SUBSURFACE CHLOROPHYLL a MAXIMA IN THE BOKA KOTORSKA BAY

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ABSTRACT
Subsurface chlorophyll maximum (SCM) are common phenomena in variety of environmental, from fresh water, brackish water, estuaries, shelf areas, the coastal zone to open ocean. It is now known that the chlorophyll maximum at the subsurface layer occurs widely in water where the water column is stratified. In permanent stratified tropical water, therefore, the SCM is expected to be a year round phenomenon. In higher latitudes, where there are occasional vertical mixing of the water column due to seasonal surface cooling or strong winds, the SCM will be restricted during a particular period of the year, such as in the summer or between spring and autumn. The Boka Kotorska Bay is semi-enclosed basin, located in the south-easter Adriatic Sea (Mediterranean Sea). The weekly changes in chlorophyll a and physico-chemical parameters were investigated in the period September 2008 to March 2009. Maximum phytoplankton biomass (11.13 mg m⁻³ chlorophyll a) was observed on 2m depth in February nearly the pycnocline. The good correspondence of SCM with occurrence of nitrate clearly indicates that the phytoplankton that make up the SCM depended highly on nitrate, which is supplied mainly by diffusional process from depth below the SCM.

Keywords: subsurface chlorophyll a maximum, nutrients, Adriatic Sea

Introduction
In the water column primary producer are generally not homogeneously distributed with depth but they often form a layer of maximum concentration. Chlorophyll accumulation at subsurface depths has been recognized in several vertical profiles of chlorophyll at various locations in the ocean (7). The subsurface chlorophyll maximum (SCM) commonly occurs at depths between 30 and 100 m (9), in the subtropical regions, this maximum occur between 60 and 120 m (1), while in the temperate regions and shelf seas the SCM occurs only seasonally and at more shallow depths (6). The subsurface maximum concentration of chlorophyll in the Adriatic Sea is often present in the depths between 50 and 75 m (3). The subsurface maximum concentration of chlorophyll (a) may become: 1. with sinking cells, 2. with movements of cells i.e. floating on appropriate depth, 3.with differential predation, 4. with rapid growth of population in the layer where there is the optimal balance of nutrients concentration and intensity of light. Accumulation of pigments is often a maximum along the bottom surface, mixing layer and just above nitracline (10). In the Mediterranean Sea have been found three types of chlorophyll distribution and in water column (12). The underling mechanisms that lead to the formation of SCM’s are understood (4, 6): two opposing resource gradients (temperature, nutrients) combined with a vertically heterogeneous turbulent mixing render only a certain region of the water column amenable for survival, but our understanding is limited if we attempt to explain the underlying mechanisms which determine the species composition within SCM, the relative dominance of a particular phytoplankton group or the transition from one dominant group to another.

The aim of this paper was to determine whether there is SCM in Kotor Bay which is the most jagged Bay in eastern Adriatic coast with greatly influenced by great influx of fresh water from numerous water streams and submarine springs.

Materials and Methods
Description study area
The coast line of Montenegro is 292.5 km long and located in the SE part of the Adriatic Sea, bordering the Croatian and Albanian coast. The Boka Kotorska Bay occupies an area of 87.3 km², a volume of 2.4 x 10⁶ m³, and coastline length of 105.7 km. The Boka Kotorska Bay is the most jagged coastline of the Dinar's seashore. It consists of four small bays. All four bays are attached and connected to each other.
(Herceg Novi Bay, Tivat Bay, Risan Bay and Kotor Bay). The Boka Kotorska Bay also has two areas. The first one connects open sea with Herceg Novi Bay and the second one connects Tivat Bay and Risan Bay with Kotor Bay.

The inner part of Kotor Bay comprised 27% of area and 26% of volume of the whole Bay. There are visible and strong streams, strong springs and underground spring waters in the Boka Kotorska Bay. They are mostly concentrated in the inside part of the Boka Kotorska Bay and less present in the outside part of the Bay. In the inside part of the Bay (that is Kotor Bay and Risan Bay) they are present during the whole coast line and they are powered with water from Lovcen and Orijen massif. These massifs in winter months cause cooling and ice appearance on surface.

Boka Kotorska is also area of high annual precipitation with point of the highest precipitation (4584 mm per year) in Europe on nearly Crkvice. Small rivers, streams and underground spring waters are seasonal in those massifs due to presents of underground lakes. Their activity starts in the late fall and sometimes little earlier than fall and is effected by atmospheric conditions. Ljuta's and Sopot's activities are maximal in the winter or early spring and by the late April most of them dry out. On the other hand Gurdic's activity in Kotor Bay and some streams and springs activities in Risan Bay are active during the whole year with less capacity in the summer.

**Sample collection and data analyses**

Seawater samples were taken with a HydroBios sampler, at one station in the inner part of the Boka Kotorska Bay (Fig. 1). The sampling was weekly from September 2008. to March 2009.

![Fig. 1. Location of the sampling station](image)

Samples for physical, chemical and biological analyses were taken from five depth: 0, 2, 5, 10 and 15 m. Temperature and salinity were measured *in situ* by multi LINE P4 - UNIVERSAL METER. Oxi-Guard handy GAMMA was used for measured 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 needs to be done the same day after sampling, or nutrients freeze. Nitrates and phosphates were determined at sea by using standard methods (8). The absorbance reading was made on Perkin Elmer UV/VIS spectrophotometer (Lambda 2), on 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. (2). Grapher 7.0 and Statistica 7 were used for data analysis and statistical calculations. One-way ANOVA and post hoc Duncan tests in Statistica 7.0 were used to test the significance of physico–chemical parameters and chlorophyll *a*.

**Results and Discussion**

Temperature followed year solar cycles with minimum 8.6°C in December on surface and maximum 23.9°C in September on 2m depth in investigated period (Table 1).
TABLE 1

Average (avg), minimum (min), maximum (max), standard deviation (SD), and number of samples (n) for physicochemical and biological parameters in the Boka Kotorska Bay

<table>
<thead>
<tr>
<th>Parameter</th>
<th>avg</th>
<th>min</th>
<th>max</th>
<th>SD</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temp. (°C)</td>
<td>16.53</td>
<td>8.60</td>
<td>23.90</td>
<td>3.63</td>
<td>93</td>
</tr>
<tr>
<td>Sal. (%)</td>
<td>29.06</td>
<td>3.00</td>
<td>36.40</td>
<td>8.77</td>
<td>93</td>
</tr>
<tr>
<td>Con. O₂ (mg L⁻¹)</td>
<td>7.53</td>
<td>6.30</td>
<td>9.60</td>
<td>0.57</td>
<td>93</td>
</tr>
<tr>
<td>NO₃⁻ (μmol L⁻¹)</td>
<td>6.46</td>
<td>0.00</td>
<td>23.91</td>
<td>6.15</td>
<td>93</td>
</tr>
<tr>
<td>PO₄³⁻ (μmol L⁻¹)</td>
<td>0.06</td>
<td>0.00</td>
<td>0.35</td>
<td>0.10</td>
<td>93</td>
</tr>
<tr>
<td>Transp. (m)</td>
<td>7.00</td>
<td>5.50</td>
<td>10.00</td>
<td>1.33</td>
<td>19</td>
</tr>
<tr>
<td>Chl a (mg m⁻³)</td>
<td>2.09</td>
<td>0.12</td>
<td>11.13</td>
<td>1.66</td>
<td>93</td>
</tr>
</tbody>
</table>

A statistically significant difference was found between the mean monthly values of temperature. Post hoch Duncan test did not show significant statistical differences between maximum average monthly values of temperatures (Fig. 2a) in the first week of September (20.5 °C) and values by the end of October (22.1-19.6 °C), as well as from the end of November (14.3°C) until mid March (12.4°C). The highest temperature changes in water column are recorded in December and isothermia appears in February (Fig. 2a). Post hoch Duncan test has shown statistical significant difference between average values of temperature on surface (14.3°C) and other depth (16-9-17.1°C). Extreme low salinity of 3 PSU on surface was measured in December and maximum was 36.4 PSU on 0.5 m in September. There is not significant difference in Post hoch Duncan test between average salinity values, but test confirmed statistical significant differences between average values on the surface (15.9 PSU) and other depths (29.5-33.6 PSU ). Halocline usually appears around 2 m or sometimes 5 m (Fig. 2 b). The thermocline and halocline divided water column on cooler and warmer layer and thus hinder mixing nutrients in upper layer. Oxygen concentration ranged from 6.3 mg L⁻¹ in February on 15m depth and 9.6 mg L⁻¹ on surface in November (Fig. 2c). The maximum average value of oxygen concentration was measured in November (8.3 mg L⁻¹) and it is statistically different from other average values during test period (7-7.7 mg L⁻¹). With the depths values of oxygen concentration decreased and Duncan test has shown statistical significant differences between the average value on the surface, 2 m and 5 m (7.8-7.5 mg L⁻¹) and depths of 10 and 15 m (7.3-7.1 mg L⁻¹).

The minimal value for nitrate concentration was under detection in October on 2m depth. Maximal value is recorded in September 23.9 μmol L⁻¹ where is also presented high concentration in every depth in that period (Fig. 3a). During research period, maximum secondary values measured during the first two frequency in September (21.65 - 14.09 μmol L⁻¹) do not show statistical significant differences with values in the last two weeks in December (16.01 - 12.3 μmol L⁻¹) , while there is statistical significant differences with all other average values concentration of nitrate (0.47-8.6 μmol L⁻¹). With the depths, values of nitrate concentration increased and Duncan test has shown statistical significant differences of average values of nitrate on the surface 4.5 μmol L⁻¹ and other depths (6.7-7.3 μmol L⁻¹). Phosphate concentration (Fig. 3b) ranged from minimum 0.01 μmol L⁻¹ which appeared several times in September to maximum 0,34 μmol L⁻¹ in February. Average values of phosphate during the first week in September, the first week in December, last week in January and early March (0.29 μmol L⁻¹ - 0.13 μmol L⁻¹) are statistical different from others. With the depths increased values of phosphate and Duncan test has shown statistical significant differences of average values of phosphate on the surface (0.09 μmol L⁻¹) and other depths (0-0.005μmol L⁻¹).

The transparency ranged between 5.5 m in January and 10 m in September (Fig. 4b). The minimal value of chlorophyll a concentration was 0.118 mg m⁻³ in November on surface and maximal value was 11.13 mg m⁻³ in February on 2m depth (Fig. 4a).

Maximum average value of chlorophyll a 4.93 mg m⁻³ has been determined in the beginning of February and it is statistically significant different from all other average values during research period (1.09-3.8 mg m⁻³). Also, average value of chlorophyll a that was measured by the end of December (3.8 mg m⁻³) was statistically significant different from all other values during research period except in November (2.8 -2.1 mg m⁻³) and in early February (4.93 mg m⁻³). As well as in the case of value for oxygen concentration there is statistically significant differences between average values of chlorophyll a on the surface, 2 m and 5 m (2.9 - 2.3 mg m⁻³) and 10 and 15 m (1.4-1.2 mg m⁻³).

A subsurface Chlorophyll a maximum represented by total chlorophyll a concentration of 11.13 mg m⁻³ was observed on 2m depth in February, whereas no such high subsurface maximum were found in the investigated period. Moreover, it was observed at nearly the same depth pycnocline in February.
The good correspondence of SCM with occurrence of nitrate clearly indicates that the phytoplankton that makes up the SCM depended highly on nitrate, which is supplied mainly by diffusional processes from depth below the SCM. The SCM depth is probably determined by a possible balancing mechanism between three processes: nutrient supply from the deep water, light penetration from the surface and the growth rate of phytoplankton (9). The phytoplankton community was considered to belong to the subsurface Chlorophyll a maximum. Large phytoplankton of > 10μm size accounted for about 80% of the total Chlorophyll a and primary productivity at the subsurface Chlorophyll a maximum.

Diatoms are reportedly the dominant large phytoplankton (11), and it can therefore be said that large phytoplankton, especially diatoms, significantly contribute to the subsurface Chlorophyll a maximum in the Kotor Bay. As one of the critical limited nutrients to phytoplankton growth, nitrate and phosphate distributions have opposite patterns to the phytoplankton growth. Nitrate is assumed as a nutrient only replenished by external sources while phosphate is a kind of recyclable nutrient. In the period when observed subsurface chlorophyll a maximum nitrate and phosphate have been almost depleted. High nutrient, ie nitrate concentrations are recorded in the same period when thermocline expressed, in December. As the phosphorus biggest limiting factor of the eastern Mediterranean, the only period when there is slightly greater its concentration is just before appearance of subsurface chlorophyll maxima. Relatively high salinity in the thermocline beneath the bloom area presumably indicates relatively high nutrient supply,
while low nitrate indicate relatively high nutrient utilization and productivity (5) what is recorded in February in Boka Kotorska Bay, too. Assuming that the difference is due to phytoplankton, these results suggest adaptation of the cells to low light by an increase in their chlorophyll content; the increase is well within the range of values reported from physiological studies of pigment concentration adaptation by algae.

![Chlorophyll and transparency changes](image)

**Fig. 4.** Temporal changes of chlorophyll $a$ (a) and transparency (b) from September 2008 to March 2009 in the Boka Kotorska Bay

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**REFERENCES**