

## **Algal pigments distribution and phytoplankton group assemblages in coastal transitional environment – Boka Kotorska Bay (South eastern Adriatic Sea)**

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*Phytoplankton assemblages and pigment distribution were determined in the transitional Boka Kotorska Bay. Samples were collected four times from three stations in the inner part of the Bay between April 2008 and March 2009. Microscopic analysis revealed that the phytoplankton was composed of marine diatoms, dinoflagellates, cryptophytes, chlorophytes and coccolithophorids. Aside from fucoxanthin as the dominant biomarker pigment, alloxanthin, 19'-hexanoyloxyfucoxanthin, peridinin and chlorophyll b provided indicative contributions. Fucoxanthin and alloxanthin showed significant correlation with the phytoplankton chlorophyll a biomass throughout the investigated period. In November 2008, the diatoms were outcompeted by coccolithophorids, which probably efficiently absorbed nutrients during a period of their reduced supply. Due to the reduced nutrient input, in the summer, the phytoplankton community in the Bay was composed mostly of marine dinoflagellates, cryptophytes, and chlorophytes.*

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**Key words:** Pigments distribution, seasonality, Boka Kotorska Bay

## INTRODUCTION

The presence of pigments and phytoplankton assemblages in water samples, as well as their qualitative and quantitative diversity, depends on the interaction of numerous environmental factors. In particular, pigment quality emerges from the species composition of phytoplankton occurring in a given geographical region and specific season (STÓN *et al.* 2002). Phytoplankton is known to exhibit rapid responses to changes in environmental conditions and is therefore commonly acknowledged as a bioindicator of environmental seasonal changes (RIMET & BOUCHEZ, 2012).

The use of adequate pigments to characterize phytoplankton communities has been found to be useful in estimating the numbers of small and fragile cells usually underestimated by other procedures (JEFFREY *et al.* 1997). Fucoxanthin, peridinin, chlorophyll b, zeaxanthin and alloxanthin are the main photosynthetic pigment characterizing diatoms, dinoflagellates, chlorophytes, cyanobacteria and cryptophytes, respectively (BARLOW *et al.*, 2008). However, pigment data interpretation can be difficult as some pigments are present in several algal groups (MENDES *et al.*, 2011). For instance, fucoxanthin, which is a major pigment in diatoms, is also present in chrysophytes and prymnesiophytes (WRIGHT & JEFFREY, 2006).

Automated measurements of pigment concentrations using high-performance liquid chromatography (HPLC) allow for highly reproducible analysis. Many studies have demonstrated a correlation between phytoplankton abundance and pigment biomarkers (AHEL & TERZIĆ, 1998; CARRETO *et al.*, 2003; VILIČIĆ *et al.*, 2008; AGIRBAS *et al.*, 2017). However, the main issue when using pigments for quantitative taxonomy is the overlapping presence of several pigments in different phytoplankton group. The chemotaxonomic software CHEMTAX was developed to overcome this problem (MACKAY *et al.*, 1996; LEWITUS *et al.*, 2005). There is a large amount of literature concerning the use of high-performance liquid chromatography (HPLC) pigment analysis and software CHEMTAX for marine systems

(MANDU *et al.*, 2014; COUPEL *et al.*, 2015; mendes *et al.*, 2015; ARAUJO *et al.*, 2017).

Boka Kotorska Bay is a deeply indented bay located in the southeastern part of the Adriatic Sea, where the effect of anthropogenic impact has been substantial, especially in its inner part (KRIVOKAPIĆ *et al.*, 2011; DRAKULOVIĆ *et al.*, 2016). Studies have been undertaken to investigate the spatial and temporal distribution of physical, chemical and biological oceanographic properties, phytoplankton, nutrients and coloured dissolved organic matter in Boka Kotorska Bay (KRIVOKAPIĆ *et al.*, 2009; CAMPANELLI *et al.*, 2009; KRIVOKAPIĆ *et al.*, 2011; DRAKULOVIĆ *et al.*, 2012; BOSAK *et al.*, 2012; DAUTOVIĆ *et al.*, 2012; MARINI *et al.*, 2015; DRAKULOVIĆ *et al.*, 2016).

The aim of this work is to present, for the first time, the detailed seasonal distribution of particular pigments obtained by HPLC with the distribution of individual phytoplankton groups against the background of changes in physico-chemical parameters, in Boka Kotorska Bay (in the SE Adriatic Sea).

## MATERIAL AND METHODS

### Investigated area

The study is focused on the southeast part of the Adriatic Sea; the inner part of Boka Kotorska Bay, Kotor Bay. Boka Kotorska Bay (Fig. 1) is one of the most important transitional areas in the region, from both an environmental and a socio-economic point of view. It is formed by three indented branches. There are two innermost embayments to the southeast and northwest (Kotor and Morinj-Risan Bays, respectively) and Tivat Bay spreading to the south from the narrow Verige Strait. The Kumbor Strait connects Tivat Bay to Herceg Novi Bay and the open Adriatic Sea (BELLAFIORE *et al.*, 2011).

The surface area of Boka Kotorska Bay is 87.3 km<sup>2</sup>. The total volume of Boka Kotorska Bay is 2.4 x 10<sup>6</sup> m<sup>3</sup>. The inner part, Kotor Bay, comprises 27% of the area and 26% of the volume of the whole Bay.

In the study area, there are two small rivers: the Škurda, an active river during the whole year

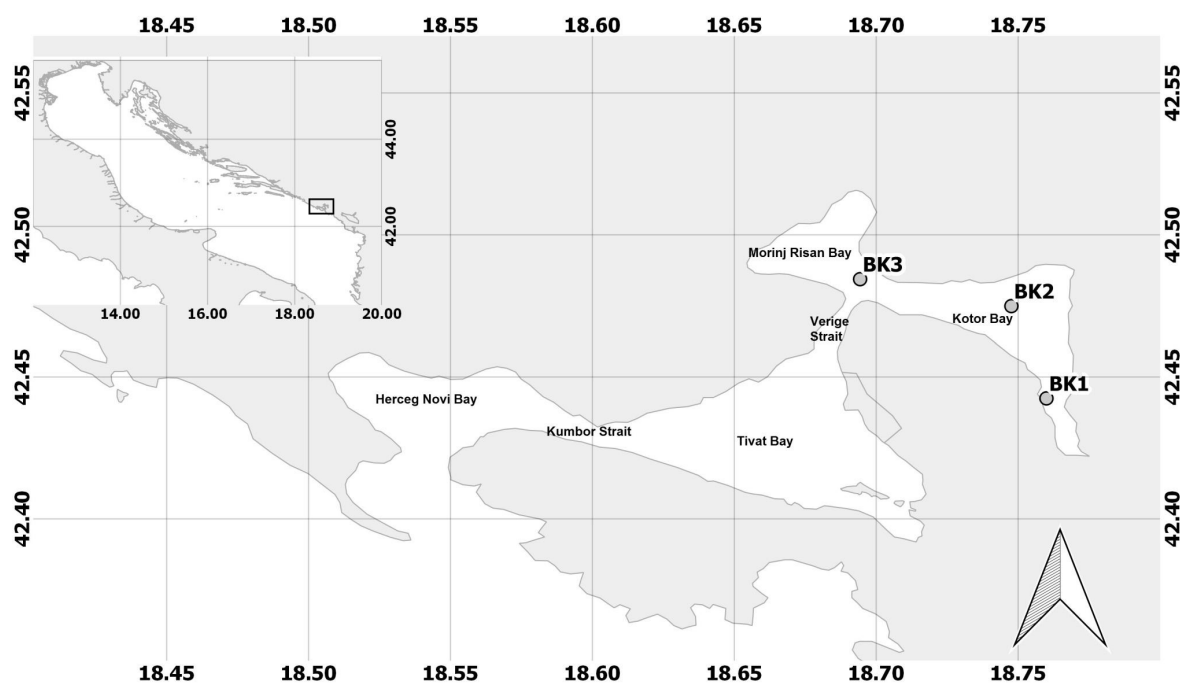


Fig. 1 Map of sampling stations

and the Ljuta, which is active only during the late fall, winter and early spring. The Škurda and the Ljuta empty into Kotor Bay. The discharge of freshwater sources can reach values up to 30 and 330 m<sup>3</sup>/s, respectively (BELLAFIORE *et al.*, 2011). Kotor Bay is greatly influenced by freshwater coming from karstic coastal and underwater springs. There is an extremely high annual precipitation quantity; the 4584 mm it received is Europe's maximum, recorded near Crkvice (IHMZ). Due to its characteristics, the Bay is considered a ROFI (Region of Freshwater Influence)

### Sample collection and data analysis

Seawater samples were taken using a 5l Niskin bottle sampler, from three stations in the inner part of the Bay (see Fig. 1) seasonally in April 2008, July 2008, November 2008 and March 2009. At the innermost station near the Institute of Marine Biology (BK1), samples for physical, chemical and biological analysis were taken from five depths: 0, 2, 5, 10 and 15m. At the central stations of Kotor (BK2) and Perast (BK3) samples were taken from seven depths: 0, 2, 5, 10, 15, 20, and 25 m. The hydrological

parameters (temperature and salinity) at BK1 station were taken on a weekly basis from February 2008 to March 2009 (a total of 47 samplings).

Temperature and salinity were measured *in situ* using a multi LINE P4 – UNIVERSAL METER while an Oxi-Guard Handy GAMMA was used for measuring oxygen concentrations. The nutrient samples were taken using 5 L Niskin bottles and stored in polyethylene bottles. The determination of nutrient concentrations was done the same day, directly after the sampling. The concentration of nitrates, nitrites, phosphates and silicates were determined using standard methods (STRICKLAND & PARSONS, 1972). The absorbance readings were made on a Perkin Elmer UV/VIS spectrophotometer (Lambda 2), at different wavelengths for each nutrient.

The most prominent biomarkers such as fucoxanthin, peridinin, 19-hexanoyloxyfucoxanthin, alloxanthin, chlorophyll *b* were chosen to illuminate the temporal and spatial variability of the diatoms, dinoflagellates, coccolithophorids, cryptophytes, chlorophyta respectively (AHEL & TERZIĆ, 1998; VILIČIĆ *et al.*, 2008). Filters containing phytoplankton were extracted in 4 ml of cold

Table 1. Taxonomic pigments used in this study

Phytoplankton groups	Phytoplankton pigments / abb	References
Diatoms	Fucoxantin / fuco	Ston <i>et al.</i> , 2002; Viličić <i>et al.</i> , 2008; Vidussi <i>et al.</i> , 2001
Dinoflagelate	Peridin /per	Ston <i>et al.</i> , 2002; Viličić <i>et al.</i> , 2008; Vidussi <i>et al.</i> , 2001
Cryptophyceae	Alloxantin / allo	Ston <i>et al.</i> , 2002; Viličić <i>et al.</i> , 2008; Vidussi <i>et al.</i> , 2001
Coccolithophorides	Hexa-fucoxantin / hex-fuco	Viličić <i>et al.</i> , 2008
Chlorophyta	Chlorophyll <i>b</i> / chl <i>b</i>	Viličić <i>et al.</i> , 2008

90% acetone using sonication, and then centrifuged to clarify the extract. The pigments were separated by reversed-phase high-performance liquid chromatography (HPLC) (BARLOW *et al.*, 1993). In short, extracts were mixed (1:1 v/v) with 1 M ammonium acetate and injected into a HPLC system incorporating a C18 3  $\mu$ m Pecosphere column (3.3 $\times$ 0.45 cm, Perkin Elmer). A binary linear gradient was used to separate the pigments. Solvent A consisted of 80:20 (v/v) methanol: 1 M ammonium acetate and solvent B contained 60:40 (v/v) methanol:acetone. Chlorophyll and carotenoids were detected by absorbance at 440 nm (Spectra Physics, Model UV 2000). Qualitative and quantitative analysis of the individual pigments was performed by external standard calibration using authentic pigment standards (VKI, Denmark).

For the enumeration of phytoplankton cells, 200 mL samples were preserved with 2% (final concentration) hexamine-buffered formaldehyde. The cells were identified and enumerated using an inverted microscope (a Zeiss Axiovert 200) operating with phase contrast and bright field optics (UTERMÖHL 1958; LUND *et al.*, 1958; VILIČIĆ *et al.*, 2008). The references for phytoplankton identification were: HUSTEDT (1959), HASTLE & SYVERTSEN (1997), BERARD-THERIAULT *et al.*, (1999) and THRONDSSEN *et al.*, (2007).

The chemotaxonomic correspondence of the HPLC-determined pigments (Table 1) can be used to study the composition of the phytoplankton community (STÓN *et al.*, 2002; VILIČIĆ *et al.*, 2008; VIDUSSI *et al.*, 2001).

The diagnostic pigments (DP) are defined as the sum of seven diagnostic pigments.

DP= zea/lut+ chl *b*+ allo+ 19'-HF+ 19'-BF+ fuco+ peri (VIDUSSI *et al.*, 2001)

For data sorting, numerical and graphical processing as well as for basic statistical analysis, Microsoft Excel (Microsoft Corporation 2007), Grafer 7, Ocean Data View 4 and Statistica 7.0 computer packages were used. RDA analysis was performed using the R package "vegan" for community ecology and R software. In order to assess the influence of each set of environmental and chemical variables on phytoplankton abundance and the concentration of adequate pigments, a redundancy analysis (RDA) was performed. RDA calculations were based on log-transformed phytoplankton abundance in order to reduce the effect of uneven density distributions. The arrows representing explanatory variables indicated the direction of maximum change of these variables across the diagram and the cosine of the angle between the arrows gives the correlation between the corresponding explanatory variables.

Additionally, a non-parametric Spearman correlation coefficient was used to confirm correlations between phytoplankton groups or phytoplankton pigments with environmental and chemical conditions. The data were log transformed for this analysis.

## RESULTS

### Hydrographic parameters and nutrients

The hydrographic parameters (temperature and salinity) from weekly sampling at BK1 station (Fig. 2) showed the presence of stratifica-

tion during the investigated period. In period from June to August, a thermocline was presented, while in the periods from February to July and October to March, the investigated area was under fresh water influence and an intensive halocline was generated, as confirmed by the coefficient of variation (>30%) shown in Table 2.

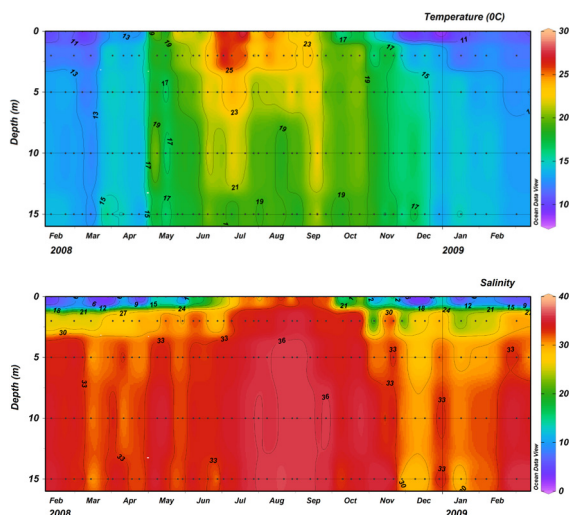


Fig. 2. Temporal variability of the temperature (a) and salinity (b) at BK1 station at weekly intervals

The temperature and salinity showed large seasonal fluctuations. The greatest mean value for temperature reached  $22.32 \pm 3.91^\circ\text{C}$  in the summer and salinity was also greatest in the summer ( $33.01 \pm 3.79$ ) (Table 2).

The lowest mean value of temperature was in the winter ( $12.48 \pm 0.86$ ) while salinity was lowest in the spring ( $28.05 \pm 10.64$ ) (Table 2).

Increasing median values of nutrient concentrations were detected in the spring and the

autumn (Fig. 3). During the summer, nitrogen ( $\text{NO}_2^-$ ,  $\text{NO}_3^-$  and NTOT) and silicate ( $\text{SiO}_4^-$ ) concentrations were lower while total phosphate (PTOT) showed its greatest value. The temporal distribution of nutrient concentration showed significant seasonal variability ( $p < 0.05$ ) for  $\text{NO}_2^-$ ,  $\text{NO}_3^-$ ,  $\text{PO}_4^-$  and  $\text{SiO}_4^-$ .

## Pigments and phytoplankton

A total chlorophyll *a* maximum was recorded at the surface, in November 2008. Chlorophyll *a* concentrations decreased from the surface to the bottom layers (Fig. 4). The greatest median of chlorophyll *a* concentration was reported in April 2008.

The HPLC pigment data revealed seasonal changes in the phytoplankton community structure. Some diagnostic pigments were seen to covary with the TChl *a* biomass, whereas some others exhibited very specific variations (Fig. 4. and Fig. 5). The water column integrated (0-25m) total chlorophyll *a* concentration (Tchl*a*) was linearly related to the integrated diagnostic pigment (Figure 6). The relationship is significant ( $r=0.921$ ;  $p < 0.001$ ) which makes this diagnostic pigment a valid estimator of Tchl*a*. In this study, fucoxanthin and alloxanthin covaried with Tchl *a* and showed a strong significant correlation (0.826 and 0.776 respectively) while the other diagnostic pigments showed different seasonal variations.

The maximum fucoxanthin concentration was registered in November 2008 ( $1324 \mu\text{g m}^{-3}$ ). Greater concentrations of fucoxanthin did not coincide with increased diatom abundance.

Table 2. Mean, Standard Deviation and Coefficient of variation of the temperature and salinity during sampling periods at the three investigated stations

Parameter	Sampling periods	Mean $\pm$ SD	CV%
Temperature ( $^\circ\text{C}$ )	April 2008	$14.83 \pm 0.34$	2.29
	July 2008	$22.32 \pm 3.91$	17.52
	November 2008	$17.42 \pm 1.62$	9.31
	March 2009	$12.48 \pm 0.86$	6.92
Salinity	April 2008	$28.05 \pm 10.64$	37.94
	July 2008	$33.01 \pm 3.79$	11.50
	November 2008	$28.56 \pm 10.60$	37.12
	March 2009	$31.16 \pm 8.54$	27.39



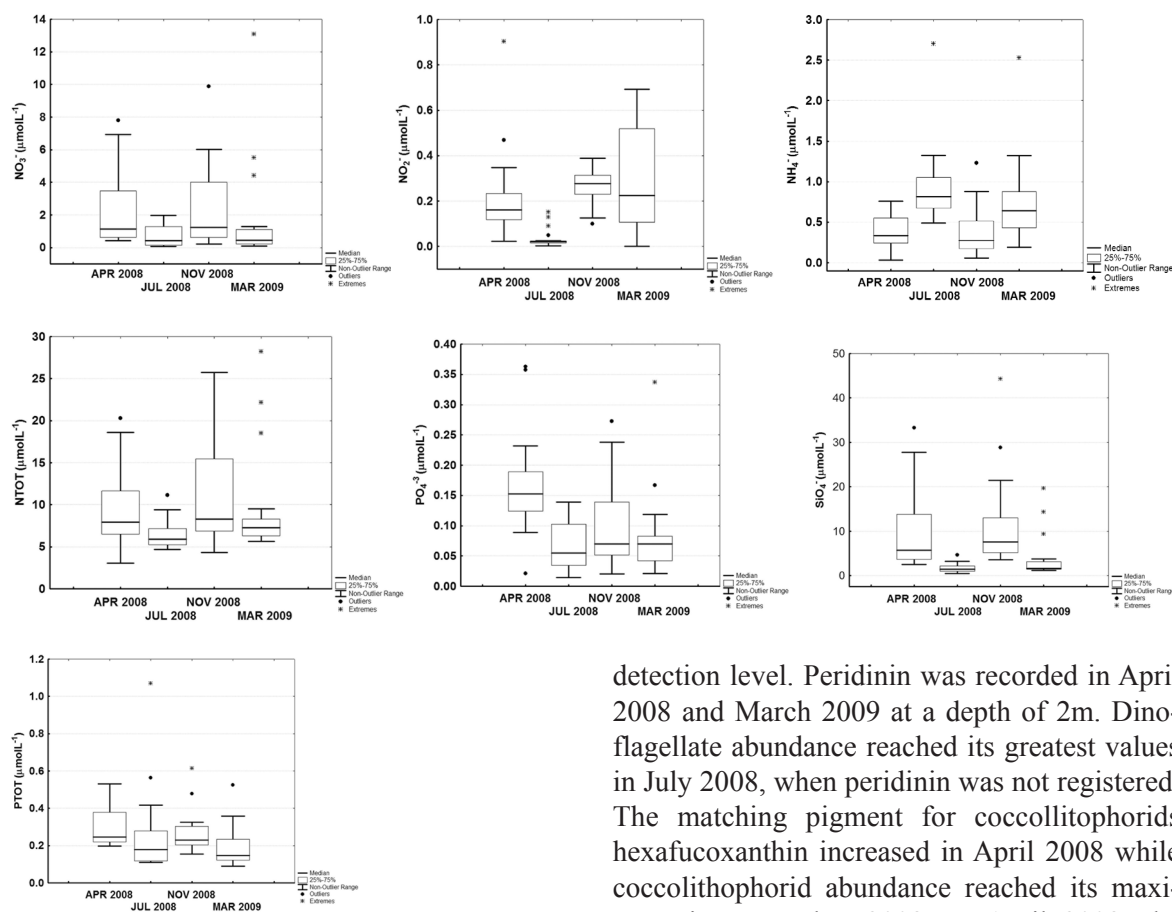


Fig. 3. Box plot diagram of seasonal distribution of a) nitrates, b) nitrites, c) ammonium, d) total nitrogen, e) phosphates, f) total phosphorus and g) silicates concentrations

The greatest diatom abundance of  $2.9 \times 10^6$  cells  $L^{-1}$  was recorded in April 2008. Peridinin was recorded only in two samples, while in other samples the concentrations remained below the

detection level. Peridinin was recorded in April 2008 and March 2009 at a depth of 2m. Dinoflagellate abundance reached its greatest values in July 2008, when peridinin was not registered. The matching pigment for coccolithophorids hexafucoanthin increased in April 2008 while coccolithophorid abundance reached its maximum in November 2008. In April 2008, the greatest abundance of cryptophyceae coincided with the greatest concentration of alloxanthin, but its maximum concentration was recorded in the autumn. Chlorophyte abundance and chlorophyll *b* concentration reached a maximum during the winter.

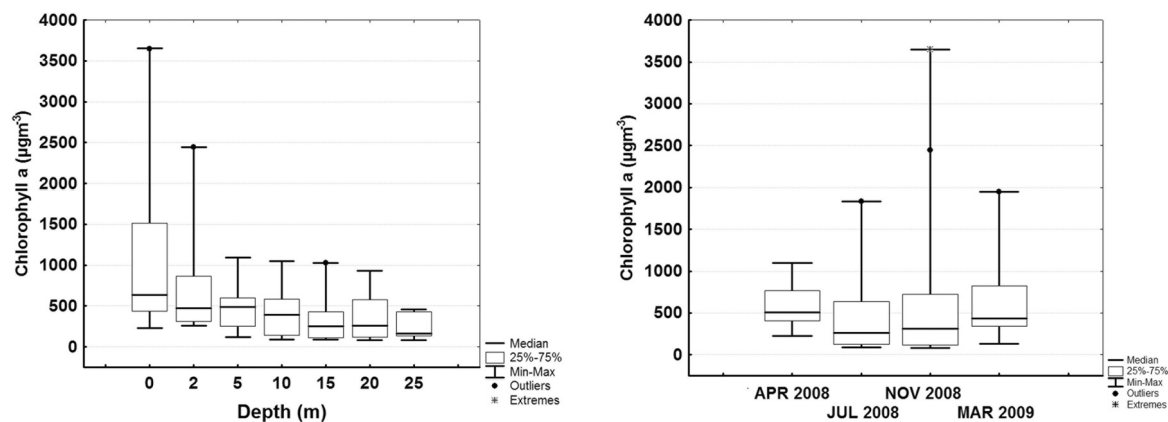
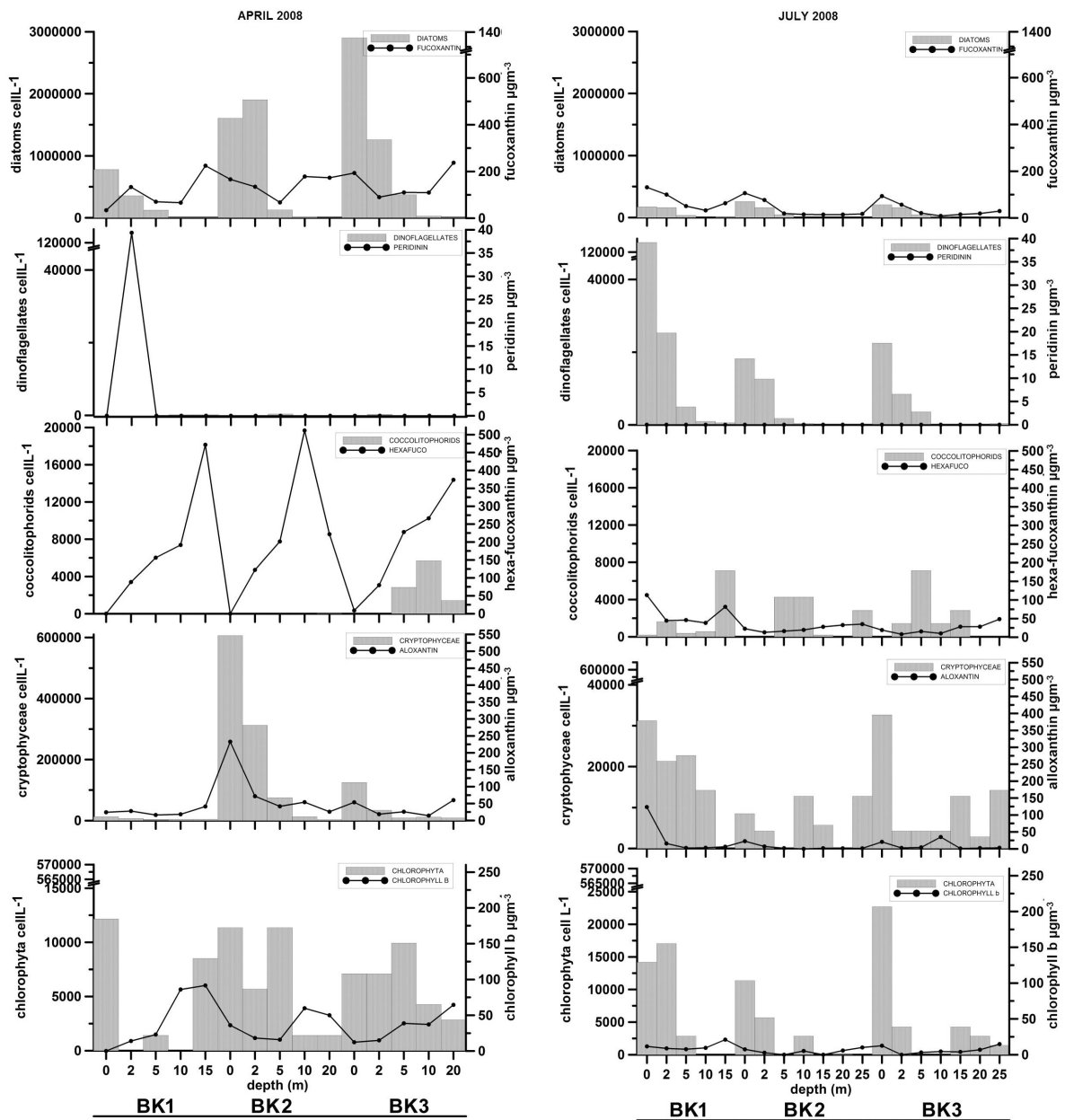


Fig. 4. Box plot diagram of chlorophyll *a* concentration by a) sampling periods and b) depth



(Fig. 5. Distribution of phytoplankton abundance and biomarker pigments in Boka Kotorska Bay over four sampling periods (April 2008; July 2008; November 2008; March 2009) stavite prvo Nov 2008 pa Mart 2009)

The abundance of phytoplankton groups and the concentration of adequate pigments was partly explained by the hydrographic and chemical variables considered in the RDA analysis (Fig. 7 a and Fig. 7 b).

The first two axes of the ordination analysis explained 26.99% of the phytoplankton group abundances and 55.99% of the pigment concen-

trations. The contributions of the axes were not equal, as indicated by their eigenvalues (0.357 and 0.174 for the phytoplankton groups and 2.21 and 1.59 for the phytoplankton pigments respectively, for axes 1 and axes 2). The analysis captured 100% of the phytoplankton groups and pigment variations that could be explained by the considered variables. The main explana-

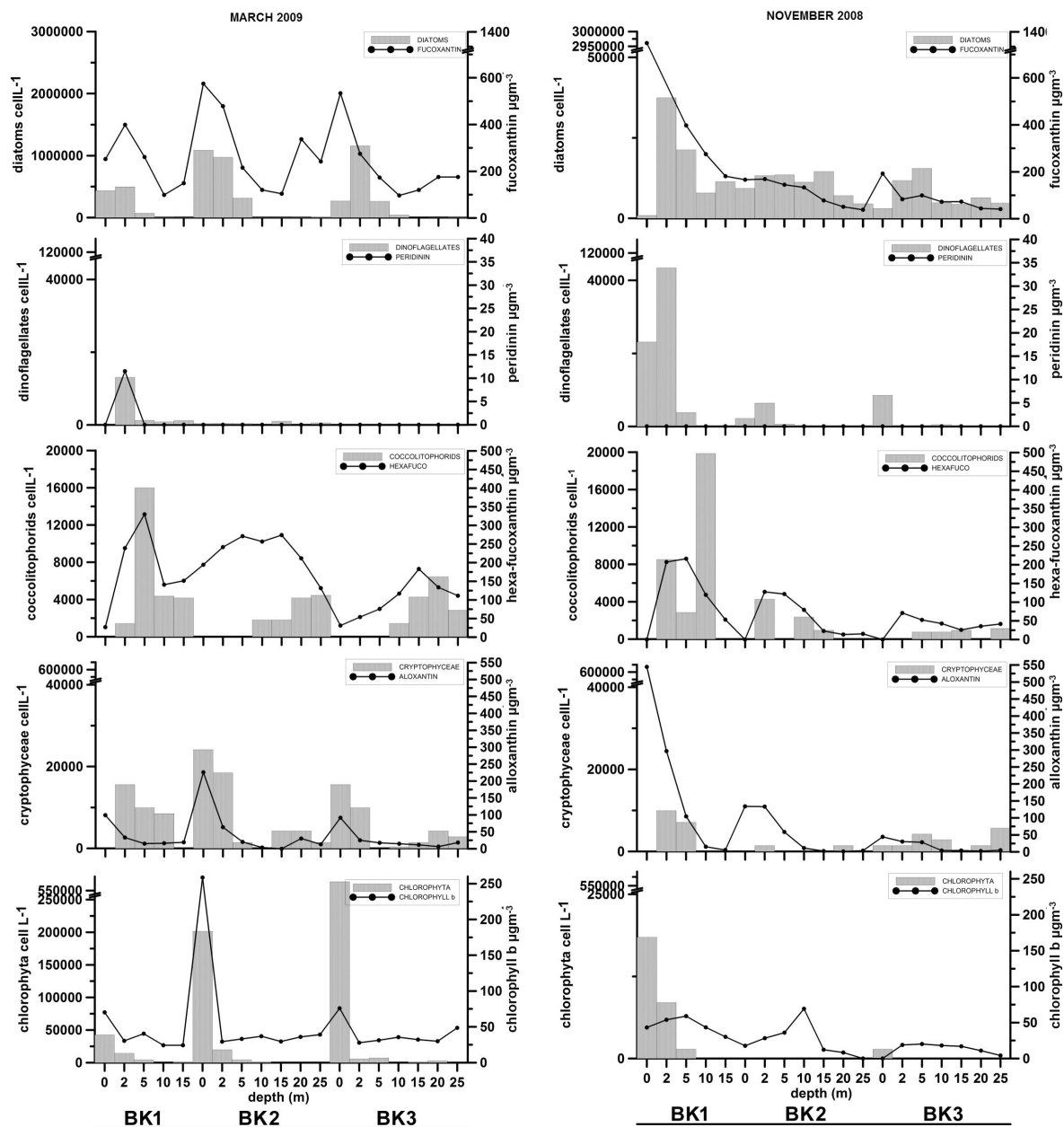


Fig. 5. Distribution of phytoplankton abundance and biomarker pigments in Boka Kotorska Bay over four sampling periods (April 2008; July 2008; November 2008; March 2009)

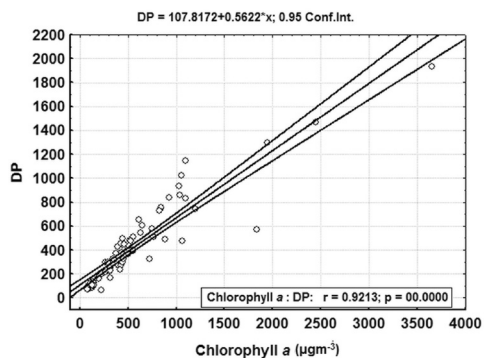


Fig. 6. Relationship between the water column integrated 0-25m concentration of total chlorophyll a and the diagnostic pigment (DP).



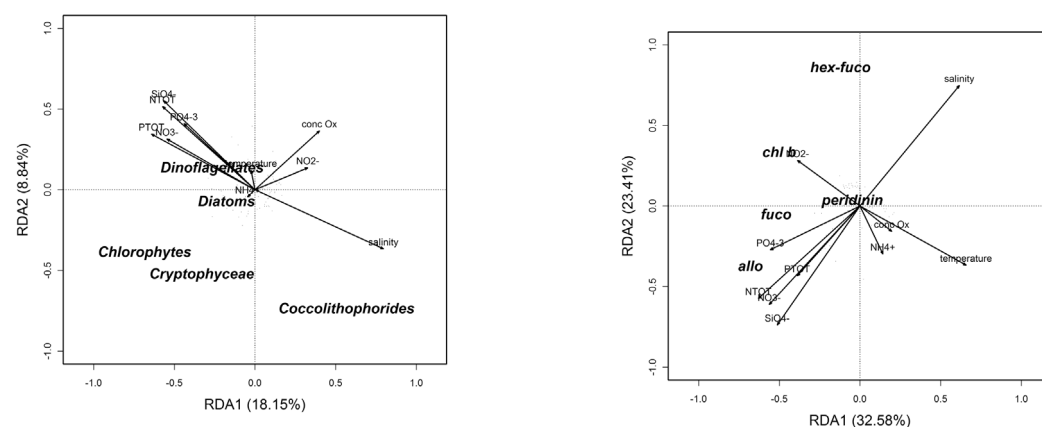


Fig. 7. Redundancy analysis (RDA) showing the bi-dimensional ordination of a) phytoplankton groups and b) phytoplankton pigments. Superimposed vectors represent explanatory variables: temperature, salinity, oxygen concentration (Ox), nutrients (SiO<sub>4</sub><sup>-</sup>, PO<sub>4</sub><sup>-3</sup>, NO<sub>3</sub><sup>-</sup>, NO<sub>2</sub><sup>-</sup>, PTOT, NTOT). In order to make their scales comparable, both biological and hydrological variables were scaled to unit variance.

Table 3. Spearman's rank order correlation of physic-chemical parameters with a) phytoplankton groups and b) phytoplankton pigments ( $p < 0.05$ ;  $n = 68$ )

a)

Variables	Diatoms	Dinoflagellates	Coccolithophorides	Criptophyceae	Chlorophyta
Temperature	-0.18	<b>0.34</b>	<b>-0.37</b>	-0.06	-0.18
Salinity	<b>-0.54</b>	-0.15	<b>0.37</b>	<b>-0.44</b>	<b>-0.33</b>
Conc O <sub>x</sub>	<b>-0.42</b>	-0.12	0.09	<b>-0.30</b>	-0.15
NO <sub>3</sub> <sup>-</sup>	<b>0.47</b>	-0.02	<b>-0.31</b>	<b>0.38</b>	0.23
NO <sub>2</sub> <sup>-</sup>	-0.08	-0.12	<b>0.26</b>	-0.06	-0.05
NH <sub>4</sub> <sup>-</sup>	0.01	0.05	-0.11	-0.01	0.23
NTOT	<b>0.41</b>	0.09	<b>-0.30</b>	<b>0.31</b>	<b>0.34</b>
PO <sub>4</sub> <sup>-3</sup>	<b>0.45</b>	0.08	-0.22	<b>0.45</b>	0.10
PTOT	<b>0.31</b>	<b>0.37</b>	<b>-0.28</b>	<b>0.28</b>	0.12
SiO <sub>4</sub> <sup>-</sup>	<b>0.48</b>	0.00	<b>-0.33</b>	<b>0.49</b>	0.09

b)

Variables	fuco	peridinin	hex-fuco	allo	chl b
Temperature	<b>-0.35</b>	-0.10	<b>-0.49</b>	-0.18	<b>-0.45</b>
Salinity	<b>-0.47</b>	-0.03	0.22	<b>-0.65</b>	<b>-0.25</b>
Conc O <sub>x</sub>	0.21	0.05	-0.18	0.20	-0.10
NO <sub>3</sub> <sup>-</sup>	<b>0.38</b>	-0.01	-0.20	<b>0.54</b>	<b>0.26</b>
NO <sub>2</sub> <sup>-</sup>	0.19	-0.14	0.21	0.06	0.17
NH <sub>4</sub> <sup>-</sup>	0.11	-0.09	<b>-0.27</b>	0.15	0.03
NTOT	<b>0.47</b>	0.03	-0.18	<b>0.59</b>	<b>0.38</b>
PO <sub>4</sub> <sup>-3</sup>	0.19	<b>0.37</b>	-0.03	<b>0.42</b>	<b>0.25</b>
PTOT	<b>0.34</b>	0.11	-0.14	<b>0.48</b>	0.11
SiO <sub>4</sub> <sup>-</sup>	<b>0.40</b>	0.06	-0.20	<b>0.60</b>	0.14

tory variable is salinity, which showed a strong positive correlation with coccolithophorides and a strong negative correlation with diatoms, cryptophytes and chlorophytes. These phytoplankton groups followed an inverse trend with the nutrients ( $\text{SiO}_4^-$ ,  $\text{NO}_3^-$ , NTOT, PTOT,  $\text{PO}_4^{3-}$ ) when compared with salinity. Adequate pigments concentrations had a similar relationship with the explanatory variables but it was weaker than for the phytoplankton groups. The second explanatory variable is temperature, which shows a positive trend only with dinoflagellates among the phytoplankton groups and a negative correlation with all the phytoplankton pigments except peridinin. These results were confirmed by the nonparametric Spearman correlation coefficient (Table 3).

## DISCUSSION

The redundancy analysis (RDA) showed that salinity represents the main environmental factor influencing the distribution of the main phytoplankton groups. Salinity was  $<30$  in the whole water column during November and December as well as at depths greater than 5m for most of the year. The distribution of coccolithophorides along the salinity gradient is presented as a negative correlation. The same was found by OVIEDO *et al.* (2015) across the Mediterranean, including the South Adriatic. Coccolithophorides are of marine origin in Boka Kotorska Bay and usually distributed as a nano size fraction below the halocline, as was the case in previous research (BOSAK *et al.*, 2012). Other taxa can tolerate lower salinity. Regarding diatoms, there was a negative correlation with salinity (also seen in BALZANO *et al.*, 2010, ADENAN *et al.*, 2013, TOTTI *et al.*, 2000). A negative correlation with salinity was characteristic of chlorophytes (also seen in NASSAR *et al.* 2014) for the southern Mediterranean coast. Cryptophytes correlated negatively with salinity and *Dynobryon* sp. preferred the upper, nutrient rich and less saline layer (as was also found in BOSAK *et al.*, 2012).

The concentration of nutrients in Boka Kotorska Bay was generally higher in April 2008 and November 2008 and favorable for phytoplank-

ton development, especially that of diatoms in April 2008. Negative correlations between salinity and the main nutrients indicated that nutrients entered the sea by freshwater inflow. In this study, the maximum chlorophyll *a* concentration was recorded in November 2008. These results coincided with trends in the northern Adriatic where greater values of chlorophyll *a* concentration were registered in the whole water column in the autumn (MOZETIČ *et al.* 1998; TEDESCO *et al.* 2007; MOZETIČ *et al.* 2010), and in the middle Adriatic basin (TOTTI *et al.*, 2000) and the southern Adriatic (KORLEVIĆ *et al.*, 2015).

As is generally known from the Adriatic, the most dominant biomarker pigment throughout the investigated area were fucoxanthin (VILIČIĆ *et al.* 2008) and alloxanthin (ŠUPRAHA *et al.* 2014) which was very well correlated with chlorophyll *a*. Microscopic analysis confirmed diatoms as the most numerous microphytoplankton group, as was reported previously in Spanish estuaries (ANSOTEGUI *et al.*, 2001; SEOANE *et al.*, 2005) as well as in the Black Sea (AGIRBAS *et al.*, 2017). The greatest abundances of diatoms are normally observed during the spring and the autumn in the Adriatic Sea (TOTTI *et al.*, 2000).

The correlation between the determined diatom cell density and fucoxanthin was good ( $p=0.009$ ), once a few outliers were ignored. The concentration of fucoxanthin was found to be greatest in November 2008 which is similar to data noted for the Black Sea (AGIRBAS *et al.*, 2017) where greater values were found during the spring and the autumn, while it differs from the data given by ANSOTEGUI *et al.* (2003) when the greatest fucoxanthin concentration was in the late winter–early spring period in the estuaries of the Spanish coastline. These findings might support the idea that the spatio-temporal distribution of the phytoplankton community is closely related to its environmental conditions (e.g. nutrients, light and so on) as was reported by BUZANČIĆ *et al.*, (2016).

Pigments concentrations in the cells normally vary with different light quality and intensity, nutrients availability and the physiological status of the cell (MOROVIĆ *et al.*, 2012). A weak presence of peridinin was detected in

the HPLC analysis, which eluted in very low concentrations, although a large population of dinoflagellates was found by microscopy. The dinoflagellate species composition from the same period of research has been presented by BOSAK *et al.* (2012). The discrepancy between peridinin concentration and dinoflagellate abundance may be caused either by the dominance of heterotrophic dinoflagellate species (LORET *et al.*, 2000), such as *Protoperidinium* spp. or *Diplopsalis* spp. (BOSAK *et al.*, 2012) or the presence of dinoflagellates that possess plastids containing phycobilin rather than peridinin, such as one of the dominant genera *Dinophysis* spp. (TAKISHITA *et al.*, 2002).

In November 2008, the diatoms were out-competed by coccolithophorids, probably due to the lower supply of nutrients and their efficient absorption by coccolithophorids (FURNAS, 1990). Coccolithophorids characteristic of coastal and brackish areas contain chlorophyll C<sub>1</sub> rather than hexa-fucoxanthin. Taxa that contain hexa-fuco, are always characteristic of open-coastal, rather than littoral, locations (VAN LENNING *et al.*, 2004). It may be for this reason that, in this paper, the concentrations of hex-fuco were lower than those of fucoxanthin and alloxanthin. This pigment, however, proved to be a very good indicator of the influence of the salinity gradient on phytoplankton composition. Hex-fuco increased with increasing salinity, clearly indicating a marine origin of the species carrying this pigment – probably prymnesiophytes (ZAPATA *et al.*, 2004). This is proved in the current paper where salinity showed a positive correlation with coccolithophorids and hexa fucoxanthin.

The correlation of cryptophytes with alloxanthin was similar with correlation in the eastern Adriatic, Zrmanja estuary (VILIČIĆ *et al.*, 2008), where it was noticed compatibility between abundances of cryptophytes and concentration of alloxanthin above halocline in winter season and below halocline in summer season. Cryptophytes containing alloxanthin were found in a greater concentration in April 2008 and a maximum concentration in November 2008, mainly in the surface layer. A greater concentration of alloxanthin and an abundance of cryptophytes was also recorded in the surface layer of the Krka

estuary (ŠUPRAHA *et al.*, 2014). The shift from diatom-dominant blooms to cryptophytes-dominant bloom confirms the hypothesis that the phytoplankton community is undergoing changes due to eutrophication (ŠUPRAHA *et al.*, 2014) and that cryptophytes are the more dominant group in anthropogenically – influenced area. The noted correlation between salinity on the one hand and cryptophytes and alloxanthin on the other was negative. A similar situation was noticed where cryptophytes containing alloxanthin were found mainly at lower salinities (18–35), with their maxima in the surface layer of the middle estuary and below the halocline in the upper estuary (VILIČIĆ *et al.*, 2008).

Chlorophyll *b* as pigment contained by chlorophytes was greatest in March 2009, which is different from the results given by ANSOTEGUI *et al.* (2003) where Chl *b* followed a clear seasonal pattern, with its greatest values during warmer periods, and increasing after the winter–spring transition. The noted correlation between chlorophyte abundance and chlorophyll *b* concentration and temperature was negative.

## CONCLUSION

Boka Kotorska Bay represents a collector of the freshwater supply coming from the springs and karstic structures along its border. Higher concentrations of nutrients in spring and autumn are favorable for phytoplankton development, and most especially diatom growth in the spring. The phytoplankton assemblages were composed of microphytoplanktonic diatoms, as well as nanoplanktonic/ picoplanktonic cryptophytes, dinoflagellates, prymnesiophytes, and chlorophytes (SIEBURTH *et al.*, 1978). The predominant biomarker throughout the investigated area was fucoxanthin, with the greatest concentration in the autumn. It seems that this differs from the point of greatest diatom abundance (in the spring) which can be accounted for by the fact that the peak of fucoxanthin may have originated from nanoplanktonic non-diatom species. Coccolithophorids dominated in the autumn, which differs from the peak of adequate hex-fuco pigment that was greatest in the spring.

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## Raspodjela algalnog pigmenta i fitoplanktonskih zajednica u obalnom prijelaznom okruženju - Boka Kotorska (jugoistočni Jadran)

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### SAŽETAK

Prisutnost zajednica fitoplanktona i rasprostranjenost pigmenata utvrđivani su u tranzicijskim vodama Bokokotorskog zaljeva. Uzorci su prikupljeni četiri puta na tri lokaliteta u unutrašnjosti Zaljeva u periodu između travnja 2008. i ožujka 2009. godine. Mikroskopskom analizom utvrđeno je da fitoplankton čine morske dijatomeje, dinoflagelati, kriptofiti, klorofiti i kokolitoforidi. Pored fukoksantina kao dominantnog biomarkerskog pigmenta, bili su zastupljeni i aloksantin, 19'-heksanoiloksifukoksantin, peridinin i klorofil *b*. Fukoksantin i aloksantin su pokazali značajnu korelaciju sa klorofilom *a* kao indikatorom biomase fitoplanktona tijekom čitavog perioda ispitivanja. U studenom 2008. godine, dijatomeje su nadjačale kokolitoforide, koji su vjerovatno efikasno apsorbirali nutrijente tijekom perioda u kojem je njihova dostupnost bila smanjena. Kao rezultat smanjenog unosa nutrijenata, u ljetnom periodu, zajednicu fitoplanktona u Bokokotorskom zaljevu sačinjavali su uglavnom morski dinoflagelati, kriptofiti i klorofiti.

**Ključne riječi:** raspodjela pigmenta, fitoplanktonska zajednica, Bokokotorski zaljev