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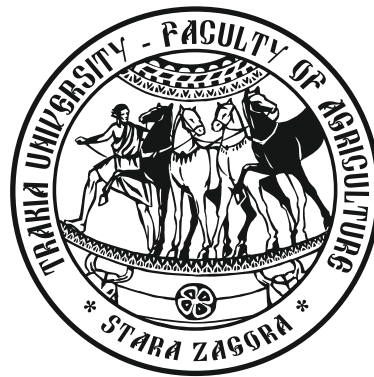
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## Ecological assessment of the phytoplankton community in the Bulgarian Black Sea coastal waters

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**Abstract.** The purpose of this article was to study and make an ecological assessment of the phytoplankton community in the Bulgarian Black Sea coastal waters for the period 2008 – 2010. Taxonomic distribution in the phytoplankton community is traced and analyzed on the basis of 389 quantitative and qualitative seawater samples. Dominant and subdominant phytoplankton species, qualitative and quantitative monthly and seasonal dynamics of the main groups in phytoplankton have been identified as well as their importance for the ecological status of the Black Sea ecosystem. The obtained results for phytoplankton demonstrated a comparative reduction of the trophic degree of the Bulgarian coastal waters, dominating natural factors of the environment over the anthropogenic ones and the present status of "good" and "very good" of the researched coastal area, according to the explored parameters. The phytoplankton annual development is accordingly with the climatic conditions. Also three seasonal phytoplankton successions were defined throughout the year.

**Keywords:** Black Sea, coastal waters, phytoplankton, taxa, ecological assessment

**Abbreviations:** b – dominating in biomass, R/V – Research Vessel, WFD – Water Framework Directive

### Introduction

Unicellular phytoplankton forms the base of food chains in nearly all ocean ecosystems and serves as a primary food source for the marine fauna. Phytoplankton accounts for 50% of the photosynthetic activity on Earth. The status of marine phytoplankton affects commercial fishing, the amount of carbon dioxide absorbed by the ocean and the way in which ocean reacts to climate change (NASA, 2009).

Typifying marine biota according to the condition of a complex of key hydrobionts is a current trend in modern hydrobiology. By definition, the dominant species are a small part of the organic community characterizing the specific biocoenosis (Angelov, 1981). Ecological assessments of marine ecosystem can be made regarding their structure, quantity and distribution of the main taxa in phytoplankton coenosis. When changing the anthropogenic load or climate change, phytoplankton community responds with a change in the dominant complexes and changes in species diversity, numbers and biomass. These indicators are involved in all modern assessments for ecological categorization of marine waters (WFD EU, 2000; Moncheva and Boicenko, 2011; Petrova et al., 2012). The Water Framework Directive (WFD) of EU (2000) requires member states to assess the ecological status of their coastal marine and estuarine waters. This assessment should include certain elements of the phytoplankton communities, such as composition, abundance and biomass (Deliverable, 2012).

The aim of this study was to investigate and make an ecological assessment of the phytoplankton community in the Bulgarian Black Sea coastal waters, based on the dominant and subdominant phytoplankton species, qualitative and quantitative monthly and seasonal dynamics of the major groups in the phytoplankton and their importance for the ecological status of the Black Sea ecosystem.

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### Material and methods

#### *Studied aquatic area*

The study was carried out within the period 2008 – 2010 in the Bulgarian coastal aquatory – up to 30 m depth (Todorova and Moncheva, 2013) and in the shelf zone (up to 200 m) of the Black Sea (Figure 1).

#### *Sampling and preparation*

The scientific expeditions were conducted on board the R/V Prof. Al. Valkanov. 389 phytoplankton samples were collected from 71 stations (Figure 1) at standard horizons (0, 10, 25, 50, 75 and 100 m) by bathometers type Niskin-5L or in shallow water (up to 15 m depth) at surface – bottom horizons. The samples were fixed onboard the ship in 2% formalin solution and concentrated by the sedimentary method (Morozova-Vodyanitskaya, 1954).

#### *Methods of analysis*

The qualitative and quantitative analyses of the samples were performed with a light microscope Nikon E400 in counting cells *Sedgewick Rafter* – 1 ml and *Palmer – Maloney* – 0.05 ml, using standard methods (Moncheva and Parr, 2010).

Microalgae forming trichomes and colonies are presented with the total number of cells in a camera. The cell volume was calculated by geometric formulas (Edler, 1979). Software *Phytomar 2.0* (IFR - Varna 2008) and *Excel 12* (Microsoft Office 2007) were used for calculations and graphs.

Dominant and subdominant phytoplankton species are determined according to the criteria adopted by Lobo and Leighton, (1986) and Colton et al. (2008). According to them, the number of dominant species is  $\geq 50\%$  of the total number in the sample. Subdominant species' number is  $< 50\%$  or  $\geq 25\%$ . By default, all other taxa are defined as "present." The same percentage measure



**Figure 1.** Map of the studied aquatic area

is used for dominance by biomass.

Classification of phyla and classes of phytoplankton microalgae is based on Temniskova and Stoyneva (2011). This distribution of species in classes is according to electronic databases "WoRMS" (WoRMS, 2013) and "Algaebase" (Guiry and Guiry, 2013).

Monthly data and mean values used in the article were averaged over the entire array of data collected in order to analyze general trends in the pelagic part of the Bulgarian coastal waters.

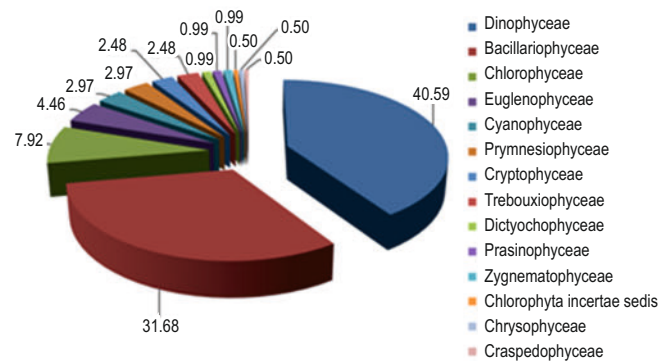
#### Ecological assessment of the hydroecosystem

The ecological assessment of the hydroecosystem in the studied area was made on the basis of the taxonomic composition – dominant and subdominant taxa and phytoplankton dynamics by months and seasons.

## Results and discussion

### Phytoplankton taxonomic composition

In collected 389 phytoplankton samples were identified 202 species and forms of algae, divided into 14 classes (Guiry and Guiry, 2013; WoRMS, 2013). Most important in the marine phytoplankton groups were *Dinophyceae* (40.59%) and *Bacillariophyceae* (31.68%), dominating with 72.28% in the taxonomic composition. The defined percentage was in line with the trend of increasing species diversity of phytoplankton communities in direction of representatives of "other" taxonomic classes established for the entire Black Sea basin since 2000 (Nesterova et al., 2008; Moncheva et al., 2010, 2012). The rest microalgae were distributed in the other 27.72% as the "green" algae subkingdom *Chlorobionta* reached 12.87% (Kozhuharova et al., 2011). The small taxonomic groups were represented by a limited number of species (Figure 2).



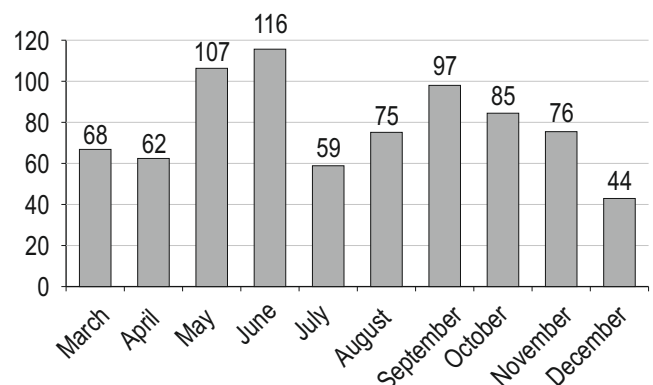
**Figure 2.** Percentage of microalgae taxonomic classes in phytoplankton qualitative composition, 2008 – 2010 (Guiry and Guiry, 2013; WoRMS, 2013)

### Phytoplankton dynamics

Phytoplankton in its development follows a series of time focused consequential changes, succession, as a process of self-organization of the community (Margalef, 1958, 1963, 1967; Holligan and Harbour, 1977; Zaitsev et al., 2006). Stability is necessary for normal development of the succession (Angelov, 1981). In areas with high stability, especially in the warm ocean waters, the cycle can be repeated many times (Raymond, 1980).

The classic scheme of succession begins with development of small diatoms (first stage), then follow large diatoms (second stage) and ends with the dominance of peridines (third stage) (Zaitsev et al., 2006). The development of succession stages of the phytoplankton in the north - western part of the Black Sea does not coincide with the changes described by Margalef (1958) but corresponds to the succession scheme proposed by Raymond (Zaitsev et al., 2006). According to Raymond (1980), in the general case phytoplankton succession begins with upwelling or mixing that enriches the eutrophic zone with nutrients, then small diatoms and flagellates dominate. After that develop *Chaetoceros*, followed by *Rhizosolenia*, coccolithophorids and finally dinoflagellates (Raymond, 1980). Registering the dynamics in the number of phytoplankton algae (2008 – 2010) we found out that the richest in species were June and September while April, July and December were the poorest in species composition. The main pattern in the annual dynamics of phytoplankton was a decline in the number of species. In that general trend three seasonal cycles of phytoplankton development were outlined: increasing the number of microalgae from March to June, from July to September and gradually decreasing towards the end of the year (Figure 3).

The composition of the dominant and subdominant diatoms



**Figure 3.** Dynamics in the number of the identified phytoplankton species by months, 2008 – 2010

**Table 1.** Dominant and subdominant phytoplankton complexes in different months of the year, 2008 – 2010 (Bacillariophyceae /d/, Dinophyceae /p/ and "Others" /o/)

Month	By abundance, %	By biomass, %	July	By abundance, %	By biomass, %
March					
<i>Skeletonema costatum</i> Cleve, 1873 (d)	78.90	75.04	<i>Prorocentrum micans</i> (p)	2.09	39.33
<i>Skeletonema subsalsum</i> Bethge, 1928 (d)	33.90	48.21	<i>Polykrikos schwarzii</i> Bütschli, 1873 (p)	1.11	36.01
<i>Dactyliosolen fragilissimus</i> Hasle, 1996 (d)	60.00	79.74	small <i>Flagellates</i> (o)	67.80	22.68
<i>Gyrodinium lachryma</i> Kof.et Swezy, 1921(p)	1.96	78.68	<i>Merismopedia</i> sp. (o)	64.66	0.29
<i>Gyrodinium spirale</i> Kof.et Swezy, 1921(p)	2.33	73.92	<i>Oscillatoria</i> sp. (o)	81.10	3.53
<i>Protoperdinium granii</i> Balech, 1974 (p)	2.00	65.77	August		
<i>Neoceratium tripos</i> (Müller) Gomez, Mor. et L.-Garcia (p)	1.01	59.45	<i>Proboscia alata</i> (d)	83.33	98.33
<i>Heterocapsa triquetra</i> Stein, 1883 (p)	11.01	29.34	<i>Protoperdinium pellucidum</i> Bergh, 1882 (p)	3.13	42.82
small <i>Flagellates</i> (o)	91.54	66.51	<i>Gymnodinium</i> sp. (p)	1.75	40.12
<i>Merismopedia</i> sp. Meyen, 1839 (o)	63.90	2.29	<i>Prorocentrum micans</i> (p)	1.29	29.00
<i>Apedinella radians</i> Campbell, 1973 (o)	45.70	42.20	<i>Merismopedia elegans</i> Bra. ex Küt., 1849 (o)	55.17	1.82
April			<i>Merismopedia</i> sp. (o)	45.21	0.43
<i>Pseudo-nitzschia delicatissima</i> Heiden, 1928 (d)	68.49	36.63	small <i>Flagellates</i> (o)	85.86	33.45
<i>Cerataulina pelagica</i> Hende, 1937 (d)	3.65	28.75	<i>Oscillatoria</i> sp. (o)	64.52	1.73
<i>Protoperdinium divergens</i> Balech, 1974 (p)	0.74	62.57	September		
<i>Polykrikos schwarzii</i> Bütschli, 1873 (p)	0.15	30.98	<i>Cyclotella caspia</i> Grunow, 1878 (d)	29.20	11.65
small <i>Flagellates</i> (o)	28.88	16.11	<i>Chaetoceros affinis</i> Lauder, 1864 (d)	26.42	2.99
May			<i>Ps.-nit. delicatissima</i> (d)	26.40	5.31
<i>Ps.-nit. delicatissima</i> (d)	82.10	22.74	<i>Prorocentrum micans</i> (p)	8.00	70.09
<i>Pseudosolenia calcar-avis</i> Sundström, 1986 (d)	0.34	65.12	<i>Gyrodinium spirale</i> Kof. & Sw., 1921 (p)	1.09	44.29
<i>Chaetoceros curvisetus</i> Cleve, 1889 (d)	24.23	67.05	<i>Neoceratium furca</i> F.Gomez, D.Mor. & P.Lop., 2009 (p)	1.54	39.00
<i>Dactyliosolen fragilissimus</i> Hasle, 1996 (d)	8.56	28.47	small <i>Flagellates</i> (o)	93.48	67.50
<i>Protoperdinium marie-lebouriae</i> Balech 1974 (p)	0.79	59.04	<i>Merismopedia</i> sp. (o)	91.57	2.53
<i>Prorocentrum micans</i> Ehrenberg, 1833 (p)	2.71	38.78	October	54.63	12.05
<i>Emiliana huxleyi</i> Hay & Mohler, 1967 (o)	67.03	30.06	<i>Skeletonema costatum</i> (d)		
small <i>Flagellates</i> (o)	28.17	11.83	<i>Cerataulina pelagica</i> Hende, 1937 (d)	28.46	47.76
<i>Oscillatoria</i> sp. V. ex Gom., 1892	31.41	0.60	<i>Peridinium</i> sp. (p)	2.78	35.00
June			<i>Protoperdinium granii</i> (p)	0.93	31.91
<i>Ps.-nit. delicatissima</i> (d)	76.82	19.83	small <i>Flagellates</i> (o)	69.35	35.63
<i>Ps. calcar-avis</i> (d)	0.43	55.53	November		
<i>Proboscia alata</i> Sund., 1986 (d)	5.14	55.37	<i>Proboscia alata</i> (d)	32.84	63.95
<i>Cer. pelagica</i> (d)	9.65	44.28	<i>Pseudo-nitzschia seriata</i> Per., 1900 (d)	43.24	14.35
<i>Ch. curvisetus</i> (d)	17.68	27.17	<i>Prorocentrum micans</i> (p)	5.88	56.76
<i>Prorocentrum cordatum</i> Dodge, 1975 (p)	36.79	34.18	<i>Gyrodinium spirale</i> (p)	6.67	62.08
<i>Protoperdinium granii</i> (p)	2.08	66.41	small <i>Flagellates</i> (o)	78.57	18.69
<i>Gymnodinium</i> sp. (p)	3.85	63.29	<i>Merismopedia</i> sp. (o)	51.95	1.44
<i>Emiliana huxleyi</i> (o)	91.76	56.60	December		
small <i>Flagellates</i> (o)	94.12	70.33	<i>Skeletonema costatum</i> (d)	62.23	22.45
July			<i>Neoceratium fusus</i> F.Gomez, D.Mor. & P.Lop., 2009 (p)	0.43	34.63
<i>Pr. alata</i> (d)	49.07	84.54	<i>Protoperdinium granii</i> (p)	0.44	33.90
<i>Chaetoceros insignis</i> Pr.- Lav., 1955 (d)	35.48	53.10	<i>Cochlodinium</i> sp. Schütt, 1896 (p)	1.09	31.88
<i>Ps.-nit. delicatissima</i> (d)	33.49	7.03	small <i>Flagellates</i> (o)	90.84	68.77
<i>Scrippsiella trochoidea</i> Bal. ex Loeb. III, 1965 (p)	4.19	28.77	<i>Merismopedia</i> sp. (o)	40.00	3.31

involved with a high percentage in the biomass changed over the year from species with small cell volume (*Skeletonema costatum* Cleve, 1873 and *Skeletonema subsalsum* Bethge, 1928) in March to large-sized (*Pseudosolenia calcar-avis* Sundström, 1986) in May and June (*Ps. calcar-avis* and *Proboscia alata* Sund., 1986). There was a change in July from small cell volume species *Pseudo-nitzschia delicatissima* Heiden, 1928 and *Chaetoceros insignis* Pr. - Lav., 1955 to large-sized *Pr.alata* in August. From September to November the dominance of diatoms with increasing volumes of cells resumed: September – *Cyclotella caspia* Grunow, 1878; *Chaetoceros affinis* Lauder, 1864; *Pseudo-nitzschia delicatissima*;

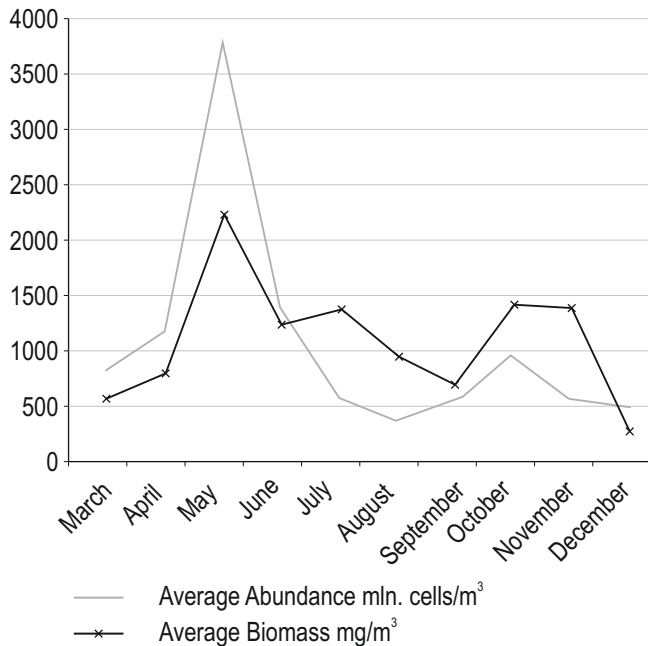
November – *Pr. alata*. In December only *Sk. costatum* dominated and subdominated in number (Table 1).

Throughout the hydrobiological year for the period 2008 – 2010, in the phytoplankton dominated only diatoms, blue-green algae and small flagellates with number  $\geq 50\%$ , whereupon in July and September diatoms dropped out of the group  $\geq 50\%$  (Table 1).

In April, May and June in the group of dominant and subdominant in number diatoms ( $\geq 25\%$ ) occurred only *Pseudo-nitzschia delicatissima*. Along with it among the dominant species were met *Emiliana huxleyi* Hay and Mohler, 1967 and cryptophytes

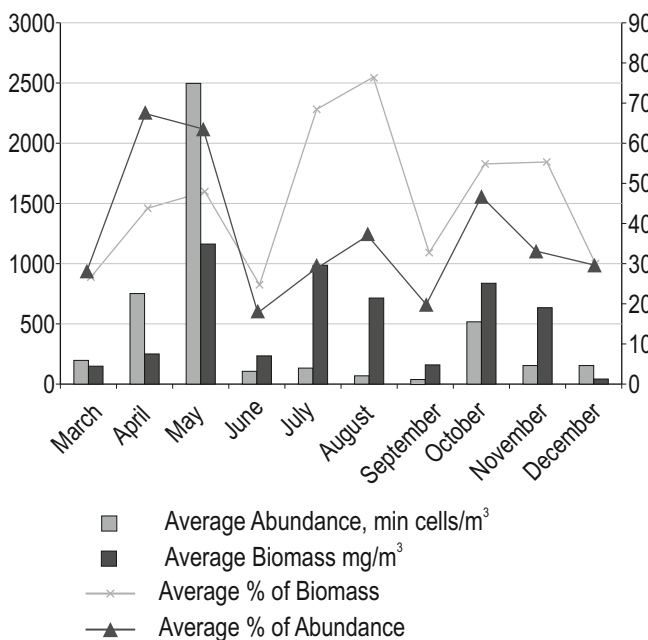
(small *Flagellates*) (Table 1).

According to the traditional understanding of the annual dynamics of phytoplankton stock in temperate latitudes (Raymond, 1980), bimodality in the quantitative values (number and biomass) of phytoplankton was registered in May, October and November (Figure 4). Trimodality of biomass ( $\text{mg}/\text{m}^3$ ) was observed in diatoms with a maximum in May, July and October. In the annual



**Figure 4.** Average monthly quantitative values (number – mln. cells/ $\text{m}^3$ ; biomass –  $\text{mg}/\text{m}^3$ ) of the phytoplankton community, 2008 – 2010

development in number (mln cells/ $\text{m}^3$ ) bimodality was recorded in May and October (Figure 5). The tendency in class Bacillariophyceae was to increase its participation in the total annual biomass



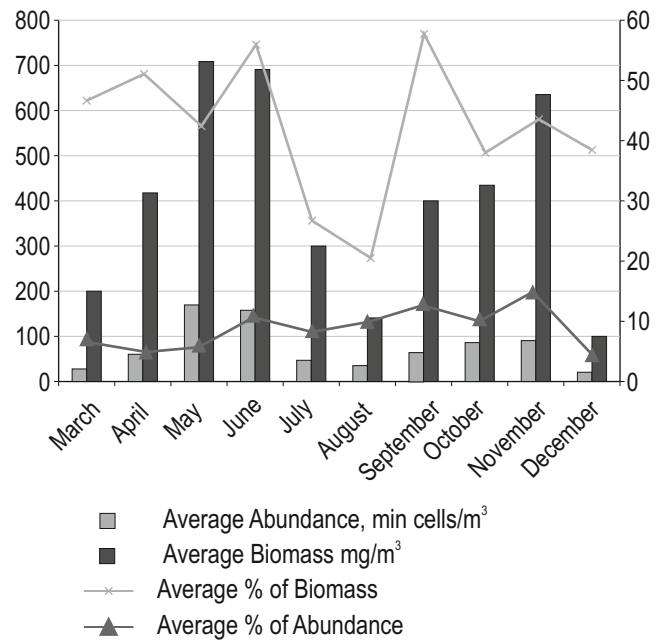
**Figure 5.** Percent of participation (%), average number (mln. cells/ $\text{m}^3$ ) and biomass ( $\text{mg}/\text{m}^3$ ) of class Bacillariophyceae in 2008 – 2010

and reduce its share in the total number (Figure 5).

In 2008 – 2010, the percent of participation (%) of diatoms in the community was modulated by three cycles of development during the year: from March to May (up to 48,38% b, May), from June to August (up to 77.11% b, August) and from September to November (up to 55,71% b, November), in December the cycle started again (Figure 5).

The other major class in the coastal phytoplanktonocenosis *Dinophyceae* was developing with less than 25% participation in number (Figure 6). Only in one sample (Varna Bay) was registered development of *Prorocentrum cordatum* (Ostenfeld, 1901) Dodge, 1975 in the subdominant species with 36.79% in June.

The general trend of class *Dinophyceae* during the year was an

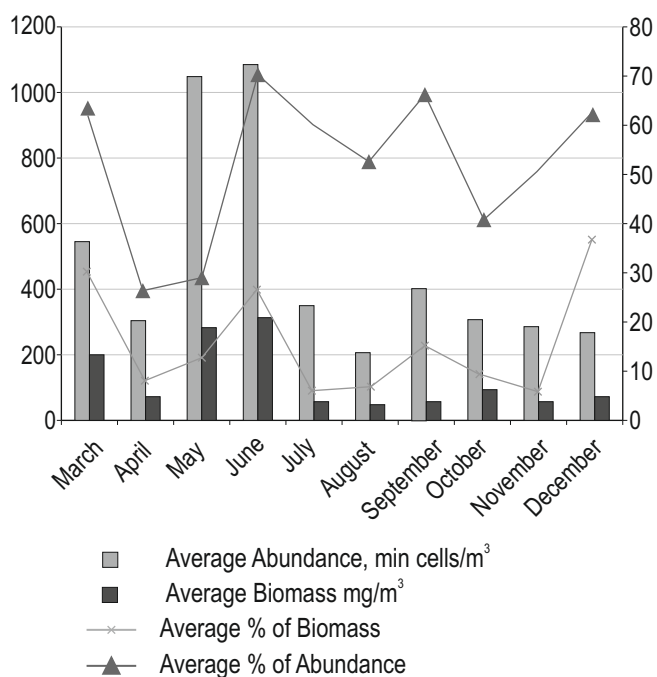


**Figure 6.** Percent of participation (%), average number (mln. cells/ $\text{m}^3$ ) and biomass ( $\text{mg}/\text{m}^3$ ) of class *Dinophyceae* in 2008 – 2010

opposite to the development of diatoms: the percent of participation in biomass decreased while number increased. The annual cycle of development of *Dinophyceae* in number (mln. cells/ $\text{m}^3$ ) and biomass ( $\text{mg}/\text{m}^3$ ) was bimodal with two almost equal maxima in spring (May, June) and autumn (November) (Figure 6). The peaks in percentage shares of peridines in biomass (June – up to 51,25% b and September – to 52,31% b), confirmed the existence of three successive seasonal cycles in the development of phytoplankton community whereupon the third stage ended up with development of peridines (Margalef, 1958; Raymond, 1980; Zaitsev et al., 2006) (Figure 6).

Five species small flagellates *Cryptophyceae* from the group of "Others" dominated in the phytoplankton. Their number was the highest in March and June (Figure 7 and Table 1). *Cyanophyceae* species occurred throughout the year. The most abundant was *Merismopedia* sp. Meyen, 1839 in September. Class *Dictyochophyceae* subdominated in March with *Apedinella radians* (Lohm.) Campbell, 1973 (syn. *A.spinifera*). Class *Prymnesiophyceae* was the most exuberant in May and June (*Emiliania huxleyi*) (Figure 7).

The annual cycle of development of the group "Others" in



**Figure 7.** Percent of participation (%), average number (mln. cells/m<sup>3</sup>) and biomass (mg/m<sup>3</sup>) of class "Others" in 2008 – 2010

number (mln. cells/m<sup>3</sup>) and biomass (mg/m<sup>3</sup>) had one pronounced maximum in March, May and June. The peaks of percent participation in phytoplankton coenocenos of the group of "Others" were in March (up to 31.44% b), July (up to 27.73% b), September (up to 15.53% b) and December (up to 38.05 % b). June and September were the months when spring succession changed into summer and summer into fall. An existence of a winter succession cycle in the development of phytoplankton might be suggested (Figure 7).

From our analysis and the established monthly biodiversity dynamics in 2008 – 2010 (Figures 3, 5, 6, 7 and Table 1) we have identified the presence of three seasonal successions in phytoplankton development in the Bulgarian coastal waters: spring (March, April, May, June), summer (June, July, August, September) and autumn (September, October, November, December). We can reasonably assume the existence of a fourth (winter) phytoplankton succession lasting from December to March. Since in this study the smallest unit in the time-line monitoring is equal to a month, it is not possible to differentiate with higher accuracy the time of passing of one seasonal succession into another.

Throughout the period 2008 – 2010 nine species were defined as dominant in number: *Bacillariophyceae* – 4 species (*Dactyliosolen fragilissimus* Hasle, 1996; *Proboscia alata* Sund., 1986; *Pseudo-nitzschia delicatissima* Heiden, 1928; *Skeletonema costatum* Cleve, 1873); *Cryptophyceae* – small flagellates (*Microflagellates*); *Cyanophyceae* – 3 species (*Merismopedia elegans* Braun ex Küt., 1849; *Merismopedia* sp. Meyen, 1839; *Oscillatoria* sp. Vauch. ex Gom., 1892); *Prymnesiophyceae* – 1 species (*Emiliania huxleyi* Hay & Moh, 1967). Total of 19 species were defined as dominant in biomass: *Dinophyceae* – 9 species; *Bacillariophyceae* – 8; *Prymnesiophyceae* – 1 and *Cryptophyceae* – a group of small flagellates (Table 1).

The total number of dominant and subdominant species according to Lobo and Leighton (1986), includes 57 species and taxa representing 28.22% of the total taxonomic composition. All

microalgae involved in the dominant group are included in the group of subdominant species as well. Studies on marine ecosystem during the eutrophication period of 70s, 80s and 90s show disturbances in its annual cycle of development, in succession cycles and availability of a great number of 'blooming' species which produce large amounts of biomass dominated by peridines in spring.

In this study, we registered a pronounced annual cycle (appropriate to the weather conditions) with relatively low quantitative values similar to those pointed out for a reference period (Regulation No H-4/14.09.2012, SG, No 22, 2013). A regular periodicity was observed and strict adherence of phytoplankton successions to the seasonal weather changes which confirmed the dominance of natural environmental factors over the anthropogenic ones and demonstrated current "good" and "very good" status of the studied water area according to those indicators.

## Conclusion

In the region of the Bulgarian Black Sea coastal and shelf zone within the period 2008 – 2010 were registered 202 species and microalgae forms, divided into 14 classes. Most important in marine phytoplankton were the groups of *Bacillariophyceae* (31.68%) and *Dinophyceae* (40.59%), dominating with 72.28% of the total taxonomic composition. The group of "Others" was represented by 27.72%. Three seasonal phytoplankton succession were defined throughout the year (from March to June – spring one, from June to September – summer and from September to December – autumn) whereupon we also assumed the existence of a winter succession from December to March. Synchronously with the development of the spring and summer successions increased the number of microalgae species. Their number gradually decreased with the development of the autumn phytoplankton succession. The highest quantitative indicators of the phytoplankton stock (number and biomass) were registered in May and October. The quantity of those maxima corresponded to the normal for the temperate latitudes bimodal annual cycle in the development of phytoplankton. General trend of class *Bacillariophyceae* during the year was to increase its share in the biomass and reduce its participation in the number. The general trend in the development of class *Dinophyceae* during the year was an opposite to the development of diatoms: the percent of participation in biomass decreased while the number increased. The group "Others" exhibited maxima in phytoplankton composition in March, June, September and December, i.e. the months when changing seasonal successions. The entire array of phytoplankton data for the period 2008 – 2010 demonstrated a comparative reduction of the trophic degree of the Bulgarian coastal waters according to the indicators of phytoplankton abundance and biomass, and showed a favorable advantage of diatoms in the structural ratio diatoms/peridines.

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