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Biomass, protein- and carbohydrate-composition of phytoplankton in Varna Bay, Black Sea

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Abstract

It was shown that phytoplankton from the Varna Bay, Black Sea, has significantly more suspended carbohydrates, proteins and biomass in July than in April. The dominant species were Bacillariophyceae and Dinophyceae. Electrophoretic and fluorescent spectra have shown the main differences in molecular weight and stability of phytoplankton proteins. Phytoplankton included specific proteins distributed over a limited range of molecular weights between 14 and 72 kilodaltons (kDa). The most abundant protein constituents in phytoplankton samples collected in April were around 45–55 kDa. The seasonal variations of the environment influence the quantitative and qualitative changes in phytoplankton.

Introduction

The interaction of phytoplankton with the environmental factors in the Bulgarian Black Sea coastal zone is well documented (Caspers, 1951; Petrova-Karadjova, 1971, 1973; Moncheva & Krastev, 1997; Shtereva et al., 1998; Moncheva et al., 2002). Changes in the biodiversity of phytoplankton species as well as structural and succession aspects of phytoplankton development in the close coastal zone of Bulgarian Black Sea area have been studied, providing enough evidence of best expressed perturbations in spring and summer (Petrova-Karadjova, 1990; Moncheva et al., 1991; Moncheva, 1992). Phytoplankton has all necessary components, such as proteins, amino acids, lipids (triglycerides, galactolipids and phosphotides), carbohydrates (mostly polysaccharides), minerals and vitamins (Fernandez et al., 1994; Gajewski & Chrost, 1995; Lin et al., 1996; Debrous, 1998). A mixing with deeper layers leads to an adequate supply of all plant nutrients and as result to a rapid growth of phytoplankton. The above mentioned favorable conditions like sufficient supply of sunlight and nutrients are not permanent values: they are undergoing seasonal changes (Danovaro & Fabiano, 1997). Therefore, it is very important to know how these seasonal changes influence the growth of phytoplankton and its quantitative and qualitative composition. It is no information in the literature on the seasonal variations of phytoplankton assemblage structure, its suspended carbohydrates and protein properties. Therefore, it was decided to study during April and July in Varna Bay of the Black Sea: (a) the quantitative structure of phytoplankton assemblage (species composition and abundance); (b) the quantitative and qualitative changes of proteins, using electrophoretic separation and fluorescence.

Materials and methods

Materials

Phytoplankton samples were collected in Varna Bay, Black Sea, during 2 years (1996–1998) in two seasons – starting from middles of April and July-weekly sampling. The study area is the Bulgarian shelf in the Western Black Sea to 40 miles offshore (Moncheva et al., 2001). The samples were taken by RV 'Academik' from the following areas: (station coordinates: Lat. (N) 43° 09′ Long. (E) 28° 40′; distance from the shore- 3 n.m.; sampling depths 0, 10, 25 m (Shtereva et al., 1998). The samples were collected by CTD-rozete system with attached 10 l bottles. Bottle samples from standard sampling depths were processed by the Utermöhl method (Utermöhl, 1958) on inverted microscope. Phytoplankton species larger than 5 μm are enumerated. The cell counts and identification to species level were done on light microscope (Olympus-phase contrast and fluorescence were used when necessary) in a Palmer-Maloney counting chamber. Cell volume (geometrical volume) was used to calculate the biomass for the common species, and standard individual weights for the rare ones.

Sample preparation for protein analysis

About 10 l of each sample were filtered through a Whatmann No. 1 filter. The phytoplankton was then collected from the filter using phosphate buffer. The phytoplankton proteins were precipitated by ammonium sulphate. Then the precipitate was dialyzed extensively against water using cellulose dialysis tubing (Sigma) with a Mw cutoff of 2000. The liquid was lyophilized and kept at 4 °C until use. Composition of various species was determined by elemental analysis.

Protein content

The protein content was measured by the procedure of Lowry et al. (1951) and bovine serum albumin (BSA) was usedas a standard. The protein concentration was determined by measuring the absorbance at 280 nm.

Electrophoretic separation

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) was done according to Laemmli (1970) with our modifications (Gorinstein et al., 2001) in 15% PAAG. The run was done for 2 h, and the gel was fixed, stained with Coomassie Brilliant Blue R-250 and destained.

Fluorescence spectra

Fluorescence measurements were done using a Model FP-770 Jasco spectrofluorometer. Proteins were dissolved in 0.01 M phosphate buffer, pH 7.2. For fluorescence measurements protein solutions were 0.15 mg ml^{-1}. The temperature of the samples was maintained at 30 °C with a thermostatically controlled circulating water bath. Fluorescence emission spectra for all samples were taken at excitation wavelengths (nm) of 274 and 295 and recorded over the frequency range from the excitation wavelength to a wavelength of 500 nm (Gorinstein et al., 2001).

Statistics

To verify the statistical significance of studied parameters the Student *tp* test was used. Means (M) of 5 replicate measurements analyzed samples ± standard deviation (SD) were calculated and then according to the formula $M_2 - M_1 / \sqrt{m_1{}^2 + m_2{}^2}$ the value of t was defined. The Table 'Significance Limits of the Student Distribution' allowed to find out the significance of p. The p values of <0.05 were adopted as statistically significant.

Results

Phytoplankton composition (biomass, carbohydrates, proteins)

The taxonomic composition observed during spring–summer months showed that phytoplankton was represented by species from the following classes: Cyanophyceae, Bacillariophyceae, Dinophyceae, Chrysophyceae, Euglenophyceae and Chlorophyceae. Nonetheless during this period phytoplankton assemblages were dominated by diatoms (Detonula pimula, Skeletonema costatum) and dinoflagellates (Prorocentrum minimum), recorded in blooming densities. Table 1 shows the average number and biomass of the different species of Bacillariophyceae recorded in April and July. The same information regarding Dinophyceae is reported in Table 2. Phytoplankton taxonomic dominance (Bacilariophyceae: Dinophyceae biomass ratio) is shown in this report as well as in our recent publications (Moncheva et al., 2002).

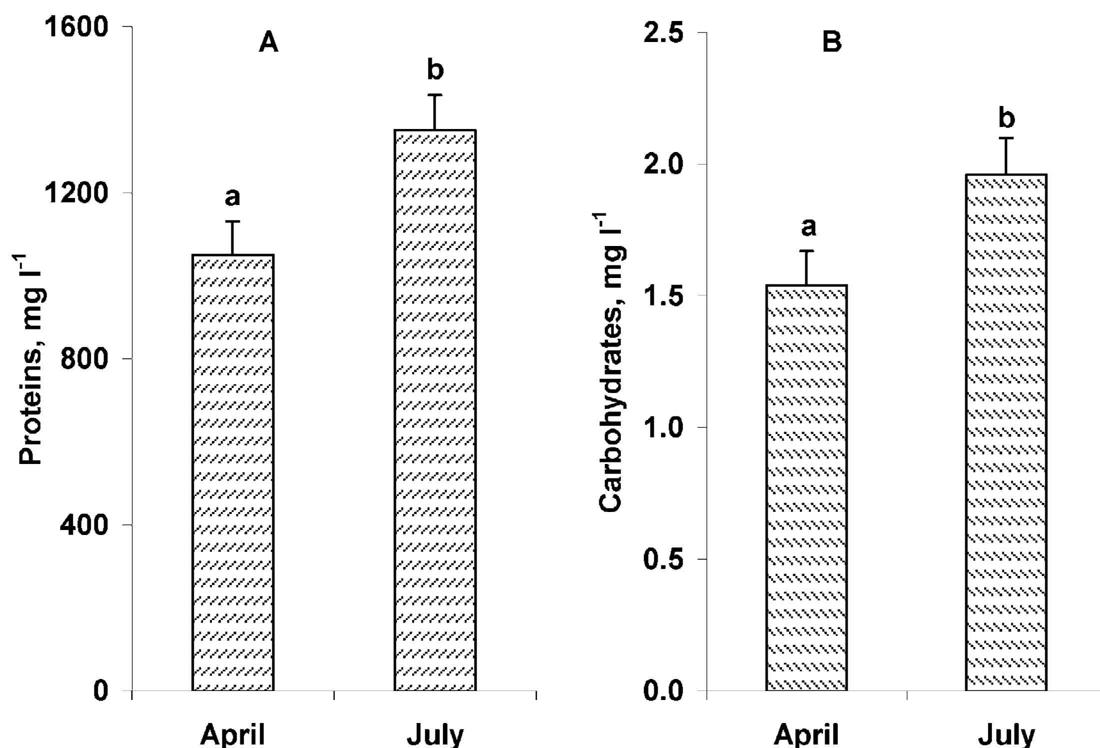

Figure 1. Changes in the contents of the suspended: (A) proteins; (B) carbohydrates. Means±SD (vertical lines). Bars with different letters are significantly different ($p < 0.05$).

Table 1. The quantity of various species of the Bacillariophyceae in April and July in Varna Bay, Black Sea

April			July		
Species	cells l^{-1}	mg l^{-1}	Species	cells l^{-1}	mg l^{-1}
Chaetoceros subsecundus	2800 ± 112	0.0112 ± 0.001	*Cyclotella caspia*	187200 ± 12321	0.041184 ± 0.0029
Dytilum brightwellii	2800 ± 112	0.098 ± 0.01	*Erataulina palagica*	74880 ± 4341	1.64736 ± 0.1432
Licmophora ehrenbergii	1400 ± 101	0.063 ± 0.001	*Licmophora ehrenbergii*	2880 ± 167	0.1296 ± 0.01212
Navicula sp.	5600 ± 313	0.0056 ± 0.001	*Thalassiosira subsalina*	23040 ± 1536	0.140544 ± 0.01322
Total	25200 ± 2430	0.2619 ± 0.02	Total	288000 ± 27420	1.958688 ± 0.21243

Values are means ± SD of 5 measurement.

Table 2. The quantity of various species of Dinophyceae in April and July in Varna Bay, Black Sea

April			July		
Species	cells l^{-1}	mg l^{-1}	Species	cells l^{-1}	mg l^{-1}
Cyst (20 u)	1400 ± 131	0.0266 ± 0.016	*Alexandrium monilatum*	11520 ± 979	0.2304 ± 0.021
Gymnodinium fungiforme	1400 ± 131	0.00077 ± 0.0001	*Gymnodinium splendens*	8640 ± 796	0.1728 ± 0.013
Gyrodinium fusiforme	2800 ± 211	0.098 ± 0.012	*Gyrodinium fusiforme*	2880 ± 213	0.1008 ± 0.012
Gyrodinium lachryma	11200 ± 978	0.4704 ± 0.008	*Gymnodinium uberrimum*	8640 ± 796	0.11232 ± 0.012
Heterocapsa triquetra	14000 ± 989	0.0938 ± 0.011	*Heterocapsa triquetra*	11520 ± 979	0.077184 ± 0.00098
Prorocentrum minimum	5600 ± 521	0.00437 ± 0.0005	*Prorocentrum minimum*	105120000±10012131	81.9936 ± 7.8897
Gymnodinium najadeum	1400 ± 131	0.0182 ± 0.001	*Scrippsiella trochoidea*	11520 ± 979	0.08064 ± 0.00724
Total	56000 ± 5220	1.11534 ± 0.1104	Total	105174720 ± 99255786	82.76774 ± 7.9445

Values are means ± SD of 5 measurements.

Suspended proteins increased in July *versus* April (Fig. 1A). This increase was statistically significant. The same pattern ($p < 0.05$ in both cases) was in the increase of suspended carbohydrates (Fig. 1B).

Electrophoretic patterns

Average intensity bands for the sample of phytoplankton which was collected in middle of July (sample 1) with better resolution (lane 2) presented in Figure 2A. Diffused and very weak bands were fixed for the sample of the middle of April (lane 3). This reduced sample migrated in several bands with an apparent molecular weight of about 14, 29, 40, 48, 60 and 72 kDa. The main bands were at 14 and 72 kDa.

Fluorescence spectra

Lyophilized material of phytoplankton proteins was used for the fluorescence studies. An average fluorescence spectrum of the extracts obtained from the two periods of collected samples is shown in Figure 2B.

Discussion

Phytoplankton composition (biomass, carbohydrates, proteins)

Bacillariophyceae assemblage highlighted a different structure in April and July. *Licmophora ehrenbergii* was the only species common for both periods of time. In July the dominant species were *Cyclotella caspia* in the numerical abundance (cells l^{-1}) and *Cerataulina pelagica* in the biomass (mg l^{-1}). The quantitative analysis indicates that in July the phytoplankton is significantly more abundant in terms of both cells l^{-1} and biomass (mg l^{-1}) than in spring ($p < 0.0005$ in both cases). Even Dinophyceae showed a different composition: only the species *Gyrodinium fusiforme*, *Heterocapsa triquetra* and *Prorocentrum minimum* were present in April as well in July. In July were found the species *Alexandrium monilatum, Gymnodinium splendens*, *Gymnodinium uberrimum* and *Scrippsiella trochoidea*. The dominant dinoflagellate was *Prorocentrum minimum*, recorded in blooming density, typical for the summer season in the area. The quantitative analysis indicates that in the summer phytoplankton there are statistically significant more cells l^{-1} and more mg l^{-1} than in April phytoplankton ($p < 0.0005$ in both cases). The seasonal variations of

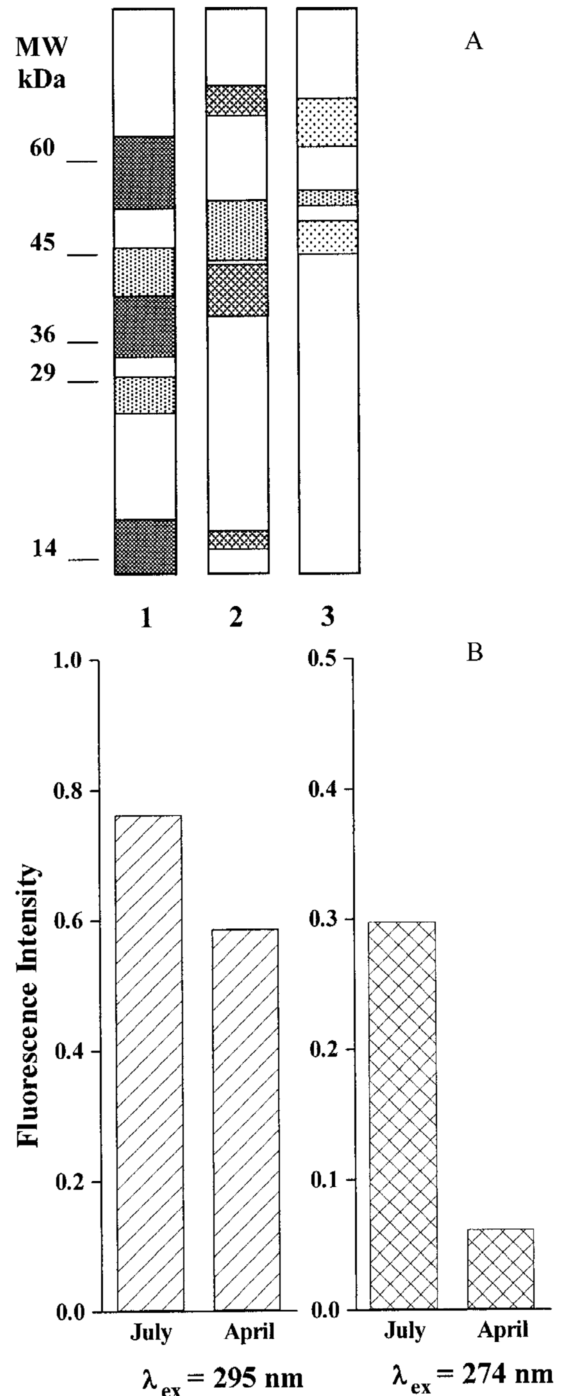

Figure 2. (A) SDS–PAGE separation of phytoplankton proteins in 15% PAAG. (1), molecular weight marker (MW) in kDa: 14.2; 20.1; 24.0; 29.0; 36.0; 45.0 and 66. (2), sample collected in July, (3), collected in April. (B) Fluorescence emission spectra of proteins extracted from phytoplankton. Sample collected in July, sample collected in April. Excitation at 295 nm and 274 nm.

the environmental conditions such as nutrient availability have to be taken for the explanation in the changes of biomass values. The principal nutrients (μg l^{-1}) and their ionic rations at c. Galata were the following: NO_3=37.1;PO_4=11.0; SiO_4=354.5; Si: N=6.3; Si: P=33.8; N: P=5.2. While nitrogen and phosphorus are the two most important nutrients governing overall algal growth (Hecky & Kilham, 1988), the ratios of nutrients present (Tilman et al., 1982) and the variability of Si concentration can regulate the taxonomic composition of phytoplankton assemblages (Edge & Aksnes, 1992). Our results are in accordance with others (Tilman et al., 1982; Hecky & Kilham, 1988; Petrova-Karadjova, 1990; Mikaelyan, 1997) that the increased nutrient loading causes an increase in phytoplankton production, especially diatoms as they are regarded as species with fast response rates to induced environmental changes.

Electrophoretic patterns

April sample had aggregated subunits mostly in average molecular weight around 45–55 kDa (Fig. 2A). Similarity in the bands of the two samples was not found. Our results of the protein separation are similar to the investigation on proteins (Tanoue, 1992, 1996). In these reports, two different groups of proteins were shown with the molecular weight of 45 and 66 kDa.

Fluorescence spectra

Fluorescence spectra in this report were measured without a fluorescent prove, based only on the properties of aromatic amino acids. At the excitation wavelength of 295 nm the fluorescent spectra of the samples were in the interval of 346–368 nm. It was a typical emission for tryptophan containing proteins with the maximum at 346 nm (Determann et al., 1998; Gorinstein et al., 2001). Fluorescence intensity for July sample collection ($I_o = 0.7630$) and for April collection ($I_o = 0.5842$). The displacement shows that tryptophan is in a more polar environment, possibly implying that the protein is in a more opened or relaxed state. The spectrum of July sample has also a shoulder at 398 nm with fluorescence intensity of 0.6400. The emission spectrum for July sample showed the maximum at 398 nm ($I_o = 0.64174$), and for April sample at 340 nm ($I_o = 0.1276$). Proteins from July sample showed high contents of phenylalanine plus tyrosine in comparison with April. Slight shoulders corresponded to tyrosine emission, were seen in April sample and bigger one for July sample proteins at an excitation wavelength of 274 nm (Fig. 2B). At an excitation wavelength of 295 nm tryptophan appears to be the only aromatic amino acid that absorbs light (Gorinstein et al., 2001). For July, sample protein (the maximum at 346.0 nm) may be the contribution of tryptophan and tyrosine or mostly tryptophan itself. In the literature, there are no data available for fluorescent properties of phytoplankton proteins, therefore we can make a linkage in our studies with the changes in the suspended proteins. A maximum of tryptophan emission for vicilin and other plant globulins were around 347 nm, which is in agreement with obtained results on phytoplankton proteins (346 nm). The fluorescence intensity of July sample was higher than for April sample at 295 nm (Fig. 2B) as well as at 274 nm. These results are in correspondence with the changes in the suspended proteins. Our results are similar to Determann et al. (1998). The intensity of emission band was at 340 nm (λ_{ex}= 230 nm). The measurement of the UV-excited fluorescence reveals occurrence of certain aromatic amino acids. Therefore by using the methods, which we have applied to other materials (Gorinstein et al., 2001), we can postulate that the protein material extracted from phytoplankton, has a typical fluorescence spectra.

Conclusion

Our results presented quantitative and qualitative changes in July versus April phytoplankton. Phytoplankton samples collected in July showed significantly more suspended carbohydrates, more biomass cells of the Bacillariophyceae and the Dinophyceae groups than in April phytoplankton ($p < 0.0005$ in both cases). These quantitative changes determine the overall increase in these indices in the July phytoplankton and were similar to other reports (Determann et al., 1998). April and July phytoplankton samples have shown some differences in electrophoresis, which obviously are related to the above described seasonal changes in the phytoplankton assemblage structure, suspended carbohydrates and proteins. Electrophoretic separation of phytoplankton proteins does not give the estimation for their classification. The seasonal changes were shown in the quantity and quality of the protein bands. Differences in the emission peak response and fluorescence intensity in phytoplankton proteins were found. It is tempting to speculate that the changes in the pro-

tein composition of phytoplankton species observed by fluorescence measurements can result from the partial seasonal changes. Originally favorable conditions for phytoplankton growth like sufficient supply of sunlight and nutrients are not permanent values: they are undergoing seasonal changes. The established phytoplankton seasonal quantitative and qualitative changes to a great extent could be related to the seasonal changes in the chemical and physical parameters of the environment, the recurrent summer blooms of the species and anthropogenic eutrophication in the coastal Black Sea zone (Moncheva & Krastev, 1997).

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