

DISTRIBUTION OF NON-PIGMENTED BACTERIA AND PROCHLOROCOCCUS IN THE COASTAL AREA OF BOKA KOTORSKA BAY

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ABSTRACT

Flow cytometry was used for the first time to determine the abundance of picoplankton in the Boka Kotorska Bay, the Montenegrin part of Adriatic Sea. The abundance of non-pigmented bacteria and Prochlorococcus were determined at three coastal stations from August 2009 to January 2011. The firstly recorded occurrences of Prochlorococcus were obtained. Non-pigmented bacteria abundance ranged from 10^5 to 10^6 cells mL^{-1} . Prochlorococcus abundance ranged from 0 to 10^4 cells mL^{-1} . The seasonal distribution of non-pigmented bacteria showed an increase in abundance during the warmer period and decrease during the winter. The seasonal distribution of Prochlorococcus showed an increase in abundance during the late summer to mid-autumn 2009 and spring 2010. The highest abundance of bacteria and Prochlorococcus was found in Orahovac, Kotor Bay. The abundance of bacteria and Prochlorococcus was influenced by temperature at the surface layers.

Key words: Non-pigmented bacteria, *Prochlorococcus*, Boka Kotorska Bay

INTRODUCTION

Natural bacteria account for a huge portion of the living biomass on earth. In the oceans 80% of the particulate organic carbon is in the form of bacterial cells (Cho & Azam, 1988). They constitute an important share of

plankton biomass, and their activity impacts ecosystem metabolism and function (Jochem et al., 2004).

The introduction of flow cytometry into the analysis of planktonic bacteria (Li et al., 1995; Marie et al., 1997) has greatly improved our capability to estimate standing stocks of bacterioplankton in marine systems (Gasol & del Giorgio, 2000) and has permitted discrimination within cyanobacterial cells.

Photosynthetic picoplankton appears to be an essential component of marine ecosystems and significantly contribute to phytoplankton biomass, especially in oligotrophic areas like the Mediterranean Sea (Magazzù & Decembri, 1995; Agawin & Agustí, 1997). Picophytoplankton is represented primarily by two cyanobacteria genera: *Prochlorococcus* (Chisholm et al., 1992) and *Synechococcus* (Waterbury et al., 1979). Genus *Prochlorococcus* has adapted itself to oligotrophy by minimizing the resources necessary for life through a drastic reduction of cell and genome size (Partenskym & Garczarek, 2010). Nevertheless, it is not strictly restricted to oligotrophic area, but can be found in mesotrophic conditions as well.

Much of the literature concerning the distribution and dynamics of non-pigmented cells within the coastal and open sea area of the southern, central and northern Adriatic. There are only a few reports of *Prochlorococcus* abundance (Fuks et al., 2005; Radić et al., 2009; Šilović et al., 2011; Šantić et al., 2011), and simultaneous observations of *Prochlorococcus* and non-pigmented bacteria in central and southern Adriatic (Šantić et al., 2013).

For the Montenegrin part of the Adriatic Sea, especially for the Boka Kotorska Bay there is lack of picoplankton investigations. The data

for abundance of non-pigmented bacteria and *Prochlorococcus* in this area has not been previously reported. In the investigation of Bosak et al. (2012) *Prochlorococcus* cells has not been detected in the samples.

The goal of this paper was to determine the presence of genus *Prochlorococcus* in Boka Kotorska Bay and if it is present to study it's seasonal cycle and abundance. The second goal was to study distribution and dynamics of non-pigmented cells within the three coastal area of Boka Kotorska Bay.

MATERIALS AND METHODS

The Boka Kotorska Bay represent unique karstic coastal environment described by Krivokapić et al. (2011) as an oligo-mesotrophic system on the basis of chlorophyll *a* and nutrient concentrations. A significant fresh water influx from numerous karstic streams and submarine springs contributes to the unique ecological characteristics of the Bay, especially in its inner part (Kotor Bay). The total surface area is 87.3 km² and the maximum depth is 60 m.

Water samples were taken twice a month from August 2009 to January 2010 and monthly from February 2010 to January 2011. Niskin bottles (5 L) were used for sampling between the surface and the near bottom layer at three stations located near shellfish farms along the coast of Kotor and Tivat Bay. In Kotor and Orahovac (Kotor Bay) samples were taken at depths of 0, 2, 5, 10 and 20 m, and from Sveta Nedjelja (Tivat Bay) at depths of 0, 2, 5, 10, 20 and 30m. Seawater temperature and salinity were recorded *in situ* with universal meter (Multiline P4; WTW).

For chlorophyll *a* measurement samples (1 L) were first filtered through Watman GF/F filters, and then the pigment extraction was

performed in 90 % acetone. Chlorophyll *a* concentrations were determined by measurement of absorbance with a Perkin-Emer UV/VIS spectrophotometer, and calculated according to Jeffrey *et al.* (1997).

Abundances of *Prochlorococcus* and non-pigmented bacteria were determined using flow cytometry (Marie *et al.*, 1997). For flow cytometry counts of autotrophic cells, 2ml of preserved samples in 0.5 % formaldehyde were kept until treatment in the laboratory, while samples for bacteria were preserved in 2 % formaldehyde and stored at 4 °C until analysis. Samples of 1ml without replicas were analyzed on a Beckman Coulter EPICS XL-MCL with a high flow rate from 1 to 1.2 $\mu\text{L sec}^{-1}$. Samples were stained for 30 minutes in the dark with SYBR Green I to the final dilution 1: 10 000. Fluorescence beads were added to calibrate the cells' fluorescence intensity (Level-II Epics Division of Coulter Corporation Hialeah, Florida).

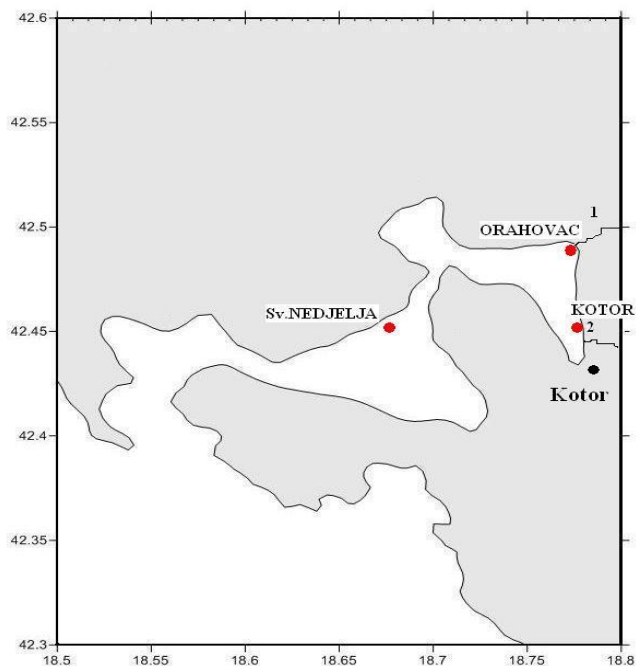


Fig. 1. Study area with sampling stations

Relationships between abiotic and biotic factors and the abundance of *Prochlorococcus* and non-pigmented bacteria were determined using the Person rank correlation index.

RESULTS AND DISCUSSION

Environmental conditions

In the investigated area, thermal stratification and low salinity was noticed almost throughout the whole investigated period mostly due to influence of river or stream discharge near stations.

The temperature at station Kotor ranged from 10.7°C in January 2010 to 27.7°C in August 2010 (Fig. 2). Due to strong influence of river Škurda up to 5m, isothermal period was obtained only from March 2010 to April 2010. In the deeper water the isothermal period lasted longer. It was obtained from October 2009 to April 2010 and again from November 2010 to January 2011. Thermal stratification formed in May 2010 and lasted throughout the warm period. Very low salinity lasted throughout the whole investigated period with the lowest value in February 2010 and December 2010 (2.5 and 1.8, respectively). The highest salinity (38.1) was in January 2011 in the bottom layer.

The temperature at station Orahovac ranged from 10.5°C in January 2010 to 27.6°C in July 2010 (Fig. 3). Isothermal period was in October 2009, in February-April 2010 and in January 2011. Inverse thermal stratification was recorded during the winter 2009 (November 2009 to January 2010) and autumn-winter 2010 (October 2010 to December 2011). Very Low salinity due to stream discharge was recorded in April 2010 and December 2010 (1 and 0.6, respectively). The highest salinity (38.2) was in September 2010 in the bottom layer.

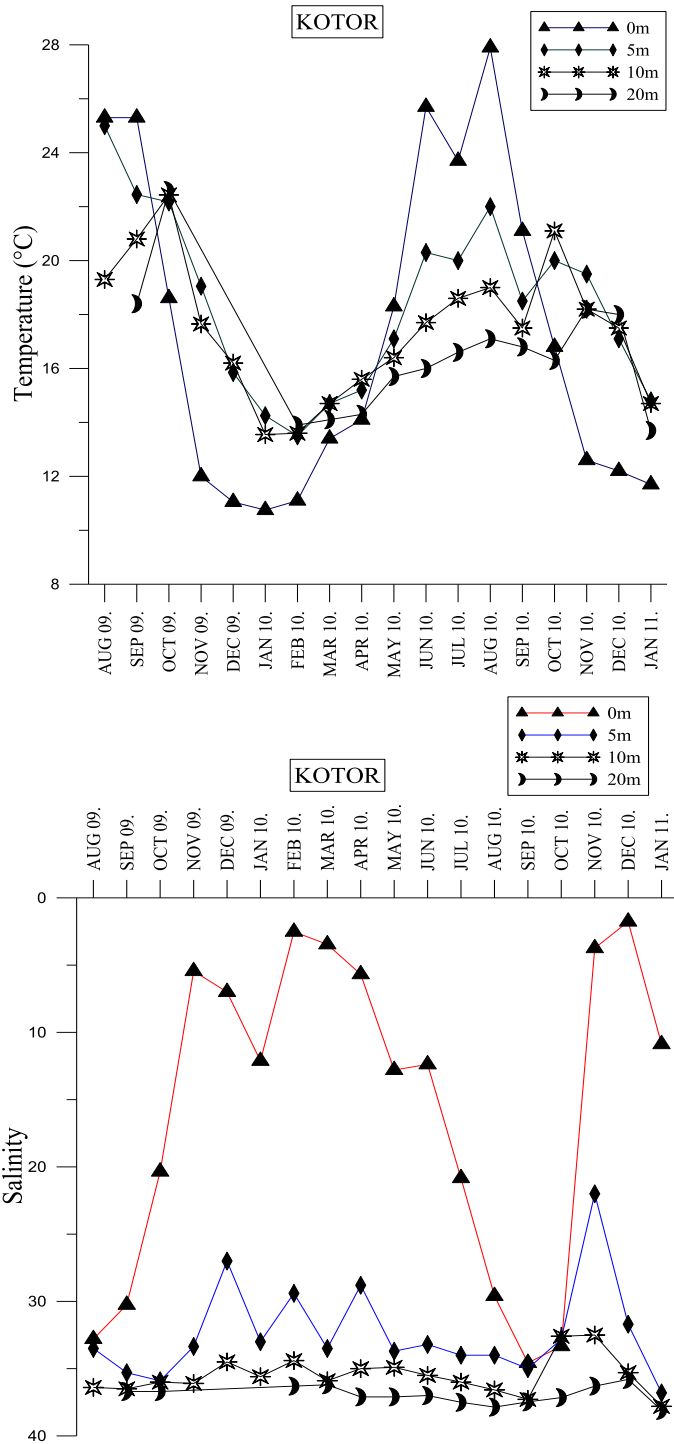


Fig. 2. Temperature (°C) and salinity at station Kotor

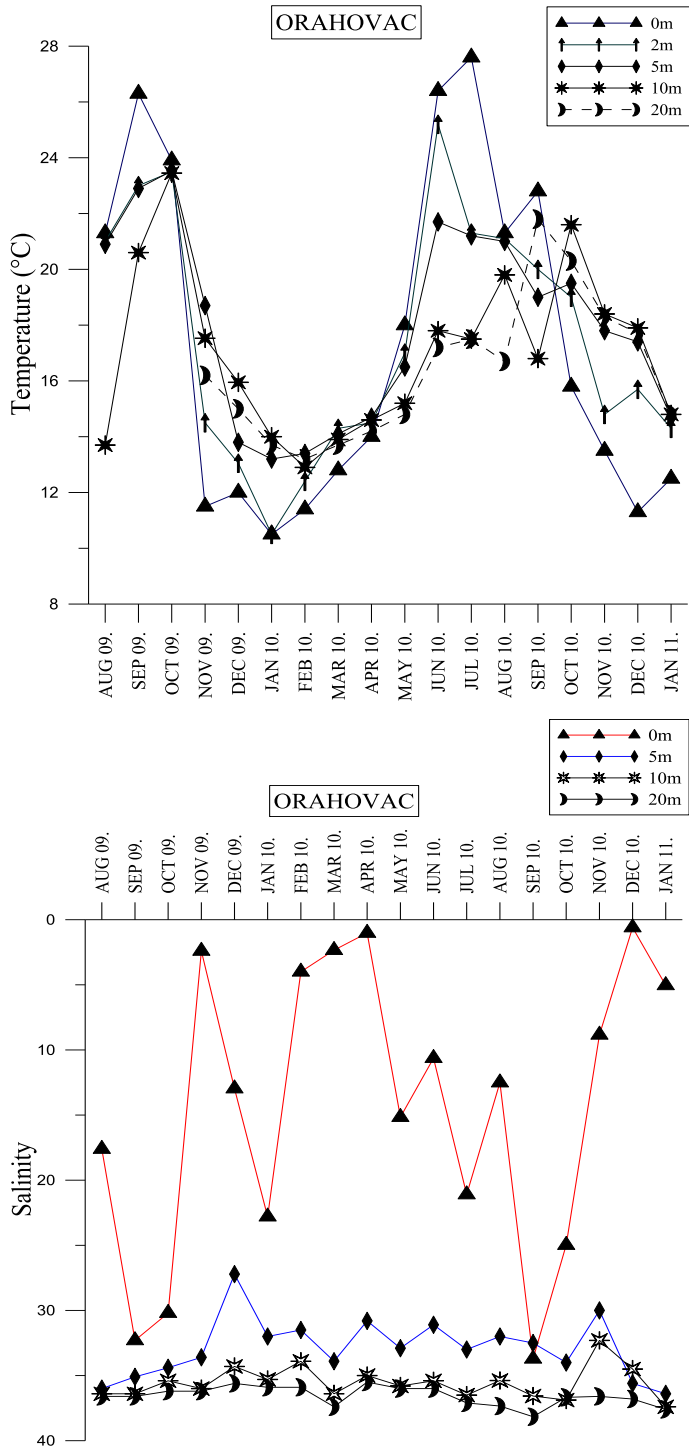


Fig. 3. Temperature (°C) and salinity at station Orahovac

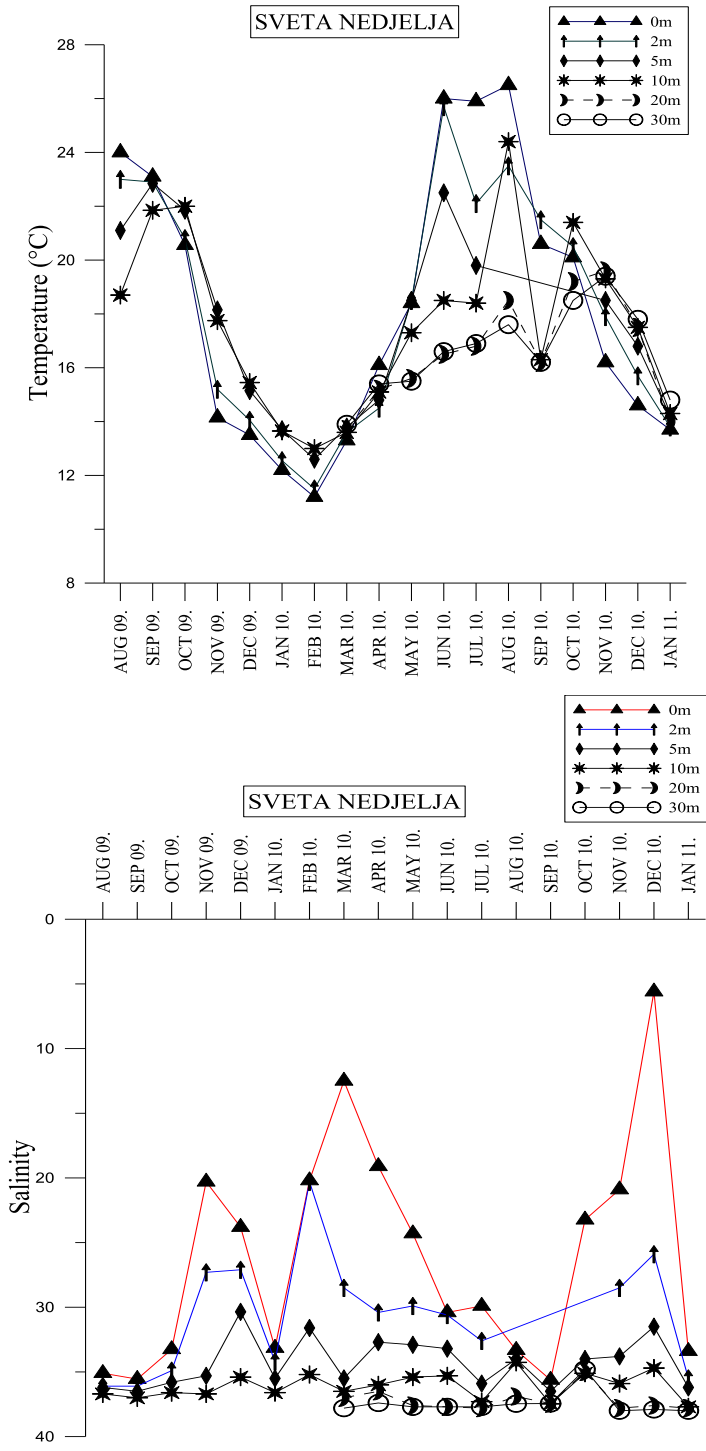


Fig. 4. Temperature (°C) and salinity at station Sveta Nedjelja

At station Sveta Nedjelja the temperature ranged from 11.2°C in February 2010 to 26.5°C in August 2010 (Fig. 4). The isothermal period began in September 2009 and lasted to April 2010. The thermal stratification formed in May and lasted until January when the isothermal period began again. The lowest salinity (5.6) was recorded in December 2010, and the highest (38) was in October 2010 in the bottom layer.

Abundance of non-pigmented bacteria

Average monthly abundance of non-pigmented bacteria, obtained as the average value from the surface to the bottom layers of the Kotor station ranged from 0.35×10^6 to 1.32×10^6 cells mL⁻¹ (Fig. 5), with a maximum in May 2010 at 0 m (1.98×10^6 cells mL⁻¹). The correlation with temperature was positive ($r = 0.59$, $p < 0.0001$, $n = 82$) and the most significant correlation was at 0 m ($r = 0.47$, $p = 0.000004$, $n = 23$) (Table 1). The correlation between bacteria abundance and salinity was $r = 0.31$ and $p = 0.004$ ($n = 82$). Positive correlation with concentration of bacteria and chlorophyll *a* was determined only at 0m - surface layer ($r = 0.47$, $p = 0.03$, $n = 21$).

At the Orahovac station, average monthly abundance ranged from 0.37×10^6 to 1.12×10^6 (Fig. 6), with a maximum in Jun 2010 at 2 m (1.89×10^6 cells mL⁻¹). The correlation with temperature was positive ($r = 0.59$, $p < 0.0001$, $n = 90$) with most significant correlation at 2 m (Table 2). The correlation between bacteria abundance and salinity was $r = 0.39$ and $p = 0.00011$ ($n = 89$).

At the Sveta Nedjelja station average monthly abundance of non-pigmented bacteria ranged from 0.31×10^6 to 0.95×10^6 cells mL⁻¹ (Fig. 7)

with maximum in October 2010 at 2 m depth (1.18×10^6 cells mL⁻¹). The correlation with temperature was positive ($r = 0.46$, $p < 0.0001$, $n = 81$) with most significant correlation at 2 m depth (Table 3). The correlation with salinity and concentration of chlorophyll *a* was not determined.

Table 1. Correlation between non-pigmented bacteria abundance and sea temperature at station Kotor ($n = 23$)

Depth (m)	r	P
0	0.8	<0.0001
5	0.27	non significant
10	0.28	non significant
20	0.53	non significant

Table 2. Correlation between non-pigmented bacteria abundance and sea temperature at station Orahovac ($n = 21$)

Depth	r	p
0	0.72	<0.001
2	0.84	<0.001
5	0.7	<0.05
10	0.35	non significant
20	-0.27	non significant

Table 3. Correlation between non-pigmented bacteria abundance and sea temperature at station Sveta Nedjelja ($n = 21$)

Depth	r	p
0	0.54	<0.05
2	0.66	<0.05
5	0.49	<0.05
10	0.31	non significant
20	0.25	non significant
30	0.25	non significant

Comparing all investigated stations, the highest numbers of non-pigmented bacteria were found in the Kotor with maximum in October 2009 and August 2010. During the investigation, seasonal distribution in the bacterial community in those three coastal areas was determined with

maximum in the spring-summer period and minima during the winter. This is consistent with previous reports for the central and southern Adriatic (Krstulović 1992; Šolić et al. 2001; Šantić et al. 2013), but these three stations have one more maxima in average bacterial abundance in October 2010.

Abundance of Prochlorococcus

Average monthly abundance of *Prochlorococcus*, obtained as the average value from the surface to the bottom layers of the Kotor station ranged from 0.8×10^3 to 3.2×10^4 cells mL⁻¹(Fig. 5). Higher abundance was observed in warmer seasons with maximum in October 2009 at 20 m and May 2010 at 5 m. The correlation between *Prochlorococcus* and temperature was $r = 0.29$ and $p = 0.007$ ($n = 83$). In the surface layer, positive correlation was between *Prochlorococcus* abundance and concentration of chlorophyll *a* ($r = 0.6$, $p < 0.05$, $n = 21$). The correlation with salinity was $r = 0.7$ and $p = 0.00006$ ($n = 23$) only in the surface layer.

At station Orahovac, average monthly abundance of *Prochlorococcus* ranged from 0 to 5.4×10^4 cells mL⁻¹(Fig. 6). Higher abundance was observed in warmer seasons, with maximum in October 2009 at 5 m. The correlation between *Prochlorococcus* and temperature was $r = 0.21$ and $p = 0.04$ ($n = 92$), and with salinity was $r = 0.29$ and $p = 0.005$ ($n = 90$).

At station Sveta Nedjelja, average monthly abundance of *Prochlorococcus* ranged from 0 to 2.3×10^4 cells mL⁻¹(Fig. 7). Higher abundance was observed in late summer, October 2009 with maximum at 0 m, and spring (March to May 2010) with maximum abundance at deeper layer (10 – 20 m). Unlike the other two positions, at this station one more peak in abundance was observed in November 2010. The correlation

between *Prochlorococcus* and temperature was $r = 0.28$ and $p = 0.03$ ($n = 64$)

In this study, the presence of *Prochlorococcus* cells in the water column was observed during all four seasons. This is consistent with results reported by Šantić *et al.* (2011) for the central Adriatic Sea. Its abundance rapidly decreases from October to the beginning of winter until its almost complete disappearance in December. It reappeared from January to June when abundance rapidly decreased again. A general increasing trend from June to October and drastically reduced number in December are in concordance with data in North Adriatic (Radić *et al.*, 2009).

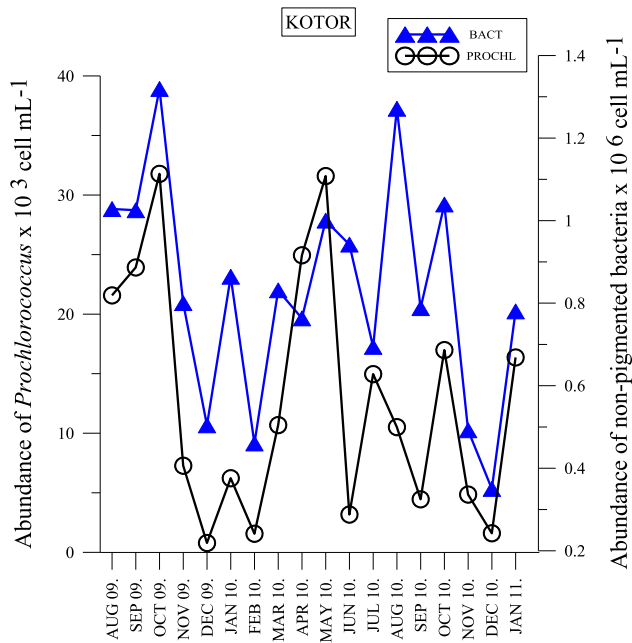


Fig. 5. Average monthly abundance of bacteria and *Prochlorococcus* in water column at station Kotor

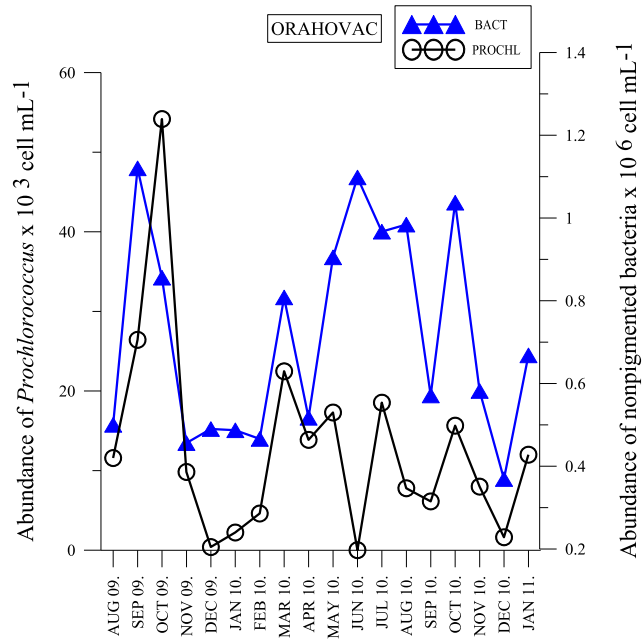


Fig. 6. Average monthly abundance of bacteria and *Prochlorococcus* in water column at station Orahovac

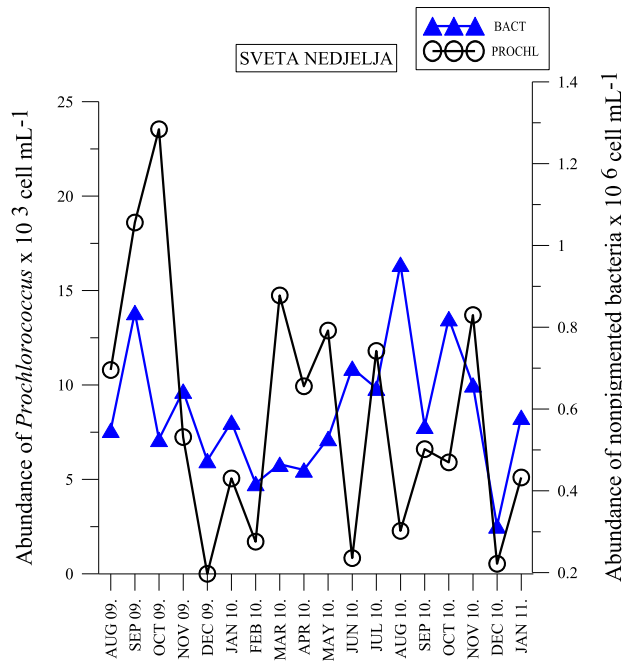


Fig. 7. Average monthly abundance of bacteria and *Prochlorococcus* in water column at station Sveta Nedjelja

Prochlorococcus is the most abundant photosynthetic organism in the open ocean but it can be also found in coastal areas and bays. In this study, their abundance ranged from undetectable ($< 0.1 \times 10^3$ cells mL⁻¹) to 101×10^3 cells mL⁻¹ which is considerably lower than most available data from the world's oceans (Partensky *et al.*, 1999). This is consistent with the abundance recorded in the central Adriatic Sea (Šantić *et al.* 2011), Mediterranean coastal and open sea (Sommaruga *et al.*, 2005; Garczarek *et al.*, 2007) and with Pan *et al.* (2007) who found that average abundance of *Prochlorococcus* in Changjiang Estuary and adjacent coastal waters was from 0 to 210×10^3 cells mL⁻¹, and for bacteria average abundance from 0.41 to 2.53×10^6 cells mL⁻¹.

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