Acta Bot. Croat. 68 (1), 45–55, 2009

CODEN: ABCRA 25
ISSN 0365–0588

Seasonal variations of phytoplankton biomass and environmental conditions in the inner Boka Kotorska Bay (eastern Adriatic Sea)

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Nutrients and chlorophyll *a* concentrations were analysed at five sampling stations in the inner part of the Boka Kotorska Bay (southern Adriatic Sea), in the period September 2003 to August 2004. Maximum concentrations of nitrates (14.4 μmol L^{-1}), nitrites (3.5 μmol L^{-1}), phosphates (0.84 μmol L^{-1}), and silicates (4.5 μmol L^{-1}) preceded maximum phytoplankton biomass (5.07 to 6.47 mg m^{-3} chlorophyll *a*) in December. Chlorophyll *a* was positively correlated with oxygen, nitrites and silicates, but negatively correlated with temperature, salinity, transparency and nitrates. Kotor Bay is the most eutrophied environment in the eastern coastal Adriatic Sea.

Key words: Chlorophyll *a*, nutrients, seasonality, eutrophication, Adriatic Sea

Introduction

Eutrophication in the Mediterranean Sea has been mainly related to local extensive use of the littoral zone, maritime activities and the increasing development of tourism. The frequency of high chlorophyll *a* concentrations, blooms of toxic algae, occurrences of anoxia, hypoxia and changes in the composition of planktonic and benthic communities steadily increases along the Mediterranean coast, especially in coastal embayments into which elevated nutrient loads are discharged (VILIČIĆ, 1989, VOLLENWEIDER 1992, LE PAPE et al. 1995, DEGOBBIS et al. 2000, MONCHEVA et al. 2001, PUIGSERVER et al. 2002, DE JONGE et al. 2002).

The Boka Kotorska Bay is located in the south eastern part of the Adriatic Sea, comprising a part of the Montenegrin coast. In the last decade, the effects of anthropogenic impact have been evident in its inner part. The Bay is greatly influenced by the great influx of fresh water from numerous streams and submarine springs. The scope of this paper is to present

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spatial and temporal changes in physicochemical parameters and phytoplankton abundance and biomass (chlorophyll *a* concentrations) in the inner part of Boka Kotorska Bay.

Materials and methods

Boka Kotorska Bay (42° 31' 00'' N – 42° 23' 32'' S; 18° 46' 32'' E – 18° 30' 29'' W) occupies an area of 87.3 km^2, a volume of 2.4×10^6 m^3, and a coastline length of 105.7 km. The inner part of Boka Kotorska Bay comprises 27% of the area and 26% of the volume of the whole bay.

Seawater samples were taken with a HydroBios sampler, at five stations in the inner part of the bay (Fig. 1), which comprises three coves: Morinj Cove in the west, Risan Cove in the north and Kotor Cove in the east. The sampling was performed on a monthly basis from September 2003 to August 2004.

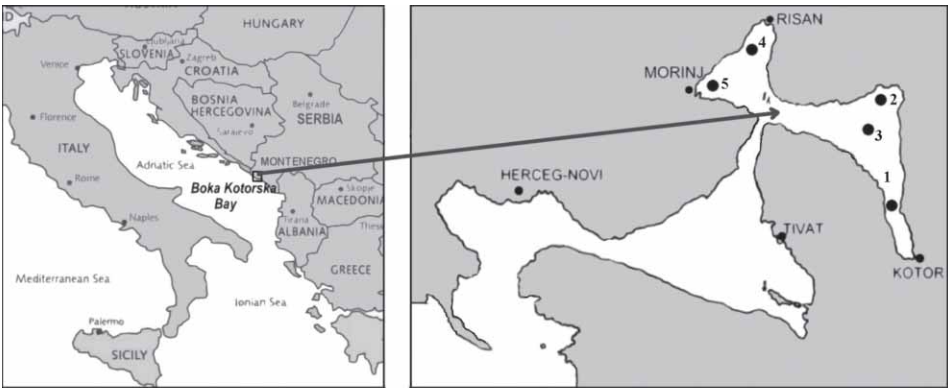

Fig. 1. Location of the sampling sites

At the innermost stations near the Institute of Marine Biology (1), Orahovac (2) and Morinj (5), samples for physical, chemical and biological analyses were taken from three depths: 0, 4, and 8 m. In the central stations of Kotor (3) and Risan (4) Coves, samples were taken from five depths: 0, 5, 10, 20, and 30 m.

Temperature and salinity were measured *in situ* by multi LINE P4 – UNIVERSAL METER. Oxi-Guard Handy GAMMA was use for measuring oxygen concentrations *in situ*. Transparency was determined with a white Secchi disk. The nutrient samples were taken with Niskin bottles and stored in polyethylene bottles. Determination of nutrients needed to be done the same day after sampling, or the else nutrients would freeze. Nitrates, nitrites, phosphates and silicates were determined at sea by using standard methods (STRICKLAND and PARSONS 1972). The absorbance readings were made on a Perkin Elmer UV/VIS spectrophotometer (Lambda 2), at at different wavelength for every nutrient.

All samples 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 measurement of absorbance with Perkin – Elmer UV/VIS spectrophotometer, and calculation according to JEFFREY et al. (1997). Abundance of phytoplankton was determined according to UTERMÖHL (1958). Statistica 7 was used for statistical analyses. Principal Component Analysis (PCA) was carried out according to S- Plus Program (getting started with S-PLUS for Windows, Insightful Corporation, Seattle, WA).

Results

Maximum monthly mean temperature was found in August, the minimum in January (Fig. 2).

The maximum salinity values appeared in July to September (Fig. 3a). Extremely low salinity of 3.9 was measured in January after heavy rains. As a result of the influence of freshwater, statistically significant differences of mean salinity values between stations 1, 2 and 5, as well as between stations 3 and 4 were found (Fig. 3b).

Oxygen concentrations ranged from 7.21 mg L^{-1} in August to 8.48 mg L^{-1} in April (Fig. 4a). The minimum oxygen concentrations were determined at stations 3 and 4 (Fig. 4 b).

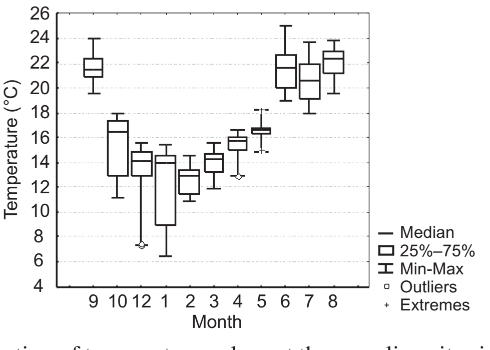

Fig. 2. Seasonal distribution of temperature values at the sampling sites in the inner part of Boka Kotorska Bay. The centre horizontal line in each box is the median value. Fifty percent of the data points lie within each box. The whiskers above and below the box indicate the 75% and the 25% percentiles.

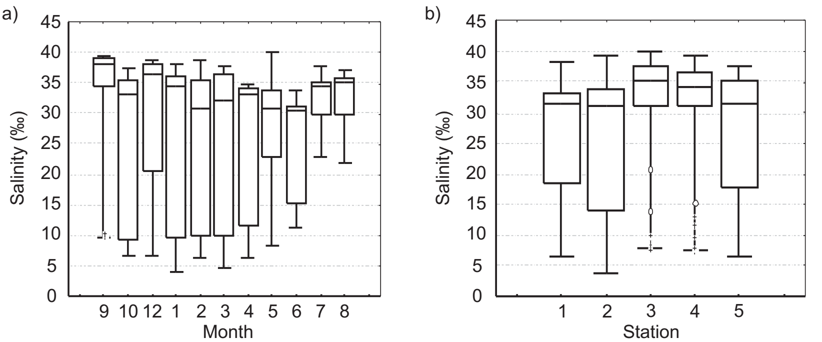

Fig. 3. Temporal (a) and spatial (b) distribution of salinity values in the inner part of Boka Kotorska Bay. For explanation of box plots, see Fig. 2.

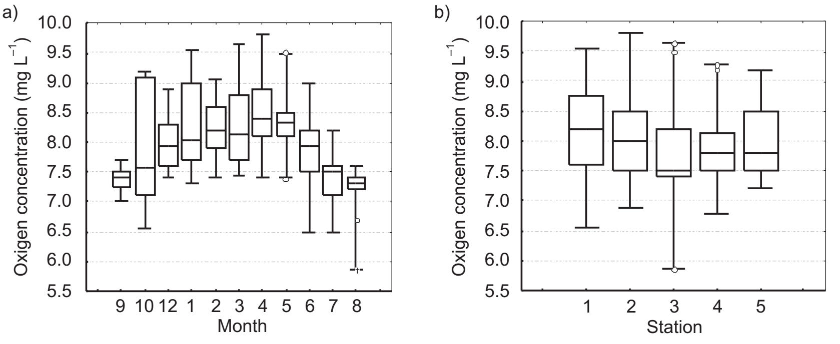

Fig. 4. Temporal (a) and spatial (b) distribution of mean oxygen concentration value in the inner part of Boka Kotorska Bay. For explanation of box plots, see Fig. 2.

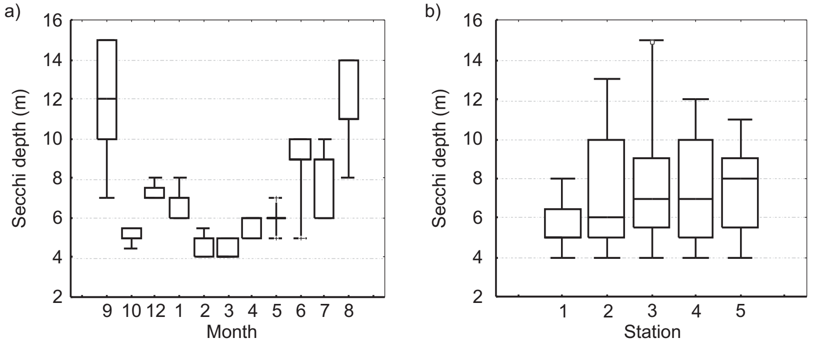

Fig. 5. Temporal (a) and spatial (b) distribution of mean transparency in the inner part of Boka Kotorska Bay. For explanation of box plots, see Fig. 2.

Tab. 1. Maximum (max), minimum (min), average (avg), standard deviation (SD), and number of samples (n) for physico-chemical and biological parameters in the inner part of Boka Kotorska Bay.

Variables	Average	Min	Max	SD	n
Temperature (C°)	16.9	6.5	25	4	198
Salinity (psu)	23.9	3.9	39.7	1	198
O_2 (mg L^{-1})	7.9	5.84	10	1	198
Secchi visibility (m)	7.3	4	15	3	198
NO_3^- (µmol L^{-1})	3.24	0	14.4	2.85	198
NO_2^- (µmol L^{-1})	0.24	0	3.5	0.35	198
PO_4^{3-} (µmol L^{-1})	0.08	0	0.23	0.11	198
SiO_2 (µmol L^{-1})	0.57	0	4.5	0.61	198
Chlorophyll a (µg L^{-1})	2.6	0.06	10	2	198
Phytoplankton abundance (10^3 cell L^{-1})	999	48	15055	1587	198

The transparency ranged between 4.39 m in March (Fig. 5a) and 11.83 m in September. A significant difference in transparency was found between station 1 and other stations (Fig. 5b).

Nutrient concentrations were low (Tab. 1) during the investigation period. Maximum concentrations of nitrates (14.4 μmol L^{-1}), nitrites (3.5 μmolL^{-1}), phosphates (0.84 μmol L^{-1}), and silicates (4.5 μmol L^{-1}) were detected in the period September to December.

The minimal monthly mean value for nitrate concentration was 0.2 μmol L^{-1} in October and the maximal of 8.06 μmol L^{-1} came in September (Fig. 6a left). A significant difference

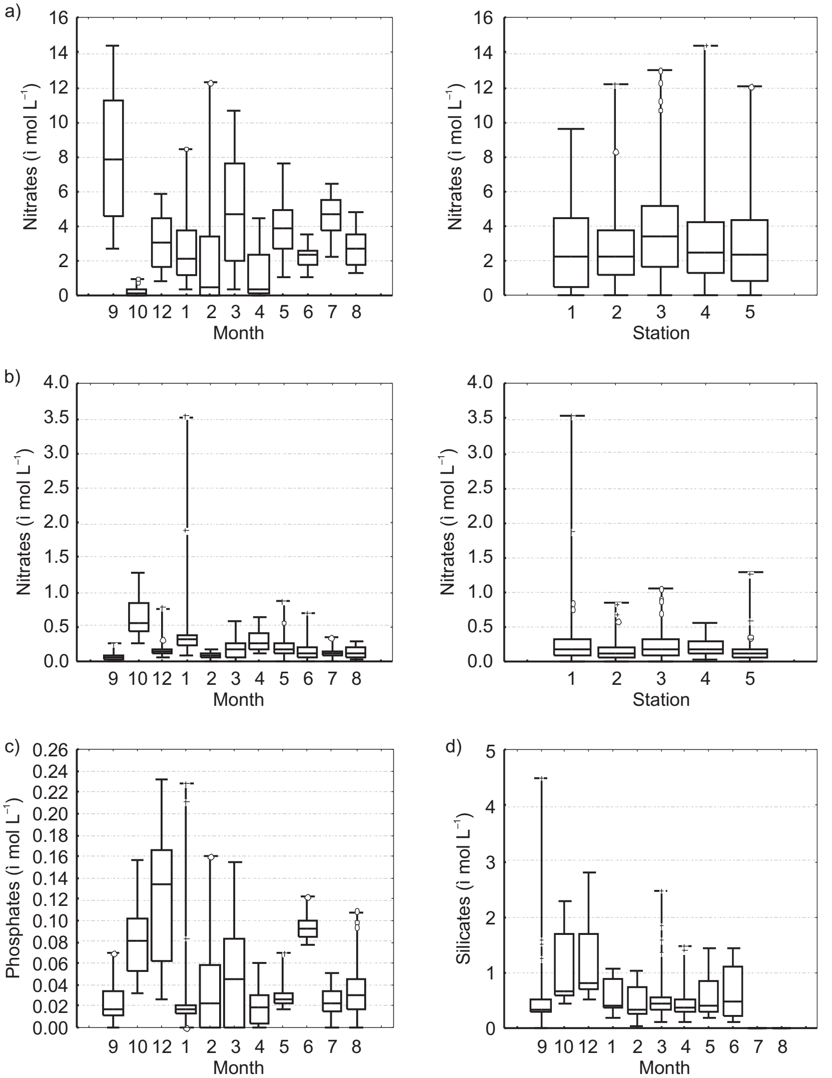

Fig. 6. Temporal and spatial distribution of nutrient concentrations: nitrates (a); nitrites (b); phosphates (c); silicates (d). For explanation of box plots, see Fig. 2.

was found between the mean value of nitrate concentration at site 3 and the other stations (Fig. 6a right). The minimal monthly value for nitrites (Fig 6b left) was determined in February (0.1 µmol L^{-1}), and the maximal in January (0.7 µmol L^{-1}). A statistically significant difference was found between the mean values of nitrite concentration at site 1 (0.43 µmol L^{-1}) and the other researched sites (Fig. 6b right). The mean monthly value of phosphate concentration (Fig. 6c) ranged from a minimal 0.01 µmol L^{-1} in April to 0.22 µmol L^{-1} in October. In the case of silicates, the maximal monthly mean value of 1.2 µmol$L^{-1}$was determined in December (Fig. 6d). In July and August the concentration of silicates was below the detection limit.

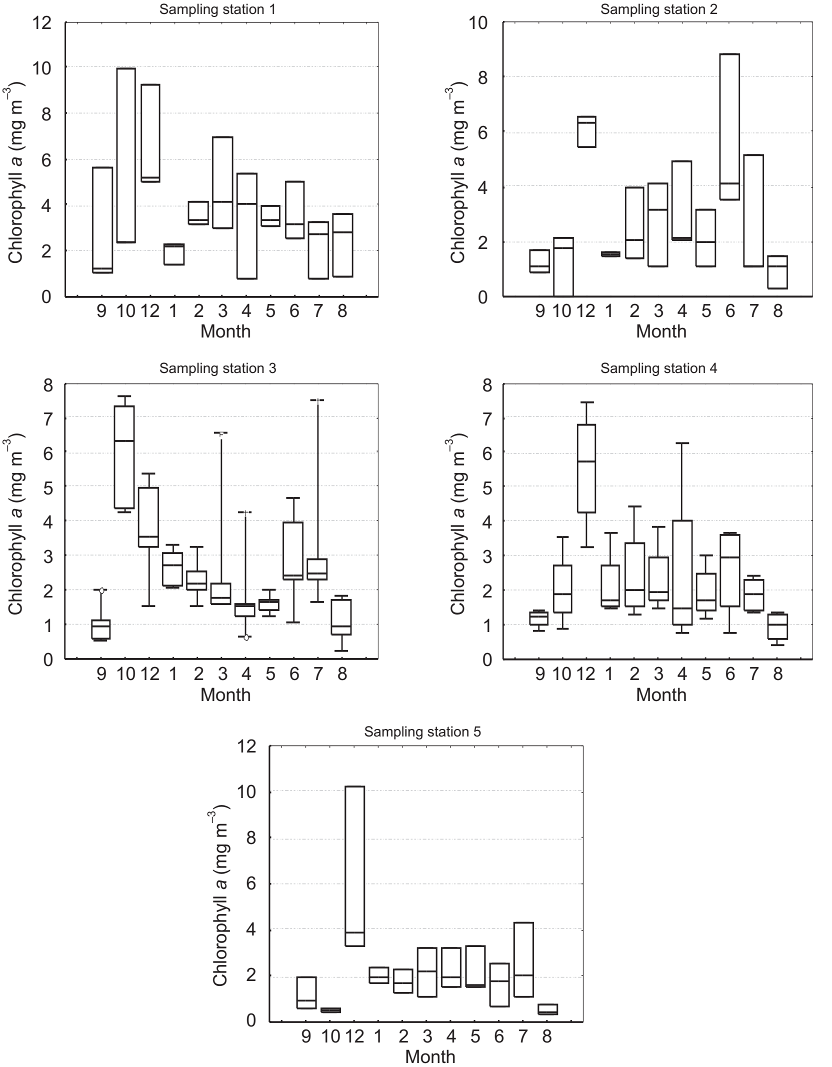

Fig. 7. Chlorophyll *a* concentrations on sampling sites 1, 2, 3, 4 and 5, in the period September 2003–August 2004. For explanation of box plots, see Fig. 2.

Phytoplankton biomass (chlorophyll *a* concentration) ranged from 0.47 mg m^{-3} (August, site 5) up to 6.47 mg m^{-3} (December, site 1) (Fig. 7).

Chlorophyll biomass provided bimodal seasonality with annual maxima mostly determined in October – December, and March – July (Figs. 7, 8).

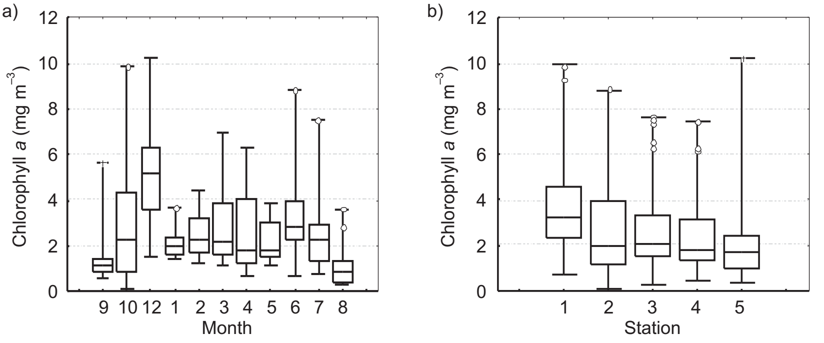

Fig. 8. Temporal (a) and spatial (b) distribution of chlorophyll *a* in the inner part of Boka Kotorska Bay. For explanation of box plots, see Fig. 2.

The phytoplankton abundance was characterised by spring maxima in March – April (2.41×10^6 cells L^{-1}) (Fig 9a). The maximum biomass and highest abundance were determined at station 1, and this station was significantly different from the other investigated stations (Figs. 8b, 9b).

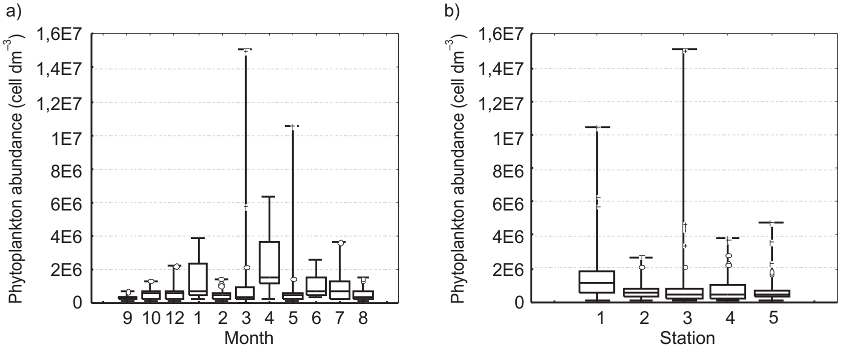

Fig. 9. Temporal (a) and spatial (b) distribution of phytoplankton abundance in the inner part of Boka Kotorska Bay. For explanation of box plots, see Fig. 2.

The PCA analysis provided 11 principal components, the first two (Fig. 10) had eigen values > 1 and explained 88.6% of the variance of the original data set. PC1 accounted for 69.38 %, PC2 19.19 %, and PC3 6.28% of total variance within data set. Salinity, nitrate and nitrite are positively correlated with first main component (Tab. 3), while oxygen concentration, oxygen saturation, silicate, phytoplankton abundance and chlorophyll *a* are negatively correlated with first main component.

Discussion

The bimodal phytoplankton seasonality is characterized by the maximum development in winter and the minimum in the warmer period of the year, as already indicated in the Adriatic Sea (NINČEVIĆ and MARASOVIĆ 1998). High concentrations of nitrites and phosphates flushed by heavy rains preceded the appearance of maximum chlorophyll *a* concentration in winter. The mean monthly values of chlorophyll *a* concentrations were high in comparison to published data elsewhere in the Mediterranean, such as the Bay of Trieste (TEDESCO et al. 2005), Ionian Sea and Morocco (CLAUSTRE et al. 2004), the north eastern coastal Adriatic (PRECALI et al. 2001, VILIČIĆ et al. 2008), but lower than values from the west port of Alexandria (DORGHAM et al. 2004), and local areas of the island of Mallorca (PUIGERVER et al. 2002).

The high chlorophyll *a* concentrations occasionally appeared during low abundances, probably due to different photosynthetic activities in different cell size fractions, different composition of phytoplankton, and the different physiological state of cells (NINČEVIĆ and MARASOVIĆ 1998). In summer, the inner part of Boka Kotorska Bay is characterized by low concentrations of nutrients, high light transparency and absence of phytoplankton blooms, which suggests summer oligotrophication, as in other eastern Adriatic nutrient enriched environments (SVENSEN et al. 2007, VILIČIĆ et al, 2008).

Chlorophyll *a* is positively correlated with phytoplankton abundance (Phy. ab.), oxygen, nitrites and silicates (Tab. 2), and negatively correlated with temperature, salinity, transparency and nitrates. High values of silicate concentration occurred during the winter-spring period, and its negative correlation with salinity indicate higher freshwater influence and its estuarine character. Nitrate concentration was positively correlated with salinity, indicating low content of nitrates in fresh waters discharging into the Kotor Bay. In contrast, salinity and nitrates show a negative correlation in other eastern Adriatic estuaries (LEGOVIĆ et al. 1994, CETINIĆ et al. 2006, BURIĆ et al. 2007).

Tab. 2. Correlation matrix for environmental variables in the sampling sites ($p<0,05$; ns-not significant).

	Temp.	Sal.	Conc.O_2	Trans.	N-NO_3^-	N-NO_2^-	P-PO_4^{3-}	Si-SiO_2	Chl.*a*	Phy.ab
Temp.	1,00									
Sal.	0,35	1,00								
Conc.O_2	–0,61	–0,67	1,00							
Trans.	0,61	0,23	–0,48	1,00						
N-NO_3^-	0,28	0,48	–0,35	0,29	1,00					
N-NO_2^-	–0,15	ns	–0,15	–0,28	–0,26	1,00				
P-PO_4^{3-}	–0,16	ns	ns	–0,16	ns	0,19	1,00			
Si-SiO_2	–0,37	–0,57	0,47	–0,16	–0,27	ns	0,17	1,00		
Chl.*a*	–0,20	–0,25	0,15	–0,24	–0,24	0,17	ns	0,36	1,00	
Phy.ab.	ns	–0,33	0,38	–0,19	–0,23	ns	ns	0,19	0,23	1,00

Water streams and submarine springs are the main external sources of silicates in the bay. The input of nutrients in spring and autumn was the main cause of increased abundance of phytoplankton and chlorophyll *a* increase, while temperature was not a limiting factor for phytoplankton growth. Salinity is an important factor that regulates phytoplankton development (DORGHAM et al. 2004), although the relationship could be negative, as in this study and elsewhere (PUIGSERVER et al.. 2002).

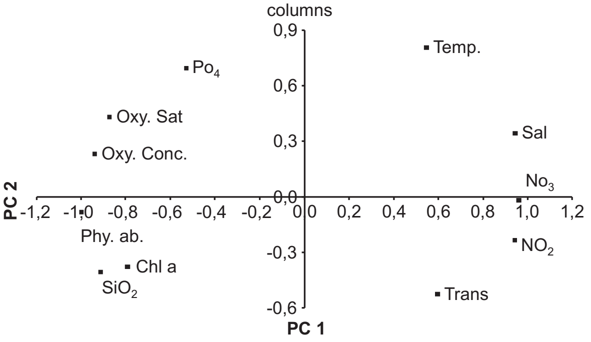

Fig. 10. Position of physical, chemical and biological parameters of Boka Kotorska Bay in space defined by Principal Components 1 and 2.

Tab. 3. Principal Component Analysis.

Variables	PC1 Loading	PC2 Loading	PC3 Loading
Temp. (C°)	0.544	0.800	–0.205
Sal. (‰)	0.938	0.341	0.035
Ox sat. (%)	–0.930	0.231	0.201
Con. O_2 (mg L^{-1})	–0.865	0.427	0.172
Tran. (m)	0.593	–0.522	0.602
NO_3^- (μmol L^{-1})	0.954	–0.018	–0.197
NO_2^- (μmol L^{-1})	0.936	–0.234	0.059
PO_4^{3-} (μmol L^{-1})	–0.527	0.693	0.309
SiO_2 (μmol L^{-1})	–0.908	–0.405	–0.106
Hl *a* (mg m^{-3})	–0.768	–0.376	–0.252
Ph. ab. (cell dm^{-3})	–0.993	–0.079	–0.058

PC, principal component
Loading of the individual variables along the PC1, PC2 and PC3.

According to chlorophyll a concentration and the criteria of HÅKANSON (1994), the area could be described as eutrophic to hypereutrophic during winter. It seems that heavy rains contribute to the increased content of nutrients in the bay during winter and consequently to the higher phytoplankton biomass.

Boka Kotorska Bay is an outstanding dynamic system with extremely variable ecological factors, where natural is more important than anthropogenic eutrophication. Therefore, for protection of Boka Kotorska Bay, a particular care should be taken of effluent discharge from the town of Kotor.

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