TROPHIC STATE OF BOKA KOTORSKA BAY (SOUTH-EASTERN ADRIATIC SEA)

Slađana Krivokapić[1,]*, Branka Pestorić[2], Sunčica Bosak[3], Grozdan Kušpilić[4] and Christian Wexels Riser[5]

[1]Department of Biology, Faculty of Science, University of Montenegro, Podgorica, Montenegro
[2]Institute of Marine Biology, Kotor, Montenegro
[3] University of Zagreb, Faculty of Science, Division of Biology, Zagreb, Croatia
[4]Institute of Oceanography and Fisheries, Split, Croatia
[5]Department of Arctic and Marine Biology, University of Tromsø, Tromsø, Norway

ABSTRACT

Boka Kotorska bay is a unique coastal ecosystem in the south-eastern Adriatic Sea that has been recently under the increasing influence of human activities. In this paper, we investigated the temporal distribution of biological and environmental parameters and the trophic state of the Bay. Sampling was conducted weekly from March 2008 to February 2009 in the inner part of the Bay (Kotor Bay). The phytoplankton biomass ranged between < 1 to > 4 μg l^{-1} chlorophyll *a* (Chl *a*), reaching maximum values in the late winter and spring period. On the basis of Chl *a* and nutrient concentrations, the study area can be defined oligo-mesotrophic. Two indicators of the trophic state, trophic index - TRIX and the pigment ratio - Fp were calculated from the available physiochemical and biological data. The TRIX value ranged from 3.02 to 5.58 (average 4.11 $\pm$ 0.66) while the Fp ratio varied between 0.05 and 0.33 (average 0.17 $\pm$ 0.08). These results indicate a system that is in a good trophic state, where natural eutrophication still dominates over eutrophication of an anthropogenic origin.

KEYWORDS:
nutrients, chlorophyll *a*, eutrophication, TRIX, Fp, Adriatic Sea

INTRODUCTION

Eutrophication is probably one of the most important factors affecting coastal marine environments [1]. The Mediterranean Sea is known as one of the most oligotrophic areas in the world [2]. As in many coastal environments around the world, the coastal regions of the Mediterranean are regions of rapid population growth and changing land-use patterns that have led to increased nutrient loadings and changes in fresh water flow patterns to coastal waters, particularly during the last few decades. The Adriatic region is characterized by intensive land-based and sea-based activities, including urban growth and development, commercial and recreational fisheries, tourism and multinational commerce. Changes in these activities are widely believed to have significantly reduced the water quality of the area, manifested as oxygen depletion of bottom water, harmful algal blooms, outbreaks of gelatinous zooplankton, invasions of non-indigenous species, loss of habitat and instability of fisheries [3-5].

Boka Kotorska Bay is a relatively large (87 km^2) semi-enclosed bay situated in the south-eastern part of the Adriatic Sea. It is surrounded by high karstic mountains with a population of about 50,000 inhabitants living along its coasts. Accelerated urbanization of the coastal zone, along with some years without a developmental strategy and a lack of spatial planning, and the disrespect of existing regulations, has led to destruction of the coastal zone and substantially increased nutrient loadings. Monitoring the changes taking place in the marine environment is quite difficult because of the lack of historical data on marine biological communities [6]. In addition to the human influence, 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). Given that this is one of the most interesting transitional areas of the Adriatic Sea for its environmental and strategic characteristics, very little information is available on the influence of the hydrographical conditions on the biological communities [7, 8].

There is a large amount of literature concerning hydrographic conditions and hydro-chemical properties in relation to phytoplankton communities in northern and middle parts of the eastern Adriatic Sea [7-16]. Several studies used the elemental ratios of dissolved nutrients (N, P and Si) in marine environments to determine which elements were likely to be limiting phytoplankton growth in the Mediterranean Sea [17, 18]. They suggested that productivity in the SE Mediterranean might be limited by the availability of phosphorus. A recent Lagrangian phosphorus addition experiment on surface water of the ultra-oligotrophic

* Corresponding author

Cyprus gyre confirmed the idea that the system is phosphorus-starved [19, 20]. Furthermore, phosphorous limitation was found to be significant in coastal areas of the Adriatic Sea [21]. Identification of limiting nutrients is essential for selecting appropriate nutrient control measures to mitigate the effects of eutrophication. In addition, in order to assess the trophic status of the coastal marine system it is necessary to apply effective tools that are comparable across waters of different trophic conditions. Measurements of chlorophyll *a* concentration and oxygen saturation enable basic but insufficient information to be gathered on the trophic status, so more complex indicators were developed. Vollenweider *et al.* [22] introduced a complex trophic index (TRIX) based on biological (chlorophyll *a* concentration) and physiochemical parameters (oxygen saturation, minerals and total nitrogen and phosphorus). We chose to use the TRIX index because it is easy to calculate and contains useable parameters for describing the trophic state of a system. Deviations of the chlorophyll *a* concentration and oxygen level from saturation directly express productivity, whilst nutritional parameters (total and available nutrient concentrations in the water column) provide supplementary information on water quality (transparency). Claustre [23] proposed the Fp ratio, which only takes into account biological parameters such as phytoplankton pigments. The Fp is the ratio of large phytoplankton pigment biomass (mainly diatoms and dinoflagellates) to total diagnostic pigment biomass [23] and it is an estimator of the ratio between new production versus total production [24]. Both indices present complementary information as Fp indicates the influence of environmental conditions on phytoplankton community composition and TRIX indicates the general trophic state.

The objective of our work was to determine whether or not the system of Boka Kotorska Bay is dominated by eutrophication of a natural origin or by eutrophication of an anthropogenic origin by: (i) investigating temporal variations in the environmental conditions and phytoplankton biomass (chlorophyll *a*) and (ii) assessing the trophic state using two trophic indices, TRIX and the Fp ratio.

2. MATERIALS AND METHODS

Weekly sampling was carried out at one monitoring station (BK 1) in the inner part of Boka Kotorska Bay (Figure 1), from March 2008 to February 2009. Samples were collected at five depths (0, 2, 5, 10, and 15 m) using 5 l Niskin bottles (Hydro Bios, Germany). Temperature and salinity were measured *in situ* using a universal meter (Multiline P4; WTW, Germany). Oxygen concentrations and saturation values were determined using an oxygen electrode (Oxy Guard Handy Gamma). Transparency was determined using a Secchi disc. Dissolved nutrient concentrations (phosphate and nitrate) were determined according to the methods described by Strickland and Parsons [25]. Water samples (1 L) for chlorophyll *a* measurement were pre-filtered through a 330 μm mesh net to remove large zooplankton. After filtration through Whatman GF/F pigment extraction was performed in 90% acetone and chlorophyll *a* concentrations were determined by measuring the absorbance with a Perkin-Elmer UV/VIS spectrophotometer, and performing calculations according to Jeffrey *et al.* [26].

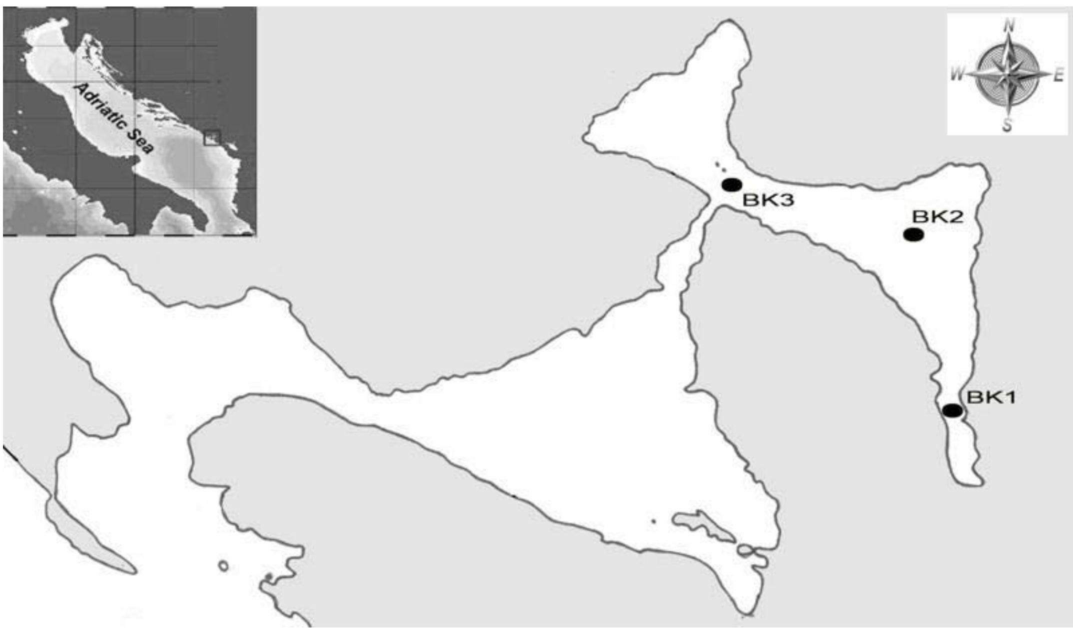

FIGURE 1 - Location of the sampling stations in the Boka Kotorska Bay.

In addition to weekly sampling at one station (BK1), samples were also taken seasonally, in April, July, and November 2008 and at the beginning of March 2009 at two additional stations (BK2, BK3). Analysis of dissolved nutrients (nitrate, nitrite, ammonia, total dissolved nitrogen, phosphate, total dissolved phosphate and silicate) and phytoplankton pigment biomarkers was performed on samples from these stations. Nutrient analyses for these seasonal samples were performed using a Seal AutoAnalyser, with conventional automated methods [27]. Samples for total nitrogen and phosphorus were analyzed after wet oxidation (REF) as nitrate and phosphate on the Seal AutoAnalyser. The concentrations of organic nitrogen and phosphorus were calculated as the difference between total nitrogen or phosphorus and their inorganic fractions. Phytoplankton pigments were analyzed by reversed-phase high-performance liquid chromatography (HPLC) [28]. Water samples (1 l) were filtered through Whatman GF/F filters and immediately frozen until analyzed. The samples were later extracted in 4 ml of cold 90% acetone using sonication and then centrifuged to clarify the extract. The extracts were mixed (1:1 v/v) with 1 M ammonium acetate and injected into an HPLC system incorporating a C18 3 µm Pecosphere column (3.3×0.45 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 *a* was detected by absorbance at 440 nm (Spectra Physics, Model UV 2000). Qualitative and quantitative analyses of individual pigments were performed by external standard calibrations using authentic pigment standards (VKI, Denmark).

The Fp ratio was calculated as described by Claustre [23] using pigment concentrations: $Fp= (fuc + per) / (fuc + per + hex + but + zea + chlb + allo)$ where *fuc* represents fucoxanthin, *per* is peridinin, *hex* is 19'-hexanoyloxyfucoxanthin, *but* is 19'-butanoyloxyfucoxanthin, *zea* is zeaxantin, *chl b* is chlorophyll b and *allo* is alloxanthin.

The trophic index TRIX was calculated according to Vollenweider *et al.* [22]:

$$TRIX = \log (Chla \times aD\%O \times N \times P) - (-1.5) / 1.2$$

where $Chla$ is the chlorophyll *a* concentration (µg l^{-1}), $aD\%O$ is the absolute (%) deviation of oxygen from saturation (ABS│100-%O│), N is total inorganic nitrogen (µg l^{-1}) and P is total phosphorus (µg l^{-1}). The index is scaled from 0 to 10.

The relationships between nutrient concentrations, salinity and phytoplankton biomass were statistically analyzed using Pearson's coefficient of correlation (r). The best way to interpret the r value was to calculate the coefficient of determination (r^2), which represents the fraction of variance in the two variables that are shared. The coefficient of determination was used to propose simple linear models of nutrient concentrations as a function of salinity in the upper water column (0-2 m) and chlorophyll *a* biomass as a function of the nutrient concentrations in the whole water column. All statistical analyses were provided using the programs STATISTICA (data analysis software system), version 8.0. (www.statsoft.com) and StatSoft, Inc. (2007).

3. RESULTS

The results of temporal changes in temperature from the weekly sampling at the BK1 station (Figure 2a) showed a clear temporal pattern, with temperatures below 15 °C throughout the water column until mid-April, when a gradual heating of the surface water was recorded, resulting in a maximum of 28.2 °C in July. Cooling of the surface water started in August/September, resulting in a minimum of 8.6 °C in the surface water in December, while the temperatures below a 5 m depth remained >13 °C. Water of a reduced salinity (<25) was recorded in the uppermost 2 m of the water column for most of the sampling period, except for the period from mid-July to the end of September (Figure 2b). The lowest salinity (<5) was detected in the surface layer in March, whereas the highest salinity was found in Aug/Sep (36.5). The upper 15 m of the water column was fully or over-saturated (>100%) with regard to oxygen (O_2) from mid-May to mid-September (Figure 2c). From mid-September onwards a gradual decrease in O_2 saturation was recorded throughout the water column and the lowest O_2 saturation was found in early February at a 15 m depth.

The phytoplankton biomass, expressed as chlorophyll *a* (Chl *a*), was found to vary in time and space in the weekly samplings at the BK1 station. The highest Chl *a* concentrations were detected in the winter-spring period (January-March) and reached >10 µg l^{-1} (Figure 3a). March was characterized by enhanced Chl *a* concentrations (3-4 µg l^{-1}) throughout the water column, while the Chl *a* concentrations later in the season were generally lower and associated with the surface layer (upper 2 m). The transparency, as measured with a Secchi disk, varied considerably over the sampling period. The transparency in March and April varied between 5 and 6 m depth. The lowest transparency was found in May (3 m) and the highest transparency of 10 m was recorded in September and varied greatly over time from September to December.

The nutrient concentrations in the weekly samplings at the BK1 station varied considerably during the one-year sampling period. The phosphate concentrations were below or close to the detection limit (<0.1 µmol l^{-1}) at all depths from August to February. Higher concentrations were present from March to May, with concentrations > 0.4 µmol l^{-1} (Figure 3b). March and April were characterized by nitrate concentrations <2.5 µmol l^{-1} at all depths (Figure 3c). This short period of reduced nitrate concentration was followed by a long period with concentrations >7 µmol l^{-1} throughout the entire water column (May-January). The maximum concentrations of nitrate were measured in December and coincided with the winter pulses of fresh water, enriched with nitrate.

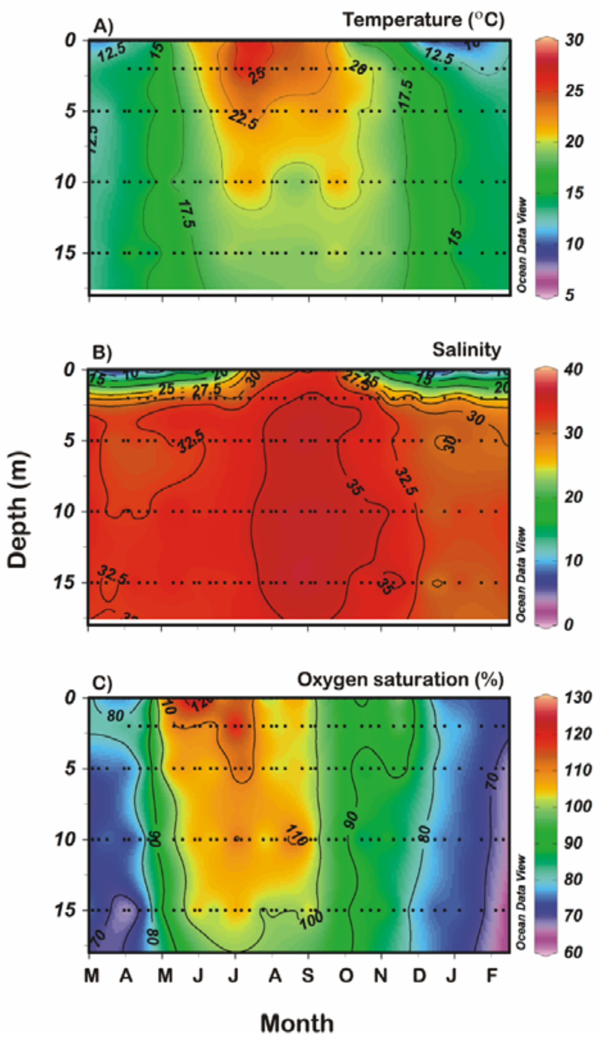

FIGURE 2 - Temporal changes in temperature (A), salinity (B) and oxygen saturation (C) from March 2008 to February 2009 at BK1 station in the Boka Kotorska Bay.

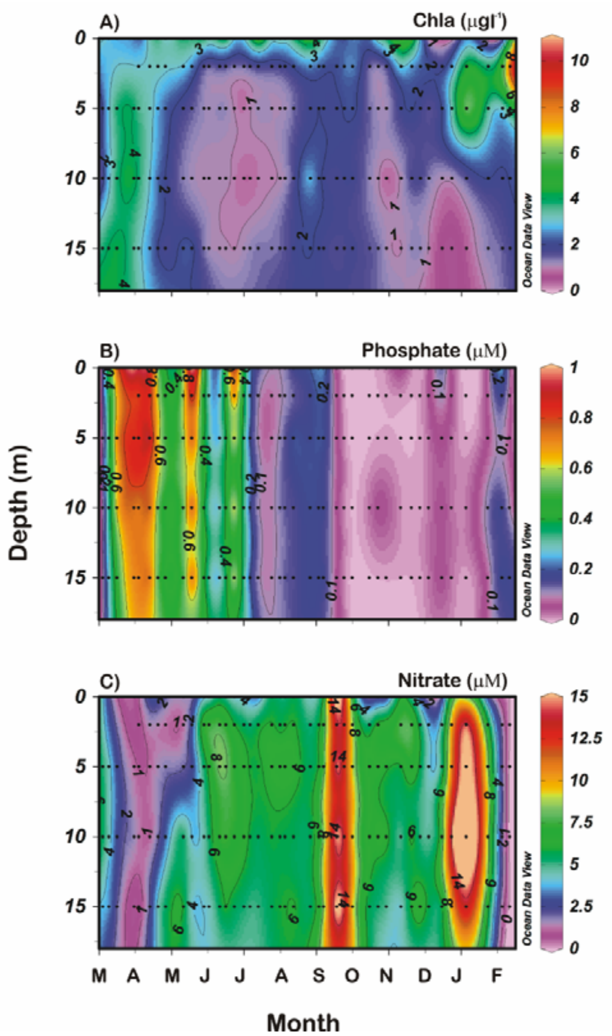

FIGURE 3 - Temporal changes in chlorophyll *a* (A), phosphate (B) and nitrate (C) concentrations from March 2008 to February 2009 at BK1 station in the Boka Kotorska Bay.

The results of the nutrient analyses from the seasonal investigations, presented as an average state for all of the investigated stations, indicated a well-defined nutricline for all inorganic and organic forms of nutrients with depths that coincided with the halocline depth (Figure 4). A similar vertical distribution was determined for the chlorophyll biomass and the N/P ratio, whereas the N/Si ratio throughout the water column was relatively uniform. Studies on the relationship between salinity and the investigated parameters in the upper (0 to 2 m depth) fresh water-influenced layer revealed a statistically significant negative correlation between salinity and nitrate, dissolved inorganic nitrogen (DIN), total dissolved nitrogen (NTOT), silicate ($p<0.001$) and nitrite, dissolved organic nitrogen (NORG) and Chl *a* at the significance level of $p<0.05$, respectively (Table 1). The correlation coefficients presented in Table 1 also indicate statistically significant relationships between some of the nutrients, but a correlation between the nutrients and phytoplankton biomass was not established.

Regression analysis for salinity and nutrients ($p<0.01$) indicated a linear relationship between these parameters (Figure 5). The annual averages of concentrations of nitrate and silicate in the fresh water are given in the linear regression equations as y-intercept values (Figure 5).

Further analysis of the data also indicated an influence of fresh water on the composition of total nitrogen (TN) and total phosphorus (TP) in this layer. To prove this hypothesis, we divided all of the data (stations BK1, BK2 and BK3) into three ranges of salinity (6.2 to 10, 10.3 to 19.3 and 22.9 to 31.5) and found that under conditions of a strong fresh water influence (salinity range 6.2 to 10) the portions of the inorganic and organic fractions in TN and TP were almost equal (Figure 6), while under a weaker fresh water influence (salinity ranges 10.3 to 19.3 and 22.9 to 31.5) the organic fraction dominated, both for TN (65 and 75%) and TP (57 and 62%).

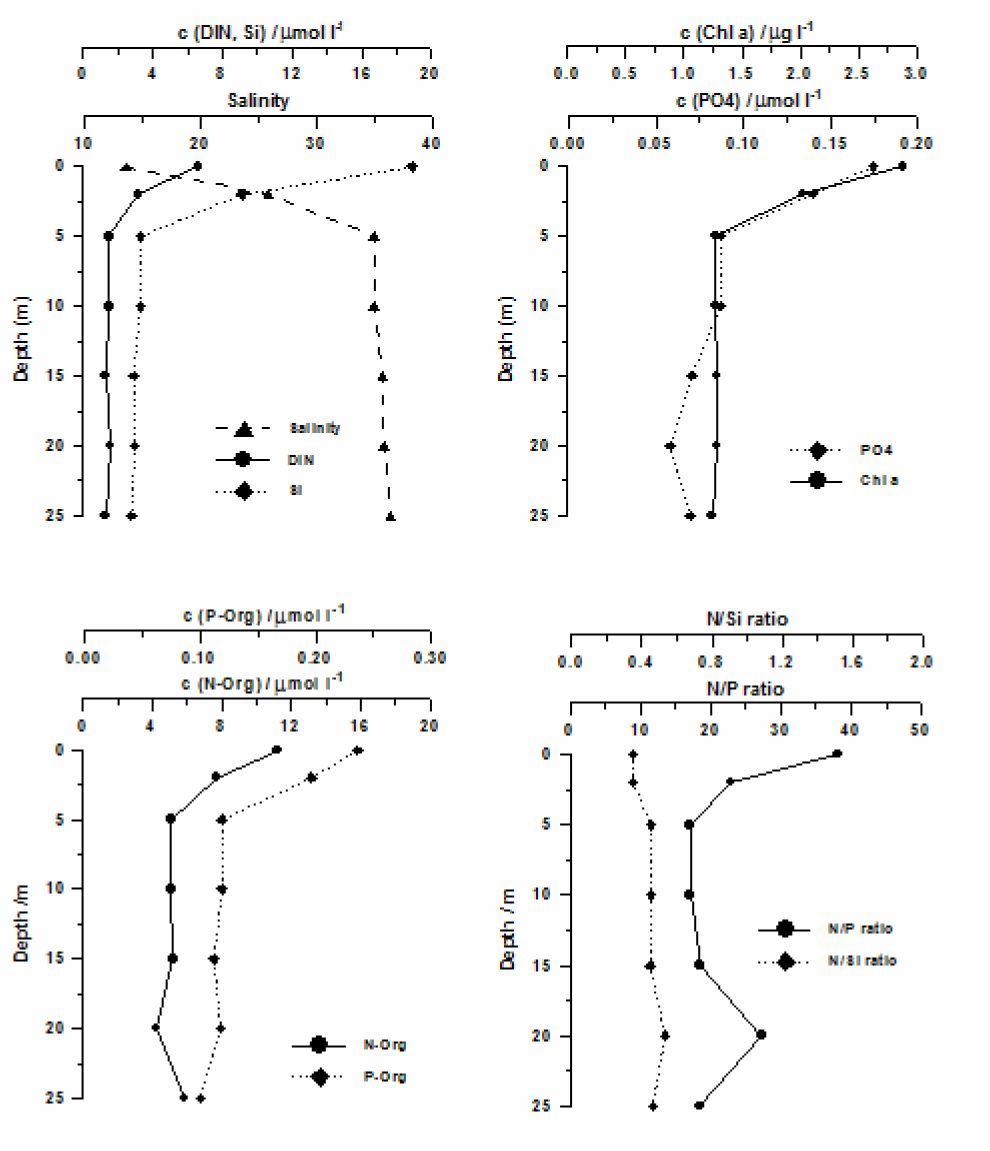

FIGURE 4 - Average vertical distribution of salinity and nutrient concentrations in the Boka Kotorska Bay.

TABLE 1 - Pearson's correlation coefficients between salinity and the nutrient concentrations (NO_3^-, nitrate; NO_2^-, nitrite; NH_4^+, ammonia; DIN, dissolved inorganic nitrogen; NTOT, total dissolved nitrogen; NORG, dissolved organic nitrogen; PO_4^{3-}; phosphate: PTOT, total dissolved phosphorus, PORG, dissolved organic phosphorus; Si, silicate) and the phytoplankton chlorophyll *a* concentrations (Chl *a*, chlorophyll *a*) in the upper (0 to 2m depth) water layer.

	Salinity and nutrient and chlorophyll *a* concentrations										
	SAL	NO_3^-	NO_2^-	NH_4^+	DIN	NTOT	NORG	PO_4^{3-}	PTOT	PORG	Si
NO_3^-	-0,868 ***										
NO_2^-	-0,487 *	0,457 *									
NH_4^+	-0,245	0,430 *	0,146								
DIN	-0,846 ***	0,992 ***	0,464 *	0,538 *							
NTOT	-0,816 ***	0,851 ***	0,352	0,425 *	0,851 ***						
NORG	-0,575 *	0,497 *	0,155	0,209	0,490 *	0,875 ***					
PO_4^{3-}	-0,361	0,469 *	0,205	0,213	0,467 *	0,474 *	0,357				
PTOT	-0,122	0,092	-0,021	0,255	0,119	0,135	0,114	0,253			
PORG	0,046	-0,127	-0,118	0,158	-0,098	-0,086	-0,052	-0,213	0,891 ***		
Si	-0,885 ***	0,831 ***	0,419 *	0,029	0,780 ***	0,716 **	0,469 *	0,384	0,073	-0,106	
Chl *a*	-0,425 *	0,235	0,357	0,129	0,243	0,349	0,355	0,410	0,350	0,161	0,350

*-significant correlation at p< 0.05; **-significant correlation at p< 0.01; ***-significant correlation at p< 0.001.

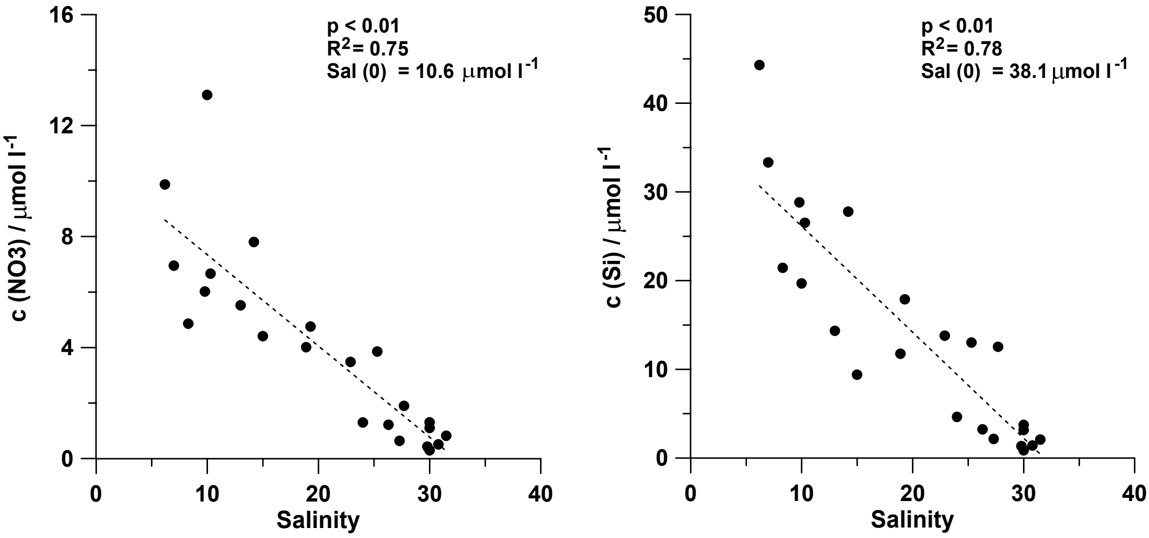

FIGURE 5 - Relationship of salinity and nutrients (NO_3^- and Si) on the surface layer (0-2 m) in the Boka Kotorska Bay.

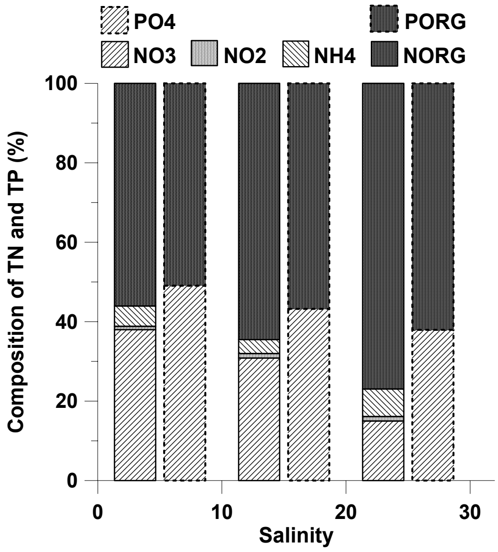

FIGURE 6 - Average composition total nitrogen and phosphorus for different salinity ranges (0 to 10, 10 to 20 and 20 to > 30) in the Boka Kotorska Bay.

Although statistically significant relationships between chlorophyll *a* and nutrients were not established in this layer, additional data, including for the entire water column, indicated significant positive correlations between Chl *a* and most of nutrients (Table 2). The linear relationships between chlorophyll *a* concentrations and some of the investigated nutrients (NTOT, PO4, PTOT and Si) are shown in Figure 7.

Table 3 shows the seasonal distributions of the major phytoplankton pigment concentrations that were used to calculate the Fp ratios. The seasonal distribution of the calculated indices: TRIX and the Fp ratios (Figure 8), showed minimum values in the summer (3.02 and 0.05, respectively) whereas maximum values were reached in autumn (5.58 and 0.33, respectively).

TABLE 2 - Pearson's correlation coefficients between the concentration of phytoplankton chlorophyll *a* and nutrient concentrations (Chl *a*, chlorophyll *a*; NO_3^-, nitrate; NO_2^-, nitrite; NH_4^+, ammonia; DIN, dissolved inorganic nitrogen; NTOT, total dissolved nitrogen; NORG, dissolved organic nitrogen; PO_4^{3-}, phosphate; PTOT, total dissolved phosphorus; PORG, dissolved organic phosphorus; Si, silicate) established in the whole water column.

	Chlorophyll *a* and nutrient concentrations									
	Chl *a*	NO_3^-	NO_2^-	NH_4^+	DIN	NTOT	NORG	PO_4^{3-}	PTOT	PORG
NO_3^-	0.440 **									
NO_2^-	0.011	-0.001								
NH_4^+	0.039	0.269 *	-0.214							
DIN	0.421 **	0.986 ***	0.033	0.413 **						
NTOT	0.475 ***	0.838 ***	-0.065	0.193	0.816 ***					
NORG	0.404 **	0.535 ***	-0.123	-0.014	0.491 ***	0.904 ***				
PO_4^{3-}	0.533 ***	0.577 ***	-0.006	0.041	0.548 ***	0.632 ***	0.548 ***			
PTOT	0.500 ***	0.404 **	-0.147	0.199	0.403 **	0.464 ***	0.402 **	0.448 ***		
PORG	0.257 *	0.125	-0.161	0.199	0.140	0.161	0.138	-0.066	0.863 ***	
Si	0.481 ***	0.873 ***	0.007	0.032	0.825 ***	0.778 ***	0.562 ***	0.541 ***	0.401 **	0.142

*-significant correlation at p< 0.05; **-significant correlation at p< 0.01; ***-significant correlation at p< 0.001.

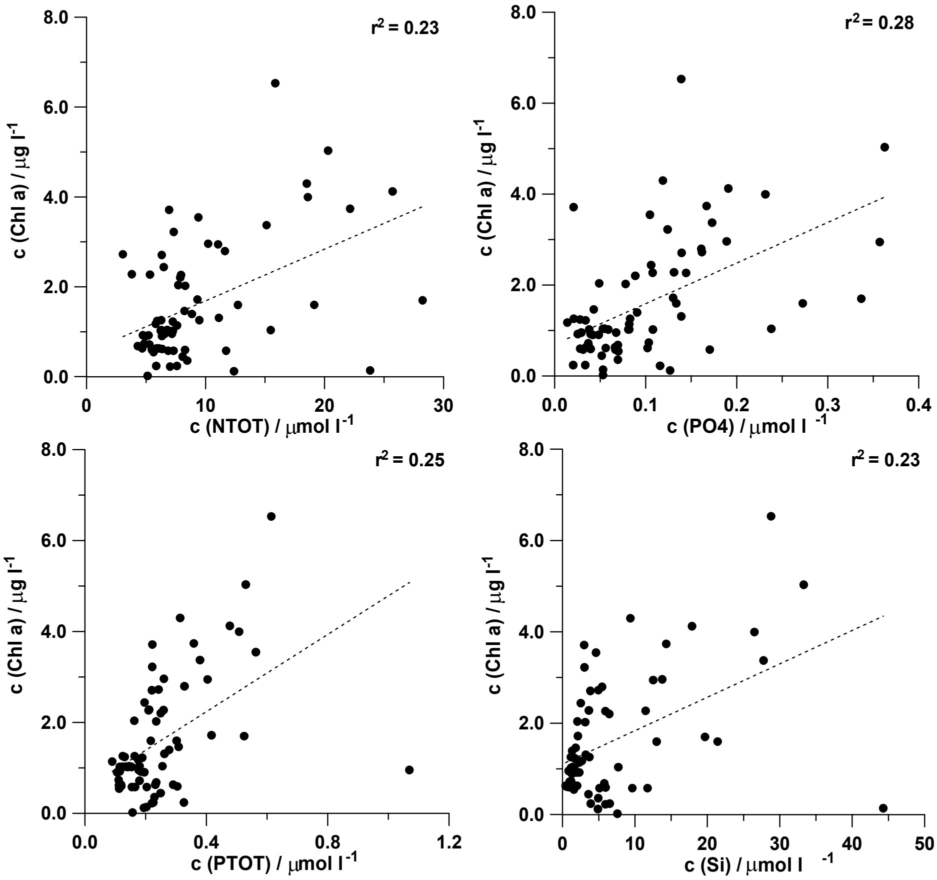

FIGURE 7 - Relationships of chlorophyll *a* and nutrients (NTOT, PO_4^{3-}, PTOT and Si) in the Boka Kotorska Bay.

TABLE 3 - The maximum and the mean (plus standard deviation) concentrations (ng l^{-1}) major accessory pigments for different seasons. But-Fuco, 19'-butanoyloxyfucoxanthin; Fuco, fucoxantine; Hex-Fuco, 19'-hexanoyloxyfucoxanthin; Allo, alloxanthin; Zea/Lut, zeaxanthin/lutein; Chl *b*, chlorophyll *b*.

	Spring		Summer		Autumn		Winter	
	Max	Mean ± SD	Max	Mean ± SD	Max	Mean ± SD	Max	Mean ± SD
But-Fuco	146.46	51.47 ±40.51	19.35	5.08 ±5.78	11.07	27.67 ±7.73	31.02	15.14 ±9.96
Fuco	237.37	137.25 ±57.19	130.88	46.53 ±37.98	1324.29	232.49 ±324.64	575.04	251.61 ±148.79
Hex-Fuco	669.45	236.42 ±172.87	113.14	34.19 ±25.85	216.015	81.41 ±59.08	329.53	167.08 ±87.71
Allo	232.60	47.07 ±48.05	123.88	18.76 ±34.38	544.59	75.22 ±135.67	225.93	43.34 ±54.20
Zea/Lut	151.78	59.84 ±41.59	231.87	77.53 ±66.74	30.02	10.11 ±7.27	43.89	20.60 ±10.01
Chl b	91.63	43.78 ±27.81	21.07	7.77 ±5.51	69.21	23.44 ±19.97	258.67	49.11 ±52.61

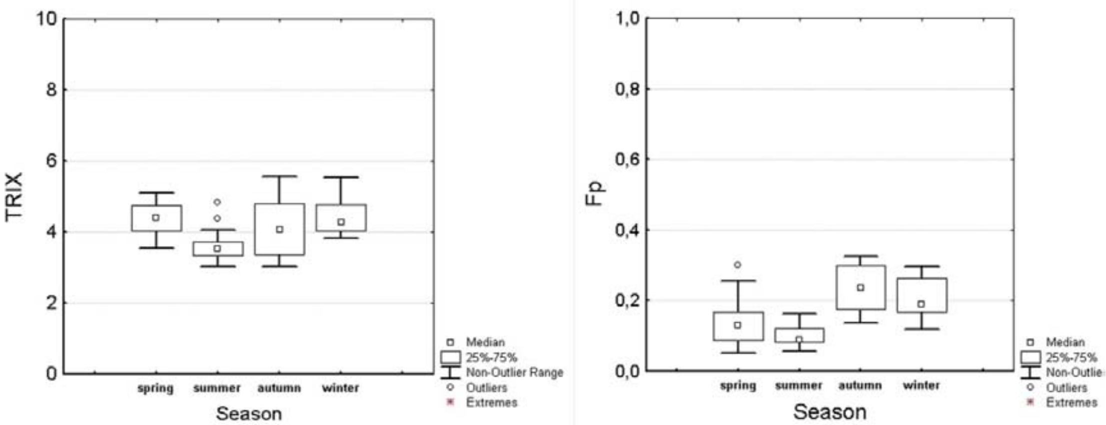

FIGURE 8 - Box & Whisker representation of seasonal TRIX and Fp index in the Boka Kotorska Bay.

4. DISCUSSION

As the Boka Kotorska area is the one of the rainiest parts in Europe [29], significant fresh water runoff and a low average tidal amplitude (28.5 cm) [30] throughout the autumn-spring period generates a pronounced halocline between 2 and 5 m depth. In our study, minimum salinity values were recorded in the surface layer in March due to winter and early spring precipitation events. In spite of the high phytoplankton production, dissolved oxygen was generally low in the entire water column with a relatively small vertical gradient. Lower concentrations of dissolved oxygen in summer are attributed to the oxidation of organic matter and higher water temperatures prevailing in summer [31]. Nutrient concentrations in Boka Kotorska Bay were generally high during the investigated period. The recorded values were higher in comparison to the values reported for the Zrmanja river estuary [5, 13], where phosphorous was detected as a limiting factor throughout the whole year and nitrogen sporadically in the summer. In the SE Mediterranean [17] and middle Adriatic [32], phosphate is known as the limiting nutrient instead of nitrate, which is in contrast to many other marine environments that agree with the results in the current study. According to the coefficients of determination (r^2) obtained in upper layer (0-2m), 75 to 78 % of the nutrient variations could be explained by variations of salinity, i.e. by the inflow of freshwater to this part of the water column.

The mean monthly values of the chlorophyll *a* concentrations were high compared to data published elsewhere in the Mediterranean, such as for the Bay of Trieste [14], the Ionian sea [33] and Morocco [34], and the north-eastern Adriatic coast [35, 5], but they were lower than values from the western port of Aleksandria [31] and the local area of the island of Mallorca [36].

Chlorophyll *a* concentrations, as an indicator of phytoplankton biomass, are often higher after rainfall, particularly if the rain has flushed nutrients into the water. Chlorophyll *a* seasonality is characterized by the distinctive maximum appeared in late winter (February) which was in concordance with the results for the middle part of Adriatic Sea [37] and in northeastern Mediterranean [38, 39] where chlorophyll *a* concentration increased in late winter. The coefficients of determination (r^2) obtained in current study indicate that 23 to 28 % of the variations of chlorophyll *a* in the Boka Kotorska Bay could be explained by variations in nutrient concentrations. However, the environmental factors (temperature, insolation, microelements, etc) could be responsible for additional variations which effect the phytoplankton community. Also, Chl *a* is significant negatively correlated with salinity ($p<0.05$), which suggests the importance of enhanced winter run-off on the annual phytoplankton biomass balance.

According to the Chl *a* concentration and the criteria of Ignatiades et al. [40] and Håkanson [41], the area in the present study could be described as oligo-mesotrophic. According to TRIX classification criteria, the investigated area is in a good trophic state, with moderately productive waters and occasional water turbidity, anomalous water colors and bottom water hypoxia episodes. The values of TRIX and Fp were similar to those described in the Gulf of Trieste [42, 43], the northern Adriatic [44], the Gulf of Gabes [45] and the Ionian Sea [33], indicative of slightly eutrophic conditions. It seems that these two indicators are complementary, and we agree with Flander-Purtle and Malev's (2003) [46] suggestion that in new studies the TRIX trophic index needs to include Chl *a* degradation products as an indication of the physiological status of the phytoplankton community. In this study, we did not include chlorophyll *a* degradation products but we used the Fp ratio, which indicated a large phytoplankton pigment biomass (diatoms and dinoflagellates) to the total diagnostic pigment biomass. The large phytoplankton species are known to be the main factors responsible for new production [47], so the basic hypothesis used herein is as follows: the higher the Fp, the more eutrophic the ecosystem [48].

It seems that in Boka Kotorska Bay changes in physical, chemical and biological parameters are mainly related to island-sea water interactions, which mainly depend on natural factors such as the pattern of rainfall. We argue that natural eutrophication is still dominant over that of anthropogenic eutrophication in Boka Kotorska Bay.

ACKNOWLEDGEMENTS

This study was funded by the Norwegian Cooperation Program on Research and Higher Education with the countries of the Western Balkans: Marine science and coastal management in the Adriatic, Western Balkans, an education and research network (2006-2009). The authors gratefully acknowledge Dr Marijan Ahel (Ruđer Bošković Insitute, Croatia), in whose laboratory the HPLC analysis of the phytoplankton pigments was performed. Also, the authors thank the anonymous referees who provided valuable comments on the manuscript.

REFERENCES

[1] GESAMP (1991) The state of the marine environment. Oxford: Blackwell. p. 146.

[2] Krom, M.D., Brenner, S., Kress, N., Neori, A. and Gordon, L.I. (1993) Nutrient distribution during an annual cycle across a warm-core eddy from eastern Mediterranean sea. Deep-Sea Res. 40, 805-825.

[3] Barić, A., Marasović, I. and Gačić, M. (1992) Eutrophication Phenomenon with Special Reference to the Kaštela Bay. Chem. Ecol. 6, 51-68.

[4] Marasović, I., Ninčević, Ž., Kušpilić, G., Marinović, S. and Marinov S. (2005) Long-term changes of basic biological and chemical parameters at two stations in the middle Adriatic. J. Sea Res. 54, 3-14.

[5] Viličić, D., Terzić, S., Ahel, M., Burić, Z., Jasprica, N., Carić, M., Mihalić, C.K. and Olijić G. (2008) Phytoplankton abundance and pigment biomarkers in the oligotrophic, eastern Adriatic estuary. Environ. Monit. Assess. 142, 199-218.

[6] Mačić, V., Antolić, B., Thibaut T. and Svircev Z. (2010) Distribution of the most common Cystoseira C. Agardh species (Heterokontophyta, Fucales) on the coast of Montenegro (South-East Adriatic sea). Fresen. Environ. Bull. 19(6), 1191-1198.

[7] Krivokapić, S., Stanković, Ž. and Vuksanović N. (2009) Seasonal variation of phytoplankon biomass and environmental conditions in the inner Boka Kotorska Bay (eastern Adriatic Sea). Acta Bot. Croat. 68(1), 45-55.

[8] Campanelli, A., Bulativić, A., Cabrini, M., Grilli, F., Kljajić, Z., Mosetti, R., Paschini, E., Penna, P. and Marini M. (2009) Spatial distribution of physical, chemical and biological oceanographic properties, phytoplankon, nutrients and Coloured Dissolved Organic Matter (CDOM) in the Boka Kotorska Bay (Adriatic Sea). Geofizika 26(2), 215-228.

[9] Viličić, D. (1989) Phytoplankton population density and volume as indicators of eutrophication in the eastern part of the Adriatic Sea. Hydrobiologia 174, 117-132.

[10] Giani, M., Savelli, F. and Boldrin A. (2003) Temporal variability of particulate organic carbon, nitrogen and phosphorus in the Northern Adriatic Sea. Hydrobiologia 494, 319-325.

[11] Vadrucci, M.R., Catalano, G. and Basset A. (2005) Spatial and seasonal variability of fractionated phytoplankton biomass and primary production in the frontal region of the Northern Adriatic Sea. Medit. Mar. Sci. 6 (1), 5-15.

[12] Cetinić, D., Viličić, D., Burić, Z. and Olujić G. (2006) Phytoplankton seasonality in a highly stratified karstic estuary (Krka, Adriatic Sea). Hydrobiologia 555, 31-40.

[13] Burić, Z., Cetinić, I., Viličić, D., Mihalić, C., Carić, M. and Olijić G. (2007) Spatial and temporal distribution of phytoplankon in a highly stratified estuary (Zrmanja, Adriatic Sea). P.S.Z.N.I :Mar. Ecol. 28, 169-177.

[14] Tedesco, L., Socal, G., Bianchi, F., Acri, F., Veneri, D. and Vichi M. (2007) NW Adriatic Sea variability in relation to chlorophyll-a dynamics in the last 20 years (1986–2005). Biogeosciences 4, 651-685.

[15] Svensen, C., Viličić, D., Wassmann, P., Arashkevich, E. and Ratkova T. (2007) Plankton distribution and vertical flux of biogenic matter during high summer stratification in the Krka estuary (Eastern Adriatic). Estuar. Coast. Shelf S. 71, 381-390.

[16] Bosak, S., Burić, Z., Djakovac, T. and Viličić D. (2009) Seasonal distribution of plankon diatoms in Lim Bay, northeastern Adriatic Sea. Acta Bot. Croat. 68 (2), 351-365.

[17] Krom, M.D., Kress, N. and Brenner S. (1991) Phosphorus limitation of primary productivity in the eastern Mediterranean Sea. Limnol. Oceanogr. 36 (3), 424-432.

[18] Zohary, T. and Robats R.D. (1998) Experimental study of microbial P limitation in the eastern Mediterranean. Limnol. Oceanogr. 43(3), 387-395.

[19] Thingstad, T.F., Krom, M.D., Mantoura, R.F.C., Flaten, G.A.F., Groom, S., Herut, B., Kress, N., Law, C., Pasternak, A., Pitta, P., Psarra, S., Rassoulzadegan, F., Tanaka, T., Tselepides, A., Wassmann, P., Woodward, E.M.S., Wexels Riser, C., Zodiatis, G. and Zohary T. (2005) Nature of P limitation in the ultraoligotrophic Eastern Mediterranean. Science 309, 1068-1071.

[20] Krom, M.D., Thingstad, T.F., Brenner, S., Carbo, P., Drakopoulos, P., Fileman, T.W., Flaten, G.A.F., Groom, S., Herut, B., Kitidis, V., Kress, N., Law, C.S., Liddicoat, M.I., Mantoura, R.F.C., Pasternak, A., Pitta, P., Polychronaki, T., Psarra, S., Rassoulzadegan, F., Skjoldal, E.F., Spyres, G., Tanaka, T., Tselepides, A., Wassmann, P., Wexels Riser, C., Woodward, E.M.S., Zodiatis, G. and Zohary T. (2005) Summary and overview of the CYCLOPS P addition Lagrangian experiment in the Eastern Mediterranean. Deep-Sea Res. II. 52, 3090-3108.

[21] Vukić Lušić, D., Peršić, V., Horvatić, J., Viličić, D., Traven, L., Djakovac, T. and Mićović, V. (2008) Assessment of nutrient limitation in Rijeka Bay, NE Adriatic Sea, using miniaturized bioassay. J. Exp. Mar. Biol. Ecol. 358, 46-56.

[22] Vollenweider, R.A., Giovanardi, F. and Montanari G. (1998) Characterization of the trophic conditions of marine special reference to the NW Adriatic Sea : proposal for trophic scale, turbidity and generalized water quality index. Environmetrics 9, 329-357.

[23] Claustre, H. (1994) The trophic status of various oceanic provinces as revealed by phytoplankton pigment signature. Limnol. Oceanogr. 39, 1206-1210.

[24] Eppley, R.W. and Peterson B.J. (1979) Particulate organic matter flux and planktonic new production in the deep ocean. Nature 282, 677-680.

[25] Strickland, J.D.H. and Parsons T.R. (1972) A Practical Handbook of Seawater Analysis. Bull. Fish. Res. Board Can. 167, 1-310.

[26] Jeffrey, S.W., Mantoura, R.F.C. and Wright S.W. (1997) Phytoplankton pigments in Oceanography. UNESCO, Paris.

[27] Grasshoff, K. (1976) Methods of Seawater Analysis. Verlag Chemie, Weinheim. 307pp.

[28] Berlow, R.G, Mantoura, R.F.C., Gough, M.A. and Fileman, T.W. (1993) Pigment signatures of the phytoplankton composition in the northeastern Atlantic during the 1990 spring bloom. Deep-Sea Res. II 40**,** 459-477.

[29] Magaš, D. (2002) Natural-geographic characteristics of the Boka Kotorska area as the basis of development. Geoadria 7 (1), 51-81.

[30] Anon. (1989) Report on the tide-gauge measurements along the Yugoslav coast of the Adriatic. Hydrographic Institute of the Yugoslav Navy, HI-OCO 53, 1-70. (In Serbo-croatian).

[31] Dorgham, M., Abdel-Aziz, N.E., El-Deeb, K.Z. and Okbah M.A. (2004) Eutrophication problems in the Western Harbour of Alexandria, Egypt. Oceanologia 46, 25-44.

[32] Vukadin, I. and Stojanoski L. (2001) Phosphorus versus nitrogen limitation in the middle Adriatic Sea. Rapp. Comm. int Mer Médit. 36, 174.

[33] Nikolaidis, G., Moschandreou K. and Patoucheas D.P. (2008) Application of a trophic index (TRIX) for water quality assessment at Kalamitsi coasts (Ionian sea) after the operation of the wastewater treatment plant. Fresen. Environ. Bull. 17 (11b), 1938-1944.

[34] Claustre, H., Hooker, S.B., Van Heukelem, L., Berthon, J.F., Barlow, R. and Ras J. (2004) An intercomparision of HPLC phytoplankton pigment methods using in situ samples: aplication to remote sensing and datebase activities. Mar. Chem. 85, 41-61.

[35] Precali, R., Daković, T., Smodlaka, N. and Ivančić I. (2001) Long-term changes of nutrient concentration and phytoplankton biomass in the Northern Adriatic Sea. Rapp. Comm. Int. Mer. Médit. 36, 156.

[36] Puigserver, M., Ramon, G., Moýa, G. and Martinez-Taberner A. (2002) Planktonic chlorophyll a and eutrophication in two Mediterranean littoral system (Mallorca Island, Spain). Hydrobiologia 475/476, 493-503.

[37] Ninčević, Ž. and Marasović I. (1998) Chlorophyll a and primary production of size fractionated phytoplankton in the middle Adriatic Sea. Rapp. Comm. Int. Mer. Médit. 35, 472-473.

[38] Ediger, D. and Yilmaz A. (1996) Characteristics of deep chlorophyll maximum in the northeastern Mediterranean with respect to environmental conditions. J. Mar. Syst. 9, 291-303.

[39] Polat, S. (2002) Nutrients, Chlorophyll a and Phytoplankon in the Iskenderun bay (Northeastern Mediterranean). P.S.Z.N.I: Mar. Ecol. 23(2), 115-126.

[40] Ignatiades, L., Karydis, M. and Vounatsou P. (1992) A possible method for evaluating oligotrophy and eutrophication based on nutrient concentration scales. Mar. Pollut. Bull. 24, 238-243.

[41] Håkanson, L. (1994) A review of effect-dose-sensitivity models for aquatic ecosystems. Int. Rev. Hydrobiol. 79, 621-667.

[42] Turk, V., Mozetič, P. and Malej A. (2007) Overview of eutrophication- related events and other irregular episodes in Slovenian sea (Gulf of Trieste, Adriatic sea). Ann. Ser. Hist. Nat. 17 (2), 197-216.

[43] Malej, A., Mozetič, P., Malačič, V., Terzić, S. and Ahel M. (1995) Phytoplankton responses to freshwater inputs in a small semi-enclosed gulf (Gulf of Trieste, Adriatic Sea), Mar. Ecol. Prog. Ser. 120, 111-121.

[44] Pečar, O. and Precali R. (2004) Long-term changes of the Northern Adriatic trophic conditions. Rapp. Comm. Int. Mer. Médit. 37, 234.

[45] Hassen, M.B., Drira, Z., Hamza, A., Ayadi, H., Akrout, F. and Issaoui H. (2008) Summer phytoplankton pigments and community composition related to water mass properties in the Gulf of Gabes. Estuar. Coast. Shelf S. 77, 645-656.

[46] Flander-Purtle, V. and Malev A. (2003) The trophic state of coastal waters under the infuence of antropogenic sources of nutrients (fish farm, seawage outfalls). Period. Biol. 105(4), 359-365.

[47] Dugdale, R.C. and Goering J.J. (1967) Uptake of new and regenerated forms of nitrogen in primary productivity. Limnol. and Oceanogr. 12 (2), 196-206.

[48] Avril, B. (2002) DOC dynamics in the northwestern Mediterranean Sea (DYFAMED site). Deep-Sea Res. II 49, 2163-2182.

Received: September 23, 2010
Revised: March 18, 2011
Accepted: April 14, 2011

CORRESPONDING AUTHOR

Slađana Krivokapić
Department of Biology
Faculty of Science
University of Montenegro
Cetinski put bb
81 000 Podgorica
MONTENEGRO

Phone: + 382 68 048 969
Fax: + 382 20 243 816,
E-mail: sladjana69@yahoo.com

FEB/ Vol 20/ No 8/ 2011 – pages 1960 - 1969