Small-scale distribution, accuracy determination and comparability of abundance and diversity of phytoplankton in two Bulgarian reservoirs

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Received: September 20, 2004 ▷ Accepted: February 25, 2005

Abstract. The small-scale frequency distribution of phytoplankton numerical, biomass abundance and individual species diversity were investigated in two Bulgarian reservoirs, differing considerably in size, thermal stratification, trophy and algal species composition. Sampling of about 30 replicates at each site visit allowed the distinguishing between a normal and skewed frequency distribution shape. The coefficient of variation (CV) of phytoplankton abundance variables was 2 to 4 times larger than CV of the diversity index. Significance of application in diversity formula of logarithmic operation and portions of single algal species abundances from the total phytoplankton abundance for explaining these differences was discussed.

Key words: freshwater phytoplankton, reservoirs, sample size, seasons, statistical analysis

Introduction

Distribution of organisms in space could be considered as random, regular and grouping (aggregative, contagious) according to Odum (1986). Distribution is random in a homogeneous environment, described by a symmetric curve, close to the shape of the normal distribution. Conversely, the curve of grouping distribution is skewed to the left or to the right, which means prevalence of small or large groups of individuals correspondingly. As generally known, this type of distribution is responsible for the formation of phytoplankton patchiness. Spatial distribution of phytoplankton could be considered on different scales ranging from a few centimeters or metres (Lund & al. 1958; Harris & Smith 1977; Sandusky & Horne 1978; Venrick 1978; Bakanov 1984; Irish & Clarke 1984; Donaghay & al. 1992; Kils 1992; Holliday & al. 2003) to several metres or 100 km (George & Edwards 1976; Nasev & al. 1978b; Therriault & Platt 1981; Abraham 1998; Olsen & al. 2000; Fennel 2001). Micro, meso and macro scale distribution have been studied by contemporary remote sensing optic and acoustic techniques for environment observation (Donaghay & al. 1992; Kils 1992; Holliday & al. 2003) and by the new model approaches to explaining horizontal phytoplankton patchiness (Abraham 1998; Olsen & al. 2000; Fennel 2001).

Spatial distribution of numerical and biomass abundance of phytoplankton is closely connected to its diversity distribution (Margalef 1978; Washington 1984) and to feeding behaviour of zooplankton and small fishes (Donaghay & al. 1992; Kils 1992). However, despite some publications on the subject (Lund & al. 1958; Nasev & al. 1978a, b; Venrick 1978; Bakanov 1984; Irish & Clarke 1984) there are still no generally accepted recommendations relating to small-scale phytoplankton frequen-

cy distribution and to its connection to accuracy determination of numerical, biomass abundance, diversity, saprobity and other quantitative phytoplankton variables in nature. According to the literature a great variety of methods and approaches have been applied when comparing the above mentioned phytoplankton variables in different basins, sampling sites, seasons etc.

This study investigates abundance and diversity frequency distribution of phytoplankton in two Bulgarian reservoirs of different size, thermal regime and trophy. The large number of sample replicates has made it possible to estimate reliably the accuracy of determination and comparability of phytoplankton diversity, numerical and biomass abundances, to draw conclusions about their normal or skewed character of distribution.

Materials and methods

The phytoplankton was sampled from the reservoirs at depth of 2 m (Iskur) and 1 m (Pchelina), taking into account the different transparency and trophy of the reservoirs. The chosen sampling depths presumed to be closest to the maximum of primary production vertical profile, i.e. at approximately optimal light conditions for phytoplankton development. The measurements in Iskur reservoir (Kalchev 1994) and unpublished data from Pchelina reservoir showed that the depth maxima of primary production in the first reservoir were located between 1–2 meter below the surface, while in the second they fluctuated between 0 and 1 m depth. Small, brownish, partly darkened glass bottles with volume of 143 ml were used for collecting samples in spring, summer and autumn in Iskur and in the summer season of the Pchelina reservoirs. The samples were preserved with Lugol's solution. Additionally we took 1-l samples from Pchelina reservoir. They were collected in plastic bottles and preserved with formaldehyde (4% final concentration). Each sampling visit was presented by 27 to 30 replicates (Table 3), taken by separate successive samples with an opaque 1,8-l plastic water sampler of Friedinger type.

In the laboratory, the phytoplankton was allowed to settle in the bottles for 1–2 weeks. Then the upper layers were siphoned with caution and the rest, if necessary was additionally concentrated by centrifugation (2000–3000 rpm). The phytoplankton was counted as individuals (a colony, single cells or filaments stood for one individual). The small algae were counted in a Bürker haemocytometer chamber under a normal microscope, while the large ones in Utermöhl 5-ml pipe chambers under an inverted microscope. The algal biomass was determined by the routine methodology approximating the shape of algal individuals to simple geometric bodies and assuming that the gravity of 1 cubic centimeter is equal to 1 g. Individual species diversity was calculated by Shannon's formula after Begon & al. (1989). Owing to the lack of a sufficient number of replicates we were not able to determine reliably the exact kind of the distribution and remain at the level of distinguishing between the normal and skewed character of the frequency distribution as practiced by other authors (Lund & al. 1958; Irish & Clarke 1984) for a significance level of P = 0,95. The coefficient of variation (CV) was calculated as usually by ratio between standard deviation and arithmetic mean, multiplied by 100. The non-parametric Mann-Whitney U-test was also applied.

Results

Morphometry and nutrient characteristics

The sampling sites were chosen far enough from the shores and river inflow (Fig. 1). Thus, they represent a truly lacustrine part of reservoirs. The volume difference between reservoirs is one order of magnitude in favor of Iskur reservoir (Table 1), however, the sampling sites have approximately the same depth (Fig. 2). Despite the large reservoir volume, the sampling station of Iskur reservoir shows much weaker temperature stratification in all three seasons, as compared to the temperature depth profile of summer season in Pchelina reservoir.

Table 1 shows also the trophic differences between the reservoirs by means of concentrations and their ratio of three important nutrients for phytoplankton growth. Phosphorus is the limiting element in all four sampling cases; however, its limitation is lowest in Pchelina reservoir. Seasonally, the Iskur reservoir spring samples were relatively more silica- than nitrogen-limited, while the summer and autumn samplings were identical and showed the opposite relation between silica and nitrogen.

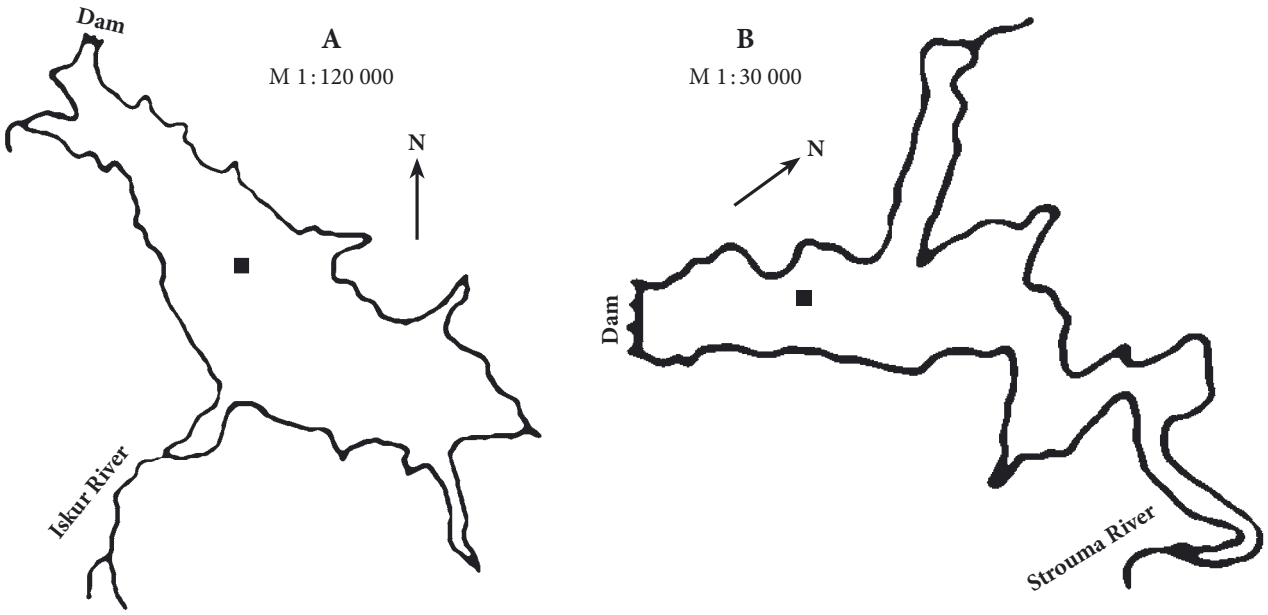

Fig. 1. Schemes according to Ivanov & al (1964) for the Iskur (**A**) and according to Beshkova (1995) for the Pchelina (**B**) reservoirs with the location of sampling sites (■).

Table 1. Some characteristics of Iskur and Pchelina reservoirs and their sampling sites.

Variables	Reservoirs			
	Iskur			Pchelina
North latitude	42° 26′			42° 55′
East longitude	23° 38′			23° 17′
Altitude (m)	820*			620**
Volume, m^3 10^6	673*			54**
Surface, m^2 10^4	3018*			538**
Maximum depth (m)	66*			19,7**
Date of sampling	12.05.1987 (Spring)	25.08.1987 (Summer)	08.10.1987 (Autumn)	03.07.1991 (Summer)
Depth of sampling (m)	2	2	2	1
Water temperature at depth of sampling, °C	9	20,3	16	20,4
Secchi depth (m)	2,5	4,0	2,75	1,45
Wind conditions	no wind	weak to moderate	strong wind	no wind
$\Sigma N_{inorganic}$ $mg.m^{-3}$	1,175	0,404	0,392	4,193
SiO_2-Si, $mg.m^{-3}$	1,3	1,8	1,8	5,843
PO_4-P, $mg.m^{-3}$	0,0049	0,003	0,003	0,068
N:Si:P, rel. units	531:294:1	298:623:1	289:663:1	137:95:1

* data after Ivanov & al. (1964)
** data after Beshkova (1995)

Species composition, abundance and diversity of phytoplankton

Bacillariophyta species prevailed in number in the spring samples and in Iskur reservoir as a whole (Table 2). The number of *Cyanoprocaryota* species was high in summer and autumn seasons for Iskur reservoir and in the summer for Pchelina reservoir. *Chlorophyta* were represented by the highest number of species in summer season for both reservoirs (Table 1). The Iskur reservoir was richer in algal species (22–25) than the Pchelina reservoir (17, Table 2).

Table 2. Species composition of phytoplankton in Iskur and Pchelina reservoirs.

Name of species (genera) in the corresponding divisions	Iskur			Pchelina
	Spring	Summer	Autumn	Summer
1	2	3	4	5
Cyanoprokaryota				
Anabena spiroides Kleb.			+	+
A. scheremetievi Elenkin		+	+	+
Aphanizomenon flos-aquae (L.) Ralfs		+	+	
Microcystis aeruginosa Kütz.	+	+	+	+
Bacillariophyta				
Asterionella formosa Hassall	+	+	+	+
A. gracillima (Hantzsch.) Heib.	+	+	+	
Aulacoseira granulata (Ehrenb.) Simonsen	+	+	+	+
Cocconeis placentula Ehrenb.	+			
Cyclotella radiosa (Grunov) Lemmerm.	+	+	+	
Cymbella ventricosa Kütz.	+			
Eunotia sp.	+			
Fragilaria arcus var. arcus (Ehrenb.) Cleve			+	
F. crotonensis Kitton	+	+	+	+
Gomphonema angustatum Agardh	+			
Melosira varians Agardh	+			+
Navicula sp.	+	+	+	
N. lanceolata (Agardh) Ehrenb.	+			
Nitzschia dissipata (Kütz.) Grunov	+			
N. palea (Kütz.) W. Sm.	+			
Rhoicosphenia curvata (Kütz.) Grunov	+			
Synedra acus Kütz.		+	+	
S. ulna (Nitzsch) Ehrenb.	+			
Pyrrhophyta				
Ceratium hirundinella (O. F. Müll.) Schrank		+	+	
Cryptomonas sp.	+	+	+	
Chlorophyta				
Ankyra judayi (G. M. Sm.) Fott				+
Closterium sp.			+	+
Coelastrum microporum Nägeli	+	+		+
Cosmarium sp.	+	+	+	
Crucigenia quadrata Morren				+
Elakatothrix lacustris Korsh.				+
Kirchneriella lunaris (Kirchn.) K. Möbius	+	+	+	
Monoraphidium arcuatum (Korshikov) Hindák	+	+		
M. contortum (Thur.) Komárk.-Legn.	+	+		
Oocystis sp.		+		+
Pandorina morum (O. F. Müll.) Bory				+
Pediastrum boryanum (Turpin) Menegh.		+		
P. duplex Meyen				+
P. tetras (Ehrenb.) Ralfs		+	+	
Scenedesmus sp.	+	+	+	
S. arcuatus var. *platydiscus* G. M. Sm.				+
S. bijugatus Schmidle				+
S. communis (Turpin) E. H. Hegew.	+	+	+	
Schroederia setigera (Schröd.) Lemmerm.		+	+	
Spirogyra sp.		+		
Staurastrum gracile Ralfs		+	+	
Total	**25**	**25**	**22**	**17**

The numerical abundance in Pchelina was one order of magnitude and the biomass abundance 4 to 5 times higher than in Iskur reservoir (Table 3), which indicated once again the strong trophic differences between them. The numerical abundance in Iskur reservoir decreased considerably towards autumn, while the biomass increased negligibly in the same direction. These seasonal changes were caused by a shift from small to large phytoplankton individuals (Table 3). Another comparison offered by Pchelina reservoir was between small and large volume samples compared qualitatively and quantitatively. It is generally known that large-volume samples contain more species than small ones. The occurrence of *Pandorina morum* in all 1-l samples and only in one of 143 ml samples confirmed that fact. Its large dimensions contributed substantially to the large biomass value in 1-l samples. Logically the average individual weight of the large samples was higher than of the small ones. The individual species diversity in the reservoirs was inversely related to their phytoplankton abundances i.e. high diversity is coupled with low abundance and vice versa. Another important point was that the CV of abundance values was 2 to 4 times higher than the CV of diversity indices. Thus the diversity could be measured more accurately than the abundance, irrespective of the fact that is was based on species abundance values.

The type of frequency distribution of phytoplankton numerical abundance coincided with that of biomass abundance in spring samples of Iskur reservoir and in small samples of Pchelina reservoir, while the summer and autumn samples of Iskur reservoir and large samples of Pchelina reservoir for both variables show different distributions half of which are skewed (Table 3). The skewed distribution is typical for organisms in heterogeneous, partitioned environment. Conversely, most of the corresponding diversity distributions calculated with numerical abundance showing skewed distribution did not differ significantly

Table 3. Abundance, diversity and distribution characteristics of phytoplankton in Iskur and Pchelina reservoirs in different seasons and of different sample volumes.

A, Abundances and Diversity: **AM** – arithmetic mean, **CV** – coefficient of variation

Variables		Reservoirs				
		Iskur			Pchelina	
Time of sampling, seasons		Spring	Summer	Autumn	Summer	
Sample volume, dm^3		0.143	0.143	0.143	0.143	1.0
Numerical Abundance	AM, Ind. dm^{-3}	$8.51\ 10^6$	$2.56\ 10^6$	$0.54\ 10^6$	$1.2\ 10^7$	$1.01\ 10^7$
	CV, %	15.3	19.2	45.6	19.8	12.8
Biomass Abundance	AM, mg.dm^{-3}	7.01	8.25	9.51	34.48	45.0
	CV, %	17.1	30.4	31.4	14.4	25.2
Diversity	AM, bits	2.52	2.58	2.63	1.52	1.6
	CV, %	7.9	7.8	10	5.7	6.3
Average individual weight, ng.ind^{-1}		0.82	3.22	17.6	2.87	4.45
Number of replicates		27	27	27	30	30

B, Distributions: **N** – normal, → – skewed right, ← – skewed left

Numerical abundance	N	→	←	N	N
Biomass abundance	N	←	N	N	←
Diversity	→	N	N	→	N

from the normal type i.e. were not skewed. Similarly, the frequency distribution of phytoplankton numerical abundance in spring samples of Iskur reservoir and in small samples of Pchelina reservoir could not be distinguished statistically significantly from the normal distribution, while the diversity of the same samplings showed a skewed distribution. Small- and large-volume samples showed also distribution differences, which indicated to the significance of sampled volumes.

Discussion

The prevalence of *Bacillariophyta* species in spring, as compared to summer and autumn seasons in the Iskur reservoir and in the Iskur reservoir, as compared to the Pchelina reservoir could be explained by the reduced stratification in spring season and in the Iskur reservoir. Conversely, the number of *Cyanoprocaryota* species increased with strengthening stratification within seasons and reservoirs. Such alternations between both algal divisions based on stratification differences between spring and summer seasons were frequently reported by many authors (Beshkova 1995, 1998; Kusel-Fetzmann 1998). The shift from *Bacillariophyta* to *Cyanoprocaryota* species was also reflected in the nutrient limitation changes presented by the N:Si:P ratio (Teubner & Dokulil 2002) (Table 1) and remained uninfluenced by concomitant wind conditions. Obviously, the stratification differences between the reservoirs are due to their different altitude and the influence of highest mountain of Bulgaria (Rila, with Mousala peak of 2925 m), to which the Iskur reservoir is close. When comparing the phytoplankton of the two reservoirs we should not forget the strong trophic differences between them. The sampling site of the Iskur reservoir was estimated as mesotrophic by chlorophyll-a and phytoplankton productivity and as eutrophic by phytoplankton biovolume, while the Pchelina sampling site was correspondingly classified as eutrophic and hypereutrophic (Kalchev & al. 1996).

The species composition has hardly any influence or other connection to the kind of frequency distribution of the total abundance and diversity of phytoplankton. The comparison of the Iskur reservoir spring sampling to the small sample summer sampling of the Pchelina reservoir confirms this thesis. Both samplings showed similar types of frequency distribution for abundance and diversity but had very different species composition.

A comparison of frequency distributions of numerical and biomass abundance between the seasons (Iskur reservoir) and between small and large sample volume (Pchelina reservoir), as already mentioned, has shown no differences in either abundance variables in spring or in small samples. However, with the occurrence of large individuals in summer, autumn and in 1-l samples the frequency distributions of numerical and biomass abundances became different. Obviously, availability of large algae favored by increasing stratification and large-sample volume caused the observed frequency distribution differences between numerical and biomass abundances. The significance of diameter size of Microcystis colonies for successful distribu-

tion modeling of this species also was emphasized (Olsen & al. 2000).

The skewed distributions in summer in both reservoirs and especially in autumn in Iskur reservoir were most probably due to the influence of factors of a biological, not physical nature (Sandusky & Horne 1978; Therriault & Platt 1981; Fennel 2001). Donaghay & al. (1992), Kils (1992) showed the connection of plankton patchy distribution with chemical data distribution and the effective feeding of zooplankton and young small fishes of *Clupea harengus*. Unfortunately neither our data for nutrients described their spatial distribution character nor do we have available simultaneous samplings for zooplankton density and its distribution.

Unfortunately, we could not find any considerations of the frequency distribution of phytoplankton diversity indices in the available literature except one restricted treatment of their relation to phytoplankton abundance (Margalef 1978). Therefore we should restrict the discussion of this aspect to our data. A comparison of distribution of numerical abundance to that of Shannon diversity index showed in four of the five cases inverse changes: when abundance distribution was symmetric, diversity distribution was skewed and vices versa. This could be explained by the application of logarithm on base 2 in the Shannon diversity formula. As it is generally known, logarithmic transformation of skewed distribution of data is a mean to convert this distribution in a symmetric (normal) one. Conversely, log-transformation of normally distributed data makes them asymmetrically distributed.

Our data and some literature sources showed that the frequency distribution of different phytoplankton variables – numerical and biomass abundance (Lund & al. 1958; Abraham 1998), diversity, chlorophyll (George & Edwards 1976; Harris & Smith 1977; Donaghay & al. 1992; Fennel 2001), production to biomass ratio (Therriault & Platt 1981)) is highly variable over time and space. Differently, Nasev & al. (1978a, b) transported the observed log-normal distribution of algal individuals from the counting chambers to natural conditions in all considered cases. Bakanov (1984) also assumed log-normal distribution for phytoplankton, when deriving his nomogram for number of samplings. However, other authors made clear distinction between distribution of algae in the counting chamber and in the nature, showing also that distribution in the nature may change frequently between symmetric and asymmetric and that the component of counting from total variance is much smaller than the variance caused by taking samples from the natural phytoplankton populations (Lund & al. 1958; Irish & Clarke 1984). Despite the scientists have not arrived to one and the same opinion, the distribution problem has been taken into account when analyzing phytoplankton variables with parametric statistical methods, requiring normally distributed data and equal variances (Nasev & al. 1977, 1978a, b; Bakanov 1984; Irish & Clarke 1984). Thus the distribution problem now is still far from being solved in a universal manner, i.e. we are not able to define what kind of distribution might occur under different circumstances. Even the log-transformation of phytoplankton data for normalizing them did not seem to be a universal means, considering the fact that phytoplankton variables from different sample volumes, seasons and water ecosystems might have different distribution. Therefore, phytoplankton data should be tested always for normality. If the number of measurement is under 100 or insufficient to allow a reliable testing we should recommend non-parametric analyses as practiced by Nasev & al. (1977) and others.

Thus, three season samplings of Iskur reservoir and the small and large samples of Pchelina reservoir were compared by the non-parametric Mann-Whitney U-test. All but the differences of diversity values between the three seasons of Iskur reservoir appear to be statistically significant. The significant differences between small and large samples underline once more the importance of sampling volume. Obviously, the larger samples are closer to the reality.

Another curious observation was the 2- to 4-times lower CV of diversity than the CVs of numerical and biomass abundance. Despite the single species abundances involved in diversity formula the resulting diversity values were characterized by a much lower variability. We suppose that the low CV of diversity is due to inclusion of a logarithm operation in the diversity calculations, which reduced the width spread of data variation. The use of portions of single species of the total phytoplankton abundance in the diversity calculation might be another reason for the observed reduction of diversity CV. In our sample replicates, the dominant species proportions vary to a lesser extent, than their absolute

abundances, while the rare species show a reverse trend: their portions of the total abundance varied more strongly than their absolute values. According to the diversity index formula, the contribution of the dominant species portions is greater than that of rare species and this is another reason to obtain lower variability around the diversity mean value (CV) than around the mean value of absolute abundances. Similarly small CV-s were obtained for another index, not comprising a logarithm operation, but based on relative or absolute portions of single algal species – the saprobity index. This index was calculated for some of the data sets presented here. However we have not published the saprobity values in this article. All this led us to the conclusion that proportions of dominant algal species from the total phytoplankton abundance are less variable, i.e. more stable, than their absolute abundances.

Conclusion

The numerical and biomass abundance of phytoplankton and diversity data sets showed different kinds of small-scale frequency distributions, varying between normal (symmetric) and aggregative, contagious (skewed) shape and influenced by seasons, sample size, water basins etc. Therefore non-parametric statistical methods should be preferred for comparing phytoplankton samples presented by less than 100 replicates or if the normality of data is not proven reliably. The CV (coefficient of variation) and hence the error of phytoplankton abundance determination is 2–4 times larger than the error of diversity determination. Probably this is due to logarithmic operation and application of single species abundance portions from the total phytoplankton abundance involved in the calculation of diversity.

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