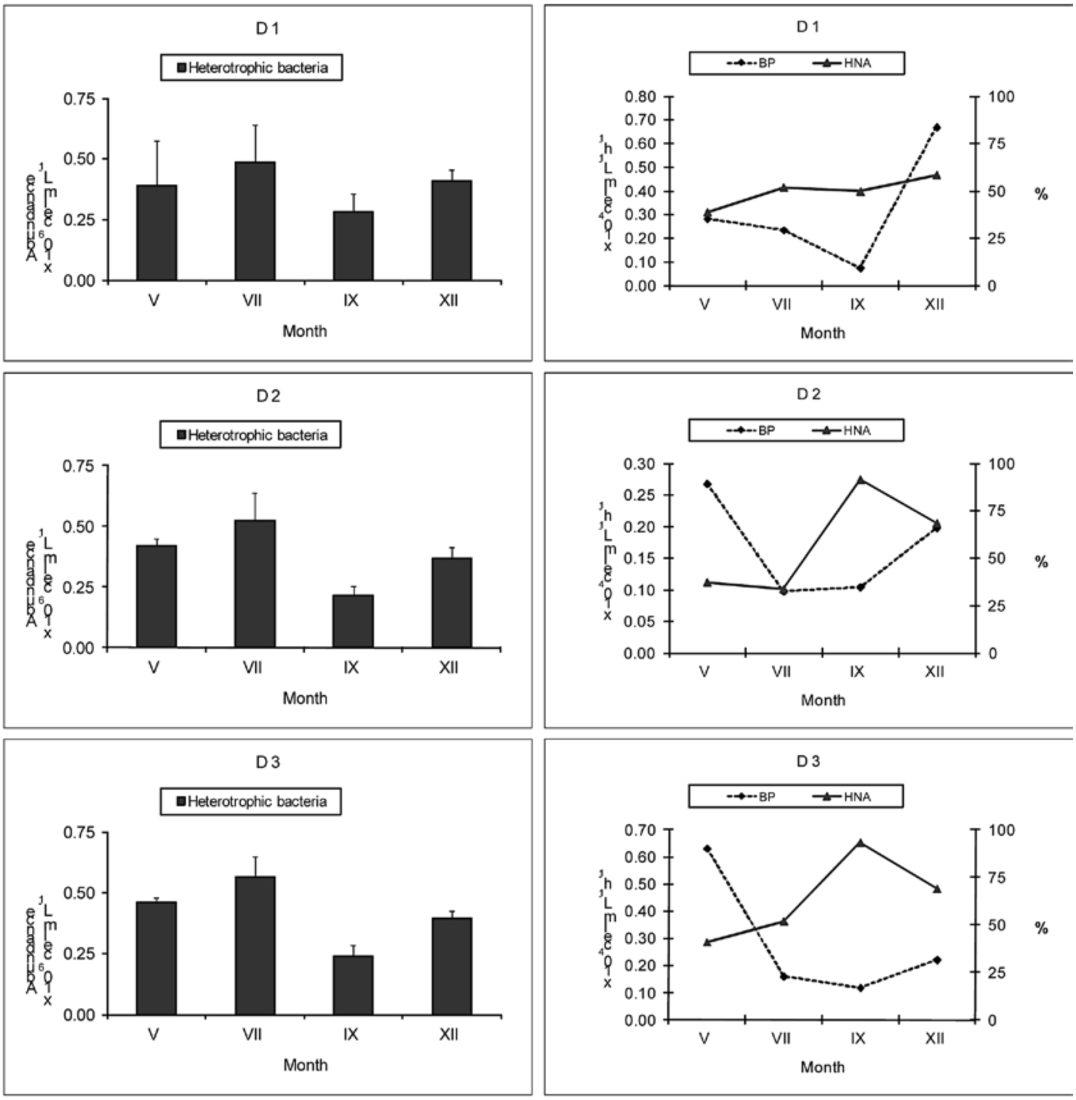

Fig. 7. Seasonal fluctuations of heterotrophic bacteria, HNA bacteria and bacterial production in the area of Dubrovnik. Average values (column) and positive standard deviation (bars) are presented

productivity generally decreased from the eutrophic to oligotrophic areas.

Bacterial activity in this study was observed through seasonal distribution of HNA and LNA bacterial groups in relation to bacterial productivity. The analysis of HNA and LNA groups in relation to bacterial productivity at most of the coastal and open sea stations showed a simultaneous increase in the percentage of the LNA group and bacterial production (Fig. 2 - Fig. 8) which is consistent with previous results from the Atlantic (MORÁN *et al.,* 2002; MORÁN *et al.,* 2004). On the contrary, in the Šibenik area, which is affected by the Krka River (Š 1 and Š 2), and in Kaštela Bay, 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). Data in the literature for the vertical distribution of HNA and LNA bacteria in the oligotrophic Gulf of Mexico, the eutrophic Bay of Biscay and the mouth of the Mississippi show the prevalence of LNA groups in surface layers and greater proportions of HNA bacteria in the euphotic zone during warmer seasons (JOCHEM, 2001; CALVO-DÍAZ & MORÁN, 2006). In

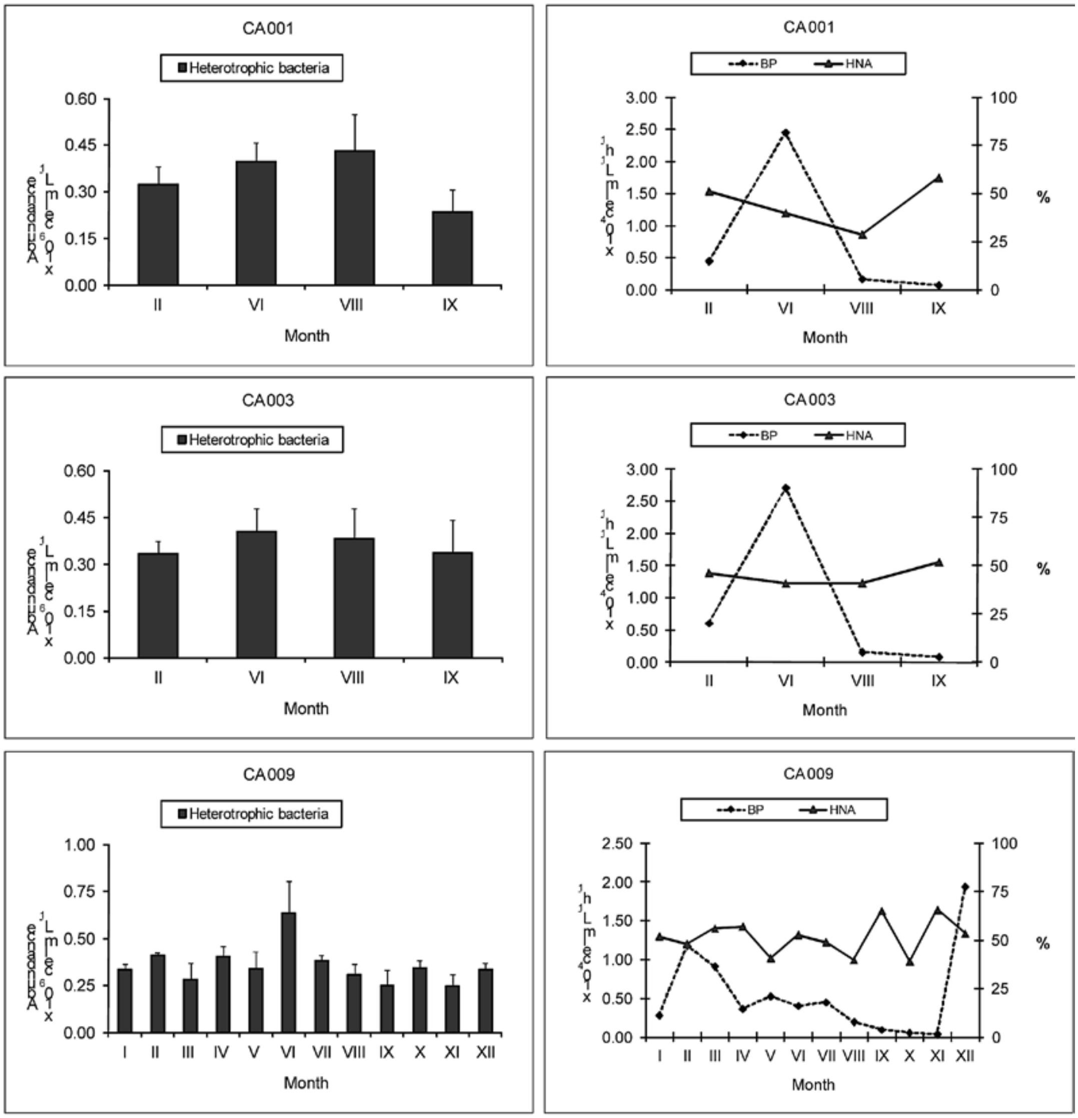

Fig. 8. Seasonal fluctuations of heterotrophic bacteria, HNA bacteria and bacterial production in the open sea area. Average values (column) and positive standard deviation (bars) are presented

this study, the percentages of HNA and LNA groups were almost equal or the HNA group was slightly dominant in the surface layers of the open sea stations during the cold season. However, during warmer periods when the bacterial production values were highest the LNA group was dominant over HNA bacteria in the surface layers (Fig. 9).

When comparing all of the area investigated in a one-year survey at the coastal area with the greatest bacterial abundance and production, the HNA bacterial group was dominant in the bacterial population, whereas in the open sea area with a low bacterial abundance and high bacterial productivity the LNA bacterial group was prevalent in the bacterial population (Fig. 10). Research into the abundance of HNA and LNA bacteria showed that HNA bacteria dominate in eutrophic and mesotrophic areas or in areas directly influenced by inflowing rivers (LI *et al.,* 1995; CASOTTI *et al.,* 2000; CALVO-DÍAZ *et al.,* 2004; JOCHEM *et al.,* 2004; ANDRADE *et al.,* 2007; ŠOLIĆ *et al.,* 2009) whereas it was found that LNA bacteria were dominant in the open seas and oligotrophic systems such as the Celtic Sea, Gulf of Mexico and the Atlantic Ocean

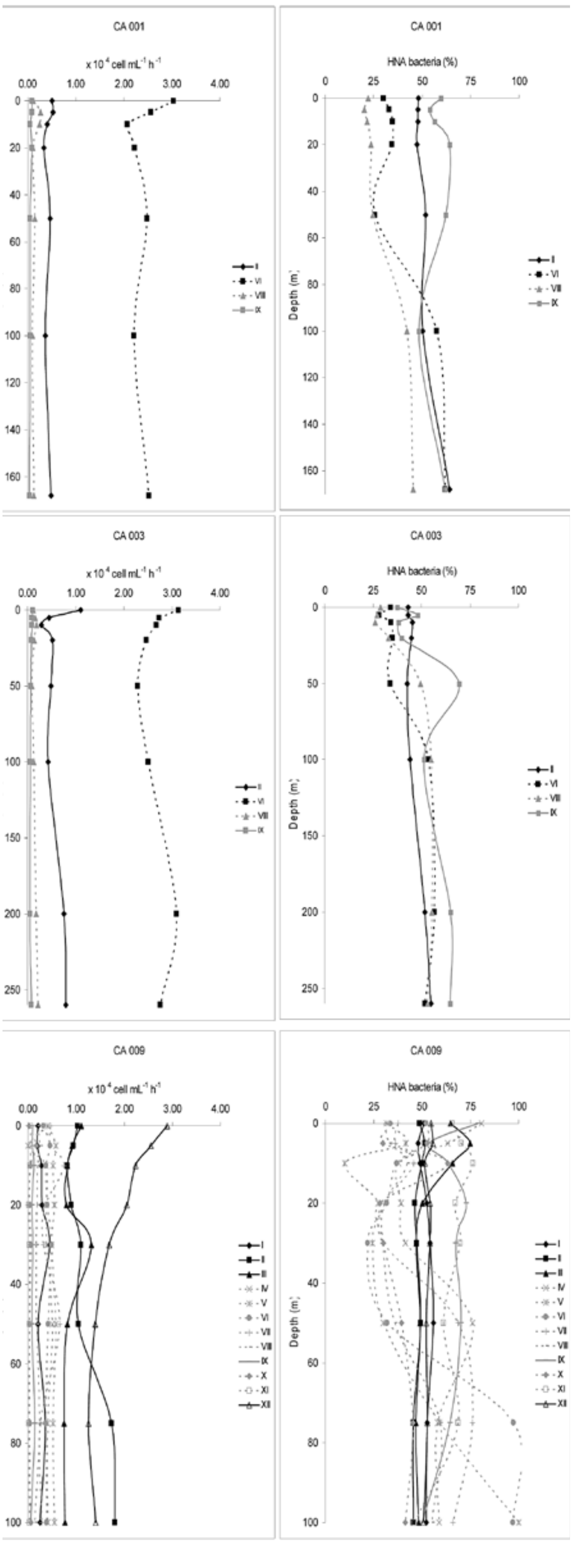

Fig. 9. Vertical fluctuations of heterotrophic bacteria abundance, HNA bacteria and bacterial production in the open sea area. Average values (line) are presented

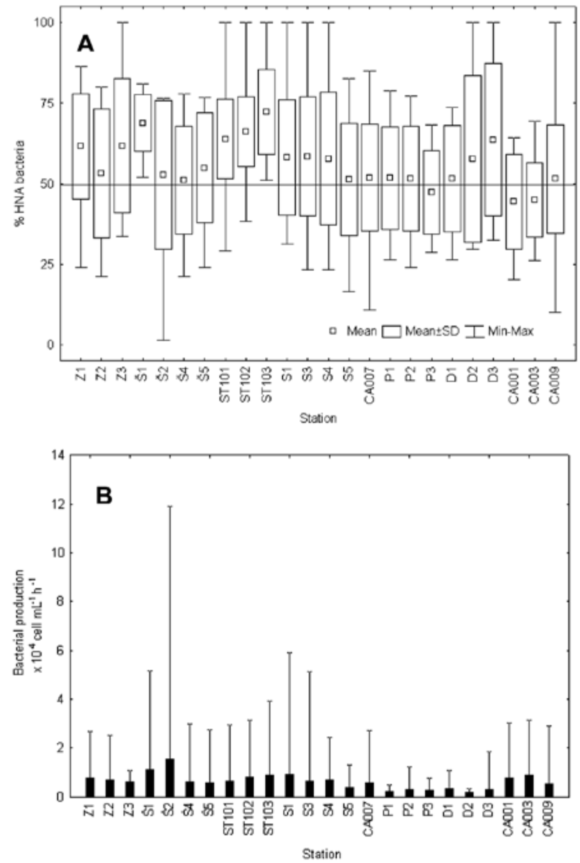

Fig. 10. (A) Contribution of HNA bacteria. Average values (dot), positive and negative standard deviation (box), and minimum and maximum values (bars) are presented; (B) Bacterial production. Average annual values (column) and maximum values (bars) are presented

(ZUBKOV *et al.,* 2001; JOCHEM *et al.,* 2004; MARY *et al.,* 2006; ANDRADE *et al.,* 2007). The predominance of the LNA group under oligotrophic conditions can be explained by the high surface area to cell volume ratio (BUTTON, 1998; JOCHEM *et al.,* 2004). Apart from the fact that LNA cells have a more favourable ratio of surface area to cell volume, ZUBKOV *et al.* (2001) found that in oligotrophic environments the LNA group of bacteria has a higher specific growth rate compared to the HNA group, which also indicates the successful survival of this group in oligotrophic environments.

The results of this study showed a simultaneous increase in the contribution of the HNA group and bacterial abundance from oligotrophic to eutrophic areas and these results agree with the results of previous research studies in the same area during the 1997 – 2006 period (ŠOLIĆ *et al.,* 2009).

CONCLUSIONS

This paper highlights the importance of both HNA and LNA bacteria in relation to the activity of heterotrophic bacteria in the areas of the Adriatic Sea that were studied. The analysis of HNA and LNA groups in relation to bacterial productivity showed simultaneously high values of bacterial production and domination of LNA bacteria in the microbial community in most of the oligotrophic areas. In contrast, in coastal areas designated as mesotrophic or eutrophic areas (areas influenced by rivers), HNA bacteria were dominant in the microbial community during the period of highest bacterial production. These results indicate the importance of both bacterial groups in the activity of heterotrophic bacteria in the marine ecosystems of the areas studied.

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REFERENCES

ALLMAN, R., R. MANCHEE & D. LLOYD. 1993. Flow cytometric analysis of heterogeneousbacterial populations, In: D. Lloyd (Editors). Flow cytometry in microbiology. Springer-Verlag, London, U.K., pp. 27-47.

ANDRADE, L., A.M. GONZALES, C.E. REZENDE, M. SUZUKI, J.L. VALENTIN & R. PARANHOS. 2007. Distribution of HNA and LNA bacterial groups in the Southwest Atlantic Ocean. Braz. J. Microbiol., 38: 330-336.

AZAM, F. & R.E. HODSON. 1977. Size distribution and activity of marine microheterotrophs. Limnol. Oceanogr., 22: 492–501.

BUTTON, D.K. 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., X.A.G. MORÁN, E. NOGUEIRA, A. BODE & M. VARELA. 2004. Picoplankton community structure along the northern Iberian continental margin in late winter–early spring. J. Plankton Res., 26 :1069–1081.

CALVO-DÍAZ, A. & X.A.G. MORÁN. 2006. Seasonal dynamics of picoplankton in shelf waters of the southern Bay of Biscay. Aquat. Microb. Ecol., 42: 159–174.

CASOTTI, R., C. BRUNET, B. ARONNE & M.R. D'ALCALÀ. 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.

COLLIER, J.L. & L. CAMPBELL. 1999. Flow cytometry in molecular aquatic ecology. Hydrobiologia., 401:33–53.

COTNER, J.B. & B. A. BIDDANDA. 2002. Small players, large role: microbial influence on auto-heterotrophic coupling and biogeochemical processes in aquatic ecosystems. Ecosystems., 5: 105–121.

DARZYNKIEWICZ, Z. & H.A. CRISSMAN. 1990. Flow Cytometry. In: Methods in Cell Biology, Z. Darzynkiewicz & H.A. Crissman (Editors). Academic Press, New York, Vol. 33:15-17.

DAVEY, H. & D. KELL. 1996. Flow cytometry and cell sorting of heterogeneous microbial populations: the importance of single-cell analysis. Microbiol. Rev., 60: 641-696.

DEL NEGRO, P., M. CELUSSI, E. CREVATIN, A. PAOLI, F. BERNARDI AUBRY & A. PUGNETTI. 2008. Spatial and temporal prokaryotic variability in the northern Adriatic Sea. Mar. Ecol., 29: 375-386.

FOUCHET, P., C. JAYAT, Y. HECHARD, M.H. RATINAUD & G. FRELAT. 1993. Recent advances in flow cytometry in fundamental and applied microbiology. Biochem. Cell Biol., 78: 95–109.

FUHRMAN, J.A. & F. AZAM. 1982. Thymidine incorporation as a measure of heterotrophic bacterioplankton production in marine surface waters. Mar. Biol., 66: 109-120.

GASOL, J.M. & X.A.G. MORÁN. 1999. Effects of filtration on bacterial activity and picoplankton community structure as assessed by flow cytometry. Aquat. Microb. Ecol., 16: 251–264.

GASOL, J.M., U.L. ZWEIFEL, F. PETERS, J.A. FURHMAN & Å. HAGSTRÖM. 1999. Significance of size and nucleic acid content heterogeneity as assessed by flow cytometry in natural planktonic bacteria. Appl. Environ. Microbiol., 65: 4475–4483.

GASOL, J.M. & P.A. DEL GIORGIO. 2000. Using flow cytometry for counting natural planktonic bacteria and understanding the structure of planktonic bacterial communities. Sci. Mar., 64: 197-224.

GRASSHOF, K. 1976. Methods of Seawater Analysis.Verlag Chemie, Weinhein. 307 p.

GRÉGORI, G., A. COLOSIMO & M. DENIS. 2001. Phytoplankton group dynamics in the Bay of Marseilles during a 2-year survey based on analytical flow cytometry. Cytometry, 44: 247–256.

GROB, C., O. ULLOA, W.K.W. LI, G. ALARCÓN, M. FUKASAWA & S. WATANABE. 2007. Picoplankton abundance and biomass across the eastern South Pacific Ocean along latitude 32.5°S. Mar. Ecol. Prog. Ser., 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. Aquat. Microb. Ecol., 25: 179-194.

JOCHEM, F.J., P.J. LAVRENTYEV & M.R. FIRST. 2004. Growth and grazing rates of bacteria groups with different apparent DNA content in the Gulf of Mexico, Mar. Biol., 145: 1213–1225.

KRSTULOVIĆ, N. 1992. Bacterial biomass and production rates in the central Adriatic. Acta Adriat., 33: 49-65.

KRSTULOVIĆ, N., T. PUCHER-PETKOVIĆ & M. ŠOLIĆ. 1995. The relation between bacterioplankton and phytoplankton production in the mid Adriatic Sea. Aquat. Microb. Ecol., 9: 41-45.

KRSTULOVIĆ, N., M. ŠOLIĆ & I. MARASOVIĆ. 1997. Relationship between bacteria, phytoplankton and heterotrophic nanoflagellates along the trophic gradient. Helgöland. Meeresuntersuch., 51: 433-443.

KRSTULOVIĆ, N. & M. ŠOLIĆ. 2006. Ekologija morskog bakterioplanktona. Sveučilišni udžbenik (Ecology of marine bacterioplankton. Academic textbook.) Institute of Oceanograhy and Fisheries, Split, 317 pp.

LI, W.K.W., J.F. JELLETT & P.M. DICKIE. 1995. DNA distribution in planktonic bacteria stained with TOTO or TO-PRO. Limnol. Oceanog., 40: 1485-1495.

LI, W.K.W. & P.M. DICKIE. 2001. Monitoring phytoplankton, bacterioplankton and virioplankton in a coastal inlet (Bedford Basin) by flow cytometry. Cytometry, 44: 236–246.

LONGNECKER, K., B.F. SHERR & E.B. SHERR. 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. Microbiol., 71: 7737–7749.

MARASOVIĆ, I, Ž. NINČEVIĆ-GLADAN, S. SKEJIĆ & M. BUŽANČIĆ. 2006. Biološke osobine u Kušpilić i sur. Kontrola kakvoće obalnog mora (Projekt Vir-Konavle 2005). Studije i elaborati Instituta za oceanografiju i ribarstvo, Split, pp. 68-81.

MARIE, D., F. PARTENSKY, S. JACQUET & D. VAULOT. 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., J.L. HEYWOOD, B.M. FUCHS, R. AMANN, P.H. BURKILL, G.A. TARRAN & M.V. ZUBKOV. 2006. SAR11 dominance among metabolically active low nucleic acid bacterioplankton in surface waters along an Atlantic meridional transect. Aquat. Microb. Ecol., 45: 107–113.

MORÁN, X.A.G., J.M. GASOL, C. PEDRÓS-ALIÓ & M. ESTRADA. 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, X.A.G., E. FERNÁNDEZ & V. PÉREZ. 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, X.A.G., A. BODE, L.Á. SUÁREZ & E. NOGUEIRA. 2007. Assessing the relevance of nucleic acid content as an indicator of marine bacterial activity. Aquat. Microb. Ecol., 46: 141-152.

PORTER, J., D. DEERE, M. HARDMAN, C. EDWARDS & R. PICKUP. 1997. Go with the flow: use of flow cytometry in environmental microbiology. FEMS Microbiol. Ecol., 24: 93–101.

RIEMANN, B., P.K. BJORSEN, S. NEWELL & R. FALLON. 1987. Calculation of cell production of coastal marine bacteria based on measured incorporation of (H)thymidine. Limnol. Oceanol., 32: 471-476.

SHAPIRO, H. M. 1995. Practical flow cytometry, 3rd edition. Wiley-Liss, New York, USA, 542 pp.

SHERR, E.B., SHERR, B.F. & K. LONGNECKER. 2006. Distribution of bacterial abundance and cell-specific nucleic acid content in the Northeast Pacific Ocean. Deep Sea Res. Part I., 53: 713–725.

STRICKLAND, J.D.H. & T.R. PARSONS. 1972. A practical handbook of seawater analysis. Bull. Fish. Res. Bd. Can., 167: p.310.

ŠOLIĆ, M. & N. KRSTULOVIĆ. 1994. Role of predation in controlling bacterial and heterotrophic nanoflagellates standing stocks in the coastal Adriatic Sea: Seasonal patterns. Mar. Ecol. Prog. Ser., 114: 219-235.

ŠOLIĆ, M., N. KRSTULOVIĆ & S. ŠESTANOVIĆ. 2001. The roles of predation, substrate suply and temperature in controlling bacterial abundance: interaction between spatial and seasonal scale. Acta Adriat., 42: 35-48.

ŠOLIĆ, M., N. KRSTULOVIĆ, I.VILIBIĆ, G. KUŠPILIĆ S. ŠESTANOVIĆ, D. ŠANTIĆ & M. ORDULJ. 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., N. KRSTULOVIĆ, I. VILIBIĆ, N. BOJANIĆ, G. KUŠPILIĆ, S. ŠESTANOVIĆ, D. ŠANTIĆ & M. ORDULJ. 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.

ŠOLIĆ, M., N. KRSTULOVIĆ, G. KUŠPILIĆ, Ž. NINČEVIĆ GLADAN, N. BOJANIĆ, S. ŠESTANOVIĆ, D. ŠANTIĆ & M. ORDULJ. 2010. Changes in microbial food web structure in response to changed environmental trophic status: A case study of the Vranjic Basin (Adriatic Sea). Mar. Environ. Res.,70: 239-249.

TROUSSELLIER, M., C. COURTIES & A. VAQUER. 1993. Recent applications of flow cytometry in aquatic microbial ecology. Biol. Cell., 78: 111–121.

VALENCIA, J., J. ABALDE, A. BODE, A. CID, E. FERNÁNDEZ, N. GONZÁLEZ, J. LORENZO, E. TEIRA & M.VARELA. 2003. Variations in planktonic bacterial biomass and production, and phytoplankton blooms off A Coruña (NW Spain). Sci. Mar., 67: 143–153.

VAQUÉ, D., E.O. CASAMAYORA & J.M. GASOL. 2001. Dynamics of whole community bacterial production and grazing losses in seawater incubations as related to the changes in the proportions of bactera with different DNA content. Aquat. Microb. Ecol., 25: 163-177.

ZUBKOV, M.V., B.M. FUCHS, P.H. BURKILL & R. AMANN. 2001. Comparison of cellular and biomass specific activities of dominant bacterioplankton groups in stratified waters of the Celtic Sea. Appl. Environ. Microbiol., 67: 5210–5218.

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HNA i LNA bakterije u odnosu na aktivnost heterotrofnih bakterija

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SAŽETAK

Jednogodišnje istraživanje HNA i LNA heterotrofnih bakterija u odnosu na bakterijsku proizvodnju provedeno je na području srednjeg i južnog Jadrana. Prosječna bakterijska brojnost na istraživanom obalnom području srednjeg i južnog Jadrana iznosila je od 0.24×10^6 do 2.39×10^6 st mm^{-1}, te na otvorenom moru od 0.23×10^6 to 0.63×10^6 st ml^{-1}. Najviše vrijednosti utvrđene su na obalnom području Šibenika i Kaštelanskom zaljevu. Prosječna bakterijska brojnost na području otvorenog mora iznosila je od 0.23×10^6 do 0.63×10^6 st ml^{-1}. Prosječni udio HNA bakterija na istraživanom priobalnom području iznosio je u rasponu od 21.48 do 93.18%, dok je na otvorenom moru HNA iznosio od 28.62 do 65.62%. Sezonska je raspodjela tijekom proljeća i ljeta na obalnom području uglavnom pokazala istovremeno prevladavanje LNA grupe u bakterijskoj zajednici i visoke vrijednosti bakterijske proizvodnje. Prevladavanje HNA grupe u bakterijskoj zajednici utvrđeno je tijekom hladnijeg razdoblja, zajedno s niskim vrijednostima bakterijske proizvodnje. Iznimke su utvrđene na područjima najviše bakterijske aktivnosti (područja pod utjecajem rijeka), gdje su HNA bakterije prevladavale u vodenom stupcu tijekom sve četiri sezone. Na području otvorenog mora sezonska i vertikalna raspodjela je za vrijeme visokih vrijednosti bakterijske proizvodnje pokazala prevladavanje LNA bakterija. Navedeno upućuje na važnost obiju bakterijskih grupa u morskom ekosustavu istraživanog područja.

Ključne riječi: heterotrofne bakterije, HNA, LNA, bakterijska proizvodnja, Jadransko more