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Spatial distribution of physical, chemical and biological oceanographic properties, phytoplankton, nutrients and Coloured Dissolved Organic Matter (CDOM) in the Boka Kotorska Bay (Adriatic Sea)

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The temporal variations of temperature, salinity, fluorescence, dissolved oxygen concentration, Coloured Dissolved Organic Matter (CDOM) and of chemical (nutrients, chlorophyll *a*) and biological (phytoplankton composition) parameters in the Boka Kotorska Bay were observed during two periods. CDOM regulates the penetration of UV light into the sea and therefore plays an important role in many hydrological and biogeochemical processes in the sea surface layer including primary productivity.

In the framework ADRICOSM-STAR it was possible to investigate the Boka Kotorska Bay during May and June 2008 in order to increase an understanding of optical and chemical characteristics and their evolution during these periods. In both periods station KO (located furthest from the open sea) presented different physical, chemical and biological characteristics with respect to the other stations inside the Boka Kotorska Bay.

A positive correlation was found between CDOM and chlorophyll *a* ($R = 0.7$, $P < 0.001$, $n = 15$) and this implies that in this area, similarly to the open sea, the primary source of CDOM should be the biological production from phytoplankton. This is probably due to the fact that the rivers entering the Boka Kotorska Bay are not severely impacted by man.

Keywords: CDOM, nutrients, hydrological data, phytoplankton, Adriatic Sea.

1. Introduction

Light entering the ocean is absorbed by water, living and detrital particles, and dissolved materials (< 0.2 μm). Absorption by the latter component, also known as coloured dissolved organic matter (CDOM), is almost exclusively attributable to humic substances.

It is well known, that the abundance and distribution of CDOM for many coastal waters is dominated by terrestrial inputs from rivers and runoff as decomposition of terrestrial organic matter yields light-absorbing compounds such as, humic and fulvic acids (Højerslev, 1982; Carder et al., 1989; Del Vecchio and Blough, 2004). In particular, CDOM is produced near the surface of the open ocean as a result of a heterotrophic process (Nelson et al., 1998, 2004; Steinberg et al., 2004; Yamashita and Tanoue, 2004) and is destroyed by solar bleaching in stratified waters (Determan et al., 1996; Vodacek et al., 1997; Siegel et al., 2002, 2005; Del Vecchio and Blough, 2004). Despite this, the optical activity of CDOM is (almost) never completely eliminated by solar bleaching or other natural processes, indicating the presence of a pool of CDOM that is at least partially resistant to solar bleaching and microbial degradation.

CDOM regulates the penetration of UV light into the sea and mediates photochemical reactions, therefore playing an important role in many biogeochemical processes on the ocean surface including primary productivity and the air-sea exchange of radiatively important trace gases (e.g. Mopper et al., 1991; Arrigo and Brown, 1996; Zepp et al., 1998; Toole and Siegel, 2004). The absorption of blue light by CDOM overlaps the phytoplankton absorption peak near 440 nm, resulting in a competition between CDOM and phytoplankton for light in this region of the visible spectrum (Wrigley et al., 1988; Davies-Colley, 1992; Kirk, 1994). To define the relationship between phytoplankton abundances and the absorption by dissolved materials, CDOM absorption coefficients ($a_{\mathrm{CDOM}}(\lambda)$) were compared with chlorophyll concentrations (Bricaud et al., 1991; Davies-Colley, 1992). Significant correlations between chlorophyll a and $a_{\mathrm{CDOM}}(\lambda)$ have been observed in eutrophic waters (Kopelevich and Burenkov, 1977). Generally, however, $a_{\mathrm{CDOM}}(\lambda)$ does not covary linearly with instantaneous estimates of pigment concentrations or phytoplankton productivity in coastal regions (Nelson et al., 1998). Bricaud et al. (1981) hypothesized that such a covariation might exist if biological activity were averaged over a seasonal time period.

The Boka Kotorska Bay is a semi-enclosed basin of Montenegro, situated in the south-eastern Adriatic Sea (Mediterrean Sea), sometimes called Europe's southernmost fjord. Kotor, Tivat and Herceg Novi represent the most populated cities. Boka Kotorska Bay represents a drowned valley initially shaped during the Pliocene period and later by tectonic down-warping. The Kotor and Risan bays are characterized by karstic rivers and underground springs, which influence temperature, density and salinity of sea water (Lepe-

tić, 1965; Milanović, 2007). The freshwater runoff from these rivers probably modifies the optical and chemical properties of seawater during the different seasons.

The enrichment of water with nutrients (primarily nitrogen, silicon and phosphorus compounds) may result in the growth of algal biomass. In particular, light and nutrient levels in the surface layer were sufficient to sustain active phytoplankton growth in similar basins, but when the residence time of surface water is short, then most of this production may be exported to the outer basin (Mikee et al., 2002). These nutrients may originate from either land surface runoff within a watershed (rivers and underground water discharges) and/or from direct urban inputs (sewage treatment plant outflows, industrial and storm water drains).

Variations in the stratification regimes and mixing depths in basins of this type have interesting effects on phytoplankton growth. However, Mikee et al. (2002) found that a high CDOM content in fresh water runoff was the most obvious factor limiting production in a fjord situated on the west coast of Scotland. They found that if CDOM concentrations in the surface layer were reduced, then the euphotic zone would extend to the bottom and conditions would be favourable for substantial growth of the phytoplankton population.

The aim of the present study is to understand and assess the optical, chemical and biological characteristics and their spatial and temporal evolution in the Boka Kotorska Bay, an area characterized by fresh water inputs and large biomass growth.

2. Methods

Measurements were collected in the Boka Kotorska Bay at four stations inside the Boka Kotorska Bay (KO, TV, HN and 1) and at station 4 in the open sea (Fig. 1) during two cruises, carried out aboard the R/V G. Dallaporta in May (10–17) and June (24–29), 2008.

The CTD (Conductivity-Temperature-Depth) data were collected at all the stations with a SeaBird Electronics SBE 911-plus CTD, equipped with additional sensors for dissolved oxygen (SBE43) and *in situ* fluorescence + turbiditimetry (Turner-SCUFA). The 24 Hz CTD data were processed according to UNESCO (1988) standards, and pressure-averaged to 0.5 db intervals. Water samples were obtained by the upcasts with a SeaBird Carousel rosette water sampler equipped with 10-litre Niskin bottles.

Samples of CDOM, chlorophyll *a*, nutrients and phytoplankton at the surface and at 10 m depth were collected at all stations.

To measure CDOM absorption, water samples were filtered through 0.2 μm Nucleopore membrane filters, then stored in the dark under refrigeration (4 °C to 8 °C) and analysed on board within 24 hours using a Perkin Elmer

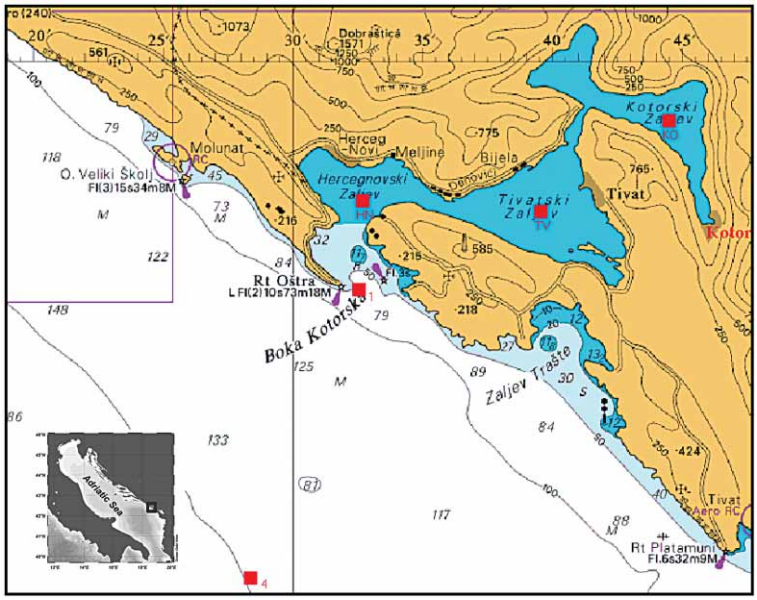

Figure 1. The study area and the sampling stations.

spectrophotometer 550A model (10 cm cuvette pathlength). Absorbance data were converted to absorption coefficient (a_{CDOM}) according to Mitchell et al. (2003):

$$a_{CDOM}(\lambda) = (2.303/l)[AB_s(\lambda) - AB_{bs}(\lambda) - AB_{null}(\lambda)]$$

where l is the cuvette pathlength, $AB_s(\lambda)$ is the optical density of the filtrate sample relative to purified water, $AB_{bs}(\lambda)$ is optical density of a purified water blank treated like a sample relative to purified water, and $AB_{null}(\lambda)$ is the apparent residual optical density at a long visible or near infrared wavelength where absorption by dissolved material is assumed to be zero.

Chlorophyll *a* was measured by filtering 3l samples through 47 mm GF/F filters and immediately extracted with 5 ml of acetone at –22 °C. The analyses were carried out at the ISMAR-CNR laboratory with a Dionex HPLC equipped with a GP50 gradient pump, a PDA100 Photodiode Array Detector (wavelength range: 190–800 nm), a C18 reversed phase column (4.6 mm × 250 mm, 5 μm particle size), an AS50 Autosampler and a 300 μl sample injection loop. Pigment concentrations were determined employing a modification of the procedure developed by Wrighit et al. (1991).

Nutrient samples were filtered (GF/F Whatman), stored at –20 °C in polyethylene vials and analysed at the ISMAR-CNR laboratory of Ancona. The nutrients (nitrate-NO_3, orthophosphate-PO_4 and orthosilicic acid-$Si(OH)_4$) were

analysed with a Bran+Luebbe Autoanalyzer QUAATRO system, and the resulting data processed with the AACE 6.0 (Automated Analyzer Control and Evaluation) software. Nutrient concentrations were determined applying a modification of procedures developed by Strickland and Parsons (1972).

Micro and nanophytoplankton samples (250 ml) were fixed with $Ca(HCO_3)_2$ buffered formaldehyde (4 % final concentration). Samples were processed using sedimentation chambers according to Utermöhl (1958) (Zingone et al., 1990) and observed with a light inverted microscope (Leitz, mod. Labovert) at 320 magnifications to determine and count all the cells belonging to both fractions.

3. Results and discussion

3.1. Physical and chemical oceanographic properties

Figure 2 and 3 show profiles of temperature, salinity, dissolved oxygen and fluorescence concentrations for the four stations inside the Boka Kotorska Bay (KO, TV, HN and 1) and for station 4 in the open sea during the two cruises.

In May, station KO showed a higher temperature (18 °C) and a lower water salinity (28.5) in the first five metres compared to the other stations inside the bay. Despite being influenced by river runoff as all other stations in the bay, station KO was the station furthest one from the open sea and this most probably influenced mixing processes along the water column because of poor open sea exchange.

The fluorescence and oxygen concentrations were even more different between station KO and the other stations. At stations TV, HN and 1 oxygen saturation and fluorescence concentration profiles were virtually the same: fluorescence was homogeneous in the entire water column (0.7–0.8 A.U.) with a slight increase on the bottom layer (0.9–1 A.U.), whilst oxygen saturation showed an opposite pattern by decreasing close to the bottom.

KO station was the only one that showed high values of fluorescence at the surface (1.4 A.U.) in correspondence to the maximum of oxygen (101 %), and in the middle layer (1.2 A.U. at 22 m depth), coupled with minimum oxygen values (89.4 % at 25 m depth). The minimum of oxygen saturation below the layer of maximum fluorescence indicated a probable predominance of mineralization processes over primary production processes and the absence of substantial vertical mixing.

Station 4 waters had the same characteristics of the Adriatic coastal waters. Salinity was approximately constant from the surface to the bottom with a slight increase along the water column (from 38 to 38.8); the surface layer began warming as expected for this period (Russo and Artegiani, 1996; Artegiani et al., 1997a; 1997b); fluorescence and oxygen profiles showed the maxi-

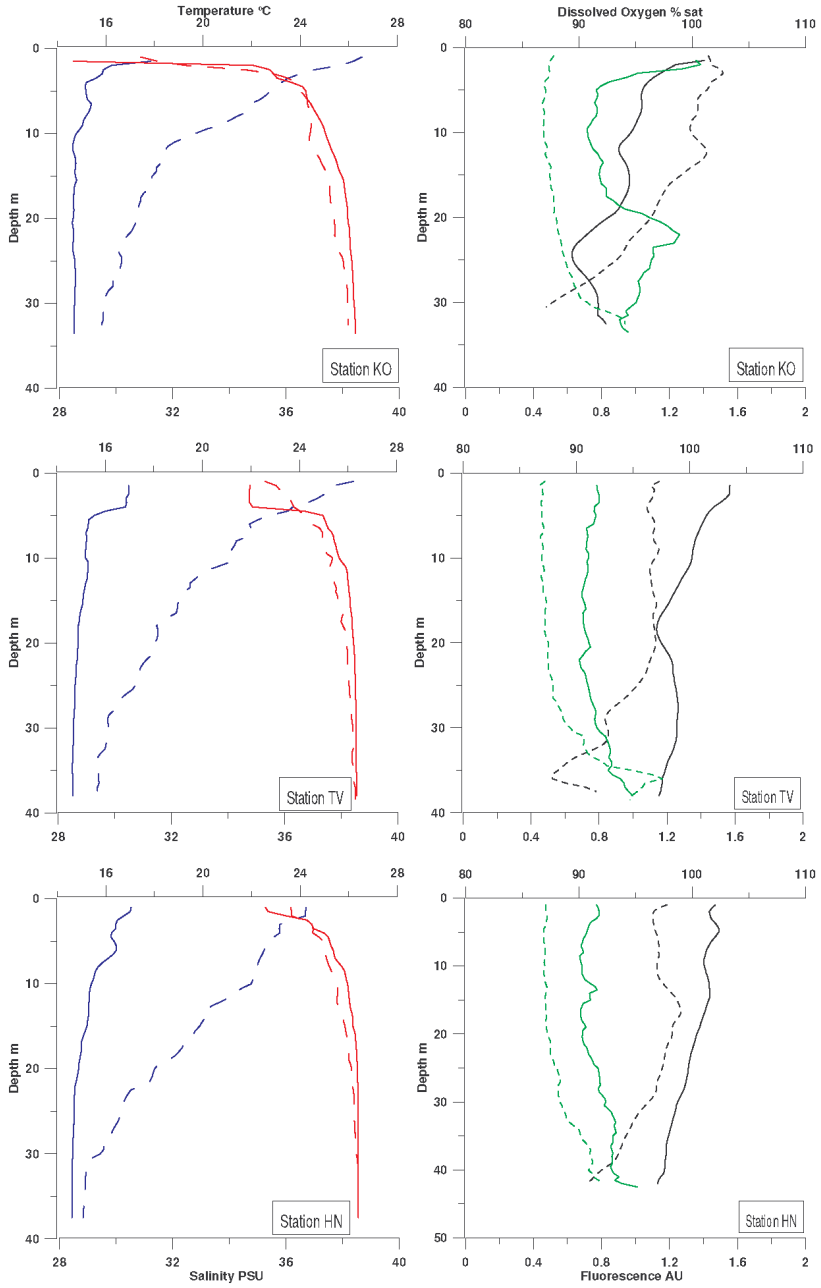

Figure 2. Profiles of temperature (blue line), salinity (red line), dissolved oxygen (black line) and fluorescence (green line) at the stations KO, TV, HN during May (solid line) and June (dotted line) 2008.

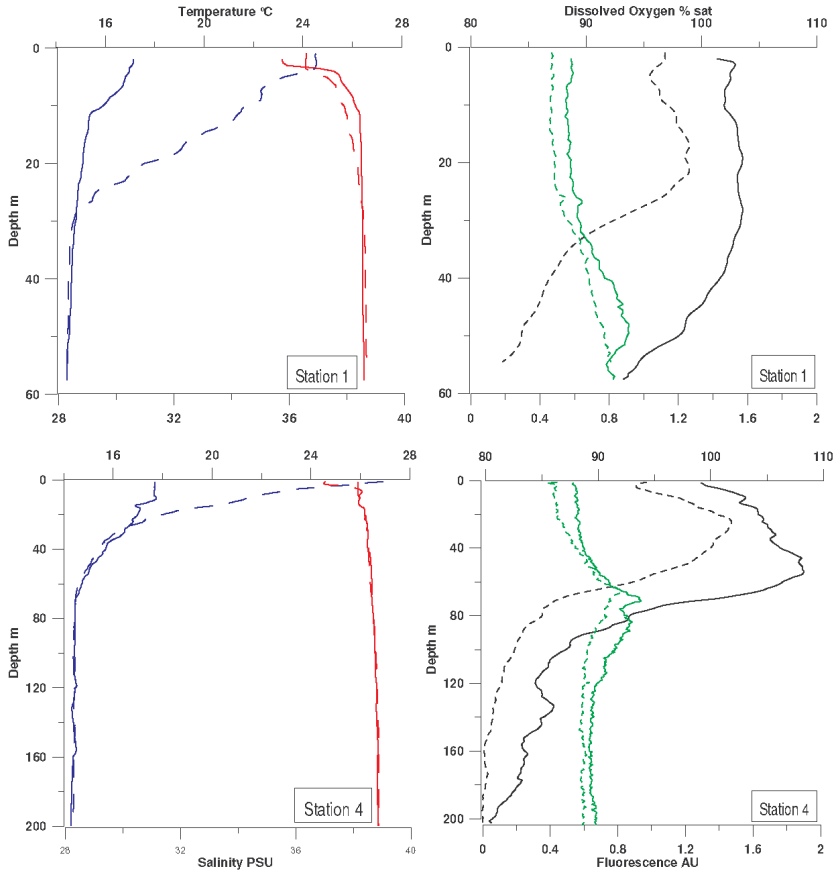

Figure 3. Profiles of temperature (blue line), salinity (red line), dissolved oxygen (black line) and fluorescence (green line) in the stations 1 and 4 during May (solid line) and June (dotted line) 2008.

mum values in the middle deep layer (50–80 m) where light conditions were good for primary production (Socal et al., 1999, Zavatarelli et al., 1998).

In June, temperature increased in all stations in particular at the 30 m depth layer. This increase with respect to the previous measurements was around 7 °C (at stations HN and 1) and 9 °C (at stations TV and 4). Salinity increased at the surface by 1–2 units at all the stations inside the bay, probably due to poorer river runoff present in June.

Station 4 showed an opposite trend with a surface decrease by 1 unit and this is due to the East Adriatic Current that transports the freshwater input from the coastal southernmost rivers (i. e. Bojana – Buna) northwards (Artegiani et al., 1997).

Oxygen saturation values were lower (by 5–10 %) in June compared to May because during the summer period vertical mixing is generally reduced (Olsen et al., 1998).

In June as well, station KO showed some differences with respect to the other stations. Oxygen saturation increased by 7–8 % compared to May. This increment was coupled with a decrease in fluorescence concentration indicating the increase in oxygen saturation may not have been due to primary production.

Major changes occurred in terms of fluorescence and oxygen saturation at stations KO, TV and HN in June compared to May and, in particular, for station KO which, once again, was very different from other stations.

3.2. Biological properties, nutrients and coloured dissolved organic matter (CDOM)

The distribution of CDOM, nutrients, chlorophyll *a* and phytoplankton concentrations (phytoflagellates, diatoms, dinoflagellates and others nanoflagellates) at the five monitored stations in the two periods is summarized in Table 1.

The phytoplankton community was dominated by diatoms (Chaetoceros decipiens, C. socialis and Pseudo.nitzschia spp.), nanoflagellates, both autotrophic and heterotrophic, and dinoflagellates. The latter had lower abundances, but high diversity, being characterized by species such as Ceratium tripos, Dinophysis caudata, D. fortii and Prorocentrum micansi, as previously documented by Vuksanović and Krivokapić (2005) in Boka Kotorska Bay.

The nutrient and phytoplankton concentrations were distributed differently at station KO compared to the others. In particular, orthosilicate concentrations and diatom abundances showed different patterns. In May, the orthosilicate concentrations were about 5 μmol l^{-1} at station KO coupled with a diatom abundance of about 3000–3500 cell l^{-1}. In June an increase in the cell number of diatoms at 10 m depth (9040 cells l^{-1}) and a reduction in orthosilicate concentration (down to 0.5 μmol l^{-1}) were observed. At the other stations inside the bay and in open sea the opposite relationships were observed. In fact, orthosilicate concentrations increased by about 1 μmol l^{-1} with respect to May and diatom concentration was reduced drastically (e.g. from 3900 cells l^{-1} to 380 cells l^{-1} at the surface of station TV). In June station KO thus appeared to have better physical and biochemical condition for diatoms growth.

Nitrate and orthophosphate concentrations increased from May to June in almost all stations; this was coupled with an increase of phytoflagellate populations and a decrease of dinoflagellates. With the exception of station KO, the total values of phytoplanktonic populations were observed to have maintained similar abundances between the two measurement periods. In addition, the chlorophyll *a* concentrations were not significantly different between May and June in most station and the values ranged between 0.06 and 3.13 μg l^{-1}. At 10

Table 1. Temperature (T °C), salinity (S), nitrate (NO_3 μmol l^{-1}), orthosilicate ($Si(OH)_4$ μmol l^{-1}), orthophosphate (PO_4 μmol l^{-1}), CDOM absorbance at 440 nm (a_{CDOM} 440 m^{-1}), chlorophyll a (chl a μg l^{-1}), phytoflagellates (cell l^{-1}), diatoms (cell l^{-1}), dinoflagellates (cell l^{-1}) and other spp (cell l^{-1}) at the five sampling stations (KO, TV, HN, 1 and 2) during May and June 2008.

		St. KO		St. TV		St. HN		St. 1		St. 4	
		May	June	May	June	May	June	May	June	May	June
T °C	Surface	17.83	26.55	16.96	26.21	17.04	24.25	17.13	24.50	17.69	26.90
	10 m	14.70	20.70	15.19	21.21	15.40	22.05	16.03	22.13	17.65	21.58
	Bottom	14.69	15.87	14.65	15.68	14.59	14.80	14.44	14.49	14.30	14.38
S	Surface	28.49	30.86	34.77	35.30	35.27	36.17	35.76	36.59	38.15	37.01
	10 m	37.44	36.90	37.92	37.39	38.17	37.71	38.25	37.80	38.17	38.18
	Bottom	38.45	38.18	38.53	38.49	38.55	38.58	38.59	38.67	38.85	38.89
NO_3 μM	Surface	0.86	0.63	0.02	0.50	0.02	0.10	0.02	1.11	0.46	0.73
	10 m	0.02	0.13	0.02	0.39	0.02	1.63	0.02	0.55	0.81	3.17
	Bottom	0.35	0.02	0.02	0.20	0.02	1.69	1.29	3.24	6.71	3.48
$Si(OH)_4$ μM	Surface	4.73	1.49	1.64	1.75	0.93	1.88	1.16	2.13	1.13	1.63
	10 m	5.17	0.54	0.91	1.37	0.52	1.58	0.81	1.55	1.12	2.96
	Bottom	2.43	3.36	1.04	2.64	1.22	1.74	1.59	4.05	5.40	4.81
PO_4 μM	Surface	0.03	0.32	0.04	0.19	0.02	0.03	0.03	0.03	0.04	0.12
	10 m	0.05	0.09	0.05	0.04	0.03	0.16	0.03	0.04	0.02	0.09
	Bottom	0.20	0.16	0.03	0.07	0.03	0.04	0.02	0.03	0.15	0.35
a_{CDOM} 440 m^{-1}	Surface	0.18	0.99	1.01	1.13	1.33	1.31	0.99	0.55	—-	0.87
	10 m	0.90	2.19	0.94	1.29	0.11	0.87	0.41	1.10	—-	0.64
Chl *a* μg l^{-1}	Surface	0.50	0.23	0.49	0.69	0.47	0.54	0.36	0.09	—-	0.44
	10 m	0.17	3.13	0.06	0.21	0.07	0.08	1.96	0.16	—-	0.51
Phytoflagellates Cell l^{-1}	Surface	1000	1200	1880	2600	1000	2800	1000	1600	800	2200
	10 m	160	2600	2200	2400	1800	2800	1600	2000	1400*	2400*
Diatoms cell l^{-1}	Surface	2980	160	3900	380	1120	400	1880	640	1260	180
	10 m	3515	9040	100	380	200	380	1340	40	500*	100*
Dinoflagellates Cell l^{-1}	Surface	1400	120	680	300	500	100	300	180	380	260
	10 m	280	220	420	40	300	140	100	0	540*	0*
Others spp. Cell l^{-1}	Surface	0	0	160	160	0	0	0	20	1220	20
	10 m	20	0	720	1000	220	0	40	880	1620*	1060*

* 50 m depth

m depth the maximum value (3.13 μg l^{-1}) was observed in June at station KO and this was coupled with high diatom concentrations.

Absorbance of coloured dissolved organic matter (CDOM) at 440 nm ranged between 0.11 and 2.19 m^{-1}.

In May higher values of CDOM were measured at the surface compared to 10 m depth. This feature was probably due to the input organic matter into the bay consequent of the major freshwater runoff, as shown by surface salinity. In fact, rivers are the primary source of CDOM (mainly soil-derived) and the groundwater near the coastlines, but coastal waters may also contain plankton-derived CDOM which is produced in rivers and estuaries as well contained in man-made compounds (Coble, 2007). A particular feature was observed at station KO where surface absorbance was low (0.18 m^{-1}) and it may be that the different physical conditions observed at this station improved the sink process of CDOM in terms of photobleaching (Mopper and Kieber, 2000) and microbial decomposition (Moran et al., 2000; Boyd and Osburn, 2004). Destruction of CDOM by exposure to sunlight releases compounds used for organism growth (Miller and Moran, 1997); amongst these is nitrate that, in fact, was found to be higher (0.86 μM) at station KO than elsewhere (Mopper and Kieber, 2002).

Compared to May, June values of CDOM at 10 m depth increased and the CDOM values between the surface and the 10 m depths were found to be more homogenous. In station KO a high CDOM absorbance (2.19 m^{-1}, 10 m depth) was observed and this was coupled with high chlorophyll *a* and diatom abundances and low nutrient concentrations (chl *a* = 3.13 μg l^{-1}; $Si(OH)_4$ = 0.54 μM; NO_3 = 0.13 μM and PO_4 = 0.09 μM).

CDOM was correlated with salinity and chlorophyll *a* to try to understand its primary source. Whilst salinity and CDOM did not show a significant relationship in the two periods as is often observed in coastal areas, on the other hand there was a significant positive relationship between CDOM and chlorophyll *a* ($R = 0.7$, $P < 0.001$, $n = 15$). These features support the hypothesis that biological production was the primary source of CDOM in this area. A review by Coble (2007) highlighted that, as demonstrated by numerous field studies, all the lower trophic groups (phytoplankton, grazers, viruses and bacteria) are involved in the production of CDOM, and in many locations a positive correlation has been found between CDOM and chlorophyll *a*. In this region phytoplankton should appears to be the primary source of CDOM, instead of terrestrial input.

4. Conclusion

Considering the paucity of published literature regarding Boka Kotorska, this current work contributes towards the improvement of our knowledge of a previously unknown area.

The Boka Kotorska Bay, from these preliminary data, appeared to be characterized in spring by a lower salinity surface layer and a phytoplankton community dominated by phytoflagellates and diatoms, with lower dinoflagellate abundance, and the near absence of other species. The open sea station showed similar phytoflagellate, diatom and dinoflagellate abundances but also higher abundances of other species probably due to different physical characteristics (i.e. higher salinity). Nutrient and chlorophyll *a* concentrations were not so different between the bay and the open sea. This feature may imply that the different phytoplankton populations found were attributable to different physical conditions, such as salinity, rather than to differences in chemical characteristics.

In both periods station KO, located furthest from the open sea, presented different physical and chemical characteristics when compared to the other stations inside the Boka Kotorska Bay. This was due to the Karst river inputs and the lower water exchange with the open sea. These features may enhance phytoplankton growth during some periods, such as in June for diatoms.

The positive correlation found between CDOM and chlorophyll *a* implies that in this area, similarly to the open sea, the biological production from phytoplankton appears to be the primary source of CDOM. This feature is very interesting and supports the hypothesis that the terrestrial inputs in the bay were not so rich in organic matter, probably due to the fact that the rivers entering the Boka Kotorska Bay are not severely impacted by man.

These preliminary results are important towards the evaluation of the role of the chemical, physical and biological parameters with respect to anthropogenic impact in order to characterise the overall hydrological properties of the entire Bay.

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SAŽETAK

Prostorna raspodjela fizikalnih, kemijskih i bioloških oceanografskih karakteristika, fitoplanktona, hranjivih tvari i organske materije (CDOM) u Bokokotorskom zaljevu na Jadranu

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U Bokokotorskom zaljevu su mjerene vremenske promjene temperature, saliniteta, fluorescencije, koncentracije otopljenog kisika, obojene otopljene organske materije (CDOM) i kemijskih (hranjive soli, klorofil *a*) i bioloških (sastav fitoplanktona) parametara tijekom dva razdoblja (svibanj i lipanj 2008. godine). CDOM određuje prodiranje UV svjetlosnih zraka u more i stoga igra vrlo važnu ulogu u mnogim hidrološkim i biogeokemijskim procesima u površinskom sloju mora koji uključuje primarnu produkciju.

Unutar ADRICOSM-STAR projekta, bilo je moguće istražiti Bokokotorski zaljev tijekom svibnja i lipnja 2008. godine radi povećanja razumijevanja optičkih i kemijskih karakteristika i njihovog razvoja kroz ova razdoblja. Zbog dotoka krških rijeka i smanjenja razmjene s otvorenim morem, u oba razdoblja postaja KO (smještena najdalje od otvorenog mora) je pokazala različite fizikalne, kemijske i biološke karakteristike u odnosu na postaje unutar Bokokotorskog zaljeva.

Pronađena je pozitivna korelacija između CDOM i klorofila *a* ($R = 0.7$, $P < 0.001$, $n = 15$) što upućuje na to da bi u ovom području, slično otvorenom moru, primarni izvor CDOM trebao biti biološka produkcija od fitoplanktona. To je vjerojatno zbog toga što dotoci rijeka u Bokokotorskom zaljevu nisu ozbiljnije ugroženi ljudskim djelovanjem.

Ključne riječi: CDOM, hranjive tvari, hidrološki podaci, fitoplanktoni, Jadran.

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