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## **ABSTRACT**

Distribution of phytoplankton and their composition were presented in Boka Kotorska Bay, small semi-enclosed bay, situated in south-eastern part of Adriatic Sea. Samples were taken at seven stations in the whole Boka Kotorska Bay, on monthly interval from April to September 2010. Abundance was higher in inner part - Kotor Bay and decreasing in outer part - Tivat Bay, especially in the most opened part of Bay - Herceg Novi Bay. Diatoms prevailed throughout all investigated period, with maximum in summer (1.2 x 10<sup>6</sup> cells L<sup>-1</sup>). Domination of nutrient preferred species: *Thalassionema nitzschioides, Pseudo-nitzschia spp., Leptocylindrus danicus, Nitzschia longissima* was noticed.

Due to the anticipated increase of human impact in the area, this study can serve as a basis for future environmental studies in Boka Kotorska Bay.

Keywords: Phytoplankton distribution, Boka Kotorska Bay, South Adriatic Sea

## **INTRODUCTION**

The Mediterranean Sea is an oligotrophic ecosystem (Azov, 1991), but nowadays Mediterranean coastal ecosystems are undergoing rapid alteration since they are under the combined pressure of climate change and human impact (Turley, 1999; Bianchi & Morri, 2000; Béthoux et al., 2002).

The Adriatic Sea is the northernmost basin in the Mediterranean, divided into three parts: northern, middle and southern part of Adriatic Sea. There are many studies which are focused on hydro-chemical properties and phytoplankton distribution and dynamics in northern Adriatic Sea (Revelante & Gilmartin, 1976, 1980; Revelante et al. 1984, Možetić et al. 2002, Totti et al. 2005), in middle Adriatic (Caroppo et al. 1999, Totti et al. 2000) and southern part of Adriatic Sea (Viličić et al. 1995, Jasprica & Carić, 2001).

Boka Kotorska Bay is a semi-enclosed Bay with surface area of 87.3 km<sup>2</sup>. In this Bay, especially in its inner part, anthropogenic impact has been substantial. The Bay is not under high influx of fresh waters, except in the period of precipitation, when streams start their activities and input of nutrients to sea ecosystems increase.

The literature concerning phytoplankton and impact of hydrographical conditions on phytoplankton communities in Bay is scarce.

The purpose of this study is to analyze phytoplankton assemblages and to estimate possible changes and with all that to contribute to our knowledge of the southern Adriatic Sea - Boka Kotorska Bay.

## MATERIALS AND METHODS

Sampling was carried out at seven stations in the whole of Boka Kotorska Bay. It comprises three positions in Kotor Bay: IBM, Orahovac and

Kotor-central position, in Risan Bay one position, in Tivat Bay two positions: Sveta Nedelja and Tivat-central position and in Herceg -Novi Bay one position (Fig.1.).

Water samples for analyses were collected from April 2010 to September 2010, on a monthly basis at three depths: surface, middle and bottom, using 5 L Niskin bottle (Hydro Bios).

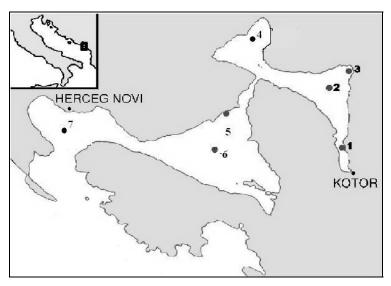


Figure 1. Sampling positions (1-IBM, 2-Kotor-central, 3-Orahovac, 4- Risan, 5-Sveta Nedelja, 6- Tivat-central, 7-Herceg Novi)

Physical parameters such as temperature, salinity and oxygen concentration were measured *in situ* with universal meter (Multiline P4). Oxygen concentrations and saturation values were determined with an oxygen electrode (Oxy Guard Handy Gamma). Transparency was determined with a Secchi disc (30cm).

Concentration of nitrates, nitrites and phosphates was determined in accordance with methods proposed by Strickland and Parsons 1972.

Absorbances were detected on a Perkin-Elmer UV/VIS spectrophotometer (Lambda 2), at a different wavelength for each nutrient.

Water samples for measurement of chlorophyll *a* were first filtered through Whatman GF/F filters, and then the pigment was extracted in 90% acetone. Finally, chlorophyll *a* concentrations were determined by measurment of absorbances on a Perkin-Emer spectrophotometer and calculated according to Jeffrey et al. (1997).

Samples for phytoplankton investigation were preserved in a 3% neutralized formaldehyde solution and analyzed on a Leica DMI4000 B inverted microscope according to Utermöhl 1958. Determination of phytoplankton was done by using the keys for phytoplankton identification such as: Hustedt (1959), Hasle & Sylvertsen (1997), Round et al. (1990) and Throndsen et al. (2007).

## RESULTS AND DISCUSSION

Physical parameters (temperature, salinity) varied during the investigated period. Temperature was from 13.40  $^{\circ}$ C to 29.40  $^{\circ}$ C, while salinity showed minimum of 1.02 PSU and maximum of 38.16 PSU (Tab. 1).

The highest nutrient concentrations (for nitrate 9.32 μmol L<sup>-1</sup>, nitrite 2.71 μmol L<sup>-1</sup>, phosphate 0.98 μmol L<sup>-1</sup>) were determined during the pre-bloom period (May) and have been considered as the available nutrient stock.

The nutrient stock in aquatic ecosystems significantly decreases when a phytoplankton bloom occurs, being transformed into biomass. In the same way, nutrient availability usually limits species growth at the end of the bloom (Howarth, 1988; Roelke et al. 1999).

Concentration of nutrients in the period of highest phytoplankton growth were lower as a results of phytoplankton consumption and ranged from

maximum of 1.759  $\mu$ mol L<sup>-1</sup> for nitrate and 0.624  $\mu$ mol L<sup>-1</sup> for phosphate. Only value for silicate (42.442  $\mu$ mol L<sup>-1</sup>) were the highest and this is in contrast with general opinion that lower values of silicate are related to typical bloom formed by diatoms. It can be explained that silicate concentration before consuming of diatoms was higher than 42.442  $\mu$ mol L<sup>-1</sup> and decreased after consuming. There is no paper to make comparison with, so we will try to deal with this issue in future investigation.

Table 1. Physical, chemical and biological parameters measured during investigated period

	Min	Mean	Max	SD	n
Temperature (°C)	13.40	18.54	29.40	3.70	126
Salinity (PSU)	1.02	32.91	38.16	7.88	126
PO <sub>4</sub> <sup>3-</sup> (μmol L <sup>-1</sup> )	0.00	0.25	0.98	0.19	126
$NO_2$ ( $\mu$ m of $L^{-1}$ )	0.00	0.21	2.71	0.37	126
$NO_3$ ( $\mu$ m of $L^{-1}$ )	0.00	1.17	9.32	1.85	126
Si (OH)4 (μmol L <sup>-1</sup> )	0.00	5.43	42.44	5.45	126
Chlorophyll a (mg m <sup>-3</sup> )	0.102	0.89	2.68	0.49	126

Maximum mean values of microplankton ranged from 5.2 x 10<sup>4</sup> to 4.1 x 10<sup>5</sup> cells L<sup>-1</sup> (on surface layer maximum were 1.39 x 10<sup>5</sup> to 1.2 x 10<sup>6</sup> cells L<sup>-1</sup>). The both highest values were found in Kotor Bay. In Tivat Bay and Herceg Novi Bay highest values were in order up to 10<sup>3</sup> to 10<sup>4</sup> cells L<sup>-1</sup>. Higher values in Kotor Bay are results of structure of this Bay which is more confined and with less water dynamics. Also this Bay is under higher influences of costal area than Tivat and Herceg Novi Bay which are more open. It was noticed that abundance decreased going from inner part to opener part, so values in Herceg Novi Bay were lower almost through the entire investigated period (Fig.2). The same results that maximum was in summer period and in inner part of Bay, were noticed by Drakulović et al. (2010a, b). These results are in contrast with general opinion that phytoplankton shows bimodal cycle with two maximum: one in spring and second in autumn (Ninčević and Marasović 1998) and low

values in warmer period. Data noticed by Kefi et al. 2005 showed a clear autumnal maximum, often higher than the winter-spring bloom. The autumnal blooms are a common feature in the western Mediterranean coastal areas (Zingone et al. 1995). It is important to emphasize that our data only comprise six months period, so for autumn and winter we do not have data and we are not able to compare. In summer, Kotor Bay (Krivokapić et al. 2009) was characterized by low concentration of nutrients, high light transparency and absence of phytoplankton blooms. These suggested summer oligotrophication, as in other eastern Adriatic environments (Svensen et al. 2007, Viličić et al. 2008). However this is in contrast with current study, in which the highest values were in summer, being the period of stratification. This can be explained as a result of human impact.

Values found in this work, namely mean abundance of 10<sup>4</sup> and 10<sup>5</sup> cells L<sup>-1</sup> coincide with data from northeastern Adriatic (Viličić et al. 2009, Bosak et al. 2009), but are higher than values found for northeastern Mediterranean which is classified as oligotrophic area (Balkis 2009).

Maximum mean values of nanoplankton ranged from 6.7 to  $7.2 \times 10^5$  cells L<sup>-1</sup> (on surface layer maximum was  $1.2 \times 10^6$  cells L<sup>-1</sup> in Tivat Bay and in middle layer  $1.02 \times 10^6$  cells L<sup>-1</sup> in Kotor Bay). High nanoplankton values usually appear one month before or after maximum microplankton abundances (Fig.2). It is due to higher capacity of nanoplankton cells to absorb lower nutrient concentrations (Thingstad & Sakshag 1990).

The size component smaller than 20  $\mu$ m, commonly defined as nanoplankton, was mainly constituted of small dinoflagellates, coccolithophorids and small solitary diatom species. Small nanoflagellates are the dominant group in terms of cell numbers most of the year in oligotrophic Mediterranean Sea waters (Revelante & Gilmartin, 1976; Malej et al., 1995; Totti et al., 1999; Decembrini et al., 2009).

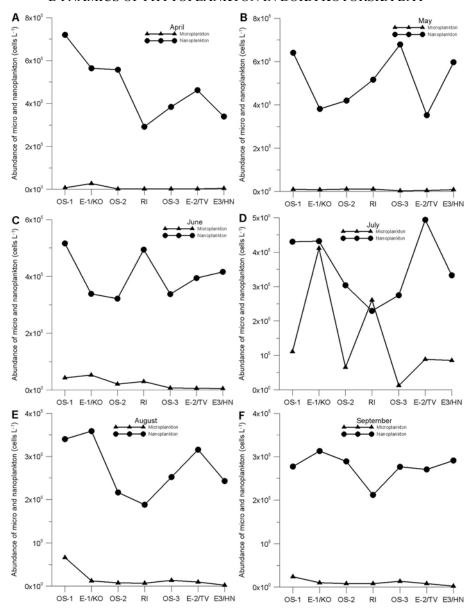


Figure 2 (A, B, C; D, E, F) Mean abundance of micro and nanoplankton on sampling stations

Biomass was estimated by the concentration of chlorophyll a. Maximum concentration of chlorophyll a ranged from 2.089 mg m<sup>-3</sup> in August to 2.682 mg m<sup>-3</sup> in May, both values were noticed in Kotor Bay. These values

do not coincide with maximum of phytoplankton in July. The same results were found by Polat 2002 for eastern Mediterranean. The non-coinciding relation between phytoplankton abundance and concentration of chlorophyll a was also found in paper of Drakulović et al. 2010b, and can be explained by different photosynthetic activities, different cell sizes, different composition of phytoplankton, and different physiological states of the cells (Ninčević and Marasović 1998). Maximum of chlorophyll a found in spring coincide with result for Zrmanja estuary (Burić et al. 2007).

Classification of seawater area by chlorophyll *a* concentration differs among investigators. According to studies by Giovanardi and Tromellini (1992) this area is classified as oligotrophic while according Babin et al. 1996 as mesothrophic.

Maximum mean abundance of chlorophyll *a* concentration in the investigated period was in Kotor Bay (Fig.3). These mean values are lower in comparison with results in Kotor Bay (Krivokapić et al. 2009), especially in comparison to Mediterranean, for example port of Alexandria (Dorgham et al. 2004) and Mallorca (Puigerver et al. 2002). However, these values are to data in northern Adriatic, such as Bay of Trieste (Tedesco et al. 2005), northeastern Adriatic Sea (Precali et al. 2001).

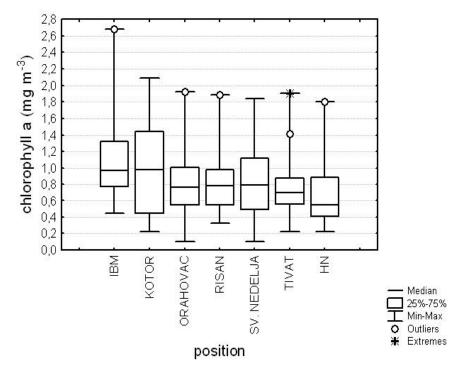


Figure 3. Mean values of concentration of chlorophyll a on investigated locations in Boka Kotorska Bay

Diatoms prevailed almost the entire period of investigation, with increased abundance in summer period (maximum value was 1.2 x 10<sup>6</sup> cells L<sup>-1</sup>, in July). The maximum mean abundance of diatoms was in inner part of Boka Kotorska Bay - Kotor Bay, while the minimum mean value was in opener part - Herceg Novi Bay and also low values were in Tivat Bay (Fig.4 A). This situation is expected having in mind that Herceg Novi is under higher influence of the open sea (Vuksanović 2003).

The highest mean abundances of dinoflagellates through the investigated period were also in Kotor Bay (Fig. 4 B). Maximum of dinoflagellates was in June and August (1.66 and 1.67 x 10<sup>4</sup> cells L<sup>-1</sup>). This phytoplankton group usually shows higher values in warmer period, when they tend to predominate over diatoms. Diatoms are predominant phytoplankton

group most of the year, while dinoflagellates prevail only in oligotrophic conditions, in summer period (Bernardi Aubry et al. 2004). In this study diatoms prevailed over dinoflagellates the entire period of investigation especially in summer when we noticed diatoms bloom which is in correlation with findings of Drakulović et al. 2010b, at the same area. This results from the increased input of nutrients which provide high phytoplankton growth in summer stratification period.

As for other groups (silicoflagellates, coccolithophorids and euglenophyta), maximum mean abundance was in Kotor Bay (Fig.4 C). The maximum mean abundance of this fraction was 5.468 x 10<sup>3</sup> cells L<sup>-1</sup> (maximum was on surface 1.3 x 10<sup>4</sup> cells L<sup>-1</sup> in August). This peak was made mostly of coccolithophorids which dominate on other groups during the investigated period. Silicoflagellates prefer colder period, so we cannot make comparison because our investigation did not comprise colder period. We can only say that in the period that we investigated abundance of silicoflagellates was low.

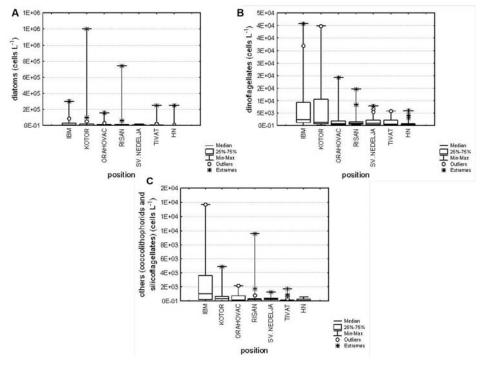


Figure 4. Mean values of abundance of A) diatoms, B) dinoflagellates and C) others (silicoflagelates and coccolithophorids)

Maximum of microplankton fraction was made of pennatae diatoms Thalassionema nitzschioides. At recent time, this pennatae have prevailed and become the most abundant and frequent. Also pennatae diatom Pseudonitzschia spp. was often present. This diatom is interesting because it produces domoic acid. Determination to species is impossible with light microscope, so we have done determination to genus. Centric diatoms were presented with species: Chaetoceros affinis, Chaetoceros spp., Coscinodiscus perforatus, Leptocylindrus mediterraneus, Melosira nummuloides, Proboscia alata, Skeletonema spp. Pennatae diatoms were presented with species: Amphora spp., Cocconeis scutellum, Diploneis bombus, Licmophora flabellata, Navicula spp., Nitzschia longissima, Pleurosigma elongatum, Pseudo-nitzschia spp., and Thalassionema nitzschioides. Majority of noticed diatoms belong to species

which grow better in area rich with nutrients (Pucher-Petković and Marasović 1980).

Cloern (2001) indicated that changes from oligotrophic to eutrophic conditions lead to changes in the species composition and food web and he emphasised the importance and usefulness of the above mentioned indicators as tools for assessment of the eutrophication status

Dinoflagellates were presented with some frequent species such as: Ceratium furca, Ceratium fusus, Dinophysis acuminata, Dinophysis fortii, Gonyaulax spp., Gyrodinium fusiforme, Gymnodinium spp, Prorocentrum micans, Prorocentrum minimum, Protoperidinium diabolus, Protoperidinium globulum, Protoperidinium spp., Scrippsiella sp.

Toxic dinoflagellates (e.g. *Dinophysis fortii*) were also noticed. However their concentration was not so high to become harmful for the environment or human health.

Among coccolithophorids the most frequent were species Calyptosphaera oblonga, Helicosphaera walichii, Rhabosphaera tignifera, Syracosphaera pulchra. Silicoflagellates were presented with species Dictyocha fibula.

The dendrogram for different ecological parameters by months showed the highest similarity between salinity and temperature on one side which and between nutrient concentration and chlorophyll a on the other side. The highest similarity between salinity and temperature was expected because both parameters depend of weather conditions. On the other hand, concentration of chlorophyll *a* depends on nutrient concentration (Fig.5.)

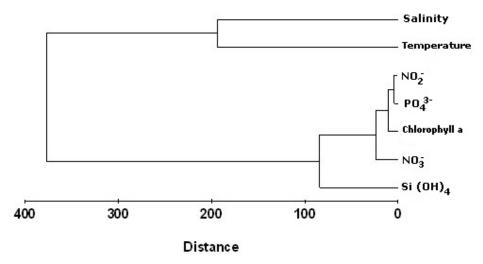


Figure 5. Tree diagram for different parameters in different months (complete linkage Euclidean distance).

## **CONCLUSION**

Considering the results mentioned above, namely abundance of  $10^6$  cells  $L^{-1}$  and presence of nutrients preferred species, it is obvious that this area is exposed to increased human impact. This impact is higher in the inner part of Boka Kotorska Bay as in the outer part of the Bay is lower as a result of the influence of the open sea.

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