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Using sediment diatom assemblages in the assessment of environmental changes in high-altitude lakes, Rila Mts, Bulgaria

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Abstract. Sediment cores were collected from nine high-altitude lakes located in three different cirques of the Rila Mts, Southwest Bulgaria, as a part of a broader Pan-European study of mountain lakes. Diatoms were analyzed from the top (present-day) and bottom (pre-industrial) sediment samples. A total of 213 diatom taxa were recorded. The total number of species found in the different cirques ranges between 115 and 183. Alkalinity, pH and conductivity were the main chemical characteristics which determined the distribution of diatom taxa in this data set of the studied lakes. In the group of lakes located in the Musalenski Cirque pH changes were detected over the last 150 years.

Key words: climate change, diatoms, mountain lakes, palaeolimnology, pH changes

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Introduction

Many palaeolimnological studies have focused on the immediate past (i.e. the last 100–200 years) of some remote mountain lakes, owing to the particular interest in the effect of atmospheric deposition of pollutants in the mountainous areas (Sorvari & Korhola 1998; Cameron & al. 1999; Korchola & Weckström 2000; Catalan & al. 2002; Sporka & al. 2002; König & al. 2002; Clarke & al. 2005). Presently, such studies in the high mountains of Bulgaria are scarce. For example, Ognjanova-Rumenova (2001) and Stephanova & al. (2003) presented taxonomic and palaeoecological descriptions of diatoms from lake Dulgoto in the Pirin Mts. Similarly, Lotter & Hofmann (2003) studied the lake evolution and climate change in the lake Sedmo Rilsko in the Rila Mts.

The project European Mountain Lake Ecosystems: Regionalisation Diagnostics and Socio-Economic Evaluation (EMERGE), supported by the European Commission (Framework Program 5), was aimed at assessing the ecological status of remote high-altitude and high-latitude mountain lakes across Europe. Bulgaria was one of the 12 lake districts which were included in the project. Sedimentary diatom assemblages were studied to evaluate the applicability of diatoms for this monitoring program. Sediment cores were collected from nine lakes situated above the natural timberline in the highest massif on the Balkan Peninsula – the Rila Mts (peak Mousala, 2925 m a.s.l.). For most of the chosen lakes, relevant limnological data should be available, preferably for several years, so as to use these present-day environmental data to estimate the environmental optima and tolerances of the diatom taxa (Leutelt-Kipke 1935; Botev 2000). Diatoms were analyzed from the top (present-day) and bottom (pre-industrial) sediment samples. This ‘top/bottom’ comparison approach is commonly used in the palaeolimnological studies for presenting a ‘snapshot’ of the environmental changes and for assessment of limnological conditions before the identified effects of industrialazion, recent climate change and extensive catchment land-use changes (e.g. Cumming & al. 1992; Dixit & al. 1999; Clarke & al. 2005).

The primary objectives of this study were to identify and document the diatom taxa in the sediment samples of the nine investigated lakes located in three different cirques of the Rila Mts, and to assess which of the environmental variables explains best the variation in the diatom sediment assemblages in the lake development.

Material and methods

Study sites

The first step was to choose a set of mountain lakes that encompass the environmental variables of interest and the range of environmental conditions in their limnological history that we wish to reconstruct. The chosen lakes should cover a sufficient gradient in altitude, bedrock type and limnological characteristics. Nine lakes were considered as good representative ecosystems of the environmental gradients (Table 1). The lakes (with their EMERGE site codes) – Okoto (RI0008), Bubreka (RI0009), Sulzata (RI0010), Bliznaka (RI0011), Alekovo (RI0067), Ledeno (RI0070), Karakashevo (RI0071), Dolno Marichino (RI0075) and Gorno Marichino (RI0076) – are situated in three cirques (Fig. 1). The first group consists of Sulzata, Okoto, Bubreka and Bliznaka lakes, within the Sedemte Ezera Cirque in the northwestern part of the mountain. These lakes are part of the river Struma drainage basin, which drains into the Aegean Sea. The Ledeno, Alekovo and Karakashevo lakes are in the Musalenski Cirque, in the eastern part of the mountain. The main river drainage basin of this area is the river Iskur, which drains into the Danube River. The Dolno Marichino and Gorno Marichino lakes are located in the Marishki Cirque, also in the eastern part of the mountain, but the main river drainage basin here is the river Maritsa, which drains into the Aegean Sea.

All studied lakes are located above the timberline and their altitudes range from 2709 to 2243 m a.s.l. (Table 1). Lake Ledeno is the second highest natural

Table 1. Geographic, morphometric, shore and catchment characteristics of the nine lakes investigated in July and September 2000.

Lake name	Sulzata	Okoto	Bubreka	Bliznaka	Ledeno	Alekovo	Karakashevo	Gorno Marichino	Dolno Marichino
Lake code	RI0010	RI0008	RI0009	RI0011	RI0070	RI0067	RI0071	RI0076	RI0075
Main river basin	Struma	Struma	Struma	Struma	Danube	Danube	Danube	Maritsa	Maritsa
Latitude N (degrees)**	42.1975	42.1996	42.2056	42.2012	42.18206	42.19025	42.1935	42.1611	42.1641
Longitude E (degrees)**	23.3103	23.3058	23.3068	23.3150	23.5891	23.5831	23.5906	23.5962	23.5961
Altitude (m a.s.l.)*	2535	2440	2282	2243	2709	2545	2391	2378	2368
Unique catchment area($\times 10^5 m^2$)*	1.8	3.6	5.6	10	2	1.4	3	11	4
Total catchment area($\times 10^5 m^2$)*	1.8	3.6	5.6	21	2	6.8	11	11	15
Valley orientation*	North	North	North	North	North	North	North	Northeast	Northeast
Surface area ($\times 10^4 m^2$)*	0.7	6.8	8.5	9.1	1.8	2.39	2.62	2.15	1.09
Water volume ($\times 10^3 m^3$)*	15	860	1170	590	97	135.5	80.5	92.3	20
Maximum depth (m)*	4.5	37.5	28.0	27.5	16.4	14.5	6.6	10.8	5.5
Mean depth (m)*	2.1	12.5	13.7	6.5	5.4	5.7	3.1	4.3	1.8
Maximum length (m)*	150	390	456	790	180	210	270	230	148
Maximum width (m)*	70	240	250	190	125	155	150	142	145
Mean width (m)*	47	180	190	116	100	114	97	93	74
Circumference of shore line (m)*	360	1100	1380	2000	495	578	646	651	452
Inlet	no	yes	yes	yes	yes	yes	yes	yes	yes
Outlet	yes	yes	yes	yes	yes	yes	yes	yes	yes
Seepage	no	no	no	no	yes	yes	yes	yes	yes
Anthropogenic impact	h,t	h,s,t	c,f,h,s,t	c,f,h,s,t	h,t	t	h,t	f,t	t

* Data after Ivanov & al. (1964); **Data after http://www.mountain-lakes.org/emerge; anthropogenic impact: c = cattle; f = fishing; h = horses; s = sheep; t = tourism.

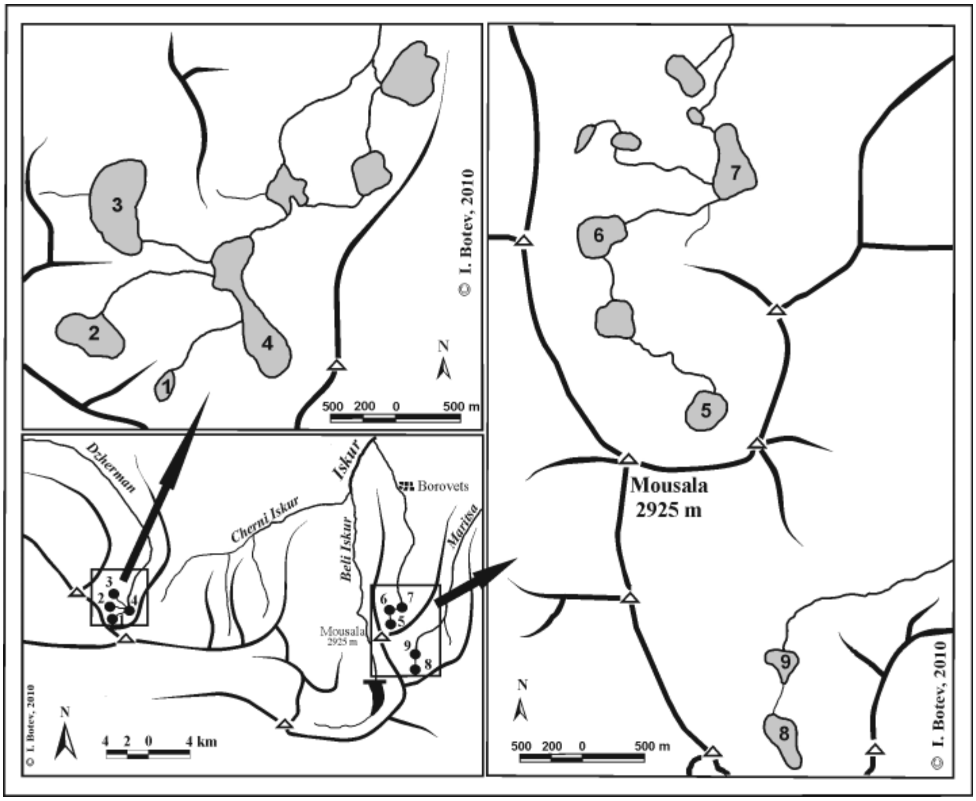


Fig. 1. Map of the Rila Mts, with location of the investigated lakes as follows: **1** – Sulzata (RI0010), **2** – Okoto (RI0008), **3** – Bubreka (RI0009), **4** – Bliznaka (RI0011), **5** – Ledeno (RI0070), **6** – Alekovo (RI0067), **7** – Karakashevo (RI0071) **8** – Gorno Marichino (RI0076), and **9** – Dolno Marichino (RI0075).

lake in Bulgaria (2709 m a.s.l.). Bliznaka is the fifth largest Bulgarian lake, Okoto is the deepest and Bubreka has the third highest water volume among the natural Bulgarian lakes.

Catchment areas of the lakes range from 1.8×10^5 m^2 to $21\times8\times10^5$ m^2 (Table 1). Watersheds of the lakes are predominantly formed of granites but the watersheds of Bubreka, Okoto and Bliznaka (Sedemte Ezera Cirque) also comprise gneisses and amphibolites. Soil percentage cover is above 50 % for the lakes in the Sedemte Ezera Cirque and Marishki Cirque, and below 32 % for the lakes in the Musalenski Cirque. The remaining terrain consists of bare rocks, moraine and debris.

Sampling

Diatom assemblages were analyzed from the 'top' and 'bottom' of each sediment core extracted from the deepest part of the nine surveyed lakes in July and September 2000 by means of a Glew gravity corer (Glew 1991). Core subsamples were taken from each sediment, from the 0–2 cm surface sample and the 15–17 cm slice, and were saved for diatom analysis. Only in lake Dolno Marichino the bottom sample was taken at about 14–16 cm, depending on the core length and the local sediment accumulation rate. Typically, in high-altitude lake systems sediment accumulation rates are low and, therefore, it was assumed that the bottom sample would represent pre-industrial lake conditions. This assumption was tested by analysis of the Sphaeroidal Carbonaceous Particles (SCP) from the bottom sediments of a core extracted in the deepest part of lake Bubreka and determined as a 'master' core. The core was extruded in the field and the section between 0–5 cm was sliced into 2.5 mm intervals, while the section below 5 cm was sliced into 5 mm intervals. From each slice, subsamples for different analyses (physical sediment properties, dating, SCP, cladoceran, chironomid, pigments and diatom remains) were collected. The bottom sample was considered older than *c.* 1800 AD. The first traces of SCP in the profile of the 'master' core were identified in the 1950s (Ognjanova-Rumenova & al. 2009b).

Environmental variables

Present-day samples for environmental parameters were taken from the surface water (0.5 m by means of one-litre Friedenger sampler) at the deepest part of each lake in the course of investigation. The sampling and chemical analyses followed the sampling and analysis protocols described by Mosello & Wathne (1997) and Mosello & al. (1997).

Diatom analysis

Diatom samples were cleaned by the standard methods of Battarbee (1986). Cleaned diatoms were identified and counted under oil immersion, at magnification of ×800 or ×2000, using an Amplival, Carl Zeiss, Jena light microscope. A minimum of 500 valves was counted in each sample (Renberg 1990). In general, the nomenclature of Krammer & Lange-Bertalot (1986–1991) and Lange-Bertalot & Metzeltin (1996) was followed. For those taxa not listed by them, other taxonomic reference works were used, e.g. Reichardt (1999; 2001) for some *Gomphonema* taxa, as well as Alles & al. (1991) for the *Eunotia* taxa. Some of the *Navicula* species sensu lato (*N. pseudosilicula* Hust., *N. detenta* Hust., *N. digitulus* Hust., and *N. accomoda* Hust.) were correlated with type slides held in the Hustedt. Collection, Bremerhaven. Autecology with respect to physical chemistry of the identified diatom taxa was mainly based on Lowe (1974), Krammer & Lange-Bertalot (1986–1991) and Van Dam & al. (1994).

Numerical analysis

Relative abundances of the diatom species were calculated and only those >2 % in any single sample were used for further statistical analysis. The ordination technique – Detrended Correspondence Analysis (DCA) – based on detrending by segments (Hill & Gauch 1980; ter Braak 1995) was used to assess the variations in diatom assemblages, separately in the top and bottom sediment samples. The third DCA was used to assess the changes in species composition between the top and bottom sediment samples within each lake. The plot assemblage scores were averaged for Axes 1 and 2 of the third DCA, so as to evaluate the average changes associated with these axes. The statistical package CANOCO, version 4 (ter Braak & Šmilauer 1998), was used to run all DCAs. Sample scores from two separate DCAs of the top and bottom diatom assemblages and environmental data from Table 1 were correlated by means of the Pearson product-moment coefficients, so as to assess the effect of selected environmental factors on the diatom assemblage composition. Correlations were carried out with the STATISTICA program (StatSoft Inc. 2001).

Results

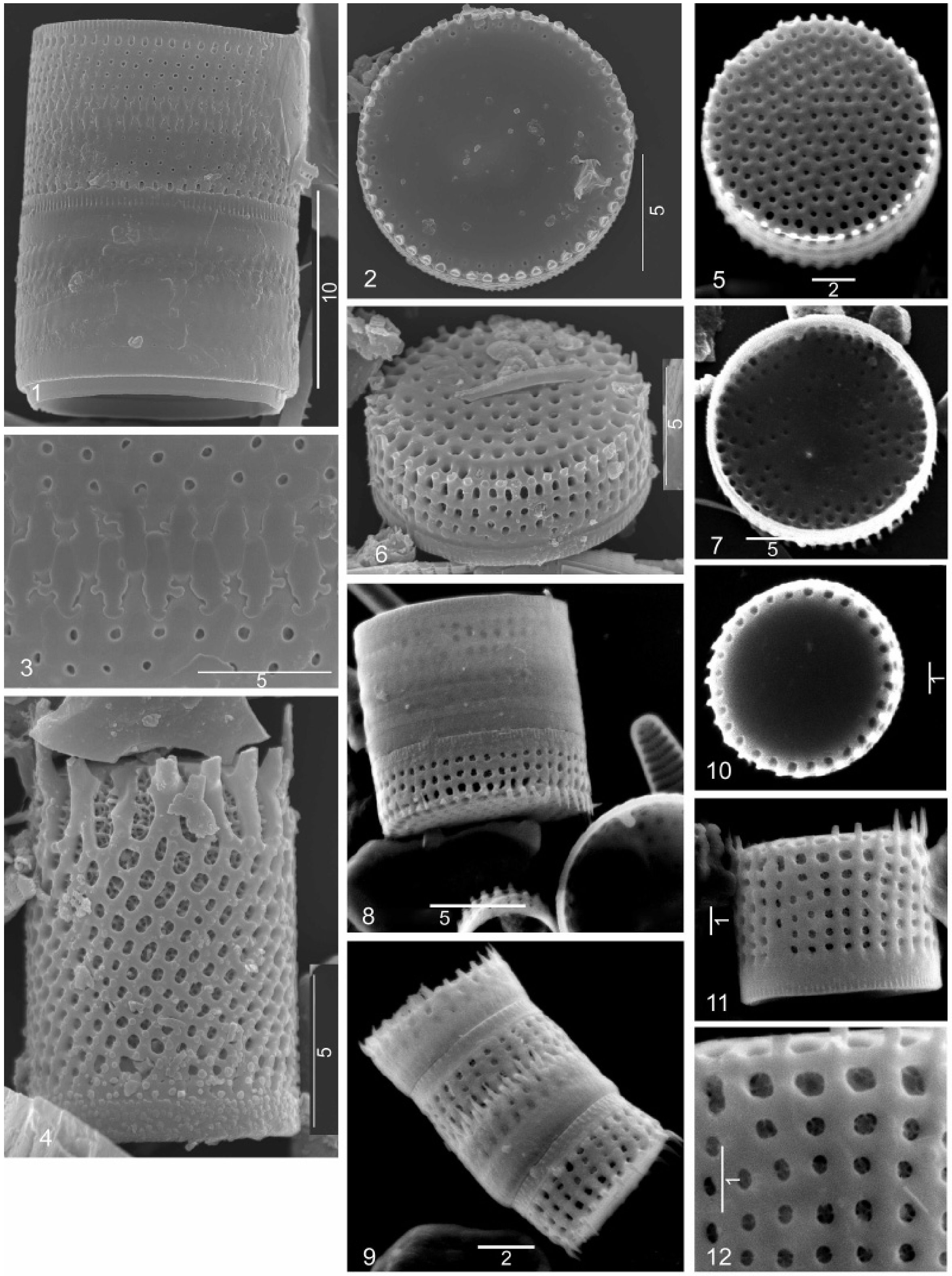
Environmental variables

The pH values varied from 5.61 to 6.76. Alkalinity varied from 21 to 205 μeq/l. Changes in conductivity registered within the range of 14.4 μS/cm to 31.1 μS/cm, and changes in Ca^{2+} from 1.51 mg/l to 4.56 mg/l. All lowest values of these parameters were for lake Ledeno (RI0070) and the highest ones for lake Bliznaka (RI0011). The detailed values of the physical and chemical parameters are given in Ognjanova-Rumenova & al. (2009a).

Diatom assemblages: structure, diversity and relationship with environmental conditions

A total of 213 taxa were recorded, belonging to 36 genera. Only 6.1 % of the diatom taxa belonged to the class Coscinodiscophyceae (Round & al. 1990). The genus *Aulacoseira* Thw. was the most varied and was represented by eight taxa. Within this genus, *Aulacoseira alpigena* was the most frequently occurring species and was recorded in the lakes Sulzata, Alekovo and Karakashevo, where it occurred at a maximum relative abundance of 21.2 %. Three more *Aulacoseira* species (*A. pfaffiana*, *A. valida*, and *Aulacoseira* sp.) were present in the Ledeno and Dolno Maritchino lakes. Among these, *Aulacoseira* sp. (a species with unresolved taxonomical status) occurred with a maximum abundance of 35 % (Plate I). In general, the classes *Fragilariophyceae* and *Bacillariophyceae* dominated the assemblages (93.9 %). The most species-rich genus was *Pinnularia* Ehrenb. (29 taxa), followed by *Eunotia* Ehrenb. (23), *Cymbella* C.Agardh sensu lato (20), *Achnanthes* Bory sensu lato (19), *Fragilaria* Lyngbye sensu lato (18), and *Navicula* Bory sensu lato (17). The other genera were represented by less than 10 species each. Harmonization of diatom taxonomy presented certain difficulties in this wider Pan-European study and involved confirmation of the identity of some critical taxa and recording of species, in order to conform to the used DIATCODE system [http://www.ecrc.ucl.ac.uk/amphora/db]. Four species were unique for the Rila region and they were not included in the DIATCODE system: *Neidium levanderi* (Hust.) Lange-Bert. & Metzeltin, *Navicula pseudosilicula* Hust., *Pinnularia microstauron* var. *brebissonii* f. *diminuta* , *P. pulchra* var. *angusta* (Cl.) Krammer.

Plate I.



Figs 1-12 SEM; scale bars in µm. **Figs 1-3.** *Aulacoseira alpigena* (Grunow) Krammer. **Fig. 1.** External girdle view. **Fig. 2.** External valve view. **Fig. 3.** Detail – linking spines. **Fig. 4.** *Aulacoseira valida* (Grunow) Krammer. **Figs 5-8.** *Aulacoseira pfaffiana* (Reinsch.) Krammer. **Figs 5-6.** External valve view. **Fig. 7.** Internal valve view, note the rimoportulae. **Fig. 8.** External girdle view. **Figs 9-12.** *Aulacoseira* sp. **Fig. 9, 11, 12.** Girdle views. **Fig. 10.** External valve view.

The diatom flora consisted of oligohalobous species, with prevalence of indifferent (57.4 %) and halophobous species (39.8 %). There were a few halophilous species (2.8 %): *Actinocyclus normanii* (Greg.) Hust., *Luticola mutica* (Kütz.) D.G. Mann, *Mastogloia smithii* var. *lacustris* Grunow, and *Thalassiosira bramaputrae* (Ehrenb.) Håkansson. & Locker, with very low abundance in the sediment samples.

With respect to the pH indicator species (182 taxa/85.4 %), indifferent taxa accounted for 36.8 %, followed by alkalophilous (35.2 %), and acidophilous (23.6 %) species. Acidobiontics and alkalibiontics were present far more seldom (2.2 % both).

Biogeographical information was available for 191 taxa (89.7 %). The cosmopolitian species were most common (75.9 %), followed by the nordic-alpine and boreal species (24.1 %). With respect to habitat preference (Lowe 1974; Krammer & Lange-Bertalot 1986–1991; Van Dam & al. 1994), periphytic species dominated the flora, but there were also planktonic and euplanktonic species (7 %), such as *Aulacoseira alpigena, A. valida,* and *Asterionella formosa*. There was an increase in the abundance of planktonic and euplanktonic species over the period of the past *c*.150 years.

One hundred and 95 of the 213 identified taxa (91.5 %) are listed in the German Red List of Diatoms (Lange-Bertalot & Steindorf 1996). Three species – *Achnanthes suchlandtii* Hust., *Pinnularia platycephala* (Ehrenb.) Cleve and *P. brevicostata* Cleve – are considered Extremely Rare, while 38 (19.5 %) are listed as Endangered to a different degree.

Patterns in diatom assemblages

The total number of species found in the different cirques (Sedemte Ezera, Musalenski and Marishki) ranged between 115 and 183. The lowest number (45 taxa) was recorded in lake Ledeno (Musala Cirque) – the second highest natural lake in Bulgaria.

Results of DCA ordination diagram of the top samples are given in Fig. 2. The eigenvalues of the first four axes were λ_1=0.691, λ_2=0.342, λ_3=0.111, λ_4=0.023 (the sum total of all eigenvalues was 2.645) and the length of gradients 3.74, 3.01, 1.28, and 1.66 SD respectively. The variance explained by the first four axes is 26.1 %, 13.0 %, 4.1 %, and 0.9 %. Higher scores for Axis 1 were recorded for several *Fragilaria* species, especially *F. brevistriata*, *F. construens* and *F. pinnata*, as well as for such alkalophilous species as *Asterionella formosa, Surirella tenera* and *Aulacoseira valida,* and such indifferent species as *Navicula radiosa*, *Diatoma mesodon*, *Gomphonema minutum*, and *Cymbella cymbiformis.* These species had the greatest relative abundance in more alkaline lakes, such as Bliznaka, Bubreka, Okoto (Sedemte Ezera Cirque), and Dolno Marichino and Gorno Marichino (Marishki cirque), plotted on the right side of the diagram (Fig. 2). The lowest score along Axis 1 belonged

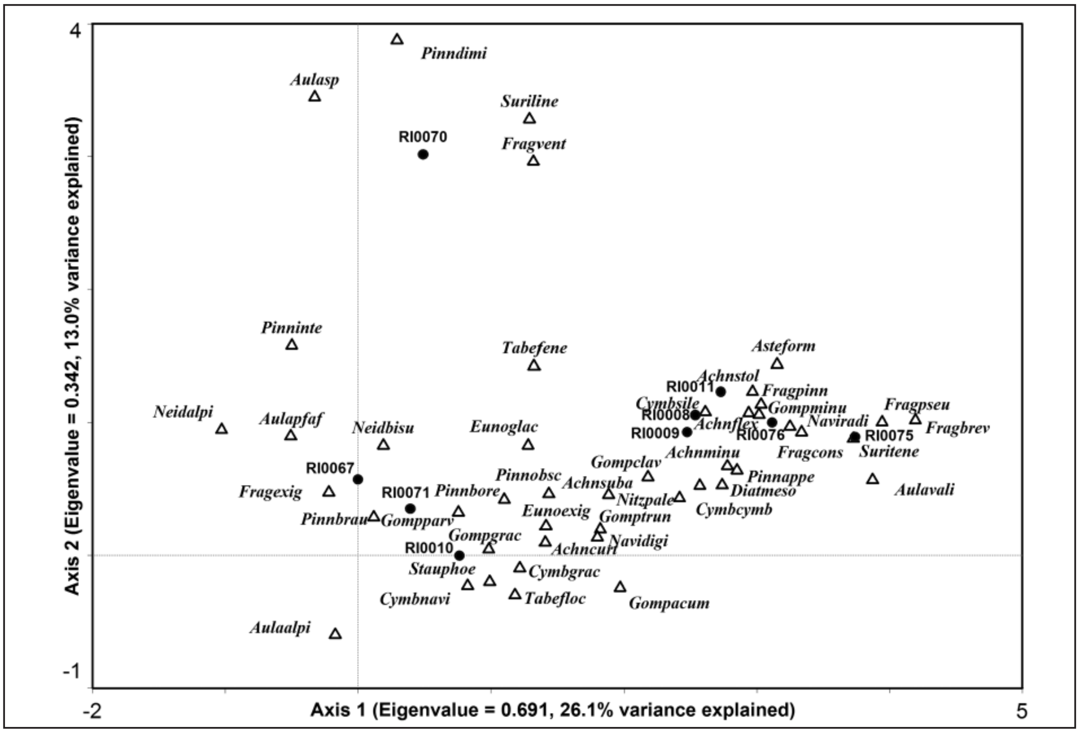


Fig. 2. Ordination diagram based on the Detrended Correspondence Analysis (DCA) of the sediment top diatom samples from nine Rila Mts lakes. For lake codes, see Table 1 and for species names and codes see Appendix 1.

to two *Pinnularia* species (*P. interrupta* f. *minutissima* and *P. braunniana* var. *amphicephala*) which frequently occurr in acidic environments (Ciniglia & al. 2007), as well as to the acidophilous species *Aulacoseira alpigena*, *A. pfaffiana*, *Neidium alpinum*, *N. bisulcatum*, and *Fragilaria exigua*. These species are characteristic for the lakes Sulzata, Alekovo and Karakashevo, plotted on the left side of the diagram. The second DCA axis sets lake Ledeno distinctly apart from all other lakes. This lake is characterized mainly by the occurrence of *Aulacoseira* sp., *Pinnularia microstauron* var. *brebissonii* f. *diminuta*, *Surirella linearis* and *Fragilaria construens* f. *venter* in its surface assemblage.

Results of the DCA ordination diagram of the bottom samples are given in Fig. 3. The eigenvalues of the first four axes were λ_1=0.610, λ_2=0.280, λ_3=0.010, λ_4=0.003 (the sum total of all eigenvalues was 2.416) and the length of gradients was 3.70, 2.42, 2.02, 2.02 SD respectively. The variance explained by the first four axes was 25.2 %, 11.6 %, 0.4 %, and 0.2 %. Higher scores along Axis 1 were recorded for *Aulacoseira* sp., *A. pfaffiana*, *A. alpigena, Fragilaria exigua*, *Pinnularia interrupta* f. *minutissima*, and *Frustulia rhomboides* var. *saxonica*, characteristic of the species assemblages in lakes Ledeno, Alekovo, Karakashevo, and Sulzata plotted on the right side of the diagram (Fig. 3). These lakes were also dominated by the nordic-alpine taxa, i.e. *Cymbella gaeumannii*, *C. gracilis*. The highest scores along the second axis were recorded for *Aulacoseira valida*, *Caloneis bacillum*, *Fragilaria brevistriata*, *F. construens* f. *venter, Surirella tenera*, *S. linearis*, *Cymbella cymbiformis*, and *Sellaphora pupula* species typical for the lakes Dolno Marichino and Gorno Marichino plotted at the top of diagram.

Results of the DCA ordination using both the 'top' and the 'bottom' samples are shown in Fig.4, where the direction of change in assemblage composition is marked by arrows. The eigenvalues of the first four axes were λ_1=0.650, λ_2=0.296, λ_3=0.114, λ_4=0.068 (the sum total of all eigenvalues was 3.382) and the length of gradients 4.19, 2.09, 1.97, and 1.67 SD respectively. The variance explained by the first four axes was 19.2 %, 8.8 %, 3.3 %, and 2.0 %. The average changes associated with the first two axes were 1.672 and 0.549. The more acidic lakes located in the Musalenski Cirque, including RI 0070 (Ledeno), RI0067 (Alekovo) and RI0071 (Karakashevo), with pH values of 5.89, 6.01 and 6.25 respectively, are recorded on the right side of the diagram

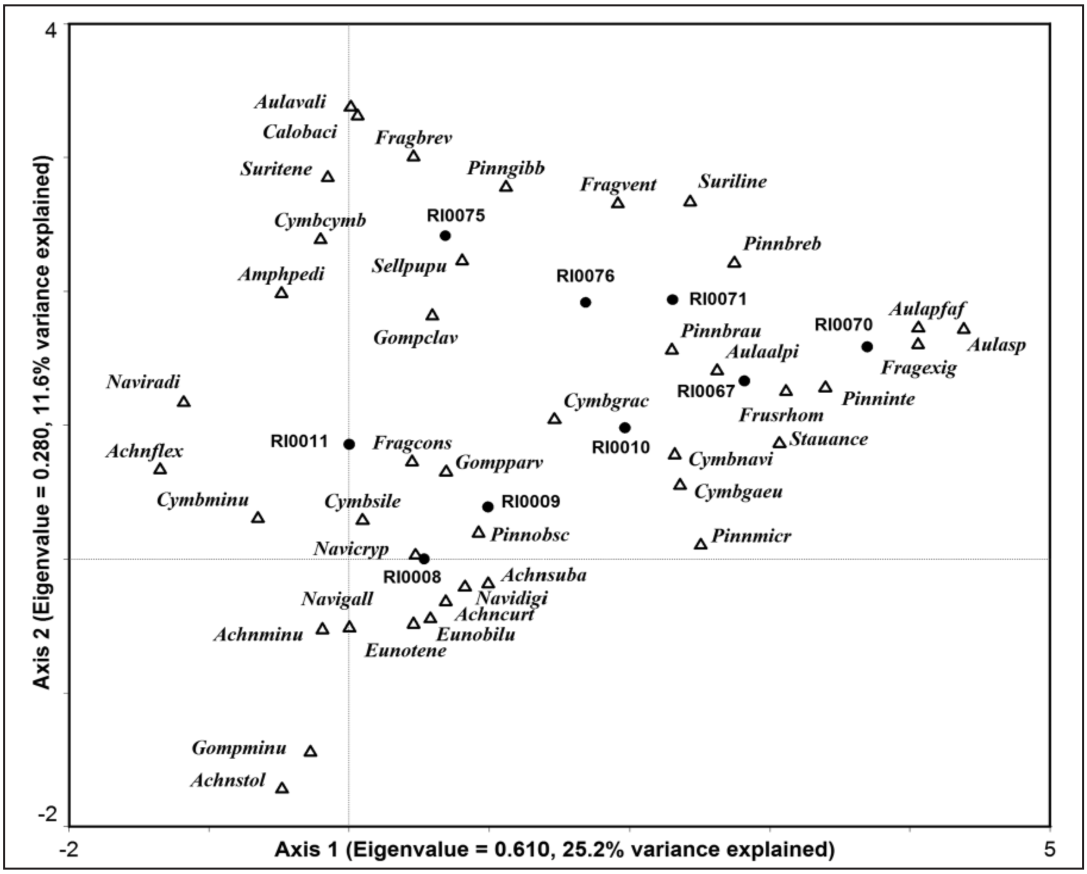


Fig. 3. Ordination diagram based on the Detrended Correspondence Analysis (DCA) of the sediment bottom diatom samples from nine Rila Mts lakes. For lake codes, see Table 1 and for species names and codes see Appendix 1.

Discussion

Alkalinity, pH and conductivity summarize the main chemical characteristics of the lakes. According to these parameters, the lakes were divided manly into two groups. The first one comprising Ledeno (RI0070), Alekovo (RI0067) and Karakashevo (RI0071) lakes contained the lowest values of these parameters, whereas the rest exhibited higher values and the highest ones were recorded in Bliznaka (RI0011) (Table 1). This distinction was mainly conditioned by the catchment areas of the lakes, the influence of the soil cover and the impact on nutrients supply (Psenner 1994; Marchetto & al. 1995; Kopáčeck & al. 1996; Kopáčeck & al. 2000). In the first group, soils constituted a small fraction (8÷32 %) of the entire catchment areas featured by bare rocks, moraine and debris, while in the other group they accounted for 88–95 % and were stronger affected by the anthropogenic influence. On the other hand, all lakes had an Acid Neutralizing Capacity (ANC) range between 20 and 50 μeq/l and only one lake (Alekovo, RI0067) exceeded the critical load of acidification, under the condition that the critical value of ANC_{crit} was assumed to be 20 μeq/l (Curtis & al. 2005). As the gradient length of the first axis for two DCAs was close to 4 SD, there were species in the data (top and bottom) that showed unimodal response along the axis, and the lakes at the opposite ends of the first axis had hardly any species in common (ter Braak 1995; ter Braak & Prentice 1988; ter Braak & Šmilauer 1998). In the first group of lakes, the diatom assemblages dominated by planktonic acidophilic forms (*Aulacoseira pfaffiana*, *A. alpigena*, *Aulacoseira* sp.) were associated with such acidophilic periphytic species as *Frustulia rhomboides* var. *saxonica*, *Fragilaria exigua*, *Cymbella gaeumannii,* and *Pinnularia microstauron*. According to Cingilia & al. (2007), most *Pinnularia* species tolerated well the temperatures and differed in their ability to grow at low pH. *Pinnularia interrupta* f. *minutissima,* a dominant in the surface assemblage from lake Alekovo (RI0067) showed a lower pH limit of 1.5. The abundant and common forms in the second group of lakes were dominated by *Aulacoseira valida* and *Asterionella formosa*, as well as by diverse benthic *Fragilaria* species *(F. pinnata*, *F. brevistriata*, *F. construens*) typical for the littoral and sublittoral of lakes with higher pH. *Fragilaria pseudoconstruens* was also abundant, but it was characteristic of highly oligotrophic cold-water lakes, with high content of dissolved organic carbon (Schmidt & al. 2004). This differentiation was in conformity with the results of two DCAs of the top and bottom diatom samples: the high gradient length and great variance explained the percentage of the first axis, as compared to the others, in particular to the third and fourth (Figs 2, 3). It may represent the pH/alkalinity gradient, i. e. the differentiation of the higher-alkalinity lakes RI0008, RI0009, RI0011, RI0075 and RI0076 from RI0067, RI0070 and RI0071. This was confirmed by the statistical significance correlations between the sample scores of the first DCA axes of two ordinations analyses and the modern environmental variables, as follows: alkalinity (0.76, P=0.017, n=9); Ca^{2+} (0.73, P=0.026, n=9); pH (0.69, P=0.040, n=9) for the top samples, and alkalinity (–0.93, P=0.000, n=9); pH (–0.93, P=0.000, n=9); and Ca^{2+} (–0.91, P=0.001, n=9) for the bottom samples.

The top and bottom samples of the lake region could be seen in Fig. 4, showing the DCA ordination plot of all samples. The direction of changes in every lake is marked by arrows, with the longer arrow denoting the greater change, and can be associated with Axis 1 or 2. By comparing the modern environmental variables for the surface samples located in the opposite ends on the plot, interferences of the variables represented by Axes 1 and 2 can be made (Clarke & al. 2005). The higher degree of change for lake Gorno Marchino (RI0076) and the smaller one for Bubreka (RI0009) and Dolno Marichino (RI0075) are explaining the high average change (1.672) associated with Axis 1 (Fig. 4). Axis 1 may reflect (as it was already mentioned above) a pH/alkalinity gradient. These lakes, with pH and alkalinity of 6.41, 6.47, 6.32 and 120, 149, 117 μeq/l (Table 1) respectively, in contrast to lakes Alekovo (RI0067), Ledeno (RI0070) and Karakashevo (RI0071), with pH values of 5.90, 5.61 and 6.24 respectively, and alkalinity measurements of 34, 21 and 63 μeq/l (Table 1), suggest that the changes occurring in Gorno Marchino (RI0076), Bubreka (RI0009) and Dolno Marichino (RI0075) lakes could have been caused by pH changes in these lakes.

The average change associated with Axis 2 is lower (0.549), but some lakes (Alekovo, Ledeno, Karakashevo, Gorno Marchino, and to a lesser extent Sulzata) have shown changes associated with that axis. The relatively great percentage variance is explained by Axis 2, with its relatively high eigenvalue, suggesting

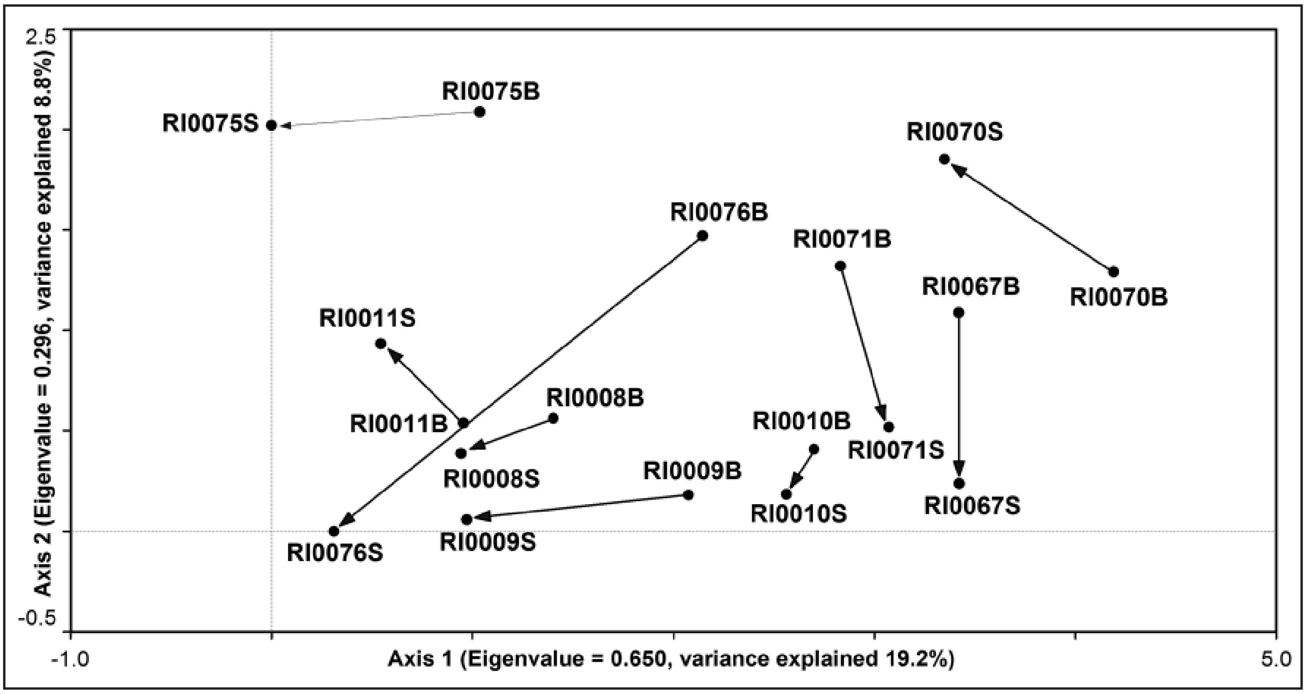


Fig. 4. DCA plot of the top and bottom sediment diatom samples of from nine Rila Mts lakes. For lake codes see Table 1. The suffixes B and S are used for bottom and top sediment samples from each site.

that there are important secondary gradients determining the diatom variance. It is difficult to identify which variables are associated with this axis by means of comparing the modern environmental variables for the surface samples located in the opposite ends of the plot. Some lakes have positive plankton abundance changes from bottom to top within the cores (Fig. 5). At the right side of the diagram the highest altitude lakes are combined (such as Alekovo and Ledeno), where the more prolonged ice-cover regimes affect the diatom assemblage composition (Lotter & Bigler 2000; Catalan & al. 2002; Clarke & al. 2005). Changes in the plankton abundance could be also related to an increase in nitrogen deposition in the area, but this will require further monitoring investigations in the region.

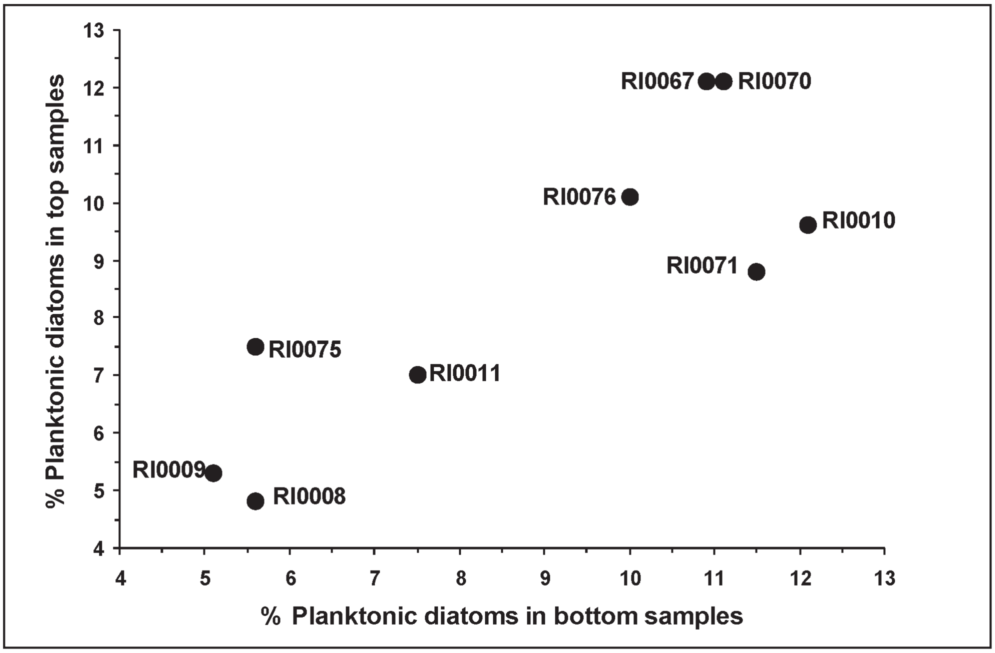


Fig. 5. A diagram of the changes in plankton abundance from top to bottom within the studied cores. For lake codes see Table 1.

Appendix 1. List of the sediment diatoms (surface and bottom) > 2 % in any single sample used in the multivariate analyses with their abbreviated names.

Achnanthes *curtissima* Carter	Achncurt
A. flexella (Kütz.) Brun	Achnflex
A. minutissima Kütz.	Achnminu
A. stolida (Krasske) Krasske	Achnstol
A. subatomoides (Hust.) Lange-Bert. & Archibald	Achnsuba
Asterionella *formosa* Hassall	Asteform
Amphora *pediculus* (Kütz.) Grunow	Amphpedi
Aulacoseira *alpigena* (Grunow) Krammer	Aulaalpi
A. ambigua (Grunow) Simonsen	Aulaambi
A. granulata (Ehrenb.) Simonsen	Aulagran
A. pfaffiana (Reinsch) Krammer	Aulapfaf
A. valida (Grunow) Krammer	Aulavali
Aulacoseira sp.	Aulasp
Brachysira *neoexilis* Lange-Bert.	Bracneoe
Caloneis *bacillum* (Grunow) Cleve	Calobaci
Cymbella *cymbiformis* C. Agardh	Cymbcymb
C. gaeumannii Meister	Cymbgaeu
C. gracilis (Ehrenb.) Kütz.	Cymbgrac
C. minuta Hilse ex Rabenh.	Cymbminu
C. naviculiformis (Auersw.) Cleve	Cymbnavi
C. silesiaca Bleisch 1864	Cymbsile
Diatoma *mesodon* (Ehrenb.) Kütz.	Diatmeso
Eunotia *exigua* (Breb. ex Kütz.) Rabenh.	Eunoexig
E. glacialis Meister	Eunoglac
E. bilunaris (Ehrenb.)Mills	Eunobilu
E. tenella (Grunow)Hust.	Eunotene
Fragilaria *brevistriata* Grunow	Fragbrev
F. construens (Ehrenb.) Grunow	Fragcons
F. construens f. *venter* (Ehrenb.) Hust.	Fragvent
F. exigua Grunow	Fragexig
F. pinnata Ehrenb.	Fragpinn
F. pseudoconstruens Marciniak	Fragpseu
F. tenera (W. Sm.) Lange-Bert.	Fragtene
F. ulna var. *amphirchynchus* (Ehrenb.) Valeva & Temniskova	Fragamph
Frustulia *rhomboides* var. *saxonica* (Rabenh.) DeToni	Frusrhom
Gomphonema *acuminatum* Ehrenb.	Gompacum
G. clavatum Ehrenb.	Gompclav
G. gracile Ehrenb.	Gompgrac
G. minutum (C. Agardh) C. Agardh	Gompminu
G. parvulum (Kütz.) Kütz.	Gompparv
G. truncatum Ehrenb.	Gomptrun
Navicula *cryptocephala* Kütz.	Navicryp
N. digitulus Hust.	Navidigi
N. gallica var. *perpusilla* (Grunow)Lange-Bert.	Navigall
N. radiosa Kütz.	Naviradi
Neidium *alpinum* Hust.	Neidalpi
N. bisulcatum (Lagerst.) Cleve	Neidbisu
Nitzschia *palea* (Kütz.) W. Smith	Nitzpale
Orthoseira *roeseana* (Rabenh.) O'Meara	Orthroes
Pinnularia *appendiculata* (Ag.) Cleve	Pinnappe
P. borealis Ehrenb.	Pinnbore
P. brauniana var. *amphicephala* (Mayer) Hust.	Pinnbrau
P. gibba Ehrenb.	Pinngibb
P. interrupta f. *minutissima* Hust.	Pinninte
P. microstauron (Ehrenb.) Cleve	Pinnmicr
P. microstauron var. *brebissoni* f. *diminuta* Grunow	Pinndimi
P. obscura Krasske	Pinnobsc
Sellaphora *pupula* (Kütz.) Mann	Sellpupu
Stauroneis *phoenicenteron* (Nitzsch) Ehrenb.	Stauphoe
S. anceps Ehrenb.	Stauance
Surirella *linearis* W. Smith	Suriline
S. tenera Greg.	Suritene
Tabellaria *fenestrata* (Lyngb.) Kütz.	Tabefene
T. flocculosa (Roth) Kütz.	Tabefloc

Conclusions

The represented top-bottom comparison approach method can be used in the assessment of regional diatom assemblage changes across the Bulgarian high-altitude mountain lakes. Alkalinity, pH and conductivity summarize the main chemical characteristics of the studied lakes. In the group of lakes located in the Musalenski Cirque (Ledeno, Alekovo and Karakashevo), pH changes were detected over the last 150 years. The increase in planktonic diatoms in the lakes could be affected by changes in the ice-cover regimes. Owing to the limited number of sites (9) and the limited gradients from which our lakes have been chosen (i.e. oligotrophic, bedrock type lakes), our conclusions for the changes in ice-cover regimes could be considered preliminary. Further monitoring is needed to evaluate the direction and size of past and future changes, and to investigate the effects of the increasing load of atmospheric pollutants.

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References

Alles, E., Nörpel-Schempp, M. & Lange-Bertalot, H. 1991. Zur Systematik und Ökologie characteristioschen *Eunotia* Arten (*Bacillariophyceae*) in elektrolytamen Bachoberläufen. – Nova Hedwigia, **53**: 171-213.

Battarbee, R. 1986. Diatom analysis. – In: **Berglund, B.** (ed.), Handbook of Holocene Paleoecology and Palaeohydrology: 527-570. John Wiley & Sons, Inc., Chichester.

Botev, I. 2000. Chemical composition and its vertical distribution in seven glacial high- altitude lakes in the Rila Mountains (Southwest Bulgaria). – In: **Golemansky, V. & Naidenow, W.** (eds), Biodiversity and Evolution of Glacial Water Ecosystems in the Rila Mountains, pp. 89-103. Prof. Marin Drinov Acad. Publ. House", Sofia.

Cameron, N., Birks, H.J., Jones, V., Berge, F., Catalan, J., Flower, R., Garcia, J., Kawecka, B., König, K., Marchetto, A., Sánchez-Castillo, P., Schmidt., R., Šiško, M., Solovieva, E., Štefková, E. & Toro, M. 1999. Surface-sediment and epilithic diatom pH calibration sets for remote European mountain lakes (AL:PE project) and their comparison with the Surface Waters Acidification Programme (SWAP) calibration set. – J. Paleolimnol., **22**: 291-317.

Catalan, J., Pla, S., Rieradevall, M., Felip, M., Ventura, M., Buchaca, T., Camarero, L., Brancelj, A., Appleby, P., Grytnes, A., Agustí-Panareda, A. & Thompson, R. 2002. Lake Redo ecosystem response to an increasing warming in the Pyrenees during the twentieth century. – J. Paleolimnol., **28**: 129-145.

Ciniglia, C., Cennamo, P., De Stefano, M., Pinto, G., Caputo, P. & Pollio, A. 2007. *Pinnularia obscura* Krasske (*Bacillariophyceae, Bacillariophyta*) from acidic environments: characterization and comparison with other acid-tolerant *Pinnularia* species. – Fundam. Appl. Limnol., **170** (1): 29-47.

Clarke, G., Kernan, M., Marcheto, A., Sorvari, S. & Catalan, J. 2005. Using diatoms to assess geographical patterns of change in high-altitude European lakes from pre-industrial times to present day. – Aquatic Sci., **67**(3): 224-236.

Cumming, B., Smol, J., Kingston, J., Charles, D., Birks, H.J., Camburn, K., Dixit, S., Uutala, A. & Selle, A. 1992. How much acidification has occurred in Adirondack Region Lakes (New York, USA) since pre-industrial times? – Canad. J. Fish. Aquatic Sci., **49**: 128-141.

Curtis, C., Botev, I., Camarero, L., Catalan, J., Hughes, M., Kernan, M., Kopáček, J., Korhola, A., Mosello, R., Psenner, R., Stuchlik, E., Veronesi, M. & Wright, R. 2005. Acidification in European mountain lake districts: a regional assessment of critical loadexceedance. – Aquatic Sci., **67**(3): 237-252.

Dixit, S., Smol, J., Charles, D., Hughes, R., Paulsen, S. & Collins 1999. Assessing water quality changes in the lakes of the northeastern United States using sediment diatoms. – Canad. J. Fish. Aquatic Sci., **56**: 131-152.

Glew, P. 1991. Miniature gravity corer for recovering short sediment cores. – J. Paleolimnol., **5**: 285-287.

Hill, M. & Gauch, H. 1980. Detrended correspondence analysis: an improved ordination technique. – Vegetatio, **42**: 47-58.

Ivanov, K., Sotirov, A., Rozhdestvenski, A. & Vodenicharov, D. 1964. Lakes in Bulgaria. Works of the Institute of Hydrology and Meteorology, Nauka i Izkustvo, Sofia, pp. 237. (in Bulgarian)

König, K., Kamenik, C., Schmidt, R., Agustí-Panareda, A., Appleby, P., Lami, A., Prazakova, M., Rose, N., Schnell, O., Tessadri, R., Thompson, R. & Psenner, R. 2002. Environmental changes in an alpine lake (Gossenköllesee, Austria) over the last two centuries – the influence of air temperature on biological parameters. – J. Paleolimnol., **28**: 147-160.

Kopáček, J., Stuchlik, E., Vyhnálek, V. & Závodsky, D. 1996. Concentration of nutrients in selected lakes in the High Tatra Mountains, Slovakia: Effect of season and watershed. – Hydrobiologia, **319**: 47-55.

Kopáček, J., Stuchlik, E., Straškrabová, V. & Pšenáková, P. 2000. Factors governing the nutrients status of mountain lakes in the Tatra Mountains. – Freshwater Biol., **43**: 369-383.

Korchola, A. & Weckström, J. 2000. A quantitative Holocene climate record from diatoms in Northern Fennoscandia. – Quatern. Res., **54**: 284-294.

Krammer, K. & Lange-Bertalot, H. 1986-1991. Bacillariophyceae. – In: **Ettl, J., Gerloff, H. & Mollenhauer, D.** (eds), Süßwasserflora von Mitteleuropa, 1986, 2/1, Teil 1; 1988, 2/2, Teil 2; 1991, 2/3, Teil 3; 1991, 2/4, Teil 4. G. Fisher, Stuttgart & New York.

Lange-Bertalot, H. & Metzeltin, D. 1996. Indicators of oligotrophy. – In: **Lange-Bertalot, H.** (ed.), Iconogr. Diatomol., 2. Koeltz Scientific Books, Koenigstein.

Lange-Bertalot, H. & Steindorf, A. 1996. Rote Liste der Limnischen Kieselalgen (Bacillariophyceae) Deutschlands. – Schriftenreihen Vegetationsk, **28**: 633-677.

Leutelt-Kipke, S. 1935. Hydrographische und hydrochemische Untersuchungen an Hochgebirgsseen des Bulgarischen Rilo Dag. – Arch. Hydrobiol., **28**: 415-436.

Lotter, A. & Bigler, C. 2000. Do diatoms in the Swiss Alps reflect the length of ice-cover? – Aquatic Sci., **62**: 125-141.

Lotter, A. & Hofmann, G. 2003. The development of the Late Glacial and Holocene diatom flora in lake Sedmo Rilsko (Rila Mountains, Bulgaria). – In: **Tonkov, S.** (ed.), Aspects of Palynology and Palaeoecology, pp. 171-185. Pensoft, Sofia-Moscow.

Lowe, R. 1974. Environmental Requirements and Pollution Tolerance of Freshwater Diatoms. Bowling Great State Univ., Cincinati, Ohio.

Marchetto, A., Mosello, R., Psenner, R., Benedetta, G., Boggero, A., Tait, D. & Tartari,. G. 1995. Factors affecting water chemistry of alpine lakes. – Aquatic Sci., **57**(1): 81-89.

Mosello, R. & Wathne, B. 1997. Surface water. Chemical analysis of major ions and nutrients. Analytical quality control. – In: **Wathne, B.M.** (ed.), MOLAR. Measuring and Modelling the Dynamic Response of Remote Mountain Lake Ecosystem to Environmental Change: A Programme of Mountain Lake Research. MOLAR Project Manual, pp. 24-45. NIVA Report 0.96061.

Mosello, R., Wathne, B. & Van der Berg, S. 1997. Surface water. Sampling and analysis protocol. – In: **Wathne, B.M.** (ed.), MOLAR. Measuring and modelling the Dynamic Response of Remote Mountain Lake Ecosystem to Environmental Change: A Programme of Mountain Lake Research., MOLAR Project Manual, pp. 20-23. NIVA Report 0.96061.

Ognjanova-Rumenova, N. 2001. Diatom analysis of Upper Quaternary lake sediments from Dalgoto Ezero in Northern Pirin Mountains: I. Taxonomic remarks. – Phytol. Balcan., **7**(2): 181-194.

Ognjanova-Rumenova, N., I. Botev & M. Kernan. (2009a): Benthic diatom flora in relation to chemical and physical factors in high mountain lakes in the Rila Mountains (Southwestern Bulgaria). – Fundam. Appl. Limnol./Spec. Issues Advanc. Limnol., **62**: 153-166.

Ognjanova-Rumenova, N., Botev, I., Velle, G., Rose, N., Appleby, P., Brancelj, A., Lami, A. & Musazzi, S. 2009b. Environmental changes in the Rila Mountains, Southwestern Bulgaria as recorded by sediment of a remote lake. – Fundam. Appl. Limnol./Spec. Issues Advanc. Limnol., **62**: 295-318.

Psenner, R. 1994. Environmental impacts on freshwaters: acidification as a global problem. – Sci. Total Environm., **143**: 53-61.

Reichardt, E. 1999. Zur Revision der Gattung *Gomphonema*. – In: **Lange-Bertalot, H.** (ed.), Iconogr. Diatomol., **8**: 1-203.

Reichardt, E. 2001. Revision der Arten um *Gomphonema truncatum* and *G. capitatum*. – In: **Jahn, R., Kociolek, J., Witkowski, A. & Compere, P**. (eds), Lange-Bertalot Festschrift: Studies on Diatoms, pp. 187-225. A.R.G. Gentner Verlag K.G.

Renberg, I. 1990. A procedure for preparing large sets of diatom slides from sediment cores. – J. Paleolimnol., **4**: 87-90.

Round, F., Crawford, R. & Mann, D. 1990. The Diatoms. Biology & Morphology of Genera. Cambridge Univ. Press, Cambridge.

Schmidt, R., Kamenik, C., Lange-Bertalot, H. & Klee, R. 2004. *Fragilaria* and *Staurosira* (*Bacillariophyceae*) from sediment surfaces of 40 lakes in the Austrian Alps in relation to environmental variables, and their potential for palaeoclimatology. – J. Limnol., **63**(2): 171-189.

Sorvari, S. & Korhola, A. 1998. Recent diatom assemblage changes in subarctic Lake Saanajärvi, NW Finish Lapland, and their palaeoenvironmental applications. – J. Paleolimnol., **20**: 205-215.

Šporka, F., Štefková, E., Bitušik, P., Thompson, R., Agustí-Panareda, A., Appleby, P., Grytnes, J., Kamenik, C., Krno, I., Lami, A., Rose, N. & Shilland, N. 2002. The palaeolimnological analysis of sediments from high mountain lake Nizne Terianske Pleso in the High Tatras (Slovakia). – J. Paleolimnol., **28**: 95-109.

StatSoft Inc. 2001. STATISTICA (data analysis software system), version 6. www.statsoft.com.

Stefanova, I., Ognjanova-Rumenova, N., Hofmann, W. & Ammann, B. 2003. Late Glacial and Holocene environmental history of the Pirin Mountains (SW Bulgaria): a palaeolimnological study of Lake Dalgoto (2310 m). – J. Paleolimnol., **30**: 95-111.

ter Braak, C.J.F. 1995. Ordination. – In: **Jongman, R.H.G., ter Braak, C.J.F & Tongeren, O.F.R.** (eds.), Data Analysis in Community and Landscape Ecology. pp. 1-173. Cambridge Univ. Press, Cambridge CB2 2RU: 9.

ter Braak C.J.F. & Prentice, I.C. 1988. A theory of gradient analysis. – Advances in Ecol. Res., **18**: 271-317.

ter Braak, C.J.F. & Šmilauer, P. 1998. CANOCO Reference Manual and User's Guide to Canoco for Windows: Software for Canonical Community Ordination (version 4). Microcomputer Power, Ithaca, NY, USA.

Van Dam, H., Mertens, A. & Sinkelda, J. 1994. A coded checklist and ecological indicator values of freshwater diatoms from the Netherlands. – Netherlands J. Aquatic Ecol., **28**(1): 117-133.