CHLOROPHYLL *a* DISTRIBUTION IN THE ROMANIAN BLACK SEA INNER SHELF WATERS IN 2009

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Abstract. In 2009, seasonal and spatial variability of surface chlorophyll *a* in Romanian inner shelf waters was studied on the basis of samples collected during four seasonal cruises conducted both in the Danube mouths and Constanta areas. The seasonal distribution showed three peaks; the first one was recorded in February (6.22 ± 2.63 µg/l), followed by a more pronounced one in early May (9.47 ± 6.88 µg/l) corresponding to the Danube's higher discharges in spring. After a relatively low chlorophyll *a* in July, there was found the third peak in September (10.29 ± 8.18 µg/l) as a result of a strong diatoms bloom in the Portita area (south of the Danube mouths). Except for winter, the spatial distribution of surface chlorophyll *a* was characterized by high variability; the chlorophyll *a* concentrations measured in the Danube mouths area, especially in shallower stations along the Portita profile (> 20 µg/l in May and September), were much higher than those recorded on transect East-Constanta.

Key words: Black Sea, Danube's mouths, Chlorophyll *a*

1. INTRODUCTION

Chlorophyll *a* is a commonly used parameter for the estimation of phytoplankton biomass and primary production. Because of its presence in all phytoplankton cells and its faster and easier determination as compared to algal biomass and primary production, chlorophyll *a* was included in the list of indicators of etrophication within European Water Framework Directive and Marine Strategy Directive.

The first measurements of chlorophyll *a* in the Black Sea waters were made in 1961 (Finenko, 1965). Since then, many studies concerning spatial, seasonal and inter-annual changes in chlorophyll *a* and primary production levels have been carried out in different regions of the Black Sea (Vedernikov *et al.*, 1983; Yunev *et al.,* 1987; Vedernikov, 1989; Yunev, 1989, Krupatkina *et al.,* 1991; Berseneva, 1993; Vedernikov and Demidov, 1993, Yilmaz *et al.*, 1998, Bologa *et al.,* 1999, Demidov, 1999). In Romanian coastal waters, sporadic chlorophyll *a* measurements were performed since the 1970s (Bologa, 1977, 1978; Bologa *et al.*, 1985). More frequently, chlorophyll measurements were done after 1980, when this biological parameter started to be monitored within the framework of Romanian Monitoring Program (Mihnea, 1988, 1992) and other national or international projects in order to emphasize trends in development of phytoplankton biomass, as well as frequency and distribution of algal blooms.

In literature, there were described a wide range of analytical methods applied to the Black Sea, according to national and international monitoring guidelines. Since the 1990s, besides *in situ* measurements, chlorophyll *a* data sets have been obtained from satellite (CZCS and SeaWiFS) images. Although the observations of near-coastal chlorophyll with remote sensing satellites provide important information on potential relationships with climate and nutrient enrichment in the Black Sea, SeaWiFS seems to overestimate chlorophyll *a* concentrations by a factor of 4 in the Romanian shelf waters, as a result of the large optical complexity of this ecosystem (Oguz and Ediger, 2006; McQuatters-Gollop *et al.*, 2008). However, the comparison between satellite-derived and in-situ chlorophyll data is encouraging and suggests that satellite data provide a potential tool for chlorophyll monitoring in Romanian shelf waters (BSC, 2008).

The main aim of present paper was to describe seasonal and spatial distribution of surface chlorophyll *a* in relation to some environmental factors variability, based on data collected in 2009 in the Romanian inner shelf waters, within Romanian National Monitoring Program.

2. MATERIALS AND METHODS

During 2009, four seasonal cruises (February, May, July and September) were conducted onboard R/V *Steaua de Mare*, in Romanian inner shelf waters. There were collected 47 surface samples from 16 stations distributed along 4 transects: Sulina, Sf. Gheorghe, Portița and East-Constanța (Fig.1).

Temperature was measured using a reversing thermometer. Salinity, measured by the titrimetric method of Mohr-Knudsen (Grasshoff *et al.*, 1999) was done onboard. Nutrient samples were frozen and kept at -20ºC until their subsequent analysis in laboratory. PO_4-P, NO_3-N, NO_2-N, NH_4-N and SiO_4-Si were determined according to standard methods (Grasshoof *et al.*, 1999).

For chlorophyll *a* measurements, variables volumes (1-5 l) of seawater were filtered onboard through 0.7µm GF/F Whatman filters. The filters were preserved at -20ºC until subsequent analysis. In laboratory, the pigment on the filters was extracted with 90% acetone and measured spectrophotometrically (CECIL CE-2020 spectrophotometer), concentrations being calculated using the SCORE - UNESCO equations (UNESCO, 1966).

Phytoplankton samples were preserved with 4% formaldehyde seawater buffered solution. Qualitative and quantitative phytoplankton determinations were made according to standard methods (Morozova-Vodyanitskaya, 1948, 1954; Bodeanu, 1987-1988).

Physical-chemical and biological data were processed with the program Ocean Data View 4 (Schlitzer, 2009).

Statistical data processing was done using the program XLSTAT 7.5.2. Analysis of variance (ANOVA) and Fisher (LSD) test were used to examine seasonal chlorophyll *a* variation. Testing significance of differences between chlorophyll *a* means recorded in the Danube mouths and Constanta areas was performed by using t test (Student) at the significance level of 0.05. The same significance level was used for testing the significance of correlation between chlorophyll *a* and some variables.

3. RESULTS

Seasonal and spatial variability of surface chlorophyll *a* in Romanian inner shelf waters is examined in relation to the sea surface temperature, salinity and nutrients. In terms of spatial distribution of chlorophyll and others parameters we refer below, on the one hand, to Constanta area (transect East-Constanta) and, on the other hand, to the Danube mouth area (including the profiles Sulina, Sf.Gheorghe and Portita).

Chlorophyll a, total phytoplankton biomass and abundance

In 2009, surface chlorophyll *a* ranged between 0.13 and 26.57 µg/l in Romanian inner shelf waters.

The seasonal pattern of surface chlorophyll *a* distribution showed quite low variability; one-way ANOVA, followed by multiple comparison Fisher (LSD) test for the variable month emphasized a significant difference only between means recorded in summer and late summer/early fall (Table 1). However, surface chlorophyll *a* recorded relatively high values in February (6.22 ± 2.63 µg/l) and especially in early May (9.47 ± 6.88 µg/l) corresponding to the Danube's maximum flow. After a slight decrease recorded in July (5.52 ± 3.46 µg/l), chlorophyll *a* peaks in September (10.29 ± 8.18 µg/l) with maximum concentrations measured in the Portita area.

Table 1 Fisher (LSD) / Analysis of the differences between groups with a confidence range of 95.00 %

Categories	Difference	Standardized difference	Critical value	Pr. > Diff	Significant
Sep ~ July	4.769	2.025	2.018	0.049	Yes
Sep ~ Feb	4.065	1.496	2.018	0.142	No
Sep ~ May	0.814	0.340	2.018	0.736	No
May ~ July	3.955	1.756	2.018	0.086	No
May~ Feb	3.251	1.237	2.018	0.223	No
Feb ~ July	0.705	0.272	2.018	0.787	No

Sorting and grouping categories:

Categories	Means	Groupings	
September	10.287	A	
May	9.473	A	B
February	6.223	A	B
July	5.518		B

Total phytoplankton biomass and abundance in the surface layer varied between 409.13 and 9292.1 mg/m^3 and $165 \cdot 10^3$ and $16624 \cdot 10^3$ cell/l, respectively. Moreover, total phytoplankton biomass exhibited a quite strong linear relationship with chlorophyll *a* (Pearson, $r = 0.642$, $p < 0.05$, $n = 23$) during the whole period of study, showing similar seasonal dynamics. Thus, the average seasonal phytoplankton biomass emphasized two maximum values, in early May (4540.41 ± 2960.12 mg/m^3) and September (3892.2 ± 3011.8 mg/m^3).

Regarding the spatial distribution of surface chlorophyll *a* in the study area, Student's test ($t = -3.312$, $p = 0.001$, $t_{crit} = 2.024$) suggests significant differences between Constanta and the Danube mouths areas during the study period.

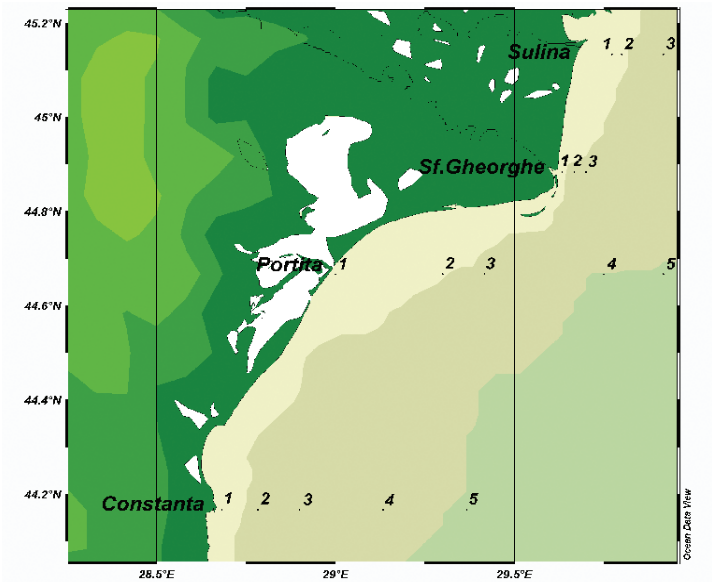

Figure 1 Location of sampling stations during seasonal cruises performed in Romanian inner shelf waters, in 2009.

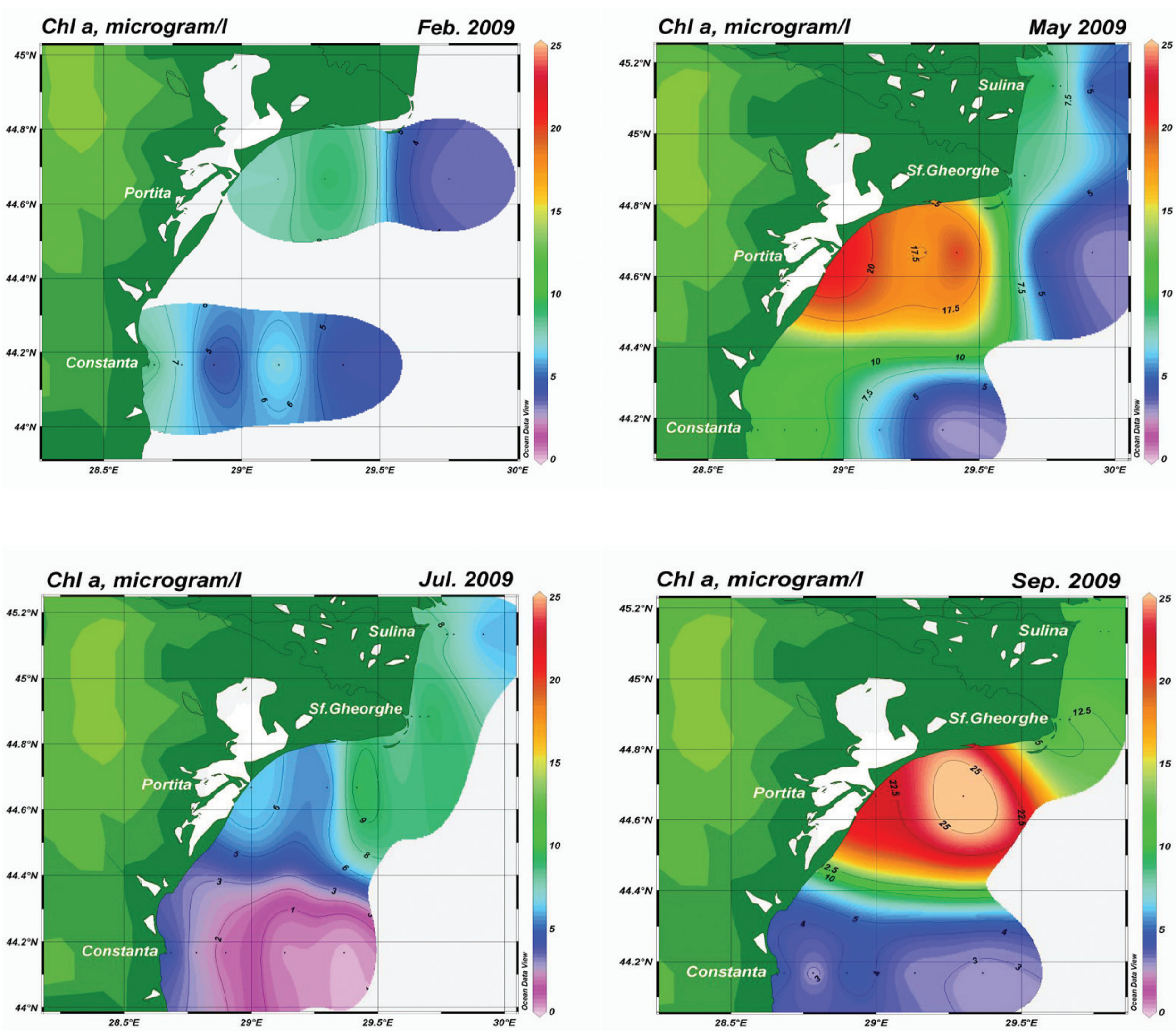

Figure 2 Spatial distribution of surface chlorophyll a in Romanian inner shelf waters, in 2009.

In February, in the absence of data from stations located on transects Sulina and Sf.Gheorghe, surface chlorophyll *a* distribution was quite homogeneous, ranging between 3.19 and 9.21 µg/l. The highest values were recorded in shallower stations both on East-Constanta (9.05 µg/l - East-Constanța-1) and Portita profile (9.21 µg/l - Portița-2) (Fig.2), where the maximum phytoplankton biomass (1809.98 mg/m^3 - Portița-2) was also found.

In early May, surface chlorophyll *a* values ranged between 2.45 and 21.92 µg/l. Student's test ($t = -0.587$, $p = 0.569$, $t_{crit} = 2.210$) showed no significant differences between Constanta and the Danube mouths areas. Higher concentrations were measured on East-Constanta and Portita profiles in stations located between the shoreline and 30 m isobath (Fig.2).

Higher total phytoplankton abundance and biomass, as well as their surface distribution suggested an intense algal bloom in early May (biomass ranged between 4048.6 and 9186.8 mg/m^3 and abundance between $6603 \cdot 10^3$ and $16624 \cdot 10^3$ cell/l) in shallower waters in Portita and Constanta areas (Fig.3).

In July, chlorophyll *a* values recorded significant reduction in the surface layer, varying between 0.13 and 9.94 µg/l, most likely due to increased grazing pressure. Thus, total phytoplankton biomass showed much lower values as compared to those recorded in spring, the regional mean reaching 767.21 ± 272.88 mg/m^3.

In terms of spatial distribution, the summer season was characterized by statistically significant differences (t test, $t = -5.177$, $p = 0.001$, $t_{crit} = 2.299$) between the chlorophyll *a* values recorded in the Danube mouths and Constanta areas; the highest values were measured in front of Sulina and Sf.Gheorghe mouths (Fig.2).

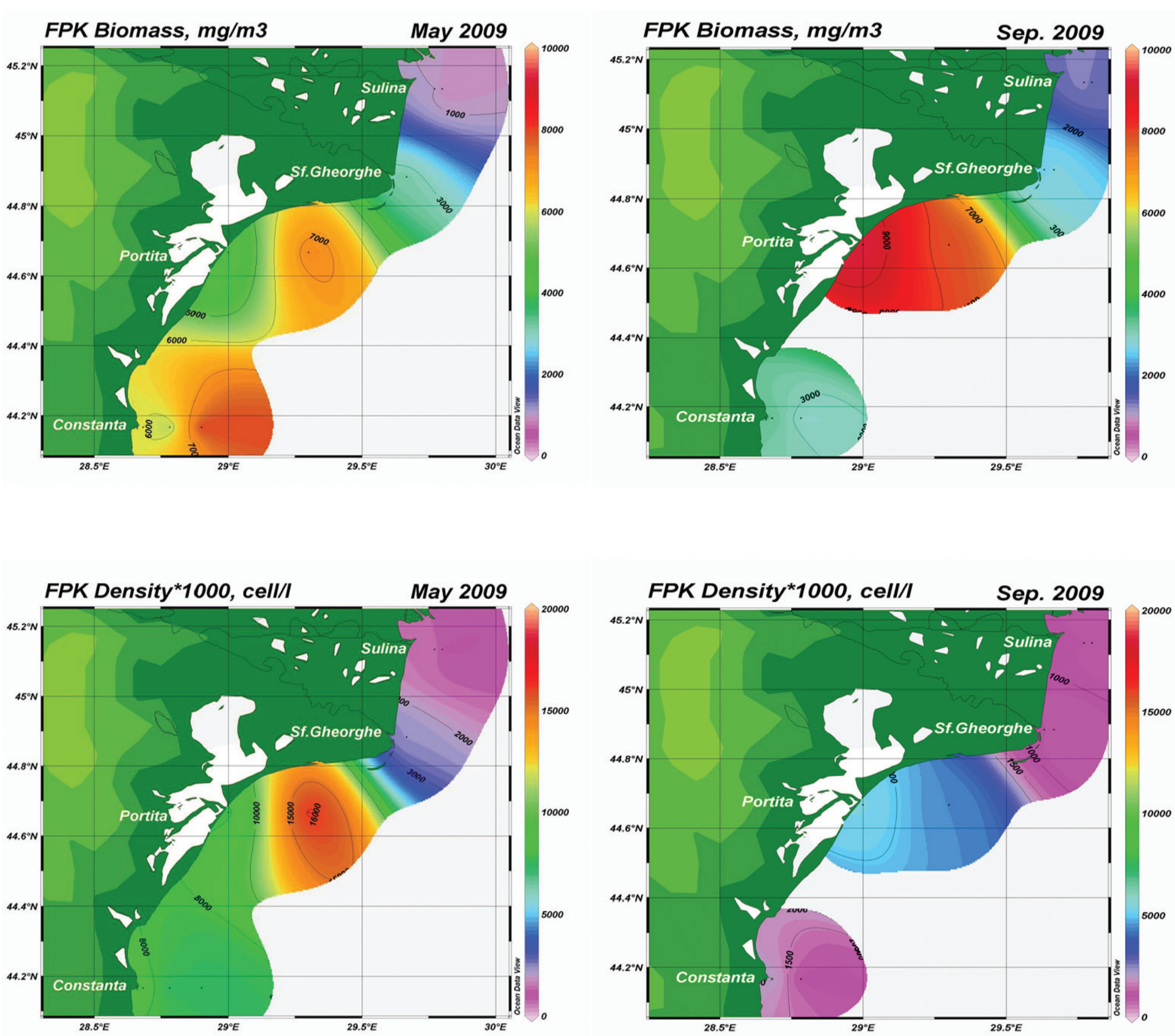

Figure 3 Spatial distribution of surface phytoplankton biomass and abundance in Romanian inner shelf waters, in 2009

In September, surface chlorophyll *a* concentrations varied between 1.06 and 26.57 µg/l. The spatial distribution of surface chlorophyll showed statistically significant differences (t test, t = -4.286, p = 0.005, t_{crit} = 2.460) between the Danube mouths and Constanta areas, much higher chlorophyll *a* concentrations being found in the Portita area (Portita-1 - 22.01 µg/l and Portita-2 - 26.57 µg/l) (Fig.2). Moreover, the highest total phytoplankton biomasses during the whole study period were recorded in this month, in shallower stations on Portita profile (9292.1 mg/m^3 at Portita-1 and 7860.2 mg/m^3 at Portita-2).

Sea surface temperature and salinity.

Sea surface temperature (SST) exhibited a predictable seasonal fluctuation with a minimum in February (3.8°C), a maximum in July (26.8°C) and no significant differences (t test, t = -1.351, p = 0.183, t_{crit} = 2.011) between Constanta and the Danube mouths areas during the study period.

Sea surface salinity fluctuated between 0.25 and 17.13 psu and exhibited large variability, both seasonal and spatial, according to seasonal variation of the Danube's flow and wind regime. The seasonal changes of salinity showed the lowest values (regional mean of 9.54 ± 2.95 psu) in early May and the highest ones in September (11.93 ± 5.41 psu).

In terms of spatial distribution, Student's test (t = 3.182, p = 0.003, t_{crit}= 2.011) reveals significant differences between values recorded on transect East-Constanta and in the Danube mouths area during the study period; the lowest salinities were found in the Danube area, more exactly at stations located just in front of Sulina mouth, in July and September (Fig.4).

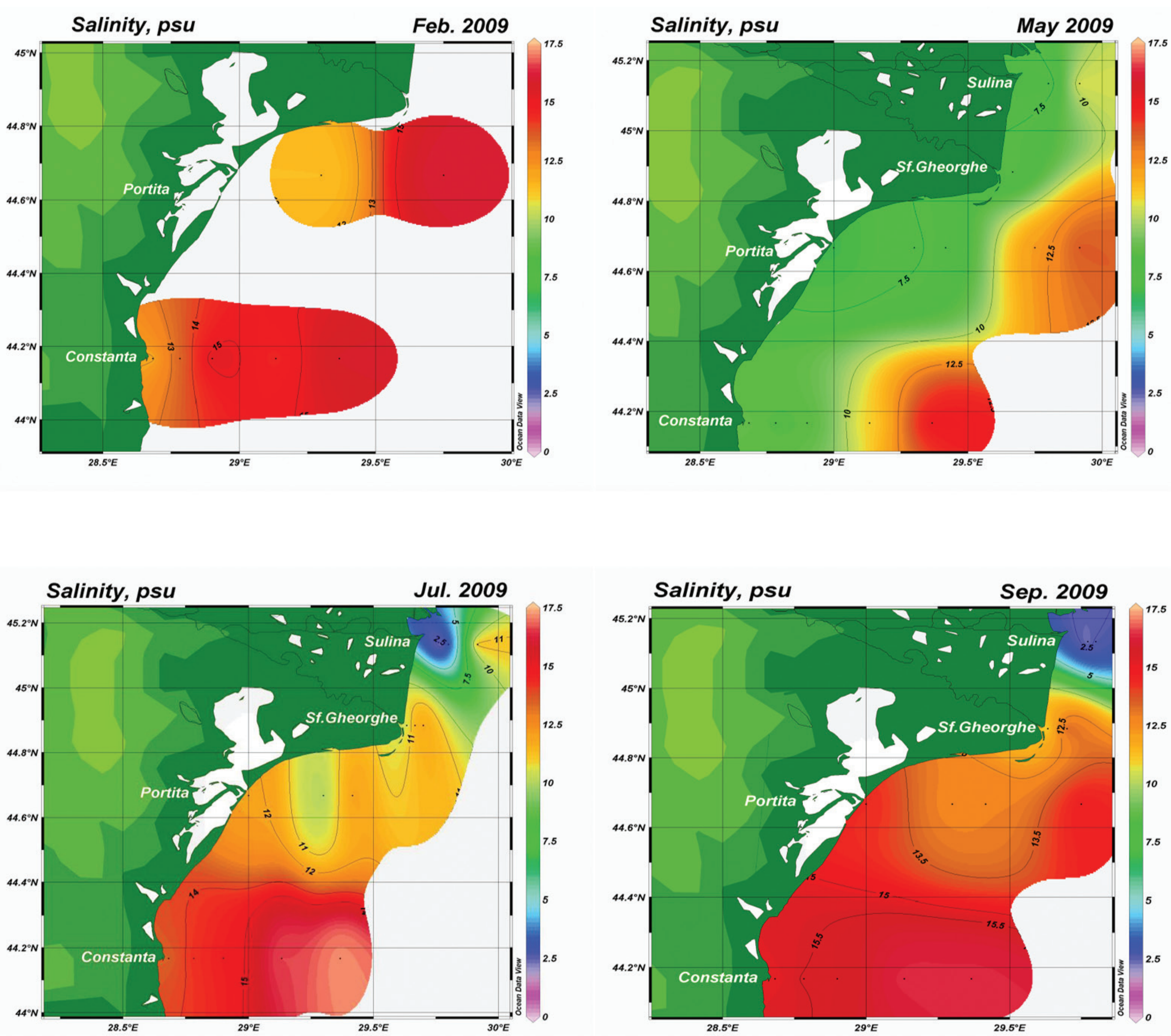

Figure 4 Spatial distribution of sea surface salinity in Romanian inner shelf waters, in 2009

Nutrients

PO_4-P ranged between values below detection limit and 4.0 µM. The seasonal variation of phosphate in the surface layer highlighted the lowest value in May (regional mean of 0.23 ± 0.07 µM) and the highest one in July (0.52 ± 1.03 µM). Student's test ($t = - 2.356$, $p = 0.025$, $t_{crit}= 2.039$) showed significant differences between surface PO_4-P concentrations recorded in the two areas during the study period, in spite of a relatively low variability in spring. The higher spatial variability was observed in the warm season, the highest surface PO_4-P was measured at stations located in front of the Danube mouths (Fig. 5).

SiO_4-Si concentrations were characterized by high seasonal variability; the highest regional mean in the study area was recorded in winter (16.46 ± 9.48 µM) and the lowest ones in May (8.20 ± 6.35 µM) and September (7.22 ± 11.06 µM). Also surface silicate showed high spatial variability during each season, especially in July (0.50 – 97.60 µM) and September (0.80 – 51.20 µM), the highest values being recorded at stations in front of Sulina mouth both in July and September (Fig.6).

NO_3-N in inner shelf waters ranged between 0.68 and 20.92 µM. The regional mean of nitrate concentrations in the surface layer recorded the highest values in February (9.54 ± 7.41 µM) and early May (9.13 ± 4.68 µM), when the nitrate was the main inorganic nitrogen form. During the warm period, nitrate concentrations decreased to values around 2 µM (regional means of 2.03 ± 0.89 µM in July and 2.04 ± 0.84 µM in September, respectively). Student's test ($t = 0.144$, $p = 0.886$, $t_{crit}= 2.011$) showed no significant differences between surface NO_3-N concentrations recorded in those two areas dur-

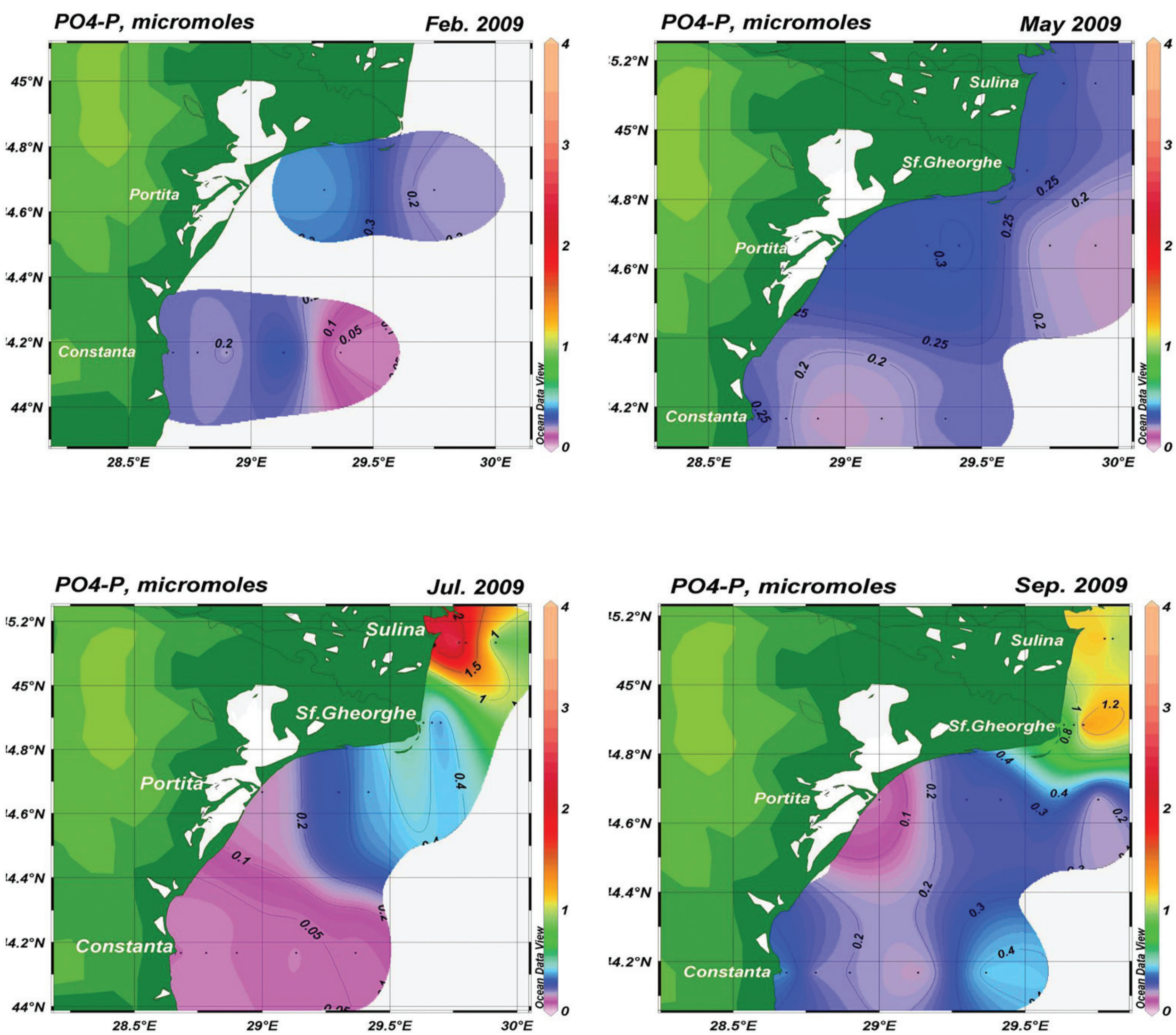

Figure 5 Spatial distribution of PO_4-P in Romanian inner shelf waters, in 2009

ing the study period. Slightly higher values were registered at shallower stations in the Danube mouths area (Fig. 7).

The seasonal distribution of ammonium showed lower concentrations in February (regional mean of 1.75 ± 1.19 µM), followed by increasing values since May (3.27 ± 2.29 µM) till summer/late summer (July 3.62 ± 3.85 µM and September 3.70 ± 3.77 µM, respectively), when ammonium represented up to 70-75 % of total inorganic nitrogen, both in the Danube mouths area and on transect East-Constanta. There were found no significant differences between surface ammonium concentrations recorded in those two areas (t test, t= -0.307, p = 0.76, t_{crit}= 2.011); the highest concentrations were measured during the warm season, at shallower stations from both the Danube mouths and Constanta areas.

4. DISCUSSION

The Romanian inner shelf area is a distinct ecosystem whose productivity is linked to both the Danube discharges and climatic processes through changes in temperature, wind patterns and the Danube's flow (McQuatters-Gollop *et al.*, 2008; Bodeanu *et al.*, 2004). The Danube's freshwater and nutrients discharges seem to be the main factor controlling the seasonal and spatial surface chlorophyll *a* distribution in Romanian inner shelf waters. Chlorophyll *a* is related to seasonal variation of the Danube's flow through its significant inverse relationship with salinity (Pearson, r = -0.426, p < 0.05, n = 46) over the whole study period. Moreover, there was found a significant linear correlation between chlorophyll *a* and the total inorganic nitrogen (Pearson, r = 0.503, p < 0.05, n = 46) and no significant correlation between chlorophyll and phosphate and silicates, respectively, probably as a result of P and

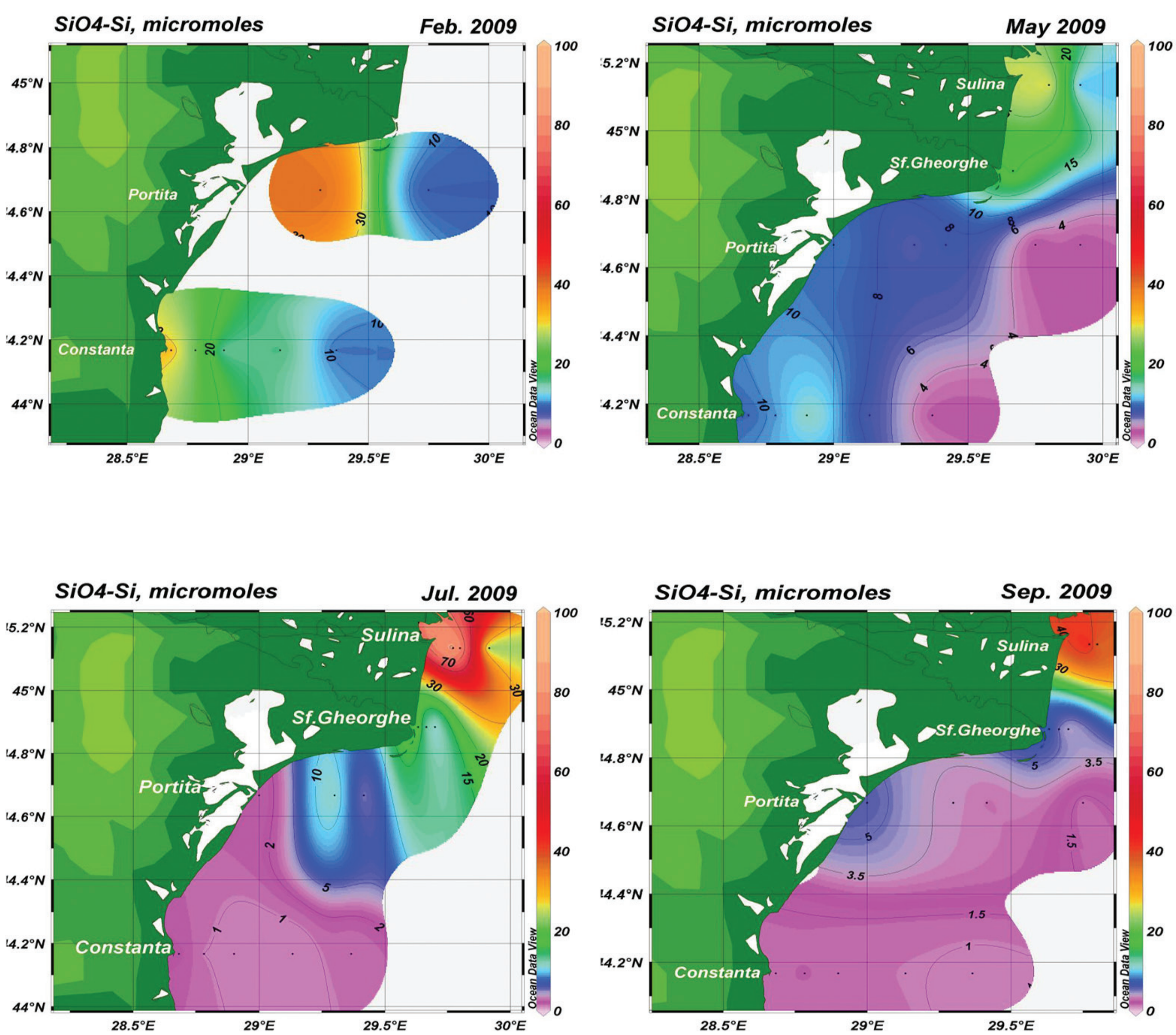

Figure 6 Spatial distribution of SiO_4-Si in Romanian inner shelf waters, in 2009

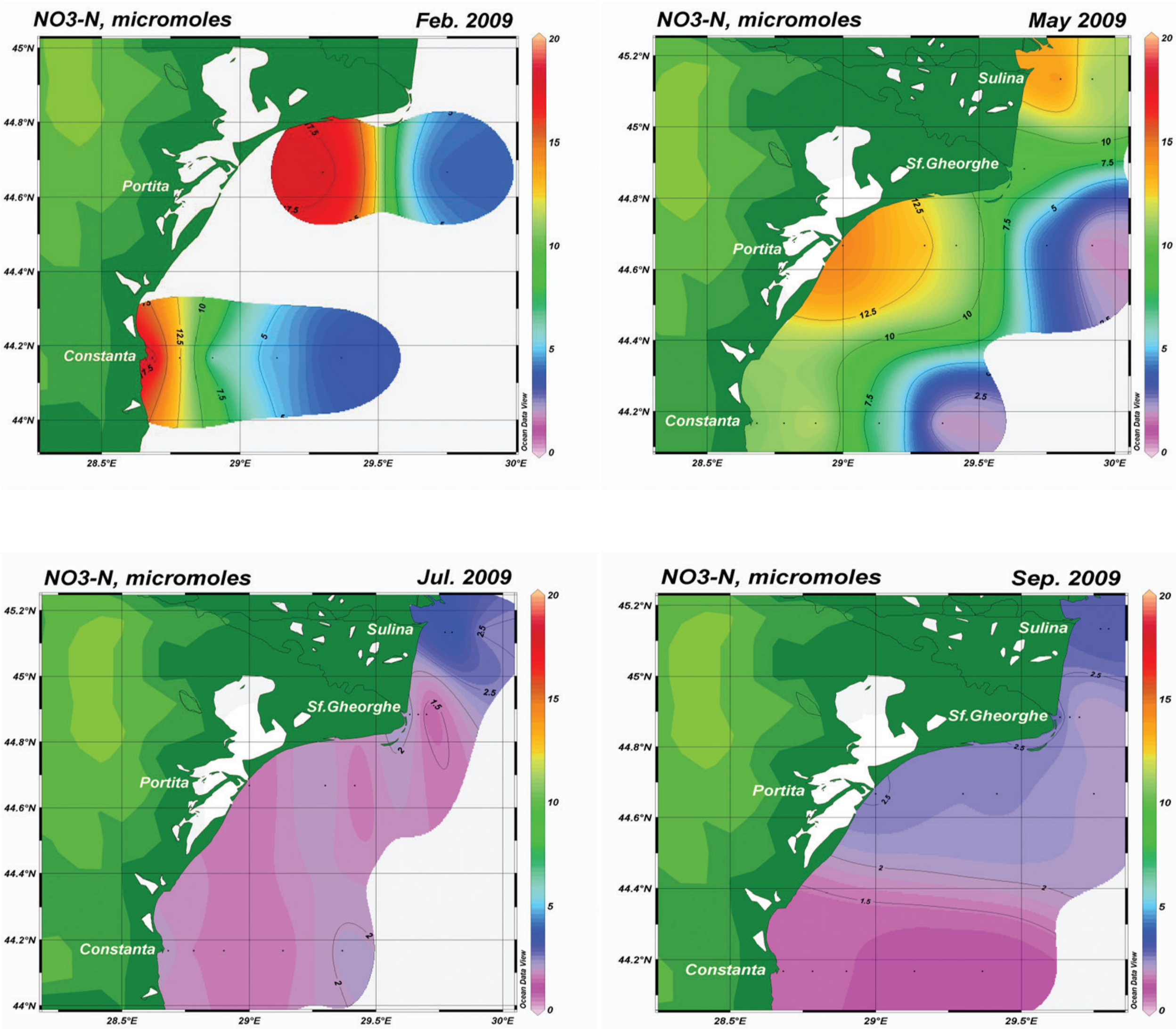

Figure 7 Spatial distribution of NO_3-N in Romanian inner shelf waters, in 2009

Si consumption in the growth of diatoms, which prevail over the populations in the phytoplankton community in Romanian inner shelf waters (SESAME, 2010).

In 2009, the seasonal pattern of chlorophyll *a* distribution was typical for north-western Black Sea inner shelf waters, showing three peaks during the study period (Table 1). The first peak, relatively weak, occurred in February, when the water mixing processes, more intense during the cold season, as well as the higher nutrient discharges from the Danube, led to favorable nutrient conditions in the surface layer (Fig. 5, 6 and 7). According to SESAME FP6 Project (2010), the nitrates and silicates inputs from the Danube were higher in February 2009 (35.5 ktones/month and 57.5 ktones/month, respectively) and there were found strong linear correlations between chlorophyll *a* and salinity (Pearson, $r = -0.963$, $p < 0.0$, $n = 8$), nitrates (Pearson, $r = 0.878$, $p < 0.05$, $n = 8$), silicates (Pearson, $r = 0.939$, $p < 0.05$, $n = 8$) and no correlation with phosphates, which suggests the influence of the Danube's discharges on surface chlorophyll *a* variability.

The second peak occurred in early May, when favorable nutrient and haline conditions (Fig.4, 5, 6 and 7) correlated with sea surface temperature rise led to high values of surface chlorophyll *a*, which varied between 2.45 and 21.92 µg/l.

Despite no significant differences between means recorded in the Danube and Constanta areas, surface chlorophyll *a* showed a rather large spatial variability as a result of the Danube's higher flow and predominance of winds from the northern sector. Thus, it can be remarked a thin surface layer, characterized by low salinity (Fig. 4), high turbidity and nutrient-rich that stretched southwards between the shoreline and 30 m isobaths. The significant relationships between chlorophyll *a* and salinity (Pearson, $r = -0.667$, $p < 0.05$, $n = 13$), nitrates (Pearson, $r = 0.641$, $p < 0.05$, $n = 13$) and phosphates (Pearson, $r = 0.599$, $p < 0.05$, $n = 13$) indicate that low salinity surface layer is a favorable environment for intense phytoplankton growth. On the other hand, there was no significant correlation between chlorophyll and silicates, probably as a result of increased Si consumption, as suggested by the high proportion of diatoms biomass and abundance (about 95 % and 98 % of total biomass and abundance, respectively).

Summarizing, during late winter-spring period, when the Danube's discharges exhibited highest values, surface chlorophyll *a* regime is significantly related to freshwater and nutrients inputs.

In July, chlorophyll *a* values recorded significant reduction in the surface layer, varying between 0.13 and 9.94 µg/l. There was found a weak, but significant inverse linear correlation between chlorophyll *a* and salinity (Pearson, $r = -0.589$, $p < 0.05$, $n = 14$), suggesting the significant influence of freshwater inputs on chlorophyll *a* regime. The highest surface chlorophyll *a* values were recorded in front of the Danube mouths (Sulina and Sf.Gheorghe) (Fig.2) as a result of higher Danube's flow in July (21.65 km^3/month, much larger than the monthly means recorded in July, during this decade) (SESAME, 2010).

Unlike the winter and spring, in July there were observed no significant correlations between surface chlorophyll *a* and nutrients. On the other hand, the total mesozooplankton biomass and abundances reached the highest values in this period (11410.61 mg/m^3 and 407363 ind/m^3, respectively) (Timofte *et al.*, 2010), suggesting the major role that grazing processes played in surface chlorophyll *a* regime.

The third peak occurred in September, when chlorophyll *a* recorded the highest values for the whole study period (1.06 - 26.57 µg/l).

There was found no significant correlations between surface chlorophyll *a* and salinity and nutrients. The absence of any correlation between surface chlorophyll *a* and these variables is due to very high surface chlorophyll *a* concentrations measured in the shallower stations in the Portita area, where the local conditions (reduced action of winds, currents) were favorable to intense phytoplankton growth. Moreover, in these stations were also recorded the highest phytoplankton biomasses and abundances (Fig.3). In spite of the big mesozooplankton quantities recorded in this area (Timofte *et al.*, 2010), the very high values of surface chlorophyll *a*, phytoplankton biomass and abundance (Fig.2, and 3) showed that the primary production exceeded phytoplankton losses through grazing processes.

In spite of relatively similar values of mesozoplankton biomass and abundance (Timofte *et al.*, 2010), as well as of nutrients concentrations measured on Portita and East-Constanta profiles, it is worth mentioning the significant difference between chlorophyll concentrations recorded in the two areas, showing the marked influence of local conditions in the Portita area on the surface chlorophyll distribution.

Unlike rather high surface chlorophyll *a* values recorded in front of Sulina and Sf. Gheorghe mouths, lower values of phytoplankton biomasses were measured in these areas (Fig.3), suggesting the interference of chlorophyll *a* degradation products from resuspended detrital material.

Therefore, considering the lack of significant correlations between surface chlorophyll and nutrients during the warm season, as we mentioned above, one may conclude that the influence of the Danube's nutrients discharges on surface chlorophyll *a* regime decreases in summer and early fall, the biological and physical processes playing an increased role in the quantitative structure of phytoplankton community.

5. CONCLUSIONS

In 2009, surface chlorophyll *a* values in Romanian inner shelf waters varied between 0.13 and 26.57 µg/l.

The seasonal distribution of surface chlorophyll *a* in NW Black Sea inner shelf waters, exhibited three peaks. The first one, relatively weak, occurred in February and was followed by a more pronounced one in early May. These two peaks are strongly related to the Danube's higher freshwater and nutrients discharges during late winter – spring period. After a rather low chlorophyll *a* level recorded in summer due to increased grazing pressure, the third peak was found in September, which is mainly due to very high surface chlorophyll *a* values measured in Portita area as a result of favorable local conditions.

Except for winter, the spatial distribution of chlorophyll a was characterized by high variability. Unlike in May, when the highest values of chlorophyll and biomasses were found in low salinity surface layer on transects East-Constanta and Portita, during the warm season (July and September) the highest values were measured in the Danube mouths area, the maximum being recorded in September, at shallower stations in the Portita area (> 20 $\mu g/l^{-1}$).

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